



Experimental winter warming modifies thermal performance and primes acorn ants for warm weather



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ABSTRACT

The frequency of warm winter days is increasing under global climate change, but how organisms respond to warmer winters is not well understood. Most studies focus on growing season responses to warming. Locomotor performance is often highly sensitive to temperature, and can determine fitness outcomes through a variety of mechanisms including resource acquisition and predator escape. As a consequence, locomotor performance, and its impacts on fitness, may be strongly affected by winter warming in winter-active species. Here we use the acorn ant, *Temnothorax curvispinosus*, to explore how thermal performance (temperature-driven plasticity) in running speed is influenced by experimental winter warming of 3–5 °C above ambient in a field setting. We used running speed as a measure of performance as it is a common locomotor trait that influences acquisition of nest sites and food in acorn ants. Experimental winter warming significantly altered thermal performance for running speed at high (26 and 36 °C) but not low test temperatures (6 and 16 °C). Although we saw little differentiation in thermal performance at cooler test temperatures, we saw a marked increase in running speed at the hotter test temperatures for ants that experienced warmer winters compared with those that experienced cooler winters. Our results provide evidence that overwintering temperatures can substantially influence organismal performance, and suggest that we cannot ignore overwintering effects when forecasting organismal responses to environmental changes in temperature.

1. Introduction

Global climate change is increasing the frequency of warm winter days in temperate, polar and alpine habitats (Stocker et al., 2013). Most climate change research focuses on warming during the growing season when environmental temperatures approach or exceed the upper tolerance limits of organisms, but winter warming can also have strong effects on thermal performance and fitness (Williams et al., 2015b). On one hand, warmer winters could be detrimental to ectothermic species that undergo winter diapause as warmer temperatures increase metabolism and cause organisms to expend excess energy that would otherwise be conserved under cooler conditions (Sorvari et al., 2011; Williams et al., 2015a). However, warmer winters could benefit less cold tolerant species by limiting their exposure to cold stress and even priming them to emerge earlier in the spring.

Running speed is a widely used locomotor performance trait, and is a key determinant of resource acquisition in many organisms (Irschick et al., 2014). This trait is highly sensitive to temperature (Shapley,

1924; Angilletta et al., 2008; Angilletta, 2009): running speed is determined by both immediate effects of the temperature at which an organism is running and long-term effects (weeks to months) of the temperature to which organisms are acclimated prior to running. In response to warmer winters, we can examine how thermal performance in locomotion is affected by acclimation at warmer or cooler winter temperatures. Growing season studies on running speed show a range of thermal performance curve response to acclimation temperature (Andrew et al., 2013; Clusella-Trullas et al., 2010). Generally, running speed increases with increasing test temperatures, but results differ as to whether warmer or intermediate acclimation temperatures yield faster running speeds (Clusella-Trullas et al., 2010; Huey and Berrigan, 1996; Leroi et al., 1994), and whether the magnitude of increase in running speed under warmer test temperatures depends on acclimation temperature (Gibert et al., 2001). While running speed and other locomotor performance metrics have been used extensively to explore responses to increased temperatures under climate change during the growing season (Huey et al., 2009; Sinervo et al., 2010), we know little

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about how locomotor performance is affected by winter warming, especially concerning the transition from winter to spring and the growing season.

Here we explore how thermal performance of running speed responds to field-based experimental winter warming in the acorn ant, *Temnothorax curvispinosus*. This widespread, thermophilic ant species exhibits thermal sensitivity in activity and behavior, including active thermoregulation inside their nests to exploit even small increases in temperature (Karlik et al., 2016; Penick et al., 2017). Ant colonies, like most animals, are often resource-limited both with respect to food (Traniello et al., 1984) and nest sites. *Temnothorax curvispinosus* must move its nest site often in a bid to compete with other cavity-nesting ant species (DeHeer and Herbers, 2004; Herbers, 1989) and to escape colony invasion by social parasite species (Foitzik et al., 2004). Locomotor performance traits like running speed can therefore govern the acquisition of limited nest sites and food resources. Colonies overwinter above ground in acorns or other similar structures, and as a consequence experience little buffering from changes in air temperature (Herbers and Johnson, 2007; Mitrus, 2013). While foraging activities occur during winter (Stuble et al., 2013) it is at a much reduced rate compared to warmer months (Fellers, 1989), and resource requirements likely decrease as larvae enter diapause (Penick et al., 2017). Despite this, nest sites are frequently moved during the winter months, with 20–35% of colonies relocating over winter (Herbers and Johnson, 2007).

Using a large-scale experimental climate warming array positioned near the center of the latitudinal range of *T. curvispinosus*, we simulated winter temperature regimes under ambient conditions and with +3 and +5 °C of warming—a range of warming that mimics the IPCC projections for changes in mean temperature during winter over the next century (Stocker et al., 2013). After a nine week overwintering acclimation period at each treatment, we tested ant running speed at four temperatures (6, 16, 26 and 36 °C) that span the range from typical winter days to very warm days (and/or very warm microclimates at the boundary layer, (Kaspary et al., 2015)) to develop thermal performance of running speed for each ambient and warmed field acclimation temperature treatment. Because previous work with *T. curvispinosus* under experimental warming conditions demonstrated a net positive response to warmer temperatures (Diamond et al., 2012), we expected that acclimation to increases in temperature over winter would improve performance. Specifically, we hypothesized that winter warming would allow *T. curvispinosus* ants to achieve faster running speeds at higher test temperatures compared with ambient acclimated ants, which, in turn, could allow colonies to take better advantage of foraging and nest relocation on warm winter days.

2. Methods

2.1. Study species and experimental warming array

Temnothorax curvispinosus is a geographically widespread species of ant found in the leaf litter of the eastern United States; this species is a cavity nester such that entire colonies are housed inside hollowed acorns, hickory nuts, or small twigs (Alloway et al., 1982). Workers are typically small (~2.5 mm in length and weigh between 0.45 and 0.55 mg) and live in small colonies (~100 workers) that fit inside a single acorn or hickory nut. We collected acorns containing *T. curvispinosus* colonies from a wooded area surrounding Lake Raleigh in Raleigh, North Carolina in November of 2010 (35.7630°N, 78.6847°W, 100 m). We then counted the number of workers per acorn and noted whether the queen was present.

Upon collection of each colony, we removed all of the brood (all colonies initially contained brood), and returned the workers and queen to their acorns. We placed the colonies in a sealed container with their original acorn and gave the ants five days to re-acclimate to their nest before being placed into an overwintering treatment. Each acorn was

placed between a layer of leaf litter in a fine mesh-bottomed 90 mL cup; the top was lined with white petrolatum to prevent escape from the top of the cup (Fisher Scientific, Waltham, MA). The leaf litter and associated detritus contained sufficient food resources for overwintering *T. curvispinosus* colonies during the nine week acclimation portion of the experiment and served to mimic the thermal environment outside the cup (Mitrus, 2013). We placed the cups on the ground and surrounded them with leaf litter in one of six chambers in the Duke Forest experimental warming array (Clark et al., 2014; Pelini et al., 2011).

The warming array contained multiple open-topped chambers under natural forest cover. Control chambers (N = 2) were held at ambient temperature, and the warmed chambers were heated to target temperatures of either +3 °C (N = 2) or +5 °C (N = 2) above ambient at both the soil layer and air through buried heating cables and hydronic radiators (Clark et al., 2014). Because of variation in the ability of the chambers to achieve their target temperatures, we collected data from air temperature sensors housed in solar radiation shields resting on the ground within the chambers. We computed the average winter air temperature (air temperatures are recorded hourly) in each chamber for the study period (December 2010 through February 2011, Fig. 1), and used these average winter air temperatures in our analyses of running speed and survival.

Four to five colonies were placed in each of the six open-topped chambers on December 5, 2010, immediately following the first frost. The colonies remained in the chambers until the second week in February when maximum daily air temperatures were consistently above 10 °C. The colonies were removed from half of the chambers on February 8, 2011 and the other half on February 12, 2011 to prevent a long re-acclimation period to laboratory conditions prior to the assessment of thermal performance.

2.2. Running speed assessment

To assess thermal performance, we measured the running speed of surviving worker ants. Running speed was tested at four temperatures: 6, 16, 26, or 36 °C. Each ant was assessed at each of the four temperatures in a quasi-randomized order. The order of the lowest three temperatures was randomized, but because of its potentially detrimental effects on ant condition, the warmest temperature was always last. A period of thermal recovery was allotted between each trial (a minimum of 2 h) wherein each ant was held individually in a

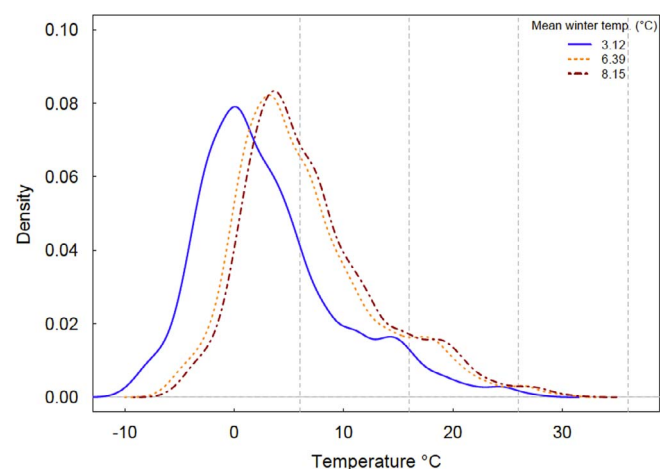


Fig. 1. Distributions of the air temperatures experienced at nest height from an ambient chamber, a +3 °C target temperature chamber, and a +5 °C target temperature chamber for the months (December 2010–February 2011) over which the winter acclimation experiment occurred (distributions are smoothed using kernel density estimation). The dashed vertical lines represent the four running speed test temperatures (6, 16, 26 and 36 °C).

microcentrifuge tube at room temperature (~ 21 °C) without access to additional food or water. Ants generally do not require access to food or water within the timeframe of this experiment, and *Temnothorax* ants are highly resistant to starvation and desiccation even over long timescales (Hood and Tschinkel, 1990; Modlmeier et al., 2013; Oberg et al., 2012; Rueppell and Kirkman, 2005). Order had no effect on performance (GLM, $N_{\text{individuals}} = 375$, $\chi^2 = 0.002$, $P = 0.96$) confirming that the recovery period was sufficient to mitigate any carryover effects of prior temperature exposure. We recorded the time elapsed for an individual ant to run between markers placed 2 cm apart on an isothermic aluminum plate; running speed was expressed as millimeters per second. Ants were given 10 s to acclimate before the start of each trial, and if an ant failed to move at the beginning of the trial, it was gently stimulated with a paintbrush.

2.3. Statistical analyses

We examined running speed as functions of test temperature, winter warming treatment, and their interaction using a linear mixed effects model. Running speed was natural log + 1 transformed to meet model assumptions of normality. We considered winter temperature as a continuous predictor. Although our test temperature predictor is also technically continuous, because we only have four test temperatures, we cannot formally evaluate differences in curve shape of the thermal reaction norm. For this reason, we considered test temperature as a factor in our global model, and used post hoc pairwise comparisons to examine specific differences in running speed responses to test and winter temperatures. To account for repeated measures on individuals and colony-level autocorrelation, we included individual identity nested within colony identity as a random intercept. All analyses were performed using R statistical software (R Core Team, 2016).

3. Results

We were able to recover 21 of the 30 colonies we placed in the warming arrays: $n = 4$ for both of the ambient chambers; $n = 4$ for both of the +3 °C chambers; and $n = 2$ and $n = 3$ for the +5 °C chambers. The mean number of workers we were able to recover per colony likewise remained high: (initial number of workers per colony: range = 4–136, mean = 56.9, se = 9.2; final number of workers per colony: range = 0–117, mean = 47.0, se = 7.9). Note that initial colony size of the colonies we recovered did not systematically vary with chamber ($F_{5,15} = 1.60$; $P = 0.22$) or target chamber temperature ($F_{2,18} = 0.04$; $P = 0.96$). With the exception of one of the +3 °C above ambient target temperature chambers (average winter temperature of 6.39 °C) that had sample sizes of 4 individuals per test temperature for the running speed trials, all other chambers contained at least 56 individuals for testing at each trial temperature. Because the responses for the chamber which contained the colony with low replication ($n = 4$ individuals) were not outliers compared with better-replicated chambers, and because our interpretations are not entirely contingent on this chamber, we retained these data in our analyses. Across the entire experiment, sample sizes of the number of individuals tested in each warming treatment and running speed temperature combination ranged from 4 to 80, with a mean of 61 workers.

Temnothorax curvispinosus exhibited considerable variation in thermal performance for running speed, and this variation depended on both test temperature and winter acclimation temperature. Overall, ant workers exhibited faster running speeds at higher test temperatures (Fig. 2), demonstrating a plastic response to test temperature. Further, the magnitude of this effect depended on the temperature of the overwintering environment, which demonstrates an acclimation response to overwintering temperatures. There was little variation in running speed among ants reared in the ambient and warmed overwintering environments when tested at cooler temperatures (6 and 16 °C); however, ants that overwintered in warmed environments

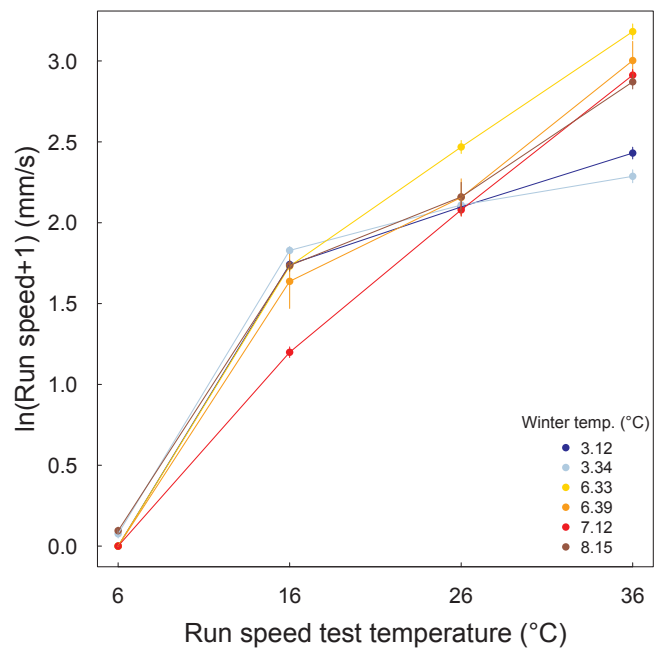


Fig. 2. Mean running speed (\ln , mm/s) \pm SE as a function of test temperature, with separate lines for each winter warming acclimation temperature (°C); blue shades indicate ambient temperature treatments, yellow/orange shades indicate +3 °C target temperature treatments, and red/burgundy shades indicate +5 °C target temperature treatments (the actual measured temperatures are reported for each chamber in the legend).

exhibited faster running speeds at warm test temperatures (26 and especially 36 °C) compared with ants overwintered in ambient environments. Our global linear mixed effects model detected a significant interaction between overwintering temperature and running speed test temperatures (Test temperature $F_{3,4030} = 18,914$, $P < 0.001$; Overwinter temperature $F_{1,14} = 1.64$, $P = 0.221$; Test*Overwinter temperature $F_{3,4030} = 14.7$, $P < 0.001$) and our post hoc tests revealed significant positive effects of overwintering temperature on running speed at 26 and 36 °C ($\beta = 0.0311$, SE = 0.0148, $F_{1,14} = 4.40$, $P = 0.0544$, and $\beta = 0.0323$, SE = 0.00110, $F_{1,14} = 8.62$, $P = 0.00361$), but not at 6 or 16 °C ($\beta = -0.00310$, SE = 0.00429, $F_{1,14} = 0.522$, $P = 0.482$, and $\beta = 0.00215$, SE = 0.00254, $F_{1,14} = 0.00720$, $P = 0.934$); see Table S1 for pairwise post hoc comparisons.

Indeed, we saw qualitatively different patterns in thermal performance for running speed among ants from warmed and ambient acclimation treatments: the relationship between running speed and test temperature consistently increased for warm-acclimated ants, while thermal performance for ambient-acclimated ants exhibited more modest increases at test temperatures in the 26–36 °C range compared with the 6–16 °C range. In other words, ants reared at ambient temperatures realized a lower increase in speed between 26 and 36 °C relative to ants that were acclimated to warmer winter temperatures.

We did not detect effects of winter warming on worker survival. Of the initial 30 colonies placed in the chambers, only 16 still had their queen intact upon removal from the warming experiment. Loss of the queen was evenly distributed across treatments; $n = 2$ in each of the ambient chambers, $n = 2$ and $n = 3$ in both the +3 °C chambers and the +5 °C chambers. In order to remove the potentially confounding effect of loss of the queen, we removed these from our analysis of worker survival leaving us with 16 colonies. We found no significant relationship between mean temperature during the acclimation period and worker survival (GLM, $N = 16$ colonies, $\chi^2 = 1.64$, $P = 0.20$) with the median percent worker survival at 75% across all treatments (Fig. S1).

4. Discussion

The effects of climate change extend throughout the year, but few studies focus on how organisms respond to changes in climate during the winter (but see Williams et al., 2015b). For winter-active species, winter warming may increase the amount of climatically suitable time available for foraging (Bale et al., 2002). Here we used the acorn ant, *T. curvispinosus* to explore how thermal performance in running speed, a key determinant of foraging and colony expansion in acorn ants, respond to experimental winter warming in a field setting. We found evidence that winter acclimation temperatures significantly altered the thermal performance in running speed. Specifically, while warmer winters had little effect on running speed at cooler test temperatures, warmer winters contributed to faster running speed at warm temperatures in excess of 26 °C. Positive effects of winter warming on running speed at high temperatures suggest that winter warming may prime *T. curvispinosus* colonies for spring activity, including foraging and nest relocation.

In the southeastern United States and across many locations globally, winter temperatures are expected to rise by several degrees Celsius over the next century (Stocker et al., 2013), a range of temperature change that was captured by our experimental climate warming array. We found that acclimation to future warmer winter conditions afforded *T. curvispinosus* faster running speed at higher temperatures (Fig. 2). However, the benefit of acclimation to warmer winters was not realized until the test temperatures reached the 26–36 °C range. Given that these ants tend to exhibit only limited activity below 20 °C (Penick et al., 2017) this result has two implications: first, *T. curvispinosus* colonies reared under warmed winter conditions will have increased performance at higher temperatures that occur sporadically during the winter season (Fig. 2), and second, they are better primed for spring activity. Functionally, these effects translate to an increase of 1–4 mm/s at 26 °C or 8–15 mm/s faster at 36 °C when ants were overwintered in the warmed treatments compared with the ambient treatments. To put this into context, and ant traveling 4 mm/s faster on a trip 1 m from the nest would return in approximately four minutes, and an ant traveling at 15 mm/s would return in just over one minute. Just as these seemingly modest increases in speed translate into large differences in ground covered, the modest differences in air temperature (near acorn ant nest height) increases the frequency of high temperatures (> 26 °C) by an order of magnitude during the December through February period over which our study occurred (i.e., from 4–5 h above 26 °C during this period to 21–28 h, Fig. 2). These warming effects could be enhanced by microclimatic heterogeneity, especially light gaps in the forest cover, which can raise temperatures on the leaf litter surface where acorn ant nests and foragers are located to several degrees Celsius above air temperature (Hemmings and Andrew, 2017; Kaspari et al., 2015). Further, positive impacts of warmer winters on running speed may correlate with other physiological changes associated with increased performance at high temperatures.

In the southeastern United States, where the climate array was located, *T. curvispinosus* is a relatively thermophilic ant species (Diamond et al., 2012) and has a mean critical thermal maximum of 44 °C (Diamond et al., 2012, 2013). In this context, the fact that this species exhibits positive responses in locomotor performance to warmer acclimation and test temperatures is perhaps unsurprising. However, the ability to take advantage of these warmer conditions comes at a cost. Many ant species move underground over winter where temperatures are much less variable than on the surface (Bale and Hayward, 2010). The surface-nesting habits of *Temnothorax* results in this group of ants experiences the full range of fluctuations in air temperature throughout the winter. Although surface-nesting allows *Temnothorax* to engage in resource acquisition activities quickly following a short-term increase in temperature (Herbers and Johnson, 2007), surface-nesting exacts large costs in terms of mortality over the course of the winter by

exposing ants to cooler air temperatures (Mitrus, 2013). The nature of this tradeoff can be modulated by winter temperatures, as interannual variation in winter temperature is strongly correlated with overwintering survival in *Temnothorax* (Mitrus, 2015). Global climate change may similarly diminish this tradeoff by reducing the high survival cost of overwintering above ground. While we did not see a benefit of warmer winters on colony survival in this study, warmer winters may yield improved survival for acorn ant colonies in cooler regions and/or allow earlier access to nest and food resources. Alternatively, the trend of increasing variance in colony survival with increasing winter temperature (Fig. S1) is potentially suggestive of higher metabolic costs to overwintering at higher temperatures (Williams et al., 2015a) which may offset any benefits from increased foraging. How warmer winters cumulatively impact metabolism, foraging and colony fitness is clearly an area in need of greater exploration.

What is unclear from our study is if the increase in running speed is simply the result of physiological acclimation to higher temperatures or if it reflects a larger phenological shift in overwinter dormancy to extend the growing season (Williams et al., 2015b). Without further study employing measures of metabolic rate, we are unable to state which phenomena is actually occurring (Williams et al., 2015a). Predictions about how temperate ant communities will respond to climate warming suggest that thermophilic species, such as *T. curvispinosus*, are limited by the short length of their growing season (Penick et al., 2017). Warming will inevitably increase the number of warm days available to forage for thermophilic species, which could, in turn, increase competition for heat-intolerant species. At our study site, *T. curvispinosus* has a relatively short activity season from June to September (Penick et al., 2017), and acclimation to warmer winters would likely expand their activity in spring.

We might generally expect winter-active thermophilic ectotherms to benefit from winter warming, at least with respect to resource acquisition traits like running speed and foraging ability, but more work is needed to determine whether winter warming may produce deficits in other traits. For example, while faster running speeds may permit increased foraging ability, if there are insufficient resources available to support increased overwintering activity, then cumulative performance and fitness may decline (Miller-Rushing et al., 2010). While our results support classic patterns of temperature-driven plasticity in performance, they also highlight the potential for the shape of thermal reaction norms to be altered by climate change in overwintering organisms. Specifically, we found that the performance consequences of a key resource-acquisition trait are contingent upon acclimation conditions. These findings provide a better understanding of how a winter-active species responds to temperature change and suggest that ecological forecasts of organismal responses to temperature change may be improved by the inclusion of warming impacts during winter.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jinsphys.2017.05.010>.

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