How vulnerable are pollen-specialist solitary bees to temperature-mediated shifts in the timing of food availability?

Gabriel Gauthier
Supervised by Dr. Jessica R. K. Forrest

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Department of Biology
Faculty of Science
University of Ottawa

Statement

This thesis has been prepared as a manuscript for publication, except for the following sections: Acknowledgements, Résumé, and Conclusion. To fulfil the requirements of the specialization in Environmental Sustainability, I briefly discuss policy implications in the conclusion.

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Proverbs 16:16 — How much better to get wisdom than gold, to get insight rather than silver

Abstract

Rising temperatures are advancing the seasonal timing of flowering and pollinator activity in numerous systems. However, if the phenologies of mutualistic partners shift at different rates, a change in temporal overlap may arise. We know that experimental bee–plant mismatch can reduce solitary bee fitness, but we remain unaware whether such mismatches occur in nature—or the extent to which bee–plant synchronization impacts bee populations in real-world conditions. I studied populations of *Osmia iridis*, a specialist solitary bee dependent on flowers of the plant tribe Fabeae, in the Colorado Rocky Mountains. I used a dataset on timing of bee emergence, bee reproductive output, flowering phenology, and site-level temperatures collected at multiple study sites between 2008 and 2019. I found that the timing of flowering of *Lathyrus lanszwertii*, the most abundant Fabeae species (which normally peaks after most bees have emerged), is more thermally sensitive than the timing of *O. iridis* emergence. As a result, *L. lanszwertii* phenology advances to a greater extent than *O. iridis* phenology at higher temperatures, leading to greater synchronization of bee emergence with flowering. Contrary to expectations, variation in synchrony between the partners does not predict bee per-capita reproductive output. The increased synchrony observed under warmer conditions is associated with higher bee reproductive output at the population level, but this effect is mainly driven by the highest-elevation site. This site experiences little nesting, likely because bees emerge too early relative to the flowers, thereby preventing large-scale colonization. At the other sites, bee populations seem largely insensitive to variation in the level of bee–plant synchrony. However, warming could eventually result in bees emerging after median flowering at certain sites—an

outcome that would likely be damaging for bee populations if they fail to adapt. Understanding the extent to which bees can tolerate shifts in synchrony is paramount given that both species have different phenological responses to temperature and that regional mean spring temperatures are rising quickly.

Résumé

L’augmentation des températures fait avancer la période saisonnière de floraison et d’activité des pollinisateurs dans de nombreux systèmes. Cependant, si les phénologies des partenaires mutualistes se déplacent à des rythmes différents, un changement de chevauchement temporel peut survenir. Nous savons qu’une incompatibilité (ou « mismatch ») abeille–plante expérimentale peut réduire l’aptitude phénotypique de l'abeille solitaire, mais nous ne savons toujours pas si de telles incompatibilités se produisent dans la nature, ou dans quelle mesure la synchronisation abeille–plante affecte les populations d'abeilles dans les conditions du monde réel. J'ai étudié des populations d'*Osmia iridis*, une abeille solitaire spécialisée qui dépend des fleurs de la tribu des Fabeae, dans les montagnes Rocheuses du Colorado. J'ai utilisé un ensemble de données comprenant le temps d'émergence des abeilles, le rendement reproductif des abeilles, la phénologie de floraison et les températures des sites, recueillies à plusieurs sites d'étude entre 2008 et 2019. J'ai trouvé que le temps de floraison de *Lathyrus lanszwertii*, l'espèce de Fabeae la plus abondante (qui atteint normalement son pic après l'émergence de la plupart des abeilles), est plus sensible à la température que le temps d’émergence d’*O. iridis*. Par conséquent, la phénologie de *L. lanszwertii* progresse plus rapidement que celle d'*O. iridis* à des températures plus élevées, ce qui permet de mieux synchroniser l'émergence des abeilles avec la floraison. Contrairement aux attentes, la variation de synchronie entre les partenaires ne permet pas de prédire le rendement reproductif par abeille. L’augmentation de la synchronie observée aux sites ayant des températures plus élevées est associée à une augmentation du rendement reproductif des abeilles au niveau de la population, mais cet effet est principalement

attribuable au site le plus élevé. Ce site connait peu de nidification, probablement parce que les abeilles émergent trop tôt par rapport aux fleurs, ce qui empêche une colonisation à grande échelle. Aux autres sites, les populations d’abeilles semblent insensibles aux variations du niveau de synchronisation abeille–plante. Cependant le réchauffement pourrait éventuellement faire émerger les abeilles après la floraison médiane à certains sites—un résultat qui serait probablement nuisible pour les populations d’abeilles si elles ne parviennent pas à s’adapter. Il est primordial de comprendre dans quelle mesure les abeilles peuvent tolérer les changements de synchronisme, étant donné que les deux espèces ont des réponses phénologiques différentes à la température et que les températures printanières régionales augmentent rapidement.

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Introduction

Climate change is shifting the timing of biological events in many organisms including plants, insects, birds and mammals (Inouye et al. 2000, Parmesan 2006, McKinney et al. 2012, CaraDonna et al. 2014, Rafferty 2017, Cohen et al. 2018, Kharouba et al. 2018). In numerous systems, phenologies are shifting earlier in the season with warming (Fitter and Fitter 2002, Donnelly et al. 2011, Rafferty and Ives 2011, CaraDonna et al. 2014, Thackeray et al. 2016). Changes in seasonal cues or drivers (e.g. temperature) may pose a threat to species that have historically relied on these cues for proper synchronization of biological events (Miller-Rushing et al. 2010, Forrest and Thomson 2011, Ovaskainen et al. 2013, Kudo 2014, CaraDonna et al. 2018). Phenological synchronization is particularly important for dietary specialists and mutualistic partners that rely on the co-occurrence of certain life history-events for survival (Forrest 2015, Rafferty et al. 2015). Pollinator–plant mutualisms are no exception. Phenological shifts that occur in parallel between wild bees and floral host-plants would likely have no impact on mutualistic interactions (Bartomeus et al. 2011, Ovaskainen et al. 2013). However, if one partner shifts more than the other, a change in temporal overlap between the partners could affect plant or bee fitness and population dynamics (Memmott et al. 2007, Hegland et al. 2009, Kudo and Ida 2013, Forrest 2015). If the change in temporal overlap improves synchrony between partners, one or both partners could benefit; but a reduction in temporal overlap could have detrimental effects on individual fitness and population dynamics, especially in the case of strict specialists (Miller-Rushing et al. 2010, Willmer 2012, Reed et al. 2013, Forrest 2015, Rafferty 2017, Farzan and Yang 2018, Schenk et al. 2018).

In many temperate environments, temperature is a key environmental predictor of both pollinator emergence and plant flowering (Doi et al. 2008, Hegland et al. 2009, Forrest and Thomson 2011, Olliff-Yang and Mesler 2018). Studies investigating the effects of climate variation on plant and pollinator phenologies have suggested that warmer springs may modify temporal overlap by differentially shifting phenologies of mutualistic partners (Aldridge et al. 2011, Kudo 2014). If mutualists have historically used temperature for the synchronization of life-history events, they may be particularly affected by climate warming if their physiological requirements do not perfectly coincide (e.g., if heat-accumulation requirements vary between species) (Forrest and Thomson 2011, Fründ et al. 2013). For example, if the phenology of a specialist bee is more sensitive than that of its floral host-plant to spring temperature variation, its emergence may shift earlier at a faster rate than host-plant flowering under climate warming, possibly resulting in bees emerging too early. By the time flowers become available, bees may be past their reproductive peak. They may experience reduced offspring production for reasons such as physiological senescence, physical wear, or exhaustion by the time flowers become available (cf. Moore and Moore 2001, O'Neill et al. 2015). On the other hand, if the phenology of the floral host-plant is more sensitive to temperature variation, flowering may shift earlier at a faster rate than the specialist bee's emergence phenology. If this leads to improved synchrony between the bees and their host-plant, warmer springs may favour bee reproductive output. However, if floral phenology advances to the point of preceding bee emergence, bees will miss much of the seasonal floral availability, resulting in reproductive losses for the bees. Some research points towards greater temperature-sensitivity of plant flowering phenology (Forrest and Thomson 2011, Kudo and Ida 2013, Kehrberger and Holzschuh 2019), while bee phenology appears to be more temperature-sensitive in other systems (Robbirt et al. 2014, Olliff-Yang and Mesler 2018). Overall, we know little about the ecological consequences of pollinator–plant desynchronization for pollinators (Hegland et al. 2009, Forrest 2015). Schenk et al. (2018) experimentally determined that bees deprived of access to floral resources in flight cages, even for a few days, suffer major reproductive losses and reduced survival. Furthermore, experimental shifts in bee phenology by Farzan and Yang (2018) demonstrated the importance of emergence timing in predicting fitness, including survival and offspring production in the field. These experiments are the first to explore the influence of phenological synchronization on bee fitness. However, because both studies artificially shifted bee phenology relative to plant phenology, it is not clear whether real-world climate change would alter pollinator fitness by modifying pollinator–plant synchrony in nature. To my knowledge, this is the first study to address this question.

I studied an oligolectic (dietary specialist) solitary bee species, *Osmia iridis*, that specializes on pollen from the plant tribe Fabeae (Fabaceae) (Forrest and Chisholm 2017). I tested whether variation in spring temperature predicts offspring production in *O. iridis* by driving variation in synchrony between the bee and its primary floral host-plant, *Lathyrus lanszwertii* (henceforth "*Lathyrus*"). For multiple study sites over several years, I determined median bee emergence dates and median flowering dates of the floral host, and tested whether the difference between these two dates ("bee precedence") predicted bee reproductive output in the field (see Figure 1A–B). Bee precedence is the number of days by which median bee emergence precedes median flowering at a site; it can be negative if median flowering precedes median bee emergence.

Earlier work on these species showed that phenological heat-accumulation requirements differed between *O. iridis* and *Lathyrus* and suggested that flowering time of the plant was more sensitive than emergence time of the bee to temperature variation (Forrest and Thomson 2011). I thus predicted a negative relationship between spring temperature and bee precedence (Figure 1C). I also predicted a negative quadratic relationship between bee precedence and bee reproductive output, reflecting some optimal level of bee precedence and reduced offspring production when bees emerge too early or too late relative to the plant (Figure 1D). I did not expect a bee precedence of 0 (representing perfect synchrony between bee emergence and flowering) to be optimal because, in this scenario, by the time the average bee emerges and finds a mate and suitable nesting location, over 50% of flowering at the site would have already occurred. I evaluated my predictions using field data on 226 individual bees and I use my results to forecast likely outcomes for *O. iridis* populations under future warming scenarios.

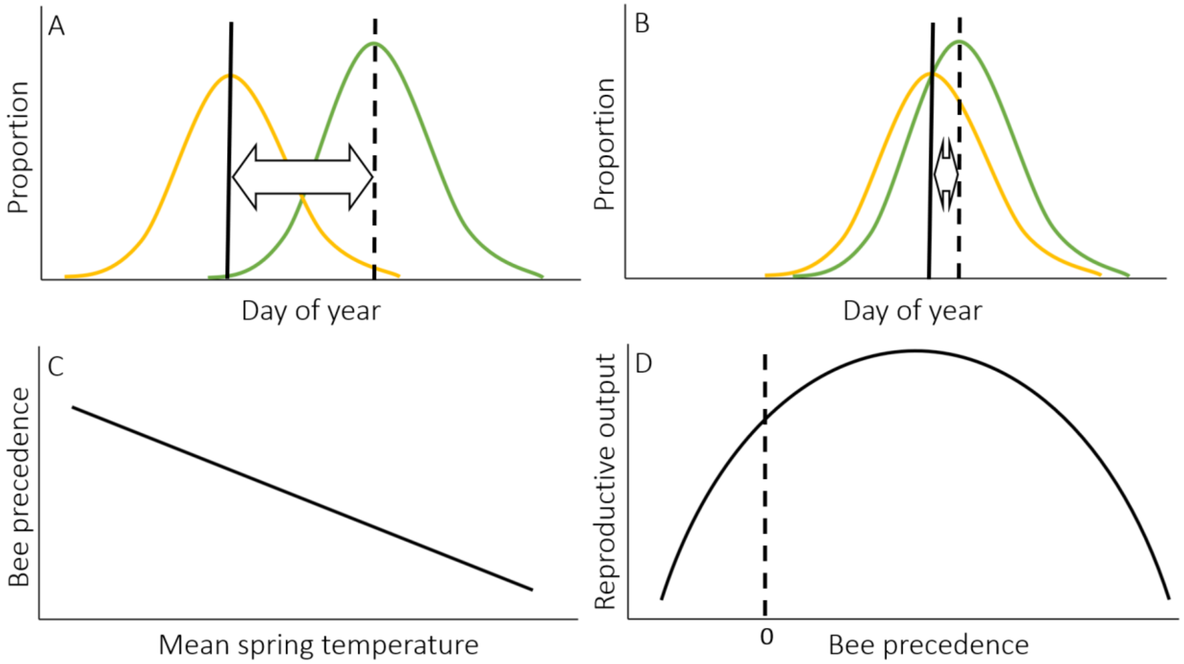

Figure 1. (A, B) Bee precedence is defined as the number of days by which median bee emergence (solid line) precedes median flowering (dashed line) as shown by the double-sided arrow. Bee emergence phenology is drawn in yellow, floral phenology in green. Bee precedence is high in (A) and low in (B). (C) I predicted a negative relationship between mean spring temperature and bee precedence because Forrest and Thomson (2011) predicted greater temperature sensitivity in *Lathyrus* phenology than *O. iridis* phenology. (D) I predicted a negative quadratic relationship between bee precedence and *O. iridis* reproductive output given the hypothesis of a reproductive maximum. High bee precedence could reduce reproductive output through reproductive senescence and old age by the time flowers become available; low bee precedence could reduce reproductive output if bees emerge when flowering is nearly finished and there is little food left for reproduction.

Methods

Study system and approach

Osmia (Hapsidosmia) iridis Cockerell & Titus (Hymenoptera: Megachilidae), a solitary mason bee native to western North America, collects pollen almost exclusively (>95%) from the plant tribe Fabeae (Fabaceae) (Forrest and Chisholm 2017). In my study area, this legume tribe is only represented by two plant species: *Lathyrus lanszwertii* (Kellogg), and the less common *Vicia americana* Muhl. Ex Willd. ("*Vicia*"). Flowers of both plant species are visited by many other species of bees including *Bombus* spp. During the spring and early summer, *O. iridis* females find linear above-ground cavities in dead wood in which to nest (Figure 2). Females work independently on one nest cell at a time. A cell consists of an egg laid on top of a lump of pollen and nectar and is sealed with walls of mud and leaf pulp (Cane et al. 2007). A nest generally consists of several successive cells and an outer vestibulum (an empty cell), protected by a thick outer wall. Females can construct several nests in sequence throughout the reproductive season. Eggs hatch and the larvae consume the pollen-and-nectar provisions over the course of the summer. *Osmia iridis* females in our study area typically exhibit a two-year life-cycle, emerging as adults two summers after hatching from the egg, although some will occasionally emerge a year earlier or later (Forrest et al. 2019).

Figure 2. (A) Female *O. iridis* building brood cells in a paper tube. (B) *Lathyrus lanszwertii*, commonly known as the Nevada sweet pea, in flower. (C) Artificial nesting habitat with 10 cavities allowing for 10 potential nests. (D) Virginia Basin, a high-elevation site (3440 m) on a steep incline.

My goal is to determine whether springtime temperature indirectly affects bee reproductive output by modifying temporal overlap between *O. iridis* and *Lathyrus*. I focus on the interaction between *O. iridis* and *Lathyrus* exclusively because of the far greater abundance of *Lathyrus* (80% of the seasonal Fabeae availability over the period 2014–2019, Table S1) than *Vicia*. Over the years, the Forrest lab has collected data on *O. iridis* emergence phenology (10 sites in 2009, 2010, 2018 and/or 2019) and per capita reproductive output (7 sites between 2014 and 2019), *Lathyrus* flowering phenology (16 sites in 2008, 2009 and/or 2014–2019), and site-specific hourly temperatures in all cases. Some of these data (2008–2015) were used in previously published analyses of emergence and flowering phenology (Forrest & Thomson 2011) and of bee reproductive output (Forrest & Chisholm 2017). Here, using the sites and years for which I had emergence data, I first generated a phenological model to predict the timing of emergence of female *O. iridis* using seasonal heating unit (degree-day) accumulation; this allowed me to estimate median bee emergence dates for all site–years. Forrest and Thomson (2011) demonstrated the importance of heat-accumulation relative to other cues in predicting bee emergence and flowering phenologies in my research area. They created similar models for numerous species, including for *O. iridis* and *Lathyrus*—all 2008–2010 data are drawn from this study. Median *Lathyrus* flowering dates were determined empirically for all site–years. Bee precedence—the number of days by which median bee emergence precedes median flowering—was used as a proxy for temporal overlap and was determined for all site–years (see Appendix A1 for a discussion of overlap metrics and to visualize actual temporal overlap scenarios). Then, using among-year and among-site (primarily elevational) variation in temperature, I tested for a relationship between expected bee precedence and mean spring temperature. Finally, I tested the relationships between expected bee precedence and (i) per capita and (ii) site-level (i.e., summed across all individuals at a site) bee reproductive outputs. I used per capita and site-level reproductive outputs to discriminate between individual fitness and population demography; these attributes may respond differently to phenological change (cf. Reed et al. 2013). Detailed methods are described below.

Study sites

Data were collected at study sites established along the edge of natural subalpine meadows around the Rocky Mountain Biological Laboratory (RMBL; Crested Butte, Colorado, USA). This study area typically experiences long, snowy winters, early-summer droughts, and late-summer monsoons. Sites were established between 2007 and 2014 along an elevational gradient (see Table S2 for details about each site's location, elevation, and the type of data collected). At each site, 10 to 24 softwood nesting blocks ("trapnests") were established to track bee emergence phenology and individual reproductive progress (Figure 2). Nesting blocks were attached to trees (aspen or spruce, usually dead or dying), 0.2–1.5 m above ground, facing meadows with abundant wildflowers. Each nesting block contained 10 cavities (thus, 10 potential nests per block), each approximately 14 cm deep with diameters ranging from 6.4 to 9.5 mm. Hardboard roofing on each block increased resistance to precipitation. Translucent paper straws were inserted in each cavity. Bees built nests in these straws, which allowed observers to briefly remove nests from blocks to record the number of brood cells completed by each bee throughout the season. A shaded HOBO pendant data-logger (Onset Computer, Bourne, Massachusetts, USA; accuracy ±0.5°C) was fastened underneath a block near the centre of every site to record hourly temperatures. Sites were visited every 3 to 8 days, with more frequent visits (every 3–4 days) during peak *O. iridis* activity and *Lathyrus* flowering at most sites.

Bee emergence phenology

Bee emergence dataset – Emergence date was recorded for 253 female bees in total: 2 at one site in 2009, 175 at six sites in 2010, 20 in ten experimental emergence boxes (see below) in 2018, and 56 at five sites in 2019. In all cases, site-specific hourly temperatures were recorded. In 2009, 2010 and 2019, bees emerged in traps (plastic vials) attached to the front of their nests in the field. Traps were checked semi-weekly to weekly for new emergence, depending on site and year. Emergence dates were inferred using bee status (alive or dead) and the number of days elapsed since the last visit (e.g. dead bees had likely emerged earlier than bees found alive at the time of observation and were thus assigned an earlier emergence date). For detailed methods regarding the 2009 and 2010 bees, see Forrest and Thomson (2011); the same methods were used in 2019. See Figure S1 for actual emergence phenology curves at six sites. In 2018, I systematically divided 64 *O. iridis* cocoons in 10 groups; each cocoon was placed in a separately sealed petri dish and originated from nests constructed at field sites in 2015 or (mostly) 2016. All cocoons had previously experienced the same overwintering conditions in a plastic container equipped with a HOBO recording hourly temperatures, located in a shaded outdoor enclosure. For emergence, each group of cocoons was placed in a separate HOBO-equipped wooden box (Figure S2). These boxes were exposed to a range of temperature regimes starting on 15 May 2018 by using natural variation in sunlight exposure along the north, south, east and west sides of buildings at the RMBL. Cocoons were checked daily in the evening for new emergence. Emerged bees were frozen and later pinned to confirm species identity and sex. On 13 June 2018, when most bees had emerged, wooden boxes were aggregated and placed in full sun for two additional weeks to promote the emergence of any remaining bees. Out of the 64 cocoons, 20 females emerged—the other cocoons produced males or no emergence for various reasons (e.g. they were parasitized, dead or immature).

Best bee emergence model and median dates – I defined median bee emergence date as the date by which 50% of a site's female bees have emerged; I estimated these by fitting a logistic model to emergence data (as in Forrest and Thomson 2011). Forrest and Thomson's (2011) best phenology model for *O. iridis* suggested that median emergence required approximately 114 degree-days of accumulated heat above a base temperature of 8°C, starting on 11 May. In this model and all other phenological models described herein, degree-days are defined as the cumulative sum of hourly heating units beginning after the threshold date (e.g. 11 May) divided by 24 (the number of hours in a day). An hourly heating unit equals that hour's temperature reading (T) minus the threshold temperature (T_{thresh}; e.g., 8°C) if $T>T_{thresh}$; otherwise, "0" heating units were assigned (see Figure S3 for a visual example of degree-day accumulation). Forrest and Thomson's (2011) *O. iridis* model included both males and females. However, because only the phenology of females is directly relevant to a bee population's reproductive output, I modelled the phenology of females alone, using data from all emerged females from 2009, 2010, 2018 and 2019. I determined which heat-accumulation start date and base temperature combination best fit the female emergence data by modelling daily bee status (0 = non-emerged, 1 = emerged) for all bees from day of year 144 to 182 (the period covering all emergences +/- 1 day) as a function of cumulative degree-days using generalized linear mixed-models with random intercepts (glmer, from the lmerTest package in R; Bates et al. 2015, Kuznetsova et al. 2017), with the composite variable “site–year” as a random term with 22 levels (as in Forrest and Thomson 2011). My glmer models could not accommodate an additional ‘bee ID’ term nested within site-year; however, this should not affect model selection because of the equal representation of all bees in each model (39 days/bee). I determined the best logistic model by selecting the one that had the lowest AIC score from a pool of 667 models testing every start date and base temperature combination from day of year 1 (1 Jan) to day of year 141 (21 May) at 5-day intervals, and from –10°C to 12°C at 1°C intervals (Table A2–1). Based on this analysis, the best phenology model for female *O. iridis* emergence predicts median bee emergence at approximately 227 degree-days above a base temperature of 4°C, beginning on day of year 126 (6 May, $R^2_m = 0.81$; $R^2_c = 0.90$, Figure 3). The robustness of this model relative to other models is evaluated in Appendix A2, where I also discuss an effect of year on emergence phenology. Median bee emergence dates for each site and year were then obtained by converting site-specific hourly temperatures to degree-days and determining the date on which 227 degree-days have accumulated (beginning 6 May). I estimated median bee emergence dates for a total of 52 site–years between 2008 and 2019—all site–years for which empirical *Lathyrus* median flowering dates were available (see Table S2). Although this modelling approach carries its own limitations, it allows me to estimate site-specific *O. iridis* median emergence dates using only site-specific temperature data (Appendix A2, Forrest and Thomson 2011).

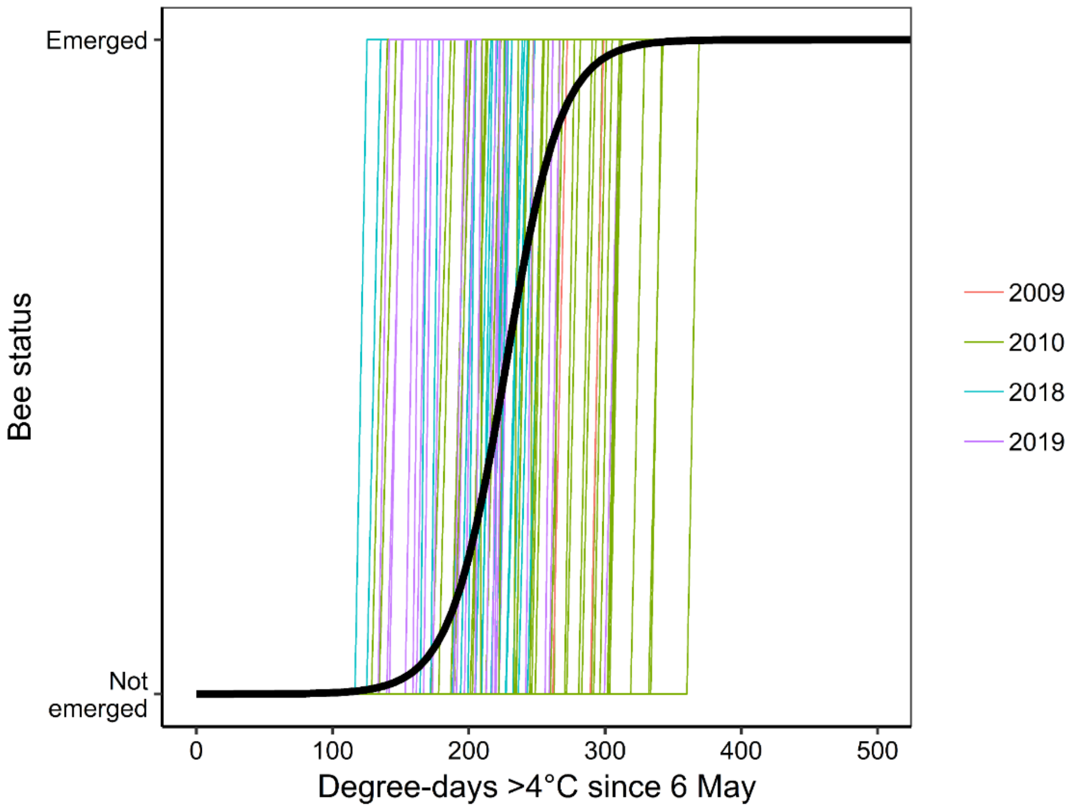

Figure 3. The phenological model that best predicts female *O. iridis* springtime emergence suggests that 227 degree-days of heat above 4°C starting on day of year 126 (6 May) are required to reach median emergence. This model had the lowest AIC score from a pool of 667 models testing every start date and temperature combination from day of year 1 (1 Jan) to day of year 141 (21 May) at 5-day intervals, and from −10°C to 12°C at 1°C intervals. Coloured lines represent the emergence profiles of individual bees that emerged in the field in 2009 (N=2), 2010 (N=175), 2019 (N = 56), or in experimental emergence boxes experiencing natural variation in sun exposure in 2018 (N=20). The black curve represents the best-fit logistic mixed-effects model. See text for details.

Floral phenology

Field methods – *Lathyrus* floral availability was recorded, typically twice per week, at most sites since 2008 (see Table S2). However, the method for counting flowers varied across years. In 2008 and 2009, open *Lathyrus* flowers were counted within fixed belt transects (Forrest and Thomson 2011). Floral density was obtained by dividing the total flower count by the total surveyed area. Beginning in 2013, *Lathyrus* density was estimated by walking in progressively larger circles in a spiral-like motion starting at the site centre and counting to 100 open flowers (as in Forrest and Chisholm 2017). Density was obtained by dividing 100 flowers by the circular surveyed area (πr^2). In 2018, in addition to the "spiral counts", four belt transects were set up at each site as in 2008 and 2009 (Forrest and Thomson 2011) to ensure that pre-2013 and post-2013 methods for obtaining floral densities gave comparable results. Both methods yielded similar phenology curves (see Figure S4), and I gave preference to the spiral-search method in 2018 for consistency. In all cases, the surveyed area ended 100 m from the site centre for feasibility and because *O. iridis* are unlikely to forage much past this distance: solitary bees with body sizes comparable to *O. iridis* typically forage within this radius (Zurbuchen et al. 2010).

Median dates – I used the loess function in R (R Core Team 2018) to interpolate daily floral densities from semi-weekly to weekly density estimates. I adjusted the function's span and degree parameters to 0.5 and 2 because these gave the best visual fit to the actual densities (see Figure S4). I determined median flowering date for each of the 52 site–years as the day of year on which the seasonal cumulative daily floral density exceeded 50%.

Best flowering model – Forrest and Thomson's (2011) best phenological model for *Lathyrus* suggested that 579 degree-days above a threshold temperature of 0°C, beginning 21 April, are required to reach median flowering. I re-fit this model, using all 2008–2018 *Lathyrus* phenology data, to verify that mid-April is the period when heat-accumulation becomes important for predicting *Lathyrus* phenology; this information is necessary for choosing an appropriate definition of 'mean spring temperature' (used in subsequent analyses). I fit the cumulative proportion of flowering to degree-day accumulation using the same approach as with *O. iridis*, although this time treating empirical site–year cumulative seasonal flowering proportions (rather than bee status) as the response variable. The best-fit model for *Lathyrus* flowering suggests that 457 degree-days above a base temperature of 2°C, beginning on day of year 101 (11 April), are required to reach median flowering (Figure S5)—confirming that heat-accumulation beginning in mid-April is important in predicting *Lathyrus* phenology. The goodness-of-fit for this model was tested in Appendix A2, where I also show that *Lathyrus* and *O. iridis* differ significantly in the nature of their phenological responses to temperature, based on their respective phenological models.

Bee reproductive output

Field data collection – At every site visit (1–2 times per week), each cavity was checked for nesting progress. When an *Osmia* nest was discovered, an observer waited for the bee to return to the nest to determine species identity and to place a unique enamel paint-mark combination on its mesosoma (or to determine if the bee had previously been marked). Visual inspection was generally sufficient to determine whether the bee was *O. iridis*; however, pollen samples were taken from every nest (as in Forrest and Chisholm 2017) to confirm that the pollen indeed originated from the plant tribe Fabeae—thus validating bee species identity. In addition, every *O. iridis* brood cell was opened (as in Forrest and Chisholm 2017) to check for the presence of a host egg and any parasite (*Sapyga* sp.) eggs. Observers attempted to attribute each nest to an individually-marked bee. Nests that were initiated and completed entirely between two site visits were recorded as having been constructed by an unknown female unless a reasonable inference could be made (e.g. if a marked bee had completed a nest in the same nesting block just before the appearance of the "unknown" nest and proceeded to construct another nest in the same block immediately after).

Per capita reproductive output – Potential per capita reproductive output was calculated for each bee by summing all completed brood cells containing host eggs (parasitized and non-parasitized) for each individually marked bee. Similarly, realized per capita reproductive output was calculated for each bee by summing only non-parasitized cells with host eggs. In both cases, "unknown" bees were omitted. While some marked bees may have completed other cells in natural cavities before or after completing nests in artificial habitats, I assume that the likelihood of such occurrences was similar across sites and years. Hence, although my definition of "per capita reproductive output" may underestimate true lifetime offspring production, this measure allows for comparisons among site–years. Per capita reproductive output is used as a proxy for bee fitness.

Site-level reproductive output – I also calculated total site-level reproductive output by summing all parasitized and non-parasitized brood cells (= potential reproductive output) and all non-parasitized brood cells (= realized reproductive output) produced by all *O. iridis* individuals (including "unknown" individuals) for each site–year. However, because the number of available cavities at each site varied somewhat among years and sites but was typically 120 in recent years (Table S3), I calculated a standardized measure of site-level reproductive output by dividing the total number of cells by the total number of cavities available at the beginning of the season and then multiplying by 120. Site-level reproductive output is used as an indicator of population success.

Temperature calculations

Mean spring temperature – Site-specific hourly temperatures were averaged between day of year 105 and day of year 196 (15 April and 15 July in common years) to obtain mean spring temperature. I focussed on this date range because the *Lathyrus* phenological model suggests that heat-accumulation becomes important around mid-April (11 April, Figure S5), and because all bees accumulate heat (beginning 6 May) and emerge within this mid-April to mid-July period. *Lathyrus* continues to bloom past mid-July in some site–years.

Long-term climate trends – I used air temperature data from a National Oceanic and Atmospheric Administration (NOAA; Climate Data Online: https://www.ncdc.noaa.gov/cdo-web/) weather station located approximately 5 km from the nearest study site (GHCND: USC00051959, Crested Butte, CO) to quantify temperature trends between 1975 and 2018. I calculated mean annual temperatures by averaging all daily temperature minima and maxima throughout the year. I also calculated mean spring temperatures by averaging minima and maxima between days of year 105 and 196 (April 15 and July 15 in common years). Years 1977, 1978, 1979, 1981 and 2009 were omitted due to missing data. I interpolated either minimum temperatures, maximum temperatures, or both in a total of 53 dates for which observations were missing across all 39 remaining years. I tested for a linear relationship between year and mean annual temperature, and between year and mean spring temperature, using the lm function in R (R Core Team 2018).

Analysis

Phenology and temperature – I tested for a relationship between (i) median bee emergence date (inferred from the *O. iridis* phenology models) and site mean spring temperature, and (ii) median flowering date and temperature, using the lmer function of the lmerTest package in R (Bates et al. 2015, Kuznetsova et al. 2017). Site and year were included as random variables. I also tested for an interaction between mean spring temperature and the categorical variable "species", again using the lmer function, to determine whether *O. iridis* emergence and *Lathyrus* flowering respond differently to variation in spring temperature. I further tested the relationship between bee precedence and temperature using the lmer function and included site and year as random variables. All marginal and conditional R-squared values (R^2_m and R^2_c) were obtained using the r.squaredGLMM function from the MuMIn package in R (Barton 2019) and are reported in Table S4.

Synchrony and bee reproduction – I tested for a relationship between potential per capita reproductive output and bee precedence, and between potential site-level reproductive output and bee precedence. I repeated the same analyses with realized (non-parasitized only) reproductive outputs. Reproductive outputs were square-root-transformed to improve the normality of residuals. In all models, I again used the lmer function of the lmerTest package in R and included site and year as random variables. Bee precedence was included as the variable poly(bee precedence,2) in all models because of my expectation of a negative quadratic (second-degree) relationship (i.e. I expected that a certain degree of bee precedence would lead to a reproductive maximum). When the second-order bee precedence term was non-significant, I re-ran a simpler model with bee precedence as a first-order term only and checked for significance. To determine the relative importance of direct vs. indirect (via bee precedence) effects of temperature on bee fitness and population success, I tested the same four models as described previously, but with temperature as a first-order term only replacing bee precedence. R^2_m and R^2_c values were extracted from every model (Table S5).

Results

Long-term climate trends

Both mean annual temperature and mean spring temperature are rising in the study area (Annual: t_{37} = 3.95; P = 0.0003, Spring: t_{37} = 3.35; P = 0.0019, Figure S6). Between 1975 and 2018, mean spring temperatures have risen by 1.7°C on average and will reach 3°C by 2050 if this trend continues.

Phenology and temperature

The phenologies of both *O. iridis* and *Lathyrus* are strongly predicted by mean spring temperature (Figure 4A; inferred median *O. iridis* emergence: $t_{48.2} = -21.35$, $P < 2\times10^{-16}$, R^2_m = 0.87; observed median *Lathyrus* flowering: $t_{25.8} = -15.03$, $P < 3\times10^{-14}$, R^2_m = 0.82; Table S4). With increasing spring temperatures, both species' phenologies shift earlier in the season, with median bee emergence generally preceding median flowering. *Lathyrus* is more sensitive to spring temperature variation than is *O. iridis* ($t_{91.9}$ = 4.05, P = 0.0001). Consequently, as spring temperatures increase, bee precedence is reduced (Figure 4B; $t_{21.3} = -2.99$, P = 0.007, R^2_m = 0.17), meaning that median *Lathyrus* flowering is shifted closer to median *O. iridis* emergence in sites and years experiencing higher spring temperatures.

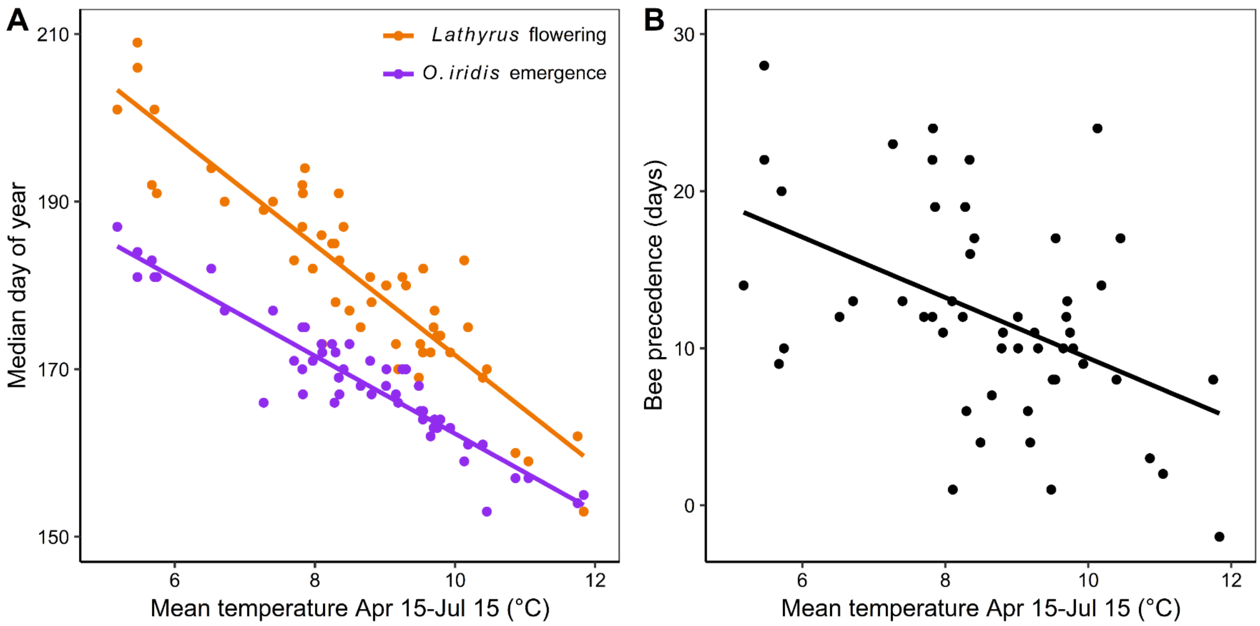

Figure 4. (A) The relationship between median *Lathyrus* flowering date (orange), median *O. iridis* emergence date (purple), and mean hourly temperature between days of year 105 and 196 (15 April and 15 July). All temperature and interaction coefficients in the linear mixed-effects models are significant (*P* <0.001). N=52 site–years. (B) The relationship between bee precedence and mean spring temperature at the same site–years. Bee precedence is the difference between the median *Lathyrus* flowering date and median *O. iridis* emergence date. The temperature coefficient in the linear mixed-effects model is significant (*P* = 0.007) and negative (Table S4).

Synchrony and bee reproduction

The significance of the relationship between bee precedence and reproductive output depends on the scale at which the latter is measured. First, none of the second-order bee precedence terms were significant—indicating a lack of support for a negative quadratic relationship (Table S5). I therefore reran models with bee precedence as a first-order term only; these are the results I report here. I found no significant association between bee precedence and per capita reproductive output (potential per capita reproductive output: $t_{14.2} = -0.98$, $P = 0.34$, $R^2_m = 0.006$, Figure 5A; realized per capita reproductive output: $t_{14.9} = -0.90$, $P = 0.38$, $R^2_m = 0.005$, Figure S7A). Reduced bee precedence is associated with increased site-level reproductive output (potential site-level reproductive output: $t_{29.6} = -3.60$, $P = 0.0011$, $R^2_m = 0.25$, Figure 5B; realized site-level reproductive output: $t_{29.8} = -3.71$, $P = 0.0008$, $R^2_m = 0.26$, Figure S7B); however, removing our highest-elevation site ('VB') renders the trend non-significant (Table S6).

Temperature, like bee precedence, does not predict per capita reproductive output (Figure S9A–B). However, temperature is positively correlated with site-level reproductive output in a model that also accounts for site and year (potential: $t_{15.7} = 2.71$, $P = 0.016$, $R^2_m = 0.24$, Figure S9C; realized: $t_{15.9} = 2.89$, $P = 0.011$, $R^2_m = 0.26$, Figure S9D); this trend also becomes non-significant when the highest-elevation site is removed (Table S6).

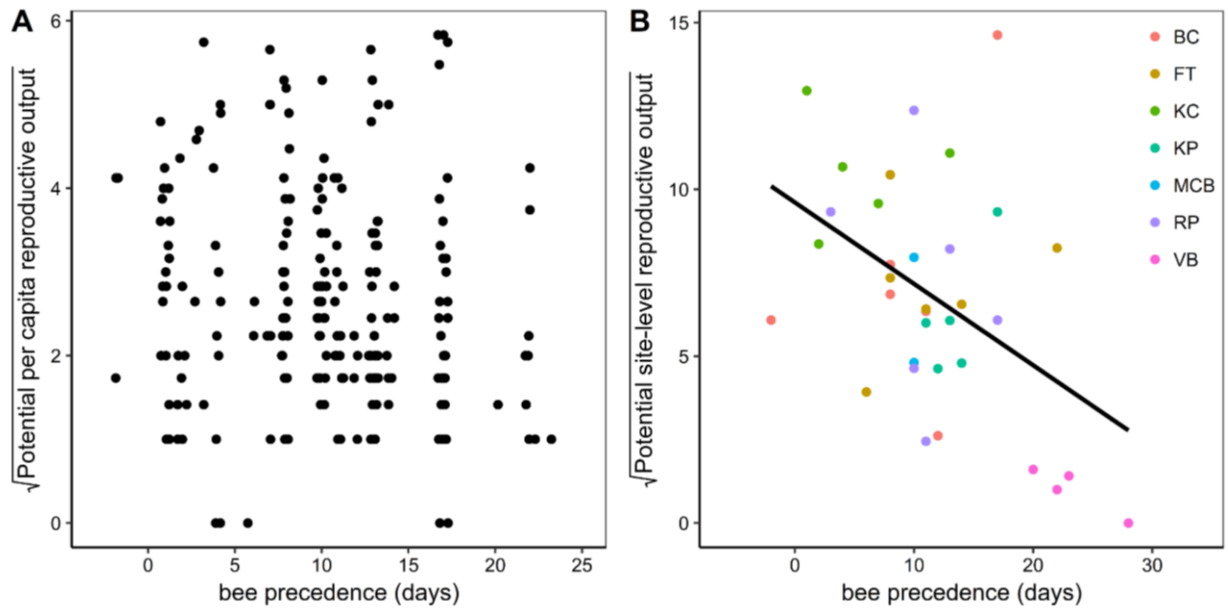

Figure 5. The relationship between potential *O. iridis* reproductive output and bee precedence—the number of days by which median *O. iridis* emergence precedes median *Lathyrus* flowering. Potential reproductive outputs include all brood cells containing a host-egg, whether parasitized or not. Reproductive outputs were square-root-transformed to improve normality. (A) Per capita reproductive outputs. N = 226 bees. The bee precedence coefficient in the linear mixed-effects model is non-significant (*P* = 0.34). Points jittered by up to 0.3 days for clarity. (B) Site-level reproductive outputs standardized to a 10 cavity per trapnest x 12 trapnest per site design. Points are colour-coded by site. Overall, with increasing bee precedence we observe reduced site-level reproduction. N=34 site–years; *P* = 0.001 (for the bee precedence coefficient in the linear mixed-effects model). See Figure S7 for realized reproductive outputs and Figure S8A for panel B colour-coded by year.

Discussion

Following global trends, temperatures in my research area are rising (IPCC 2014, Figure S6). Warmer sites and years experienced earlier emergence of the solitary bee *O. iridis* and earlier flowering of its primary host-plant, *Lathyrus*. Using data from 14 sites, each monitored for up to 8 years, I determined that *Lathyrus* phenology is more thermally-sensitive than *O. iridis* phenology, leading to a narrowing of the gap between bee and flower median dates at higher temperatures. Bee precedence does not predict per capita *O. iridis* offspring production; however, lower bee precedence is associated with increased offspring production by *O. iridis* at the site level. Decreasing the gap between the two median dates thus appears to promote population success, although this trend only holds if we include our coolest and highest-elevation site ('VB'), which experiences overall high bee precedence and little *O. iridis* nesting. My results suggest that well-established *O. iridis* populations will be insensitive to rising temperatures as long as bee precedence remains positive (i.e. median bee emergence occurs before median flowering). Rising temperatures would likely benefit *O. iridis* at high-elevation sites like VB by reducing the extent to which their emergence precedes flowering of their floral host-plant. Below, I elaborate on these findings and discuss what they mean for the future of this plant–pollinator interaction.

The indirect effect of temperature on bee fitness

I expected greater temperature sensitivity in the phenology of *Lathyrus* because of its lower threshold for heat-accumulation (0^{o}C) than *O. iridis* (8^{o}C) as presented by Forrest and Thomson

(2011). Higher and more frequent spring temperatures within the range of 0°C to 8°C would therefore advance the phenology of *Lathyrus* faster. In my analyses, I found that the difference in heat-accumulation thresholds between the two species is much smaller than was previously noted (2°C vs. 4°C), probably partly due to the exclusion of *O. iridis* males in the new phenology model; *Osmia* spp. phenologies can be sex-specific, as is the case for this species (i.e. males tend to emerge earlier than females). However, *Lathyrus* tends to begin heat-accumulation much earlier than *O. iridis* (11 April vs. 6 May), as was also found by Forrest and Thomson (2011). This means that warming within this period can advance the phenology of *Lathyrus* without advancing the phenology of *O. iridis*, which can drive greater apparent temperature sensitivity in the plant than in the bee.

Bee per capita reproduction appears to be insensitive to changes in bee–plant synchronization, likely thanks to the large variability in emergence dates within a site: bees can emerge over a period spanning >20 days (Figure S1). Regardless of the level of bee precedence of a site as a whole, some bees will emerge at a better time than other and therefore benefit reproductively. The variability in emergence dates could be driven by within-site variability in climatic conditions (e.g. snow cover varies with nest height, sun exposure varies by location; both can affect nest temperature and thus emergence timing within a site), by body condition (e.g. bees in better condition may emerge earlier), by a bet-hedging strategy, or even by genetic polymorphism. If I had been able to document emergence dates of individual bees, I might have observed the expected negative quadratic relationship between an individual's phenology and its reproductive output (cf. Reed et al. 2013). Although plotting per capita bee precedence and per capita offspring production would be interesting, data collection would be virtually impossible, because of the requirement to track both the emergence timing and lifetime reproductive output of individual bees. The question is also less relevant given that, to project trends under climate warming (which of course affects entire sites), we want to know how *site-level* synchrony affects bee fitness.

At the site level, my analysis suggests that greater synchronization of bee emergence with median flowering (through decreased bee precedence) benefits bee populations. However, the negative relationship between bee precedence and site-level reproductive output is largely driven by the coolest and highest-elevation of my study sites ('VB'). Consistent with the per capita results, running the same analysis without the latter site renders the relationship largely non-significant, suggesting that bee populations at the other sites are insensitive to variation in synchrony. Although few *O. iridis* ever nest at VB, I suspect that this site has the potential to host large *O. iridis* populations: *Lathyrus* flowers abundantly (although later in the season) and nesting cavities are widely available; the site hosts large populations of other species of cavity-nesting bees. Based on databased occurrence records for *O. iridis* (Discover Life 2019), the elevation of the VB site represents the upper altitudinal range limit of *O. iridis*. My results suggest that bees occurring at the species upper range margin are likely limited by a lack of synchronization with floral resources because of high bee precedence. Conversely, my results show that sites with bee precedence within the –2 to +18-day range are largely unaffected by variation in synchrony. Unfortunately, none of my sites capture incidences of bee precedence less than –2, but it seems likely that bees at warmer and lower-altitude sites may experience a mismatch if they emerge too late relative to *Lathyrus*.

As with bee precedence, temperature does not predict per capita reproductive output, a result also reported by Forrest and Chisholm (2017). Furthermore, the positive relationship between temperature and site-level reproduction is largely driven by the same high-elevation site (VB). Hence, site-level *O. iridis* reproduction appears to be insensitive to changes in temperature, except at site VB, which experiences lower temperatures and lower site-level reproduction overall. It is unclear how this effect can be explained except as a result of mismatch. Although temperature affects *Sapyga* parasitism rates in this system (Forrest and Chisholm 2017), including parasitism in my models of reproductive output changed none of my statistical conclusions (Table S5) and, in any case, parasitism would be expected to negatively affect bees nesting at warmer sites – the opposite of the trend observed here.

The bigger picture

Previous experimental studies have shown that simulated mismatches with floral host-plants can reduce bee fitness. Schenk et al. (2018) showed that preventing bees' access to nectar and pollen, even for a few days, can have severe consequences, including decreased survival and brood cell production. However, even bees that are highly specialized with respect to their pollen usage tend to be generalized in their consumption of nectar (Wcislo and Cane 1996); they can potentially obtain sugar from a wide variety of flowers (and even non-floral sources, e.g. Meiners et al. 2017). The patterns documented by Schenk et al. (2018) may have been driven by the complete absence of nectar in their experimental cages—a situation that is unlikely in most natural settings. In my system, *O. iridis* bees that emerge significantly before *Lathyrus* flowering should be able to consume nectar from earlier-flowering species to meet their energetic needs. Nevertheless, Farzan and Yang (2018) demonstrated that, even in a more natural setting, experimental shifts in solitary bee emergence can affect bee survival and reproduction. However, their experiment varied the timing of emergence of one bee species relative to an otherwise unmanipulated community, so it is unclear whether the magnitude of the mismatch they generated would actually be observed naturally. Here, in contrast, I studied natural variation in bee–plant synchronization and its relationship with bee fitness and population success. My study complements the experiments described above, in that, for the first time, we have studied the effect of bee–plant synchronization on bees in nature. At most of our sites, bees appear to be insensitive to variation in synchronization; however, the study is limited to one highly-specialized solitary bee in one high-elevation environment. Knowing that distinct taxa exhibit varying levels of specialization and experience phenological shifts of differing magnitudes and directions (Thackeray et al. 2016, Cohen et al. 2018, Kharouba et al. 2018), studies of other systems are needed to determine whether the observed patterns are general.

My study focuses exclusively on the gap between median dates (of emergence and flowering) in measuring synchrony because of its potential for detecting mismatches. Within the –2 to +18-day bee precedence range, bee reproductive output appears unaffected by changes in the level of synchrony. Hence, all else being equal, bees should be resilient to changes in bee precedence within that range. However, other phenological characteristics may be impacted by warming, such as the duration of flowering of *Lathyrus*, the total number of flowers available, the spread of *O. iridis* emergence dates, and the co-occurrence of *Vicia* (a secondary floral resource for *O. iridis*). Rising temperatures could well shorten the duration of flowering and compress the bee emergence period, thanks to faster heat accumulation and more rapid floral senescence (cf. Gordo and Sanz 2010, Kehrberger and Holzschuh 2019). Bees could be disadvantaged if their foraging seasons are shortened. In addition, Forrest and Chisholm (2017) demonstrated that *O. iridis* bees produce more offspring when a greater number of flowers are available at a site. A change in floral density with climate change (a possibility that has not yet been investigated for *Lathyrus*) could modify per capita bee reproductive output by changing a bee's time and energy allocation (e.g. when flowers are less abundant, more time and energy are required to build a brood cell). Increasing temperatures could further modify the shape of co-flowering patterns of *Lathyrus* and later-flowering *Vicia*, possibly by increasing time between flowering peaks of the two species (cf. Aldridge et al. 2011). We do not know how this would impact *O. iridis*.

I centred my analysis on the interaction between *O. iridis* and *Lathyrus* because *Vicia* is much less abundant at most sites and should therefore be less important in regulating *O. iridis* populations. *Vicia* may offer an adequate alternative food for *O. iridis* when *Lathyrus* flowering is over, in spite of its relative scarcity. In late 2019, an observer even witnessed an *O. iridis* bee visiting a non-native *Trifolium* flower when Fabeae flowers were no longer available (Lydia Wong, personal communication). *Trifolium* is only available at one of my study sites; yet, it is conceivable its pollen was used by this bee and thus permitted a longer nesting season than *Lathyrus* and *Vicia* alone would have allowed. My initial hypothesis was that perfect synchronization between bee emergence and *Lathyrus* flowering (i.e. a bee precedence of “0”) would not be optimal, but in fact I observed high site-level reproduction under these conditions. Use of *Vicia* may explain why sites with a bee precedence of “0” are able to maintain healthy bee populations. If bees are at their best when they emerge (as suggested by the negative effect of age on *O. iridis* nesting progress by Forrest and Chisholm 2017), then having an abundant flower supply upon emergence, followed by a period of gradually declining floral abundance, could be advantageous. *Osmia iridis* is highly specialized, and my failing to detect a robust relationship between synchronization and bee reproduction suggests that even such a specialized organism can be resilient to changes in temporal overlap.

Implications for a warming climate

It is generally assumed that obligate, specialized mutualisms are less likely than more facultative or generalized mutualisms to experience a mismatch because of the high selective pressure to maintain partner synchrony that these organisms have presumably experienced. As such, the interaction partners should respond to similar climatic cues (Rafferty et al. 2015). Yet, my research demonstrates that *Lathyrus* and its specialist visitor *O. iridis* respond at different rates to warming, suggesting that a change in temporal overlap between the mutualistic partners is possible in a warmer world. It is also often assumed that climate change is a threat to bee populations in general (Potts et al. 2010, Goulson et al. 2015). However, my results do not point towards any imminent mismatches for this pollinator if bee precedence remains within the –2 to +18-day range. All else being equal, high-elevation sites that currently experience little *O. iridis* activity would likely see growing bee populations with warming as a result of decreasing bee precedence. Further warming at lower-elevation sites, however, could decrease bee precedence to the point that bees emerge after median *Lathyrus* flowering. With less food available, bees would require more time and energy to find pollen while also experiencing shorter reproductive seasons and therefore decreased population success. In such a situation, selection should favour bees whose phenologies are more thermally sensitive to keep up with the fast-shifting *Lathyrus* phenology. However, evolutionary adaptation to such rapid warming may be hindered by *O. iridis'* typically slow two-year life cycle combined with the interannual climatic variability of our study area (cf. Kingsolver and Buckley 2015).

Conclusion

Climate change, in combination with other threats, is exerting significant pressure on pollinator communities around the world (Vanbergen et al. 2013, Goulson et al. 2015). Many pollinator species, like *O. iridis*, use climate-related cues to determine when to emerge. Phenological synchronization is particularly important for mutualistic partners that rely on each other for survival. Many researchers have speculated about possible pollinator–plant mismatches and their potential consequences for one or both partners, and we know that artificial mismatches can have detrimental consequences for solitary bee survival and reproduction. However, I am the first to study how pollinator–plant synchronization impacts pollinators in nature. I demonstrated that the phenology of *Lathyrus*, *O. iridis*' preferred flowering plant, is more temperature-sensitive than that of the bees. Higher site temperatures therefore lead to improved synchrony in this pair of mutualistic partners. Using data on bee per capita reproductive output collected at multiple sites over several years, I also showed that the level of synchrony does not predict bee fitness or population success, except at one marginal site with extreme bee precedence. All else being equal, *O. iridis* populations should not be threatened by warming as long as bee precedence remains within the range observed in this study. However, rising temperatures may impact bee populations if it leads to most bees at a site emerging after the middle of the flowering season, that is, if they cause negative bee precedence.

Our understanding of how wild pollinators are impacted by climate change is limited (Memmott et al. 2007, Hegland et al. 2009, Rafferty et al. 2015). Yet, robust, healthy pollinator–plant networks significantly contribute to the sustainability of our ecosystems and offer important pollination services to humans (Potts et al. 2010, 2016, Gill et al. 2016). Generating knowledge on the impacts of climate change on ecological communities takes time, and typically requires extensive datasets and significant funding (U.S. Long Term Ecological Research Network (LTER) 2007, Clutton-Brock and Sheldon 2010). Therefore, underfunding can prevent the generation of the much-needed datasets that can answer important ecological questions such as the one addressed in this study (cf. Kareiva 2011, Martin 2011). As such, government policies should ensure ecologists have access to longstanding, reliable funding for ecological research, while also encouraging researchers to answer questions about the sustainability of our ecosystems in the context of the many current threats to pollinator communities. Newly generated knowledge will help to direct the development of future environmental mitigation and adaptation strategies in a changing world.

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Supplementary material

Supplementary figures

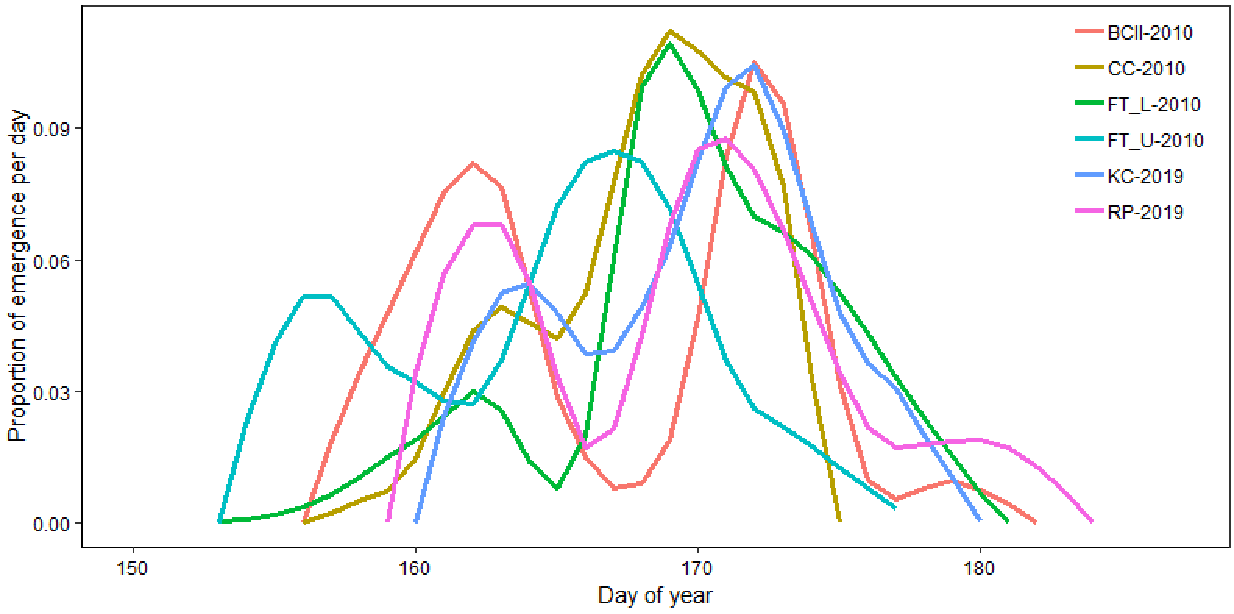

Figure S1. Actual female *O. iridis* emergence curves for six site–years where at least 15 bee emergences were recorded. Interpolated curves were obtained by Loess regression using a span of 0.5 and a degree of 2 in R (R Core Team 2018). Curves were rescaled so that the total cumulative daily proportion of emergence equals 1. Overall, curves tend to be normally distributed although distinctive weather events (cold, rain) can induce bimodality. The spread between first and last emergence appears relatively constant across site–years.

Figure S2. (A) A 2018 bee emergence box deployed in the field. (B) View inside a bee emergence box. HOBO is in the left section; *O. iridis* cocoons are in individually-marked petri dishes in the right section. Insects in the Eppendorf tubes are unrelated to this study.

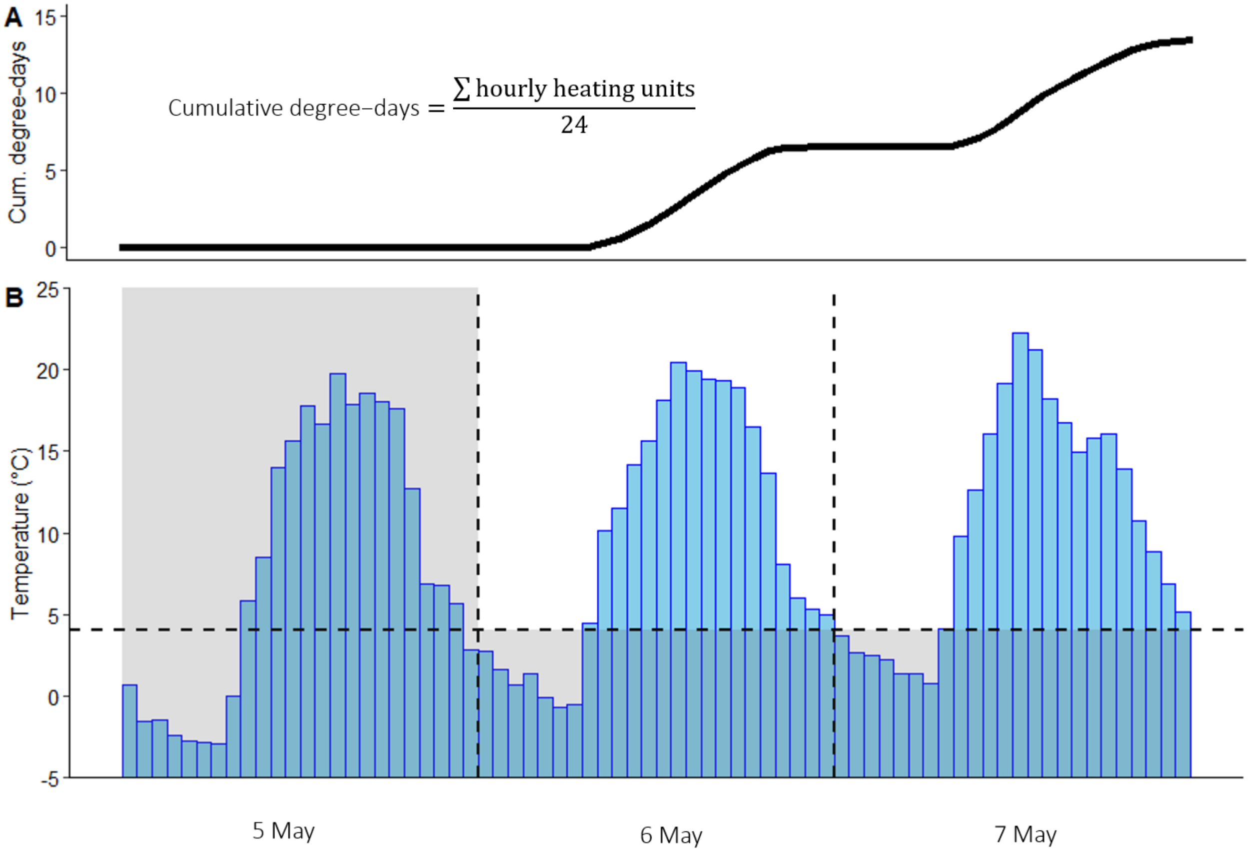

Figure S3. Example of degree-day accumulation between 5 May and 7 May in a model where heat accumulation begins 6 May at temperatures above 4°C (the best model predicting *O. iridis* emergence; see Figure 3). (A) Cumulative degree-days equal the sum of all accumulated hourly heating units divided by 24 hours/day. (B) An hourly heating unit is defined as the difference between the hourly temperature and the model's base temperature (4°C) if the temperature is greater than the base temperature, and "0" if the hourly temperature is lower than the base temperature. Hourly heating unit accumulation begins on the model's start date (6 May). Unshaded bars show relevant hourly heating units. Temperature sample originates from site RP in 2018; according to this model, median emergence occurred on 6 June 2018 (when 227 degree-days have accumulated).

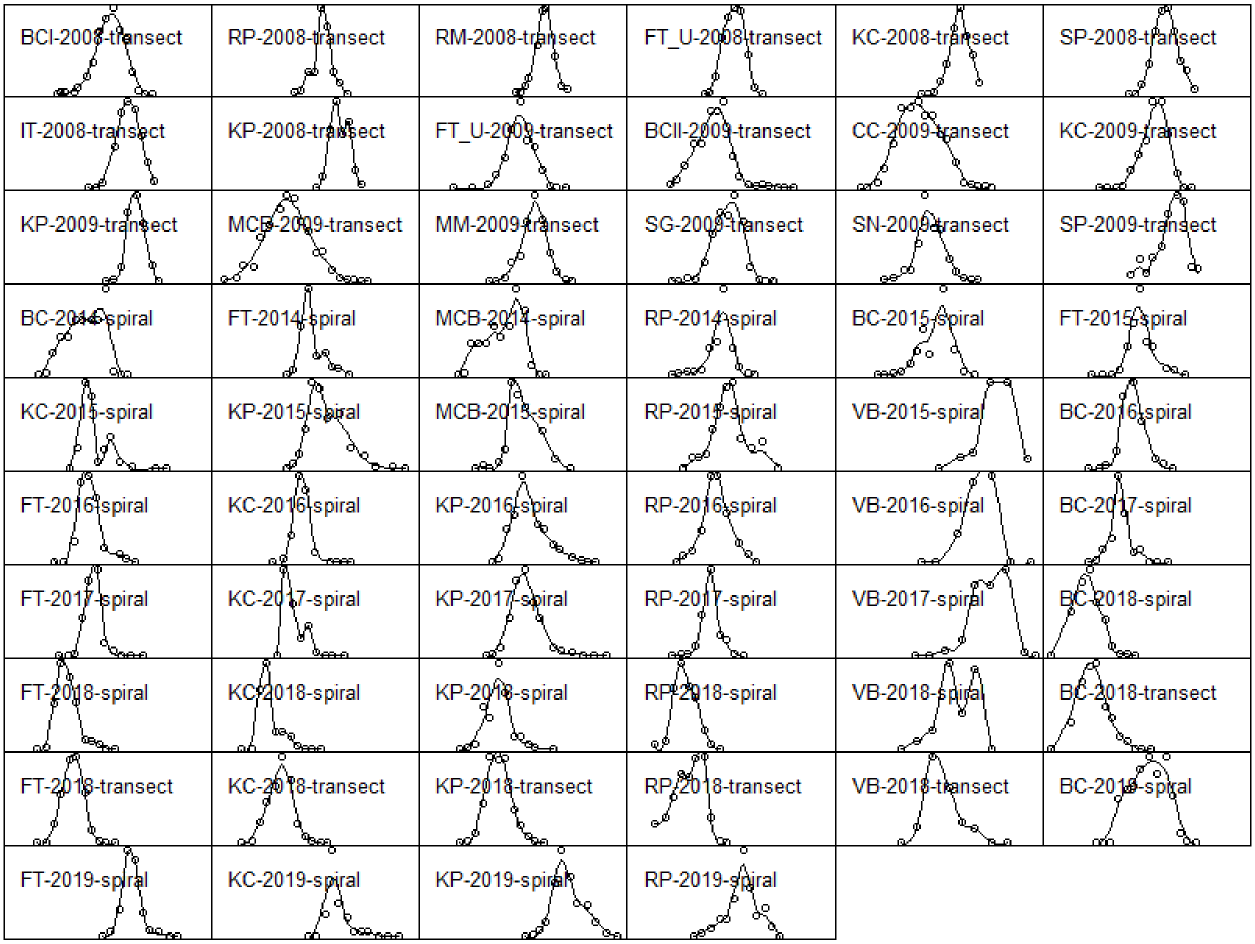

Figure S4. Interpolated *Lathyrus* floral phenology curves at all 52 site–years. Sites in 2018 have two curves, one for each sampling method (spiral and transect). Day of year is on the x-axis and ranges from 137 to 226 in each panel (17 May to 14 Aug). Floral density is on the y-axis and ranges from 0 m^2 to the site–year's maximum floral density. Actual floral densities are denoted by circles. Interpolated curves were obtained by Loess regression using span and degree parameters of 0.5 and 2 respectively in R (R Core Team 2018).

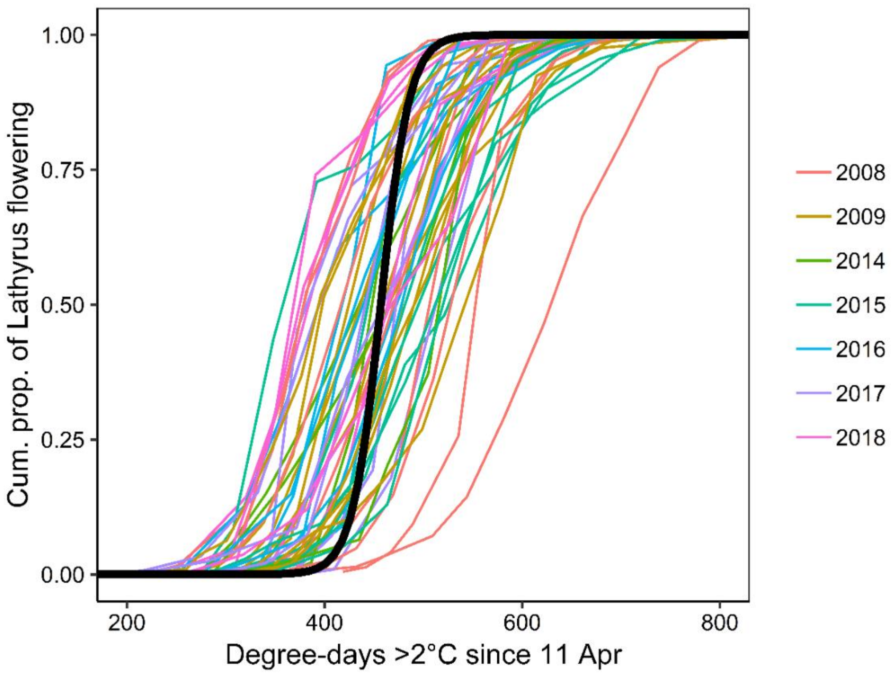

Figure S5. The phenological model that best predicts *Lathyrus* springtime flowering suggests that 457 degree-days of heat above 2°C starting on day of year 101 (11 Apr) are required to reach median flowering. This model had the lowest AIC score from a pool of 667 models testing every start date and temperature combination from day of year 1 (1 Jan) to day of year 141 (21 May) at 5-day intervals, and from −10°C to 12°C at 1°C intervals. Coloured lines represent the flowering profiles of *Lathyrus* in individual site–year combinations, colour-coded by year (N=47). Cumulative proportion of flowering was determined for every site–year using floral density (0 = flowering has not yet begun, 0.5 = median flowering; half of all the season's cumulative daily floral density has occurred, 1 = all flowering has occurred). *Lathyrus* density was estimated using a transect-based approach in 2008–2009 and using a spiral-search method in 2014–2018 (2019 omitted in this analysis). The black curve represents the best-fit logistic mixed-effects model. See text for details.

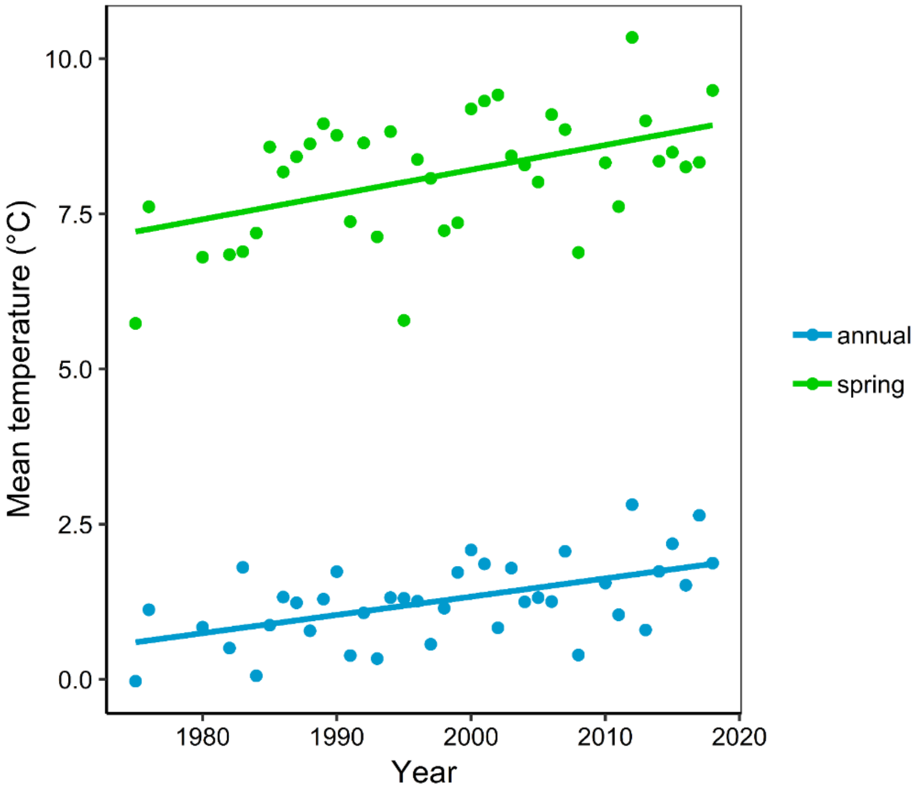

Figure S6. Long-term trends in mean annual and spring temperatures between 1975 and 2018. Data obtained from the National Oceanic and Atmospheric Administration weather station in Crested Butte (5 km from the nearest site). Mean temperatures were obtained by averaging daily temperature minima and maxima throughout the entire year for annual temperatures, and between day of year 105 and 196 (15 April and 15 July in common years) for spring temperatures. Years 1977, 1978, 1979, 1981 and 2009 were omitted due to missing data. N= 39 years. Annual: slope = 0.029; *SE* = 0.0074; *P* = 0.0003. Spring: slope = 0.040; *SE* = 0.012; *P* = 0.0019.

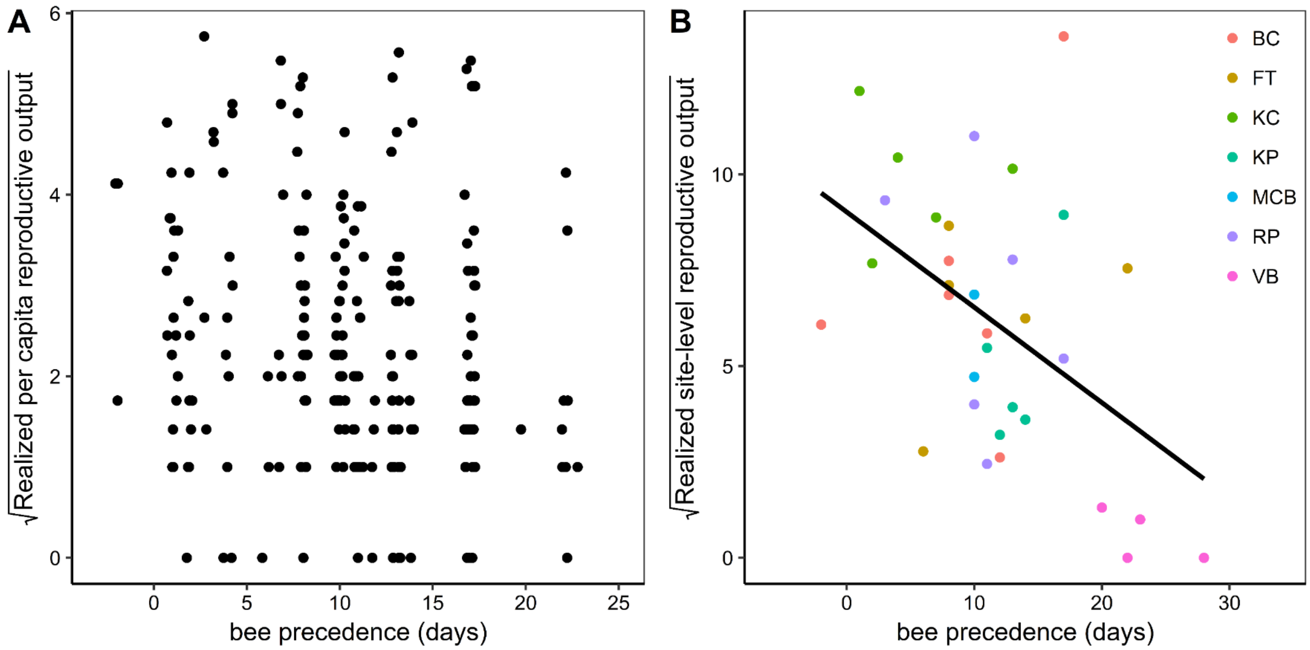

Figure S7. The relationship between realized *O. iridis* reproductive output and bee precedence—the number of days by which median *O. iridis* emergence precedes median *Lathyrus* flowering. Realized reproductive outputs only include non-parasitized brood cells containing a host-egg. Reproductive outputs were square-root-transformed to improve normality. (A) Per capita reproductive outputs. N = 226 bees. The bee precedence coefficient in the linear mixed-effects model is non-significant (*P* = 0.38). Points jittered by up to 0.3 days for clarity. (B) Site-level reproductive outputs standardized to a 10 cavity per trapnest x 12 trapnest per site design. Points are colour-coded by site. Overall, with increasing bee precedence we observe reduced site-level reproduction. N=34 site–years; *P* = 0.0008 (for the bee precedence coefficient in the linear mixed-effects model). See Figure S8B for panel B colour-coded by year.

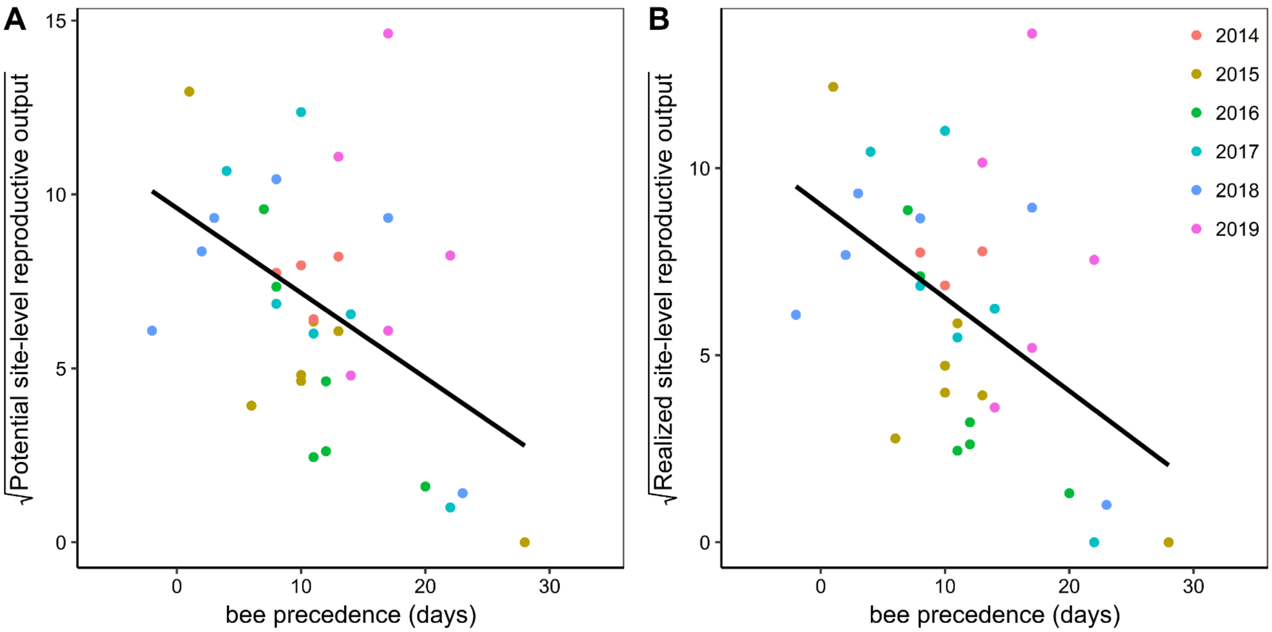

Figure S8. (A) Idem to Figure 5B but with points colour-coded by year. (B) Idem to Figure S7B but with points colour-coded by year.

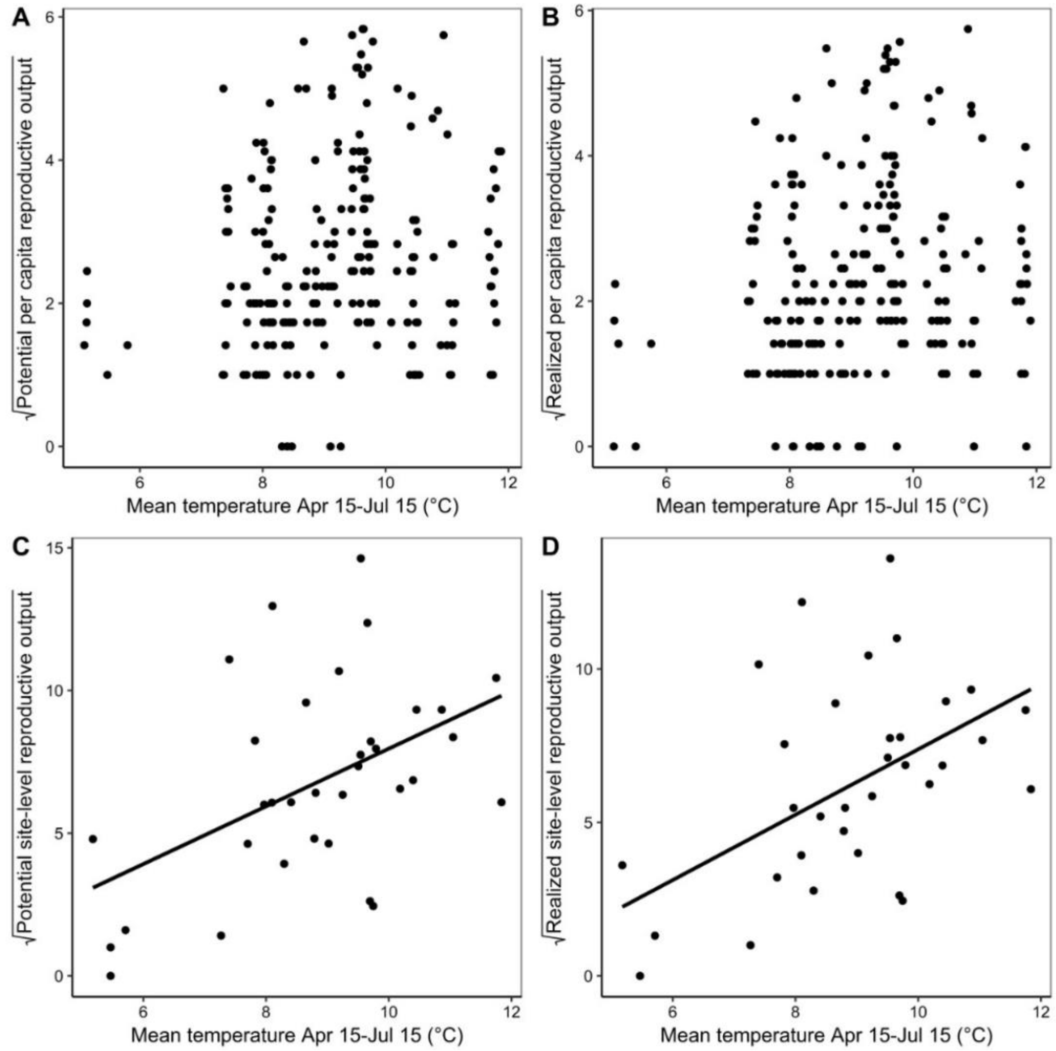

Figure S9. The relationships between *O. iridis* reproductive output and mean spring temperature. Reproductive outputs were square-root-transformed to improve normality. (A) Potential and (B) realized *O. iridis* per capita reproductive outputs. Points jittered by up to 0.1°C for clarity. Relationships are non-significant. N=226 bees. (C) Potential and (D) realized *O. iridis* site-level reproductive outputs standardized to a 10 cavity per trapnest x 12 trapnest per site design. Overall, with increasing mean spring temperature, sites experience higher reproductive outputs. N=34 site–years. See Table S5 for statistics.

Supplementary tables

Table S1. The seasonal cumulative daily floral densities of Fabeae species (*Lathyrus lanszwertii* and *Vicia americana*) at sites where *O. iridis* reproductive outputs were collected. Cumulative daily floral densities were calculated by summing all interpolated daily floral densities for each species throughout the flowering season at a site. The last two columns show the relative contributions of *Lathyrus* and *Vicia* to total Fabeae floral density for that site–year. The averages across all site–years are included in the last row.

Site	Year	*Lathyrus* cumulative daily floral density (flowers/m²)	*Vicia* cumulative daily floral density (flowers/m²)	Proportion *Lathyrus*	Proportion *Vicia*
BC	2014	135.99	7.19	0.950	0.050
FT	2014	12.59	1.78	0.876	0.124
MCB	2014	56.88	4.28	0.930	0.070
RP	2014	10.04	5.29	0.655	0.345
BC	2015	65.77	30.14	0.686	0.314
FT	2015	20.32	4.39	0.822	0.178
KC	2015	31.35	8.73	0.782	0.218
KP	2015	20.29	2.93	0.874	0.126
MCB	2015	8.20	11.53	0.416	0.584
RP	2015	5.33	17.04	0.238	0.762
VB	2015	45.84	0.47	0.990	0.010
BC	2016	96.30	77.90	0.553	0.447
FT	2016	68.02	2.82	0.960	0.040
KC	2016	234.05	17.25	0.931	0.069
KP	2016	28.30	1.14	0.961	0.039
RP	2016	10.75	8.83	0.549	0.451
VB	2016	27.55	0.80	0.972	0.028
BC	2017	79.29	17.42	0.820	0.180
FT	2017	40.88	1.56	0.963	0.037
KC	2017	67.73	6.67	0.910	0.090
KP	2017	16.35	0.94	0.946	0.054
RP	2017	26.00	8.51	0.753	0.247
VB	2017	46.81	0.48	0.990	0.010
BC	2018	77.22	2.19	0.972	0.028
FT	2018	36.14	1.78	0.953	0.047
KC	2018	30.70	8.99	0.773	0.227
KP	2018	16.64	0.94	0.947	0.053

RP	2018	3.34	2.89	0.536	0.464
VB	2018	27.27	0.54	0.981	0.019
BC	2019	117.00	22.13	0.841	0.159
FT	2019	29.39	3.29	0.899	0.101
KC	2019	18.01	7.32	0.711	0.289
KP	2019	12.74	1.20	0.914	0.086
RP	2019	2.46	9.25	0.210	0.790
Average		44.87	8.78	0.802	0.198

Table S2. Sites and years of data collection, site coordinates and elevation, and type of data collected at each location.

Site code	Site name	Years	Latitude (N)	Longitude (W)	Elevation (m)	Hourly spring temperature	Female bee emergence	*Lathyrus* floral density	*Vicia* floral density	Median bee emergence date (estimated)	Per capita bee reproductive output
BC	Brush Creek	2008	38.8609	106.9196	2730	x		x		x	
		2014-2019				x	x[a]	x	x	x	x
BCII	Brush Creek II	2009	38.8633	106.915	2743	x		x		x	
		2010				x	x				
CC	Cement Creek	2009	38.8216	106.8694	2682	x		x		x	
		2010				x	x				
EXP	Emergence Experiment	2018	38.9591	106.9894	2888	x	x				
FT	401 Trail	2014-2019	38.9708	106.9906	3020	x	x[a]	x	x	x	x
FT_L	401 Trail-Lower	2009-2010	38.9616	106.9849	2970	x	x				
FT_U	401 Trail-Upper	2008-2009	38.9616	106.9849	2970	x		x		x	
		2010				x	x				
IT	Irwin Triangle	2008	38.856	107.0896	3009	x		x		x	
KC	Kebler Clearing	2008-2009	38.858	107.061	2957	x		x		x	
		2010				x	x				
		2015-2019				x	x[a]	x	x	x	x
KP	Kebler Pass	2008-2009	38.8525	107.101	3037	x		x		x	
		2015-2019				x	x[a]	x	x	x	x
MCB	Mt Crested Butte	2009	38.8892	106.9623	2879	x		x		x	
		2014-2015				x		x	x	x	x
MM	Marmot Meadow	2009	38.9779	106.9992	2938	x		x		x	
RM	Research Meadow	2008	38.956	106.9821	2929	x		x		x	
RP	Rosy Point	2008	38.9327	106.9702	2900	x		x		x	
		2014-2019	38.9328	106.9687	2879	x	x[a]	x	x	x	x

SG	South Gothic	2009	38.9546	106.9852	2926	x		x		x	
SN	Snodgrass	2009	38.921	106.9712	2999	x		x		x	
		2010				x	x				
SP	Splain’s Gulch	2008-2009	38.8567	107.0746	2967	x		x		x	
VB	Virginia Basin	2015-2018	38.977	106.9762	3440	x		x	x	x	x

[a] emergence data collected in 2019 only

Table S3. Number of artificial nesting blocks and available cavities at the site–years where *O. iridis* reproductive outputs were recorded. Each nesting block had 10 cavities.

Site	Years	Number of blocks	Number of cavities
BC	2014-2016	14	140
	2017-2019	12	120
MCB	2014-2015	14	140
RP	2014-2015	24	240
	2016	14	140
	2017-2019	12	120
FT	2014-2016	14	140
	2017-2019	12	120
KC	2015-2016	14	140
	2017-2019	12	120
KP	2015-2016	14	140
	2017-2019	12	120
VB	2015-2016	14	140
	2017-2018	12	120

Table S4. Models testing the relationship between *O. iridis* median emergence date, *Lathyrus* median flowering date, and bee precedence, and the predictor variable mean hourly spring temperature. Site and year are included as random terms in all models. Coefficients and coefficient p-values are included, along with marginal and conditional R^2 values. Significant p-values are marked with an asterisk (*).

Response variable	Predictor variable	Coefficient (slope)	*t*	df	*P*	N	R^2_m	R^2_c
O. iridis median emergence date	Mean spring temperature	-4.43	-21.35	48.22	$<2\times10^{-16}$*	52	0.870	0.927
Lathyrus median flowering date		-6.57	-15.03	25.75	3×10^{-14}*	52	0.817	0.818
Bee precedence		-1.71	-2.99	21.29	0.0069*	52	0.171	0.330

Table S5. Models testing the relationship between *O. iridis* reproductive output as the response variable and bee precedence or mean spring temperature as predictor variables. Each model with bee precedence was tested twice: (i) using bee precedence as both a first- and second-order term, and (ii) using bee precedence as a first-order term only. Per capita and site-level reproductive outputs were square-root-transformed to improve normality. Potential outputs include all cells with host egg, whether parasitized or not. Realized outputs only include non-parasitized cells with host egg. Site and year are included as random terms in all models. Marginal and conditional R-squared values are included. P-values test the significance of predictor coefficients. Significant p-values are marked with an asterisk (*). To test the robustness of statistical conclusions, models marked with a (†) are tested again after the removal of site VB, our highest-elevation site; results are reported in Table S6.

Response variable	Predictor 1	*t*	df	*P*	Predictor 2	*t*	df	*P*	N	R^2_m	R^2_c
√(Potential per capita reproductive output)	Bee precedence	-0.92	213.4	0.36	(Bee precedence)^2	-1.334	218.4	0.18	226	0.012	0.24
	Bee precedence	-0.98	14.2	0.34					226	0.0062	0.25
	Mean spring temp	1.09	5.9	0.32					226	0.0083	0.25
√(Realized per capita reproductive output)	Bee precedence	-0.88	12.4	0.40	(Bee precedence)^2	-0.624	97.3	0.53	226	0.0063	0.26
	Bee precedence	-0.90	14.9	0.38					226	0.0051	0.27
	Mean spring temp	1.56	5.2	0.18					226	0.014	0.26
√(Potential site-level reproductive output)	Bee precedence	-3.51	29.1	0.0015*	(Bee precedence)^2	-1.043	30.7	0.31	34	0.26	0.42
	Bee precedence	-3.60	29.6	0.0011*†					34	0.25	0.42
	Mean spring temp	2.71	15.7	0.016*†					34	0.24	0.56
√(Realized site-level reproductive output)	Bee precedence	-3.61	29.3	0.0011*	(Bee precedence)^2	-0.693	30.8	0.49	34	0.26	0.41
	Bee precedence	-3.71	29.8	0.00084*†					34	0.26	0.42
	Mean spring temp	2.89	15.9	0.011*†					34	0.26	0.57

Table S6. See caption of Table S5. After the removal of site VB, none of the significant statistical conclusions hold. All significant relationships are therefore driven by site VB.

Response variable	Predictor	*t*	df	*P*	N	R^2_m	R^2_c
√(Potential site-level reproductive output)	Bee precedence	-0.22	21.5	0.83	30	0.002	0.17
	Mean spring temp	0.99	10.5	0.35	30	0.04	0.27
√(Realized site-level reproductive output)	Bee precedence	-0.19	22.3	0.85	30	0.001	0.25
	Mean spring temp	1.29	10.6	0.23	30	0.07	0.34

Appendix A1: Synchrony

Metric for synchrony – I considered two metrics for examining synchrony between female *O. iridis* emergence and *Lathyrus* flowering: (i) bee precedence (the number of days by which median bee emergence precedes median flowering), and (ii) remaining proportion of flowering. Remaining proportion of flowering is the proportion of cumulative daily floral density available after the median bee emergence date at a site. Figure 1 of the thesis provides a visual description of bee precedence, and Figure A1–1 a visual description of remaining proportion of flowering. I ultimately used bee precedence as my only metric of temporal overlap because both metrics are strongly correlated, because there is greater variability in the bee precedence metric, and because the expected relationship with bee fitness is more straightforward for bee precedence (Figure A1–2).

Actual phenological overlap scenarios – I also plotted actual phenology curves of *O. iridis*, *Lathyrus* and *Vicia* to visualize actual temporal overlap scenarios. Figure A1–3 shows empirical phenological curves of all three species at two sites. Panel A shows the phenologies at site KC in 2019, with *Lathyrus* being more abundant than *Vicia*, the later-flowering species. Panel B shows the phenological curves for the most atypical site–year, RP-2019, where *Vicia* is more abundant than *Lathyrus*. On average, *Lathyrus* makes up 80% of the Fabeae floral densities at my sites.

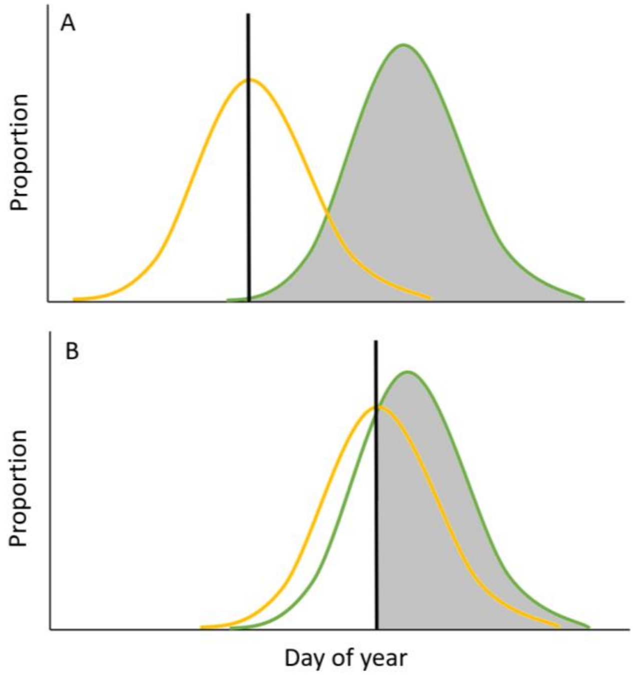

Figure A1–1. The remaining proportion of flowering is the remaining daily cumulative proportion of seasonal floral density left after median bee emergence (solid line). The yellow curve represents the emergence phenology of *O. iridis*, and the green curve, the flowering phenology of *Lathyrus*. The remaining proportion of flowering is shaded in grey. Remaining proportions of flowering are approximately 1 in (A) and 0.7 in (B).

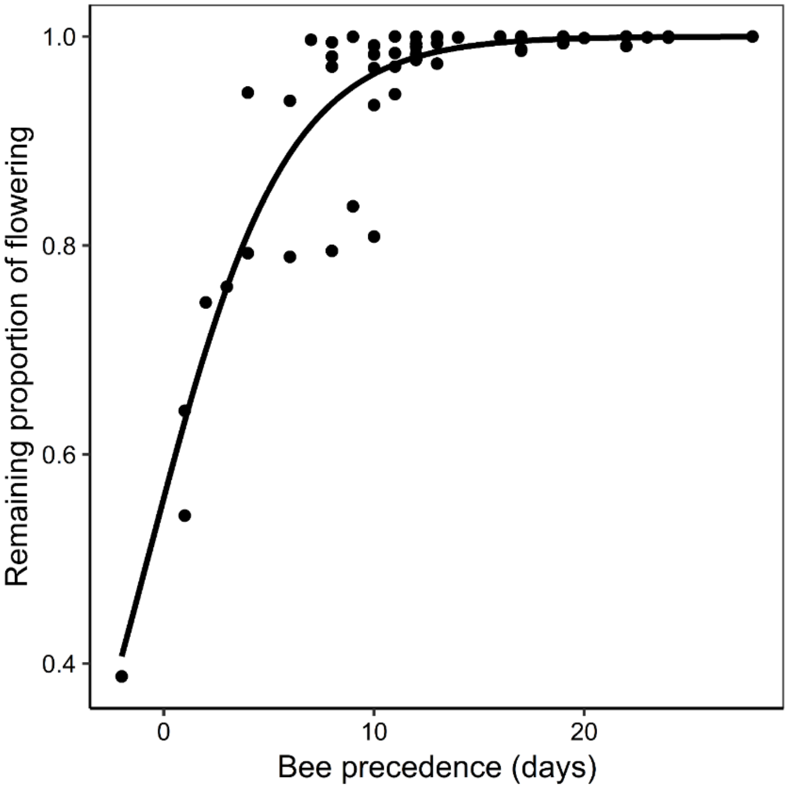

Figure A1–2. The relationship between bee precedence and remaining proportion of flowering. N=52 site–years. I focussed on bee precedence for analysis for four main reasons: (1) both overlap metrics are strongly correlated, (2) there is higher variability in the bee precedence metric (most values for remaining proportion of flowering are between 0.75 and 1), (3) when bee precedence is greater than 8, remaining proportions are almost all 1 (although it is evident that a precedence of 20 days, for example, should be worse for the bees than a precedence of 8 days), and (4) formulating fitness vs. overlap hypotheses is more straightforward using bee precedence (see Figure 1D of thesis).

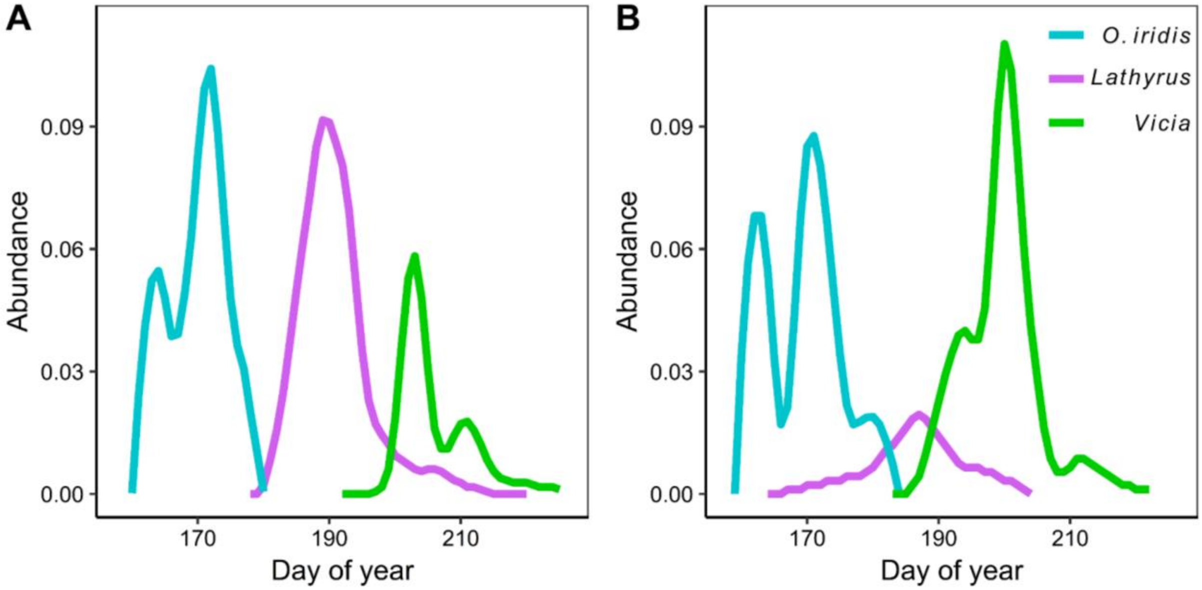

Figure A1–3. Actual phenological curves for female *O. iridis* emergence (blue), and *Lathyrus* (purple) and *Vicia* (green) flowering phenologies at two sites in 2019. Interpolated curves were obtained by Loess regression using a span of 0.5 and a degree of 2 in R (R Core Team 2018). Curves were rescaled so that the total cumulative daily proportion of emergence/flowering equals 1. The curve of the less abundant floral resource was further adjusted according to its measured density relative to the more abundant floral resource (cf. Table S1). (A) Phenologies at site KC in 2019. This is a typical site, where *Lathyrus* is the most abundant floral resource. (B) Phenologies at site RP in 2019. This is the most atypical site–year, where *Vicia* is much more abundant than *Lathyrus*.

Appendix A2: Phenological modelling analyses

I performed secondary analyses on the best phenological models for *O. iridis* and *Lathyrus* to test their robustness or goodness-of-fit to actual median dates. I also tested whether the two taxa differ significantly in their phenological responses to temperature by comparing their best-fit models. Table A2–1 contains the ΔAIC values of all 667 *O. iridis* phenological models.

Robustness of bee model – To assess the robustness of the best phenological model for *O. iridis* (median emergence at 227 degree-days above 4°C starting day of year 126) relative to models using different starting dates and temperature thresholds, I compared all 52 site-specific median emergence dates predicted by the best model with the average of the median emergence dates predicted by the 10 models with the lowest AIC scores (including the best model). I plotted the estimated median date from the best model against the average estimated median date of the top 10 models. The relationship is highly significant and shows a quasi 1:1 relationship (Figure A2–1). For every site–year, the difference between the median emergence date predicted by the best model and the averaged date from the 10 best models was always less than 24h except in one case where the difference was slightly greater than a day, supporting the use of a single best model.

A post-hoc analysis treating site as a random factor and year as a categorical variable with 4 levels instead of using the composite site–year variable as a random factor revealed a significant effect of year. However, with thousands of data points (9867 observations) in each model and because certain years had few recorded emergences (e.g. 2009), we may have power to detect unimportant year effects. Comparing the best model's marginal and conditional R-squared values (Figure 3 of the main text) suggests that the year effect is relatively trivial: cumulative degree-days explain 81% of the variance in emergence dates; adding site-year as a random factor only improves the goodness-of-fit of the model (R^2_c) to 90%.

Goodness-of-fit of floral model – To determine how well phenological models predict actual median dates, I estimated median *Lathyrus* flowering dates using the *Lathyrus* phenological model at 47 site–years and tested the relationship between predicted and actual median *Lathyrus* flowering dates fitted on the same site–years. The relationship is highly significant (t = 14.77, df = 24.55, $P = 1\text{x}10^{-13}$, $R^2_m = 0.84$) and is described by the equation $y = 1.042x-5.127$, suggesting that the model is good at predicting median dates (Figure A2–2). I could not reproduce this analysis with bee emergence due to the limited size of the empirical bee emergence dataset.

Phenological responses to temperature – I wanted to test whether the phenological models I developed for *O. iridis* and *Lathyrus* independently were in fact meaningfully different from one another. In the case of *O. iridis*, the best model had threshold conditions of 6 May and 4°C; I wanted to ensure that using these thresholds provided a model that was significantly better at predicting bee phenology than the thresholds used for *Lathyrus* (11 April and 2°C), and vice versa for *Lathyrus*. To do this, I calculated the AIC values of each species' best model using their own best thresholds. I also calculated the AIC value of the model fitting *Lathyrus*' best thresholds on *O. iridis* emergence data, and the AIC value of the model fitting *O. iridis*' best thresholds on

Lathyrus flowering data. AIC values for models using each species' own best thresholds were at least 13 units lower than the AIC values of models using the other species' best thresholds (Table A2–2). Hence, I concluded that the two species differ significantly in the nature of their phenological responses to temperature.

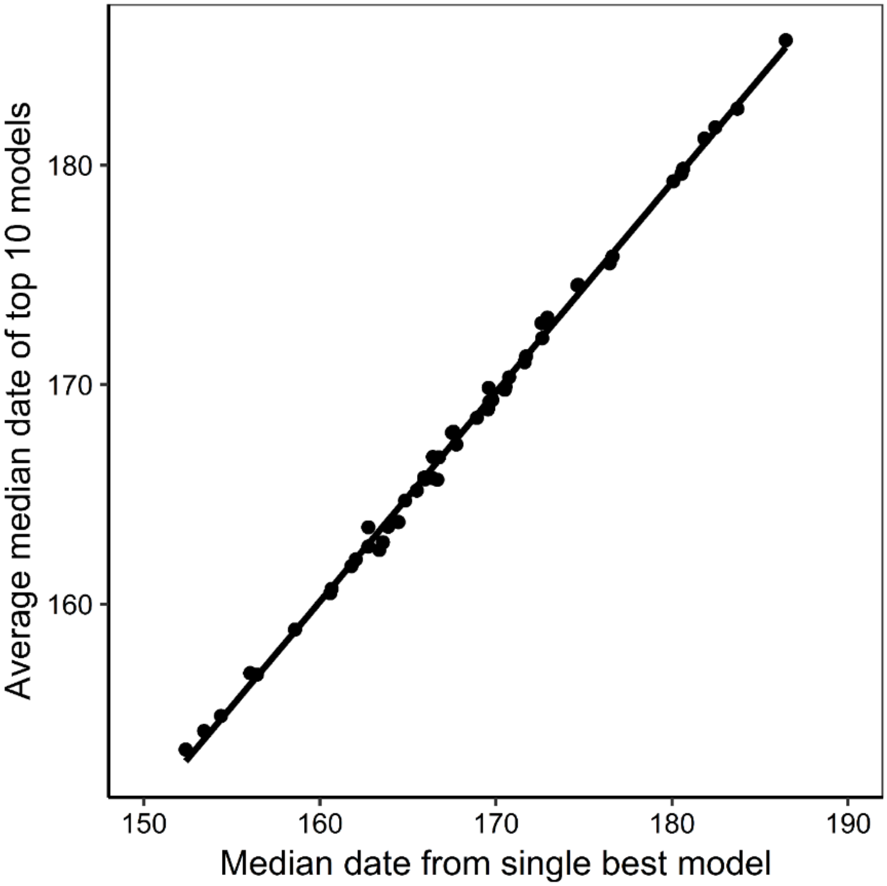

Figure A2–1. The relationship between the median *O. iridis* emergence date from the single best model and the averaged median *O. iridis* emergence date from the top 10 models with lowest AIC scores. The strong relationship between the two variables supports the use of a single best phenological model in predicting *O. iridis* median emergence dates in my analyses. N = 52 site–years, $P < 2 \times 10^{-16}$, $R^2_m = 0.997$, $y = 0.954x + 7.32$.

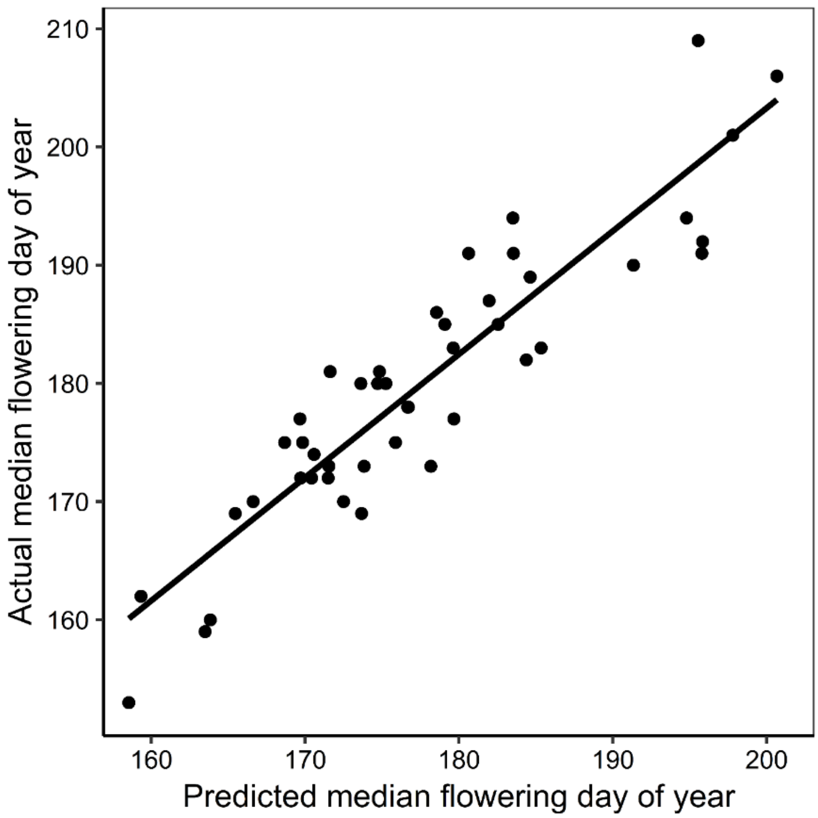

Figure A2–2. The relationship between actual median flowering dates and median flowering dates predicted by the *Lathyrus* phenological model (fitted on the same site–years). N = 47 site–years (2019 omitted), t = 14.77, df = 24.55, $P = 1\text{x}10^{-13}$, R^2_m = 0.84, y = 1.042x-5.127.

Table A2–1. ΔAIC values of all 667 *O. iridis* phenology models. Lowest ΔAIC values (best models) are in green, highest in red. Each model used a different heat-accumulation start date and temperature threshold combination between days of year 1 and 141, and –10 to 12^{o}C. The best model predicts emergence using heat accumulation above 4^{o}C beginning on day of year 126. A model using a threshold of 8^{o}C and day of year 131 (as in Forrest and Thomson 2011) gives a much worse fit to the data (ΔAIC = 13).

Model threshold day of year	Model threshold temperature (^{o}C)																						
	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8	9	10	11	12
1	35	32	29	27	25	25	25	26	29	32	33	31	29	28	27	27	28	29	32	37	43	52	63
6	33	31	28	26	25	24	25	26	29	32	33	31	29	27	27	27	28	29	32	37	43	51	63
11	33	31	28	26	24	24	25	26	28	31	32	30	28	27	26	27	27	29	32	36	43	51	62
16	32	29	26	24	23	23	24	25	27	30	32	29	27	26	25	26	26	28	31	36	42	51	62
21	30	28	26	24	23	23	23	25	27	30	31	28	26	25	25	25	26	27	31	35	42	50	62
26	28	26	24	22	21	22	23	24	27	30	31	28	26	25	25	25	26	27	31	35	42	50	62
31	27	25	22	21	20	20	22	23	26	29	30	28	26	24	24	24	25	27	30	35	41	50	61
36	29	27	24	22	21	21	21	23	25	28	30	27	25	24	24	24	25	26	30	35	41	50	61
41	29	27	24	22	21	21	21	23	25	28	29	27	25	24	23	24	25	26	30	35	41	50	61
46	30	27	24	22	21	20	21	22	25	27	29	27	25	24	23	24	24	26	30	35	41	50	61
51	30	27	25	23	21	20	20	22	24	27	29	26	24	23	23	23	24	26	29	34	41	50	61
56	27	25	23	21	20	19	20	22	24	27	28	26	24	23	23	23	24	26	29	34	41	50	61
61	26	24	22	20	19	18	19	21	23	26	28	25	23	22	22	22	23	25	29	34	40	50	61
66	24	22	20	19	18	18	18	20	22	25	27	24	22	21	21	21	22	24	28	33	40	49	61
71	24	22	21	19	18	18	18	19	21	24	26	23	22	21	20	21	22	24	28	33	39	49	61
76	25	23	21	19	18	17	17	18	20	22	25	22	20	19	19	20	21	22	27	32	39	48	60
81	23	21	19	18	17	16	16	17	18	21	23	21	19	18	17	18	19	21	25	31	38	47	59
86	24	22	20	19	18	17	16	16	18	20	22	20	18	17	16	17	18	20	25	30	37	47	59
91	25	23	21	19	17	16	15	15	16	18	20	18	16	15	14	15	16	18	23	29	36	46	58
96	27	25	23	21	19	18	17	16	16	17	19	17	15	14	14	15	16	18	23	29	36	46	58
101	28	26	24	22	21	19	17	16	16	16	18	16	14	13	12	13	14	16	21	27	35	45	57
106	28	26	24	22	20	18	16	15	14	14	15	13	11	10	9.4	10	11	13	18	24	32	43	56
111	27	25	23	20	18	16	14	12	11	11	12	9.3	7.1	5.6	4.9	5.1	6.1	8.4	13	20	29	40	54
116	27	25	23	20	18	16	14	12	10	10	10	7.7	5.6	4.1	3.5	3.7	4.6	7.0	12	19	27	39	53
121	29	26	24	22	19	17	14	12	10	8.9	8.4	6.1	4.0	2.6	2.1	2.4	3.5	5.9	11	18	26	38	52
126	29	27	24	22	19	17	14	11	9	7.4	6.5	4.1	1.9	0.6	0.0	0.4	1.6	4.2	9.3	16	25	37	51
131	31	29	26	24	21	18	15	13	10	7.5	5.8	3.5	1.7	0.8	0.9	2.0	4.0	7.2	13	20	29	41	54
136	35	33	31	29	26	24	21	18	16	14	12	10	9.0	8.7	9.3	11	13	16	21	28	36	46	58
141	38	36	34	32	30	27	25	22	19	17	15	13	12	12	13	14	16	19	24	30	38	47	59

Table A2–2. Best models predicting *O. iridis* emergence and *Lathyrus* flowering phenologies, and their AIC values (*). I also calculated the AIC value of the model fitted using the best thresholds for the other species (†). The difference in AIC values is calculated as (†) minus (*). Differences are greater than 13 AIC units, and so I concluded that the two species differ significantly in the nature of their phenological responses to temperature.

Best model	Day of year threshold	Date threshold (common year)	Temperature threshold (°C)	Degree-days required to reach median according to best model	AIC values	
					Model fitted on *O. iridis* emergence data	Model fitted on *Lathyrus* flowering data
m_4_126 (best for *O. iridis*)	126	6 May	4	227.0	4175.72*	204.37†
m_2_101 (best for *Lathyrus*)	101	11 April	2	456.9	4189.41†	188.37*
Difference in AIC					13.69	16.00