Timing of colony phenology and foraging activity in honey bees

Zeitliche Koordination von Koloniephänologie und Sammelaktivität bei Honigbienen

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Table of contents

Affidavit

I hereby confirm that my thesis entitled '*Timing of colony phenology and foraging activity in honey bees*' is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

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Hiermit erkläre ich an Eides statt, die Dissertation „*Timing of colony phenology and foraging activity in honey bees*" eigenständig, d.h. insbesondere selbstständig und ohne Hilfe eines kommerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

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Summary

I. Timing is a crucial feature in organisms that live within a variable and changing environment. Complex mechanisms to measure time are wide-spread and were shown to exist in many taxa. These mechanisms are expected to provide fitness benefits by enabling organisms to anticipate environmental changes and adapt accordingly. However, very few studies have addressed the adaptive value of proper timing. The objective of this PhD-project was to investigate mechanisms and fitness consequences of timing decisions concerning colony phenology and foraging activity in the honey bee (*Apis mellifera*), a social insect species with a high degree of social organization and one of the most important pollinators of wild plants and crops. In chapter II, a study is presented that aimed to identify the consequences of disrupted synchrony between colony phenology and the local environment by manipulating the timing of brood onset after hibernation. In a follow-up experiment, the importance of environmental factors for the timing of brood onset was investigated to assess the potential of climate change to disrupt synchronization of colony phenology (Chapter III). Chapter IV aimed to prove for the first time that honey bees can use interval time-place learning to improve foraging activity in a variable environment. Chapter V investigates the fitness benefits of information exchange between nest mates via waggle dance communication about a resource environment that is heterogeneous in space and time.

II. In the study presented in chapter II, the importance of the timing of brood onset after hibernation as critical point in honey bee colony phenology in temperate zones was investigated. Honey bee colonies were overwintered at two climatically different sites. By translocating colonies from each site to the other in late winter, timing of brood onset was manipulated and consequently colony phenology was desynchronized with the local environment. Delaying colony phenology in respect to the local environment decreased the capability of colonies to exploit the abundant spring bloom. Early brood onset, on the other hand, increased the loads of the brood parasite *Varroa destructor* later in the season with negative impact on colony worker population size. This indicates a timing related trade-off and illustrates the importance of investigating effects of climate change on complex multi-trophic systems. It can be concluded that timing of brood onset in honey bees is an important fitness relevant step for colony phenology that is highly sensitive to climatic conditions in late winter. Further, phenology shifts and mismatches driven by climate change can have severe fitness consequences.

III. In chapter III, I assess the importance of the environmental factors ambient temperature and photoperiod as well as elapsed time on the timing of brood onset. Twenty-four hibernating honey bee colonies were placed into environmental chambers and allocated to different combinations of two temperature regimes and three different light regimes. Brood onset was identified non-invasively by tracking comb temperature within the winter cluster. The experiment revealed that ambient temperature plays a major role in the timing of brood onset, but the response of honey bee colonies to temperature increases is modified by photoperiod. Further, the data indicate the involvement of an internal clock. I conclude that the timing of brood onset is complex but probably highly susceptible to climate change and especially spells of warm weather in winter.

IV. In chapter IV, it was examined if honey bees are capable of interval time-place learning and if this ability improves foraging efficiency in a dynamic resource environment. In a field experiment with artificial feeders, foragers were able to learn time intervals and use this ability to anticipate time periods during which feeders were active. Further, interval time-place learning enabled foragers to increase nectar uptake rates. It was concluded that interval time-place learning can help honey bee foragers to adapt to the complex and variable temporal patterns of floral resource environments.

V. The study presented in chapter V identified the importance of the honey bee waggle dance communication for the spatiotemporal coordination of honey bee foraging activity in resource environments that can vary from day to day. Consequences of disrupting the instructional component of honey bee dance communication were investigated in eight temperate zone landscapes with different levels of spatiotemporal complexity. While nectar uptake of colonies was not affected, waggle dance communication significantly benefitted pollen harvest irrespective of landscape complexity. I suggest that this is explained by the fact that honey bees prefer to forage pollen in semi-natural habitats, which provide diverse resource species but are sparse and presumably hard to find in intensively managed agricultural landscapes. I conclude that waggle dance communication helps to ensure a sufficient and diverse pollen diet which is crucial for honey bee colony health.

VI. In my PhD-project, I could show that honey bee colonies are able to adapt their activities to a seasonally and daily changing environment, which affects resource uptake, colony development, colony health and ultimately colony fitness. Ongoing global change, however, puts timing in honey bee colonies at risk. Climate change has the potential to cause mismatches with the local resource environment. Intensivation of agricultural management with decreased resource diversity and short resource peaks in spring followed by distinctive gaps increases the probability of mismatches. Even the highly efficient foraging system of honey bees might not ensure a sufficiently diverse and healthy diet in such an environment. The global introduction of the parasitic mite *V. destructor* and the increased exposure to pesticides in intensively managed landscapes further degrades honey bee colony health. This might lead to reduced cognitive capabilities in workers and impact the communication and social organization in colonies, thereby undermining the ability of honey bee colonies to adapt to their environment.

Zusammenfassung

I. Zeitliche Koordination ist äußerst wichtig für Organismen, die in einer variablen und sich wandelnden Umwelt leben. Komplexe Mechanismen, die das Messen von Zeit ermöglichen, sind weit verbreitet und wurden bei vielen Taxa aufgezeigt. Es wird generell angenommen, dass diese Mechanismen Fitnessvorteile verschaffen, indem sie es Organismen ermöglichen, Umweltveränderungen vorherzusehen und sich entsprechen anzupassen. Allerdings gibt es bisher nur sehr wenige Studien zum adaptiven Wert einer guten zeitlichen Koordination. Ziel dieses Dissertations-Projekts war es, Mechanismen der zeitlichen Koordination bei Honigbienen (*Apis mellifera*) zu erforschen und deren Bedeutung für die Fitness des Honigbienenvolks zu identifizieren. In Kapitel II präsentiere ich meine Studie über die Konsequenzen eines falsch gewählten Zeitpunkts für den Brutbeginn am Ende des Winters und der daraus folgenden gestörten Synchronisation zwischen der Phänologie von Honigbienenvölkern und der lokalen Umwelt. In einem Folgeexperiment wurde die Bedeutung von Umweltfaktoren für das Timing des Brutbeginns untersucht (Kapitel III). Die Studie in Kapitel IV zielt darauf ab, erstmalig den Beweis zu erbringen, dass Honigbienen das „Intervall time-place learning“, d.h. die Fähigkeit, Zeitintervalle zwischen Ereignissen zu lernen und mit deren räumlichen Lage zu assoziieren, beherrschen und, dass diese Fähigkeit beim Sammeln von Ressourcen vorteilhaft ist. Kapitel V untersucht die Fitnessvorteile, die aus dem Austausch von Informationen über ein raumzeitlich heterogenes Ressourcenumfeld zwischen Stockgenossinnen mit Hilfe des Schwänzeltanzes gezogen werden.

II. In der Studie, die in Kapitel II präsentiert wird, wurde die Bedeutung des Brutbeginns als entscheidender Punkt für die Phänologie von Honigbienenvölkern in den gemäßigten Breiten untersucht. Honigbienenvölker wurden an zwei klimatisch unterschiedlichen Standorten überwintert. Indem ein Teil der Völker im Spätwinter zwischen den Standorten ausgetauscht wurde, wurde deren Brutbeginn manipuliert und dadurch die Phänologie bezüglich der lokalen Umwelt desynchronisiert. Das verzögern der Phänologie der Völker verminderte deren Fähigkeit die üppige Frühjahrsblüte zu nutzen. Ein früher Brutbeginn andererseits erhöhte die Belastung der Völker durch den Brutparasiten *Varroa destructor* im Verlauf der Saison, was sich negativ auf die Menge der Arbeiterinnen im Volk auswirkte. Es gibt also entscheidende gegensätzlich wirkende Faktoren, die den optimalen Zeitpunkt des Brutbeginns bestimmen. Die Studie zeigt zudem warum es wichtig ist, die möglichen Folgen des Klimawandels in einem

multitrophischen System zu betrachten statt sich auf einfache Interaktionen zu beschränken. Man kann allgemein folgern, dass das Timing des Brutbeginns einen bedeutenden fitnessrelevanten Schritt in der Phänologie von Honigbienenvölkern darstellt, der stark von klimatischen Bedingungen im Spätwinter beeinflusst wird. Verschiebungen und Fehlanpassungen des Brutbeginns, und damit der Phänologie, durch den Klimawandel können ernsthafte negative Konsequenzen für die Fitness von Honigbienenvölkern haben.

III. In Kapitel III beleuchte ich die Bedeutung der Umweltfaktoren Umgebungstemperatur und Photoperiode sowie der verstrichenen Zeit auf das Timing des Brutbeginns. Vierundzwanzig überwinternde Honigbienenvölker wurden in Klimakammern untergebracht und auf sechs unterschiedliche Kombinationen von Temperatur- und Lichtregimes verteilt. Der Brutbeginn wurde nicht-invasiv über den Temperaturverlauf auf der Wabe innerhalb der Wintertraube festgestellt. Das Experiment hat gezeigt, dass die Umgebungstemperatur eine entscheidende Rolle beim Timing des Brutbeginns spielt. Allerdings wurde die Reaktion der Völker auf einen Temperaturanstieg vom jeweils vorherrschenden Lichtregime beeinflusst. Zudem deuten die Daten auf die Beteiligung einer inneren Uhr hin. Ich folgere, dass das Timing des Brutbeginns durch ein komplexes System geregelt wird, das wahrscheinlich anfällig für Einflüsse durch den Klimawandel und insbesondere durch Warmwetterphasen im Winter ist.

IV. In Kapitel IV meiner Dissertation wird eine Studie präsentiert, die untersucht ob Bienen die Befähigung zum „Intervall time-place learning“ besitzen und ob diese Fähigkeit die Sammeleffizienz in einem dynamischen Ressourcenumfeld verbessert. In einer Feldstudie mit künstlichen Futterquellen zeigten Sammelbienen, dass sie in der Lage waren, Zeitintervalle zu lernen und das Wissen zu nutzen, um die Zeiten vorherzusehen zu denen die Futterquellen aktiv waren. Dieses Lernverhalten ermöglichte es den Sammelbienen, ihre Nektaraufnahmerate zu steigern. Es wurde gefolgert, dass „Intervall time-place learning“ Sammelbienen dabei helfen kann, sich in einem Blühressourcenumfeld mit komplexen und variablen Zeitmustern zurechtzufinden.

V. Diese Studie, die in Kapitel V präsentiert wird, untersuchte die Bedeutung der Schwänzeltanzkommunikation der Honigbienen für die raumzeitliche Koordination der Sammelaktivität des Volkes innerhalb eines Ressourcenumfelds, das täglich variieren kann. Die Folgen der Störung der instruktiven Komponenten des Schwänzeltanzes wurden in acht unterschiedlich komplex strukturierten Landschaften innerhalb der gemäßigten Breiten ermessen. Während kein Einfluss auf den Nektarsammelerfolg festgestellt werden konnte, wurde jedoch gezeigt, dass der Pollensammelerfolg, unabhängig von der raumzeitlichen Komplexität der Landschaft, stark von der Schwänzeltanzkommunikation profitiert. Der Grund dafür liegt vermutlich darin, dass Honigbienen vorzugsweise Pollen in halbnatürlichen Habitaten sammeln, die eine hohe Ressourcenvielfalt bieten, aber in intensiv agrarwirtschaftlich genutzten Landschaften eher selten und relativ schwer zu finden sind. Die Studie lässt schließen, dass die Schwänzeltanzkommunikation dabei hilft, eine ausreichende und diverse Pollenernährung zu gewährleisten und damit eine große Rolle für die Gesundheit von Honigbienenvölkern spielt.

VI. Ich konnte in meinem Dissertationsprojekt zeigen, dass Honigbienen in der Lage sind ihre Aktivitäten an eine sich jahreszeitlich und täglich verändernde Umwelt anzupassen. Eine gute zeitliche Koordination hat Einfluss auf Sammelerfolg, Volksentwicklung, Gesundheit und letztlich auf die Fitness des Volkes. Allerdings gefährdet der voranschreitende globale Wandel die zeitliche Koordination der Honigbienenvölker. Der Klimawandel hat das Potenzial, zeitliche Anpassungen an die lokale Umwelt zu stören. Die Intensivierung der Landwirtschaft und der damit einhergehende Verlust von Pflanzenvielfalt sowie die kurzen Zeiträume von extrem hohem Ressourcenangebot, gefolgt von einer ausgeprägten Blühlücke, erhöht die Wahrscheinlichkeit, dass zeitlich Fehlanpassungen auftreten. In einer derartigen Umwelt könnte selbst das höchst effiziente Ressourcensammelsystem der Honigbienen nicht mehr genügen, um eine ausreichende, vielfältige und gesunde Ernährung zu gewährleisten. Die globale Verbreitung der parasitischen Varroamilbe durch den Menschen und die erhöhte Belastung durch Pestizide verschlechtert zusätzlich den Gesundheitszustand der Honigbienen. Das wiederum kann sich negativ auf das Lernvermögen und des Weiteren auf die Kommunikation und soziale Organisation der Völker auswirken und dadurch deren Fähigkeit, sich an eine veränderliche Umwelt anzupassen unterwandern.

I. General Introduction

Timing and fitness

Proper timing, or ‘being in the right place (or state) at the right time’, is a crucial feature for fitness in all organisms. Charles Darwin famously defined an individual’s relative fitness by its contribution to the genepool of the next generation, either via its own offspring or by supporting relatives (Darwin 1963). Any successful strategy that improves an individual’s adaption to its environment and thereby its fitness will be favored by evolution and passed on to coming generations. However, the environmental conditions tend not to be stable. Climatic conditions for example can change drastically from favorable to unfavorable within an organism’s live time. Changes of abiotic climatic factors like temperature, humidity and radiation patterns do not only affect organisms directly but will most probably also impact the biotic environment, including resources as well as protagonistic or antagonistic interaction partners. Such a variable environment puts stress on organisms (Harrison 1979). An organism’s capability to synchronize its activities with time patterns of a changing environment determines the chance to find sufficient food, the success of mating and raising brood, the chance to synchronize with mutualistic interaction partners and the likelihood to escape antagonist interactions or harmful environmental events and is therefore critical for the fitness of organisms.

Temporarily adverse conditions force organisms to apply strategies that help to endure or evade these ‘dog times’, while restricting most activities to times when conditions are most advantageous (Gwinner 2012; Heldmaier et al. 2004). That means that organisms are often most active during times of the day or year when resources are relatively abundant and climatic conditions favorable. On the other hand, they decrease activity when resources are scarce or climatic conditions adverse. This is especially obvious in the temperate zones with distinct winter periods. Many organisms enter a state of dormancy in winter and start reproduction in spring, when days become longer and warmer and availability of vital resources is good. This can be observed across many taxa, including plants, invertebrates and vertebrates (Badeck et al. 2004; Denlinger & Lee 1991; Heldmaier et al. 2004).

During periods of adverse conditions, many animals seek out protective shelters, like hibernacula in mammals (Perry 2012) or the often very sophisticated and complex nests in social insects (Jones & Oldroyd 2006). Other organisms, like migratory birds, leave the deteriorating habitats to seek out more favorable sites (Alerstam & Lindström 1990) often to return once previous habitats become suitable again. In addition, many organisms undergo physiological or behavioral changes that enable them to endure the unfavorable conditions (Denlinger & Lee 1991; Heldmaier et al. 2004). These include for example building up internal or external resource storages, entering a state of decreased metabolic rate to reduce resource needs, halting reproduction, or increasing amounts of chemical components in cells that prevent freezing. Most of these strategies cannot be applied *ad hoc*. Instead organisms need time for preparation (Gwinner 2012). For example, a sufficiently long period of extensive foraging activity is needed to build up storages (Körtner & Geiser 2000; Meyer et al. 2016; Seeley & Visscher 1985); hibernacula or nest sites need to be discovered or built and prepared (Jones & Oldroyd 2006); physiological conditions that enable to endure the hardship of long-range migration need to be established (Gwinner 2012); and mating needs to be timed so that offspring can develop and then emerge and grow once resources are abundant and conditions generally favorable enough to ensure survival (Gwinner 2012; Johnson et al. 2017; Visser et al. 2004). In order to start preparations in time, organisms need to be able to anticipate the changes of environmental conditions more or less precisely. Often conditions change in diurnal and seasonal cycles, generally in conjunction with the earth's position relative to the sun, or in monthly cycles, connected to the moons position relative to the earth. Such cyclic events are predictable. Complex internal clocks that allow organisms to measure time and anticipate these cyclic events evolved several times in the history of life and were shown to exists across many taxa (Bell-Pedersen et al. 2005; Rosbash 2009). So far, the circadian clock is the best investigated variant of internal clocks. As the name indicates, circadian clocks have an internal pacemaker with a period of around, but not precisely, 24 h. This rhythm can be kept without any external stimuli and is compensated for temperature effects. External cues, so called Zeitgebers, can then be used to entrain the clock to the actual period of the perceived environment (Dobson 2014). Light and temperature are generally used as Zeitgebers for diurnal oscillations (Johnson et al. 2003). Internal clocks of other than daily rhythms might well be based on altered mechanisms of the circadian clock (Yerushalmi & Green 2009). The wide distribution of these complex but still mechanistically similar systems that allow measurement of time lets us draw conclusions on the importance of timing in organisms and heavily suggests an adaptive value (Yerushalmi & Green 2009). To date chronobiologists have gained a lot of insight into the mechanisms of internal clocks. However, although the significance of proper timing and internal clocks for fitness is generally assumed, there are relatively few studies that actually addressed the adaptive value of timing (O'Donnell et al. 2011). Those studies that investigated effects of a disrupted timing, for example due to consequences of climate change (Both et al. 2006; Inouye 2008; Parmesan 2006; Visser & Both 2005) or manipulations of the internal clock (Yerushalmi & Green 2009), clearly demonstrate the importance of timing and negative consequences of mistiming for fitness.

This PhD-project was part of the collaborative research center ‘Insect timing: mechanisms, plasticity and interactions’ which aimed to investigate the importance of timing and underlying mechanisms in a number of insect species and on many different levels, from the molecular base of internal clocks to the impact of mistiming in food webs.

Honey bees

My PhD-thesis specifically focuses on fitness consequences of timing decisions in Western honey bee (*Apis mellifera* L.) colonies. Honey bees are eusocial insects. That means that they have a complex social organisation with reproductive division of labour (as well as division of labour in other tasks), overlapping generations within the nest, and cooperative caring for the offspring of reproductive animals (Crespi & Yanega 1995; Wilson 1971; Wilson & Hölldobler 2005). The honey bee and its complex social system that makes a huge number of individuals apparently act in harmony for the benefit of the colony has intrigued and fascinated humanity for centuries. At first, the interest was certainly motivated by the name-giving ability of honey bees to produce honey from plant nectar. Honey bees have been kept as livestock in order to harvest honey for at least 8000 years (Roffet-Salque et al. 2015). Honey bees produce and store large amounts of honey to survive periods of low resource availability. The storages allow the honey bee to maintain colonies with a queen and several thousand workers the whole year round and even during temperate zone winters (Seeley & Visscher 1985). Very few other social insects species are able to sustain intact colonies during winter (Jones & Oldroyd 2006). This life-history strategy is highly demanding for the social organisation of honey bees. The colony phenology needs to be adapted to the seasonally changing climatic conditions and resource availability. Further, the colony’s forager force needs to be effectively coordinated to allow for an efficient exploitation of floral resources that vary considerably in quality and quantity not only seasonally but also on a daily basis (Fründ et al. 2011; Miller-Rushing et al. 2010). Honey bees were shown to have a highly developed time memory, which could be a consequence of social organisation and at the same time cause for the success of sociality (Bloch 2010). The combination of a sophisticated social organisation within honey bee colonies, their adaption to a changing environment, and the fact that honey bee colonies can be relatively easily observed and manipulated (Seeley 1995) makes the honey bee a very appealing model organism. Studying the honey bee can help us to understand how proper timing is achieved in complex societies as well as to identify the importance of timing for fitness.

Further, the honey bee is one of the most important pollinator species for crops and wild plants worldwide and is therefore of very high economical and ecological value (Klein et al. 2007; Steffan-Dewenter & Tscharntke 2000). About 37% of the yield of human food crops and 73% of the food crop species depend on honey bee pollination services (Klein et al. 2007). These services are at risk, as this invaluable and fascinating species is declining. Manifestations of recent global change, i.e. the intensivation of agricultural management with more intensive land-use practices and pesticide use, the global spread of invasive species, including the highly relevant honey bee brood parasite *Varroa destructor*, and climate change, have profound negative impacts on honey bees and other pollinators (Potts et al. 2010; Potts et al. 2016). I hypothesize and aim to illustrate in this thesis that many of the negative consequences of global change for honey bee fitness are associated with aspects of timing in colonies and its disruption. Only if we understand how honey bees achieve an optimal timing in respect to a changing environment and know the impact of disrupted timing on the fitness of colonies can we truly assess risks of global change for this important pollinator species and identify ways to mitigate negative consequences.

In my PhD-project I investigated timing in honey bee colonies on two different scales: timing of colony phenology on a seasonal scale as well as timing and coordination of foraging activity on a daily scale.

Timing of colony phenology

Phenology describes recurring events in nature (Badeck et al. 2004). The winter in temperate zone environments, when ambient temperatures are low, photoperiod is short, and resources are generally very scarce or not available at all, is a major driver of phenology (Williams et al. 2015). A good synchronization of phenology with the seasonally changing abiotic and biotic conditions of the environment is critical for organisms (Schenk et al. 2017; van Asch & Visser 2007; Visser et al. 2004). Especially the timing of reproduction is of major importance (Inouye 2008). A mismatch between reproduction and the seasonality of the environment can prevent success in finding mates and in matching the high resource demands with temporal peaks in resource availability.

During winter, when honey bee workers are unable to forage, colonies remain mostly inactive. This strategy is not unlike hibernation in mammals and only possible due to the habit of honey bees to store up large amounts of nectar within their combs during spring and summer (Seeley 1995) and their unique capability of social thermoregulation (Jones & Oldroyd 2006). During cold days, the workers cluster up in the so-called winter cluster, which helps to reduce colony heat loss (Southwick 1985). Within the cluster individual workers actively produce heat by flight muscle shivering to keep the cluster core temperature above ambient temperature and the temperature of the cluster periphery high enough to prevent workers from becoming hypothermic and falling off (Esch 1964; Stabentheiner 2005). This form of active thermoregulation is highly energy demanding (Stabentheiner et al. 2010). Demands of thermoregulation increase even further if colonies rear brood (Fahrenholz et al. 1989; Jones et al. 2004; Kronenberg & Heller 1982). To save resources while foraging is not possible, honey bee colonies refrain from large-scale brood rearing during temperate zone winters. During spring, when resources are most abundant, a high number of workers is bred and colonies also start to rear reproductive animals, i.e. drones and queens (Seeley 1995). The timing of the onset of brood rearing is assumed to be of major importance for colony fitness (Seeley & Visscher 1985). Brood rearing in honey bee colonies already recommences in late winter, before resources are available. This way honey bee colonies are able to build up a strong and rejuvenated worker population, anticipating the availability of mass flowering in spring (Avitabile 1978; Seeley & Visscher 1985). Once brood onset took place energy consumption of colonies increases largely (Seeley & Visscher 1985; Stabentheiner et al. 2010). In case of a premature brood onset this could cause colonies to exhaust resource stocks and disrupt the synchrony between the colony phenology and flower resources. An early onset of brood rearing in colonies due to warmer winter periods may also benefit the reproduction of the honey bee brood parasite *Varroa destructor*. Varroa-mites have a strong effect on colony health and winter mortality (Genersch et al. 2010; Le Conte et al. 2010). A late brood onset, on the other hand, might reduce a colony's capability to exploit the abundant spring bloom and again can have negative effects on colony reproduction

(Seeley & Visscher 1985). Regardless of direction, desynchronization with the local environment can have profound negative consequences for the fitness of honey bee colonies.

In chapter II, the consequences of a mismatch of brood onset with the environment, which can be a consequence of global change, will be further explored. Temperature increase and especially changing winter conditions, caused by global or regional climate changes have been shown to affect the seasonal timing of terrestrial organisms (Parmesan 2006; Williams et al. 2015). Mismatches between interacting species can occur if interaction partners react differently to changing abiotic conditions. Long-term studies indicate that climate warming significantly advances honey bee worker activity which may lead to mismatches with flowering phenology (Gordo & Sanz 2005; Gordo & Sanz 2006). Until now little is known about the effects of late winter conditions on the phenology of honey bee colonies and their parasites. In addition, consequences of phenological mismatches between honey bee colonies and their local environment for resource exploitation are not well-investigated. In this study, we assessed for the first time the role of seasonal timing of brood rearing activity for resource-consumer-antagonist interactions in the Western honey bee by experimentally inducing mismatches between colony phenology and local environmental conditions. We shifted colony phenology by translocating colonies from a cold to a warm location in late winter and *vice versa* (Fig. I.1). During the following growth season, we measured consequences of delayed or advanced phenology on the development of honey bee colonies and the brood parasite *V. destructor*.

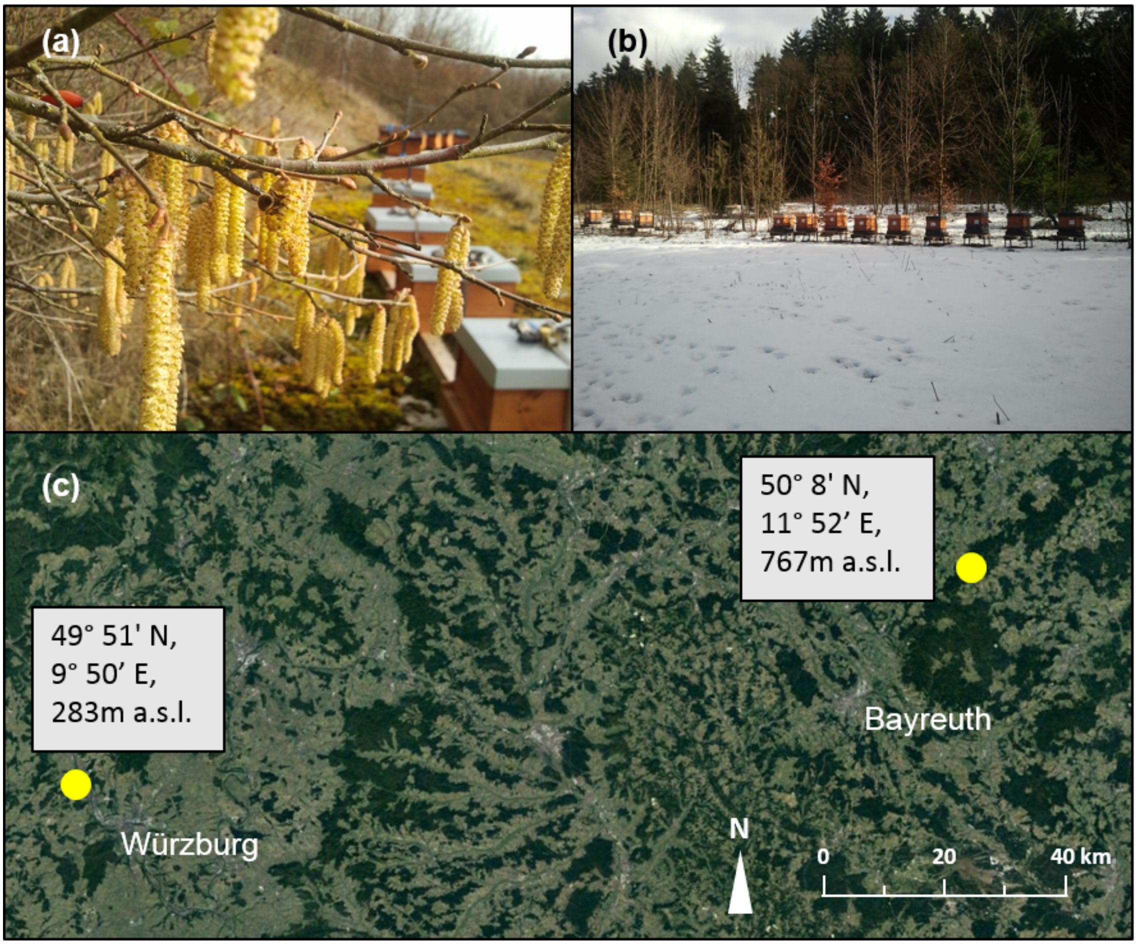

Figure I.1.

Two climatically different study sites were chosen for an experiment on the effects of phenology shifts on honey bee colony fitness. At the lowland site close to Würzburg (a) winters were considerably shorter than at the highland site in the Fichtelgebirge, north-east of Bayreuth (b). By translocating half of the colonies from each site to the other during late winter, brood onset and consequently colony phenology was experimentally shifted in respect to the local environment. (c) Geographical position of the two study sites (source: Google Earth).

Chapter III addresses how honey bee colonies achieve an optimal timing of brood onset and what role environmental factors play as potential cues. Many taxa are known to use ambient temperature and length of photoperiod to time phenological events like emergence after hibernation or reproduction (Bradshaw & Holzapfel 2007; Körtner & Geiser 2000; Visser 2013). These factors are also generally assumed to affect brood rearing activity in hibernating honey bee colonies. Empirical evidence for effects of ambient temperature or photoperiod on brood rearing in winter, however, is still lacking.

In an experimental setup, 24 hibernating honey bee colonies were placed into two environmental chambers (Fig I.2) and equally distributed among six different combinations of temperature and light regimes. The daily temperature variation within the winter clusters was tracked. This allowed us to draw conclusions on the state of brood rearing and how it is affected by ambient temperature and photoperiod.

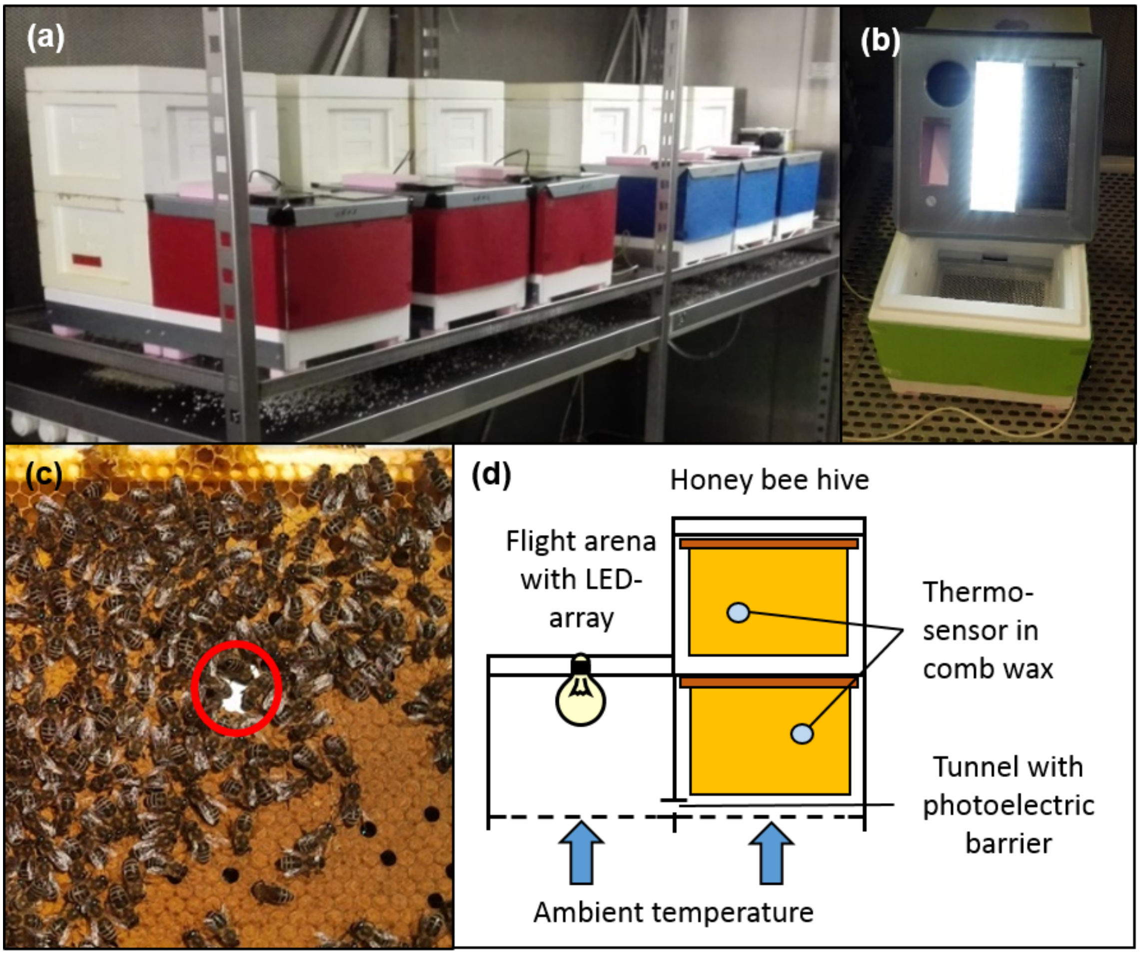

Figure I.2.

Experimental setup to test the effects of environmental factors on timing of honey bee brood onset. (A) Honey bee colonies were placed into experimental hive boxes that were connected to a third box that served as flight arena via a short tunnel. (B) An array of LEDs was installed into each flight arena and allowed to implement individual light regimes for each colony. (C) Thermo-sensors were installed into the comb wax to track temperature within the winter cluster, which allowed to determine the timing of brood onset. (D) Within each hive level thermo-sensors on consecutive combs were installed in alternating order, either between the left and the middle third of the comb or between the right and the middle third of the comb. This pattern was reversed on the other hive level, to maximise the area covered by thermo-sensors. All colonies were placed into two dark environmental chambers. A wire mesh bottom in the flight arena and hive box and metal lid on the flight arena top facilitated temperature exchange through convection and conduction to make sure that the honey bee colonies were not isolated from ambient temperatures.

Coordination of foraging activity in space and time

In addition to the seasonal timing of honey bee colony activity, this PhD-project also aimed to investigate how colonies cope with spatiotemporal heterogeneity of the resource environment on a day-to-day basis. Advertisement of pollen and nectar in flowering plants is often restricted to certain times of the day. Further, opening and closing times of flowers can differ between plant species (Linnaeus 1783). Species specific differences in opening and closing times or flowers even inspired Linné to create a floral clock based on these differences.

An internal representation of the local floral clock would clearly benefit foragers in a resource environment of considerable spatial and temporal heterogeneity. If individual foragers can learn spatiotemporal patterns of floral resources and hence predict periods of resource advertisement in different floral resources at various locations, they can increase their foraging efficiency by concentrating activities on places and times of high resource availability. In fact, the ability to associate fixed times of day with the location of events, i.e. time-place learning, was shown across many taxa and is based on the circadian clock (Mulder et al. 2013; Smarr et al. 2014). However, opening and closing times of flowers are not completely fixed to definite times of the day but variable to a certain degree. Periods of resource advertisement in flowering plants depend on a combination of environmental factors, like light and temperature, endogenous rhythms and biotic interactions with pollinators and can differ from day to day (Fründ et al. 2011; van Doorn & Kamdee 2014). These variable floral rhythms increase the challenges honey bee foragers face and demand for even more sophisticated learning abilities. Vertebrates, that can face similar variable resource environments, were shown not only to be able to associate fixed times of the day with locations of events, but can learn time intervals to anticipate events at defined locations that are variable but linked in time (García-Gallardo & Carpio 2016).

Chapter IV presents an experiment that aimed to investigate if honey bees are also capable of interval time-place learning. In a field experiment, free-flying colonies were trained to three spatially separated feeder locations that were activated at different times of the day but were linked by fixed time intervals between activation of successive feeders. Feeder visitation rates, nectar consumption at feeders and communication about the resource environment within the hive were tracked to identify signs for interval time-place learning in honey bees and associated fitness benefits.

Communication plays a very important role in the coordination of the vast worker force in social insects (Hölldobler & Wilson 2009; Wilson 1971) and is expected to help honey bee colonies to efficiently exploit temporally restricted resources (Seeley 1995). The honey bees in the genus *Apis* have evolved a highly sophisticated device of communication to exchange information about their resource environment. Depending on the current needs of the colony and the relative value of a discovered resource patch, a successful honey bee forager may decide to advertise a discovered resource patch using the so-called waggle dance (Seeley 1995). The waggle dance is a unique recruitment behavior that conveys not only information about the presence and identity of a rewarding resource but also encodes the relatively exact position of the resource patch (Menzel et al. 2011; von Frisch 1967). The dance is performed as a repetitive figure-of-eight taking place on the vertical combs of the dark hive. A dancing forager communicates the distance to a resource patch *via* the duration of the waggle run. This waggle run is a component of the waggle dance during which the dancer moves in a straight line while waggling its abdomen quickly on a plane parallel to the dance floor, before it turns either left or right and circles back to the starting position. The flight angle relative to the sun's current azimuth is encoded as the angle between direction of the waggle run and the opposite vector of gravitation. This direction information is determined via a sun-compass system and the circadian clock of the honey bee worker enables it to compensate the sun-compass for the movement of the sun (von Frisch 1967).

Studies on the Western honey bee revealed that the waggle dance is highly efficient in recruiting foragers to artificial food sources (Sherman & Visscher 2002; von Frisch 1967) and enables honey bee colonies to concentrate their foraging efforts to the most rewarding resources available at the moment (Schmickl & Crailsheim 2004; Seeley 1986; Seeley 1995; Seeley et al. 1991). Continuous information exchange about variable resource patches could increase resource uptake rates of honey bee colonies significantly (Donaldson-Matasci & Dornhaus 2012), and provide fitness advantages (Brown 1988; Dyer 2010; Seeley & Visscher 1988). Nevertheless, the importance of dance communication for an efficient use of resource patches under natural conditions is still unclear. Benefits of waggle dancing for colony fitness were found to be highly dependent on resource density, quality and distribution (Donaldson-Matasci & Dornhaus 2012; Donaldson-Matasci & Dornhaus 2014; Dornhaus & Chittka 1999; Dornhaus & Chittka 2004; Dornhaus et al. 2006; Okada et al. 2012; Sherman & Visscher 2002).

In chapter V of this thesis, I present my studies on fitness benefits provided by the waggle dance as a device to coordinate foraging activities of honey bee colonies in space and time. In a field experiment the information value of dance communication was experimentally manipulated in 24 honey bee colonies. Nectar and pollen uptake rates of colonies with either impaired or intact dance communication were measured in eight different landscapes around Würzburg, Germany, to identify benefits of waggle dance communication in differently structured landscapes (Fig. I.3).

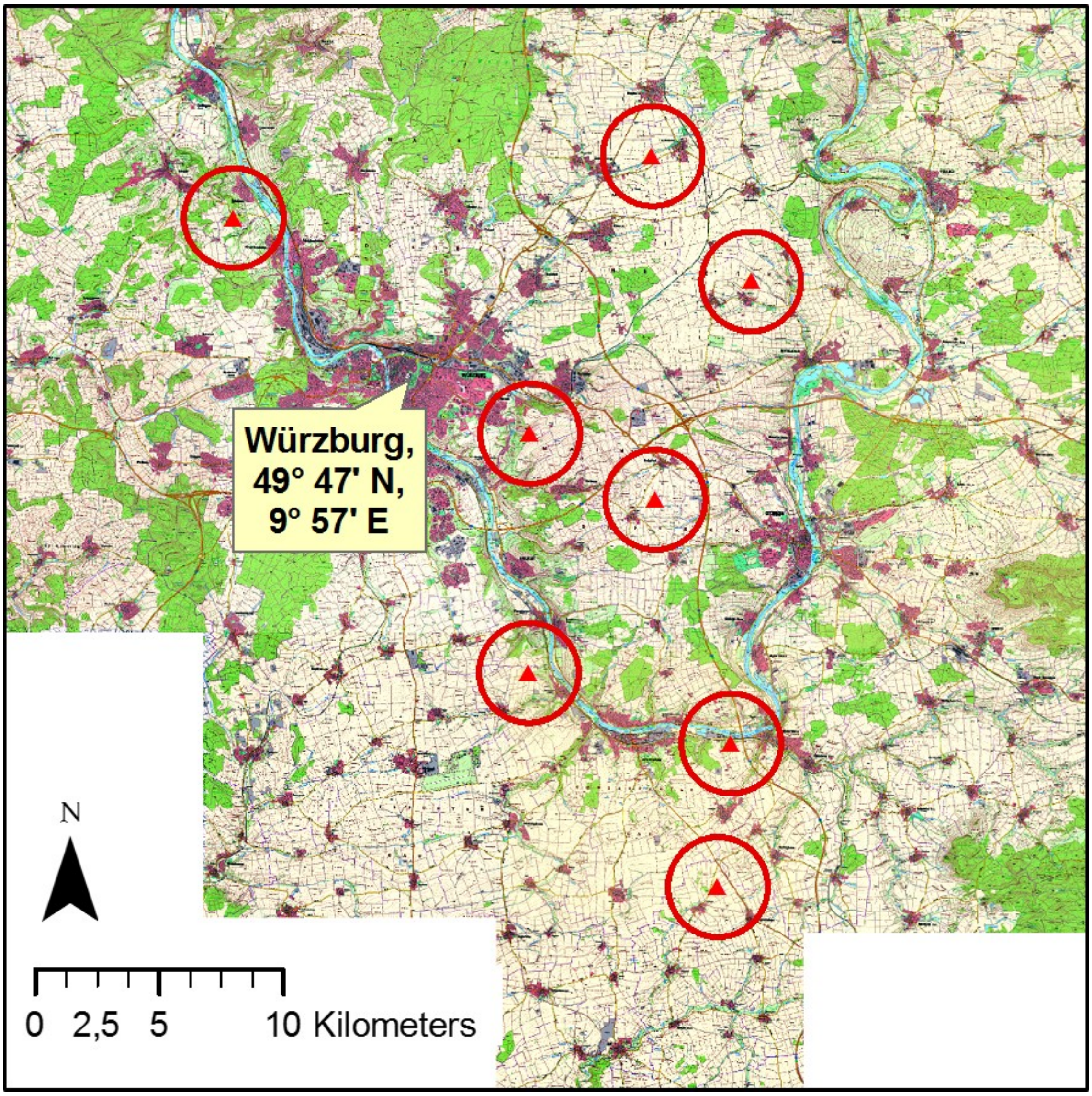

Fig. I.3

Map of study sites around Würzburg, Germany, that were used during the study on benefits of waggle dance communication in honey bee colonies. Chosen sites differed in the complexity of the resource environment within a radius of 2 km. Three experimental colonies were placed for four days at each site and then moved to another site until all colonies were tested at all sites. Each time the waggle dance communication in one randomly chosen colony at each site was disrupted by rotating the colony by 90° so that combs were now in a horizontal position and foragers could not use the gravitational vector to orient their dances.

Methods

All experiments were performed with the Western honey bee *A. mellifera carnica*. Details of bee keeping procedures, study sites, experimental methods, materials and statistical analyses are specific to the individual experiments and are at large described in the respective chapters II, III, IV, and V.

II. Seasonal timing in honey bee colonies: phenology shifts affect foraging success and parasite load

This chapter is submitted as: Nürnberger F, Härtel S, and Steffan-Dewenter I. Seasonal timing in honey bee colonies: phenology shifts affect foraging success and parasite reproduction. *BMC Ecology*

Running title: Seasonal timing affects colony performance

Abstract

Climate change can alter species interactions if trophic levels differ in their phenological response to increasing temperatures or extreme weather events but empirical data are scarce. In temperate regions, more frequent warm periods during winter have the potential to cause mismatches between the phenology of honey bee (*Apis mellifera*) colonies and their floral food resources. Warmer winter periods may also affect colony brood rearing activity and consequently the reproduction of the invasive honey bee brood parasite *Varroa destructor*. Until now little is known about the effects of late winter conditions on the phenology of honey bee colonies and their parasites. In addition, consequences of phenological mismatches between honey bee colonies and their local environment for resource exploitation are not well-investigated. We performed a reciprocal translocation experiment with honey bee colonies to simulate climate change-induced shifts in resource-consumer-antagonist phenologies.

Honey bee brood phenology was highly sensitive to climatic conditions in late winter. Colonies in which phenology was experimentally delayed relative to the local environment had smaller worker populations in early spring and reduced amounts of stored honey later in the season. However, later in the season, the parasite load in colonies with advanced phenology was five times higher than in colonies with delayed and about 50% higher than in colonies with non-shifted phenology. High parasite loads during summer reduced worker population size before the next winter.

Our results indicate a strong timing related trade-off for colony performance. We conclude that brood onset is a crucial step in the seasonal phenology of honey bee colonies, as it affects the capability to exploit floral resources and the growth of parasite populations. In temperate regions, climate change related phenology shifts may challenge the seasonal timing and fitness of honey bees with possible negative consequences for beekeeping and pollination of crops and wild flowers.

Keywords: Climate change, translocation experiment, phenological desynchronization, plant-pollinator interactions, host-parasite interactions, resource-consumer-antagonist phenology, brood rearing activity, *Apis mellifera*, *Varroa destructor*

Introduction

Temperature increase and especially changing winter conditions, caused by global or regional climate changes have been shown to affect the seasonal timing of terrestrial organisms (Parmesan 2006; Williams et al. 2015). Mismatches between interacting species can occur if interaction partners react differently to changing abiotic conditions. This can result in significant fitness losses in one or both interaction partners (Both et al. 2009; Hegland et al. 2009; Parmesan 2006; Visser & Both 2005). The limited empirical evidence for phenology mismatches is based on very few long-term studies on climate warming induced shifts in resource-consumer interactions of birds and pollinators on an evolutionary time-scale (Bartomeus et al. 2011; Visser & Both 2005), whereas data for tri-trophic interactions are mainly lacking. Long-term studies are restricted to already present conditions and models can only reliably interpolate within the boundaries of empirical data. Unlike long-term studies, experimental approaches generally do not work on time scales that allow for evolutionary adaption of organisms, but they make it possible to explore possible future climate scenarios and help to understand the consequences of phenological mismatches in biotic interactions (Alexander et al. 2016; Dawson et al. 2011).

Phenological desynchronization in plant-pollinator systems may cause pollination limitation in plants and reduced survival and reproduction in pollinators (Kudo et al. 2004; Miller-Rushing et al. 2010). So far the few experimental studies are limited to solitary bees (Forrest & Thomson 2011) where mismatches with flowering resources were shown to cause severe fitness losses (Schenk et al. 2017). The social honey bee (*Apis mellifera*) is one of the most important pollinator species for crops and wild plants worldwide (Potts et al. 2010; Potts et al. 2016). Long-term studies indicate that climate warming significantly advances honey bee worker activity which may lead to mismatches with flowering phenology (Gordo & Sanz 2005; Gordo & Sanz 2006). The honey bee maintains colonies with a high number of adult workers the whole year round. Therefore the flight activity of foragers can respond fast and flexible to climatic conditions (Gordo & Sanz 2006). However, honey bee colonies reduce their brood rearing activity or even stop it during winter in temperate regions to save energy while environmental conditions prevent foraging (Avitabile 1978; Bodenheimer 1937; Seeley & Visscher 1985). Brood rearing is resumed in late winter, long before flower resources are available. This way honey bee colonies are able to build up a rejuvenated worker population anticipating the availability of mass flowering in spring (Avitabile 1978; Seeley & Visscher 1985). Once brood onset took place energy consumption of colonies increases largely (Seeley & Visscher 1985). In case of a very early activity this could give rise to exhausted stocks and consequently to a disruption between colony phenology and flower resources.

Honey bees and interacting plant species do not necessarily rely in the same way on environmental factors like temperature and photoperiod to time their seasonal phenology. In changing climates, physiological control mechanisms that evolved to ensure the synchronisation of interacting species can become inadequate (Visser et al. 2004) and may lead to mismatches between colony phenology and floral resource phenology with negative consequences for colony survival and reproduction.

Not only mutualistic but also antagonistic interactions can be altered by global change (Tylianakis et al. 2008). Changing interactions with mutualists or antagonists alter the net-effect of climate change on individual species (Straka & Starzomski 2014; Williams et al. 2015). The relatively recent global spread of the invasive parasitic mite *Varroa destructor* in colonies of *A. mellifera* facilitated by human activity has the potential to alter the consequences of global change related phenology shifts in honey bees. Varroa mites are identified as a main risk for European honey bee stocks (Le Conte et al. 2010) and are in particular responsible for accelerated overwintering mortality (Genersch et al. 2010). Furthermore, varroa is also an important driver of honey bee colony losses in many other parts of the world (Amdam et al. 2004; van Dooremalen et al. 2012; van Engelsdorp et al. 2009). The parasite development in colonies depends directly on brood rearing activity of honey bees (Rosenkranz et al. 2010). Therefore, extended brood rearing activity under warmer climatic conditions could reinforce negative impacts of these important parasites on honey bee colonies.

In this study, we assessed for the first time the role of seasonal timing of brood rearing activity for resource-consumer-antagonist interactions in the Western honey bee by experimentally inducing mismatches between colony phenology and local environmental conditions. We shifted colony phenology by translocating colonies from a cold to a warm location in late winter and *vice versa*. During the following growth season, we measured consequences of delayed or advanced phenology on the development of honey bee colonies and their main parasite *V. destructor.* We hypothesised that (i) delayed brood phenology impairs the capability of colonies to exploit floral resource peaks in spring, with negative impacts on colony development, and (ii) reduces the growth of parasite populations whereas (iii) advanced brood phenology accelerates population growth of parasites, again with ramifications for colony development, and (iv) increases the risk of colonies to starve if environmental climatic conditions do not allow for foraging.

Methods

Study sites and honey bee colonies

The study was performed in Franconia, a region in the northeast of Bavaria, Germany, from 2013 to 2015. Twenty-four equally sized colonies of *A. mellifera carnica* headed by sister-queens from a professional breeder (Schüler, Münster, Germany) in nine-frame hive boxes (Zander measure) were established in 2013 at the apiary of the University of Würzburg, Germany. During September and October 2013, Colonies were adjusted and fed with sugar syrup (Apiinvert, Südzucker) to make sure that they contained approximately the same amounts of workers, pollen stores and honey stores. Colonies were then divided into two random subgroups of twelve and each group was moved to one of two study sites on the 9th of October 2013: site one (referred as lowland site) was situated in the lowland close to Würzburg, Germany, 283m a.s.l. (49° 51' N, 9° 50' E), and site two (referred as highland site) was situated at a fenced experimental site of the Bayreuth Centre of Ecology and Environmental Research in the Fichtelgebirge, 767m a.s.l. (50° 8' N, 11° 52' E). Climate at the lowland site was warmer (mean daily temperatures from December 2013 to March 2014 differed by 3.9°C in mean) and winters were shorter than at the highland site, but there was almost no difference in day length (0.8 minutes at 27th February). During 2014 additional hive boxes with empty comb frames were added to the colonies to make sure that colonies always had sufficient space to build worker and drone combs. Swarming was prevented by removal of all queen cells before they were capped. In total only three queen cells were detected during the experiment. Colonies were treated against varroosis by sprinkling the winter cluster with oxalic acid on 16th December 2013 and 17th December 2014.

Shifting colony phenology

In order to shift colony phenology, including brood onset, in relation to the local environment six randomly selected colonies at each study site were translocated to the other study site in late winter during night-time from 27th to 28th February 2014. By translocating colonies from the warmer site to the colder site, we advanced the phenology of these colonies in relation to the local environment. *Vice versa*, we produced colonies with delayed colony phenology by translocating colonies from the cold to the warm site. Colonies that were not translocated were also moved for at least one hour before putting them back to their previous location to control for possible effects of colony movement. Brood rearing activity in colonies was tracked by nine thermo-sensors (Maxim Integrated DS1921G-F5 Thermochron iButton; 0.5°C resolution) that were embedded into the central wax layer of combs. Day of brood onset of a colony was defined as the first day since the start of hibernation at which temperature on at least one occupied comb remained constantly over 30°C with maximum 1.5°C daily temperature variation (Kronenberg & Heller, 1982).

Colony development

Colony development of all colonies was recorded between 20th March 2014 and 16th October 2014. Every 21 days for a total of eleven times each colony was opened and checked for the presence of the queen. The comb area filled with open and closed worker and male sexual brood cells, honey and pollen, and the number of adult workers were estimated, using the Liebefelder method (Imdorf et al. 1987). All colonies at one site were recorded on the same day and the colonies at the different sites were recorded on consecutive days. Three colonies were lost by natural causes during the first winter at the highland site in the group that was not moved. This may have reduced the power of tests that included this group, but tests with the three remaining colonies were still statistically viable. Further two colonies, one at each site, were lost by vandalism in October 2014. At this point the experiment was terminated.

Flower phenology

Flower phenology in radius of 2 km around each study sites was recorded between 1st April and 14th June 2014, covering the spring bloom in the surrounding of the hives. We selected nine relevant habitat types in the landscapes around each site, based on digital land use data and validated by field inspections: settlements, hedgerows, grassland, meadow orchards, vineyards, rape fields, forest, forest borders and roads and forest plantation areas. Inflorescences of flowering plants were mapped within at least three fixed 100 m² plots of each habitat type at each site and mean flower cover per ha was determined for each habitat type (Scheper et al. 2015). Total area covered by each habitat type was computed for a circle of 2 km diameter around the study sites using a geographical information system (Arc-GIS). The product of mean flower cover per habitat type and total cover of each habitat type were used to calculate total flower cover at each site. In order to keep track of changes in resource distributions over time the mapping of inflorescences and calculation of total flower cover was done every two weeks during the recording period.

Parasite load

V. destructor mite deadfall in debris was monitored for every colony to keep track of parasitic load within the colonies (Calatayud & Verdu 1993). On removable bottom boards, dead mites were collected on paper towels soaked with plant oil to avoid varroa removal by ants. Counts of dead mites took place twelve times between May and July 2014 in intervals of 11-21 days. After each count all debris was removed and paper towels were exchanged. Daily mite deadfall was calculated by dividing the number of dead mites at each count by the number of days that passed since the last count.

Statistics

We used linear mixed-effects models in R Version 3.2.0 (R Core Team 2017) with the package lme4 (Bates et al. 2014) to test for effects of shifting colony phenology on colony development and varroa loads of colonies over the whole observation period. We included identity of colony and time as random term were included in each model to take the time-series character of the data into account. *P*-values, degrees of freedom and *F*-values were obtained using the R-package lmerTest applying the Satterthwaite approximation to compute denominator degrees of freedom (Kuznetsova et al. 2015). Data on brood rearing activity, worker force and nectar storages were square root-transformed and data on varroa mite fall, drone brood area and pollen storage were log-transformed after adding a small value to all data (Berry 1987) to meet the prerequisite of normal distribution for linear models. Model residuals were inspected visually for violation of assumptions of normality and homoscedasticity. Student’s t-test was used to check if means of the different measures of colony development differed significantly between synchronized and shifted colonies at the same site at single time points. Person’s product moment correlation tests were applied to examine how fast changes in flower availability during the season translate to changes in brood rearing activity. We used linear interpolation to match the available data on flower cover to estimates of brood rearing activity with time lags of 0 to 8 weeks. To check if varroa loads of colonies affected development of worker populations, we performed Person’s product moment correlation tests for correlations between relative worker population growth rate from the first to the last estimate and varroa mite fall at different time points. Additionally, we applied a linear model with relative worker population growth rate as dependent variable and varroa mite fall at the time of maximum brood rearing as independent factor.

Results

Brood onset

Brood onset of honey bee colonies was shifted by relocating them in late winter (effect of translocation treatment on day of year of brood onset: $F_{3, 17}=31.53$; $p<0.001$). Brood onset in colonies that were left at the lowland site started 14 days earlier than brood onset in colonies that were left at the highland site. Brood onset in colonies that were relocated from highland to lowland was significantly delayed by 10 days compared to colonies that were left at the lowland site. Relocating colonies from lowland to highland significantly advanced the brood onset by 13 days in comparison to colonies that were overwintered and left at the highland site (Fig. II.1).

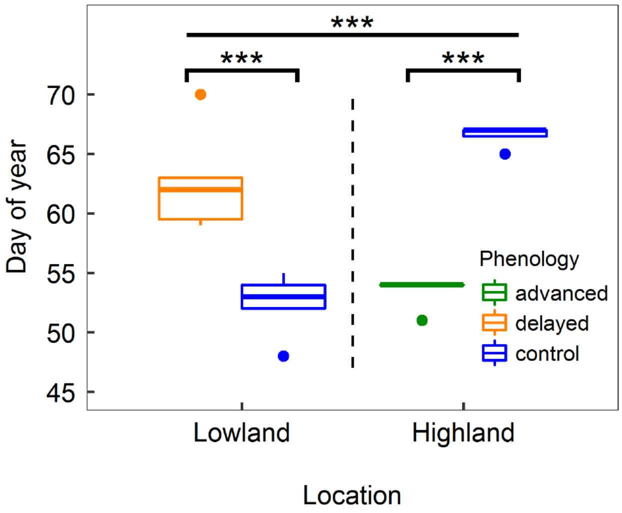

Figure II.1.

Results of experimental shifts of colony phenology. Day of year of brood onset in control colonies that were in synchrony with the respective environment or in which colony phenology was either advanced or delayed by translocating them from the warmer lowland site to the colder highland site or vice versa. Student's t-test and Tukey HSD post-hoc tests were used to check for between group differences. Centre bands show medians; lower and upper box hinges show first and third quartiles; 'whiskers' show highest and lowest data points; dots mark data outliers (>1.5 times the inter-quartile range). ***: $p<0.001$.

Colony development and consequences of phenology-shifts

Worker brood rearing activity

Worker brood rearing activity in lowland site colonies in which phenology was delayed did not differ significantly from that in colonies that were not shifted ($F_{1,\ 11.9}=0.61$; $p=0.451$). Worker brood rearing activity of colonies at the lowland site increased steadily during spring and peaked in the second half of May (Fig. II.2a). Worker brood rearing activity of colonies at the highland site did not differ significantly between shifted colonies with advanced phenology and those in which phenology was not shifted over the course of the year ($F_{1,\ 9.4}=0.001$; $p=0.993$). Worker brood rearing activity of colonies at the highland site increased from March to the beginning of May, declined again during May and then reached its highest rate in mid-June (Fig. II.2b).

Rearing of male sexuals

Male sexuals were mainly reared in June. Rearing of male sexuals started about three weeks later in the colonies at the highland site than in the colonies at the lowland site (Fig. II.2c,d). No male sexuals were reared in any colony after July. Brood area of male sexuals on combs did not differ between colonies with delayed ($F_{1, 21.3}$=3.19; p=0.088) or advanced phenology ($F_{1, 7.0}$=2.27; p=0.176) and control colonies.

Development of the worker population

The development of the worker population size over time in colonies at the lowland site followed a bimodal curve with a steep population rise from April to the second half of May followed by a quick decline in mid-June and another smaller peak between mid-June and mid-November, when worker population sizes were smallest (Fig. II.2e). At the first observation (21st March 2014) worker populations of colonies at the lowland site with delayed phenology were significantly smaller than of control colonies (t(9.95)=-2.54; p=0.030) but this difference vanished within 3 weeks. The mid-June break down of the worker population was significantly less pronounced in colonies in which phenology was delayed than in colonies in which phenology was not shifted (t(7.42)=2.98; p=0.019). When considering the whole observation period mean population size in colonies with advanced phenology did not significantly differ from the mean population size in control colonies ($F_{1, 11.9}$=1.68; p=0.220).

At the highland site, the bimodal shape of the worker population-time curve was less pronounced (Fig. II.2f). The worker populations grew slower and peaked later. Worker population sizes of control colonies did not differ significantly from colonies with advanced or delayed phenology ($F_{1, 9.8}$=0.93; p=0.358).

Honey storage

At the lowland site colony honey storages steeply increased beginning in April and peaked during the second half of May (Fig. II.2g). The effect of shifting colony phenology on honey stores depended on season (interaction: $F_{10, 74.9}$=2.44; p=0.014). Honey storages of colonies at the lowland site did not differ during the beginning or end of the observation. However, during spring colonies with delayed phenology at the lowland site gathered less resources than control colonies. Honey storages in colonies at the highland site strongly increased from beginning of May, peaked around mid-June and slowly decreased during the rest of the year (Fig. II.2h). Colonies at the highland site in which phenology was advanced and control colonies did not differ significantly in their amounts of stored honey ($F_{1,\ 10.5}$=1.29; p=0.281).

Pollen storage

The pollen storage of colonies at the lowland site increased steeply within three weeks from beginning to end of May after which it declined fast (Fig. II.2i). Pollen storages of colonies with delayed phenology did not differ significantly from those of control colonies ($F_{1,\ 12.0}$=0.02; p=0.898). Pollen storages of colonies that were situated at the highland site increased from early May till end of July, interrupted by a short period of decline in June, peaked in July and then decreased steadily (Fig. II.2j). Pollen storages of colonies with advanced phenology did not differ significantly from control colonies ($F_{1,\ 9.8}$=2.45; p=0.149).

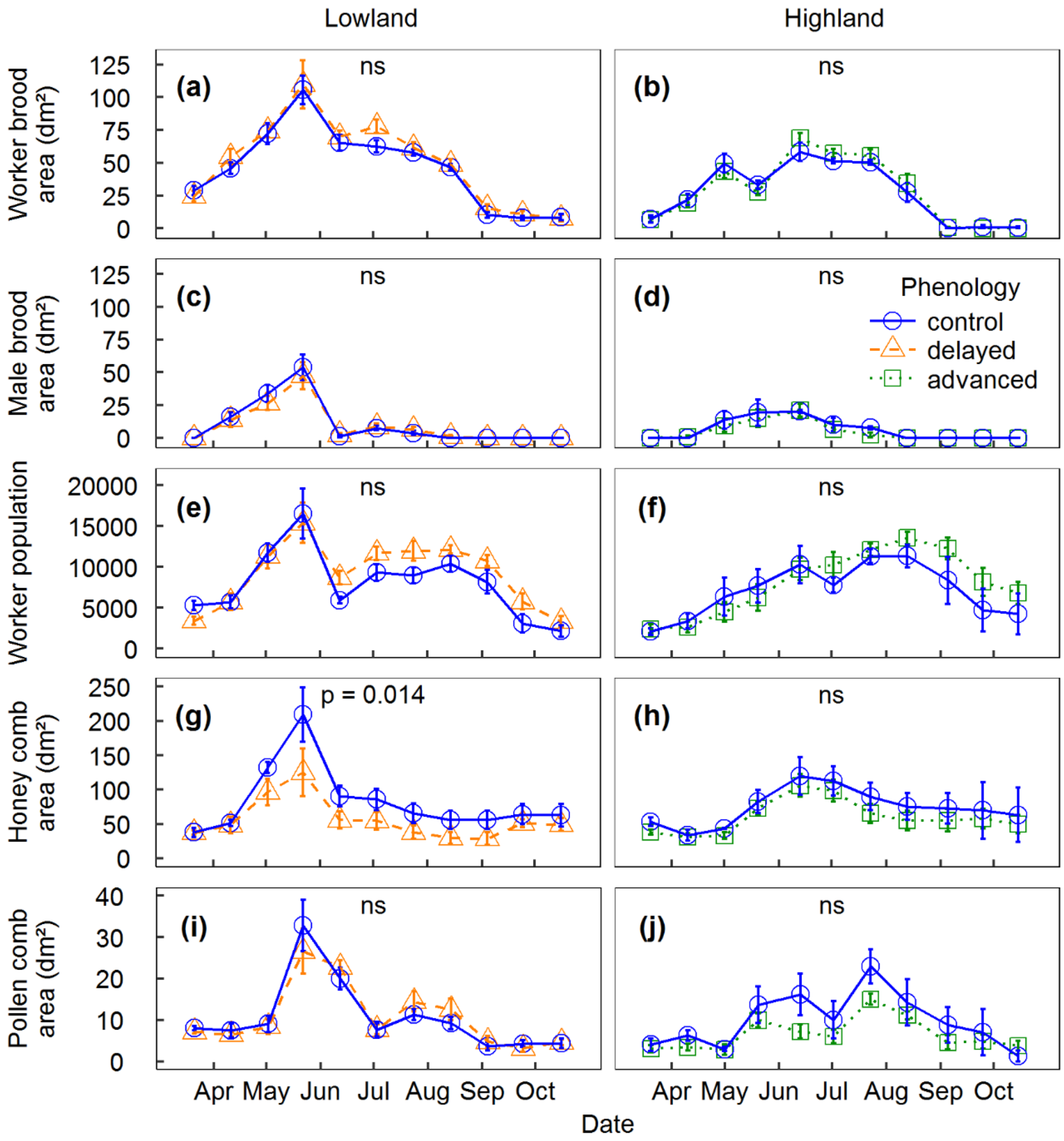

Figure. II.2.

Effects of phenology shifts on development of honey bee colonies. Mean comb area of worker brood (a,b) and male sexuals brood (c,d), number of workers (e,f), comb area of honey storages (g,h) and comb area of pollen storages (i,j) in honey bee colonies from March to October 2014 (± s.e.). The left column shows colonies that were kept at a warm lowland site close to Würzburg, Germany, with colony phenology being either not shifted (control) or in which phenology was delayed. The right column shows colonies that were kept at a colder highland site in the Fichtelgebirge, Germany, with phenology being either not shifted or advanced. *P*-values of the effect of phenology shifts on the respective dependent variable derived from linear mixed-effect models are shown.

Flower phenology

Spring flower abundance in 2 km around the apiary at the lowland site was considerably higher and peaked about two weeks earlier than at the highland site (Fig. II.3). Overall spring bloom at the lowland site lasted about 4 weeks and was more condensed than the bloom at the highland site which was more constant throughout the season. Flower abundance at both sites was positively correlated with worker brood output of honey bee colonies at the respective sites 5 to 6 weeks later (Fig. II.4a,b,c).

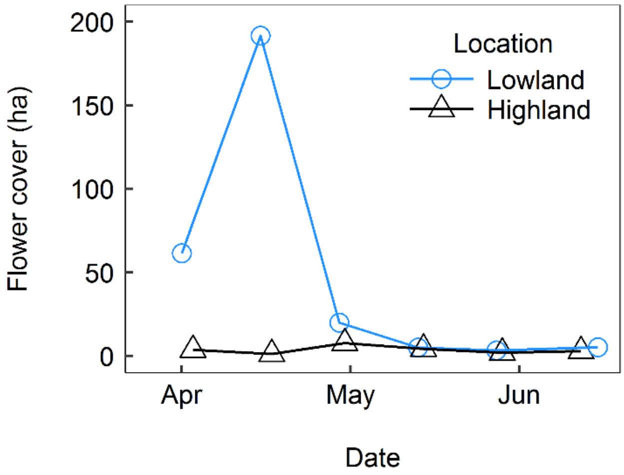

Figure II.3.

Change of flower availability over time. Total flower cover estimated within 2 km of the apiaries at the lowland site and the highland site from April to June 2014.

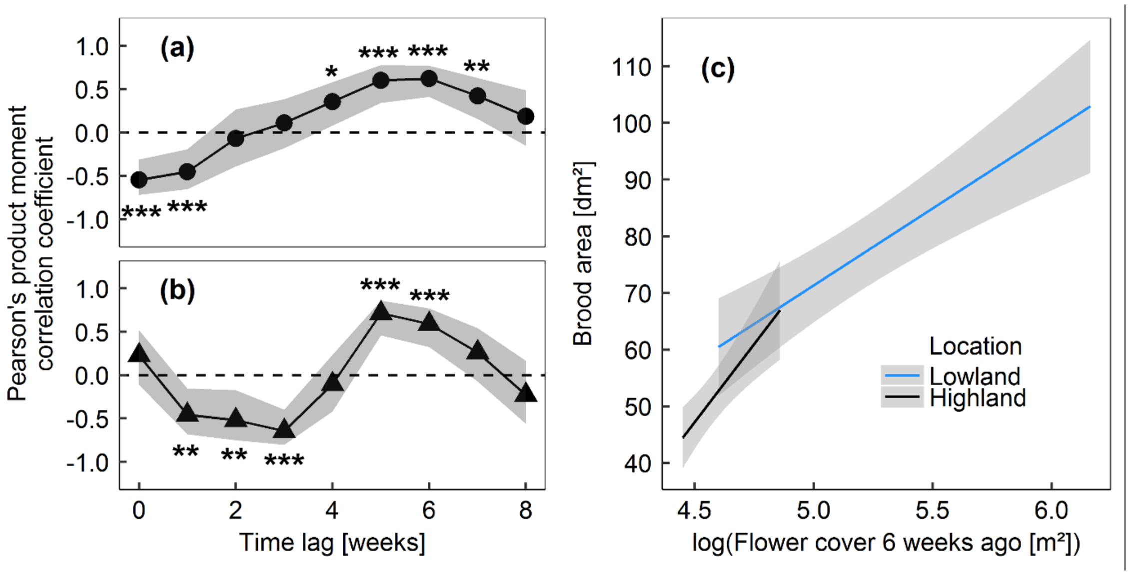

Figure. II.4.

Correlation between flower availability and colony worker brood output. Pearson's product moment correlation coefficients for correlation between total flower cover estimated within 2 km and colony worker brood output at (a) the lowland site and (b) the highland site. Pearson's product moment correlation coefficients were calculated for time lags between resource availability and worker brood output of 0 to 8 weeks. (c) Effect of total flower cover 6 weeks ago on worker brood output in honey bee colonies that were kept at the lowland site or at the highland site. Regression lines fitted with linear model. Grey area: 95% confidence interval. *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$.

Varroa mite deadfall in honey bee colonies

Natural deadfall of *V. destructor*, a common estimator for varroa loads, increased exponentially in all colonies over the observation period (Fig. II.5a,b). At the lowland site the deadfall of varroa mites increased faster in control colonies than in colonies with delayed phenology ($F_{1, 13.7}=5.74$; $p=0.031$). Across all dates, mite deadfall in colonies at the highland site did not differ significantly between colonies in which phenology was advanced and control colonies ($F_{1, 9.6}=0.23$; $p=0.642$).

At both sites colonies with high natural varroa deadfall during the time period of highest reproductive output in colonies also showed significantly reduced net growth rates of colony worker population between the first observation in March and the last observation at the end of the season in October (lowland site: $F_{1,7}$=18.11; p=0.004; highland site: $F_{1,6}$=13.30; p=0.011; Fig. II.6a,b,c).

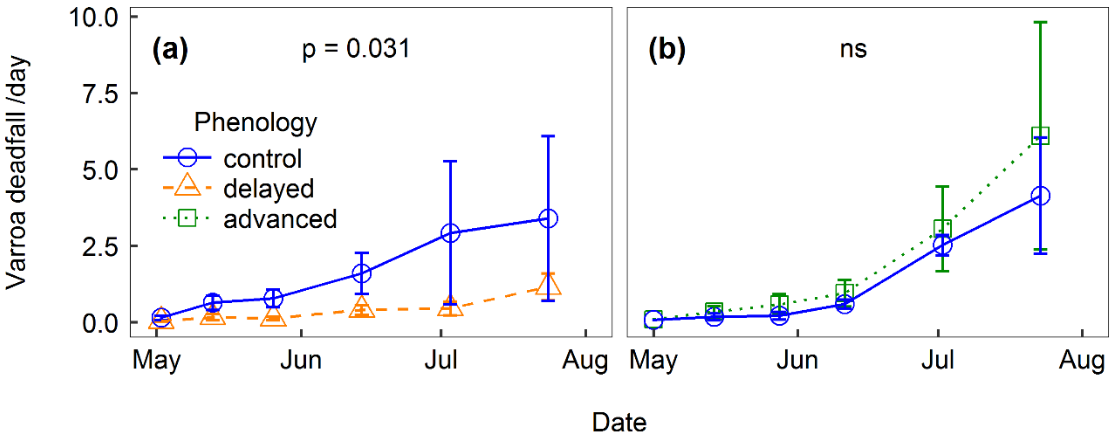

Figure. II.5.

Effects of phenology shifts on varroa loads of honey bee colonies. Varroa mite deadfall in debris of (a) colonies that were kept at the lowland site with phenology being either not shifted (control) or in which phenology was delayed and (b) within colonies that were kept at the highland site with phenology being either not shifted or in which phenology was advanced. May to July 2014. *P*-value of the effect of phenology shifts on varroa mite deadfall derived from linear mixed-effect models are shown.

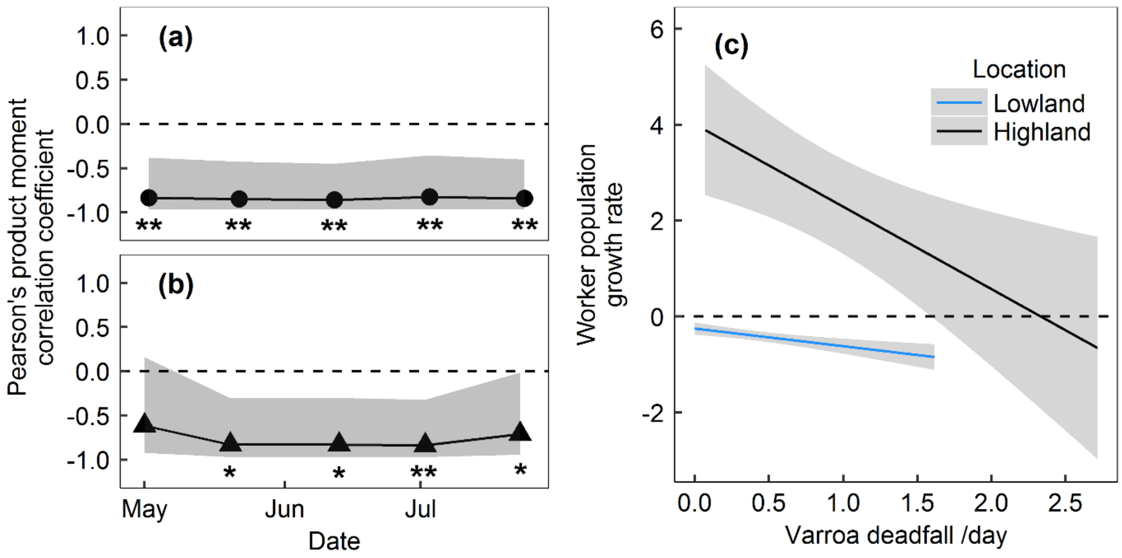

Figure II.6.

Effect of varroa mite infestation on honey bee colony growth. Pearson's product moment correlation coefficients for correlation between varroa mite infestation (measured as daily mite deadfall) during different times of the season and worker population growth rate of honey bee colonies at (a) the lowland site and (b) the highland site from March to November 2014. (c) Effect of varroa mite infestation in colonies at the lowland site and highland site during times of highest worker brood output on worker population growth rate of honey bee colonies from March to November 2014. Grey area: 95% confidence interval. *: $p \leq 0.05$; **: $p \leq 0.01$.

Discussion

Using an experimental approach, we could show that late winter conditions have a strong effect on the timing of brood onset after hibernation and therefore colony phenology of *Apis mellifera*. Further, our results demonstrate that shifting colony phenology relative to the local environment can have profound negative consequences for colony development, foraging success, and parasite pressure.

We manipulated resource-consumer-antagonist phenologies, by translocating honey bee colonies from a warm to a cold site and *vice versa* in late winter. We successfully manipulated brood onset and shifted colony phenology in respect to the environment. We created scenarios of strongly advanced or delayed brood onset leading to substantial mismatches between colony phenology and environmental conditions. Our experimental approach enabled us to empirically assess the vulnerability and resilience of honey bee colonies to climate change related phenology shifts and mismatches. This would not have been possible by extrapolation from long-term studies, that are limited to current conditions (Alexander et al. 2016). It is important to bear in mind that the experimental approach could not account for evolutionary adaption to changing environments over larger time scales, but only for phenotypic plasticity within the used honey bee strain. A comparable approach was previously used to assess the effects of novel climatic conditions on plant communities and solitary bees along elevational gradients (Alexander et al. 2015; Forrest & Thomson 2011) and butterfly species along geographic ranges (Pelini et al. 2009). However, to the best of our knowledge this study is first using a translocation approach for a multi-trophic plant-pollinator-parasite system. The development of honey bee worker populations and colony reproduction strongly rely on timing of brood onset (Seeley & Visscher 1985). We were able to show that delaying colony phenology resulted in decreased numbers of workers, but only for a brief period early in the season. This had a surprisingly strong and long lasting negative effect on honey stores during the season, which are highly relevant for colony survival (Seeley & Visscher 1985). This effect might have been caused by a less smooth turnover from long-living winter bees to more productive but short-living summer bees. The simulated delayed phenology scenario could occur, if changing weather conditions, with more frequent extreme (warm or cold) weather events, affect honey bee and plant phenology in different and no longer adequate ways (Visser et al. 2004). Adverse weather conditions in early spring have the potential to prevent foraging and hamper colony growth but not spring flower bloom. We found no effect of advancing colony phenology on the development of colonies. While our data indicate that brood onset seems to be irreversible, the ability to store resources that ensure survival when environmental conditions prevent foraging presumably buffers honey bee colonies against negative impacts of a premature brood onset to a certain degree. However, this depends on the foraging success during the previous season (Seeley & Visscher 1985). As long as resource storages are sufficient, honey bees may be relatively well adapted to the scenario of advanced phenology. It is not uncommon that spells of warm weather trigger brood onset early in winter. We are not aware of any comparable study on effects of advancing brood onset and colony phenology.

Spring bloom at both study sites was dominated by the bloom of the mass-flowering crop *Brassica napus.* It lasted only for a brief period, which is typical for intensively managed agricultural landscapes with a high amount of mass-flowering crop fields (Danner et al.

2014; Danner et al. 2016; Requier et al. 2015). Resource input is probably a main driver of colony brood rearing activity and thus colony development during the season (Keller et al. 2005). In our study, resource availability correlated with brood rearing activity, but with a time lag of five to six weeks. As a result, worker populations of honey bee colonies in both climates peaked several weeks after the peak of flower availability, when flowering resources were already scarce again. Honey bee colony phenology is probably not well adapted to the flowering phenology in intensively managed agricultural landscape and the synchrony between worker populations and depending mass-flowering crops already seems to be poor. This limits the capability to exploit this resource bonanza sustainably. Narrow bell-shaped abundance curves, like the one we observed especially at the lowland site, are known to increase the chances for significant mismatches between interaction partners (Durant et al. 2007; Miller-Rushing et al. 2010). Therefore honey bee colonies in landscapes with floral resource peaks in spring might be especially vulnerable to further seasonal decoupling (Singer & Parmesan 2010).

Not only bottom-up but also top-down control may influence phenology of species (Both et al. 2009). In our experiment, advanced honey bee colony phenology resulted in a 50% increase of loads of the invasive brood parasite *Varroa destructor* compared to control colonies and even five-times higher varroa numbers compared to colonies with delayed brood phenology later in the season. High varroa loads in late summer have profound consequences on winter survival of honey bee colonies in temperate zones (Amdam et al. 2004; van Dooremalen et al. 2012; van Engelsdorp et al. 2009). We could show, that high varroa loads in colonies at times of high brood rearing activity had a negative impact on worker population growth during the season. This was probably due to decreased worker longevity (Amdam et al. 2004). Decreased worker population size is associated with reduced colony survival during the following winter (Requier et al. 2016). This indicates that there is a strong trade-off between the necessity of an early brood onset to synchronize and keep up with advancing flower phenology and negative effects of increased brood parasite pressure.

Conclusions

Our study illustrates the necessity to consider not only simple two-species interactions but complex multi-trophic systems, including interactions with both mutualists and antagonists, to assess fitness consequences of phenology shifts due to climate change scenarios. We also demonstrate that the anthropogenic introduction of a new parasite, a common result of globalisation, imposes strong trade-offs with impacts on the fitness consequences of timing decisions and phenology shifts in one of the globally most relevant bee pollinator species. Different consequences of global change, like mismatches with mutualistic interaction partners, increasing parasite pressure and introduction of alien species, may have synergistic negative effects on pollinators (Didham et al. 2007; González-Varo et al. 2013; Potts et al. 2010; Schweiger et al. 2010). The honey bee provides a good example of how global change might affect seasonal timing decisions of a pollinator and how several conflicting pressures within a resource-consumer-antagonist system are shaping the consequences of these decisions. The trade-off between advantages of early versus late brood onset that our study revealed might constrain the degree of synchrony between honey bee phenology and flowering phenology. The honey bee might not be able bridge contrasting adaptive requirements under ongoing climate change. We conclude that climate change related phenology shifts can reduce the fitness and survival rates of honey bee colonies with possible negative consequences for beekeeping and the provision of highly valuable pollination services.

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III. The influence of temperature and photoperiod on thermoregulation and brood onset in hibernating honey bee colonies

This chapter is prepared for submission as: Nürnberger F, Härtel S, and Steffan-Dewenter I. The influence of temperature and photoperiod on thermoregulation and brood onset in honey bee winter clusters.

Running title: Environmental conditions affect winter cluster activity

Abstract

For hibernating honey bee colonies (*Apis mellifera*), the onset of brood rearing is a crucial timing decision because brood rearing is accompanied by energy demanding thermoregulation. Colonies must reassume brood rearing already during the winter to build up a sufficient worker force that allows them to exploit floral resources in spring. Ongoing global climate change has the potential to cause a mistiming of brood onset, if temperature is the main driver of brood activity without modulation by photoperiod. This could lead to an early depletion of energy reservoirs and starvation.

To study the unknown importance of ambient temperature and photoperiod as potential regulating factors for brood rearing activity in hibernating colonies, we overwintered 24 honey bee colonies within environmental chambers. The colonies were assigned to two different temperature treatments and three different photoperiod treatments in a way that allowed us to evaluate the individual and interacting effects of temperature and photoperiod. Tracking in-hive temperature as indicator for brood rearing activity revealed that increasing ambient temperature triggered brood onset. Under cold conditions, photoperiod alone did not affect brood onset, but the light regime altered the impact of higher ambient temperature on brood rearing activity. Further the probability of brood rearing also increased with elapsed time indicating a role of an internal clock. We conclude that timing of brood onset is mainly driven by temperature but modulated by day length. Climate warming might change the interplay of these factors and result in mismatches of brood phenology and environmental conditions.

Keywords: phenology, brood rearing activity, *Apis mellifera*, climate change

Introduction

The timing of life-history events, such as flowering in plants, insect emergence, and reproduction, in respect to the changing abiotic and biotic conditions of the environment is critical for most organisms (van Asch & Visser 2007; Visser et al. 2004). In temperate regions, environmental conditions during winter are important drivers of phenology (Williams et al. 2015). During winter organisms need to cope with low temperature conditions and often drastically reduced resource availability. Most ectotherms hibernate in a state of dormancy at different stages of development. Endothermic mammals generally keep their body temperature actively above ambient temperature, but often go into a state of reduced metabolism, i.e. hibernation or daily torpor, to reduce energy expenditure and tend not to reproduce during winter (Körtner & Geiser 2000). Due to their capability of social thermoregulation, honey bees (*Apis mellifera* L.) are able to maintain colonies over the whole year (Jones & Oldroyd 2006), using a strategy analogous to hibernation in mammals. Much like mammals that undergo hypothermic phases during hibernation, the honey bee colony is effectively heterothermic. When the colony experiences cold stress the workers of a colony tend to remain relatively inactive and cluster up densely in the so-called winter cluster to reduce colony heat loss (Southwick 1985), while individual workers actively produce heat by flight muscle shivering to keep the cluster core temperature above ambient temperature (Esch 1964; Stabentheiner 2005). In brood rearing honey bee colonies, the degree and accuracy of thermoregulation is exceptionally high (Fahrenholz et al. 1989; Jones et al. 2004; Kronenberg & Heller 1982). This is necessary as the larvae of honey bees require a higher and more stable temperature than workers to survive and develop well. Even minor deviations from the optimal temperature-window during development can lead to decreased fitness in adult workers (Jones et al. 2005; Tautz et al. 2003). Thermoregulation is highly energy demanding (Stabentheiner et al. 2010). To save resources while foraging is not possible, honey bee colonies refrain from large-scale brood rearing during temperate zone winters. Anticipating resource availability in spring, colonies reassume brood rearing already in late winter. The timing of brood onset is highly critical for colony fitness (Seeley & Visscher 1985)(see also chapter II). Premature brood onset increases the risk of starvation before spring bloom and can lead to increased loads of the brood parasite *Varroa destructor*. Late brood onset decreases the ability to exploit spring bloom. In both ways, wrong timing of brood onset can result in reduced colony growth, colony reproduction, and survival of swarms as well as increased winter mortality. Emergence from hibernation before resources are available is also seen in several mammal species. Increased risk of predation and starvation are hazarded in order to reproduce early so that the offspring has sufficient time to develop and build up resource storages or fat-tissue before the next winter (Körtner & Geiser 2000; Meyer et al. 2016).

To date, very little is known how honey bee colonies achieve an optimal timing of brood onset and what role environmental factors during winter do play as potential triggers. Increasing ambient temperature and length of photoperiod are used across many taxa to time phenological events like emergence after hibernation or reproduction (Bradshaw & Holzapfel 2007; Körtner & Geiser 2000; Visser 2013). In addition, endogenous circannual clocks can control the timing of hibernation (Körtner & Geiser 2000). Nothing is known about the role of internal clocks for timing of brood onset in honey bees. But it is generally assumed that ambient temperature does affect brood rearing activity in honey bee colonies in winter and it has been shown that photoperiod can affect brood rearing activity in summer (Kefuss 1978). Empirical evidence for effects of ambient temperature or photoperiod on brood rearing in winter, however, is still lacking. This is probably because tracking the status of brood rearing within the winter cluster is difficult and generally highly invasive. In the light of ongoing climate change, well-founded information on the impact of environmental conditions on honey bee phenology is critically needed if we want to assess potential consequences of climate change for one of the ecologically and economically most important pollinators (Potts et al. 2016). Climate change and especially changing winter conditions have already been shown to alter timing of life history-stages in many organisms (Williams et al. 2015) and resulting mismatches with the environment can lead to severe fitness losses in wild bees (Schenk et al. 2017).

We aimed to investigate the effects of ambient temperature, photoperiod and elapsed time on the brood rearing status within the winter cluster of honey bee colonies in a minimal invasive way. By tracking the daily temperature variation within the cluster we drew conclusions on the state of brood rearing. Twenty-four honey bee colonies were assigned to two environmental chambers that differed in temperature regimes and to three light regimes. We expected ambient temperature to have a major effect on timing of brood onset that is modulated by photoperiod and elapsed time.

Methods

Study organism

Twenty-four equally sized colonies of *A. mellifera carnica* headed by sister-queens were established in July 2014. Queens were artificially inseminated with 8-10 µl sperm of ten drones all belonging to the same drone population in cooperation with the Institut für Bienenkunde, Oberursel, Frankfurt University. Artificial swarms with 600g of workers and a queen were placed into two-storied miniPlus-hive boxes with 12 empty wax-sheet frames and fed with sugar syrup (Apiinvert, Südzucker) during August to October 2014 to enable comb construction and ensure sufficient honey stores. It was confirmed that all colonies successfully reared worker brood before hibernation and all colonies were adjusted in September 2014 to make sure that they contained approximately the same amounts of workers and honey stores. All colonies were placed into two environmental chambers in December 2014 (12 colonies in each chamber) and kept at 0°C daily mean temperature with daily oscillation from -3°C during midnight to +3°C at noon and under constant short-day conditions of 8h photoperiod. Within the environmental chambers, each colony was connected to a separate flight arena with an individually controllable LED light source (36 cold white (6500K) LEDs and 6 UV-LEDs; ~2000 lx illuminance), diffused with a sandblasted glass cover (Supplemental Fig. III.S1). Honey bees could enter the flight arena via a short tunnel. The tunnels were covered with reflective aluminium foil to increase the amount of light that passes from the arena into the hive box to be perceived by honey bees in the winter cluster. To identify effects of ambient temperature and photoperiod on brood rearing activity, individual temperature and light regimes were started at 28th January.

Temperature regimes

To investigate the effects of ambient temperature on brood rearing in honey bee winter clusters colonies were distributed equally to two temperature treatments (Fig. III.1):

(a) In environmental chamber A the temperature remained at constant cold conditions of 0°C ± 3°C for 78 days after start of the experiment as a control.

(b) Imitating a spell of warm weather, ambient temperature in environmental chamber B was gradually upregulated to 11°C ± 3°C after day 30 after a warm period of 15 days, ambient temperature dropped again to cold conditions.

At day 78 and day 75 respectively the experiment was terminated and all colonies were released from the environmental chambers and placed outside on a meadow at the campus of the University of Würzburg at 6th March 2015.

Light regimes

To check for effects of total day length and day length changes on brood rearing activity colonies were assigned to three different photoperiod treatments (Fig. III.1):

(a) Constant photoperiod: short-day conditions with 8h photoperiod.

(b) Increasing photoperiod: steadily increasing day length, starting at 8h photoperiod with daily increase of 2min 40sec.

(c) Peaking photoperiod: day length starting at 8h photoperiod with a steady increase of 10min 40sec each day for 45 days to a maximum of 16h photoperiod, followed by a steady decrease of 10min 40sec each day until the end of the experiment.

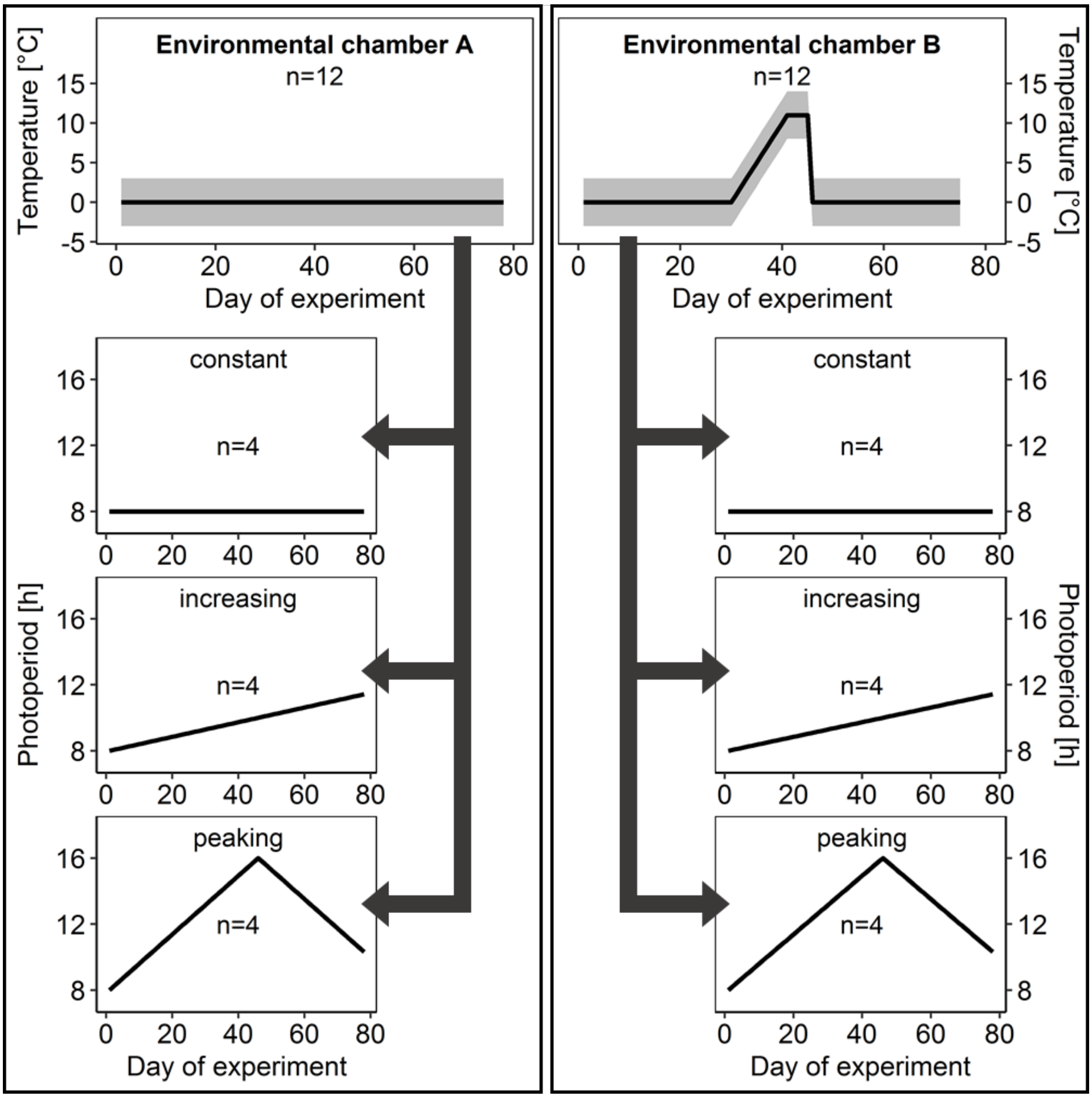

Figure III.1.

Temperature and light regimes. At the start of the experiment, 24 honey bee colonies within experimental hive boxes were distributed equally among two environmental chambers (environmental chamber A and B) that differed in ambient temperature regime. Each colony was connected to its own flight arena with individually controllable light regime and distributed among three different light regimes (constant, increasing and peaking photoperiod) independently from ambient temperature regime. This allowed us to test for effects of ambient temperature and photoperiod in isolation as well as for interacting effects on brood rearing activity in honey bee winter clusters. Grey area: daily amplitude of ambient temperature.

Tracking of comb temperature

Comb temperature in each colony was tracked by eight thermo-sensors (Maxim Integrated DS1921G-F5 Thermochron iButton; 0.5°C resolution) that were embedded into the central wax layer of combs to keep track of winter cluster activity (Supplemental Fig. III.S1). Temperature was measured in 3-hour intervals. At each interval, the sensor that measured the highest temperature was considered as being closest to the centre of the winter cluster and used in the statistical analyses as measure for comb temperature. When daily temperature variation was not higher than 1.5 °C, colonies were defined as brood rearing (Kronenberg & Heller 1982). Ambient temperature for each colony was tracked via a thermos-sensor in the respective flight arena.

Statistics

The statistical software R version 3.4.0 (R Core Team 2017) was used for data analysis. For each observation day colonies were classified as brood rearing if the comb temperature was stable with a daily amplitude of comb temperature ≤ 1.5°C. A linear-mixed effects model was used to test for the effects of ambient temperature and comb temperature variability on mean comb temperature. Data was square root transformed to meet requirements of normal distribution. A contrast matrix was used post hoc to test for differences between individual factor levels. We used a generalized linear mixed-effects model for binomial data to test for interacting effects of temperature phase and light regime on the probability of brood rearing to occur, i.e. the proportion of days during which brood rearing was detected in colonies for each temperature phase and light regime combination. Only data from environmental chamber B was used to analyse interactions between the environmental factors. Temperature in chamber A remained constant at all times, making its data inadequate to assess interactions. Differences between individual levels of factors were tested post hoc using Tukey’s test. The effect of photoperiod duration on the proportion of colonies that were rearing brood for each day was tested, using a generalized linear mixed-effects model for binomial data. A linear mixed-effects model was used to test for effects of direction of photoperiod change on probability of brood rearing. We used a linear mixed-effects model to test for the effect of the direction of change of photoperiod on the probability of brood rearing. Only data from colonies that were kept at constant low temperature conditions was used to test for effects of photoperiod duration or direction of change of photoperiod on brood rearing status. The effect of time spent within the experiment on proportion of colonies that reared brood was tested using a generalized linear-mixed effects model for binomial data. This was done for a subset of colonies under constant cold and short-day conditions, as well as for all colonies, regardless of treatment combination. Colony ID was included as random factor in all models. Benjamini-Hochberg correction for multiple testing was applied for all post hoc tests (Benjamini & Yekutieli 2001). Model residuals were inspected visually for violation of assumptions of normality and homoscedasticity. For all models, a significance level (α) of 0.05 was considered.

One colony under constant cold temperature and peaking photoperiod conditions was removed from the statistical analyses because the temperature profiles revealed that it was still rearing brood at the beginning of the experiment and continued to rear brood during the whole experiment. Three colonies within environmental chamber A and one colony within environmental chamber B were removed from the analyses because they died early in the experiment. This left the treatment combination of constant cold temperature and increasing photoperiod with only two colonies. As data from all colonies within chamber A were combined to analyze effects of photoperiod, this should not have compromised statistical analysis. All other treatment combinations were left with at least three colonies. Four colonies were lost during the second half of the experiment. Observation days from these colonies were included into the analyses until temperature profiles became unstable and eventually dropped to ambient temperature level. A total of 1325 observation days from 19 colonies contributed to the statistical analysis.

Results

Variability of comb temperature

Stability of comb temperature and mean ambient temperature had interacting effects on mean comb temperature measured in the winter cluster (interaction: stability of comb temperature × mean ambient temperature: $F_{1,\,1271.85} = 8.26$; $p = 0.004$; $n = 1325$ observation days from 19 colonies; Fig. III.2). When comb temperature was stable (i.e.

daily amplitude of comb temperature ≤ 1.5 °C) mean comb temperature was significantly higher than when comb temperature was variable (i.e. daily amplitude of comb temperature > 1.5°C; $z = 6.19$, $p < 0.0001$) and no longer affected by ambient temperature (Tukey's post hoc test: $z = 1.60$, $p = 0.111$). This state of stable comb temperature was considered a strong indicator of brood rearing activity. Stable comb temperature was used to identify brood rearing activity in colonies for all following analyses. When comb temperature was variable, mean comb temperature was negatively correlated with ambient temperature (Tukey's post hoc test: $z = -3.35$, $p = 0.001$). Colonies were considered to not rear significant amounts of brood in this state. According to the comb temperature data, all but one colony, which was placed under constant cold temperature and peaking photoperiod conditions, were not rearing brood at the beginning of the experiment.

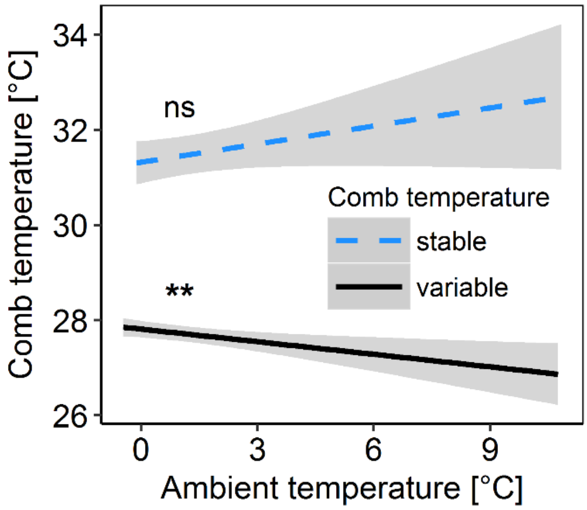

Figure III.2.

Decreased daily variation of comb temperature in honey bee colonies is accompanied by a significant increase of mean comb temperature which is also no longer significantly affected by ambient temperature. Linear mixed-effects model: stability of comb temperature × ambient temperature: $F_{1,\ 1271.85} = 8.255$; $p = 0.004132$**.** Blue line: variable comb temprature, defined by daily amplitude of comb temperature > 1.5 °C; black line: stable comb temperature, defined by daily amplitude of comb temperature ≤ 1.5°C. Grey areas: 95% confidence intervals. $n = 1325$ observation days from 19 colonies. Tukey's test with Benjamini-Hochberg correction for post hoc analysis of effect of ambient temperature on mean comb temperature. **: $p < 0.01$; ns: $p > 0.05$.

Effects ambient temperature and light regime on brood rearing activity

There was a significant interaction between the effects of ambient temperature and light regime on the probability that observed colonies reared brood (i.e. daily amplitude of comb temperature ≤ 1.5°C; data from environmental chamber B; temperature conditions x light regime: $F_{4, 34} = 2.26$, $p < 0.023$; $n = 752$ observation days from 11 colonies; Fig. III.3). Under short-day conditions, the probability of brood rearing increased when the ambient temperature was increased (11°C ± 3°C; Tukey's post hoc test: $z = 4.34$, $p < 0.001$). A drop of ambient temperature back to 0°C ±3°C after the warm period did not significantly reduce the brood rearing activity (Tukey's post hoc test: $z = -1.85$, $p = 0.146$). Surprisingly, there was no significant effect of ambient temperature on brood rearing under conditions of increasing or peaking photoperiod.

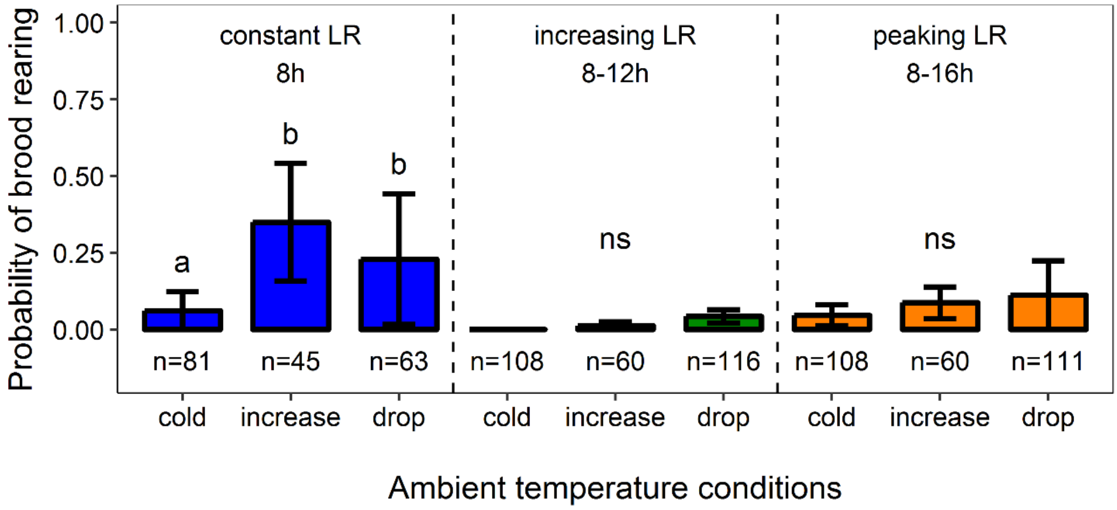

Figure III.3.

Ambient temperature conditions affected the probability of colonies to rear brood (daily amplitude of comb temperature ≤ 1.5°C) depending on light regime. Cold: Colonies were kept at constant cold conditions for 30 days. Increase: a spell of warm ambient temperature for 15 days. Drop: after the phase of temperature increase, ambient temperature dropped again to cold conditions. Constant LR: constant short-day light regime with 8 h photoperiod (*n* =189 observation days from three colonies); increasing LR: gradually increasing photoperiod starting at 8 h photoperiod (*n* =284 observation days from four colonies); peaking LR: fast increase of photoperiod, starting at 8 h photoperiod, peaking at 16 h photoperiod and followed by a fast decrease of photoperiod at the same point of time when temperature dropped again (*n* =279 observation days from four colonies). See Fig. III.1 for more information. Generalized linear mixed-effects model. Tukey's test with Benjamini-Hochberg correction for post hoc analysis of diffrences between factors within light regimes. Letters: statistical groups. ns: $p > 0.05$. Means ± standard errors.

Under constant low temperature conditions of 0°C ±3°C within environmental chamber A the duration of photoperiod had no significant effect on the proportion of colonies that reared brood ($F_{1,570} = 0.10$, $p = 0.755$; $n = 573$ observation days from eight colonies; Fig. III.4). The direction of change of photoperiod had no significant effect on the proportion of days during which colonies reared brood ($F_{2,8.09} = 1.72$, $p = 0.238$; $n =$ 573 observation days from eight colonies; Fig. III.5).

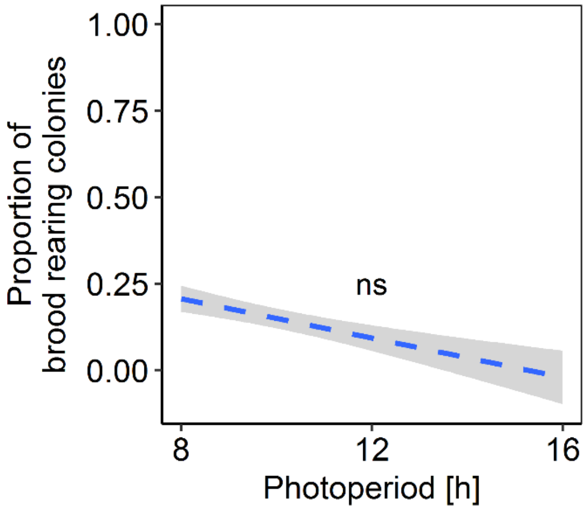

Figure III.4.

The proportion of brood rearing honey bee was not significantly correlated with the duration of photoperiod under constant cold conditions. *n* = 573 observation days from eight colonies. Generalized linear mixed-effects model ns: $p > 0.05$. Grey area: 95% confidence interval.

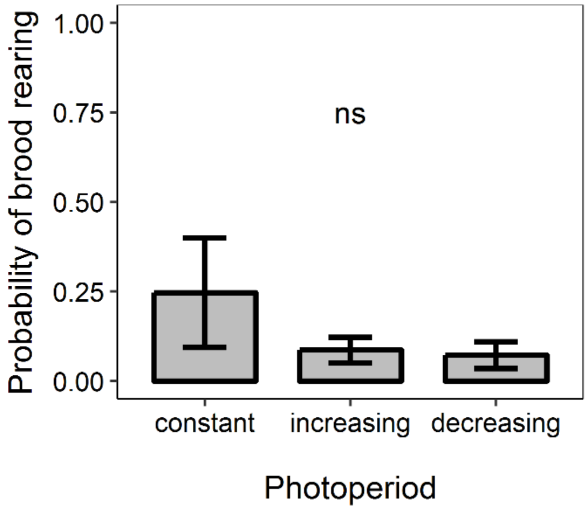

Figure III.5.

The probability of honey bee colonies to rear brood was not significantly affected by the direction of change of photoperiod under constant cold conditions. Means ± standard errors. Constant photoperiod: n = 225 observation days from three colonies; increasing photoperiod: n = 279 observation days from five colonies; decreasing photoperiod: n = 69 observation days from three colonies. Linear mixed-effects model. ns: $p > 0.05$.

Independent of the tested environmental factors, the probability of colonies to start brood rearing (i.e. daily amplitude of comb temperature ≤ 1.5 °C) significantly increased over time in both a subset of colonies that were all kept at constant cold and short-day conditions without further environmental triggers ($F_{1, 222} = 3.81$, $p = 0.045$; n = 225 observation days from three colonies; Fig. III.6) as well as in the complete data set ($F_{1, 1320} = 24.47$, $p < 0.0001$; n = 1325 observation days from 21 colonies; Fig III.6).

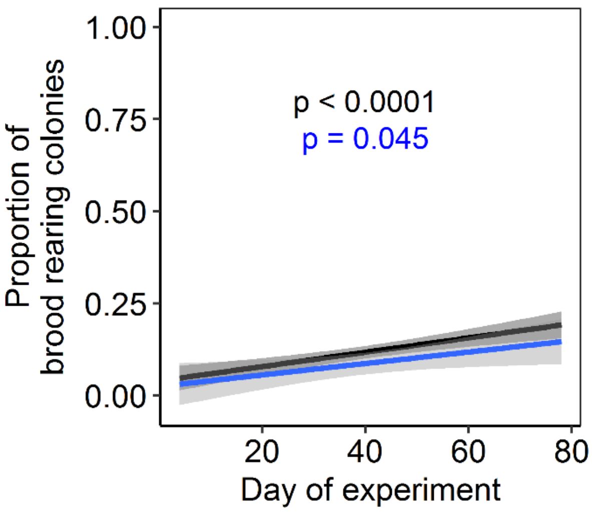

Figure III.6.

The proportion of brood rearing honey bee colonies increased significantly with the time spent within the experiment. Black: *n* = 1325 observation days from 21 colonies regardless of temperature and light regime; blue: *n* = 225 observation days from three colonies under constant cold and short-day conditions. Generalized linear mixed-effects model. Grey areas: 95% confidence interval.

Discussion

We could show that onset of brood rearing in honey bee winter clusters is affected by environmental conditions with ambient temperature playing a major role. In our experimental setting, an increase of ambient temperature after a cold period significantly increased the probability of honey bee colonies to rear brood. Neither duration of photoperiod nor the direction of daily change of photoperiod alone had a significant effect on brood rearing activity within winter clusters. However, the light regime did affect the response of winter clusters to temperature changes. There was only a significant response to temperature increase in colonies that were kept at constant short-day. While interacting effects of different abiotic conditions could help to minimise the risk of premature brood onset, our results suggest that increasing winter temperatures and more frequent spells of warm weather due to global climate change could result in advanced timing of brood onset. This might cause mismatches with the environment with negative consequences for honey bee colony fitness and pollination services. Independent of the measured environmental factors, onset of brood rearing also became more probable with time, which could indicate the involvement of an internal clock.

This study is, to the best of our knowledge, the first where individual and combined effects of ambient temperature and photoperiod on honey bee winter cluster activity were investigated under controlled conditions. Our experimental design allowed us to keep track of honey bee colony thermoregulation and thereby brood rearing activity under defined environmental conditions and without disturbing the colonies. We provide an alternative approach to earlier studies that were either extremely invasive (Avitabile 1978) or not conducted under winter conditions (Fluri & Bogdanov 1987; Harris 2009; Kefuss 1978). Indirectly detecting brood rearing by tracking thermoregulatory activity via thermo-sensors within the comb wax allowed us to investigate honey bee colonies under winter conditions without severely affecting honey bee behaviour and colony health. By analysing patterns of daily comb temperature variation, we could identify days where colonies performed intensive thermoregulation. A daily comb temperature amplitude within the winter cluster of maximally 1.5°C, despite a considerably higher ambient temperature amplitude, was accompanied by an increase of mean comb temperature to more than 30°C. Such conditions were previously measured in the presence of capped brood within the winter cluster (Kronenberg & Heller 1982). Further, mean comb temperature within the cluster was independent of ambient temperature during these days of reduced temperature variation, but was negatively correlated with ambient temperature during days when colonies did allow for higher temperature variation and lower comb temperature within the cluster. This coincides with previous findings that comb temperature is negatively correlated with ambient temperature only while colonies were brood-less (Fahrenholz et al. 1989). When no brood is reared, thermoregulation serves to prevent workers in the cluster periphery from becoming immobile and falling off the cluster. In this state, it is less important to regulate cluster core temperature. Therefore, density and hence isolation of the winter cluster as well as heat production within the cluster can be decreased when ambient temperatures increase. This explains the observed negative relationship between ambient temperature and cluster core temperature. When colonies rear brood, the cluster core temperature is highly important and needs to be stable to allow for a proper development of brood (Jones et al. 2005; Tautz et al. 2003). We conclude that daily temperature amplitude measured within the winter cluster is a good predictor for brood rearing activity. Unlike earlier findings (Fahrenholz et al. 1989), there was no significant positive correlation between comb temperature and ambient temperature during times when colonies were expected to rear brood. This could be explained by the fact that we tested colonies at lower temperature and a shorter temperature gradient. It is important to keep in mind that the spatial resolution of temperature data was limited and small brood nests might not have been detected in all cases. In fact, it is possible that even in temperate zones continuous brood rearing during winter could be common, albeit at very limited extent (Avitabile 1978; Harris 2009; Szabo 1993). The honey bee queen probably lays eggs the whole year round, while the workers decide whether to care for the brood or to remove it (Mattila & Otis 2006). The constant activity of queens enables a rapid onset of large scale brood rearing if environmental conditions are beneficial. Once the brood nest grows and colonies start to rear brood at considerable amounts, this can be expected to be reflected in the temperature data obtained from our experimental setting. Although some uncertainty about the status of the colony will remain, we argue that this indirect method is preferable over the much more invasive method of disrupting the cluster to visually assess brood status.

In our experiment brood rearing activity was rarely detected under cold environmental conditions. Once ambient temperature increased the probability of colonies to start rearing brood also increased. Reactions could already be observed during the first days of temperature increase, when daily maximum of ambient temperature was still below 10°C. The effect of ambient temperature on brood rearing activity is not surprising. The energy demand of thermoregulation necessary for brood rearing increases with decreasing ambient temperature (Kronenberg & Heller 1982). As the resources needed to fuel thermoregulation are strongly limited, honey bee colonies should refrain from brood rearing under cold environmental conditions (Seeley & Visscher 1985; Southwick 1991). With increasing ambient temperature thermoregulation, and hence brood rearing, becomes less cost intensive and more viable, even when colonies need to solely rely on storages. Ambient temperature was previously also shown to have a strong effect on timing of increased thermoregulation after hibernation in ants of the *Formica*-group (Rosengren et al. 1987) as well as timing of hibernation and emergence in mammals (Körtner & Geiser 2000; Meyer et al. 2016; Mrosovsky 1990; Ruf et al. 1993). After colonies started to rear brood, a drop of ambient temperature did not immediately cause them to stop. The mere presence of capped brood might have stimulated the workers to continue keeping the brood combs warm, even when mean ambient temperature was as cold as 0°C. This may cause honey storages to run out quickly and leave colonies starving. It is possible that, once triggered, only a disruption of honey or pollen stores will ultimately force a stop of brood rearing activity. It is important to note that, despite a relatively large increase of ambient temperature, probability of brood rearing in our experiment only increased by about 30%. This reaction was weaker than expected and suggests that further factors are involved in the timing of brood onset.

Our data revealed that photoperiod in isolation had no effect on brood rearing activity. Neither duration of photoperiod nor direction of change of photoperiod affected brood rearing under cold conditions. It might be possible that honey bees are not able to measure photoperiod when densely packed within the winter cluster. It has been suggested for mammals which hibernate in shelters and therefore have limited access to day light, that ambient temperature would be the most appropriate stimulus or zeitgeber for timing of emergence after hibernation (Davis 1977; Körtner & Geiser 2000; Michener 1977; Mrosovsky 1980; Murie & Harris 1982). However, in our experiment light regime did alter the response of honey bee colonies during warmer conditions, when winter clusters were probably less dense and workers could leave the cluster. Adult emergence, reproduction and oviposition in the marine midge *Cluneo marinus* is also known to be controlled by two environmental factors that need to occur in unison (Kaiser & Heckel 2012). Increasing ambient temperature affected brood onset only at constant short-day conditions of 8h photoperiod, but not in the other two light regimes in which photoperiod was considerably longer (about 12h to 18h, depending on light regime) and increasing. These findings are not in line with suggestions that a short photoperiod elicits cannibalization of eggs and hence inhibits brood rearing activity (Cherednikov 1967; Woyke 1977). Several studies proposed that , irrespective of current duration of photoperiod, an increase in photoperiod has a positive effect on brood rearing activity while a decrease of photoperiod negatively affect brood rearing (Avitabile 1978; Kefuss 1978). The negative effect of photoperiod on brood rearing under warm conditions in our study also does not support these findings. However, most of the previous studies that investigated the effect of photoperiod on brood rearing activity either did not investigate brood rearing activity in winter (Fluri & Bogdanov 1987; Kefuss 1978) or did not control for other environmental conditions that might have affected brood rearing activity like ambient temperature (Avitabile 1978; Fluri & Bogdanov 1987). It was previously shown that brood rearing activity in colonies that were kept at constantly low mean ambient temperature of 6°C were not affected by photoperiod (Harris 2009). This supports our findings, that photoperiod matters only under warm conditions. Fluri & Bogdanov (1987) failed to find an effect of photoperiod under warm conditions, but investigated the effect of artificial shortening of day length in summer when colonies were already rearing large amounts of brood. Under these circumstances the effect of photoperiod might be reduced (but see Kefuss (1978)). Due to the experimental settings, we cannot disentangle if it was the longer duration of photoperiod or the fact that photoperiod increased that reduced brood rearing under warm conditions. It also remains to be investigated if a decrease of photoperiod during a warm period would affect brood rearing. As we expected photoperiod was used as additional cue and might help to prevent premature brood onset due to spells of warm weather. Our results are still surprising. According to our hypothesis, short photoperiod was expected to inhibit brood rearing while increasing photoperiod should have promoted brood rearing activity and not *vice versa*. This illustrates that further experiments on combined effects of temperature and photoperiod are needed.

Our results indicate that in addition to photoperiod and ambient temperature also elapsed time affected brood rearing in the honey bee colonies. Brood rearing activity was detected with increasing frequency over time and we observed brood rearing activity in one colony even at constant short-day and cold conditions. This suggests that colonies recommence brood rearing at some point regardless of environmental conditions. It has been shown for mammals that a circannual rhythmicity underlies the timing of hibernation and seasonal torpor, which can be entrained by photoperiod, ambient temperature and food-availability, but does not rely on these external zeitgebers (Collins & Cameron 1984; Heldmaier & Steinlechner 1981; Körtner & Geiser 2000; Mrosovsky 1986; Steinlechner et al. 1983; Wang 1988). Timing of honey bee brood rearing activity might also be controlled by an internal clock. Another reason for increased probability of brood onset over time might be the build-up of moisture within colonies. It was proposed that the humidity in colonies affects brood rearing activity and brood may serve to bind moisture generated by the metabolic activity of colonies which may otherwise be harmful (Omholt 1987). Humidity within the different colonies might have varied and was not tracked during the experiment. The availability of resources might be another highly important factor for timing of brood rearing. Colonies that were supplemented with pollen in spring were previously found to start brood rearing earlier in the year (Mattila & Otis 2006). It has also been shown that the nutritional status of individuals and food-availability can affect the response to environmental cues for timing of hibernation in mammals (Norquay & Willis 2014; Ruf et al. 1993) and increase of thermoregulation in *Formica*-ants (Rosengren et al. 1987).

We conclude that brood rearing activity in hibernating honey bee colonies is highly sensitive to climatic conditions with ambient temperature as important trigger for brood onset. Therefore, climate change and associated more frequent warm weather events during winter (IPCC 2014) have the potential to disrupt the synchronization between the seasonal timing of brood onset in honey bee colonies and flowering phenology. This can have profound negative consequences for colony fitness.

Acknowledgements

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IV. Interval time-place learning in honey bees

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Running title: Interval time-place learning in honey bees

Abstract

The exploitation of resources that vary in space and time is a fundamental challenge for animals (Gallistel 1990). Vertebrates and invertebrates are able to learn a specific resource availability at a certain place that recur at a fixed time of day (circadian time-place learning (Beling 1929; Biebach et al. 1989; Boulos & Logothetis 1990; Chouhan et al. 2015; Reebs 1996; Schatz et al. 1994)). However, environmental variation and biotic interaction can lead to a more complex daily and seasonal resource dynamics, which require other forms of spatiotemporal orientation and associative learning (Jones & Agrawal 2017). For example, the time of day when a resource is available at a certain place might change, e.g. depending on temperature, in a systematic and predictable way(van Doorn & Kamdee 2014). In such dynamic resource environments, vertebrates are able to associate spatiotemporal resource sequences with flexible starting times where only the time intervals between resource events are fixed (interval time-place learning)(Carr & Wilkie 1997; Henderson et al. 2006; Thorpe et al. 2012). This cognitive ability is highly adaptive but thought to be completely absent in invertebrates. Here we show for the first time that foraging honey bees are able to learn resource patterns that are timed by time intervals but not time points. In a field experiment, foragers concurrently learned multiple intervals to anticipate feeder opening times, feeder reward time, and the end of feeding intervals at different resource sites. This led to a higher foraging success, but bee communication was not commenced before the resource was available. Our results show that *A. mellifera* use not only circadian time memories but also measure the duration of elapsed time for spatiotemporal orientation. We conclude from our experiment that foraging honey bees use interval time-place

learning skills similar to vertebrates which provide significant evolutionary benefits for resource exploitation.

Time perception can be explained by two distinct ways of measuring time: circadian timing, representing time of day with circadian oscillators with a period close to 24h; and interval timing, as an estimation of elapsed time durations between events in the range of seconds to hours (Gibbon et al. 1997). Accurate orientation in time and space benefits fitness, as it allows animals to restrict activities to times and places with high resource availability. However, daily resource patterns are often variable and can be modulated by both abiotic environmental conditions and biotic interactions (Fründ et al. 2011; van Doorn & Kamdee 2014). Vertebrates including humans, birds and rats, have adapted to more complex but still predictable spatiotemporal resources patterns, as they are able to associate interval time measures to places with variably occurring resource events (Mulder et al. 2013; Smarr et al. 2014; Wahl 1932). Despite facing similar environmental variations in resource availability, interval time-place learning has never been shown before in insects or any other invertebrate.

Here we used a simple feeder experiment to test whether free-flying honey bees, *Apis mellifera,* have the capability to measure the passage of elapsed time so as to associate spatiotemporal resource regularities (i.e. interval time-place learning). We established three feeder locations within 300 m to 500 m distance of the test colony (Extended Data Fig. IV.2). For five consecutive days, the time of the start event (= Feeder 1 activation with sugar-water) was arbitrarily reset every day. Two spatially separated feeders (= Feeder 2 and 3) were activated sequentially with a fixed but unequal interfood-interval of 60 and 50 minutes after closing the first and second feeder, respectively (Figure IV.1 and exemplary training schedule in Extended Data Fig. IV.1). Video monitoring of 12,135 feeder visits of foraging bees from four observation colonies were statistically analysed. During the first two days, foraging bee colonies did not express any interval time-place learning guided behaviours (Figure IV.2a, see also Extended Data Table IV.1 for statistics). In contrast, during test days three, four and five, the number of bee visits increased significantly at Feeder 3, 10 min before the feeder was rewarded (Figure IV.2b, Extended Data Table IV.1). The ability to use interval timing to anticipate spatiotemporal patterns of food availability is unique for bees and all other invertebrates. Nevertheless, honey bees can show similar food anticipatory behaviour for circadian-timed resource events (Beling 1929; von Frisch 1967; Wahl 1932). In our experiment, the number of training days until foraging honey bees used an elapsed duration of time as temporal reference to foresee resource availability matches' data for circadian time-place learning of honey bee colonies

(Koltermann 1974), indicating a similar memory formation pace for circadian and interval time-place learning (Menzel 1999). Additionally, we analysed feeder visitation rates during a sixth consecutive day of observations without offering a sugar-water reward at Feeders 2 and 3 (Figure IV.2c). After the arbitrary set of sugar-water reward at Feeder 1, it turned out that despite the missing reward the frequency of marked bee visits increased again significantly at the end of both interfood intervals (50 or 60 minutes; Figure IV.2c, Extended Data Table IV.1). Learning the duration of the interval length between a delivered reinforcement and the next reward is widely accepted as evidence for interval learning in vertebrate studies (Gallistel & Gibbon 2000). In our experiment foraging bee colonies clearly associated the elapsed duration of interfood time with an anticipated resource at two feeder locations, which differed in interfood interval length (Figures IV.1, IV.2c, Extended Data Table IV.1, Extended Data Fig. IV.1). At the sixth observation day, the colonies showed another strong interval timing effect at Feeder 2. The visitation rate at the unrewarded feeder during the scheduled feeding interval significantly increased (Figure IV.2c, Feeder 2). Furthermore, at this testing day, foragers did not only include the measurement of the interfood and feeding interval into their foraging behaviour, but they also learned the putative end of the active feeding interval (20 minutes, Figure IV.2c, Feeder 2; Extended Data Table IV.1). All these results show for the first time that honey bee colonies can concurrently perceive and memorize multiple time intervals to exploit flexible but predictable resource patterns while foraging. In our experiment, all behavioural decisions when to visit or leave a feeder are decoupled from a specific daytime. Our data illustrate that honey bee colonies can obviously integrate interval time related temporal information in a comparable way as they use circadian timing for exploitation of food resources with limited temporal availability. Moreover, an anticipated end of reward (Beling 1929; Wahl 1932), (Figure IV.2c, Feeder 2; Extended Data Table IV.1), can clearly be decoupled from both time of day and resource availability. This indicates that honey bee foragers can measure cessation of food resources only based on interval time memories. Monitoring the timing of foraging bees in our experiment suggests that our view on time perception of invertebrates was incomplete. It looks like that at least honey bees can also use time intervals to orientate in space and time. Nevertheless, with these data, we made only the very first step in understanding the different levels of time duration measurements. Future research can now focus on extent and functioning of interval time-place learning in bees and other invertebrates.

In order to study the role of honey bee communication in the described interval time-place learning task, we also recorded and decoded N = 635 dances of feeder-specific marked bees in the tested observation hives. The frequency of other dances by non-marked bees (N = 1292) during our experimental phase were not affected by feeder activation times (Figure IV.S4). Significantly more waggle dances of feeder experienced marked bees occurred when feeders where active during day 1-5 (Figure IV.3, Extended Data Table IV.2). In contrast, this was never the case in the anticipatory phase, indicating that feeder-experienced foragers in the hive did not recruit nest mates to food resources in advance (Van Nest et al. 2016), (Figure IV.3, Extended Data Table IV.2). The dance communication results of the sixth observation round (open-loop approach) were less clear (Figure IV.3, Extended Data Table IV.2). During both: arbitrary start event and proposed feeder activation times, we never observed a significant increase in dance activity (Figure IV.3, Extended Data Table IV.2). The non-increase in recruitment for the rewarded feeding time is most likely related to differences in the baseline dance activity compared to the preceding days (Figure IV.3, Feeder 1: day 1-2, day 3-5, Extended Data Table IV.2). Therefore, we cannot exclude that further factors underlie the missing dance activity increase for unrewarded feeding times within the open-loop experiment.

We were also interested in the evolutionary benefits of interval time-place learning for resource exploitation. We quantified foraging success by measuring sugar-water uptake at each feeder. The data revealed a significant increase in net uptake of sugar-water from day 1-2 to day 3-5 by 66% (Figure IV.4a, Extended Data Table IV.3). This indicates, in combination with the feeder visitation pattern, an adaptive foraging benefit of learning to utilize fixed-time intervals measures when exploiting spatiotemporally distributed food resources. Furthermore, we found an increasing total sugar-water consumption rate from Feeder 1 to Feeder 3 (Figure IV.4b, Extended Data Table IV.3), suggesting a more efficient exploitation of resources that were linked in a predictable way by fixed-time intervals, compared to the first feeding event with arbitrary rewarding times. The data on foraging success and the memorized measurement of start and end of resource availability (Figure IV.2b,c) underline the enhanced efficiency of foraging by using interval time-place learning guided behaviour. We hypothesise that interval time-place learning is an important feature to achieve optimal foraging behaviour (Stephens & Krebs 1986) in honey bees. By learning time intervals foragers can further maximize their net rate of energy intake, time portioning among feeding sites, and consequently the fitness of the whole colony. Since we did not analyse the foraging efficiency at random opened feeders, it would greatly aid the interpretation of the present results if further work evaluated the relative foraging success benefits at interval-timed resources.

Why should honey bees keep track of elapsed time intervals? Flexible environmental cues such as insolation, humidity and temperature are among the main factors influencing the daily timing of pollen dehiscence and nectar secretion in flowering plants (Pacini 2000; Willmer 2011). In an environment with high spatial and temporal variation of pollen and nectar presentation, more complex associative learning skills are predicted to be evolutionary beneficial (Dukas 2008; Jones & Agrawal 2017). Under constant weather conditions and a relatively uniform landscape structure, circadian time-place learning may be sufficient to time efficient foraging trips. In contrast, to perform timely flower visits in dynamic resource environments is highly challenging when animals only rely on circadian time perception. To respond to flexible occurring resource patterns, a stopwatch-like time sense to measure time intervals in a range of an hour is a perfect addition to adjust behaviour to the temporal structure of the environment. Our data suggest that in predictably cycling floral resource environments, the bees can use an arbitrary occurring floral food patch as reference to anticipate food availability at other resource sites.

The measurement of time is a basic issue for animals. Honey bees show time-place sensing skills that until now have only been reported from vertebrates. The evolutionary success of this species in all major biomes of the world is almost certainly related to the combined advantages of its unique communication behaviour (Seeley 1985; von Frisch 1967) and advanced spatiotemporal learning and memory skills as demonstrated in this study. The honey bee remains an inspiration for ecological, behavioural, and neurobiological sciences and challenges future interdisciplinary research on the underlying mechanisms of time perception and its ecological fitness consequences in variable and globally changing resource environments.

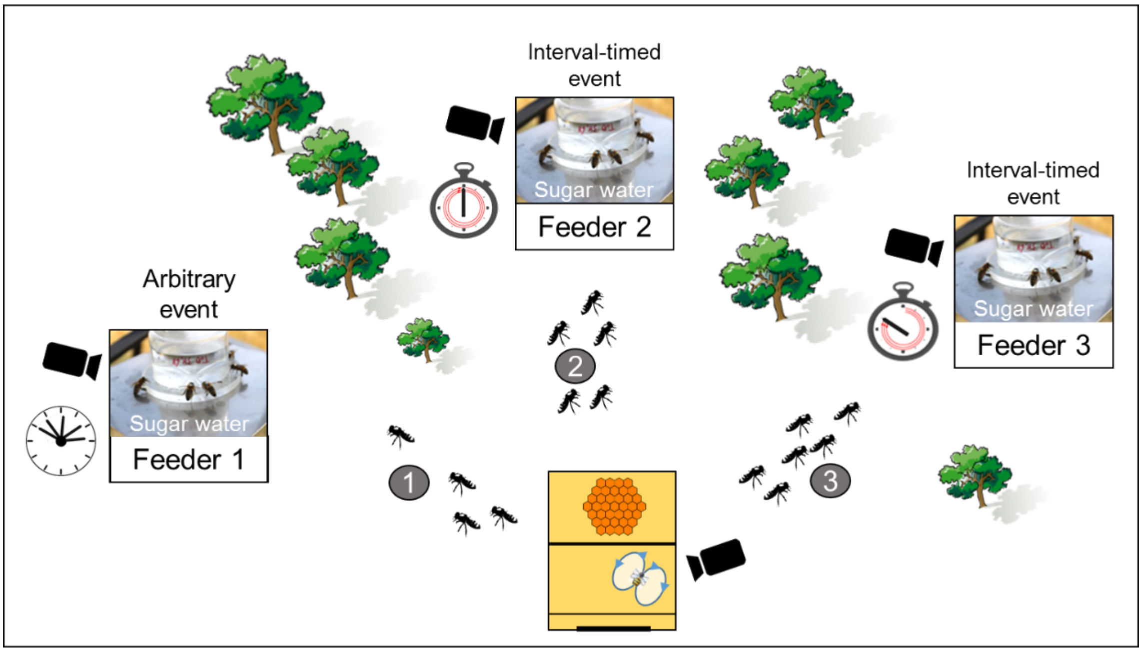

Figure IV.1.

Experimental set up to reveal interval time-place learning in honey bees. At an arbitrary time of day, a feeding event (sugar-water reward) was set at Feeder site 1. After a fixed 60 min interfood interval, a spatially separated second feeder became rewarded. This was followed by a further fixed interfood interval of 50 min until a third feeder was activated at a different location. Dance activity and feeder visits were recorded over the whole experimental time. Sugar water uptake at all feeders was used as measure for foraging success. The experimental set up was applied at five consecutive days, followed by a sixth round without sugar-water rewards at Feeder 2 and 3 representing an open-loop approach. Four *Apis mellifera* colonies were tested following the same fixed interfood interval scheme but with different arbitrary starting times at the first feeder. This approach represented conditioning to time intervals instead of fixed time of day events. Prior to the core experiment, the bees were trained to the three feeder locations for one day. All feeder locations were in between 300 m to 500 m distance from the hive and approximately 100 m distance to each other (Extended Data Fig. IV.2).

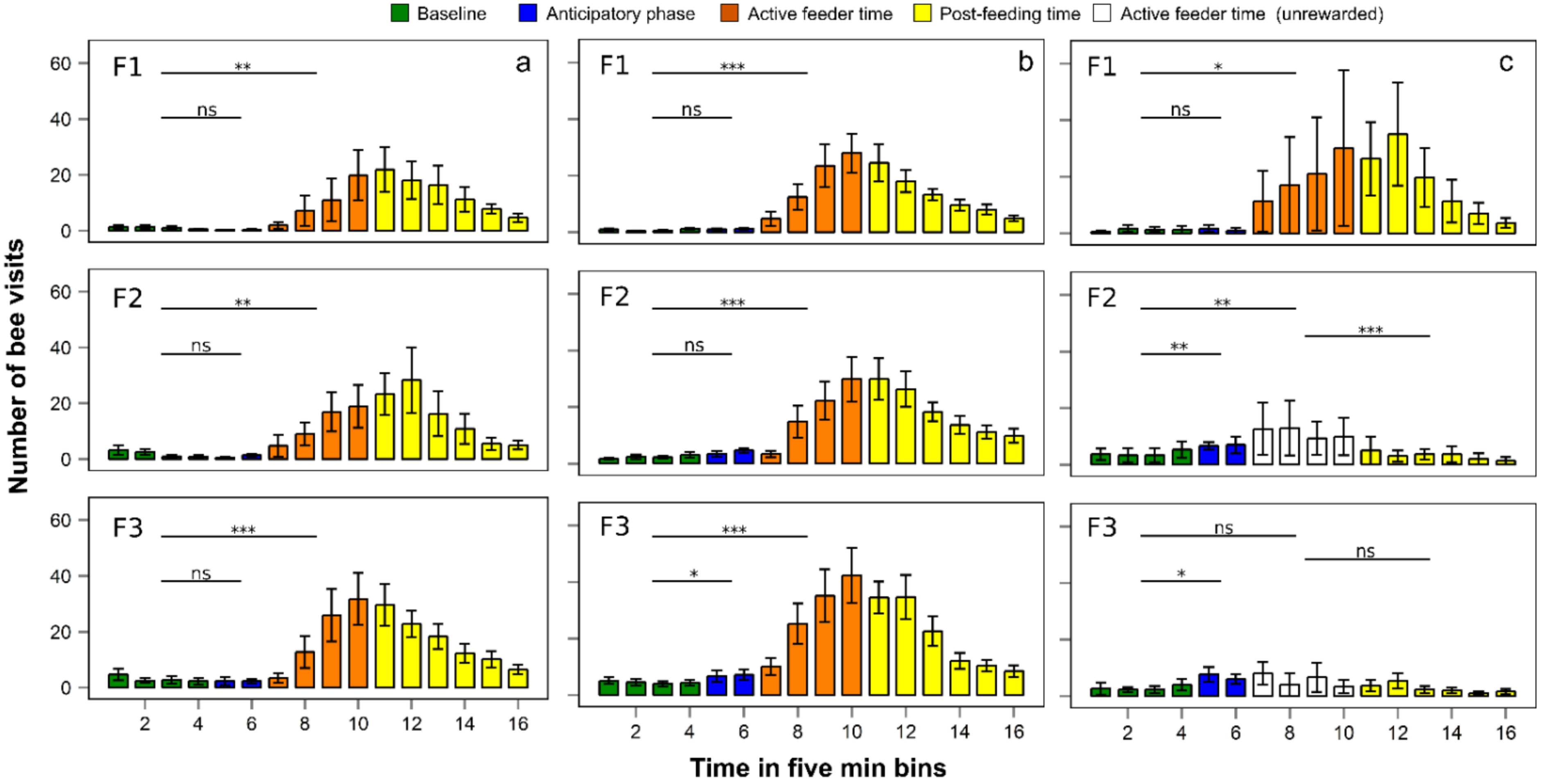
🟩 Baseline
🟦 Anticipatory phase
🟧 Active feeder time
🟨 Post-feeding time
☐ Active feeder time (unrewarded)
a
b
c
F1
F2
F3
**
***
*
ns
Number of bee visits
0
20
40
60
2
4
6
8
10
12
14
16
Time in five min bins

Figure IV.2.

Visitation rates of honey bee foragers at interval-timed sugar-water feeders. Visitation rates of bees at three spatially separated artificial feeders (F1-F3). Experimental time was divided into four distinct phases: baseline phase, 30-10 minutes before feeder activation; anticipatory phase, 10-0 minutes before active feeder time; active feeder time; and post-feeding phase, 0-30 min after feeder activation. a) Feeder visits during the first and second day. b) Feeder visits within days 3-5. c) Feeder visits at day six conducted as open-loop experiment with unrewarded feeders F2 and F3. The statistics for the comparison active feeder time vs post-feeding time is only included for unrewarded feeders (more statistical information in Extended Data Table IV.1). N=12,135 feeder visits of marked bees, error bars represent ± s.e.m., Linear Mixed-Effects Models, asterisks indicate significance levels *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$, ns= non-significant.

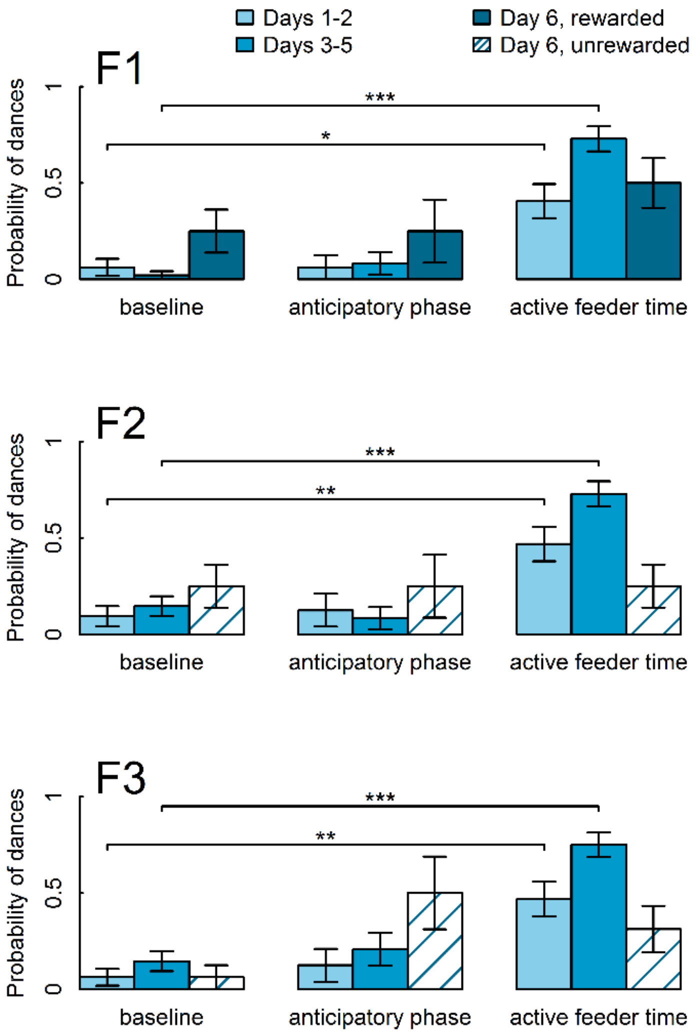

Figure IV.3.

Honey bee dance activity during baseline, anticipatory, and feeding phases. Analyses of in-hive recruitment behaviour of feeder experienced (marked) bees in parallel to the feeder-activation (F1-F3) schedule of the interval time-place experiment. The in-hive recruitment system did not respond in an anticipatory way to future rewards. N=635 decoded dances of marked bees, Generalised Linear Mixed-Effects Model, error bars represent ± s.e.m, Tukey's test and Benjamini-Hochberg correction, more statistical information in Extended Data Table IV.2, asterisks indicate significance levels *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$.

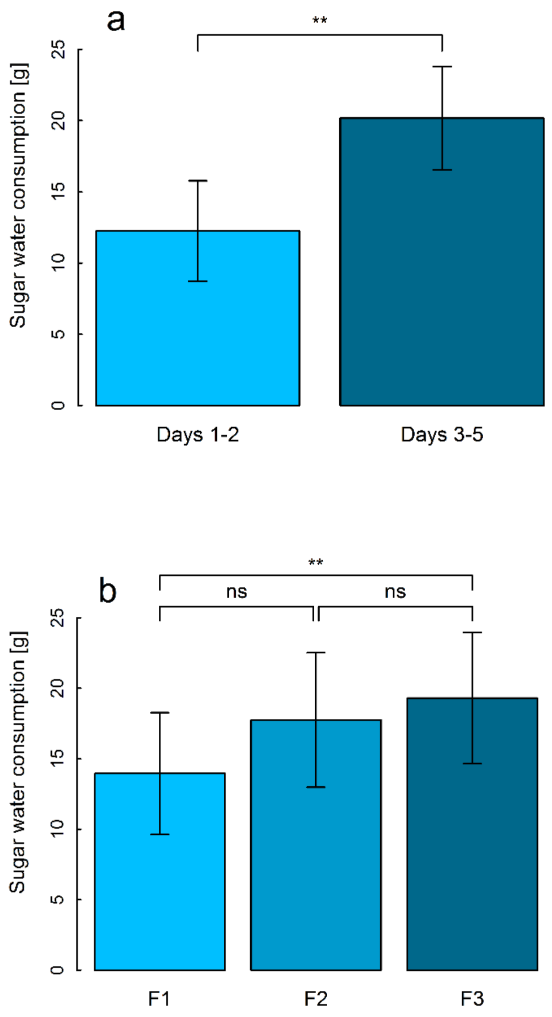

Figure IV.4.

Foraging success of honey bees at interval-timed food resources. a) Sugar-water uptake of four honey bee colonies at interval-timed sugar-water feeders during the first two days and in days 3-5. b) Accumulated foraging success for interval-timed sugar-water rewards. Foraging uptake at a time-arbitrary occurring food resource (F1) and at two subsequent interval-timed food resources (F2, F3) in the course of the experiment (days 1-5). Error bars represent ± s.e.m., Generalised Linear Mixed-Effects Model, Tukey's test and Benjamini-Hochberg correction, more statistical information are available in Extended Data Table IV.3, asterisks indicate significance levels (**=p<0.01), ns=non-significant.

Materials and Methods

Study sites and experimental design

In an agricultural landscape near Würzburg, Germany, we selected three spatially separated study sites to analyse the foraging behaviour of four *Apis mellifera carnica* colonies (Extended Data Fig. IV.2). Behavioural tests on two colonies located at the same site were performed sequentially, one after another. At the beginning of each trial, we established three artificial feeders at 300 m to 500 m distance from the hive. Each feeder, when open, offered the same sugar-water reward (see next section for further detail). The feeders were allocated at different sides of a row of trees or hedges and spatially separated from each other by approximately 100 m (Extended Data Fig. IV.2). Over five consecutive test days, the first feeder (=Feeder 1 or F1) was activated for exactly 20 minutes, beginning and running at an arbitrarily chosen, different and non-overlapping time period each day. When this 20-minute period ended, the feeder was replaced by a blank and after 60 min of interfood-interval the second feeder (F2) was activated and sugar-water was provided again for 20 min, followed by a 50 min interfood-interval until the third feeder (F3) was activated for 20 min (see also Extended Data Figures IV.1, IV.3). On the sixth and last consecutive day of testing, only the first feeder was activated, Feeders 2 and 3 remained empty but camera recordings were maintained, creating an open-loop experiment. Test schedules were designed in a way that avoided an overlap of feeder activation times at any of the test days. Later in the year, when general bee activity decreased, active feeder periods for the last tested colony were extended to 35 min. To make data from this colony with an elongated feeding phase compatible with the ones before, only the first four five-minute bins of the feeder advertisement periods were used in the analyses. Investigators were not blinded to allocation during experiments and outcome assessment.

Honey bee colonies and feeder set up

Behavioural tests on bees were performed in August-September 2015, in a period with few alternative flowering resources available in the landscape. The observation hive colonies had a size of about 3000 worker bees (two Zander frames) and were free-flying. Queens were sisters, inseminated with 8-10 µl sperm of 10 drones all belonging to the same drone population. Artificial insemination was performed in cooperation with the Institut für Bienenkunde, Oberursel, Frankfurt University. Artificial feeders (60 ml) were placed on a tray at 1 m height and filled with diluted Apiinvert sugar solution (31% sucrose, 30% glucose, 39% fructose) that was scented with rosemary (Gies Kerzen GmbH Glinde, Germany), lavender or anis (LaVita Kempen, Germany), (concentration: 30 µl/l). Within the individual interval timing tests only one type of scent was used for each colony. Empty feeders presented at interfood intervals or during the open loop test were carefully handled scent free. Prior to the behavioural test, bees were trained to the feeder locations for one day at a time of the day that was not also used as an active feeder phase during following test days. We assigned colonies randomly to the environments in which the behavioural experiments were conducted.

Data collection

Honey bee activity at feeders and dance activity inside of the hive were video recorded. Cameras were started 60 min before the first feeder was activated and stopped 60 min after the last feeder had been closed (Extended Data Fig. IV.3). All recordings were performed with Sony HD-Camcorders (Sony HDR-CX240). When analysing the videotapes, a visit was counted if a bee either landed on the feeder or visibly circuited it. Additionally, the active feeder was observed and all visiting bees were marked with feeder-specific marker pens (Uni Posca PC-5M). At day six only video recordings were performed. Before and after the active feeder phase, the filled feeder was weighed on a field scale to determine sugar water consumptions. In order to analyse the dance behaviour, all returning foragers were directed to one side of the hive by a diagonal wooden block (Danner et al. 2014; Danner et al. 2016). In addition, all further gaps between combs close to the hive entrance were also blocked to further restrict the dance behaviour to one side near to the hive entrance (Danner et al. 2014; Danner et al. 2016). All dances with at least five repetition rounds were taken into account (Danner et al. 2014; Danner et al. 2016).

Statistical analyses

Statistical analyses were performed using the software R 3.4.0 for Windows (R Core Team 2017). For all statistical analyses of feeder visits and dance behaviour, we distinguished the following time periods: baseline phase (post-reinforcement pause) 30 - 10 minutes before feeder activation time; reward anticipatory phase 10 - 0 minutes before feeder activation; active feeder phase; and post-feeding phase (0 - 30 minutes after feeder deactivation) (see also Figures IV.1, Extended Data Figures IV.1, IV.3). All statistically analysed data on feeder visit and dances were from feeder-marked bees. The variable day was divided into three groups: (i) first two days of conditioning, when bees were not expected to show interval time place learning based on time required for honey bees to acquire circadian time-place memory (Koltermann 1974); (ii) days 3-5; and (iii) day 6, the open-loop experiment. Linear Mixed-Effects Models were fitted for each combination of feeder and day group to test whether the number of bee visits increased over time between baseline and either the anticipatory phase, the active feeder phase or the post feeding phase. Data on bee visits were log-transformed after adding a small value to all data points (Berry 1987). Time in five-minute bins was modelled as continuous explanatory variable. One open-loop test day was discarded in line with low flight activity due to windy and cold weather conditions at the testing day. In general, only few dances could be observed per five-minute bins, therefore count data were converted to presence/absence data to investigate the probability of observing dances during a specified observation phase. A Generalized Linear Mixed-Effects Model using a distribution from the binomial family was fitted to the data with the interaction effect of phase and day number as categorical explanatory variable. The effect of observation day number and feeder number on the sugar water consumption was tested by fitting a Linear Mixed-Effects Model to the square root-transformed data with day group and feeder number as categorical explanatory variables. To account for variation between colonies, the Colony-ID was included as a random factor in all mixed-effects models. Denominator degrees of freedom were calculated using the Satterthwaite approximation (Satterthwaite 1946). Model residuals were inspected visually for violation of assumptions of normality and homoscedasticity. For all models, a significance level (α) of 0.05 was considered. Differences between individual levels of factors were tested post hoc using Tukey’s tests followed by Benjamini-Hochberg correction for multiple testing (Benjamini & Yekutieli 2001). Colony positions and feeder locations were plotted into land-use maps (Extended Data Fig. IV.2, Geobasisdaten © Bayerische Vermessungsverwaltung) using the software ArcGIS 10.3 (ESRI, 2011). No statistical methods were used to predetermine sample size.

Acknowledgements

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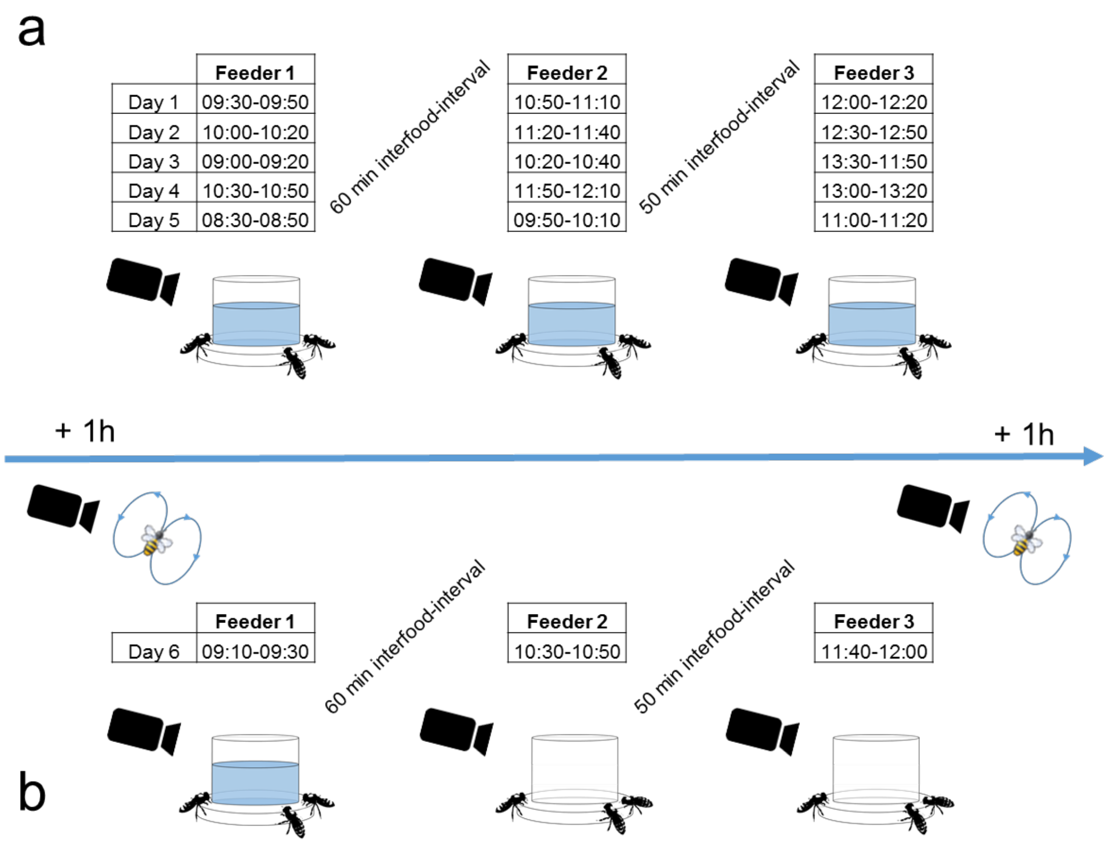
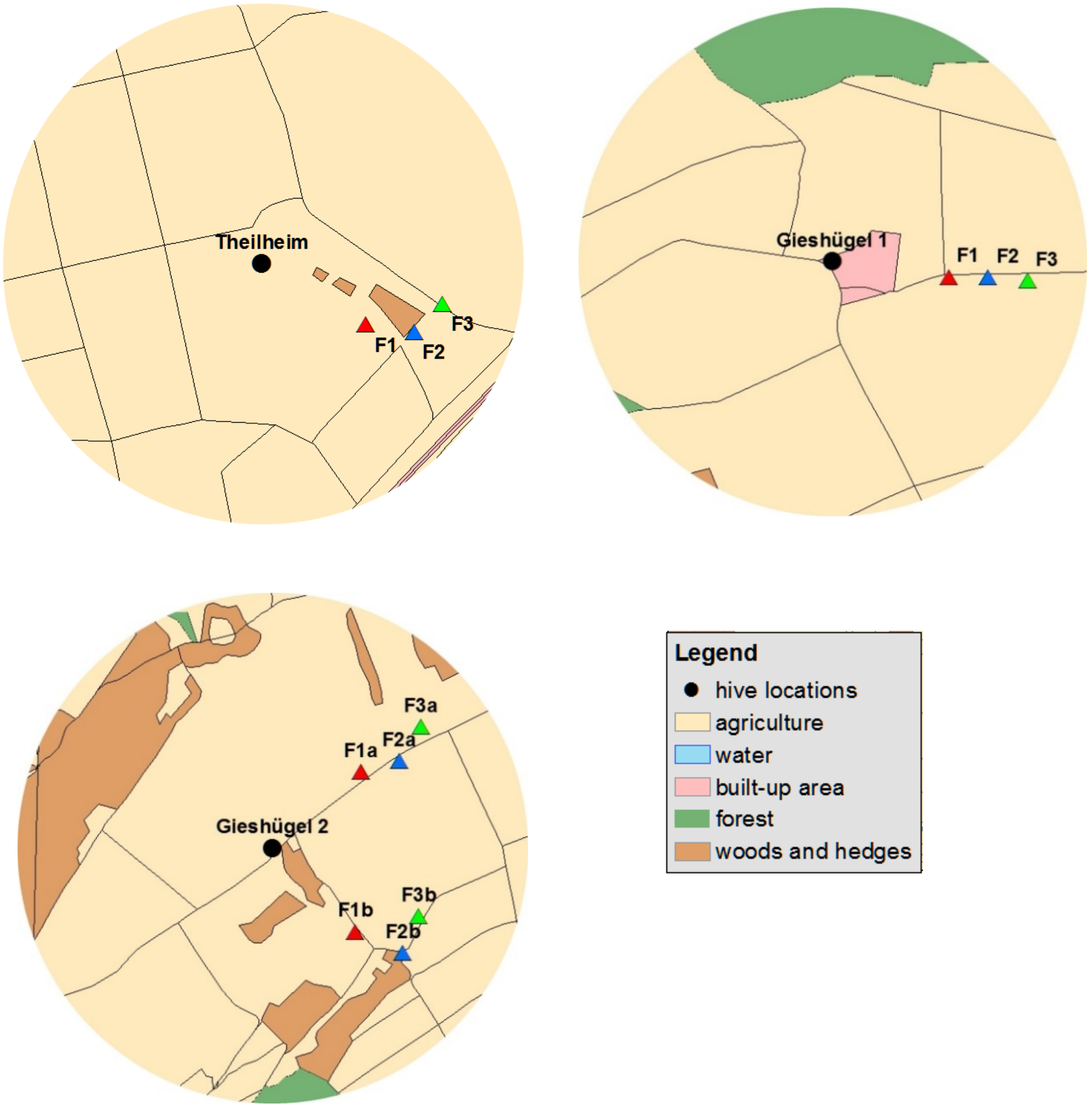


	Feeder 1	Feeder 2	Feeder 3
Day 1	09:30-09:50	10:50-11:10	12:00-12:20
Day 2	10:00-10:20	11:20-11:40	12:30-12:50
Day 3	09:00-09:20	10:20-10:40	13:30-11:50
Day 4	10:30-10:50	11:50-12:10	13:00-13:20
Day 5	08:30-08:50	09:50-10:10	11:00-11:20

	Feeder 1	Feeder 2	Feeder 3
Day 6	09:10-09:30	10:30-10:50	11:40-12:00

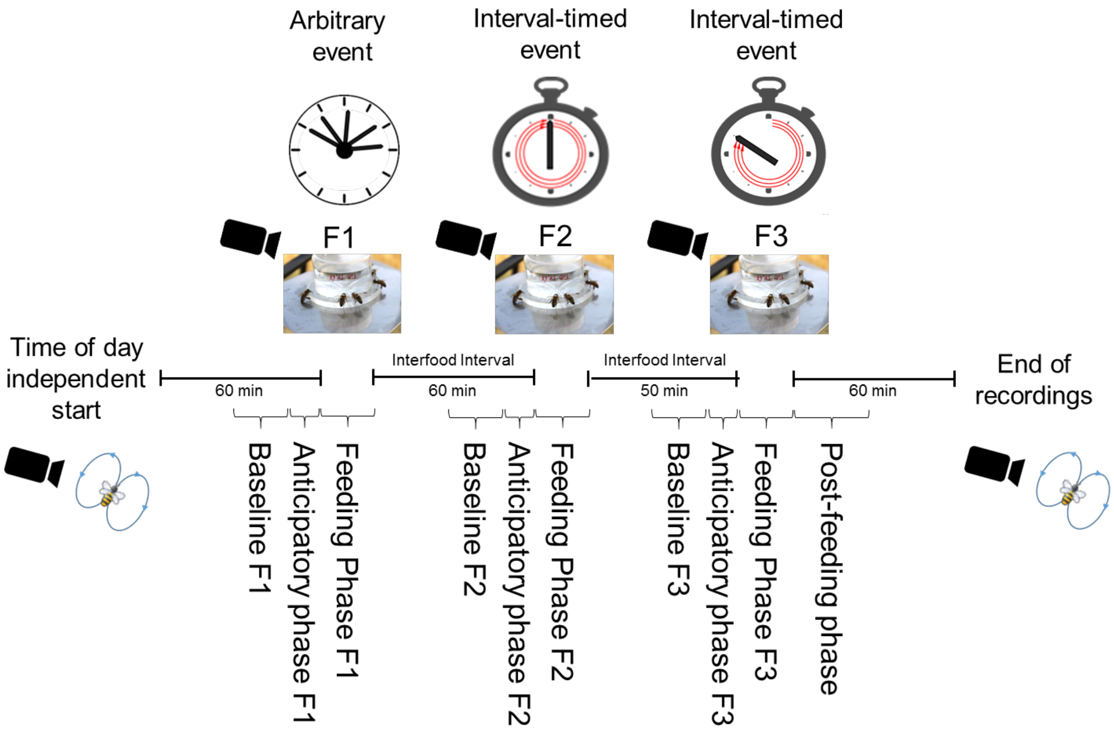
Extended Data Figure IV.1.

Exemplary schedule for testing routines. a) In order to test interval time-place learning skills of honey bees we combined an arbitrary time of day feeding event with further spatially separated but interval time linked sugar-water rewards for five consecutive days. At every test day, the first feeder (Feeder 1) was activated for 20 minutes at different and non-overlapping times of day. This was followed by a non-rewarding 60 minutes interfood-interval before Feeder 2 supplied a sugar water reward for 20 minutes. After an interfood-interval of 50 minutes the last feeder (Feeder 3) was activated for 20 minutes. b) At day six of the experiment, we performed an open-loop test with arbitrary activation of Feeder 1, but Feeders 2 and 3 remained non-rewarded for the whole testing day including the interval scheduled feeder activation times. Four colonies were tested following the same time scheme but with different arbitrary starting events at Feeder 1. Video recordings of feeders and dance floor areas within the hives ranged from one hour before the activation of the Feeder 1 until one hour after closing of Feeder 3.



Extended Data Figure IV.2.

Agricultural study locations close to Würzburg, Germany. Three study sites with the position of the observation hive (black dots), the surrounding landscape within a radius of 0.75 km and three distinct feeder locations (triangles F1, F2, F3) in 300 m to 500 m distance to the hive are shown. At the site Gieshügel 2, two colonies were tested successively, each on a different set of feeders. All four interval timing tests were conducted without interference with other honey bee colonies.



Extended Data Figure IV.3.

Experimental set up: assignment of test phases. Experimental time was divided into four distinct phases: baseline phase, 30 - 10 minutes before feeder (F) activation; anticipatory phase, 10 - 0 minutes before active feeding time; active feeding time; and post-feeding phase, 0 - 30 min after feeder activation.

Extended Data Table IV.1.

Effects of day number and phase on the number of bee visits at interval-timed feeder activation times. Results from Linear Mixed-Effects Models for changes in honey bee feeder visits over time from baseline to anticipatory phase, from baseline to active feeder phase and active feeder phase to post-feeding phase for all feeders and day groupings are shown (see also Figures IV.1, Extended Data Fig. IV.1 for further details).

Phase		**Feeder**	**Estimate**	***F*-value**	**numDF**	**denDF**	***p*-value**
Baseline –							
Anticipatory	Days 1-2	F1	-0.1683	1.9883	1	48	0.165
		F2	-0.1382	1.224	1	44	0.275
		F3	-0.1758	2.3972	1	42.74	0.129
	Days 3-5	F1	0.1621	3.3841	1	68	0.070
		F2	0.14295	2.8607	1	65.83	0.096
		F3	0.12781	4.9803	1	65.95	0.029
	Day 6	F1	-0.08502	0.21229	1	15	0.652
		F2	0.3508	10.693	1	15	0.005
		F3	0.366	4.752	1	15	0.046
Baseline –							
Active feeder	Days 1-2	F1	0.24909	13.129	1	76	0.0005
		F2	0.21824	10.649	1	76	0.002
		F3	0.27335	25.955	1	74.88	< 0.0001
	Days 3-5	F1	0.45073	88.782	1	112.9	< 0.0001
		F2	0.27491	47.36	1	113.93	< 0.0001
		F3	0.30641	137.69	1	113.96	< 0.0001
	Day 6	F1	0.25124	7.534	1	27	0.011
		F2	0.17451	11.594	1	27	0.002
		F3	0.01791	0.041015	1	27	0.841

Phase		**Feeder**	**Estimate**	***F*-value**	**numDF**	**denDF**	***p*-value**
Active feeder –							
Post-feeding	Days 1-2	F1	0.21870	11.836	1	76	0.0009
		F2	0.05674	0.94654	1	74.95	0.334
		F3	0.07315	2.9642	1	76	0.089
	Days 3-5	F1	0.02875	0.40497	1	113.76	0.526
		F2	0.08670	4.9836	1	113.95	0.028
		F3	-0.08664	12.234	1	116	0.0007
	Day 6	F1	0.1242	1.9881	1	27	0.170
		F2	-0.2902	18.383	1	27	0.0002
		F3	-0.08515	1.9894	1	27	0.170

Extended Data Table IV.2.

Effects of day and phase on dance activity of bees for interval-timed resources. A Generalized Linear Mixed-Effects Model for binomial distributed data revealed interacting effects of day and phase on dance activity ($F_{16, 692}$=2.077, p=0.002). Results of a Tukey's test applied post hoc on the Generalized Linear Mixed-Effects Model are shown. Benjamini-Hochberg correction of *p*-values was applied.

Phase		**Feeder**	**Estimate**	***z*-value**	***p*-value**
Baseline – Anticipatory	Days 1-2	F1	0.001	0.001	0.999
		F2	0.324	0.334	0.949
		F3	0.765	0.726	0.696
	Days 3-5	F1	1.454	1.162	0.442
		F2	-0.633	-0.749	0.696
		F3	0.436	0.670	0.696
	Day 6	F1	-0.0001	-0.001	0.999
		F2	-0.00002	-0.000	0.999
		F3	2.729	2.174	0.076
Baseline - Active feeder	Days 1-2	F1	2.345	2.874	0.012
		F2	2.161	3.067	0.008
		F3	2.601	3.198	0.006
	Days 3-5	F1	4.872	4.588	< 0.0001
		F2	2.785	5.307	< 0.0001
		F3	2.895	5.459	< 0.0001
	Day 6	F1	1.110	1.446	0.296
		F2	-0.0004	-0.001	0.999
		F3	1.931	1.654	0.221

Extended Data Table IV.3.

Foraging success on interval-timed food resources (sugar water uptake). Results from a Linear Mixed-Effects Model for sugar water uptake at feeders and post hoc Tukey's test for differences between feeders are shown. Benjamini-Hochberg correction of *p*-values was applied to the Tukey's test.

Linear mixed-effects model	**Explanatory variable**	**Estimate**	***SE***	***F*-value**	**numDF**	**denDF**	***p*-value**
	Days 1-2 vs. 3-5	0.8683	0.2958	8.6188	1	19.613	0.008
	Feeder No.		0.2307	4.833	2	51.694	0.012

Tukey's test	**Linear hypothesis**	**Estimate**	***SE***	***z*-Value**	***p*-value**
	F2 - F1 = 0	0.3358	0.2307	1.455	0.146
	F3 - F1 = 0	0.7169	0.2307	3.107	0.006
	F3 - F2 = 0	0.3812	0.2307	1.652	0.146

V. Combined effects of waggle dance communication and landscape heterogeneity on nectar and pollen uptake in honey bee colonies

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Running title: Effects of dance communication on foraging success

Abstract

The instructive component of waggle dance communication has been shown to increase resource uptake of *Apis mellifera* colonies in highly heterogeneous resource environments, but an assessment of its relevance in temperate landscapes with different levels of resource heterogeneity is currently lacking. We hypothesized that the advertisement of resource locations via dance communication would be most relevant in highly heterogeneous landscapes with large spatial variation of floral resources.

To test our hypothesis, we placed 24 *Apis mellifera* colonies with either disrupted or unimpaired instructive component of dance communication in eight Central European agricultural landscapes that differed in heterogeneity and resource availability. We monitored colony weight change and pollen harvest as measure of foraging success.

Dance disruption did not significantly alter colony weight change, but decreased pollen harvest compared to the communicating colonies by 40%. There was no general effect of resource availability on nectar or pollen foraging success, but the effect of landscape heterogeneity on nectar uptake was stronger when resource availability was high. In contrast to our hypothesis, the effects of disrupted bee communication on nectar and pollen foraging success were not stronger in landscapes with heterogeneous compared to homogenous resource environments.

Our results indicate that in temperate regions intra-colonial communication of resource locations benefits pollen foraging more than nectar foraging, irrespective of landscape heterogeneity. We conclude that the so far largely unexplored role of dance communication in pollen foraging requires further consideration as pollen is a crucial resource for colony development and health.

Keywords: *Apis mellifera*, foraging behaviour, orientation, recruitment, waggle dance, landscape ecology

Introduction

Communication is a key feature in social insect colonies, and allows them to allocate the colony's work force effectively to necessary tasks at hand (Hölldobler & Wilson 2009; Seeley 1995; Wilson 1971). An example of this is the recruitment for collaborative foraging, where successful scouts guide idle or unsuccessful nest mates to valuable resource locations (Biesmeijer & de Vries 2001; Dechaume-Moncharmont et al. 2005; Seeley 1983). The honey bee waggle dance found in the genus *Apis* is a unique, highly sophisticated and well-studied recruiting behaviour. Honey bees are also capable of spreading information about the resource environment via dance-independent behaviours, e.g. by offering samples of gathered nectar to nest mates via trophallaxis (Farina et al. 2005; Grüter et al. 2006). The waggle dance, however, does not only provide a motivational component that includes information about the presence and identity of rewarding resources. It also includes the well-known instructive component (Menzel et al. 2011; von Frisch 1967). A dancing forager communicates the distance and flight angle relative to the sun's current azimuth, and hence the relatively precise spatial position of a rewarding food source (von Frisch 1967).

Studies on the western honey bee (*A. mellifera* L.) revealed that waggle dances are highly efficient in recruiting foragers to artificial food sources (Sherman & Visscher 2002; von Frisch 1967) and enable honey bee colonies to concentrate their foraging efforts to the most rewarding resources (Schmickl & Crailsheim 2004; Seeley 1986; Seeley 1995; Seeley et al. 1991). Continuous information exchange about variable resource patches could increase resource uptake rates of honey bee colonies significantly (Donaldson-Matasci & Dornhaus 2012), and provide fitness advantages (Brown 1988; Dyer 2010; Seeley & Visscher 1988). Dance communication might also allow for selective pollen foraging by allocating the colony worker force to preferred pollen sources (Danner et al. 2016), which enables more consistent exploitation of high-quality resources (Donaldson-Matasci & Dornhaus 2014). Pollen quality may differ significantly between plant species (Haydak 1970), and pollen quality and diversity are important factors for honey bee health (Alaux et al. 2017; Alaux et al. 2010; Di Pasquale et al. 2016; Di Pasquale et al. 2013).

Nevertheless, the importance of dance communication for an efficient use of nectar or pollen resources in agricultural landscapes is still unclear. Benefits of spatial information conveyed by the instructive component of waggle dancing for colony fitness were found to be highly dependent on resource density, quality and distribution (Donaldson-Matasci & Dornhaus 2012; Donaldson-Matasci & Dornhaus 2014; Dornhaus & Chittka 1999;

Dornhaus & Chittka 2004; Dornhaus et al. 2006; Okada et al. 2012; Sherman & Visscher 2002). Dornhaus & Chittka (2004) were able to show a significant effect of dance communication on resource uptake in a complex tropical environment. However, there were no detectable benefits of the instructive component of dance communication in human-modified temperate regions, where distribution of resource patches was less complex (Dornhaus & Chittka 2004). Within a temperate landscape the benefit of dance communication may change with shifting resource conditions over the seasons (Sherman & Visscher 2002). Landscapes may differ in the number, proportion and spatial arrangement of different habitat types (Tscharntke et al. 2005), which affects resource distributions. Human-modified temperate landscapes are often dominated by intensively used arable land (Benton et al. 2003; Robinson & Sutherland 2002). They are characterized by few large habitat patches (Tscharntke et al. 2005), including mass-flowering crops that provide plenty of easily available resources (Holzschuh et al. 2016; Westphal et al. 2003). Such areas have low landscape heterogeneity and form simple resource environments, with easy to find resource patches. This can reduce the value of instructive information exchange between foragers (Beekman & Lew 2008). In addition, landscapes may contain varying amounts of semi-natural habitats for which pollen foragers show a strong preference (Danner et al. 2016; Steffan-Dewenter & Kuhn 2003; Steffan-Dewenter et al. 2002). Semi-natural habitats increase the complexity of a resource environment by generating a more heterogeneous landscape. Here mean patch sizes are more variable and generally smaller (Beekman & Ratnieks 2000; Steffan-Dewenter et al. 2002). Increased heterogeneity and decreased patch size raise the value of instructive information exchange among honey bee foragers (Beekman & Lew 2008). Accordingly, the dance frequency of honey bee foragers increases with higher proportion of semi-natural habitats (Steffan-Dewenter and Kuhn 2003).

The design of previous studies may have obscured some beneficial effects of waggle dance communication (Schürch & Grüter 2014). Honey bee colonies in previous studies remained at the same location during the whole experimentation time. This means that foragers were able to gather and exchange information about resources during phases when communication was not disturbed (Sherman and Visscher 2002; Dornhaus and Chittka 2004b; Donaldson-Matasci and Dornhaus 2012; Donaldson-Matasci et al. 2013) and were probably able to profit from this information while dance communication was disrupted.

The aim of our study was to investigate the importance of the information about resource locations conveyed by honey bee dance communication for nectar and pollen foraging success of colonies exposed to landscapes with varying resource heterogeneity. We experimentally disrupted the instructive component of dance communication in honey bee colonies and measured nectar and pollen uptake rates. Unlike previous studies, we performed this in a number of spatially separated human-modified temperate landscapes featuring a variety of levels of complexity and resource availability. For the first time in this context, we used landscape heterogeneity, i.e. heterogeneity in the spatial arrangement of resource patches within a landscape, which describes the complexity of the resource environment on the landscape level and independently from the amount of available resources. We expected that the value of dance communication for colony performance would increase with decreasing resource availability and increasing landscape heterogeneity.

Material and Methods

Study region

The study was conducted in Central Europe, in the vicinity of Würzburg, Germany. Within the study region, simple landscapes, dominated by intensive agriculture, and complex landscapes with a mixture of arable land, woodland, hedgerows, meadows and settlements can be delineated. In order to assess the role of waggle dance communication in different resource environments we selected eight circular landscapes (distances among landscapes ranged from 5.0 to 31.2 km) with differing proportions of intensively used arable land and semi-natural habitats (Tab. V.1). Landscapes were analysed within a radius of 2 km (1265.64 ha area), because mean bee foraging distances under comparable circumstances were shown to lie well within this range (Steffan-Dewenter & Kuhn 2003), and more than 90% of pollen foraging recruitments advertise patches within this distance to the colony (Danner et al. 2014). The experiment took place in late summer 2013 (18th July – 18th August 2013).

Table V.1.

Landscape parameters of the eight selected landscapes for a 2000 m radius buffer around experimental colonies. Flower cover is given for the two distinct mapping periods. Means ± standard errors and ranges.

Landscape parameter	**Mean ± *se***	**Range**
% Semi-natural habitat	7.8 ± 2.5	0.4 - 16.6
% Arable land	71.5 ± 5.7	51.1 - 89.6
Flower cover - Period A [ha]	23.9 ± 2.9	11.1 - 33.3
Flower cover - Period B [ha]	12.5 ± 3.2	3.4 - 26.0
Mean patch size [ha]	1.5 ± 0.2	0.8 - 2.3

Landscape-level floral resource availability and heterogeneity

Resource availability in each of the eight study landscapes was assessed in two steps. Firstly, we distinguished between habitats that provided noteworthy plant resources for honey bees and those that were unlikely to be utilized for foraging. Resource providing habitats were hedgerows, intensively or extensively used grassland, fallows, meadow orchards, maize fields, sunflower fields, legume fields (including alfalfa, white and red clover and legume mixtures) and non-flowering crop fields (predominantly weeds in beet and cereal fields and vineyards). The relative cover of each habitat type was computed using a geographical information system (Arc-GIS) and digital land use data, which was validated by field inspections. Secondly, we estimated total flower cover on the 2000m scale. For this purpose, flower cover was assessed in at least three randomly selected 100m² plots in each habitat type that provided measurable amounts of resources (Scheper et al. 2015). Total flower cover was extrapolated by summing estimations of mean flower cover per area multiplied by the relative cover of each habitat type across all habitat types in each landscape. In order to keep track of changes in resource distributions over time, the assessment of the flower cover was done twice. The two discrete timespans for which flower cover was assessed in this study were named period A and period B. Period A lasted from 17th July to 2nd August 2013, while vegetation period B lasted from 3rd August to 18th August 2013. Mean patch size of resource-providing habitats, a configurational measure of landscape complexity, was used as proxy for resource heterogeneity in the landscape. Heterogeneous and more complex resource environments are characterized by small mean patch sizes. Flower cover (resource availability) and mean patch size (landscape heterogeneity) were not correlated significantly ($r = -0.30$; $t = -1.16$, $df = 14$, $p = 0.265$).

Study organism

Twenty-four colonies of *Apis mellifera carnica* were established on 11th July 2013 by making nucleus colonies that were equal in size. Each colony was provided with three fully occupied brood combs, two food combs (Zander measure) and a mated queen. All queens were sister-queens from a professional breeder (Schüler, Münster, Germany). Nucleus colonies were inserted into hive boxes with nine frames. The empty space was filled with two empty combs and two wax sheet frames. Sets of three honey bee colonies were placed in the centre of each study landscape on individual levelled tables.

Disruption of waggle dance communication

The hive box design enabled us to disrupt the instructive component of waggle dance communication using a method following the established approach of Sherman & Visscher (2002) and Dornhaus & Chittka (2004). The hive boxes were placed on levelled tables, and rotation of hives by 90° allowed for combs to be positioned horizontally, preventing bees from orienting their dances in a specific angle to the gravitational cue. All incoming foragers in rotated hives were forced to enter the hive box via the top frame next to the window, to encourage them to dance there (Dornhaus & Chittka 2004). Combs were held in place by a tight-fitting slot system that prevented tilting while hive boxes were rotated. In a dark hive without additional cues, dances are performed in random directions and no longer provide consistent spatial information about resource locations (Dornhaus & Chittka 2004; Sherman & Visscher 2002; von Frisch 1967). The successful disruption of waggle dance orientation on horizontal combs in our experimental hive boxes was confirmed by in-hive video recordings (Fig. V.S1). Dance orientation on horizontal combs can be re-established if dancers are allowed to see the sun, blue sky or any directional light source (Sherman & Visscher 2002; von Frisch 1967). As an additional treatment we attempted to restore dance orientation by providing a directional light source in form of a closable circular window of 2.5 cm in diameter. However, dance observations revealed that dance orientation could not be fully restored (Fig V.S1). Therefore, we do not report results of this treatment.

We analysed groups of colonies with (1) disrupted communication: combs were positioned horizontally and dances were disoriented, in order to investigate the impact of disrupted dance communication; and (2) intact communication: combs were positioned vertically in a dark hive, allowing for unimpaired dances.

All sets of three colonies were moved between the eight landscapes every fourth day during night time. This was repeated seven times, so that each set of colonies was placed in each of the eight landscapes for four days by the end of the experiment. For the statistical analysis, each four-day period was regarded as a distinct time step. All communication treatments were randomly re-assigned to the three colonies in each landscape at each time step. All 24 colonies were tested in each treatment and landscape. Minimum distance between consecutive colony locations was ten kilometres to prevent foragers from returning to former colony sites (mean = 19.2 km, *SD* = ±6.9 km). At the same time, this procedure reduced the value of information about the resource environment that was previously acquired by foragers. This prevented carry-over effects from masking the influence of waggle dance communication on resource uptake. The spatial arrangement of resource patches and of landmarks that could guide workers during foraging flights differed considerably between landscapes. Foragers were shown to perform waggle dances advertising resource patches in up to 4.4 km distance on the first day after moving to a new environment (Danner et al. 2014). Due to the methodology, it was not possible to record data on colonies blindly.

Colony development

The presence of the marked queen and brood in the colonies was confirmed every eight days and the total brood area was estimated. In one colony, a queen had to be replaced by a reserve sister queen, because she died in the course of the experiment. Data obtained from this colony were not excluded from the presented models, as excluding data did not significantly change model outcomes.

Colony weight

Colony weight change is supposed to reflect resource uptake on colony level. Nectar is the main factor influencing colony weight changes on a daily basis (Meikle et al. 2008; Seeley 1995). A portable platform balance (Kern EOB35K10) was used to weigh the colonies. Each colony was weighed at the beginning of the experiment and on the first and fourth day at each site. Weighing took place during night time, when all foragers were back in their nest and there was no further resource uptake.

Pollen uptake

The complete pollen forage of each colony was sampled throughout the first day that colonies spent in a new landscape. A total of 192 pollen samples was collected. The pollen was gathered using pollen traps with removable perforated plates (5 mm diameter holes) in front of the colony entrance (Keller et al. 2005). Pollen traps were activated during night time after moving the colonies. Deactivation and pollen collection occurred during the consecutive night, following the weighing of colonies.

The pollen samples were stored in a -20°C freezer. Later on, pollen samples were vacuum-dried, cleaned from insect parts and other artefacts and weighed to the nearest 0.01 g using a lab scale (Kern Type 430-33). Mean weight-loss (± *se*) by vacuum drying was 17.5% ± 1.3%.

Statistics

We used linear mixed-effects models in R version 3.4.0 (R Core Team 2017) with the package lme4 (Bates et al. 2014) to test for effects of instructive dance communication, flower cover and mean patch size, as well as respective interactions, on colony weight change and dry-weight of pollen harvest. Effects on colony weight change were only tested during times when pollen traps were not active. Identity of colony, site and time step were included as random factors in each model to address pseudo-replication and design imbalances. *P*-values, degrees of freedom and *F*-values were obtained using the R-package lmerTest using the Satterthwaite approximation for degrees of freedom (Kuznetsova et al. 2015). Minimum adequate models were identified using ANOVA-tests. *P*-values of factors that were not included in the minimum adequate models but that were relevant for the hypotheses were calculated by adding the respective factor to the minimum adequate model. As integral part of the main hypothesis, the effects of communication treatments on dependent variables were always shown in the figures, regardless of statistical significance. Data on pollen dry-weight were cubic-root transformed to meet the assumption of normal distribution for linear models. Model residuals were visually inspected for spatial autocorrelation and violation of assumptions of normality and homoscedasticity. See Tab. V.2 for an overview of tested factors and interactions.

Results

Effects of dance communication on foraging success

Mean daily colony weight changes did not differ significantly between colonies with disrupted or intact dance communication (Fig. V.1; see Tab. V.2 for statistics). Due to the scarcity of floral resources in late summer in the region of Lower Franconia, Germany, all colonies lost weight over the study period and during most time steps (mean weight change = -37.87 g/day, *se* = ±6.04 g/day, *n* = 192; Fig. V.1).

Rotating the combs to a horizontal position and thereby disrupting dance communication significantly reduced the dry-weight of pollen harvest by 40.25% (Fig. V.2; Tab. V.2).

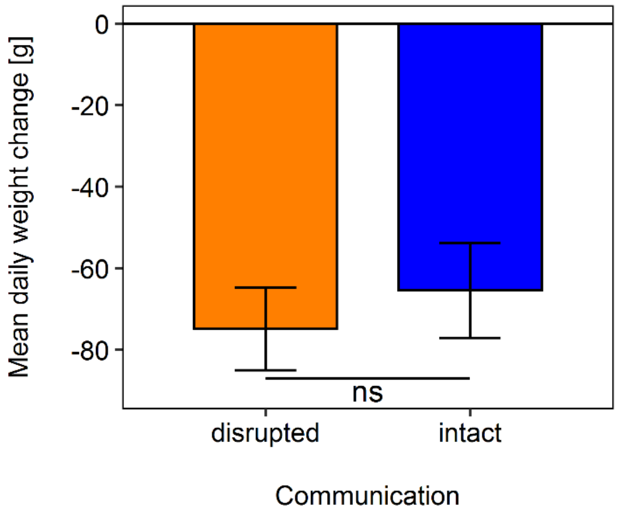

Figure V.1.

Effects of dance communication on mean daily weight change (± *se*) of honey bee colonies. Disrupted: colonies with horizontal comb position and disoriented dances; and intact: colonies with non-affected dance communication on vertically positioned combs. ns: $p > 0.05$.

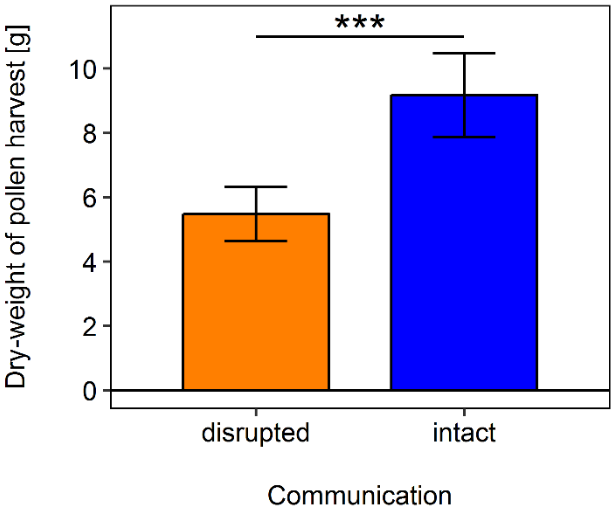

Figure V.2.

Effects of dance communication on mean dry-weight (± *se*) of pollen harvest collected by honey bee colonies. For treatments see Fig. V.1. ***: $p \leq 0.001$.

Effects of flower cover and mean patch size

Mean flower cover of habitat types ranged from 0 – 85.2 % (mean = 8.4 %; *se* = ± 1.6 %). The flower cover in the studied landscapes varied considerably, both among the eight landscapes and between the two distinct mapping periods (Tab. V.1). In every landscape and during each mapping period we recorded highly rewarding patches of nectar-providing crops like sunflower and legume fields, flower-rich areas promoted by agri-environmental schemes, or flower-rich grasslands. Overall there was no significant effect of flower cover on colony weight change (Fig V.3; Tab. V.2) or dry-weight of pollen harvest (Fig. V.4; Tab. V.2).

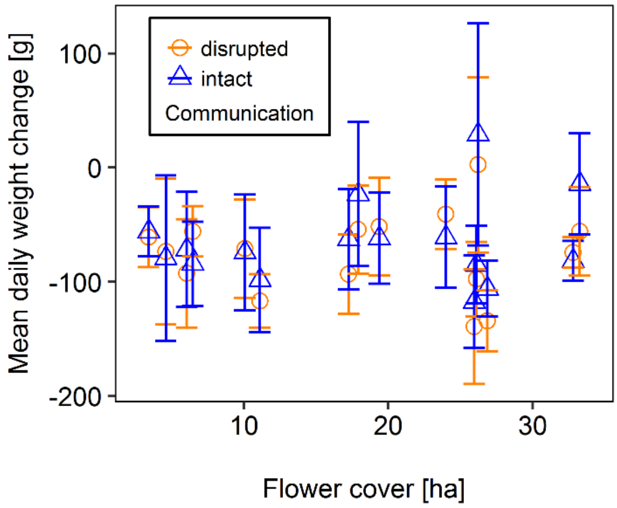

Figure V.3.

The relationship between flower cover and mean daily weight change (± *se*) of honey bee colonies. For statistics see Tab. V.2.

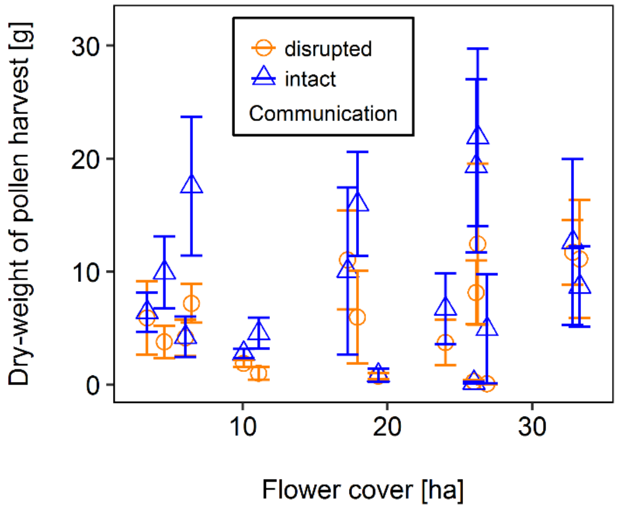

Figure V.4. The relationship between flower cover and mean dry-weight (± *se*) of pollen collected by honey bee colonies.

For statistics see Tab. V.2.

We used mean patch size in a landscape to define landscape heterogeneity (see Tab. V.1 for patch size range), with higher mean patch size in landscapes with lower heterogeneity. Mean patch size was significantly positively correlated with colony weight change (Fig. V.5; Tab. V.2) and dry-weight of pollen harvest (Fig. V.6; Tab. V.2). Additionally, flower cover affected the impact of mean patch size on colony weight change, with stronger effects of mean patch size when flower cover was high (Fig. V.7; Tab. V.2).

There was no significant interaction between flower cover or mean patch size of the studied landscapes and the effect of dance communication on foraging success (Fig. V.3–6; Tab. V.2).

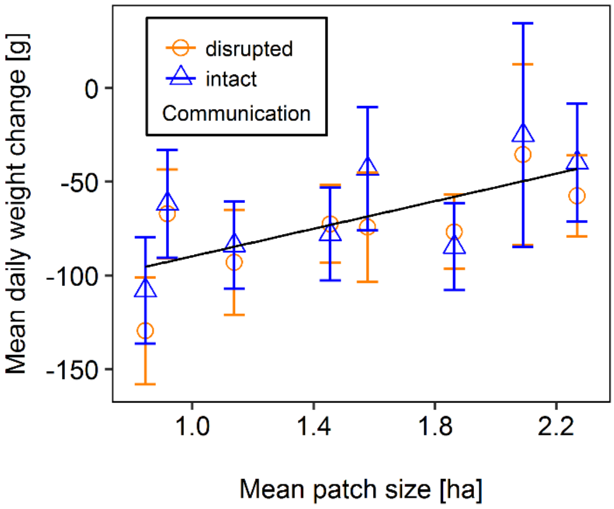

Figure V.5. The relationship between mean patch size and mean daily weight change (± *se*) of honey bee colonies.

Regression line fitted with linear model. For statistics see Tab. V.2.

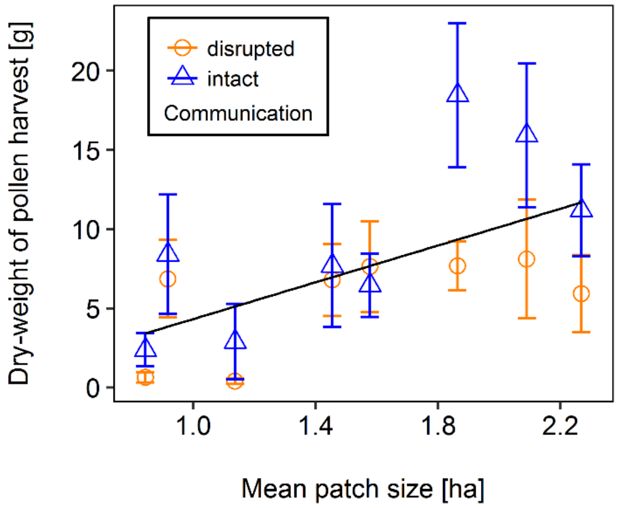

Figure V.6.

The effect of mean patch size within landscapes on mean dry-weight (± *se*) of pollen collected by honey bee colonies. Regression line fitted with linear model. For statistics see Tab. V.2.

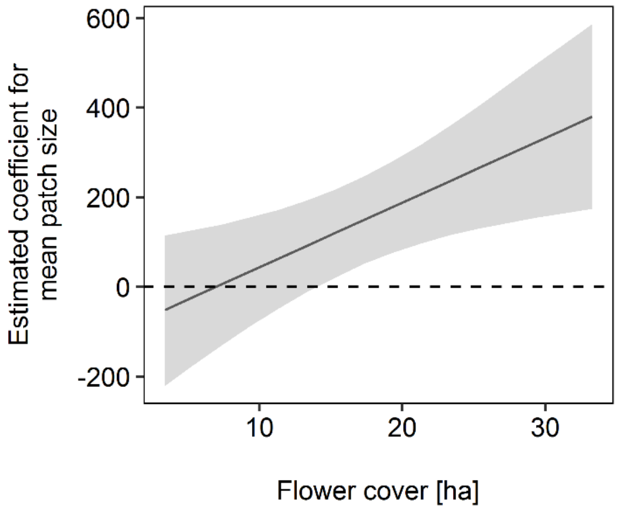

Figure V.7.

The effect of mean patch size on mean daily weight change of honey bee colonies depending on flower cover within the landscape. Grey area: 95% confidence interval. For statistics see Tab. V.2.

Table V.2.

Results of linear mixed effects models relating colony weight change and dry-weight of pollen harvest to explanatory variables. *n* = 8 landscapes, *n* = 24 colonies, *n* = 127 colony weight measurements, *n* = 127 pollen samples.

Explanatory variables	**nDF**	**dDF**	***F***	***p***
Colony weight change [g]				
Communication (Comm)	1	111.93	1.03	0.312
Flower cover	1	125.84	2.64	0.107
Mean patch size (MPS)	1	8.93	10.35	**0.011**
Comm × flower cover	1	111.37	0.40	0.528
Comm × MPS	1	111.43	0.01	0.924
Flower cover × MPS	1	114.93	7.25	**< 0.001**
Comm × flower cover × MPS	1	111.11	0.39	0.532
Dry-weight of pollen harvest [g]				
Comm	1	111.99	11.02	**0.001**
Flower cover	1	107.25	0.52	0.473
MPS	1	7.816	8.18	**0.022**
Comm × flower cover	1	110.75	0.001	0.977
Comm × MPS	1	110.32	1.28	0.260
Flower cover × MPS	1	97.57	1.96	0.164
Comm × flower cover × MPS	1	110.72	1.17	0.282

nDF: numerator degrees of freedom; dDF: denominator degrees of freedom

Discussion

In this study, we analysed the interplay between the instructive component of dance communication and landscape structure, with regard to colony foraging success. Contrary to our hypothesis, we found that honey bee communication about locations of rewarding floral resources did not promote the nectar intake of bee colonies in temperate agricultural landscapes. The amount of pollen collected in colonies within hives that were rotated in order to disrupt dance orientation was reduced by 40%, indicating an important role of instructive communication in pollen foraging. Our data reveal that the amount of brood reared by a colony which is a main driver of pollen foraging activity was not affected by hive rotations (Fig V.S2) but we cannot exclude that the horizontal comb position has further unknown effects on brood rearing behaviour or pollen foraging and storage. Landscape heterogeneity affected nectar and pollen foraging success, but in contrast to our expectation, the benefits of instructive dance communication were not modulated by the complexity of the resource environment. Resource availability within the tested landscapes had no direct effects on nectar or pollen foraging success, but altered effects of landscape heterogeneity on nectar foraging success.

It is important to keep in mind that we, and others, disrupted only the instructive information in waggle dance recruitment behaviour. Waggle dances also include information about the presence of rewarding nectar or pollen sources, as well as about their identity (von Frisch 1967; von Frisch 1968). Dancing foragers are also known to activate idle foragers as well as to reactivate experienced but currently unemployed foragers (Grüter & Farina 2009), so that dancing generally increases forager recruitment (Gilley 2014; von Frisch 1968). Thus dancing can have a positive effect on resource uptake rates that is unrelated to communication of resource location directions.

In our study, colony weight change was not impacted by manipulation of dance communication, although we deliberately placed colonies in experimentally selected environments where effective communication should offer advantages for foraging success. We tested a number of different landscapes that varied significantly in resource availability and heterogeneity. The study was conducted during late summer, when resources in the study region were generally scarce and colonies lost in weight, but some resource-rich patches were still available and information exchange was expected to be valuable (Okada et al. 2012; Sherman & Visscher 2002). Additionally, repeatedly moving the colonies to a new environment created an exceptionally short-living resource environment. This forced foragers to repeatedly update information about locations of profitable resources instead of making good use of previously acquired information, which might have masked effects in earlier experiments (Schürch & Grüter 2014). In contrast to our hypothesis the high temporal turnover and the spatial heterogeneity of resource patches experienced by foragers did not increase the importance of communication. In temperate landscapes the instructive component of waggle dance communication might only prove to be advantageous for nectar foraging in environments under very specific conditions, like strong intra- or interspecific competition (Donaldson-Matasci & Dornhaus 2012; Seeley & Visscher 1988) or during specific seasonal resource distributions (Sherman & Visscher 2002). While the conditions were deliberately chosen in order to identify the specific conditions under which communication of resource location would be beneficial, it is important to note that these conditions are not representative for the whole flowering period. In early spring, for example, resources would also be scarce but possibly much more patchily distributed in form of few flowering trees and scrubs, which may increase the value of directional dance communication. Additionally, if instructive dance communication does only outweigh dancing costs if advertised resources can be used over extended time periods (Schürch & Grüter 2014), repeatedly moving colonies every four days prevented us from identifying these long-term benefits. This should be addressed in future field experiments. Contrary to our findings in nectar foraging, our data show that the disruption of instructive dance communication had a strong negative effect on pollen foraging. To our knowledge, only two related studies also investigated the effect of instructive dance communication directly on pollen forage instead of colony weight change (Donaldson-Matasci & Dornhaus 2012; Donaldson-Matasci & Dornhaus 2014). However, the studies were restricted to Sonoran Desert scrub and grassland habitats. In these non-temperate landscapes, dance communication increased pollen uptake rates independently of resource availability, but only if resource distribution was patchy. Additionally, instructive dance communication also proved to be advantageous, depending on resource conditions (Donaldson-Matasci & Dornhaus 2012). Due to the study design foragers could make use of information on resource locations gathered before communication was disrupted (Schürch & Grüter 2014) or ignore available dance information in favour of previously acquired information on resource locations (Grüter & Ratnieks 2011). Therefore, these studies possibly failed to reveal the actual extent of the effect of dance communication. As colonies in our study were moved to unknown landscapes with considerably different spatial features at the same time at which treatments in individual colonies were changed, we prevented that foragers profited from previously acquired information on resource locations. This allowed us to assess the total benefits of directional dance information under the given conditions. Our findings for temperate landscapes under the conditions of sub-optimal resource availability do not support the hypothesis that resource distribution affects the value of directed dance communication in honey bee colonies. It remains to be confirmed if this is also true when foragers can profit from the directional dance information for a longer period of time, as we only investigated effects on short-term benefits. The fact that in the tested temperate landscapes dance communication always improved pollen foraging, but never nectar foraging, is remarkable. We suspect that this is related to the circumstance that honey bee colonies exploit a higher diversity of plant species for pollen than for nectar (Requier et al. 2015). The identity and diversity of pollen sources may have a strong effect on colony health (Alaux et al. 2010; Di Pasquale et al. 2016; Di Pasquale et al. 2013). Dance communication may allow for a selective and diverse but still effective pollen foraging, but may be less important for effective nectar foraging in temperate landscapes. In fact, it was shown that waggle dance communication affects the composition of pollen forage (Donaldson-Matasci & Dornhaus 2014). A mechanistic explanation for the differences in our findings between nectar and pollen foraging might be, that pollen foragers are more motivated to follow dances and make use of the instructive component of the dances, e.g. of scouts that advertise novel resource patches. It has been shown that previous experience in the field and in-hive olfactory information affect the way foragers deal with available dance information (Farina et al. 2012). In addition, pollen foragers were shown to have a preference for pollen collected from plant species found in semi-natural habitats (Danner et al. 2016) which are generally relatively small, scarce, patchily distributed and probably quickly depleted. Therefore pollen foragers could profit more from the instructive component of dance communication than nectar foragers that commonly forage in presumably more easy to find mass-flowering crop fields or other floral resources with abundant nectar supply (Beekman & Lew 2008). Additionally, pollen advertisement in plants can be more limited in time than nectar advertisement and pollen within inflorescences can be rapidly depleted (Herrera 1990; Stone et al. 1999). High ephemerality of pollen sources and possibly increased competition would increase the benefits of effective communication (Dornhaus & Chittka 2004; Seeley & Visscher 1988). We cannot rule out the possibility that additional factors affected pollen foraging activity, as disrupting dance communication coincided with hive rotation (Sherman &

Visscher 2002). It could be argued that rotating the hives affected the brood, brood-provisioning behaviour or brood rearing activity which is known to be strongly correlated with pollen foraging activity (Al-Tikrity et al. 1972; Dreller & Tarpy 2000; Free 1967; Pankiw et al. 1998). While a small proportion of larvae may be malformed, brood rearing in general and egg-laying activity of queens are not known to be affected by horizontal comb position (Chauvin 1960). In our study the amount of reared brood seemed unaffected by comb position (Fig IV.S2). We cannot exclude that other components of brood rearing activity are affected by hive rotation and further research on this might help to confirm that indeed disruption of the instructive dance communication caused the observed effects on pollen foraging. However, the random exchange of treatments every four days combined with a considerably longer development time of bee brood minimised possible effects of comb rotation on brood rearing. Although our treatment to control for effects of hive rotation by restoring directed dances on horizontal combs did not work, we therefore conclude that the measured effects of comb orientation on pollen foraging success were most probably due to the disrupted instructive dance information. Incoming pollen was only sampled during the first day within a new environment, in order not to disrupt protein supply and hence brood rearing. To which extent our findings can be extrapolated to longer time periods needs further investigation.

Studies on landscape-related foraging patterns of honey bee colonies are still rare (Couvillon et al. 2014; Danner et al. 2014; Danner et al. 2016; Härtel & Steffan-Dewenter 2014). In our study, variation in the generally low resource availability within late summer in temperate landscapes had no direct effect on foraging success. Irrespective of overall resource availability, foragers probably concentrated their efforts on few but most valuable resource patches. However, especially in the most resource rich landscapes, landscape heterogeneity had a strong effect on foraging success. Foraging was most successful in landscapes that contained flower-rich, large and easy to find resource patches, like mass-flowering crop fields. With increasing landscape heterogeneity, i.e. decreasing patch sizes, colony foraging success decreased. Foragers presumably spent less time within the smaller, quickly depleted patches (Cresswell & Osborne 2004) and hence probably more time on travelling between the scattered patches. This may reduce foraging efficiency (Westphal et al. 2006).

Conclusions

Although there is an increasing number of theoretical studies and field experiments addressing the possible benefits of the instructive component of waggle dance communication (Beekman & Lew 2008; Donaldson-Matasci et al. 2013; Donaldson-Matasci & Dornhaus 2012; Donaldson-Matasci & Dornhaus 2014; Dornhaus & Chittka 2004; Dornhaus et al. 2006; Okada et al. 2012; Schürch & Grüter 2014; Sherman & Visscher 2002), this study demonstrates that we still lack some essential knowledge regarding its actual relevance on colony level. Even in heterogeneous temperate landscapes and under specific conditions that were expected to increase the benefits of advertisement of resource locations, there were no short-term benefits of instructive dance communication for nectar foraging. In an unknown environment, individual search abilities of honey bee foragers and newly established knowledge of resource locations may be sufficient to secure colony foraging success. It is possible that communicating nectar resource locations in temperate landscapes will only provide benefits on the long-term (Schürch & Grüter 2014), which was prevented in our study. Importantly, our data indicate that, within temperate landscapes, waggle dancing plays a far more important role in pollen foraging than in nectar foraging. As pollen is the major protein source in honey bee hives, dance communication can be expected to have significant effects on colony development and health. This underpins the potential evolutionary advantage of dance communication and suggests that future research should focus more on pollen foraging ecology of honey bees.

Acknowledgements

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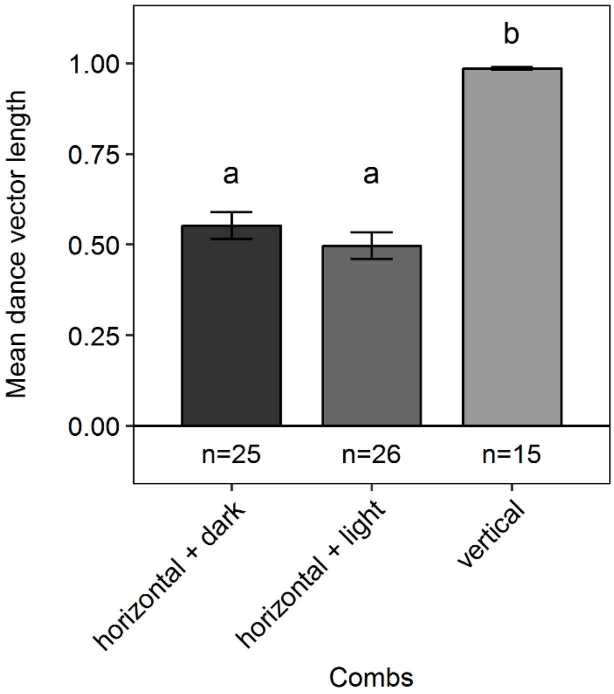
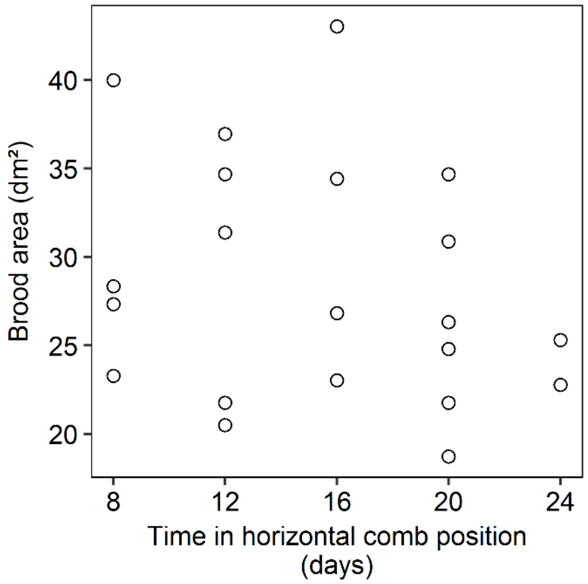


Supplemental Figure V.S1.

Mean vector lengths (± *se*) of waggle dances were used as measure for waggle dance directionality in three initial treatments. Waggle dances were recorded from two honey bee colonies within experimental hive boxes in July - August 2016. Recordings were performed during several days and a number of different weather conditions. Only dances that consisted of more than 5 waggle runs were considered and each first and last waggle run was ignored. Colonies were either left unimpaired (vertical; $n = 15$), i.e. with vertical comb position, or were rotated, so that combs were positioned horizontally. When combs were in horizontal position, we either provided a light source in form of an opened window of 2.5 cm in diameter on top of the hive box (horizontal + light; $n = 26$) or kept the combs in dark with closed window (horizontal + dark; $n = 25$). Mean vector lengths of waggle dances were computed by vector addition of the individual waggle run vectors. A maximum mean vector length of 1 would occur, if runs were directed into the exactly same direction with no variation (maximum directionality). Mean vector length decreases with increasing variation in waggle dance run directions, i.e. decreasing directionality, to a minimum of 0 (no directionality). Treatments differed significantly in mean vector length of waggle dances (Kruskal-Wallis test: $Chi^2 = 34.72$; $df = 2$; $p < 0.001$). Dunn's test revealed that dances on horizontal combs with ($z = -5.59$; $p = < 0.001$) or without light source ($z = -4.96$; $p = < 0.001$) were significantly less directional than dances on vertical combs, which were highly directional. There was no significant difference in the directionality of dances on horizontal combs with either a light source or without light source ($z = 0.69$; $p = 0.264$).



Supplemental figure V.2.

Time spent in horizontal comb position had no significant effect on brood rearing activity in honey bee colonies within 24 days. The area of brood cells (open and capped) was estimated for all colonies after 24 days using the Liebefelder method (Imdorf et al. 1987) and correlated with the time colonies had spent in horizontal comb position. Linear model: $F_{1, 22} = 1.7245$, p=0.2026.

VI. General Discussion

In my PhD-thesis, I investigated the relevance of timing of colony phenology and foraging activity for fitness of honey bee colonies. Further, I identified mechanisms that enable colonies to synchronize their activities with the temporal patterns of the environment. In chapter II, I have shown that late winter conditions have a strong effect on the timing of brood onset after hibernation and therefore colony phenology of *Apis mellifera*. Further, the results of the presented study demonstrate that shifting colony phenology relative to the local environment, e.g. as a result of climate change, can have profound negative consequences for colony development, foraging success, and parasite pressure. To identify the relevant factors for the timing of brood onset a follow up experiment was conducted. The study presented in chapter III demonstrated that brood rearing activity in honey bee winter clusters is affected by environmental conditions. The experiment revealed that ambient temperature plays a major role in the timing of brood onset, but its impact on brood rearing activity is modified by photoperiod. Further, the data indicated the involvement of an internal clock in the timing of honey bee brood onset. The high importance of ambient temperature for the timing of brood onset suggests that global change, with increasing mean temperatures and more frequent events of warm weather in winter, has the potential to disrupt the synchronization between colony phenology and flowering phenology and cause severe fitness losses.

In the following chapters IV and V, I presented two experiments that investigated the relevance and mechanisms of timing honey bee foraging activity within a spatiotemporally heterogeneous resource environment. Honey bee foragers have evolved sophisticated devices that are based on an internal circadian clock and enable them to coordinate activities in a highly variable environment. The experiment presented in chapter IV revealed that honey bees are capable of interval time-place learning and can use it to efficiently exploit temporally restricted resources at different locations within a variable environment. This complex cognitive task was previously only described for vertebrates. In chapter V, it was shown that the unique waggle dance behaviour helps to coordinate foraging activity of colonies even in relatively simple temperate zone resource environments. Disrupting the instructional component of waggle dances had a strong impact on pollen foraging efficiency of colonies, regardless of landscape complexity.

Timing of colony phenology

Timing of brood onset during hibernation proved to be a decisive point for honey bee colony phenology with strong impacts on colony fitness. By experimentally manipulating and desynchronizing brood onset with the local environment in late winter, I could show that timing of brood onset affected colony development, parasite loads and consequently colony fitness. Colonies that were in synchrony with the phenology of the resource environment started to rear brood already in late winter, when there were no resources available yet. These colonies were able to build up larger honey stores during the spring bloom than colonies in which brood onset was delayed. This is pivotal for honey bee fitness. High resource availability is key for successful reproduction (Inouye 2008; Johnson et al. 2017; Seeley & Visscher 1985) and honey stores ensure the survival of colony during the next winter (Seeley 1995; Seeley & Visscher 1985). In a previous study, it was also shown that an early start of brood rearing activity in late winter allows colonies to swarm early in the year and increases survivability of swarms (Seeley & Visscher 1985). On the other hand, an early brood onset was associated with increased loads of the brood parasite *Varroa destructor*. This led to a decreased growth of the worker population, which might reduce winter survivability. As brood rearing is highly energy demanding, it can further be expected that early brood onset also increases the risk of depleting resource storages and lead to starvation of colonies. This kind of trade-off between risks and advantages of starting the reproductive phase already during late winter when no resources are available has also been shown for mammals. Here early emergence after hibernation increases risk of predation and starvation but allows to reproduce early so that the offspring has sufficient time to develop and build up resource storages or fat-tissue before the next winter (Körtner & Geiser 2000; Meyer et al. 2016).

Until now, very little is known about how honey bees achieve a well-timed brood onset that balances risk and rewards of early breeding. Using environmental chambers and a non-invasive method to track winter cluster activity, I was able to demonstrate that timing of brood onset seems to be driven by a complex interaction of at least two environmental factors: ambient temperature and photoperiod. While increasing temperature seemed to be the major trigger of brood onset, the light regime altered the response to a temperature increase. This makes sense, as capped brood demands a relatively high and stable comb temperature, which would be extremely energy demanding at low ambient temperatures. The involvement of photoperiod as additional Zeitgeber might help to prevent a premature brood onset during early winter and decrease the risk of starvation due to mistiming. How exactly ambient temperature and photoperiod interact, however, still requires further investigation. Additionally, the data suggested the involvement of an internal clock in the timing of brood onset. This coincides with findings in mammals. Here timing of hibernation and seasonal torpor is controlled by a combination of a circannual clock and different environmental factors like photoperiod, ambient temperature and food-availability (Collins & Cameron 1984; Heldmaier & Steinlechner 1981; Körtner & Geiser 2000; Mrosovsky 1986; Steinlechner et al. 1983; Wang 1988). Such a complex system is probably necessary to achieve a proper timing in a variable environment where seasonal long-term changes can be superimposed by short-term weather fluctuations (Troein et al. 2009).

However, the adaptive value of this timing system may be at risk. Recent climate change affects winter temperatures with the potential to cause phenology shifts in all organisms that use temperature as a Zeitgeber. Photoperiod on the other hand is not affected by climate change. Different species can rely differently strong on temperature and photoperiod as Zeitgebers. Hence reactions on climate change can also differ. This can lead to mismatches between interacting species and cause substantial fitness losses as was demonstrated by the here presented study as well as several other studies (Both et al. 2009; Hegland et al. 2009; Parmesan 2006; Schenk et al. 2017; Visser & Both 2005). Mismatches between pollinator and flower phenologies for example can reduce fitness of pollinators (Schenk et al. 2017) but also affect reproduction of pollinator dependent flowering species (Kudo et al. 2004; Miller-Rushing et al. 2010). Tracking worker population of the experimental honey bee colonies and local flower availability within the surrounding intensively managed landscapes revealed that the maximum worker population was only reached several weeks after flower availability peaked. When worker population strength peaked, resource availability was very low again. Apparently, honey bee colony phenology is not well adapted to the flowering phenology in intensively managed agricultural landscapes with extremely fluctuating resource abundances. The synchrony between worker populations and depending mass-flowering crops already seems to be poor, limiting the capability to exploit the rich resource offer. Narrow bell-shaped abundance curves, like those observed in floral resources at the two study sites, increase the chances for significant mismatches between interaction partners (Durant et al. 2007; Miller-Rushing et al. 2010). Honey bee colonies in landscapes with short floral resource peaks in spring followed by resource gaps in summer might be especially vulnerable to further seasonal decoupling (Singer & Parmesan 2010). Additionally, the global introduction of the invasive honey bee brood parasite *V. destructor* as unwanted side-effect of globalisation has a major impact on fitness consequences of timing decisions in honey bee colonies. *V. destructor* has strong effects on colony health and is a major driver of winter losses in honey bees (Le Conte et al. 2010; van Dooremalen et al. 2012). This demonstrates the importance to not only focus on consequences of phenology shifts in two interacting species, but to assess consequences in a multi-level interaction web. The benefits of an early brood onset that allows to build up a sufficient worker force to exploit spring bloom and swarm early (Seeley & Visscher 1985) as well as the need to keep track of the forward shift of flowering phenology due to climate change (Williams et al. 2015) conflict with the necessity to keep varroa loads low, which selects for a late brood onset. The honey bee might not be able bridge contrasting adaptive requirements under ongoing climate change.

Coordination of foraging activity in space and time

The presented studies investigated the mechanisms and fitness benefits of coordination of honey bee foraging activity in a spatiotemporally heterogeneous resource environment on two levels. The level of the individual forager and the level of the colony. On the level of the individual forager, experience and knowledge about the spatial and temporal patterns in the resource environment were shown to increase the foraging success. It is known that honey bee workers can combine time memory and spatial memory to learn and associate locations of food sources with fixed times of day when food sources are expected to be active, which is called time-place learning (von Frisch 1967; Wahl 1932). The study presented in chapter IV showed for the first time, that honey bee workers can not only learn fixed times of the day. They are also capable of measuring time intervals to anticipate temporally linked events and different locations. This complex cognitive task called interval time-place learning allows to adapt activity patterns to an environment where variations in abiotic conditions and biotic interactions can lead to more complex daily and seasonal resource dynamics (Jones & Agrawal 2017) and was so far only shown for vertebrates (Carr & Wilkie 1997; Henderson et al. 2006; Thorpe et al. 2012). Interval time-place learning can expected to be a highly valuable ability for honey bee foragers as it allows to adapt activity patterns to the variable spatiotemporal patterns of the floral resource environment (Fründ et al. 2011). Restricting foraging attempts to times when resource patches are anticipated to be rewarding can save energy and make foraging more efficient. The study presented in Chapter IV showed that in a resource environment with predictable cycles honey bee foragers were able use an arbitrary occurring food patch as reference to anticipate food availability at other resource sites. Once foragers got a hang of the spatiotemporal patterns and could successfully anticipate rewarding periods at different feeder sites, net nectar uptake increased considerably. The experiment demonstrated that individual honey bee foragers can use interval time-place learning to optimize timing of foraging activity in a variable resource environment, which is expected to benefit resource uptake and hence fitness of the whole colony.

Honey bee foragers do not function as solitary individuals but act as part of a well organised colony. Communication is used to coordinate a colony’s forager force in space and time. Once a forager discovers a rewarding food patch, it can use the waggle dance communication to guide idle or unsuccessful nest mates to valuable resource locations (Biesmeijer & de Vries 2001; Dechaume-Moncharmont et al. 2005; Seeley 1983; von Frisch 1967). In chapter V, it was shown that the ability to communicate locations of currently active resource patches greatly increased foraging efficiency on colony level. Previous studies could demonstrate that instructive waggle dance communication increases nectar foraging efficiency in a tropical landscape with patchily distributed and ephemeral resources (Donaldson-Matasci & Dornhaus 2012; Dornhaus & Chittka 2004). Benefits on nectar uptake in temperate landscapes, on the other hand, could only be shown under very specific resource conditions (Sherman & Visscher 2002). The study presented in this thesis also failed to reveal any positive impact of instructive dance communication on nectar foraging efficiency within the studied agriculturally used temperate landscapes, regardless of landscape complexity level. However, waggle dance communication greatly benefitted pollen uptake of honey bee colonies, even within the simplest tested resource environments. Pollen advertisement in plants can be more ephemeral than nectar advertisement and pollen within inflorescences can be rapidly depleted (Herrera 1990; Stone et al. 1999). This could explain the increased importance of dance communication in pollen foraging (Dornhaus & Chittka 2004; Seeley & Visscher 1988). Pollen is the main protein and vitamin source for honey bee colonies and identity and diversity of pollen sources can have a strong impact on colony health (Alaux et al. 2017; Alaux et al. 2010; Di Pasquale et al. 2016; Di Pasquale et al. 2013). Honey bee colonies are known to exploit a higher diversity of plant species for pollen than for nectar (Requier et al. 2015) and prefer to forage pollen from plant species found in semi-natural habitats instead of mass-flowering crop fields (Danner et al. 2016). In intensively managed agricultural landscapes, semi-natural habitats that feature a high diversity of resource species tend to be small and variable (Beekman & Ratnieks 2000; Steffan-Dewenter et al. 2002) and probably hard to find. Further, the high diversity of plant species foraged for pollen creates more variable and complex temporal patterns in the resource environment that will challenge foragers. These conditions further increase the value of information exchange (Beekman & Lew 2008). Dance communication might be critical to ensure a healthy pollen diet and instructive dance communication could even be more important for pollen foraging in extremely simple agriculturally used temperate landscapes than in more diverse tropical landscapes.

The spatiotemporal coordination of foraging activity on the level of the individual and the colony are clearly closely linked. The ability of foragers to learn and predict spatiotemporal patterns of resource availability within the environment makes them highly efficient scouts for the colony. In chapter IV, we have seen that a small fraction of experienced foragers starts to visit resource patches persistently several minutes before they are expected to be active, but rarely waste energy to check resources when they are not expected to be rewarding. Most of the experienced foragers however stay within the nest. These idle foragers were shown to use their time memory to approach the colony’s dance floor when they anticipate the persistent foragers to come back and confirm that a known resource patch is active again (Van Nest et al. 2016). Once a resource patch becomes rewarding, but not earlier, the scouts then start to recruit nest mates and the colony can react and exploit the limited resources very quickly. Thanks to the effective waggle dance recruitment system, only relatively few scouting individuals are needed to keep track of the status of discovered but temporally unrewarding resources, which probably saves a lot of energy. Additionally, integrating the information conveyed from many active foragers enables honey bee colonies to concentrate their foraging efforts to the most rewarding resources (Schmickl & Crailsheim 2004; Seeley 1986; Seeley 1995; Seeley et al. 1991).

The evolutionary success of this species in all major biomes of the world is certainly related to the combined advantages of its unique communication behavior (Seeley 1985; von Frisch 1967) and advanced spatiotemporal learning and memory skills. The common and widely use of pesticides in intensive agriculture, however, could interfere with cognitive abilities and dance communication of honey bee foragers (Belzunces et al. 2012; Desneux et al. 2007; Tison et al. 2016) and hence reduce the capability of colonies to coordinate foraging activity effectively. Additionally, if ongoing intensivation of agricultural practices further reduces the diversity of available flowering resources even the sophisticated foraging system of honey bee colonies will eventually fail to ensure an adequate diet. Especially during periods of resource dearth in summer, which are distinctive for intensively managed landscapes, sufficient provision of nectar and pollen might not be ensured and colony fitness can suffer (Horn et al. 2016).

Conclusions

It can be concluded that honey bee colonies are well adapted to an environment that changes on seasonal and daily basis. The ability to anticipate changes within the environment and the capacity to synchronize with temporal patterns of the environment benefits foraging success, colony health, growth, reproduction, and ultimately colony fitness. Ongoing global change, however, puts colonies at risk as it has the potential to disrupt synchronization of colony phenology with the local resource environment, increase fitness losses due to pathogens, reduce the diversity of flowering resources and thereby disrupt the continuous availability of sufficiently diverse resources. Additionally, intensivation of agricultural practices increases the exposure to harmful pesticides affecting learning and communication performance of workers that are necessary to adapt to a variable environment. Various consequences of global change may even have synergistic negative effects on honey bees (Didham et al. 2007; González-Varo et al. 2013; Potts et al. 2010; Schweiger et al. 2010). While the different studies in this thesis provide valuable insight into the mechanisms and relevance of timing of colony phenology and foraging activities, there are still a lot of gaps in our knowledge. I stress that we urgently need to improve our understanding about timing processes in honey bees in order to identify and mitigate negative effects of global change on this highly important pollinator species.

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Chapter II: Nürnberger F, Härtel S & Steffan-Dewenter I (submitted to *BMC Ecology*). Seasonal timing in honey bee colonies: phenology shifts affect foraging success and parasite reproduction.

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Study Design	FN	ISD	SH		
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Data Collection	FN				
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Methods Development	SH	ISD			
Data Collection	AAM				
Data Analysis and Interpretation	FN	AAM	SH		
Manuscript Writing					
Writing of Introduction	SH	AAM	FN	ISD	
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Fig 7	FN	SH	ISD		
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Tab 2	FN	SH	ISD		
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Härtel S, Mayer AA, **Nürnberger F**, and Steffan-Dewenter I (submitted to *Nature Communications*). Interval time-place learning in honey bees.

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