

Antifreeze proteins in Alaskan insects and spiders

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Abstract

Prior to this study, antifreeze proteins (AFPs) had not been identified in terrestrial arthropods from the Arctic or anywhere in Alaska. The hemolymph of 75 species of insects and six spiders from interior and arctic Alaska were screened for thermal hysteresis (a difference between the freezing and melting points), characteristic of the presence of AFPs. Eighteen species of insects and three spiders were shown to have AFPs. Ten of the insects with AFPs were beetles including the first species from the families Chrysomelidae, Pythidae, Silphidae and Carabidae. In addition, the first Neuropteran to have AFPs was identified, the lacewing *Hemerobius simulans* together with the second and third Diptera (the first Tipulids) and the second and third Hemiptera, the stinkbug *Elasmostethus interstinctus* (the first Pentatomid), and the water strider *Limnoporus dissortis* (the first Gerrid). Prior to this study, 33 species of insects and three spiders had been reported to have AFPs. Most AFP-producing terrestrial arthropods are freeze avoiding, and the AFPs function to prevent freezing. However, some of the AFP-producing insects identified in this study are known to be freeze tolerant (able to survive freezing) to very low temperatures (−40 to −70 °C).

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1. Introduction

Antifreeze proteins (AFPs), first discovered in the blood of Antarctic fish (DeVries, 1971), lower the freezing point of water in the presence of ice while not affecting the melting point. This produces a difference between the freezing and melting points which is termed thermal hysteresis (DeVries, 1986). AFPs are unique in their ability to produce thermal hysteresis and consequently it is diagnostic for their presence. The magnitude of thermal hysteresis is dependant on the specific activity and concentration of AFPs, and in some cases the presence of enhancers of AFP activity (Duman, 2001; Duman and Serianni, 2001). AFPs lower the freezing point by a non-colligative mechanism whereby the AFPs adsorb onto preferred surfaces of potential seed ice crystals (Raymond and DeVries, 1977; Raymond et al., 1989; Brown and

Sönnichsen, 2002). Consequently, growth of the crystal (addition of water molecules to the crystal surfaces) can only occur between the adsorbed AFPs and in high radius of curvature fronts (high surface free energy), rather than the preferred low radius of curvature fronts (low surface free energy). Therefore, according to the Kelvin effect, the temperature must be lowered below the colligative melting point for growth to proceed.

AFPs have been identified in many species of high latitude marine teleost fishes, where the AFPs have evolved independently multiple times (Cheng and DeVries, 2002; Brown and Sönnichsen, 2002). Five structurally different types of AFPs have been described in fish (Brown and Sönnichsen, 2002). AFPs are also present in numerous terrestrial arthropods including spiders (Duman, 1979a; Husby and Zachariassen, 1980), mites (Block and Duman, 1989; Sjørnsen and Sömme, 2000), centipedes (Tursman et al., 1994; Tursman and Duman, 1995), and of course insects (Duman, 1977, 2001). Most terrestrial arthropods which produce AFPs are freeze avoiding. This occurs in overwintering larvae of the Pyrochroid beetle *Dendroides canadensis*

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where AFPs function (1) to inhibit inoculative freezing initiated by external ice across the cuticle (Olsen et al., 1998) and (2) to mask ice nucleators, both in the gut and hemolymph (Olsen and Duman, 1997a,b; Duman et al., 2002). While AFPs are more often present in freeze avoiding species, they are also found in certain freeze tolerant (able to survive freezing) species (Tursman and Duman, 1995; Duman, 2001). While recrystallization inhibition by AFPs does occur in these situations, additional AFP functions are likely and, consequently, their function is not well understood in freeze tolerant species.

Prior to this study 33 species of insects including Coleoptera, Collembola, Plecoptera, Orthoptera, Hemiptera, Mecoptera, Lepidoptera and Diptera had been shown to produce AFPs, usually through the presence in the hemolymph of the distinctive thermal hysteresis activity (Table 1). Note the comparatively large number of beetles known to have AFPs, and the

relatively small number of Lepidoptera and Diptera (one species each). Also, at this time no Hymenoptera had been shown to have AFPs. AFPs had not previously been identified in a single species of terrestrial arthropod from the Arctic or from anywhere in Alaska. There are several possible explanations for this. (1) While representative species from several families of insects are known to produce AFPs, the majority of known AFP-producing insects are beetles. However, while nearly half of the named species of insects in the world are beetles, the Coleoptera are less well represented in the Arctic (Danks, 1981). In contrast, among the Diptera, which are very well represented in the Arctic, only one species is known to have AFPs (Li et al., 2000). It may be that the lack of AFP-producing insect species in the Arctic simply reflects the decreasing percentage of beetles and increasing percentage of Diptera at high latitude. (2) Another possibility is that at very low temperatures AFPs might

Table 1
A list of the published antifreeze producing arthropods

Taxon		Species	Reference
<i>I. Insects</i>			
Collembola	Isotomidae	<i>Vertagopus westerlundii</i>	Zettel, 1984
		<i>Isotomurus schaefferi</i>	Zettel, 1984
		<i>Agrenia bidenticulata</i>	Zettel, 1984
		<i>Isotoma hiemalis</i>	Zettel, 1984
		<i>Isotoma propingua</i>	Zettel, 1984
		<i>Isotoma</i> sp.	Zettel, 1984
	Entomobryidae	<i>Entomobrya nivalis</i>	Meier and Zettel, 1997
Plecoptera	Perlodidae	<i>Arcynopteryx compacta</i>	Gehrken and Sömme, 1987
Orthoptera	Blattidae	<i>Parcoblatta pennsylvanica</i>	Duman, 1979b
Hemiptera	Lygaeidae	<i>Oncopteltus fasciatus</i>	Patterson et al., 1981
Mecoptera	Boreida	<i>Boreus westwoodi</i>	Husby and Zachariassen, 1980
Lepidoptera	Tortricidae	<i>Choristoneura fumiferana</i>	Hew et al., 1983
Diptera	Cecidomiidae	<i>Thecodiplosis japonensis</i>	Li et al., 2000
Coleoptera	Tenebrionidae	<i>Tenebrio molitor</i>	Ramsay, 1964
		<i>Meracantha contracta</i>	Patterson and Duman, 1978
		<i>Uloma impressa</i>	Duman, 1977
		<i>Platyedema</i> sp.	Duman, 1979b
	Elateridae	<i>Ampedus lineatus</i>	Duman, 1979b
		<i>Ampedus</i> sp.	Duman, 1979b
		<i>Lepidotus discoideus</i>	Duman, 1979b
		<i>Melanotus</i> sp.	Duman, 1979b
	Cucujidae	<i>Cucujus clavipes</i>	Duman, 1979b
	Pyrochroidae	<i>Dendroides canadensis</i>	Duman, 1979b
	Lampyridae	<i>Photinus</i> sp.	Duman et al., 1982
	Coccinellidae	<i>Coccinella novemnotata</i>	Duman et al., 1982
	Scolytidae	<i>Ips acuminatus</i>	Gehrken, 1984
	Cerambycidae	<i>Rhagium inquisitor</i>	Bremdal and Zachariassen, 1988
<i>II. Non-insect arthropods</i>			
Arachnida			
Araneae	Thomisidae	<i>Philodromus</i> sp.	Duman, 1979a
	Clubionidae	<i>Clubiona</i> sp.	Duman, 1979a
	Linyphiidae	<i>Bolyphantes</i> sp.	Husby and Zachariassen, 1980
Acari	Oribatidae	<i>Alaskozetes antarcticus</i>	Block and Duman, 1989
		<i>Phauloppia</i> sp.	Sjursen and Sömme, 2000
Chilopoda	Lithobiomorpha	<i>Lithobius forficatus</i>	Tursman and Duman, 1995

act as ice nucleators to initiate spontaneous ice formation and inhibit supercooling. This would, of course, be lethal in freeze avoiding species. This suggestion stems from the adsorption–inhibition mechanisms of action of AFPs whereby, AFPs bind to the surface of ice. However, when not bound to ice, AFPs may structure water in an ice-like fashion on their surface, and at sufficiently low temperatures this ordered water may become large enough to function as an ice nucleator and seed the hemolymph. This would appear to exclude the possibility that freeze avoiding species exposed to very low temperatures could use AFPs. (3) A third possibility is that, as concluded by Zachariassen (1985), the percentage of insects which survive winter by becoming freeze tolerant increases at high northern latitudes. Consequently, fewer AFP-producing insects might be expected in the higher northern latitudes. (4) Another possibility is that investigators have simply not looked for AFPs in far northern species. The last option is supported by the study of Alaskan species described here which identifies numerous AFP-producing species of insects and three species of spiders.

2. Materials and methods

2.1. Study sites

Field collections were made in three primary sites in Alaska. These are near (1) Fairbanks, (2) Wiseman and (3) the Toolik Field Station. Fairbanks and Wiseman are located in the interior of Alaska and have a continental climate where minimum winter temperatures routinely reach -40 to -50 °C. Both sites lie in boreal forest. Wiseman is approximately 100 km north of the Arctic Circle on the south side of the Brooks Range within 50 km of the latitudinal tree line. The most northerly site is Toolik Field Station ($68^{\circ} 38'N$), run by the Institute of Arctic Biology, University of Alaska, Fairbanks. Toolik is located in the northern foothills of the Brooks Range, and as it is a tundra site, it has a much different terrestrial arthropod fauna than the two more southerly sites which are located in the taiga.

The insects reported in this study were collected in late winter/early spring (late March and April) or in September (1999–2003). While temperatures in April had started to warm, air temperatures of -20 to -30 °C were not uncommon. At the Toolik site snow cover and subzero temperatures persist through May. In September, temperatures have begun to cool but air temperatures only occasionally dip below freezing. Some insects collected in September were cold acclimated for 1 month in the dark in a Tenney Series 942 Environmental Chamber according to the following protocol. On days 1–3 the insects were held at 0 °C, days 4–6 at -1 °C, days 7–9 at -2 °C, days 10–14 at

-3 °C, days 15–21 at -4 °C, and days 22–30 at -4.5 °C. A few species reported here were also collected in January and February when air temperatures of -35 to -50 °C are common in arctic and interior Alaska.

2.2. Thermal hysteresis measurements

Hemolymph was taken by puncturing the cuticle of the insect or spider with a 28-gauge needle, generally in an anterior section along the dorsal midline. Hemolymph (1–8 μ l) was then taken into a 10 μ l glass capillary tube, and the opposite end sealed in a flame. The hemolymph was then centrifuged into the sealed end of the tube, and the open end was sealed with mineral oil, leaving a small air space between the hemolymph and the oil. The sample was then frozen at -20 °C or below for later analysis. The difference, if any, between the freezing and melting points of the hemolymph (thermal hysteresis) was determined using the technique of De Vries (1986). The solution in the capillary is partially frozen with spray freeze and the temperature slowly raised within a translucent temperature controlled bath to melt the ice until a very small ice crystal (~ 0.25 mm in diameter) is just visible under the stereomicroscope. This is the melting point (equilibrium freezing point) of the sample. If the temperature is then lowered 0.01–0.02 °C, the crystal will begin to grow if AFPs are not present (i.e. melting point = freezing point). However, if AFPs are present the crystal will not grow until the temperature has been lowered to the hysteretic freezing point (i.e. melting point \neq freezing point) whereupon the crystal grows rapidly. Note that a seed crystal is present in the sample as the temperature is lowered, thus the temperature where the crystal grows (hysteretic freezing point) is not the nucleation temperature (supercooling point) of the sample. The seed crystal size is quite important in these measurements. As shown previously (Zachariassen and Husby, 1982; Zachariassen et al., 2002), there is often an inverse relationship between crystal size and thermal hysteresis. Consequently, thermal hysteresis values obtained by the ‘capillary technique’ described above are not directly comparable to those determined by the Clifton nanoliter osmometer as the latter employs a smaller seed crystal (see Duman, 2001 for a comparison of the techniques).

3. Results

The hemolymph of 75 species of insects from the three Alaskan study sites were screened for the presence of thermal hysteresis activity. Of these 75 species, 18 (24%) were positive indicating the presence of antifreeze proteins, 44 (59%) were negative, and 13

(17%) were questionable. In the questionable group the level of thermal hysteresis was low (generally less than 0.3 °C), but the freezing and melting points were not identical as they would be in the absence of antifreeze protein activity. Further work must be done with these species, and they are not included as having AFPs in this study. In addition, six species of spiders were screened, and three of these were positive. The thermal hysteresis positive species are listed in Tables 2–4, except for those species (mostly larvae) that could not be identified.

3.1. Coleoptera with AFPs

Of the 41 species tested for hemolymph thermal hysteresis that were beetles 10 (22.7%) were positive, 22 were negative and nine were questionable. The positive beetles which could be identified are shown in Table 2. Except for *Cucujus clavipes*, which had previously been studied in Indiana and Michigan and shown to have AFPs (Duman, 1979a), none of the beetles listed in Table 2 were previously known to have AFPs. In addition, this is the first report of AFPs in species from

the families Chrysomelidae, Pythidae, Silphidae, and Carabidae. The Carabids are the first members of the suborder Adephaga known to produce AFPs. In addition to the beetles listed in Table 2, an unidentified larval Cerambycid had hemolymph thermal hysteresis. In some cases, such as *Cucujus clavipes* and *Hypnoidus bicolor*, the levels of thermal hysteresis are fairly substantial, although not greater than seen previously in species from more moderate climates. However, it is important to note that mid-winter field collected individuals are not generally shown in Table 2. In contrast, many of the beetles shown in Table 2 had low levels of thermal hysteresis (less than 1 °C). Some of these are known from previous studies to be freeze tolerant, and it is possible that other insects with low hemolymph thermal hysteresis activity shown here are also freeze tolerant. *Phratura* sp. (Miller, 1982), *Upis ceramboides* (Miller, 1978) and *Pterosticus brevicornis* (Miller, 1969) demonstrate AFP activity and are freeze tolerant. Individuals of these species collected in January had less than 0.7 °C of thermal hysteresis in the hemolymph.

Table 2

Alaskan beetles found to have antifreeze proteins as indicated by the presence of hemolymph thermal hysteresis (a difference between the freezing and melting points)

Species	Location	Time or treatment	Stage	N	mp (°C)	fp (°C)	Thermal hysteresis (°C)
*Carabidae							
<i>Pterosticus brevicornis</i>	Fairbanks	cold accl. 2002	adult	P	−4.32	−4.75	0.43
	Wiseman	cold accl. 2002		P	−4.07	−4.60	0.53
	Fairbanks	January 2003		P	−4.35	−4.95	0.60
<i>Pterosticus laevis</i>	Fairbanks	cold accl. 2001	adult	2	−1.58	−2.14	0.56
*Silphidae							
<i>Thanatophilus sagax</i>	Toolik	cold accl. 2001	adult	1	−2.10	−2.40	0.30
Elateridae							
<i>Hypnoidus bicolor</i>	Toolik	August 30 2001	adult	P	−1.68	−4.60	2.92
<i>Athou</i> sp.	Fairbanks	April 1999	larva	2	−2.35	−3.75	1.40
		cold accl. 2002		2	−2.00	−2.30	0.30
Tenebrionidae							
<i>Upis ceramboides</i>	Fairbanks	April 1999	adult	7	−1.75	−2.18	0.43
		cold accl. 1999		2	−2.65	−3.31	0.66
		cold accl. 2001		11	−3.39	−3.80	0.41
		January 2003		3	−2.13	−2.55	0.42
		March 2003		8	−2.04	−2.85	0.54
*Pythidae							
<i>Pytho niger</i>	Fairbanks	cold accl. 2001	adult	2	−1.85	−2.15	0.30
Cucujidae							
<i>Cucujus clavipes</i>	Fairbanks	April 1999	larva	3	−0.91	−4.46	3.54
		September 1999		4	−0.83	−4.69	3.86
		cold accl. 1999		P	−3.30	−7.92	4.62
*Chrysomelidae							
<i>Phratura</i> sp. (possibly <i>P. interstitialis</i>)	Fairbanks	April 1999	adult	P	−2.10	−2.90	0.80
		September 1999		P	−1.60	−2.20	0.60
		cold accl. 1999		P	−2.42	−2.58	0.46

The collection site, time of collection and the overwintering stage collected are indicated. Some insects collected in September were cold acclimated for 1 month as described in the text. “N” provides the sample size. “P” indicates a pooled sample. * indicates taxa that were not previously known to have antifreeze protein producing members. The only species listed previously known to produce AFPs is *Cucujus clavipes*.

Table 3

Alaskan insects (non-beetles) found to have antifreeze proteins as indicated by the presence of hemolymph thermal hysteresis (a difference between the freezing and melting points)

Species	Location	Time or treatment	Stage	N	mp (°C)	fp (°C)	Thermal hysteresis (°C)
Hemiptera							
*Pentatomidae							
<i>Elasmostethus interstictus</i>	Fairbanks	September 1999	adult	2	−0.90	−1.70	0.80
*Gerridae							
<i>Limnopus dissortis</i>	Fairbanks	cold accl. 2001	adult	1	−0.75	−1.27	0.52
*Neuroptera							
Hemerobiidae							
<i>Hemerobius simulans</i>	Fairbanks	September 1999	adult	2	−0.92	−1.60	0.68
		cold accl. 1999		P	−2.40	−3.60	1.20
Diptera							
*Tipulidae							
<i>Tipula</i> sp.	Fairbanks	cold accl.	larva	1	−0.80	−1.30	0.50
<i>Limonia</i> sp.	Fairbanks	cold accl.	larva	P	−1.07	−1.50	0.43

The collection site, time of collection, and the overwintering stage collected are indicated. Some insects collected in September were cold acclimated for 1 month as indicated. “N” provides the sample size. “P” indicates a pooled sample. * indicates taxa that were not previously known to have antifreeze protein producing members.

Table 4

Alaskan spiders (collected near Fairbanks) found to have antifreeze proteins as indicated by the presence of hemolymph thermal hysteresis (a difference between the freezing and melting points)

Species	Time or treatment	N	mp (°C)	fp (°C)	Thermal hysteresis (°C)
<i>Philodromus</i> sp. (Philodromidae)	April 1999	3	−1.74	−3.13	1.39
	cold accl. 1999		−3.47	−4.93	1.46
<i>Pardosa</i> sp. (*Lycosidae)	cold accl. 2001	1	−3.40	−3.65	0.25
<i>Gnaphosa</i> sp. (*Gnaphosidae)	cold accl. 2001	1	−3.90	−4.75	0.85

All were immatures, and therefore could not be identified to species. The collection site and the time of collection are indicated. Some spiders collected in September were cold acclimated for 1 month as indicated. “N” provides the sample size. “P” indicates a pooled sample. * indicates taxa that were not previously known to have antifreeze protein producing members.

3.2. Non-Coleoptera with AFPs

Table 3 lists five additional insects, all non-Coleoptera, which had hemolymph thermal hysteresis activity. These include the first Neuropteran known to have AFPs, the lacewing *Hemerobius simulans*, a species known to be freeze tolerant (Miller, 1982). Previously, only one Hemipteran, the milkweed bug *Oncopeltus fasciatus* (Patterson et al., 1981) and one Dipteran, the midge *Thecodiplosis japonensis* (Li et al., 2000), have been shown to have AFPs. Therefore, the stinkbug *Elasmostethus interstictus* is the first known AFP-producing Pentatomid, and the water strider *Limnopus dissortis* (Drake and Harris) is the first known AFP-producing Gerrid. *E. interstictus* is known to be freeze avoiding (Barnes et al., 1996). In addition, the *Tipula* sp. and the *Limonia* sp. are the first Tipulids and just the second and third Diptera shown to have AFPs. These genera represent two of the three sub-families of Tipulids (Tipulinae and Limoniinae).

3.3. Spiders with AFPs

Table 4 identifies three AFP-producing spiders. Only three spiders have previously been shown to have AFPs

(Duman, 1979b; Husby and Zachariassen, 1980). The Alaskan *Pardosa* sp. is the first Lycosid and the *Gnaphosa* sp. the first Gnaphosid species known to have AFPs. A *Philodromus* sp. of crab spider from Indiana was previously shown to have AFPs (Duman, 1979b). Because there are seven species of *Philodromus* with ranges extending from Indiana to Alaska it is possible that the Alaskan *Philodromus* sp. shown in Table 4 is the same species as studied previously in Indiana. All three of these spiders were juveniles, and therefore they could not be identified to species.

4. Discussion

This study of Alaskan insects and spiders adds considerably to the list of known AFP-producing terrestrial arthropods by identifying 17 new species of insects and three species of spiders with AFPs, thus increasing the number of insects known to have AFPs by over 50% and doubling the number of known AFP-producing spiders. The first Neuropteran, the lacewing *Hemerobius simulans*, was shown to have AFPs. Previously, only one Hemipteran and one Dipteran were known to produce AFPs, and this work has added two new species to each

group, all in new families. Also, representatives from four new beetle families and two new families of spiders were shown to produce AFPs. Altogether, 25.9% of the Alaskan terrestrial arthropods tested, including arctic species, demonstrated thermal hysteresis activity suggesting that AFPs are a widespread adaptation to high latitudes, especially in interior Alaska.

Most AFP-producing insects and spiders exhibit hemolymph thermal hysteresis activities of 2–5 °C, with some individuals having as much as 8–9 °C (Duman, 2001). While this level of antifreeze protein activity is greater than that seen in either fish (~0.5–2.0 °C of thermal hysteresis) or plants (~0.2–0.5 °C) (Duman et al., 1993; Duman, 2001), this 2–5 °C would not be sufficient to provide much protection from the temperature extremes which many overwintering insects must endure. However, the functional value provided by the insect AFPs, by inhibiting inoculative freezing by external ice and by inhibiting ice nucleators, greatly exceeds the 2–5 °C of thermal hysteresis seen in the hemolymph of midwinter insects (Olsen et al., 1998; Duman, 2001, Duman et al., 2002). For example, AFPs from the beetle *Dendroides canadensis* at a concentration which produced ~2° of thermal hysteresis completely inhibited the ice nucleating activity of ice nucleating active bacteria or hemolymph protein ice nucleators, if the synergistic effect of the AFP enhancer glycerol was present.

Many of the species listed in Tables 2–4 have only low levels of thermal hysteresis (less than 1 °C). There are a number of potential reasons for this. Some species were collected only in early September. While moderately low temperatures were persistent at this time, it may have been too early in the season for more significant accumulation of AFPs. In many cases September collected individuals were subjected to cold acclimation in the laboratory. While this often led to an increase in thermal hysteresis activity, even when none was seen prior to cold acclimation, the acclimation conditions may have been insufficient to trigger the full AFP production characteristic of winter. However, the low hemolymph melting points often measured in the cold acclimated animals indicate the accumulation of polyols and polyol production typically requires more rigorous acclimation conditions than does AFP production (Duman et al., 1991a). Also, some of the species collected in early September (i.e. *Cucujus clavipes* from Fairbanks and Wiseman, and *Hypnoidus bicolor* from Toolik) already have relatively high thermal hysteresis activity.

Another possibility is that some of these species with low levels of thermal hysteresis are freeze tolerant. While AFPs in low concentrations may improve survival of freeze tolerant species, high levels of AFPs and/or high thermal hysteresis activity may actually be counterproductive in an organism that is adapted to

freeze. The inhibition of ice nucleators by AFPs, with the resulting extended supercooling and/or the fast ice crystal growth that follows freezing in the presence of high levels of thermal hysteresis may be deleterious in freeze tolerant systems (Tursman et al., 1994). The cold tolerance adaptations of some of the more common insects found in the vicinity of Fairbanks have been previously studied by Keith Miller and John Baust (see Miller, 1982 for a review of these species), including the especially well studied freeze tolerant beetles *Pterosticus brevicornis* (lower lethal temperature of –70 °C) (Miller, 1969, 1982; Baust and Miller, 1970), *Upis ceramboides* (LLT of –60 °C) (Miller, 1978, 1982; Miller and Smith, 1975) and *Phratora* sp. (LLT of –45 °C or less) (Miller, 1982), and also the lacewing *Hemerobius simulans* (LLT of –40 °C or less) (Miller, 1982). Hemolymph thermal hysteresis activities of all four species are comparatively low (Tables 2 and 3). This combination of low thermal hysteresis and freeze tolerance is reminiscent of plants which produce AFPs (Duman et al., 1993; Duman and Olsen, 1993; Griffith et al., 1997) and of the centipede *Lithobius forficatus* (Tursman et al., 1994). Addition of appropriate AFPs to centipede (Tursman and Duman, 1995) or plant (Newton, 1999) cells provides protection from damage resulting from freezing. The mechanism of this protection is unknown. However, AFPs of all types inhibit recrystallization, the process whereby water molecules migrate from the surfaces of ice crystals with higher surface free energies (i.e. smaller crystals with greater radii of curvature) to those with lower surface free energy (i.e. larger crystals) (Knight and Duman, 1986). AFPs, even at very low concentrations, prevent recrystallization and the potential damage to tissue which can result (Knight and Duman, 1986; Tursman et al., 1994). Another possible protective effect is suggested by studies with fish AFPs where specific AFPs have been shown to provide membrane stabilization at low temperatures (reviewed by Tomczak and Crowe, 2002). Fish type-1 AFPs can alter the phase transition temperature of cell membranes, and fish glycoprotein antifreezes stabilize membranes during phase transitions, thereby preventing leaking. Thus, fish AFPs bind to, and thereby stabilize cell membranes. While they are also free in the extracellular fluid, AFPs appear to associate with the cell membrane in both the centipede, *L. forficatus* (Tursman and Duman, 1995), and the plant, *Solanum dulcamara* (Newton, 1999). However, it is not known if the AFPs are able to stabilize the cell membranes in these systems. The function(s) of the AFPs in those freeze tolerant insects listed in Tables 2 and 3 is unknown, but it is unlikely that they would perform the usual antifreeze function typical of AFPs in freeze avoiding animals. For one, the level of antifreeze activity appears to be too low to be of consequence, and secondly it is not clear why a freeze tolerant insect, adapted to survive freezing and often with extracellular

ice nucleators to inhibit supercooling, would benefit from the usual antifreeze function of AFPs.

In spite of the above discussion, the most important finding of this study is not the magnitude of the thermal hysteresis activity found in these Alaskan insects and spiders, but rather the large proportion of species with the presence of thermal hysteresis, which signifies that they produce AFPs. Successful overwintering, especially in environments such as the interior or arctic Alaska where temperatures are extreme and the winters may be 7–9 months in duration, requires an extensive suite of adaptations, whether the organism is freeze tolerant or freeze avoiding (Duman et al., 1991c; Lee and Denlinger, 1991; Storey and Storey, 1988; Zachariassen, 1985; Leather et al., 1993). It is apparent that AFPs comprise an important component of these adaptations in both freeze avoiding and freeze tolerant Alaskan arthropods.

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