

# Variation in growth and developmental responses to supraoptimal temperatures near latitudinal range limits of gypsy moth *Lymantria dispar* (L.), an expanding invasive species

LILY M. THOMPSON<sup>1</sup>, TREVOR M. FASKE<sup>2</sup>, NANA BANAHENE<sup>1</sup>, DOMINIQUE GRIM<sup>2</sup>, SALVATORE J. AGOSTA<sup>2,3</sup>, DYLAN PARRY<sup>4</sup>, PATRICK C. TOBIN<sup>5</sup>, DEREK M. JOHNSON<sup>2</sup> and KRISTINE L. GRAYSON<sup>1</sup>

<sup>1</sup>Department of Biology, University of Richmond, Richmond, Virginia, U.S.A., <sup>2</sup>Department of Biology, Virginia Commonwealth University, Richmond, Virginia, U.S.A., <sup>3</sup>Center for Environmental Studies, Virginia Commonwealth University, Richmond, Virginia, U.S.A., <sup>4</sup>Department of Environmental and Forest Biology, College of Environmental Science and Forestry, State University of New York, Syracuse, New York, U.S.A. and <sup>5</sup>School of Environmental and Forest Sciences, University of Washington, Seattle, Washington, U.S.A.

**Abstract.** Variation in thermal performance within and between populations provides the potential for adaptive responses to increasing temperatures associated with climate change. Organisms experiencing temperatures above their optimum on a thermal performance curve exhibit rapid declines in function and these supraoptimal temperatures can be a critical physiological component of range limits. The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Erebidae), is one of the best-documented biological invasions and factors driving its spatial spread are of significant ecological and economic interest. The present study examines gypsy moth sourced from different latitudes across its North American range for sensitivity to high temperature in constant temperature growth chamber experiments. Supraoptimal temperatures result in higher mortality in northern populations compared with populations from the southern range extent (West Virginia and coastal plain of Virginia, U.S.A.). Sublethal effects of high temperature on traits associated with fitness, such as smaller pupal mass, are apparent in northern and West Virginia populations. Overall, the results indicate that populations near the southern limits of the range are less sensitive to high temperatures than northern populations from the established range. However, southern populations are lower performing overall, based on pupal mass and development time, relative to northern populations. This suggests that there may be a trade-off associated with decreased heat sensitivity in gypsy moth. Understanding how species adapt to thermal limits and possible fitness trade-offs of heat tolerance represents an important step toward predicting climatically driven changes in species ranges, which is a particularly critical consideration in conservation and invasion ecology.

**Key words.** Forest pest, heat tolerance, invasion front, latitudinal gradient, local adaptation, thermal performance, warming climate.

Correspondence: Kristine L. Grayson, Department of Biology, Gottwald Science Center, University of Richmond, 28 Westhampton Way, Richmond, Virginia 23173, U.S.A. Tel.: +1 840 484 1623; e-mail: kgrayson@richmond.edu

## Introduction

Physiological limits to temperature are a fundamental factor defining the distributions and range boundaries of species. As average global surface temperatures continue to rise, understanding how the range boundaries of species are altered in response to high temperatures is a fundamental concern. This is especially true for many insects, which, in addition to being a crucial component of most ecosystems, are poikilotherms that respond directly to changes in ambient temperature (Butterfield, 1996; Addo-Bediako *et al.*, 2000; Vanhanen *et al.*, 2007; Lee *et al.*, 2009; Hill *et al.*, 2011; Bennie *et al.*, 2013).

Poikilothermic organisms experience a sharp decline in developmental rate above an optimal temperature, whereas the decline in the developmental rate below this threshold is more gradual (Logan *et al.*, 1991; Kingsolver & Woods, 1997). Supraoptimal temperatures (i.e. those above the developmental optimum) can negatively affect fitness through these changes in development time, as well as by impacting fecundity and survival (Kingsolver & Woods, 1997; Deutsch *et al.*, 2008; Zizzari & Ellers, 2011). Therefore, supraoptimal temperatures form a narrow, yet highly important, component of the temperature performance curve that can impose abrupt physiological restrictions on the range of a species (Chown & Gaston, 1999; Vanhanen *et al.*, 2007; Sinclair *et al.*, 2012).

Variation in thermal performance curves within a species may allow for differential selection across a geographical range that drives evolutionary responses to temperature regimes (Ayres & Scriber, 1994). Adaptive responses to higher temperatures are important to consider in economically important species, particularly those that are non-native and invasive, because changes in climate that alter exposure to supraoptimal temperatures could open new habitats to invasion or impose environmental barriers to further spread (Logan *et al.*, 2003; Deutsch *et al.*, 2008).

The gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Erebidae) is an extensively studied forest defoliator that arrived in Massachusetts, U.S.A., from Europe in 1869. It has subsequently spread west to Minnesota, U.S.A., as far south as North Carolina, U.S.A., and north into Canada, covering approximately 13° of latitude (Tobin *et al.*, 2012, 2016). The gypsy moth detrimentally impacts forest ecosystems through periodic outbreaks that result in intensive defoliation events (Lovett *et al.*, 2006). Implementation of the Slow-the-Spread programme by the United States Department of Agriculture (USDA) is reported to have reduced the rate of gypsy moth spread in North America at the same time as providing one of the most complete range-wide species distribution datasets in existence (Sharov & Liebhold, 1998; Tobin & Blackburn, 2007).

The potential for further range expansion by gypsy moth is in large part determined by the availability of hosts and suitable climate. Gypsy moth feeds on almost 300 tree and shrub species, of which approximately 79 are considered to be primary hosts (Liebhold *et al.*, 1995). The univoltine life cycle of gypsy moth requires an obligate winter diapause and the necessary duration of cold winter temperatures to complete this diapause, as well as the susceptibility of eggs to subfreezing temperatures, is well characterized (Madrid & Stewart, 1981; Gray *et al.*, 1991; Bale, 1993; Gray, 2004). Less is known about the potential for

high temperature to limit continued southern range expansion in North America.

Recent analysis of spread rates near the southern edge of the established gypsy moth range in West Virginia and Virginia, U.S.A., reveals unexpected range dynamics occurring at the southern invasion front (Tobin *et al.*, 2014). Most notably, range retraction is occurring in a region much farther north than the predicted southern limits of the gypsy moth range based on requirements for winter diapause (Gray, 2004) and in the presence of ample host density (Tobin *et al.*, 2014). In some areas of the current southern range limit, late-stage larvae and pupae are exposed to summer temperatures that often approach or exceed the developmental optimum for this species (Logan *et al.*, 1991; Tobin *et al.*, 2014). Tobin *et al.* (2014) report that the rate of gypsy moth spread decreases as the number of hours of supraoptimal temperature (above 28 °C) increases and this is seen most clearly in the warm coastal plain of Virginia. By contrast, southern range expansion has continued unabated in the cooler Central Appalachian Mountain region of West Virginia and Virginia, whereas a pattern of stasis is evident in the climatically intermediate Piedmont region of Virginia (Tobin *et al.*, 2014). The correlation between range retraction and increased supraoptimal temperatures suggests that negative consequences of high temperature exposure could be an important factor in determining the southern range edge of gypsy moth and, in addition, continued climatic warming may further inhibit invasion in southern North America.

The majority of physiological studies on gypsy moth are performed using a single laboratory strain (Logan *et al.*, 1991; Yocum *et al.*, 1991; Lindroth *et al.*, 1997; Broderick *et al.*, 2006), which has been under continual captive rearing without outcrossing and maintained at a constant temperature environment from 1967 onward (Keena & ODell, 1994). The thermal performance curve for the laboratory strain of gypsy moth is well characterized, with peak developmental rates occurring at approximately 28 °C (Logan *et al.*, 1991). However, recent work reports that there are performance differences between laboratory strain individuals and individuals from wild populations when reared on different diets (Grayson *et al.*, 2015). In natural populations, there is the potential to adapt to warmer temperatures as gypsy moth continues to spread south in the U.S.A.; yet, no studies have compared gypsy moth performance across their wide latitudinal range or addressed the potential for local adaptation at the leading edge of the invasive range.

The present study aims to quantify geographical variation in lethal and sublethal effects of supraoptimal temperatures on growth and development of gypsy moth using natural populations sourced from the current latitudinal range in North America. The study is composed of two complementary experiments conducted in constant temperature growth chambers: one comparing performance across the latitudinal range of the gypsy moth invasion in North America and a second assessing differences specifically at the southern invasion front, where the range edge is expanding and contracting in different geographical regions. Together, these experiments increase our understanding of the role that temperature plays in limiting the potential extent of the gypsy moth range in North America.

## Materials and methods

### Study populations

Gypsy moth has one non-overlapping generation per year where the entire complement of eggs is laid in a single clutch by adult females in summer. Eggs overwinter and hatch in the subsequent spring around bud break of the preferred host plants. Overwintering egg masses were obtained from wild populations in two separate collections for use in the two experiments. Variation in local population densities, host quality and environment at the collection site can influence maternal effects and potentially confound comparisons among populations (Rossiter, 1991). To minimize maternal effects, individuals from all populations were maintained under the same temperature conditions and larvae from all populations were reared on the same diet for at least one generation before being used experimentally.

For the latitudinal experiment, populations were collected from Quebec, Canada (46.9090°N, 70.80611°W), New York, U.S.A. (43.0806°N, 75.9813°W) and North Carolina, U.S.A. (36.4491°N, 76.0247°W) in 2012. The first generation from these sources was reared outdoors on local red oak (*Quercus rubra* L.) foliage in accordance with its phenology near Syracuse, New York, U.S.A. (43.0481°N, 76.1474°W). Second-generation egg masses entered diapause in Syracuse and then were transferred to Virginia Commonwealth University, Richmond, Virginia, U.S.A., where they completed diapause in a refrigerator at 4–6 °C. After 180 days, 10–15 egg masses from each population were removed from the refrigerator, gently crumbled to release individual eggs from the mass, mixed together to maximize genetic diversity, and allowed to hatch at room temperature.

For the southern invasion front experiment, egg masses from natural populations were collected from the Appalachian mountains of West Virginia, U.S.A. (37.5462°N, 81.2184°W) and from the coastal plain of Virginia (36.6350°N, 76.5078°W) in 2013. The first generation from these sources was reared indoors with ambient light and temperature on locally-collected red oak (*Q. rubra*) foliage in accordance with its phenology in Richmond, Virginia, U.S.A. (37.5365°N, 77.4553°W). Second-generation egg masses were given 60–80 days to embryonate before entering a refrigerator at 4–6 °C to complete diapause. Egg masses spent 180 days in the cold before 20 egg masses from each population were removed from the refrigerator, mixed together as described above to maximize genetic diversity, and allowed to hatch at room temperature.

The gypsy moth larval period is relatively short, lasting approximately 6–8 weeks, which poses time constraints and other challenges for studies using natural populations. Instead of using larvae from wild populations, laboratory strains of larvae cultivated on artificial diet are often used in experiments because of the year-round availability of life stages. The primary colony available for research, maintained by the USDA, Animal and Plant Health Inspection Service (APHIS), Center for Plant Health Science and Technology (CPHST, Otis Air National Guard Base, Massachusetts), is derived from the New Jersey Standard Strain (NJSS) of gypsy moth (Keena & ODell, 1994). As a result of the widescale use of this colony in

fundamental gypsy moth research, this population was included in both experiments to provide comparisons with wild populations. Post-diapause egg masses were sourced from this laboratory colony (NJSS) within 2 weeks of the start date for each experiment.

### Response to heat across the latitudinal range

In spring 2014, larvae sourced from Quebec, Canada, New York and North Carolina, U.S.A., as well as from the NJSS, were reared individually ( $n = 40$  per population per treatment) in plastic 30-mL cups filled to half capacity with the standard wheat germ based artificial diet formulated for gypsy moth (prepared by CPHST, Otis Air National Guard Base, Massachusetts) (Bell *et al.*, 1981). When the majority of larvae across the experiment reached fourth instar, each larva was transferred to a 74-mL cup filled to half capacity with artificial diet from the same source to provide more space for growth.

Populations were compared at three constant temperature treatments: 28, 31 and 34 °C. Treatment temperatures were selected based on the conditions under which development time is fastest (28.6 °C) (Logan *et al.*, 1991) and supraoptimal temperatures that could be experienced by gypsy moth in the southern portion of their North American range. Temperatures were maintained in three Percival Growth Chambers (Model I-36VL) under an LD 14 : 10 h photocycle and a target relative humidity of 60–80%. A data logger (HOBO® Pro v2 logger, Model U23-001; Onset Computer Corp., Bourne, Massachusetts) was placed in each chamber to record temperature and humidity every 5 min. Larvae were checked daily for survival and instar transitions (molted cuticle present). Mass for each instar was recorded within 24 h of each transition and at pupation.

### Response to heat across the southern invasion front

The southern invasion front experiment was conducted in spring 2015 using the NJSS and populations sourced from the West Virginia Appalachian Mountain region and the Virginia coastal plain to test for physiological differences across this invasion front. Three temperature treatments were used, although the supraoptimal temperatures were reduced to 29 and 32 °C as a result of extremely high mortality at 34 °C in the latitudinal experiment and, instead of the temperature for optimum development time (28 °C), 26 °C was used to reflect the standard rearing temperature of NJSS. These temperatures were maintained in three Powers chambers (Model DROS33SD, Level 2 and Level 4; Powers Scientific, Inc., Warminster, Pennsylvania) under an LD 14 : 10 h photocycle and a target relative humidity of 60–80%. A data logger (HOBO® temp/RH logger, Model UX1000-003; Onset Computer Corp.) was placed in each chamber to record temperature and humidity every 5 min.

Gypsy moth larvae were reared in groups of 10 individuals on 2-mL cubes of artificial diet (USDA, Hamden Formula Gypsy Moth Diet #F9630B; Frontier Agricultural Sciences, Newark, Delaware) in plastic 74-mL cups. Our experimental unit was a cup and each temperature treatment chamber contained 10 cups

of 10 larvae for each population. Every 3–4 days, the diet was changed and survival was assessed. When the majority of larvae in a cup were fourth instar, all larvae in that cup were transferred to a plastic 177.5-mL cup with 20 mL of cubed diet.

Pupation was checked daily and sclerotized pupae were weighed, placed singly into 74-mL plastic cups with a plastic lid, and housed in their respective treatment chambers. Pupal mass is a useful surrogate for fitness because it strongly correlates with adult mass and female fecundity (Honěk, 1993; Myers *et al.*, 2000; Calvo & Molina, 2005). Pupae were checked daily for adult emergence and sex was recorded based on the distinct sexually dimorphic colouration of adults. Growth metrics for each individual include time to pupation (larval duration), pupal mass and pupal duration.

The diet for this experiment was a slightly different formulation from the latitudinal experiment; however, diet was consistent within an experiment. Although differences in diet are known to impact larval and egg characteristics (Keena *et al.*, 1995), no quantitative comparisons were made between the two experiments.

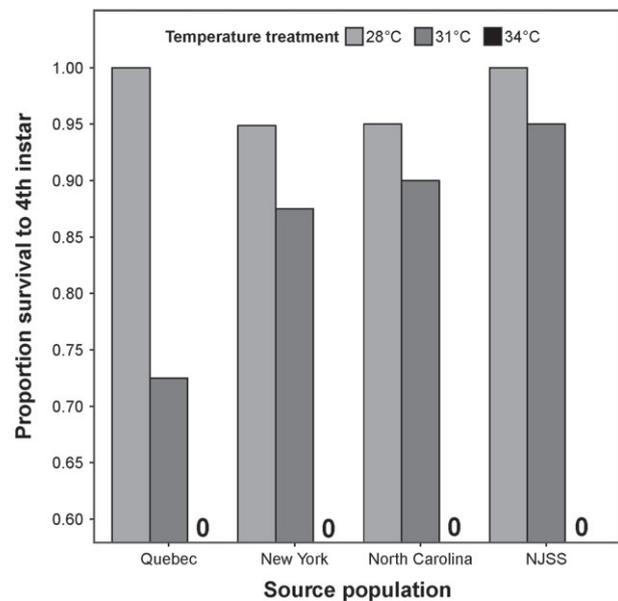
### Statistical analysis

Chamber temperatures recorded by data loggers were analyzed and all were, on average, within 0.2 °C of the target treatment temperature. Humidity in the latitudinal experiment was very high after fourth instars were moved to 74-mL cups resulting in some pupal malformations. Therefore, the fourth instar is used as the end point of the latitudinal experiment.

Survival for the latitudinal experiment was analyzed using a logistic regression (*car* package in R, version 3.3.1) to examine differences among source populations and temperature in the number of individuals surviving to fourth instar. Survival for the southern invasion front experiment based on source population and temperature treatment was analyzed using a linear mixed model (*lme4* package in R, version 3.3.1) with a random effect of cup. The dependent variable was the arc sine square root transformation of the proportion alive per cup regardless of sex.

All growth metrics were analyzed separately for each experiment to determine the effects of source population, temperature treatment, and the interaction of population and treatment. Independent two-way ANOVAs (*stats* package in R, version 3.3.1) were used to test for variation in fourth-instar mass and time to fourth instar for the latitudinal experiment. Degrees of freedom were calculated using a Satterthwaite approximation to account for unequal sample sizes as a result of mortality. To avoid violating assumptions of normality, fourth-instar mass was transformed using natural log.

Growth metrics from the southern invasion front experiment were analyzed as cup means in a two-way generalized mixed effects model (*lme4* package in R, version 3.3.1) with fixed effects of population and treatment and a random effect of number of individuals per cup. Male and female growth metrics for the southern invasion front experiment were analyzed independently because of the sexual dimorphism typical at late developmental stages of gypsy moth. Satterthwaite approximation of degrees of freedom was used to account for unequal sample sizes



**Fig. 1.** Proportion of larvae surviving to the fourth instar for each population at each temperature in the latitudinal experiment. Each bar represents the number of individuals alive divided by the total number of individuals in each treatment and population combination ( $n = 40$ ). The light grey bars represent 28 °C and the darker grey bars represent 31 °C. The temperature treatment at 34 °C had 100% mortality.

as a result of mortality in the analyses of pupal mass, larval duration and pupal duration.

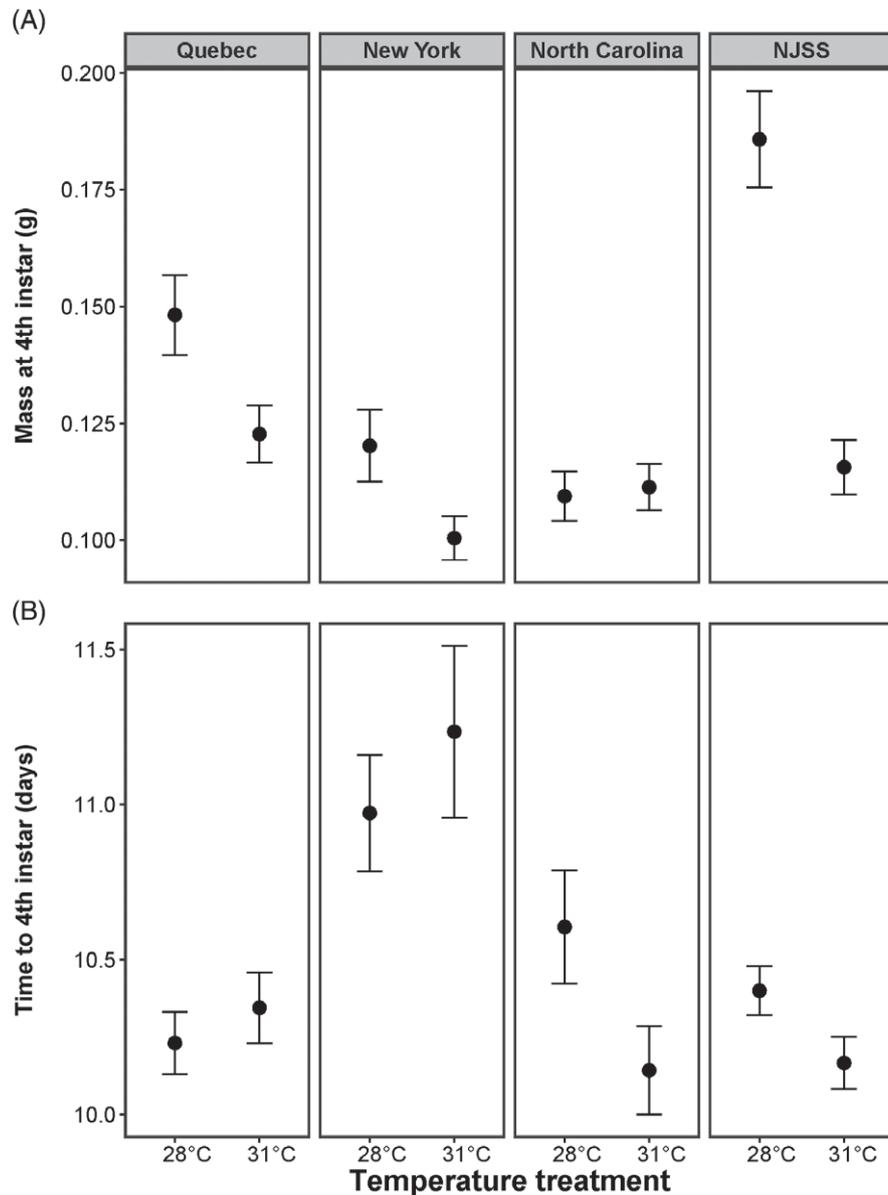
## Results

### Response to heat across the latitudinal range

Temperature significantly impacted survival in this experiment (Wald  $\chi^2 = 7.5390$ ,  $P = 0.006$ ). All populations had 94–100% survival at 28 °C, whereas all larvae died before the fourth instar at 34 °C. Survival at 31 °C increased with a decreasing latitude of the population source (Quebec = 72.5%, New York = 87.5%, North Carolina = 90%; Wald  $\chi^2 = 9.7557$ ,  $P = 0.021$ ). At 31 °C, the NJSS laboratory population had the highest survival (95%) (Fig. 1).

Source population and temperature had an interactive effect on the mass of fourth-instar larvae ( $F = 8.008$ , d.f. = 3,243.21,  $P < 0.001$ ). The NJSS laboratory strain attained much greater mass than all other populations at 28 °C and a greater or equivalent mass relative to all other populations at 31 °C. Differences between mass at 28 and 31 °C were most pronounced for the NJSS population. All populations had a smaller mass at 31 °C than at 28 °C, except for the North Carolina population, for which the masses were equivalent at both temperature treatments (Fig. 2A).

Source population had a significant effect on the amount of time that it took larvae to reach fourth instar ( $F = 12.3744$ , d.f. = 3,280,  $P < 0.001$ ), although there was no significant effect of temperature treatment, nor a significant interaction between



**Fig. 2.** Mass at fourth instar (A) and time to reach fourth instar (B) in the latitudinal experiment. Each point represents the mean  $\pm$  SE for each temperature treatment and population combination ( $n = 29\text{--}40$ ). The temperature treatment at 34 °C was not included because of 100% mortality.

the main effects of population and temperature treatment. Larvae from the New York population took an average of 1 day longer to reach fourth instar relative to any other population (Fig. 2B).

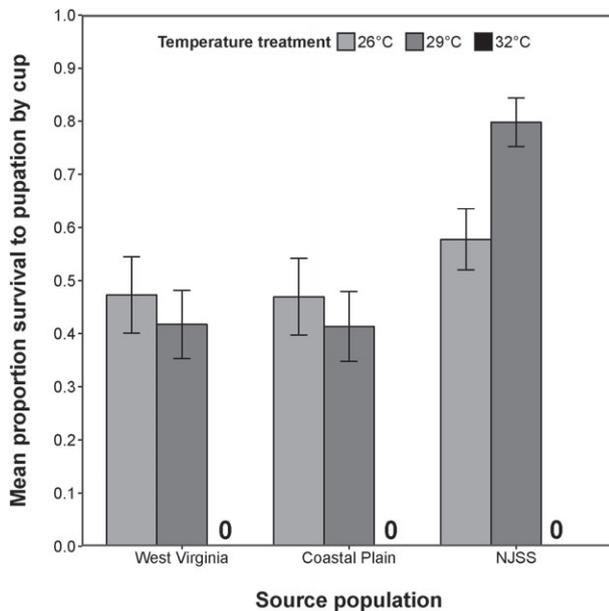
#### *Response to heat across the southern invasion front*

Source population and temperature had an interactive effect on survival among populations (NJSS, West Virginia and Virginia coastal plain;  $F = 5.3206$ , d.f. = 4,81,  $P < 0.001$ ). West Virginia and Virginia coastal plain populations exhibited similar survival probabilities based on cup means, each with approximately 47% survival at 26 °C and approximately 41% survival at 29 °C. The

NJSS strain generally had higher survival than the wild sourced populations and had higher survival at 29 °C than at 26 °C (72% and 57% average cup survival, respectively) (Fig. 3). All larvae reared at 32 °C died before pupation.

Source population but not temperature was a significant predictor of female pupal mass ( $F = 3.352$ , d.f. = 2,54,  $P = 0.04$ ). There was a significant interaction between temperature treatment and source population for male pupal mass ( $F = 4.265$ , d.f. = 2,50,  $P = 0.03$ ). For both sexes, pupal mass was greater or equal at 26 °C compared with 29 °C (Fig. 4A).

Females reared at 29 °C took less time to pupate than those reared at 26 °C ( $F = 18.704$ , d.f. = 1,54,  $P < 0.001$ ) and source population had a significant effect on female larval duration



**Fig. 3.** Proportion of individuals surviving to pupation in the southern invasion front experiment. Cup means were calculated by dividing the number of adults by the sum of the number of adults and the number of recorded deaths for each population at each temperature ( $n = 10$  cups of 10 individuals). The light grey points represent 26 °C and the darker grey points represent 29 °C. The temperature treatment at 32 °C had 100% mortality.

( $F = 34.564$ , d.f. = 2,54,  $P < 0.001$ ). Source population but not treatment temperature significantly affected larval duration for males ( $F = 40.743$ , d.f. = 2,50,  $P < 0.001$ ) (Fig. 4B).

## Discussion

The present study highlights the role of high summer temperatures in shaping the range limits of the invasive gypsy moth in North America. Specifically, abrupt declines in survivorship and performance across a narrow range of supraoptimal temperatures demonstrate the sensitivity of gypsy moth to small differences in temperature. Relative to long-established northern populations, individuals in the present study from populations along the currently established southern range front have superior performance at constant supraoptimal temperatures. These results have important implications for gypsy moth risk assessments and projections of its future range expansion in North America.

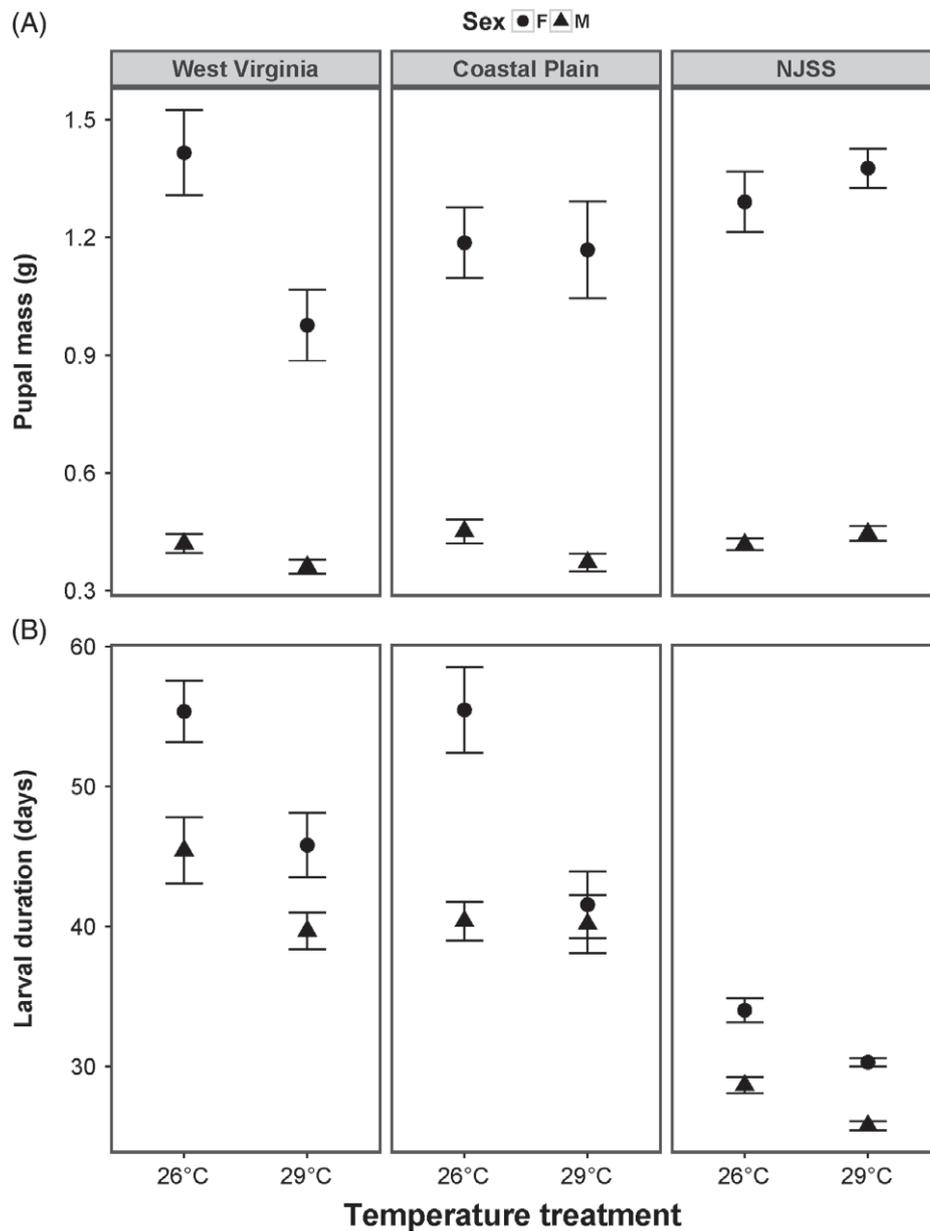
Across temperature treatments for all populations in both experiments, survival generally decreases with increasing temperature, and the hottest treatment temperature tested is lethal to all individuals from any population. In both experiments, final mass (fourth instar or pupal mass) is lower for northern populations at higher temperatures, which can lead to decreased fecundity (Honěk, 1993; Myers *et al.*, 2000; Calvo & Molina, 2005). Interestingly, this effect is not seen in the pupal mass of males from West Virginia or the pupal mass of males and females from the Virginia coastal plain. Collectively, this suggests that high

temperatures do not reduce fitness for the Virginia coastal plain population to the same extent as more northerly located populations, a pattern that may reflect an adaptive response to the higher temperatures at the southern range edge.

Logan *et al.* (1991) report the fastest larval developmental times at approximately 28 °C when using the NJSS population. In both the latitudinal and southern invasion front experiments, mean development time for the NJSS population is consistent with the developmental times reported by Logan *et al.* (1991). The New York population in the latitudinal experiment also develops at a rate consistent with the findings of Logan *et al.* (1991); in contrast, the other populations develop faster or similarly at supraoptimal temperatures. Methodological limitations of the latitudinal and southern invasion front experiments may explain the mixed results. For example, using fourth instar as the terminal stage of development in the latitudinal experiment likely compressed the magnitude of differences in development across temperature treatments by excluding effects on later instars and pupae. Additionally, the 24-h intervals between observations are too coarse to detect differences in development occurring at finer time scales. In the southern invasion front experiment, a faster development time at 29 °C compared with 26 °C could be because 29 °C is close to the optimal temperature (28.6 °C) (Logan *et al.*, 1991). An important caveat of optimal developmental temperature is that, although the development rate is maximized at this temperature, other performance traits important for survival and fecundity may be maximized at other temperatures. For example, it is plausible that another temperature, likely one lower than the optimum of 28 °C for developmental rate (Logan *et al.*, 1991), may result in the largest pupal mass. This is consistent with the result of larger pupal mass at 26 °C compared with 29 °C in the southern invasion front experiment.

The results from the present study reinforce a recent finding that wild populations perform differently from the NJSS population (Grayson *et al.*, 2015). These differences are not surprising considering the NJSS laboratory strain of gypsy moth is reared on artificial diet, having been in isolation for more than 70 generations, and is selected for rapid development and reproductive capacity, a desirable trait for mass production (Keena & ODell, 1994). The NJSS population has likely never experienced supraoptimal temperatures subsequent to its initial founding, yet it has higher survival at 29 and 31 °C than all other populations, even those from the southern range extent that have historically experienced these temperatures. This relative success may be attributed to NJSS performing better when reared on artificial diet than the wild sourced populations (Grayson *et al.*, 2015).

Lower performance at higher temperatures in the present study provides a mechanistic explanation for the results of Tobin *et al.* (2014), where supraoptimal temperatures (i.e. those above 28 °C) are associated with gypsy moth range retraction at its southern range front (Virginia, U.S.A.). The increase in performance of the southern sourced populations at higher temperatures compared with the northern populations may be indicative of local adaptation in these areas through widening or shifting the breadth of their thermal performance curve (Huey & Kingsolver, 1993; Sinclair *et al.*, 2012). There is little difference in survival observed between gypsy moths sourced from West



**Fig. 4.** Pupal mass (A) and larval duration (B) for both sexes (female, circle; male, triangle) in the southern invasion front experiment. Each point represents the mean  $\pm$  SE of cup means for each temperature treatment and population ( $n = 10$  cups of one to seven individuals). The temperature treatment at 32°C was not included because of 100% mortality.

Virginia, U.S.A. and the coastal plain of Virginia, U.S.A. when exposed to supraoptimal temperatures. This could imply that local adaptation to supraoptimal temperature is occurring on a latitudinal scale but is not, or is less detectable, at the smaller regional scale of the southern invasion front. Although pupal mass in southern populations does not vary significantly with temperature, it is smaller overall, suggesting that fecundity of southern populations may be lower than more northerly populations. Thus, there may be a fitness trade-off associated with adaptation to high temperatures that results in a lower overall performance (Huey & Kingsolver, 1993).

Generalist herbivores, such as gypsy moth, could be overlooked when studying climate and habitat change because their wide range of potential host trees is assumed to represent generally high plasticity, which may provide a buffer against environmental change. Even in widely distributed generalist species, however, climate change can alter insect–host plant interactions (Uelmen *et al.*, 2016). The data from this study indicate that there may be geographical differences in how gypsy moth populations across North America respond to temperature. This is consistent with other studies demonstrating local adaptation in populations near range edges, even when gene

flow is limited (Dlugosch & Parker, 2008; Sexton *et al.*, 2009; Hill *et al.*, 2011).

Although southern populations demonstrate the potential for local adaptation to supraoptimal temperatures, extrapolating results from constant temperature environmental chambers to complex natural environments should be carried out with caution. For example, it is unclear whether a subset of performance measures derived in an artificial laboratory setting is representative of the complex spatiotemporal interactions with climate found in natural gypsy moth populations. Conceivably, using fluctuating temperatures that more closely reflect natural conditions could be a better basis for understanding whether the survival and performance implications of the present study translate to perceptible differences in the wild (Fischer *et al.*, 2011). Quantifying population-specific thermal performance curves would, additionally, provide increased precision for region specific models of population performance. Lastly, more direct metrics of fitness, such as egg production and hatching viability, would more accurately capture the effects of supraoptimal temperatures on gypsy moth performance.

Upper thermal limits can be an important factor in shaping range boundaries of invasive insects and it is critical to understand how these organisms will respond to a warming climate. Previous studies in insects show that warmer temperatures can facilitate range expansion in some species (Hagen *et al.*, 2007; de la Giroday *et al.*, 2012), potentially accelerate invasion speed in others (Seiter & Kingsolver, 2013; Lantschner *et al.*, 2014), as well as negatively affect populations of other species (Hickling *et al.*, 2005; Esper *et al.*, 2007; Johnson *et al.*, 2010; Haynes *et al.*, 2014). This variation in thermal response prohibits generalizing the response of invasive insects to climate change and necessitates further research into how individual species and populations will perform in a warming environment.

Studies that examine range-wide response to climate often utilize latitudinal gradients to test for local adaptation across geographical ranges (Ayres & Scriber, 1994; Parry *et al.*, 2001; Santamaría *et al.*, 2003; Schmidt *et al.*, 2005; Zakharov & Hellmann, 2008; Lieferting *et al.*, 2009). The experiments reported here emphasize range edge populations of gypsy moth and the effect of supraoptimal temperatures on performance. For an invasive species such as gypsy moth, the interplay of demographic and adaptive processes at the range boundaries drives further spread. Therefore, the present study focuses primarily on representative populations from these areas, instead of sampling across the entire geographical range.

The gypsy moth is considered classic example of a generalist, invasive species whose success is a result, in part, of plasticity in performance. Few studies in the gypsy moth consider the potential for adaptation in range-edge populations. Yet, evolution occurring at edges of expansion is common both in insect and other systems (Butterfield, 1996; Phillips *et al.*, 2006; Dlugosch & Parker, 2008; Kunin *et al.*, 2009; Sexton *et al.*, 2009; Hill *et al.*, 2011). Adaptive shifts in physiological tolerance could have large impacts for invasion potential and future spread, particularly in the context of climate change. Studies of heat tolerance in gypsy moth provide not only information to facilitate better informed management decisions, but also insight into how

range dynamics could be altered by climate change for other invasive insects and declining native species.

## Acknowledgements

We are grateful for the generous support of the Biology Departments at Virginia Commonwealth University and the University of Richmond. We thank the USDA APHIS CPHST Laboratory, particularly Hannah Nadel and Christine McCallum, for providing NJSS gypsy moth eggs. We also thank the following individuals for providing the egg masses used in these experiments: Ken Gooch (Massachusetts Department of Conservation and Recreation), Louis Morneau (Ministère des Ressources Naturelles et de la Faune du Québec), Kerry Bailey (West Virginia Department of Agriculture), Chris Elder, and Randy Copeland (NCDA & CS Gypsy Moth program). We are grateful to Diane Jennings at Virginia Commonwealth University for the use of environmental chambers and research space. Special thanks are extended to Katrina Khalil, Kennesha Myrick, Joseph Neale, Taylor Price, Melisa Quiroga-Herrera, Sharon Woosley and Amber Yang for their diligent assistance in rearing gypsy moths used for the present study. Funding for this research was provided by the USDA National Institute of Food and Agriculture (Grant Number 2014-67012-23539 to K.L.G.), the United States Forest Service (Grant Number 13-CA-11420004-231 to D.M.J.), the Virginia Commonwealth University Presidential Research Quest Fund (to S.J.A.) and the Gypsy Moth Slow-the-Spread Foundation, Inc. (Grant Number A106307 to P.C.T.). Additional support was provided by the HHMI undergraduate program (Grant Number 52007567) and the School of Arts and Sciences at the University of Richmond. The authors declare that they have no conflicts of interest. There are no ownership disputes of the data reported in the present study and all contributions have been properly attributed.

## References

- Addo-Bediako, A., Chown, S.L. & Gaston, K.J. (2000) Thermal tolerance, climatic variability and latitude. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **267**, 739–745.
- Ayres, M.P. & Scriber, J.M. (1994) Local adaptation to regional climates in *Papilio canadensis* (Lepidoptera: Papilionidae). *Ecological Monographs*, **64**, 465–482.
- Bale, J.S. (1993) Classes of insect cold hardiness. *Functional Ecology*, **7**, 751–753.
- Bell, R.A., Owens, C.D., Shapiro, M. & Tardif, J.R. (1981) Mass rearing and virus production. *The Gypsy Moth: Research Towards Integrated Pest Management*. Technical Bulletin 1581 (ed. by C.C. Doane and M.L. McManus), pp. 599–655. United States Forest Service, U.S. Department of Agriculture, Washington, District of Columbia.
- Bennie, J., Hodgson, J.A., Lawson, C.R. *et al.* (2013) Range expansion through fragmented landscapes under a variable climate. *Ecology Letters*, **16**, 921–929.
- Broderick, N.A., Raffa, K.F. & Handelsman, J. (2006) Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 15196–15199.
- Butterfield, J. (1996) Carabid life-cycle strategies and climate change: a study on an altitude transect. *Ecological Entomology*, **21**, 9–16.

- Calvo, D. & Molina, J.M. (2005) Fecundity-body size relationship and other reproductive aspects of *Streblote panda* (Lepidoptera: Lasiocampidae). *Annals of the Entomological Society of America*, **98**, 191–196.
- 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.
- Deutsch, C.A., Tewksbury, J.J., Huey, R.B. *et al.* (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 6668–6672.
- Dlugosch, K.M. & Parker, I.M. (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, **17**, 431–449.
- Esper, J., Büntgen, U., Frank, D.C. *et al.* (2007) 1200 years of regular outbreaks in Alpine insects. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **274**, 671–679.
- Fischer, K., Kölzow, N., Höltje, H. & Karl, I. (2011) Assay conditions in laboratory experiments: is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? *Oecologia*, **166**, 23–33.
- de la Giroday, H.C., Carroll, A.L. & Aukema, B.H. (2012) Breach of the northern Rocky Mountain geoclimatic barrier: initiation of range expansion by the mountain pine beetle. *Journal of Biogeography*, **39**, 1112–1123.
- 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**, 155–171.
- 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 pre-diapause development. *Environmental Entomology*, **20**, 1645–1652.
- Grayson, K.L., Parry, D., Faske, T.M. *et al.* (2015) Performance of wild and laboratory-reared gypsy moth (Lepidoptera: Erebidae): a comparison between foliage and artificial diet. *Environmental Entomology*, **44**, 864–873.
- Hagen, S.B., Jepsen, J.U., Ims, R.A. & Yoccoz, N.G. (2007) Shifting altitudinal distribution of outbreak zones of winter moth *Operophtera brumata* in sub-arctic birch forest: a response to recent climate warming? *Ecography*, **30**, 299–307.
- Haynes, K.J., Allstadt, A.J. & Klimetzek, D. (2014) Forest defoliator outbreaks under climate change: effects on the frequency and severity of outbreaks of five pine insect pests. *Global Change Biology*, **20**, 2004–2018.
- Hickling, R., Roy, D.B., Hill, J.K. & Thomas, C.D. (2005) A northward shift of range margins in British Odonata. *Global Change Biology*, **11**, 502–506.
- Hill, J.K., Griffiths, H.M. & Thomas, C.D. (2011) Climate change and evolutionary adaptations at species' range margins. *Annual Review of Entomology*, **56**, 143–159.
- Honěk, A. (1993) Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, **66**, 483–492.
- Huey, R.B. & Kingsolver, J.G. (1993) Evolution of resistance to high temperature in ectotherms. *The American Naturalist*, **142**, S21–S46.
- Johnson, D.M., Büntgen, U., Frank, D.C. *et al.* (2010) Climatic warming disrupts recurrent Alpine insect outbreaks. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 20576–20581.
- Keena, M. A. & Odell, T.M. (1994) *Effects of Laboratory Rearing on Gypsy Moth (Lepidoptera: Lymantriidae)*. General Technical Report No NE-181. Northeastern Forest Experiment Station, United States Forest Service, Newtown Square, Pennsylvania.
- Keena, M.A., Odell, T.M. & Tanner, J.A. (1995) Effects of diet ingredient source and preparation method on larval development of laboratory-reared gypsy moth (Lepidoptera, Lymantriidae). *Annals of the Entomological Society of America*, **88**, 672–679.
- Kingsolver, J.G. & Woods, H.A. (1997) Thermal sensitivity of growth and feeding in *Manduca sexta* caterpillars. *Physiological Zoology*, **70**, 631–638.
- Kunin, W.E., Vergeer, P., Kenta, T. *et al.* (2009) Variation at range margins across multiple spatial scales: environmental temperature, population genetics and metabolomic phenotype. *Proceedings of the Royal Society of London Series B, Biological Sciences*, **276**, 1495–1506.
- Lantschner, M.V., Villacide, J.M., Garnas, J.R. *et al.* (2014) Temperature explains variable spread rates of the invasive woodwasp *Sirex noctilio* in the southern hemisphere. *Biological Invasions*, **16**, 329–339.
- Lee, J.E., Janion, C., Marais, E. *et al.* (2009) Physiological tolerances account for range limits and abundance structure in an invasive slug. *Proceedings of the Royal Society of London B Biological Sciences*, **276**, 1459–1468.
- Liebholt, A.M., Gottschalk, K.W., Muzika, R-M. *et al.* (1995) *Suitability of North American tree species to the gypsy moth: a summary of field and laboratory tests*. General Technical Report No NE-211. Northeastern Forest Experiment Station, United States Forest Service, Newtown Square, Pennsylvania.
- Liefting, M., Hoffmann, A.A. & Ellers, J. (2009) Plasticity versus environmental canalization: population differences in thermal responses along a latitudinal gradient in *Drosophila serrata*. *Evolution*, **63**, 1954–1963.
- Lindroth, R.L., Klein, K.A. *et al.* (1997) Variation in temperature and dietary nitrogen affect performance of the gypsy moth (*Lymantria dispar* L.). *Physiological Entomology*, **22**, 55–64.
- Logan, J.A., Casagrande, R.A. & Liebhold, A.M. (1991) Modeling environment for simulation of gypsy moth (Lepidoptera: Lymantriidae) larval phenology. *Environmental Entomology*, **20**, 1516–1525.
- Logan, J.A., Régnière, J. & Powell, J.A. (2003) Assessing the impacts of global warming on forest pest dynamics. *Frontiers in Ecology and the Environment*, **1**, 130–137.
- Lovett, G.M., Canham, C.D., Arthur, M.A. *et al.* (2006) Forest ecosystem responses to exotic pests and pathogens in eastern North America. *BioScience*, **56**, 395–405.
- 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.
- Myers, J.H., Malakar, R. & Cory, J.S. (2000) Sublethal nucleopolyhedrovirus infection effects on female pupal weight, egg mass size, and vertical transmission in gypsy moth (Lepidoptera: Lymantriidae). *Environmental Entomology*, **29**, 1268–1272.
- Parry, D., Goyer, R.A. & Lenhard, G.J. (2001) Macrogeographic clines in fecundity, reproductive allocation, and offspring size of the forest tent caterpillar, *Malacosoma disstria*. *Ecological Entomology*, **26**, 281–291.
- Phillips, B.L., Brown, G.P., Webb, J.K. & Shine, R. (2006) Invasion and the evolution of speed in toads. *Nature*, **439**, 803.
- Rossiter, M.C. (1991) Maternal effects generate variation in life history: consequences of egg weight plasticity in the gypsy moth. *Functional Ecology*, **5**, 386.
- Santamaría, L., Figuerola, J., Pilon, J.J. *et al.* (2003) Plant performance across latitude: the role of plasticity and local adaptation in an aquatic plant. *Ecology*, **84**, 2454–2461.
- Schmidt, P.S., Matzkin, L., Ippolito, M. & Eanes, W.F. (2005) Geographic variation in diapause incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. *Evolution*, **59**, 1721–1732.

- Seiter, S. & Kingsolver, J. (2013) Environmental determinants of population divergence in life-history traits for an invasive species: climate, seasonality and natural enemies. *Journal of Evolutionary Biology*, **26**, 1634–1645.
- Sexton, J.P., McNyre, P.J., Angert, A.L. & Rice, K.J. (2009) Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 415–436.
- Sharov, A. & Liebhold, A. (1998) Model of slowing the spread of gypsy moth (Lepidoptera: Lymantriidae) with a barrier zone. *Ecological Applications*, **8**, 1170–1179.
- Sinclair, B.J., Williams, C.M. & Terblanche, J.S. (2012) Variation in thermal performance among insect populations. *Physiological and Biochemical Zoology*, **85**, 594–606.
- Tobin, P.C. & Blackburn, L.M. (2007) *Slow the Spread: A National Program to Manage the Gypsy Moth*. General Technical Report No NRS-6. Northeastern Forest Experiment Station, United States Forest Service, Newtown Square, Pennsylvania.
- Tobin, P.C., Bai, B.B., Eggen, D.A. & Leonard, D.S. (2012) The ecology, geopolitics, and economics of managing *Lymantria dispar* in the United States. *International Journal of Pest Management*, **58**, 195–210.
- 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**, 813–823.
- Tobin, P.C., Cremers, K.T., Hunt, L. & Parry, D. (2016) All quiet on the western front? Using phenological inference to detect the presence of a latent gypsy moth invasion in northern Minnesota. *Biological Invasions*, **18**, 3561–3573.
- Uelmen, J.A., Lindroth, R.L., Tobin, P.C. *et al.* (2016) Effects of winter temperatures, spring degree-day accumulation, and insect population source on phenological synchrony between forest tent caterpillar and host trees. *Forest Ecology and Management*, **362**, 241–250.
- Vanhanen, H., Veteli, T.O., Päävinen, S. *et al.* (2007) Climate change and range shifts in two insect defoliators: gypsy moth and nun moth – a model study. *Silva Fennica*, **41**, 621–638.
- Yocum, G.D., Joplin, K.H. & Denlinger, D.L. (1991) Expression of heat shock proteins in response to high and low temperature extremes in diapausing pharate larvae of the gypsy moth, *Lymantria dispar*. *Archives of Insect Biochemistry and Physiology*, **18**, 239–249.
- Zakharov, E.V. & Hellmann, J.J. (2008) Genetic differentiation across a latitudinal gradient in two co-occurring butterfly species: revealing population differences in a context of climate change. *Molecular Ecology*, **17**, 189–208.
- Zizzari, Z.V. & Eilers, J. (2011) Effects of exposure to short-term heat stress on male reproductive fitness in a soil arthropod. *Journal of Insect Physiology*, **57**, 421–426.

Accepted 13 February 2017