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 1- and 2-year life cycles. One-year bees reach adulthood quickly but die if they fail to complete pupation before winter; 2-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 birthdates increased the frequency of 1-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 1-year life cycles—particularly those developing from late-laid eggs—will be <100%; consequently, we expect the life cycle polymorphism to persist.

Keywords: adaptive plasticity, bet hedging, cohort splitting, Megachilidae, thermal time, voltinism.

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, 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 (Rathcke and Lacey 1985; Tauber et al. 1986; Gyllström and Hansson 2004).

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; Slatkin 1974; Seger and Brockmann 1987; Philippi and Seger 1989). 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 (Seger and Brockmann 1987; Philippi and Seger 1989; 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 become warmer and longer (IPCC 2014). These novel conditions may allow short-lived ectotherms to complete more generations per year because of both the prolonged opportunity for growth and the faster growth rates that can be achieved under warmer

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conditions (Deutsch et al. 2008). Indeed, in many insects, voltinism—the number of generations per year—does respond to changes in temperature (Neff and Simpson 1992; Altermatt 2010; Buckley et al. 2015), and the number of generations per year has increased in recent decades (reviewed in Forrest 2016). Similarly, longer growing seasons may allow organisms that currently develop over more than 1 year to compress their life cycle into a single year. However, a short-term (plastic) response to longer summers requires (1) that organisms receive cues indicating that the season will be long enough and (2) 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 (Fye 1965; Torchio and Tepedino 1982; Forrest and Thomson 2011). Intriguingly, some populations—and even some individual nests—display both 1- and 2-year life cycles, a phenomenon termed "parsivoltinism" (Torchio and Tepedino 1982). A mason bee that pursues a typical 1-year (univoltine) life cycle hatches and develops into an adult within one growing season (year t_0) and then remains in its cocoon through the winter and emerges the following spring (t_1; fig. 1). In a 2-year (semivoltine) life cycle, the bee completes larval development in the first growing season (t_0) and then pupates and develops into an adult the following summer (t_1), emerging the next spring (t_2; fig. 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 1 or 2 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 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 2 years could experience siblicide from their 1-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 because of depletion of metabolic reserves (Bosch and Kemp 2004; Bosch et al. 2010; Sgolastra et al. 2011; CaraDonna et al. 2018) and increases the risk of mortality from extrinsic threats, such as accidental nest destruction. Taken together, fitness of 2-year bees should be much less than that of 1-year bees, making the high proportion of 2-year bees in the Utah populations (as high as 57% in *Osmia californica*) surprising. At our higher-elevation study sites in the Rocky Mountains of Colorado, several *Osmia* species, including *Osmia iridis*, are overwhelmingly semivoltine, with 93% taking 2 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 1-year life cycle and shorter, cooler seasons favoring a 2-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 1-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 1-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 (1) to test the effects of summer temperature and duration on generation times and (2) 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 (3) to determine the viability of 1- and 2-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, (4) 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

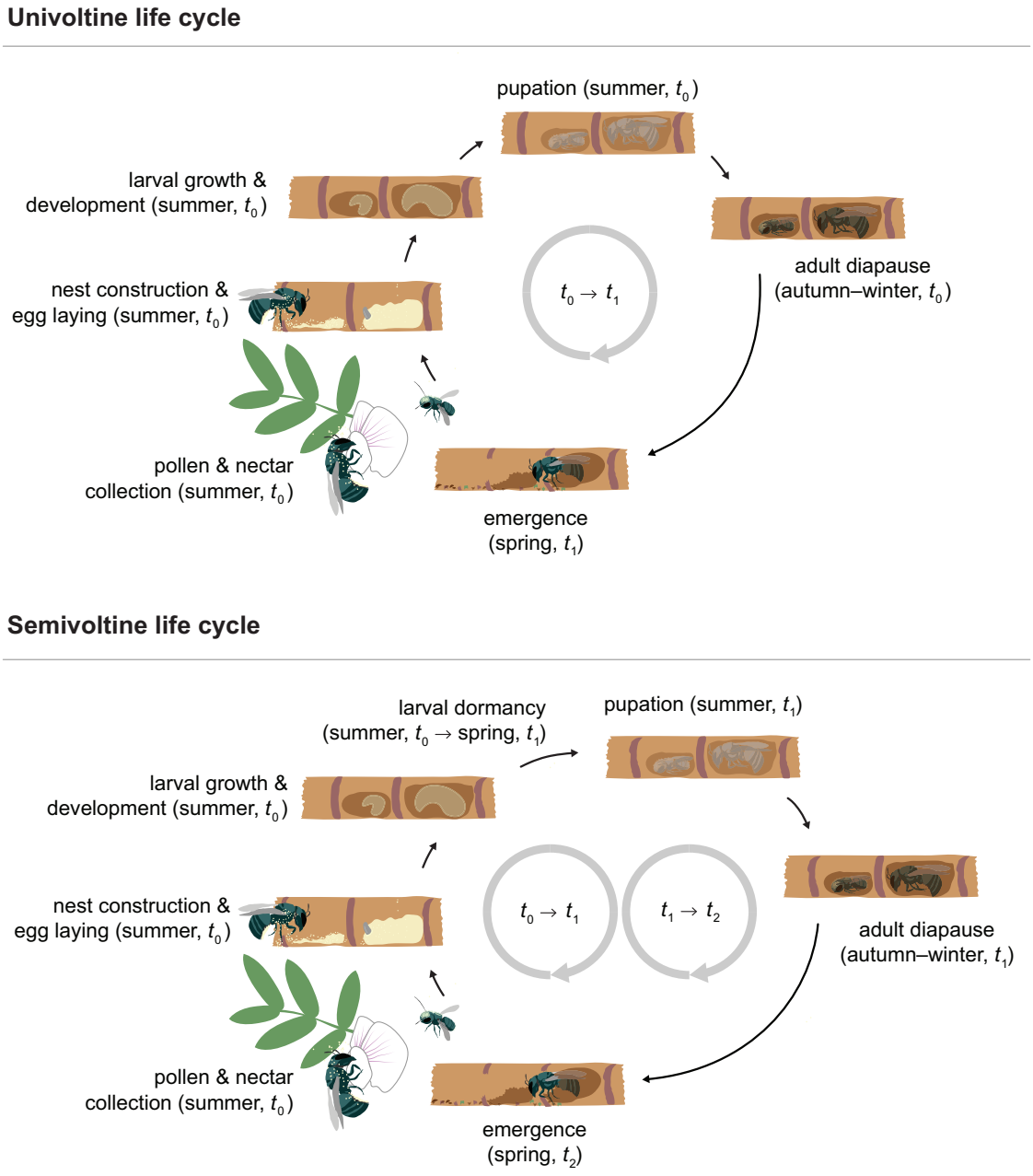

Figure 1: Two life cycles observed in *Osmia iridis*. Note the existence of an overwintering larval (prepupal) stage in the semivoltine (2-year) life cycle. Illustration by P. J. CaraDonna.

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 Rocky Mountain Biological Laboratory (RMBL), Colorado (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 sep-

arated by walls of sand and macerated leaves (fig. 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, available online) 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 (for detailed methods, see Forrest and Chisholm 2017). Hourly temperature data were recorded at each of the six field sites using HOBO pendant data loggers (Onset Computer, Bourne, MA; 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; fig. 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 the appendix (available online).

Summer Duration Experiment. If summer duration is an important (proximate) determinant of generation time, we

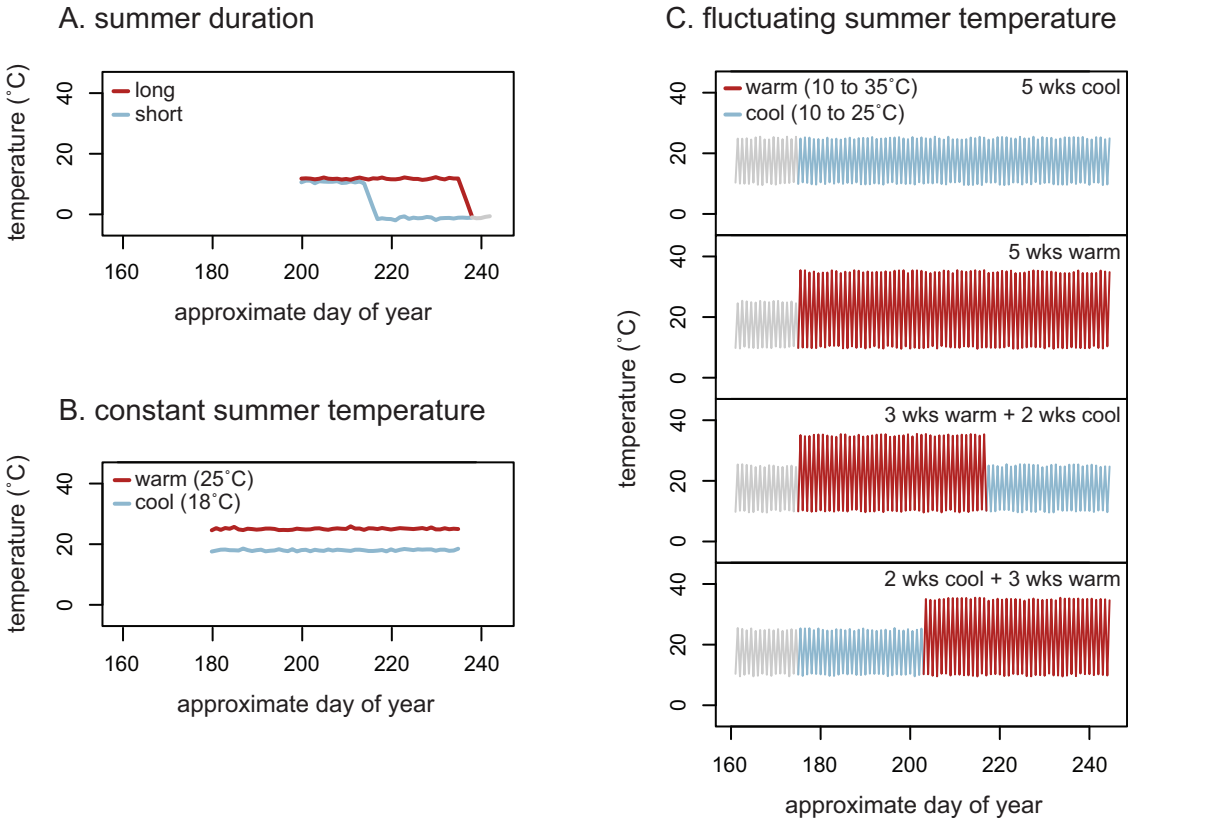

Figure 2: Conceptual overview of incubator experiments for *Osmia iridis* from field sites near Rocky Mountain Biological Laboratory in Colorado. *A*, Summer duration experiment with long and short summer duration treatments. *B*, Constant summer temperature experiment with warm and cool summer treatments. *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. Gray lines indicate times of the experiment when bees are in the incubator but there is no difference between treatments.

expected to observe a greater proportion of 1-year bees under long summer conditions than under shorter summer conditions. The initial summer duration experiment (fig. 2*A*) 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. Before transfer to winter conditions, bees were cooled at a rate of 4°C day^{-1} over 3 days. Bees in the short summer treatment were placed in the 0°C incubator on September 13, 2013 (t_0); long summer bees were placed at 0°C on October 25. Starting on April 30, 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 day^{-1}. 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"). This experiment was repeated in 2014 with several minor differences (described in the appendix).

Constant Summer Temperature Experiment. If summer temperature is an important (proximate) determinant of generation time, we expected to observe a greater proportion of 1-year bees under warmer summer conditions than under cooler summer conditions. We conducted a 5-week, two-treatment experiment in summer 2015 (fig. 2*B*) to test the effect of larval developmental 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 July 24, 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 days in their assigned treatment and that were <11 days 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").

Fluctuating Summer Temperature Experiment. If summer temperature is a key determinant of generation time, and if the timing of warm temperatures is also important, then we expected to find a difference in the proportion of 1-year bees between those that experienced warm temperatures early in development and those that experienced warm temperatures later. (As before, we also expected more 1-year bees under generally warmer conditions.) In 2016, we conducted a 6-week experiment with four summer temperature treatments (fig. 2*C*). We used growth chambers (Percival E-41HO, Perry, IA; henceforth, incubators) on a daily temperature cycle, mimicking field conditions more accurately than we had in 2015 (fig. A1; figs. A1–A3 are available online). The warm incubator cycled from 10° to 35°C daily (mean temperature = 22.5°C) and the cool incubator from 10° 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 plus 2 weeks cool, 2 weeks cool plus 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 plus 2 weeks cool treatment and the 5 weeks warm treatment were moved into the warm incubator on day 7, while bees in the 2 weeks cool plus 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 (fig. 2*C*). At the end of the experiment (day 42), all bees were placed in the cool incubator.

Observations of Development

To estimate the degree-day 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 h (2015) or 120 h (2016) for nests in the laboratory. We distinguished the following developmental stages (fig. A2): egg, feeding (predefecating) 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 (July 29) 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: (1) warm incubator (~25°C), (2) cool incubator (~18°C), and (3) field conditions in 2015; and (4) 5 weeks warm cycle (10°C–35°C), (5) 3 weeks warm cycle plus 2 weeks cool cycle, (6) 2 weeks cool cycle plus 3 weeks warm cycle, and (7) 5 weeks cool cycle (10°C–25°C) in 2016.

Analysis of Experimental Data

All analyses were conducted in R version 3.4.2 (R Development 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 a bee achieved a 1-year life cycle (a binary variable). However, because no bees in the summer duration experiments reached adulthood in 1 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 two or four levels, depending on the experiment (fig. 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 1-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 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 nonlinearly to temperature (Beck 1983); however, degree-day models are still widely used (e.g., Sato and Sato 2015; Uelmen et al. 2016; Geng and Jung 2018) 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, that is, accounting for the number of days each individual spent in the field before being transported to the lab. Hourly temperature data for both 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 and Thomson 2011). We then fit the developmental progression of all 275 bees in our data set 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 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 max-

imum temperatures from a National Oceanic and Atmospheric Association (NOAA; Climate Data Online: https://www.ncdc.noaa.gov/cdo-web/) weather station (GHCND: USC00051959, Crested Butte, CO), 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, that is, those calculated from more frequent temperature measurements. We fitted the latter two values using 4 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 6 years in which data were completely missing for seven or more dates between June 1 and September 30 (2 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 May 31 in 6 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 on the basis of 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, June 27; calculated from our 2015–2016 data set) and October 31 against DD12 in the first 6 weeks following the mean date of egg laying.

Model Validation

We used data on the natural life cycle of *O. iridis* to qualitatively test whether the proportion of 1-year bees developing from a given year's nests can be predicted from that year's summer temperature (specifically, total June–July DD12). 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 populations, and Forrest and Thomson (2011) reported data from nests constructed in 2008 in several Colorado populations around RMBL (approximately 500 km from the Utah populations). In addition, two of us have separately monitored natural emergence from trap nests established around RMBL, following methods of Forrest and Thomson (2011), from nests constructed in 2012–2015. 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 approximately 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 are provided in the appendix. Because of methodological inconsistencies among years and sites, we view this analysis as a qualitative test only. All data files are deposited in the Dryad Digital Repository (https://dx.doi.org/10.5061/dryad.r2dm56s; Forrest et al. 2019), and voucher specimens will be deposited at the Canadian National Collection, Ottawa.

Results

Summer Duration Experiment

Regardless of summer duration (fig. 2*A*), no *Osmia iridis* in this experiment pupated in its first summer (t_0). Despite having experienced at least 440 DD12 before 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, that is, 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 3 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, $P > .25$). However, the strongly male-biased sex ratio of emerging bees in both treatments (approximately 6 : 1) suggests greater mortality of females during development (since the expected sex ratio for this species is approximately 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 female) having higher mortality ($r = -0.74$, $P = .023$, $N = 9$ 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

Table 1: Years of pupation and emergence for bees in summer duration experiments

Year of experiment initiation and treatment	Year 0		Year 1		Year 2		Year 3[a]
	No. pupated	No. dead first 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
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
Total	0	37	0	38	28	62	16

Note: Year 0 = 2013 for 2013 bees, 2014 for 2014 bees; year 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.

[a] We have never observed 3-year life cycles in nature, but they may occur occasionally.

(treatment: $z = 37.3$, $N = 55$ bees from 24 nests, $P < .0001$; fig. 3*A*, 3*B*). This was also true in the more realistic cycling temperature treatments (fig. 2*C*). 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, $P = .00053$; fig. 3*C*, 3*D*). However, because of small sample sizes, no treatments differed significantly in adjusted pairwise comparisons ($z \leq 2.0$, $P \geq .14$).

In both summer temperature experiments, the date on which an egg was laid was a significant predictor of year of pupation (constant temperature experiment: $z = -2.3$, $P = .023$; fluctuating temperature experiment: $z = -2.6$, $P = .009$): eggs laid later in the summer were less likely to follow a 1-year developmental pathway (fig. 3*A*, 3*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 date), but experimental artifacts 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 ($\chi^2 = 11.1$, df = 1, $P = .0009$) but not in the fluctuating summer temperature experiment ($\chi^2 = 0.01$, df = 1, $P = .93$).

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 (fig. 4). Across all 275 bees observed in field or laboratory conditions, a base temperature of 12°C for degree-day calculation was best supported (fig. A3). Bees that pupated in t_0 did so on average after 43 days (SD = 7.4) and after having accumulated 432 DD12 (SD = 35.2). 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 quanti-

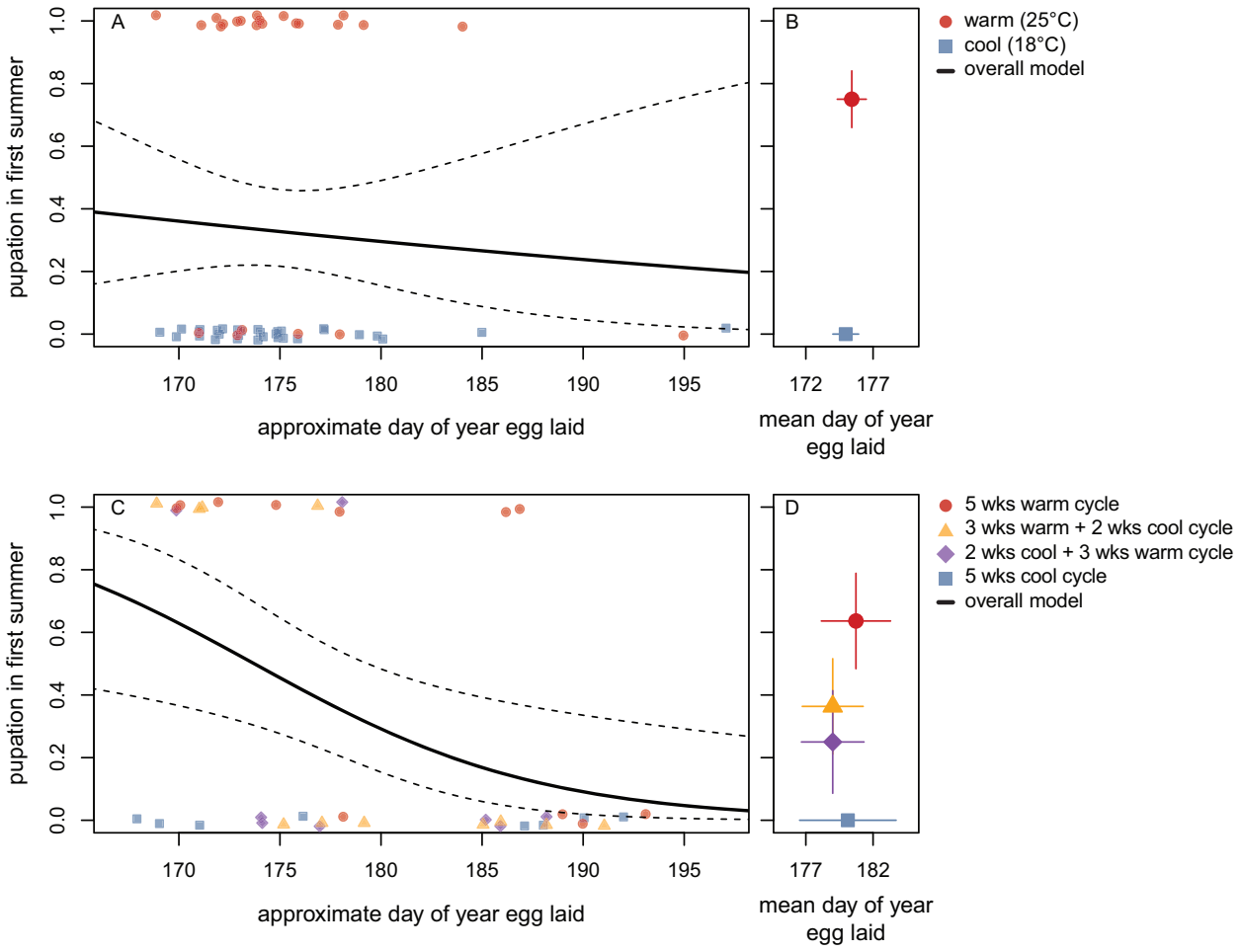

Figure 3: Pupation in first summer (year 0) as a function of treatment and date laid in constant summer temperature experiment (*A*, *B*) and fluctuating summer temperature experiment (*C*, *D*). Bees that pupated in year 0 have a *Y*-axis value of 1. *A* and *C* show individual data points, which have been jittered to reduce overlap. *B* and *D* show treatment means with error bars (±1 SE). Treatment abbreviations: 5 wks warm cycle = 5 weeks on a warm daily cycle (35°/10°C); 5 wks cool cycle = 5 weeks on a cool daily cycle (25°/10°C); 3 wks warm + 2 wks cool cycle = 3 weeks on warm cycle followed by 2 weeks on cool cycle; 2 wks cool + 3 wks warm cycle = 2 weeks on cool cycle followed by 3 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.

ties 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 days 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 DD12 (the amount necessary to reach the pupal stage) accumulated between June 1 and October 31 in Crested Butte, Colorado. However, only 20% of years (12 of 60) accumulated this much heat between June 27—the average date on which *O. iridis* eggs were laid in our 2015–2016 data set—and October 31 (fig. 5). Finally, only 7% (4 years) accumulated more than 580 DD12 between June 1 and October 31—an amount potentially sufficient for bees to reach adulthood (i.e., 432 degree-days to reach the pupal stage plus a minimum of 149 degree-days as a pupa). For bees developing from eggs laid on June 27 (day of year 178), degree-days accumulated in the first 6 weeks of life (by August 8) strongly predicted total summer degree-days (slope = 1.6, $r^2 = 0.80$, $n = 60$ years, $P < .0001$).

Model Validation

Data from 6 years and two study areas support the hypothesis that warmer temperatures during the first sum-

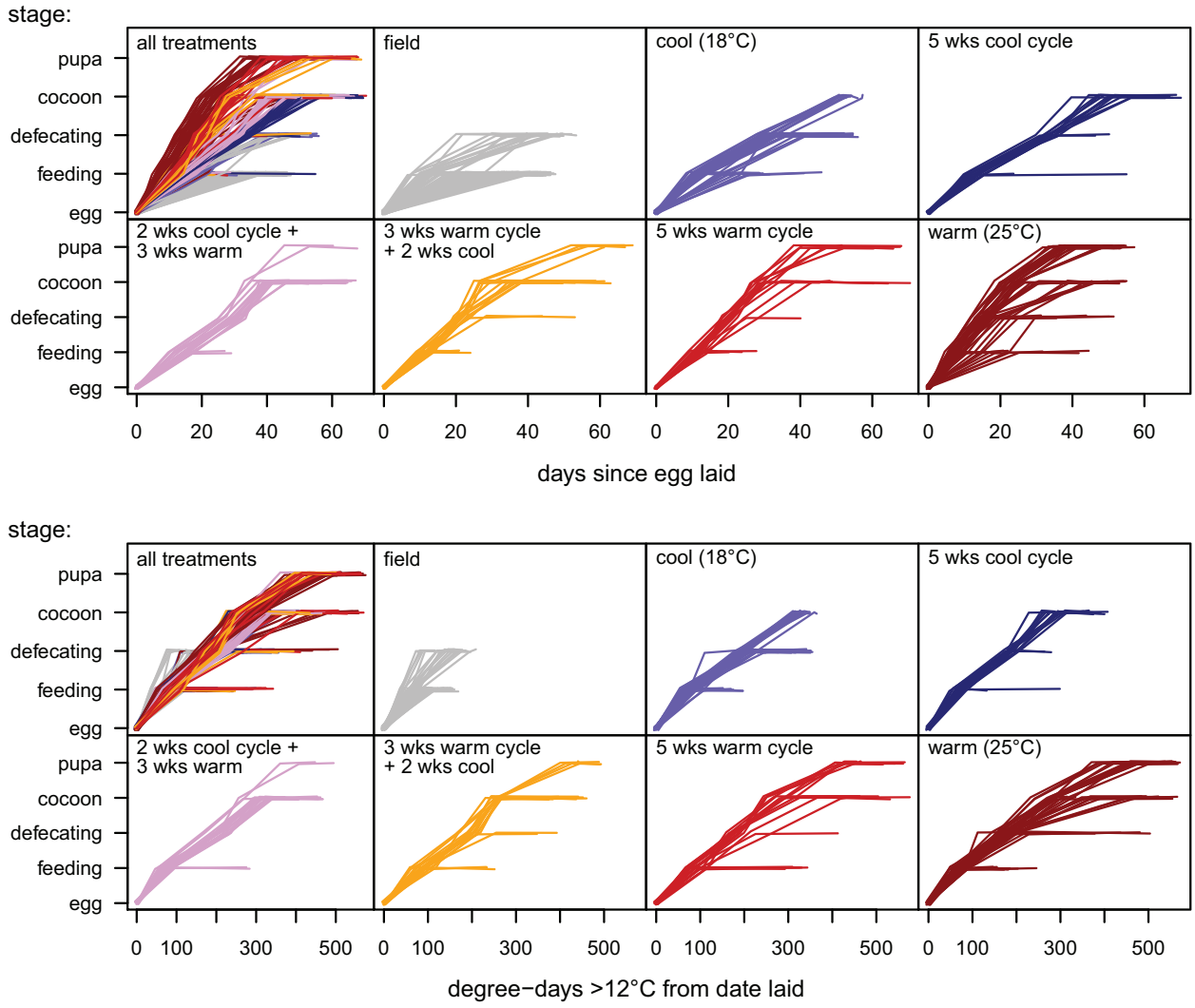

Figure 4: Developmental progression of *Osmia iridis* as a function of days since egg was laid (*top*) and degree-days above a base temperature of 12°C (*bottom*). Each line represents a single bee and starts at the beginning of development (the day on which the egg is estimated to have been laid) and extends until death or until the last observation of the summer (whichever came first). Data points have been jittered by 0.5 days (*top*) or 5 degree-days (*bottom*) 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 ± SD); the remaining bees were subjected to experimental rearing temperatures in the laboratory (treatments as in fig. 3). Sample sizes (in parentheses): field (106), cool (50), 5 weeks cool cycle (16), 2 weeks cool cycle plus 3 weeks warm (19), 3 weeks warm cycle plus 2 weeks cool (18), 5 weeks warm cycle (19), warm (47).

mer (t_0) increase the likelihood of a 1-year life cycle in *O. iridis* (fig. 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—2 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 (1-year) life cycle. This plasticity explains why a minority of bees in our study area naturally exhibit a 1-year life cycle, giving rise to the observed developmental polymorphism—

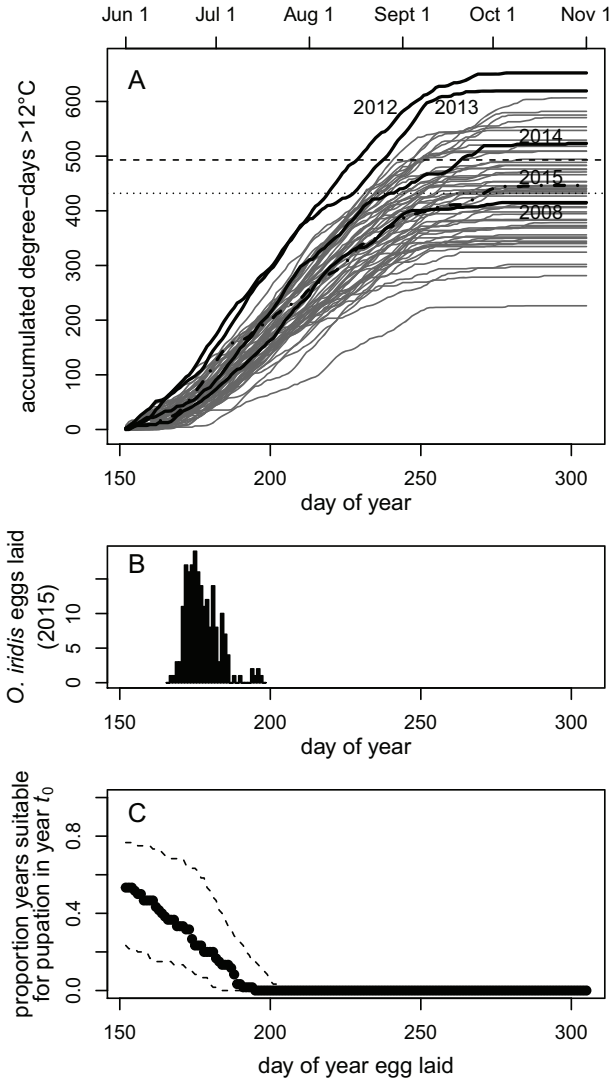

Figure 5: Seasonal patterns of heat accumulation and oviposition in *Osmia iridis*. *A*, Long-term (1950–2015) variation in summer heat sums, based on data from the National Oceanic and Atmospheric Association Crested Butte weather station, located near our lowest-elevation field site. Gray lines represent accumulated degree-days above a base temperature of 12°C (DD12) from June 1 (day of year 152) to November 1 (day of year 305) in each of 60 years; the uppermost (warmest) year is 2012, and the lowest (coolest) is 1975. Black lines represent heat accumulation in the years for which we have local data on bee voltinism (fig. 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 data set) 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*, Distribution of dates on which *O. iridis* eggs were laid in summer 2015 at our six field sites. *C*, 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 bottom dashed line represents the fraction of years with ≥502 DD12 (mean DD12 to pupation + 2 SD); the top dashed line represents the fraction of years with ≥362 DD12 (mean DD12 to pupation − 2 SD).

parsivoltinism. Our experiments provide 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 2-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 1-year life cycle, and a minority adopt the lower-risk, lower-reward 2-year strategy—reflecting a diversified bet-hedging strategy.

Voltinism in *O. 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 but 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 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

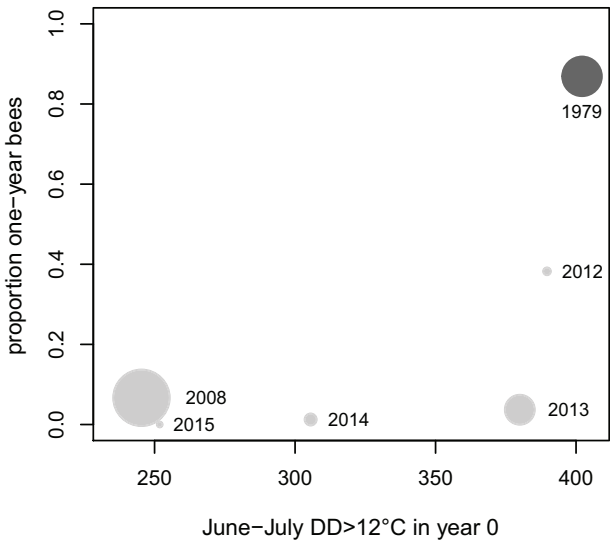

Figure 6: Proportion of *Osmia iridis* bees exhibiting a 1-year life cycle as a function of summer heat accumulation in the first year of development. Data are from several sources: 1979 data point (dark gray circle) is from Utah (Torchio and Tepedino 1982); data points from 2008 and later (light gray circles) are from Colorado (Forrest and Thomson 2011; this study). Circle size is roughly proportional to the number of bees observed (i.e., sample size), with the smallest circle representing 13 bees and the largest representing 555 bees.

photoperiod; Danks 1987; Grevstad and Coop 2015). Yet a role of photoperiod seems unlikely in *O. iridis*, which spends its entire preadult 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 nonphotoperiodic 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 (fig. 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 2-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 also appears 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 *O. 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; Tepedino and Parker 1986; Tanaka 1994).

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 3 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; $P = .057$ 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 ($P = .033$, $N = 100$ 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 3-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 3-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 (figs. 5, 6). Conditions in the two preceding years may have been responsible. Specifically, 2011 was warm (fig. 5*A*, fourth line from top), so it should have yielded numerous univoltine bees. However, 2012 was likely a devastating year for nesting bees in our study area because of 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 4 decades (Ogilvie 2017; J. E. Ogilvie and D. W. Inouye, unpublished data), 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. J. CaraDonna, unpublished data). 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 (fig. 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 1-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 1 year, and Torchio and Tepedino (1982) found that *Osmia* females (the larger sex) were more likely than males to undergo a 2-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 1-year bees.

How will life cycles of *O. 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 1-year life cycle. Provided these warmer summers are accompanied by warm autumns, we expect these 1-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 1-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 2 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 overwintering 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 have 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.

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"In the economy of our wild bees we see the manifestation of a wonderful instinct, as well as the exhibition of a *limited reason*." *a*, "Cells of *Osmia simillima*, the common green Mason-bee, built in the deserted gall of the Oak-gall Fly." *b*, "The Leaf-cutter Bee (*Megachile*), on a rose-leaf, in the act of cutting out a circular piece." *c*, "*Xylocopa Virginica*, the Carpenter Bee." From "The Home of the Bees" by A. S. Packard Jr. (*The American Naturalist*, 1867, 1:364–378).