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Winter warming facilitates range expansion: cold tolerance of the butterfly *Atalopedes campestris*

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Abstract Our ability to predict ecological and evolutionary responses to climate change requires an understanding of the mechanistic links between climate and range limits. The warming trend over the past half-century has generated numerous opportunities to develop much-needed case studies of these links. Species that are only limited by climatic factors are likely to shift range quickly during periods of warming. Such species directly impact recipient communities and indicate trends that will become more widespread. Because minimum temperature (T_{\min}) is rising at twice the rate of maximum temperature, species with this range-limiting factor may be especially responsive to global warming. In this study, I test the hypothesis that rising T_{\min} has directly affected the range of a skipper butterfly. *Atalopedes campestris* has moved northward rapidly this century, recently colonizing eastern Washington where January T_{\min} has risen 3°C in 50 years. The results show that:

1. *A. campestris*' range lies completely within the -4°C January average minimum isotherm, and that recently colonized areas were below this threshold earlier this century.
2. In acute cold stress experiments, -4 to -7°C proved to be a critical thermal limit: median supercooling point was -6.3°C , and minimum lethal temperature (LT_{50} with 12-h exposure) was -5.7°C .
3. In chronic cold stress experiments, survivorship declined sharply in diurnally fluctuating thermal regimes typical of the current range edge. High mortality occurred under constant 0°C conditions as well as in fluctuating regimes, implying that thermal insulation from snow would not protect *A. campestris*.
4. There was no evidence of evolution in cold tolerance at the range margin, despite strong selection. Thus,

winter warming was apparently a prerequisite for the range expansion. Characteristics of this species that seem to be associated with its rapid response are that it is an opportunistic species, it is not habitat or dispersal limited, and it is constrained by T_{\min} .

Keywords Chill injury · Climate change · Lethal temperature · Range limits

Introduction

Global temperatures are rising, especially in winter: average winter minimum temperature rose 2.9°C while summer maximum temperature rose 1.3°C in the Northern Hemisphere from 1951 to 1990 (Karl et al. 1993). The scientific consensus is that this trend is likely to accelerate over the next century due to the increasing concentration of greenhouse gases in the atmosphere (IPCC 2001). The consequences of asymmetric warming for wildlife are still largely unexplored (Mooney et al. 1994, but see Alward et al. 1999). Historically, most species have shifted their geographic ranges individually during periods of climate change (e.g., Davis 1976; Coope 1977; Atkinson et al. 1987; COHMAP 1988; Cronin and Schneider 1990; Overpeck et al. 1992; Webb and Bartlein 1992; Graham et al. 1996). Some of these shifts may directly reflect bioclimatic requirements, while others are secondary responses to changing biotic conditions. Species that react to climate change at the fastest rates initiate a cascade of consequences for the whole environment. Species that are limited by minimum temperature (T_{\min}) have good dispersal ability and wide-ranging host/habitat distributions are likely to be vanguard species. There are few non-pest model systems in which specific range-limiting factors are known (Lawton 1995), and even fewer known to be limited by T_{\min} . Observations of a correlation between T_{\min} and range limits suggest that many species may be limited by this variable (Root 1988b), but few studies test mechanistic hypotheses

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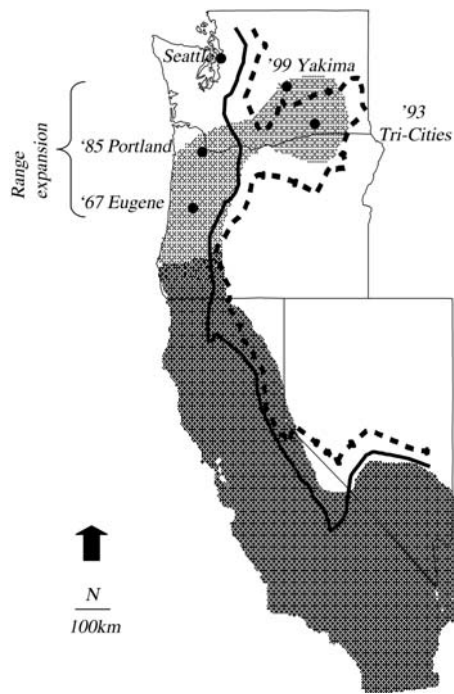


Fig. 1 Overwintering range of *Atalopedes campestris* (shaded) in Washington, Oregon, California, and Nevada from Opler (1999), modified to include the western range expansion (lighter shading). Colonization dates of *A. campestris* by four cities in Oregon and Washington show the chronology of the range expansion. Contour lines represent the January average minimum -4°C isotherm from 1950–1959 (solid) and 1990–1998 (dotted) (NCDC 2000)

linking the two (but see Pigott 1975; Nobel 1980; Root 1988a, 1991).

The sagem skipper, *Atalopedes campestris* (Lepidoptera: Hesperiiidae), is a common generalist butterfly across the southern United States and Mexico (Opler 1999). It is typically a southern and coastal butterfly with tropical origins and no known hibernial diapause. *A. campestris* has expanded its range up the west coast of the United States over the past 40 years (Fig. 1), recently colonizing areas where winter minimum temperature has risen 3°C since 1950. These observations indicate that *A. campestris*' current range may be limited by winter temperature. This study investigates a potential mechanistic link between climatic change and range change in this species. If *A. campestris*' range edge coincides with its minimum lethal temperature, then warming winters would be a necessary precondition for the recent range expansion.

Approach

I first explored the large-scale association between January minimum temperature and *A. campestris*' range edge. A striking correlation with the -4°C isotherm emerged from this analysis. In order to test whether -4°C represents a physiological threshold for *A. campestris*, I then studied the direct impact of acute cold stress on

caterpillar mortality using supercooling point (SCP) and minimum lethal temperature (LT_{50}) assays. Since freezing is fatal for *A. campestris*, the SCP reflects the minimum temperature it could theoretically survive. I used the SCP technique to compare the cold tolerance of populations, developmental stages, and acclimation regimes to identify the lowest SCP likely to occur in the wild. There are some limitations to the SCP technique, however. First, the standard rate of cooling is much faster than organisms would actually experience in the field, which might affect survivorship. Second, in some species mortality occurs prior to actually freezing (Bale 1987, 1991). I used the LT_{50} technique with an ecologically relevant rate of cooling and exposure time (overnight) to verify the relevance of SCP for the survival of extreme cold in the wild.

I supplemented these standard techniques with a more extensive study of cumulative injury due to sub-lethal cold exposure by mimicking average winter conditions as closely as possible. Although most studies do not incorporate cumulative hazard estimates into their field survivorship predictions, acute stress experiments may not accurately predict field survivorship for several reasons. Sub-lethal injury accumulates over the entire season and can affect longevity and fecundity, despite initial survival (Parish and Bale 1993; Bradshaw et al. 1998). Furthermore, small-scale spatial heterogeneity in ground conditions increases the variance in the thermal environment experienced by individual caterpillars, which is not estimated in the SCP or LT_{50} techniques. Finally, caterpillar behavior might ameliorate some stress. Not accounting for sub-lethal injury would lead to an overestimate of field survivorship, while ignoring heterogeneity and behavior would lead to an underestimate.

I used walk-in environmental chambers to re-create the thermal regime most frequently encountered at ground level near *A. campestris*' range limit, while caterpillars were settled more naturally in their microhabitat. These month-long diurnally fluctuating treatments provide a better estimate of the variance around the minimum physiological tolerance to be expected in the field than is possible from the standard cold tolerance techniques. Finally, I used the laboratory results to develop a model of mortality as a function of cold stress. This model predicts field survivorship under variable conditions over winter.

Materials and methods

Study system

Atalopedes campestris (Lepidoptera: Hesperiiidae), the sagem skipper, is an abundant generalist butterfly native from the southern United States through the neotropics. Larvae develop on many grass species (Poaceae), including native prairie, lawn, and weed grasses. Adults take nectar from a wide variety of common flowers, including alfalfa (*Medicago sativa*), thistles (*Cirsium* spp.), and many Compositae. *A. campestris* expands its range seasonally in the Midwest and Northeast US in late summer, dying back over winter (Opler 1999). *A. campestris* expanded its range into the Pacific Northwest from California in the mid-1960s (Fig. 1),

establishing a persistent population in Eugene, Oregon in 1967 (Dornfeld 1980). It progressed 180 km northwards during the 1970s and 1980s, colonizing the Portland, Ore. environs in 1985. Western Oregon has a Mediterranean climate, so summer warming may be more important for this part of the range expansion. However, in the 1990s *A. campestris* colonized eastern Washington, which has a continental climate due to the presence of the Cascade Mountain Range. *A. campestris* has maintained a population in the Tri-Cities (consisting of Richland, Kennewick, and Pasco) since 1993. In 1998–1999, it spread up the Yakima River and across the Columbia Basin as far as Ritzville, Wash. (Crozier, unpublished data).

Range edge analysis

I analyzed *A. campestris*' overwintering range as depicted by Opler (1999), modified to include the western range expansion (Fig. 1). Early spring sampling and overwinter field experiments in western Oregon and southern Washington demonstrate the persistence of these populations (Crozier, unpublished data; Hinchliff, collection records). I analyzed average January minimum temperature for 1950–1959 and 1990–1998 for all 1221 stations in the Historical Climatological Network (NCDC 2000). I treated the range status of each station (based on the field guide map) as a binomial variable in a generalized linear model (GLM; range status~January temperature) with a logit link function. I determined the amount of variation explained with an Analysis of Deviance using a χ^2 significance test.

Insects for cold tolerance experiments

I collected field-mated *A. campestris* females from the Tri-Cities, Wash. weekly through September and October, 1999 and 2000. The larvae from wild-caught females grew on 25×50×4 cm flats of live Kentucky bluegrass (*Poa pratensis*) in environmentally controlled chambers. The temperature in the chambers cycled continuously between daily minimum and maximum temperature through a 24-h triangular wave. Rearing temperature mimicked an average September in the Tri-Cities (25°C/10°C; WRCC 1999). Although this species is not thought to have a winter diapause (Scott 1979), day length was kept short to try to induce diapause, if possible (8 h light:16 h dark). Unless otherwise stated, larvae and pupae acclimated to a 12°C/4°C triangular temperature regime (typical of early November) for 2 weeks prior to each experiment.

Supercooling point

To determine the SCP of *A. campestris*, I cooled caterpillars in 1.5 ml Eppendorf vials at 0.1°C/min from 5°C to –20°C in an ethylene glycol/water bath (Neslab RTE-110; see Neven 1999 for further details). I measured the temperature of each caterpillar every 30 s with a 0.01 mm copper-constantin thermocouple probe, recorded by a Campbell Scientific 21X datalogger. A positive temperature spike resulting from release of the latent heat of fusion indicated caterpillar freezing.

Developmental stage

In winter, insects in the temperate zone typically enter dormancy and enhance their cold tolerance (for reviews, see Uvarov 1931; Danks 1978; Lee and Denlinger 1991). Freeze-intolerant species usually lower their SCP in the overwintering developmental stage in response to the appropriate environmental cue. To determine whether caterpillars are more cold-tolerant in any particular developmental stage, I measured the SCP of 20 larvae/instar. Adults had access to food (20% honey water solution) upon emergence, and then acclimated at 12°C/4°C without food for 2 days prior to experiments.

Acclimation treatment

I varied both the duration of exposure to the 12°C/4°C regime, and the temperature at which larvae were acclimated prior to SCP determination. I assessed the SCP of third instar larvae after 0, 14, or 60 days at 12°C/4°C, and after 2 weeks in 12°C/4°C, 8°C/0°C or 4°C/–4°C regimes. In a separate experiment, 40 caterpillars lived outdoors on two grass flats in Seattle until January, before testing SCP.

Source population

I evaluated the potential for recent cold adaptation in the Washingtonian population by comparing both the SCP and the LT₅₀ of insects from Californian and Washingtonian populations. I chose to compare the SCP of the Tri-Cities population with that of a population from Sacramento, Calif. for two primary reasons. Firstly, the Sacramento Valley is very likely to be the source of the original northwest immigrants, and hence may represent the ancestral state of this population. Secondly, the Sacramento population is not under strong selection for cold tolerance. Average minimum January air temperature in Sacramento is 7°C warmer than the Tri-Cities (4.2°C versus –3.2°C; WRCC 1999), and the extreme minimum air temperature very rarely drops below –5°C. I measured the SCP of 30 larvae/population over six replicates during their third instar.

Lower lethal temperature

I determined the temperature at which 50% of the caterpillars died (LT₅₀) by chilling them at 2°C/h, holding the test temperature for 12 h, and slowly warming them to room temperature over 5 hr. Cooling rates measured in the field range from 0 to 7°C/h, where sustained cold frequently lasted 12 h. I defined fatality as lack of movement within 24 h after warming up. Test temperatures were determined for each larva by individual thermocouples, because the temperature inside the test tubes was ~1.6°C warmer than the bath temperature. This technique also allowed SCP monitoring during the LT₅₀ experiments. Bath temperatures for the third instar experiment were –2°C, –4°C, –6°C, –7°C, –8°C, –9°C, and –10°C; fifth instars were tested at –4°C, –6°C, and –8°C. I compared the LT₅₀ of the Sacramento and Tri-Cities populations with two replicates of five caterpillars/population/trial.

I fitted a hyperbolic tangent function to the data with a least squares goodness of fit criterion to estimate the LT₅₀. I tested the hypothesis that Washingtonian caterpillars have greater cold tolerance than Californian caterpillars by modeling survivorship as a generalized linear model function of temperature and population (including interaction effects), separating the data by instar. I compared the SCP of each population in each experiment by a Kruskal-Wallis rank sum test because the SCP was not normally distributed.

Winter simulation

I designed this experiment to characterize the impact of typical winter conditions within and beyond the current range as naturally as possible. Treatments are comparable to the 30-year average January diurnal temperature regimes of: (1) the Sacramento Valley, Calif. (12°C/4°C); (2) the Willamette Valley, Ore. (8°C/0°C); and (3) the upper Yakima Valley, just beyond the current range edge (4°C/–4°C, WRCC 1999). I placed 40 larvae (split into four age classes) in each of 60 grass flats (25×25×5 cm). Flats had 20 cm of dirt, allowing natural caterpillar behavior at the thatch/ground boundary. The 4,800 caterpillars in these experiments had one week to settle into the flats with all three environmental chambers at 25°C/10°C, then 2 weeks under acclimation conditions (12°C/4°C) before the first sample. Each sample consisted of destructively searching 5 flats/chamber and occurred weekly for 4 weeks. The

following year I added 2°C/–6°C and 0°C/–8°C regimes. Sampling frequency was adjusted in the colder treatments to capture the faster mortality: 0, 3, 7, 14 days (2°C/–6°C); 0, 3, 5 days (0°C/–8°C). Field measurements of ground temperature in eastern Washington confirmed that 8°C is the average diurnal variation (Crozier, unpublished data).

A common characteristic of high elevation and high latitude is a persistent snow pack over winter, under which the temperature hovers near 0°C for months at a time. I determined the effect of this prolonged chilling in additional constant temperature treatments: 0°C±1°C (sampled weekly), and –3°C±1°C (sampled after 0, 3, 7, and 14 days).

I tested the hypothesis that the degree of cold stress had a significant effect on mortality after arcsine square root transforming survivorship per flat (N_t/N_0). I built generalized linear models stepwise by adding day, treatment temperature/chamber and caterpillar cohort (i.e., age) as predictor variables. I controlled for a significant environmental chamber effect on survival by conducting an analysis of variance (ANOVA) of survivorship to the first sample after the 21-day acclimation period, which was the same for all treatments. All analyses were performed on S Plus 6.0 software (Insightful Corp).

Hazard-rate model

To describe survivorship as a continuous function of temperature and aid in comparison of the results from different techniques, I developed a hazard rate model of cold stress. I estimated the mortality rate for each treatment as the slope of a linear regression of $\ln(\text{number of surviving caterpillars per flat})$ against time, starting with the first sample period, and compared the slopes (λ) across treatments. Each sample involved caterpillars in different flats, and thus was an independent estimate of the hazard rate (λ). I modeled survivorship as a hyperbolic tangent function of temperature. I used a least squares criterion of goodness of fit to select parameter values. Although the exact function of this curve is arbitrary, alternatives are constrained by the need to approach 0 at *A. campestris*' lower lethal temperature, and to approach 100% survival in the absence of cold stress. I used the same functional shape to model the results from the LT_{50} experiment.

Results

Range edge analysis

January temperature is a highly significant predictor of whether a location is inside or outside the range of *Atalopedes campestris* (Fig. 1): 63% of the deviance explained in 1950, $\chi^2=783.7$, $P<0.001$, and 65% in 1990. The average January minimum temperature is warmer than –4°C at 98.3% of all weather stations within the range. All those below –4°C are at high elevation and are unlikely to actually be occupied. However, 21% of stations outside the range were warmer than –4°C in 1950 (23% in 1990), and are mostly in the southeastern United States. This may reflect the fact that other parts of the range map have not been updated recently, but it is more likely that warmer winter temperatures are a necessary, but not sufficient, condition for presence. If there is a physiological basis underlying the observation that *A. campestris* is not found in cold locations, then there should be a significant difference in survivorship at temperatures above and below this threshold.

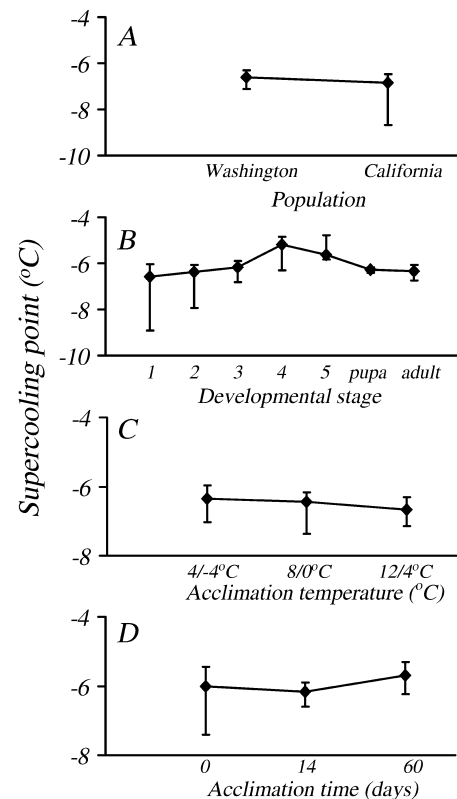


Fig. 2 Supercooling point of *Atalopedes campestris* comparing source population (Sacramento, Calif. v. Richland, Wash.) (A), developmental stage (B), acclimation temperature (C), and acclimation duration (D). Median + 1st and 3rd quartiles are shown. There were no significant differences between treatments ($P>0.05$) except for developmental stage

Insects for cold tolerance experiments

Larvae never ceased development during rearing, and commenced eating whenever it was warm enough. Larvae outside from August to January exhibited similar behavior. This indicates that *A. campestris* is not entering diapause over winter in Washington, nor does it have a specialized overwintering stage.

Supercooling point

Median SCP ranged from –6.8°C to –5.2°C across all variables tested (grand median –6.3°C, grand mean –6.8°C, extreme values ranged from –17.3°C to –3.4°C; Fig. 2). I used a Kruskal-Wallis rank sum test to compare groups because the data had an average skew of –1.9. There are no significant differences ($P>0.05$) as a result of source population, acclimation temperature or duration. There are significant differences between the developmental stages ($\chi^2=42.9$, $df=6$, $n=132$, $P<0.001$). The lowest median SCP was in the first instar (–6.6°C), which also had the widest variance ($SD=3.5$). Second and third instars, pupae, and adults had median SCP between –6.4°C and –6.2°C (SD 0.4–2.6). Fourth instars had the

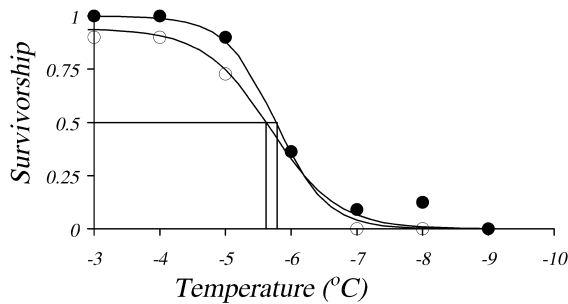


Fig. 3 Lethal temperature for 50% of the sample (LT_{50}) for Californian (open circles) and Washingtonian (closed circles) third instar larvae with a hyperbolic tangent curve fit. Estimated LT_{50} is -5.6°C (Calif.), and -5.8°C (Wash.) (drop-down lines). The difference between populations is not significant ($P>0.05$)

highest SCP (-5.2°C), and fifth instars were intermediate (-5.6°C).

Lower lethal temperature

LT_{50} after 12 h of exposure for third instar caterpillars was between -5°C and -6°C , for both Californian and Washingtonian larvae. Survivorship dropped off rapidly between -4°C and -7°C (95% to 5%; Fig. 3). The hyperbolic tangent LT_{50} estimate is slightly higher for Californian larvae (-5.6°C) than Washingtonian larvae (-5.8°C ; Fig. 3), but the difference is not significant: there is neither a population main effect ($\chi^2=1.5$, $P=0.2$, $n=120$), nor an interaction between population and temperature ($\chi^2=0.15$, $P=0.7$, $n=120$). As in the SCP experiments described above, SCP measured during the LT_{50} experiment did not differ between populations ($\chi^2=0.34$, $P=0.5$, $n=73$). No caterpillars survived -10°C for any duration ($n=40$). All caterpillars that froze died. I did not detect an SCP in 19% of the caterpillars that died, suggesting that there may be some injury due to cold stress other than freezing. But this did not occur consistently at temperatures above the median SCP.

LT_{50} for fifth instars was between -3°C and -4°C for Californian caterpillars, -5°C to -6°C for Washingtonian caterpillars. There is a main effect of population source ($\chi^2=5.3$, $P=0.02$, $n=60$) and a significant interaction between population and test temperature ($\chi^2=10.5$, $P=0.001$, $n=60$). These results indicate that fifth instar Californian caterpillars had a higher mortality rate overall, as well as a stronger reaction to cold temperatures. Visual inspection of the data indicates that the

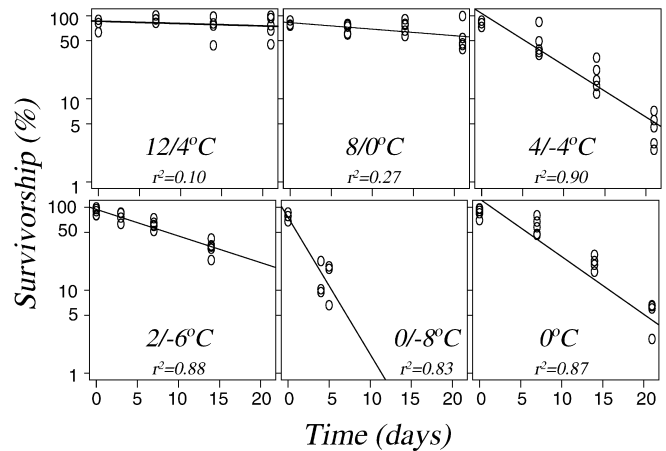


Fig. 4 Survivorship (%) of caterpillars in simulated winter experiment: lines represent linear regressions of $\log_{10}(\text{survivorship}/\text{flat})$ on time. Statistical analyses were computed on $\ln(\text{number of survivors}/\text{flat})$

largest difference between the populations occurred at -4°C , when 100% of the Washingtonian caterpillars survived compared with 38% of the southern caterpillars. However, the SCP of Californian fifth instars is not significantly higher (Kruskal Wallis $\chi^2=3.45$, $P=0.063$, $n=27$). There is a significant three-way interaction in the generalized linear model between instar, population, and test temperature ($\chi^2=8.3$, $P=0.004$) when the two experiments are pooled, reflecting the fact that fifth instars have a higher LT_{50} than third instars, and that the population effect is only manifest in the fifth instar.

Winter simulation

There is a strong interaction effect of temperature and exposure duration, indicating that cold significantly reduces survivorship over time ($F_{1,119}=54.1$, $P<0.001$, Fig. 4, Table 1). Both treatment and duration are also significant as main effects (Table 1). Treatment temperature and duration combined account for 70% of the total variance in survivorship.

Survivorship was high (94% after 3 weeks) in the warmest treatment, comparable to Californian average conditions ($12^{\circ}\text{C}/4^{\circ}\text{C}$). It was slightly lower (71%) in the next cooler treatment ($8^{\circ}\text{C}/0^{\circ}\text{C}$), but there was a clear drop (survivorship of 4%) to the next treatment level ($4^{\circ}\text{C}/-4^{\circ}\text{C}$). Survivorship in the $2^{\circ}\text{C}/-6^{\circ}\text{C}$ treatment is statistically indistinguishable from $4^{\circ}\text{C}/-4^{\circ}\text{C}$, although

Table 1 Analysis of deviance from a generalized linear model of survivorship-day \times treatment (Trt) in the winter simulation experiments. Flat was not significant as a nested effect and was eliminated from the model. The full model explained 70% of the variation in survivorship

	<i>df</i>	Deviance	Resid. <i>df</i>	Resid. Dev.	<i>F</i>	<i>P</i> value
NULL	122	17.79				
Day	1	4.55	121	13.25	100.89	<0.001
Trt	1	5.44	129	7.80	120.69	<0.001
Day \times Trt	1	2.44	119	5.36	54.11	<0.001

regression slopes indicate lower mortality. Survivorship was clearly lower in 0°C/−8°C conditions: only 16% survived the first 4 days. The constant temperature treatments are not statistically different from the fluctuating treatments with the same mean temperature ($P>0.5$).

Age significantly affects survivorship ($F_{1,212}=7$, $P=0.008$). Average survivorship of third and fourth instars was $0.16\pm 0.09SE$ higher than that of first and second instars across all treatments and sample periods.

Discussion

Physiological constraints on the range

Range shifts may correlate with climate change for a variety of reasons, from coincidental to causal. Given a causal relationship, the physiological constraint may be either: (1) a higher likelihood of extreme exposure; or (2) a longer duration of moderately unfavorable conditions. The strong correspondence between temperatures measured at the range end of *Atalopedes campestris* and temperatures causing severe declines in their survival (with all three cold tolerance techniques) suggests a physiological constraint at the current range edge. The specific constraint may be a combination of acute and chronic stress. SCP and LT_{50} experiments produced nearly synonymous results, demonstrating a steep population mortality gradient from −4°C to −7°C. In this temperature range, the probability that individual caterpillars will freeze increases sharply, and therefore the isotherm correlation corresponds to the risk of acute stress. However, in regions where January minimum temperature is below −4°C there is a good chance that snow will accumulate and insulate the ground from extreme lows, reducing the risk of acute stress. The high mortality observed at temperatures above the SCP in the winter simulation experiment (96% by the end of 3 weeks at 4°C/−4°C, and 92% at 0°C) suggests that chilling injury from prolonged exposure to moderate stress is equally deadly for this species. This implies that insulation by snow pack would not be sufficient to protect *A. campestris* from cold because it usually persists for at least one month, long enough to induce significant mortality. Snow pack also tends to reduce spatial variation in ground temperature, further limiting potential refugia that could protect *A. campestris* from cold. Therefore, the minimum lethal temperature by all three measures is close to the observed isotherm at the range limit, but for slightly different reasons. The implication of *A. campestris*' thermal constraint in the context of global warming is therefore mixed. Pacific Northwest regional climate models predict increasing winter precipitation over the next century (Leung and Ghan 1999). Increased snowfall will decrease the probability of persistence. However, warming temperatures at the northern range edge will generally enhance persistence in this species, especially where snow transforms into rain.

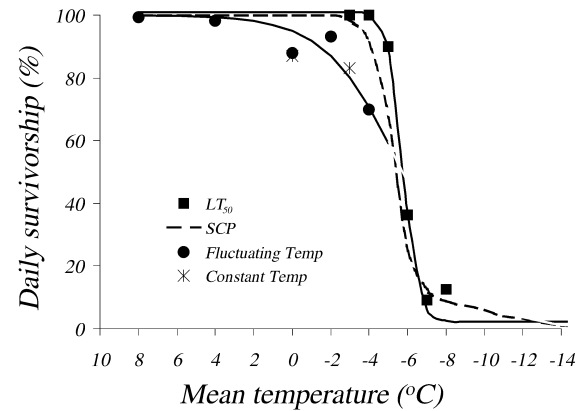


Fig. 5 Survival curves estimated by: (1) the cumulative frequency distribution of supercooling points (SCP); (2) hyperbolic tangent curve fit to the LT_{50} experiment; and (3) hyperbolic tangent curve fit to regression estimates of $\ln(\text{number of survivors/flat})$ on time in the simulated winter experiment. Symbols depict fluctuating temperature regression slopes (circles) and constant temperature regression slopes (stars)

The inflection points of survivorship curves based on data from SCP, LT_{50} , and winter simulation results are quite similar and correspond to temperature isotherms at the range edge (Fig. 5). However, the corresponding model functions have different slopes, and therefore different predictions of actual field survivorship under variable conditions. Specifically, the slope of the survivorship curve based on the winter simulation results was shallower than slopes of the LT_{50} and SCP data (Fig. 5). The winter simulation model thus effectively adds variance to the basic mechanistic relationship revealed in the LT_{50} and SCP experiments. This difference may be interpreted as allowing more caterpillars to survive the occasional extremely cold night, but higher mortality due to chill injury.

Other studies have demonstrated that some species are more tolerant of chronic stress than others. For instance, in the autumnal moth *Epirrita autumnata*, freezing is essentially the only source of winter mortality, and information on the SCP, local topography and weather patterns are sufficient to describe the probability of a population outbreak (Tenow and Nilssen 1990). For other species, including several aphids, e.g., *Myzus persicae* (Bale et al. 1988), and *Sitobion avenae* (Knight et al. 1986), and the beech weevil (*Rhynchaenus fagi*), substantial mortality occurs at temperatures above the SCP, and a more thorough study of their cold tolerance is required to predict field survivorship (Bale 1991). Several butterflies are in this latter group, including the peacock (*Inachis io*), small tortoiseshell (*Aglais urticae*), and monarch (*Danaus plexippus*) (Pullin and Bale 1989, Troyer et al. 1996). Chill injury is most likely to be important for those species with range edges in between regions requiring freeze tolerance or depressed SCP (where average minimum temperature is less than −5°C), and the subtropics, where development is possible year round. This thermal boundary includes much of the

southern United States and Europe, and will likely move northward over the next century (IPCC 2001).

Evolution at the range edge

Why do physiological constraints limit ranges at all? Why don't organisms adapt to range edge conditions, and then expand until they hit another type of constraint? In some cases, such as the European corn borer (*Diatraea grandiosella*), cold tolerance has evolved during range expansion, allowing species to colonize environments radically different from their historical conditions (McCauley et al. 1995). In this case study, however, there was no evidence of phenotypic adaptation at the range edge. The Californian LT_{50} was higher in the fifth instar than the Washingtonian LT_{50} , but this does not indicate evolution in cold tolerance at the range margin: mortality was higher in the Californian caterpillars at all temperatures, suggesting some factor other than temperature is sufficient to explain the observed shift in LT_{50} . Moreover, the cold tolerance of third instars is probably much more important for population persistence for several reasons: (1) it has a lower SCP (Fig. 2B); (2) larvae reared in cooler conditions never passed the third instar in the laboratory due to high mortality (constant 15°C: Crozier 2001; 20°C/12°C: Crozier, unpublished data); and (3) this stage is prevalent in the field in fall due to the timing of the largest adult flight period (Crozier, unpublished data). There was no significant difference in LT_{50} between Washingtonian and Californian third instars (Fig. 3). This is surprising given the extremely strong selection that must be operating over winter in eastern Washington. It is possible that cold tolerance is actually increasing, but the effect is not yet detectable. However, the failure of *A. campestris* to survive winter in the Midwest suggests a limited ability to adapt to severe cold.

There are two potential explanations for the lack of physiological adaptation at range edges (Hoffmann and Blows 1994). Either the edge population lacks heritable variability for the trait in question (without costs that override its selective value); or gene flow from larger populations in a different environment swamps local adaptation (Kirkpatrick and Barton 1997). In this case study, either or both of these processes may be limiting adaptation. *A. campestris* may lack heritable variation in this trait at the species or population level. The genus *Atalopedes* is historically Neotropical (Burns 1989): all four other species in the genus are endemic to small areas in the Caribbean and northern South America. When this genus split from *Hesperia* (which has many species in the temperate zone) it may have lost its cold tolerance, or the ancestral species may not have been cold adapted. Alternatively, the deficiency might exist only in the Washingtonian population, perhaps because founding individuals lacked this trait by chance. Consistent with this hypothesis, SCP variance was higher in the Californian population (Fig. 2A).

There may also be costs to cold tolerance. For example, small size is associated with lower SCP because water content is reduced (Salt 1961). Accordingly, most of the variation in SCP in this study occurred in first and second instars. However, these stages performed more poorly than third and fourth instars during the winter simulation experiment, possibly because older instars have more body fat reserves to cope with prolonged stress and better-insulated nests (Crozier, personal observation). More generally, smaller individuals may be selected against because usually size is positively correlated with fecundity (Garcia-Barros 2000). Finally, gene flow from other populations may be overwhelming local adaptation. The rapid colonization of eastern Washington after Portland was colonized (≤ 8 years) suggests that the Cascade Mountains are not an insurmountable barrier to dispersal. Regardless of the exact mechanism inhibiting evolution, enhanced cold tolerance fails to explain *A. campestris*' rapid range expansion.

Implications for responses to climate change

Many species are already responding to climate change (Parmesan and Yoho 2003; Root et al. 2003). Understanding crucial factors in individual cases may help to define groups of functional responses to climate change. The key features in this case are that *A. campestris* is: (1) an opportunistic species; (2) not dispersal or habitat limited; and (3) minimally cold tolerant. It is clear that opportunistic species respond the fastest to climate change, regardless of their specific range-limiting factors (Pollard 1988; Pollard et al. 1995; Thomas 2001). It is not known how many species are limited by winter temperature. Efforts have been made to estimate range-limiting factors from correlations with distributions (Root 1988b; Sutherst et al. 1995; Beaumont and Hughes 2002). Unfortunately, many factors (biotic and abiotic) tend to be correlated, so without laboratory and field experiments it is difficult to determine which may be most important during range change (Repasky 1991; Loehle and Leblanc 1996). However, a comparative analysis of niche evolution in closely related birds, mammals, butterflies (Peterson et al. 1999) and plants (Huntley et al. 1989) suggests that environmental conditions at range edges tend to be conserved over evolutionary time scales, at least below the family level. Thus, there may be phylogenetic patterns in responses to climate change.

There are several implications of this case study that could be useful for conservation and land managers. The first is that the influx of lower latitude species has begun and may drive resident species poleward. The second is that because the climatic envelope for this species has shifted geographically, the same may be true for other species with more restricted dispersal or habitat requirements (such as endangered species). If climatic envelopes move much faster than species can shift their ranges, they may be left in increasingly stressful locations. On the other hand, new territories outside historic ranges may

soon be suitable for introductions of endangered species and therefore worth preserving regardless of the status of resident flora and fauna. Clearly, natural responses to climatic change have begun and will cause many ecological upheavals in the near future.

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