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Brent J. Sinclair
Western University, bsincla7@uwo.ca

Caroline M. Williams
University of Florida, carolinewilliams@ufl.edu

Wesley D. Chick
Western University

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A cross-seasonal perspective on local adaptation: Metabolic plasticity mediates responses to winter in a thermal-generalist moth

Caroline M. Williams[a]*, Wesley D. Chick[b], Brent J. Sinclair[b]

[1]Department of Entomology and Nematology, University of Florida, Gainesville, USA

[2]Department of Biology, University of Western Ontario, London, Canada

*Corresponding author: Caroline Williams: carolinewilliams@ufl.edu

Running title: Local adaptation to winter conditions

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Summary

1. Local adaptation determines responses to climate change, but is not well-explored for terrestrial animals, particularly in the context of winter.
2. The physiological and ecological impact of the thermal environment across life-stages can result in tradeoffs that determine fitness and population dynamics. Understanding mechanisms and consequences of local adaptation for any organism that overwinters requires taking a cross-seasonal perspective.
3. We used a trait-based approach to distinguish variation among ecotypes in ecological and physiological responses to overwintering conditions. We used fall webworms (*Hyphantria cunea*; Lepidoptera: Arctiidae) from Ottawa, Ontario and Columbus Ohio, representing the centre and periphery of the native range.
4. We hypothesized that populations would be locally adapted to their overwintering environments, with fitness maximised under natal overwintering conditions. We predicted that this local adaptation would result from modulation of rates of energy use, growth and development.
5. The Ohio ecotype was larger at pupation, and entered dormancy two weeks earlier than the Ontario ecotype.
6. Each ecotype had higher overwinter survival in their natal compared to non-natal winter environment, and this was associated with larger pupal mass, size and carbohydrate reserves at the end of winter. This suggests that the ecotypes are locally adapted to winter conditions. Larger adults laid more eggs, but there was no effect of ecotype or environment on fecundity.

7. Pupae that overwintered at warm, energetically demanding southern temperatures facultatively suppressed their metabolism in autumn, and developed more quickly in the spring, compensating for the increased energetic demands of warmer winters. Northern ecotypes had lower thermal sensitivity of metabolism, leading to higher metabolic rates at cool temperatures and faster post-winter development.
8. This local adaptation to winter conditions suggests it is simplistic to expect performance of peripheral populations to be enhanced by warming winters, and that predicted decoupling of winter and growing season temperatures may have negative fitness consequences for ectotherms.

Key-words:

bioenergetics, climate change, energy drain, fitness, insect, Lepidoptera, metabolic rate, overwintering, temperature compensation, tradeoff

Introduction

Temperature regulates the performance and evolution of ectotherms through thermodynamic effects on biochemical processes (Clarke & Fraser 2004). Global climate change is altering operative temperatures for ectotherms (Dillon, Wang & Huey 2010), and is also decoupling the relationship between growing season and winter temperatures (Bonsal & Kochtubjada 2009). Ectotherms can compensate physiologically for changes in temperature, facilitating the colonisation of diverse thermal environments (Hochachka & Somero 2002; Clarke 2003). However, the role of among-population variation in temperature responses is underexplored, particularly for terrestrial ectotherms, despite its importance in determining species' responses to climate change (Sinclair, Williams & Terblanche 2012).

Local adaptation (higher fitness of a population at its native site compared to other populations) will determine a population's response to climate change by determining a species' ability to respond to conditions that change across the geographic range. If responses to the environment are invariant across a species' range, then central populations will be better adapted to their environment than peripheral populations (assuming that range limits are set by environmental factors). If climate change makes environmental conditions at the periphery more like central conditions (e.g. poleward range limits in a warming climate), then peripheral populations will be enhanced. Conversely, if all populations are adapted to their current environment (e.g. peripheral populations have enhanced environmental tolerance compared to central populations), climate change may cause global fitness declines as all populations are disturbed from local fitness optima (Hellmann, Prior & Pelini 2012).

The response of a population to environmental conditions can be described by reaction norms that relate a phenotype expressed by a genotype to the environment in which that phenotype is expressed (Stearns 1992). The slope of a reaction norm estimates the environmental sensitivity (phenotypic plasticity) of the phenotype. Steep reaction norms that are parallel among genotypes indicate that a species responds to environmentally-heterogeneous environments primarily through phenotypic plasticity. Conversely, divergent reaction norm slopes indicate that the degree or direction of plasticity has evolved (a genotype-by-environment interaction). This evolution of plasticity may lead to local adaptation if fitness is higher for genotypes in their natal environment, relative to non-adapted genotypes (Kawecki & Ebert 2004). To detect local adaptation, multiple populations must thus be assessed under more than one environmental condition, and a reaction norm constructed for each population (Kawecki & Ebert 2004).

Our ability to predict the impacts of climate change is thus impeded by lack of information on local adaptation to temperature in terrestrial animals. Of 74 field studies of local adaptation, Hereford (2009) identified only four on terrestrial animals, of which only one assessed local adaptation to temperature (Qualls 1997). Local adaptation was present in 71% of remaining local adaptation studies, with substantial fitness advantages, so the dearth of knowledge on terrestrial animals is troubling. Inclusion of laboratory studies (e.g. simulated reciprocal transplants, or common garden experiments with more than one acclimation treatment), and studies using fitness proxies such as size or growth and development ratesreveals several convincing demonstrations of local adaptation to temperature in terrestrial animals including butterfly larvae, frog tadpoles, and adult flies (e.g. Ayres & Scriber 1994; Berrigan & Partridge 1997; Laugen *et al.* 2003; Rotvit & Jacobsen 2014). Thus, that local adaptation to temperature may be common in terrestrial animals.

Insects in temperate regions can spend more than half of their lives dormant (Koštál 2006), subsisting on metabolic reserves which must also fuel pre-feeding development and reproduction in spring (Hahn & Denlinger 2007). Metabolic rates during diapause are suppressed but still temperature-sensitive: an increase in temperature elicits an increase in metabolic rate and can hasten energy depletion (e.g. Bosch & Kemp 2004; Williams, Hellmann & Sinclair 2012), imposing selection for strategies that enhance energy conservation (e.g. Williams, Shorthouse & Lee 2003; Williams, Hellmann & Sinclair 2012; Williams *et al.* 2012). Local adaptation to winter conditions has been described for traits related to dormancy (e.g. Bradshaw & Holzapfel 2001), and thermal tolerance (e.g. Kukal, Ayres & Scriber 1991; Lyytinen, Mappes & Lindström 2012). However, few studies have examined local adaptation in overwintering energetics of terrestrial ectotherms (but see Pelini *et al.* 2009; Williams *et al.* 2012), and none have taken a cross-seasonal perspective (Williams, Henry & Sinclair in press).

Higher order traits such as fecundity or viability are determined by nutrient allocation strategies at the physiological level (Zera & Harshman 2001). Thus, studying physiological traits can advance a mechanistic understanding of local adaptation (Woods & Harrison 2002; Schulte, Healy & Fangue 2011). Because the consequences of season-specific physiological performance are integrated across the lifecycle, a cross-seasonal perspective is essential to realise the full fitness consequences of variation in physiological traits (Potter & Woods 2012). For example, caterpillars with high metabolic rates and thermal sensitivity benefit from faster growth and development during the summer growing season (Ayres & Scriber 1994), but individuals with high metabolic rates consume more energy reserves during winter (Pelini *et al.* 2009; Williams *et al.* 2012). Since winter temperatures are predicted to change more than summer temperatures (Bonsal & Kochtubjada 2009), it is important to understand whether alterations to metabolism are induced by winter or are a carryover from growing season conditions, and whether this relationship is modulated by local adaptation.

Here, we experimentally decouple growing season and winter temperatures in the laboratory to separate the effects of growing season temperatures from those of overwintering temperatures. We construct thermal reaction norms for multiple physiological and life-history traits related to energy metabolism, testing for signatures of local adaptation and plasticity in overwintering energetics. We use Fall webworms (*Hyphantria cunea* Drury; Lepidoptera: Arctiidae; Fig. 1), a widespread moth species, from populations at the northern edge and centre of their native North American range. This system is ideal for several reasons: 1) Fall webworms inhabit thermal environments from sub-tropical to cool temperate, implying they are masters of temperature compensation; 2) adults do not feed post-winter, thus, reproductive capacity depends solely on juvenile-derived nutrients making them vulnerable to negative fitness consequences of energy depletion (Gomi 2000) and 3) larvae live communally in nests, each of which is the entire reproductive output of a singly-inseminated female (Jaenike & Selander 1980), facilitating a split-brood design. *Hyphantria cunea* species has traits which promote genetically-based local adaptation: moderate dispersal (Yamanaka, Tatsuki & Shimada 2001), genetic structure across their native range (Gomi, Muraji & Takeda 2004), and high genetic diversity (Tao *et al.* 2009). Local adaptation of development time, critical photoperiod for diapause induction, and number of larval instars has been detected in *H. cunea* populations in Japan (Gomi & Takeda 1996; Gomi, Inudo & Yamada 2003; Gomi 2007; Gomi *et al.* 2007).

We thus hypothesise that *H. cunea* populations will be locally adapted to their overwintering thermal environment, generating non-parallel reaction norms for fitness-related life-history traits, such that fitness is maximised in natal overwintering conditions. We predict that this local adaptation will stem from divergence of overwintering metabolism between populations, which will alter reaction norms for energy use, growth and post-winter development.

Materials and methods

OVERVIEW OF STUDY DESIGN

We employ a reciprocal common-garden design, using populations of *H. cunea* from the northern edge and centre of their native range, wild-collected at the end of the larval growing season, and housed in the laboratory at temperatures approximating the northern range edge and range centre. Since the majority of development occurred in the field prior to collection, population effects are due not only to the genetic background, but are also a result of developmental effects prior to collection, as well as maternal effects (Nijhout & Davidowitz 2009). We will refer to the source populations as “ecotypes”, to emphasise the joint impacts of genotype and environment in determining the phenotypes of each population.

MICROCLIMATE DATA

We collected microclimate temperatures (±0.5°C) at hourly intervals from October 2008 to May 2009 using iButton thermochron data loggers (Model DS1922L, Maxim-Dallas Semiconductor; Sunnyvale, CA, USA) (Sinclair *et al.* 2013). We placed the data loggers in 10 mL plastic containers filled with silica gel to protect them from moisture damage, and deployed three loggers on the ground beneath the leaf litter in one woodlot near Ottawa, Ontario (dominated by black walnut [*Juglans nigra*], ash [*Fraxinus spp.*], and cherry [*Prunus spp.*]), and one near Athens, Ohio (black walnut). *H. cunea* were present in these woodlots, and overwintered beneath the leaf litter similar to the logger placement. We calculated bi-weekly mean daily maxima and minima for each location from the microclimate data, and used these to determine the temperature regimes used in the laboratory experiments. We also summed the total degrees above a threshold of -10°C for each logger over the whole period of recording (Oct – May) to give an index of the amount of heat accumulated at each site (and compared these accumulated heat units between sites using a t-test). We inferred snow cover when microclimate temperatures remained close to 0°C with little daily variation.

STUDY SPECIES AND REARING

The native range of *Hyphantria cunea* extends from Mexico to northern Canada across the breadth of North America (Wagner 2005), with an invasive range encompassing much of Asia (Gomi *et al.* 2007). Larvae are polyphagous, feeding on >400 species of woody plants (Wagner 2005). The *H. cunea* larvae used in this study were black-headed, although there is a sympatric sibling sub-species of red-headed larvae with markedly different ecology (Takeda 2005). Fall webworms overwinter in pupal diapause in the leaf litter, and adults emerge in early summer (Takeda 2005) (Fig. 1). We collected late-instar larvae in August 2009 by removing 20 entire nests per site from walnut trees in Columbus, Ohio, USA (40.06°N, 82.57°W) and Ottawa, Ontario, Canada (45.23°N, 75.43°W). We transported the larvae to the Biotron Experimental Climate Change Facility at the University of Western Ontario, where we counted them and reared them to pupation on *ad libitum* freshly cut local black walnut leaves in 3.7 L plastic containers (one nest per container) in temperature-controlled chambers (EGC-TC2, Environmental Growth Chambers, Chagrin Falls, Ohio, USA) under short daylength (12L:12D), 20:12°C 80 % RH.

We checked the larvae daily for pupation, and upon pupation broods were split between warm (Ohio-like) or cool (Ontario-like) overwintering treatment giving four treatment groups (Ecotype/winter environment): Ohio /warm, Ontario/warm, Ohio /cool, and Ontario/cool. Remaining larvae were discarded in late October when the host plant leaves began to senesce. All larvae that successfully pupated were considered to have survived the larval period, while larvae that did not pupate before 28 October were included in larval mortality estimates. Although pupae from each family were allocated evenly between overwinter environments, some families were underrepresented in some treatments by the end of winter due to mortality.

The pupae were kept in the dark in 6-well cell culture plates with a moist paper towel on the lid to maintain high humidity, in MIR-153 incubators (Sanyo Scientific, Bensenville, IL, USA) at temperatures fluctuating between the mean daily maximum and minimum microclimate temperatures for Ontario and Ohio calculated from hourly microclimate data (Fig. 2). The incubators were reset every two weeks to track seasonal changes in microclimate temperatures. We weighed the pupae in November and April (MX5 microbalance, Mettler-Toledo, Columbus, OH, USA; d=0.1 µg) and measured their length (± 0.5 mm) using digital calipers (Mastercraft, Toronto, Ontario, Canada). In November and April, 20 pupae from each treatment group were flash-frozen in liquid nitrogen and stored at -80°C for body composition analysis. At the beginning of April, all pupae were placed on moist vermiculite, and transferred to EGC-TC2 chambers on a long day photoperiod (16L:8D) under a 25°C:15°C thermocycle, at 80% relative humidity. Emergence was checked daily, and, when adult moths emerged, time taken to emerge following transfer to 25°C was recorded, the moths were killed at -20°C, and the length of the right forewing was measured from the proximal wing attachment point to the apex.

ENERGY RESERVE ASSAYS

To determine the effects of source population and overwintering environment on energy reserves, we measured storage lipids, total carbohydrates, and protein in overwintering pupae at the beginning (November) and end (April) of winter. We determined the sex of each pupa by the presence (female) or absence (male) of a line intersecting the first abdominal sternite. We validated this method of sexing pupae by sexing 77 pupae that were subsequently allowed to develop into adults, and sexed by the presence (males) or absence (females) of claspers and feathered antennae (Resh & Cardé 2009), with a success rate of 95%. We assayed triglycerides, carbohydrates and protein as previously described (Williams *et al.* 2011; Williams, Hellmann & Sinclair 2012). We expressed triglycerides, carbohydrate and protein concentrations in µg·mg DM^{-1}, then scaled them up to whole-animal values by multiplying by total DM. We subtracted whole-animal TAG and carbohydrate from DM to give lipid- (and carbohydrate-) free DM.

RESPIROMETRY

To assess plasticity and local adaptation in the temperature-metabolic rate relationship, we measured the CO_2 emission of six pupae from each treatment group over a range of temperatures in November (beginning of winter) and April (end of winter). We measured each individual pupa five times: at 5, 10, 15, 20 and 25°C. The order of temperature and time of day of measurement (between 8am and 8pm) were randomized, and there was no less than 48 hours between measurements on any individual. Pupae were weighed before each measurement.

We measured CO_2 emission as a proxy for metabolic rate using a Sable Systems flow-through respirometry system (Sable Systems International [SSI], Las Vegas, Nevada) with a Li7000 infrared CO_2 analyser (LiCor; Lincoln, NE, USA) as previously described (Williams *et al.* 2010). The flow rate was 50 mL·min^{-1} through a 4 cm^3 chamber. We controlled the temperatures (± 0.1°C) using a PELT-5 temperature-controlled cabinet (SSI) in which all chambers were contained. Data were acquired at 1s frequency with a UI2 interface (SSI). Resulting data were converted into energy used per unit time (Supporting information).

DATA ANALYSIS

All statistical analyses were performed in R v2.15.1. Preliminary data analysis was performed using a standardised data exploration protocol (Zuur, Ieno & Elphick 2010), and our general modelling approach was to start with the saturated model and drop non-significant terms sequentially (confirming the improved fit by ANOVA) until the minimal adequate model was reached (Crawley 2007). The fit of each model was then assessed by plotting residuals against fitted values to check for mean residual deviation of zero and constant variance. Where non-significant terms are retained in a final model, the distribution of residual variance was strongly preferable in the model presented compared to the simplified model.

We calculated larval and pupal survival for each family as the proportion surviving to pupation and adulthood respectively. We compared larval survival among ecotypes using a binomial regression, pupal survival using a generalised linear mixed model (*nlme* package) (Pinheiro *et al.* 2013) with binomial errors; for all other variables we used general linear mixed models (*lme4* package) (Bates, Maechler & Bolker 2011) with Gaussian errors using maximum likelihood parameter estimation. We used family as a random factor in all cases apart from larval survival (for which each family was represented by only one value [proportion survival] since the broods had not yet been split), with the fixed factors ecotype (larval and pupal survival), ecotype and sex (date of diapause) or ecotype, environment, and sex (all other univariate analyses). Fecundity analysis was performed only on females so sex was omitted as a factor and pupal mass added as a covariate due to an observed strong correlation between pupal mass and fecundity. For metabolite analyses, lipid-free dry mass (calculated by subtracting estimated lipid mass from dry mass) was used as a covariate to control for body size.

To examine direct correlations among life-history traits, we used data all females that survived to adulthood and constructed network graphs based on partial correlation matrices (pairwise Pearson's correlations conditioned on all other life-history variables) using the *qgraph* package (Epskamp *et al.* 2012), where two traits were connected by an edge if they had a significant partial correlations (FDR < 0.05) (Benjamini & Hochberg 1995).

Results

MICROCLIMATE DIFFERENCES AMONG SITES

Mean microclimate temperatures in Ohio were warmer and accumulated more heat units over winter than those in Ontario (t_1=18.3, p=0.035; Table S1; Fig. 2). In Ohio, the data loggers were covered by snow for only a few weeks in January, while in Ontario there was some snow in late November, and continuous cover (leading to low thermal variability) from mid-December to late March (Fig. 2A, Table S1). In months without snow cover, thermal variability of microclimates at the two sites was similar (Table S1). Incubator temperature regimes calculated from these data reflected what we regard as the salient features of the thermal environment at each site: specifically, the longer period of low and stable temperatures in Ontario, and the greater thermal variability and accumulation of heat in Ohio (Fig. 2C).

LIFE HISTORY MEASUREMENTS

The Ontario ecotype had significantly higher larval survival rates than the Ohio ecotype (Ontario: 26.8 % of 2418 larvae from 15 nests survived; Ohio: 17.5 % of 3637 larvae from 20 nests survived; $z = 7.64$, $p < 0.0001$). The Ohio ecotype had higher mass than the Ontario ecotype at pupation (Fig. 3A, Table S2, Table S3). By the end of winter, pupae from the two ecotypes were more similar in mass, but the responses to the environment differed among ecotypes: each ecotype lost more mass over the winter in the non-natal compared to natal environment, such that Ontario ecotypes were larger than Ohio ecotypes in the cool environment, while Ohio ecotypes were larger than Ontario ecotypes in warm environments (Fig. 3B, Table S2, Table S3). Reaction norms for pupal length in April revealed a similar interaction between ecotype and environment, except that in this case the pupal size was similar in cool environments, while Ohio pupae were considerably larger than Ontario pupae in the warm environment (Fig. 3C, Table S2, Table S3). By adulthood, Ohio ecotypes were larger and there were no effects of overwintering environment (Fig. 3D, Table S2, Table S3). Females were larger in all size and mass measurements (Table S2, Table S3).

Ohio ecotypes entered dormancy on average two weeks earlier than Ontario ecotypes (Ohio: 14 Sep ± 12 days; Ontario: 29 Sep ± 16 days; Table S2, Fig. 4A). Emergence from dormancy was governed by both ecotype and environment: Ontario ecotypes and individuals in warm environments emerged a few days earlier than Ohio ecotypes and those in cool environments respectively (Fig. 4B, Table S2). Fecundity was positively related to mass, and thus larger Ohio ecotypes tended to lay more eggs than did Ontario ecotypes (Fig. 4C, Table S2). However, there was no effect of ecotype or environment on fecundity once size was controlled for (Table S2). Each ecotype survived to adulthood better under their natal overwintering conditions than did the non-natal ecotype (ecotype × environment $z = 1.966$, $p = 0.049$; Fig. 4D).

There were significant partial correlations among size measurements within each life stage, but no direct significant correlations across life-stages in size measurements (Fig. 5). However, we did detect correlations between pupal size measurements and fecundity (estimated by egg number), and a negative partial correlation between egg number and egg size (Fig. 5).

PHYSIOLOGICAL MEASUREMENTS

Water content at the beginning of winter was higher in females and Ohio ecotypes (Fig. 6A, Table S4). By the end of winter, water content had decreased considerably and did not differ by ecotype or environment, although females had a higher water content than did males (Fig. 6B, Table S4). Triglycerides at the beginning of winter were higher in females (Fig. 6C), and the warm environment showed a trend toward reducing triglyceride stores in October (Fig. 6C, Table S4). Triglycerides at the end of winter were natural-log-transformed to improve normality. Ohio ecotypes in both environments and Ontario ecotypes in the warm environment had similar (relatively high) triglyceride levels, but Ontario ecotypes in the cool environment had very low triglyceride levels (Fig. 6D, Table S4). Carbohydrates at the beginning and end of winter were square-root-transformed to improve normality. For females at the beginning of winter, carbohydrate concentrations were higher for natal compared to non-natal ecotypes ($t_{1,7}$=2.33, p=0.044). At the end of winter, carbohydrate content was positively related to lipid-free dry mass (females: $t_{1,7}$=2.57, p=0.037; males: $t_{1,9}$=6.18, p<0.001) and Ontario ecotype females had higher carbohydrate content at the end of winter (Table S5), while for males there was no effect of ecotype or environment on carbohydrate content at the end of winter. Soluble protein was higher in females at both the beginning and end of winter (Table S4, Table S5). Lipid-free dry mass was higher for females than for males, but did not differ by ecotype or environment at either the beginning or the end of winter (Table S4, Table S5).

All pupae respired continuously (i.e. did not exhibit discontinuous or cyclic gas exchange) at all measurement temperatures (Fig. S1). Metabolic rate was $\log_{10}$-transformed prior to analysis to meet assumptions of normality. There were no effects of measurement order on metabolic rate at either time point (beginning of winter: $F_{1,118}$=0.261, p=0.610; end of winter: $F_{1,117}$=0.1147, p=0.735). At the end of winter, the 15°C measurement for one individual from the Ontario ecotype in the cool environment was lost due to equipment malfunction. We interpolated to this value using a linear regression of measurement temperature on $\log_{10}$ metabolic rate for that individual. At the beginning of winter, metabolic rate was positively correlated with measurement temperature and negatively correlated with mass, and was lower in pupae that were overwintering in the warm environment (Table S4, Fig. 7A). At the end of winter, metabolic rate remained positively temperature-dependent and was subject to a significant measurement temperature × ecotype interaction, such that the thermal sensitivity of metabolic rate was lower in individuals from Ontario (Fig. 7B).

Discussion

Metabolic responses to changes in winter conditions have diverged between populations of *Hyphantria cunea*, and these altered responses at the physiological level give rise to differences in fitness-relevant traits that suggest adaptation to local winter thermal conditions. This local adaptation appears to be driven by among-population variation in rates of energy use, growth and development and increases survival to adulthood in the natal overwintering environment for each population.

EVIDENCE FOR LOCAL ADAPTATION – A CROSS-SEASONAL PERSPECTIVE

Increased performance of natal compared to non-natal ecotypes within each environment is a characteristic signature of local adaptation (Kawecki & Ebert 2004). We found this signature of local adaptation in overwinter survival: mortality of each ecotype was lowest in their natal environment. We note that this pattern may also be generated by developmental or maternal effects, so we use the term local adaptation as an hypothesis requiring further experiments to test. Looking to the physiological level to explain the mechanisms for this local adaptation, we found similar ecotype-by-environment interactions in fitness-relevant traits including pupal mass, size and storage lipid and carbohydrate reserves at the end of winter, thermal sensitivity of metabolism in the spring, and mortality. For all of these traits (except storage lipids), performance was higher for each ecotype at “home” compared to “away”. Thus, it appears that the higher survival of each ecotype in their natal winter conditions is mediated by alterations to intermediary metabolism that allow them to retain larger size and greater energy reserves throughout winter. This suggests that if winter temperatures become decoupled from growing season temperatures, negative fitness consequences could result for both ecotypes.

Local adaptation to temperature in terrestrial animals has been shown in life-history traits including body size and growth and development rates (Conover, Duffy & Hice 2009), but few studies have measured traits at both the physiological and life-history level, across multiple life-stages and seasons. In particular, we have shown that local adaptation is mediated across seasons – energetic responses to the overwintering environment influence performance and fitness the following spring, emphasising the importance of taking a cross-seasonal perspective to understanding the impacts of climate change on terrestrial organisms (Williams, Henry & Sinclair in press). Many of these impacts will be mediated through the effects of energetics on seasonal timing.

The timing of entry into and exit from dormancy will interact with energetics to determine performance and fitness. All else being equal, a longer overwintering period relative to growing season will reduce fitness due to increased energetic costs of winter, or reduced opportunity for resource accumulation. We found that Ohio ecotypes enter dormancy on average two weeks earlier than Ontario ecotypes, likely due to a combination of earlier spring emergence and faster rates of larval growth and development due to warmer temperatures (Morris & Fulton 1970a). The threshold temperature for pupal development in *H. cunea* is 11°C (Morris & Fulton 1970a; Gomi, Inudo & Yamada 2003) - our microclimate data show that mean temperatures would cross this threshold in March in the range centre, but not until April at the northern range edge (Fig. 2A). This suggests that adult emergence would occur earlier in Ohio than in Ontario, and indeed spring phenology is generally correlated with latitude, with more southerly populations having earlier spring phenology (Hodgson *et al*. 2011). Earlier entry into dormancy in autumn can have negative fitness consequences, since it increases the length of dormancy and leads to energy drain in this species (Gomi 2000), and other insects (Bosch & Kemp 2004). However, the Ohio ecotype also accumulated greater lipid, protein and carbohydrate reserves and attained larger pupal mass and length, which appeared to offset any energetic costs of longer dormancy, since fecundity and adult size were higher in the Ohio ecotype.

Shorter growing seasons at high latitudes limits the time available for foraging and growth, and thus final size that can be obtained, resulting in body size clines towards smaller size at high latitudes (converse Bergmann clines), particularly in ectotherms with long generation times relative to season length (Blanckenhorn & Demont 2004). Our data are consistent with a converse Bergmann cline in this species, which at the latitudes we collected from have 1-2 generations per year (Wagner 2005). Seasonal time constraints at high latitudes drive differential selection on growing season energetics which can lead to countergradient variation in growth and development rates (Blanckenhorn & Demont 2004). Consistent with this hypothesis, we observed faster development in the Ontario ecotype. *Hyphantria cunea* populations have been previously shown to differ in their heat requirement for post-winter pupal development post-winter, with populations from relatively cool continental environments in Canada having lower pupal heat requirements post-winter than do coastal populations, enabling early emergence in cool environments (Morris & Fulton 1970a). Post-winter pupal development in this species is highly heritable and influences fitness (Morris & Fulton 1970b). Frog tadpoles, dragonfly larvae and butterfly larvae from poleward populations also develop faster at a common temperature than do more central populations (Ayres & Scriber 1994; Laugen *et al.* 2003; Śniegula, Johansson & Nilsson-Örtman 2012; Muir *et al.* 2014).

We propose that increased low-temperature anabolism at the end of winter could underlie early development in these and other ectotherms adapted to high temperate latitudes: since it is likely that development had resumed by May when the end-of-winter measurements were taken, the metabolism we measured likely included costs of synthesising adult tissue, and the increased metabolic rate in Ontario ecotypes at low temperatures may reflect an increase in anabolic processes - consistent with selection for early emergence in short, cool growing seasons. Global patterns in the relationship between thermal sensitivity of growth, development and metabolism have been mixed, with various studies finding either negative (MacKay 1982; Addo-Bediako, Chown & Gaston 2002; Terblanche *et al.* 2009), positive (Rao & Bullock 1954), or no relationship (Scholander *et al.* 1953) between thermal sensitivity and environmental temperatures. Some authors have suggested that these idiosyncrasies may relate to microclimate temperatures available to the organism, whereby cold-adapted organisms that have access to more frequent hot, sunny periods might be expected to have higher thermal sensitivity relative to warm-adapted organisms, while those in permanently cool and cloudy environments might have reduced thermal sensitivity (Addo-Bediako, Chown & Gaston 2002). Our study species overwinters on the ground beneath the leaf litter in wooded areas, and microclimate temperatures in Ontario remain below 10°C until late April. Thus, reduced thermal sensitivity that prevents large reductions in metabolic and development rates at low temperatures may be most beneficial (and are supported by our data). By measuring both metabolism and development rates, the present study provides evidence linking the physiological mechanism (increased metabolic rate) to the life-history consequence (faster post-winter development) under laboratory conditions.

Local adaptation will determine species' responses to climate change: if poleward populations are metabolically adapted to local climate conditions, then warming may disproportionately impact these populations by increasing overwinter mortality. This, in turn, could lead to range contraction, or the failure to colonise newly suitable poleward climates. It remains to be seen how widespread such metabolic local adaptation to winter climate may be among ectotherms or hibernators. If such local adaptation to winter conditions is common, it may require us to rethink the paradigm of peripheral enhancement for poleward populations under climate warming scenarios.

EFFECTS OF THE OVERWINTERING ENVIRONMENT

The warm overwintering environment induced a plastic metabolic suppression in pupae from both ecotypes at the beginning of winter. Plastic changes to phenotypes may be adaptive, maladaptive, or neutral, depending on their fitness consequences (Ghalambor *et al.* 2007). The plastic metabolic response to warm winters may be an example of adaptive phenotypic plasticity

(DeWitt & Scheiner 2004), since it was in the predicted direction, expressed similarly by two separate populations, and prevented pupae from experiencing energy drain from warmer winters. Adaptive phenotypic plasticity can facilitate adaptation to novel environments, by reducing directional selection and allowing time for organisms to respond to environmental change (Ghalambor *et al.* 2007). Global climate change is modifying winter conditions rapidly, and the capacity for adaptive phenotypic plasticity to buffer some of the negative effects will be an important predictor of species responses to climate change (Williams, Henry & Sinclair in press). The presence of substantial phenotypic plasticity in energy use will decrease the vulnerability of *H. cunea* to energy drain as a result of winter warming. *Hyphantria cunea* pupae also show pronounced metabolic suppression and no detectable decline in energy reserves over the course of a winter in the field (Li *et al.* 2001). However, many dormant ectotherms do experience energy drain as a result of winter warming (Williams, Shorthouse & Lee 2003; Williams, Hellmann & Sinclair 2012; Muir *et al.* 2013), suggesting that metabolic plasticity is not universal and may be a useful predictor of vulnerability to climate change.

Since broods experienced identical conditions up until the point of transfer into overwintering treatments, we can definitively say that the metabolic suppression resulted from thermal conditions experienced during the dormant, overwintering stage. Metabolic suppression is a common component of winter dormancy both in insects (Koštál 2006) and in other hibernating or torpid animals (Storey & Storey 2004), but here we illustrate that the depth of suppression can be modulated by conditions experienced after the onset of dormancy. The depth of metabolic suppression in an overwintering insect can also be increased by increasing thermal variability (Williams *et al.* 2012).

CONCLUSIONS

We detected a signature of local adaptation to the overwintering environment such that survival was maximised in natal environments by both ecotypes, as a result of alterations to intermediary metabolism. These alterations to overwintering metabolism impacted not only survival but also performance in spring. This suggests that any changes to overwintering conditions could have negative impacts on populations across the range of *H. cunea*, rather than enhancing poleward populations. Since the data available suggest that local adaptation may be common in terrestrial animals, and winter conditions are changing rapidly, more research effort should be expended to assessing cross-seasonal consequences of local adaptation to thermal conditions in terrestrial animals. Current evidence for local adaptation to thermal conditions in terrestrial animals is sufficient to suggest that the population is the appropriate unit for conservation.

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Data Accessibility

Data are archived in Dryad data repository (doi: xxxxxxx).

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Figure captions

Figure 1 – Life cycle of temperate univoltine populations of *Hyphantria cunea*. Adults lay eggs in late spring, which hatch and feed communally in nests during the summer until they reach the final (6th) instar (larval developmental stage) in the autumn. They then disperse into the leaf litter and pupate, overwintering in cocoons beneath the leaf litter. Photos provided by Dr. Andrei Sourakov, McGuire Center for Lepidoptera and Biodiversity, Gainesville FL.

Figure 2 - Microclimate temperatures for sites near Ottawa, Ontario (ON) and Columbus, Ohio (OH), and incubator temperatures derived from those data (A) Representative traces of microclimate temperatures from under the leaf litter in woodlots where *Hyphantria cunea* occur in ON or OH from October 2008 to May 2009; measured by paired iButton data loggers. Horizontal lines below indicate the period of continuous snow cover at each site, determined by continuous zero temperatures and low thermal variability. **(B)** Accumulated degrees above -10°C (close to the close to the minimum temperature experienced at either site) between October and May in Ohio and Ontario. Data are mean ± SEM of two loggers at each site. **(C)** Temperatures of incubators used to house *H. cunea* under conditions approximating OH (warm) or ON (cool). Incubator temperatures were derived from fortnightly mean daily minima and maxima for Oct 2008 - May 2009, calculated from microclimate temperatures from two iButtons per site.

Figure 3 - Size measurements of *Hyphantria cunea* from Ohio or Ontario, overwintered at warm or cool temperatures in the lab in a simulated reciprocal transplant. Pupal mass at the beginning **(A)** and end **(B)** of winter; pupal length at the end of winter **(C);** and adult mass in the spring **(D).** Values (± SEM) are predicted from models provided in Table S2, thus taking into account the effects of family and any significant covariates. See Table S3 for raw data.

Figure 4 – Life history reaction norms of *Hyphantria cunea* from Ohio or Ontario, overwintered at warm or cool temperatures in the lab in a simulated reciprocal transplant. **(A)** Date of entering diapause in the fall; **(B)** days at 25°C prior to adult emergence in the spring; **(C)** number of eggs per female and **(D)** percent survival. Values (± SEM) are predicted from models provided in Table S2, thus taking into account the effects of family and any significant covariates.

Figure 5 – Partial correlations among life-history traits across life-stages of *Hyphantria cunea*. Beg = beginning of winter, End = end of winter. We found consistent direct correlations within life stages, but few among-stage correlations. Notably, we did not demonstrate any relationship between adult size and fecundity.

Figure 6 – Body composition measurements of *Hyphantria cunea* from Ohio or Ontario, overwintered at warm or cool temperatures in the lab in a simulated reciprocal transplant. Water at the beginning **(A)** and end **(B)** of winter; and triglycerides at the beginning **(C)** and end **(D)** of winter. Values (± SEM) are predicted from models provided in Table S2, thus taking into account the effects of family and any significant covariates. See Table S5 for raw data.

Figure 7 - Metabolic rates of diapausing *Hyphantria cunea* pupae from Ohio or Ontario, overwintered at warm or cool temperatures in the lab in a simulated reciprocal transplant. Metabolic rate was measured in (A) October (beginning of winter) or (B) April (end of winter) using flow-through respirometry. The trend lines indicate the predictions of linear models (Table S2). Pupae kept under warm winter conditions had decreased metabolic rates at the beginning of winter, while at the end of winter pupae from Ontario had less temperature-sensitive metabolism.

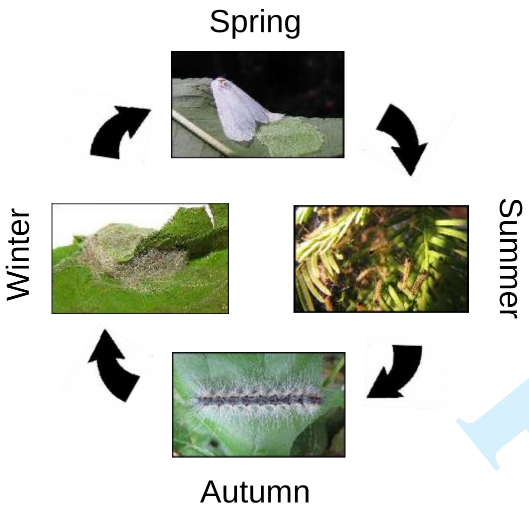


Figure 1

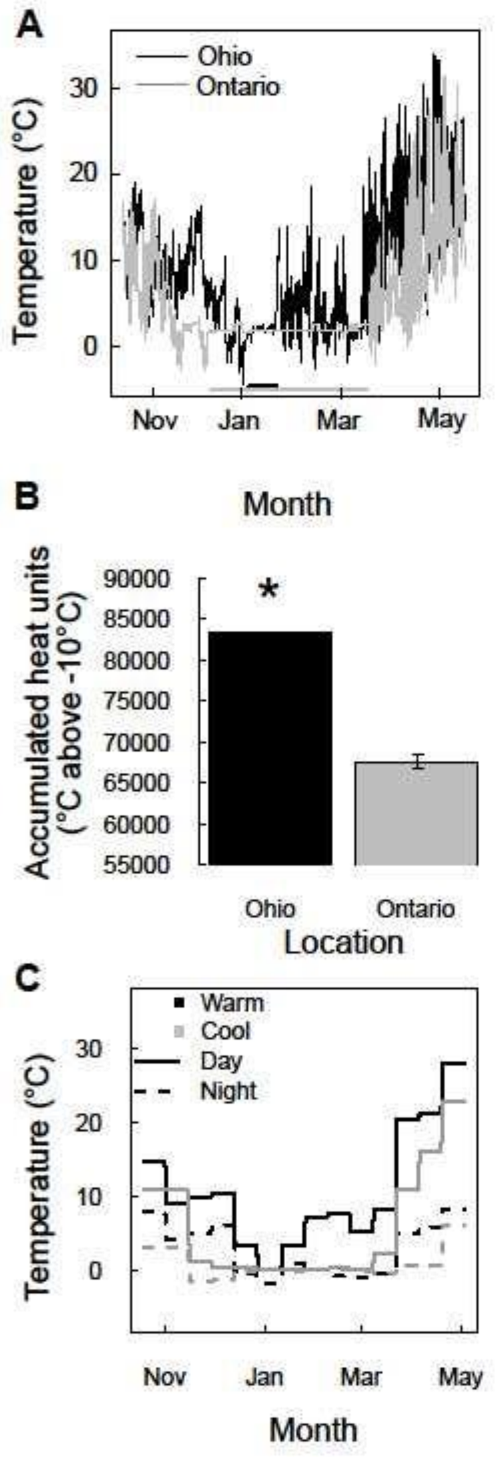
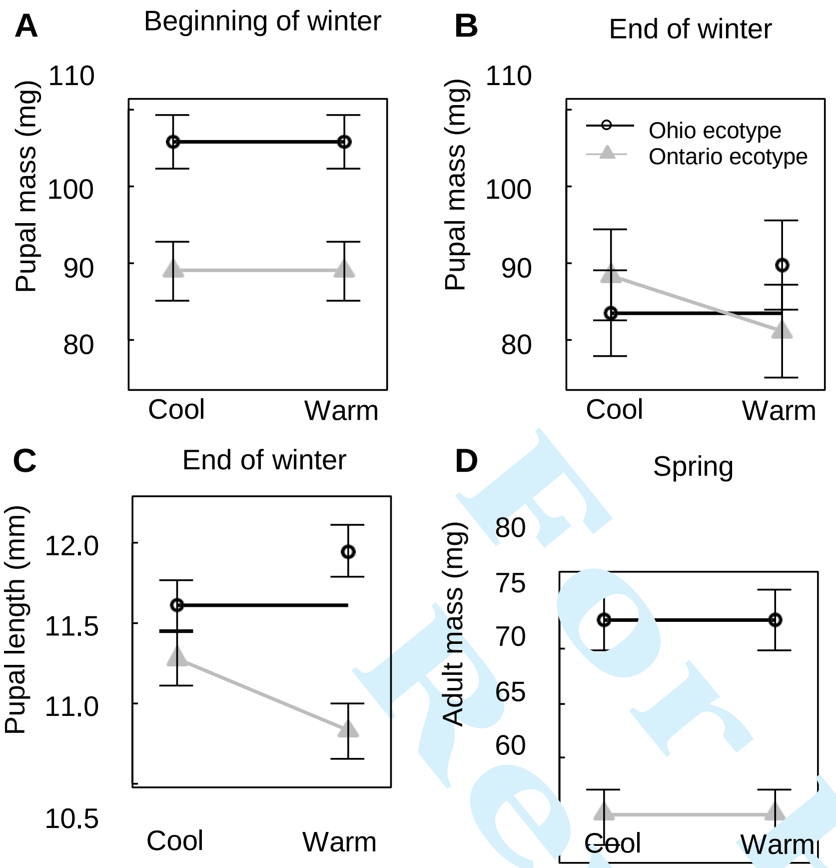

A
Ohio
Ontario
Temperature (°C)
30
20
10
0
Nov
Jan
Mar
May
Month
B
Accumulated heat units (°C above -10°C)
90000
85000
80000
75000
70000
65000
60000
55000
*
Ohio
Ontario
Location
C
■ Warm
■ Cool
Day
Night
Temperature (°C)
30
20
10
0
Nov
Jan
Mar
May
Month

Figure 2



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703 Figure 3

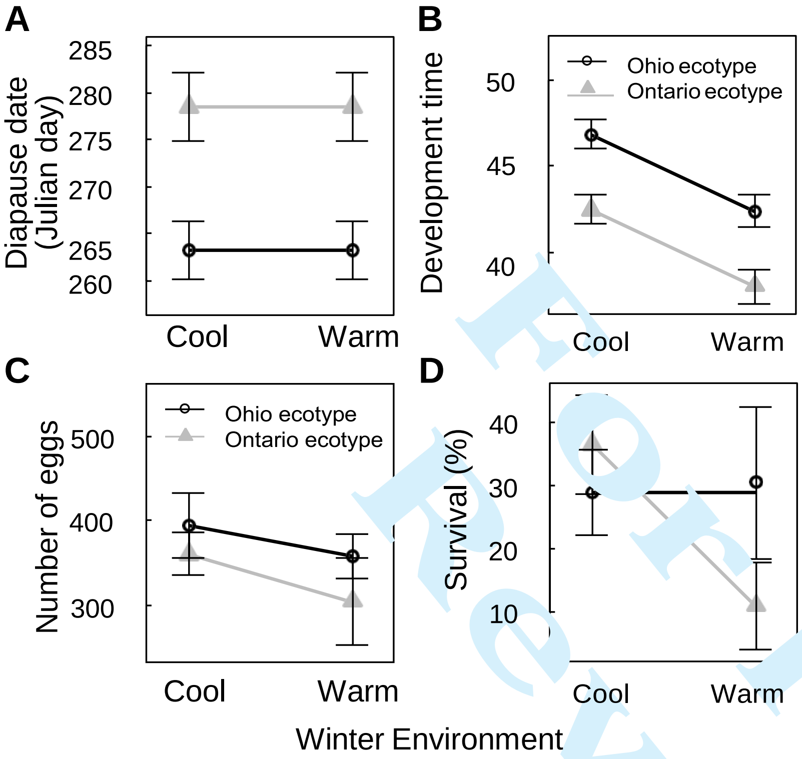
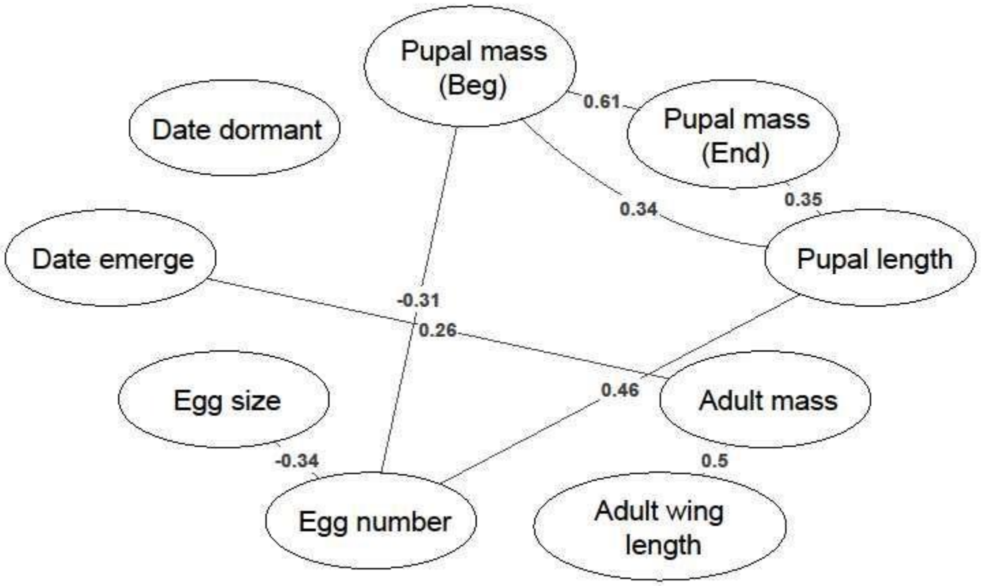

A
Diapause date (Julian day)
285
280
275
270
265
260
Cool
Warm
B
Development time
50
45
40
Ohio ecotype
Ontario ecotype
Cool
Warm
C
Number of eggs
500
400
300
Ohio ecotype
Ontario ecotype
Cool
Warm
D
Survival (%)
40
30
20
10
Cool
Warm
Winter Environment

Figure 4



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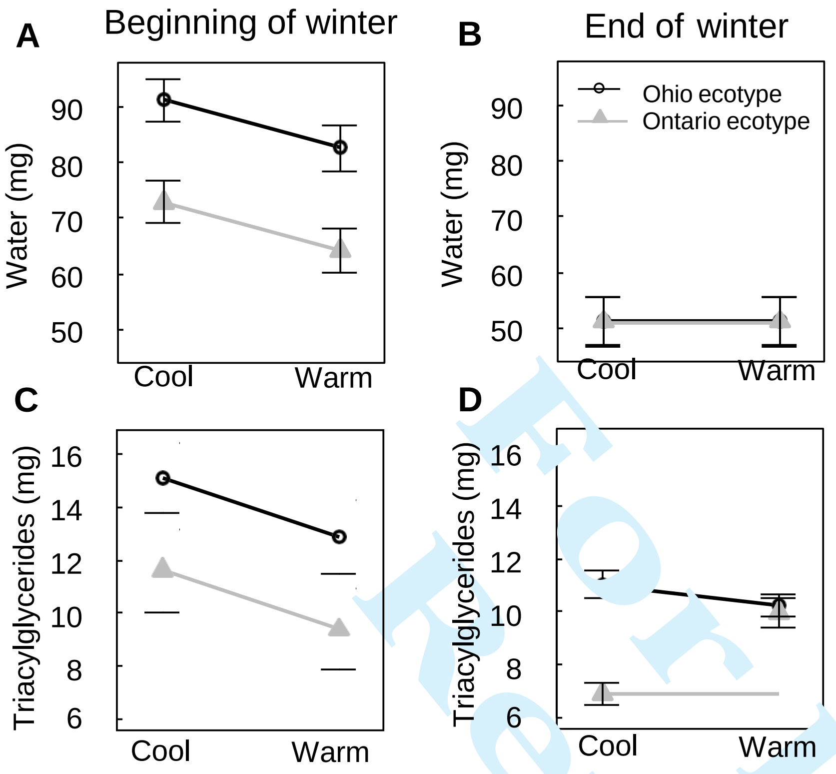
710 Figure 5



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713 Figure 6

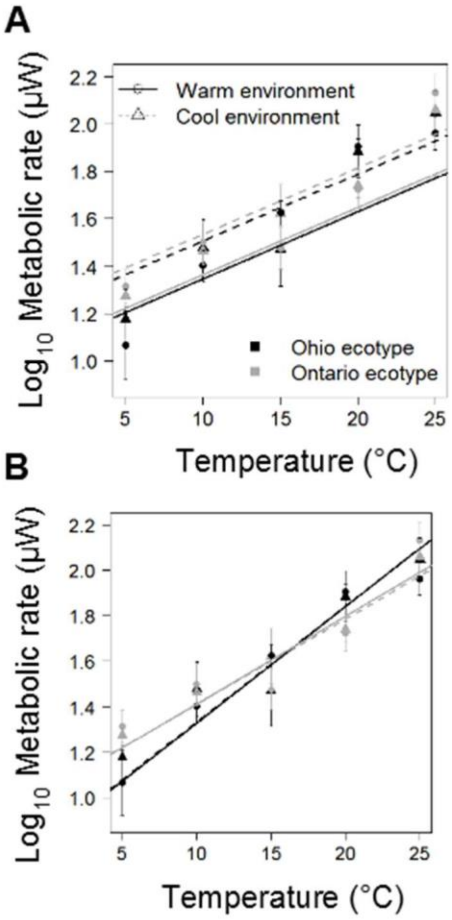
A
Log$_{10}$ Metabolic rate (µW)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
Warm environment
Cool environment
Ohio ecotype
Ontario ecotype
5
10
15
20
25
Temperature (°C)
B
Log$_{10}$ Metabolic rate (µW)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
5
10
15
20
25
Temperature (°C)

Figure 7

Supporting information

SUPPORTING METHODS

Respirometry data processing

We drift-corrected water and CO_2 measurements to the baseline chamber, then converted into CO2 production using the following equation (Lighton 2008):

$$\dot{V}CO_2 = FR_i(F_eCO_2 - F_iCO_2) \times FR \qquad (1)$$

Where VCO_2 is the rate of CO_2 production in $mL{\cdot}min^{-1}$; FR_i is the incurrent flow rate in $mL{\cdot}min^{-1}$, and F_eCO_2 and F_iCO_2 are the fractional concentrations of excurrent and incurrent CO_2 respectively.

We measured VCO_2 of each pupa over a 40 min period after a minimum of 1 h acclimation and calculated mean VCO_2 emission over the final 30 minutes of recording to allow accumulated gases to wash through the system. We converted VCO_2 to VO_2 (rate of O_2 consumption) assuming a respiratory exchange ratio (RER) of 0.8:

$$VO_2 = VCO_2 / RER \qquad (2)$$

and then converted VO_2 into metabolic rate in Watts ($J{\cdot}sec^{-1}$) using the oxyjoule equivalent (Lighton 2008):

$$\text{oxyjoule equivalent} = 16 + (5 \times RER) \qquad (3)$$

$$\text{Metabolic rate} = (VO_2 \times \text{oxyjoule equivalent}) / 60 \qquad (4)$$

As RERs of non-assimilating organisms vary from 0.7 - 1 depending on the metabolic substrate, some error (-3 to +5%) will be introduced by an incorrect assumption of RER in equation 2 (Lighton 2008). However, as the value of the oxyjoule equivalent also depends on RER (equation 3), and the error introduced at this step is in the opposite direction, the assumption of an RER of 0.8 throughout will cause less than 0.6 % error in metabolic rate estimates over the entire physiological range of RER (Lighton 2008).

SUPPORTING REFERENCES

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SUPPORTING FIGURES

Figure S1 - Representative CO_2 emission traces from 6 female overwintering *Hyphantria cunea* pupae, weighing 0.057, 0.089, 0.065, 0.057, 0.0069, and 0.043g (left to right) and measured at 20°C. ‘b’ indicates baseline measurements from an empty cuvette, conducted at the beginning and end of each run.

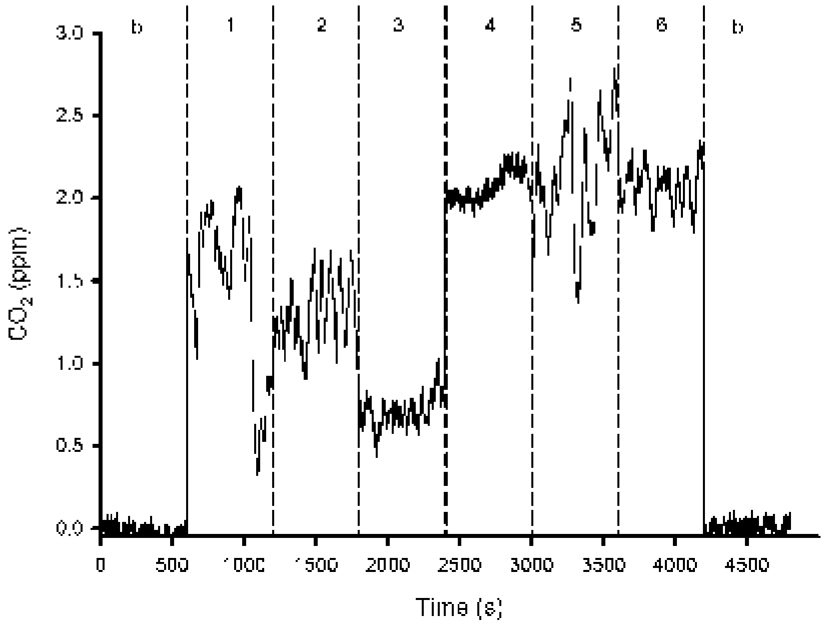
b
1
2
3
4
5
6
b
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CO_2 (ppm)
0
500
1000
1500
2000
2500
3000
3500
4000
4500
Time (s)

Figure S1

SUPPORTING TABLES

Table S1 - Microclimate temperatures from *H. cunea* habitat in Ottawa, Ontario or Athens, Ohio. Data are soil surface temperatures in°C (monthly mean ± SEM) for the 2008 – 2009 winter, from iButton data loggers in the leaf litter. N= number of loggers per site; Snow = days of snow cover.

Location	**Ontario** 45.2°N, 75.4°W				**Ohio** 39.2°N, 82.0°W			
N	2				2			
	Minimum	Mean	Maximum	Snow	Minimum	Mean	Maximum	Snow
October	0.4 ± 2.2	6.0 ± 3.5	18.7 ± 4.2	0	2.3 ± 3.5	11.3 ± 4.2	20.0 ± 2.7	0
November	-5.2 ± 3.3	2.9 ± 4.3	18.5 ± 5.8	0	-0.5 ± 2.2	6.9 ± 3.9	16.3 ± 2.2	0
December	-4.4 ± 1.5	0.0 ± 1.0	2.1 ± 0.6	18	-7.2 ± 5.2	3.3 ± 6.3	18.8 ± 5.4	0
January	-0.4 ± 0.2	0.0 ± 0.2	0.4 ± 0.2	31	-5.0 ± 2.1	1.6 ± 3.3	14.8 ± 4.3	28
February	0.1 ± 0.1	0.3 ± 0.2	0.6 ± 0.2	28	-6.1 ± 2.5	2.8 ± 4.3	19.8 ± 4.4	6
March	-4.7 ± 1.5	1.7 ± 3.9	23.7 ± 5.4	25	-5.8 ± 2.5	4.0 ± 5.5	25.8 ± 8.9	5
April	-2.4 ± 3.0	8.7 ± 7.5	39.2 ± 8.1	0	2.8 ± 2.8	12.7 ± 5.5	33.5 ± 7.7	0
May	3.9 ± 2.6	13.7 ± 5.7	33.7 ± 7.2	0	9.3 ± 2.2	17.2 ± 4.1	33.7 ± 5.5	0
Absolute min	-5.4				-9.1			
Absolute max	42.1				34.8			
Length of snow cover	14.5 weeks				5.5 weeks			

Table S2 – Influences on life-history of overwintering *Hyphantria cunea*. General linear mixed effects models of the effects of ecotype, overwintering environment, and sex on Fall webworms from Columbus, Ohio (OH) or Ottawa, Ontario (ON) overwintered in the laboratory at warm or cool microclimate temperatures in a simulated reciprocal transplant. Mass = pupal mass, Development = days to emerge after transfer to 25°C. The factor level associated with higher values of the response variable is indicated in parentheses unless interactions were detected, and the direction of the slope for significant covariates is indicated in parentheses. Q-values were calculated using a table-wide FDR-correction (Benjamini & Hochberg 1995).

Variable	Parameter	df	T statistic	P value	Q value
Mass_{Nov}	Sex (F)	511	8.65	<0.001	<0.001
	Ecotype (OH)	29	2.48	0.019	0.021
Mass_{Apr}	Sex (F)	144	4.11	<0.001	<0.001
	Ecotype × Environment	144	2.09	0.038	0.038
Pupal $\text{length}_{\text{Apr}}$	Sex (F)	141	4.28	<0.001	<0.001
	Ecotype × Environment	141	3.22	0.002	0.003
Adult mass	Sex (F)	59	8.58	<0.001	<0.001
	Ecotype (OH)	19	5.11	<0.001	<0.001
Wing length	Sex (F)	55	4.78	<0.001	<0.001
Diapause date	Ecotype (ON)	30	3.2	0.003	0.004
Development	Ecotype (ON)	16	3.89	0.001	0.001
	Environment (Cool)	48	4.76	<0.001	<0.001
Fecundity	Mass (+)	10	4.97	<0.001	0.001

Table S3 - Size of Fall webworms originating from Ohio or Ontario and overwintered at warm (shaded) or cool temperatures in a simulated reciprocal transplant experiment.

Values are mean ± SEM, sample sizes are in parentheses.

			Pupal measurements			Adult measurements	
Ecotype	**Environment**	**Sex**	**Autumn mass (mg)**	**Spring mass (mg)**	**Length (mm)**	**Mass (mg)**	**Wing length (mm)**
Ohio	warm	M	88.2 ± 3.0 (73)	74.8 ± 6.8 (22)	11.6 ± 0.2 (22)	51.1 ± 5.8 (10)	12.7 ± 0.5 (8)
		F	102.3 ± 3.4 (61)	92.2 ± 4.6 (21)	11.9 ± 0.1 (21)	79.7 ± 2.8 (13)	14.4 ± 0.7 (13)
	cool	M	84.1 ± 2.5 (71)	79.5 ± 3.9 (21)	11 ± 0.2 (21)	45.4 ± 5.1 (12)	12.2 ± 0.4 (10)
		F	108.6 ± 2.9 (73)	89.3 ± 4.8 (27)	11.8 ± 0.2 (27)	78.9 ± 4.4 (10)	13.4 ± 0.7 (10)
Ontario	warm	M	76.9 ± 2.7 (57)	69.8 ± 4.6 (16)	10.5 ± 0.2 (16)	31.7 ± 4.7 (5)	11.8 ± 0.5 (4)
		F	91.1 ± 2.9 (74)	79.7 ± 4.4 (21)	10.9 ± 0.2 (21)	61.5 ± 7.3 (6)	13.5 ± 0.7 (6)
	cool	M	81.3 ± 1.8 (74)	70.9 ± 2.7 (22)	10.8 ± 0.1 (21)	35.3 ± 4.2 (9)	11.3 ± 0.6 (9)
		F	91.4 ± 2.5 (60)	90.3 ± 4.7 (16)	11.2 ± 0.2 (14)	57 ± 4.0 (16)	13.7 ± 0.3 (16)

Table S4 – Influences on physiology of overwintering *Hyphantria cunea*. General linear mixed effects models of the effects of ecotype, overwintering environment, and sex on Fall webworms from Columbus, Ohio (OH) or Ottawa, Ontario (ON) overwintered in the laboratory at warm or cool microclimate temperatures in a simulated reciprocal transplant. Mass = pupal mass, LFDM = lipid-free dry mass, Met. rate = metabolic rate, Temp. = measurement temperature for metabolic thermal performance curves. The factor level associated with higher values of the response variable is indicated in parentheses unless interactions were detected, and the direction of the slope for significant covariates is indicated in parentheses. Q-values were calculated using a table-wide FDR-correction (Benjamini & Hochberg 1995).

Variable	Parameter	df	T statistic	P value	Q value
Water$_{\text{Nov}}$	Sex (F)	21	2.25	0.035	0.049
	Ecotype (OH)	15	4.59	<0.001	0.001
	Environment (Cool)	21	2.15	0.044	0.057
Water$_{\text{Apr}}$	Sex (F)	20	2.76	0.012	0.023
Triglycerides$_{\text{Nov}}$	Sex (F)	21	2.32	0.030	0.045
	Ecotype (OH)	15	2.14	0.049	0.057
	Environment (Cool)	21	1.74	0.096	0.096
Triglycerides$_{\text{Apr}}$	Sex (F)	18	3.90	0.001	0.003
	Ecotype × Environment	18	2.49	0.023	0.037
Carbohydrates$_{\text{Nov}}$	Sex × Ecotype × Environment	18	2.04	0.057	0.060
Carbohydrates$_{\text{Apr}}$	Sex × Ecotype × Environment	17	2.06	0.055	0.060
Protein$_{\text{Nov}}$	Sex (F)	24	4.45	<0.001	<0.001
Protein$_{\text{Apr}}$	Sex (F)	23	4.61	<0.001	<0.001
	Ecotype (OH)	23	2.10	0.046	0.057
LFDM$_{\text{Nov}}$	Sex (F)	22	5.40	<0.001	<0.001
LFDM$_{\text{Apr}}$	Sex (F)	20	3.50	0.002	0.006
Met. rate$_{\text{Nov}}$	Temp. (+)	94	10.45	<0.001	<0.001
	Mass (-)	94	2.64	0.010	0.021
	Incubator (Cool)	22	2.99	0.007	0.016
Met. rate$_{\text{Apr}}$	Temp. (+)	94	13.77	<0.001	<0.001
	Temp. × Ecotype	94	2.47	0.015	0.026

Table S5 – Body composition of Fall webworms originating from Ohio or Ontario and overwintered at warm (shaded) or cool temperatures in a simulated reciprocal transplant experiment. Values are mean ± SEM, sample sizes are in parentheses. TAG = triglycerides, Carb = carbohydrates, LFDM = lipid- and carbohydrate-free dry mass.

			Autumn measurements (November)					**Spring measurements (April)**				
Ecotype	**Environment**	**Sex**	Water (mg)	TAG (mg)	Carb (mg)	Protein (mg)	LFDM (mg)	Water (mg)	TAG (mg)	Carb (mg)	Protein (mg)	LFDM (mg)
Ohio	warm	M	77.3 ± 4.5 (5)	10.9 ± 1.4 (5)	0.074 ± 0.044 (5)	13.7 ± 0.8 (3)	22.0 ± 2.5 (5)	30.7 ± 10 (4)	10.9 ± 1.4 (5)	0.062 ± 0.034 (3)	14.6 ± 2.3 (5)	19.9 ± 1.7 (4)
		F	80.3 ± 5.1 (4)	12.3 ± 2.4 (4)	0.257 ± 0.042 (6)	8.1 ± 0.4 (6)	28.3 ± 2.8 (4)	50 ± 7.1 (7)	12.3 ± 2.4 (4)	0.033 ± 0.020 (6)	7.2 ± 0.6 (6)	27.5 ± 3.1 (7)
	cool	M	76.2 ± 2.8 (6)	11.2 ± 0.9 (6)	0.253 ± 0.067 (4)	12.6 ± 1.1 (7)	19.1 ± 1.7 (6)	39 ± 11.2 (6)	11.2 ± 0.9 (6)	0.122 ± 0.052 (7)	11.7 ± 1.3 (4)	21.2 ± 2.7 (6)
		F	96.4 ± 3.5 (5)	16.5 ± 2.5 (5)	0.221 ± 0.040 (5)	10.4 ± 1.7 (4)	28.1 ± 1.7 (5)	51.2 ± 8.5 (3)	16.5 ± 2.5 (5)	0.095 ± 0.014 (4)	8.5 ± 0.9 (5)	26.8 ± 1.5 (3)
Ontario	warm	M	54.4 ± 8.4 (6)	6.7 ± 1.6 (6)	0.273 ± 0.097 (5)	10.6 ± 0.6 (5)	17.2 ± 1.2 (6)	34 ± 2.8 (5)	6.7 ± 1.6 (6)	0.159 ± 0.014 (5)	11.6 ± 0.9 (5)	21.6 ± 2.5 (5)
		F	63 ± 8.9 (4)	9.7 ± 4.1 (4)	0.179 ± 0.081 (5)	7.2 ± 0.9 (5)	22.6 ± 2.3 (4)	66.8 ± 7.2 (4)	9.7 ± 4.1 (4)	0.146 ± 0.055 (5)	8.4 ± 1.4 (5)	25.8 ± 2.6 (4)
	cool	M	68.2 ± 4 (5)	10.4 ± 1.2 (5)	0.124 ± 0.066 (4)	10.9 ± 1.2 (4)	17.9 ± 0.9 (5)	49.8 ± 2.3 (5)	10.4 ± 1.2 (5)	0.306 ± 0.087 (4)	10.1 ± 1.5 (4)	15.9 ± 1.6 (5)
		F	70.4 ± 5.3 (5)	11.6 ± 1.4 (5)	0.134 ± 0.048 (6)	9.2 ± 0.8 (5)	28.8 ± 3.2 (5)	49.9 ± 9.6 (5)	11.6 ± 1.4 (5)	0.081 ± 0.028 (5)	7.7 ± 1.3 (6)	24.2 ± 1.3 (5)