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[Short Communication]

Heat susceptibility of ice nucleators limiting supercooling ability of the house spider, *Achaearanea tepidariorum* (Araneae: Theridiidae)

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Abstract — Supercooling ability of the house spider, *Achaearanea tepidariorum*, is depressed by exogenous ice nucleators ingested with prey animals. To characterize the ice nucleators, the effect of heat exposure on the ice nucleating activity of the nucleators was examined. Spiders that fed on a heated isopod's gut (80–90°C) had a lower supercooling point (SCP) or temperature of crystallization than those that fed on an unheated gut. This result suggests that the ice nucleators limiting the supercooling ability of the predator are organic in origin.

Key words — Ice nucleators; Supercooling point; Spider; Heat susceptibility

The house spider, *Achaearanea tepidariorum*, is a freezing intolerant predator and avoids lethal tissue freezing by extensive supercooling (Tanaka 1993). The supercooling ability is depressed by exogenous ice nucleators taken from prey animals (Tanaka 1994, 2001, 2005; Tanaka & Watanabe 2003). In the body of prey animals such as *Porcellio scaber*, ice nucleators limiting the supercooling ability of the spiders exist at least in the gut (Tanaka 2005), thus suggesting that prey animals intake them by feeding. As ice nucleators have been shown to remain active in the alimentary canal of *Acheta domestica* at least for 12 h (Tanaka 2005), they will be transferred from the environment to predators via their prey. At present, however, little is known about the nature of the ice nucleator.

Because of the ubiquity, both inorganic (e.g. mineral dusts or soil particles) and organic (e.g. microorganisms) ice nuclei can be the candidates as the ice nucleators (e.g. Costanzo et al. 2000). The objective of the present study is therefore to determine if the nucleators limiting supercooling ability of *A. tepidariorum* are organic or inorganic in origin. Because organic ice nucleators are more susceptible to heat than inorganic ones (e.g. Costanzo et al. 2000), the effect of heat exposure on the ice nucleating activity of the nucleators was examined.

Laboratory stocks of *A. tepidariorum* were established from females collected in May 2004 from the campus of Miyagi Gakuin Women's University, Sendai (38°16′N), Japan. Newly hatched nymphs were reared individually in a glass tube (15 mm in diameter and 70 mm in height) plugged with cotton wool and were placed at 26°C under a diapause-avoiding photoperiod (16 h light: 8 h dark; Tanaka 1991). They were fed daily on hatchlings of the cricket, *A. domestica*, cultured on an ice nucleator-free artificial diet, Insect Feed (Oriental Yeast, Tokyo) (Tanaka 2001). A preliminary experiment showed that crickets reared on this diet do not influence supercooling point (SCP) or temperature of crystallization of spiders, which remains as low as −15°C, indicating that they could be regarded as ice nucleator-free prey. Spider nymphs about 40–50 days old (average 8.0 mg wet weight) were used for the present study. Each of them was given a selected prey item 12 h before SCP determination. Methods used for SCP determination were substantially the same to that used in the previous study (Tanaka 2005).

The terrestrial isopod, *Porcellio scaber*, was chosen as an experimental prey animal. This crustacean is a natural prey for *A. tepidariorum* and is known to have efficient exogenous ice-nucleating agents reducing the ability of spiders to supercool in the gut (Tanaka 1989, 2001, 2005). Juveniles of the isopod were collected on the campus of Hokkaido University, Sapporo, in July 2005 and were divided into five groups; four of the five were heated to various temperatures from 60 to 90°C at 10°C intervals and the remainders at 25°C (control) both for 1h by using a controlled temperature (±1°C) water bath (E-2, Yazawa Sci. Technol., Tokyo, Japan). After the treatment, they were dissected and the gut was removed. *A. tepidariorum* is a web-builder and did not eat the dissected gut supplied. Therefore, isolated guts were fed to crickets (*A. domestica*) starved for more than 72 h at 25°C. Twelve hours after feeding, the crickets were given to the spider.

Figure 1 compares SCPs of the spiders that ingested indirectly either heated (60–90°C) or unheated (25°C) isopod's gut by feeding on crickets (*A. domestica*) that had consumed the isopods gut. The heat treatment reduced significantly the effect of the ice nucleators on the supercooling ability of the spiders; indirect feeding on heated isopod's gut lowered the SCPs (ANOVA, $F_{4,26}=154.9$, $P<0.0001$). SCPs of the spiders that fed on isopod's gut after exposure, for 1 h, to 80°C or higher was significantly lower than that of the spiders given mildly heated (60–70°C) or unheated gut (Tukey-Kramer test, $P<0.05$). No difference was found in mean SCP between spiders of the latter three groups (Tukey-Kramer test, $P>0.05$).

The present experiment indicates that the ice nucleators reducing the supercooling ability of the spiders are susceptible to heat of 80–90°C. This means that they are organic. Although various organic materials are capable of serving as ice nuclei (Vali 1995; Hirano & Upper 1995), bacteria are the most probable candidate. The biological ice nucleators

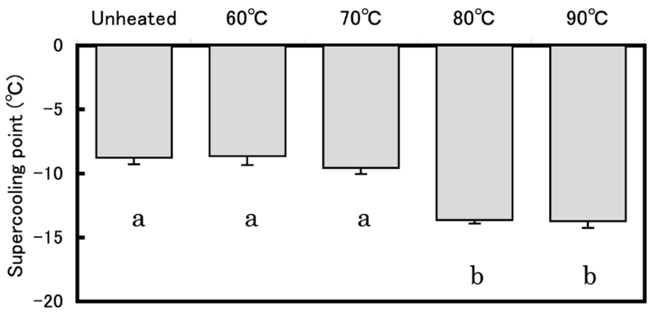

Fig. 1. Effect of 1 h heat treatment on ice nucleating activity of ice nucleators in the gut of prey (*Porcellio scaber*). The ice nucleation activity is estimated by mean SCP (±SE) of *A. tepidariorum* that fed on either heated (60–90°C) or untreated (25°C) gut of field-collected *P. scaber* through crickets (*Acheta domestica*). The crickets were reared on artificial diet free from ice nucleators and given heated or unheated isopod gut just before offering to the spider. Sample size is 5, 11, 4, 7 and 7 from left to right. Bars with the same letter are not significantly different (Tukey-Kramer test, $P > 0.05$)

occur commonly in the environment, particularly on dead leaf or detritus, the main food of the prey, *P. scaber* (Hirano & Upper 1995). It has also recently been found that the SCP of spiders ingested ice-nucleating bacteria (*Pseudomonas syringae*) is in the range of that for the summer-active spiders taken from the field (Tanaka & Watanabe 2003).

However, several features of ice nucleating active bacteria so far reported do not agree with the trend noted in the present study. For example, ice nucleating activity of *P. syringae* is destroyed or inactivated by heating to 65°C for 5 min (Maki et al. 1974). In *Pseudomonas* sp., ice nucleating activity decreases in two steps, above 30°C and above 80°C (Pouleur et al. 1992). In the present study, however, ice nucleating activity of the nucleators in the gut of prey was stable up to 70°C (Fig. 1). Thus, we can not exclude the possibility that organic ice nucleators other than bacteria lower the supercooling capacity of spiders. A more extensive study is now in progress to identify the factor causing ice nucleation in the alimentary canal of the spiders.

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