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E-ARTICLE

Trade-Offs in Cold Resistance at the Northern Range Edge of the Common Woodland Ant *Aphaenogaster picea* (Formicidae)

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ABSTRACT: Geographic variation in low temperatures at poleward range margins of terrestrial species often mirrors population variation in cold resistance, suggesting that range boundaries may be set by evolutionary constraints on cold physiology. The northeastern woodland ant *Aphaenogaster picea* occurs up to approximately 45°N in central Maine. We combined presence/absence surveys with classification tree analysis to characterize its northern range limit and assayed two measures of cold resistance operating on different timescales to determine whether and how marginal populations adapt to environmental extremes. The range boundary of *A. picea* was predicted primarily by temperature, but low winter temperatures did not emerge as the primary correlate of species occurrence. Low summer temperatures and high seasonal variability predicted absence above the boundary, whereas high mean annual temperature (MAT) predicted presence in southern Maine. In contrast, assays of cold resistance across multiple sites were consistent with the hypothesis of local cold adaptation at the range edge: among populations, there was a 4-min reduction in chill coma recovery time across a 2° reduction in MAT. Baseline resistance and capacity for additional plastic cold hardening shifted in opposite directions, with hardening capacity approaching zero at the coldest sites. This trade-off between baseline resistance and cold-hardening capacity suggests that populations at range edges may adapt to colder temperatures through genetic assimilation of plastic responses, potentially constraining further adaptation and range expansion.

Keywords: climate gradient, trade-offs, range limit, ants.

Introduction

The geographic range of a species encompasses the suitable abiotic and biotic environment for populations to persist (Sexton et al. 2009; Geber 2011). For ectotherms, environmental temperature is a primary determinant of growth, metabolic rate, and activity (Stevenson 1985; Angiletta 2009), and it is expected to be an important determinant of species' range boundaries (Sunday et al. 2011). The thermal physiology of ectotherms generally reflects their latitudinal ranges (Addo-Bediako 2000; Calosi 2010; Lancaster 2016). Temperatures at the warm-edge boundaries of a geographic range tend to be less extreme than predicted by organismal physiological limits, suggesting that biotic interactions and other factors constrain warm-edge boundaries (Sunday et al. 2012). In contrast, temperatures at the cold-edge boundaries of ectotherm geographic ranges tend to match their physiological resistance of cold, consistent with a range limit that is set abiotically by cold temperatures (Sunday et al. 2012; Andersen et al. 2015).

Extreme thermal environments impose selective pressures on marginal populations distinct from those experienced in the center of the geographic range (Bridle and Vines 2007). Over evolutionary time, adaptive changes in thermal physiology at range boundaries can permit further expansion (Kirkpatrick and Barton 1997; Chevin and Lande 2011; Lancaster 2016; Szűcs et al. 2017). Whether edge populations can adapt to local conditions depends on dispersal ability, strength, and direction of selection as well as the underlying genetic architecture (Lande and Arnold 1983; Bridle and Vines 2007; Kawecki 2008; Wood and Brodie 2016). Asymmetric gene flow into marginal populations may swamp countervailing selective forces, which may constrain local adaptation (Kirkpatrick and Barton 1997; Bridle et al. 2009; Paul et al. 2011). Conversely, population fragmentation and limited dispersal from the center of the range may lead to either constrained or enhanced potential for local adaptation, depending on the extent of genetic and phenotypic diversity present within

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marginal populations (Brown 1984; Eckert et al. 2008; Hardie and Hutchings 2010; Vergeer and Kunin 2013).

Patterns of covariation among traits may also influence adaptive potential in response to environmental extremes. Organisms in poleward environments must cope with both cooler average temperatures and greater thermal fluctuation across diurnal and seasonal timescales (Marshall and Sinclair 2012). In ectotherms, these patterns of temperature fluctuation are associated with distinct physiological response mechanisms (Terblanche 2006; Hadamová and Gvoždík 2011; Elkinton et al. 2017; Noh et al. 2017). Constitutive cold resistance provides increased protection from continuous or unpredictable cold stress (Teets et al. 2011) but is energetically expensive and can depress individual growth rates (Marshall and Sinclair 2012). In contrast, induced resistance enhances performance when cold exposure is predictable (e.g., seasonal acclimation; Koštál et al. 2011) or encountered repeatedly over hourly or daily timescales (cold hardening; Lee et al. 1987; Kelty and Lee 2001; Findsen et al. 2013), but it may lead to reduced fecundity (Marshall and Sinclair 2010). The extent and direction of genetic dependence among these mechanisms will dictate the ability of the population to adapt to correlated selective pressures: positive genetic covariation would promote both baseline and inducible cold resistance, whereas negative genetic covariation would inhibit simultaneous adaptation to both environmental components and potentially limit range expansion. Our understanding of the genetic architecture of cold resistance mechanisms—and therefore the potential for simultaneous adaptation to poleward extremes—is still limited (Hoffmann et al. 2013; Gerken et al. 2015).

In eastern North America, the ant genus *Aphaenogaster* includes a complex of approximately 23 species (Demarco and Cognato 2016) that are mostly ground dwelling and inhabit deciduous forests from Florida to Maine. This species complex is a useful model system for understanding how the genetic architecture of thermal resistance mechanisms influences physiological performance and range limits. The northernmost of these species, *Aphaenogaster picea*, has diverged from its southern sister taxa and expanded into northern postglacial regions in the eastern United States (Demarco and Cognato 2016). Physiological resistance to cold temperatures varies across the species' geographic range (L. Chick, in preparation), suggesting that populations have responded adaptively to local conditions and are sensitive to cold temperatures. From museum records (Ellison and Gotelli 2009; Ellison 2012) and field observations (A. M. Ellison, personal observation), the northern range boundary of *A. picea* in New England occurs in central Maine at a latitude of approximately 45°N, even though there is ample suitable nesting habitat (mixed hardwood-deciduous forest) much farther north in Maine and Canada.

In this study, we combined evidence from ecological niche modeling and physiological resistance assays to characterize the location and habitat characteristics of the northern range boundary of *A. picea*. We identified which climate factors may act as selective agents and whether marginal populations can respond adaptively to multivariate climatic conditions present at the range boundary. We conducted extensive presence/absence surveys across central Maine and identified key climatic variables associated with its occurrence using decision tree modeling (De'ath 2002). We used a short-term common-garden experiment to quantify intrinsic cold resistance and capacity for rapid cold hardening of colonies collected from sites at varying distances from the northward range boundary. To determine the degree of genetic correlation between baseline and hardening of cold performance, we estimated the broad-sense variance-covariance (**G**) matrix (Kingsolver et al. 2001, 2004, 2015) of cold performance and further decomposed **G** using principal component analysis (PCA) at the population level.

Methods

Field Surveys and Niche Modeling

Aphaenogaster picea is a common forest ant species that ranges from the high elevations of Virginia to northern Minnesota and Maine. Its northern range boundary—estimated from georeferenced museum specimens—occurs in Maine near 45°N (Ellison and Gotelli 2009; Ellison 2012; fig. 1*D*). To further characterize the northern range boundary of *A. picea*, we combined presence/absence data from previous field surveys at 27 sites (1991–2015; Ellison and Gotelli 2009) with new data collected for this study in July and August 2015 from 75 additional sites. These 75 sites were sampled randomly along a 65-km east-west belt transect centered on 45°N latitude and running across all of central Maine. For the July 2015 survey of 32 sites, two researchers searched each site haphazardly for *A. picea* colonies for 20 min in deciduous and mixed hardwood forests. For the August 2015 survey of 43 additional sites, two researchers established 50 × 50-m plots and searched them for colonies for 20 min each (40 person-minutes per plot).

We used classification and regression tree (CART) analysis, implemented in the rpart package (ver. 4.1-10) in R version 3.4.2 (R Development Team 2017), to determine which of the 19 bioclimatic variables (at 2.5-m resolution) downloaded from WorldClim (http://www.worldclim.org/bioclim) best predicted the occurrence of *A. picea*. To obtain the optimal regression tree and to avoid overfitting the data, we pruned the tree so that it had the lowest complexity parameter and smallest cross-validated error. For each cross validation, CART models were fitted to a training set and then used to predict presence or absence in the testing set. In total, we analyzed 10 independent cross validations.

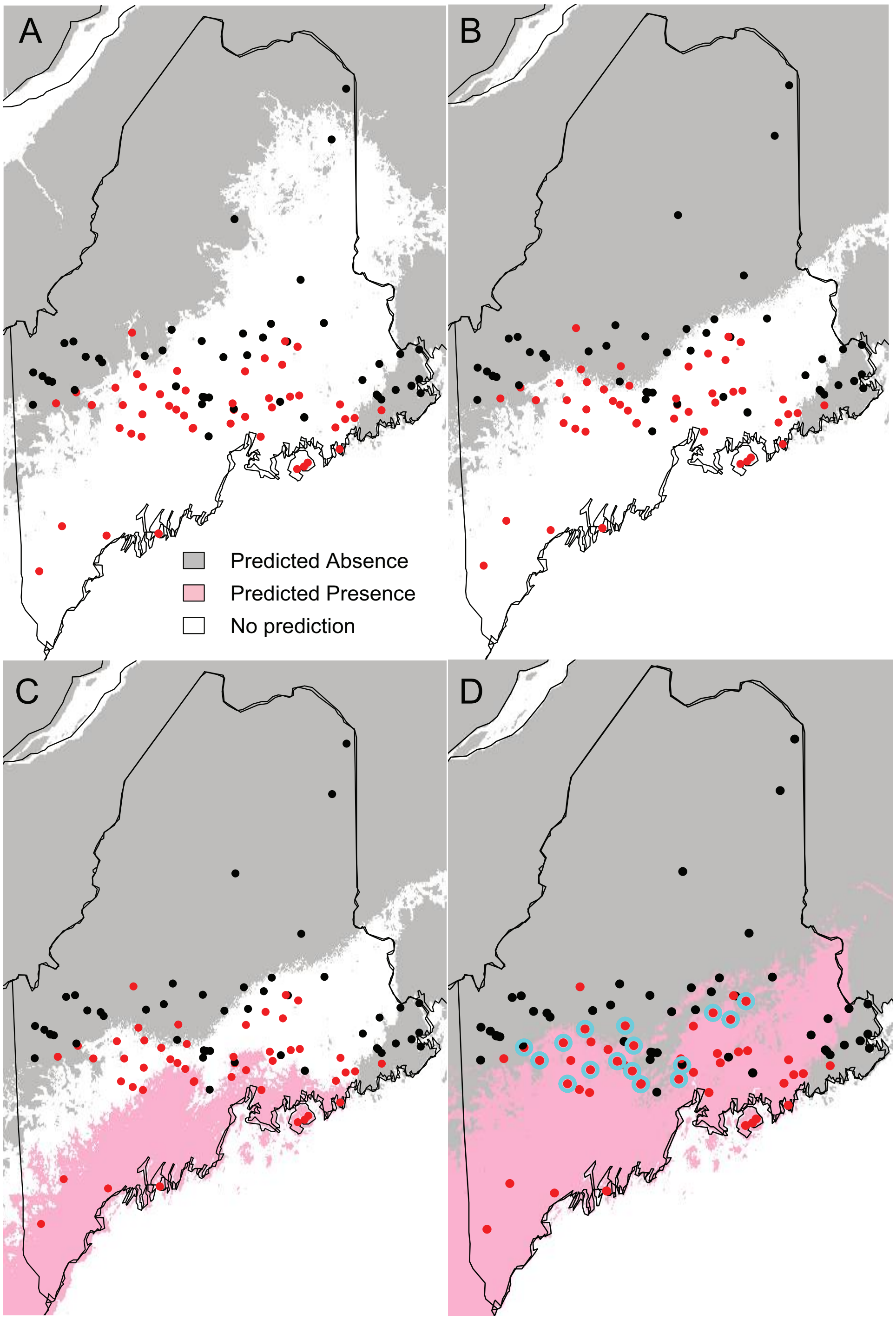


Figure 1: Overlay of classification tree model predictions on empirical surveys of presence/absence. Gray background color represents cumulative regions where the model predicts absences, pink background color represents cumulative regions where the model predicts presences, and unshaded regions are unspecified for a given predictor variable. Points indicate empirical absences (black) or presences (red). T_{max} (maximal temperature of the warmest month), temperature seasonality (standard deviation of the range of annual temperatures × 100), mean annual temperature (MAT), and precipitation at warmest quarter (PwarmQ) represented four out of 19 bioclimatic variables (http://www.worldclim.org/bioclim) that predicted presence/absence with 86% accuracy. Sites with T_{max} of <25°C predict absences (*A*), and sites with T_{max} of >25°C and temperature seasonality of >10.05 predict absences (*B*). Sites with a MAT of >6.5 predict presences (*C*), and sites with a MAT of <6.5 and a PwarnQ of <261 and >270 mm predict presences (*D*). Ant colonies were collected and measured for chill coma recovery time from sites outlined in cyan (*D*).

Field Collecting and Rearing Conditions in a Common Garden

To test for local adaptation in cold performance, we collected 16 colonies from 16 unique sites along the range boundary in July 2015 (table A1; fig. 1*D*; tables A1, A2 are available online). At each site, we collected whole colonies, including workers, larvae, pupae, and queens (when possible). Collected colonies were housed in 22 × 16-cm plastic containers and maintained under a 12L∶12D photoperiod at ≈50% humidity and 25°C (i.e., within the range of optimal development; Penick et al. 2017). To minimize the contribution of the source environment, colonies were laboratory acclimated under these conditions for at least 1 month before any physiological measurements were taken. Ant workers typically live from a few weeks to several months. Colonies readily nested within glass test tubes that were plugged with water-saturated cotton to maintain humidity. Each colony was fed 100 μL of 20% honey in water and one bisected meal worm three times each week.

Constructing Cold-Performance Curves

We exposed ants to a series of cold pretreatments, recovery treatments, and subsequent temperature treatments to construct cold-performance curves for adult workers from each laboratory-acclimated colony (figs. 1*D*,2). Cold resistance was measured as the chill coma recovery time (CCRT) in seconds (Terblanche et al. 2011; Andersen et al. 2015; Sinclair et al. 2015): the time in seconds needed for an individual ant worker to orient itself in an upright position and take one step after a 1-h exposure to −5°C. We focused on CCRT rather than other commonly used measures of cold resistance (such as critical thermal minimum [CT_{min}] or survival) because CCRT is both straightforward to measure and likely to be ecologically relevant under low-temperature extremes. Both CCRT and CT_{min} have been shown to vary latitudinally with temperature in aquatic (e.g., Wallace et al. 2014) and terrestrial ectotherms (e.g., Karl et al. 2008; Sisodia and Singh 2010; Ransberry et al. 2011; Gaitán-Espitia et al. 2013). However, the onset of chill coma at CT_{min} occurs at relatively mild low temperatures, whereas CCRT is measured following more severe low-temperature conditions, when irreversible damage is a higher risk and additional physiological mechanisms are likely to be involved (Macdonald et al. 2004). In two temperate ant species, only CCRT displayed significant latitudinal variation, whereas CT_{min} was invariant across 17° in latitude (Maysov 2014).

To assess basal cold recovery and cold-hardening ability, we measured the CCRT of ants that were pretreated for 1 h at one of four temperature treatments: 25°, 5°, 0°, or −5°C (fig. 2). The 25°C pretreatment is the control that represents basal cold resistance, and the −5°, 0°, and 5°C pretreatments represent cold hardening at different temperature levels. All ants were

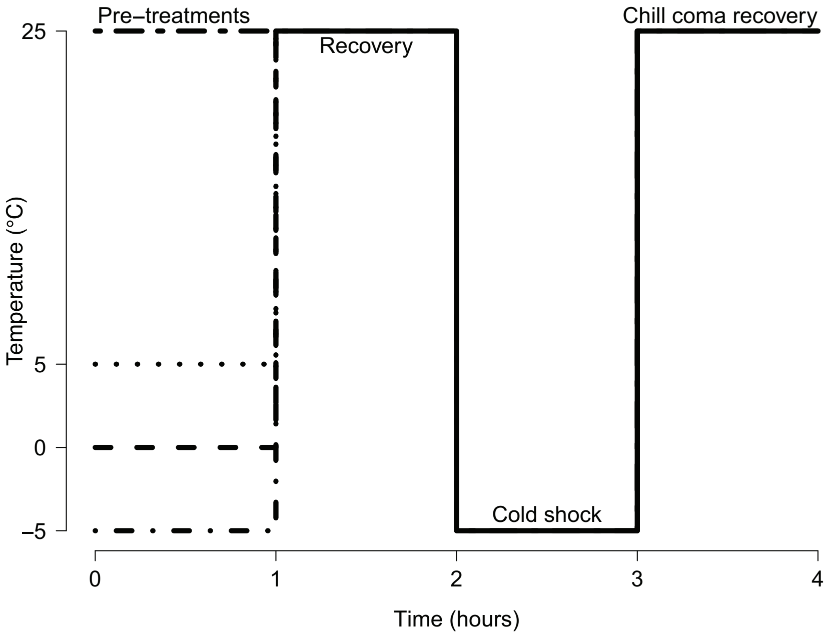


Figure 2: Experimental design for measuring cold-performance curves for four pretreatment temperatures (−5°, 0°, 5°, and 25°C). Basal cold resistance was defined as performance at 25°C pretreatment temperature and was used as a baseline for measuring cold-hardening responses (−5°, 0°, and 5°C pretreatment temperatures).

temperature treated in a circulating water bath (Polyscience) that contained a 50% ethylene glycol solution to prevent freezing. For each colony, four ants per pretreatment were placed in a sealed 15 × 160-mm glass test tube for 1 h at the pretreatment temperature. After 1 h, the ants were removed and placed into a test tube and allowed to recover at 25°C for 1 h (fig. 2), then placed in the −5°C treatment for another hour. After this second hour, ants were placed in glass tubes with water-soaked cotton plugs. CCRT was measured by an observer without prior knowledge of pretreatment groups. We excluded 24 of 272 ants tested that were lost or did not survive handling.

Evaluating Trade-Offs in Cold Performance

We adapted multivariate methods from quantitative genetics (Kingsolver et al. 2001, 2004, 2015) to estimate the correlation structure between basal cold resistance and cold hardening in *A. picea*. In Kingsolver's original analyses, the data consisted of performance traits that were measured for replicated genotypes. For n measured traits, the resulting $n \times n$ **G** matrix measures the additive genetic variance in the traits along the diagonal and the additive covariances between pairs of traits in the off-diagonal elements. In our analyses of whole-colony variation, the **G** matrix reflects among-colony variances and covariances in the responses to different pretreatment temperatures. The trait data consisted of CCRT that was measured at four different pretreatment temperatures for four replicate workers from each of the 16 colonies. Performance at each pretreatment temperature was considered a "trait," where baseline performance corresponded to the 25°C treatment and 5°, 0°, and −5°C traits measured the combined effects of baseline resistance and hardening on performance at progressively lower temperatures. We estimated the colony-level 4 × 4 variance-covariance matrix by first fitting a mixed effects model and then extracting variance and covariance components using the lme4 package in R (Paccard et al. 2016):

$$Y_{ijk} = \mu + C_{jk} + \epsilon_{ijk},$$

where Y_{ijk} represents the value of CCRT for ant worker i of colony j measured for each pretreatment temperature k, μ is the fixed effect of the intercept, C_{jk} is the random effect of colony j at pretreatment temperature k, and ϵ_{ijk} represents the residual error. Colony-level and residual within-colony effects were treated as random effects with an unconstrained covariance structure (Paccard et al. 2016). We extracted variance and covariance components that make up the 4 × 4 matrix with the VarCorr() function from the mixed effects model.

Estimates of the variance-covariance matrix were calculated from untransformed data because each trait (CCRT under each pretreatment temperature) has the same units. We then decomposed **G** using PCA to produce orthogonal eigenvectors, which represent independent axes of genetic correlations between traits (Kingsolver et al. 2001, 2004, 2015). The first principal component, $\mathbf{g}_{\max}$, is the eigenvector that explains most of the variation in the **G** matrix. The pattern of loadings for $\mathbf{g}_{\max}$ can be biologically interpreted as falling within one of three broad scenarios (Kingsolver et al. 2001, 2015). In the first scenario, if colonies with higher baseline performance were also able to mount equivalent or greater cold-hardening responses, resulting in consistent differences in performance across all pretreatment temperatures (additive variation), then the $\mathbf{g}_{\max}$ loadings would be all negative or all positive (fig. 3*A*, 3*B*). In the second scenario,

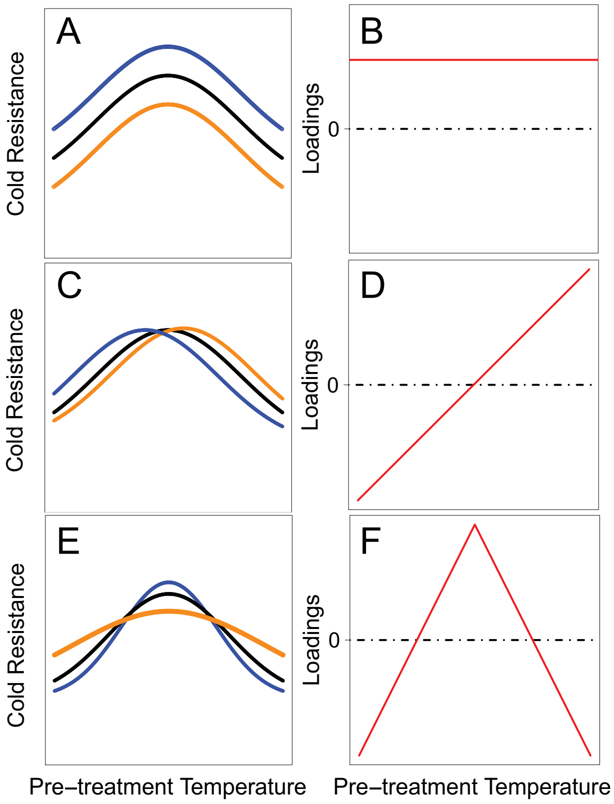


Figure 3: Predictions for three biological patterns of performance curves using multivariate quantitative genetic methods, adapted from figures 4 and 5 in Kingsolver et al. (2001). Colonies are expected to differ in three different ways with respect to pretreatment temperature (*A*, *C*, *E*). For each, the loadings of the dominant eigenvector from the principal component analysis decomposition of their variance-covariance (**G**) matrix provides estimates of the correlation between basal cold resistance and levels of hardening ability on a continuous scale (*B*, *D*, *F*). Colonies exhibiting overall additive responses across pretreatment temperatures (*A*) will also have constant loading patterns across pretreatment temperatures (*B*). Colonies whose peak performance differs from cooler to warmer pretreatment temperatures (*C*) will have either a negative or a positive relationship between loadings and pretreatment temperatures (*D*). Last, colonies exhibiting generalist-specialist differences (*E*) will have will have positive loadings at intermediate temperatures and negative loadings at extreme cold or warm pretreatment temperatures (*F*).

if baseline recovery and hardening ability were negatively correlated, resulting in shifts in relative cold performance between low and high pretreatment temperatures (cooler-warmer variation), then the $\mathbf{g}_{\text{max}}$ loadings would shift in sign from low to high pretreatment temperatures (fig. 3*C*, 3*D*). In the third scenario, if increased hardening responsiveness to cold shock manifested as heightened performance at intermediate pretreatment temperatures (generalist-specialist variation), then the $\mathbf{g}_{\text{max}}$ loadings would shift in sign between intermediate and both extreme pretreatment temperatures (fig. 3*E*, 3*F*).

Determining the Relationship between Cold Resistance and Climate

To explore how variation in cold resistance traits was related to the local thermal environment at the range margin, we tested for an effect of local mean annual temperature (MAT) on baseline cold resistance and cold-hardening capacity. To calculate baseline cold resistance, we transformed CCRT so that higher values indicated greater cold resistance. Specifically, the maximum CCRT value in the entire data set (CCRT_{max}) was treated as a reference level, and each CCRT measurement (CCRT_{obs}) was subtracted from CCRT_{max} ($\text{CCRT}_{\text{max}} - \text{CCRT}_{\text{obs}}$) to measure relative cold resistance. To calculate cold-hardening capacity, we used the same data transformation and for each colony subtracted the average CCRT at each pretreatment temperature (−5°, 0°, 5°C) from the average CCRT at the 25°C pretreatment temperature (−5°, 0°, and 5°C; $\text{CCRT}_{25^\circ\text{C}} - \text{CCRT}_{\text{pretreatment temperature}}$). To detect simple linear and nonlinear relationships between local temperature and cold resistance or cold hardening, we fit a regression model with cold resistance or cold hardening as the response variable and a linear and a quadratic term for temperature as the predictor variable. We used Akaike information criterion model selection to determine whether the quadratic term should be retained or dropped from the final model.

Results

Climatic Predictors of the Distribution of Aphaenogaster picea

The geographic distribution data included 102 georeferenced localities in Maine, consisting of 52 presences and 50 absences (table A1). CART successfully predicted the distribution of *A. picea* with 86% accuracy (fig. A1, available online) with balanced error rates, correctly identifying 42 absences out of 50 (88% specificity) and 46 presences out of 52 (84% sensitivity; fig. A1). Measures of environmental temperature were most strongly associated with *A. picea*'s distribution: CART sequentially identified the maximal temperature of the warmest month (T_{max}), annual temperature seasonality (SD), annual mean temperature (MAT), and precipitation in the warmest quarter (PwarmQ) as the best set of predictors of the distribution of *A. picea* (fig. 1). *Aphaenogaster picea* was not found at sites below a T_{max} of 25°C, which coincides with areas in Maine along the east coast and along a southeast to northwest inland axis (fig. 1*A*, gray shaded area). In locations where T_{max} was >25°C, *A. picea* was not found where SD was greater than or equal to 10.05, corresponding to areas of central and northern Maine (fig. 1*B*, gray shading). If SD was below 10.05, *A. picea* was predicted to occur when MAT was ≥6.5°C, a combination of conditions present in southern Maine (fig. 1*C*, pink). The remaining area, characterized by sufficiently low SD but MAT below 6.5°C, formed an east-west band along the northern range edge within which presence was dependent on precipitation in the warmest quarter. Presence was predicted when PwarmQ was >270 or <261 mm (fig. 1*D*).

Cold Performance in Marginal Populations

Colonies collected from 16 sites within the range-edge region and maintained under common-garden conditions exhibited substantial variation in their CCRT performance curves (fig. 4*A*). Overall, cold resistance was significantly affected by pretreatment temperature (ANOVA: $F_{1,3} = 18.95, P < .001$; fig. 4*B*). Significant cold hardening occurred when individuals were pretreated at 0°C (Tukey's HSD pairwise comparisons: all comparisons vs. 0°C, $P < .01$), with negligible enhancements that did not differ significantly from room-temperature controls when exposed to −5°C and +5°C (fig. 4*B*). When we decomposed the colony-level variance-covariance **G** matrix (table A2) with a PCA, the first principal component, $\mathbf{g}_{\text{max}}$, accounted for 92% of the variation in **G**. In support of a baseline-hardening trade-off (fig. 3*C*, 3*D*), $\mathbf{g}_{\text{max}}$ had opposite loading patterns between the 0° and 25°C pretreatment temperatures (fig. 4*C*); ants from colonies with high cold resistance at the 0°C pretreatment temperature had lower cold resistance at the 25°C pretreatment temperature (fig. 4*C*) and vice versa. Hardening in the most basally cold-resistant colonies improved CCRT by ≈5% (33 s), whereas the least basally cold-resistant colonies improved CCRT through hardening by up to ≈160% (504 s).

Patterns of Local Adaptation in Cold Performance

Across the 16 range-edge sites, MAT varied by 2°C, with correlated changes in both winter low temperature extremes (T_{max}, $R^2 = 0.91$) and short-term temperature variability (MDR, $R^2 = -0.31$), which increased as MAT and T_{max} declined (fig. 5). Local environmental temperatures differentially affected baseline and inducible components of cold performance. There was a significant negative linear effect of MAT on baseline cold resistance ($F_{1,15} = 8.21$, $P < .02$; fig. 6*A*). In contrast, cold-hardening ability ($\text{CCRT}_{25^\circ\text{C}} - \text{CCRT}_{0^\circ\text{C}}$)

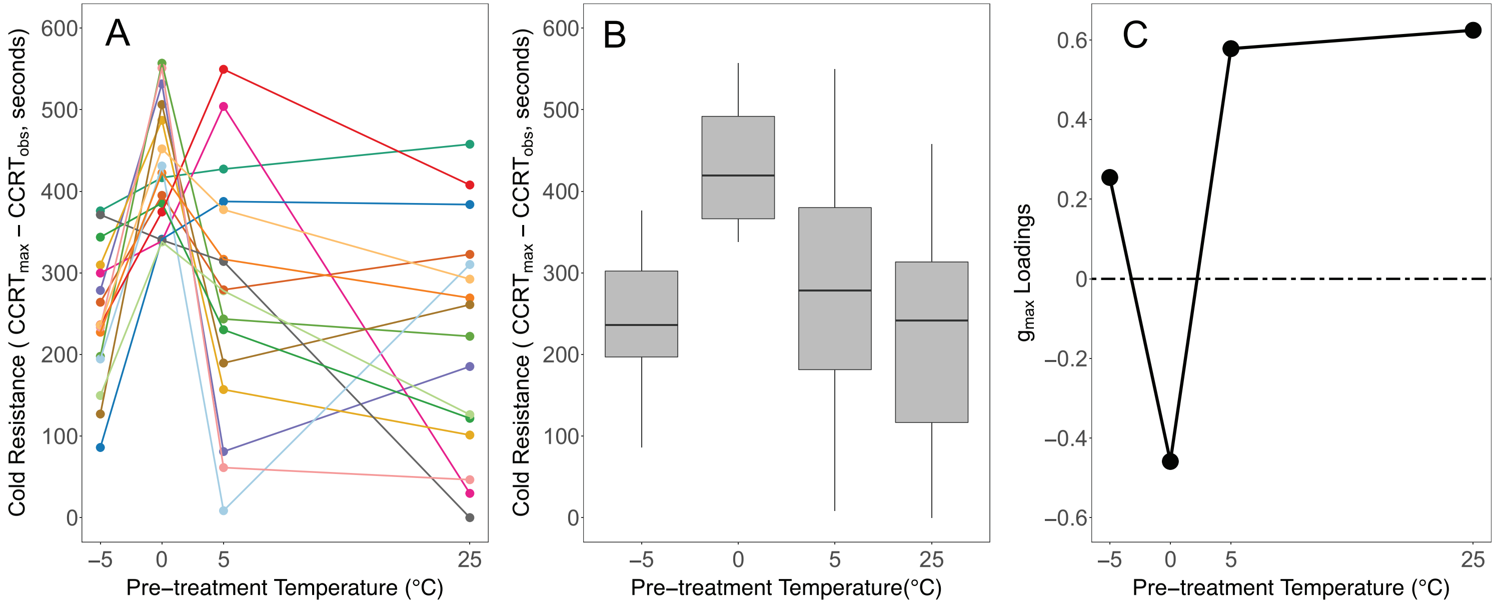
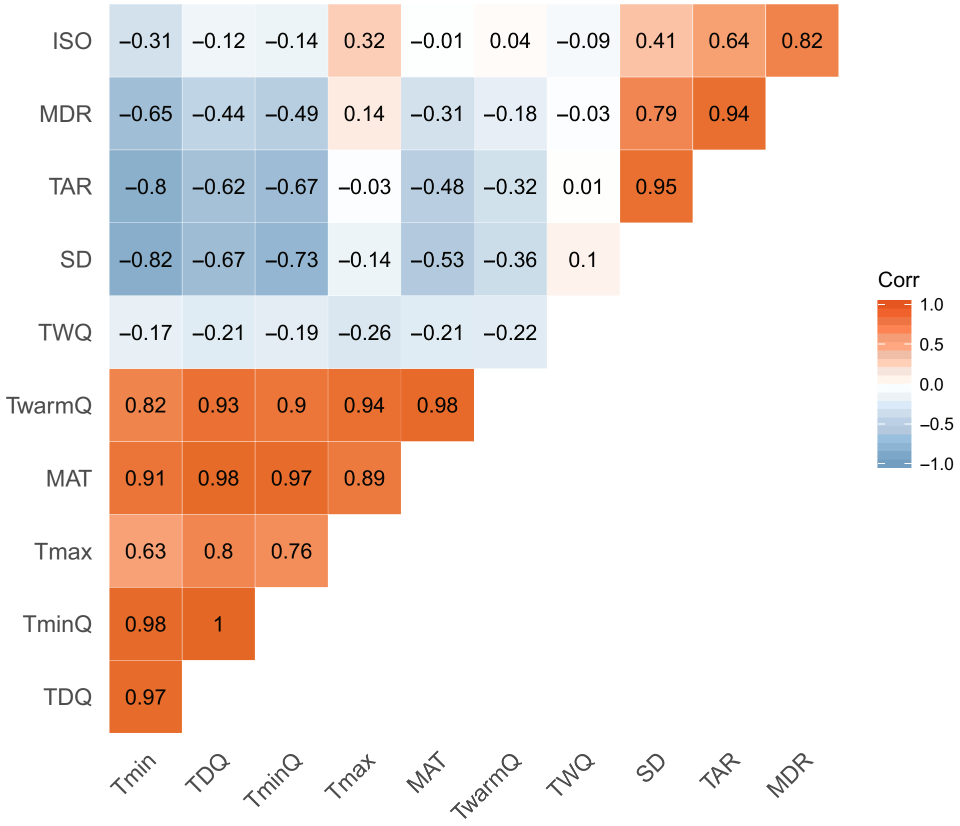


Figure 4: Patterns of cold resistance as a function of pretreatment temperature and how they are correlated. Cold resistance (chill coma recovery time [CCRT], seconds) is shown as a function of pretreatment temperature (°C) for 16 individual colonies (*A*) and for the whole population (*B*). Principal component loadings of the among-colony variance-covariance matrix are plotted as a function of pretreatment temperatures (°C) to visualize the genetic constraints (*C*). Loadings for the first eigenvector, $\mathbf{g}_{max}$, explained 92% of the variation in **G**.



	Tmin	TDQ	TminQ	Tmax	MAT	TwarmQ	TWQ	SD	TAR	MDR
ISO	−0.31	−0.12	−0.14	0.32	−0.01	0.04	−0.09	0.41	0.64	0.82
MDR	−0.65	−0.44	−0.49	0.14	−0.31	−0.18	−0.03	0.79	0.94	
TAR	−0.8	−0.62	−0.67	−0.03	−0.48	−0.32	0.01	0.95		
SD	−0.82	−0.67	−0.73	−0.14	−0.53	−0.36	0.1			
TWQ	−0.17	−0.21	−0.19	−0.26	−0.21	−0.22				
TwarmQ	0.82	0.93	0.9	0.94	0.98					
MAT	0.91	0.98	0.97	0.89						
Tmax	0.63	0.8	0.76							
TminQ	0.98	1								
TDQ	0.97									

Figure 5: Correlation matrix of 10 bioclimatic variables related to temperature and temperature variation for the 16 common-garden sites in Maine. ISO = isothermality; MDR = mean diurnal range temperature; TAR = temperature annual range; SD = temperature seasonality; TWQ = temperature at wettest quarter; TwarmQ = mean temperature at warmest quarter; MAT = mean annual temperature; $T_{\max}$ = maximum temperature of warmest month; $T_{\min}$ = minimum temperature of coldest month; TminQ = mean temperature at coldest quarter; TDQ = temperature at driest quarter.

displayed a nonlinear response to MAT, with no clear relationship at higher MAT values but an increasingly steep negative relationship at colder temperatures that intersected with zero at the coldest sites (MAT^2, $F_{2,14} = 5.28$, $P < .02$; fig. 6*B*). There was a negative relationship between baseline and hardening cold resistance ($R^2 = 0.87$, $P < .001$; fig. 6*C*).

Discussion

We paired two approaches to investigate the northern range limit of *Aphaenogaster picea*. Occupancy modeling of presence/absence data suggests that abiotic factors are likely to be important drivers of species occupancy but that low winter temperatures may not be the sole or primary factor determining the range limit. Nevertheless, populations sampled from different distances to the range edge exhibited variation in physiological cold resistance that matched the local thermal regime. Collectively, these results suggest that poleward range edge environments present a multifaceted set of selective pressures that together shape the characteristics and ecological limits of marginal populations.

Consistent with the results of many studies across a wide range of taxa (Sunday et al. 2012; Lancaster et al. 2015; Angert et al. 2017), species occupancy at the northern range limit of *A. picea* was strongly associated with abiotic factors, with an overall prediction accuracy of 84% (fig. 1). Despite the expectation that low temperatures would be a major driver of the range limit, the minimum monthly temperature did not emerge as the most significant predictor of occupancy. Two variables, $T_{\max}$ and precipitation during the warmest quarter, significantly predicted the northwest range boundary as well as occurrence within the range edge region (fig. 1*B*), suggesting that conditions during the summer growing season are critical for overall colony performance. Because poleward populations have fewer total degree-days available for growth, ectotherms potentially face a minimal threshold for the amount of time required to successfully rear a cohort of offspring to adulthood or a winter-tolerant juvenile stage (Yamahira and Conover 2002). In populations more distant from this northwest boundary, both higher mean and lower variance in annual temperatures were stronger predictors of occurrence than summer conditions, suggesting

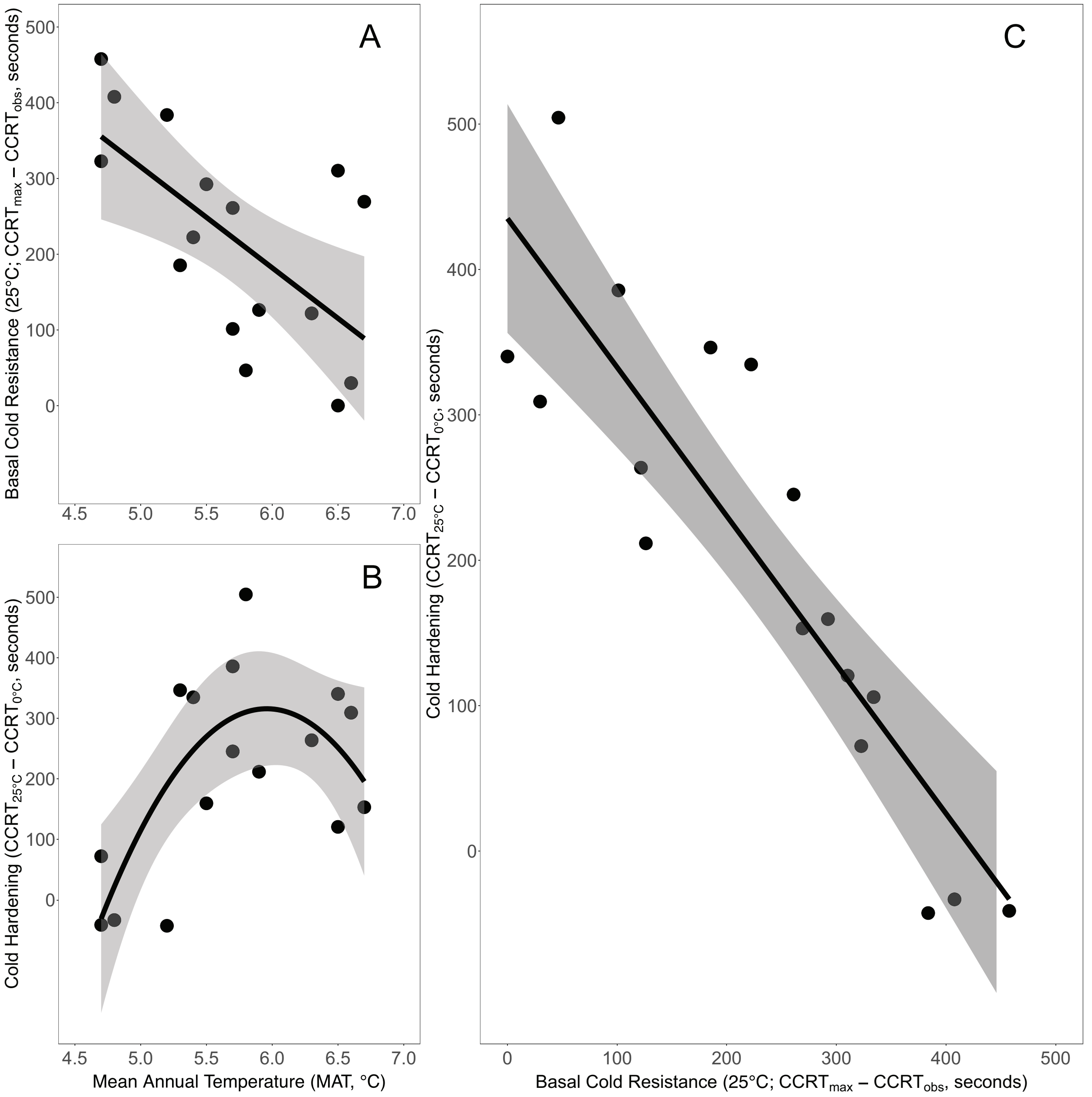


Figure 6: Variation in both cold resistance (*A*) and cold hardening (*B*) as a function of temperature. Cold resistance was measured as $\mathrm{CCRT}_{\mathrm{max}} - \mathrm{CCRT}_{\mathrm{obs}}$, and cold hardening was measured as $\mathrm{CCRT}_{25°\mathrm{C}} - \mathrm{CCRT}_{0°\mathrm{C}}$. There was a negative correlation between mean annual temperature (MAT) and cold resistance (*A*), a quadratic relationship between MAT and cold-hardening ability (*B*), and negative correlation between cold resistance and cold-hardening ability (*C*). CCRT = chill coma recovery time.

that the duration or intensity of low temperatures may act as a secondary environmental filter once minimal growth temperatures are achieved (fig. 1*B*, 1*C*). Low or variable temperature regimes can limit the foraging season in early spring and autumn, while brief cold snaps and extended low temperatures can induce chill injury and reduce survival during overwintering (Teets et al. 2011). These results are consistent with other studies demonstrating that simple cold resistance is not the only factor limiting poleward range boundaries (e.g., Cunningham et al. 2016). It is important to note, however, that niche models are correlative and should ideally be confirmed by the results of transplant experiments and independent measures of physiological resistance (Parmesan et al. 2005). Even abiotic variables that strongly predict field occurrences may not be causally related to persistence, and they may not accurately represent the abiotic conditions experienced by individuals at small spatial scales (Spicer et al. 2017; Baudier et al. 2018).

In contrast to the niche-modeling results, direct measurement of cold resistance traits suggested a more compelling role for temperature extremes in driving ecological and evolutionary outcomes at the range edge (fig. 6). In *A. picea*, marginal populations did not appear to be constrained in their ability to adapt to local conditions either by swamping effects from the core of the geographic range or by depletion of genetic variation at the range margin. Baseline cold resistance improved linearly with MAT, with an overall reduction in CCRT of over 4 min across an environmental gradient of 2°C (fig. 6*A*).

Thermal resistance limits can be adjusted via local adaptation or plasticity; positive selection is expected for both mechanisms of response as species approach their poleward range boundary (Karl et al. 2008; Hardie and Hutchings 2010). Although range-edge sites were progressively both colder and more variable in temperature (fig. 5), baseline CCRT in *A. picea* was negatively correlated with the magnitude of cold hardening, with little to no hardening ability evident at the coldest locations (fig. 6*B*). Developmental and seasonal acclimation, which were not measured here, may also influence the expression of thermal traits (Bowler and Terblanche 2008); however, $\mathrm{CT}_{\min}$ for *A. picea* does not appear to be impacted by acclimation temperature (L. Chick, in preparation), and the magnitude of their effects in other taxa are mixed (Ransberry et al. 2011; Hadamová and Gvoždík 2011; Bacigalupe et al. 2018; de Jong and Saastamoinen 2018).

Two alternative scenarios may explain the contrasting patterns of baseline and induced cold resistance. First, plasticity may be less physiologically relevant under harsher temperatures, particularly if overwintering colonies are insulated by significant snowpack that dampens temperature variability. As a consequence, thermal limits may evolve toward constitutive protection and away from unused and potentially costly hardening capacity. Alternatively, plasticity may decline as an indirect consequence of selection on baseline resistance if the two traits are negatively genetically correlated. Genetic correlations between traits can either facilitate or impede adaptation along the optimal multivariate vector favored by selection (Barton and Partridge 2000; Barton and Keightley 2002; Agrawal and Stinchcombe 2009). This relationship can be captured by a **G** matrix analysis (Kingsolver et al. 2015), in which factor loadings can reveal both positive correlations (additive variation: fig. 3*A*, 3*B*) and various forms of trade-offs between traits (specialist-generalist: fig. 3*C*, 3*D*; cooler-warmer: fig. 3*E*, 3*F*). Across range-edge sites, *A. picea* displayed opposite loading patterns of baseline CCRT and cold hardening, consistent with a cooler-warmer trade-off. Notably, there was a stronger negative relationship between traits (fig. 6*C*) than between hardening capacity and MAT, which was curvilinear in shape and declined only at the lowest temperatures (fig. 6*B*).

Ultimately, the genetic correlation between baseline resistance and the increase in resistance conferred by hardening should be determined by the extent to which they share common molecular pathways and physiological modes of action (Williams et al. 2014; Saltz et al. 2017). Investigations into the degree of association between thermal resistance and hardening ability more generally have produced inconsistent results. In *Drosophila melanogaster*, artificial selection on baseline cold resistance had no indirect impact on induction capacity and yielded mixed results for other thermal performance traits (Andersen et al. 2015; Gerken et al. 2016). Similarly, quantitative genetic analysis of standing variation in basal and induced cold resistances revealed a weakly negative correlation, whereas quantitative trait locus analysis identified distinct genetic loci underlying each trait (Gerken et al. 2015). In contrast, an analysis of natural variation in cold resistance across the clade, which includes a wider range of environmental temperatures and multiple evolutionary transitions in thermal niches, revealed a pervasive negative association between baseline and inducible mechanisms (Nyamukondiwa et al. 2011).

The solution to this paradox may lie in whether and how genetic correlations respond to selective regimes at range margins (Wilson et al. 2006; Lande 2009). As the increasing strength of selection experienced in poleward populations depletes independent genetic variance, correlated variance may remain as the primary means of adaptive response that forces environmental compromise and trade-offs not evident in the core of the range (Berger et al. 2013). If so, the complete loss of cold hardening at the coolest sites may indicate a hard limit on evolutionary response to selection at the northern range boundary of *A. picea*. Genetic assimilation (Waddington 1953) of the hardening response may be a particularly costly form of adaptation to low temperatures because the cellular processes involved in hardening can have negative impacts on survival, growth, and reproduction, potentially cancelling the fitness benefits of enhanced cold resistance (Pigliucci 2006; Basson et al. 2012; Hoffmann et al. 2013; Everman et al. 2018). Further work investigating within-population genetic architecture of cold resistance traits may help to resolve how ecological conditions interact with genetic architecture to determine species range boundaries.

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Data and Code Availability

Raw data files with metadata and scripts are located on the Harvard Forest data archive (HF323; http://harvardforest.fas.harvard.edu:8080/exist/apps/datasets/showData.html?id = HF323).

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