

Inertia in physiological traits: *Embryonopsis halticella* caterpillars (Yponomeutidae) across the Antarctic Polar Frontal Zone

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Abstract

Geographic variation is characteristic of many physiological traits at the population and species levels. However, several recent studies have suggested that population-level variation is either limited or that it is mostly a consequence of phenotypic plasticity. Here we show that there is considerable physiological inertia in cold hardiness, upper thermal tolerance limits and desiccation resistance in caterpillars of the sub-Antarctic moth *Embryonopsis halticella* Eaton, such that populations from two climatically different islands are physiologically very similar. Both populations are moderately chill tolerant, with no difference in the supercooling points of caterpillars (−17 to −20 °C). Within their host plants caterpillars of both populations freeze at substantially higher, and statistically equivalent temperatures (−9.5 to −11.5 °C). The populations also have similar upper lethal limits (38 °C), and survival times of dry conditions (6–170 h depending on mass). The previously inexplicably low freezing point of caterpillars at the climatically less severe Marion Island seems likely a consequence of physiological inertia given that the freezing point of caterpillars within their hosts is only a few degrees below absolute minima at the older, and colder, Heard Island. Lack of adaptive geographic variation in physiological traits has consequences for models of range limits, and highlights the importance of exploring phenotypic plasticity as a response to climatic variation.

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1. Introduction

Geographic variation is a hallmark of environmental physiology. Most physiological traits display substantial variation between species and/or populations living in different areas (Spicer and Gaston, 1999), and such patterns have often been used as a basis for arguments in favour of the adaptive nature of physiological variation (see Bartholomew, 1987). In insects, geographic variation has been recorded for a wide variety of physiological traits between populations and species at several spatial scales, ranging from the microgeographic to the macrophysiological (Chown et al., 2004). These traits include metabolic rate (Massion, 1983; Schultz et al.,

1992; Addo-Bediako et al., 2002), water balance characteristics (Juliano, 1986; Hoffmann and Parsons, 1989; Gibbs et al., 1991), cold hardiness and low temperature tolerance (Parsons, 1977; Gibert and Huey, 2001; Williams et al., 2002; Sinclair et al., 2003), high-temperature tolerance (Addo-Bediako et al., 2000; Hoffmann et al., 2002), and development rate (Honěk, 1996). Such geographic variation has also routinely been documented for insects of the sub-Antarctic islands. Substantial differences have been reported between species and populations, both across islands which differ substantially in climate, and across altitudes on particular islands, in metabolic rates (Chown, 1997; Chown et al., 1997; Todd, 1997), thermal tolerances (Worland et al., 1992; van der Merwe et al., 1997), and water balance characteristics (Todd and Block, 1997; Chown and Klok, 2003). One of the most detailed

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studies to have made a thorough statistical comparison of variation within and among islands compared the critical thermal minima and maxima (temperatures of cold stupor and heat coma, respectively) of ten species and 31 populations from Marion and Heard Islands (Klok and Chown, 2003). Here, a small portion of the variation in critical thermal limits was found between species, and c. 22% of the variation was found between islands, which differ substantially in climate because of their position on either side of the Antarctic Polar Frontal Zone. These studies reconfirm the fact that geographic variation in physiological traits is as much a feature of insects as it is of other animals.

Despite the apparent ubiquity of geographic variation in physiological traits, not all species show such variation, and there are considerable differences between traits in the extent to which they vary geographically. In the former case, Hoffmann et al. (2001) examined variance partitioning of desiccation, starvation and cold resistance, body size and lipid content in *Drosophila melanogaster* among regions, populations, and strains in Australia. Differences between regions and populations accounted for only 0–28% of the variance in these traits, with strain and individual (including error) differences accounting for the bulk of the variance (71–97%). Similarly, despite the among species and among island differences, Klok and Chown (2003) also found that most of the variance in critical temperatures was explained by acclimation effects or by among individual (including error) differences (60–75%). Likewise, as far as differences between traits are concerned, it has long been appreciated that upper thermal limits tend to be less variable geographically and less responsive to acclimation than lower ones (see Kingsolver and Huey, 1998; Chown, 2001 for review and Hoffmann et al. (2002) for an alternative view).

Nonetheless, the prevalence of limited geographic variation in physiological traits, or physiological inertia (see Huey et al., 2003), is not clear. There are two reasons why this might be the case. First, much of the work on geographic variation in physiological traits has been done within a strong adaptationist framework (reviews in Bartholomew, 1987; Spicer and Gaston, 1999). Whilst the importance of evolution as a theme underlying all of biology means there are good reasons why this should be the case, this approach is also likely to lead an under-reporting of non-significant results, except perhaps where specific hypotheses (such as that of metabolic cold adaptation) are being tested (e.g. Nylund, 1991). This is a classic file-drawer problem (Csada et al., 1996). Second, the extent to which acclimatization might account for such geographic or interspecific variation, especially at the population level, is not widely explored, with the exception perhaps of studies of *Drosophila* (e.g. Watson and Hoffmann, 1996; Berrigan and Hoffmann, 1998; Hoffmann et al., 2001,

2005; Ayrinhac et al., 2004; but see also Gibbs et al., 1991). Thus, apparently adaptive variation might rather be a consequence of phenotypic plasticity. Here we address both issues by comparing thermal tolerance and desiccation resistance of caterpillars of the sub-Antarctic moth, *Embryonopsis halticella* (Yponomeutidae) from Marion and Heard Islands. These two islands are widely separated and have very different climates as a consequence of the fact that Marion Island lies to the north, and Heard Island to the south of a major climatic boundary in the Southern Ocean: the Antarctic Polar Frontal Zone (APFZ) (see Knox, 1994; Klok and Chown, 2003). Nonetheless, initial data suggested that the populations of the moth species differ only slightly in several of their physiological characteristics.

2. Materials and methods

2.1. Study animal and study sites

E. halticella Eaton is a flightless moth endemic to the South Indian Ocean Province Islands (Prince Edward Islands, Crozet archipelago, Kerguelen Islands, Heard Island) of the Southern Ocean (Crafford and Scholtz, 1986). Its larvae feed almost exclusively on the grass *Poa cookii*, with which they have an intimate association, starting as leaf miners in the early instars and later becoming concealed feeders (*sensu* Gaston et al., 1991). The species is most abundant in highly manured areas, and at Marion Island has a life cycle of approximately 1 year with overlapping generations (Crafford and Scholtz, 1986).

The climate on Marion Island (46°54'S, 37°45'E, 1230 m at its highest point) is cool, wet and windy with little seasonal temperature variation (monthly mean Stevenson Screen values vary between 2°C and 7°C over the year with a mean of approximately 5°C), though mean annual temperatures have increased by slightly more than 1°C over the past 50 years (Schulze, 1971; Smith, 2002). Microclimate (soil surface) temperatures are lower, with absolute temperatures varying between –4.8 and 29.2°C, and a mean of 5.7°C at 25 m above sea level (over a full year, data provided in Table 1 of Klok and Chown, 1998). Temperatures within *Poa cookii* tussocks and tillers are generally higher (in autumn, tussock: minimum –0.5°C and maximum 12.5°C; in summer tiller: minimum –1.2°C and maximum 33.8°C) (Klok and Chown, 1998) (see also Table 1). Heard Island (53°06'S, 73°30'E) lies south of the APFZ and rises to 2745 m a.s.l. It is approximately 80% glaciated, and, as a consequence of its southerly position has a much colder and more seasonal climate than Marion Island. An Argos transmitting automatic weather station showed that between 1997 and 2002 mean annual temperature at Atlas Cove was 2.0°C, with an

Table 1

Microclimate measurements in *Poa cookii* stands on Marion Island (from Klok and Chown, 1998) and Heard Island (October–December 2000)

Temperatures (°C)	Mean	Mean daily min.	Mean daily max.	Absolute min.	Absolute max.
Marion Island, Trypot Beach area, <i>P. cookii</i> stand surrounding an albatross nest					
Summer data ^a	9.4	1.5	21.7	−0.2	33.8
May 1997					
Air at 30 cm	6.3	2.4	11.0	−2.0	14.5
Within tussock	6.1	2.9	9.6	−0.5	12.5
Within tiller	6.0	2.5	10.1	−1.2	12.9
Heard Island—Atlas Cove, mixed <i>P. cookii</i> / <i>Pringlea antiscorbutica</i> stand, Mt. Aubert de la Rue					
Air				−5.9	17.2
Heard Island-Spit Bay					
Air				−5.9	18.1

^aSummer temperature data were extracted from Chown and Crafford (1992) from temperatures measured inside the bases of *P. cookii* tillers from November 1987 to March 1988.

absolute minimum of -7.6°C and an absolute maximum of 15.8°C . Although tiller temperatures have not been measured at Heard Island, microclimate temperatures within mixed *P. cookii*/*Pringlea antiscorbutica* stands indicate temperatures varying between -5.9°C and 18.1°C over the summer months (Table 1). Collection and laboratory protocols for caterpillars at Marion Island are described fully in Klok and Chown (1998).

Facilities at the field camp at Atlas Cove on Heard Island were basic. Here, host tillers were collected during mid-summer (December 2000–February 2001) from around the Atlas Cove camp or from mixed *P. cookii*/*P. antiscorbutica* stands at Mt. Aubert de la Rue and returned to the makeshift laboratory within an hour of collection. The same protocols as those used on Marion Island were followed to obtain caterpillars and caterpillars within tillers. These were subsequently held outside, but in a shaded area, against the wall of the makeshift laboratory (an abandoned building of the old scientific station) at natural day length (*c.* 16L:8D) and temperature (*c.* $1-4^{\circ}\text{C}$). As was the case at Marion Island, these ‘field-fresh’ caterpillars were used for experiments within 24 h of collection. Owing to the absence of reliable incubation facilities only a single acclimation trial was undertaken. Caterpillars were held at 0°C for 7 days by keeping them on melting ice in an insulated container at natural day length. During the acclimation period caterpillars were supplied with fresh filter paper (moistened) and grass cuttings every 3 days.

2.2. Thermal biology and desiccation resistance

The full protocols for the work at Marion Island are provided by Klok and Chown (1998). These protocols were followed at Heard Island as closely as possible, with only a few modifications to accommodate the less extensive facilities. For the determination of supercooling points (SCP, or temperature of crystallization)

the methods of Klok and Chown (1997, 1998) were followed. The SCPs of individual field-fresh caterpillars, caterpillars starved at 100% relative humidity for 7 days at ambient temperature outside the laboratory, caterpillars acclimated at 0°C , field-fresh caterpillars inside *P. cookii* tillers, and field-fresh caterpillars inside moist filter paper were determined. Individual caterpillars (field fresh and 0°C acclimated) were placed tightly in pipette tips with a 40 gauge copper-constantan thermocouple inserted between the specimen and the pipette tip wall. For caterpillars inside *P. cookii* tillers or moist filter paper the thermocouples were inserted between the caterpillar and the surrounding grass tissue or moist filter paper. The specimens in the pipette tips were placed in air-filled vials and submerged in a Grant LTC12 waterbath and cooled from 0°C at $0.1^{\circ}\text{C min}^{-1}$. The thermocouples were connected to a Campbell Scientific CR10 datalogger which measured temperatures every second and calculated and logged the mean for each specimen every 15 s. The temperature logged directly prior to the freezing exotherms was noted as the supercooling point (Lee, 1991). Mean SCP was calculated for each experimental group. However, because frequency distributions of SCPs generally revealed a non-normal (and perhaps bimodal, see also Klok and Chown, 1998) distribution of the data, means (\pm S.E.) were derived for each group (a high and a low group) and the distribution of specimens in the two SCP groups (using -15°C as the threshold), was expressed as the ratio $R = \text{LG}/(\text{HG} + \text{LG})$, where LG and HG are the numbers of specimens in each group, respectively (Block, 1982). Single classification analyses of variance (ANOVA) were used for comparisons among islands, or within islands among treatments for each of the low and high groups. In addition, because the bimodality of the data in the Heard Island population was not as clear as for the Marion Island population, the ANOVAs for the among island comparisons were also undertaken with the combined SCP data.

To determine upper and lower lethal temperatures similar protocols were followed on Marion and Heard Islands. For lower lethal limits, groups of ten caterpillars were placed in 1.5 cc eppendorf vials on a dry strip of filter paper, to absorb any potential condensation, and submerged in the Grant LTC12 waterbath. Caterpillars were kept for a 15 min equilibration period at 0 °C after which the temperature was lowered at 0.1 °C min⁻¹ until the first temperature, -6 °C, was reached. After 1 h, one vial was removed from the bath and the specimens were given 24 h to recover at laboratory temperature (8 ± 1 °C at Heard Island, 2 ± 0.5 °C at Marion Island). The temperature was then lowered 1 °C at 0.1 °C min⁻¹, and the above procedure repeated (Worland et al., 1992). This was continued to -25 °C. After 24 h, caterpillars were assessed for survival and only those with full locomotor function were considered survivors. These survivors were reassessed after seven days. To determine upper lethal temperatures, vials each containing ten caterpillars on *P. cookii* cuttings were warmed at 0.1 °C min⁻¹ to 30 °C (25 °C at Marion Island), where the experiment commenced. Ten caterpillars were removed after 1 h and the temperature raised by 1 °C at 0.1 °C min⁻¹ and the procedure repeated. This was continued to 40 °C (43 °C at Marion Island). Caterpillars were assessed for survival after 24 h at laboratory temperature, and survivors were reassessed after 7 days.

For the desiccation resistance trials, 40 field-fresh caterpillars were held for 24 h with access to water only at 8 ± 1 °C (2 ± 0.5 °C at Marion Island) to allow them to clear their digestive tracts. They were numbered, weighed (to the nearest 0.0001 mg using a Mettler UMT2 electronic microbalance) and placed in desiccation chambers containing silica gel at 0.66 kPa saturation deficit (5% relative humidity) at 8 ± 1 °C and natural day length (2 ± 0.5 °C; 9L:15D at Marion Island). The caterpillars were weighed every 12 h until 100% mortality. Mortality was assessed by turning the caterpillars on their backs and the inability to right themselves constituted death. Maximum tolerable water loss (mg), the percentage fresh mass lost (% FM), time

to maximum tolerable water loss (h), and rate of water loss expressed as % FM h⁻¹ and mg h⁻¹ were calculated from the mass recorded at the time interval directly preceding death in each caterpillar. These measures were used because water loss due to cuticular and respiratory transpiration could not be determined separately. The caterpillars produced no frass, therefore we assumed that mass loss was approximately equivalent to incidental and respiratory water loss, with a small effect of substrate loss owing to catabolism. To determine the effects of starvation, the same procedure was followed except that caterpillars were placed in chambers with moistened filter paper to maintain 100% relative humidity. This experiment was conducted at the same time, and with the same conditions (except for relative humidity) as the desiccation experiment, and the survival of all specimens were checked at the time of death of the last remaining caterpillar in the desiccation experiments. Comparisons among island populations were made using analyses of variance or covariance as appropriate.

3. Results

Throughout this section most attention is drawn to the new results for caterpillars on Heard Island (hereafter HI), and comparisons are then made with the population on Marion Island (hereafter MI), the results for which are reported in full by Klok and Chown (1998).

In the supercooling point experiments (Table 2) no caterpillars survived freezing. A single classification ANOVA of the SCPs of the field-fresh caterpillars and those acclimated at 0 °C indicated that the acclimation treatment had no significant effect on SCP ($F_{(1,54)} = 3.51$, $p = 0.067$). Frequency distributions of SCPs also indicated that caterpillars in both above groups showed non-normal distributions, that could be considered bimodal when compared with the MI population (see Fig. 1 in Klok and Chown, 1998). These distributions, when divided into high and low groups

Table 2

Summary statistics for the supercooling points (SCP) of *Embryonopsis halticella* caterpillars and their host, *P. cookii*, from Heard Island (see text for details)

SCP of	Mean ± S.E.	Minimum	Maximum	n
Field fresh caterpillars	-17.6 ± 0.9	-26.1	-5.2	40
Starved caterpillars	-15.4 ± 1.6	-25.0	-4.0	20
Caterpillars acclimated at 0 °C	-20.5 ± 1.1	-25.1	-7.3	16
Caterpillars within <i>P. cookii</i> tillers	-9.5 ± 0.7	-16.4	-3.1	25
Caterpillars within moist filter paper	-6.7 ± 0.5	-8.2	-3.2	10
Field fresh <i>P. cookii</i>	-12.8 ± 0.7	-19.7	-5.3	20
<i>P. cookii</i> containing caterpillars	-6.7 ± 0.5	-14.6	-2.5	25
Moist filter paper	-5.2 ± 0.3	-7.4	-3.4	20

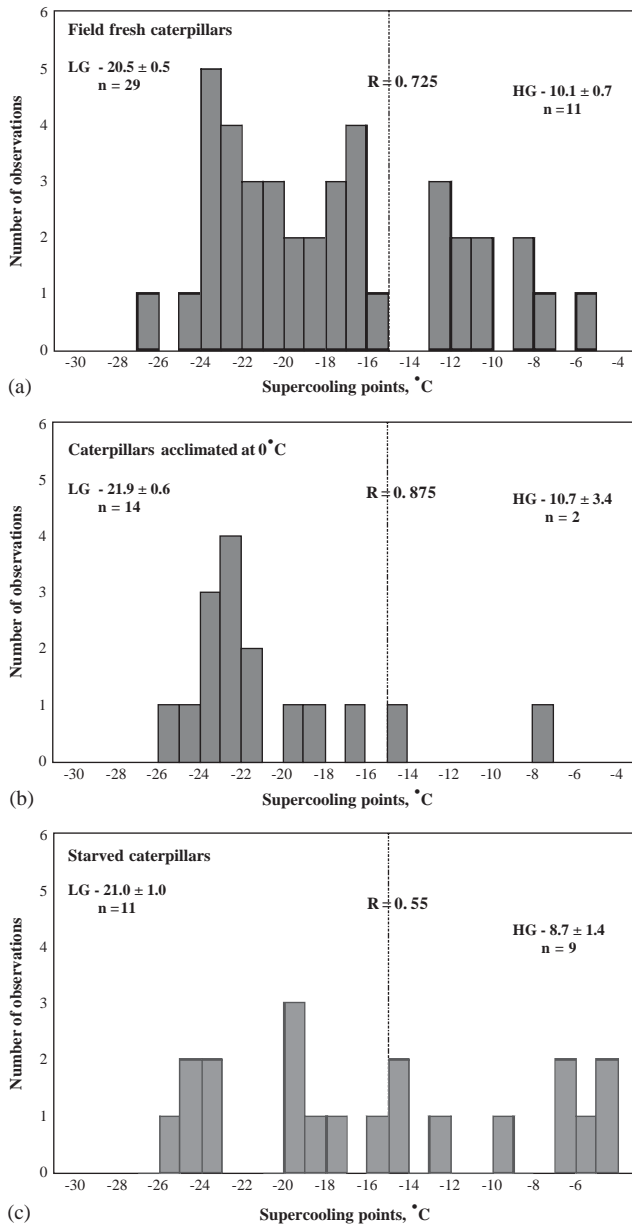


Fig. 1. Frequency distributions of supercooling points for *E. halticella* caterpillars from Heard Island: (a) field-fresh caterpillars, (b) caterpillars acclimated at 0°C, (c) caterpillars starved for 7 days. Means \pm S.E., sample size (n) for the high group (HG) and low group (LG) values and the relationship between these ($R = LG/(LG + HG)$) for each experiment are provided. The division between the HG and LG is -15°C . See text for further detail.

had similar R values ($R_{\text{FF}} = 0.725$ and $R_{0^\circ\text{C Acccl}} = 0.875$; see Fig. 1). There was no difference between the field-fresh and acclimation groups when high ($F_{(1,11)} = 0.069$, $p = 0.797$) and low ($F_{(1,41)} = 2.689$, $p = 0.1087$) group caterpillars were compared. The MI population likewise showed no difference in SCPs between field-fresh caterpillars and those acclimated to 2°C (Klok and Chown, 1998). At HI, there was no difference between the SCPs of field-fresh individuals and those that had been starved for 7 days ($F_{(1,58)} = 1.68$, $p = 0.20$).

Starved caterpillars also showed a somewhat bimodal SCP distribution although their ratio was more balanced between HG and LG ($R = 0.55$; Fig. 1). Once again this result is very similar to that found at MI, where SCPs did not differ between starved and fed groups. Indeed, these similarities are born out by direct comparisons of the LG and HG SCPs of the populations from each island. In no instances were there significant differences among the MI and HI populations for LG and HG field-fresh, low temperature acclimated, and starved caterpillars (Table 3). This was also the case for the low temperature acclimated caterpillars when the full distributions were compared. However, when this was done for the field fresh and starved caterpillars, the MI population had significantly lower SCPs than the HI population in both cases (Table 3).

As was the case for the MI experiments, on HI SCPs of caterpillars within *P. cookii*, and those of the grass itself, were readily distinguishable during assessments of the SCPs of caterpillars within the grass (Fig. 2). Because of its larger size, and hence larger absolute amount of water, the excised *P. cookii* leaf showed a broader exotherm than the caterpillar, making distinction between caterpillar and grass freezing points relatively easy. Moist filter paper in contact with caterpillars gave similar patterns. On HI, the SCP of the field-fresh *P. cookii* was significantly lower than that of *P. cookii* containing caterpillars (Table 2, $F_{(1,43)} = 29.59$, $p < 0.0001$). Field-fresh caterpillars froze at significantly lower temperatures than did the caterpillars within the *P. cookii* tillers or in contact with moist filter paper, but the latter two groups did not differ significantly ($F_{(2,72)} = 37.50$, $p < 0.0001$; Tukey's Honest Significant Difference). No significant relationship was found between the freezing point of *P. cookii* and that of the caterpillars ($F_{(1,23)} = 1.97$, $p = 0.174$), but there was a significant relationship between the freezing point of *P. cookii* and the difference in freezing point of *P. cookii* and the freezing point of the caterpillar (Difference = $6.918 + 0.622 P. cookii$ SCP, $r^2 = 0.188$, $F_{(1,23)} = 5.333$, $p < 0.03$). This suggests that the influence of the host on the SCP of the caterpillars becomes more marked at lower temperatures. These results show both similarities and differences to those of the Marion Island population. In general, caterpillars of both populations show an increase in SCP when held within tillers, and the effect of the host becomes more pronounced at low temperatures in both cases. However, the host SCP was much lower on HI than on MI when free of caterpillars, and the effect of caterpillars on the host was stronger on HI than it was on MI (Table 3). Although the high and low group SCPs differed among island populations for the caterpillars within host tillers, the magnitude of these differences was relatively small, and this remained the case when the entire distributions were analysed (Table 3).

Table 3

Outcomes of the statistical comparisons of supercooling points of several categories of *E. halticella* caterpillars and their *P. cookii* host plant from Marion Island (autumn 1997) and Heard Island (summer 2000)

Categories	Mean SCP \pm S.E.		ANOVA		
	Marion	Heard	F-ratio	df	p
<i>Low groups</i>					
Field fresh	-20.9 ± 0.6	-20.5 ± 0.5	0.933	1, 50	0.339
Low temp. accl. ($2^\circ\text{C}^{\text{Marion}}$; $0^\circ\text{C}^{\text{Heard}}$)	-20.5 ± 0.6	-21.9 ± 0.6	2.485	1, 28	0.126
Starved caterpillars	-22.1 ± 0.7	-21.0 ± 1.0	0.844	1, 26	0.367
Caterpillars within <i>P. cookii</i> tillers	-15.4 ± 0.1	-16.4 ± 0.1	75.797	1, 5	<0.0003
<i>High groups</i>					
Field fresh	-12.9 ± 1.2	-10.1 ± 0.7	2.362	1, 11	0.153
Low temp. accl. ($2^\circ\text{C}^{\text{Marion}}$; $0^\circ\text{C}^{\text{Heard}}$)	-12.7 ± 1.0	-10.7 ± 3.4	0.700	1, 4	0.459
Starved caterpillars	-11.6 ± 2.3	-8.7 ± 1.4	1.199	1, 10	0.299
Caterpillars within <i>P. cookii</i> tillers	-10.7 ± 0.6	-8.5 ± 0.5	7.967	1, 41	<0.007
<i>Entire distribution</i>					
Field fresh	-20.5 ± 0.9	-17.6 ± 0.7	5.856	1, 63	0.018
Low temp. accl. ($2^\circ\text{C}^{\text{Marion}}$; $0^\circ\text{C}^{\text{Heard}}$)	-19.0 ± 0.9	-20.5 ± 1.1	1.141	1, 34	0.293
Starved caterpillars	-20.5 ± 1.4	-15.4 ± 1.4	6.776	1, 38	0.013
Caterpillars within <i>P. cookii</i> tillers	-11.5 ± 0.6	-9.5 ± 0.6	4.738	1, 48	<0.035
Field fresh <i>P. cookii</i>	-9.4 ± 0.9	-12.8 ± 0.7	6.602	1, 38	<0.014
<i>P. cookii</i> containing caterpillars	-9.3 ± 0.6	-6.7 ± 0.5	9.071	1, 48	<0.004

Due to the bimodality of the SCPs, comparisons were made between low groups and high groups (see text for details).

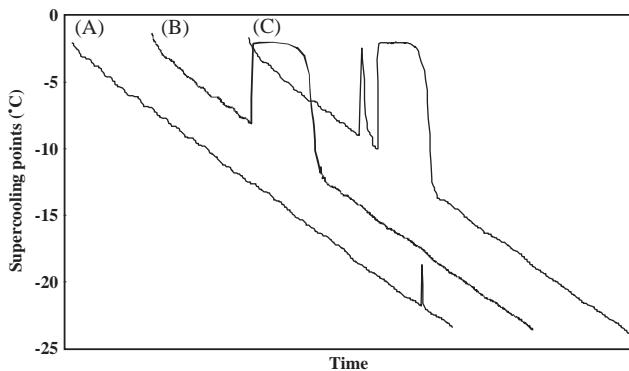


Fig. 2. Three samples of temperature recording traces of supercooling experiments (temperature ramp of 0.1°C in each case). Trace A represents the temperature recording of a single *E. halticella* caterpillar. The small peak at the lower end of the trace is the exotherm. Trace B represents the temperature recording for a cutting of *P. cookii*. Note the broadened exotherm. Because the grass cutting is much larger than the caterpillar there is a greater volume of fluid to undergo the phase transition from liquid to solid and, as a result, a greater quantity of latent heat is released over a longer time during the freezing of grass. Trace C represents the temperature recording of both a cutting of *P. cookii* and an embedded caterpillar. Both the grass and the caterpillar were in contact with the thermocouple. Because of the distinctive exotherms given by the grass and the caterpillar (A and B) the respective supercooling points can readily be distinguished.

In the lower lethal limits experiments caterpillar mortalities on Heard Island corresponded closely to the distributions of the SCP values for field-fresh caterpillars (Fig. 3a). There was a distinct drop in survival from -12°C (although the groups at -16°C only had 40% mortality) reaching 0% survival from -20°C onwards. This suggests that over a short

timescale there are no prefreeze mortalities in *E. halticella* caterpillars and that the SCP represents their lower lethal temperature. In the determination of upper lethal temperatures, survival dropped markedly above 38°C (Fig. 3b). These results could not be compared statistically with those from the MI population. However, the figures indicate that the upper lethal temperatures are virtually identical for both populations, and that LT100 values for lower lethal temperatures are also the same. However, there is some variation in the way that the LT100 values are approached, with the Heard Island individuals having a lower initial survival than Marion Island individuals at the same temperatures.

Summary statistics for maximum tolerable water loss, rate of water loss and time to maximum water loss for the HI population are provided in Table 4. The caterpillars lost approximately a third of their body mass over a large range of survival times. Rate of water loss, maximum tolerable water loss, and time to maximum water loss were significantly related to initial body mass (Table 4). All caterpillars in the starvation experiment were still alive at the end of the desiccation experiment at 175 h. By comparison with the MI population, the HI caterpillars used for the water loss experiments were generally larger (5.27 ± 0.77 vs. 1.43 ± 0.18 mg, respectively; $F_{(1,77)} = 19.9$, $p < 0.0001$), perhaps as a consequence of differences in seasonal responses (see Section 4). In consequence, maximum tolerable water loss was greater for the HI population (ANCOVA $F_{(1,73)} = 5.127$, $p < 0.027$), but so was water loss rate (ANCOVA $F_{(1,73)} = 5.00$, $p < 0.028$). However, the upshot was that the most important determinant of

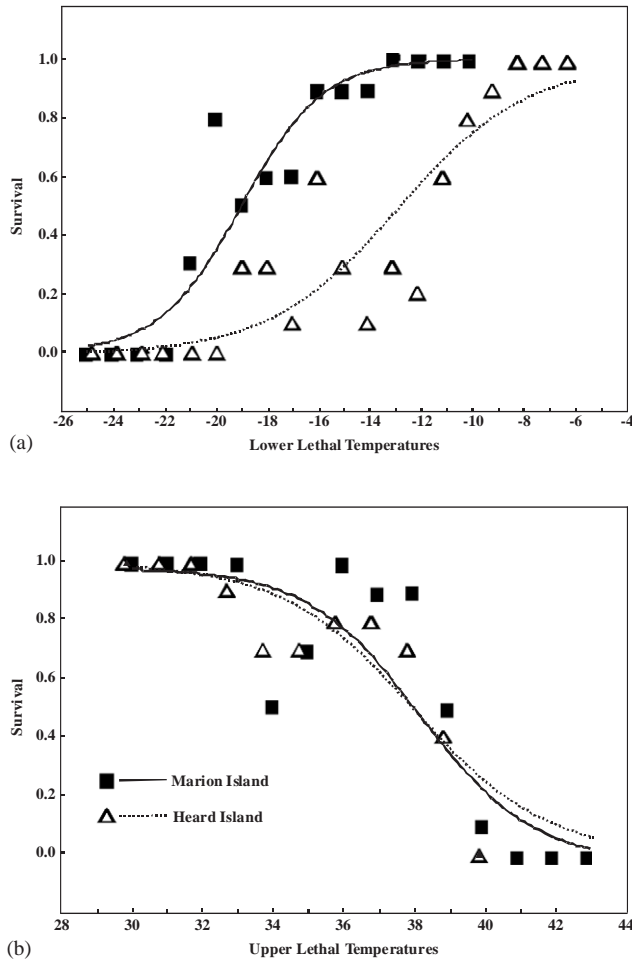


Fig. 3. The decline in survival at a. lower and b. upper lethal temperatures (in °C) for *E. halticella* caterpillars. The fitted lines are logistic regression curves (all significant at $p < 0.05$), with the following equations: LLT Heard: Mortality = $\text{EXP}(-5.1156 - 0.39696 * \text{LLT}) / (1 + \text{EXP}(-5.1156 - 0.39696 * \text{LLT}))$; LLT Marion: Mortality = $\text{EXP}(-11.993 - 0.63047 * \text{LLT}) / (1 + \text{EXP}(-11.993 - 0.63047 * \text{LLT}))$; ULT Heard: Mortality = $\text{EXP}(-20.301 + 0.535821 * \text{ULT}) / (1 + \text{EXP}(-20.301 + 0.535821 * \text{ULT}))$; ULT Marion: Mortality = $\text{EXP}(-23.75 + 0.622562 * \text{ULT}) / (1 + \text{EXP}(-23.75 + 0.622562 * \text{ULT}))$.

desiccation resistance, survival time, did not differ among the populations (ANCOVA $F_{(1,73)} = 3.27$, $p = 0.075$). The temperature difference between the trials on the two islands (6 °C) therefore had little effect on survival time (even taking differences in mass of the caterpillars into account).

4. Discussion

The cold hardiness strategy of *E. halticella* on HI appears to be identical to that of the population on MI. Both populations are freeze avoiding and over the short term show no prefreeze mortality. In Bale's (1993, 1996) terminology they can be considered chill tolerant, and perhaps moderately so. Although the investigations of acclimation effects were in no way as extensive on HI as they were on MI, owing to poor laboratory conditions on the former, the low-temperature acclimation trial gave identical results for both populations. Thus, it seems unlikely that acclimatization to the coming winter in the MI population (the work was undertaken in late autumn (April–May) for that population, see Klok and Chown, 1998), but summer acclimatization in the HI population (the experiments were done between December and February) could explain the absence of differences between the populations. It is well known that there is a pronounced cold hardening response by freeze avoiding insects to approaching winter conditions, which is usually accompanied by lowered freezing points and changes in body chemistry (especially upregulation of cryoprotectants) (reviews in Lee and Denlinger, 1991; Chown and Nicolson, 2004). However, the data from the field-fresh and low temperature acclimated populations suggest that both populations maintain substantial cold hardiness irrespective of the season. Of course, a fairer comparison might have been one made between summer-acclimatized individuals

Table 4

Summary statistics for starting mass, maximum tolerable water loss, rate of water loss and time to maximum water loss in the desiccation experiment, and the relationships between initial mass and maximum tolerable mass loss, rate of water loss and survival time, in *E. halticella* caterpillars from Heard Island

Variable	Mean \pm S.E.	Minimum	Maximum	n
Starting mass (mg)	5.2698 \pm 0.7681	0.3690	13.4980	40
Maximum tolerable loss (mg)	1.8816 \pm 0.2901	0.0320	4.6330	37
Maximum tolerable loss (%)	30.56 \pm 1.37	8.67	45.83	37
Rate (mg h ⁻¹)	0.0359 \pm 0.0115	0.0034	0.4300	37
Rate (% h ⁻¹)	1.22 \pm 0.25	0.19	6.54	37
Time (h)	73.95 \pm 10.04	6.0	175.0	37
Regression analyses				
Mass loss = 0.35–0.072 starting mass	$r^2 = 0.967$	$p < 0.0001$	$F_{(1,35)} = 1035.3$	
Rate = 0.0173 + 0.003 starting mass	$r^2 = 0.057$	$p < 0.153$	$F_{(1,35)} = 2.1$	
Time = 16.69 + 10.3 starting mass	$r^2 = 0.694$	$p < 0.0001$	$F_{(1,35)} = 79.24$	

from both islands. Although such data are not available for Marion Island, the SCPs of caterpillars from the 15 °C acclimation trial on MI did not differ from field-fresh individuals on HI (All SCP values: $F_{(1,58)} = 1.72$, $p = 0.195$, mean \pm S.E. MI: -15.5 ± 1.4 °C, HI: -17.6 ± 0.9 °C; High group values: $F_{(1,19)} = 0.0017$, $p = 0.968$, MI: -10.1 ± 0.7 °C, HI: -10.1 ± 0.7 °C; Low group values: $F_{(1,37)} = 0.0273$, $p = 0.605$, MI: -21.0 ± 0.9 °C, HI: -20.5 ± 0.5 °C). However, these results belie the movement of individuals between the high and low groups. In this particular instance for the 15 °C acclimation trial on MI, there were 10 individuals in each group, whereas in the field-fresh individuals on HI, there were 11 individuals in the high group and 29 in the low group. This suggests that if there were to be pronounced differences between the populations, they would be found in the proportion of individuals in the high and low groups, given that there is such malleability in movement between the groups (Klok and Chown, 1998). The analyses of the full SCP distributions and those of the high and low groups for the field fresh and starved caterpillars lend support to this idea. For both the field fresh and starved caterpillars the high and low groups did not differ between the populations, but when the entire distributions were analyzed the populations differed substantially, mostly as a consequence of larger numbers of individuals in the high group on Heard Island (compare Fig. 1. here with Fig. 1 of Klok and Chown, 1998).

Acknowledging that the two studies were undertaken at different times of the year, and that the acclimation trials were not as sophisticated as they might have been at Heard Island, these results nonetheless suggest that differences between the populations are mostly a consequence of phenotypic plasticity, rather than strict adaptation. This is likewise reflected in the differences in the shape of the lower lethal temperature curve (Fig. 3a). It is also in keeping with findings for weevils across the two islands (Klok and Chown, 2003), with large-scale latitudinal variation in low temperature recovery times in *D. melanogaster*, which appear to be a consequence of plasticity rather than adaptation (Ayrinhac et al., 2004), and with other traits in the latter species (Hoffmann et al., 2001, 2005).

Although bimodality of SCPs in the HI caterpillar population was not as clear as that on MI, the distributions were distinctly non-normal, and certainly resembled the bimodality in the MI population. Quite why there should be animals with 'high' and those with 'low' SCPs has long been a subject of contention in the literature (Baust and Rojas, 1985; Parish and Bale, 1990; Sømme and Block, 1991), and is thought by some to be a consequence of gut evacuation and dehydration. By contrast, based on little difference between the SCPs of starved and fed individuals on MI, as was found again for the HI population, and on the habit of the

caterpillars of retaining food in their guts for long periods (Crafford and Scholtz, 1986), Klok and Chown (1998) implied that the SCP division into two groups might represent some kind of physiological bet-hedging strategy. In the context of the unpredictable and generally cold climates of the sub-Antarctic (Sinclair et al., 2003), such a strategy does seem plausible (see also Bale et al., 2001). However, Klok and Chown (1998) also noted that the cold hardiness of the caterpillars was considerably better developed than might be expected from microclimates at MI (see their Tables 1 and 7, and Table 1 here), and attributed this considerable cold hardiness to the accumulation of solutes that might assist in making this species so highly desiccation resistant. Although bet-hedging seems unlikely given the latter, Sinclair and Chown (2002) demonstrated that the haemolymph osmolality of *E. halticella* (mean: 358–370 mOsm) is no different to that of most other insects, and questioned the interpretation provided by Klok and Chown (1998), leaving the problem of considerable cold hardiness in *E. halticella* unresolved.

The present data from HI go some way to providing a solution. As was the case on MI, the SCPs (and hence the lower lethal temperatures) of the caterpillars were substantially higher when caterpillars were ensheathed within their host leaves. Moreover, it is clear that the lower the temperature at which the host freezes, the more likely it is to inoculate freezing in the caterpillars in both populations, with equivalence at approximately -10 °C for the HI population. Thus, caterpillars within leaves freeze at approximately -9.5 °C at HI, and are very likely inoculated by their hosts at temperatures below this value. These temperatures are reasonably close to the microclimatic limits found at Heard Island. Although long-term climatic data are not available, what records there are demonstrate that absolute minimum air temperatures can reach -8.4 °C in the summer months, and can decline to -5.9 °C even within *P. cookii* tussocks (Table 1). From this perspective, the tolerance limits of *E. halticella* (Table 2) seem readily explicable, given that they closely match those of the extremes of their natural environment, i.e. the likely situation within *P. cookii* tillers.

If it is presumed that the physiological tolerances of *E. halticella* are conservative, as seems likely given similarities in cold hardiness, upper lethal limits and desiccation resistance of the MI and HI populations, then the extreme cold hardiness of the population at MI might simply be a consequence of physiological inertia (Huey et al. (2003) provide a discussion of drive vs. inertia in evolution), or a lack of change in their tolerances since arriving at the island (there is no evidence for the occurrence of *P. cookii* at Marion Island during the last glacial maximum, $18,000 \pm 2000$ years before present, Scott, 1985). Such an explanation based on inertia presumes that the population of

E. halticella on MI is more derived than the one on HI. Although no phylogeographic data for this species are available, geological and biogeographic studies indicate that this is likely to be the case. Not only is Marion Island geologically much younger (<0.5 Ma) than the islands on the Kerguelen Plateau (>40 Ma) (Heard and Kerguelen) (LeMasurier and Thomson, 1990; McDougall et al., 2001; Wallace et al., 2002), but its biota is also clearly derived from that group (Kuschel and Chown, 1995; Greve et al., 2005).

Thus, it seems likely that the extreme cold hardiness of the population on MI is a consequence of physiological inertia, rather than of the extreme desiccation resistance of the species. Although this considerable desiccation resistance is not in question, and is plausibly a response to high saturation deficits and a lack of boundary conditions owing to high winds (Klok and Chown, 1998 provide a comprehensive explanation), it now seems clear that it has had little effect on the cold hardiness of the caterpillars, and the two traits are probably not as closely linked as was suggested by Klok and Chown (1998). The bet-hedging strategy, whereby individuals are capable of altering their SCPs, thus remains plausible, but to gain support it would need considerably greater exploration in the context of seasonal variation at either of the islands, as has been done for several other insects (e.g. Chen et al., 1991; Sinclair, 1997; Bale et al., 2000, 2001). The question of the extent of physiological inertia also deserves further scrutiny to ascertain how common it is in other traits in this species. It certainly does not appear to extend to life history traits in *E. halticella*, given that the population at MI appears to have overlapping generations, whilst the one at HI appears to be more synchronized. Nonetheless, it may be characteristic of other physiological variables such as metabolic rates and critical thermal limits.

In conclusion, this study has demonstrated that there is considerable inertia in cold hardiness, upper lethal limits and desiccation resistance of populations of *E. halticella* across two islands that differ considerably in their climates. This finding is in keeping with at least one other group in the region (Klok and Chown, 2003), and is similar to recent findings for *D. melanogaster* (Hoffmann et al., 2001; Ayrinhac et al., 2004), where population differences are largely a consequence of phenotypic plasticity (but see also Hoffmann et al., 2005). The prevalence of such a lack of adaptive geographic variation at the population level needs to be more widely explored. It is in keeping with several models of the way in which limits to species ranges are set (which presume that gene flow reduces adaptive differences between populations—review in Butlin et al., 2003), and also suggests that phenotypic plasticity plays an important role by enabling populations to survive better under marginal conditions (see also Watson and

Hoffmann, 1996; Hoffmann and Parsons, 1997; Klok and Chown, 2003).

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