Conserved and narrow temperature limits in alpine insects: Thermal tolerance and supercooling points of the ice-crawlers, Grylloblatta (Insecta: Grylloblattodea: Grylloblattidae)

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Abstract

For many terrestrial species, habitat associations and range size are dependent on physiological limits, which in turn may influence large-scale patterns of species diversity. The temperature range experienced by individuals is considered to shape the breadth of the thermal niche, with species occupying temporally and/or geographically stable climates tolerating a narrow temperature range. High-elevation environments experience large temperature fluctuations, with frequent periods below 0°C, but Grylloblatta (Grylloblattodea: Grylloblattidae) occupy climatically stable microhabitats within this region. Here we test critical thermal limits and supercooling points for five Grylloblatta populations from across a large geographic area, to examine whether the stable microhabitats of this group are associated with a narrow thermal niche and assess their capacity to tolerate cold conditions. Thermal limits are highly conserved in Grylloblatta, despite substantial genetic divergence among populations spanning 1500 m elevation and being separated by over 500 km. Further, Grylloblatta show exceptionally narrow thermal limits compared to other insect taxa with little capacity to improve cold tolerance via plasticity. In contrast, upper thermal limits were significantly depressed by cold acclimation.

Grylloblatta maintain coordinated movement until they freeze, and they die upon freezing. Convergence of the critical thermal minima, supercooling point and lower lethal limits point to adaptation to a cold but, importantly, constant thermal environment. These physiological data provide an explanation for the high endemism and patchy distribution of Grylloblatta, which relies on subterranean retreats to accommodate narrow thermal limits. These retreats are currently buffered from temperature fluctuations by snow cover, and a declining snowpack thus places Grylloblatta at risk of exposure to temperatures beyond its tolerance capacity.

Keywords:
Physiological tolerance
Climate variability hypothesis
Plasticity
Ectotherm
Lethal limits
Freeze avoidance

1. Introduction

The variability and predictability of temperature fluctuations are considered to be important components shaping the thermal niches of insects (Chown and Nicolson, 2004). In its simplest form, the climate variability hypothesis posits that the thermal tolerance breadth of a species should reflect the variability (diurnal and/or seasonal) in the climate to which it is exposed (Janzen, 1967; Stevens, 1989). Generally considered in a macroecological framework, in which the thermal limits (including plasticity of these limits) of species and/or populations across a latitudinal gradient are compared (review: Bozinovic et al., 2011), the climate variability hypothesis can also be applied to predict microhabitat-associated and/or seasonal variation in thermal tolerance breadth (Molina-Montenegro and Naya, 2012).

High elevation climates are typically highly variable, both daily and seasonally, with ambient temperatures reaching or exceeding the thermal limits of many insect taxa (Hoffmann et al., 2013). Nevertheless, high elevation species do not consistently have lower thermal limits than low-elevation relatives (Semme and Zachariassen, 1981; Gaston and Chown, 1999; Buse et al., 2001; Buckley et al., 2014). This raises the fundamental problem of how these taxa persist in high elevation habitats if they are not adapted to extreme cold.

Edwards (1986) argued that, for small ectotherms, tolerance to temperature extremes is not a necessary adaptation to alpine environments. Instead, the capacity to maintain activity at low temperatures (~0°C) and avoid or tolerate freezing should determine a
species’ ability to persist in these areas (Mani, 1968; Edwards, 1986). Further, Sinclair et al. (2003) suggested that the mechanisms by which individuals survive sub-zero temperatures are, at least in part, a reflection of the predictability of their environment; freeze tolerance (survival of internal ice formation) predominates in unpredictably cold environments, while freeze avoidance (depression of the freezing point, with ice formation lethal) is more common in predictable, moderately cold environments.

The insect genus Grylloblatta (Grylloblattodea: Grylloblattidae), commonly known as the ice-crawlers, provide an important case study in the interrelationship between thermal tolerance and distribution due to their high level of local endemism, habitat specificity, and yet broad latitudinal occurrence (36–59°N) throughout western North America (Schoville and Graening, 2013; Fig. 1). These flightless, long-lived, and relatively large-bodied insects are often found in rocky subterranean habitats on alpine talus slopes. These habitats are thermally stable. Where ambient temperatures in surrounding alpine and sub-alpine areas can range from −40 to 40 °C across a year, Kamp (1973) recorded Grylloblatta microhabitat temperatures between −3 and 6 °C, with little spatial or seasonal variation and no rapid temperature fluctuations. Unlike most alpine insects, Grylloblatta also inhabit low elevation ice caves and riverine canyons (Kamp, 1963; Schoville and Roderick, 2010). These microhabitats are more thermally variable (diurnally and annually) than those at higher elevations and Grylloblatta are often seasonally transient, moving to more moderate conditions beneath rocks and snowpack during the winter (Kamp, 1973). Throughout their distribution, Grylloblatta are, however, active throughout the year and lab experiments using a thermal gradient indicate a near-zero preferred temperature in a remarkable example of cold stenothermy (Henson, 1957).

The thermally stable microclimates (geographically and temporally) occupied by Grylloblatta suggest that individuals should have narrow thermal limits across their range, with minimal plasticity (according to the climate variability hypotheses). Previous studies of Grylloblatta suggest that this is the case (Mills and Pepper, 1937; Edwards and Nutting, 1950; Kamp, 1973; Morrissey and Edwards, 1979), but variation in experimental design and experimental objectives makes comparisons among datasets challenging. This study implements an experimental design to test for conserved thermal limits by establishing a standardized protocol, experimentally determining the influence of prior acclimation, and directly estimating the effect of population/species origin. In addition, we predict that, given microclimate stability, Grylloblatta should survive sub-zero temperatures via a freeze avoidance mechanism (Sinclair et al., 2003). Here we test these predictions by measuring critical thermal limits and supercooling points in Grylloblatta from three alpine, one sub-alpine and one cave population. Within the sub-alpine population, we also test the extent to which variation in thermal limits is plastic with respect to (a) age, (b) gender, and (c) acclimation. We use thermal ramping to ensure that our data provide ecologically relevant measures of thermal tolerance (Terblanche et al., 2011) and remain comparable to a broad set of insect studies.

2. Methods

2.1. Sample sites, collection time, and maintenance prior to experiments

Samples of Grylloblatta were collected from five sites (Fig. 1): a subalpine habitat on Mt. Spokane, Washington (47.92°N, −117.11°W, 1200 m a.s.l.), alpine habitats on Mt. Rainier, Washington (46.85°N, −121.76°W, 2000 m a.s.l), Mt. Adams, Washington (46.20°N, −121.49°W at 2200 m a.s.l), and South Sister, Oregon (44.10°N, −121.77°W, 2000 m a.s.l), and a cave population from near Cougar, Washington (46.11°N, −122.21°W, 640 m a.s.l). Three alpine/subalpine populations represent undescribed taxa (Jarvis and Whiting, 2006; Schoville and Graening, 2013), while the alpine South Sister population represents Grylloblatta rothi Gurney, 1953 and the cave population represents Grylloblatta chirurgica Gurney, 1961. These five taxa are distinguishable based on genetic data (see below), but because three taxa are formally undescribed, we refer to them as populations throughout. Adults and nymphs were collected from Mt. Spokane in mid-October 2013, and mid-October and early-November 2014. Adults from Mt. Rainier, Mt. Adams, and South Sister were collected in June and July 2014. Adults and nymphs were collected from Cougar in late-November 2014. Adults and nymphs are active throughout the year, with no dormant period.

All specimens were caged individually in 2 oz. plastic containers, with a moistened cotton round providing moisture and dried mealworms (Tenebrio molitor) for food, and refreshed regularly. After capture, specimens were maintained in cool (<10 °C) conditions, in constant darkness (grylloblattids are photonegative and become agitated upon exposure to light) for 1–3 weeks while being transported to the laboratory in Madison, Wisconsin.

Samples from Mt. Rainier (n = 3 males, 7 females), Mt. Adams (n = 3 females), Cougar (n = 1 male, 2 females, 2 nymphs), and South Sister (n = 3 males, 12 females) were maintained in a 5 °C cold-room for 2 weeks before experiments began. Samples from Mt. Spokane were divided into three treatment groups. Group one consisted of laboratory-acclimated adults (n = 1 males, 10 females) collected in 2013 and maintained for 9 months at a

![Fig. 1. Map of the Grylloblatta taxa used in this study (green circles) and all known populations (black diamonds, data from Schoville and Graening (2013)), overlaid on a map of annual temperature range in western North America (data from Worldclim, Hijmans et al. (2005)). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)](image-url)
constant temperature of 5 °C. Group two consisted of field-captured adults (n = 1 males, 10 females), 3rd–4th instar nymphs (n = 9), and 1st–2nd instar nymphs (n = 8) collected in October 2014 and maintained at a constant temperature of 5 °C for 1 week before experimentation. Group three consisted of cold-acclimated adults (n = 1 male, 4 female), 3–4th instar nymphs (n = 10), and 1–2nd instar nymphs (n = 6) collected in October 2014 and maintained at a constant temperature of −1 °C for 1 week before experimentation.

2.2. Genetic divergence

Sequence data for 742 base pairs of the mitochondrial cytochrome oxidase subunit II (COII) gene was obtained for samples representing each population following isolation, amplification and sequencing protocols in Schoville and Roderick (2010). The data comprise 13 individuals from Mt. Spokane, 6 individuals from Mt. Rainier, 5 individuals from Mt. Adams, and 2 individuals of G. rothi from South Sister (data deposited as GenBank Accessions KP972433–KP972441). These data were combined with published data (Jarvis and Whiting, 2006; Schoville, 2012), including that of G. chirurgica, to examine genetic divergence among Grylloblatta taxa endemic to the Pacific Northwest region (Accessions DQ457339–DQ457349, DQ457359–DQ457368, JN612961–JN612964). Manually edited and aligned sequence data were analyzed in the software MRMODELTEST2 v2.3 (Nylander, 2004) to estimate the most likely substitution model based on the Akaike Information Criterion (Akaike, 1973). Model GTR + I + G was selected as the best model. A Bayesian phylogeny was estimated in BEAST v3.1.2 (Drummond and Rambaut, 2007) based on two independent runs with the following conditions: 1 billion steps with genealogies sampled every 100,000 steps. TRACER v1.6 was used to assess convergence of the two runs, ensuring that ESS values for each parameter were greater than 200, and to estimate an appropriate burn-in period (Rambaut and Drummond, 2009). A 20% burn-in was selected before calculating the maximum clade credibility tree in TREEANNOTATOR v2.1.2 (Drummond and Rambaut, 2007).

2.3. Thermal ramping and critical thermal limits

Cold and heat tolerance limits were measured using a ramping protocol (Mitchell and Hoffmann, 2010; Overgaard et al., 2011). The experimental apparatus consisted of a water bath (TX150 R2, Grant Instruments, UK) that circulated a 50:50 propylene glycol/water mix around six cylindrical chambers (4 cm diameter, 8 cm high). In each experimental run, one individual was placed into each chamber and responses to cold and heat were scored by direct observation. In both cold and heat tolerance experiments, the chambers were pre-cooled to 5 °C and individuals were allowed 10 min at this temperature to equilibrate. The temperature was decreased at 0.2 °C/min for cold tolerance trials. For heat tolerance, the temperature was increased at 0.5 °C/min to 20 °C, and 0.2 °C/min thereafter. Critical thermal limits (CTmin, CTmax) were defined as a loss of righting ability, corresponding, respectively, to the ‘chill coma’ temperature (Mellanby, 1939) and ‘heat movement’ threshold (Hazell et al., 2008). Individuals were removed from the testing chambers immediately upon reaching their critical thermal limits, and returned to either 5 °C (Lab & Field groups) or −1 °C (Cold group) to recover. All individuals were tested first for cold tolerance and then allowed 1 week to recover before being tested for heat tolerance. A week after the heat tolerance test, they were transferred to the supercooling experiment.

2.4. Supercooling protocol

Many insects from cold climates survive sub-zero temperatures by supercooling (Sinclair and Chown, 2005a) and the supercooling point (SCP), defined as the temperature at which ice forms in the body, can be well below the critical thermal minimum measured by behavioral assays (Sinclair, 1999). As ice formation is an exothermic reaction, the SCP can be detected by continuous measurement of body temperature, where a spike in body temperature indicates ice formation. To measure SCPs in Grylloblatta, each individual was wrapped in a thin layer of cotton, with a K-type thermocouple (Jaycar Electronics, Australia) held against the abdomen. The individual was then placed in a small tube which was held in a water bath. After 10 min at 5 °C to equilibrate, the temperature of the water bath was decreased at 0.2 °C/min. Thermocouples were attached to a data logger (TC-08, Pico Technology, UK) which recorded temperature every two seconds, and the SCP was defined as the lowest body temperature immediately preceding a rapid rise in body temperature which indicates ice formation. After all individuals had reached their SCP, the experiment was terminated and individuals were returned to 4 °C. After 24 h, no individuals had recovered from the experiment. All individuals were starved for 2 days prior to measuring the SCP as gut contents can influence ice formation (Salt, 1966; Block and Semin, 1982; Salin et al., 2000).

2.5. Statistical analysis

Means and variances were estimated for each group, where a group represented either a discrete population, life-stage or treatment. Although sample sizes are small for some groups, and unequal across the study design, Levene’s test showed that among-group variation was similar and Shapiro–Wilks tests showed that the data do not deviate from a normal distribution. Therefore, we employed analysis of variance (ANOVA) to test for statistical differences among groups. All analyses were done in R 3.0.1 (R Core Team, 2014) and the data are presented as mean ± standard deviation (S.D.) unless stated otherwise.

3. Results

3.1. Genetic divergence among populations

Analysis of the mitochondrial COII gene suggested substantial genetic divergence among the five populations, as well as other Grylloblatta taxa from the Pacific Northwest region (see Fig. 2). Each population examined in this study is minimally 10% different with G. chirurgica the most closely related to a sample of Grylloblatta campodeiformis from southern Montana and placed within the same clade as the Mt. Rainier population. These relationships have high posterior probability support (>0.90) in the phylogeny. The Mt. Adams population is very closely related to G. rothi and is placed in a well-supported clade with G. chirurgica clade is estimated as sister to the G. campodeiformis clade. Finally, the South Sister population is very closely related to G. rothi, and is a member of a deeply split southern clade in the Grylloblatta phylogeny.

3.2. Variation in critical thermal limits and supercooling points among populations

Across all populations, thermal limits were between −4.0 ± 0.8 °C (n = 41) and 27.0 ± 0.7 °C (n = 31) (Fig. 3a and e; see Inline
Supplementary Table 1). *Grylloblatta* were able to maintain coordinated movement until they froze, with the SCP (−3.9 ± 1.0 °C, n = 23) almost identical to the CTmin, but ice formation was lethal (Fig. 3c). We separately analyzed data with and without the cave population (Cougar), as the number of adults was small and the later collection date (November) might have allowed populations to seasonally acclimate. There was no among-population variation in any thermal tolerance trait measured when the cave population was not included (CTmin: F3,33 = 0.587, p = 0.628; CTmax: F2,25 = 1.110, p = 0.365; SCP: F2,15 = 0.247, p = 0.862; see Inline Supplementary Table 2). However, the cave population had slightly lower thermal limits than the other populations, and there was a marginally significant difference in CTmax among populations when it was included in the analysis (CTmin: F3,33 = 2.669, p = 0.047; CTmax: F4,28 = 1.127, p = 0.364; SCP: F1,18 = 1.178; p = 0.354; see Inline Supplementary Table 2).

### 3.3. Variation in critical thermal limits and supercooling points among age-classes

Three age-classes were defined for individuals from Mt Spokane: adults (80–135 mg body mass), 3rd–6th instar nymphs...
(15–63 mg) and 1st–2nd instar nymphs (4–17 mg). We found no variation among life-stages for either cold tolerance measure ($CT_{\text{min}}$: $F_{2,44} = 0.429, p = 0.654$; SCP: $F_{2,31} = 0.604, p = 0.553$; Fig. 2b and d; see Inline Supplementary Tables 1 and 3). However, $CT_{\text{max}}$ increased with age ($F_{2,31} = 13.767, p < 0.001$; Fig. 3f), the 1st–2nd instar nymphs having an average $CT_{\text{max}}$ of 26.2 ± 1.5 °C ($n = 9$) which was significantly lower than both late-instar nymphs (27.0 ± 1.4 °C, $n = 18$) and adults (28.5 ± 0.8 °C, $n = 11$).

3.4. The influence of acclimation on critical thermal limits and supercooling points

Acclimation for 1 week at 0 °C depressed both lower and upper thermal limits ($CT_{\text{min}}$: $F_{1,44} = 5.589, p = 0.023$; $CT_{\text{max}}$: $F_{1,31} = 94.043, p < 0.001$; Fig. 3b; see Inline Supplementary Tables 1 and 3). This effect was stronger for upper thermal limits, where cold-acclimated individuals lost coordinated movement an average 2.3 °C cooler than individuals acclimated to 5 °C. A 0 °C acclimation improved cold tolerance by an average 0.9 °C. In contrast, acclimation had no consistent effect on the SCP ($F_{1,31} = 0.984, p = 0.329$). The length of acclimation affected heat tolerance ($t = 5.728, p < 0.001$) but neither measure of cold tolerance ($CT_{\text{min}}$: $t = 0.593, p = 0.560$; SCP: $t = 0.562, p = 0.584$). Individuals kept for 9 months at 5 °C reached their $CT_{\text{max}}$ an average 2 °C lower than those acclimated for 1 week.

4. Discussion

The temperature regime of the environment occupied by a population or species is thought to be a key component shaping the limits and breadth of its thermal niche (Clusella-Trullas et al., 2011; Hoffmann et al., 2013). Temperature range, seasonality, and predictability can each impose selection pressure for thermal adaptation (Sinclair et al., 2003; Angilletta, 2009; Hoffmann et al., 2013). Despite occurring primarily in alpine areas – an environment characterized by large temperature fluctuations, high seasonality and often rapid and unpredictable temperature changes (Mani, 1968), Grylloblatta occupy cold, but thermally stable, microhabitats across their range (Kamp, 1973). It is thus, perhaps, unsurprising to find a remarkably narrow thermal niche (~31 °C), supporting the predictions of the climate variability hypothesis. This thermal niche was also strongly conserved among populations, despite significant genetic divergence, 1500 m difference in elevation, and geographical separation of up to 560 km.

One short-coming of our experimental design is that we could not estimate the effect of developmental plasticity, which is known to influence thermal tolerance in other insects (Colinet et al., 2015). Viable strategies to rear and cross Grylloblatta populations have not been developed, and due to their long lifespan, may not be feasible. Given our experimental constraints, we have attempted to measure lab acclimation, seasonal acclimation, and compare five genetically distinct populations to test for the influence of genetic background and environmental variation. We were able to detect significant effects of acclimation. If developmental differences did exist among populations, we would have expected them to be obvious in comparisons among populations. However, such effects appear to be small (or at least, smaller than variation due to acclimation effects). We therefore do not expect a strong bias in our conclusion that the thermal niche is highly conserved among genetically distinct Grylloblatta populations.

Among insects for which both cold and heat tolerance have been measured, thermal tolerance breadth is typically greater than 35 °C and increases towards higher latitudes (Addo-Bediako et al., 2000; Hoffmann et al., 2013). Variation in thermal tolerance breadth is driven primarily by changes in cold tolerance, while heat tolerance remains relatively fixed (49.0 ± 4.9 °C) (Addo-Bediako et al., 2000; Hoffmann et al., 2013). In Grylloblatta, however, we see a remarkably low heat tolerance threshold. This is likely to be, if anything, an overestimate of heat tolerance, as Kamp (1973) recorded lipid melting points below the $CT_{\text{max}}$ recorded here. This result is perhaps even more notable as Grylloblatta are placed within the Polyneoptera (Schoville, 2014), a group that includes species with exceptionally high thermal tolerances (e.g., 49 °C in alpine Kosciuscola in Australia, R.A. Slatyer unpublished data; Whyard et al., 1986) and preferred temperatures frequently above 30 °C (Forsman, 2000; Harris et al., 2013).

We note that our design employed repeated measurements on individuals for different thermal traits ($CT_{\text{max}}$, $CT_{\text{min}}$, and SCP), which could influence successive thermal tolerance measurements (Brown et al., 2004). Prior exposure to stress could have sub-lethal effects that reduce subsequent thermal tolerance (Brown et al., 2004; Sinclair and Chown, 2005b), or alternatively, could enhance thermal tolerance due to hardening (Lee et al., 1987). It is challenging, without extensive knowledge of fitness effects or the latency of hardening, to minimize this problem, especially in a species where samples sizes are limited. We have tried to address this problem based on standards in the field (Terblanche et al., 2011), which suggest that choosing a reasonable time period (in our case, 1 week) between repeated measures will minimize hardening effects and that clear description of the experimental design be provided to facilitate future interpretations.

4.1. Acclimation and developmental stage have small but significant effects on thermal limits

Thermal plasticity is considered an integral component of how ectotherms overcome temperature stress, with many studies showing substantial plasticity in response to short-term acclimation and strong seasonal variation in cold tolerance (Chown and Nicolson, 2004; Chown and Terblanche, 2006). We found limited thermal plasticity with respect to cold acclimation with a small shift in $CT_{\text{min}}$ (0.9 °C) but, notably, a considerable shift in $CT_{\text{max}}$ (2.3 °C). A similar lowering of $CT_{\text{max}}$ was also evident following prolonged lab-acclimation, and in comparisons among age classes. These three results indicate, unusually, greater plasticity in upper compared to lower thermal limits (Hoffmann et al., 2013).

Seasonal acclimation is common among insects, with some of the most striking examples involving shifts in the supercooling point (e.g., from ~7 °C in summer to ~26 °C in winter for the stag beetle Cerinus piceus: Neven et al., 1986). The thermal regimes of alpine and sub-alpine Grylloblatta habitats are remarkably stable throughout the year, but cave areas become considerably colder during the winter months (Kamp, 1973). We observed lower $CT_{\text{min}}$ among individuals from the low-elevation cave site, which were collected close to winter. However, with very small sample sizes and only a single population, we are unable to separate potential differences due to habitat (i.e., a more variable thermal regime within the cave microhabitat) from those of seasonal acclimation.

4.2. Cold stenothermy and freeze avoidance: how does Grylloblatta persist in alpine habitats?

In discussing the biology of Grylloblatta, Edwards (1987) noted that these species rely on microhabitat choice to avoid exposure to extreme cold temperatures, using insulating layers of snow and subterranean retreats to find relatively temperature-constant environments – temporally and spatially. Rather than being governed by extremes, he argued that Grylloblatta specialize on cold, yet constant low temperatures. Several field observations of
Grylloblatta foraging activity have reported temperatures ranging from −2 to 7 °C (Campbell, 1949; Kamp, 1963; Schoville and Graening, 2013), while Grylloblatta choose temperatures between −3.5 and 5 °C in thermal gradient experiments (Mills and Pepper, 1937; Henson, 1957; Clusella-Trullas et al., 2011). An unusual aspect of the Grylloblatta thermal niche revealed in this study is the relationship between CT_{min} and SCP. Across all populations and life stages, and regardless of acclimation treatment, limits for the cessation of coordinated movement are virtually identical to the supercooling point – that is, individuals retain coordination until the point at which their body tissues freeze. Among insects for which both thresholds have been measured, few other examples like this are documented, though Sinclair et al. (2006) note that the sub-Antarctic Collembola Isotoma kovs-tadi often freezes while still active. More commonly, the SCP is several degrees (sometimes over 20 °C) lower than the CT_{min} (e.g., Klok and Chown, 2000; Terblanche et al., 2005).

Freeze tolerance and freeze avoidance are two alternative strategies for survival in sub-zero temperatures, with the latter considered to predominate in moderately cold and predictable thermal environments (Lee, 1991; Sinclair et al., 2003; Sinclair and Chown, 2005a). According to the definitions of these strategies, Grylloblatta are freeze-avoidant; activity is maintained below 0 °C and individuals are killed on freezing (Edwards, 1986; this study). Considering the convergence of CT_{min}, SCP, and the lower lethal temperature, and a thermal preference approaching 0 °C, our data support Edwards’ (1986) conclusion that Grylloblatta are physiologically specialized to a (moderately) cold and stable thermal environment. These features of Grylloblatta cold tolerance bear a close resemblance to the annelid ice worm, Mesenchytraeus solifugus, which is found in glacial ice in the northwest USA (Edwards, 1986). Both taxa maintain activity and complete their life cycles at temperatures close to 0 °C but have relatively high SCP/lethal points (~6.8 °C for ice worms) when compared with other freeze-avoidant arthropods (Edwards, 1986; Hoffmann et al., 2013). Napolitano and Shain (2004) found convergent shifts in energy metabolism among ice worms, fungi, bacteria and algae from glacial habitats, suggesting an important pathway for cold adaptation. To the best of our knowledge, similar shifts have not been detected in insects, but Grylloblatta appears to be a useful candidate system in which to explore specialization to persistent (rather than extreme) cold.

What are the implications of these results for predicting climate change responses in alpine insects? Improving our understanding of thermal limits in high-elevation insects could explain their limited geographical and elevational distributions, as well as the risks that high-elevation communities face from climate change (Mani, 1968; Pepin and Lundquist, 2008; Bässler et al., 2010). It is clear for Grylloblatta, at least, that microhabitat conditions, especially the availability of cold-temperature retreats, will be critical for long-term persistence. The thermal stability of their subterranean habitat is likely to be dependent on snowpack (Mani, 1968), which is declining in the mountains of western North America as a direct result of global warming (Mote et al., 2005; Casola et al., 2009). If Grylloblatta populations are presently isolated in disjoint microhabitats, their presumably low dispersal ability (flightlessness) combined with climate warming and/or drying may eventually lead to local extirpation and significant contraction of species ranges, some of which appear to be very restricted geographically.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.jinsphys.2015.04.014.

**References**


