Insect adaptations to cold and changing environments[1]

H.V. Danks

Biological Survey of Canada (Terrestrial Arthropods), Canadian Museum of Nature, P.O. Box 3443, Station D, Ottawa, Ontario, Canada K1P 6P4 (e-mail: hdanks@mus-nature.ca)

Abstract—A review of insect adaptations for resistance to cold and for life-cycle timing reveals the complexity of the adaptations and their relationships to features of the environment. Cold hardiness is a complex and dynamic state that differs widely among species. Surviving cold depends on habitat choice, relationships with ice and water, and synthesis of a variety of cryoprotectant molecules. Many aspects are time-dependent and are integrated with other factors such as taxonomic affinity, resource availability, natural enemies, and diapause. Timing adaptations reflect the fact that all environments change over many different time frames, from days to thousands of years. Environments differ in severity and in the extent, nature, variability, and predictability of change, as well as in how reliably cues indicate probable conditions in the future. These differences are reflected by a wide range of insect life-cycle systems, life-cycle delays, levels of responsiveness to various environmental signals, genetic systems, and circadian responses. In particular, the degree of environmental change, its predictability on different time frames, and whether it can be monitored effectively dictate the balance between fixed and flexible timing responses. These same environmental features have to be characterized to understand cold hardiness, but this has not yet been done. Therefore, the following key questions must be answered in order to put cold hardiness into the necessary ecological context: How much do conditions change? How consistent is the change? How reliable are environmental signals?

Résumé—La présente rétrospective des adaptations pour la résistance au froid et l'ajustement temporel des cycles biologiques illustre la complexité des adaptations et leurs relations aux caractéristiques du milieu. La résistance au froid est un état dynamique et complexe qui varie considérablement d'une espèce à l'autre. La survie au froid dépend du choix de l'habitat, des relations avec la glace et l'eau et de la synthèse d'une gamme de molécules cryoprotectrices. Plusieurs des aspects sont reliés au temps et sont aussi intégrés à d'autres facteurs, tels que l'affinité taxonomique, la disponibilité des ressources, les ennemis naturels et la diapause. Les adaptations d'ajustement temporel sont reliées au fait que tous les milieux changent à des échelles temporelles multiples, allant de jours à plusieurs millénaires. Les milieux différent entre eux quant à la rigueur et à l'étendue, la nature, la variabilité et la prévisibilité du changement; de plus, les indices des conditions futures probables y sont plus ou moins fiables. Ces différences se reflètent dans les systèmes de cycles biologiques des insectes, les délais dans les cycles biologiques, les degrés de réaction aux divers signaux environnementaux, les systèmes génétiques et les réponses circadiennes. En particulier, le degré de changement environnemental, sa prévisibilité aux différentes échelles temporelles et la possibilité d'en faire un suivi efficace déterminent l'équilibre entre un ajustement temporel fixe et un flexible. Ces mêmes caractéristiques de l'environnement doivent être définies de manière à pouvoir comprendre la résistance au froid, mais ce n'est pas encore fait. Pour replacer la résistance au froid dans son contexte écologique obligé, les questions essentielles à poser sont donc : quelle est l'importance du changement de conditions? ce changement est-il uniforme? les signaux de l'environnement sont-ils fiables?

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Introduction

Insects display a remarkable range of adaptations to cold and changing environments. The many selective pressures and combinations of traits make for complex systems of adaptation. In particular, temperate insects face two sorts of challenges. First, they must cope with the cold of winter (as well as hot dry summers in typical continental climates with cold winters). Second, they must time their life cycles not only so that resistant stages are present to cope with seasonal severity, but also so that developmental and reproductive stages coincide with suitable conditions of temperature, moisture, resource availability, food quality, and other potential constraints.

Here I review the suites of adaptations for resistance to the cold of winter and for the timing of the life cycle and their relationships to environmental changes on seasonal and other temporal scales. Viewing these aspects together reveals instructive parallels. In particular, life-cycle adaptations accord with the extent and consistency of environmental changes and how reliably future conditions can be predicted from available signals about environmental quality. The onset and extent of cold hardiness must also accord with these same features of the environment, but cold hardiness has not yet been examined sufficiently in an ecological context.

Responses to cold

Cold hardiness

Insect cold hardiness has been studied in increasing detail over the years; sample reviews are those by Cannon and Block (1988), Duman *et al.* (1991), Lee and Denlinger (1991), Storey and Storey (1992), Danks (1996, 2000*b*, 2005*a*), Sømme (1999), and Bale (2002). These studies have led to the discovery of more and more elements that contribute to insect cold hardiness, as summarized in Table 1.

The conditions of cold actually experienced depend on region and microhabitat, which are modified by insect movement and habitat choice, and on the effects of cold temperatures, which are modified chiefly by physiological and biochemical means. During cold, temperate winters, a few species remain active beneath or even above the snow (*e.g.*, Aitchison 1979, 1989; Soszynska and Durska 2002), but most species are inactive. Potential challenges include the mechanical impact of external ice (water expands by about 4% on freezing), disruption of biochemical processes, and damage to cells and membranes. Beyond the effects of habitat, three core factors have proven to be especially significant: ice crystallization (controlled by nucleators), water relations (controlled by water status and availability), and molecules used for cryoprotection.

Habitat effects

Often overlooked because of the interest in biochemical mechanisms is the importance of climate and weather, coupled with the choice of microhabitat (Table 1). Most species choose protected sites beneath litter, soil, or snow. Such substances, especially when air is trapped within them, provide good insulation against low ambient temperatures, so that below the snow temperatures seldom fall far below freezing, even in cool temperate winters (Danks 1991*a*). Sheltered microhabitats also reduce the risk of ice damage or ice inoculation. In particular, they slow the rate of temperature change. This is potentially significant because some species survive freezing, or at least survive in greater numbers, only after very slow cooling (fractions of degrees per minute) (Miller 1978; Shimada and Riihimaa 1988; Bale *et al.* 1989). The choice of overwintering sites is therefore very important. Although anecdotal evidence suggests that sites are carefully chosen on the basis of light, temperature, moisture, touch, gravity, and other factors (Danks 1991*a*), few quantitative experiments have been done. Relatively small-scale dispersal to more favourable sites is a common strategy or partial strategy (*e.g.*, Knülle 2003). However, the alternative — long-distance displacement or migration away from areas with cold winters — is relatively rare among insects, especially in cool temperate regions with large tracts of similar terrain, and therefore is not considered further here.

Ice and nucleation

The two main types of cold hardiness customarily recognized differ in whether or not water in the insect freezes. Freezing-resistant species supercool, the body fluids remaining unfrozen even well below 0 °C, whereas freezing-tolerant species survive the formation of extracellular ice within the body. These categories are somewhat artificial: for example, some species die at temperatures above 0 °C, some species that supercool are killed at temperatures above the supercooling point, and

Table 1. Elements of insect cold hardiness.

Element	Sample features	Main effects on cold hardiness
Region	Climate, weather	Temperature and its variation, freezing rate
Microhabitat	Choice, site features, modification	Insulation, protection against inoculative freezing, freezing rate, effects of ice
Surface phenomena	Cocoons, cuticle	Ice inoculation, external mechanical effects
Crystallization	Freezing resistance, freezing tolerance	Absence or presence of ice
	External nucleators, internal nucleators (*cf.* feeding), ice-nucleating proteins	Ice formation
Water relations	Status, availability, dehydration	Ice amount, "unfreezable" water, water management and solute interactions, supercooling by dehydration
Cryoprotectants	Solutes, antifreeze proteins, shock proteins	Inhibition or modification of ice or injury
Other molecules	Enzymes, antioxidants	Modification of function or injury

populations may differ in the supercooling point and in the level of injury at different temperatures above the supercooling point (*e.g.*, Knight *et al.* 1986; Bale 1987; Turnock *et al.* 1990, 1998; Turnock and Boivin 1997). Nevertheless, the two customary categories serve to highlight the major significance of ice formation.

Water assembles into a hexagonal crystal configuration during ice formation; it freezes easily when a nucleus is available to maintain that configuration. Once a single ice crystal forms, it can nucleate others. In the absence of suitable nuclei, ice crystals develop when the water molecules are moving slowly enough to maintain the configuration for a finite time. Movement is slow enough only at low temperatures, and in small droplets of pure water such spontaneous or homogeneous nucleation does not take place until the temperature falls to about –40 °C (Vali 1995; Matsumoto *et al.* 2002). Freezing-resistant species apparently have ways to mask internal heterogeneous nucleators, and other adaptations, notably reduced water content, limit homogeneous nucleation (Zachariassen *et al.* 2004; see also Wilson *et al.* 2003 and review by Danks 2005*a*).

Insects can reduce the chance of freezing by avoiding external nucleators such as dust particles, surface ice crystals, food fragments, and ice-nucleating bacteria (see Strong-Gunderson *et al.* 1989; Lee *et al.* 1995; Lee and Costanzo 1998). In some species, overwintering cocoons significantly protect against inoculation by ice (*e.g.*, Sakagami *et al.* 1985; review by Danks 2004*b*). Special, tightly applied winter cocoons of some chironomid midges are believed to give mechanical protection against surrounding ice in pond substrates (see review by Danks 1971). The structure of the cuticle also influences resistance to ice inoculation (Kelty and Lee 2000).

To remain unfrozen, insects must eliminate or mask nucleators in the gut and tissues. However, if a supercooled insect begins to freeze, ice will spread very rapidly and thus dangerously because the temperature is now well below 0 °C. Therefore, in contrast to most freezing-resistant insects, which eliminate nucleators, most (but not all) freezing-tolerant insects manufacture ice-nucleator proteins for the winter (Zachariassen and Hammel 1976; review by Duman 2001). These nucleators ensure that freezing will take place at relatively high, and hence less dangerous, subfreezing temperatures. Several species survive after freezing by inoculation but not after freezing at lower temperatures in the absence of surface ice (*e.g.*, Tanno 1977; Fields and McNeil 1986; review by Duman *et al.* 1991); such species may lack ice-nucleating proteins. Nevertheless, a few species can survive freezing after extensive supercooling (Ring 1982, for a pythid beetle). Moreover, some species remain supercooled even at –60 °C, a temperature well below the homogeneous nucleation point of water (Ring 1981, 1982, 1983; Ring and Tesar 1981; Miller 1982; Miller and Werner 1987).

A few species are known that survive cold temperatures either by supercooling or by freezing tolerance, perhaps depending on conditions in a given year (Horwath and Duman 1984; Duman *et al.* 1991). Some freezing-tolerant species include individuals that reduce their supercooling points after being frozen and thawed. Consequently, their subsequent re-freezing is less likely, but these individuals are more likely to die if they are frozen again (Bale *et al.* 2000, 2001; Brown *et al.* 2004). Bale (2002) suggested that this strategy might reflect the greater costs of repeated freezing and thawing compared with a single freezing.

Water relations

The behaviour of water and ice within an insect depends on water status and availability. Water can be in one of a number of states, including water in matrix molecules (a very small percentage), water closely associated with biological structures and surfaces, and storage water not closely associated with the cells (Danks 2002*a*). Ice is another potential state in freezing-tolerant species. Typically, water in an insect is in dynamic balance among the various states, depending on the condition of the insect and the nature of the environment. Water bound more or less tightly to cellular constituents has been termed "unfrozen water" or "osmotically inactive water" (also, less usefully, "unfreezable water" or "bound water"). Wolfe *et al.* (2002) pointed out that the amount of unfrozen water may exceed the potentially "unfreezable" amount because equilibrium is not reached over normal time frames at low temperatures; see Block (2002, 2003) for further discussion. Unfrozen water comprises 10%–30% of the total water in different species (Storey and Storey 1988; Block 1996), and typically the proportion that will not freeze increases in preparation for winter (Storey *et al.* 1981; Storey and Storey 1988).

Under normal conditions, water is managed internally by solute or ion transport across membranes (water follows by solvent drag). Higher concentrations of solutes (see below) affect the behaviour of water in solutions. At extreme solute concentrations, achieved especially by drying, viscous rubbers and glasses are produced in which molecular kinetics are extremely slow, preventing physical and chemical interactions including nucleation (Danks 2000*a*).

Water balance is used as a means of remaining unfrozen when surrounded by ice during winter by some springtails (Holmstrup and Sømme 1998; Worland *et al.* 1998) and other invertebrates (Holmstrup 1992; Holmstrup and Zachariassen 1996; Sømme and Birkemoe 1997; Holmstrup *et al.* 2002). In these species, water is lost from the animal to external ice, a loss that is favoured because ice has a lower vapour pressure than water at a given temperature. Thus dehydrated, with corresponding reductions in melting point and supercooling point, the organism cannot freeze at the given temperature, provided its cuticle is permeable enough to transfer water out relatively rapidly. Of course, such a mechanism requires an ability to survive drying, which causes stresses similar to those created by internal ice formation. Protection against drying comes especially from certain solutes such as trehalose (Ring and Danks 1998; Danks 2000*a*). In insects, such solutes have most commonly been referred to as cryoprotectants (Ring and Danks 1994, 1998).

Cryoprotectants and other adjustments

Several kinds of cryoprotectant molecules that may increase in winter in cold-hardy species mediate effects on internal water and ice. Solutes of low molecular weight (especially glycerol, sorbitol, and other polyhydric alcohols, but also trehalose and other sugars and a few other substances; see review by Lee 1991) reduce the melting point and hence the freezing point by osmotic effects (for other roles, see below). Such effects depend on the number of solute molecules, such that numerous small molecules are most efficient. Many species manufacture more than one kind of these solutes at the same time (Miller and Smith 1975; Morissey and Baust 1976; Ring 1977; and see Lee 1991, Table 2.1).

Antifreeze proteins, or thermal hysteresis proteins (see review by Duman 2001 for insects and by Fletcher *et al.* 2001 for fishes), are large molecules that inhibit ice formation by binding in specific orientations to the ice surface at the ice–water interface or by binding to and reducing the efficiency of nucleating sites (*e.g.*, Wilson and Leader 1995; Madura *et al.* 2000; Daley *et al.* 2002; Wilson *et al.* 2003; Wathen *et al.* 2003). Glycerol and other small solutes enhance these actions of antifreeze proteins, apparently by disabling nucleators (Li *et al.* 1998; Duman 2002; Duman and Serianni 2002).

Recrystallization inhibitors prevent potentially injurious ice recrystallization in frozen tissues. Such growth or change of form of ice crystals is a common occurrence as temperatures change, especially in the presence of solutes (which make available more intervening water molecules to facilitate transfer between ice crystals). Antifreeze proteins and other large molecules can serve as recrystallization inhibitors, and smaller specific recrystallization inhibitors that are effective at very low concentrations have been reported from a nematode (Ramløv *et al.* 1996). Such phenomena have been little studied in insects.

Shock proteins, or stress proteins (Morimoto *et al.* 1994; Goto and Kimura 1998; Denlinger *et al.* 2001), appear very rapidly in many species in response to marked temperature shock by heat or cold, but for the most part their roles in cold hardiness or in the very rapid cold-hardening responses described in some species have not been determined (see review by Danks 2005*a*). Other molecules manufactured or modified for cold hardiness include enzymes, which may be altered to change the temperature at which they are activated or damaged, and antioxidants, which serve to repair or protect cells from some of the products of freezing damage (*e.g.*, Joanisse and Storey 1996, 1998).

Despite much work on cryoprotectants, much remains to be understood, given the multiple modes of protective action of these substances and their interactions. For example, some species that are very cold hardy lack the usual cryoprotectants (Ring 1981, 1983; Storey *et al.* 1993).

Adaptive changes in cell membranes also appear to be associated with cold hardiness. In the fly *Chymomyza costata* (Zetterstedt) (Diptera: Drosophilidae), significant changes in phospholipid composition are associated with freezing tolerance (Košt'ál *et al.* 2003).

Degradation of mitochondria has been associated with cold hardiness (Kukal *et al.* 1989, for the high Arctic moth *Gynaephora groenlandica* (Wocke) (Lepidoptera: Lymantriidae)). The amount of mitochondrial DNA in overwintering individuals is thereby reduced, but in both *G. groenlandica* and *Eurosta solidaginis* (Fitch) (Diptera: Tephritidae), stable RNAs are stored over winter, allowing mitochondrial proteins to be restored rapidly when temperatures rise (Levin *et al.* 2003).

Cold hardiness and time

Many aspects of cold hardiness are time-dependent (Table 2). These temporal effects influence the effectiveness of adaptations. For example, whether or not freezing occurs during supercooling depends on the temperature and on the duration of the supercooled state, because nucleation is time-dependent and any nucleation event will precipitate freezing of the insect. Once the insect is frozen, temperature and time also determine the likelihood and rate of recrystallization. Cryoprotectants serve to mitigate these rearrangements as well as protect against the freezing process. Water balance and the dynamics of internal water status and availability are also time-dependent. Moreover, cryoprotectants and other molecules accumulate before winter, but in some species they are also adjusted during winter (Baust and Lee 1982; Baust and Nishino 1991). Such changes occur in response to environmental conditions including low temperatures (Baust 1982; Layne and Kuharsky 2000), photoperiod (*e.g.*, Meier and Zettel 1997), and dehydration (*e.g.*, Layne and Kuharsky 2000), but some may simply accord with developmental status or the passage of time. Year-round cold hardiness is known in a few species from polar climates (*e.g.*, Kukal *et al.* 1988; Sinclair and Chown 2003).

The fact that the environmental effects of temperature and the status and responses of most overwintering insects change over time in these ways greatly complicates the assessment of cold hardiness. Cooling rate and, separately, warming rate influence survival (Baust and Nishino 1991). Survival of even freezing-tolerant species is both temperature-dependent and time-dependent (*e.g.*, Danks 1971 for high Arctic midge larvae). Moreover, the fact that in at least some species site seeking and cryoprotectant synthesis are triggered by different environmental cues and are controlled by hormones (*e.g.*, Danks 1996, p. 391) and that multiple cryoprotectant substances can be adjusted during winter suggests that cold hardiness is best regarded as a dynamic programme linked to environmental conditions. These ongoing changes are not consistent with the older concept of cold hardiness as some sort of static, "hardened" state for winter protection. Consequently, interpreting the many time-dependent elements of cold hardiness requires detailed assessments of microhabitat temperatures in nature over relatively long periods.

Integration of cold hardiness with other factors

Sheltering from or withstanding cold is not the only requirement for survival in cold

Table 2. Key temporal effects influencing insect cold hardiness.

Element	Temporal effects
Region	Seasonality Life cycles Control of development
Microhabitat	Habitat selection Winter *versus* spring demands
Surface phenomena	Supercooling
Crystallization	Supercooling Recrystallization
Water relations	Availability Status
Cryoprotectants and other molecules	Acclimation in fall Modulation in winter

Table 3. Some other elements related to insect cold hardiness (for additional details see Table 2 in Danks 1996).

Element	Relevant sample features in addition to cold hardiness
Region	Phylogeny, evolutionary pathway, faunal composition
Microhabitat	Humidity, temperature, natural enemies
Surface phenomena	Heat gain, camouflage, water loss, natural enemies, mechanical strength
Water relations	Desiccation tolerance and resistance, including water acquisition, and conservation by both cuticular and molecular means
Energy relations	Energy storage, respiration, metabolism, development, reproduction
Timing	Life cycles, including diapause or quiescence responding to seasonal elements that include resource availability, dryness, and food quality

environments. Many other features are integrated with insect adaptations to cold (Table 3). The fauna of cold environments is dominated by groups that evolved in cold conditions and habitats, at a variety of taxonomic levels. Thus, the order Diptera among the insects, the family Chironomidae among the Diptera, and the genus *Spilogona* among the Muscidae are very well represented in Arctic faunas. They constitute 61%, 46%, and 80% of the high Arctic insects, flies, and muscids, respectively, compared with only 24%, 7%, and 24% of these taxa in Canada as a whole (Danks 1992*b*, 1993). Only groups with coordinated adaptations persist at the highest latitudes. Such related species may be especially helpful in understanding how particular features of cold hardiness arose. For example, chironomid midges, the dominant family in the high Arctic, are supposed to have evolved in seasonally frozen habitats (Brundin 1966), and freezing tolerance is especially widespread in this family (Danks 1971).

Microhabitat and the behaviour associated with its choice determine much more than exposure to cold; they also determine (at particular times of year) moisture availability, developmental temperature, and exposure to natural enemies. If seasonal microhabitat change is not feasible, winter and summer needs may conflict. For example, the sites most sheltered from cold are slowest to warm up in spring, and consequently Arctic species that develop early tend to overwinter in cold, exposed places (review by Danks 2000*b*). Species in cold sites also conserve energy (*e.g.*, Parry 1986; and see below).

Cocoons and cuticular structures that might enhance cold hardiness serve both active and inactive individuals, providing heat gain, camouflage, water retention, and protection against disturbance or attack by natural enemies (Danks 2002*b*, 2004*b*). The thick walls of cocoons protect against many adverse conditions simultaneously, but such a feature must be balanced with the cocoon's other uses because the construction of shelters has a measurable cost (*e.g.*, Stevens *et al.* 1999, 2000). However, the durability and effectiveness of cocoons for multiple purposes, such as water permeability and resistance to mechanical damage, predators, and ice inoculation, has not been measured (Danks 2004*b*). Protective structures around the eggs may play similar roles (*cf.* Danks 2002*b*; Rivers *et al.* 2002).

Securing and retaining enough water is one of the greatest challenges for terrestrial insects, and many adaptations are known. These include maintaining high basic water content, surviving drying, drinking, eating wet food, absorbing atmospheric water, metabolizing water from food or stored reserves, and limiting water loss by ecological, mechanical, and physiological–biochemical means (Danks 2000*a*). Some mechanisms are not available to individuals that are immobile in winter. Moreover, storage, reduction, or change in status of an organism's water during winter changes its availability to participate in freezing, desiccation, fluid and ionic balance, excretion, transport, and other processes. For example, water content (as assessed by drying) usually drops in winter, commonly from about 75% to 60%, as the amount of "unfreezable" water increases. This 15% change represents the "loss" of fully half of the water in the body (Hadley 1994, p. 31) and coincides with increased cold hardiness. Reduced cuticular permeability in winter (*e.g.*, by increased lipid layers: Danks 1987, p. 23; Yoder *et al.* 1992; Nelson and Lee 2004) reflects the need for additional desiccation resistance during inactivity, but all such changes must be integrated with demands at other times of the year.

Energy budgeting throughout the year is coordinated with winter survival. Energy demands for summer feeding, development, reproduction, and flight, for example, are balanced with other needs for energy. Those needs pertain not just to cold hardiness (*e.g.*, the manufacture of cryoprotectants) but also to requirements to survive inactivity in the winter shelter and to survive in fall and spring when it is warm enough to metabolize significant reserves (Irwin and Lee 2000, 2003). Reserves typically are stored as large amounts of fat (see review by Danks 1987). At the same time, reserves may be converted to glycerol and other cryoprotectants (Storey and Storey 1988). Respiration and cold hardiness are linked in some habitats because organisms encased in ice in spring respire anaerobically (Conradi-Larsen and Sømme 1973*a*, 1973*b*; Sømme 1974*a*, 1974*b*; Meidell 1983).

Metabolism is completely suppressed during anhydrobiosis, a condition known in chironomids (Hinton 1960), springtails (Greenslade 1981), and other small invertebrates (Barrett 1991; Sømme 1996; Ricci 2001). Anhydrobiosis allows these species to withstand very low temperatures and even immersion in liquid nitrogen, as well as extreme drying. Anhydrobiosis requires specific energy-dependent preparation (see review by Danks 2000*a*) and so, as already noted for cold hardiness, is not simply a form of static resistance.

In some species, cold hardiness does not appear to be linked with diapause (*e.g.*, Danks 1987, p. 41; van der Woude and Verhoef 1988), but a linkage between the two is now known in an increasing number of other species (Pullin and Bale 1989; Denlinger 1991; Hodková and Hodek 1994, 2004; Pullin 1994; Watanabe and Tanaka 1998*a*, 1998*b*, 1999), and several species must even be in diapause before they can develop cold hardiness (*e.g.*, Šlachta *et al.* 2002). Diapause puparia of *Sarcophaga crassipalpis* Macquart (Diptera: Sarcophagidae) are more resistant to inoculative freezing than non-diapause puparia (Kelty and Lee 2000), independent of the surface lipids. Vernon and Vannier (2002) have even proposed from limited evidence that freezing tolerance occurs only in individuals in diapause. Whether or not cold hardiness and diapause are linked, the major environmental cues that induce them, photoperiod and temperature, are similar. Of course, diapause and other life-cycle programmes might be linked to an inability to be active or to find or assimilate food during cold winters at least as much as to dangerously low temperatures. Other aspects of life-cycle timing are considered in the next section.

Responses to change

Changing environments

All environments change over many different time frames (Table 4). This wide range contrasts with a current preoccupation among scientists and the general public with change over intermediate periods (*e.g.*, 50 years) that might be tied to human activity. Climatic norms include characteristic levels of regular and irregular variation as well as average values. For

Table 4. Time frames of climate change.

Time frame	No. of years	Sample pattern of conditions	Explanation	Sample response
Long-term	1000s	Climatic norms, climate trends, severity	Long-term average of all conditions, long-term trends	Genetic systems
Supra-annual	2 to 100s	Variability and change	Differences among years, mid-term trends	Patterns of genetic variation
Annual	1	Seasonality	Patterns within each year	Life-cycle controls
Sub-annual	<1	Degree of unpredictability	Differences within years	Flexible development
Daily	0.0027	Amplitude, variability, unpredictability	Diel patterns of light, temperature, and moisture driven by changes in solar elevation	Circadian pattern of activities

example, mean July temperature is not exactly the same from one year to the next, the minimum temperature on a given date usually differs from the long-term average, and annual extremes can differ widely from year to year.

Key elements of climates (Danks 1999) according to the time frames shown in Table 4 include severity (persistent conditions that limit life, such as low temperatures or limited heat for growth, and very low winter temperatures), variability (changes in temperature or other potential constraints from year to year), seasonality (changes on an annual time frame), and unpredictability (short-term changes within a given year). The potential complexity of these different patterns makes it difficult to describe changes in detail. Organisms would be expected to respond to trends, which are directional changes in significant features over many years, including changes in extremes as well as mean values. Nevertheless, because organisms are adapted to “normal” patterns of change on various time frames and their responses are complicated by relationships with habitat and other biota, as explained below, the results of a given trend may not be conspicuous.

The effects of current variability and of any potential future trends therefore differ according to the species and the time frame. At the longest time frame, major astronomical, topographical, and latitudinal features (global rotational shifts, orogeny and continental drift, differential solar heating) dictate regional climates. Marked variability from year to year punishes fixed life-cycle patterns such as emergence at a given time of year, which exposes individuals to unsuitable conditions in cold years. Seasonality restricts the time at which development, activity, and reproduction, each of which has different requirements, can be successful. These impacts drive seasonal life cycles, even though (as already noted) the need to survive while inactive, especially during cold winters, has attracted particular study. Unpredictable changes within the year force adaptations for flexibility. Changes on a daily time frame dictate patterns of activity. Habitat is especially important because it governs immediate physical and biotic effects.

The adaptations required depend also on whether resources, especially temperature, are close to the limits for life. For example, a rapid change from 8 °C to 2 °C in the high Arctic would be common and would inhibit the activity of species there. The same 6 °C change

Table 5. Core elements of life-cycle systems in insects that respond to the nature of change in climates or habitats.

Element	Alternative features	Description	Typical climate or habitat
Duration of life cycle	Days	Multivoltine life cycle	Prolonged favourable
	One year	Strictly univoltine	Reliably seasonal
	Years	Slow life cycle, spread across more than one season	Severe and intermittently favourable
	Variable	Individuals have life cycles of different durations	Variable and unpredictable
Number of available life-cycle routes	One	Fixed or simple life cycle	Predictable
	Many	Multiple alternative pathways, complex life cycles	Variable or unpredictable
Default developmental response	Active development	Development unless signalled otherwise	Suitable, or environmental signals reliable
	Passive delay	Delay unless signalled otherwise ("obligate diapause", *etc.*)	Severe and unpredictable, or environmental signals unreliable

from 28 °C to 22 °C would have far less impact in the temperate region, where a rapid change of the same relative magnitude with respect to freezing (28 °C to 7 °C) would be unusual.

The significance of change depends too on whether environmental signals can reliably predict future environments. For example, short days precede the onset of the cold temperatures of the winter season, but there is no reliable signal to predict the onset of several years of drought. Climates, habitats, and environmental signals therefore interact to govern the responses of insects to changes on various scales. These responses comprise basic life-cycle systems, various life-cycle delays and how they are controlled in response to environmental signals, as well as long-term genetic and short-term circadian responses.

Life-cycle systems

The core elements of insect life cycles are duration, complexity, and whether basic developmental responses are active or passive (Table 5).

When habitats are favourable for long periods, especially during summer, abbreviated life cycles are possible. Species of several taxa characterized by rapid development and small size, such as mites, parasitic Hymenoptera, and aphids, can complete a generation in as little as a few days at high temperatures (*e.g.*, Campbell and Mackauer 1975; Tillman and Powell 1991; Sabelis and Janssen 1994) and are multivoltine. In cool temperate regions, where seasonal patterns are reliable but resources do not always allow multiple generations, many species are strictly univoltine (Danks and Foottit 1989; Danks 2002*a*). Harsh or intermittently favourable habitats may prolong life cycles over several seasons, especially in large species (see review by Danks 1992*a*). Severity includes not only short, cool, or unpredictable summers, but also patchy or unreliable food; food of low quality, such as wood (Danks 1992*a*); and the presence of natural enemies that would exact severe losses on populations that appeared every year, as suggested for periodical cicadas (*e.g.*, Lloyd and Dybas 1966; Williams and Simon 1995; Itô 1998) and some moths (*e.g.*, Wipking and Mengelkoch 1994; Lafontaine and Wood 1997).

When environments are variable or unpredictable, different individuals may have life cycles of different durations. In some species, individuals from even the same cohort emerge in different seasons (cohort splitting: *e.g.*, Townsend and Pritchard 1998; Aoki 1999). More commonly, individuals from unpredictable habitats are spread out within and between seasons, typically through variable and delayed development, sometimes in multiple stages (see below). The means by which such delays are controlled are considered in later subsections.

Predictable habitats, whether or not they are strongly seasonal, allow simple or fixed life cycles. Individuals of species in predictable habitats typically follow the same fixed life cycle — for example, developing in summer and entering diapause in winter. However, in many species from less predictable habitats, different individuals follow different routes among a variety of alternative life-cycle pathways. Different routes — including slow or rapid development, diapause or non-diapause, and quiescence or its absence — are available through genetic polymorphism (see below) or environmental responses. Sample sets of pathways are figured by Danks (1987, 1991*b*, 1994). For example, the stored-product mite *Lepidoglyphus destructor* (Schrank) (Acarina: Glycyphagidae) varies greatly in genetic propensity for both the onset and duration of diapause in a specialized hypopal stage (Knülle 1987, 1991). High-quality food reduces the incidence of diapause in intermediate genotypes, and temperature and humidity change its duration. After diapause ends, development stops unless humidity is high. These genetic and environmental responses result in life-cycle pathways ranging very widely from direct development (*i.e.*, without a hypopal stage) to very long delays. Even in species in which the population as a whole reproduces continuously, there may be cryptic dormant individuals (Spieth 2002).

Finally, the core structure of life cycles depends on whether development or delay is the default condition (Danks 2001, 2002*a*). When the default is active, development proceeds unless delays are specifically signalled. Such responses accord with habitats that are suitable for activity or with habitats that (even if intermittently unsuitable) supply environmental signals that reliably indicate when conditions will deteriorate, allowing development to be adjusted adaptively. Under such circumstances, a passive response would cause safe opportunities for development to be missed.

When the default is passive, development stops and does not continue unless signalled to resume by changed conditions. Passive responses are prevalent in species from more severe and unpredictable habitats or from habitats where environmental signals do not reliably indicate future suitability. Such responses, including fixed diapause and very narrow requirements for development, are conservative. Active responses in difficult habitats would probably expose vulnerable individuals to dangerous conditions.

The existence of passive systems has led to many difficulties in understanding life cycles. This is especially true of so-called "obligate" diapause, which often has been seen as an inevitable programmed arrest of development. However, many such diapauses simply end as soon as there is a required change in cues, typically in photoperiod (for examples, see Danks 1987, Table 23) but also in temperature (Worland *et al.* 2000) or food (Glitho *et al.* 1996). Passive defaults permit very conservative, slow, or complex life cycles, especially when there are multiple requirements for development to resume. Passive or active defaults can be used to govern not only diapause, but also other seasonal developmental responses such as wing morph and instar number (Danks 2002*a*).

Life-cycle delays

Insect life cycles are controlled especially by delaying development. Delays take four forms (Table 6): directly limited growth; reduced growth rates under indirect control by cues; directly arrested development when conditions are unsuitable (quiescence); and programmed suppression of development (diapause). These adjustments serve to avoid adversity, exploit favourable conditions, or enforce univoltinism.

Ordinary environmental variations govern the rate of growth directly — for example, through the influence of temperature on the rate of metabolism. However, many species also modify the rate of growth by using environmental conditions as cues or indirect signals, notably photoperiod (for examples in 80 species of insects see Danks 1987, Table 33) and density (Danks 1987, Table 36). Growth or development can be adjusted continuously or successively in response to these signals, allowing development to be optimized despite seasonal conditions that change within and between years. For example, some species slow development by increasing the number of instars in response to photoperiod (Danks 1987, Table 35). In several different taxa, growth-rate responses

Table 6. Types of delays in insect life cycles that respond to the nature of change in climates or habitats.

Element	Features or alternative features	Description	Typical climate or habitat
Directly modified growth rate		Slower growth at lower temperatures	Ordinary and seasonal variations
Indirectly modified growth rate	Cued effects	Slower (or more rapid) growth according to photoperiod, *etc.*	Ongoing adjustments to variable habitats
	Continuous, plastic responses	Ongoing response to cues, variable number of instars, stationary moults	Complex seasonal patterns and variation
Quiescence		Complete arrest through direct action of a single factor, usually temperature	Season with limited adversity
Diapause	Programmed response, but single, limited	Development but not activity suppressed	Moderately adverse conditions
	Single, fixed, relatively short duration	Development and activity suppressed during a single adverse season; diapause ends at a standard rate	Predictably seasonal
	Staged	Several components in a single diapause	Seasonal patterns and variation (responses allow ongoing control)
	Re-induction	Successive entries into equivalent diapauses in the same stage	Seasonal patterns and variation (responses allow ongoing control)
	Adjacent diapauses	Successive delays immediately after one another	Seasonal patterns and variation (responses allow ongoing control)
	Multiple diapauses	Life-cycle programme with diapauses at intervals	Seasonal patterns and variation (responses allow ongoing control)
	Complex diapause-ending response	Multiple requirements; diapause can end at an accelerated rate	Seasonal patterns and variation (responses allow ongoing control)
	Long or very long duration	Prolonged diapause for more than one season, duration usually variable	Unreliable

to photoperiod reverse direction during development (*e.g.*, Ryan 1975; Norling 1976; Khaldey 1977; Tanaka 1983).

When conditions are not especially variable or adverse, advance preparations for cold hardiness or energy conservation are not necessary. In such circumstances, development may stop simply by directly controlled quiescence. Nevertheless, especially in temperate regions, the life cycles of most species include at least one of the programmed delays known as diapause.

Diapauses structure life cycles in many ways, ranging — according to environment — from a single limited suppression of development to multiple and long-term responses (Table 6). The trend shown in Table 6 is that a more complex or longer-lasting diapause is correlated with greater seasonal variation or less predictable habitats and allows more precise seasonal coincidence or programming. For example, a single diapause can include several component stages (*e.g.*, Danks 1987; Lavenseau and Hilal 1990); adult diapause can be re-induced (*e.g.*, Hodek and Hodková 1992); several diapauses can follow one another within a stage (Ingrisch 1986; Niva and Becker 1998) or between stages (*e.g.*, Nishizuka *et al.* 1998); multiple diapauses can intervene at different stages throughout the life cycle (*e.g.*, Danks 1991*b*); and diapause-ending signals can act in more than one way (*e.g.*, Hodek 1981, 1983; Košt'ál *et al.* 2000). Therefore, diapause intensity is highly variable (Masaki 2002).

Diapause can even last for two or more seasons, a trait that is strongly correlated with unreliable conditions. Most species with prolonged diapause, although belonging to diverse taxa, live in unstable habitats such as the Arctic or deserts or feed on erratic resources such as the cone crops of evergreen trees (Danks 1987, Table 27; Danks 1992*a*, Table 5; Hanski 1988).

Responsiveness to environmental signals

A critical element of adaptations to changing environments is how insects respond to the presence and reliability of environmental signals, not just the environmental conditions themselves. Insects use one or a combination of photoperiod, temperature, thermoperiod, moisture, food, and other factors to assess the current or future suitability of habitats for development or reproduction. The best cues are reliable, frequent, and easily recognized (Table 7). Therefore, photoperiod is normally the most useful external factor because it is highly informative about seasonal time, can be monitored daily, and is easily visible in most habitats. Thermoperiod shares some of the same features. However, which cues can be used by a particular species depends on the species' habitat and its life cycle. For example, it would be difficult for insects concealed in opaque substrates to detect photoperiod reliably, even with very low thresholds for light detection. The signal given by temperature is too noisy for precise use in many places and is most useful in habitats buffered against daily variations. For details of the seasonal correlations, variability, and other features of various cues, see Danks (1987, ch. 7).

Although environmental signals indicate seasonal time, photoperiod and many other factors are not themselves significant selective forces but simply serve as a proxy for climate and other ultimate factors. Depending on the species, its habitat, and its cues, the degree of correlation of environmental signals with the ultimate selective forces might be different after a change in climate.

Whatever the cue or cues used to monitor environments, several patterns of response can be identified (Table 8). Patterns of sensitivity include direct regulation only (without any use of indirect signals or token stimuli) and fixed delays in development that are insensitive to cues. Most common is the integration of multiple signals to govern complex life cycles that include diapause and adjustments of growth (see above). It is significant that even when the reliable, frequent, and recognizable signals from photoperiod are available, most species integrate information from temperature or food to modify this photoperiodic control (*e.g.*, Goehring and Oberhauser 2002; Luker *et al.* 2002). Evidently, even a small contribution of additional evidence from other cues about likely future environmental changes has adaptive value (Danks 2002*a*).

Many species respond to cues in a simple qualitative manner: their responses differ either side of an absolute threshold such as a critical photoperiod or differ according to the presence or absence of a cue such as food or rainfall. However, in some species the level of the cue sets a quantitative response. In such species, the actual photoperiod or temperature can determine the rate of growth (*e.g.*, Wassmer and Page 1993), the incidence of diapause (Kimura 1990), and diapause intensity during induction

Table 7. Features of environmental signals used by insects (after Danks 2001).

Element	Description	Sample factors and properties
Reliable	Correlated with seasonal position and predictability of seasonal change	Photoperiod, thermoperiod reliable; food often reliable; other factors mostly unreliable
Frequent	Available for regular monitoring	Temperature, moisture, photoperiod, thermoperiod frequent; other factors mostly intermittent or variable
Recognizable	Sensors available and seasonal rate of change high	Sensors available for most factors but most factors with low rates of change

Table 8. Types of responsiveness to environmental signals by insects (for further details see Danks 1987).

Element	Alternative features	Description
Type of sensitivity	Direct regulation only	Temperature-controlled growth, quiescence
	Fixed pattern	"Obligate" diapause
	Indirect, multiple factors	Diapause and related responses
Type of response to cues	Qualitative, fixed	Presence/absence or absolute thresholds
	Quantitative	Actual value or duration of cue affects response; responses may change through stage
Stage of sensitivity and response	One instar sensitive	Signals monitored in only one instar
	Many instars or stages sensitive	Long-term integration of environmental signals
	Response soon after sensitive stage	More or less immediate response
	Response long after sensitive stage	Response only after a delay, even in next generation

(Nakamura and Numata 2000; Kalushkov *et al.* 2001) or during diapause (Nakamura and Numata 2000). Moreover, in a few species changing photoperiods have different effects from stationary ones on the duration of diapause or the rate of development (Canard and Grimal 1993; Košt'ál and Hodek 1997; Kemp 2000).

The stages at which cues are monitored and at which responses take place vary among species; the range of possibilities is shown in the lower part of Table 8. Stages sensitive to diapause-inducing cues are more commonly active (larvae, adults) than passive (eggs, pupae), apparently because active stages have better-developed sense organs and greater exposure to the environment, favouring monitoring (Danks 1987, p. 80). Normally, diapause is governed by the stage that immediately precedes it, apparently because environmental information is more reliable the closer it is to the event being predicted. Nevertheless, a common pattern adds sensitivity in preceding stages (allowing environments to be monitored for a longer period), even though the most recent information is given more weight. Sensitivity in the preceding generation is not uncommon (see reviews by Danks 1987, Table 14; Mousseau and Dingle 1991); for example, maternal conditions influence egg diapause. However, very early commitment to developmental delays is relatively rare, although there are some spectacular cases such as the silkworm *Bombyx mori* (L.) (Lepidoptera: Bombycidae), in which egg diapause is induced in the eggs of the preceding generation.

Most species have only one dormant stage per generation. Stages most often dormant are larvae, especially during developmental pauses such as the prepupal stage, and adults, especially in the pre-reproductive stage. Also prevalent are stages in a given taxon that characteristically are resistant to adverse conditions. Finally, dormant stages correspond with

Table 9. Genetic systems in insects that respond to the nature of change in climates or habitats.

Element	Alternative features	Description	Typical climate or habitat
Patterns of genetic variation	Limited	Little variation, normally distributed; canalization	Many
	Extensive	Wide and retained variation	Variable
	Polymorphism	Different morphs in one cohort	Unpredictable habitats without reliable environmental signals
Parthenogenesis	Obligate	Reproduction always parthenogenetic	Severe and variable over the long term (with other factors)
	Dominant	Occasional sexual reproduction	Severe
	Cyclic	Parthenogenesis and sexual reproduction alternate seasonally	Alternating habitats
	Intermittent	Occasional parthenogenesis, but usually reproduce sexually	Unknown

other timing requirements such as the availability of food plants at the correct stage or the need for synchronous emergence of reproductive adults. All of these adaptations provide both precision to ensure survival and reproduction and flexibility to cope with change. As a result of this complexity, there is an enormous range of timing possibilities both among and within species. Consequently, environmental changes can create unexpected effects both directly and through the signals that govern development.

Genetic systems

Environmental responses, whereby a given genotype allows a range of plastic reactions to different environments, act in concert with genetic variation (Table 9). All organisms show individual variation. Traits that are critical to survival may be canalized so that important adaptations will not be lost through variation away from the mean (*e.g.*, Stearns and Kawecki 1994). By the same token, canalization limits adaptation to change. On the other hand, extensive variation makes it possible to produce individuals adapted to variable habitats. Such variation tends to be genetic rather than environmental in two circumstances: first, when habitats — or at least different regional habitats — are stable, and second, when habitats change and the changes cannot be predicted from environmental cues, preventing adequate plastic adjustments. A wide range of variation then spreads developmental or other adaptive types through time. For example, staggered egg hatch or asynchronous development is characteristic of species from habitats with unpredictable supplies of water (Hynes and Hynes 1975; Campbell 1986; Moreira and Peckarsky 1994; Zwick 1996), streams with unpredictable spates (Courtney 1991), areas with late winter storms (Neal *et al.* 1997), and so on. In many species the range of this genetic variation is not continuous, but is subdivided into two or more discrete sets (which has the advantage of synchronizing the population within each set, even if the sets do not cover the full potential range of variation). Such polymorphism produces a set of different genotypes within a cohort to cope with different unpredictable circumstances. Common examples are bimodal emergence (*e.g.*, Biron *et al.* 1999; review by Danks 1987, Table 27) and prolonged diapause (see above).

Such bet-hedging or risk-spreading adaptations have been recognized in several settings as responses to unpredictability (see Frank and Slatkin 1990 for sample theory). Moreover, beyond striking examples of polymorphism, general variation is preserved in all species by a number of mechanisms, suggesting that all habitats — even those that seem stable and favourable — have some level of unexpected risk. Many temperate species include occasional individuals that vary greatly from the mean — for example, in size, developmental time, diapause incidence and duration, dispersal, and resistance to physical or chemical adversity — and clearly they result from adaptations rather than from accidents (Danks 1983). Part of the preserved variation undoubtedly arises simply because environments are heterogeneous, so no one genotype suits them all: interactions between genotype and environment help to maintain variation (Iriarte and Hasson 2000; Jia *et al.* 2000). Variation is also retained because not all genes, especially in diploid and polyploid organisms, are exposed to selection in any given generation. A pool of dormant individuals also shelters some genotypes from selection (Hairston and De Stasio 1988). In addition, specific genetic mechanisms exist to retain variation. Consequently, it is very difficult to eliminate diapause completely in most species even by very stringent selection against it over many generations (Danks 1987, Table 30).

In summary, variation tends to be retained because it is adaptive in real environments, which are characterized by change and by differences in both the nature of the change itself and the availability of environmental signals to predict the change.

Parthenogenesis is more prevalent in Arctic environments than elsewhere (Downes 1965; Danks 1981), apparently because it buffers change. In such severe habitats the relatively rapid selection to current circumstances that would follow recombination would remove genotypes that are best adapted to the long-term severity. In some Arctic species, as in many plants, parthenogenesis is dominant but not universal. Systems in which parthenogenesis dominates may retain the advantages of sexual reproduction in allowing recombination but constrain its potential for dangerous change. Cyclical or annual parthenogenesis allows exploitation of different habitats that become available alternately throughout the year. In many common species of aphids, for example, ephemeral host plants are rapidly exploited by parthenogenetic viviparae in summer, but the overwintering egg stage is produced through sexual reproduction. Finally, rare parthenogenesis occurs even in species that normally reproduce sexually (*e.g.*, Grodhaus 1971 for unfertilized chironomid eggs), although its significance is not known.

Circadian responses

The timing of daily activity depends on biological features: visual predators are active by day, for example, whereas many species sensitive to desiccation are active only at night.

In parallel with most seasonal responses, circadian timing is controlled by levels of light, temperature, and moisture, by internal daily rhythms, or by internal programmes that can be modified by external environments. Circadian rhythms are typical of stable temperate regions. However, in the high Arctic, daily photoperiod (whether or not it can be detected from the daily changes in light intensity during 24 h daylight) is not a useful indicator of what is important for insect existence in places where temperature is often limiting (Danks and Oliver 1972). Instead, direct responses to temperature govern the daily activity of most species there (see review by Danks 1981, pp. 271–272; Lankinen and Riihimaa 1997). Unfortunately, most of the recent work on circadian responses has focussed on timing mechanisms rather than on ecological relevance (Danks 2003, 2005*b*).

Conclusions

Insects survive in seasonal climates by adaptations to resist adversity, including inactivity when appropriate, as well as by adaptations to ensure that activity or reproduction coincide with suitable conditions. Withstanding cold requires complex adaptations, and different species use different elements among a wide range of possibilities (Table 1). Comparable complexity and diversity characterize the means by which life cycles are timed to cope with environmental changes on a range of scales (Table 5). Moreover, cold hardiness and life-cycle timing interact. For example, many high Arctic species overwinter in exposed places, where they require extreme cold hardiness, because these places warm up earliest in spring, a major benefit when summers are very short and cool. In some species, adaptations such as cold hardiness and diapause are clearly linked.

However, we do not yet know why different species have different modes of cold hardiness. For example, different species supercool to different degrees or freeze through inoculation by external ice, through freezing of the gut contents, or through haemolymph nucleators. Only recently has a start been made towards understanding such differences by considering the evolution of freezing tolerance (Vernon and Vannier 2002; Voituron *et al.* 2002) and the types of cold hardiness. Nevertheless, "classifications" of cold hardiness (Bale 1993, 1996; Sinclair 1999; Nedvěd 2000), although they document the fact that cold temperatures can be harmful in the absence of freezing, are otherwise of limited value because of the multifactorial nature of the adaptations (see Danks 1987, ch. 2, for classifications of diapause).

Care is required in developing generalizations because one element can serve in many different ways. For example, in one or another circumstance or species, certain key molecules (cryoprotectants) can have one or more effects among multiple possibilities: they appear to favour supercooling or disable nucleators, resist or protect against desiccation, protect frozen membranes or other cell constituents directly, modify the freezing process, mitigate cellular damage from ice crystallization or recrystallization while tissues are frozen or while they are thawing, and repair damage after thawing. In the same way, developmental delays can serve multiple purposes: they may conserve energy, protect against adversity, synchronize the feeding stage with food resources, optimize the timing of reproduction, synchronize individuals with one another, prevent a risky generation late in the year, or assist in further life-cycle programming by allowing the environment to be monitored for a longer period (Danks 2002*a*). Moreover, there is more than one way to achieve the same end: selection is insensitive to exactly how a functional requisite is met. Consequently, successful species may have different cryoprotectants or different diapause stages not only to serve different ends but also because they have different evolutionary histories. Different species in the same place can have widely divergent strategies (*e.g.*, Worland and Block 2003). Therefore, multiple simultaneous experimental approaches will be needed to identify the components of such strategies. For both cold hardiness and life cycles there is particular interest in how the patterns of variation (including genetic components of prolonged diapause, for example) are maintained in response to long-term patterns of environmental variation.

However, cold-hardiness adaptations have scarcely been analyzed in the context of microenvironments or environmental change because of an emphasis on summer conditions, such as mean summer temperature or duration of the growing season (*e.g.*, Hodkinson *et al.* 1998; Danks 2004*a*). The major reason for this neglect is that, with limited exceptions (*e.g.*, Sinclair 2001), winter habitats have not been adequately studied (Danks 1978, 1991*a*, 2000*b*). Information on life cycles shows that habitat features are the key to understanding responses to change, but comparable insights are not yet possible for cold hardiness because we do not know the conditions experienced by overwintering insects, even in species for which cryoprotectant profiles are known. Without detailed knowledge of microhabitat conditions we cannot predict the likely effects of environmental changes on winter survival, especially for the time frames of intermediate duration that are of particular current interest. Such knowledge would also inform experimental work, suggesting appropriate cooling rates, temperature patterns to test survival or energy use, and preconditioning requirements, for example (Danks 2005*a*).

The parallels between cold hardiness and life-cycle control — the complexity of adaptations, the multiplicity of roles, and the influence of key elements of the habitat — confirm that both sets of adaptations are driven by the same kinds of environmental elements. Consequently, we now need to ask for cold hardiness too: How much do the conditions experienced change on various temporal and spatial scales? How consistent is the change on given (especially annual) time frames? And how reliably do the available environmental signals forecast coming changes? To answer these questions, more detailed knowledge about environments, not just about insects, is required.

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