Bimodal life cycle of the burying beetle *Nicrophorus quadripunctatus* in relation to its summer reproductive diapause

TOMOYOSI NISIMURA[1], MASAHIRO KON[2] and HIDEHARU NUMATA[1] [1]Department of Bio- and Geosciences, Graduate School of Science, Osaka City University and [2]School of Environmental Science, University of Shiga Prefecture, Japan

Abstract. 1. Under natural conditions in Kyoto, Japan, the reproductive activities of *Nicrophorus quadripunctatus* Kraatz (Coleoptera: Silphidae) decreased in summer and the species showed a bimodal life cycle.

2. In the laboratory, most adult pairs raised at 20 °C under a LD 12:12 h regime reproduced when provided with a piece of chicken. In adults raised at 20 °C under a LD 16:8 h regime, however, both reproductive behaviour and ovarian development were reduced. It is concluded that these adults entered a reproductive summer diapause.

3. High temperature (25 °C) also suppressed the reproductive behaviour even under a favourable LD 12:12 h regime. In the field, therefore, adults reduce their reproductive activity in summer because of diapause induced by long-day photoperiods and direct inhibition of reproduction by high temperatures.

4. When the temperature was changed from 20 °C to 25 °C immediately after hatching of larvae, they reached the wandering stage in 95% of adult pairs. When the temperature was changed from 20 °C to 25 °C immediately after oviposition, however, no larvae hatched in 85% of pairs. Egg mortality was significantly higher at 25 °C than at 20 and 22.5 °C; no eggs hatched at 27.5 °C. The physiological mechanisms for reducing reproduction probably prevent the beetles from inefficient oviposition in summer.

Key words. Bimodal life cycle, burying beetle, *Nicrophorus quadripunctatus*, photoperiod, reproductive behaviour, summer diapause.

Introduction

Burying beetles *Nicrophorus* spp. (Coleoptera: Silphidae) use small vertebrate carcasses for reproduction. A carcass is often discovered by several beetles but is eventually monopolised and buried by a single male/female pair. Within a few days of the discovery of a carcass, the female lays eggs in the surrounding soil. The pair remains with the larvae and provides extensive parental care, such as regurgitating predigested carcass to the young and defending against competitors and predators until the larvae cease feeding (Pukowski, 1933; Wilson & Fudge, 1984; Scott, 1998). It is thought that this parental care evolved because resources such as carcasses are valuable and unpredictable and must be defended from a diverse group of organisms (Tallamy & Wood, 1986; Scott, 1998). The seasonal pattern of reproductive activities in burying beetles has been explained by competition with congeners for the resource (Wilson *et al.*, 1984; Trumbo, 1990a; Scott, 1998). For example, *N. defodiens* Mannerheim and *N. orbicollis* Say co-exist in the U.S.A., and both the seasonal pattern of reproductive activity and the habitat of these two species overlap. In Michigan, *N. defodiens* has a bimodal seasonal pattern of reproductive activities, shown with baited traps (Wilson *et al.*, 1984). Wilson *et al.* suggested that this bimodal pattern is because

Correspondence: Hideharu Numata, Department of Bio- and Geosciences, Graduate School of Science, Osaka City University, Osaka 558-8585, Japan. E-mail: numata@sci.osaka-cu.ac.jp

N. defodiens, the smaller species, avoids competition with *N. orbicollis*.

Many insects pass a seasonally adverse period in a physiologically controlled state of arrested development, i.e. diapause. Photoperiod and temperature have been shown to be the primary factors controlling diapause in many species (Danks, 1987). Reproductive diapause has been shown in some burying beetles (Anderson, 1982; Wilson *et al.*, 1984; Müller & Eggert, 1987; Trumbo, 1990a), although the environmental factors that control diapause have not been examined. It is, therefore, difficult to determine whether the seasonal pattern of reproductive activities in burying beetles results from competition with other species or adaptation to abiotic environmental conditions.

In the work reported here, the seasonal reproductive activities of *N. quadripunctatus* Kraatz were monitored using baited traps in Kyoto, Japan, the effects of temperature and photoperiod on the reproduction of this species were examined in laboratory experiments, and the factors determining the seasonal pattern of *N. quadripunctatus* were discussed.

Materials and methods

Collection of insects in the field

Adults of *N. quadripunctatus* were trapped by plastic cup traps (12.2 cm diameter, 11.1 cm deep) baited with a piece of chicken (40 g), which were installed in the site 1.5 m above the ground on the trunks of trees. Four traps were installed ≤50 m apart in a deciduous forest in Kyoto City (35°01′N, 135°44′E). Every 7 days, all trapped insects were removed and the traps were restocked with new bait from May to October 1991 and 1992. In May and September 1995–1997, the traps were checked and trapped insects were collected twice a week. Adults trapped in 1995–1997 and their progeny were used for the laboratory experiments.

Observation of reproduction

The reproductive behaviour of adult pairs and development of the progeny larvae were observed in plastic cups (12.2 cm diameter, 11.1 cm deep) under a short-day (LD 12:12 h) or a long-day (LD 16:8 h) photoperiod at $20 \pm 1\,°C$ or $25 \pm 1\,°C$. First, the plastic cup was filled with soil to ≈5 cm depth and a hole (≈5 cm diameter, ≈5 cm deep) was made on the soil surface. A piece of chicken (≈25 g) was used as larval food. A male/female pair was placed in the plastic cup with a piece of chicken.

The temporal order in a typical example of reproduction at 20 °C is shown in Fig. 1. After setting in a plastic pot (day 0), the parent beetles carried the chicken into the hole and secreted brown fluid from their mouth and anus onto the surface of the chicken. On day 3, the preparation of food for the progeny, as above, was completed. On day 4, the female laid eggs in soil around the chicken. On day 6, larvae hatched, moved to the chicken, and the parents began to help their progeny to feed. On day 12, third-instar larvae ceased feeding and left the chicken to pupate. These larvae were transferred to a plastic pot (15.5 cm diameter, 8.5 cm deep) filled with soil to ≈5 cm depth for pupation. They pupated in cells in the soil, and pupal–adult ecdysis occurred on day 20. New adults emerged on the soil on day 37. The following four events were recorded every day: preparation, completion of preparation of food by the adult pair for the progeny; oviposition, finding the first egg from outside the plastic cup; hatching, finding the first larva on the chicken; wandering, cessation of feeding, and disappearance of the larvae from the chicken. If hatching was not observed for 13 days after setting, the experiment was terminated. The statistical difference between two proportions was examined by Fisher's exact probability test unless stated otherwise.

Rearing of larvae and adults

Larvae were reared as indicated above under long- or short-day conditions at 20 °C. Adults were kept as single
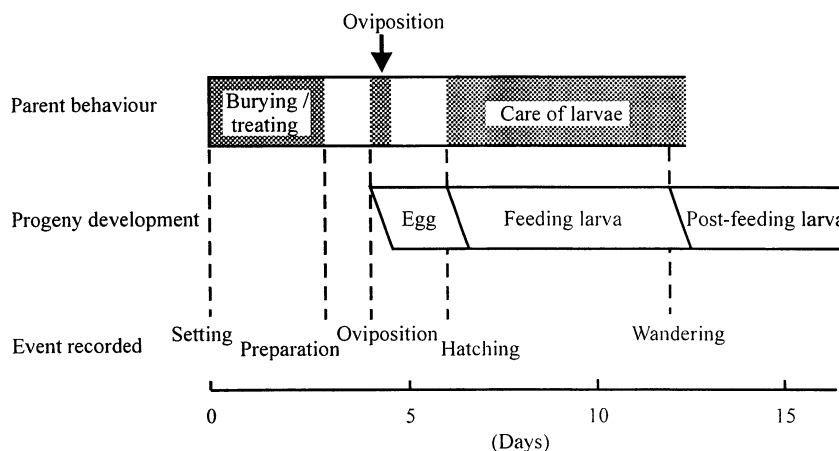

Fig. 1. Temporal order in a typical example of reproduction at 20 °C in *Nicrophorus quadripunctatus*. On setting, a male/female pair was provided with a piece of chicken. See text for further explanation.

male/female pairs in plastic cups (9.7 cm diameter, 4.0 cm deep) with a little soil on the bottom, and five third-instar larvae of the blow fly *Protophormia terraenovae* (Robineau-Desvoidy) were provided as food every 3 days.

Ovarian stage

After measuring the body weight of female adults, their ovaries were dissected in 0.9% NaCl solution. Each ovary consisted of 12 ovarioles, the development of which was not synchronous. The length *L* and width *W* of the largest oocyte in each ovary were measured using a stereoscopic microscope. From these values, the volume *V* was calculated assuming the oocyte to be an ellipsoid: $V = \pi LW^2/6$. The larger volume of the two oocytes was adopted as the volume for each individual. It was also recorded whether or not yolk had accumulated in the oocytes.

Egg mortality

Eggs laid by adults reared continuously under short-day conditions at 20 °C were dug out carefully within 24 h of oviposition and transferred onto wet cotton wool in Petri dishes (6.0 cm diameter, 1.5 cm deep). They were kept at 20, 22.5, 25, or 27.5 °C under short-day conditions, and the larvae hatching were counted every day for 3 days. To exclude the effect of individual variation, eggs laid by each female were divided equally among the temperatures used.

Results

Phenology

The seasonal occurrence of *N. quadripunctatus* is shown in Fig. 2. Clear bimodal patterns were observed for both 1991 and 1992. The numbers of trapped *N. quadripunctatus* were reduced from June to August and the second peak was observed 4 months after the first peak.

Effects of photoperiod and temperature on reproduction of field-collected adults

Reproductive behaviour in adult pairs collected in the field in May 1995 was observed under long- or short-day conditions at 20 °C and under long-day conditions at 25 °C, and in pairs collected in September 1995 under long- and short-day conditions at 20 °C and under short-day conditions at 25 °C. At 20 °C, all adult pairs used the chicken as a reproductive resource and their progeny became wandering larvae after consuming it regardless of photoperiod (Fig. 3a,b). At 25 °C, however, the proportion of oviposition was significantly lower than that under the same photoperiod at 20 °C (*P* < 0.001 and *P* < 0.01 under long- and short-day conditions respectively). Under long-day conditions at 25 °C, no females laid eggs (Fig. 3a). Under short-day conditions at 25 °C, three out of 10 females laid eggs
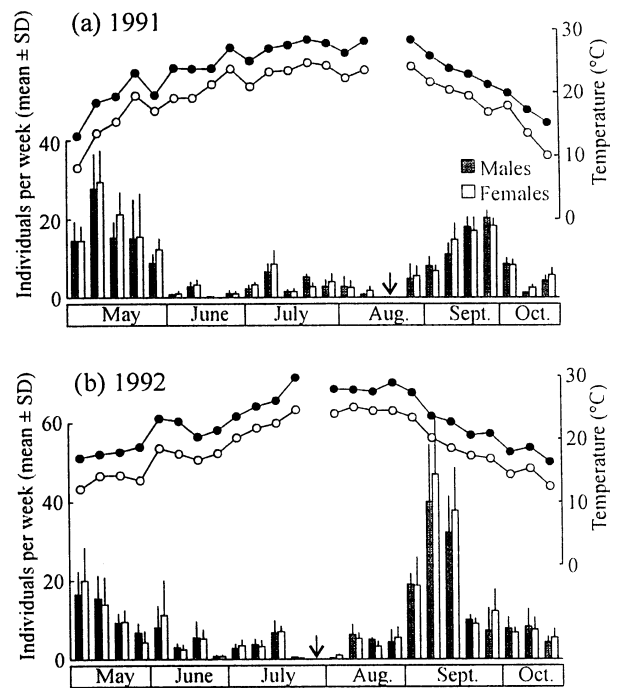

Fig. 2. Seasonal changes in average numbers of *Nicrophorus quadripunctatus* adults captured by four traps, and air temperatures in (a) 1991 and (b) 1992 in Kyoto, Japan. ● weekly average temperatures of the average of the daily high and low, ○ weekly average of daily lowest temperatures, ↓ no data.

but no larvae hatched (Fig. 3b). After 13 days at 25 °C, both male and female adults were alive in all pairs under long-day conditions and seven out of 10 pairs under short-day conditions, so the failure of reproduction at 25 °C was not due to the death of parents. The living pairs were then transferred from 25 °C to 20 °C without changing the photoperiod. After 3 days at 20 °C, they were provided with a new piece of chicken and the reproductive behaviour was observed again at 20 °C. Under long-day conditions, eight out of 10 pairs completed preparation and seven pairs succeeded in reproducing (Fig. 3a). Under short-day conditions, all pairs completed preparation and five out of seven pairs succeeded in reproducing (Fig. 3b).

Effects of photoperiod and temperature on the reproduction of adults reared in the laboratory

Insects were reared under long- or short-day conditions at 20 °C from eggs, and reproductive behaviour was observed in adult pairs 12 days after emergence at 20 or 25 °C without changing the photoperiods. Most pairs did not complete preparation under long-day conditions at 25 °C whereas some pairs completed preparation and laid eggs under short-day conditions at 25 °C (Fig. 4). The difference in the proportion of preparation between the two photoperiods was significant (*P* < 0.01), however no larvae hatched under either photoperiod at 25 °C.
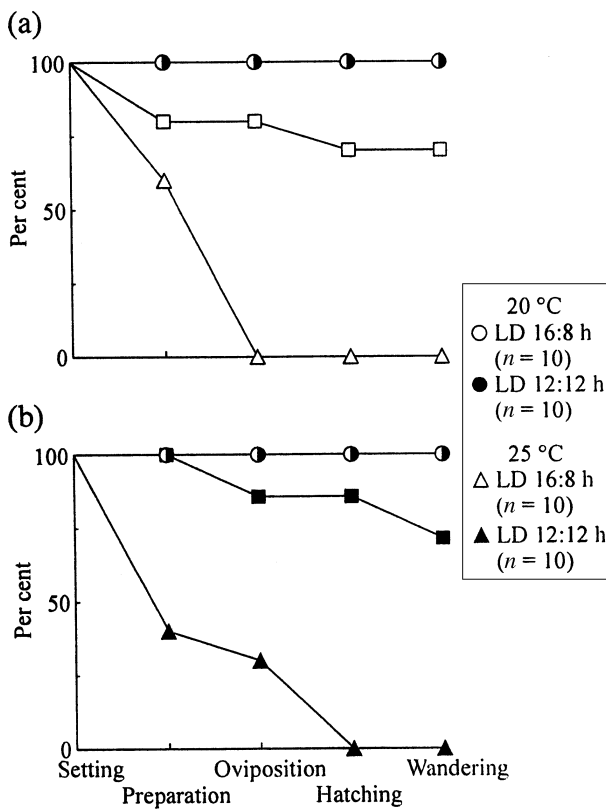
(a)

(b)

Fig. 3. The percentage of pairs in which each stage of reproduction was observed, when adult pairs of *Nicrophorus quadripunctatus* were collected in the field in (a) May and (b) September 1995. Thirteen days after starting the first experiment at 25 °C (△, ▲), the pairs were transferred to 20 °C without changing the photoperiod, and 3 days after the transfer they were given a new reproductive opportunity at 20 °C (□, LD 16:8 h, $n = 10$; ■ LD 12:12 h, $n = 7$). See Fig. 1 for stages in the sequence of reproduction.

Under long-day conditions at 20 °C, ≈70% of adult pairs that completed preparation laid eggs, and the progeny in ≈90% of pairs laying eggs became wandering larvae. Under short-day conditions at 20 °C, all adult pairs completed preparation and laid eggs, and the progeny of ≈90% of pairs became wandering larvae (Fig. 4). The difference in the proportion of preparation between the two photoperiodic conditions was significant ($P < 0.001$).

Reproductive behaviour was then observed in adult pairs 24 days after emergence at 20 °C. About 95% of pairs completed preparation under long- or short-day conditions, and wandering of progeny larvae was observed in 90 and 70% of pairs under long- and short-day conditions respectively ($n = 20$ or 21). There was no significant difference in the proportion of wandering to setting between the photoperiodic conditions ($P > 0.05$).

High temperatures, therefore, suppressed reproductive behaviour both in the field-collected adults and in adults raised in the laboratory, although rearing under long-day conditions also delayed reproductive maturity. If adults show reproductive behaviour only in a certain period in a
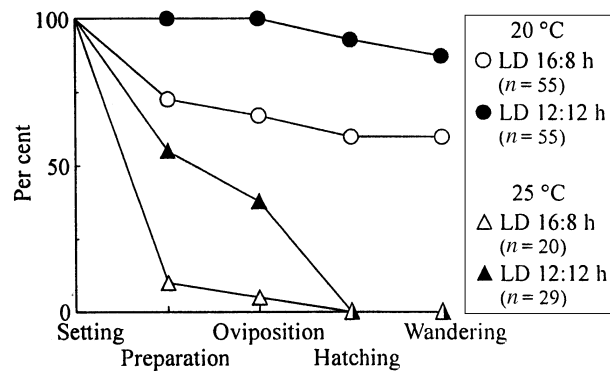

Fig. 4. The percentage of pairs in which each stage of reproduction was observed under constant photoperiod and temperature, when adult pairs of *Nicrophorus quadripunctatus* were raised at 20 °C. See Fig. 1 for stages in the sequence of reproduction.

day, only high temperatures during the active period could affect their behaviour. It was therefore examined whether high temperature during photophase or scotophase would inhibit reproductive behaviour. Reproductive behaviour was observed in adult pairs 12 days after emergence under short-day and thermoperiodic conditions. A square-wave type thermoperiod was used, with temperature alternating between 20 °C and 25 °C every 12 h. As a control, reproductive behaviour at a constant 22.5 °C was also observed. Larvae reached the wandering stage in most pairs at 22.5 °C. Under thermoperiodic conditions in which high temperature coincided with dark, 95% of pairs completed preparation and laid eggs (Fig. 5). The proportion of oviposition differed significantly between the two thermoperiodic conditions ($P < 0.05$), however under thermoperiodic conditions progeny larvae hatched in only 5 or 10% of pairs, but they became wandering larvae (Fig. 5). High temperature
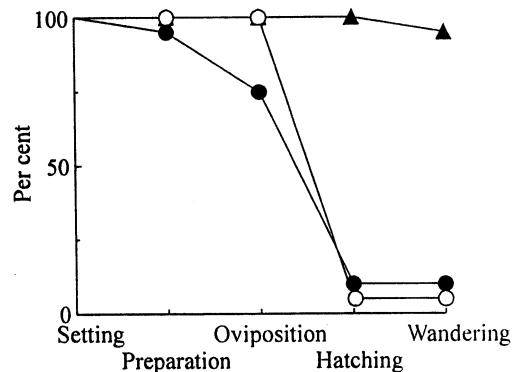

Fig. 5. The percentage of pairs in which each stage of reproduction was observed under thermoperiodic conditions, when adult pairs of *Nicrophorus quadripunctatus* were raised at 20 °C under a LD 12:12 h regime. Temperature was changed between 20 and 25 °C at light-on and light-off. ○ high temperature coinciding with light ($n = 20$), ● high temperature coinciding with dark ($n = 20$), ▲ constant 22.5 °C ($n = 20$). See Fig. 1 for stages in the sequence of reproduction.

during the scotophase suppressed oviposition a little, and high temperature in the photophase or scotophase had a lethal effect on eggs or larvae just after hatching.

The stage during which high temperature inhibits reproduction was then examined. Adult pairs were provided with chicken 12 days after emergence at 20 °C. When the pairs were transferred to 25 °C immediately after oviposition, no larvae hatched in 85% of pairs (Fig. 6). In two out of three pairs in which larvae hatched, the progeny became wandering larvae. When the pairs were transferred to 25 °C immediately after hatching, however, the progeny became wandering larvae in 95% of pairs (Fig. 6). The proportion of hatching was significantly higher in the pairs that were transferred to 25 °C immediately after hatching ($P < 0.001$). It is, therefore, probable that high temperature had a lethal effect on eggs.

Effects on reproduction of high temperature in the adult stage

In all the above experiments, adults were reared at 20 °C until reproductive behaviour was observed. In subsequent experiments, the effect of high temperature during the pre-reproductive adult stage was examined. Insects were reared from eggs at 20 °C then transferred to 25 °C at adult emergence without changing the photoperiods. Reproductive behaviour was observed 12 days after emergence at 20 °C. The period from setting to preparation was significantly longer than for adults maintained at constant 20 °C (*t*-test, $P < 0.05$), however there was no significant difference in the proportion of preparation and that of oviposition between these adult pairs (Fig. 7) and those reared continuously at 20 °C (Fig. 4) under either photoperiodic condition ($P > 0.05$). In the pairs kept at 25 °C in the adult stage, the proportion of preparation was significantly lower under long-day conditions than under short-day conditions ($P < 0.05$; Fig. 7).
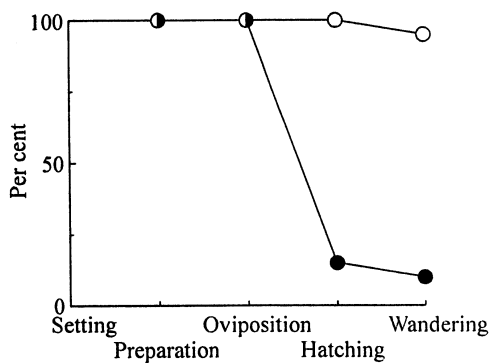

Fig. 6. The percentage of pairs in which each stage of reproduction was observed, when adult pairs of *Nicrophorus quadripunctatus* were raised at 20 °C under a LD 12:12 h regime and transferred to 25 °C during reproduction. ● transfer immediately after oviposition ($n = 20$), ○ transfer immediately after hatching of the progeny larvae ($n = 20$). See Fig. 1 for stages in the sequence of reproduction.
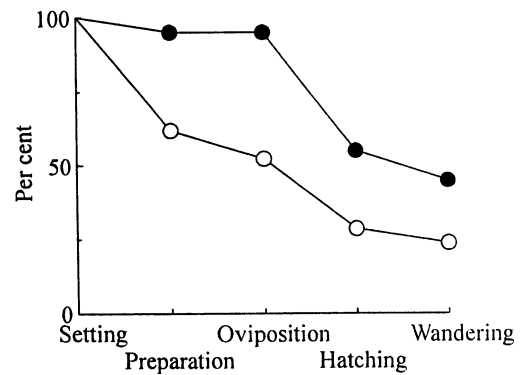

Fig. 7. The percentage of pairs in which each stage of reproduction was observed, when adult pairs of *Nicrophorus quadripunctatus* were raised at 20 °C and exposed to high temperature in the adult stage. Adult pairs raised at 20 °C under LD 16:8 h (○, $n = 21$) or LD 12:12 h (●, $n = 20$) were kept for 12 days at 25 °C from adult emergence, and their reproduction was observed at 20 °C without changing the photoperiods. See Fig. 1 for stages in the sequence of reproduction.

Effects of photoperiod and temperature on ovarian development

In the above experiments, high temperatures and long-day conditions showed an inhibitory effect on reproduction. Whether this inhibition was related to direct suppression of reproductive behaviour or to suppression of ovarian development was then examined. Insects were reared under long-day or short-day conditions at 20 °C from eggs to adult emergence, then some were transferred to 25 °C without changing the photoperiods. Females were dissected 12 days after adult emergence.

The oocytes of females reared under short-day conditions were significantly larger than those of females under long-day conditions at 20 °C (Mann–Whitney *U*-test, $P < 0.001$; Fig. 8a,b). Yolk was deposited in the oocytes of $\approx 94\%$ of females that were reared under short-day conditions but in only $\approx 45\%$ of females reared under long-day conditions ($P < 0.001$; Fig. 8a,b).

In females transferred to 25 °C, the oocytes were also significantly larger in females under short-day conditions than in females under long-day conditions (Mann–Whitney *U*-test, $P < 0.001$; Fig. 8c,d). The proportion of yolk deposition was significantly higher under short days than under long days ($P < 0.01$; Fig. 8c,d). Furthermore, the oocytes of females transferred to 25 °C were significantly smaller than the oocytes of females reared continuously at 20 °C (Mann–Whitney *U*-test, $P < 0.001$ for both photoperiods; Fig. 8c,d, see also Fig. 8a,b), and the proportion of yolk deposition was significantly lower in females transferred to 25 °C than in females reared continuously at 20 °C ($P < 0.01$ and $P < 0.001$ for long- and short-day conditions respectively; Fig. 8c,d, see also Fig. 8a,b). This indicates that ovarian development is reduced by high temperature and long-day conditions.
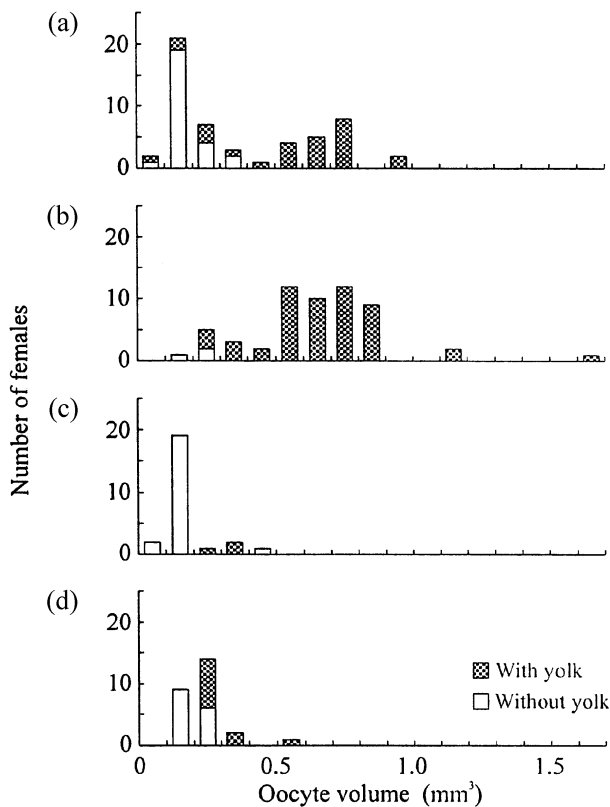
Fig. 8. Effects of photoperiod and temperature on oocyte development of *Nicrophorus quadripunctatus*. Insects were reared continuously under (a) LD 16:8 h or (b) LD 12:12 h conditions at 20 °C, or transferred from 20 °C to 25 °C at adult emergence under (c) LD 16:8 h or (d) LD 12:12 h conditions. The volume of the largest oocytes was measured and the occurrence of yolk deposition was examined 12 days after adult emergence.

The increase in body weight was compared between females reared continuously at 20 °C and those transferred to 25 °C at adult emergence. The rates of increase in body weight were significantly lower in females transferred to

Table 1. Effects of photoperiod and temperature on body weight in *Nicrophorus quadripunctatus* during the first 12 days in the adult stage.

| Conditions† | Number of pairs | Relative body weight, mean ± SD‡ | |
		Male	Female
20 °C, LD 16:8 h	53	1.26 ± 0.10 a	1.25 ± 0.11 a
20 °C, LD 12:12 h	57	1.26 ± 0.13 a	1.28 ± 0.11 a
25 °C, LD 16:8 h	25	1.23 ± 0.12 a	1.24 ± 0.12 a
25 °C, LD 12:12 h	26	1.12 ± 0.09 b	1.13 ± 0.10 b

†Conditions in the adult stage are shown. Insects were reared at 20 °C under LD 16:8 h or LD 12:12 h from eggs. Some were transferred to 25 °C without changing the photoperiods at adult emergence.
‡Given as values relative to those at adult emergence. Means followed by the same letter in each column are not significantly different (Tukey test, $P > 0.05$; Zar, 1999).

25 °C under short-day conditions than in females under the other three conditions, however there was no significant difference among the other three conditions (Table 1). Therefore, adults can gain weight under long-day conditions at 25 °C as at 20 °C, even though ovarian development is markedly reduced at 25 °C.

Effect of temperature on egg mortality

At 20 and 22.5 °C, ≈60% of eggs hatched. At 25 °C, the percentage of hatching was significantly lower than at the two lower temperatures. No eggs hatched at 27.5 °C (Fig. 9). This indicates that high temperatures have a lethal effect on *N. quadripunctatus* eggs.

Discussion

Physiological mechanisms underlying the bimodal life cycle

Ovarian weight in adults of *N. orbicollis* and *N. tomentosus* Weber increased two- to three-fold within 48 h of discovery of a suitable resource for reproduction (Wilson & Knollenberg, 1984; Scott & Traniello, 1987; Trumbo *et al.*, 1995). Although adult diapause has usually been defined by reduced ovarian development in females (Danks, 1987), reproductively active adults of *Nicrophorus* may have immature ovaries before discovery of a resource for reproduction. In *N. quadripunctatus*, when adult pairs were kept at 25 °C in the adult stage, ≈70% of adult pairs completed preparation at 20 °C (Fig. 7) even though most females had small oocytes without yolk deposition (Fig. 8). Some of the females with small oocytes probably developed their ovaries rapidly after discovery of carcasses and then laid eggs. Therefore, diapause status cannot be determined only by immature ovaries in this species.

Insects in diapause do not usually respond immediately to conditions that normally allow growth or reproduction (Danks, 1987). Adult pairs of *N. quadripunctatus* showed no reproductive behaviour at 25 °C but began breeding immediately after transfer to 20 °C (Fig. 3). Moreover, non-diapause adults raised under short-day conditions at 20 °C also showed no reproductive behaviour at 25 °C (Fig. 4). Therefore, the reduction in reproductive behaviour at 25 °C is not caused by diapause but derives directly from high temperature; however adults that have immature ovaries and do not show reproductive behaviour even at a favourable temperature of 20 °C can be regarded as in reproductive diapause. Under long-day conditions at 20 °C, ≈30% of pairs did not complete preparation of a carcass for reproduction (Fig. 4), and no yolk was deposited in ≈55% of females (Fig. 8). Twenty-four days after emergence, however, adult pairs kept under long-day conditions at 20 °C showed the same level of reproductive behaviour as those kept under short-day conditions. It was concluded that these adults had entered diapause, although it terminated spontaneously within 24 days after adult emergence.

Such faint diapause has been reported in adults of *Drosophila melanogaster* Meigen and *Orius insidiosus* Say (Saunders & Gilbert, 1990; Ruberson *et al.*, 2000). According to the criterion of Masaki (1980), diapause induced under long-day conditions is appropriately called summer diapause. Summer adult diapause with reduction of ovarian development has been shown in several other species (Stewart *et al.*, 1967; Braby, 1995; Kida *et al.*, 1997).

In many species, high temperature favours the induction of summer diapause (Masaki, 1980). In *N. quadripunctatus*, females transferred to 25 °C had smaller oocytes than those kept continuously at 20 °C (Fig. 8). Even under short-day conditions, ovarian development was reduced by high temperatures in the adult stage (Fig. 8), but this reduction may result from metabolic consumption because the body weight of these adults did not increase (Table 1). Moreover, high temperatures in the adult stage did not decrease the proportion of pairs that completed preparation at 20 °C under either photoperiod (Fig. 7, see also Fig. 4). Therefore, in *N. quadripunctatus*, high temperature in the adult stage does not affect the incidence of diapause. Contrary to short-day conditions, the body weight of adults increased under long-day conditions even at high temperature (Table 1). In some species, in spite of high temperature during aestivation, the metabolic rate is maintained at an extremely low level, as measured by oxygen consumption (Stewart *et al.*, 1967; Tombes, 1971; Masaki, 1980). Differences in the increase of body weight may therefore be related to diapause status, however most adults increased their body weight at 25 °C although the proportion of adults in diapause was assumed to be only ≈30% under long-day conditions. It is necessary to measure oxygen consumption to determine the relationship between metabolic rate and diapause in this species.

In the field in Kyoto, overwintering adults begin reproduction in May (M. Kon, unpublished). The average development time from providing adults with chicken to adult emergence of their progeny was 37 days at 20 °C, which corresponds to average temperatures in May in the field. Therefore, adults of the first generation emerge in June and July. These adults do not show reproductive behaviour because of summer diapause induced by long-day conditions and direct suppression of reproductive behaviour by high temperature. Consequently, the number of adults captured by baited traps decreased from July to August.

Summer diapause induced by long-day conditions terminated in 24 days, although the ovarian stage at this age was not examined. It is concluded, therefore, that in August summer diapause in *N. quadripunctatus* has already terminated. In the laboratory, reproductive behaviour was suppressed at a constant 25 °C (Fig. 4). In Kyoto, the average lowest temperature for a week is lower than 25 °C in late August (Fig. 2), so the increase in adults captured by baited traps in late August can be explained by a cessation of direct suppression of reproductive behaviour by high temperature. Thus, *N. quadripunctatus* produces two generations a year, between which there is a reproductively inactive period, i.e. summer diapause.

Ecological significance of the bimodal life cycle

It has been reported that interspecific competition affects the seasonal activity patterns in some burying beetles (Wilson *et al.*, 1984; Trumbo, 1990a; Scott, 1998). Carcasses of vertebrates are extremely rich in nitrogenous compounds and usually provide more than enough nutrients for development of insects, but are widely dispersed and ephemeral. There are not always enough carcasses. To utilise such resources, burying beetles are often compelled to engage in inter- and intra-specific competition (Tallamy & Wood, 1986). The bimodal distribution of seasonal reproductive success in *N. defodiens* is caused by interspecific competition with congeners in a northern part of Michigan, U.S.A. (45°34′N). In the same locality, *N. orbicollis*, which is larger than *N. defodiens*, shows a one-peak pattern in seasonal activity (Wilson *et al.*, 1984). In inter- and intra-specific competition of burying beetles for carcasses, large individuals win against smaller individuals (Wilson & Fudge, 1984; Otronen, 1988). Therefore, *N. orbicollis* can displace *N. defodiens* on a carcass, but requires warm temperatures to find a carcass. In the field, *N. defodiens* may find a carcass successfully only when night temperatures become too cool for *N. orbicollis*, although *N. defodiens* appears to reproduce on a small carcass throughout the summer (Wilson *et al.*, 1984; Trumbo, 1990b).

In Hokkaido, interspecific competition for resources again causes spatio-temporal segregation among some sympatric species of *Nicrophorus* (Katakura & Ueno, 1985; Ohkawara *et al.*, 1998). In Kyoto, however, adults of larger species were trapped together with adults of *N. quadripunctatus*. Adults of *N. maculifrons* Kraatz were trapped from May to July and in October, and adults of *N. concolor* Kraatz were trapped continuously from May to October. Moreover, the number of captured adults in these two species was not higher in July and August when adults of *N. quadripunctatus* were rarely trapped (Trumbo *et al.*, in press). Therefore, it is not probable that the bimodal distribution of seasonal activity in *N. quadripunctatus* in Kyoto results primarily from competition with congeners.

No larvae were observed when a small number of females laid eggs at 25 °C (Figs 3b and 4). Furthermore, when adult pairs were transferred to 25 °C immediately after oviposition, no larvae were observed in most cases (Fig. 6). In contrast, larvae developed to the post-feeding stage even after transfer to 25 °C (Fig. 6). Direct evidence that 25 °C or higher temperatures are lethal for eggs of *N. quadripunctatus* was also obtained (Fig. 9). In Kyoto, adult pairs of *N. quadripunctatus* bury a carcass into a shallow nest beneath the leaf litter ≤5 cm deep (M. Kon, unpublished), and the weekly average of daily lowest temperatures reaches 24 °C in August (Fig. 2). Moreover, a high temperature of 25 °C during either the photophase or scotophase was lethal for eggs (Fig. 5). Therefore, if adult pairs occupied a carcass and laid eggs in mid-summer, their eggs would not hatch. In *N. quadripunctatus*, it is concluded that the physiological mechanisms reducing reproduction effectively prevent the beetles from inefficient oviposition in mid-summer.
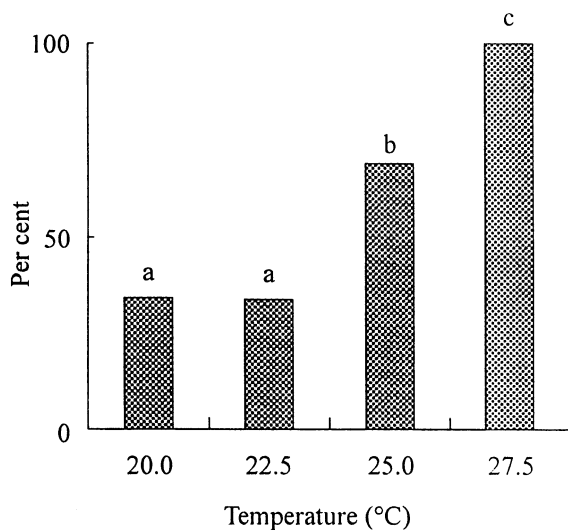
Fig. 9. Effects of temperature on mortality in the egg stage of *Nicrophorus quadripunctatus*. Eggs laid by adults reared at 20 °C under LD 12:12 h conditions were kept at various temperatures under the same light:dark regime. The percentages with the same letter are not significantly different (Tukey-type multiple comparison test for proportions, $P > 0.05$; Zar, 1999; $n = 86$–88).

In more northerly Hokkaido, *N. quadripunctatus* is active from June to September, with a peak in late July and August (Katakura & Ueno, 1985; Ohkawara *et al.*, 1998). The monthly average temperature in August in Sapporo, Hokkaido (43°03′N) is 21.7 °C (National Astronomical Observatory, 1998). Therefore, even if the eggs of *N. quadripunctatus* in Hokkaido have the same characteristics as those in Kyoto, this species may reproduce in Hokkaido in mid-summer because the eggs would not die as a result of high temperatures in this period.

Tolerance to high temperature in eggs of *N. quadripunctatus* may be restricted by phylogeny. Both population densities and species diversity of the genus *Nicrophorus* are higher in northern localities (Anderson, 1982; Wilson & Fudge, 1984; Scott *et al.*, 1987; Trumbo, 1990a), so it is likely that this genus is of northern origin (Peck & Anderson, 1985).

Sota (1985) reported the role of interspecific interactions in addition to climatic factors in the seasonal activity patterns of some carabid beetles, however it is difficult to determine whether interspecific differences in the seasonal activity pattern have resulted from competition or merely from divergences in physiology or other features evolved in other circumstances (Danks, 1987). In *N. quadripunctatus*, a phylogenetic constraint rather than interspecific competition probably caused the bimodal seasonal activity, as discussed above, although interspecific competition has not been examined experimentally. Although *N. tomentosus* overwinter as prepupae and emerge as adults in late June, adults are not found on resources for reproduction until late August in southern Michigan (42°34′N), U.S.A. (Wilson *et al.*, 1984). Wilson *et al.* assumed that the newly emerging adults enter a summer reproductive diapause, however it is still unclear which factors, e.g. photoperiod, temperature, genetic differences, are the mechanism responsible for this diapause. In contrast to *N. quadripunctatus*, competition with *N. orbicollis* is suggested to be a primary factor for the evolution of reproductive diapause in *N. tomentosus* (Wilson *et al.*, 1984; Trumbo, 1990a). Nevertheless, it is also possible that in *N. tomentosus* the seasonal activity is influenced by physiological constraints, as shown in *N. quadripunctatus* in this study. Physiological studies of more species are necessary to explain the seasonal activity and the distribution of burying beetles.

Acknowledgements

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