Environmental Entomology, 46(4), 2017, 1012–1023
doi: 10.1093/ee/nvx111
Advance Access Publication Date: 4 July 2017
Research

Physiological Ecology

Effects of Temperature on Development of *Lymantria dispar asiatica* and *Lymantria dispar japonica* (Lepidoptera: Erebidae)

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Subject Editor: Kelly Johnson

Received 18 January 2017; Editorial decision 1 June 2017

Abstract

Periodic introductions of the Asian subspecies of gypsy moth, *Lymantria dispar asiatica* Vnukovskij and *Lymantria dispar japonica* Motschulsky, in North America are threatening forests and interrupting foreign trade. Although Asian gypsy moth has similar morphology to that of European and North American gypsy moth, it has several traits that make it a greater threat, the most important being the flight capability of females. Asian gypsy moth is not yet established in North America; however, infestations have been detected multiple times in Canada and the United States. To facilitate detection and eradication efforts, we evaluated the effect of a range of temperatures on development time, survivorship, and fertility of eight populations of Asian gypsy moth. There were significant impacts of temperature and population on these life history characteristics. The larval developmental rate increased with temperature until it reached an optimum at 29 °C. Larvae experienced significant molting problems at the highest and lowest temperatures tested (10 °C and 30 °C). At 30 °C, female fitness was markedly compromised, as evidenced by reduced fecundity and fertility. This suggests that development and survival of Asian gypsy moth may be limited by summer temperature extremes in the Southern United States. We also determined the degree-day requirements for two critical life stages and two populations of Asian gypsy moth, which represent the extremes in latitude, to predict the timing for biopesticide application and adult trap deployment. Our data will benefit pest managers in developing management strategies, pest risk assessments, and timing for implementation of management tactics.

Key words: Asian gypsy moth, temperature, development time, degree-day

Temperature has significant impacts on survival, growth, development, fecundity, spread, and migration of ectotherms, such as insects (Beck 1983, Walther et al. 2009). Temperature influences insects' basic physiological processes, such as metabolic and respiratory rates, as well as functions of the nervous and endocrine systems (Neven 2000). There is increasing interest in investigating insect responses to changing temperature to determine their success under climate change senarios, shifts in distribution, and phenology predictions. The majority of insects are well adapted to their native range and have evolved several mechanisms, such as diapause or behavioral adaptations, to survive under extreme thermal conditions, but they may struggle to survive in a new environment to which they are not adapted. The recent rise in mean annual temperatures associated with global climate change and frequent introductions of exotic species owing to trade and other human activities have increased the likelihood of establishment of exotic insects (Huang et al. 2011, Ponti et al. 2014). Exotic species can become pests that cause substantial disturbances to forest and agricultural ecosystem, threaten biodiversity, and can inflict significant economic impacts.

The gypsy moth (*Lymantria dispar* L.) found in North America is an exotic species that originated from Western Europe (Wu et al. 2015). The larval stage of gypsy moth is a serious forest pest capable of completely defoliating numerous tree species throughout the northern hemisphere (Elkinton and Liebhold 1990). This polyphagous pest can consume >300 host species (Liebhold et al. 1995), although it prefers oaks (*Quercus* spp.) over other species (Raupp et al. 1988, Shields et al. 2003, Foss and Rieske 2003). This subspecies of gypsy moth, introduced into Medford, MA, in 1869, is expanding its range at the rate of 6–18 km/yr (Tobin et al. 2007). It is currently found as far south as North Carolina, as far west as Wisconsin (U.S. Department of Agriculture–Animal and Plant Health Inspection Service [USDA-APHIS] 2015), and has established populations in the southern parts of Canada east to Lake Superior (Régnière et al. 2009). Since 1970, gypsy moth has defoliated 81 million acres of forest (Gypsy Moth Digest 2013). The more recent incursion of two other subspecies of *L. dispar*, collectively known as Asian gypsy moth (*Lymantria dispar asiatica* Vnukovskij and *Lymantria dispar japonica* Motschulsky), threatens establishment of these subspecies in North America and many other parts of the world (Matsuki et al. 2001, Pitt et al. 2007).

Lymantria dispar asiatica is found east of the Ural Mountains in Russia, throughout China and Korea, and in parts of Japan, whereas *L. dispar japonica* occurs in patches on all islands in Japan (Pogue and Schaefer 2007). In North America, an infestation of an Asian subspecies was first found in British Columbia in 1991 (Bogdanowicz et al. 1993, Savotikov et al. 1995), but an immediate eradication effort removed the pest successfully. Since then, Asian gypsy moth has been detected and eradicated on multiple occasions and is thus far not known to have established in North America.

Asian gypsy moth is considered a greater threat to forest and agricultural resources than their European counterparts because of some distinct behavioral and ecological characteristics. Unlike the European subspecies, Asian gypsy moth females are capable of sustained flight (Wallner et al. 1995, Keena et al. 2008). They have a broader reported host range, including more coniferous species (Baranchikov and Sukachev 1989). Asian gypsy moths are attracted to lights, including well-lit port areas, where they oviposit on cargo and ships (Wallner et al. 1995), thus causing delays at ports of entry for regulatory disinfestation and affecting international trade. Eggs of the Asian subspecies from some geographic areas also require shorter chilling to be ready to hatch (Keena 1996, 2015). These characteristics may increase their potential to establish in new areas, increase their dispersal capability, and result in extensive damage to multiple forest types. Also, the Asian and European subspecies can successfully hybridize and the initial hybrids of European and Asian gypsy moth are able to glide varying distances, indicating better dispersal capabilities in hybrid offspring than the nonflying European parent (Keena et al. 2001). Therefore, the introduction of the Asian subspecies in areas where the European subspecies is already present could make limiting the spread of gypsy moth difficult, requiring more aggressive management strategies than currently employed.

Despite efforts to intercept and eradicate Asian gypsy moth, the pest has been introduced into new areas through international trade (Gibbons 1992, Matsuki et al. 2001, U.S. Department of Agriculture [USDA] 2015). A recent study on potential global distribution using ecological niche modeling suggests that Asian gypsy moth is capable of occupying almost all temperate regions of the world (Peterson et al. 2007). However, survival and establishment of the Asian subspecies in a new area depend on several factors. For example, larval development is influenced by temperature, foliage suitability, geographical origin, larval density, and sex (Hough and Pimentel 1978, Campbell 1978, Casagrande et al. 1987, Honěk 1996, Jarošík and Honěk 2007). Because insects are ectothermic, temperature is likely the primary driving factor influencing larval gypsy moth development rate (Johnson et al. 1983, Casagrande et al. 1987). Studies on the effect of temperature on European subspecies exist, but none on Asian subspecies. Predicting phenology of the Asian gypsy moth is critical for monitoring and management. For instance, *Bacillus thuringiensis kurstaki* (*Btk*), a preferred treatment for Asian gypsy moth control, is most effective if applied to the early second instar (Reardon et al. 1994). Currently, phenology of the gypsy moth is predicted based on the European subspecies and by climate matching, which may not be accurate for the Asian subspecies.

In the laboratory, we reared eight geographical populations of Asian gypsy moth at five constant temperatures to quantify the effects of temperature on the development rate of the larvae and determine degree-day requirements for each instar and stage. Because Asian gypsy moth is not an established pest in North America, major efforts are made to prevent its introduction through international trade, and, if an infestation is detected using pheromone traps, its geographical extent is delimited and triggers immediate eradication measures. Temperature-dependent effects on development of this pest can be used to develop phenology models to predict timing for biopesticide application and trap deployment. Further, this study will assist development of improved risk assessments for this pest around the world and help predict the potential distribution of this pest should it become established in new areas.

Materials and Methods

Gypsy Moth Populations

Six geographical populations of *L. d. asiatica* (C1, C2, C3, R1, R2, and Sk) and two populations of *L. d. japonica* (J1 and J2) from a broad range of latitudes were selected for this study to capture potential variation in development rates between these two subspecies and among populations (Table 1). Voucher specimens for each population were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT.

Larval Rearing at Different Temperatures

Ten egg masses from each population were placed individually in 150- by 15-mm sterile petri dishes (model 351058, Falcon, Tewksbury MA) and held inside a water box at 25 °C and 60% relative humidity (RH) with a photoperiod of 16:8 (L:D) h to initiate hatch. The eggs were placed in environmental chambers at five constant temperatures—10°C, 15°C, 20°C, 25°C, 30 °C—with a photoperiod of 16:8 (L:D) h; humidity in these chambers was 70 ± 5%, 70 ± 10%, 80 ± 5%, 60 ± 5%, and 45 ± 5% RH, respectively. Once >80% of larvae hatched from each egg mass, individual larvae were transferred to 30-ml clear plastic cups (model 9051, Frontier Agricultural Sciences, Newark, DE) with paper tab lids (model 9049, Frontier Agricultural Sciences, Newark, DE); each cup contained 15 ml of high wheat germ artificial diet (Bell et al. 1981). The diet was made with Wesson salt mix without iron and with 2 g of amorphous ferric phosphate added per liter of prepared diet (Riedel-deHaen AG, Germany) to provide a source of iron that is bioavailable to gypsy moth (Keena 2005). Rearing was done on artificial diet instead of host plant material to avoid confounding effects of variations in plant quality on larval development.

Ten larvae from each of the 10 egg masses per population were selected randomly and assigned to one of the temperature

Table 1. Approximate location (latitude and longitude), designation of the source population, collection date, and laboratory generation of gypsy moth evaluated in this study, arranged by latitude from north to south

Population	Country	Collection location	Collection date[a]	Laboratory generation	Latitude	Longitude	Plant hardiness zone[b]
R1	Russia	Shira, Khakassi	Aug. 1994	30	54.41° N	90.00° E	2
C1	China	Harbin, Heilongjiang	Aug. 2012 (Oct. 2012)	3	45.78° N	126.61° E	4
R2	Russia	Mineralni, Primorski	Aug. 1992	30	44.10° N	133.15° E	5
C3	China	Sandeli, Liaoning	Sept. 2011 (Oct. 2012)	4	41.51° N	122.37° E	6
C2	China	Yanzikou, Beijing, Takizawa, Morika, Nishine	Aug. 2011 (Oct. 2012)	4	40.32° N	116.15° E	7
J1	Japan	Hachimantai City, Northern Iwate District, Honshu	Oct. 2005(Nov. 2014)	15	39.73° N	141.08° E	7a
Sk	Korea	Samhwa-Dong, Gangovon-Do	Aug. 2009 (Nov. 2014)	9	37.49° N	129.06° E	6
J2	Japan	Nagoya, Honshu	Mar. 1996	28	35.15° N	137.08° E	8b

[a] Date in parentheses indicates when the population was received at the Ansonia quarantine facility.

[b] Plant hardiness zones according to the Davis Landscape Architecture.

treatments, ensuring a sample size of 100 larvae per population per temperature. However, at 30 °C, populations J2 and C2 had unexpectedly poor survival, so we added some larval replicates with neonates 21–25 d later. Egg masses used for the additional larvae originated from the same sources as the failed replicates, held at 10 °C until use.

Manipulations of the insects were always carried out under sterile conditions in a laminar flow hood to prevent contamination. Individual larvae were reared in the same 30-ml container until they reached the fourth instar, when they were transferred to 118-ml clear plastic containers (Model ME4, Solo Cup Company, Lake Forest, IL) with a paper tab lid to provide more space and were supplied with 40 ml of artificial diet. Larvae were provided with fresh diet as needed until they pupated. Molting, survival, diet condition, disease, and molting problems were recorded daily. If a larva was unable to shed its head capsule or exuviae within 2–4 d after molting began, it was declared dead. Larvae with this condition always abstained from feeding and died of apparent starvation.

Once fully sclerotized, pupae were sexed and weighed, then transferred individually to 236-ml paper cups (U508N, Solo Cup Company, Lake Forest, IL) covered with a plastic bag (15 by 15 cm) held in place with a rubber band. Pupae were held until adult eclosion at the temperature at which they were reared and checked daily for adult emergence. Once adults eclosed, they were weighed within the first 24 h and any abnormalities were noted.

The 10 °C treatment was ended after 120 d because a large number of larvae died as early instars and development was so slow that they would not have reached adulthood in nature before lethal winter temperatures. To obtain the sex of the surviving larvae, therefore, these insects were transferred to 25 °C to complete development.

Statistical Analyses

Statistical analyses were performed using SAS 9.3 (SAS Institute Inc. [2011], Cary, NC). PROC UNIVARIATE was used to assess the distributional fit of the data. Normality of the data was evaluated using the Shapiro–Wilk and the Anderson–Darling tests. Graphical plots were also examined to evaluate the distribution of the data.

Temperature, population, and their interactions were examined for effects on survivorship of *L. d. asiatica* and *L. d. japonica* to the pupal and adult stages. The proportion of larval survival was calculated for each egg mass within each population at each temperature (15°C, 20°C, 25°C, and 30 °C). Survival at 10 °C was excluded from this analysis because, as explained earlier, a large number of larvae died as early instars and larval development rate was too slow to expect pupation and survival in nature before the onset of winter. The fixed effects of temperature, population, and their interactions on survival from one life stage to the next were evaluated using a generalized linear model via PROC GLIMMIX with maximum likelihood technique. The model was fitted using a beta distribution with logit link function. Because the limits of the beta distribution are zero and one, 0% survival was coded as 0.000001 and 100% survival as 0.999999. The Tukey–Kramer post hoc analysis was used to determine differences among means at $\alpha = 0.05$. Residuals were also evaluated for normality and homogeneity of variance.

To determine if the responses of the Asian populations to temperature could be categorized into distinct groups, the HPSPLIT procedure was used to build a separate classification tree for each tested temperature using individuals from all populations. Duration of the first instar was used as the predictor variable at 10 °C, and total duration of the larval and pupal stages was used at the remaining temperatures. Entropy was used in the GROW statement to set the splitting criteria, cost-complexity in the PRUNE statement to specify the pruning method, and the SEED value was set at 100. A maximum of two branches per node was allowed, and the number of nodes was set at the value that minimized the average squared error in the cost-complexity cross-validation analysis in an initial unlimited run. This provided the smallest tree that adequately categorized the data. Based on this analysis, the two populations that represented the extremes of variation in response to temperature across all temperatures were used to determine the upper and lower developmental temperature thresholds and degree-day requirements.

PROC GLIMMIX was used to evaluate the fixed effects of temperature, population, sex, and their interactions on the duration of each instar and development time from hatch to pupa. To evaluate effects on pupal and adult body weight (g), the fixed effect of ultimate instar was added to the model. Duration of development to adult was evaluated with the fixed effects of temperature, population, ultimate instars, and their interactions. The model was fitted to a negative binomial distribution with a log link function because the response variables had long right tails. Differences among means were determined by the Tukey–Kramer post hoc analysis at $\alpha = 0.05$. For each model, residuals were evaluated for normality, and the homogeneity of variance was assessed by Levene's test.

Only the developmental time from egg to second instar and from egg to adult are presented in the results section because these are the critical stages of the gypsy moth life cycle when treatment and monitoring should be initiated, respectively. Information on the other instars and stages is provided in Supp. Tables (online only). The relationship between temperature and pupal weight by population (only for the two populations that represented the extremes), sex, and ultimate instar was fitted to Lysyk model using PROC NLIN and Marquardt convergence method (Table 2).

We determined the degree-day requirements for the onset of the second instar and adult eclosion. The relationship between temperature and rate of development from hatch to the second instar by population (R1 and C2) was estimated using a linear model. Two nonlinear (Shi/performance and Logan) and a linear model were used to evaluate the relationship between temperature (excluding 10 °C) and development rate to reach the adult stage by population (R1 and C2) and by sex. Mathematical models used to fit the relationship between developmental rate and temperature are presented in Table 2. PROC REG was used to examine the linear model in SAS, and the two nonlinear models were fitted using MATLAB R2015a (Mathworks 2001). These relationships were used to calculate the lower temperature thresholds ($T_{\min}$) for the development of R1 and C2 larvae to the second instar, and the lower ($T_{\min}$), upper ($T_{\max}$), and optimum temperature (T_o) for development of the R1 and C2 populations to adulthood by sex.

Degree-days (DD) required for development from egg hatch to second instar by population and to adult by population and sex were estimated using the function: $DD = [\text{constant temperature} - T_{\min}] \times Dt$, where Dt is the total number of days required by individual larvae to reach the second instar or to reach adulthood. The relationships between accumulated degree-days and cumulative proportion of individuals to reach second instar or adult were estimated using the Gompertz function, $P = \exp\,[-\exp\,(-b * DD + a]$, with the Marquardt convergence method. Accumulated degree-days required by 10%, 50%, 90%, and 95% of the R1 and C2 larvae to reach the second instar and to reach the adult stage were calculated.

To evaluate the effect of temperature and population on Asian gypsy moth fitness, we first examined the proportion of insects that was unable to eclose normally, as well as the proportion with adult deformity, by egg mass for each population at each temperature (15°C, 20°C, 25°C, and 30 °C). We also examined effects of temperature (25°C and 30 °C) and population on fertility. Fertility was determined by counting the number of embryonated and unembryonated eggs produced by each female. Because survival of larvae at 30 °C was poor and the majority of adults reared at this temperature were deformed, few matings of moths reared at this temperature were possible. Therefore, because we were unable to rear more larvae to adult at 30 °C and we wanted to evaluate the effects of 30 °C on fertility, we compared moths reared and mated at 25 °C with those reared at 25 °C until pupation and transferred to 30 °C for the duration of the pupal stage, adult emergence, and mating. A minimum of 20 single-pair matings per population per temperature was included in the fertility test.

We estimated overall performance of moths reared from each egg mass by population at each temperature. The estimated overall performance was defined as mean weight gain per day multiplied by survival, and calculated for each population and temperature as: $P_{\mathrm{surv}} \times \mathrm{rate} \times P_{\mathrm{wt}}$, where P_{surv} is the proportion of larvae reared from each egg mass surviving to the pupal stage, rate is their mean developmental rate (1 per d) to pupation, and P_{wt} is their mean pupal weight (g; Lee et al. 2006). PROC GLIMMIX with maximum likelihood estimation technique was used to evaluate fixed effects of temperature, population, and their interactions on estimated performance of Asian gyspy moth populations at 15°C, 20°C, 25°C, and 30 °C. The model was fitted to a beta distribution with a logit link function. The Tukey–Kramer post hoc analysis (at $\alpha = 0.05$) was used to determine differences among means. Residuals were also evaluated for normality and the homogeneity of variance.

Results

Survival

The mean percentage of larvae that survived to the pupal ($F = 3.27$; df = 21, 288; $P < 0.0001$; Fig. 1a) and adult stages ($F = 4.74$; df = 21, 288; $P < 0.0001$; Fig.1b) were significantly affected by the temperature × population interaction. Mean percentage survivorship until pupation for the C1, C2, J1, J2, and Sk populations was significantly higher at 15°C, 20°C, and 25 °C than at 30 °C. There were no significant differences among populations at 15°C, 20°C, and 25 °C, except for the Japanese populations at 15 °C, which had significantly lower survival to pupation than the R2 population at 15°C and 20 °C.

Similarly, no significant differences in the mean proportion of survivors to adult were evident within a population at 20°C and 25 °C, but there was significantly higher mean percentage survivorship at both of these temperatures than at 30 °C. Some populations struggled more at 30 °C than others and some populations struggled to survive to adulthood at 15 °C as well. For example, ≤13% (mean 9.89 ± 2.9%) of J2 survived to adulthood at 15 °C, which was significantly lower than the survival of larvae from the remaining populations at this temperature. However, the larvae of some Chinese populations (C1 and C3) survived well at the lower temperature, and their survival at 15 °C was not significantly different from that at 20°C and 25 °C.

Development Time From Hatch to Second Instar and to Adult Emergence

The criteria used, the developmental time (D1 and DA) criteria for splitting at each node, and the resulting percentage of individuals from each population in each node of a classification tree at four different temperatures are given in Supp. Table 1 (online only). Each temperature classification tree had three to four nodes, with the first node representing the fastest development and the last node the slowest (for example, <44 d and ≥73 d to complete the first instar at 10 °C). Overall, at five temperatures, a greater proportion of R1 individuals consistently completed development earlier than the rest of the populations, while a greater proportion of C2 individuals completed development more slowly than the rest of the populations. R1 and C2, therefore, were selected to represent the extremes of development time for the tested populations; thus, most of the results presented and discussed below focus on these two populations. Information for development time spent in different life stages of all populations is provided in Supp. Tables 2–9 (online only).

As a main effect, the mean number of days spent in each life stage was significantly different among instars ($F = 14451$; df = 9, 12073; $P < 0.001$), e.g., the number of days spent in the first instar was significantly lower than the number of days spent in the ultimate larval instar. Therefore, development time was compared only within each instar or stage. The mean number of days from larval hatch to second instar was affected by the interactions of temperature, population, and sex ($F = 1.90$; df = 66, 2569; $P < 0.0001$; Table 3). There was no significant difference in development time between populations R1 and C2 and between sexes within a temperature. However, when compared across temperatures, both populations developed significantly faster to the second instar at temperatures >10 °C. There was no significant difference in development time to the second instar between populations or sexes

Table 2. Mathematical models used to describe relationships between temperature and developmental rate of Asian gypsy moths

Model	Function[a]	Reference
Linear regression[b]	$r = aT + b$	
Logan[c]	$r = a\left(\exp(cT) - \exp\left(cT_{\max} - \left(\frac{T_{\max}-T}{b}\right)\right)\right)$	Logan et al. (1976)
Shi/performance[d]	$r = c(1-\exp(-a(T-T_{\min})))*(1-\exp(b(T-T_{\max})))$	Shi et al. (2011)
Lysyk[e]	$y = a(T-T_{\min})(T_{\max}-T)$	Lysyk (2001)

[a] For each function, r represents the rate of development and T represents holding temperature. $T_{\min}$ and $T_{\max}$ represent minimum and maximum temperature thresholds, respectively.

[b] The parameters a and b in a linear regression represent slope and intercept of a line, respectively.

[c] In the Logan model, "a" represents the developmental rate at a base temperature above the lower developmental threshold, "b" is the high temperature boundary layer, and "c" is the rate increase to optimum temperature.

[d] In the Shi/performance model, parameters a and b represent rate of increase and rate of decrease compared with optimum temperature, and c is a constant.

[e] In the Lysyk model, y represent pupal weight parameters and a is an empirical constant.

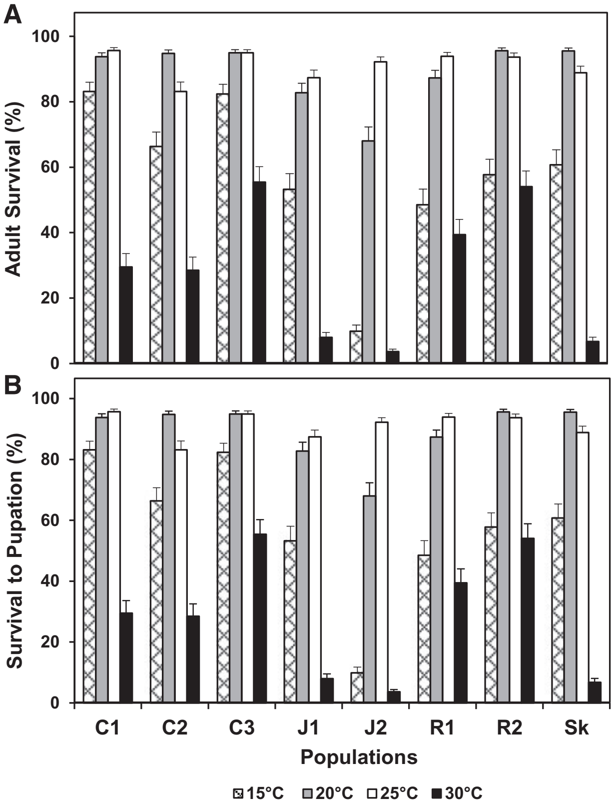

Fig. 1. Percentage (mean ± SE) survivorship of *L. dispar asiatica* neonates to the pupal stage (**b**) and to adult (**a**) reared at four constant temperatures. The mean percentage survivorship of larvae to pupae ($F = 3.27$; df = 21, 288; $P < 0.0001$) and adult stages ($F = 4.74$; df = 21, 288; $P < 0.0001$) were significantly affected by the temperature and population interaction. Error bars represent the 95% confidence interval.

among the other temperatures (15°C, 20°C, 25°C, and 30 °C), except for R1 males, which when reared at 30 °C developed significantly faster than all other populations at 15°C and 20 °C of both sexes.

Similarly, the mean number of days from egg hatch to adult was affected by the interaction of temperature, population, and ultimate instar attained ($F = 1.98$; df = 66, 2019; $P < 0.0001$; Table 4). Larvae completed development to adulthood significantly faster as temperature increased. At 15 °C, larvae completed development to adulthood more slowly than at higher temperatures regardless of population and ultimate instar attained. However, development time at 25°C and 30 °C was not significantly different between populations. Larvae pupated after various numbers of instars, ranging from four to eight, but most C1 underwent six instars, while R1 most commonly reached only five instars. Seven and eight instars occurred rarely and only at 15°C and 30 °C, temperatures at which survival was lower. R1 was the only population to pupate after four instars. R1 completed development faster than C2.

Rate of Larval Development and Estimated Degree-Day Requirements

A linear relationship was evident between temperature and development rate for larvae from hatch to second instar in both R1 ($F = 1862$; df = 1, 455; $P < 0.0001$) and C2 ($F = 6716$; df = 1, 479; $P < 0.0001$; Table 5; Fig. 2a); in general, larvae developed faster as temperature increased. Lower temperature thresholds calculated from this relationship indicated that the first instar of C2 has a slightly lower temperature threshold (7.77 °C) than that of R1 (8.51 °C). A significant relationship between development rate to adult eclosion and temperature was seen in all three models (Linear, Shi/performance, and Logan). Lower and upper developmental temperature thresholds obtained from different models were comparable. As an example, the lower temperature thresholds for male C2 estimated from linear and Shi/performance models were 7.10°C and 7.24 °C, respectively. The models also indicated that C2 larvae had a lower temperature threshold than R1. However, the upper temperature thresholds for larvae of these two populations were comparable. Also, the larval temperature thresholds for both sexes were similar.

R1 required fewer degree-days than C2 to reach the second instar, and the difference in DD requirements increased with the proportion of the population reaching that stage (Table 6; Fig. 3a). To determine accumulated degree-days to reach adulthood by population and sex, we used the lower thresholds produced by the Shi/performance model (Table 5; Fig. 2b). R1 reached the adult stage with fewer degree-days than C2 (Table 6; Fig. 3b). Also, females had higher degree-day requirements than males (Table 6).

Pupal and Adult Body Weight of *L. d. asiatica*

Mean pupal weights (g) were significantly affected by interaction of temperature, population, sex, and ultimate instar attained ($F = 3.73$; df = 120, 2379; $P < 0.0001$; Fig. 4). Mean pupal weights of males were not significantly different from each other as a function of temperature, population, and ultimate instar; however, variations in

Table 3. Mean [± SE (*n*)] time spent (d) by *L. dispar asiatica* in the first instar at different temperatures by sex and population

Sex	Population	Temp (°C)				
		10	15	20	25	30
F	R1	50.9 ± 1.91a (14)	14.4 ± 0.57b (42)	7.7 ± 0.41b (46)	4.9 ± 0.31bc (49)	4.2 ± 0.57bc (14)
F	C2	66.6 ± 4.73a (4)	14.7 ± 0.48b (63)	9.0 ± 0.39b (57)	6.1 ± 37bc (49)	4.9 ± 0.78bc (9)
M	R1	51.7 ± 1.86a (15)	13.5 ± 0.50b (54)	7.4 ± 0.40b (46)	4.9 ± 0.34bc (48)	3.9 ± 0.40c (32)
M	C2	78.33 ± 5.13a (3)	16.5 ± 0.73b (31)	10.0 ± 0.52b (37)	6.5 ± 0.42bc (36)	5.7 ± 0.51bc (23)

Means followed by a different letter are significantly different from each other at $P < 0.05$ using Tukey–Kramer post hoc test. Sample size (*n*) is the number of survivors.

Table 4. Mean [± SE (*n*)] time spent (d) by *L. dispar asiatica* from egg hatch to adult at different temperatures, by population, and ultimate instar attained

Population	Ultimate instar	Temp (°C)			
		15	20	25	30
C2	5	110.5 ± 3.4 ab (10)	74.1 ± 1.5 cd (36)	52.2 ± 1.3 de (35)	47.9 ± 3.1 def (5)
C2	6	120.1 ± 1.5 a (60)	78.2 ± 1.3 c (55)	54.0 ± 1.1 de (45)	52.4 ± 1.7 de (18)
C2	7	137 ± 11.7 a (1)	NA	NA	59.5 ± 5.5 cde (2)
C2	8	NA	NA	NA	71.0 ± 6.0 cd (2)
R1	4	NA	58.3 ± 5.5 cde (2)	36.3 ± 2.0 fg (9)	30.9 ± 2.8 g (4)
R1	5	98.3 ± 1.5 b (46)	61.0 ± 0.9 cd (71)	39.6 ± 0.7 fg (81)	34.7 ± 1.1 g (27)
R1	6	112.9 ± 7.5 ab (2)	72.0 ± 6.0 cd (2)	44.8 ± 4.7 defg (2)	40.8 ± 2.9 efg (5)
R1	7	NA	NA	NA	44.4 ± 6.6 defg (1)

Means followed by different letters are significantly different from each other at $P < 0.05$ using Tukey–Kramer post hoc test. Sample size (*n*) is the number of survivors.

Table 5. Parameter values for linear and nonlinear models used to describe the relationship between temperature (°C) and developmental rate of Asian gypsy moth life stages by population and sex

Development period	Temp range (°C)	Population	Sex	Model	a	b	c	$T_{\min}$	$T_{\max}$	T_{o}[a]	*n*	$_{\text{Adj}}R^2$
Days in first instar	10–30	C2	Both	Linear	0.0093	−0.072	NA	7.77	NA	NA	455	0.80
	10–30	R1	Both	Linear	0.0120	−0.102	NA	8.51	NA	NA	479	0.93
Days to adult	15–25	C2	M	Linear	0.0011	−0.008	NA	7.10	NA	NA	89	0.96
	15–25	C2	F	Linear	0.0010	−0.007	NA	7.05	NA	NA	148	0.96
	15–25	R1	M	Linear	0.0016	−0.015	NA	8.97	NA	NA	107	0.97
	15–25	R1	F	Linear	0.0015	−0.013	NA	8.95	NA	NA	104	0.97
	15–30	C2	M	Shi	0.002	2.33	0.558	7.24	30.7	28.9	112	0.93
	15–30	C2	F	Shi	0.0009	2.86	1.10	7.13	30.6	29.1	157	0.95
	15–30	R1	M	Shi	0.001	2.52	1.75	9.02	30.8	29.2	133	0.95
	15–30	R1	F	Shi	0.001	2.63	1.53	9.01	30.7	29.2	119	0.95
	15–30	C2	M	Logan	0.0028	0.087	0.077	NA	30.7	NA	112	0.92
	15–30	C2	F	Logan	0.0025	0.124	0.078	NA	30.14	NA	157	0.96
	15–30	R1	M	Logan	0.0027	0.145	0.091	NA	30.18	NA	133	0.96
	15–30	R1	F	Logan	0.0025	0.095	0.090	NA	30.11	NA	119	0.96

See methods for details of the analysis.

[a] T_{o} represents the optimum temperature for development calculated from the Shi/performance model.

pupal weights were observed among females. Within each temperature, pupal weight was not significantly affected by population or by number of instars attained. However, differences were observed within populations when female weights were compared across temperatures. For example, female C2 reared at 25 °C that attained six instars were significantly heavier than C2 females reared at 15 °C that attained seven instars and C2 females reared at 30 °C that attained eight instars (Supp. Table 10 [online only]). Females were considerably heavier than males; however, at 15°C and 30 °C, female weights were more comparable with males. The relationship between temperature and pupal weight for each sex and population is shown in Fig. 4 (see Table 7 for model fit). The $T_{\min}$ and $T_{\max}$ temperatures for the C2 and R1 populations range between 9°C and 11 °C and 34–36 °C, respectively. Temperature had a stronger effect on female than male pupal weights, and the C1 population tended to be heavier near the optimum temperature for both sexes.

The interaction between populations, temperature, and sex was not significant ($F = 0.57$; df = 51, 1943; $P < 0.99$), but an interaction between temperature and sex did significantly affect mean adult weight ($F = 116$; df = 7, 1999; $P < 0.0001$; Fig. 5). Adult females were significantly heavier than males. Male weight was not affected by temperature, but females reared at 20°C or 25 °C were significantly heavier than females reared at 30°C or 15 °C.

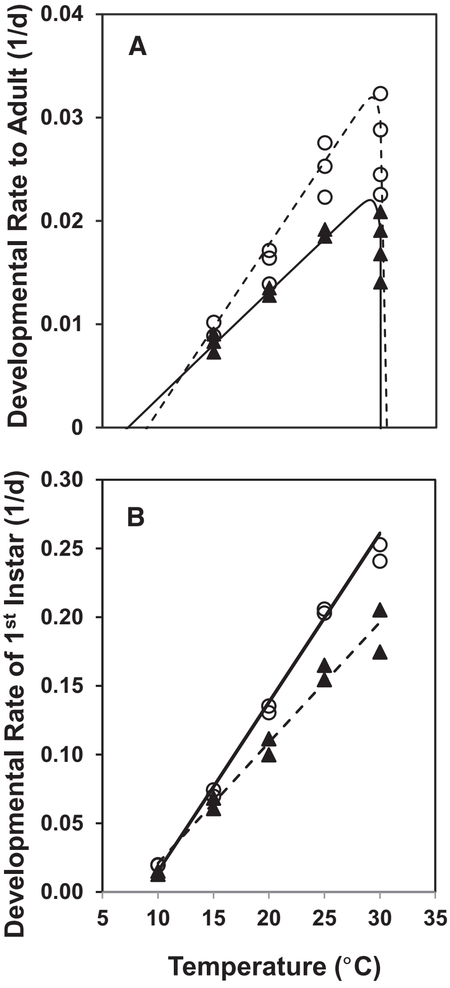

Fig. 2. Developmental days to second instar (**b**) and to adult (**a**) of *L. dispar asiatica* larvae from the R1 and C2 populations in response to constant temperatures. The solid and dashed lines represent R1 and C2, respectively, and were fitted to linear regression (b) and Shi/performance models (a). Data points at each temperature represent mean developmental rate of each sex (b) and by ultimate instar (a), open circles for the R1 population, and solid triangles for the C2 population (See methods for detail).

Effect of Temperature and Population on Pupal and Adult Fitness

Both pupal mortality and proportions of deformed adults were affected by temperature, especially at 15 °C but sometimes also at 30 °C. Pupae that failed to eclose often appeared normal, but some had attached exuvia or had very short or missing wing pads. Adult abnormalities included wings that failed to expand properly or stuck exuvia. Pupal mortality was significantly affected by the interaction between temperature and population ($F = 4.16$; df = 21, 280; $P < 0.0001$; Table 8). Within the C1, C2, C3, and R2 populations, the proportion of pupae that eclosed was similar across temperatures (15°C, 20°C, 25°C, and 30 °C). However, temperature significantly affected pupal mortality within the J1, J2, R1, and Sk populations. Within temperature, no difference in the proportion of pupae that died was evident among populations at 20°C and 25 °C. However, at 15 °C, significantly more J2 pupae died than did pupae from the C1, C3, Sk, and R2 populations. At 30 °C, J1, J2, and Sk also suffered significantly more pupal mortality than C1, C2, and R1.

The proportion of deformed adults was also significantly affected by the interaction between temperature and population ($F = 1.81$; df = 21, 271; $P < 0.0181$; Table 8). All populations had a significantly higher proportion of deformed adults when reared at 15 °C compared with 25 °C, except R2. R2 deformity was not affected by rearing temperature. The proportions of deformed adults were similar across populations at 20°C, 25°C, and 30 °C.

The estimated overall performance of Asian gypsy moth was significantly affected by an interaction of temperature and population ($F = 4.50$; df = 21, 280; $P < 0.0001$; Table 8). In general, the estimated performance at 20°C and 25 °C was not different among populations, except R1, R2, and J2 had significantly better performance at 25 °C than at 20 °C. Performance at 15°C and 30 °C was consistently poorer than at 20°C and 25 °C across all populations. Although most populations performed equally poorly at the highest and coldest temperatures, J2 had significantly reduced performance at the highest temperature.

An interaction of temperature and population affected the mean proportion of embryonated eggs produced by females at 25°C and 30 °C ($F = 2.61$; df = 6, 280; $P < 0.0177$; Supp. Table 11 [online only]). When moths were reared at 25 °C until pupation and transferred to 30 °C for emergence and mating, the mean percentage of their embryonated eggs was significantly lower compared with eggs produced by females reared only at 25 °C. The proportion of embryonated eggs produced by females reared at 25 °C did not differ across populations. However, R1 produced a significantly greater proportion of embryonated eggs compared with other populations held at 25°C then at 30 °C. In contrast, no eggs became

Table 6. Estimated accumulated degree-days (±SE) required to reach second instar and adult emergence by *L. d. asiatica* larvae from egg hatch for 10%, 50%, 90%, and 99% of the population

Life stage reached	Population	Sex	Degree-day (±SE) requirement for larvae (%) to reach different life stages after egg hatch				R^2_{Adj}
			10%	50%	90%	99%	
Second instar	R1	Both	68.97 ± 0.47	81.49 ± 0.10	101.1 ± 1.69	125.7 ± 3.68	0.98
Second instar	C2	Both	78.58 ± 1.33	106.0 ± 0.04	150.7 ± 1.96	205.4 ± 4.47	0.99
Adult	R1	M	564.1 ± 2.31	619.3 ± 0.12	705.8 ± 3.95	813.8 ± 8.73	0.99
Adult	R1	F	607.3 ± 1.88	657.4 ± 0.45	736.1 ± 4.13	834.2 ± 8.72	0.99
Adult	C2	M	858.3 ± 4.75	936.6 ± 0.23	1060 ± 8.04	1213 ± 17.8	0.98
Adult	C2	F	912.5 ± 1.98	979.6 ± 0.22	1084 ± 3.68	1216 ± 7.99	0.99

Lower temperature thresholds are presented in Table 5 for each life stage, population, and sex. The lower temperature threshold for adults was obtained from Shi/performance model. R^2_{Adj} value is based on the relationship between degree-days and cumulative proportion to adulthood using the Gompertz function, $P = \exp[-\exp(-b * DD + a]$.

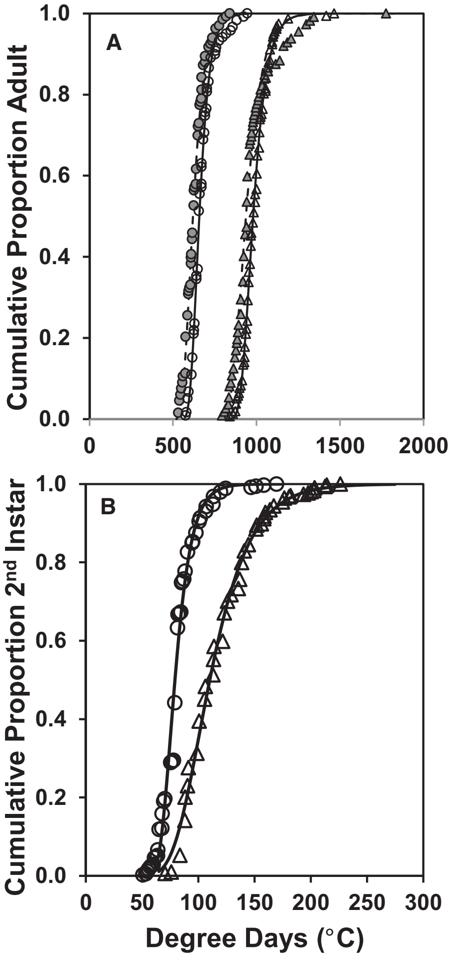

Fig. 3. Cumulative proportion of *L. dispar asiatica* R1 and C2 individuals to reach the second instar reared at 10 °C, 15 °C, 20 °C, 25 °C, and 30 °C (**b**) and to complete development to adult reared at 15 °C, 20 °C, 25 °C, and 30 °C (**a**) over accumulated degree-days. Circles represent R1 and triangles represent C2 individuals (ab). Open markers for females and solid for males (a). Solid lines were fitted to cumulative proportion of individuals (b) and of female and dotted lines to males (a) using Gompertz function, $P = \exp[-\exp(-b * DD + a]$.

embryonated when females were reared and mated at 30 °C. Females produced an average of 303.8 ± 71.96 eggs; 61.8% of these eggs remained unembryonated, while the rest were only partially embryonated.

Discussion

Temperature had a significant effect on the life history of Asian gypsy moth regardless of geographical origin. As expected, temperature significantly affected survivorship, development rate, and fitness, as evidenced by effects on moth fertility. Developmental rate increased with temperature from 10°C to 25 °C for most populations and instar or stages, and declined at 30 °C. Survival to adult declined at 15°C and 30 °C compared with 20°C and 25 °C. At 30 °C, fitness and fertility were also negatively affected. The variable response to temperature among the populations of Asian gypsy moth was expected because insects are adapted to their local thermal environment (Honěk 1996, Roy et al. 2015, Porcelli et al. 2016). Our results show that populations from colder plant hardiness zones (two to five) tended to develop faster than those from

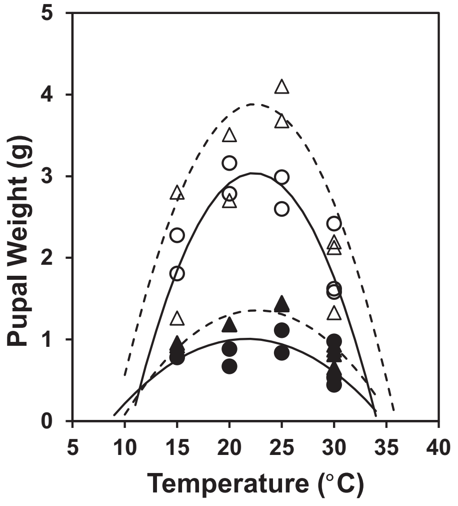

Fig. 4. Body weight (mean ± SEM) of male and female *L. dispar asiatica* pupae reared at different constant temperatures. Different data points represent the ultimate instars within each sex (open markers female and solid markers male) for two populations of *L. dispar asiatica*, circles for the C2, and triangles for the R1. Solid lines were fitted to female weight and dotted lines to male weight using the function: $y = a\,(T - T_{\min})\,(T_{\max} - T)$ (Lysyk 2001).

Table 7. Parameter values for Lysyk models used to describe the relationship between temperature (°C) and pupal weight (g) of Asian gypsy moth by population and sex

Temp range (°C)	Population	Sex	a	$T_{\min}$	$T_{\max}$	n	$_{\text{Adj}}R^2$
15–30	C2	M	0.0080	9.62	35.6	126	0.43
15–30	C2	F	0.0214	8.98	35.9	175	0.32
15–30	R1	M	0.0058	8.52	34.8	177	0.26
15–30	R1	F	0.0220	10.6	34.1	150	0.52

See methods for details of the analysis.

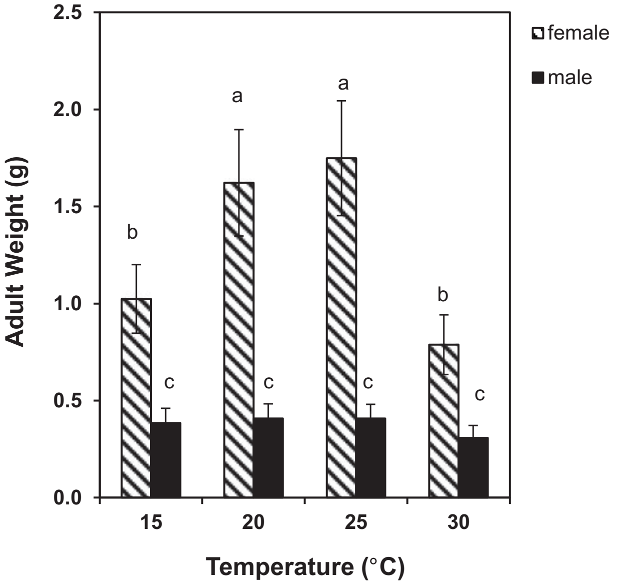

Fig. 5. Body weight (mean ± SEM) of male and female *L. dispar asiatica* adults reared at different constant temperatures. Different letters above bars represent significant differences at $P < 0.05$ using Tukey–Kramer post hoc test.

Table 8. Percentage (mean ± SE [n]) of *L. dispar asiatica* pupal mortality, deformed adults, and estimated overall performance by temperature

Fitness index	Population	Temp (°C)			
		15	20	25	30
Pupal mortality	C1	19.1 ± 5.70defgh (15)	9.03 ± 2.88fgh (4)	7.22 ± 2.33gh (2)	6.76 ± 2.29gh (4)
	C2	37.0 ± 9.01abcdef (23)	8.05 ± 2.59fgh (3)	8.15 ± 2.62fgh (5)	9.56 ± 3.05fgh (5)
	C3	15.1 ± 4.65efgh (12)	6.01 ± 1.95h (0)	6.56 ± 2.12h (1)	19.6 ± 5.85defgh (11)
	J1	37.0 ± 9.02abcdef (19)	9.12 ± 2.92fgh (4)	10.5 ± 3.33fgh (5)	74.8 ± 7.11ab (14)
	J2	81.5 ± 5.55a (65)	26.2 ± 7.31cdefgh (20)	7.22 ± 2.33gh (2)	72.3 ± 9.80abc (4)
	R1	50.1 ± 9.75abcde (48)	15.9 ± 4.89efgh (17)	10.3 ± 3.26fgh (5)	11.3 ± 3.57fgh (9)
	R2	33.2 ± 8.52bcdefg (40)	9.20 ± 2.94fgh (7)	10.4 ± 3.30fgh (6)	24.8 ± 7.03cdefgh (12)
	Sk	29.0 ± 7.85bcdefgh (26)	8.09 ± 2.60fgh (4)	8.21 ± 2.64fgh (6)	67.3 ± 10.1abcd (16)
Adult deformity	C1	63.8 ± 9.30abcdef (47)	16.3 ± 5.04g (15)	9.42 ± 3.00g (2)	18.8 ± 6.02efg (9)
	C2	67.8 ± 8.69abcd (51)	44.6 ± 10.1bcdefg (33)	20.0 ± 6.04efg (17)	48.1 ± 10.2bcdefg (11)
	C3	65.5 ± 9.04abcde (50)	13.8 ± 4.31g (11)	11.7 ± 3.71g (5)	34.3 ± 9.02cdefg (20)
	J1	79.3 ± 6.21abc (39)	32.2 ± 8.68cdefg (21)	29.9 ± 8.26defg (16)	49.1 ± 12.2abcdefg (5)
	J2	89.6 ± 3.67a (9)	49.1 ± 10.2abcdefg (34)	19.8 ± 5.99efg (18)	7.9 ± 4.56g (0)
	R1	73.1 ± 7.67abcd (32)	29.7 ± 8.23defg (15)	16.1 ± 4.97g (12)	27.1 ± 7.71defg (12)
	R2	35.7 ± 9.22cdefg (24)	18.5 ± 5.63fg (11)	15.8 ± 4.90g (11)	12.5 ± 3.92g (8)
	Sk	83.6 ± 5.05ab (52)	43.6 ± 10.0bcdefg (32)	13.8 ± 4.31g (10)	31.0 ± 10.9cdefg (7)
Estimated performance	C1	17.8 ± 2.14a (96)	34.9 ± 3.03a (94)	50.2 ± 3.622a (97)	13.1 ± 1.91ab (51)
	C2	24.3 ± 2.52a (94)	43.3 ± 3.37a (94)	63.4 ± 4.05ab (85)	7.39 ± 1.31bc (32)
	C3	17.2 ± 2.10a (90)	36.0 ± 3.07a (94)	61.8 ± 4.01ab (95)	22.1 ± 2.40bd (67)
	J1	14.0 ± 1.88a (73)	34.5 ± 3.01a (83)	50.2 ± 3.62a (81)	9.04 ± 1.47ac (26)
	J2	14.9 ± 1.95a (75)	44.2 ± 3.40a (92)	65.3 ± 4.11ab (91)	1.96 ± 0.68d (7)
	R1	18.0 ± 2.15a (96)	39.9 ± 3.23a (92)	70.6 ± 4.27ab (97)	14.3 ± 1.90ab (46)
	R2	23.1 ± 2.45a (99)	45.4 ± 3.45a (100)	73.8 ± 4.36b (95)	23.8 ± 2.49b (66)
	Sk	20.2 ± 2.29a (89)	48.9 ± 3.57a (96)	66.6 ± 4.15ab (89)	15.3 ± 2.36ab (33)

Means followed by different letters are significantly different at $P < 0.05$ (Tukey–Kramer post hoc test). Sample size (*n*) is the number of survivors.

warmer zones (seven to eight). Also, the population from the coldest zone, R1, tolerated both the lower and upper temperatures evaluated better than the other populations.

Each insect has an optimum range of temperatures in which normal functioning of their physiological systems is achieved. Temperature influences protein structure, enzymatic activies, cell membrane structure, which ultimately affect basic physiological functions in insects (Neven 2000). Outside of their normal thermal range, insects experience thermal-stress, which include negative impacts on their performance such as growth and development, reproduction, and longevity (Piyaphongkul et al. 2012). Extreme thermal conditions can be fatal to insects if irreversible physiological changes occur. Developmental abnormalities such as wing deformity and impaired abdomen segmentation have been reported owing to high temperature exposure for *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), which directly influenced fitness (Roberts and Feder 1999). In another study, *Helicoverpa punctigera* Wallengren (Lepidoptera: Noctuidae) experienced changes in wing shape as the indicator of cold stress (Hoffmann et al 2002). We also observed wing and abdominal deformities in pupae and adults of Asian gypsy moth at 15°C and 30 °C in our study. Larvae experienced problems during molting at the highest and lowest temperatures tested (10°C and 30 °C), leading to high mortality at these temperatures. Overall survivorship to pupa and adult stages was greatest at 20°C and 25 °C and lowest at 30 °C. At 15 °C, larvae often successfully reached the pupal stage; however, a significant proportion of pupae failed to eclose to adult. Of those that did eclose, a large number were deformed, indicating that although the moth can complete development at lower temperatures, higher temperatures are required for normal adult emergence. This may reflect stage-specific tolerance to low temperature, suggesting that the larval stage may be more cold tolerant than the pupal stage (Kingsolver et al. 2011). This also highlights the importance of phenological synchrony of different life stages with the appropriate season for successful establishment outside of the native range.

Taken together, our findings suggest that Asian gypsy moth populations may struggle in regions where temperatures stay >30°C or below 15 °C after egg hatch, but the degree of the impact will depend on the population. Based on our results on survivorship and deformity, the Japanese population appeared to struggle more at extreme temperatures (15°C and 30 °C) than the Chinese and Russian populations.

Across the populations tested, Asian gypsy moth caterpillars required 55–85 d at 10 °C and 4–6 d at 30 °C to reach second instar. Similarly, the larvae completed development to adult after a significantly longer period at 15 °C than at higher temperatures. Some variation across populations was evident when developmental times to adult were compared. The Russian R1 population developed the fastest and the Chinese C2 population the slowest. Given the cold hardiness zones in the United States, R1 may be more cold tolerant than C2 and should survive minimum temperatures throughout North America, except for certain locations in Alaska and Northern Canada, whereas C2 may be limited in its ability to tolerate minimum temperatures across the same range (Table 1). Although insects tend to be adapted to local thermal conditions (Honěk 1996, Roy et al. 2015), the Russian population from the coldest climate tolerated the highest temperature better than the Chinese population that was from a much warmer climate. Females from this Russian population (R1) also have the highest proportion of strong fliers (Keena et al. 2008) and a portion of their eggs have lower thermal requirements for egg hatch (Keena 2015). In our study, R1 females produced a significantly greater proportion of embryonated eggs compared with other populations held at 25°C than at 30 °C. Thus, the R1 population may be resilient enough to establish outside its native range, spread rapidly, and potentially cause greater damage than the other Asian populations evaluated. Although the Russian (R1 and R2) and a Japanese (J2) population have been reared in the laboratory for an extended period of time (28–30 generations) on artificial diet, which could alter the performance of the moth compared with other tested populations (Grayson et al. 2015), our laboratory routinely maintains genetic diversity in these populations by starting each generation with a mixture of 100 egg masses from each population and harvesting nearly 100% of the pupae each generation. In addition, quality control records for these populations show that the speed of development has remained stable over time (M. K., unpublished data).

The Asian gypsy moth populations developed to the second instar at a rate similar to the North American gypsy moth population at 15°C, 20°C, 25°C, and 30 °C (Casagrande et al. 1987). However, at lower temperatures, the North American population appears to develop faster to second instar than the Asian gypsy moth. At 10 °C, the fastest growing Asian gypsy moths (R1) required >50 d to reach second instar, while the North American population took only 38.06 ± 3.43 (mean ± SD) d. However, at 20°C, 25°C, and 30 °C, the R1 Russian population that pupated after four instars completed development to adult faster than is reported for the North American population, while the C2 Chinese population developed slower. Thus, it appears that detection of Asian populations may require expanding the normal trapping season used for the North American populations to both earlier and later dates than is standard now. However, care should be taken when extrapolating laboratory data based on constant temperatures and artificial diet to natural environments, as many other factors can influence development, such as host foliage and topography. Also, the larvae from the North American population used in this comparison were reared on white oaks instead of artificial diet, which would also affect developmental rates.

As expected, the rate of development of Asian gypsy moth larvae increased with rearing temperature until it reached the upper temperature threshold for development. The upper and lower temperature thresholds of Asian gypsy moth are predicted to be 30–31 °C and 7–9 °C, respectively. Variations in lower and upper temperature thresholds by population were minor. All of the models used in this study predicted similar temperature thresholds for Asian gypsy moth by life stage and population.

Despite several introductions since 1991, Asian gypsy moth is not yet established in North America. Thus, the focus of Asian gypsy moth management practices have been on monitoring and eradication procedures. In this study, we determined timing for the second instar and adult stages because these are critical stages for monitoring and control treatments. Therefore, degree-day requirements to reach these stages were determined to predict the timing for control and monitoring activities. The fastest and slowest developing populations were selected for degree-day calculation to represent the range of variation evident in the tested populations. Degree-day estimations were determined for each sex separately, even though we did not observe significant differences in development time by sex within any temperature and population. However, despite insignificant differences, we acknowledge that male Asian gypsy moth usually completed development a few days ahead of females. For example, after egg hatch, at 30 °C, the first male and female R1 emerged after 29 and 32 d, while C2 emerged after 45 and 48 d, respectively. The pattern of early male emergence was consistent across all holding temperatures, and the sex differences between first emergence dates increased at lower temperatures. Therefore, during monitoring for flight and application of pheromone traps, the degree-day model for males should be used to ensure capture of early emerging male moths.

Asian gypsy moth populations had a variable number of molts when reared at different temperatures, and the effects also varied by sex. In general, females pupated at the sixth instar and males pupated at the fifth; however, males had four to seven instars and females five to eight instars. The variation in the total number of molts by sex is in alignment with North American gypsy moths (Casagrande et al. 1987). The only population with individuals that could pupate at the fourth instar was the R1 from Russia. The moths tended to complete their development faster when they pupated at earlier rather than later instars. This suggests that the ability to pupate in an earlier instar may be an adaptation that enabled R1 to develop faster than the other populations. Additionally, during the study supernumerary molts (seven or eight) were observed only at 15°C and 30 °C, which could indicate thermal stress and abnormal functioning of endocrine systems at those temperatures (Neven and Rehfield 1995, Neven 2000). Generally, the larvae that underwent the unusual seventh or eighth molt before pupation tended to be smaller and darker in color, which has been reported for other insects that are experiencing thermal stress.

Female Asian gypsy moth fitness was compromised when rearing was at 15°C and 30 °C. These females had reduced pupal and adult weights compared with those reared at 20°C and 25 °C. The lower weights correlate with lower fecundity and reduced fitness at these temperatures. Sublethal effects from heat stress during the juvenile or adult stage can reduce reproductive performance in insects (Porcelli et al. 2016). A single hot day is reported to stress diamondback moth (*Plutella xylostella* (L.), Lepidoptera: Plutellidae) females and reduce their fecundity and fertility (Zhang et al. 2013). We found that female Asian gypsy moths produced fewer eggs when reared at 30 °C compared with 25 °C; these eggs were also unembryonated or only partially embryonated with little chance for eclosion. We observed that when reared at 30 °C or exposed to 30 °C as a pupa, there were detrimental effects on fertility. These findings suggest that Asian gypsy moth development and survival may be limited by hot, prolonged summer temperatures in the southern United States. Also, at lower temperatures (10 °C), Asian gypsy moth populations developed very slowly, indicating these subspecies have a lower probability of establishing in areas where the temperature stays <10 °C during the gypsy moth larval development time frame. However, global warming has resulted in distributional shifts in organisms, and many species have expanded their geographical range to higher latitudes and altitudes (Bale et al. 2002, Hughes 2000). Increases in summer temperatures and longer spring and summer seasons may present an opportunity for Asian gypsy moth to expand its range toward Northern Eurasia and for European subspecies toward the Northern range of the continental United States. Also, the Southern range of the current distribution of gypsy moth may become inhospitable to both subspecies owing to extreme heat (Bale et al. 2002).

Asian gypsy moth is considered a high-risk pest in North America. To prevent its establishment, aggressive control and eradication treatments are employed following detection. Timing is critical for monitoring and eradication of this pest and so far it has been based on North American population developmental data or trapping data in the native range, which likely does not provide adequate estimates. Our data can provide better estimates for these critical time points and be used to develop an Asian gypsy moth-specific phenology model, which is in progress. Such a model would benefit pest managers in modifying management strategies and developing risk assessments. Also, these data can be combined with climate data to predict the current and future (under different climate change scenarios) potential distributions of Asian gypsy moth populations throughout the world.

Acknowledgments

We thank Jessica Richards, Paul Moore, Greg Bradford, and Vicente Sánchez for providing assistance in rearing Asian gypsy moth larvae in a laboratory. Funding was provided in part by USDA APHIS Plant Protection and Quarantine Science and Technology Cooperative Agreement 14-8130-0451-CA to K.H.

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