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Do northern populations of *Reticulitermes speratus* (Kolbe) possess an additional physiological capacity to cold-acclimate that enhances cold tolerance during the winter?

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ABSTRACT

We measured and compared different carbohydrates, the total lipids, and the body water content between summer- and winter-acclimatized *Reticulitermes speratus* (Kolbe) in three latitudinal regions to determine whether northern populations possess an additional physiological capacity to acclimate to cold temperatures. The results of supercooling points and the lower lethal temperatures of summer- and winter-acclimatized *R. speratus* workers revealed that these termites are adapted to cold climates by altering their carbohydrate metabolites (e.g., glucose and trehalose), and balanced their body water content and total lipids to improve their survivability by adapting the physiological strategies to tolerate inclement environment conditions. We observed a mean SCP of −5.1 °C for summer-acclimatized *R. speratus*, which is below the observed mean lower lethal temperature; their SCP slight increase to −6.9 °C with winter-acclimatized *R. speratus*. Within three contrasting locations, *R. speratus* populations in Kyoto had higher levels of carbohydrate metabolites alteration, lower supercooling points, and lower lethal temperatures. The results of this study support the null hypothesis that termite adapted the physiological strategies to tolerate cold environment. However, the geographical range of *R. speratus* is not necessarily dependent on only its physiological strategies. Instead, we conclude that it appears to rely on a combination of behavioral, physiological, and other competitive adaptations that ensure the survival of the whole colony under low, non-optimal temperatures. Additionally, the northern populations of *R. speratus* do not possess an additional physiological capacity to cold-acclimate that enhances cold tolerance during the winter.

Introduction

In general, insects living at higher latitudes tend to be more tolerant to low temperatures than their counterparts because temperature variation increases with latitude (Addo-Bediako et al., 2000; Sinclair et al., 2003; Sunday et al., 2012)e This hypothesis may reflect the evolution of a suite of biological adaptations in higher latitude organisms to cope with temperature variation. This hypothesis has also led to many studies seeking to understand the mechanisms of thermal tolerance of insects (Bale, 2002; Chown et al., 2004; Danks, 2000; Singh and Khan, 2009; Storey and Storey, 1991; Teets and Denlinger, 2013) including termites (Cabrera and Kamble, 2004; Clarke et al., 2013; Lacey et al., 2010; Sponsler and Appel, 1991).

A classic example of a termite behavioral trait is habitat exploration. Hu and Appel (2004) reported that *Reticulitermes* spp. were able to adjust their minimum critical temperature to survive the seasonal change in soil temperature. Furthermore, their ability to excavate enables them to relocate their nest to deeper ground, where soil temperature is buffered from the climate (Clarke et al., 2013; Hu and Song, 2007; Strack and Myles, 1997). In natural settings, the habitat for termites is often underneath stumps and large pieces of decayed logs, (Grace, 1996) whereas in urban areas they can be found near heated structures and in sewer systems (Myles and Grace, 1991; Scaduto et al., 2012). In contrast to studies on the behavioral strategies of termites, much research has been published on the physiological adaptations and changes in termites in response to a gradual or abrupt decrease in temperature (Cabrera and Kamble, 2004; Davis and Kamble, 1994; Hu and Appel, 2004; Sponsler and Appel, 1991; Strack and Myles, 1997; Woodrow et al., 2000). Lacey et al. (2010) recently reviewed the research on the physiological strategies of the order Isoptera (termites) and revealed that two dampwood termites, *Porotermes adamsoni* Froggatt and *Stolotermes victoriensis* Hill, enhanced their cold tolerance by synthesizing (i.e., increasing) trehalose and unsaturated lipids which function as cryoprotectants. A disaccharide and non-reducing sugar, trehalose, has

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been found to be a cryoprotectant in many insect species (Fuller, 2004; Rudolph and Crowe, 1985; Storey and Storey, 1991; Thompson, 2003) that acts by stabilizing insect membranes during hypertonic exposure. Trehalose usually constitutes 1–2% of the sugars in hemolymph insects (Becker et al., 1996) including termites (Itakura et al., 2006; Lacey et al., 2010). The biosynthesis of trehalose (Arrese and Soulages, 2010; Candy, 1985; Nomura and Ishikawa, 2001) uses glycogen reserves and occurs when insects are under temperature stress. Cold induction can also affect other metabolites that serve as cryoprotectants, and cold tolerant insects can also acclimate to cold temperatures by altering their lipid content (Overgaard et al., 2007; Roy et al., 1991) and glucose (Costanzo et al., 1993; Storey and Storey, 1985) to increase their survivability during slower and less abrupt seasonal changes in temperature. Although the above researches show that many physiological adaptations may allow termites to survive at cold temperatures, we are unaware of research demonstrating whether physiological adaptations play a key role in limiting their range of distribution.

Reticulitermes speratus (Kolbe) is a well-known native termite species that is considered to be an urban pest in Japan due to its wide range of distribution throughout the tropical rainforest (Okinawa) and humid continental (Hokkaido) climate zones. Based on its taxonomy, *R. speratus* can be subdivided into five subspecies: *R. speratus speratus*, *R. speratus leptolabralis*, *R. speratus kyushuensis*, *R. speratus okinawanus*, and *R. speratus yaeyamanus* (Morimoto, 1968). Of these subspecies, *R. speratus* and *R. speratus kyushuensis* are the most important in economic terms in Japan. Several Japanese studies (Ikehara, 1966; Matsumoto, 1983; Morimoto, 1975) reported that the northern range limit of *R. speratus* corresponds with the region where the average January temperature does not dip below −4.0 °C. However, a well-established *R. speratus* colony is present in Asashigawa, Hokkaido (JWPA, 2013) where the mean daily temperature and the mean minimum temperature in January are −7.9 and −12.7 °C, respectively (JMA, 2014). In the same genus, *Reticulitermes flavipes* (Kollar) has been observed inhabiting the southernmost tip of Ontario, Canada which is well north of its native range in North America (Raffoul et al., 2011). The distribution of *Reticulitermes* spp. would indicate that *R. speratus* – similar to other termite species – can survive winter conditions by adapting to the cold using one or more biological adaptation. (Cabrera and Kamble, 2001; Davis and Kamble, 1994; Lacey et al., 2010).

The objectives of this study were 1) to measure the supercooling points and the lower lethal temperatures of summer-acclimatized *R. speratus* (as controls) and winter-acclimatized *R. speratus* from three contrasting locations and 2) to compare the physiological biochemical traits – including the carbohydrate metabolites (glycogen, glucose, and trehalose), body water content, and total lipid content – in termites to determine whether *R. speratus* has evolved physiological adaptations to cope with temperature variation. Finally, we aimed to project the potential distribution of *R. speratus* across Japan and worldwide.

Materials and methods

Collection of termites

Termites from three colonies of *R. speratus* were collected from each of three regions distributed across a latitudinal gradient in summer and winter: 1) Sapporo City, Hokkaido, northern Japan (43.071°N, 141.341°E: within a humid continental climate zone based on the Köpper–Geiger climate classification); 2) Uji City, Kyoto, central Japan (34.909°N, 135.797°E: within a humid subtropical climate zone); and 3) Hioki City, Kagoshima, southern Japan (31.513°N, 130.331°E: within a humid subtropical climate zone). Termites were collected from colonies in tree stumps, fallen logs and branches in the forest at the sampling sites in each region in early summer (end of July) and winter (end of November). Collected termites were packed in plastic containers with ground soil from the different areas and sent to the laboratory. During the course of two days of travel, all samples were kept within a temperature range similar to that of their natural environment (3.0 °C). After the arrival of the samples, the termites from each region were immediately removed from the transported decayed logs and branches for determination of supercooling points, lower lethal temperatures, carbohydrate metabolites, total lipids and body water content.

Bioassays

For the analysis of the supercooling points (SCPs) of the termites, we prepared five replicates (10 termites/replicate) for each colony from the three locations for each season (five replicates per colony × three colonies × three regions × two seasons = 90 replicates). To determine the SCPs, we used contact thermocouple thermometry, as described by Hanson and Venette (2013), but with a slight modification. A 24-gauge copper–constantan thermocouple was formed into a spiral and placed in a 2 mL syringe tube; termite specimens were placed in gelatin capsules (110 × 80 × 200 mm) and attached to the coiled thermocouple. The thermocouple wire leading out of the syringe was connected to a computer via a thermocouple interface, and SoftThermo E830 software (Technol Seven, Tokyo, Japan) was used for the data analysis. The thermocouples with termites were cooled to −20.0 °C at a constant of −1.0 °C per minute inside an incubator (Eyela KCL-2000A, Tokyo, Japan). The SCP was recorded from the temperature plots as the lowest temperature reached before the release of latent heat of fusion.

For the analysis of the lower lethal temperatures (LLTs) of the termites, the same experimental design for determination of the SCPs (Rust et al., 1997) was used in which there were a total of 90 replicates (10 termites/replicate). For each replicate, termites were placed in a glass Petri dish over a moistened Whatman™ number 2 filter paper (diameter 5.5 cm with 0.5 mL of distilled water) and then placed in an Eyela KCL-2000A incubator at natural temperature conditions for *R. speratus*. Both the summer and winter samples underwent a cold-shock process in which termites were exposed to a series of temperature settings (−4, −5, −6, −7, −8, −9, −10, −11.0, −12.0 and −13.0 °C). After the designated temperature was reached (at a rate of −1 °C/min), it was maintained for 30 min. After the 30 min period of cold-shock, the Petri dishes were removed and left for 2 h at room temperature for termites to either recover from chill coma or die; they were then left overnight in a conditioning chamber (at 23 °C with a relative humidity of 75%). Next, the indices of mortality were determined by separating and counting the live and dead termites after overnight exposure. The LLT_{50} and the LLT_{95} were calculated using the mortality data once mortality had reached 100%.

Measurement of carbohydrate metabolites

For the analysis of carbohydrate metabolites 36 replicates (300 termites/replicate) were prepared (two replicates per colony × three colonies × three regions × two seasons = 36 replicates). For each replicate, termites were placed in glass Petri dishes over Whatman™ number 2 filter paper and then transferred to an oven (Eyela NDO-600ND, Rikakikai, Tokyo, Japan) for drying at 100 °C for 10 min followed by additional drying at 60 °C for 48 h. The procedure described by Itakura et al. (2006) was used in sample preparation. Each sample was homogenized by combining 100 mg of dried termites with 3.0 mL and placing it in a Potter-Elvehjem ice-bath homogenizer (Sigma-Aldrich, St. Louis, MO, USA). Homogenized samples were divided into three aliquots for measurement of glycogen, glucose, and trehalose content. Each aliquot was centrifuged at 15,000 *g* for 10 min (Kubota 3500, Kubota, Tokyo, Japan).

For the glycogen analysis, 1.0 mL of 0.3 mol of trichloroacetic acid (TCA) was added to 1.0 mL of the supernatant, and the mixture was refrigerated (4 °C) for 2 h. It was then centrifuged for 20 min at 15,000 *g*. Next, 2.0 mL of the supernatant was removed and placed in a 15 mL centrifuge tube, to which 8 mL of ethanol was added. The mixture was kept