Commentary

Plasticity in arthropod cryotypes

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Summary

Low-temperature acclimation and acclimatization produce phenotypic changes in arthropods at multiple levels of biological organization from the molecular to the behavioural. The role and function of plasticity – where a constitutive, reversible change occurs in the phenotype in response to low temperature – may be partitioned hierarchically at evolutionary scales according to cryoprotective strategy, at macrophysiological scales according to climatic variability, and at meso- and micro-scales according to ecological niche and exposure. In correspondence with these scales (which are interdependent rather than mutually exclusive), a hierarchical typology of interaction between thermal history and organism is proposed, descending, respectively, from what we define as 'cryotype' (class of cryoprotective strategy) to genotype and, ultimately, phenotype. Alternative (and sometimes complementary) strategies to plasticity include specialization, generalization, bet-hedging, cross-resistance and convergence. The transition of cryotypes from basal to derived states is a continuum of trait optimization, involving the fixation of plasticity and/or its alternatives.

Key words: arthropod, cold tolerance, cryotype, cryoprotection, acclimation, acclimatization, phenotype.

Introduction

Animal physiology in the real world is dynamic – it must respond to variability at multiple temporal and spatial scales. This is particularly true of ectotherms, which are physiologically coupled to their environments across both ecological and metabolic levels of organization: from the niche and range of populations to the catalytic pathways of enzymes. In this sense, all arthropods are 'plastic' in their physiology, with reaction norms over lesser or greater ranges of temperature. But some arthropods are more plastic in their physiology than others. Here, we examine the role of plasticity in the cryoprotection of arthropods at low temperatures.

Plasticity may be defined as a measure of organism malleability (Huey and Berrigan, 1996). Phylogenetically, it may be partitioned at a number of levels from the single phenotype (phenotypic plasticity) to multiple phenotypes (genotypic plasticity) to comparisons across taxa that share the same evolutionary adaptation to an environmental variable (cryotypic – as the variable in this case is low temperature – plasticity). As a physiological phenomenon it has two distinguishing characteristics: firstly, it 'stretches' the limits of physiological response; secondly, it is impermanent – i.e. the extension is a temporary response to transient conditions (Huey and Berrigan, 1996). If one imagines that an arthropod's physiology is a rubber band, and low temperature the 'hand' or stimulus stretching it, plasticity is the measure of how far it can be stretched at a certain point in time. Just as the rubber band's elasticity depends on the type of rubber band and the stimulus it is given, so the plasticity of an arthropod's response varies in response to endogenous (e.g. life stage, state of acclimation/acclimatization, phenotype, species) and exogenous (environmental) factors. Scheiner thus describes phenotypic plasticity as 'the change in the expressed phenotype of a genotype as a function of the environment' (Scheiner, 1993).

Basal physiological responses become physiologically plastic when a constitutive change in the phenotype takes place. As the boundaries between basal and plastic responses can be fuzzy – from an evolutionary perspective, it is probably best described as a continuum – here we concentrate on changes that are most markedly constitutive. In the case of the cold-hardiness trait or, rather, 'trait complex' (*sensu* Scheiner, 1993), this is expressed as the acquisition of the cold-hardy state, which may be associated with both qualitative and quantitative changes at various levels (morphological, behavioural, physiological). Although DeWitt and Scheiner (DeWitt and Scheiner, 2004) have noted the potential 'breadth of scope' in definitions of plasticity, behaviour is included here, not so much for this reason as for the fact that the cryoprotective response in arthropods is expressed in a multivariate and interdependent manner at all levels of biological organization from the behavioural (e.g. habitat selection) to the molecular (e.g. protein expression).

There is considerable burgeoning interest in the role of plasticity in evolutionary pathways. Linking the physiological,

ecological and, ultimately, temporal (evolutionary fitness) range of a species through plasticity is certainly intuitively attractive. However, ascribing adaptiveness to traits is problematic – see, for example, Gould and Lewontin's critique of indiscriminate appellations of 'adaptation' (Gould and Lewontin, 1979).

Storey distinguishes between two types of cold adaptation: firstly, adaptations involved in cellular preservation at sub-zero temperatures (cryoprotective adaptation) and, secondly, adaptations involved in the maintenance of normal physiological function at sub-zero temperatures (metabolic adaptation) (Storey, 1984). Although there may be debate about the latter (e.g. Clarke, 1991; Clarke, 1993; Addo-Bediako et al., 2002; Hodkinson, 2003), and the expression of both may depend to varying degrees on interactions between one another, there is a general consensus that – except in instances of cross-resistance/convergence – *cold tolerance is an adaptation to low temperatures*. Fitness may be accrued at multiple levels, however the most fundamental measure of it is survival; at low temperatures this is impossible without some form of cryoprotection. Here, therefore, we argue from the opposite direction – a trait or 'trait complex' that is known to be adaptive – and examine its plasticity across various scales of biological significance. Although the transition from basal tolerance to plasticity offers some interesting questions in itself, here our discussion focuses on instances where phenotypic variation is self-evidently associated with low-temperature adaptation, e.g. alpine and polar species and temperate overwinterers.

Plasticity and cryotype

We adopt the term 'cryotype' to refer to the type of cold tolerance strategy an organism adopts. Arthropods survive low temperature by one of four strategies according to how they manage body water at freezing temperatures: (1) freeze avoidance (cannot survive internal ice formation); (2) freeze tolerance (can survive internal ice formation); (3) non-freezing (obviate the problem of internal ice formation by removing body water or vitrifying) and (4) a 'mixed' strategy. It should be noted, however, that nomenclature can be problematic and requires careful qualification. This is largely because strategies are dispersed heterogeneously through evolutionary time and are therefore not expressed as singularities but continuities with many interchangeable components. For example, both freeze-avoiding and freeze-tolerating cryotypes have evolved antifreeze proteins. Although their seemingly 'contradictory' presence in freeze-tolerant organisms may facilitate the management of ice nucleation by inhibiting re-crystallization (Knight and Duman, 1986), they are clearly not necessary to the tolerance of extracellular ice, as is shown by their absence in species like *Hemideina maori* (Ramløv et al., 1996). Nor, indeed, are they a prerequisite of freeze avoidance, as their absence in many species shows. Such variations may reflect the diverse trajectories of evolved low-temperature adaptation; certainly, they emphasize the ways in which cryotypes may not be related uniformly or even predictably.

Freeze-avoiding cryotype

Freeze avoiders are unable to tolerate the freezing of body water and so 'avoid' ice nucleation by supercooling (Bale, 2002). This is the strategy most commonly adopted by arthropods and, by argument of parsimony, probably represents the basal state (Sinclair et al., 2003). Likewise, the extent to which freeze avoiders express a basal or derived degree of cold tolerance also varies. Although constitutive changes in the phenotype can be associated with behavioural and morphological changes, for most arthropods the change is primarily physiological. The acquisition of the cold-hardy phenotype is accomplished by a range of mechanisms that work to depress the supercooling point (SCP) or temperature of crystallization (T_c). Three main physiological processes operate to achieve this: (1) the removal or inactivation of ice-nucleating agents (Zachariassen, 1985; Cannon and Block, 1988); (2) the accumulation of low-molecular-mass solutes such as polyhydric alcohols, which increase the concentration (osmolality) of body fluids (Zachariassen, 1985; Zachariassen, 1991), and (3) the production of antifreeze proteins, which adsorb to ice at the molecular level to inhibit its growth (Duman, 2001). [However, none of these countermeasures are universal – antifreeze proteins, in particular (e.g. Duman et al., 2004)].

In addition, other collaborative factors – like antifreeze proteins, not exclusive to freeze-avoiders – operate to facilitate physiological function at low temperatures. For example, heat shock proteins (Hsps) are often upregulated in arthropods at low temperatures (e.g. Joplin et al., 1990; Rinehart et al., 2006). These proteins act as molecular chaperones, overseeing the protein assembly process by binding to the surfaces of immature proteins during their synthesis and preventing 'incorrect interactions' with the stressed cellular milieu that might otherwise lead to unfolding/denaturation (Ellis, 1993). Also, some arthropods show evidence of membrane phospholipid desaturation at low temperatures (e.g. Bennett et al., 1997; Koštál et al., 2003). Desaturation promotes membrane fluidity, extending the gel phase of the cellular–extracellular interface to ensure that communication between the two environments remains selective during low-temperature exposure (Hazel, 1995).

Different classes of these freeze-susceptible cryotypes vary in their ability to prevent cold injury (Bale, 1993; Bale, 1996). Thus, although within a cryotype they are classified as ones that employ supercooling, for those that experience chill injury or mortality – i.e. experience injury and/or mortality as a result of physiological malfunction prior to the crystallization of body fluids (cf. Bale, 1993; Bale, 1996) – it is not their capacity to supercool so much as the maintenance of other levels of physiological organization that is the determinant of low-temperature survival. Koštál et al., for example, have demonstrated the importance of maintaining ion gradients at sub-zero temperatures in mitigating pre-freeze mortality (Koštál et al., 2004). Whether this might be an argument for the necessity of possessing both cryoprotective and metabolic adaptations (*sensu* Storey, 1984) for robust tolerance of low temperature needs to be determined. However, one might distinguish here between taxa and species that express a true 'cold-hardy phenotype' (e.g. chill-tolerant species that show little pre-freeze mortality) and those that express an acclimated or acclimatized phenotype that shows varying degrees of improvement in low-temperature tolerance (i.e. some degree of 'hardening') along the continuum from basal to derived responses. Survival of extreme sub-zero temperatures by freeze-avoiding cryotypes is only achieved by supercooling. Thus,

although SCPs may not always have physiological or ecological relevance to many freeze avoiders, in that the degree of correlation between SCPs and lower lethal temperatures (LLTs) (i.e. the extent of chill tolerance) is an expression of evolutionary derivation in freeze avoiders, the SCP remains an indispensable measurement.

Freeze-tolerant cryotype

Although localized survival of intracellular freezing has been documented in the fat body of the gall fly, *Eurosta solidaginis* (Salt, 1962; Lee et al., 1993), to date the only organism known to survive organism-level intracellular ice formation is the Antarctic nematode *Panagrolaimus davidi* (Wharton and Ferns, 1995). Freeze-tolerant arthropods survive low temperatures by the tolerance of ice formation within the extracellular matrix. For most but not all arthropods (e.g. Fields and McNeil, 1986; Mugnano et al., 1996), this is achieved by ice-nucleating proteins (INPs) and lipoproteins in the haemolymph that facilitate crystallization in a manner and at a temperature that can be managed without injurious effects (Zachariassen and Hammel, 1976; Duman, 2001). Thus, the majority of freeze-tolerant arthropods freeze at high sub-zero temperatures (but see Sinclair, 1999). This enables cells to reach osmotic equilibrium with the extracellular matrix as ice forms in its spaces (Zachariassen and Hammel, 1976), as well as reducing demands on energy stores by lowering metabolic rate (Storey et al., 1981). Direct cryoprotective measures such as the expression of INPs are, in turn, supported by measures that are indirectly involved in cryoprotection but directly involved in mitigating the physiological stresses produced by the frozen state – e.g. ion transport (Zachariassen et al., 2004; Kristiansen and Zachariasen, 2001) and ischemia (Morin et al., 2005). Little is known about the extent to which freeze-tolerant arthropods are adapted to tolerate sub-zero (i.e. chilling) temperatures above their SCP.

There is considerable variability in the extent to which freeze-tolerant species show plasticity in their low-temperature adaptation. Quite a few species show permanent freeze tolerance (albeit with reduced LLTs in the summer), e.g. the caterpillars of *Gynaephora groenlandica* (Kukal, 1991). Others may acquire their tolerance seasonally, e.g. the gallfly *E. solidaginis* (Morrisey and Baust, 1976), or lose it in response to repetitive sub-zero stress, e.g. the hoverfly *Syrphus ribesii* (Brown et al., 2004). Some may only express tolerance under specific conditions; the beetle *Upis ceramboides*, for example, only tolerates internal ice nucleation at cooling rates of <0.3 deg. min^{-1} (Miller, 1978). From an ecological perspective (i.e. the prediction of parameters for population persistence), it makes sense that the gradient in 'strength' of freeze tolerance be considered in terms of lower lethal temperature (Sinclair, 1999). However, variability in the proportion of frozen body water that can be tolerated by different species is also of note; Ramløv suggests that there are two independent gradients – tolerance of low temperature and tolerance of internal ice (Ramløv, 1998). The New Zealand alpine weta, *H. maori*, for example, which only survives a few degrees below its SCP, can survive freezing of up to 82% of body water, compared with many freeze-tolerant species that tolerate ~65% (Ramløv and Westh, 1993).

Species with permanent freeze tolerance (e.g. Kukal, 1991)

show relatively little phenotypic variation in cold hardiness. In this sense they are the direct opposite of freeze avoiders, with the expression of plasticity inversely related to departure from the basal state (Fig. 1). This may seem contradictory given the general correlation between environmental heterogeneity and plasticity (Doughty and Reznick, 2004) – indeed, climatic variability at both seasonal (northern latitudes) and aseasonal (southern latitudes) timescales has been implicated in the evolution of freeze tolerance (Sinclair et al., 2003). However, freeze tolerance in this context may be seen as expressing an alternative evolutionary solution – namely, specialization (DeWitt and Langerhans, 2004). Thus, DeWitt and Scheiner (DeWitt and Scheiner, 2004) note that if '*a single phenotype is best in all circumstances, then environmentally induced deviation away from the best phenotype only reduces fitness*'.

Non-freezing cryotype

Anhydrobiosis

Anhydrobiosis confers considerable cold tolerance on a number of invertebrate taxa through cross resistance. These include Rotifera (Tunnicliffe and Lapinski, 2003), Tardigrada (Sømme, 1996), Nematoda (Wharton, 2003), Oligochaeta (Holmstrup et al., 2002a; Holmstrup, 2003), Branchiopoda (Clegg, 2001), Collembola (Poinsot-Balaguer and Barra, 1983) and, among the Insecta, larvae of the 'sleeping' chironomid *Polypedilum vanderplanki* (Watanabe, 2006). Lesser magnitudes of desiccation are employed by many species of arthropods as a component of their cold-hardening strategies (e.g. Zachariassen, 1991). To date, however, the only arthropod that is known to employ desiccation at low temperatures – to the extent of anhydrobiosis – as a cold-tolerance strategy is the Arctic collembolan *Onychiurus arcticus* (Worland, 1996; Worland et al., 1998; Holmstrup and Sømme, 1998; Worland and Block, 2001). Water loss is temperature dependent in practice, so it may be either 'partial' (Worland et al., 1998) or nearly identical to what has been called anhydrobiosis elsewhere – *O. arcticus* has been shown to lose up to 90% water content (Holmstrup and Sømme, 1998) compared with the anhydrobiotic *P. vanderplanki*, which
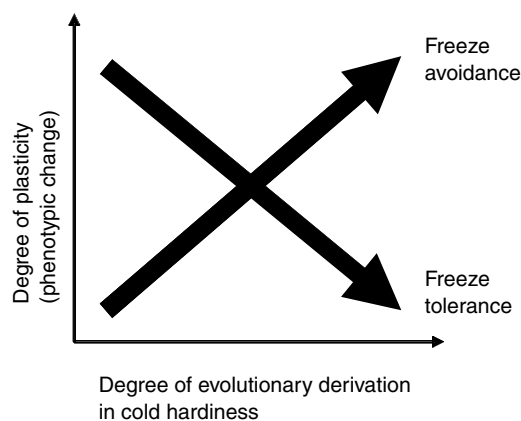

Fig. 1. Generalized relationship between the degree of evolutionary derivation of cold-hardening strategy and the degree of plasticity (phenotypic variation) in freeze-tolerance and freeze-avoiding cryotypes.

loses 92% (Hinton, 1960). Thus, although in its habitat, *O. arcticus* experiences dehydration as more of a continuum than species traditionally described as exhibiting anhydrobiosis *sensu strictu*, the culmination of its acclimatization strategy is nonetheless almost complete desiccation. Tunnicliffe and Lapinski have noted elsewhere the problems of anhydrobiosis as a term generally (Tunnicliffe and Lapinski, 2003). Pending further understanding of this strategy in *O. arcticus*, it seems sensible to utilize, with qualification, already flexible nomenclature rather than invent new terminology.

O. arcticus therefore represents a third alternative to the threat of body water nucleation in arthropods, i.e. its passive removal by desiccation. At sub-zero temperatures it loses water across the vapour pressure gradient established between its supercooled body fluids and the ice in its surroundings. Morphologically, this is attended by progressive 'shrivelling' (Fig. 2). Initially, this causes a concentration of body fluids, reduction of osmotically active water and depression of the supercooling point, but ultimately when osmotically active water is removed, it enters a dehydrated state in which there is no supercooling point as it can no longer be 'frozen' by ice nucleation (Holmstrup and Sømme, 1998). The ability to tolerate such extreme desiccation is facilitated in particular by increases in trehalose – a disaccharide known to protect membrane and proteins against desiccation (Rudolf and Crowe, 1985; Crowe and Crowe, 1986) and prevalent in a range of anhydrobiotic organisms including bacteria, yeast, fungal spores, invertebrates and resurrection plants (for a review, see Clegg, 2001). Over a 3-week exposure to decreasing temperatures from 0 to –5.5°C in the presence of ice, for example, trehalose concentration in *O. arcticus* increased from 0.9 to 94.7 μg mg^{-1} fresh mass (Worland et al., 1998).

Vitrification

An alternative strategy for internal water management at sub-zero temperatures is vitrification – where water forms a glassy rather than a crystalline state (Baust and Nishino, 1991). Found in seeds and twigs, this strategy obviates the physical and osmotic injuries of freeze damage by the incorporation of extra- and/or intracellular solutes into the glassy state (Storey and Storey, 2004). Although sugars, such as trehalose, that are typically involved in anhydrobiosis undergo glassy-state formation – suggesting some continuity between the strategies – vitrifying organisms undergo glassy change without such extensive dehydration (Storey and Storey, 2004). Little is known about its prevalence in arthropods – it has been documented in the freeze-tolerant insect *E. solidaginis* (Wasylyk and Baust, 1989) and implicated recently in the overwintering strategies of *Cucujus clavipes* (Bennett et al., 2005). Notwithstanding continuities between strategies (especially in the former, where vitrification occurs after an initial crystallization event), like anhydrobiosis, it is fundamentally a 'non-freezing' strategy, replacing crystallization with glass formation.

Mixed-strategy cryotype

A fourth cryoprotective strategy is the employment of a 'mixed strategy'. These are species that do not belong exclusively to one or another cryotype but may employ more than one strategy.
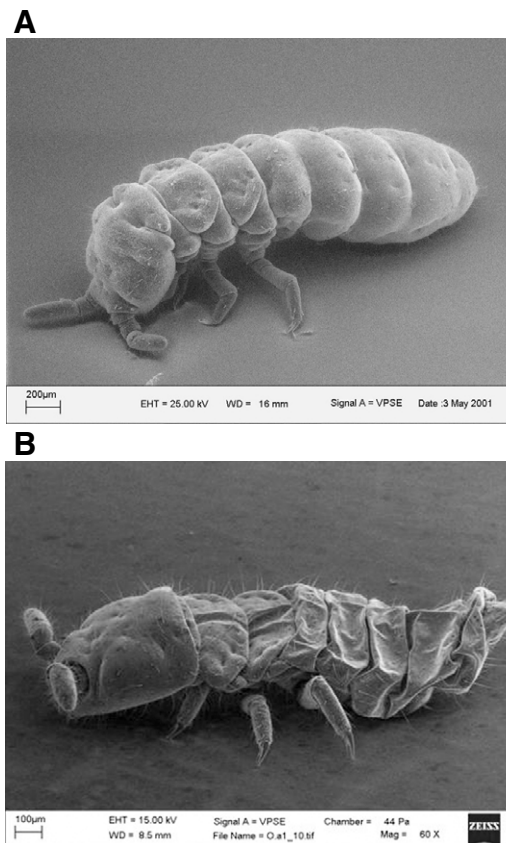
Fig. 2. Comparison of morphological changes in *O. arcticus* associated with dehydration: (A) hydrated; (B) desiccated at sub-zero temperatures.

Thus, although some freeze-tolerant species may be freeze avoiding until they are acclimated or acclimatized to their cold-hardy phenotype (e.g. Miller, 1969; Morrisey and Baust, 1976), the species that employ a mixed strategy are those for which the cold-hardy phenotype may itself belong to more than one cryotype. This phenomenon has to date only been encountered *in situ* in two species of beetle – *Dendroides canadensis* and *C. clavipes* (Duman, 1984; Horwath and Duman, 1984; Kukal and Duman, 1989). *C. clavipes*, for example, switched from freeze tolerance in the winter of 1978–1979 to freeze avoidance in the winter of 1982–1983 (Duman, 1984).

Is this a 'real world' manifestation of the bet-hedging strategy experimentally induced in some freeze-tolerant species (Bale et al., 2001)? Or is it an example of remarkable plasticity? As the former it might be considered an alternative to plasticity (see below) (Table 1); as the latter, either accidental or adaptive plasticity. Voituron et al. (Voituron et al., 2002) hypothesize that either energy levels and/or variation in winter conditions are responsible. Certainly, these 'switchers' suggest an intriguing middle ground between the evolution of the freeze-avoiding and freeze-tolerating cryotypes.

In this context, it is worth noting that, in addition to continuity between different cryotypic strategies, there may also be continuity between the physiological components of strategies. There may be instances where the nomenclature of cryotypes is determined more by quantitative, than qualitative, variation:

Table 1. *Alternative (and/or complementary) strategies to phenotypic plasticity in arthropods at low temperatures*

Strategy	Definition	Example
Specialization	Life stage specialized for overwintering	Eggs, larvae, pupae (Danks, 1981; Danks, 1999; Leather et al., 1993)
	Specialized physiology	Freeze tolerance (see text)
Generalization	'General purpose' phenotype is produced	*Hypogastrura tullbergi* (Hawes et al., 2006) (see text)
Bet-hedging	Production of more than one phenotype to spread risk	Speckled wood butterfly (*Pararge aegeria*) produces two overwintering phenotypes – one that diapauses; one that is winter-active and feeds on grasses during mild winter temperatures (Lees and Tilly, 1981)
Cross-resistance/overlapping adaptation	Tolerance achieved by adaptation to other physiological stress	Desiccation tolerance (Ring and Danks, 1994) Diapause (e.g. Denlinger, 1991; Pullin, 1996) Anaerobic metabolism (Coulson and Bale, 1991; Storey and Storey, 2004)
Convergence/'accidental'	Tolerance achieved by other (non-stress) adaptation	Cold hardiness of moulting animals – e.g. *Tullbergia antarctica* (Worland, 2005); *Alaskozetes antarcticus* (Hawes et al., in press)

desiccation, for example. As already noted, desiccation forms an important component of many cold-tolerance strategies. In the Collembola, particularly, where this assumes the status of a separate strategy in *O. arcticus*, there are good grounds for proposing the existence of a continuum between the use of desiccation as a component of the acquisition of cold tolerance in freeze-avoiders and its employment as a means of completely removing osmotically active water as a nucleating substrate. The cuticular permeability of Collembola makes them phylogenetically pre-disposed to the evolution of desiccation resistance as an adaptation (Hopkin, 1997). As dehydration can confer cold tolerance through cross resistance, even species with little obvious low-temperature adaptation can increase their cold tolerance by drought acclimation (e.g. Bayley et al., 2001; Holmstrup et al., 2002b). While psychrophilic or 'cold-loving' species may include desiccation as a component of their supercooling strategies. The Antarctic springtail *Cryptopygus antarcticus*, for example, also desiccates under ice in its habitat and accumulates trehalose over winter (Cannon et al., 1985; Montiel, 1998), although it is not capable of managing water loss to the extent of *O. arcticus* (Worland and Block, 2003). Indeed, *O. arcticus* is *sensu strictu* a freeze-avoider until it voids all of its osmotically active body water and no longer has a supercooling point. (Its distinct nomenclature is however justified in that the 'anhydrobiotic' phenotype represents the culmination of its acclimation/acclimatization.)

Plasticity and genotype

Although much of the phenotypic variation in arthropod cold tolerance can be attributed to their evolutionary solutions to low temperatures (cryotypes), variation is also evident at other scales. Many species exhibit genotypic plasticity – i.e. variation in the relative physiological limits of different phenotypes. Although there has been promising research into the selection of cold-tolerant phenotypes in *Drosophila* (e.g. Hoffmann et al., 2003), much less is known about selection for cold tolerance *in situ*. Also, geography can operate as a substitute for evolutionary time: for example, species widely distributed in space (latitude) or separated by topography (e.g. islands, mountains) may experience great variation in the degree to which low temperature impinges

on their fitness. As a result, they may be differentiated in their degree of low-temperature adaptation. The epedaphic springtail *Orchesella cincta*, for example, demonstrates geographic variation in a number of stress-resistance traits – including cold-shock resistance – along a 2000 km north–south transect of Europe (Bahrndorff et al., 2006).

Selection for cold-tolerance traits may be determined in part by the plasticity of developmental pathways (e.g. Zeilstra and Fischer, 2005). In addition, traits that do undergo selection will have potentially been filtered through an array of competing biotic and abiotic selective pressures. In this sense, species occupying comparatively 'simple' environments (e.g. high latitudes) where selective pressures are reduced numerically (but not necessarily in magnitude) may provide particularly good models of selection for cold tolerance. (Although the dominance of temperature as a selective pressure may be traded off to some extent against low energy budgets and long generation times.) Whether phenotypes along a gradient of genotype–environment interactions become fixed at an optimal physiological strategy or remain plastic is an area of both theoretical and practical enquiry, as are the conditions that promote either outcome (e.g. Berrigan and Scheiner, 2004). Plasticity may potentially serve as an evolutionary outcome itself (adaptation) or as a route to a 'non-plastic' evolutionary outcome (catalyst for adaptation).

In arthropod cryobiology, the best example of specialization (fixation of an optimal strategy) is probably freeze tolerance, as it is expressed in species like *G. groenlandica* (Kukal, 1991). On the other hand, the best examples of genotypic plasticity are probably to be found in the growing numbers of cases documenting differences in low-temperature capabilities of species over latitudinal gradients (e.g. Baust and Lee, 1981; Addo-Bediako et al., 2000; Gibert and Huey, 2001; David et al., 2003; Chen and Kang, 2004). Clinal variability in the cold tolerance of different species remains a topical field of interest and will no doubt continue to provoke fresh insights into the ways in which physiological variability is expressed in insects. From an applied perspective, particularly, genotypic plasticity (or lack of it) has important implications for understanding the dynamics of species range expansion and contraction under past and future scenarios of climate change (e.g. Crozier, 2003).

However, neither cryotype nor genotype can account for all of the physiological variability apparent in insect cold tolerance. Indeed, some species show little evidence of genotypic plasticity at all. For example, comparisons of desiccation resistance and upper and lower thermal limits of the moth *Embryonopsis halticella* from two islands with very different climates (Heard Island and Marion Island in the sub-Antarctic) found no evidence of adaptive geographic variation (Klok and Chown, 2005). Additional variability must therefore also be partitioned at the level of the individual phenotype.

Plasticity and phenotype

Phenotypic changes in cold tolerance occur in response to acclimation or acclimatization processes. The genetic contribution to these responses is determined over evolutionary time by selection for a particular cryotype and over geographic and climatic clines by genotypic plasticity. However, for some species, the phenotype-specific contribution to physiological variation may also be significant. Thus, for example, when Hoffman et al. partitioned the genotype–environment interaction into the relative effects of inheritance and environment on *Drosophila melanogaster* stress resistance across a climatic cline in Australia, environmental differences (phenotypic plasticity) were 1.5 times greater than heritable differences (genotypic plasticity) (Hoffman et al., 2005).

From the point of view of arthropod ecophysiology, the effect of the immediate environment on phenotypic variation is most influenced by interactions between phenotypes and factors like ecological niche and habitat exposure. For example, studies of meso-scale gradients – e.g. altitude (Gaston and Chown, 1999) or the terrestrial–marine interface (Deere et al., 2006) – have provided good evidence of plasticity in operation. Contrastingly, some species may be constrained in this regard: gall-formers, for example, have specialized life-history strategies that demand overwintering in exposed sites – many are therefore also specialized for low temperatures [e.g. freeze tolerant (Humble, 2006) or with extremely low SCPs (Miller and Werner, 1987)]. Indeed, *E. solidaginis* are preferentially adapted to such exposure; demonstrating reduced emergence and potential fecundity when overwintering at ground-level in buffered environments (Irwin and Lee, 2003).

Superplasticity

In addition to seasonal phenotypic changes, some species are capable of rapidly changing their physiological phenotype. Lee et al. first drew attention to the ability of some insects to 'rapidly cold harden' (RCH) after just a few hours of acclimation at sub-lethal temperatures (Lee et al., 1987). The extent of cold tolerance acquired by rapidly cold-hardened arthropods may vary across different taxa and latitudes; however, at their most extreme level of expression (and indeed in many laboratory inductions of the response in temperate species) they may be distinguished from standard 'labile' responses (*sensu* Scheiner, 1993) by the fact that they operate at temporal and/or physiological scales in excess of environmental variation – hence the attribution of 'superplasticity'. RCH has been observed in a variety of arthropods (e.g. Lee et al., 1987) including one freeze-tolerant species (Lee et al., 2006). However, the most dramatic examples of these changes to date

– and epitomizing the notion of superplasticity – are found in Antarctic terrestrial micro-arthropods. Diurnal sampling by Worland and Convey demonstrated that the chill-tolerant Antarctic collembolan *C. antarcticus* was capable of changing its supercooling point from 'winter' to 'summer' modes over a matter of hours (Worland and Convey, 2001). Recently, laboratory experiments on the Antarctic mite *Halozetes belgicae*, which feeds on crustose lichens on extremely exposed rock surfaces, have shown that a warm-acclimated phenotype is capable of changing to a cold-hardy winter-acclimated phenotype after just 2 h of low-temperature acclimation (Hawes et al., 2007) – in terms of the rate and depression of median lethal temperature (>15°C), this is the most potent RCH response yet induced in any arthropod. It is the kind of change that most other arthropods require an entire season to achieve.

Alternatives to plasticity

So far, low-temperature adaptations have been considered exclusively in the context of plasticity. However, it should be noted that although plasticity represents a widely distributed strategy among the Arthropoda, other strategies are also employed. In other contexts, much work has gone, and continues to go, into evaluating and modelling the costs and benefits of plasticity as a mode of performance (e.g. Berrigan and Scheiner, 2004). This is, however, relatively new ground for low-temperature biology. Rather than entering into too much speculation, it is worth just noting the alternatives and providing a few examples that exemplify these scenarios (Table 1). The evolutionary 'popularity' of these strategies varies – there are, for example, a large number of species that have adopted specialization in one form or another (e.g. freeze tolerance, overwintering life stages); but much less is known about the prevalence of strategies like bet-hedging. It should also be noted that plasticity or its 'alternatives' are not necessarily mutually exclusive strategies – indeed, as with species that employ a mixed-strategy cryotype, there may be numerous evolutionary 'cocktails' of plastic and fixed traits.

The Arctic collembolan *Hypogastrura tullbergi*, for example, is a moderately chill-tolerant freeze avoider that acquires a cold-hardy phenotype over winter. It shows little flexibility in summer cold-tolerance levels (Hawes et al., 2006), and overwintering mortality is greatly increased by manipulations of winter stress (Coulson et al., 2000). Thus, although on the one hand it does show seasonal phenotypic plasticity, its inflexibility outside typical environmental parameters suggests that this seasonal strategy is itself 'fixed' at a general level of tolerance appropriate to predictable summer and winter temperatures, rather than acclimating or acclimatizing to novel stress regimes (Coulson et al., 2000; Hawes et al., 2006). By contrast, the sub-Antarctic beetle *Hydromedium sparsutum* is specialized for freeze tolerance, but when low temperature stress is increased (through repetition of sub-zero exposure) plasticity catalyzes the expression of a bet-hedging strategy in which a proportion of the population retain their fixed strategy of freezing at high sub-zero temperatures while a proportion lower their supercooling point to low sub-zero temperatures (Bale et al., 2001). One determinant of the character of such cocktails may be the scale at which organisms experience

variability – and therefore require plastic rather than fixed responses.

Conclusion

Arthropods at low temperature provide a good model for looking at the diversity of evolutionary responses to a common stress. Plasticity forms a crucial, but not exclusive, component of these responses. Both its expression and its absence provide informative routes into disentangling the relationships between selection and environment in arthropods adapted to low temperature.

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