

Egg diapause in *Alydus calcaratus* (L.) (Heteroptera: Alydidae): Photoperiodic induction under natural temperature conditions

Dmitry L. Musolin,^{1, 2,*} Andrey V. Maysov³ and Aida H. Saulich³

¹Department of Bio- and Geosciences, Graduate School of Science, Osaka City University, Osaka 558–8585, Japan

²Department of Zoology, St. Petersburg State Forest Technical Academy, Institutskiy per., 5, St. Petersburg, 194021, Russia

³Laboratory of Entomology, Biological Research Institute of St. Petersburg State University, Oranienbaumskoye shosse, 2, Sary Peterhof, St. Petersburg, 198904, Russia

(Received 27 January 1999; Accepted 11 June 1999)

Abstract

Field observations and an experiment were conducted to clarify the seasonal cycle of *Alydus calcaratus* (Heteroptera: Alydidae) in the forest-steppe zone of Russia and to test the role of day length in the control of seasonal development of this species. Insects were reared under a constant photoperiod and at natural temperature. Two alternative photoperiodic regimes were used: short (14L–10D) and long (18L–6D) days. No significant differences in duration of nymphal development were observed between these conditions. Females laid eggs under both photoperiods, although preoviposition period was significantly shorter under long-day (11.6 ± 1.4 days) than under short-day (13.8 ± 1.5 days) conditions. Eggs were kept under the same photoperiodic conditions at a constant temperature of 24°C for two months. In the long-day regime, nymphs from 37.4% of eggs hatched on 14.1 ± 1.3 days after oviposition (hatchability in the egg samples laid by one female varied between zero and 85.7%). During the same period, no nymphs hatched from the eggs laid and maintained under short-day conditions. These observations suggested that day length plays a principal role in diapause induction in this species.

Key words: Egg diapause, seasonal development, life cycle, Alydidae, Heteroptera

INTRODUCTION

Seasonal development of any insect species is a complex phenomenon. The final structure of the seasonal pattern realized by the species as a whole or by its local population may be determined by external (environmental) or internal factors or by a species-specific combination of these factors.

In Heteroptera, seasonal cycles with winter diapause at the egg stage are known in many species of Miridae, Nabidae and Rhopalidae. Also, some examples of such life cycles are known from Coreidae, Tingidae and Pentatomidae (Southwood and Leston, 1959; Kerzhner and Jaczewski, 1964; Putschkov, 1972, 1987). This evidence came mostly from phenological observations or records of museum collections. There is little experimental evidence concerning the nature of egg diapause in heter-

opterans. In the mirids *Leptopterna dolabrata* and *Calocoris roseomaculatus*, it was shown that all combinations of laboratory regimes resulted in diapause in 100% of the eggs. All attempts to induce non-diapause development failed, and diapause terminated only in response to low temperature treatment (Braune, 1971, 1980). In the predatory pentatomid *Picromerus bidens*, egg diapause was considered to be obligatory, but recently it was shown that nymphs can emerge from some (up to 20%) eggs even without cold treatment if batches are kept at temperatures favorable for embryogenesis (Musolin and Saulich, 1998). Environmental control of induction of egg diapause was demonstrated in the mirid *Adelphocoris lineolatus* (Ewen, 1966) and in the oriental chinch bug *Cavelerius saccharivorus* (Hokyo et al., 1983). Böcher (1975) showed that egg diapause in the arctic-alpine lygaeid *Nisius*

*To whom correspondence should be addressed at: E-mail: musolin@sci.osaka-cu.ac.jp or musolin@DM1037.spb.edu

groenlandicus is facultative and controlled by photoperiodic response of females. In the mirid *Trigonotylus coelestialium*, egg diapause is also induced by a short day length experienced by the parental generation, and it was demonstrated that maternal age has some influence on the incidence of egg diapause, too (Okuyama, 1982; Kudô and Kurihara, 1988, 1989).

Alydus calcaratus (L.) (Alydidae) is a Holarctic species, largely restricted to the north (Schaefer and Schaffner, 1994). The species was reported to produce two generations per year in the temperate zone of Eurasia and to overwinter at the egg stage (Southwood and Leston, 1959; Kerzhner and Jaczewski, 1964). A similar seasonal scheme was described for three other *Alydus* species (*A. conspersus*, *A. eurinus* and *A. pilosulus*) from North America (Yonke and Medler, 1968). This pattern of seasonal development may be realized only if the egg diapause is controlled by environmental conditions.

The present study was performed to clarify the seasonal cycle of *A. calcaratus* in the forest-steppe zone of Russia and test experimentally whether day length controls the induction of egg diapause in this species.

MATERIALS AND METHODS

Field phenological observations were carried out in the natural reserve "Forest on the River Vorskla" (50°N, 36°E, forest-steppe zone, Belgorod region, Russia) in 1994, 1995 and 1996. An experiment was carried out in the same reserve in 1996. For the experiment, adults of the first summer generation were collected in the

field in the second part of June 1996 and kept under laboratory conditions consisting of long day (photophase 18 h–scotophase 6 h, 18L–6D) and constant temperature of $24 \pm 1^\circ\text{C}$. Eggs laid were kept in plastic Petri dishes under the same conditions. Nymphs hatched between 3 and 9 July and were used for experiments on the day of eclosion.

To determine whether day length plays a role in the induction of egg diapause, we used a special semi-automatic device that allowed rearing of insects under conditions of constant photoperiod and natural daily temperature rhythm (Braun and Goryshin, 1984). Special two-layer frame cowls covered the glass containers containing insects and thus provided darkness during the scotophase. Electric lamps were used to extend the natural day length up to 18 h in the long-day regime. The insects experienced natural ambient temperature (Table 1; the framed cowls did not increase temperature significantly during the scotophase). Two alternative photoperiodic regimes were used: short (14L–10D) and long (18L–6D) day. Ambient temperature was recorded with a daily thermograph and checked using min and max thermometers.

Nymphs were kept in 1 l glass containers covered with gauze netting. The initial density of the insects (25–30 first stadium nymphs per container) was gradually reduced to 5–6 insects per container when nymphs reached the last (fifth) stadium. Adult emergence was recorded daily. After emergence, adults were kept in pairs (one male and one female) in 0.25 l glass containers covered with gauze netting under the

Table 1. Ambient temperature in summer 1996 and natural day length in the experimental station

		Temper- ature, °C ^a	Day length, h.min ^b			Temper- ature, °C ^a	Day length, h.min ^b			Temper- ature, °C ^a	Day length, h.min ^b
June,	1–5	16.9	16.45	July,	1–5	17.5	17.03	August,	1–5	23.2	15.53
	6–10	18.1	16.54		6–10	23.3	16.56		6–10	15.3	15.37
	11–15	20.0	17.01		11–15	24.2	16.48		11–15	16.7	15.21
	16–20	15.3	17.05		16–20	18.2	16.37		16–20	19.8	15.04
	21–25	21.3	17.07		21–25	14.8	16.26		21–25	20.7	14.46
	26–30	17.6	17.05		26–31	19.9	16.11		26–31	14.8	14.27

^a Mean periodic (five- or six-day) temperature.

^b Mean natural day length including half of civil twilight (after Sharonov, 1945).

same experimental conditions. The start of oviposition was checked daily. Females were kept for 20–30 days from the day of registration of the first eggs. The eggs laid were removed, counted and transferred to the laboratory conditions (same photoperiod, but constant temperature of $24 \pm 1^\circ\text{C}$) every other day. These eggs were checked every other day during the next two months and then twice a month for four more months.

No clear evidence of host plant or food preferences has been reported in *A. calcaratus* in the forest-steppe zone, and it has been suggested that this species may be associated with ants and ant-hills or may feed on living or dead insects or other small animals (Southwood and Leston, 1959). Most (if not all) species of Alydidae live on legumes or grasses (Schaefer, 1980; Schuh and Slater, 1995). In our experiment, we fed the insects with the following: fresh bunches of different wild grasses (such as chee reed grass *Calamagrostis epigeios*, timothy grass *Phleum pratensis*, blue grass *Poa pratensis*, field brome *Bromus arvensis*, alfalfa *Medicago sativa*, cow clover *Trifolium pratensis*) in small vials with water, dry grains of soy bean *Glycine max* and lentil *Lens* sp., fresh pods of acacia black locust *Robinia pseudoacacia*, pupae of ants *Lasius niger* and *Formica polyctena*, larvae of the wax moth *Galleria mellonella*, and larvae of the chrysomelid beetle *Haltica saliceti*. The food was replenished every other day.

RESULTS

Phenological observations

Adults of the first generation appeared from the middle of June–beginning of July (the earliest record was 12 June). After a short period of feeding, the insects began reproduction and females of the first generation laid eggs from the beginning of July. These eggs were non-diapause and nymphs of the second generation emerged within several days. Nymphal development of this generation took approximately one month and new adults of the second generation appeared from the beginning or middle of August. Females of this generation laid eggs, but new nymphs hatched from these eggs only the following summer.

Nymphal development

In the experiment, the period of nymphal development took approximately the same time under both regimes and for each sex. Under long-day conditions, both females and males developed within a slightly shorter time (Table 2), although the difference between the two regimes was not significant for either sex (t -test; $p > 0.05$).

Parameters of reproduction

The duration of the preoviposition period differed significantly between the two photoperiodic regimes (t -test; $p < 0.05$): i.e. two days

Table 2. Quantitative characteristics of nymphal development, reproduction and hatchability of nymphs in *Alydus calcaratus* under different photoperiodic conditions and natural temperatures

Regime	Nymphal development, days ^a		Preoviposition period, days ^a	Fecundity		Hatchability, % ^c [range]	Embryonic development, days at 24°C ^a
	Females	Males		Eggs per female ^a	Rate of oviposition, eggs/day ^{ab}		
14L–10D	30.6 ± 2.1 (32)	30.1 ± 2.3 (30)	13.8 ± 1.5 (27)	31.5 ± 13.9 (27)	2.7 ± 0.8 (27)	0.0	—
18L–6D	29.6 ± 1.8 (29)	29.1 ± 1.4 (29)	11.6 ± 1.4 (29)	50.9 ± 21.8 (29)	3.7 ± 1.2 (29)	37.4 [0.0–85.7]	14.1 ± 1.3 (552)

^a Mean ± SD (n).

^b Rate of oviposition was calculated as number of eggs laid during the experiment/duration of the period of egg production (eggs/day).

^c Hatchability of nymphs during the two-month period from the date of oviposition is given in %; 100% is the total number of eggs laid during the experiment.

longer under the short-day regime than under the long-day regime. Also, under short-day conditions females laid fewer eggs and the rate of oviposition (calculated as the ratio between the number of eggs laid during the experiment and duration of the period of egg production in the experiment) was lower (Table 2).

Hatchability of nymphs

Under the long-day regime, some nymphs hatched 14.1 ± 1.3 days after oviposition. During the two-month observation period, nymphs hatched from 37.4% of the eggs and hatchability varied between zero (2 of 29 females) and 85.7% in the samples of eggs laid by individual females. In most samples, hatchability ranged between 10 and 60%. At the same time, no nymphs hatched from the eggs laid and kept under short-day conditions during the two-month observation period. Under this regime, only a few nymphs hatched during the sixth month of the experiment.

DISCUSSION

While egg diapause is typical to some families of Heteroptera, environmental control of diapause induction has been studied in only a few species of true bugs. As mentioned, photoperiodic conditions experienced by the parental generation determine induction of facultative egg diapause in the mirids *Adelphocoris lineolatus* (Ewen, 1966) and *Trigonotylus coelestialium* (Okuyama, 1982; Kudô and Kurihara, 1988, 1989) and the lygaeid *Nysius groenlandicus* (Böcher, 1975).

Our observations indicated that under natural conditions, day length is an environmental cue that controls the induction of egg diapause in *A. calcaratus*, and this species has a long-day type photoperiodic response. Hatchability of nymphs recorded in our experiment did not reach the level of 100% under long-day conditions. For as yet unknown reasons, many eggs laid by females in the long-day regime (those, from which nymphs did not hatch) and in the short-day regime were not fertilized. Nevertheless, 37.4% of nymphs (the progeny of 27 of 29 females examined in this experiment) hatched soon after oviposition in the long-day regime, while no nymphs hatched within two months

from the date of oviposition in the alternative (short-day) regime. It should be mentioned that in two other cases when the heteropterans with egg diapause were experimentally studied, a high incidence of unfertilized eggs and consequently a low level of hatchability were reported: in *Alydus eurinus* only 48.5% out of 3,022 eggs hatched (Yonke and Medler, 1968) and 38% of the eggs of *Nysius groenlandicus* were infertile (Böcher, 1975). Based on laboratory observations of reproductive behavior in three *Alydus* species, Yonke and Medler (1968) reported that single pairs of bugs copulated up to 21 times. It is probable that the laboratory conditions are not always satisfactory for the bugs to achieve full realization of their sexual behavior, and 100% fertilization of the eggs merely cannot be achieved in the laboratory experiments. It also seems possible that embryos in non-diapause eggs may require a special combination of humidity and temperature (because females naturally scatter eggs over the ground) and some embryos died under the laboratory conditions. Nevertheless, the results suggest that photoperiod is very important in diapause induction in this species.

In our experiment, the preoviposition period was longer in the short-day regime than in the long-day one and the two-day difference might also have been related to egg diapause in this species: production of diapause eggs should require more energy and resources.

The results obtained are in agreement with the phenology of this species in the region. Insects of the first generation grow and lay eggs in the first half of the summer when the day is long (Table 1). The second generation develops when day length becomes shorter. Similarly to many other insect species, *A. calcaratus* utilizes this environmental cue to ensure induction of diapause in time. On the other hand, this preliminary study did not provide answers to questions such as whether day length is the only environmental factor used by this species in diapause induction, and which generation and developmental stage(s) (maternal or daughter generations and adults or embryos) are sensitive to this/these environmental signal(s). Our results also did not indicate the critical photoperiod for diapause induction, or whether some fraction of

the eggs laid under long-day conditions in June–July may be in diapause and remain dormant until the next spring. Further studies are needed to clarify the details of the seasonal development in this species.

ACKNOWLEDGEMENTS

We would like to thank Professor H. Numata (Graduate School of Science, Osaka City University) for thoughtful reading and improvement of the manuscript. The study was partly supported by the Russian Foundation of Basic Research (project # 96-15-97897, A.S.) and the Ministry of Education, Science, Sports and Culture of Japan (Grant-in-Aid for JSPS Fellows # 98116, D.M.). Dmitry Musolin is a postdoctoral fellow supported by the Japan Society for the Promotion of Science.

REFERENCES

- Böcher, J. (1975) Notes on the reproductive biology and egg-diapause in *Nysius groenlandicus* (Zett.) (Heteroptera: Lygaeidae). *Vidensk. Medd. Dansk. Naturhist. Foren. Kbhøbenhavn* 138: 21–38.
- Braun, V. A. and N. I. Goryshin (1984) Device for photoperiodic research under conditions of natural temperature rhythms. *Zool. Zh.* 63: 272–276 (in Russian).
- Braune, H.-J. (1971) Der Einfluss der Temperatur auf Eidiapause und Entwicklung von Weichwanzen (Heteroptera, Miridae). *Oecologia* (Berl.) 8: 223–266.
- Braune, H.-J. (1980) Ökophysiologische Untersuchungen über die Steuerung der embryonalen Diapause bei Insekten am Beispiel von *Leptopterna dolabrata* (Heteroptera, Miridae). *Zool. Jb. Syst.* 107: 32–112.
- Ewen, A. B. (1966) A possible endocrine mechanism for inducing diapause in the eggs of *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae). *Experientia* 22: 470.
- Hokyo, N., H. Suzuki and M. Murai (1983) Egg diapause in the oriental chinch bug, *Cavelerius saccharivorus* Okajima (Heteroptera: Lygaeidae). 1. Incidence and intensity. *Appl. Entomol. Zool.* 18: 382–391.
- Kerzhner, I. M. and T. L. Jaczewski (1964) 19. Order Hemiptera (Heteroptera)—True bugs. In *Keys to the Insects of the European USSR: in 5 vols, Vol. 1. Apterygota, Palaeoptera, Hemimetabola* (G. Ya. Bei-Bienko ed.). Nauka (Academy of Sciences of the USSR, Zoological Institute), Moscow-Leningrad, pp. 655–845 (in Russian).
- Kudō, S. and M. Kurihara (1988) Seasonal occurrence of egg diapause in the rice leaf bug, *Trigonotylus coelestialium* Kirkaldy (Hemiptera: Miridae). *Appl. Entomol. Zool.* 23: 365–366.
- Kudō, S. and M. Kurihara (1989) Effect of maternal age on induction of egg diapause in the rice leaf bug, *Trigonotylus coelestialium* Kirkaldy (Heteroptera, Miridae). *Jpn. J. Entomol.* 57: 440–447.
- Musolin, D. L. and A. H. Saulich (1998) Two periods of dormancy in the seasonal cycle of *Picromerus bidens* (Heteroptera, Pentatomidae): The results of laboratory and field experiments. In *Book of Abstracts, VIth Eur. Congr. Entomol.* (V. Bunnhofer and T. Soldán eds.). Inst. of Entomology, České Budějovice, pp. 281–282.
- Okuyama, S. (1982) Role of day-length in the oviposition of dormant eggs by the rice leaf bug, *Trigonotylus coelestialium* Kirkaldy, and conditions for release from the dormancy. *Ann. Rep. Plant Prot. North Japan* 33: 89–92 (in Japanese).
- Putchkov, V. G. (1972) Order Hemiptera (Heteroptera)—True bugs. In *Insects and Acari—Pests of Agricultural Plants: in 4 vols, Vol. 1. Hemimetabola* (O. L. Krizhanovskii and E. M. Danzig eds.). Nauka (Academy of Sciences of the USSR, Zoological Institute), Leningrad, pp. 222–262 (in Russian).
- Putchkov, V. G. (1987) Order true bugs—Hemiptera. In *Pests of Agricultural Plants and Forests: in 3 vols, Vol. 1. Pest Nematodes, Molluscs, Arthropods* (V. P. Vasiliev ed.). Urozhay, Kiev, pp. 279–305 (in Russian).
- Schaefer, C. W. (1980) The host plants of the Alydinae, with a note on heterotypic feeding aggregations (Hemiptera: Coreoidea: Alydidae). *J. Kansas Entomol. Soc.* 53: 115–122.
- Schaefer, C. W. and J. C. Schaffner (1994) *Alydus calcaratus* in North America (Hemiptera: Alydidae). *Proc. Entomol. Soc. Wash.* 96: 314–317.
- Schuh, R. T. and J. A. Slater (1995) *True Bugs of the World (Hemiptera: Heteroptera): Classification and Natural History*. Cornell University Press, Ithaca and London. 338 pp.
- Sharonov, V. V. (1945) *The Tables for Calculation of Natural Illumination and Visibility*. Academy of Sciences of the USSR, Moscow-Leningrad. 198 pp. (in Russian).
- Southwood, T. R. E. and D. Leston (1959) *Land and Water Bugs of the British Isles*. Frederick Warne and Co., London and New York. 436 pp.
- Yonke, T. R. and J. T. Medler (1968) Biologies of three species of *Alydus* in Wisconsin. *Ann. Entomol. Soc. Am.* 61: 526–531.