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Brood translocation and circadian variation of temperature preference in the ant *Camponotus mus*

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Summary. 1) When a thermal gradient (20–40° C) was established along a laboratory nest, *Camponotus mus* nurse workers showed a photoperiodic circadian rhythm of temperature preferences for brood rearing. Two different temperatures were daily selected to translocate the brood, i.e. 30.8° C selected at the middle of the photophase, and 27.5° C selected during the scotophase, 8 h later. 2) The daily temperature response of nurse workers consisted of paired high and low-temperature translocations, with a 8 hs-interval in between: high-temperature translocation was shown to be entrained by the photophase length, whereas low-temperature translocation was shown to be dependent on the precedent one. 3) Prey deprivation to the colony modified the brood transport behaviors resulting in translocations of only cocoons and large (ripe) larvae, stages in which the pupation processes are triggered. Small larvae and eggs remained located at 27.5° C. 4) Evaluation of pupa developmental time as well as percentage of pupa mortality at different temperature regimes allowed to construct an efficiency index relating *pupa survival* and *cocoon developmental time*. In the range of temperatures selected by nurses, the index reached its maximal values. 5) The ecological significance of these results is discussed.

Key words: Ants – *Camponotus mus* – Circadian rhythm – Temperature – Brood development

Nest-temperature control by ants is known to be achieved by selecting a suitable place to locate the nest and by translocating the brood from one part to another according to its developmental stage and the variations in nest climate (Steiner 1929; Vanderplank 1960; see revision in Heinrich 1981).

Studies with laboratory colonies in which a thermal gradient was established along their nest allowed brood transport behaviors and temperature preferences to be accurately measured. Using such experimental device, Protomastro (1973) found in *Camponotus mus* nurse workers a daily rhythm of brood translocation from low to high temperatures. Brian (1973) and Ceusters (1977) reported the temperature choice in *Myrmica rubra* and *Formica polyctena* respectively, although data of circadian variation of such preferences, if present, are not available from these studies.

In this paper brood translocation and temperature preferences in the ant *Camponotus mus* were recorded along a thermal gradient under different light/dark cycles and in relation to prey supplied to the colony. Furthermore, pupal development at different thermal programs was measured.

Materials and methods

Two colonies of *Camponotus mus* were used during this study. The founding queens were captured after the nuptial flight and were reared in artificial glass nests (40 cm × 10 cm × 2 cm). Colonies were fed with 50% w/w sucrose solution and freshly-killed *Drosophila* flies ad libitum, and kept under a light/dark cycle of 12 h/12 h (light-on: 8 a.m.; 100 lux).

In one experimental colony, a thermal gradient was established along the nest by placing a heater with a parabolic screen at one end (Fig. 1), which maintained it at 40° ± 1° C. The other nest-end was at room temperature, 20° ± 2° C.

Temperature records were taken on the nest floor with a thermistor-thermometer (accuracy 0.1° C) introduced through the existing holes in the 2 cm-wide zones marked on the nest cover. Mercury thermometers placed into the nest at the two ends allowed further control. A kymographic cammera located 50 cm over the nest was used to

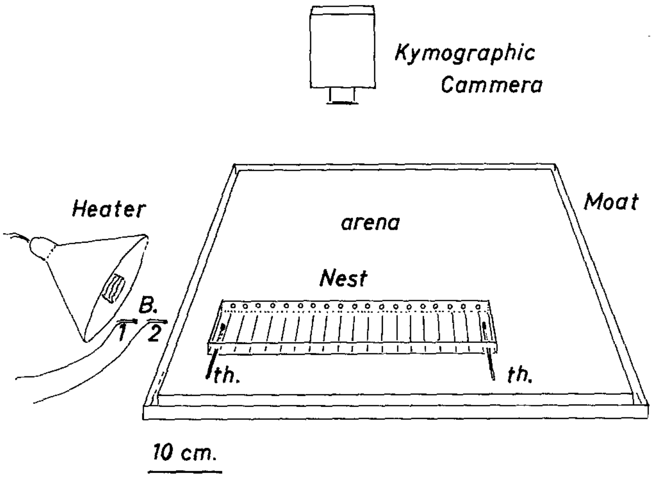

Fig. 1. Diagrammatic view of the experimental arrange. *B* heater bimetallic control, *th* thermometers

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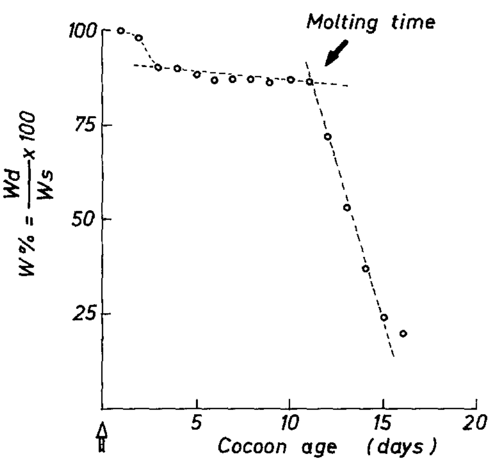

Fig. 2. Weight change of a cocoon with the enclosed insect as a percentage of weight 24 h after spinning. The curve is presented to show how the *molting time* value could be obtained. *Abcissae* cocoon age in days. *Ordinates* $W\% = \frac{Wd}{Ws} \cdot 100$
where Wd = weight at age *d*
Ws = weight at spinning
The *white arrow* marks the cocoon spinning

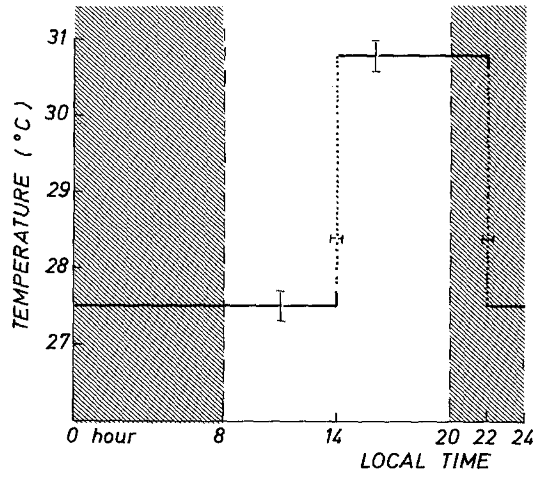

Fig. 3. Daily pattern of brood location along the thermal gradient. *Broken lines* mark times when brood translocations were performed by nurse workers. *Full lines* mark selected temperatures to translocate the brood (see text). *Dashed areas* mark the scotophase (L/D cycle 12 h/12 h)

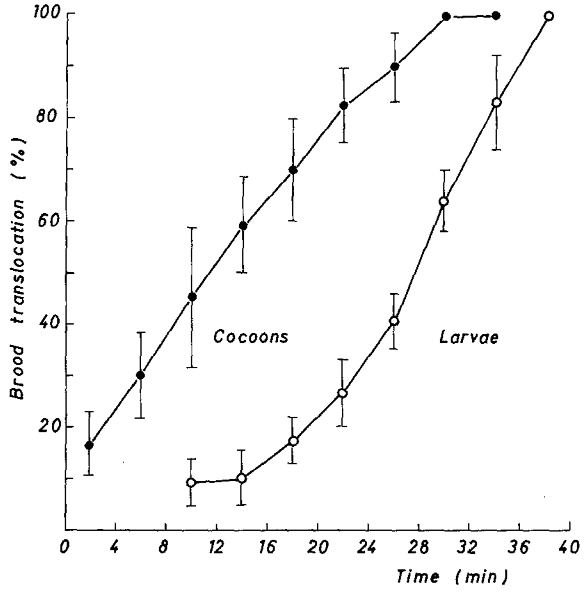

Fig. 4. Dynamics of brood translocation. *Abcissae* time elapsed from the start of brood translocations. *Ordinates* cumulative percentage of brood translocated. *Closed circles* cocoon translocation, *open circles* larva translocation. *Dots* are mean ± SE for six replicates. Egg carrying (not plotted) was always the last translocation recorded

record the position of the brood along the thermal gradient. Pictures were taken every 15 min.

Effects of the colony nutrimental condition on the brood transport behaviors and temperature preferences were analysed by presenting the colony with two different food supply programs of 30 days each, alternating: *Feeding*: with a daily supply of freshly-killed *Drosophila* flies, just covering colony requirements for a day, i.e. all flies were dissected and eaten; *Starvation*: without this supply. A 50% w/w sucrose solution was available ad libitum during both food supply programs.

Effects of temperature programs on pupal development were assessed by rearing cocoons in brood chambers at different constant temperatures, i.e. 20°, 25°, 30° and 35° C, and under a variable temperature regime of 16:8 h/ 27.5°:31° C (mean constant temperature = 28.6° C). Newly spun cocoons were removed from the colonies, placed in the chambers and daily weighted at the nearest 0.01 mg. The obtained cocoon weight-age curves at each temperature were used to determine the *molting time* (see Fig. 2 as an example), the time when a sharply increase in the slope was evident.

Results

a) Circadian rhythm of brood translocation

When the thermal gradient was established in the nest, the brood location along it was not static. Nurse workers, which comprised 5–6 individuals of a total of 70–80 ants inhabiting the nest, showed a circadian rhythm of temperature preferences to carry and locate the brood. Figure 3 depicts the daily pattern of selected temperatures and times when brood translocations were recorded. Each day, two translocations of all brood were performed by nurse workers. At the day times noted in the figure, i.e. nearly 2 p.m. and 10 p.m., nurses currently resting over the brood started to move vigorously along the nest, running back and forth. Brood transport behaviors were immediately initiated by them. At 2:05 p.m. ± 0:10 min (mean ± SE, $N = 15$) the brood, till this time located at 27.5° ± 0.2° C ($N = 15$), was translocated to 30.8° ± 0.2° C ($N = 15$). At 10:10 p.m. ± 0:10 min ($N = 15$), conversely, it was picked-up and carried to 27.5° C. Note that the brood translocation to 30.8° C was performed six hours after light-on, i.e. at the middle of the photophase. The translocation to 27.5° C, performed 8 h later, took place during the scotophase, 2 h after light-off.

The dynamics of brood translocation is shown in Fig. 4. Cocoons were first translocated, followed by larva and finally by egg carrying. The translocation of all brood, that in that moment comprised 30 cocoons, 14 larvae and 2 egg

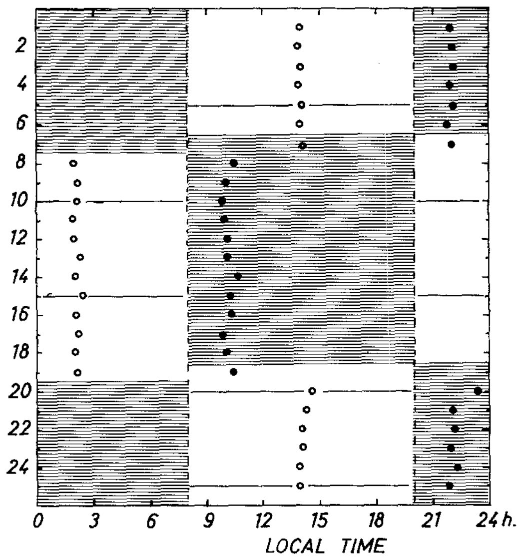

Fig. 5. Day-by-day brood translocation activity. *Open circles* start of brood translocations to 30.8° C. *Closed circles* start of translocations to 27.5° C. *Dashed areas* mark the scotophase (L/D cycle 12 h/12 h)

clusters, was performed by nurse workers in approximately 40 min.

b) Brood translocation under different light/dark cycles

The day-by-day brood translocation activity under a 12 h/12 h L/D cycle is shown in Fig. 5. The daily activity data were expressed as open circles, when the brood translocation to 30.8° C was started, and as closed circles, when the translocation to 27.5° C occured. As Fig. 5 shows, at the end of the 6th day the L/D cycle was 12 h shifted, followed two days later by a concomitant shift in the brood translocation activity. When the initial L/D cycle was restored, activity changed concomitantly two days later (see day 19th, Fig. 5).

Figure 6 compares the translocation activity of day 6th and day 19th, when the 12 h-shift of the L/D cycle was imposed. At the day 6th, the shift ocurred by a 12 h-enlargement of the scotophase (Fig. 6a), whereas at the day 19th it was obtained by a 12 h-enlargement of the photophase (Fig. 6b). It could be noted that 12 h-scotophase-enlargement showed no effect on the following translocation activity, which remained entrained to the precedent light cycle. On the other hand, 12 h-photophase-enlargement damped the following translocation activity. Finally, the succeeding translocations were shown to be entrained to the new light cycle (Fig. 6a, b). It could be also noted that in all situations, the 8 h-interval between the two translocations was maintained.

The activity record presented in Fig. 7 shows the brood translocation events when the photophase was shortened, i.e. the 12 h/12 h L/D cycle was changed to 8 h/16 h. In this experiment, the regime was changed at the end of the 10th day, and a shift in the brood translocation activity was evinced three days later. The 2 p.m.-translocation turned out to be at 4 p.m., i.e. 6 h after light-on, whereas the 10 p.m.-translocation changed to nearly midnight, i.e. 8 h later, 6 h after light-off. As Fig. 7 also illustrates, eleven days after the L/D cycle was changed (day 21th) brood translocations to 30.8° C shifted to 2 p.m. were recorded, followed immediately by retro-translocations to 27.5° C, noted as triangles in the figure. Close observations showed that some nurses started to carry cocoons at 2 p.m., placing them at 30.8° C. At the same time, other nurses picked-up the recently carried cocoons and returned them to 27.5° C. However, at 4 p.m. all the brood was carried to 30.8° C. Retro-translocation events were recorded between day 21th and day 44th. The percentage of shifted translocations is shown in Fig. 8, where open circles mark the shifting to 2 p.m. (without retro-translocations) and closed circles mark the shifting to 10 p.m. response which was shown to be delayed (Fig. 8). From the day 45th, 34 days after the shortening of the photophase, all brood translocations to 30.8° C were shifted to 2 p.m., i.e. at the middle of the photophase, 4 h after light-on. Translocations to 27.5° C occured eight hs later at 10 p.m., 4 h after light-off.

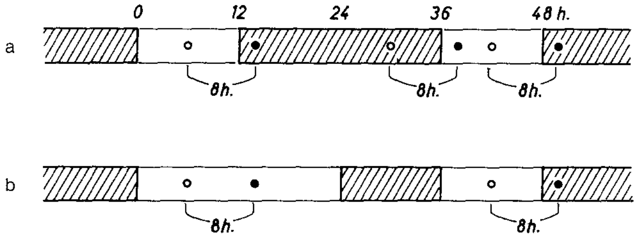

Fig. 6a, b. Comparison of translocation activity corresponding to data of Fig. 5. **a** Shift by 12 h-scotophase-enlargement (day 6th). **b** Shift by 12 h-photophase-enlargement (day 19th). *Circles* as in Fig. 5

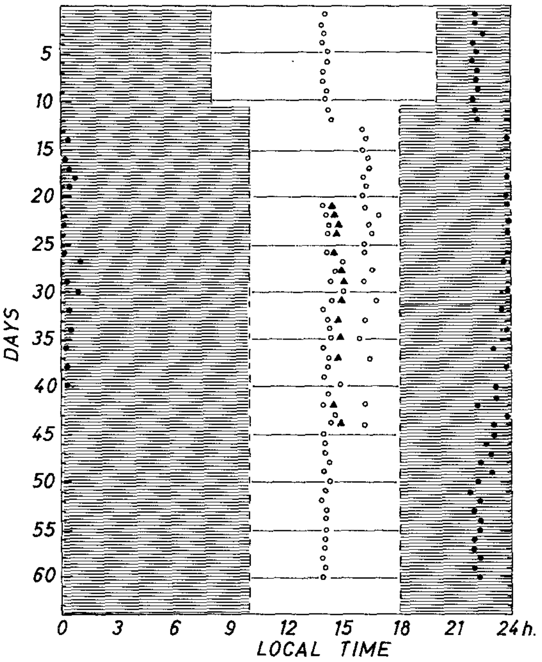

Fig. 7. Brood translocation activity during the experiment of photophase shortening (from L/D cycle 12 h/12 h to 8 h/16 h). *Circles* as in Fig. 5. *Triangles* represent retro-translocations, i.e. translocations to 27.5° C of the recently carried cocoons (for further explanation see text)

c) Prey supply effects

During the *Feeding* program, all brood was translocated as described in Fig. 3. During the *Starvation* program, on

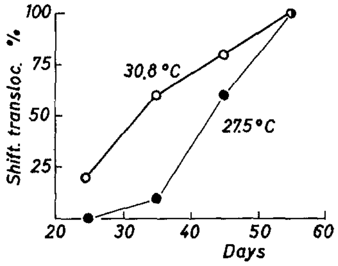

Fig. 8. Data of shifted translocations from day 21th, corresponding to the experiment of photophase shortening. Circles correspond to five-days pooled data. *Open circles* 30.8° C-translocation shifted to 2 p.m. (without retro-translocations). *Closed circles* 27.5° C-translocation shifted to 10 p.m.

the other hand, nurses responded with a delay of 3.8 ± 0.5 ($N=4$) days by translocating only cocoons and large (ripe) larvae, which were observed spinning their cocoons a few days later. Small larvae and eggs remained located at 27.5° C as the *Starvation* program lasted. There was no delay in translocate small larvae and eggs when the next *Feeding* program was started ($N=4$).

Interruption of only sugar solution supply had no effects on the brood translocation activity, that remained as in the *Feeding* program.

d) Thermal program simulation

A simulated daily thermal program was imposed in the experimental nest, which resembled the circadian rhythm of temperature preferences of nurse workers. A switch in the heater bimetallic control allowed the temperature at the place where the brood was located to be changed from 27.5° to 31° C with a roughly 1 h delay. Therefore, the immobile brood experienced a daily thermal program similar to the program received as a consequence of translocations by nurse workers. Under such simulation, no translocations were observed along the gradient neither during *Feeding* nor during *Starvation* programs ($N=16$).

e) Temperature effects on pupal developmental time

As expected, temperature influenced both developmental time and survival of cocoon-enclosed pupae. Figure 9 summarizes the developmental time values (open circles – broken line) for cocoons reared at different constant temperatures, as well as for cocoons reared under a variable temperature regime of 16:8 h/27.5°:31° C (mean constant temperature = 28.6° C). This regime resembled the daily thermal program achieved by cocoons through the nurse workers' thermopreferences. For cocoons kept at constant temperatures, the relationship between temperature (T) and molting time (M) is described by $M = 115243.T^{-2.6687}$ ($N=19$, $r=-0.946$, standard error of estimate $=0.262$, $P<0.0001$). The M value for cocoons kept under the variable regime stood up the 0.95% confidence interval of the curve, i.e. there were no significant differences if compared with the M value for cocoons kept at the corresponding mean constant temperature.

Figure 9 also shows mortality percentage of cocoon-enclosed pupae at the different temperature regimes (closed circles–full line). Cocoon mortality occurred because imaginal molting took no place. The heavy line curve (triangles) expresses an efficiency index obtained as the quotient of *pupa survival* and *cocoon developmental time.*

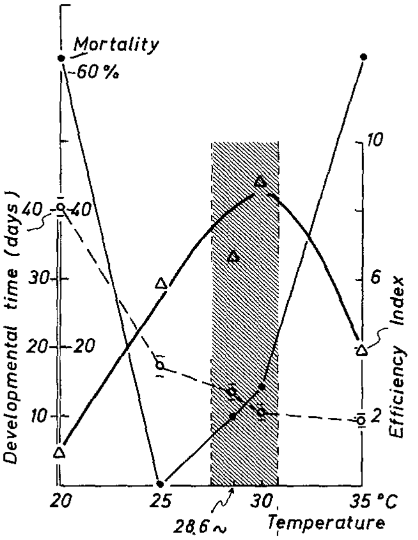

Fig. 9. Cocoon developmental parameters as a function of rearing temperature. 28.6° C is the mean (constant) temperature for cocoons reared under the variable temperature regime of 16:8 h/27.5°:31° C. *Broken line* (*open circles*) developmental time values for cocoon-enclosed pupae (20° C: $N=3$; 25°: $N=7$; 30°: $N=6$; 35°: $N=3$; 28.6°: $N=9$). *Full line* (*closed circles*) pupa mortality percentage (20° C: $N=8$; 25°: $N=7$; 30°: $N=7$; 35°: $N=8$; 28.6°: $N=10$). *Heavy line* (*triangles*) efficiency index $= \frac{\text{pupa survival}}{\text{cocoon developmental time}}$. *Dashed area* range of temperatures selected by nurses to translocate the brood

Discussion

There have been several papers on daily rhythms of behavioral responses in ants, focused either on activity patterns of individuals (McCluskey 1958, 1965; North 1987) or on temporal organization of colony social activities outside the nest (Whitford and Ettershank 1975; Rosengren 1977; Gano and Rogers 1983; Gordon 1983a, b). However, the existence of daily rhythms of brood translocation associated to temperature was only once reported (Protomastro 1973).

The results presented in this paper show three essentially new findings on brood rearing behavior in ants.

First, the existence of a photoperiodic circadian rhythm of temperature preferences for brood rearing in *Camponotus mus* nurse workers, with two different temperatures daily selected. One translocation to 30.8° C attained at the middle of the photophase, and the another one to 27.5° C during the scotophase, eight hours after the precedent translocation (Figs. 3–5).

Second, the entrainment of the high-temperature translocation response by the photophase length (Fig. 7). Although the light-on event was used by nurses as a cue which initially entrain their response (Figs. 6, 7, day 13th), photophase length finally accounted for the long-term adjustment, which occurred from a nurse worker to another (Fig. 7, between day 21th and 44th, and Fig. 8). The low-temperature translocation response was shown to depend on the precedent translocation, with a 8 hs-interval in between.

Third, the temperature effects on developmental rate and on pupa survival, which suggests that in the narrow range of preferred temperatures, a maximal efficiency is obtained on emerged adults per unity of time invested by nurses in cocoon tending (see efficiency index, Fig. 9).

It is known that variable temperature regimes experienced by larvae of the ant *Leptothorax acervorum* are necesary for their further pupation (Buschinger 1973). In the present study, the effects of oscillating temperatures on pupal development was not possible to assess. Cocoons reared under the variable temperature regime (which resembled the program selected by nurses when translocate the brood) showed a molting time value not significantly different of that for cocoons reared at the corresponding mean constant temperature. In crickets, developmental time was shown to be drastically reduced by the possibility to bask in sunshine at self-determined intervals (Remmert 1985). This was not the case for *Camponotus mus* pupae. Considering the concept developed by Ratte (1984) distinguishing temperature action on quantitative (growth) and qualitative (differentiation) aspects of development, it could be argumented that temperature selection by *Camponotus mus* nurse workers results in an optimization of other physiological parameters than growth.

Pupation processes in ants mainly depend on the amount of proteinic food ingested by larvae. Once it has started, development was shown to be highly sensitive to temperature programs (Fig. 9). In this sense, *Camponotus mus* nurse workers showed a photoperiodic circadian rhythm of temperature preferences to rear cocoons and large (ripe) larvae (developmental stages in which the pupation is already triggered) independent of prey supplied to colony. During prey deprivation, but not during sugar solution deprivation, nurses showed no temperature-related translocations of small larvae and eggs whose further development is arrested. They can continue to develop when preys are again available (Roces and Protomastro 1988), suggesting a colony strategy adapted to the fluctuating condition of temperature and food availability in a temperate habitat.

On both food supply programs, brood translocations did not occur when brood (and nurse workers) experienced a daily thermal fluctuation as they receive due to the translocations along the stable gradient. It is proposed the hypothesis that in their habitat, *Camponotus mus* colonies will select to locate their nests places which undergo a daily thermal fluctuation as nurse workers actively seek along the stable gradient in the laboratory. On account that brood translocations were recorded at day times when ambient temperature nears its maximal and minimal values, brood temperature would be under control if nurse workers pay attention to temperature only at these two critical times, "expecting" that after the photophase-translocation, ambient temperature will decrease, and after the scotophase-translocation, it will not decrease much more. If daily thermal fluctuations at the selected place do not fit the program expected by nurses, brood translocations will occur, as have been observed when the stable thermal gradient was established along the nest. If fit, translocations will be not neccesary, a finding obtained during the simulation experiment. A similar interpretation of a photoperiodic program matching rhythmical ambiental parameters was proposed by Núñez (1977) for honeybee flight activity. Therefore, honeybees and ants seem to use highly predictive ambiental factors, i.e. daily temperature fluctuations dependent on day/night cycles, to program social activities as water supply to the hive and temperature control for ant brood rearing.

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