

Cold-Hardiness of the Most Common Soil Invertebrates in Northeast Asia. 2. Cold-Hardiness as an Adaptation to Climate

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Abstract—Data on the occurrence and adaptive potential of three mechanisms of cold-hardiness are analyzed in different taxa of soil invertebrates under the conditions of the cold climate of Northeast Asia. The relationship between low temperature resistance and overwintering conditions in selected species is discussed. It is shown that taxonomically close species may have different mechanisms and values of cold-hardiness. The effect of winter low temperature resistance on the biotopical distribution and formation of the soil invertebrate fauna in permafrost regions is assessed.

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The first part of our article (Berman and Leirikh, 2017) deals with cold-hardiness of 64 soil-dwelling invertebrates of the Asian Northeast that belong to 6 taxonomic groups. Such a number of the species studied is clearly insufficient for building the general picture of adaptation of invertebrates to extremely low overwintering temperatures. However, further accumulation of material would be impractical without interpretation of the results already available. The objective of the present study is comparative characterization of the cold-hardiness of soil invertebrates and assessment of its role in shaping the biotopical distribution and geographic ranges of these animals.

EFFECTIVENESS AND OCCURRENCE OF THE MECHANISMS OF COLD-HARDINESS

As it was stated in the first part of this work (Berman and Leirikh, 2017), soil invertebrates in Northeast Asia exhibit all the three cold-hardiness strategies known to date: cryoprotective dehydration, supercooling, and extracellular freezing of liquids (Fig. 1). Apparently, **cryoprotective dehydration** may be considered as the most effective mechanism of low temperature resistance, which, in extreme cases, ensures survival of earthworm *Dendrodrilus rubidus tenuis* (Eisen 1874) egg cocoons at a temperature of -196°C (Berman et al., 2010a) (Fig. 2). Among the taxa studied, the fraction of invertebrates that utilize this mecha-

nism is fairly low: besides earthworm cocoons, these are eggs of three species of the slug and larvae of several species of the click beetle. They have small bodies and water-permeable integuments, which, presumably, is instrumental to this strategy of protection from cold. Another compulsory requirement is the ability to accumulate polyols (in earthworm cocoons) or polyols plus reserve lipids (in click beetle larvae).

Freeze tolerance may ensure survival of invertebrates at the temperatures down to -83°C (Miller, 1982; Li, 2011) and probably even lower. In our studies, only the groundhopper *Tetrix fuliginosa* (Zetterstedt, 1828) and the click beetle *Denticollis varians* (Germar, 1846) larva withstood temperatures below -40°C . Resistance of other freeze-tolerant organisms ranged from -5°C (the earthworm *Aporrectodea caliginosa* (Savigny, 1826)) to -35°C (the amphipod *Traskorchestia ditmari* (Derzhavin, 1923)). This mechanism is found in a greater number of taxa than the previous one; it is also utilized by all the earthworms that survive at below-zero temperatures (5 species out of 13 studied), the click beetle *Hypnoidus hyperboreus* (Gyllenhal, 1827), 4 species of myriapods, and 2 slug species.

The ability of earthworms to survive freezing is brought about by polyol and glucose synthesis from reserve glycogen. However, only a few species can accumulate it in significant amounts, due to the specific-

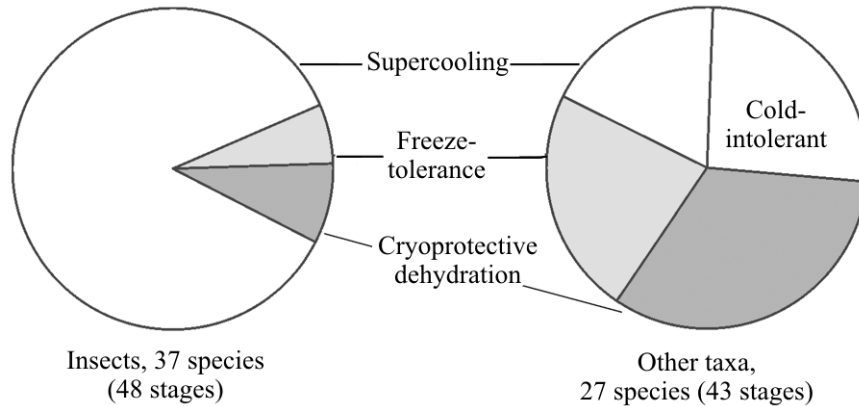


Fig. 1. Distribution of cold-hardiness strategies in the overwintering stages of soil invertebrates studied in Northeast Asia.

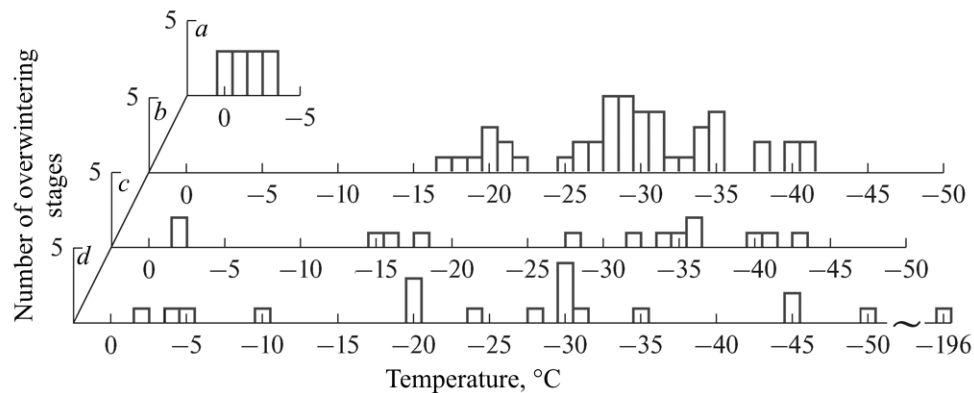


Fig. 2. Distribution of the lower lethal temperature (LT100) in 91 overwintering stages (64 species) of soil invertebrates: cold-intolerant (a), supercooling (b), freeze-tolerant (c), and dehydrating (d).

ties of the structure of the chloragogen tissue, and initially the reserve glycogen probably ensured survivorship during summer diapause (Byzova, 1977).

Supercooling is the predominant mechanism in soil invertebrates. Due to low supercooling points (SCP), freezing does not occur in overwintering ants, grasshopper and harvestmen eggs, most click beetle larvae, the scale insect *Arctorthezia cataphracta* (Shaw, 1794), and terrestrial shelled gastropods. Effectiveness of this mechanism is comparable to the former two: the lowest SCPs of certain species approach $-40\text{--} -44^{\circ}\text{C}$, whereas the minimum values lie below -50°C , such as in the ants *Leptothorax acervorum* (Fabricius, 1793), *L. muscorum* (Nylander, 1846), and *Camponotus herculeanus* (Linnaeus 1758). The bulk of the studied species has mean SCPs around -30°C . Lower resistance is found in most shelled gastropods and in 4 out of 5 *Formica* species. It is in this state that all the insect eggs studied to date overwinter.

SEASONAL DYNAMICS OF COLD-HARDINESS

The pattern of seasonal changes in cold-hardiness differs even among animals that utilize the same mechanism, let alone those realizing different mechanisms.

The seasonal propensity for supercooling was tracked in detail in ants. Changes in the SCP from summer to autumn amounted to $6\text{--}12^{\circ}\text{C}$ in *Formica* species and $22\text{--}30^{\circ}\text{C}$ in other species of ants. The decrease in SCP was accompanied by significant stabilization of the supercooled state in *Formica*: in summer, one-day exposure to a temperature $10\text{--}12^{\circ}\text{C}$ higher than the SCP is lethal to ants; in winter, only $3\text{--}5^{\circ}\text{C}$ higher. Cold-hardiness was increasing gradually from August till December, doing so at a greater pace in larvae than in workers. Throughout the following 4 months, SCPs remained steadily low and increased only after the nest temperature had reached -10°C .

Species of soil invertebrates which are able to tolerate temperatures below -35°C

Species, stage	Strategy, LT100	Chemical constitution, % of wet mass		
		polyols	sugars	dehydration
<i>Camponotus herculeanus</i> , A, L	Supercooling, -38°C	10–15	2	10
<i>Leptothorax acervorum</i> , A, L	Supercooling, -40°C	10–15	1	10
<i>Eisenia nordenskioldi</i> , worms	Freezing, -36°C	0.4	0.4	20
<i>E. nordenskioldi</i> , cocoons	Dehydration, -46°C	+	n/a	30
<i>Tetrix fuliginosa</i> , A, L	Freezing, -43°C	0.3	0.3	3
<i>Traskorchestia ditmari</i> , A, L	Freezing, -41°C	0.3	0.04	n/a
<i>Denticollis varians</i> , L	Freezing, below -42°C	0.4	0.2	0

Notes: A, adults; L, larvae.

Measurements of cryoresistance parameters in other invertebrates, as a rule, were carried out in midsummer (July) and at the end of winter (March). The availability of a sufficient number of animals allowed for additional measurements in August–September. Invertebrates that overwintered in the supercooled state during postembryonic developmental stages showed seasonal changes in SCP and in its ratio to lethal temperatures that were within the range given above for ants. The smallest changes were observed in species with low winter cold-hardiness (shelled gastropods) or in those showing summer non-specific resistance (the scale insect). The changes were greater in click beetle larvae. In grasshoppers and harvestmen, the difference between SCP values of newly laid and overwintering eggs is no more than 3°C and is probably determined not by the pattern of temperature decrease in autumn but by the diapausing stage.

Earthworms of the 9th genetic lineage of the nominate subspecies of *Eisenia nordenskioldi* (Eisen, 1879), which is the only lineage in the Northeast (Shekhovtsov et al., 2015), do not survive freezing in summer; this ability is attained by the end of August. By midwinter, the earthworms have accumulated up to 0.3% of glycerol and survive cooling down to -35°C . On the contrary, the centipede *Lithobius steingeri* Bollman 1893, which also overwinters in the frozen state, withstands cooling down to 2°C below the SCP even in summer and more than 30°C below the SCP in winter.

Seasonal changes in cold-hardiness of slug eggs, which overwinter in the state of cryoprotective dehydration, differ from the changes described above for supercooled grasshopper eggs. In the latter, the SCP changes only slightly due to acclimation, whereas

cold resistance of *Deroceras reticulatum* (Müller 1774) eggs that have undergone acclimation (-30°C) is more than twice that of non-acclimated eggs (Berman et al., 2011).

CHEMICAL BASES OF MECHANISMS OF COLD-HARDINESS

The chemical bases of the three cold-hardiness mechanisms mentioned above may be either similar or different even within the same family of invertebrates. The lowest mean SCPs (about -40°C) are typical of 3 ant species: *Leptothorax acervorum*, *L. muscorum*, and *C. herculeanus*; these combine the significant supercooling ability with accumulation of 16–20% of polyols (Berman et al., 2010) (table). The population of *C. herculeanus* from the upper reaches of the Kolyma has greater cold-hardiness than the population from a more southerly and warmer region of North America (Pincher Creek, Alberta, Canada, 50°N , 114°W), where the mean SCP was -28.7°C and glycerol content was 5.8% (Sømme, 1964). Among the previously studied *Camponotus* species, the mean SCPs were the lowest for adults from the Kolyma population (Berman et al., 2017), i.e., cold-hardiness in these ants is probably close to the maximum for the genus. Apparently, the Kolyma population lives at the limit of its physiological capacities, as attested for by significant winter mortality in nearly each of the 20 nests examined.

Survival of the representatives of other taxa that tolerate temperatures below -30°C is provided by other mechanisms. *E. nordenskioldi*, the ground-hopper, amphipods, and myriapods are freeze-tolerant; earthworm cocoons utilize cryoprotective dehydration. Cryoprotective agents may be represented by polyols,

such as in the earthworms (Berman and Leirikh, 1985), similar to the situation found in ants, or by compounds unidentified by us, such as in the groundhopper, the amphipod, and *D. varians* click beetle larvae (Berman et al., 1989; 1990; 2013).

Animals with moderate cold-hardiness exhibit no less diverse mechanisms behind it. These are mostly individuals that overwinter in the supercooled state with the SCP of about -30°C . The decrease of the SCP in winter may also be accompanied by accumulation of polyols and sugars, although in smaller amounts than in the first group, with a maximum of 10% in the ants *Myrmica kamtschatica* Kupianskaya, 1986 (Berman et al., 2012). Low SCP values in overwintering grasshopper and harvestmen eggs are facilitated by the thick egg shell that prevents inoculation of ice from the outside and by the absence of internal crystallization nuclei; this is probably why these eggs do not need to accumulate antifreezes (Leirikh et al., 2009). Click beetle larvae overwinter both in the dehydrated and in the supercooled state; both mechanisms in these animals may be associated with an increase in concentration of polyols or may take place in the absence thereof (Berman et al., 2013).

The least cold-hardy species have SCPs within $-17... -22^{\circ}\text{C}$ and contain small amounts of low-molecular-weight antifreezes; cold-hardiness is achieved, in particular in *Formica* ants, due to greater stability of the supercooled state than in summer (Berman et al., 1984, 1987, 2010).

A number of freeze-tolerant species contain characteristic glycoproteins that act as cryoprotectors that can prevent recrystallization of ice (Denlinger and Lee, 2010). These compounds, which earlier were found in *Lithobius forficatus* (Linnaeus, 1758) (Tursman and Duman, 1995), are presumably involved in cold-hardiness maintenance in the myriapod species studied by us as well as in the groundhopper and the amphipod. This is indirectly indicated by the absence of increase in the concentration of low-molecular-weight cryoprotectors from summer to winter.

The species that can utilize any of the three mechanisms of survival at below-zero temperatures but are cold-resistant due to accumulation of polyols probably rely on the presence of reserve glycogen by the onset of negative temperatures as a means of attaining the maximal cold-hardiness. However, the reserves accumulated by autumn may become spent on energy metabolism before the freezing of the

overwintering substrate. It may be hypothesized that in cases of a prolonged transition period in autumn or overwintering at low positive temperatures (which is fairly often the case in the European part of Russia), the species-specific maximal resistance to cold may not be realized. Year-to-year changes in cold-hardiness parameters are apparently related to differences in summer and autumn weather, which determine the conditions for accumulation and expenditure of reserves. A convincing argument supporting these statements is that the mean SCPs in ants are highly variable among sites with different overwintering conditions within the same area (Berman and Zhigul'skaja, 1995; Berman et al., 2010).

INTERPOPULATION DIFFERENCES IN COLD-HARDINESS

In view of the foregoing, one can hardly expect that animals from populations inhabiting different climates could have similar cryoresistance; that is likelier to be found in the species which overwinter at the egg stage. In the Northern Hemisphere, over 450 species have been studied that overwinter in the supercooled state. The SCPs of two or more geographically remote populations are known for only 25 species (Turnock and Fields, 2005). Thermal parameters of the overwintering sites for the vast majority of these species are not reported. Only three species that overwinter in the tree crowns or under bark (i.e., above the snow level) show a correlation between SCP and environmental temperature. In general, the data available are absolutely insufficient for drawing any conclusion on the geographic variation of cold-hardiness in supercooled invertebrates.

Unfortunately, only a few species addressed by us were also studied from other regions: the already mentioned ants *Camponotus herculeanus* (Sømme, 1964), slugs *Deroceras laeve* (Müller, 1774) (Storey et al., 2007) and *D. reticulatum* (Bale, 1985; Cook, 2004), the earthworm *Eisenia nordenskioldi* (Holmstrup and Petersen, 1997), and the grasshopper *Chorthippus fallax* (Zubovski, 1900) (Hao and Kang, 2004). In these species, except for the eggs of *Ch. fallax*, Northeastern populations show a significantly higher resistance to below-zero temperatures.

However, it should be pointed out that it is almost impossible to carry out a correct comparison because different authors, as a rule, use different methodological techniques for sampling and rearing, as well as

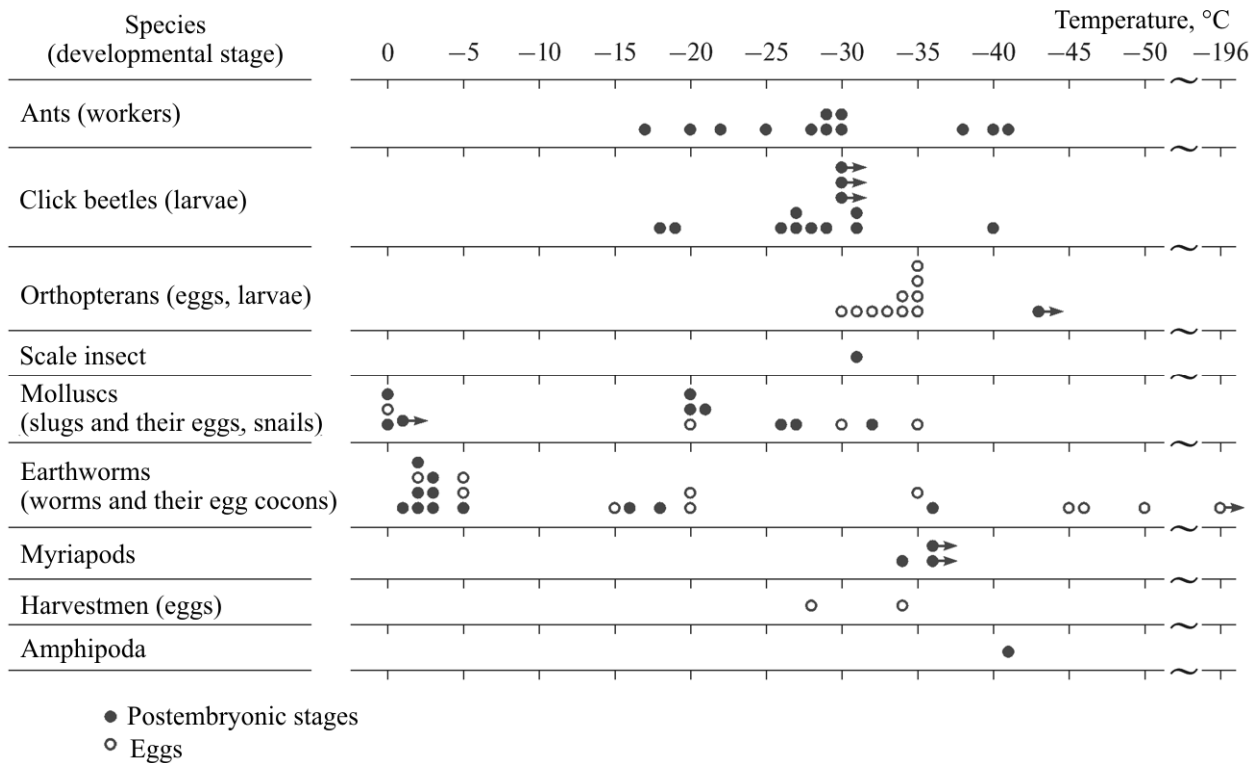


Fig. 3. Lower lethal temperature (LT100) in different species of studied groups of soil invertebrates (64 species). Arrows point towards undetermined values.

different acclimation regimes, cooling and heating rates, etc.

We also studied several invertebrate species that overwinter in biotopes differing in soil temperature minima within the same region and in climatically dissimilar regions. In the coldest of these regions, in the upper reaches of the Kolyma, 2 series of 8 *Leptothorax acervorum* nests in each showed a statistically significant difference in the SCPs of adult (2.0°C) and larval ants (6.2°C), while the difference in the temperature minima at the depth of location of winter chambers amounted to 8–10°C (Berman et al., 2010). Much greater differences in SCP that correlated with overwintering conditions were revealed in the ants *L. acervorum* (up to 20°C) and three *Formica* species (up to 12°C): *Formica exsecta* Nylander, 1846; *F. gagatoides* Ruzsky, 1904, and *F. lemani* Bondroit, 1917, from the populations of the upper Kolyma and the environs of Magadan, and from Finland (Berman et al., 2010). However, in *Myrmica* ants, no such relationship between SCP and environmental temperature was found (Berman et al., 2012).

It is even more difficult to assess interpopulational differences in cold-hardiness for invertebrate species that overwinter in the frozen state, because the methods and assessment criteria are even less standardized and the number of the species studied is small. According to our data, the earthworms *Dendrobaena octaedra* and their egg cocoons have similar cold-hardiness values in different climatic zones (Meshcheryakova and Berman, 2014). Also similar are lethal low temperatures for *Lumbricus rubellus* cocoons that overwinter in the state of cryoprotective dehydration. Presumably, geographically remote populations of freeze-tolerant species from other groups would also show similar values of analogous parameters.

TAXONOMIC POSITION, DEVELOPMENTAL STAGES, AND COLD-HARDINESS MECHANISMS

Mechanisms of cold-hardiness and its possible limits are unrelated to the taxonomic position of the studied animals (Fig. 3). Below are the most telling examples. Cold-hardiness of 5 species of *Formica* that

inhabit the Asian Northeast appeared to be different: their mean SCPs range from -17 to -30°C . On the contrary, 4 species of *Myrmica* have almost the same degree of resistance. As a reminder, all ants share the same mechanism of cold-hardiness, namely, supercooling (Berman et al., 2010).

Another insect family, click beetles (Elateridae), exhibits all the three cold-hardiness mechanisms with a very similar outcome: the larvae tolerate temperatures of about -25°C , except for the larvae of *Denticollis varians* that survive temperatures below -40°C in the frozen state (Berman et al., 2013).

Two slug species belonging to the same genus have different cold-hardiness mechanisms (as well as different life cycles): frozen *Deroceras laeve* survives cooling down to -28°C , whereas *D. reticulatum* successfully overwinters at the egg stage in the state of cryoprotective dehydration at a temperature as low as -35°C (Berman et al., 2011).

Earthworms of the 9th genetic lineage of the nominate subspecies of *Eisenia nordenskioldi* overwinter at both developmental stages and survive cooling down to -35°C (Berman and Leirikh, 1985); another species of this genus, *E. fetida*, does not tolerate below-zero temperatures at any stage (Berman et al., 2009). On the contrary, the earthworms from different orders *Dendrobaena octaedra* (Savigny, 1826) (Opisthopora, Lumbricidae) and *Drawida ghilarovi* Gates, 1969 (Moniligastrida, Moniligastridae) overwinter in the frozen state and have a similar lethal temperature of about -16°C (Berman et al., 2002, 2010a).

Eggs of various invertebrates studied by us do not tolerate freezing (Leirikh et al., 2009; Berman et al., 2011), as do all the eggs studied to date (over 70 species): those of insects, mites, spiders (Sømme, 1982), enchytraeids (Bauer et al., 2001), and earthworms (Holmstrup et al., 2002; Meshcheryakova and Berman, 2014). They can only overwinter in the supercooled (insect and harvestmen eggs) or dehydrated state (earthworm and slug eggs).

Overwintering Northeastern insect larvae follow different strategies of cold-hardiness. Larvae of some species can supercool (ants, the Arctic scale insect, click beetles *Orithales serraticornis* (Paykull, 1800), *Sericus brunneus* (Linnaeus, 1758), *Limonius koltzei* Reitter, 1895, *Selatosomus melancholicus* Fabricius, 1798, *S. gloriosus* (Kishii, 1955), *S. impressus* (Fabri-

cius, 1792), *Prosternon sericeum* (Gebler, 1824), and *Ampedus* sp.). Larvae of others can freeze, such as the click beetle *Denticollis varians*, or dehydrate to a significant extent, such as the click beetles of the genus *Oedostethus* and *Hypnoidus hyperboreus*.

Adults of ants, scale insects, and some click beetles (*Orithales serraticornis*, *Selatosomus melancholicus*, and *Sericus brunneus*) can supercool, whereas the adult click beetle *H. hyperboreus* is capable of freezing, and so are all the postembryonic stages of the groundhopper.

Freeze-tolerance and cryoprotective dehydration predominate in representatives of the remaining invertebrate taxa studied (myriapods, an amphipod, slugs and their eggs, earthworms and their egg cocoons) (Fig. 1). Only the small terrestrial shelled gastropods and harvestmen eggs overwinter in the supercooled state. Centipedes, namely, both species of lithobiids and one geophilid, can withstand cooling down to $-25\ldots -31^{\circ}\text{C}$ in the frozen state. The amphipod *Traskorchestia ditmari* also freezes (at -6°C) and successfully overwinters in this state at the temperatures as low as -35°C .

Earthworms belonging to 5 out of 13 taxa studied by us withstand below-zero temperatures due to freeze tolerance, whereas their egg cocoons survive in the state of cryoprotective dehydration. However, not all the species exhibit efficient dehydration of egg cocoons, and dehydration does not always guarantee cold-hardiness (Meshcheryakova and Berman, 2014).

COLD-HARDINESS AND BIOTOPICAL DISTRIBUTION

According to the degree of adaptedness to winter conditions of the continental part of the Asian Northeast, the animals studied can be divided into several groups.

The first group is composed of species with high cold-hardiness that tolerate temperatures of $-30\ldots -35^{\circ}\text{C}$ and lower. Their biotopical distribution is practically unlimited by winter thermal conditions and therefore they predominate in background landscapes and biotopes that share the following common features: high position of the permafrost level (50–60 cm), excessive moisture content in the soil, and predominance of the moss and lichen cover. This group includes 2 ant species (*Leptothorax acervorum* and *Camponotus herculeanus*), the groundhopper *Tetrix fuliginosa*, and the click beetle *Denticollis vari-*

ans. These insects overwinter in the subsurface soil layer, under leaf litter and snow that noticeably buffer the ambient temperature (from -50°C in the air to $-20\text{...}-25^{\circ}\text{C}$ at a depth of 5 cm in the soil).

Some species of this group, while having equally high cold-hardiness, occur locally due to constraints imposed by summer conditions (moisture distribution, pH, etc.). These include the earthworm *Eisenia nordenskioldi*, the amphipod *Traskorchestia ditmari*, and the ant *Leptothorax muscorum*.

Two lithobiid species with LT50 of -30°C may also be included in this group; their lower thermal limits lie below -35°C , but precise values could not be determined due to the absence of the equipment required. Thus, we assign 9 out of the 64 species studied to the group of the most cold-resistant invertebrates. Among these, freeze-tolerant organisms predominate. There are species not included in the group but still worth mentioning; these have egg cold-hardiness below -35°C but the postembryonic stages are less resistant, such as in the earthworms *Dendrobaena octaedra* and *Dendrodrilus rubidus tenuis* and the slug *Deroceras reticulatum*. They are absent in the continental part of Northeast Asia due to a very short growing season for an “egg-to-egg” life cycle to be completed.

The second group includes 36 species that tolerate winter temperatures down to $-20\text{...}-25^{\circ}\text{C}$. It is mostly comprised by insect species that overwinter at different stages in the supercooled state: adults and larvae of *Myrmica* ants, adult *Formica gagatoides*, grasshopper eggs, the scale *Arctorthezia cataphracta*, and most click beetle larvae (9 out of 13 species). Other taxa are represented by harvestmen that overwinter as eggs and the snail *Vertigo modesta* (Say, 1824). The freeze-tolerant geophilid *Escaryus japonicus* Attems 1927 and the slug *Deroceras laeve* may also be included here. The abovementioned ants and click beetle larvae overwinter at a somewhat greater depth, i.e., under milder conditions than in the species of the first group. The biotopical distribution of all these invertebrates is almost unlimited by winter temperatures in the sparse forest zone; they are abundant and are among the dominant species in their assemblages. Part of the species occurs locally but their distribution is determined not by insufficient cold-hardiness but by unfavorable summer conditions: low amount of heat, potential depth of winter chambers in ants, etc.

The third group comprises species with limited cold-hardiness that tolerate temperatures of $-10\text{...}-15^{\circ}\text{C}$. These include 4 out of 5 *Formica* ant species and 4 species of terrestrial shelled gastropods. All of these overwinter in the supercooled state and occur locally in warmer patches. Some ant species occupy habitats that quickly and deeply thaw through during spring and retain higher-than-ambient temperatures of soil horizons in winter due to the presence of the snow cover.

Similar cold-hardiness is found in several animal species that inhabit the coast of the Sea of Okhotsk (the environs of Magadan) with a milder climate but do not penetrate into the continental parts of the area. These include the earthworm *Dendrobaena octaedra*, which overwinters in the frozen state and withstands temperatures down to -14°C , and two invasive slug species: *Deroceras altaicum* (Simroth, 1886) and *D. agreste* (Linnaeus, 1758).

Finally, two earthworm species occur in the Northeast in the anthropogenic habitats only: *Eisenia fetida* and *Dendrodrilus rubidus tenuis*. The former, due to its inability to survive below-zero temperatures at both stages, inhabits exclusively non-freezing sites: along poorly insulated heating pipelines, in the “streams” of continuous industrial warm water discharge, in year-round heated greenhouses, etc. Cocoons of the latter species tolerate extremely low temperatures but the life cycle cannot be completed in the natural habitats due to the shortness of the growing season. Greenhouses that are heated in spring and thus prolong the growing season by August may harbor many hundreds of *D. rubidus tenuis* earthworms and tens of thousands of their egg cocoons per 1 m^2 .

Cold-hardiness is clearly not the only adaptation of Northeast Asian invertebrates that facilitates their thriving under the extreme climatic conditions. A number of other adaptations can also be observed. In ants, these include decreased degree-day requirements during summer at the expense of life-cycle modifications: the absence of rapid brood and prolongation of larval development over 2–3 years (Zhigul'skaya et al., 1989, 1992). In addition, the least cold-hardy species persist in relatively warm (at the depth of location of winter chambers) patches that generally occupy a small area (Berman et al., 2010).

Overwintering at the immature stages, which is typical of the groundhopper *Tetrix fuliginosa* and the slug *Deroceras laeve*, allows prolonging development

over two or more seasons and thus becoming independent of weather conditions of the short northern summer (Berman et al., 1989; 2011). The groundhopper has an adult diapause as well (Berman et al., 1989), but this was not found in the slugs. In England, the slugs *D. reticulatum* overwinters at different ages, and the population typically contains two overlapping generations (Bale, 1985). Under the conditions of Magadan, the life cycle of this species is seasonally synchronized: the slugs die out during the first cold snaps, whereas the eggs enter embryonic diapause. In contrast, *Deroceras laeve* can tolerate below-zero temperatures during the postembryonic stages only, and so it would be natural to expect the existence of imaginal diapause in this species.

Survival of grasshoppers in Northeast Asia is indubitably facilitated by their multiyear embryonic diapause (“superpause”) (Bey-Bienko and Mishchenko, 1951; Uvarov, 1966; Berman and Leirikh, 2006). In southern regions, this property ensures survival of the population in drought years (Uvarov, 1966); in the north, it allows surviving cold or excessively rainy years.

COLD-HARDINESS AND GEOGRAPHIC RANGES

The role of cold-hardiness in shaping the geographic distribution of animals could be assessed for selected species from a few groups, because, for most species studied, there was no information on cold resistance in the south of their distribution ranges.

Ants. High cold-hardiness of the ants of the Low Arctic species complex allows them to survive in the upper soil horizons extremely harsh winters across the entire northern Eurasia, including the Pole of Cold of the Northern Hemisphere—Oymyakon and Verkhoyansk. On the contrary, the limited ability to supercool in *Formica* ants explains depauperation of their fauna in the upper Kolyma and even more so in the upper reaches of the Yana and Indigirka, where winter temperatures are lower still.

In general, the cold-hardiness characteristics obtained for most species are likely to be close to the limits possible for ants. This is indirectly suggested by the above mentioned depauperation of their fauna in the upper Indigirka basin relative to the upper Kolyma basin and even more so to Central Yakutia (Berman et al., 2010). Harsher winter conditions in Northeast Yakutia accompanied by the lower amount of heat in summer in comparison with the abovementioned re-

gions appear to be insurmountable for many *Formica* species, and perhaps also *Myrmica*.

Thus, insufficient resistance of ants to low temperatures is a strong factor of faunogenic processes along with the shortage of summer heat required for the completion of development, biotic relationships, and historical circumstances.

Slugs. Three out of four species (except for *Deroceras laeve*) studied by us are introduced in the area and are encountered locally and separately from each other around Magadan. They are not found farther inland, although egg cold-hardiness, at least in *D. reticulatum*, is more than sufficient for successful overwintering in the continental regions. The absence of the latter species is explained by circumstances unrelated to the overwintering conditions, primarily the short frost-free period. The limiting factors seem to be insurmountable, since the invasion has probably lasted no less than 75–80 years, starting from the onset of intensive exploitation of the region (Berman et al., 2011).

Harvestmen. Cold-hardiness revealed in the overwintering harvestmen eggs shows that *Mitopus morio* (Fabricius, 1799) is not restricted by low winter temperatures either in the environs of Magadan or in many biotopes of the continental parts of Magadan Province, and *Homolophus arcticus* Banks, 1893, is even less so (Leirikh et al., 2009). The preliminary data on developmental rates indicate that both *M. morio* and *H. arcticus* have enough time and heat for completion of the life cycle within one season; warm years may even provide a certain reserve. In cool summers, part of the animals probably fails to lay eggs in time before the beginning of winter. On the whole, both harvestmen species are common every year and their populations are stable in the environs of Magadan.

Earthworms. The species diversity of earthworms on the Russian Plain declines northward and eastward. Only one or two species are found almost everywhere to the east of Western Siberia, barring the valleys of big rivers, mountains (the Altai-Sayan system, the Sikhote-Alin, etc.), and disturbed territories (Vsevolodova-Perel, 1997).

The most cold-hardy earthworm is the 9th genetic lineage of *Eisenia nordenskioldi nordenskioldi*, which inhabits the Asian North and Yakutia; it is not restricted by the thermal conditions of overwintering. The relation of other lineages of the *Eisenia norden-*

skioldi complex (Berman and Meshcheryakova, 2013; Shekhovtsov et al., 2015, 2016; Berman et al., 2016) to low below-zero temperatures has not been studied. *Eisenia sibirica* Perel et Graphodatsky, 1984 tolerates temperature not lower than -12°C , and the limited capabilities for overwintering at low temperatures prevent this endemic species from expanding its range (Berman et al., 2016). Increasing winter severity from the west to the east prevents invasion of the cosmopolitan species *Dendrobaena octaedra* in Siberian tundra and taiga beyond the soil minimum isotherm of $-12... -14^{\circ}\text{C}$ at a depth of 3 cm (Berman et al., 2002). The overwintering conditions similarly control the northward expansion from the south of Khabarovsk Territory in the earthworm *Drawida ghilarovi*, which in the frozen state withstands temperatures down to -16°C (Berman et al., 2010b). The distribution of *Aporrectodea caliginosa* is limited by cold resistance of the adult stage (-5°C) and possibility for the completion of its ontogeny, including egg cocoon production, within one season. The distribution range of the species with highly cold-tolerant egg cocoons (*Dendrodrilus rubidus tenuis*, *Octolasion lacteum*, *Lumbricus castaneus*, and *L. rubellus*) is limited in the east to the biotopes that either do not freeze through during winter (the adult stage does not survive temperatures below zero) or are warm enough during summer so that the life cycle can be completed. The distribution of the species that do not tolerate cooling down to $-2... -5^{\circ}\text{C}$ at any developmental stage is also restricted to the biotopes that do not freeze from the surface (*Eisenia fetida*, *Eiseniella tetraedra*, and *Aporrectodea rosea*) or (*Lumbricus terrestris*) to deeper soil horizons (Meshcheryakova and Berman, 2014).

This study provides an unequivocal answer regarding the primary role of either winter or summer climatic conditions on the assembly of local faunas of the studied groups. In particular, the substantial similarity of winter thermal (and permafrost) conditions in the upper Kolyma basin and Central Yakutia and the striking difference in their faunas suggests that depauperation of the species diversity of upper Kolyma ants and orthopterans is primarily caused by the shortage of heat during summer. The diversity of slugs and earthworms in the continental parts of the Asian Northeast is impoverished not only due to the insufficient resistance of overwintering stages but also because of the short duration of the growing season, which prevents them from reaching the cold-resistant

stages before the onset of cold (such stages may be different in different species). The role of historical circumstances also cannot be excluded: there is little probability of recovery in a species that has gone extinct from the vast territory of Northeast Asia, for example, due to an anomalously cold and snowless winter, since there is no nearby source of secondary (remedial) invasion, in contrast to Central Yakutia.

The most important conclusion that can be drawn from our work is that taxonomically related species may have different mechanisms and values of cold-hardiness. Different developmental stages of a species can also have different resistance to negative temperatures, which is again underpinned by different mechanisms. Consequently, so far it has not been possible to predict cold-hardiness for a certain species, even knowing it in some representatives of that genus. At best, one can guess, but not predict, the mechanism of cold-hardiness.

This pessimistic conclusion, does not, of course, inevitably call for total analysis of all invertebrates, but it necessarily obliges us to seek heuristic approaches. We would like to draw attention to four of them, which seem to be promising in research on the most general aspects of cold-hardiness.

(1) The origins of cold-hardiness were not discussed in the article. The available data on the occurrence of strategies in different invertebrate taxa are insufficient to build hypotheses regarding their evolution (Chown and Sinclair, 2010).

Significant variations in resistance within genus may be viewed as the signs of both the trait's recency and conservatism. It is obviously tempting to link the origin of cold-hardiness to the last glaciation. One specific evidence in favor of this idea in our material is high cryoresistance of earthworms of the tropical genus *Drawida* (Berman et al., 2010b) that has penetrated into Primorskii Territory. The origin of cryoresistance in *D. ghilarovi* may be elucidated by studying this trait in its relatives that inhabit Central China and more southerly regions of that country.

Cold-hardiness may be viewed as an ancient trait that mirrors the species' existence in cold regions in the past. Ice ages ("cryochrons") are no rare events in the history of the Earth. Only during the Pleistocene (1.7 million years), at least 16 "great" glaciations are recognized. However, the evolutionary age of most invertebrates we deal with is much older. Furthermore,

the morphological traits of Pleistocene insects that have been preserved in the permafrost are indistinguishable from those in modern-day species (Kiselev, 1981; Matthews, 1977a, 1977b).

Ice ages are known to have occurred long before the Pleistocene (*Winters...*, 1979). Localization of the glaciations was hardly the same, which determined the differences in the distribution of landscapes, dispersal of animals, and eventually the formation of cold-hardiness, which is of primary interest to us. Thus, development of adaptive strategies in relation to cold may have had a long history, and then it would be more correct to speak about adaptations as originating outside the discussed timeframe.

Among such cases, there would be preadaptations, i.e., novelties that emerged as byproducts of adaptation to a factor other than cold. For example, it seems evident that cryoprotective dehydration may have emerged during adaptation to aridity.

(2) It is shown in the corresponding section of this article and in previous publications (Meshcheryakova and Berman, 2014) that cryoresistance of earthworms and that of their cocoons are by no means linked and may come in whatever combination. As a reminder, *Dendrodrilus rubidus* cocoons survive submersion into liquid nitrogen, whereas the earthworms do not withstand cooling below zero, although both the worms and the cocoons overwinter in the same layer—in leaf litter. On the contrary, both stages of *Eisenia nordenskioldi* of the 9th genetic lineage are highly tolerant to low temperatures.

The absence of relationship is explained by essentially different mechanisms behind cold-hardiness. Earthworms of most of the taxa studied (11 out of 15) remain unfrozen only down to $-5...-3^{\circ}\text{C}$ (i.e., they are supercooled); further cooling leads to their freezing through and death. Representatives of the remaining 4 taxa that belong to different genera, in contrast, survive freezing, i.e., formation of ice inside the body.

Conversely, cocoons of all the species, except for *Eisenia fetida* (Savigny, 1986), exhibit cryoprotective dehydration. Low temperature tolerance in this case is promoted by loss of nearly all of the osmotically active water. Switching from cryoprotective dehydration in cocoons to freeze tolerance or supercooling ability in active worms occurs instantaneously during hatching. Apparently, it is the emergence of crystallization nuclei on the earthworm body surface that triggers this process.

To what extent are the experimentally obtained cryoresistance values, both for earthworms and their cocoons, variable and do they depend on the thermal conditions of overwintering? In other words, is the measured cold-tolerance varying, temperature-dependent, and characteristic of adaptive “behavior” within the reaction norm, or is it a species-specific, fixed trait? Our material on earthworm resistance in geographically remote populations suggests that cold-hardiness is a species-specific trait that varies negligibly, i.e., is evolutionarily fixed. Therefore, the evolution of low temperature tolerance in earthworms and their cocoons followed different pathways, even though both developmental stages occur in the same environment, i.e. in the soil.

The case discussed above is another example of independent evolution of developmental stages in the same organism (embryo and adult), which was earlier shown for insect larvae and adults that live in the soil and above ground, respectively (Ghilarov, 1949).

With respect to the above statements, it seems quite interesting and important to study deeply and in parallel the relation to below-zero temperatures in preimaginal stages and adults of the same species that utilize different mechanisms of cold-hardiness.

(3) On the basis of the material presented and the synthesis of scarce literature sources it may be hypothesized that animals surviving negative temperatures in the supercooled state and freeze-tolerant species have different patterns of geographic variation of cold-hardiness.

Invertebrates that overwinter above the snow level mostly tolerate freezing, which occurs, as a rule, at moderate subzero temperatures of $-5...-10^{\circ}\text{C}$. It seems as though that their maximum resistance is initially “tuned” to extremely low air temperatures both in the temperate and ultracontinental climate.

On the contrary, cold-hardiness of animals that overwinter in the supercooled state depends on the current environmental temperature: the lower the latter, the lower the SCP, and the greater cold-hardiness. It varies even among soil invertebrates living in adjacent biotopes with different thickness of the snow cover, i.e., depending on the degree of insulation of the overwintering sites (Berman and Zhigulskaja, 1995). It remains unclear to what extent the ability to promptly respond to a temperature change differs between animals from regions with mild and harsh win-

ter climate (or, equivalently, how large is the genetic component acted upon by natural selection).

In order to test this hypothesis, it would be worthwhile to compare cold-hardiness in peripheral (westernmost and easternmost) populations of widespread freeze-tolerant and supercooling species, since the January isotherms are mostly known to show a longitudinal pattern. In the broadest terms, the problem is reduced to determining cold-resistance in two groups of animals that originate from extremely different climatic regions. The populations from the continental parts of Northeast Asia studied by us may serve as a starting point.

(4) It should be borne in mind that caution should be exercised in uncovering geographic variation of cold-hardiness, since it may be confounded by fluctuations caused by weather conditions not only during winter but also during previous seasons. This is due to the dynamics of accumulation as well as timing and pattern of expenditure of reserves utilized by the organism for the synthesis of cryoprotectants. Their accumulation during summer indubitably depends on hygrothermal conditions. If overwintering most of the time takes place at positive or near-zero temperatures, reserves may be spent on vital processes. Glycogen is a universal reserve substance, from which both the sugars (trehalose) and polyols may be synthesized in the insect organism. Glycerol is the most widespread and metabolically efficient of polyols; the pathways of polyol and sugar synthesis from glycogen are addressed in detail by Storey and Storey (1991, 2012). An early onset of temperatures that suppress metabolism triggers the conversion of reserves into cryoprotectants (Storey and Storey, 1991, 2012), which additionally enhance cold-hardiness. Figuratively speaking, accumulator capacity is limited, and its power may be spent on different purposes, but every time at the expense of some other purpose. This idea is supported by a wealth of evidence, which is unfortunately scattered over the literature and is obtained in different times and for different organisms. Thus, it cannot be strictly proved, as of now, but seems quite promising. The described approach seems to be more applicable to supercooled rather than frozen animals.

Part of the above conclusions heavily depends on the selection of the animal groups studied. Such are the conclusions on the occurrence of cold-hardiness mechanisms (supercooling, freeze tolerance, and cryoprotective dehydration), biotopical distribution as af-

ected by cold-hardiness, “fine-tuning” of soil invertebrates in general in accordance with soil temperature levels, etc. A different composition of the studied groups could yield somewhat different conclusions. In particular, inclusion of dipteran larvae, an entirely disregarded yet abundant component of almost any type of soil fauna, may exert an especially significant effect on the results. The vast majority of beetle families have not been studied in terms of cold-hardiness of overwintering stages. Undoubtedly, it would be inappropriate to combine the inhabitants of different strata in the same dataset: soil, leaf litter, subnival and above-snow vegetation.

In conclusion, we would like to emphasize that it is not possible to predict the shifts in species distribution ranges under different scenarios of climate change without assessing cold-hardiness. Unfortunately, we are yet far from being able to solve such problems.

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