Environmental Entomology, 48(4), 2019, 903–910
doi: 10.1093/ee/nvz051
Advance Access Publication Date: 30 May 2019
Research

A Phenology Model for Asian Gypsy Moth Egg Hatch

David R. Gray[1,3] and Melody A. Keena[2,]

[1]Natural Resources Canada, Canadian Forest Service—Atlantic Forestry Centre, P.O. Box 4000, Fredericton, NB, Canada E3B 5P7, [2]USDA Forest Service, Northern Research Station, Hamden, CT 06514, and [3]Corresponding author, e-mail: david.gray@canada.ca

Subject Editor: Colin Brent

Received 6 December 2018; Editorial decision 17 April 2019

Abstract

Phenology models are useful tools in pest management interventions, biosecurity operations targeting alien invaders, and answering questions regarding the potential for range expansion/shift. The Gypsy Moth Life Stage model (*GLS*) has been used to predict the invasive range of the North American gypsy moth (*Lymantria dispar dispar* Linnaeus [Lepidoptera: Erebidae]) in North America and New Zealand. It has been used to examine the role of supra-optimal temperatures in range expansion/stasis/retraction. However, *GLS* has also been used where the target organism is the Asian subspecies *L. d. asiatica* Vnukovskij, despite observed differences between the predominant phenotypes of the two subspecies in the temperature requirements for egg hatch and the absence of egg phenology model parameters specific to the Asian phenotype. Here we describe the results of temperature and exposure duration on the timing of Asian gypsy moth egg hatch, and we present phenology model parameters for the Asian phenotype. Sum of squared differences (observed minus predicted day of median egg hatch) was reduced from 7,818 d^2 (North American parameters) to 178 d^2. Days of simulated median egg hatch differed from the observed days by 0–7 d ($\bar{x} = 0.2$; SD = 3.1). The pattern of simulated egg hatch closely mimicked the irregular pattern of observed egg hatch from the temperature regimes of our experiment. Egg hatch is arguably the most important life cycle event in gypsy moth population suppression/eradication interventions and in estimating their potential invasive range. The model parameters described here produce accurate predictions of Asian gypsy moth egg hatch.

Key words: Asian gypsy moth, phenology, egg hatch, diapause

The North American gypsy moth, *Lymantria dispar dispar* [Linnaeus] (Lepidoptera: Erebidae) (Pogue and Schaefer 2007), was accidentally released in 1868 or 1869 in Medford, MA, United States (Liebhold et al. 1989). It has gradually expanded its range north to New Brunswick, Canada, south to North Carolina, United States and west to Minnesota, United States and Ontario, Canada, where it has become a major forest and urban pest. Heavy defoliation by gypsy moth larvae can cause tree mortality and shifts in forest composition (Campbell and Sloan 1977); defoliation damages urban landscapes where the cost of control can be substantial (Bigsby et al. 2014) and the urticating larval hairs can pose a significant health hazard (Etkind et al. 1982). Isolated populations of gypsy moth have been detected in all of the contiguous states of the United States (National Agriculture Pest Information System [NAPIS 2018]) and all Canadian provinces (Canadian Food Inspection Agency 2009). The established populations in eastern North America are—likely without exception—progeny of the original Medford, MA population that originated from France. Populations in western North American have been either transplanted North American gypsy moth populations, or international introductions of one of the Asian gypsy moth subspecies, *L. d. asiatica* Vnukovskij or *L. d. japonica* (Motschulsky) native from Siberia to Japan and China. One of the important tools in the management of this invasive species is a phenology model to predict the timing of critical life cycle events.

Phenology is the study of the relationship between environmental factors (especially temperature, photoperiod, moisture, and nutrition) and cyclical/seasonal biological phenomena (e.g., flowering, insect emergence) (Schwartz 2003). Temperature is the strongest determinant for phenological development of poikilotherms and the only known determinant in the case of gypsy moth egg hatch (Leonard 1974). Predictions of phenology are an important component of many gypsy moth management and research activities. Interventions with biological or chemical agents to eradicate isolated populations of North American gypsy moth and Asian gypsy moth must be timed to coincide with the susceptible second-instar larvae, but empirical observations of phenology—useful in guiding the treatment timing—do not exist in areas without a history of a resident population. Potential range limits of North American gypsy moth and Asian gypsy moth under historical and future climate scenarios have been estimated by assessing climatic suitability, where climatic suitability is the likelihood that the intra- and interannual variations in temperature satisfy the seasonal requirements of the life cycle (Régnière and Nealis 2002, Gray 2004, Pitt et al. 2007, Régnière et al. 2009, Gray 2014). In a series of papers, Gray (2016a,

2016b, 2017) demonstrated how a phenology model can estimate pathway risk of Asian gypsy moth introductions via international maritime trade. All of these examples used the Gypsy Moth Life Stage (a.k.a. Gray/Logan/Sheehan) (*GLS*) phenology model (Gray 2004). But *GLS* has been properly parameterized only for the predominant phenotype of North American gypsy moth. A phenotype exists in all *L. dispar* subspecies—rare in the North American phenotype, but predominant in Asian gypsy moth—that is able to hatch after less exposure to cold temperature than the predominant phenotype of North American gypsy moth (Keena 2016).

Gypsy moth is a univoltine species throughout its range. Oviposition occurs in early to mid-summer (Leonard 1981). Embryos complete three developmental phases (prediapause, diapause, and postdiapause) in the egg stage. Embryos are initially more responsive to higher temperatures and complete the prediapause phase in approx. 16 d at 25°C or 48 d at 15°C (Gray et al. 1991). Well developed embryos then enter a diapause phase that typically lasts several months (Leonard 1968) and is more quickly terminated by exposure to cold temperatures (Masaki 1956, Pantyukhov 1964, Giese and Cittadano 1977). In the terminal postdiapause phase, embryos are again more responsive to higher temperatures (Gray 2009). Larvae emerge from the eggs in early spring, and mature adults appear by late summer to mate and oviposit the next generation (Leonard 1981).

The *GLS* model describes the entire gypsy moth life cycle by its individual life-stage component parts. The three phases of embryonic development (leading to egg hatch) are described by Gray et al. (1991, 2001) and Gray (2009). Post-hatch life stages are described by Logan et al. (1991) and Sheehan (1992). Timing of egg hatch is arguably the most critical life cycle event in the planning of population interventions because emergence of the susceptible second instar follows very soon after egg hatch (Leonard 1981). Timing of egg hatch is also, arguably, the most critical event in the estimation of potential range limits based on climatic suitability: in areas that are climatically suitable for population persistence, phenological progression in the egg stage must not be so rapid that eggs transition from the cold-hardy diapause phase until lethal freezing temperatures have ended, but eggs must hatch early enough that subsequent adult emergence, mating, and oviposition occur in time for eggs to reach diapause before the onset of the next winter (Gray 2004). Unfortunately, although timing of egg hatch may be the most important of the phenological predictions, *GLS* egg hatch has been properly parameterized for the North American gypsy moth only.

Differences among gypsy moth populations from different regions in their temperature-duration requirements for egg hatch were noted at least as early as 1932 (Goldschmidt 1932, 1943). In the first rigorous comparison of the temperature-duration requirements, Keena (1996) observed that the requirements were satisfied in an Asian gypsy moth population from the Russian Far East by fewer cold days (≤5°C) than required by a North American gypsy moth population from Massachusetts, United States. When cold-day exposures were sufficient for both populations, the Asian gypsy moth eggs hatched sooner than the North American gypsy moth eggs. The reduced temperature-duration requirement for Asian gypsy moth egg hatch was shown to be inherited via a single dominant gene that is not exclusively found in the Asian populations nor present in all Asian populations (Keena 2016). It is important to note that none of the aforementioned studies were able to ascribe the requirement differences to the diapause phase specifically, or to the diapause and postdiapause phases combined.

The egg hatch model of Gray et al. (1991, 2001) and Gray (2009) is the prevailing egg hatch model for gypsy moth. In the diapause phase of the model, the authors invoked a hypothetical enzyme-like agent to explain the highly variable observations from a complex experiment where the developmental responses to a given temperature varied over the duration of the phase. The hypothetical agent is more active (i.e., more strongly inhibitory) at high than at low temperature. It inhibits developmental progression and is more rapidly removed by colder temperatures. The agent does not need to be completely removed for diapause to be completed; but a reduction in agent concentration permits a higher developmental response to warmer temperatures. In this way, developmental response to high temperature becomes greater as the spring approaches. This phenomenon has been termed 'sensibilization' by Zaslavski (1988). According to this model, there is no specific number of 'cold days' to which an egg must be exposed for diapause completion. In the postdiapause phase of the model, the developmental response to temperature is also age-dependent and increases steadily with increasing age, but not uniformly across the range of temperatures.

The importance of Asian gypsy moth as a potential forest and urban pest in North America, and the ongoing threat of Asian gypsy moth introduction via international trade, make it vital to derive model parameters that can predict the phenological development leading to egg hatch of the Asian gypsy moth whose egg hatch requirements are satisfied with less cold exposure than North American gypsy moth. Here, we describe the results of our experiments to derive those parameters.

Materials and Methods

Egg masses of *L. d. asiatica* were collected from three sources. Twenty eggs masses (hereafter, the 'RM' strain) were collected from Mineralni, Russia (44.10 N, 133.15 E). Four egg masses were collected from Kraznoyarsk, Russia (56.03 N, 92.94 E) and randomly interbred with the RM strain (hereafter, the 'RR' strain). More than 100 egg masses (hereafter, the 'AA' strain) were collected from a ship in Vancouver, Canada, that had just arrived from the Russian port of Nakhodka (42.82 N, 132.88 E). All strains were reared for two generations before beginning the experiment.

Following the larval emergence of the third generation, larvae were reared in cohorts of 8–10 individuals at 25°C, 60% relative humidity and a 16:8 (L:D) h photoperiod. Adults were collected daily and paired randomly within the strain for mating. Egg masses were collected 10 d after mating. All egg masses were reared at 25°C for 27–32 d to ensure that prediapause was completed (Gray et al. 1991), but that virtually no diapause development would occur (Gray et al. 2001). Thirty-one treatments (Fig. 1) described below all began after the completion of prediapause.

Median diapause developmental rate in *GLS* is modeled as two interacting processes (Gray et al. 2001). One process is similar to the common temperature-dependent developmental rate paradigm ($rate = f(T)$) except that the realization of this potential developmental rate is inhibited by the presence of a hypothetical enzyme-like inhibiting agent. The strength of the inhibition at any given time and temperature is a product of the inhibiting agent concentration and its temperature-dependent activation level. The second process is the temperature-dependent depletion of the inhibiting agent. The actual developmental response to temperature in the diapause phase is a function of the inhibiting agent concentration, the activation level of the inhibiting agent, and the potential developmental rate (in the absence of inhibition). Median postdiapause developmental rate in *GLS* is modeled as a temperature- and age-dependent process (Gray 2009). Our experimental treatments of the RM strain were chosen

days / trt. # & population	1–14	15–30	31–44	45–50	51–55	56–57	58–60	61–62	63	64	65	66–74	75–90	91–93	94	95–96	97–98	99–104	105–120	121–150	151–180	181–210	211–hatch
1 RM (md)							7.5												25				
2 RM (md)	-5									7.5											25		
3 RM (val)	-15									7.5											25		
4 RM (md)	20									7.5											25		
5 RM (md)	25									7.5											25		
6 RM (val)		7.5	-5								7.5										25		
7 RM (md)		7.5	15								7.5										25		
8 RM (md)		7.5	20								7.5										25		
9 RM (md)		7.5	25								7.5										25		
10 RM (md)			7.5				-5							7.5							25		
11 RM (val)			7.5		15							7.5								25			
12 RM (md)			7.5		20						7.5									25			
13 RM (val)			7.5		25						7.5									25			
14 RM (md)				7.5						-5					7.5						25		
15 RM (md)				7.5					15					7.5						25			
16 RM (val)				7.5				20				7.5							25				
17 RM (val)				7.5				25				7.5							25				
18 AA (dis)													25										
19 AA (dis)		5												25									
20 AA (md)				5												25							
21 AA (md)							5												25				
22 AA (md)										5											25		
23 AA (md)											5											25	
24AA (md)												5											25
25 AA (val)												5											25
26 RR (dis)													25										
27 RR (dis)													20										
28 RR (md)													15										
29 RR (dis)				15												25							
30 RR (md)				10												25							
31 RR (val)				5												25							

Fig. 1. After completion of prediapause, samples of eggs were subjected to one of the 31 temperature treatments shown here. Eggs within each treatment were exposed to the temperatures of the treatment during the experimental days indicated on the top row. Observations of egg hatch from 18 randomly selected treatments were used for model development (md); eight treatments were used for model validation (val); five treatments were discarded (dis) due to low egg hatch.

to maximize the effect that different temperatures, applied for different durations at different times in the diapause and postdiapause phases, would have on the timing of egg hatch. The treatments of the RR and AA strains, and their related observations, were part of an experiment conducted for a different purpose by Keena (2016) and are well suited to further explore the timing of egg hatch under a variety of temperature regimes.

Thirty egg masses of the RM strain were randomly selected, and eggs were mixed together and divided into 17 more-or-less equal sized samples (>550 eggs each) required for the study. One sample of the RM eggs was then reared at 7.5°C for 90 d, followed by an incubation period at 25°C until egg hatch was completed (i.e., no further hatch had occurred for at least 30 d). The 16 remaining samples of RM eggs were also reared at 7.5°C for 90 d, but with an additional intervening exposure period of 14, 7, 5, 3 or 2 d to −5, 15, 20 or 25°C, followed by incubation at 25°C until egg hatch was complete. Exposure periods began on day 1, 31, 51, or 61 of the treatment regime (i.e., after completion of prediapause) (Fig. 1). Thirty egg masses of the AA and RR strains were randomly selected. Each egg mass of the AA strain was divided into eight more-or-less equal samples. One sample of the AA eggs was reared at 5°C for each of 0, 30, 60, 90, 120, 150, 180, or 210 d, followed by incubation at 25°C until egg hatch was complete. Each egg mass of the RR strain was divided into six more-or-less equal samples. One sample of RR eggs was reared at each of 15, 20, or 25°C until egg hatch was complete. One sample of RR eggs was reared at each of 5, 10, or 15°C for 60 d, followed by incubation at 25°C until egg hatch was complete. The temperature regimes of the 31 treatments are summarized in Fig. 1.

Egg hatch was monitored in each sample a minimum of three times per week, and as often as daily. Cumulative egg hatch was calculated from the observations within each treatment. Day of median egg hatch in each treatment was estimated by interpolation between the two successive observations that spanned 50% cumulative egg hatch. Insufficient egg hatch occurred in the AA samples reared at constant 25°C (treatment #18) or at 5°C for 30 d (treatment #19), in the RR samples reared at constant 25°C or constant 20°C (treatments #26 and #27, respectively), and in the RR sample reared at 15°C for 60 d (treatment #29) (Fig. 1). These five treatments were removed before analysis.

We used the observations of egg hatch from 18 randomly chosen treatments for model development (hereafter, model development treatments), and preserved the observations of egg hatch from the remaining eight treatments for model validation (hereafter, validation treatments) (Fig. 1). Egg hatch within each treatment was normalized to a uniform total to eliminate the bias that unequal sample sizes among treatments would create. All subsequent references to observed hatch refer to the normalized observations.

The two interacting processes in *GLS* diapause are described by three parameters:

1. *PotDiapRate*: the temperature-dependent potential developmental rate of diapause (i.e., developmental rate when the concentration of the inhibiting agent has been reduced to zero, or when the temperature renders the agent inactive);
2. *EFF_RES*: the temperature-dependent activation level of the inhibiting agent; and
3. Δ*INHIB*: the temperature-dependent depletion rate of the inhibiting agent.

Postdiapause in *GLS* is described by *PostdiapRate*: the age- and temperature-dependent postdiapause developmental rate.

We found the best Asian gypsy moth-specific parameter values for *PotDiapRate*, *EFF_RES*, Δ*INHIB*, and *PostdiapRate* by iterative searches of their respective potential parameter spaces. The initial search space was $^1/_4$–4 × the North American gypsy moth parameter values in 45 geometric increments (>4.1 mil combinations). The best combination of parameter values was chosen based on the

smallest sum of squared differences between observed and predicted day of median egg hatch. Each subsequent search began with the best combination of parameter values of the previous search and was of a progressively smaller search space with progressively more incremental steps: $^{2}/_{3}$ $^{3}/_{2}$ × the current best values in 45 geometric increments; $^{4}/_{5}$ $^{5}/_{4}$ × the current best values in 60 geometric increments (60^4 combinations); and $^{3}/_{4}$ $^{4}/_{3}$ × the current best values in 65 geometric increments (65^4 combinations). Further searches did not produce a smaller sum of squared differences.

Within-population developmental rate variability during the egg stage is modeled in *GLS* with a unique three-parameter cumulative Weibull function, $F(x) = 1 - \exp\left[-\left(\frac{x-\gamma}{\beta}\right)^{\alpha}\right]$, for each egg phase (prediapause, diapause, and postdiapause). The best Asian gypsy moth parameter values for developmental rate variability were found by iterative searches of the α, β, and γ parameter spaces in the diapause and postdiapause phases. The initial search space was ±0.5 × the North American gypsy moth values in 12 even increments (12^6 combinations). The best combination of α, β, and γ values for diapause and postdiapause was selected based on the smallest sum of squared differences between the observed cumulative egg hatch and the cumulative egg hatch predicted with the Asian gypsy moth rate parameters found above (*PotDiapRate*, Δ*INHIB*, *EFF_RES*, and *PostdiapRate*) and α, β and γ values for diapause and postdiapause. A second search was ±0.25 × the best values of the first search in 11 even increments. A third search did not produce a smaller sum of squared differences.

A validation test of the estimated Asian gypsy moth parameter values (*PotDiapRate*, Δ*INHIB*, *EFF_RES*, *PostdiapRate*, and α, β, and γ [for diapause and postdiapause variability]) was conducted by running *GLS* with the Asian gypsy moth-specific parameter values under the temperature regimes of each of the eight treatments reserved for model validation (Fig. 1). Cumulative predicted egg hatch was compared to cumulative observed egg hatch in each treatment.

Results

Model Development Treatments

A total of 25,597 eggs hatched from the 18 model development treatments. The first egg hatch (one egg) occurred in treatment #28 (RR, constant 15°C) 49 d after prediapause completion (Fig. 2). The last egg hatch occurred 224 d after prediapause completion in this same treatment. Ninety-five percent of the total observed egg hatch (2.5–97.5%) occurred 76–185 d after prediapause completion. Treatment conditions were considerably less favorable for development in some treatments than in others, producing several periods during which almost no hatch occurred (e.g., between day 120–130; day 135–155; day 160–185) (Fig. 2).

The North American gypsy moth values for *PotDiapRate*, Δ*INHIB*, *EFF_RES*, *PostdiapRate*, and α, β and γ (all egg phases) produced predictions of the day of median egg hatch that are 3–76 d ($\bar{x} = 14.3$; SD = 16.9) later than the observed day of median egg hatch (Fig. 3). One treatment (#30) could not produce simulated egg hatch: simulated eggs remained in diapause. The first search of the rate parameter space (*PotDiapRate*, Δ*INHIB*, *EFF_RES*, and *PostdiapRate*) reduced the sum of squared differences between observed and predicted day of median egg hatch from 7,818 d^2 (with the original North American gypsy moth parameters) to 178 d^2. Successive searches reduced the sum of squared differences

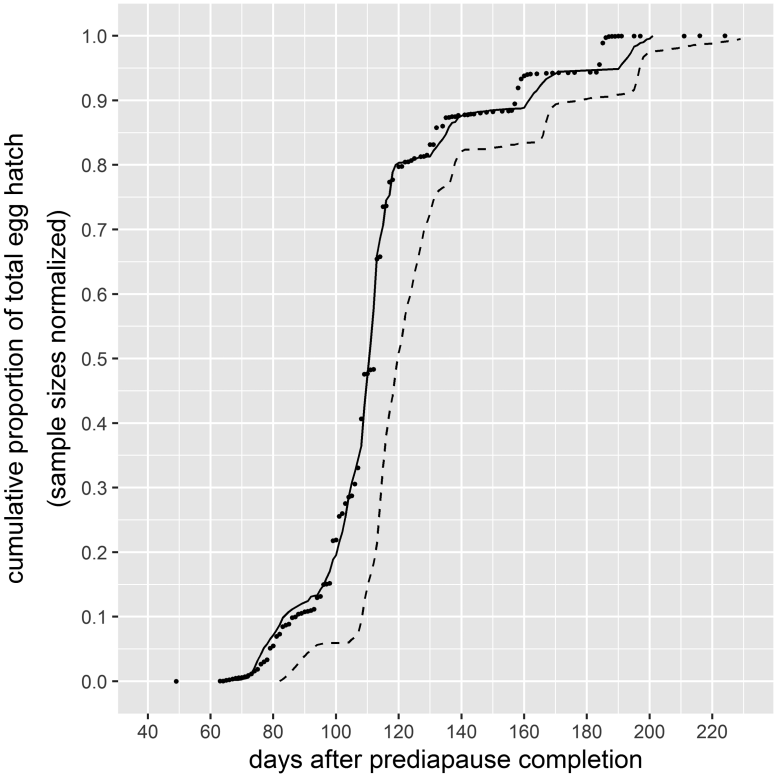

Fig. 2. Observed (●) and predicted cumulative proportion of total egg hatch in 18 model development treatments. Predictions shown by the dotted line were generated with the North American gypsy moth rate and α, β, and γ variability parameters. Predictions shown by the solid line were generated with the best *PotDiapRate*, Δ*INHIB*, *EFF_RES*, and *PostdiapRate* values and α, β, and γ variability parameters. Total hatch in each treatment has been standardized to eliminate bias from unequal sample sizes.

between observed and predicted day of median egg hatch to 170, 168, and 167 d^2. Days of median egg hatch predicted with the final Asian gypsy moth values for *PotDiapRate*, Δ*INHIB*, *EFF_RES*, and *PostdiapRate* differ from the observed days by 0–7 d ($\bar{x} = 0.2$; SD = 3.1) (Fig. 3). Successive searches of the diapause and postdiapause rate variability parameters (α, β and γ) reduced the sum of squared differences between observed and predicted cumulative egg hatch from 0.270 (with the North American gypsy moth values) to 0.073 and 0.059 (Fig. 2). Using the final Asian gypsy moth values for *PotDiapRate*, Δ*INHIB*, *EFF_RES*, and *PostdiapRate*, all treatments produced simulated egg hatch. The first simulated egg hatch occurred 63 d after prediapause completion; the last egg hatch occurred 192 d after prediapause completion. Ninety-five percent of the total simulated egg hatch (2.5–97.5%) occurred 75–192 d after prediapause completion.

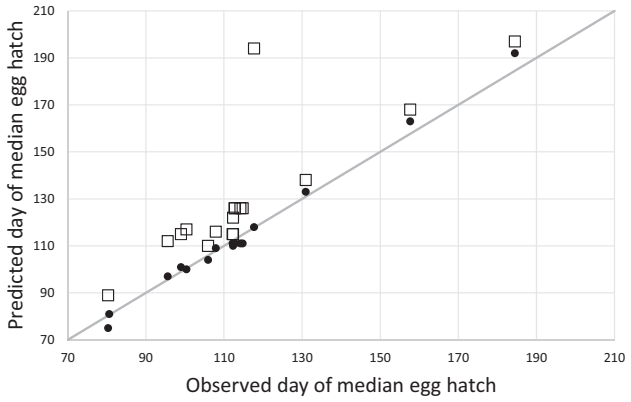

Fig. 3. Observed and predicted day of median egg hatch in 18 model development treatments: North American gypsy moth parameters (□) and the final Asian gypsy moth parameters (●).

Model Validation Treatments

A total of 7,721 eggs hatched from the eight validation treatments. The first egg hatch occurred in treatment #31, 67 d after prediapause completion (Fig. 4). The last ≈15% of total egg hatch occurred after a 95-d period during which there was no egg hatch in any of the validation treatments. This final 15% of total egg hatch occurred in treatment #25 where the temperature was maintained at 5°C for 210 d. Ninety-five percent of the total observed egg hatch (2.5–97.5%) occurred 70–217 d after prediapause completion. Total simulated egg hatch was ≈98% of the simulated population as a result of less than 92% egg hatch under treatment #13 conditions. Ninety-five percent of the total simulated egg hatch occurred 63–228 d after prediapause completion. The obvious divergence between observed and predicted egg hatch (between days 75–95; Fig. 4) is discussed below.

The best model parameters for Asian gypsy moth egg hatch suggest that the temperature-dependent potential developmental rate of diapause (*PotDiapRate*) is approx. 2× faster in Asian gypsy moth than in North American gypsy moth. The temperature-dependent activation level of the inhibiting agent (*EFF_RES*) is virtually the same in the two phenotypes, but the inhibiting agent is removed (Δ*INHIB*) >2× faster in Asian gypsy moth than in North American gypsy moth. The age- and temperature-dependent postdiapause developmental rate (*PostdiapRate*) is 1.8× faster in Asian gypsy moth than in North American gypsy moth. A complete description of the calculations of *PotDiapRate*, Δ*INHIB*, *EFF_RES*, and *PostdiapRate* is given in the Supp. Appendix. Rate variability functions (α, β, and γ)

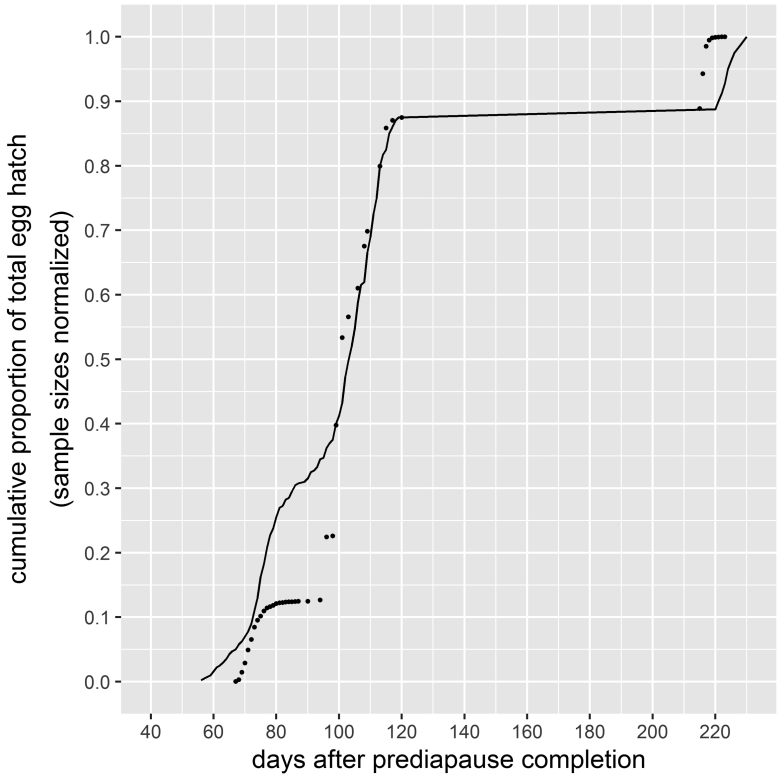

Fig. 4. Observed (●) and predicted (solid line) cumulative proportion of total egg hatch in eight model validation treatments. Predictions of egg hatch were generated with the best *PotDiapRate*, Δ*INHIB*, *EFF_RES*, and *PostdiapRate* values and α, β, and γ variability values. Total hatch in each treatment has been standardized to eliminate bias from unequal sample sizes.

for Asian gypsy moth diapause and postdiapause are also given in the Supp. Appendix.

Discussion

The most obvious discrepancy between observed and predicted egg hatch in the validation treatments occurred 75–95 d after prediapause completion during which just 2.5% of the total observed egg hatch occurred (cumulative proportion of total increased 0.1–0.125), but simulated egg hatch was 19% of total (cumulative proportion of total increased 0.15–0.34) (Fig. 4). This discrepancy is almost entirely attributable to treatments #13 and #17, with a small contribution from treatment #16. And the discrepancy is actually produced by a small (1–4 d) error in simulating the transition from diapause to postdiapause. A detailed examination of the simulated daily phenological progression in each treatment (results not shown here) showed that simulated eggs in treatments #13, #16, and #17 completed diapause in response to the first day of the 5-d exposure to 25°C, the 3-d exposure to 20°C, and the 2-d exposure to 25°C respectively. All these simulated eggs were thus able to progress quickly in postdiapause and hatch due to the remaining exposure to 20 or 25°C acting on the postdiapause phase. We manipulated the computer code of *GLS* to delay the diapause to postdiapause transition until the last day of exposure to 20 or 25°C: 4 d (treatment #13), 2 d (treatments #16), or 1 d (treatment #17). When simulated eggs did not transition from diapause to postdiapause in time to benefit from the remaining exposure to 25°C, simulated egg hatch in treatment #13 was delayed by 32 d (Fig. 5a), and simulated egg hatch in treatment #17 was delayed by 25 d (Fig. 5c). When simulated eggs in treatment #16 did not transition from diapause to postdiapause in time to benefit from the short exposure to 20°C, simulated egg hatch was delayed by 17 d (Fig. 5b). Simulated hatch of all the validation treatments (using the transition delays described above) is in Fig. 6. Thus, a small error (1–4 d) in predicting the time of transition from diapause to postdiapause produced the large error in predicted egg hatch. But this small error in predicting the transition from diapause to postdiapause will produce the large error in predicted hatch seen here only when the predicted diapause to postdiapause transition coincides with a dramatic, and unnatural, change in temperature.

Egg hatch is arguably the most critical life cycle event vis-à-vis the timing of the other life cycle events, the probability of introductions from international maritime traffic, and the probability that an introduction can lead to population establishment. Population suppression with a biocide application must target the most vulnerable second-instar larvae, which occurs only days after egg hatch, for maximum efficacy. Maritime vessels that may be carrying egg masses pose a threat of introduction if eggs hatch while the vessel is in port. Following an introduction, population establishment is possible only where the intra- and interannual variations in temperature satisfy the life cycle requirements; the timing of egg hatch has been shown to be a critical event in determining the climatic suitability of a location (Gray 2004).

The *GLS* phenology model has been used extensively to predict phenological events and to estimate probabilities of introduction and of establishment (Régnière and Nealis 2002, Gray 2004, Pitt et al. 2007, Régnière et al. 2009, Gray 2016a). However, the target organism has sometimes been Asian gypsy moth, a subspecies with a predominant phenotype for which the egg hatch model (Gray et al. 1991, Gray et al. 2001, Gray 2009) had not been parameterized. The work described here establishes a valid egg hatch model

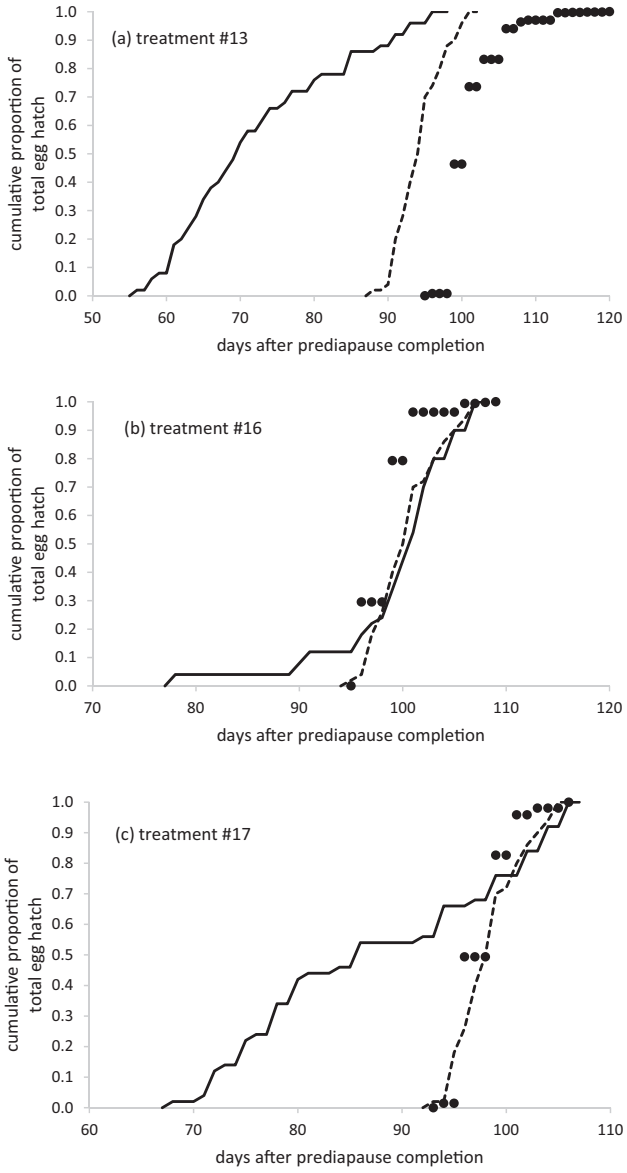

Fig. 5. Observed (●) and predicted (solid line) cumulative proportion of total egg hatch in treatments #13 (a), #16 (b), and #17 (c). Delaying simulated transition from diapause to postdiapause by 4 d (#13), 2 d (#16), or 1 d (#17) would result in egg hatch predictions shown by the dotted line.

for the dominant *L. dispar asiatica* phenotype. Egg hatch predictions by *GLS* with Asian gypsy moth phenotype rate and variability values closely mimicked observations of egg hatch in 18 temperature regimes used for model development (Fig. 2). There was strong agreement between observed and simulated egg hatch in the eight temperature regimes reserved for model validation when simulated diapause to postdiapause transition was delayed by 1–4 d in three treatments (Fig. 6). Ninety-five percent of total observed egg hatch occurred 76–185 d (18 model development treatments) (Fig. 4) or 70–217 d (eight model validation treatments) after prediapause completion (Fig. 6). Ninety-five percent of total simulated egg hatch occurred 75–192 d (model development treatments) (Fig. 4) or 73–227 d (model validation treatments with delayed 1–4 d delay in diapause to postdiapause transition in three treatments) (Fig. 6) after prediapause completion. Predictions of introduction probability (as

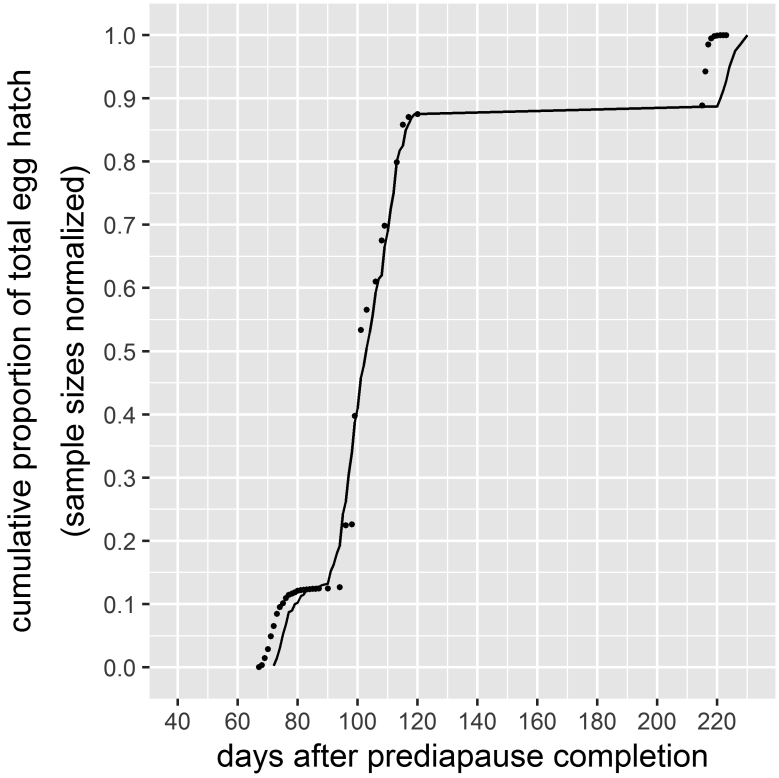

Fig. 6. Observed (●) and predicted (solid line) cumulative proportion of total egg hatch in eight model validation treatments when simulated transition from diapause to postdiapause was delayed to occur after exposure to 20 or 25°C: 4 d (treatment #13), 2 d (treatment #16), or 1 d (treatment #17).

per the methodology of Gray 2016b, 2017) and establishment (by estimating climatic suitability; Gray 2004) in areas at risk to Asian gypsy moth can be made with greater confidence.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

References Cited

Bigsby, K. M., M. J. Ambrose, P. C. Tobin, and E. O. Sills. 2014. The cost of gypsy moth sex in the city. Urban For. Urban Greening 13: 459–468.

Campbell, R. W., and R. J. Sloan. 1977. Forest stand responses to defoliation by the gypsy moth. For. Sci. Monograph. 19: 34.

Canadian Food Inspection Agency. 2009. Plant Protection Survey Report, pp. 107. http://publications.gc.ca/site/archivee-archived.html?url=http://publications.gc.ca/collections/collection_2017/acia-cfia/A101-11-2009-eng.pdf. Accessed 17 May 2019.

Etkind, P. H., T. M. Odell, A. T. Canada, S. K. Shama, A. M. Finn, and R. Tuthill. 1982. The gypsy moth caterpillar: a significant new occupational and public health problem. J. Occup. Med. 24: 659–662.

Giese, R. L., and M. L. Cittadano. 1977. Relationship of the gypsy moth to the physical environment. II. Diapause. Staff Paper Series No. 6. Department of Forestry, University of Wisconsin, Madison, WI. pp. 13.

Goldschmidt, R. 1932. Research on the genetics of geographic variation V.: analysis of diapause as an adaptation characteristic. Wilhelm Roux' Archiv für Entwicklungsmechanik der Organismen. 126: 674–768.

Goldschmidt, R. 1943. Lymantria. Bibliographia Genetica. 11: 1–186.

Gray, D. R. 2004. The gypsy moth life stage model: landscape-wide estimates of gypsy moth establishment using a multi-generational phenology model. Ecol. Model. 176: 155–171.

Gray, D. R. 2009. Age-dependent postdiapause development in the gypsy moth (Lepidoptera: Lymantriidae) life stage model. Environ. Entomol. 38: 18–25.

Gray, D. R. 2014. Unwanted spatial bias in predicting establishment of an invasive insect based on simulated demographics. Int. J. Biometeorol. 58: 949–961.

Gray, D. R. 2016a. Risk reduction of an invasive insect by targeting surveillance efforts with the assistance of a phenology model and international maritime shipping routes and schedules. Risk Anal. 36: 914–925.

Gray, D. R. 2016b. Climate change can reduce the risk of biological invasion by reducing propagule pressure. Biol. Invasions. 19: 913–923.

Gray, D. R. 2017. Risk analysis of the invasion pathway of a known forest invader. Biol. Invasions 19: 3259–3272.

Gray, D. R., J. A. Logan, F. W. Ravlin, and J. A. Carlson. 1991. Toward a model of gypsy moth egg phenology: using respiration rates of individual eggs to determine temperature-time requirements of prediapause development. Environ. Entomol. 20: 1645–1652.

Gray, D. R., F. W. Ravlin, and J. A. Braine. 2001. Diapause in the gypsy moth: a model of inhibition and development. J. Insect Physiol. 47: 173–184.

Keena, M. A. 1996. Comparison of the hatch of *Lymantria dispar* (Lepidoptera: Lymantriidae) eggs from Russia and the United States after exposure to different temperatures and durations of low temperature. Ann. Entomol. Soc. Am. 89: 564–572.

Keena, M. A. 2016. Inheritance and world variation in thermal requirements for egg hatch in *Lymantria dispar* (Lepidoptera: Erebidae). Environ. Entomol. 45: 1–10.

Leonard, D. E. 1968. Diapause in the gypsy moth. J. Econ. Entomol. 61: 596–598.

Leonard, D. E. 1974. Recent developments in ecology and control of the gypsy moth. Annu. Rev. Entomol. 19: 197–229.

Leonard, D. E. 1981. Bioecology of the gypsy moth, pp. 9–29. *In* C. C. Doane and M. L. McManus (eds.), The gypsy moth: research toward integrated pest management. USDA Tech. Bull. 1584. United States Department of Agriculture, Forest Service, Newtown Square, PA.

Liebhold, A. M., V. C. Mastro, and P. Schaefer. 1989. Learning from the legacy of Leopold Trouvelot. Bull. Entomol. Soc. Am. 35: 20–22.

Logan, J. A., P. A. Casagrande, and A. M. Liebhold. 1991. Modeling environment for simulation of gypsy moth (Lepidoptera: Lymantriidae) larval phenology. Environ. Entomol. 20: 1516–1525.

Masaki, S. 1956. The effect of temperature on the termination of diapause in the egg of *Lymantria dispar* Linne (Lepidoptera: Lymantriidae). Jpn J Appl. Zoology. 21: 148–157.

National Agriculture Pest Information System (NAPIS). 2018. Survey status of gypsy moth - *Lymantria dispar* (all years). Purdue University. http://pest.ceris.purdue.edu/map.php?code=ITAXAIA&year=alltime. Accessed 10 April 2019.

Pantyukhov, G. A. 1964. The effect of low temperatures on different populations of the brown-tail moth *Euproctis chrysorrhoea* (L.) and the gypsy moth *Lymantria dipsar* (L.) (Depidoptera: Orgyidae). Entomological Reviews. 43: 47–55.

Pitt, J. P. W., J. Régnière, and S. Worner. 2007. Risk assessment of the gypsy moth, *Lymantria dispar* (L), in New Zealand based on phenology modelling. Int. J. Biometeorol. 51: 295–305.

Pogue, M. G., and P. W. Schaefer. 2007. A review of selected species of *Lymantria* Hubner [1819] (Lepidopter: Noctuidae: Lymantriinae) from subtropical and temperate regions of Asia, including the descriptions of three new species, some potentially invasive to North America. United States Department of Agriculture, Forest Service, Washington, DC. pp. 221.

Régnière, J., and V. Nealis. 2002. Modelling seasonality of gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), to evaluate probability of its persistence in novel environments. Can. Entomol. 134: 805–824.

Régnière, J., V. G. Nealis, and K. B. Porter. 2009. Climate suitability and management of the gypsy moth invasion into Canada. Biol. Invasions 11: 135–148.

Schwartz, M. D. 2003. Introduction, pp. 1–5. *In* M. D. Schwartz (ed.), Phenology: an integrative environmental science. Kluwer Academic Publishers, Boston, MA.

Sheehan, K. A. 1992. User's guide for GMPHEN: gypsy moth phenology model. General Technical Report INT-GTR-336. United States Department of Agriculture, Forest Service, Washington, DC. pp. 29.

Zaslavski, V. A. 1988. Insect development. Photoperiods and temperature control, Springer-Verlag, Berlin, Germany.