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Physiological Ecology

Evidence for an adult summer diapause in mountain pine beetle (Coleoptera: Curculionidae) that varies geographically and among haplogroups

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Identifying dormancy traits is important for predicting insect population success, particularly in a changing climate that could disrupt evolved traits. The mountain pine beetle (*Dendroctonus ponderosae* Hopkins) is native to North America, is responsible for millions of acres of tree mortality, and is expanding northward in Canada. Research has identified thermal traits important to epidemic-phase ecology that vary among populations. Genomic research identified 3 mountain pine beetle haplogroups representing Pleistocene glacial refugia. Significant variation in generation timing aligning with the haplogroups has been observed. The adult stage was previously identified as the likely cause of differences among populations, although the mechanism(s) remain unclear. We tested for an adult summer diapause that varies among populations from 2 haplogroups, southern Colorado (CO) (central haplogroup) and southern Idaho (ID) (eastern haplogroup) using respirometry and reproduction experiments. Warm temperatures (25 °C) resulted in reduced respiration rates of central haplogroup mountain pine beetle compared to a cool temperature treatment (15 °C), whereas respiration of the eastern haplogroup did not differ between the treatments. Mated pairs of central haplogroup mountain pine beetle reared/held at 15 °C were more likely to be classified with a higher reproductive success rating compared to pairs reared/held at 25 °C. These results support a facultative summer adult diapause in southern CO central haplogroup mountain pine beetle. Manifestation of this diapause was low/absent among adults from the northerly ID location. This diapause likely serves to maintain univoltinism shown to be important for mountain pine beetle epidemic-phase ecology. The variation occurring among haplogroups highlights the long-term, evolved processes driving local adaptations in mountain pine beetle.

Key words: insect seasonality, summer diapause, phenology, bark beetle ecology, local adaptation

Introduction

Mountain pine beetle (*Dendroctonus ponderosae* Hopkins) (Coleoptera: Curculionidae: Scolytinae) is a bark beetle species native to North America. In recent decades, mountain pine beetle population outbreaks and extensive tree mortality have occurred across the western United States and Canada due to a variety of biotic and abiotic factors (Raffa et al. 2008, Hicke et al. 2020). Although favorable forest stand composition and structure are necessary for population success (Fettig et al. 2014), temperatures that reduce cold-induced mortality and promote synchronized brood development can facilitate population growth to epidemic levels. Mountain pine beetle epidemic-phase ecology is associated with thermal conditions that result in the overwintering of cold tolerant late-stage larvae, seasonally appropriate and synchronized adult emergence needed to overwhelm host tree defenses, and a univoltine lifecycle (1 generation per year) (Logan and Bentz 1999, Powell and Logan 2005, Safranyik et al. 2010).

Mountain pine beetle has an extensive distribution across *Pinus* forests from northern Baja, California to northern British Columbia and eastward to Nebraska, South Dakota, and eastern Alberta (Dowle et al. 2017, Shegelski et al. 2021). What is now taxonomically considered mountain pine beetle was originally described as 2 species: *D. monticolae* west of the Great Basin, and *D. ponderosae* east of the Great Basin (Hopkins 1909). The 2 species were later synonymized to *D. ponderosae* (mountain pine beetle) based on morphology (Wood 1963). Recent genomic analyses, however,

separates mountain pine beetle into 3 distinct haplogroups based on Y loci, similar to Hopkins' (1909) original description, likely a result of historic contraction into separate Pleistocene glacial refugia (Bracewell et al. 2017, Dowle et al. 2017). Phylogeographic groups were identified as "western" (California, Oregon, Washington), "central" (Great Basin sky islands, Arizona [AZ]), and "eastern" (Idaho [ID], Montana, northern Utah [UT], northern Colorado [CO]) haplogroups (Dowle et al. 2017). Only 2 populations from the mountain pine beetle distribution in Canada (north central British Columbia and eastern Alberta) were included in the Dowle et al.'s (2017) analyses, and these were placed in the eastern haplogroup. Additional samples are required to further delineate haplogroup boundaries throughout Canada, in addition to non-sampled areas in the western United States. For example, recent collections and analyses place populations from southern CO in the central haplogroup (R.B. Bracewell, personal communication).

Previous research showed that evolved thermal developmental traits differ across the mountain pine beetle distribution in the United States (Bentz et al. 2001, Bracewell et al. 2013, Soderberg et al. 2021) and these differences align with the boundaries of the recently described haplogroups. For example, in common garden experiments at constant 22.5 °C, median time from infestation of host material to brood adult emergence was longer (149 d) for mountain pine beetle from AZ (central haplogroup), whereas that of mountain pine beetle from southern ID (eastern haplogroup) was 69 d (Bracewell et al. 2013). Similar results were observed in a translocation experiment wherein northern UT mountain pine beetle (eastern haplogroup) were reciprocally translocated with AZ mountain pine beetle (central haplogroup) in field trials. Mountain pine beetle sourced from northern UT had the fastest generation time at both the cooler UT site and warmer AZ site, while AZ mountain pine beetle had the slowest generation time at both field sites, highlighting genetic and environmental sources of variation in physiological strategies influencing generation time (Soderberg et al. 2021). The delay in generation timing at warm central haplogroup sites is likely an evolved response to local climates facilitating a seasonally appropriate univoltine lifecycle (Logan and Bentz 1999, Bentz et al. 2011).

Other than during adult dispersal, the mountain pine beetle lifecycle occurs entirely in the phloem beneath the bark of host trees. Mountain pine beetle has 4 larval instars including a transition stage (i.e., prepupae) at the end of the fourth instar wherein the gut is voided prior to pupation. A facultative diapause that varies among populations can occur in prepupae (Bentz and Hansen 2018), a stage that is considered among the most cold-hardy (Safranyik and Carroll 2006), particularly relative to the cold-intolerant egg (Bleiker et al. 2017) and pupal stages (Bleiker and Smith 2019). Following pupation, new adults are not fully sclerotized (i.e., teneral adult), and spend time beneath the bark maturation feeding on associated mycangial fungi (Bleiker and Six 2014) before emerging from the host tree to disperse and attack a new tree where mating occurs. A univoltine lifecycle is most common wherein larvae, typically late-stage larvae, overwinter and pupation and the adult stage occur in early to mid-summer. Adult emergence and dispersal to new trees are regulated by temperature and can vary among geographic locations, but typically occurs in mid to late summer (Reid 1962, Safranyik and Carroll 2006, Bentz et al. 2014, Soderberg et al. 2021).

To evaluate lifestage(s) responsible for the observed generation time differences among populations, McManis et al. (2018) conducted lab experiments of central haplogroup mountain pine beetle from central AZ and found that lifestage-specific developmental rates from oviposition through pupation to an adult varied from ID mountain pine beetle (Régnière et al. 2012), an eastern haplogroup population, but not sufficiently to account for the overall difference in observed generation time. These results suggest that overall developmental time differences between populations from each region occur during the adult stage. The adult stage, however, has been difficult to measure because of inability to determine eclosion dates in experimental bolts, where individuals are cryptic until emergence, or in phloem sandwiches, from which new adults are unable to emerge. Although the mechanism was not investigated, using field and laboratory based data, Wangen et al. (2024) recently documented that adult central haplogroup mountain pine beetle from AZ develop through the adult stage slower than eastern haplogroup beetles from ID.

While it is clear that populations are locally adapted and differences in generation timing are likely in the adult lifestage, the mechanism(s) remains unclear. One factor that could result in slow adult development timing in one population and not another is dormancy (i.e., diapause or quiescence). Diapause is a dynamic stage-specific neurohormonally mediated state of low metabolic activity that is genetically determined and associated with resistance to environmental extremes (Tauber et al. 1986). Quiescence is a developmental arrest that, unlike diapause, can occur at any developmental stage within a single species and is entered and exited immediately and in direct response to prevailing environmental conditions (Denlinger 2023). An adult diapause has been hypothesized for multiple *Dendroctonus* species based on observations of delayed adult emergence and/or lack of reproductive capacity in the absence of a considerable cold period (Massey and Wygant 1954, Ryan 1959, Langor and Raske 1987, Safranyik et al. 1990, Gent et al. 2017, Bleiker and Willsey 2020). Lester and Irwin (2012) suggested a diapause occurs in post-reproductive mountain pine beetle parent adults that was associated with cold-hardening and winter survival. Pre-reproductive adults (i.e., brood adults), however, typically do not overwinter and instead can be observed from early to late summer, depending on the geographic location (Reid 1962, Safranyik and Carroll 2006, Bentz et al. 2014, Soderberg et al. 2021). Although less well-represented in the literature, summer diapause is common in species of temperate insects worldwide including among insects with ecological requirements for univoltinism (Masaki 1980, Numata and Shintani 2023). Summer diapause occurs most frequently at the adult stage in Coleoptera and is maintained by warm temperatures and completed with falling temperatures, thereby serving to delay gonad maturation and synchronize population lifestages (Masaki 1980, Tzanakakis and Veerman 1994). Moreover, adult diapause can suppress mating activity (Hodek 2012). Among investigated Coleopterans, about 90% of studied species have an adult diapause, most conspicuously among a few families including Curculionidae (Hodek 2012). There are also examples of species wherein summer diapause has been found and it is nearly absent in northern parts of a range but with increasing incidence and duration of developmental arrest at more southerly locations (Masaki 2002, Yoshimura et al. 2021). Moreover, there are examples of relatively high temperatures inducing adult summer diapause and slowing diapause development, whereas low temperatures prevent diapause induction (Masaki 1980). Adult diapause in mountain pine beetle could serve to delay emergence, reproductive maturity, and mating, thereby maintaining the univoltine cycle in locations with warm mid to late summer temperatures.

Insect phenology models are important for determining timing of management activities and predicting population success under climate warming scenarios (Bentz and Jönsson 2015, Bentz et al. 2019, Crimmins et al. 2020). Efforts to model life cycle timing can be substantially confounded, however, if a dormancy syndrome is

manifested and geographically variable but not included in model parameterization. Our goal was to evaluate if central haplogroup mountain pine beetle have an adult summer diapause. We compared a central haplogroup population from southern CO to an eastern haplogroup population from southern ID, hypothesizing that central haplogroup adults are more likely than eastern haplogroup adults to enter a summer diapause. We hypothesized that warm temperatures would induce summer adult diapause in central haplogroup adults while cool temperatures would avert it. Endocrine control of a summer diapause, including reduced metabolism and development, has been found to be like that controlling winter diapause when the diapause occurs in the same lifestage (Masaki 1980, Ding et al. 2003, Denlinger et al. 2012). We used respirometry to investigate CO_2 production (i.e., a measure of metabolic activity) among central and eastern haplogroup mountain pine beetle at warm and cool temperatures across a range of ages after adult eclosion. Low CO_2 production is an indication of metabolic suppression and being in a state of diapause (Tauber et al. 1986). We also investigated the reproductive success of central haplogroup mountain pine beetle reared at the same warm and cool temperatures to clarify respirometry results and evaluate the potential for diapause effects on reproductive fitness.

Materials and Methods

CO_2 Production

Beetle Sources

Mountain pine beetle for the central haplogroup were obtained from an infested ponderosa pine (*Pinus ponderosa* Lawson & C. Lawson) from the San Juan National Forest, Colorado (37.71 °N, 108.67 °W; 2,424 m), felled 25 September 2020. At the time of felling, egg galleries were 5–10 cm long, suggesting infestation ~1 wk earlier, and no eggs were apparent in limited sampling. Eastern haplogroup mountain pine beetle were sourced from an infested lodgepole pine (*Pinus contorta* Douglas var. *latifolia* (Engelm.)) felled 12 November 2020, from the Sawtooth National Forest, Idaho (43.54 °N, 114.82 °W; 1,840 m). In limited sampling, egg galleries were >30 cm long with a majority of second-instar larvae present. Beetles from this tree were used only for the respirometry experiment and we did not test eastern haplogroup beetles for reproductive success under varying temperature treatments (described below).

Bolts were transported to the Rocky Mountain Research Station (RMRS) Laboratory in Logan, Utah. Bolt ends were sealed with wax to reduce desiccation and stored in walk-in coolers in complete darkness at near 0 °C until use. After about 1 wk in the cooler, central haplogroup respirometry bolts were transferred to 2 incubators at 20 °C with LD 12:12. On 16 November 2020, after most brood attained the prepupal and pupal stages, we stripped the bolts and made ~150 phloem discs (see below). For the eastern haplogroup respirometry, bolts were transferred to room temperature on 4 January 2021 in anticipation of completion of central haplogroup respirometry. On 3 February 2021, we made ~180 phloem discs using eastern haplogroup prepupae and pupae.

Phloem Discs

Our experimental design required determination of eclosion dates for each tested beetle. We have decades of experience with glass/Plexiglas "phloem sandwiches" which allow visual inspection of individuals to monitor timing of egg eclosion, instar molting, pupation, and adult eclosion (e.g., Bentz et al. 1991, Hansen et al. 2001, McManis et al. 2018). For the respirometry study, we needed to observe the adult eclosion date and conduct repeated measurements on individual beetles, i.e., keep record of individual beetles and keep them alive over multiple weeks of measurements. Instead of a 150 × 150 mm glass/Plexiglas sandwich with multiple individuals, we used 50 × 11 mm polystyrene Petri dishes to enclose a 50 mm diameter bark and phloem disc removed from the bolts using a hole saw. Bark on ponderosa pine bolts was shaved with a draw knife to leave a bark and phloem thickness similar to the depth of the Petri dish, allowing a close-fitting margin between the phloem and dish lid. For the thin-barked lodgepole pine, we added sawdust under the bark and phloem disc to get a snug fit to the lid.

Discs were removed from bolts and we targeted 1 pupa per Petri dish, removing any other lifestages. The pupa was placed in a mountain pine beetle larvae-created pupal chamber, when available, and the lid secured with transparent medical tape to allow access for weekly respirometry. Some sandwiches were made with prepupae and some of the discs had only feeding tunnels and egg galleries without a pupal chamber. The latter were populated with extra pupae from discs with more than 1 pupa. Petri dishes were housed in plastic desiccators (Bel-Art Products, Fisher Scientific) that were fashioned into humidity chambers by including a salt solution (400 ml water, 40 ml salt) that maintained ~95% relative humidity in the chamber. Chambers were placed in incubators (Percival Scientific, Inc., Perry, IA, USA) at the treatment temperatures immediately after disk construction and the Petri dishes were examined daily for eclosion to the adult stage. *Dendroctonus* spend their lifecycle beneath the bark of host trees and thermoperiod rather than photoperiod has been a considered critical cue for diapause (Hansen et al. 2001, 2011). Therefore, chambers containing the disks were in incubators with complete darkness, and disks were placed horizontally in the chambers with the bark side down. We did not note any attempts of beetles to chew through the bark to emerge, but this anecdote is confounded by a potential geotropic response to the position of the disk.

Treatments

Data from Soderberg et al. (2021) suggest that, among central haplogroup mountain pine beetle, constant 25 °C induces summer diapause while diapause is averted or reduced at 18 °C. We chose 25 °C as the potential "diapause-inducing" treatment and 15 °C as the "diapause-averting" treatment. Development rate data are available for pre-adult lifestages of central haplogroup mountain pine beetle at both temperatures (McManis et al. 2018). These temperatures were applied to discs in the Petri dishes starting with the prepupal/pupal stage so that adult eclosion would occur at the treatment temperature.

Additional treatments tested the timing of diapause induction, if any, resulting from exposure to constant 25 °C. In these treatments, adult beetles that had been at 25 °C as a prepupae/pupae were exposed to 25 °C for 20, 40, or 60 d after eclosion then transferred to constant 15 °C. Additionally, after 2 weekly measurements at 15 °C, one-half of the beetles that had been transferred remained at 15 °C while the other one-half was returned to 25 °C. Thus, our treatments were:

1) Constant 15 °C;
2) Constant 25 °C;
3) Constant 25 °C for 20 d post-eclosion then transfer to 15 °C (hereafter, "Transfer 20d");
4) Constant 25 °C for 40 d post-eclosion then transfer to 15 °C ("Transfer40d");
5) Constant 25 °C for 60 d post-eclosion then transfer to 15 °C ("Transfer60d");

6) Constant 25 °C for 20 d post-eclosion, transfer to 15 °C for 2 wk, then returned to 25 °C ("Transfer20d_return");
7) Constant 25 °C for 40 d post-eclosion, transfer to 15 °C for 2 wk, then returned to 25 °C ("Transfer40d_return").

Note that cumulative mortality reduced the samples sizes to unacceptably low values for a potential "Transfer60d_return" treatment.

Respirometry

We assumed mortality would become problematic over multiple weeks of repeated sampling, so we started the experiment with 14 replicates per treatment and additional discs of each treatment were available as replacements for any deaths. Over the 80 d of the experiment, approximately 70% of individuals in disks were exchanged with additional individuals from the same treatment conditions. We conducted weekly respirometry starting within a few days of eclosion to detect reduced respiration as an indication of diapause. Respiration of individuals included in transfer treatments was measured 1 wk after transfer to the new temperature.

On sample days, beetles were removed from incubators at the treatment temperature (i.e., 15 or 25 °C) and equilibrated at 20 °C for 1 h before conducting constant volume respirometry (PTC-1 temperature cabinet and PELT-3 controller, Sable Systems International, North Las Vegas, NV, USA). That is, all measurements were conducted at a standardized temperature, not the treatment temperature. Thus, any differences in respiration rate are assumed to be a result of physiological changes from the treatment temperature rather than the temperature during respirometry.

Beetles were placed individually into glass respirometry chambers attached to a CO_2 analyzer (LI-COR LI-6251, LI-COR, Lincoln, NE, USA). The Sable multiplexer has 8 channels and, with one reserved as the baseline, can measure 7 individuals per cycle. Respiration, expressed as CO_2 output, was measured at 20 °C after an additional 20 min with a flow rate of 50 ml/min of ambient air depleted of water vapor and carbon dioxide using columns of Drierite (W. A. Hammond DRIERITE Co. LTD, Xenia, OH, USA) and Ascarite (Thomas Scientific, Swedesboro, NJ, USA). Data acquisition and constant volume calculations were made with Expedata-P software and a custom macro (Sable Systems International). Beetles were weighed (±0.001 mg; Cahn C-33, Thermo Orion, Beverly, MA, USA) to calculate weight-standardized expression of respiration (ml CO_2 g^{-1} h^{-1}).

Reproductive Success

Beetle Sources

Central haplogroup mountain pine beetle were obtained from an infested ponderosa pine from the San Juan National Forest, Colorado (37.71 °N, 108.67 °W; 2,420 m), about 1 km from the location of the population used in respirometry experiments. The tree was felled 21 September 2022. At the time of felling, egg galleries were 10–15 cm long, suggesting infestation 1–2 wk earlier, but no eggs were apparent in limited sampling. Bolts were transported to the RMRS Laboratory in Logan, Utah. Bolt ends were sealed with wax to reduce desiccation and stored in walk-in coolers in constant darkness at near 0 °C until use. Bolts were placed at room temperature on 26 September 2022. Most brood therein attained the fourth instar by late October when we made ~400 phloem discs, allowing for adequate replication despite anticipated losses to mortality and unbalanced sex ratios. An un-infested ponderosa pine was cut from the Dixie National Forest, Utah (37.55 °N, 112.62 °W; 2,472 m), on 26 October 2022, to provide green bolts for female–male pairs to establish egg galleries. Eastern haplogroup MPB were not evaluated for reproductive success.

Treatments

We chose 15 and 25 °C as treatment temperatures to match the respirometry testing. Again, these temperatures were applied to discs in the Petri dishes starting with the prepupal or pupal stage so that adult eclosion would occur at the treatment temperature. Individual beetles were reared in separate petri dishes in one of the 2 temperatures and checked daily for eclosion. At target intervals of 15, 30, or 45 d post-eclosion, the adult was removed and gender was determined. Because individual beetles eclosed on a continuum of dates, we paired females and males that were within 2 d post-eclosion age of each other. That is, replicate pairs were not necessarily exactly aged at the target classes. In anticipation of higher mortality rates, additional phloem discs were allocated to the 25 °C treatment. We intended a minimum of 5 replicates per treatment/post-eclosion age class but utilized all available female–male pairs, resulting in additional replicates in some cases. Female/male pairs were manually inserted into the phloem of un-infested bolts for mating. Bolts were kept at either 15 or 25 °C in 12:12 LD until the bolts were sampled to quantify reproductive success. Treatments were:

1) Constant 15 °C from before eclosion until bolt sampling;
   a. Female/male pairs mated 15 d post-eclosion;
   b. Female/male pairs mated 30 d post-eclosion;
   c. Female/male pairs mated 45 d post-eclosion;
2) Constant 25 °C from before eclosion until bolt sampling;
   a. Female/male pairs mated 15 d post-eclosion;
   b. Female/male pairs mated 30 d post-eclosion;
   c. Female/male pairs mated 45 d post-eclosion;
3) 15 °C from before eclosion until mating/infestation, infested bolt held at 25 °C;
   a. Female/male pairs mated 15 d post-eclosion;
   b. Female/male pairs mated 30 d post-eclosion;
   c. Female/male pairs mated 45 d post-eclosion;
4) 25 °C from before eclosion until mating/infestation, infested bolt held at 15 °C.
   a. Female/male pairs mated 15 d post-eclosion;
   b. Female/male pairs mated 30 d post-eclosion;
   c. Female/male pairs mated 45 d post-eclosion.

Using data from McManis et al. (2018, 2019), we estimated the 50th percentile egg would hatch about 16 and 30 d, respectively, after infestation at 25 and 15 °C. That is, these temperature-dependent intervals were considered adequate for oviposition and, at least, some portion of egg eclosion which confirms egg viability. Therefore, beetles reared at 25 °C then transferred to bolts at 15 °C after mating were kept at 15 °C for 30 d prior to sampling, and beetles reared at 15 °C then transferred to 25 °C after mating were kept at 25 °C for 16 d prior to sampling. Individual galleries were opened after those intervals to measure gallery length and count eggs and larvae. For galleries with eggs but no larvae, we put the eggs on moistened filter paper in a Petri dish and monitored for eclosion to first-instar larvae at room temperature. We found no evidence that eggs were nonviable.

Analyses

CO_2 Production

Statistical analyses were made using generalized linear mixed models (PROC GLIMMIX, SAS Institute, Inc., Cary, NC, USA; Littell et al.

2006). The main effects tested were treatment, post-eclosion age, and their interaction. The sex of each individual was included as a controlled variable. The response error distribution was specified as gamma using the log link function and degrees of freedom were calculated using the Kenward–Roger method. Random effects included the multiplexer channel number (i.e., the first beetle measured in each cycle had 1 h at the equilibration temperature, whereas the seventh beetle had about 1 h 25 min at the equilibration temperature), the technician operating the respirometer during each cycle (operator bias in calibration of the CO_2 analyzer is possible although none was detected in preliminary analyses), and the model residuals for each individual beetle (autoregressive lag 1 type as most beetles were repeatedly sampled).

Note that the transfer treatments are identical to the constant 25 °C treatment during the initial time steps. For example, Transfer20d beetles were at constant 25 °C for the first 3 weekly time steps, and Transfer40d beetles were at constant 25 °C for the first 6 weekly time steps. This artifact of the design lends itself to increased sample size for the constant 25 °C treatment.

Pairwise comparisons of treatment least squares means were made using SAS PROC PLM, including comparisons treatment means at selected post-eclosion ages. *P*-values were adjusted for family-wise error using the Bonferroni method. For displaying results, we specified a 95% confidence interval from modeled output. In separate models, the Transfer20d_return and Transfer40d_return treatments were compared only to the Transfer20d and Transfer40d treatments, respectively.

Reproductive Success

Because the response variable can be ranked (0 = no eggs, 1 = eggs only, 2 = at least some larvae, i.e., egg hatch), we analyzed these data with an ordinal logistic regression model and confirmed the proportional odds assumption (Hosmer et al. 2013). We again used SAS PROC GLIMMIX but with a multinomial response distribution and the cumulative logit link function (Littell et al. 2006). Replicate within treatment was specified as a random variable, degrees of freedom were calculated using the Kenward–Roger method, and we requested output of the odds ratios. We consider a lack of oviposition to be evidence of a diapause that influences reproductive fitness albeit some observations could simply be failures of manual mating (see Discussion).

Results

Central Haplogroup Respirometry

Treatment by post-eclosion age interaction was significant in our model ($F_{4,550} = 5.29$, $P < 0.001$), whereas treatment ($F_{4,482} = 1.55$, $P = 0.185$), age ($F_{1,643} = 0.48$, $P = 0.487$), and sex were not ($F_{2,150} = 0.50$, $P = 0.607$). Comparing the constant 15 and 25 °C treatments, respiration rates initially were not significantly different but quickly diverged, the model indicating divergence by 14 d post-eclosion ($\alpha = 0.05$) (Fig. 1, Table 1). The timing from this linear model result, however, potentially misses actual timing of treatment separation. In preliminary analyses, data were parsed into weekly age classes and the 2 treatments did not become significantly different until the fourth week ("age class 24 d").

Within the transfer treatments, the first respiration measurement was taken 1 wk following placement at 15 °C from 25 °C. Respiration rates of Transfer20d beetles were statistically indistinguishable from those in the constant 15 °C treatment 1 wk following transfer (Fig. 2A, Table 1). As with the constant 15 °C beetles, Transfer20d beetles had higher respiration rates than constant 25 °C beetles throughout the interval of comparison. Beetles in the Transfer40d treatment responded somewhat slower to the transfer to 15 °C. Initial measurements taken 1 wk after transfer were not significantly different than those of either the constant 15 or 25 °C treatments. Within a few days, however, rates of Transfer40d beetles became statistically indistinguishable from rates of constant 15 °C beetles and significantly higher than those of constant 25 °C beetles (Fig. 2B). Similarly, respiration rates of Transfer60d beetles were intermediate to, but not significantly different than, rates of constant 15 and 25 °C beetles during the first measurement 1 wk following transfer to 15 °C. Thereafter, Transfer60d beetles had respiration significantly higher than that of constant 25 °C beetles and not significantly different than that of constant 15 °C beetles (Fig. 2C, Table 1).

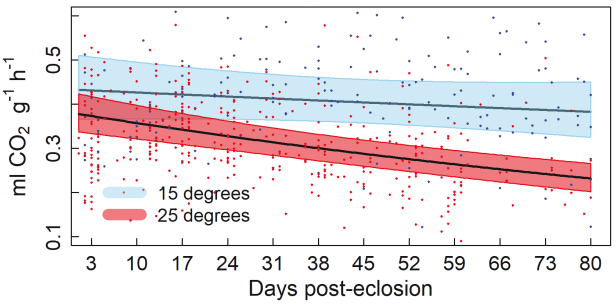

Fig. 1. Predicted means, as a function of post-eclosion age, and 95% confidence intervals of respiration rates for southern Colorado central haplogroup mountain pine beetles treated at constant 15 and 25 °C. All measurements were taken at 20 °C after 1 h equilibration from the treatment temperature. The scatterplot data are the actual observations.

In a model comparing respiration rates of Transfer20d and Transfer20d_return beetles, the treatment by post-eclosion age interaction was marginally significant ($F_{1,39} = 3.76$, $P = 0.060$), whereas treatment ($F_{1,93} = 1.68$, $P = 0.198$) and age were not ($F_{1,59} = 1.24$, $P = 0.271$). Sex was marginally significant in this model ($F_{2,21} = 3.35$, $P = 0.054$) and a pairwise comparison found females to have marginally higher respiration rates than males (gamma-scale estimate of the difference 0.300, SE 0.116, $t_{21} = 2.59$, $P|t| = 0.017$, $P_{adj} = 0.068$). Respiration rates of Transfer20d_return beetles declined after return to 25 °C but never became significantly different than those of Transfer20d beetles (Supplementary Fig. S1A, Supplementary Table S1).

In the model comparing respiration rates of Transfer40d and Transfer40d_return beetles, the treatment by post-eclosion age interaction was significant ($F_{1,70} = 3.98$, $P = 0.050$), whereas treatment ($F_{1,69} = 1.77$, $P = 0.188$), age ($F_{1,67} = 0.00$, $P = 0.945$), and sex were not ($F_{1,20} = 0.27$, $P = 0.608$). Respiration rates 1 wk after return to 25 °C found no significant difference among Transfer40d and Transfer40d_return beetles. Thereafter, rates of beetles in the treatments diverged, with Transfer40d_return beetles having significantly reduced respiration rates than those of Transfer40d beetles (Fig. 3B, Supplementary Table S1).

Eastern Haplogroup Respirometry

Treatment ($F_{4,424} = 3.23$, $P = 0.013$) and the treatment by post-eclosion age interaction were significant in our model ($F_{4,438} = 2.73$, $P = 0.029$), whereas age ($F_{1,619} = 0.55$, $P = 0.457$) and sex were not ($F_{2,171} = 2.46$, $P = 0.088$). Pairwise comparisons, however, found no significant differences among the constant 15 and 25 °C treatments at any age (Fig. 3, Table 2). Moreover, the only significant difference among the transfer and constant temperature treatments was

Table 1. Pairwise comparisons of southern CO central haplogroup mountain pine beetle respirometry treatments least squares means at selected post-eclosion ages

Treatment pair	Age	Estimate	SE	*t*-value	P_t	P_{adj}
15–25 °C	0	0.130	0.080	1.63	0.105	1
15–25 °C	20	0.223	0.056	3.95	<0.001	0.002
15–25 °C	40	0.316	0.049	6.49	<0.001	<0.001
15–25 °C	60	0.408	0.063	6.50	<0.001	<0.001
15–25 °C	80	0.501	0.089	5.64	<0.001	<0.001
15 °C – Transfer20d	20	−0.057	0.090	−0.63	0.528	1
15 °C – Transfer20d	40	−0.109	0.067	−1.63	0.104	1
15 °C – Transfer20d	60	−0.161	0.081	−1.98	0.049	1
15 °C – Transfer20d	80	−0.213	0.121	−1.76	0.079	1
Transfer20d – 25 °C	20	0.280	0.078	3.59	<0.001	0.009
Transfer20d – 25 °C	40	0.425	0.058	7.30	<0.001	<0.001
Transfer20d – 25 °C	60	0.569	0.077	7.43	<0.001	<0.001
Transfer20d – 25 °C	80	0.714	0.117	6.13	<0.001	<0.001
15 °C – Transfer40d	40	0.081	0.093	0.87	0.388	1
15 °C – Transfer40d	60	−0.068	0.075	−0.91	0.363	1
15 °C – Transfer40d	80	−0.217	0.122	−1.78	0.076	1
Transfer40d – 25 °C	40	0.235	0.088	2.66	0.008	0.204
Transfer40d – 25 °C	60	0.476	0.069	6.93	<0.001	<0.001
Transfer40d – 25 °C	80	0.718	0.114	6.28	<0.001	<0.001
15 °C – Transfer60d	60	0.180	0.125	1.44	0.150	1
15 °C – Transfer60d	80	−0.006	0.126	−0.05	0.960	1
Transfer60d – 25 °C	60	0.228	0.121	1.88	0.060	1
Transfer60d – 25 °C	80	0.507	0.116	4.37	<0.001	<0.001

P-values were adjusted using the Bonferroni method.

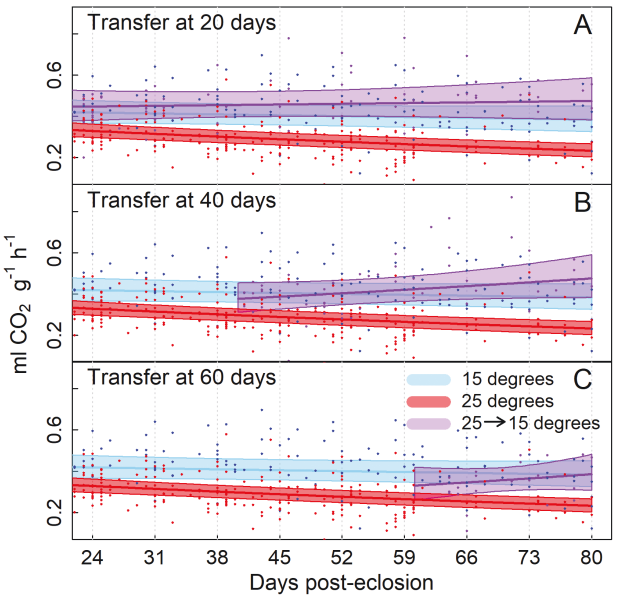

Fig. 2. Predicted means, as a function of post-eclosion age, and 95% confidence intervals of respiration rates for southern Colorado central haplogroup mountain pine beetles treated at constant 15 and 25 °C and additional populations transferred from 25 to 15 °C after 20 (A), 40 (B), and 60 (C) days post-eclosion. All measurements were taken at 20 °C after 1 h equilibration from the treatment temperature. The scatterplot data are the actual observations.

between Transfer40d and constant 25 °C beetles at age 40 d wherein Transfer40d beetles had significantly higher respiration rates than constant 25 °C beetles (Table 2).

The generalized linear mixed model found differences among the Transfer20d and Transfer20d_return treatments: age ($F_{1,52}$ = 6.45, P = 0.014) and the treatment by age interaction were significant ($F_{1,60}$ = 4.19, P = 0.045), whereas treatment ($F_{1,66}$ = 1.75, P = 0.191) and sex were not ($F_{1,21}$ = 0.69, P = 0.416). Pairwise comparisons at selected ages indicate no significant differences between the treatments within a few days after the Transfer20d_return beetles were returned to 25 °C but rates significantly declined among Transfer20d_return beetles as the experiment progressed (Supplementary Fig. S2, Supplementary Table S2). In the model comparing respiration rates in the Transfer40d and Transfer40d_return treatments, only post-eclosion age was significant ($F_{1,49}$ = 4.22, P = 0.045); the effects of treatment ($F_{1,53}$ = 0.16, P = 0.689), the treatment by age interaction ($F_{1,50}$ = 0.01, P = 0.912), and sex ($F_{2,20}$ = 0.07, P = 0.929) were not significant. Pairwise comparisons at selected ages revealed no significant differences among the treatments (Supplementary Table S2).

Central Haplogroup Reproductive Success

Rearing ($F_{1,96}$ = 19.42; P < 0.0001) and post-mating ($F_{1,96}$ = 6.28; P = 0.0139) temperature treatments were significantly related to the likelihood of being classified with a higher reproductive success rating (i.e., eggs present instead of no eggs or larvae present instead of eggs only) (Fig. 4). Mated pairs reared at 15 °C were 10.5 times more likely to be classified with a higher reproductive success rating compared to pairs reared at 25 °C (95% confidence interval: 3.7–30.5) (Fig. 4A, B). Mated pairs held at 15 °C after mating (Fig. 4A, C) were 3.7 times more likely to be classified with a higher reproductive success rating compared to pairs held at 25 °C after mating (Fig. 4B, D) (95% confidence interval: 1.3–10.5). Post-eclosion age class was not significant ($F_{2,90.3}$ = 0.100; P = 0.9636) nor were any interactions of rearing temperature, post-mating temperature, and post-eclosion age. Among most unsuccessful matings, female–male pairs were not observed to have done anything more than advance short (<5 cm) feeding tunnels during the 16–30 d after infestation. As evidenced by the presence of larvae within 20 d post-eclosion in

15 °C-treated central haplogroup beetles (Fig. 4A, B), some beetles were reproductively mature within a few days of eclosion. These results suggest that reproductive organs may become mature soon after adult eclosion although additional research is needed to better understand the timing and contributing factors.

Discussion

Summer diapause is induced by warm temperatures and terminated by cool temperatures, and, in adults, is followed by reproduction thereby serving to synchronize seasonal timing (Masaki 1980). We provide evidence for an adult summer diapause in a central haplogroup mountain pine beetle population from southern CO based on several lines of evidence. The diapause was induced at 25 °C, a temperature near optimal for pre-adult lifestage development and oviposition in this population (McManis et al. 2018, 2019), and terminated when exposed to 15 °C. In the absence of diapause, respiration should be at or near maximal rates at 25 °C yet we observed reduced respiration at that temperature when applied starting at the prepupal/pupal stage and measured for up to 80 d post-eclosion. Reduced respiration from this treatment strongly indicates suppressed metabolism resulting from the diapause syndrome. Summer diapause has a shorter period for completion than winter diapause (Masaki 1980) and distinguishing between summer diapause and quiescence can be difficult. For example, the onion maggot completed summer diapause within 5 d at 16 °C following induction at 24 °C (Ishikawa et al. 2000). Similarly, we found that when mountain pine beetle adults with reduced respiration at 25 °C (i.e., in diapause) were transferred to 15 °C, respiration measured 1 wk following transfer increased and was similar to levels of individuals kept at 15 °C, suggesting that diapause was terminated. Low temperature was also found to accelerate summer diapause termination in the cabbage armyworm (Ding et al. 2003). Metabolic arrest associated with quiescence occurs immediately following exposure to an unfavorable condition (Tauber et al. 1986, Diniz et al. 2017), often within an hour (Hand and Podrabsky 2000). In our experiments, all individuals in temperature treatments were held for 1 h at 20 °C prior to respirometry measurements, and despite this we found reduced respiration among 25 °C-treated beetles, relative to those at 15 °C (Figs. 1–3, Table 1, Supplementary Table S1), suggesting physiological changes caused by the temperature treatments. In the absence of diapause, respiration was expected to be identical among the temperature treatments.

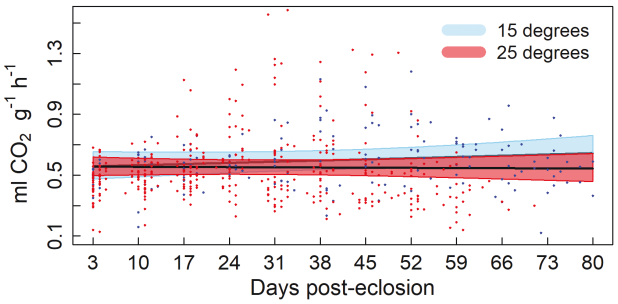

Fig. 3. Predicted means, as a function of post-eclosion age, and 95% confidence intervals of respiration rates for southern Idaho eastern haplogroup mountain pine beetles (i.e., sourced from Idaho) treated at constant 15 and 25 °C. All measurements were taken at 20 °C after 1 h equilibration from the treatment temperature. The scatterplot data are the actual observations.

Respirometry results suggesting adult diapause for the southern CO central haplogroup population are further supported by the reproductive success results. Central haplogroup mountain pine beetle reared and/or held post-mating at 15 °C were several times more likely to have galleries with eggs and larvae than those reared and/or held post-mating at 25 °C (Fig. 4). Again, 25 °C is near the optimal rate of development for all pre-adult lifestages, including oviposition (McManis et al. 2018, 2019). The lack of any apparent

Table 2. Pairwise comparisons of southern ID eastern haplogroup mountain pine beetle respirometry treatments least squares means at selected post-eclosion ages

Treatment pair	Age	Estimate	SE	t_{637}-value	P_t	P_{adj}
15–25 °C	0	−0.003	0.091	−0.03	0.978	1
15–25 °C	20	0.041	0.059	0.69	0.489	1
15–25 °C	40	0.085	0.051	1.66	0.099	1
15–25 °C	60	0.128	0.074	1.73	0.086	1
15–25 °C	80	0.172	0.111	1.55	0.122	1
15 °C – Transfer20	20	−0.147	0.113	−1.30	0.195	1
15 °C – Transfer20	40	−0.061	0.073	−0.83	0.409	1
15 °C – Transfer20	60	0.026	0.102	0.25	0.801	1
15 °C – Transfer20	80	0.112	0.168	0.67	0.505	1
Transfer20 – 25 °C	20	0.188	0.103	1.81	0.071	1
Transfer20 – 25 °C	40	0.145	0.068	2.14	0.034	0.829
Transfer20 – 25 °C	60	0.103	0.103	1.00	0.319	1
Transfer20 – 25 °C	80	0.060	0.169	0.35	0.724	1
15 °C – Transfer40	40	−0.319	0.117	−2.72	0.007	0.169
15 °C – Transfer40	60	0.050	0.088	0.56	0.573	1
15 °C – Transfer40	80	0.419	0.168	2.50	0.013	0.319
Transfer40 – 25 °C	40	0.404	0.114	3.54	<0.001	0.011
Transfer40 – 25 °C	60	0.078	0.088	0.89	0.375	1
Transfer40 – 25 °C	80	−0.247	0.168	−1.47	0.143	1
15 °C – Transfer60	60	0.375	0.214	1.75	0.081	1
15 °C – Transfer60	80	0.334	0.209	1.60	0.110	1
Transfer60 – 25 °C	60	−0.247	0.213	−1.16	0.248	1
Transfer60 – 25 °C	80	−0.162	0.206	−0.79	0.432	1

P-values were adjusted using the Bonferroni method.

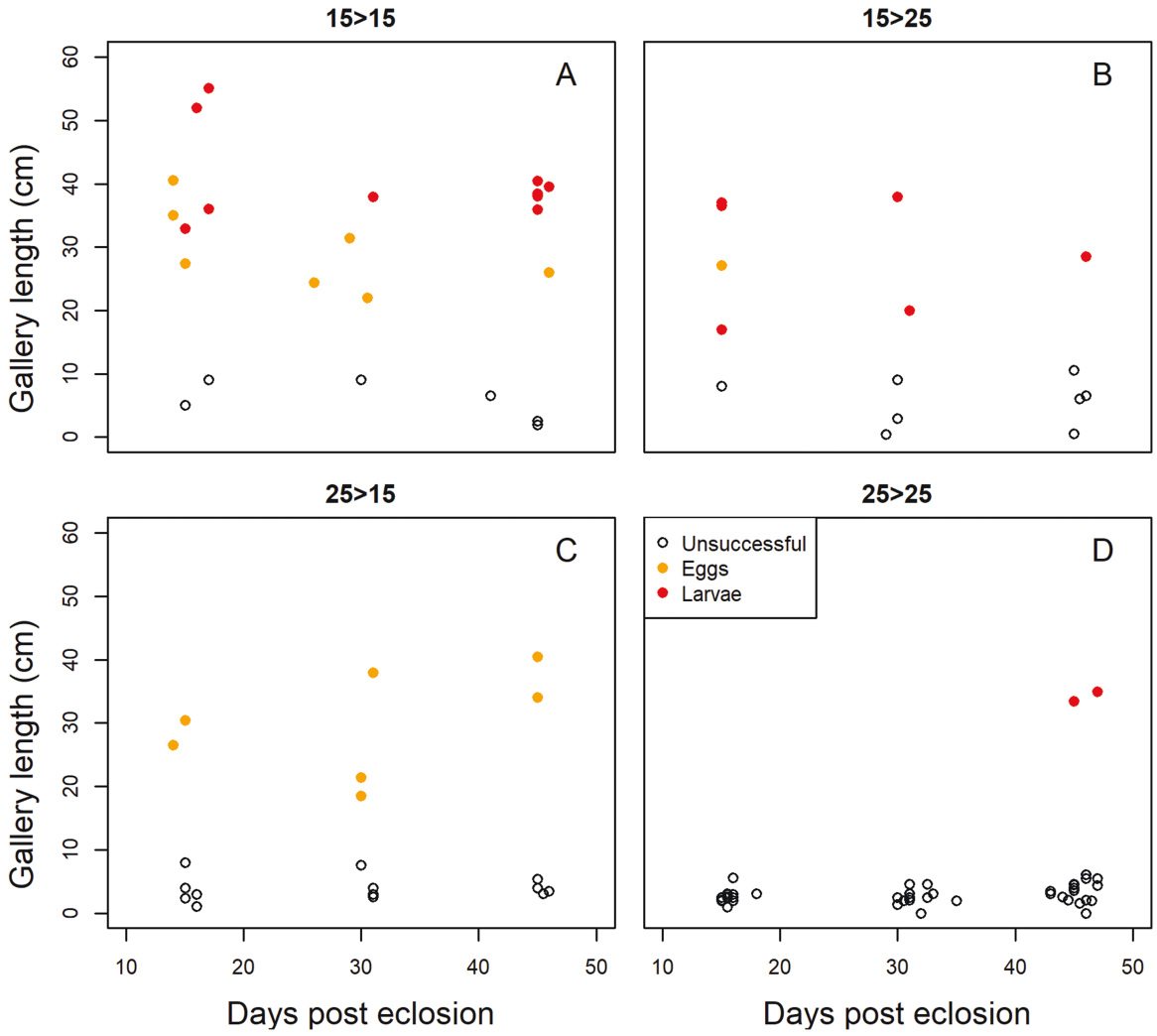

Fig. 4. Southern Colorado central haplogroup mountain pine beetle reproductive success and egg gallery length by rearing and post-mating temperatures. A) 15 > 15: beetles reared at constant 15 °C and remained at 15 °C after mating. B) 15 > 25: beetles reared at constant 15 °C and placed at 25 °C after mating. C) 25 > 15: beetles reared at constant 25 °C and placed at 15 °C after mating. D) 25 > 25: beetles reared at constant 25 °C and remained at 25 °C after mating. The interval between infestation of the bolts and destructive sampling was extended 16 d for the 15 °C post-mating treatments and 30 d for the 25 °C post-mating treatments in effort to allow equivalent oviposition and brood development. In our ordinal logistic regression model, unsuccessful galleries were coded as 0, eggs only as 1, and galleries with at least some larvae as 2.

mating activity among unsuccessful infestations is consistent with the suppression of mating behavior related to diapause (Hodek 2012). Sensitive stages for induction of summer diapause have been found to be in the lifestage just prior to the stage where diapause occurs (Ishikawa et al. 2000, Liu et al. 2016). Although multiple lifestages were not tested, our data suggest that pupae or early adults may be the induction sensitive stage. Adults are the most common stage for summer diapause in Coleoptera (Masaki 1980), and a diapause in pre-reproductive adults has been suggested for multiple bark beetle species (Massey and Wygant 1954, Ryan 1959, Safranyik et al. 1990, McKee and Aukema 2015, Gent et al. 2017, Bleiker and Willsey 2020), in accord with our finding that the diapause is in the adult stage in central haplogroup mountain pine beetle from southern CO. Our results also suggest that the diapause is facultative rather than obligatory and that it influences reproductive success.

Both males and females entered the summer diapause, thereby providing a mechanism to synchronize emergence and mating as was found for the soybean pod borer (Yoshimura et al. 2021). Unlike quiescence, diapause is an evolved trait. Quantitative trait loci (QTL) that span most of the X chromosome and influence development time were found between AZ (central haplogroup) and UT (eastern haplogroup) populations, with the shorter development time in UT mountain pine beetle dominant over the longer development time in AZ mountain pine beetle (C. Pushman, personal communication). The finding of QTL for development time highlights that directional selection has occurred (Mathias et al. 2007) across the haplogroup boundaries, further suggesting the dormancy we observed is genetically based rather than a short-term response to an environmental condition.

In contrast to results for the southern CO central haplogroup, we found little evidence for manifestation of an adult diapause in an eastern haplogroup population from ID. Respiration rates were statistically indistinguishable among the treatments with the exception of the Transfer20_return compared to Transfer20 beetles. Decades of research with this haplogroup have not yielded any anomalous phenological observations with respect to the adult stage (Bentz et al. 1991, Régniére et al. 2012) such as were observed for the AZ central haplogroup in the field and common gardens (Bentz et al.

2001, Bracewell et al. 2013, Soderberg et al. 2021). The ID eastern haplogroup population has a more northerly distribution than the AZ central haplogroup (Dowle et al. 2017), thus this result is consistent with examples of species wherein summer diapause incidence is lower in more northerly populations (Masaki 1980, Spieth et al. 2011). Note, however, that the central haplogroup includes a disjunct population in the Black Hills, South Dakota which has a similar latitude to central Idaho (Dowle et al. 2017). The South Dakota population, however, also had delayed generation time at a relatively high temperature (e.g., 27 °C) in common garden experiments (Bentz et al. 2011). Additional investigations are needed to describe the propensity for adult diapause among populations across the mountain pine beetle range and haplogroups.

The adult summer diapause in central haplogroup southern CO mountain pine beetle can be completed within 1–2 wk by relatively cool temperature. Beetles held at 25 °C for 40 and 60 d before transfer to 15 °C showed that respiration rates recovered, within 1–2 wk, to levels like those of beetles held at constant 15 °C. As found in another insect with a summer diapause (Ding et al. 2003), short exposure to cooler temperatures with, possibly, a longer interval of cool temperatures was required to complete diapause after 60 d of exposure to 25 °C. That cooler temperatures can complete diapause development within 1–2 wk is corroborated by the reproductive success results. Successful matings of beetles held at 25 °C then transferred to 15 °C after mating had eggs but not larvae when the bolts were sampled at fixed intervals post-mating (Fig. 4C). These eggs later hatched at room temperature, consistent with delayed oviposition because of the timing needed for adult diapause completion. Although reproductive success following temperature treatments was not investigated, Bleiker and Willsey (2020) found that *D. rufipennis* adults from a British Columbia population exposed continuously to 22 °C for more than 20 d did not emerge from bolts, although emergence was relatively rapid after exposure to a substantial cold period. The adult diapause syndrome may be similar among *Dendroctonus* species, although it will vary depending on species, geographic location, and adult timing.

Central haplogroup southern CO beetles that were transferred to 15 °C from 25 °C after 40 d and then returned to 25 °C had reduced respiration relative to beetles that remained at 15 C (Supplementary Fig. S1). This result suggests a sensitivity to diapause-inducing conditions in this population and that diapause may be entered a second time during the same lifestage. There was not a similar reduction in respiration of eastern haplogroup southern ID beetles that were exposed to the same treatment (Supplementary Fig. S2). Repeated diapause has been noted in adult grasshoppers (*Oedipoda miniata*) (Orshan and Pener 1979), mites (*Amblyseius potentillae*) (Van Houten 1989), and bean bugs (*Riptortus clavatus*) (Numata 1990), as well as larval stages of the curculionid *Curculio elephas* (Soula and Menu 2005) and several lepidopterans (Harvey 1967, Lounibos and Bradshaw 1975, Wipking 1988, Nealis 2005). Our results suggesting repeated sensitivity to diapause-inducing conditions in central haplogroup mountain pine beetle warrants additional research.

While we found mating and reproduction nearly arrested at 25 °C (Fig. 4D; only 2/25 "'25 > 25" pairs produced brood), McManis et al. (2019) observed maximal oviposition rates at 27 °C for a central haplogroup from AZ. A key difference is that McManis et al. used beetles reared at 20 °C and allowed to naturally emerge, i.e., those adult beetles were reared at a temperature that potentially does not induce diapause, and those beetles were fully mature rather than mated according to our post-eclosion timing. We speculate that the beetles in McManis et al. (2019) had developed beyond the point wherein diapause induction was possible.

We considered several factors that may have influenced our results. Firstly, the respirometry results could have been an artifact of heat stress on beetles treated at 25 °C. Insect growth and development occur over a broad temperature range with increasing rates positively correlated with increasing temperatures up to a critical upper limit (Neven 2000). For example, laboratory experiments show that most lifestages of central haplogroup mountain pine beetle (sourced from central AZ) have a lower developmental threshold near 6 °C and an upper threshold near 31 °C with optimal growth rates in the range 25–27 °C (McManis et al. 2018). Respiration responds similarly as a function of temperature including rapid decreases in rates near the upper threshold (Neven 2000). We do not think our warm temperature introduced heat stress on our test beetles, given that 25 °C is near the optimal development rate for lifestages from oviposition through pupation (McManis et al. 2018, 2019) and that full generations (i.e., oviposition through brood adult emergence) have been successfully reared at constant 25 °C (Soderberg et al. 2021). Moreover, similar numbers of central haplogroup mountain pine beetle from AZ emerged from bolts held in the laboratory at constant 18 °C (1,012 adults, median time 115 d) and 25 °C (1,035 adults, median time 114 d) (Soderberg et al. 2021). Secondly, the fact that some beetles reared and/or held post-mating at 15 °C did not produce successful galleries is likely due, in part, to the artificial conditions of our experiment. Our decades of rearing scolytines in this manner typically results in some small proportion of unsuccessful matings, anecdotally on the order of ~10%. The "higher than typical" proportions of unsuccessful matings, however, suggest the possibility that even some beetles reared and held at 15 °C post-mating might not have been reproductively mature. Our experiments lack the resolution to determine the temperatures that allow beetles to avoid/bypass diapause. Instead, our results simply confirm that central haplogroup mountain pine beetle have an adult diapause. Finally, although the CO and ID populations were held at near 0 °C for different intervals immediately following field harvest, observed differences in diapause induction between central and eastern haplogroups were likely not influenced by this treatment to eggs and early instars, given that our results suggest summer diapause is induced by warm temperatures and that the induction sensitive stage is the pupa or early adult lifestage(s).

Conclusions

Collectively, our results for an adult mountain pine beetle summer diapause in a central haplogroup population are consistent with descriptions of summer diapause outlined by Masaki (1980): (i) relatively high temperatures induced diapause and cool temperature terminated diapause, (ii) relatively cool temperatures averted diapause induction, (iii) the reproductive phase was delayed until after diapause completion, and (iv) diapause manifestation was high among adults from a relatively southern location (i.e., southern CO central haplogroup) and low/absent among adults from a relatively northern location (i.e., southern ID eastern haplogroup). Although we consider our results to be compelling evidence for a facultative mountain pine beetle summer diapause that varies geographically, they have poor resolution for describing the induction sensitive lifestages and temperature thresholds. For example, our experiments did not parse results from warm and cool temperature exposure during the fourth instar/prepupa, pupa, and "early" to "mid" adult. Nor did we test any temperatures between 15 and 25 °C. We also cannot describe the rate of diapause completion other than to note

that transfer of beetles from 25 to 15 °C appears to remove diapause within 2 wk. Thus, more work is needed to parameterize a phenology model that includes this diapause. Regardless, these results are satisfying in that they explain the discrepancy in generation lengths, under laboratory conditions and the field, among mountain pine beetle from differing geographies (Bracewell et al. 2013, Soderberg et al. 2021, Wangen et al. 2024) despite all lifestages other than adult having comparable temperature-dependent development rates (McManis et al. 2018). Moreover, they help to explain the propensity of mountain pine beetle to the univoltine cycle across wide geographies despite substantially different thermal inputs among those locations (Bentz et al. 2014).

Phenotypic plasticity and genetic variation play important roles in observed developmental strategy differences among mountain pine beetle populations (Bentz et al. 2011, Soderberg et al. 2021). In addition to variation in adult diapause manifestation reported here, populations of the central haplogroup and eastern haplogroup also vary in manifestation and induction temperatures for a facultative prepupal diapause (Bentz and Hansen 2018). The dual facultative diapauses in different lifestages serve to maintain seasonality, and likely limit a shift from univoltinism to bivoltinism across its current range even in a warming climate (Bentz and Powell 2014, Bentz et al. 2022, Wangen et al. 2024). Latitudinal variation in physiological traits that influence generation time are common among insects (Addo-Bediako et al. 2000), including bark beetles with widespread distributions (McKee and Aukema 2015, Schebeck et al. 2017). Our results showing variation in thermal traits among mountain pine beetle haplogroups further highlights the long-term, evolved processes driving local adaptations in this species.

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Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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Author contributions

Earl Hansen (Conceptualization [lead], Data curation [lead], Formal analysis [equal], Investigation [lead], Methodology [lead], Project administration [lead], Visualization [lead], Writing—original draft [lead]), Barbara Bentz (Conceptualization [supporting], Supervision [lead], Writing—original draft [supporting], Writing—review & editing [lead]), and Larry Baggett (Formal analysis [equal], Validation [lead], Writing—review & editing [supporting])

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