Daily Temperature Cycle Induces Daily Hatching Rhythm in Eastern Lubber Grasshoppers, Romalea microptera

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Daily temperature cycle induces daily hatching rhythm in Eastern Lubber Grasshoppers, *Romalea microptera*

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Abstract

In nature, Eastern Lubber Grasshoppers, *Romalea microptera*, tend to hatch in the morning, thus exhibiting a daily hatching rhythm. They also show high intra-pod hatching synchrony. We tested the hypotheses that both diel-hatching rhythmicity and intra-pod hatching synchrony are elicited by a daily temperature cycle. In the laboratory, we exposed egg pods to either a daily 15.26 °C temperature cycle (12 h at each temperature) or a constant 26 °C temperature regimen. Under the daily temperature cycle, hatching was strongly rhythmic, with peak hatching at 3 h after the beginning of the warm phase. Under the constant temperature, hatching rhythmicity was greatly reduced and eggs hatched throughout the 24-h period. There was little intra-pod hatching synchrony under either treatment. We propose that in nature both daily hatching rhythm and intra-pod hatching synchrony are controlled by a thermal threshold for hatching.

Key words

hatching, egg hatch, daily rhythm, circadian rhythm, grasshopper, threshold temperature

Introduction

Virtually all animals exhibit daily behavioral and physiological rhythms (Zaslawski 1988; Koukkari & Sothern 2006; Nelson et al. 2010). Daily rhythms can be expressed by individuals, such as when foraging, sexual, or roosting behaviors are repeated at the same time each day by an individual (Saunders 1976; Loher 1989). Daily rhythms can also be a property of the population, as with once-in-a-lifetime events, such as hatching, adult eclosion, or oviposition (Saunders 1976; Matthews & Matthews 2010; Nelson et al. 2010).

In general, daily rhythms in animals result from one of two main influences. In some cases the rhythmic activity is stimulated or elicited exogenously by an acute rhythmic environmental factor, such as when a threshold level of light, temperature, moisture, or mechanical disturbance is reached (Lockwood & Story 1985; Tomioka et al. 1991; Danks 2005; Bradshaw & Holzapfel 2010). In other cases, the timing of the activity is determined endogenously by an internal biological clock, which itself was previously set by a zeitgeber (an external oscillating environmental factor that entrains a biological rhythm) (Bünning 1967; Danks 2006; Köstál 2011; Saunders & Bertossa 2011). Often, both exogenous and endogenous processes work together to determine the specific pattern; i.e., acute, current environmental stimuli alter the expression of existing circadian rhythms (Lockwood & Story 1985; Tomioka et al. 1991).

Insect hatching often exhibits a strong diurnal rhythm. Some insect species only hatch at night (Beck 1980; Raina 1982), some during the day (Michiyo 1989; Ramachandran 1989), some primarily at or just before dawn (Beck 1980; Itoh & Sumi 2000; Schilman et al. 2009), and others mainly at or just after dusk (Corbet 1962). In other species, hatching time is random, or it varies over the 24-h period, based on some non-rhythmic environmental factor (Chapman 1998; Vitek & Livdahl 2009).

Little is known about daily hatching rhythms in the approximately 11,100 species of grasshopper, despite the importance of hatching biology in gaining a broader understanding of the population dynamics of pest grasshoppers and locusts. Most of the few and often anecdotal reports about hatching patterns suggest that grasshoppers hatch in mid- to late morning (Kelly & Middlekauff 1961; Uvarov 1966, 1977; Farrow 1975). However, there are also scattered observations of grasshopper populations hatching primarily at or before dawn (Ashall & Ellis 1962; Wardhaugh et al. 1969; Hunter-Jones 1970), throughout the entire day (Pickford 1966), or with appreciable hatching after dark (Pickford 1976). It appears that immediate environmental factors such as temperature and moisture can greatly affect this timing (Ashall & Ellis 1962; Bernays 1971; Uvarov 1977). For example, the same population of Siberian grasshoppers hatched in late morning on a warm and sunny day, but hatched in the afternoon on a cool and cloudy day (Rubtsof 1935). A sudden strong decrease or increase in temperature, due to a sudden cooling rain or warming due to cloud dissipation, can terminate or initiate hatching of grasshoppers in the field (Pickford 1966, 1976; Uvarov 1977). In some desert grasshopper species, rain is reported to cause immediate hatching (Uvarov 1977), but this needs confirmation. Even mechanical stimuli can elicit hatching (Bernays 1971; Farrow 1975). Hence, at this time, we do not understand the importance or priority of environmental factors that influence the timing of hatching in grasshoppers. In this laboratory study, we examine hatching rhythms in Eastern Lubber Grasshoppers, *Romalea microptera* (Beauvois), from the Everglades region of south Florida.

The Eastern Lubber Grasshopper ranges across the southeastern USA (Rehn & Grant 1961). They are univoltine, depositing underground egg pods in the summer and fall, containing anywhere from 15 to 78 eggs (Stauffer & Whitman 2007; Taylor & Whitman 2010). In the vertical egg pods, individual eggs can lie from 1.1 to 9 cm below the ground (Stauffer & Whitman 2007). In south Florida, the hatching period extends from the beginning of February through mid-March, but peaks at the end of February (Stauffer & Whitman 2007). Average daily low and high air temperatures for February in the Everglades area are 8.4 and 26.0 °C (NOAA). In the field,
lubbers tend to hatch in the late morning (Whitman, unpub.). In addition, there is a tendency for the eggs in a single pod to hatch synchronously in the wild. For example, it is not uncommon for 15 to 25 insects to emerge from a single pod within a 30-min period (Whitman, unpub.). In nature, synchronous hatching would be highly advantageous. Lubbers are toxic to most vertebrates, and individuals benefit by hatching in unison, because most naïve vertebrate predators would not attack more than a single grasshopper (Whitman & Vincent 2008). Likewise, synchronous hatching could overwhelm the feeding capacity of those predators that are less deterred by lubber toxins, such as spiders (Whitman & Vincent 2008). In contrast to field observations, in the laboratory under fairly constant 26 °C, the grasshoppers tend to hatch individually and randomly throughout the 24-h day, rather than synchronously at a certain time of day. This suggests that in the field, daily cyclical environmental conditions may stimulate daily synchronous hatching rhythms in lubber grasshoppers, but that both rhythmicity and synchronicity of hatching are eliminated under uniform laboratory conditions. We therefore proposed and tested the following hypotheses and predictions:

**Hypothesis 1:** Daily hatching rhythms in lubber grasshoppers are set by daily temperature cycles.

**Prediction 1:** When reared under a 12:12-h alternating temperature cycle, lubber eggs will exhibit a 24-h hatching cycle. When maintained at a constant temperature, eggs will exhibit no diel-hatching rhythm.

**Hypotheses 2:** Daily temperature rhythms stimulate synchronous hatching within individual egg pods of lubber grasshoppers.

**Prediction 2:** When reared under a 12:12-h fluctuating temperature cycle, eggs within individual egg pods will tend to hatch in unison. Egg pods reared at a constant temperature will not show intra-pod hatching synchrony.

**Methods**

**Study insect.**— *Romalea microptera* (Beauvois) grasshoppers (fam.: Romaleidae) were collected from the Everglades wetlands region in south Florida in 2008 and thereafter cultured continuously at Illinois State University as per Matuszak and Whitman (2001). We used eggs from laboratory-reared females because it allowed us to standardize their conditions and efficiently obtain high numbers of pods.

**Egg cups.**— We allowed gravid females to oviposit into 1-liter plastic cups filled ⅔ full with damp sand (Chladny & Whitman 1997; 1998). Each cup contained a single egg pod consisting of approximately 20 to 75 eggs. Egg pods were sealed with a tight-fitting lid and stored on open shelves in the laboratory at 26 ± 1.5 °C; we periodically opened and added small amounts of tap water to the cups to maintain the sand at approximately 7% moisture level. In the laboratory, pods begin to hatch 4.5 to 6.5 months after laying, and individual pods may continue hatching for 1 to 3 weeks (Stauffer et al. 2011). Individual eggs can be as deep as 9 cm under the ground, and this depth causes some verniform (fresh-hatched) nymphs to die before reaching the surface. We therefore removed approximately 2/3 of the sand from each cup, which made it easier for nymphs to survive the transit through the soil. For the experiment, we chose 80 egg cups that ranged from 4 to 6 months old. Hence, some of the oldest cups had already started hatching, whereas the youngest pods were not due to hatch for another month. Treatments 1 and 2 (see below) each received 40 cups, balanced for age. Note that each of the pods used in our experiment had been maintained in individual cups, at room temperature, on open laboratory shelves for 4-6 months prior to the start of the experiment.

**Experimental design.**— We employed three treatments, each in a temperature-controlled environmental chamber, with constant light:

**Treatment 1:** 40 egg cups, each containing a single egg pod, maintained for 12 d at a 12:12-h thermoperiod of 15:26°C. Incubator temperature change was at 8 am and 8 pm, local time.

**Treatment 2:** 40 egg cups, maintained for 8 d at 26 ± 0.5°C.

**Treatment 3:** At the completion of Treatment 2, the conditions for the Treatment 2 egg cups were changed to Treatment 1 conditions, for 4 d — i.e., the egg cups were held for 8 d at constant 26°C, and then held for 4 d at a 12:12-h thermoperiod of 15:26°C.

We used 26°C as our warm temperature because it is far above the estimated threshold temperature for hatching (~ 15°C), but far below the maximum soil surface temperature that can occur in Florida. We selected 15°C for our low temperature because we believe it to be near the hatchling threshold temperature and because sub-soil temperatures in south Florida are generally below this temperature during the winter.

During the 12 d experiment, we recorded and removed all newly hatched nymphs from each cup, at the beginning of each hour. It is important to note that these data reflect the times that new hatchings reached the soil surface, and not when they hatched. There is an approximately 30-min time lag between actual hatching from the egg, buried several centimeters under the sand, and when the struggling vermiform nymph finally reaches the soil surface. Hence, the actual hatching of the eggs occurs (on average) approximately 1 h prior to the recorded emergence time.

For Treatment 1, we also recorded the rate of temperature change inside the soil at the exact position of the eggs: beaded thermocouple probes were inserted into the soil of 5 sealed cups held inside the environmental chamber. Wire leads were threaded through a small hole to the outside of the chamber and connected to a Bailey BAT-12 digital thermometer (Physitemp Instruments, Clifton, NJ). This allowed us to determine how long it took the eggs to thermally equilibrate following the change in the environmental-chamber temperature change from 15 to 26 °C, or vice versa.

**Statistical analysis.**— For each treatment, we combined the data for all days into 24 consecutive 1-h bins. We used binomial tests to compare the number of individuals hatched during the warm phase vs. the cold phase for Treatments 1 and 3, and the equivalent time periods (from 8 am to 8 pm vs. from 8 pm to 8 am, local time) for Treatment 2.

**Results**

Treatment 1 egg pods, kept under a 15:26 °C daily temperature cycle and constant light, showed a strong daily hatching rhythm (Fig. 1A). During the 12 d of this treatment, significantly more eggs hatched during the warm than the cold phase (84 vs. 77 eggs; binomial test, p < 0.0001). The daily hatching pattern was expressed as a highly right-skewed normal distribution with peak “hatching” recorded at 3 h after the beginning of the warm phase (i.e., at the 11am counting) (Fig. 1A). As noted in the Methods section these data represent when new hatchings were observed on the soil surface; actual egg hatch (ecdlosion from the egg) was likely about 1 h earlier.

Treatment 2 egg pods kept at constant 26 °C and in constant light also showed a daily hatching rhythm (Fig. 1B), with significantly more eggs hatching between 8 am and 8 pm than between 8 pm and 8 am (59 vs. 32 eggs; binomial test, p = 0.006). However, rhythmic-
ity in Treatment 2 was much muted compared to Treatment 1. For example, 60% of Treatment 1 eggs hatched between 9 am and 1 pm, vs. only 19% of Treatment 2 eggs during the same 4-h period. When these Treatment 2 egg pods were switched from a constant 26 °C temperature to a cyclical 15:26 °C daily temperature regime (Treatment 3), a very strong diel-hatching rhythm appeared, with 82 eggs hatching during the warm phase and no eggs hatching during the cool phase (binomial test, p < 0.0001) (Fig. 1C); this is similar to the hatching pattern seen in Treatment 1 (Fig. 1A). Furthermore, 56 of these hatchings (68%) occurred in the first day following the temperature regime change. This suggests that hatching rhythms can respond rapidly in response to changes in temperature cycles.

None of the three treatments exhibited high hatching synchrony. If we arbitrarily define hatching synchrony as eight insects hatching from a single pod within a 3-h period, we find that of the 40 cups in each treatment, only 3 Treatment 1 cups (during 12 d), 1 Treatment 2 cup (during 8 d), and 2 Treatment 3 cups (during 4 d) exhibited synchronous hatching. Hence, the conditions in our laboratory experiment failed to produce the intra-pod hatching synchrony observed in the field.

The sand inside the sealed egg cups, at the position of the buried eggs, required 47 ± 8.0 min (mean ± SD) to reach the new temperature, following a change in incubator air temperature from warm to cold or vice versa (i.e., 26 °C to 15 °C or 15 °C to 26 °C). However, half of the temperature difference (i.e., 20.5 °C) was achieved after only 16.0 ± 4.5 min.

Discussion

Our results support the hypothesis that daily hatching rhythms in lubber grasshoppers are set by daily temperature cycles. Eggs maintained under a daily thermal rhythm (Treatment 1) showed a pronounced daily hatching rhythm, whereas eggs maintained at a constant warm temperature (Treatment 2) showed low hatching rhythmicity. The results from Treatment 3 show that hatching patterns in the same egg pods can be rapidly changed from low to high rhythmicity by simply imposing a temperature cycle. Whether these conspicuous hatching rhythms derive primarily from endogenous or exogenous control, or their interaction, remains unexplored. However, the rapidity of rhythm establishment in Treatment 3 (i.e., within hours of switching the eggs from a constant to a fluctuating temperature, a very strong daily hatching rhythm was established, with no eggs hatching during the cold-phase), implies that acute (current) temperatures can immediately alter lubber hatching patterns.

Acute, direct thermal control of hatching has been shown for some insect species (Miyazaki et al. 2011; Tanaka & Watari 2011), including some grasshopper species (Parker 1929; Shotwell 1929, 1941; Rubtzov 1935; Padgham 1981). The physiological mechanism underlying such a process in temperate species may involve a thermal threshold for hatching. Hatching involves a series of behaviors, and, as such, requires muscle contraction, which, itself, is strongly temperature-dependent (Josepshon 1981). Indeed, thermal-performance studies on the closely related Western Lubber Grasshopper, *Taeniopoda eques* (Burmeister), show that nymphs will not initiate molting below 25 °C, and die if moved to 21 °C after molting has started, because they are unable to complete the behavior at that low temperature (Whitman & Orsak 1985; Chappel & Whitman 1990). Hatching is similar to molting in that both require muscle activity, and both involve emerging from an external “covering”. To hatch, the insect must break the egg shell, wiggle out of the egg shell, struggle up through the soil to reach the surface, and then
molt out of the provisional cuticle (Uvarov 1966). These behaviors are all muscle-based and require relatively warm temperatures for these cold-blooded insects.

Because muscle power, contraction rate, and contraction speed all decline precipitously at cold temperatures (Josephson 1981), it would be disastrous for grasshopper eggs to hatch at cold temperatures. At low soil temperatures, the tiny vermiform nymphs would die, because they would be unable to push their way through several centimeters of soil or egg-pod foam (Stauffer & Whitman 1997) to reach the surface. Hence, as is the case with molting, there is probably a low-temperature limit (threshold temperature), below which R. microptera refuse to, or are unable to hatch. As such, we propose that in nature, lubber hatching is triggered acutely when mature embryos reach the temperature threshold for effective muscular activity. We further propose that embryos develop at both cool and warm soil temperatures, but then upon reaching full maturity, wait until a proper temperature to initiate hatching. This does not preclude an additional or dominant endogenous circadian component. As shown in many insects (Edwards 1964; Lockwood & Story 1985) and some grasshoppers (Rubtzov 1935; Padgham 1981), acute temperatures may interact with entrained circadian rhythms to determine time and pattern of hatching. Our hypothesized low-temperature threshold for lubber hatching must be close to 15°C, based on the fact that in our experiment only three eggs hatched at that low temperature (Fig. 1A). Note that in Fig. 1A, the three new hatchlings found at the 9 pm recording period (1 h after the temperature changed from cold to warm), probably initiated hatching while their surrounding soil was still warm, and hence, likely hatched before the change from 26 °C to 15 °C.

We were surprised to observe a statistically significant hatching rhythm in Treatment 2 egg cups held under constant temperature (26 °C) and light in an environmental chamber (Fig. 1B). Over the 8 d of this treatment, 59 eggs hatched during the local “day” (8 am – 8 pm), and 32 during the local “night” (8 pm – 8 am). We offer two hypotheses for this observation: (1) The eggs were entrained to a daily rhythm prior to being placed into constant conditions in the environmental chamber, and subsequently expressed rhythmicity, due to an internal biological clock. (2) The eggs buried in sand, inside sealed containers, inside an environmental chamber, received some sort of external stimulus associated with the outside diel pattern. We would not expect the first explanation, because the day/night temperature difference in our lab, where the eggs cups were kept for approximately 5 months prior to this experiment, was only 1 °C. Alternatively, the eggs could have been entrained by the strong daily photoperiod in our lab. But we would not expect grasshopper eggs, which, in nature, are buried under ground (Stauffer & Whitman 1997), to have evolved to respond to light cycles.

Our experiment did not support the hypothesis that daily temperature rhythms stimulate synchronous hatching within individual egg pods. Indeed, we found very low levels of synchronous hatching among our 80 egg cups. Thus, our laboratory results were different than what occurs in nature. We suggest the following explanation: in many temperate grasshopper species, the eggs overwinter and are thus exposed to low temperatures for months (Uvarov 1977). Presumably, embryonic development proceeds at these low winter temperatures (Chapman 1998). Hence, by early spring, many of the buried lubber eggs (embryos) are mature and ready to hatch, but do not do so, because temperatures at that depth are below the threshold for hatching. When spring arrives, sub-soil temperatures begin to increase. Eventually, temperatures at the depth of the buried eggs surpass the thermal-threshold for hatching, eliciting synchronous intra-pod hatching in those eggs. In this scenario, the hatching threshold temperature acts as a thermal gate: over time, it retains a large number of mature embryos. When soil temperatures finally exceed the low-thermal threshold for hatching, the gate is opened, allowing large numbers of eggs to hatch synchronously from that pod. Likewise, when many nearby pods reach the thermal-hatching threshold simultaneously, multiple pods begin to hatch, producing inter-pod hatching synchrony, as well. In the field, this condition occurs in mid- to late morning, after heat from the morning sun conducts down to the level of the buried eggs. In contrast, in our laboratory experiment, eggs were exposed to optimal hatching temperatures daily, allowing daily hatching of any mature embryos. As such, there was little build-up of mature embryos behind a “thermal gate,” and thus, little intra-pod hatching synchrony.

The above scenario explains both the rhythmic and synchronous hatching pattern observed in lubber grasshoppers in the Everglades region of south Florida. In mid-February in the Everglades, daily high air temperatures average approximately 26 °C, whereas night temperatures reach lows of 0 to 14.4 °C (NOAA). Hence, soil surface temperatures are coolest at dawn, but then begin to warm as solar radiative heating and air temperatures increase in the morning. Eventually those warmer temperatures are conducted to the level of the buried eggs, stimulating the late morning hatching peak that we observed in nature. Although their habitat is densely vegetated, lubbers tend to lay in open, less shaded habitats (Stauffer & Whitman 2007), where the soil would be heated by direct sunlight. In such cases, the soil surface can heat far in excess of the air temperature (e.g., Whitman 1987; Stauffer & Whitman 2007). These hot, sun-exposed soils could conceivably elicit hatching, even on cold days. Thus, it is possible for some Florida lubbers to hatch in early February when soil temperatures in sunny spots are hot, but air temperatures are actually below the low-thermal hatching threshold. Note that this laboratory study eliminated the chaotic conditions that exist in nature, where individual females and egg pods experience different and varying conditions. Future field studies will sort out these variables, as well as help to identify the fitness consequences of variation in hatching time of lubbers in nature.

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