The effects of land use and nutritional state on the behaviour, physiology and health of honey bees

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Zusammenfassung

In den letzten Jahrzehnten haben anthropogene Aktivitäten zu einem erheblichen Verlust und einer Fragmentierung von Lebensräumen geführt, was die Qualität der Nahrungsräume und die Ernährungsgrundlage zahlreicher Tierarten, einschließlich Bestäuber wie der Honigbiene (*Apis mellifera*), maßgeblich beeinflusst hat. Diese Dissertation konzentriert sich auf die Honigbiene als Modellorganismus eines sozialen Insekts, um zu untersuchen, wie Landnutzung, Futtermangel und Nahrungsreserven das Verhalten, die Morphologie und die Physiologie von Honigbienen beeinflussen. Das Überleben und die Gesundheit von Honigbienenvölkern hängen von ausreichenden Honigvorräten ab. Diese Vorräte sind entscheidend, um das Volk in Zeiten von Nahrungsmangel zu unterstützen, die kontinuierliche Brutaufzucht zu ermöglichen und die allgemeine Stabilität sowie Produktivität aufrechtzuerhalten. In **Kapitel 1** untersuchten wir, ob die Honigreserven die Schwänzeltanz Kommunikation und die individuelle Gesundheit beeinflussen, indem wir die Honigvorräte in Beobachtungsvölkern manipulierten. Die Studie zeigte, dass die Anzahl Schwänzeltänze zunimmt, wenn Honigbienen wenig Honig haben. Darüber hinaus wiesen Völker mit wenig Honig eine erhöhte Expression des Defensin-1 Gens auf, einem wichtigen Indikator für das Überleben von Bienenvölkern im Winter. Diese Ergebnisse zeigen, dass interner Futterstress die Schwänzeltanz Kommunikation und die Gesundheit von Honigbienen beeinflusst. In **Kapitel 2** wurde der Einfluss der Landschaftszusammensetzung und der Jahreszeit auf die Physiologie und Morphologie von Honigbienen untersucht. Honigbienen wurden während mehrerer Monate in landwirtschaftlichen, urbanen und gemischten Lebensräumen im Südwesten Deutschlands gesammelt. Die Studie zeigte, dass Honigbienen in städtischen und gemischten Lebensräumen im Sommer kleiner waren, stärkeren Flügelverschleiß aufwiesen, aber mehr Fett speicherten als Bienen in landwirtschaftlichen Gebieten. Mit dem Herannahen des Herbstes verringerte sich der Fettvorrat der Bienen in städtischen und gemischten Gebieten ebenfalls. Diese Ergebnisse zeigen, dass Honigbienen morphologische und physiologische Veränderungen als Reaktion auf die Landnutzung und saisonale Schwankungen durchlaufen, was ihr Sammelverhalten und ihr Überleben im Winter beeinflussen könnte. In **Kapitel 3** untersuchten wir weiter, wie Honigbienen ihre Kommunikationsstrategien als Reaktion auf

Veränderungen in der Landnutzung anpassen. Wir analysierten 17 Honigbienenvölker entlang eines ländlich-städtischen Gradienten im Südwesten Englands, wobei wir das Gesamtgewicht der Völker und zentrale Merkmale der Schwänzeltanz Kommunikation im Sommer und Herbst mithilfe eines neuartigen maschinellen Lernalgorithmus zur automatisierten Tanzdekodierung aufzeichneten. Wir stellten fest, dass im Herbst, wenn die Völker an Gewicht zunahmen, die Tänze 47 % mehr Nachtänzerinnen hatten als im Sommer. Außerdem beobachteten wir einen positiven Zusammenhang zwischen Gewichtszunahme und sowohl der Häufigkeit als auch der Intensität der Schwänzeltänze. Unsere Ergebnisse deuten darauf hin, dass die Umweltbedingungen rund um die Honigbienenvölker eine entscheidende Rolle auf das Kommunikationsverhalten und den allgemeinen Ernährungszustand des Volkes spielen. Zusammenfassend erweitert diese Dissertation unser Verständnis darüber, wie sowohl die intern als auch extern bedingten Nahrungsmittelverhältnisse entscheidend für das Verhalten, die Physiologie und damit die Gesundheit und das Überleben der Kolonie sind. Unsere Ergebnisse zeigen, dass gezielte Maßnahmen, wie die Ausweitung halbnatürlicher Lebensräume, das Anpflanzen einer Vielzahl von bestäuberfreundlichen Pflanzenarten und die Förderung ökologischer Anbaumethoden, Honigbienenvölker unterstützen können. Der Einsatz von automatisierter Tanzdekodierung und maschinellem Lernen zur Untersuchung der Sammelmuster von Honigbienen hat sich als vorteilhaft erwiesen und sollte weiter genutzt werden. Diese Werkzeuge ermöglichen die Erfassung und Analyse ökologisch relevanter Daten in größerem Maßstab und tragen zu einem besseren Verständnis der Bienengesundheit und der Nachhaltigkeit der Völker in sich verändernden Landschaften bei.

Summary

Human activities have led to a widespread loss and fragmentation of habitats in recent decades, impacting the foraging habitat quality and nutritional landscape of various animals, including pollinators such as the honey bee (*Apis mellifera*). This thesis focuses on the honey bee as a social insect model to investigate how land use, nutritional stress, and food stores affect honey bee behaviour, morphology, and physiology. The survival and health of honey bee colonies rely on the presence of sufficient honey stores. These stores are crucial in supporting the colony during periods of food scarcity, sustaining continuous brood rearing, and maintaining overall stability and productivity. In **Chapter 1**, we explored whether the food stores affect waggle dance communication, an important communication behaviour in honey bees, and bee health by manipulating the honey storage in observation hives. The study revealed that the number of waggle dances increases when honey bees were deprived of honey. Additionally, honey-starved colonies exhibited higher expression of the *defensin 1* gene, an important indicator of overwinter survival. These findings demonstrate that internal nutritional stress affects waggle dance communication and honey bee health. In **Chapter 2**, the influence of landscape composition and the time of year on honey bee body condition was explored. Honey bees were sampled in agricultural, urban, and mixed habitats throughout a foraging season in southwest Germany. The study revealed that honey bees in urban and mixed habitats were smaller during summer, exhibited greater wing wear, but stored more fat compared to bees in agricultural areas. As autumn approached, bees in urban and mixed areas also experienced a decrease in fat stores. These findings demonstrate that honey bees undergo morphological and physiological changes in response to land use and seasonal variations, which could impact their foraging behaviour and winter survival. In **Chapter 3**, we further explored how honey bees adjust their communication strategies in response to changes in land use. We studied 17 honey bee colonies across a rural-urban gradient in southwest England, recording the overall weight of the colonies and key features of waggle dance communication during summer and autumn using a novel machine-learning algorithm to automate dance decoding. We found that in autumn when colonies gained weight, dances had 47% more followers than in summer. Additionally, we observed a positive relationship between weight gain and both the frequency and intensity of waggle dances. Our findings suggest that environmental conditions surrounding honey bee colonies play a critical role in shaping their communicative behaviour and the overall nutritional state of the colony. In summary, this dissertation expands our understanding of how both the hive-internal and -external nutritional conditions are vital for bee behaviour, physiology and, therefore, colony health and survival. Our findings indicate that targeted actions can support honey bee colonies, such as expanding semi-natural habitats, planting a variety of pollinator-friendly species, and encouraging organic farming methods. The use of automated dance decoding and machine learning in studying honey bee foraging patterns has proven to be beneficial and should be further utilized. These tools enable the collection and analysis of ecologically relevant data on a larger scale, contributing to a better understanding of honey bee health and colony sustainability in changing landscapes.

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General Introduction

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Biodiversity is facing significant pressure in the Anthropocene, driven by climate change, intensified pesticide application, and the introduction of non-native species (De la Rúa et al., 2009; Powney et al., 2019; Soroye et al., 2020). These challenges are further compounded by nutritional deficiencies stemming from habitat loss and fragmentation (Seddon et al., 2016). In the last decades, the emergence and spread of intensive agriculture (Aizen et al., 2008; Otto et al., 2018; Plourde et al., 2013), the demand for materials for industry, and rapid urbanisation (Mcdonald et al., 2008; Seto et al., 2012a) have destroyed habitats and altered the distribution, diversity, quality, and temporal availability of resources in these habitats. This threatens the health and survival of many organisms, including social insects (Crist et al., 2017). In social insects, communication, however, has evolved to help them solve the challenges they encounter and adapt efficiently to environmental change (Alves et al., 2023). Eusocial bees, including honey bees (Apini), are particularly notable for their sophisticated communication methods, which have evolved to help them overcome environmental challenges. The best-studied method is the waggle dance, a fascinating behaviour that enables honey bees to convey information about the location of highly beneficial food resources, water sources, and new nest sites (von Frisch, 1967; Lindauer, 1955). Uniquely, humans can decode this communication behaviour, gaining insights into the costs and benefits of inhabiting different land uses and the impacts of land use changes on bee behaviour and survival.

Land-use change in the Anthropocene

Anthropogenic habitat conversion and agricultural intensification are major drivers of landscape changes and habitat loss (Foley et al., 2005; Graitson et al., 2020). Over 75% of terrestrial ecosystems exhibit direct evidence of historical or ongoing transformation (Sanderson et al., 2002; Ellis & Ramankutty, 2008; Ellis et al., 2021), with just over 50% (~70 million km^2) currently utilised by humans (Hooke et al., 2012). Of this area, approximately 44% is allocated to agriculture and forestry, while around 7% is dedicated to infrastructure, including urban areas (Hooke et al., 2012). On a global scale, population growth is driving agricultural intensification, which negatively impacts various ecosystem services, such as pollination (IPBES, 2016; United Nations, 2019). These land-use changes significantly affect insect communities. For example, agricultural expansion (Aizen et al., 2008; Plourde et al., 2013; Otto et al., 2018) and urbanisation (Mcdonald et al., 2008; Seto et al., 2012a) have been shown to reduce pollinator diversity through diminishing floral and nesting resources (Hernandez et al., 2009; Forrest et al., 2015; Crist et al., 2017).

Effects of urbanisation

The impact of urbanisation on food availability for pollinators is multifaceted and complex (Liang et al., 2023). As humans continue to modify landscapes and increase urbanisation, we create a mosaic-like environment in terms of resource distribution and clustering. In addition to habitat loss, urban growth leads to noise, light and air pollution, and increased habitat fragmentation (Goines & Hagler, 2007; He et al., 2014; Santana Marques et al., 2020). Urban and suburban areas are frequently associated with declines in pollinator populations, with species typically exhibiting significantly lower diversity and abundance compared to rural areas (McKinney, 2008; Bates et al., 2011; Millard et al., 2021; Liang et al., 2023). Moreover, the reduction in the number of interactions performed by pollinating flower visitors in urban and suburban settings indicates the sensitivity of pollination processes to urbanisation (Geslin et al., 2013). However, some studies have reported that certain urban land uses can support significant pollinator populations (Hunter & Hunter, 2008; Lovell & Taylor, 2013; Aronson et al., 2017; Bennett & Lovell, 2019). For example, in Denmark, honey bee colonies in predominantly urban areas were significantly more productive than those in predominantly agricultural or mixed areas (Lecocq et al., 2015). Similarly, in the UK, honey bees in Brighton showed a preference for foraging almost entirely in urban areas, even when rural countryside was within their foraging range, indicating that urban areas can provide sufficient forage for bees (Garbuzov et al., 2015). Urban areas, including residential gardens and allotments, can create pollinator "hotspots" where certain species thrive and find “refuge” compared to intensively managed agricultural lands (Normandin et al., 2017; Hall et al., 2017; Baldock et al., 2019). Additionally, suburban gardens have been shown to increase foraging activity in bees to even higher levels than in their natural environments (Kaluza et al., 2016; Tew et al., 2021, 2022).

Effects of agricultural intensification

Agricultural intensification leads to a decreased plant diversity and increased chemical inputs, which significantly impact pollinators both directly, by increasing mortality, and indirectly, by reducing resource availability (Potts et al., 2010; Roulston & Goodell, 2011). Intensively farmed crops create hostile environments for pollinators, as they fail to provide adequate forage year-round (Dolezal et al., 2019; Wenzel et al., 2020). For example, large-scale monocultures have numerous negative effects on honey bees, including replacing diverse habitats (Liu et al., 2018; de Groot et al., 2021), reducing forage availability when the crops' bloom period ends, leading to "green deserts" (Altieri, 2009), and increasing nutritional stress, which makes bees more susceptible to diseases and parasites (Branchiccela et al., 2019; St. Clair et al., 2020; Cohen et al., 2021). In such areas, mechanical management and treatments of the land often involve chemical applications, such as fungicides, herbicides, and fertilizers, which are commonplace in much of the modern world (Benton et al., 2002, 2003). Neonicotinoid pesticides, in particular, have been shown to restrict colony growth and queen production in bumble bees and limit the foraging success and survival of honey bees (Henry et al., 2012; Whitehorn et al., 2012). Nitrogen fertilizers and herbicides can affect bees indirectly by reducing plant diversity (Kleijn et al., 2009) and thus foraging resources (Roulston & Goodell, 2011). In response to declining insect populations (Steffen et al., 2015; Seibold et al., 2019), some governments have implemented initiatives to support the creation of pollinator-friendly habitats on agricultural lands (Dicks et al., 2016), For example, the agri-environment scheme (AES) has been found to benefit insect biodiversity in Germany (Boetzl et al., 2021) and included areas have been shown to be particularly attractive to honey bees in the UK (Couvillon et al., 2014c). These programs support insects by supplementing nutritional resources available throughout the season (Scheper et al., 2015; Sidhu & Joshi, 2016; Grab et al., 2018).

Honey bees (*Apis mellifera*) in the Anthropocene

The western honey bee (*Apis mellifera*), is the most widespread species of honey bee worldwide and a highly adaptable and vital pollinator species for global agriculture and ecosystem functioning (Ashman et al., 2004; Venturini et al., 2017). They are among the most important pollinators of crops and wildflowers; 70%-90% of flowering plants rely on animal pollination, and roughly 30%-50% of crop species benefit from pollination by honey bees (Bawa, 1990; Fontaine et al., 2005; Klein et al., 2007; Ollerton et al., 2011; Potts et al., 2016; Ollerton, 2017; Kleijn et al., 2015; Bushmann & Drummond, 2020). Human dependence on insect pollinators continues to grow even as pollinators, including bees, face global declines (Potts et al., 2010; Potts et al., 2016; Otto et al., 2018; Osterman et al., 2021). Honey bee colonies have decreased in many European countries, and North America (vanEngelsdorp et al., 2008; Moritz et al., 2010; Potts et al., 2010; Osterman et al., 2021; Phiri et al., 2022), but have increased in number in South America and Asia (Phiri et al., 2022). For example, since Colony Collapse Disorder (CDD) was first described in late 2006, high annual losses of managed colonies have been observed in the United States (Oldroyd, 2007; vanEngelsdorp et al., 2007), and Europe (Bacandritsos et al., 2010; Dainat et al., 2012; Breeze et al., 2012). This decline of honey bee colonies in some areas brought global attention to honey bee health and spurred a wave of research into potential causes, including pesticides, diseases, and environmental stressors. While no strong evidence has clearly explained CCD, it is evident that bee health is under pressure in many human-modified areas (vanEngelsdorp & Meixner, 2010; van der Zee et al., 2012, 2015; Ollerton, 2021) due to a cocktail of stressors, including pesticides (Tosi et al., 2017; Thompson et al., 2019; Obregon et al., 2021; Cappa et al., 2022), pathogen infestations (Higes et al., 2008; Guzmán-Novoa et al., 2010), climate change (Soroye et al., 2020; Goulson & Nicholls, 2022; Outhwaite et al., 2022), habitat fragmentation and loss (Klein et al., 2007; Goulson & Nicholls, 2022). The interplay between these factors further exacerbates the health challenges that bees face (Nazzi et al., 2012; Blanken et al., 2015; Kulhanek et al., 2017).

Waggle dance communication

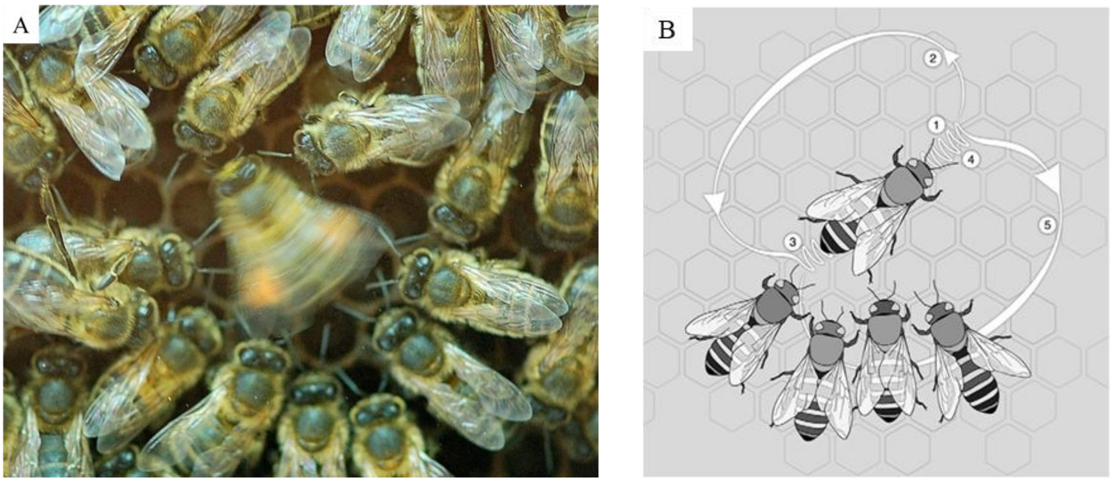


Figure intro. 1. The waggle dance. An *Apis mellifera* forager performing a waggle dance (A, photo by C. Grüter). A honey bee waggle dance observed by four following bees (B, From Grüter and Farina, 2009). The central zigzag represents the waggle run (① and ④), Once complete, the dancer will turn left (②) or right (⑤) to return and start a new waggle run ③.

"When a bee discovers a source of food, it returns to the hive and performs a lively and animated dance, tracing circles or half-circles, which seems to excite its companions to leave the hive." Swiss entomologist François Huber was the first to write about the bee dance in his work *New Observations on Bees* in 1792 (translated version). Around 150 years later, the waggle dance was decoded and described by Karl von Frisch, a milestone in animal communication research and the behaviour has been extensively studied ever since (Fig intro 1B) (von Frisch, 1967; Gould, 1975; Dyer, 2002; I'Anson Price & Grüter, 2015; I'Anson Price et al., 2019). Returning foragers perform a figure-of-eight dance with a central waggle phase (Fig intro 1B) to recruit nestmates to high-quality food resources or nest sites (von Frisch, 1967; Seeley et al., 2000). During this waggle dance pattern (Fig intro 1B), the dancer shakes its body from side to side along a straight line on the vertical comb surface inside the hive. It then turns its body either right or left and circles back to its original location, completing the second phase of the dance, the return phase. Subsequently, another waggle phase follows, during which the bee, upon reaching the end, turns its body in the opposite direction of the previous return phase before returning to its original position. The dancer can perform this waggle dance pattern between 1 and over 100 times, depending on the profitability of the resource (von Frisch, 1967; Seeley, 1986; Seeley et al., 1991, 2000; Grüter & Farina, 2009; Couvillon et al., 2012). The higher the profitability of a resource, the greater the number of individual waggle phases a bee will produce in its dance upon returning from the location (Seeley, 1986; Seeley et al., 1991, 2000).

The waggle dance communicates distance and directional information through cues within the waggle phase to recruit other bees and assist them in finding the advertised resource (von Frisch, 1967). Bees dancing on vertical wax combs transpose the angle between the food source and the azimuth of the sun to the angle between the direction of gravity and the direction of the waggle phase. Honey bees dancing on horizontal surfaces (e.g. *Apis florea*) use the sun and landmarks as reference points to waggle directly towards the target (von Frisch, 1967; Lindauer, 1955; Dyer, 2002; Beekman et al., 2015; Alves et al., 2023). The duration of the waggle phase indicates the distance to the resource, with a longer waggle phase signifying resources that are further away (von Frisch, 1967). Bees can recruit nestmates to the best food resources within a radius of up to 10 km around the nest (von Frisch 1967). Olfaction also plays a crucial role in recruitment, as the release of pheromones and floral odours clinging to the body of the dancer help recruits pinpoint the exact resource indicated by the foragers (Thom et al., 2007; Grüter et al., 2008). Thus, the waggle dance serves as a multi-component signal system for recruitment, including (1) advertising the location of beneficial food resources, (2) activating experienced foragers, (3) sharing food odour and quality information (Grüter & Farina, 2009).

Dance followers position themselves behind and around the dancer, continuously running with the dance and frequently making contact with the dancer using their antennae to obtain information about the dance (von Frisch, 1967; Rohrseitz & Tautz, 1999; Hadjitofi & Webb, 2024). The more waggle phases a recruit follows, the higher their flight accuracy (Mautz, 1971; Tanner & Visscher, 2009). Studies indicate that a minimum of 5-6 waggle phases must be followed for a bee to successfully acquire enough information to locate the advertised resource (Esch & Bastian, 1970; Mautz, 1971). Waggle dances attract both naive and experienced foragers (Seeley, 1983; Biesmeijer & de Vries, 2001; Gil & Farina, 2002; Biesmeijer & Seeley, 2005; Grüter et al., 2008; Grüter & Farina, 2009). Experienced foragers decide whether to follow and interpret the dance to use its vector information or to revisit a food source location that was memorized during previous foraging trips, depending on the colony's needs and the spatiotemporal distribution of food sources (Biesmeijer & de Vries, 2001; Dornhaus & Chittka, 2004; Beekman & Lew, 2008; Grüter & Ratnieks, 2011; Schürch & Grüter, 2014; I'Anson Price et al., 2019).

The interest of dance followers in the spatial information conveyed by the dance can be gauged by the number of followers and the number of waggle runs they follow (Grüter & Ratnieks, 2011). Most bees follow dances only briefly and without decoding its spatial information (Grüter & Farina, 2009). Following more waggle runs indicates that bees attempt to decode the dance and it increases the accuracy of the information (Tanner & Visscher, 2009). The number of followers and the extent to which they follow a waggle dance can vary depending on the time of year, and resource availability around the hive (I'Anson Price et al., 2019), suggesting that the following behaviour may be influenced by land-use effects as well.

Bee size in relation to nutrition

Body size is a fundamental trait that influences animal physiology, ecology, and evolution (LaBarbera, 1989): it affects many physiological processes, such as oxygen consumption (Callier & Nijhout, 2014), determines or constrains fertility, mortality, and ecological processes, including competitive interactions between individuals or species. In social insects, body size is also linked to caste differentiation, division of labour, and colony efficiency. For example, queens are generally larger than workers due to their reproductive role. Size variation among workers in a colony is evident in many species, including ants (Hölldobler & Wilson, 1990), termites (Noirot & Pasteels, 1987), bumble bees (Goulson et al., 2002), and stingless bees (Grüter et al., 2012). In the stingless bee *Tetragonisca angustula*, different worker types—guards, foragers, and nest-cleaners—show distinct morphologies (Grüter et al., 2012; Hammel et al., 2016). Guards are the largest, with relatively larger legs, whereas foragers are smaller but have relatively larger heads. In bumble bees (*Bombus* spp.), workers exhibit even greater size variation, with the largest workers being up to ten times larger than the smallest (Couvillon & Dornhaus, 2010). Larger bumble bees tend to perform foraging tasks, while smaller workers are more inclined to undertake within-nest tasks (Goulson et al., 2002; Jandt & Dornhaus, 2009; Spaethe & Weidenmüller, 2002). Conversely, Roulston & Cane (2000) found that *Apis mellifera* shows the smallest variation in body size among the 31 bee species they studied. Waddington (1989) suggested that low size variation among foragers reduces mistakes in communicating foraging distances through the waggle dance. Thus, a smaller size variation among worker bees could be selected to enhance the accuracy of vector information transmission. Despite this, there is some size variation, and it has been shown that worker size influences the age at which workers begin foraging and the size of workers produced by colonies increased during the foraging season in a mixed habitat in Switzerland (Kerr & Hebling, 1964; Sauthier et al., 2017).

Bergmann (1847) was the first to describe a positive relationship between body size and environmental stressors, such as temperature, in endotherms ("Bergmann's rule"). In some species of bees, there is a trend for bees to be larger in colder habitats, such as in the solitary bee *Osmia bicornis* and in some bumble bees (Radmacher & Strohm, 2010; Osorio-Canadas et al., 2016; Fitzgerald et al., 2022), which is likely related to their thermoregulatory abilities and partial endothermy (Pereboom & Biesmeijer, 2003; Gérard et al., 2018). Furthermore, Geist (1987) described that body size is proportional to food availability during the growing season. Bees can grow larger in a resource-rich environment but stay smaller when resources are limited. In social bees that store food inside their nest, body size is also likely to depend on food stores. For example, in the stingless bees *Melipona flavolineata*, the body size increased with the food stores in a colony (Veiga et al., 2013). Smaller body size can indicate nutritional stress, lower floral resource availability (Kim, 1999), and reduced the quality of pollen and nectar in the larval diet (Burkle & Irwin, 2009).

Given the links between food source availability and body size, it is likely that land-use indirectly affects bee size in many species. Anderson et al. (2024) found that the body size of the solitary bee *Habropoda laboriosa* decreased with increasing urban development and overall cropland. Bumble bees have been shown to exhibit varying body sizes in habitats with different levels of urbanization. These patterns do not seem to be consistent across species as sites with increased urbanization were associated with larger *B. impatiens* and smaller *B. pensylvanicus* bees (Theodorou et al., 2021; Austin et al., 2022). Mayes et al. (2019) also reported that habitat fragmentation affects stingless bees, with body size increasing in response to greater amounts of deforestation in Brazil. However, Grab et al. (2019) found that declines in bee body size were buffered by habitat enhancements, such as the addition of floral resources. These enhancements can support bees in intensively managed agricultural landscapes across various cropping systems and regions (Grab et al., 2018, 2019). Bee size, in turn, affects foraging ranges, with larger bees foraging at greater distances (Gathmann and Tscharntke, 2002; Greenleaf et al., 2007; Gueedot et al, 2009; Zurbuchen et al, 2010; Kendall et al., 2022; Grüter & Hayes 2022). This highlights that bee size has implications for pollination services on an ecosystem scale (Jauker et al., 2016; Chole et al., 2019).

Nutritional stress and wing wear

Nutritional stress has been shown to lead to a range of behavioural changes, such as an early onset of foraging in honey bees (Schulz et al., 1998, 2002) or more intense communication about resources (Rinderer, 1982; Wu et al., 2024), thus potentially affecting the foraging load of nutritionally stressed bees. Bees will acquire and accumulate wing damage due to foraging (Foster & Cartar, 2011a) and aging (Mueller & Wolf-Mueller, 1993; Higginson & Barnard, 2004), which may further increase foraging effort and reduce lifespan (Schmid-Hempel & Wolf, 1988; Johnson & Cartar, 2014; Vance & Roberts, 2014). Furthermore, Higginson and Barnard (2004) found that wing damage affected the foraging decisions and reduced nectar foraging efficiency in the honey bee (see also Foster & Cartar, 2011b). Studying the solitary bee *Agapostemon virescens*, Brasil et al. (2023) observed that the wing wear of bees increased during spring and decreased during summer, indicating seasonal changes in foraging effort. Given that different land uses and seasons can influence the availability and quality of floral resources, it seems plausible that land use and season affect the level of wing wear found in honey bee foragers as well.

Fatty acids content and bee nutrition

The fat body is a crucial tissue in insects, serving major roles in nutrient storage, energy metabolism, innate immunity, and detoxification (Stanley-Samuelson et al., 1988; Canavoso et al., 2001; Arrese & Soulages, 2010; Buchon et al., 2014). It is a dynamic, loose tissue composed of three primary nutritional molecules: lipids, glycogen, and proteins. It is located beneath the integument and surrounds the gut and reproductive organs in the abdomen (Dean et al., 1985; Law & Wells, 1989). Triglycerides are the predominant form of lipids, comprising about 90% of the stored lipids (Bailey, 1975; Canavoso et al., 2001). Glycogen and triglycerides serve as energy reserves in fat body cells. Glycogen can be rapidly degraded to provide glycolytic fuel (Steele, 1982), while triglycerides, which have a higher caloric content per unit of weight, are used for energy production through β-oxidation (Athenstaedt & Daum, 2006). During flight, the fat body supplies the energy needed to sustain high metabolic rates in the muscles. For instance, locusts and moths rely on fat oxidation for long-term flight (van Handel, 1974; Sacktor, 1975), while tsetse flies and Colorado potato beetles oxidize proline for flight energy (Weeda et al., 1979; Gäde & Auerswald, 2002; Mantilla et al., 2017). In contrast, bees primarily use carbohydrates stored in their crop for flight (Suarez et al., 2005). The fat body is also vital for innate immune responses, producing antimicrobial peptides (Lemaitre & Hoffmann, 2007; Li et al., 2007; Buchon et al., 2014; Skowronek et al., 2021). Additionally, it has been reported that the fat body of insects, such as honey bees, contains magnetite (Fe_3O_4), which may be related to the earth's magnetic fields and aid in navigation (Kuterbach et al., 1982).

Foraging behaviour and fat Stores in bees

A reduction in body fat stores correlated with foraging behaviour in various species of ants, bees, and wasps (Blanchard et al., 2000; Toth & Robinson, 2005). Nurse honey bees, for example, maintain high levels of abdominal fat stores, while foragers typically have very low fat stores (Toth et al., 2005; Scofield & Amdam, 2024). The decline in stored fat can influence the age at which honey bees begin foraging, often leading them to forage precociously (Toth et al., 2005; Toth & Robinson, 2005).

Fatty acid composition and bee nutrition

The dominant fatty acids found in bees include oleic acid (C18:1) and palmitoleic acid (C16:1) among monounsaturated fatty acids, palmitic acid (C16:0) and stearic acid (C18:0) among saturated fatty acids, and linoleic acid (C18:2) among polyunsaturated fatty acids (Giri & Dillon, 2012; Ghosh et al., 2017; Wu et al., 2024). Palmitic acid, the most common saturated fatty acid in pollen, is poorly absorbed by bees (Manning, 2006). Linoleic acid, an essential fatty acid, is absorbed and allocated most efficiently among the three fatty acids tested in bumble bees. In contrast, oleic acid is absorbed and oxidized at lower rates compared to linoleic acid.

Pollen is a particularly good dietary source of unsaturated fatty acids (Domínguez et al., 2024). Balanced lipid diets are crucial for honey bees' learning abilities, brood production, and overall health (Arien et al., 2015; Bennett et al., 2022). Arien et al. (2015), for instance, found that honey bees fed unbalanced diets exhibited significantly reduced learning abilities and sucrose sensitivity (see also Bennett et al., 2022). Habitat fragmentation and monoculture landscapes can lead to reduced pollen diversity and, therefore, an unbalanced lipid diet. Dolezal et al. (2019), for example, found that bees in intensively farmed monocultures had reduced fat stores and colony weight when food sources became scarce, both of which affect survival. However, the links between land use, food availability and lipid stores require further study.

Immunity and bee nutrition

Bees, like other insects, rely on an innate immune system to defend against pathogens. This system includes physical barriers, humoral defence mechanisms, and various cellular processes (Larsen et al., 2019). Pathogens and xenobiotics must first overcome these physical barriers—such as the exoskeleton, tracheal tubes, and intestinal mucosa—to infect the bee. Viruses often breach these defences with the assistance of vectors (Shen et al., 2005; Nazzi & Conte, 2016). For instance, many viruses are transmitted to honey bees by the mite *Varroa destructor*, which penetrates these barriers and facilitates viral infection (Shen et al., 2005; Di Prisco et al., 2016; Amiri et al., 2018).

Humoral and cellular immune responses in bees are activated when pathogen-recognition receptors in body cells and haemocytes detect an invader (Wang et al., 2019). Cellular immunity, mediated by haemocytes in the haemolymph, encompasses phagocytosis, nodulation, encapsulation, clot formation, and melanisation (Lavine & Strand, 2002; Strand, 2008; Marringa et al., 2014; Negri et al., 2014). The humoral response is regulated by the immune deficiency (Imd), Toll, Janus kinase-signal transducer and activator of transcription (Jak/Stat), and c-Jun N-terminal kinase (JNK) pathways (Chen et al., 2014; Ryabov et al., 2016; Wang et al., 2017; Zanni et al., 2017; Brutscher et al., 2017; McMenamin et al., 2018). In *Bombus pascuorum*, the humoral response can be detected within 24 to 48 hours post-infection

(Riddell et al., 2009). This response leads to the production of antimicrobial peptides (AMPs), defensive enzymes, and complement-like proteins (Meister et al., 1997; Huang et al., 2015).

These small, conserved proteins (12-50 amino acids), mainly synthesized in the fat body, are released into the haemolymph in response to bacterial, fungal, and occasionally viral infections (Schlüns & Crozier, 2007; Gätschenberger et al., 2013). While over 170 AMPs have been identified in insects, honey bees produce fewer humoral effectors compared to *Drosophila* and *Anopheles* (Brutscher et al., 2015). AMPs such as apidaecin, abaecin, hymenoptaecin, lysozyme, and defensin 1 have been linked to pollen nutrition stress, microorganisms, and xenobiotic compounds (Vannette et al., 2015a; Danihlík et al., 2018a; Castelli et al., 2020a). Relish was found to be involved in the regulation of these antimicrobial peptides (Schlüns & Crozier, 2007; Brutscher et al., 2015). Rahnamaeian et al. (2015), for instance, found that AMPs have been shown to act in synergy to provide greater antimicrobial additive effects in *B. pascuorum* and *B. terrestris*. Bees also employ antiviral mechanisms, including RNA interference (RNAi) in the innate immune system, which targets double-stranded RNA (dsRNA) to inhibit the expression of protein-coding genes (Niu et al., 2014; Brutscher et al., 2015).

In addition to the innate immune system, honey bees and other social insects have developed specialised group-level behaviours known as social immunity to enhance colony health. These behaviours include raising the nest temperature to control pathogenic fungi such as *Ascosphaera apis* (Starks et al., 2000), using mandibles and legs for self-grooming and social grooming to remove external parasites like *Varroa destructor* (De Roode & Lefèvre, 2012; Pritchard, 2016), detecting and removing diseased or parasitized brood from comb cells, and gathering propolis to prevent or minimize the development of pathogenic bacteria and fungi (Simone et al., 2009; Simon-Delso et al., 2014). Additionally, sick bees often leave the colony to die outside the brood nest, thereby reducing the risk of disease transmission to their nestmates (Rueppell et al., 2010).

Nutritional stress and diet play a crucial role in shaping the immunocompetence of bees, influencing the expression of immune-related genes (Alaux et al., 2010; Porrini et al., 2011; DeGrandi-Hoffman et al., 2016; Corona et al., 2023). Research indicates that nutritional stress affects both the expression of immune genes (Alaux et al., 2010; Corby-Harris, Maes, et al., 2014) and susceptibility to various pathogens (DeGrandi-Hoffman et al., 2010; Di Pasquale et al., 2013; Tritschler et al., 2017). For instance, *defensin 1* is upregulated in older bees from pollen-restricted colonies, which face increased health risks (Corona et al., 2023). Additionally, pollen nutrition stress impacts the expression of several antimicrobial peptides (AMPs) in adult bees (Danihlík et al., 2018; Castelli et al., 2020). Based on these findings, we might predict that genes crucial for immunocompetence will show increased expression in colonies experiencing nutritional stress.

The aims of my dissertation

In recent decades, honey bee colony losses reported worldwide have been associated with nutritional stress resulting from the anthropogenic transformation of landscapes into urban and intensive agricultural land (Baldock, 2020; Evans et al., 2018). This transformation reduces suitable habitats and diminishes the abundance and diversity of resources. Yet how honey bees respond to land use changes at different biological levels, from behaviour and body size to physiology and immune investment remains poorly understood. In this thesis, we explore links between behavioural traits, mainly waggle dance communication, physiological traits, the nutritional state of colonies and land use. The knowledge gained from the experiments in this thesis can enhance our understanding of how honey bees adapt their communication strategies and physiological traits to environments characterized by nutritional deficiencies.

Previous studies have demonstrated that nutritional stress can affect the lifespan, foraging activity, and dance communication of bees (Rinderer, 1982; Seeley, 1986; Schulz et al., 1998, 2002). Honey depletion produces more precocious foragers and increases the number of foraging trips (Seeley, 1986; Schulz et al., 1998, 2002). How honey depletion affects waggle dance communication is less well understood. Rinderer (1982) found that dance frequency and dance follower number depended on the amount of empty comb space, with bees dancing more and attracting more followers in colonies with more empty comb areas. We manipulated the honey stores of colonies to understand how honey depletion impacted the heath of foragers and the behaviour of dancers and followers. We hypothesised that waggle dance frequency and follower number would increase in honey-starved colonies as foragers seek more information about high-quality food sources (**Chapter 1**). We predicted that genes known to be important for immunocompetence would be expressed more, and the fat stores in bees would decrease when colonies experience an acute honey shortage (**Chapter 1**).

Food storage is significantly linked to the food availability in the surrounding habitat and summer is known to be a challenging period for colonies in temperate habitats, both agricultural and urban, due to a scarcity of flowers (Nürnberger et al., 2017; Dolezal et al., 2019; I'Anson Price et al., 2019; Czekońska et al., 2023). During this time, bees may need to forage further away from their hives (Couvillon et al., 2014b). These seasonal changes in different landscape types could impact bee body size, foraging effort and fat stores. In **Chapter 2**, we assessed the effects of land use and time of year on honey bees, by sampling bees in three types of habitats across three meteorological seasons in southwestern Germany: 16 agricultural sites, 16 urban sites, and 15 mixed habitat sites. We predicted that honey bees captured in these mixed sites are larger and store more fat. We also expected bees to be smaller in summer due to a lack of food sources (Mandelik et al., 2012; Couvillon et al., 2014b; I'Anson Price et al., 2019; Timberlake et al., 2019).

In **Chapter 3**, we further investigated how bees adapt their dance communication in different habitats and seasons in the south-west of England. We for the first time employed machine learning to automatically detect and decode waggle dances under field conditions to test how honey bees alter the characteristics of their waggle dance communication in response to varying land-use and seasonal changes. We placed 17 colonies at 10 different sites, where the urban area varied from 3.4% to 95.2%. We hypothesised that seasonal changes will significantly influence waggle dance communication, waggle dance frequency and follower number will increase in autumn due to better foraging conditions (Couvillon et al., 2014b; Danner et al., 2017; Samuelson et al., 2022). We also predicted that waggle phase durations are likely to decrease in autumn due to increased resource availability and in urban areas due to more stable foraging conditions (Couvillon et al., 2014b; Danner et al., 2016; Samuelson et al., 2022). Additionally, we hypothesised that there may be an interaction between season and build-up area because urban sites in the study region provide more stable foraging than rural sites (Timberlake et al., 2019; Tew et al., 2021, 2022).

Chapter 1

Low food stores affect dance communication and health-related gene expression in honey bees

Abstract

Honey bees, *Apis mellifera*, are important pollinators, and they face many natural and anthropogenic challenges that affect their ability to collect the resources needed to maintain the colony. Foragers can make use of a remarkable repertoire of communication behaviours that help colonies to exploit their environment successfully. Food source availability is a key factor for colony success and, therefore, survival and reproduction. Few studies have investigated how food stores impact forager communication strategies and bee physiology. We experimentally manipulated honey stores and (1) quantified the production and following of waggle dances, (2) quantified the expression of immune-related genes using qPCR and (3) analysed fatty acids from bee abdomens using GC-MS 6 days after the experimental manipulation. We found that the number of waggle dances increased by about 60% when honey bees were starved of honey. The number of followers per dance, however, decreased, which may be due to a switch to proactive, solitary foraging or to the occurrence of more waggle dances. Waggle dance duration, the number of waggle phase followers that were followed and foraging distances were not affected by the treatments. Bees in starved colonies showed a higher expression of the gene *defensin 1*, which is an important predictor of overwinter survival, but there was no treatment effect on fatty acid content. Our results show that the amount of honey stored in hives affects communication behaviours and the investment in immunocompetence of bees, possibly to counter the negative health effects of nutritional stress. However, fat content does not seem to be affected in the time span of the study.

Key words: fatty acid content, follower, honey bee, immune system, nutrition stress, waggle dance

Introduction

Bees play a critical role in pollinating agricultural crops (Hristov et al., 2020; van der Sluijs & Vaage, 2016) as well as wild flowers (Garibaldi et al., 2013; Hung et al., 2018) but their health is under pressure in many human-modified areas (Ollerton, 2021; van Engelsdorp & Meixner, 2010) due to a cocktail of stressors, including pesticides, pathogens, climate change and habitat loss (Soroye et al., 2020; Goulson & Nicholls, 2022; Outhwaite et al., 2022). The conversion of natural habitat into urban or intensively managed agricultural land, in particular, is thought to negatively impact the health of honey bee, *Apis mellifera,* colonies and contribute to the decline of wild bees in some areas (Naug, 2009; Branchiccela et al., 2019; Liang et al., 2023). These changes in land use can lead to an inadequate and unbalanced nutrition, with negative effects on the growth and development of honey bee colonies and individual bees (Brodschneider & Crailsheim, 2010; Di Pasquale et al., 2013). For example, insufficient pollen nutrition can weaken the bees' immune system (Alaux et al., 2010), affect their pesticide tolerance (Barascou et al., 2021) and increase the risk of colony death (Goulson et al., 2015). While the effects of pollen shortages have been relatively well studied, research on the effects of low honey stores remains scarce. We aimed to explore the consequences of low honey stores on three aspects: (1) foraging behaviour, specifically the waggle dance communication, (2) immune-relevant gene expression and (3) bee physiology, measured as their fatty acid stores.

Honey bees use the unique waggle dance behaviour to convey information about the presence, smell and location of important resources (von Frisch, 1967; Dyer, 2002; Grüter & Farina, 2009; Couvillon, 2012). Studies have suggested that the characteristics and value of waggle dance communication depend on ecological factors, such as the availability and distribution of food sources (Dornhaus et al. 2006; Couvillon, 2012; Couvillon et al. 2014b, I'Anson Price & Grüter 2015). I'Anson Price et al. (2019), for example, found that dance following increased over time in an environment with few food sources. However, even under constant environmental conditions, dance followers vary greatly in their interest in dances, that is, the number of waggle phases they follow, depending on whether they seek spatial information to locate the advertised food source or whether they primarily seek olfactory information that helps foragers decide whether to resume foraging at previously exploited food sources (von Frisch, 1967; Grüter et al., 2008, 2013; Grüter & Farina, 2009). Rinderer (1982) found that dance frequency and dance follower number also depended on the amount of empty comb space, with bees dancing more in colonies with more empty comb area. Based on these different observations, we hypothesized that waggle dance frequency and follower number will increase in honey-starved colonies as foragers seek more information about high-quality food sources. We also tested whether follower interest, measured as the duration of dance following, and foraging distances, measured as waggle phase duration, are also affected by honey store depletion.

Nutritional stress and diet can shape the immunocompetence of bees, including immune-related gene expression (Alaux et al., 2010; Corona et al., 2023). Innate immunity is an important line of defence against pathogens, which includes humoral immune responses associated with antimicrobial peptides such as defensin 1 (Casteels-Josson et al., 1994), hymenoptaecin (Casteels et al., 1993) and abaecin (Casteels et al., 1990) and cellular responses (Strand & Pech, 1995). Several studies show that nutritional stress affects both the expression of immune genes (Alaux et al., 2010; Corby-Harris et al., 2014) and susceptibility to different pathogens (DeGrandi-Hoffman et al., 2010; Di Pasquale et al., 2013; Tritschler et al., 2017). Moreover, defensin 1 was found to be upregulated in old bees in pollen-restricted colonies, which are likely to be exposed to greater health risks (Corona et al., 2023). We predicted that genes known to be important for immunocompetence will be expressed more when colonies experience an acute honey shortage. Fatty acid stores in the bee's fat body, a tissue with an essential role in energy storage, metabolism and immunity, can also be an indicator of the bee's nutritional condition (Stanley-Samuelson et al., 1988). Nutritional stress can significantly reduce the fatty acid stores, while lipids in pollen or commercial supplements will increase the bees' lipid and essential fatty acids stores (Arien et al., 2020). Dolezal et al. (2019) found that bees inhabiting intensively farmed monocultures experienced a reduction in fat stores and colony weight when food sources became scarce, both of which affect survival (Dolezal et al., 2019). We explored whether a reduction in honey stores leads to a reduction in fat stores in the short to medium term, that is, within a few days.

Methods

Study Site and Study Animals

The study was conducted between May and August 2020 on three pairs of observation hives (OH1-OH6) with *A. m. carnica* honey bees, each comprising 3000 - 4000 workers, food stores, brood and a naturally mated queen. The study took place in the apiary of the Johannes Gutenberg University in Mainz, Germany. All observation hives were kept in a wooden shed. Bees could reach the outside of the shed and visit natural food sources by passing through a plastic tube that led to the outside.

Experimental Procedure

We used a paired design with two observation hives being tested at a time. We created each hive a few days before the experiment began and designed them to have three ‘Deutsch Normal’ frames: a honey store frame at the top, a brood frame in the middle and a mixed frame (containing honey, pollen and empty space in similar proportions) at the bottom. The two hives of a pair were first left unchanged for 6 days. The two treatments were as follows: (1) we replaced the top honey frame in one of the paired hives with an empty frame (EH treatment); (2) we selected a full honey frame (FH treatment) from a colony in the apiary to replace the existing top honey frame in the other hive. This ensured both hives experienced the opening and replacement of a frame. Subsequently, for 6 days one hive experienced the EH treatment while the other experienced the FH treatment. The 6-day treatment period was short enough to ensure that EH colonies would not die from starvation. After another 6-day recovery period with a full honey frame in both hives, the treatments were reversed for another 6 days (Fig. S1.1). We filmed the ‘dance floor’ near the entrance (i.e. the area where dancing is most intense; von Frisch 1967) on both sides of the observation hives for 2 h in the morning and 2 h in the afternoon throughout the treatment period using digital cameras (Panasonic video HC-V180). For the last pair (OH5 and OH6), we recorded only 90 min in the morning and 90 min in the afternoon because the shorter daytime and cooler temperatures in August reduced the duration of foraging activity. In the event of short periods of rain, we adjusted the recording schedule to maintain the filming duration. At the end of each treatment period (day 6), we collected nine returning foraging bees from the entrance of the observation hive, and immediately placed them into liquid nitrogen. They were then stored at −80 °C until we analysed the expression of immune-related genes and fatty acid content in the fat body.

Behavioural Observations

To determine the overall dancing activity of a colony, we used a scan sampling approach and counted the waggle dances that occurred during 2 min every 20 min of video recording. For each of the counted waggle dances, we also counted the waggle phase number as a measure of waggle dance duration. To assess dance follower interest in a standardized way, we selected the first five waggle dances with at least five waggle phases from scans each day and counted the followers and waggle phases that these bees followed. We identified followers as bees that (1) faced the dancer, (2) were within an antenna's length during the waggle phase and (3) followed the dancer's movements during at least one waggle phase of the dance (Al Toufailia et al., 2013). Data on the number of dance followers were collected starting from the third waggle phase from the start of a dance (W3) for three waggle phases (W3-5; I'Anson Price et al., 2019). The follower number per dance was calculated as the average number of followers across these three waggle phases. We counted the waggle runs followed per follower present at W3 to assess the motivation of bees to follow an individual dance (I'Anson Price et al., 2019). In addition, we quantified the distance of the food sources visited by the dancers by examining the average waggle phase time frame by frame, from the second to fifth waggle phase, based on the universal calibration curve (Couvillon et al., 2012; Schürch et al., 2019).

Immune Genes Expression

The expression of several antimicrobial peptide (AMP) genes, namely *abaecin, apidaecin, hymenoptaecin* and *lysozyme,* has been shown to be related to pollen nutrition stress in adult bees (Danihlík et al., 2018; Castelli et al., 2020). *Defensin 1* and *apimisin* are also AMPs and were found to be highly expressed in the nectar-processing tissues, hypopharyngeal gland and mandibular glands of forager bees, which may help protect them against microorganisms and xenobiotic compounds acquired while foraging (Vannette et al., 2015). *Relish* was found to be involved in the regulation of these AMPs (Schlüns & Crozier, 2007; Brutscher et al., 2015).

We used real-time quantitative PCR (qPCR) to investigate whether the expression of these seven immune genes (*abaecin, apidaecin, apimisin, relish, hymenoptaecin, defensin 1* and *lysozyme*) increases in foragers, captured on day 6 of the treatment period, when colonies experience a depletion in honey stores. We used four bees per colony and treatment (*N*=24 from the EH treatment; *N*=24 from the FH treatment). Total RNA was extracted from whole bees using RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA quality and quantity were assessed using a Qubit spectrophotometer (Thermo-Fisher Scientific, Foster City, CA, U.S.A.); 0.8 µg total RNA for each reaction were used. cDNA was synthesized using a Quanti Tect Reverse Transcription Kit (Qiagen) following the manufacturer's instructions.

We performed qPCR on a mic qPCR cycler (Bio Molecular Systems, Upper Coomera, QLD, Australia) using the Blue S'Green qPCR mix Separate ROX (BioZyme, St Joseph, MO, U.S.A.). Each reaction volume of 10 µl contained 5 µl Blue S'Green qPCR mix, 0.25 µM of each primer, 1 µl cDNA and DNase/RNase free distilled water. The following cycling parameters were used: 95 °C for 2 min; 40 cycles of 95 °C for 5 s and 60 °C for 20 s. The fluorescence signal was measured at the end of each extension step at 60 °C. Quantification cycle (Cq) values were determined at the same fluorescent threshold for each gene by the micPCR Version 2.6 software (Bio Molecular Systems). Gene primers of tested immune genes were based on published sequences (Table 1.1). The transcript levels of the target genes were expressed as normalized transcript abundance using *GAPDH* and *β-actin* as internal reference genes (Chen et al., 2005; Reim et al., 2013; Peng et al., 2021). We found that combining the two reference genes was more stable than using a single reference gene.

Table 1.1. The primer of the selected immune genes

Immune genes	Sequence (5'-3')	Reference
Abaecin	ATCTTCGCACTACTCGCCAC	
	AGCCTTGAGGCCATTTAATTTTCG	(Zhao et al. 2019)
Apidaecin	GGCACGAGAAGAATTTTGCCT	
	GAAGGCGCGTAGGTCGAGTA	(Zhao et al. 2019)
Hymenoptaecin	CTCTTCTGTGCCGTTGCATA	
	GCGTCTCCTGTCATTCCATT	(Zhao et al. 2019)
Defensin 1	TGCGCTGCTAACTGTCTCAG	
	AATGGCACTTAACCGAAACG	(Zhao et al. 2019)
Apisimin	TGAGCAAAATCGTTGCTGTC	
	AACGACATCCACGTTCGATT	(Evans, 2006)
Lysozymes	GGAGGCGAGGATTCTGACTCAATG	
	TGTTGCATATCCCTCCGCTGTG	(Aronstein, 2010)
Relish	GCAGTGTTGAAGGAGCTGAA	
	CCAATTCTGAAAAGCGTCCA	(Evans, 2006)
GAPDH	ACC TTC TGC AAA ATT ATG GCG A	
	CAC CTT TGC CAA GTC TAA CTG TTA AG	(Reim et al. 2013)
β.Actin	TGCCAACACTGTCCTTTCTGGAGGT	
	TTCATGGTGGATGGTGCTAGGGCAG	(Francis et al. 2013)

Fatty Acids Extraction

Fatty acid contents were extracted from the abdomen of bees using 1 ml of a chloroform:methanol mixture, 2:1 (v/v), over a period of 24 h (Folch et al., 1957). The samples were evaporated to dryness under gentle nitrogen flow and then redissolved in 250 µl of a 2:1 dichloromethane:methanol (v/v) mixture. We added 2 µg of nonadecanoic acid (dissolved in 10 µl DCM/MeOH) as internal standard. After vortexing, we moved 15 µl of this solution into a new glass vial and evaporated it to dryness under a gentle nitrogen flow. Finally, we added 20 µl trimethylsulphonium hydroxide (TMSH; 0.25 M in MeOH, Sigma-Aldrich, Munich, Germany) to samples to derivatize to fatty acid methyl esters (FAMEs) and analysed them with a 7890A gas chromatograph (Agilent) coupled to a 5975C mass-selective detector (Agilent, GC/MS). Helium was used as the carrier gas at a flow rate of 1.2 ml/min. The temperature of the GC oven started at 60°C for 1 min, then increased by 15°C/min to 150°C, followed by an increase to 200 °C with a heating rate 3°C/ min, and finally increased by 10°C/min to 320°C, where it was held constant for 10 min. The separated FAMEs were transferred to the MS and electron ionization mass spectra were recorded at 70 EV from m/z 40 to 650. Resulting peak areas were integrated manually using the software MSD Chem Station G1701EA E.02.02.1431 (Agilent) and identified from diagnostic ions, retention time and the molecular peak. Only fatty acids with abundance >1% were included in our analyses (Rosumek et al., 2017). This method can detect fatty acids between C10 and C20, but only chain lengths of C16 to C19 were found.

Statistical analyses

Behaviour: waggle dances and followers

The data were analysed in R 4.2.2 (R Core Team, 2022). We used general and generalized linear mixed-models (LMEs and GLMMs), with hive ID and hive pair ID included as random effects to control for the nonindependence of data from the same colony (Zuur et al., 2009). Our fixed effects were honey storage (EH versus FH) and day of treatment (2-6, the 1st treatment day was not considered and allowed bees to adapt to the new honey storage condition). Dance frequency data were zero-inflated, so we ran a zero-inflated model with a Poisson distribution to perform the GLMMs. We used the 'lme4' and 'lmerTest' packages to calculate *P* values for fixed effects (Bolker et al., 2009). We tested whether our fixed effects affected the (1) waggle dance frequency, (2) waggle dance duration, (3) average number of waggle dance followers per dance, (4) average number of waggle phases followed per dance and (5) waggle phase duration. We first used likelihood ratio tests (LRT) to determine whether to retain both random effects in the model. To simplify the model structure, pair ID was removed from the random effects if it was not significant, while colony ID was always retained as a random effect to account for nonindependence of observations due to the shared hive environment. Significance of fixed effects and their interaction was tested by comparing models with and without the fixed effects of interest using LRTs. The 'DHARMa' (Hartig, 2022) package was used to check whether model assumptions were met (Zuur et al., 2009). If necessary, we log-transformed response variables to achieve a Gaussian distribution of the model residuals. We used *Z* scores to check for outliers in the data (Shiffler, 1988).

Gene expression

The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method and the following formula: normalized target gene = $2^{-(CqTarger\ -\ CqReference)}$ (Schmittgen & Livak, 2008). PCR efficiency (E) values were calculated by the software micPCR Version 2.6 (Bio Molecular Systems) for each gene from the slope following the running standard curves and the formula: $E=2^{-1/\mathrm{slope}}-1$ (Taylor et al., 2010). We used LMEs with gene expression as the response variable. The fixed effect was the honey storage condition (EH versus FH). Random effects were colony ID and pair ID, as described above.

Fatty acids

We tested for differences in the absolute quantity of fatty acids as well as the proportions of saturated and di-unsaturated fatty acids (tri-unsaturated acids were not detected) by normalizing the values using the quantity of the internal standard. The remaining fatty acids, the monounsaturated fatty acids, are equal to 1-(saturated + double unsaturated fatty acids). We used LMEs to compare the fatty acid quantities in bees from different treatments (EH versus FH), again including colony ID and pair ID as a random effect, following the procedure described above. Pair ID was removed as a random effect if it was not significant. To compare the relative proportions of different types of fatty acids, we used nonmetric multidimensional scaling (NMDS; command metaMDS, package ‘vegan’; Oksanen et al., 2022). The permutational multivariate analysis of variance (PERMANOVA) used the ‘adonis’ function (package ‘vegan’) to randomly rearrange the proportions of different types of fatty acids within the levels of the ‘Treatment’ variable while keeping the structure of the ‘Colony’ grouping intact to assess the significance of the results by permutations.

Since treatment effects on physiological traits could also depend on the treatment order (first EH versus first FH), we tested whether treatment order interacted with honey storage condition to affect gene expression and fatty acid quantities. However, we found no significant interaction between treatment order and honey storage condition on any of the physiological and immune-related traits ($P > 0.05$). Since we had no a priori interest in treatment order effects and to avoid unnecessarily complex models, we did not include treatment order as a predictor to assess whether honey storage condition affected gene expression and fatty acid quantities.

Results

Communication behaviour in relation to honey stores

Waggle dance frequency

We performed 743 scans and observed 1400 dances and 8581 waggle phases. We found that the waggle dance frequency was significantly higher in honey-depleted hives than in hives with full honey stores (GLMMs: zero-inflation model; EH mean (95% confidence interval, CI) = 2.33 (2.00 - 2.65); FH = 1.45 (1.21 - 1.68); $Z = 2.66$, $P = 0.008$; Fig. 1.1). In addition, there was an increase in dance frequency from second to last treatment day (second day of treatment (95% CI) = 1.47 (1.02 - 1.91); last day of treatment = 2.56 (2.03 - 3.09); $Z = -4.120$, $P < 0.001$). No interaction was found between treatment day and treatment ($Z= 0.74$, $P = 0.46$).

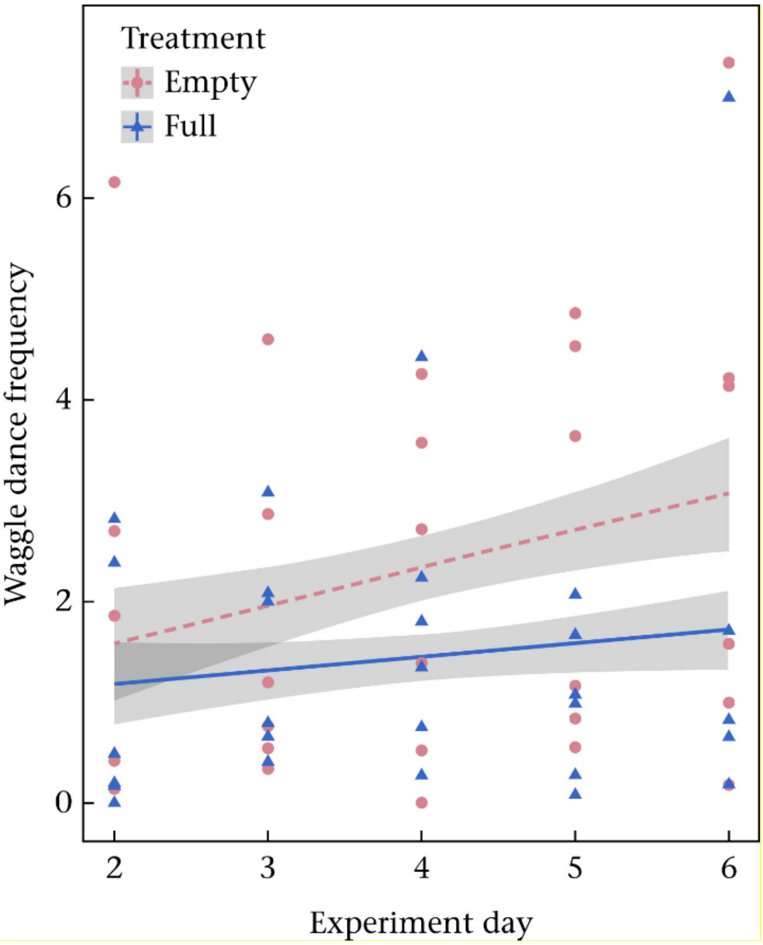


Figure 1.1. Number of waggle dances counted for 2 min every 20 min of video recording in relation to treatment from the second to sixth day of the treatment period. Treatments were empty and full honey store hives. Dots represent the average waggle dance number of each colony. The lines depict the predicted linear trend of each treatment (FH and EH). Grey areas delimit 95% confidence intervals.

We found no significant differences in the dance duration (*N* = 58) depending on the treatment or treatment day (LME; treatment: 6.09 waggle phase per dance (5.04 - 7.13) versus FH = 6.47 (5.13 - 7.81); $F_{1,49.22}$ = 0.873, *P* = 0.35; treatment day: 6.09 waggle phase per dance (5.04 - 7.13) versus FH = 6.47 (5.13 - 7.81); $F_{1,49.13}$ = 0.008, *P* = 0.93), and no interaction was found between days and treatments ($F_{1,49.13}$ = 1.50, *P* = 0.23).

Waggle dance follower behaviour

The number of dance followers was lower when the honey stores were low (LME: EH = 2.82 (2.58 - 3.06) versus FH =3.43 (3.21 - 3.66); $F_{1,190.65}$ = 17.3, *P* < 0.001; Fig. 1.2). Treatment day had no effect (first day of video recording, day 2 = 3.00 (2.62 - 3.38) versus last day of video recording, day 6 = 2.96 (2.55 - 3.37); $F_{1,192.16}$ = 0.32, *P* =0.58) and no interaction was found between days and treatments ($F_{1,188.76}$ =2.47, *P* =0.12).

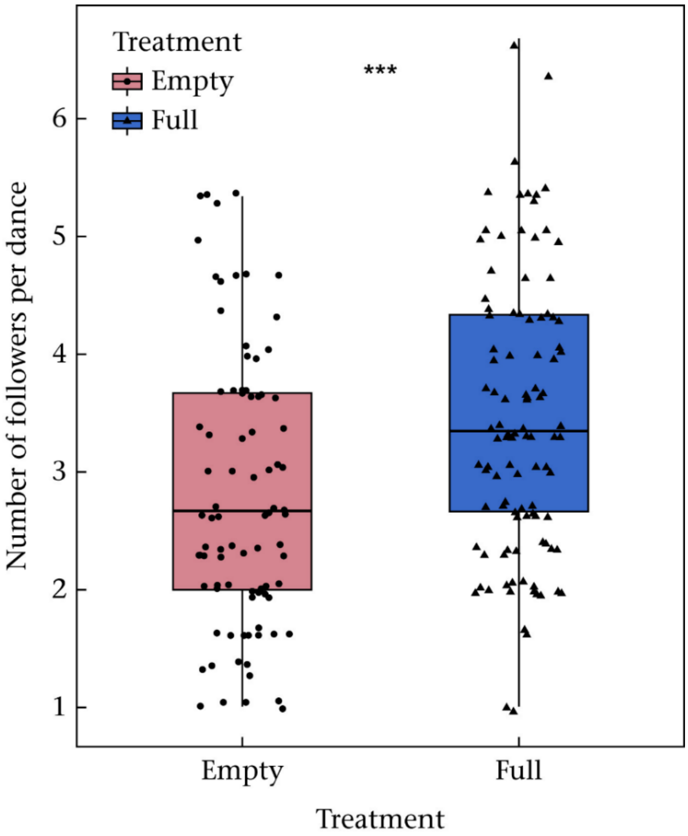


Figure 1.2. Number of waggle dance followers per dance during the experimental period in hives with empty and full honey stores. Box plots show the median, 25th and 75th quartiles and the 5th and 95th percentiles. ***P < 0.001. Individual data points are represented by the black points and triangles.

We also analysed the interest of the followers to follow a waggle dance but found no difference in the number of the waggle phases followed by dance followers in FH hives compared to EH hives (LME: EH = 4.23 waggle phase followers followed (3.67 - 4.79) versus FH = 4.22 (3.82 - 4.61); $F_{1,184.57} = 0.05$, $P = 0.83$). The number of waggle phases followed by followers was not affected by the treatment day (FD = 4.73 (4.03 - 5.44) versus LD = 4.45 (3.70 - 5.20); $F_{1,186.55} = 0.49$, $P = 0.48$). No interaction was found between treatment day and treatment ($F_{1,183.90} = 0.16$, $P = 0.69$).

We found no difference in foraging distance, measured as the waggle phase duration, between FH and EH hives (LME: EH = 1.05 s (0.93 - 1.17 s) versus FH = 1.21 s (1.07 - 1.35 s); this corresponds to ~0.76 km versus ~0.88 km; $F_{1,182.68} = 1.87$, $P = 0.17$). We found a borderline nonsignificant trend of treatment day to affect the foraging distance (FD = 1.18 s (0.88 - 1.48 s) versus LD = 1.22 s (1.04 - 1.40 s); 0.86 km versus 0.88 km; $F_{1,185.44} = 3.42$, $P = 0.066$). No interaction was found between treatment day and treatment ($F_{1,181.53} = 2.40$, $P = 0.12$).

Immune gene expression

We found that the expression of *defensin 1* was significantly higher in honey-depleted hives (LME: $F_{1,46} = 4.77$, $P = 0.035$; Fig. 1.3), while the other six genes (*abaecin, apidaecin, apimisin, relish, hymenoptaecin* and *lysozyme*) showed no change in expression due to the removal of honey stores (*abaecin*: $F_{1,46} = 1.16$, $P = 0.29$; *apidaecin*: $F_{1,46} = 0.21$, $P = 0.65$; *apimisin*: $F_{1,46} = 0.86$, $P = 0.36$; *relish*: $F_{1,46} = 0.07$, $P = 0.79$; *hymenoptaecin*: $F_{1,46} = 0.25$, $P = 0.62$; *lysozyme*: $F_{1,46} = 2.26$, $P = 0.14$; Fig. 1.3), compared to hives with a full honey frame (FH).

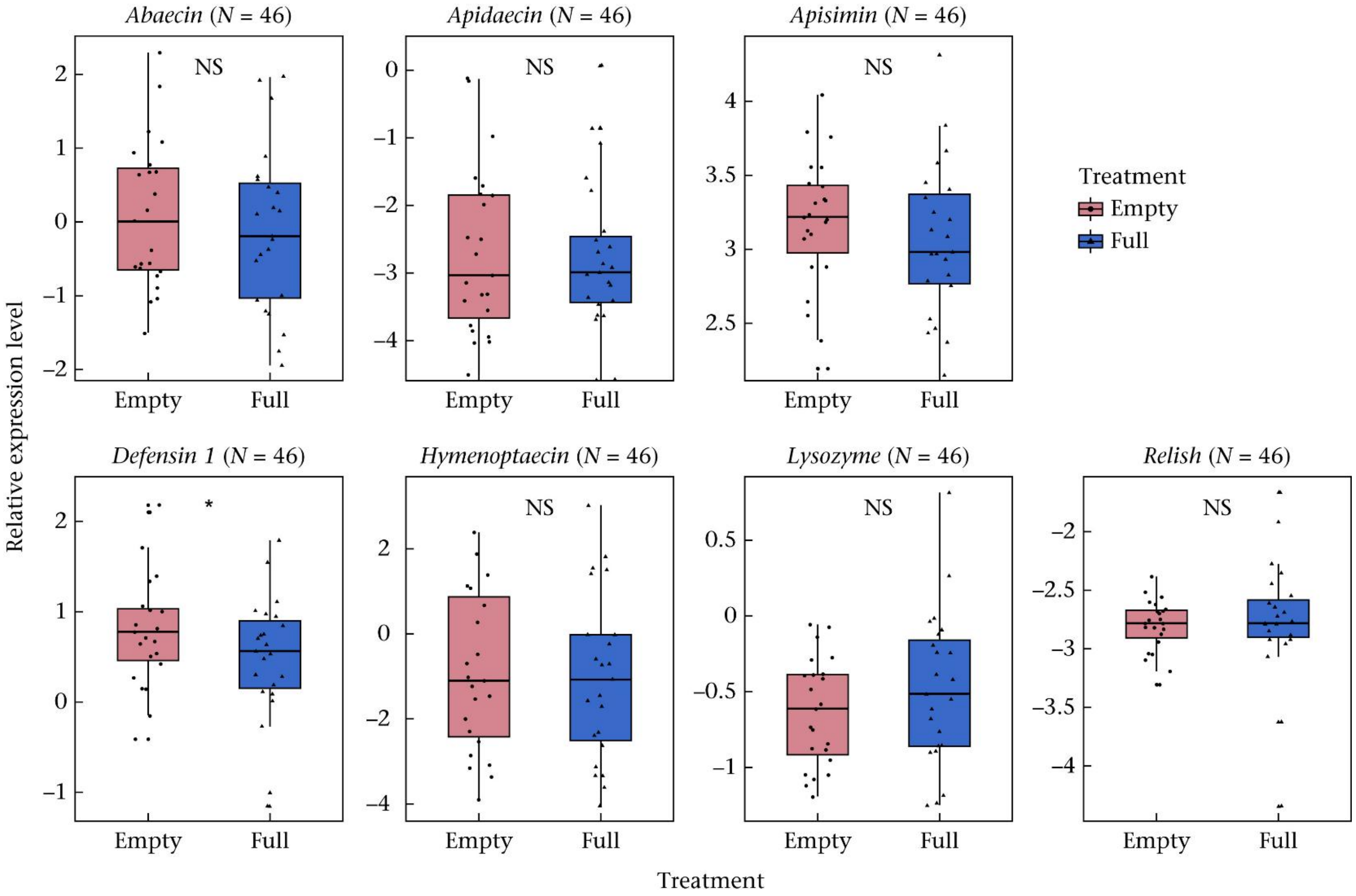


Figure 1.3. Expression of the seven tested immune genes in hives with empty and full honey stores. Box plots show the median, 25th and 75th quartiles and the 5th and 95th percentiles. $*P \leqslant 0.05$.

Fatty acids

We identified five main fatty acids: palmitic acid (C16:0), a monounsaturated C16 acid (probably palmitoleic acid, C16:1), stearic acid (C18:0), oleic acid (C18:1) and a di-unsaturated C18 acid (probably linoleic acid, C18:2). Palmitic acid, stearic acid and oleic acid can be biosynthesized by the bees and are most abundant in their bodies. Palmitoleic acid can be converted from palmitic acid in the fat body but is only present in small amounts. Linoleic acid, on the other hand, has to be acquired from the diet (Stanley-Samuelson et al., 1988). Rosumek et al. (2017) found that linoleic acid (C18:2) can quickly accumulate through dietary supplement.

We found no effect of our treatment on the absolute quantity of fatty acids (LME: FH versus EH: $\chi^2_1 = 0.009$, $P = 0.92$; Fig. 1.4). When we assessed the proportions of different types of fatty acids, saturated fatty acids and di-unsaturated fatty acids, we again found no difference between treatments in saturated fatty acids ($\chi^2_1 = 0.05$, $P = 0.83$) or di-unsaturated fatty acids ($\chi^2_1 = 0.006$, $P = 0.93$).

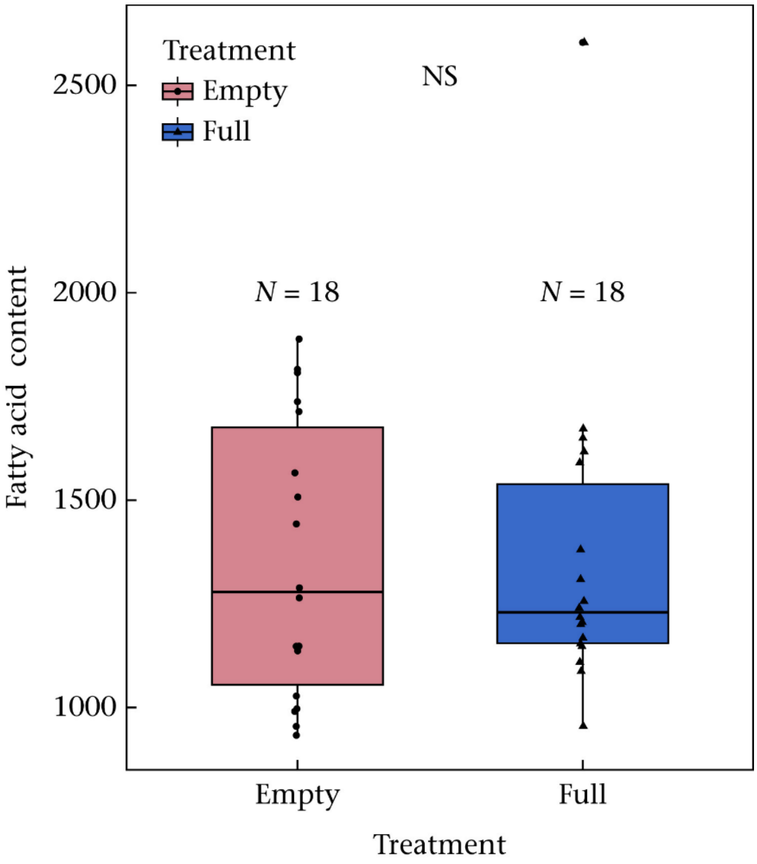


Figure 1.4. Absolute quantity (μg) of fatty acids in bee abdomens in hives with empty and full honey stores. Box plots show the median, 25th and 75th quartiles and the 5th and 95th percentiles.

Discussion

We found that the waggle dance frequency increased by ~60% when colonies had very little honey compared to colonies with full honey stores (Fig. 1.1); thus, workers were providing more information about foraging opportunities to nestmates under these conditions. Furthermore, dancing increased during our experimental period. Several reasons could explain this finding. First, starvation causes bees to start foraging at a younger age, which may temporarily increase the forager pool (Schulz et al., 1998, 2002). Furthermore, foraging activity increased after honey removal in both stingless bees (*Melipona fasciculata*) and honey bees (Schulz et al., 1998, 2002; Gostinski et al., 2017), suggesting that foragers may have switched to a more proactive foraging strategy when honey stores are depleted. In addition, increased storage space reduced the production of stop signals, which inhibit waggle dancing (Kietzman & Visscher, 2021). These processes, possibly acting in concert, could explain why we found more dances after the experimental reduction in honey stores. Rinderer (1982) found that empty cells could be used as an information cue by foragers to assess the nutritional state of the colony and thereby affect the number of dances. At a physiological level, the perception of low honey stores is likely to modulate the sucrose response threshold of foragers, which is linked to a range of foraging- and communication-related behaviours, such as learning(Scheiner et al., 2003, 2004), foraging division of labour (Pankiw & Page Jr, 2000) and dancing (George et al., 2020).

We did not find a difference in the average duration of waggle dances, which often correlates with dance motivation (von Frisch 1967; Seeley et al., 2000). This might indicate that our treatment did not increase average dance motivation. However, if low food stores caused bees that would not normally dance to perform short dances, average duration of all dances might remain constant despite a general increase in dance motivation in the forager population. Overall, our results indicate that starved colonies are likely to become less selective than colonies with full honey stores. We found no effect of honey removal on foraging distances indicated by dancing bees; colonies foraged at an average distance of ~0.76 km (starved) to ~0.88 km (full). Colonies will often collect nectar and pollen at greater distances if food sources are scarce (Couvillon et al., 2014b; Ohlinger et al., 2022), but since our treatment did not affect food source abundance and bees will often return to the same food sources for several days

(Butler et al., 1943; von Frisch, 1967; Al Toufailia et al., 2013), a lack of an effect of honey stores on foraging distances is not surprising.

The number of followers per dance decreased by ~20% when colonies were honey depleted, suggesting that fewer forgers relied on spatial information about resources, which is consistent with foragers switching to a proactive, solitary foraging strategy (I'Anson Price et al. 2019). An alternative explanation is that forager interest in dances remained constant, but individual bees had more options to follow waggle dances since there were more dances overall, leading to a reduction in dance followers per dance. As a result of this reduction in dance follower number and the concurrent increase in dance number, starved colonies might spread out and exploit a larger number of different food sources. Our finding contrasts somewhat with Rinderer (1982) who found that dancers had more followers in colonies with more available storage space. However, Rinderer (1982) manipulated storage space rather than honey stores, which could explain the different outcomes.

Food stores had no effect on the number of waggle phases that followers followed. The number of waggle phases a bee follows is an indication of how a bee uses the dance information. Longer dance following suggests bees try to decode the vector information, whereas shorter dance following indicates that bees are using it for reactivation or confirmation that a previously visited food source is still available (Biesmeijer & Seeley 2005; Grüter & Ratnieks 2011; Grüter et al. 2013). On average, interest in spatial information seems to have remained constant in those bees that did follow dances.

We found that the expression of the immune-related gene *defensin 1* increased when colonies had low honey stores, while the other tested genes did not respond to our treatment in the time window we studied. *Defensin 1* plays an important role in social immunity, it regulates AMPs expression and affects antimicrobial and antifungal activity (Ilyasov et al., 2013). Previous studies found that low pollen reserves in hives increased the expression of *defensin 1* in honey bees, and increased expression was a reliable predictor for colony survival over winter (Barroso-Arévalo et al., 2019, Corona et al., 2023). An increased *defensin 1* expression suggests an increased investment in immunity in response to nutritional stress. In turn, *defensin 1* expression can be used to monitor the health of honey bee colonies (Barroso-Arévalo et al., 2019). The other tested genes (*abaecin, apidaecin, apimisin, relish,* *hymenoptaecin* and *lysozyme*), which can be activated by abundant pollen, play crucial roles in individual immunity against a broad range of microorganisms, including bacteria, fungi, parasites and viruses (Castelli et al., 2020; Corona et al., 2023; Danihlík et al., 2018). One possible explanation for a lack of a treatment effect on the expression of these genes might be that they are activated in response to pollen availability rather than honey stores.

Previous studies have found that fat stores predict overwintering survival and foraging activity in honey bees (Toth et al., 2005; Toth & Robinson, 2005; Döke et al., 2015). The composition of fatty acids can be affected by diet; for instance, linoleic acid can only be acquired through the diet (Rosumek et al., 2017; Arien et al., 2020). However, the relative proportions of fatty acids and total fatty acid content did not differ between treatments after 6 days of honey depletion (Fig. 1.4). It is possible that body fat content and composition depend more on the pollen diet of bees, the main source of lipids (Brodschneider & Crailsheim, 2010), rather than on honey. Accordingly, pollen lipid profiles affected the amount of total fatty acids and essential fatty acids in bees (Arien et al., 2020). Alternatively, our experimental period of 6 days may have been too short to cause changes in fat stores. Physiological changes do not happen as quickly as behavioural changes, which are often the first response to environmental changes (Wong & Candolin, 2015). Our findings suggest that body fat stores are unlikely to change in response to brief periods of low nectar availability, for example those caused by several days of bad weather. More studies of fatty acid content in pollinators are needed to better understand how fat stores relate to landscape level changes, nutritional challenges and health. Nutritional stress is likely to have varied impacts on behaviour, the immune system and the physiology of pollinators, with knock-on effects for their survival. Our study is a step towards a better understanding of how nutritional stress caused by honey depletion influences foraging behaviours, the expression of immune-related genes and physiological changes.

Acknowledgements

Data availability

The data set used for this study is available on the DOI: https://doi.org/10.1016/j.anbehav.2024.07.017

Supplementary materials

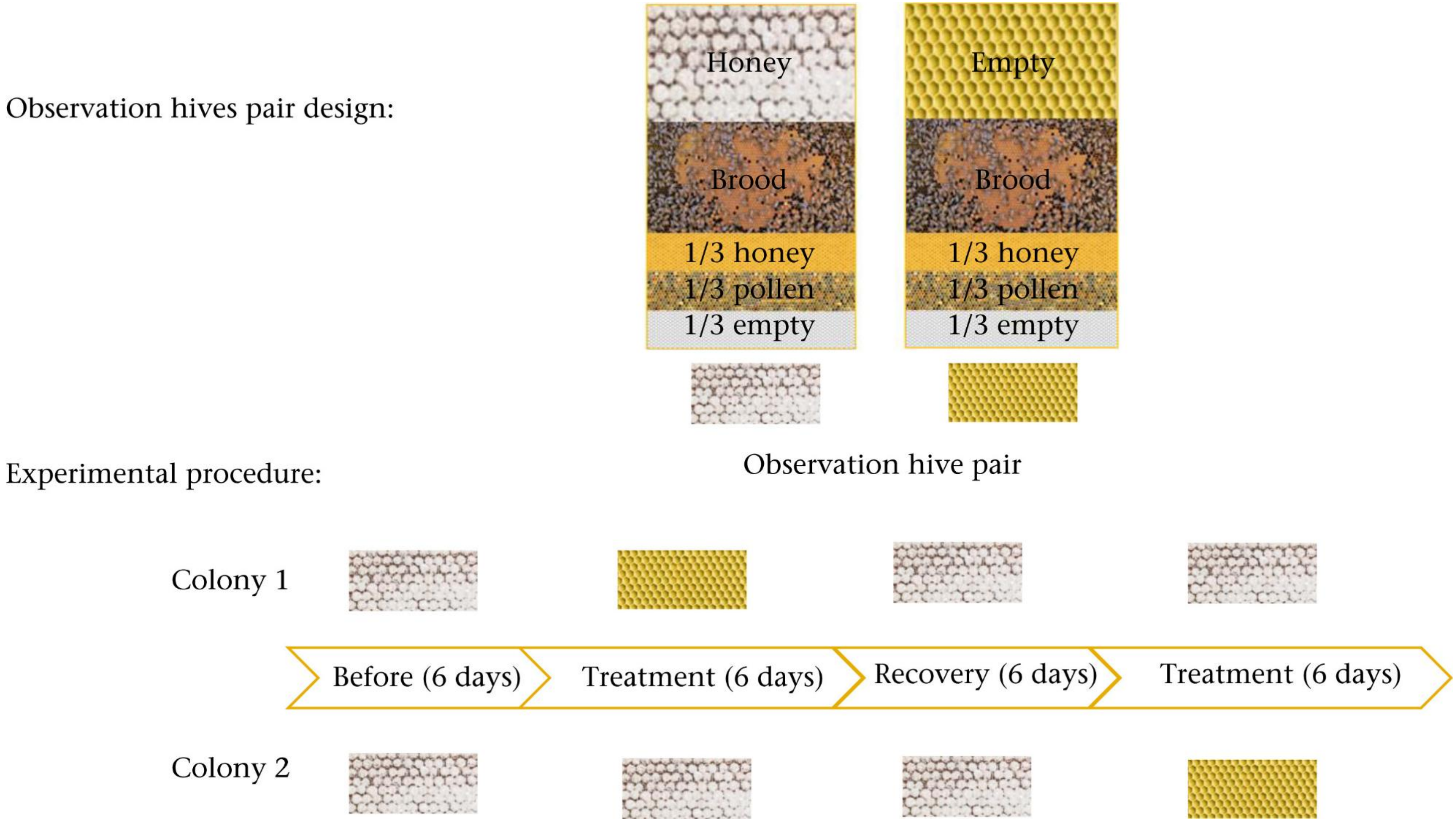


Figure S1.1. (a) The paired design with two observation hives. Each hive had a honey store frame, a brood frame and a mixed frame with honey, pollen and empty space. (b) Experimental procedure. The hives were first left for 6 days. Then we replaced the top honey frame in one hive with an empty frame (EH treatment) and in the other with a full honey frame from another colony (FH treatment) The treatments lasted 6 days. After another 6-day recovery period with a full honey frame in both hives, we reversed the treatments for another 6 days.

Chapter 2

Land use and season interactively affect honey bee (*Apis mellifera*) body size and fat stores

Abstract

The loss and fragmentation of habitats caused by anthropogenic activities in the last decades has affected foraging habitat quality and, therefore, foraging success (food quantity, quality and foraging range) of many animals, including many pollinators. Honey bees (*Apis mellifera*) are important pollinators of many plant species, and habitat change has also affected their ability to collect the resources they need to maintain the colony. Two important biological traits that might be affected by land use are body size and fat stores, which have the potential to affect body condition, and therefore success, and colony health. However, few studies have investigated these traits in different landscapes and different times of year. We surveyed 47 sites in three different landscape types (agriculture, urban and mixed habitats) in south-western Germany. We measured honey bee body size, wing wear and analysed body fat quantity and composition using GC-MS in the spring, summer and autumn. We found that summer honey bees were smaller in urban and mixed habitats, they showed the greatest wing wear, but they had 18.4-21.3% larger fat stores compared to agricultural sites. Bees in agricultural habitats experienced a drop in fat stores in summer, while body size remained unaffected. In autumn, just before honey bees enter the inactive winter period, bees in urban and mixed areas experienced a drop in fat stores. Wing length decreased from spring to autumn irrespective of habitat type. Our findings indicate that bees in agricultural settings experience physiological challenges in a central European region in summer, possibly because urban and mixed habitats provide better nutritional conditions during summer. Our findings, thus, confirm that honey bees undergo morphological and physiological changes in response to land use and season, which could impact their physiological condition and winter survival.

Key words: body size, fatty acids content, head width, honey bee, land-using, wing wear

Introduction

Most flowering plants, including wild and cultivated species, rely on animal pollination (70%-90% of all angiosperm species) and bees, in particular, play important roles as pollinators (Bawa, 1990; Fontaine et al., 2005; Ollerton et al., 2011; Potts et al., 2016; Ollerton, 2017). In the last decades, however, anthropogenic activity has created landscapes that are increasingly dominated by agricultural monocultures (Aizen et al., 2008; Plourde et al., 2013; Otto et al., 2018) and urban habitat (Mcdonald et al., 2008; Seto et al., 2012a). This conversion of natural habitat into urban or intensively managed agricultural land can lead to food shortages, either in terms of overall quantity or diversity, in some of these landscapes, leading to seasonal foraging challenges (e.g. Couvillon et al., 2014b; I'Anson Price et al., 2019; Timberlake et al., 2019). Poor nutrition, in turn, has different negative effects on bees, e.g. reduced body size, poor immunity, and lower fat stores (Roulston & Cane, 2002; Li et al., 2012; Alaux et al., 2010; Ruedenauer et al., 2020). For example, pollen protein content and diversity were lower in areas of high crop intensification (Donkersley et al., 2014) and honey bee (*Apis mellifera*) colonies lost a considerable amount of weight when crops stopped blooming, causing food scarcity and a reduction in individual bee fat stores (Dolezal et al., 2019). The effects of urbanisation on food availability appear to be complex (Liang et al., 2023). Some urban areas were found to offer better foraging conditions for honey bees, based on an analysis of foraging distances and flower visitation rates (Theodorou et al., 2020; Samuelson et al., 2021). However, increases in hive numbers in urban areas, due to the increased popularity of urban beekeeping (Alton and Ratnieks 2013; Lorenz & Stark, 2015; Stevenson et al., 2020) can lead to a food shortage (Casanelles-Abella & Moretti, 2022). There is evidence that urbanization also affects pathogen loads, with some studies reporting increasing pathogen levels (Youngsteadt et al., 2015; Chau et al., 2023), while others show lower pathogen levels in urban habitats (Samuelson et al., 2020).

Another important trait affected by land use is body size: bumblebees (*Bombus*) in urban habitats have been shown to exhibit different body sizes, though these patterns were not consistent across species (Theodorou et al., 2021; Austin et al., 2022). Body size can show considerable intra-specific variation and is linked to fitness in both solitary and social bees (Goulson et al., 2002; Bosch, 2008). Smaller body size can be an indicator of nutritional stress, decreased floral resource availability (Kim, 1999), and reduced quality of pollen and nectar in the larval diet (Burkle & Irwin, 2009). Stingless bees, for example, adjust worker body size according to hive food stores (Veiga et al., 2013), foraging competition (Segers et al., 2016) and time of year (Quezada-Euán et al., 2011). Bee size, in turn, can affect foraging ranges, with larger bees foraging at greater distances (Greenleaf et al., 2007; Kendall et al., 2022; Gruter & Hayes 2022).

Fatty acids stored in the bee's fat body – a tissue with an essential role in energy storage, metabolism and immunity – can also be an indicator of the bee’s nutritional condition (Beenakkers et al., 1985; Stanley-Samuelson et al., 1988). Nutritional stress caused by food shortages can significantly reduce fat stores. For example, Dolezal et al. (2019) found that bees from intensively farmed monocultures experienced reduced fat stores and colony weight when food sources became scarce, both of which can affect survival (Dolezal et al., 2019). Therefore, a better understanding of the links between land use and bee fat stores would help us better understand the links between habitat and bee health.

Nutritional stress has been shown to lead to a range of behavioural changes, such as an early onset of foraging in honey bees (Schulz et al., 1998, 2002) or more intense communication about resources (Rinderer, 1982; Wu et al., 2024), thus potentially affecting the foraging load of nutritionally stressed bees. This, in turn, could have an impact on wing damage: bees will acquire and accumulate wing damage due to foraging (Foster & Cartar, 2011a) and aging (Mueller & Wolf-Mueller, 1993; Higginson & Barnard, 2004), which may further increase foraging effort, reduce lifespan (Schmid-Hempel & Wolf, 1988; Johnson & Cartar, 2014; Vance & Roberts, 2014) and nectar foraging efficiency (Higginson & Barnard, 2004; Foster & Cartar, 2011b). Given that land use and season affect the availability and quality of floral resources, it is possible that they also affect the level of wing wear found in honey bee foragers.

We aimed to gain a better understanding of how different landscape types and seasons affect the three previously discussed traits with links to nutrition: (1) body size, assessed by measuring head width and wing length, (2) wing wear, a common measure of overall foraging activity (Toth et al., 2009) and (3) bee fatty acid stores, an important physiological trait that has been linked to colony winter survival

(Dolezal et al. 2019). We captured free-flying honey bees visiting flowers in 47 sites in south-western Germany (states of Hesse and Rhineland-Palatinate; Fig. 2.1). We focused on three different landscape types: (1) predominantly urban habitats, (2) predominantly agricultural habitats and (3) mixed habitats. In response to declining insect populations (Steffen et al., 2015; Seibold et al., 2019), some governments have implemented initiatives that provide support for the creation of pollinator-friendly habitats on agricultural lands (Dicks et al., 2016), such as agri-environment schemes (AES) which were found to benefit insect biodiversity in Germany (Boetzl et al., 2021) and were particularly attractive for honey bees in the UK (Couvillon et al., 2014c). These programmes support insects by supplementing nutritional resources available throughout the season (Scheper et al., 2015; Sidhu & Joshi, 2016; Grab et al., 2018). Therefore, our third land use type "mixed" included areas that were part of an AES (Kennartenprogramm Rhineland-Palatinate). We predicted that honey bees captured in these mixed sites are larger and store more fat. We also expected bees to be smaller in summer due to a lack of food sources in many European habitats (Mandelik et al., 2012; Couvillon et al., 2014b; Timberlake et al., 2019; I'Anson Price et al., 2019).

Materials and methods

Study sites and study animals

We sampled foraging honey bees (*Apis mellifera*) at 47 sites located in the states of Hesse and Rhineland-Palatinate, Germany, from April to October 2021. We distinguished between urban (N=16), agricultural (N=16) and mixed (N=15) sites (Fig. 2.1). Land use data was extracted from the "Landcover classification map of Germany 2021 based on Sentinel-2 data" (mundialis GmbH & Co. KG (2022); Fig. 2.2 and Table S2.1). ArcGIS (Version 10.7.1, Esri) quantified land use within a radius of 1.5 km (most foraging happens within this distance from the hive; Steffan-Dewenter & Kuhn, 2003; Couvillon et al., 2014b), with the sampling location as the centre. Urban sites included, for example, the cities of Mainz, Wiesbaden and Frankfurt and the proportion of build-up was 55.6% on average (range: 31.3%-72.2%) (U01-U16; Fig.2.2; Table S3.1), while agricultural sites (A01-A16; Fig.2.2; Table S1) were dominated by agricultural land (on average 57.3%; range: 28.8%-85.3%). Finally, mixed sites (M01-

M15; Fig. 2.2; Table S2.1) included more diverse types of habitats, including forests (on average 44.5%; range: 19.3%-74.6%), urban habitats (on average 11.1%; range: 1.0%-27.3%) and habitat reserved for the agri-environment scheme (AES) “Kennartenprogram” of the state Rhineland-Palatinate. AES have been found to increase insect biodiversity in the neighbouring state of Bavaria (Boetzl et al., 2021) (Fig. S2.1). The distance between most sampled locations was at least >3.0 km (Fig. 2.1), but there were 2 agricultural sites and 7 urban sites with a minimum distance of 2.0 km. Honey bee foraging distances were shorter in urban environments in Samuelson et al. (2021), suggesting that urban hives have smaller foraging ranges.

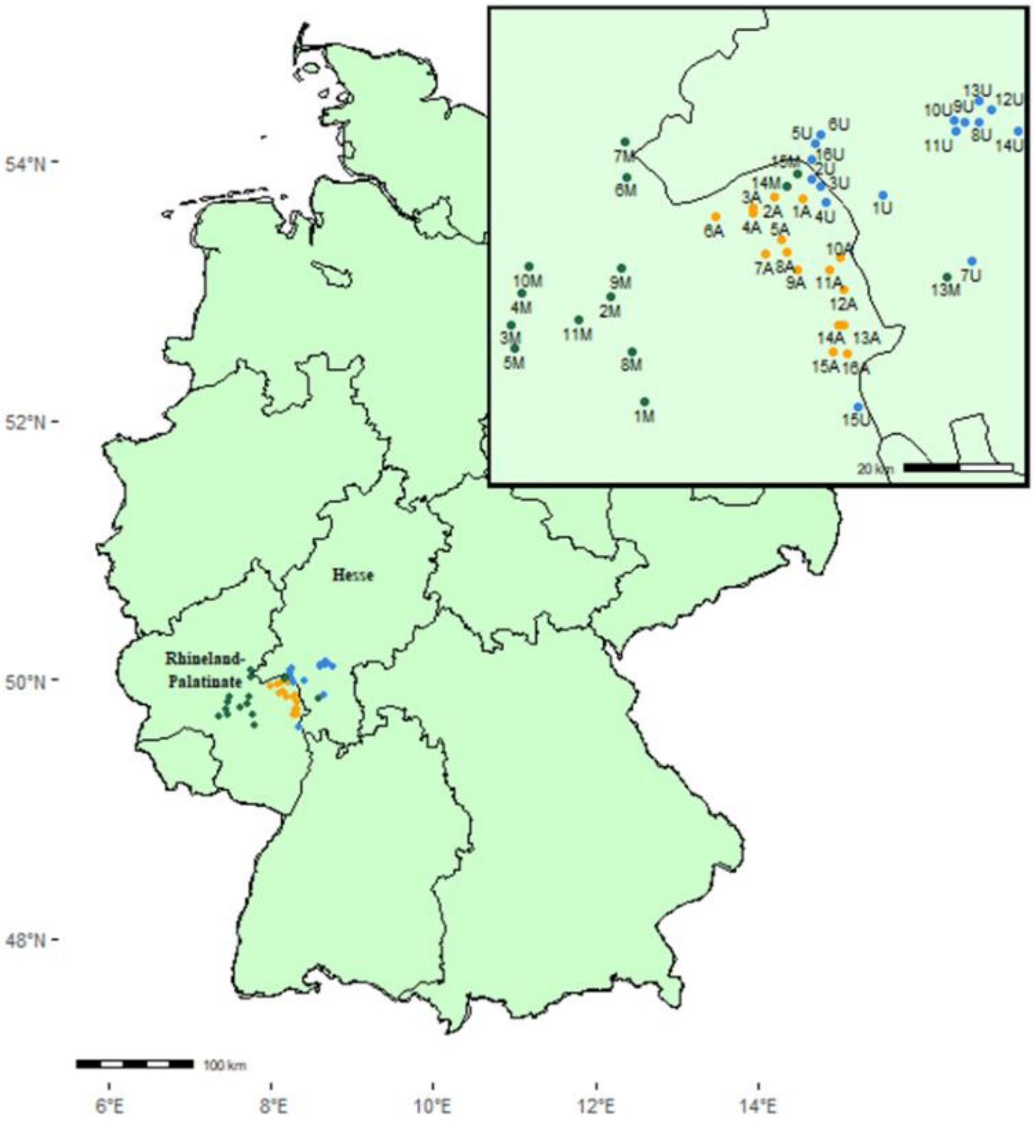


Figure. 2.1. Locations of sampled bees. Dots represent individual sites in the states of Hesse and Rhineland-Palatinate, south-western Germany. U (blue) = urban landscapes, A (orange) = agricultural landscapes, and M (green) = Mixed-habitats.

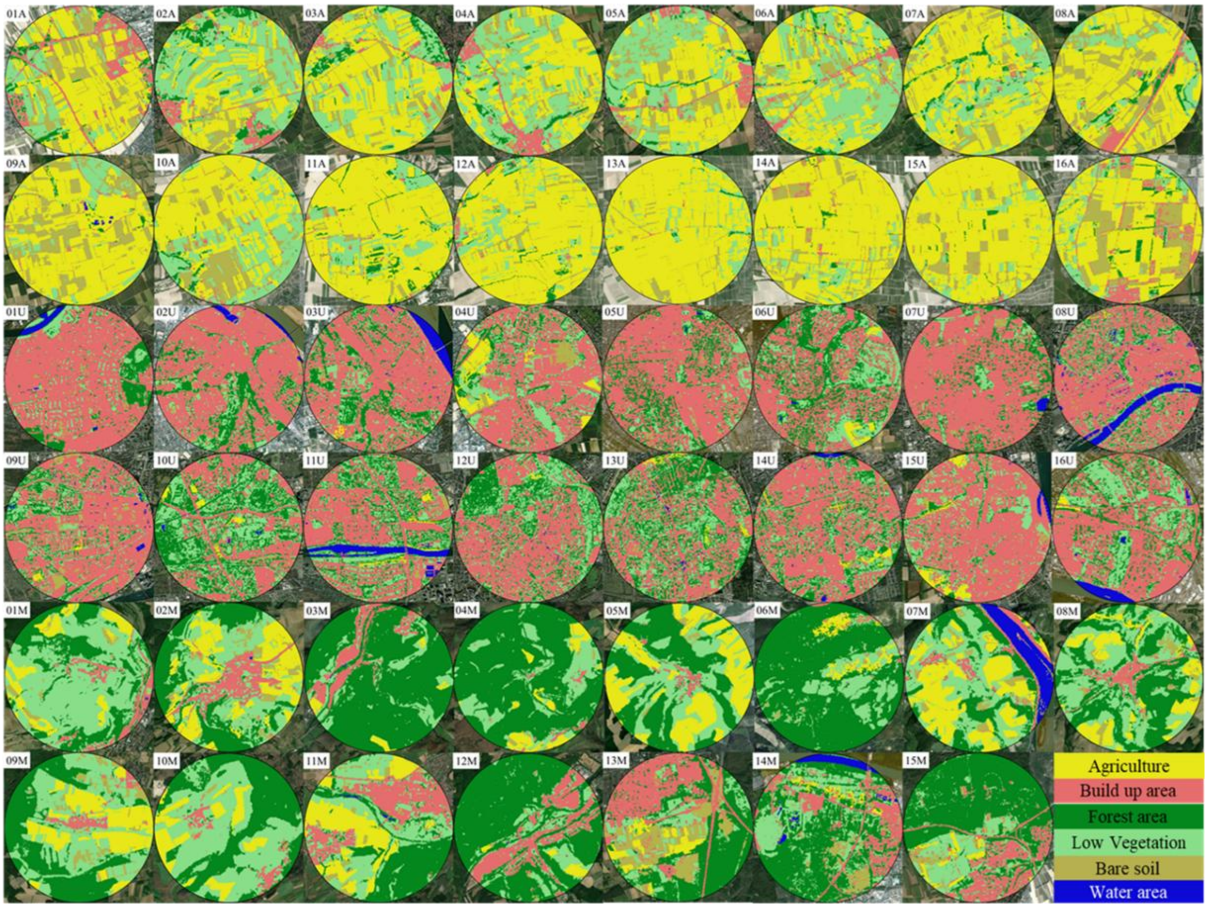


Figure. 2.2. Land use classification of sites (r = 1.5 km from the location of bee capture), extracted from the “Landcover Classification Map of Germany 2021 based on Sentinel-2 Data” (mundialis GmbH & Co. KG, 2022): Agriculture (yellow): cultivated areas, including non-irrigated and irrigated arable land, crop fields, temporary bare soils (e.g., fallow lands), and areas with vines. Build-up area (red): surfaces altered by human construction, replacing natural surfaces with artificial materials (Malinowski et al., 2020). This includes mixed urban and suburban areas like residential, commercial, industrial, transportation, communication, and utilities. Forest area (dark green): broadleaf tree cover land and coniferous tree cover land. Low vegetation (soft green.): herbaceous vegetation, both natural, low productivity grassland and managed grassland, used for grazing and/or mowing. Also includes low growing vegetation with closed cover and with predominately shrub and bushy vegetation (limited herbaceous species allowed). Bare soil (moderate yellow): any natural surface material, including consolidated, mostly impervious surfaces formed by natural materials with a solid surface. This includes surfaces modified by human processes like extraction sites, as well as loose mineral particles from natural sedimentation or human activity, such as mountain slope debris, glacier moraines, river pebble banks, beaches, sand dunes (unvegetated), and quarries. Water area (blue.): water bodies (natural or artificial).

Bee collection

We sampled 6 foraging honey bees at each site in three different meteorological seasons (see below) with a 15ml Falcon tube. We located bees on flowers, starting our search from the centre of the selected sites. Each tube was used to catch one honey bee, and tubes were immediately put in an insulated portable cooling bag containing reusable ice packs (frozen before use) to stop bees from moving. Cooled bees were kept in a -20°C freezer until further measurements took place. Each site was visited three times corresponding to three different meteorological seasons, once in spring (April and early May), summer (June and July) and autumn (September). Thus, 18 bees were collected at each site. Four of six bees per site and time point were used to measure the fatty acid content, while head width and wing measurements were taken from all six bees.

Head width and wing length measurements

Head width (HW, Fig S2.2A) and left forewing length (WL, Fig. S2.2B) were used as measures of bees size as they reliably correlate with overall body size (Bullock 1999; Grüter et al., 2012; Sauthier et al., 2017). In our study, HW and WL were significantly correlated (Pearson correlation coefficients: $r = 0.455$, $P < 0.001$), and both were considered because land use and season might affect them differently. To explore this further, we also calculated the HW/WL ratio. Body parts were placed on laminated graph paper (wings were flattened under a microscope slide) and pictures were taken using an Axiocam 208 microscopy camera mounted on a Stemi 305 (Carl Zeiss, Jena, Germany) stereomicroscope. Subsequently, measurements were performed using ImageJ version 1.54h (Abràmoff et al., 2004).

Wing damage

Wing damage can often be found in the form of cuts or missing areas. We classified wing damage using three levels according to Mueller & Wolf-Mueller (1993): wing margins are (1) hardly damaged (wear < 10%), (2) considerably damaged (10% < wing margins wear < 80%) or (3) extensively damaged (80% < wing margins wear (Fig S2.3)). This classification is based on the proportion of the wing margins affected by damage. We assessed the level of wing wear in honey bee samples in relation to land use type and season.

Fatty acids extraction

The most commonly found fatty acids in bee bodies are the *saturated fatty acids* (palmitic acid (C16:0), stearic acid (C18:0)) and the *unsaturated fatty acids* (palmitoleic acid (C16:1), oleic acid (C18:1), linoleic acid, (C18:2)) (Wu et al., 2024). Palmitic acid, stearic acid and oleic acid can be biosynthesized by the bees and are most abundant in their bodies. Palmitoleic acid can be converted from palmitic acid in the fat body, but is only present in small amounts. Linoleic acid, on the other hand, has to be acquired from the diet (Stanley-Samuelson et al., 1988).

Fatty acids were extracted from entire bee abdomens using 1 ml of a chloroform: methanol mixture, 2:1 (v/v) over a period of 24 h (Folch et al., 1957; Wu et al.,2024). The samples were evaporated to dryness under gentle nitrogen flow and then redissolved in 250 µl of a 2:1 dichloromethane: methanol (v/v) mixture. We added 1.6 µg of nonadecanoic acid (dissolved in 10µl DCM/MeOH) as internal standard. After vortexing, we moved 5 µl of this solution into a new glass vial and evaporated to dryness under a gentle nitrogen flow. Finally, we added 20 µl trimethylsulfonium hydroxide (TMSH; 0.25 M in MeOH, Sigma-Aldrich, Munich, Germany) to derivatize to fatty acid methyl esters (FAMEs) and analyzed them with a 7890A gas chromatograph (Agilent) coupled to a 5975C mass-selective detector (Agilent) (GC/MS). Helium was used as the carrier gas at a flow rate of 1.2 ml per minute. The temperature of the GC oven started at 60 °C for 1 min, then increased by 15 °C/min to 150°C, followed an increase to 200 °C with a heating rate 3 °C/ min, and finally increased by 10 °C/min to 320 °C, where it was held constant for 10 min. The separated FAMEs were transferred to the MS and electron ionization mass spectra were recorded at 70 eV from 40 to 650 m/z. Resulting peak areas were integrated manually using the software MSD ChemStation G1701EA E.02.02.1431 (Agilent) and identified based on diagnostic ions, retention time and the molecular peak. Only fatty acids with abundance >1% were included in our analyses (Rosumek et al., 2017). This method can detect fatty acids between C10 and C20, but only chain lengths of C16 to C19 were found.

Statistical analyses

Head width, wing length, and the ratio between the head width and wing length

All data were analyzed in R 4.3.2 (R Core Team, 2023). We used general linear mixed-effects models (LMEs), with sampling sites as random effect to control for the non-independence of data from the same sites (bees from one site could be from the same hive) (Zuur et al., 2009). Our fixed effects were *landscape type* (agricultural, urban and mixed) and *season* (spring, summer and autumn). We used the “lme4” and “lmerTest” packages for model fitting and estimation of p-values for fixed effects (Bolker et al., 2009). The package "emmeans" was use to estimate p-values for pairwise comparisons (Lenth, 2023). We tested if our fixed effects affected the head width and wing length. The head width data caused a singular fit due to low variation in our random effects, therefore we also used general linear models (LM) without random effects. The p-values for both types of models were very similar and only LME outputs are shown. Significance of fixed effects and their interaction was tested by comparing models with and without each fixed effect (or interaction) using likelihood ratio tests (LRTs). We used the “rcompanion” packages for calculating the means and their confidence intervals (CIs) for fixed effects (Mangiafico, 2023). The “DHARMa” (Hartig, 2022) package was used to check whether model assumptions were met (Zuur et al., 2009). We used Interquartile Range (IQR) to check for outliers in the data (Dekking et al., 2005).

Wing wear

We analysed wing damage as an ordinal response variable (1 to 3) and used Cumulative Link Mixed Models (CLMM). We used the “ordinal” package for model fitting and estimation of p-values for fixed effects (Christensen, 2023). Model structure and significance testing followed the procedure described above.

Fatty acids

We tested for differences in the absolute quantity of fatty acids as well as the proportions of saturated and di-unsaturated fatty acids (tri-unsaturated acids were not detected) by normalizing the values using the quantity of the internal standard. The remaining fatty acids, the monounsaturated fatty acids, are equal to 1 - (saturated + double unsaturated fatty acids). We used LMEs to compare fatty acid quantities in bees between different seasons and landscapes following the procedure described above. To compare the relative proportions of different types of fatty acids, we used non-metric multidimensional scaling (NMDS) (command *metaMDS,* package *vegan*) (Dixon, 2003). The permutational multivariate analysis of variance (PERMANOVA) used the *adonis* function (package *vegan*) to assess the significance of 'landscapes' and 'seasons' as fixed effects while accounting for "sample sites" as a random effect. This was done by randomly rearranging the proportions of different types of fatty acids within the levels of the fixed effects, while keeping the structure of the random effect intact. This approach was used to determine the significance of the observed patterns through permutations.

Results

Body size difference between landscapes and seasons

Head width differences in different landscapes and seasons

We measured a total of 840 bees. We found a significant interaction between the fixed effects landscape and season (LME, *LRT* = 11.72, *P* = 0.020), therefore, we separated the data according to landscape to further explore this interaction. In urban sites, bees were smaller in summer than in spring and autumn (Table S2.2; Fig 2.3A). Similarly, bees in mixed habitats were smaller in summer than in spring (HW_{Sum} = 3.88 mm (3.86 to 3.89) *vs* HW_{Spr}=3.91mm (3.90 to 3.92), *t* = 3.51, *P* = 0.002; Table S2.2; Fig 2.3A). Autumn bees were intermediate in size (Fig. 2.3A). There was no significant difference between seasons in agricultural areas (LME: *df* = 2, *F* = 0.63, *P* = 0.53; Table S2.2; Fig 2.3A).

Wing length differences in different landscapes and seasons

We found significant differences in wing length depending on season (LME: *df* = 2, *F* = 4.08, *P* = 0.017), but not landscape type (LME: *df* = 2, *F* = 0.15, *P* = 0.86), and there was no significant interaction between landscape and season (*LRTs* = 2.86, *P* = 0.58). Wing lengths were shorter in autumn than in spring (LME: WL_{Aut} = 8.48 mm (8.46 to 8.49) *vs* WL_{Spr} = 8.51 mm (8.49 to 8.53); *t* = -2.85, *P* = 0.012; Table S2.3, Fig. 2.3B), with summer bees having intermediate wing lengths (Table S2.3, Fig. 2.3B).

Ratio between head width and wing length in different landscapes and seasons

We also tested if the ratio between head width and wing length depended on season and landscape type to explore possible morphological changes, with a larger ratio indicating a relatively larger head/shorter wing. We found a significant interaction between landscape and season ($LRT = 10.86$, $P = 0.03$). To explore this further, we analysed the landscape types separately. We found that in urban habitats, the HW/WL ratio was smaller in summer than in spring and autumn, meaning that summer bees had relatively smaller heads (Table S2.4, Fig. 2.3C). In agricultural landscapes, on the other hand, the ratio was larger in the autumn than in the spring (Table S2.4, Fig. 2.3C), meaning that autumn bees had relatively smaller wing size. There was no seasonal effect in mixed habitats (LME: $df = 2$, $F = 1.0$, $P = 0.36$).

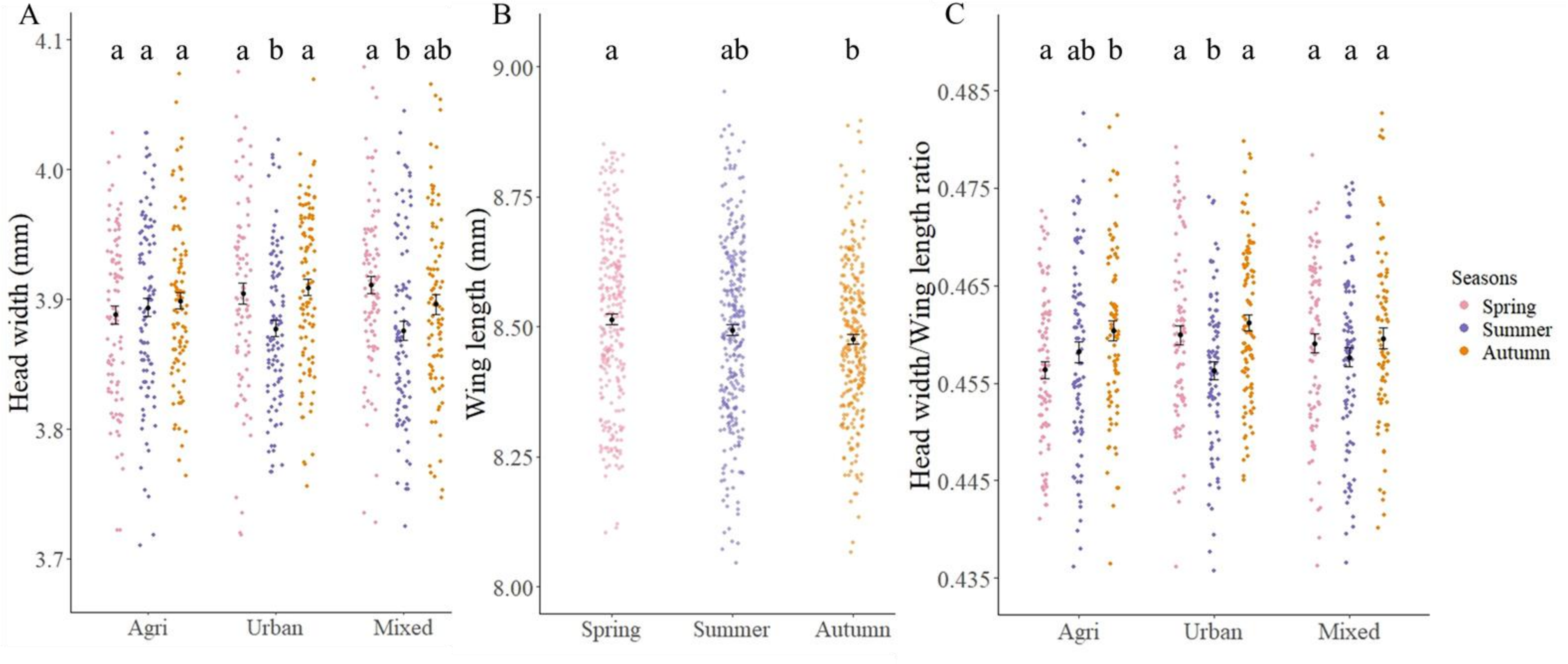


Figure. 2.3. Head width of sampled bees (A). Wing length of bees in the study area (B). The ratio of the Head Width (HW)/ Wing Length (WL) (C). Dots represent individual bees in different seasons (pink = spring, blue = summer and Orange = autumn) and landscapes (Agri = Agricultural, Urban = Urban and Mixed = Mixed habitats). Error bars show the mean value and the standard error. Lowercase letters indicate statistical significance following pair-wise t-test comparisons ($p < 0.05$).

]

Wing wear depending on landscape and season

We found significant differences in wing wear depending on season (*LRTs* = 27.6, *P* < 0.001), but not landscape (*LRTs* = 4.0, *P* = 0.14), and there was no significant interaction between landscape and season (*LRTs* = 4.49, *P* = 0.34). Wing wear was significantly larger in summer compared to spring and autumn (CLMM: *Z* = -3.4, P = 0.002; *Z* = -4.9, P < 0.001; Fig 2.4; Table S2.5), but there was no significant difference between wing wear in autumn and spring (CLMM: *Z* = 0.034, P = 1.0).

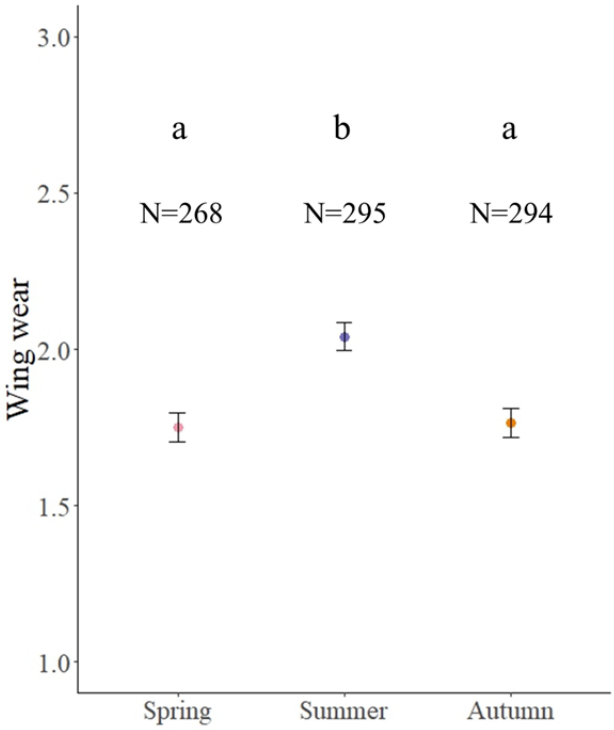


Figure. 2.4. Wing wear in the different seasons. Dots represent the mean, whiskers represent standard errors. a and b lower case letters show statistical difference ($p < 0.05$) in tested group.

Fatty acid content in relation to landscape and season

The five main fatty acids identified from the abdomen of individual honey bees were palmitic acid (C16:0), a monounsaturated C16 acid (probably palmitoleic acid, C16:1), stearic acid (C18:0), oleic acid (C18:1) and a di-unsaturated C18 acid (probably linoleic acid, C18:2). We analysed the absolute quantity of fatty acid content and the proportions of different types of fatty acids of honey bees in different landscapes and seasons.

The absolute quantity of fatty acid content

We found a significant interaction between landscape type and season (LME: *LRT* = 29.41, *P* < 0.001). Therefore, we analysed the different landscapes separately. In agricultural landscapes, we found a lower quantity of fatty acids (16.3%) in summer than in the autumn (*t* = -3.82, *P* < 0.001; Table S2.6; Fig. 2.5), with spring bees having intermediate levels of fatty acids. In contrast, we found that fatty acid content was higher in summer than in autumn in urban and mixed landscapes (9.1% and 9.7%, respectively) (Table S2.6; Fig. 2.5). When separating the different seasons, we found that fatty acid content was lower in agricultural sites than in urban (18.4%) and mixed (21.3%) sites in summer, but there was no significant difference in spring and autumn (Table S2.6; Fig. 2.5).

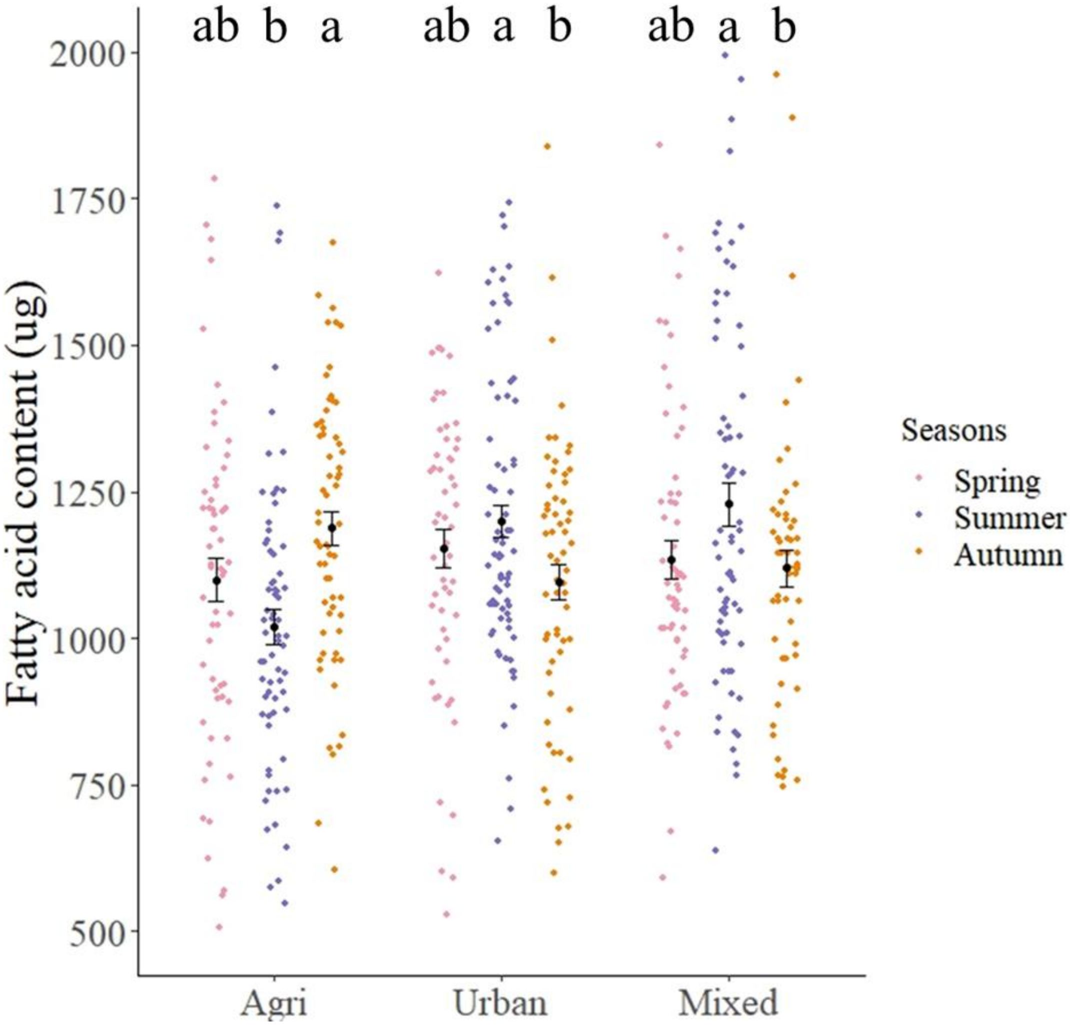


Figure. 2.5. Absolute quantity of fatty acids in bee abdomen captured in different landscape types (agriculture, urban and mixed) and seasons (pink= spring, blue= summer and orange= autumn). Dot and whisker represent the mean, and the standard error. Lowercase letters indicate statistical significance following pair-wise t-test comparisons (p < 0.05).

The proportion of different types of fatty acids in different landscapes and seasons

We found significant differences in the proportion of di-unsaturated C18 acid (probably linoleic acid, C18:2) between seasons (LME: $df = 2$, $X^2 = 485.36$, $P < 0.001$), but not landscapes (LME: $df = 2$, $X^2 = 4.24$, $P = 0.12$). There was no interaction between the landscape and season ($LRTs = 6.48$, $P = 0.17$). Bees had a higher proportion of doubly unsaturated fatty acids in spring than in summer and autumn (Fig S2.4; Table S2.7). Furthermore, we found significant differences in the proportion of monounsaturated fatty acids between seasons (LME: $df = 2$, $X^2 = 219.11$, $P < 0.001$), but not between the landscapes (LME: $df = 2$, $X^2 = 0.60$, $P = 0.74$). There was no interaction between the landscapes and seasons ($LRTs = 4.47$, $P = 0.35$). The proportion of monounsaturated fatty acids was lower in spring than in summer and autumn (Table S2.7). However, we found no significant differences in the saturated fatty acids depending on landscape (LME: $df = 2$, $X^2 = 1.31$, $P = 0.52$), and season (LME: $df = 2$, $X^2 = 1.83$, $P = 0.40$), and there was no significant interaction between landscape and season ($LRTs = 5.72$, $P = 0.22$).

Relationship between head width and fatty acids content in honey bee

We also tested if the total fatty acid content of a bee is related to body size. However, we found no significant relationship between head width and absolute fatty acid content (LME: $df = 1$, $X^2 = 0.2465$, $P = 0.62$).

Discussion

We found that land use type and season affected bee size and fat stores, often interactively. As predicted, bees had smaller heads in summer compared to spring and autumn, but this was only found in urban and mixed habitats, whereas bee size remained constant in agricultural sites (Fig. 2.3A). In contrast, we found that wing lengths decreased from spring to autumn (Fig 2.3B). Summer is known to be a challenging period for colonies in temperate European habitats, both agricultural and urban, due to a scarcity of flowers (Nürnberger et al., 2017; Dolezal et al., 2019; I'Anson Price et al., 2019; Czekońska et al., 2023) before the bloom of ivy (*Hedera helix*) in autumn improves the foraging conditions for bees (Garbuzov & Ratnieks, 2014; Knoll et al., 2024). The relative scarcity of food sources during summer may force bees to forage further away from their hives (Couvillon et al., 2014b). This might explain why wing wear was highest in summer. Wing wear directly impacts the flight ability and reflects cumulative foraging effort (Mueller & Wolf-Mueller, 1993). There is evidence that increased wing wear reduces lifespan and foraging efficiency of honey bees (Foster & Cartar, 2011b; Vance & Roberts, 2014). The challenging summer conditions are consistent with our finding that honey bees were smaller in summer, but it is somewhat unexpected that this effect was only found in urban and mixed landscapes, but not in agricultural landscapes (Fig. 2.3A). One explanation could be that some agricultural areas experience a short-term feast in late spring and early summer (Dolezal et al., 2019), benefitting bee size later in summer. Alternatively, producing smaller bees in summer might allow colonies that do well to increase brood production rate and boost colony population faster (Ramalho et al., 1998). This could reflect different maternal investment strategies in response to food resource availability in spring and early summer (Kim & Thorp, 2001) and deserves further study. Furthermore, high temperatures in summer might affect larval growth rate and, thus, the body size of bees: Kelemen and Rehan (2021) found that individuals of *Ceratina calcarata* were smaller at warmer temperatures (see also Sibly & Atkinson, 1994).

The relatively large size of spring bees should be interpreted with caution because the long lifespan of winter bees (Fukuda & Sekiguchi, 1966; Smedal et al., 2009) means it is possible that bees collected in spring were actually winter bees that developed in autumn of the previous year. As mentioned before, foraging conditions are often good in autumn due to the availability of ivy flowers, leading to improved larval diet for the production of winter bees (Garbuzov & Ratnieks, 2014; Knoll et al., 2024). In contrast, Sauthier et al. (2017) found a general tendency of bees to become larger during the foraging season, but their study included only two sites (compared to our 47) and their results could be driven by local foraging conditions.

The reduction of wing length over time was unexpected (Fig. 2.3B) and future research could explore if this impacts the flight performance of bees. This could be the result of an accumulation of pathogens or an increase in temperature fluctuations (Es'kov & Es'kova, 2013; Janczyk & Tofilski, 2021; Tafi et al., 2024). Our results also contrast with the findings of Es'kov and Es'kova (2013) who found that honey bees in a Russian habitat increased in wing size by the end of summer (Table S2.3, Fig. 2.3B) (Es'kov & Es'kova, 2013). To further explore these morphological changes, we studied the effects of landscape type and seasons on the ratio *head width/wing length* (HW/WL). We found that in urban areas, the HW/WL ratio was smaller in summer than in spring and autumn (Table S2.4, Fig. 2.3C), indicating that summer bees had relatively smaller head size in urban environments. In agricultural landscapes, on the other hand, autumn bees had relatively smaller wings (Table S2.4, Fig. 2.3C).

Our data on fat stores again found that bees in agricultural sites show different patterns than bees in urban and mixed habitats. However, the direction of the effect was opposite of what we expected. Although being smaller, summer bees stored more fat in urban and mixed habitats (18.4% and 21.3%, respectively) than in agricultural habitats (Fig. 2.5). Urban and mixed habitats are likely to offer a greater diversity of food in summer (Danner et al., 2017; Baldock et al., 2019; Tew et al., 2021), which could allow bees to store more fat in these habitats. In autumn, however, bees carried 9% less fat in urban and mixed areas. Conversely, in agricultural habitats, bees increased their fat content by 16.3% from summer to autumn achieving fat levels similar to those of bees in urban and mixed habitats (Fig. 2.5). These results highlight the lack of a positive correlation between bee size and fat stores. Several reasons could explain why bees in urban and mixed habitats store more fat than bees in agricultural areas during summer. Firstly, urban and mixed areas may provide pollen types with higher protein content. Donkersley et al. (2014) found that pollen protein content was lower in arable and horticultural farmland and correlated positively with the presence of natural grassland, broadleaf woodlands, and built-up areas. This, however, might change in autumn when urban and forested land covers offered the least valuable sources for pollinators in a study by Richardson et al. (2023). In agricultural areas, bees might experience an increase in pollen foraging diversity after the summer gap and before winter, helping them to build up more fat stores (Knoll et al., 2024).

The most common fatty acids in bees, including both saturated and unsaturated fatty acids, are stored in fat and only about 5% are components of cell membranes (Ruess & Chamberlain, 2010; Stanley-

Samuelson et al., 1988). Palmitoleic acid (C16:1), oleic acid (C18:1), and linoleic acid (C18:2) are additionally related to antimicrobial defence and cognitive functions (Ramanathan et al., 2018; Arien et al., 2018; Kim et al., 2020; Domínguez et al., 2024). We found that the doubly unsaturated fatty acids (most likely linoleic acid, which can only be acquired through the diet, Rosumek et al., 2017; Arien et al., 2020) were 47% - 49% higher in spring than in summer and autumn, while the monounsaturated acids fatty (probably palmitoleic acid, C16:1 and oleic acid (C18:1)) were 8% lower in spring than in summer and autumn (Fig S2.4; Table S2.7). Our findings align with the general observation that increasing dietary polyunsaturates are associated with higher proportions of polyunsaturated fatty acids and lower proportions of monounsaturated fatty acids in tissues (Stanley-Samuelson et al., 1988). This suggests that honey bees collect and consume more pollen that includes significant amounts of linoleic acid, such as dandelion (14% linoleic acid) in spring (Standifer, 1966).

Conclusions

We found that landscape and season interactively affect honey bee body size, wing wear and fat stores. Agricultural landscapes can provide honey bees with ample mono-floral nectar and pollen for short periods, however, we found that in summer bees in these areas experience greater nutritional and physiological challenges compared to bees in urban and mixed habitats. Despite their slightly smaller body size, bees in urban and mixed habitats seem to benefit from a better nutritional conditions in summer, as evidence by increased fat stores. This supports the view that urban and mixed habitats can be a refuge during the particularly challenging summer months. More research is needed to understand the behavioural and health implications of our findings. Our findings suggest that to improve overwintering success and prevent colony losses, management decisions should aim to increase the nutritional diversity and availability of food for bees: (1) in agricultural habitats during early summer, and (2) in urban and mixed habitats at the start of autumn.

Acknowledgements

Supplementary materials

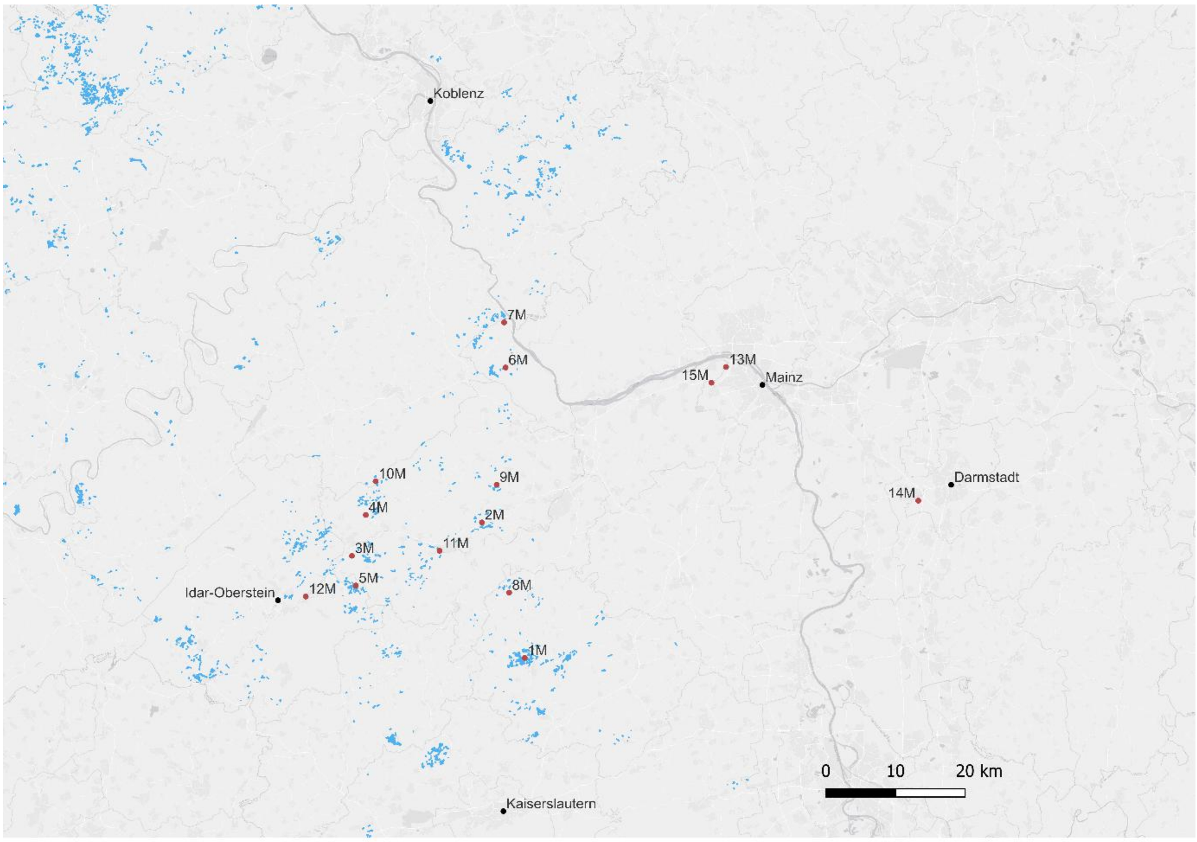


Figure S2.1. Blue area represent the agri-environmental scheme (AES). Pink dots show our study sites. Black dots show the cities around the study sites.

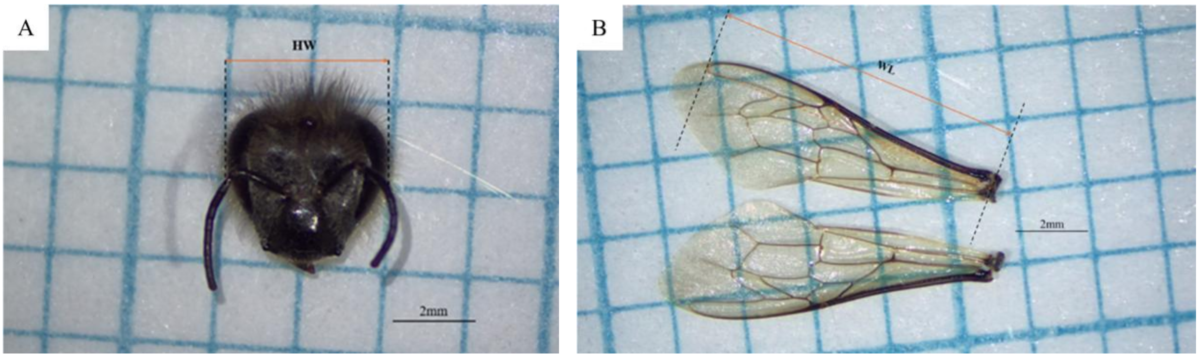


Figure S2.2. The head width (mm) of the bees (A, 3-17A-12).The wing length(mm) of the bees (B, 3-03A-01).

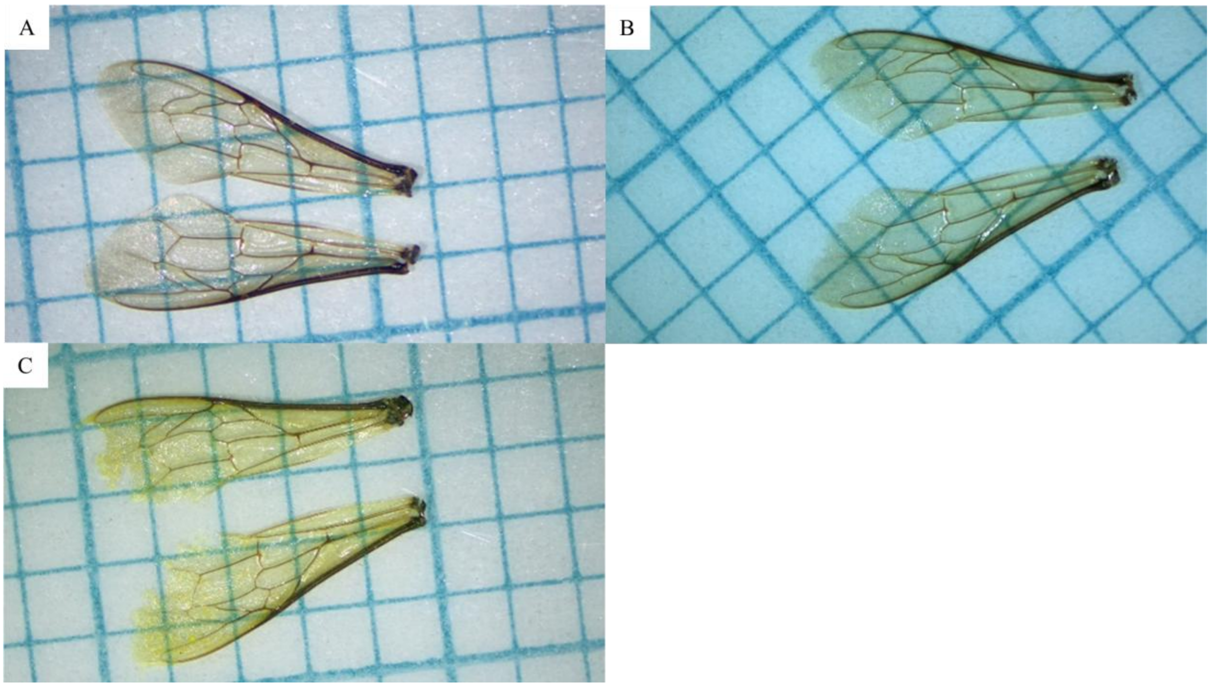


Figure S2.3. A level: wing margins wear < 10% (A, 3-03A-01). B level: 10% < wing margins wear % < 80% (B, 1-12M-06). C level: 80% < wing margins wear (C, 1-05M-10).

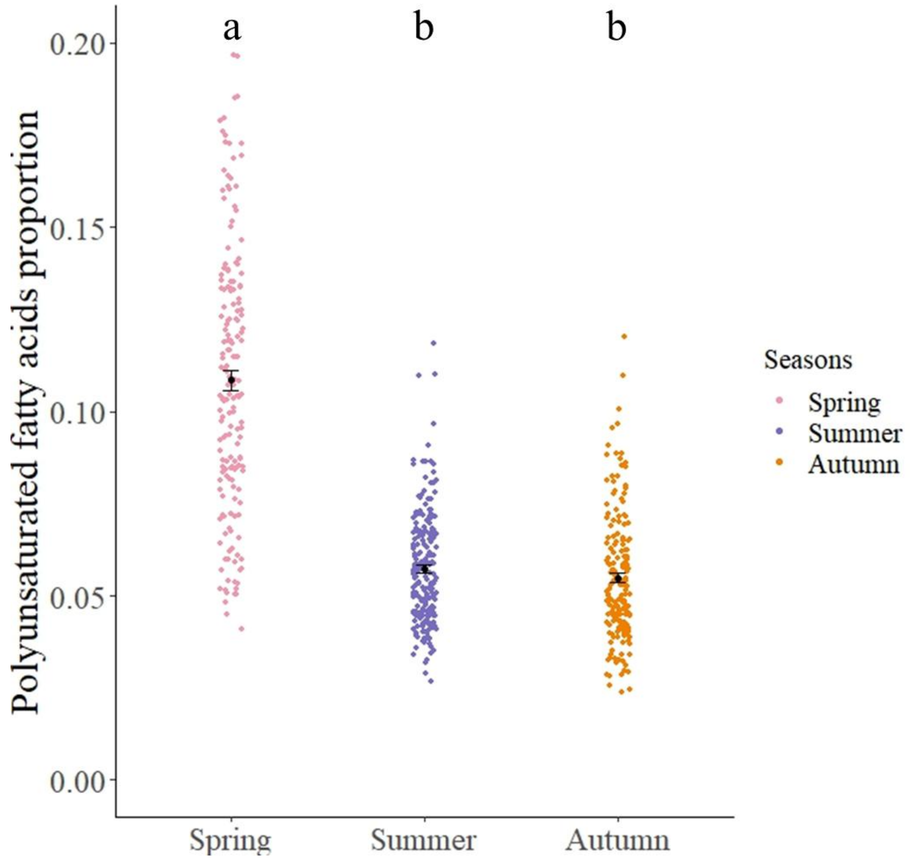


Figure S2.4. Proportion of polyunsaturated fatty acids in captured bee abdomen. Dot and whisker represent the mean, and the standard error. Lowercase letters indicate statistical significance following pair-wise t-test comparisons ($p < 0.05$).

Table 2.1. Land cover categories and coordinates for each sampling site

Site	Forest	Low vegetation	Water	Built-up	Bare soil	Agriculture	Coordinate
01A	4.654802	16.134122	0.01529	13.595237	19.76024	45.685767	49°58'56.97"N,8°12'50.69"E
02A	7.813544	42.897825	0	6.695157	13.673206	28.76434	49°58'58.59"N, 8° 8'16.98"E
03A	5.194981	27.279383	0	3.763307	11.246455	52.405202	49°58'15.49"N, 8° 5'45.84"E
04A	3.554946	39.179658	0	7.479177	10.675238	38.97548	49°57'13.49"N,8° 4'49.00"E
05A	2.547698	33.35549	0.005668	7.086811	16.246262	40.622137	49°54'48.20"N,8° 9'31.30"E
06A	2.632303	42.26885	0	4.803327	12.482926	37.631546	49°57'6.90"N, 7°59'21.55"E
07A	4.308505	22.715304	0	1.172815	13.687037	57.962038	49°53'17.31"N,8° 7'1.07"E
08A	4.149972	12.969785	0.042509	5.487144	21.457555	55.753492	49°53'26.86"N,8°10'25.02"E
09A	1.213329	10.299139	0.349989	1.372566	25.924872	60.675134	49°51'46.56"N, 8°12'7.43"E
10A	2.083882	27.917312	0.011336	1.536142	17.992101	50.278724	49°52'51.88"N,8°18'44.33"E
11A	5.158456	13.736506	0.002834	1.066043	4.517797	75.360485	49°51'43.51"N, 8°16'54.39"E
12A	3.099251	11.85345	0	1.44083	5.84674	77.584891	49°49'45.86"N,8°19'8.10"E
13A	1.027729	8.15549	0	0.432945	4.874962	85.33833	49°48'6.15"N,8°19'5.89"E
14A	1.271317	8.045996	0	1.863783	11.643092	77.015005	49°46'17.26"N, 8°18'20.36"E
15A	1.068668	7.030358	0	1.178779	11.664365	78.874447	49°44'31.20"N, 8°17'33.50"E
16A	4.46803	11.054655	0.001417	10.021296	20.988281	53.303266	49°43'21.87"N, 8°19'51.92"E
01U	12.093431	12.11235	1.813288	71.487441	2.327657	0	49°59'18"N， 8°25'21"E
02U	16.956265	12.641087	1.495175	65.569996	3.228551	0	50° 0'49.72"N, 8°14'8.13"E
03U	18.656382	14.555111	3.884037	59.835826	2.574293	0.373638	50° 0'1.52"N, 8°15'28.41"E
04U	11.824523	21.672625	0	53.345824	5.693747	7.299154	49°58'31.70"N, 8°16'28.81"E
05U	19.149686	13.668327	0.239468	64.733387	2.09706	0	50° 4'21.92"N, 8°14'37.54"E
06U	27.723555	25.206936	0.116617	43.415296	1.26571	2.158835	50° 5'20.43"N, 8°15'32.36"E
07U	14.576955	9.76518	0.701553	72.216571	2.580308	0	49°52'38.69"N, 8°39'5.69"E
08U	12.49634	15.723514	6.864639	62.967527	1.593155	0.201822	50° 6'35.77"N, 8°40'18.67"E
09U	14.07679	17.541086	0.867692	61.444163	5.415569	0.585242	50° 6'27.75"N, 8°38'5.68"E
10U	34.118437	30.887493	0.323087	31.298434	1.829591	1.459214	50° 6'44.32"N, 8°36'26.60"E

Continued

11U	21.266991	23.14335	5.93502	45.418165	2.524881	1.605515	50°05'36"N， 8°36'35"E
12U	25.0903	25.271275	0.292974	47.593678	1.385734	0.21681	50° 7'47.14"N, 8°42'10.11"E
13U	30.569836	26.900601	0.416614	38.623647	2.095337	1.251282	50° 8'40.87"N, 8°40'8.86"E
14U	18.861692	20.071938	0.869885	56.423066	2.001444	1.610378	50° 5'41.92"N, 8°46'13.50"E
15U	6.220436	16.366706	0.944295	67.836968	4.804782	3.668485	49°38'7.17"N, 8°21'19.18"E
16U	18.584698	26.648518	3.155129	48.880767	1.938103	0.655231	50° 2'53.76"N, 8°14'13.44"E
01M	25.280509	54.792102	0.079342	6.379093	2.532258	10.773495	49°38'35.21"N, 7°48'22.51"E
02M	27.443441	30.850767	0.042503	11.289628	5.66087	24.551546	49°49'1.10"N, 7°42'59.25"E
03M	74.645783	13.118043	0.096332	9.624018	0.294547	2.07276	49°46'14.89"N,7°27'30.46"E
04M	70.475126	20.510919	0	1.416471	1.031289	6.408215	49°49'25.91"N,7°29'4.04"E
05M	37.109244	28.607567	0.00425	2.174558	1.46004	30.485142	49°43'56.79"N,7°28'3.48"E
06M	74.351411	18.285961	0	1.053262	1.130405	5.017858	50° 1'2.99"N,7°45'30.68"E
07M	19.319917	29.53616	10.45627	6.718953	4.872582	28.930461	50° 4'32.42"N,7°45'14.94"E
08M	36.590215	38.466563	0	5.778234	3.832515	15.157641	49°43'37.06"N,7°46'23.58"E
09M	31.617533	36.939386	0	3.874415	6.101516	21.291345	49°51'57.84"N,7°44'40.71"E
10M	45.126924	38.472192	0.002833	3.480158	2.232129	10.525525	49°52'3.53"N,7°30'10.36"E
11M	25.661861	29.534217	0.307432	19.926807	3.60251	20.796	49°46'45.93"N,7°37'59.16"E
12M	65.260784	14.782752	0.042498	17.166493	0.766813	1.820578	49°43'01"N,7°22'05"E
13M	45.40902	25.670259	3.660794	17.556616	5.549706	2.026253	49°51'5.39"N,8°35'14.02"E
14M	33.991517	15.118679	0.028341	27.370117	17.370029	5.966666	50° 1'19.35"N, 8°12'1.85"E
15M	55.88632	20.394094	0	16.947462	3.613776	2.992618	50° 0'5.44"N,8°10'17.72"E

A = Agriculture, U = Urban, M = mixed habitat

Table S2.2. Head width (mm) differences in different seasons and landscapes

Landscapes	Seasons(A)	Season(B)	Difference of Means	SE	df	t	p
Agri	Spring	Summer	0	0.00959	267	-0.586	0.8276
	Summer	Autumn	-0.01	0.00954	266	-0.542	0.8508
	Autumn	Spring	0.01	0.00961	268	1.122	0.5014
Urban	Spring	Summer	0.02	0.00972	274	2.785	0.0157
	Summer	Autumn	-0.03	0.00939	270	-3.375	0.0024
	Autumn	Spring	0.01	0.00962	269	0.479	0.8812
Mixed	Spring	Summer	0.03	0.01	260	3.514	0.0015
	Summer	Autumn	-0.02	0.0101	261	-2.007	0.1125
	Autumn	Spring	-0.01	0.0101	260	-1.478	0.0967

Agri = Agriculture, Urban = Urban, Mixed = mixed habitat

Table S2.3. Wing length (mm) differences in different seasons

Season(A)	Season(B)	Difference of Means	SE	df	t	p
Spring	Summer	0.02	0.01	769	1.57	0.26
Summer	Autumn	0.01	0.01	775	1.33	0.38
Autumn	Spring	0.03	0.01	773	-2.85	0.01

Table S2.4. Ratio between wing length and head width in different landscapes and seasons

Landscapes	Seasons(A)	Season(B)	Differene of Means	SE	df	t	p
Agri	Spring	Summer	0.00986	0.00648	248	1.521	0.283
	Summer	Autumn	0.00922	0.00661	250	-1.395	0.3451
	Autumn	Spring	-0.01908	0.00667	251	-2.862	0.0127
Urban	Spring	Summer	-0.01625	0.00612	250	-2.653	0.023
	Summer	Autumn	0.02258	0.00593	248	-3.808	0.0005
	Autumn	Spring	-0.00634	0.00587	247	-1.08	0.5272
Mixed	Spring	Summer	-0.00689	0.00653	233	-1.055	0.5428
	Summer	Autumn	0.00897	0.00669	234	-1.341	0.3738
	Autumn	Spring	-0.00208	0.00681	236	-0.306	0.9497

Agri = Agriculture, Urban = Urban, Mixed = mixed habitat

Table S2.5. Wing wear differences in different seasons

Season(A)	Season(B)	Difference of Means	SE	z	p
Spring	Summer	0.02	0.23	-3.4	0.002
Summer	Autumn	0.01	0.16	4.85	< 0.001
Autumn	Spring	0.03	-0	-0.03	1

Table S2.6. Absolute fatty acid content (AbsFA) in different landscapes and seasons

Fixed effects	Season(A)	Season(B)	Differene of Means	SE	df	t	p
Agri	Spring	Summer	67	46.4	172	1.451	0.3172
	Summer	Autumn	-167	42.5	169	-3.925	0.0004
	Autumn	Spring	100	46.9	174	2.121	0.0886
Urban	Spring	Summer	-67	48.4	125	-1.379	0.3549
	Summer	Autumn	109	38.3	170	2.8	0.0157
	Autumn	Spring	-42	50	134	-0.834	0.6823
Mixed	Spring	Summer	-104	50.9	156	-2.032	0.1079
	Summer	Autumn	120	46.9	165	2.553	0.0309
	Autumn	Spring	-16	54.3	156	-0.3	0.9517
Spring	Agri	Mixed	-38	66.1	48	-0.579	0.832
	Urban	Agri	44	67	48.4	0.656	0.7899
	Mixed	Urban	-6	66.3	50.1	-0.085	0.996
Summer	Agri	Mixed	-217	62.5	51.4	-3.467	0.003
	Urban	Agri	187	62	50.4	3.011	0.0111
	Mixed	Urban	30	61.7	50.5	0.487	0.8776
Autumn	Agri	Mixed	72	51.6	45	1.164	0.4808
	Urban	Agri	-90	50.3	44.1	-1.492	0.3045
	Mixed	Urban	18	52	44.5	0.288	0.9554

Agri = Agriculture, Urban = Urban, Mixed = mixed habitat

Table S2.7. Proportion of each fatty acid content in different seasons

Types	Seasons (A)	Seasons (B)	Differene of Means	SE	df	t	p
SFA	Spring	Summer	0.003	0.00236	468	1.404	0.3394
	Summer	Autumn	-0.002	0.00212	510	-1.065	0.536
	Autumn	Spring	-0.001	0.00245	479	-0.433	0.9019
PUFA	Spring	Summer	0.0505	0.00256	467	19.713	<0.0001
	Summer	Autumn	0.0025	0.00231	510	1.086	0.5234
	Autumn	Spring	-0.053	0.00266	478	-19.943	<0.0001
MUFA	Spring	Summer	-0.054	0.00392	466	-13.753	<0.0001
	Summer	Autumn	-0.00027	0.00353	511	-0.076	0.9968
	Autumn	Spring	0.054	0.00407	477	13.322	<0.0001

SFA = saturated fatty acids, PUFA = polyunsaturated fatty acids,
MUFA = monounsaturated fatty acids

Chapter 3

Automated dance decoding reveals how ecological factors affect honey bee dance communication

Abstract

Communication is a fundamental feature of animal societies, helping organisms mitigate challenges and adapt to new environments efficiently. The waggle dance is an important communicative behaviour in honey bees, allowing bees to communicate about high-quality foraging opportunities. Rapid environmental change, particularly due to human activities, poses significant challenges to bees. Understanding how honey bees adjust their communication strategies in response to land use modifications is crucial to understanding the links between behaviour and nutritional challenges. We studied 17 honey bee colonies across a rural-urban gradient in South-West England, recording the overall weight of the colony and key features of waggle dance communication during late summer and autumn. Using a novel machine-learning algorithm to automate dance decoding, we detected and analysed the characteristics of nearly 10,000 waggle runs and 2,214 waggle dances. We found that in autumn colonies gained weight and dances had 47% more followers than in summer, when colonies lost weight. Autumn dances also had a higher number of followers in rural areas than in urban areas, a trend not observed in summer. There were no differences in how long followers interacted with a dancer. We also found a positive relationship between weight gain and both the frequency and intensity of waggle dances. Waggle phase durations, which indicate foraging distances, revealed that bees travelled on average ~107m further during autumn than in late summer. This was unexpected because colonies are thought to forage closer to the hive when foraging conditions are more favourable. Our results show automated dance decoding can be used in field studied to explore how season, land-use and colony nutrition are linked honey bee communication.

Key words: followers, honey bee, land-use, land-cover, waggle dance, waggle duration

Introduction

Animal pollinators play a pivotal role in ecosystems worldwide by facilitating the pollination of wildflowers and crops (Klein et al., 2007; Ollerton et al., 2011; Kremen, 2018). However, environmental change has led to declining and stagnating numbers of pollinators, raising concerns about the impact on pollination services (Naug, 2009; Becher et al., 2013; Smith et al., 2013). Pollinators face numerous challenges, including climate change, increased pesticide use, and the introduction of non-native species (De la Rúa et al., 2009; Powney et al., 2019; Soroye et al., 2020). Arguably, the most significant challenge for pollinators is the anthropogenic transformation of natural and semi-natural landscapes into intensive agricultural land and urban (Evans et al., 2018; Baldock, 2020). This transformation reduces suitable habitats and diminishes the abundance and diversity of resources. For example, agricultural intensification leads to decreased plant diversity and increased chemical inputs, impacting pollinators directly by increasing mortality and indirectly by decreasing resource availability (Potts et al., 2010; Roulston & Goodell, 2011). In addition, intensively farmed crops create environments that do not provide efficient forage year-round (Dolezal et al., 2019; Wenzel et al., 2020). The effects of urbanisation on resource availability appear to be complex (Liang et al., 2023). Urban and suburban land-use shifts often result in reductions in pollinator diversity and abundance, and decreased flower-pollinator visits (Bates et al., 2011; Geslin et al., 2013; Liang et al., 2023). Conversely, pollinators are often abundant in certain urban locations like allotments and gardens which can serve as pollinator "hotspots" (Tommasi et al., 2004; Plascencia & Philpott, 2017; Baldock, 2020; Tew et al., 2021, 2022), where pollinators may even outperform colonies in less advantageous rural areas, such as agricultural sites (Garbuzov et al., 2015; Lecocq et al., 2015; Normandin et al., 2017; Baldock et al., 2019). Accordingly, urban honey bee colonies in London, UK, foraged closer to their hive than rural colonies, presumably because city colonies found good food sources closer to their hive than rural colonies (Samuelson et al., 2022). Complex habitats with higher percentages of semi-natural areas enhance species richness, diversity, pollinator populations, and services (Morandin & Kremen, 2013; Holland et al., 2017; Fijen et al., 2019). Steffan-Dewenter and Kuhn (2003) found that bees foraged over greater distances in simple, crop-dominated landscapes compared to complex. These changes in land use affect resource diversity and availability, impacting the foraging efficacy of pollinators.

Furthermore, seasonal fluctuations in resource availability throughout the year present additional challenges. Summer months provide limited foraging opportunities compared to spring or autumn when keystone species such as ivy and oilseed rape bloom (Abrol, 2007; Couvillon et al., 2014a, 2014b; Garbuzov & Ratnieks, 2014; Danner et al., 2016; Knoll et al., 2024). Since flying is energetically costly, bees prefer to forage close to the colony, but limited summer resources often force them to fly farther away from the hive (Couvillon et al., 2014a, 2014b; Samuelson et al., 2022). These ecological variations represent a significant challenge to bees and test the impact of their environment on their communicative behaviours.

Communication is a fundamental feature of animal societies, as exemplified by the waggle dance, an iconic communication signal used by honey bees (Apini) to recruit nestmates to profitable food sources, thereby facilitating the efficient exploitation of resources (von Frisch, 1967; Seeley et al., 1991, 2000; Alves et al., 2023). Previous studies have found that colonies in areas with high species richness, where high-quality resource patches are widely spaced and harder to locate, benefit most from dance communication (Dornhaus & Chittka, 2004; Donaldson-Matasci & Dornhaus, 2012; I'Anson Price & Gruter 2015; Shackleton et al., 2023). However, dance communication is time-consuming, and bees in some temperate habitats achieve better foraging success by reducing their reliance on it and adopting solitary foraging strategies (Beekman & Lew, 2008; I’Anson Price et al., 2019).

Foragers need to follow a minimum of 5-6 waggle phases, the vector information-encoding component of the waggle dance (von Frisch, 1967), to locate the advertised resource, and following more waggle phases exhibits higher flight accuracy (Mautz, 1971; Tanner & Visscher, 2009; Grüter & Farina, 2009; Al Toufailia et al., 2013). However, even after following several waggle phases, bees often fail to find the communicated resource and return for more information, investing energy and time (Seeley & Visscher, 1988; Grüter & Leadbeater, 2014). Many dance followers follow dances only briefly (2-4 waggle phases), subsequently ignoring the social information. Instead, they may be attracted by the floral odours present on the body of the dancer, which can trigger memories of previously visited food source locations (Reinhard et al. 2004; Grüter et al., 2008; Grüter & Leadbeater, 2014; I'Anson Price & Grüter, 2015). Recent studies have showed that the number of followers per dance decreases in colonies with dysfunctional waggle dances and starved colonies (I'Anson Price et al., 2019; Wu et al., 2024). Moreover, in changing environments, dance information can become unreliable and outdated (Giraldeau et al., 2002). Thus, the value of dance communication depends on colony's need and the habitat, and there are likely to be conditions where using spatial information from waggle communication is not the most efficient foraging approach (Dornhaus et al. 2006; I'Anson Price & Grüter, 2015; I'Anson Price et al. 2019).

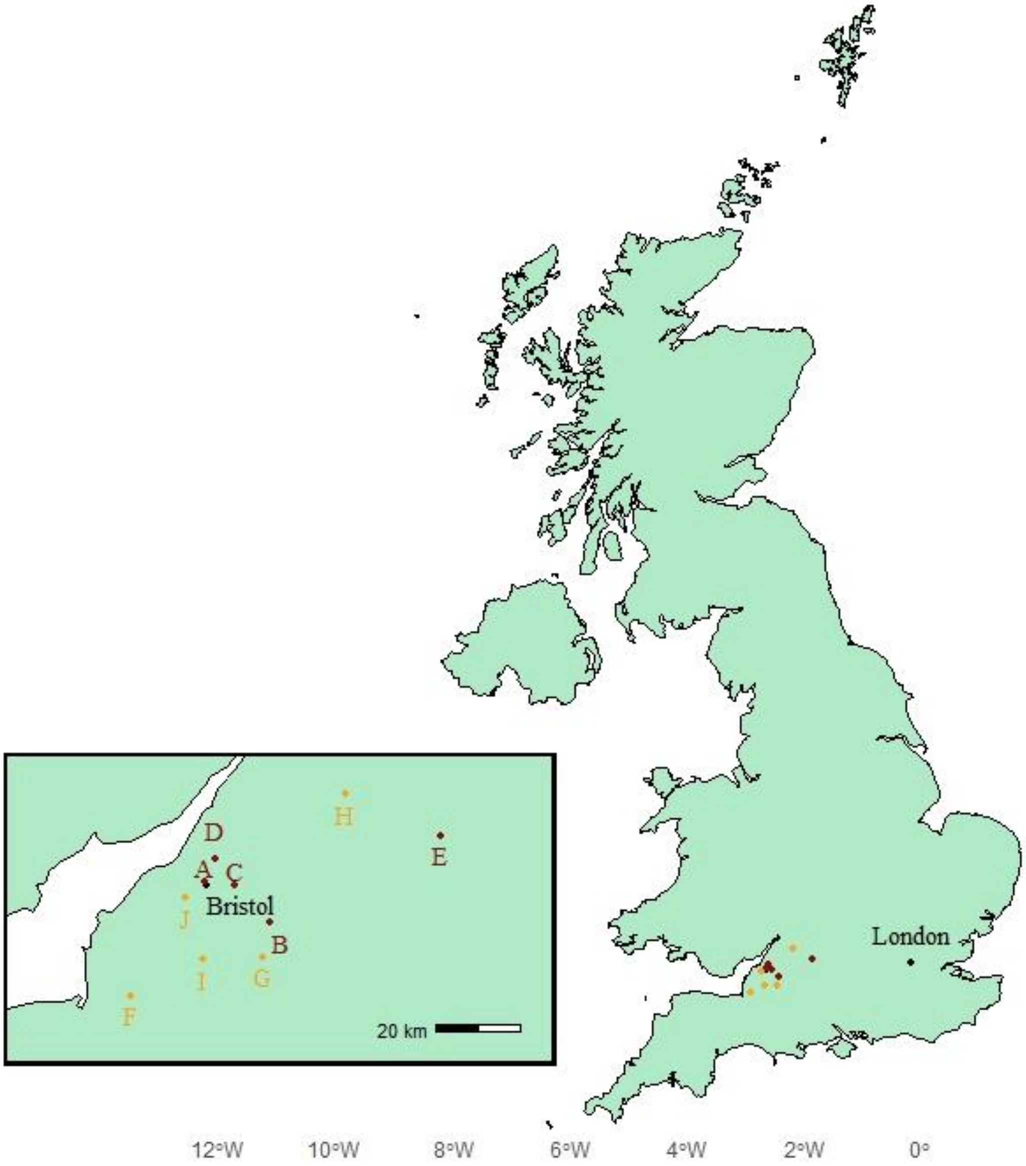
Behavioural plasticity is often an organism's first response to environmental change and is frequently a sign of stress, e.g. due to modifications to land use (Wong & Candolin, 2015; Cerini et al., 2023). However, behavioural changes may not always compensate for environmental change and could even be counterproductive, leading to fitness loss and "evolutionary traps" (Schlaepfer et al., 2002; Rodewald et al., 2011; Robertson et al., 2013). Thus, environmental and land use changes pose a significant challenge. However, how honey bees adjust their communication and foraging behaviours in response to seasonal variations and anthropogenic environmental modifications remains inadequately understood. In this study, we employ both manual dance decoding and a machine learning algorithm to detect and analyse waggle dances and reveal key characteristics of dance communication. The machine learning algorithm in our study, originally developed by Wario et al. (2017) and significantly improved since, is used for the first time in field conditions to detect and analyse waggle dances. We aim to gain a better understanding of how dance communication is affected by land use and seasonal changes by investigating: 1) the interests of followers, gauged by the number of followers and the number of waggle runs they follow (Grüter & Ratnieks, 2011), 2) foraging distances, decoded by waggle phase duration (Schürch et al., 2019) and 3) waggle dance activity. We monitored the weight of colonies to assess how bees perform in their environment. As the resources closer to the hive become more profitable due to the energetic costs of flying (Shackleton et al., 2023), we hypothesized that waggle dance frequency and follower number will increase in autumn due to better foraging conditions. We also predicted that waggle phase durations are likely to decrease in autumn due to an increased resource availability.

Study sites and data collection

We studied honey bee colonies (predominately of *Apis mellifera mellifera*) headed by a naturally mated queen and an estimated population ranging between 20,000 to 40,000 bees, kept in Langstroth hives comprising two 10-frame medium boxes placed on top of each other. The front of the hive contained a glass window allowing us to observe the first frame closest to the entrance (Fig. 3.1). The hive box was modified so returning foragers were forced to walk across the first frame before reaching other frames. This ensured the first frame acted as a "dance floor", so the majority of waggle dances were visible through the window, as previously described by I'Anson Price et al., (2019). Hive boxes that were situated in complete sunlight were covered from the back with a small tent to prevent direct sunlight on the windows. *BeeWatch* ("Home") hive scales were attached to each hive to continuously monitor weight fluctuations (± 20g), with measurements recorded hourly to quantify colony foraging success. Colony weight change was determined by identifying the daily change in weight in the 7 days before the video recording by subtracting the value at midnight from the previous day. A single mean weight change per day was then calculated.

Figure. 3.1. Image showing the Langstroth hives comprising of two medium boxes used. An observational frame is used at the front and a *BeeWatch* hive scale is attached below.

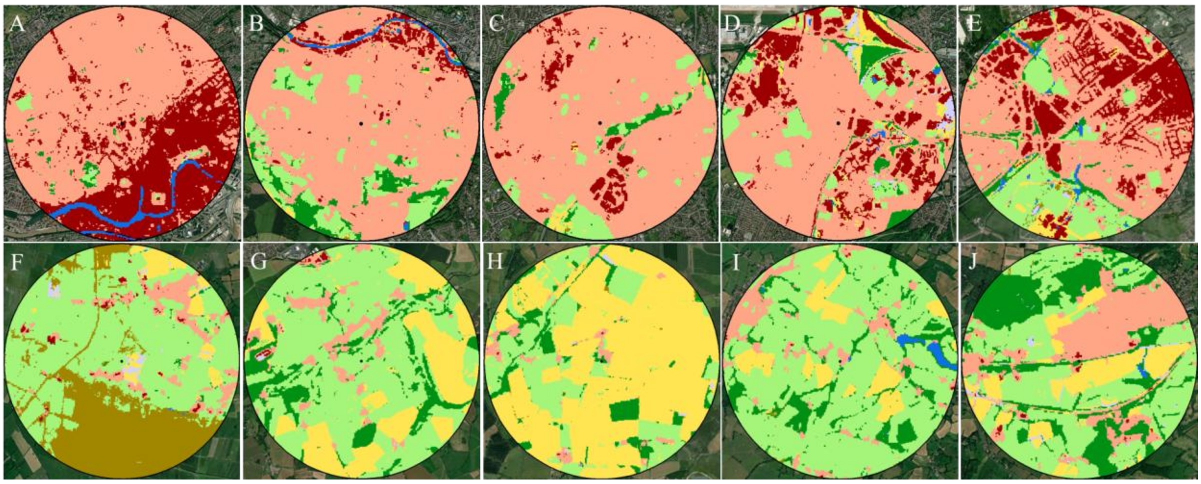
Data were collected from ten sites in the South-West region of England. These sites were categorized into five predominantly rural and five predominantly urban locations (Fig. 3.2). Predominantly urban sites were located in Bristol, Bath and Swindon. The classification of a site as rural or urban was determined based on the percentage of urban and suburban land use within the vicinity (radius = 1.5km), comprising an overall percentage build-up (Fig. 3.3). We obtained these values using ArcGIS (Esri, 2024).



Sites: A – LSB, B – Monksdale, C – Hollyguest, D – Mortimer, E – Westcott, F – Tucker, G – Holmes, H – Haslers, I – Gay, J - Fenswood

Figure. 3.2. Map depicting the geographical distribution of the ten sites where data were collected. Sites A, G and I contained one colony, whereas the remaining seven contained two. Predominantly urban sites are denoted by markers in red, while rural sites are indicated by markers in yellow.

Among the ten sites, three (A, G and I) were home to a single colony, while the remaining seven sites accommodated two colonies each, resulting in a total of 17 distinct colonies sampled (Fig. 3.2). All colonies originated from an apiary near the University of Bristol and were brought to their respective sites a few weeks before data collection started. Colonies were managed in a standardised way by members of the research team. Data collection spanned two different assessment periods: Assessment Period 1, encompassing the late summer (31/08/22 – 14/09/22); and Assessment Period 2, during the autumn season (03/10/22 – 15/10/22). These two periods were chosen as they allowed us to compare a period when bees notoriously struggle, due to lack of resources (summer), with a period when bees tend to perform better, due to important flowering species coming to bloom (autumn), such as common ivy (*Hedera helix*) (Couvillon et al., 2014b; Danner et al., 2016; Samuelson et al., 2022).



Sites: A – LSB, B – Monksdale, C – Hollyguest, D – Mortimer, E – Westcott,
F – Tucker, G – Holmes, H – Haslers, I – Gay, J - Fenswood

Figure. 3.3. Land use classification of sites (r = 1.5 km from the location of hive), extracted from landcover map 2021 from the UK Centre for Ecology & Hydrology (UKCEH) through ArcGIS (Version 10.7.1, Esri). Forest areas in *dark green* includes broadleaf and coniferous woodland; arable and horticulture area is in *yellow*; improved grassland area is in *light green*; semi-natural grassland is highlighted in brown includes neutral, calcareous and acid grassland and fen; mountain, heath and bog includes heather, heather grassland, bog and inland rock are depicted in *mauve*; water is in *blue*; coastal region includes supralittoral rock and sediment, littoral rock and sediment and saltmarsh are in *black*; urban area is in *mahroon* and suburban area is in *peach* colour.

Dance recording

Before each recording session, we conducted a preliminary examination of the observation frame. If the bee population observed was below a threshold of approximately 50% coverage of the frame, the observation frame and the immediately adjacent frame were interchanged with frames located deeper within the hive. This approach was adopted because a lower bee density on the observation frame would not produce sufficient heat, potentially leading to mist formation within the observation panel and thus obscuring the bees from view, resulting in unusable recording footage. Furthermore, it would make sure that returning foragers would find a similarly occupied first frame as they entered the hive. In the event of a frame substitution, a 15-minute acclimation period for the bees was allowed before beginning the recording.

Figure. 3.4. Image showing the view from our camera during the recording of one colony. The top and bottom observational frames are in full view and weights affixed to two thin strings provide a vertical frame of references.

We used a NexiGo N660P webcam mounted atop a tripod for the recordings. The camera was linked to a laptop, which allowed us to view its field of vision. The top and bottom corners of the frames were aligned with the camera field of vision and the distance from the camera to the hive was measured each time to be between 38-41cm (Fig. 3.4). All videos were recorded in 60 frames per second (as required by the automated decoding software). Behind the camera, we erected three metal poles and attached a large black cloth across them to eliminate any reflections of sunlight on the glass window of the hive. To provide a vertical frame of reference for the direction of gravity, we suspended two thin strings, each with a weight affixed to one end, along the top of the hive. The weights were out of sight during the recordings and the two strings appeared as thin white lines in the video. Each colony was recorded for 90 minutes per session, with recording taking place during daylight hours (10:00-15:00). In cases where rain occurred during a recording session, we paused the session and covered the laptop and camera until the rain had ceased, at which point we resumed the recording.

Video analysis of dance following

Videos were analysed to determine the average number of bees that followed a waggle dance, as well as their level of interest in the dance as indicated by the duration of their following behaviour. The first eight dances observed in each recording were selected for manual analysis, as we believed this would give a good representation of the characteristics of waggle following within the colony whilst also mitigating the time constraints of attempting to analyse follower behaviour of each dance. Due to the well-known significant variability in the initial waggle phases of a dance (Couvillon et al., 2012), we analysed the 3rd, 4th, and 5th waggle phases of a dance in accordance with previous studies (I'Anson Price et al., 2019; Wu et al., 2024). To calculate the number of dance followers per dance, we analysed the number of bees that stood facing the dancer with their heads within an antenna length during the dancer's waggle phase (Al Toufailia et al., 2013). Moreover, we analysed the movement of these followers during the subsequent waggle phase to ensure they were interested in the dance and not simply standing near it.

To quantify the level of interest in the dance, we examined the individual bees that followed the 3rd waggle phase and determined the number of subsequent waggle phases they followed (I'Anson Price et al., 2019; Wu et al., 2024). We consider a follower to have stopped following a dance when they turned and walked away from the dancer (Al Toufailia et al., 2013). For the recordings of 'Site F, Period 1', 'Site F, Period 2' and 'Site J, Period 2' we were unable to analyse eight individual waggle dances due to either a lack of dances in the recording or a high density of bees within the frames that made accurate tracking challenging. Under such circumstances, we analysed the maximum number of waggle dances possible with the highest accuracy achievable, thus giving us a total dance sample size of n=242 waggle dances.

Waggle Dance Detector software

We used the 'Waggle Dance Detector' software (hereafter called the WDD) to detect the individual waggle phases from each recording (software can be found in Wario et al., 2017; however, it has been largely rewritten and improved since this publication, improved software can be found at https://github.com/BioroboticsLab/bb_wdd2). The software detects waggle runs through three layered processes. Initially, each pixel in the frame of a video is analysed and the brightness is tracked. If the change in a pixel's brightness matches the waggle frequency (the range of frequencies bees move at during their dance) (Landgraf et al., 2011) it is marked as a potential dance. Next, adjacent pixels are marked together into clusters and finally, the position of a dancer is connected across frames to form a potential waggle run (Wario et al., 2017). The software is implemented through Python code, and after processing input videos, it generates a set of files containing detected waggle phases. Each file includes a series of images representing frames of the waggle phase and a metafile, containing information about the characteristics of the phase, such as its timestamp, duration and angle. The WDD contains an adjustable threshold level that controls its sensitivity: a higher threshold enhanced detection precision (*i.e.* fewer false positives) but risked not detecting all waggle phases (*i.e.* more false negatives), while lower thresholds increased the likelihood of detecting phases but also yielded more false positives. False positives occurred when the software detected a movement that is detected wrongly as a waggle phase, such as repetitive body swaying and shaking, bees falling off the frame and most commonly rapid wing movements during ventilation.

To determine which threshold to use, we compared manually decoded videos with WDD results using a range of thresholds. We analysed 20 different clips, ranging from 30 to 60 seconds. These clips were chosen with the majority containing one or more waggle dances; however, some also included none. The clips were chosen from different sites and assessment periods to cover a wide range of conditions. We manually analysed each video following Couvillon et al., (2012) and then compared the output from the software at different threshold settings with our manual analysis, thus giving us information on how many waggle phases the software failed to detect (false negatives) and how many non-waggle phases were detected and reported by the software (false positives). The detection sensitivity threshold of 12.8 detected 72.4% of all waggle phases, with a 46.8% false positive rate. We chose this threshold, as it was low enough the detect the majority of waggle phases; however, lowering it any further would have been impracticable due to a substantial increase in false positives, which needed to be removed manually after WDD processing.

The WDD detected waggle phases from the first flickering of a pixel of a bee beginning its waggle phase until the last, which provides information about the distance of the food source. We compared this value to manual decoding, by counting the number of frames of a waggle phase (Couvillon et al., 2012) and subsequently calculated its duration. We choose dances from the same 20 clips, analysing a minimum of 4 waggle phases per dance (Couvillon et al., 2012). A Spearman's correlation coefficient showed a significant positive relationship between the values obtained by manual and automated decoding (N = 29, rs = 0.526, p=0.003), suggesting that the waggle phase duration values created by the WDD are a good indicator of actual waggle phase durations. However, we observed that in a limited number of cases (3%), longer waggle phases were split into two files. This most likely occurred if, during a waggle phase, a bee's movement dipped below the detection threshold of the WDD before its movement became stronger again later in the phase. This suggests that long waggle phases are therefore likely to be underrepresented. Overall, the WDD provided shorter waggle phase values than manual decoding (mean difference = 0.63s, median difference (mdn) = 0.28s), which needs to be considered when estimating foraging distances. As a result, we did not use data from automated dance decoding to estimate foraging ranges, and only used it to analyse relative foraging distances in relation to land use and assessment period.

After these quality control and threshold selection steps, we processed each recording with the WDD software. Due to their large size, recordings were split into three thirty-minute segments. The WDD output was then checked manually for false positives which were removed. A custom-made Python script was used to run through every output file and metafile to create a database containing key characteristics of every waggle phase. Another custom-made Python script grouped similar waggle phases into individual dances. We set three thresholds that allowed us to group dances: waggle phases within a 30-degree angle, ±1.13 seconds in duration and within the subsequent minute, would be grouped into dances. These thresholds were based on the variation in waggle phase duration and angle within a dance observed in previous work (Al Toufailia et al., 2013, unpublished raw data). Comparisons with manual dances, which were assessed by analysing 5 subsequent waggle phases within a dance, showed good correspondence between manual decoding and grouping based on our algorithm.

Data analysis

To analyse the data we ran both linear mixed-effects models (LME) and generalised linear mixed-effects models (GLMM) using Rstudio (RStudio Team, 2024) and the R packages ‘Lme4’ (Bates et al., 2015) and ‘Lmertest’ (Kuznetsova et al., 2017). To test the distribution of our data, we used the R package ‘DHARMa’ (Hartig, 2022). We created five different models to test five dependent variables: the number of followers, follower interest, waggle phase duration (as a proxy of foraging distance), number of waggle phases per dance and number of waggle phases per minute (as a proxy of dancing frequency in the hives). In each model, the dependent variables were measured against the fixed effects “assessment period” (1-2), “percentage build-up” (0-100%) and “average daily colony weight change”. We accounted for the non-independence of observations from the same colony by including "colony" as a random effect (Zuur et al., 2009).

Furthermore, we hypothesised there may be an interaction between assessment period and build-up because urban sites in the study region provide more stable foraging than rural sites (Timberlake et al., 2019; Tew et al., 2021, 2022). This was tested using a Likelihood ratio test (LRT). If necessary, the dependent variables were transformed with a log or square-root transformation. Non-significant interactions were removed from the model before the model was rerun (Zuur et al., 2009). Throughout our analysis, we used random intercept models; moreover, in one case (waggle phases per dance), we fitted a negative binomial distribution due to the distribution of our data.

Results

Number of followers

We explored the effects of assessment period, percentage build-up and colony weight change on the average number of individual dance followers. We analysed followers of 242 dances across 17 colonies.

The interaction term between assessment period and percentage build-up was significant (LRT = 0.02, $p < 0.001$). To further explore this interaction, we split up the data into the two models corresponding to the different assessment periods and subsequently tested the effects of percentage build-up and weight change. For late summer, there was no statistically significant effect of percentage build-up (LME: $t = -1.28$, $p = 0.22$) or weight change ($t = 0.22$, $p = 0.83$) on the number of followers. However, during autumn, build-up exhibited a significant negative impact on the number of dance followers (t- = -2.87, $p = 0.01$). Dances in areas with a higher percentage of build-up had fewer followers (Fig. 3.5). There was no significant effect of weight change ($t = 0.27$, $p = 0.79$).

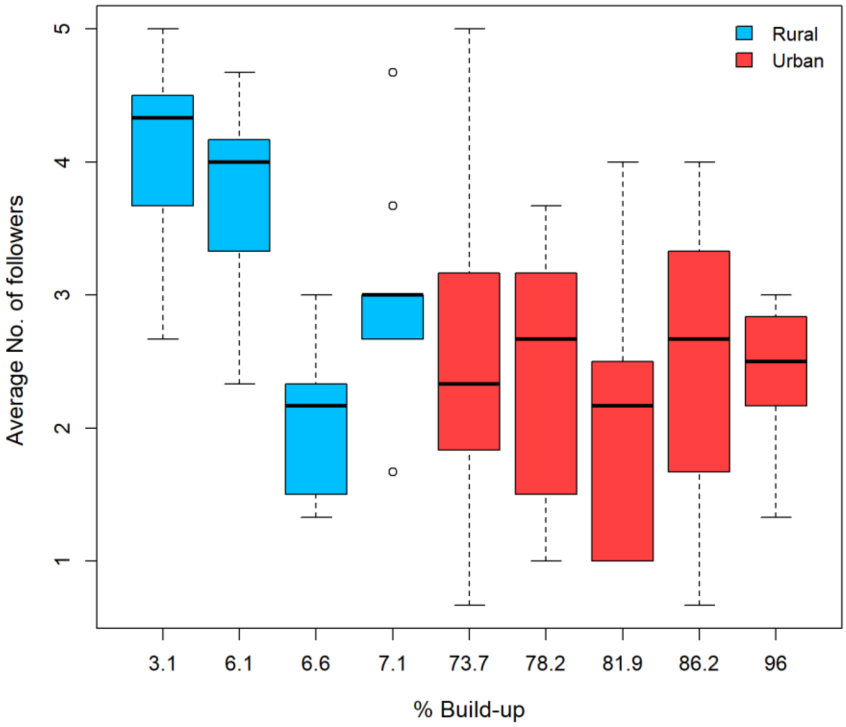


Figure. 3.5. The average number of individual bees following a waggle dance (3rd, 4th, and 5th waggle phases) in colonies of differing levels of urban build-up during assessment period 2 (October 2022). Boxplots show the median, 25% and 75% quartile and the 5% and 95% percentile and are colour-coded to show rural and urban locations.

An analysis that included only assessment period and weight change as predictors revealed that waggle dances in autumn exhibited a significantly higher number of followers compared to late summer, with dances in period 2 having on average 47% more followers than in period 1 (2.74 ± 1.10 vs. 1.83 ± 0.92; mean ± standard deviation) (Fig. 3.6) (LME: $t = 4.90$, $p < 0.001$). However, there was no statistically significant effect of weight change on the average number of waggle dance followers (t-value = 0.66, p

= 0.51). Interestingly, in late summer, three dances contained zero followers which did not occur for a single dance in autumn. Moreover, each of these dances occurred in colonies declining in weight. In contrast, each dance containing the maximum number of followers observed (5), occurred within colonies increasing in weight and during autumn.

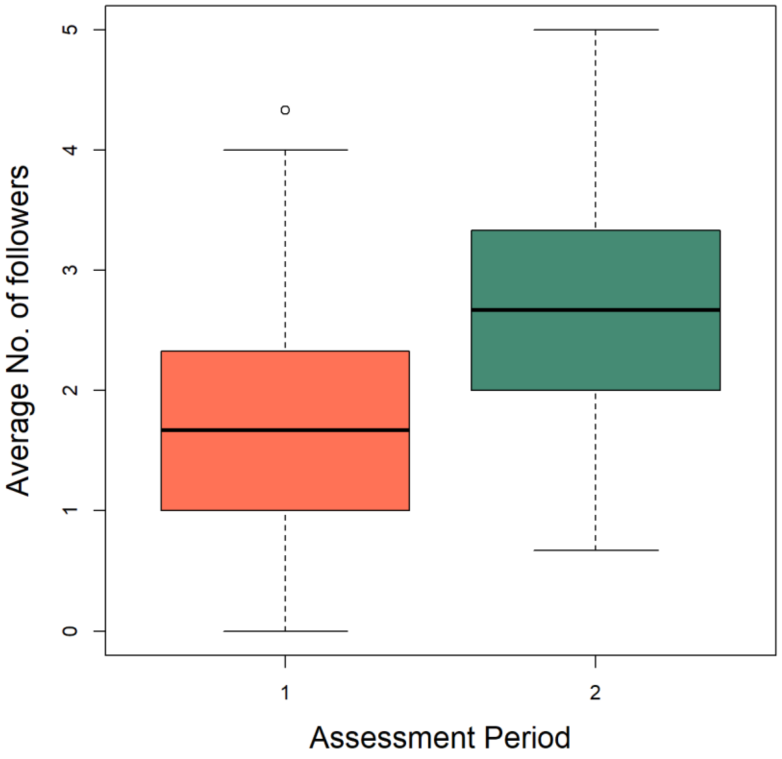


Figure. 3.6. The average number of individual bees following a waggle phase (3rd,4th, and 5th waggle phases of a dance) during two assessment periods. Assessment period data collection taken from late summer to autumn (period 1 = late summer, 2 = autumn). Boxplots show the median, 25% and 75% quartile and the 5% and 95% percentile.

Follower interest

We explored whether the time of year, land use and colony weight change affected the level of interest followers had in waggle dances, by analysing the number of waggle phases a follower followed for. Our model showed that the fixed effects of assessment period (LME: t = -1.19, p = 0.24), percentage build-up (t = 0.77, p = 0.45), their interaction (LRT = 0.56, p = 0.96), and colony weight change (t = -0.40, p = 0.69) had no significant effect on follower interest. Interestingly, however, urban areas did contain many of the dances with the highest follower interest, with the 11 dances where followers followed the most waggle phases occurring in urban areas.

Waggle phase duration

Overall, the WDD detected 9,696 waggle phases, which were grouped into 2,214 dances. We tested if the average waggle phase duration per dance differed across assessment periods, percentage build-up and colony weight changes. An LRT showed a significant interaction between the variables of assessment period and percentage build-up (LRT = 4.92, $p = 0.03$). However, analysis of individual assessment periods showed no significant effects, therefore we concluded that the interaction term does not have an overly strong effect and decided to continue without the interaction term. The duration of waggle phases per dance was significantly affected by season (LME: $t = 4.49$, $p < 0.001$). Waggle phases during late summer were 0.16 seconds shorter compared to autumn (0.57s ± 0.28 vs. 0.73s ± 0.41) ($t = 4.49$, $p < 0.001$) (Fig. 3.7). Moreover, the longest waggle phase was observed in autumn, lasting 8.1s, and this period contained the 7 longest runs.

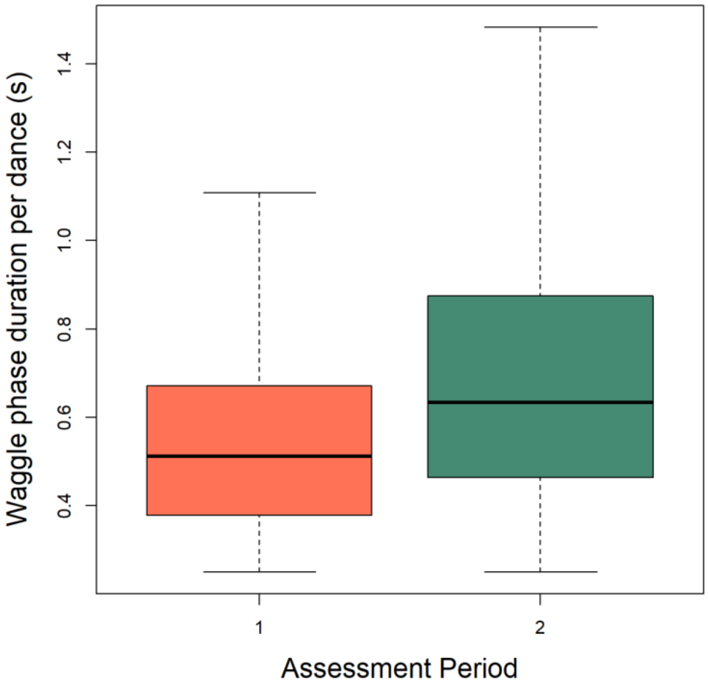


Figure. 3.7. The average waggle phase duration per dance of dances analysed during late summer (assessment period 1) and autumn (assessment period 2). The duration of each waggle phase was quantified using the 'Waggle dance detector' (Wario et al., 2017) and averaged across a whole dance.

We used the universal calibration curve by Schürch et al., (2019) to estimate the average and median foraging distances as communicated by the bees through their waggle phase duration. Bees foraged further during autumn with an average distance travelled of 969m (±302) (Mdn= 668m) compared to the late summer (920 ± 267) (Mdn= 886m). The average duration of waggle phases per dance was not significantly affected by percentage build-up (t = 0.68, p = 0.51) or weight change (t = 1.31, p = 0.19).

Phases per dance

We assessed whether there was a change in the average number of waggle phases per dance in different assessment periods, percentage build-up and colony weight change. Our model showed there was a significantly positive relationship between colony weight change and the number of waggle phases produced per dance (t = 4.00, p < 0.001) (Fig. 3.8a). The longest dances (up to 73 phases per dance) all occurred during autumn in assessment period 2, in predominately urban colonies. However, we found no statistically significant difference between the fixed effects of assessment period (t = -0.46, p = 0.65), percentage build-up (t = 1.25, p = 0.21) and their interaction (p = 0.054) on waggle phases per dance.

Phases per minute

We sought to identify any changes in the number of waggle phases per minute, or dance intensity, with season, land use and weight change. There was no significant effect of assessment period (t = -0.36, p = 0.73), percentage build-up (t = 0.57, p = 0.58) or their interaction (p = 0.65) on dance intensity. Nevertheless, we observed a significant positive relationship between dance intensity and weight change (t = 2.74, p = 0.01) (Fig. 3.8B).

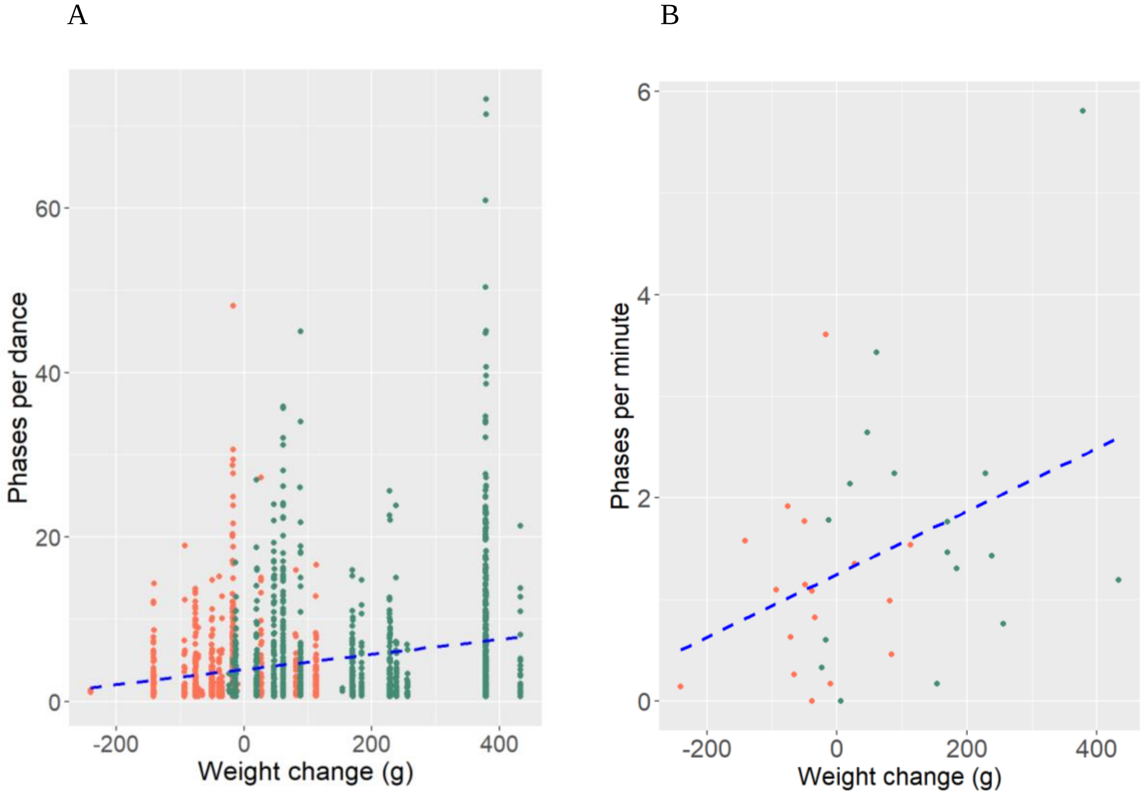


Figure. 3.8. Relationship between the average number of waggle phases per dance (A) and waggle phases per minute (B) in relation to colony weight change. Colony weight change was measured over two assessment periods (orange = late summer, green = autumn). A linear regression line is added to show the overall trend between weight change and runs per dance (A) and runs per minute (B). In Figure A, the individual data points represent dances, whereas in Figure B, the data points represent a single recording session at a colony.

Weight change and assessment period

Finally, we tested if there was a relationship between colony weight change and assessment period. We also aimed to identify whether there was any effect of percentage build-up on colony weight change. Build-up did not affect weight change (t = -0.31, p = 0.76), however, assessment period showed a significant effect on colony weight change (t = 4.95, p < 0.001). On average colonies in late summer lost 36g (± 196.3) per day, compared to during autumn, where colonies gained 126g (± 307.6) daily. This resulted in colonies gaining on average 162g (± 32.8) more weight during autumn than late summer (t = -5.00, p < 0.001) (Fig. 3.9). Interestingly, although colonies performed better in autumn, they were also more unpredictable, displaying significantly more variation than in late summer (t = 1.87, p = 0.007).

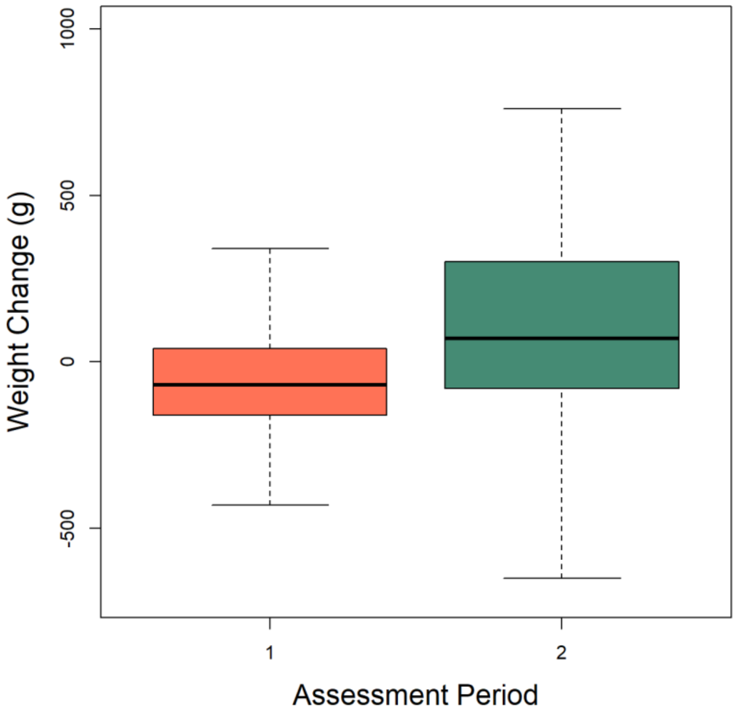


Figure. 3.9. The difference in colony weight over the two assessment periods (orange = late summer, green = autumn). Colony weight is taken by the change in overall colony weight each day for 7 days prior to recording, with a single average value calculated afterwards.

Discussion

The honey bee waggle dance is a unique and crucial communication display that allows colonies to exploit food sources in changing landscapes. Our study indicates how honey bees may adapt their communication and, consequently, foraging behaviour to seasonal changes, fluctuations in the nutritional state of the colony, and human modifications of the environment.

Our results indicated an overall increase in waggle dance followers from late summer to autumn. This increase coincided with the increase in colony weight in autumn compared to late summer, indicating that colonies performed better later in the year, with more honey bees foraging on good food, likely resulting in the increased number of waggle followers. Furthermore, we observed a significant positive relationship between weight change and dance activity, specifically waggle phases per minute and waggle phases per dance. Colonies in autumn gained more weight and were more active in terms of waggle dances, with these dances containing a higher number of followers. This is consistent with feeder experiments showing that bees dance more when food sources are more profitable (von Frisch, 1967).

Interestingly, although colonies gained more weight in autumn, they exhibited higher variability in weight change than in late summer. This increased variability may be attributed to the more variable weather patterns in autumn, with warmer days from summer and colder days approaching winter. Colonies often perform better in autumn due to ivy blooming during this season. Ivy is a crucial species for pollinating insects in Europe, especially honey bees, and blooms abundantly in both rural and urban locations (Al Toufailia et al., 2013; Garbuzov & Ratnieks, 2014). The seasonal flowering of this essential plant could lead to a substantial increase in honey bee foraging behaviour in autumn, leading to an increased dance frequency, as bees tend to dance more circuits for higher reward resources (Seeley, 1986; Seeley et al., 1991, 2000). It is also probable that higher dance frequency resulted in the increased dance intensity observed. However, the opposite could also be true, and as bees danced for longer, there were more dances per minute. Further research is needed to understand if one of these factors is influencing the other. Ultimately, colonies gaining weight showed an increase in dance frequency and intensity.

Although we observed an increase in the number of waggle dance followers, there was no corresponding increase in the number of phases bees followed. Thus, an overall increase in followers does not necessarily equate to an increase in following duration, as recruiting more bees does not mean these individual bees will follow for longer.

Interestingly, our results indicated that although dances had more followers in autumn, there was also a negative relationship between build-up and the number of dance followers. This effect may be attributed to the availability of the flowering species surrounding these hives. For example, hives in highly built-up areas could have had abundant resources nearby in residential gardens and allotments. Plenty of resources close to the hive could result in foragers having less interest in spending time following dances, preferring to fly out on their own. This is supported by computer simulations showing that dance communication is less beneficial when food sources are abundant (Dornhaus et al., 2006; I'Anson Price et al., 2019). Additionally, the overall increase in colony weight observed in autumn may reduce the incentive for individuals to follow waggle dances, as finding resources was less of a necessity. However, this is unlikely since there was a general increase in dance following in autumn. Additionally, food sources may have been more clustered and variable in quality during autumn, which, according to computer simulations, would favour dance communication (Dornhaus et al., 2006). This variability would also explain why there was a larger variation in overall colony weight gain in autumn compared to late summer. The contradiction between increased overall dance following and decreased following in highly built-up areas suggests a complex relationship between resource distribution and foraging strategies. This interaction may require further analysis to understand its effects fully.

We did not observe any significant difference in the average waggle phase duration, and therefore the distance bees travelled, between different land use types. This finding contrasts with other studies; for example, Samuelson et al. (2022) showed that honey bees travel significantly less distance to food sources in an urban environment than in rural areas. However, their urban sites were located in London, a city with greater levels of urbanisation than the urban locations we used (Bristol, Bath and Swindon), which may have influenced waggle distances (Clark, 2024). The exact location of the colonies in urban areas may result in differences in waggle phase duration as well, such as their proximity to allotments and gardens, often described as diversity hotspots (Baldock et al., 2019).

However, we did observe a significant increase in waggle phase duration during autumn compared to late summer. These results could be interpreted as bees having had more difficulty finding good food sources during autumn, as the longer waggle phase duration indicated bees were travelling further from the hive. Our results contrast with earlier studies that have found that autumn typically coincides with bees foraging closer to the hive compared to the summer months with limited resources and longer waggle phases. For example, mean foraging distances increased from spring to summer before decreasing again in autumn (Couvillon et al., 2014b; Danner et al., 2016; Samuelson et al., 2022). Conversely, the lack of this relationship in our experiment could be due to the location of our colonies. Honey bees have been shown to forage great distances if the food resource is of high quality (Beekman & Ratnieks, 2000). However, it is likely the relationship between foraging conditions and waggle dance distances is not as straightforward as expected, and more emphasis should be put on other environmental factors than waggle distances. Environmental conditions and resource availability in specific locations highlight the importance of considering local environmental circumstances when interpreting waggle dance data, signifying resource distribution and colony placement impact foraging behaviour.

Moreover, this effect may be more prominent when analysing longer time periods in summer and autumn, rather than individual months. For example, Garbuzov et al. (2015) identified differences in foraging distances between summer and autumn, but no significant differences between August, September, and October, the same months as our data collection. Furthermore, their study was conducted in Brighton, a city more similar in urbanisation to Bristol (Clark, 2024), suggesting seasonal trends in foraging behaviour may vary depending on regional environmental factors. Similarly, Couvillon et al. (2014) found no differences in foraging distances between August and September; however, foraging distances were lower in October. Thus, our focus on specific months rather than entire seasons might have obscured seasonal trends in waggle dance behaviours related to foraging distances.

The conditions throughout the study period may affect results. The aforementioned experiments were all completed in different years than ours. Our study year, 2022, was comparatively warm, with the summer impacted by an “extreme” heat wave, leading to the summer months averaging higher temperatures than six of the previous seven years (GOV.UK, 2024; Holley and Lee, 2022). Studies have shown ambient temperatures can affect foraging negatively and that increased ambient temperatures, over 30°C, negatively impact honey bee density and foraging behaviours (Abou-Shaara et al., 2017; Blažytė-Čereškienė et al., 2010; Le Conte and Navajas, 2008) These temperatures were consistently recorded during the summer of 2022 (GOV.UK, 2024), possibly explaining the decreased foraging behaviour during summer. However, they do not explain why we observed decreased foraging distances in summer compared to autumn. Ultimately, this unexpected relationship was puzzling and may warrant further research to understand its causes.

Bees followed, on average, between four and six waggle runs, which is less than some previous studies have reported. For instance, other studies found that successful recruits follow 15 to 20 waggle runs (Menzel et al., 2011; Wray et al., 2012). However, these previous observations were conducted as field experiments involving the manipulation of bees by training them to specific food sources. In contrast, our experiment allowed bees to forage freely on natural food sources. Studies observing waggle following in naturally performing colonies have found a similar average number of waggle phases followed as in our study (Biesmeijer & Seeley, 2005; Al Toufailia et al., 2013; I'Anson Price et al., 2019). A returning forager might spike interest in other hive bees for various reasons, such as reactivation. Experienced bees, upon smelling a familiar odour from previous interactions, briefly follow dances before returning to the familiar site (Gil & Farina, 2002; Biesmeijer & Seeley, 2005; Grüter et al., 2008). Additionally, bees returning to the hive with a familiar food scent attract more followers during dancing, likely because a familiar odour indicates a highly profitable plant species (Grüter & Farina, 2009). Furthermore, dancing also attracts nectar receivers; hence, some followers may not be interested in the resource location (Farina, 2000).

Conclusion

Our results highlight the critical role of environmental conditions surrounding honey bee colonies in shaping their communicative behaviour and the overall nutritional state of the colony. This underscores the behavioural plasticity in waggle dance communication, where nestmates adjust their dancing efforts in response to seasonal changes and varying environmental factors. For example, we found that honey bees followed fewer waggle dances in autumn in more urban sites. A similar conclusion was proposed by I'Anson Price et al. (2019), who suggested that under challenging conditions, waiting for dance information reduces foraging success, leading bees to change their strategy to focus less on dances. In certain environments during autumn, it was likely more beneficial for bees to forage using individual experience information rather than relying on waggle dance communication.

This research emphasises the complexity of honey bee communication and foraging strategies, highlighting the necessity of developing approaches to support honey bee populations. Crucial strategies include implementing additional semi-natural lands, planting diverse pollinator-friendly species, and promoting organic farming practices. Additionally, the use of automated dance decoding and machine learning to study honey bee foraging patterns is extremely beneficial. These methods allow the collection and analysis of ecologically relevant data on a much larger scale that was not possible previously. These tools will greatly aid and improve research into honey bee health and colony sustainability in changing landscapes.

General Discussion

Yongqiang Wu

Main findings

This thesis aimed to investigate the effects of internal nutritional stress, influenced by the unavailability of food stores within colonies, on dance communication and the health of honey bees (**Chapter 1**). Additionally, it explored whether honey bees exhibit distinct physiological and morphological traits when residing in different land-use habitats (**Chapter 2**), and how honey bee colonies adapt their dance communication strategies across varying land-use habitats (**Chapter 3**). We found that the depletion of honey stores significantly impacted waggle dance communication among foragers, with workers providing more information about foraging opportunities to nestmates under these conditions. Furthermore, bees from starved colonies exhibited higher expression of the immune gene *defensin 1*, a key predictor of overwinter survival. However, there was no effect on fatty acid content (**Chapter 1**). Land use and season interactively influenced honey bee body size and fat stores. Bees in agricultural habitats experienced a reduction in fat stores during summer, while body size remained unaffected. In contrast, bees in urban and mixed habitats had increased fat stores in summer, suggesting better nutritional conditions in these habitats (**Chapter 2**). Honey bees exhibited behavioural plasticity in waggle dance communication, adjusting their dancing efforts in response to seasonal changes and varying environmental factors. Bees followed fewer waggle dances in autumn in more urban sites, suggesting a shift towards individual foraging strategies under challenging conditions (**Chapter 3**).

Nutritional stress inside the hive on bee health and dance communication

Food stores are vital for the survival and health of honey bee colonies, and they critically depend on the availability of food sources in the environment. Honey stores, in particular, ensure that the colony can withstand periods of food scarcity, support continuous brood rearing, and maintain overall colony stability and productivity. Scofield and Mattila (2015) found that pollen-stressed larvae become poor foragers, who are lighter and short-lived and dance less precisely.

Waggle dance communication is a unique behaviour in honey bees that allows them to convey information about high-quality resources (von Frisch, 1967; Dyer, 2002; Grüter & Farina, 2009;

Couvillon, 2012; Alves et al., 2023). Previous studies have suggested that the characteristics and value of waggle dance communication depend on ecological factors, such as the availability and distribution of food sources (Dornhaus et al. 2006; Couvillon 2012; Couvillon et al. 2014b, I'Anson Price & Grüter 2015). I'Anson Price et al. (2019), for example, found that dance following increased over time in an environment with few food sources. In addition, Rinderer (1982) found that the empty comb space affected dance frequency and dance follower number, with bees dancing more in colonies with more empty comb areas. However, our understanding of the effects of honey depletion on dance communication and colony health remains limited. We explored if the food stores affect the communication between the foragers by manipulating the honey storage in observation hives (**Fig S1.1**), We found that the number of waggle dances increased significantly when honey bees were honey-starved, therefore providing more information about foraging opportunities to nestmates (**Fig 1.1**). We also found a decreased number of followers per dance, which may be due to a switch to pro-active, solitary foraging or due to the presence of a larger number of waggle dances (**Fig 1.2**).

Nutritional stress and diet were reported to shape the immunocompetence and affect the fatty acid stores of bees (Alaux et al., 2010; Corona et al., 2023). Corona et al. (2023), for example, found that pollen restriction affects immune gene expression, and Dolezal et al. (2019) found that bees inhabiting intensively farmed monocultures experienced a reduction in fat stores (Dolezal et al., 2019). We investigated how low honey stores affected the health of bees, and we found that bees in starved colonies showed a higher expression of the gene *defensin 1*, which is an important predictor of overwinter survival (**Fig 1.3**), but there was no effect on fatty acid content (**Fig 1.4**). It is possible that body fat content and composition depend more on the pollen diet of bees (Brodschneider & Crailsheim, 2010) than on honey, or that the 6-day experiment period was too short to impact fat stores. Our findings support that physiological changes do not happen as quickly as behavioural changes, which are often the first response to environmental changes (Wong & Candolin, 2015). Brief periods of low nectar availability, such as during bad weather, are unlikely to affect body fat stores. This study enhances our understanding of how honey depletion impacts foraging, immune gene expression, and physiology.

Nutritional links between land use and body condition of bees

Food availability in the external environment of honey bee colonies plays a critical role in the bees' ability to collect the resources they need to maintain the colony. Anthropogenic activities, particularly agricultural monocultures and urbanization, have significantly altered natural habitats, leading to food shortages and seasonal foraging challenges (Aizen et al., 2008; Mcdonald et al., 2008; Seto et al., 2012b; Plourde et al., 2013; Otto et al., 2018). Two key biological traits that may be influenced by land use are body size and fat stores. Previous research has demonstrated that bumble bees exhibit varying body sizes in urban habitats (Theodorou et al., 2021; Austin et al., 2022), Austin et al. (2022), for instance, found that habitats with higher levels of urbanization were associated with larger *Bombus impatiens* and smaller *Bombus pensylvanicus* bees (Theodorou et al., 2021; Austin et al., 2022). Additionally, Dolezal et al. (2019) reported that bees residing in intensively farmed monocultures exhibited reduced fat stores. However, for honey bees (*Apis mellifera*), while body size variation is minimal, there is a lack of studies addressing how these biological traits may change across different habitats.

We sampled honey bees in agricultural habitats, urban habitats and mixed habitats throughout a foraging season in south-western Germany (**Fig 2.1 and 2.2**) to further explore the effects of landscape and season on bees' fat storage, body size, and wing wear. We found that landscape and season interactively affect honey bee body size, wing wear and fat stores. Agricultural landscapes can provide honey bees with ample mono-floral nectar and pollen for short periods, however, we found that in summer bees in these areas experience greater nutritional and physiological challenges compared to bees in urban and mixed habitats (**Fig 2.5**). Despite their slightly smaller body size, bees in urban and mixed habitats seem to benefit from better nutritional conditions in summer, as evidenced by increased fat stores (**Fig 2.3 and 2.5**). This supports the view that urban and mixed habitats can be a good environment for honey bees during the particularly challenging summer months. Our findings also confirm that honey bees undergo morphological and physiological changes in response to land use and season, which could impact their physiological condition and winter survival, as well as highlights that to improve overwintering success and prevent colony losses, management decisions should aim to increase the nutritional diversity and availability of food for bees: (1) in agricultural habitats during early summer, and (2) in urban and mixed habitats at the start of autumn.

Adaptive dance communication amid environmental nutritional stress

Behavioural plasticity is often an organism's initial response to environmental change and is frequently a sign of population stress, increasingly observed due to human modifications to land use (Wong and Candolin, 2015; Cerini et al*.,* 2023). Honey bees rely on waggle dance communication to recruit nestmates to profitable food sources, but in the face of major environmental changes, they face challenges in finding sufficient food resources (von Frisch, 1967; Seeley et al., 1991, 2000; I'Anson et al., 2019). Recent studies (Donaldson-Matasci and Dornhaus, 2012; Dornhaus and Chittka, 2004; I'Anson et al*.,* 2019) show waggle dance communication may not always be beneficial in modern urban landscapes. Consequently, studies exploring how honey bees adjust their communication behaviour to suit the land use characteristics of their colony's habitat are limited. Understanding how honey bees adjust their communication strategies in response to land use modifications is crucial to understanding the links between behaviour and nutritional challenges. To explore how dance communication is affected by land use and seasonal changes, we studied 17 honey bee colonies across a rural-urban gradient in South-West England (**Fig 3.2 and 3.3**), recording the overall weight of the colonies and key features of waggle dance communication during summer and autumn using a novel machine-learning algorithm to automate dance decoding. Our data highlight the critical role of environmental conditions surrounding honey bee colonies in shaping their communicative behaviour and the overall nutritional state of the colony (**Chapter 3**). This underscores the behavioural plasticity in waggle dance communication, where nestmates adjust their dancing efforts in response to seasonal changes and varying environmental factors. For example, we found that honey bees followed fewer waggle dances in autumn in more urban sites (**Fig 3.6**). A similar conclusion was proposed by I'Anson Price et al. (2019), who suggested that under challenging conditions, waiting for dance information reduces foraging success, leading bees to change their strategy to focus less on dances. In certain environments during autumn, it was likely more beneficial for bees to forage relying on individual experience rather than relying on waggle dance communication.

Taking together the findings of the three chapters, this thesis emphasizes the importance of hive-internal and -external nutritional conditions for honey bee behaviour, physiology, and colony health. The findings highlight the need for strategies to support honey bee populations, such as implementing additional semi-natural lands, planting diverse pollinator-friendly species, and promoting organic farming practices (Holzschuh et al., 2008; Holland et al., 2017; Buhk et al., 2018). The use of automated dance decoding and machine learning in studying honey bee foraging patterns has proven to be beneficial and should be further utilized. These tools enable the collection and analysis of ecologically relevant data on a larger scale, contributing to a better understanding of honey bee health and colony sustainability in changing landscapes.

Outlook

While this thesis provides valuable insights into the effects of nutritional stress and land use on honey bees, there are still unanswered questions that require further research. In **Chapter 1**, we demonstrated that internal nutritional stress affected the behaviours of dance communication and health of honey bees. Scofield and Mattila (2015) found that pollen-stressed larvae become poor foragers, which were lighter and shorter-lived, and Schulz et al. (1998) found honey-starved colonies produce more precocious foragers. Future research could assess the life span and health of these precocious foragers, as they have a shorter development time. We found that one important immune gene was expressed more in honey-starved colonies, but we need further study about whether this is linked to disease resistance and we should investigate other nutritional markers, such as oxidative stress. In **Chapter 2,** we demonstrate that body size and fat stores are affected in different land-use habitats and seasons. But we still do not know if the foragers' body size and fat stores linked to their hive’s food stores or their foraging distance? We did not test immune genes expression, pesticide levels and the virus titres of our sampled bees, and this should be added in to future studies. In **Chapter 3**, we found that land use and season affect honey bee communication, demonstrating potentially adaptive behavioural responses to their environment.

Due to time constraints, we assessed only two periods, late summer and autumn. Future research should consider extending the study period to cover the full foraging year or multiple years for a more comprehensive understanding of waggle dance patterns (see Couvillon et al., 2014b). Additionally, frame adjustments during filming might have influenced dance frequency data, and future studies should avoid this manipulation. Finally, addressing issues with the Waggle Dance Detector (WDD) and improving detection rates could refine analyses. With additional data, such as solar positioning, dance maps could be created to explore preferred foraging sites across landscapes and seasons.

References

Abou-Shaara, H. F., Owayss, A. A., Ibrahim, Y. Y., & Basuny, N. K. (2017). A review of impacts of temperature and relative humidity on various activities of honey bees. *Insectes Sociaux*, *64*(4), 455–463. https://doi.org/10.1007/s00040-017-0573-8

Aizen, M. A., Garibaldi, L. A., Cunningham, S. A., & Klein, A. M. (2008). Long-term global trends in crop yield and production reveal no current pollination shortage but Increasing pollinator dependency. *Current Biology*, *18*(20), 1572–1575. https://doi.org/10.1016/j.cub.2008.08.066

Al Toufailia, H., Couvillon, M. J., Ratnieks, F. L. W., & Grüter, C. (2013). Honey bee waggle dance communication: Signal meaning and signal noise affect dance follower behaviour. *Behavioral Ecology and Sociobiology*, *67*(4), 549–556. https://doi.org/10.1007/s00265-012-1474-5

Alaux, C., Dantec, C., Parrinello, H., & Le Conte, Y. (2011). Nutrigenomics in honey bees: Digital gene expression analysis of pollen's nutritive effects on healthy and varroa-parasitized bees. *BMC Genomics*, *12*(1), 496. https://doi.org/10.1186/1471-2164-12-496

Alaux, C., Ducloz, F., Crauser, D., & Le Conte, Y. (2010). Diet effects on honeybee immunocompetence. *Biology Letters*, *6*(4), 562–565. https://doi.org/10.1098/rsbl.2009.0986

Altieri, M. A. (2009). *Green deserts: Monocultures and their impacts on biodiversity.* Sweden; FIAN International.

Alves, D. A., George, E. A., Kaur, R., Brockmann, A., Hrncir, M., & Grüter, C. (2023). Diverse communication strategies in bees as a window into adaptations to an unpredictable world. *Proceedings of the National Academy of Sciences*, *120*(24), e2219031120. https://doi.org/10.1073/pnas.2219031120

Amiri, E., Kryger, P., Meixner, M. D., Strand, M. K., Tarpy, D. R., & Rueppell, O. (2018). Quantitative patterns of vertical transmission of deformed wing virus in honey bees. *PLOS ONE*, *13*(3), e0195283. https://doi.org/10.1371/journal.pone.0195283

Anderson, S. E., Hahn, P., Gonzalez, G., & Mallinger, R. E. (2024). Land-use change alters specialist bee diet and drives body size declines. *Landscape Ecology*, *39*(6), 115. https://doi.org/10.1007/s10980-024-01893-1

Arien, Y., Dag, A., & Shafir, S. (2018). Omega-6:3 ratio more than absolute lipid level in diet affects associative learning in honey bees. *Frontiers in Psychology*, *9*, 1001. https://doi.org/10.3389/fpsyg.2018.01001

Arien, Y., Dag, A., Yona, S., Tietel, Z., Lapidot Cohen, T., & Shafir, S. (2020). Effect of diet lipids and omega-6:3 ratio on honey bee brood development, adult survival and body composition. *Journal of Insect Physiology*, *124*, 104074. https://doi.org/10.1016/j.jinsphys.2020.104074

Arien, Y., Dag, A., Zarchin, S., Masci, T., & Shafir, S. (2015). Omega-3 deficiency impairs honey bee learning. *Proceedings of the National Academy of Sciences*, *112*(51), 15761–15766. https://doi.org/10.1073/pnas.1517375112

Aronson, M. F., Lepczyk, C. A., Evans, K. L., Goddard, M. A., Lerman, S. B., MacIvor, J. S., Nilon, C. H., & Vargo, T. (2017). Biodiversity in the city: Key challenges for urban green space management. *Frontiers in Ecology and the Environment*, *15*(4), 189–196. https://doi.org/10.1002/fee.1480

Arrese, E. L., & Soulages, J. L. (2010). Insect fat body: energy, metabolism, and regulation. *Annual Review of Entomology*, *55*(1), 207–225. https://doi.org/10.1146/annurev-ento-112408-085356

Ashman, T.L., Knight, T. M., Steets, J. A., Amarasekare, P., Burd, M., Campbell, D. R., Dudash, M. R., Johnston, M. O., Mazer, S. J., Mitchell, R. J., Morgan, M. T., & Wilson, W. G. (2004). Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology*, *85*(9), 2408–2421. https://doi.org/10.1890/03-8024

Athenstaedt, K., & Daum, G. (2006). The life cycle of neutral lipids: Synthesis, storage and degradation. *Cellular and Molecular Life Sciences*, *63*(12), 1355–1369. https://doi.org/10.1007/s00018-006-6016-8

Austin, M. W., Tripodi, A. D., Strange, J. P., & Dunlap, A. S. (2022). Bumble bees exhibit body size clines across an urban gradient despite low genetic differentiation. *Scientific Reports*, *12*(1), 4166. https://doi.org/10.1038/s41598-022-08093-4

Bacandritsos, N., Granato, A., Budge, G., Papanastasiou, I., Roinioti, E., Caldon, M., Falcaro, C., Gallina, A., & Mutinelli, F. (2010). Sudden deaths and colony population decline in Greek honey bee colonies. *Journal of Invertebrate Pathology*, *105*(3), 335–340. https://doi.org/10.1016/j.jip.2010.08.004

Bailey, E. (1975). Biochemistry of Insect Flight. In D. J. Candy & B. A. Kilby (Eds.), *Insect Biochemistry and Function* (pp. 89–176). Springer Netherlands. https://doi.org/10.1007/978-94-009-5853-1_2

Baldock, K. C. (2020). Opportunities and threats for pollinator conservation in global towns and cities. *Current Opinion in Insect Science*, *38*, 63–71. https://doi.org/10.1016/j.cois.2020.01.006

Baldock, K. C. R., Goddard, M. A., Hicks, D. M., Kunin, W. E., Mitschunas, N., Morse, H., Osgathorpe, L. M., Potts, S. G., Robertson, K. M., Scott, A. V., Staniczenko, P. P. A., Stone, G. N., Vaughan, I. P., & Memmott, J. (2019). A systems approach reveals urban pollinator hotspots and conservation opportunities. *Nature Ecology & Evolution*, *3*(3), 363–373. https://doi.org/10.1038/s41559-018-0769-y

Barascou, L., Sene, D., Barraud, A., Michez, D., Lefebvre, V., Medrzycki, P., Di Prisco, G., Strobl, V., Yañez, O., Neumann, P., Le Conte, Y., & Alaux, C. (2021). Pollen nutrition fosters honeybee tolerance to pesticides. *Royal Society Open Science*, *8*(9), 210818. https://doi.org/10.1098/rsos.210818

Barroso-Arévalo, S., Vicente-Rubiano, M., Puerta, F., Molero, F., & Sánchez-Vizcaíno, J. M. (2019). Immune related genes as markers for monitoring health status of honey bee colonies. *BMC Veterinary Research*, *15*(1), 72. https://doi.org/10.1186/s12917-019-1823-y

Bates, A. J., Sadler, J. P., Fairbrass, A. J., Falk, S. J., Hale, J. D., & Matthews, T. J. (2011). Changing Bee and Hoverfly Pollinator Assemblages along an Urban-Rural Gradient. *PLoS ONE*, *6*(8), e23459. https://doi.org/10.1371/journal.pone.0023459

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using **lme4**. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Bawa, K. S. (1990). Plant-pollinator interactions in tropical rain forests. *Annual Review of Ecology and Systematics*, *21*(1), 399–422. https://doi.org/10.1146/annurev.es.21.110190.002151

Becher, M. A., Osborne, J. L., Thorbek, P., Kennedy, P. J., & Grimm, V. (2013). REVIEW: Towards a systems approach for understanding honeybee decline: a stocktaking and synthesis of existing models. *Journal of Applied Ecology*, *50*(4), 868–880. https://doi.org/10.1111/1365-2664.12112

Beekman, M., & Lew, J. B. (2008). Foraging in honeybees—When does it pay to dance? *Behavioral Ecology*, *19*(2), 255–261. https://doi.org/10.1093/beheco/arm117

Beekman, M., Makinson, J. C., Couvillon, M. J., Preece, K., & Schaerf, T. M. (2015). Honeybee linguistics—A comparative analysis of the waggle dance among species of Apis. *Frontiers in Ecology and Evolution*, *3*. https://doi.org/10.3389/fevo.2015.00011

Beekman, M., & Ratnieks, F. L. W. (2000). Long-range foraging by the honey-bee, *Apis mellifera* L. *Functional Ecology*, *14*(4), 490–496. https://doi.org/10.1046/j.1365-2435.2000.00443.x

Beenakkers, M.Th., Van der Horst, A. D. J., & Van Marrewijk, W. J. A. (1985). Insect lipids and lipoproteins, and their role in physiological processes. Progress in Lipid Research, 24(1), 19–67. https://doi.org/10.1016/0163-7827(85)90007-4

Bennett, A. B., & Lovell, S. (2019). Landscape and local site variables differentially influence pollinators and pollination services in urban agricultural sites. *PLOS ONE*, *14*(2), e0212034. https://doi.org/10.1371/journal.pone.0212034

Bennett, M. M., Welchert, A. C., Carroll, M., Shafir, S., Smith, B. H., & Corby-Harris, V. (2022). Unbalanced fatty acid diets impair discrimination ability of honey bee workers to damaged and healthy brood odors. *Journal of Experimental Biology*, *225*(7), https://doi.org/10.1242/jeb.244103

Benton, T. G., Bryant, D. M., Cole, L., & Crick, H. Q. P. (2002). Linking agricultural practice to insect and bird populations: A historical study over three decades. *Journal of Applied Ecology*, *39*(4), 673–687. https://doi.org/10.1046/j.1365-2664.2002.00745.x

Benton, T. G., Vickery, J. A., & Wilson, J. D. (2003). Farmland biodiversity: Is habitat heterogeneity the key? *Trends in Ecology & Evolution*, *18*(4), 182–188. https://doi.org/10.1016/S0169-5347(03)00011-9

Biesmeijer, J. C., & de Vries, H. (2001). Exploration and exploitation of food sources by social insect colonies: A revision of the scout-recruit concept. *Behavioral Ecology and Sociobiology*, *49*(2–3), 89–99. https://doi.org/10.1007/s002650000289

Biesmeijer, J. C., & Seeley, T. D. (2005). The use of waggle dance information by honey bees throughout their foraging careers. *Behavioral Ecology and Sociobiology*, *59*(1), 133–142. https://doi.org/10.1007/s00265-005-0019-6

Blanchard, G. B., Orledge, G. M., Reynolds, S. E., & Franks, N. R. (2000). Division of labour and seasonality in the ant *Leptothorax albipennis*: Worker corpulence and its influence on behaviour. *Animal Behaviour*, *59*(4), 723–738. https://doi.org/10.1006/anbe.1999.1374

Blanken, L. J., van Langevelde, F., & van Dooremalen, C. (2015). Interaction between *Varroa destructor* and imidacloprid reduces flight capacity of honeybees. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1820), 20151738. https://doi.org/10.1098/rspb.2015.1738

Blažytė-Čereškienė, L., Vaitkevičienė, G., Venskutonytė, S. and Būda, V., (2010). Honey bee foraging in spring oilseed rape crops under high ambient temperature conditions. Zemdirbyste-Agriculture, 97(1), pp.61-70.

Boetzl, F. A., Krauss, J., Heinze, J., Hoffmann, H., Juffa, J., König, S., Krimmer, E., Prante, M., Martin, E. A., Holzschuh, A., & Steffan-Dewenter, I. (2021). A multitaxa assessment of the effectiveness of agri-environmental schemes for biodiversity management. *Proceedings of the National Academy of Sciences*, *118*(10), https://doi.org/10.1073/pnas.2016038118

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.S. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, *24*(3), 127–135. https://doi.org/10.1016/j.tree.2008.10.008

Bosch, J. (2008). Production of undersized offspring in a solitary bee. *Animal Behaviour*, *75*(3), 809–816. https://doi.org/10.1016/j.anbehav.2007.06.018

Branchiccela, B., Castelli, L., Corona, M., Díaz-Cetti, S., Invernizzi, C., Martínez de la Escalera, G., Mendoza, Y., Santos, E., Silva, C., Zunino, P., & Antúnez, K. (2019). Impact of nutritional stress on the honeybee colony health. *Scientific Reports*, *9*(1), 10156. https://doi.org/10.1038/s41598-019-46453-9

Brasil, S. N. R., Ayers, A. C., & Rehan, S. M. (2023). The effect of urbanisation and seasonality on wild bee abundance, body size and foraging efforts. *Ecological Entomology*, *48*(4), 499–507. https://doi.org/10.1111/een.13243

Breeze, Roberts, S. P.M, & Potts, S. G. (2012). *The decline of England's bees: Policy review and recommendations* (In Friends of the Earth Report).

Brodschneider, R., & Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie*, *41*(3), 278–294. https://doi.org/10.1051/apido/2010012

Brutscher, L. M., Daughenbaugh, K. F., & Flenniken, M. L. (2015). Antiviral defense mechanisms in honey bees. *Current Opinion in Insect Science*, *10*, 71–82. https://doi.org/10.1016/j.cois.2015.04.016

Brutscher , L. M., Daughenbaugh, K. F., & Flenniken, M. L. (2017). Virus and dsRNA-triggered transcriptional responses reveal key components of honey bee antiviral defense. *Scientific Reports*, *7*(1), 6448. https://doi.org/10.1038/s41598-017-06623-z

Buchon, N., Silverman, N., & Cherry, S. (2014). Immunity in Drosophila melanogaster—From microbial recognition to whole-organism physiology. *Nature Reviews Immunology*, *14*(12), 796–810. https://doi.org/10.1038/nri3763

Burkle, L., & Irwin, R. (2009). Nectar sugar limits larval growth of solitary bees (Hymenoptera: *Megachilidae*). *Environmental Entomology*, *38*(4), 1293–1300. https://doi.org/10.1603/022.038.0441

Bushmann, S. L., & Drummond, F. A. (2020). Analysis of pollination services provided by wild and managed bees (Apoidea) in wild blueberry (vaccinium angustifolium aiton) production in maine, USA, with a literature review. *Agronomy*, *10*(9), 1413. https://doi.org/10.3390/agronomy10091413

Buhk, C., Oppermann, R., Schanowski, A., Bleil, R., Lüdemann, J., & Maus, C. (2018). Flower strip networks offer promising long term effects on pollinator species richness in intensively cultivated agricultural areas. BMC ecology, 18, 1-13. https://doi.org/10.1186/s12898-018-0210-z

Butler, C. G., Jeffree, E. P., & Kalmus, H. (1943). The behaviour of a population of honeybees on an artificial and on a natural crop. *Journal of Experimental Biology*, *20*(1), 65–73. https://doi.org/10.1242/jeb.20.1.65

Callier, V., & Nijhout, H. F. (2014). Plasticity of insect body size in response to oxygen: Integrating molecular and physiological mechanisms. *Current Opinion in Insect Science*, *1*, 59–65. https://doi.org/10.1016/j.cois.2014.05.007

Canavoso, L. E., Jouni, Z. E., Karnas, K. J., Pennington, J. E., & Wells, M. A. (2001). Fat metabolism in insects. *Annual Review of Nutrition*, *21*(1), 23–46. https://doi.org/10.1146/annurev.nutr. 21.1

Cappa, F., Baracchi, D., & Cervo, R. (2022). Biopesticides and insect pollinators: Detrimental effects, outdated guidelines, and future directions. *Science of The Total Environment*, *837*, 155714. https://doi.org/10.1016/j.scitotenv.2022.155714

Casanelles-Abella, J., & Moretti, M. (2022). Challenging the sustainability of urban beekeeping using evidence from Swiss cities. *Npj Urban Sustainability*, *2*(1), 3. https://doi.org/10.1038/s42949-021-00046-6

Casteels, P., Ampe, C., Jacobs, F., & Tempst, P. (1993). Functional and chemical characterization of Hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honeybee (Apis mellifera). *Journal of Biological Chemistry*, *268*(10), 7044–7054. https://doi.org/10.1016/S0021-9258(18)53143-4

Casteels, P., Ampe, C., Riviere, L., Damme, J., Elicone, C., Fleming, M., Jacobs, F., & Tempst, P. (1990). Isolation and characterization of abaecin, a major antibacterial response peptide in the honeybee (Apis mellifera). *European Journal of Biochemistry*, *187*(2), 381–386. https://doi.org/10.1111/j.1432-1033.1990.tb15315.x

Casteels-Josson, K., Zhang, W., Capaci, T., Casteels, P., & Tempst, P. (1994). Acute transcriptional response of the honeybee peptide-antibiotics gene repertoire and required post-translational conversion of the precursor structures. *Journal of Biological Chemistry*, *269*(46), 28569–28575. https://doi.org/10.1016/S0021-9258(19)61943-5

Castelli, L., Branchiccela, B., Garrido, M., Invernizzi, C., Porrini, M., Romero, H., Santos, E., Zunino, P., & Antúnez, K. (2020). Impact of nutritional stress on honeybee gut microbiota, immunity, and nosema ceranae infection. *Microbial Ecology*, *80*(4), 908–919. https://doi.org/10.1007/s00248-020-01538-1

Cerini, F., Childs, D. Z., & Clements, C. F. (2023). A predictive timeline of wildlife population collapse. *Nature Ecology & Evolution*, *7*(3), 320–331. https://doi.org/10.1038/s41559-023-01985-2

Chau, K. D., Samad-zada, F., Kelemen, E. P., & Rehan, S. M. (2023). Integrative population genetics and metagenomics reveals urbanization increases pathogen loads and decreases connectivity in a wild bee. *Global Change Biology*, *29*(15), 4193–4211. https://doi.org/10.1111/gcb.16757

Chen, Y. P., Higgins, J. A., & Feldlaufer, M. F. (2005). Quantitative Real-Time Reverse Transcription-PCR Analysis of Deformed Wing Virus Infection in the Honeybee (*Apis mellifera* L.). *Applied and Environmental Microbiology*, *71*(1), 436–441. https://doi.org/10.1128/AEM.71.1.436-441.2005

Chen, Y. P., Pettis, J. S., Corona, M., Chen, W. P., Li, C. J., Spivak, M., Visscher, P. K., DeGrandi-Hoffman, G., Boncristiani, H., Zhao, Y., vanEngelsdorp, D., Delaplane, K., Solter, L., Drummond, F., Kramer, M., Lipkin, W. I., Palacios, G., Hamilton, M. C., Smith, B., … Evans, J. D. (2014). Israeli Acute Paralysis Virus: Epidemiology, Pathogenesis and Implications for Honey Bee Health. *PLoS Pathogens*, *10*(7), e1004261. https://doi.org/10.1371/journal.ppat.1004261

Chole, H., Woodard, S. H., & Bloch, G. (2019). Body size variation in bees: Regulation, mechanisms, and relationship to social organization. *Current Opinion in Insect Science*, *35*, 77–87. https://doi.org/10.1016/j.cois.2019.07.006

Clark, D., (2024). Statista. Largest urban agglomerations in the United Kingdom in 2023. Available at: https://www.statista.com/ (Accessed on 07/04/2024)

Cohen, H., Smith, G. P., Sardiñas, H., Zorn, J. F., McFrederick, Q. S., Woodard, S. H., & Ponisio, L. C. (2021). Mass-flowering monoculture attracts bees, amplifying parasite prevalence. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1960). https://doi.org/10.1098/rspb.2021.1369

Corby-Harris, V., Jones, B. M., Walton, A., Schwan, M. R., & Anderson, K. E. (2014). Transcriptional markers of sub-optimal nutrition in developing Apis mellifera nurse workers. *BMC Genomics*, *15*(1), 134. https://doi.org/10.1186/1471-2164-15-134

Corby-Harris, V., Maes, P., & Anderson, K. E. (2014). The Bacterial Communities Associated with Honey Bee (Apis mellifera) Foragers. *PLoS ONE*, *9*(4). https://doi.org/10.1371/journal.pone.0095056

Corona, M., Branchiccela, B., Alburaki, M., Palmer-Young, E. C., Madella, S., Chen, Y., & Evans, J. D. (2023). Decoupling the effects of nutrition, age, and behavioral caste on honey bee physiology, immunity, and colony health. *Frontiers in Physiology*, *14*. https://doi.org/10.3389/fphys.2023.1149840

Couvillon, M., Fensome, K. A., Quah, S. K. L., & Schürch, R. (2014a). August foraging leaves honey bees empty-handed. *Communicative & Integrative Biology*, *7*. https://doi.org/10.4161/cib.2882

Couvillon, M. J. (2012). The dance legacy of Karl von Frisch. *Insectes Sociaux*, *59*(3), 297–306. https://doi.org/10.1007/s00040-012-0224-z

Couvillon, M. J., & Dornhaus, A. (2010). Small worker bumble bees (Bombus impatiens) are hardier against starvation than their larger sisters. *Insectes Sociaux*, *57*(2), 193–197. https://doi.org/10.1007/s00040-010-0064-7

Couvillon, M. J., Riddell Pearce, F. C., Harris-Jones, E. L., Kuepfer, A. M., Mackenzie-Smith, S. J., Rozario, L. A., Schürch, R., & Ratnieks, F. L. W. (2012). Intra-dance variation among waggle runs and the design of efficient protocols for honey bee dance decoding. *Biology Open*, *1*(5), 467–472. https://doi.org/10.1242/bio.20121099

Couvillon, M. J., Schürch, R., & Ratnieks, F. L. W. (2014c). Dancing Bees Communicate a Foraging Preference for Rural Lands in High-Level Agri-Environment Schemes. *Current Biology*, *24*(11), 1212–1215. https://doi.org/10.1016/j.cub.2014.03.072

Couvillon, M., Schürch, R., & Ratnieks, F. (2014b). Waggle dance distances as integrative indicators of seasonal foraging challenges. *PLoS ONE*, *9*(4), https://doi.org/10.1371/journal.pone.009349

Crist, E., Mora, C., & Engelman, R. (2017). The interaction of human population, food production, and biodiversity protection. *Science*, *356*(6335), 260–264. https://doi.org/10.1126/science.aal2011

Czekońska, K., Łopuch, S., Miścicki, S., Bańkowski, J., & Szabla, K. (2023). Monitoring of hive weight changes in various landscapes. *Apidologie*, *54*(3), 30. https://doi.org/10.1007/s13592-023-0101

Dainat, B., vanEngelsdorp, D., & Neumann, P. (2012). Colony collapse disorder in Europe. *Environmental Microbiology Reports*, *4*(1), 123–125. https://doi.org/10.1111/j.17582229.2011

Danihlík, J., Škrabišová, M., Lenobel, R., Šebela, M., Omar, E., Petřivalský, M., Crailsheim, K., & Brodschneider, R. (2018). Does the pollen diet influence the production and expression of antimicrobial peptides in individual honey bees? *Insects*, *9*(3), 79. https://doi.org/10.3390/insects9030079

Danner, N., Keller, A., Härtel, S., & Steffan-Dewenter, I. (2017). Honey bee foraging ecology: Season but not landscape diversity shapes the amount and diversity of collected pollen. *PLOS ONE*, *12*(8), e0183716. https://doi.org/10.1371/journal.pone.0183716

Danner, N., Molitor, A., Schiele, S., Härtel, S., & Steffan-Dewenter, I. (2016). Season and landscape composition affect pollen foraging distances and habitat use of honey bees. *Ecological Applications : A Publication of the Ecological Society of America*, *26 6*, 1920–1929. https://doi.org/10.1890/15-1840.1

de Groot, G. S., Aizen, M. A., Sáez, A., & Morales, C. L. (2021). Large-scale monoculture reduces honey yield: The case of soybean expansion in Argentina. *Agriculture, Ecosystems & Environment*, *306*, 107203. https://doi.org/10.1016/j.agee.2020.107203

De la Rúa, P., Jaffé, R., Dall'Olio, R., Muñoz, I., & Serrano, J. (2009). Biodiversity, conservation and current threats to European honeybees. *Apidologie*, *40*(3), 263–284. https://doi.org/10.1051/apido/2009027

De Roode, J. C., & Lefèvre, T. (2012). Behavioral immunity in insects. *Insects*, *3*(3), 789–820. https://doi.org/10.3390/insects3030789

Dean, R. L., Locke, M., & Collins, J. V. (1985). Structure of the fat body. In *Integument, Respiration and Circulation* (pp. 155–210). Elsevier. https://doi.org/10.1016/B978-0-08-030804-3.50011

DeGrandi-Hoffman, G., Chen, Y., Huang, E., & Huang, M. H. (2010). The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (Apis mellifera L.). *Journal of Insect Physiology*, *56*(9), 1184–1191. https://doi.org/10.1016/j.jinsphys.2010.03.017

DeGrandi-Hoffman, G., Chen, Y., Rivera, R., Carroll, M., Chambers, M., Hidalgo, G., & de Jong, E. W. (2016). Honey bee colonies provided with natural forage have lower pathogen loads and higher overwinter survival than those fed protein supplements. *Apidologie*, *47*(2), 186–196. https://doi.org/10.1007/s13592-015-0386-6

Dekking, F. M., Kraaikamp, C., Lopuhaä, H. P., & Meester, L. E. (2005). *A modern introduction to probability and statistics*. Springer London. https://doi.org/10.1007/1-84628-168-7

Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L. P., Decourtye, A., Kretzschmar, A., Suchail, S., Brunet, J.-L., & Alaux, C. (2013). Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? *PLoS ONE*, *8*(8). https://doi.org/10.1371/journal.pone.0072016

Di Prisco, G., Annoscia, D., Margiotta, M., Ferrara, R., Varricchio, P., Zanni, V., Caprio, E., Nazzi, F., & Pennacchio, F. (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*, *113*(12), 3203–3208. https://doi.org/10.1073/pnas.1523515113

Dicks, L. V., Viana, B., Bommarco, R., Brosi, B., Arizmendi, M. del C., Cunningham, S. A., Galetto, L., Hill, R., Lopes, A. V., Pires, C., Taki, H., & Potts, S. G. (2016). Ten policies for pollinators. *Science*, *354*(6315), 975–976. https://doi.org/10.1126/science.aai9226

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

Döke, M. A., Frazier, M., & Grozinger, C. M. (2015). Overwintering honey bees: Biology and management. *Current Opinion in Insect Science*, *10*, 185–193. https://doi.org/10.1016/j.cois.2015.05.014

Dolezal, A. G., Clair, A. L. S., Zhang, G., Toth, A., & O’Neal, M. (2019). Native habitat mitigates feast–famine conditions faced by honey bees in an agricultural landscape. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 25147–25155. https://doi.org/10.1073/pnas.1912801116

Domínguez, E., Giardini, P. M., Quintana, S., de la Paz Moliné, M., Chierichetti, M., Medici, S. K., Gende, L. B., & Damiani, N. (2024). Fatty acid profile of Apis mellifera workers in the face of an immune challenge. *Apidologie*, *55*(1), 15. https://doi.org/10.1007/s13592-024-01059-7

Donaldson-Matasci, M. C., & Dornhaus, A. (2012). How habitat affects the benefits of communication in collectively foraging honey bees. *Behavioral Ecology and Sociobiology*, *66*(4), 583–592. https://doi.org/10.1007/s00265-011-1306-z

Donkersley, P., Rhodes, G., Pickup, R. W., Jones, K. C., & Wilson, K. (2014). Honeybee nutrition is linked to landscape composition. *Ecology and Evolution*, *4*(21), 4195–4206. https://doi.org/10.1002/ece3.1293

Dornhaus, A., & Chittka, L. (2004). Why do honey bees dance? *Behavioral Ecology and Sociobiology*, *55*(4), 395–401. https://doi.org/10.1007/s00265-003-0726-9

Dornhaus, A., Klügl, F., Oechslein, C., Puppe, F., & Chittka, L. (2006). Benefits of recruitment in honey bees: Effects of ecology and colony size in an individual-based model. *Behavioral Ecology*, *17*(3), 336–344. https://doi.org/10.1093/beheco/arj036

Dyer, F. C. (2002). The biology of the dance language. *Annual Review of Entomology*, *47*(1), 917–949. https://doi.org/10.1146/annurev.ento.47.091201.145306

Ellis, E. C., Gauthier, N., Klein Goldewijk, K., Bliege Bird, R., Boivin, N., Díaz, S., Fuller, D. Q., Gill, J. L., Kaplan, J. O., Kingston, N., Locke, H., McMichael, C. N. H., Ranco, D., Rick, T. C., Shaw, M. R., Stephens, L., Svenning, J.-C., & Watson, J. E. M. (2021). People have shaped most of terrestrial nature for at least 12,000 years. *Proceedings of the National Academy of Sciences*, *118*(17), e2023483118. https://doi.org/10.1073/pnas.2023483118

Ellis, E. C., & Ramankutty, N. (2008). Putting people in the map: Anthropogenic biomes of the world. *Frontiers in Ecology and the Environment*, *6*(8), 439–447. https://doi.org/10.1890/070062

Esch, H., & Bastian, J. A. (1970). How do newly recruited honey bees approach a food site? *Zeitschrift Für Vergleichende Physiologie*, *68*(2), 175–181. https://doi.org/10.1007/BF00297693

Es'kov, E. K., & Es'kova, M. D. (2013). Factors influencing wing size and body weight variation in the western honeybee. *Russian Journal of Ecology*, *44*(5), 433–438. https://doi.org/10.1134/S1067413613050056.

Evans, J. D. (2006). Beepath: an ordered quantitative-PCR array for exploring honey bee immunity and disease. Journal of invertebrate pathology, 93(2), 135-139. https://doi.org/10.1016/j.jip.2006.04.004

Evans, E., Smart, M., Cariveau, D., & Spivak, M. (2018). Wild, native bees and managed honey bees benefit from similar agricultural land uses. *Agriculture, Ecosystems & Environment*, *268*, 162–170. https://doi.org/10.1016/j.agee.2018.09.014

Farina, W. M. (2000). The interplay between dancing and trophallactic behavior in the honey bee Apis mellifera. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, *186*(3), 239–245. https://doi.org/10.1007/s003590050424

Fijen, T. P. M., Scheper, J. A., Boekelo, B., Raemakers, I., & Kleijn, D. (2019). Effects of landscape complexity on pollinators are moderated by pollinators' association with mass-flowering crops. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1900), 20190387. https://doi.org/10.1098/rspb.2019.0387

Fitzgerald, J. L., Ogilvie, J. E., & CaraDonna, P. J. (2022). Ecological Drivers and Consequences of Bumble Bee Body Size Variation. *Environmental Entomology*, *51*(6), 1055–1068. https://doi.org/10.1093/ee/nvac093

Folch, J., Lees, M., & Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*(1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., Chapin, F. S., Coe, M. T., Daily, G. C., Gibbs, H. K., Helkowski, J. H., Holloway, T., Howard, E. A., Kucharik, C. J., Monfreda, C., Patz, J. A., Prentice, I. C., Ramankutty, N., & Snyder, P. K. (2005). Global consequences of land use. *Science*, *309*(5734), 570–574. https://doi.org/10.1126/science.-1111772

Fontaine, C., Dajoz, I., Meriguet, J., & Loreau, M. (2005). Functional diversity of plant–pollinator Interaction webs enhances the persistence of plant communities. *PLoS Biology*, *4*(1), e1. https://doi.org/10.1371/journal.pbio.0040001

Forrest, J. R. K., Thorp, R. W., Kremen, C., & Williams, N. M. (2015). Contrasting patterns in species and functional-trait diversity of bees in an agricultural landscape. *Journal of Applied Ecology*, *52*(3), 706–715. https://doi.org/10.1111/1365-2664.12433

Foster, D. J., & Cartar, R. V. (2011a). What causes wing wear in foraging bumble bees? *Journal of Experimental Biology*, *214*(11), 1896–1901. https://doi.org/10.1242/jeb.051730

Foster, D. J., & Cartar, R. V. (2011b). Wing wear affects wing use and choice of floral density in foraging bumble bees. *Behavioral Ecology*, *22*(1), 52–59. https://doi.org/10.1093/beheco/arq160

Fukuda, H., & Sekiguchi, K. (1966). Seasonal change of the honeybee worker longevity in sapporo, north japan, with notes on some factors affecting the life-span. *Japanese Journal of Ecology*, *16*(5), 206–212. https://doi.org/10.18960/seitai.16.5_206

Gäde, G., & Auerswald, L. (2002). Beetles' choice—proline for energy output: Control by AKHs. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *132*(1), 117–129. https://doi.org/10.1016/S1096-4959(01)00541-3

Garbuzov, M., & Ratnieks, F. L. W. (2014). Ivy: An underappreciated key resource to flower-visiting insects in autumn. *Insect Conservation and Diversity*, *7*(1), 91–102. https://doi.org/10.1111/icad.12033

Garbuzov, M., Schürch, R., & Ratnieks, F. (2015). Eating locally: Dance decoding demonstrates that urban honey bees in Brighton, UK, forage mainly in the surrounding urban area. *Urban Ecosystems*, *18*, 411–418. https://doi.org/10.1007/s11252-014-0403-y

Garibaldi, L. A., Steffan-Dewenter, I., Winfree, R., Aizen, M. A., Bommarco, R., Cunningham, S. A., Kremen, C., Carvalheiro, L. G., Harder, L. D., Afik, O., Bartomeus, I., Benjamin, F., Boreux, V., Cariveau, D., Chacoff, N. P., Dudenhoffer, J. H., Freitas, B. M., Ghazoul, J., Greenleaf, S., … Klein, A. M. (2013). Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*, *339*(6127), 1608–1611. https://doi.org/10.1126/science.1230200

Gätschenberger, H., Azzami, K., Tautz, J., & Beier, H. (2013). Antibacterial immune competence of honey bees (apis mellifera) is adapted to different life stages and environmental risks. *PLoS ONE*, *8*(6), e66415. https://doi.org/10.1371/journal.pone.0066415

Geist, V. (1987). Bergmann's rule is invalid. *Canadian Journal of Zoology*, *65*(4), 1035–1038. https://doi.org/10.1139/z87-164

George, E. A., Bröger, A., Thamm, M., Brockmann, A., & Scheiner, R. (2020). Inter-individual variation in honey bee dance intensity correlates with expression of the *foraging* gene. *Genes, Brain and Behavior*, *19*(1). https://doi.org/10.1111/gbb.12592

Gérard, M., Vanderplanck, M., Franzen, M., Kuhlmann, M., Potts, S. G., Rasmont, P., Schweiger, O., & Michez, D. (2018). Patterns of size variation in bees at a continental scale: Does Bergmann's rule apply? *Oikos*, *127*(8), 1095–1103. https://doi.org/10.1111/oik.05260

Geslin, B., Gauzens, B., Thébault, E., & Dajoz, I. (2013). Plant pollinator networks along a gradient of urbanisation. *PLoS ONE*, *8*(5), e63421. https://doi.org/10.1371/journal.pone.0063421

Ghosh, S., Jung, C., Choi, K., Choi, H. Y., Kim, H. W., & Kim, S. (2017). Body fatty and amino acid composition of a native bumblebee, Bombus ignitus relative to B. terrestris of foreign origin in Korea. *Journal of Apiculture*, *32*(2), 111–117. https://doi.org/10.17519/apiculture.2017.06.32.2.111

Gil, M., & Farina, W. (2002). Foraging reactivation in the honeybee Apis mellifera L.: Factors affecting the return to known nectar sources. *Naturwissenschaften*, *89*(7), 322–325. https://doi.org/10.1007/s00114-002-0323-1

Giraldeau, L., Valone, T. J., & Templeton, J. J. (2002). Potential disadvantages of using socially acquired information. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *357*(1427), 1559–1566. https://doi.org/10.1098/rstb.2002.1065

Giri, S., & Dillon, M. (2012). Seasonal and altitudial variation in fatty acid composition of native bees. *The UW National Parks Service Research Station Annual Reports*, *35*, 23–30. https://doi.org/10.13001/uwnpsrc.2012.3919

Goines, L., & Hagler, L. (2007). Noise pollution: a modern plague: *Southern Medical Journal*, *100*(3), 287–294. https://doi.org/10.1097/SMJ.0b013e3180318be5

Gostinski, L. F., de Albuquerque, P. M. C., & Contrera, F. A. L. (2017). Effect of honey harvest on the activities of *Melipona (Melikerria) fasciculata* Smith, 1854 workers. *Journal of Apicultural Research*, *56*(4), 319–327. https://doi.org/10.1080/00218839.2017.1329795

Gould, J. L. (1975). Honey Bee Recruitment: The dance-language controversy: Unambiguous experiments show that honey bees use an abstract language for communication. *Science*, *189*(4204), 685–693. https://doi.org/10.1126/science.1154023

Goulson, D., & Nicholls, E. (2022). Anthropogenic influences on bee foraging. *Science*, *375*(6584), 970–972. https://doi.org/10.1126/science.abn0185

Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*, *347*(6229), 1255957. https://doi.org/10.1126/science.1255957

Goulson, D., Peat, J., Stout, J. C., Tucker, J., Darvill, B., Derwent, L. C., & Hughes, W. O. H. (2002). Can alloethism in workers of the bumblebee, Bombus terrestris, be explained in terms of foraging efficiency? *Animal Behaviour*, *64*(1), 123–130. https://doi.org/10.1006/anbe.2002.30

Grab, H., Brokaw, J., Anderson, E., Gedlinske, L., Gibbs, J., Wilson, J., Loeb, G., Isaacs, R., & Poveda, K. (2019). Habitat enhancements rescue bee body size from the negative effects of landscape simplification. *Journal of Applied Ecology*, *56*(9), 2144–2154. https://doi.org/10.1111/1365-2664.13456

Grab, H., Poveda, K., Danforth, B., & Loeb, G. (2018). Landscape context shifts the balance of costs and benefits from wildflower borders on multiple ecosystem services. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1884), 20181102. https://doi.org/10.1098/rspb.2018.1102

Graitson, E., Ursenbacher, S., & Lourdais, O. (2020). Snake conservation in anthropized landscapes: Considering artificial habitats and questioning management of semi-natural habitats. *European Journal of Wildlife Research*, *66*(3), 39. https://doi.org/10.1007/s10344-020-01373-2

Greenleaf, S. S., Williams, N. M., Winfree, R., & Kremen, C. (2007). Bee foraging ranges and their relationship to body size. *Oecologia*, *153*(3), 589–596. https://doi.org/10.1007/s00442-007-0752-9

Grüter, C., Balbuena, M. S., & Farina, W. M. (2008). Informational conflicts created by the waggle dance. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1640), 1321–1327. https://doi.org/10.1098/rspb.2008.0186

Grüter, C., & Farina, W. M. (2009). The honeybee waggle dance: Can we follow the steps? *Trends in Ecology & Evolution*, *24*(5), 242–247. https://doi.org/10.1016/j.tree.2008.12.007

Grüter, C., & Leadbeater, E. (2014). Insights from insects about adaptive social information use. *Trends in Ecology & Evolution*, *29*(3), 177–184. https://doi.org/10.1016/j.tree.2014.01.004

Grüter, C., Menezes, C., Imperatriz-Fonseca, V. L., & Ratnieks, F. L. W. (2012). A morphologically specialized soldier caste improves colony defense in a neotropical eusocial bee. *Proceedings of the National Academy of Sciences*, *109*(4), 1182–1186. https://doi.org/10.1073/pnas.11133981

Grüter, C., & Ratnieks, F. L. W. (2011). Honeybee foragers increase the use of waggle dance information when private information becomes unrewarding. *Animal Behaviour*, *81*(5), 949–954. https://doi.org/10.1016/j.anbehav.2011.01.014

Grüter, C., Segers, F. H. I. D., & Ratnieks, F. L. W. (2013). Social learning strategies in honeybee foragers: Do the costs of using private information affect the use of social information? *Animal Behaviour*, *85*(6), 1443–1449. https://doi.org/10.1016/j.anbehav.2013.03.041

Guzmán-Novoa, E., Eccles, L., Calvete, Y., Mcgowan, J., Kelly, P. G., & Correa-Benítez, A. (2010). *Varroa destructor* is the main culprit for the death and reduced populations of overwintered honey bee (*Apis mellifera*) colonies in Ontario, Canada. *Apidologie*, *41*(4), 443–450. https://doi.org/10.1051/apido/2009076

Hadjitofi, A., & Webb, B. (2024). Dynamic antennal positioning allows honeybee followers to decode the dance. *Current Biology*, *34*(8), 1772-1779.e4. https://doi.org/10.1016/j.cub.2024.02.045

Hall, D. M., Camilo, G. R., Tonietto, R. K., Ollerton, J., Ahrné, K., Arduser, M., Ascher, J. S., Baldock, K. C. R., Fowler, R., Frankie, G., Goulson, D., Gunnarsson, B., Hanley, M. E., Jackson, J. I., Langellotto, G., Lowenstein, D., Minor, E. S., Philpott, S. M., Potts, S. G., … Threlfall, C. G. (2017). The city as a refuge for insect pollinators. *Conservation Biology*, *31*(1), 24–29. https://doi.org/10.1111/cobi.12840

Hammel, B., Vollet-Neto, A., Menezes, C., Nascimento, F. S., Engels, W., & Grüter, C. (2016). Soldiers in a stingless bee: work rate and task repertoire suggest they are an elite force. *The American Naturalist*, *187*(1), 120–129. https://doi.org/10.1086/684192

Hartig F (2022). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.6, <https://CRAN.R-project.org/package=DHARMa>.

He, C., Liu, Z., Tian, J., & Ma, Q. (2014). Urban expansion dynamics and natural habitat loss in China: A multiscale landscape perspective. *Global Change Biology*, *20*(9), 2886–2902. https://doi.org/10.1111/gcb.12553

Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J.-F., Aupinel, P., Aptel, J., Tchamitchian, S., & Decourtye, A. (2012). A common pesticide decreases foraging success and survival in honey bees. *Science*, *336*(6079), 348–350. https://doi.org/10.1126/science.1215039

Higes, M., Martín-Hernández, R., Botías, C., Bailón, E. G., González-Porto, A. V., Barrios, L., del Nozal, M. J., Bernal, J. L., Jiménez, J. J., Palencia, P. G., & Meana, A. (2008). How natural infection by *Nosema ceranae* causes honeybee colony collapse: Natural Nosema ceranae infection. *Environmental Microbiology*, *10*(10), 2659–2669. https://doi.org/10.1111/j.1462-2920.2008.01687.x

Higginson, A. D., & Barnard, C. J. (2004). Accumulating wing damage affects foraging decisions in honeybees (*Apis mellifera* L.). *Ecological Entomology*, *29*(1), 52–59. https://doi.org/10.1111/j.0307-6946.2004.00573.x

Holland, J. M., Douma, J. C., Crowley, L., James, L., Kor, L., Stevenson, D. R. W., & Smith, B. M. (2017). Semi-natural habitats support biological control, pollination and soil conservation in Europe. A review. *Agronomy for Sustainable Development*, *37*(4), 31. https://doi.org/10.1007/s13593-017-0434-x

Holzschuh, A., Steffan-Dewenter, I., & Tscharntke, T. (2008). Agricultural landscapes with organic crops support higher pollinator diversity. Oikos, 117(3), 354-361. https://doi.org/10.1111/j.2007.0030-1299.16303.x

Hölldobler, B., & Wilson, E. O. (1990). *The Ants*. Harvard University Press.

Hooke, R. LeB., & Martín-Duque, J. F. (2012). Land transformation by humans: A review. *GSA Today*, *12*(12), 4–10. https://doi.org/10.1130/GSAT151A.1

Hristov, P., Neov, B., Shumkova, R., & Palova, N. (2020). Significance of Apoidea as main pollinators. ecological and economic impact and implications for human nutrition. *Diversity*, *12*(7), 280. https://doi.org/10.3390/d12070280

Huang, J.H., Jing, X., & Douglas, A. E. (2015). The multi-tasking gut epithelium of insects. *Insect Biochemistry and Molecular Biology*, *67*, 15–20. https://doi.org/10.1016/j.ibmb.2015.05.004

Hung, K.L. J., Kingston, J. M., Albrecht, M., Holway, D. A., & Kohn, J. R. (2018). The worldwide importance of honey bees as pollinators in natural habitats. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1870), 20172140. https://doi.org/10.1098/rspb.2017.2140

Hunter, M. R., & Hunter, M. D. (2008). Designing for conservation of insects in the built environment. *Insect Conservation and Diversity*, *1*(4), 189–196. https://doi.org/10.1111/j.17524598.200-8.00024.x

I'Anson Price, R., Dulex, N., Vial, N., Vincent, C., & Grüter, C. (2019). Honeybees forage more successfully without the "dance language" in challenging environments. *Science Advances*, *5*(2), eaat0450. https://doi.org/10.1126/sciadv.aat0450

I'Anson Price, R., & Grüter, C. (2015). Why, when and where did honey bee dance communication evolve? *Frontiers in Ecology and Evolution*, *3*. https://doi.org/10.3389/fevo.2015.00125

Ilyasov, R. A., Gaifullina, L. R., Saltykova, E. S., Poskryakov, A. V., & Nikolaenko, A. G. (2013). Defensins in the honeybee antiinfectious protection. *Journal of Evolutionary Biochemistry and Physiology*, *49*(1), 1–9. https://doi.org/10.1134/S0022093013010015

Janczyk, A., & Tofilski, A. (2021). Monthly changes in honey bee forewings estimated using geometric morphometrics. *Journal of Apicultural Science*, *65*(1), 139–146. https://doi.org/10.2478/jas-2021-0002

Jandt, J. M., & Dornhaus, A. (2009). Spatial organization and division of labour in the bumblebee Bombus impatiens. *Animal Behaviour*, *77*(3), 641–651. https://doi.org/10.1016/j.anbehav.2008.11.019

Jauker, F., Speckmann, M., & Wolters, V. (2016). Intra-specific body size determines pollination effectiveness. *Basic and Applied Ecology*, *17*(8), 714–719. https://doi.org/10.1016/j.baae.2016.07.004

Johnson, S. A., & Cartar, R. V. (2014). Wing wear, but not asymmetry in wear, affects load-lifting capability in bumble bees *Bombus impatiens*. *Canadian Journal of Zoology*, *92*(3), 179–184. https://doi.org/10.1139/cjz-2013-0229

Kaluza, B. F., Wallace, H., Heard, T. A., Klein, A., & Leonhardt, S. D. (2016). Urban gardens promote bee foraging over natural habitats and plantations. *Ecology and Evolution*, *6*(5), 1304–1316. https://doi.org/10.1002/ece3.1941

Kelemen, E. P., & Rehan, S. M. (2021). Opposing pressures of climate and land-use change on a native bee. *Global Change Biology*, *27*(5), 1017–1026. https://doi.org/10.1111/gcb.15468

Kendall, L. K., Mola, J. M., Portman, Z. M., Cariveau, D. P., Smith, H. G., & Bartomeus, I. (2022). The potential and realized foraging movements of bees are differentially determined by body size and sociality. *Ecology*, *103*(11). https://doi.org/10.1002/ecy.3809

Kerr, W. E., & Hebling, N. J. (1964). Influence of the weight of worker bees on division of labor. *Evolution*, *18*(2), 267–270. https://doi.org/10.1111/j.1558-5646.1964.tb01599.x

Kietzman, P. M., & Visscher, P. K. (2021). The influence of available comb storage space on the performance of honey bee communication signals that regulate foraging. *Apidologie*, *52*(1), 133–140. https://doi.org/10.1007/s13592-020-00803-z

Kim, J. (1999). Influence of resource level on maternal investment in a leaf-cutter bee (Hymenoptera: *Megachilidae*). *Behavioral Ecology*, *10*(5), 552–556. https://doi.org/10.1093/beheco/10.5.552

Kim, J., & Thorp, R. W. (2001). Maternal investment and size-number trade-off in a bee, *Megachile apicalis*, in seasonal environments. *Oecologia*, *126*(3), 451–456. https://doi.org/10.1007/-s004420000540

Kim, Y.G., Lee, J.H., Park, J. G., & Lee, J. (2020). Inhibition of *Candida albicans* and *Staphylococcus aureus* biofilms by centipede oil and linoleic acid. *Biofouling*, *36*(2), 126–137. https://doi.org/10.1080/08927014.2020.1730333

Kleijn, D., Kohler, F., Báldi, A., Batáry, P., Concepción, E. D., Clough, Y., Díaz, M., Gabriel, D., Holzschuh, A., Knop, E., Kovács, A., Marshall, E. J. P., Tscharntke, T., & Verhulst, J. (2009). On the relationship between farmland biodiversity and land-use intensity in Europe.

Proceedings of the Royal Society B: Biological Sciences, *276*(1658), 903–909. https://doi.org/10.1098/rspb.2008.1509

Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L. G., Henry, M., Isaacs, R., Klein, A.-M., Kremen, C., M'Gonigle, L. K., Rader, R., Ricketts, T. H., Williams, N. M., Lee Adamson, N., Ascher, J. S., Báldi, A., Batáry, P., Benjamin, F., Biesmeijer, J. C., Blitzer, E. J., … Potts, S. G. (2015). Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nature Communications*, *6*(1), 7414. https://doi.org/10.1038/ncomms8414

Klein, A.-M., Vaissière, B. E., Cane, J. H., 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: Biological Sciences*, *274*(1608), 303–313. https://doi.org/10.1098/rspb.2006.3721

Knoll, S., Fadda, V., Ahmed, F., & Cappai, M. G. (2024). The nutritional year-cycle of Italian honey bees (*Apis mellifera ligustica*) in a southern temperate climate. *Agriculture*, *14*(5), 730. https://doi.org/10.3390/agriculture14050730

Kremen, C. (2018). The value of pollinator species diversity. *Science*, *359*(6377), 741–742. https://doi.org/10.1126/science.aar7614

Kulhanek, K., Steinhauer, N., Rennich, K., Caron, D., Sagili, R., Pettis, J., Ellis, J., Wilson, M. E., Wilkes, J. T., Tarpy, D., Rose, R., Lee, K., Rangel, J., & vanEngelsdorp, D. (2017). A national survey of managed honey bee 2015–2016 annual colony losses in the USA. *Journal of Apicultural Research*, *56*, 328–340. https://doi.org/10.1080/00218839.2017.1344496

Kuterbach, D. A., Walcott, B., Reeder, R. J., & Frankel, R. B. (1982). Iron-containing cells in the honey bee (*Apis mellifera*). *Science*, *218*(4573), 695–697. https://doi.org/10.1126/science.218.-4573.695

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). **lmerTest** Package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13). https://doi.org/10.18637/jss.v-082.i13

LaBarbera, M. (1989). Analyzing body size as a factor in ecology and evolution. *Annual Review of Ecology and Systematics*, *20*, 97–117.

Landgraf, T., Rojas, R., Nguyen, H., Kriegel, F., & Stettin, K. (2011). Analysis of the waggle dance motion of honeybees for the design of a biomimetic honeybee robot. *PLoS ONE*, *6*(8), e21354. https://doi.org/10.1371/journal.pone.0021354

Larsen, A., Reynaldi, F. J., & Guzmán-Novoa, E. (2019). Bases del sistema inmune de la abeja melífera (Apis mellifera). Revisión. *Revista Mexicana de Ciencias Pecuarias*, *10*(3), 705–728. https://doi.org/10.22319/rmcp.v10i3.4785

Lavine, M. D., & Strand, M. R. (2002). Insect hemocytes and their role in immunity. *Insect Biochemistry and Molecular Biology*, *32*(10), 1295–1309. https://doi.org/10.1016/S09651748-(02)00092-9

Law, J. H., & Wells, M. A. (1989). Insects as biochemical models. *Journal of Biological Chemistry*, *264*(28), 16335–16338. https://doi.org/10.1016/S0021-9258(19)84707-5

Lecocq, A., Kryger, P., Vejsnæs, F., & Jensen, A. B. (2015). Weight watching and the effect of landscape on honeybee colony productivity: investigating the value of colony weight monitoring for the beekeeping industry. *PLoS ONE*, *10*. https://doi.org/10.1371/journal.pone.0132473

Le Conte, Y., & Navajas, M. (2008). Climate change: impact on honey bee populations and diseases. Revue Scientifique et Technique-Office International des Epizooties, 27(2), 499-510.

Lemaitre, B., & Hoffmann, J. (2007). The Host Defense of *Drosophila melanogaster*. *Annual Review of Immunology*, *25*(1), 697–743. https://doi.org/10.1146/annurev.immunol.25.022106.141615

Li, C., Xu, B., Wang, Y., Feng, Q., & Yang, W. (2012). Effects of dietary crude protein levels on development, antioxidant status, and total midgut protease activity of honey bee (*Apis mellifera ligustica*). *Apidologie*, *43*(5), 576–586. https://doi.org/10.1007/s13592-012-0126-0

Liang, H., He, Y., Theodorou, P., & Yang, C. (2023). The effects of urbanization on pollinators and pollination: A meta-analysis. *Ecology Letters*, ele.14277. https://doi.org/10.1111/ele.14277

Lindauer, M. (1955). Schwarmbienen auf Wohnungssuche. *Zeitschrift für Vergleichende Physiologie*, *37*(4), 263–324. https://doi.org/10.1007/BF00303153

Liu, C. L. C., Kuchma, O., & Krutovsky, K. V. (2018). Mixed-species versus monocultures in plantation forestry: Development, benefits, ecosystem services and perspectives for the future. *Global Ecology and Conservation*, *15*, e00419. https://doi.org/10.1016/j.gecco.2018.e00419

Lorenz, S., & Stark, K. (2015). Saving the honeybees in Berlin? A case study of the urban beekeeping boom. *Environmental Sociology*, *1*(2), 116–126. https://doi.org/10.1080/23251042.2015.1008383

Lovell, S. T., & Taylor, J. R. (2013). Supplying urban ecosystem services through multifunctional green infrastructure in the United States. *Landscape Ecology*, *28*(8), 1447–1463. https://doi.org/10.1007/s10980-013-9912-y

Malinowski, R., Lewiński, S., Rybicki, M., Gromny, E., Jenerowicz, M., Krupiński, M., Nowakowski, A., Wojtkowski, C., Krupiński, M., Krätzschmar, E., & Schauer, P. (2020). Automated production of a land cover/use map of europe based on sentinel-2 imagery. *Remote Sensing*, 12(21), 3523. https://doi.org/10.3390/rs12213523

Mandelik, Y., Winfree, R., Neeson, T., & Kremen, C. (2012). Complementary habitat use by wild bees in agro-natural landscapes. *Ecological Applications*, *22*(5), 1535–1546. https://doi.org/10.1890/11-1299.1

Mantilla, B. S., Marchese, L., Casas-Sánchez, A., Dyer, N. A., Ejeh, N., Biran, M., Bringaud, F., Lehane, M. J., Acosta-Serrano, A., & Silber, A. M. (2017). Proline metabolism is essential for *Trypanosoma brucei brucei* survival in the Tsetse Vector. *PLOS Pathogens*, *13*(1), e1006158. https://doi.org/10.1371/journal.ppat.1006158

Marringa, W. J., Krueger, M. J., Burritt, N. L., & Burritt, J. B. (2014). Honey Bee Hemocyte Profiling by Flow Cytometry. *PLoS ONE*, *9*(10), e108486. https://doi.org/10.1371/journal.pone.0108486

Mautz, D. (1971). Der Kommunikationseffekt der Schwänzeltänze bei Apis mellifica carnica (Pollm.). *Zeitschrift fär Vergleichende Physiologie*, *72*(2), 197–220. https://doi.org/10.1007/BF0029782

Mayes, D. M., Bhatta, C. P., Shi, D., Brown, J. C., & Smith, D. R. (2019). Body size influences stingless bee (Hymenoptera: Apidae) communities across a range of deforestation levels in Rondônia, Brazil. *Journal of Insect Science*, *19*(2). https://doi.org/10.1093/jisesa/iez032

Mcdonald, R. I., Kareiva, P., & Forman, R. T. T. (2008). The implications of current and future urbanization for global protected areas and biodiversity conservation. *Biological Conservation*, *141*(6), 1695–1703. https://doi.org/10.1016/j.biocon.2008.04.025

McKinney, M. L. (2008). Effects of urbanization on species richness: A review of plants and animals. *Urban Ecosystems*, *11*(2), 161–176. https://doi.org/10.1007/s11252-007-0045-4

McMenamin, A. J., Daughenbaugh, K. F., Parekh, F., Pizzorno, M. C., & Flenniken, M. L. (2018). Honey bee and bumble bee antiviral defense. *Viruses*, *10*(8), 395. https://doi.org/10.3390/v10080395

Meister, M., Lemaitre, B., & Hoffmann, J. A. (1997). Antimicrobial peptide defense in *Drosophila*. *BioEssays*, *19*(11), 1019–1026. https://doi.org/10.1002/bies.950191112

Menzel, R., Kirbach, A., Haass, W.D., Fischer, B., Fuchs, J., Koblofsky, M., Lehmann, K., Reiter, L., Meyer, H., Nguyen, H., Jones, S., Norton, P., & Greggers, U. (2011). A common frame of reference for learned and communicated vectors in honeybee navigation. *Current Biology*, *21*(8), 645–650. https://doi.org/10.1016/j.cub.2011.02.039

Millard, J., Outhwaite, C. L., Kinnersley, R., Freeman, R., Gregory, R. D., Adedoja, O., Gavini, S., Kioko, E., Kuhlmann, M., Ollerton, J., Ren, Z.-X., & Newbold, T. (2021). Global effects of land-use intensity on local pollinator biodiversity. *Nature Communications*, *12*(1), 2902. https://doi.org/10.1038/s41467-021-23228-3

Morandin, L. A., & Kremen, C. (2013). Hedgerow restoration promotes pollinator populations and exports native bees to adjacent fields. *Ecological Applications*, *23*(4), 829–839. https://doi.org/10.1890/12-1051.1

Moritz, R. F. A., de Miranda, J., Fries, I., Le Conte, Y., Neumann, P., & Paxton, R. J. (2010). Research strategies to improve honeybee health in Europe. *Apidologie*, *41*(3), 227–242. https://doi.org/10.1051/apido/2010010

Mueller, U. G., & Wolf-Mueller, B. (1993). A method for estimating the age of bees: Age-dependent wing wear and coloration in the Wool-Carder beeAnthidium manicatum (hymenoptera: Megachilidae). *Journal of Insect Behavior*, *6*(4), 529–537. https://doi.org/10.1007/BF0104953

Naug, D. (2009). Nutritional stress due to habitat loss may explain recent honeybee colony collapses. *Biological Conservation*, *142*(10), 2369–2372. https://doi.org/10.1016/j.biocon.2009.04.007

Nazzi, F., Brown, S. P., Annoscia, D., Del Piccolo, F., Di Prisco, G., Varricchio, P., Della Vedova, G., Cattonaro, F., Caprio, E., & Pennacchio, F. (2012). Synergistic Parasite-Pathogen Interactions Mediated by Host Immunity Can Drive the Collapse of Honeybee Colonies. *PLoS Pathogens*, *8*(6), e1002735. https://doi.org/10.1371/journal.ppat.1002735

Nazzi, F., & Conte, Y. L. (2016). Ecology of Varroa destructor, the Major Ectoparasite of the Western Honey Bee, Apis mellifera. In *Annual Review of Entomology* (Vol. 61, Issue Volume 61, 2016, pp. 417–432). https://doi.org/10.1146/annurev-ento-010715-023731

Negri, P., Maggi, M., Szawarski, N., Lamattina, L., & Eguaras, M. (2014). *Apis mellifera* haemocytes *in-vitro* , What type of cells are they? Functional analysis before and after pupal metamorphosis. *Journal of Apicultural Research*, *53*(5), 576–589. https://doi.org/10.3896/IBRA.1.53.5.11

Niu, J., Meeus, I., Cappelle, K., Piot, N., & Smagghe, G. (2014). The immune response of the small interfering RNA pathway in the defense against bee viruses. *Current Opinion in Insect Science*, *6*, 22–27. https://doi.org/10.1016/j.cois.2014.09.014

Noirot, C., & Pasteels, J. M. (1987). Ontogenetic development and evolution of the worker caste in termites. *Experientia*, *43*(8), 851–860. https://doi.org/10.1007/BF01951642

Normandin, É., Vereecken, N. J., Buddle, C. M., & Fournier, V. (2017). Taxonomic and functional trait diversity of wild bees in different urban settings. *PeerJ*, *5*, e3051. https://doi.org/10.7717/peerj.3051

Nürnberger, F., Steffan-Dewenter, I., & Härtel, S. (2017). Combined effects of waggle dance communication and landscape heterogeneity on nectar and pollen uptake in honey bee colonies. *PeerJ*, *5*, e3441. https://doi.org/10.7717/peerj.3441

Obregon, D., Guerrero, O. R., Stashenko, E., & Poveda, K. (2021). Natural habitat partially mitigates negative pesticide effects on tropical pollinator communities. *Global Ecology and Conservation*, *28*, e01668. https://doi.org/10.1016/j.gecco.2021.e01668

Ohlinger, B. D., Schürch, R., Silliman, M. R., Steele, T. N., & Couvillon, M. J. (2022). Dance-communicated distances support nectar foraging as a supply-driven system. *Biology Letters*, *18*(8), 20220155. https://doi.org/10.1098/rsbl.2022.0155

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, <https://CRAN.R-project.org/package=vegan>.

Oldroyd, B. P. (2007). What's killing american honey bees? *PLoS Biology*, *5*(6), e168. https://doi.org/10.1371/journal.pbio.0050168

Ollerton, J. (2017). Pollinator diversity: Distribution, ecological function, and conservation. *Annual Review of Ecology, Evolution, and Systematics*, *48*(1), 353–376. https://doi.org/10.1146/annurev-ecolsys-110316-022919

Ollerton, J. (2021). *Pollinators & pollination: Nature and society*. Pelagic Publishing.

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, *120*(3), 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Osorio-Canadas, S., Arnan, X., Rodrigo, A., Torné-Noguera, A., Molowny, R., & Bosch, J. (2016). Body size phenology in a regional bee fauna: A temporal extension of Bergmann's rule. *Ecology Letters*, *19*(12), 1395–1402. https://doi.org/10.1111/ele.12687

Osterman, J., Aizen, M. A., Biesmeijer, J. C., Bosch, J., Howlett, B. G., Inouye, D. W., Jung, C., Martins, D. J., Medel, R., Pauw, A., Seymour, C. L., & Paxton, R. J. (2021). Global trends in the number and diversity of managed pollinator species. *Agriculture, Ecosystems & Environment*, *322*, 107653. https://doi.org/10.1016/j.agee.2021.107653

Otto, C. R. V., Zheng, H., Gallant, A. L., Iovanna, R., Carlson, B. L., Smart, M. D., & Hyberg, S. (2018). Past role and future outlook of the Conservation Reserve Program for supporting honey bees in the Great Plains. *Proceedings of the National Academy of Sciences*, *115*(29), 7629–7634. https://doi.org/10.1073/pnas.1800057115

Outhwaite, C. L., McCann, P., & Newbold, T. (2022). Agriculture and climate change are reshaping insect biodiversity worldwide. *Nature*, *605*(7908), 97–102. https://doi.org/10.1038/s41586-022-04644-x

Pankiw, T., & Page Jr, R. E. (2000). Response thresholds to sucrose predict foraging division of labor in honeybees. *Behavioral Ecology and Sociobiology*, *47*(4), 265–267. https://doi.org/10.1007/s002650050664

Peng, T., Derstroff, D., Maus, L., Bauer, T., & Grüter, C. (2021). Forager age and foraging state, but not cumulative foraging activity, affect biogenic amine receptor gene expression in the honeybee mushroom bodies. *Genes, Brain and Behavior*, *20*(4). https://doi.org/10.1111/gbb.12722

Pereboom, J. J. M., & Biesmeijer, J. C. (2003). Thermal constraints for stingless bee foragers: The importance of body size and coloration. *Oecologia*, *137*(1), 42–50. https://doi.org/10.1007/s00442-003-1324-2

Phiri, B. J., Fèvre, D., & Hidano, A. (2022). Uptrend in global managed honey bee colonies and production based on a six-decade viewpoint, 1961–2017. *Scientific Reports*, *12*(1), 21298. https://doi.org/10.1038/s41598-022-25290-3

Plascencia, M., & Philpott, S. M. (2017). Floral abundance, richness, and spatial distribution drive urban garden bee communities. *Bulletin of Entomological Research*, *107*(5), 658–667. https://doi.org/10.1017/S0007485317000153

Plourde, J. D., Pijanowski, B. C., & Pekin, B. K. (2013). Evidence for increased monoculture cropping in the Central United States. *Agriculture, Ecosystems & Environment*, *165*, 50–59. https://doi.org/10.1016/j.agee.2012.11.011

Porrini, M. P., Sarlo, E. G., Medici, S. K., Garrido, P. M., Porrini, D. P., Damiani, N., & Eguaras, M. J. (2011). *Nosema ceranae* development in *Apis mellifera*: Influence of diet and infective inoculum. *Journal of Apicultural Research*, *50*(1), 35–41. https://doi.org/10.3896/IBRA.1.50.1.04

IPBES(2016). Potts, S. G., Imperatriz-Fonseca, V., & Hien T, N. *The assessment report on pollinators, pollination and food production*. Secretariat of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services.

Potts, S. G., Imperatriz-Fonseca, V., Ngo, H. T., Aizen, M. A., Biesmeijer, J. C., Breeze, T. D., Dicks, L. V., Garibaldi, L. A., Hill, R., Settele, J., & Vanbergen, A. J. (2016). Safeguarding pollinators and their values to human well-being. *Nature*, *540*(7632), 220–229. https://doi.org/10.1038/nature20588

Potts, S. G., Roberts, S. P. M., Dean, R., Marris, G., Brown, M. A., Jones, R., Neumann, P., & Settele, J. (2010). Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research*, *49*(1), 15–22. https://doi.org/10.3896/IBRA.1.49.1.02

Powney, G. D., Carvell, C., Edwards, M., Morris, R. K. A., Roy, H. E., Woodcock, B. A., & Isaac, N. J. B. (2019). Widespread losses of pollinating insects in Britain. *Nature Communications*, *10*(1), 1018. https://doi.org/10.1038/s41467-019-08974-9

Pritchard, D. J. (2016). Grooming by honey bees as a component of varroa resistant behavior. *Journal of Apicultural Research*, *55*(1), 38–48. https://doi.org/10.1080/00218839.2016.1196016

Quezada-Euán, J. J. G., López-Velasco, A., Pérez-Balam, J., Moo-Valle, H., Velazquez-Madrazo, A., & Paxton, R. J. (2011). Body size differs in workers produced across time and is associated with variation in the quantity and composition of larval food in Nannotrigona perilampoides (Hymenoptera, *Meliponini*). *Insectes Sociaux*, *58*(1), 31–38. https://doi.org/10.1007/s00040-010-0113-2

Radmacher, S., & Strohm, E. (2010). Factors affecting offspring body size in the solitary bee *Osmia bicornis* (Hymenoptera, Megachilidae). *Apidologie*, *41*(2), 169–177. https://doi.org/10.1051/apido/2009064

Rahnamaeian, M., Cytryńska, M., Zdybicka-Barabas, A., Dobslaff, K., Wiesner, J., Twyman, R. M., Zuchner, T., Sadd, B. M., Regoes, R. R., Schmid-Hempel, P., & Vilcinskas, A. (2015). Insect antimicrobial peptides show potentiating functional interactions against Gram-negative bacteria. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1806), 20150293. https://doi.org/10.1098/rspb.2015.0293

Ramalho, M., Imperatriz-Fonseca, V. L., & Giannini, T. C. (1998). Within-colony size variation of foragers and pollen load capacity in the stingless bee Melipona quadrifasciata anthidioides Lepeletier (Apidae, Hymenoptera). *Apidologie*, *29*(3), 221–228. https://doi.org/10.1051/apido:19980302

Ramanathan, S., Ravindran, D., Arunachalam, K., & Arumugam, V. R. (2018). Inhibition of quorum sensing-dependent biofilm and virulence genes expression in environmental pathogen Serratia marcescens by petroselinic acid. *Antonie van Leeuwenhoek*, *111*(4), 501–515. https://doi.org/10.1007/s10482-017-0971-y

Reim, T., Thamm, M., Rolke, D., Blenau, W., & Scheiner, R. (2013). Suitability of three common reference genes for quantitative real-time PCR in honey bees. *Apidologie*, *44*(3), 342–350. https://doi.org/10.1007/s13592-012-0184-3

Richardson, R. T., Conflitti, I. M., Labuschagne, R. S., Hoover, S. E., Currie, R. W., Giovenazzo, P., Guarna, M. M., Pernal, S. F., Foster, L. J., & Zayed, A. (2023). Land use changes associated with declining honey bee health across temperate North America. *Environmental Research Letters*, *18*(6), 064042. https://doi.org/10.1088/1748-9326/acd867

Riddell, C., Adams, S., Schmid-Hempel, P., & Mallon, E. B. (2009). Differential Expression of Immune Defences Is Associated with Specific Host-Parasite Interactions in Insects. *PLoS ONE*, *4*(10), e7621. https://doi.org/10.1371/journal.pone.0007621

Rinderer, T. E. (1982). Regulated Nectar Harvesting by the Honeybee. *Journal of Apicultural Research*, *21*(2), 74–87. https://doi.org/10.1080/00218839.1982.11100519

Robertson, B. A., Rehage, J. S., & Sih, A. (2013). Ecological novelty and the emergence of evolutionary traps. *Trends in Ecology & Evolution*, *28*(9), 552–560. https://doi.org/10.1016/j.tree.2013.04.004

Rodewald, A. D., Shustack, D. P., & Jones, T. M. (2011). Dynamic selective environments and evolutionary traps in human-dominated landscapes. *Ecology*, *92*(9), 1781–1788. https://doi.org/10.1890/11-0022.1

Rohrseitz, K., & Tautz, J. (1999). Honey bee dance communication: Waggle run direction coded in antennal contacts? *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, *184*(4), 463–470. https://doi.org/10.1007/s003590050346

Rosumek, F. B., Brückner, A., Blüthgen, N., Menzel, F., & Heethoff, M. (2017). Patterns and dynamics of neutral lipid fatty acids in ants – implications for ecological studies. *Frontiers in Zoology*, *14*(1), 36. https://doi.org/10.1186/s12983-017-0221-1

Roulston, T. H., & Cane, J. H. (2000). The Effect of Diet Breadth and Nesting Ecology on Body Size Variation in Bees (Apiformes). *Journal of the Kansas Entomological Society*, *73*(3), 129–142.

Roulston, T. H., & Cane, J. H. (2002). The effect of pollen protein concentration on body size in the sweat bee Lasioglossum zephyrum (Hymenoptera: Apiformes). *Evolutionary Ecology*, *16*(1), 49–65. https://doi.org/10.1023/A:1016048526475

Roulston, T. H., & Goodell, K. (2011). The Role of Resources and Risks in Regulating Wild Bee Populations. *Annual Review of Entomology*, *56*(1), 293–312. https://doi.org/10.1146/annurev-ento-120709-144802

Ruedenauer, F. A., Raubenheimer, D., Kessner-Beierlein, D., Grund-Mueller, N., Noack, L., Spaethe, J., & Leonhardt, S. D. (2020). Best be(e) on low fat: Linking nutrient perception, regulation and fitness. *Ecology Letters*, *23*(3), 545–554. https://doi.org/10.1111/ele.13454

Rueppell, O., Hayworth, M. K., & Ross, N. P. (2010). Altruistic self-removal of health-compromised honey bee workers from their hive. *Journal of Evolutionary Biology*, *23*(7), 1538–1546. https://doi.org/10.1111/j.1420-9101.2010.02022.x

Ruess, L., & Chamberlain, P. M. (2010). The fat that matters: Soil food web analysis using fatty acids and their carbon stable isotope signature. *Soil Biology and Biochemistry*, *42*(11), 1898–1910. https://doi.org/10.1016/j.soilbio.2010.07.020

Ryabov, E. V., Fannon, J. M., Moore, J. D., Wood, G. R., & Evans, D. J. (2016). The Iflaviruses Sacbrood virus and Deformed wing virus evoke different transcriptional responses in the honeybee which may facilitate their horizontal or vertical transmission. *PeerJ*, *4*, e1591. https://doi.org/10.7717/peerj.1591

Sacktor, B. (1975). Biochemistry of insect flight. *Insect Biochemistry and Function* (pp. 1–88). Springer US. https://doi.org/10.1007/978-1-4899-3204-4_1

Samuelson, A. E., Gill, R. J., & Leadbeater, E. (2020). Urbanisation is associated with reduced Nosema sp. Infection, higher colony strength and higher richness of foraged pollen in honeybees. *Apidologie*, *51*(5), 746–762. https://doi.org/10.1007/s13592-020-00758-1

Samuelson, A. E., Schürch, R., & Leadbeater, E. (2022). Dancing bees evaluate central urban forage resources as superior to agricultural land. *Journal of Applied Ecology*, *59*(1), 79–88. https://doi.org/10.1111/1365-2664.14011

Sanderson, Eric. W., Jaiteh, M., Levy, Marc. A., Redford, Kent. H., Wannebo, Antoinette. V., & Woolmer, G. (2002). The human footprint and the Llst of the wild. *BioScience*, *52*(10), 891–904.

Santana Marques, P., Resende Manna, L., Clara Frauendorf, T., Zandonà, E., Mazzoni, R., & El-Sabaawi, R. (2020). Urbanization can increase the invasive potential of alien species. *Journal of Animal Ecology*, *89*(10), 2345–2355. https://doi.org/10.1111/1365-2656.13293

Sauthier, R., I'Anson Price, R., & Grüter, C. (2017). Worker size in honeybees and its relationship with season and foraging distance. *Apidologie*, *48*(2), 234–246. https://doi.org/10.1007/s13592-016-0468-0

Scheiner, R., Barnert, M., & Erber, J. (2003). Variation in water and sucrose responsiveness during the foraging season affects proboscis extension learning in honey bees. *Apidologie*, *34*(1), 67–72. https://doi.org/10.1051/apido:2002050

Scheiner, R., Page, R. E., & Erber, J. (2004). Sucrose responsiveness and behavioral plasticity in honey bees (Apis mellifera). Apidologie, 35(2), 133-142. https://doi.org/10.1051/apido:2004001

Scheper, J., Bommarco, R., Holzschuh, A., Potts, S. G., Riedinger, V., Roberts, S. P. M., Rundlöf, M., Smith, H. G., Steffan-Dewenter, I., Wickens, J. B., Wickens, V. J., & Kleijn, D. (2015). Local and landscape-level floral resources explain effects of wildflower strips on wild bees across four European countries. *Journal of Applied Ecology*, *52*(5), 1165–1175. https://doi.org/10.1111/1365-2664.12479

Schlaepfer, M. A., Runge, M. C., & Sherman, P. W. (2002). Ecological and evolutionary traps. *Trends in Ecology & Evolution*, *17*(10), 474–480. https://doi.org/10.1016/S0169-5347(02)02580-6

Schlüns, H., & Crozier, R. H. (2007). Relish regulates expression of antimicrobial peptide genes in the honeybee, *Apis mellifera* , shown by RNA interference. *Insect Molecular Biology*, *16*(6), 753–759. https://doi.org/10.1111/j.1365-2583.2007.00768.x

Schmid-Hempel, P., & Wolf, T. (1988). Foraging effort and life span of workers in a social insect. *The Journal of Animal Ecology*, *57*(2), 509. https://doi.org/10.2307/4921

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. *Nature Protocols*, *3*(6), 1101–1108. https://doi.org/10.1038/nprot.2008.73

Schulz, D. J., Huang, Z.-Y., & Robinson, G. E. (1998). Effects of colony food shortage on behavioral development in honey bees. *Behavioral Ecology and Sociobiology*, *42*(5), 295–303. https://doi.org/10.1007/s002650050442

Schulz, D. J., Vermiglio, M. J., Huang, Z. Y., & Robinson, G. E. (2002). Effects of colony food shortage on social interactions in honey bee colonies. *Insectes Sociaux*, *49*(1), 50–55. https://doi.org/10.1007/s00040-002-8279-x

Schürch, R., & Grüter, C. (2014). Dancing Bees Improve Colony Foraging Success as Long-Term Benefits Outweigh Short-Term Costs. *PLoS ONE*, *9*(8), e104660. https://doi.org/10.1371/journal.pone.0104660

Schürch, R., Zwirner, K., Yambrick, B. J., Pirault, T., Wilson, J. M., & Couvillon, M. (2019). Dismantling Babel: Creation of a universal calibration for honey bee waggle dance decoding. *Animal Behaviour*, *150*, 139–145. https://doi.org/10.1016/j.anbehav.2019.01.016

Scofield, S., & Amdam, Gro. V. (2024). *Fat body lipogenic capacity in honey bee workers is affected by age, social role, and dietary protein*. https://doi.org/10.1101/2024.03.24.586478

Seddon, N., Mace, G. M., Naeem, S., Tobias, J. A., Pigot, A. L., Cavanagh, R., Mouillot, D., Vause, J., & Walpole, M. (2016). Biodiversity in the Anthropocene: Prospects and policy. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1844), 20162094. https://doi.org/10.1098/rspb.2016.2094

Seeley, T., Camazine, S., & Sneyd, J. (1991). Collective decision-making in honey bees: How colonies choose among nectar sources. *Behavioral Ecology and Sociobiology*, *28*(4). https://doi.org/10.1007/BF00175101

Seeley, T. D. (1983). Division of labor between scouts and recruits in honeybee foraging. *Behavioral Ecology and Sociobiology*, *12*(3), 253–259. https://doi.org/10.1007/BF00290778

Seeley, T. D. (1986). Social foraging by honeybees: How colonies allocate foragers among patches of flowers. *Behavioral Ecology and Sociobiology*, *19*(5), 343–354. https://doi.org/10.1007/BF00295707

Seeley, T. D., Mikheyev, A. S., & Pagano, G. J. (2000). Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, *186*(9), 813–819. https://doi.org/10.1007/s003590000134

Seeley, T. D., & Visscher, P. K. (1988). Assessing the benefits of cooperation in honeybee foraging: Search costs, forage quality, and competitive ability. *Behavioral Ecology and Sociobiology*, *22*(4), 229–237. https://doi.org/10.1007/BF00299837

Segers, F. H. I. D., von Zuben, L., & Grüter, C. (2016). Local differences in parasitism and competition shape defensive investment in a polymorphic eusocial bee. *Ecology*, *97*(2), 417–426. https://doi.org/10.1890/15-0793.1

Seibold, S., Gossner, M. M., Simons, N. K., Blüthgen, N., Müller, J., Ambarlı, D., Ammer, C., Bauhus, J., Fischer, M., Habel, J. C., Linsenmair, K. E., Nauss, T., Penone, C., Prati, D., Schall, P., Schulze, E.-D., Vogt, J., Wöllauer, S., & Weisser, W. W. (2019). Arthropod decline in grasslands and forests is associated with landscape-level drivers. *Nature*, *574*(7780), 671–674. https://doi.org/10.1038/s41586-019-1684-3

Seto, K. C., Güneralp, B., & Hutyra, L. R. (2012a). Global forecasts of urban expansion to 2030 and direct impacts on biodiversity and carbon pools. *Proceedings of the National Academy of Sciences*, *109*(40), 16083–16088. https://doi.org/10.1073/pnas.1211658109

Seto, K. C., Reenberg, A., Boone, C. G., Fragkias, M., Haase, D., Langanke, T., Marcotullio, P., Munroe, D. K., Olah, B., & Simon, D. (2012b). Urban land teleconnections and sustainability.

Proceedings of the National Academy of Sciences, *109*(20), 7687–7692. https://doi.org/10.1073/pnas.1117622109

Shackleton, K., Balfour, N. J., Al Toufailia, H., James, E., & Ratnieks, F. L. W. (2023). Honey bee waggle dances facilitate shorter foraging distances and increased foraging aggregation. *Animal Behaviour*, *198*, 11–19. https://doi.org/10.1016/j.anbehav.2023.01.009

Shen, M., Yang, X., Cox-Foster, D., & Cui, L. (2005). The role of varroa mites in infections of Kashmir bee virus (KBV) and deformed wing virus (DWV) in honey bees. *Virology*, *342*(1), 141–149. https://doi.org/10.1016/j.virol.2005.07.012

Shiffler, R. E. (1988). Maximum z scores and outliers. *The American Statistician*, *42*(1), 79–80. https://doi.org/10.1080/00031305.1988.10475530

Sibly, R. M., & Atkinson, D. (1994). How rearing temperature affects optimal adult size in ectotherms. Functional Ecology, 486-493. https://doi.org/10.2307/2390073

Sidhu, C. S., & Joshi, N. K. (2016). Establishing wildflower pollinator habitats in agricultural farmland to provide multiple ecosystem services. *Frontiers in Plant Science*, *7*. https://doi.org/10.3389/fpls.2016.00363

Simon-Delso, N., San Martin, G., Bruneau, E., Minsart, L.-A., Mouret, C., & Hautier, L. (2014). Honeybee Colony Disorder in Crop Areas: The role of pesticides and viruses. *PLoS ONE*, *9*(7), e103073. https://doi.org/10.1371/journal.pone.0103073

Simone, M., Evans, J. D., & Spivak, M. (2009). Resin collection and social immunity in honey bees. *Evolution*, *63*(11), 3016–3022. https://doi.org/10.1111/j.1558-5646.2009.00772.x

Skowronek, P., Wójcik, Ł., & Strachecka, A. (2021). Fat body—Multifunctional insect tissue. *Insects*, *12*(6), 547. https://doi.org/10.3390/insects12060547

Smedal, B., Brynem, M., Kreibich, C. D., & Amdam, G. V. (2009). Brood pheromone suppresses physiology of extreme longevity in honeybees (*Apis mellifera*). *Journal of Experimental Biology*, *212*(23), 3795–3801. https://doi.org/10.1242/jeb.035063

Smith, K. M., Loh, E. H., Rostal, M. K., Zambrana-Torrelio, C. M., Mendiola, L., & Daszak, P. (2013). Pathogens, Pests, and Economics: Drivers of Honey Bee Colony Declines and Losses. *EcoHealth*, *10*(4), 434–445. https://doi.org/10.1007/s10393-013-0870-2

Soroye, P., Newbold, T., & Kerr, J. (2020). Climate change contributes to widespread declines among bumble bees across continents. *Science*, *367*(6478), 685–688. https://doi.org/10.1126/science.aax8591

Spaethe, J., & Weidenmüller, A. (2002). Size variation and foraging rate in bumblebees (Bombus terrestris). *Insectes Sociaux*, *49*(2), 142–146. https://doi.org/10.1007/s00040-002-8293-z

St. Clair, A. L., Zhang, G., Dolezal, A. G., O'Neal, M. E., & Toth, A. L. (2020). Diversified Farming in a Monoculture Landscape: Effects on Honey Bee Health and Wild Bee Communities. *Environmental Entomology*, *49*(3), 753–764. https://doi.org/10.1093/ee/nvaa031

Standifer, L. N. (1966). Some lipid constituents of pollens collected by honeybees. *Journal of Apicultural Research*, *5*(2), 93–98. https://doi.org/10.1080/00218839.1966.11100140

Stanley-Samuelson, D. W., Jurenka, R. A., Cripps, C., Blomquist, G. J., & de Renobales, M. (1988). Fatty acids in insects: Composition, metabolism, and biological significance. *Archives of Insect Biochemistry and Physiology*, *9*(1), 1–33. https://doi.org/10.1002/arch.940090102

Starks, P. T., Blackie, C. A., & Seeley, T. D. (2000). Fever in honeybee colonies. *Naturwissenschaften*, *87*(5), 229–231. https://doi.org/10.1007/s001140050709

Steele, J. E. (1982). Glycogen phosphorylase in insects. *Insect Biochemistry*, *12*(2), 131–147. https://doi.org/10.1016/0020-1790(82)90001-4

Steffan-Dewenter, I., & Kuhn, A. (2003). Honeybee foraging in differentially structured landscapes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1515), 569–575. https://doi.org/10.1098/rspb.2002.2292

Steffen, W., Richardson, K., Rockström, J., Cornell, S. E., Fetzer, I., Bennett, E. M., Biggs, R., Carpenter, S. R., de Vries, W., de Wit, C. A., Folke, C., Gerten, D., Heinke, J., Mace, G. M., Persson, L. M., Ramanathan, V., Reyers, B., & Sörlin, S. (2015). Planetary boundaries: Guiding human development on a changing planet. *Science*, *347*(6223), 1259855. https://doi.org/10.11-26/science.1259855

Stevenson, P. C., Bidartondo, M. I., Blackhall-Miles, R., Cavagnaro, T. R., Cooper, A., Geslin, B., Koch, H., Lee, M. A., Moat, J., O'Hanlon, R., Sjöman, H., Sofo, A., Stara, K., & Suz, L. M.

(2020). The state of the world's urban ecosystems: What can we learn from trees, fungi, and bees? *PLANTS, PEOPLE, PLANET*, *2*(5), 482–498. https://doi.org/10.1002/ppp3.10143

Strand, M. R. (2008). The insect cellular immune response. *Insect Science*, *15*(1), 1–14. https://doi.org/-10.1111/j.1744-7917.2008.00183.x

Strand, M. R., & Pech, L. L. (1995). Immunological Basis for Compatibility in Parasitoid-Host Relationships. *Annual Review of Entomology*, *40*(1), 31–56. https://doi.org/10.1146/annurev.en.40.010195.000335

Suarez, R. K., Darveau, C.-A., Welch, K. C., O'Brien, D. M., Roubik, D. W., & Hochachka, P. W. (2005). Energy metabolism in orchid bee flight muscles: Carbohydrate fuels all. *Journal of Experimental Biology*, *208*(18), 3573–3579. https://doi.org/10.1242/jeb.01775

Tafi, E., Nanetti, A., Cilia, G., Bortolotti, L., & Bogo, G. (2024). Pathogens may affect wing morphology in *Apis mellifera* (L.) workers. *Journal of Apicultural Research*, 1–9. https://doi.org/10.1080/00218839.2024.2328472

Tanner, D., & Visscher, K. (2009). Does the body orientation of waggle dance followers affect the accuracy of recruitment? *Apidologie*, *40*(1), 55–62. https://doi.org/10.1051/apido:2008074

Taylor, S., Wakem, M., Dijkman, G., Alsarraj, M., & Nguyen, M. (2010). A practical approach to RT-qPCR—Publishing data that conform to the MIQE guidelines. *Methods*, *50*(4), S1–S5. https://doi.org/10.1016/j.ymeth.2010.01.005

Tew, N. E., Baldock, K. C. R., Vaughan, I. P., Bird, S., & Memmott, J. (2022). Turnover in floral composition explains species diversity and temporal stability in the nectar supply of urban residential gardens. *Journal of Applied Ecology*, *59*(3), 801–811. https://doi.org/10.1111/1365-2664.14094

Tew, N. E., Memmott, J., Vaughan, I. P., Bird, S., Stone, G. N., Potts, S. G., & Baldock, K. C. R. (2021). Quantifying nectar production by flowering plants in urban and rural landscapes. *Journal of Ecology*, *109*(4), 1747–1757. https://doi.org/10.1111/1365-2745.13598

Theodorou, P., Baltz, L. M., Paxton, R. J., & Soro, A. (2021). Urbanization is associated with shifts in bumblebee body size, with cascading effects on pollination. *Evolutionary Applications*, *14*(1), 53–68. https://doi.org/10.1111/eva.13087

Theodorou, P., Radzevičiūtė, R., Lentendu, G., Kahnt, B., Husemann, M., Bleidorn, C., Settele, J., Schweiger, O., Grosse, I., Wubet, T., Murray, T. E., & Paxton, R. J. (2020). Urban areas as hotspots for bees and pollination but not a panacea for all insects. *Nature Communications*, *11*(1), 576. https://doi.org/10.1038/s41467-020-14496-6

Thom, C., Gilley, D. C., Hooper, J., & Esch, H. E. (2007). The scent of the waggle dance. *PLoS Biology*, *5*(9), e228. https://doi.org/10.1371/journal.pbio.0050228

Thompson, H., Overmyer, J., Feken, M., Ruddle, N., Vaughan, S., Scorgie, E., Bocksch, S., & Hill, M. (2019). Thiamethoxam: Long-term effects following honey bee colony-level exposure and implications for risk assessment. *Science of The Total Environment*, *654*, 60–71. https://doi.org/10.1016/j.scitotenv.2018.11.003

Timberlake, T. P., Vaughan, I. P., & Memmott, J. (2019). Phenology of farmland floral resources reveals seasonal gaps in nectar availability for bumblebees. *Journal of Applied Ecology*, *56*(7), 1585–1596. https://doi.org/10.1111/1365-2664.13403

Tommasi, D., Miro, A., Higo, H. A., & Winston, M. L. (2004). Bee diversity and abundance in an urban setting. *The Canadian Entomologist*, *136*(6), 851–869. https://doi.org/10.4039/n04-010

Tosi, S., Nieh, J. C., Sgolastra, F., Cabbri, R., & Medrzycki, P. (2017). Neonicotinoid pesticides and nutritional stress synergistically reduce survival in honey bees. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1869), 20171711. https://doi.org/10.1098/rspb.2017.1711

Toth, A. L., Bilof, K. B. J., Henshaw, M. T., Hunt, J. H., & Robinson, G. E. (2009). Lipid stores, ovary development, and brain gene expression in Polistes metricus females. *Insectes Sociaux*, *56*(1), 77–84. https://doi.org/10.1007/s00040-008-1041-2

Toth, A. L., Kantarovich, S., Meisel, A. F., & Robinson, G. E. (2005). Nutritional status influences socially regulated foraging ontogeny in honey bees. *Journal of Experimental Biology*, *208*(24), 4641–4649. https://doi.org/10.1242/jeb.01956

Toth, A. L., & Robinson, G. E. (2005). Worker nutrition and division of labour in honeybees. *Animal Behaviour*, *69*(2), 427–435. https://doi.org/10.1016/j.anbehav.2004.03.017

Tritschler, M., Vollmann, J. J., Yañez, O., Chejanovsky, N., Crailsheim, K., & Neumann, P. (2017). Protein nutrition governs within-host race of honey bee pathogens. *Scientific Reports*, *7*(1), 14988. https://doi.org/10.1038/s41598-017-15358-w

United Nations. (2019). *Independent Group of Scientists appointed by the Secretary-General, Global Sustainable Development Report 2019: The Future is Now – Science for Achieving Sustainable Development*.

van der Sluijs, J. P., & Vaage, N. S. (2016). Pollinators and global food security: The need for holistic global stewardship. *Food Ethics*, *1*(1), 75–91. https://doi.org/10.1007/s41055-016-0003-z

van der Zee, R., Gray, A., Pisa, L., & de Rijk, T. (2015). An observational study of honey bee colony winter losses and their association with varroa destructor, neonicotinoids and other risk factors. *PLOS ONE*, *10*(7), e0131611. https://doi.org/10.1371/journal.pone.0131611

van der Zee, R., Pisa, L., Andonov, S., Brodschneider, R., Charrière, J.-D., Chlebo, R., Coffey, M. F., Crailsheim, K., Dahle, B., Gajda, A., Gray, A., Drazic, M. M., Higes, M., Kauko, L., Kence, A., Kence, M., Kezic, N., Kiprijanovska, H., Kralj, J., … Wilkins, S. (2012). Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008–9 and 2009–10. *Journal of Apicultural Research*, *51*(1), 100–114. https://doi.org/10.3896/IBRA.1.51.1.12

van Handel, E. (1974). Lipid utilization during sustained flight of moths. *Journal of Insect Physiology*, *20*(12), 2329–2332. https://doi.org/10.1016/0022-1910(74)90020-1

Vance, J. T., & Roberts, S. P. (2014). The effects of artificial wing wear on the flight capacity of the honey bee *Apis mellifera*. *Journal of Insect Physiology*, *65*, 27–36. https://doi.org/10.1016/j.j-insphys.2014.04.003

vanEngelsdorp, D., Hayes, J., Underwood, R. M., & Pettis, J. (2008). A Survey of Honey Bee Colony Losses in the U.S., Fall 2007 to Spring 2008. *PLoS ONE*, *3*(12), e4071. https://doi.org/-10.1371/journal.pone.0004071

vanEngelsdorp, D., & Meixner, M. D. (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–S95. https://doi.org/10.1016/j.jip.2009.06.011

Vanengelsdorp, Underwood,R., Caron,D., & Hayes,J.,Jr.,. (2007). *An estimate of managed colony losses in the winter of 2006-2007: A report commissioned by the Apiary Inspectors of America. 147*(7), 599–603.

Vannette, R. L., Mohamed, A., & Johnson, B. R. (2015). Forager bees (*Apis mellifera*) highly express immune and detoxification genes in tissues associated with nectar processing. *Scientific Reports*, *5*(1), 16224. https://doi.org/10.1038/srep16224

Veiga, J. C., Menezes, C., Venturieri, G. C., & Contrera, F. A. L. (2013). The bigger, the smaller: Relationship between body size and food stores in the stingless bee *Melipona flavolineata*. *Apidologie*, *44*(3), 324–333. https://doi.org/10.1007/s13592-012-0183-4

Venturini, E. M., Drummond, F. A., Hoshide, A. K., Dibble, A. C., & Stack, L. B. (2017). Pollination reservoirs for wild bee habitat enhancement in cropping systems: A review. *Agroecology and Sustainable Food Systems*, *41*(2), 101–142. https://doi.org/10.1080/21683565.2016.1258377

von Frisch, K. (1967). *The Dance Language and Orientation of Bees*. Harvard University Press. https://doi.org/10.4159/harvard.9780674418776

Waddington, K. D. (1989). Implications of variation in worker body size for the honey bee recruitment system. *Journal of Insect Behavior*, *2*(1), 91–103. https://doi.org/10.1007/BF01053620

Wang, H., Smagghe, G., & Meeus, I. (2017). The role of a single gene encoding the Single von Willebrand factor C-domain protein (SVC) in bumblebee immunity extends beyond antiviral defense. *Insect Biochemistry and Molecular Biology*, *91*, 10–20. https://doi.org/10.1016/j.ibmb.2017.10.002

Wang, Q., Ren, M., Liu, X., Xia, H., & Chen, K. (2019). Peptidoglycan recognition proteins in insect immunity. *Molecular Immunology*, *106*, 69–76. https://doi.org/10.1016/j.molimm.2018.12.021

Wario, F., Wild, B., Rojas, R., & Landgraf, T. (2017). Automatic detection and decoding of honey bee waggle dances. *PLOS ONE*, *12*(12), e0188626. https://doi.org/10.1371/journal.pone.0188626

Weeda, E., de Kort, C. A. D., & Th Beenakkers, A. M. (1979). Fuels for energy metabolism in the Colorado potato beetle, *Leptinotarsa decemlineata* Say. *Journal of Insect Physiology*, *25*(12), 951–955. https://doi.org/10.1016/0022-1910(79)90108-2

Wenzel, A., Grass, I., Belavadi, V. V., & Tscharntke, T. (2020). How urbanization is driving pollinator diversity and pollination – A systematic review. *Biological Conservation*, *241*, 108321. https://doi.org/10.1016/j.biocon.2019.108321

Whitehorn, P. R., O'Connor, S., Wackers, F. L., & Goulson, D. (2012). Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science*, *336*(6079), 351–352. https://doi.org/10.1126/science.1215025

Wong, B. B. M., & Candolin, U. (2015). Behavioral responses to changing environments. *Behavioral Ecology*, *26*(3), 665–673. https://doi.org/10.1093/beheco/aru183

Wray, M. K., Klein, B. A., & Seeley, T. D. (2012). Honey bees use social information in waggle dances more fully when foraging errors are more costly. *Behavioral Ecology*, *23*(1), 125–131. https://doi.org/10.1093/beheco/arr165

Wu, Y., Peng, T., Menzel, F., & Grüter, C. (2024). Low food stores affect dance communication and health-related gene expression in honey bees. *Animal Behaviour*, *216*, 131–139. https://doi.org/10.1016/j.anbehav.2024.07.017

Youngsteadt, E., Appler, R. H., López-Uribe, M. M., Tarpy, D. R., & Frank, S. D. (2015). Urbanization increases pathogen pressure on feral and managed honey bees. *PLOS ONE*, *10*(11), e0142031. https://doi.org/10.1371/journal.pone.0142031

Zanni, V., Galbraith, D. A., Annoscia, D., Grozinger, C. M., & Nazzi, F. (2017). Transcriptional signatures of parasitization and markers of colony decline in Varroa-infested honey bees (*Apis mellifera*). *Insect Biochemistry and Molecular Biology*, *87*, 1–13. https://doi.org/10.1016/j.ibmb.2017.06.002

Zuur, Alain F, Elena N. Ieno, Neil J. Walker, Anatoly A. Saveliev, & Graham M. Smith. (2009). *Mixed effects models and extensions in ecology with R* (Vol. 574). New York: springe