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Alternating Low and High Temperatures Increases the Hatching Rate of *Eupolyphaga sinensis* (Blattaria: Polyphagidae) Eggs

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ABSTRACT

The hatching rates and developmental durations of *Eupolyphaga sinensis* Walker eggs were studied under variable combinations of low temperatures (5 °C and 10 °C) and high temperatures (25 °C and 30 °C). Our findings were intended to provide new evidence on the effect of temperature on the hatching of insect eggs. The results showed that both low temperatures and high temperatures significantly affected the hatching rate and time of *E. sinensis* eggs. Further analysis revealed a significant interactive effect between low temperatures and high temperatures. The 5 °C + 30 °C treatment achieved the highest egg hatching rate (77.33%) and the shortest hatching time (44.11 ± 1.55 d), which was significant compared with three other temperature treatments. The effects of high temperatures treatments on the hatching rate and the hatching time were more significant than that in the low temperature treatments. Hatching began earlier at 5 °C + 30 °C and 10 °C+30 °C temperatures, and single-day hatching first increased rapidly before declining afterwards within a short period. Comparatively, the hatching of eggs under the (5 °C+25 °C) and (10 °C+25 °C) temperatures was initially significantly delayed, including the peak single-day hatching rate. Under these treatments, the hatching rate tended to be gradual in terms of the temporal dynamics without showing any prominent peak. These results showed that low and high temperatures significantly affect the hatching rates of *E. sinensis* eggs. This study provided basis for the large-scale breeding of *E. sinensis.*

Keywords: Egg hatching, developmental duration, *Eupolyphaga sinensis*, variable temperature.

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INTRODUCTION

For most insects, eggs are the first developmental stage, and they directly affect the post-embryonic development of insects. In this sense, the development of insect eggs occupies a particularly critical place in research on insect ontogeny (Heming, 2003). Apart from adult foods, environmental factors, especially temperature, are the main factors affecting the development of insect eggs (Raza et al, 2015; Harrison, Woods,& Roberts, 2012; Gillespie, Picker, Dallas & Day, 2018). In particular, in middle and high-latitude regions with noticeable seasonal changes, the germination and hatching of insect eggs are highly affected by low temperatures and high temperatures (Liu et al, 2020). Exploring the effects of temperature change on the hatching of insect eggs not only provides a theoretical basis for the large-scale breeding of insects but is also a great reference for an in-depth understanding of the dynamic relationship between insect development and temperature.

Eupolyphaga sinensis Walker (Blattaria: Polyphagidae) is a traditional medicinal insect and has been included in the list of traditional Chinese medicine in the Pharmacopoeia of the People's Republic of China (Xie et al, 2020; Kim, Jim, Park, & Song, 2022). In recent years, studies have revealed that *E. sinensis* are rich in proteins, unsaturated fatty acids, fat-soluble vitamins, and a variety of nutrients, including amino acids and trace elements, essential for the human body (Zhang et al, 2014). Inspired by the folk eating experience, people have processed this insect into food (Li, Yan, & Guo, 2000). The dual use of *E. sinensis* as medicine and food has driven its market demands to continue increasing year by year. While the artificial culture technology of *E. sinensis* rearing is becoming increasingly advanced, the low hatching rate of its oothecae remains a notable challenge, particularly in large-scale culture (Li, Duan, Wu, Wang, & Wu, 2003; Jin, Tang, Wang, Wu, & Wu, 2005; Jin et al, 2006; Jin, Wu, Tang, Wang, & Wu, 2007; Wang & Liang, 2017). Regarding factors affecting the hatching of *E. sinensis* eggs, existing studies have only reported the hatching of *E. sinensis* eggs in natural environments or under different constant temperatures and soil moisture contents (Li, Tang, Zhang, Wu, & Wu, 2003; Jin et al, 2007; Wang & Liang, 2012, 2017). To date, the effect of temperature change on the hatching of *E. sinensis* eggs remains unclear. In the present study, we investigate the effects of different combinations of low-temperature (germination temperatures) and high-temperature (hatching temperatures) on the hatching of *E. sinensis* eggs. Our results deepen our understanding of the effect of varied temperatures on the hatching of *E. sinensis* eggs in natural environments, providing scientific guidance for the large-scale breeding of *E. sinensis*.

MATERIALS AND METHODS

Insect source

E. sinensis used in our experiment were provided by Yuchen Special Culture Base in Bozhou, Anhui Province. The same batch of newly emerged adults was reared at a room temperature of 25 ± 2 °C under a soil moisture content of about 20% (Wang & Zhang, 2000) until a large number of female adults had oothecae hanging on their abdomens. Before the experiment, 720 oothecae, characterized by regular appearance, bright shells, and even size, were selected from the oothecae laid on 21 July 2021. They were soaked in a 4% neutral formaldehyde for disinfection, rinsed with distilled water, and dried for use later (Jin et al, 2005).

Nest mud (hatching soil)

Nest mud (hatching soil) was prepared from humus soil, cow dung, and rice bran by mixing the three in a ratio of 1:1:1 (Jin et al, 2007).

Temperature treatments

The factorial experimental design (2 × 2) was adopted to set up four experimental treatments; two low-temperatures (5 °C and 10 °C) and two high-temperature (25 °C and 30 °C) treatments: (i) low-temperature at 5 °C + high-temperature at 25 °C (5 °C + 25 °C); (ii) low-temperature at 10 °C + high-temperature at 25 °C (10 °C + 25 °C); (iii) low-temperature at 5 °C + high-temperature at 30 °C (5 °C + 30 °C); (iv) low-temperature at 10 °C + high-temperature at 30 °C (10 °C + 30 °C).

The low temperature of 5 °C was achieved using a refrigerator (model: BCD-215STPD; manufacturer: Qingdao Haier Co., Ltd.). The low temperature of 10 °C and the high temperature of 25 °C and 30 °C were provided by a microcomputer artificial climate chamber (model: SPX-400; manufacturer: Shanghai Boxun Medical Biological Instrument Corp.). The temperature ranges were as follows: 5 °C ± 0.5 °C, 10 °C ± 1.0 °C, 25 °C ± 1.0 °C, 30 °C ± 1.0 °C. Each experimental treatment was composed of three replicates of 60 oothecae (one day old). The replicates were conducted in different refrigerators and chambers. The experimental oothecae were placed in a plastic square box (25. 0 × 25. 0 ×15. 0 cm in size) containing hatching soil (about 12.0 cm thick) under alternating low and high-temperature treatments. The boxes were first placed in the proper set refrigerator and microcomputer artificial climate chamber for seven days at low-temperature, and then transferred to the properly set microcomputer artificial climate chamber for high-temperature treatment until the completion of egg hatching. The relative humidity of hatching soil was maintained at 25 ± 5% during the experiment by spraying water three times every day.

Hatching rate

The number of hatched eggs was regularly recorded daily at 14:00. It started from the appearance of the first nymph in the oothecae of each box and ended when no new nymph was observed (when no new nymph hatched from the oothecae for five consecutive days, egg hatching would be deemed as completed).

Data analysis

A homogeneity test was conducted each time before the statistical analysis without data conversion. Two-way ANOVA was used to test the effects of temperatures and high temperatures on the hatching of *E. sinensis* eggs. One-way ANOVA was performed to compare the effects of different temperature treatments on the developmental duration, hatching rate, and single-day hatch rate of *E. sinensis* eggs. All data were analyzed using SPSS version 25.0. Continuous normally distributed data were expressed as mean ± standard error (X ± SE).

RESULTS

Effects of different temperatures on the cumulative hatching rate of *E. sinensis* eggs

The results indicated that the cumulative hatch rate of *E. sinensis* eggs varied significantly across four variable temperature combinations (F = 580.718, $P < 0.01$; Fig. 1). The highest hatching rate occurred under the 5 °C + 30 °C temperature (77.33 ± 2.52%), followed by the 10 °C + 30 °C temperature (66.00 ± 2.65%), the 5°C + 25°C treatment (23.33 ± 1.53%), and the 10 °C + 25 °C treatment (15.00 ± 2.00%) in this order. There was no difference in the number of nymphs hatched from each ootheca under different treatment conditions. The results indicated that there was a strong interaction between low and high temperatures, which significantly affected the hatching rate of *E. sinensis* eggs. Particularly, low temperatures had significant effect on the hatching rate of *E. sinensis* eggs (F = 61.239, $P < 0.01$). At the same combined high temperature (25 °C or 30 °C), the egg hatching rate at the combined low temperature of 5 °C was significantly higher than that at 10 °C ($P < 0.01$). At the same time, high temperatures also significantly affected the hatching rate of *E. sinensis* (F = 1,785.332, $P < 0.01$). At the same combined low temperature (5 °C or 10 °C), the egg hatching rate at the combined high temperature of 30 °C was significantly higher than that at 25 °C ($P < 0.01$). The results showed that the combined temperature (5 °C + 30 °C) significantly increased the hatching rate of *E. sinensis* eggs.

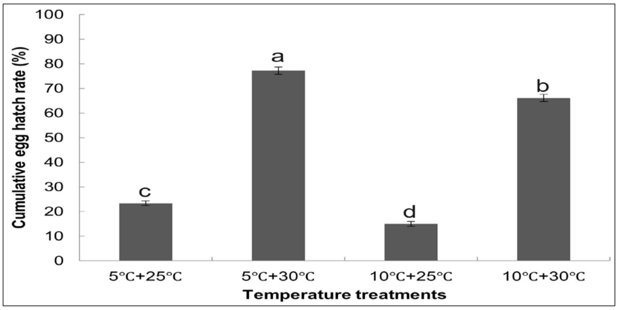

Figure 1. Effects of different temperatures on the cumulative hatching rate of *E. sinensis* eggs. Different lowercase letters above the bars indicate significant differences in cumulative hatching rate ($P \leq 0.05$, Tukey's test).

Developmental durations of *E. sinensis* eggs under different temperature treatments.

Oothecae treated at low temperatures for seven days (starting and ending time: 22:00, 21 July 2021–22:00 to 28 July 2021) were transferred to a microcomputer artificial climate chamber for high-temperature treatment on 28 July 2021 at 22:00. Under the 5 °C + 30 °C treatment, the first oothecae hatched on 31 August 2021, and no more ootheca hatching occurred after 8 September. The hatching period was 9 d, and the average developmental duration of eggs was 44.11 ± 1.55 d (Range: 41 – 49 d). Under the 10 °C + 30 °C treatment, oothecae hatching began on 2 September 2021, and no more hatching was observed after 9 September. The hatching period was 8 d, and the average developmental duration of eggs was 45.31 ± 1.57 d (Range: 43–50 d). Under the (5 °C + 25 °C) treatment, the oothecae began to hatch on 13 September 2021, and there was no hatching occurred after 27 September. The hatching period was 12 d, and the average developmental duration of eggs was 58.72 ± 3.78 d (Range: 54 – 65 d). Under the 10 °C + 25 °C treatment, oothecae began to hatch on 16 September 2021, and no more ootheca hatching was observed after 24th September. The hatching period was 9 d, and the average developmental duration of eggs was 60.97 ± 2.87 d (Range: 57 – 65 d). The results indicated that the developmental duration of *E. sinensis* eggs varied significantly across different temperature treatments (F = 422.774, $P < 0.05$; Fig. 2). Compared with low temperatures, high temperatures more significantly affected the developmental rate of eggs. The developmental duration of eggs at 30 °C was significantly shorter than 25 °C ($P < 0.01$).

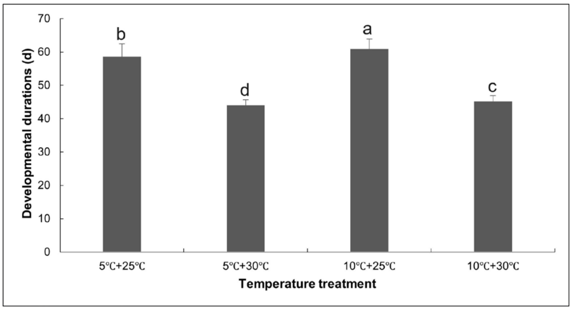

Figure 2. Developmental durations of *E. sinensis* eggs under different temperature treatments. Different lowercase letters above the bars indicate significant differences in the developmental durations ($P \leq 0.05$, Tukey's test).

Temporal dynamics of the egg hatch rate of *E. sinensis* under the same variable-temperature treatment.

The egg hatching rate fluctuated significantly from day to day under a given temperature (Fig. 3 and Fig. 4). With the extension of hatching time, the egg hatching rate of *E. sinensis* under the 5 °C + 30 °C and 10 °C + 30 °C first increased rapidly, then started to decline. Under 5 °C + 30 °C, the peak single-day hatching rate of eggs (25.56 ± 1.15%), counted from the beginning day of hatching (F = 187.858, $P < 0.001$; Fig. 4: A), occurred on the 4th day. Under 10 °C + 30 °C, the peak single-day hatching rate of eggs (17.22 ± 0.58%), counted from the beginning day of egg hatching (F = 162.890, $P < 0.001$; Fig. 4: C), occurred on the 5th day. In comparison, the eggs under 5 °C + 25 °C and 10 °C + 25 °C displayed a gradual hatching rate and had no pronounced peak hatching day. Under the 5 °C + 25 °C temperature, the peak single-day hatching rate of eggs (3.89 ± 0.58%), counted from the beginning day of egg hatching (F = 4.111, P = 0.002; Fig. 4: B), occurred on the 14th day. Under the 10 °C + 25 °C temperature, the peak single-day hatching rate of eggs (2.78 ± 0.58%), counted from the beginning of egg hatching (F = 3.551, P = 0.012; Fig. 4: D), was observed on the 22nd day.

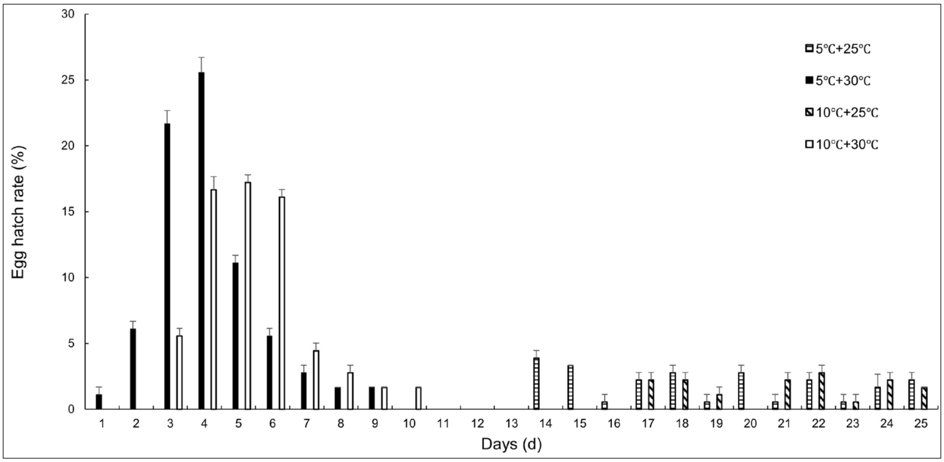

Figure 3. Single-day hatching rate of *E. sinensis* eggs under different temperature treatments. "Day 1" indicated the beginning day of egg hatching, the same below. ns: no significance. Asterisks above the bars indicate a significant difference in Single-day hatch rate (P ≤ 0.05, Tukey's test).

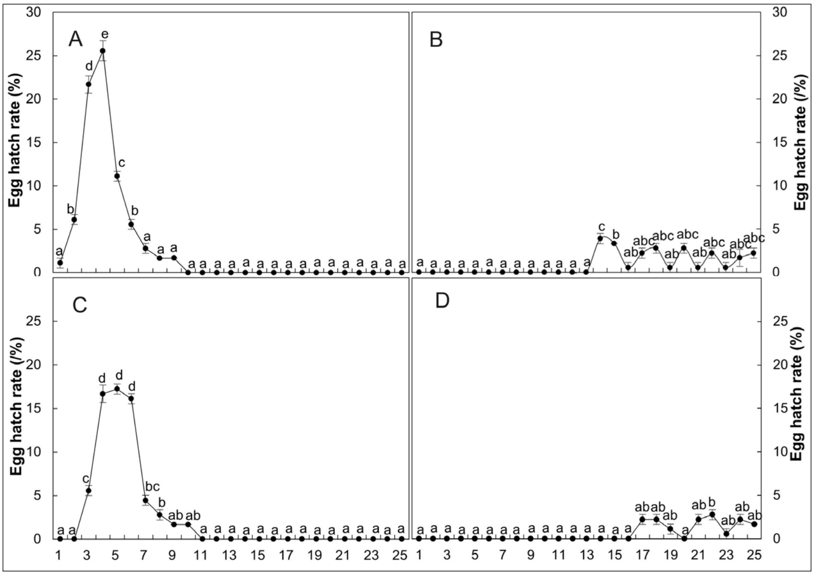

Figure 4. Temporal dynamics of the egg hatching rate of *E. sinensis* under different temperature treatments. A: 5 °C + 30 °C; B: 5 °C + 25 °C; C: 10 °C + 30 °C; D: 10 °C + 25 °C. Different lowercase letters indicate significant differences in the hatching rate (P ≤ 0.05, Tukey's test).

DISCUSSION

The low hatching rate of oothecae has remained a major constraint in the large-scale artificial culture of *E. sinensis*. According to the survey by Li et al. (2003), some farmers had complained of the extremely low hatching rate of oothecae (30 – 40%), which fell far short of the promise (80 – 90%) by some companies. Jin et al (2007) reported that the average hatching rate of *E. sinensis* oothecae under a temperature of 30 °C and a relative humidity of 95% was 64.7 ± 6.7%. A related study by Wang & Liang (2017) showed that the hatching rate of *E. sinensis* oothecae peaks at 26 °C and 30 °C, reaching 56.5% and 52.8%, respectively. Our findings revealed that the peak hatching rate of *E. sinensis* oothecae occurs in 5 °C + 30 °C treatment, reaching 77.33 ± 2.52%. In production practice, oothecae are first incubated at a low temperature of 5 °C for 7 d and then transferred to a high temperature of 30 °C to hatch. In theory, this approach can significantly improve the hatch rate of *E. sinensis* oothecae.

Temperature change affects the hatching rates of insect eggs and the survival rates of larvae, pupae, and adults (Pan, Chen, Xiao, Ji, & Xie, 2014). Compared with constant temperatures, variable temperatures can improve the survival rate of insects and prolong their survival time. For example, the hatching rate of *Gryllus bimaculatus* egg at a constant temperature of 20 °C is only 18% but rises to 49 – 65% under the diurnally alternating temperatures condition of 8 h (8.00 to 16.00) at 28 °C: 16 h at 16 °C (Behrens, Hoffmann, Kempa, & Merkel-wallner, 1983). The emergence rates of *Trichogramma japonicum*, *Trichogramma Conflum*, and *Trichogramma nubilale* improved after variable temperature treatment relative to their counterparts incubated under thermophilic conditions only (Zhu & Zhang, 1987). The emergence rate of *Aphidius gifuensis* after variable-temperature treatment is higher than that under a constant low temperature. Thus, the variable-temperature storage technology may be more suitable for the breeding and release of *A. gifuensis* (Tang et al, 2011). However, some studies have shown that low temperatures reduce the hatching rate of insect eggs. For example, Liu et al (2020) reported that low temperatures (4 °C and 7 °C) significantly lowered the egg hatch rate of *Acheta domesticus*. The authors believe that low temperature reduces the metabolism in *A. domesticus* eggs, making it impossible for them to either form a structure resistant to low temperature or to undergo complete embryonic development. Consequently, the egg-hatching rate declines under low temperatures. According to the findings of this study, low temperatures (5 °C and 10 °C) significantly increased the hatching rate of *E. sinensis* eggs. Clearly, the effect of temperature change on the hatching of insect eggs varies significantly across different species.

Generally, our findings concluded that low temperatures and high temperatures both significantly affect the hatching of *E. sinensis* eggs. Specifically, compared with low temperatures, high temperatures more significantly affected the hatching rate of *E. sinensis* eggs, which generally increased the hatching rate. Studying the effect of temperature change on the embryonic development and hatching of insect eggs provides solid evidence on the artificial breeding of insects. It should be pointed out that, in addition to temperature, other abiotic factors, such as humidity, photoperiod, latitude, and altitude, may also affect the hatching of *E. sinensis* eggs to varying degrees. Moreover, a strong interaction and synergy may exist between them and temperature. Therefore, it is indispensable to identify the biotic and abiotic factors affecting the hatching of *E. sinensis* eggs, as well as the interaction and synergy between various environmental factors in the future.

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