Timing of wild bee emergence: mechanisms and fitness consequences

Zeitliche Abstimmung des Bienenschlupfes: Mechanismen und Fitnesskonsequenzen

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Affidavit

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Summary

Solitary bees in seasonal environments have to align their life-cycles with favorable environmental conditions and resources. Therefore, a proper timing of their seasonal activity is highly fitness relevant. Most species in temperate environments use temperature as a trigger for the timing of their seasonal activity. Hence, global warming can disrupt mutualistic interactions between solitary bees and plants if increasing temperatures differently change the timing of interaction partners. The objective of this dissertation was to investigate the mechanisms of timing in spring-emerging solitary bees as well as the resulting fitness consequences if temporal mismatches with their host plants should occur. In my experiments, I focused on spring-emerging solitary bees of the genus *Osmia* and thereby mainly on *O. cornuta* and *O. bicornis* (in one study which is presented in Chapter IV, I additionally investigated a third species: *O. brevicornis*).

Chapter II presents a study in which I investigated different triggers solitary bees are using to time their emergence in spring. In a climate chamber experiment I investigated the relationship between overwintering temperature, body size, body weight and emergence date. In addition, I developed a simple mechanistic model that allowed me to unite my different observations in a consistent framework. In combination with the empirical data, the model strongly suggests that solitary bees follow a strategic approach and emerge at a date that is most profitable for their individual fitness expectations. I have shown that this date is on the one hand temperature dependent as warmer overwintering temperatures increase the weight loss of bees during hibernation, which then advances their optimal emergence date to an earlier time point (due to an earlier benefit from the emergence event). On the other hand I have also shown that the optimal emergence date depends on the individual body size (or body weight) as bees adjust their emergence date accordingly. My data show that it is not enough to solely investigate temperature effects on the timing of bee emergence, but that we should also consider individual body conditions of solitary bees to understand the timing of bee emergence.

In Chapter III, I present a study in which I investigated how exactly temperature determines the emergence date of solitary bees. Therefore, I tested several variants degree-day models to relate temperature time series to emergence data. The basic functioning of such degree-day models is that bees are said to finally emerge when a critical amount of degree-days is accumulated. I showed that bees accumulate degree-days only above a critical temperature value (~4°C in *O. cornuta* and ~7°C in *O. bicornis*) and only after the exceedance of a critical calendar date (~10th of March in *O. cornuta* and ~28th of March in *O. bicornis*). Such a critical calendar date, before which degree-days are not accumulated irrespective of the actual temperature, is in general less commonly used and, so far, it has only been included twice in a phenology model predicting bee emergence. Furthermore, I used this model to retrospectively predict the emergence dates of bees by applying the model to long-term temperature data which have been recorded by the regional climate station in Würzburg. By doing so, the model estimated that over the last 63 years, bees emerged approximately 4 days earlier.

In Chapter IV, I present a study in which I investigated how temporal mismatches in bee-plant interactions affect the fitness of solitary bees. Therefore, I performed an experiment with large flight cages serving as mesocosms. Inside these mesocosms, I manipulated the supply of blossoms to synchronize or desynchronize bee-plant interactions. In sum, I showed that even short temporal mismatches of three and six days in bee-plant interactions (with solitary bee emergence before flower occurrence) can cause severe fitness losses in solitary bees. Nonetheless, I detected different strategies by solitary bees to counteract impacts on their fitness after temporal mismatches. However, since these strategies may result in secondary fitness costs by a changed sex ratio or increased parasitism, I concluded that compensation strategies do not fully mitigate fitness losses of bees after short temporal mismatches with their food plants. In the event of further climate warming, fitness losses after temporal mismatches may not only exacerbate bee declines but may also reduce pollination services for later-flowering species and affect populations of animal-pollinated plants.

In conclusion, I showed that spring-emerging solitary bees are susceptible to climate change as in response to warmer temperatures bees advance their phenology and show a decreased fitness state. As spring-emerging solitary bees not only consider overwintering temperature but also their individual body condition for adjusting emergence dates, this may explain differing responses to climate warming within and among bee populations which may also have consequences for bee-plant interactions and the persistence of bee populations under further climate warming. If in response to climate warming plants do not shift their phenologies according to the bees, bees may experience temporal mismatches with their host plants. As bees failed to show a single compensation strategy that was entirely successful in mitigating fitness consequences after temporal mismatches with their food plants, the resulting fitness consequences for spring-emerging solitary bees would be severe. Furthermore, I showed that spring-emerging solitary bees use a critical calendar date before which they generally do not commence the summation of degree-days irrespective of the actual temperature. I therefore suggest that further studies should also include the parameter of a critical calendar date into degree-day model predictions to increase the accuracy of model predictions for emergence dates in solitary bees. Although our retrospective prediction about the advance in bee emergence corresponds to the results of several studies on phenological trends of different plant species, we suggest that more research has to be done to assess the impacts of climate warming on the synchronization in bee-plant interactions more accurately.

Zusammenfassung

Solitäre Bienen aus gemäßigten Breiten müssen ihre Lebenszyklen vorteilhaften Umweltbedingungen und –ressourcen angleichen. Deshalb ist ein gutes Timing ihrer saisonalen Tätigkeit von höchster Relevanz. Die meisten Arten aus gemäßigten Breiten nutzen Temperatur als Trigger um ihre saisonale Aktivität zeitlich abzustimmen. Aus diesem Grund kann der Klimawandel die mutualistischen Interaktionen zwischen Bienen- und Pflanzenarten stören, falls steigende Temperaturen das Timing der Interaktionspartner unterschiedlich verändern. Das Ziel dieser Doktorarbeit war es, die Timing-Mechanismen von Frühlingsbienenarten zu untersuchen, sowie die resultierenden Fitnessfolgen, falls zeitliche Fehlabstimmungen zu ihren Wirtspflanzen eintreten sollten. In meinen Experimenten konzentrierte ich mich auf Frühlingsbienenarten der Gattung *Osmia* (Mauerbienen) und dabei vor allem auf zwei spezielle Arten, nämlich *O. cornuta* und *O. bicornis* (in meiner Studie, die ich im Kapitel IV meiner Doktorarbeit präsentiere, untersuchte ich zusätzlich noch eine dritte Bienenart: *O. brevicornis*).

Kapitel II präsentiert eine Studie, in der ich verschiedene Trigger untersuchte, die solitäre Bienen nutzen um ihren Schlupfzeitpunkt im Frühjahr festzulegen. Dazu untersuchte ich in einem Klimakammerexperiment den Zusammenhang zwischen Überwinterungstemperaturen, Körpergröße, Körpergewicht und Schlupftag. Zusätzlich entwickelte ich ein einfaches mechanistisches Modell, welches mir ermöglichte, meine verschiedenen Ergebnisse in einem einheitlichen Rahmen zusammenzufügen. In Kombination mit den empirischen Daten deutet das Modell stark darauf hin, dass Bienen einen strategischen Ansatz verfolgen und genau an dem Tag schlüpfen, der für ihre individuelle Fitnesserwartung am sinnvollsten ist. Ich konnte zeigen, dass dieser gewählte Schlupftag einerseits temperaturabhängig ist, da wärmere Temperaturen den Gewichtverlust der Bienen während der Überwinterung steigern, was wiederum den optimalen Schlupftag auf einem früheren Zeitpunkt verschiebt, andererseits konnte ich ebenfalls zeigen, dass der optimale Schlupfzeitpunkt von der individuellen Körpergröße bzw. dem Körpergewicht der Biene abhängt, da diese ihren Schlupftag

danach abstimmen. Meine Daten zeigen, dass es nicht reicht alleinig Temperatureffekte auf das Timing der solitären Bienen zu untersuchen, sondern dass wir ebenfalls die Körperkonditionen der Bienen beachten sollten, um die zeitliche Abstimmung des Bienenschlupfes besser verstehen zu können.

In Kapitel III präsentiere ich eine Studie, in der ich den Temperatureinfluss auf den Schlupftermin solitärer Bienen detailreicher untersuchte. Dazu habe ich verschiedene Varianten von Temperatursummen-Modellen getestet, um Temperaturzeitreihen auf Schlupftermine zu beziehen. Die grundlegende Funktionsweise solcher Temperatursummen-Modelle ist, dass der Bienenschlupf auf den Tag prognostiziert wird an dem die Bienen eine bestimmte Menge an Temperatursummen aufsummiert haben. Ich konnte zeigen, dass Bienen Temperatursummen erst ab bestimmten Temperaturen bilden (ab circa 4°C bei *O. cornuta* und circa 7°C bei *O. bicornis*) und erst nach Erreichen eines bestimmten Kalendertages (circa 10.März bei *O. cornuta* und circa 28.März bei *O. bicornis*). Solch ein bestimmter Kalendertag, vor dessen Erreichen und unabhängig von der aktuellen Temperatur keine Temperatursummen gebildet werden, wird grundsätzlich recht selten verwendet und in Phänologie-Modellen zur Vorhersage des Bienenschlupfes, bis heute auch nur zwei Mal. Zusätzlich benutzte ich mein Modell, um rückwirkend den Bienenschlupf über die letzten Jahrzehnte vorherzusagen. Dazu wandte ich das Modell auf Langzeit-Temperaturdaten an, die von der regionalen Wetterstation in Würzburg aufgezeichnet wurden. Das Modell prognostizierte rückwirkend, dass im Verlauf der letzten 63 Jahre die Bienen ungefähr 4 Tage früher schlüpfen.

In Kapitel IV präsentiere ich eine Studie, in der ich untersuchte, inwieweit zeitliche Fehlabstimmungen in Bienen-Pflanzen-Interaktionen die Fitness der solitären Bienen beeinflussen. Dazu führte ich ein Experiment mit großen Flugkäfigen durch, die als Mesokosmos dienten. Innerhalb jedes dieser Mesokosmen manipulierte ich das Angebot an Blüten um Bienen-Pflanzen-Interaktionen wahlweise zu synchronisieren oder zu desynchronisieren. Zusammengefasst konnte ich dabei aufzeigen, dass sogar kurze zeitliche Fehlabstimmungen von drei oder sechs Tagen bereits genügen (Bienen schlüpften zeitlich vor dem Erscheinen der Pflanzen) um bei den Bienen fatale

Fitnessfolgen zu verursachen. Nichtsdestotrotz konnte ich bei den Bienen verschiedene Strategien erkennen, mit denen sie Auswirkungen auf ihre Fitness nach zeitlichen Fehlabstimmungen entgegenwirken wollten. Allerdings könnten diese Strategien zu sekundären Fitnessverlusten folgen da sie zu einem veränderten Geschlechterverhältnis oder einem stärkeren Prasitierungsgrad führen. Deshalb konnte ich zusammenfassend feststellen, dass nach zeitlichen Fehlabstimmungen zu den entsprechenden Wirtspflanzen, die Kompensationsstrategien der Bienen nicht ausreichen, um Fitnessverlusste zu minimieren. Im Falle des weiter voranschreitenden Klimawandel könnten die Fitnessverluste der Bienen nicht nur das momentane Bienensterben weiter verschärfen, sondern auch ihren Bestäubungsdienst an später blühenden Arten minimieren und dadurch Populationen von tierbestäubten Pflanzen beeinträchtigen.

Zusammenfassend konnte ich zeigen, dass Frühlingsbienenarten anfällig für Klimawandel sind, da sie nach warmen Überwinterungstemperaturen früher schlüpfen und einen geringeren Fitnesszustand aufweisen. Da Frühlingsbienenarten bei der zeitlichen Abstimmung ihres Schlupftages nicht nur Überwinterungstemperaturen, sondern auch ihren individuellen Fitnesszustand beachten, könnte dies unterschiedliche Reaktionen innerhalb oder zwischen Bienenpopulationen auf den Klimawandel erklären. Dies könnte ebenfalls Folgen für Bienen-Pflanzen Interaktionen haben und das weitere Bestehen von Bienenpopulationen gefährden. Falls, durch den Klimawandel bedingt, Pflanzenarten ihre Phänologie nicht in Einklang mit der Phänologie der Bienen verschieben, dann könnten Bienen zeitliche Fehlabstimmungen mit ihren Wirtspflanzen erleben. Da Bienen keine einzige Kompensationsmaßnahme aufzeigen, die erfolgreich Fitnessverlusten entgegenwirken konnte, wären in einem solchen Fall die Folgen für Frühlingsbienenarten fatal. Darüber hinaus konnte ich feststellen, dass Frühlingsbienen einen bestimmten Starttag im Jahr beachten, vor dessen Erreichen sie keine Temperatursummen bilden, unabhängig von der aktuellen Temperatur. Ich schlage deshalb vor, dass weitere Studien ebenfalls einen solchen Starttag in Temperatursummen-Modelle einbauen sollten, um die Genauigkeit zur Berechnung des Bienenschlupfes weiter zu verbessern. Obwohl meine retrospektive Vorhersage zum verfrühten Bienenschlupf ziemlich genau den Ergebnissen von verschiedenen Studien zu den phänologischen Verschiebungen von Pflanzenarten entspricht, schlagen wir vor, dass zusätzliche Untersuchungen konzipiert werden müssen um präzisere Aussagen über die Folgen des Klimawandels auf die Synchronisation der Bienen-Pflanzen-Interaktionen liefern zu können.

Chapter I: General Introduction

Plant-pollinator interactions

Almost 90% of flowering plant species rely on animal pollinators (Ollerton, Winfree & Tarrant 2011), whereby 75% of the main crops used directly for human consumption in the world, show increased fruit or seed set with animal pollination (Klein *et al.* 2007). The total economic value of pollination worldwide is said to be €153 billion annually (Gallai *et al.* 2009). Many plants could not set seed and reproduce without pollinators but at the same time, many animal populations would decline without plants to provide pollen and nectar (Kearns, Inouye & Waser 1998). Therefore, plants and animals share a mutualistic relationship in which both partners benefit from each other (Fig.1).

Fig.1 (A) *Bombus terrestris* collecting nectar and (B) *Cetoniinae* on *Echinacea purpurea*. Photos: Mariela Schenk

Many different animal species, for example, hummingbirds, bats, rodents and small marsupials, pollinate plant species (Townsend, Begon & Harper 2008). However, with 16,000 different species (Michener 2000) bees are among the primary pollinators for the majority of flowering plants (Ollerton, Winfree & Tarrant 2011). Therefore, bees are of particular value for ecological systems as well as agriculture. It has been shown that wild insects pollinate crops even more effectively than honey bees (Garibaldi *et al.* 2013). This underpins the importance of wild insect pollinators, like solitary bees, in addition to managed honey bees. Yet the abundance and diversity of wild bees as well as the abundance of honeybees are now declining and some species are clearly at risk (Biesmeijer *et al.* 2006). The causes include habitat fragmentation and other changes in land use, agriculture and grazing, pesticide and herbicide use, and the introduction of alien species (Kearns, Inouye & Waser 1998). Another cause of the current decline of bees may be the advancing climate change. In the last century, global average temperature raised by 0.74°C and the rate of warming even increased in the last decades (Trenberth *et al.* 2007). Increased temperatures and especially changing winter conditions, caused by global or regional climate changes have been shown to influence the seasonal timing of terrestrial organisms (Parmesan 2006; Williams, Henry & Sinclair 2015), shifting the phenologies of most species to an earlier date in the year (Menzel *et al.* 2006; Visser 2013). As some species respond more to climate warming than others (Parsche, Fründ & Tscharntke 2011; Willmer 2012; Posledovich *et al.* 2015; Thackeray *et al.* 2016), temporal mismatches between interacting species are likely to occur (Visser & Both 2005; Memmott *et al.* 2007; Kudo & Ida 2013; Petanidou *et al.* 2014; Schmidt *et al.* 2016). However, it remains unknown if plants or pollinators will shift their phenologies more in response to climate warming. Some studies suggest that plants advance their phenology more than bees in response to early-spring warmth or snowmelt (Forrest & Thomson 2011; Kudo & Ida 2013), and some have reported equivalent shifts among plant and bee species (Hegland *et al.* 2009; Bartomeus *et al.* 2011; Rafferty & Ives 2011). In contrast, other studies have shown that insect phenology has shifted more rapidly than plant phenology over the last several decades (Gordo & Sanz 2005; Parmesan 2007; Willmer 2014). So far, we know little about the fitness consequences of such temporal mismatches. Research effort has mostly focused on the fitness consequences for plants but to date fitness consequences have not been investigated for bees (Forrest 2015). A certain “sit-and-wait-strategy” has been suggested for many plant species when pollinators are lacking (Huang, Takahashi & Dafni 2002), but it remains unknown if bees are also able to simply outlast several days when food plants are lacking. This knowledge gap should definitely be closed as fitness losses to bees that result from temporal mismatches with their food resources could exacerbate the current decline in bees and pollination services in many regions which could have negative consequences for economically relevant plant species (Potts *et al.* 2010; Gonzalez-Varo *et al.* 2013).

Mechanisms of timing

As life-cycles of organisms have to be aligned with favorable environmental conditions and resources (Van Asch & Visser 2007; Donoso *et al.* 2016), a proper timing of their phenological events is highly fitness relevant (Bradshaw & Holzapfel 2007). Pollinating animals like solitary bees, for example, have to time their emergence in order that their activity period matches the phenology of their food plants (and vice versa).

Most of the knowledge on the timing of species involved in interactions comes from studies focusing on individual species (Yang & Rudolf 2010). It has been shown that different species use different environmental triggers to time their phenology. For example, some species use rainfall (Danforth 1999; Penuelas *et al.* 2004) and others use photoperiod in addition to temperature (Caffarra, Donnelly & Chuine 2011) to time their phenological event. However, in temperate environments where there exist different seasons, most species use temperature as a trigger for the timing of their seasonal activity (Fründ, Zieger & Tscharntke 2013; Visser 2013). A basic distinction of the mechanisms in timing between plants and bees in temperate climates may be that plant development mostly accelerates with increasing length of photoperiod and increasing temperature sums, but it can also be decelerated when cold temperatures are missing during winter (Henderson, Shindo & Dean 2003). In contrast, the timing of bee emergence is mainly triggered by temperature (Bosch & Kemp 2003), though chilling temperatures can be needed to initiate winter diapause (Sgolastra *et al.* 2010). However, solitary bees show a considerable variability in emergence dates even if they overwinter at the same location with exactly the same overwintering temperature (Westrich 2011). To our knowledge, the mechanisms underlying this variation cannot be explained yet. If bees would (in addition to temperature) also consider their individual body condition for their emergence decision, the causes of their high variability in emergence dates might be explained. Furthermore, it is said that, in contrast to many plant species, most bee species might be unable to use photoperiod as a trigger for the emergence decision as the cocoons located in above-ground or soil cavities might provide isolation from light stimuli (Wasielewski *et al.* 2011). Due to climate change, global temperature will still increase in the future but day lengths, in contrast, will stay unchanged. Thus, it might be conceivable that some bee and plant species will be desynchronized in the future as the phenologies of bees might shift more pronounced than the phenologies of plants due to their exclusively use of temperature for the timing of their seasonal activity. In opposition to bees, the usage of day lengths in addition to temperature might slightly buffer the phenological shifts in plants in response to climate warming.

In order to investigate how exactly certain environmental triggers influence the phenology of different species, statistical model approaches can be used to relate these triggers to specific phenological events. Such model approaches generally use different parameters like, for example, precipitation rates, wind speed, day lengths or temperature-related parameters to predict the timing of distinct phenological events in different species (Richter *et al.* 2008; Green 2017; Pettit & O'keefe 2017). For several plant species all over the world many of such models have already been developed (Diekmann 1996; Cave *et al.* 2013; Olsson & Jonsson 2014). In contrast, there have been just very few statistical models for predicting emergence dates in solitary bees, and these studies used exclusively temperature-related parameters (White, Son & Park 2009; Ahn, Park & Jung 2014). Here we propose, however, that bees that additionally consider a critical calendar date before which temperatures are irrelevant for the emergence decision, are at an advantage as this may prevent them from emerging much too early in case of spells of warm temperatures early in the season. To date there exists no study that additionally applied such a degree-day model for bees to long-term temperature data (which have been recorded, for example, by a regional climate station), to retrospectively predict the emergence dates of bees over the last several decades. However, such applications of statistical model predictions could be used to assess the long-term effects of climate warming on the phenology of solitary bees more accurately.

Model organism *Osmia* bees

Bees of the genus *Osmia* (Hymenoptera: Apiformes: Megachilidae) occur mainly in temperate climate zones on the northern hemisphere (Michener 1979). Several species of *Osmia* bees are managed artificially, mostly for fruit tree pollination (Yamada *et al.* 1971; Torchio 1981; Bosch 1994). Partly due to their high economic value, there have been several studies on the ecology of different *Osmia* bee species. These studies investigated, for example, the development, winter survival, emergence and longevity of *O. bicornis*, *O. cornifrons*, *O. cornuta* and *O. lignaria* (Bosch & Kemp 2000; Bosch & Kemp 2004; Bosch, Sgolastra & Kemp 2010; Ahn, Park & Jung 2014; Giejdasz & Fliszkiewicz 2016). However, most of these experiments have not primarily aimed to understand the mechanisms and fitness consequences of timing under natural conditions but their main aim was to artificially synchronize the emergence dates of bees to the bloom of fruit trees. This resulted in studies with experimental treatments that did not reflect natural conditions. Therefore, precise ecological implications of their results can only be concluded with inaccuracy.

Seasonal average temperatures reveal that annual warming is clearly evident in winter and spring seasons (Schwartz, Ahas & Aasa 2006). This might lead to the assumption that bee species emerging in early spring are most susceptible to climate change. In accordance, early-flowering plants have already been shown to shift their phenologies more pronounced than late-flowering plants (Fitter & Fitter 2002; Willmer 2012; Ovaskainen *et al.* 2013). As the danger of emerging in the absence of any potential interaction partners is highest in very early spring (Forrest & Thomson 2011), we expected, that bee species emerging in early spring must be better adapted to cope with such circumstances than bee species emerging in late spring. Therefore, I investigated different species of spring-emerging solitary bees, which emerge either in early spring (among the very earliest bee species) or later in spring to finally compare their responses to a warming climate.

In my studies I focused on three different spring-emerging solitary bees of the genus *Osmia*: The hornfaced mason bee *O. cornuta* (Fig.2 A) with an activity period from March until May, the red mason bee *O. bicornis* (Fig.2 B) with an activity period from early April until June and the wallflower mason bee *O. brevicornis* (Fig.2 C) with an activity period from late April until June (Westrich 2011). *O. cornuta* naturally occurs in central and southern Europe as well as in parts of the Middle East (Peters 1977), *O. bicornis* occurs in large sections of Europe and also in northern regions of Africa (Peters 1977), and *O. brevicornis* occurs in some regions of central and southern Europe (Westrich 2011).

Fig.2 Female bee of *O. cornuta* (A), *O. bicornis* (B), and *O. brevicornis*. Photos: Mariela Schenk

Solitary bees becoming active in late spring or summer overwinter as prepupae in the cocoon and complete their development during the following spring and early summer (O'neill *et al.* 2011), while bees becoming active in early spring eclose as adults in autumn and overwinter as adults in the cocoon (Bosch & Kemp 2000). Therefore, spring-emerging solitary bees are able to emerge as soon as temperatures are rising in spring. Male bees in general emerge at least some days if not weeks before the females of the same species (Raw 1972; Westrich 2011). After emergence, bees immediately start to reproduce. Females are able to store the transferred sperm in the receptaculum seminis (seminal vesicle) for several days and thereby their eggs can be fertilized selectively (Krombein 1967). Females of *O. cornuta*, *O. bicornis* and *O. brevicornis* build their nests in pre-established holes, for example, in hollowed plant stems, drilled holes of beetles or abandoned constructions of other insects (Westrich 2011). Inside a nest, bees build up to 20 brood cells in a row (Michener 2000). Thereby, bees first construct brood cells with female (fertilized) eggs and then they construct brood cells with male (unfertilized) eggs more outwardly of the nest. Single brood cells are generally separated via partition walls (but not in all species) (Fig. 3).

Fig. 3 Opened nest of *O. bicornis* with six brood cells in a row, each of which containing an egg (white, oval structure) and pollen provision (yellow). Brood cells are separated by loam partition walls (brown). At the entrance of the nest (at the right), there exists a closure made out of loam to prevent parasites from retrospectively entering the nest. Photo: Paul Westrich

Male brood cells are generally equipped with less pollen provision than female brood cells (Seidelmann, Ulbrich & Mielenz 2010). The birth weight of an individual bee is solely determined by the amount of provision that was stocked by the mother inside its brood cell (and which the larva fully consumes before pupating) (Bosch & Kemp 2002). Body size (or body weight) is a key component of fitness in solitary bees with large individuals having a higher fitness state than small individuals (Larsson 1990; Kim 1997).

Thesis outline

In my thesis, I raised the following questions:

- Do overwintering temperatures and individual body conditions of the bees influence the emergence date of solitary bees? Do solitary bees emerge at a time that maximizes their (expected) fitness? (Chapter II)
- Which particular parameters (lower temperature threshold, degree-day requirements and starting date) should indeed be included in a statistical model for predicting the date of bee emergence? And based on the best identified models for both species and sexes, what is the prognosticated retrospective phenological shift in solitary bees during the last 50 years? (Chapter III)
- What are the resulting fitness consequences in solitary bees after short temporal mismatches with their food plants? And do solitary bees have strategies to mitigate fitness losses when food plants are completely lacking? (Chapter IV)

Chapter II: Overwintering temperature and body condition shift emergence dates of spring-emerging solitary bees

This Chapter is under Revision as: Schenk M, Mitesser O, Hovestadt T and Holzschuh A (*under Revision*) Overwintering temperature and body condition shift emergence dates of spring-emerging solitary bees.

Abstract

Solitary bees in seasonal environments have to align their life-cycles with favorable environmental conditions and resources; the timing of their emergence is highly fitness relevant. Overwintering temperature influences the emergence date and body weight at emergence in several bee species. A high variability in emergence dates among specimens overwintering at the same temperatures suggests that the timing of emergence also depends on individual body conditions. However, possible causes for this variability such as individual differences in body size or weight have hardly been studied.

In a climate chamber experiment with two spring-emerging mason bees (*Osmia cornuta* and *O. bicornis*) we investigated the relationship between temperature, body size, which is not affected by overwintering temperature, body weight and emergence date. Our study shows that body weight declined during hibernation more strongly in warm than in cold overwintering temperatures. Although bees emerged earlier in warm than in cold overwintering temperatures, at the time of emergence, bees in warm overwintering temperatures had a lower body weight than bees in cold temperatures (all except male *O. cornuta*). Among specimens that experienced the same overwintering temperatures, small and light bees emerged later than their larger and heavier conspecifics. By means of a simple mechanistic model we are able to reveal that spring-emerging solitary bees follow a strategic approach and emerge at a date that is most promising for their individual fitness expectations.

Our results suggest that increased overwintering temperature reduces bee fitness because it decreases body weight at emergence. For adjusting emergence dates, bees do not only use temperature but also their individual body condition as triggers. This may explain differing responses to climate warming within and among bee populations and may have consequences for bee-plant interactions and the persistence of bee populations under climate change.

Introduction

In seasonal environments, climate has a powerful influence on the timing of many spring events like the flowering of plants, the breeding of birds and the arrival of migrant species (Walther *et al.* 2002; Parmesan & Yohe 2003; Gordo & Sanz 2010; Aldridge *et al.* 2011). As life-cycles of organisms have to be aligned with favorable environmental conditions and resources (Van Asch & Visser 2007; Donoso *et al.* 2016), a proper timing of phenological events is highly fitness relevant (Bradshaw & Holzapfel 2007). Pollinating insects like solitary bees, for example, have to time their emergence in order that their activity period matches the phenology of their food plants (and vice versa). In the course of spring, the mean ambient temperature and the availability of flower resources are increasing (Schwartz & Karl 1990), leading to increasingly favorable emergence conditions for spring-emerging solitary bees. However, just waiting for the most favorable environmental conditions may not be the best strategy as waiting carries an opportunity cost. Waiting too long may needlessly shorten the overall time available for reproduction and with an increasing number of bees emerged, intra-specific competition for mating partners (in the case of males) and inter- and intra-specific competition for nesting sites (in the case of females) are increasing. Individuals emerging earlier may thus gain fitness benefits (Poethke, Hovestadt & Mitesser 2016). Choosing the right moment for emergence may therefore require balancing these different risks and benefits.

Temperature is generally regarded to have a strong influence on the timing of bee emergence in temperate systems; statistical modelling approaches have already shown that the emergence date of solitary bees can be explained by including temperature-related factors like lower temperature thresholds and degree days requirements (White, Son & Park 2009; Forrest & Thomson 2011). However, solitary bees show a considerable variability in emergence dates even if they overwinter at the same location with the same overwintering temperature (Westrich 2011). To our knowledge, the mechanisms and causes underlying this variation cannot be explained with certainty, but it is speculated to be a (maternal) bet-hedging strategy, which can be expected to pay off in environments with unpredictable environmental variability (Danforth 1999; Hopper 1999; Childs, Metcalf & Rees 2010; Poethke, Hovestadt & Mitesser 2016). With our study we try to understand the ultimate and proximate causes of this high variability in emergence dates of solitary bees.

It has been shown in various species that life-history strategies can be dependent on individual conditions, especially on body size. For example, natal movement in juvenile Atlantic salmon represents a body size-dependent strategy with larger individuals staying closer to the nest than their smaller conspecifics (Einum *et al.* 2012). Another example is that larger males of the red fox show greater presence at boundaries than their lighter conspecifics and therefore have larger territories (Iossa *et al.* 2008). In bees, larger individuals of the same species are able to forage and to collect pollen also when ambient temperatures are low (Stone 1993; Stone 1994). Large and heavy bees may have a higher probability to survive starving periods after emergence in which insects in general rely on fat reserves (Arrese & Soulages 2010; Weissel *et al.* 2012), because larger bees with a high body weight may have larger fat reserves than their smaller conspecifics. In addition, large individuals have more offspring than their smaller conspecifics, which makes body size (or body weight) a key component of fitness in solitary bees (Larsson 1990; Kim 1997). Hence, we expect that within a population of spring-emerging bees, larger individuals are better able to cope with the risks of harsh weather conditions and low food availability, which are associated with early emergence than their smaller conspecifics and therefore emerge earlier to seek potential benefits of early emergence. We thus assume that solitary bees do not only consider environmental factors like overwintering temperature but also their own body condition for adjusting emergence dates. As overwintering temperature also has an influence on the loss of body weight during winter (and therefore on the fitness) of spring-emerging solitary bees (Fliszkiewicz *et al.* 2012; Fründ, Zieger & Tscharntke 2013), we want to disentangle the (partly) mutual relationship between body weight and body size, overwintering temperature and the emergence date of solitary bees. This will enable us to assess the impacts of climate warming on the timing and the fitness of solitary bees more accurately, and to provide more precise predictions about the persistence of these species.

To evaluate these issues, we performed an experiment in three climate chambers in which we focused on the weight loss of bees during hibernation, on their emergence date and on their body size and weight at the time of emergence under average, above average and below average overwintering temperatures. We focused on two solitary bee species of the genus *Osmia* that emerge in early and in mid-spring, respectively. The following questions were addressed: 1) Is the decline of body weight during overwintering steeper under warm than under cold temperatures and are bees that overwinter under warm temperatures at the time of emergence lighter than bees that overwinter under cold temperatures? 2) How do overwintering temperature and body size (or body weight) influence emergence dates? In addition, we developed a simple mechanistic model that allows us to unite our different observations in a consistent framework, and thus to explain the (potential) adaptive benefits of different responses to the environment.

Material and methods

Bees

In our study we focused on two spring-emerging solitary bee species (Hymenoptera: Apiformes: Megachilidae): the hornfaced mason bee *Osmia cornuta* has an activity period from March until May whereas the red mason bee *Osmia bicornis* has an activity period from early April until June (Westrich 2011). Like most solitary bee species that emerge in early spring, *O. cornuta* and *O. bicornis* overwinter in their cocoons as already fully developed adults that remain inside their brood cells; they finally emerge in spring when temperatures are rising (Bosch & Kemp 2002). The initial body weight of a bee (and therefore its initial amount of internal fat reserves) is solely determined by the amount of pollen provision that was stocked by the mother inside its brood cell in the previous season (and which the larva fully consumes before pupating) (Bosch & Vicens 2002). During the overwintering period *O. cornuta* and *O. bicornis* are not provided with food anymore (Westrich 2011) and thus have to live from their internal fat reserves. Male bees in general emerge at least some days if not weeks before the females of the same species (Raw 1972; Westrich 2011). Cocoons of both species were purchased from “WAB Mauerbienenzucht“ (Konstanz, Germany), a commercial supplier of solitary bees. From October 2013 until the start of the experiment in December 2013, cocoons were stored inside a climate chamber at constant 4°C.

Experimental design

In three climate chambers (Panasonic Cooled Incubator MIR-254-PE), we established three overwintering temperature treatments that were based on data on the long-term (65 years) daily means from the regional climate station in Würzburg, Germany (DWD Climate Data Center CDC 2016). For each month between December and June we calculated the monthly mean temperatures that were used to regulate standard temperatures in the climate chambers. On the basis of these values, we implemented the following temperature treatments: (1) warm overwintering temperature (=monthly mean + 3°C), (2) medium overwintering temperature (=monthly mean) and (3) cold overwintering temperature (=monthly mean - 3°C). Temperatures were shifted monthly in all treatments, but were kept constant within months (Table A1). We defined each month to last 30 days. The experiment started on the 1st of December 2013 and lasted until the last bee emerged in the following spring (11th June 2014). To control the accuracy of climate chambers, we recorded temperature and humidity inside the chambers every 20 minutes. For this purpose, sensors (Driesen & Kern DK390 ECH20 HumiLog GP "rugged") were attached in the center of each chamber.

In each climate chamber, initially 600 cocoons per bee species were kept. The cocoons (3600 in total) were individually placed in ID-labelled plastic tubes that were sealed with cotton wool (Fig. A1)

Data recording

Half of the cocoons were used to assess the change in bees’ dry weights during overwintering (300 cocoons x 3 chambers x 2 species =1800 cocoons in total): at the starting date of the experiment and at the last day of each month (every 30 days), we removed 22 cocoons (11 females and 11 males) per species and treatment. Thereto, cocoons were randomly and successively removed from the experiment and opened until we had collected 11 male and 11 female bees; superfluous bees (>22) were dismissed. Directly after removal, bees were killed in a freezer at -80°C. Dry weight was determined after drying specimens for 48 h at 60 °C by weighing dried specimens within 10 min after taking them from the drying oven to avoid humidity absorption. Afterwards, we measured the head width of specimens, as this measure has previously been shown to be a reliable correlate of body size (Bosch & Vicens 2002; O'neill, Delphia & O'neill 2014). To correct dry weight for body size, we calculated the body mass index of bees as representative fitness value (body mass index = dry weight / head width^2).

The other half of the cocoons was assigned to stay inside the chamber until emergence. These bees were used to assess emergence dates and emergence weights. Starting in February, we daily checked and recorded the emergence of bees; emerged bees were removed from climate chambers, killed and dried to determine their body mass index (BMI) as described above. From the initially 300 cocoons per species and treatment, 240-290 bees ultimately emerged (Table A2).

Statistical analyses

For statistical analysis of the data we used software R (R Core Team 2015). All models were calculated for each bee species separately. To test whether the decline of body weight during overwintering is steeper under warm than under cold temperatures, we used a general linear model with temperature treatment (medium vs. warm vs. cold overwintering temperature), sex and time during winter and all their interactions as predictors and with body weight measured monthly as BMI [mg/mm^2] of bees as response variable. For each monthly BMI measurement new specimens were used (see above). Non-significant predictors ($p > 0.05$) were removed from the model in a manual stepwise model selection (Crawley 2007). To detect differences in the BMI at the time of emergence among temperature treatments, we used separate general linear models for males and females with BMI as response variable and temperature treatment as predictor. Temperature treatments were compared using treatment contrasts (Crawley 2007). To assess how overwintering temperature and body size (or BMI) affect the emergence date, we used two general linear models with emergence date as response variable and treatment, sex and body size (or BMI) and all their interactions as predictors. Body size was measured as head size. Non-significant predictors ($p > 0.05$) were removed from the models in a manual stepwise model selection (Crawley 2007). Model residuals were inspected for violation of assumptions or normality and homoscedasticity.

Results

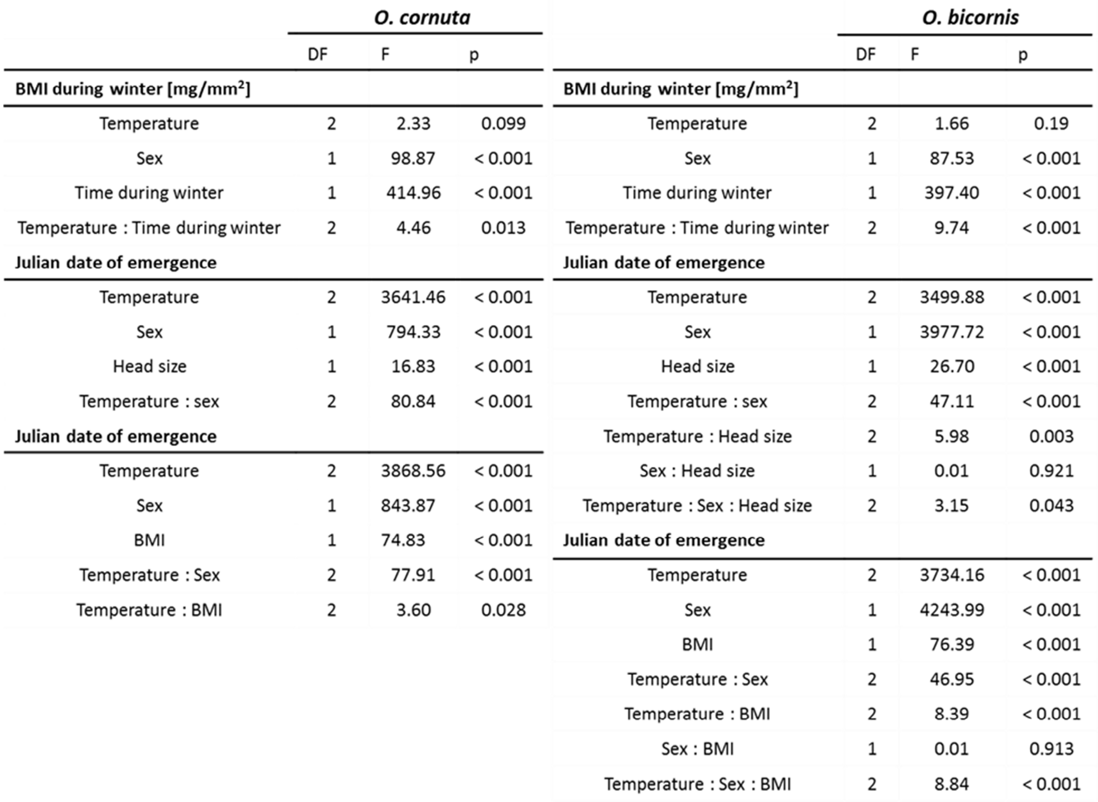
The body mass index BMI [mg/mm^2] of *O. cornuta* and *O. bicornis*, males and females, decreased over time during overwintering. The significant interaction between temperature treatment and time during winter revealed that the slope of this relationship depends on temperature treatment (warm vs. medium vs. cold): The BMI of both species and sexes decreased most strongly under warm temperature and least under cold temperature, with the medium temperature in between (Table 1, Fig. 1).

At emergence, females of *O. cornuta* and males and females of *O. bicornis* had a reduced BMI in the warm temperature treatment in comparison to the cold temperature treatment; and females of both species also had a reduced BMI at emergence in the warm temperature treatment in comparison to the medium temperature treatment. The BMI of male *O. cornuta* was not significantly influenced by temperature treatment (Table 2, Fig. 2).

Temperature treatment (warm vs. medium vs. cold) had an influence on the emergence dates of *O. cornuta* and *O. bicornis* (Table 1). Males and females of both species emerged earlier under warm temperature and later under cold temperature in comparison to medium temperature treatment (Fig. 3).

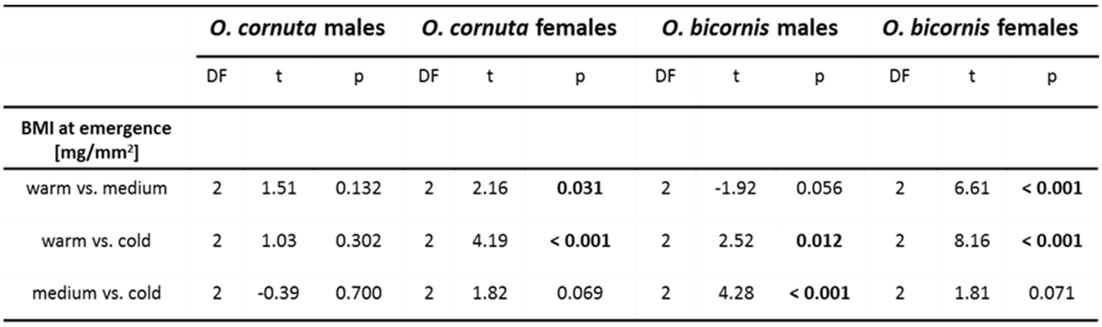
Emergence date and head size (which is a reliable measurement for body size and which is not affected by overwintering temperatures) were negatively related for both species and sexes: individuals with a large head size emerged earlier than individuals with a low head size (Table 1, Fig. 3). In *O. bicornis*, the significant interaction between temperature treatment and head size revealed that the slope of this relationship depends on temperature treatment (warm vs. medium vs. cold), though without a clear pattern among temperature treatments. Emergence date and BMI at emergence were also negatively related for both species and sexes. In all temperature treatments (warm, medium and cold), individuals with a high BMI emerged before individuals with a low BMI (Table 1, Fig. A2). The significant interaction between temperature treatment and BMI in both species revealed that the slope of this relationship depends on temperature treatment (warm vs. medium vs. cold), though without a clear pattern among temperature treatments. Although BMI and head size were overall highly significantly related to the emergence date of bees (Table 1), we occasionally observed 'mass-emergence events' where many bees with differing BMIs emerged on the same day (e.g. on day 120 86 of 113 *O. cornuta* males emerged in the cold temperature treatment and 120 of 156 *O. bicornis* females emerged in the warm temperature treatment).

Table 1 Effects of temperature (warm vs. medium vs. cold overwintering temperature treatment), sex and time during winter on the bee body weight at emergence (measured as BMI [mg/mm2]), and of temperature, sex and head size (body size) on the Julian date of emergence (day 100 ≙ 10th of April). Non-significant predictors ($p > 0.05$) were removed from the model in a manual stepwise model selection.



	O. cornuta				***O. bicornis***		
	DF	F	p		DF	F	p
BMI during winter [mg/mm²]				**BMI during winter [mg/mm²]**			
Temperature	2	2.33	0.099	Temperature	2	1.66	0.19
Sex	1	98.87	< 0.001	Sex	1	87.53	< 0.001
Time during winter	1	414.96	< 0.001	Time during winter	1	397.40	< 0.001
Temperature : Time during winter	2	4.46	0.013	Temperature : Time during winter	2	9.74	< 0.001
Julian date of emergence				**Julian date of emergence**			
Temperature	2	3641.46	< 0.001	Temperature	2	3499.88	< 0.001
Sex	1	794.33	< 0.001	Sex	1	3977.72	< 0.001
Head size	1	16.83	< 0.001	Head size	1	26.70	< 0.001
Temperature : sex	2	80.84	< 0.001	Temperature : sex	2	47.11	< 0.001
Julian date of emergence				Temperature : Head size	2	5.98	0.003
Temperature	2	3868.56	< 0.001	Sex : Head size	1	0.01	0.921
Sex	1	843.87	< 0.001	Temperature : Sex : Head size	2	3.15	0.043
BMI	1	74.83	< 0.001	**Julian date of emergence**			
Temperature : Sex	2	77.91	< 0.001	Temperature	2	3734.16	< 0.001
Temperature : BMI	2	3.60	0.028	Sex	1	4243.99	< 0.001
				BMI	1	76.39	< 0.001
				Temperature : Sex	2	46.95	< 0.001
				Temperature : BMI	2	8.39	< 0.001
				Sex : BMI	1	0.01	0.913
				Temperature : Sex : BMI	2	8.84	< 0.001

Table 2 Results of linear models testing differences among temperature treatments. Shown are treatment contrasts between warm, medium and cold overwintering temperatures. Dependent variable is the BMI [mg/mm^2] of bees at the time of emergence. P-values in bold indicate significant results ($p < 0.05$).



	***O. cornuta* males**			***O. cornuta* females**			***O. bicornis* males**			***O. bicornis* females**		
	DF	t	p	DF	t	p	DF	t	p	DF	t	p
BMI at emergence [mg/mm²]												
warm vs. medium	2	1.51	0.132	2	2.16	**0.031**	2	-1.92	0.056	2	6.61	**< 0.001**
warm vs. cold	2	1.03	0.302	2	4.19	**< 0.001**	2	2.52	**0.012**	2	8.16	**< 0.001**
medium vs. cold	2	-0.39	0.700	2	1.82	0.069	2	4.28	**< 0.001**	2	1.81	0.071

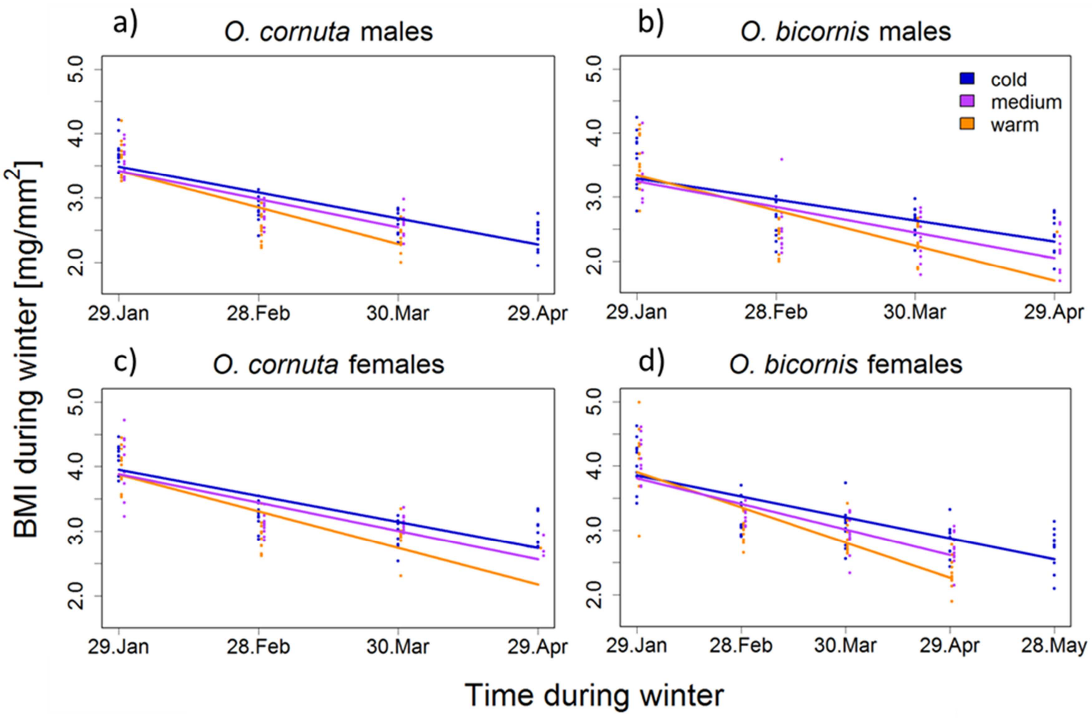


Fig.1 Influence of time during winter on the body mass index BMI [dry weight/head width^2] of a) *O.cornuta* males, b) *O.bicornis* males, c) *O. cornuta* females and d) *O. bicornis* females during overwintering. Measurements at each date are taken from a new group of 22 bees per temperature treatment (orange: warm, purple: medium, blue: cold temperature treatment). Points show the raw data and regression lines represent the results of the general linear model.

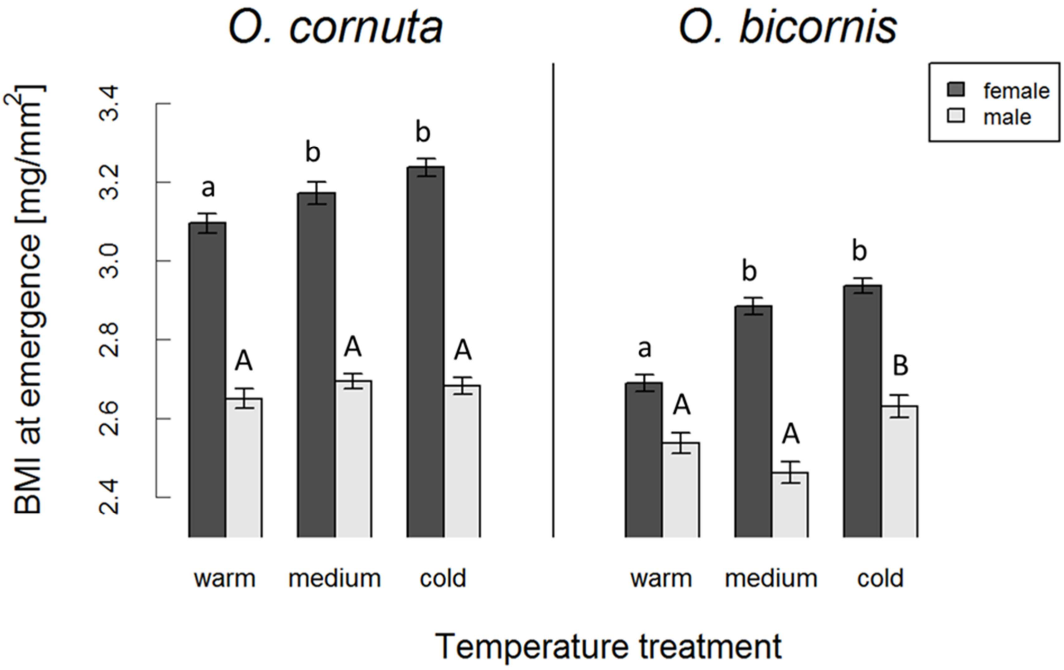


Fig.2 Influence of temperature treatment (warm, medium and cold temperature treatment) on the body mass index BMI [dry weight/head width^2] at emergence of *O.cornuta* and *O.bicornis* males and females. Different letters above bars (means ± SE) indicate significant differences among temperature treatments ($p < 0.05$).

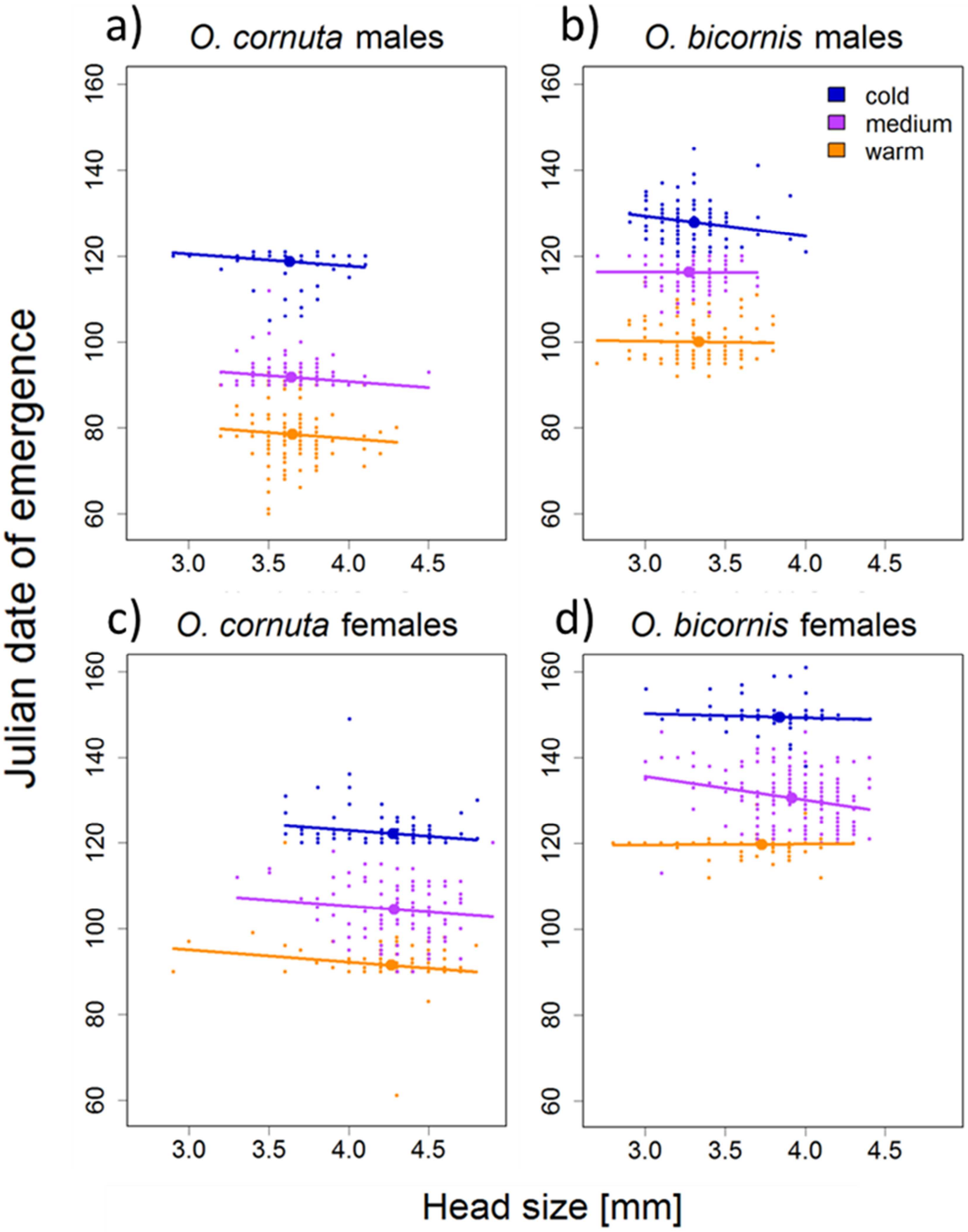


Fig.3 Influence of temperature treatment (orange: warm, purple: medium, blue: cold temperature treatment) and head size (body size) on the Julian date of emergence (e.g. day 100 ≙ 10th of April) of a) *O. cornuta* males, b) *O. bicornis* males, c) *O.cornuta* females and d) *O.bicornis* females. Small points show the raw data and regression lines represent the results of general linear models. Bold points show the mean head size and the mean Julian date of emergence of each temperature treatment.

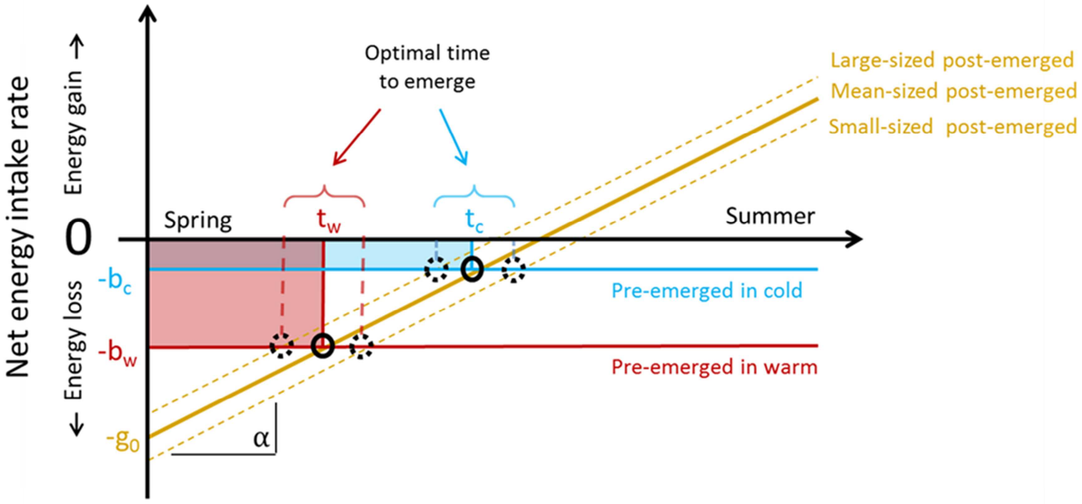


Fig.4 Schematic presentation of mechanistic model predicting the optimum emergence date for bee individuals with different body condition and under different overwintering temperatures. $-b_c$: net energy intake rate for inactive (pre-emerged) bee in cold overwintering temperature. $-b_w$: net energy intake rate for inactive (pre-emerged) bee in warm overwintering temperature. $-g_0$: net energy gain for active (post-emerged) bee from natural resources. α: (daily) increase in net energy intake rate for active (post-emerged) bee in spring. t_w: optimal time to emerge for bee in warm overwintering temperatures. t_c: optimal time to emerge for bee in cold overwintering temperatures. The larger red area represents the cumulated (integrated) amount of weight loss for bees in warm and the smaller blue area represents the cumulated amount of weight loss for bees in cold overwintering temperatures; for further explanations see Appendix 2.

Discussion

We showed that spring-emerging solitary bees lost body weight during hibernation and that the slope of decline over time was steeper in warm than in cold overwintering temperatures. These results are in accordance with previous studies on solitary bees that suggested negative effects of increased overwintering temperatures (Fliszkiewicz *et al.* 2012; Fründ, Zieger & Tscharntke 2013): In warm temperatures metabolic functions are faster and overall energy expenditure is thus higher than in cold temperatures (Vesterlund & Sorvari 2014). Further, at the time of emergence, bees overwintering in warm temperatures had, despite emerging about a month earlier than bees in cold temperatures, a lower body weight than bees overwintering in medium (females of both species) or cold temperatures (all except male *O. cornuta*). This indicates that the fat reserves saved by shortening the overwintering period till emergence did not fully compensate for the higher monthly weight loss in warm temperatures. As fitness depends on body weight (or body size) in solitary bees (Seidelmann, Ulbrich & Mielenz 2010), we assume that bees in warm overwintering temperatures do not only emerge earlier with a decreased body weight but also have lower fitness expectations than bees kept in cold overwintering temperatures. Consistent therewith, it has already been shown that solitary bees show signs of decreased survival and decreased longevity when overwintered in warm temperatures (Bosch & Kemp 2003). Therefore, we conclude that climate warming may strongly threaten the persistence of spring-emerging solitary bees.

As expected on the basis of previous studies (Bosch & Kemp 2003; Bosch & Kemp 2004; Fründ, Zieger & Tscharntke 2013), both bee species emerged earlier in warm than in cold overwintering temperatures. Our study further showed that in all temperature treatments, emergence date was negatively related to body size (which is unaffected by overwintering temperatures), and to the body mass index (BMI) for both species and sexes. Among specimens that experienced the same overwintering temperatures, small (as measured by their head size) and light bees (as measured by their BMI) emerged later than their larger and heavier conspecifics. We are aware of only one single other study that finds, for a desert bee species, a relationship between body weight and emergence date (Danforth 1999). However, the trend reported in that study is just opposite to the one we found: after the onset of annual rainfall period, which marks the onset of food plant flowering, individuals with a low body weight were more likely to emerge than individuals with a high body weight. The half of the population, which was characterized by a high average body weight, remained in diapause for another year. This was interpreted as a bet-hedging strategy in which only the heaviest individuals have enough energy resources for postponing emergence for one year (Danforth 1999). In contrast, our study focused on the variation of emergence dates within one spring season. In temperate climates, larger individuals from the same species have been shown to be able to forage already at cooler temperatures (Stone 1993; Stone 1994) and they might also have larger fat reserves than smaller individuals to sustain life during starving periods outside the nest. Therefore, we conclude that within a population of spring-emerging solitary bees, larger individuals emerge earlier in order to seek potential benefits of early emergence as they may be better able to cope with the harsher weather conditions and the low food availability that occur early in spring.

To further develop this argument we present in the following a simple mechanistic model that provides a coherent and strategic explanation for all our different observations by predicting the optimum emergence dates for bee individuals with different body conditions and under different overwintering temperatures. We will explain the model step by step with a graphical presentation provided in Fig. 4; in the appendix we provide an analytical formulation of the model (Appendix 2). The model is based on our results and on facts known from literature and builds on the assumption that bees take a strategic decision on when to emerge based on balancing fitness expectations associated with either remaining in the cocoon or emerging and becoming active. We first integrated the observed steeper weight loss of bees in warm than in cold overwintering temperatures into our model via the net energy intake rate of pre-emerged bees in warm overwintering temperatures being more negative than the net energy intake rate of pre-emerged bees in cold overwintering temperatures (indicated by the red and the blue horizontal lines in Fig.4). If food resources outside the nest are scarce or absent, a post-emerged, active bee would certainly lose more energy than a pre-emerged, inactive bee in its cocoon. The availability of flower resources is increasing with the onset of spring (Schwartz & Karl 1990) and likely so the probability of successful foraging trips for bees. Therefore, the potential net energy intake rate of post-emerged bees should gradually increase during spring (an effect indicated by the orange upward sloping line in Fig.4). At some moment the negative net energy intake rates of pre-emerged bees in warm and cold overwintering temperatures will both intersect (yet at different points in time) with this orange line; a bee should emerge when its (expected) net energy intake rate becomes larger outside than inside the cocoon. In Fig. 4, these optimal moments of emergence are in each case marked by the black-circled intersection point of lines; our model indicates an earlier optimal emergence date for bees kept under warm compared to those kept under cold overwintering temperatures, which is consistent with our empirical results. In fact, this effect would even be enhanced if warmer temperatures (at the same time) also indicated an earlier availability of flowers (which would shift the line for the potential net energy intake rate of post-emerged bees to the left). Furthermore, the model confirms our results that the weight loss of bees until emergence is greater in warm than in cold overwintering temperatures even though warm temperatures advance the emergence event. This is indicated in Fig. 4 by the differently-sized and differently-colored areas: The larger red area represents the cumulated (integrated) amount of weight loss for bees in warm and the smaller blue area the cumulated amount of weight loss for bees in cold overwintering temperatures; for further explanations on this topic see Appendix 2. To account for the better foraging efficiency of large-sized bees (Seidelmann, Ulbrich & Mielenz 2010), we adjust their respective potential net energy intake rates accordingly: the orange dotted line of the net energy intake rate for large-sized bees lays above the one for small-sized post-emerged bees (Fig. 4). Consequently, the model predicts that the optimal date of emergence occurs earlier for large-sized bees than for small-sized bees under identical environmental conditions as large-sized bees benefit already at an earlier time point from emerging (compare black dotted circled intersection point of lines of large- and small-sized bees, Fig.4). Consistently, we indeed observed earlier emergence of large-sized bees than of small-sized bees. It has not escaped our notice that under certain circumstances ‘mass emergence events’ occurred with many bees with differing BMIs emerging on the same day (Fig. A2). Our model is capable to explain the reasons for these ‘mass emergence events’ and the reasons why these events do not always occur after an abrupt change of temperature (for more details see Fig. A3).

By means of our mechanistic model we are thus able to explain all our different observations as well as facts known from the literature by a consistent theoretical scheme. In combination with the empirical data the model strongly suggests that solitary bees follow a strategic approach and emerge at a date that is most profitable for their individual fitness expectations. Therefore, we conclude that solitary bees do not emerge earlier in warm overwintering temperatures because they cannot afford to wait longer due to their increased weight loss. Instead, solitary bees in warm overwintering temperatures emerge earlier because they benefit already at an earlier date from the emergence event than bees in cold overwintering temperatures. Among specimens that experienced the same overwintering temperatures, larger individuals tend to emerge earlier than their smaller conspecifics, because they benefit already at an earlier date from the emergence event than their smaller conspecifics. Ecological implications of our results may be diverse and depend on the trend of synchronization or desynchronization between bees and plants under climate warming. For example, if under climate warming bees advance their phenologies more strongly than plants (Willmer 2014), our results suggest that particularly large-sized bee individuals will be desynchronized with their food plants, because they emerge earlier than their smaller conspecifics. Bees emerging only six days before the flowering onset of their food plants fail to produce offspring (Schenk, Krauss & Holzschuh 2017). An increased risk of desynchronization for large individuals, which are expected to have higher reproductive output than small individuals (Larsson 1990; Kim 1997), may enhance the negative effects of desynchronization on bee populations. An alternative scenario is that, in response to climate warming, bees and plants show equivalent shifts in their phenologies (Bartomeus *et al.* 2011) or that plants advance their phenologies even faster than bees (Forrest & Thomson 2011; Kudo & Ida 2013). If this scenario is accompanied by resource scarcity this would lead to reduced body sizes (or body weights) in the bee progeny (Bosch & Vicens 2002). Possible causes for resource scarcity and thus reduced pollen resources are habitat loss (Clough *et al.* 2014) or changed precipitation patterns (Rafferty, Bertelsen & Bronstein 2016). A lack of nest cavities leads to the usage of suboptimal smaller nesting tubes which then also leads to smaller offspring (Seidelmann, Bienasch & Prohl 2016). As small-sized bees emerge later than their larger conspecifics, emergence dates of bees could then be delayed which in turn may increase the danger that bees cannot keep pace with the phenology shift in plants and plant pollination decreases.

Conclusion

Our empirical data and our mechanistic model clearly suggest that bees emerge at a time that maximizes their (expected) fitness. We have shown that this date is on the one hand temperature dependent as warmer overwintering temperatures increase the weight loss of bees during hibernation, which then advances their optimal emergence date to an earlier time point (due to an earlier benefit from the emergence event). On the other hand our findings also suggest that the optimal emergence date depends on the individual body size (or body weight) as bees adjust their emergence date according to their foraging ability and possibly their ability to cope with harsh conditions early in the season. We therefore suggest that it is not enough to solely investigate temperature effects on the timing of bee emergence, but that we should also consider individual body conditions of solitary bees to understand the timing of bee emergence. Only then, we will be able to give more precise predictions about the risks and consequences of temporal mismatches between bees and food plants and the persistence of these bee species in times where environmental conditions are changing.

Supporting information

Appendix 1

Table A1 Monthly values of the four temperature treatments. Temperature treatments were based on long-term (65 years) daily means from the regional climate station in Würzburg, Germany: constant warm (=mean + 3°C), constant medium (=mean) and constant cold (=mean - 3°C). Temperatures changed monthly in all treatments, but were constant within months.

	Temperature [°C]						
Treatment	**December**	**January**	**February**	**March**	**April**	**May**	**June**
Warm	3.5	2.5	4.0	8.0	12.5	16.5	20.0
Medium	0.5	-0.5	1.0	5.0	9.5	13.5	17.0
Cold	-2.5	-3.5	-2.0	2.0	6.5	10.5	14.0

Table A2 Number of emerged females and males of *O.cornuta* and *O.bicornis* from each temperature treatment.

	Number of emerged bees		
	Females	**Males**	**Total**
O. cornuta			
Warm	124	129	253
Medium	151	104	255
Cold	127	113	240
O. bicornis			
Warm	156	120	276
Medium	192	94	286
Cold	172	118	290

Fig. A1 Cocoons were stored inside plastic tubes that were sealed with cotton wool. Female *Osmia cornuta* that left its cocoon (=emerged).

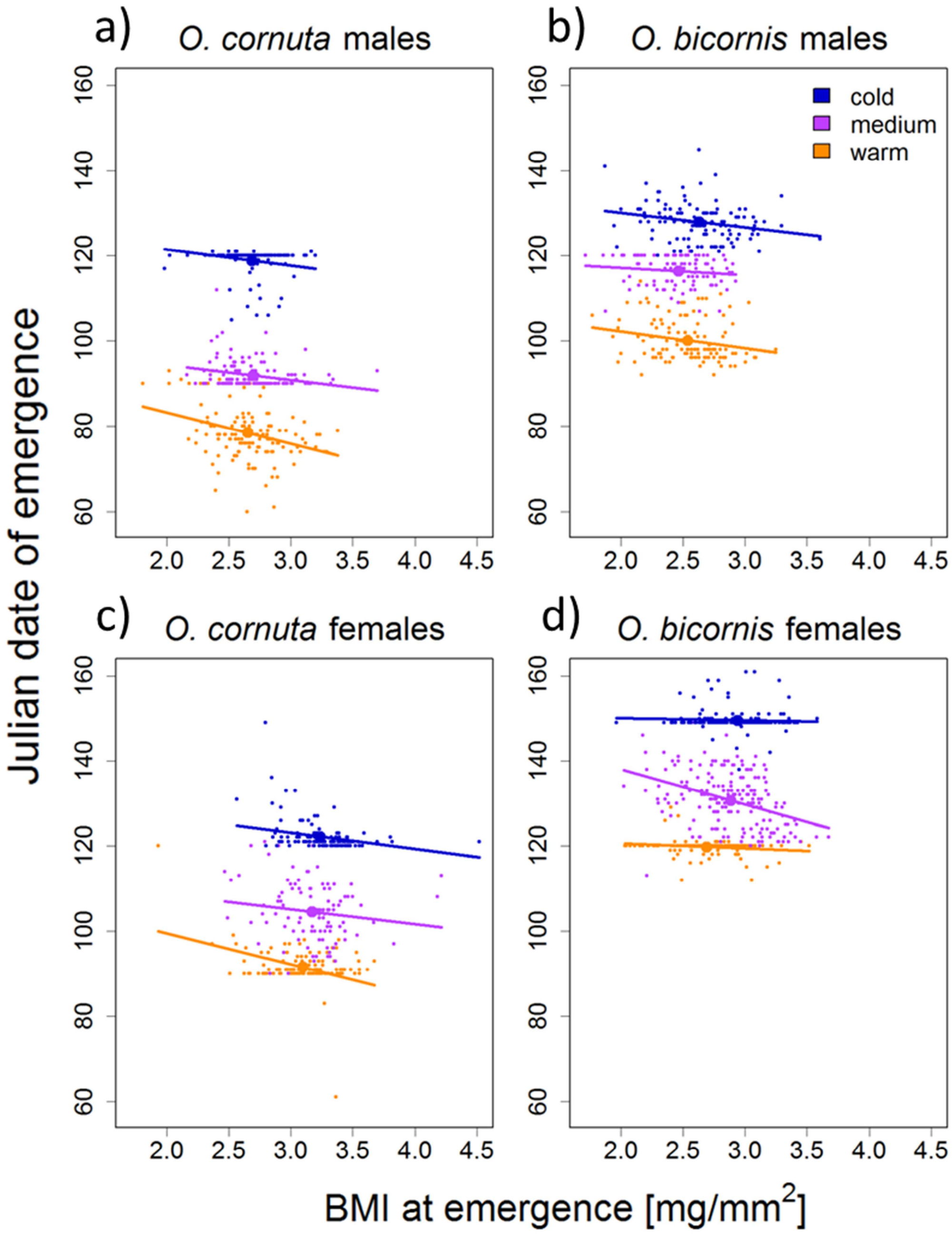


Fig. A2 Influence of temperature treatment (orange: warm, purple: medium, blue: cold temperature treatment) and body mass index BMI [dry weight/head width^2] on the Julian date of emergence (day 100 ≙ 10th of April) of a) *O. cornuta* males, b) *O. bicornis* males, c) *O.cornuta* females and d) *O.bicornis* females. Small points show the raw data and regression lines represent the results of general linear models. Bold points show the mean BMI and the mean Julian date of emergence of each temperature treatment.

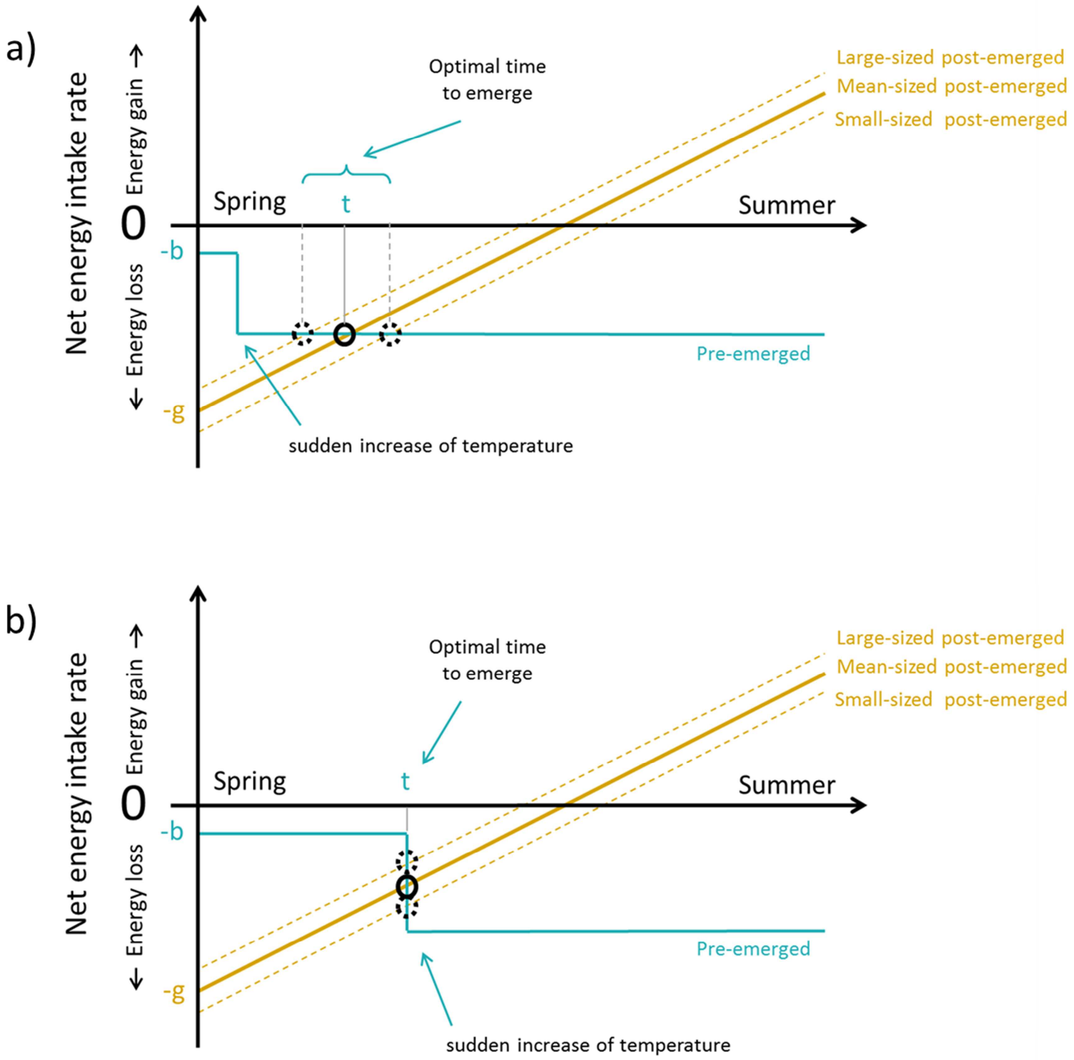


Fig. A3 Mechanistic model predicting the optimum emergence date for bee individuals for a sudden increase in temperature (a) soon enough before bees start to emerge and (b) right before bees start to emerge. -b: net energy intake rate for inactive (pre-emerged) bee. -g: net energy gain for active (post-emerged) bee from natural resources. t: optimal time to emerge.

Explanation: It has not escaped our notice that under certain circumstances 'mass emergence events' occurred with many bees with differing body sizes and BMIs emerging on the same day (Fig. 2 and Fig. A2). As such events were exclusively observed on day 90 and on day 120 after the start of the experiment, we attribute them to the triggering effect of the monthly temperature adjustment (which we performed every 30 days: at day 0, 30, 90, 120 and at day 150 after the start of the experiment). Our model is capable to explain both, the reason for the occurrence of such ‘mass emergence events’ and also the reason why such events do not occur always after an abrupt change of temperature (this further underpins the credibility of our model): In case of a sudden temperature increase (which increases the energy loss of bees during overwintering) soon enough before bees start to emerge (a), the temperature increase has no effect on the natural variability in emergence dates of bees (large-sized bees emerge before small-sized bees). However, in case of a sudden temperature increase right before bees start to emerge (b), many bees with differing body sizes emerge on the same date.

Certainly, the amount of inter- or intraspecific competition for nesting sites and the current amount of available nesting sites or mating partners may also play a role in determining the most profitable emergence date of a bee and our mechanistic model could indeed be extended to account for such additional factors. Nevertheless, already in its current form our model is able to explain all our different results, signaling that we included the most important factors, namely ambient winter temperature and body size (or body weight) affecting the timing of emergence.

Appendix 2

In the following we provide a very simple but mechanistically based model that is built only on three assumptions: (1.) that the metabolic rate and thus fat consumption depends on environmental temperature and is higher under warm than under cool conditions, (2.) that in spring the (expected) net-energy intake rate of an active bee would increase as the season progresses, and (3.) that larger body size improves foraging performance (under cool temperatures) and thus net-energy intake rate. Assumptions (1.) and (3.) were already tested independently as explained in the main text, the second must, in seasonal habitats, at least qualitatively be true for very obvious reasons.

Based on these assumptions we construct a model that is capable to unify and explain the different empirical observations. Note that in principle, the passage of time should be measured from the beginning of hibernation viz. the end of maternal food provisioning in last season. However, as we are interested in explaining the **differences** in energy consumption and timing of emergence as sparked by the experimental treatment, we only consider the winter phase were individuals are exposed to different temperature conditions; before setting up the experiments all bee cocoons were kept under the same conditions.

For clarity of argumentation, we only contrast the situation with regard to two different temperature regimes ('cold' and 'warm') but the model applies to any gradual shift in temperature conditions. We define two net-energy intake rates $-b_C$ applying to inactive (pre-emergent) bees kept under cold conditions and $-b_W$ applying to bees kept under warm conditions. b_C and b_W (or at least the difference between the two) are assumed to be constant throughout the winter time. According to assumption (1.) defined above we specify $-b_W < -b_C < 0$, i.e. inactive bees loose energy at a faster rate if ambient temperatures are warm. The two intake rates are shown as the two blue and red horizontal lines in Figure 4.

Assumption (2.) is accounted for in the model by letting the net-energy intake rate of an active, viz. emerged, bee increases linearly as the season progresses from early spring to late spring and summer, i.e. $b_{act} = -g_0 + \alpha \cdot t$ where α specifies the daily increase in net-energy intake rate for active bees. We (trivially) assume that $-g_0 < -b_W$, i.e. that in mid-winter an active (foraging) bee would lose energy at a faster rate than any inactive bee as there are no resources available at this time. The relationship is indicated by the yellow line in Figure 4.

The two simple assumptions above directly define the optimal moment of emergence in spring; any bee should emerge at just that moment/date (t_C respectively t_W) when for the first time the (expected) net energy intake rate if becoming active surpasses the net-energy intake rate when remaining inactive, that is when

$$\alpha \cdot t - g_0 \geq -b_C \Rightarrow t_C \geq \frac{g_0 - b_C}{\alpha}$$

(1a)

and analogously $$t_W \geq \frac{g_0 - b_W}{\alpha}.$$

(1b)

Because of $-b_W < -b_C$ we can directly conclude that individuals with higher net energy intake rate when inactive should emerge later than those with lower rate, i.e. $t_C > t_W$ as is observed in the data; the expected time lag in emergence is $(b_W - b_C)/\alpha$.

Assumption (3.) indicates that larger bodied bees (or bees that are fitter for other reasons) are more efficient foragers than smaller bees. We can account for this effect in the model simply by shifting the yellow line in figure 4, i.e. by assuming a smaller value for g_0 for large bodied bees (a similar effect would also emerge if they would expect a larger α), and inversely for small bodied bees a larger value for g_0. From equations (1) we can directly conclude that reducing g_0 will result in moving the optimal time of emergence forward as was observed in the data.

A further question is, whether the later emerging bees exposed to cooler temperatures will have consumed more or less fat at the time of emergence than those bees that emerged earlier but were exposed to higher temperature and thus had lower net energy intake rate in the winter.

The earlier emerging bees (those raised in warm winter conditions) will have lost more (or at least as much) energy at the time of emergence than the later emerging bees kept under cold conditions if

$$b_W \cdot t_W \geq b_C \cdot t_C \text{ or } \quad b_W \frac{g_0 - b_W}{\alpha} \geq b_C \frac{g_0 - b_C}{\alpha}$$

(2)

rearranged to

$$g_0(b_W - b_C) \geq b_W^2 - b_C^2 = (b_W + b_C) \cdot (b_W - b_C)$$

and thus

$$g_0 \geq b_C + b_W \qquad (3)$$

we can directly conclude that the slope parameter α does not affect this inequality.

Whether inequality (3) is true or not thus simply depends on whether g_0 is smaller or larger than $b_C + b_W$. Note that g_0 should not be interpreted as a true net energy intake rate that is valid at t_0 – it is indeed unlikely that the net intake rate of an active bee would be the lowest just at the onset of winter. Instead, g_0 is a fictive intersection point that defines the length of the period before the earlier emerging bee become active in relation to the time interval until also the later emerging bees become active.

Both, b_C and b_W must by definition be smaller than g_0 but this does not necessarily hold for the sum of both. We can conclude that if b_W were close to g_0 (that is the bees emerge after a brief overwintering time), b_C needs to be very small to fulfill the condition. However, it is unreasonable to assume that bees could find food resource in the middle of an even mild winter under mid-European conditions (this would be the implication of b_W being close to g_0) and we thus conclude that eq. (3) should typically hold and consequently that bees kept under warm conditions should burn more fat reserves until the moment of emergence than the later emerging bees kept under cold conditions.

Chapter III: Temperature and date are crucial for predicting emergence dates in solitary bees

This chapter is prepared for submission as: Schenk M, Kehrberger S, Mitesser O, Hovestadt T and Holzschuh A (*in preparation*) Temperature and date are crucial for predicting emergence dates in solitary bees.

Abstract

Although several studies already demonstrated an advancement of bee emergence under warmer spring temperatures, just little is known about exact long-term effects of climate warming on the phenology of solitary bees. As standardized long-term studies on the emergence date of solitary bees are widely lacking, statistical model predictions about the emergence dates of solitary bees could be applied to long-term temperature date to retrospectively predict the emergence date of bees over the last several decades.

We performed experiments in the field and within climate chambers where we recorded emergence dates of two spring-emerging mason bees (*Osmia cornuta* and *O. bicornis*) that had overwintered at different temperature conditions. Based on these data we tested several variants degree-day models to relate temperature time series to emergence data. Models included the degree-day requirements, and a starting date or only two or one of these parameters. For both species and sexes the best model was the most complex model including all three variables. This demonstrates that in addition to temperature-related parameters, spring-emerging solitary bees use a critical starting date before which they generally do not start the summation of degree-days irrespective of the actual temperature. Based on temperature data of the time period 1949 to 2012, our model estimates that both species advanced their phenology by 3.31 days per 1°C temperature increase, and at a rate of 0.63 days per decade.

Our results suggest that further studies should also include the parameter of a starting date into degree-day model predictions to increase the accuracy of model predictions

for emergence dates in solitary bees. Applying such model predictions to temperature data of climate change projections would then lead to more precise predictions of phenological shifts in solitary bees.

Introduction

If pronounced temporal mismatches between interaction partners occur, populations are more likely to go extinct (Both *et al.* 2006). Therefore, a proper timing of phenological events is highly fitness relevant (Bradshaw & Holzapfel 2007). Pollinating insects like solitary bees, for example, have to time their activity period and thus their emergence in accordance to the phenology of their host plants (and vice versa). However, predicting the optimal emergence date is difficult from a bee's perspective. During hibernation, when solitary bees have not emerged yet and still remain inside their natal nests, they receive just few triggers from the environment. So far, it has been shown that the emergence date of solitary bees depends on ambient air temperature, with warmer overwintering temperatures leading to an advancement of emergence dates (Bosch & Kemp 2003; Bosch & Kemp 2004; Skandalis *et al.* 2011; Fründ, Zieger & Tscharntke 2013).

During the last decades effects of climate warming became evident in many regions worldwide, especially during winter and spring (Schwartz, Ahas & Aasa 2006). Several studies described an advancement of bee emergence under warmer spring temperatures (Gordo & Sanz 2006; Skandalis *et al.* 2011). However, just little is known about exact long-term effects of climate warming on the phenology of solitary bees as these studies did not investigate the phenology of bees in relation to time. To our knowledge only one study has focused on that topic, showing a mean advancement of bee emergence of 10.4 ± 1.3 days over the past 130 years (Bartomeus *et al.* 2011). This study relied on insect collections data from museums because standardized long-term studies on the emergence date of solitary bees are lacking. However, data from museum collections only allow a rough estimation of long-term shifts in bee phenology because generally only one or few individuals are collected during the activity period of any population. Additionally, data on bee phenology can be biased if they are based on bee specimens collected on flowers and therefore are not independent of the plants' phenology (Forrest & Thomson 2011). One possibility to deal with the lack of standardized long-term studies on the emergence dates of solitary bees is to use statistical model predictions about the emergence dates of bees. In contrast to studies that simply describe an advancement of bee emergence under warmer temperatures, statistical model approaches allow exact predictions about the emergence date of bees for any possible temperature profile. This enables generalizable statements about the shift in bee emergence under climate warming by applying the models to long-term temperature data.

Statistical models in general use different variables like, for example, precipitation rates, wind speed, day lengths or temperature-related parameters to predict the timing of distinct phenological events in different species (Richter *et al.* 2008; Green 2017; Pettit & O'keefe 2017). For plant species many such models have already been developed to predict, for example, the timing of bud burst or flowering onset (Diekmann 1996; Cave *et al.* 2013; Olsson & Jonsson 2014). In contrast, just very few statistical models for predicting emergence dates in solitary bees have been published. These studies in general used only temperature-related factors like lower temperature thresholds and degree-day requirements to predict the emergence dates of bees (White, Son & Park 2009; Ahn, Park & Jung 2014). A lower temperature threshold describes the temperature value above which bees are predicted to accumulate degree-days, and degree-day requirements describe the threshold for summed degree-days at which bees are predicted to finally emerge. Here we propose, however, that bees that additionally consider a critical starting date before which they do not start the summation of degree-days irrespective of the actual temperature, are at an advantage as this may prevent them from emerging much too early in case of spells of warm temperatures early in the season. In accordance, a recent study suggested that it might be important to include the parameter of a starting date into degree-day models in solitary bees as starting dates later than 1st January improved model fit (Forrest & Thomson 2011). Another study also used a set of starting dates to calculate growing degree-hour requirements and likewise suggested that later starting dates can improve the accuracy of the model (Kraemer & Favi 2010). To our knowledge, the relevance of starting date has otherwise not been tested in models predicting emergence in solitary bees. Therefore, we investigated if this additional parameter in fact improves the fit of degree-day models by directly comparing degree-day models with and without the use of a starting date.

Knowing the lower temperature threshold and the starting date for degree-day accumulation in solitary bees, would then allow us to give more precise predictions about the effects of climate change on the phenology of these species as effect size of climate change clearly varies depending on these values. In Fig.1, we describe the functional principle of a fictional degree-day model which is composed of the three parameters: lower temperature threshold (T_L), degree-day requirement (=higher temperature threshold; T_H) and starting date (D_S). Furthermore, to our knowledge there exists no study that additionally applied such a degree-day model for bees to long-term temperature data to retrospectively predict the emergence dates of bees.

To precisely predict bee phenology under natural and artificial conditions via statistical modelling, we performed experiments in the field and also within climate chambers where we recorded emergence dates of bees that had overwintered at different temperature conditions. We focused on the two solitary bee species *Osmia cornuta* and *O. bicornis*, which typically emerge in early and in mid-spring, respectively. We tested several variants degree-day models to relate temperature time series to emergence data. The most complex model of our approach accounts for all the three investigated variables (lower temperature threshold, degree-day requirements and starting date) with potential sub-models including only two or one parameter. Based on the best identified models for both species and sexes we retrospectively estimated bee emergence dates during the last 63 years (1949-2012) by utilizing historical temperature data for the location Würzburg, Germany.

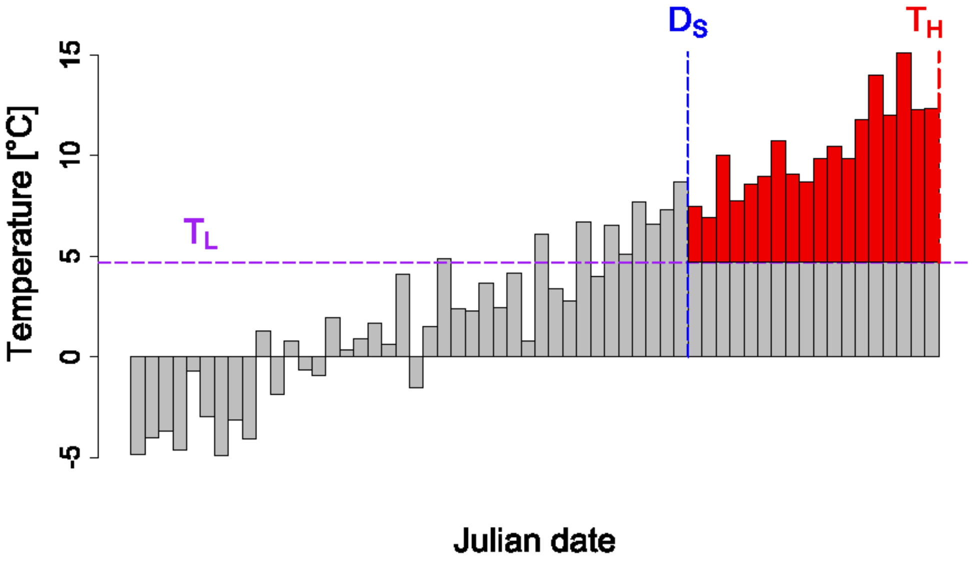


Fig.1 Functional principle of a fictional degree-day model which includes three parameters: (1) a lower temperature threshold (T_L), (2) a starting date (D_S) and (3) a critical degree-day requirement (=higher temperature threshold; T_H). Julian date describes the count of days since January 1st (≙ Julian day 1) until December 31st (≙ Julian day 356) within one year. T_L defines the temperature threshold above which bees are predicted to accumulate degree-days, D_S describes a critical date -before this date bees do not start the accumulation irrespective of the actual temperature, and T_H describes the threshold for the summed degree-days at which bees are predicted to finally emerge. This threshold is indicated by the red area.

Material and methods

To fit the statistical model we used emergence data from forty data sets. Twenty data sets from experiments, which were conducted in 2014 within different climate chambers (ThermoTec climate chamber, Panasonic Cooled Incubator MIR-254-PE, CLF Plant Climatics I-66L4VL). Twenty field data sets were collected in 2015 in different regions around Würzburg and Karlstadt in Lower Franconia, Germany. In all experimental setups, we recorded the ambient air temperature and the related emergence dates of solitary bees. Data recording was started on December 1st.

Bees

We focused on two spring-emerging species of solitary bees (Hymenoptera: Apiformes: Megachilidae): the hornfaced mason bee *Osmia cornuta* with an activity period from March until May, and the red mason bee *Osmia bicornis* with an activity period from early April until June. Both species are food generalists and nest in above-ground cavities. Single cocoons of *O. cornuta* and *O. bicornis* were purchased from WAB Mauerbienenzucht (Konstanz, Germany), a commercial supplier of solitary bees. Between October and the start of the experiment, cocoons were stored inside a climate chamber at constant 4°C.

Temperature recording

For each location, either in the lab or in the field, a temperature time-series was recorded via temperature loggers (iButton temperature logger DS1922L, Maxim Integrated, USA). From the start of each time series (start of measurement December 1st) until their end (when the last bee from the respective location had emerged), ambient temperature was recorded in 2h intervals with an accuracy of ± 0.0625°C. Inside climate chambers temperature loggers were placed in the middle of the chamber. In the field, temperature loggers were attached to wooden posts at 90 centimeter height and in the shadow of a plastic device that was attached above temperature loggers.

Emergence data

Concerning field data sets, emergence of bees were recorded every 2-3 days. Therefore, single cocoons of bees (100 cocoons of *O. cornuta* and *O. bicornis* each) were brought to the respective field locations and stored inside hard plastic tubes (50 cocoons per tube, tube length: 25.5cm, inner diameter: 7cm) which were attached at wooden posts one meter above ground. Bees that emerged from their cocoon were trapped inside a net. After determination of the sex, bees were released. Concerning data sets from experimental setups with climate chambers, emergence dates of bees were recorded on a daily basis. For this purpose, bees were stored inside small plastic tubes sealed with cotton wool (to trap bees after emergence). Emerged bees were released after the determination of sex.

Day degree models

We tested several day degree models to relate temperature time series to emergence data. The most general model version transforms a sequence of temperature measurements $\tau_i^{(k)}$ at site k into cumulative values $T_d^{(k)}$ after subtraction of a temperature threshold T_L. Values being less than 0 after subtraction are ignored. Summation starts at a specific date D_S and is calculated for any date $d \geq D_S$ within a future period of sufficient duration.

$$(1) \qquad T_d^{(k)} = \sum_{i=D_S}^{d} \quad max(0, \tau_i^{(k)} - T_L)$$

When $T_d^{(k)}$ exceeds a threshold value T_H the corresponding value d indicates the phenological event, i.e. bee emergence $\epsilon^{(k)}$.

$$(2) \qquad \epsilon^{(k)} = min\{d: T_d^{(k)} \geq T_H\}$$

Model parameters T_L, D_S, and T_H are chosen such that the sum of squared deviations Δ^2 between observed date of emergence within the test set $y^{(k)}$ and predicted dates $\epsilon^{(k)}$ for observation is minimized.

$$(3) \qquad \Delta^2 = \sum_{k \in Y_0} \quad (y^{(k)} - \epsilon^{(k)})^2$$

In all analyses the unit of time is 2h as measured from December 1st.

Observations were randomly assigned to a test set Y_0 for parametrization of the model and a validation set Y_1. The ratio of observations in each of the sets was chosen as approx. 2 : 1, i.e. $n_0 = 27$ and $n_1 = 13$.

The most complex model of our approach includes all 3 parameters. However, more parsimonious models might be a better choice with respect to the trade-off between the goodness of fit and the complexity of the model. Thus, we also calculated AIC values for model selection between all potential submodels with 3, 2 or 1 parameter. The fixed parameters of these sub-models were chosen as $T_L = 0$, $D_S = 1$ (corresponding to the start of measurement December 1^{st}), and $T_H = 0.1$.

It is important to note that in any of the reduced models these parameters are still included; they are, however, in part fixed to trivial values. For example, a model version with $T_H \approx 0$ would predict that the bees emerge as soon as either the critical temperature threshold T_L is passed for the first time or the critical start date D_S is reached depending on which of the two parameters is fitted. If both, then the bees would emerge as soon as the critical temperature threshold is passed for the first time after date D_S has passed.

For further quantitative evaluation of models we also calculated the level of explained variance (EV).

$$(4) \qquad EV = 1 - \frac{\sum_{k \in Y_0} \quad (y^{(k)} - \epsilon^{(k)})^2}{(y^{(k)} - \bar{y})^2}$$

EV typically yields values between 0 and 1. However, negative values are possible, if a model increases variance instead reducing it (Parker *et al.* 2011).

We tested the effect of predictors year, species, and sex on the predicted date of emergence via model selection based on a general linear model (Crawley 2007).

Climate data

Based on the best identified models for both species and sexes we retrospectively predicted bee emergence dates during the last 63 years by utilizing historical temperature data for the location Würzburg. Climate data are provided by the German Weather Service (DWD) since 1949.

Results

As expected, *O. cornuta* and *O. bicornis* emerged at different times during spring. In our experiments, *O. cornuta* emerged approximately 25 days (mean value) before *O.*

bicornis. In both species males emerged approximately 13.70 ± 3.08 (mean ± SD) days before their female conspecifics (Fig. 2).

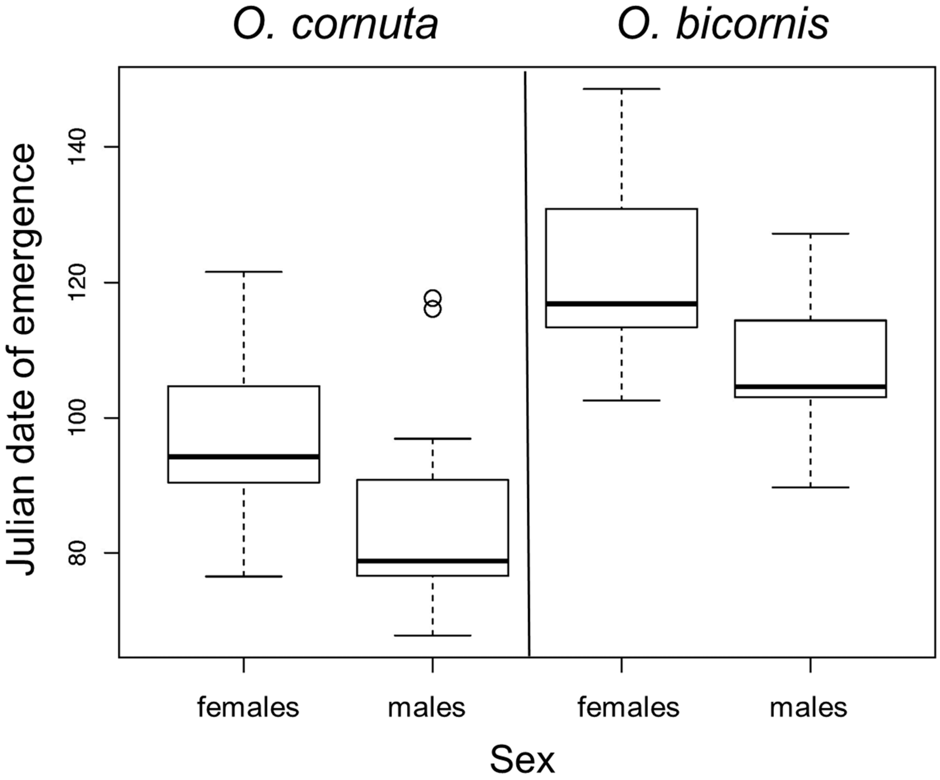


Fig.2 Julian date of emergence of *O. cornuta* and *O. bicornis* in 40 different locations (20 data sets from climate chamber setups and 20 field data sets). Julian date describes a continuous count of days since January 1st (≙ Julian day 1) until December 31st (≙ Julian day 356) within one year (e.g. day 100 ≙ 10th of April). Boxes bound the upper and lower quartile of the data; the horizontal line represents the median and the whisker the 95% confidence interval of the data.

All degree-day models were parameterized by choosing model parameters such that summed quadratic deviation between model prediction and empirical values was minimized for a randomly chosen subset of the data including 27 out of the 40 different settings. The best model was determined by comparison of AIC values. For both species and both sexes the best model was always the complete model with specific values for degree-day requirements (T_H), lower temperature threshold (T_L), and starting day threshold (D_S) fitted. In any case, AIC distance from the second best model was greater than 2 indicating the relevance of all three predictors (Hilborn & Mangel 1997). Observed and predicted emergence dates were highly correlated (Table 1, Fig.3). The fraction of variance explained by the model (EV) ranged from 0.80 to 0.90. Applying the model to the test data (13 out of 40 time series) yielded values for explained variance (EV2) above 0.80 (Table 1).

Table 1 Results of the different degree-day models that were compared by their AIC values. T_L gives the lower temperature threshold above which bees are predicted to accumulate degree-days, T_H (=higher temperature threshold) is the amount of summed degree-days at which bees are predicted to finally emerge (T_H/12=degree-day) and D_S describes the starting date before which bees do not start the summation of degree-days irrespective of the actual temperature ((D_S/12) – 31 ≙ Julian date). Models either included all of the three investigated parameters ($T_HT_LD_S$), two (T_HT_L, T_HD_S, T_LD_S) or one of these parameters ($T_{H;}$ T_L, D_S). EV: explained variance of the data for parameter estimation (n=27); EV2: explained variance of the validation data (n=13); corfit: pearson correlation coefficient between observation and prediction of the data for parameter estimation; cortsts: pearson correlation coefficient between observation and prediction of the validation data.

species	sex	model	EV	AIC	T_H	T_L	D_S	corfit	cortst	EV2
O. cornuta	males	T_H	-2.116	156.282	4326	0.1	1	0.880	0.930	-0.870
O. cornuta	males	T_L	-27.670	216.207	0.1	15.8	1	-0.357	-0.706	-25.526
O. cornuta	males	D_S	-0.002	125.644	0.1	0.1	1381	0.044	NA	0
O. cornuta	males	T_HT_L	0.241	120.168	625	5.2	1	0.946	0.967	0.594
O. cornuta	males	T_HD_S	0.729	92.312	1751	0.1	937	0.861	0.994	0.966
O. cornuta	males	T_LD_S	0.851	76.199	0.1	8.6	1297	0.926	0.980	0.941
O. cornuta	males	$T_HT_LD_S$	0.939	54.193	146	5	1225	0.972	0.987	0.966
O. cornuta	females	T_H	-0.988	145.784	5571	0.1	1	0.903	0.926	-2.216
O. cornuta	females	T_L	-35.317	224.226	0.1	19.8	1	-0.321	-0.785	-50.364
O. cornuta	females	D_S	-0.008	127.443	0.1	0.1	1537	-0.142	NA	0
O. cornuta	females	T_HT_L	0.434	113.877	1255	4.6	1	0.935	0.951	0.085
O. cornuta	females	T_HD_S	0.829	81.502	2961	0.1	841	0.917	0.975	0.869
O. cornuta	females	T_LD_S	0.226	122.325	0.1	8.6	1513	0.477	0.774	0.574
O. cornuta	females	$T_HT_LD_S$	0.858	78.511	1041	3.6	1201	0.926	0.943	0.850
O. bicornis	males	T_H	-2.171	143.415	6686	0.1	1	0.906	0.916	-2.987
O. bicornis	males	T_L	-78.429	230.374	0.1	19.8	1	-0.437	-0.787	-91.865
O. bicornis	males	D_S	-0.003	112.330	0.1	0.1	1657	NA	NA	-0.007
O. bicornis	males	T_HT_L	0.092	111.652	1906	4.6	1	0.945	0.972	0.005
O. bicornis	males	T_HD_S	0.787	72.474	3311	0.1	1201	0.891	0.974	0.934
O. bicornis	males	T_LD_S	0.598	89.678	0.1	11.9	1609	0.800	0.945	0.193
O. bicornis	males	$T_HT_LD_S$	0.905	52.614	526	7	1417	0.958	0.959	0.818
O. bicornis	females	T_H	-0.238	131.344	8546	0.1	1	0.927	0.766	-5924.584
O. bicornis	females	T_L	-65.431	238.88	0.1	19.8	1	-0.501	-0.800	-51.770
O. bicornis	females	D_S	-0.001	125.601	0.1	0.1	1825	NA	NA	-0.020
O. bicornis	females	T_HT_L	0.623	101.229	3115	4.6	1	0.938	0.975	0.847
O. bicornis	females	T_HD_S	0.775	87.337	5361	0.1	1153	0.884	0.962	0.813
O. bicornis	females	T_LD_S	0.792	85.154	0.1	12.8	1729	0.895	0.963	0.941
O. bicornis	females	$T_HT_LD_S$	0.909	64.973	1376	7.2	1441	0.955	0.937	0.886

Table 2 Estimated parameters of the best model for both species and sexes. T_L gives the lower temperature threshold above which bees are predicted to accumulate degree-days, T_H (=higher temperature threshold) is the amount of summed degree-days at which bees are predicted to finally emerge (T_H/12=degree-day), D_S describes the starting date before which bees do not start the summation of degree-days irrespective of the actual temperature ((D_S/12) – 31 ≙ Julian date) and Julian date describes this starting date (D_S) converted into the Julian date (1 ≙ 1st January; 365 ≙ 31th December).

species	sex	model	T_H	T_L	D_S	≙ Julian date
O. cornuta	males	$T_HT_LD_S$	146	5	1225	71
O. cornuta	females	$T_HT_LD_S$	1041	3.6	1201	69
O. bicornis	males	$T_HT_LD_S$	526	7	1417	87
O. bicornis	females	$T_HT_LD_S$	1376	7.2	1441	89

The lower temperature threshold T_L was lower for *O. cornuta* (males: 5.0°C; females: 3.6°C) than for *O. bicornis* (males: 7.0°C; females: 7.2°C) with comparably small differences between the sexes (Table 2). Besides, the model estimates a lower starting date D_S for *O. cornuta* (males: 1225; females: 1201) than for *O. bicornis* (males: 1417; females: 1441) with comparably small differences between the sexes (≙ 2 days for both species). Julian date describes the value of D_S converted into the Julian date ((D_S/12)-31 ≙ Julian date). Julian date describes a continuous count of days since January 1st (≙ Julian day 1) until December 31st (≙ Julian day 356) within one year (e.g. day 100 ≙ 10th of April). The model predicts that *O. cornuta* females in general do not start the summation of degree-days before 10th of March (≙ Julian day 69) with *O. cornuta* males two days afterwards (12th of March); and that *O. bicornis* males in general do not start the summation of degree-days before 28th of March (≙ Julian day 87) with *O. bicornis* females two days afterwards (30th of March). For both species the model estimates a lower T_H for males than for females, and in general lower T_H values for *O. cornuta* than for *O. bicornis*: *O. cornuta* males: 146; *O. cornuta* females: 1041; *O. bicornis* males: 526; *O. bicornis* females: 1376. For a better understanding of the amount of required degree-days, we here present an exemplary calculation: a degree-day requirement of 146 with a lower temperature threshold of 5°C (which is the case for *O. cornuta* males), means that after the achievement of the starting date, *O. cornuta* males already emerge after four days with a mean temperature of 8.1°C or after two days with a mean temperature of 11.1°C (for more information on these calculations see Supporting information).

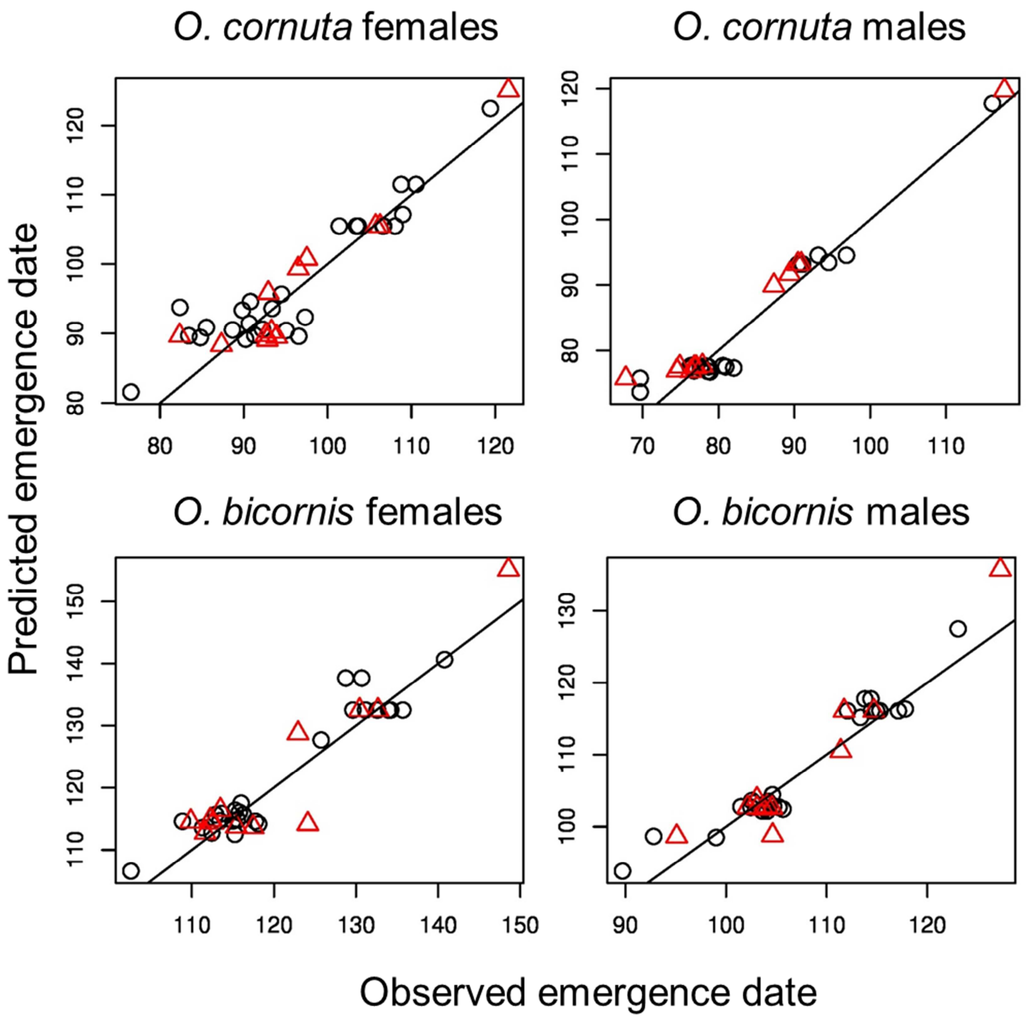


Fig.3 Observed Julian emergence date in relation to the emergence date predicted by the best model. Black lines represent a strong correlation between the observed and the predicted emergence dates. Black circles show data for model parametrization (n=27) and red triangles show the validation data set (n=13).

The retrospective estimation of emergence data shows a shift of bee emergence towards earlier dates by 3.37 days between 1949 and 2012, indicating that the emergence of both bee species has been advanced at a rate of 0.53 days per decade. The lack of significant interactions between year, sex and species identity indicates that the level of protandry stayed constant despite the change in emergence date and that both species shifted their emergence dates to the same extent.

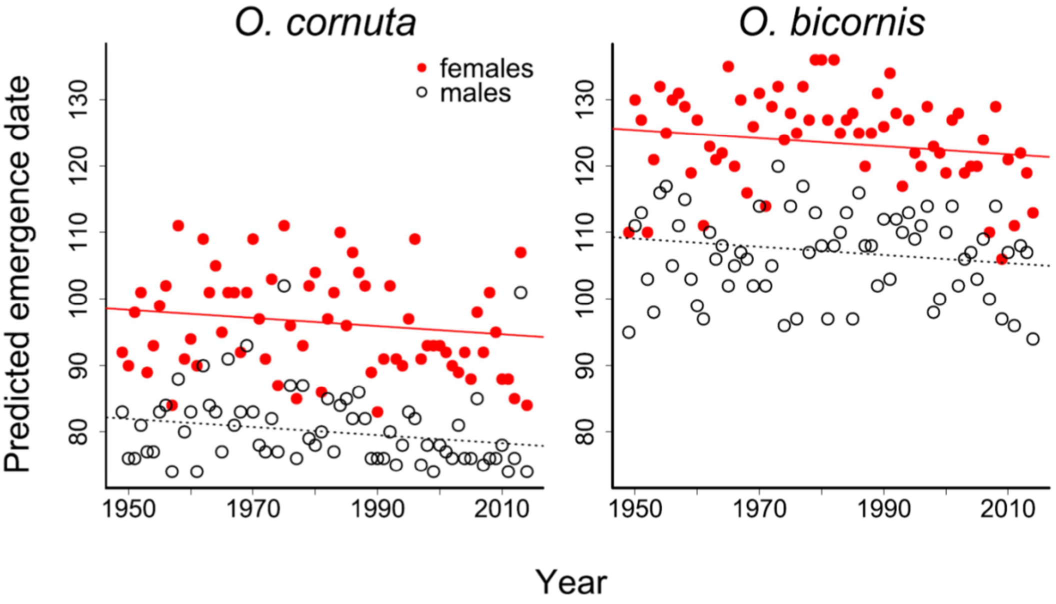


Fig.4 Retrospectively predicted Julian date of emergence for *O. cornuta* and *O. bicornis* based on best model and temperature data since 1949 at location Würzburg, Germany. Julian date describes a continuous count of days since January 1st (≙ Julian day 1) until December 31st (≙ Julian day 356) within one year. Black points show the mean annual emergence dates of males and red circles show the mean annual emergence dates of females. Regression lines represent the results of general linear models.

Discussion

We showed that the best model for predicting emergence dates in spring-emerging solitary bees is the most complex model which includes all three investigated parameters (lower temperature threshold, degree-day requirements and starting date). We thereby showed that the usage of a starting date in addition to temperature-related parameters improves the accuracy of degree-day model predictions, which confirms the suggestion of a recent study (Forrest & Thomson 2011). Hence, we conclude that solitary bees use a critical starting date before which they generally do not commence the summation of degree-days irrespective of the actual temperature: This strategy might safe-guard them against emerging too early in case of spells of warm temperatures early in the season. We thus suggest that future studies should also include the parameter of a critical starting date into degree-day model predictions to increase the accuracy of model predictions for emergence dates in solitary bees. This would make it possible to assess the impacts of climate warming on the synchronization in bee-plant interactions more precisely.

However, it remains unclear how such a critical starting date for degree-day accumulation can be determined by the bees. It is conceivable that solitary bees have a sense for the actual date via an internal clock; internal clocks are an attribute of many organisms from different taxa (Akesson *et al.* 2017; Chapman *et al.* 2017). In solitary bees, their pupation or the termination of their winter diapause might thereby generate as a start impulse for the internal clock. In addition, increasing day lengths might have an effect on the determination of a starting date as well as an internal clock might need light stimuli for its entrainment. However, it has been assumed that cavity-nesting bees do not use photoperiod as trigger of emergence (Kraemer & Favi 2010; Sgolastra *et al.* 2010), although the effects of photoperiod on the circannual emergence of bees has not been tested, so far. We suggest that further studies should try to understand how spring-emerging solitary bees are able to identify a critical starting date for degree-day accumulation.

We showed furthermore that the lower temperature threshold, the critical starting date and the critical degree-day requirement that provided the best fit in the model were in each case lower for *O. cornuta* than for *O. bicornis*. Therefore, our model predicts that *O. cornuta* accumulates a lower amount of degree-days already at lower temperatures starting at an earlier calendar date than *O. bicornis*. We assume that these differences between the species explain the earlier (mean) emergence dates of *O. cornuta* in comparison to *O. bicornis* (March vs. April). The critical starting date for degree-day accumulation is estimated in mid-March for *O. cornuta* (males: 12th of March; females: 10th of March) and end of March in *O. bicornis* (males: 28th of March; females: 30th of March). After exceeding these calendar dates and the respective lower temperature threshold (O.cornuta males: 5°C, females: 3.6°C; O. bicornis males: 7°C, females:

7.2°C), bees start to accumulate degree-days. Our model predicts that males of both species have a quiet low critical degree-day requirement compared to the females (degree-day requirements for *O. cornuta* males: 146, *O. cornuta* females: 1041; degree-day requirements for *O. bicornis* males: 526, *O. bicornis* females: 1376). This indicates that, in case of relatively warm temperatures, males will rather quickly emerge once their critical starting date is exceeded (mean time gap between the exceedance of the critical starting date and the emergence event for *O. cornuta* males: 10 days, *O. cornuta* females: 27 days, for *O. bicornis* males: 19 days, *O. bicornis* females: 35 days). Our model therefore predicts that in comparison to females, the emergence dates of males are more strongly determined by the actual date than by the long-term tracking of ambient temperatures.

This might lead to the assumption that due to climate warming males and females of both species will differently shift their emergence dates, which might lead to temporal mismatches between the sexes. However, our retrospective estimation of emergence data suggests a consistent trend not only for both species but, more importantly, also for both sexes within species. Over the entire time period studied, from 1949 to 2012, mean temperatures were increasing about 1.2°C. For that time period the regression of the model predicts an advance in bee emergence of *c.* 4 days, indicating that spring-emerging solitary bees have been advancing their phenology by 3.3 days per 1°C temperature increase or at a rate of 0.63 days per decade, respectively. The only study (to our knowledge) that focused on long-term effects of climate warming on the phenology of solitary bees, demonstrated a pretty similar advancement of emergence dates in spring-emerging solitary bees, namely a rate of 0.8 days per decade (Bartomeus *et al.* 2011). In accordance with our study, they also showed that male and female solitary bees were shifting their phenology at similar rates. This indicates a further persistence of protandry in solitary bees under climate warming. As standardized long-term studies on the emergence dates of solitary bees are widely lacking, Bartomeus et al. (2011) had to rely on data derived from museums' insect collections whereas we applied our degree-day model to long-term temperature data from the regional climate station. It is assuring that these fundamentally different methods resulted in consistent estimations about the past phenological trend of spring-emerging solitary bees under climate warming; this not only underpins the accuracy of these predictions but also the significance of our degree-day model. Nonetheless, only long-term empirical records of phenology data can ultimately proof the correctness of this model; at the moment such data do, to our knowledge, not exist.

A study which investigated the phenology of plants in temperate regions (USA and UK) showed that first flowering was advanced by 4 days per 1°C mean temperature increase (Memmott *et al.* 2007). Another study (USA) showed that over the last 100 years, plant phenology was advancing at a rate of 0.8 days per decade and for 3.9 days per 1°C temperature increase in mean spring temperature, respectively (Primack *et al.* 2004). A third study (USA) showed that over a 30-year period, plant phenology was advancing at a rate of 0.8 days per decade (Abu-Asab *et al.* 2001). Comparing these results to our prediction that spring-emerging solitary bees have been advancing their phenology at a rate of 0.63 days per decade and for 3.31 days per 1°C temperature increase, demonstrates that phenology of bees and plants due to climate warming seem to shift more or less in synchrony. However, estimates of phenological response have the potential to be substantially influenced by the choice of study design and statistical methodology (Parmesan 2007). Therefore, comparing our predictions to other studies on phenological trends of different plant species might form a completely different forecast about the future synchronization in bee-plant interactions. In accordance, several studies demonstrated that bees and plants were not shifting their phenologies to the same but to different extents (Gordo & Sanz 2005; Parmesan 2007; Forrest & Thomson 2011; Kudo & Ida 2013; Willmer 2014), indicating conflicting evidence. Therefore, a concordant prediction about the further persistence of bee-plant interactions does not yet exist. We suggest conceiving more studies in which phenological shifts of bee and plants species are simultaneously investigated. Only then, we will be able to give more precise predictions about the risks and consequences of temporal mismatches between bees and food plants and the persistence of these species in times where environmental conditions are changing.

Conclusion

We showed that spring-emerging solitary bees use a critical starting date before which they generally do not start the summation of degree-days irrespective of the actual temperature. To increase the accuracy of model predictions, we suggest that further degree-day model predictions about the phenology in spring-emerging solitary bees should also include the parameter of a critical starting date. Our findings also suggest that in comparison to females, the emergence dates of males are more strongly determined by the actual date than by the long-term tracking of ambient temperatures. Nonetheless, our retrospective estimation of emergence data of spring-emerging solitary bees showed a consistent decreasing trend not only for both species investigated but also for both sexes within species indicating that under climate warming the level of protandry stays constant despite the change in emergence date. Over the entire time period studied, from 1949 to 2012, the regression of the model predicts that spring-emerging solitary bees have been advancing their phenology for 3.31 days per 1°C temperature increase and at a rate of 0.63 days per decade. Although these predictions match the results of several studies on phenological trends of plant species, we suggest that more research has to be done to finally answer the question if bee-plant interactions will still be synchronized under further climate warming.

Supporting information

For *O. cornuta* males, the model predicts a lower temperature threshold of 5°C and a degree-day requirement of 146. This means that after the achievement of their starting date and if temperatures exceeded 5°C, the difference between the actual temperature value and the value of the lower temperature threshold is summed every two hours. As soon as the sum results 146, the model predicts the bees to emerge.

Example 1: mean temperature of 8.1°C for several days. Then, the model sums 8.1 - 5 = 3.1 every two hours. → 1 day ≙ 12 x 3.1 = 37.2. And 37.2 x 4 days = 148.8 which means that the degree-day requirement of 146 is exceeded after 4 days.

Or: mean temperature of 11,1°C or several days. Then the model sums 11.1 – 5 = 6.1 every two hours. → 1 days ≙ 12 x 6.1 = 73.2. And 73.2 x 2 days = 146.4 which means that the degree-day requirement of 146 is exceeded after two days.

Chapter IV: Desynchronizations in bee-plant interactions cause severe fitness losses in solitary bees

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Abstract

Global warming can disrupt mutualistic interactions between solitary bees and plants when increasing temperature differentially changes the timing of interacting partners. One possible scenario is for insect phenology to advance more rapidly than plant phenology. However, empirical evidence for fitness consequences due to temporal mismatches is lacking for pollinators and it remains unknown if bees have developed strategies to mitigate fitness losses following temporal mismatches.

We tested the effect of temporal mismatches on the fitness of three spring-emerging solitary bee species, including one pollen specialist. Using flight cages, we simulated (i) a perfect synchronization (from a bee perspective): bees and flowers occur simultaneously, (ii) a mismatch of three days and (iii) a mismatch of six days, with bees occurring earlier than flowers in the latter two cases.

A mismatch of six days caused severe fitness losses in all three bee species, as few bees survived without flowers. Females showed strongly reduced activity and reproductive output compared to synchronized bees. Fitness consequences of a three day mismatch were species-specific. Both the early-spring species Osmia cornuta and the mid-spring species Osmia bicornis produced the same number of brood cells after a mismatch of three days as under perfect synchronization. However, O. cornuta decreased the number of female offspring, whereas O. bicornis spread the brood cells over fewer nests, which may increase offspring mortality e.g. due to parasitoids. The late-spring specialist Osmia brevicornis produced fewer brood cells even after a mismatch of three days. Additionally, our results suggest that fitness losses after temporal mismatches are higher during warm than cold springs, as the naturally occurring temperature variability revealed that warm temperatures during starvation decreased the survival rate of O. bicornis.

We conclude that short temporal mismatches can cause clear fitness losses in solitary bees. Although our results suggest that bees have evolved species-specific strategies to mitigate fitness losses after temporal mismatches, the bees were not able to completely compensate for impacts on their fitness after temporal mismatches with their food resources.

Keywords: conditional sex allocation, emergence, mitigation strategies, mutualism, phenological shift, pollination, species interactions

Introduction

Species interactions depend on synchronization of the partner species; a mismatch in their timing results in the disruption of the interaction (Miller-Rushing *et al.* 2010). Most species in temperate environments use temperature as a trigger for the timing of their seasonal activity (Fründ, Zieger & Tscharntke 2013; Visser 2013). Thus, global warming shifts the phenologies of most of these species to an earlier date in the year (Menzel *et al.* 2006; Visser 2013). As some species respond more to climate warming than others (Parsche, Fründ & Tscharntke 2011; Willmer 2012; Posledovich *et al.* 2015; Thackeray *et al.* 2016), temporal mismatches between interacting species are likely to occur (Visser & Both 2005; Memmott *et al.* 2007; Kudo & Ida 2013; Petanidou *et al.* 2014; Schmidt *et al.* 2016). The negative impact of desynchronization between interacting partners is expected to be highest for temperate species occurring either very early or very late in the season (early spring or late autumn), when the danger of emerging in the absence of any potential interaction partners is highest (Forrest & Thomson 2011). Some studies suggest that plants advance their phenology more than bees in response to early-spring warmth or snowmelt (Forrest & Thomson 2011; Kudo & Ida 2013), and some have reported equivalent shifts among plant and bee species (Hegland *et al.* 2009; Bartomeus *et al.* 2011; Rafferty & Ives 2011). In contrast, other studies have shown that insect phenology has shifted more rapidly than plant phenology over the last several decades (Gordo & Sanz 2005; Parmesan 2007; Willmer 2014). Most solitary bee species that emerge in early spring overwinter as already full-fledged adults, but still inside their brood cells. Thus, these bees could respond quickly to a brief period of warm weather in spring, potentially leading to temporal mismatches with their host plants. So far, we know little about the fitness consequences of such temporal mismatches. Research effort has mostly focused on the fitness consequences for plants but to date fitness consequences have not been investigated for bees (Forrest 2015). The few studies available on adult food limitation in pollinating insects examined bumblebees and butterflies in the laboratory (Murphy, Launer & Ehrlich 1983; Boggs & Ross 1993; Vesterlund & Sorvari 2014). They indicated that fecundity and/or longevity are reduced, implying severe fitness losses for these species. Bees are considered to be the most important pollinators of many agricultural crops and wild plants (Kearns, Inouye & Waser 1998; Potts *et al.* 2010). Fitness losses to bees that result from temporal mismatches with their food resources could exacerbate the current decline in bees and pollination services in many regions, which could have negative consequences for economically relevant plant species (Potts *et al.* 2010; Gonzalez-Varo *et al.* 2013).

We investigated the effects of temporal mismatches with food plants on the survival, the activity and the reproductive output of spring-emerging solitary bee species. In addition, we examined how increasing temperatures modify the impact of temporal mismatches on the fitness of bees. Since in warm conditions metabolic functions are faster and overall energy expenditure is higher than in cold conditions (Vesterlund & Sorvari 2014), temporal mismatches and therefore starvation during periods of warm temperatures could be greater than during cold periods. As temporal mismatches can also occur due to interannual temperature fluctuations, we cannot neglect the possibility that bees could have evolved strategies to mitigate fitness losses when they are desynchronized with their host plants. In early spring, when plant diversity is low, bees cannot easily switch to another (previously less or non-important) interaction partner when their preferred interaction partners are absent. One strategy of spring bees to mitigate a reduction in reproductive output after a temporal mismatch could be to counterbalance a period of initially reduced activity by increasing their activity towards the end of their lives. Other strategies could involve switching the sex ratio of their offspring towards males, the less costly sex (Trivers & Willard 1973), or neglecting time-consuming protection against parasitoids to make up for periods of reduced activity.

We performed an experiment with large flight cages serving as mesocosms. We manipulated the supply of blossoms inside the mesocosms to synchronize or desynchronize bee-plant interactions. Fitness parameters were recorded for three spring-emerging solitary bee species of the genus *Osmia,* synchronized, or with a mismatch of either three or six days. Thackeray et al. (2016) predicted an average temporal mismatch of about three days between primary consumers (e.g. bees) and primary producers (e.g. plants) under different emission scenarios by the 2050s. We assumed, therefore, that the temporal mismatches we chose represented a reliable scenario under future climate warming. For the experiment we chose two polylectic and one oligolectic bee species that emerge between early and late spring. We measured their survival rates, their activity over their lifetimes, the number of brood cells and nests produced, and the sex of their offspring. The following questions were addressed: 1) Is there a negative impact of a temporal mismatch with their food plants on the survival rate, total activity and reproductive output of solitary bees? 2) Do solitary bees have strategies that mitigate fitness losses when food plants are completely lacking? 3) Does the ambient temperature modify the impact of desynchronization on the fitness of solitary bees after emergence?

We showed that temporal mismatches in bee-plant interactions of three or six days cause tremendous fitness losses to solitary bees even though bees have strategies to mitigate associated impacts on their fitness. Additionally, our results suggest that fitness losses after temporal mismatches are higher during warm than during cold springs.

Material and methods

Experimental design

We established 36 mesocosms (= flight cages, Fig. 1 A & B) to test effects of synchronized and desynchronized plant-bee interactions on the fitness of three solitary bee species. Inside the cages we simulated either a perfect synchronization between solitary bee emergence and plant flowering or temporal mismatches of three or six days where bees were kept in the flight cages without food resources (Fig. 1 C). The experiment was conducted in spring and summer of 2014. Flight cages were placed in a grassland near the University of Würzburg, Germany. The flight cages were 3 x 2 x 2m in size to offer adequate living space with a mesh width of 0.8mm to prevent bees and other insects from entering and leaving the cages. For each bee species we conducted between five and ten survey rounds (for more details see section 'Bees'). Survey rounds began on different days to cover different temperature conditions, as they may modify the effects of temporal mismatches on bees. In each survey round (see Fig. 1C), we manipulated three cages, each of which represented one of the three treatments: (1) perfect synchronization (from a bee perspective): bees and flowers were placed simultaneously in the cage, (2) bees were added three days before flowers were placed in the cage, (3) bees were added six days before flowers were placed in the cage. Treatments were randomly assigned to cages.

Flight cages were equipped with trap nests to record the reproductive output of female bees. In the center of each cage, trap nests were attached to a pole at a height of one metre. Each bee species was provided with trap nests consisting of nesting tubes of their preferred size. We supplied O. cornuta and O. brevicornis with one trap nest each but O. bicornis with two different trap nest types. O. cornuta and O. bicornis received one trap nest from Oxford Bee Company (Schrewsbury, England) containing approximately 120 paper tubes of 8mm diameter and a tube length of 20cm. O. bicornis and O. brevicornis received one trap nest from the University of Würzburg (approximately 100 20cm long reed internodes inside plastic tubes, which were accessible from two sides). Diameters of reed internodes ranged from 3 to 8mm. We recorded the temperature in each flight cage once per hour. Temperature-sensors (Maxim Integrated DS1921G-F5 Thermochron iButton; 0.5°C resolution) were attached at a height of one metre on the north side of the trap nest pole to avoid direct sunlight. Flight cages were also equipped with flowering plants, either together with the bees (synchronized) or 3 or 6 days after the bees were added (see below). We also equipped each flight cage with a small pot of 7 x 5.5 x 5.5cm size. These pots were filled with sandy loam which we moistened once per day during the whole length of the experiments to make the sandy loam accessible for bees and also to provide them with water.

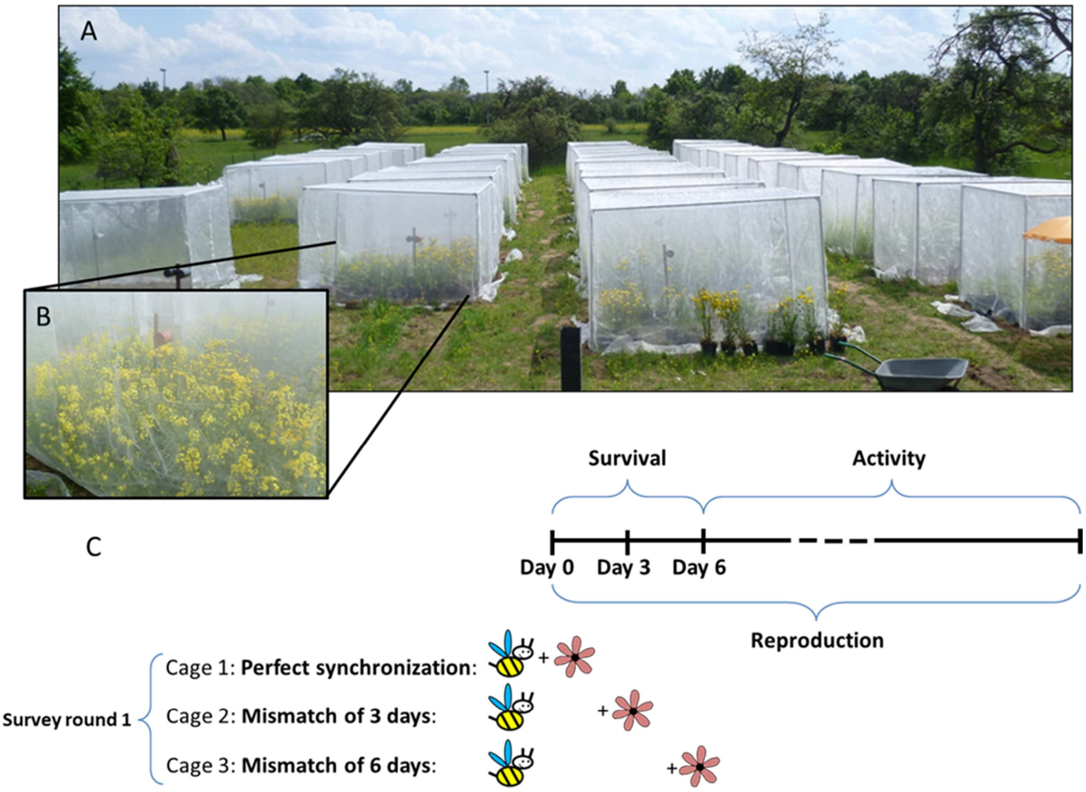

Fig. 1 (A) View of the experimental setup. (B) Supply of blossoms inside flight cages (C) Illustrative description of the practical implementation of one survey round. Further survey rounds – each with three cages – started at later dates. It is shown when bees and plants were added to the cages depending on the treatment and during which time periods survival and activity of bees were recorded. Reproduction was possible during the whole survey round.

Bees

We selected three spring-emerging species of solitary bees as study species (Hymenoptera: Apiformes: Megachilidae). We chose study species according to their seasonal appearance during spring to cover a range from early to late spring-emerging species and to cover a spectrum of food preferences. The hornfaced mason bee Osmia cornuta is a food generalist with an activity period from March until May, the red mason bee Osmia bicornis is a food generalist with an activity period from early April until June, and the wallflower mason bee Osmia brevicornis is a solitary bee species specialized on Brassicaceae, with an activity period from late April until June

(Westrich 2011). Single cocoons of O. cornuta and O. bicornis were purchased from WAB Mauerbienenzucht (Konstanz, Germany), a commercial supplier of solitary bees. Nests from O. brevicornis were collected from trap nests (reed internodes inside plastic tubes) that had been exposed in the field in 2013 around Würzburg, Germany. From October 2013 until spring 2014 nests and single cocoons overwintered inside a climate chamber at constant 4°C. In spring 2014, cocoons were incubated successively in the laboratory at 21-23°C until emergence. For each survey round we incubated a new group of individuals. The required incubation time was known from pilot studies (Schenk, personal observations). To start a survey round, bees that had emerged in the laboratory during the previous 24 hours were placed in three flight cages. For O. cornuta and O. bicornis, we placed seven females and four males per cage. For O. brevicornis we placed 5.6 ± 1.5 females (mean ± SD) and 3.4 ± 0.8 males (mean ± SD) per cage with 3 females and 2 males minimum and 7 females and 4 males maximum, whereby female and male abundances per cage did not differ among treatments within a survey round. The male bees were placed inside the cages to ensure the fertilization of females. Fertilization generally took place in the flight cages shortly after the start of the experiment, independent of the occurrence of flower resources (personal observations). Data collection was focused on female bees only because females are the demographically limiting sex (Goulson et al. 2010).

We tested the three solitary bee species in succession following their natural appearance time during spring. Survey rounds of O. cornuta started between the third and the 30th of April 2014, survey rounds of O. bicornis started between the 14th of May and the 4th of July 2014 and survey rounds of O. brevicornis started between the 27th of May and the 10th of June 2014. O. cornuta was tested in eight cages per treatment (total 24 cages), O. bicornis was tested in ten cages per treatment (total 30 cages) and O. brevicornis was tested in five cages per treatment (total fifteen cages). Since the survey rounds did not completely match the natural flight periods of the bees (O. cornuta: March to May, O. bicornis and O. brevicornis: April to June), we compared temperatures measured in the cages to long-term temperature data (1990-2013), which were measured during the natural flight periods at the regional climate station in Würzburg (DWD Climate Data Center CDC 2016). The mean cage temperatures measured during the experiment (O. cornuta: 14.97°C, O. bicornis: 19.13°C, O. brevicornis: 18.65°C) were within the range of long-term (1990-2013) temperatures measured during the natural flight periods for all species (mean ± SD; O. cornuta (March-May): 9.95°C ± 5.29, O. bicornis and O. brevicornis (April-June): 13.85°C ± 4.86). The monthly temperatures measured in the cages during the experiment were on average 0.93°C higher than temperatures measured at the regional climate station at the same time.

Plants

We provided cages of O. cornuta and O. bicornis with Prunus spinosa, Prunus avium, Pyrus (spp.), Prunus domestica, Sinapsis arvensis, Brassica napus, Crepis biennis, Matricaria chamomilla, Chrysanthemum segetum, Campanula glomerata, Campanula persicifolia, Campanula rotundifolia, Campanula rapunculoides, Campanula rapunculus and Helianthus annuus. All plant species were visited by these two generalist bee species (personal observations). Plant composition differed among survey rounds, but was standardized for the three cages within a survey round. O. brevicornis, a solitary bee species specialized on Brassicaceae, was exclusively provided with Sinapsis arvensis and Brassica napus. Flowering Brassica napus was collected from a nearby agricultural field and flowering branches of Prunus spp. and Pyrus spp. were cut in orchard meadows surrounding the study site. Seeds of the other plant species were purchased from Rieger-Hofmann® GmbH (Blaufelden-Raboldshausen, Germany) and sown in spring 2013 and in spring 2014 respectively, depending on the plant species. We provided 50-70 flower pots of 17 x 17 x 17cm size per cage. Each pot contained approximately 65 ± 19 (mean ± se) blossoms. Flowering branches of Prunus spp. and Pyrus spp. were put inside three water buckets that were buried into the soil per species and cage. Each bucket contained approximately 1200 ± 94 (mean ± se) blossoms. The surface of the water was covered with bottle corks to avoid drowning of bees. We checked the condition of plants inside the cages once per day. Plants with faded blossoms were exchanged immediately to maintain consistent flower supply. Cages belonging to the same survey round were provided with the same number of flower pots consisting of the same plant composition. Generally, each cage was filled with potted plants until its ground area was entirely covered with flowering plants (Fig. 1 B).

Data recording

Bees were placed inside the cages at day 0 of each survey round. Plants were added – depending on the treatment – either on the same day (perfect synchronization) or 3 or 6 days later (temporal mismatch of 3 or 6 days, Fig. 1 C). For the analysis we recorded three measures of bee fitness: the survival rate, an activity index and the reproductive output. For determining the survival rate and the activity index, we counted all visible active and non-active females every second day for three minutes per cage from outside the cages starting at day 6 of each survey round and continuing until the last bee in the cage had died. Females were considered to be active if they were flying, visiting the flowers, walking on the mesh tent or mating with males. Each individual was counted only once per observation date. This was ensured by determining the maximum number of active females that could be observed simultaneously. To calculate the variable "Survival rate [%]" per cage we divided the maximum number of females observed in the cage on day 6 by the number of females placed in the cage at day 0 and multiplied the value by hundred. To calculate the variable "Activity Index" per cage, we divided the number of active females observed in the cage at each observation day by the total number of females placed in that cage at day 0, and summed these values for each cage starting with day 6 of the survey round until the death of the last bee within that cage. To receive an index value between 0 and 1, we divided this value by the number of observation days. To investigate additionally whether activity changed over time and whether these changes differed among treatments, we split the observation dates into two halves (early activity: days 6-26, late activity: days 27-52).

Reproductive output included the "Number of nests", "Number of brood cells" and "Number of female offspring" that had been produced per cage. After the death of all bees within a cage, trap nests were removed from the flight cages and placed under field conditions inside a mesh tent (mesh widths ca. 0.8mm) to exclude other trap-nesting insects. At the end of October 2014, trap nests were brought into the laboratory and stored inside a climate chamber at constant 4°C. During the winter, the number of nests was counted and nests were opened to record the number of brood cells. The sex of the offspring was determined after opening the cocoons that contained adult bees.

To investigate interacting effects of treatment and temperature on the survival rate, we measured daytime temperature hourly between 7am and 9pm, from day 0 to day 6 of each survey round, and averaged these temperatures for each cage. As two of the temperature-sensors failed to record data, we had to exclude one data point for O. cornuta and one for O. bicornis, both from the treatment with perfect synchronization.

Statistical analyses

For statistical analysis of the data we used the software RStudio (R version 3.0.2) and the ‘nlme’ package (Pinheiro et al. 2015). Models were calculated for each bee species separately. To detect differences in the survival rate, the activity index, the number of brood cells, the number of nests and the number of female offspring among treatments (synchronized vs. three-day mismatch vs. six-day mismatch), we used linear mixed-effects models with treatment as a fixed factor and survey round number as random factor. Treatments were compared using treatment contrasts (Crawley 2007). To detect differences in the survival rate in relation to temperature in cages with synchronized vs. cages with three-day mismatch, we used linear mixed-effects models with treatment, temperature of the first six days and their interaction as fixed factors and survey round number as random factor. Cages with a mismatch of six days were excluded because too few females survived the first six days. To test the combined effects of time and treatment on the activity of bees we used linear mixed-effects models with treatment, time period (early: day 6-26 vs. late: day 27-52) and their interaction as fixed factors and survey round number as random factor. Model residuals were inspected for violation of assumptions or normality and homoscedasticity.

Results

Survival rates and activity

No specimen of the late-spring specialist O. brevicornis and very few individuals of the mid-spring generalist O. bicornis or the early-spring generalist O. cornuta survived a temporal mismatch of six days. This caused decreased activity of all three bee species after a temporal mismatch of six days in comparison to perfect synchronization. Mismatches of three days reduced the survival rate and the activity of both O. bicornis and O. brevicornis compared to perfect synchronization. The survival rate and activity of O. cornuta were not significantly affected after a mismatch of three days (Table 1, Fig. 2 a-f).

The effect of treatment (mismatch of three days vs. perfect synchronization) on survival rate of O. bicornis was temperature-dependent (Table 2, Figure 3 b). Increasing temperature decreased the survival rate of O. bicornis after a mismatch, but not after perfect synchronization. The interaction between treatment and temperature was not significant for the other two species, O. cornuta and O. brevicornis (Table 2, Figure 3 a & c).

Activity was lower in the second half of adult life compared to the first half of adult life in all treatments in O. cornuta and O. bicornis, but we found a significant interaction between time of activity (first vs. second half of adult life) and treatment (perfect synchronization vs. temporal mismatch of three or six days; Table 2) for these two species. For O. brevicornis, this interaction was marginally significant. The interaction between time of activity and treatment shows that the decline of activity in the second half of adult life was smaller after a mismatch of three days than after perfect synchronization (Fig. 2 g-i). The late activity of O. cornuta was even enhanced after a mismatch of three days compared to perfect synchronization (Fig. 2 g), indicating that bees were able to recover after the mismatch. The activity of all species was generally highly reduced after a mismatch of six days.

Reproductive output

The number of brood cells and the number of nests of O. brevicornis were reduced after a mismatch of three days and of six days compared to perfect synchronization (Table 1, Fig. 4 c & f). The number of female offspring of O. brevicornis did not differ significantly among treatments (Table1, Fig. 4 i). For O. bicornis, the number of brood cells and the number of female offspring were reduced after a mismatch of six days, while the number of nests was reduced after a mismatch of only three days (Table 1, Fig. 4 b, h, and e). The number of brood cells and the number of nests of O. cornuta were not significantly affected by a mismatch of three days, but were reduced after a mismatch of six days (Table 1, Fig. 4 a & d). The number of female offspring of O. cornuta was reduced after mismatches of both three days and of six days compared to perfect synchronization (Table 1, Fig. 4 g).

Table 1 Results of linear mixed effect models testing differences among treatments. Shown are treatment contrasts for ‘perfect synchronization of bees and flowers’ (0), ‘mismatch of three days’ (3) and ‘mismatch of six days’ (6). Dependent variables were the survival rate [%] of females, the activity index of females, the number of brood cells, the number of nests and the number of female offspring per cage. P-values in bold indicate significant results ($p < 0.05$).

	O. cornuta			***O. bicornis***			***O. brevicornis***		
Dependent variable	DF	t	p	DF	t	p	DF	t	p
Survival rate [%]									
0 vs. 3	15	-1.68	0.115	19	-3.59	**0.002**	8	-4.07	**0.004**
0 vs. 6	15	-9.10	**< 0.001**	19	-6.65	**< 0.001**	8	-5.31	**< 0.001**
3 vs. 6	15	-7.42	**< 0.001**	19	-3.06	**0.007**	8	-1.24	0.250
Activity Index									
0 vs. 3	15	-0.29	0.779	19	-4.20	**< 0.001**	8	-4.27	**0.003**
0 vs. 6	15	-7.22	**< 0.001**	19	-7.82	**< 0.001**	8	-5.42	**< 0.001**
3 vs. 6	15	-6.94	**< 0.001**	19	-3.62	**0.002**	8	-1.15	0.283
No. of brood cells									
0 vs. 3	15	0.65	0.523	19	-0.99	0.332	8	-3.26	**0.012**
0 vs. 6	15	-4.07	**0.001**	19	-4.46	**< 0.001**	8	-4.66	**0.002**
3 vs. 6	15	-4.72	**< 0.001**	19	-3.47	**0.003**	8	-1.40	0.198
No. of nests									
0 vs. 3	15	1.10	0.289	19	-2.53	**0.021**	8	-2.43	**0.042**
0 vs. 6	15	-4.24	**< 0.001**	19	-6.13	**< 0.001**	8	-3.93	**0.004**
3 vs. 6	15	-5.34	**< 0.001**	19	-3.60	**0.002**	8	-1.51	0.171
No. of female offspring									
0 vs. 3	15	-2.89	**0.011**	19	-0.49	0.633	8	-1.85	0.101
0 vs. 6	15	-3.02	**0.009**	19	-2.43	**0.025**	8	-1.94	0.088
3 vs. 6	15	-0.12	0.910	19	1.95	0.102	8	-0.09	0.932

Table 2 Interacting effects of treatment (perfect synchronization vs. mismatch of three days vs. mismatch of six days) and temperature (Temp) on the survival rate [%] of females, and of treatment and time (early vs. late) on the activity of females. Results are calculated per cage and come from linear mixed effect models. P-values in bold indicate significant results ($p < 0.05$).

	O. cornuta				***O. bicornis***				***O. brevicornis***			
Dependent variable	**nDF**	**dDF**	**F**	**p**	**nDF**	**dDF**	**F**	**p**	**nDF**	**dDF**	**F**	**p**
Survival rate [%]												
Treatment	1	5	3.34	0.127	1	7	15.66	**0.005**	1	2	19.10	**0.049**
Temp	1	5	2.66	0.163	1	7	5.60	**0.049**	1	2	0.12	0.766
Treatment : Temp	1	5	0.24	0.642	1	7	9.88	**0.016**	1	2	7.71	0.109
Activity Index												
Treatment	2	36	31.53	**< 0.001**	2	46	34.51	**< 0.001**	2	20	24.71	**< 0.001**
Time (early vs. late)	1	36	48.60	**< 0.001**	1	46	17.52	**< 0.001**	1	20	3.87	0.063
Treatment : Time	2	36	6.85	**0.003**	2	46	5.95	**0.005**	2	20	4.24	0.064

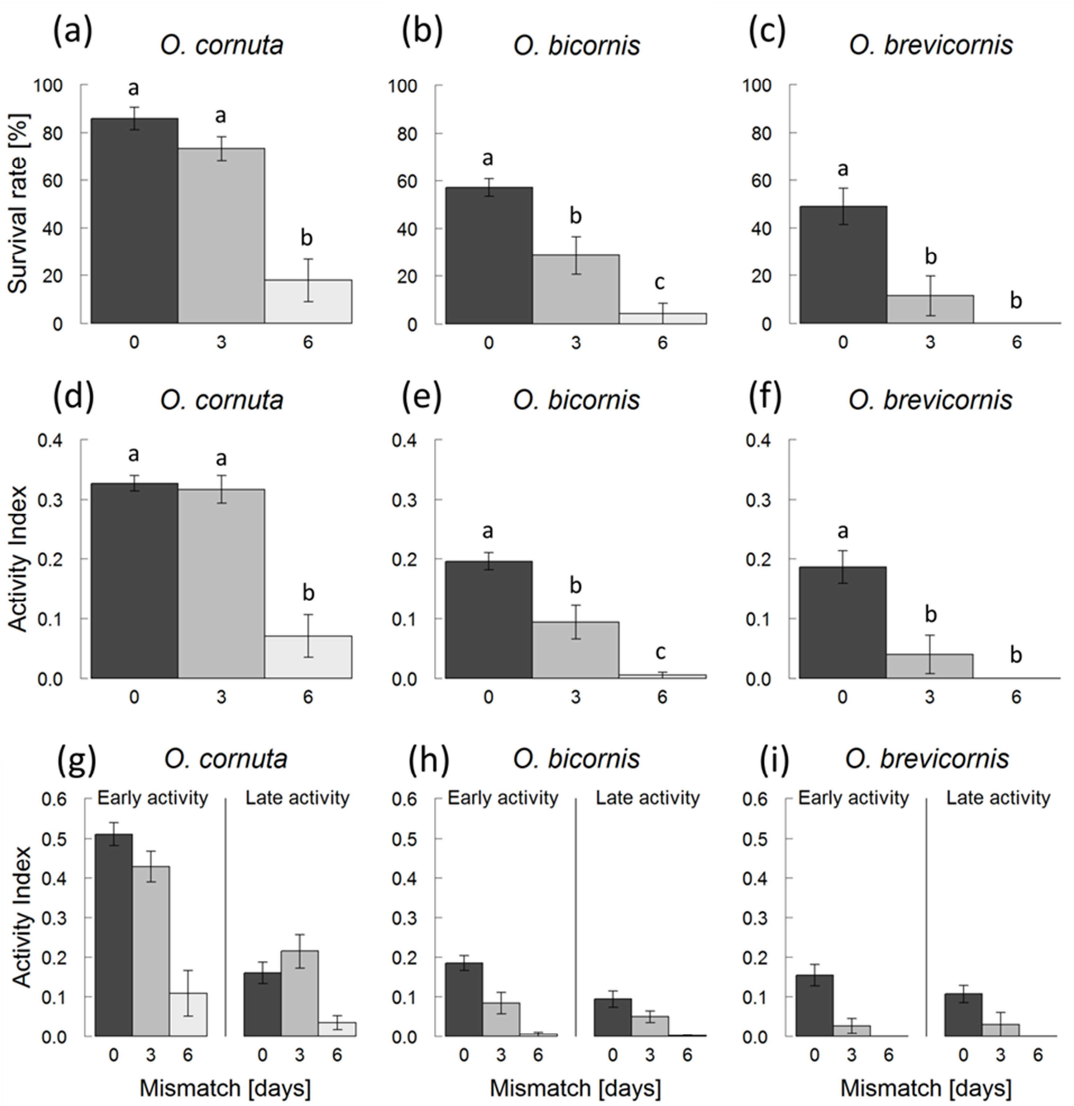

Fig. 2 Influence of temporal mismatches on the survival rate per cage (a-c) and the activity per cage (d-i) of the females of three bee species. To calculate the variable "Survival rate [%]" per cage we divided the maximum number of females observed in the cage on day 6 by the number of females placed in the cage at day 0 and multiplied the value by hundred. To calculate the variable "Activity Index" per cage, we divided the number of active females observed in the cage at each observation day by the total number of females placed in the cage at day 0, and summed these values for each cage starting with day 6 of the survey round until the death of the last bee within that cage. To receive an index value between 0 and 1, we divided this value by the number of observation days. Depending on the treatment bees emerged 0, 3 or 6 days before flowering onset. Different letters above bars (means ± SE) indicate significant differences among treatments ($p < 0.05$).

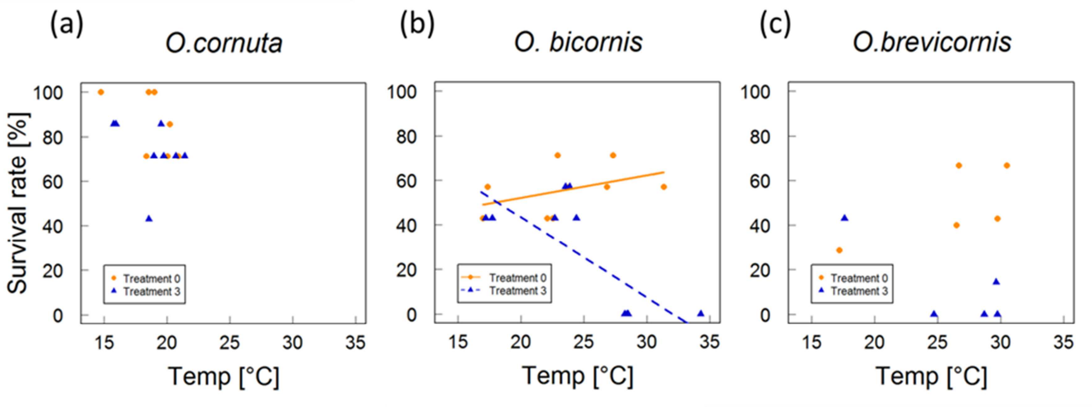

Fig. 3 Influence of temperature (Temp) on the survival rate [%] of females per cage of three bee species (a-c). Depending on the treatment bees emerged 0 or 3 days before flowering onset. Cages with a mismatch of six days were excluded because too few individuals survived the first six days. Regression lines represent the results of linear mixed effect models in case of significant interaction.

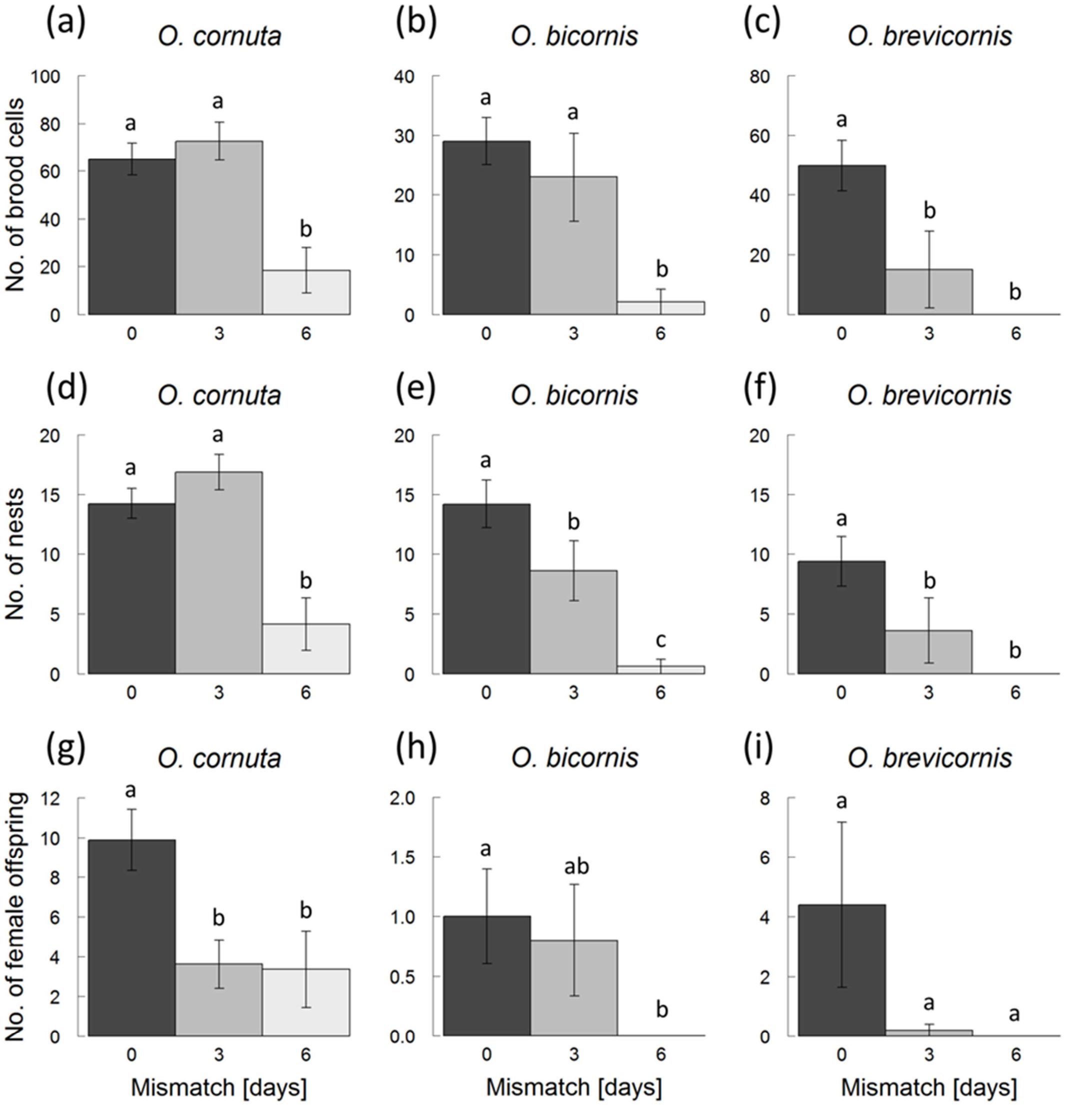

Fig. 4 Influence of temporal mismatches on the number (No.) of brood cells per cage (a-c), the number of nests per cage (d-f), and the number of female offspring per cage (g-i) of three bee species. Depending on the treatment bees emerged 0, 3 or 6 days before flowering onset. Different letters above bars (means ± SE) indicate significant differences among treatments ($p < 0.05$).

Discussion

Our study showed that in bee-plant interactions a temporal mismatch of six days caused tremendous fitness losses in all three bee species. No individual of the late-spring specialist *Osmia brevicornis* and very few individuals of the early- and mid-spring generalists *Osmia cornuta* and *Osmia bicornis* survived six days without flower resources. The low survival rates for all three bee species resulted in strongly reduced numbers of brood cells.

A temporal mismatch of three days caused species-specific changes in reproductive output. Depending on the bee species, one or several of the following effects were observed: (1) a reduction in survival rate, (2) reduction in activity, (3) reduction in the number of (female) brood cells and (4) reduction in the number of nests. After a temporal mismatch of three days, the early-spring generalist *O. cornuta* showed the same survival rate and the same activity as under perfect synchronization. In contrast, only a few individuals of the mid- and late-spring species *O. bicornis* and *O. brevicornis* survived a temporal mismatch of three days, and they subsequently showed reduced total activity. Emerging before flower occurrence forces bees to live from their internal energy reserves because adult insects in general rely on fat reserves to sustain life during starvation periods (Arrese & Soulages 2010; Weissel et al. 2012). It has been shown in ants that the overall survival rate increases with larger fat body resources (Sorvari, Haatanen & Vesterlund 2011) and larger species are considered to be able to survive long periods of starvation better than smaller species (Gergs & Jager 2014). As the early-spring generalist *O. cornuta* is larger than *O. bicornis* and *O. brevicornis* (Westrich 2011) and presumably also has more fat reserves, this may explain why the survival success of *O. cornuta* during periods of starvation is higher than that of *O. bicornis* and *O. brevicornis*. The larger body size and the presumably larger fat reserves of *O. cornuta* could be an adaption to its higher risk of emergence before potential interaction partners, as this is more likely to occur in early spring (Forrest and Thomson 2011).

Although *O. cornuta* showed the same total activity after a temporal mismatch of three days as after perfect synchronization, its activity immediately after a temporal mismatch was reduced in comparison to synchronized bees. *O. cornuta* compensated for this decline in activity in the first half of life with increased activity in the second half of life. This indicates that *O. cornuta* was able to recover from a short temporal mismatch. Nevertheless, *O. cornuta* produced fewer female offspring after a temporal mismatch. The shift towards male offspring was not caused by a lack of mated females, as mating occurred on the first day of the experiment in all treatments. Females of solitary bee species are able to determine the sex and the size of each offspring depending on their individual condition (Rosenheim, Nonacs & Mangel 1996; Seidelmann, Ulbrich & Mielenz 2010; Wogin et al. 2013). Females in poor condition produce fewer female offspring and shift the sex ratio towards the less costly sex (males in this case) (Trivers & Willard 1973). Possibly due to this "making the best of a bad lot" strategy of females in poor individual condition (Fisher 1930), female *O. cornuta* produced fewer female offspring after a temporal mismatch than after perfect synchronization. Consequently, we conclude that the early-spring generalist *O. cornuta* mitigates negative effects of a temporal mismatch of three days on reproductive output with relatively high activity levels towards the end of its lifetime, as well as by shifting the sex ratio towards male offspring to stabilize brood cell numbers. As females are the demographically limiting sex (Goulson et al. 2010), a reduced number of female offspring could lead to population declines.

Surprisingly, a mismatch of three days did not significantly reduce the number of brood cells produced by the mid-spring generalist *O. bicornis,* although its survival rate, activity and number of nests were reduced compared to synchronized bees. Our results suggest that *O. bicornis* was able to mitigate negative effects of reduced activity by distributing brood cells over fewer nests than under perfect synchronization. Searching for new nest cavities and learning the cavity position in orientation flights are costly in terms of time (Michener & Retten-Meyer 1956; Schönitzer & Klinksik 1990; Miliczky 2008; Rezkova et al. 2012). By decreasing the number of nests, bees may increase their efficiency and the number of brood cells that can be produced in a given amount of time. However, this strategy comes at a cost because it reduces protection against parasitoids and may increase offspring mortality in the nest. High parasitism risk is generally regarded as the main reason for construction of multiple nests, because distribution of brood cells over multiple nests decreases the probability that a natural enemy enters all brood cells of the female (Vinson & Frankie 1988). Our results suggest that *O. bicornis* females have evolved a strategy that helps to stabilize brood cell numbers even if the environmental conditions are suboptimal. In populations with low parasitism risk, this strategy may compensate for fitness losses after short temporal mismatches. In populations with high parasitism risk, the fitness benefits of this strategy may be reduced by an increase in offspring mortality. This is equally applicable for other negative events, such as accidental damage to the nest, fungal infection and predation (e.g. by birds). But *O. bicornis* was able to use its mitigation strategy only under cold temperatures, because under warm temperatures no females survived three days without plants. We showed that high ambient temperatures enhanced the negative effect of a temporal mismatch on the survival rate of *O. bicornis*. Temperature-dependent survival during starvation periods has also been documented for bumblebees and can be explained by more rapid metabolic function and concomitant higher overall energy expenditure in warm than cold conditions (Vesterlund & Sorvari 2014). The temperature-independent survival rate of *O. bicornis* individuals in perfect synchronization with their food plants (meaning that energy intake was possible) suggests that not only overall energy expenditure but also overall energy intake is higher in warm than cold conditions. As warm temperatures enhanced the negative impact of temporal mismatches on the survival of *O. bicornis*, we conclude that increasing spring temperatures due to climate warming may have severe consequences for bee-plant interactions. However, this conclusion supposes that the phenological advancement of solitary bee species due to warming temperatures cannot keep pace with the increase in ambient temperatures.

The late-spring specialist *O. brevicornis* that experienced a temporal mismatch of three days produced fewer brood cells than under perfect synchronization. This finding reflects the result that its survival rate and activity were reduced after a temporal mismatch of three days. *O. brevicornis* did not exhibit any observable strategies to mitigate fitness losses after temporal mismatches.

The danger of emerging in the absence of any potential interaction partners is highest in early spring and late autumn (Forrest & Thomson 2011). We expected, therefore, that bee species emerging in early spring must be better adapted to cope with such circumstances than bee species emerging in late spring. This expectation was confirmed by our results showing that the severity of fitness losses corresponded to the chronological sequence of species emergence. The negative impact of desynchronization was least obvious for the early-spring species *O. cornuta* and most obvious for the late-spring species *O. brevicornis,* with the mid-spring species *O. bicornis* in between. This result also includes the observation that the (late-spring) pollen specialist *O. brevicornis* was less well adapted to temporal mismatches than the (earlier emerging) generalist species. The assumption that specialists are less likely to become phenologically disrupted than generalist species (Rafferty, Caradonna & Bronstein 2015) may possibly explain the disparate ability of our specialist and generalist species to cope with temporal mismatches. This raises the question if future climate warming will further desynchronize plant-pollinator interactions, causing temporal mismatches with severe fitness losses even to species that have not been forced yet to evolve mitigation strategies. Further studies on this topic are needed to assess the impacts of temporal mismatches more precisely.

Flight-cage experiments are a useful contribution to our understanding of the consequences of plant-pollinator mismatches. Nevertheless, care must be taken in extrapolation from flight-cage results to global consequences for species interactions. The spatial scale of these mesocosms is inevitably small relative to the spatial scale over which bees normally forage. In nature, bees are likely to have access to habitats that vary slightly in their flowering phenology. Thus, it is conceivable that at least some bees may be able to fly far enough to reach well-timed flowering patches before initiating nesting which would lead to less severe fitness consequences than those observed in our experiments. On the other hand, long-distance flights to search for flower resources would deplete the energy reserves of the bee, potentially leading to even higher fitness losses than those seen in our cage experiment.

Conclusion

Ours is the first study of how temporal mismatches in bee-plant interactions can affect the fitness of solitary bees. We showed that even short temporal mismatches of three and six days in bee-plant interactions (with solitary bee emergence before flower occurrence) can cause severe fitness losses in solitary bees. We detected different strategies by solitary bees to counteract impacts on their fitness after temporal mismatches. However, since these strategies may result in secondary fitness costs by a changed sex ratio or increased parasitism we conclude that compensation strategies do not fully mitigate fitness losses of bees after short temporal mismatches with their food plants. As bees showed strongly decreased survival rates after mismatches of three or six days, we assume that bees are unable to use a “sit-and-wait-strategy” (Huang, Takahashi & Dafni 2002), a compensation strategy suggested for many plant species when pollinators are lacking. Bees may depend on the availability of nectar and pollen for survival and reproduction on a shorter time-scale than plants (Benadi *et al.* 2014). In the event of further climate warming, fitness losses after temporal mismatches may not only exacerbate bee declines but may also reduce pollination services for later-flowering species and affect populations of animal-pollinated plants. Several studies have focused on temporal mismatches in mutualistic interactions and on the question of whether these are more likely to occur due to further climate warming (Parmesan 2006; Hegland *et al.* 2009; Bartomeus *et al.* 2011; Burkle & Alarcon 2011), but we should also investigate the extent of resulting fitness losses of involved species (Colautti, Agren & Anderson 2017). This would make it possible for us to assess the impacts of temporal mismatches more accurately and to make more precise and even species-specific predictions. We suggest that the impacts of global warming on the persistence of mutualistic species interactions may prove to be more urgent and of greater magnitude than previously expected.

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Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.doi:10.5061/dryad.rm317

Supporting information

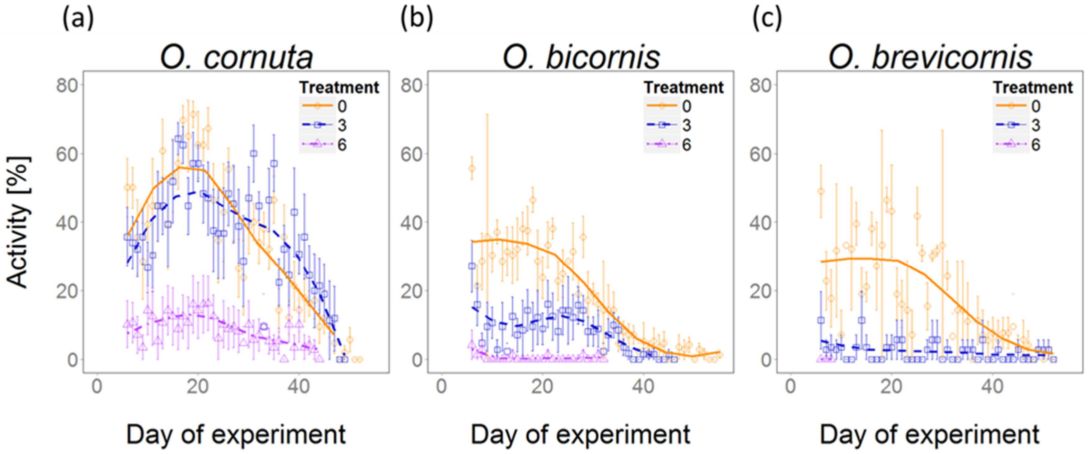

Fig. S1 Influence of the day of experiment on the "Activity [%]" of females per treatment of three bee species. To calculate the variable "Activity [%]" we divided the number of active females that have been observed in the cage per day by the number of females that have been placed in the cage at day 0 and multiplied the value by hundred. Depending on the treatment bees emerged 0, 3 or 6 days before flowering onset.

Chapter V: General Discussion

In my PhD-thesis I investigated different triggers that influence the timing of emergence dates in spring-emerging solitary bees and the resulting fitness consequences of the bees if due to climate change short temporal mismatches with their food plants should occur.

I have shown that not only external but also internal triggers are influencing the phenology of spring-emerging solitary bees. On the one hand, I have shown that overwintering temperatures influence the emergence date and also the fitness state of the bees, and on the other hand, I have also shown that the individual body size (or body weight) of the bees influences the timing of bee emergence (Chapter II). Additionally, I investigated how exactly overwintering temperatures are influencing the emergence dates in spring-emerging solitary bees (Chapter III). Therefore, I developed a statistical model that is able to correctly predict the emergence dates of two different spring-emerging bee species. As standardized long-term studies on the emergence date of solitary bees are widely lacking, I additionally applied this model to long-term temperature data, and thereby showed that the model estimated that over the last 63 years, bees emerged approximately 4 days earlier. If due to climate warming bees and plants do not shift their phenologies harmoniously, I showed furthermore, that even short temporal mismatches of only three days, will lead to severe fitness losses in spring-emerging solitary bees (Chapter IV).

Mechanisms of timing

Most species in temperate environments use temperature as a trigger for the timing of their seasonal activity (Fründ, Zieger & Tscharntke 2013; Visser 2013). Thus, global warming shifts the phenologies of most of these species to an earlier date in the year (Menzel *et al.* 2006; Parmesan 2006). Chapter II presents a study in which I have shown that warm overwintering temperatures advance the emergence date and decrease the body weight in spring-emerging solitary bees. Therefore, I conclude that climate warming may not only lead to earlier emergence dates but also to reduced fitness states in spring-emerging solitary bees. This observation raises the question if pollination services will still be maintained under further climate warming as bees with a lower fitness state also transfer a lower amount of pollen (Seidelmann, Ulbrich & Mielenz 2010). In addition, if climate warming differently changes the timing of bees and plants, interacting partners might, moreover, be desynchronized. This, on the one hand might, further minimize pollination services and, on the other hand, also lead to population declines. Besides, I showed that among bee specimens that experienced the same overwintering temperatures, there exists a negative relationship between body weight (or body size) and the emergence date of solitary bees, a trend that has never been observed before: large-sized spring-emerging bees with a high body weight emerged earlier than their smaller conspecifics with a low body weight. In bees, larger individuals of the same species are able to forage and to collect pollen also when ambient temperatures are low (Stone 1993; Stone 1994), they have a higher probability to survive starving periods after emergence in which insects in general rely on fat reserves (Arrese & Soulages 2010; Weissel *et al.* 2012), and they have more offspring than their smaller conspecifics, which makes body size (or body weight) a key component of fitness in solitary bees (Larsson 1990; Kim 1997). Therefore, I conclude that spring-emerging bees adjusted their emergence dates not only according to overwintering temperatures but also according to their foraging ability at low temperatures and their ability to cope with harsh conditions early in the season as bees with a higher fitness state (large-sized or heavy bees) emerge earlier than their conspecifics with a lower fitness state (small-sized or light bees). This condition dependence in the emergence dates of solitary bees may explain, at least partially, the proximate causes of the high variability in emergence dates of spring-emerging solitary bees which is speculated to be a (maternal) bet-hedging strategy that can be expected to pay off in environments with unpredictable environmental variability (Danforth 1999; Hopper 1999; Childs, Metcalf & Rees 2010; Poethke, Hovestadt & Mitesser 2016). Interestingly, bees in cold overwintering temperatures emerged later with a higher fitness state than bees in warm overwintering temperatures which seems to contradict the observation stated beforehand. By means of a simple mechanistic model, I explained these different observations as well as facts known from the literature by a consistent theoretical scheme. This mechanistic model showed that spring-emerging solitary bees follow a strategic approach and emerge at a date that is most promising for their individual fitness expectations. This date is on the one hand temperature dependent as warmer overwintering temperatures increase the weight loss of bees during hibernation, which then advances their optimal emergence date to an earlier time point (due to an earlier benefit from the emergence event). On the other hand our findings suggest that the optimal emergence date also depends on the individual body size (or body weight) as spring-emerging bees adjusted their emergence dates accordingly. Ecological implications of these results may be diverse and their extents also depend on the trend of synchronization or desynchronization in bee-plant interactions under further climate warming. For example, if bees will shift their phenologies more than plants under climate warming, especially the large-sized bees will be desynchronized with their host plants. This would more strongly decrease the total amount of bee offspring than if plants would shift their phenologies more than bees as this would lead to the fact that only the small-sized bees (which anyway produce less offspring) would be desynchronized with their host plants (for more details on this statement see Discussion of Chapter II). If we take into account that in bee-plant interactions not only the trend of their future phenological shifts but also the respective fitness states of species involved is important to evaluate their further existence under climate warming more accurately, the dimension of fitness losses due to climate change may turn out to be bigger than previously expected.

Chapter III presents a study in which I have shown how exactly overwintering temperatures influence the timing of bee emergence. Therefore, I tested several variants degree-day models to relate temperature time series to emergence data. I showed that bees accumulate degree-days only above a critical temperature value (~4°C in *O. cornuta* and ~ 7°C in *O. bicornis*) and only after the exceedance of a critical calendar date (~10th of March in *O. cornuta* and ~28th of March in *O. bicornis*). Such a critical calendar date, before which degree-days are not accumulated irrespective of the actual temperature, is in general less commonly used and, so far, it has only been included twice in a phenology model predicting bee emergence. My results suggest that further studies should also include the parameter of a calendar date into degree-day model predictions to increase the accuracy of model predictions for emergence dates in solitary bees. Many studies described an advancement of bee emergence under warmer overwintering temperatures but there exists only one study, so far, that describes long-term effects of climate warming on the phenological shift in solitary bees (Bartomeus *et al.* 2011). This study indicates that spring-emerging bees from northeastern North America are advancing their phenology at a rate of 0.8 days per decade. However, this study had to rely on insect collections data from museums as standardized long-term studies on the emergence date of solitary bees are lacking. Besides, there exists no further study about long-term effects of climate warming on the timing of solitary bees from any other part of the world. To at least partially fill this knowledge gap about the advancement of bee emergence over the last decades, I additionally applied the statistical model which I already introduced above, to regional long-term temperature data to retrospectively predict the emergence dates of bees during the last 63 years for the region of Würzburg, Germany. Thereby, I showed that spring-emerging solitary bees have been advancing their phenology for 3.31 days per 1°C temperature increase and at a rate of 0.63 days per decade, which pretty much corresponds to the results of Bartomeus et al. (2011). Although my predictions also correspond to the results of several studies on phenological trends of plant species (Abu-Asab *et al.* 2001; Primack *et al.* 2004; Memmott *et al.* 2007), other studies demonstrated that bees and plants were not shifting their phenologies to the same but to different extents (Gordo & Sanz 2005; Parmesan 2007; Forrest & Thomson 2011; Kudo & Ida 2013; Willmer 2014). Therefore, we suggest that more research has to be done to finally answer the question if bee-plant interactions will still be synchronized under further climate warming. More studies should be concieved in which phenological shifts of local occurring bee and plants species are investigated simultaneously. Only then, we will be able to give more precise predictions about the risks and consequences of temporal mismatches between bees and food plants and the persistence of these species in times where environmental conditions are changing.

Fitness consequences after temporal mismatches

To date, we still know little about the exact dimensions of phenological shifts in different species of temperate climates in response to global warming. Some studies have shown that insect phenology has shifted more rapidly than plant phenology over the last several decades (Gordo & Sanz 2005; Parmesan 2007; Willmer 2014). In accordance with this, a recent study predicted an average temporal mismatch of about 3 days between primary consumers (e.g. solitary bees) and primary producers (e.g. plants) under different emission scenarios by the 2050s (Thackeray *et al.* 2016). However, we still know little about the resulting fitness consequences in bees after such temporal mismatches. Research effort has mostly focused on the fitness consequences for plants but to date fitness consequences have not been investigated for bees (Forrest 2015).

Chapter IV presents a study in which I showed that even short temporal mismatches of few days (with bee emergence before flower occurrence) can cause clear fitness losses in spring-emerging solitary bees. A temporal mismatch of six days caused tremendous fitness losses with very low survival rates and strongly reduced numbers of brood cells. After a temporal mismatch of three days, I detected species-specific changes in reproductive output and also species-specific strategies to counteract impacts on their fitness. However, as these strategies resulted in secondary fitness costs by a changed sex ratio or increased parasitism risk, I conclude that compensation strategies do not fully mitigate fitness losses of bees after short temporal mismatches with their food plants. Therefore, if in response to further climate warming, spring-emerging bees will shift their phenologies more pronounced than their food plants, the persistence of the bees might be endangered. As the danger of emerging in the absence of any potential interaction partners is highest in early spring (Forrest & Thomson 2011), we expected, that bee species emerging in early spring must be better adapted to cope with such circumstances than bee species emerging in late spring. This expectation was confirmed by our results showing that the severity of fitness losses corresponded to the chronological sequence of species emergence. However, as stated above, fitness losses due to short temporal mismatches of few days anyway resulted in severe fitness losses also for bee species emerging in early spring. This indicates that solitary bees might not (yet) be sufficiently adapted to their phenological shifts in response to a warming climate.

Differing responses of bee species to climate change

Seasonal average temperatures reveal that annual warming is clearly evident in winter and spring seasons (Schwartz, Ahas & Aasa 2006). This might lead to the assumption that species emerging in spring are in general susceptible to climate change. In accordance, I showed that both, the early- (*O. cornuta*) and the mid-spring bee species (*O. bicornis*), advanced their emergence dates and also showed a decreased fitness state after warm overwintering temperatures. However, in spite of a general air warming trend during spring, climate conditions during that period are in general still quiet harsh as mean temperatures are still quite low and the probability of late cold snaps or even late frost events is still quiet high. If such strong weather deteriorations occur after the bees have already emerged, this might further reduce their survivability as spring-emerging bee species are only able to fly and to collect nectar above a particular temperature threshold (Vicens & Bosch 2000). Therefore, at quiet low temperatures spring-emerging bees might suffer from starvation as their fat reserves do not last for a long time. Earlier emergence dates of spring-emerging bees in response to climate change may thereby even lead to an increased risk of such late frost events in spite of a general air warming trend, as the timing of late frost events is not changing (Kreyling *et al.* 2012). Therefore, spring-emerging bees are generally in danger if they show a bad timing in their emergence decision. In accordance, I showed that both, the early- and the mid-spring bee species showed adaptations to these harsh weather conditions: in both species, large sized bees with a high fitness state emerged before their smaller conspecifics with a lower fitness state. In temperate climates, larger individuals from the same bee species have been shown to be able to forage already at cooler temperatures (Stone 1993; Stone 1994) and they might also have larger fat reserves than smaller individuals to sustain life during starving periods outside the nest. Therefore, we conclude that within a population of spring-emerging solitary bees, larger individuals emerge earlier in order to seek potential benefits of early emergence as they may be better able to cope with the harsher weather conditions and the low food availability that occur early in spring.

The danger of emerging in the absence of any potential interaction partners is highest in very early spring (Forrest & Thomson 2011). I expected, therefore, that bee species emerging in early spring must be better adapted to cope with such circumstances than bee species emerging in late spring. This expectation was confirmed by our results showing that the severity of fitness losses corresponded to the chronological sequence of species emergence. The negative impact of desynchronization was least obvious for the early-spring species *O. cornuta* and most obvious for the late-spring species *O. brevicornis,* with the mid-spring species *O. bicornis* in between. This raises the question if future climate warming will further desynchronize plant-pollinator interactions, causing temporal mismatches with severe fitness losses even to species that have not been forced yet to evolve mitigation strategies. Further studies on this topic are needed to assess the impacts of temporal mismatches more precisely.

In contrast, during summer environmental conditions are more stable and the total amount of potential interaction partners is also quiet high. Therefore, one might expect that bee species emerging in summer will not have to suffer from temporal mismatches with their host plants. In addition, and as already mentioned: seasonal average temperatures reveal that annual warming is clearly evident in winter and spring seasons (Schwartz, Ahas & Aasa 2006). This might lead to the assumption that bee species emerging in summer are less susceptible to climate warming than bee species emerging in spring. In accordance, a recent study showed that in contrast to spring-emerging solitary bees, bee species emerging in summer neither accelerated their emergence dates nor showed a decreased fitness state (a decreased body weight) after warm overwintering temperatures (Fründ, Zieger & Tscharntke 2013). The underlying causes of such differences between bee species emerging in spring or summer might be that their developmental stages differ during the overwintering period which might lead to disparate susceptibilities to rising overwintering temperatures: bee species emerging in summer overwinter as prepupae in the cocoon and complete their development during the following spring and early summer (O'neill *et al.* 2011), whereas bees becoming active in spring eclose as adults in autumn, overwinter as adults in the cocoon and they finally emerge directly after temperatures are rising in spring (Bosch & Kemp 2000). However, warmer and drier summers can produce midsummer floral scarcity (Aldridge *et al.* 2011) and this outcome is exacerbated by climate change (Willmer 2012). Therefore, fitness losses of summer-emerging bees due to climate change may be different to the ones in spring but nevertheless, they should not be neglected.

Conclusion

It can be concluded that spring-emerging solitary bees are susceptible to climate change as in response to warmer overwintering temperatures bees advance their phenology and show a decreased fitness state. If in response to climate warming plants do not shift their phenologies according to the bees, then bees would not only emerge with a decreased fitness state but also experience temporal mismatches with their host plants. As a consequence, resulting fitness consequences would be exacerbated for spring-emerging solitary bees as they failed to show a single compensation strategy that was entirely successful in mitigating fitness consequences after short temporal mismatches with their food plants. Furthermore, spring-emerging solitary bees do not only consider overwintering temperature but also their individual body condition for adjusting emergence dates. This may lead to differing responses to climate warming within and among bee populations which may also have consequences for bee-plant interactions and the persistence of bee populations under further climate change. Therefore, I suggest that it is not enough to solely investigate temperature effects on the timing of bee emergence, but that we should also consider individual body conditions of solitary bees to understand the timing of bee emergence. Additionally, we should also conceive more studies in which phenological shifts of local occurring bee and plants species are investigated simultaneously. Only then, we will be able to give more precise predictions about the risks and consequences of temporal mismatches between bees and food plants and the persistence of these species in times where environmental conditions are changing. I additionally suggest that further degree-day models should also be developed for several different species. This would help to understand the phenological shifts of different species due to climate change on a community level.

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Study Design	MS	AH	TH	OM
Methods Development	MS	AH	TH	OM
Data Collection	MS			
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