

Variations in the Embryonic Stages of Overwintering Eggs of Eight Grasshopper Species (Orthoptera: Acrididae) in Inner Mongolian Grasslands

Yun-Xian Zhao, Shu-Guang Hao, and Le Kang*

State Key Laboratory of Integrated Management of Pest Insects and Rodents, Institute of Zoology, Chinese Academy of Sciences, Beijing 100080, China

(Accepted September 2, 2005)

Yun-Xian Zhao, Shu-Guang Hao, and Le Kang (2005) Variation in the embryonic stages of overwintering eggs of eight grasshopper species (Orthoptera: Acrididae) in Inner Mongolian grasslands. *Zoological Studies* 44(4): 536-542. The embryologic developmental stages at which the eggs overwinter were investigated in 8 dominant grasshopper species in Inner Mongolian grasslands in 2000 and 2001. Eggs deposited by adult grasshoppers in different seasons enter the winter at different embryological developmental stages. The mean development stages of the early-period species, *Dasyhippus barbipes*, *Myrmeleotettix palpalis*, and *Omocestus haemorrhoidalis*, were at about embryonic stage 19. Among mid-period species, *Calliptamus abbreviatus*, *Oedaleus decorus asiaticus*, and *Angaracris barabensis*, embryonic development reached stages 17, 13, and 10, respectively; developmental stages of late-period species, *Chorthippus dubius* and *Ch. fallax*, were respectively at about the stages 11 and 3. Embryonic stage 19 is regarded as the diapause stage of these grasshoppers for 2 reasons: no embryos developed beyond stage 19 without hibernating before the onset of winter, and for 2 late-period species, *Ch. dubius* and *Ch. fallax*, most of the eggs remained at stage 19 when incubated at 25°C for 100 d. No significant difference was found between the supercooling points (SCPs) of eggs in a diapause or pre-diapause status, except for *Om. haemorrhoidalis*, *Ch. dubius* and *Ch. fallax*, for which the SCPs were higher in diapause eggs (stage 19) than in pre-diapause eggs. Relationships between embryological developmental stages of overwintering eggs and the grasshoppers' hatching sequences are discussed. <http://zoolstud.sinica.edu.tw/Journals/44.4/536.pdf>

Key words: Grasshoppers, Embryological development, Overwintering eggs, Supercooling point, Hatching sequence.

Grasshoppers in Inner Mongolian grasslands are univoltine and overwinter as eggs in the soil (Lockwood et al. 1994). The hatching time of 1st instar nymphs is species-specific and runs from mid-May to early July (Li and Kang 1991). These species have a fixed egg-hatching sequence from year to year. The grasshopper species can be divided into 5 groups according to their egg-hatching times and the dominant period of the adults: early-period, pre-medium period, medium-period, post-medium-period, and late-period species (Table 1). Several factors may influence the hatching time of grasshoppers

including soil temperature, oviposition site, pod depth, pod orientation, soil moisture, and the embryonic stage of the overwintering eggs (Pepper and Hasting 1952, Gage et al. 1976, Anderson et al. 1979, Gillis and Possai 1983, Kemp 1986, Kemp and Sanchez 1987). Fisher (1994) reported that *Melanoplus differentialis* has a slower embryonic development curve than *M. sanguinipes* and *M. bivittatus*. In field observations, the hatching time of *M. differentialis* eggs was 2-4 wk later than those of *M. sanguinipes* and *M. bivittatus*. However, comparison studies on *Omocestus haemorrhoidalis*, *Calliptamus abbreviatus*

*To whom correspondence and reprint requests should be addressed. Tel: 86-10-62558304. Fax: 86-10-62565689. E-mail: lkang@ioz.ac.cn

viatus, *Angaracris barabensis*, *Oedaleus decorus asiaticus*, *Chorthippus dubius*, and *Ch. fallax* indicated that post-diapause embryonic developmental rates and accumulated heat did not help to explain the different hatching sequences of these species (Hao and Kang 2004a b). Cherrill and Begon (1991) found that oviposition dates influence the overwintering embryonic stage of *Ch. brunneus* and the hatching time the following year. Oviposition times of grasshoppers in Inner Mongolian grasslands are species-specific. For example, early-period species, such as *Dasyhippus barbipes* and *Myrmeleotettix palpalis*, oviposit in July. In contrast, the late-period grasshopper species, *Ch. dubius* and *Ch. fallax*, oviposit from late Sept. to early Oct. It is suggested that eggs of grasshoppers deposited in different periods of the season may overwinter in different embryological stages. Eggs of early-period species may accumulate sufficient heat to develop to an advanced stage (Fig. 1), while embryos of late-period species may enter winter at an earlier stage. However, egg hatching of all species is delayed until the following spring. In early-period species, this is caused by embryonic diapause (Fisher et al. 1996). Low temperatures (Fig. 1) preclude the possibility of embryological development during the winter season, and the occurrence of diapause in the other species has yet to be established.

Moore (1948) investigated variations in embryological development in 3 grasshopper

species entering the winters of 1941 to 1947 in different areas of the Prairie Region of Canada. He showed that the eggs of *Melanoplus bivittatus* Say, *M. mexicanus mexicanus* Sauss, and *Camnula pellucida* Scud vary considerably in the stage of embryological development at which they enter the winter. In comparison, little is known about the overwintering embryological stage of grasshoppers on the Asian Steppe.

In this study, we present variations in development stages of overwintering eggs of 8 dominant grasshopper species in Inner Mongolian grasslands (Table 1). The supercooling points of eggs in different development stages were also examined to determine if all eggs can safely survive the winter.

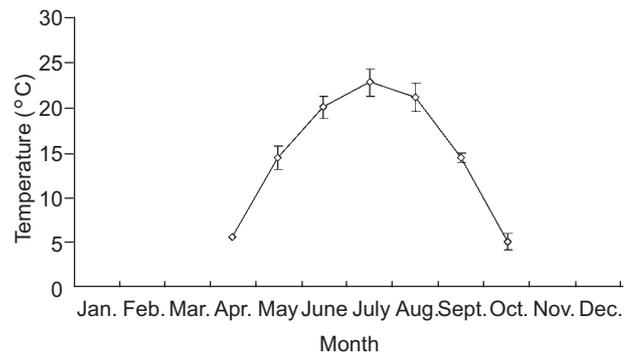


Fig. 1. Mean (±SD) soil temperatures (5 cm under the surface) at Xilin, Inner Mongolia.

Table 1. Mean embryonic stages of eggs for 8 grasshopper species of Inner Mongolia grasslands before the onset of winter. *Significantly different between years ($p < 0.05$). Significant differences between species in 2001 are indicated by different letters (Kruskal-Wallis, $p < 0.001$)

Species	Cohorts	Timing of adults [†]	Mean stage (±SD)	
			15 Oct. 2000	21 Oct. 2001
<i>Dasyhippus barbipes</i>	Early-period	June-July	19.43 ± 0.51	-
<i>Myrmeleotettix palpalis</i>	Early-period	Mid-July	19.00	19.02 ± 1.12 ^a
<i>Omocestus haemorrhoidalis</i>	Early-period	Mid-July	-	19.38 ± 0.55 ^a
<i>Calliptamus abbreviatus</i>	Mid-period	Mid-July	18.07 ± 1.31	16.94 ± 3.45 ^b
<i>Oedaleus decorus asiaticus</i> *	Mid-period	Late July	17.29 ± 0.46	12.41 ± 2.56 ^c
<i>Angaracris barabensis</i>	Mid-period	Early Aug.	-	10.70 ± 5.41 ^d
<i>Chorthippus dubius</i>	Late-period	Late Aug.	-	11.50 ± 8.55 ^{cd}
<i>Chorthippus fallax</i>	Late-period	Early Sept.	3.32 ± 4.43	2.59 ± 5.54 ^e

[†]Kang and Chen (1992). Means in column with the same letter are not significantly different at 0.05 level.

MATERIALS AND METHODS

Collection of grasshoppers and their eggs

During 2000-2001, sexually mature adults of 8 grasshopper species were collected in July, Aug., and Sept. from Xilin, the Grassland Ecosystem Research Station of Inner Mongolia, Chinese Academy of Sciences (N43°26'-44°08', E116°04'-117°05'). Adults of each grasshopper species were divided into 2 groups. One was placed in a cage (1 x 1 x 0.5 m) outdoors. The other was caged (0.5 x 0.5 x 0.45 m) in a rearing room maintained at $28 \pm 1^\circ\text{C}$ in the daytime and $25 \pm 1^\circ\text{C}$ at night, with a photoperiod of 14: 10 h (L: D). Both groups were allowed to oviposit directly in the soil. Fresh food was provided daily.

The egg pods deposited outdoors were collected on Oct. 15 in 2000 and Oct. 20 in 2001, then they were stored in wet sand at 5°C . All eggs were examined within 1 wk after collection. The egg pods oviposited in the rearing room were collected every 5 d, and then were stored in wet sand at 5°C to prevent morphogenesis until initiation of the experiments.

Identifying the development stages of the embryos

To identify the embryological development stage, embryos were examined by cutting off the micropylar end of the egg and squeezing out the contents onto a microscope slide as described by Slifer (1932). Eggs of each grasshopper species collected in 2000 were removed from their pods and randomly selected to examine the embryonic stage. No fewer than 25 eggs were examined for each species. Eggs collected in 2001 were examined by pods. Ten pods were examined for each grasshopper species.

Grasshopper embryonic stages were classified by comparing them with the diagrams of the morphological stages of other grasshoppers (Slifer 1932, Riegert 1961, Van Horn 1966). Differences in the median development stage of each grasshopper species was analyzed using Kruskal-Wallis analysis of variation by ranks (SPSS 10.0, Inc.).

Determination of diapause stages of 2 late-period grasshopper species

For the 2 late-period grasshopper species, *Ch. dubius* and *Ch. fallax*, eggs collected in the

rearing room were removed from their pods, and 450 eggs of each species were randomly selected. Then they were placed into 3 plastic cups (150 eggs/cup) with moistened sand (10% water to sand by weight) and sealed with petroleum jelly to ensure that they were airtight. The cups were incubated at a temperature of $25 \pm 1^\circ\text{C}$ with a photoperiod of 12: 12 h (L: D).

Fifteen to 20 eggs were sampled and dissected at intervals during development. The sampling interval reflected the embryonic development rate. For the 1st 28 d, eggs were sampled at 2 d intervals. The sample interval was then increased to 5 d. After a total of 58 d, the sample interval was increased to 10 d. The experiment was terminated after 108 d, and all eggs were dissected to determine their developmental stage. Embryos were removed from their egg membranes to determine the embryonic development stage with a standard binocular microscope (Van Horn 1966).

Supercooling point (SCP) determination

Adults of each grasshopper species were collected in the field and reared indoors so they would oviposit. Eggs were collected and incubated in moist sand at $25 \pm 1^\circ\text{C}$ with a photoperiod of 12: 12 h (L: D). Samples of eggs were collected at intervals of 5 d to include a range of developmental stages. The supercooling points of eggs were determined according to the method described by Zhao and Kang (2000). Cold exposure began at room temperature. Cooling resulted in a non-linear cooling rate of about $1^\circ\text{C}/\text{min}$ from 0 to -40°C . Eggs were dissected after determination of SCPs to identify the embryological development stage. At least 15 eggs were tested for each grasshopper species. Statistical differences between the SCPs of eggs in pre-diapause and diapause within species were determined by *t*-tests.

RESULTS

Embryological development stages

Considerable variations occurred in the embryological development stages between different species (Kruskal-Wallis test, $H = 785.29$, $df = 17$, $p < 0.001$, Table 1). The mean development stages of *D. barbipes*, *M. palpalis*, and *Om. haemorrhoidalis* was all at about embryonic stage 19, while *C. abbreviatus*, *O. d. asiaticus*, *A. barabensis*, *Ch. dubius*, and *Ch. fallax* reached

stages 17, 13, 10, 11, and 3, respectively. Although different sample methods were used in 2000 and 2001, embryological development did not differ between the 2 yr, except for *O. d. asiaticus* (Table 1).

Stage-frequency distributions of each grasshopper species

The overwintering development stages of eggs differed for each grasshopper species (Fig. 2). Almost all eggs of *D. barbipes*, *M. palpalis*, and *Om. haemorrhoidalis* overwintered at stage 19 or 20. In fact, morphogenesis is a continuous process without distinct steps, and the degree of morphological changes between stage 19 and 20 is minor. Thus, in later analyses, we considered stage 19 and 20 as the same stage. The embryological development stages of *C. abbreviatus*, *O. d. asiaticus*, and *A. barabensis* ranged from stage 0 to 19. For 2 late-period species, *Ch. fallax* and *Ch. dubius*, the development stages could be divided into 2 groups. One was concentrated at the beginning of development, while the other was at about stage 15.

Determination of diapause stages of 2 late-period grasshopper species

About 33.3% and 28.6% of eggs developed to stage 19 after 16 and 18 d of incubation for *Ch. fallax* and *Ch. dubius*, respectively. The percentage of eggs in stage 19 increased rapidly. More than 1/2 of the eggs had reached stage 19 after 24 d. More than 80% eggs still remained at stage 19, although the embryos had developed for 100 d at 25°C (Fig. 3). However, 3 nymphs of *Ch. dubius* and 7 nymphs of *Ch. fallax* hatched after 68-78 d.

Supercooling points (SCPs)

Mean SCPs were lower than -23°C for eggs of each grasshopper species. The lowest SCPs, of about -32.7°C, were detected in *A. barabensis* diapause eggs. There were no significant differences in the mean SCPs between pre-diapause (< stage 17) and diapause (stage 19) eggs of any grasshopper species, except for *Om. haemorrhoidalis*, *Ch. dubius*, and *Ch. fallax* (Fig. 3). Much lower SCPs were detected in diapause eggs than in pre-diapause eggs in *Om. haemorrhoidalis* ($p < 0.01$) and *Ch. fallax* ($p < 0.05$). In *Ch. dubius*,

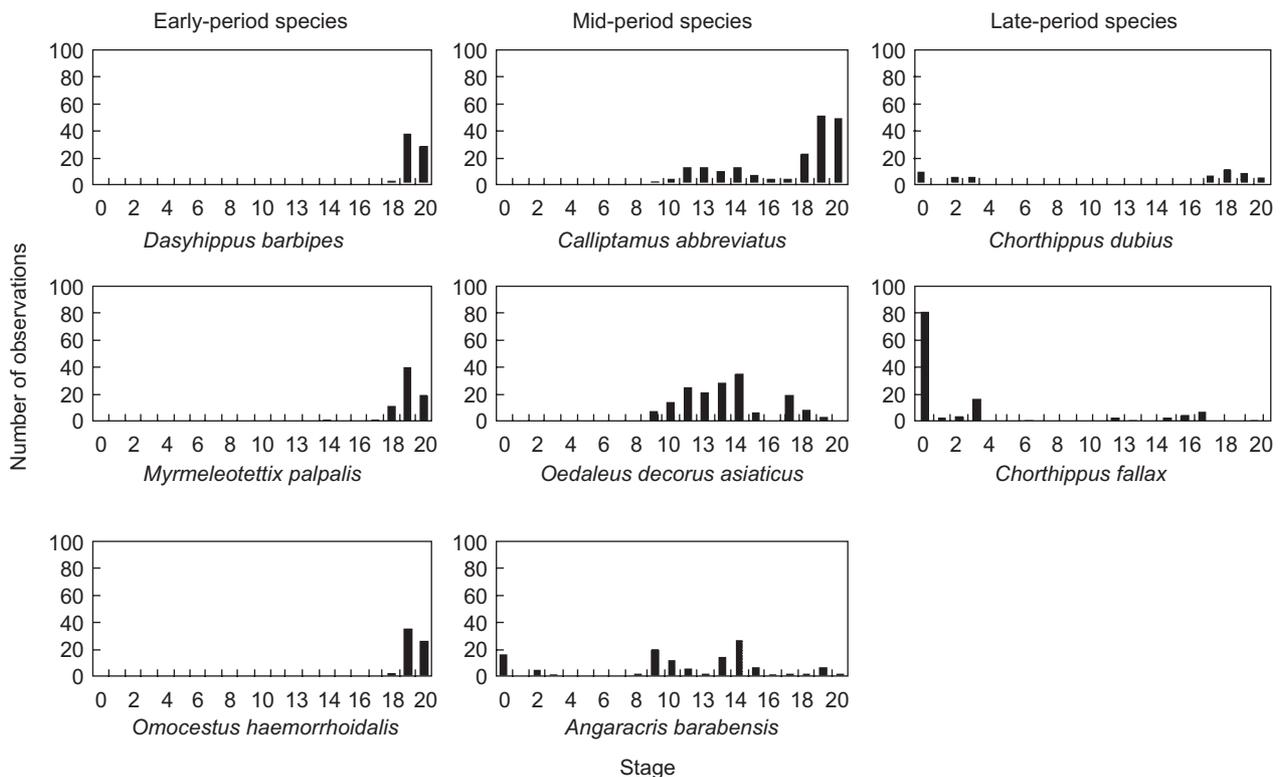


Fig. 2. Stage-frequency distributions of eggs laid by 8 grasshopper species in Inner Mongolia grasslands (sampled on 21 Oct. 2001).

SCPs of pre-diapause eggs were lower than those of diapause eggs ($p < 0.05$).

DISCUSSION

There was great variation in embryonic stages of the overwintering eggs of grasshopper species in Inner Mongolian grasslands. Generally, early-period species were able to reach a much more advanced stage of development before the onset of winter than late-period ones due primarily to the accumulation of heat (Fig. 1). For species with early summer adults, such as *D. barbipes*, *M. palpalis*, and *Om. haemorrhoidalis*, there was sufficient heat accumulation to allow their embryological development to stage 19 before the onset of winter (Table 1). The development stage at which the eggs of a species entered the winter greatly varied within a population of mid-summer adults, such as *O. d. asiaticus*, and *A. barabensis* (Fig. 2). Although more than 1/2 of their eggs developed to stage 13 or 14, some eggs may have developed as far as stage 19, and some eggs still remained at an early development stage. That is to say, the embryos of the mid-period species did not always reach stage 19 before development was stopped by low winter temperatures. Such variations in development were even greater in late-period species. For instance, nearly 1/2 of *Ch. dubius* eggs remained at less than stage 10, while 1/2 of the eggs reached stage 19 (Table 1). Similar results could also be found for *Ch. fallax*, another late-period species. Two reasons could be responsible for such a result. First, the adults of these 2 species were collected twice: once at the beginning of their oviposition season (mid-Aug.), and once at the end of their egg lying season (late

Sept.). Second, the temperature drops rapidly in fall in Inner Mongolian grasslands (Fig. 1). Eggs deposited in mid-Aug. have sufficient heat to complete pre-blastokinesis (stage 19) development, while eggs deposited in late Sept. might not meet the thermal requirement to reach stage 19. In that case, most eggs of late-period grasshoppers cannot enter blastokinesis before the onset of winter. Similar results were obtained by Cherrill and Begon (1991), who found that in NW England, *Ch. brunneus* eggs laid prior to Sept. entered diapause before winter and hatched relatively synchronously the following spring. In contrast, eggs laid after that time failed to reach the diapause stage, and hatched later without entering diapause.

In this study, no eggs of these grasshoppers were observed to have embryos beyond stage 19. Thus, they obviously enter a very definite diapause at stage 19 before winter. Similar results were found by Moore (1948): embryos of *Camnla pellucida* Scud. appeared to enter diapause just before blastokinesis, although they did not always reach this stage before development was stopped by winter temperatures. Our data suggest that grasshoppers in Inner Mongolian grasslands may enter diapause in stage 19 (before blastokinesis). Two observations support this conclusion. First, the most-advanced embryological development stage remained at stage 19/20 in all species studied. Eggs of early period and/or mid period species are deposited in July and Aug. They may accumulate sufficient heat to develop beyond stage 19. However, it did not happen in the field for the reason of diapause. Second, the diapause induction experiment of 2 late period grasshopper species, *Ch. dubius* and *Ch. fallax*, showed that most embryos remained at embryonic stage 19 even after 100 d of incubation at 25°C (Fig. 3).

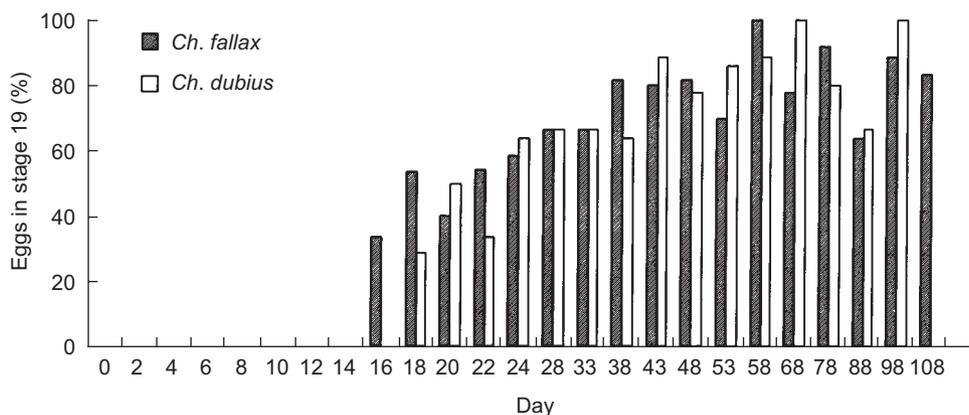


Fig. 3. Diapause induction in 2 late-period species, *Chorthippus fallax* and *Ch. dubius* embryos subjected to 25°C.

The results of this study show that not all grasshopper eggs overwinter in diapause (stage 19). Eggs in both the pre-diapause and diapause stages experience the same low temperatures in winter. This overwintering survival strategy may be based on an increased capacity for supercooling enhanced by natural dehydration in their soil surface habitat (Block et al. 1995). The cold hardiness of pre-diapause and diapause eggs did not significantly differ in many grasshoppers in terms of supercooling points, results similar to those obtained by Hao and Kang (2004b), and this suggests that both pre-diapause and diapause eggs can overwinter safely. In previous work, evidence was found both for and against a relationship between diapause and cold tolerance (Pullin 1996, Jing et al. 2005). In some species, cold-hardiness is likely to be an integral part of the diapause syndrome, while in others it can occur completely independent of diapause. A close relation between these adaptive mechanisms implies that they are controlled by the same factors (Denlinger 1991). Because fall temperatures decrease gradually, cold acclimation is another important factor enhancing insect cold tolerance (Milonas and Savopoulou-Soultani 1999, Jing and Kang 2003).

The stage of development of embryos within a single pod was quite uniform in all species studied, while the development stage between pods differed significantly (Fig. 2). Synchronic development within egg pods enhances survival: eggs at the base of the pod may die if the upper eggs

above do not hatch first. Differences in development stages between pods may be caused by different oviposition times. Embryological development did not differ between 2000 and 2001, except for *O. d. asiaticus* (Table 1). In contrast, in a study by Moore (1948), development within a population differed from 1 location to another and from 1 year to the next, which he attributed to climatic variations.

In the experiment of diapause stage determination, some nymphs did hatch after incubation at 25°C for 68 d, although they did not experience low temperatures. One reason is that chilling is often not a prerequisite for completion of hibernation diapause, and diapause development progresses well at intermediate or high temperatures; sometimes it is even stimulated by high or increasing temperatures (Hodek and Hodková 1988). The other reason is that eggs that have not entered diapause may exist in these grasshopper egg pods. Previous studies have shown that some grasshoppers exhibit such a phenomenon. When eggs of *Aulocara ellioti* and *Ageneotettix deorum* were incubated at 30°C without low-temperature treatments, 6.1% of *A. ellioti* hatched after 36 d of incubation and 1.5% of *A. deorum* hatched after 46 d of incubation (Fisher 1997). We also observed some eggs of grasshoppers which hatched at 25 °C without low-temperature treatment (unpubl. data).

The present study has shown that the different overwintering embryonic stages of grasshop-

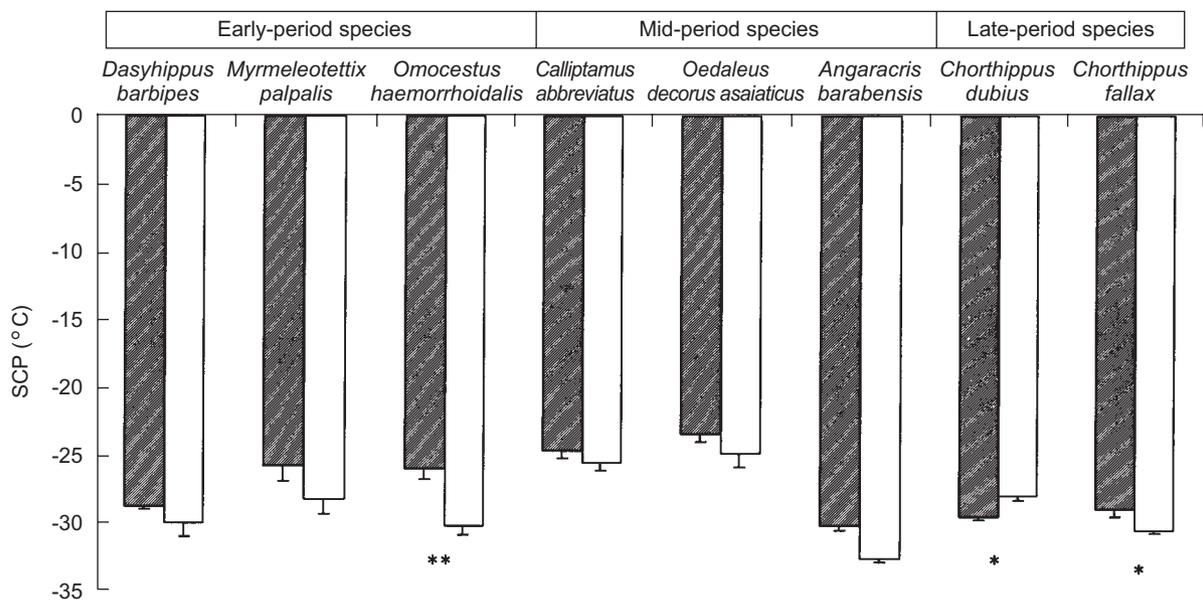


Fig. 4. Supercooling points (SCPs) (mean ± SE) of pre-diapause (shade bars) and diapause (empty bars) eggs of grasshoppers in Inner Mongolia grasslands (* $p < 0.05$, ** $p < 0.01$).

pers may lead to different hatching times of 1st-instar nymphs and produce a hatching sequence the following year. However, if the eggs of late-period species, such as *Ch. fallax*, cannot develop to diapause before winter, do they enter diapause development in the next spring? Are there any differences between diapause-terminating conditions required by early-period species and those of late-period species? What are the ecological and evolutionary advantages of both diapause and non-diapause eggs coexisting in same population? These issues should be researched further.

Acknowledgments: The authors want to extend thanks to Mr. Ke-Zheng Ma and Dr. Yong Gao for their great help in insect collection. This research was supported by grants from Innovation Projects of Chinese Academy of Sciences (KSCX1-08 and KSCX2-SW-105).

REFERENCES

- Anderson RV, CR Tracy, Z Abramsky. 1979. Habitat selection in two species of short-horned grasshoppers: the role of thermal and hydric stress. *Oecologia* **38**: 359-374.
- Block W, HC Li, R Worland. 1995. Parameters of cold resistance in eggs of three species of grasshoppers from Inner Mongolia. *CryoLetter* **16**: 73-78.
- Cherrill A, M Begon. 1991. Oviposition date and pattern of embryogenesis in the grasshopper *Chorthippus brunneus* (Orthoptera: Acrididae). *Holarctic Ecol.* **14**: 225-233.
- Denlinger DL. 1991. Relationship between cold hardiness and diapause. In DE Lee, DL Denlinger, eds. *Insects at low temperature*. New York: Chapman and Hall, pp. 174-198.
- Fisher JR. 1994. Temperature effect on postdiapause development and survival of embryos of three species of *Melanoplus* (Orthoptera: Acrididae). *Ann. Entomol. Soc. Am.* **87**: 604-608.
- Fisher JR, WP Kemp, FB Pierson. 1996. *Aulocara elliotti* (Orthoptera: Acrididae): diapause termination, postdiapause development, and prediction of hatching. *Environ. Entomol.* **25**: 1158-1166.
- Fisher JR. 1997. Embryonic diapause in *Aulocara elliotti* and *Ageneotettix deorum* (Orthoptera: Acrididae): low-temperature relationships. *Environ. Entomol.* **26**: 906-911.
- Gage SH, MK Mukerji, RL Randell. 1976. A predictive model for seasonal occurrence of three grasshopper species in Saskatchewan (Orthoptera: Acrididae). *Can. Entomol.* **108**: 245-253.
- Gillis JE, KW Possai. 1983. Thermal niche partitioning in the grasshoppers *Arphia conspersa* and *Trimerotropis suffusa* from a montane habitat in central Colorado. *Ecol. Entomol.* **8**: 155-161.
- Hao SG, L Kang. 2004a. Effects of temperature on post-diapause development and hatching of eggs of three grasshopper species (Orthoptera: Acrididae). *J. Appl. Entomol.* **128**: 95-101.
- Hao SG, L Kang. 2004b. Supercooling capacity and cold hardiness of the eggs of the grasshopper *Chorthippus fallax* (Orthoptera: Acrididae). *Eur. J. Entomol.* **101**: 231-236.
- Hodek I, M Hodková. 1988. Multiple roles of temperature during insect diapause: a review. *Entomol. Exp. Appl.* **49**: 153-165.
- Jing XH, L Kang. 2003. Geographical variation in egg cold hardiness: a study on the adaptation strategies of the migratory locust *Locusta migratoria* L. *Ecol. Entomol.* **28**: 151-158.
- Jing XH, XH Wang, L Kang. 2005. Chill injury in the eggs of the migratory locust, *Locusta migratoria* (Orthoptera: Acrididae): the time-temperature relationship with high-temperature interruption. *Insect Science* **12**: 171-178.
- Kang L, YL Chen. 1992. Temporal and spatial heterogeneity of grassland grasshoppers. *Res. Grassland Ecosyst.* **4**: 109-123. (in Chinese with English summary)
- Kemp WP. 1986. Thermal regulation in three grasshopper species. *Can. Entomol.* **118**: 335-343.
- Kemp WP, NE Sanchez. 1987. Differences in post-diapause thermal requirements for eggs of two rangeland grasshoppers. *Can. Entomol.* **119**: 653-661.
- Li HC, L Kang. 1991. The eco-geographical conditions for grassland insects in Inner Mongolia. In Y Ma, HC Li, L Kang, eds. *The grassland insects of Inner Mongolia*. Beijing: Tianze Eldonejo, pp. 1-99. (in Chinese with English summary)
- Lockwood JA, Li HC, JL Dodd, SE Williams. 1994. Comparison of grasshopper (Orthoptera: Acrididae) ecology on the grasslands of the Asian Steppe in Inner Mongolia and the Great Plains of North America. *J. Orthop. Res.* **2**: 4-14.
- Milonas PG, M Savopoulou-Soultani. 1999. Cold hardiness in diapause and non-diapause larvae of the summer fruit tortrix, *Adoxophyes orana* (Lepidoptera: Tortricidae). *Eur. J. Entomol.* **96**: 183-187.
- Moore HW. 1948. Variations in fall embryological development in three grasshopper species. *Can. Entomol.* **80**: 83-88.
- Riegert PW. 1961. Embryological development of a nondiapause form of *Melanoplus bilituratus* Walker (Orthoptera: Acrididae). *Can. J. Zool.* **39**: 491-494.
- Pepper JH, E Hastings. 1952. The effects of solar radiation on grasshopper temperatures and activities. *Ecology* **23**: 96-103.
- Pullin AS. 1996. Physiological relationships between insect diapause and cold tolerance: Coevolution or coincidence? *Eur. J. Entomol.* **93**: 121-129.
- Slifer EH. 1932. Insect development IV. External morphology of grasshopper embryos of known age and with a known temperature history. *J. Morph.* **53**: 1-21.
- Van Horn SN. 1966. Studies on the embryogenesis of *Aulocara elliotti* (Thomas) (Orthoptera, Acrididae) I. External morphogenesis. *J. Morphol.* **120**: 83-114.
- Zhao YX, L Kang. 2000. Cold tolerance of the leafminer *Liriomyza sativae* (Diptera: Agromyzidae). *J. Appl. Entomol.* **124**: 185-189.