POINT OF VIEW

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Insect cold tolerance: How many kinds of frozen?

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Abstract. Insect cold tolerance mechanisms are often divided into freezing tolerance and freeze intolerance. This division has been criticised in recent years; Bale (1996) established five categories of cold tolerance. In Bale's view, freezing tolerance is at the extreme end of the spectrum of cold tolerance, and represents insects which are most able to survive low temperatures. Data in the literature from 53 species of freezing tolerant insects suggest that the freezing tolerance strategies of these species are divisible into four groups according to supercooling point (SCP) and lower lethal temperature (LLT): (1) Partially Freezing Tolerant-species that survive a small proportion of their body water converted into ice, (2) Moderately Freezing Tolerant-species die less than ten degrees below their SCP, (3) Strongly Freezing Tolerant-insects with LLTs 20 degrees or more below their SCP, and (4) Freezing Tolerant Species with Low Supercooling Points which freeze at very low temperatures, and can survive a few degrees below their SCP. The last 3 groups can survive the conversion of body water into ice to an equilibrium at sub-lethal environmental temperatures. Statistical analyses of these groups are presented in this paper. However, the data set is small and biased, and there are many other aspects of freezing tolerance, for example proportion of body water frozen, and site of ice nucleation, so these categories may have to be revised in the future. It is concluded that freezing tolerance is not part of Bale's (1996) continuum, but rather a parallel, alternative strategy of cold tolerance.

INTRODUCTION

Research into insect cold tolerance is underpinned by the paradigm that there are two strategies of cold survival: (1) Freeze intolerance, the survival of sub-zero temperatures by preventing the formation of ice crystals within the body and (2) Freezing tolerance, the survival of sub-zero temperatures by withstanding the formation of ice in the body (Lee, 1991; Storey & Storey, 1988). This paradigm provided the framework for a number of comparative and single species studies (for example, those reviewed by Lee, 1991), in which the supercooling point (SCP: the temperature at which an animal freezes) was emphasised as an indicator of "strategy", of lower lethal temperature, and, by proxy, of cold hardiness (Baust & Rojas, 1985). Criticism by Baust & Rojas (1985) of the SCP as a phenomenological measure, and a subsequent reply by Bale (1987) have led to more scepticism of the freezing tolerance/intolerance dichotomy as the sole descriptor of cold hardiness. Most important among these criticisms was the observation that SCP was not necessarily related to cold hardiness in those animals which were freezing intolerant (Bale, 1987, 1991, 1993; early work by Salt is reviewed by Ring & Riegert, 1991).

Bale (1996) proposed a new categorisation of insect cold tolerance, ranging in scope from least to most cold hardy, based upon when mortality occurs:

Opportunistic species cannot enter a dormant state, and die when temperatures are too low to maintain normal metabolism.

Chill-susceptible species die after brief chilling at moderate to high sub-zero temperatures.

Chill-tolerant (highly or moderately) describes those animals that die after prolonged chilling at moderate to low sub-zero temperatures.

Freeze avoiding insects are those that can survive extensive periods in the supercooled state, but die when they freeze (i.e., at the SCP).

Freeze tolerant insects are all those that can survive the formation of ice in their body tissues.

Bale (1996) argues that the last group will predominate in the more extreme alpine and polar environments. Essentially, Bale (1996) has removed the dichotomy of freezing-tolerance/avoidance and replaced it with a continuum of cold hardiness. He describes freezing tolerance as being a "relatively clear" situation: animals in this group "usually freeze between –5 and –10°C through the action of ice nucleating agents, but some freeze at lower temperatures... Once frozen, they can be cooled to much lower temperatures (e.g., –50°C) and on warming, they thaw, recover and show (as far as is known) normal developmental, reproductive and behavioural capabilities" (Bale, 1993). Bale (1996) notes that there are some exceptions to this rule, but states criteria leading to a description of freezing tolerance as above.

In spite of this re-classification, it is still common for authors (e.g., Neufeld & Leader, 1998) to make the distinction between freezing tolerance and freeze intolerance, suggesting a disinclination to give up this dichotomy. There is also suggestion in the literature that freezing tolerance does not constitute a single strategy, but may be a continuum in itself. For example, Ring (1982) published a paper entitled "Freezing tolerant insects with low supercooling points"; Worland et al. (1997) described the alpine cockroach *Celatoblatta quinquemaculata* as "moderately freezing tolerant" and Hart & Bale (1997) described *Syrphus ribesii* as "strongly freeze-tolerant". Klok & Chown (1997), in their discussion of the sub-Antarctic caterpillar *Pringleophaga mari-*

oni suggested that Bale's (1993) approach of considering less cold-hardy freeze avoiders separately could be usefully applied to freezing tolerant species as well. Ramløv (1998), however, clearly feels that such divisions are neither supported by data, nor necessary. In his words "...the insects are freeze-tolerant and that should be sufficient to describe them ... researchers in the field should read the original literature to get a description of the actual freeze tolerance of that particular insect".

In this paper I will argue (1) that freezing tolerance is not a part of a continuum with freezing intolerance, but rather a distinct, parallel and therefore *alternative* strategy from those insects that cannot survive freezing; (2) that there are clearly defined groups within freezing tolerance that represent different environmental tolerances and provide useful foci for comparative research; and (3) that this view may be integrated into Bale's (1996) description of categories of freeze avoidance.

Like Bale (1996), my rationale rests upon the relationship between the freezing point (the SCP), and the "observed limits of ... cold tolerance" (Bale, 1996): the lower lethal temperature (LLT).

METHODS

Freezing points and lower lethal temperatures for freezing tolerant insect species were collated from the literature and from unpublished data (Sinclair, unpubl.). Species were divided into groups by SCP and LLT according to the suggestions or implications of some authors (e.g., Hart & Bale, 1997; Worland et al., 1997; Ring, 1982). These groups were examined for integrity using Canonical Discriminant Function analysis and ANOVA with Tukey's HSD post-hoc comparisons, and the relationships within groups examined using linear regression and compared using multiple t-tests. Independence of taxon and category was tested using MANOVA with Type III sums of squares. Statistical analyses were performed using SAS/STAT (SAS Institute Inc., 1989).

RESULTS AND DISCUSSION

A dataset comprising LLT and SCP for 53 freezing tolerant insect species was compiled and is presented in Table 1. There is bias toward several groups (for example, the New Zealand stenopelmatid weta), although both hemimetabolous and holometabolous insects are represented.

On the basis of LLT and SCP, four groups were identified among the freezing tolerant insects: Partially Freezing Tolerant, Moderately Freezing Tolerant, Strongly Freezing Tolerant and Freezing Tolerant with a Low SCP (Table 1, Fig. 1).

Partial Freezing Tolerance

These are species that can survive some formation of ice in the body, but apparently do not survive if ice formation goes to an equilibrium at (or above) the SCP (eg: *Tipula paludosa* (Todd & Block, 1995), *Hemideina thoracica* (Sinclair et al., 1999). They freeze at a relatively high temperature, and die before their body temperature equilibrates with the environment, but will survive if warmed part way through the freezing process. Mortality in any insect species appears to be dependent upon the proportion of body water converted into ice, although truly freezing tolerant species can withstand the formation of ice to an equilibrium at a sublethal temperature (Zachariassen, 1991). Partially Freezing Tolerant species do not appear to survive an equilibrium proportion of ice (represented by the completion of the exotherm at a given temperature). It is unclear whether this case (survival of partial freezing) represents the norm among freeze avoiding insects that die at the SCP, or whether Partial Freezing Tolerance constitutes a separate group that is a freezing tolerant strategy. Further work on species that are considered to be freeze avoiding would serve to refine this distinction. The ecological significance of this group is likely to be negligible, although it is possible to envisage situations where transient sub-zero temperatures could be experienced [for example, lowland *Hemideina* species at high altitudes in New Zealand's North Island (Sinclair et al., 1999)]. Partial Freezing Tolerance is, however, fascinating from an evolutionary point of view, because it is suggestive of an intermediate position between Freeze Avoidance (no survival of ice in the body) and Freezing Tolerance (survival of an equilibrium proportion of ice in the body).

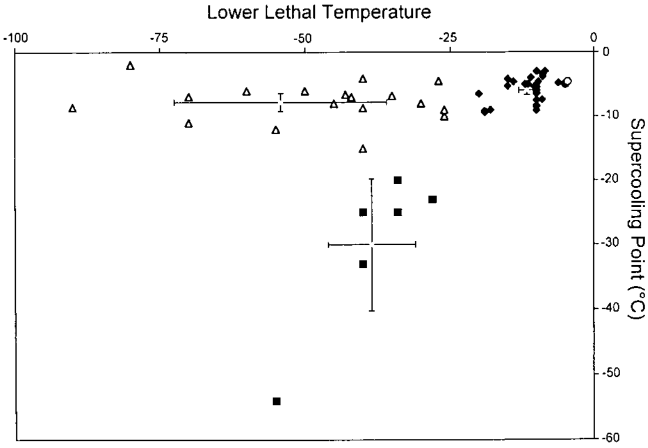

Fig. 1. Relationships between supercooling point (SCP) and Lower Lethal Temperature (LLT) for 53 species of freezing tolerant insects. Groups were defined a priori: ○ = Partially Freezing Tolerant; ◆ = Moderately Freezing Tolerant; △ = Strongly Freezing Tolerant; ■ = Freezing Tolerant with a Low Supercooling Point. Error bars represent 95% confidence intervals around the means for each group.

The next three categories include all those insects that can survive the conversion of an equilibrium proportion of body water into ice at a given (sub-lethal) temperature. This was assumed if they could survive completion of the exotherm, and usually survival for more than 24 h was tested.

Moderate Freezing Tolerance

These are species similar to those alluded to by Worland et al. (1997) and Klok & Chown (1997), that freeze at a high temperature, and die less than ten degrees below their SCP, without any further observable freezing events. They are able to survive long periods frozen (e.g., *Cryptocercus punctulatus* can survive 205 days frozen at −10°C (Hamilton et al., 1985), tend to come from milder

Table 1. Freezing tolerant insect species used in this analysis. Groups defined a priori: p = Partially Freezing Tolerant, m = Moderately Freezing Tolerant; s = Strongly Freezing Tolerant; l = Freezing Tolerant with a Low SCP.

Order	Family	Species	Life stage	Habitat	SCP	LLT	Group	Reference
Coleoptera	Carabidae	*Damaster blaptoides rugipennis*	adult	decaying wood – Northern Japan	–6.3	–10	m	Ohyama & Asahina, 1972
		Pterostichus brevicornis	adult	Rotting wood in Arctic Alaska	–11	–70	s	Miller, 1982
		Pterostichus orientalis	adult	decaying wood – Northern Japan	–7.5	–10	m	Ohyama & Asahina, 1972
	Chrysomelidae	*Melasoma collaris*	adult	alpine Scandinavia	–4.5	–27	s	Gehrken & Southon, 1997
		Phratora sp.	adult	under loose bark, Alaska	–8	–45	s	Miller, 1982
		Phyllodecta laticollis	adult	beneath bark	–7	–42	s	van der Laak, 1982
	Cucujidae	*Cucujus clavipes*	larva	under bark in northern temperate forests	–10	–26	s	Duman, 1984
	Ostomatidae	*Ostoma ferruginea*	adult	under loose bark	–12	–55	s	Miller, 1982
	Perimylopidae	*Hydromedion sparsutum*	larva	sub-antarctic	–3.8	–8.9	m	Worland et al., 1992
		Hydromedion sparsutum	adult	sub-antarctic	–3	–8.5	m	Worland et al., 1992
		Perimylops antarcticus	larva	sub-antarctic	–5.4	–10.4	m	Worland et al., 1992
		Perimylops antarcticus	adult	sub–antarctic	–4.6	–9.7	m	Worland et al., 1992
	Pyrochroidae	*Dendroides canadensis*	larva	beneath bark	–9	–26	s	Horwath & Duman, 1984
	Pythidae	*Pytho deplanatus*	larva	Canadian alpine	–54	–55	l	Ring, 1982
		Pytho americanus	larva	Canadian Arctic – decaying wood	–4.1	–40	s	Ring & Tesar, 1980
		Pytho americanus	adult	Canadian Arctic – decaying wood	–8.7	–40	s	Ring & Tesar, 1980
	Tenebrionidae	*Bolitophagus reticulatus*	adult	high latitude temperate forest	–6.5	–20	m	Gehrken et al., 1991
		Upis ceramboides	adult	under bark in Arctic Alaska	–6	–60	s	Miller, 1982
	Silphidae	*Phosphuga atrata*	adult	decaying wood – Northern Japan	–8.3	–10	m	Ohyama & Asahina, 1972
Dictyoptera	Blattidae	*Celatoblatta quinquemaculata*	nymphs and adults	New Zealand alpine grasslands	–3.4	–8.9	m	Sinclair, 1997
		Periplaneta japonica	nymphs	decaying wood, Northern Japan	–7.4	–9	m	Tanaka & Tanaka, 1997
	Cryptocercidae	*Cryptocercus punctulatus*	adult	decaying logs in Northern US	–5.5	–10	m	Hamilton et al., 1985
Diptera	Bibionidae	*Bibio rufiventris*	larva	in soil, Japan	–3	–10	m	Sakagami et al., 1983
	Chironomidae	*Belgica antarctica*	larva	maritime Antarctic	–5.3	–15	m	Baust & Edwards, 1979
	Drosophilidae	*Chymomyza costata*	diapausing larva	Northern Hemisphere	–2	–80	s	Shimada & Riihimaa, 1988
	Mycetophilidae	*Mycetophila* sp.	adult	under bark	–33	–40	l	Miller, 1982
	Syrphidae	*Syrphus ribesii*	larva	UK Temperate leaf litter	–6.8	–35	s	Hart & Bale, 1998
	Tephritidae	*Eurosta solidaginis*	larva	Goldenrod galls, inland North America	–6	–50	s	Baust & Lee, 1981
	Tipulidae	*Tipula excisa*	larva	mid alpine Norway 1,200 m	–4.8	–4.8	p	Todd & Block, 1995
		Tipula montana	larva	montane heathland (UK) 500 m	–4.7	–4.7	p	Todd & Block, 1995
		Tipula paludosa	larva	upland pasture UK 500 m and lowland habitats	–4.6	–4.6	p	Todd & Block, 1995
		Tipula trivittata	larva	decaying wood – inland North America	–8	–30	s	Duman et al., 1985
	Xylophagidae	*Xylophagus* sp.	larva	Arctic	–6.6	–43	s	Ring, 1982
	Sciaridae	*Sciara* sp.	prepupa	inside stems, Japan	–4.2	–15	m	Tanno, 1977

TABLE 1 (continued).

Order	Family	Species	Life stage	Habitat	SCP	LLT	Group	Reference
Hymenoptera	Formicidae	*Camponotus obscuripes*	adult	decaying wood – Northern Japan	–8.5	–10	m	Ohyama & Asahina, 1972
	Ichneumonidae	*Chasmias* sp.	adult	decaying wood – Northern Japan	–6.5	–10	m	Ohyama & Asahina, 1972
		Hoplismenus pica japonica	adult	decaying wood – Northern Japan	–9.1	–10	m	Ohyama & Asahina, 1972
		Pterocormus molitorius	adult	decaying wood – Northern Japan	–6	–10	m	Ohyama & Asahina, 1972
	Tenthredinidae	*Trichiocampus populi*	prepupa	hanging on trees, Japan	–8.6	–196	s	Shimada, 1989
	Vespidae	*Vespula maculata*	adult queens	hibernacula in Northern temperate forests (rotten logs)	–4.6	–14	m	Duman & Patterson, 1978
Lepidoptera	Arctiidae	*Ctenucha virginica*	larva	northern North America	–9	–18	m	Fields & McNeil, 1988
	Lymantriidae	*Gynaephora groenlandica*	larva	high Arctic	–6.9	–70	s	Kukal et al., 1988
	Noctuidae	*Agrotis* sp.	larva	alpine Hawaii	–4.8	–6.2	m	Duman & Montgomery, 1991
		Ostrinia nubilalis	larva	north temperate pasture lands	–23	–6.2	l	Grubor-Lajsic et al., 1992
	Nymphalidae	*Polygonia* sp.	adult	hibernacula	–25	–34	l	Miller, 1982
		Nymphalis antiopa	adult	hibernacula	–20	–34	l	Miller, 1982
	Oecophoridae	*Martyrhilda ciniflonella*	adult	beneath bark	–25	–40	l	Miller, 1982
	Tineidae	*Pringleophaga marioni*	larva	sub-Antarctic	–5	–11.5	m	Klok & Chown, 1997
Neuroptera	Hemerobiidae	*Hemerobius simulans*	adult	beneath bark	–15	–40	s	Miller, 1982
Orthoptera	Acrididae	*Arphia conspersa*	nymphs	Rocky Mountain montane meadows	–9.2	–19	m	Alexander, 1967
		Sigaus australis	adult	NZ alpine	–4	–11	m	Sinclair, unpubl.
		Xanthippus corallipes	nymphs	Rocky Mountain montane meadows	–9.4	–19	m	Alexander, 1967
	Stenopelmatidae	*Deinacrida connectens*	nymph	alpine zone (NZ)	–5	–12	m	Sinclair, unpubl.
		Deinacrida parva	adult	NZ alpine	–5	–5	p	Sinclair, unpubl.
		Hemideina maori	adult	NZ alpine zone	–5	–10	m	Ramløv et al., 1992
		Hemideina thoracica	adult	NZ alpine	–5	–5	p	Sinclair, unpubl.

(often alpine or sub-Antarctic, cf. Bale, 1996) environments, and the lower lethal temperature is close to the minimum environmental temperature. For example, *Celatoblatta quinquemaculata* has an LLT of –8.9°C, and a minimum recorded microenvironmental temperature of –7.3°C for that year (Sinclair, 1997).

Strong Freezing Tolerance

Hart & Bale (1997) describe Strongly Freezing Tolerant species as those whose LLT is 30°C or more below the SCP. They contrast these with species where there is only a few degrees difference between SCP and LLT – the species I have described as Moderately Freezing Tolerant. My grouping of Strong Freezing Tolerance includes those species whose SCP is above –15°C and their LLT below –20°C. Essentially, these species freeze at a relatively high temperature, yet can survive considerably lower temperatures than Moderately Freezing Tolerant species. The mean SCP of the Strongly Freezing Tolerant group (–7.8°C) does not differ significantly from that of Moderately Freezing Tolerant species (mean SCP = –5.9°C, Tukey's HSD $P > 0.05$). This group includes the "classic" model of freezing tolerant insects described by Bale (1996), for example, *Eurosta solidaginis* (Baust & Nishino, 1991) and *Syrphus ribesii* (Hart & Bale, 1997).

Freezing Tolerant with Low SCP

As described by Ring (1982), this represents a group of species with extremely low SCP (–25°C and below), but which can survive freezing to temperatures a few degrees below their SCP. For example, *Pytho deplanatus* has an SCP of –54°C, yet survives freezing to about –55°C (Ring, 1982).

Integrity of categories

Canonical Discriminant Function analysis on SCP and LLT for the four groups identified a priori defined a first canonical axis (eigenvalue 2.903) which explained 78.6% of variation. Log-likelihood ratios for the two axes con-

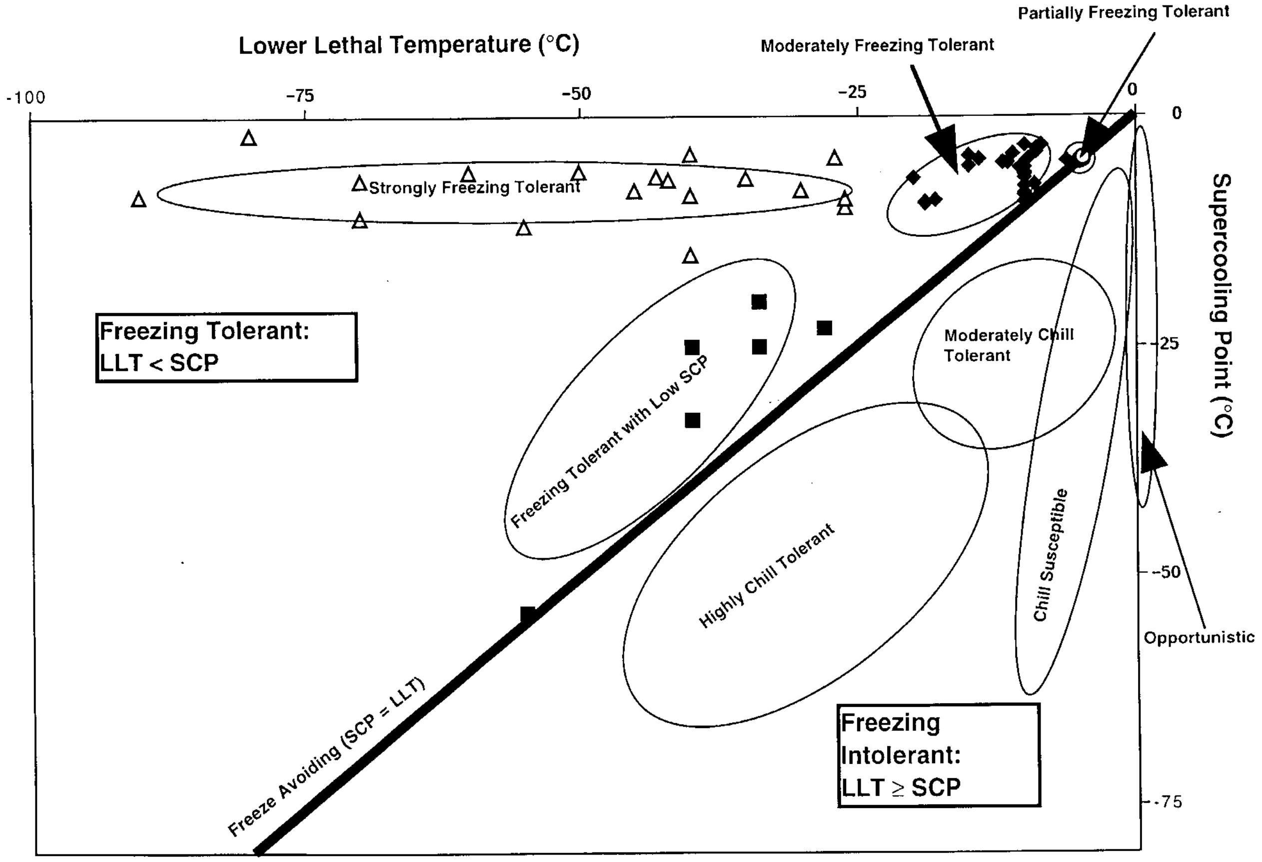

Fig 2. Schematic showing relationship between supercooling point and Lower Lethal Temperature for four freezing tolerant groups described in this paper, and five freezing intolerant groups described by Bale (1996).

firm that both axes significantly discriminate between the four groups (canonical axis 1 ratio = 0.143, approximate $F_{6,102} = 27.91$ $P < 0.0001$; canonical axis 2 ratio = 0.559, approximate $F_{2,52} = 20.50$ $P < 0.0001$). All members of the Partial Freezing Tolerant and Freezing Tolerant with Low SCP groups were correctly classified. 25% of Moderately Freezing Tolerant species were reclassified as Partially Freezing Tolerant, however, the Partial Freezing Tolerant group was defined a priori on the inability to survive the completion of the exotherm; this information was not included in the discriminant analysis as it is meaningless for all but this group. 22% of Strongly Freezing Tolerant species were reclassified as Moderately Freezing Tolerant, suggesting that there may be some overlap between the two groups. This may be indicative of a continuum between Moderately and Strongly Freezing Tolerant categories.

The four groups are found to differ significantly on the basis of both SCP ($F_{3,52} = 50.06$, $P < 0.001$) and LLT ($F_{3,52}=15.02$, $P < 0.001$). Post-hoc analysis shows that SCP does not differ significantly between Partially, Moderately or Strongly Freezing Tolerant groups, but that the SCP of these all differ significantly from the SCP of the Freezing Tolerant with Low SCP group ($P < 0.05$, Tukey's HSD). Moderately and Partially Freezing Tolerant species both had mean LLT significantly different to the Strongly Freezing Tolerant group ($P < 0.05$, Tukey's HSD).

Microhabitat minimum temperatures were available from the literature for 31 of the species in Table 1. There is a strong correlation between LLT and minimum temperature in the animal's environment ($R^2 = 0.643$, $P < 0.01$) for these species.

A multivariate General Linear Model solved for Type III sums of squares, using taxon (in this case, Order) and category to model SCP and LLT showed that taxon and category explained the same amount of variation regardless of their position in the model. This suggests that the category into which a species falls is independent of taxon and, by inference, category is therefore independent of phylogeny.

What is Freezing Tolerance, then? An integration with Bale's (1996) categorisation

Freezing tolerant insects, then, are species that can survive ice formation in their tissues, while the species that cannot are freezing intolerant (Fig. 2). LLT of both strategies covers roughly the same range – the highest LLT in a species that may be described as freezing tolerant is –6.2°C in *Agrotis* sp. (Duman & Montgomery, 1991), compared to –6.9°C for the freeze intolerant adults of *Myzus persicae* (Bale, 1991). Thus there is considerable overlap in cold tolerance, yet on a graph of SCP and LLT, the strategies occur independently. Bale's (1996) statement "freeze tolerant species are undoubtedly the most cold hardy insects" thus requires rearrangement: in fact, the most cold hardy insects are undoubtedly freeze tolerant, but the reverse is not necessarily true. There is not a large enough data set of the information used by Bale (1996) to plot this, but the expected relationships from his work have been indicated in Fig. 2. It is clear that freezing tolerance represents an alternative strategy to freeze intolerance under similar environmental conditions, and this can be used to provide a comparative framework between the two. The view presented in Fig. 2 allows the understanding of the two strategies in an integrative setting. It must be remembered that this only includes insects, and that the inclusion of other freezing tolerant invertebrate and vertebrate groups may produce new categories, clarify the existing categories, or prove these categories to be artifactual. In any case, the dichotomy between freezing tolerance and freeze avoidance is certain to exist, and there is reasonable evidence of a continuum, if not actual groups within the freezing tolerance category.

Limitations of this analysis

This classification is based almost entirely on SCP and LLT, and omits data that were not available for all or even some species. Other pieces of information that may be important include:

(1) The proportion of body water converted to ice. This is the major distinguishing feature of Partially Freezing Tolerant species, and of course, it is the factor that divides freezing tolerant from freeze intolerant (which have no body water frozen) strategies. Ramløv (1998) presses for a distinction between what he describes as the level of freezing tolerance (the amount of body water frozen) and the level of cold tolerance (the temperatures that are actually withstood). The proportion of water frozen may be important if there is a difference between partially freezing tolerant and freezing intolerant species. It may also be important if it can be shown that further ice formation is the cause of low temperature mortality in freezing tolerant insects, or that survival is directly dependent upon ice content. Of the species for which data are available (Table 2), there is no clear relationship between the proportion of water frozen and temperature survived, if freezing goes to completion. It is much more likely that the proportion of water converted into ice is dependent on the composition of the insect, and probably therefore phylogeny. I would predict that related animals of the same size (for example alpine and lowland tree weta, *Hemideina*) would have similar proportions of body water converted to ice at a given temperature, irrespective of their respective survival of freezing.

TABLE 2. Lower Lethal Temperature compared to percentage body water converted to ice in three freezing tolerant insects.

	LLT	Ice content
Eurosta solidaginis[1]	ca. –50°C	ca. 65%
Hemideina maori[2]	ca. –10°C	ca. 82%
Celatoblatta quinquemaculata[3]	–8.9°C	ca. 74%

[1] Lee, 1991; Lee et al., 1993; Lee & Lewis, 1985.
[2] Ramløv et al., 1992; Ramløv & Westh, 1993.
[3] Sinclair, 1997; Block et al., 1998.

(2) Mode and location of ice nucleation. Within both the Strongly and Moderately Freezing Tolerant groups, there are several sites of ice nucleation ranging from inoculative (exogenous) nucleation through the cuticle, to nucleators in the gut or haemolymph. Freezing tolerant insects with low supercooling points appear not to require nucleators at all. Indeed, Sinclair et al. (1999) and Worland et al. (1997) show that the apparent presence of a haemolymph ice nucleator does not necessitate its ecological relevance. Again, more data on ice nucleation may reveal a new range of categories that makes these proposed ones obsolete, or may reveal that differences in the mode and site of nucleation represent different solutions to the same problem, leaving the category unaffected.

(3) Cryoprotectant systems. There are nearly as many different cryoprotectant systems as there are freezing tolerant insects, and these systems may explain some or all of the observed groupings. Zachiariassen (1985) has suggested that the species I have included in the Freezing Tolerant with Low SCP group have SCPs depressed by extremely high glycerol concentrations, and that these concentrations confer a degree of freezing tolerance upon the insects as well. Similarly, Zachariassen (pers. comm.) has suggested that the difference between Moderate and Strong Freezing Tolerance may lie in the insect's ability to produce high concentrations of polyol cryoprotectants. In addition to this, the use by some insects of amino acids [e.g., proline in *Hemideina maori* (Ramløv, 1993)] or thermal hysteresis factors (Duman et al., 1991) as part of their mechanisms of freezing tolerance may provide a different (physiological) basis for separating groups within the strategy of freezing tolerance.

(4) Life stage, body size, diet, evolutionary history. There are a whole host of ecological and evolutionary parameters that may be built into a model of freezing tolerance strategies. The actual physical make up of larval and adult holometabolous insects is quite different, and body size affects both the freezing process (via thermal inertia and concentration gradients (Ramløv & Westh, 1993), and the temperature at which freezing takes place (Zachariassen, 1991). In addition, the evolutionary history may have an effect; for example, a group that migrated into a very cold area in the space of a few (hundred) generations may exhibit different strategies to groups whose exposure to cold has increased over hundreds of thousands or millions of years. In addition, some groups may have repeatedly colonised cold environments (e.g., New Zealand alpine weta *Hemideina maori* and the giant scree weta *Deinacrida connectens*), while others may all be descended from a single, cold-hardy ancestor.

(5) Problems with the data set. The data set does not include species that are not cold hardy (unless Partially Freezing Tolerant species fall under this description), nor does it include all groups, and there is bias towards certain groups, whether because these groups are most likely to be freezing tolerant, or because they are most likely to be studied. I have not made distinction between the cold hardy life stages, although I have used the overwintering life stage when given a choice. In addition, the descrip-

tion of these groups using statistics may lead sceptics to question their validity. The only answer is to investigate even more freezing tolerant species, encompassing a wider geographical and environmental range, in even further detail!

Freezing tolerance groups as foci for comparative research

One of the advantages of this classification system is that it provides a framework for comparative studies. For example, a question might be framed with reference to Moderately and Strongly Freezing Tolerant groups: "Why can Moderately Freezing Tolerant species survive only a few degrees below their SCP, when Strongly Freezing Tolerant species can survive a long way below their SCP?"; between those groups with high SCP (Moderately and Strongly Freezing Tolerant) and Freezing Tolerant species with Low SCP "Why must these groups initiate ice formation at high temperatures, when Freezing Tolerant species with Low SCP do not require this?"; and within groups "Is there a fundamental difference between the freezing process in Strongly Freezing Tolerant species that require inoculative freezing and those with endogenous nucleators?". This categorisation also highlights the difference between freezing tolerant and freeze avoiding species: "Is there any difference between a "Partially Freezing Tolerant" species (such as those described here) and a freezing intolerant species?" Thus the classification serves a scientific question, and can be used to generate a number of falsifiable hypotheses for research in a comparative environment.

CONCLUSIONS

Freezing tolerant insects constitute their own group and freezing tolerance represents a parallel, alternative strategy to freeze intolerance. This view can be accommodated in Bale's (1996) classification by a shift away from a direct continuum to include a dichotomy.

Four categories within the freezing tolerant strategy (Partial, Moderate and Strong Freezing Tolerance and Freezing Tolerance with Low SCP) have been identified within the freezing tolerant insects, and have some statistical support. Further research may show the groups to be part of a continuum, but they may be nevertheless useful for framing hypotheses in future comparative studies.

This classification is based upon the relationship between SCP and LLT, and as such may need to be heavily revised in the face of further data. The categorisation itself is open to discussion and rearrangement.

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