



MATE GUARDING BEHAVIOUR IN RESPONSE TO TEMPERATURE IN PARTHENIUM BEETLE *ZYGOGRAMMA BICOLORATA* PALLISTER

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ABSTRACT

Insects are ectothermic organisms in which most of the biochemical, physiological and behavioral processes may depend on thermal conditions of surrounding environments. Here, we anticipated that the copulatory parameters may also depend on different rearing conditions. So, it was hypothesized that developmental thermal conditions might play crucial role in modulation of mate guarding as well as reproductive outputs of *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae). In result, deaths of larval instars and pupa were observed along with underdeveloped adults at extreme developmental thermal conditions (15°C and 35°C). On the other hand, maximum time to commencement of mating (TCM) was observed at 20°C and minimum at 25°C. But latent period (LP) and mate guarding duration (MGD) were maximum at 30°C and minimum at 20°C and 25°C respectively. Further, result showed increase level of reproductive output at 20°C. So, it can be concluded that developmental temperature significantly influenced mate guarding and reproductive attributes.

Key words: Thermal regimes, reproductive behavior, copulatory parameters, TCM, LP, MGD, reproductive attributes, fecundity, percent eggs viability, *Z. bicolorata*

A fundamental query in behavioral ecology and evolution is why different behavioral activities varies in across the group of organisms; and it has long been known that biotic and abiotic factors play crucial role for all such activities. The biotic factors include any living species which is associated with other species for different behavioural patterns whereas major abiotic factors are photoperiod, humidity, CO₂, and temperature (Gracia-Roa et al., 2020). Among them, temperature is a prominent abiotic factor that modifies a wide range of physiological, morphological, and behavioral patterns (Gracia-Roa et al., 2020). It affects individuals and populations on a wide range of taxonomy such as invertebrates (Leith et al., 2021) to the vertebrates phyla (Zhao et al., 2022). All across the phyla, arthropod represents one of the largest and diverse forms of life on the earth, in which members of Insecta are cosmopolitan in distribution (Kikuchi, 2009), due to its highly adaptive efficiency (Darwin, 1859). Since, insects are mostly ectothermic their behavioural and physiological functions are decided by the surrounding temperature (Fields, 2001). Sexual selection is also modulated by temperature like other behaviour (Gracia-Roa et al., 2020).

In sexual selection, mate guarding is one of the most important mechanism of post copulatory sexual selection

which offers maximum paternity success towards the guarding male (Elgar et al., 2000; Simmons, 2001). Mate guarding is a prolonged period of mating which is beyond the time period required for the ejaculate transfer (Simmons, 2001). Such post mating interactions might have several profits for engaged partners (Alcock, 1994) by minimizing the sperm competition (Vahed et al., 2011). It can be done either by preventing female re-mating (Parker, 1984) or by inserted aedeagus in female genitalia to prevent sperm ejection/ flushing removal after the mating (Chaudhary et al., 2015). It might be affected by different factors which includes male resistivity (Rowe, 1992), receptivity of female (Mossinson and Yuval, 2003), degree of sperm transfer (Dallai et al., 2013), male density (Wada et al., 1999), mating intervals (Carroll, 1991), predation (Cothran, 2004), age and body size of the partners (Amin et al., 2012) etc.

For the current study, the model organism *Zygogramma bicolorata* Pallister has been used, which is known as parthenium beetle. It is one of the most effective biological control agents of *Parthenium hysterophorus* L. Several studies have been performed on this beetle regarding the biocontrol activity (Cowie et al., 2019; Hasan et al., 2020; Patel et al., 2020; Bhusal et al., 2020), diapause behaviour (Bali et al.,

2022), reproductive behaviour (Bali et al., 2022), paternity success (Afaq and Omkar, 2017) and post copulatory mate guarding (Bhaisare et al., 2021). After review of literature, the effect of temperature on time to commencement of mating (TCM) and latent period (LP), post copulatory mate guarding have not yet been studied. It is one of the best model to address the question arose in the present study as knowledge on post copulatory mate guarding has well established in this beetle (Bhaisare et al., 2021). So, present study aims to examine the influence of different thermal regimes on TCM, LP, post copulatory mate guarding, fecundity and percent egg viability. It was hypothesized that, variation in the developmental thermal condition may modulate the pre-copulatory parameters and reproductive outputs. These results will provide valuable evidences on the potential effects of thermal regimes on the reproductive behaviour and output of this beetles.

MATERIALS AND METHODS

Adults of *Z. bicolorata* were collected from the agricultural fields of Amarkantak (22°40'N, 81°45'E), India, to establish a laboratory stock. The beetles were fed with ad libitum fresh excised *Parthenium hysterophorus* leaves in plastic petri dishes (14.5×1.5 cm) and maintained in a BOD incubator (25±2°C, 65±5% RH and 14L: 10D) and adults were paired in petri dish for the oviposition. The withered leaves were replaced with fresh ones on a regular basis. Newly hatched larvae were developed in new petri dishes until they reached the fourth instar. Fourth instar larvae were then transfer to 500 ml glass beakers filled with moist sand for pupation. Newly emerged 10-day-old sexually mature adults were paired again for the oviposition and newly hatched larvae used for the further experimentation. First instar larvae from the stock were reared till the adult's emergence at 15, 20, 25, 30 and 35°C. After the emergence, adult male and female individually reared in separate petri dish, until they reach to their sexual maturity. After that, adult male and female of respective developmental thermal condition were kept in petri dish for mating and mating parameters such as time to commencement of mating (TCM), latent period (LP) (Bhaisare et al. 2021) and mate guarding duration (MGD) were recorded with Magnus stereoscopic microscope. After the natural disengagement, the female was isolated in new petri dish for further observation of reproductive outputs such as fecundity and egg viability. All the sets of experiment were performed in 10 replicates. The data were checked for normality and homogeneity of the variances using

Kolmogorov-Smirnov and Bartlett's tests, respectively. TCM, LP, MGD, fecundity and % eggs viability as a dependent factor were subjected to one-way ANOVA, followed by Tukey's post hoc comparisons of means, considering temperature as independent factor. All the statistical tests were performed using MINITAB 16 (Minitab Inc., Pennsylvania, USA).

RESULTS AND DISCUSSION

The deaths of larval instars and pupa were observed along with underdeveloped adults at extreme developmental thermal conditions (15 and 35°C). Unfortunately, adults for further experimentation had not been able to rear at these thermal conditions (Fig. 1A). One-way ANOVA revealed significant effect of temperature on TCM ($P=0.040$, $df=2$, $F=3.63$), MGD ($P=0.001$, $df=2$, $F=10.11$), fecundity ($P=0.000$, $df=2$, $F=16.96$) and eggs viability ($P=0.000$, $df=2$, $F=34.36$) but showed insignificant effects on the LP ($P=0.630$, $df=2$, $F=0.47$). Graphs showed maximum TCM (8.35 ± 1.09 min) at 30°C and it was minimum (4.59 ± 0.66 min) at 25°C (Fig. 1A). Further results also revealed that the LP was increasing with temperature (29.65 ± 3.81 sec, 31.78 ± 5.39 sec and 34.6 ± 5.91 sec) from 20 to 30°C respectively (Fig. 1B) but did not show significant effect. MGD was maximum (818.5 ± 72.31 min) at 30°C and it was minimum (383 ± 32.06 min) at 25°C (Fig. 1C). However, the reproductive output was opposite to the pre-copulatory parameters—it was found maximum at 20°C and subsequently decreased at 25 and 30°C. The maximum fecundity (26.88 ± 1.64 eggs) (Fig. 1D) and eggs viability (91.14 ± 1.66 % larvae hatched) (Fig. 1E) were observed at 20°C.

In the present study, extreme developmental thermal conditions (15 and 35°C) had not been tolerated by the larval, pupal and pre-mature adult stages leading to death. The intolerance of thermal shock might be because of the decrease in the titer of obligate bacterial endosymbionts which manipulate the thermal tolerance of insects host species as observed in aphids (Zhang et al., 2019). Further, approximately average temperature of the geographical area (22° 40' N, 81° 45' E) of collected beetle ranged between 20 to 30°C (Malviya and Dwivedi, 2015) which may be adopted by the beetle through the epigenetic changes, as temperature is one of the factor for the changes in the genome at epigenetic level (Richard et al., 2019). Hence, either positive or negative shift in this ambient temperature range may disturb the physiological and biochemical activities of this beetle which ultimately leads to intolerance.

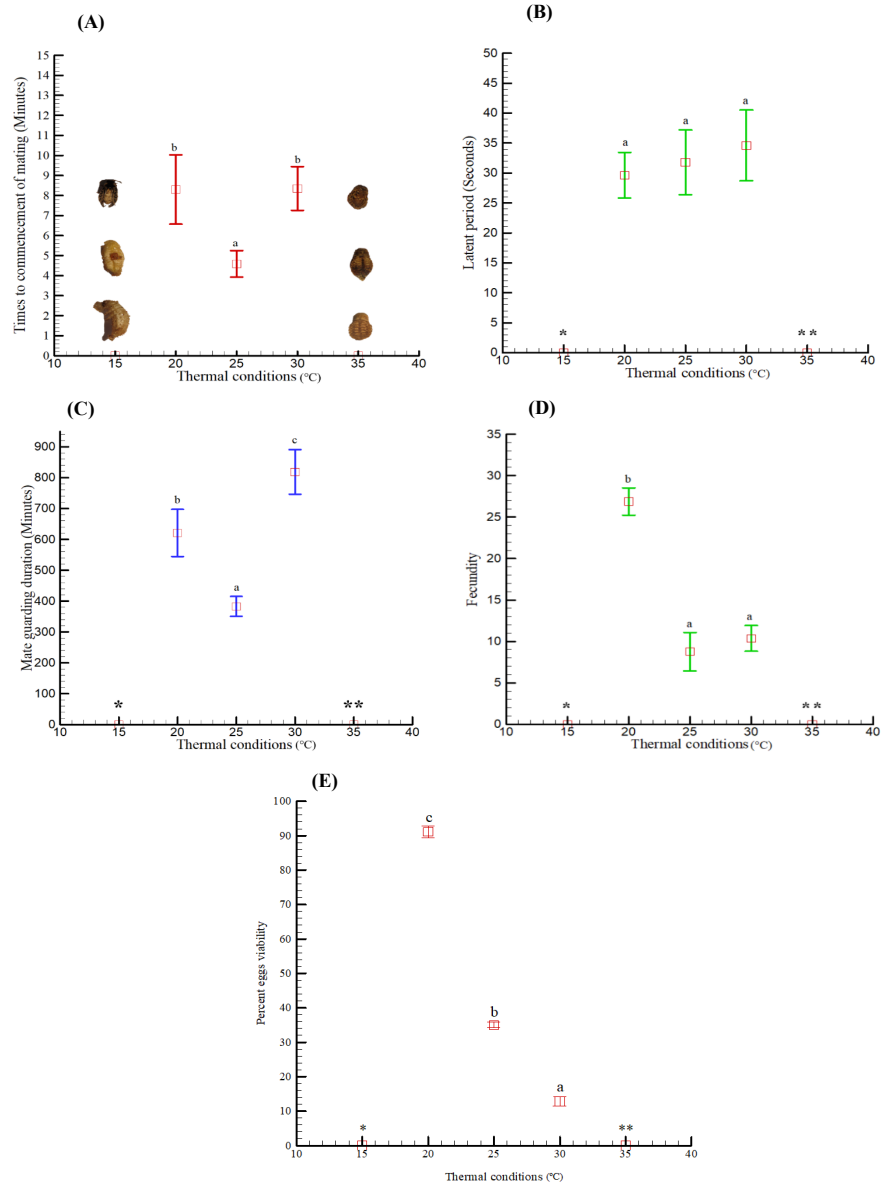


Fig. 1. Effects of developmental thermal conditions on: (A) times to commencement of mating, (B) latent period, (C) mate guarding duration, (D) fecundity and (E) eggs viability of *Z. bicolorata* (Values Mean± SE; Small letters represents comparisons of mean; Similar letters indicates lack of significant difference): *Death of different life stages at 15°C and **Death of different life stages at 35°C.

Further, results states that the maximum TCM was observed at 30°C and minimum at 25°C. It might be because of the mean time of the sex hormone release, which is progressive at low temperature in compare to the high temperature (Sower et al., 1971). It has been also studied that the release of sex pheromone is faster at the low temperature than at high temperature (Mbata, 1986; Sower et al., 1971). Apart from this, we also observed that LP increased with increase in temperature from 20 to 30°C but statistical analysis

showed insignificant effect of temperature on it. This might be because, LP is very short period of time, which implies no significant difference in all the thermal conditions. The similar findings were also reported by Spieth and Ringo (1983) and Ferveur et al. (1996) in *D. melanogaster*. The maximum mate guarding duration was recorded at 30°C and reduced at low temperature. The decrease in the mate guarding duration might be because it is physically difficult for males to transfer sperm or mate for longer duration at low temperatures

(Horton et al., 2002). The other finding also shows that low temperature reduces the intensity of biochemical and physiological processes (Kostal et al., 2004; Overgaard et al., 2007). Similar results were reported in *D. pseudoobscura* by Parsons and Kaul (1966) and in *Callosobruchus chinensis* (Katsuki and Miyatake, 2009).

Maximum fecundity was observed at 20°C. Similar results were recorded in *Scathophaga stercoraria* which showed that warmer temperatures give rise to smaller ovarioles due to which fecundity decreases (Blanckenhorn and Henseler, 2005). Another possibility is that under the thermal stress condition decrease in fecundity due to the direct cessation of eggs deposition during the oviposition period (Huang et al., 2007). Although the slight increase in temperature is non-lethal to adults but it may produce adverse effects for many physiological functions, particularly reproductive systems. Similar findings have been reported in *Stomoxys* (Gilles et al., 2005), *N. osculatus* (Ren et al., 2002) and in acarophagous ladybird beetle *Stethorus gilvifrons* (Aksit et al., 2007) and also some other insects such as *Eurosta solidaginis* (Irwin and Lee Jr., 2000), *D. melanogaster* (Hercus et al., 2003), and *Diplolepis spinosa* (Williams et al., 2003). A similar trend was also recorded for the eggs viability which was high at 20°C and it significantly decreases at higher temperatures. This decrease with increased temperature was possibly due to the inhibition of spermatogenesis or mortality of sperm in the spermathecae of female at high temperature (Ponsonby and Copland, 1998). In support, similar findings were also been reported in the *Anoplophora glabripennis* (Coleoptera: Cerambycidae) by Keena (2006). Apart from this, the warmer temperature might give rise to smaller testicular size which anticipated in the formation of smaller spermatophore which directly affect the fertilizations which leads in the reduction of egg viability (Blanckenhorn and Henseler, 2005). Thus, the highest egg viability was recorded at 20°C, indicating that this might be the optimum temperature for fertilization.

In conclusion, the temperature changes during development affect traits that are associated with pre- and postcopulatory sexual selection of *Z. bicolorata*. Thus, long-term variation in temperature can have significant effects on the fitness consequences for this beetle, via the interactions between temperature and pre- and postcopulatory processes. Moreover, the ambient temperature during development and mating can affect female mating because the number of sperms

transferred to females is sensitive to the temperature. Thus, slight positive or negative variation in the ambient temperature during development significantly modulate the reproductive performance, post copulatory mate guarding and reproductive outputs in *Z. bicolorata*. However, further study needs to be conducted to know the exact mechanism by which temperature modulate the sexual selection and reproductive attributes in this beetle and other ectotherm's.

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AUTHORS CONTRIBUTION STATEMENT

LYB Conduct of experiment, Data curation, writing - original draft, visualization, investigation. DDC conceptualization, methodology, software, validation, supervision, writing - review and editing.

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CONFLICT OF INTEREST

No conflict of interest.

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