7-11-2018

Geographic Variation in Larval Metabolic Rate Between Northern and Southern Populations of the Invasive Gypsy Moth

Carolyn May
University of Richmond

Noah Hillerbrand
University of Richmond

Lily M. Thompson
University of Richmond, lthomps2@richmond.edu

Trevor M. Faske

Eloy Martinez

See next page for additional authors

Recommended Citation

May, Carolyn, Noah Hillerbrand, Lily M. Thompson, Trevor M. Faske, Eloy Martinez, Dylan Perry, Salvatore J. Agosta, and Kristine L. Grayson. "Geographic Variation in Larval Metabolic Rate Between Northern and Southern Populations of the Invasive Gypsy Moth." *Journal of Insect Science* 18, no. 4 (July 2018): 1-7.

Journal of Insect Science, (2018) 18(4): 5; 1–7
doi: 10.1093/jisesa/iey068
Research

Geographic Variation in Larval Metabolic Rate Between Northern and Southern Populations of the Invasive Gypsy Moth

Carolyn May,[1] Noah Hillerbrand,[1] Lily M. Thompson,[1] Trevor M. Faske,[2] Eloy Martinez,[3] Dylan Parry,[4] Salvatore J. Agosta,[2,5] and Kristine L. Grayson[1,6]

[1]Department of Biology, University of Richmond, 28 Westhampton Way, Richmond, VA 23173, [2]Department of Biology, Virginia Commonwealth University, 1000 West Cary Street, Richmond, VA 23284, [3]Department of Biological Sciences, Eastern Illinois University, 600 Lincoln Avenue, Charleston, IL 61920, [4]Department of Environmental and Forest Biology, State University of New York, College of Environmental Science and Forestry, 109 Illick Hall, 1 Forestry Drive, Syracuse, NY 13210, [5]Center for Environmental Studies, Virginia Commonwealth University, 1000 West Cary Street, Richmond, VA 23284, and [6]Corresponding author, e-mail: kgrayson@richmond.edu

Subject Editor: Louis Hesler

Received 29 March 2018; Editorial decision 25 June 2018

Abstract

Thermal regimes can diverge considerably across the geographic range of a species, and accordingly, populations can vary in their response to changing environmental conditions. Both local adaptation and acclimatization are important mechanisms for ectotherms to maintain homeostasis as environments become thermally stressful, which organisms often experience at their geographic range limits. The spatial spread of the gypsy moth (*Lymantria dispar* L.) (Lepidoptera: Erebidae) after introduction to North America provides an exemplary system for studying population variation in physiological traits given the gradient of climates encompassed by its current invasive range. This study quantifies differences in resting metabolic rate (RMR) across temperature for four populations of gypsy moth, two from the northern and two from southern regions of their introduced range in North America. Gypsy moth larvae were reared at high and low thermal regimes, and then metabolic activity was monitored at four temperatures using stop-flow respirometry to test for an acclimation response. For all populations, there was a significant increase in RMR as respirometry test temperature increased. Contrary to our expectations, we did not find evidence for metabolic adaptation to colder environments based on our comparisons between northern and southern populations. We also found no evidence for an acclimation response of RMR to rearing temperature for three of the four pairwise comparisons examined. Understanding the thermal sensitivity of metabolic rate in gypsy moth, and understanding the potential for changes in physiology at range extremes, is critical for estimating continued spatial spread of this invasive species both under current and potential future climatic constraints.

Key words: invasive species, metabolism/life process, physiological ecology, thermal biology

As invasive species spread across landscapes, their range may encompass increasingly novel habitats that impose divergent climatic constraints. Physiological tolerance to temperature is often a driving factor determining range limits, particularly in ectothermic species such as insects (Vanhanen et al. 2007, Sexton et al. 2009, Sinclair et al. 2012). As a species expands its latitudinal range, populations at the northern and southern margins are exposed to diverging thermal regimes that may impact physiological performance. In response, selection may drive adaptive change and increased performance under local conditions (Addo-Bediako et al. 2000, Castañeda et al. 2004, Chown and Terblanche 2007, Angilletta 2009). Local adaptation to temperature in invasive species has been found in several insect systems, where some populations have accrued significant physiological or morphological changes after only a few decades (e.g., springtails: Chown et al. 2007, *Fiorinia externa* (Ferris) (Hemiptera: Diaspididae): Preisser et al. 2008, *Drosophila subobscura*: Huey and Pascual 2009).

In addition to adaptive responses among populations, acclimation to current environmental conditions through behavioral or physiological plasticity may also be advantageous in range edge populations, as well as being generally important for species in seasonal environments (e.g., Rako and Hoffmann 2006, Rajamohan and Sinclair 2008, Ransberry et al. 2011, Colinet and Hoffmann 2012). Indeed, invasive species are often thought to be successful in novel habitats because of their capacity for tolerance and inherently greater plasticity (Sultan 2001, Parker et al. 2003, Yeh and

Price 2004, Davidson et al. 2011). Given that further spread by many invasive species may largely be a function of abiotic limitation, particularly climate, they can serve as useful systems for testing the interplay of acclimation and adaptation across environmental gradients.

The expansion of the gypsy moth (*Lymantria dispar* L.) (Lepidoptera: Erebidae) across Eastern North America is one of the best studied invasions and its spatial spread has been well documented (Tobin et al. 2012, Grayson and Johnson 2018). It was first introduced to the United States after accidental release in Medford, Massachusetts in 1869, and the source has been shown to originate from western Europe (Wu et al. 2015), where the gypsy moth is found climates with sufficient cold for its obligate overwinter diapause (Tauber et al. 1990). The gypsy moth has spread as far south as coastal North Carolina and north to Quebec, Canada (Liebhold et al. 1992, Sharov et al. 2002). Although gypsy moth likely experienced a genetic bottleneck at introduction (Bogdanowicz et al. 1997, Wu et al. 2015), its rapid increase in population size combined with the diverse thermal regimes it now inhabits provides the opportunity for population divergence and local adaptation in physiological traits. While the thermal performance of a laboratory strain of gypsy moth has been well characterized (Logan et al. 1991), few studies have evaluated population variation in ecologically important traits among wild populations across the invasive range (but see Grayson et al. 2015 and Thompson et al. 2017).

Metabolism is an important metric of organismal performance (Chown and Terblanche 2006), and can provide insights into bioenergetic processes (Martinez et al. 2017). For ectotherms, metabolic rate, as well as all other biological processes, is dependent upon environmental temperature (Chown and Gaston 1999, Angilletta 2009). A wide variety of studies have examined interspecific variation in the thermal sensitivity of metabolic rate, particularly in the context of comparative analyses across wide latitudinal gradients (e.g., Steffensen et al. 1994, Irlich et al. 2009). The metabolic cold adaptation (MCA) hypothesis predicts that organisms from colder climates will have higher metabolic rates at a given temperature than those from warmer locations (Addo-Bediako et al. 2002). According to the MCA, higher metabolic rate is adaptive in localities where the organism must complete development, reproduction, and growth under thermally constrained conditions such as low degree-day accumulation or compressed growing seasons. Empirical studies examining differences in metabolic rate among species have found mixed support for this hypothesis (e.g., Addo-Bediako et al. 2002). Fewer studies have examined metabolic rate variation among populations across a range of environmental conditions; those that have found inconsistent support for higher metabolic rates in more northern populations (Scholander et al. 1953, Nielsen et al. 1999, Lardies et al. 2004). In thermal acclimation experiments, it is hypothesized that metabolism will follow predictions that align with the MCA hypothesis, where acclimation to colder rearing temperatures results in metabolic compensation to increase the performance of individuals in a cold environment (Angilletta 2009, Chown et al. 2016).

We used gypsy moth populations from the northern and southern portions of its invasive range in North America to test for intraspecific variation and acclimation in larval metabolic rate in response to temperature. Two separate experiments were conducted, each comparing different northern and southern populations. In accordance with the MCA hypothesis, we predicted that populations from colder, more northern climates would have higher metabolic rates at a given temperature compared with those from warmer, more southern climates. Within all populations, we predicted that at a given assay temperature, larvae acclimated to lower temperatures would have higher metabolic rates than those acclimated to higher temperatures. Examining variation in thermal sensitivity of metabolic rate in response to climate among and within North American gypsy moth populations can provide evidence for the roles of local adaptation and acclimation in the invasion process.

Methods

Study Populations

Gypsy moths are univoltine, with one generation per year. The entire reproductive compliment of 200–1,000 eggs are deposited as a single mass by adult females in summer, which then overwinter and hatch the following spring. The populations used in our experiments were obtained from two separate field collections of egg masses. Three of the populations used in this study were collected from the field in 2012 from Milford, Massachusetts (MA; 42.1399° N, 71.5163° W), Quebec, Canada (QC; 46.9090° N, 70.80611° W), and North Carolina (NC; 36.4491° N, 76.0247° W). The MA population is located 72 km from the original introduction site of the gypsy moth invasion. A population from the Great Dismal Swamp in the Coastal Plain of Virginia (VA; 36.6350° N, 76.5078° W) was collected in 2013.

To obtain climatological data for our source populations, we downloaded daily maximum and minimum air temperatures for each collection site from 1980 to 2015 from NASA's Daily Surface Weather and Climatological Summaries (DAYMET) database (Thornton et al. 1997). We calculated monthly means for daily maximum and minimum temperatures over this 35-yr period. To determine the relevant period for larval development in each location, we used seasonal estimates of gypsy moth life stage timing based on the Gray phenology model (Gray 2009) interpolated with yearly NOAA temperature and precipitation data over a digital elevation model using BioSIM software (Régnière and Sharov 1998). We extracted 10 yr of estimated dates for the 5th percentile of egg hatch and 95th percentile of male moth flight from data available from the Slow-the-Spread Program for 2003–2012 (Slow-the-Spread Decision Support System 2018).

The MA, QC, and NC populations were reared outdoors under ambient temperatures on local red oak foliage (*Quercus rubra* L.) at Lafayette Road Field Station in Syracuse, New York (43.0481° N, 76.1474° W) for two generations and were then transferred in fall 2014 to the University of Richmond, Virginia where they completed diapause in a 4–6°C refrigerator before experiment 1 in 2015. The first generation of the VA population was reared on local red oak foliage under ambient laboratory conditions in Richmond, Virginia, in accordance with local tree phenology. In the summer of 2015, MA and VA populations were reared outdoors at the University of Richmond, Virginia on local red oak foliage and were overwintered in a 4–6°C refrigerator to complete diapause prior to experiment 2 with these populations in 2016.

Larval Rearing and Acclimation Temperatures

In experiment 1, conducted in 2015, eggs from the QC and the NC populations (N = 10 egg masses per population) were removed from refrigeration after 158 d (1 March 2015). Equal sized egg masses from each population were broken up and thoroughly mixed together to evenly sample the available gene pool. We removed sequential batches of eggs over 1–2 wk to hatch at room temperature and haphazardly selected hatched larvae to stock our experiments. Larvae were reared in environmental chambers (model I-22VL, Percival Scientific, Perry, IA) at 22 ± 1°C and 28 ± 1°C until reaching second instar as determined by daily monitoring.

In 2016, we conducted experiment 2, using populations from MA (26 egg masses) and VA (32 egg masses). Eggs were removed from refrigeration after 151 d (29 January 2016) and each population mixed as described above and allowed to hatch at room temperature. Larvae were reared using the same environmental chambers as is in experiment 1, but at 20 ± 1°C and 30 ± 1°C, until reaching third instar as determined by daily monitoring.

In both experiments, larvae were reared in groups of 10 individuals until reaching the desired instar for metabolic measurement. Groups were reared on approximately 2 ml of artificial diet that was either poured directly into 74 ml cups (experiment 1) or cut into cubes and then placed in the cups (experiment 2) (USDA, Hamden Formula Gypsy Moth Diet #F9630B, Frontier Agricultural Sciences, Newark, DE).

Measurement of Resting Metabolic Rate

We measured the resting metabolic rate (RMR) of larvae as a proxy for baseline levels of metabolism needed to sustain minimal levels of activity. Larvae used were selected for respirometry within 24 hr after fully transitioning into the desired experimental instar. We used a stop-flow respirometry system to measure RMR using established methodology (see Lighton and Felden 1995, Klok et al. 2004, Lighton 2008, Martinez and Agosta 2016). A field metabolic system (Sable Systems International, Las Vegas, NV) was placed inside a custom-made temperature-controlled chamber (described in Martinez and Agosta 2016). Two eight-channel multiplexers (model RM-8, Sable Systems International) were connected to the Field Metabolic System (FMS), and each channel was fitted with a 50-ml chamber. Prior to respirometry, individuals were held without food for 3 hr and then weighed to the nearest 0.001 g and placed inside separate 50 ml chambers. Although some food processing and digestion may have occurred during respirometry trials, essentially no frass and very little movement was observed by larvae in the chambers during the experimental assays. The entire air space of each chamber was flushed at a rate of 100 ml/min for 3 min, every 60 min for a period of 16 hr. After being held for one hour at the assay temperature, 15 hourly measurements (hours 2–16) were taken and averaged to represent the RMR value for each individual larvae. Purged air was routed to analyzers using inert tubing (Bev-a-Line; Cole Parmer, Vernon Hills, IL) and analyzed for CO_2 emission. To ensure that temperatures inside the chambers matched the desired assay temperature, one of the three baseline chambers was fitted with a temperature probe and monitored by the field metabolic system. Gas analyzers and sampling systems were controlled by ExpeData software. A total of eight assays were run between the two experiments with new larvae used in each assay. Assay temperatures were 18, 22, 28, and 32°C for QC and NC populations (experiment 1) and 20, 28, 30, and 35°C for MA and VA populations (experiment 2).

RMR Data Processing and Extraction

Raw CO_2 emission traces were processed and integrated according to Lighton (2008). All equations and methodologies correspond to a push-mode, stop-flow respirometry system coupled with a water scrubber column (magnesium perchlorate). This configuration allowed a more simplified calculation of gas volumes, and reduces potential errors arising from the dilution effect of CO_2 with water vapor. CO_2 was zeroed for baseline correction using a two-marker baseline correction method (Lighton 2008). Raw CO_2 signals were lag corrected, and smoothed by averaging each data point with the nearest 10 values.

The bolus integration was performed by first transforming excurrent gas fractional content to sample rates (M_s) for CO_2 for each data point (M_s CO_2= FR × CO_2, where FR is the standard temperature and pressure-corrected flow rate, which is recorded by the FMS). After the sample rates were calculated for each data point, the total sample volume of CO_2 emitted ($Volco_2$) was calculated by integrating under each M_s CO_2 peak. The resulting gas volume was divided by the enclosure time and wet mass to calculate CO_2 emission rate in milliliters per hour per gram of wet mass. The details and commands used in the ExpeData software are available in the supplement. Sample sizes ranged from 3 to 10 individuals for each temperature regime (Supp Tables S1 and S2 [online only]).

Data Analysis

Statistical analyses were conducted separately for each experiment as rearing and respirometry temperatures and the instars used differed between the experiments. The effect of population origin and respirometry temperature on metabolic rate was tested using a two-way generalized linear model for each of the rearing temperatures. We also calculated a Q_{10} temperature coefficient (the magnitude at which the rate of a given process changes with a 10°C increase in temperature) for the increase in mean metabolic rate between minimum and maximum test temperatures in each experiment (18 and 32°C for QC and NC in experiment 1 and 20 and 35°C for MA and VA in experiment 2). To test the effect of rearing temperature and respirometry temperature on metabolic rate, we used a two-way generalized linear model for each of the four populations. We compared the effect of rearing temperature on larval mass using two-sample *t*-tests for each population. Given the low statistical power resulting from sample sizes at each respirometry temperature, analyses were separated to focus on the main effect of either population or rearing temperature. Analyses were performed using a Satterthwaite approximation for degrees of freedom to account for unequal sample sizes with a significance threshold of $\alpha = 0.05$. All analyses were conducted using the stats and lme4 libraries in R version 3.3.1 (R Core Team 2016).

Results

Population Comparisons

Typical onset of hatching in their natural localities occurs around 14 March for the NC and VA populations (±1.3–2.0 d SE), 23 April ± 1.7 d for the MA population, and 29 May ± 3.8 d for the QC population. While daily maximum and minimum temperatures at hatch are similar for the four locations in our study, the southern populations experience higher temperatures as larval development progresses (Supp Table S3 [online only]).

Respirometry temperature had a significant impact on mass-specific RMR ($F_{1,21} > 64.429$, $P < 0.0001$), with metabolic rate increasing linearly as respirometry temperature increased for all populations (Supp Tables S1 and S2 [online only]; Fig. 1). For all comparisons, population had no significant effect on mass-specific RMR ($F_{1,21} < 3.863$, $P > 0.059$). There were no significant interactions between population and respirometry temperature ($F_{1,21} < 1.674$, $P > 0.210$), with RMR increasing linearly and at a similar rate for each population across the range of test temperatures. Q_{10} temperature coefficients for metabolic rate ranged from 1.47 to 2.04 across populations and acclimation temperatures (Supp Tables S1 and S2 [online only]).

Acclimation Response to Rearing Temperature

Mass of larvae reared at lower temperatures was less than larvae reared at higher temperatures for the QC ($t = -5.2714$, $P < 0.001$), NC ($t = -6.5241$, $P < 0.001$), and VA ($t = -2.7455$, $P = 0.011$)

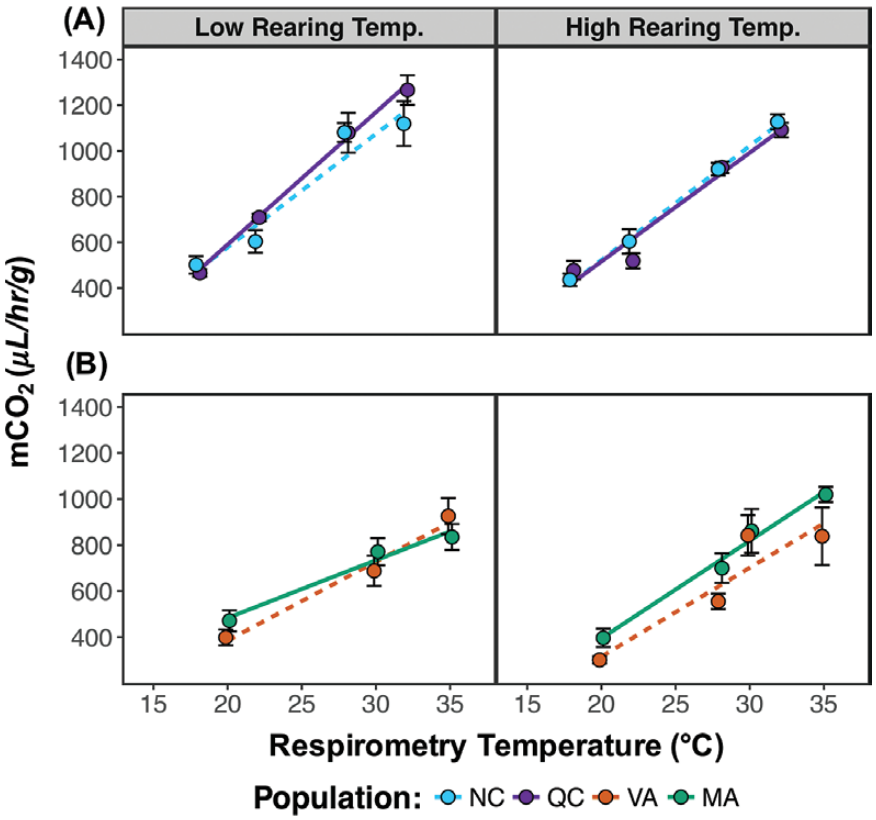

Fig. 1. Comparison of population response of mass-specific RMR at different respirometry temperatures for each rearing category. In experiment 1 (A), larvae from NC (blue/dashed line) and QC (purple/solid line) populations were reared at a low temperature (22°C) or a high temperature (28°C) and measured at four respirometry temperatures (18, 22, 28, 32°C). In experiment 2 (B), larvae from VA (orange/dashed line) and MA (green/solid line) populations were reared at a low temperature (20°C) or a high temperature (30°C) and measured at three or four respirometry temperatures (20, 28, 30, 35°C). Sample size ranged from 3 to 11 independent larvae tested at each respirometry temperature for each population and rearing temperature (see Supp Tables S1 and S2 [online only]).

populations (Fig. 2). There was not a significant difference in body mass between the two rearing temperatures for the MA population (t = –1.3734, P = 0.1812; Fig. 2).

There was a significant increase in mass-specific RMR with increasing respirometry temperature for all populations ($F_{1,25}$ > 49.041, P < 0.0001; Supp Tables S1 and S2 [online only]; Fig. 3). In the QC and NC populations, individuals reared at the lower temperature had significantly higher RMR than those reared at higher temperature (QC: $F_{1,52}$ = 13.990, P < 0.001, NC: $F_{1,59}$ = 4.543, P = 0.037). In the VA population, metabolic rate was unaffected by the rearing temperatures. In contrast, there was a significant interaction between rearing temperature and respirometry temperature ($F_{1,25}$ = 5.969, P = 0.022) for larvae from MA, as well as a significant main effect of rearing temperature ($F_{1,25}$ = 4.804, P = 0.0379). For the other three populations, there were no significant interactions between rearing temperature and respirometry temperature.

Discussion

The general expectation under the MCA hypothesis is that ectothermic species from colder climates will have higher metabolic rates than those from warmer climates when compared at the same ambient temperature (Chown and Gaston 1999, Lardies et al. 2004). In our study, there was no difference in metabolic rates between gypsy moth populations from northern and southern regions of their introduced range, and our results provide little support for the intraspecific application of the MCA hypothesis. Extending the principles of the MCA to an acclimation response, we expected larvae from the same population reared at colder temperatures to have a higher metabolic rate than those reared at warmer. Our results support this expectation in the first experiment, but not in the second.

Under the MCA, the higher metabolic rate shown by species from colder climates allows important physiological processes, such as development, growth, and reproduction, to be accelerated in their environment with shorter periods of optimal conditions (Chown and Gaston 1999, Lardies et al. 2004). However, a global meta-analysis of 346 insect species found that although the MCA was supported, the overall relationship between local annual temperature and metabolic rate was weak (Addo-Bediako et al. 2002). In contrast to expectations under the MCA, the "Hotter is Better" hypothesis suggests that species or populations from warmer locations may have higher thermal optimums (Kingsolver and Huey 2008). If applied to the thermal performance of physiological traits (Angilletta et al. 2009), this hypothesis expects that metabolic rates would be higher in organisms from warmer climates.

While the majority of studies evaluating geographic differences in metabolic rate have focused on interspecies comparisons, several recent studies have tested for intraspecific latitudinal variation in metabolic physiology. Schaefer and Walters (2010) found differing responses among northern and southern populations of two species of topminnow with metabolic rates congruent with the MCA hypothesis in one species, but not the other. Among populations of woodlice collected from four localities across a wide latitudinal gradient, variation in metabolic rates was opposite of that expected

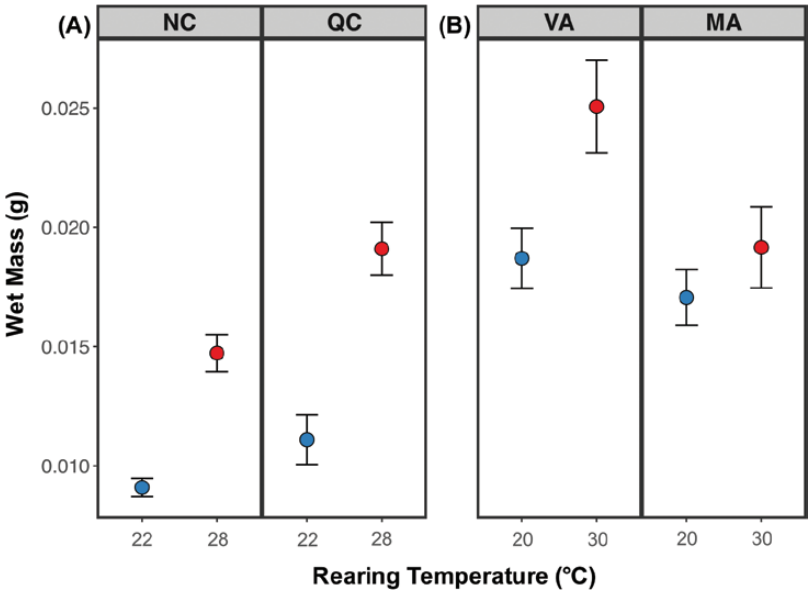

Fig. 2. Comparison of body mass of larvae reared at high and low temperatures. In experiment 1 (A), larvae from NC and QC populations were reared at a low temperature (22°C) or a high temperature (28°C) to the second instar. In experiment 2 (B), larvae from VA and MA populations were reared at a low temperature (20°C) or a high temperature (30°C) to the third instar. Wet mass was measured to the nearest 0.001 g after a 3-hr fasting period and before entering respirometry chambers. Sample size ranged from 12 to 34 independent larvae tested for each population and rearing temperature (see Supp Tables S1 and S2 [online only]).

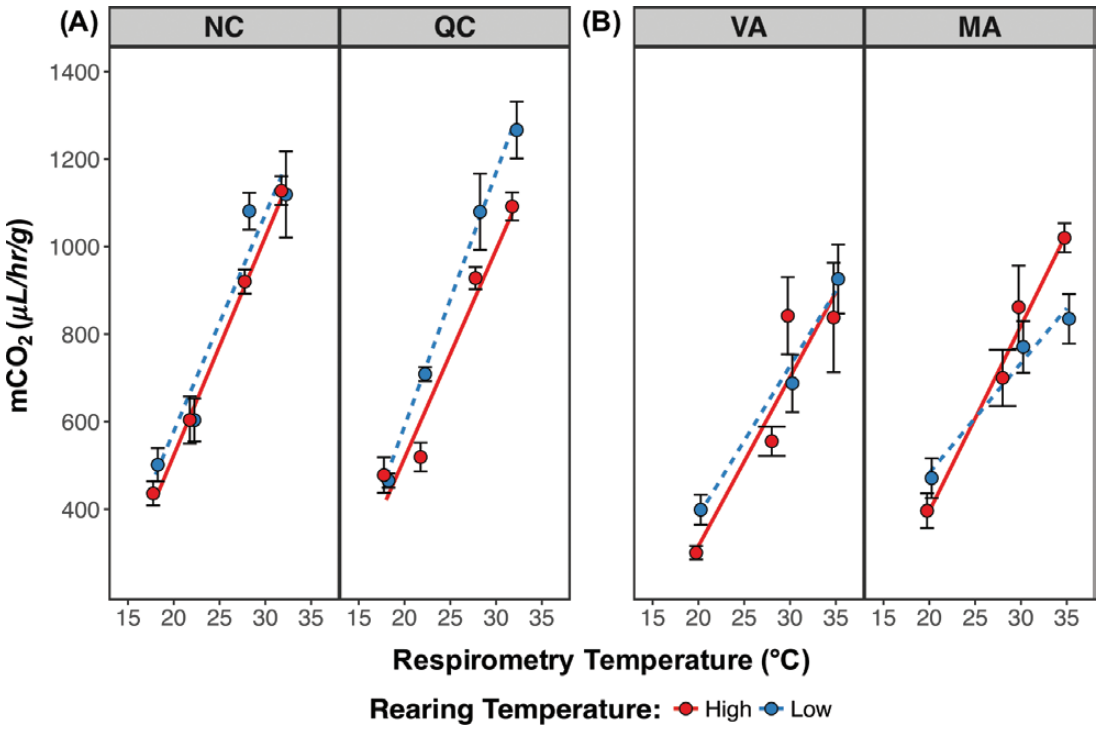

Fig. 3. Comparison of acclimation response to rearing temperature of mass-specific RMR at different respirometry temperatures for each population. In experiment 1 (A), larvae from NC and QC were reared at either 22 (low temperature, dashed line) or 28°C (high temperature, solid line) and measured at four respirometry temperatures (18, 22, 28, 32°C). In experiment 2 (B), larvae from VA and MA were reared at either 20 (low temperature, dashed line) or 30°C (high temperature, solid line) and measured at three or four respirometry temperatures (20, 28, 30, 35°C). Sample size ranged from 3 to 11 independent larvae tested at each respirometry temperature for each population and rearing temperature (see Supp Tables S1 and S2 [online only]).

by the MCA framework, with slightly higher metabolic rates in lower latitude populations (Lardies et al. 2004). A common-garden experiment of three invasive snail populations by Gaitán-Espitia and Nespolo (2014) found no significant difference in metabolic rate among the populations, despite a latitudinal gradient of 1300 km. Similar to this study, we did not find support in gypsy moth for latitudinal variation in metabolic rate that would align with either the MCA or "Hotter is Better" hypothesis.

Characteristics of the initial invasion and subsequent spread of gypsy moth may provide a rationale for the lack of latitudinal

patterns and high degree of plasticity in thermal sensitivity of metabolic rates observed in our study. The introduction of gypsy moth to North America was likely associated with a profound founder effect and associated genetic bottleneck, which could act as a constraint on adaptive potential. Analysis of microsatellite allele frequencies shows reduced genetic diversity across populations in North America compared with putative source populations in southern Europe (Wu et al. 2015). However, this does not preclude the possibility of local adaptation, as other invasive species have shown adaptive evolutionary responses to novel conditions (e.g., Butin et al. 2005, Hoffmann 2017, Pélissié 2018). Future studies that incorporate a wider gradient of regions, more populations, or more extreme temperatures could provide additional insights on physiological divergence during invasion. Alternatively, temperature may not be acting as a strong selective pressure on larval metabolic rates. Temperature differences in late spring and early summer during larval development in these regions are naturally not as pronounced as differences in overwinter temperatures. Studies that examine other life stages, such as overwintering eggs, could provide additional insights regarding the potential for population variation in gypsy moth physiology.

In addition to adaptive variation in metabolic rate among populations, plasticity in metabolic rate and the ability to acclimate allow organisms to modify physiological performance in response to environmental conditions. Under acclimation to colder or warmer temperatures, the general expectation is that biochemical processes will change to enhance performance with respect to the new environment (Angilletta 2009). In terms of metabolic rate, however, there are competing predictions for whether organisms will experience a relative increase in metabolic rate under cold or warm acclimation. Similar to the predictions of MCA, a relative increase in metabolic rate is expected under cold acclimation due to short-term temperature compensation. However, increases in metabolic rate under cold acclimation may be measured because of reductions in metabolic rate to conserve water when individuals are acclimated to higher temperatures, or increases in metabolic rate following a short-term acclimation to low temperature (Chown et al. 2016). Conversely, other studies have found elevated metabolic rates associated with high-temperature acclimation (Clarke 1993, Lardies et al. 2004). Terblanche et al. (2005) found that metabolic rates in the flea beetle, *Chirodica chalcoptera* (Germar) (Coleoptera: Chrysomelidae), were 1.5 times higher for individuals held at 25°C than those at 12°C. These responses to higher temperatures can be explained by energetically expensive and thermosensitive cellular processes (e.g., Abele et al. 2002, Martinez et al. 2017).

The low- and high-temperature acclimation environments in our experiment generally resulted in reduced body mass for larvae reared at low temperatures. This result was unsurprising given that the high-temperature environments were near the developmental optimum for this species and the low temperatures were suboptimal for growth (Logan et al. 1991, Thompson et al. 2017). In our comparisons of metabolic rate between low and high acclimation temperature treatments, we found either higher metabolic rates under low-temperature acclimation or equivalent rates between the two. Results from the first experiment using populations from Quebec and North Carolina show higher metabolic rates for larvae reared at 22°C than those reared at 28°C. However, results from the second experiment using populations from Massachusetts and Virginia, which used more extreme rearing temperatures of 20 and 30°C, did not find clear evidence of an acclimation response. It is unclear if these differing results in acclimation response were driven by the instars used, low sample sizes in the Massachusetts and Virginia populations, or the differences in rearing temperatures. Experiments using additional populations with a greater number of acclimation temperatures could provide further insight into these physiological responses. For gypsy moth in North America, our results suggest that geographic variations seen in other developmental traits (e.g., Thompson et al. 2017) are not apparent in metabolic physiology for the populations we tested.

Hypotheses regarding the evolution of metabolic performance are far from having unified empirical support. Across the literature, inter- and intraspecific patterns in metabolic rate depend on the species, the climate and habitat of the organism, and the experimental design. While intraspecific comparisons of metabolic variation are increasing, few studies have examined these relationships in invasive species (but see Gaitán-Espitia and Nespolo, 2014). While the outcomes of our study, like many others, do not provide consistent evidence for a single hypothesis or geographic pattern, our work highlights the importance of examining range-wide variation in organismal responses to temperature change. Invasive species in particular can be compelling systems for testing the potential for physiological divergence during range expansion, which can have implications for managing future spread.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

Acknowledgments

We are very grateful to R. St-Amant (Canadian Forest Service) for providing phenological prediction layers for gypsy moth and advice from P. Tobin (University of Washington) and L. Blackburn (USDA Forest Service). S. Powers provided helpful comments on the manuscript. We thank K. Gooch (Massachusetts Department of Conservation and Recreation), L. Morneau (Ministère des Ressources Naturelles et de la Faune du Québec), and C. Elder and R. Copeland (NCDA and CS Gypsy Moth program) for collecting egg masses used in this experiment. We thank the Biology Departments at the University of Richmond and Virginia Commonwealth University for their support and for use of their facilities. This work was funded by the School of Arts and Sciences at University of Richmond and a Presidential Research Quest Fund to S. Agosta from Virginia Commonwealth University. Gypsy moths were transported and housed under USDA permits P526P-17-03720 (D.P.) and P526P-15-00488 (K.G.).

References Cited

Abele, D., K. Heise, H. O. Pörtner, and S. Puntarulo. 2002. Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. J. Exp. Biol. 205: 1831–1841.

Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2000. Thermal tolerance, climatic variability and latitude. Proc. Biol. Sci. 267: 739–745.

Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2002. Metabolic cold adaptation in insects: a large scale perspective. Funct. Ecol. 16: 332–338.

Angilletta, Jr., M. J. 2009. Thermal adaptation: a theoretical and empirical analysis. Oxford University Press, New York, NY.

Angilletta, M. J., Jr, R. B. Huey, and M. R. Frazier. 2009. Thermodynamic effects on organismal performance: is hotter better? Physiol. Biochem. Zool. 83: 197–206.

Bogdanowicz, S. M., V. C. Mastro, D. C. Prasher, and R. G. Harrison. 1997. Microsatellite DNA variation among Asian and North American gypsy moths (Lepidoptera: Lymantriidae). Ann. Entomol. Soc. Am. 90: 768–775.

Butin, E., A. H. Porter, and J. Elkinton. 2005. Adaptation during biological invasions and the case of *Adelges tsugae*. Evol. Ecol. Res. 7: 887–900.

Castañeda, L. E., M. A. Lardies, and F. Bozinovic. 2004. Adaptive latitudinal shifts in the thermal physiology of a terrestrial isopod. Evol. Ecol. Res. 6: 579–593.

Chown, S. L., and K. J. Gaston. 1999. Exploring links between physiology and ecology at macro-scales: the role of respiratory metabolism in insects. Biol. Rev. Camb. Philos. Soc. 74: 87–120.

Chown, S. L., and J. S. Terblanche. 2006. Physiological diversity in insects: ecological and evolutionary contexts, pp. 50–152. *In* S. J. Simpson (ed.), Advances in insect physiology, vol. 33. Elsevier, Sydney, Australia.

Chown, S. L., S. Slabber, M. McGeouch, C. Janion, and H. P. Leinaas. 2007. Phenotypic plasticity mediates climate change responses among invasive and indigenous arthropods. Proc. Biol. Sci. 274: 2531–2537.

Chown, S. L., T. M. Haupt, and B. J. Sinclair. 2016. Similar metabolic rate-temperature relationships after acclimation at constant and fluctuating temperatures in caterpillars of a sub-Antarctic moth. J. Insect Physiol. 85: 10–16.

Clarke, A. 1993. Seasonal acclimatization and latitudinal compensation in metabolism: do they exist? Funct. Ecol. 7: 139–149.

Colinet, H., and A. A. Hoffmann. 2012. Comparing phenotypic effects and molecular correlates of developmental, gradual and rapid cold acclimation responses in Drosophila melanogaster. Funct. Ecol. 26: 84–93.

Davidson, A. M., M. Jennions, and A. B. Nicotra. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. Ecol. Lett. 14: 419–431.

Gaitán-Espitia, J. D., and R. Nespolo. 2014. Is there metabolic cold adaptation in terrestrial ectotherms? Exploring latitudinal compensation in the invasive snail *Cornu aspersum*. J. Exp. Biol. 217: 2261–2267.

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

Grayson, K. L., D. Parry, T. M. Faske, A. Hamilton, P. C. Tobin, S. J. Agosta, and D. M. Johnson. 2015. Performance of wild and laboratory-reared gypsy moth (Lepidoptera: Erebidae): a comparison between foliage and artificial diet. Environ. Entomol. 44: 864–873.

Grayson, K. L., and D. M. Johnson. 2018. Novel insights on population and range edge dynamics using an unparalleled spatiotemporal record of species invasion. J. Anim. Ecol. 87: 581–593.

Hoffmann, A. A. 2017. Rapid adaptation of invertebrate pests to climatic stress? Curr. Opin. Insect Sci. 21: 7–13.

Huey, R. B., and M. Pascual. 2009. Partial thermoregulatory compensation by a rapidly evolving invasive species along a latitudinal cline. Ecology. 90: 1715–1720.

Irlich, U. M., J. S. Terblanche, T. M. Blackburn, and S. L. Chown. 2009. Insect rate-temperature relationships: environmental variation and the metabolic theory of ecology. Am. Nat. 174: 819–835.

Kingsolver, J. G., and R. B. Huey. 2008. Size, temperature, and fitness: three rules. Evol. Ecol. Res. 10: 251–268.

Klok, C. J., B. J. Sinclair, and S. L. Chown. 2004. Upper thermal tolerance and oxygen limitation in terrestrial arthropods. J. Exp. Biol. 207: 2361–2370.

Lardies, M. A., L. D. Bacigalupe, and F. Bozinovic. 2004. Testing the metabolic cold adaptation hypothesis: an intraspecific latitudinal comparison in the common woodlouse. Evol. Ecol. Res. 6: 567–578.

Liebhold, A. M., J. A. Halverson, and G. A. Elmes. 1992. Gypsy moth invasion in North America: a quantitative analysis. J. Biogeogr. 19: 513–520.

Lighton, J. R. B. 2008. Measuring metabolic rates: a manual for scientists. Oxford University Press, New York, NY.

Lighton, J. R. B., and L. J. Fielden. 1995. Mass scaling of standard metabolism in ticks : a valid case of low metabolic rates in sit-and-wait strategists. Physiol. Zool. 68: 43–62.

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

Martinez, E., and S. J. Agosta. 2016. Budget-limited thermal biology: design, construction and performance of a large, walk-in style temperature-controlled chamber. J. Therm. Biol. 58: 29–34.

Martinez, E., M. A. Menze, and S. J. Agosta. 2017. Reduced mitochondrial efficiency explains mismatched growth and metabolic rate at supraoptimal temperatures. Physiol. Biochem. Zool. 90: 294–298.

Nielsen, M. G., G. W. Elmes, and V. E. Kipyatkov. 1999. Respiratory Q10 varies between populations of two species of Myrmica ants according to the latitude of their sites. J. Insect Physiol. 45: 559–564.

Parker, I. M., J. Rodriguez, and M. E. Loik. 2003. An evolutionary approach to understanding the biology of invasions: local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. Conserv. Biol. 17: 59–72.

Pélissié, B., M. S. Crossley, Z. P. Cohen, and S. D. Schoville. 2018. Rapid evolution in insect pests: the importance of space and time in population genomics studies. Curr. Opin. Insect Sci. 26: 8–16.

Preisser, E. L., J. S. Elkinton, and K. Abell. 2008. Evolution of increased cold tolerance during range expansion of the elongate hemlock scale *Fiorinia externa* Ferris (Hemiptera: Diaspididae). Ecol. Entomol. 33: 709–715.

R Core Team. 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rajamohan, A., and B. J. Sinclair. 2008. Short-term hardening effects on survival of acute and chronic cold exposure by *Drosophila melanogaster* larvae. J. Insect Physiol. 54: 708–718.

Rako, L., and A. A. Hoffmann. 2006. Complexity of the cold acclimation response in *Drosophila melanogaster*. J. Insect Physiol. 52: 94–104.

Ransberry, V. E., H. A. MacMillan, and B. J. Sinclair. 2011. The relationship between chill-coma onset and recovery at the extremes of the thermal window of *Drosophila melanogaster*. Physiol. Biochem. Zool. 84: 553–559.

Régnière, J., and A. Sharov. 1998. Phenology of *Lymantria dispar* (Lepidoptera : Lymantriidae), male flight and the effect of moth dispersal in heterogeneous landscapes. Int. J. Biometeorol. 41: 161–168.

Schaefer, J., and A. Walters. 2010. Metabolic cold adaptation and developmental plasticity in metabolic rates among species in the *Fundulus notatus* species complex. Funct. Ecol. 24: 1087–1094.

Scholander, P. F., W. Flagg, V. Walters, and L. Irving. 1953. Climatic adaptation in arctic and tropical poikilotherms. Physiol. Zool. 26: 67–92.

Sexton, J. P., P. J. McIntyre, A. L. Angert, and K. J. Rice. 2009. Evolution and ecology of species range limits. Annu. Rev. Ecol. Evol. Syst. 40: 415–436.

Sharov, A. A., D. S. Leonard, A. M. Liebhold, E. A. Roberts, and W. Dickerson. 2002. Slow the spread: a national program to contain the gypsy moth. J. For. 100: 30–35.

Sinclair, B. J., C. M. Williams, and J. S. Terblanche. 2012. Variation in thermal performance among insect populations. Physiol. Biochem. Zool. 85: 594–606.

Slow the Spread Decision Support System. 2018. Phenology baselayers. STS Found. Oper, Raleigh, NC. http://yt.ento.vt.edu/da/.

Steffensen, J. F., P. G. Bushnell, and H. Schurmann. 1994. Oxygen consumption in four species of teleosts from Greenland: no evidence for metabolic cold adaptation. Polar Biol. 14: 49–54.

Sultan, S. E. 2001. Phenotypic plasticity for fitness components in *Polygonum* species of contrasting ecological breadth. Ecology. 82: 328–343.

Tauber, M. J., C. A. Tauber, J. R. Ruberson, A. J. Tauber, and L. P. Abrahamson. 1990. Dormancy in *Lymatria dispar* (Lepidoptera: Lymantriidae): analysis of photoperiodic and thermal responses. Ann. Entomol. Soc. Am. 83: 494–503.

Terblanche, J. S., B. J. Sinclair, C. Jaco Klok, M. L. McFarlane, and S. L. Chown. 2005. The effects of acclimation on thermal tolerance, desiccation resistance and metabolic rate in *Chirodica chalcoptera* (Coleoptera: Chrysomelidae). J. Insect Physiol. 51: 1013–1023.

Thompson, L. M., T. M. Faske, N. Banahene, D. Grim, S. J. Agosta, D. Parry, P. C. Tobin, D. M. Johnson, and K. L. Grayson. 2017. Variation in growth and developmental responses to supraoptimal temperatures near latitudinal range limits of gypsy moth *Lymantria dispar* (L.), an expanding invasive species. Physiol. Entomol. 82: 181–190.

Thornton, P. E., S. W. Running, and M. A. White. 1997. Generating surfaces of daily meteorological variables over large regions of complex terrain. J. Hydrol. 190: 214–251.

Tobin, P. C., B. B. Bai, D. A. Eggen, and D. S. Leonard. 2012. The ecology, geopolitics, and economics of managing *Lymantria dispar* in the United States. Int. J. Pest Manag. 58: 195–210.

Vanhanen, H., T. O. Veteli, S. Päivinen, S. Kellomäki, and P. Niemelä. 2007. Climate change and range shifts in two insect defoliators: gypsy moth and nun moth-a model study. Silva Fenn. 41: 621–638.

Wu, Y., J. J. Molongoski, D. F. Winograd, S. M. Bogdanowicz, A. S. Louyakis, D. R. Lance, V. C. Mastro, and R. G. Harrison. 2015. Genetic structure, admixture and invasion success in a Holarctic defoliator, the gypsy moth (*Lymantria dispar*, Lepidoptera: Erebidae). Mol. Ecol. 24: 1275–1291.

Yeh, P. J., and T. D. Price. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. Am. Nat. 164: 531–542.