RESEARCH ARTICLE

Desynchronizations in bee–plant interactions cause severe fitness losses in solitary bees*

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Abstract

1. Global warming can disrupt mutualistic interactions between solitary bees and plants when increasing temperature differentially changes the timing of interacting partners. One possible scenario is for insect phenology to advance more rapidly than plant phenology.

2. However, empirical evidence for fitness consequences due to temporal mismatches is lacking for pollinators and it remains unknown if bees have developed strategies to mitigate fitness losses following temporal mismatches.

3. We tested the effect of temporal mismatches on the fitness of three spring-emerging solitary bee species, including one pollen specialist. Using flight cages, we simulated (i) a perfect synchronization (from a bee perspective): bees and flowers occur simultaneously, (ii) a mismatch of 3 days and (iii) a mismatch of 6 days, with bees occurring earlier than flowers in the latter two cases.

4. A mismatch of 6 days caused severe fitness losses in all three bee species, as few bees survived without flowers. Females showed strongly reduced activity and reproductive output compared to synchronized bees. Fitness consequences of a 3-day mismatch were species-specific. Both the early-spring species *Osmia cornuta* and the mid-spring species *Osmia bicornis* produced the same number of brood cells after a mismatch of 3 days as under perfect synchronization. However, *O. cornuta* decreased the number of female offspring, whereas *O. bicornis* spread the brood cells over fewer nests, which may increase offspring mortality, e.g. due to parasitoids. The late-spring specialist *Osmia brevicornis* produced fewer brood cells even after a mismatch of 3 days. Additionally, our results suggest that fitness losses after temporal mismatches are higher during warm than cold springs, as the naturally occurring temperature variability revealed that warm temperatures during starvation decreased the survival rate of *O. bicornis*.

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

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1 | INTRODUCTION

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

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

We performed an experiment with large flight cages serving as mesocosms. We manipulated the supply of blossoms inside the mesocosms to synchronize or desynchronize bee–plant interactions. Fitness parameters were recorded for three spring-emerging solitary bee species of the genus *Osmia*, synchronized, or with a mismatch of either 3 or 6 days. Thackeray et al. (2016) predicted an average temporal mismatch of about 3 days between primary consumers (e.g. bees) and primary producers (e.g. plants) under different emission scenarios by the 2050s. We assumed, therefore, that the temporal mismatches we chose represented a reliable scenario under future climate warming. For the experiment, we chose two polylectic and one oligolectic bee species that emerge between early and late spring. We measured their survival rates, their activity over their lifetimes, the number of brood cells and nests produced, and the sex of their offspring. The following questions were addressed: (i) Is there a negative impact of a temporal mismatch with their food plants on the survival rate, total activity and reproductive output of solitary bees? (ii) Do solitary bees have strategies that mitigate fitness losses when food plants are completely lacking? (iii) Does the ambient temperature modify the impact of desynchronization on the fitness of solitary bees after emergence?

We showed that temporal mismatches in bee–plant interactions of 3 or 6 days cause tremendous fitness losses to solitary bees even though bees have strategies to mitigate associated impacts on their fitness. Additionally, our results suggest that fitness losses after temporal mismatches are higher during warm than during cold springs.
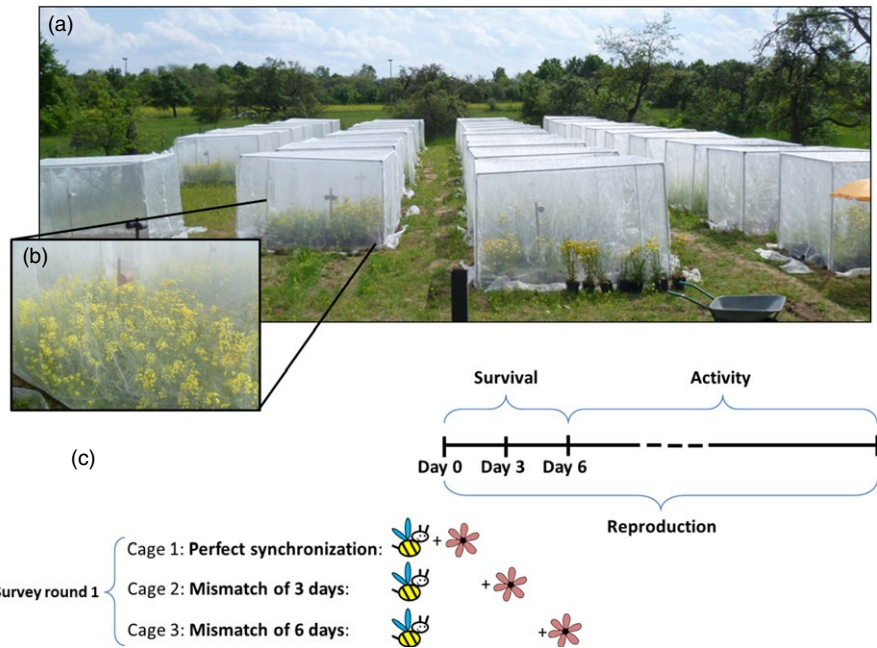
FIGURE 1 (a) View of the experimental setup. (b) Supply of blossoms inside flight cages. (c) Illustrative description of the practical implementation of one survey round. Further survey rounds—each with three cages—started at later dates. It is shown when bees and plants were added to the cages depending on the treatment and during which time periods survival and activity of bees were recorded. Reproduction was possible during the whole survey round [Colour figure can be viewed at wileyonlinelibrary.com]

2 | MATERIALS AND METHODS

2.1 | Experimental design

We established 36 mesocosms (=flight cages, Figure 1a and b) to test effects of synchronized and desynchronized plant–bee interactions on the fitness of three solitary bee species. Inside the cages we simulated either a perfect synchronization between solitary bee emergence and plant flowering or temporal mismatches of 3 or 6 days where bees were kept in the flight cages without food resources (Figure 1c). The experiment was conducted in spring and summer of 2014. Flight cages were placed in a grassland near the University of Würzburg, Germany. The flight cages were 3 × 2 × 2 m in size to offer adequate living space with a mesh width of 0.8 mm to prevent bees and other insects from entering and leaving the cages. For each bee species, we conducted between 5 and 10 survey rounds (for more details see section "Bees"). Survey rounds began on different days to cover different temperature conditions, as they may modify the effects of temporal mismatches on bees. In each survey round (see Figure 1c), we manipulated three cages, each of which represented one of the three treatments: (i) perfect synchronization (from a bee perspective): bees and flowers were placed simultaneously in the cage; (ii) bees were added 3 days before flowers were placed in the cage; and (iii) bees were added 6 days before flowers were placed in the cage. Treatments were randomly assigned to cages.

Flight cages were equipped with trap nests to record the reproductive output of female bees. In the centre of each cage, trap nests were attached to a pole at a height of 1 m. Each bee species was provided with trap nests consisting of nesting tubes of their preferred size. We supplied *Osmia cornuta* and *Osmia brevicornis* with one trap nest each but *Osmia bicornis* with two different trap nest types. *Osmia cornuta* and *O. bicornis* received one trap nest from Oxford Bee Company (Schrewsbury, England) containing approximately 120 paper tubes of 8 mm diameter and a tube length of 20 cm. *Osmia bicornis* and *O. brevicornis* received

one trap nest from the University of Würzburg (approximately one hundred 20-cm-long reed internodes inside plastic tubes, which were accessible from two sides). Diameters of reed internodes ranged from 3 to 8 mm. We recorded the temperature in each flight cage once per hour. Temperature-sensors (Maxim Integrated DS1921G-F5 Thermochron iButton; 0.5°C resolution) were attached at a height of 1 m on the north side of the trap nest pole to avoid direct sunlight. Flight cages were also equipped with flowering plants, either together with the bees (synchronized) or 3 or 6 days after the bees were added (see below). We also equipped each flight cage with a small pot of 7 × 5.5 × 5.5 cm size. These pots were filled with sandy loam which we moistened once per day during the whole length of the experiments to make the sandy loam accessible for bees and also to provide them with water.

2.2 | Bees

We selected three spring-emerging species of solitary bees as study species (Hymenoptera: Apiformes: Megachilidae). We chose study species according to their seasonal appearance during spring to cover a range from early to late spring-emerging species and to cover a spectrum of food preferences. The hornfaced mason bee *O. cornuta* is a food generalist with an activity period from March until May, the red mason bee *O. bicornis* is a food generalist with an activity period from early April until June and the wallflower mason bee *O. brevicornis* is a solitary bee species specialized on Brassicaceae, with an activity period from late April until June (Westrich, 2011). Single cocoons of *O. cornuta* and *O. bicornis* were purchased from WAB Mauerbienenzucht (Konstanz, Germany), a commercial supplier of solitary bees. Nests from *O. brevicornis* were collected from trap nests (reed internodes inside plastic tubes) that had been exposed in the field in 2013 around Würzburg, Germany. From October 2013 until spring 2014, nests and single cocoons overwintered inside a climate chamber at constant 4°C. In spring 2014, cocoons were incubated successively in the

laboratory at 21–23°C until emergence. For each survey round, we incubated a new group of individuals. The required incubation time was known from pilot studies (M. Schenk, pers. obs., April–July 2014). To start a survey round, bees that had emerged in the laboratory during the previous 24 hr were placed in three flight cages. For *O. cornuta* and *O. bicornis*, we placed seven females and four males per cage. For *O. brevicornis*, we placed 5.6 ± 1.5 females (mean ± *SD*) and 3.4 ± 0.8 males (mean ± *SD*) per cage with three females and two males minimum and seven females and four males maximum, whereby female and male abundances per cage did not differ among treatments within a survey round. The male bees were placed inside the cages to ensure the fertilization of females. Fertilization generally took place in the flight cages shortly after the start of the experiment, independent of the occurrence of flower resources (M. Schenk, pers. obs., April–July 2014). Data collection was focused on female bees only because females are the demographically limiting sex (Goulson et al., 2010).

We tested the three solitary bee species in succession following their natural appearance time during spring. Survey rounds of *O. cornuta* started between 3 and 30 April 2014, survey rounds of *O. bicornis* started between 14 May and 4 July 2014 and survey rounds of *O. brevicornis* started between 27 May and 10 June 2014. *Osmia cornuta* was tested in 8 cages per treatment (total 24 cages), *O. bicornis* was tested in 10 cages per treatment (total 30 cages) and *O. brevicornis* was tested in 5 cages per treatment (total 15 cages). As the survey rounds did not completely match the natural flight periods of the bees (*O. cornuta*: March to May, *O. bicornis* and *O. brevicornis*: April to June), we compared temperatures measured in the cages to long-term temperature data (1990–2013), which were measured during the natural flight periods at the regional climate station in Würzburg (DWD Climate Data Center CDC, 2016). The mean cage temperatures measured during the experiment (*O. cornuta*: 14.97°C, *O. bicornis*: 19.13°C and *O. brevicornis*: 18.65°C) were within the range of long-term (1990–2013) temperatures measured during the natural flight periods for all species (mean ± *SD*; *O. cornuta* (March–May): 9.95°C ± 5.29, *O. bicornis* and *O. brevicornis* (April–June): 13.85°C ± 4.86). The monthly temperatures measured in the cages during the experiment were on average 0.93°C higher than temperatures measured at the regional climate station at the same time.

2.3 | Plants

We provided cages of *O. cornuta* and *O. bicornis* with *Prunus spinosa*, *Prunus avium*, *Pyrus* (spp.), *Prunus domestica*, *Sinapsis arvensis*, *Brassica napus*, *Crepis biennis*, *Matricaria chamomilla*, *Chrysanthemum segetum*, *Campanula glomerata*, *Campanula persicifolia*, *Campanula rotundifolia*, *Campanula rapunculoides*, *Campanula rapunculus* and *Helianthus annuus*. All plant species were visited by these two generalist bee species (M. Schenk, pers. obs., April–July 2014). Plant composition differed among survey rounds, but was standardized for the three cages within a survey round. *Osmia brevicornis*, a solitary bee species specialized on Brassicaceae, was exclusively provided with *Sinapsis arvensis* and *Brassica napus*. Flowering *Brassica napus* was collected from a nearby agricultural field and flowering branches of *Prunus* spp. and *Pyrus* spp. were cut in orchard meadows surrounding the study site. Seeds of the

other plant species were purchased from Rieger-Hofmann® GmbH (Blaufelden-Raboldshausen, Germany) and sown in spring 2013 and in spring 2014 respectively, depending on the plant species. We provided 50–70 flower pots of 17 × 17 × 17 cm size per cage. Each pot contained approximately 65 ± 19 (mean ± *SE*) blossoms. Flowering branches of *Prunus* spp. and *Pyrus* spp. were put inside three water buckets that were buried into the soil per species and cage. Each bucket contained approximately 1200 ± 94 (mean ± *SE*) blossoms. The surface of the water was covered with bottle corks to avoid drowning of bees. We checked the condition of plants inside the cages once per day. Plants with faded blossoms were exchanged immediately to maintain consistent flower supply. Cages belonging to the same survey round were provided with the same number of flower pots consisting of the same plant composition. Generally, each cage was filled with potted plants until its ground area was entirely covered with flowering plants (Figure 1b).

2.4 | Data recording

Bees were placed inside the cages at day 0 of each survey round. Plants were added—depending on the treatment—either on the same day (perfect synchronization) or 3 or 6 days later (temporal mismatch of 3 or 6 days, Figure 1c). For the analysis, we recorded three measures of bee fitness: the survival rate, an activity index and the reproductive output. For determining the survival rate and the activity index, we counted all visible active and non-active females every second day for 3 min per cage from outside the cages starting at day 6 of each survey round and continuing until the last bee in the cage had died. Females were considered to be active if they were flying, visiting the flowers, walking on the mesh tent or mating with males. Each individual was counted only once per observation date. This was ensured by determining the maximum number of active females that could be observed simultaneously. To calculate the variable "Survival rate (%)" per cage, we divided the maximum number of females observed in the cage on day 6 by the number of females placed in the cage at day 0 and multiplied the value by 100. To calculate the variable "Activity Index" per cage, we divided the number of active females observed in the cage at each observation day by the total number of females placed in that cage at day 0, and summed these values for each cage starting with day 6 of the survey round until the death of the last bee within that cage. To receive an index value between 0 and 1, we divided this value by the number of observation days. To investigate additionally whether activity changed over time and whether these changes differed among treatments, we split the observation dates into two halves (early activity: days 6–26, late activity: days 27–52).

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

were opened to record the number of brood cells. The sex of the offspring was determined after opening the cocoons that contained adult bees.

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

2.5 | Statistical analyses

For statistical analysis of the data, we used the software RStudio (R version 3.0.2) and the NLME package (Pinheiro, Bates, Debroy, & Sarkar, 2015). Models were calculated for each bee species separately. To detect differences in the survival rate, the activity index, the number of brood cells, the number of nests and the number of female offspring among treatments (synchronized vs. 3-day mismatch vs. 6-day mismatch), we used linear mixed-effects models with treatment as a fixed factor and survey round number as random factor. Treatments were compared using treatment contrasts (Crawley, 2007). To detect differences in the survival rate in relation

to temperature in cages with synchronized vs. cages with 3-day mismatch, we used linear mixed-effects models with treatment, temperature of the first 6 days and their interaction as fixed factors and survey round number as random factor. Cages with a mismatch of 6 days were excluded because too few females survived the first 6 days. To test the combined effects of time and treatment on the activity of bees, we used linear mixed-effects models with treatment, time period (early: day 6–26 vs. late: day 27–52) and their interaction as fixed factors and survey round number as random factor. Model residuals were inspected for violation of assumptions or normality and homoscedasticity.

3 | RESULTS

3.1 | Survival rates and activity

No specimen of the late-spring specialist *O. brevicornis* and very few individuals of the mid-spring generalist *O. bicornis* or the early-spring generalist *O. cornuta* survived a temporal mismatch of 6 days. This caused decreased activity of all three bee species after a temporal mismatch of 6 days in comparison to perfect synchronization. Mismatches of 3 days reduced the survival rate and the activity of both *O. bicornis* and *O. brevicornis* compared to perfect

TABLE 1 Results of linear mixed effect models testing differences among treatments. Shown are treatment contrasts for "perfect synchronization of bees and flowers" (0), "mismatch of 3 days" (3) and "mismatch of 6 days" (6). Dependent variables were the Survival rate (%) of females, the activity index of females, the number (No.) of brood cells, the number of nests and the number of female offspring per cage. *p*-values in bold indicate significant results (*p* < .05)

Dependent variable	Osmia cornuta			Osmia bicornis			Osmia brevicornis		
	df	t	p	df	t	p	df	t	p
Survival rate (%)									
0 vs. 3	15	−1.68	.115	19	−3.59	**.002**	8	−4.07	**.004**
0 vs. 6	15	−9.10	**<.001**	19	−6.65	**<.001**	8	−5.31	**<.001**
3 vs. 6	15	−7.42	**<.001**	19	−3.06	**.007**	8	−1.24	.250
Activity Index									
0 vs. 3	15	−0.29	.779	19	−4.20	**<.001**	8	−4.27	**.003**
0 vs. 6	15	−7.22	**<.001**	19	−7.82	**<.001**	8	−5.42	**<.001**
3 vs. 6	15	−6.94	**<.001**	19	−3.62	**.002**	8	−1.15	.283
No. of brood cells									
0 vs. 3	15	0.65	.523	19	−0.99	.332	8	−3.26	**.012**
0 vs. 6	15	−4.07	**.001**	19	−4.46	**<.001**	8	−4.66	**.002**
3 vs. 6	15	−4.72	**<.001**	19	−3.47	**.003**	8	−1.40	.198
No. of nests									
0 vs. 3	15	1.10	.289	19	−2.53	**.021**	8	−2.43	**.042**
0 vs. 6	15	−4.24	**<.001**	19	−6.13	**<.001**	8	−3.93	**.004**
3 vs. 6	15	−5.34	**<.001**	19	−3.60	**.002**	8	−1.51	.171
No. of female offspring									
0 vs. 3	15	−2.89	**.011**	19	−0.49	.633	8	−1.85	.101
0 vs. 6	15	−3.02	**.009**	19	−2.43	**.025**	8	−1.94	.088
3 vs. 6	15	−0.12	.910	19	1.95	.102	8	−0.09	.932
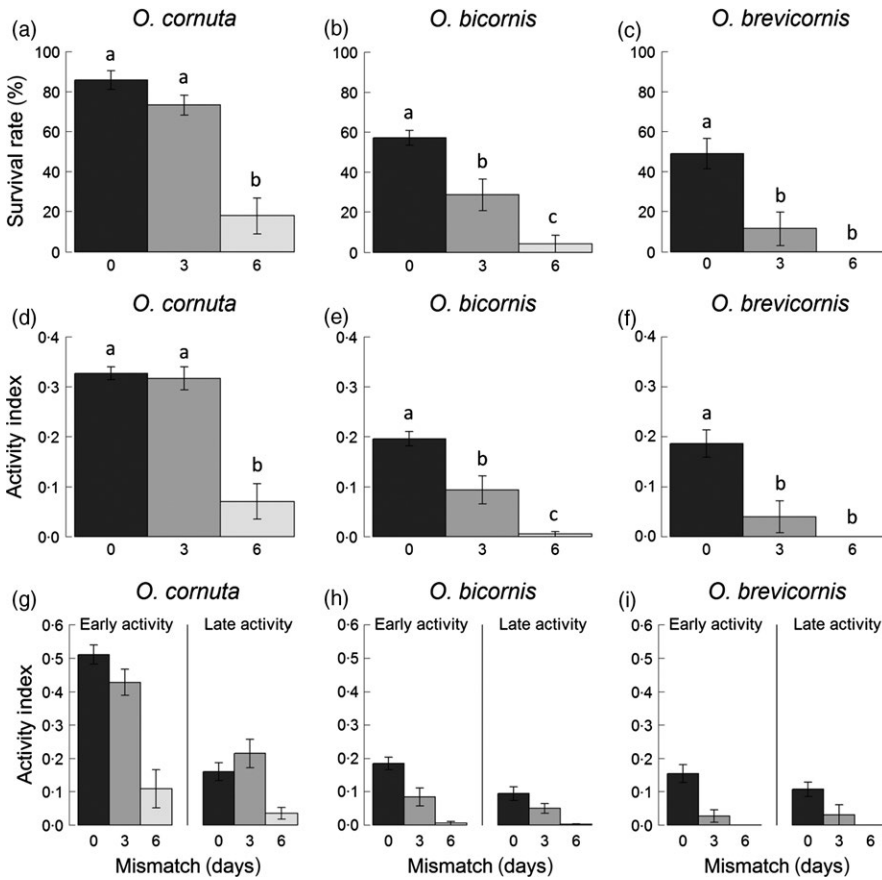
FIGURE 2 Influence of temporal mismatches on the survival rate per cage (a–c) and the activity per cage (d–i) of the females of three bee species. To calculate the variable "Survival rate (%)" per cage, we divided the maximum number of females observed in the cage on day 6 by the number of females placed in the cage at day 0 and multiplied the value by 100. To calculate the variable "Activity Index" per cage, we divided the number of active females observed in the cage at each observation day by the total number of females placed in the cage at day 0, and summed these values for each cage starting with day 6 of the survey round until the death of the last bee within that cage. To receive an index value between 0 and 1, we divided this value by the number of observation days. Depending on the treatment, bees emerged 0, 3 or 6 days before flowering onset. Different letters above bars ($M \pm SE$) indicate significant differences among treatments ($p < .05$)

TABLE 2 Interacting effects of treatment (perfect synchronization vs. mismatch of 3 days vs. mismatch of 6 days) and temperature (Temp) on the Survival rate (%) of females, and of treatment and time (early vs. late) on the activity of females. Results are calculated per cage and come from linear mixed effect models. p-values in bold indicate significant results ($p < .05$)

Dependent variable	Osmia cornuta				Osmia bicornis				Osmia brevicornis			
	ndf	ddf	F	p	ndf	ddf	F	p	ndf	ddf	F	p
Survival rate (%)												
Treatment	1	5	3.34	.127	1	7	15.66	**.005**	1	2	19.10	**.049**
Temp	1	5	2.66	.163	1	7	5.60	**.049**	1	2	0.12	.766
Treatment: Temp	1	5	0.24	.642	1	7	9.88	**.016**	1	2	7.71	.109
Activity Index												
Treatment	2	36	31.53	**<.001**	2	46	34.51	**<.001**	2	20	24.71	**<.001**
Time (early vs. late)	1	36	48.60	**<.001**	1	46	17.52	**<.001**	1	20	3.87	.063
Treatment: Time	2	36	6.85	**.003**	2	46	5.95	**.005**	2	20	4.24	.064

synchronization. The survival rate and activity of *O. cornuta* were not significantly affected after a mismatch of 3 days (Table 1; Figure 2a–f).

The effect of treatment (mismatch of 3 days vs. perfect synchronization) on survival rate of *O. bicornis* was temperature-dependent (Table 2; Figure 3b). Increasing temperature decreased the survival rate of *O. bicornis* after a mismatch, but not after perfect synchronization. The interaction between treatment and temperature was not

significant for the other two species, *O. cornuta* and *O. brevicornis* (Table 2; Figure 3a and c).

Activity was lower in the second half of adult life compared to the first half of adult life in all treatments in *O. cornuta* and *O. bicornis*, but we found a significant interaction between time of activity (first vs. second half of adult life) and treatment (perfect synchronization vs. temporal mismatch of 3 or 6 days; Table 2) for these two species. For *O. brevicornis*, this interaction was marginally significant. The
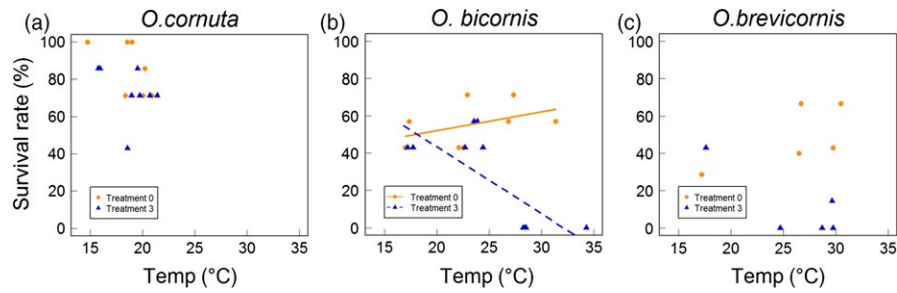
FIGURE 3 Influence of temperature (Temp) on the Survival rate (%) of females per cage of three bee species (a–c). Depending on the treatment, bees emerged 0 or 3 days before flowering onset. Cages with a mismatch of 6 days were excluded because too few individuals survived the first 6 days. Regression lines represent the results of linear mixed effect models in case of significant interaction [Colour figure can be viewed at wileyonlinelibrary.com]

interaction between time of activity and treatment shows that the decline of activity in the second half of adult life was smaller after a mismatch of 3 days than after perfect synchronization (Figure 2g–i). The late activity of O. cornuta was even enhanced after a mismatch of 3 days compared to perfect synchronization (Figure 2g), indicating that bees were able to recover after the mismatch. The activity of all species was generally highly reduced after a mismatch of 6 days.

3.2 | Reproductive output

The number of brood cells and the number of nests of O. brevicornis were reduced after a mismatch of 3 days and of 6 days compared

to perfect synchronization (Table 1; Figure 4c and f). The number of female offspring of O. brevicornis did not differ significantly among treatments (Table 1; Figure 4i). For O. bicornis, the number of brood cells and the number of female offspring were reduced after a mismatch of 6 days, while the number of nests was reduced after a mismatch of only 3 days (Table 1; Figure 4b, h, and e). The number of brood cells and the number of nests of O. cornuta were not significantly affected by a mismatch of 3 days, but were reduced after a mismatch of 6 days (Table 1; Figure 4a and d). The number of female offspring of O. cornuta was reduced after mismatches of both 3 days and of 6 days compared to perfect synchronization (Table 1; Figure 4g).
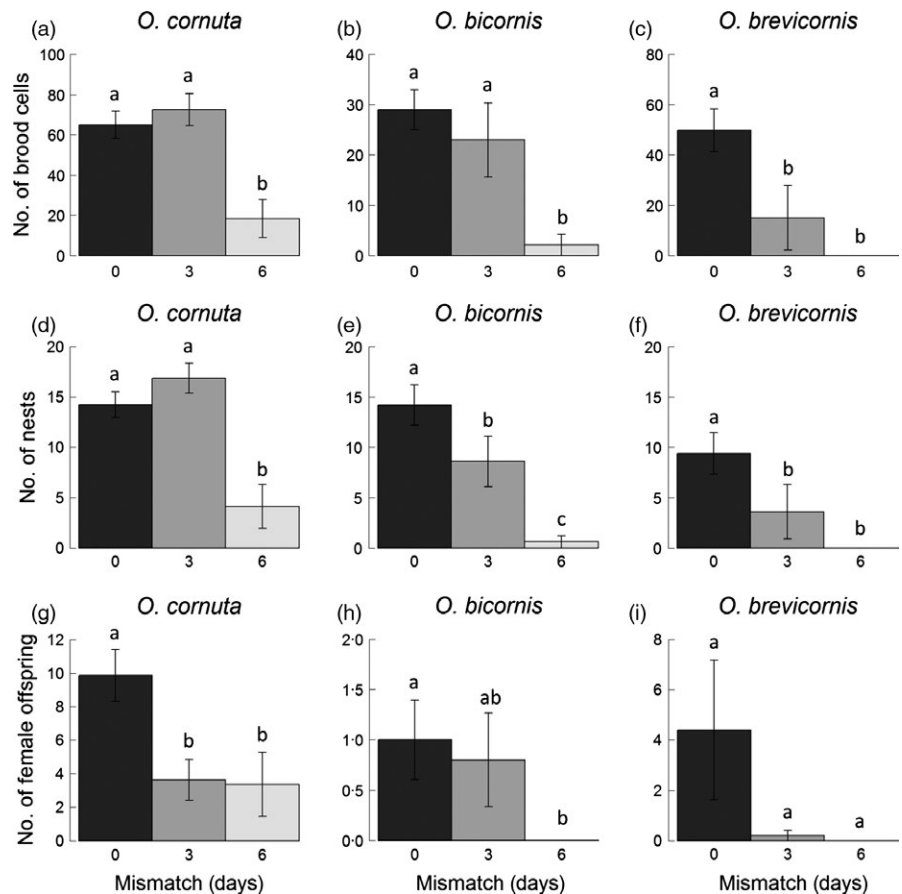
FIGURE 4 Influence of temporal mismatches on the number (No.) of brood cells per cage (a–c), the number of nests per cage (d–f) and the number of female offspring per cage (g–i) of three bee species. Depending on the treatment, bees emerged 0, 3 or 6 days before flowering onset. Different letters above bars (means ± SE) indicate significant differences among treatments (p < .05)

4 | DISCUSSION

Our study showed that in bee–plant interactions a temporal mismatch of 6 days caused tremendous fitness losses in all three bee species. No individual of the late-spring specialist *O. brevicornis* and very few individuals of the early- and mid-spring generalists *O. cornuta* and *O. bicornis* survived 6 days without flower resources. The low survival rates for all three bee species resulted in strongly reduced numbers of brood cells.

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

Although *O. cornuta* showed the same total activity after a temporal mismatch of 3 days as after perfect synchronization, its activity immediately after a temporal mismatch was reduced in comparison to synchronized bees. *Osmia cornuta* compensated for this decline in activity in the first half of life with increased activity in the second half of life. This indicates that *O. cornuta* was able to recover from a short temporal mismatch. Nevertheless, *O. cornuta* produced fewer female offspring after a temporal mismatch. The shift towards male offspring was not caused by a lack of mated females, as mating occurred on the first day of the experiment in all treatments. Females of solitary bee species are able to determine the sex and the size of each offspring depending on their individual condition (Rosenheim, Nonacs, & Mangel, 1996; Seidelmann, Ulbrich, & Mielenz, 2010; Wogin, Gillespie, Haye, & Roitberg, 2013). Females in poor condition produce fewer female offspring and shift the sex ratio towards the less costly sex (males in this case) (Trivers & Willard, 1973). Possibly due to this "making the best of a bad lot" strategy of females in poor individual condition (Fisher, 1930), female *O. cornuta* produced fewer female offspring after a temporal mismatch than after perfect synchronization. Consequently, we conclude that the early-spring generalist *O. cornuta* mitigates negative effects of a temporal mismatch of 3 days on reproductive output with relatively high activity levels towards the end of its lifetime, as well as by shifting the sex ratio towards male offspring to stabilize brood cell numbers. As females are the demographically limiting sex (Goulson et al., 2010), a reduced number of female offspring could lead to population declines.

Surprisingly, a mismatch of 3 days did not significantly reduce the number of brood cells produced by the mid-spring generalist *O. bicornis*, although its survival rate, activity and number of nests were reduced compared to synchronized bees. Our results suggest that *O. bicornis* was able to mitigate negative effects of reduced activity by distributing brood cells over fewer nests than under perfect synchronization. Searching for new nest cavities and learning the cavity position in orientation flights are costly in terms of time (Michener & Retten-Meyer, 1956; Miliczky, 2008; Rezkova, Zakova, Zakova, & Straka, 2012; Schönitzer & Klinksik, 1990). By decreasing the number of nests, bees may increase their efficiency and the number of brood cells that can be produced in a given amount of time. However, this strategy comes at a cost because it reduces protection against parasitoids and may increase offspring mortality in the nest. High parasitism risk is generally regarded as the main reason for construction of multiple nests, because distribution of brood cells over multiple nests decreases the probability that a natural enemy enters all brood cells of the female (Vinson & Frankie, 1988). Our results suggest that *O. bicornis* females have evolved a strategy that helps to stabilize brood cell numbers even if the environmental conditions are suboptimal. In populations with low parasitism risk, this strategy may compensate for fitness losses after short temporal mismatches. In populations with high parasitism risk, the fitness benefits of this strategy may be reduced by an increase in offspring mortality. This is equally applicable for other negative events, such as accidental damage to the nest, fungal infection and predation (e.g. by birds). But *O. bicornis* was able to use its mitigation strategy only under cold temperatures, because under warm temperatures, no females survived 3 days without plants. We showed that high ambient temperatures enhanced the negative effect of a temporal mismatch on the survival rate of *O. bicornis*. Temperature-dependent survival during starvation periods has also been documented for bumblebees and can be explained by more rapid metabolic function and concomitant higher overall energy expenditure in warm than cold conditions (Vesterlund & Sorvari, 2014). The temperature-independent survival rate of *O. bicornis* individuals in perfect synchronization with their food plants (meaning that energy intake was possible) suggests that not only overall energy expenditure but also overall energy intake is higher in warm than cold conditions. As warm temperatures enhanced the negative impact of temporal mismatches on the survival of *O. bicornis*, we conclude that increasing spring temperatures due to climate warming may have severe consequences for bee–plant interactions. However, this conclusion supposes that the phenological advancement of solitary bee species due to warming temperatures cannot keep pace with the increase in ambient temperatures.

The late-spring specialist *O. brevicornis* that experienced a temporal mismatch of 3 days produced fewer brood cells than under perfect synchronization. This finding reflects the result that its survival rate

and activity were reduced after a temporal mismatch of 3 days. *Osmia brevicornis* did not exhibit any observable strategies to mitigate fitness losses after temporal mismatches.

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

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

5 | CONCLUSIONS

Ours is the first study of how temporal mismatches in bee–plant interactions can affect the fitness of solitary bees. We showed that even short temporal mismatches of 3 and 6 days in bee–plant interactions (with solitary bee emergence before flower occurrence) can cause severe fitness losses in solitary bees. We detected different strategies by solitary bees to counteract impacts on their fitness after temporal mismatches. However, as these strategies may result in secondary fitness costs by a changed sex ratio or increased parasitism we conclude that compensation strategies do not fully mitigate fitness losses of bees after short temporal mismatches with their food plants. As bees showed strongly decreased survival rates after mismatches of 3 or 6 days, we assume

that bees are unable to use a "sit-and-wait-strategy" (Huang, Takahashi, & Dafni, 2002), a compensation strategy suggested for many plant species when pollinators are lacking. Bees may depend on the availability of nectar and pollen for survival and reproduction on a shorter time-scale than plants (Benadi, Hovestadt, Poethke, & Blüthgen, 2014). In the event of further climate warming, fitness losses after temporal mismatches may not only exacerbate bee declines but may also reduce pollination services for later-flowering species and affect populations of animal-pollinated plants. Several studies have focused on temporal mismatches in mutualistic interactions and on the question of whether these are more likely to occur due to further climate warming (Bartomeus et al., 2011; Burkle & Alarcon, 2011; Hegland et al., 2009; Parmesan, 2006), but we should also investigate the extent of resulting fitness losses of involved species (Colautti, Agren, & Anderson, 2017). This would make it possible for us to assess the impacts of temporal mismatches more accurately and to make more precise and even species-specific predictions. We suggest that the impacts of global warming on the persistence of mutualistic species interactions may prove to be more urgent and of greater magnitude than previously expected.

AUTHORS' CONTRIBUTIONS

A.H. and J.K. conceived the ideas; A.H. and M.S. designed the study; M.S. collected and analysed the data, and drafted the manuscript. A.H. and J.K. critically revised the manuscript and all authors approved the final version of the manuscript.

DATA ACCESSIBILITY

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.rm317 (Schenk, Krauss, & Holzschuh, 2017).

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