

# Temperature-dependent phototaxis in overwintering adults of the grasshopper *Patanga japonica* (Orthoptera, Acrididae)

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## Abstract

In central Japan, adult *Patanga japonica* (Bolívar) grasshoppers overwinter as adults while in reproductive diapause. At this local, February nights fall as low as  $-7^{\circ}\text{C}$ , whereas days can exceed  $16^{\circ}\text{C}$ . Adults respond to the diel thermal cycle with daily vertical movements out of and back into leaf litter. This paper documents and discusses the significance of this interesting winter behavior. Temperature strongly influenced the daily vertical movements. Time of morning emergence, duration of aboveground occupancy, and number of adults emerging all highly correlated with current and maximum daily temperatures. In January, adults were immobile at  $< -1^{\circ}\text{C}$  but could stand up when their body temperatures reached  $\sim 3.7^{\circ}\text{C}$ . In contrast, adults held outdoors in semi-natural conditions emerged from the litter at  $\sim 14^{\circ}\text{C}$ , suggesting threshold temperatures of  $\sim 14^{\circ}\text{C}$  for morning emergence. The numbers of adults emerging or hiding varied over the winter season. Light also influenced movements. Adults held in horizontal transparent tubes, each with half covered with black paper (D-area) and the other half exposed to light (L-area), moved into the L-area during the day and returned to the D-area in the afternoon. In both cases, movement was into a colder microhabitat, implying that the direction of daily movements was possibly via phototaxis, not thermotaxis. Further experiments suggested that increasing temperatures elicited positive phototaxis, and decreasing temperatures elicited negative phototaxis and that the phototaxis was controlled by the direction, magnitude, and absolute range of the temperature change in *P. japonica*.

## Keywords

body temperature, daily activity, diapause, thermoregulation

## Introduction

The overwintering biology of insects has been intensively studied in two aspects: diapause and cold hardiness (Tauber et al. 1986, Danks 1987, 2006, Leather et al. 1993, Saunders et al. 2002, Denlinger 2022). Many insects overwinter in a state of diapause in response to photoperiod, temperature, humidity, food quality, and others depending on the species. Diapause is typically induced before the arrival of winter and ends during winter under the influence of low temperature. Post-diapause development then proceeds

depending on the ambient temperature in the spring. Cold hardiness consists of three major components, including cold acclimation, freeze tolerance, and freeze avoidance (Salt 1961). Numerous studies have focused on the cryoprotective polyols, ice nucleating agents, and other molecules associated with diapause and overwintering (Danks 1987, Lee and Constanzo 1998). However, we know relatively little about the behavior of overwintering insects in diapause. For example, insects in diapause are generally characterized by low metabolic rates, suppressed reproduction, and vegetative activity (Tauber et al. 1986). Therefore, they are expected to remain inactive to save the fat reservoir accumulated before diapause. This paper investigates the interesting case of a Japanese grasshopper, *Patanga japonica* (Bolívar, 1898) (also known as *Nomadacris*), which makes daily vertical movements out of and back into leaf litter during the overwintering period.

Phototaxis refers to the directed movement of organisms toward (positive phototaxis) or away (negative phototaxis) from a light source (Fraenkel and Gunn 1973). Many studies have analyzed the wavelengths and intensities of light to which insects respond positively or negatively using LED lights during the active season (Castrejon and Rojas 2010, Zheng et al. 2014, Zhang et al. 2016, Park and Lee 2017, Kim et al. 2019, Kühne et al. 2019, Komatsu et al. 2020, Takikawa et al. 2021). The information obtained from such studies is important in terms of pest management, and the studies were mainly carried out during the active season of insects by putting light sources in the field or in the laboratory and counting the number of insects attracted. In contrast, few empirical data are available on the phototaxis in overwintering insects. In this study, I examined the involvement of phototaxis in controlling the daily vertical movements of *P. japonica*.

*P. japonica* is a grasshopper occurring in Asian countries including India, Bangladesh, Pakistan, Indonesia, Malaysia, Vietnam, China, Taiwan, Korea, and Japan (Ichikawa et al. 2006, Murai and Ito 2011, Cigliano et al. 2022). In temperate central Japan, it is univoltine, with adults overwintering under leaf litter or between stalks of wilted plants (Murai and Ito 2011). Adults enter a state of dormancy from late fall to the end of winter. In the laboratory, adults reared at  $30^{\circ}\text{C}$  remained without reproducing for 4

months, suggesting that they undergo a reproductive diapause and exhibit some requirements for the termination of diapause (Tanaka and Okuda 1996). My unpublished observations suggested that adults might require a period of low temperature to end reproductive diapause and ovarian development initiates at 25°C. Adults that overwinter begin mating in March, followed by oviposition from April through July, and then death by mid-August. Most eggs hatch in July and August, and most nymphs reach adulthood by November (Tanaka 2023). Although *P. japonica* shows some density-dependent polyphenism in body color similar to locusts (Pener 1991, Pener and Simpson 2009, Tanaka 2023, Tanaka and Kayukawa 2024), it is not considered a locust in the strictest sense because it does not aggregate or migrate.

Many insects are known to be active even under low temperatures (Danks 1981), and some polar chironomids can continue to grow through the winter in ice-covered lakes (Welch 1976). In contrast, information about the behavior of adult insects overwintering in a state of diapause is limited. This might be partly because they are inactive and seldom seen flying by researchers during the cold season and because diapause insects typically maintain reduced metabolism (Denlinger 2022).

While studying Japanese *P. japonica*, I noticed that overwintering adults were sluggish but showed an interesting pattern of daily behavior in an outdoor cage: they hid under leaves at night but moved up on the screen walls of the cage in the late morning and stayed there during the day. In the afternoon, they returned to beneath the leaves. Hence, they moved daily from hiding to exposure and back again. These movements seemed to depend on current air temperatures. Below -1°C, adults were immobile. Between approximately 0 and 1°C, they could make sluggish movements when stimulated but did not move from beneath the leaves until their body temperature increased to 14°C, on average. These anecdotal observations led me to investigate the behavior of overwintering adults in more detail. Factors that might influence their behaviors could include temperature, light, gravity, humidity, pathogen/parasite load, predator threat, hunger, thirst, endogenous daily rhythms, acclimation, and genetically programmed ontogeny or seasonal responses, etc. Specific environmental cues could regulate general activity levels, including undirected (random) locomotion (kinesis), directed locomotion (tropism), or both. In this study, I employed a series of experiments to investigate the effects of temperature and light on adult activities during winter. The present paper describes the results of these observations and discusses the significance of phototaxis and overwintering activity in *P. japonica*.

## Materials and methods

**Location and climate.**—Experiments were conducted in Tsukuba (36.1°N, 140.1°E), Ibaraki Prefecture, Japan. Mean high daily air temperature during January and February 2022 was 9.4°C (range, 2.9–16.4°C), and mean low daily temperature in January and February was -3.2°C (range, -7.2–3.0°C) (Japan Meteorological Agency 2022). A total of 10 cm of snow and 88 mm of rain fell during the 2 months (Japan Meteorological Agency 2022). On warm days, sun-exposed ground surface temperature could reach several degrees higher than adjacent air temperatures. The times of sunrise and sunset on February 1 were 06:41 and 17:06, respectively.

**Insects.**—*P. japonica* adults were either reared in outdoor cages (Tanaka 2023) or collected in a grassy area in Tsukuba in the fall of 2021. They were housed in nylon screen-covered cages with a front acrylic door (42 × 24 × 42 cm) and placed in an outdoor wood

deck where they were exposed to sunlight during the day. Stalks and leaves of *Bromus catharticus* Vahl were provided as shelter and placed in a water bottle for food.

**Righting time.**—I measured time to stand (= righting time) for cold-immobilized adults in January. Animals were kept overnight in outdoor cages. At 08:00 h (~70 min after sunrise), 5 male and 5 female adults were gently removed using forceps from below the leaf litter of their outdoor cage and placed with their sides down on a cork floor of an open plastic container (22 × 30 × 10 cm) in a shady area outdoors (Suppl. material 1). The plastic container was placed near the cages outdoors in shade before and during the observation period. At this time of day, most individuals were cold-immobilized because of the cold overnight temperatures and because temperatures in the cages were still cold. Individuals were separated by at least 3 cm to prevent them from touching one another. I recorded the exact time that each individual stood up on all 6 legs and measured the pronotum and floor temperatures with a THI-500 infrared (IR) thermometer (Tasco Japan Co. Osaka, Japan) positioned ~3 cm above the subject. At this distance, the IR sensor scans an area of ~3 mm in diameter. I repeated the experiment each day from January 20 to 29, 2022, selecting animals randomly from among the 30 adults in the outdoor cages. I used a Fuso LM-8000 multi-environmental instrument (A-Gas Japan Co., Tokyo, Japan), equipped with an internal thermistor to measure air temperatures inside the open plastic container.

To determine if live and dead individuals differed in body temperature during morning heating, 10 adults killed in a freezer beforehand were kept outdoors overnight in a covered 9 cm diameter plastic Petri dish. At 08:30 on Dec. 28, 2022, I transferred the 10 dead and 10 live cold-immobilized adults onto the shaded cork floor and then recorded their temperatures as described above.

**Behavior in cage.**—In the morning of Dec. 27, 2021, two groups of ~20 adults were moved from the outdoor cages to two screen-covered cages (40 × 16 × 40 cm). Each cage contained fresh leaves of the grass *B. catharticus* with their basal ends placed in a vertical water bottle and their long leaves lying in a thick mass on the floor. One cage was kept outdoors in an exposed area on the wood deck, and the other cage was placed indoors near a large window that extended to the floor (Suppl. material 2). Although the indoor room was not directly heated, the indoor cage was slightly warmer than the outdoor cage during the night because the indoor room was connected to an adjacent heated room. During the day, the indoor cage heated rapidly because direct sunlight passed into the room via the large window. The following morning (Dec. 28), all adults in the outdoor cage were found hiding under the long leaves of the grass on the floor. In contrast, most individuals in the indoor cage were on the sides of the cage where they had apparently remained overnight. At 08:00, the latter were gently picked up with fingers and placed on the cage floor. The number of adults on the walls of each cage was then counted every hour from 08:00 to 20:00 on December 28 and 29, and the temperature on the floor of each cage was monitored every hour with an Ondotori Jr. TR-52i equipped with a thermistor probe (T&D Co., Nagano, Japan). After dusk, a red LED lamp (CP-1950B, GENTOS, Tokyo, Japan) was used for observations. On December 29, insects in the indoor cage were not moved to the cage floor in the morning.

**Behavior in tubes Experiment 1.**—To observe the daily behavior of adults under simplified conditions, transparent tubes (76 cm long, 9 cm diameter) were made of four cut PET bottles joined together.

One half of each tube was covered with black paper (= dark area or D-area), and the other half was covered with white paper only in the lower portion (= light area or L-area) (Fig. 1). Temperatures at both ends of one tube were recorded using thermistor probes attached to Ondotori TR-52i temperature recorders.

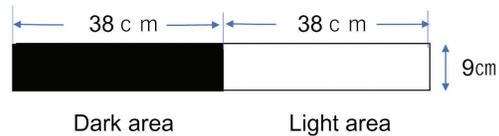


Fig. 1. Tube used to observe daily behavior of *P. japonica* adults.

At 08:00 on both December 31, 2021, and January 1, 2022, 10 cold-immobilized adults from an outdoor cage were put in the D-area of one tube, and another 10 were placed into the L-area of a different tube. Thereafter, the number of adults observed in the L-area of each tube was recorded every hour until 18:00 under outdoor conditions (Suppl. material 3). The same insects were used on both days. Light intensity (lux) was measured hourly from 08:00 to dark at a point 3 cm away from the L-area of the tube using a TR-74Ui light meter (T&D, Nagano, Japan) and included times when tubes were under either shade or direct sunlight. After dusk, a red LED lamp was used for the observations.

*Behavior in tubes Experiment 2: Long-term trial.*—From December 22, 2021 to January 6, 2022, I conducted a 16-day experiment to determine how temperature influenced movements between the L- and D-areas of the tubes. I used one tube from December 22 to January 2, and two tubes from January 3–6, each containing 10 adults that had previously been held in an outdoor cage. For each day, I recorded the temperature between 08:00 and 18:00 in both the D- and L-areas as well as the maximum number of grasshoppers occupying the L-area. Test adults were changed every 2–3 days after 20:00.

*Behavior in tubes Experiment 3: Cardboard screen.*—The above experiment showed that in the afternoon, grasshoppers moved from cool L-areas into warmer D-areas, suggesting that movement was possibly stimulated by positive thermotaxis. To test this hypothesis, a cardboard screen was placed in front of the D-area of a tube to block the sunlight at 13:00, which made the D-areas cooler than the L-areas. Ten adults were placed in the L-areas of each tube at 13:00, and the number of adults observed in the L-area was recorded every hour until 20:00. The temperatures at both ends of the tube were recorded as described above, and the intensity of illumination (< 20,000 lux) 3 cm from the L-area of one of the tubes was measured with the TR-74Ui light meter. The experiment was repeated the next day using the same insects.

*Behavior in tubes Experiment 4: Artificial heating.*—The above hypothesis was tested again by comparing the movement of grasshoppers into the D-area in the afternoon when the L-area was either heated or not heated by a 40-W incandescent bulb covered with aluminum foil and placed close to the end of the L-area (Suppl. material 4). This outdoor experiment consisted of 1 control and 3 replicates of the experimental group. Ten adults were placed into each tube at 20:00 on the night before the experiments. In each experimental tube, heating was initiated when the first individual moved from the L- to D-area in the afternoon. The number of grasshoppers in the L-area was recorded hourly during the experiment. The experiments were conducted from January 20 to February 9.

*Behavior in tubes Experiment 5: Effect of temperature change.*—The final tube experiment tested whether an increase in temperature could elicit movement from dark to light at a time of day (early evening) when it is already dark outside, temperatures are low and declining, and winter adults have already moved from light to dark. In this experiment, Treatment individuals experienced a large temperature increase, and Control insects experienced a small one.

Two tubes, each containing 10 adults, were kept outdoors at night under cold, natural temperatures. The Control tube was moved into the indoor conditions and placed by a large window at 08:00 on January 3 and at 15:00 on January 8. The mean temperature experienced by the Control grasshoppers from 09:00 to 19:55 on January 3 was 18.5°C (range, 15.9–25.5°C) and that from 16:00 to 19:55 on January 8 was 19.1°C (range, 19.1–22.1°C). Conversely, on both days, the Treatment tube remained outdoors where the average temperature during the above periods was 11.0°C (range, 2.7–25.4°C) on January 3 and 1.5°C (range, -0.3–6.2°C) on January 8. At 20:00 on each day (when it was already dark outside), both Treatment and Control adults were moved to a temperature-controlled room where temperature was ~ 20°C under artificial illumination (1,900–2,200 lux) by 3 incandescent lamps and a fluorescent lamp. At this time, all adults were moved into the D-areas of their respective tubes by tilting the tubes vertically. Starting at 20:00, the number of individuals appearing in the L-area was recorded every 15 min.

*Behavior in an outdoor enclosure.*—In winter, adults typically hid beneath leaf litter during the night and moved to the litter surface during the day. However, the time of and numbers moving varied greatly day by day. I studied the relationships between changing environmental factors and these movements in a semi-natural environment consisting of a wood-framed enclosure (48 × 80 × 30 cm) placed outside on the ground and exposed to direct sunlight as described previously (Tanaka 2023). Cage walls were covered with nylon mesh with a glass top during the day for observation and replaced with nylon mesh during the night for natural ventilation and rainfall. The floor of the enclosure was layered 12 cm deep with leaf litter (wilted Kudzu leaves and stems obtained from a habitat of this grasshopper). A water bottle holding the leaves of *B. catharticus* (changed every 2 or 3 days) was placed near the south wall to supply food. Grasshoppers ate them occasionally during the observation period. The temperatures at the bottom (T1) and surface (T2) of the litter layer, together with air temperature (AT) in shade just outside the east wall of the cage, were recorded every hour. The levels of both ultraviolet light (L1) and full wavelength illumination (L2) were measured hourly inside the enclosure using the TR-74Ui light meter. Ten female and

10 male adults were introduced to the enclosure on January 10, 2022, and their numbers on the litter surface were recorded every hour from 08:00 to 18:00 until April 13, when it became so warm that most grasshoppers stopped hiding beneath the litter at night and instead remained above the litter for 24 h. Observations were interrupted four times to check mortality: no mortality was observed except on February 4 when one male was found dead under the litter and replaced with a live male.

The relationships between the daily maximum number of adults on or above the litter and the corresponding T1, T2, and AT were analyzed for February 6 to 25. In addition to the average temperature between 08:00 and 18:00, the average temperatures between 11:00 and 16:00, 11:00 and 14:00, 11:00 and 13:00, the daily maximum temperature and the temperatures 1 h before and at the time when the maximum number of adults occurred on the litter on each day were tested as variables in regression/correlation analyses. The daily maximum intensities of ultraviolet light (L1) and full wavelength illumination (L2) and the average of each of these light intensities between 11:00 and 13:00 were also included as variables.

At 07:00 on February 4, when grasshoppers were cold-immobilized, the litter was removed little by little to determine the depth of litter at which adults had stayed that night. The distance from the compound eyes of each adult to the top of the litter layer was measured using a digital caliper. After the measurements, the leaf litter was put back to the enclosure. The thickness of the litter layer before and after the measurements was 12.2 and 11.2 cm, respectively ( $t$ -test;  $p > 0.05$ ,  $N = 10$ ).

I used the THI-500 infrared thermometer (described previously) to measure the thoracic temperatures of individuals that had just emerged from under the leaf litter, those that had started moving for the purpose of hiding under the litter, and those that were sitting on the litter during the day from January 17 to February 7. Litter surface temperature < 1 cm from each individual was also recorded. On exceptionally warm days, a few individuals flew out of the enclosure during measurements but were captured and put back into the enclosure.

**Statistical analyses.**—The grasshoppers' body and floor temperatures were compared with a paired-sample  $t$ -test or Tukey's multiple comparison test. Pearson's correlation coefficient and linear regression were used to analyze the relationships between body and floor temperatures and between the number of grasshoppers on the litter and temperatures. An independent  $t$ -test was applied to the comparisons between the body temperatures after emergence from the litter and those before hiding under the litter and

between the temperature changes during 1 h before emerging and hiding under the litter. The mean number of adults on the litter among different observation periods was compared using the Steel-Dwass test. These analyses were performed using a statistics service available at <http://www.gen-info.osaka-u.ac.jp/MEPHAS/kaiseiki.html>, Descriptive Statistics (Excel, Microsoft Office 365) or StatView (SAS Institute Inc., NC, USA). Differences were judged as significant when  $p < 0.05$ .

## Results

**Righting time.**—On January mornings, grasshoppers were usually motionless due to the prevailing low morning temperatures. When placed sideways on the cork floor at 08:00 (Suppl. material 1), most adults remained motionless for >10 min when floor temperatures at 08:00 were < 0°C. In contrast, most animals that were tested when floor temperatures at 08:00 were > 1°C stood within 10 min (Fig. 2A). These adults actually stood up immediately after being placed sideways. They apparently responded to the handling made at 08:00. This response was not manifested at temperatures below 0°C, suggesting that the thermal threshold for simple behaviors was ~1°C.

Of the 52 individuals that did not stand immediately after being placed sideways at 08:00 (Fig. 2A), 37 spontaneously got up between 09:00 and 10:00, and some stood only after 10:30 (Fig. 2B). The average body temperature at standing was 3.7°C ( $N = 40$ ), which was significantly higher than the average floor temperature at the moment of standing (mean = 3.3°C,  $N = 40$ ;  $t = 5.29$ ,  $DF = 78$ ,  $p < 0.001$ ; Fig. 2C).

When 10 cold-immobilized live adults were placed side-down onto the cork floor at 08:30 on December 28, a significant difference was observed between body and floor temperatures at standing (mean  $\pm$  SD = 0.4  $\pm$  0.1°C;  $t = 8.94$ ,  $DF = 9$ ,  $p < 0.0001$ , Suppl. material 5), as observed above. In contrast, no significant difference was observed between body and floor temperatures for 10 dead adults measured at 08:40 (mean  $\pm$  SD = 0.1  $\pm$  0.1°C;  $t = 0.22$ ,  $DF = 9$ ,  $p = 0.83$ ; Suppl. material 5), suggesting that live insects generated heat even when they were motionless.

**Behavior in cages.**—In the morning of December 27, 2021, 20 adults were placed into an outdoor cage and 22 into an indoor cage. At 08:00 the following morning, grasshoppers in both cages were sluggish due to low overnight temperatures.

In the outdoor cage, adults began to emerge from beneath the grass and climb the walls at 11:00, ~4 h after sunrise (Fig. 3A). By

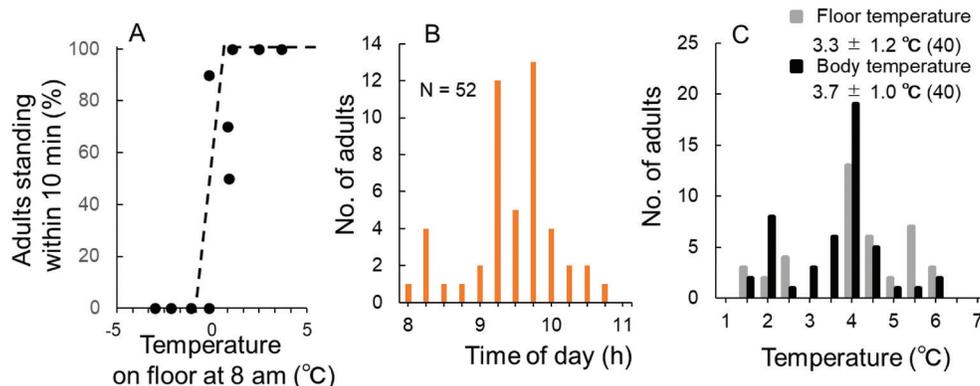


Fig. 2. The proportion of *P. japonica* adults that stood within 10 min after being placed with their sides down at 08:00 ( $N = 8$ –12) on the cork floor under outdoor conditions from Jan. 20–29 (A), the time of day when those adults that remained motionless for >10 min after being placed with their sides down at 08:00 stood spontaneously (B), and the floor and their body temperatures when standing (C).

noon, ~ half of the individuals were on the walls and appeared to be basking in sunlight. At 14:00, the outdoor adults began to move toward the floor, and by 17:00, all individuals in the outside cage were hiding under *B. catharticus* leaves.

In contrast, in the indoor cage at dawn on December 28, most of the insects were on the walls where they had presumably spent the night. At 08:00, these sluggish insects were gently moved to the floor of their cage. As the indoor cage heated, these insects warmed and began to return to the cage walls. By 16:00, all indoor adults occupied walls, where they presumably remained overnight (Fig. 3A).

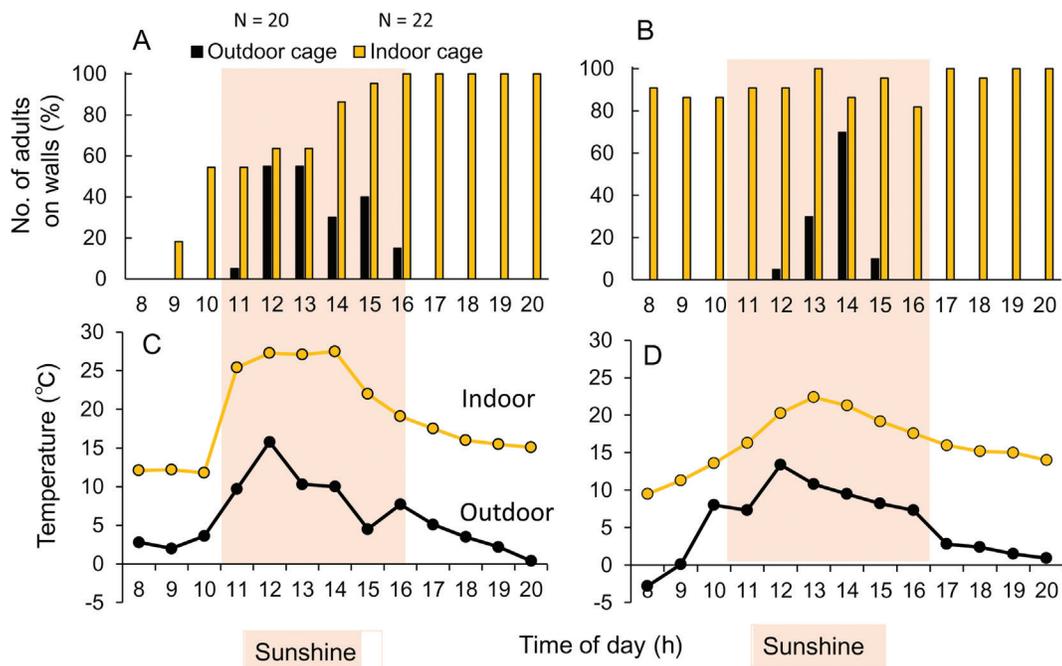
The morning of December 29 was similar to that of the previous day (above), with outdoor adults hiding under litter while indoor adults occupied cage walls. However, unlike December 28, the indoor insects were allowed to remain on the walls. The results on December 29 were similar to those of December 28 (Fig. 3A, B). On both days, the adults in the cold outdoor cage performed daily cyclical vertical movements and hid under litter during the night, whereas those in the warmer indoor cage did not. In both cages, adults moved upwards as both temperature and light intensity were increasing. In the outdoor cage, but not the indoor cage, adults moved downward when temperatures and illumination levels were decreasing. Throughout this 2-day experiment, the outdoor cage was always colder than the indoor cage, and all outdoor insects hid under grass at night, but no indoor ones did. This suggests that absolute temperature influences hiding. Note also that upward movement generally occurred after both temperature and light levels had increased, but that downward movement in the outdoor cage generally began before light and temperatures declined substantially (Fig. 3C, D). This suggests a lag effect for upward movement but an anticipatory movement for descent. In aggregate, these results suggest that vertical movements and hiding are primarily controlled by temperature with a possible influence from light (Fig. 3).

*Behavior in tubes Experiment 1.*—Adults placed outdoors in horizontal plastic tubes exhibited similar daily movement patterns as

observed in the cage experiment (above). On December 31, all 10 adults that were placed in the light (L) area of Tube 2 at 08:00 remained there until 14:00 when the first individual moved to the dark half (D) of the tube (Fig. 4A). In contrast, in Tube 1, the 10 individuals placed in the D-area of the tube at 08:00 remained there until 11:00 when a few began to move into the L-area of the tube. Ultimately, 6 of 10 adults in Tube 1 moved into the L-area. By 16:00, all individuals in both tubes had moved into the D-area of their tubes (Fig. 4A).

The experiment was repeated on January 1, with similar results (Fig. 4B). On both days, tube temperatures were at or below 0°C in the early morning, increased to a maximum of 14–16°C near midday, then returned to 0°C by 20:00 (Fig. 4C, D). The D-areas of the tubes tended to be slightly warmer than the L-areas between 11:00 and 16:00 (Fig. 4C, D). On both days, light intensity rapidly increased at 10:00 when direct sunlight fell on the tubes and fell in the afternoon when the tubes came into shadow (Fig. 4E, F). On December 31, illumination and temperature dropped at 12:00 when clouds blocked the sun (Fig. 4C, E).

In this experiment, adults placed in the L-area of tubes behaved differently from those placed in the D-area of tubes. The former remained in the light during most of the day period, whereas about half of the grasshoppers placed in the D-areas of tubes moved midday into the L-areas, well after both temperatures and light levels had risen (Fig. 4B). Conversely, in the early afternoon, adults in both tubes moved from L-areas to D-areas while both temperature and light intensity were still relatively high but starting to fall (Fig. 4), suggesting afternoon descent is anticipatory. These results suggest that changing temperature and/or light levels might control the alternative movements between the D- and L-areas. The fact that the tubes were horizontal implies that adults can perform daily movements from dark to light and back without using gravity to orient. On January 1, the grasshoppers moved from the D-area to the L-area even though the latter was slightly cooler (Fig. 4B, D), suggesting that they were not orienting toward heat.



**Fig. 3.** The numbers of *P. japonica* adults on the walls of cages kept under outdoor and indoor conditions on Dec. 28 (A) and 29 (B). All adults were placed on the floor at 08:00 on the first day in the indoor cage. Temperature on the floor was monitored hourly (C, D). Pale orange areas indicate the time of sunlight on cages.

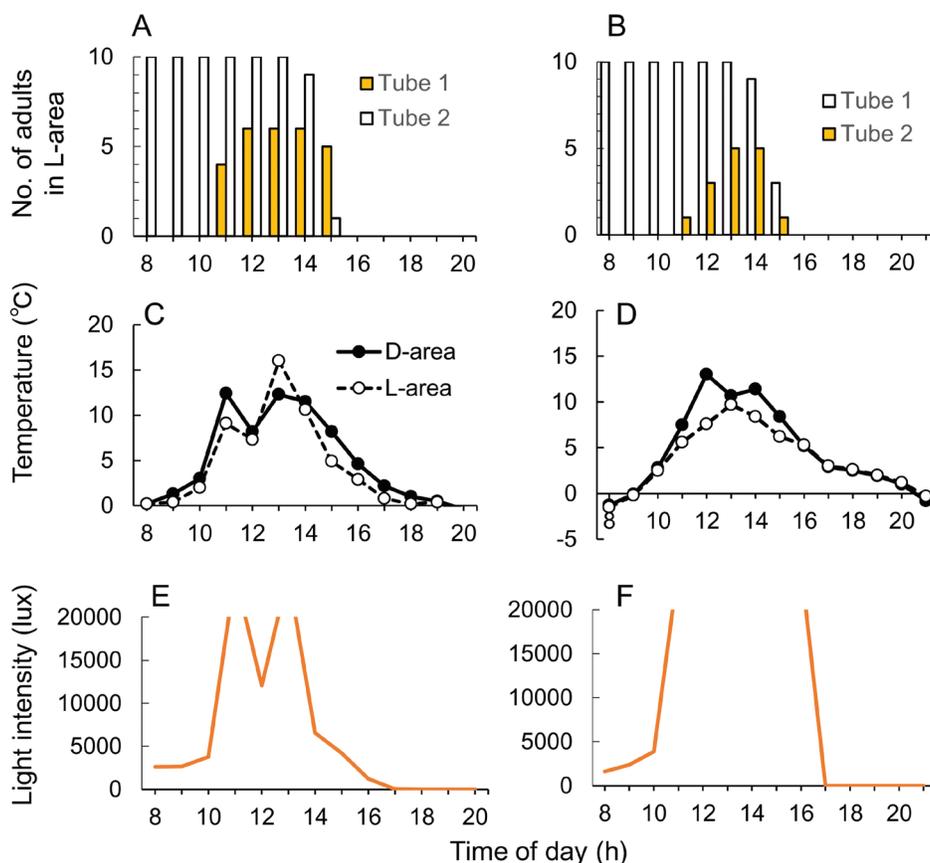


Fig. 4. The numbers of *P. japonica* adults in the L-area of tubes (A, B), temperatures in the L- and D-area (C, D) and light intensities (< 20,000 lux, E, F) under outdoor conditions on Dec. 31 (A, C, E) and Jan. 1 (B, D, F). Ten adults were placed in the D- or L-area of each tube at 08:00.

*Behavior in tubes Experiment 2: Long-term trials.*—The daily maximum number of adults observed in the L-area varied greatly from December 22 to January 6 (Fig. 5A), as did the mean temperatures in each of the L- and D-areas recorded from 08:00 to 18:00 (Fig. 5B). The maximum number of adults observed in the L-area on each day positively correlated with both the daily mean temperatures in the D-area ( $r = 0.76$ ,  $p < 0.001$ ,  $N = 20$ ) and the L-area ( $r = 0.68$ ,  $p < 0.001$ ,  $N = 20$ ), suggesting that more adults moved to the L-area on days when it was warmer. On January 4, 5, and 6, when mean day temperatures were below 6°C, only 1 of 60 insects exited the D-areas of tubes (Fig. 5A, B), suggesting that the threshold temperature for this behavior was around 6°C.

A high positive correlation was also observed when the daily maximum number of adults in the L-area was plotted against the daily maximum temperatures (Fig. 5C, D) instead of the mean temperatures. The tubes were considerably heated in the sunlight on a few days, and the temperature increased beyond 25°C. Heated grasshoppers in the L-area jumped actively inside the tubes, and some got into the D-area by accident, lowering the maximum number of adults in the L-area (Fig. 5C, D). If the data on such days were excluded, the correlation with the temperatures in the D- and L-areas increased to 0.81 ( $R^2 = 0.65$ ,  $N = 18$ ;  $p < 0.001$ ) and 0.84 ( $R^2 = 0.71$ ;  $N = 17$ ;  $p < 0.001$ ), respectively.

Adults moved from the D- to L-area while the temperature was increasing. During this transitional period, a significant correlation was observed between the number of adults in the L-area and temperature in both the L-area ( $r = 0.71$ ,  $N = 65$ ,  $p < 0.001$ ) and the D-area ( $r = 0.67$ ,  $N = 65$ ,  $p < 0.001$ ; data not shown). The mean

temperature in the L-area during this period (10.1°C,  $N = 65$ ) was significantly lower than that in the D-area (11.7°C,  $N = 65$ ;  $t = 4.22$ ;  $DF = 64$ ,  $p < 0.0001$ ), suggesting that the grasshoppers moved against the temperature gradient in the morning.

In contrast, while adults were moving from the L- to D-area in the afternoon, the number of adults in the L-area gradually decreased as the temperature decreased (data not shown). During this transitional period, the mean temperature was significantly higher in the D-area (9.9°C,  $N = 61$ ) than in the L-area (8.9°C,  $N = 61$ ;  $t = 3.84$ ;  $DF = 60$ ,  $p < 0.0001$ ). This difference might suggest the possibility that a positive thermal gradient was required for adult afternoon movement.

*Behavior in tubes Experiment 3: Cardboard screen.*—To test the hypothesis that a positive thermal gradient was required for adult afternoon movement into the D-area of the tube, a cardboard screen was placed in front of the D-area (Fig. 6A) to block the sunlight at 13:00, and all insects were placed in the L-area at the same time. As expected, the temperature in the D-area became lower than in the L-area until around 15:45, when the light intensity near the tube dropped below 5,000 lux and L- and D-area temperatures equilibrated (Fig. 6B,C). However, some adults still moved from the L- to D-area before this time, falsifying the hypothesis that, in the afternoon, a positive thermal gradient is required for adult afternoon movement.

*Behavior in tubes Experiment 4: Artificial heating.*—The above hypothesis was tested by another experiment in which the L-area of

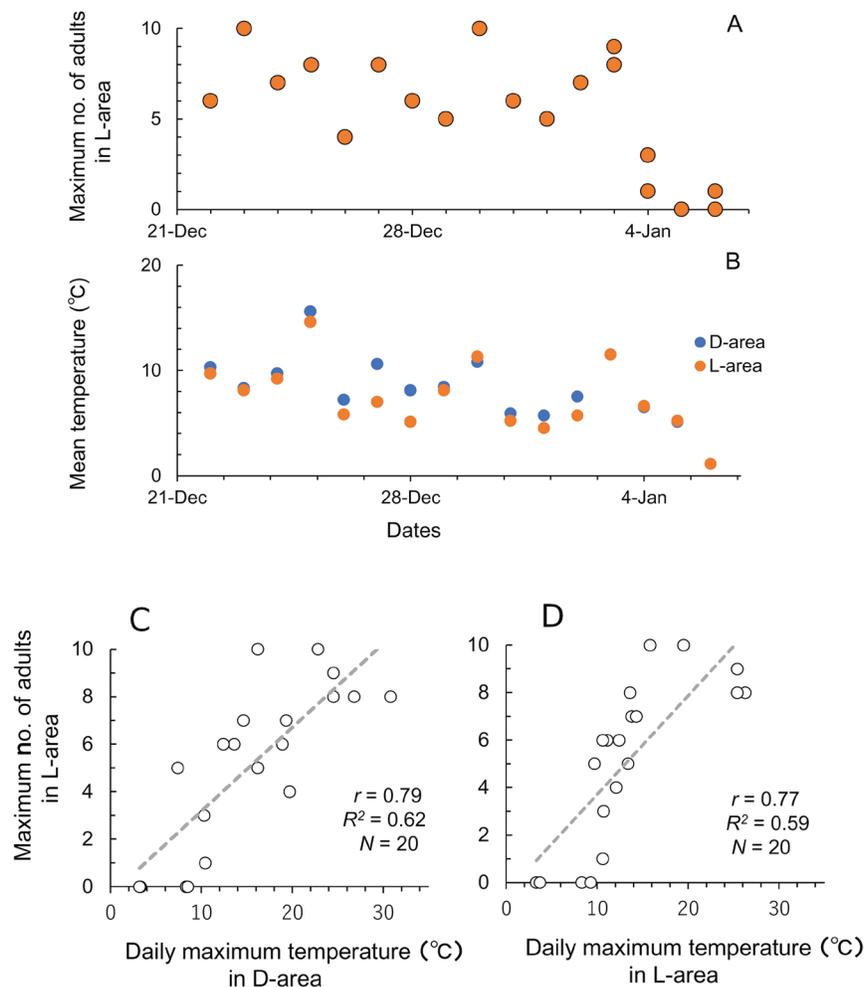


Fig. 5. A, B. The daily maximum number of *P. japonica* adults in the L-area of tubes (A) and mean temperatures from 08:00–18:00 (B). C, D. The relationship between the daily maximum number of adults in the L-area of tubes and daily maximum temperature in the D-area (C) and L-area (D) under outdoor conditions during the period from Dec. 22, 2021 to Jan. 6, 2022.

the tube was heated by a foil-covered 40-W incandescent lamp in the afternoon (Suppl. material 4). Although the L-area was significantly warmer than the D-area, adults returned to the D-area in the afternoon in all treatments including the unheated control (Fig. 6D–G), again falsifying the hypothesis that a positive thermal gradient is required for adult afternoon movement. Among the remaining hypotheses to explain afternoon movement are declining temperature, declining illumination, and negative phototaxis.

*Behavior in an outdoor enclosure.*—Twenty adults were maintained in a nylon mesh enclosure (Fig. 7) under outdoor conditions, and their behaviors, body temperatures, and environmental temperatures were observed from January 12–April 13, 2022, except for four short interruptions to check mortality.

Adult behaviors changed over the course of this 3-month experiment in conjunction with changing season, temperature, day length, and intensity of illumination. In general, adults almost always hid under leaf litter during the night, emerged from the litter during the day (Fig. 8A), and returned to the litter in the afternoon (Fig. 8B).

The daily maximum proportion of adults observed on the litter surface fluctuated mostly below 60% in January and February, whereas it increased to more than 80% in March and April except for a few cold days (Fig. 8C). This change in behavior was

also revealed when the daily maximum proportions of adults on the litter in the five observation periods were compared: the mean proportion was similar in the first three periods (Steel-Dwass test;  $p > 0.05$ ; Fig. 8C) but significantly increased in the last two periods ( $p < 0.05$ ).

The fluctuations in daily maximum number of adults on the litter were correlated with various mean temperatures between 08:00 and 18:00 (Fig. 8C, D). They were best explained by the air temperature ( $r = 0.79$ ,  $R^2 = 0.62$ ,  $N = 76$ ,  $p < 0.001$ ), followed by the temperature on the litter surface ( $r = 0.59$ ,  $R^2 = 0.35$ ,  $N = 87$ ,  $p < 0.001$ ) and that at the bottom of the litter ( $r = 0.57$ ,  $R^2 = 0.34$ ,  $N = 87$ ,  $p < 0.001$ ).

Fig. 9A–E summarizes the hourly changes in the proportion of adults on the litter between 08:00 and 18:00 in each observation period. In January and February, most or all adults remained under the litter in the morning and started appearing above the litter at noon (Fig. 9A–C). The number of individuals above the litter reached a maximum at 14:00 and then declined in the afternoon. In March and April, adults appeared earlier than in the previous months, and substantial numbers of individuals remained on the litter at 18:00 (Fig. 9D, E). Night inspections using a red light revealed that the grasshoppers were inactive during the night, suggesting that most of those observed at 18:00 in March and April remained on top of the litter until the following morning.

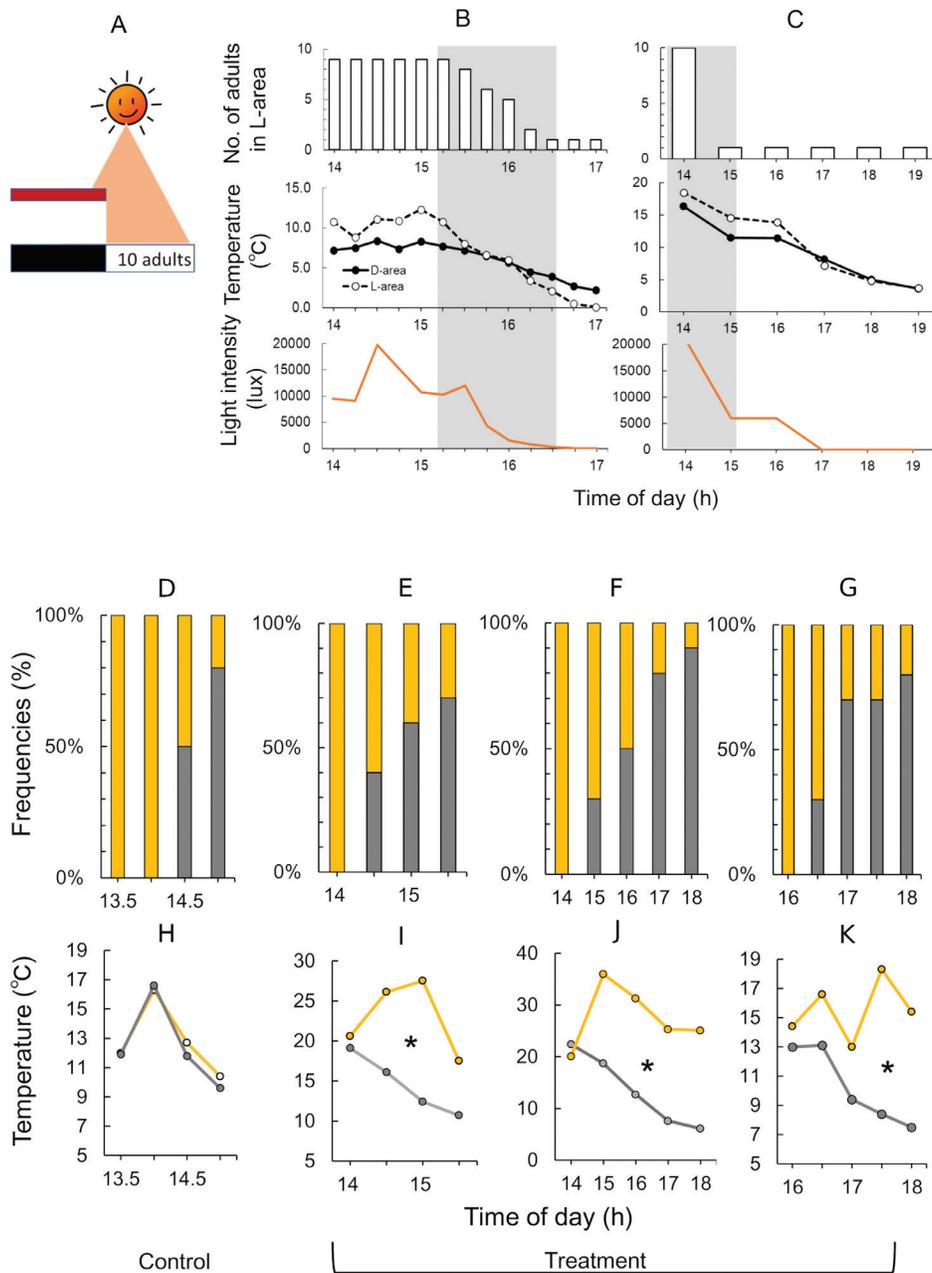


Fig. 6. A–C. The effect of blocking the sunlight in the D-area (A) on the number of *P. japonica* adults in the L-area and temperatures of the two areas (B, C). Cardboard screen was placed in front of the D-area at 13:00. Grey areas show the period during which adults were moving from L- to D-areas. D–K. The effect of heating of the L-area on the behavior of *P. japonica* adults. In D–G, yellow and black bars indicate the number of adults in the L- and D-areas. In H–K, yellow and black lines indicate the temperatures in the L- and D-areas. Asterisks indicate a significant difference with a t-test at the 5% level.

On February 4, the depth at which adults stayed in the litter was determined at 07:00 when the adults were still cold-immobilized (Suppl. material 6). Most adults were found at a depth of 3–9 cm but some were found against the cage floor at the very bottom of the litter (12 cm), suggesting that they might have descended even further if the litter layer was deeper. All individuals were found with their ventral side downward.

To understand how emerging behavior was controlled during the winter, I analyzed the relationships between daily maximum number of adults on the litter and various temperatures from February 6 to 25. The results showed that the daily maximum temperature had a consistently high  $R^2$  and  $r$  with the daily maximum

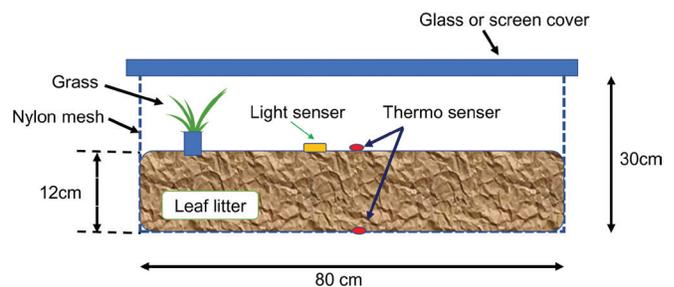
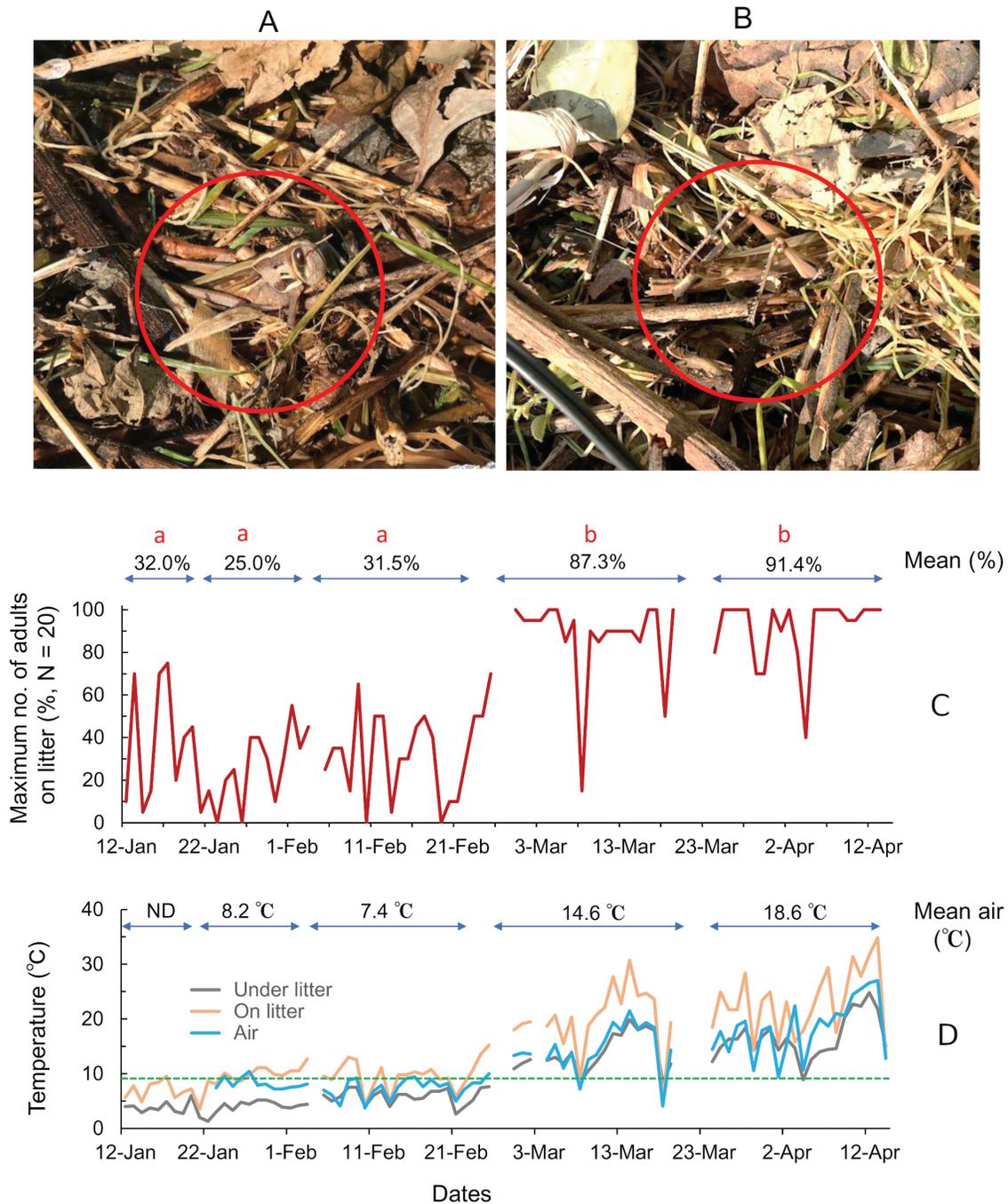


Fig. 7. Outdoor enclosure (48 × 80 × 30 cm) for observation of behavior of *P. japonica* adults.



**Fig. 8.** A, B. *P. japonica* adult emerging from the litter (A) and beginning to hide under the litter (B). C, D. The daily maximum number of *P. japonica* adults above litter surface in the outdoor enclosure from Jan. 12 to Apr. 13, 2022 (C) and temperatures under litter, on litter, and air temperature in shade (D). The mean values for short periods are given on top of each panel. Different letters above the means in C indicate significant differences with Steel-Dwass test at the 5% level.

number of adults observed on the litter ( $R^2 > 0.57$ , Fig. 9F;  $r > 0.75$ , nos.1–4 in Table 1). The air temperature (AT) at which the maximum number of adults on the litter occurred ( $r = 0.77$ , no. 11) and the average temperature on the litter (T2) between 11:00 and 16:00 ( $r = 0.76$ , no. 20) also showed a high correlation coefficient. The maximum light intensity ( $r = 0.61$ , no. 28) and the average value between 11:00 and 13:00 ( $r = 0.60$ , no. 29) also showed a significant correlation with the daily maximum number of adults on the litter. During the 20-day observation period, all but one adult appeared at least once on the litter ( $N = 20$ ) with a

mean frequency of 6.6 times (range, 0–14 times; SD = 3.9 times; Suppl. material 7), suggesting that adults appeared above the litter about every 3 days on average.

As mentioned, the number of adults above the litter at 18:00 increased from February to April and showed a positive correlation with the temperatures at 18:00 (Fig. 9G). The highest  $R^2$  was observed with the temperature on the litter surface (T2) followed by AT and T1. The  $r$  and  $R^2$  gradually decreased as the number of adults at 18:00 regressed on the temperature recorded earlier than 18:00 (Table 2), suggesting that adults appeared to make the final

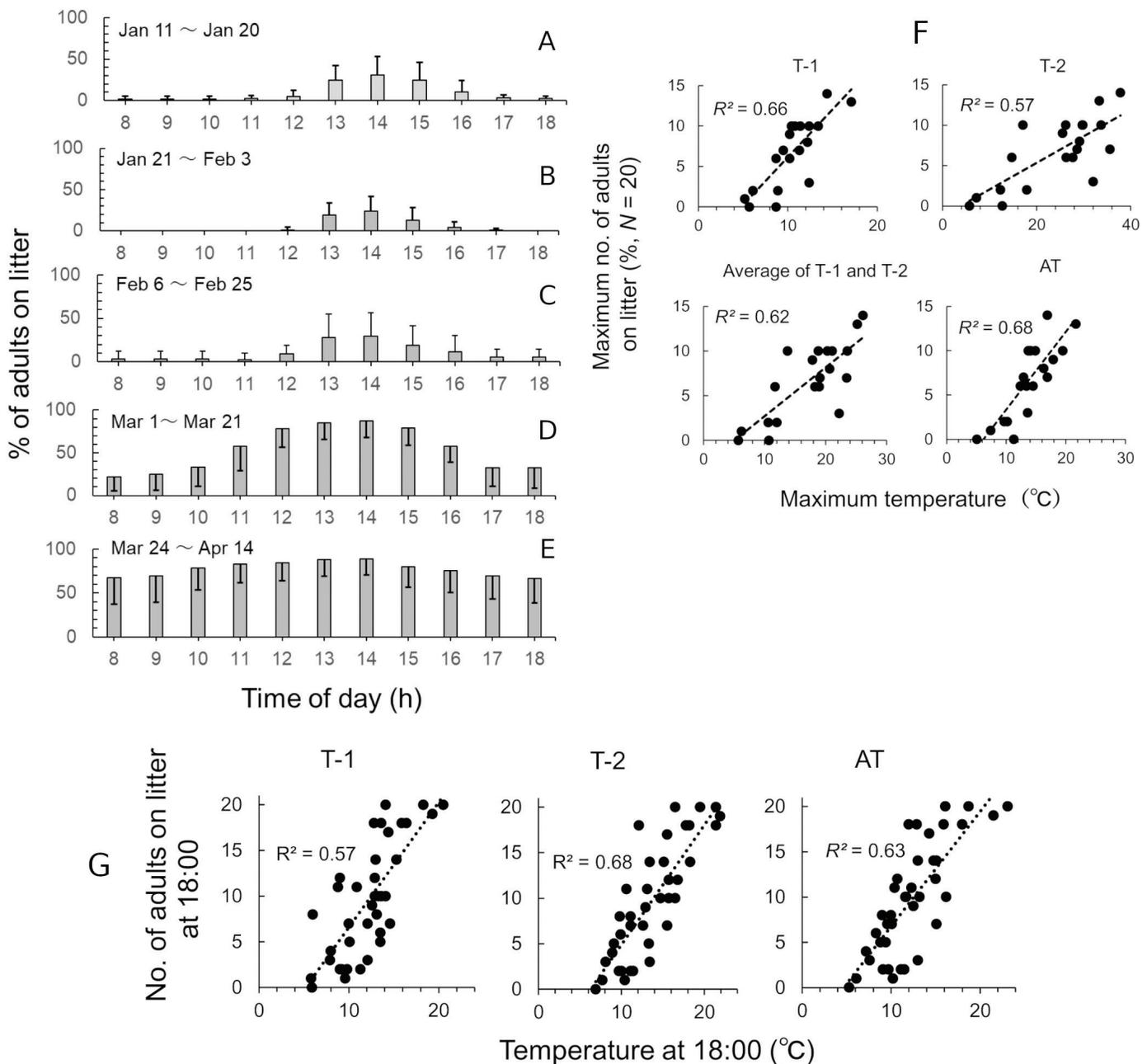


Fig. 9. A–E. Daily changes in the percentage of *P. japonica* adults above litter in the outdoor enclosure containing 20 individuals. Each histogram represents the mean percentage of insects at that hour averaged over all the days of that specific period. Bars indicate one SD. F. The relationship between the daily maximum number of *P. japonica* adults above the litter surface and various daily maximum temperatures in the outdoor enclosure from Feb. 6 to 25. T-1 = temperature at the bottom of litter; T-2 = temperature on litter; AT = air temperature, shade. G. The relationship between the numbers of *P. japonica* adults above litter and temperatures at 18:00 in the outdoor enclosure from Feb. 27 to Apr. 12. T-1, temperature at the bottom of litter; T-2, temperature on litter; AT, air temperature, shade.

decision to stay above the litter or not by monitoring the temperature in the late afternoon or evening.

Adult body temperatures were positively correlated with adjacent litter surface temperatures ( $r = 0.86$ ,  $N = 236$ ,  $p < 0.001$ ; Fig. 10A). The body temperatures (mean = 19.4°C, SD = 8.1°C,  $N = 236$ ) were significantly higher than the temperatures on the litter surface (mean = 15.5°C, SD = 5.3°C,  $N = 236$ ;  $t = -13.80$ , DF = 235,  $p < 0.001$ ). Indeed, some adults sitting on the litter achieved body temperatures up to 17°C above the adjacent litter surface (Fig. 10A), showing that they are efficient thermoregulators. The mean body

temperatures of adults immediately after emerging from the litter (14.1°C, SD = 4.4°C, range, 9.8–24.6°C,  $N = 17$ ) and those shortly before hiding under the litter (14.0°C, SD = 3.2°C, range, 8.8–20.9°C,  $N = 37$ ) were almost identical ( $t = 0.07$ , DF = 24,  $p = 0.94$ ). Adults sitting on the litter had a mean value of 21.1°C (SD = 8.3°C,  $N = 182$ ) with a wide range of 7.2–43.0°C. These results show that grasshopper body temperatures are similar to litter temperatures when emerging, but that once above the litter, adults can greatly exceed ambient temperatures via thermoregulation. Interestingly, both emerging and hiding behaviors occur at around 14°C.

**Table 1.** The relationships between the daily maximum numbers of *Patanga japonica* adults observed on or above the litter in the outdoor enclosure and temperatures from February 6 to 25.

Analysis no.	Parameter	<i>r</i>	<i>n</i>	<i>R</i> <sup>2</sup>	<i>p</i>
1	T-1 Maximum	0.81	20	0.66	< 0.0001
2	T-2 Maximum	0.75	20	0.57	< 0.0001
3	Average of T-1 and T-2 maximum (T3)	0.79	20	0.62	< 0.0001
4	Maximum air temperature (AT)	0.82	20	0.68	< 0.0001
5	T-1 when max no. occurred	0.67	17	0.44	< 0.01
6	T-1 1 h before max no. occurred	0.52	17	0.27	< 0.05
7	T-2 when max no. occurred	0.68	17	0.46	< 0.01
8	T-2 1 h before max no. occurred	0.46	17	0.22	0.06
9	T-3 when max no. occurred	0.76	17	0.58	< 0.001
10	T-3 1 h before max no. occurred	0.51	17	0.26	< 0.05
11	AT when max no. occurred	0.77	16	0.60	< 0.05
12	AT 1 h before max no. occurred	0.34	17	0.12	0.19
13	LI1 when max no. occurred	0.04	17	0.00	0.89
14	LI1 1 h before max no. occurred	0.30	17	0.09	0.24
15	LI2 when max no. occurred	0.38	17	0.15	0.13
16	LI2 1 h before max no. occurred	0.36	17	0.13	0.16
17	Average of T-1 between 0800–1800	0.53	17	0.28	< 0.05
18	Average of T-2 between 0800–1800	0.64	17	0.41	< 0.05
19	Average of T-1 between 1100–1600	0.56	18	0.32	< 0.05
20	Average of T-2 between 1100–1600	0.76	18	0.58	< 0.001
21	Average of T-1 between 1100–1300	0.15	18	0.02	0.056
22	Average of T-2 between 1100–1300	0.68	18	0.46	< 0.01
23	Average of AT between 0800–1800	0.40	18	0.16	0.106
24	Average of AT between 1100–1400	0.67	18	0.45	0.078
25	Average of AT between 1100–1300	0.43	18	0.18	< 0.01
26	Maximum light intensity (LI1)(mw/cm <sup>2</sup> )	0.44	20	0.19	0.054
27	Average of LI1 between 1100 and 1300	0.39	18	0.15	0.115
28	Maximum light intensity (LI2)(Lux)	0.61	20	0.37	< 0.01
29	Average of LI2 between 1100 and 1300	0.60	18	0.36	< 0.01

T-1, temperature under litter; T-2, temperature on litter; T-3, average of T-1 and T-2; AT, air temperature in shade; LI1, intensity of ultraviolet light; LI2, intensity of illumination. Bold figures indicate  $p < 0.05$ .

The above results raised a question: If *P. japonica* adults emerged from the litter and started hiding under the litter at similar body temperatures, how was the behavioral difference (moving up vs. moving down) brought about? Fig. 10B, C compares the temperature changes on the litter during the 1 h before the first individual appeared on the litter with those before the first individual hid under the litter. The temperature increased in all cases ( $N = 39$ ) in the former, whereas it decreased in 92.5% ( $N = 40$ ) in the latter. The mean change was 8.9°C and -5.0°C, respectively, with the difference being highly significant ( $t = 10.67$ ,  $DF = 56$ ,  $p < 0.001$ ). The results suggested that a change in temperature triggers the daily vertical movements, with a positive temperature change eliciting positive phototaxis out of the litter and a negative temperature change eliciting negative phototaxis back beneath the litter.

*Behavior in tubes Experiment 5: Effect of temperature change.*—To test the hypothesis that a positive change in temperature triggers a positive phototaxis in winter adults, Treatment grasshoppers were

**Table 2.** The correlations between the numbers of adults on or above litter at 18:00 in the outdoor enclosure and the temperatures at 14:00–18:00 from Feb 27 to Apr12.

Time	<i>r</i>	<i>R</i> <sup>2</sup>	<i>N</i>	<i>p</i>
T-1				
14:00	0.28	0.08	38	0.0900
15:00	0.40	0.02	40	0.0950
16:00	0.59	0.35	39	<0.0001
17:00	0.73	0.53	40	<0.0001
18:00	0.76	0.57	39	<0.0001
T-2				
14:00	0.17	0.03	38	0.3142
15:00	0.47	0.22	40	0.0020
16:00	0.64	0.41	39	<0.0001
17:00	0.79	0.62	40	<0.0001
18:00	0.83	0.68	39	<0.0001
AT				
14:00	0.49	0.24	38	0.0016
15:00	0.51	0.26	39	0.0009
16:00	0.64	0.40	39	<0.0001
17:00	0.72	0.52	40	<0.0001
18:00	0.79	0.63	39	<0.0001

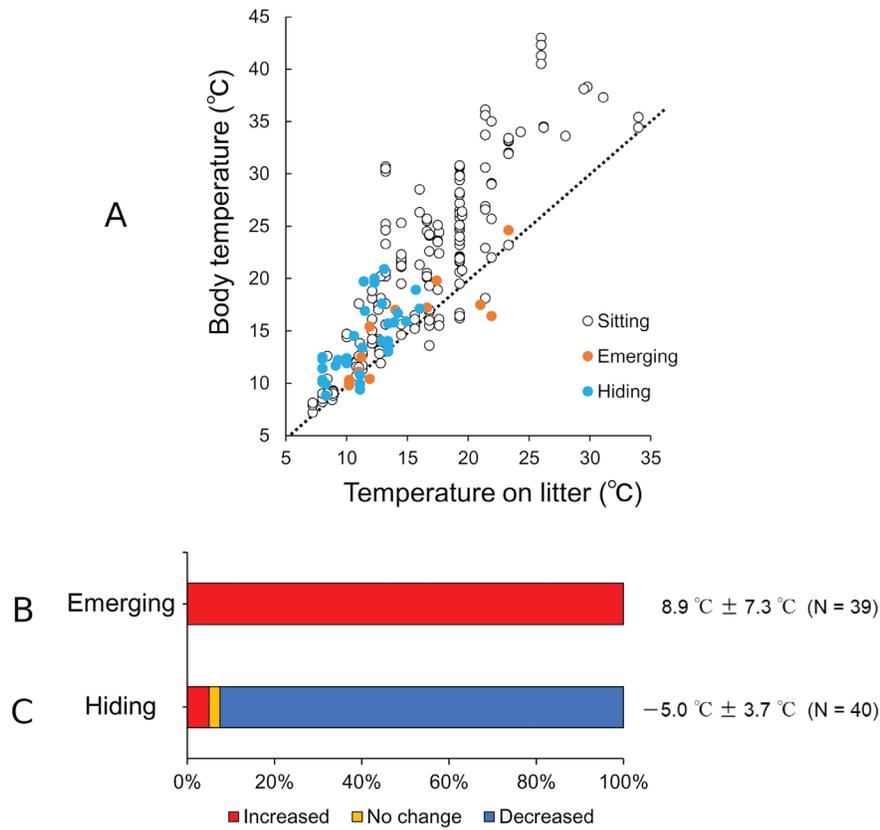
T-1, temperature under litter; T-2, temperature on litter; AT, air temperature in shade. Bold figures indicate  $p < 0.05$ .

transferred from cold outdoor conditions to 20°C to expose them to a large increase in temperature late in the afternoon when they normally have moved into the D-areas. The Control insects that were transferred from indoor fluctuating temperatures to steady 20°C experienced a small increase in temperature. When Treatment adults were rapidly heated, 50 to 70% moved into the L-area within 1 h, suggesting that rapid heating triggered positive phototaxis (Fig. 11). In contrast, all Control insects remained in the D-area. This suggests that (1) an increase in temperature can trigger positive phototaxis, but the magnitude of the temperature change is important, and (2) current temperature changes take precedence over both biological rhythms and light stimulation in determining movement from dark into light.

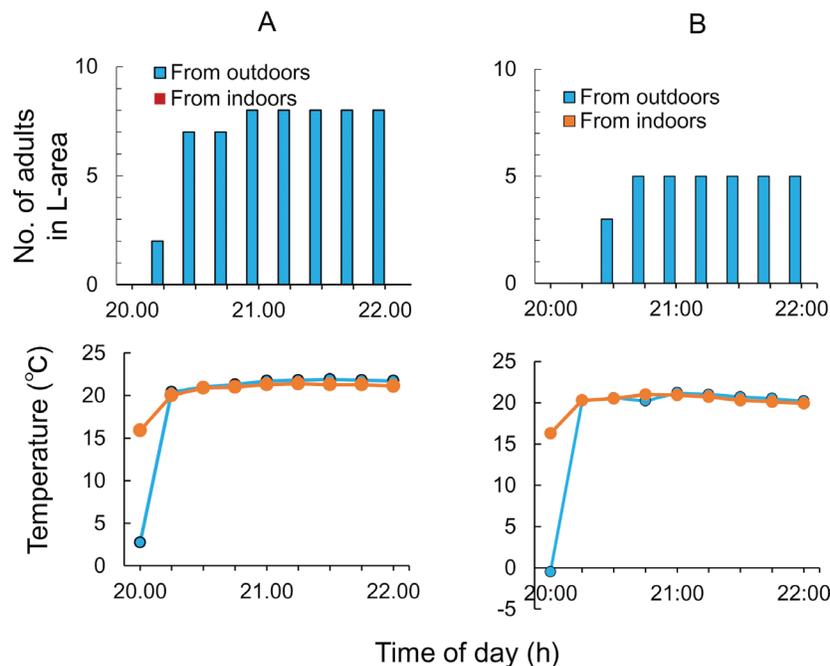
## Discussion

The present study showed that, in central Japan in winter, *Patanga japonica* adults exhibited a daily cyclical pattern of vertical movements. They hid under leaf litter during the night, crawled to the surface in mid- to late morning, then descended back under the litter in early to late afternoon. At night, some grasshoppers descended as deep as 12 cm below the litter surface, and on sunny days, many basked in the sunlight. On warm winter days, adults climbed the cage walls and occasionally warmed enough to fly.

The experimental results suggest that temperature and light control the vertical movements. In the morning, adults were immobile at subzero temperatures but could move their appendages in a sluggish way at above 0°C when handled. Adults placed side-down on the floor got up and assumed a normal posture when body temperature reached 3.7°C on average (Fig. 2C). In contrast, under semi-natural conditions, adults stayed under leaves in the morning and emerged around noon at ambient temperatures above 10°C (Fig. 3). Hence, although winter adults possess the ability to right themselves at 3.7°C (with some standing at



**Fig. 10.** A. The relationship between body temperatures of *P. japonica* adults and temperatures on litter in the outdoor enclosure from Jan. 17 to Feb. 7. Sitting, adults sitting on litter; emerging, those that just emerged from litter; hiding, those that started moving to hide under litter. Dotted lines indicate that the two temperatures are similar. B, C. The proportions of days when the temperature on litter increased, remained unchanged, and decreased during 1 h before the first *P. japonica* emerged (B) and before the first adult hid under litter (C) in the outdoor enclosure from Jan. 12 to Feb. 25.



**Fig. 11.** The number of *P. japonica* adults that appeared in the L-areas of tubes at  $20 \text{ } ^\circ\text{C}$  under artificial illumination on Jan. 3 (A) and Jan. 8 (B). Treatment tubes were transferred to warm indoors from the cool outside at 20:00, causing them to rapidly heat. Control tubes experienced only a mild temperature increase because they had already been indoors for several hours. All adults were placed in D-areas at 20:00. Temperatures in the L-areas are shown. Note that no Control insects moved into the L-areas on both days.

-0.3°C), under semi-natural conditions, they generally remained under leaves until the air temperature exceeded 10°C. These results show that the morning emergence of overwintering adults is not simply dependent on whether temperature was high enough for them to be able to move.

Insects control their development and life cycle in response to various physical and biological factors (Tauber et al. 1986, Danks 1987, Leather et al. 1993, Saunders et al. 2002, Denlinger 2022). Adults in winter diapause often show a negative phototaxis by which they can hide in a hibernaculum (de Wilde 1954, Stoffolano and Matthyse 1967, Pienkowski 1976, Vinogradova 2007, Gill et al. 2017). In mosquitoes, approximately 50% of diapausing adults change their location every 6 days searching for more favorable temperature conditions in the hibernacula (Onyeka and Boreham 1987), but the relation of this behavior to phototaxis is unknown. In the present study, overwintering *P. japonica* adults held in tubes were in the D-area in the morning but appeared in the L-area at around noon and then returned to the D-area in the late afternoon (Fig. 4). This pattern of behavior is similar to what was observed in the outdoor cage mentioned above, suggesting the possibility that the daily behavior of overwintering adults was controlled by phototaxis.

The number of *P. japonica* adults observed in the L-areas of tubes fluctuated during the winter months and showed a positive correlation with the daily maximum tube temperature: more adults occupied L-areas when ambient temperatures were higher (Fig. 5A–D). However, while they were moving from the D- to L-area, the temperature in the latter was lower (Fig. 4), suggesting that moving to the L-area is controlled by a positive phototaxis independently of the temperature gradient.

In the late afternoon, *P. japonica* adults held in tubes returned to the D-area and remained there until the following day under outdoor conditions. The fact that D-areas were slightly warmer (Fig. 4) suggested that perhaps the insects were attracted to the warmer area in the afternoon when ambient temperatures normally decrease. However, adults still moved into the D-area when it was made colder than the L-area (Fig. 6A–K), suggesting that returning to the D-area is controlled by a negative phototaxis independently of the temperature gradient.

To understand the significance of the changes in phototaxis in *P. japonica*, the daily behaviors of overwintering adults were observed in an outdoor enclosure. They appeared on the litter during the day and hid under the litter during the night (Fig. 8A, B). The daily maximum number of adults appearing on the litter fluctuated mostly below 60% in January and February, whereas it remained above 80% in March and April except for several days. This fluctuation was highly correlated with the air temperature (Figs 8C, D, 9A–E). Indeed, the number of adults appearing on the litter in February was highly correlated with the daily maximum air temperature, implying that adults decide to emerge from litter in response to rising temperature in nature (Table 1, Fig. 9F). The body temperature immediately after emerging from the litter was 14.1°C on average. During the day, they were inactive, and many spent several hours bathing in direct sunlight. In the afternoon, they began to hide under the litter. The body temperature immediately before hiding was 14.0°C, which was virtually the same as the body temperature at emergence from the litter (Fig. 10A). But how can adults with the same body temperature show a positive phototaxis in the morning and a negative phototaxis in the afternoon? Perhaps the direction of temperature change determines a subsequent positive or negative phototaxis. Indeed, adults experienced an increase in temperature before emerging from the litter in the morning and a decrease in temperature before hiding under the litter in the after-

noon (Fig. 10B, C). This hypothesis was tested by subjecting adults to either a large or a small temperature increase in the late afternoon when they would normally move downward into leaf litter. The results (Fig. 11) revealed that adults exposed to large increasing temperatures in the afternoon moved into light, but those exposed to small increasing temperatures remained in the dark. From this and other experimental results described above, it may be concluded that the induction of phototaxis depends not only on the direction but also on the magnitude and absolute range of the temperature change. In other words, the phototaxis of *P. japonica* is triggered based on the monitoring of changing temperatures and the integration of the information. Once phototaxis is triggered by a temperature change, the grasshoppers appear to follow it independently of the temperature gradient in *P. japonica*. However, their behavior is not rigid but flexible. The final decision of whether they hide or remain on the litter is made by monitoring the temperature on the litter in the evening (Table 2, Fig. 9G).

Other arthropods exhibit a change in phototaxis in morning vs. evening. For example, the pill bug, *Armadillidium vulgare* (Latreille, 1804), is positively phototactic during the daytime but negatively phototactic in the evening (Warburg 1964). Pill bugs move toward warm sunlight when it is cold and ambient temperature is increasing, and seek a shelter when sunlight is too warm. This phototaxis is known to be influenced by light intensity, temperature, humidity, and thigmotaxis (Warburg 1964, 1968, Refinetti 1984). However, how the phototaxis is induced has not been determined.

*P. japonica* adults in Japan enter a reproductive diapause in late fall and do not mate or develop eggs until spring (Tanaka 2023). Adults remain in reproductive diapause for 4 months, even when maintained at 30°C. This observation may suggest that they have some requirement, such as a period of low temperature, to terminate diapause (Tanaka and Okuda 1996). Control of this diapause has not been elucidated.

Other insect species perform similar cyclical daily movements out of and back into refugia. For example, some grasshopper species shelter under plants or leaf litter or crawl into rodent holes or cracks in rocks or soil, descending as deep as 90 cm to escape cold, heat, or desiccation (Uvarov 1977). In winter, *Schistocerca gregaria* (Forskål, 1775) locusts hid in clumps of grass at night but emerged daily for 2 hours around noon (Volkonsky 1941). Other acridid species bury themselves under sand during extreme temperatures, including during winter, or enter water on nights when water temperatures are warmer than air temperatures (Uvarov 1977). In contrast, a great many grasshoppers perform daily vertical movements in the opposite direction. Many roost in plants at night and then descend daily to forage on the ground (Uvarov 1977). An example is the desert grasshopper *Taeniopoda eques* (Burmeister, 1838), which almost always roosts at the very tops of desert shrubs at night but forages on the ground during the day (Whitman 1987). However, following a sudden freezing rain, the grasshoppers did not ascend bushes but immediately buried themselves beneath cut hay and stayed there throughout the cold night, insulated from the colder air temperatures. This anecdotal observation shows that grasshoppers are both highly attuned and responsive to temperature as well as flexible in their behavioral responses (D. Whitman, unpublished observation). Grasshoppers are richly endowed with thermosensilla and will move to the warmest spot under complete darkness (Chapman 1955). What makes *P. japonica* unique is that it performs these daily vertical movements in the middle of winter and that this behavior does not seem to be for foraging, escaping from predators, or avoiding extreme temperatures or desiccation, as discussed below.

During the present study, *P. japonica* adults were observed on leaf litter at the study site in February. Although adult body color is cryptic against the litter or ground (Fig. 8A, B) (Tanaka and Kayukawa 2024), it seems dangerous for them to appear on the ground in the winter because they cannot move quickly or fly when threatened by predators such as birds and some small mammals that forage in winter. Why do they appear on the ground? Bathing in the sun is probably not the top priority for this behavior because, in the outdoor enclosure, there were always some adults staying in shade, and adults emerged from the litter even on cloudy days. Overwintering adults in cages were sometimes observed feeding on *Bromus* leaves during the day, but this plant is an introduced species, and usually most domestic herbaceous plants in their natural habitat are wilted in winter. Therefore, it is unlikely that they appeared on the ground for feeding.

In November of 2020, I kept two groups of 20 *P. japonica* adults with *Bromus* plants planted in soil in plastic containers (30 × 40 × 45 cm) and piled up the containers on a deck where the roof prevented them from getting direct sunlight and rainfall. Each container had a lid with small screen windows, but only the top container had some ventilation through these windows. In March 2021, I observed that the plants were still alive in both containers, but only 14 and 1 adults were alive in the top and bottom containers, respectively (Tanaka S, unpublished observation). Some of the dead were moldy. After this experience, I succeeded in keeping adults alive for the present study by housing them in screen-covered cages with *Bromus* plants for overwintering. Grasshoppers are well known to be highly susceptible to pathogens during cold, wet weather (Joern and Gaines 1990, Streett and McGuire 1990). It is thus possible that overwintering adults frequently appear on the ground to dry and warm their bodies to reduce fungus infection. Periodic warming probably also temporarily increases overall metabolism, which may benefit the grasshoppers. The phototaxis seems to provide diapausing *P. japonica* adults with an effective means of controlling their daily activity during the winter.

Biogenic amines serve as important neuromodulators, neurohormone, and neurotransmitters and are involved in phototactic behaviors in insects (Neckameyer et al. 2001, Thamm et al. 2010, Riemensperger et al. 2011, Zhang et al. 2016). In locusts, the application of octopamine can dishabituate the habituated response of descending movement detector interneurons to repetitive visual stimuli (Bacon et al. 1995). In the honey bee *Apis mellifera* (Linnaeus, 1758), responsiveness to light is modulated by octopamine and tyramine (Scheiner et al. 2014). The underlying mechanism controlling the change in phototaxis is unknown in *P. japonica*. It would be interesting to explore the possible involvement of biogenetic amines in this phenomenon.

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### Supplementary material 1

Author: Seiji Tanaka

Data type: jpg

Explanation note: **fig. S1**. Photograph showing the setup for measuring the time for *Patanga japonica* adults to stand.

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Link: <https://doi.org/10.3897/jor.33.102749.suppl1>

### Supplementary material 2

Author: Seiji Tanaka

Data type: jpg

Explanation note: **fig. S2**. Photograph showing indoor and outdoor cages housing *Patanga japonica* adults.

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Link: <https://doi.org/10.3897/jor.33.102749.suppl2>

**Supplementary material 3**

Author: Seiji Tanaka

Data type: jpg

Explanation note: **fig. S3**. Transparent tubes used to observe the behavior of *P. japonica* adults. Ten adults were placed either in the dark or light (L) area at 08:00 (A), and the number of individuals in the L-area (B) was recorded every hour until 18:00. Note thermistor probes inserted into opposite ends of tube to record temperatures in the light and dark areas.

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Link: <https://doi.org/10.3897/jor.33.102749.suppl3>

**Supplementary material 4**

Author: Seiji Tanaka

Data type: jpg

Explanation note: **fig. S4**. Plastic tube heated by an incandescent lamp covered with aluminum foil at the end of the light area.

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Link: <https://doi.org/10.3897/jor.33.102749.suppl4>

**Supplementary material 5**

Author: Seiji Tanaka

Data type: jpg

Explanation note: **fig. S5**. Body and floor temperatures of dead and live *P. japonica* adults after 30 min exposure to outdoor conditions in early morning. The temperature difference between body and floor temperatures amounted to 0.1 and 0.4°C for dead and live adults, respectively. Asterisk indicates that the difference was significant at  $p < 0.05$  with *t*-test ( $N = 10$  each).

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Link: <https://doi.org/10.3897/jor.33.102749.suppl5>

**Supplementary material 6**

Author: Seiji Tanaka

Data type: jpg

Explanation note: **fig. S6**. The depths of litter at which *P. japonica* adults were found in the outdoor enclosure at 07:00 on Feb 4, 2022.

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Link: <https://doi.org/10.3897/jor.33.102749.suppl6>

**Supplementary material 7**

Author: Seiji Tanaka

Data type: jpg

Explanation note: **fig. S7**. The number of days each *P. japonica* adult appeared above the litter in the outdoor enclosure during the period from Feb. 6 to 25.

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