TWO-YEAR BEE, OR NOT TWO-YEAR BEE? HOW VOLTINISM IS AFFECTED BY TEMPERATURE AND SEASON LENGTH IN A HIGH-ELEVATION SOLITARY BEE

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Abstract

Organisms must often make developmental decisions without complete information about future conditions. This uncertainty—for example, about the duration of conditions favorable for growth—can favor bet-hedging strategies. Here, we investigated the causes of life-cycle variation in *Osmia iridis*, a bee exhibiting a possible bet-hedging strategy with co-occurring one- and two-year life cycles. One-year bees reach adulthood quickly but die if they fail to complete pupation before winter; two-year bees adopt a low-risk, low-reward strategy of postponing pupation until the second summer. We reared larval bees in incubators in various experimental conditions and found that warmer—but not longer—summers, and early birth dates, increased the frequency of one-year life cycles. Using *in situ* temperature measurements and developmental trajectories of laboratory- and field-reared bees, we estimated degree-days required to reach adulthood in a single year. Local long-term (1950–2015) climate records reveal that this heat requirement is met in only ~7% of summers, suggesting that the observed distribution of life cycles is adaptive. Warming summers will likely decrease average generation times in these populations. Nevertheless, survival of bees attempting one-year life cycles— particularly those developing from late-laid eggs—will be <100%; consequently, we expect the life-cycle polymorphism to persist.

Introduction

Organisms must often commit to particular developmental pathways on the basis of incomplete information. For example, amphibians inhabiting temporary pools must determine when to metamorphose, in the face of uncertainty in when their aqueous larval habitat will disappear (Newman 1992); copepods must determine when to produce diapausing eggs without prior knowledge of when fish predation will reach catastrophic levels (Hairston and Munns 1984); and plants must determine when to flower, despite uncertainty in when the first frost will put an end to growth or reproduction (Inouye 2000). Trade-offs between survival and extended growth or reproduction are often inherent in these developmental decisions (see Cohen 1970; Cohen 1971). In many organisms (including insects, plants, and zooplankton), some life-stages cannot survive cold or dry periods, so these organisms have been selected to avoid initiating developmental pathways (e.g., metamorphosis, germination, or hatching) that can "trap" them in an inviable life stage when the unfavorable season begins (Gyllström and Hansson 2004; Rathcke and Lacey 1985; Tauber et al. 1986).

Bet-hedging life-history strategies reduce variance in fitness at the expense of lower mean fitness (i.e., maximizing geometric mean fitness at the expense of reduced arithmetic mean fitness; Philippi and Seger 1989; Seger and Brockmann 1987; Slatkin 1974). These strategies can be advantageous in situations where future environmental conditions are unpredictable (Simons 2011). With a diversified bet-hedging strategy, multiple phenotypes are produced, maximizing the chance that at least some will be well-suited to the future conditions (Cohen 1966; Simons 2011); with conservative bet-hedging, organisms "play it safe" by invariably exhibiting a low-

risk but also low-reward phenotype (Philippi and Seger 1989; Seger and Brockmann 1987; Simons 2011).

In general, it is unclear whether organisms' evolved life-history strategies will be well-suited to novel climates experienced under climate change (cf. Van Dyck et al. 2015). Climate change is causing growing seasons to get warmer and longer (IPCC 2014). These novel conditions may allow short-lived ectotherms to complete more generations per year, due both to the prolonged opportunity for growth and the faster growth rates that can be achieved under warmer conditions (Deutsch et al. 2008). Indeed, in many insects, voltinism—the number of generations per year— does respond to changes in temperature (Altermatt 2010; Buckley et al. 2015; Neff and Simpson 1992), and the number of generations per year has increased in recent decades (reviewed by Forrest 2016). Similarly, longer growing seasons may allow organisms that currently develop over more than one year to compress their life cycle into a single year. However, a short-term (plastic) response to longer summers requires (a) that organisms receive cues indicating that the season will be long enough, and (b) an evolutionary history of association between such cues and long summers (i.e., the cues must have been reliable in the past for a plastic response to have evolved). Furthermore, voltinism can be affected by factors other than plastic responses to climatic variation, including local adaptation (Stoks et al. 2014), photoperiod (Grevstad and Coop 2015), larval food supply (Shintani et al. 2017), and maternal cues (Danks 1987).

Mason bees (*Osmia* spp.) are normally univoltine (one generation per year or season), but longer life cycles have been documented in high-latitude and high-altitude populations of several species (Forrest and Thomson 2011; Fye 1965; Torchio and Tepedino 1982). Intriguingly, some populations—and even some individual nests—display both one- and two-year life cycles, a

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phenomenon termed "parsivoltinism" (Torchio and Tepedino 1982). A mason bee that pursues a typical one-year (univoltine) life cycle hatches and develops into an adult within one growing season (year t_0), then remains in its cocoon through the winter and emerges the following spring (t_1; figure 1). In a two-year (semivoltine) life cycle, the bee completes larval development in the first growing season (t_0), then pupates and develops into an adult the following summer (t_1), emerging the next spring (t_2; figure 1). As far as is known, bees cannot successfully overwinter as pupae; thus, bees must choose between a rapid developmental trajectory that allows them to reach adulthood in their first summer, and a slow trajectory in which they spend their first winter as a late-instar larva (prepupa). It is currently unknown what factors dictate whether an individual takes one or two years to reach adulthood.

Torchio and Tepedino (1982) proposed that parsivoltinism in *Osmia* bees might be a bet-hedging strategy (implicitly, a diversified bet-hedging strategy)—potentially an adaptation to uncertainty in the quality or quantity of the following summer's floral resources. However, as they acknowledged, the evidence from their study populations in Utah, U.S.A., did not entirely fit this interpretation, in that mean fitness would likely differ markedly between the different life-histories. In particular, prolonged dormancy was accompanied by substantial mortality risk, because bees overwintering for two years could experience siblicide from their one-year counterparts emerging from behind them in the nest (cf. *Osmia texana*, Tepedino and Frohlich 1984). Even in the absence of siblicide, prolonged summer dormancy generally decreases survival in *Osmia*, likely due to depletion of metabolic reserves (Bosch and Kemp 2004; Bosch et al. 2010; CaraDonna et al. 2018; Sgolastra et al. 2011), and increases the risk of mortality from extrinsic threats such as accidental nest destruction. Taken together, fitness of two-year

bees should be much less than that of one-year bees, making the high proportion of two-year bees in the Utah populations (as high as 57% in *O. californica*) surprising. At our higher-elevation study sites in the Rocky Mountains of Colorado, USA, several *Osmia* species, including *O. iridis*, are overwhelmingly semivoltine, with 93% taking two years to develop (Forrest and Thomson 2011)—a level that seems inconsistent with a diversified bet-hedging strategy.

Here, we hypothesize that generation time in these bees represents an adaptive response to local growing-season length or temperature (or both), with longer, warmer seasons favoring a one-year life cycle, and shorter, cooler seasons favoring a two-year life cycle. Because developing bees lack precise information on how long a given growing season will be, we expect the modal generation time to be adaptive under local, historical conditions. However, because season lengths vary from year to year, and because bees can assess some aspects of the environment (notably temperature) during development, we expect that bees may adjust their developmental trajectories in response to conditions experienced during early larval development. For example, if warmth during early development is a good indicator that the growing season will be sufficiently long and warm, bees may respond plastically by committing to a one-year life cycle. In addition, we expect that eggs laid early in the season should be more likely than those laid later to develop into bees with a one-year life cycle, since individuals that begin growth early in the season should generally have a better chance of completing development in a single year (cf. Seger and Brockmann 1987).

We examine these hypotheses first by conducting a series of laboratory experiments with field-collected *O. iridis*, to better understand the proximate determinants of generation time in these

insects. These experiments allowed us (i) to test the effects of summer temperature and duration on generation times, and (ii) to estimate the heating units (degree-days) required for bees to reach particular developmental stages. We then analyzed long-term climate records from our study area (iii) to determine the viability of one- and two-year life cycles under historic (1950–2015) climates and thereby to investigate the ultimate causes of the observed phenotype distribution—that is, to explain the existence of parsivoltinism. Finally, (iv) we used field observations of natural bee life cycles, across multiple sites and years, combined with local temperature records, to qualitatively test the ability of our developmental model to predict life-histories in the field. These experiments and observations allow us to better understand how bees decide on a developmental pathway, and even to predict future shifts in voltinism as the climate changes.

Methods

Study species and sites

Osmia iridis (Megachilidae) is a solitary bee species that is a pollen-specialist on legumes (Fabaceae, tribe Fabeae) and a common occupant of "trap-nests" in subalpine meadows around the Rocky Mountain Biological Laboratory (RMBL), Colorado, USA (Forrest and Chisholm 2017), where their nesting period extends from early June to late July or early August. Females normally nest in holes in wood such as abandoned insect burrows in trees (Cane et al. 2007) and will often construct several nests in succession (Forrest and Chisholm 2017). Nests consist of a series of brood cells, constructed sequentially over a period of several days (median = 5 d) and separated by walls of sand and macerated leaves (figure 1). Each cell contains a pollen-and-nectar provision and an egg. By providing artificial nesting holes in wood ("trap-nests") lined

with paper straws, and allowing these to be colonized by nesting females, we are able to observe bee nesting behavior and subsequent offspring development in the field, and to collect completed nests for laboratory experiments.

Six field sites around RMBL (table A1) were visited and bee nesting progress recorded every 3–6 d throughout the nesting season, which allowed us to estimate the date on which each egg was laid (see Forrest and Chisholm 2017 for detailed methods). Hourly temperature data were recorded at each of the six field sites using HOBO pendant data-loggers (Onset Computer Corp., Bourne, MA, USA; accurate to ±0.5°C) attached to the underside of one trap-nest at each site.

Incubator experiments

In the following, we use the word "bees" to refer to individuals at any stage of development, not only adults. Between 2013 and 2016, we conducted a series of three experiments with developing bees in which we manipulated either the timing of onset of winter-like temperatures (*summer-duration experiment*), or the summer temperatures experience by bees (*constant-summer-temperature* and *fluctuating-summer-temperature experiments*) (figure 2). These experiments allowed us to test whether total accumulated heat (summer duration) or thermal cues experienced early in development (summer temperature) influenced voltinism, and also to quantify the degree-day requirements for development. For the summer-duration experiment, bee nests were collected from the field at the end of summer (August 2013 and 2014); for the summer-temperature experiments, nests were collected as soon as they were completed (June–July 2015 and 2016). Developing bees were kept in the dark (i.e., without lighting in the incubators), to simulate their natural habitat. Additional details on these experiments are provided in Online Appendix A: Supplementary Methods.

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Summer-duration experiment. If summer duration is an important (proximate) determinant of generation time, we expected to observe a greater proportion of two-year bees under long-summer conditions than under shorter-summer conditions. The initial summer-duration experiment (figure 2A) took place in 2013. Completed *O. iridis* nests were divided into individual brood cells, which were placed in gelatin capsules and stored in a Fisher Scientific 307A low-temperature incubator set to 12°C, the recorded mean August–September temperature from our field sites in 2013–2015. A second incubator set at 0°C simulated "winter". Brood cells were alternately allocated to "short summer" (early winter onset) or "long summer" (late winter onset) treatments. Prior to transfer to winter conditions, bees were cooled at a rate of 4°C/d over three days. Bees in the "short summer" treatment were placed in the 0°C incubator on 13 September 2013 (t_0); "long summer" bees were placed at 0°C on 25 October. Starting on 30 April 2014, bees were warmed to 18°C for emergence. This and all subsequent transitions in and out of winter conditions were made at a rate of ~6°C/d. No bees emerged during summer 2014 (t_1); all were therefore overwintered a second time ($t_1 \rightarrow t_2$) and subsequently monitored for adult emergence (again at 18°C). In July 2015 (t_2), all remaining cocoons were opened to assess the developmental stage of their (deceased) occupants (see *Observations of development*, below). This experiment was repeated in 2014 with several minor differences (described in Online Appendix A: Supplementary Methods).

Constant-summer-temperature experiment. If summer temperature is an important (proximate) determinant of generation time, we expected to observe a greater proportion of one-year bees under warmer summer conditions than under cooler summer conditions. We conducted a 5-week, two-treatment experiment in summer 2015 (figure 2B) to test the effect of larval developmental

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temperature on voltinism. Bee nests were separated into individual brood cells, placed in gelatin capsules, and allocated to experimental treatments. Bees in these nests ranged from 0 to 24 days old (i.e., days since the egg was laid) at their time of entry into the experiment. Cells were alternately assigned to two treatments: a "cool" incubator (Fisher Scientific 307A) set to 18°C, and a "warm" incubator at 25°C. This "warm" temperature represents a typical daily maximum summer temperature in our study region; the "cool" temperature is between the typical daily maximum and the daily mean (table A1). On 24 July 2015 (t_0), the experiment was concluded and all bees were placed in the "cool" incubator. Because bees were brought to the lab on different dates, but the experiment ended on the same date for all of them, bees varied in how long they spent in their assigned experimental treatment (up to 35 d). Only bees that spent at least 7 d in their assigned treatment and that were < 11 d old at the start of the experiment were included in analysis of treatment effects on development. All bees were included in analysis of degree-day requirements for development (see *Observations of development*, below).

Fluctuating-summer-temperature experiment. If summer temperature is a key determinant of generation time, and the timing of warm temperatures is also important, then we expected to find a difference in the proportion of one-year bees between those that experienced warm temperatures early in development and those that experienced warm temperatures later. (As before, we also expected more one-year bees under generally warmer conditions.) In 2016, we conducted a 6-week experiment with four summer-temperature treatments (figure 2C). We used growth chambers (Percival E-41HO; Perry, IA, USA; henceforth "incubators") on a daily temperature cycle, mimicking field conditions more accurately than we had in 2015 (figure A1). The "warm" incubator cycled from 10°C to 35°C daily (mean temperature = 22.5°C), and the

"cool" incubator from 10°C to 25°C (mean = 17.5°C). The high temperature setting in the warm incubator (35°C) was within the range of maximum summer temperatures experienced by bee nests at our study sites (table A1). The four treatments—5 weeks warm, 3 weeks warm + 2 weeks cool, 2 weeks cool + 3 weeks warm, and 5 weeks cool—were achieved by moving bees between incubators at different times. Bees typically began the experiment on the seventh day after the egg was laid (day 7). This gave sufficient time for the nest to be completed and for us to retrieve the nest from the field and allocate cells to treatments. All bees began the experiment in the cool incubator. Bees in the 3 weeks warm + 2 weeks cool and the 5 weeks warm treatments were moved into the warm incubator on day 7, while bees in the 2 weeks cool + 3 weeks warm treatment were transferred to the warm incubator on day 21; bees in the 5 weeks cool treatment remained in the cool incubator for the duration of the experiment (figure 2C). At the end of the experiment (day 42), all bees were placed in the cool incubator.

Observations of development

To estimate the degree-days requirements for development of *O. iridis*, we monitored development rates during the summers of 2015 and 2016, using the specimens in the summer-temperature experiments (described above) as well as nests remaining in the field (2015 only). For all nests, we cut a small flap (~1 × 2 mm) at each cell in a straw, using a sterilized scalpel and microscissors, to observe the bee's developmental stage and survival with a hand-lens or dissecting microscope. These flaps were closed and covered by label tape between observations. Observations were made at every site visit for nests remaining in the field (every 3–6 days) and every 48 hours (2015) or 120 hours (2016) for nests in the laboratory. We distinguished the following developmental stages (figure A2): egg, feeding (pre-defecating) larva, defecating larva

(i.e., with frass pellets, though potentially still feeding), and cocoon (i.e., larva no longer visible through silk). Once a cocoon was completed, we left it until the end of the experimental treatments in 2015 (29 July), or for 10–14 days in 2016, before slicing a small flap in the cocoon to allow further observations.

Observations from the field and summer-temperature experiments together provide data on the approximate number of days required to reach each development stage under seven temperature regimes: (i) warm incubator (~25°C), (ii) cool incubator (~18°C), and (iii) field conditions in 2015; and (iv) 5 weeks warm cycle (10°C–35°C), (v) 3 weeks warm cycle + 2 weeks cool cycle, (vi) 2 weeks cool + 3 weeks warm cycles, and (vii) 5 weeks cool cycle (10°C–25°C) in 2016.

Analysis of experimental data

All analyses were conducted in R v. 3.4.2 (R Core Team 2017). We excluded from analysis individuals that were parasitized (mainly by sapygid wasps) or that failed to hatch. This left 53 bees from 27 nests in the 2013 summer-duration experiment, 168 bees from 45 nests in the 2014 summer-duration experiment, 82 bees from 27 nests in the 2015 constant-summer-temperature experiment, 61 bees from 19 nests in the 2016 cycling-temperature experiment, and 106 bees from 35 nests in the field in 2015.

We initially analyzed each experiment separately. For each, our primary response variable was whether or not a bee achieved a one-year life cycle (a binary variable). However, because no bees in the summer-duration experiments reached adulthood in one year (see Results), we did not analyze these data except to test whether summer-duration treatment affected survival. We did this using generalized linear mixed models (GLMMs) with binomial error structure in the R

package "lme4" (Bates et al. 2015), with source nest included as a random factor and bee status (alive or dead at the end of the first winter) as the response variable. We analyzed the effects of the summer-temperature treatments on voltinism in the same way, using GLMMs with binomial error, and we included in analysis only those bees that survived to pupation ($N = 55$ bees from 24 nests in 2015, $N = 38$ bees from 13 nests in 2016). Treatment was the primary fixed factor of interest, with 2 or 4 levels, depending on the experiment (figure 2). We also included day of year on which the egg was estimated to have been laid as a continuous covariate, to test whether eggs laid earlier in the season were more likely to undergo a one-year developmental pathway. We did not include the interaction between treatment and day of year because we had no a priori prediction of an interaction, and likelihood-ratio tests showed that it did not improve model fit. Nest identity was included as a random factor. We used Wald z-tests to evaluate significance of the two fixed effects in the constant-summer-temperature experiment. Because of the greater number of treatment levels in the fluctuating-temperature experiment, we used a likelihood-ratio test to evaluate significance of the overall treatment effect in that experiment. Differences among treatment levels were tested using the 'glht' function of the package "multcomp" (Hothorn et al. 2008). Finally, for each summer-temperature experiment, we tested whether nests differed significantly from one another using likelihood-ratio tests of models (with treatment and date-laid terms) with and without the random source-nest term.

Next, to determine the way in which heat accumulation drives bee development, we calculated the number of degree-days required to reach each developmental stage in each treatment of the summer-temperature experiments and in the field. Here, we expected that—regardless of experimental treatment—all bees would follow a similar developmental trajectory with respect to

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degree-day accumulation, provided degree-days were calculated with respect to the appropriate base (threshold) temperature. We recognize that this expectation may be simplistic, as responsiveness to temperature can vary over the course of development (Manel and Debouzie 1997; Salis et al. 2016), and development rate can respond non-linearly to temperature (Beck 1983); however, degree-day models are still widely used (e.g., Geng and Jung 2018; Sato and Sato 2015; Uelmen et al. 2016) because they generally provide a good fit to insect phenology data, despite their limitations.

For each individual, we calculated the number of degree-days (above a given base temperature) experienced by the bee from the date the egg was estimated to have been laid until the observed start of each developmental stage, i.e. accounting for the number of days each individual spent in the field before being transported to the lab. Hourly temperature data, both for field sites and incubators, were taken from data-loggers, described above. We calculated accumulated degree-days until each developmental transition using every possible integer base temperature between 5 and 15°C (following Forrest & Thomson 2011). We then fit the developmental progression of all 275 bees in our dataset to the accumulated degree-days using mixed-model ordinal logistic regression (i.e., proportional-odds mixed models, function 'clmm' in R package "ordinal"; Christensen 2015), fitted separately using each of the 11 possible base temperatures. Because our aim was to generalize across all treatments, we included each bee's source nest and treatment as random factors in the model; "treatment" here had seven levels (warm, cool, and field in 2015, plus the four fluctuating-summer-temperature treatments). We then selected the "best" base temperature as the one that yielded the model with the lowest AIC (Akaike Information Criterion) value.

Historical climate data

We compared the accumulated degree-days necessary to reach each developmental stage
(calculated as above) to long-term records (1950–2015) of daily minimum and maximum
temperatures from a U.S. National Oceanic and Atmospheric Association (NOAA; Climate Data
Online[1]) weather station (GHCND:USC00051959, Crested Butte), located ~5 km from, and ~30
m lower in elevation than, our lowest-elevation field site. We estimated mean daily temperature
using the formula of Dall'Amico and Hornsteiner (2006), which requires daily minima and
maxima, daily sunset times, and two parameters fitted from "true" daily mean temperatures, i.e.
those calculated from more frequent temperature measurements. We fitted the latter two values
using four years (2008, 2009, 2014, 2015) of nearly complete (≥362 days per year) hourly
temperature data recorded by data-loggers at our lowest-elevation field site. Because a base
temperature of 12°C provided the best fit to the bee developmental data (see Results), we used
this threshold in subsequent calculations. First, we calculated the daily degree-days above 12°C
(DD12) for each year from June 1 (day of year 152) through October 31, excluding six years in
which data were completely missing for ≥7 dates between 1 June and 30 September (two years
missing dates in October were retained, as October degree-day sums averaged <5°C). We used
linear interpolation to infer temperatures for an additional 34 dates (across the 60 remaining
years) on which one or both daily measurements were missing. We did not consider May
temperatures, as we have never observed *O. iridis* nesting earlier than 31 May, in six years of
study. We then determined, for each summer day in each year from 1950 to 2015, the number of

remaining DD12 in summer, and compared these degree-day sums to the amounts required for

O. iridis to reach the pupal stage, based on our 2015–2016 results. Finally, to test whether

heating units experienced early in development could be used by bees as reliable indicators of

the total heat they would experience in a summer, we ran a linear regression of the total summer

DD12 between the mean date *O. iridis* eggs were laid (day of year 178, 27 June; calculated from

our 2015–2016 dataset) and 31 October against DD12 in the first six weeks following the mean

date of egg-laying.

Model validation

We used data on the natural life cycle of *Osmia iridis* to qualitatively test whether the proportion

of one-year bees developing from a given year's nests can be predicted from that year's summer

temperature (specifically, total June–July degree-days above 12°C). Data on natural life-cycle

variation in *O. iridis* were available from two published sources: Torchio and Tepedino (1982)

reported voltinism of *O. iridis* from nests constructed in 1979 in two Utah, USA, populations,

and Forrest and Thomson (2011) reported data from nests constructed in 2008 in several

Colorado populations around the RMBL (approx. 500 km from the Utah populations). In

addition, two of us have separately monitored natural emergence from trap-nests established

around the RMBL, following methods of Forrest and Thomson (2011), from nests constructed in

2012, 2013, 2014, and 2015. For Colorado, we based degree-day calculations on the daily mean

temperatures calculated from the Crested Butte NOAA weather station data and corrected as

described above (the corrected means are approx. 2°C higher than the means of the recorded

daily minima and maxima). For Utah, we had to use uncorrected means of daily minima and

maxima recorded at nearby NOAA weather stations. Details on the nests and the weather stations

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are provided in Online Appendix A: Supplementary Methods. Because of methodological

inconsistencies among years and sites, we view this analysis as a qualitative test only.

All data files will be deposited in Dryad Digital Repository upon manuscript acceptance, and

voucher specimens will be deposited at the Canadian National Collection, Ottawa, Canada.

Results

Summer-duration experiment

Regardless of summer duration (figure 2A), no *Osmia iridis* in this experiment pupated in its first

summer (t_0). Despite having experienced at least 440 degree-days >12°C prior to the start of

"winter" (mean = 747 in 2013, 707 in 2014), all 131 bees that survived to pupation pupated in

their second summer or later (31 from 2013, 100 from 2014; table 1). All 66 bees that

successfully emerged as adults did so at least two winters (and two growing seasons) after larval

eclosion, i.e., in year t_2 or later (22 from 2013, 44 from 2014). Sixteen of these, all from 2014

and representing 36% of the emerging bees from that year's cohort, emerged after three years (in

2017, i.e., t_3), having pupated only after their second winter (in summer 2016). There was no

significant difference in mortality between summer-duration treatments (binomial GLMMs, p >

0.25). However, the strongly male-biased sex ratio of emerging bees in both treatments (approx.

6:1) suggests greater mortality of females during development (since the expected sex ratio for

this species is approx. 1.7:1; Torchio and Tepedino 1982). This interpretation is supported by the

fact that mortality rate (i.e., the proportion of larvae not surviving to the end of their first winter)

was correlated with brood cell position within nests, with inner brood cells (which are more often

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female) having higher mortality ($r = -0.74$, $p = 0.023$, $N = 9$ brood cell positions; 2013 and 2014 data combined).

Summer-temperature experiments

Warmer temperatures during larval development increased the likelihood of a bee reaching adulthood after a single winter (i.e., pupating in t_0). Specifically, 18 of 24 bees (75%), including both males and females, reared in constant warm conditions (25°C) pupated in year t_0, whereas 0% of their 31 siblings reared in cooler conditions (18°C) pupated in t_0 (treatment: $z = 31.1$, $N = 55$ bees from 24 nests, $p < 0.0001$; figure 3A, B). This was also true in the more realistic cycling-temperature treatments (figure 2C). Here, 7 of 11 bees (64%) in the "5 weeks warm" treatment pupated in year t_0, whereas none of those in the "5 weeks cool" treatment did so, and an intermediate proportion (25–36%) of bees that experienced 3 weeks of the warm cycle pupated in year t_0, whether the warm temperatures occurred early or late in larval development (main effect of treatment: $\chi^2 = 17.6$, $N = 38$ bees from 13 nests, $p = 0.00053$; figure 3C, D). However, because of small sample sizes, no treatments differed significantly in adjusted pairwise comparisons ($z < 2.0$, $p > 0.15$).

In both summer-temperature experiments, the date on which an egg was laid was a significant predictor of year of pupation (constant-temperature experiment: $z = -2.3$, $p = 0.022$; fluctuating-temperature experiment: $z = -2.6$, $p = 0.009$): eggs laid later in the summer were less likely to follow a one-year developmental pathway (figure 3A, C). In the constant-temperature experiment, the latter effect may simply reflect the fact that bees from later-laid eggs were subjected to the experimental treatments for less time (since the experiment ended on a fixed

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date), but experimental artefacts cannot explain this result for the fluctuating-temperature experiment.

Additionally accounting for the identity of the nest from which eggs were taken significantly improved model fit in the constant-summer-temperature experiment (χ^2 = 11.6, df = 1, p = 0.0007), but not in the fluctuating-summer-temperature experiment (χ^2 = 0.01, df = 1, p = 0.93).

Degree-day requirements

Accumulated degree-days since the estimated day on which an egg was laid provided a good fit to the bee development data (figure 4). Across all 275 bees observed in field or laboratory conditions, a base temperature of 12°C for degree-day calculation was best supported (figure A3). Bees that pupated in t_0 did so, on average, after 43 days (s.d. = 7.4) and after having accumulated 432 (s.d. = 35.2) degree-days above 12°C. These values likely overestimate the time or heat required to reach the pupal stage, because we may not have observed pupae until a few days after pupation occurred. On the other hand, additional time and heat must be necessary for bees to complete pupation and reach adulthood. We cannot properly estimate these quantities from our data, as no bees reached adulthood during our observations, but two individuals had darkened and were likely about to shed their pupal exuviae after an additional 25–30 d and 149–191 DD12 as pupae (both were observed to be adults the following spring).

Historical data

In 53% of years (32 of 60) between 1950 and 2015, at least 432 degree-days > 12°C (the amount necessary to reach the pupal stage) accumulated between 1 June and 31 October in Crested Butte, CO. However, only 20% of years (12 of 60) accumulated this much heat between 27

June—the average date on which *Osmia iridis* eggs were laid in our 2015–2016 dataset—and 31

October (figure 5). Finally, only 7% (4 years) accumulated more than 580 degree-days > 12°C

between 1 June and 31 October—an amount potentially sufficient for bees to reach adulthood

(i.e., 432 degree-days to reach the pupal stage + a minimum of 149 degree-days as a pupa). For

bees developing from eggs laid on 27 June (day of year 178), degree-days accumulated in the

first 6 weeks of life (by 8 August) strongly predicted total summer degree-days (slope = 1.6, r^2 =

0.80, n = 60 years, p < 0.0001).

Model validation

Data from six years and two study areas support the hypothesis that warmer temperatures during

the first summer (t_0) increase the likelihood of a one-year life cycle in *O. iridis* (figure 6).

Discussion

Many organisms must make developmental decisions without complete information about future

conditions, and such uncertainty can favor bet-hedging life-history strategies. Our results with

Osmia iridis are consistent with the hypothesis that the most commonly observed generation

time in our study area—two years—is adaptive in the context of the recorded historical climate,

in which most summers have been too cool or too short to support a complete generation.

Nevertheless, we find that individuals can plastically adjust their developmental schedule in

response to temperatures experienced early in life, and that those developing from eggs laid

earlier in the season are more likely to undergo a univoltine (one-year) life cycle. This plasticity

explains why a minority of bees in our study area naturally exhibit a one-year life cycle, giving

rise to the observed developmental polymorphism—parsivoltinism. Our experiments provide

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insight into the potential mechanisms underlying this life-history strategy. Individuals experiencing suboptimal developmental conditions (cool temperatures) or arising from late-laid eggs uniformly undergo a two-year life cycle, and therefore do not exhibit diversified bet-hedging. On the other hand, among individuals experiencing warmer developmental conditions and arising from early-laid eggs, most undertake a one-year life cycle, and a minority adopt the lower-risk, lower-reward two-year strategy—reflecting a diversified bet-hedging strategy.

Voltinism in *Osmia iridis* is clearly highly plastic, responding strongly to incubation temperatures during egg and larval development. This responsiveness to early-life temperatures is likely adaptive, in that early summer warmth is a strong predictor of whole-summer heating units, and, therefore, the capacity for a given growing season to support development through pupation to adulthood. A response of developmental rate to temperature is, on its own, unsurprising: ectotherms generally develop more rapidly under warmer conditions, presumably because of the temperature dependence of the underlying metabolic processes (Gillooly et al. 2002). Here, however, we show that temperature differences not only influenced developmental rates, they also set larvae on different developmental pathways, with cool temperatures causing bees to pause development at the prepupal stage, and warm temperatures causing bees to proceed directly from larval growth to pupation, entering diapause only as adults. These results also point to a limitation of the degree-day modeling approach, which treats time and temperature as equal, multiplicative components of the same currency—yet, in *O. iridis*, degree-days accumulated over a long time period (as in the long-summer treatments) did not have the same developmental effect as an equal number of degree-days experienced in a shorter interval. Furthermore, although we were able to model development to pupation as a simple function of accumulated

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heat, several bees arrested development before the pupal stage. Both results show that heat accumulation on its own is insufficient to describe or predict bee development. Our findings add to other warnings about the application of degree-day models, which are often used to forecast ectotherm responses to climate change without critical evaluation of the models' assumptions and limitations (Moore et al. 2012).

We do not know the precise mechanism by which early-life temperatures trigger the developmental switch between direct development and prepupal diapause. In many taxa, photoperiod plays an important role in such developmental decisions. For example, diapause may be initiated when a certain developmental stage coincides with a given daylength (the critical photoperiod; Danks 1987; Grevstad and Coop 2015). Yet, a role of photoperiod seems unlikely in *O. iridis*, which spends its entire pre-adult life in the darkness of a sealed nest in wood. Furthermore, the bees in our first summer-temperature experiment experienced constant temperatures, such that even indirect photoperiod cues (i.e., thermoperiod) were unavailable. Thus, we suspect non-photoperiodic mechanisms. Also, our two summer-temperature experiments yielded similar results in terms of voltinism, yet involved very different temperature regimes for the warmest and coolest treatments (figure 3); this suggests that the developmental trigger cannot have been something as simple as crossing a fixed temperature threshold. This leads us to the tentative conclusion that the insects can sense their own developmental rate (e.g., by comparing developmental progress to a temperature-independent internal clock) and make decisions about diapause stage accordingly. Biological clocks maintaining circadian rhythms are well-known in insects (Denlinger et al. 2017), but biological "calendars" that maintain seasonal

timing in the absence of photoperiod cues—though well studied in vertebrates (e.g., Åkesson et al. 2017)—are not well documented in insects (Danks 2006).

Although incubation temperatures had a strong influence on voltinism in our experiments, some variation in voltinism was still unexplained by our temperature treatments. In each iteration of the summer-temperature experiment, some bees even in the warmest treatments underwent a two-year life cycle. Environmental cues experienced in the field before the nest was brought to the lab may explain some of the variation within treatments, and the day of year on which an egg was laid appears also to be an important influence. Additional (e.g., genetic or maternal) factors—suggested by the significant effect of nest identity in the constant-summer-temperature experiment—may also play a role. Experiments that control environmental conditions from the moment of egg-laying would be required to demonstrate a role for such factors, and such experiments would be logistically challenging for *Osmia iridis*, which we are unable to rear in captivity. Nevertheless, the apparent influence of egg-laying date is intriguing, since our summer-duration experiments show that bees do not respond to season length per se. Instead, they apparently respond to calendar date—even though the larvae seemingly lack access to photoperiod cues. It seems likely that maternal cues about how much of the season remains are somehow transmitted to the offspring (perhaps via signals of maternal age, or by maternal perception of photoperiod). Indeed, maternal control of offspring diapause has been demonstrated in other insect taxa, including another megachilid bee (Parker 1979; Parker and Tepedino 1982; Tanaka 1994; Tepedino and Parker 1986).

Unexpectedly, a substantial fraction of the bees in the second overwintering experiment (i.e., from nests constructed in 2014), but none of the bees in the first overwintering experiment (from

2013 nests), took three years to reach adulthood. Incubation temperatures experienced in the second summer of development (t_1) were the same in both experiments, so these cannot explain the difference between years. Part of the explanation may again lie in the dates on which these bees were laid, since bees from 2013 were laid, on average, 12 days earlier than those from 2014. Furthermore, date laid was a marginally significant predictor of year of pupation for the bees from 2014 nests: eggs laid later in 2014 were slightly less likely to pupate in 2015 (as opposed to 2016 or 2017; $p = 0.057$ in a GLMM with date of winter onset as an additional factor). However, duration of the first summer also appears to play a role: a later winter onset in t_0 significantly increased the probability of pupation in t_1 ($p = 0.033$, $N = 100$ bees from 31 nests). This suggests that bees are able to accommodate an early onset of winter in their first year (at least if they have not begun pupation) by completing their within-cocoon larval development in their second summer. In other words, there are at least three possible developmental trajectories for these bees, all influenced by conditions in their first summer. We have no evidence that three-year life cycles occur naturally in our study area—we have never documented live but dormant bees remaining in nests at the end of their third summer (t_2)—but we suspect it may occur occasionally. A three-year life cycle has previously been documented in one other bee species, from a different family (Danforth 1999), and even more complex life cycles, with multiple alternative pathways dictated by combinations of temperature and photoperiod, have been documented in other insect orders (reviewed by Danks 1991).

The scarcity of univoltine bees from nests constructed in 2013 is surprising, given that 2013 was one of the warmest years recorded in our study area (figure 5, 6). Conditions in the two preceding years may have been responsible. Specifically, 2011 was warm (fourth line from top in

figure 5A), so it should have yielded numerous univoltine bees. However, 2012 was likely a devastating year for nesting bees in our study area, owing to drought and frost damage to flowers. Peak numbers of *Lathyrus lanszwertii* and *Vicia americana* flowers (the only pollen sources for *O. iridis*) in long-term study plots were the lowest recorded in four decades (data from J. Ogilvie and D. Inouye; Ogilvie 2017) , and few *O. iridis* nests were produced that year: nine sites that were sampled in 2012 and 2013 had, overall, 83% fewer nests in 2012 (P. CaraDonna, unpublished). Thus, the 2012 drought may have selected against phenotypes that responded strongly to warmth in 2011. If voltinism is heritable in this species, this episode of selection could have produced greater semivoltinism in the 2013 cohort. Genetic differences among populations could also contribute to the differences in voltinism between the Utah and Colorado populations (figure 6). Although the latter could be explained as purely plastic responses to environmental cues that differ between regions, we cannot rule out local adaptation.

There is another way in which the 2012 drought may have led to a greater proportion of one-year bees in that year than in 2013. In this study, we did not test for an effect of provision size (i.e., amount of pollen and nectar per larva) on voltinism. However, it seems plausible that only well-provisioned larvae would have sufficient reserves to survive an entire summer (t_1) without feeding, and, conversely, that larvae with relatively little food would pupate sooner (see Helm et al. 2017). Indeed, Danforth (1999) found that heavier *Perdita* (=*Macrotera*) *portalis* bees were more likely to remain dormant for more than one year, and Torchio and Tepedino (1982) found that *Osmia* females (the larger sex) were more likely than males to undergo a two-year life cycle. While our experiments show that differences in temperature alone are sufficient to produce variation in voltinism, provision size may also play a role in nature, and it is possible that

provisions were generally smaller in 2012 due to the scarcity of flowers. On the other hand, if floral scarcity causes bees to take longer to provision brood cells, egg-laying might tend to occur later in the season, which could decrease the proportion of one-year bees.

How will life cycles of *Osmia iridis* change as the climate continues to warm? The answer depends, in part, on how the warming is distributed seasonally. Our results suggest that warmer summers could increase the proportion of bees attempting a one-year life cycle. Provided these warmer summers are accompanied by warm autumns, we expect these one-year bees to be successful. Furthermore, if warmer springs lead to earlier emergence of adults and earlier egg-laying, we would also expect a greater proportion of bees attempting a one-year life cycle (provided mothers transmit information about day of year, rather than about maternal age). Critically, however, we also expect that environmental unpredictability under climate change will continue to favor genotypes that hedge their bets, at least partially, by producing some fraction of offspring that take two years to emerge.

The developmental plasticity demonstrated by *O. iridis* and other *Osmia* (Torchio and Tepedino 1982) may be an advantage in the cold and climatically variable high-elevation environment occupied by these bees (see Danks 1992). The evolution of parsivoltinism in these bees apparently required the insertion of a prepupal diapause into the *Osmia* life cycle, which normally includes only an adult over-wintering diapause. In our experience, other megachilids (*Megachile*, *Hoplitis*, *Stelis*, *Coelioxys*), which diapause as prepupae, are inflexibly univoltine even at these higher elevations; a secondary adult diapause has seemingly never evolved in these genera. Interestingly, carabid beetles exhibit the reverse pattern: only the lineages that ancestrally overwinter as larvae have evolved semivoltinism (by adding an adult overwintering stage) and

thus been able to colonize high latitudes and altitudes (Sota 1994). The developmental flexibility exhibited by *Osmia* may similarly have contributed to their success in these challenging environments.

Acknowledgements

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Table 1. Years of pupation and emergence for bees in summer-duration experiments. t_0 = 2013 for 2013 bees, 2014 for 2014 bees; t_3 = 2017 for 2014 bees. N = 53 bees from 27 separate nests in 2013 experiment; N = 168 bees from 45 nests in 2014 experiment. Numbers in table do not add up to the reported sample size because several individuals died after the first winter, and emerging individuals are listed under year of pupation as well as year of emergence. Individuals that died before adulthood could not be sexed.

Year of experiment initiation	Treatment	Year 0		Year 1		Year 2		Year 3[1]
		No. pupated	No. dead 1st winter	No. emerged	No. pupated	No. emerged	No. pupated	No. emerged
2013	Short summer:							
	males	0	–	0	10	7	0	0
	females	0	–	0	1	1	0	0
	unknown	0	7	0	2	1	0	0
	Long summer:							
	males	0	–	0	13	11	0	0
	females	0	–	0	3	2	0	0
	unknown	0	3	0	2	0	0	0
	2013 total:	*0*	*10*	*0*	*31*	*22*	*0*	*0*
2014	Short summer:							
	males	0	–	0	9	7	16	13
	females	0	–	0	2	2	2	0
	unknown	0	21	0	4	1	21	0
	Long summer:							

males	0	–	0	16	14	7	3
females	0	–	0	4	4	0	0
unknown	0	16	0	3	0	16	0
2014 total:	*0*	*37*	*0*	*38*	*28*	*62*	*16*

[1]We have never observed three-year life cycles in nature, but they may occur occasionally.

Figure captions

Figure 1. The two life cycles observed in *Osmia iridis*. Note the existence of an overwintering larval (prepupal) stage in the semivoltine (two-year) life cycle. Illustration by P. CaraDonna.

Figure 2. Conceptual overview of incubator experiments for *Osmia iridis* from field sites near the Rocky Mountain Biological Laboratory in Colorado, USA: (A) summer duration experiment with long and short summer-duration treatments; (B) constant-summer-temperature experiment with warm and cool summer treatments; (C) fluctuating-summer-temperature experiment with variable timing of warm and cool cycle treatments. The *x*-axis in all cases represents an approximate (hypothetical) day of year for the timing of the experiment; depending on the experiment, individual bees can be in treatments at different times. Grey lines indicate times of the experiment when bees are in the incubator, but there is no difference between treatments.

Figure 3. Pupation in first summer (year 0) as a function of treatment and date laid, in (A, B) constant-summer-temperature experiment and (C, D) fluctuating-summer-temperature experiment. Bees that pupated in year 0 have a y-axis value of 1. Panels A and C show individual datapoints, which have been jittered to reduce overlap. Panels B and D show treatment means with error bars (± 1 s.e.). "5 wks warm cycle" = five weeks on a "warm" daily cycle (35°C/10°C); "5 wks cool cycle" = five weeks on a "cool" daily cycle (25°C/10°C); "3 wks warm + 2 wks cool cycle" = three weeks on warm cycle followed by two weeks on cool cycle; "2 wks cool + 3 wks warm cycle" = two weeks on cool cycle followed by three weeks on warm cycle. Lines (with 95% confidence intervals) represent binomial fits to the model *Pupation.yr.0 ~ Day.of.year.laid*. N = 55 bees from 24 nests in A and B; N = 38 bees from 13 nests in C and D.

Effects of treatment and day of year are significant in mixed-effects logistic models including both fixed factors and nest identity as a random term.
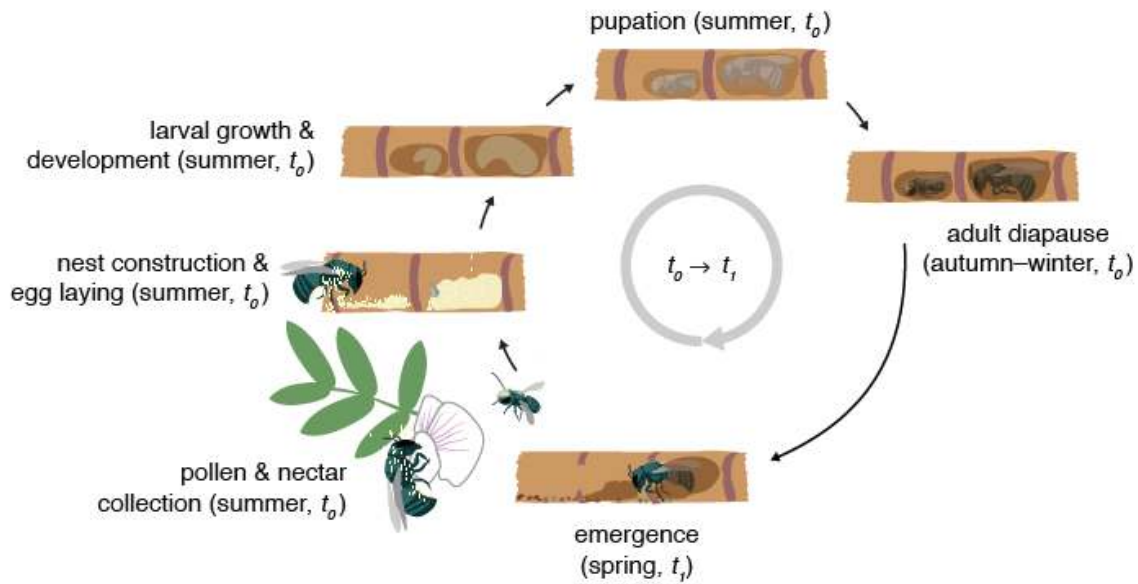
Figure 4. Developmental progression of *Osmia iridis* as a function of days since egg was laid (two upper rows of panels), and degree-days above a base temperature of 12°C (two lower rows of panels). Each line represents a single bee and starts at the beginning of development (the day on which the egg is estimated to have been laid) and extends until death or until the last observation of the summer (whichever came first). Datapoints have been jittered by 0.5 days (upper panels) or 5 degree-days (lower panels) to reduce overlap. The two top-left panels of each group include the data from all treatments, showing that developmental trajectories are similar across rearing conditions when plotted as a function of accumulated degree-days. The remaining panels show data for each treatment separately. "Field" bees experienced ambient outdoor temperatures of 13.9 ± 6.8°C (mean ± s.d.); the remaining bees were subjected to experimental rearing temperatures in the laboratory (treatments as in figure 3). Sample sizes are (in parentheses): field (106), cool (50), 5 weeks cool cycle (16), 2 weeks cool cycle + 3 weeks warm (19), 3 weeks warm cycle + 2 weeks cool (18), 5 weeks warm cycle (19), warm (47).

Figure 5. Seasonal patterns of heat accumulation and oviposition in *Osmia iridis*. (A) Long-term (1950–2015) variation in summer heat sums, based on data from the NOAA Crested Butte weather station, located near our lowest-elevation field site. Solid grey lines represent accumulated degree-days above a base temperature of 12°C (DD12) from 1 June (day of year 152) to 1 November (day of year 305) in each of 60 years; the uppermost (warmest) year is 2012, and the lowest (coolest) is 1975. Thicker black lines represent heat accumulation in the years for which we have local data on bee voltinism (figure 6); 2015 is represented by a dashed line to
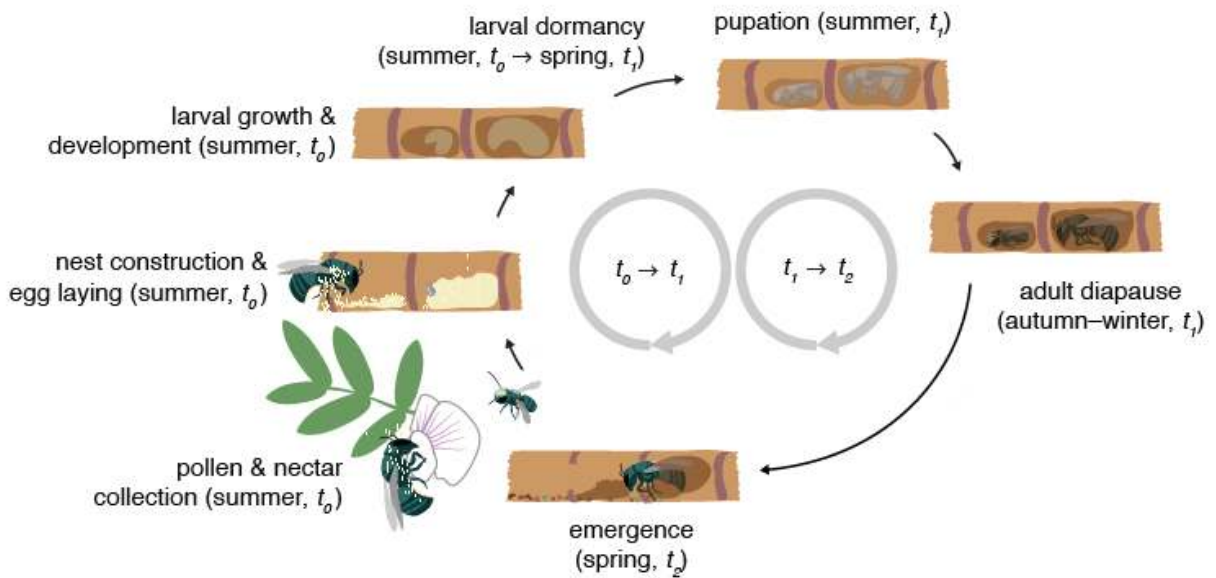
distinguish it from the other years. The dotted horizontal line indicates 432 DD12; the dashed

horizontal line indicates 493 DD12—the total amount of summer heat required for a bee that

began life on day of year 178 (the mean date *O. iridis* eggs were laid in our 2015–2016 dataset)

and thus missed, on average, 61 DD12 in early June. Note that this is the estimated amount of

heat required to reach the pupal stage; additional heat would be required to reach adulthood. (B)

The distribution of dates on which *O. iridis* eggs were laid in summer 2015 at our six field sites.

(C) The fraction of years (out of 60) in which sufficient heat (DD12) could be accumulated for

an individual *O. iridis* to reach the pupal stage, if it began heat accumulation on a given day of

the year. Filled points represent the proportion of years with ≥432 DD12. The lower dashed line

represents the fraction of years with ≥502 DD12 (= mean DD12 to pupation + 2 s.d.); the upper

dashed line represents the fraction of years with ≥362 DD12 (= mean DD12 to pupation − 2 s.d.).

Figure 6. Proportion of *Osmia iridis* bees exhibiting a one-year life cycle as a function of

summer heat accumulation in the first year of development. Data are from several sources: 1979

datapoint (dark gray circle) is from Utah (Torchio and Tepedino 1982); datapoints from 2008

and later (light gray circles) are from Colorado (Forrest and Thomson 2011 and this study; see

text for details). Bubble size is roughly proportional to the number of bees observed (i.e., sample

size), with the smallest bubble representing 13 bees and the largest representing 555 bees.
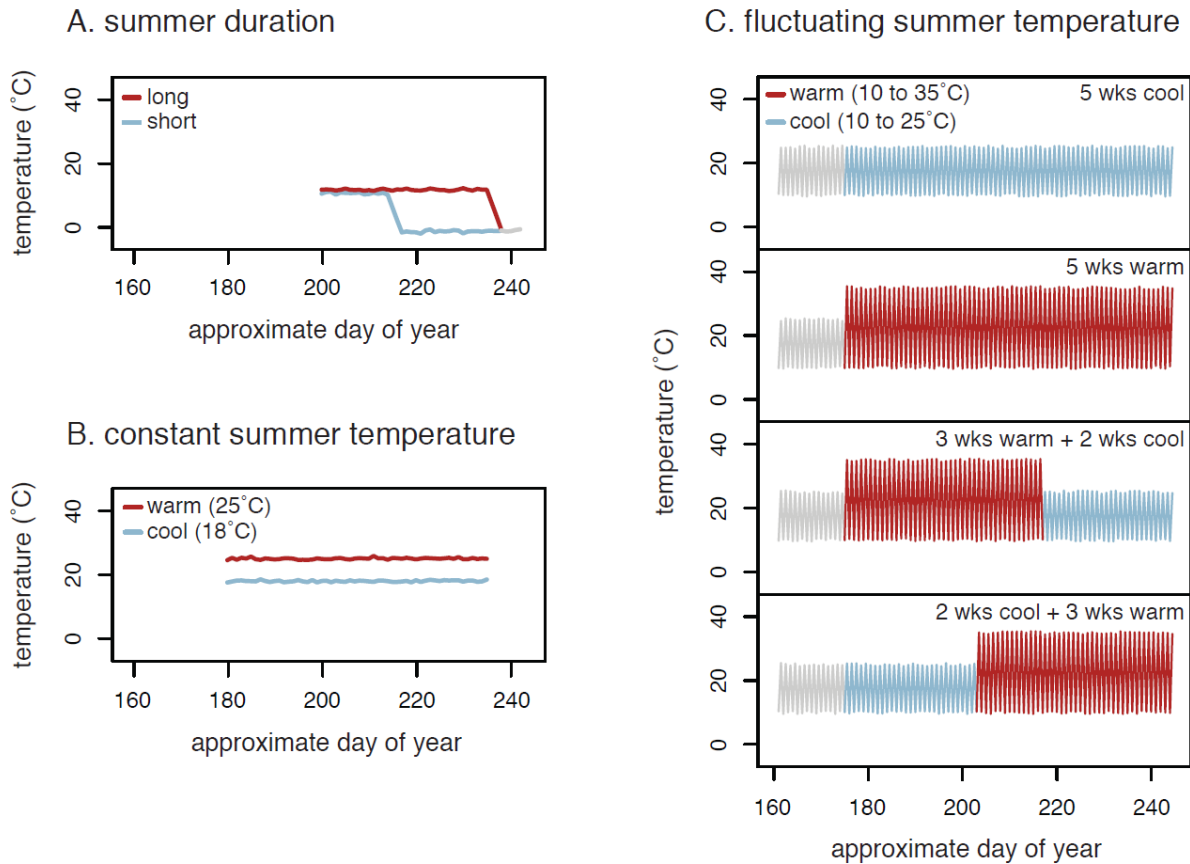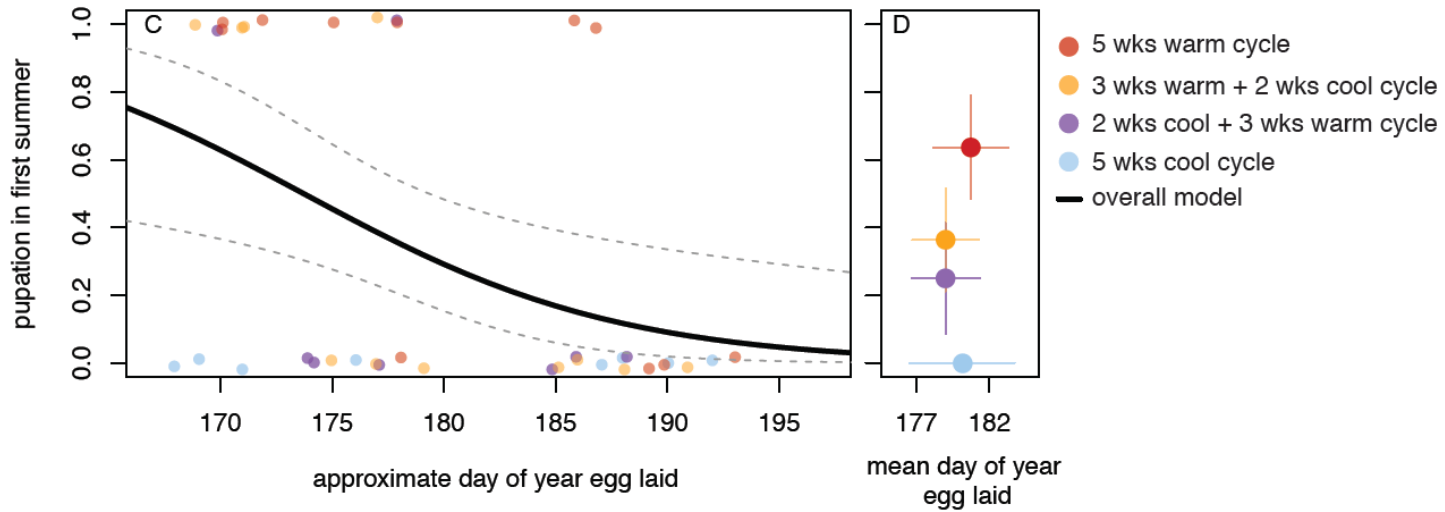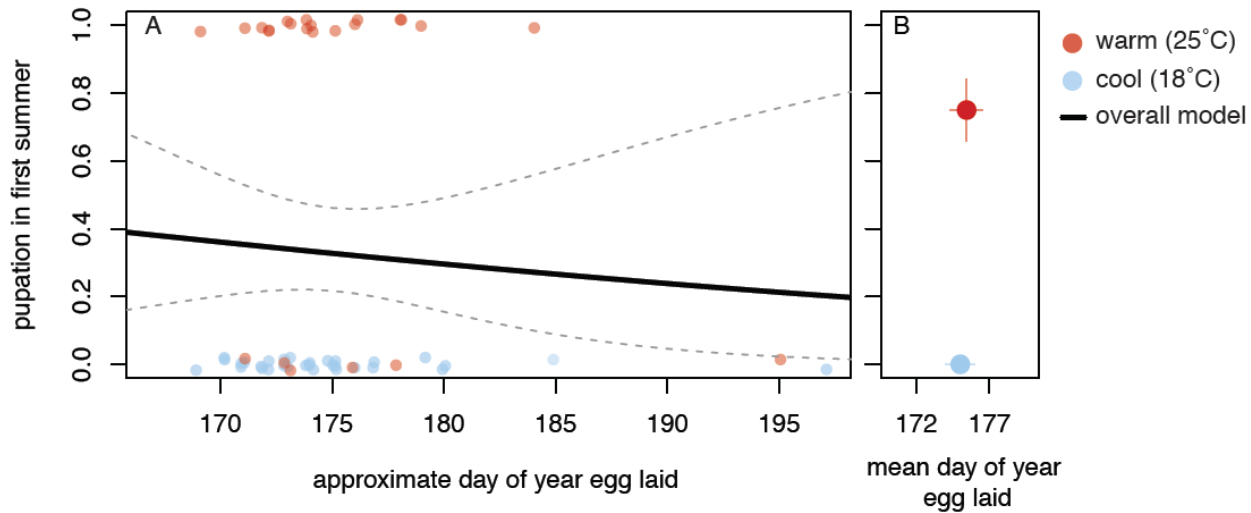
Univoltine life cycle

pupation (summer, t_0)

larval growth & development (summer, t_0)

nest construction & egg laying (summer, t_0)

$t_0 \to t_1$

adult diapause (autumn–winter, t_0)

pollen & nectar collection (summer, t_0)

emergence (spring, t_1)

Semivoltine life cycle

larval dormancy (summer, $t_0 \to$ spring, t_1)

pupation (summer, t_1)

larval growth & development (summer, t_0)

nest construction & egg laying (summer, t_0)

$t_0 \to t_1$ $t_1 \to t_2$

adult diapause (autumn–winter, t_1)

pollen & nectar collection (summer, t_0)

emergence (spring, t_2)

1

2 **Figure 1**. The two life cycles observed in *Osmia iridis*. Note the existence of an overwintering

3 larval (prepupal) stage in the semivoltine (two-year) life cycle. Illustration by P. CaraDonna.
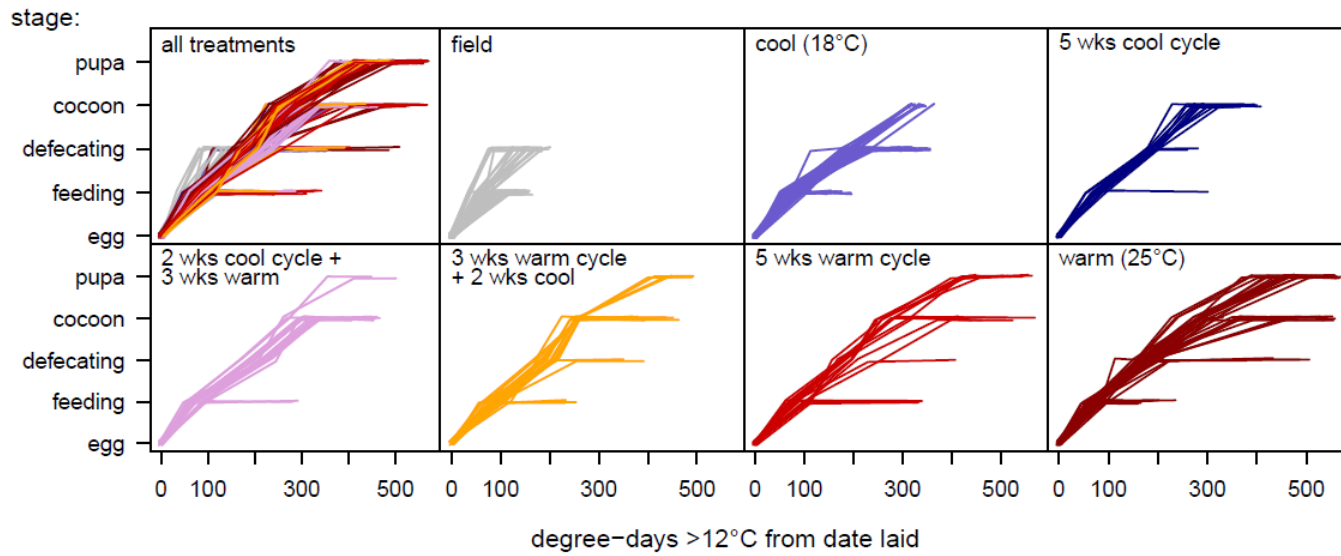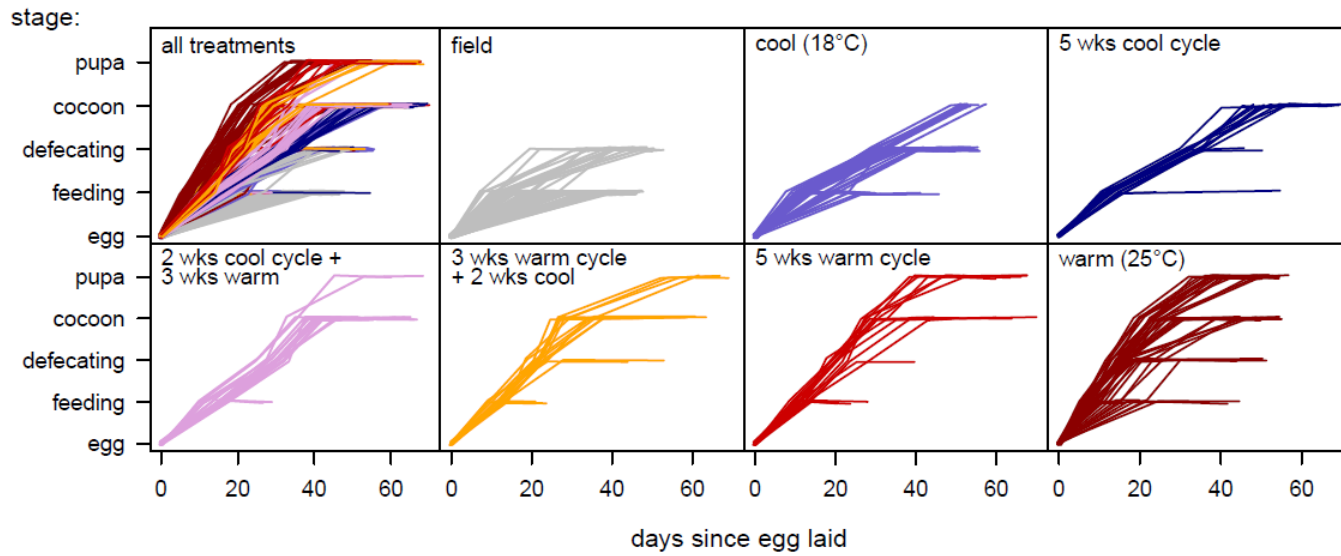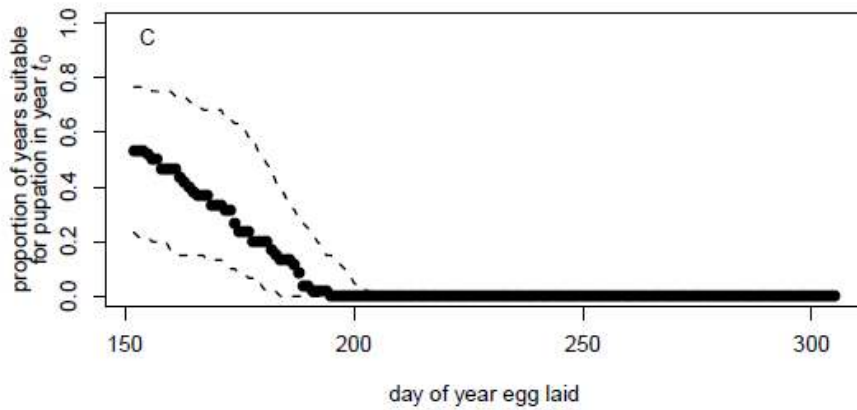
4

A. summer duration

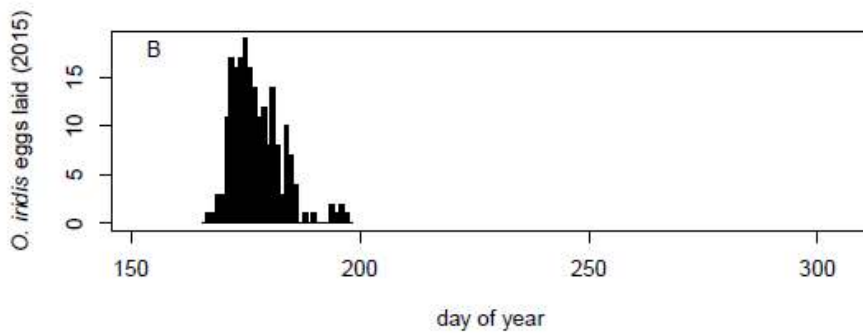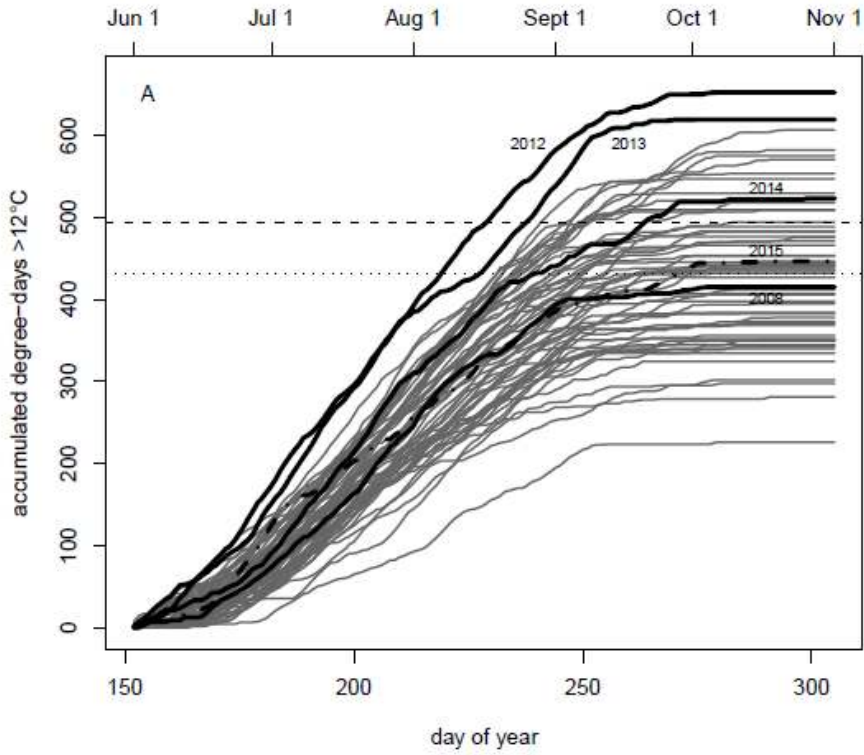B. constant summer temperature

C. fluctuating summer temperature

5

Figure 2. Conceptual overview of incubator experiments for *Osmia iridis* from field sites near

the Rocky Mountain Biological Laboratory in Colorado, USA: (A) summer-duration experiment

with long and short summer-duration treatments; (B) constant-summer-temperature experiment

with warm and cool summer treatments; (C) fluctuating-summer-temperature experiment with

variable timing of warm and cool cycle treatments. The *x*-axis in all cases represents an

approximate (hypothetical) day of year for the timing of the experiment; depending on the

experiment, individual bees can be in treatments at different times. Grey lines indicate times of

the experiment when bees are in the incubator, but there is no difference between treatments.
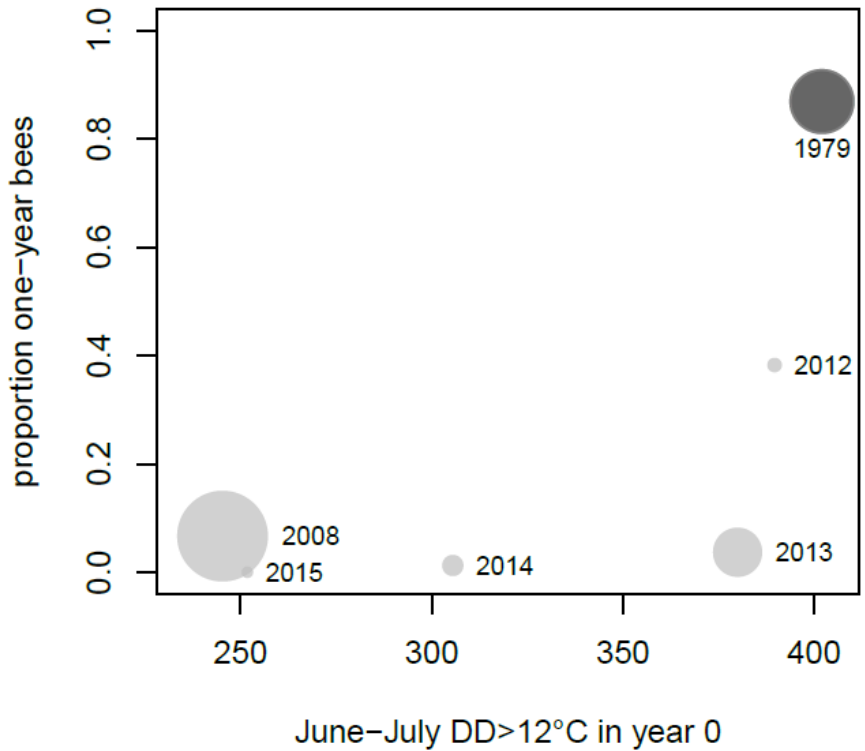
15   **Figure 3**. Pupation in first summer (year 0) as a function of treatment and date laid, in (A, B) constant-summer-temperature

16   experiment and (C, D) cycling-summer-temperature experiment. Bees that pupated in year 0 have a y-axis value of 1. Panels A and C

17   show individual datapoints, which have been jittered to reduce overlap. Panels B and D show treatment means with error bars ($\pm$ 1

18   s.e.). "5 wks warm cycle" = five weeks on a "warm" daily cycle (35°C/10°C); "5 wks cool cycle" = five weeks on a "cool" daily cycle

19   (25°C/10°C); "3 wks warm + 2 wks cool cycle" = three weeks on warm cycle followed by two weeks on cool cycle; "2 wks cool + 3

20   wks warm cycle" = two weeks on cool cycle followed by three weeks on warm cycle. Lines (with 95% confidence intervals) represent

21   binomial fits to the model *Pupation.yr.0 ~ Day.of.year.laid*. N = 55 bees from 24 nests in A and B; N = 38 bees from 13 nests in C and

22   D. Effects of treatment and day of year are significant in mixed-effects logistic models including both fixed factors and nest identity as

23   a random term.

stage:

all treatments | field | cool (18°C) | 5 wks cool cycle
2 wks cool cycle + 3 wks warm | 3 wks warm cycle + 2 wks cool | 5 wks warm cycle | warm (25°C)

days since egg laid

stage:

all treatments | field | cool (18°C) | 5 wks cool cycle
2 wks cool cycle + 3 wks warm | 3 wks warm cycle + 2 wks cool | 5 wks warm cycle | warm (25°C)

degree−days >12°C from date laid

24

Figure 4. Developmental progression of *Osmia iridis* as a function of days since egg was laid (two upper rows of panels), and degree-days above a base temperature of 12°C (two lower rows of panels). Each line represents a single bee and starts at the beginning of development (the day on which the egg is estimated to have been laid) and extends until death or until the last observation of the summer (whichever came first). Datapoints have been jittered by 0.5 days (upper panels) or 5 degree-days (lower panels) to reduce overlap. The two top-left panels of each group include the data from all treatments, showing that developmental trajectories are similar across rearing conditions when plotted as a function of accumulated degree-days. The remaining panels show data for each treatment separately. "Field" bees experienced ambient outdoor temperatures of 13.9 ± 6.8°C (mean ± s.d.); the remaining bees were subjected to experimental rearing temperatures in the laboratory (treatments as in figure 3). Sample sizes are (in parentheses): field (106), cool (50), 5 weeks cool cycle (16), 2 weeks cool cycle + 3 weeks warm (19), 3 weeks warm cycle + 2 weeks cool (18), 5 weeks warm cycle (19), warm (47).

Figure 5. Seasonal patterns of heat accumulation and oviposition in *Osmia iridis*. (A) Long-term

(1950–2015) variation in summer heat sums, based on data from the NOAA Crested Butte

weather station, located near our lowest-elevation field site. Solid grey lines represent

accumulated degree-days above a base temperature of 12°C (DD12) from 1 June (day of year

152) to 1 November (day of year 305) in each of 60 years; the uppermost (warmest) year is 2012,

and the lowest (coolest) is 1975. Thicker black lines represent heat accumulation in the years for

which we have local data on bee voltinism (figure 6); 2015 is represented by a dashed line to

distinguish it from the other years. The dotted horizontal line indicates 432 DD12; the dashed

horizontal line indicates 493 DD12—the total amount of summer heat required for a bee that

began life on day of year 178 (the mean date *O. iridis* eggs were laid in our 2015–2016 dataset)

and thus missed, on average, 61 DD12 in early June. Note that this is the estimated amount of

heat required to reach the pupal stage; additional heat would be required to reach adulthood. (B)

The distribution of dates on which *O. iridis* eggs were laid in summer 2015 at our six field sites.

(C) The fraction of years (out of 60) in which sufficient heat (DD12) could be accumulated for

an individual *O. iridis* to reach the pupal stage, if it began heat accumulation on a given day of

the year. Filled points represent the proportion of years with ≥432 DD12. The lower dashed line

represents the fraction of years with ≥502 DD12 (= mean DD12 to pupation + 2 s.d.); the upper

dashed line represents the fraction of years with ≥362 DD12 (= mean DD12 to pupation − 2 s.d.).

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Figure 6. Proportion of *Osmia iridis* bees exhibiting a one-year life cycle as a function of

summer heat accumulation in the first year of development. Data are from several sources: 1979

datapoint (dark gray circle) is from Utah (Torchio and Tepedino 1982); datapoints from 2008

and later (light gray circles) are from Colorado (Forrest and Thomson 2011 and this study; see

text for details). Bubble size is roughly proportional to the number of bees observed (i.e., sample

size), with the smallest bubble representing 13 bees and the largest representing 555 bees.

Supplementary Methods

Temperature settings for incubator experiments. Starting in August 2014, a HOBO data-logger in each incubator was used to verify that actual internal temperatures did not deviate by more than 2°C (on average) from the set temperatures; unless otherwise indicated, reported temperatures for the summer-duration and constant-summer-temperature experiments are the set temperatures. For the constant-summer-temperature experiment, the "cool" incubator set at 18°C had an actual mean temperature (± s.d.) of 18.6 (± 0.2)°C; the "warm" incubator at 25°C had an actual mean temperature of 25.2 (± 1.6)°C. For the fluctuating-summer-temperature experiment, each chamber was set to 10°C at 06:00, ramped to its maximum at 15:00, and ramped back down to reach 10°C at 06:00 (figure A1). Realized temperatures in both incubators were within 0–0.4°C of their set temperatures at their minima and maxima.

Summer-duration experiments: The nests used in the 2013 experiment were completed between 12 June and 21 July 2013 and were brought to the lab between 20 and 22 August. No bees from the 2013 experiment emerged during summer 2014 (t_1); all were therefore overwintered a second time from 1 October 2014 (t_1) to 8 June 2015 (t_2) and subsequently monitored for adult emergence (again at 18°C). Bee positions within incubators were rotated weekly throughout each winter.

For the 2014 iteration of the experiment, nests were completed between 18 June and 14 August 2014 and were brought to the lab between 5 and 20 August. Brood cells were stored in an 18°C incubator upon collection from the field (instead of 12°C, as in the 2013 experiment) and then

alternately assigned to short or long summer treatments. For the purposes of another study, these bees were further allocated, in a factorial design, to an early (9 May 2015) or late (8 June) spring-onset treatment; we do not consider the spring treatments here. Bees were cooled at 6°C/d to their winter temperature (0°C) starting on 29 September 2014 for the short-summer treatment and 29 October for the long-summer treatment. Bees were checked weekly to assess developmental stage (via small flaps cut in cocoons) in summer 2015 (t_1). They were then overwintered a second time from 15 October 2015 (t_1) to 15 June 2016 (t_2), transferred to a 20°C incubator, and again monitored for adult emergence. Developmental status was assessed once in June 2016 (t_2) and again in late August for bees that had not yet emerged.

Summer-temperature experiments: For the constant-summer-temperature experiment, laboratory observations began on the day we collected each nest from the field (starting on 19 June 2015), and finished on 14 August 2015, when we left the field station. These nests were completed between 18 June and 15 July 2015 (the first half of the nesting period for that year, during which 86% of eggs were laid) and were brought to the lab as soon as possible once complete, between 19 June and 17 July. On 24 July 2015 (t_0), the experiment was concluded and all bees were placed in the "cool" incubator, where they stayed until 13 October, at which point temperature was gradually lowered to 0°C (as in the summer-duration experiment). Starting on 13 June 2016 (t_1), these bees were gradually raised to 20°C for emergence; on 24 June they were transferred to a growth chamber on a 25°C–10°C cycle (as in the fluctuating-temperature experiment).

The four treatments for the 2016 fluctuating-temperature experiment (5 weeks cool cycle, 5 weeks warm cycle, 3 weeks warm + 2 weeks cool, and 2 weeks cool + 3 weeks warm) were chosen based on the results from the 2015 constant-temperature experiment. Specifically, we

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expected the first 3 weeks of the fluctuating-temperature experiment (i.e., days 7–28) to correspond roughly with the egg-to-defecating-larva phase of development. We expected the latter 3 weeks (days 21–42) to correspond with the defecation-to-cocoon phase, including potential pupation.

For the fluctuating-temperature experiment, observations began on 26 June 2016 and ended on 25 August 2016. Nests for this experiment were completed between 16 June and 13 July 2016 (the first half of the nesting period for that year, during which 87% of eggs were laid) and were brought indoors as soon as possible, between 21 June and 18 July 2016. Bees older than 7 days when brought to the lab were randomly assigned to either the 2 weeks cool + 3 weeks warm or the 5 weeks cool treatments. These were excluded from analysis of treatment effects but included in analysis of degree-day requirements for development. On 25 August 2016, all bees in the fluctuating-temperature experiment were transferred (along with all remaining bees from the 2015 constant-summer-temperature experiment) to an opaque container in a shaded outdoor location for overwintering. Bees from both summer-temperature experiments were returned to the "cool" cycling incubator (25°C–10°C) on 10 June 2017.

For both experiments, bee status (development stage and survival) was assessed at the beginning (early June) and end (mid- to late August) of the second summer (t_1), and bee emergence was monitored throughout the summer.

Model validation: We used data on the natural life cycle of *Osmia iridis* to qualitatively test whether the proportion of one-year bees developing from a given year's nests can be predicted from that year's summer temperature. Data on natural life-cycle variation in *O. iridis* were available from two published sources: Torchio and Tepedino (1982) reported voltinism of *O.*

3

iridis from nests constructed in 1979 in two Utah, USA, populations (separated by 33 km), and

Forrest and Thomson (2011) reported data from nests constructed in 2008 in several Colorado

populations around the RMBL (all within 20 km of one another and approx. 500 km from the

Utah populations). In addition, two of us have separately monitored natural emergence from

trap-nests established around (within 16 km of) the RMBL, following methods of Forrest and

Thomson (2011), from nests constructed in 2012 (P.J.C.), 2013 (J.F. & P.J.C.), 2014 (J.F.), and

2015 (J.F.). We included in the 2013 and 2014 data bees used in the summer-duration

experiments, as these had experienced most of the summer in the field but emerged indoors

(similar to the 1979 bees; Torchio and Tepedino 1982). For some of these sources, we do not

have information on dates when individual nests were constructed, or the nest(s) to which

individual emerging bees belonged. In addition, for nests that were not brought to the lab (all

those from 2008, 2012, and 2015, and a portion of those from 2013 and 2014), our observations

are only of bees that survived to adulthood and emergence; i.e., the values for those years may

underestimate the number of bees that attempted to pupate in t_0. For Colorado, we based degree-

day calculations on the daily mean temperatures calculated from the Crested Butte NOAA

weather station data and corrected as described above (the corrected means are approx. 2°C

higher than the means of the recorded daily minima and maxima). For Utah, we had to use

uncorrected means of daily minima and maxima recorded at nearby NOAA weather stations. We

chose four weather stations, each located 51 km or less from both Utah study sites, to

collectively represent the climate at the two sites (Hardware Ranch, USC00423671; Logan Utah

St U, USC00425186; Logan 5 SW Experimental Farm, USC00425194; and Pineview Dam,

USC00426869). We used these four stations because the two stations nearest to the sites (within

20 km) were >130 m lower in elevation, while weather stations slightly further away were more similar in elevation (within 105 m). For each day in 1979, we calculated the mean daily temperature across all four sites, and used these across-site mean temperatures to calculate degree-days.

Table A1. Sites at which trapnests were established around the Rocky Mountain Biological Laboratory, and summer temperatures at those sites, as recorded by HOBO pendant data-loggers. Temperatures are reported as ranges, across available years of data, of means, mean daily minima, mean daily maxima, and summer maxima.

| Site name	Site code	Latitude (N)	Longitude (W)	Elevation (m)	Years of temp. data	Mean temp. (°C), June–Aug.	Mean daily min. (°C), June–Aug.	Mean daily max. (°C), June–Aug.	Max. temp. (°C), June–Aug.
Brush Creek	BC	38° 51.656′	106° 55.177′	2730	2013–2016	13.5–14.8	4.7–5.9	23.8–25.0	29.9–32.5
Mt. Crested Butte	MCB	38° 53.324′	106° 57.722′	2870	2013–2015	13.8–14.7	5.1–6.2	27.6–30.0	38.4–41.9
Rosy Point	RP	38° 55.966′	106° 58.122′	2880	2013–2016	13.3–14.8	4.2–5.8	23.5–26.7	29.5–34.3
Kebler Clearing	KC	38° 51.48′	107° 3.66′	2960	2014–2016	13.0–13.3	5.0–5.7	24.6–26.9	32.9–40.4
401 Trail	FT	38° 58.247′	106° 59.434′	3020	2013–2016	12.9–14.3	4.9–6.1	24.6–28.0	33.3–36.9
Kebler Pass	KP	38° 51.15′	107° 6.06′	3040	2014–2016	13.3–13.6	4.5–5.7	24.3–27.4	32.2–34.9
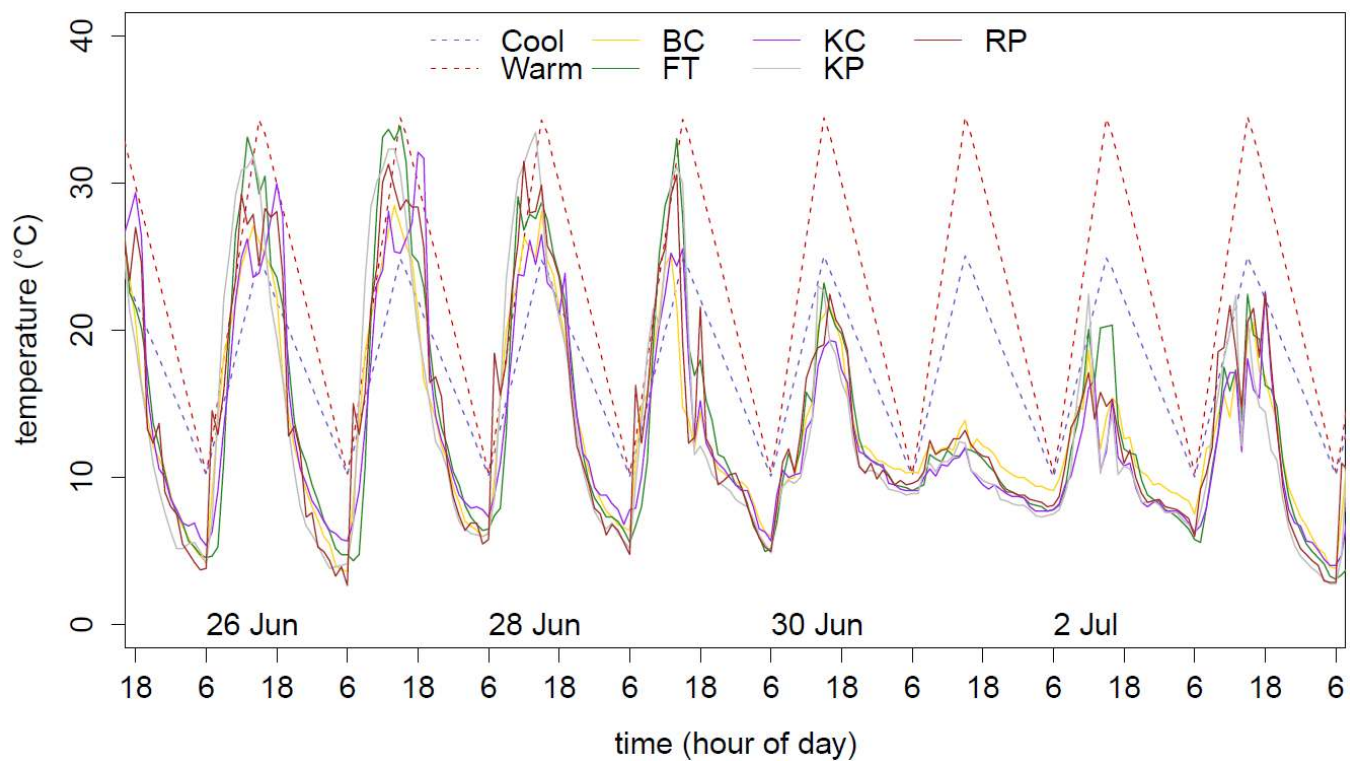
Figure A1. Temperatures recorded hourly by HOBO data-loggers at five field sites and two growth chambers ("cool" and "warm") between 26 June and 4 July 2016, illustrating typical diel temperature variation.
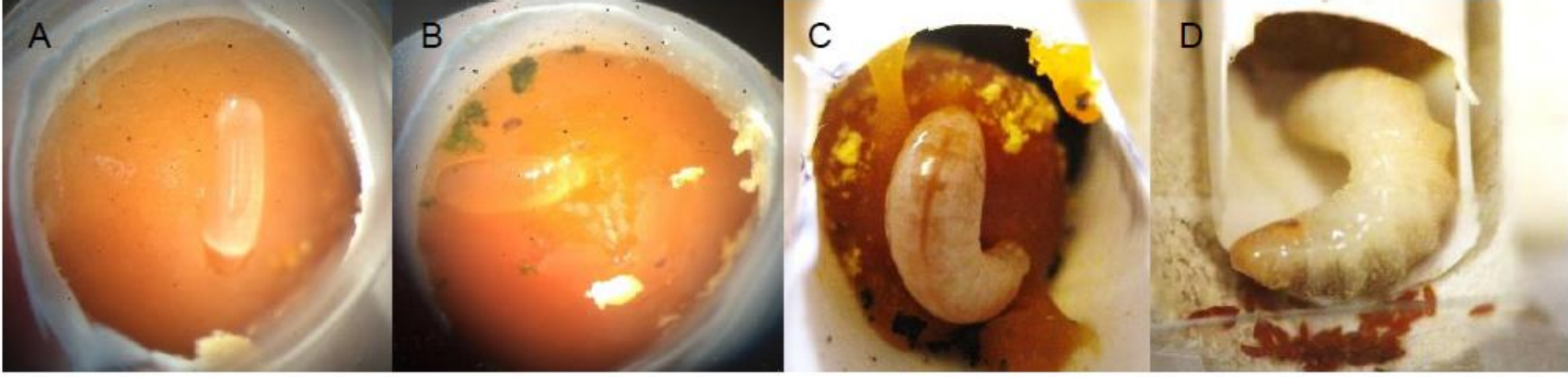
Figure A2. Photographs of *Osmia iridis* at different developmental stages. (a) Egg, (b) young feeding larva, (c) older feeding (but still pre-defecating) larva, and (d) defecating larva (note brown frass in foreground). Photos (a) and (b) by R. Cross, (c) by J. Forrest, (d) by M. McAulay. Photos (c) and (d) were taken during a separate study.
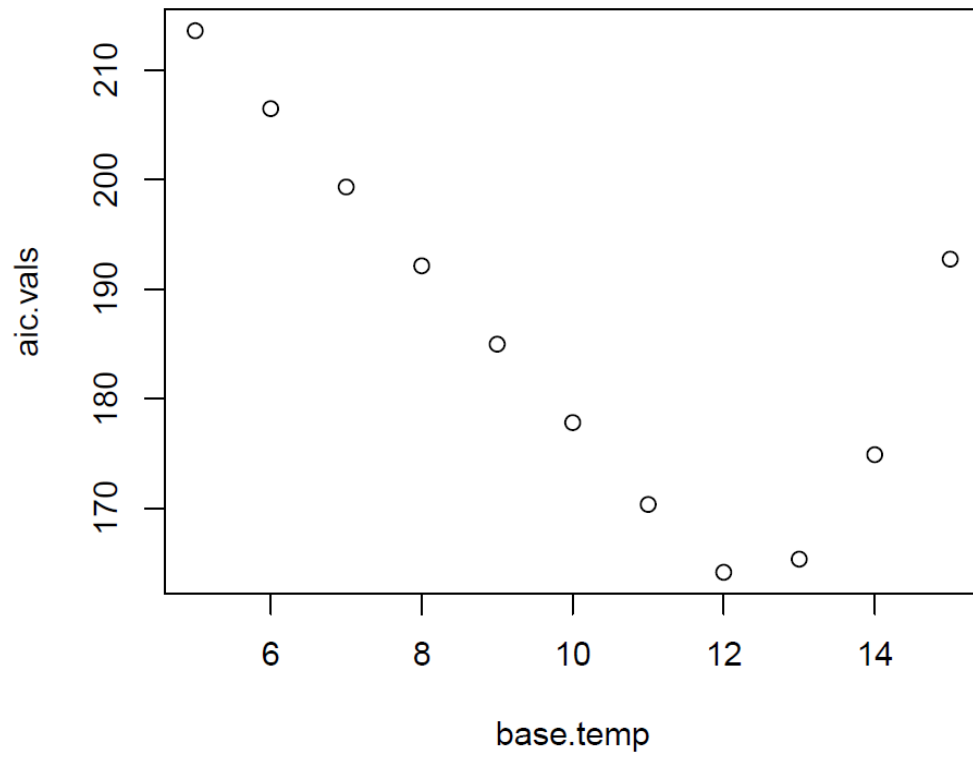
Figure A3. AIC (Akaike Information Criterion) values for mixed-model ordinal logistic

regression models of *O. iridis* development (developmental stage vs. accumulated degree-days),

as a function of base temperature (in °C) used for degree-day calculation.