Effects of climate warming on the timing of flowering and emergence in a tritrophic relationship: plants - bees - parasitoids

Auswirkungen der Klimaerwärmung auf die zeitliche Regulierung der Blüte und des Schlupfes in einer tritrophischen Beziehung: Pflanzen - Bienen - Parasitoide

Dissertation zur Erlangung des

naturwissenschaftlichen Doktorgrades

der Julius-Maximilians-Universität Würzburg

vorgelegt von

Sandra Kehrberger

geboren in Feuchtwangen

Würzburg, 2020

Submitted/Eingereicht am: 28.05.2020

Members of the Doctoral Commitee/ Mitglieder der Promotionskommission:

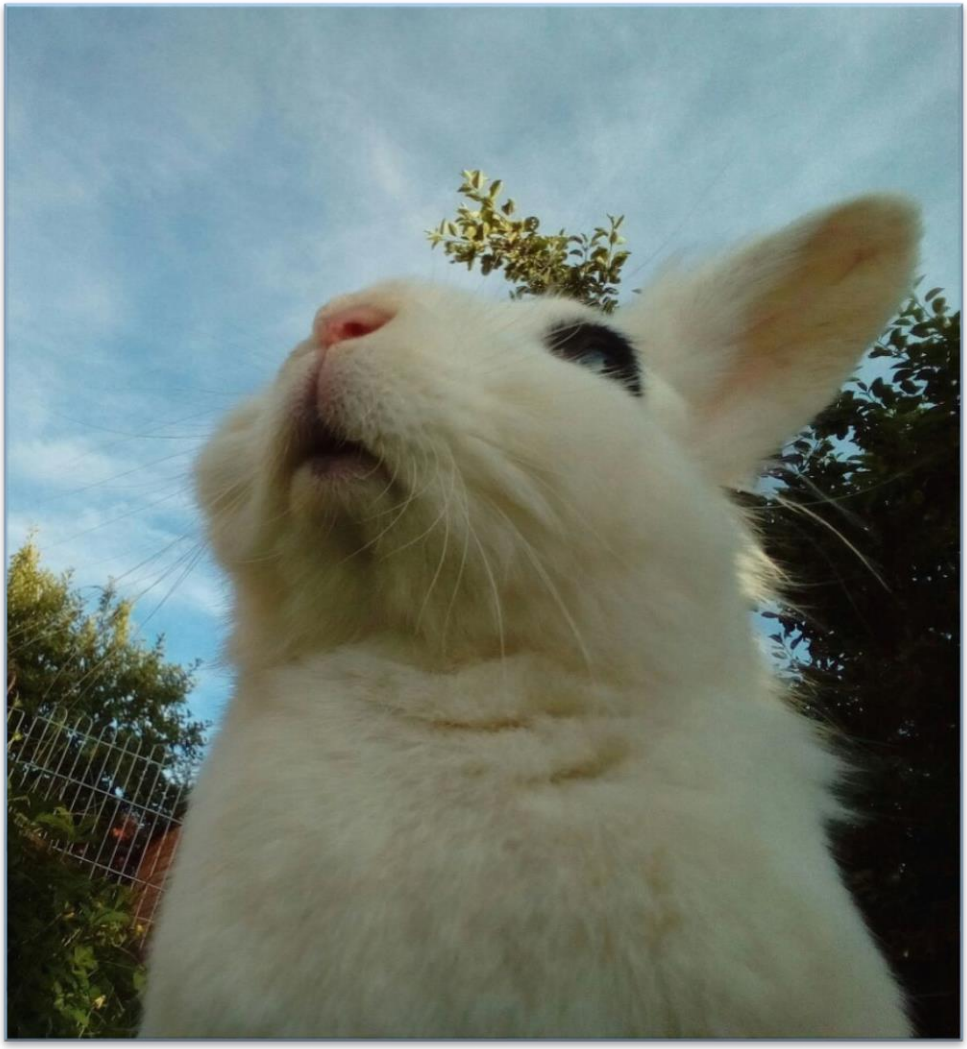
Chair/Vorsitzender: Prof. Dr. Charlotte Förster

Referee/Gutachter: AR PD Dr. Andrea Holzschuh

Referee/Gutachter: Prof. Dr. Ricarda Scheiner

Defence/Tag des Promotionskolloquiums: 21.10.2020

Receipt of Certificates/Doktorurkunde ausgehändigt am:



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Klaus Albers

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Summary

The right timing of phenological events is crucial for species fitness. Species should be highly synchronized with mutualists, but desynchronized with antagonists. With climate warming phenological events advance in many species. However, often species do not respond uniformly to warming temperatures. Species-specific responses to climate warming can lead to asynchrony or even temporal mismatch of interacting species. A temporal mismatch between mutualists, which benefit from each other, can have negative consequences for both interaction partners. For host-parasitoid interactions temporal asynchrony can benefit the host species, if it can temporally escape its parasitoid, with negative consequences for the parasitoid species, but benefit the parasitoid species if it increases synchrony with its host, which can negatively affect the host species. Knowledge about the drivers of phenology and the species-specific responses to these drivers are important to predict future effects of climate change on trophic interactions. In this dissertation I investigated how different drivers act on early flowering phenology and how climate warming affects the tritrophic relationship of two spring bees (*Osmia cornuta* & *Osmia bicornis*), an early spring plant (*Pulsatilla vulgaris*), which is one of the major food plants of the spring bees, and three main parasitoids of the spring bees (*Cacoxenus indagator*, *Anthrax anthrax*, *Monodontomerus*).

In Chapter II I present a study in which I investigated how different drivers and their change over the season affect the reproductive success of an early spring plant. For that I recorded on eight calcareous grasslands around Würzburg, Germany the intra-seasonal changes in pollinator availability, number of co-flowering plants and weather conditions and studied how they affect flower visitation rates, floral longevity and seed set of the early spring plant *P. vulgaris*. I show that bee abundances and the number of hours, which allowed pollinator foraging, were low at the beginning of the season, but increased over time. However, flower visitation rates and estimated total number of bee visits were higher on early flowers of *P. vulgaris* than later flowers. Flower visitation rates were also positively related to seed set. Over time and with increasing competition for pollinators by increasing numbers of co-flowering plants flower visitation rates decreased. My data shows that a major driver for early flowering dates seems to be low interspecific competition for pollinators, but not low pollinator abundances and unfavourable weather conditions.

Chapter III presents a study in which I investigated the effects of temperature on solitary bee emergence and on the flowering of their food plant and of co-flowering plants in the field. Therefore I placed bee cocoons of two spring bees (*O. cornuta* & *O. bicornis*) on eleven calcareous grasslands which differed in mean site temperature. On seven of these grasslands the early spring plant *P. vulgaris* occurred. I show that warmer temperatures advanced mean emergence in *O. cornuta* males. However, *O. bicornis* males and females of both species did not shift their emergence. Compared to the bees *P. vulgaris* advanced its flowering phenology more strongly with warmer temperatures. Co-flowering plants did not shift flowering onset. I suggest that with climate warming the first flowers of *P. vulgaris* face an increased risk of pollinator limitation whereas for bees a shift in floral resources may occur.

In Chapter IV I present a study in which I investigated the effects of climate warming on host-parasitoid relationships. I studied how temperature and photoperiod affect emergence phenology in two spring bees (*O. cornuta* & *O. bicornis*) and three of their main parasitoids (*C. indagator*, *A. anthrax*, *Monodontomerus*). In a climate chamber experiment with a crossed design I exposed cocoons within nest cavities and cocoons outside of nest cavities to two different temperature regimes (long-term mean of Würzburg, Germany and long-term mean of Würzburg + 4 °C) and three photoperiods (Würzburg vs. Snåsa, Norway vs. constant darkness) and recorded the time of bee and parasitoid emergence. I show that warmer temperatures advanced emergence in all studied species, but bees advanced less strongly than parasitoids. Consequently, the time period between female bee emergence and parasitoid emergence decreased in the warm temperature treatment compared to the cold one. Photoperiod influenced the time of emergence only in cocoons outside of nest cavities (except *O. bicornis* male emergence). The data also shows that the effect of photoperiod compared to the effect of temperature on emergence phenology was much weaker. I suggest that with climate warming the synchrony of emergence phenologies of bees and their parasitoids will amplify. Therefore, parasitism rates in solitary bees might increase which can negatively affect reproductive success and population size.

In this dissertation I show that for early flowering spring plants low interspecific competition for pollinators with co-flowering plants is a major driver of flowering phenology, whereas other drivers, like low pollinator abundances and unfavourable weather conditions are only of minor importance. With climate warming the strength of

different drivers, which act on the timing of phenological events, can change, like temperature. I show that warmer temperatures advance early spring plant flowering more strongly than bee emergence and flowering phenology of later co-flowering plants. Furthermore, I show that warmer temperatures advance parasitoid emergence more strongly than bee emergence. Whereas temperature changes can lead to non-uniform temporal shifts, I demonstrate that geographic range shifts and with that altered photoperiods will not change emergence phenology in bees and their parasitoids. In the tritrophic system I investigated in this dissertation climate warming may negatively affect the reproductive success of the early spring plant and the spring bees but not of the parasitoids, which may even benefit from warming temperatures.

Zusammenfassung

Der richtige Zeitpunkt von phänologischen Ereignissen ist maßgeblich für das Überleben und die Fortpflanzung einer Art. Arten sollten eine möglichst hohe Synchronisation mit Mutualisten aufweisen, aber eine möglichst geringe mit Antagonisten. Die Klimaerwärmung führt dazu, dass sich bei vielen Arten phänologische Ereignisse verfrühen. Allerdings reagieren Arten unterschiedlich auf wärmere Temperaturen. Artspezifische Reaktionen auf die Klimaerwärmung können zu Asynchronität oder sogar zu zeitlicher Diskrepanz bei interagierenden Arten führen. Eine zeitliche Diskrepanz zwischen Mutualisten, die voneinander profitieren, kann sich negativ auf beide Interaktionspartner auswirken. Bei Wirt-Parasitoid Beziehungen kann der Wirt von einer zeitlichen Diskrepanz profitieren, wenn er seinem Parasitoid zeitlich entfliehen kann, was wiederum negative Folgen für den Parasitoid haben kann. Jedoch kann der Parasitoid profitieren, wenn er die Synchronisation mit seinem Wirt erhöhen kann, was wiederum den Wirt negativ beeinflussen kann. Das Wissen über die Treiber von phänologischen Ereignissen und die artspezifischen Reaktionen auf diese Treiber sind von Bedeutung um die Auswirkungen des Klimawandels auf trophische Beziehungen vorherzusagen. In meiner Doktorarbeit habe ich untersucht, wie verschiedene Treiber mit einer frühen Blüte zusammenhängen und wie der Klimawandel die tritrophische Beziehung von zwei Frühlingsbienen (*Osmia cornuta* & *Osmia bicornis*), einer Frühlingspflanzenart (*Pulsatilla vulgaris*), die eine der wichtigen Futterpflanzen der Bienen ist, und der drei Hauptparasitoiden der Frühlingsbienen (*Cacoxenus indagator*, *Anthrax anthrax*, *Monodontomerus*) beeinflusst.

In Kapitel II präsentiere ich eine Studie, in der ich den Einfluss verschiedener Treiber und ihre saisonale Veränderung auf den Fortpflanzungserfolg einer Frühlingspflanzenart untersucht habe. Dazu habe ich auf acht Kalkmagerrasen bei Würzburg (Deutschland) die innersaisonalen Veränderungen der Bestäuberverfügbarkeit, der Anzahl an gleichzeitig blühenden Pflanzenarten und die Wetterbedingungen aufgezeichnet. Des Weiteren habe ich erforscht wie diese Faktoren die Blütenbesuchsrate, die Blütenlanglebigkeit und den Samenansatz der Frühlingspflanze *P. vulgaris* beeinflussen. Ich konnte zeigen, dass die Anzahl an Bienen und die Anzahl an Stunden, die ein Furagieren von Bestäubern ermöglicht hätten, am Anfang der Saison niedrig waren und mit der Zeit zunahmen. Jedoch war die Blütenbesuchsrate und die geschätzte Anzahl an Bienenbesuchen höher bei frühen als bei späten *P. vulgaris* Blüten. Die Blütenbesuchsrate wirkte sich auch

positiv auf den Samenansatz aus. Die Blütenbesuchsrate nahm mit der Zeit und mit zunehmender Konkurrenz um Bestäuber durch eine zunehmende Anzahl an gleichzeitig blühenden Pflanzenarten ab. Meine Daten zeigen, dass ein Hauptreiber von frühen Blühzeitpunkten die geringe zwischenartliche Konkurrenz um Bestäuber ist, aber nicht die niedrige Bestäuberanzahl und ungünstige Wetterbedingungen.

Kapitel III präsentiert eine Studie, in welcher ich die Auswirkungen der Temperatur auf den Schlupf von Solitärbienen und die Blüte ihrer Futterpflanzen und gleichzeitig blühenden Pflanzen im Freiland untersucht habe. Dafür habe ich Bienenkokons von zwei Frühlingsbienen (*O. cornuta* & *O. bicornis*) auf elf Kalkmagerrasen, die sich in der mittleren Flächentemperatur unterschieden, platziert. Auf sieben dieser Kalkmagerrasen kam die Frühlingspflanzenart *P. vulgaris* vor. Ich konnte zeigen, dass wärmere Temperaturen den mittleren Schlupf von *O. cornuta* Männchen verfrühen. Die Männchen von *O. bicornis* und die Weibchen beider Arten haben ihren Schlupfzeitpunkt jedoch nicht verschoben. Im Vergleich zu den Bienen verfrühte *P. vulgaris* seine Blühphänologie bei warmen Temperaturen stärker. Die gleichzeitig blühenden Pflanzenarten verschoben ihren Blühbeginn nicht. Die Daten zeigen, dass wärmere Temperaturen den Bienenschlupf weniger stark verfrühen als die Blüte ihrer Futterpflanze. Das lässt darauf schließen, dass mit dem Klimawandel die ersten Blüten von *P. vulgaris* ein erhöhtes Risiko haben nicht bestäubt zu werden, während die Bienen möglicherweise auf andere Blühressourcen ausweichen müssen.

Kapitel IV beschreibt eine Studie, in welcher ich die Auswirkungen der Klimaerwärmung auf Wirt-Parasitoid Beziehungen untersucht habe. Dabei habe ich die Auswirkungen von Temperatur und Photoperiode auf die Schlupfphänologie zweier Frühlingsbienen (*O. cornuta* & *O. bicornis*) und drei ihrer Hauptparasitoide (*C. indagator*, *A. anthrax*, *Monodontomerus*) erforscht. In einem Klimakammerexperiment mit gekreuztem Design habe ich Kokons in Nesthöhlen und Kokons außerhalb von Nesthöhlen, zwei verschiedenen Temperaturregimen (Langzeitmittel von Würzburg, Deutschland und Langzeitmittel von Würzburg + 4 °C) und drei Photoperioden (Würzburg, Deutschland contra Snåsa, Norwegen contra Dauerdunkel) ausgesetzt und die Zeitpunkte des Bienen- und Parasitoidenschlupfes aufgezeichnet. Ich konnte zeigen, dass warme Temperaturen in allen untersuchten Arten den Schlupfzeitpunkt verfrühten, jedoch bei den Bienen weniger stark als bei den Parasitoiden. Eine Folge daraus ist, dass sich die Zeitspanne zwischen dem Schlupf der Bienenweibchen und dem Schlupf der Parasitoide im warmen

Temperaturregime im Vergleich zum kalten verkürzte. Die Photoperiode hatte auf den Zeitpunkt des Schlupfes nur in Kokons außerhalb von Nisthöhlen einen Effekt (außer beim Schlupf von *O. bicornis* Männchen). Die Daten zeigen auch, dass der Effekt der Photoperiode auf die Schlupfphänologie im Vergleich zu dem Effekt der Temperatur viel schwächer war. Daraus schließe ich, dass sich im Zuge der Klimaerwärmung die Synchronisation der Schlupfphänologien von Bienen und ihren Parasitoiden verstärken wird. Eine Folge davon könnten erhöhte Parasitierungsraten bei Solitärbienen sein, welche den Reproduktionserfolg und die Populationsgröße negativ beeinflussen können.

In dieser Doktorarbeit habe ich gezeigt, dass einer der Haupttreiber einer frühen Blüte bei Frühlingspflanzen geringe zwischenartliche Konkurrenz um Bestäuber mit später gleichzeitig blühenden Pflanzenarten ist, während andere Treiber, wie geringe Bestäuberabundanzen und ungünstige Wetterbedingungen nur von geringer Bedeutung sind. Im Zuge des Klimawandels könnte sich die Stärke verschiedener Treiber, die den Zeitpunkt von phänologischen Ereignissen beeinflussen, verändern. Ich konnte außerdem zeigen, dass wärmere Temperaturen die Blüte von frühen Frühlingspflanzen stärker verfrühen, als den Schlupf von Bienen und die Blüte von später gleichzeitig blühenden Pflanzenarten. Des Weiteren zeigte ich, dass wärmere Temperaturen den Schlupf von Parasitoiden stärker verfrühen als den Schlupf der Bienen. Ich konnte zeigen, dass während Temperaturveränderungen zu verschieden starken zeitlichen Verschiebungen führen können, Verschiebungen von geografischen Verbreitungsgebieten und damit geänderten Photoperioden die Schlupfphänologie von Bienen und ihren Parasitoiden wahrscheinlich nicht ändern werden. In dem tritrophischen System, das ich in dieser Doktorarbeit untersucht habe, könnte die Erwärmung des Klimas den Fortpflanzungserfolg der frühen Frühlingspflanze und der Frühlingsbienen negativ beeinflussen, aber wahrscheinlich nicht die der Parasitoide, die vielleicht sogar davon profitieren können.

Chapter I: General Introduction

I.1 Species interactions

The right timing of phenological events is crucial for species fitness (Austen et al. 2017). Animal pollinated plants, which constitute almost 88 % of all flowering plants worldwide depend on pollinators for reproduction (Ollerton et al. 2011). One of their main pollinators are wild- and honeybees (Garibaldi et al. 2013), which comprise around 17.500 species worldwide (Michener 2007). For survival and reproduction bees depend on flowering resources, which provide nectar and pollen (Vaudo et al. 2015). As both animal pollinated plants and bees benefit from each other they share a mutualistic relationship (Kearns et al. 1998). Reproductive success and population size of both mutualists increases with increasing abundance of their interaction partner (Ohara and Higashi 1994; Roulston and Goodell 2011; Garibaldi et al. 2013). Besides mutualists, species may also interact with competitors and antagonists. Competition for resources can have negative consequences for reproduction (Jakobsson et al. 2009; Hudewenz and Klein 2015). Plants may compete with co-flowering plants for pollinators (Sargent and Ackerly 2008), and bees may compete for floral resources (Hudewenz and Klein 2015). Besides competitors, antagonists, like parasites and parasitoids, can also negatively affect reproductive success and, furthermore, population size in bees (Roulston and Goodell 2011).

To ensure high reproductive success species should maximize temporal overlap with mutualists or hosts but minimize temporal co-occurrence with competitors and antagonists (Elzinga et al. 2007; Roulston and Goodell 2011; Pauw 2013; Schenk et al. 2018a; Damien and Tougeron 2019). However, besides biotic drivers, the time of phenological events is also selected by different abiotic drivers with the net outcome of those drivers depending on the strengths and directions of them (Ehrlén and Münzbergová 2009; Wolkovich et al. 2014). So far, little is known about the joint effects of abiotic and biotic drivers on the timing of phenological events.

In Chapter II I focused on the abiotic driver temperature and the biotic drivers interspecific competition and mutualist availability and how the timing of a phenological

Fig. I.1. *P. vulgaris* flower

event affects reproductive success. As a model organism I chose *Pulsatilla vulgaris* (Fig. I.1), which is an herbal plant that starts flowering as the first plant at the beginning of the season on the studied sites. To set seeds it depends on bees for pollination (Wells and Barling 1971). However, at the beginning of the season bees are scarce and foraging activity is limited by prevailing low temperatures. Besides, competition for pollinators by other plants is very low for a certain period. As the gradient of those drivers changes over the flowering season of *P. vulgaris* their joint effects and the changes in their impact over time can be studied to investigate how the timing of flowering affects reproductive success.

I.2 Climate warming

Climate warming is one of the major threats to biodiversity and affects the phenology, distribution and interspecific relationships of many species (Scheffers et al. 2016). Global mean temperature increased by 0.85 °C from 1880 to 2012 and is projected to further increase between 0.3 to 4.8 °C until 2100 (IPCC 2014).

I.2.1 Phenology

In many plant species recent climate warming led to advances in spring and summer phases, like bud burst, flowering, leaf unfolding and fruiting, however, early spring species are most affected and show the strongest responses (Fitter and Fitter 2002; Menzel et al. 2006). Also, in many vertebrate and invertebrate species phenological events are advanced in response to climate warming, like migration in birds and emergence in insects (Roy and Sparks 2000; Parmesan and Yohe 2003; Gordo and Sanz 2005). Those phenological advances are not uniformly across species but differ in their strength and direction depending on the species-specific phenological climate sensitivity which can cause temporal desynchronization (Thackeray et al. 2016). Negative or positive

consequences of a temporal mismatch with an interaction partner may be particularly pronounced at the beginning of the season, when other potential interaction partners are not yet present that could replace the original interaction partner (Forrest and Thomson 2011). Therefore, the right timing of phenological events is important to maximize the temporal overlap with mutualists, but also to minimize the temporal overlap with competitors and antagonists (Elzinga et al. 2007; Pauw 2013; Evans et al. 2013). Reductions in temporal synchrony or temporal mismatches can have severe negative consequences for survival and reproductive success of mutualistic interaction partners, like plants and bees (Hegland et al. 2009; Schenk et al. 2018a). As a result of a temporal mismatch with its pollinators, plants may receive reduced visitation rates and pollen deposition, whereas for bees a temporal mismatch with the plants they forage on can decrease the availability of food resources (Hegland et al. 2009). The effects of climate warming on plant-pollinator synchrony vary between studies, probably due to species-specific differences in the responses to warming temperatures. Some studies found that plants have advanced their phenology more strongly than bees in response to climate change (Kudo et al. 2004; Forrest and Thomson 2011; Kudo and Ida 2013; Pyke et al. 2016) others that bees have advanced more strongly (Gordo and Sanz 2005; Burkle et al. 2013; Robbirt et al. 2014; Olliff-Yang and Mesler 2018) or showed no difference in the phenological shift of plants and bees (Bartomeus et al. 2011; Ovaskainen et al. 2013). The majority of these studies focused on the synchrony between plant phenology and activity of bumble bees (Kudo et al. 2004; Kudo and Ida 2013; Ovaskainen et al. 2013; Pyke et al. 2016) or used museum collections providing flight activity data to study the synchrony between plant phenology and flight activity of solitary bees (Bartomeus et al. 2011; Burkle et al. 2013; Robbirt et al. 2014), whereas field studies on the synchrony between plant phenology and solitary bee emergence are still scarce (but see Forrest and Thomson 2011). Field studies on the effect of temperature on the timing of bee emergence and plant flowering can help to understand how temperature affects the synchrony of pollinators and plants and provide the basis for predicting effects of future climate warming on plant-pollinator interactions.

For competing interaction partners shifts in temporal synchrony can be beneficial if the degree of competition is reduced, but adverse if competition for resources increases (Sherry et al. 2007). For co-flowering plants that compete for pollinators reductions in co-flowering patterns could increase reproductive success, whereas increases in temporal

co-occurrence may decrease reproductive success (Bell et al. 2005). With climate warming flowering onset of many plant species is advanced (Gordo and Sanz 2005; Menzel et al. 2006), however, species vary greatly in their degree of response (CaraDonna et al. 2014). Besides, flowering durations can also be affected by climate warming, when flowering onset and end shift non-uniformly (CaraDonna et al. 2014). Consequently, the temporal co-flowering patterns in sequentially flowering plant species can be altered by climate warming, which may affect competitive interactions (Sherry et al. 2007; CaraDonna et al. 2014; Theobald et al. 2017).

Fig. I.2. *O. cornuta* male and *O. bicornis* female

Fig. I.3. Emergence tubes filled with bee cocoons of *O. cornuta* and *O. bicornis* on a calcareous grassland with flowering *P. vulgaris*

In Chapter III I assessed how temperature affects emergence phenology of two solitary-spring bees and flowering phenology of an early spring plant, which interacts mutualistically with the bees. Besides, I aimed to study if the sequentially co-flowering pattern of an early spring plant is affected by climate warming. Therefore, I investigated how warmer temperatures affect, besides flowering onset, the flowering duration of *P. vulgaris* and the flowering onset of its later co-flowering plants with which *P. vulgaris* competes for pollinators. For that I placed bee cocoons of the two bee species *Osmia cornuta* and *Osmia bicornis* (Fig. I.2) on calcareous grasslands with a temperature gradient in mean site temperature and recorded flowering of naturally occurring *P. vulgaris* populations, of later co-flowering plant species and emergence of both bee species (Fig. I.3).

In antagonistic interactions changes in temporal synchrony can reduce parasitism rates if hosts can temporally escape parasitoid activity (Evans et al. 2013) but increase parasitism rates when parasitoids increase phenological synchrony with hosts (Nouhuys and Lei

2004). Wild bees interact with predators, parasites and parasitoids, which can all reduce reproductive success and population size (Krunić et al. 2005; Roulston and Goodell 2011; Felicioli et al. 2017). Among important antagonists of wild bees are parasitoid flies and wasps (Krunić et al. 2005; Felicioli et al. 2017). So far, the effects of climate warming on parasitoid phenology and on the temporal synchrony between bees and parasitoids are hardly studied (Farzan and Yang 2018). However, to predict future effects of climate warming on wild bee persistence not only possible temporal shifts in mutualistic interactions should be considered but also antagonistic interactions.

I.2.2 Environmental cues

To predict when environmental conditions are most suitable for a phenological event organisms use different environmental cues (Visser et al. 2010). The cues used and the strength of the response to them can differ among species (Visser et al. 2010; Forrest and Thomson 2011). To allow temporal synchrony interacting species should use the same or similar cues (Kraemer and Favi 2010; Bartomeus et al. 2011). If interacting species respond to different cues, different combinations of cues or to different extents to the same cues, changes in used cues could result in different phenological responses and alter temporal co-occurrence patterns (Menzel et al. 2006; Memmott et al. 2007; Kudo and Ida 2013). In wild bees temperature is suggested to be the main cue for emergence (Forrest and Thomson 2011; Ovaskainen et al. 2013), with warmer temperatures advancing the time of emergence (Bosch and Kemp 2003; Fründ et al. 2013; Schenk et al. 2018b). Parasitoids of wild bees probably use the same or similar cues as their hosts to trigger emergence and thereby ensure synchrony. However, studies on the environmental cues utilized by wild bee parasitoids to time their emergence are still rare and mainly focus on wasp species (Kraemer and Favi 2010; Forrest and Thomson 2011). Many insects have also been shown to use photoperiod as an additional cue to time phenological events (Bradshaw and Holzapfel 2007). Besides shifts in the timing of phenological events, species have also been shown to shift their geographical ranges towards the poles as a response to climate warming (Parmesan et al. 1999; Parmesan and Yohe 2003). With these geographic range shifts species may be exposed to the same temperature regimes as before climate warming, however, to an altered photoperiod. In wild bees and their parasitoids photoperiod used as an additional cue, besides temperature, could prevent too early emergences during time periods when environmental conditions are not yet suitable

for emergence despite prevailing warm temperatures. However, the effects of photoperiod on the circannual timing of bee and parasitoid emergence have not yet been studied.

In Chapter IV I assessed the effects of climate warming and exposures to new photoperiods due to geographical range shifts on the emergence phenology of two solitary-spring bees (*O. cornuta* & *O. bicornis*, Fig. I.3) and three of their main parasitoids (*Cacoxenus indagator*, *Anthrax anthrax* and *Monodontomerus*, Fig. I.4) and the

Fig. I.4. *A. anthrax, C. indagator* and *Monodontomerus*

synchrony between them. I therefore exposed cocoons within nest cavities and cocoons that had been removed from the nests to two temperature regimes (long-term mean of Würzburg and long-term mean of Würzburg + 4 °C) and three photoperiods (Würzburg 49° 47´ N vs. Snåsa 64° 14´ N vs. constant darkness) and recorded the time of emergence of bees and parasitoids.

Chapter II: How does timing of flowering affect competition for pollinators, flower visitation and seed set in an early spring grassland plant?

This chapter is published as: Kehrberger S., Holzschuh A. (2019) How does timing of flowering affect competition for pollinators, flower visitation and seed set in an early spring grassland plant? *Sci Rep* **9,** 15593. doi:10.1038/s41598-019-51916-0

II.1 Summary

Knowledge on how the timing of flowering is related to plant fitness and species interactions is crucial to understand consequences of phenological shifts as they occur under climate change. Early flowering plants may face advantages of low competition for pollinators and disadvantages of low pollinator abundances and unfavourable weather conditions. However, it is unknown how this trade-off changes over the season and how the timing affects reproductive success. On eight grasslands we recorded intra-seasonal changes in pollinators, co-flowering plants, weather conditions, flower visitation rates, floral longevity and seed set of *Pulsatilla vulgaris*. Although bee abundances and the number of pollinator-suitable hours were low at the beginning of the season, early flowers of *P. vulgaris* received higher flower visitation rates and estimated total number of bee visits than later flowers, which was positively related to seed set. Flower visitation rates decreased over time and with increasing number of co-flowering plants, which competed with *P. vulgaris* for pollinators. Low interspecific competition for pollinators seems to be a major driver for early flowering dates. Thus, non-synchronous temporal shifts of co-flowering plants as they may occur under climate warming can be expected to strongly affect plant-pollinator interactions and the fitness of the involved plants.

II.2 Introduction

The optimal timing of flowering is crucial for plant fitness (Austen et al. 2017). The timing of flowering depends on abiotic factors like temperature and the availability of water, nutrients and light (Rathcke and Lacey 1985; Ehrlén 2015). Besides, interspecific

interactions with mutualists, like pollinators, and competitors, like co-flowering plants, have been suggested to affect the timing of flowering (Rathcke and Lacey 1985; Elzinga et al. 2007; Ehrlén 2015). In many plants that depend on animal pollination seed set increases with increasing pollinator visitation rates (Ohara and Higashi 1994; Garibaldi et al. 2013). However, plants that are poor competitors for pollinators may receive reduced pollinator visitation rates in the presence of competing co-flowering plant species, which are more attractive to pollinators (Mosquin 1971). Previous studies indicated that plant species can mitigate negative effects of low pollinator visitation by elongating their floral longevity, which increases the probability of pollinator visitation, but warm temperatures may hinder elongation (Yasaka et al. 1998; Arroyo et al. 2013). If plants cannot mitigate for low pollinator visitation rates, then competition for pollinators could drive poor competing plants to shift their flowering phenology to times with less competition (Mosquin 1971). So competing plant species can achieve coexistence by temporal niche separation (Jensen et al. 2019). However, the options for plant species to shift flowering to periods with less competing plants being present, like at the beginning of the flowering season, are limited by pollinator availability (Oertli et al. 2005; Leong et al. 2016), temperatures allowing flower survival (Thomson 2010) and foraging activity of pollinators (Kevan and Baker 1983; Totland 1994). The net outcome of those different drivers on the selection of flowering phenology depends not only on their direction, but also on their strength (Ehrlén and Münzbergová 2009). So far, we know little about the joint effects of temperature, pollinator availability and competition by co-flowering plants on pollinator visitation and the resulting reproductive success of plant species. We would expect a directional selection towards earlier flowering dates, if reproductive success would increase with earlier flowering, but a more stabilizing selection if first and last flowers have lowest reproductive success compared to peak flowers.

Plant species flowering at the beginning of the season constitute good model organisms to study how these drivers and their joint effects change over time, if their flowering period covers a sufficiently long period with a strong change in the gradient of the drivers. Early flowering plants may face the risk of low pollinator availability and low temperatures. However, they may also have the advantage of a flowering onset in the absence of co-flowering plants, and thereby in the absence of interspecific competition for pollinators for a certain time span of flowering. Previous studies focusing on plants

flowering as the first plant species in the season in forests and subalpine grasslands showed that the first flowers had a reduced seed or fruit set compared to later flowers. This was suggested to be caused by low pollinator availability due to either low pollinator abundances or impaired foraging activity by low temperatures (Schemske 1977; Motten et al. 1981; Mahoro 2002; Kudo et al. 2004; Thomson 2010; Kudo and Ida 2013).

This raises the question why plant species don´t start flowering later, especially grassland plants, which are not restricted by canopy closure, like plants in deciduous forests. One explanation could be that competition with later co-flowering plants for pollinators may reduce reproductive success. For plant species in the Mediterranean, which start flowering in winter, it has been shown that pollinator visitation was lower after the flowering onset of co-flowering plant species, which were suggested to withdraw pollinators (Vesprini and Pacini 2010). We suggest that competition with co-flowering plants for pollinators is lowest at the beginning of the season, but increases over time, which in turn decreases reproductive success. However, the net outcome of the advantages of low interspecific competition for pollinators and the disadvantages of low pollinator abundances and low numbers of hours with temperatures suitable for pollinator foraging on flower visitation rates and reproductive success and their change over time has not yet been studied. With climate warming temporal co-flowering patterns may change due to species-specific phenological responses to warmer temperatures (CaraDonna et al. 2014). This could alter the strength and/or the direction of the drivers acting on flower phenology and reproductive success. We suggest that to predict future effects of climate warming on plant communities knowledge about the current drivers and their strength and direction on flower phenology is necessary.

In this study we focused on the red-list spring plant *Pulsatilla vulgaris*, which is the first plant species to flower on semi-natural calcareous grasslands in Germany. On eight sites, we studied how the timing of flowering affected pollinator availability, competition for pollinators, pollinator visitation rates, floral longevity, the number of hours with temperatures allowing pollinator activity, the estimated total number of bee visits, and seed set.

We tested the following hypothesis:

1. Flower visitation rates of *P. vulgaris* flowers increase with increasing bee abundance but decrease with increasing competition for pollinators with co-

flowering plant species.

2. Later dates of bud opening shorten floral longevity but increase the flower-specific number of pollinator-suitable hours with temperatures allowing pollinator activity and the estimated total number of bee visits per flower of *P. vulgaris*.
3. *P. vulgaris* benefits from insect pollination and seed set increases with an increase in the estimated total number of bee visits per flower and is higher for early than for late dates of bud opening.

II.3 Materials and Methods

II.3.1 Study sites

The study was conducted on eight calcareous grasslands around the city of Würzburg, Germany (49° 47' 28" N, 9° 57' 12" E). Grasslands had a minimum size of one hectare and were located in an area of about 116 km^2 with a distance of 2.5 to 28.6 km between them. Calcareous grasslands comprise high biodiversity and rare species but are threatened by land use change as maintenance of grasslands depends on regular management (WallisDeVries et al. 2002). Seven of the eight studied calcareous grasslands were managed by extensive sheep grazing, one was not managed. The population size of *Pulsatilla vulgaris* ranged between 15 and 600 individuals, depending on the site.

From 6th February to 30th May 2015 we hourly recorded air temperature with two temperature loggers per site (iButton temperature logger DS1922L, Maxim Integrated, USA; resolution: 0.0625 °C; Note II.S2).

II.3.2 Pulsatilla vulgaris

The common pasque flower (*Pulsatilla vulgaris*; Ranunculaceae) is a perennial herb, which grows on calcareous grasslands (Wells and Barling 1971). On the studied sites it was the first herbal plant species that started flowering. *P. vulgaris* is listed as a threatened plant species in the red list of threatened plant species of Germany (Ludwig and Schnittler 1996). It reproduces sexually as well as vegetatively (Wells and Barling 1971). Flowering occurs between March and April (Hensen et al. 2005). During the flowering season *P.*

vulgaris mostly produces one to three flowers per plant, which are hermaphrodite and protogynous (Wells and Barling 1971). Each flower is characterized by six purple-violet petals and numerous carpels and stamens, whereby the outer stamens are sterile and secreting nectar (Jonsson et al. 1991). The main flower visitors of *P. vulgaris* are bees (Kratochwil 1988). The produced seeds have a long feathery style and are dispersed by wind (Wells and Barling 1971).

II.3.3 Data recording

To detect flowering onset of *P. vulgaris* populations we walked across each site between 6th February and 4th March 2015 every fourth to tenth day and after 4th March 2015 every second to third day. *P. vulgaris* populations started flowering between 13th and 18th March depending on site and the last population ended flowering on 5th May 2015. Bee and plant surveys were conducted between 17th March and 5th May 2015. During the sampling period, the phenology of *P. vulgaris*, of other than *P. vulgaris* flowering plant species and of bees (Apiformes) was recorded every second to third day on each site. Bees and the phenologies of other plant species than *P. vulgaris* were not recorded on days with daylong rain, but phenology of *P. vulgaris* was recorded. We conducted 21 bee surveys on five sites, 19 on two sites and 18 on one site. Due to different flowering durations of the *P. vulgaris* populations, *P. vulgaris* phenology surveys ranged from 13 to 24 surveys per site.

For each bee and plant survey, a variable transect of 100 m^2 (Westphal et al. 2008) containing the highest abundance of *P. vulgaris* flowers was chosen. We conducted bee and plant surveys until the flowering period of *P. vulgaris* populations had ended on all sites. If there were no more flowering *P. vulgaris* plants on the studied site, transects with the highest abundance of other flowering plants were chosen. We walked transects for 14 - 36 minutes (mean: 25 min.) per survey and recorded the number of bees and whether they were encountered on a *P. vulgaris* flower or at another location. Bees were captured if possible and individuals that could not be identified in the field were taken to the lab for further identification. To avoid multiple counts of single individuals, all in the field identified bees were released not until the end of the survey. Bees that could not be captured, were, if possible, classified to the genus level. We excluded bees that could not be assigned to a genus and the genera *Halictus* and *Lasioglossum* from the data set,

because bees of these genera mostly never touched the carpels and may not have provided pollination services in our study. The lack of pollen transfer from *Halictus* and *Lasioglossum* bees to the carpels of *P. vulgaris* was confirmed by camera recordings of 56 *Halictus* and *Lasioglossum* bees (unpublished data). After each transect walk the number of other than *P. vulgaris* flowering plant species on the transect was recorded and the abundance of flowers or flower heads for each plant species including *P. vulgaris* estimated.

For *P. vulgaris* we calculated for every survey the day-specific flower visitation rate (per flower and hour), by dividing the number of bees on *P. vulgaris* flowers on the transect by the abundance of *P. vulgaris* flowers on the transect and the duration of the transect walk. The day-specific bee abundance (per hour) for every survey was calculated by dividing the number of all recorded bees on the transect by the duration of the transect walk. The weekly mean flower visitation rate on *P. vulgaris* flowers and the weekly mean bee abundance on the transect were calculated as the mean of the day-specific flower visitation rates and day-specific bee abundances, respectively. The day-specific number of other flowering plant species on the transect and the day-specific flower abundance of other plant species on the transect were highly correlated (Pearson rank correlation coefficient: $cor = 0.83$; $p < 0.001$), so we used only day-specific number of other flowering plant species on the transect for further analysis. The weekly total number of other flowering plant species was the cumulated number of other flowering plant species recorded on that site during one week.

II.3.4 Pollination treatments and quantifying seed set

To identify the relative influence of wind, insect and optimal pollination on the seed set of *P. vulgaris* three pollination treatments were compared: (1) pollinator exclusion (wind and self-pollination only, mesh width of nets: 1mm), (2) open flowers (wind, self- and insect pollination), (3) open hand-pollinated flowers (optimal pollination, wind, self-, insect and hand pollination). Every second or third day we randomly selected at least three *P. vulgaris* individuals per site, one for each treatment, and marked one up to two days old flower or bud per individual. The age of the flowers was determined by visual inspection. Young flowers are erect with a short stalk, have deep purple petals and are not yet fully opened (Wells and Barling 1971; Kratochwil 1988). We recorded the date of bud

opening and the end of flowering for each marked flower or bud. For the pollinator exclusion treatment, we netted only closed flower buds. For the open and hand pollination treatments flowers were up to two days old. For the hand pollination treatment, we randomly selected flowers of other *P. vulgaris* individuals with available pollen and removed the pollen with a brush. The pollen was deposited on the carpels of the hand pollinated flower by sweeping six times across the carpels with the pollen loaded brush. Pollen donator plants were marked and not used for further treatments. When no flower buds were available anymore, we ended treatments on that site. Treatments started at 16th March 2015 and ended on four sites between 14th and 17th April 2015 and on three sites between 20th and 24th April 2015. On one site we could carry out treatments only on two days, 17th and 22nd March 2015 due to the small population size. In total we marked 81 flowers for the pollinator exclusion treatment, 80 flowers for the open pollination treatment and 78 flowers for the hand pollination treatment (Table II.S3). Because of strong wind some nets opened during the study and, some flowers and seeds were broken off or partly eaten by animals, thus reducing the number of replicates to 45 flowers in the pollinator exclusion treatment, to 64 flowers in the open pollination treatment and 65 flowers in the hand pollination treatment. End of May we harvested the ripe seeds produced by the marked flowers and counted the number of fertilized and non-fertilized seeds, with non-fertilized seeds having shorter styli than fertilized (Kratochwil 1988). Seed set (%) per flower was calculated by dividing the number of fertilized seeds by the sum of fertilized and non-fertilized seeds, which represents the number of ovules. Pollen limitation was calculated by dividing the seed set of hand pollinated flowers by the seed set of open pollinated flowers which had the same bud-opening date.

For each flower from the open pollination treatment we calculated the floral longevity, as the difference between the recorded date of flowering end and the recorded date of bud opening. The relationship between temperature during bee survey and day-specific flower visitation rate showed that bees were only observed at an air temperature warmer than 11.3 °C on *P. vulgaris* flowers. One exception was a single bumble bee queen, which was recorded at a temperature of 7.9 °C (Fig. II.S4). This indicates that mainly temperatures warmer than 11.3 °C were suitable for bees to visit *P. vulgaris* flowers during our study. For every open pollinated flower, we calculated the flower-specific number of pollinator-suitable hours, as the sum of hours equal or warmer than 11.3 °C between 8.00 am and 8.00 pm during flower life. We also calculated the flower-specific mean temperature as

the mean of the hourly recorded temperatures during flower life. Furthermore, we calculated the flower-specific mean flower visitation rate (per hour) as the mean of the day-specific flower visitation rates recorded during surveys with an air temperature equal or warmer than 11.3 °C during flower life. We estimated the total number of bee visits per flower for each open pollinated flower, by multiplying the flower-specific number of pollinator-suitable hours with the flower-specific mean flower visitation rate. For this estimation of the potential total number of bee visits a flower receives during flower life we assumed that the attractiveness of a flower stays constant until flowering end and that all hours equal or warmer than 11.3 °C were suitable for pollinators to forage.

II.3.5 Statistical analyses

All statistical analyses were performed using the software R version 3.6.1 (R Core Team 2019). For the linear-mixed effects models we used the nlme package (Pinheiro et al. 2019) and present p-values for Wald tests. To determine if the time of flowering affects the weekly mean bee visitation rate on *P. vulgaris* flowers (we used only weeks and sites with more than one *P. vulgaris* flower), the weekly mean bee abundance and the total number of other flowering plant species, we used linear mixed-effects models with week of the year as fixed factor and site as random factor. Out of 115 bee surveys, 58 took place when other plant species than *P. vulgaris* flowered on the transects. Using these surveys we tested the effect of the fixed factors day-specific number of other flowering plant species and Julian date on the response variable day-specific flower visitation rate on *P. vulgaris* flowers (only surveys with more than one *P. vulgaris* flower were used) with a linear mixed-effects model with site as random factor. Julian date was removed from the model because it had no additional explanatory power ($p > 0.05$) suggesting that it had no direct effects in addition to indirect effects via the number of other flowering plant species.

To test if floral longevity, flower-specific number of pollinator-suitable hours and the estimated total number of bee visits per flower of *P. vulgaris* are affected by bud opening we used linear mixed-effects models with site as random factor and Julian date of bud opening as fixed factor. As Julian date and mean temperature were positively correlated, we used a separate linear-mixed effects model with site as random factor, to analyse the effect of flower-specific mean temperature on the response variable floral longevity of *P.*

vulgaris. When using both Julian date and flower-specific mean temperature as fixed factors, the latter had no additional explanatory power ($p > 0.05$). Furthermore, we analysed the effect of the fixed factor flower-specific mean temperature on the response variable floral longevity of *P. vulgaris* with a linear-mixed effects model with site as random factor.

We analysed if *P. vulgaris* depends on animal pollination with a linear mixed-effects model by comparing the effects of the fixed factor pollination treatment (pollinator exclusion vs. open vs. hand pollination) on the response variable seed set with site as random factor. The effect of pollination treatment was examined with the contrasts between the mean values of the three factor categories. To estimate the differences the glht function in the R-package multcomp (Hothorn et al. 2008) was used. P-values of multiple comparisons were corrected by the Holm correction. To assess whether the seed set of open pollinated flowers was related to pollen limitation, we used linear mixed-effects models with seed set as fixed factor, pollen limitation as response variable and site as random factor. The effect of the fixed factor estimated total number of bee visits per flower on the response variable seed set of *P. vulgaris* was analysed with a linear mixed-effects model with site as random factor. When using both estimated total number of bee visits per flower and Julian date of bud opening as fixed factors in the same model, Julian date of bud opening had no additional explanatory power ($p > 0.05$) suggesting that date had an indirect effect by affecting the total number of bee visits. Furthermore, we tested the effect of the fixed factors Julian date of bud opening, pollination treatment (hand vs. open) and their interaction on the response variable seed set of *P. vulgaris* with a linear mixed-effects model with site as random factor. In a second step we tested the effect of the fixed factor Julian date of bud opening on the response variable seed set separately for open and hand pollinated *P. vulgaris* flowers. We visually inspected model residuals for violation of assumptions of normality and homoscedasticity.

II.4 Results

The flowering period of naturally occurring *Pulsatilla vulgaris* populations started in week 11 or 12 of the year, depending on site, showed peak flowering in week 13 or 14 and ended in week 15 to 19. In total we observed 929 bee individuals, from 54 bee species and 11 genera during transect walks. *P. vulgaris* was visited by 80 bees comprising 12

species and four genera (excluding *Halictus* and *Lasioglossum*, which are no pollinators of *P. vulgaris*, and unidentified bees). The managed honeybee *Apis mellifera* was most common with 52.50 % of the bee visits on *P. vulgaris*, followed by the genera *Osmia* (18.75 %), *Bombus* (17.50 %) and *Andrena* (11.25 %).

In week 12 of the year, at the beginning of the flowering period of *P. vulgaris*, weekly mean flower visitation rate on *P. vulgaris* flowers was highest and decreased over time (linear mixed-effects model (lme): $F_{1,38} = 5.3$, $p = 0.027$, Fig. II.1a). Contrary weekly mean bee abundance on the transect was lowest in week 12 and increased over time (lme: $F_{1,55} = 6.0$, $p = 0.018$, Fig. II.1b). In week 14 other plant species than *P. vulgaris* started flowering. Besides *P. vulgaris* we recorded 20 flowering plant species during *P. vulgaris* flowering and 40 flowering plant species during the sampling period. The most common flowering plant species during *P. vulgaris* flowering were - besides *P. vulgaris* - *Potentilla neumanniana,* which occurred on all grasslands, followed by *Taraxacum officinale*, which occurred on seven grasslands, and *Euphorbia cyparissias* and *Viola* , which occurred on six grasslands. The total number of other flowering plant species increased over time (lme: $F_{1,32} = 29.9$, $p < 0.001$, Fig. II.1c). With increasing day-specific number of other flowering plant species day-specific flower visitation rate on *P. vulgaris* flowers declined (lme: $F_{1,106} = 6.9$, $p = 0.010$, Fig. II.1d).

Floral longevity of *P. vulgaris* shortened with later bud opening (lme: $F_{1,55} = 78.5$, $p < 0.001$, Fig. II.2a), as well as with increasing flower-specific mean temperature (lme: $F_{1,55} = 22.4$, $p < 0.001$). Despite an increasing number of flower-specific pollinator-suitable hours with later bud opening (lme: $F_{1,55} = 11.1$, $p = 0.002$, Fig. II.2b), the estimated total number of bee visits per flower marginally decreased with later bud opening (lme: $F_{1,55} = 3.6$, $p = 0.065$, Fig. II.2c).

The pollination experiment showed that pollinator exclusion (wind and self-pollination only) resulted in less than 20% of the seed set produced by open flowers (wind, self- and insect pollination), whereas there was no difference between open and hand pollinated flowers in seed set (lme: $F_{2,164} = 43.2$, $p < 0.001$; post-hoc test: netted vs. open or hand: $p < 0.001$, open vs. hand: $p = 0.643$, Fig. II.3a). Pollen limitation decreased with increasing seed set of open pollinated flowers ($F_{1,31} = 10.4$, $p = 0.003$, after removing one outlier: $F_{1,30} = 31.2$, $p = <0.001$, Fig. II.S1). Seed set increased with increasing estimated total number of bee visits for open pollinated flowers (lme: $F_{1,55} = 4.1$, $p = 0.047$, Fig. II.3b).

A model testing the effects of Julian date of bud opening, pollination treatment (hand vs. open) and their interaction on seed set of *P. vulgaris* showed no significant effects (lme: Julian date of bud opening: $F_{1,118} = 2.5$, $p = 0.113$; treatment: $F_{1,118} = 0.2$, $p = 0.664$; Julian date of bud opening*treatment: $F_{1,118} = 0.6$, $p = 0.426$). However, when analysing seed set of hand and open pollinated flowers in separate models, seed set of open pollinated flowers marginally decreased with a later bud opening (lme: $F_{1,55} = 2.8$, $p = 0.098$, Fig. II.3c), while seed set of hand pollinated flowers did not change with date of bud opening (lme: $F_{1,56} = 0.6$, $p = 0.460$).

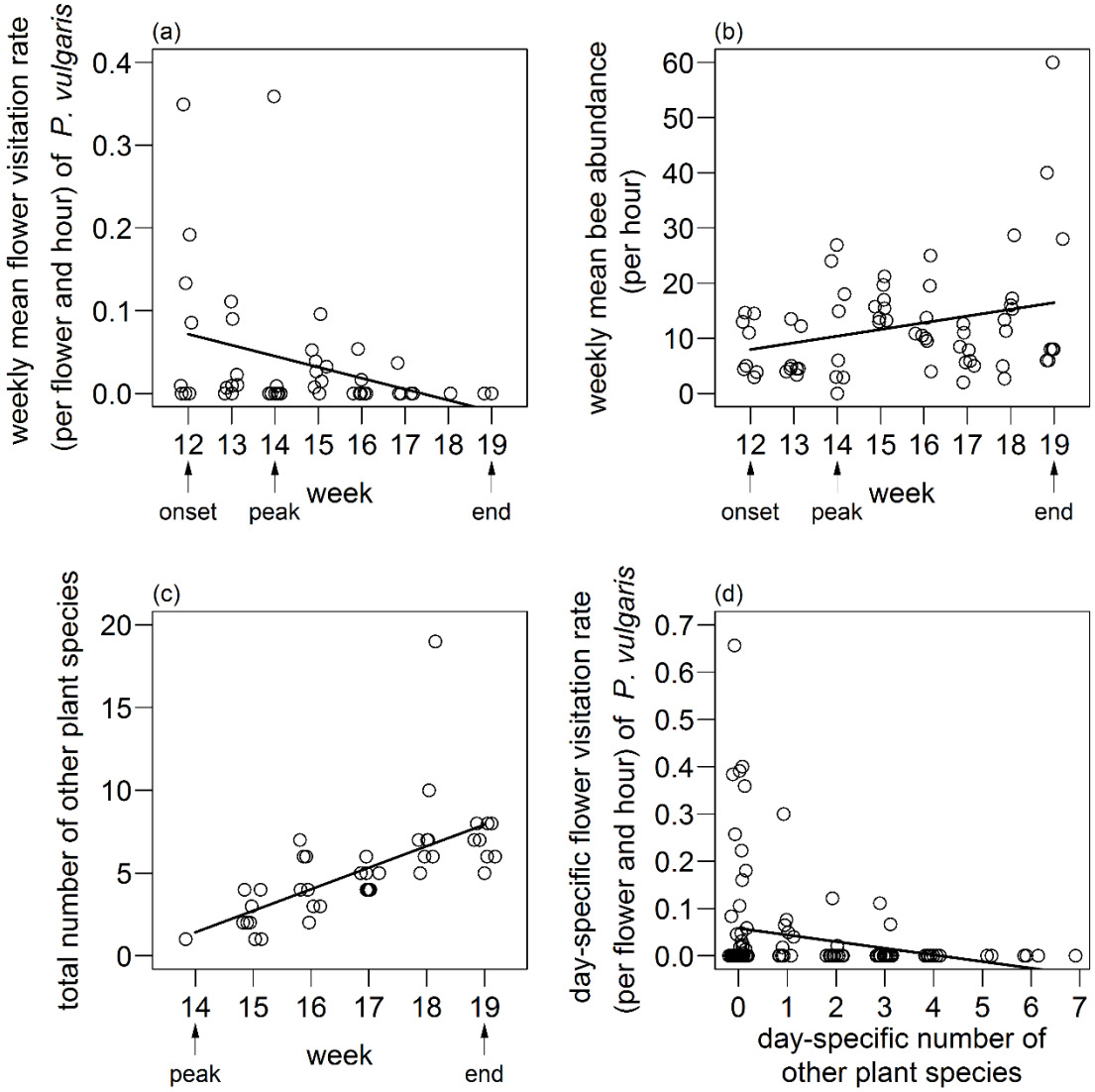

Fig. II.1. Relationship between week of the year and (**a**) weekly mean flower visitation rate (per flower and hour) of *P. vulgaris* flowers, (**b**) weekly mean bee abundance (per hour) on the transect and (**c**) total number of other flowering plant species on the transect. (**d**) Relationship between day-specific number of other flowering plant species and day-specific flower visitation rate (per flower and hour) of *P. vulgaris*. Results are from linear mixed effects models. Solid lines show significant relationships ($p < 0.05$). Arrows show flowering onset (onset), peak flowering (peak) and flowering end (end) of *P. vulgaris*. A horizontal jitter was added to separate overlying data points.

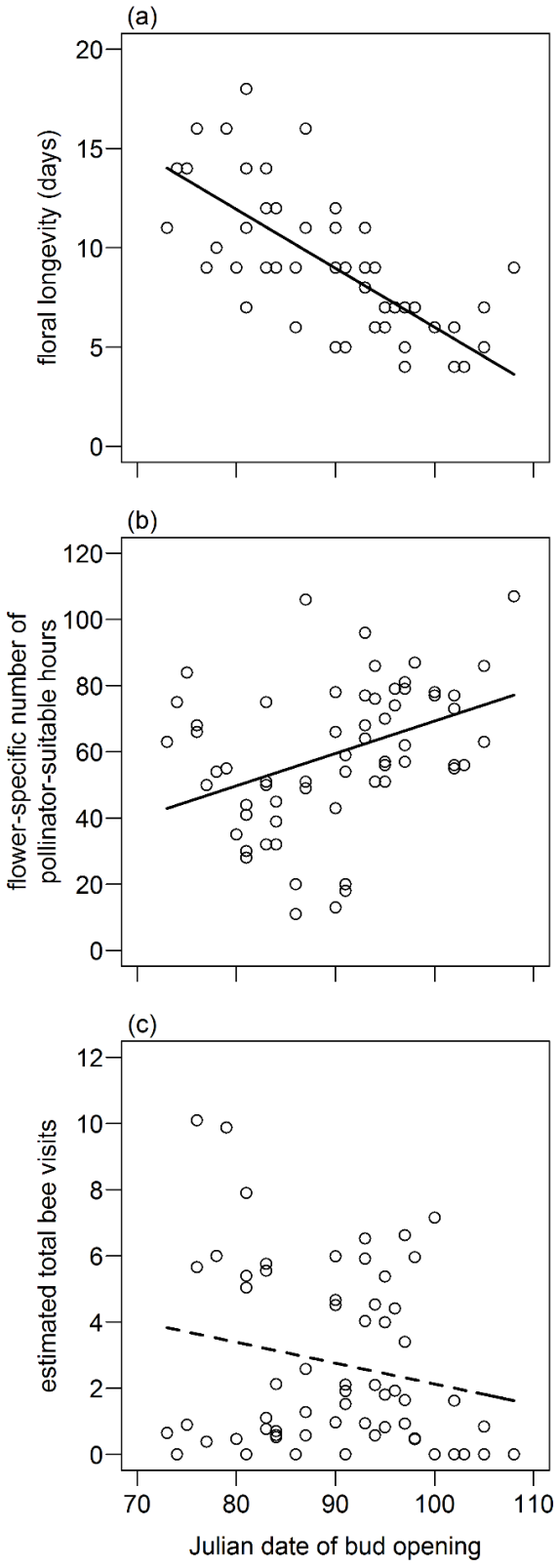

Fig. II.2. Relationship between Julian date of bud opening and (**a**) floral longevity (days), (**b**) flower-specific number of pollinator-suitable hours and (**c**) estimated total number of bee visits per flower of *P. vulgaris*. Solid lines show significant relationships ($p < 0.05$), dashed lines marginal significant relationships ($p < 0.1$).

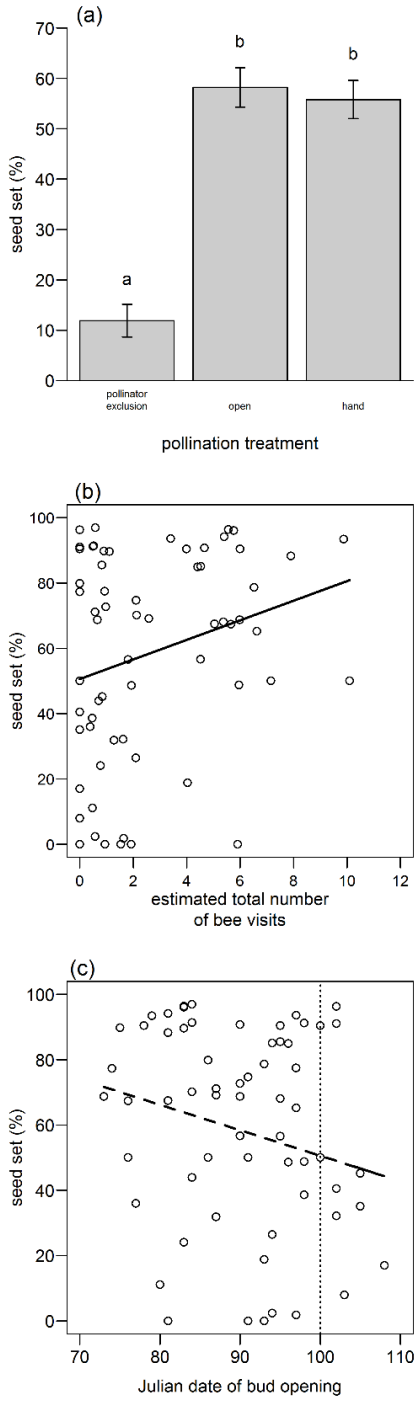

Fig. II.3. Relationship between (**a**) pollination treatment (pollinator exclusion (wind and self-pollination), open (wind, self- and insect pollination), hand (wind, self-, insect and hand pollination) and mean seed set (±SE) of *P. vulgaris*. Relationship between (**b**) estimated total number of bee visits per *P. vulgaris* flower and (**c**) Julian date of bud opening and seed set of *P. vulgaris*. Different letters indicate significant differences ($p < 0.05$). The solid line shows a significant relationship ($p < 0.05$), the dashed line a marginal significant relationship ($p < 0.1$). The dashed, vertical line in (**c**) demonstrates the mean Julian date of flowering onset of other plant species.

II.5 Discussion

Our study showed, that although bee abundance increased over time, flower visitation rates on *Pulsatilla vulgaris* flowers declined as the flowering season progressed. Furthermore, visitation rates on *P. vulgaris* flowers declined with increasing number of co-flowering plant species. The high attractiveness of *P. vulgaris* at the beginning of the flowering period was probably due to the absence of co-flowering plants and therefore of alternative food resources. The number of pollinator visits a flowering plant receives depends not only on the abundance of pollinators, but also on the attractiveness of the plant itself and of its co-flowering plants (Sargent and Ackerly 2008; Lázaro et al. 2009; Mitchell et al. 2009). Our results show that the negative effect of the growing number of co-flowering plants and therefore the increase in interspecific competition for pollinators of *P. vulgaris* with its co-flowering plants could not be compensated by the increase in bee abundance, which led to an overall reduction of the flower visitation rates on *P. vulgaris* flowers over time.

Although the number of hours, which were suitable for pollinators to forage and therefore to visit a flower, increased over the season, the estimated total number of bee visits a *P. vulgaris* flower could receive during its flowering period did not increase, but marginally decreased. This decrease was caused by the decrease of flower visitation rates as well as the decrease of floral longevity over the season. The estimated value of the total number of bee visits for a *P. vulgaris* flower involves the flower-specific number of pollinator-suitable hours, the flower-specific mean flower visitation rate and the floral longevity and indicates how much visits would have been possible for a flower during its lifetime. However, the actual number of visits a flower received was probably lower, as the attractiveness of a flower to pollinators could have decreased over time or after successful pollination, due to a decline in floral scent or nectar production or due to withering (van Doorn 1997). Nevertheless, our results show that *P. vulgaris* could not enhance the floral longevity of late flowers when visitation rates were low. This is in contrast to other studies which showed that flowers can mitigate negative effects of low pollinator visitation rates by elongating their longevity (Castro et al. 2008; Aronne et al. 2015). We suggest that the shortage of the floral longevity of later *P. vulgaris* flowers seems to be imposed by the warming temperatures over the course of flowering, which probably enhance physiological processes like, flower respiration and transpiration, leading to a faster flower senescence (Arroyo et al. 2013).

The decrease of the estimated total number of bee visits for a *P. vulgaris* flower over the season suggests that a higher number of pollinator-suitable hours and higher bee abundances later in the season could not compensate for the disadvantages arising during the season, namely the increasing competition for pollinators and the decrease of floral longevity. As a consequence of this, seed set of *P. vulgaris* was highest for the first flowers and marginally decreased over time. Contrary to our study, the seed set of herbal plants in deciduous forests and subalpine meadows was lowest in the first flowers but increased over time (Schemske 1977; Motten et al. 1981; Mahoro 2002; Kudo et al. 2004; Thomson 2010; Kudo and Ida 2013). In our study, negative effects on flower visitation were overcompensated by the positive effect of low competition for pollinators at the beginning of the season. Our data strongly suggest that there is a causal relationship between the number of co-flowering plant species, pollinator visitation and seed set of *P. vulgaris,* however, we cannot exclude that other reasons than causal links might have resulted in the observed relationships. In general, flowering during periods of suboptimum flower visitation can be a bet-hedging strategy, where plants spread their flowering onset over time to buffer negative consequences of low visitation rates due to absent pollinator activity for the first flowers or due to pollinators drawn away by competing co-flowering plants for the last flowers. Our study shows for the first time that in cool temperate regions flowering as the first plant species of the season does not have to be negative for the reproductive success of early flowers, instead the last flowers were negatively affected. However, late flowers could act as insurance against rare, extreme cold weather events at the beginning of the flowering period, because the lifetime fitness of individuals is influenced by the reproductive success within multiple flowering seasons.

Seed set of open pollinated plants was more than five times as high as of plants were pollinators had been excluded. This confirms previous results that *P. vulgaris* strongly depends on insect pollination (Wells and Barling 1971; Kratochwil 1988). Self-fertilization in *P. vulgaris* is limited by the protogynous flowering schedule (Zimmermann 1935). Pollen limitation was low if seed set of open pollinated flowers was high and vice versa suggesting that low seed set of open flowers was caused by high pollen limitation at that time. The seed set of *P. vulgaris* was positively correlated to the estimated total bee visits per flower. A previous study on *P. vulgaris*, which focused on 20 *P. vulgaris* flowers, indicated that 20 pollinator visits to a flower ensure a seed set of 90% (Kratochwil 1988). Our data suggest a threshold value of three total bee visits to ensure a

seed set of more than 50 %. If a flower received less than three total bee visits during its flowering period, there was a high variance in the probability of successful and sufficient pollination. This high variance could be attributed to the circumstance that the amount of produced seeds is not only defined by the number of pollinator visits a flower receives, but also by the effectiveness (Ne'eman et al. 2010) and the functional differences of the flower visiting pollinators (Woodcock et al. 2019).

We conclude that for plant species flowering at the beginning of the season in grasslands the limiting factor for reproduction seems to be low pollinator visitation imposed by interspecific competition for pollinators by co-flowering plants and not the low abundance of pollinators nor the limited time span for pollinators to forage due to unfavourable weather conditions. Climate warming, which advances the flowering onset of many plant species (Menzel et al. 2006), could negatively affect the reproductive success of *P. vulgaris* if co-flowering plants advance their flowering onset more strongly than *P. vulgaris* (CaraDonna et al. 2014). Our results suggest, that future studies focussing on the effects and consequences of climate change on the reproductive success of plant species in a community, should not only consider non-parallel phenological shifts of plants and their pollinators (Schenk et al. 2018a), but also changes in interspecific competition for pollinators. Another threat to the reproductive success of *P. vulgaris* could be the ongoing decline of pollinators (Potts et al. 2010; Powney et al. 2019) and non-synchronous temporal shifts of flowering onset and pollinator emergence (Kehrberger and Holzschuh 2019a).

II.6 Data availability

All relevant data are deposited on Dryad at https://doi.org/10.5061/dryad.4b8gtht7t.

II.7 Acknowledgements

We thank Burkhard Biel, Christiane Brandt, Franz Dunkel, Jürgen Faust and Lenz Meierott for help with site selection and Ann-Kathrin Kiesel for her valuable help in data recording. Funding was provided by the German Research Foundation (DFG), collaborative research centre SFB 1047 'Insect timing'. Additional funding for Open

Access Publishing was provided by the German Research Foundation (DFG) and the University of Würzburg in the funding programme Open Access Publishing.

II.8 Supplementary

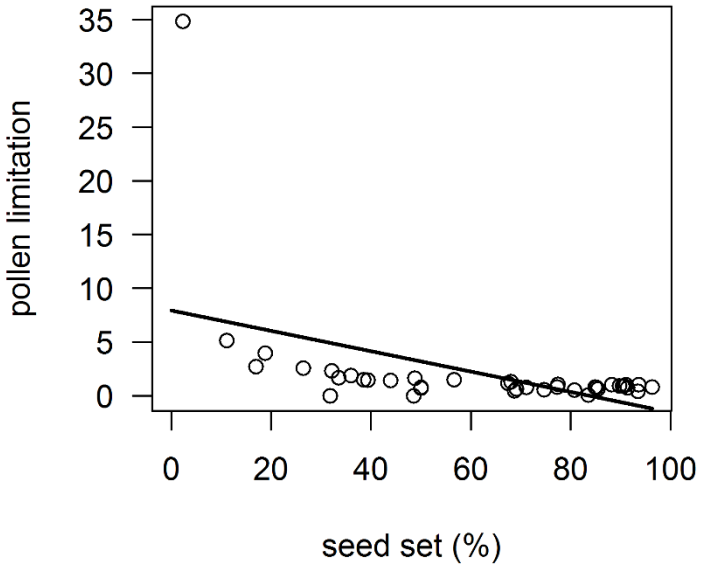

Fig. II.S1. Relationship between seed set (%) and pollen limitation. Pollen limitation decreased with increasing seed set of open pollinated flowers ($F_{1,31} = 10.4$, $p = 0.003$, after removing one outlier: $F_{1,30} = 31.2$, $p = <0.001$).

Note II.S2. Methods of temperature recording: Loggers were fixed on two wooden posts each, 90 cm above ground, underneath a plastic tube, which was used for a separate experiment, and facing to south. The distance between the posts was five to 100 meters, depending on the site. For each site we calculated the hourly and the daily mean temperature as the mean of the two loggers. One logger failed on one site between 6th February and 21st March 2015 and one on another site between 6th February and 27th March 2015. Therefore, the mean temperature for the two sites and time periods comprises only the temperature obtained from one logger.

Table II.S3. Mean, minimum (min.) and maximum (max.) number of marked flowers per site and marking days per site for the three pollination treatments pollinator exclusion, open pollination and hand pollination.

	marked flowers per site			marking days per site		
treatment	mean	min.	max.	mean	min.	max.
pollinator exclusion	10.1	2	14	9.5	2	13
open pollination	10.0	2	14	9.6	2	13
hand pollination	9.8	2	14	8.8	2	13

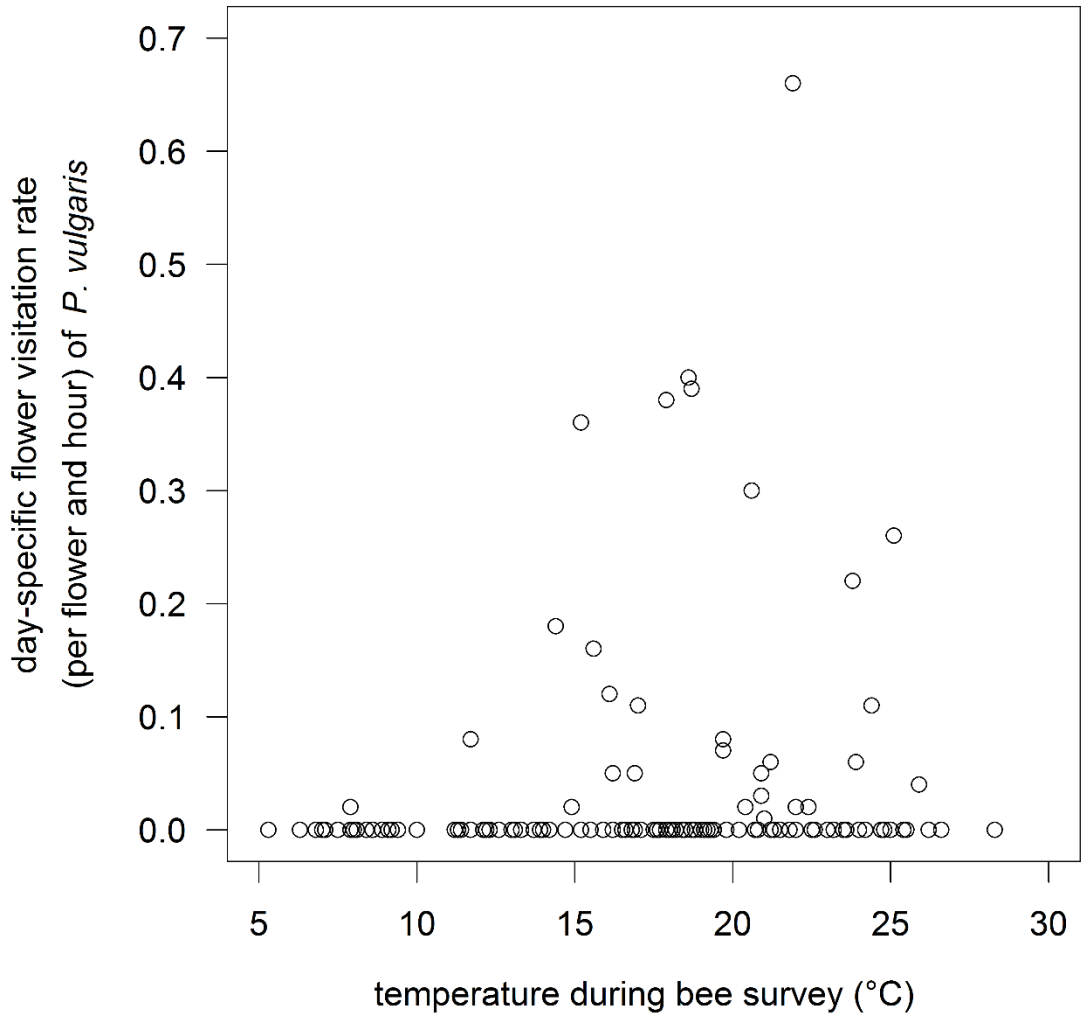

Fig. II.S4. Relationship between temperature (°C) during bee survey and day-specific flower visitation rate (per flower and hour) on *P. vulgaris*.

Chapter III: Warmer temperatures advance flowering in a spring plant more strongly than emergence of two solitary spring bee species

This chapter is published as: Kehrberger S., Holzschuh A. (2019) Warmer temperatures advance flowering in a spring plant more strongly than emergence of two solitary spring bee species. *PLoS ONE* 14(6): e0218824. https://doi.org/10.1371/journal.pone.0218824

III.1 Abstract

Climate warming has the potential to disrupt plant-pollinator interactions or to increase competition of co-flowering plants for pollinators, due to species-specific phenological responses to temperature. However, studies focusing on the effect of temperature on solitary bee emergence and the flowering onset of their food plants under natural conditions are still rare.

We studied the effect of temperature on the phenology of the two spring bees *Osmia cornuta* and *Osmia bicornis*, by placing bee cocoons on eleven grasslands differing in mean site temperature. On seven grasslands, we additionally studied the effect of temperature on the phenology of the red-list plant *Pulsatilla vulgaris*, which was the first flowering plant, and of co-flowering plants with later flowering.

With a warming of 0.1 °C, the abundance-weighted mean emergence of *O. cornuta* males advanced by 0.4 days. Females of both species did not shift their emergence. Warmer temperatures advanced the abundance-weighted mean flowering of *P. vulgaris* by 1.3 days per 0.1 °C increase but did not shift flowering onset of co-flowering plants. Competition for pollinators between *P. vulgaris* and co-flowering plants does not increase within the studied temperature range. We demonstrate that temperature advances plant flowering more strongly than bee emergence suggesting an increased risk of pollinator limitation for the first flowers of *P. vulgaris*.

III.2 Introduction

Species-specific phenological shifts in response to climate warming can alter the temporal overlap among mutualistic but also antagonistic partners, and as a consequence also the

structure of whole communities (Memmott et al. 2007; Garcia et al. 2014). For animal pollinated angiosperms, which constitute 78% of all angiosperms in the temperate zones (Ollerton et al. 2011), wild- and honeybees are the main pollinators (Garibaldi et al. 2013). For both plants and pollinators, a temporal mismatch with their interaction partners can have negative consequences for survival and reproductive output and can furthermore affect population dynamics (Hegland et al. 2009; Schenk et al. 2018a). Whereas for plants temporal mismatches with pollinators can lead to reduced visitation rates and reduced pollen deposition, for pollinators a temporal mismatch with their forage plants can reduce the availability of nectar and pollen (Hegland et al. 2009). Negative consequences of a temporal mismatch may be particularly high at the beginning of the season, when other potential interaction partners are not yet available that could replace the original interaction partner (Forrest and Thomson 2011). On the other hand, species may benefit from temporal mismatch, if their interaction with competitors is desynchronized by non-parallel phenology shifts. However, non-parallel phenology shifts of co-occurring plant species can also increase competition, e.g. if those shifts result in a prolonged period of flowering overlap with co-flowering plants and thus enhanced competition for pollinators (Bell et al. 2005; Sherry et al. 2007). Therefore, the right timing of phenological events is important to maximize the temporal overlap with mutualists, but also to minimize the temporal overlap with competitors (Elzinga et al. 2007; Pauw 2013). However, if interaction partners respond to different cues, different combinations of cues or the same cues but to different extents, a decoupling of temporal synchrony could arise (Menzel et al. 2006; Memmott et al. 2007).

Temperature is an important trigger of wild bee emergence (Forrest and Thomson 2011; Ovaskainen et al. 2013) and often the main driver of flowering phenology in temperate regions (Hegland et al. 2009; Forrest and Thomson 2011). However, the flowering phenology of plants can also be affected by other environmental cues, like precipitation, photoperiod or time of snowmelt (Rathcke and Lacey 1985; Körner and Basler 2010). For bees the temperature experienced during overwintering influences the timing of emergence, with spring bees incubated under warmer temperatures emerging earlier than spring bees incubated under colder temperatures (Bosch and Kemp 2004; White et al. 2009; Fründ et al. 2013). Also, many plant species advance flowering onset in response to climate warming (Gordo and Sanz 2005; Menzel et al. 2006), however, the degree of response varies greatly among species (CaraDonna et al. 2014). Studies investigating the

effects of climate warming on plant-pollinator synchrony differ in their results, probably due to species-specific differences in the response to environmental cues. Some studies showed that bees have advanced their phenology more strongly than plants in response to climate change (Gordo and Sanz 2005; Burkle et al. 2013; Robbirt et al. 2014; Olliff-Yang and Mesler 2018), others found that plants have advanced more strongly (Kudo et al. 2004; Forrest and Thomson 2011; Kudo and Ida 2013; Pyke et al. 2016) or found no difference in the phenological shift of plants and bees (Bartomeus et al. 2011; Ovaskainen et al. 2013). However, most of these studies focused on the synchrony between plant phenology and activity of bumble bees (Kudo et al. 2004; Kudo and Ida 2013; Ovaskainen et al. 2013; Pyke et al. 2016) or used museum collections providing flight activity data to study the synchrony between plant phenology and flight activity of solitary bees (Bartomeus et al. 2011; Burkle et al. 2013; Robbirt et al. 2014), whereas field studies on the synchrony between plant phenology and solitary bee emergence are still scarce (but see Forrest and Thomson 2011). A disadvantage of using flight activity data is that it can be biased by the detectability of flying bees, which depends on their abundance and the presence of mutualistic species (Benadi et al. 2014). So, the absence of flowering plants at the beginning of the season or the low abundance of bees during the first days of emergence or during the last days of the flying season can lead to missed bees. Missed individuals would not only underestimate the actual flying season length of the studied bees but could also mask a potential temporal mismatch between the first bees and the first flowers if bees are active before flowering onset of the first flowers but are not detected due to missing floral resources. These problems can be avoided by recording emergence dates instead of flight activity. Field studies on the effect of temperature on the timing of bee emergence and flowering can help to understand how an environmental cue affects the synchrony of pollinators and plants in a variable environment and provide the basis for predicting effects of future climate warming on plant-pollinator interaction. Plant-pollinator interactions are not only affected by species-specific shifts of flowering phenology, but also by changes in the flowering duration (Hegland et al. 2009). The flowering duration of a plant species can be compressed or elongated if flowering onset and end shift non-uniformly (CaraDonna et al. 2014). Furthermore, species-specific shifts of flowering duration can alter the temporal co-flowering patterns in sequentially flowering plant species, which can also modify interspecific interactions (Sherry et al. 2007; CaraDonna et al. 2014; Theobald et al. 2017).

Besides temporal mismatches among interacting species due to climate warming, temporal mismatches among mating partners of a species could also occur. Many pollinators show protandry, which is the emergence of males prior to females (Morbey and Ydenberg 2001). Protandry is supposed to maximize reproductive success for males and to reduce the risk of pre-reproductive death for females (Fagerström and Wiklund 1982). A laboratory study showed that in some solitary bee species warmer overwintering temperatures reduce the degree of protandry (Fründ et al. 2013). On the contrary, long-term phenological data from museum specimen records showed that warmer temperatures shift the flying season of solitary bees at a similar rate for both sexes, indicating no change in the degree of protandry (Bartomeus et al. 2011). However, experimental evidence on the effect of temperature on the degree of protandry in solitary bees under field conditions is still lacking.

In this study we tested the effect of temperature on the timing of emergence in the spring bee species *Osmia cornuta* and *Osmia bicornis,* by placing bee cocoons on eleven calcareous grasslands that differed in the mean temperature. We assessed if temperature alters the degree of emergence protandry of the studied bees. Furthermore, we tested the influence of temperature on the flowering onset, flowering end and flowering duration of seven populations of the red-list perennial spring plant *Pulsatilla vulgaris*, which is the first herbal plant species to flower on the studied grasslands and on the flowering onset of the co-flowering plant species of *P. vulgaris*. Specifically, we asked (1) how temperature shifts the timing of emergence and the degree of protandry in *O. cornuta* and *O. bicornis*, (2) how temperature shifts flowering onset and end of *P. vulgaris* and flowering onset of co-flowering plant species and (3) whether temperature affects the flowering duration of *P. vulgaris* and the time span of *P. vulgaris* flowering in the absence of co-flowering plant species. We hypothesize that warmer temperatures affect the phenology of male and female bees, *P. vulgaris* and co-flowering plants, to different extents.

III.3 Material and methods

III.3.1 Study sites

We studied eleven calcareous grasslands in an area of about 840 km^2 in the vicinity of Würzburg (49° 48´ N, 9° 56´ E), Germany, with a minimal distance of 2.5 km between study sites (Table III.S1). Grasslands were at least 1 ha in size and the only sites in the region where *Pulsatilla vulgaris* populations of more than 50 individuals were expected to occur. However, on one site we found only 15 *P. vulgaris* individuals and on three sites no flowering individuals and therefore used seven sites in the plant analyses and eleven sites in the bee analyses. The population sizes of *P. vulgaris* on the study sites ranged between 50 and 600 individuals.

Grasslands differed by their exposition to sun and had differing mean temperatures. We hourly recorded air temperature with two temperature loggers per site (iButton temperature logger DS1922L, Maxim Integrated, USA; resolution: 0.0625 °C). Loggers were fixed on two posts, 90 cm above ground, underneath the bee tubes (see below) and facing to south. Temperature recordings started on 6th February 2015 and ended on 30th May 2015. For each site, we calculated the mean temperature of the two loggers and the whole recording period. On one site, one logger failed between 6th February and 21st March 2015 and on another site, one logger failed between 6th February and 27th March 2015. For the two sites and time periods we used only the temperature obtained from one logger. The difference in mean temperature was 1.05 °C between the warmest and the coldest of the eleven sites and 0.28 °C between the warmest and the coldest *P. vulgaris* site.

III.3.2 Timing of bee emergence

We studied the two spring bee species *Osmia cornuta* and *Osmia bicornis* (Hymenoptera: Apiformes: Megachilidae). Both species overwinter as imagines in the cocoon, are univoltine and polylectic. *O. cornuta* males emerge from beginning of March to end of April, while females emerge from beginning of March to beginning of June. *O. bicornis* males emerge from beginning of April to mid-May, females from beginning of April to end of July (Westrich 1989). The foraging range of *O. cornuta* is 100-200 m (Vicens and Bosch 2000) and up to 600 m for *O. bicornis* (Gathmann and Tscharntke 2002). During

the flight period of *O. cornuta*, *Pulsatilla vulgaris* was the only food plant flowering on the study sites. At landscape scales, the only other potential food plant at this time was *Salix caprea,* which occasionally occurred within the bee foraging range. During the flight period of *O. bicornis*, several food plants started flowering.

Timing of bee emergence was studied by placing 1100 cocoons of *O. cornuta* and 1100 cocoons of *O. bicornis* on the study sites. Bee cocoons were purchased from WAB Mauerbienenzucht (Konstanz, Germany), which is a commercial supplier of wild bee species. Bee cocoons were stored in a climate chamber at constant 4 °C between October 2014 and 19th January 2015. Then, in the lab, bee cocoons were filled in plastic tubes (length: 25.5 cm, diameter: 7 cm), whose open ends were closed with gauze (mesh width: 1mm). Each tube contained 50 bee cocoons. In total there were 22 tubes with *O. cornuta* and 22 with *O. bicornis* cocoons. Filled tubes were stored at an exterior area at the University of Würzburg until the 4th or 5th February 2015, when the tubes were brought to the study sites.

To predict phenological events of insects, like emergence, degree-day models can be used (van Asch and Visser 2007). Those models take into account the length of a period (e.g. in days) in which a certain temperature threshold has been exceeded and the temperature experienced during that period. After a certain value in degree-days has been reached the phenological event takes place (van Asch and Visser 2007). Previous studies on emergence dates of several solitary bee species have suggested that bees only accumulate degree-days above a temperature-threshold between 8 °C – 14 °C , and after a specific starting date of degree-day accumulation (White et al. 2009; Forrest and Thomson 2011; Ahn et al. 2014). Accumulation of degree-days does not start before the starting date even if temperatures are above the temperature-threshold before the starting date (Forrest and Thomson 2011). Pre-wintering temperatures have not been found to affect the timing of emergence in solitary bees (Kemp and Bosch 2001; Bosch and Kemp 2004).

We placed two tubes per species in each site. Tubes were fixed on two wooden posts at one meter above ground with open ends directed east west. One tube per species was fixed on the north side of a post and the other one on the south side of the other post. The two posts were 5 to 100 m apart from each other, depending on the size of the study site. Tubes were checked for emerged bees between 6th February and 4th March 2015 every fourth to tenth day on each site and between 4th March and 15th May 2015 every second

to third day on each site. On 29th May, all tubes were checked for the last time, however, no more bees had emerged. Remaining cocoons were then removed. For each emerged bee, we recorded species, sex and date of recording, which was taken as the date of emergence. For the analyses, we used for each site, species and sex the first and the last Julian date of emergence as well as the abundance-weighted mean date of emergence, which is the arithmetic mean of all days on which a bee of this species and sex had emerged on this site, weighted by its abundance on each date on this site (Benadi et al. 2014). The degree of protandry is the difference between females and males of a species in the first date, the last date and the abundance-weighted mean date of emergence. Due to a severe storm, we lost one tube containing *O. bicornis* cocoons on one site, after only one male bee had emerged. Thus for this site we used only recordings from one tube for analysis of the last Julian date of emergence for males and of the first, abundance-weighted mean and last Julian date of emergence for females of *O. bicornis*, as well as for the analysis of the degree of protandry for abundance-weighted mean and last date of emergence.

III.3.3 Plant phenology

Pulsatilla vulgaris (Ranunculaceae) was the first and only plant species flowering on the study sites when the study bees started to emerge. *P. vulgaris* is a perennial herb restricted to calcareous grasslands and listed as a threatened plant species on the red lists of threatened plant species of Germany and Bavaria (Ludwig and Schnittler 1996; Scheuerer and Ahlmer 2003). Reproduction is vegetative as well as sexual with bees being the main flower visitors (Kratochwil 1988). The most abundant wildflower visitors were *O. cornuta* and *O. bicornis*, responsible for 39 % of the visits by wild bees, followed by bumblebees (37%) and bees of the genus *Andrena* (24%). The managed honeybee *Apis mellifera* was responsible for 53 % of the total bee visits.

Flowering phenology of *P. vulgaris* populations and of co-flowering plant species was recorded between 6th February and 4th March 2015 every fourth to tenth day on each site and every second to third day on each site from 4th March to 8th May 2015. We stopped recording after detecting no more *P. vulgaris* flowers on all sites at consecutive recording dates. For each recording we walked across the study sites to look for *P. vulgaris* flowers and then defined a variable transect of 100 m^2 containing the highest abundance of *P.*

vulgaris flowers. For each transect the abundance of *P. vulgaris* flowers was estimated. For the analyses, we used for each site the first and the last Julian date of *P. vulgaris* flowering as well as the abundance-weighted mean date of flowering, which is the arithmetic mean of all dates on which *P. vulgaris* flowered at this site, weighted by its abundance on each date on this site (Benadi et al. 2014). The flowering duration of each *P. vulgaris* population was calculated as the difference between the Julian date of flowering end and the Julian date of flowering onset of *P. vulgaris* on the site.

We also recorded the Julian date when the first plant species other than *P. vulgaris* started to flower. During the flowering period of *P. vulgaris* we recorded three up to ten co-flowering plant species per study site, however, plant species identity differed partly between sites. In total, we recorded 20 different co-flowering plant species. We hypothesize that the co-flowering plants compete with *P. vulgaris* for pollinators and therefore calculated the time span of *P. vulgaris* flowering in the absence of co-flowering plant species, which represents the flowering period in which only *P. vulgaris* flowered, as the difference between the first co-flowering plant species and *P. vulgaris* in their Julian date of flowering onset.

III.3.4 Statistical analyses

To test how temperature affects emergence dates and protandry of *O. cornuta* and *O. bicornis*, we used linear models with number of emerged bee individuals and site temperature as predictors and phenological variables or degree of protandry as response variables. Phenological variables were the first, the abundance-weighted mean and the last Julian date of emergence. The number of emerged bee individuals only had a significant positive effect on the abundance-weighted mean emergence of *O. bicornis* females, in all other models there was no significant effect and hence we excluded the number of emerged bee individuals from those models. Emergence models were calculated separately for each sex and species, protandry models for each species.

To test how temperature affects the flowering phenology and the total flowering duration of *P. vulgaris* we used linear models with population size and site temperature as predictors. However, there was no effect of population size and therefore we excluded population size from the models. We also tested if temperature has an effect on the

flowering onset of co-flowering plant species and the time span of *P. vulgaris* flowering in the absence of co-flowering plant species with linear models with site temperature as predictor. We visually inspected model residuals for violation of assumptions of normality and homoscedasticity. All statistical analyses were performed using the software R (R Core Team 2019).

III.4 Results

Bees emerged from 83.0 % of all *Osmia cornuta* cocoons and from 82.2 % of all *Osmia bicornis* cocoons, with 44.8 % males in *O. cornuta* and 57.3 % males in *O. bicornis*. Emergence of *O. cornuta* started - depending on site - between 6th and 18th March for males and between 8th and 20th March for females and ended between 25th March and 7th April for males and between 7th and 18th April for females. Emergence of *O. bicornis* started between 26th March and 10th April for males and between 10th and 16th April for females and ended between 20th April and 5th May for males and between 4th and 15th May for females. In total, only 20 *O. cornuta* males and one *O. cornuta* female emerged before the flowering onset of *P. vulgaris* (all males on the four warmest *P. vulgaris* sites, the female on the third warmest site). Flowering of *Pulsatilla vulgaris* started between 13th and 18th March and ended between 18th April and 05th May. On the two warmest sites *P. vulgaris* started flowering before the first female bees had emerged. On all sites, the first male bees had been emerged before or emerged on the same day when *P. vulgaris* started flowering. Whereas warmer temperatures did not change the time lag between the first emerged *O. cornuta* male and the first *P. vulgaris* flower, the time lag between the last emerged female *O. bicornis* and the last *P. vulgaris* flower increased by 6.6 days per 0.1 °C temperature increase (Table III.1). The first co-flowering plant species, which all attracted bees and potentially competed with *P. vulgaris* for pollinators, were *Potentilla neumanniana* (three sites), *Viola* (three sites) and *Primula veris* (one site) with a flowering onset between 9th and 11th April, and *Adonis vernalis* (one site) with a flowering onset on 3rd April.

A temperature increase of 0.1 °C advanced the first emergence date of *O. cornuta* males by 1.2 days and the abundance-weighted mean date by 0.4 days , while for *O. bicornis* males, a temperature increase of 0.1 °C advanced the first emergence date by 1.3 days but had no significant effect on the abundance-weighted mean date of emergence .

Temperature did neither affect the first emergence date nor the abundance-weighted mean date of females in both species. The last emergence date was not affected by temperature in either sex or species (Table III.1, Fig. III.1).

The difference between the first dates of female and male emergence increased by 1.6 days per 0.1 °C temperature increase for *O. cornuta* and by 1.4 days for *O. bicornis*. Differences in the abundance-weighted mean dates and in the last emergence dates between female and male of both species were not affected by temperature (Table III.1, Fig. III.2).

Warmer temperatures advanced the flowering onset of *P. vulgaris* by 1.9 days per 0.1 °C temperature increase, abundance-weighted mean flowering by 1.3 days and flowering end by 6.7 days. Temperature had no significant effect on the flowering onset of the first co-flowering plant species. A temperature increase of 0.1 °C shortened flowering duration of *P. vulgaris* by 4.8 days but did not alter the time span of *P. vulgaris* in the absence of co-flowering plant species (Table III.1, Fig. III.3).

Table III.1. Site temperature effects on *O. cornuta* and *O. bicornis* emergence on flowering phenology of *P. vulgaris* and co-flowering plants and on time lag between first bee and first flower and last bee and last flower. Slopes and 95% confidence levels (CL) are shown for models with $p < 0.1$.

Response	*df*	*F*	*P*	*slope*	*Lower CL (95 %)*	*Upper CL (95 %)*
O. cornuta males						
first emergence	9	22.6	**0.001**	- 1.2	- 1.8	- 0.7
wmd of emergence	9	9.8	**0.012**	- 0.4	- 0.6	- 0.1
last emergence	9	1.5	0.246	- 0.7	- 1.9	0.5
O. cornuta females						
first emergence	9	0.8	0.387	0.4	- 0.6	1.4
wmd of emergence	9	0.6	0.477	- 0.3	- 1.0	0.5
last emergence	9	1.9	0.203	- 0.4	- 1.2	0.3
O. bicornis males						
first emergence	9	11.3	**0.008**	- 1.3	- 2.2	- 0.4
wmd of emergence	9	4.2	0.070	- 0.2	- 0.5	- 0.0
last emergence	9	1.8	0.213	- 0.7	- 1.8	0.5
O. bicornis females						
first emergence	9	0.2	0.683	0.1	- 0.4	0.6
wmd of emergence *	8	0.5	0.503	- 0.4	- 0.8	- 0.1
last emergence	9	0.0	0.984	- 0.0	- 1.0	1.0
protandry *O. cornuta* (days)						
first emergence	9	13.1	**0.006**	1.6	0.6	2.7
wmd of emergence	9	0.1	0.742	0.1	- 0.7	0.9
last emergence	9	0.1	0.762	0.2	- 1.4	1.8
protandry *O. bicornis* (days)						
first emergence	9	17.8	**0.002**	1.4	0.7	2.2
wmd of emergence	9	3.4	0.097	- 0.2	- 0.5	0.0
last emergence	9	0.1	0.789	- 0.2	- 1.9	1.5
P. vulgaris						
flowering onset	5	14.2	**0.013**	- 1.9	- 3.3	- 0.6
wmd of flowering	5	8.9	**0.031**	- 1.3	- 2.4	- 0.2
flowering end	5	16.8	**0.009**	- 6.7	- 10.9	- 2.5
flowering duration	5	13.4	**0.015**	- 4.8	- 8.1	- 1.4
time span of *P. vulgaris* flowering in the absence of co-flowering plant species	5	0.9	0.390	1.5	- 2.7	5.7
co-flowering plant species						
flowering onset	5	0.1	0.763	- 0.4	- 3.7	2.9
time lag						
time lag between first *O. cornuta* male and first *P. vulgaris* flower	5	0.4	0.557	- 0.8	- 4.0	2.4
time lag between last *O. bicornis* female and last *P. vulgaris* flower	5	6.9	**0.047**	6.6	0.1	13.0

Effects of site temperature on the Julian date of first, abundance-weighted mean (wmd) and last emergence of *O. cornuta* and *O. bicornis* males and females, the degree of protandry, calculated as the difference between males and females in date of first, wmd and last emergence, flowering onset, wmd of flowering, flowering end, flowering duration and the time span of *P. vulgaris* flowering in the absence of co-flowering plant species and flowering onset of co-flowering plant species. The number of emerged bee individuals was significant in one model (*) and was otherwise removed from the models.

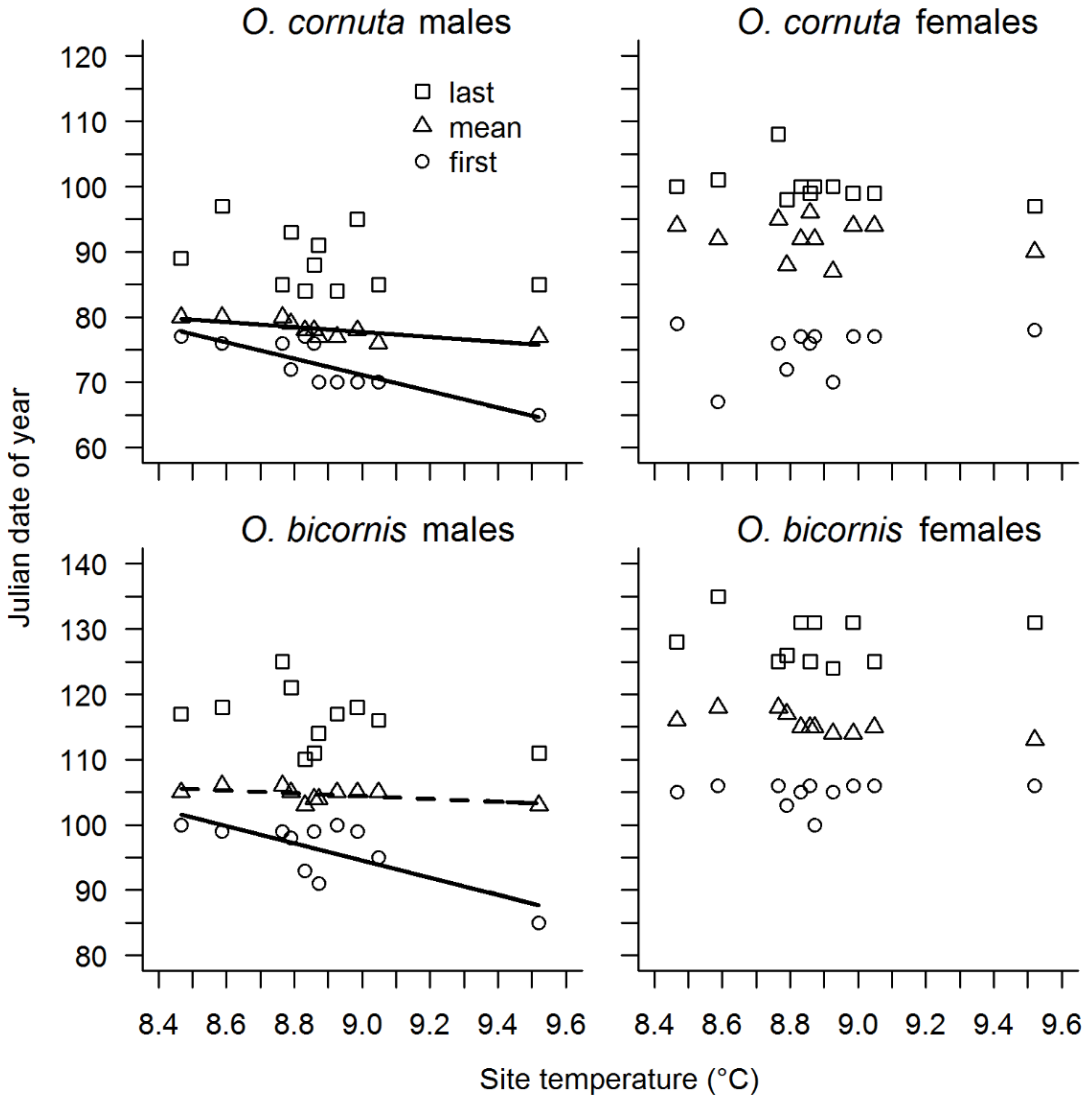

Fig. III.1. Site temperature effects on *O. cornuta* and *O. bicornis* emergence. Effect of site temperature on Julian date of first emergence (first), abundance-weighted mean emergence (mean) and last emergence (last) for *O. cornuta* males, *O. cornuta* females, *O. bicornis* males and *O. bicornis* females. Solid lines indicate significant relationships ($P < 0.05$), dashed lines marginal significant relationships ($P < 0.1$).

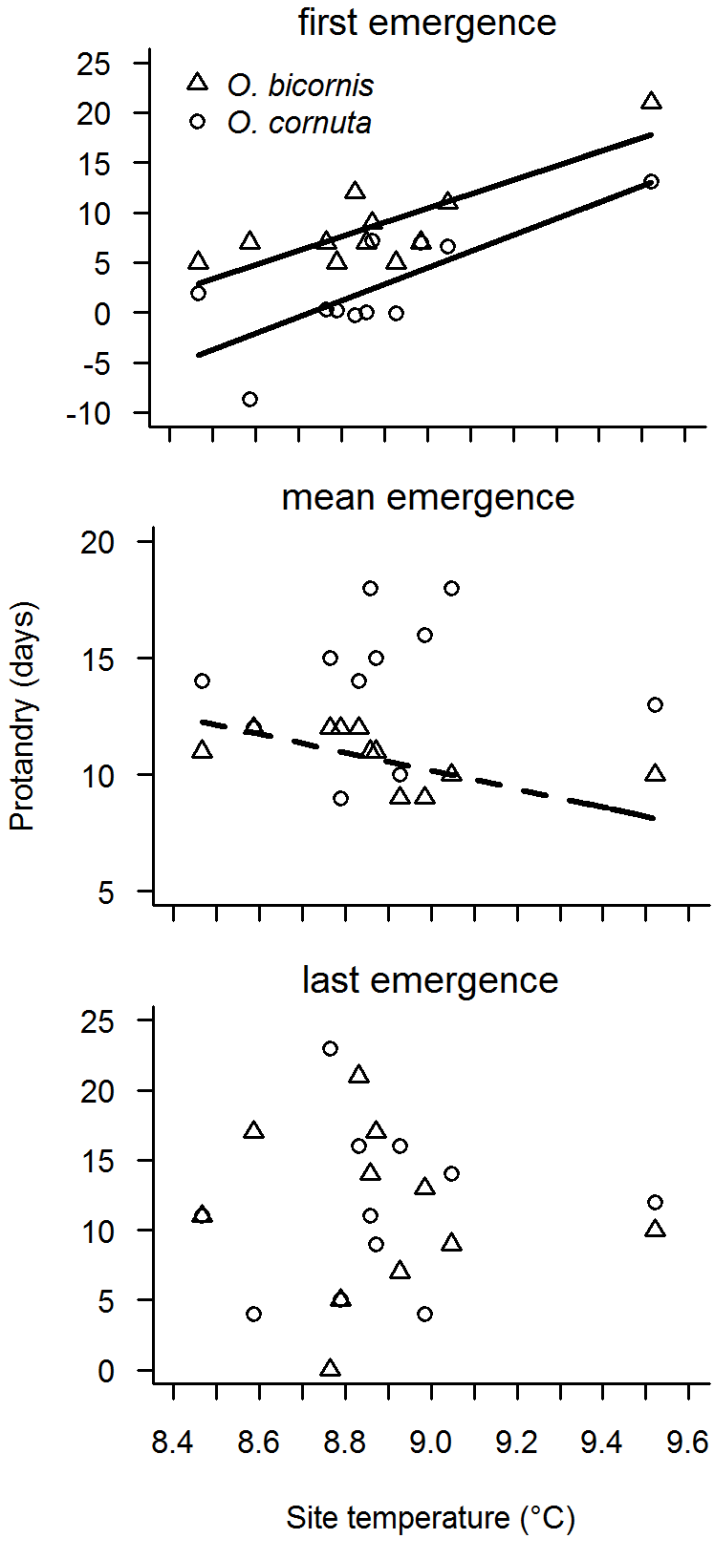

Fig. III.2. Site temperature effects on protandry levels of *O. cornuta* and *O. bicornis*. Effect of site temperature on the level of protandry calculated as the difference between females and males of *O. cornuta* and *O. bicornis* in first emergence,abundance-weighted mean emergence and last emergence. Solid lines indicate significant relationships ($P < 0.05$), dashed lines marginal significant relationships ($P < 0.1$).

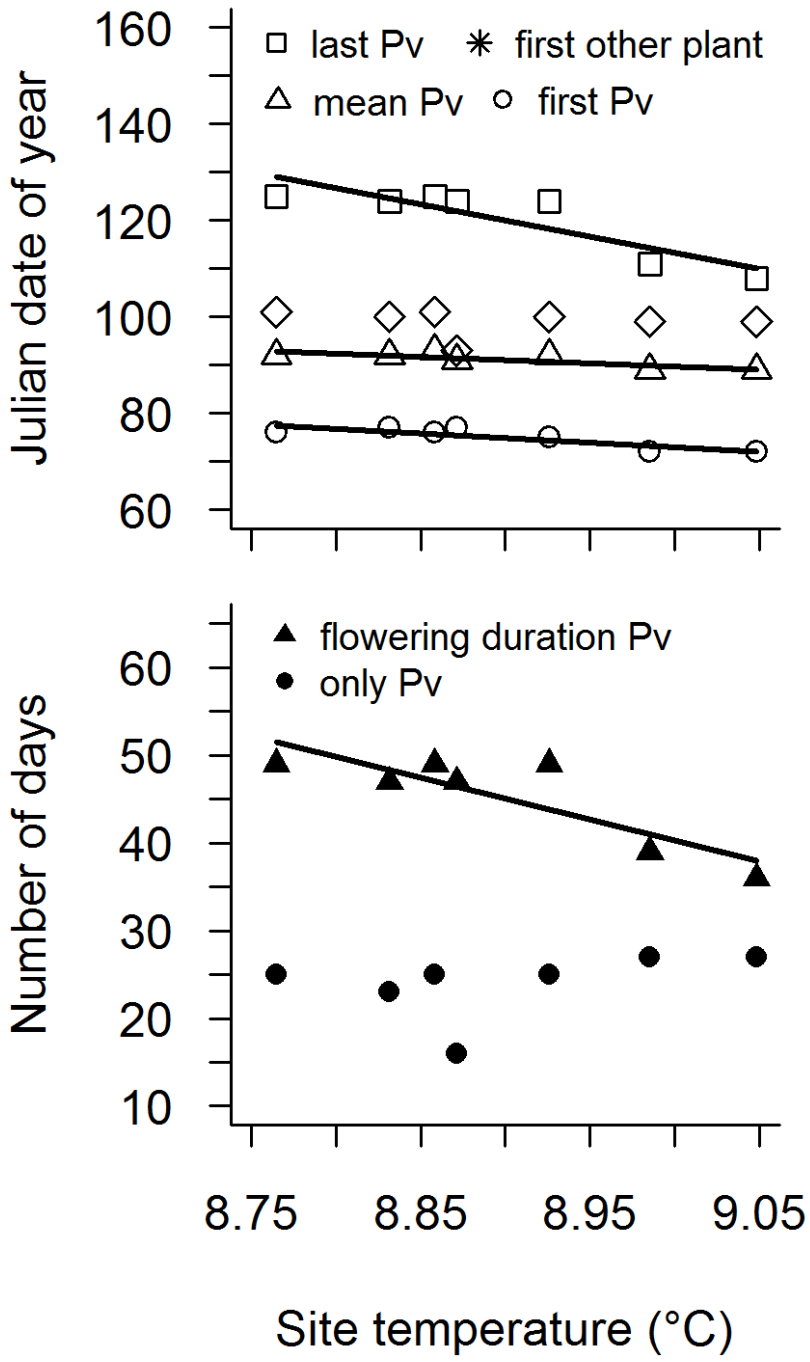

Fig. III.3. Site temperature effects on flowering phenology of *P. vulgaris* and co-flowering plants. Effect of site temperature on the Julian date of flowering onset (first Pv), abundance-weighted mean flowering (mean Pv) and flowering end (last Pv) for *P. vulgaris* and flowering onset of co-flowering plants (first other plant) and on the number of days of the time span of *P. vulgaris* flowering in the absence of co-flowering plant species (only Pv) and of the flowering duration of *P. vulgaris* (flowering duration Pv). Solid lines indicate significant relationships ($P < 0.05$).

III.5 Discussion

We showed that warmer temperatures accelerated the timing of emergence in the first *Osmia cornuta* and *Osmia bicornis* males, but not in the last. Female bees of both species were not affected by warmer temperatures. Flowering end of *Pulsatilla vulgaris* advanced 3.5 times stronger than flowering onset with warmer temperatures. Plant flowering shifted more strongly than bee emergence.

An increase of 0.1 °C advanced the abundance-weighted mean emergence of *O. cornuta* males by 0.4 days. A less strong advance of mean emergence by about 0.1 days per 0.1 °C increase was found in a laboratory study for *O. cornuta*, which might be explained by the study design with constant instead of naturally fluctuating temperatures as in our study (Fründ et al. 2013). Analyses of museum specimen records showed for the flight activity of North American spring bees a similar shift towards earlier dates as we found, ranging between 0.18 to 0.50 days per 0.1 °C increase (Bartomeus et al. 2011). However, for *Andrena nigroaenea* a much stronger advance of mean flight activity was found, with 0.74 days per 0.1 °C increase for flight records, and 1.15 days per 0.1 °C increase for museum collection data (Robbirt et al. 2014). The differences in temperature dependence could be either species-specific or due to the different methods used, with emergence data monitoring every individual of a population compared to flight activity data, which depends on the detectability of flying bees and can therefore lead to missed bees. The shift of the abundance-weighted mean flowering of *P. vulgaris*, which advanced by 1.3 days per 0.1 °C increase, was three times as strong as the shift in abundance-weighted mean emergence of *O. cornuta* males.

The first date of emergence of *O. cornuta* and *O. bicornis* males as well as the first flowering date of *P. vulgaris* advanced more strongly than the abundance-weighted mean date of emergence and flowering with warmer temperatures, respectively. At the beginning of the season the first individuals in a population have the highest risk of a temporal mismatch with mutualistic interaction partners. In the study year, on the four warmest sites, 20 *O. cornuta* males and one female emerged before the first *P. vulgaris* flowered on the respective site. 97.8 % of *O. cornuta* males and 99.9 % of females emerged at or shortly after the flowering onset of *P. vulgaris* suggesting that bees and plants are currently well synchronized. We expect that the bee emergence dates we recorded did not differ from emergence dates of bees naturally nesting on the studied

sites, because a reciprocal transplant experiment on trap-nesting bees showed that site of origin and therefore adaptations to site conditions had no effect on emergence phenology (Forrest and Thomson 2011). The advance of the first flowering date of *P. vulgaris*, with 1.9 days per 0.1 °C increase was stronger than the advance of the first date of emergence of *O. cornuta* and *O. bicornis* males, with 1.2 and 1.3 days per 0.1 °C increase, respectively. Our data thus suggest, that warm temperatures involve the risk that *P. vulgaris* starts flowering before the emergence of its main pollinator *O. cornuta*, which is the first cavity-nesting species on the studied sites. On the two warmest sites *P. vulgaris* already started flowering before the first *O. cornuta* female had been emerged. Female bees play a more important role in plant pollination than male bees which do not collect pollen for nest provision (Ne'eman et al. 2006). Pollinator limitation can result in reduced reproductive success and consequently have a negative effect on population size (Sargent and Ackerly 2008). Plants ideally compensate for a temporal mismatch with their pollinators, by elongating their floral longevity, however, warm temperatures can restrict the ability to enhance floral longevity independently of pollination (Arroyo et al. 2013).

Also, in our study we found that warmer temperatures shortened the flowering duration of *P. vulgaris* populations, due to a less strong advance of flowering onset compared to flowering end. We suggest that the shorter flowering duration of *P. vulgaris* populations with warmer temperatures is due to shorter floral longevities of individual flowers, induced by warmer temperatures. Contrary to our results, in early flowering montane plant species, warmer temperatures delayed the last date of flowering and lengthened the flowering season (CaraDonna et al. 2014; Theobald et al. 2017). A compressed flowering period in response to warmer temperatures may negatively affect the reproductive success of a plant species, because it decreases the probability that the plant is visited by pollinators. Another way for *P. vulgaris* to compensate for pollinator limitation is to switch to vegetative reproduction if pollination fails (Wells and Barling 1971), but this can reduce the genetic variability of the population and the adaptive plasticity to respond to environmental variation (Holsinger 2000). Besides compensation mechanisms implemented by the pollinator-limited plant itself, the plant can also mitigate negative effects resulting from non-parallel phenology shifts of plants and pollinators by shifting to other pollinators, which fulfil the same function (Bartomeus et al. 2013). Other early pollinators we could observe on *P. vulgaris* at the beginning of flowering were honeybees and bumble bee queens. Both, emergence of bumble bee queens from hibernation

(Bartomeus et al. 2011) and the first appearance of honeybees (Gordo and Sanz 2005) have previously been found to advance with warmer temperatures, however, less strong than flowering onset of *P. vulgaris* in our study.

The last date of emergence in both bee species and sexes did not shift with warmer temperatures, whereas the last date of flowering of *P. vulgaris* advanced by 6.7 days per 0.1 °C increase. We suggest that for *O. bicornis*, which emerges towards the end of the flowering period of *P. vulgaris*, the earlier flowering end of *P. vulgaris* with warmer temperatures, reduces the abundance of *P. vulgaris* flowers to forage on, with probable negative effects on the reproductive output of the bee if other food resources are also scarce.

Our results show not only a difference in the response to warmer temperatures between plants and pollinators, but also different responses between the sexes of bee species. In contrast to male bees of both species, female bees did not advance the first emergence in response to warmer temperatures within the studied temperature range. We suggest that this difference is due to different reproductive pressures and strategies of male and female bees. Whereas female bees are mostly monandrous, males are polygynous (Seidelmann 1999). As a consequence, males can only increase their reproductive success by mating with as many receptive, unmated females as possible, whereas the reproductive success of females depends on successful construction and provisioning of brood cells (Seidelmann 1999). This imposes the selective pressure on male bees to emerge earlier than competing conspecific males and to increase the probability to encounter receptive, unmated females. We suggest that with warmer temperatures males that emerge at the beginning of the emergence period can increase their reproductive success by emerging even earlier. This leads to males in a population that encounter a risky reproductive strategy, despite the threats of bad weather conditions, absent flowering plants and desynchronization with females that come along with a disproportionate early emergence. However, with warmer temperatures this reproductive strategy can become even more risky if it coincides with a desynchronization with food plants, as a survival without food is more difficult at warmer temperatures than at colder temperatures (Schenk et al. 2018a). The risk of desynchronization with females for the first emerged males is shown in the increased protandry of the first emerged individuals in both species, while protandry was not significantly affected by temperature when we focus on abundance-weighted mean emergence dates of the populations.

Whereas warmer temperatures advanced the flowering onset of *P. vulgaris*, flowering onset of its co-flowering plants did not change. However, this did not result in a significantly longer time span in which *P. vulgaris* flowered without co-flowering plant species. The reproductive success of a plant species can be strongly decreased by co-flowering plant species, which withdraw pollinators (Mosquin 1971). Thus, there may be a strong selective pressure on *P. vulgaris*, to start flowering prior to co-flowering plant species (Elzinga et al. 2007). Our results indicate that - within the study range - warmer temperatures will not increase competition for pollinators between *P. vulgaris* and its co-flowering plants.

III.6 Conclusion

As warm temperatures advance the emergence of spring bees less strongly than the flowering of an early plant, with warming temperatures early pollinator-dependent plants are at the risk to face pollinator limitation, with negative consequences for their reproductive success. On our four warmest study sites 20 *O. cornuta* males and one female emerged before the flowering onset of *P. vulgaris*, which could negatively affect the reproductive success of the bee population. However, for the threatened *P. vulgaris* with climate warming and its stronger shift in response to warmer temperatures compared to *O. cornuta*, this temporal mismatch could be reversed to the first flowers of *P. vulgaris* flowering without pollinators being present. Although phenological asynchrony of plants and pollinators can be mitigated by different compensation mechanisms, like alternative reproductive strategies, species complementarity or range shifts, warming temperatures impose a critical threat on mutualistic interaction partners. Non-parallel phenology shifts of bees and plant species can reduce the diversity and alter the composition of flowering plant communities, where bees can forage on during their flight season (Burkle et al. 2013), with negative effects for bee larval development and reproductive success (Sedivy et al. 2011; Schenk et al. 2018a). For the threatened plant species *P. vulgaris* a decrease of seed production, imposed by pollinator limitation, could reduce the genetic variability of the population through increased vegetative reproduction (Holsinger 2000). Reduced viability and reproductive success can negatively affect the population size and on a long-term scale even push a species to extinction.

III.7 Data availability

All relevant data are deposited on Dryad at DOI:10.5061/dryad.5tq5dn6.

III. 8 Acknowledgements

We thank Burkhard Biel, Christine Brandt, Franz Dunkel, Jürgen Faust and Lenz Meierott for help with site selection and Ann-Kathrin Kiesel for her valuable help in data recording.

III. 9 Supplementary

Table III.S1. Coordinates of study sites.

study site	latitude	longitude
1	50°00'19.5"N	9°48'21.5"E
2	49°49'44.6"N	9°51'07.0"E
3	49°51'55.9"N	9°47'20.8"E
4	49°46'52.0"N	9°48'27.3"E
5	49°46'42.0"N	9°41'59.4"E
6	49°58'27.9"N	9°49'13.9"E
7	49°51'15.8"N	9°50'03.3"E
8	49°58'11.8"N	9°47'12.1"E
9	49°59'54.9"N	9°42'06.7"E
10	50°01'38.1"N	9°47'58.0"E
11	49°47'26.2"N	9°45'14.9"E

Chapter IV: How do temperature and photoperiod affect the seasonal synchrony between wild bees and their parasitoids?

This chapter is submitted as: Kehrberger S., Steffan-Dewenter I., Holzschuh A. How do temperature and photoperiod affect the seasonal synchrony between wild bees and their parasitoids? Functional Ecology

IV.1 Abstract

Climate warming can expose species to altered temperatures or photoperiods if species ranges shift towards the poles. If responses differ among interacting species the degree of synchrony between them could change. In wild bees the interaction with parasitoids affects their reproductive success. However, studies addressing how new combinations of temperature and photoperiod in the course of climate change affect the seasonal synchrony between wild bees and their parasitoids are missing.

In our study we investigated the effects of temperature and photoperiod on the emergence phenology of the two early-season, solitary bees *Osmia cornuta* and *Osmia bicornis* and their three main parasitoids *Anthrax*, *Monodontomerus* and *Cacoxenus indagator*. We exposed cocoons within nest cavities and cocoons that had been removed from the nests to two temperature regimes (long-term mean of Würzburg, Germany and long-term mean of Würzburg + 4 °C) and three photoperiods (Würzburg 49° 47´ N vs. Snåsa, Norway 64° 14´ N vs. constant darkness) and recorded the date of bee and parasitoid emergence.

Warmer temperatures advanced emergence in all species, however, parasitoids showed a stronger advance than bees. This resulted in a shorter time period between *O. bicornis* female and parasitoid emergence in the warm temperature treatment than in the colder one. Photoperiod affected the emergence date only in cocoons removed from the nest (except *O. bicornis* male emergence) and its effect was much weaker than that of temperature.

Our results suggest that due to the stronger advance of parasitoid than female bee emergence climate warming enhance the synchrony of host-antagonist phenologies and

thereby increase parasitism rates of solitary bees, with potential negative consequences for their reproductive success and population size.

IV.2 Introduction

Climate warming is one of the major threats to biodiversity (Scheffers et al. 2016). Species may respond to climate warming with seasonal shifts of phenological events to track temperature changes (Duchenne et al. 2020; Walther et al. 2002) or with geographical range shifts towards the poles (Chen et al. 2011). Non-synchronous phenological shifts can lead to temporal mismatches of interacting species (Both et al. 2009; Hegland et al. 2009) which can negatively affect their reproductive success (Kehrberger and Holzschuh 2019b; Schenk et al. 2018a;). Depending on the species-specific responses to climate warming temporal synchrony between hosts and parasitoids can increase, with positive effects for the reproductive success of the parasitoids (Nouhuys and Lei 2004) or decrease, which benefits the reproductive success of the host (Wetherington et al. 2017). Hence, to predict the effects of climate warming on host-parasitoid relationships is challenging, as the response of both interaction partners and the effect on their interaction need to be considered (Tougeron et al. 2019).

Parasitoid species act as key regulators of arthropod population dynamics (Godfray 1994) and are important biocontrol agents for pests in agriculture (Chidawanyika et al. 2019). However, parasitoids can also negatively affect reproductive success and decrease population size of bees (Roulston and Goodell 2011), which are important pollinators of crops (Garibaldi et al. 2013) and wild plants (Kremen et al. 2007). Therefore, it is important to know how climate warming affects bee and parasitoid phenology and their interaction. Besides direct effects of warmer temperatures on bees, like warmer overwintering temperatures leading to lighter bees than colder overwintering temperatures (Schenk et al. 2018b), also indirect effects through altered biotic interactions are possible. Studies on the effect of climate warming on the synchrony of wild bees and their interaction partners so far focused mainly on the interaction between bees and plants (Bartomeus et al. 2011; Burkle et al. 2013; Robbirt et al. 2014; Schenk et al. 2018a; Olliff-Yang and Mesler 2018; Kehrberger and Holzschuh 2019a). In contrast, the effects of climate warming on emergence phenologies and interactions of bees and natural enemies have – to our knowledge – not yet been studied. So far, it was shown that warmer

temperatures increase parasitism rates of solitary bees due to higher activity rates of parasitoids (Forrest and Chisholm 2017). Experimental phenological shifts of bees but not of their parasitoids altered bee parasitism rates with the degree depending on the parasitoid species (Farzan and Yang 2018). However, it is unknown how parasitoids of wild bees respond to warmer temperatures and if they shift phenology in parallel with their hosts.

Species that aim to synchronize their activity period with interaction partners should use the same environmental cues for timing their emergence. However, if they use different cues or respond to varying degrees to the same cues, temporal synchrony can be changed. The best studied trigger for emergence in bees is temperature (Forrest and Thomson 2011; Ovaskainen et al. 2013) with several studies showing that warmer temperatures advance the date of emergence (Bosch and Kemp 2003; Fründ et al. 2013; Schenk et al. 2018b; Kehrberger and Holzschuh 2019a). However, it has been rarely studied how parasitoids of wild bees respond to temperature regarding their emergence phenology and the main focus has been on parasitoid wasps (Kraemer and Favi 2010; Forrest and Thomson 2011). Besides temperature, other possible environmental triggers of emergence phenology are also hardly studied, except one study which showed that precipitation can trigger emergence in a desert bee (Danforth 1999). A cue, used by many insects to time phenological events, is photoperiod (Bradshaw and Holzapfel 2007). Wild bees and their parasitoids could use photoperiod as an additional cue to prevent very early emergence dates under warm temperatures and very late emergence dates under cold temperatures. So far, the effects of photoperiod have only been studied concerning the effects on the circadian timing of wild bees, while the effects on the circannual timing have not been investigated yet. Photoperiod has been found to synchronize circadian emergence of the solitary bee *Megachile rotundata* if there was no additional temperature cue (Bennett et al. 2018), but not of the bee *Osmia bicornis* (Beer et al. 2019).With climate warming species may be exposed to altered photoperiods if they shift their geographical range towards the poles to avoid increased temperatures. This response to warmer temperatures, which has already been observed in many species (Parmesan et al. 1999; Parmesan and Yohe 2003), results in an exposition to altered photoperiods, while temperatures resemble the temperatures in the original range (Nürnberger et al. 2018). While species that emerge around solstice, where photoperiods at different latitudes are similar, might not be

affected by an altered photoperiod, the emergence of later species might be affected by it.

In a climate chamber experiment with a crossed design we examined how temperature (long term mean vs. long term mean + 4 °C) and photoperiod (Würzburg, Germany vs. Snåsa, Norway vs. constant darkness) affect the emergence date of solitary bees and their parasitoids. We focused on the two solitary early-season bees *Osmia cornuta* and *Osmia bicornis*, which are commercially used as crop pollinators, and three of their main parasitoids the flies *Anthrax anthrax* and *Cacoxenus indagator* and the wasp *Monodontomerus.* We additionally assessed if temperature and photoperiod affect the time period between bee and parasitoid emergence. We exposed cocoons inside nesting cavities and cocoons that had been removed from nesting cavities to the long term mean temperature of Würzburg, Germany and the analogous photoperiod. To simulate climate warming in Germany we established a treatment with 4 °C warmer temperatures but unchanged photoperiod. A geographical range shift to track temperature changes was simulated by a treatment where species were exposed to a northward photoperiod, but temperature was unchanged. An extreme climate warming event was simulated by exposing species to a northward photoperiod and additionally an increase in temperature by 4 °C. To test if photoperiod is used as a cue to time emergence, we additionally exposed species to constant darkness in both temperature treatments. In our study we tested the following hypotheses: (1) Warmer temperatures advance the date of emergence in solitary bees and their parasitoids; (2) Photoperiod alters emergence dates in bees and parasitoids; (3) Temperature and photoperiod affect the synchrony of solitary bees and their parasitoids.

IV.3 Material and Methods

IV.3.1 Study organisms

In our study we focused on the two spring-emerging solitary bee species *Osmia cornuta* and *Osmia bicornis* (Hymenoptera: Apiformes: Megachilidae). Both species are univoltine, show a low degree of protandry and overwinter as imagines in the cocoon. *O. cornuta* emerges from March to June, *O. bicornis* from April to July (Westrich 1989). Parasitoids of both species are among others the cleptoparasitoid *Cacoxenus indagator*

(Diptera: Drosophilidae) and the endoparasitoids *Anthrax anthrax* (Diptera: Bombyliidae), *Monodontomerus obscurus* (Hymenoptera: Torymidae) and *M. aeneus* (= *M. obsoletus*, Hymenoptera: Torymidae) (Bosch 1992; Krunić et al. 2005). As the genus *Monodontomerus* comprises several species in Germany which are hard to determine, we refer to *Monodontomerus* in this study.

The flies *C. indagator* and *A. anthrax* lay their eggs in the open bee brood cells before they have been closed by a loam wall (Krunić et al. 2005; Felicioli et al. 2017). *Monodontomerus* penetrates closed nests with its ovipositor when the bee larvae have developed to a prepupae or a pupae inside the cocoon (Krunić et al. 2005). All three parasitoid species parasitize also other bee species (Gathmann and Tscharntke 1999; Filella et al. 2011; Pereira-Peixoto et al. 2016).

From artificial trap nests, we collected 1248 bee nests that had a high probability to contain *O. cornuta* or *O. bicornis* (Note IV.S1). Each nest was placed in a glass test tube (16 cm length, 1.5 cm diameter) which was closed with cotton wool. Nests were then stored at a shaded, exterior area at the University of Würzburg until the start of the experiment.

In addition, we purchased from a commercial supplier 2688 *O. cornuta* cocoons, 2688 *O. bicornis* cocoons and 1920 bee cocoons that were – according to the supplier – parasitized with *A. anthrax* or *Monodontomerus* (WAB Mauerbienenzucht, Konstanz, Germany). Those cocoons were individually placed in a transparent plastic tube (volume: 2 ml) sealed with cotton wool and stored in a climate chamber with constant 4 °C until the start of the experiment.

IV.3.2 Experimental setup

The experiment had a crossed design with two temperature regimes and three photoperiod regimes. The temperature regimes were based on (1) the weekly mean temperatures of Würzburg, Germany, that were calculated from hourly measurements at the local climate station between 1948 and 2016 (hereafter "cold treatment", DWD Climate Data Center CDC 2017) and (2) these weekly mean temperatures increased by 4 °C as expected based on climate-change scenarios (hereafter "warm treatment", IPCC 2014). Temperatures oscillated daily between mean temperature minus 3 °C at midnight, mean temperature at

6:00 am and pm and mean temperature plus 3 °C at noon with an hourly temperature change of 0.5 °C. The two temperature regimes were crossed with the photoperiod regimes: (1) the photoperiod of Würzburg, Germany (49°48'0" N, 9°55'48" E), (2) the photoperiod of Snåsa, Norway (64°15'0" N, 12°23'0" E), and (3) constant darkness. Daily photoperiods of Würzburg and Snåsa were obtained from the NOAA solar calculator (National Oceanic & Atmospheric Administration, U.S. Department of Commerce), and a one hour period of dawn and dusk, in which the light intensity slowly increased or decreased, respectively. The combination of mean temperature and photoperiod in Germany were the baseline treatment, because it corresponds to the past conditions experienced by the study organisms. The photoperiod of Snåsa, Norway was chosen, because species could shift their range northwards to avoid the increased temperatures if climate warming enhances temperatures by 4 °C. Snåsa, Norway has a similar annual temperature cycle as Würzburg, Germany, but approximately 4 °C colder. Constant darkness was used as control treatment to assess whether the studied species use photoperiod as an environmental cue.

The experiment was conducted in two climate chambers, which were constantly dark. In each chamber we placed six Mini Plus hive boxes, two for each photoperiod treatment (Note IV.S2). The boxes with the German and Norwegian photoperiod were equipped with an individually controllable LED light source (6500 K; 400lm/meter; ~2000 Lux) that were programmed with daily photoperiods (National Oceanic & Atmospheric Administration, 2017). In each box, we placed 104 glass tubes with bee nests and 76 plastic tubes with individual cocoons of the commercial supplier per layer (hereafter "cocoons outside of nests"): 28 *O. cornuta* cocoons, 28 *O. bicornis* cocoons and 18 - 19 parasitized *O. bicornis* cocoons. Nests were placed in the boxes on 11th November (hereafter "cocoons in nests") cocoons outside of nests on 21st December.

IV.3.3 Data recording

We daily checked the boxes for emerged individuals during the main emergence period (as long as at least one individual emerged at the current day or the previous four days). During the remaining period we checked emergence every three to four days (Note IV.S3). Due to the small size of *C. indagator* and *Monodontomerus* we only recorded the

first day of their emergence for every nest. For every emerged individual we recorded date of emergence, species and the sex in the case of bees.

IV.3.4 Statistical analysis

All statistical analysis were performed using the program R version 3.6.1 (R Core Team 2019). Separate models were calculated for cocoons inside and outside of nests. To test if temperature and photoperiod affect the emergence dates of bees and parasitoids we used linear models with the predictors temperature, photoperiod and their interaction, and with the response variable Julian date of emergence. Separate models were calculated for males and females of *O. cornuta* and *O. bicornis* and for *C. indagator, A. anthrax* and *Monodontomerus.* In a second step we tested if temperature and photoperiod affect the emergence dates of *O. bicornis* females and their parasitoids differently by applying linear models with emergence dates of both bees and parasitoids as response variables and temperature, photoperiod, trophic level (*O. bicornis* female vs. parasitoid species) and their interaction as predictors. Models were tested separately for each parasitoid species. Predictors that did not significantly contribute to the model ($p > 0.05$) were removed with a stepwise backward procedure. To test for the differences between the photoperiod categories we used the Tukey HSD test.

For *O. bicornis* females and their parasitoids we calculated the time period between emergence, as the difference in days between the mean emergence of *O. bicornis* females and the mean emergence of *C. indagator, Monodontomerus* and *A. anthrax*. With linear models, we analysed if the time period between the emergence of *O. bicornis* females and their parasitoids was affected by temperature. With general additive models (package "mgcv"; Wood 2011), we calculated curves that estimate the relationship between Julian date and number of emerged individuals for *O. bicornis* females and its parasitoids and of *O. bicornis* females that reached their maximum life expectancy, which was set to 64 days (Giejdasz and Wasielewski 2017).

IV.4 Results

IV.4.1 Cocoons inside nests

Out of in total 1248 nests, 1858 bees emerged from 798 nests. The parasitoids *Anthrax* and *Cacoxenus indagator* emerged from 112 nests with 52 *A. anthrax* and out of 60 nests *C. indagator* (Table IV.S4). *Monodontomerus* emerged from 10 nests and was not further analysed (but see section "cocoons outside nest cavities").

O. cornuta emerged between 8th March and 25th April, *O. bicornis* between 27th March and 14th May, *C. indagator* between 26th April and 23rd May and *A. anthrax* between 06th June and the last one on 26th July.

Emergence of all bee and parasitoid species advanced in the warm temperature treatment compared to the cold one (Table IV.1, Fig. IV.1). A temperature increase of 4 °C advanced emergence in *O. cornuta* males by 16.2 days, in *O. cornuta* females by 20.1 days, in *O. bicornis* males by 17.0 days, in *O. bicornis* females by 16.2 days, in *C. indagator* by 22.1 days and in *A. anthrax* by 30.0 days. Temperature had a stronger effect on parasitoids than on *O. bicornis* females, which is shown by a significant temperature-species interaction in models for *O. bicornis* females and either *C. indagator* or *A. anthrax* ($p < 0.001$ in both cases).

Photoperiod did not affect the date of emergence, except for *O. bicornis* males. *O. bicornis* male emergence was in the photoperiod of Germany 0.8 and 0.7 days later compared to Norway ($p = 0.026$) and constant darkness ($p = 0.049$).

A temperature increase of 4 °C increased the synchrony of hosts and parasitoids because it reduced the time period between the mean emergence of *O. bicornis* females and of *C. indagator* by 5.8 days and between the mean emergence of *O. bicornis* females and of *A. anthrax* by 13.8 days. The first *C. indagator* emerged three days after the last *O. bicornis* female in the cold treatment and 15 days before the last *O. bicornis* female in the warm treatment. The first *A. anthrax* in the cold treatment emerged 58 days after the last *O. bicornis* female and six days before the estimated end of life of the last *O. bicornis* female. In the warm treatment the first *A. anthrax* emerged 26 days after the last *O. bicornis* female, and 38 days before the estimated end of life of the last *O. bicornis* female (Fig. IV.2).

IV.4.2 Cocoons outside of nests

Bees and parasitoids emerged from 91.5 % of all cocoons that had been removed from nest cavities. Bee emergence started earlier in cocoons outside of nests but ended both earlier and later than in cocoons inside of nests, depending on the treatment. *A. anthrax* emergence started earlier and later outside of nests, depending on the treatment and ended later than in cocoons inside of nests. *Monodontomerus* emerged between 7th June and 15th August (Table IV.S4).

In cocoons outside of nests both temperature and photoperiod affected the emergence date in all species and both bee sexes (Fig. IV.3, Table IV.2, Table IV.S5). Warm temperature advanced the emergence of *O. cornuta* similarly as for cocoons inside nests (males: 16.7, females: 19.2 days). In both temperature treatments, *O. cornuta* males and females emerged 1.5 days earlier in the photoperiod of Germany and 1.3 (males) or 1.7 days (females) earlier in the photoperiod of Norway compared to constant darkness.

O. bicornis male emergence was affected by the interaction of temperature and photoperiod. In the cold treatment, emergence was 2.9 days earlier in the photoperiod of Germany than in constant darkness, with Norway in between. In the warm treatment, emergence dates did not differ among photoperiod treatments, and were 15.8 days earlier in the photoperiod of Germany, 17.9 days earlier in the photoperiod of Norway and were generally earlier in the cold treatment. *O. bicornis* females emerged 2.1 and 1.7 days earlier in the photoperiods of Germany and Norway compared to constant darkness. Warm temperature advanced the male and female emergence approximately to the same degree as in the case of cocoons inside nests (male: 17.0 days, female: 18.4 days).

Both *A. anthrax* and *Monodontomerus* emergence were affected by the interaction of temperature and photoperiod. In the cold temperature treatment, *A. anthrax* emergence was 6.1 days earlier in the photoperiod of Germany than in the constant darkness treatment and did neither differ among other treatments nor in the warm treatment. *Monodontomerus* emergence in the cold treatment was in the photoperiods of Germany and Norway 7.8 and 7.5 days earlier than in constant darkness. In the warm treatment, Monodontomerus emerged 7.2 days earlier in the photoperiod of Norway than in constant darkness, with the photoperiod of Germany in between (Fig. IV.3).

Temperature had a stronger effect on *A. anthrax* than on *O. bicornis* females, which is shown by a significant interaction between temperature and trophic level in the model for *O. bicornis* females and *A. anthrax*. Both temperature and photoperiod had a stronger effect on *Monodontomerus* than on *O. bicornis* females (Table IV.2). With a temperature increase of 4 °C, host-parasitoid synchrony increased, because the time period between the mean emergence of *O. bicornis* females and of *A. anthrax* decreased by 14.5 days. The first *A. anthrax* emerged 39 days (cold) and 29 days (warm) after the last *O. bicornis* female and 25 days (cold) and 35 days (warm) before the last estimated end of life of *O. bicornis* females (Fig. IV.4). The time period between the mean emergence of *Monodontomerus* and of *O. bicornis* females decreased by 14.1 days in the warm treatment compared to the cold. The first *Monodontomerus* emerged 83 days (cold) and 73 days (warm) after the first *O. bicornis* female, the last emerged 79 days (cold) and 66 days (warm) after the last *O. bicornis* females

Table IV.1. Effect of photoperiod and temperature on the emergence date of *O. cornuta* and *O. bicornis* males and females and *A. anthrax* and *C. indagator* from nest cavities and effect of temperature, species and their interaction on the synchrony of the emergence of *O. bicornis* females and *A. anthrax* and *C. indagator*.

Cocoons inside nests	df	F	p
***O. cornuta* male emergence**			
temperature	1, 682	2819.3	< 0.001
***O. cornuta* female emergence**			
temperature	1, 192	1676.5	< 0.001
***O**. bicornis* male emergence			
temperature	1, 888	5062.4	< 0.001
photoperiod	2, 888	4.1	0.017
***O. bicornis* female emergence**			
temperature	1, 86	158.2	< 0.001
***C. indagator* emergence**			
temperature	1, 67	1693.0	< 0.001
***A. anthrax* emergence**			
temperature	1, 50	541.3	< 0.001
O. bicornis* females – *A. anthrax			
temperature	1, 136	1275.5	< 0.001
species	1, 136	4383.3	< 0.001
temperature:species	1, 136	51.4	< 0.001
O. bicornis* females – *C. indagator			
temperature	1, 153	659.7	< 0.001
species	1, 153	127.4	< 0.001
temperature:species	1, 153	14.8	< 0.001

Table IV.2. Effect of photoperiod and temperature on the emergence date of *O. cornuta* and *O. bicornis* males and females and *A. anthrax* and *Monodontomerus* outside of nest cavities and effect of temperature, photoperiod and species on the synchrony of the emergence of *O. bicornis* females and *A. anthrax* and *Monodontomerus*.

Cocoons outside of nests	df	F	p
***O. cornuta* male emergence**			
temperature	1, 1224	2646.9	< **0.001**
photoperiod	2, 1224	8.9	< **0.001**
***O. cornuta* female emergence**			
temperature	1, 1237	3610.1	< **0.001**
photoperiod	2, 1237	10.9	< **0.001**
***O. bicornis* male emergence**			
temperature	1, 1208	4432.4	< **0.001**
photoperiod	2, 1208	16.1	< **0.001**
temperature*photoperiod	2, 1208	8.5	< **0.001**
***O. bicornis* female emergence**			
temperature	1, 1252	4697.0	< **0.001**
photoperiod	2, 1252	22.5	< **0.001**
***A. anthrax* emergence**			
temperature	1, 650	1865.2	< **0.001**
photoperiod	2, 650	9.6	< **0.001**
temperature*photoperiod	2, 650	3.0	**0.048**
***Monodontomerus* emergence**			
temperature	1, 497	5228.5	< **0.001**
photoperiod	2, 497	104.9	< **0.001**
temperature*photoperiod	2, 497	4.5	**0.011**
O. bicornis* females – *A. anthrax			
temperature	1, 1908	6671.1	< **0.001**
species	1, 1908	54281.1	< **0.001**
temperature*species	1, 1908	461.5	< **0.001**
O. bicornis* females – *Monodontomerus			
temperature	1, 1749	10900.4	< **0.001**
photoperiod	2, 1749	142.3	< **0.001**
species	1, 1749	78370.7	< **0.001**
temperature*photoperiod	2, 1749	4.1	**0.016**
temperature*species	1, 1749	778.5	< **0.001**
photoperiod*species	2, 1749	44.2	< **0.001**

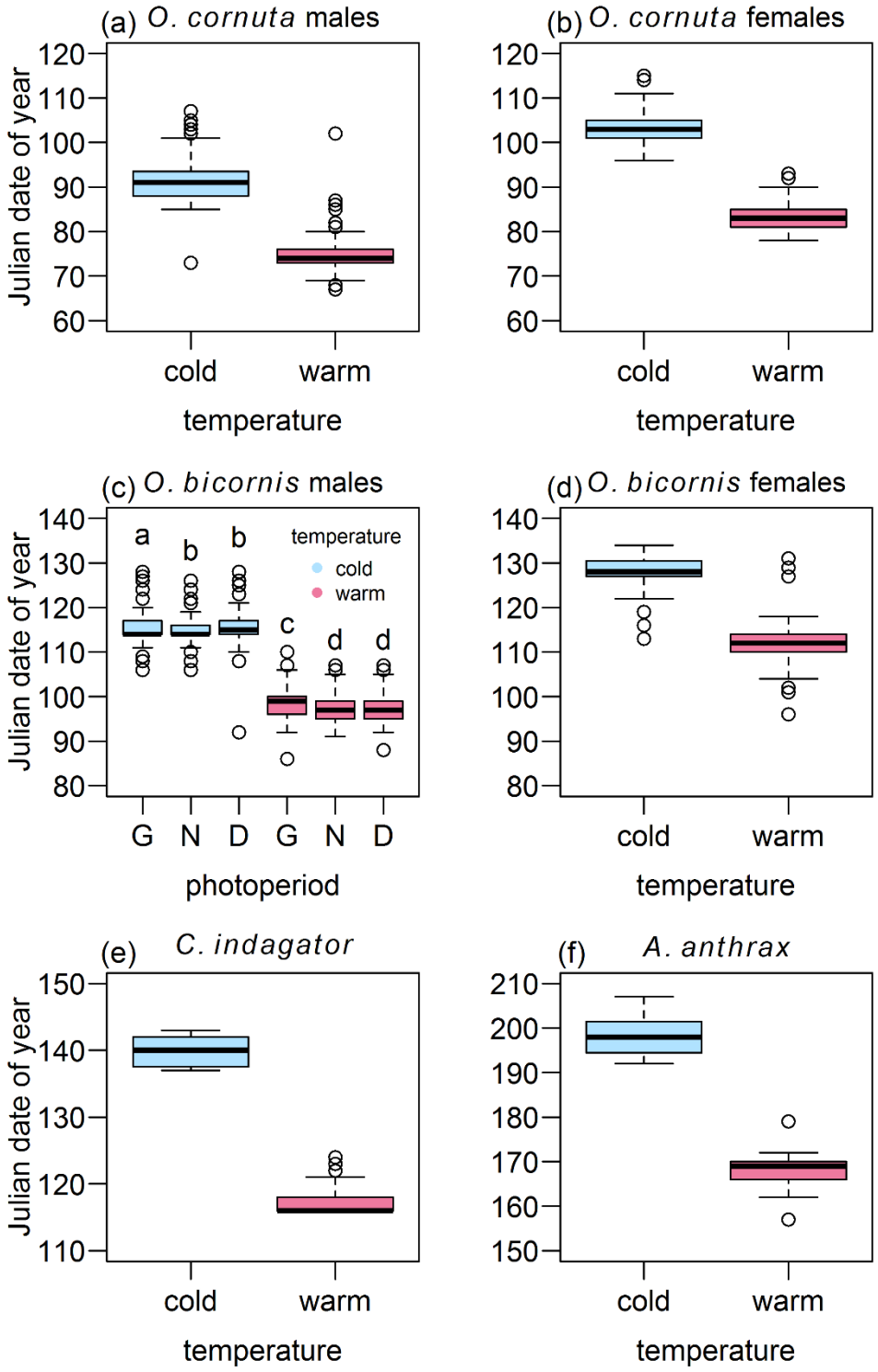

Fig IV.1. Effect of the two temperature treatments warm vs. cold (4 °C difference) on the Julian date of emergence for bees (a-d) and parasitoids (e-f) that emerged from cocoons inside of nest cavities. For *O. bicornis* males (c) the effect of photoperiod is shown. Different letters indicate significant differences ($p < 0.05$) between emergence dates (G: Germany, N: Norway, D: constant darkness).

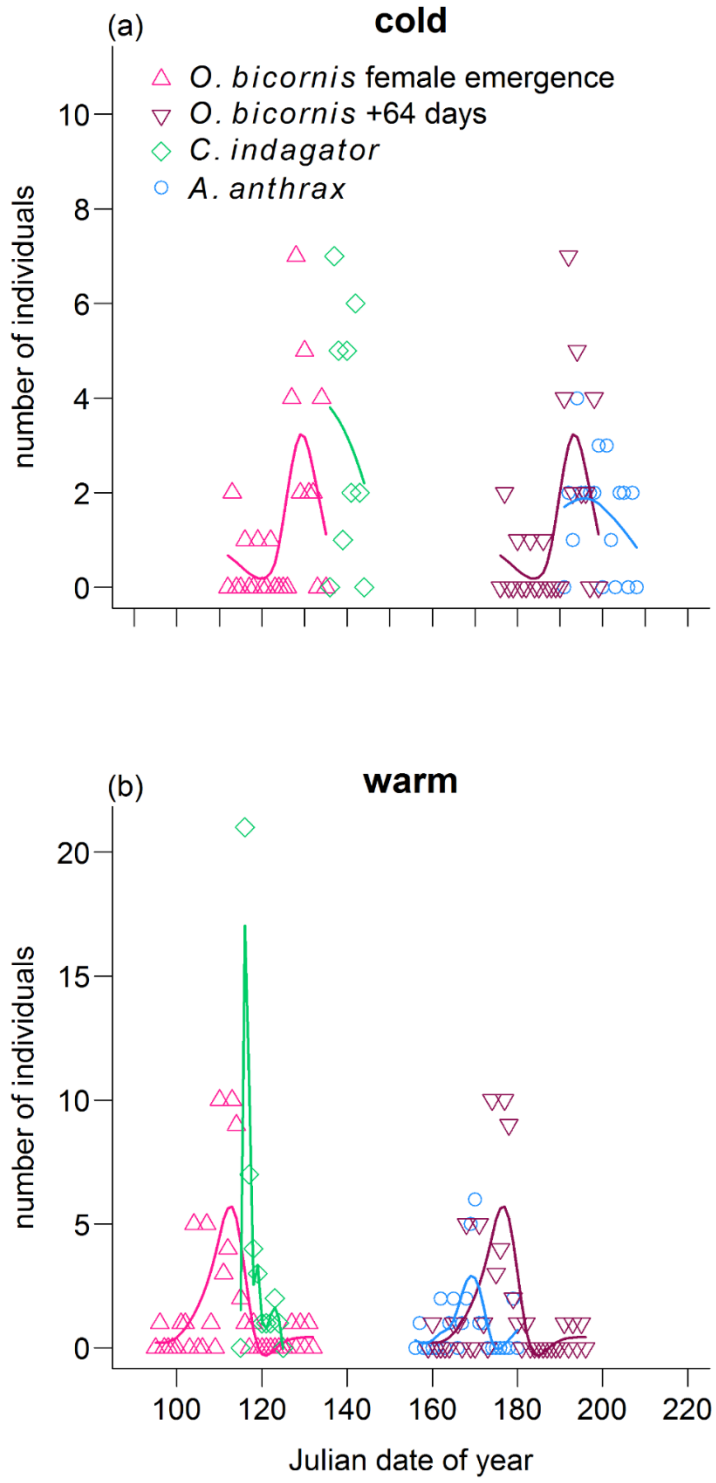

Fig. IV.2. Relationship between Julian date of year and number of emerged individuals from nest cavities for female bees of *Osmia bicornis*, and the parasitoids *Anthrax anthrax* and *Cacoxenus indagator* under (a) cold and (b) warm temperatures (4°C difference). For *O. bicornis* females, additionally the number of individuals estimated maximum life expectancy of 64 days after emergence is shown. Curves were fitted with general additive models.

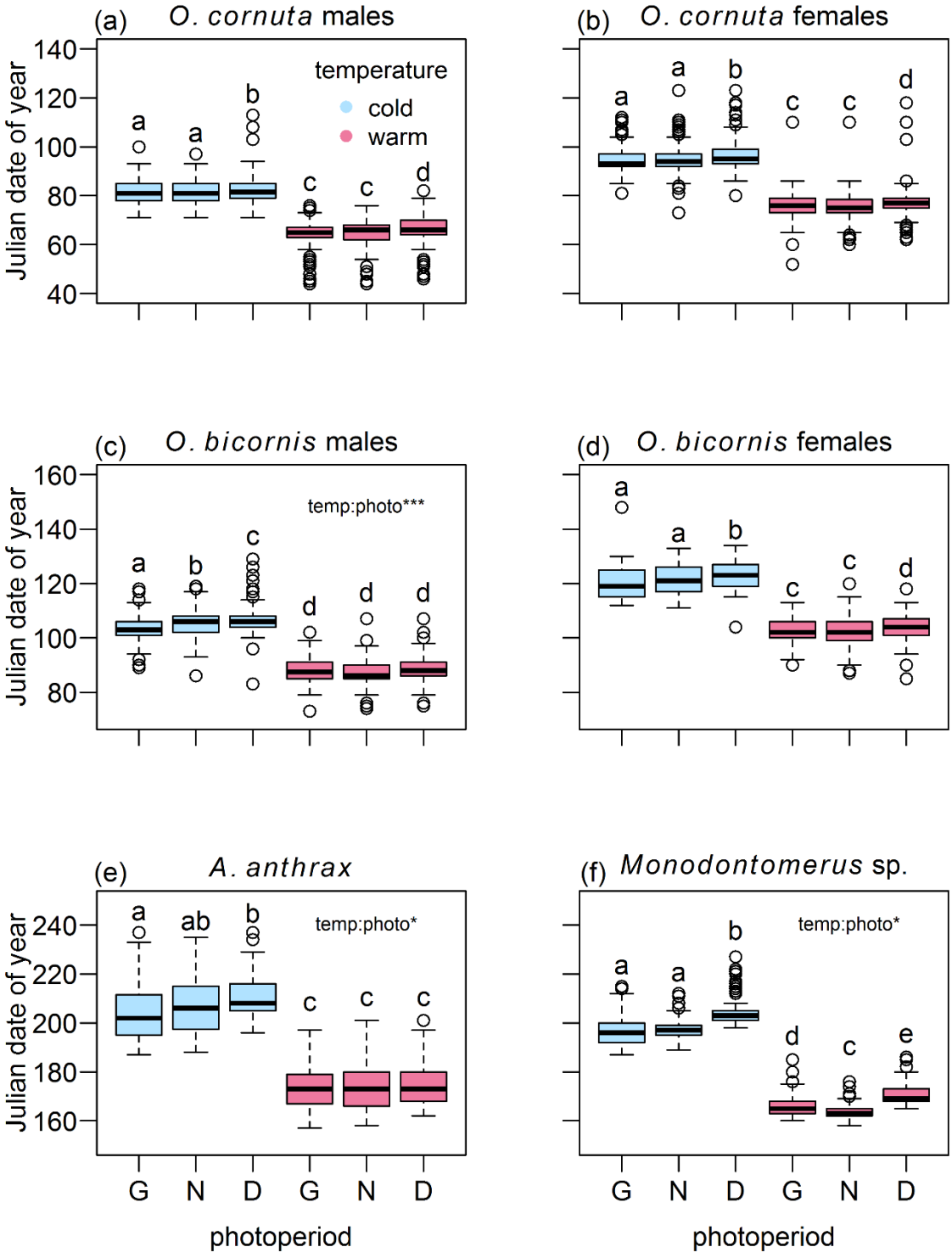

Fig. IV.3. Effect of the two temperature treatments warm vs. cold (4 °C difference) and three photoperiod treatments (G: Germany, N: Norway, D: constant darkness) on the Julian date of emergence for bees (a-d) and parasitoids (e- f) that emerged from cocoons outside of nest cavities. For significant interactions of temperature and photoperiod (temp:photo) the level of significance is indicated (***: $p < 0.001$, *: $p < 0.05$).

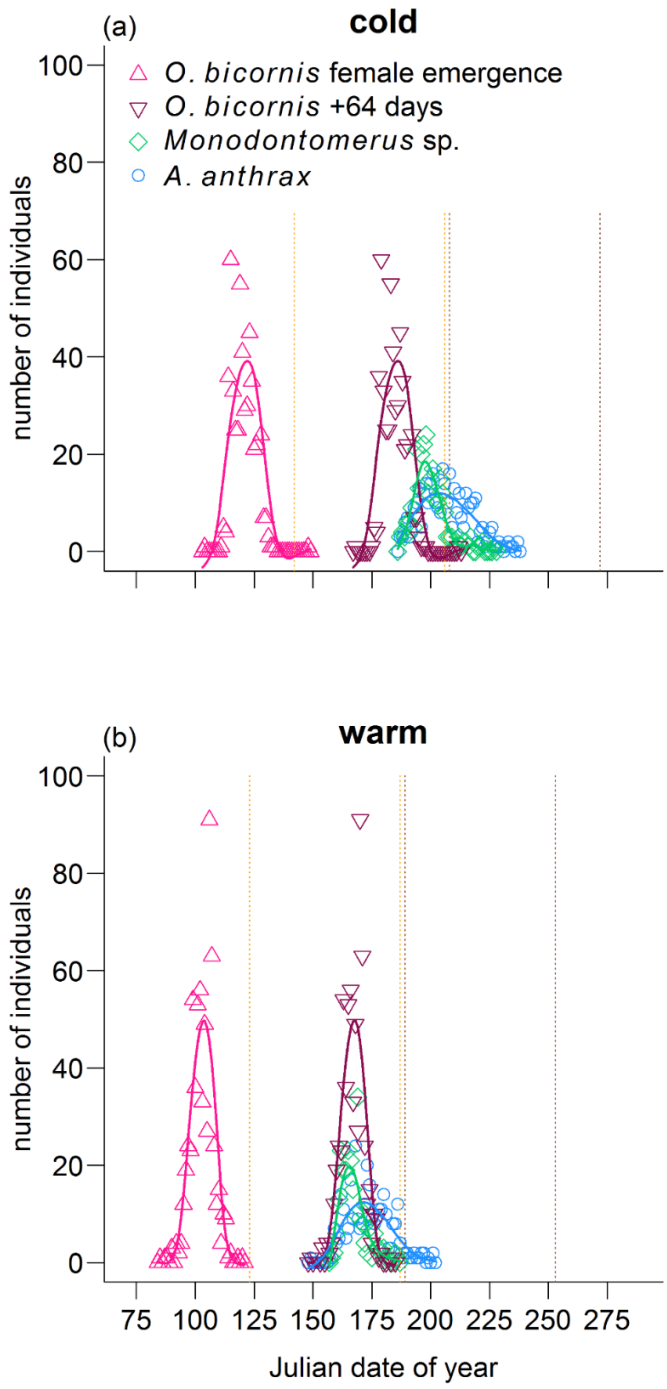

Fig. IV.4. Relationship between Julian date and number of emerged individuals from cocoons removed from nest cavities for female bees of *O. bicornis*, and the parasitoids *A. anthrax* and *Monodontomerus* under (a) cold and (b) warm temperatures (4°C difference). For *O. bicornis* females, additionally the estimated number of individuals based on a maximum life expectancy of 64 days after emergence is shown. Curves were fitted with general additive models. The orange vertical lines indicate the time period of the larval cocoon spinning phase (38 days), the brown vertical line the time period of the prepupae and pupae phase (66 days) between the first and last nest.

IV.5 Discussion

Our study showed that warmer temperatures advanced the date of emergence in *Osmia cornuta*, *Osmia bicornis* and their three main parasitoids. Our data suggest that emergence of both bee species advances by 4 to 5 days per 1 °C temperature increase. This is in accordance to a field study where an advance of 4 days per 1 °C temperature increase was found for *O. cornuta* males (Kehrberger and Holzschuh 2019a). A laboratory study showed a slower advance of 1 - 2 days per 1 °C temperature increase (Fründ et al. 2013), however, this difference could be attributed to the different study design with constant temperatures instead of fluctuating temperatures. All parasitoid species in our study advanced their emergence more strongly than the bees. *Cacoxenus indagator* emergence advanced by 5.5 days per 1 °C temperature increase, *Anthrax anthrax* emergence by 7.5 to 8.9 days and *Monodontomerus* emergence by 7.7 to 8.5 days depending on the photoperiod treatment. The differences between bees and parasitoids in their response to temperature could be explained by differences among the studied species in how they use temperature as trigger of emergence. To predict phenological events like emergence dates degree-day models are often used (van Asch and Visser 2007). Those models base on the assumption that a phenological event takes place as soon as the individual has experienced a species-specific temperature sum. Temperature values are summed up when a lower temperature threshold has been exceeded (van Asch and Visser 2007). Possible explanations for different emergence dates in bees and parasitoids are species-specific differences in the lower temperature threshold or in the temperature sum. Another explanation could be that there is a species-specific date before which temperatures are irrelevant for the timing of emergence.

Compared to temperature, photoperiod had a very small effect on the date of emergence of bees and their parasitoids in cocoons outside of the nest. All species emerged earlier under the photoperiod treatments than under constant darkness. Depending on the species, emergence was 0.5 to 7.8 days earlier under a photoperiod than in constant darkness. Our data suggest that bees and their parasitoids can use photoperiod as an additional cue to fine-tune the date of emergence, but that temperature is the dominating cue for timing of emergence. We only detected an effect of photoperiod on emergence in cocoons outside of nests. We expected that photoperiod also affects emergence in nest cavities by light that transmits into brood cells at the margins of the loam walls closing the brood cells. However, emergence in cocoons enclosed in nest cavities was not affected by

photoperiod. Under natural conditions cocoons enclosed in nest cavities might receive photoperiod only through damaged nest partitions produced by already emerged bees or parasitoids or by weathering.

In the cold treatment, *C. indagator* and *Monodontomerus* were well synchronized with the developmental state of *O. bicornis* that they parasitize, while large parts of the *A. anthrax* population emerged too late for a successful parasitation. Both parasitoid flies *C. indagator* and *A. anthrax* lay or shoot their eggs in a bee brood cell as long as the cell has not been closed with a loam wall (Krunić et al. 2005). Female bees start building brood cells a few days after emergence until their death (Bosch and Vicens 2005). The first *C. indagator* emerged three days and the first *A. anthrax* 39 days after the last *O. bicornis* females in the cold temperature treatment suggesting that all *C. indagator* had many opportunities to parasitize *O. bicornis* nests, even the last emerged, while only few female bees might have survived when *A. anthrax* started to emerge. In the warm treatment the time period between the emergence dates of *O. bicornis* females and *C. indagator* decreased by 1.5 days per 1 °C temperature and by 3.4 days for *A. anthrax*. In consequence, *C. indagator* was still well synchronized with its host, *A. anthrax* improved its synchrony and might thereby has enhanced opportunities to parasitize *O. bicornis* nests. In contrast to the parasitoid flies, the wasp *Monodontomerus* penetrates closed nests with its ovipositor when the bee larvae have developed to a prepupae or pupae inside cocoons (Krunić et al. 2005; Filella et al. 2011), i.e. approximately between day 38 and day 104 after the bee laid the egg (Raw 1972). In the cold treatment, the first *Monodontomerus* emerged 83 days after the first *O. bicornis* females and thus might have many opportunities to parasitize bee nests, as well as the last emerged *Monodontomerus*, which emerged 79 days after the last female bee. In the warm temperature treatment, the time period between the emergence dates of *O. bicornis* females and *Monodontomerus* decreased by 3.5 days per 1 °C temperature increase. Thus, the parasitism by *Monodontomerus* may increase under warmer temperatures. Our results suggesting that under the long-term mean temperatures in Germany (our cold treatment), large parts of *C. indagator* and *Monodontomerus* populations, but not of the *A. anthrax* population are well synchronized with *O. bicornis*, are in accordance with field data from Germany. This data showed very low parasitism rates of *O. bicornis* nests with *A. anthrax* (< 0.1 %; Steffan-Dewenter and Schiele 2008) and higher parasitism rates of *C. indagator* (6.1 %) and *Monodontomerus obscurus* (1.1 %, Seidelmann et al. 2016). In general, our findings

suggest that increasing temperatures will enhance the reproductive success of parasitoids and decrease the reproductive success of the bees. Similar to our results, phenological synchrony of host and parasitoid emergence increased with temperature in a butterfly - parasitic wasp interaction with negative effects on the host and positive effects on the parasitoid (Nouhuys and Lei 2004). However, a too strong increase in synchrony between host and parasitoid can also have negative consequences for both host and parasitoid if it leads to local extinction of the host due to the increased parasitoid pressure (Jeffs and Lewis 2013) followed by the extinction of the parasitoid (Hance et al. 2007). As parasitoids advanced their emergence to a stronger degree than their hosts a temperature increase beyond 4 °C, which was tested in this study, could lead to parasitoids emerging even before the first hosts. This could release hosts from parasitoids, but negatively affect parasitoid populations. This was shown for a cereal leaf beetle and its parasitoid wasp, where warmer temperatures reduced the parasitism rates of the beetles, as warmer temperatures delayed egg hatching of the beetle but did not affect parasite phenology, which led to parasites emerging before hosts being present (Evans et al. 2013). The reproductive success of the parasitoids could also remain unaffected by the degree of synchrony with one bee species if the generalist parasitoids parasitize different host species under cold than under warm temperatures (Damien and Tougeron 2019).

We conclude from our results that climate warming will change the synchrony in bee-parasitoid interactions with probable negative consequences for bees and neutral or positive consequences for parasitoids. The relationships between synchrony and reproductive success must be assessed in further studies as well as whether it is a general pattern that parasitoids respond more strongly to temperature changes than their hosts. Effects of temperature on the date of emergence were much stronger than the effect of photoperiod. Thus, changes in synchrony will rather take place if bees and parasitoids remain in their current range under climate warming than if they shift towards the poles to avoid increasing temperatures, and experience different photoperiod-temperature constellations. Further studies will have to reveal whether both bees and parasitoids will be able to shift their species range in response to climate change.

IV.6 Acknowledgements

We thank Katharina Merten and Kübra Degirmenci for their valuable help in data recording and Thomas Igerst for implementing and maintaining the set-up of the climate chambers.

IV.7 Supplementary

Note IV.S1. Bee nests

We collected 1248 bee nests from artificial trap nests a nesting site located near the University of Würzburg. The trap nests consisted of shelves with various plastic tubes filled with common reed (*Phragmites australis*). We selected reeds with a diameter ranging between 5 to 11 mm and sealed by loam, which have a high probability to be colonized by either *Osmia cornuta* or *Osmia bicornis*. The occurrence of parasitoids could not be predicted. Each reed internode filled with brood cells represented one nest. Nests had a length between 1.8 and 13.7 cm and contained 1 to 7 brood cells. The estimated number of brood cells per nest was the nest length divided by 2 cm, which was approximately the length of a brood cell.

After opening all nests at the end of the experiment, it became apparent that 383 nests (30.7 %) were built by *O. cornuta*, 568 (45.5 %) by *O. bicornis* and 15 (1.2 %) *by Osmia adunca*. For 297 nests (23.8 %) the bee species could not be identified, because no inhabitants had developed properly.

Note IV.S2. Boxes for photoperiod treatments

In each chamber we placed six two-storied Mini Plus hive boxes (Polystyrene, 216 x 160 mm) two for each photoperiod treatment. Stories were not separated by a barrier. For the constant darkness treatment we covered the boxes with the original Mini Plus roof. The boxes with the German and Norwegian photoperiod were covered with a metal roof with an individually controllable LED light source (6500 K; 400lm/meter; ~2000 Lux). The light sources were programmed with daily photoperiods (NOAA solar calculator, 2017). A one-hour period of dawn and dusk was simulated, by slowly increasing or decreasing light intensity after sunrise or before sunset, respectively. To avoid light transmission

through the walls, all boxes were additionally covered with black cardboard. In each box we placed 16 metal mash layers (21.5 x 20.5 cm; mesh width: 1 x 1cm). On the eight lower layers we placed 13 glass tubes with nests per layer. When assigning the nests to the boxes, we tried to avoid systematic differences among boxes in number of brood cells per nest, nest diameter and former compass directions of the nest entrances at the natural site. On the upper eight layers we placed 76 plastic tubes with individual cocoons of the commercial supplier per layer (hereafter "cocoons outside of nests"): 28 *O. cornuta* cocoons, 28 *O. bicornis* cocoons and 18 - 19 parasitized *O. bicornis* cocoons. Nests were placed in the boxes on 11th November (hereafter "cocoons in nests") cocoons outside of nests on 21st December.

Note IV.S3. Data recording

In the warm chamber we started to check for emergence on 13th February and in the cold chamber on 5th March the last check was on 13th September 2018 for both chambers. To check for emerged individuals we took out all layers and illuminated every layer with a red light LED (11 lm). When a brood-cell exit was broken or an emerged individual was visible, we removed the emerged individual and cut the reed half-open until the next closed brood cell before putting the nest back into the box. Due to the small size of *C. indagator* and *Monodontomerus* we did not remove individuals of those species and only recorded the first day of its emergence for every nest. In the case of cocoons outside of nests, the tube was removed from the experiment when an individual had emerged.

Table IV.S4. Total and mean number per treatment ± SE of emerged individuals from nests and individuals from cocoons that had been removed from nest cavities for the species *O. cornuta*, *O bicornis*, *C. indagator*, *A. anthrax* and *Monodontomerus*.

		number of emerged individuals from nests		number of emerged individuals from cocoons outside of nests	
species	sex	total	mean ± SE per treatment	total	mean ± SE per treatment
O. cornuta	male	684	114 .0 ± 0.8	1228	204.7 ± 0.6
O. cornuta	female	194	32.3 ± 0.4	1241	206.8 ± 0.6
O bicornis	male	892	148.7 ± 0.3	1214	202.3 ± 0.3
O bicornis	female	88	14.7 ± 0.6	1256	209.3 ± 0.3
C. indagator	NA	60	11.5 ± 0.4	0	0
A. anthrax in *O. cornuta* cocoons	NA	NA	NA	26	4.3 ± 0.2
A. anthrax in *O. bicornis* cocoons	NA	NA	NA	657	109.5 ± 0.7
Total *A. anthrax*	NA	52	8.7 ± 0.4	683	56.9 ± 2.2
Monodontomerus in *O. cornuta* cocoons	NA	NA	NA	36	6.0 ± 0.5
Monodontomerus in *O. bicornis* cocoons	NA	NA	NA	503	83.3 ± 0.3
Total *Monodontomerus*	NA	11	1.8 ± 0.3	539	44.9 ± 1.8

Table IV.S5. Results of Tukey HSD tests. Differences between photoperiod treatments in the date of emergence from cocoons outside of nests. Models included either temperature and photoperiod or additionally their interaction. P-values < 0.05 are bold.

Cocoons outside of nests	p
***O. cornuta* male emergence**	
Norway vs. Germany	0.811
constant darkness vs. Germany	**< 0.001**
constant darkness vs. Norway	**0.004**
***O. cornuta* female emergence**	
Norway vs. Germany	0.875
constant darkness vs. Germany	**< 0.001**
constant darkness vs. Norway	**< 0.001**
***O. bicornis* male emergence**	
warm	
Norway vs. Germany	0.787
constant darkness vs. Germany	0.883
constant darkness vs. Norway	0.151
cold	
Norway vs. Germany	**0.011**
constant darkness vs. Germany	**< 0.001**
constant darkness vs. Norway	**0.031**
***O. bicornis* female emergence**	
Norway vs. Germany	0.292
constant darkness vs. Germany	**< 0.001**
constant darkness vs. Norway	**< 0.001**
***A. anthrax* emergence**	
warm	
Norway vs. Germany	1.000
constant darkness vs. Germany	0.899
constant darkness vs. Norway	0.879
cold	
Norway vs. Germany	0.229
constant darkness vs. Germany	**< 0.001**
constant darkness vs. Norway	0.116
***Monodontomerus* emergence**	
warm	
Norway vs. Germany	**0.035**
constant darkness vs. Germany	**< 0.001**
constant darkness vs. Norway	**< 0.001**
cold	
Norway vs. Germany	**1.000**
constant darkness vs. Germany	**< 0.001**
constant darkness vs. Norway	**< 0.001**

Chapter V: General Discussion

In my PhD-thesis I investigated how the timing of flowering affects plant reproductive success and how abiotic and biotic drivers shape that relationship. Furthermore, I investigated how temperature affects the emergence of two solitary-spring bees (*Osmia cornuta* & *Osmia bicornis*), flowering of one of their food plants (*Pulsatilla vulgaris*) and emergence of three of their main parasitoids (*Cacoxenus indagator*, *Anthrax anthrax*, *Monodontomerus*). I also investigated if altered photoperiods, as they would result from geographical range shifts, affect emergence of two solitary-spring bees and three of their main parasitoids.

In Chapter II I have shown that flowering at the beginning of the season can ensure high reproductive success due to low interspecific competition for pollinators, which overcompensates low pollinator abundances and low numbers of time spans suitable for pollinators to forage. The study presented in Chapter III demonstrates that warmer temperatures advance flowering onset of an early spring plant more strongly than bee emergence which suggests an increased risk of pollinator limitation for the first flowers. Furthermore, the data indicates that competition for pollinators between an early spring plant and co-flowering plants does not increase within the studied temperature range. In Chapter IV I present a study that investigated the effects of temperature and photoperiod on the timing of emergence of two solitary-spring bees and their three main parasitoids. Species may be exposed not only to warming temperatures as a result of climate warming, but range shifts may also occur, which expose species to new photoperiods. The data showed that warmer temperatures advanced parasitoid emergence more strongly than bee emergence leading to increased temporal synchrony which may reduce reproductive success in bees. Furthermore, photoperiod advanced bee and parasitoid emergence only in cocoons that had been removed from cavity nests but not in cavity nests and to a much lower degree than temperature.

V.1 Timing of flowering

The optimal timing of flowering is crucial for the reproductive success of plant species (Austen et al. 2017) and is affected by abiotic factors like temperature (Rathcke and Lacey 1985; Ehrlén 2015) and biotic factors like interspecific interactions with mutualists and

competitors (Rathcke and Lacey 1985; Elzinga et al. 2007; Ehrlén 2015). In Chapter II I have shown that the negative effect of the growing number of co-flowering plants and with that the increase in interspecific competition for pollinators of an early spring plant with its co-flowering plants could not be compensated by the increase in bee abundance, which led to an overall reduction of the flower visitation rates over time. The number of pollinator visits a flowering plant receives depends on the abundance of pollinators and on the attractiveness of the plant itself and of its co-flowering plants (Sargent and Ackerly 2008; Lázaro et al. 2009; Mitchell et al. 2009). Therefore, I suggest that the high attractiveness at the beginning of the flowering period of plants flowering at the beginning of the season is due to the absence of co-flowering plants and with that of alternative food resources. I have also shown that early flowering grassland plants cannot elongate floral longevity of late flowers when visitation rates are low. This is in contrast to other studies which showed that flowers are able to mitigate negative effects of low pollinator visitation rates by elongating their longevity (Castro et al. 2008; Aronne et al. 2015). The data suggests that the shortage of the floral longevity of later flowers were imposed by the warming temperatures over the course of flowering, which probably enhance physiological processes like, flower transpiration and respiration, which leads to a faster flower senescence (Arroyo et al. 2013). The decline in flower visitation rates over the flowering period was reflected in the seed set which was highest for the first flowers and marginally decreased over time. Contrary, the seed set of herbal plants in subalpine meadows and deciduous forests was lowest in the first flowers but increased over time (Schemske 1977; Motten et al. 1981; Mahoro 2002; Kudo et al. 2004; Thomson 2010; Kudo and Ida 2013). When plants flower during periods with suboptimum flower visitation the spread of flowering onset over time to buffer negative consequences of low visitation rates by absent pollinator activity for the first flowers or pollinators being drawn away by competing co-flowering plants for the last flowers can be considered a bet-hedging strategy. I have shown for the first time that in cool temperate regions flowering as the first plant species of the season does not have to be negative for the reproductive success of early flowers, instead the last flowers were negatively affected. However, late flowers could act as insurance against rare, extreme cold weather events at the beginning of the flowering period, because the lifetime fitness of individuals is influenced by the reproductive success within multiple flowering seasons. I conclude that for plant species flowering at the beginning of the season in grasslands the limiting factor for reproduction seems to be low pollinator visitation imposed by interspecific competition for pollinators

by co-flowering plants but not the low abundance of pollinators nor the limited time span for pollinators to forage due to unfavourable weather conditions.

V.2 Phenological shifts in plant-pollinator interactions

Climate warming, which advances the flowering onset of many plant species (Menzel et al. 2006), could negatively affect the reproductive success of at the beginning of the season flowering plants if co-flowering plants advance their flowering onset more strongly (CaraDonna et al. 2014) or if plants and their pollinators respond with non-parallel phenological shifts of flowering and emergence. So far field studies on the synchrony between plant phenology and solitary bee emergence are still scarce (Forrest and Thomson 2011). In a follow up study, I assessed in Chapter III how warmer temperatures affect the timing of flowering of an early spring plant and its co-flowering plants and the emergence of its main pollinators on grasslands differing in mean site temperature. I have shown that warmer temperatures advanced plant flowering more strongly than bee emergence. Additionally, I demonstrated that the first date of male emergence of solitary-spring bees as well as the first flowering date of an early spring plant advanced more strongly than the mean date of emergence and flowering with warmer temperatures, respectively. The first individuals in a population especially at the beginning of the season face the highest risk of a temporal mismatch with mutualistic interaction partners. The stronger advance of an early spring plant compared to two of its main pollinators suggests that warm temperatures involve the risk that early flowering plants start flowering before the emergence of their pollinators. The limitation of pollinators can reduce reproductive success and consequently have a negative effect on population size (Sargent and Ackerly 2008). A way to compensate for a temporal mismatch with pollinators can be the elongation of floral longevity (Arroyo et al. 2013). I have shown that due to a less strong advance of flowering onset compared to flowering end warmer temperatures shortened the flowering duration of an early flowering plant. The shorter flowering durations with warmer temperatures are probably due to shorter floral longevities of individual flowers, induced by warmer temperatures. Contrary, warmer temperatures delayed the last date of flowering and lengthened the flowering season in early flowering montane plant species (CaraDonna et al. 2014; Theobald et al. 2017). Compressed flowering periods may decrease the probability that a plant is visited

by pollinators, which can negatively affect its reproductive success. Another way to compensate for pollinator limitation is to switch to vegetative reproduction if pollination fails (Wells and Barling 1971), however, this can reduce the genetic variability of the population and the adaptive plasticity to respond to environmental variation (Holsinger 2000). Besides compensation mechanisms implemented by the pollinator-limited plant itself, the plant can also mitigate negative effects resulting from temporal mismatches of plants and pollinators by switching to alternative pollinators, which fulfil the same function (Bartomeus et al. 2013). Other early pollinators at the beginning of the season can be honeybees and bumble bee queens.

Flowering of the early spring plant advanced not only more strongly than emergence of its pollinators but also more strongly than flowering onset of its later co-flowering plants. But this did not elongate the time span of flowering without co-flowering plant species. However, in a North-American plant community it was shown that warming changes the periods of temporal overlap of reproductive stages (Sherry et al. 2007). Co-flowering plant species, which withdraw pollinators can strongly decrease the reproductive success of a plant species (Mosquin 1971). This suggests that there may be a strong selective pressure for early flowering plants which are less competitive to start flowering prior to co-flowering plant species (Elzinga et al. 2007).

I conclude that with climate warming early animal pollinated plants are at the risk to face pollinator limitation, as warming temperatures advance flowering of early plants more strongly than emergence of spring bees. This can have severe negative consequences for plant reproductive success. Non-parallel phenology shifts of bees and plants can decrease diversity and change the composition of flowering plant communities, where bees can forage on during their flight season (Burkle et al. 2013), with possible negative effects for bee larval development and reproductive success (Sedivy et al. 2011; Schenk et al. 2018a).

V.3 Phenological shifts in host-parasitoid interactions

Besides plants, bees interact with antagonists like parasites and parasitoids, which can reduce reproductive success and population size (Krunić et al. 2005; Roulston and Goodell 2011; Felicioli et al. 2017). However, studies focusing on the effect of climate

warming on the temporal synchrony between wild bees and their parasitoids are still rare. In Chapter IV I conducted an experiment where cocoons within nest cavities and cocoons that had been removed from the nests were exposed to two temperature regimes (long-term mean of Würzburg (cold) and long-term mean of Würzburg + 4 °C (warm)) and three photoperiods (Würzburg vs. Snåsa vs. constant darkness) to record how temperature and photoperiod affect the timing of emergence of bees and their parasitoids. I show for the first time, that warmer temperatures advanced both bee and parasitoid emergence, with parasitoids exhibiting a stronger advance than bees. As a consequence, the time period between female bee and parasitoid emergence was shorter under warm than colder long term mean temperatures. The data shows that bee emergence (*O. cornuta* and *O. bicornis*) advanced by 4 to 5 days per 1 °C temperature increase, whereas parasitoids advanced their emergence by 5.5 days per 1 °C temperature increase (*C. indagator*) or between 7.5 to 8.9 days per 1 °C temperature increase (*A. anthrax* and *Monodontomerus*) depending on the species. The stronger response of parasitoids to temperature compared to bees could be explained by differences in the temperature sensitivity of the species. A common way to predict phenological events like emergence dates is the usage of degree-day models (van Asch and Visser 2007). Those models take into account the length of a period (e.g. in days or hours) in which a lower temperature threshold has been reached and multiply that period with the temperature experienced during that period. After a species-specific value in degree-days or hours has been exceeded the phenological event takes place (van Asch and Visser 2007). Bees and parasitoids may differ in the parameters used in the model or in the length of their diapause.

The data shows that under the current temperatures prevailing in Germany large parts of *C. indagator* and *Monodontomerus* populations are synchronised with *O. bicornis* populations, whereas large parts of *A. anthrax* populations are not synchronised. This could explain the very low parasitism rates of *O. bicornis* nests with *A. anthrax* (< 0.1 %, Steffan-Dewenter and Schiele 2008) and the higher parasitism rates of *C. indagator* (6.1 %) and *Monodontomerus obscurus* (1.1 %) found in Germany (Seidelmann et al. 2016).

Both studied flies *C. indagator* and *A. anthrax* depend on open bee nests for reproduction, in which they lay or shoot their eggs on nest provisioning of single brood cells (Krunić et al. 2005). The wasp *Monodontomerus*, however, depends on bee prepupae or white bee pupae inside cocoons in which it lays its eggs (Krunić et al. 2005; Filella et al. 2011). To locate its host *Monodontomerus* uses chemical volatiles emitted from the frass and cocoon

of bee prepupae but not emitted directly from bee prepupae (Filella et al. 2011). *O. bicornis* bee larvae start spinning their cocoon approximately 38 days after egg laying and stay in the prepuae and pupae phase for 66 days altogether (Raw 1972). The data shows that the first *C. indagator* emerged three days, the first *A. anthrax* 39 to 58 days and the first *Monodontomerus* 83 days after the first *O. bicornis* females under cold temperatures. The short time period between *C. indagator* and *O. bicornis* female emergence suggests a high temporal synchrony between them and many opportunities for *C. indagator* to parasitize *O. bicornis* nests. The same seems to be the case for *Monodontomerus* as they emerge after the first bee nests already contain prepuae and pupae of bees. Furthermore, they may also parasitize some *O. cornuta* cocoons which could still be in the pupae phase. For *A. anthrax* the data suggests that due to their late emergence compared to *O. bicornis* females they can only parasitize few *O. bicornis* nests that are still under construction. However, I demonstrate that the prevailing parasitism rates of *O. bicornis* in Germany may increase with climate warming, as the time period between female bee and parasitoid emergence decreased with warmer temperatures. The strongest decrease in the time period between female bee and parasitoid emergence was found for *A. anthrax* and *Monodontomerus* with a mean decrease of 3.6 days per 1 °C temperature increase and the weakest decrease for *C. indagator* with 1.5 days per 1 °C temperature increase. In consequence, *C. indagator* and *Monodontomerus* were still well synchronized with their host and *A. anthrax* improved its synchrony and might thereby enhanced opportunities to parasitize *O. bicornis* nests. In general, my findings suggest that increasing temperatures will decrease the reproductive success of the bees but enhance the reproductive success of the parasitoids. Similar to my results, phenological synchrony of host and parasitoid emergence increased with temperature in a butterfly - parasitic wasp interaction with negative effects on the host and positive effects on the parasitoid (Nouhuys & Lei, 2004). However, a too strong increase in synchrony between host and parasitoid can also have negative consequences for both host and parasitoid if it leads to local extinction of the host due to the increased parasitoid pressure (Jeffs & Lewis, 2013) followed by the extinction of the parasitoid (Hance et al., 2007). A stronger advance of parasitoid emergence compared to host emergence could even lead to parasitoids emerging before the first hosts which would release hosts from parasitoids, but negatively affect parasitoid populations. This was shown for a cereal leaf beetle and its parasitoid wasp, where warmer temperatures reduced the parasitism rates of the beetles, as warmer temperatures delayed egg hatching of the beetle but did not affect

parasite phenology, which led to parasites emerging before hosts being present (Evans et al., 2013). The reproductive success of the parasitoids could also remain unaffected by the degree of synchrony with one bee species if the generalist parasitoids parasitize different host species under cold than under warm temperatures (Damien & Tougeron, 2019).

V.4 Photoperiod as an emergence cue in bee-parasitoid interactions

Besides altered temperatures, climate warming can expose species also to changed photoperiods if species ranges shift towards the poles. If interacting species differ in their phenological response to warmer temperatures or altered photoperiods temporal synchrony between them can be changed. I demonstrate that photoperiod affected the time of emergence of bees and their parasitoids when the cocoons had been removed from the cavity nest, while photoperiod had no effect – except for *O. bicornis* males – on cocoons within cavity nests. Bees emerged earlier in the photoperiod treatments compared to the constant darkness treatment but showed no difference in time of emergence between the photoperiods. I also showed that the response to photoperiod was much weaker than the response to temperature. I suggest that bees and their parasitoids use photoperiod, if they receive it, as an additional cue to fine-tune the time of emergence and to secure that they do not emerge too early in the season, but temperature is the dominating cue used for timing of emergence. Under natural conditions, where cocoons are enclosed in cavity nests, bees and their parasitoids might receive photoperiod through damaged nest partitions produced by already emerged bees or parasitoids or by weathering. Altered photoperiods entailed by geographic range shifts may alter the timing of emergence in both bees and their parasitoids and therefore the time period between their emergences, however, the effects may be more pronounced with climate warming. I suggest that besides warming temperatures also geographic range shifts have the potential to evoke temporal shifts between bee and parasitoid emergence.

V.5 Conclusion

It can be concluded that climate warming has the potential to disrupt temporal synchrony of interacting species over multiple trophic levels due to species-specific response to

warmer temperatures. Early spring plants advance flowering more strongly than their pollinating bees, which could result in pollinator limitation for the first flowers. However, bees advance their emergence less strongly than their parasitoids in response to warmer temperatures which can increase parasitism rates. Both non-parallel phenological shifts of wild bees with food plants and parasitoids can negatively affect reproductive success and population size of bees and plants, whereas reproductive success and population size of parasitoids may increase. To predict further effects of climate warming on species I suggest that not only species-specific responses to climate change are considered, but also the responses of all of their interaction partners to consider changes in interactions. With that cascading effects of climate warming over multiple trophic levels could be more precisely predicted which could improve predictions of species persistence under climate warming.

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Author contributions

Statement of individual author contributions and of legal second publication rights

Chapter II: Kehrberger S., Holzschuh A. (2019) How does timing of flowering affect competition for pollinators, flower visitation and seed set in an early spring grassland plant? *Sci Rep* **9,** 15593. doi:10.1038/s41598-019-51916-0 Corresponding author: Sandra Kehrberger		
Participated in	**Author Initials, Responsibility decreasing from left to right**	
Study Design	AH	SK
Methods Development	SK	AH
Data Collection	SK	
Data Analysis & Interpretation	SK	AH
Writing of First Draft	SK	
Editing the Manuscript	SK	AH

Chapter III: Kehrberger S., Holzschuh A. (2019) Warmer temperatures advance flowering in a spring plant more strongly than emergence of two solitary spring bee species. *PLoS ONE* 14(6): e0218824. https://doi.org/10.1371/journal.pone.0218824		
Participated in	**Author Initials, Responsibility decreasing from left to right**	
Study Design	AH	SK
Methods Development	SK	AH
Data Collection	SK	
Data Analysis & Interpretation	SK	AH
Writing of First Draft	SK	
Editing the Manuscript	SK	AH

Chapter IV: Kehrberger S., Steffan-Dewenter I., Holzschuh A. (submitted as) How do temperature and photoperiod affect the seasonal synchrony between wild bees and their parasitoids? Functional Ecology			
Participated in	**Author Initials, Responsibility decreasing from left to right**		
Study Design	ISD	AH	SK
Methods Development	ISD	AH	SK
Data Collection	SK		
Data Analysis & Interpretation	SK	AH	
Writing of First Draft	SK		
Editing the Manuscript	SK	AH	ISD

Funding

Funding was provided by the German Research Foundation (DFG) in the framework of the Collaborative Research Center 1047 - Insect Timing: mechanisms, plasticity and fitness consequences, SFB 1047, Project C4.

Publication list

Kehrberger S., Holzschuh A. (2019) Warmer temperatures advance flowering in a spring plant more strongly than emergence of two solitary spring bee species. *PLoS ONE* 14(6): e0218824. https://doi.org/10.1371/journal.pone.0218824

Kehrberger S., Holzschuh A. (2019) How does timing of flowering affect competition for pollinators, flower visitation and seed set in an early spring grassland plant? *Sci Rep* **9,** 15593. doi:10.1038/s41598-019-51916-0

Acknowledgements

First, I would like to thank Andrea Holzschuh and Ingolf Steffan-Dewenter for giving me the opportunity to work on this exciting topic and for funding me. I would like to thank my supervisor Andrea Holzschuh for her great supervision and support. Thank you for always having an open door and the motivation during experiments and manuscript writing. I would also like to thank my second supervisor Prof. Dr. Ricarda Scheiner for her valuable feedback during our annual meetings and for reviewing my PhD-thesis.

I want to thank the Zoo III team for the wonderful working atmosphere. Thanks to those that shared lunch in the department kitchen, especially Veronika and Sarah and for our nice and refreshing conservations. Thanks to Fabian B. for his support with R. I would also like to thank Benjamin for sharing an office with me. Thanks to the members of the green commission for our discussions and actions trying to make the department a bit more sustainable. I want to thank the Living Campus team and especially Sarah, Hannah, Martina, Jessica and Holger for their help in monitoring wild bees on the campus.

Thanks to Thomas Igerst for his technical support and for the effort and time he invested in constructing the system for the climate chamber experiment and keeping it working. Thanks also to Norbert Schneider for helping with the climate chamber experiment. I want to thank Beate, Gudrun and Suse for their help during experiments, especially for helping with the bee cocoons. I want to thank Fabian N and Gemma for the great cooperation during the climate chamber experiment. Thanks to Birgit and Michaela for their help with administration.

Next I want to thank Katharina, Kübra, Jana and Tobias for doing their thesis with me. Thank you for handling hundreds of bee cocoons and emerged bees. Thanks also to my Hiwis, especially Dominik, for your great work.

I want to thank Rose Liebert for giving me the opportunity to be part of the Scientia Mentoring Program. The workshops were a great help. A huge thank goes to Karin Günter for being my mentor during the program. I really enjoyed our talks and am very greatful for your support and advises. I also would like to thank my peer group Julia, Inci, Sarah, Valentina and Annika for our meetings and mutual support.

Finally, I would like to thank my family and friends for their support and motivation. Thank you for always believing in me and encouraging me to pursue my way.