Commentary

Physiological variation and phenotypic plasticity: a response to 'Plasticity in arthropod cryotypes' by Hawes and Bale

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Summary

In a recent publication, Hawes and Bale provide an extended discussion of phenotypic plasticity in the context of low temperature responses of animals. They argue that phenotypic plasticity may be partitioned phylogenetically at several levels and go on to explore these levels, and cold hardiness strategies that they term cryotypes, which in their view constitute cryotypic plasticity. Here we argue that this attempt to partition plasticity is misleading, that the term 'genotypic plasticity' is potentially highly confusing and a misnomer for physiological variance, and that the term 'superplasticity' should not be used. We also show that a definition of strategies as cryotypes is not useful and that the hypothesis about the relationship between evolutionary derivation and extent of plasticity in freeze-avoiding *vs* freeze-tolerant species is not supported by current evidence.

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Introduction

Recently, the ecological implications of physiological diversity and its underlying mechanisms have risen to the fore, particularly as physiologists are increasingly being called on to help address some of humanity's most pressing environmental problems (Spicer and Gaston, 1999; Helmuth et al., 2005; Chown and Gaston, 2008). The significance of phenotypic plasticity as a major component of this variation is now being widely recognized, and in consequence has grown as a research focus in physiology (e.g. Angilletta et al., 2002; Franklin et al., 2007; Kristensen et al., 2008), so augmenting the substantial, and sometimes controversial, literature that already exists on the topic (for reviews, see West-Eberhard, 2003; DeWitt and Scheiner, 2004a; Ghalambor et al., 2007). Given this increasing interest in the plasticity of physiological traits, we examine here the recent commentary on phenotypic plasticity in arthropod cryotypes by Hawes and Bale (Hawes and Bale, 2007). In particular, we focus on the conceptual approach to and definitions of plasticity outlined in Hawes and Bale, which we will argue differ substantially from much of the modern literature; consider their perspectives on the extent of phenotypic plasticity [*sensu* West-Eberhard, p. 34 (West-Eberhard, 2003)] of cold hardiness strategies (their 'cryotypes'), for which, in our view, considerable evidence is to the contrary; and draw attention either to alternative conceptual approaches or to alternative interpretations for the proposals they have made.

Phenotypic plasticity?

Hawes and Bale [(see p. 2585 of Hawes and Bale (Hawes and Bale, 2007)] define phenotypic plasticity as 'a measure of organism malleability,' and then go on to argue that 'phylogenetically' it may be partitioned at several 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).' Later (on p. 2589), they define genotypic plasticity as '…variation in the relative physiological limits of different phenotypes.' They also argue (p. 2590) that the genetic contribution to phenotypic changes in cold tolerance that occurs in response to acclimation or acclimatization processes '…is determined over evolutionary time by selection for a particular cryotype and over geographic and climatic clines by genotypic plasticity.'

Defining plasticity as a measure of organismal malleability stems most recently from Huey and Berrigan [see p. 207 of Huey and Berrigan (Huey and Berrigan, 1996)], and is in keeping with other definitions of plasticity, such as those of West-Eberhard [see p. 34 of West-Eberhard (West-Eberhard, 2003)]: '…the ability of an organism to react to an environmental input with a change in form, state, movement, or rate of activity,' and DeWitt and Scheiner [see p. 2 of DeWitt and Scheiner (DeWitt and Scheiner, 2004b)]: '…the environmentally sensitive production of alternative phenotypes by given genotypes.' The problem arises with Hawes and Bale's 'phylogenetic partitioning' of the term, which we think is not only incorrect, but if adopted will lead to renewed confusion in a field that has only just emerged from a siege of semantic and theoretical difficulties (for reviews, see Stearns, 1989; West-Eberhard, 2003; DeWitt and Scheiner, 2004a). We hold this view for several reasons.

First, the term 'single phenotype' to our minds means a particular character state or form of a trait, or form of a complex of traits, at a given point in time. A 'single phenotype' cannot be characterized

by 'malleability' or be included in other common definitions of plasticity. As soon as the phenotype changes, for argument's sake in response to a low temperature event, multiple phenotypes are involved.

Second, to define genotypic plasticity as '...variation in the relative physiological limits of different phenotypes' ignores well-established theory that total phenotypic variance is partitioned as:

$$V_P = V_G + V_E + V_{G \times E} + V_{error} , \qquad (1)$$

which includes genetic effects (V_G), a systematic environmental effect (V_E), a genotype by environment interaction ($V_{G \times E}$) [the extent and form of which may be heritable (e.g. Via and Lande, 1985; Ghalambor et al., 2007)] and error (Via and Lande, 1985; DeWitt and Scheiner, 2004b). In consequence, 'genotypic plasticity' is a misnomer for physiological variation or total physiological variance. These latter terms are entirely apt and we see no reason why variation or variance should now be termed genotypic plasticity, when in fact variation can result in several ways.

Third, we cannot understand why a particular, presumably broad, physiological response (or set of similar responses), that is consistent amongst different taxa (recalling that the term taxa covers species to kingdoms) should be labelled plasticity (cryotypic plasticity in this instance). Such a response or set of responses is likely to be the outcome of a range of evolutionary pathways. These might vary from some form of phylogenetic conservatism (or signal) [we avoid use of the term constraint (for details, see Ketterson and Nolan, 1999; Roff and Fairbairn, 2007)] to convergent evolution. The latter, in turn, might have arisen from selection for a fixed strategy under all environmental conditions to selection for marked phenotypic plasticity (see Lively, 1986; Moran, 1992; Scheiner, 1993; Tufto, 2000; Berrigan and Scheiner, 2004; Ghalambor et al., 2007). In our view, labelling such a set of responses 'plasticity' precludes any sensible use of the term.

Recognizing that physiological and life history responses typically form a continuum, and frequently vary over a range of temporal and spatial scales (Chown, 2001; Hoffmann et al., 2003; Gaston et al., 2008), but that categorization, or a framework of concepts, can often promote scientific understanding of complex variation (Mayr, 1982), we think that broadly similar physiological responses could more simply be termed 'strategies' or 'categories'. This has long been the usage both in the physiological (e.g. Salt, 1961; Bale, 1993; Hadley, 1994; Sømme, 1995; Willmer et al., 2000) and life-history (Southwood, 1977; Southwood, 1988) literature. Such categorization may later outlive its usefulness [e.g. Roff, pp. 77–79, for *r*- and *K*-selection (Roff, 2002)], but if it is beset from the start with terminological and theoretical ambiguity it is likely to confound substantially the field of study. Hawes and Bale (Hawes and Bale, 2007) equate cryotype and strategy (p. 2586), but earlier insist that cryotypes represent a form of plasticity.

In our view, at least part of the confusion stems from a commonly held, but incorrect, view that genetic and environmental effects are exclusive entities. As DeWitt and Scheiner [see p. 3 of DeWitt and Scheiner (DeWitt and Scheiner, 2004b] have so clearly pointed out, the question of whether variation is plastic or genetic is 'enduring and perennially misleading'. Further difficulty may also have arisen because the terms plasticity and G×E interactions apply at the level of both individual genotypes and populations of genotypes (Pigliucci, 2005). Because plasticity is defined as the ability of an organism to react to an environmental input, a slope (positive or negative) in the environment–phenotype space indicates plasticity at the individual level, and plasticity at the

population level if the average difference among environments across genotypes is considered.

Inappropriate analogies and terminology

Hawes and Bale (Hawes and Bale, 2007) describe phenotypic plasticity using a rubber band analogy, arguing that low temperature is the 'hand' that stretches the band – or physiology – and that plasticity is a measure of how far the band/physiology can be stretched. The analogy is used to illustrate the characteristics of plasticity, which according to Hawes and Bale are that it 'stretches the limits of physiological response' and is 'impermanent', but the analogy is misleading. Although, where present, phenotypic plasticity will alter the physiological phenotype, such plasticity need not increase the degree of low temperature tolerance, but may well reduce it. Moreover, plasticity does not require that the optimal condition is constant, i.e. that the rubber band is attached to some point from which the hand may stretch it. Rather it is likely that it moves the 'operative' temperature range (e.g. Huey and Kingsolver, 1993; Pörtner, 2001; Pörtner, 2002). To avoid confusion, it may be better, rather, simply to use the term reaction norm, with the form (slope in continuous characters) of the reaction norm indicating the extent of plasticity [see figure 1 in Ghalambor (Ghalambor et al., in press)].

Later, Hawes and Bale (Hawes and Bale, 2007) argue that '....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.' This description is not clear, particularly when compared with previous definitions of plasticity, such as 'the change in the expressed phenotype of a genotype as a function of the environment,' provided by Scheiner (Scheiner, 1993), and used in the same paragraph of Hawes and Bale. Moreover, Hawes and Bale's statement suggests that acclimation and acclimatization are somehow independent of the environment experienced by the individual, which is contrary to all previous definitions thereof [e.g. p. 9 of the study by Willmer and colleagues (Willmer et al., 2000)]. Likewise, restricting plasticity to transient responses contrasts completely with previous literature (e.g. Huey and Berrigan, 1996; DeWitt and Scheiner, 2004a). Developmental plasticity is often not reversible [e.g. in dispersal polymorphisms (reviewed by Zera and Denno, 1997); and adult size in insects (Atkinson, 1994)], and no logical reason exists to exclude non-reversible phenotypic changes from definitions of plasticity (for details, see Wilson and Franklin, 2002; Piersma and Drent, 2003) (Ghalambor et al., in press). Hawes and Bale's suggestion that 'Basal physiological responses become physiologically plastic when a constitutive change in the phenotype takes place,' is similarly confusing because the sources of variation in the phenotype are not adequately distinguished (see above).

Similarly, it is our view that the term 'superplasticity', which Hawes and Bale (p. 2590) (see also Hawes et al., 2007) have coined for cases of high levels of plasticity that are 'distinguished from standard "labile" responses,' and that 'operate at temporal and/or physiological scales in excess of environmental variation,' is not useful for several reasons. Perhaps most significant among these is that, to date, little evidence exists that the rapid and sometimes large responses described by Hawes and Bale as 'superplasticity' really do exceed environmental variation. This is the justification for the use of the term, because their 'standard labile responses' refer to Scheiner's definition [see p. 38 of Scheiner (Scheiner, 1993)] of a labile trait as one where '...the phenotype of the individual can change at least as fast as the environment...'. Hawes and Bale use two examples to justify the use of this term. The first, by Worland

and Convey (Worland and Convey, 2001), includes microclimate data indicating concurrent rapid change in temperature and physiology. Indeed, Worland and Convey [see p. 515 of Worland and Convey (Worland and Convey, 2001)] claim that they have documented '…a hitherto unrecognized capacity to alter cold hardiness in summer in response to environmental temperature cues over a shorter timescale than previously thought…'. The second example from their own work (Hawes et al., 2007) includes no relevant data on short-term variation in temperature, with the exception of reference to an earlier paper (Hawes et al., 2006), which does not include such explicit data either.

Unpredictable and substantial temperature changes are a hallmark of many maritime Antarctic and other southern hemisphere sites and the extent of physiological change typically reflects the rate and magnitude of these changes (Walton, 1984; Pugh and MacAlister, 1994; Kennedy, 1995; Worland and Convey, 2001; Sinclair et al., 2003a; Sinclair et al., 2003b; Sinclair and Chown, 2005). Moreover, Scheiner's (Scheiner, 1993) definition of labile traits suggests that the change is 'at least as fast as the environment,' and therefore includes responses that are faster. In consequence, it is our view that the existing terminology in both the phenotypic plasticity and rapid cold hardening (RCH) literature is adequate and that the term 'superplasticity' is redundant. No need exists for special terminology to distinguish among different degrees of plasticity, including RCH, because the degree of plasticity will change on a continuous scale with environmental conditions and the traits in question. As one of us has argued elsewhere (Loeschcke and Sørensen, 2005), the terminology used is perhaps of less interest and importance than the requirement to report clearly, and where feasible to control carefully, the state/stage/age of the organisms, the traits investigated, and the experimental treatments applied. Indeed, we agree with Hawes and Bale (p. 2588) that 'it seems sensible to utilize, with qualification, already flexible nomenclature rather than invent new terminology.'

Finally, we disagree with Hawes and Bale (p. 2586) that the most fundamental measure of fitness in relation to low temperatures is survival. Endler [see pp. 33–50 of Endler (Endler, 1986)] provides a comprehensive discussion of fitness as a concept, and defines it as '…the degree of demographic difference among phenotypes…'. Clearly, survivorship is one component of fitness, but it is not the only one. If a broader view of fitness is taken than the one Endler (Endler, 1986) has proposed then it might also be argued that, while survival to first reproduction or between reproductive bouts is a necessary component of fitness, it is not sufficient without that reproduction (e.g. Sibly and Calow, 1986; Roff, 2002). Moreover, although survival is often used as a convenient estimate of how a trait might contribute to fitness, it may also neglect significant sublethal effects (Layne and Peffer, 2006). Survival traits ignore all processes and effects that occur before mortality sets in and reduce fitness to a binominal state. This does not fit well with an ecological reality where organisms will be exposed to continuous changes in temperature, and reproductive or behavioural traits important for reproduction might be strongly affected well before survival itself is influenced, such as is seen for exposure to both low (Shreve et al., 2004) and high temperatures (Fasolo and Krebs, 2004; Jørgensen et al., 2006).

Strategies and plasticity

The 'cryotypes' listed by Hawes and Bale reflect a classification scheme for cold hardiness that has a venerable and controversial history (e.g. Salt, 1961; Block, 1982; Baust and Rojas, 1985; Bale, 1993; Bale, 2002; Sømme, 2000; Nedvěd, 2000). Recent work has

been at pains to point out the considerable variation within each of the more traditional strategies (freeze avoiding and freeze tolerance) and to demonstrate that they incorporate a wide range of responses and exclude some others (Bale, 1987; Bale, 1993; Sinclair, 1999; Holmstrup et al., 2002). Indeed, the diversity of mechanisms employed (reflecting, no doubt, the fact that insects have invaded and re-invaded cold habitats on multiple occasions) is substantial, and thus far no single biochemical or physiological adaptation has been identified that is both necessary and sufficient for any of the cold tolerance strategies that have been described. For example, even the ice nucleating proteins (INPs) described as an important component of freeze tolerance by Hawes and Bale (p. 2587) are neither necessary [in many cases, even species that have INPs have much more potent nucleators within the material in their gut (e.g. Worland et al., 1997)] nor sufficient [some species with these haemolymph INPs are not freeze tolerant (Sinclair et al., 1999)] for survival of freezing. In consequence, the recognition of a variety of responses to low temperature that constitute a continuum of those possible under any strategy [see figure 5.17 of Chown and Nicolson (Chown and Nicolson, 2004)] has enabled these responses to be interpreted in the light of the extent and predictability of the environmental variation encountered by the animals concerned (see also Zachariassen, 1985; Sinclair and Chown, 2005). For this reason we cannot see why this variation should now be collapsed back into cryotypes, and that statements should be made about 'true' cold-hardy phenotypes, when it is not clear what a false cold-hardy phenotype might constitute. If the latter refers to acclimatization, it is apparent that hardening, acclimation, acclimatization and seasonal responses to low temperature are part of a continuum of responses that are difficult to distinguish. Seasonal responses to low temperature can be considered an acclimatization response, just as rapid responses to temperature change can. Indeed, the work by Hawes and colleagues (Hawes et al., 2007), Sinclair and colleagues (Sinclair et al., 2003b) and Worland and Convey (Worland and Convey, 2001) suggests that the responses thought previously to be restricted to seasonal temperature changes may be expressed more rapidly. For these reasons we find the discussion of cryotypes unhelpful and potentially misleading.

In their figure 1 and the accompanying text, Hawes and Bale (Hawes and Bale, 2007) further make the case that freeze tolerance is relatively non-plastic (we interpret their statements to imply that 'plasticity' in this case is a change in lower lethal temperature, LLT), and that the more evolutionarily derived, the less plasticity will be expressed. Little is known about the evolution of freeze tolerance in insects and other arthropods. Freeze tolerance has arisen on multiple occasions (Sinclair et al., 2003a), and it is generally assumed that many of the physiological and biochemical mechanisms are convergent between the species that survive freezing. Hawes and Bale suggest that while increasing cold hardiness (which they assume to indicate a more evolutionarily derived state) is associated with increased plasticity in freeze avoiders, the reverse is true for species that are freeze tolerant. It is unclear how Hawes and Bale determine 'plasticity', but in our Fig. 1 we examine Hawes and Bale's hypothesis using 15 diverse species for which seasonal variation in cold hardiness (measured as LLTs) is available. Both freeze-tolerant and freeze-avoiding species trace a trajectory similar to that illustrated by the 'freeze avoidance' line in Hawes and Bale's figure 1 (Hawes and Bale, 2007), suggesting that little basis exists to suppose freeze-tolerant species to be either less phenotypically plastic or more specialized than freeze avoiders. In addition, we note that significant and rapid
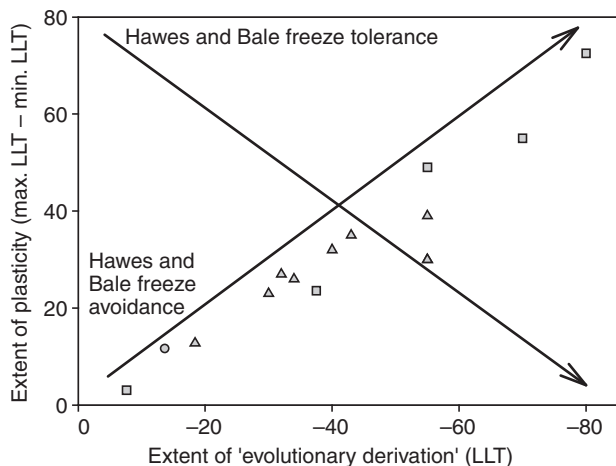
Fig. 1. Data applied to test Hawes and Bale's (Hawes and Bale, 2007) hypotheses. Fifteen arthropod species for which seasonal variation in cold hardiness are available were used (see supplementary material Table S1 for the species list and data). Freeze-tolerant species are indicated with squares, freeze-avoiding species with triangles, and a solitary chill-susceptible species (the house spider *Acharaenae tepidariorum*) with a circle. Note that two points, for *Rhabdophaga strobiloides* and *Mayetiola rigidae*, overlap completely. The arrows indicate the hypotheses erected in figure 1 of Hawes and Bale (Hawes and Bale, 2007). No phylogenetic correction was applied to these data, although they represent seven orders and 11 families. Following Hawes and Bale's description, the extent of 'evolutionary derivation' was taken as the extreme lower lethal temperature (LLT, in °C), and the 'extent of plasticity' as the summer (or non-acclimated) LLT minus winter (or acclimated) LLT (in °C). Note that this procedure might introduce an element of non-independence of the axes (see Brett, 2004), which would have to be given consideration in further tests of the hypothesis. The greater variation in LLT of the freeze-tolerant than in the freeze-avoiding species is likely because of homogeneous nucleation in the latter (see also Zachariassen et al., 2004): no insect supercooling points below –54°C have been reported in either strategy (Addo-Bediako et al., 2000), although freeze-tolerant species can survive well below their supercooling point.

short-term phenotypic plasticity [a RCH response (see Lee et al., 1987)] has been described in many freeze-avoiding species (e.g. Chown and Nicolson, 2004) and at least one freeze-tolerant species (Lee et al., 2006), suggesting no absolute limit to the rate or extent of phenotypic change in freeze-tolerant *vs* freeze-avoiding species. Using a dataset of 53 freeze-tolerant species, Sinclair (Sinclair, 1999) showed that the range of LLTs of freeze-tolerant species paralleled that of freeze avoiders, and argued that freeze tolerance, far from being a specialized strategy, is simply an alternative way of tolerating any given range of sub-zero temperatures, although circumstances may exist in which one strategy or another might be particularly advantageous (Zachariassen, 1985; Voituron et al., 2002; Sinclair et al., 2003a). Thus, the available evidence of variation and phenotypic plasticity in freeze-tolerant species does not fit with Hawes and Bale's view of freeze tolerance as an ultra-specialized strategy whose capacity for plasticity declines with increasing 'evolutionary derivation', and we recommend that the hypothesis presented in their figure 1 be rejected.

Conclusion

After a considerable period of neglect, the significance of phenotypic plasticity as a source of variation and its role in the evolution of responses to the environment is once again being recognized (West-Eberhard, 2003; Grether, 2005; Suzuki and

Nijhout, 2006). This renewed interest in both adaptive and non-adaptive plasticity (Ghalambor et al., 2007) has grown most rapidly in the case of life history traits and development. Whilst in a dynamic field such as this one disagreements continue to flourish, many early debates and sources of potential confusion have been clarified (Via et al., 1995; West-Eberhard, 2003; DeWitt and Scheiner, 2004a). As recognition of the importance of phenotypic plasticity grows in other fields, such as low temperature physiology, a risk exists that these very same early problems and sources of confusion may be re-introduced. Careful consideration of the broader literature on plasticity, especially those works where problems have been resolved or clearly articulated, should help avoid such a situation. Moreover, adopting the conventions and terminology now agreed by the broader field will provide prophylaxis against theoretical confusion. Clearly, consideration of phenotypic plasticity in traits previously not examined may change theory more generally. However, to be useful the changes should not be narrowly discipline specific or semantically confusing.

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