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Physiological Ecology

Variability in spruce beetle (Coleoptera: Curculionidae, Scolytinae) adult diapause and evidence for oocyte development prior to winter in a Colorado population

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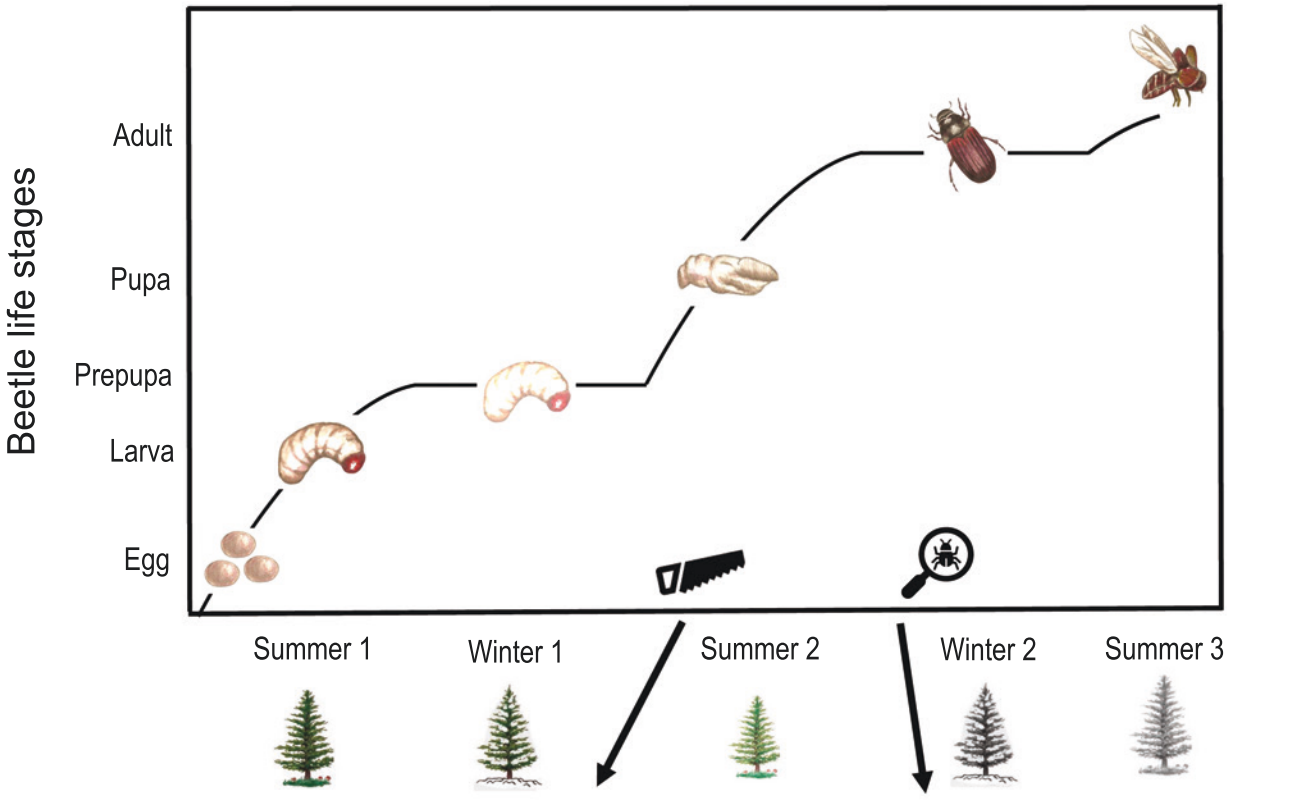
Diapause regulates seasonal insect life cycles and may be highly variable within and among populations due to genetic and environmental variability. Both types of variation may influence how populations respond plastically or evolutionarily to changing climates. We assessed diapause variability in spruce beetle *Dendroctonus rufipennis* Kirby (Coleoptera: Curculionidae, Scolytinae), a major forest pest whose life cycle timing is regulated by both prepupal and adult diapauses. Using mating studies and ovary dissections, we tested for variability in adult diapause within and between collection sites in Colorado and Wyoming, USA. Ovary morphology suggested that most females from both sites enter diapause prior to egg formation (oogenesis) when reared at warm temperatures. Though previous studies suggested that adult diapause is obligate, we found that a small proportion of females from both populations terminated diapause without winter chilling in the lab. Moreover, we found that most female beetles sampled at the Colorado field site had mature ovaries relatively early in the fall, suggesting that transient exposure to low temperatures may potentiate pre-winter reproductive development. Adult diapause may act primarily as a block to prevent offspring production late in the season but not necessarily as an overwintering phenotype. Overall, our data do not suggest imminent life cycle shifts mediated by adult diapause, but if the observed variability is heritable, diapause regulation may evolve in response to changing environmental conditions.

Key words: overwintering, life cycle, bark beetle

Graphical Abstract



Introduction

Seasonal environments impose strong natural selection to synchronize insect development and reproduction with benign, resource-rich conditions, while mitigating exposure to harsh or resource-poor conditions. Diapause is a type of insect dormancy and is a common adaptive strategy facilitating synchronization via regulation of the timing of diapause entry and exit. Diapause may occur in any life-history stage and is typically characterized by reduced metabolism and increased stress hardiness (Denlinger 2022). The central, defining feature of diapause is a clear developmental delay that is not

simply the result of thermal thresholds for morphogenic development (Koštál 2006). Diapause induction and termination are cued by environmental variables such as photoperiod, thermoperiod, and resource scarcity (Tauber et al. 1986). Moreover, diapause timing is often variable within and among populations (Masaki 1961, 2002, Ragland et al. 2019). Thus, changes in environmental conditions can cause both plastic and evolutionary changes in seasonal timing (Bradshaw and Holzapfel 2008, Tobin et al. 2008).

Many studies document how recent changes in climate are altering the timing of life history events within a season, which, in turn, may affect trophic interactions and range expansion (Visser and Both 2005, Altermatt 2010, Forrest and Thomson 2011, Buckley et al. 2015, Bentz et al. 2019). Longer and warmer growing seasons associated with climate change can cause faster development, which can also allow populations to complete more generations in a year (increased voltinism; Jacques et al. 2019), assuming temperatures do not exceed the threshold for optimal development (Deutsch et al. 2008). However, there are alternative scenarios where longer growing seasons simply extend the duration of certain life cycle stages, or where interactions between thermal sensitivity of development rate and photoperiodic diapause induction could actually reduce voltinism (reviewed in Forrest 2016).

In addition to effects on developmental timing, changes in temperature can alter the timing of diapause entry or exit. In particular, exposure to relatively warm temperatures during a sensitive life stage can prevent facultative diapause induction (Dyer and Hall 1977, Nunes and Saunders 1989), even in insects where diapause appears to be obligate under typical, cooler temperatures in the field (Dambroski and Feder 2007). This can result in additional generations if conditions are permissive but could also result in high mortality if the environment does not allow growth, development, and/or reproduction necessary to attain a life stage capable of surviving winter later in the life cycle (Dambroski and Feder 2007). The consequences of averted diapause may be particularly difficult to predict in insects capable of overwintering in multiple life stages. For example, some species of bark beetles have life cycles that vary between semivoltine (one generation every 2 yr) and univoltine (one generation per year) across geography, depending on the environmental sensitivity of two or more possible overwintering stages (Schebeck et al. 2017). Even in species where there is one primary overwintering stage, geographic variation in environmental sensitivity may render different populations effectively obligate diapausers (univoltine) or facultative diapausers that may have multiple generations per year when conditions are permissive (Schebeck et al. 2022).

The spruce beetle (*Dendroctonus rufipennis* Kirby, Coleoptera: Curculionidae, Scolytinae) is a native disturbance agent of North American spruce (*Picea* spp.) with a flexible diapause/overwintering strategy that may be particularly sensitive to warming climates. It occurs throughout the range of its host trees across western North America from sea level at northerly latitudes (e.g., Alaska) to over 3,600 m in the southern US Rocky Mountains (e.g., northern New Mexico; Holsten 1999). Generation time can vary from 1 to 3 yr across this range, where populations experiencing warmer mean temperatures generally have shorter generation time (Knight 1961, McCambridge and Knight 1972). Spruce beetle voltinism is regulated by overwintering stages that can occur in both the prepupal stage, or final larval instar (Dyer and Hall 1977, Hansen et al. 2001, 2011) and in the adult stage (**Fig. 1**) (Massey and Wygant 1954, Safranyik et al. 1990, Bleiker and Willsey 2020). We are not aware of any clear, published evidence establishing the precise, additional life cycle stage that may overwinter in the rare, 3-yr life cycle.

Spruce beetles can enter a facultative, prepupal diapause that is relatively well studied and is induced by temperatures consistently below 15°C in populations sampled from sites ranging from British Columbia south through Colorado and Utah (Dyer and Hall 1977, Hansen et al. 2001, 2011). Consistent exposure to temperatures above 15°C during late summer will avert the facultative prepupal diapause with development progressing to the adult stage prior to winter (Hansen et al. 2011). Adults emerge the following summer resulting in a univoltine life cycle. However, when prepupal diapause is induced by relatively cool temperatures, the first winter is spent as a prepupa and a second winter is typically spent as an adult leading to a semivoltine lifecycle (Hansen et al. 2001).

Research has been comparatively limited on adult overwintering, but the evidence is generally consistent with a diapause state that is mostly, but perhaps not completely obligate and that suppresses reproduction until after winter (Safranyik et al. 1990). An early natural history study by Massey and Wygant (1954) compared adults that had overwintered as prepupae and then developed to adulthood (i.e., no chilling as an adult) with adults that had overwintered in the host tree (i.e., experienced chilling as an adult). Adults that had overwintered readily reproduced, whereas the majority of adult beetles that had not overwintered did not bore into fresh phloem to initiate egg galleries. However, a few non-overwintered beetles did reproduce, suggesting that at least some proportion of adults were reproductively viable without requiring overwintering. Likewise, Schebeck et al. (2017) notes unpublished observations of some spruce beetle male-female pairs producing eggs without cold exposure, particularly from a more southerly location. Finally, Bleiker and Willsey (2020) conducted a large, laboratory experiment showing that exposing adult spruce beetles to cold temperatures followed by warm temperatures greatly reduced time to emergence from infested logs. Although they did not investigate reproductive potential, the authors suggested that the results support an adult diapause that requires extended chilling to terminate. However, some adult beetles did emerge from infested logs without any simulated winter cooling, with an even smaller, but non-zero proportion emerging without a pronounced delay. Interpreting these observations is somewhat complicated because adults that have developed from overwintered prepupae will often emerge from infested trees the following fall and relocate to a lower position near the base of the tree where they overwinter (Massey and Wygant 1954). Nevertheless, results from these studies suggest that some proportion of adult spruce beetles may avert or complete diapause prior to winter and could be reproductively mature and produce offspring without previously overwintering. There is precedent for this type of variability in the closely related species *Dendroctonus simplex*; a portion of non-overwintered beetles from a population at the southernmost edge of the species range were found to avert the obligate diapause observed in more northern populations (McKee and Aukema 2015).

Flexibility of adult diapause in spruce beetles has important implications for population dynamics with changing climates. The univoltine life cycle for this species occurs when favorable late summer temperatures fail to induce prepupal diapause, allowing for pupation and adult eclosion before the onset of winter (Hansen et al. 2001, 2011). Consecutive warm summers have been correlated with regionalized outbreaks (Berg et al. 2006), likely due to the exponential increases in population growth rate observed when populations transition from semivoltine to univoltine (Hansen and Bentz 2003, Werner et al. 2006). If adult diapause can also be averted or completed without obligatory winter chilling, that could provide additional routes to changes in voltinism. Warming temperatures, however, could also result in fractional or asynchronous voltinism as seasonality and

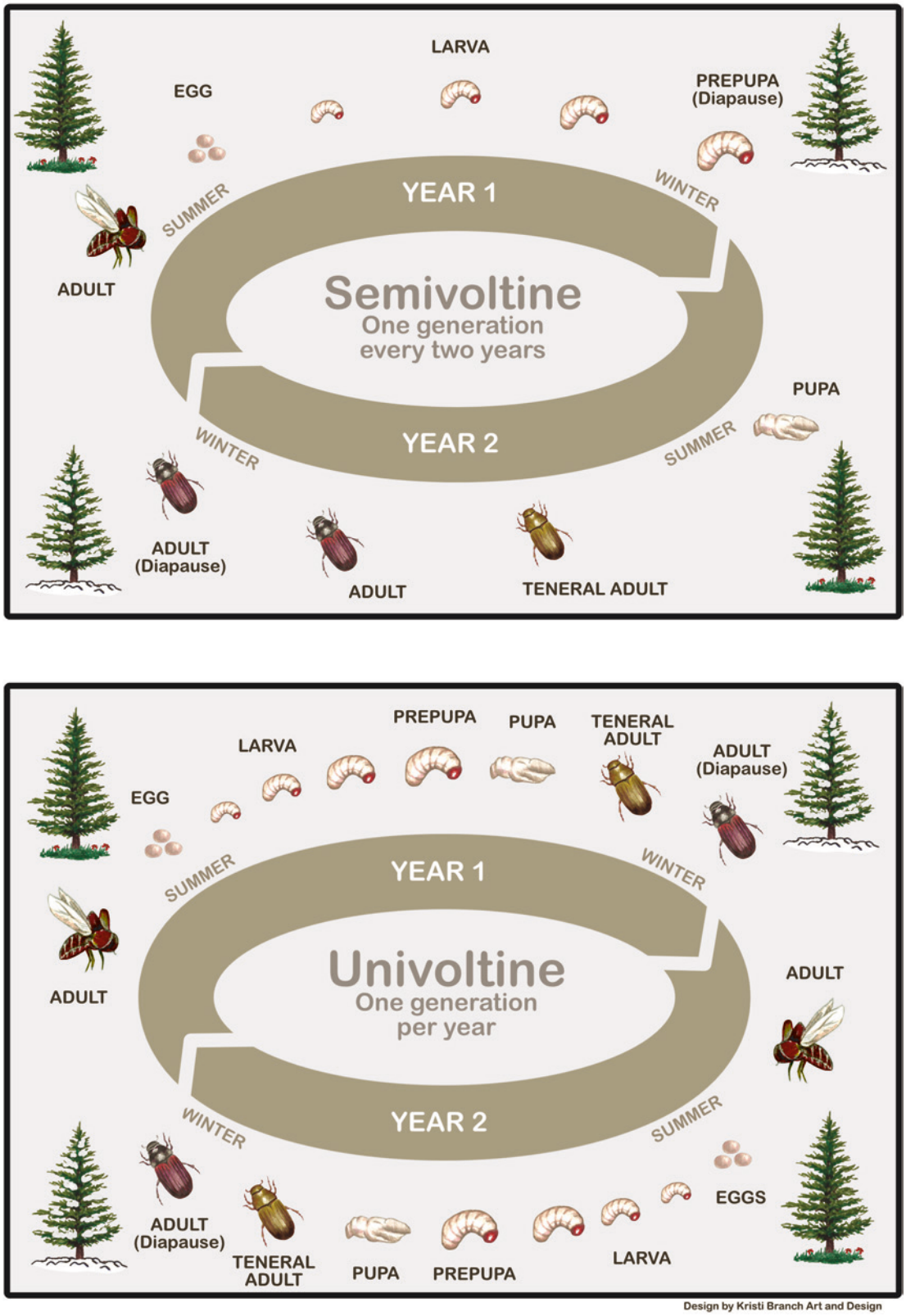


Fig. 1. Typical semivoltine (top) and univoltine (bottom) life cycles of *Dendroctonus rufipennis*. Semivoltine beetles overwinter twice, once as a prepupa, and once as an adult, whereas univoltine beetles develop directly to an adult overwintering stage by averting prepupal diapause.

diapause cues are disrupted (Van Dyck et al. 2015, Bentz et al. 2022). Even if aversion or completion of adult diapause without chilling is relatively rare, evolutionary responses to warming climates could shift lifecycle timing if variation within populations is heritable.

We used a combination of laboratory manipulations and field observations to investigate the developmental stage and potential variability in early aversion or completion of adult female diapause. Laboratory experiments determined the stage of ovarian

development in which female beetles arrest development during diapause and quantified variability in diapause through ovarian morphology and reproductive assays. We also sampled beetles from the field in fall and spring to determine whether female beetles overwinter at the same stage of ovarian development identified as the diapause stage in the laboratory studies. Diapause-related traits are often highly variable across geography in many insects including bark beetles (Masaki 1961, Schebeck et al. 2017). We thus conducted the laboratory experiments using spruce beetles collected at 2 geographic sites in Colorado and Wyoming, USA varying by more than 4°C latitude. However, we conducted the field study of overwintering beetles only at the Colorado field site.

Materials and Methods

Field Collection of Infested and Green Trees

We harvested spruce beetle-infested Engelmann spruce (*Picea engelmannii* Parry ex Engelmann) from a southern location (Guanella Pass, Colorado; N 39.6°, W 105.7°; 3,479 m elevation) and a northern location (Togwotee Pass, Wyoming; N 43.8°, W 110.2°; 2,596 m elevation) (**Fig. 2**). The locations were chosen based upon data from the USDA Forest Service Aerial Detection Survey (ADS) and 2017 ground surveys showing that both areas had high levels of beetle activity. In June and July 2018, we cut one Engelmann spruce at each site that had been infested the previous year by spruce beetles (Supplementary **Table S1**). Only bolts from a minimum of 50 cm from the ground were kept to avoid sampling potentially re-emerged parent adults that had previously mated and relocated to the base of the tree (Massey and Wygant 1954). Hansen and Bentz (2003) reported that surviving broods from 4–5 female–male pairs introduced into 15 × 25 cm phloem sections produced about 50 offspring, or about 10 offspring per female at infestation densities approximating natural conditions. Therefore, the 4,000+ beetles reared out of each tree (see Results) likely represent hundreds of beetle pairs, a large sample of each geographic population. Nevertheless, we avoid making any definitive inferences about differences between sites because the host trees could affect some or all of the measured traits, and we did not sample replicate trees within geographic sites.

Prior to cutting, trees at both sites were sampled to observe current life stages. The CO tree had a large proportion of overwintered pre-pupae and the WY tree had a majority of teneral (not fully sclerotized) adults. No eggs or fully sclerotized adults were observed at either site, suggesting the trees were attacked and colonized the previous year and that beetles were developing on a semivoltine lifecycle

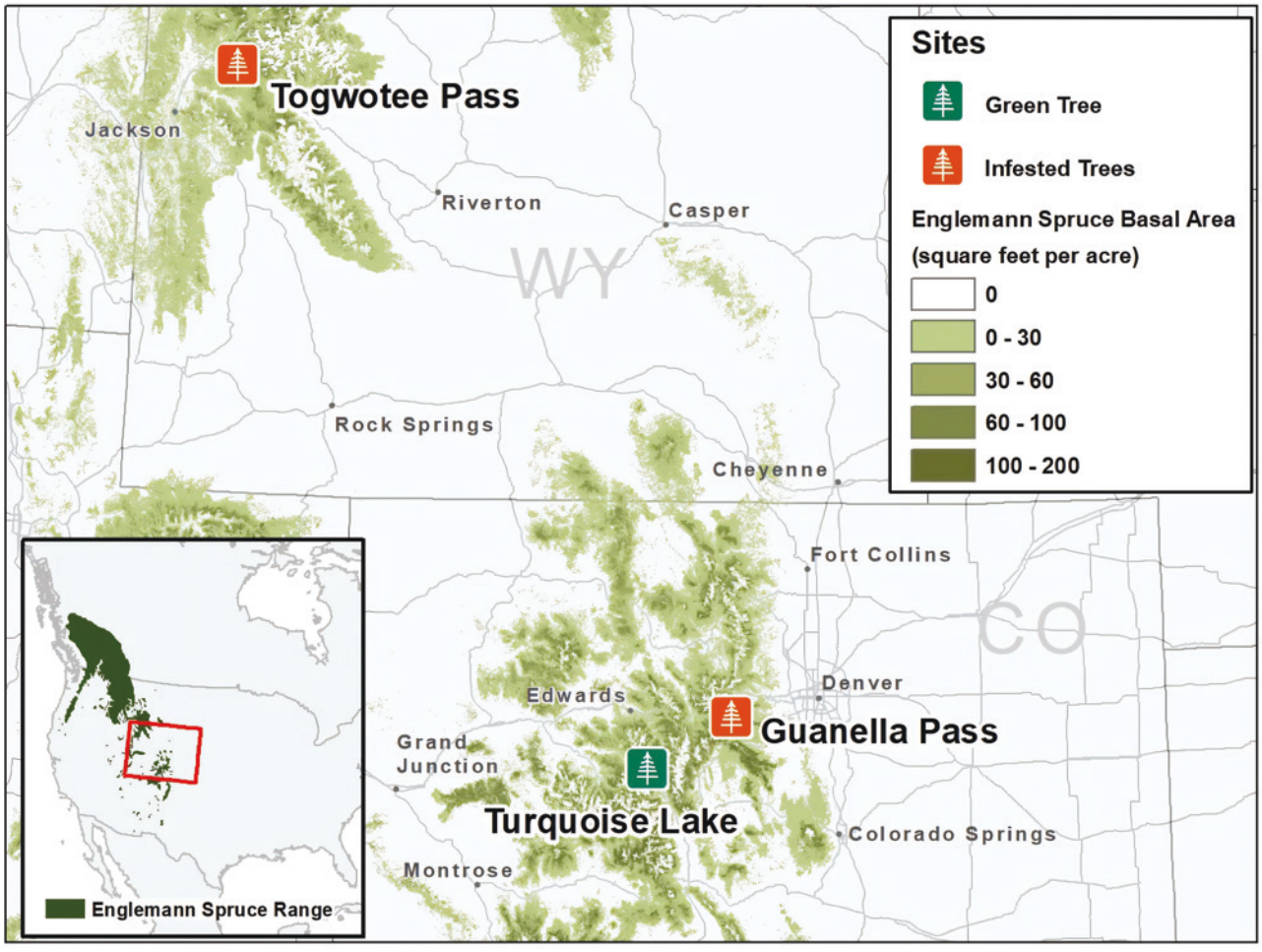


Fig. 2. Spruce beetle-infested Engelmann spruce were harvested from a northern population in Wyoming and southern population in Colorado (Togwotee Pass and Guanella Pass, respectively). An un-infested Engelmann spruce was harvested from a site in Colorado (Turquoise Lake), and was used for mating experiments. Distribution of Engelmann spruce in North American shown in inset (data from https://databasin.org/datasets/8f64f9f5e793419eb38ca15580a29d7c/), and Engelmann spruce basal area is shown in monochromatic green (data from https://www.arcgis.com/home/item.html?id=4ebf103ddeeb4766a72e58cb786d3ee2).

(Hansen et al. 2001). On arrival in the laboratory, infested bolts were immediately stored in temperature-controlled rooms at 22°C ± 1°C, a relatively warm temperature approximating mean temperature at the warmest part of the growing season. This temperature matches the 'warm' condition applied by Bleiker and Willsey (2020) and we chose it primarily to impose a strong environmental signal likely to maintain a diapause state that would be terminated by exposure to colder temperatures. Each bolt was placed in an individual mesh emergence enclosure to monitor emergence (**Fig. 3**).

To provide bolts for beetle mating trials, we harvested one uninfested Engelmann spruce near Turquoise Lake on the San Isabel National Forest in August 2018. We selected the tree from a stand with no evidence of spruce beetle activity based on ADS data and ground surveys (**Fig. 2**). After felling, 15 bolts approximately 30–35 cm in length were cut and transported to the University of Colorado Denver. The ends of all bolts were sealed with paraffin wax to reduce desiccation and stored at 4°C ± 1°C for 34–56 days.

Data from the closest weather stations to each site were used to compare thermal conditions between the 2 collection sites over the last 20 yr (1999–2019). The Colorado site had relatively warmer monthly mean temperatures on average (Supplementary **Fig. S1**) and more annual degree days > 3°C (929 ± 64) compared to the Wyoming site (789 ± 92).

Diapause Status of Laboratory-reared Beetles

We monitored adult emergence from infested bolts from both sites, collecting beetles every other day from the start of the experiment (day 0; when bolts were placed at 22°C) until 5 days passed with no newly emerged beetles (day 246). Beetles from Colorado started emerging after 25 days, while Wyoming-origin beetles started emerging immediately. However, after a pulse of beetle emergence ($n = 188$) on day 1, no more beetles emerged from the Wyoming-origin bolts until day 25. Though our inspection of infested trees prior to felling revealed no sclerotized adult beetles, there is a small possibility that this initial pulse of emerged adults may have included a few re-emerged parent beetles that overwintered following oviposition (Hansen and Bentz 2003). As an additional measure to avoid sampling parent beetles, no beetles emerging prior to day 25 were used for any subsequent experiments or observations. We collected all beetles emerging after day 25 and separated them by sex (Lyon 1958). Beetles sampled for ovary dissections were frozen, while beetles sampled for reproductive assays were collected live (see details below).

To assess diapause status, we scored the extent of ovarian development of adult female beetles emerging from infested bolts. We reasoned that in many cases adult insects overwinter prior to investing in ovary maturation (Hodek 2011) and that lack of ovary development could be a signature of adult (female) diapause. We assessed ovarian development by dissecting 83 Colorado and 94 Wyoming female beetles that emerged from infested bolts. Beetles were randomly sampled across the emergence distribution after day 100 such that sample sizes were approximately proportional to the number of adults that emerged at each time point (see example for CO beetles in Supplementary **Fig. S2**). Prior to day 100, beetle sampling was infrequent enough that many beetles had died prior to collection, compromising internal tissue morphology. All samples collected at 100 days or later for ovary dissection were confirmed alive at the time of freezing. Live-frozen female beetles were removed from the freezer and placed onto a dissecting dish with phosphate-buffered saline. Elytra, wings and heads were removed, openings were made in the metathorax and ventral sternites, and ovaries were removed. Determination of development status followed Ryan (1959). We scored ovaries as undeveloped if no oocytes were present in the ovarioles (**Fig. 4a**) and developed if oocytes were present in at least one ovariole (**Fig. 4b**).

Presence of oocytes in the ovaries does not guarantee that female beetles can successfully mate. Therefore, we also measured reproductive success of 45 male–female pairs of beetles from both sites that emerged between 54 and 116 days after being placed at 22°C. These beetles were sampled immediately or stored in petri dishes at 4°C with moist filter paper until use (mean of 4 days of storage). We inserted male–female pairs into holes drilled into the phloem on the cut-side of green, uninfested bolts spaced at 6.35–8.89 cm. We first inserted a female into the starter hole, followed by a male after the female disappeared into the phloem. If a male hesitated to join the female, he was replaced with another male. Holes were covered with mesh screen, the bolts inverted and fully enclosed in mesh screen, and placed at 22°C. Sixty days after a bolt was manually infested, it was moved to an incubator set at 4°C to halt development. Cold storage prevented continued beetle movement into galleries from other mating pairs and slowed fungal growth (Eidson et al. 2018). This sampling allowed more than enough time for successful mating and reproduction at 22°C (Hansen et al. 2001). After 6 to 76 additional days (convenience sampling) we carefully removed the bark from each bolt, keeping galleries intact, then counted individual larval feeding galleries from each of the parental galleries. We recorded (i) mating success or failure, (ii) parental gallery length (cm), (iii) total sum of eggs and larval offspring, and (iv) pronotal width of females as a metric of body size. Reproductive success was determined by the presence of eggs or larval galleries. In unsuccessful pairs, no vertical gallery was present, or the pair of adult beetles were dead with no evidence of eggs or larval feeding galleries.

Diapause Status of Field-sampled Beetles

The laboratory experiments described above tested whether adult spruce beetles could develop oocytes and reproduce in constant warmth without cold temperature cues, but they do not reflect field-relevant conditions. To assess the developmental status of female beetles in the field, we collected overwintering adult female beetles directly from infested trees at Guanella Pass, Colorado during fall 2019 and from fall 2021 to spring 2022. Initially, we collected beetles in fall with the goal to establish a time point where all female beetles were clearly in diapause (Fig. 3). However, after our first collection in 2019, we realized that most female beetles in the field had advanced past the characteristic diapause stage (see results) and had developed oocytes in the ovaries. Thereafter, we made several more fall collections in 2019 and again in fall 2021 to confirm this observation, then followed with a spring collection in 2022 to test whether egg chambers and oocytes might be flexibly re-absorbed as has been documented in other insects under unfavorable environmental conditions (Nozaki and Matsuura 2021). This allowed us to confirm whether females that had developed past the diapause stage and invested in oocytes survive overwinter.

In the fall of 2019 and 2021, we identified 20 and 18 trees, respectively that had been attacked and colonized the previous summer by semivoltine beetles and collected 51 and 72 female adults over several collection dates in the fall of 2019 and 2021, respectively, and 29 in the spring of 2022 (Table 1). As for the infested trees sampled above, these trees contained no sclerotized adults that could represent parent beetles that had already produced offspring. We collected beetles overwintering at the base of standing, infested trees by carefully removing sections of bark, and all beetles found

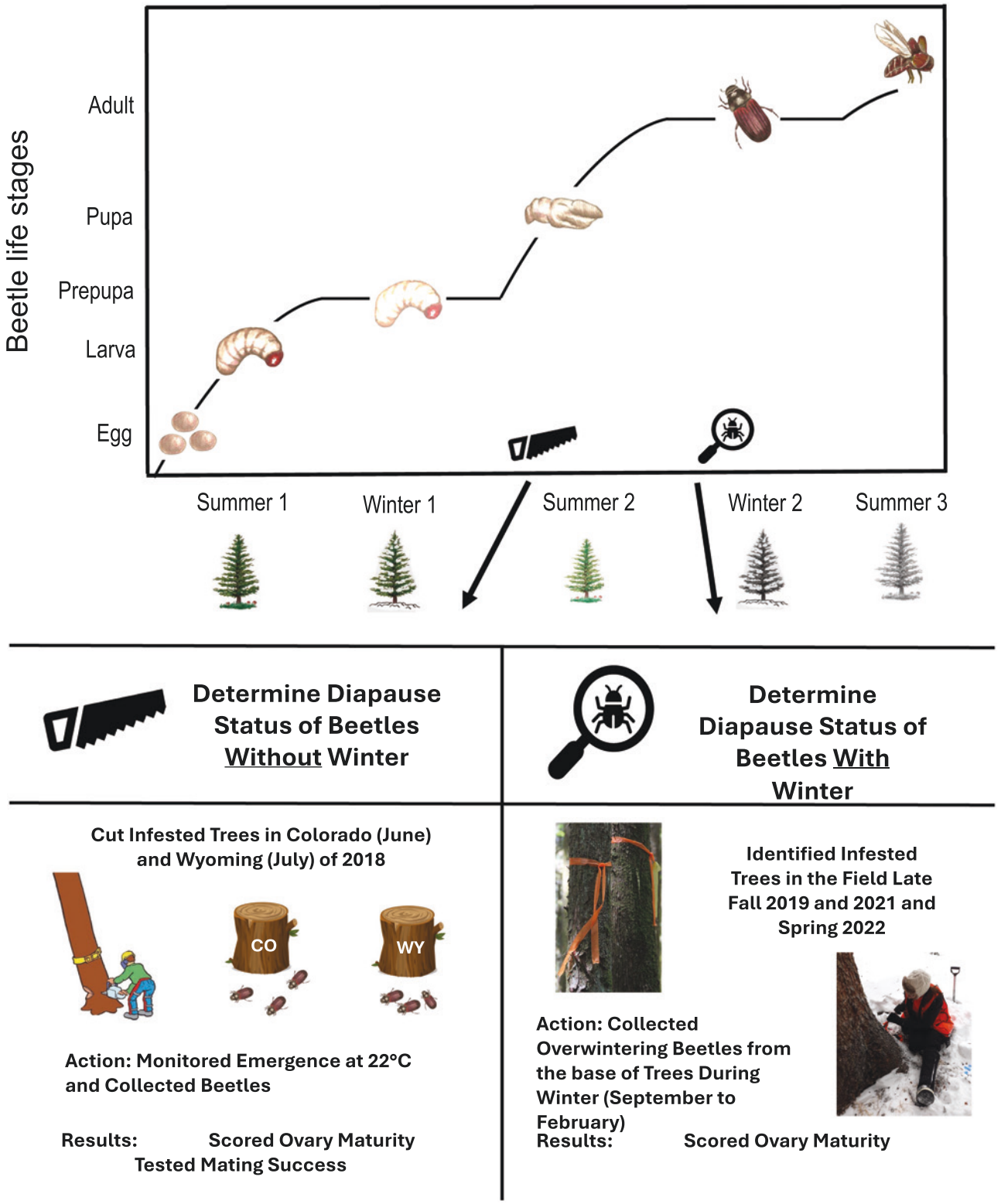


Fig. 3. Conceptual diagram of the experimental design. *Top*: Semivoltine spruce beetle life cycle. *Bottom left*: 2018 experiment details that correspond to life cycle figure with a saw icon. We cut infested trees from Colorado and Wyoming after the beetles completed prepupal diapause. *Bottom right:* 2019 experiment details that correspond to the life cycle figure with a magnifying glass icon. We field-collected overwintering adult beetles from Colorado during the winter.

within the phloem were placed in a petri dish with moist filter paper. All samples were transported to the laboratory in a cooler with an ice pack, and subsequently frozen live for storage prior to dissections to assess ovary maturity.

We also monitored field temperatures to assess whether transient exposure to cold temperatures interspersed with warmer temperatures favorable for development could explain the observation that female beetles had matured ovaries by late September. We

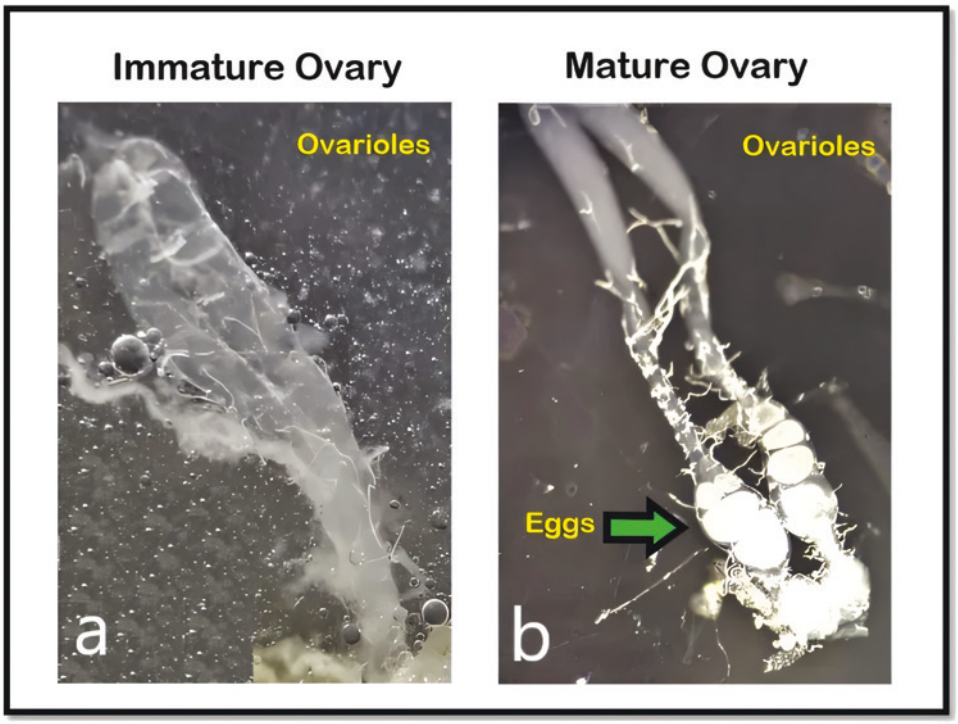


Fig. 4. Microscopic images of immature (a) and mature (b) spruce beetle ovaries. Two ovarioles (of 4 total) are shown for each beetle.

Table 1. Number of mature female spruce beetle adults field-collected from the base of infested trees. Beetles were collected from Guanella Pass, Colorado before, during and after winter.

Date collected	Number dissected	Number mature
9/27/2019	22	17
10/4/2019	6	4
10/31/2019	7	7
11/20/2019	1	1
12/4/2019	15	15
10/6/2021	31	27
11/9/2021	41	35
4/15/2022	29	25

were not able to install loggers in late summer of 2019. Instead, we installed them from 5 August 2023 to 14 October 2023, used data from 2 nearby SNOTEL stations (Jackwacker Gulch and Echo Lake) to parameterize a linear model, then projected 2019 phloem temperatures using the model. We removed a 5.1 × 7.6 cm section of phloem, inserted data loggers (Thermochron iButton DS 1922L-f5#) wrapped in parafilm and set to record temperatures in hourly increments (± 1°C), then stapled the phloem flap back in place. We placed loggers in 2 trees in close proximity to the trees from which we field-collected beetles: one on Naylor Lake Road, and one slightly downhill at Guanella Pass Campground.

Statistical Analysis

We estimated the 95% confidence intervals (CIs) and performed hypothesis tests for all reported proportions using functions in R (R version 3.4.4) to fit models to binomial response variables. We used Fisher's exact test (*fisher.test* function) to compare proportions of developed ovaries and proportions of successful reproduction between the Colorado and Wyoming collection sites. We calculated 95% CIs for all proportions from the appropriate binomial distributions using *binom.test*. We also fit a generalized linear model with a logit link function and binomial response (using *glm*) to test whether emergence time influenced the probability that a female had mature ovaries. We fit a linear model (*lm* function) to predict 2023 field phloem daily maximum and minimum temperatures from 2023 SNOTEL data. We fit separate models for each tree with an installed logger with SNOTEL data from each station as continuous covariates, then used the fitted models to predict 2019 phloem temperatures using 2019 SNOTEL data from 5 August to 27 September (the first field beetle collection date in 2019). We constructed distributions of maximum and minimum temperatures over this time period based on the mean predicted temperatures of the 2 trees.

Results

Adults Emerged Without Cold Treatment

A total of 4,208 adults emerged from Colorado-origin bolts and 4,530 from Wyoming-origin bolts. Adult emergence from the Wyoming bolts began immediately after they were placed at 22°C (**Fig. 5a**), and they continued to emerge regularly for 211 days. One emergence peak was present for Wyoming during the week of 5 September 2018 (n = 845; 55 days at 22°C). Adult emergence from Colorado bolts began 25 days after they were placed at 22°C (**Fig. 5b**), and they continued to emerge regularly for 217 days. Two emergence peaks were present for Colorado, one during the week of 26 July 2018 (n = 598; 48 days at 22°C), and the other during the week of 8 November 2018 (n = 427; 153 days at 22°C).

Most, But Not All Females Arrested Ovarian Development Prior to Oogenesis

Though beetles emerged from bolts at a high rate, most emerging female beetles from both the northern and southern collection sites

had not developed oocytes and thus were not reproductively mature. Given that these adults were kept at a constant 22°C and did not experience any chilling, this result suggests that most females entered diapause characterized by arrested ovarian development prior to oogenesis. A small proportion of adult females from each population, however, developed oocytes in the constant warm temperatures. Of the adult females dissected, 11% of Colorado beetles (9 out of 83 total, 95% CI of proportion mature; 0.051–0.20) and 1% of Wyoming beetles (1 out of 94 total, 95% CI of proportion mature; 0.00027–0.058) were mature (**Fig. 6a**). These percentages/proportions do not account for beetles that may not have emerged or died prior to emergence. The proportion appeared to be slightly higher for Colorado compared to Wyoming ($P = 0.007$, Fisher's exact test). Within the range of emergence times over which the samples were collected (100–246 days), emergence time had no discernable relationship to the probability that a female had developed oocytes based on a generalized linear model with a logit link function (CO—Wald $Z = 0.012$, $P = 0.522$; WY—Wald $Z = 0.025$, $P = 0.344$; Supplementary Fig. S3).

Some, But Few Adults Successfully Reproduced Under Constant Warm Conditions

Corroborating our observations of a low but non-zero rate of reproductive maturity based on ovarian development, some male–female pairs were able to successfully reproduce without a cold treatment. Of the 45 mating pairs from Colorado, 22% reproduced successfully (10 out of 45 pairs; 95% CI of the proportion: 0.11–0.37), and 4% of Wyoming mating pairs successfully reproduced (2 out of 45 pairs; 95% CI of the proportion: 0.0054–0.15) (**Fig. 6b**). Given that we collected bolts well above the root collar where re-emerged parents relocate (see methods), we are confident that re-emerged parents are unlikely to account for any substantial portion of the 19 and 3 total female beetles from CO and WY, respectively, that either exhibited advanced ovarian development or successfully reproduced. As was the case for ovary maturity, the proportion of successfully reproducing females was marginally higher in the Colorado population compared to the Wyoming population ($P = 0.03$, Fisher's exact test). Female size did not differ substantially between successful and unsuccessful reproducing females (Supplementary **Fig. S4**).

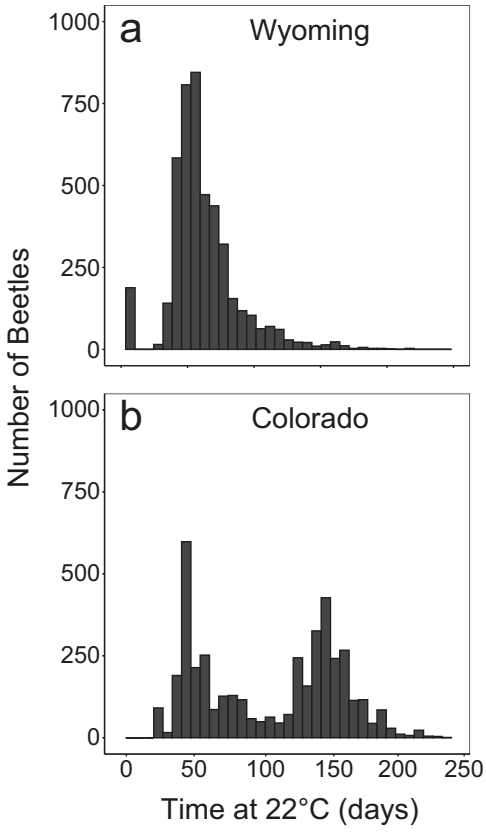


Fig. 5. Frequency distributions of emergence time for (a) Wyoming and (b) Colorado starting on the day bolts were placed in a 22°C incubator. No newly emerged beetles were observed beyond day 246.

Field-collected Adult Beetles from Colorado Develop Oocytes in Early Fall and Spring

Hibernating unmated brood adults that overwintered in clusters at the base of trees that were infested the previous year were collected in the spring and fall. Given the lifecycle timing and behavior of spruce beetles, it is unlikely that previously mated parent beetles from the previous year would still be at the base of trees (Massey and Wygant 1954). Most female brood adults collected during late summer through early spring had developed oocytes, including on the first 2019 collection date of 27 September (Table 1). From 5 August to the first beetle collection date (27 September) in 2019 predicted daily minimum phloem temperatures occasionally dipped below 4°C, while predicted daily maximum temperatures frequently surpassed 15°C (Fig. 7). It appears that the combination of occasional cold temperature exposure with temperatures permissive for growth and morphogenesis was sufficient to terminate diapause and allow ovarian development, whereas little to no ovarian development to the oocyte stage occurred in constant warm temperatures in the lab. The high percentage of spring-collected beetles with developed oocytes suggest that egg chambers and oocytes are likely not resorbed, and that beetles that have invested in oocyte production do survive winter.

Though we were not systematically targeting spermatheca as part of the dissection, we also serendipitously observed some females that had clearly mated based on presence of sperm in the spermatheca that were ruptured during dissection (27 September 2019: $n = 3$; 4 October 2019: $n = 1$; 31 October 2019: $n = 2$; 4 December 2019: $n = 5$). As mentioned above, it is unlikely that any of these beetles were parent beetles from a previous generation. Thus, the data would be consistent with some female beetles mating prior to relocation to the base of trees for overwintering. However, sample sizes were small, so we avoid drawing any strong conclusions about pre-winter mating behavior.

Discussion

Spruce Beetle Reproductive Development Is Highly Dependent on Low Temperature Exposure

Adult diapause in the spruce beetle appears to be heavily influenced by exposure to low temperature. Previously, Bleiker and Willsey (2020) showed that pulses of cold temperatures can accelerate and synchronize beetle emergence from bolts in the lab, although reproductive maturity of these insects was not evaluated. We found that large numbers of beetles emerged from infested bolts kept in constant warmth (22°C), but only 11% and 1% of emerged females, from the Colorado and Wyoming sites, respectively, had developed oocytes. Similarly, we found that a proportion of male–female pairs of emerged beetles from each population reproduced successfully; 22% of Colorado beetles and 4% of

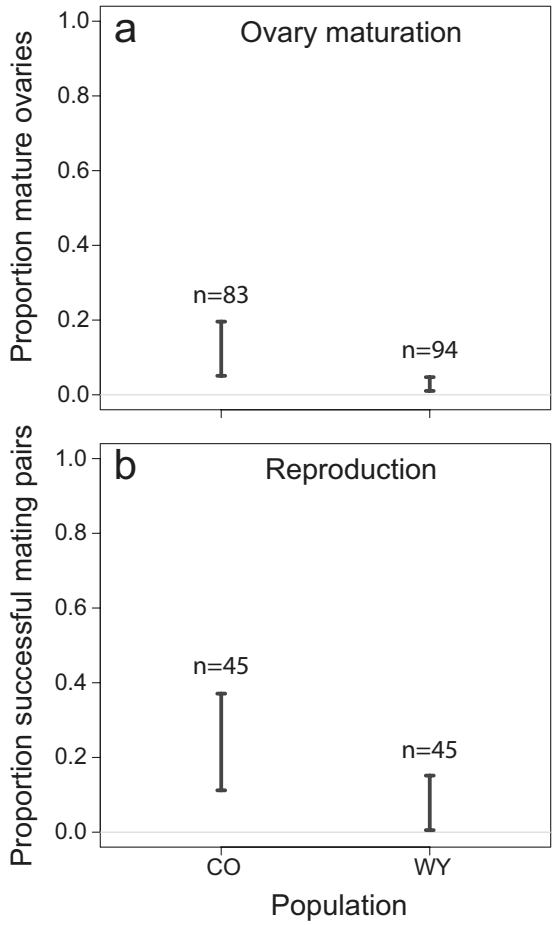


Fig. 6. 95% confidence intervals for the proportion of spruce beetles (a) with mature ovaries (presence of oocytes) and (b) the proportion of mating pairs successfully producing offspring for the Colorado (CO) and Wyoming (WY) populations. All proportions were greater than zero ($P << 0.001$) based on binomial tests of the null hypothesis of proportion mature/proportion mating = 0. Sample sizes (n) are for individual female beetles in (a) and for mating pairs of beetles (b).

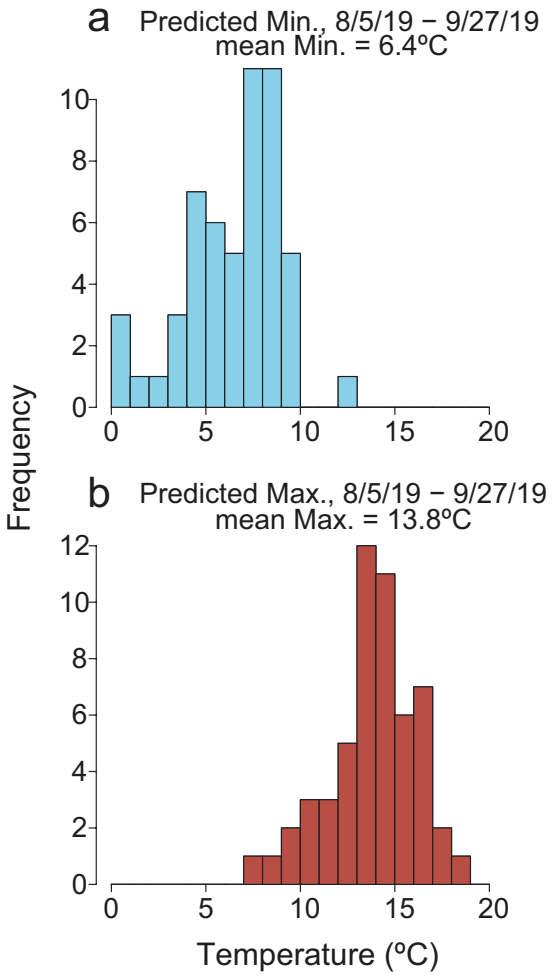


Fig. 7. Distributions of predicted daily minimum and maximum field phloem temperatures between 5 August and 27 September 2019 at the Guanella pass site where adult beetles were collected in the field. Predictions were derived from linear models predicting 2023 field phloem temperature from nearby SNOTEL data as described in the methods. Distributions represent the mean daily min/max between the two trees with temperature loggers.

Wyoming beetles. Note that these estimated percentages could be biased if beetles that died prior to emergences had different diapause propensities (see results). Nevertheless, the collective results suggest that a small proportion of female spruce beetles either averted or completed adult diapause without chilling. Though we cannot directly distinguish between aversion (not entering diapause) or entry then completion (termination) of diapause, the relatively long emergence times hint at the latter explanation. Moreover, without cold cues the extended emergence time observed in the lab (> 200 days) differed substantially from the more synchronous emergence that occurs within ~30 days in the field (McCambridge and Knight 1972). This synchronized, mass emergence, thought to be facilitated by adult diapause, is ecologically necessary for spruce beetles to effectively overcome host tree defenses (Chippendale 1982, Raffa et al. 2008). In the case of the field-collected Colorado beetles with developed oocytes as early as late September, we suspect that temperatures below the flight threshold of near 13°C (Holsten and Hard 2001) prevented dispersal and attacks on new hosts. That is, adult diapause delayed emergence during an unfavorable time of year (i.e., August and September) until simple temperature regulation was adequate to ensure synchronized emergence the following spring.

Our observation of slightly higher emergence and mating success at the more southern site (Colorado vs. Wyoming) is consistent with unpublished results (EM Hansen) summarized in Schebeck et al. (2017) suggesting that a larger proportion of spruce beetles sampled from New Mexico, USA (more southern) successfully reproduced without winter chilling compared to a population from Utah, USA (more northern). Although sampled trees at both sites were the same species and represent offspring from hundreds of attacking beetles in each population, with only one sampled tree from each, we cannot rule out the influence of host tree on emergence and mating traits.

In contrast to lab rearing in constant warmth, a majority of spruce beetles sampled from infested trees in the field during fall and early winter had developed oocytes. Though we did not test whether these field-collected beetles were capable of successful reproduction, the result shows that female beetles had developed past the clear, early arrest of oogenesis that we observed in the vast majority of female beetles in the laboratory study. This was an unexpected result because many studies suggest an extended, developmentally static dormancy in adult-diapausing beetles that suppresses oocyte development before and during winter, at least in the laboratory (Hodek 2011). Relatively short exposures to transiently (i.e., diurnally) cold

temperatures appeared to be sufficient to potentiate reproductive development. Although we do not know when during spring or summer 2019 the adult life stage was attained at our study locations, we estimate that the adults collected in mid-September experienced minimum temperatures less than or equal to 2°C on at least 4 days. It is unclear whether development is potentiated by a simple summation of cold exposure below a certain threshold, or whether there is a more complex and potentially broad reaction norm relating diapause development and temperature (Toxopeus et al. 2024).

Whatever the specific relationship between thermal variability and development through and past the initial block on oocyte development, the outcome in nature is that oogenesis is decelerated, yet clearly initiated prior to winter. Thus, beetles must survive winter and successfully synchronize emergence in spring after already investing in reproductive development. They may be functionally quiescent, suppressing further development only while temperatures remain below some lower thermal threshold for completion of reproductive maturation. However, under laboratory conditions spruce beetles require a sustained period of exposure to constant cold temperature (50 days or more at 2°C) to stimulate a transition from relatively slow to relatively rapid emergence from bolts (Bleiker and Willsey 2020). This is a much longer duration requirement of low temperature exposure than was necessary to stimulate oogenesis in our field study. Either there is some additional, subsequent block on development or emergence behavior past the initial block on oogenesis, or field conditions such as more intense cold or thermal fluctuations lead to different emergence patterns in nature (Colinet et al. 2015). In either case, overwintering after investing in oogenesis breaks the mold of pre-oogenesis overwintering established in previous studies (Hodek 2011). Overwintering pre-oogenesis is intuitive, as it prevents exposure of oocytes to potentially harsh conditions. Spruce beetle oocytes, however, are clearly cold tolerant, as the beetles can experience very low winter temperatures in the field (Miller and Werner 1987). Though investment in oogenesis will energetically deplete somatic tissues, beetles would need to make the same investment following winter if they overwintered pre-oogenesis. A careful accounting of energetic reserves pre-winter, metabolic costs pre-reproduction, and feeding post-winter could elucidate any unique features that accompany overwintering post-oogenesis.

Potential Responses to Changing Climates

Our findings that a small proportion of adult spruce beetles can become reproductively mature and potentially mate without cold temperature cues, in addition to reproductive development prior the onset of continuous cold during winter, highlight further flexibility in the spruce beetle lifecycle. In addition to a facultative prepupal diapause, a facultative adult diapause in some individuals could allow completion of a generation in a single summer assuming adult emergence occurred early in summer and temperatures were warm enough for completion of a generation. Although warm daytime temperatures with a few pulses of cold exposure could in combination enable both reproductive maturation and successful mating and reproduction in late summer or fall, life stages (e.g., eggs, early instars) that may not be as cold tolerant or sufficiently provisioned to tolerate overwinter nutrient depletion could occur in winter. This would limit the potential for 2 generations in a year. Flexibility of adult reproductive development could provide alternate pathways between semivoltinism and univoltinism, but could also lead to fractional voltinism that could have positive or negative influences on population dynamics (Van Dyck et al. 2015, Bentz et al. 2022). Further studies employing shorter chilling periods in the lab and earlier, more fine-grained field sampling would further elucidate the flexibility of adult diapause in nature.

Variation in adult diapause may also provide a pathway to evolutionary changes in the life cycle. Indeed, evidence for absolute chilling thresholds for diapause termination in insects is relatively scarce. Rather, diapausing insects that are temperature sensitive often exhibit variable responses within and across populations (Masaki 1961, Feder et al. 1997b, Schmidt et al. 2005), where cold temperatures accelerate diapause development, but are not absolutely necessary for diapause termination (Hodek 2013). Furthermore, diapause regulation and field phenology can evolve extremely rapidly (years to decades) in insects (Feder et al. 1997a, Gomi et al. 2007, Bradshaw and Holzapfel 2008, Batz et al. 2020). Though the proportion was small, some adult beetles from both Colorado and Wyoming matured ovaries and successfully reproduced without any low temperature exposure. If this variation were heritable, warming conditions could select for these currently rare phenotypes, moving the population mean response toward earlier reproductive maturity. Such evolutionary responses would need to shift beetles to a much shorter mean generation time to allow successful reproduction (and thus increase voltinism). Of course, warming conditions could also plausibly select against the warm-reproducing phenotype, e.g., if fractional voltinism leads to overwintering of life stages that cannot survive sustained and more extreme winter cold. Better understanding of these possibilities would be challenging to achieve, likely requiring both field observation and lab manipulation of temperature and tracking of phenotypic (and potentially genotypic) distributions across generations.

Reproductive Maturity, Not Emergence, Is the Most Reliable Indicator of Adult Diapause Status

Previous studies have used emergence as a measure of adult diapause termination, including those on spruce beetle (Bleiker and Willsey 2020) and the Douglas-fir beetle, *Dendroctonus pseudotsugae* (Ryan 1959). These studies correlate activity outside of the tree (e.g., being capable of flight) with reproductive maturity. However, our study shows that most emerged spruce beetles are not reproductively mature in the absence of a cold treatment. Because a high number of adults emerged from both populations but only a small proportion were reproductively viable, these results also suggest that adult emergence does not necessarily indicate reproductive maturity. Immature adults of this species are capable of activity—some proportion relocate from the upper bole to the base of the tree prior to overwintering, one year before they would normally reproduce (Knight 1961). In combination with our results, this suggests that emergence under constant warm conditions in the lab may primarily reflect relocation behavior in this species, not reproductive maturity.

We scored reproductive maturity using both ovary dissections and mating trials and found that the proportion of mating success without a cold treatment was higher than the proportion of females with developed oocytes for both Colorado and Wyoming populations. This could be due to several factors. It is possible that unemerged beetles may have had a higher proportion of developed ovaries, though we have no a priori reason to expect this. Alternatively, changes in behavior when exposed to a potential mate or a new uninfested tree (i.e., green rearing bolts) may further stimulate reproductive development. Intrapopulation variation may be another consideration as beetles used for ovary maturity assessments were sampled across the emergence distribution after 100 days at 22°C, whereas beetles used from mating experiments were sampled at the beginning of the main emergence curves, after approximately

50–70 days at 22°C. Thus, the experimental conditions and timing of sampling may influence estimates of the proportion of diapausing vs. mature bark beetle adults in a population.

Summary

Our study documents a low, but measurable rate of successful adult reproduction without chilling, and geographic variation in the thermal sensitivity of adult diapause in spruce beetle: beetles from a southern site had greater propensity to avert adult diapause than beetles from a northern site, similar to that found for *D. simplex* (McKee and Aukema 2015). Emergence was a poor predictor of reproductive maturity under warm conditions. We further showed that adult female beetles from a Colorado population can mature past the diapause stage and develop oocytes prior to winter when exposed to fluctuating warm and cool temperatures in field conditions, a new finding for this species. Further investigation of the thermal sensitivity of development and variation among and within populations could lead to better predictions about how beetle populations may respond to changing seasonal temperatures under climate change.

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Author contributions

Marianne Davenport (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Investigation [lead], Methodology [equal], Visualization [equal], Writing—original draft [lead]), Barbara Bentz (Conceptualization [equal], Formal analysis [equal], Methodology [equal], Resources [equal], Visualization [equal], Writing—review & editing [equal]), Earl Hansen (Conceptualization [equal], Formal analysis [equal], Methodology [equal], Resources [equal], Writing—review & editing [equal]), and Gregory Ragland (Conceptualization [equal], Data curation [equal], Formal analysis [equal], Funding acquisition [lead], Methodology [equal], Project administration [lead], Supervision [lead], Visualization [equal], Writing—original draft [equal])

Supplementary data

Supplementary data are available at *Environmental Entomology* online.

Data Availability

All data not provided in the main text, tables, figures, or supplement is available at https://figshare.com/s/e6130aee8151e91baa0c. Note that this is a temporary, private link for review purposes only. A public link to the repository will be provided at publication.

References

Altermatt F. 2010. Tell me what you eat and I'll tell you when you fly: diet can predict phenological changes in response to climate change. Ecol. Lett. 13(12):1475–1484. https://doi.org/10.1111/j.1461-0248.2010.01534.x

Batz ZA, Clemento AJ, Fritzenwanker J, et al. 2020. Rapid adaptive evolution of the diapause program during range expansion of an invasive mosquito. Evolution 74(7):1451–1465. https://doi.org/10.1111/evo.14029

Bentz BJ, Jönsson AM, Schroeder M, et al. 2019. Ips typographus and Dendroctonus ponderosae models project thermal suitability for intra- and inter-continental establishment in a changing climate. Front. For. Glob. Change 2. https://www.frontiersin.org/articles/10.3389/ffgc.2019.00001

Bentz BJ, Hansen EM, Davenport M, et al. 2022. 2 - Complexities in predicting mountain pine beetle and spruce beetle response to climate change. In: Gandhi KJK, Hofstetter RW, editors. Bark beetle management, ecology, and climate change. Cambridge: Academic Press; p. 31–54. https://doi.org/10.1016/B978-0-12-822145-7.00013-1

Berg EE, David Henry J, Fastie CL, et al. 2006. Spruce beetle outbreaks on the Kenai Peninsula, Alaska, and Kluane National Park and Reserve, Yukon Territory: relationship to summer temperatures and regional differences in disturbance regimes. Forest Ecol. Manag. 227(3):219–232. https://doi.org/10.1016/j.foreco.2006.02.038

Bleiker KP, Willsey T. 2020. experimental evidence supporting an obligate adult diapause for spruce beetle (Coleoptera: Curculionidae) from British Columbia. Environ. Entomol. 49(1):98–103. https://doi.org/10.1093/ee/nvz152

Bradshaw WE, Holzapfel CM. 2008. Genetic response to rapid climate change: it's seasonal timing that matters. Mol. Ecol. 17(1):157–166. https://doi.org/10.1111/j.1365-294x.2007.03509.x

Buckley LB, Nufio CR, Kirk EM, et al. 2015. Elevational differences in developmental plasticity determine phenological responses of grasshoppers to recent climate warming. Proc. Biol. Sci. 282(1809):20150441. https://doi.org/10.1098/rspb.2015.0441

Chippendale GM. 1982. Insect diapause, the seasonal synchronization of life cycles, and management strategies. Entomol. Exp. Appl. 31(1):24–35. https://doi.org/10.1111/j.1570-7458.1982.tb03116.x

Colinet H, Sinclair BJ, Vernon P, et al. 2015. Insects in fluctuating thermal environments. Annu. Rev. Entomol. 60(1):123–140. https://doi.org/10.1146/annurev-ento-010814-021017

Dambroski HR, Feder JL. 2007. Host plant and latitude-related diapause variation in *Rhagoletis pomonella*: a test for multifaceted life history adaptation on different stages of diapause development. J. Evol. Biol. 20(6):2101–2112. https://doi.org/10.1111/j.1420-9101.2007.01435.x

Denlinger DL. 2022. Insect diapause. Cambridge: Cambridge University Press.

Deutsch CA, Tewksbury JJ, Huey RB, et al. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. Proc. Natl. Acad. Sci. USA. 105(18):6668–6672. https://doi.org/10.1073/pnas.0709472105

Dyer EDA, hall PM. 1977. Factors affecting larval diapause in *Dendroctonus rufipennis* (coleoptera: scolytidae). Can. Entomol. 109(11):1485–1490. https://doi.org/10.4039/ent1091485-11

Eidson EL, Mock KE, Bentz BJ. 2018. Low offspring survival in mountain pine beetle infesting the resistant Great Basin bristlecone pine supports the preference-performance hypothesis. PLoS One 13(5):e0196732. https://doi.org/10.1371/journal.pone.0196732

Feder JL, Roethele JB, Wlazlo B, et al. 1997a. Selective maintenance of allozyme differences among sympatric host races of the apple maggot fly. Proc. Natl. Acad. Sci. USA. 94(21):11417–11421. https://doi.org/10.1073/pnas.94.21.11417

Feder JL, Stolz U, Lewis KM, et al. 1997b. The effects of winter length on the genetics of apple and hawthorn races of *Rhagoletis pomonella* (Diptera: Tephritidae). Evolution 51(6):1862–1876. https://doi.org/10.1111/j.1558-5646.1997.tb05109.x

Forrest JR. 2016. Complex responses of insect phenology to climate change. Curr. Opin. Insect Sci. 17:49–54. https://doi.org/10.1016/j.cois.2016.07.002

Forrest JRK, Thomson JD. 2011. An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. Ecol. Monogr. 81(3):469–491. https://doi.org/10.1890/10-1885.1

Gomi T, Nagasaka M, Fukuda T, et al. 2007. Shifting of the life cycle and life-history traits of the fall webworm in relation to climate change. Entomol. Exp. Appl. 125(2):179–184. https://doi.org/10.1111/j.1570-7458.2007.00616.x

Hansen EM, Bentz BJ. 2003. Comparison of reproductive capacity among univoltine, semivoltine, and re-emerged parent spruce beetles (Coleoptera: Scolytidae). Can. Entomol. 135(5):697–712. https://doi.org/10.4039/n02-109

Hansen EM, Bentz BJ, Turner DL. 2001. Physiological basis for flexible voltinism in the spruce beetle (Coleoptera: Scolytidae). Can. Entomol. 133(6):805–817. https://doi.org/10.4039/Ent133805-6

Hansen EM, Bentz BJ, Powell JA, et al. 2011. Prepupal diapause and instar IV developmental rates of the spruce beetle, *Dendroctonus rufipennis* (Coleoptera: Curculionidae, Scolytinae). J. Insect Physiol. 57(10):1347–1357. https://doi.org/10.1016/j.jinsphys.2011.06.011

Hodek I. 2011. Adult diapause in Coleoptera. Psyche J. Entomol. 2012:1–10. https://doi.org/10.1155/2012/249081

Hodek I. 2013. Controversial aspects of diapause development. EJE 99(2):163–173. https://doi.org/10.14411/eje.2002.024

Holsten EH. 1999. The Spruce Beetle. Washington (DC): USDA For. Serv. For.

Holsten EH, Hard JS. 2001. Dispersal flight and attack of the spruce beetle, *Dendroctonus rufipennis*, in south-central Alaska. USDA For. Serv. Res. Pap. PNW-RP-536.

Jacques J, Sampaio F, Santos HT, et al. 2019. Climate change and voltinism of Mythimna sequax: the location and choice of phenological models matter. Agric. For. Entomol. 21(4):431–444.

Knight FB. 1961. Variations in the life history of the Engelmann spruce beetle. Ann. Entomol. Soc. Am. 54(2):209–214. https://doi.org/10.1093/aesa/54.2.209

Koštál V. 2006. Eco-physiological phases of insect diapause. J. Insect Physiol. 52(2):113–127. https://doi.org/10.1016/j.jinsphys.2005.09.008

Lyon RL. 1958. A useful secondary sex character in Dendroctonus bark beetles. Can. Entomol. 90(10):582–584. https://doi.org/10.4039/ent90582-10

Masaki S. 1961. Geographic variation of diapause in insects. Bull. Fac. Agric. Hirosaki Univ. 7:66–98.

Masaki S. 2002. Ecophysiological consequences of variability in diapause intensity. Eur. J. Entomol. 99(2):143–154. https://doi.org/10.14411/eje.2002.022

Massey CL, Wygant ND. 1954. Biology and control of the Engelmann spruce beetle in Colorado. Washington (DC): USDA Forest Service, Circular no. 944.

McCambridge WF, Knight FB. 1972. Factors affecting spruce beetles during a small outbreak. Ecology 53(5):830–839. https://doi.org/10.2307/1934298

McKee FR, Aukema BH. 2015. Successful reproduction by the eastern larch beetle (Coleoptera: Curculionidae) in the absence of an overwintering period. Can. Entomol. 147(5):602–610. https://doi.org/10.4039/tce.2014.81

Miller LK, Werner RA. 1987. Cold-hardiness of adult and larval spruce beetles *Dendroctonus rufipennis* (Kirby) in interior Alaska. Can. J. Zool. 65(12):2927–2930. https://doi.org/10.1139/z87-444

Nozaki T, Matsuura K. 2021. Oocyte resorption in termite queens: seasonal dynamics and controlling factors. J. Insect Physiol. 131:104242. https://doi.org/10.1016/j.jinsphys.2021.104242

Nunes MV, Saunders DS. 1989. The effect of larval temperature and photoperiod on the incidence of larval diapause in the blowfly, *Calliphora vicina*. Physiol. Entomol. 14(4):471–474. https://doi.org/10.1111/j.1365-3032.1989.tb01116.x

Raffa KF, Aukema BH, Bentz BJ, et al. 2008. Cross-scale drivers of natural disturbances prone to anthropogenic amplification: the dynamics of bark beetle eruptions. BioScience 58(6):501–517. https://doi.org/10.1641/b580607

Ragland GJ, Armbruster PA, Meuti ME. 2019. Evolutionary and functional genetics of insect diapause: a call for greater integration. Curr. Opin. Insect Sci. 36:74–81. https://doi.org/10.1016/j.cois.2019.08.003

Ryan RB. 1959. Termination of diapause in the Douglas-fir beetle. Can. Entomol. 91(8):520–525. https://doi.org/10.4039/ent91520-8

Safranyik L, Simmons C, Barclay HJ. 1990. A conceptual model of spruce beetle population dynamics. Inf. Rep. - Pac. Yukon Reg. For. Can.(No. BC-X-316). https://www.cabdirect.org/cabdirect/abstract/19950609064.

Schebeck M, Hansen EM, Schopf A, et al. 2017. Diapause and overwintering of two spruce bark beetle species. Physiol. Entomol. 42(3):200–210. https://doi.org/10.1111/phen.12200

Schebeck M, Dobart N, Ragland GJ, et al. 2022. Facultative and obligate diapause phenotypes in populations of the European spruce bark beetle *Ips typographus*. J. Pest Sci. 95(2):889–899. https://doi.org/10.1007/s10340-021-01416-w

Schmidt PS, Matzkin L, Ippolito M, et al. 2005. Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. Evolution 59(8):1721–1732. https://doi.org/10.1111/j.0014-3820.2005.tb01821.x

Tauber MJ, Tauber CA, Masaki S. 1986. Seasonal adaptations of insects. USA: Oxford University Press.

Tobin PC, Nagarkatti S, Loeb G, et al. 2008. Historical and projected interactions between climate change and insect voltinism in a multivoltine species. Global Change Biol. 14(5):951–957. https://doi.org/10.1111/j.1365-2486.2008.01561.x

Toxopeus J, Dowle EJ, Andaloori L, et al. 2024. Variation in thermal sensitivity of diapause development among individuals and over time predicts life history timing in a Univoltine insect. Am. Nat. 203(6):E200–E217. https://doi.org/10.1086/729515

Van Dyck H, Bonte D, Puls R, et al. 2015. The lost generation hypothesis: could climate change drive ectotherms into a developmental trap? Oikos 124(1):54–61. https://doi.org/10.1111/oik.02066

Visser ME, Both C. 2005. Shifts in phenology due to global climate change: the need for a yardstick. Proc. Biol. Sci. 272(1581):2561–2569. https://doi.org/10.1098/rspb.2005.3356

Werner RA, Holsten EH, Matsuoka SM, et al. 2006. Spruce beetles and forest ecosystems in south-central Alaska: a review of 30 years of research. Forest Ecol. Manag. 227(3):195–206. https://doi.org/10.1016/j.foreco.2006.02.050