Climate-related geographic variation in performance traits across the invasion front of a widespread nonnative insect

Running title:
Trait variation across an insect invasion

Lily M. Thompson[1,2], Sean D. Powers[3], Ashley Appolon[1], Petra Hafker[1], Lelia Milner[4], Dylan Parry[5], Salvatore J. Agosta[4], and Kristine L. Grayson[1*]

[1]University of Richmond, Department of Biology
[2]Clemson University, Department of Forestry and Environmental Conservation
[3]Virginia Commonwealth University, Integrative Life Sciences Doctoral Program
[4]Virginia Commonwealth University, Center for Environmental Studies
[5]State University of New York, College of Environmental Science and Forestry

[*]Corresponding author: kgrayson@richmond.edu

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Abstract

Aim: Invasive species are an ideal system for testing geographic differences in performance traits and measuring evolutionary responses as a species spreads across divergent climates and habitats. The European gypsy moth, *Lymantria dispar dispar* L. (Lepidoptera: Erebidae), is a generalist forest defoliator introduced to Medford, Massachusetts, USA in 1869. The invasion front extends from Minnesota to North Carolina and the ability of this species to adapt to local climate may contribute to its continuing spread. We evaluated the performance of populations along the climatic gradient of the invasion front to test for a relationship between climate and ecologically important performance traits.

Methods: Insects from 14 populations across the US invasion front and interior of the invasive range were reared from hatch to adult emergence in six constant temperature treatments. The responses of survival, pupal mass, and larval development time were analyzed as a function of source climate (annual mean normal temperature), rearing temperature, and their interaction using multiple polynomial regression.

Results: With the exception of female development time, there were no significant interactions between source climate and rearing temperature, indicating little divergence in the shape of thermal reaction norms among populations. Source population and rearing temperature were significant predictors of survival and pupal mass. Independent of rearing temperature, populations from warmer climates had lower survival than those from colder climates, but attained larger body size despite similar development times. Larval development time was dependent on rearing temperature, but there were not consistent relationships with source climate.

Main Conclusions: Thermal adaptation can be an important factor shaping the spread of invasive species, particularly in the context of climate change. Our results suggest that *L. d. dispar* is highly plastic, but has undergone climate-related adaptation in thermal performance and life history traits as it spread across North America.

Keywords: climatic performance gradient, forest pest, gypsy moth, local adaptation, *Lymantria dispar*, thermal biology

INTRODUCTION

Species with broad geographic distributions often exhibit phenotypic variation among populations resulting from a combination of local adaptation and plasticity in performance (Bennett, Duarte, Marbà, & Wernberg, 2019; Gaston, 2009; Valladares et al., 2014). Many efforts have been made to classify the observed geographic patterns of inter-and intra-specific variation along gradients of latitude or elevation (e.g. Blois, Williams, Fitzpatrick, Jackson, & Ferrier, 2013). The characterizations of these patterns, such as Bergmann's rule, James' rule, and the metabolic cold adaptation hypothesis, provide general expectations for the types of life history and trait variation expected across geographic gradients (Horne, Hirst, & Atkinson, 2017; Shelomi, 2012; Williams et al., 2016). Climate, particularly as it relates to temperature, plays an important role in driving geographic variation in physiological performance, which contributes to shaping species' ranges and determining distributional limits (Crozier & Dwyer, 2006). Environmental temperature is especially important for ectothermic organisms, whose physiological dependence on temperature often results in strong patterns of geographic variation in thermal performance and life history traits (Addo-Bediako, Chown, & Gaston, 2000; Spicer & Gaston, 1999; Sunday et al., 2019).

Invasive species provide an ideal opportunity to both examine these processes over relatively short time-scales and to measure the evolutionary and plastic responses of a species as it spreads across divergent climates and habitats. Despite extensive knowledge of geographic and climate-related variation in performance of ectotherms, comparatively little is known about the rate at which these traits may evolve as species disperse across the landscape and expand their geographic range. Historically, successful invasive species were characterized as generalists with high degrees of plasticity in performance traits (Chown, Slabber, Mcgeoch, Janion, & Leinaas, 2007; Sakai et al., 2001). More recently, greater attention has been paid to the role of local adaptation in these systems and adaptive evolutionary change has been found in an array of invasive organisms from a broad range of taxa (Colautti & Lau, 2015; Kosmala, Brown, Christian, Hudson, & Shine, 2018; Medley, Westby, & Jenkins, 2019). Quantifying adaptation in invasive species is challenging as selection pressures are often strongest on individuals at range-edges. These range-edge populations are often prohibitively difficult to study as densities are very low, individuals are difficult to detect and range limits are often based on coarse-grained sampling methodologies (Grayson & Johnson, 2018; Kramer, Dennis, Liebhold, & Drake, 2009).

The invasion of North America by *Lymantria dispar dispar* L. (Lepidoptera: Erebidae) from Europe provides a unique example for understanding how geographic variation in performance among populations may develop when an introduced species spreads and encounters a wide range of novel habitats and climates. The North American introduction of this forest insect, commonly called the gypsy moth and denoted here as *L. d. dispar*, originated with an accidental release in Medford, MA in 1869. Genetic studies suggest that most, if not all, individuals in North America today are the descendants of this original population

(Bogdanowicz, Mastro, Prasher, & Harrison, 1997; Y. Wu et al., 2015). The geographic expansion of *L. d. dispar* across the North American landscape has been well-documented both historically and contemporaneously, with the current invasion front extending 2000 km and encompassing a wide array of climates from Virginia and North Carolina in the south, to eastern Canada in the north, and, more recently, west to Minnesota and Wisconsin (Fig. 1; Grayson & Johnson, 2018; Tobin, Gray, & Liebhold, 2014). Currently, the average spread rate is estimated to be 4 km/yr (Slow the Spread Foundation, 2016), although this varies widely among regions (Tobin et al., 2014; Tobin, Whitmire, Johnson, Bjørnstad, & Liebhold, 2007). Outbreaks can result in mass defoliation of a wide variety of host trees and the ability to consume over 300 woody species has undoubtedly contributed to the success of the *L. d. dispar* in North America (Liebhold et al., 1995).

The establishment and success of *L. d. dispar* in climatically divergent regions of North America suggests this species has expressed significant variation in thermal performance through some combination of phenotypic plasticity and local adaptation as it has spread across the landscape. Much of the foundational work on *L. d. dispar* thermal biology utilized data derived from a single population to determine how temperature can predict developmental phenology in order to implement effective management interventions that target specific life stages (e.g, Gray, 2004). Many of these studies focused on the effects of cold temperatures, particularly shortened season lengths in colder climates, the amount of cold necessary for egg hatching, and limitations on egg viability constraining northward expansion (Gray, Logan, Ravlin, & Carlson, 1991; Madrid & Stewart, 1981; Streifel, Tobin, Kees, & Aukema, 2019). More recent work has expanded these questions to include warm temperatures (Banahene et al., 2018; Limbu et al., 2017). This area of research has shown that a range retraction along the southeastern invasion

front is correlated with supraoptimal temperatures (Tobin et al., 2014) and provides support for local adaptation of fitness-related traits in southern populations (Faske et al., 2019; Friedline et al., 2019; Thompson et al., 2017). These lines of evidence suggest that while *L. d. dispar* may have become thermally constrained in the southeastern portion of the invasive range, these populations may be locally adapted to current climatic conditions.

In this study, we examined how the performance of an invasive insect may be evolving as the species expands its range into increasingly divergent North American climates using the fitness-related metrics of pupal mass, larval development time, and survival. We hypothesized that populations from colder climates would survive and grow better under cooler conditions and conversely, populations from warmer climates would have higher performance under warmer conditions (i.e. the shape of the thermal performance relationship would differ among populations). We also predicted that individuals from warmer climates of the invasion front (southern populations) would achieve larger body sizes than those from colder climates (northern populations) based on the converse-Bergmann rule that is predominant in clinal patterns of size variation in Lepidoptera (Chown & Gaston, 2010; Shelomi, 2012). Finally, we predicted that populations from colder climates would develop in a shorter amount of time relative to populations from warmer climates as a result of experiencing a shorter growing season that favors more rapid growth (e.g. Nylin & Svärd, 1991). Together, this research sheds light on the effect of climate in an active and ongoing invasion and how future climates may alter the progression of its range expansion.

MATERIALS AND METHODS

Study system and populations

Lymantria dispar has a single, non-overlapping generation per year with the majority of the life cycle spent overwintering as an egg. Larvae hatch in the spring and transition through 5 – 6 instars before pupation. Adults are sexually dimorphic; males are smaller and require fewer instars than females. In the European subspecies, flying males seek flightless females using pheromone signaling. The immobility of females limits both short and long distance dispersal to ballooning by neonates after hatch and human-mediated transport (Leonard, 1981).

In this experiment, populations were sourced from 14 locations in the eastern United States (Fig. 1, Table S1). Three of the populations were sourced from the established range (NY, MA1, and MA2), with the two Massachusetts populations collected 15.9 – 23.1 km from the original site of introduction in Medford, MA. Five populations were collected from the southern portion of the *L. dispar* invasion front, with three populations from the Appalachian Mountain region (WV1, WV2, and SWVA) and two from the North Carolina Coastal Plain region (NC1, NC2). Six populations were collected from the northern portion of the invasion front in Wisconsin and Minnesota (AL, BF, IR, WI1, WI2, and MN). These 11 populations represent both areas of active range expansion and the current extremes of the invasive range in North America (Table S1). Variation in egg provisioning, which can impact larval development, has been shown to be related to maternal environmental feeding conditions in *L. d. dispar* and other insects (Rossiter, 1991; Rossiter, Cox-Foster, & Briggs, 1993, but see Myers, Boettner, & Elkinton, 1998). While we were unable to fully control for potential differences among populations arising from non-genetic parental effects, each of the populations collected directly

from the field came from areas at the invasion front with low population density and we collected multiple separate populations from each region to serve as replicates. Insects used in this experiment were transported and housed under USDA APHIS permits P526P-17-03681 (KLG) and P526P-16-04388 (DP).

Experimental Design

In order to compare the performance of populations across a wide range of temperatures, 20 individuals from each population were reared in each of six constant temperature treatments: 15, 20, 25, 28, 30, and 32°C. These were maintained in environmental chambers (Model: I-22VL, Percival Scientific, Inc.) using a 14 hour light, 10 hour dark cycle. Prior to the experiment, each environmental chamber was carefully calibrated for both light and dark cycles at their assigned rearing temperature to ensure each chamber maintained temperature within ± 0.5°C. Temperature data loggers (HOBO U23 Pro v2, Onset Computer Corporation) were also placed inside each environmental chamber to ensure rearing temperatures remained constant for the duration of the experiment. The temperatures were selected based on previous research suggesting that the known optimum temperature for development in the lab strain of *L. d. dispar* was 29°C (Casagrande, Logan, & Wallner, 1987; Logan, Casagrande, & Liebhold, 1991) and that successful development to adulthood was impeded at temperatures below 10°C (Casagrande et al., 1987) and above 32°C (Thompson et al., 2017). Additionally, this span of temperatures was within those that are experienced in the current invasive range (Faske et al., 2019). Caterpillars were housed in individual plastic cups with cubes of artificial diet (USDA APHIS formulation) that were replaced weekly. To minimize chamber effects, the location of individuals in the chambers was randomized at the time of diet replacement. Individuals in the first two

instars were housed in 30 ml cups each with 5 ml of diet and were switched to 74 ml cups with 10 ml of diet for the remainder of their life cycle to accommodate larval growth.

Individuals were checked daily and the date of pupation and adult emergence was recorded. We measured pupal mass, which correlates with fecundity in females (Faske et al., 2019; Honěk, 1993), for each individual on the date of pupation. Based on morphology, sex was recorded for each individual at adult emergence or determined from the pupal case. Larval development time was calculated as the number of days between the start of the experiment and pupation date. Adult survival was defined by successful adult emergence from the pupal stage.

From the coordinates of the source location for each population, we obtained a mean annual 30 year (1981 – 2010) climate normal temperature calculated from the PRISM model (PRISM Climate Group, 2012) to objectively represent differences among populations in a biologically meaningful way (Fig. 1). We chose PRISM to obtain our single metric of source location climate because it has been shown to be a robust method for calculating temperature data for continuous surfaces (Scully, 2010). Mean temperature was selected because it captures variation in climate that would not be included if using the minimum or maximum temperature alone.

Data analysis

Data were analyzed using multiple polynomial regression with the "stats" and "aod" packages in R version 3.5.2 (Lesnoff & Lancelot, 2010; R Core Team, 2018). Statistical significance was assessed using $\alpha = 0.05$. Response variables of larval development time, pupal mass, and adult survival were independently modeled as a function of source climate, rearing temperature, and the quadratic effect of rearing temperature, which accounts for the curvature of

thermal reaction norms (Bodensteiner et al., 2019). Interactions of source climate with rearing temperature and with the quadratic effect of rearing temperature were also assessed to test for differences in the shape of thermal reaction norms. Given the complexity of interpreting interactions with quadratic effects, the reported results are of models with any non-significant interactions removed (Eq. 1).

$$Performance = Source\ Climate + Rearing\ Temp + Rearing\ Temp^2 \qquad \text{(Eq.1)}$$

Both source climate and rearing temperature were treated as continuous fixed effects in the model. We included data for all individuals reaching a specific development point regardless of their survival later in the experiment. Adult survival was analyzed as a binominal for individuals emerging from pupae as adults. Pupal mass and larval development time are sexually dimorphic (Leonard, 1981); therefore, analyses of these metrics were conducted separately for females and males. To conform to model assumptions, transformations were performed on the data if doing so improved normality.

RESULTS

Survival to adult emergence was generally high across moderate rearing temperatures, but low at the upper and lower rearing temperature extremes (Fig. 2). Interactions between rearing temperature and the quadratic effect of rearing temperature with source climate were not significant predictors of survival ($p < 0.05$), indicating the shape of thermal performance was similar among populations; therefore, these interactions were removed from the model. In the model without interactions, rearing temperature ($\beta = 2.41$, $p < 0.001$), the quadratic effect of rearing temperature ($\beta = -0.050$, $p < 0.001$), and source climate ($\beta = -0.039$, $p = 0.017$) were all significant predictors of adult survival ($\chi^2 = 373.5$, $df = 3$, $p < 0.001$). The highest (32°C) and

lowest (15°C) rearing temperatures resulted in the lowest survival for all populations, but populations from colder source climates survived better than those from warmer climates overall, especially at moderate temperatures (Fig. 2).

Larval development time ranged from 29 to 125 days for females and from 25 to 127 days for males across all rearing temperatures. Within a particular rearing temperature, mean development time varied very little between populations from the warmest and coolest source climates; the maximum difference was 0.2 days for males and 1.7 days for females (Fig. 3b and 3d). Populations differed significantly in the shape of thermal reaction norms for females; lower rearing temperatures resulted in longer larval development time for colder source climate populations, while warmer rearing temperatures resulted in longer development time for warmer source climate populations (Fig. 3a). This pattern is indicated by a significant interaction between source climate and rearing temperature ($\beta = 0.37$, $p = 0.022$) and between source climate and the quadratic effect of rearing temperature ($\beta = -0.007$, $p = 0.032$). Female development time was also significantly predicted by the main effects of rearing temperature ($\beta = -25.76$, $p < 0.001$), the quadratic effect of rearing temperature ($\beta = 0.46$, $p < 0.001$), and source climate ($\beta = -4.47$, $p = 0.018$). Together, these predictors explained 95% of the variance in the female data (adj $R^2 = 0.95$, $F(5, 482) = 1832$, $p < 0.001$). In contrast, we found no evidence for differences in the shape of thermal reaction norms or in development times overall for males (Fig. 3c and 3d). Since the interactions of rearing temperature and the quadratic effect of rearing temperature with source climate were not significant predictors of male larval development time ($p < 0.05$), they were removed from the model. Male larval development time was significantly predicted by rearing temperature ($\beta = -20.5$, $p < 0.001$) and the quadratic effect of rearing temperature ($\beta = 0.36$, $p < 0.001$), but not by source climate ($p > 0.05$). These predictors

explained, 94% of the variance in the data for male larval development time (adj $R^2 = 0.94$, $F(3, 564) = 3462$, $p < 0.001$).

Pupal mass ranged from 0.56 to 4.02 g in females and from 0.20 to 1.04 g in males across all rearing temperatures. Among all populations, the highest (32°C) and lowest (15°C) rearing temperatures resulted in the smallest pupal masses (Fig. 4). For both sexes, the shape of thermal performance related to pupal mass did not differ among source climates as indicated by non-significant interactions of rearing temperature and the quadratic effect of rearing temperature with source climate ($p < 0.05$; Fig. 4a and 4c); thus, these interactions and were removed from the models. In the model without interactions, female pupal mass was significantly predicted by rearing temperature ($\beta = 0.60$, $p < 0.001$), the quadratic effect of rearing temperature ($\beta = -0.013$, $p<0.001$), and source climate ($\beta = 0.044$, $p < 0.001$). These predictors explained 26% of the variance in female pupal mass (adj $R^2 = 0.264$, $F(3, 477) = 58.41$, $p < 0.001$). Similarly, male pupal mass was significantly predicted by rearing temperature ($\beta=0.11$, $p<0.001$), the quadratic effect of rearing temperature ($\beta = -0.0023$, $p < 0.001$), and source climate ($\beta = 0.0079$, $p < 0.001$). These predictors explained 20% of the variance in male pupal mass (adj $R^2 = 0.195$, $F(3, 560) = 46.53$, $p < 0.001$). Overall, pupae from warmer source climates were larger across all rearing temperatures (Fig. 4b and 4d). Within a particular rearing temperature, pupae ranged from 15 to 34% larger in the population from the warmest climate compared to the population from the coolest source climate.

DISCUSSION

A mechanistic understanding of the factors that determine geographic ranges is a fundamental goal of ecology (Gaston, 2003) and critical for predicting the dynamics of

biological invasions (Buckley & Csergő, 2017; Early & Sax, 2014). Invasive species that spread over broad geographic areas provide a unique window into the processes of adaptation and geographic structure in trait variation as a species encounters novel environments and climatic conditions. Here we used the invasion of *L. d. dispar*, which has steadily spread over the North American landscape over the last 150 years, to test for performance differences across the current climatic extremes of the invasion, including populations near the original introduction point in the interior of the established range. When reared in constant conditions at a gradient of ecologically relevant temperatures (Casagrande et al., 1987; Thompson et al., 2017), we found many of the expected differences in performance among source climates indicating the potential for local adaptation to the climatic regimes along the invasion front.

Our comparison of populations from the northern and southern extremes of the invasion front provides evidence for local adaptation in body size and survival associated with climate. Surprisingly, with the exception of small differences in female development time, we found little evidence for divergence in the shape of thermal reaction norms based on source climate over the range of rearing temperatures tested (i.e. no significant interactions between source climate and rearing temperature for the performance traits measured). Independent of temperature, we found a clear source climate-related cline for both survivorship (decreases with increasingly warmer source climate) and pupal mass (increases with increasing warmer source climate). This suggests a potential performance trade-off in which this species has poorer survival in warm climates, but grows to a larger body size. For female *L. d. dispar*, larger pupal mass is advantageous because it is highly correlated with fecundity (Faske et al., 2019). Similarly, larger males can have increased flight capacity, giving them an advantage in mate-finding (Agosta, 2010). Larger body size may also provide increased energetic efficiency and resistance to thermal stress (Chown &

Gaston, 1999; Cushman, Lawton, & Manly, 1993). Together, our results suggest that this invasive species is highly plastic, but has also undergone climate-related adaptation in thermal performance and life history traits as it has spread from its point of introduction (Friedline et al., 2019).

Although widely considered broad ranging generalists, many studies have demonstrated rapid adaptive change in invasive species (Colautti & Barrett, 2013; Huey & Pascual, 2009; Kosmala et al., 2018; Medley et al., 2019), suggesting the potential for clines in performance to develop across invasive ranges and to shape the potential for further range expansion. Across the climates of our source populations, we found pupal size variation to be consistent with the converse-Bergmann's rule, with larger individuals in lower latitude populations, where climates are warmer (Mousseau, 1997). This relationship is well-documented in ectotherms and has been found in many other species of Lepidoptera (Chown & Gaston, 2010; Shelomi, 2012). The comparison of survival in relation to source climate provides additional support for local adaptation in this system, although contrary to other study systems we found that individuals from colder climates had higher survival than those from warmer climates. Previous research in *L. d. dispar* has similarly found that populations from different regions of eastern North America are genetically distinct (Friedline et al., 2019; Y. Wu et al., 2015) and evidence suggests that these genetic differences are reflective of local adaptation in fitness-related traits at the southern invasion front (Faske et al., 2019) and along a latitudinal gradient from the point of introduction (Thompson et al., 2017).

Studies demonstrating clinal patterns in performance and life history traits in relation to latitude and elevation are common in the literature and have taught us a great deal about the evolution of geographic differences within and among species (e.g. Addo-Bediako et al., 2000;

Beck et al., 2016; DeLong et al., 2018; Keller, Alexander, Holderegger, & Edwards, 2013). These spatial attributes have been used not only to describe geographic variation, but also as a surrogate for climate (De Frenne et al., 2013; Howard-Williams, Peterson, Lyons, Cattaneo-Vietti, & Gordon, 2006). While latitudinal and elevational gradients are often correlated with changes in climate, the correlations are not always strong or linear; therefore, their role as a climate surrogate can be limited (Hawkins & Felizola Diniz-Filho, 2004). Instead, we chose to use mean annual temperature as a more direct proxy of the local climate experienced by each population (as in Bennett et al., 2019). By assessing populations using the 30 year mean annual temperature derived from each sampling location, rather than latitude, elevation, or region alone, we were able to more directly assess the hypothesis that geographic variation in performance is driven by variation in climate. As this metric is representative of the climatic environment for a particular geographic location, we aimed to measure how selection for thermal performance traits may have affected a particular population. While condensing climate into any single metric for a particular location is undoubtedly an oversimplification, our approach allowed for biologically meaningful comparisons among populations based on a metric of local temperature.

The strongest geographic pattern found in our study was that insects from warmer source climates ultimately produced larger pupae across all rearing temperatures. Body size is a fundamental life history trait that is positively correlated with an array of other fitness-related traits (Chown & Gaston, 2010). Across taxa with complex life cycles, intraspecific variation in size at metamorphosis is often driven by trade-offs with development time, where selection for a shorter larval development time can result in smaller adult body sizes (Nylin & Gotthard, 1998; Rohde, Dreher, & Hochkirch, 2015). However, despite increased pupal mass at warmer temperatures, we found no biologically meaningful relationship between source climate and

development time, with differences in development time less than 1 – 2 days among populations within a rearing temperature. Given the significantly larger size of *L. d. dispar* from warmer climates across all rearing temperatures, our results indicate that these populations have higher growth rates and can attain a greater mass in the same duration than those from colder climates, a similar pattern to that found by Friedline et al. (2019). An increased growth rate in warmer climates may be driven by differences in ingestion rates, conversion efficiency, or metabolic rates among the populations. The metabolic cold adaptation hypothesis suggests that populations from colder climates have higher metabolic rates than those from warmer climates because of adaptations that maximize physiological efficiency in cold temperatures (Shik, Arnan, Oms, Cerdá, & Boulay, 2019). May et al. (2018) did not find support for metabolic cold adaptation in *L. d. dispar*, but their study compared populations based on latitude rather than a measure of local climate. Further research on growth and metabolic rates across a broader environmental gradient and using a metric of climate rather than latitude would increase understanding of the physiological mechanisms underlying the patterns found in our study.

Numerous studies have demonstrated rapid evolutionary change in invasive species, despite constraints on local adaptation such as genetic bottlenecks during introduction (e.g. Selechnik et al., 2019; N. Wu et al., 2019). Once established, human-mediated transport can add additional introductions, contribute to long distance dispersal, and result in heterogeneous satellite populations, all processes that potentially disrupt local adaptation through continued genetic mixing (Garnas et al., 2016; Rius & Darling, 2014). Successful invasive species often undergo rapid range expansion and encounter novel habitats and climates when spreading across a landscape, which can result in local phenotypic changes despite these constraints. Local adaptation, or lack thereof, in invasive species can illustrate the capacity of organisms respond to

rapid changes in climate (Moran & Alexander, 2014; Wiens, Litvinenko, Harris, & Jezkova, 2019). Our research demonstrates how geographic differences in ecologically important traits can develop in invasive species, which can aid mitigation efforts while increasing our understanding of adaptive and plastic responses to changing climates.

DATA ACCESSIBILITY

Data associated with this article is deposited in the Dryad Digital Repository (doi:10.5061/dryad.59zw3r25j).

Figure Legends

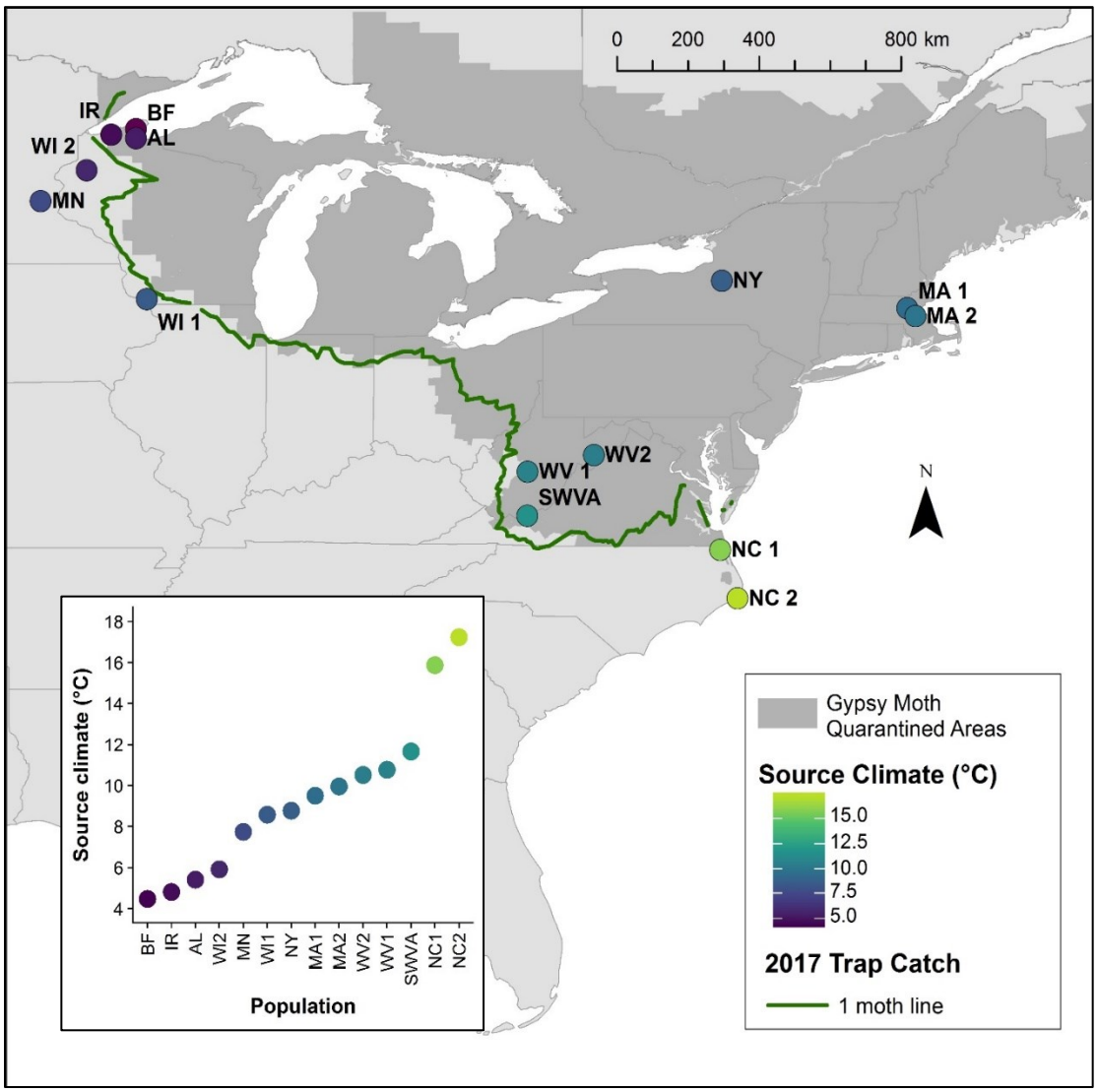
Fig. 1. Map of *Lymantria dispar dispar* population source locations in the United States including quarantined areas (darker gray shading) and the 1-moth line in 2017, which represents the invasion front based on geographically-referenced annual deployment of pheromone traps. Inset shows graphical representation of the PRISM mean annual 30-year climate normal temperature for each population location from lowest to highest. Colors of points correspond to source climate values shown in the map legend.

Fig. 2. Proportion of *Lymantria dispar dispar* individuals surviving to adult emergence by source climate and rearing temperature. The left panel visualizes our results as a thermal performance curve across rearing temperatures with a gradient of colors illustrating populations based on source climate. Populations were sourced from 14 locations in the eastern United States from the northern (n = 6) and southern (n = 5) portions of the current invasion front, as well as the established range (n = 3; see also Fig. 1, Table S1). The right panel shows the same data illustrating the linear trend across populations based on source climate, with each rearing temperature as a separate colored line. In all cases, lines represent the regression and are shaded with 95% confidence intervals.

Fig. 3. The effect of source climate and rearing temperature on *Lymantria dispar dispar* larval development time for females (a, b) and males (c, d). The left panel visualizes our results as a thermal performance curve across rearing temperatures with a gradient of colors illustrating populations based on source climate. Populations were sourced from 14 locations in the eastern

United States from the northern (n = 6) and southern (n = 5) portions of the current invasion front, as well as the established range (n = 3; see also Fig. 1, Table S1). The right panel shows the same data illustrating the linear trend across populations based on source climate, with each rearing temperature as a separate colored line. In all cases, lines represent the regression and are shaded with 95% confidence intervals.

Fig. 4. The effect of population source climate and rearing temperature on *Lymantria dispar dispar* pupal mass for females (a, b) and males (c, d). The left panel visualizes our results as a thermal performance curve across rearing temperatures with a gradient of colors illustrating populations based on source climate. Populations were sourced from 14 locations in the eastern United States from the northern (n = 6) and southern (n = 5) portions of the current invasion front, as well as the established range (n = 3; see also Fig. 1, Table S1). The right panel shows the same data illustrating the linear trend across populations based on source climate, with each rearing temperature as a separate colored line. In all cases, lines represent the regression and are shaded with 95% confidence intervals.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Source climate (°C)
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16
14
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BF IR AL WI2 MN WI1 NY MA1 MA2 WV2 WV1 SWVA NC1 NC2
Population
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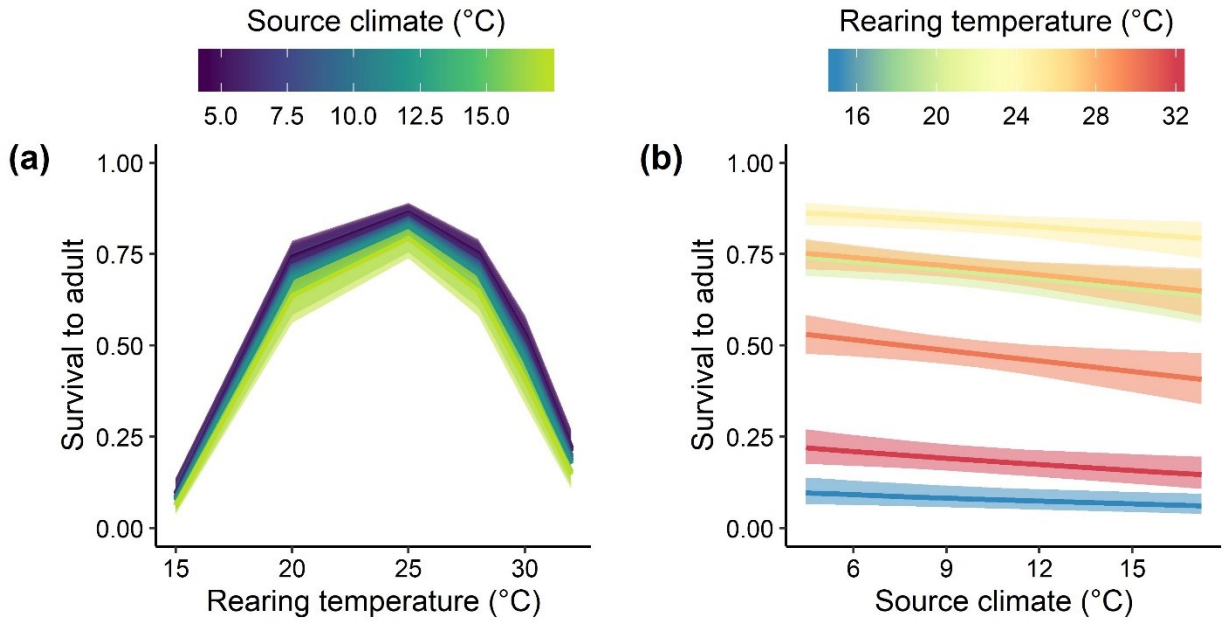
2017 Trap Catch
1 moth line

Fig. 1.


Source climate (°C)
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7.5
10.0
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15.0
(a)
1.00
0.75
0.50
0.25
0.00
Survival to adult
15
20
25
30
Rearing temperature (°C)
Rearing temperature (°C)
16
20
24
28
32
(b)
1.00
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0.50
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0.00
Survival to adult
6
9
12
15
Source climate (°C)

Fig. 2.

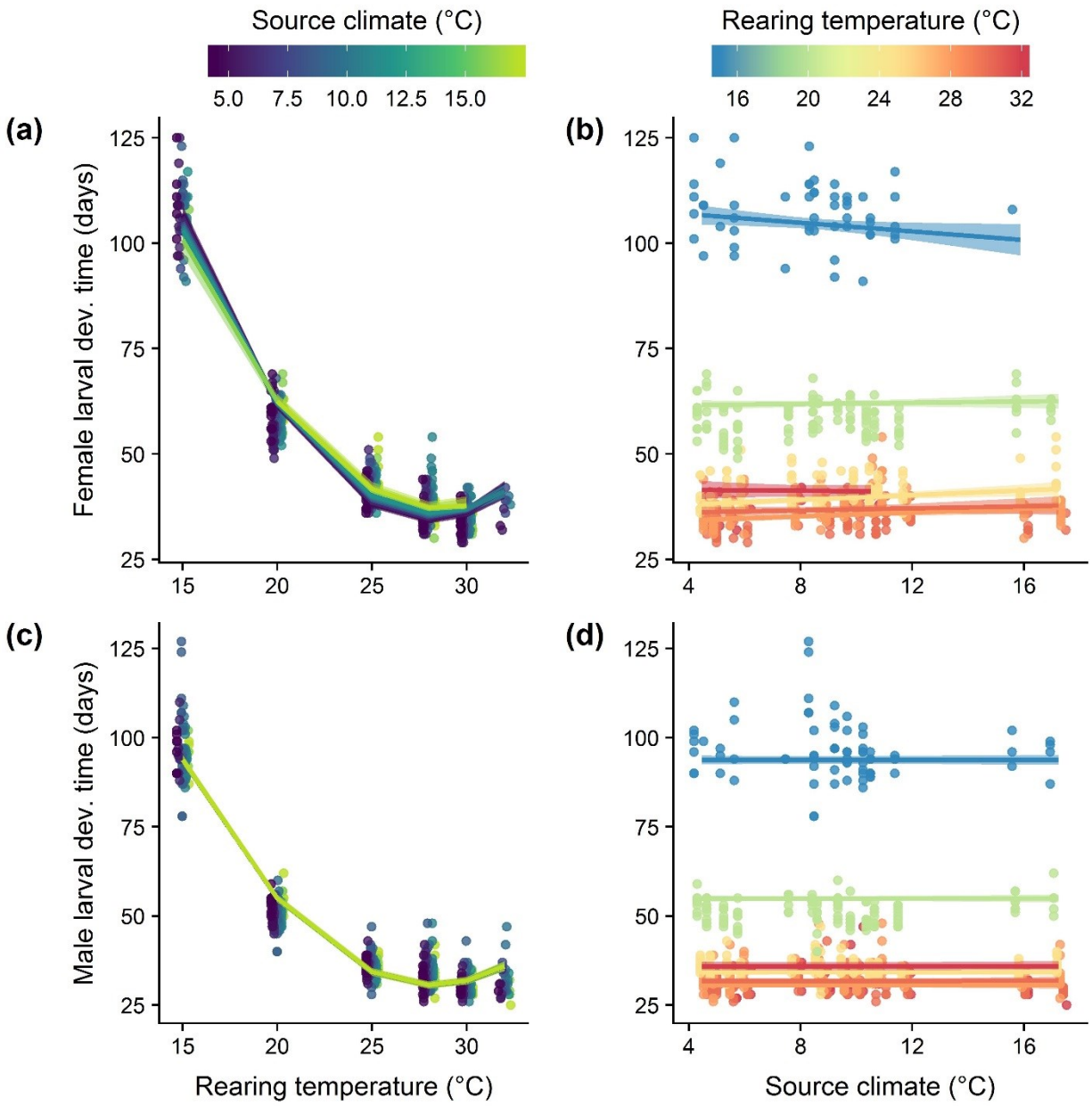
Source climate (°C)
5.0 7.5 10.0 12.5 15.0
Rearing temperature (°C)
16 20 24 28 32
(a)
(b)
(c)
(d)
Female larval dev. time (days)
Male larval dev. time (days)
125
100
75
50
25
15 20 25 30
4 8 12 16
Rearing temperature (°C)
Source climate (°C)

Fig. 3.

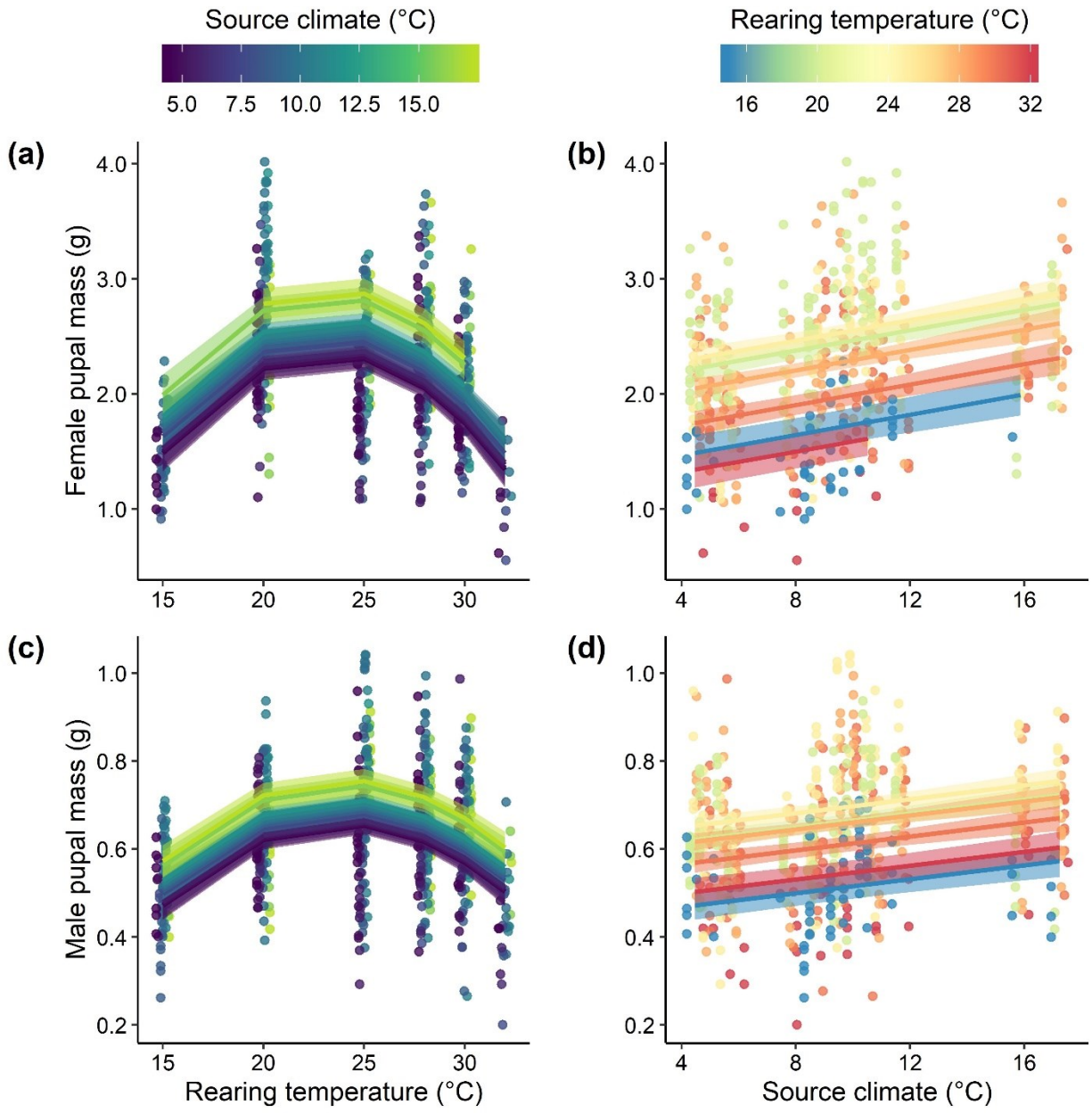
Source climate (°C)
5.0 7.5 10.0 12.5 15.0
Rearing temperature (°C)
16 20 24 28 32
(a)
(b)
(c)
(d)
Female pupal mass (g)
Male pupal mass (g)
Rearing temperature (°C)
Source climate (°C)

Fig. 4.

REFERENCES

Addo-Bediako, A., Chown, S. L., & Gaston, K. J. (2000). Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society B*, *267*(1445), 739–745. https://doi.org/DOI 10.1098/rspb.2000.1065

Agosta, S. J. (2010). Male body size and mating success and their relation to larval host plant history in the moth *Rothschildia lebeau* in Costa Rican dry forest. *Biotropica*, *42*(2), 201–207. https://doi.org/10.1111/j.1744-7429.2009.00556.x

Banahene, N., Salem, S., Faske, T. M., Byrne, H. M., Glackin, M., Agosta, S. J., … Thompson, L. M. (2018). Thermal sensitivity of gypsy moth (*Lymantria dispar* L.) (Lepidoptera: Erebidae) during larval and pupal development. *Environmental Entomology*, *47*(6), 1623–1631.

Beck, J., Liedtke, H. C., Widler, S., Altermatt, F., Loader, S. P., Hagmann, R., … Fiedler, K. (2016). Patterns or mechanisms' Bergmann's and Rapoport's rule in moths along an elevational gradient. *Community Ecology*, *17*(2), 137–148. https://doi.org/10.1556/168.2016.17.2.2

Bennett, S., Duarte, C. M., Marbà, N., & Wernberg, T. (2019). Integrating within-species variation in thermal physiology into climate change ecology. *Philosophical Transactions of the Royal Society B: Biological Sciences*, Vol. 374. https://doi.org/10.1098/rstb.2018.0550

Blois, J. L., Williams, J. W., Fitzpatrick, M. C., Jackson, S. T., & Ferrier, S. (2013). Space can be subsituted for time in predicting climate-change effects on biodiversity. *Proceedings of the National Academy of Sciences*, *110*(23), 9374–9379. https://doi.org/10.5061/dryad.d5f1r

Bodensteiner, B. L., Warner, D. A., Iverson, J. B., Milne-Zelman, C. L., Mitchell, T. S., Refsnider, J. M., & Janzen, F. J. (2019). Geographic variation in thermal sensitivity of early

life traits in a widespread reptile. *Ecology and Evolution*, *9*(5), 2791–2802. https://doi.org/10.1002/ece3.4956

Bogdanowicz, S. M., Mastro, V. C., Prasher, D. C., & Harrison, R. G. (1997). Microsatellite DNA variation among Asian and North American gypsy moths (Lepidoptera: Lymantriidae). *Annals of the Entomological Society of America*, *90*(6), 768–775.

Buckley, Y. M., & Csergő, A. M. (2017). Predicting invasion winners and losers under climate change. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(16), 4040–4041. https://doi.org/10.1073/pnas.1703510114

Casagrande, R. A., Logan, P. A., & Wallner, W. E. (1987). Phenological model for gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae), larvae and pupae. *Environmental Entomology*, *16*(2), 556–562. https://doi.org/10.1093/ee/16.2.556

Chown, S. L., & Gaston, K. J. (1999). Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. *Biological Reviews of the Cambridge Philosophical Society*, *74*, 87–120. https://doi.org/Doi 10.1017/S000632319800526x

Chown, S. L., & Gaston, K. J. (2010). Body size variation in insects: A macroecological perspective. *Biological Reviews*, *85*(1), 139–169. https://doi.org/10.1111/j.1469-185X.2009.00097.x

Chown, S. L., Slabber, S., Mcgeoch, M. A., Janion, C., & Leinaas, H. P. (2007). Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. *Proceedings of the Royal Society B*, *274*(August), 2531–2537. https://doi.org/10.1098/rspb.2007.0772

Colautti, R. I., & Barrett, S. C. H. (2013). Rapid adaptation to climate facilitates range expansion

of an invasive plant. *Science*, *342*(6156), 364–366. https://doi.org/10.1126/science.1242121

Colautti, R. I., & Lau, J. A. (2015). Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. *Molecular Ecology*, *24*(9), 1999–2017. https://doi.org/10.1111/mec.13162

Crozier, L., & Dwyer, G. (2006). Combining population-dynamic and ecophysiological models to predict climate-induced insect range shifts. *The American Naturalist*, *167*(6), 853–866. https://doi.org/10.1086/504848

Cushman, J. H., Lawton, J. H., & Manly, B. F. J. (1993). Latitudinal patterns in European ant assemblages: variation in species richness and body size. *Oecologia*, *95*(1), 30–37. https://doi.org/10.1007/BF00649503

De Frenne, P., Graae, B. J., Rodríguez-Sánchez, F., Kolb, A., Chabrerie, O., Decocq, G., … Verheyen, K. (2013). Latitudinal gradients as natural laboratories to infer species' responses to temperature. *Journal of Ecology*, *101*(3), 784–795. https://doi.org/10.1111/1365-2745.12074

DeLong, J. P., Bachman, G., Gibert, J. P., Luhring, T. M., Montooth, K. L., Neyer, A., & Reed, B. (2018). Habitat, latitude and body mass influence the temperature dependence of metabolic rate. *Biology Letters*, *14*(8). https://doi.org/10.1098/rsbl.2018.0442

Early, R., & Sax, D. F. (2014). Climatic niche shifts between species' native and naturalized ranges raise concern for ecological forecasts during invasions and climate change. *Global Ecology and Biogeography*, *23*(12), 1356–1365. https://doi.org/10.1111/geb.12208

Faske, T. M., Thompson, L. M., Banahene, N., Levorse, A., Quiroga Herrera, M., Sherman, K., … Grayson, K. L. (2019). Can gypsy moth stand the heat? A reciprocal transplant experiment with an invasive forest pest across its southern range margin. *Biological*

Invasions.

Friedline, C. J., Faske, T. M., Lind, B. M., Hobson, E. M., Parry, D., Dyer, R. J., … Eckert, A. J. (2019). Evolutionary genomics of gypsy moth populations sampled along a latitudinal gradient. In *Molecular Ecology*. https://doi.org/10.1111/mec.15069

Garnas, J. R., Auger-Rozenberg, M. A., Roques, A., Bertelsmeier, C., Wingfield, M. J., Saccaggi, D. L., … Slippers, B. (2016). Complex patterns of global spread in invasive insects: eco-evolutionary and management consequences. *Biological Invasions*, *18*(4), 935–952. https://doi.org/10.1007/s10530-016-1082-9

Gaston, K. J. (2003). *The structure and dynamics of geographic ranges*. Oxford: Oxford University Press.

Gaston, K. J. (2009). Geographic range limits: achieving synthesis. *Proceedings of the Royal Society B*, *276*(1661), 1395–1406. https://doi.org/10.1098/rspb.2008.1480

Gray, D. R. (2004). The gypsy moth life stage model: landscape-wide estimates of gypsy moth establishment using a multi-generational phenology model. *Ecological Modelling*, *176*(1–2), 155–171. https://doi.org/10.1016/j.ecolmodel.2003.11.010

Gray, D. R., Logan, J. A., Ravlin, F. W., & Carlson, J. A. (1991). Toward a model of gypsy moth egg phenology: using respiration rates of individual eggs to determine temperature-time requirements of prediapause development. *Environmental Entomology*, *20*(6), 1645–1652.

Grayson, K. L., & Johnson, D. M. (2018). Novel insights on population and range edge dynamics using an unparalleled spatiotemporal record of species invasion. *Journal of Animal Ecology*, *87*(3), 581–593. https://doi.org/10.1111/1365-2656.12755

Hawkins, B. A., & Felizola Diniz-Filho, J. A. (2004). “Latitude” and geographic patterns in species richness. *Ecography*, *27*(2), 268–272. https://doi.org/10.1111/j.0906-

7590.2004.03883.x

Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, *66*(3), 483–492.

Horne, C. R., Hirst, A. G., & Atkinson, D. (2017). Seasonal body size reductions with warming covary with major body size gradients in arthropod species. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1851). https://doi.org/10.1098/rspb.2017.0238

Howard-Williams, C., Peterson, D., Lyons, W. B., Cattaneo-Vietti, R., & Gordon, S. (2006). Measuring ecosystem response in a rapidly changing environment: The Latitudinal Gradient Project. *Antarctic Science*, *18*(4), 465–471. https://doi.org/10.1017/S0954102006000514

Huey, R. B., & Pascual, M. (2009). Partial thermoregulatory compensation by a rapidly evolving invasive species along a latitudinal cline. *Ecology*, *90*(7), 1743–1750.

Keller, I., Alexander, J. M., Holderegger, R., & Edwards, P. J. (2013). Widespread phenotypic and genetic divergence along altitudinal gradients in animals. *Journal of Evolutionary Biology*, Vol. 26, pp. 2527–2543. https://doi.org/10.1111/jeb.12255

Kosmala, G. K., Brown, G. P., Christian, K. A., Hudson, C. M., & Shine, R. (2018). The thermal dependency of locomotor performance evolves rapidly within an invasive species. *Ecology and Evolution*, *8*(9), 4403–4408. https://doi.org/10.1002/ece3.3996

Kramer, A. M., Dennis, B., Liebhold, A. M., & Drake, J. M. (2009). The evidence for Allee effects. *Population Ecology*, *51*(3), 341–354. https://doi.org/10.1007/s10144-009-0152-6

Leonard, D. E. (1981). Bioecology of the gypsy moth. In C. C. Doane & M. L. McManus (Eds.), *The Gypsy Moth: Research Toward Integrated Pest Management* (pp. 9–29). Washington, D.C.: USDA-Forest Service Technical Bulletin 1584.

Lesnoff, M., & Lancelot, R. (2010). *aod*. Retrieved from http://cran.r-project.org/package=aod

Liebhold, A. M., Gottschalk, K. W., Muzika, R.-M., Montgomery, M. E., Young, R., O'Day, K., & Kelley, B. (1995). Suitability of North American tree species to the gypsy moth: A summary of field and laboratory tests. *USDA-Forest Service*, (General Technical Report NE-211).

Limbu, S., Keena, M., Chen, F., Cook, G., Nadel, H., & Hoover, K. (2017). Effects of temperature on development of *Lymantria dispar asiatica* and *Lymantria dispar japonica* (Lepidoptera: Erebidae). *Environmental Entomology*, *46*(4), 1012–1023. https://doi.org/10.1093/ee/nvx111

Logan, J. A., Casagrande, R. A., & Liebhold, A. M. (1991). Modeling environment for simulation of gypsy moth (Lepidoptera: Lymantriidae) larval phenology. *Environmental Entomology*, *20*(6), 1516–1525.

Madrid, F. J., & Stewart, R. K. (1981). Ecological significance of cold hardiness and winter mortality of eggs of the gypsy moth *Lymantria dispar* L., in Quebec. *Environmental Entomology*, *10*, 586–589.

May, C., Hillerbrand, N., Thompson, L. M., Faske, T. M., Martinez, E., Parry, D., … Grayson, K. L. (2018). Geographic variation in larval metabolic rate between northern and southern populations of the invasive gypsy moth. *Journal of Insect Science*, *18*(4), 5; 1–7. https://doi.org/10.1093/jisesa/iey068

Medley, K. A., Westby, K. M., & Jenkins, D. G. (2019). Rapid local adaptation to northern winters in the invasive Asian tiger mosquito *Aedes albopictus*: A moving target. *Journal of Applied Ecology*, *56*(11), 2518–2527. https://doi.org/10.1111/1365-2664.13480

Moran, E. V., & Alexander, J. M. (2014). Evolutionary responses to global change: Lessons from invasive species. *Ecology Letters*, *17*(5), 637–649. https://doi.org/10.1111/ele.12262

Mousseau, T. (1997). Ectotherms follow the converse Bergmann's rule. *Evolution*, *51*(2), 630–632. https://doi.org/10.1111/j.1558-5646.1997.tb02453.x

Myers, J. H., Boettner, G., & Elkinton, J. (1998). Maternal effects in gypsy moth: Only sex ratio varies with population density. *Ecology*, *79*(1), 305–314.

Nylin, S., & Gotthard, K. (1998). Plasticity in life-history traits. *Annual Review of Entomology*, *43*(125), 63–83.

Nylin, S., & Svärd, L. (1991). Latitudinal patterns in the size of European butterflies. *Holarctic Ecology*, *14*(3), 192–202.

PRISM Climate Group. (2012). *Oregon State University*. Retrieved from http://prism.oregonestate.edu

R Core Team. (2018). *R: A language and environment for statistical computing*. Retrieved from https://www.r-project.org

Rius, M., & Darling, J. A. (2014). How important is intraspecific genetic admixture to the success of colonising populations? *Trends in Ecology & Evolution*, *29*(4), 233–242. https://doi.org/10.1016/j.tree.2014.02.003

Rohde, K., Dreher, E., & Hochkirch, A. (2015). Sex-specific phenotypic plasticity in response to the trade-off between developmental time and body size supports the dimorphic niche hypothesis. *Biological Journal of the Linnean Society*, *115*(1), 48–57. https://doi.org/10.1111/bij.12460

Rossiter, M. C. (1991). Environmentally-based maternal effects: a hidden force in insect population dynamics? *Oecologia*, *87*(2), 288–294. https://doi.org/10.1007/BF00325268

Rossiter, M. C., Cox-Foster, D. L., & Briggs, M. A. (1993). Initiation of maternal effects in *Lymantria dispar*: Genetic and ecological components of egg provisioning. *Journal of*

Evolutionary Biology, *6*(4), 577–589.

Sakai, A. K., Allendorf, F. W., Holt, J. S., Lodge, M., Molofsky, J., With, K. A., … Weller, S. G. (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics*, *31*(1), 305–332.

Scully, R. A. (2010). Intercomparison of PRISM and DAYMET Temperature. *Master's Thesis*.

Selechnik, D., Richardson, M. F., Shine, R., DeVore, J. L., Ducatez, S., & Rollins, L. A. (2019). Increased adaptive variation despite reduced overall genetic diversity in a rapidly adapting invader. *Frontiers in Genetics*, *10*(November), 1–14. https://doi.org/10.3389/fgene.2019.01221

Shelomi, M. (2012). Where are we now? Bergmann's rule sensu lato in insects. *The American Naturalist*, *180*(4), 511–519. https://doi.org/10.1086/667595

Shik, J. Z., Arnan, X., Oms, C. S., Cerdá, X., & Boulay, R. (2019). Evidence for locally adaptive metabolic rates among ant populations along an elevational gradient. *Journal of Animal Ecology*, *88*(8), 1240–1249. https://doi.org/10.1111/1365-2656.13007

Slow the Spread Foundation. (2016). Annual Accomplishment Report. Retrieved from STS Foundation Reports website: http://www.gmsts.org/freports.html.

Spicer, J. I., & Gaston, K. J. (1999). *Physiological diversity and its ecological implications*. Oxford, UK: Blackwell Science.

Streifel, M. A., Tobin, P. C., Kees, A. M., & Aukema, B. H. (2019). Range expansion of *Lymantria dispar dispar* (L.) (Lepidoptera: Erebidae) along its north-western margin in North America despite low predicted climatic suitability. *Journal of Biogeography*, *46*(1), 58–69. https://doi.org/10.1111/jbi.13474

Sunday, J., Bennett, J. M., Calosi, P., Clusella-Trullas, S., Gravel, S., Hargreaves, A. L., …

Morales-Castilla, I. (2019). Thermal tolerance patterns across latitude and elevation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1778). https://doi.org/10.1098/rstb.2019.0036

Thompson, L. M., Faske, T. M., Banahene, N., Grim, D., Agosta, S. J., Parry, D., … Grayson, K. L. (2017). Variation in growth and developmental responses to supraoptimal temperatures near latitudinal range limits of gypsy moth *Lymantria dispar* (L.), an expanding invasive species. *Physiological Entomology*, *42*(2), 181–190. https://doi.org/10.1111/phen.12190

Tobin, P. C., Gray, D. R., & Liebhold, A. M. (2014). Supraoptimal temperatures influence the range dynamics of a non-native insect. *Diversity and Distributions*, *20*(7), 813–823. https://doi.org/10.1111/ddi.12197

Tobin, P. C., Whitmire, S. L., Johnson, D. M., Bjørnstad, O. N., & Liebhold, A. M. (2007). Invasion speed is affected by geographical variation in the strength of Allee effects. *Ecology Letters*, *10*, 36–43. https://doi.org/10.1111/j.1461-0248.2006.00991.x

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M. B., Balaguer, L., Benito-Garzón, M., … Zavala, M. A. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*, *17*(11), 1351–1364. https://doi.org/10.1111/ele.12348

Wiens, J. J., Litvinenko, Y., Harris, L., & Jezkova, T. (2019). Rapid niche shifts in introduced species can be a million times faster than changes among native species and ten times faster than climate change. *Journal of Biogeography*, *46*(9), 2115–2125. https://doi.org/10.1111/jbi.13649

Williams, C. M., Szejner-Sigal, A., Morgan, T. J., Edison, A. S., Allison, D. B., & Hahn, D. A. (2016). Adaptation to low temperature exposure increases metabolic rates independently of

growth rates. *Integrative and Comparative Biology*, *56*(1), 62–72. https://doi.org/10.1093/icb/icw009

Wu, N., Zhang, S., Li, X., Cao, Y., Liu, X., Wang, Q., … Zhan, S. (2019). Fall webworm genomes yield insights into rapid adaptation of invasive species. *Nature Ecology and Evolution*, *3*(1), 105–115. https://doi.org/10.1038/s41559-018-0746-5

Wu, Y., Molongoski, J. J., Winograd, D. F., Bogdanowicz, S. M., Louyakis, A. S., Lance, D. R., … Harrison, R. G. (2015). Genetic structure, admixture and invasion success in a Holarctic defoliator, the gypsy moth (*Lymantria dispar*, Lepidoptera: Erebidae). *Molecular Ecology*, *24*, 1275–1291. https://doi.org/10.1111/mec.13103

BIOSKETCH

This research team focuses on empirically testing the effects of temperature on the performance of invasive insect populations and the relationship between local climates and the trajectory of future spatial spread in North America. Our work provides an opportunity for cross-institutional collaboration of undergraduate students, graduate students, faculty, and research professionals.

Author contributions: LMT and KLG led the writing of the manuscript. LMT led the data collection, and statistical analysis. LMT, SDP, PH, AA, and LM collected the data. DP coordinated the collection of source populations and maintained them in culture. KLG, SJA, and DP conceived the research ideas and obtained the funding for this research. All authors contributed to the revision of the manuscript.

SUPPORTING INFORMATION

Table S1. Population information for *Lymantria dispar dispar* including coordinates, elevation, number of generations reared prior to this experiment, and the number of egg masses contributing to the reared population. Egg masses were field collected from relatively low-density populations from across the invasive range at sites where populations were high enough for sampling, but well below outbreak densities. Detection of extremely low populations at the leading edge of an invasion can be prohibitively difficult, thus, some of our populations were sourced from collections made in previous years and reared annually under ambient outdoor conditions. These populations were used when we were unable to find new eggs masses at desired localities. While variation in number of previously reared generations was unavoidable, the pattern among populations is consistent between those that were recently collected and those that were reared for multiple years.

Population	**Coordinates (Lat, Long)**	**Elevation (m asl)**	**No. of previously reared generations**	**No. of egg masses contributing to 2018 experiment**
BF	46.8126°N, 90.8204°W	222	0	> 30
IR	46.6762°N, 91.4469°W	269	0	13
AL	46.5967°N, 91.0251°W	297	0	21
WI2	45.8033°N, 92.0680°W	353	0	30
MN	44.8660°N, 93.2296°W	261	0	> 30
WI1	42.6231°N, 90.5451°W	243	0	30
NY	43.0806°N, 75.9812°W	146	2	30
MA1	42.4050°N, 71.2871°W	56	1	> 40
MA2	42.2073°N, 71.0809°W	96	1	> 40

WV2	38.7851°N, 79.2228°W	525	0	30
WV1	38.3747°N, 80.9011°W	614	0	60
SWVA	37.2843°N, 80.9081°W	528	0	11
NC1	36.4491°N, 76.0246°W	3	6	20
NC2	35.2503°N, 75.5813°W	1	0 – 1*	5, ~6*

*5 egg masses were freshly collected in 2018, ~6 had been reared for 1 previous generation.