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Developmental parameters of a southern mountain pine beetle population reveal potential source of latitudinal differences in generation time

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Abstract:	Mountain pine beetle (Dendroctonus ponderosae, Hopkins) is a major disturbance agent in pine ecosystems of western North America. Adaptation to local climates has resulted in primarily univoltine generation time across a thermally diverse latitudinal gradient. We hypothesized that voltinism patterns have been shaped by selection for slower developmental rates in southern populations inhabiting warmer climates. To investigate traits responsible for latitudinal differences we measured lifestage-specific development of southern mountain pine beetle eggs, larvae and pupae across a range of temperatures. Developmental rate curves were fit using maximum posterior likelihood estimation with a Bayesian prior to improve fit stability. When compared to previously published data for a northern population (Régnière et al. 2012), optimal development of southern individuals occurred at higher temperatures, with higher development thresholds, as compared with northern individuals. Observed developmental rates of the southern and northern populations were similar across studied lifestages at 20 °C, and southern lifestages were generally faster at temperature extremes (10, 27 °C). At 25 °C southern fourth instars were significantly slower than northern fourth instars. Our results suggest that evolved traits in the fourth instar and remaining unstudied lifestage, teneral (i.e., pre-emergent) adult, likely influence latitudinal differences in mountain pine beetle generation time.

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Title: Developmental parameters of a southern mountain pine beetle population reveal potential

source of latitudinal differences in generation time

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1 **Abstract**

2   Mountain pine beetle (*Dendroctonus ponderosae,* Hopkins) is a major disturbance agent

3 in pine ecosystems of western North America. Adaptation to local climates has resulted in

4 primarily univoltine generation time across a thermally diverse latitudinal gradient. We

5 hypothesized that voltinism patterns have been shaped by selection for slower developmental

6 rates in southern populations inhabiting warmer climates. To investigate traits responsible for

7 latitudinal differences we measured lifestage-specific development of southern mountain pine

8 beetle eggs, larvae and pupae across a range of temperatures. Developmental rate curves were fit

9 using maximum posterior likelihood estimation with a Bayesian prior to improve fit stability.

10 When compared to previously published data for a northern population (Régnière *et al*. 2012),

11 optimal development of southern individuals occurred at higher temperatures, with higher

12 development thresholds, as compared with northern individuals. Observed developmental rates

13 of the southern and northern populations were similar across studied lifestages at 20 °C, and

14 southern lifestages were generally faster at temperature extremes (10, 27 °C). At 25 °C southern

15 fourth instars were significantly slower than northern fourth instars. Our results suggest that

16 evolved traits in the fourth instar and remaining unstudied lifestage, teneral (i.e., pre-emergent)

17 adult, likely influence latitudinal differences in mountain pine beetle generation time.

18

19 **Keywords:** *Dendroctonus ponderosae*, development rate, mountain pine beetle, phenology,

20 latitudinal gradient

21

22 **1. Introduction**

23   As poikilotherms, insect development rates and thresholds are temperature-dependent

24 (Taylor 1981). In seasonal environments evolved adaptations in these and related physiological

25 traits, including diapause and quiescence, serve to synchronize developmental timing with local

26 climates (Tauber and Tauber 1976; Danks 1987), and to enhance mate-finding and host plant

27 feeding (Forrest and James 2011; Li *et al*. 2011). For species that inhabit highly seasonal and

28 cold environments, these strategies can also reduce the probability that lifestages vulnerable to

29 cold-induced mortality are present during winter. Due to their significant influence on population

30 success, thermally-dependent fitness traits commonly vary with environmental conditions along

31 latitudinal gradients, particularly in species with extensive distributions (Deutsch *et al*. 2008).

32 Understanding intraspecific trait variability is key to predicting population responses in a

33 changing climate.

34   Mountain pine beetle (*Dendroctonus ponderosae,* Hopkins, Coleoptera: Curculionidae,

35 Scolytinae) is a bark beetle native to mountainous areas of western North America with an

36 expansive distribution that ranges from Baja Norte, Mexico to northern British Columbia and

37 Alberta (Dowle *et al*. 2017; Cooke and Carroll, 2017). Mountain pine beetle feeds on, reproduces

38 in, and, when populations are at outbreak levels, kills pine (*Pinus*) trees. Mountain pine beetle

39 was responsible for 5.2 Mha of pine mortality in the western United States (US) between 1997

40 and 2012 (Hicke *et al*. 2016). In addition to the availability of suitable host trees, weather that

41 supports appropriate seasonal timing and synchronous adult emergence are essential for

42 population outbreaks (Logan and Bentz 1999; Safranyik and Carroll 2006). Pines have evolved

43 resins and other defensive compounds to resist attack (Franceschi *et al*. 2005; Boone *et al*. 2011),

44 and synchronous adult emergence facilitates a mass attack on individual trees that can occur

45 more quickly than a tree can mobilize its defenses (Berryman *et al*. 1985). Successful attacks on

46 the largest and often better defended trees with the thickest phloem (i.e., food for developing

47 larvae) can lead to increased offspring and ultimately a population outbreak (Raffa *et al*. 2008).

48 In northern US mountain pine beetle, synchronous adult emergence is achieved by temperature-

49 dependent physiological strategies including a facultative prepupal diapause (Bentz and Hansen

50 2017) and lifestage-specific developmental rates and thresholds (Bentz *et al*. 1991; Powell and

51 Logan 2005; Régnière *et al*. 2012).

52   In addition to facilitating synchronicity in lifestage timing, evolved temperature-

53 dependent physiological strategies control the time required to complete a generation and

54 subsequently the number of generations that can be completed annually (i.e., voltinism). A

55 lifecycle that is appropriately timed and results in one generation per year is considered

56 univoltine, two generations in a single year is considered bivoltine, and semivoltine generations

57 occur when two years are required for a single generation. Mountain pine beetle adult emergence

58 typically occurs in mid to late summer across its range and univoltinism is considered the most

59 optimal strategy (Logan and Bentz 1999; Safranyik and Carroll 2006). Bivoltinism has not been

60 observed at the warmest or most southern extent of the current mountain pine beetle range in the

61 US (Hopkins 1909; Bentz *et al*. 2014; Bentz and Powell 2014; BJB unpublished). A combination

62 of univoltine and semivoltine strategies, however, can be found in outbreak-level populations at

63 the highest elevations (Bentz *et al*. 2014), demonstrating that semivoltinism is a viable strategy

64 in the coldest areas (Weed *et al*. 2015).

65   The pervasiveness of univoltinism found across the range of mountain pine beetle in the

66 western US masks thermally-dependent strategies that evolved as a result of climatic differences

67 across latitudes (Bentz *et al*. 2014), as revealed in common garden studies. Median generation

68   time of southern US populations was significantly longer than mountain pine beetle from

69   northern US populations when reared at the same constant temperature (Bentz *et al.* 2001; Bentz

70   *et al*. 2011; Bracewell *et al*. 2013). Countergradient variation, a type of phenotypic plasticity

71   wherein the evolutionary response to a gradient is opposite of the ecological response, is not

72   uncommon in species with large geographic ranges (Conover and Schultz 1995). The observed

73   longer generation time in southern mountain pine beetle is likely a result of selection pressure to

74   maintain univoltinism despite a warmer climate. To predict the impact of continued climate

75   warming on population success, an understanding of the strategies and lifestages responsible for

76   developmental differences between southern and northern mountain pine beetle populations is

77   critical.

78   Our goal was to describe temperature-dependent lifestage-specific developmental times

79   and thresholds for a southern US mountain pine beetle population. We then compare our results

80   with previously described developmental data for a northern US population (Régnière *et al*.

81   2012). Using maximum posterior likelihood estimation, we fit observed data on time to complete

82   each lifestage across a range of temperatures to the same seven parameter rate function used by

83   Régnière *et al*. (2012), adding a Bayesian prior to the procedure to increase stability in the model

84   fits. We also used transfer treatments to facilitate timely data collection and increase survival at

85   extreme temperatures, and developed a method of assessing the reliability and effectiveness of

86   those treatments. Our comparison of lifestage-specific thermal responses for northern and

87   southern mountain pine beetle populations provides a platform for increased understanding of

88   evolved developmental differences across latitudes, in addition to the development of a

89   phenology model for southern populations that can be used in predicting range-wide population

90   success in a changing climate.

91

2. Methods

2.1 Experimental Materials and Design

To obtain fresh phloem material to infest with mountain pine beetle parents from a southern population, one live, un-infested southwestern white pine (*Pinus strobiformis* Engelm.) was harvested on 3 May, 2016 near Flagstaff, Arizona (AZ) (35.36272, -111.7439) and cut into 50-55 cm bolts. Bolts were transported to the US Forest Service Rocky Mountain Research Station (RMRS) laboratory in Logan, Utah (UT) and bolt ends were waxed (Gulf Wax, Roswell, GA) to retain moisture and then stored at 0 °C for up to 2 months. Unmated adults for infesting the bolts of host tree material were acquired by harvesting a mountain pine beetle-infested southwestern white pine on 4 May, 2016 near Flagstaff, AZ (35.35506, -111.6132). The infested tree was cut into bolts 45-50 cm long and transported to the RMRS laboratory in Logan, UT. Bolts ends were waxed to retain moisture and stored at 0 °C when not used to produce brood. Eight bolts from the infested tree were immediately placed in incubators (Percival Scientific, Perry, IA) (4 bolts per incubator) at ~20 °C to facilitate brood development and adult emergence. Adults were collected daily and kept at 4 °C in Petri dishes for 1-7 days before use. Moistened filter paper was placed in each dish to reduce desiccation. Beetles were sexed using secondary sex characteristics on the seventh tergite (Lyon 1958).

Phloem sandwiches were used to monitor lifestage-specific development following methods found in Bentz *et al*. (1991) and Hansen *et al*. (2001). Sandwiches were initiated with eggs, and development of each individual was monitored on a daily basis until the adult stage was reached or mortality occurred. To obtain eggs, several un-infested bolts were manually infested with male/female pairs by inserting first a female then a male into holes drilled

114   vertically into the phloem. Wire mesh screen was placed over each hole to prevent parent beetle

115   escape. The bolts were inverted and incubated at room temperature for 10-12 days before peeling

116   the bark to expose egg galleries. Eggs were collected from three 1.5 cm gallery sections, starting

117   with the most recently completed gallery, and eggs were considered to be 1, 2, or 3 days old

118   respectively based on preliminary data on rate of gallery construction and oviposition (McManis

119   2018).

120   To obtain phloem for sandwiches, the outer bark was stripped from several un-infested

121   bolts with a sterilized draw knife. Phloem pieces were cut into six-inch squares using a sterilized

122   knife and carefully peeled from the bolt. Peeled phloem squares were vacuum packed

123   (FoodSaver, Sunbeam Products, Boca Raton, FL) and refrigerated for 1-2 days before use.

124   Phloem sandwiches were assembled using tools sterilized in 95% ethanol to reduce

125   contamination. On the cambial surface of the phloem, seven evenly spaced niches for eggs were

126   cut along the centerline of the phloem parallel to the grain with a sterilized dissecting probe. For

127   each phloem sandwich eggs of similar age were used (i.e., 1, 2 or 3 days old). Phloem containing

128   eggs was placed between sterilized glass and sterilized plexiglass plates, with plexiglass against

129   the bark side and glass against the cambial side with the eggs. These `sandwiches' were clamped

130   on each edge and the edges secured with tape (Nexcare 3M, St. Paul, MN) and parafilm (Bemis,

131   Neenah, WI).

132   Completed phloem sandwiches (hereafter, "plates") were numbered and placed upright in

133   racks in 26 cm diameter plastic desiccators (Bel-Art™ SP Scienceware™, Fisher Scientific,

134   Pittsburg, PA), with a 5% sodium chloride solution in the bottom to maintain constant humidity

135   (~ 93%), and the desiccators were placed in incubators. Individual eggs were numbered from 1-7

136   for each plate by writing on the glass next to the current location of the individual. There were

137 seven plates per desiccator, and two desiccators per temperature, for a total of 98 individuals at

138 each temperature, 10, 15, 20, 25, 27, 28, 29 and 30 °C. Experimental temperatures were spread

139 asymmetrically across the previously developed rate curve for a northern population (Régnière *et*

140 *al*. 2012) to ensure sufficient data to resolve the upper and lower developmental thresholds.

141 Because the slope of the development rate curve at temperatures lower than the expected optimal

142 (~25 °C) is shallow, we included temperatures every 5 °C between 10 and 25 °C. Expecting a

143 sudden sharp drop in development rate above the optimal, we included temperatures every 1 °C

144 between 27 and 30 °C.

145   Plates were inspected under a dissecting microscope every 24 hours and larval head

146 capsule width recorded. When present, discarded head capsule exuviae, indicating a recent molt

147 to a new instar, was also recorded for each individual. In the absence of head capsule exuviae,

148 increases in head capsule width of at least 0.5 mm between individual observations were

149 recorded as advancement to the next instar. An individual was considered a pupa when a loss of

150 larval body morphology and the presence of proto-wing structures were observed. An individual

151 was considered an adult when adult structures were present (e.g. legs and elytra) and

152 sclerotization began (i.e., the individual turned from a creamy white to light brown). From these

153 data, the number of days to complete each instar/lifestage at a particular temperature was

154 calculated for each individual. Individuals that failed to complete a lifestage (i.e., died or were

155 still alive at the end of the experiment) or transitioned between lifestages while hidden beneath

156 the surface of the phloem (so that the exact date of transition was unknown) were included as

157 censored data *sensu* Régnière *et al*. (2012).

158

159 *2.2 Transfer Treatment Implementation*

160   Based on estimates of development time from preliminary data, in addition to previously

161   published data on a northern population (Régnière *et al*. 2012), total development time for

162   individuals at or below 15 °C and above 27 °C would require an extended amount of time, in

163   addition to the likelihood of reduced survival. To reduce these effects, we used transfer

164   treatments. Transfer treatments assume that thermal history does not influence development time,

165   and they increase the probability of observing lower and upper thermal thresholds (Régnière *et al*.

166   2012). In transfer treatments, plates spent part of the time at the treatment temperature and part

167   of the time at 25 °C. Transfer treatments were used for 10, 15, 29 and 30 °C and included 98

168   individuals (14 plates) per temperature.

169   For each lifestage, individuals were kept at the treatment temperature for approximately

170   seven days before transfer to 25 °C. Seven days was chosen as a compromise between

171   accelerating data collection and ensuring that, even where rates were lowest, a non-trivial

172   amount of an individual's development (i.e. 10-15%) would be completed at the treatment

173   temperature. If an individual in the plate had already advanced to the next lifestage, the plate was

174   left at the treatment temperature for another seven days. Plates were transferred from 25 °C back

175   to the treatment temperature one day after the most advanced individual completed the current

176   lifestage. There were seven individuals per plate, and the individual in the most advanced

177   lifestage was used to determine if and when a plate should be transferred. This insured that all

178   individuals spent at least seven days per lifestage at the treatment temperature, although some

179   individuals spent more time.

180   For comparison, a constant temperature control at 10 °C with 49 individuals that were not

181   transferred was established. After 382 days, the majority of these individuals had not completed

182 development to the pupal stage. Data on eggs, and first, second and third instars were used to

183 compare development time of transferred and non-transferred individuals at 10 °C.

184

185 *2.3 Model Development*

186 Although Régnière *et al*. (2012) used a lognormal error distribution for northern

187 population data, a normal distribution was a better description of variability among southern

188 individual development times/rates at a given temperature. Lognormal error is multiplicative and

189 lower rates correspond to lower variance regardless of temperature (Régnière *et al*. 2012).

190 Conversely, normal error is additive and does not scale with development times/rates and was a

191 better fit to the variability among southern individuals. Using normally-distributed error also

192 allowed for the possibility that an individual's upper and lower threshold may vary relative to the

193 median threshold in the population, whereas lognormal error assumes fixed upper and lower

194 developmental thresholds for all individuals.

195 For the nth individual, there is a mismatch (ε_n) between the observed development rate

196 and the modeled mean rate due to individual variation in rate, which we assumed to be normally

197 distributed with variance σ^2. Therefore an individual's rate of development, $r_n(T, \boldsymbol{\theta})$, relates to

198 the mean rate, $r_o(T, \boldsymbol{\theta})$, as

199
$$r_n(T, \boldsymbol{\theta}) = r_o(T, \boldsymbol{\theta}) + \varepsilon_n, \ \varepsilon_n \sim N(0, \sigma^2), \tag{1}$$

200 where T is temperature, $\boldsymbol{\theta}$ is a vector of parameters, and $r_o(T, \boldsymbol{\theta})$ is the rate function

201
$$r(T, \boldsymbol{\theta}) = \psi \left[e^{\omega(T-T_b)} - \left(\frac{T_m - T}{T_m - T_b} \right) e^{-\frac{\omega(T-T_b)}{\Delta_b}} - \left(\frac{T-T_b}{T_m-T_b} \right) e^{\omega(T_m-T_b) - \frac{T_m-T}{\Delta_m}} \right], \tag{2}$$

202 previously used by Régnière *et al*. (2012). In this rate function, T_m and T_b correspond to the

203 upper and lower temperature thresholds, respectively, and the remaining parameters are shape

204   parameters. The observed development time, t_n, of individual n at constant temperature (T)

205   gives an observed rate, $r_n(T, \boldsymbol{\theta}) = \frac{1}{t_n}$, and therefore the likelihood of observing t_n is

206
$$L_n = \frac{e^{-\frac{\left(r_o(T,\boldsymbol{\theta})-\frac{1}{t_n}\right)^2}{2\sigma^2}}}{\sqrt{2\pi\sigma^2}}. \tag{3}$$

207   The negative log likelihood for the single observation, t_n, (after multiplying the numerator and

208   denominator by t_n^2) becomes

209
$$NLL_n = \frac{(r_o(T,\boldsymbol{\theta})t_n-1)^2}{2\sigma^2 t_n^2} + \frac{1}{2}\ln(2\pi\sigma^2). \tag{4}$$

210

211   *2.4 Transfer Treatment Data*

212   For individuals that were transferred between temperatures to accelerate development and

213   increase survival, fitting their data to the rate curve was more complicated. Integrating equation

214   (1) gives

215
$$\int_0^t r_n(T,\theta)dt = \int_0^t r_o(T,\theta)dt + \int_0^t \varepsilon_n dt. \tag{5}$$

216   For an individual that spent some time (t_{n1}) at a treatment temperature (T_1) and some time (t_{n2})

217   at T_2 (25 °C) this results in

218
$$\int_0^{(t_{n1}+t_{n2})} r_o(T,\theta)dt = r_o(T_1,\theta)t_{n1} + r_o(T_2,\theta)t_{n2}. \tag{6}$$

219   The integral from 0 to time of completion is always one,

220
$$\int_0^{(t_{n1}+t_{n2})} r_n(T,\theta)dt = 1, \tag{7}$$

221   and therefore

222
$$1 = r_o(T_1,\theta)t_{n1} + r_o(T_2,\theta)t_{n2} + \varepsilon_n(t_{n1} + t_{n2}). \tag{8}$$

223   Solving for ε_n and using $\varepsilon_n \sim N(0,\sigma^2)$, the negative log likelihood for observing $(t_{n1} + t_{n2})$

224   becomes

$$NLL_n = \frac{(r_o(T_1,\theta)t_{n1} + r_o(T_2,\theta)t_{n2} - 1)^2}{2\sigma^2(t_{n1} + t_{n2})^2} + \frac{1}{2}\ln(2\pi\sigma^2). \tag{9}$$

2.5 Testing Consistency of Transfer Treatment Data

Developmental rates for transferred and control individuals at 10 °C were compared based on developmental deviance. Developmental deviance (Δ_n) is a measure of how observed developmental time for an individual differs from the median time in a particular lifestage. For individuals in a transfer treatment being moved between 10 and 25 °C, we first calculated the median rate of development at 10 °C, R_{10}, using observed development rates for individuals at a constant 10 °C, and the median rate of development at 25 °C, R_{25}, using observed development rates for individuals at a constant 25 °C. The Δ_n for an individual is calculated as

$$\Delta_n = t_{10}R_{10} + t_{25}R_{25}, \tag{10}$$

where t_{10} and t_{25} are time spent at 10 °C and 25 °C, respectively. The Δ_n for individuals at a constant 10 °C who were not transferred is calculated the same way, except $t_{25} = 0$, so

$$\Delta_n = t_{10}R_{10}. \tag{11}$$

If development rate is not affected by thermal history of an individual, then the distributions of Δ_n values for individuals at a constant 10 °C and transferred individuals will not be significantly different. If development rate is affected by thermal history, then there will be a significant difference in the Δ_n values between treatment groups. Because sample sizes were relatively small and somewhat skewed, we used a non-parametric Wilcox Rank-Sum test (W) to compare groups (R Core Team 2015).

2.6 Censored Data

247   Censored data represents individuals who failed to complete their current lifestage while

248   data collection was ongoing or transitioned between lifestages while unobservable beneath the

249   phloem such that the exact duration of the lifestage was unknown. Therefore, their total

250   development time is at least as long as the observation time, but could have been longer. The

251   probability (P) the observed time for a censored data point is less than the mean development

252   time for that temperature is

253
$$P\left(t_n < \frac{1}{r_o(T,\theta)}\right) = P(r_o(T,\theta)t_n < 1) = P(r_o(T,\theta)t_n - 1 < 0). \tag{12}$$

254   Since $1 - r_o(T,\theta)t_n = \varepsilon_n t_n$ and $\varepsilon_n t_n \sim N(0, \sigma^2 t_n^2)$,

255
$$P(r_o(T,\theta)t_n - 1 < 0) = F(r_o(T,\theta)t_n;\ \sigma^2 t_n^2), \tag{13}$$

256   where F is the normal CDF with variance $\sigma^2 t_n^2$. The negative log likelihood for a censored

257   observation, t_n, is then

258
$$NLL_n = -\ln[F(r_o(T,\theta)t_n;\ \sigma^2 t_n^2)]. \tag{14}$$

259

260   *2.7 Adding a Bayesian Prior to the Negative Log Likelihood*

261   A Bayesian prior was used to improve the stability of the fit for upper and lower

262   threshold parameters. This weights the maximum likelihood fit using prior information and

263   confidence in the prior. According to Bayes' Theorem, the posterior distribution of the vector of

264   parameters, $\boldsymbol{\theta}$, satisfies

265
$$P(\boldsymbol{\theta}|t_n) = \frac{1}{P(t_n)} P(t_n|\boldsymbol{\theta})P(\boldsymbol{\theta}) \tag{15}$$

266   In this expression $P(t_n|\boldsymbol{\theta})$ is the likelihood, $P(\boldsymbol{\theta})$ is a prior distribution of the parameters and

267   $P(t_n)$ is an unknown constant that can be ignored. For example, assuming the prior distribution of

268   the upper threshold (T_u) is normal we can write the posterior likelihood as

The Canadian Entomologist

269
$$P(T_u) = e^{-NLL(\theta|t_n)} \left(\frac{e^{-\frac{(T_u-U)^2}{2\sigma_u^2}}}{\sqrt{2\pi\sigma_u^2}} \right),$$
(16)

270 where U and σ_u^2 are the mean and variance of the prior distribution. The posterior probability of

271 observing t_n is therefore

272
$$P(t_n) \propto e^{-NLL(\theta|t_n)} P(T_u)P(T_l),$$
(17)

273 where $P(T_u)$ and $P(T_l)$ are, respectively, the prior probability of particular upper and lower

274 thresholds. Assuming $T_u \sim \text{Normal}(U, \sigma_u^2)$ and $T_l \sim \text{Normal}(L, \sigma_l^2)$, the negative log posterior

275 (NLP) is

276
$$NLP(\theta|t_n) = NLL(\theta|t_n) + \frac{(T_u-U)^2}{2\sigma_u^2} + \frac{(T_l-L)^2}{2\sigma_l^2} + K.$$
(18)

277 Here K is a constant which is independent of parameter values. Prior means for the upper

278 thresholds were chosen based on high observed mortality in the data at 30°C, and prior variances

279 were set at $\sigma_u^2 = 0.125$ and $\sigma_l^2 = 0.50$, reflecting the differing steepness of the rate curves

280 approaching upper and lower thresholds. Where consistent with our data, the lower threshold

281 means for the northern population (Régnière *et al*. 2012) were used as priors for the southern

282 population. If a fit generated by minimizing NLP resulted in a steep drop off in the curve

283 unsupported by our data, the prior mean was stepped down by 0.5 °C up to six times (at most a

284 3 °C decrease), the fit was rerun, and the parameters associated with the lowest NLP was kept.

285

286 *2.8 Descriptive Statistics and Pairwise Comparison of Observed Developmental Times*

287　　　　Using our data for the southern population and data for the northern population published

288 in Régnière *et al*. (2012), we compared observed developmental time in each population for egg,

289 larva, and pupa at temperatures used in both studies (i.e., 10, 20, 25, 27 °C). Data were analyzed

290 using a generalized linear model with a Poisson distribution (SAS Institute Inc., v9.4). Post-hoc

291   pairwise comparisons were tested with a Tukey-Kramer adjustment for multiple comparisons.

292   We tested for size differences between the fourth instar with and without a fifth instar using a

293   similar analyses based on a normal distribution.

294

295   **3. Results**

296   When individuals across all treatments were considered, mortality was greatest in the

297   fourth instar (35%) and egg (29%). Mortality for all lifestages was lowest at 20 °C, and no

298   individuals survived the pupal stage at 29 or 30 °C (Fig 1). Although four instars have

299   historically been described for mountain pine beetle (Amman and Cole 1983; Rosenberger *et al*.

300   2018), Myrholm and Langor (2016) recently observed individuals with up to seven instars. We

301   observed a fifth instar in 57 individuals (~14% of fourth instar individuals) at temperatures ≥

302   15 °C. Headcapsule size of fifth instar (mean = 1.39 ± 0.09, N = 51) was larger than the size of

303   fourth instar (mean = 1.26 ± 0.08, N = 348) (χ^2 = 101.23, *P* < 0.0001). A headcapsule size was

304   not measured for those individuals inappropriately oriented within phloem sandwiches. Of those

305   individuals with a fifth instar, size of the fourth instar (mean = 1.16 ± 0.08, N = 48) was smaller

306   than fourth instars that did not molt to a fifth instar (mean = 1.27 ± 0.07, N = 300) (χ^2 = 32.50, *P*

307   < 0.0001). Due to limited data, model parameters for a fifth instar could not be estimated.

308   Developmental deviance, and therefore developmental rates, did not differ significantly

309   for second instars that were either transferred from 10 to 25 °C or kept at a constant 10 °C (*P* =

310   0.427). However, developmental deviance of eggs, first, and third instar larvae were significantly

311   different between transferred individuals and those kept at constant 10 °C (Fig. 2). We also

312   observed reduced variability among transferred eggs, relative to eggs kept at a constant

313   temperature (Fig. 2). There was insufficient constant temperature data to test for developmental

314   differences between transferred and non-transferred individuals in the fourth instar, and

315   subsequently pupa, because the majority of non-transferred fourth instars held at a constant

316   10 °C did not pupate. In contrast, a majority of the individuals transferred between 10 and 25 °C

317   did pupate. Differences in pupation rates between transferred and non-transferred individuals are

318   most likely a result of prepupal facultative diapause development that is sped up with warm

319   temperatures (Bentz and Hansen 2017). Due to observed differences, model fits were performed

320   separately for temperature data with and without transfers for all stages except second instar.

321   Using constant temperature and censored data (i.e., no transfer data), estimated

322   parameters from the fit to equation (2) differed among lifestages (Table 1). In particular, the

323   lower developmental threshold (T_b) for the fourth instar was estimated to be substantially higher

324   (~15 °C) than all other life stages which ranged from ~4.6 to 6.3 °C (Table 1). The estimated

325   upper developmental thresholds (T_m) were similar among lifestages and ranged from 30.8 to

326   31.9 °C. Optimal developmental rate was estimated to be between 24.8 and 26.5 °C for all

327   lifestages (Fig. 3). At the optimal rate, first instars developed approximately twice as fast as eggs,

328   third instars and pupae, and almost four times as fast as fourth instars. When data on individuals

329   that were transferred among temperatures were included in parameter estimation, predicted

330   development rates differed slightly from fits using constant temperature data, particularly in the

331   fourth instar (Table 2; Fig. 4). Fourth instars exposed to 25 °C during part of their development

332   (i.e., a transfer treatment) had positive development at temperatures ≤ 15 °C (Fig. 4) in contrast

333   to fourth instars that were kept at a constant 10 or 15 °C, where no development was observed

334   (Fig. 3).

335   We were interested in comparing lifestage-specific observed developmental times and

336   fitted rate curves of the southern population with those previously described for a northern

337   population (Bentz *et al*. 1991, Régnière *et al*. 2012, Bentz and Powell 2014). The same phloem

338   sandwich methodology was used to collect data for both populations. At a constant 10 °C,

339   observed development time of southern population eggs and second instars was faster than

340   northern individuals in the same lifestages, and southern third instars developed slower than

341   northern third instars (Table 3). No individuals in either population completed fourth instar

342   development (i.e., pupated) at 10 °C without some period of development at a warmer

343   temperature. There were no significant differences in observed development time between the

344   populations in any lifestage at 20 °C (Table 3). At 25 °C southern fourth instars developed

345   significantly slower than northern fourth instars, and at 27 °C southern second and third instars

346   developed faster than northern individuals (Table 3). When fitted development rate curves for

347   each population were compared, using only not-transferred data, estimated upper thresholds were

348   higher for southern compared to northern individuals across all lifestages (Fig. 5). Lower

349   thresholds were similar between the populations in all lifestages except the fourth instar, where

350   southern individuals developed at a lower temperature (Fig. 5). Estimated development rates of

351   southern second and third instars were higher than northern second and third instars at all

352   temperatures.

353

4. Discussion

355   Our goal was to describe temperature-dependent lifestage-specific developmental times

356   and thresholds for a southern mountain pine beetle population. We then compared these

357   developmental data from a southern population with previously described developmental data for

358   a northern population that was collected using the same phloem sandwich method (Régnière *et al*.

359   2012). Previous research showed that in common garden experiments, median generation time of

360 a southern population was significantly longer (~73 days) than that of a northern population at

361 22 °C (Bracewell *et al*. 2013), and we were interested in identifying the lifestage(s) and evolved

362 traits that may differ between the populations. Estimated upper developmental thresholds and

363 optimal development rates were at slightly higher temperatures for southern compared to

364 northern individuals across all lifestages. Although northern data was not collected at

365 temperatures ≥ 28 °C, the slowing trend in northern individuals at 27 °C suggests optimal

366 development occurs at slightly higher temperatures in southern individuals. Southern fourth

367 instars that were not transferred to 25 °C during development pupated (i.e., completed the fourth

368 instar) at a lower temperature than did northern fourth instars given the same treatment, a result

369 also found by Bentz and Hansen (2017). Both populations have a facultative prepupal (i.e., last

370 stage of the fourth instar) diapause, and induction can occur at higher temperatures in northern

371 (15 to 17 °C) compared with southern populations (< 15 °C) (Bentz and Hansen 2017). Southern

372 fourth instars developed slower than northern fourth instars, but only significantly so at 25 °C. At

373 all other temperatures and lifestages, southern individuals generally developed faster than

374 northern individuals.

375   Given the longer total development time from egg through adult emergence of southern

376 compared to northern individuals at a constant 22.5 °C (Bracewell *et al*. 2013), we hypothesized

377 that selection would act on one or more lifestages to slow development rates and thereby

378 maintain univoltinism despite warmer habitat temperatures. Although development was

379 generally faster in southern individuals, our result that southern fourth instars developed slower

380 (~ 5 days) than northern fourth instars at 25 °C highlights that physiological aspects of the fourth

381 instar may partially explain generation time differences between the populations. In addition to

382 the lifestages monitored in our study, total development time as reported by Bracewell *et al*.

383   (2013) included oviposition and teneral (i.e., pre-emergent) adult development through

384   emergence. McManis (2018) showed that oviposition is slightly slower in southern compared to

385   northern mountain pine beetle (18.5 vs 12 days at 21 °C). This time difference, combined with

386   the few days difference in fourth instar, contribute only marginally to the observed median

387   difference of 73 days in total generation time. A potential additional explanation for differences

388   in generation time observed by Bracewell *et al*. (2013) is trait differences between the

389   populations in the unstudied teneral adult stage. Differences in evolved traits such as adult

390   development and maturation rates, and temperature thresholds for emergence from beneath the

391   bark could result in differences in adult emergence timing and generation time. Because teneral

392   adults feed on spores of fungal associates to obtain vital nutrients prior to emergence (Six and

393   Paine 1998), differences in fungal acquisition or species composition could also play a role in

394   developmental differences between the populations (Addison *et al*. 2014).

395   A proportion of individuals (~14%) molted to a fifth instar prior to pupation. Plasticity in

396   the number of instars an insect may go through prior to pupation can be influenced by multiple

397   environmental factors including temperature and food quality and quantity (Esperk *et al*. 2007).

398   Myrholm and Langor (2016) recently observed mountain pine beetles with up to seven instars,

399   and the headcapsule sizes of the additional instars were between that of instar 3 and instar 4.

400   They suggested that inadequate nutrition results in extra instars and that larvae must attain a

401   threshold size to initiate metamorphosis (Nijhout 1994). In constrast to Myrholm and Langor

402   (2016), the size of the additional instar we observed was larger than the size of fourth instars, and

403   therefore was the last instar prior to pupation. Moreover, individuals that molted to a fifth instar

404   were smaller as a fourth instar than were individuals that pupated following the fourth instar. Our

405   results concur with Myrholm and Langor (2016) that a size threshold for pupation likely exists in

406 mountain pine beetle, and that additional instars may serve as a compensatory mechanism in

407 adverse conditions (Esperk *et al*. 2007). Collectively, however, results suggest that the timing of

408 supernumerary instars is not fixed.

409 Measuring insect development near thresholds can be difficult. We used temperature

410 transfer treatments to increase survival at extreme temperatures and reduce the time required for

411 measurements when development is slowed (Régnière *et al*. 2012). An assumption of transfer

412 treatments is that past thermal history does not affect future developmental rate. We found that

413 only second instars transferred between 10° and 25 °C met this assumption of no difference, with

414 first and third instars developing faster than those kept at a constant 10 °C and transferred eggs

415 developing more slowly. The apparent slowing of development in transferred eggs is likely due

416 to the difficulty of accurately aging eggs, rather than the transfer treatment alone. For first and

417 third instars, however, pulses of warm temperature (25 °C) during development at 10 °C resulted

418 in slightly faster development. Future study is needed to determine if this effect is due to the

419 relatively brief exposure of transferred individuals to their treatment temperature, or if it reflects

420 more complex physiological processes.

421 A physiological-based description of an organism's thermal response can provide a

422 robust framework for making predictions of potential range shifts due to climatic changes. A

423 major benefit of mechanistic, relative to statistical, models is that inherent biological behaviors

424 can emerge. Our description of developmental responses of a southern US mountain pine beetle

425 population provide a foundation for incorporating evolved geographic variation in mountain pine

426 beetle lifecycle timing into predictive models. Our findings suggest that future research should

427 focus on physiological traits in the fourth instar and teneral adult lifestages to further our

428   understanding of intraspecific fitness trait differences that drive population success across the

429   expansive mountain pine beetle range.

430

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439

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544

545   **Figure Captions:**

546   Figure 1: Proportion mortality of southern population mountain pine beetle lifestages in phloem

547   sandwiches at a range of constant temperatures (°C) throughout the experimental period.

548   Figure 2: Comparison of developmental deviance for individual eggs and first, second and third

549   instars in transfer treatments (i.e., transferred between 10 and 25 °C) versus a constant

550   temperature control at 10 °C. Based on a Wilcoxon Rank-Sum test, transferred individuals

551   developed significantly differently than individuals at a constant 10 °C in eggs (W = 2100, P

552   <0.005), and first (W = 684.5, P = 0.005) and third (W = 353, P = 0.005) instars. Boxes

553   represent the third and first quartile (25th and 75th percentiles), whiskers extend up to 1.5 times

554   the interquartile range from the top (bottom) of the box to the furthest data point, and the midline

555   is the median.

556   Figure 3. Model-predicted and observed lifestage-specific developmental rates for a southern

557   mountain pine beetle population based on constant temperature and censored data. 'Censored'

558   data represent individuals that did not complete the lifestage at a given temperature. Data for the

559   fourth instar includes prepupal rates. All point sizes are on a log scale, with larger points

560   corresponding to more highly repeated observations. Dashed lines are ±1 sigma, the variance

561   parameter associated with model fit. Note differences among plots in y-axis scale.

562   Figure 4. Model-predicted and observed lifestage-specific developmental rates for a southern

563   mountain pine beetle population based on constant temperature and transfer data. 'Transfer' data

564   represent individuals that were transferred between the treatment temperature and a constant

565   25 °C. Data for the fourth instar includes prepupal rates. All point sizes are on a log scale, with

566   larger points corresponding to more highly repeated observations. Dashed lines are ±1 sigma, the

567   variance parameter associated with model fit. Note differences among plots in y-axis scale.

568   Figure 5. Comparison of predicted development rates of northern and southern mountain pine

569   beetle populations. Southern population predictions were based on estimated parameters in the

570   current study using non-transferred constant temperature and censored data (see Figure 3).

571   Northern population predictions were published in Régnière et al. (2012; Figure 4), also using

572   non-transferred constant temperature and censored data. Estimated upper (T_m) and lower (T_b)

573   development thresholds for each population and lifestage are in Table 1. Also shown are the

574   observed median development rates (± SD) of each population at the common experimental

575   temperatures (see Table 3 for observed development times).

576 Table 1: Lifestage-specific parameters for the rate curve (equation 2) for a southern mountain

577 pine beetle population using constant temperature data and censored data (see Figure 3). Data

578 from transfer treatments was excluded from the model fits for eggs, first instar, third instar, and

579 fourth instar. T_b and T_m are lower and upper thresholds for development, Δb and Δm are the

580 width of thermal transitions from normal to negligible development at the lower and upper

581 thresholds, ω describes the expected exponential acceleration of rate with temperature, ψ is

582 proportional to the maximum development rate, and σ is the variance.

Parameter	Eggs	First Instar	Second Instar	Third Instar	Fourth Instar	Pupae
Ψ	0.0326	0.0521	0.0431	0.017	0.0545	0.0166
ω	0.2045	0.1517	0.1374	0.1856	0.1694	0.1658
T_b	6.0251	4.6029	5.9791	6.0115	14.9999	6.3504
T_m	31.9309	31.7661	31.8337	31.2656	31.4364	30.8041
Δ_b	0.5410	0.0117	0.0413	0.0	0.0	0.0
Δ_m	5.5031	5.4256	4.4534	4.3079	5.2947	3.5426
σ	0.038	-0.2182	0.1524	0.1650	0.1354	0.0673

583

584

585   Table 2: Lifestage-specific parameters for the rate curve (equation 2) for a southern mountain

586   pine beetle population including constant temperature data and data from transfer treatments (see

587   Figure 4). Second instar parameters are equivalent to those in Table 1 because there were no

588   differences between transfer and constant temperature treatments. T_b and T_m are lower and upper

589   thresholds for development, Δb and Δm are the width of thermal transitions from normal to

590   negligible development at the lower and upper thresholds, ω describes the expected exponential

591   acceleration of rate with temperature, ψ is proportional to the maximum development rate, and σ

592   is the variance.

Parameter	Eggs	First Instar	Second Instar	Third Instar	Fourth Instar	Pupae
Ψ	0.0306	0.0433	0.0431	0.0417	0.0044	0.0179
Ω	0.1914	0.1965	0.1374	0.1406	0.2791	0.1494
T_b	6.5976	4.6428	5.9791	4.3248	10.0	5.6187
T_m	31.8135	31.7810	31.8337	31.6171	31.4294	30.7638
Δ_b	0.9239	1.0369	0.0413	1.3224	0.1478	0.2520
Δ_m	5.5441	5.3945	4.4534	6.2150	3.2194	3.1587
σ	0.0349	0.1938	0.1524	0.1411	0.1139	0.0726

593

594   Table 3: Comparison of median development time (days) for southern (this study) and northern

595   (Régnière *et al*. 2012) mountain pine beetle populations at a constant 10, 20, 25 and 27 °C.

596   Shown are the median days, standard deviation (SD), sample size (N) and test results (z) using a

597   generalized linear model with a Tukey's post-hoc multiple comparison (Adjusted *P*) testing for

598   differences between populations. No individuals in either population completed instar 4 or pupal

599   development at a constant 10 °C.

Lifestage	Northern Days (SD)	Southern Days (SD)	z	Adj $P > z$
10°C				
Egg	29 (1.6) N = 20	17 (5.2) N = 32	-7.80	< 0.0001
First Instar	15 (2.9) N = 18	14 (4.1) N = 36	-1.55	1.0
Second Instar	33 (49.3) N = 13	18 (29.6) N = 27	-16.13	< 0.0001
Third Instar	63 (37.6) N = 9	166 (64.9) N = 7	17.77	< 0.0001
Fourth Instar	NA	NA	NA	NA
Pupa	NA	NA	NA	NA
20°C				
Egg	7 (0.8) N = 32	6 (0.9) N = 81	-2.54	0.9355
First Instar	3 (0.7) N = 19	4 (1.6) N = 69	1.87	0.9998
Second Instar	4 (2.0) N = 36	4 (1.2) N = 61	-0.09	1.0
Third Instar	6 (2.3) N = 46	6 (1.4) N = 60	-1.14	1.0
Fourth Instar	11 (3.5) N = 61	14 (2.5) N = 68	3.48	0.2506
Pupa	6 (1.1) N = 91	7 (0.5) N = 67	2.75	0.8326
25°C				
Egg	6 (0.6) N = 28	5 (0.7) N = 141	-1.56	1.0
First Instar	3 (0.8) N = 17	3 (2.7) N = 119	1.21	1.0
Second Instar	4 (1.4) N = 11	3 (3.1) N = 107	-1.91	0.9997
Third Instar	7 (3.3) N = 9	5 (1.9) N = 110	-1.48	0.9999
Fourth Instar	7 (4.7) N = 19	12 (6.3) N = 112	5.48	< 0.0001

Pupa	5 (1.4) N = 9	5 (0.5) N = 99	0.28	1.0
		27°C		
Egg	6 (0.5) N = 22	4 (0.9) N = 73	-2.80	0.8007
First Instar	4 (1.1) N = 21	2 (2.1) N = 63	-2.39	0.9741
Second Instar	7 (3.7) N = 16	3 (3.7) N = 61	-5.42	< 0.0001
Third Instar	17 (9.3) N = 6	5 (2.4) N = 59	-11.50	< 0.0001
Fourth Instar	NA	14 (3.6) N = 54	NA	NA
Pupa	NA	5 (0.5) N = 42	NA	NA

600
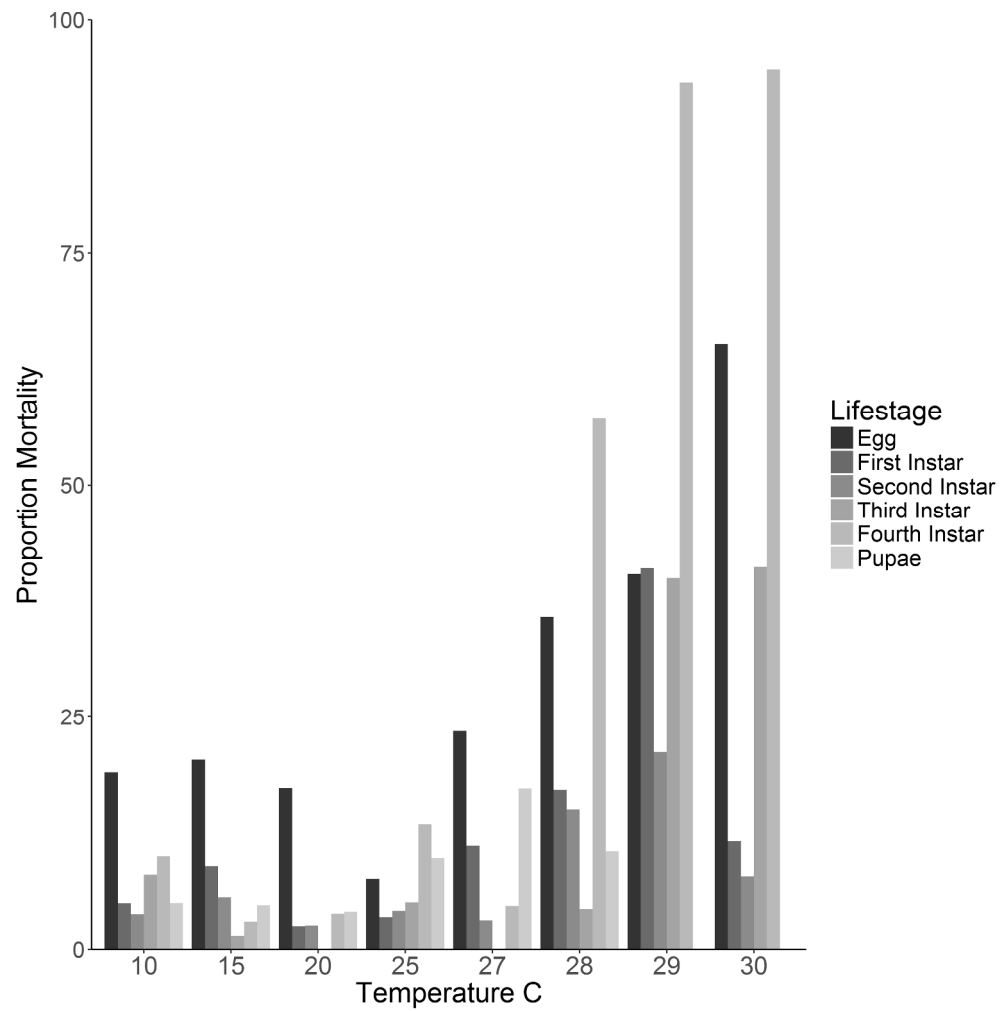
Figure 1: Proportion mortality of southern population mountain pine beetle lifestages in phloem sandwiches at a range of constant temperatures (°C) throughout the experimental period.

254x254mm (300 x 300 DPI)
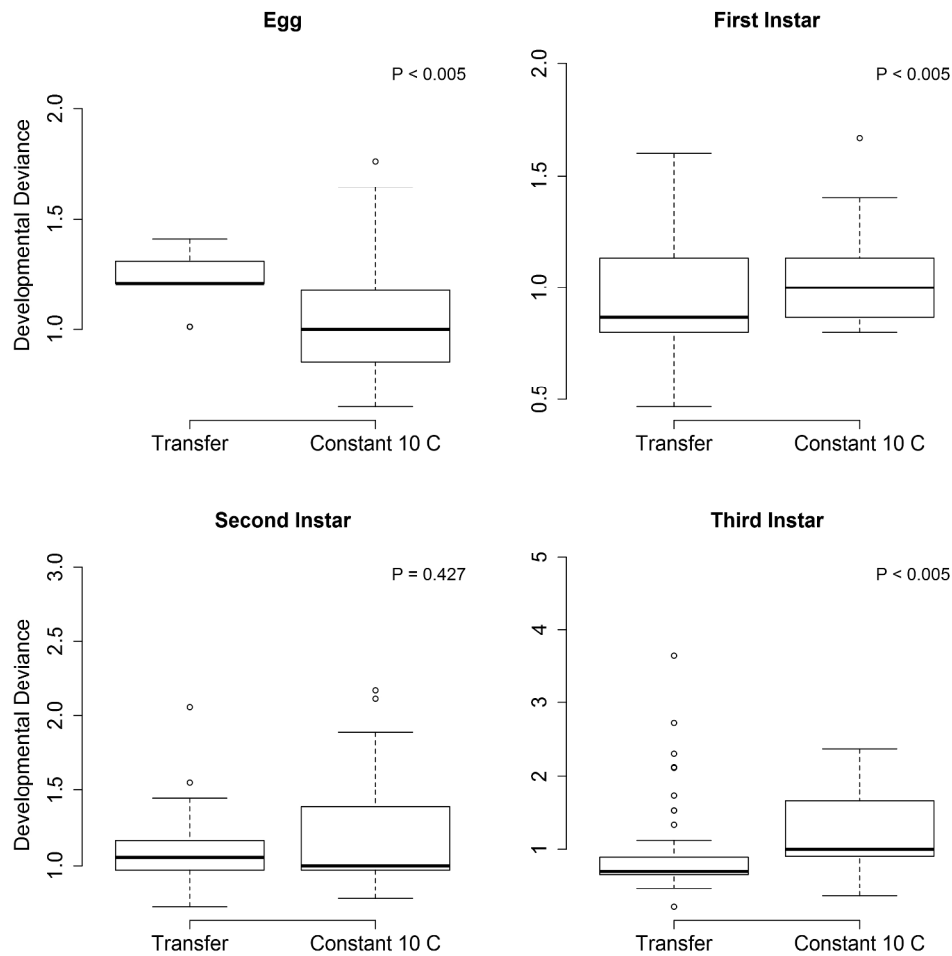
Figure 2: Comparison of developmental deviance for individual eggs and first, second and third instars in transfer treatments (i.e., transferred between 10 and 25 °C) versus a constant temperature control at 10 °C. Based on a Wilcoxon Rank-Sum test, transferred individuals developed significantly differently than individuals at a constant 10 °C in eggs (W = 2100, P <0.005), and first (W = 684.5, P = 0.005) and third (W = 353, P = 0.005) instars. Boxes represent the third and first quartile (25th and 75th percentiles), whiskers extend up to 1.5 times the interquartile range from the top (bottom) of the box to the furthest data point, and the midline is the median.
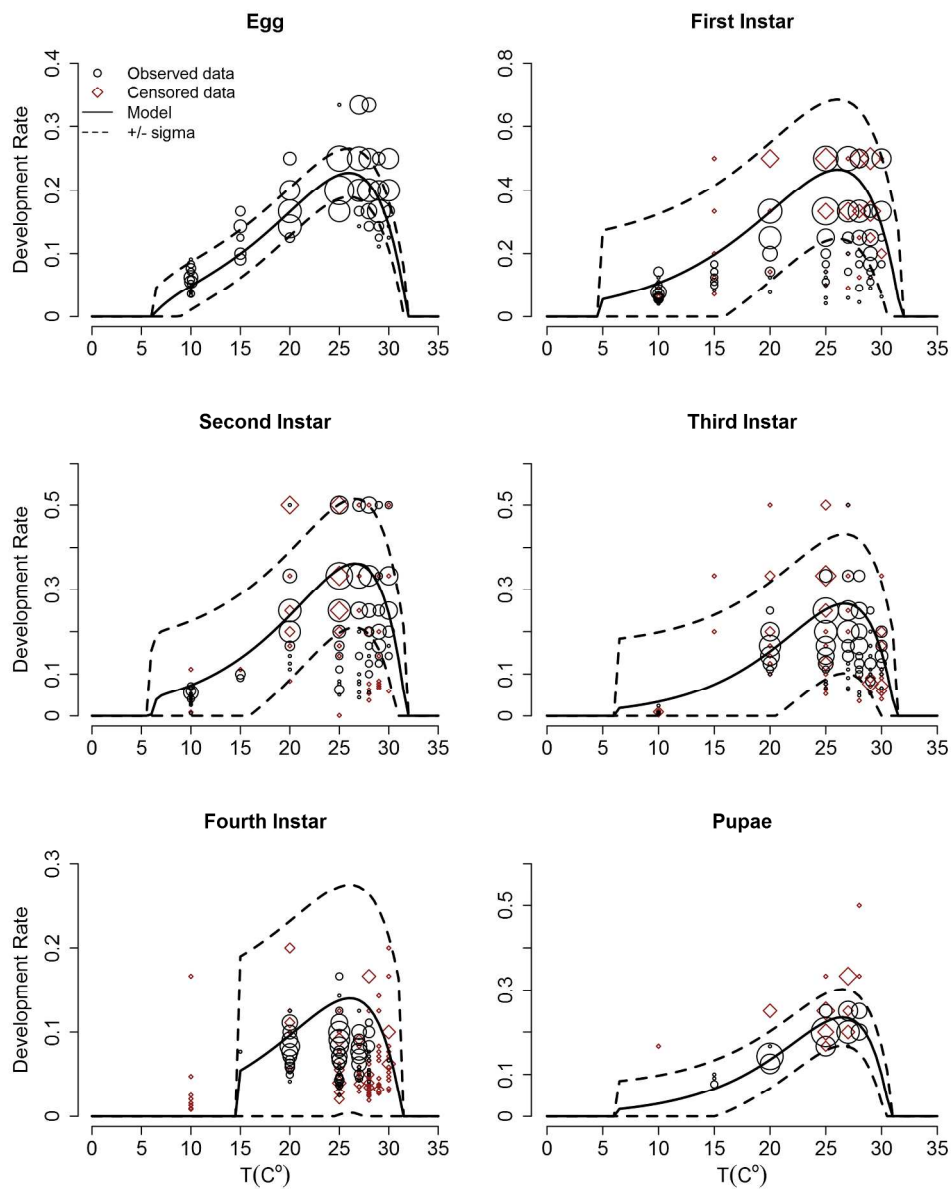
254x254mm (300 x 300 DPI)

Figure 3. Model-predicted and observed lifestage-specific developmental rates for a southern mountain pine beetle population based on constant temperature and censored data. 'Censored' data represent individuals that did not complete the lifestage at a given temperature. Data for the fourth instar includes prepupal rates. All point sizes are on a log scale, with larger points corresponding to more highly repeated observations. Dashed lines are ±1 sigma, the variance parameter associated with model fit. Note differences among plots in y-axis scale.
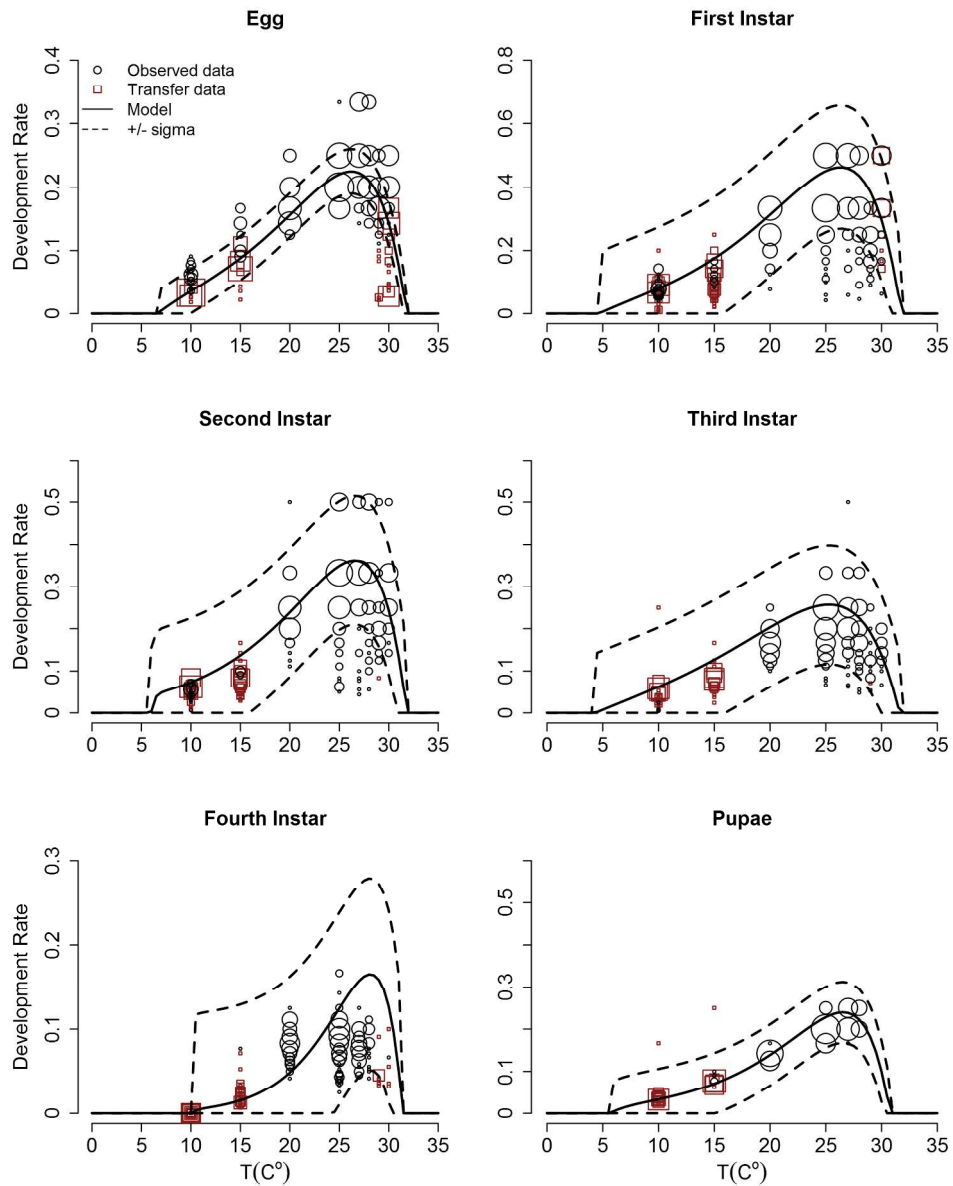
211x254mm (300 x 300 DPI)

Figure 4. Model-predicted and observed lifestage-specific developmental rates for a southern mountain pine beetle population based on constant temperature and transfer data. 'Transfer' data represent individuals that were transferred between the treatment temperature and a constant 25 °C. Data for the fourth instar includes prepupal rates. All point sizes are on a log scale, with larger points corresponding to more highly repeated observations. Dashed lines are ±1 sigma, the variance parameter associated with model fit. Note differences among plots in y-axis scale.
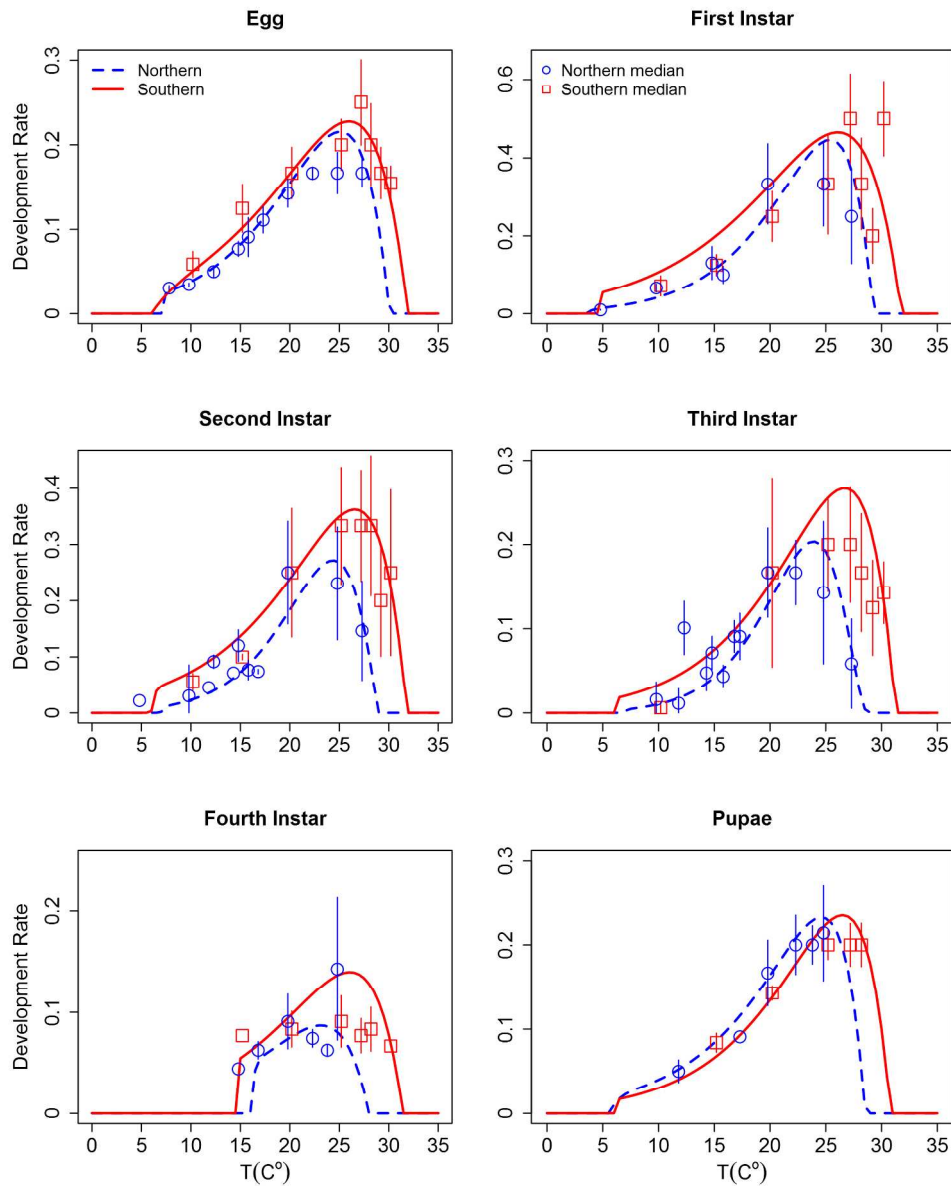
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Figure 5. Comparison of predicted development rates of northern and southern mountain pine beetle populations. Southern population predictions were based on estimated parameters in the current study using non-transferred constant temperature and censored data (see Figure 3). Northern population predictions were published in Régnière et al. (2012; Figure 4), also using non-transferred constant temperature and censored data. Estimated upper (Tm) and lower (Tb) development thresholds for each population and lifestage are in Table 1. Also shown are the observed median development rates (± SD) of each population at the common experimental temperatures (see Table 3 for observed development times).

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