Behavioral Responses of Two Subterranean Termite Species (Isoptera: Rhinotermitidae) to Instant Freezing or Chilling Temperatures

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ABSTRACT The behavioral responses to instant freezing or chilling temperatures and survivorship of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and the Eastern subterranean termite, *Reticulitermes flavipes* (Kollar), were studied using a novel experimental design that closely simulated subterranean termites' natural in-ground environment. Both termite species responded to changes in temperature by exhibiting a downward mass movement from the cold to warmer area of constant temperature. However, the degrees of response were specific to the species and temperature regimen. Approximately 88 and 96% of *R. flavipes* escaped from instant 0°C and chilling regimens (from 24 to 0°C at a rate of 1°C/h or 1°C/12 h), respectively, compared with ≈77 and 91% of *C. formosanus*. No significant difference was detected between the two cooling regimens in either termite species. Controls resulted in a relatively even distribution within test tubes in both termite species. The small portion of the termites that did not escape endured a cold coma at a 24-h 0°C and had low mortality of 2.2 and <1% in *R. flavipes* and <5.2 and <3% in *C. formosanus* at instant and chilling regimens, respectively. This result may have implications for understanding group intelligence and decision making evolved by subterranean termites to survive temporary freezing cold.

KEY WORDS *Coptotermes formosanus*, *Reticulitermes flavipes*, low temperature, cold avoidance, behavioral response

Subterranean termites (Isoptera: Rhinotermitidae) are the most destructive pest of human wooden structures. The estimate of damage and control in the United States is over three billion dollars per year (Su 2002). Of the known termite species, the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is considered the most destructive. The Formosan subterranean termite was introduced from Asia after World War II (La Fage 1985) and has infested at least 11 southeastern states in the United States (Woodson et al. 2001, Hu and Oi 2004). Originating from tropical and subtropical regions, this species was not expected to infest areas of <4°C mean January and of < −5°C mean minimum January temperature under natural conditions (Li 1991). However, a well-established *C. formosanus* colony swarmed in northern Alabama in June 2003, where the mean and minimum January temperatures that year were 2.11 and −15°C, respectively (Hu and Oi 2004). Although a few studies have been conducted on this termite species with regard to physiological cold tolerance (Sponsler and Appel 1991, Hu and Appel 2004), no study has been carried out to understand its overwintering biology.

It has long been acknowledged that winter temperature is the primary element of all environmental factors that affect termites' northern distribution (Kofoid 1934). Subterranean termites have a well-developed sensitivity and respond quickly to temperature changes (Kofoid 1934). They do not enter diapause when subjected to cold temperatures, raising the possibility that they maintain some level of activity during the winter months (Strack and Myles 1997). In a previous study, we investigated the physiological thermal tolerances of *C. formosanus* and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Hu and Appel 2004). *R. flavipes* is another damaging species in the United States, which has a broad distribution throughout temperate regions and extends into areas with severe winters (Esenther 1969, Strack and Myles 1997). *C. formosanus* showed higher values of CTMin (critical temperature minimum, the low temperature at which a termite is knocked down but will recover at favorable temperatures) than *R. flavipes.* Interestingly, both species were able to physiologically enhance their cold tolerance levels by adjusting CTMins in response to seasonal changes of habitat soil temperature at 15-cm depth (Hu and Appel 2004). However, the subsurface soil temperature in north Alabama can drop to 2°C in winter (Alabama Mesonet Weather Data), which is much lower than the reported lowest CIMin values of 7.2°C for *C. formosanus.* It has been hypothesized that subterranean termites in nature may avoid exposure to lethal low winter temperatures by descending to deep soil (Esenther 1969, Strack and Myles 1997).

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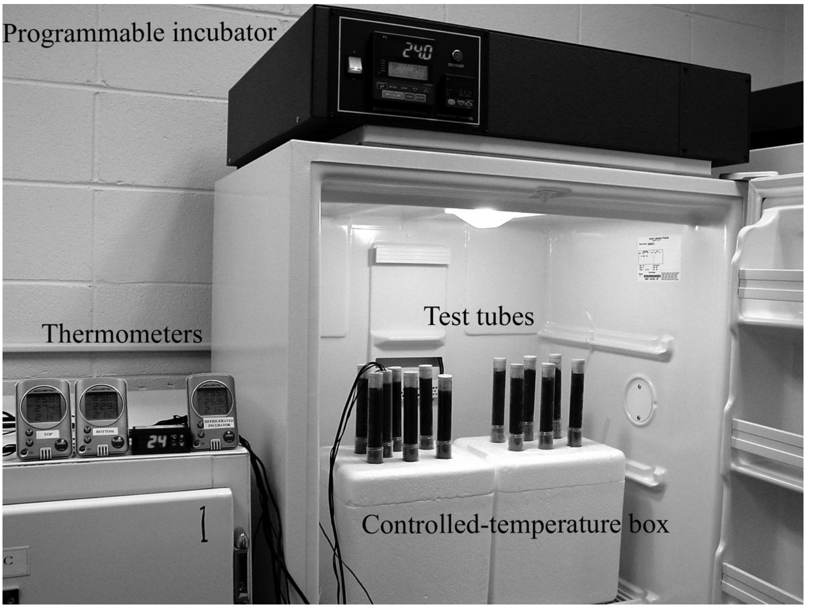

Fig. 1. Experimental apparatus for studying termite behavioral response to cool or chilling temperatures.

This study was conducted to test the hypothesis by investigating behavioral responses of *C. formosanus* and *R. flavipes* to instant freezing or chilling temperatures at different cooling rates. The experiment was designed to closely simulate subterranean termites' natural in-ground conditions to facilitate a better understanding of their behavioral mechanism to survive cold. Survivorship after exposure to the tested temperature regimens was assessed. Results from this study can provide a more comprehensive insight into how *C. formosanus* survives in the colder parts of its current distribution range.

Materials and Methods

Termite Species. Three colonies each of *C. formosanus* and *R. flavipes* were collected from field sites in central Alabama (Auburn and Opelika, Lee County) in late April by using cardboard rolls installed inside underground open-bottom plastic bucket traps (18 cm high by 15 cm diameter). The collecting sites' soil temperatures at the time of collection were between 22 and 23°C. Colonies were at least 2,000 m apart from each other and were identified using the triple mark-release-recapture technique of Su et al. (1993). Species were identified using soldier or alate characters according to Scheffrahn and Su (1994). Voucher specimens are stored in 100% ethyl alcohol in the Insect Collection of the Department of Entomology and Plant Pathology, Auburn University, Auburn, AL.

Cardboard rolls harboring termites were brought to the laboratory and maintained in clear plastic boxes (26 by 10 by 9 cm) at 24 ± 1°C. Termites were extracted by gently brushing or tapping them out of the rolls into plastic storage containers (18 by 14 by 9 cm) containing moistened pieces of No. 1 Whatman filter papers. Termites were used within 2 d of field collection.

Experimental Design. The experimental apparatus consisted of test tubes, temperature-controlled boxes, and programmable incubators (model BOD50A15; Revco, Asheville, NC; Fig. 1).

Each test tube was assembled with two translucent plastic cylinders (17.5 cm long by 2.4 cm diameter; Forestry Suppliers, Jackson, MS), held together end to end by a plastic sleeve (5 cm long by 2.5 cm in diameter). The test tube was filled with sterilized soil (210 g) moistened with distilled water (30 ml). A small roll of corrugated cardboard (1 cm long by 2 cm diameter) moistened with distilled water (1 ml) was buried with its top even with the surface of the soil at both ends to serve as food sources. The tube ends were covered with plastic lids perforated with tiny holes (<0.2 mm) to allow air flow.

Each controlled-temperature box was constructed from a Styrofoam (polystyrene) insulating box (30 by 25 by 25 cm, walls were 3 cm thick), a silicone rubber heater (5 by 12.5 cm, 50 W; Cole Parmer Instrument Co., Vernon Hills, IL) hanging on a metal wire (28 cm long) in the center of the insulating box along the long axis (the ends of the metal wire were secured in the opposite walls of the box), and a tight-fitting lid (3 cm thick). The heater was equipped with a digital temperature controller (TS-13011; Dwyer Instruments, Michigan City, IN) to maintain temperature within the box at a stable 24°C. Each box lid was perforated by six holes (2.4 cm diameter) arranged in two rows 12 cm apart and 9 cm apart within each row. Six test tubes were inserted halfway through the six holes, so that their upper portions (16 cm) were outside the box, lower portions (16 cm) were within the box, and

Table 1. Temperature regimens

Regimen	Soil-filled test tubes: Air temperature (°C) surrounding the upper portions extending outside of the controlled temperature boxes	Air temperature (°C) around the lower portions maintained within the controlled temperature boxes	Treatment duration (h)
Constant	0	24	24
Chilling at a rate of 1°C/h	24–0 followed by 24-h 0	24	48
Chilling at a rate of 1°C/12 h	24–0 followed by 24-h 0	24	312
Control (constant warm)	24	24	24, 48, or 312, corresponding to treatment regimens

middle sections with plastic sleeves (3 cm) were secured tightly in the lid.

Inside each controlled-temperature box, there was a 6-cm space between the suspended heater and each test tube. To monitor soil temperatures within the upper and lower portions, two thermometers (model 00891; ACU.RITE, China) were connected to one of the six tubes. Each thermometer was equipped with a remote flexible cable thermocouple probe (100 cm) that was inserted 5 cm into the upper or lower portion of the test tube through the plastic lid. The remote cable thermocouple probe allowed us to record soil temperatures without disturbing the bioassays. Groups of 200 termite workers (fifth to sixth instars) were introduced from the top into the test tubes.

Hereafter, the combination of a controlled-temperature box and the six test tubes attached to its lid is defined as an experimental unit. In total, we had 12 experimental units (6 of each termite species), 72 tests tubes, and 14,400 termites. All experimental units were kept under laboratory conditions (24 ± 1°C, 60% RH) for 5 d before the experiment, allowing the termites to freely tunnel to reach equilibrium in distribution within the test tubes.

Temperature Regimens. Four programmable incubators were preset to perform the following four temperature regimens: 24-h 0°C; 24-h chilling from 24 to 0°C at the rate of 1°C/h followed by a 24-h 0°C; 12-d chilling from 24 to 0°C at the rate of 1°C/12 h followed by a 24-h 0°C; and a constant 24°C that was used as control (Table 1). In field environments, cold weather may occur quickly or slowly. The instant 0°C regimen was intended to simulate a quick drop to 0°C air temperature in autumn. The two chilling regimens from 24 to 0°C at different cooling rates were intended to mimic temperature conditions from autumn to winter. When experimental units were placed within the four incubators, upper portions of the test tubes were exposed to one of the preset temperature regimens, whereas lower portions of the same test tubes were maintained at a constant 24°C within the controlled-temperature box.

Testing Procedure. Two experimental units (one of each termite species) were placed in each incubator preset to perform cold or chilling temperature regimens, and 6 U were placed in the control incubator. Experimental units of each species were randomly assigned to the test regimens.

At the end of each test, the two experimental units along with two from the control (one for each species) were carefully removed from the incubators, and the two portions of each test tube were immediately disconnected in dark (<0.1 FC, measured by DLM2 light meter; Universal Enterprises, Beaverton, OR). The laboratory light was turned on, and the disconnected test tubes were destructively sampled in separate containers (18 by 14 by 9 cm) to count termite numbers. Termites were placed in separated petri dishes (1.5 cm high by 10 cm diameter) lined with moistened filter paper at room temperature (24 ± 1°C) to record recovery time and mortality after 24 h. A termite was considered dead if it could not walk a body length on being probed with a pen brush.

Data Analysis. The number of termites extracted from the upper and lower portions within each test tube was calculated as a percentage of the 200 termites. The number of dead termites at 24 h after treatment was calculated as a percentage of termites aggregated in the corresponding portion. Percentage data were arcsine-square root transformed and tested for homogeneity of variance before analysis. Within a termite species, differences between various temperature regimes were analyzed using one-way analysis of variance, followed by Tukey honestly significant difference (HSD). Differences between termite species were analyzed using two-sample *t*-test. The 0.05 probability level was used in all tests for significance. All analyses were performed using Statistix 8 software (Analytical Software 2003).

Results

Thermometer readings indicated that the soil in upper portions did not fall to 0°C as quickly as surrounding air, probably because of the soil's buffering effect. Under the instant 0°C regimen, there was a 30- to 35-min delay before the soil reached a stable 0°C. Under the two chilling regimens, it took 5–10 min for the soil to reach a stable 0°C after air temperature fell to 0°C. The soil in lower portions of all temperature regimens remained a steady 24°C.

Behavioral Response to Different Temperature Regimens. Mean percentages of termite distribution within test tubes are presented in Fig. 2. Both termite species showed the capability to behaviorally respond to the temperature regimens by moving downward to stay in warm lower portions to avoid encountering damaging cold, whereas the controls had an even distribution of termites within the soil-filled test tubes. Significantly more termites escaped from the two

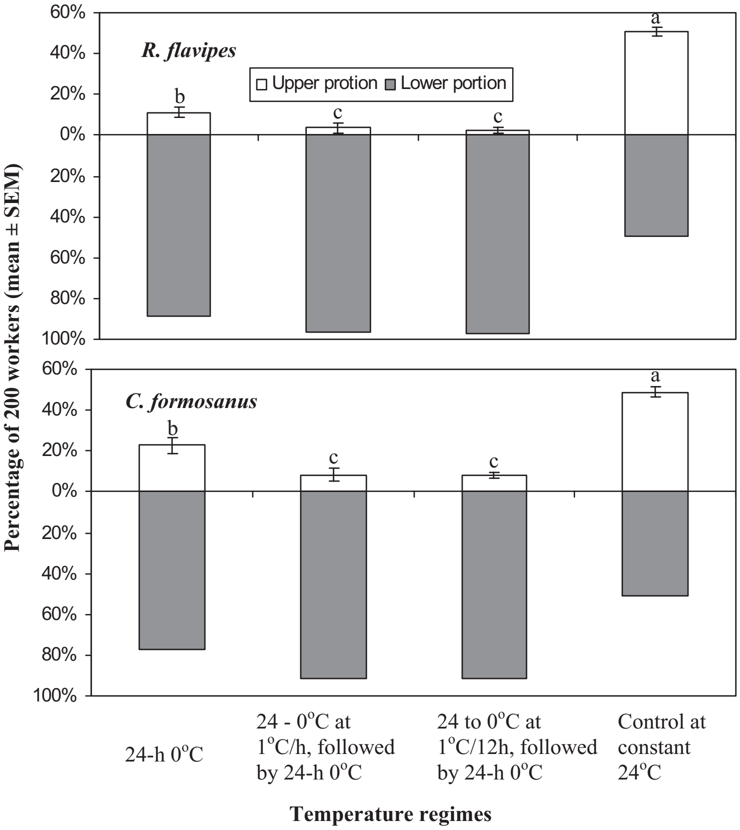

Fig. 2. Comparison of termite distributions in the upper and lower portions within the same test tubes at the end of different temperature regimens (different letters indicate significant difference between temperature regimens, $P < 0.05$).

chilling regimens than from the instant cold (*C. formosanus:* $F_{3,20} = 150$, $P < 0.001$; *R. flavipes:* $F_{3,20} = 222$, $P < 0.001$), but no significant difference was detected between the two chilling regimens (critical value for comparison = 0.069), indicating a subzero cooling acclimatization effect. Interestingly, there were always a few termites that remained in the cold upper portions, regardless of the test regimens or termite species. Close examination showed these termites were within or immediately beneath the corrugated cardboard roll.

Recovery and Survivorship of Termites Remaining in the Cold Upper Portions. When the experiment was terminated, termites extracted from the warm lower portions remained active. Those remaining in the upper portions of cold or chilling temperature regimens were in a cold coma but most of them resumed activity within 5 min at an ambient temperature of 24 ± 1°C. A few of them that did not recover after 24 h were defined as dead.

Overall, termite mortality was low (Fig. 3). Nevertheless, mortality at the direct 24-h 0°C regimen was significantly greater (*C. formosanus:* $F_{3,20} = 24.5$, $P < 0.001$; *R. flavipes:* $F_{3,20} = 7.8$, $P = 0.032$) than those at the chilling and control regimens. No difference was found between the two chilling regions. Significantly different of mortality between the two chilling and control regimens was detected in *C. formosanus* (critical value for comparison = 0.029) but not in *R. flavipes* (critical value for comparison = 0.018). Close examinations revealed that cannibalism was the primary cause of mortality from control and warm lower portions. Cannibalism was not observed in termites remaining in cold upper portions.

Species Effect. The two-sample *t*-test indicated significantly different degrees of behavioral response and mortality between the two termite species in temperature regimens, except control (instant 24-h 0°C: $T_{5,5} > 16.2$, $P \leq 0.03$; cooling at 1°C/h followed by 24-h 0°C: $T_{5,5} > 16.9$, $P \leq 0.03$; cooling at 1°C/12 h followed by 24-h 0°C: $T_{5,5} > 20.25$, $P \leq 0.02$, respectively; Figs. 2 and Fig. 3). In each case, significantly more *R. flavipes* moved away from the cold upper portion, and those staying in a cold coma suffered less mortality than *C. formosanus.*

Discussion

This study reports the first empirical evidence that proves the hypothesis that both species, *C. formosanus* and *R. flavipes,* use behavioral mechanisms to survive damaging cold, in addition to physiological mechanisms. They moved away from potentially lethal low temperatures, retreated deep in the ground, and

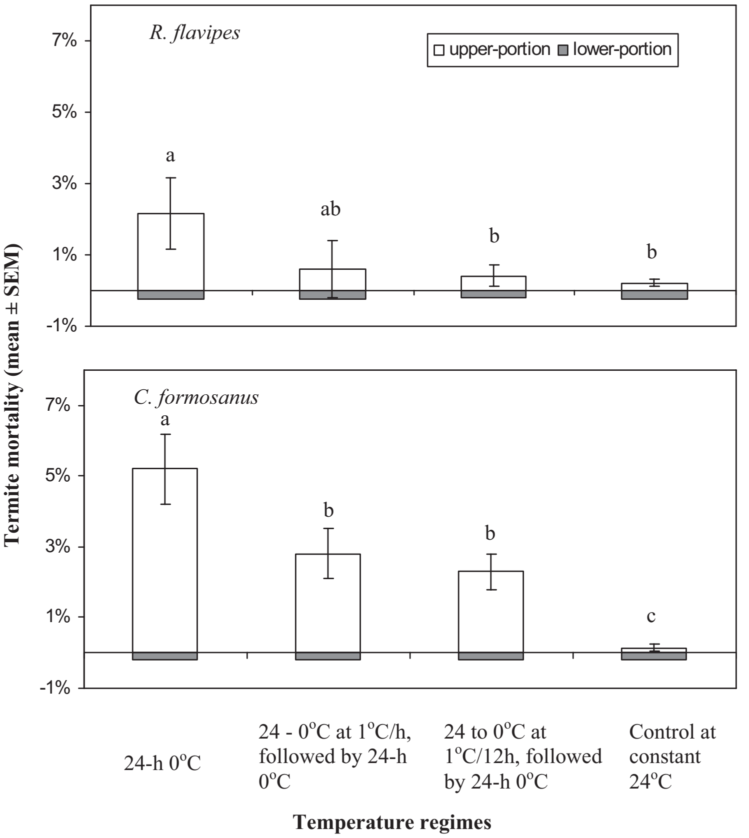

Fig. 3. Comparison of mortality of termites extracted from the upper and lower portions within the same test tubes after 24 h at the end of different temperature regimens (different letters indicate significant differences between temperature regimens, $P < 0.05$).

stayed in a warm area of constant temperature to avoid freeze mortality. The result of this study supports previous field observations that, in winter months, *R. flavipes* burrows downward, with a record depth of 1.5 m, to uniformly warm areas (Esenther 1969, Husby 1980). It also corroborates the anecdotal field observations of vertical movement of *C. formosanus* within underground tunnel network in response to daily and seasonal temperature changes (X.-P.H., unpublished data). More termites in the chilling regimens were able to escape freezing than those subjected to the instant 0°C regimen. A possible explanation is that the termites acclimate to surrounding temperature until it drops to a critical point, at which time they move downward to escape extreme cold. Denlinger and Lee (1998) pointed out that behavioral avoidance is usually the first line of defense in cold weather survival of insects. This may be particularly true for nondiapause termites that live in a diffuse network of interconnected galleries and nursery areas scattered throughout the soil and wood (Strack and Myles 1997). Behavioral avoidance of cold has been reported being more important than physiologically increasing cold tolerance for the imported red fire ant, *Solenopsis invicta* Buren, another nondiapause tropical and subtropical insect (Morrill et al. 1978).

The two termite species showed a similar but significantly different ($P < 0.05$) pattern of escaping and thereafter avoiding cold. The number of *C. formosanus* remaining in the cold upper portion and resulting mortality was about twice of that of *R. flavipes*, indicating that *C. formosanus* is less capable of behaviorally surviving harmful low temperatures than *R. flavipes.* It has been reported that *R. flavipes* is able to increase physiological cold tolerance in response to low environmental temperatures more effectively than *C. formosanus* (Hu and Appel 2004). The thermal limit to activity is reported 0°C in *R. flavipes* (Strack and Myles 1997) and 10°C in *C. formosanus* (Li 1991). These data are endorsed by the fact that *C. formosanus* is a tropical and subtropical species and *R. flavipes* is a species with a broad distribution from subtropical to temperate regions.

An intriguing finding of this study was the small percentage of termites that simply remained in the cold upper portions, regardless of the regimens and termite species. Of course, one may argue that those termites were trapped because the temperatures at which they ceased activity occurred too quickly. This possibility could partially explain the result of the instant 0°C regimen, but hardly those of the gradually chilling regimens. We reason that this observation may have implications for understanding termite group intelligence and decision making. The mass downward movement could be an indication of group response: those who escaped the cold were quick in responding to the temperature change and communicated to those in lower portions to remain there to avoid the danger of encountering potentially lethal low temperatures above. However, the fact that several individuals remained in and immediately beneath the food source (cardboard roll) at the top of the upper portions might mean that the avoidance of cold is an individual decision: those caught by the cold were slow in responding to the temperature change because they instead responded to another signal: stop and feed. This phenomenon may suggest an alternative strategy evolved by subterranean termites to cope with a temporary cold environment. Assuming that freezing temperatures remain for only a short period of time, it would be advantageous for termites to depress metabolic activity and to wait before resuming normal activities. A similar hypothesis was made by Forschler and Henderson (1995) as an evolutionary strategy for subterranean termites to survive temporary inundation. Development of explicit and elaborate experimental designs is needed to answer the question of whether the mass movement recorded in this experiment was a group response or individual decisions.

The low mortality of termites suffering a 24-h chill coma indicates the ability of the tested termites to survive short-term 0°C. The mortality represents a measurement of physiological cold hardiness and not behavioral factors. One fundamental piece of information needed for understanding termite overwintering is their ability to survive long-term exposure to cold temperatures. Cold hardiness is a result of temperature and exposure period and is a tool to measure insects' ability to survive long-term exposure to cold temperatures. *R. flavipes* is reported to have >50% survival when held at temperatures gradually decreasing from 25 to 0°C over 6 wk, including 1 wk at 0°C (Davis and Kamble 1994, Cabrera and Kamble 2001). *C. formosanus* is reported to yield 100% mortality at −1°C for 9 d (Li 2002). The lethal low limit for *C. formosanus* is lower than −5°C when held at a cooling temperature at 1°C/min (Hu and Appel 2004). A study is being undertaken to specify cold hardiness of the two species at various low temperatures for various exposure periods.

The experimental protocol we used in this study has the advantage of in-ground conditions that simulate subterranean termites' living environment. Therefore, the results are indicative of termite behavioral responses to cold temperatures they may confront in natural habitat. The cold air temperature regimens applied to the upper portions represents aboveground temperatures; the uniformly warm temperatures provided by the lower portions of the same test tubes represent in-ground temperature; and the middle section connecting the upper and lower portions represents the air–ground interface with a consequent temperature gradient. This experimental protocol would also be useful for studying other factors that affect termite movement, such as humidity and food location.

In conclusion, this study shows the empirical evidence in support of the hypothesis that subterranean termites avoid exposure to critical low temperatures by moving downward in the ground where the temperature is more uniform, a behavioral mechanism to enhance survivorship during a cold winter. To gain a complete understanding of how well *C. formosanus* can survive above its natural latitude in the United States, more research needs to be conducted on the effects of various low temperature regimens on colony growth and reproduction, cold hardiness of various castes at different low temperatures and over various extended periods, and foraging movement dynamics in the microhabitats of urban environment.

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