

Modeling mountain pine beetle (*Dendroctonus ponderosae*) oviposition

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Abstract

Mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae), is a significant forest disturbance agent with a widespread distribution in western North America. Population success is influenced by temperatures that drive phenology and ultimately the adult emergence synchrony required to mass attack and kill host trees during outbreaks. In addition to lifestage-specific developmental rates and thresholds, oviposition timing can be a source of variance in adult emergence synchrony, and is a critical aspect of mountain pine beetle phenology. Adaptation to local climates has resulted in longer generation times in southern compared to northern populations in common gardens, and the role of oviposition rate in these differences is unclear. Oviposition rates and fecundity in a northern population have been described, although data are lacking for southern populations. We assessed southern mountain pine beetle oviposition rates and fecundity in a range of temperatures using a non-destructive technique that included frequent X-ray imaging. We found that oviposition rate and fecundity vary independently such that a female with high oviposition rate did not necessarily have high fecundity and vice versa. Observed fecundity within the 30-day experimental period was lowest at the lowest temperature, although estimated potential fecundity did not differ among temperatures. Females at varying temperatures have the potential to lay similar numbers of eggs, although it will take longer at lower temperatures. Southern mountain pine beetle reared in *Pinus strobiformis* Engelm. (Pinaceae) had a higher upper threshold for oviposition, a similar lower threshold, and slightly greater potential fecundity compared to a northern population reared in *Pinus contorta* Douglas. A comparison of modeled oviposition rates between the two populations, which could be influenced by host tree, suggests that differences in oviposition rate do not explain observed differences in total generation time. Our oviposition model will facilitate development of a phenology model for southern mountain pine beetle populations.

Introduction

Mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae, Scolytinae), a bark beetle native to western North America that reproduces in pine (*Pinus* spp., Pinaceae) and kills host trees during population outbreaks, has an extensive distribution and is found in pine ecosystems from Baja California Norte, Mexico, to northern British Columbia and Alberta, Canada (Dowle et al., 2017). Rising temperatures that increase overwinter survival and benefit timely development have facilitated range expansion

northward and eastward in Canada in the past several decades (Sambaraju et al., 2012; Cooke & Carroll, 2017). Newly emerged adults attack pine hosts using a series of pheromone signals to attract conspecifics over a relatively short time frame, thereby serving to overwhelm resinous tree defenses and to allow offspring feeding that ultimately kills the host tree when at epidemic population levels (Franceschi et al., 2005; Boone et al., 2011). Timely and synchronous adult emergence is required for a successful mass attack on new host trees. When weather is favorable for temperature-dependent survival and synchronous adult emergence, attacks on large host trees may create a positive feedback loop and an eventual population outbreak in landscapes with susceptible host trees (Raffa et al., 2008).

Synchrony in mountain pine beetle populations is facilitated by quiescence in the form of developmental

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temperature thresholds that vary among life stages (Bentz et al., 1991; Powell & Logan, 2005), in addition to a facultative prepupal diapause (Bentz & Hansen, 2017). Following mating, females construct a vertical oviposition gallery, laying eggs sequentially over a period of weeks. There are at least four larval instars that feed horizontally through the phloem prior to pupation and progression to a teneral or pre-emergent adult, and ultimately a mature adult that emerges to disperse and colonize new host trees (Safranyik & Carroll, 2006). Temperature thresholds can synchronize cohort timing as early instars develop at lower temperatures than late instars, thereby allowing individuals oviposited later in the season to ‘catch up’ with more advanced larvae in their cohort (Jenkins et al., 2001; Powell & Logan, 2005). When invoked, a facultative prepupal diapause serves as a biofix that can also synchronize individuals within a cohort. Both strategies increase the probability that cold-hardy life stages (i.e., larvae) are present in winter (Bentz & Mullins, 1999; Rosenberger et al., 2017), and that adult dispersal occurs at a seasonally appropriate time (Safranyik & Carroll, 2006).

Eggs are among the most susceptible life stages to cold mortality (Bleiker et al., 2017) and both adult dispersal and oviposition timing influence their potential exposure to mortality-inducing temperatures. Oviposition timing can also create variance in development time among mountain pine beetles within a cohort that may or may not be countered by the synchronizing effect of larval developmental thresholds and diapause. Unlike other organisms in which oviposition requires little time and co-occurs with ongoing dispersal and host search (Ives, 1989; Rosenheim, 1996), mountain pine beetle oviposition is a phenologically distinct event that occurs after dispersal, with lower temperature thresholds than those required for flight (Safranyik & Carroll, 2006).

Mountain pine beetle oviposition occurs in tree phloem beneath the outer bark and can be difficult to quantify. Amman (1972) used destructive sampling to measure the average number of eggs laid over 13 days at several constant temperatures, assuming that an equal number of eggs was laid each day. These data were subsequently used to develop oviposition models based on an exponential function. Due to the structure of the data, however, variation in the rate of oviposition and the fecundity of an individual were confounded. A higher number of eggs laid in the 13-day sampling period could be due to either a higher rate of oviposition or higher fecundity, or a combination of the two factors. For modeling purposes, fecundity could be held constant and oviposition rate allowed to vary (Logan et al., 1995), or oviposition rate held constant and fecundity allowed to vary (Régnière et al., 2012). When either rate or fecundity is held constant in a mathematical

model for oviposition, observed variation in the mathematically fixed variable is counted by the model as variation in the non-fixed variable, thereby potentially exaggerating or biasing variability. Mathematical models should account for the possibility of independent variance in rate and fecundity.

Our goal was to quantify the effect of temperature on mountain pine beetle oviposition rate and fecundity simultaneously to develop a predictive oviposition model. We used a population near the southern extent of the mountain pine beetle range in Arizona (AZ, USA), whereas previous oviposition models were developed using data from a northern population in Utah (UT, USA) (Amman, 1972; Logan et al., 1995; Régnière et al., 2012). We describe a non-destructive method that uses a combination of constant temperature experiments, X-rays, and image analysis to measure oviposition rate with a 2–3-day resolution. The experimental unit was a portion of a cut bolt wherein oviposition occurred in the phloem layer beneath the outer bark. Time series data within each bolt allowed us to isolate and resolve variation in oviposition rate and fecundity separately and parametrize a model. We compare our southern US data and model with previously published information for a northern US population (Régnière et al., 2012). Divergent selection has occurred and local adaptation in development time parameters has resulted in longer generation times in southern (median = 149 days) compared to northern (median = 76 days) mountain pine beetles in 22 °C common garden experiments (Bentz et al., 2011; Bracewell et al., 2013). We evaluate how differences in oviposition timing may contribute to known phenological differences between the two latitudinally separated populations.

Materials and methods

Data collection

Unmated adult mountain pine beetles were obtained from an infested southwestern white pine, *Pinus strobiformis* Engelm. harvested on 4 May 2016 in the Kaibab National Forest near Flagstaff, AZ (35.35506, –111.6132) (hereafter ‘southern population’). *Pinus strobiformis* is the main *Pinus* species in central and southern Arizona that mountain pine beetle is currently found attacking naturally. The tree was cut into 45–50-cm-long bolts and transported to the US Forest Service Rocky Mountain Research Station (RMRS) laboratory in Logan, UT. Bolts were waxed with melted paraffin (Gulf Wax, Roswell, GA, USA) on both ends to retain moisture and stored at 0 °C for less than a week prior to use. Eight bolts were placed in incubators (Percival Scientific, Perry, IN, USA) at 20 °C to facilitate

brood development and adult emergence (four bolts per incubator). Adults were collected daily, sexed using secondary sex characteristics on the seventh tergite (Lyon, 1958), then held for 1–7 days before use at 4 °C in Petri dishes lined with moistened filter paper. To obtain material to infest with mountain pine beetle parents, a live southwestern white pine was harvested on 3 May 2016 near Flagstaff, AZ (35.36272, –111.7439) and cut into bolts of 50–55 cm. Bolts were transported to the RMRS laboratory and bolt ends were waxed to retain moisture and then stored at 0 °C for ca. 2 months, until adults emerged from the infested bolts.

Oviposition and fecundity were monitored for individual female–male pairs in ‘boards’, a section of bark, phloem, and xylem. To make individual boards, a chainsaw was used to cut sections vertically from the circumference of un-infested bolts to ca. 50–55 cm tall × 10 cm wide × 1.25 cm deep from the outer bark edge. Each board was then trimmed to 35 × 5 × 1.25 cm using a table saw, and exposed sections were waxed to retain moisture. Each board was infested with one adult mountain pine beetle pair randomly chosen from a pool of adults that emerged over several weeks on either side of peak emergence. A 2-cm-deep hole was drilled vertically into the phloem and parallel to the board’s height, and a female and then a male beetle were inserted into the hole. Mesh screen was stapled over the opening so the beetle pair could not back out. Fine wire was wrapped around the boards at one-third and two-third of the length to provide reference points for gallery construction progress in successive X-ray images.

Forty infested boards were placed upright in four large coolers (10 boards each) which were then placed in one of four incubators (Percival Scientific) set to 10, 20, 27, or 29 °C in constant darkness. These temperatures were chosen to give the broadest coverage of the oviposition rate curve, with emphasis on the upper threshold, with the limited materials available. During the 30-day incubation period each board was X-rayed 3× per week with a Faxitron X-Ray machine (Faxitron Biophysics, Tucson, AZ, USA) for 12 s at 18 kV following guidelines for insects found in Stephen & Undurraga (1976). At the end of the 30-day period (10–13 X-rays completed), boards were stored at 0 °C to halt further oviposition and egg development until the bark was peeled, after no more than 2 weeks. Phloem and outer bark were carefully peeled from each board and the location of unhatched and hatched (i.e., first instars) eggs were identified and marked. Photographs were taken of each peeled board with a ruler for scale.

Using ImageJ (Schneider et al., 2012), oviposition gallery lengths for successive days were measured using the

X-ray images, and egg/larva locations were taken from the photographs. Gallery lengths were determined by drawing a segmented line along the gallery completed up to the date of each X-ray using a scale of 52 pixels cm^{−1} for all images. Because galleries packed with frass were difficult to see on subsequent X-rays, wires at one-third and two-third of each board’s length were used to provide reference measurements of older, frass-packed galleries. Egg locations were determined using the figure calibration plug-in with ImageJ to establish a Cartesian grid over the gallery. The (0,0) point on the grid was set to the base of the gallery, egg points were marked, and the coordinates of those points were extracted. The pixels cm^{−1} scale for those images was reset each time using the ruler in the picture. On boards where parent beetles made forked galleries, or turned the gallery at the board end, the y coordinates of eggs and larvae included pre- and post-fork or turned gallery lengths to represent the total gallery length. Length and location data were combined and used to assign a date range of oviposition for each egg.

Model development

Oviposition rate. Following Régnière et al. (2012), we used an exponential functional form to model oviposition rate. We assumed that each female has a fixed potential fecundity, Ω_n , and that a female oviposits a constant proportion, r_n , of her remaining potential fecundity, F_n , per unit time (Sahota & Thomson, 1979; Régnière et al., 2012). Given that this proportion is a function of temperature (T) and a vector of parameters, θ , the rate of potential oviposition expenditure is

$$\frac{dF_n}{dt} = -r_n(T, \theta)F_n \quad (1)$$

There is a delay period between initiation of a gallery and the beginning of oviposition wherein the female mates and constructs a few centimeters of egg-free gallery, so the initial time of oviposition is $t_0 > 0$ and $F_n(t_0) = \Omega_n$. Solving (1) for F_n with this initial condition produces the following equation for remaining potential fecundity over time

$$F_n = \begin{cases} \Omega_n \left[e^{-\int_{t_0}^t r_n(T, \theta) dt} \right], & t > t_0, \\ \Omega_n, & t \leq t_0 \end{cases} \quad (2)$$

The data collected are in terms of eggs laid over time, rather than potential fecundity expended over time. Defining $O_n(t)$ as the cumulative oviposition by individual n , at time t and using $O_n(t) = \Omega_n - F_n$ gives our oviposition model

$$O_n(t) = \begin{cases} \Omega_n \left[1 - e^{-\int_{t_0}^t r_n(T, \theta) dt} \right], & t \geq t_0, \\ 0, & t < t_0 \end{cases} \quad (3)$$

The term $\left[1 - e^{-\int_{t_0}^t r_n(T, \theta) dt} \right]$ represents the fraction of potential oviposition that has been completed by time t . To specify the dependence of rate on temperature, let $r_o(T, \theta)$ be the mean rate of oviposition expenditure and, assuming a normal distribution, the rate for an individual female $[r_n(T, \theta)]$ can be written

$$r_n(T, \theta) = r_o(T, \theta) + \varepsilon_n, \varepsilon_n \sim N(0, \sigma_r^2) \quad (4)$$

here, $r_o(T, \theta)$ is the rate function

$$r(T) = \psi \left[e^{\omega(T-T_b)} - \left(\frac{T_m - T}{T_m - T_b} \right) e^{-\frac{\omega(T-T_b)}{\Delta_b}} - \left(\frac{T - T_b}{T_m - T_b} \right) e^{\omega(T_m - T_b) - \frac{T_m - T}{\Delta_m}} \right] \quad (5)$$

previously used by Régnière et al. (2012) to describe oviposition in a northern mountain pine beetle population. The parameters T_b and T_m are lower and upper thresholds for oviposition, respectively, Δ_b and Δ_m are the width of thermal transitions from normal to negligible oviposition at the lower and upper thresholds, ω describes the expected exponential acceleration of oviposition with temperature, and ψ is proportional to the maximum rate of fecundity expenditure. This curve was chosen for its inherent flexibility and also to facilitate comparison of estimates between southern and northern populations.

Independent sources of individual variation and parameter estimation. Our observations of oviposition indicated that there are two sources of individual variation around the temperature-dependent mean. Individual females vary greatly in their maximum potential fecundity, and each female appears to construct gallery either more or less rapidly than the mean individual in the population, independently of her potential fecundity. These two sources of variation must be mathematically separated to obtain unbiased estimates of oviposition rate (e.g., so that individuals with higher potential fecundity do not positively bias the rate). We proceeded by first fitting an exponential curve to observed oviposition in each board to determine individual fecundity, making no assumptions about the structure of rates in the population. Having resolved variation in fecundity, we devised a method to analyze rates

directly through ratios of individual fecundity in each board, which vary with rate alone (because the ratios normalize individual fecundity differences). We constructed a likelihood estimation procedure based on these ratios to characterize temperature-dependent oviposition rates.

Estimating individual and mean fecundity. To estimate individual fecundity, we fit the curve

$$O_n(t) = \begin{cases} \Omega_n [1 - e^{-\rho_n(t-t_0)}], & t \geq t_0, \\ 0, & t < t_0 \end{cases} \quad (6)$$

to oviposition data for each female individually using nonlinear regression and sum squared error. Note that this allows each female her own rate of oviposition, ρ_n , so the fitting procedure makes no assumption regarding individual variability in rates. Following Régnière et al. (2012), we assume that individual and population mean fecundities follow a log normal model, that is, $\Omega_n = O_o \delta_n$, and $\delta_n \sim \text{LogNormal}\left(\frac{-\sigma_o^2}{2}, \sigma_o^2\right)$. Population mean fecundity (O_o) can then be estimated directly from observations using $\mu = \text{mean}[\ln(\Omega_n)]$ and $\sigma_o = \text{stdev}[\ln(\Omega_n)]$. Population mean fecundity is then (Hilborn & Mangel, 1997):

$$O_o = e^{\mu + \frac{1}{2}\sigma_o^2} \quad (7)$$

Fecundity data were bootstrapped by generating a random sample with replacement of the fitted Ω_n values for each board and recalculating the mean using the new random subset. This was repeated 1 000× in R to determine the distribution of estimates of O_o (R Core Team, 2015).

Estimating rates using observed fecundity ratios. To isolate variation in rates from individual variation in fecundity, we normalized the data with regard to the total number of eggs laid by each female. Defining X_{nij} as the number of eggs laid by individual n at temperature T_i in time interval (t_{j-1}, t_j) , and $X_{ni(j+1)}$ as the number of eggs laid by the same individual in the next time interval (t_j, t_{j+1}) , we observed that the ratio between these quantities removes the effect of variable fecundity,

$$\frac{X_{ni(j+1)}}{X_{nij}} = \frac{O_o \delta_n}{O_o \delta_n} \cdot \frac{[e^{-r_n(t_j - t_0)} - e^{-r_n(t_{j+1} - t_0)}]}{[e^{-r_n(t_{j-1} - t_0)} - e^{-r_n(t_j - t_0)}]} \quad (8)$$

Canceling the $O_o \delta_n$ terms and substituting in (4), leaves an expression involving only the mean population rate,

$$\frac{X_{ni(j+1)}}{X_{nij}} = \frac{e^{-[r_o(T_i, \theta) + \varepsilon_n]t_j} - e^{-[r_o(T_i, \theta) + \varepsilon_n]t_{j+1}}}{e^{-[r_o(T_i, \theta) + \varepsilon_n]t_{j-1}} - e^{-[r_o(T_i, \theta) + \varepsilon_n]t_j}} \quad (9)$$

Solving for ε_n (details in Appendix I) gives

$$\varepsilon_n = \frac{1}{\Delta t_j} \cdot \ln \left[\frac{X_{nij}}{X_{ni(j+1)}} \frac{\Delta t_{j+1}}{\Delta t_j} \right] - r_o(T_i, \theta) \quad (10)$$

Here, $\Delta t_j = t_j - t_{j-1}$. The ε_n are assumed to be independent and identically distributed with $\varepsilon_n \sim N(0, \sigma_r^2)$, and the likelihood of observing a particular ratio is

$$L \left[\frac{X_{ni(j+1)}}{X_{nij}} \right] = \frac{1}{2\pi\sigma_r^2} e^{-\frac{\left\{ \frac{1}{\Delta t_j} \ln \left[\frac{X_{nij}}{X_{ni(j+1)}} \frac{\Delta t_{j+1}}{\Delta t_j} \right] - r_o(T_i, \theta) \right\}^2}{2\sigma_r^2}} \quad (11)$$

Maximizing the likelihood over all data is then equivalent to minimizing the negative log likelihood,

$$\begin{aligned} \text{NLL}(\theta) \\ = \sum_{n,i,j} \left[\frac{\left\{ \frac{1}{\Delta t_j} \ln \left[\frac{X_{nij}}{X_{ni(j+1)}} \frac{\Delta t_{j+1}}{\Delta t_j} \right] - r_o(T_i, \theta) \right\}^2}{2\sigma_r^2} + \frac{1}{2} \ln(2\pi\sigma_r^2) \right] \end{aligned} \quad (12)$$

which was done with the ‘optim’ function in R using the default Nelder–Mead method and scaling the step-size used to fit the vector of parameters, θ , according to $\text{parscale} = c(0.001, 0.0001, 1, 1, 0.001, 0.1, 0.01)$ (R Core Team, 2015). Based on the fit of this rate function to a northern mountain pine beetle population (Régnière et al., 2012), this scaling was necessary because parameters are of different orders of magnitude and disproportionally sensitive to large steps.

This procedure has the advantage not only of separating the roles of variable fecundity (so that individuals laying more eggs do not bias rate estimates) but also of increasing sample size (because the individual sample is not the board but a sampling interval on a particular board). Although the ε_n are assumed to be independent, correlations among the observations X_{nij} are handled by means of the logarithmic terms in equation 12. These terms, however, may also create difficulties in the analytical analyses. Periods during which $X_{nij} = 0$ (no oviposition observed) cannot be included because two terms would be removed from the likelihood equation 12 due to the logarithmic ratio, which is sensitive to zeros in both X_{nij} and $X_{ni(j+1)}$. In spite of these complexities, this approach represents a novel method to remove bias from estimation of oviposition rates.

Statistical analysis

We tested for significant differences in egg-free gallery length, the time delay prior to oviposition (t_o), and

potential fecundity among temperatures using a generalized linear model with a Poisson distribution and post-hoc Bonferroni test (SAS v.9.4; SAS Institute, Cary, NC, USA).

Results

Females successfully oviposited viable eggs at the four treatment temperatures (10, 20, 27, and 29 °C). Due to assumptions associated with model parameterization, 8 of the original 40 boards that were initiated with male/female pairs were removed from analyses. Fewer than 10 eggs were produced in 5 boards wherein the female abandoned the board, which we assumed was due to the artificial environment and not part of natural oviposition variability in the population. In one board, the data were linear with few non-zero entries, and therefore violated restrictions on logarithmic arguments in equation 12. Moreover, long portions of galleries with no eggs laid, as was seen in this board, is not common on naturally attacked trees. In two additional boards, more than 10 eggs were laid before the first X-ray image, due to an experimental error, masking oviposition start time and violating a model assumption of a delay time prior to oviposition. Data from the remaining 32 boards (10 °C: $n = 8$, 20 °C: $n = 9$, 27 °C: $n = 7$, 29 °C: $n = 8$) were used to fit parameters of the exponential function to describe oviposition rate.

As expected, the cumulative number of eggs laid followed a pattern of diminishing returns and the rate of oviposition declined with individual age (i.e., time since mating) at all temperatures except 10 °C (Figure 1). At 10 °C oviposition was continuing to increase, albeit at a slow rate, when the experiment was ended. Variability in the observed fecundity among individuals, within the 30-day experimental period, generally increased with increasing temperature, and the fewest eggs were laid at the lowest temperature (Figure 1). Model fits estimating potential fecundity of individual females, using observed fecundity data and equation 6, ranged from $R^2 = 0.975$ – 0.993 across the four temperatures (Figure 1). Although the fewest eggs were laid at the lowest temperature during the 30-day experimental period (i.e., realized fecundity), model estimates of individual potential maximum fecundity (Ω_n) did not differ among temperatures ($F_{3,25} = 1.78$, $P = 0.18$; Figure 2). These results suggest that potential fecundity is independent of temperature. Based on bootstrapping across the four temperatures, mean realized fecundity during the 30-day experimental period was 73.6 eggs per female (Figure 3), and mean potential population fecundity (O_o) was estimated as 91.0 eggs per female (Figure 4).

The time delay before oviposition begins, t_o , was significantly longer at 10 °C compared to all other temperatures (d.f. = 25, adjusted $P < 0.01$), and differences among all

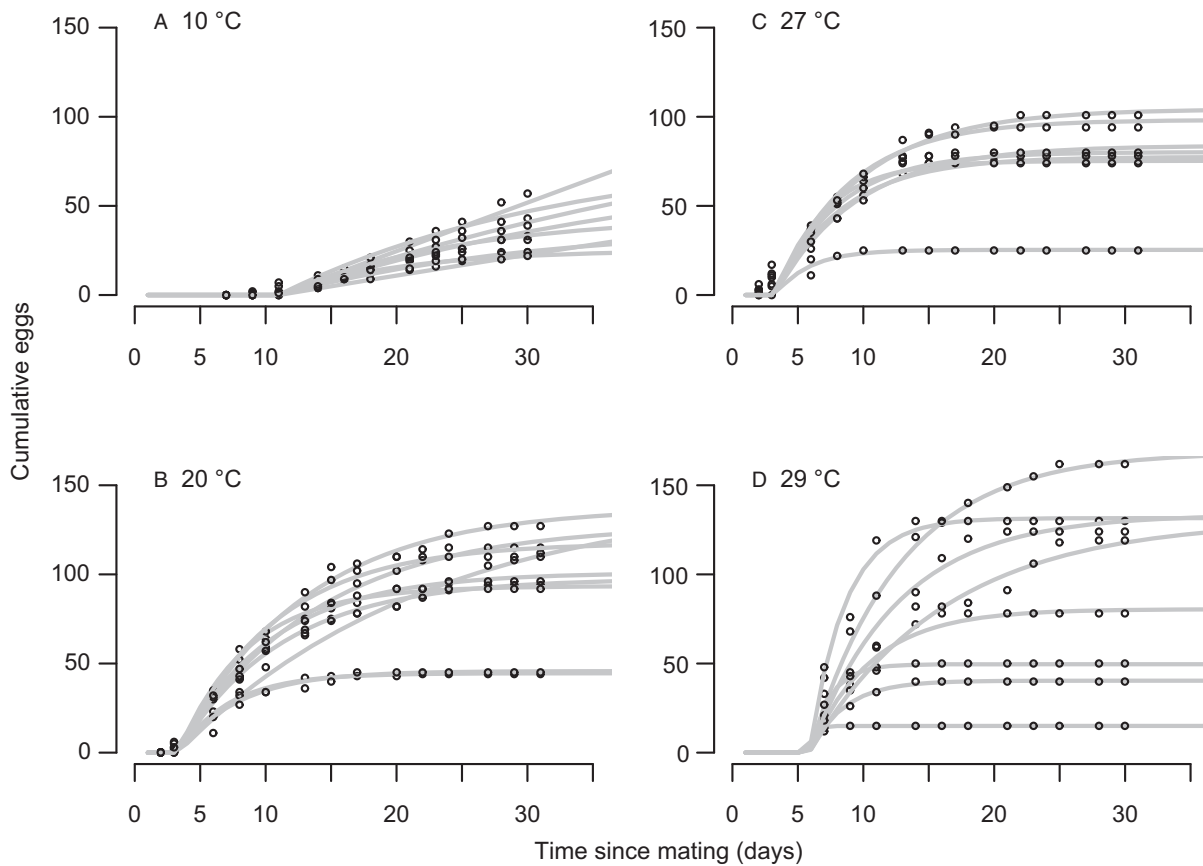


Figure 1 Observed cumulative number of eggs laid by female mountain pine beetle as a function of time since mating (days) and associated oviposition model fits describing potential fecundity at (A) 10 °C ($n = 8$), (B) 20 °C ($n = 9$), (C) 27 °C ($n = 7$), and (D) 29 °C ($n = 8$). Each line represents data from a single female.

other temperatures were not significant (Figure 5A). A similar pattern was seen in egg-free gallery length across temperatures, wherein egg-free length was the greatest at the lowest temperature, although there was considerable variability and differences were not significant ($F_{3,25} = 2.03$, $P = 0.14$; Figure 5B).

Following the procedure to isolate variation in rates from individual variation in fecundity, parameters were simultaneously estimated for oviposition rates across temperatures for the rate curve using equation 5. The predicted upper threshold for oviposition was estimated as $T_m = 30.9$ °C ($\Delta_m = 1.989$) and the lower threshold at $T_b = 6.6$ °C ($\Delta_b = 1.361$), with the peak rate estimated to be 26.2 °C (Figure 6, Table 1). A comparison of oviposition model parameters for the southern population, described here, and parameters estimated for a northern population (Régnière et al., 2012) suggest a warmer upper oviposition threshold for southern (30.9 °C) compared to northern (27.7 °C) mountain pine beetle (Table 1). The expected exponential acceleration of oviposition with temperature

was lower in southern ($\omega = 0.0309$) than in northern ($\omega = 0.2560$) populations, and both populations had similar lower oviposition thresholds at ca. 7.0 °C. Estimated potential fecundity was slightly greater in southern (91.0 eggs per female) than in northern (81.8 eggs per female) individuals (Figure 4). In addition to population differences due to local adaptation to climate, differences could have been influenced by tree species, as northern individuals came from and were reared in *Pinus contorta* Douglas and southern individuals were from *P. strobiformis*.

Discussion

Our goal was to quantify oviposition rate and fecundity of individuals from a southern mountain pine beetle population, collected from an infested *P. strobiformis*, across a range of temperatures from 10 to 29 °C. Due to difficulties in investigating oviposition patterns of this cryptic insect, the two fitness traits have previously been assumed to not vary independently. Our approach removed bias due to

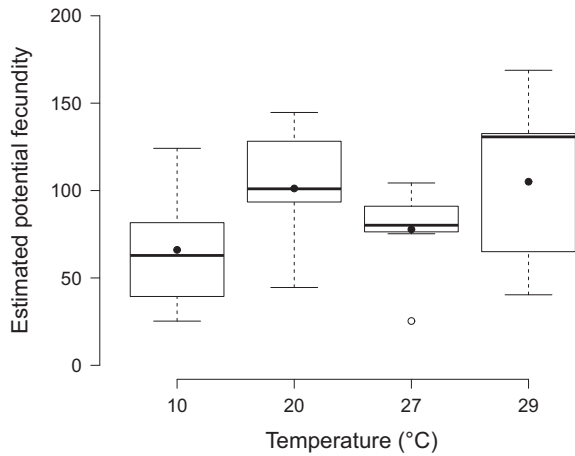


Figure 2 Model estimated potential fecundity (Ω_n , number of eggs per female) of female mountain pine beetles at four temperatures. Shown are the median (solid line) and mean (closed dot) within boxes, the interquartile range (IQR; the upper and lower boxes), and whiskers representing $1.5 \times$ IQR. The open dot indicates an outlier. Potential fecundity was not significantly different among the four temperatures. See Figure 1 for sample size at each temperature.

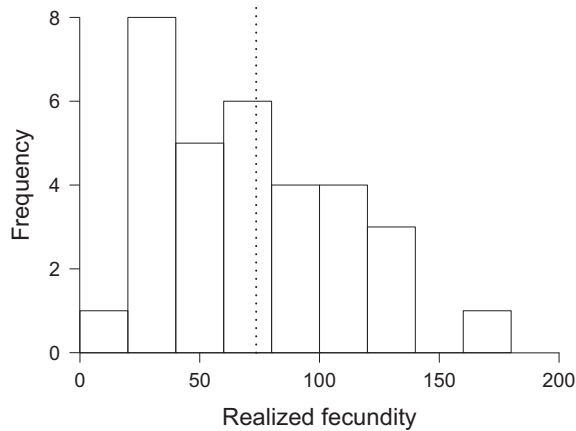


Figure 3 Realized, observed fecundity (no. eggs per female/30 days) during the 30-day experimental period using females from a southern mountain pine beetle population that were reared at each of the four constant temperatures. Mean realized fecundity was calculated as 73.56 eggs per female (dotted line) based on bootstrapped data. Even though some females continued to oviposit, the asymmetrical shape is consistent with a lognormal distribution. See Figure 1 for sample size at each temperature.

fecundity variability from oviposition rate estimation and results suggest there is independent variability in both oviposition rate and maximum potential fecundity. A female with a high oviposition rate did not necessarily have

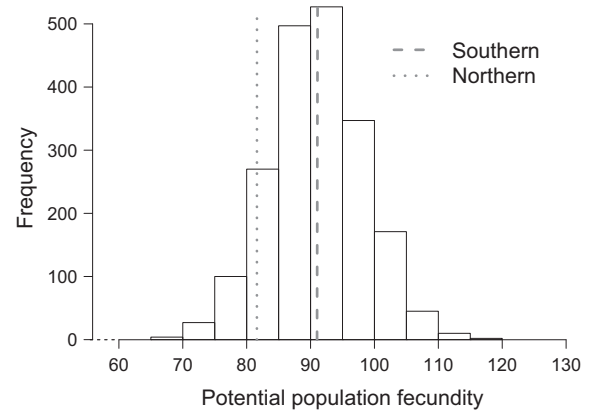


Figure 4 Distribution of estimates of mean potential population fecundity (O_o , number of eggs per female) calculated from each iterant of bootstrapped data. Mean predicted potential fecundity of the southern population (91.0 eggs; dashed line) was slightly higher than the predicted mean (81.8 eggs; dotted line) for a northern population reported by Régnière et al. (2012).

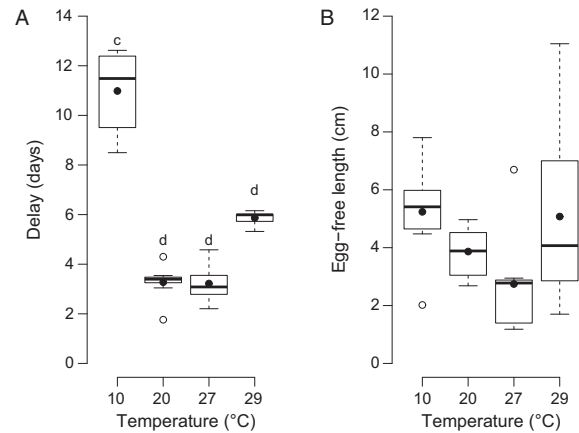


Figure 5 (A) Delay (no. days) and (B) egg-free length (cm) before the start of oviposition at each of four constant temperatures. Data for each temperature are based on 7–9 galleries. Shown are the median (solid line), mean (closed dot), interquartile range (IQR; the upper and lower boxes), and whiskers representing $1.5 \times$ IQR. The open dots indicate outliers. Means capped with different letters were significantly different (post-hoc Bonferroni test: $P < 0.05$). There were no significant differences in egg-free length across temperatures. See Figure 1 for sample size at each temperature.

high fecundity and vice versa. Despite differences in oviposition rate among temperatures, estimated maximum potential fecundity did not differ significantly among temperatures. Given sufficient phloem habitat, females at varying temperatures have the potential to lay similar

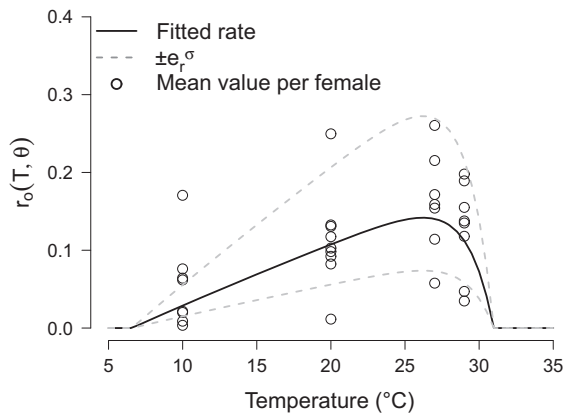


Figure 6 Oviposition rate [$r_o(T, \theta)$, days⁻¹] of southern mountain pine beetle with respect to temperature and observed rate for individual females (○), after normalization for variance in fecundity. Upper (T_m) and lower (T_b) thresholds are at 30.9 and 6.6 °C, respectively, and peak oviposition rate is 26.2 °C. Dashed lines indicate ± 1 SD in oviposition rate, capturing 68% of observed variance.

numbers of eggs, although it will take longer at lower temperatures. Similar to previous findings (Amman, 1972; Régnière et al., 2012), oviposition rate was high initially and declined with time since mating.

Mountain pine beetle fecundity can be influenced by food quantity (i.e., phloem thickness; Amman, 1972), and active induced host tree resins that affect oviposition of individuals that attack a tree first (Raffa & Berryman, 1983). Our experimental units were portions of cut bolts (i.e., boards) of a single live *P. strobiformis*, the main host tree species for mountain pine beetle in central and southern Arizona, which contained constitutive defense compounds but no active induced defenses. Although data are lacking on the influence of host tree species on fecundity and oviposition rates, we acknowledge potential variability in phloem compounds among trees of the same and different species, and that oviposition rates and fecundity in live trees may differ from our laboratory results wherein cut bolts were cold-stored prior to use. Because our goal was to quantify oviposition rate and fecundity for development

of a predictive model to describe population-scale patterns, females and males were randomly assigned to each temperature regardless of size. Although size may influence maximum fecundity (Reid, 1958; McGhehey, 1971; Honek, 1993; but see Amman, 1972), this effect is not explicit in the model.

Although the length of gallery constructed prior to the start of egg laying did not vary among temperatures, the delay in days prior to the start of oviposition took longer at the lowest temperature (i.e., 10 °C). Our finding of ca. 3.7 days delay prior to oviposition at 20 °C is similar to the 3–4-day delay observed in naturally attacked trees (Reid, 1958). Females excavated a more or less consistent length of gallery before oviposition began, but the process was longer at low temperature. This difference in time required to build the same length of egg-free gallery emphasizes that, in addition to the rate of egg laying, gallery construction, and potentially mating are also temperature-dependent phenological events.

A comparison of oviposition model parameters derived for the southern population and parameters estimated for a northern population using the same exponential form (Régnière et al., 2012) suggest similar lower oviposition thresholds and that southern mountain pine beetle can oviposit at higher temperatures than northern individuals. Estimated potential maximum fecundity for southern (mean of 91.0 eggs) individuals reared in *P. strobiformis* was slightly higher than for northern (mean of 81.8 eggs) individuals reared in *P. contorta*. To further evaluate whether differences in oviposition rate between southern and northern US mountain pine beetle could explain differences in total development time between the populations (Bentz et al., 2011; Bracewell et al., 2013), we estimated mean oviposition time for both populations at 21 °C. The northern population laid 66.8 ± 17.79 eggs per female in 13 days (Amman, 1972). At the same temperature, our model predicted that mountain pine beetle from a southern population would lay 66.8 eggs per female over 18.5 ± 4 days. Although total oviposition time predicted for a southern population is longer (6.5 days) than that of a northern population, the delay does not account for the 73 days of additional development time required by the

Table 1 Oviposition rate function parameters estimated for a southern (this study) and a northern mountain pine beetle population (Régnière et al., 2012). T_b and T_m are lower and upper thresholds (°C) for oviposition, Δ_b and Δ_m are the width of thermal transitions from normal to negligible oviposition at the lower and upper thresholds, ω describes the expected exponential acceleration of oviposition with temperature, and ψ is proportional to the maximum rate of fecundity expenditure

Population	ψ	ω	T_b	T_m	Δ_b	Δ_m	σ_r
Southern	0.0913	0.0309	6.6	30.9	1.3613	1.9889	0.32
Northern	0.0237	0.2560	7.0	27.7	0.0200	4.400	0.18

southern population when reared in a common garden at 22.5 °C (Bracewell et al., 2013). Instead, local adaptation to climate in the northern and southern mountain pine beetle populations has likely occurred in temperature-dependent developmental timing or thresholds of one or more life stages. McManis et al. (2019) investigated development rates and thresholds of the egg, larvae, and pupal life stages and also found few differences compared to a northern population except in the last instar which developed slower than northern individuals at a relatively high temperature. These results suggest that lifecycle timing differences between the populations may occur in the last instar, where a facultative diapause occurs, in addition to the unstudied teneral (i.e., pre-emergent) adult life stage.

Quantifying temperature-dependent phenological events that vary along latitudinal clines is a critical step in the development of models for predicting range-wide mountain pine beetle population success in a changing climate. Oviposition is an important part of phenology models, particularly for bark beetles that can spend several weeks laying eggs, thereby adding considerable variability to desired cohort synchrony. Using a non-destructive methodology for quantifying variance among individuals and temperatures we found non-trivial variation in both fecundity and oviposition rates of southern mountain pine beetle. We acknowledge low sample size used for model parameterization, a result of experimental logistics, and highlight the need for additional ovipositional data on populations from multiple host trees across the range of mountain pine beetle. Our analytical methodology for separating variability due to oviposition and fecundity provides a basis for further oviposition modeling. When coupled with data on temperature-dependent life stage-specific development rates of southern mountain pine beetle populations, predictions regarding range-wide population dynamics in a changing climate can be made.

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References

- Amman GD (1972) Some factors affecting oviposition behavior of the mountain pine beetle. *Environmental Entomology* 1: 691–695.
- Bentz BJ & Hansen EM (2017) Evidence for a prepupal diapause in the mountain pine beetle (*Dendroctonus ponderosae*). *Environmental Entomology* 47: 175–183.
- Bentz BJ & Mullins DE (1999) Ecology of mountain pine beetle cold hardening in the Intermountain west. *Environmental Entomology* 28: 577–587.
- Bentz BJ, Logan JA & Amman GD (1991) Temperature-dependent development of the mountain pine beetle (Coleoptera: Scolytidae) and simulation of its phenology. *Canadian Entomologist* 123: 1083–1094.
- Bentz BJ, Bracewell RR, Mock KE & Pfreder ME (2011) Genetic architecture and phenotypic plasticity of thermally-regulated traits in an eruptive species, *Dendroctonus ponderosae*. *Evolutionary Ecology* 25: 1269–1288.
- Bleiker KP, Smith GD & Humble LM (2017) Cold tolerance of mountain pine beetle (Coleoptera: Curculionidae) eggs from the historic and expanded ranges. *Environmental Entomology* 46: 1165–1170.
- Boone CK, Aukema BH, Bohlmann J, Carroll AL & Raffa KF (2011) Efficacy of tree defense physiology varies with bark beetle population density: a basis for positive feedback in eruptive species. *Canadian Journal of Forest Research* 41: 1174–1188.
- Bracewell RR, Pfreder ME, Mock KE & Bentz BJ (2013) Contrasting geographic patterns of genetic differentiation in body size and development time with reproductive isolation in *Dendroctonus ponderosae* (Coleoptera: Curculionidae, Scolytinae). *Annals of the Entomological Society of America* 106: 385–391.
- Cooke BJ & Carroll AL (2017) Predicting the risk of mountain pine beetle spread to eastern pine forests: considering uncertainty in uncertain times. *Forest Ecology and Management* 396: 11–25.
- Dowle EJ, Bracewell RR, Pfreder ME, Mock KE, Bentz BJ & Ragland GJ (2017) Reproductive isolation and environmental adaptation shape the phylogeography of mountain pine beetle (*Dendroctonus ponderosae*). *Molecular Ecology* 26: 6071–6084.
- Franceschi VR, Krokene P, Christiansen E & Krekling T (2005) Anatomical and chemical defenses of conifer bark against bark beetles and other pests. *New Phytologist* 167: 353–375.
- Hilborn R & Mangel M (1997) *The Ecological Detective: Confronting Models with Data*. Princeton University Press, Princeton, NJ, USA.
- Honek A (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos* 66: 483–492.

- Ives AR (1989) The optimal clutch size of insects when many females oviposit per patch. *American Naturalist* 133: 671–687.
- Jenkins J, Powell JA, Logan JA & Bentz BJ (2001) Low seasonal temperatures promote life cycle synchronization. *Bulletin of Mathematical Biology* 63: 573–595.
- Logan JA, Bolstad PV, Bentz BJ & Perkins DL (1995) Assessing the effects of changing climate on mountain pine beetle dynamics. Proceedings of a Joint IUFRO Working Party Conference (ed. by FP Hain, SM Salom, WF Ravlin, TL Payne & KF Raffa), pp. 86–108. Ohio State University, Wooster, OH, USA.
- Lyon RL (1958) A useful secondary sex character in *Dendroctonus* bark beetles. *Canadian Entomologist* 90: 582–584.
- McGhehey JH (1971) Female size and egg production of the mountain pine beetle, *Dendroctonus ponderosae* Hopkins. Information Report NOR-X-9, Canadian Forest Service, Northern Forest Research Centre, Edmonton, Alberta, Canada.
- McManis AE, Powell JA & Bentz BJ (2019) Developmental parameters of a southern mountain pine beetle (Coleoptera: Curculionidae) population reveal potential source of latitudinal differences in generation time. *Canadian Entomologist* 151: 1–15.
- Powell JA & Logan JA (2005) Insect seasonality: circle map analysis of temperature-driven life cycles. *Theoretical Population Biology* 67: 161–179.
- R Core Team (2015) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Raffa KF & Berryman AA (1983) The role of host plant resistance in the colonization behavior and ecology of bark beetles (Coleoptera: Scolytidae). *Ecological Monographs* 53: 27–49.
- Raffa KF, Aukema BH, Bentz BJ, Carroll AL, Hicke JA et al. (2008) Cross-scale drivers of natural disturbances prone to anthropogenic amplification: dynamics of biome-wide bark beetle eruptions. *BioScience* 58: 501–518.
- Régnière J, Powell J, Bentz B & Nealis V (2012) Effects of temperature on development, survival and reproduction of insects: experimental design, data analysis and modeling. *Journal of Insect Physiology* 58: 634–647.
- Reid RW (1958) The behavior of the mountain pine beetle, *Dendroctonus monticolae* Hopk., during mating, egg laying and gallery construction. *Canadian Entomologist* 9: 505–509.
- Rosenberger DW, Aukema BH & Venette RC (2017) Cold tolerance of mountain pine beetle among novel eastern pines: a potential for trade-offs in an invaded range? *Forest Ecology and Management* 400: 28–37.
- Rosenheim JA (1996) An evolutionary argument for egg limitation. *Evolution* 50: 2089–2094.
- Safranyik L & Carroll A (2006) The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. The Mountain Pine Beetle: A Synthesis of Biology, Management, and Impacts on Lodgepole Pine (eds. by L Safranyik & WR Wilson), pp. 3–66. Canadian Forest Service, Pacific Forestry Centre, Victoria, BC, Canada.
- Sahota T & Thomson A (1979) Temperature induced variation in the rates of reproductive processes in *Dendroctonus rufipennis* (Coleoptera: Scolytidae): a new approach to detecting changes in population quality. *Canadian Entomologist* 111: 1069–1078.
- Sambaraju KR, Carroll AL, Zhu J, Stahl K, Moore RD & Aukema BH (2012) Climate change could alter the distribution of mountain pine beetle outbreaks in western Canada. *Ecography* 35: 211–223.
- Schneider CA, Rasband WS & Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675.
- Stephen WP & Undurraga JM (1976) X-radiography, an analytical tool in population studies of the leafcutter bee, *Megachile pacifica*. *Journal of Apicultural Research* 15: 81–87.

Appendix I

Starting with equation 8, our goal is to derive an expression for $r_n = r_o(T_i, \theta) + \varepsilon_n$, the individual rate of oviposition, independent of fecundity, to use in maximum likelihood estimation (MLE)

$$\frac{X_{ni(j+1)}}{X_{nij}} = \frac{O_o \delta_n}{O_o \delta_n} \cdot \frac{[e^{-r_n(t_j - t_o)} - e^{-r_n(t_{j+1} - t_o)}]}{[e^{-r_n(t_{j-1} - t_o)} - e^{-r_n(t_j - t_o)}]} \quad (A1)$$

Canceling $O_o \delta_n$ and factoring out common r_n from numerator and denominator gives

$$\frac{X_{ni(j+1)}}{X_{nij}} = \frac{e^{-r_n(t_j - t_o)}}{e^{-r_n(t_{j-1} - t_o)}} \cdot \frac{[1 - e^{-r_n(t_{j+1} - t_j)}]}{[1 - e^{-r_n(t_j - t_{j-1})}]} \quad (A2)$$

which simplifies to

$$\frac{X_{ni(j+1)}}{X_{nij}} = e^{-r_n \Delta t_j} \cdot \frac{(1 - e^{-r_n \Delta t_{j+1}})}{(1 - e^{-r_n \Delta t_j})} \quad (A3)$$

where $\Delta t_j = t_j - t_{j-1}$. Using $1 - e^{-r_n \Delta t_j} = r_n \Delta t_j + O(r_n^2 \Delta t_j^2)$ and neglecting quadratic terms

$$\frac{X_{ni(j+1)}}{X_{nij}} = e^{-r_n \Delta t_j} \cdot \frac{r_n \Delta t_{j+1}}{r_n \Delta t_j} \quad (A4)$$

which gives

$$e^{r_n \Delta t_j} = \frac{X_{nij}}{X_{ni(j+1)}} \cdot \frac{\Delta t_{j+1}}{\Delta t_j} \quad (A5)$$

Solving for r_n

$$r_n = \frac{1}{\Delta t_j} \ln \left[\frac{X_{nij}}{X_{ni(j+1)}} \cdot \frac{\Delta t_{j+1}}{\Delta t_j} \right] \quad (A6)$$

and using $r_n = r_o(T_i, \theta) + \varepsilon_n$,

$$\varepsilon_n = \frac{1}{\Delta t_j} \ln \left[\frac{X_{nij}}{X_{ni(j+1)}} \cdot \frac{\Delta t_{j+1}}{\Delta t_j} \right] - r_o(T_i, \theta) \quad (A7)$$

As $\varepsilon_n \sim N(0, \sigma_\varepsilon^2)$, we now have an expression suitable for MLE.