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Ice nucleators in the gut of prey reduce supercooling ability of a predator, *Achaearanea tepidariorum* (Araneae: Theridiidae)

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Abstract — In many arthropod species, the gut contains exogenous ice nucleators. These substances might be transmitted to spiders through predation and cause ice formation in the spider's alimentary canal. To test this hypothesis, supercooling point (SCP) of the house spider, *Achaearanea tepidariorum* that ate either the whole body of *Porcellio scaber* or only the isolated gut by way of cricket were compared. Ingestion of the cricket that had fed isopod's gut elevated the spider's SCP from about −15°C to −9°C and this effect was comparable to that of whole body of the isopod. The supercooling ability of *A. tepidariorum* is thus limited by ice nucleators present at least in the gut of prey animals.

Key words — exogenous ice nucleator, supercooling point, gut

Introduction

Overwintering arthropods are commonly classified into either freeze susceptible or freeze tolerant based upon their ability to survive the freezing of their body water. The former is intolerant of internal freezing, thereby avoid lethal tissue freezing by supercooling, while the latter can survive the freezing of the body water (Lee 1991).

As in other spiders hitherto examined (Kirchner 1987), the house spider, *Achaearanea tepidariorum*, is intolerant of internal freezing (Tanaka 1993). The freeze avoidance is highly dependent on its supercooling ability. The supercooling point (SCP) or the temperature of ice crystallization of *A. tepidariorum* varies from −8°C in summer to −20°C in winter (Tanaka 1993). The supercooling ability of this spider is depressed by exogenous ice nucleators taken from prey animals (Tanaka 1994, 1996, 2001; Tanaka & Watanabe 2003). At present, however, it remains unknown where the ice nucleators localize in the body of prey animals.

Gut content is one of the efficient exogenous ice nucleators for many arthropod species (Cannon & Block 1988; Lee 1991). It is therefore possible that the ice nucleators in the gut of prey are transmitted to the spider and seed the formation of ice crystals in the alimentary canal. The aim of this study is to test this possibility. If ice nucleators in the gut of prey limit the ability of spiders to supercool, it should be expected that feeding solely on the isolated gut of prey causes elevation of the predator's SCP comparable to feeding on the whole body prey. In the present study, therefore, SCPs of spiders that ate either the whole body or the isolated gut of a prey were compared. As a prey animal, the terrestrial isopod, *Porcellio scaber*, was used. The alimentally canal of this isopod is relatively large, so that the gut is easily removed. This species is included in the natural diet of *A. tepidariorum* (Tanaka 1989) and has potent ice nucleators which elevate the spider's SCP after ingestion (Tanaka 1994, 2001). I here provide evidence that ice nucleators in the gut of prey animals reduce the supercooling ability of its predator.

Materials and Methods

The laboratory stocks of the house spider, *A. tepidariorum*, were established from females collected in May 2003 at the campus of Miyagi Gakuin Women's University, Sendai (38°16′N), Japan. Newly hatched spiders obtained from an egg sac produced by the female spider were reared individually in glass tubes (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, *Acheta domesticus*, being cultured with "ice nucleator-free" Insect Feed (Oriental Yeast, Tokyo) (Tanaka 2001).

Juveniles of a terrestrial isopod, *Porcellio scaber*, were collected at the experimental field of Hokkaido National Agricultural Experiment Station, Sapporo, in August 2003. They were divided into two groups; one group was given directly to spiders. The remainders were dissected and the gut was removed. *Achaearanea tepidariorum* is a web-builder and did not eat the isolated gut supplied. Therefore, the isolated gut of *P. scaber* was fed to crickets (*A. domesticus*) starved for more than 72 h at 25°C. Twelve hours after feeding, the crickets were given to the spider. Five spiders were examined in each treatment.

To determine the SCP, spiders were put individually in test tubes (10 mm in diameter and 40 mm in height) in which each specimen was in contact with the tip of a copper-constantan thermocouple connected to a recorder (Soft Thermo E830; Technol Seven, Yokohama, Japan). The cooling rate was approx. 1°C/min. The SCP was recorded as the lowest temperature reached just before the release of latent heat of fusion as body water freezes. SCP values were compared among treatments using one-factor analysis of variance (ANOVA), with means distinguished ($P < 0.05$) using Fisher's least significant difference test.

Results

Table 1 summarizes the effects of feeding whole body isopod (*P. scaber*) and only gut of isopod by way of cricket (*A. domesticus*) on the supercooling ability of the spider (*A. tepidariorum*). SCPs of spiders depended significantly on prey animals (ANOVA, $F = 74.9$, $P < 0.0001$). The spiders that fed on the whole body *P. scaber* and those ate isopod-gut-fed cricket had significantly higher SCPs than the spiders given a control cricket free of the isopod's gut (LSD, $P < 0.0001$), while the mean SCPs of the former two groups did not differ significantly ($P = 0.8657$).

Discussion

Present results indicated that ingestion of the field-collected isopod reduces the supercooling ability of its predator compared with that of the laboratory-cultured cricket (Table 1). This confirms the previous results: *P. scaber* taken from the field contains efficient ice nucleators and transfers them to the spider on feeding, while the laboratory-cultured prey is free of such substances (Tanaka 2001).

The objective of the present study is to localize such ice nucleators in the body of *P. scaber* taken from the field. Ingestion of dissected isopod's gut by way of cricket elevated the spider's SCP and this effect was comparable to that of the whole body of the isopod (Table 1). These observations clearly indicate that, in *P. scaber*, ice nucleators limiting the supercooling ability of its predator exist at least in the gut.

Because the ice nucleators in the body of *P. scaber* are of external origin (Tanaka 2001), it is likely that *P. scaber* incorporates them in the process of feeding. Under the natural conditions, this isopod mainly feeds on plant leaves, detritus or decomposed litter which are known to be rich in potent ice nucleators (Schnell & Vali 1972; Hirano & Upper 1995). Although the ice nucleators taken up by *P. scaber* on feeding have not been identified yet, the substances do not lose their ice nucleating activity even if they are in the alimentary canal of cricket for at least 12h (Table 1). This means that the ice nucleators are highly resistant to the cricket digestive enzyme. Thus, it is expected that the ice nucleators in the environments are transmitted to spiders by way of alimentary canal of the prey.

The present result does not exclude another possibility that potent ice nucleators localize in the tissues of prey animals outside the gut. In addition to the exogenous ice nucleators mentioned above, it is also known in several insect species that some endogenous ice nucleators present in haemolymph, muscle, fat body cells and other tissues (e.g. Zachariassen & Hammel 1976; Tsumuki & Konno 1991; Mugano et al. 1996). Although it remains unknown whether such endogenous ice nucleators affect the supercooling ability of its predator, the cricket-spider system used in the present study would be a useful prey-predator model system to test this possibility.

Table 1. Mean (±SE) supercooling point of *Achaearanea tepidariorum* that fed on the whole body of isopod (*Porcellio scaber*) directly and only gut of isopod by way of cricket (*Acheta domesticus*) indirectly. In column, means followed by the same letter are not significantly different ($P > 0.05$, Fisher's least significant difference test). Sample size is 5 in each treatment.

Prey	Supercooling point (°C)
Porcellio scaber	-8.9 ± 0.3^{a}
Porcellio-gut-fed cricket	-9.1 ± 0.5^{a}
Control cricket*	-15.1 ± 0.4^{b}

*Crickets were cultured on ice nucleator-free artificial diet.

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