



Latitudinal shift in thermal niche breadth results from thermal release during a climate-mediated range expansion

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ABSTRACT

Aim Climate change is currently altering the geographical distribution of species, but how this process contributes to biogeographical variation in ecological traits is unknown. Range-shifting species are predicted to encounter and respond to new selective regimes during their expansion phase, but also carry historical adaptations to their ancestral range. We sought to identify how historical and novel components of the environment interact to shape latitudinal trends in thermal tolerance, thermal tolerance breadth and phenotypic plasticity of a range-shifting species.

Location Southern and central Sweden.

Methods To evaluate phenotypic responses to changes in the thermal selective environment, we experimentally determined the upper and lower thermal tolerances of > 2000 wild-caught damselflies (*Ischnura elegans*) from populations distributed across core and expanding range-edge regions. We then identified changing correlations between thermal tolerance, climate and recent weather events across the range expansion. Niche modelling was employed to evaluate the relative contributions of varying climatic selective regimes to overall habitat suitability for the species in core versus range-edge regions.

Results Upper thermal tolerance exhibited local adaptation to climate in the core region, but showed evidence of having been released from thermal selection during the current range expansion. In contrast, chill coma recovery exhibited local adaptation across the core region and range expansion, corresponding to increased climatic variability at higher latitudes. Adaptive plasticity of lower thermal tolerances (acclimation ability) increased towards the northern, expanding range edge.

Main conclusions Our results suggest micro-evolutionary mechanisms for several large-scale and general biogeographical patterns, including spatially and latitudinally invariant heat tolerances (Brett's rule) and increased thermal acclimation rates and niche breadths at higher latitudes. Population-level processes unique to climate-mediated range expansions may commonly underpin many broader, macro-physiological trends.

Keywords

Character release, colonization and range shifts, habitat suitability, insect invasions, macroecology, *Ischnura elegans*, Maxent, species distribution model, Sweden, thermotolerance.

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INTRODUCTION

As climates continue to warm precipitously under anthropogenic influences, it is increasingly important that we understand biotic responses to such rapid environmental change

(Williams *et al.*, 2008). Such investigations can inform conservation strategies (Hannah *et al.*, 2002) and improve our understanding of how past environmental changes have shaped the current global distribution of biodiversity (Reusch & Wood, 2007). Contemporary rates of climate change can

affect organisms in two different ways, via either selection for the evolution of thermally relevant traits (Reusch & Wood, 2007) or changes in species' distributions if populations track ancestrally suitable climates by migrating to higher latitudes and altitudes (Chen *et al.*, 2011). These two sources of climatic effects have often been summarized under an 'evolve or move' model of species persistence under global change. In practice these two processes often operate simultaneously, and both can have important reciprocal effects on biogeographical distributions and adaptations of species (Davis *et al.*, 2005). We investigated the links between ongoing range expansion processes and spatial patterns in thermal tolerance in an insect, to understand both the evolutionary changes underlying climatically mediated range shifts and the effects of range expansion on geographical variation in adaptive traits.

Periods of global climate change are often modelled as the spatial movement of a given climate regime across the landscape (Loarie *et al.*, 2009). For instance, global warming represents the poleward movement of a given thermal regime. Under this model, range shifts reflect species tracking of suitable temperature regimes across space (Chen *et al.*, 2011). During periods of warming, populations just beyond the leading range edge can become established once minimal temperatures become warm enough to support demographic growth (Thomas *et al.*, 2001), while trailing-edge populations become extirpated at the rate at which maximum tolerable temperatures are exceeded (Parmesan, 2006).

Climates at the leading, poleward, range edge often differ from ancestral climate regimes, despite having become suitable for population growth. For example, higher latitudes exhibit lower minimum temperatures, increases in daily and annual thermal variation, and different patterns of precipitation and extreme weather events than lower latitudes, and these latitudinal climatic differences are not necessarily ameliorated by warming trends (Janzen, 1967; Ghalambor *et al.*, 2006). Thus, during range expansions facilitated by warming climates, organisms face novel climatic selection pressures in the new part of the range, despite tracking other aspects of their ancestrally preferred (pre-adapted) climatic envelope. Rapid changes in thermal tolerance during climate-mediated range expansions have been observed in several insect and plant species, with evidence that genetic mechanisms and local adaptation underlie at least some of these changes (Preisser *et al.*, 2008; Janes *et al.*, 2014).

Increasing interest in the ability of organisms to respond to global climate change has led to recent advances in the field of macro-physiology, which seeks to understand physiological adaptations in a biogeographical context (Clarke, 2003; Gaston *et al.*, 2009). One common macro-physiological pattern that emerges from species-level comparisons, summarized as Brett's rule (Brett, 1956; Gaston *et al.*, 2009), states that heat tolerances of ectotherms (compared with cold tolerances) are relatively invariant with geographical distance (Brett, 1956; Grigg & Buckley, 2013) and latitude (Addo-Bediako *et al.*, 2000; Sunday *et al.*, 2011; Araújo *et al.*, 2013)

and also show higher phylogenetic inertia than cold tolerances (Kellermann *et al.*, 2012; Grigg & Buckley, 2013). While various hypotheses have been suggested to explain this pattern (Araújo *et al.*, 2013; Hoffmann *et al.*, 2013; Sunday *et al.*, 2014), the potential explanatory role of recent range expansions, which are widespread among terrestrial ectotherms, has not previously been explored.

Lower overall mean and maximum temperatures at the leading range edge may result in relaxed selection on upper thermal tolerance during climate-mediated range shifts (Parmesan, 2006). While release from thermal selection has been proposed as a mechanism facilitating range shifts (Thomas *et al.*, 2001; Parmesan, 2006), the consequences of thermal release for biogeographical patterns in thermal tolerances is thus far unknown. Release from thermal selection may increase the opportunity for selection on other traits that trade-off with thermal capacity, such as habitat and resource use (Thomas *et al.*, 2001), or metabolic costs and competitive ability (Clarke, 2003), leading to rapid evolution of both thermal and non-thermal traits during climate-induced range expansions. Because most high-latitude lineages have recently undergone recent range expansions from lower latitudes and glacial refugia (Hewitt, 1999), thermal release during range expansion may therefore have left a large signal on currently observed trait variation at higher latitudes (Keller *et al.*, 2009).

As suggested by Brett's rule, upper and lower thermal tolerance limits are often physiologically and evolutionarily decoupled within lineages, such that responses to cold stress rely on different physiological mechanisms than heat-stress responses (Chown *et al.*, 2002), and may thus also evolve differently during range expansions. While upper thermal tolerances may be released from selection during a poleward expansion, natural selection on cold tolerances may remain constant or intensify, leading to local adaptation in lower (but not upper) thermal tolerances along poleward expansion fronts. For instance, if colonization events keep pace with the climate velocity of minimally suitable temperature regimes, selection on lower tolerances will remain constant during the range expansion. Selection on cold tolerance may additionally intensify during the colonization process, as organisms encounter increasingly variable climates and extreme weather events at higher latitudes (Addo-Bediako *et al.*, 2000; Ghalambor *et al.*, 2006), leading to lower absolute lower thermal tolerances and more plastic responses to cold weather challenges at the poleward expansion front (Ghalambor *et al.*, 2006). Evolutionary constraints on the rate of adaptation of cold tolerances to increased climatic variability at higher latitudes may therefore limit the rate of poleward range shifts such that, somewhat paradoxically, the evolvability of lower thermal tolerances determines the effectiveness of a range-shifting strategy to rescue species from warming-induced declines.

We hypothesized that Brett's rule can arise as a result of relaxed selection on upper thermal tolerances, combined with continued or enhanced selection on lower thermal tolerances,

during post-glacial and contemporary range expansions to higher latitudes. To identify evolutionary changes in heat tolerance, cold tolerance and thermal tolerance plasticity during an ongoing range expansion, we conducted experimental assays for upper and lower thermal-tolerance phenotypes, combined with habitat data and niche modelling to characterize selective environments, in the blue-tailed damselfly, *Ischnura elegans* (Vander Linden, 1820) (Zygoptera: Odonata). Like many insect species, this small coenagrionid damselfly is currently rapidly expanding its geographical range northwards in Europe in response to global-warming trends (Hickling *et al.*, 2005). We examined thermal tolerances and the selective environment in both a core region in southern Sweden, and at the expanding northern range limit in central Sweden. Our hypothesis for relaxation of selection on heat tolerance during range expansions led us to predict: (1) increased phenotypic variance in heat tolerance, but decreased correlation of upper thermal tolerance with climatic selective agents, towards the recently colonized range edge in comparison with the range core, and (2) that at the approach of the range edge, overall habitat suitability for the species relies less on climatic predictors of upper thermal tolerance than predictors of lower thermal tolerance. Furthermore, our hypothesis that the evolution of cold tolerance limits the rate of climatic warming-induced range expansion of species led us to predict: (1) greater tolerance to cold temperatures towards the range edge, combined with a positive correlation of cold tolerance with geographical variation in climate throughout the range, indicative of ongoing local adaptation in cold tolerance during the expansion; and (2) constant or increasing importance of climatic variables that correlate with cold tolerance to overall habitat suitability towards the range edge. Finally, if thermal plasticity is an evolved property of range expansions, individuals towards the range edge may experience more beneficial effects from recent weather events on fitness-relevant (i.e. lower) thermal tolerances.

Importantly for each of our hypotheses, our core and peripheral study regions are both situated in the northern part of the species' distribution and share post-glacial

colonization histories (Hewitt, 1999) but exhibit different colonization histories under contemporary rates of global warming. Thus, our study does not confound the effects of post-glacial expansion with more recent range shifts, allowing us to characterize phenotypic shifts under current processes. Our results provide experimental evidence in natural populations for temperature-driven evolutionary change under contemporary climate change-induced range expansion.

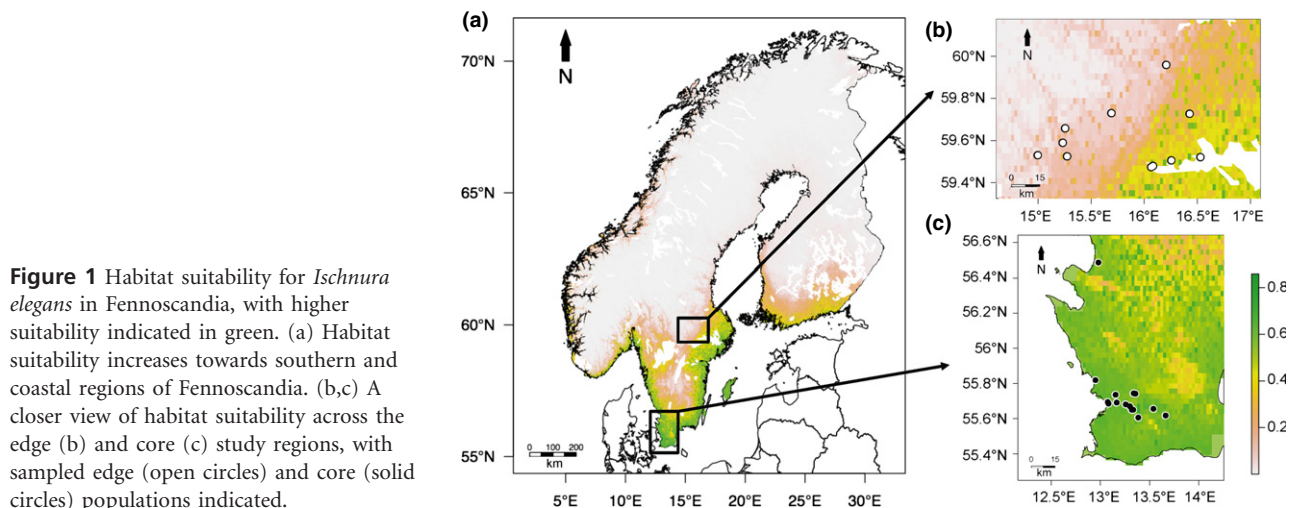
MATERIALS AND METHODS

Study system

Ischnura elegans is common across Europe and Asia, where it occupies a variety of thermal niches to which it responds with significant heritable, inter-population variation in thermal plasticity and life history (Bouton *et al.*, 2011). High levels of additive genetic variation for thermal-response traits and significant gene \times environment interactions for thermal-reaction norms across the latitudinal range of *I. elegans* suggest ample potential for future evolution of this species' thermal niche (Shama *et al.*, 2011).

Ischnura elegans is rapidly expanding its range northwards under current global warming, and has extended its northern range limit by 143 km in Great Britain between the two 10-year periods of 1960–1970 and 1985–1995, concomitantly increasing its population density in the northern part of Great Britain to occupy an additional 168 10-km² quadrats (Hickling *et al.*, 2005). We investigated altered patterns of thermal tolerance associated with this range expansion in Sweden, which is currently experiencing warming comparable with British climates and similarly facilitating rapid, contemporary range expansions of small ectotherms (Jaenson *et al.*, 2012). We sampled range-edge populations and core populations c. 500 km south of the range edge to span conservatively the current range expansion in Sweden (Fig. 1).

The leading edge of the species range was determined roughly from published records (Swedish Species Observation System, <http://www.artportalen.se>, and the Global



Biodiversity Information Facility, <http://www.gbif.org>), and identified practically from our 2013 census as the northernmost patches of freshwater habitat where populations could be found. Our study identified new populations within and beyond the previously sampled range, and beyond previously published range limits for *I. elegans* (Dijkstra & Lewington, 2006).

Sampling and thermal tolerance experiments

We collected wild-caught, adult, individual *I. elegans* for thermal tolerance experiments in June–early August of 2012 and June–late July of 2013. In 2012 we conducted experiments on wild-caught individuals from 15 populations in southern Sweden (mean latitude 55.73°, SD 0.22°; Fig. 1c), which is representative of the northern part of the species' core ancestral range (hereafter the core region). In 2013, we conducted thermal experiments on individuals from 11 populations at the leading edge of the species' range in central Sweden (hereafter the edge region; mean latitude 59.61°, SD 0.15°; Fig. 1b). In 2013 we also revisited core sites to address any potential yearly variation in adult damselfly thermal tolerances. Regions were matched for microclimate and habitat variation, and thermal tolerance phenotypes and local weather variation were highly congruent across years (see Appendix S1 in Supporting Information). At each site, we performed intensive, timed catching bouts so that capture rates could be recorded (number of individuals captured per minute per capturer) and used as a proxy for site density. Captures were balanced across the flying season and among populations within both regions. In total, we assayed 991 individuals in heat-ramping experiments and 1022 individuals in cold-ramping experiments, with a mean of 32/30 (edge) and 36/40 (core) individuals per population for the cold/heat-ramping trials, respectively. Further details on the capture and transport of damselflies back to the laboratory for thermal-tolerance trials can be found in Appendix S1.

Ramping experiments for upper and lower thermal tolerances were conducted in a Binder APT.line KB 53 (E3.1) refrigerated incubator (Binder GmbH, Tuttingen, Germany), with programmable heating- and cooling-rate settings. Within each trial, individuals were balanced by sex (determined from genitalia) and age (determined via thorax coloration, which exhibits ontogenetic colour changes; Dijkstra & Lewington, 2006). Individuals were equilibrated at 25 °C for 30 min. For the heat-ramping treatment, individuals were then heated at a rate of 0.06 °C min⁻¹ up to a maximum temperature of 43 °C, which was the temperature at which half of the individuals were knocked down (i.e. no longer standing because of thermal stress). For the cold-ramping (chill coma) treatment, individuals were chilled at the same rate of 0.06 °C min⁻¹ to 2 °C, which produced a 50% knockdown in pilot studies, and retained at 2 °C overnight. Heating and cooling rates (Fig. 2) were designed to mimic natural diurnal rates of heating and cooling (Terblanche *et al.*, 2011). See Appendix S1 for further details.

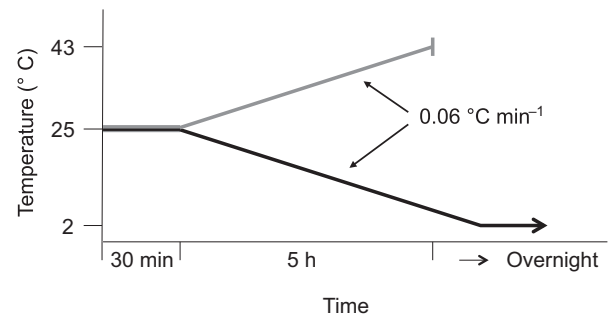


Figure 2 Schematic representation of temperatures during heat- and cold-ramping experiments to assess the thermal tolerances of Swedish *Ischnura elegans* from core and range-edge populations. After a 30-min equilibration at 25 °C, individuals were heated or cooled at a rate of 0.06 °C min⁻¹ to high (43 °C) or low (2 °C) temperatures that induced acute thermal stress. Rates of heating and cooling were chosen to approximate natural rates in the wild. Heat ramping, grey line. Cold ramping, black line. Axes not to scale.

At the end of each ramping experiment, individuals were observed for recovery times at room temperature. For cold-ramping experiments, individuals were scored as recovered when they were observed to flutter their wings in preparation for flight or other activity. During recovery from the heat-ramping experiment, individuals did not exhibit such conspicuous signs of activity, and so recovery was scored when they righted themselves from a knocked-down position. Non-knocked down individuals in the heat-ramping trials were given a recovery time of 0 s. All individuals were monitored for recovery up to a censor time of 15 min (900 s). The same equipment, observer and protocol were used in each laboratory to minimize any regional differences in experimental outcomes.

Statistical analyses

Differences between regions in inter- and intra-population variation in recovery times to heat- and cold-ramping experiments were compared using Bayesian generalized linear mixed models, fitting a censored Poisson distribution to the heat or cold ramping recovery-time data. In separate models for heat- and cold-ramping recoveries, we included region as a fixed effect and population and capture date as random effects, and fit population-level and residual variance independently across regions, while specifying zero covariance for thermal tolerance between regions (because no individual or population was sampled in both regions). For both models, we specified an uninformative inverse-gamma prior with minimal belief parameter. To optimize model performance, the Markov chain was run for 100,000 steps, retaining every 10th sample, in a Markov chain Monte Carlo (MCMC) analysis of variance of cold tolerance, and for 500,000 steps retaining every 100th sample in the analysis of heat tolerance. In each case, a 10% burn-in was discarded. Significantly different thermal response variances were identified as those

with non-overlapping highest posterior density (HPD) intervals. Models were run in the MCMCGLMM package for R 3.0.2 (Hadfield, 2010; R Core Team, 2012).

We also investigated the fixed effects of region, climate and weather on individual recovery times from heat and cold ramping, using a cox proportional hazards survival model implemented in the COXME package for R (Therneau, 2012). In these models, recovery time from thermal challenges, up to a censor time of 15 min, was assessed as the response variable in separate models for heat and cold challenge experiments, where individual damselflies were replicates. All experimental subjects were included in these models, including individuals that were unaffected, recovered or unrecovered (at 15 min) from the thermal challenge. Explanatory variables included recent field temperatures and population-specific long-term climate variables, considered as main effects and in interaction with a fixed effect for region, where the interaction effects test for regional variation in individual phenotypic responses to these environmental factors. Recent environmental temperatures were estimated as the minimum, maximum and mean temperatures from the past day, 3-day interval and 7-day interval from local weather station records (data provided by the Swedish Meteorological and Hydrological Institute, <http://www.smhi.se>; see Appendix S1 for further details). We extracted temperature-summarizing climatic variables (Bioclim BIO1–11; Hijmans *et al.*, 2005) for each population's georeferenced locale. We also included capture site and date as random effects to control for spatial and temporal variation in thermal tolerance not accounted for by climate and weather, and to address any batch effects in our experiments. During model selection, Akaike's information criterion (AIC) and the significance of fixed effects were used to distinguish among competing models. In some cases, climatic or weather variables were highly correlated and so were tested separately.

Habitat suitability model

To help predict the importance of thermally relevant traits in driving range expansions, we created a niche model for *I. elegans* that included both climatic and non-climatic variables. A maximum entropy habitat suitability model was constructed for Fennoscandia, using the Maxent algorithm (Phillips *et al.*, 2006) implemented in the DISMO package for R (Hijmans *et al.*, 2013), with default parameters and using the jackknife function to calculate variable importance. At a 1-km resolution, we included environmental layers that we considered potentially important in explaining the distribution of *I. elegans* and which were uncorrelated with each other at the 80% level (Table 2, and see Appendix S2). A total of 1767 non-overlapping occurrence points for *I. elegans* in Fennoscandia were extracted from the Global Biodiversity Information Facility (<http://www.gbif.org>), with 80% of the points used to train the model and the remaining 20% of the data used to test the model. The final model was averaged over all five possible combinations of 20%

occurrence withheld for testing. For each model run, 10,000 background points (pseudo-absences) were randomly generated across Fennoscandia for comparison with occurrence data. Model fit was evaluated using the area under the curve (AUC) of the receiver operating characteristic (ROC), a graphical representation of sensitivity versus 1 – specificity (positively identified known presences versus the false positive rate). AUC values of 0.5 or less correspond to the model performing similarly or worse than random, while AUC values of 1 – $a/2$ (where a is the relative size of the true species range within the study area) indicate a very good fit of the model to the data (Phillips *et al.*, 2006). The use of AUC has been criticized as a method of model evaluation because this measure introduces a number of biases and is not comparable among studies or analysis methods (Peterson *et al.*, 2008). Therefore we also validated our niche model with our field data, by testing the model's ability to predict population densities at our study sites (Oliver *et al.*, 2012).

RESULTS

Response to heat ramping in core and edge populations

Intra-population variance in heat-ramping recovery time in the edge region was significantly greater than intra-population variance in core regions, while inter-population variation in heat-ramping recovery was overall quite low and did not differ between the two regions (Table 1a). There were no net shifts in average recovery time between regions (effect of region on heat-ramping recovery time = 0.06 ± 0.16 SE, $z = 0.36$, $P = 0.72$). Thus, range-edge individuals took the same average amount of time to recover from heat challenge compared with core individuals, but there was higher variability between individuals in recovery times at the edge.

Table 1 Bayesian estimation of population-level and residual variance in the recovery of Swedish populations of *Ischnura elegans* from thermal challenge, with variances allowed to vary independently across regions. Non-overlapping highest posterior density (HPD) intervals indicate significantly different levels of thermal tolerance variability between inter- and intra-populations and between core and edge regions. Intra-population variability in heat tolerance was higher at the range edge than in the core region (bold).

Location	Source of variance	Variance estimate	HPD interval
(a) Regional variation in recovery from heat challenge ($n = 991$)			
Edge	Inter-population	0.56	0.05–11.18
Edge	Intra-population	329.03	209.15–545.63
Core	Inter-population	0.72	0.08–7.80
Core	Intra-population	114.01	82.49–148.64
(b) Regional variation in recovery from cold challenge ($n = 1022$)			
Edge	Inter-population	0.10	0.05–0.32
Edge	Intra-population	0.40	0.33–0.48
Core	Inter-population	0.08	0.04–0.19
Core	Intra-population	0.36	0.33–0.43

Recovery time from the heat-ramp challenge was affected by the long-term climate of an individual's capture location (BIO1, mean annual temperature), but only within the core region (BIO1 \times region effect on recovery time = -1.69 ± 0.78 , $z = -2.16$, $P = 0.03$). This interaction effect reflects changes in the degree of local adaptation at the approach of the expanding range edge. Within the core region, higher upper thermal tolerances were positively associated with a long-term history of warm climates at capture sites (effect of BIO1 on heat-ramping recovery time in core populations = -1.55 ± 0.78 , $z = -2.00$, $P < 0.05$; Fig. 3a), suggestive of local adaptation in the core region. However, in the edge region, upper thermal tolerances were uncoupled from local climates (effect of BIO1 = 0.07 ± 0.25 , $z = 0.27$, $P = 0.78$; Fig. 3b). Recent air temperatures (i.e. data from recent weather station records) on or leading up to capture dates were uncorrelated with upper thermal tolerances, either on their own or in interaction with region. Across both core and edge regions, heat-ramping recovery time was also affected by body mass, with larger individuals recovering more quickly (effect of mass on recovery time = -0.02 ± 0.006 , $z = -3.02$, $P = 0.003$). The covariate for mass did not mediate climatic effects on recovery time.

Mean (\pm SE) annual temperature (BIO1) was higher across core populations than among edge populations (core 7.90 ± 0.03 °C, edge 5.79 ± 0.01 °C; $t = 23.66$, $P << 0.0001$). However, there was no difference between regions in temperature on our specific capture days (weather station data; mean daily temperatures during the course of our study: core 16.23 ± 0.23 °C, edge 16.88 ± 0.28 °C, $t = 1.62$, $P = 0.10$; see Fig. S1a in Appendix S1).

Response to cold ramping in core and edge populations

Individual recovery times from chill coma were significantly shorter in edge than core populations (effect of region = -0.45 ± 0.09 , $z = -4.93$, $P < 10^{-7}$). However, there were no regional differences in the levels of intra- or inter-population variation in chill coma recovery (Table 1b). Thus recovery from cold challenge exhibited a shift in mean but not in variability at the expanding range edge.

Chill coma recovery rates were affected by inter-population variation in longer term climatic factors and by recent, short-term weather events. In both regions, recovery time was affected by climatic thermal variability (mean diurnal temperature range; BIO2). Individuals from populations characterized by more variable climates recovered more quickly from chill coma than individuals from less variable climates (main effect of BIO2 on recovery time = -0.55 ± 0.21 , $z = -2.61$, $P = 0.009$; Fig. 3c). The interaction of BIO2 with region was non-significant and did not improve the model AIC, and so was removed from the model. The main effect of region was no longer significant when BIO2 was included in the model, suggesting that regional differences in climatic variability explained regional

differences in cold tolerance. In addition to being explained by climatic variability, chill coma recovery was significantly correlated with the minimum temperature experienced by the population during the past 7 days (from local weather station data), although this effect varied by region (minimum 7-day temperature \times region effect on chill coma recovery time = -0.12 ± 0.04 , $z = -2.65$, $P = 0.01$). The weather \times region interaction reflected a significant hardening-type acclimation effect at the range edge, where individuals in populations recently exposed to cooler minimum temperatures in the past week experienced faster recovery times from experimental chill coma (effect of 7-day air temperature minimum on chill coma recovery time in the edge region = -0.11 ± 0.04 , $z = -2.85$, $P = 0.004$, Fig. 3e). In contrast, in the core populations, this hardening effect was not observed (minimum weekly temperature effect = -0.02 ± 0.02 , $z = -0.94$, $P = 0.35$; Fig. 3d). Body mass did not affect cold tolerance.

Average daily temperature variability (BIO2) was greater at the edge than in the core populations (edge 8.1 ± 0.07 °C, core 6.1 ± 0.05 °C, $t = 25.55$, $P << 0.0001$). Minimum weekly temperatures during the summers of our study (reflecting recent weather) were also generally lower at the edge than in our core populations (core 8.46 ± 0.20 °C, edge 6.51 ± 0.29 °C, $t = -5.23$, $P < 0.0001$; see Fig. S1b in Appendix S1).

Habitat suitability

We evaluated the climatic variables that were important predictors of thermal tolerance in a Maxent niche model that included 16 other, uncorrelated environmental variables. Our resulting habitat suitability model (Fig. 1a) had an AUC of 0.92 ± 0.01 , indicating a good fit of the model to the data. Our study populations had habitat suitability scores ranging from 0.59–0.76 in the core populations (Figs 1c & 4) to 0.04–0.55 at the range edge (Figs 1b & 4). Our average capture rates in these populations (numbers of individuals caught per minute of catching effort; a proxy for site density) were significantly correlated with predicted habitat suitability values from our species distribution model [effect of habitat suitability on $\log(\text{capture rate}) = 2.03 \pm 0.55$, $t = 3.73$, $P = 0.001$, $R^2 = 0.37$]. This independent evaluation of the model using the field dataset therefore strongly supported the niche model's predictive abilities. The relative contributions of each environmental variable to the model are shown in Table 2. Four out of the top five environmental factors contributing to habitat suitability were temperature measures, and temperature variables contributed a total of 83% of habitat suitability. In contrast, precipitation only contributed 0.1%, and biotic and abiotic features of the landscape contributed 1.8% and 15%, respectively.

Mean annual temperature, which was a good predictor of recovery rates from heat-ramping trials for core (but not edge) populations (Fig. 3a,b), was also the best predictor of habitat suitability in our niche model, explaining most (62.1%) of the variation in habitat suitability for *I. elegans*

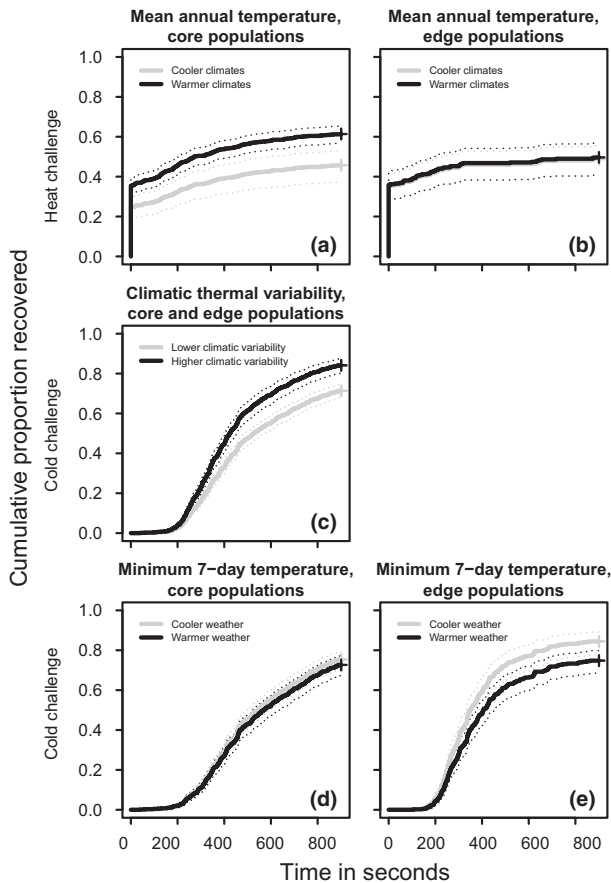


Figure 3 Climate and weather effects on recovery rates from thermal ramping experiments in Swedish populations of *Ishcnura elegans* from the core of their geographical range and at the expanding, northern, range limit. The cumulative probability of recovery is plotted as a function of time elapsed since the end of the temperature ramping trial, up to a 15-min (900-s) censor time. For visualization purposes only, continuous weather and climate predictors are plotted as two-level factors, corresponding to all above-average versus all below-average values. Dashed lines represent 95% confidence intervals for responses in these (artificially binary) categories. (a,b) Faster recovery from heat challenge corresponds positively with mean annual temperature (BIO1) in core but not edge populations. (c) Faster recovery from cold challenge corresponds with increasing climatic thermal variability (BIO2) across core and edge regions. (d,e) Recovery from a cold challenge is improved by recent exposure to cold weather in edge but not core populations.

across Fennoscandia. Habitat suitability increased with higher values of mean annual temperature, with some evidence of a shallower relationship above 6 °C (Fig. 4a). All core populations had above-average habitat suitability compared with that predicted by mean annual temperature (Fig. 4a). Among edge populations, easterly populations also experienced similar or higher habitat suitability than predicted by their mean annual temperatures, whereas westerly edge populations, which overall experienced lower habitat suitabilities (Fig. 1b), experienced lower habitat suitabilities than predicted by their yearly average temperatures (Fig. 4a).

Mean diurnal temperature range, which explained phenotypic variation in recovery from chill coma in both core and edge regions (Fig. 3e), was the third most important predictor variable in the niche model, explaining 12% of habitat suitability (Table 2). Habitat suitability was inversely correlated with temperature variability, such that habitat suitability was uniformly high when temperatures fluctuated by less than 8 °C day⁻¹, but then dropped sharply at higher levels of daily variability (Fig. 4b). Core and edge populations all experienced average habitat suitability levels for their diurnal temperature ranges (Fig. 4b).

DISCUSSION

Release from thermal selection and non-directional evolution of heat tolerance

Variation in upper thermal tolerances across core and edge populations was consistent with the hypothesis that upper thermal limits evolve under release from thermal selection during climate warming-induced range expansion. The phenotypic patterns observed in support of this hypothesis were: (1) increased intra-population variance in this trait at the range edge in comparison with the core, but without a change in mean phenotype across the recent range expansion (Table 1); and (2) a loss of local adaptation of this trait to inter-site variation in mean annual temperatures at the range edge but not in the core (Fig. 3a,b). Complementing the phenotypic data, habitat suitability differences between core and edge populations further supported the release from selection hypothesis: mean annual temperature was the strongest factor limiting the geographical distribution of *I. elegans* across Fennoscandia (Table 2) but mean annual temperature was more strongly limiting in core populations than in edge populations (Fig. 4a). As the climate warms above a minimal threshold to support demographic growth at regions just beyond the current poleward range limits, colonists escape the climatic selection on heat tolerances that characterized (warmer, established) the source populations where they had previously been adapted.

Environmental thermal variability and cold-tolerance adaptation

In contrast to upper thermal tolerances, lower thermal tolerances exhibited local adaptation in both the core and edge regions. The observed recovery from chill coma was significantly faster in edge populations than in core populations, and this effect is attributed to higher climatic thermal variability at the edge than in the core (Fig. 3c). A correspondence between the cold-hardiness of a species and the amount of environmental thermal variation it encounters has been found in comparative studies (Payne, 1926; Gaston *et al.*, 2009); however, rapid evolution of cold tolerance in response to novel climatic variability has rarely been found across geographical gradients within a species. Our habitat

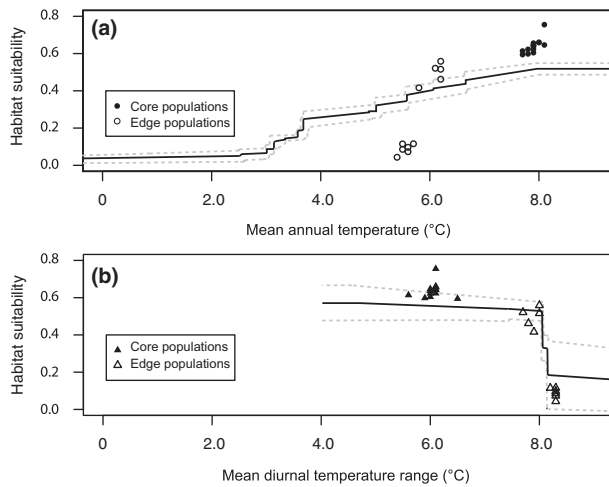


Figure 4 Partial effects of climate on habitat suitability for 26 populations of Swedish *Ischnura elegans* used in thermal tolerance experiments. (a) Core (black circles) and some edge (open circles) populations have higher than expected habitat suitability for their characteristic mean annual temperatures (BIO1), suggesting that high temperatures may limit demographic growth in the core (see also Fig. 3a). Inland edge populations (open circles) have lower than expected habitat suitabilities for their characteristic mean annual temperatures, suggesting that high temperatures are not limiting there (see also Fig. 3b). (b) Core (black triangles) and edge (open triangles) populations all experience habitat suitabilities that fall within or above predictions for habitat suitability based on their climatic daily temperature ranges (BIO2), suggesting that thermal variability is limiting in both regions (see also Fig. 3e).

suitability model indicates that selection arising from thermal variability may consistently limit demographic growth across both edge and core regions (Fig. 4b), suggesting that selection on cold tolerance remains strong during and across the leading edge of climate change-induced range shifts. Together, our thermal experiments and niche model results indicate that adaptability of cold tolerance may commonly limit a species' rate of poleward expansion during periods of global warming.

Niche breadth and range expansion

Release from selection on upper thermal tolerances combined with intensifying selection on lower thermal tolerances has resulted in an overall wider thermal niche at higher latitudes, an oft-reported pattern in the comparative literature (Janzen, 1967; Addo-Bediako *et al.*, 2000; Gaston *et al.*, 2009; Sunday *et al.*, 2011, 2014; Sheldon & Tewksbury, 2014) that finds novel support here within a species. The comparative literature suggests that thermal tolerance breadths correlate with latitude most strongly in the terrestrial Northern Hemisphere, weakly in the Southern Hemisphere, and not at all in the marine environment (Sunday *et al.*, 2011). The lack of congruence between hemispheres, and between terrestrial and marine environments, has often been hypothesized to

result from current differences in environmental thermal gradients among these regions, combined with physiological differences between terrestrial and marine organisms (Klok *et al.*, 2004). Our study supports the more parsimonious explanation that differences in glaciation history among these regions (i.e. more extensive in the Northern Hemisphere than the Southern Hemisphere and absent in the marine environment), resulting in different colonization histories and concomitant evolutionary processes, may also explain these regional differences in latitudinal patterns for thermal tolerance breadth. Increased niche breadths resulting from release from selection during range expansion have been reported for other ecological traits such as parasitism (Menéndez *et al.*, 2008) and competitive ability (Bolnick *et al.*, 2010). However, the data presented here constitute, to our knowledge, the first empirical evidence that release from thermal selection during range expansions may be responsible for broader thermal niches at higher latitudes.

Adaptive plasticity in thermal tolerance during range expansions

Lower thermal tolerance phenotypes benefited from prior experience of recent weather events in edge but not core regions. At the expanding range edge, cold-tolerant phenotypes were correlated with recent (7-day) temperature minima, but this effect was not observed in the core populations, despite the fact that experimental subjects from core and edge populations exhibited comparable variation in weekly temperature minima (see Fig. S1b in Appendix S1). Our results suggest that successful invasions may not require pre-adapted plasticity in cold-tolerance phenotypes, if plasticity can arise in the course of range expansion itself (Chevin & Lande, 2011). Adaptive environmental influences on cold tolerance may arise during range expansions as a response to predictably increasing rates of environmental and demographic change at the shifting range edge. Our selected core populations, located just 500 km from the range edge in Sweden, may have subsequently lost at least some aspects of cold-tolerance plasticity, such as acclimation to recent cold exposure, that once facilitated their previous, post-glacial range expansion into northern Europe. Loss of plasticity in established core populations implies that costs of plasticity may limit the possibility for range shifts to facilitate the evolution of ever-more invasive phenotypes.

Local adaptation and developmental plasticity

This study provides insight into changes in biogeographical patterns of correspondence between ecologically relevant wild phenotypes and their underlying selective factors. The use of wild-caught individuals that have developed under native conditions was essential for this goal but introduced an important limitation to the study. In the absence of common-garden conditions, we cannot rule out that some of the observed changes in thermal tolerance resulted from develop-

Table 2 Variable contributions to a Maxent species distribution model for *Ischnura elegans* in Fennoscandia, based on 1767 occurrence points and 10,000 pseudo-absences distributed across Fennoscandia. See Appendix S2 for further information on each environmental layer selected for analysis. Percentage contributions are calculated as permutation importance, indicating the reduction in model fit following random permutation of each variable, normalized to percentages. Contributions estimated in this way do not depend on the order in which variables are added to the model.

Predictor	Percentage contribution
Mean annual temperature	62.1
Elevation	13.7
Mean diurnal temperature range	12.1
Mean temperature of the warmest quarter	5.6
Mean temperature of the wettest quarter	2.4
Terrestrial ecoregion	1.4
Distance from the coast	1.1
Temperature seasonality	0.7
Tree cover	0.4
Soil parent material	0.2
Maximum temperature of the warmest month	0.1
Annual precipitation	0.1
Land cover	0
Mean summer greenness	0
Variation in summer greenness	0
Net primary productivity	0
Isothermality	0
Precipitation seasonality	0

mental plasticity rather than genetic change. Thus, for instance, it is plausible that increased phenotypic variability in heat tolerance at the range edge may reflect increased environmental instability during development, rather than genetic change (although this alternative mechanism should also produce increased variability in cold tolerance, which was not observed). However, survival of these phenotypically variable individuals, resulting in a loss of correlation between adult upper thermal tolerances and local climate, implicates a process of release from thermal selection at the range edge regardless of evidence for genetic change underlying the phenotypic response. Similarly, the correlation between cold tolerance and a climatic variable that limits habitat suitability in both core and edge regions implies a strong role for selection on locally adapted phenotypes in both regions, in the absence of data on how these phenotypes are produced. Furthermore, previous work indicates that genetic change and local adaptation during range shifts is the rule rather than the exception (Preisser *et al.*, 2008; Bridle *et al.*, 2014; O'Neil *et al.*, 2014) and thus it is highly unlikely that our observed patterns of phenotypic variation, which correspond so closely with expected evolutionary changes in response to underlying variation in selective regimes, have been produced by a single plastic genotype. More data on genetic correlates of thermal tolerance in this system will shed additional light on the mechanisms of thermal evolution during a range expansion.

Range expansion and adaptive potential

Brett's rule has commonly been interpreted as indicating a lower capacity for evolution in upper than in lower thermal tolerances, a prospect that is of concern under a climate-warming scenario (Kellermann *et al.*, 2012; Araújo *et al.*, 2013; Hoffmann *et al.*, 2013). In contrast, our results suggest that upper thermal tolerances do evolve non-directionally during range expansions. Such non-directional evolution can produce an illusion of evolutionary stasis if biogeographical history or geographical variation in levels of intra-population thermal tolerance variability are not accounted for in comparative analyses. The oft-reported macroecological pattern of relatively invariant heat tolerances across lineages and latitudes might reflect a shared history of range expansions out of the tropics between glaciations, a historical process that underpins most temperate biodiversity (Hewitt, 1999; Rolland *et al.*, 2014).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Additional methodological details for field capture and thermal trials, and weather variation data.

Appendix S2 Habitat suitability model environmental layers and references.

BIOSKETCH

Lesley T. Lancaster is fascinated by evolutionary processes that maintain a diversity of complex and well-adapted phenotypes, with particular interest in the roles of range shifts, niche evolution and social feedback.

Author contributions: B.H. and E.I.S. proposed the experiments, L.T.L. developed the aims and hypotheses; all four authors contributed to the sampling design; L.T.L. and R.Y.D. collected the data; L.T.L. analysed the data and wrote the paper; all four authors edited the paper.

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