Biol Invasions
https://doi.org/10.1007/s10530-018-1907-9

ORIGINAL PAPER

Can gypsy moth stand the heat? A reciprocal transplant experiment with an invasive forest pest across its southern range margin

Trevor M. Faske · Lily M. Thompson · Nana Banahene · Andi Levorse · Melisa Quiroga Herrera · Kayla Sherman · Sarah E. Timko · Banruo Yang · David R. Gray · Dylan Parry · Patrick C. Tobin · Andrew J. Eckert · Derek M. Johnson · Kristine L. Grayson

Received: 4 May 2018 / Accepted: 16 December 2018

Abstract Temperature provides important physiological constraints that can influence the distribution of an invasive species. Gypsy moth (*Lymantria dispar* L.) is a generalist defoliator in North America and supraoptimal temperatures (above the optimal for developmental rate) have been implicated in range dynamics at the southern invasion front in West Virginia and Virginia. We sourced egg masses from the Appalachian Mountains (AM), where the gypsy moth range is expanding, from the Coastal Plain (CP), where range retraction is occurring, and from a long-established population in New York (NY) and conducted a reciprocal transplant experiment to compare development and fitness components among these populations at two sites along the southern invasion front. We found evidence of sublethal effects from rearing in the CP, with decreased pupal mass and fewer eggs compared to individuals reared in the AM, but little difference between source populations in developmental traits. The AM and NY populations did experience reductions in egg viability under a southern winter at the CP site compared to control wintering conditions, while the CP egg masses had equivalent survival. This study provides empirical support for negative fitness consequences of supraoptimal

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10530-018-1907-9) contains supplementary material, which is available to authorized users.

Trevor M. Faske and Lily M. Thompson have contributed equally to this work.

T. M. Faske · A. J. Eckert · D. M. Johnson
Department of Biology, Virginia Commonwealth University, 1000 West Cary Street, Room 126, Richmond, VA, USA

L. M. Thompson · N. Banahene · A. Levorse · M. Quiroga Herrera · K. Sherman · S. E. Timko · B. Yang · K. L. Grayson (✉)
Department of Biology, University of Richmond, 138 UR Drive, Richmond, VA 23173, USA
e-mail: kgrayson@richmond.edu

D. Parry
Department of Environmental and Forest Biology, College of Environmental Science and Forestry, State University of New York, 1 Forestry Drive, Syracuse, NY 13210, USA

P. C. Tobin
School of Environmental and Forest Sciences, University of Washington, 123 Anderson Hall, 4000 15th Avenue NE, Seattle, WA 98195, USA

D. R. Gray
Canadian Forest Service – Atlantic Forestry Centre, Natural Resources Canada, P.O. Box 4000, Fredericton, NB E3B 5P7, Canada

temperatures at the southern range edge, consistent with patterns of range retraction and spread in the region, as well as suggesting the potential for local adaptation through variation in egg survival. Our work illustrates that sublethal effects from high temperature can be an important factor determining the distribution of invasive species under current and future climates.

Keywords *Lymantria dispar* · Forest defoliator · Common garden experiment · Local adaptation · Supraoptimal temperatures

Introduction

Invasive species can expand across wide regions in an introduced range, where environmental extremes can impose geographic and physiological limits on future spread (Chown and Gaston 1999; Hill et al. 2011; Sinclair et al. 2012; Vanhanen et al. 2007). Temperature is perhaps the most important environmental constraint for terrestrial poikilothermic organisms because it directly influences rates of physiological processes and performance (e.g., Addo-Bediako et al. 2000; Deutsch et al. 2008). Thermal performance is typically quantified in controlled laboratory settings (e.g., Ayres and Scriber 1994; Kingsolver and Woods 1997; Logan et al. 1991), and can then be applied to models that predict suitable environments for invasive species in novel habitats (e.g., Gray 2004; Venette et al. 2010). Field studies in natural environments can test these predictions and increase our understanding of the interactions between temperature, organismal performance, and, ultimately, current and future distributional limits.

Experiments using common garden and reciprocal transplant study designs provide insights into local adaptation and the role of environmental variation in driving phenotypic change (Kawecki and Ebert 2004; Savolainen et al. 2013). Peripheral populations occupy environments at extremes relative to the rest of a range, thus, the ability of a population situated at a range margin to perform in its local environment can determine whether a species continues to expand beyond its current range boundary (Antonovics 1976; Kawecki 2008; Kirkpatrick and Barton 1997; Sheth and Angert 2018). Understanding the relative role of plastic versus adaptive responses in range edge populations is also important for quantifying the spread potential of an invasive species. These processes are not mutually exclusive because plasticity itself can respond to selection when its genetic basis varies across individuals and this variation has fitness consequences (e.g., Chevin et al. 2010; Lande 2009, 2015). Given their prevalence and ease of experimentation, a majority of common garden and reciprocal transplant experiments on invasive species focus on plants (e.g., Eckert et al. 2008; Gibson et al. 2016; Pahl et al. 2013). These experimental approaches may also provide important insights in invasive insect systems, particularly given that local adaptation in range edge populations has been shown for several species, with significant change occurring in some systems after only a few decades (e.g., Huey and Pascual 2009; Preisser et al. 2008).

The spread of the gypsy moth in North America is perhaps one of the best-documented biological invasions. Since its introduction in 1869 to Medford, Massachusetts, USA from Europe, the gypsy moth has spread north into Canada, as far west as Minnesota, and as far south as North Carolina (Tobin et al. 2012, 2016). Detailed spatiotemporal rates of spread are available from data collected by the Slow-the-Spread program, which has deployed an extensive trapping grid across the invasion front over the last 2 decades (Grayson and Johnson 2018; Sharov et al. 2002). These data have revealed dynamic spread rates as the gypsy moth has encountered climatically diverse regions across Eastern North America (Tobin et al. 2007). While gypsy moth is classically used as a study system in invasion biology, few studies have considered the potential for population variation across the invasive range of gypsy moth in North America.

The southern invasion front of gypsy moth in North America is particularly notable for its spatial variation in spread rates across a narrow region over the last 25 years with steady progression in the Appalachian Mountains, stasis in the Piedmont region, and net range retraction in the Coastal Plain region (Tobin et al. 2014; Fig. 1). However, spread rates in the Coastal Plain are temporally variable, shifting between years of spread and retraction, even though the overall range has contracted significantly in this region. Correlational analyses showed a negative association between spread rate and the frequency of temperatures above the developmental rate optimum

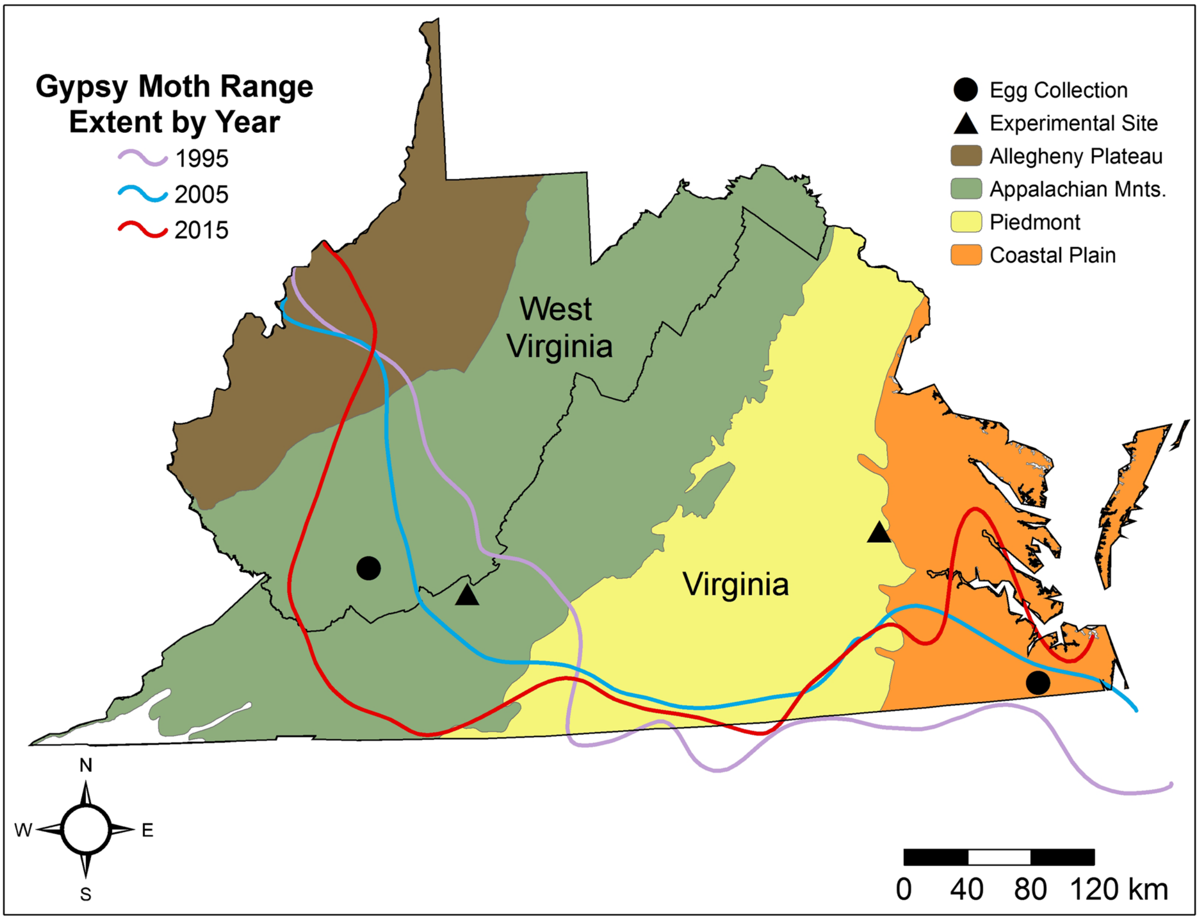

Fig. 1 Geographic variation in gypsy moth spread across the southern invasive range edge from 1995 to 2015. Range extents are the boundaries estimated from the Slow-the-Spread trap catch abundance data (Sharov et al. 1995), shown as smoothed isoclines where the mean abundance is 0.5–1 moths/trap. These patterns in spread dynamics were originally reported by Tobin et al. (2014) and redrawn with updated data by Laura Blackburn (US Forest Service)

(supraoptimal) occurring during larval and pupal stages, suggesting that heat may be imposing limits on further southward spread in this region (Tobin et al. 2014). This work highlights the opportunity to further study the mechanisms behind range dynamics seen in this region to understand the factors that determine the range edge for an invasive species and limit the relative invasion risks for portions of this area.

The majority of empirical work on thermal limits in gypsy moth has focused on the requirements for overwintering egg survival. Northern range expansion is limited by cool spring and summer temperatures that slow egg hatch and larval development, as well as the early onset of winter temperatures before embryos have entered the cold-tolerant diapause phase (Gray 2004; Régnière and Nealis 2002). The predicted southern limits of the invasive gypsy moth range in North America have been based on the occurrence of winter temperatures of sufficient cold and duration for entering and terminating the obligatory diapause (Allen et al. 1993; Gray 2004). Less is known about gypsy moth larval performance at high temperatures, and previous work used a laboratory strain to quantify the temperature-dependence of development rate under constant temperature conditions (Logan et al. 1991, but see Casagrande et al. 1987). Thompson et al. (2017) expanded our understanding of the effects of heat on laboratory and wild sourced gypsy moth populations by showing that prolonged larval exposure to constant supraoptimal temperatures (> ~ 29 °C) consistently resulted in reduced survival and mass, with the response dependent on population origin. It is unclear, however, if these results can be replicated in natural settings where temperatures fluctuate and supraoptimal temperatures may only be experienced for short durations.

Here, we empirically compare southern invasion front populations and their respective thermal regimes for gypsy moth growth and development over a complete life-cycle using a reciprocal transplant design between the Appalachian Mountains and Coastal Plain regions of Virginia. We reared populations from both regions, in addition to a long-established population from New York, at two field sites located at this southern invasion front. Our study was initiated with egg masses that overwintered at each location, and we measured development time, body mass, and survival during larval and pupal life stages during the subsequent spring and summer. To more directly compare fitness outcomes for each population at each site, egg production and overwintering success were quantified through to hatching the following spring. Our study provides the opportunity to assess whether changes in ecologically important life history traits have occurred during the gypsy moth invasion, specifically, along an invasion front experiencing highly variable spread rates. The presence of population-based adaptive shifts in physiological tolerance has important impacts for species establishment and invasion. Testing for these changes can give a critical insight into the process of range expansion, as well as our ability to make accurate predictions about subsequent spread.

Methods

Study populations and field locations

Gypsy moth has a single non-overlapping generation per year, where eggs laid in the summer overwinter and hatch the following spring. As larvae, gypsy moth feeds on nearly 300 different species of host plants (Liebhold et al. 1995). Adult moths are non-feeding, and the flightless females attract males with a sex pheromone, mate once, and then deposit their entire egg compliment in a single mass. Collecting egg masses from wild populations to test for life history differences can be confounded by variation in local resource availability and population densities, which can impact egg and larval traits (Rossiter 1991). Therefore, individuals were reared under consistent dietary and temperature conditions for at least one generation to remove potential site and maternal effects before being used in our experiment (detailed in Supplement). After mating, selected egg masses were given 60–80 days to enter diapause before being placed outdoors at one of the two sites in the reciprocal transplant experiment. To reduce relatedness and maximize genetic diversity, 20 egg masses from each population were lightly separated by hand to detach individual eggs from the mass and all eggs were mixed together. Approximately half of the eggs from each population were placed in petri dishes within a breathable protective housing and attached to a Northern red oak tree (*Quercus rubra* L.) at each site to experience local overwintering conditions.

Our reciprocal transplant experiment used wild-sourced populations from Kirkville, New York (designated as the NY population representing a long-established population of gypsy moth that dates to the 1970s, US Code of Federal Regulations, Title 7, Chapter III, Section 301.45), Raleigh County in West Virginia (designated as the AM population representing the Appalachian Mountains region), and the Great Dismal Swamp in Suffolk County, Virginia (designated as the CP population representing the Coastal Plain region). Gypsy moth populations were established at the AM and CP sites by 2007 and 1987, respectively (US Code of Federal Regulations, Title 7, Chapter III, Section 301.45). We selected a representative experimental rearing site in the AM and CP regions based on proximity to the egg collection sites, available facilities, and logistical considerations. The AM site was located at Mountain Lake Biological Station (University of Virginia, Giles County, Virginia, USA; 37.376347°N, 80.522053°W; elevation: 1184 m) in the southwestern region of Virginia, 64 km from the egg collection area in West Virginia. The CP site was located at University of Richmond (Richmond, Virginia, USA; 37.573084°N, 77.542114°W; elevation: 61 m), which is on the western edge of the Coastal Plain region in Virginia and is located 139 km from the egg collection area for the CP population (Fig. 1).

Experimental design

Overwintering eggs were removed from the trees in early spring and kept in short-term cold storage to synchronize hatch across populations with budburst of local *Q. rubra* at each site. Hatched larvae from each population were haphazardly selected and placed on host foliage in 1-l, unwaxed, paper cups with a plastic lid containing pin holes for air exchange at densities of 10 larvae/cup with 15 replicates per population. All populations were started on host foliage within 24 h at each site (CP site initiation: 16–17 April 2015, AM site initiation: 14 May 2015). When the majority of individuals were third instar (28 days for CP site, 30 days for AM site), larvae from each population were consolidated into 11 replicate 7.6-l plastic buckets and covered with a mesh fabric at densities of 10 larvae/bucket. Smaller rearing containers were initially used to better locate and enumerate larvae during early development and establish enough individuals at a consistent density in bucket replicates. All larvae were fed fresh foliage on the same day every 3–4 days by placing *Q. rubra* stems with leaves inside each rearing container in either a floral water tube (cup) or 1-l plastic flask (bucket) to maintain foliage quality. Foliage harvested for each feeding at each location was sourced from a single tree, however, leaf-collections were made from different trees across feeding dates to minimize damage to individual trees. Gypsy moth larvae have diurnal rhythms with vertical movement into the canopy to feed (Leonard 1970). Limits on the extent of vertical movement and the potential range of microhabitat temperatures available to larvae were necessary constraints of our bucket rearing design. Each experiment was conducted in a screened outdoor insect rearing pavilion shaded by full canopy cover. Thus, larvae experienced conditions that approximated ambient forest floor temperatures.

Larvae were checked daily and pupation date was recorded on the day of pupal formation. Fully sclerotized pupae were weighed within 24 h of the onset of pupal formation and stored individually in paper-lined 74-ml plastic cups with snap-on lids containing pinholes for air exchange. Pupae were checked daily for adult emergence, at which point sex and date were recorded. Within 24 h of adult emergence, males and females from within a single population at a rearing location were randomly paired. No individual was used in more than one pairing and individuals that failed to mate (no discrete egg mass) were excluded from analysis. The life history traits measured were larval development time (hatch to pupation), pupal development time (pupation to adult emergence), pupal mass, and female fecundity (total number of eggs laid by each female, see below). Survival was assessed as percentage surviving from hatch to adult emergence.

The effect of rearing location on fecundity was measured by counting the total number of eggs within an egg mass laid by an individual female, regardless of hatching or fertilization. We used 22 egg masses per population and site for these fecundity measurements. To account for variation across bucket replicates, we selected two egg masses, each with unique parents, from each bucket replicate for each population, with a random substitution was from another bucket when two were not available.

To determine if overwintering location affected egg viability, we compared hatching success for eggs that experienced a natural winter at the two experimental sites and those that overwintered under optimal laboratory conditions. Egg masses were allowed to embryonate in their respective experimental site for 60 days and then were split in half. One half was returned to the outdoor insect rearing facility to complete overwintering, and hatch under natural conditions. The other was overwintered in a 4–6 °C laboratory refrigerator for 160 days, brought to room temperature (~ 23 °C), and allowed to hatch. Neonates were counted periodically until no larvae hatched for more than 1 week, after which the remaining numbers of fertilized and unfertilized unhatched eggs were recorded.

Site temperatures

Temperature data loggers (TidbiT v2 Temp Logger UTBI-001, Onset Computer Corp.) within the rearing pavilion at each site were used to quantify the thermal regime experienced by gypsy moth during development. Hours of supraoptimal temperature exposure were estimated according to Tobin et al. (2014), where temperatures $\geq$ 29.0 °C are considered above optimal for development based on empirical development rate functions in Logan et al. (1991). We calculated the number of hours of supraoptimal temperature for each degree $\geq$ 29.0 °C during larval development (hatch—95% pupation) and total development (hatch—95% adult emergence). We also calculated the number of hours near the optimum developmental temperature (26–29 °C) and the number of hours $\leq$ 10 °C. We used 10 °C as a lower threshold given past work reporting negligible development at this temperature (Casagrande et al. 1987; Logan et al. 1991).

To compare the frequency of supraoptimal temperatures in the study year (2015) with the previous 25 years (1990–2014), we used similar methods as Tobin et al. (2014). Daily minimum and maximum air temperatures (for the entire 26 year period: 1990–2015) were generated with BioSIM v.11 (Régnière et al. 2017) using the combined records of six weather stations nearest the egg collection sites of the two southern populations (National Centers for Environmental Information 2017; Table S1). Egg collection sites were chosen instead of experimental sites in order to best estimate the phenology and source environments of these populations. Hourly temperatures were generated from these daily minimum and maximum temperatures using 24 h sine wave-interpolation (Allen 1976). The predicted dates of life history transition points (50% hatch, 95% pupation, and 95% adult emergence) for each year and location were determined using the gypsy moth life stage model (Gray 2004). The hours of supraoptimal temperatures between these predicted life history transition points were calculated from the hourly temperatures generated at each location in each year, providing a relative measure of the amount of heat in 2015 compared to previous years.

Data analysis

All analyses were conducted separately for males and females in this sexually dimorphic species (Leonard 1981). Only individuals that survived until adulthood were included in the analysis of larval and pupal characteristics. To avoid pseudoreplication, analyses were conducted using means for each container replicate using a two-way generalized linear mixed effects model including population and treatment as fixed effects and the number of individuals measured within each container as a random effect. Independent statistical analyses were performed for each response variable (pupal mass, larval development time, and pupal development time) using a Satterthwaite approximation for degrees of freedom due to unequal sample sizes based on mortality. Statistical significance was assessed using $\alpha = 0.05$.

Paired a priori comparisons of female fecundity between sites for the AM and NY populations were carried out by generalized mixed linear models. Statistical significance was assessed with $\alpha = 0.05/2$ tests, using a Bonferroni multiple test correction. The CP population was excluded from this analysis due to low female survival at the AM site.

Egg hatching success after overwintering was calculated as the proportion of total hatched larvae out of the total number of fertilized eggs and was arcsine transformed to conform to model assumptions. For each site and population, we made a priori comparisons of egg hatching success between overwintering at the reciprocal transplant site and in the laboratory using the overwintering location as the fixed effect and the maternal parent identification as a random factor. Statistical significance was assessed with $\alpha = 0.05/5$ tests, using a Bonferroni multiple test correction. The CP population at the AM site was excluded from this analysis, as were egg masses with no or extremely low hatching success (< 1%), which likely represent failed matings. All analyses were conducted using the stats and lme4 libraries in R version 3.3.1 (Bates et al. 2015; R Core Team 2016).

Results

Site temperatures

During the experiment in 2015, the mean (± SE) temperature was 17.8 (± 3.7 °C) at the AM site and was 22.1 (± 4.9 °C) at the CP site from larval hatch to adult emergence. Temperature data loggers indicated that while the CP site experienced 12 h of supraoptimal development temperatures (≥ 29 °C) during larval development and 77 h total during larval and pupal development combined, the AM site experienced none (Table 1). Temperatures at or below our lower developmental threshold of 10 °C during the larval period were greater for the CP site than the AM site. The majority of air temperatures throughout development were between 10 and 26 °C at the AM site. The CP experimental site had 159 h and 230 h of optimal temperature (26–28.99 °C) during larval and total development, respectively, while the AM site had only 5 h throughout development (Table 1).

When comparing the means (± SE, median) in the 26 years between 1990 and 2015, the weather data near the AM population source had 5.81 (± 1.83, 0) h of supraoptimal temperature and the CP population source had 73.23 (± 6.33, 78) h during the larval

Table 1 Hours of ≤ 10 °C, optimal, and supraoptimal temperature during larval and total development for the AM and CP experimental sites in 2015. Dates of the larval stage are from the start of the experiment to 95% pupation and to 95% adult emergence for total development

Experimental site	AM		CP	
	Larval	Total	Larval	Total
Hours of ≤ 10 °C	41	41	81	81
Hours of optimal temperature (26–28.99 °C)	5	5	159	230
Hours of supraoptimal temperature (≥ 29 °C)				
+ 1	0	0	12	27
+ 2	0	0	0	19
+ 3	0	0	0	16
+ 4	0	0	0	15
Total	0	0	12	77

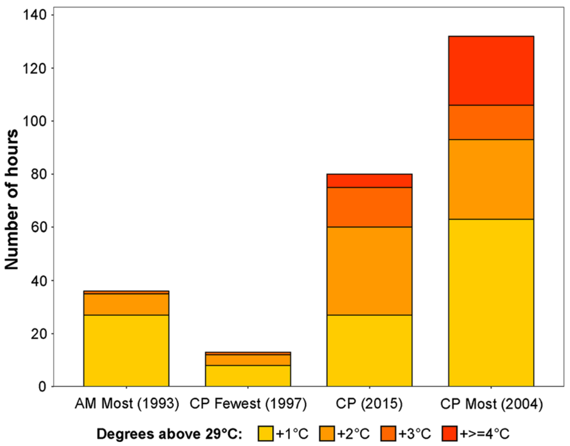

Fig. 2 The frequency of supraoptimal temperatures (above 29 °C) for gypsy moth in 2015 (the growing year of the study) and the most extreme temperature years during the larval stage from 1990 to 2015 (see methods for calculations). Weather data represent locations nearest the egg collection site for each southern population (AM, CP). The AM area had many years, including 2015, with 0 h of supraoptimal temperatures during larval development (not shown). Refer to Table S2 for the full 26-year supraoptimal temperature dataset

period (Fig. 2, Table S2 for all years). For total development time, which includes pupal development, there were 13.46 (± 3.40, 8.5) h of supraoptimal temperature for the AM and 137.50 (± 7.39, 144.5) h for the CP associated weather data (Table S3 for all years). In 2015, there were no hours of supraoptimal temperature during the predicted larval stage for the AM data, which also occurred in 8 of the 25 years between 1990 and 2014 (Table S2, S3). There were 80 h of supraoptimal temperatures in the CP weather data during larval development in 2015, making it an intermediate year in comparison with the most extreme years for this region (Fig. 2). However, early summer heat during the predicted period of pupal development, which is short in comparison to the larval stage, in the CP weather data made this one of the hotter years for total gypsy moth development (Table S3).

Growth, development, and survival

Survival from hatching to adulthood was greater at the CP site (population: NY = 95%; AM = 94%; CP = 100%) than at the AM site (population: NY = 89%; AM = 67%; CP = 36%). Differences in survival were due to infection at the AM site by the gypsy moth fungal pathogen *Entomophaga maimaiga*, which is found throughout the invasive range (Hajek et al. 1996) and known to be linked to temperature and precipitation during larval development (e.g. Hajek et al. 1990; Siegert et al. 2008; Hajek and Tobin 2011).

Experimental site had a significant effect on all developmental traits (larval and pupal development time, and pupal mass) for both sexes from all source populations (Ps < 0.0001, Fig. 3, Table 2). All individuals reared at the CP site had reduced pupal mass and shorter larval and pupal development time in days compared to the AM site (Fig. 3, summary statistics see Table S4). There was no significant effect of source population on pupal mass for either sex, but source population did have a significant effect on

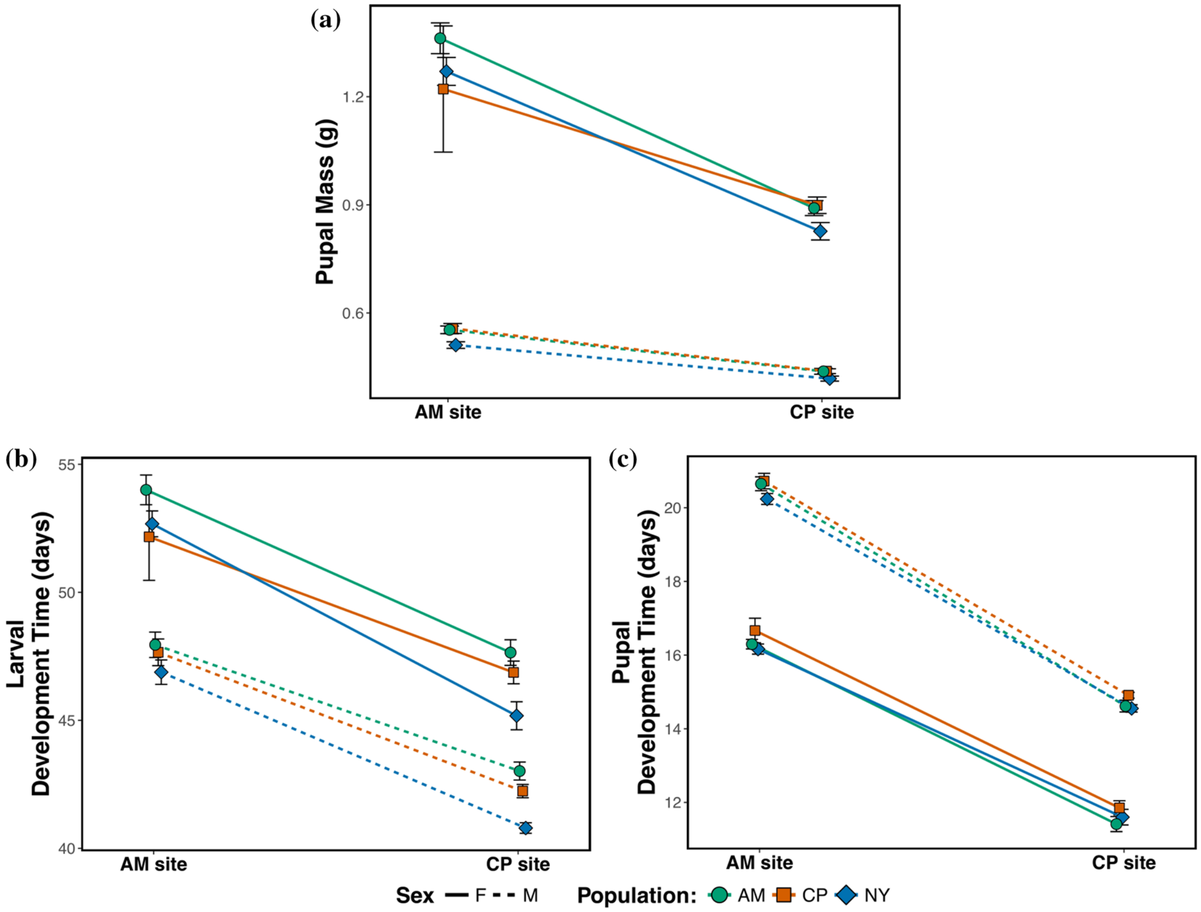

Fig. 3 Phenotypic measurements for each environmental site, population, and sex indicated by points as mean and standard error bars. **a** Pupal mass measured in grams **b** larval development time measured by time from hatch to pupation in days **c** pupal development time measured by time from pupation to adult emergence in days. Male and female (abbreviated as M and F) are indicated by solid and dashed lines, respectively. Populations are redundantly indicated by distinct point shape and color to conserve inferential power if viewed in black and white

larval development time in days for both females ($F_{2,56} = 6.62$, $P = 0.0027$) and males ($F_{2,65} = 7.36$, $P = 0.0013$). Development time of the NY population was generally shorter than the AM and CP populations. Source population had a significant effect on male pupal development time ($F_{2,65} = 3.19$, $P = 0.0476$), but not female.

Effect of overwintering location on fecundity and egg viability

Rearing location had a significant effect on female fecundity ($F_{1,134} = 50.78$, $P < 0.0001$, Fig. 4a). Total egg count at the CP site compared to the AM site was reduced by 32.7% on average for the AM and NY populations. For all females used in this portion of the study, egg number was correlated to female pupal mass ($R^2 = 0.5817$, $P < 0.0001$).

Comparing hatching outcomes between eggs overwintered outdoors at the rearing sites and the laboratory, we found that overwintering at the AM site yielded egg hatching success roughly equivalent to the ideal laboratory conditions (AM population: $F_{1,42} = 0.2237$, $P = 0.6391$; NY population: $F_{1,42} = 1.071$, $P = 0.3066$). Overwintering at the CP site had a significant effect on egg hatching success, but only for the AM and NY population (AM population: $F_{1,42} = 51.79$, $P < 0.0001$; NY population: $F_{1,42} = 14.83$, $P = 0.0003$; CP population: $F_{1,40} = 1.005$, $P = 0.3221$). Specifically, the AM

Table 2 Summary of analysis of variance (ANOVA) comparisons across developmental traits

	Female				Male			
ANOVA	MS	*F*	*df*	*P*	MS	*F*	*df*	*P*
Pupal mass (g)								
Site	2.1662	129.5	1, 56	**< 0.0001**	0.1653	79.552	1, 65	**< 0.0001**
Population	0.0216	1.292	2, 56	0.2827	0.0049	2.335	2, 65	0.1048
Site:population	0.0025	0.152	2, 56	0.8596	0.0004	0.212	2, 65	0.8093
Larval dev. time (d)								
Site	1387.7	651.23	1, 56	**< 0.0001**	485.89	140.53	1, 65	**< 0.0001**
Population	14.10	6.62	2, 56	**0.0027**	15.18	4.390	2, 65	**0.0163**
Site:population	4.03	1.89	2, 56	0.1602	4.07	1.177	2, 65	0.3146
Pupal dev. time (d)								
Site	254.34	513.27	1, 56	**< 0.0001**	541.00	2743.8	1, 65	**< 0.0001**
Population	0.720	1.45	2, 56	0.2425	0.63	3.19	2, 65	**0.0477**
Site:population	0.055	0.11	2, 56	0.8960	0.27	1.35	2, 65	0.2653

Results examine the effect of site/treatment, population, and their interaction on each of the three developmental traits for each sex (pupal mass, larval development time, and pupal development time). Bolded values indicate significance

MS Mean squared

population showed a 19.4% decrease in egg hatching success and the NY population showed a 10.9% decrease after overwintering at the CP site when compared to hatching success of eggs overwintered in the laboratory (Fig. 4b). The 4.4% decrease in egg hatching success for the CP population overwintered at the CP site was not significant (Fig. 4b).

Discussion

This study capitalized on a gradient of range spread, stasis, and retraction across Virginia to test the roles of thermal environment and population origin on gypsy moth survival and performance at the southern invasion front. Previous work found a correlation between yearly range retraction and the hours of supraoptimal temperature during the gypsy moth growing season in this southern portion of the invasive range (Tobin et al. 2014). Overall, experimental site had much stronger effects on gypsy moth performance than source population, with the AM site providing a superior climatic environment for gypsy moth development for all populations in this study. While we found no lethal effects of the warmer CP site during the growing season, there was substantial evidence for sublethal effects on larval and pupal development that resulted in fitness costs. The warmer climate in the CP site had negative consequences for overwintering egg survival and hatching success the following spring, but this difference was significant only for the AM and NY populations. These results suggest that selective pressures under warmer climates could lead to local adaptation in traits, such as egg resilience to heat, in gypsy moth populations at the southeastern invasion front. Overall, our results not only add to our understanding of organismal thermal performance, but also support previous hypotheses regarding regional range dynamics by providing empirical evidence of the negative fitness consequences of supraoptimal temperatures on gypsy moth.

The thermal regimes between the two experimental sites were dramatically different, with the AM site being much cooler overall than the CP site. The majority of temperatures at the AM site were below optimal for gypsy moth development (10–26 °C) with no supraoptimal temperatures, while there were more hours of optimal and supraoptimal temperatures at the CP site throughout development. The heat that gypsy moth experienced at the CP site was not lethal for any of the three source populations. Using 25 years of weather data from the egg collection sites to place the hours of supraoptimal temperature exposure during our study year (2015) in context, we found that the

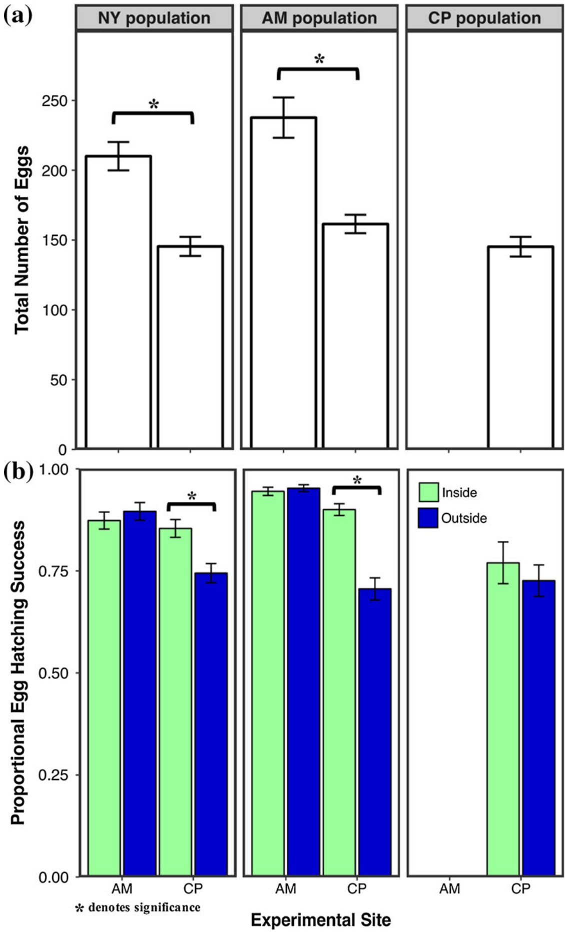

Fig. 4 Comparisons of fecundity for each site and population. Reported are the means and standard errors of each treatment by population within a site. Statistical analyses were performed for a treatment effect within each environmental location and population. Bolded bracket and stars indicate significant differences under a generalized mixed linear model. **a** Total number of eggs laid per female at each site for each population. **b** Egg hatching success calculated as the percentage of total hatched larvae of the total number of fertilized eggs. Each egg mass was split in half and treatments are indicated based on their storage/wintering location outside (eggs overwintered outdoors in natural conditions, light green) and inside (eggs overwintered indoors under known optimal conditions, dark blue)

temperatures occurring during larval development for our experiment were within the average range of temperatures for the CP region. When including temperatures over the entirety of gypsy moth development, more hours of high temperature during the relatively short pupal stage made 2015 the second hottest year compared to the historical weather data. Despite these conditions, gypsy moth can complete development in the laboratory even when reared at a constant 31–32 °C (Logan et al. 1991; Thompson et al. 2017) and gypsy moth can tolerate up to 40 °C when exposed for shorter durations of up to 2 days (Banahene et al. 2018). Thus, it is unlikely that range retraction in this area is due to direct lethal effects of high temperature and our results point to the importance of sublethal effects on growth and development in a hotter environment.

We used our reciprocal transplant experimental design to test for trait differences indicative of local adaptation among populations from the southern invasion front and the long-established range. While we found no differences between populations in pupal mass, there were significant differences in larval and pupal duration among populations. These differences were largely due to faster development in the NY population. At northern latitudes, larval and pupal development times can be constrained both by cooler temperatures during the growing season and a shortened growing season compared to those at more southerly latitudes (Roff 1980). Similar effects are common in other studies examining the effects of latitudinal and elevational clines on phenology and development (e.g., Halbritter et al. 2015; Śniegula et al. 2016). Temperature and length of growing season are well-known constraints for gypsy moth in the northern portions of its invasive range (Gray 2004; Tobin et al. 2016), thus it is reasonable that the NY population in our study has experienced selection for more rapid development compared to the AM and CP populations. This is consistent with findings by Friedline et al. (unpublished data) indicating selection for development times to be shorter in northern populations and longer in their southern counterparts.

In addition to pupal mass and development time, which are indirect measures of fitness, we assessed fitness directly using egg production and viability. There were clear differences in total egg production between sites where gypsy moths reared in the CP site laid fewer eggs compared to those in the AM site. Since size at pupation in gypsy moth was highly correlated with fecundity in this study, as has been found in other Lepidoptera (Calvo and Molina 2005; Honěk 1993), this difference was likely driven by the sublethal effect of the warmer environment on growth and pupal mass. When comparing our hatching success in the natural overwintering environment to the controlled laboratory conditions, we found that hatching success was generally lower for eggs overwintering in the natural environment at the CP site compared to the ambient laboratory control, while hatching in the AM site was equivalent. While the lack of eggs from the CP population at the AM site weakens our comparison, we observed equivalent hatching of the CP population outside at the CP site and in laboratory conditions. These differences found in overwintering egg survival based on both source population and experimental site show that eggs from the CP population were less affected by the warmer temperature conditions during the late summer and winter at the CP site than the other two populations. As an obligate univoltine species, overwintering egg survival in the gypsy moth is strongly influenced by conditions in the late summer during pre-diapause, overwintering temperatures for diapause, and spring conditions after breaking diapause. Eggs laid in the CP region experience prolonged egg exposure to summer heat compared to those laid in the Appalachian Mountains and this high temperature exposure can have detrimental physiological impacts on egg development (Allen et al. 1993; Gray et al. 1991). The low overall hatching success of the CP population suggests that there is an overall fitness trade-off between egg number and heat tolerance that could contribute to local adaptation through selective pressure for heat tolerance in eggs.

While the large trait differences between sites in our study indicate a plastic response across the gypsy moth populations, our data also provide some evidence for local adaptation, particularly with regards to reproductive capacity. In the warmer climate of the CP site, we found a slight home-site advantage for the CP population that suggests strong selective pressure in the region, which was expected based on documented patterns of recent range retraction (Tobin et al. 2014). It is notable that the effects of the CP site were not lethal, but caused reductions in fitness leading to negative demographic consequences in the next generation. All three populations exhibited similar sublethal effects in pupal size and egg number, which could indicate that range retraction will continue unless gypsy moth further adapts to a continually warming climate. Additional work taking into account genome-wide differences among populations that may affect developmental traits and fecundity in natural environments, particularly at a larger scale with more populations in a quantitative genetic study design, could provide useful insights to the adaptive potential of gypsy moth populations at a dynamic range margin with regards to increased exposure to warmer temperatures.

Field experiments using common garden designs have long been used for separating phenotypic differences based on genetics and the environment (Langlet 1971). Reciprocal transplant studies take the common garden design further by testing for comparative performance differences between two or more locations. Many studies of invasive species have used these designs to elucidate differences in comparative performance between native and introduced populations as a result of considerable abiotic and biotic variation between the native and introduced ranges (e.g., Keller and Taylor 2008). Because invasive species undergo rapid population growth and range expansion after successfully establishing, the novel environment can have vast ecological and climatic differences even within the invasive range. Reciprocal transplant studies can also provide important insights on variation in performance under varying selective pressures in these scenarios. For example, a common garden experiment with the invasive plant purple loosestrife (*Lythrum salicaria*) has shown that individuals from southern populations within its invaded range are consistently larger than all other populations regardless of the latitude at which they were grown, suggesting higher overall fitness. However, only through a reciprocal transplant was it revealed that viable reproduction of southern populations (accustomed to a long growing season) was impeded when grown under a northern (short-season) climate, implying local adaptation to latitudinal variation in the length of growing season (Colautti and Barrett 2013).

Our study is one of the few common garden-type studies that uses an invasive insect species (but see Krehenwinkel and Tautz 2013) and our reciprocal transplant design is notable in that it directly measures reproductive success by quantifying egg viability under divergent climatic conditions. The majority of reciprocal transplant studies focus on plants, and the design is rarely applied to invasive animal systems (but see examples reviewed in Colautti and Lau 2015). This study demonstrates the utility of natural field experiments that take into consideration the recent life history and ecology of the system in study and directly tests fitness, rather than substitution of correlated traits such as growth and dispersal. We found that supraoptimal temperatures at the range edge of the gypsy moth have negative sublethal effects, but provide some evidence that the population under the strongest selective regime has increased heat resilience in egg hatching. Continued selection for heat tolerance providing differential fitness outcomes could potentially alter spread rates patterns in the coastal plain region and lead to further spread into novel environments. Overall, this work adds to our knowledge of the role of thermal limits in shaping the geographic distribution of an invasive insect.

Acknowledgements Funding for this project was provided by the United States Department of Agriculture National Institute of Food and Agriculture grant (Grant Number 2014-67012-23539 to KLG), the National Science Foundation (Award Number 1556111 to PCT and DEB-1556767 to DMJ), the University of Richmond School of Arts and Sciences, and an award in Interdisciplinary Research from the Thomas F. and Kate Miller Jeffress Memorial Trust (KLG). We thank the University of Richmond Biology Department for use of research facilities and supplies. Many thanks to Mountain Lake Biological Station (University of Virginia) for the use of supplies and space. Thanks to Laura Blackburn (US Forest Service) for mapping assistance and to Sarah Bailey for rearing assistance. Thanks to Ksenia Onufrieva, Andrea Hickman, and the STS Foundation for technical guidance. Thanks to Chelsea Jahant-Miller for comments on the manuscript. We also thank Kerry Bailey (West Virginia Department of Agriculture), Chris Elder, and Randy Copeland (NCDA & CS Gypsy Moth program) for collecting the egg masses used in these experiments. Gypsy moths were transported and housed under USDA APHIS permit Numbers P526P-14-00377 (DP), P526P-12-01012 (DJ), P526P-15-00488 (KG).

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