Life stage-related differences in hardening and acclimation of thermal tolerance traits in the kelp fly, *Paractora dreuxi* (Diptera, Helcomyzidae)

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ABSTRACT

It is widely appreciated that physiological tolerances differ between life stages. However, few studies have examined stage-related differences in acclimation and hardening. In addition, the behavioural responses involved in determining the form and extent of the short-term phenotypic response are rarely considered. Here, we investigate life stage differences in the acclimation and hardening responses of the survival of a standard heat shock (SHS) and standard low temperature (or cold) shock (SCS), and the crystallization temperature (or supercooling point, SCP) of adults and larvae of the sub-Antarctic kelp fly, *Paractora dreuxi*. These stages live in the same habitat, but differ substantially in their mobility and thus environmental temperatures experienced. Results showed that neither acclimation nor hardening affected the lower lethal limits in larvae or adults. Adults showed an increase in survival of upper lethal limits after low temperature acclimation, whilst larvae showed a consistent lack of response. The acclimation × hardening interaction significantly affected the SCP in adults, but no response to either acclimation or hardening was found in the larvae. This study further demonstrates the complexities of thermal tolerance responses in *P. dreuxi*.

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1. Introduction

Acclimation and hardening responses in insects have recently become subjects of increasing interest in the literature. An ability to acclimate and/or to harden have not only been shown to increase survival (Rako and Hoffmann, 2006; Jensen et al., 2007), but also to improve reproductive potential (Shreve et al., 2004), locomotion (Deere and Chown, 2006) and shock recovery (David et al., 1998; Rako and Hoffmann, 2006). Enhanced survival or performance following acclimation and/or hardening (Chown and Nicolson, 2004) is underpinned by a wide array of biochemical mechanisms (Storey and Storey, 2005), although mostly at some metabolic cost (Hoffmann, 1995).

The extent of the response to hardening (short exposures (hours) to moderately high or low temperatures) and to acclimation (exposure over several days) can differ markedly within a species depending on the traits involved (Deere et al., 2006; Slabber et al., 2007; see Hoffmann et al., 2003 for hardening and acclimation definitions). Generally, acclimation responses in cold tolerance traits are more pronounced than those in heat tolerance traits (Kingsolver and Huey, 1998; Klok and Chown, 2003; Slabber

and Chown, 2005; Terblanche and Chown, 2006), although exceptions to this trend have been found in several species, including moths and flies (Klok and Chown, 1998; Hoffmann et al., 2003; Sinclair and Chown, 2003). Nonetheless, it is clear that considerable variation in the responses to acclimation of different thermal tolerance traits exists, which likely also reflects genetically based differences in these traits (Rako et al., 2007).

It is also widely appreciated that physiological tolerances differ depending on life stage. For example, several studies have demonstrated pronounced differences in response to low and to high temperatures among non-diapausing life stages (e.g. Levins, 1969; Czajka and Lee, 1990; Vernon and Vannier, 1996; Feder et al., 1997a; Klok and Chown, 2001; Lee et al., 2006; Rinehart et al., 2006; Cho et al., 2007, but see also Klok and Chown, 2000). However, fewer studies have examined stage-related differences in acclimation and hardening. Where such work has been done (e.g. Loeschcke and Krebs, 1996; Slabber and Chown, 2005; Lee et al., 2006; Powell and Bale, 2006; Jensen et al., 2007) variation in responses among life stages is the norm, although the form and extent of the life stage-related differences in response varies among species. For example, pronounced differences in hardening among adults and larvae were found in the Antarctic midge *Belgica antarctica* (Lee et al., 2006) and among adults and nymphs of the grain aphid *Sitobion avenae* (Powell and Bale, 2006), whilst almost no differences in acclimation response were found among adults and larvae of the rove beetle, *Halmaeus atriceps* (Slabber and Chown, 2005). Clearly, differences in acclimation and/or hardening

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might be expected for diapause vs. non-diapause individuals, or among stages that differ in the extent to which they overwinter (see e.g. Leather et al., 1993; Flannagan et al., 1998; Rinehart and Denlinger, 2000; Hayward et al., 2005).

By contrast, it is not known whether consistent, life stage-related differences in hardening or acclimation among species might exist where neither diapause nor quiescence are involved, nor what factors might underlie such differences. Obviously some trade-off between habitat use (or habitat selection by females for their offspring) and behavioural responses (including mobility) must be involved in determining the form and extent of the short-term phenotypic response (see e.g. Huey, 1991; Feder et al., 1997b; Huey et al., 2003), and it could only be similarities in this trade-off that might effect consistent stage-related differences in phenotypic plasticity among species. For example, leaf-mining or fruit-dwelling insect larvae might be expected to show a considerably greater basal tolerance than their free-living adults owing to regular exposures to extreme temperatures. Alternatively, behavioural flexibility on the part of the adults might preclude the necessity for well-developed phenotypic plasticity. Such differences will likely also vary among the traits investigated, since recovery from coma or critical limits to activity might be more significant for adult fitness, whilst absolute lethal limits might be more significant for fitness in less mobile larvae (Chown and Nicolson, 2004), and indeed these may be considered different traits (see below). However, determining whether this is the case requires explicit documentation not only of hardening and acclimation responses among life stages and traits (e.g. Deere et al., 2006; Jensen et al., 2007), but also careful consideration of differences in behavioural responses and environmental conditions faced by the different life stages (e.g. Lee et al., 2006). Only once such information is available for a reasonably wide range of species, traits and environments could any generality be sought, and in consequence tests be undertaken of hypotheses concerning the conditions under which different forms of plasticity are most likely to evolve (see Chown and Terblanche, 2007; Ghalambor et al., 2007 for review).

Here, we contribute to this requirement by investigating life stage-related differences in the acclimation and hardening responses of the survival of a standard heat shock (SHS) and standard low temperature (or cold) shock (SCS), and the crystallization temperature (or supercooling point, SCP) of a sub-Antarctic kelp fly, *Paractora dreuxi*, in which adults and larvae live in the same habitat, but differ substantially in mobility (Crafford, 1984; Crafford and Scholtz, 1987; Marais and Chown, 2008). In doing so we broaden considerably the range of treatments over which this species was previously investigated (Terblanche et al., 2007), demonstrating that responses vary amongst traits, and are more consistent among life stages for some traits (SCS, SCP) than for others (SHS).

This work complements a recent study of life stage differences in the effects of acclimation on chill coma recovery in the same species (Marais and Chown, 2008), specifically by focussing on survival of standardized high and low temperatures. Much evidence now supports the contention that knockdown traits, such as knockdown temperature or chill coma recovery, might differ substantially from survival traits, such as survival of a given heat or cold shock. Not only have clear experimental differences in these traits and their responses to acclimation, hardening and selection been demonstrated, especially at the intraspecific level (see Hoffmann et al., 2003; Chown and Nicolson, 2004; Rako and Hoffmann, 2006), but evidence is also accumulating to demonstrate that the traits may be genetically independent (Rako et al., 2007). In consequence, whilst the set of trials reported here is superficially similar to those concerning chill coma recovery undertaken by Marais and Chown (2008), in our view they represent examination of a different set of

physiological features. Such a view would be demonstrably incorrect if the responses of the two sets of traits (knockdown and survival) proved to be similar.

2. Methods

2.1. Study site and animal collection

Adults (mass c. 15–40 mg) and final instar larvae (mass c. 40 mg) of the endemic kelp-fly, *P. dreuxi mirabilis* Séguy (Diptera, Helcomyzidae), inhabit pebbled beaches on sub-Antarctic Marion Island (46°54S 37°45E), closely associated with decaying bull-kelp (*Durvillaea antarctica*) and decomposing organic matter (Crafford and Scholtz, 1987). The larval duration is approximately 2 months, the pupal stage lasts for 30–60 days, while the adult lifespan is 14–21 days (Crafford, 1984). Although brachypterous, the adults are exceptionally mobile, and are capable of rapid vertical movement through the volcanic, pebble substrate on which they are typically found. By contrast, larvae are much less active and tend to remain on decaying kelp fronds, until just prior to pupariation (Crafford, 1984; Crafford and Scholtz, 1987; Klok and Chown, 2001). Adults and larvae usually avoid the intertidal zone but can be found occasionally in the splash zone (upper eulittoral zone) 3–7 m from the shoreline (Crafford and Scholtz, 1987).

The physiological responses of this species to temperature and to water availability have previously been examined especially in the context of likely differences between adults and larvae (Klok and Chown, 2001; Terblanche et al., 2007). Larvae are moderately freeze tolerant, freezing between c. -2.8°C and -10.6°C , depending on their physiological state and the state of the environment (wet or dry), whilst adults freeze between -4.7 and -15.0°C , with no survival beyond the freezing point. Survival in larvae declines substantially between 31 and 34°C , whilst in adults the change occurs over a somewhat narrower range of temperatures (Klok and Chown, 2001; Terblanche et al., 2007). Adults are more desiccation resistant than the larvae, although both lose water at relatively high rates, and larvae are clearly capable of considerable osmoregulation (Klok and Chown, 2001).

For this investigation, larvae and adults were collected using soft tweezers and an aspirator, respectively, from Trypot Beach on the east coast of Marion Island during the April 2005 and 2006 relief periods in the late austral summer/early autumn (similar to the methods described in Klok and Chown, 2001). Individuals were kept in 500 ml plastic jars, on small pieces of kelp, at low density, and returned to the island laboratory within 2 h. The Trypot Beach site is less than 2 km from the laboratory and main weather station at the island, where temperatures just below the soil surface have been logged since June 2002 (Deere et al., 2006). Nonetheless, for the duration of one sampling period, an eight channel SQ800 Squirrel (Grant Instruments, UK) data logger was placed on Trypot Beach to record microclimate temperatures at a range of substrate depths. The data logger was set to log average temperature for 5 min intervals using temperature readings obtained by 30 s sampling. Type T 36-gauge thermocouples were connected to the data logger and thermocouple tips were attached to a pole every 10 cm. The pole with thermocouples attached was buried to 40 cm below the beach surface. Thermocouples were attached to the remaining channels of the logger and placed on top of and under round pebbles (roughly 15–20 cm diameter) at surface level to obtain an estimate of surface temperature (see also Marais and Chown, 2008).

2.2. Acclimation treatments

Individuals were returned to the laboratory where they were sorted for acclimations or experimental use immediately (usually <2 h). In the laboratory, the animals were kept in

temperature-controlled cabinets (0, 5, 10, 15 °C) with a photo-period set to match environmental day light cycles at the time of the year that the study was conducted (12:12 L:D). Temperature within the climate chambers was measured with thermochron iButtons (Model DS1921, Dallas Semiconductors, Texas) sampled at hourly frequencies during the 6-day acclimation period. Acclimation groups were staggered over consecutive days in a random order due to equipment constraints and all experiments were completed within a 3-week period during each of the years. Acclimated animals were stored in 500 ml plastic containers at low density (adults: $n = 25/\text{container}$; larvae $n = 50/\text{container}$) with moistened, fresh kelp. Kelp was replaced or added, moistened with fresh water, and containers were randomized between shelves on a daily basis. A total of 400 flies and 400 larvae were acclimated at each temperature (total $n = 1600$ per life stage). Acclimation-related mortality was estimated as the proportion of flies which died during this period.

2.3. Hardening pre-treatments

Adults and larvae from the four acclimation groups were used for experiments exploring survival at lethal temperatures and for determining SCPs. Prior to these assessments, individuals were exposed to acute, non-lethal temperatures of: 20 or 25 °C (warm), 10 °C (moderate), or –4, –2 or 0 °C (cold) temperatures for 2 h. Climate chambers were used for the 25, 20 and 10 °C treatments, while a Grant LTC 12 (Grant Instruments, UK) programmable water bath was used for the –4, –2 and 0 °C treatments. Replicated polypropylene vials (40 ml) containing 8–10 individuals per vial were used for the hardening pre-treatments.

2.4. Supercooling point determinations

Only individuals from the 0, 5 and 10 °C acclimations were used for SCP determinations because of mortality at 15 °C. A single fly or larva was placed into a 1.0 or 1.5 ml eppendorf vial, respectively, in contact with a Copper-Constantan (Type T) 40-gauge thermocouple and held in place with cotton-wool and elastic putty. Sixteen thermocouples were attached to two, eight-channel, PC-08 (Picotech, UK) data loggers connected via RS-232 serial ports to a standard desktop computer. Data were logged using Picotech PicoLog software and extracted via Microsoft Excel. Adults and larvae were cooled at 0.1 °C/min (to be consistent with a previous study of this species—Klok and Chown, 2001), using a Peltier-cooling module as described in Sinclair et al. (2003). Temperatures

of crystallization were derived from the data files as the temperature recorded just prior to the upward inflection of temperature caused by the release of latent heat of crystallization.

2.5. Survival of lower and upper lethal temperatures

For these experiments, eight individuals (either adults or larvae) were placed into a plastic vial, with three (survival of cold shock – SCS) and five (survival of heat shock – SHS) replicates for each pre-treatment from every acclimation, for each stage, with the exception of the 15 °C acclimation where fewer individuals were available. Indeed, mortality during the 15 °C acclimation was high, and in consequence the 20 and –2 °C pre-treatments were not investigated for the 15 °C acclimation. After individuals were placed into the vials, the vials were placed into a plastic bag, which was then submerged in the water bath or placed into a temperature-controlled cabinet for 2 h at the respective pre-treatment temperatures. An iButton datalogger was also placed into a plastic vial during each pre-treatment to ensure that the pre-treatment temperature was reached and maintained inside the bag. After 2 h, the bag containing the vials was placed directly into another water bath for a further 2 h. The second water bath was set at either 33.5 °C (SHS) for both larvae and adults, or –7.6 and –11.0 °C, respectively, for larvae and adults (SCS). Thereafter, individuals were returned to 10 °C in their plastic vials and survival was scored after 24 h.

2.6. Statistical analyses

Supercooling point distributions were examined for bimodality prior to analyses because such distributions are often common for SCPs (Block, 1990). Due to the generally normal form of the SCP frequency distributions (33/36 examined showed $p > 0.05$ using a Shapiro–Wilks test for normality) acclimation treatments were compared using a generalized linear model (GLZ) assuming a normal distribution with an identity link function in SAS 9.1 (SAS institute Inc., Cary, NC, USA). To examine the effect of stage, acclimation and hardening on the upper and lower lethal limits, GLZs were used assuming a binomial distribution and a logit link function in SAS (Proc Genmod), with corrections for overdispersion.

3. Results

Life stage had the largest effect on SCP, with larvae having a typically higher SCP than adults, irrespective of acclimation and hardening (Table 1, Fig. 1). The response to acclimation differed

Table 1
Generalized linear model (GLZ) results for effects of acclimation, pre-treatment, life stage and all the interactions on the supercooling point (SCP) of adults and larvae of *Paratortora dreuxi*. A normal distribution with an identity link function was used.

Factor	d.f.	Estimate ($\pm$ S.E.)	χ^2	P
SCP	528			
Acclimation	2	–0.032 $\pm$ 0.065	0.25	0.619
Pre-treatment	5	0.024 $\pm$ 0.034	0.50	0.478
Life stage	1	–5.309 $\pm$ 0.624	72.51	<0.0001
Acclimation $\times$ pre-treatment	6	–0.001 $\pm$ 0.005	0.01	0.910
Acclimation $\times$ life stage	2	0.187 $\pm$ 0.095	3.87	0.049
Pre-treatment $\times$ life stage	5	0.023 $\pm$ 0.046	0.25	0.616
Acclimation $\times$ pre-treatment $\times$ life stage	6	–0.013 $\pm$ 0.007	3.13	0.077
SCP larvae				
Acclimation	3	–0.032 $\pm$ 0.036	0.82	0.365
Pre-treatment	5	0.024 $\pm$ 0.019	1.67	0.196
Acclimation $\times$ pre-treatment	7	–0.001 $\pm$ 0.003	0.04	0.837
SCP adults				
Acclimation	3	0.155 $\pm$ 0.091	2.94	0.086
Pre-treatment	5	0.047 $\pm$ 0.041	1.36	0.243
Acclimation $\times$ pre-treatment	7	–0.013 $\pm$ 0.007	4.06	0.044

Statistically significant values are indicated in bold.

Fig. 1. Mean $\pm$ S.E. for supercooling points (SCP) of adult and larval *Paractora dreuxi* following three acclimation temperatures (0, 5, 10 °C) and pre-treatments (–4, –2, 0, 10, 20 and 25 °C).

among life stages as evidenced by the significance of life stage and the marginally significant acclimation $\times$ life stage interaction (Table 1). Separate GLZs for larvae and adults revealed that the SCP in larvae is typically insensitive both to acclimation and to hardening, whilst in adults the response is more complex (Table 1). In this stage, the hardening effect differs among acclimation groups, with SCPs following hardening differing less after 10 °C acclimation, than after the other acclimation treatments (Fig. 1). Apart from a generally low SCP following 0 °C acclimation, however, the direction of the hardening and acclimation effects was inconsistent, resulting in the hardening by acclimation interaction being marginally significant (Table 1).

Although survival of the low temperature stress varied considerably overall (Fig. 2), neither life stage, acclimation, nor hardening had significant effects thereon (Table 2). The combined analysis neglects the fact, however, that larvae were subjected to a higher temperature (–7.6 °C) than the adults (–11 °C), which means that adults have greater stress resistance than larvae. Nonetheless, even when the larvae and adults were subject to separate GLZs, no effects of acclimation or hardening were apparent for SCS (Table 2). By contrast, the effects of acclimation on the survival of a high temperature stress differed among the stages (Table 3), with adults showing a stronger acclimation response than the larvae (Fig. 3). Indeed, the response to

Fig. 2. Mean $\pm$ S.E. % survival of a fixed low temperature (–7.6 °C in larvae and –11.0 °C in adults) in adult and larval *P. dreuxi* following acclimation (0, 5, 10 and 15 °C) and pre-treatment (–4, –2, 0, 10, 20 and 25 °C). Survival was assessed after 24 h at 10 °C.

Table 2

Generalized linear model (GLZ) results for effects of acclimation, pre-treatment, life stage and all the interactions on lower lethal limits in *P. dreuxi*. A binomial distribution and a logit link function were used.

Factor	d.f.	Estimate ($\pm$ S.E.)	χ^2	P
LLT				
Acclimation	3	0.072 $\pm$ 0.045	2.60	0.107
Pre-treatment	5	−0.055 $\pm$ 0.032	3.10	0.079
Life stage	1	0.585 $\pm$ 0.529	1.23	0.268
Acclimation $\times$ pre-treatment	7	0.004 $\pm$ 0.003	1.45	0.228
Acclimation $\times$ life stage	3	0.064 $\pm$ 0.061	1.05	0.305
Pre-treatment $\times$ life stage	5	0.066 $\pm$ 0.042	2.49	0.114
Acclimation $\times$ pre-treatment $\times$ life stage	6	−0.006 $\pm$ 0.005	1.77	0.182
LLT larvae				
Acclimation	3	−0.072 $\pm$ 0.049	2.19	0.138
Pre-treatment	5	−0.056 $\pm$ 0.034	2.61	0.106
Acclimation $\times$ pre-treatment	7	0.004 $\pm$ 0.004	1.22	0.269
LLT adults				
Acclimation	3	−0.008 $\pm$ 0.039	0.04	0.841
Pre-treatment	5	0.010 $\pm$ 0.024	0.18	0.675
Acclimation $\times$ pre-treatment	7	−0.002 $\pm$ 0.003	0.55	0.460

Table 3

Generalized linear model (GLZ) results for effects of acclimation, pre-treatment, life stage and all the interactions on upper lethal limits in *P. dreuxi*. A binomial distribution and a logit link function were used.

Factor	d.f.	Estimate ($\pm$ S.E.)	χ^2	P
ULT				
Acclimation	3	0.007 $\pm$ 0.024	0.08	0.774
Pre-treatment	5	−0.011 $\pm$ 0.017	1.51	0.534
Life stage	1	0.166 $\pm$ 0.333	0.25	0.617
Acclimation $\times$ pre-treatment	7	0.001 $\pm$ 0.001	0.02	0.890
Acclimation $\times$ life stage	3	−0.140 $\pm$ 0.044	10.73	0.0011
Pre-treatment $\times$ life stage	5	−0.014 $\pm$ 0.024	0.34	0.560
Acclimation $\times$ pre-treatment $\times$ life stage	6	−0.003 $\pm$ 0.003	0.97	0.325
ULT larvae				
Acclimation	3	0.007 $\pm$ 0.025	0.08	0.781
Pre-treatment	5	−0.010 $\pm$ 0.018	0.36	0.548
Acclimation $\times$ pre-treatment	7	0.001 $\pm$ 0.001	0.02	0.894
ULT adults				
Acclimation	3	−0.136 $\pm$ 0.035	15.32	<0.0001
Pre-treatment	5	0.004 $\pm$ 0.017	0.05	0.827
Acclimation $\times$ pre-treatment	7	−0.003 $\pm$ 0.003	1.24	0.226
ULT without the 0 °C acclimation 10 °C pre-treatment groups				
Acclimation	3	0.039 $\pm$ 0.022	3.09	0.078
Pre-treatment	5	−0.020 $\pm$ 0.016	1.51	0.219
Life stage	1	0.206 $\pm$ 0.313	0.43	0.551
Acclimation $\times$ pre-treatment	7	0.001 $\pm$ 0.002	0.40	0.530
Acclimation $\times$ life stage	3	−0.140 $\pm$ 0.039	13.14	0.0003
Pre-treatment $\times$ life stage	5	0.017 $\pm$ 0.023	0.56	0.455
Acclimation $\times$ pre-treatment $\times$ life stage	6	−0.003 $\pm$ 0.003	1.21	0.270
ULT without the 0 °C acclimation 10 °C pre-treatment groups—larvae				
Acclimation	3	0.039 $\pm$ 0.022	3.03	0.082
Pre-treatment	5	−0.020 $\pm$ 0.016	1.48	0.224
Acclimation $\times$ pre-treatment	7	−0.001 $\pm$ 0.002	0.39	0.533
ULT without the 0 °C acclimation 10 °C pre-treatment groups—adults				
Acclimation	3	−0.100 $\pm$ 0.032	10.44	0.0012
Pre-treatment	5	−0.003 $\pm$ 0.016	0.03	0.862
Acclimation $\times$ pre-treatment	7	−0.002 $\pm$ 0.002	0.84	0.361

Statistically significant values are indicated in bold.

acclimation in adults was significant, but this was not the case for the larvae. In both stages, survival was highest following 0 °C acclimation and a 10 °C hardening treatment. Removal of this particular acclimation $\times$ hardening treatment from the analyses did not alter the outcome of the general acclimation by stage interaction (Table 3). Thus, it appears that adults show a much broader range of survival probabilities following low temperature acclimation than following acclimation to 10 or 15 °C, when survival is typically low (Fig. 3). By contrast, larvae tend to have

lower survival following low temperature acclimation, although this effect of acclimation was not significant, irrespective of the GLZ applied (Tables 2 and 3).

4. Discussion

Life stage-related differences in thermal tolerance have been recorded previously for *P. dreuxi*. As was the case here, Klok and Chown (2001) found that the SCP of larvae was higher than that of

Fig. 3. Mean $\pm$ S.E. % survival of a fixed high temperature (33.5 °C) in adult and larval *P. dreuxi* following acclimation (0, 5, 10 and 15 °C) and pre-treatment (–4, –2, 0, 10, 20 and 25 °C). Survival was assessed after 24 h at 10 °C. The 0 °C acclimation and 10 °C pre-treatment data points for larvae (mean: 100 $\pm$ 0%) and adults (mean: 92.5 $\pm$ 3.06%) were excluded from the graph for clarity.

the adults, and showed that this is related to moderate freeze tolerance in the larvae and freeze intolerance in the adults. These authors also found differences in the critical limits and lower and upper lethal temperatures that are more or less in keeping with what we have documented here. Life stage effects on tolerances have also commonly been documented in other taxa. For example, in the sub-Antarctic fly, *Anatalanta aptera*, the pupae and teneral adults had significantly lower SCPs than the larvae and mature adults (Vernon and Vannier, 1996). In the Antarctic midge, *B. antarctica* larval cold tolerance increased significantly after hardening, while this was not the case for adults (Lee et al., 2006). However, exceptions do exist, such as in the sub-Antarctic *Apetaenus littoralis* (Klok and Chown, 2000), where no differences among stages have been recorded. In general, stage-related differences in tolerance seem more the norm than the exception, and have been recorded from a wide variety of insect species (Bowler and Terblanche, 2008).

By contrast with the consistent variation in absolute tolerances, acclimation and hardening effects were much more variable among traits and, at times, among the life stages. In the case of survival of a fixed low temperature it is plain that neither acclimation nor hardening affected the trait either in the larvae or in the adults. To some extent, this finding was a consequence of high variances within each of the hardening $\times$ acclimation treatments, but it seems unlikely that larger sample sizes would have altered the outcome (a *post hoc* power analysis indicated that for a significant difference to be found at $p < 0.05$, three times the current sample size would be needed). Rather it appears that survival of cold shock in both adults and larvae of *P. dreuxi* shows little phenotypic plasticity over hours or days. These findings contrast somewhat with the results of our earlier study in which a narrower range of treatments was applied and where larvae acclimated to 10 °C appeared to show a small rapid cold hardening response (Terblanche et al., 2007), whereas here they did not. By contrast, our findings for the adults were consistent with the previous study, demonstrating that they show no plasticity in LLT. The supercooling point, or crystallization temperature, showed a similar lack of plasticity in the larvae, and in adults the only

significant effect was marginal, suggesting that plasticity is weak at best, and certainly not consistent in a manner that might be interpreted as beneficial (see Huey et al., 1999; Deere and Chown, 2006 for a summary of acclimation hypotheses). Thus, overall it appears that these two low temperature resistance traits show almost no plasticity in either adults or larvae.

An absence of plasticity has been recorded previously in terrestrial species from Marion Island. In terrestrial ameronothroid mites this is true both of SCP and lower lethal temperatures (Deere et al., 2006), and of locomotor performance (Deere and Chown, 2006). Likewise, Slabber et al. (2007) documented a weak effect of acclimation on SCP in indigenous springtails, while the invasive species showed a more pronounced acclimation response. This absence of plasticity has been ascribed to the island's highly unpredictable, terrestrial environment (Deere and Chown, 2006). Unpredictable environments mean low cue reliability, and most models for the evolution of plasticity show that for plastic responses to evolve, cue reliability must be high (Moran, 1992; Hazel et al., 2004; see also Chown and Terblanche, 2007). In the absence of cue reliability, plasticity is unlikely to evolve, but traits might still differ in their absolute (or basal) values (Tufto, 2000). This certainly appears to be true of low temperature resistance traits both in this fly species and in other terrestrial arthropods from Marion Island. An absence of short-term phenotypic plasticity of lower lethal temperatures has also been documented in the Arctic springtail *Hypogastrura tullbergi*, and has been ascribed to either behavioural avoidance, or the inability of the species to deal with rare, unpredictable low temperature events in summer (Hawes et al., 2006). Thus, where acclimation effects on lower lethal temperatures are absent in arthropods it appears that environmental unpredictability may form the common theme. However, this theme does seem to vary among traits.

In particular, the lack of acclimation or hardening effects on survival of a given low temperature treatment (or cold shock) found here contrasts substantially with the outcome found for recovery from chill coma in the adults and larvae of the same species. Marais and Chown (2008) found that in larvae, strong evidence for beneficial acclimation of chill coma recovery time

exists, whilst in adults, low temperature acclimation tends to provide the shortest recovery times irrespective of the treatment temperature. The substantial differences found between the survival traits (SCS, SCP) examined here and the knockdown trait (chill coma recovery time) investigated by Marais and Chown (2008) provide additional support for the idea that these two groups of traits likely reflect different mechanisms with different genetic underpinnings (Hoffmann et al., 2003; Rako and Hoffmann, 2006; Rako et al., 2007). How these differences among the groups of traits relate to environmental predictability and the significance of behavioural mediation of physiological responses is not entirely clear. Perhaps the survival traits reflect the fact that after a substantial period at extreme low temperature (i.e. -7.6 – 11.0 °C for 2 h, relative to -5 °C for 30–45 min) behaviour is no longer especially significant as a mediator of responses because neither stage would be capable of a behavioural response under extreme conditions (critical thermal minima are in the region of -2.7 – 5.1 °C, Klok and Chown, 2001).

Further reflecting variation in responses among traits, in the case of upper lethal limits, differences in plasticity among the adults and larvae were apparent. As was typical both of SCP and survival of lower lethal temperatures, survival of upper lethal temperatures varied little with acclimation in the larvae. By contrast, survival tended to be higher following low temperature acclimation in the adults. Thus, whilst larvae show a consistent lack of response to acclimation over hours or days in the resistance traits measured here, in the adults some evidence of an improvement in survival of upper lethal temperatures following low temperature treatments is apparent. Indeed, it appears that, overall, low temperature acclimation is more conducive to survival than high temperature acclimation, and this was borne out by high mortality during the acclimation period (adults at 15 °C: 65% mortality; adults at 0, 5 and 10 °C: <15% mortality). Thus, it seems that at least for this trait, 'colder is better' (see Huey and Berrigan, 1996) for the adult flies. This outcome is in keeping with that found for chill coma recovery in this stage (Marais and Chown, 2008), providing further confirmation of the fact that variation in acclimation response among the adults and larvae in this trait, and variation in the acclimation response in many of the traits examined here, is likely a consequence of the profound behavioural differences in the two stages as well as differences in the activities that adults and larvae are likely to be engaged in at high vs. subzero temperatures. At temperatures close to their lower lethal limits, it seems likely that both larvae and adults will be relatively immobile given that their critical thermal limits for activity are higher than their lower lethal limits (larvae: -5.1 and -7 °C, respectively, adults -2.7 and -11 °C, respectively, Klok and Chown, 2001; present data). By contrast, at the high temperature end of the spectrum, adults and larvae can maintain activity over the short-term to above 30 °C (Klok and Chown, 2001). However, larvae are much less mobile than are the exceptionally active, though brachypterous, adults (Crafford, 1984; Crafford and Scholtz, 1987). In consequence, it seems likely that at high temperatures, adults can readily seek out the thermal environments that will most suit survival and reproduction by moving through the temperature variable substrate (Fig. 4). Thus, rather than being exposed to unpredictably varying temperatures at the high temperature end, they simply avoid the extremes, and may even make use of low temperatures to enhance their ability to endure higher temperatures for short periods. Larvae, however, will not be able to avoid temperature extremes as readily and may well have to remain as mobile as possible under a wide range of conditions. Their broader range of critical limits for activity (40 °C, as opposed to 33 °C in adults, Klok and Chown, 2001) as well as their tendency to show beneficial acclimation for an activity-related trait (chill coma recovery, Marais and Chown, 2008) supports this contention.

Fig. 4. Temperature data from the Trypot beach study site at 0 cm (on top of the pebble substrate) and at a depth of 30 cm recorded during the sampling period (redrawn from Marais and Chown, 2008).

Nonetheless, further investigation is required to determine how general are such trait differences and the ways in which environmental variation and behavioural mediation are likely to influence the physiological response.

The single exception to these generalities was the exceptionally high survival of both adults and larvae to an acclimation of 0 °C and a pre-treatment hardening of 10 °C. Why survival was so high in both stages is not clear. Perhaps the only explanation at present is that these represent optimal conditions for the animals and therefore exposure to a high temperature stress following these conditions has the least impact on them. That individuals should show an optimum set of conditions for performance is not surprising, and indeed is well supported by a large literature on animal performance-temperature relationships (see Angilletta et al., 2002 for review). Moreover, deleterious effects of acclimation to extreme temperatures have also been documented across a wide range of insect species (Krebs and Loeschcke, 1994; Scott et al., 1997; Feder and Hofmann, 1999; Hoffmann and Hewa-Kapuge, 2000; Loeschcke and Hoffmann, 2002). Thus, it may well be that both the adults and larvae of *P. dreuxi* prefer a particular set of conditions because it enhances tolerance of extreme high temperature events. Such performance-preference co-adaptation has been documented in several ectotherms (see Angilletta et al., 2006 for review), but has not been well investigated for insects.

In conclusion, this study has demonstrated that the extent and form of phenotypic plasticity of thermal tolerance traits varies substantially among the traits themselves and among life stages of *P. dreuxi*. As has been found elsewhere (Rako and Hoffmann, 2006; Jensen et al., 2007), this variation is complex and not always simple to interpret. However, the extent to which cues are reliable and mediated behaviourally, and the ability of animals to execute specific behaviours at low vs. high temperatures appear to be prominent themes underlying the variation. Future work on other species focussing on these themes might help bring generality to what appears at present to be a large variety of interesting case studies documenting complex trait variation.

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