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Ecological Characteristics of Oviposition and Eggs in the Antlions Living in Seaside Dunes: Tolerance to High Temperature (Neuroptera: Myrmeleontidae)

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Abstract. Antlions living in seaside dunes lay their eggs at night during mid-summer below the sand-surface, where the temperature becomes extremely high in the daytime. We first observed the oviposition behavior and examined the number of eggs laid per night by *Myrmeleon bore* and *Distoleon contubernalis.* The larva of the former species is a pit builder but the latter is not. Most female antlions of both species came to the oviposition site from 8 : 00 p.m. to 9 : 00 p.m. They oviposited eggs one by one at the depth of *ca.* 7.5 mm (*M. bore*) or 10 mm (*D. contubernalis*) in the sand. The average number of eggs laid per night per female was 16.5 for *M. bore* and 17.3 for *D. contubernalis.* Secondly, we examined their hatching rates under the conditions of constant or changing temperature in order to know how their eggs are tolerant to high temperature. Under a constant temperature of 37.5℃, most eggs of *D. contubernalis* hatched successfully, while no eggs of *M. bore* hatched. At the temperature of 40℃ none of the eggs of either species hatched. Under quasi-natural conditions, the hatching rate became lower as the total duration of exposure to a temperature above 40℃ became longer. These results suggest that the female antlion selects a thermally suitable oviposition site by using an environmental indicator.

Key words: Antlion, oviposition, high temperature, dune, *Myrmeleon bore, Distoleon contubernalis.*

Introduction

The larva of antlion is an ambush predator. Most species of antlion larvae live in an open sandy area. Sand-surface temperature in the field often becomes extremely high (Marsh, 1987; van Zyl *et al.*, 1996). Therefore, it has been reported that some species of antlion larvae show a behavioral adaptation to avoid being exposed to the extremely high temperature. That is, they sometimes change their sit-and-wait sites to a shady area within a pit (Green, 1955) or show vertical migration during the day (Matsura, 1996; van Zyl *et al.*, 1996).

Because their eggs cannot react behaviorally to a high temperature, oviposition site selection by female adults is critical for the survival of the eggs. Adult antlions are active only at night and the temperature on the sand-surface is almost uniform everywhere. It appears that they cannot predict which sites are suitable thermally for eggs when they oviposit at night. Do their eggs have tolerance to extremely high temperatures? Or, are their eggs not resistant to high temperatures and female antlions select thermally suitable oviposition sites? To answer these questions, we need first to know when (season and time of day) and where they oviposit. Moreover it is necessary to know how the eggs of antlions tolerate a high temperature.

In the seaside dunes of northern Kyoto Prefecture, Japan, four species of antlion larvae, *Myrmeleon bore, Distoleon contubernalis, Heoclisis japonica* and *Glenuloides japonicus* coexist. The former two species are found in the highest density among them, and the larva of *M. bore* makes a pit but the larva of *D. contubernalis* is not a pitbuilder. Little information about the ecology of adult antlions, especially their oviposition, has been obtained (New, 1986), although numbers of ecological studies on their larvae have been carried out.

In the present study, we first examined the oviposition behavior and number of eggs oviposited per night by these two species. Then we investigated their survival rates both under various constant temperature conditions and quasi-natural conditions in order to know how their eggs are tolerant to a high temperature.

Materials and Methods

Field survey

The research site was a sandy cultivated field (*ca.* 50 m × 80 m) close to the beach at Tango-Kitsu (135.56°E 35.9°N), Kyoto Prefecture. This field was surrounded by a small forest mainly consisting of pines *Pinus thunbergii* and locusts *Robinia pseudoacacia* on two sides. The remaining two sides faced grassy plots. Pits by *M. bore* were found at a high density in peripheral areas of this field during the period from spring to late autumn excluding summer.

Adult antlions coming to this field were caught with a sweeping net by three persons at night (7 : 00 p.m. to 11 : 00 p.m.). We walked mainly along the peripheries of the field, throwing light on or over the sand-surface with an electric torch. Since both species of adults of *M. bore* and *D. contubernalis* fly slowly, it was not so difficult to find and catch them. These collections were carried out for 5 nights from 27 July to 31 July in 1995 and for 3 nights of 2 August to 4 August in 1998.

Collected antlions were kept separately in a small paper bag and brought to the field station of our university close to the research site. To determine the number of eggs laid by each antlion, we prepared cylindrical containers (13.5 cm in diameter, 12.5 cm in depth), on which bottom we put a sheet of filter paper and scattered fine sand grains (particle size, 0.25 to 0.5 mm) to a uniform depth of 5 mm on it. The upper side of the container was covered with a mesh cloth not only to facilitate air circulation but also to offer a perch for the antlion. A preliminary experiment showed that both adults of *M. bore* and *D. contubernalis* lay eggs in the sand at a depth of at least 5 mm under the sand-surface. Therefore, when fine sand was removed from the container the next morning where an adult female had been enclosed, we could find its eggs on the filter paper. Since an antlion's egg is covered with sand grains immediately after oviposition, it was probably very difficult to find its eggs in the sand unless such a method was used.

We intended to determine how deep in the sand the antlions lay their eggs by changing the depth of sand scattered on filter paper in a container. If they lay eggs at a shallower location than the depth of the sand scattered on the filter paper, the eggs would be found not on the filter paper but in the sand. We set up three grades of sand depths (7 mm, 10 mm and 15 mm) for *M. bore* and two grades (10 mm and 15 mm) for *D. contubernalis,* in addition to the 5 mm-deep sand layer described above. The number of replications for *M. bore* and *D. contubernalis* was 6 and 4, respectively.

To examine how their eggs in the habitat are exposed to a high temperature, a digital thermistor thermo-recorder (T and D Inc., TR-71) was set up for 3 days from 27 June to 29 June 1995 in the field where the adult antlions had been collected. The recording interval of time was 0.5 hour. Since this thermo-recorder had two channels, we measured two locations (shady area and open area) at the same time. The shady area was under canopy of pine trees. Each sensor of the thermistor was buried at a depth of 10 mm under the sand-surface.

Duration of egg stage under constant temperature conditions

Both species of eggs oviposited on the same day were kept under the conditions of various constant temperatures (±1℃) with a photoperiod of DD; 25℃, 30℃, 35℃, 37.5℃ and 40℃. Two to seven eggs oviposited by 9 *D. contubernalis* and 4 *M. bore* adults were allotted to each temperature condition in order to reduce the influence by their individual variations. The total number of eggs used per temperature was 15 to 18 for *M. bore* and 45 to 48 for *D. contubernalis.* Many deformed larvae were found among emerged hatchlings. We used not only normal hatchlings but also these deformed ones when calculating the incubation periods.

Hatching rate under conditions of changing temperature

We examined the hatching rates of eggs under quasi-natural conditions. Each egg of *M. bore* or *D. contubernalis* was put on the base of a small plastic tube (25 mmϕ × 40 mm) and sand was poured on it to a depth of 5 or 10 mm. One hundred and twenty tubes were prepared in total for *M. bore* and 90 tubes for *D. contubernalis.*

We prepared 3 containers (40 cm × 60 cm × 15 cm) filled with sand to the depth of 10 cm. Forty tubes containing an egg of *M. bore* and 30 tubes for *D. contubernalis* were stood on the surface of sand to adjust the surface of sand within a tube to the same level as those in the containers. Each half of three tubes contained sand to the depth of 10 or 5 mm. These 3 containers were put 1 meter apart from each other on the rooftop of a building, over which there was no cover, in the university campus. To manipulate the thermal environmental conditions, we covered the container with a wooden board (10 mm in thickness) over one container, a transparent plastic board (5 mm in thickness) over another one and none for the remaining container. These are referred hereafter as Shadow, TB and Control groups, respectively.

The sand temperature was measured by a thermistor thermo-recorder (TR-71) whose sensor had been set up under similar conditions to the plastic tube containing an egg.

Results

Oviposition behavior and number of eggs oviposited

Both *M. bore* and *D. contubernalis* came to the sandy field to oviposit after sunset. Fifty-one and 84 adults for *M. bore* and for *D. contubernalis* were collected, respectively in the 2 years of 1995 and 1998, all of them being females except for 5 males in *M. bore*. Female antlions appeared at oviposition sites most often during the period from 8 : 00 p.m. to 9 : 00 p.m. (Fig. 1); 67.4% (31/46) of female *M. bore* and 78.6% (66/84) of female *D. contubernalis* were collected during this period. During the oviposition they inserted their abdomen tip into the sand, bending it downward. At the same time they were touching the sand-surface with their legs and fluttering their wings.

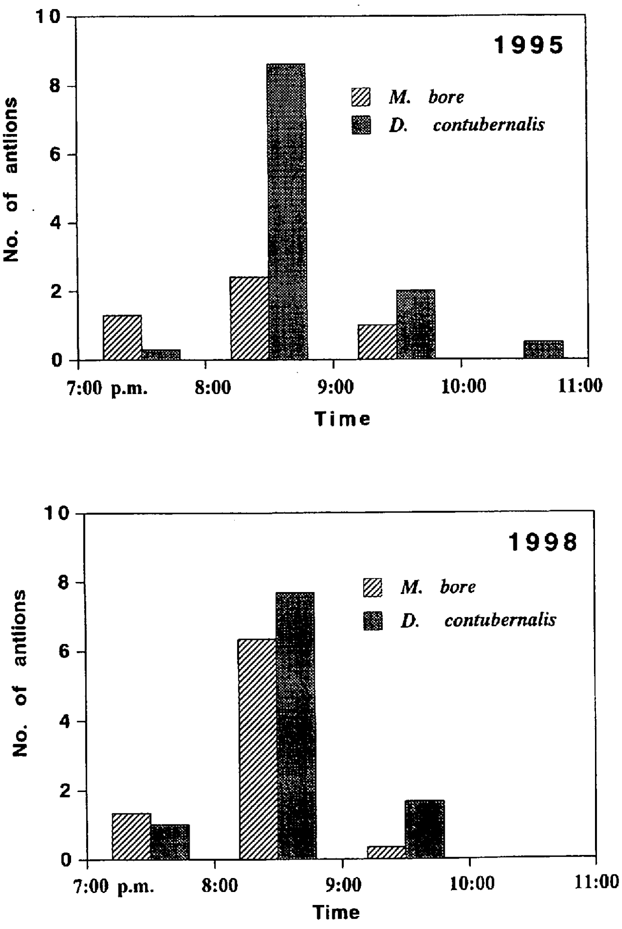

Fig. 1. Average numbers of adult antlions collected per hour from 7 : 00 p.m. to 11 : 00p.m. in mid-summer of 1995 (upper) and 1998 (lower).

Table 1. Number of eggs and oviposition rates for the two species of antlions.

	M. bore	*D. contubernalis*
No. of females used (a)	45	81
No. of females oviposited (b)	25	49
Oviposition rate (% a/b)	55.6%	60.5%
No. of eggs per female		
Mean±S.E.	16.2±11.4 (N=26*)	17.3±2.7 (N=53*)
Range	1–36	1–35

* Two and 4 females in *M. bore* and *D. contubernalis* respectively, oviposited twice.

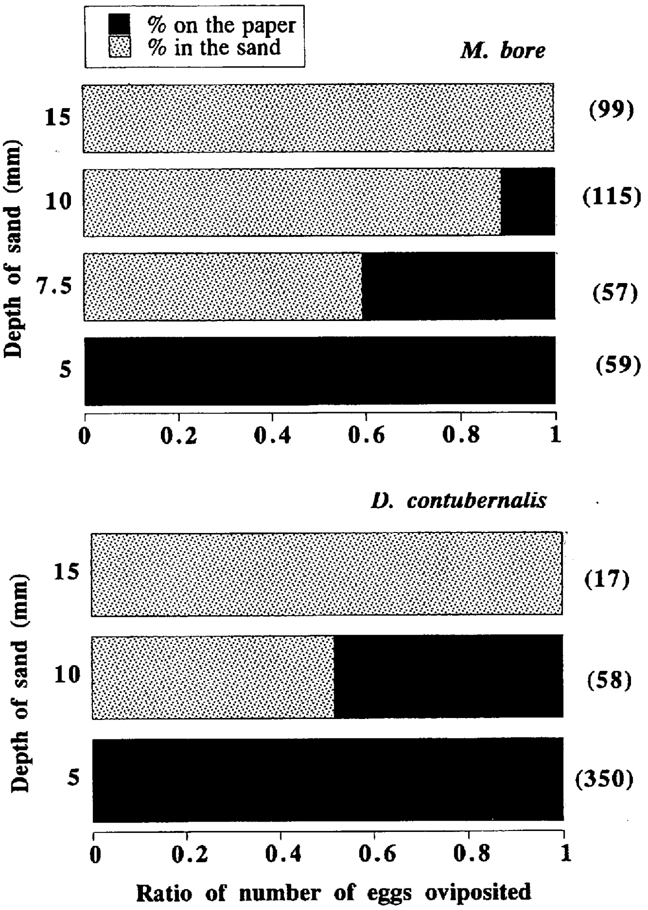

Fig. 2. Results of experiments to examine at which depth of sand antlions lay their eggs. Numbers of eggs found on the paper or in the sand were counted, when sand was supplied at various depths for each species of antlions. Numerals in parentheses show the number of eggs oviposited.

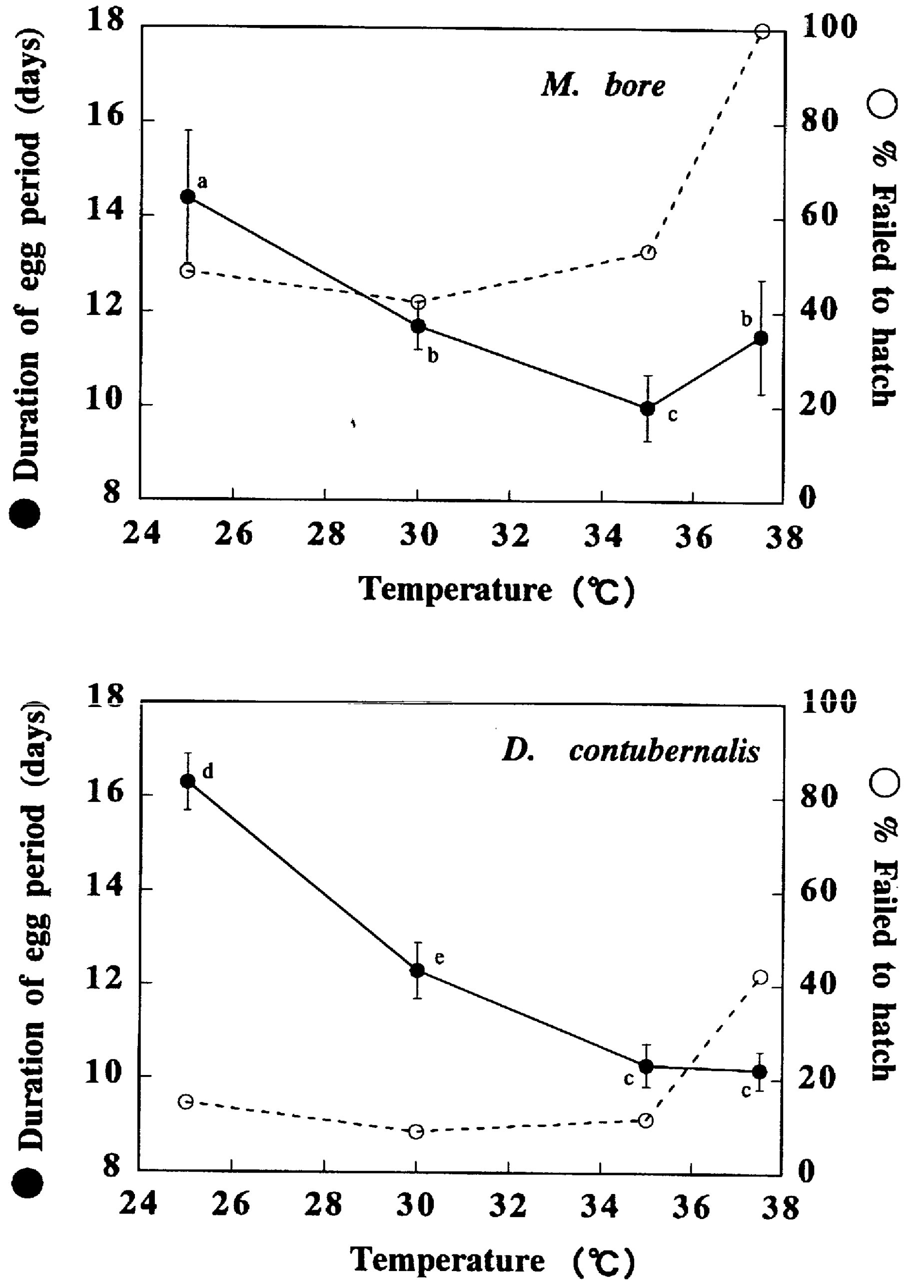

Fig. 3. Durations (days) of egg period and the failed hatching rates (see text for explanation) for *M. bore* and *D. contubernalis* under every constant temperature. Different letters among treatments show a significant difference ($P=0.0001$, Fisher's PLSD test).

Table 2. Analysis of variance testing the effect of antlion species and the temperature on the duration of egg period.

Source	df	MS	*F*	*P*
Temperature	3	142.034	354.314	<0.0001
Species	1	5.876	14.657	0.0002
Species× Temperature	3	12.087	30.153	<0.0001
Error	186	0.401		

Not all antlions collected oviposited in the laboratory, and the number of eggs laid per night varied greatly (Table 1). The maximum and average numbers of eggs oviposited per night were similar in *M. bore* and *D. contubernalis,* although the egg size of the former was slightly smaller than that of the latter (average major axis size × minor axis size, *M. bore*: 1.4 mm × 1.0 mm (N=82); *D. contubernalis*: 1.8 mm × 1.2 mm (N=225)). Most antlions which had finished oviposition ate no food, and they died without additional oviposition before the second morning after

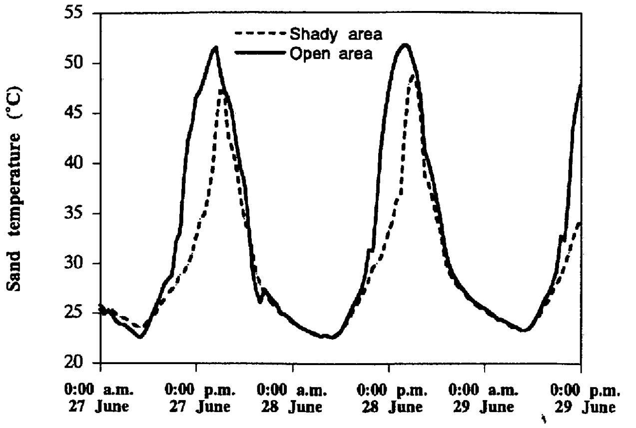

Fig. 4. Daily changes in the sand temperature (10 mm deep below sand-surface) in the field.

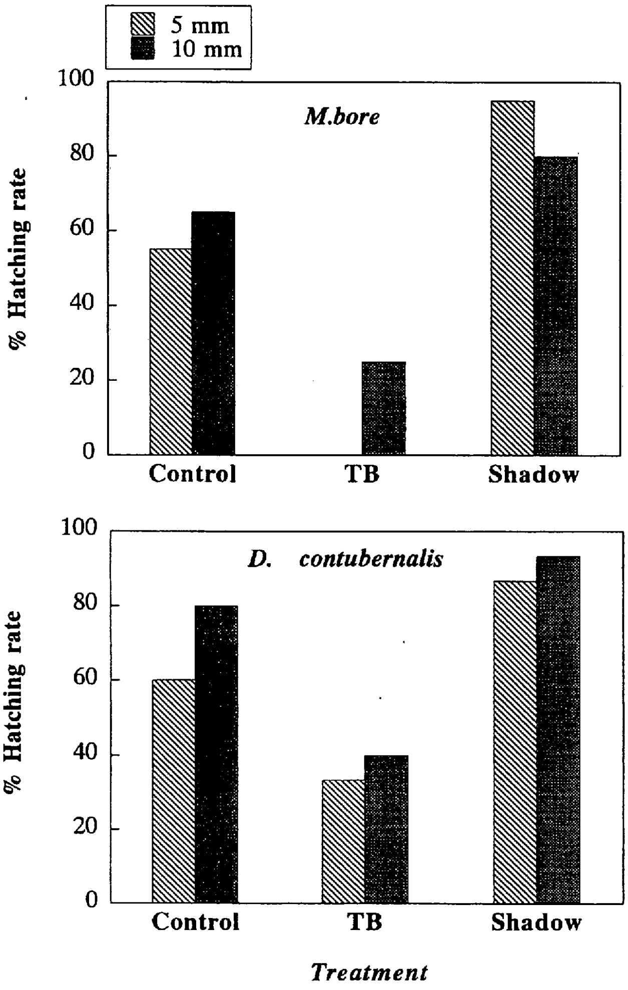

Fig. 5. Comparison of the hatching rates of the eggs (*M. bore* and *D. contubernalis*) among thermal environments. Each egg was kept in the sand at the depth of 5 mm or 10 mm. A sheet of transparent plastic board ("TB") or wooden board ("Shadow") was put over each container in which eggs were buried.

collection except for a few individuals. There were fully matured and/or unmatured eggs within ovaries in 61.3 percent of antlions out of the dissected ones (N=93; including both species).

Each egg of the two species was distributed separately on the filter paper. Thus, we could speculate that they oviposited their eggs one by one in the field, too. The surface of the eggs was sparsely covered with sand grains. When a sand layer on the filter paper was thicker (=15 mm), all the eggs were found among the sand (Fig. 2). On the other hand, all of them were found on the paper when the depth was 5 mm. These antlions probably insert their ovipositors to a depth where nearly half of the oviposited eggs are found in the sand or on the filter paper respectively, *i.e.*, about 7.5 mm for *D. contubernalis* and 10 mm for *M. bore*.

Duration of egg stage under constant temperature conditions

Table 2 shows the result of two-way ANOVA to test the effect of temperature and species on the duration of egg stage. The significant two-factor interaction reveals that the effect of temperature on the egg period was different between antlion species. Although between 25℃ and 35℃ the duration of egg stage in both species was shorter at high temperatures, that in *M. bore* at 37.5℃ was significantly prolonged than that at 35℃ (Fig. 3). This developmental delay under high temperature was not found in *D. contubernalis*. None of the eggs of the two species hatched at 40℃.

We defined "failed hatching rate" as the ratio of number of eggs that did not hatch normally, that is, this includes not only the number of eggs that died during the egg stage but also the number of deformed hatchlings. In the case of *M. bore*, the failed hatching rate was generally higher than that of *D. contubernalis*, especially none of the eggs hatched normally at 37.5℃. On the other hand, about 60 percent of eggs in *D. contubernalis* hatched successfully even at 37.5℃.

Hatching rate under conditions of changing temperature

Eggs of antlions in the field would be exposed to extremely high temperatures. Figure 4 shows the records of sand temperature at the point of 10 mm below the sand-surface in their natural habitat. The maximum temperature in the open area and the shady area was 51.8℃ (2 : 00 p.m. 28 June) and 48.6℃ (3 : 00 p.m. 28 June), respectively, whereas the minimum temperature was 22.6℃ and 22.7℃, respectively. A temperature of more than 40℃ damaged the eggs of antlions as described above. The total period that the sand temperature was more than 40℃ during the 60

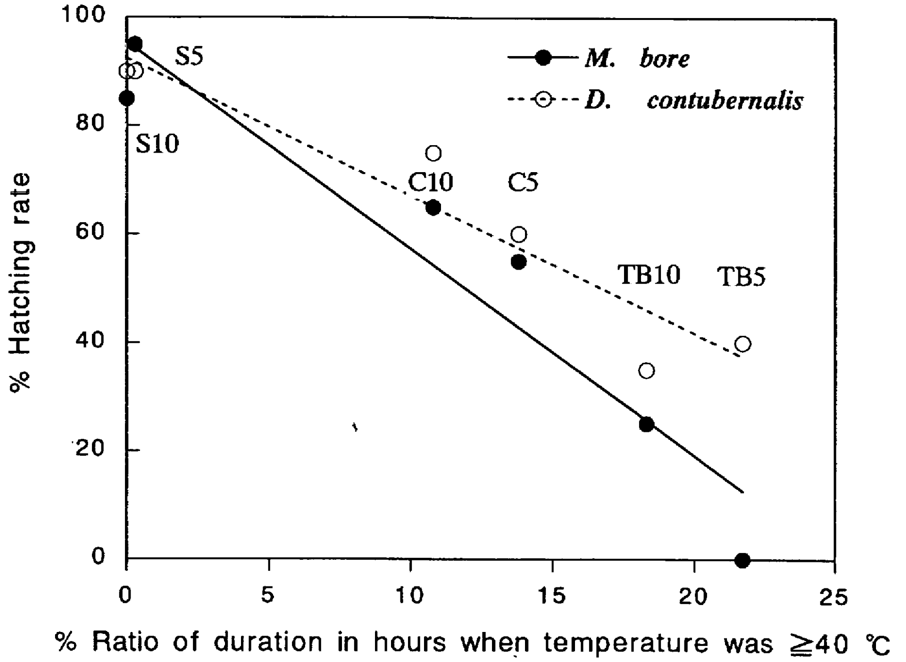

Fig. 6. Relationships of the hatching rates in *M. bore* and *D. contubernalis* and total hours that their eggs were exposed to the thermal conditions of more than 40℃. "S" and "C" denote "Shadow" and "Control", respectively (see Fig. 5). Numerals attached to the code (5 or 10) show the depth (mm) of sand where each egg was kept. *M. bore*: $y=95.449-3.817x$ ($r=-0.958$, $F=44.768$, $P=0.0026$), *D. contubernalis*: $y=92.506-2.543x$ ($r=-0.957$, $F=43.801$, $P=0.0027$).

hours of measurement was 4.5 hours (7.5%) for the shady area and 13.5 hours (22.5%) for the open area.

In the experiment on the hatching rate carried at the rooftop of a building, the ratio was in the order of Shadow > Control > TB in both species (Fig. 5). Furthermore, the hatching rate was higher in eggs left at a depth of 10 mm in the sand than at 5 mm except in the Shadow group in *M. bore*. These treatments significantly affected the hatching rate of both species of antlions (χ^2-test of independence; *M. bore* (5 mm), $P<0.0001$; *M. bore* (10 mm), $P=0.0014$; *D. contubernalis* (5 mm), $P=0.0117$; *D. contubernalis* (10 mm), $P=0.0036$). These significant differences were produced by the very low hatching rate in TB, and there was no significant difference between the Shadow and Control groups except for the case of a 5 mm depth in *M. bore* (Fisher's exact probability test, $P=0.0084$).

These different treatments inevitably exposed the eggs to different thermal conditions. We analyzed how many hours the eggs had been exposed in total to ≥40℃. Figure 6 shows the relationship between the hatching rate and the ratio of duration for which eggs were exposed to ≥40℃. The hatching rate of both species decreased as the duration of exposure to ≥40℃ became longer. The maximum sand temperature during the incubation period also gave significant negative regression coefficients on the hatching rate (*M. bore*: $r=-0.777$, $F=13.928$, $P=0.0203$; *D. contubernalis*: $r=-0.919$, $F=21.774$, $P=0.0095$).

Discussion

The life history of *M. bore* has been investigated in detail (e.g. Matsura *et al.*, 1991), while little is known about *D. contubernalis* because their larvae are not pit builders and it makes them difficult to observe. *M. bore* is a univoltine species, and their adults emerge and oviposit in summer. In the sandy field, where we collected the adult antlions, numbers of small pits made by the first instar larvae were found along the periphery facing a forest from late August to September (Personal observation). Few pits existed in the central area of the sandy cultivated field, though no crops were cultivated and the whole area became an open sandy field in summer. Did adult antlions avoid laying eggs in the central area because the sand temperature became too high in the daytime (cf. Fig. 4)? In fact, we have never observed females ovipositing in the central area, while we often found them ovipositing in the peripheral area. The results of the experiment under the conditions of changing temperature revealed that the hatching rate of the eggs in both species decreased steeply as their period of exposure to a high temperature had become longer (Fig. 6). It would be unreasonable to consider that they lay eggs wastefully at a thermally unsuitable site for them, because a female's choice of oviposition site is one of the most important factors affecting her fitness (Feder *et al.*, 1997). Thus, we should consider that antlions avoid ovipositing at a site where the sand temperature becomes too high in the daytime.

Although there was a great difference in sand temperature during the daytime between shady area and sunny area, no difference between them was detected at night. Yasserri & Parzefall (1996) showed that the antlions, *Euroleon nostras* occurring at the beach in northern Germany, preferred artificially heated sand (25℃) to uncontrolled sand as an oviposition site. This suggests that the antlions can perceive a difference of the sand temperature. They did not, however, describe the temperature difference between the heated sand and the uncontrolled sand, nor did they evaluate the hatching rate directly (they only counted the number of pits by the first instar larvae). Our preliminary data measured at the same research site as the present study showed that the average sand temperature at around 8 : 00 p.m., when many females came to the oviposition site, slightly differed between shady and open areas on clear days. All the points measured within an open area, however, were not higher than those within a shady area. Besides, there was no significant difference in the sand temperature on cloudy days. We cannot deny completely the possibility that female antlions can distinguish slight differences in the sand temperature among places, but we consider the sand temperature is not a reliable cue for their oviposition site selection.

How do they select a suitable oviposition site? The difference in water contents of sand is one of possible indicators of their environmental assessment. On a summer night, the sand surface of the seaside dune becomes wet gradually after sunset (Matsura, 1996). Whereas the surface of sand covered by the shelter such as twigs and leaves is relatively drier than that in an open area due to smaller amount of night dew. Although this difference in water content of sand decreases with the elapse of time, there would be a clear difference between shaded area and open area yet during 8 : 00 p.m.–9 : 00 p.m. when most females came to oviposit.

The hatching rate in *D. contubernalis* under constant high temperature conditions was much higher than that in *M. bore* (Fig. 3). Also, eggs of *D. contubernalis* were oviposited deeper below the sand sur face than eggs of *M. bore*. Since temperature becomes lower in general as the depth of sand becomes deeper, *D. contubernalis* might be able to oviposit even at a site where the sand temperature in the daytime becomes higher, compared with *M. bore*. That is, it appears that the range of the thermally suitable area for oviposition is wider in *D. contubernalis* than in *M. bore*. Larvae of *D. contubernalis* are waiting for prey, opening their mandibles beneath the sand-surface. Therefore, both eggs and larvae of *D. contubernalis* must be more tolerant to a high temperature than those of *M. bore*.

In order to clarify the ecology of these antlion larvae and adults occurring at the unique habitat, a seaside dune, it would be necessary to examine further the physical aspects of sand which they use ingeniously.

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