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Preliminary Observation on the Mating Behavior and Daily Rhythm of the Ussur Brown Katydid, *Paratlanticus ussuriensis* Uvarov (Orthoptera: Tettigoniidae)

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갈색여치의 일일행동패턴과 교미행동

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ABSTRACT: The ussur brown katydid, *Paratlanticus ussuriensis* (Orthoptera: Tettigoniidae), causes substantial damage in orchard areas in South Korea. Daily rhythm and mating behaviors were investigated in last instar nymphs, immature adults and mature adults of *P. ussuriensis*. The daily rhythm was repeated upon resting, moving, and feeding. The daily rhythm of the movement behavior similar in both last instar nymphs and immature adults. Last instar nymphs becoming active at 10:06 and ceasing activity at 21:12 in average. Immature adults becoming active at 11:30 and ceasing activity at 20:08 in average of totally 10 individuals. However, mature adults showed a different active time of 15:30. Generally, behavior was affected by the conditions of their environment, especially temperature. Mature adults activated later than nymphs and immature adults. Mature adults display mating behavior at a particular time of day (approximately 20:00). *P. ussuriensis* showed a mating behavioral sequence which was similar to related species in completely retaining the spermatophylax. Females took 270 min to 360 min to completely retain the spermatophylax. The daily rhythm of *P. ussuriensis*, indicating its occurrence and ecology, could be useful as basic data for managing field populations of *P. ussuriensis*.

Key words: Daily rhythm, Mating behavior, Ussur brown katydid, *Paratlanticus ussuriensis*

초 록: 갈색여치는 최근 대발생으로 과수농가에서 피해가 급격히 증가하고 있다. 갈색여치의 종령 약충과 미성숙 성충, 성숙 성충에 대한 교미행동 및 일일행동패턴에 대하여 조사하였다. 일일행동패턴은 휴식, 이동, 섭식을 반복하였다. 종령 약충과 미성숙 성충의 일일행동패턴은 유사했다. 종령 약충은 평균 10:06에 활동을 시작하고 21:12에 휴식을 취한다. 미성숙 성충은 평균 11:30에 활동을 시작하고 20:08에 휴식을 취한다. 그러나 성숙 성충의 경우 15:30에 활동을 시작하는 차이를 보였다. 성숙 성충은 특정시간(약 20:00)대에 교미행동을 보인다. 갈색여치의 교미행동은 암컷의 spermatophylax를 섭취하게 되는데 다 섭취하기까지 270-360분이 소요된다. 갈색여치의 행동패턴은 이들의 발생양상과 생태를 알 수 있어 갈색여치를 방제하기 위한 기초자료로 이용될 수 있을 것이다.

검색어: 일일행동패턴, 교미행동, 갈색여치

Climate change disturbs the habitat of pests and of beneficial species (Fuhrer, 2003; Menendez, 2007). This increased pressure on pests is expected to disturb the stability of the pests, encouraging their transitions into new niches. Some pests, such as the ussur brown katydid (*Paratlanticus ussuriensis*), spot clothing wax cicada (*Lycorma delicatula*), rice planthopper (*Nilaparvata lugens*) and oriental armyworm (*Pseudaletia separate*) have occasionally exhibited severe outbreaks of their population in South Korea, causing increased levels of crop damage in plants (Lee et al., 2009; Moon et al., 2009).

Paratlanticus ussuriensis is mainly known to inhabit broadleaf trees, especially those in Northeast China, including Ussuri, Russia, and South Korea, but it is not technically classified as a pest (Bae, 1999). *P. ussuriensis* belongs to Orthoptera: Tettigoniidae

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(Paek et al., 2010), a subfamily that includes 135 genera and 920 species worldwide (Naskrecki and Otte, 1999). However, *P. ussuriensis* katydids are omnivorous, and their search for food can target forests or nearby agricultural farms, depending on the ecology, food supply and changes in climate. *P. ussuriensis* have spread throughout the central regions of South Korea, beginning with Chungju city in 2001, followed by Chungbuk Province in 2006, and Chungbuk Province and Chungnam Province in 2007. Consequently, damage to fruit orchards and farms has also increased (Ahn et al., 2007). Research related to katydid species similar to *P. ussuriensis* was initiated several decades ago, but our knowledge of the ecology of these species remains insufficient (Simmons and Gwynne, 1991; Higaki and Ando, 1999; Castner and Nickle, 1995). Research on *P. ussuriensis* has determined its distribution in Korea, its ovipositional characteristics, the temperature effects on diapause termination in its eggs and embryonic development, its seasonal occurrence and developmental characteristics and the various ecological factors relating to control of this pest (Ahn et al., 2007; Noh et al., 2008; Bang et al., 2008; Moon et al., 2009; Bang et al., 2009; 2011; Jung et al., 2011). No report related to the diel rhythm and mating behaviors of the species is yet available.

This study was performed to understand the basic ecology of the ussur brown katydids, *P. ussuriensis*. The last nymph instars as well as immature and mature adults were compared in terms of their diel behavioral rhythms. The mating behaviors of mature male and female katydids were also studied.

Materials and Methods

Insects

The insects used were collected from the hills of Bitan-ri, Yeongdong-Gun, and Chungbuk. Nymphs were randomly captured from three different sites in May of 2007 and 2008. Nymphs were moved to the laboratory, provided fishmeal and fresh bean leaves from 4-week-old plants for food, and kept in individual plastic containers (9 cm in diameter × 5 cm height).

The last instar nymphs (7th instar) of *P. ussuriensis* and immature adults (less than 3 days after emergence) were used.

Emerged adults were classified as either male or female and kept separately in plastic containers. The containers were kept at a constant temperature of 25±1℃ with a 14:10 h (L:D) photoperiod at 60±10% relative humidity.

Daily Rhythm Observations

The diel behavioral rhythms of the last instar nymphs, immature adults and mature adults of *P. ussuriensis* were observed. Nymphs were collected from 2007 to 2008 and were reared in an insectary until they had grown to a nymph stage appropriate for observation. The diel behavioral rhythms were observed independently. Last instar nymphs were observed from the 27th of June to the 1st of July, and immature adults were observed from the 3rd of July to the 7th of July in 2008. The general daily behaviors and mating behaviors of male and female adults were observed from the 26th to the 30th of July in 2007. All experiments were performed at the same location in an outdoor net cage (2.5 × 2.0 × 1.8 m^3) in a forested area near the campus of Chungbuk National University, Cheongju, Chungbuk, Korea (N36°37′, E127°27′). Insects were introduced to the outdoor environment one day before observations to adapt the insects to the new environment. For each observation period, the behaviors of the insects, including daily rhythm, mating and movement, were continuously recorded in detail for 24 h. Oak pollen and fishmeal were provided as food, and 10 pairs of each stage of *P. ussuriensis* were released at the center of a net cage. Observations were repeated for five days. For the last instar nymphs and immature adults, investigations were conducted to determine the effects of temperature and humidity on movement and rest as well as the times for moving, feeding and resting. For mature adults, characteristics such as movement initiation time and temperature and humidity during movement and rest were determined. The temperature and humidity inside the net cage were constantly monitored using a HOBO Datalogger (Contoocook, NH, USA), which was hung on a corner of the net cage. A red-light was used for nighttime observation.

Mating Behavior of Mature Adults

A pair of mature *P. ussuriensis* adults was observed to determine the time of mating behavior procedures, such as calling song, antennal contact, licking, mounting, copulation

and consumption of the spermatophylax, and 24 h of observations were recorded.

The mating behavior of *P. ussuriensis* adults was determined in the same net cages used to determine diel behavior rhythms. The general daily behavioral rhythms and mating patterns were observed on the same day. In particular, mating behaviors were observed for male and female *P. ussuriensis* separately. During the observations, the temperature, humidity, movement and behavior of each insect were continuously recorded. Five replicate experiments were conducted.

Results

Measurement of behavioral conditions for each stage of *P. ussuriensis*

Behavioral times and environmental conditions were observed for three different stages of *P. ussuriensis*. The last instar nymphs were observed at 10:06 at the start of movement (Table 1). The average temperature at this time was about 26.1℃. The immature adults started movement and feeding at 11:30 and 15:37 respectively (Table 2). The last nymphs and immature adults had similar periods of movement throughout the day, whereas mature adults began to move later in the day. In terms of feeding, the last instar nymphs, immature adults and mature adults were found to have similar schedules; however, mature adults had a 2nd feeding time that started later than the nymphs and immature adults (Table 3). Mature adults showed significant differences with respect to the start of movement compared to the last instar nymphs and immature adults.

When the last instar nymph began to move, the temperature and humidity were 26℃ and 85.5%, respectively, and there was a significant difference (approximately 6℃) in the temperature at which immature (29.8℃) and mature adults (30℃) initiated movement and when they began resting. All stages of *P. ussuriensis* showed similar responses at feeding time. Feeding temperature was similar with moving temperature at all stage of *P. ussuriensis*. The 2nd feeding time, which occurred after mating, was observed only in mature adults. Mature adults of *P. ussuriensis* had a significantly later resting time (approximately

Table 1. Behavioral responsiveness and environmental condition to the daily rhythm of *P. ussuriensis* last nymphs

	I	II	III	IV	V	Average
No. of pairs	10	10	10	10	10	10
Activation starting temperature (℃)	27.7	22.9	24.3	28.3	27.2	26.1
Activation starting humidity (%)	74.3	94.8	86.7	84.3	87.5	85.5
Moving time	09:00	09:30	10:10	12:00	09:50	10:06
Feeding time	14:20	16:00	17:10	17:20	14:40	16:17
Resting time	19:00	21:20	22:00	21:30	22:10	21:45
Temperature at resting (℃)	19.7	17.9	20.1	19.3	20.9	19.6
Humidity at resting (%)	63.7	84.9	78.3	71.6	79.4	75.6

Table 2. Behavioral responsiveness and environmental condition to the daily rhythm of *P. ussuriensis* immature adults

	I	II	III	IV	V	Average
No. of pairs	10	10	10	10	10	10
Activation starting temperature (℃)	27.4	30.9	29.2	31.5	29.8	29.8
Activation starting humidity (%)	89.5	87.4	89.7	80.5	79.2	85.2
Moving time	13:20	12:15	10:25	11:30	10:00	11:52
Feeding time	16:15	14:30	16:00	15:20	16:00	15:53
Resting time	20:00	18:00	21:10	21:00	20:30	20:40
Temperature at resting (℃)	22.6	21.6	21.7	25.6	23.4	23.0
Humidity at resting (%)	76.4	81.2	83.5	78.4	83.7	80.6

Table 3. Behavioral responsiveness and environmental condition to the daily rhythm of *P. ussuriensis* mature adults

	I	II	III	IV	V	Average
No. of pairs	10	10	10	10	10	10
Activation starting time	15:30	16:00	15:40	14:00	15:40	15:22
Activation starting temperature (℃)	29.9	30.1	30.7	30.7	28.3	29.9
Activation starting humidity (%)	71.8	73.3	76.9	64.6	74.3	72.3
Feeding time	14:30	16:00	15:40	14:50	16:30	15:30
Calling song of male (time)	19:00	20:30	20:00	19:30	21:00	20:00
No. of attracting female	2	2	1	3	2	2
Starting time of licking	21:15	21:00	21:20	20:00	22:40	21:15
Starting time of mating	21:50	21:40	21:50	20:50	23:00	21:50
Ending time of mating	22:30	22:10	22:30	21:30	23:30	22:26
Consuming time of spermatophylax (min)	300	290	270	330	360	310
Average No. of mating	2	1	1	2	1	1.4
Resting time	3:30	3:00	2:00	3:00	5:30	3:12
Temperature at resting (℃)	23.1	24.8	25.0	22.5	22.1	23.5
Humidity at resting (%)	90.9	91.7	90.4	93.8	91.7	91.7

03:00) than the last instar nymphs and immature adults (approximately 20:00). Temperature and humidity were determined to identify their effect on *P. ussuriensis* movement. Immature adults and mature adults started to move at a similar temperature, and the resting temperature (23℃) was lower than the moving temperature (29.8℃) for both immature and mature adults of *P. ussuriensis* (Table 2, 3). Humidity was shown to have a significant effect on moving and resting. The time of movement initiation occurred at a higher humidity than the time of resting in both nymphs and immature adults. However, mature adults showed the reverse, as movement initiations occurred at lower humidity and the start of resting occurred at a higher temperature (Table 3).

Daily rhythm of *P. ussuriensis* at each stage

The daily rhythm of the immature adult stage was not found to be related to mating behavior (Fig. 1). The movement of immature adults involved actions such as foraging for food or shelter and feeding. Periods of movement were 3 h shorter in immature adults than in last instar nymphs. The resting time of immature adults was observed to last longer than that of last instar nymphs. All mature adults were observed to initially rest motionlessly in the branches but began to move at 15:20 and started feeding at approximately 15:30; these movement initiations started approximately 3 h to 5 h after the beginning in movement in last instar nymphs and immature adults. After sunset, mature adult started mating behavior, including their calling song, copulation and spermatophylax feeding, lasting until 3:00 AM. The female consumed the spermatophylax over a period of approximately 3 h, during which time males began feeding. Resting then continued until 15:00, when the mature adults started moving and feeding again.

Mating behavior

The mating behavior of the mature adult male of *P. ussuriensis* regularly began with a calling song signal to attract females (Fig. 2). Some females approached the male but did not respond immediately to the calling song, remaining motionless near the male. One female eventually approached the male and tried to come into contact with its antenna and then moved behind the male and licked the dorsal part under the male's wings. Within minutes, the female mounted the male and attempted copulation. Licking and copulation took some time, after which the calling song began again. Copulation of the male and female adult took approximately 30 min to 40 min. After copulation was finished, the male transferred the spermatophores, which are attached externally, to the genitalia of the female.

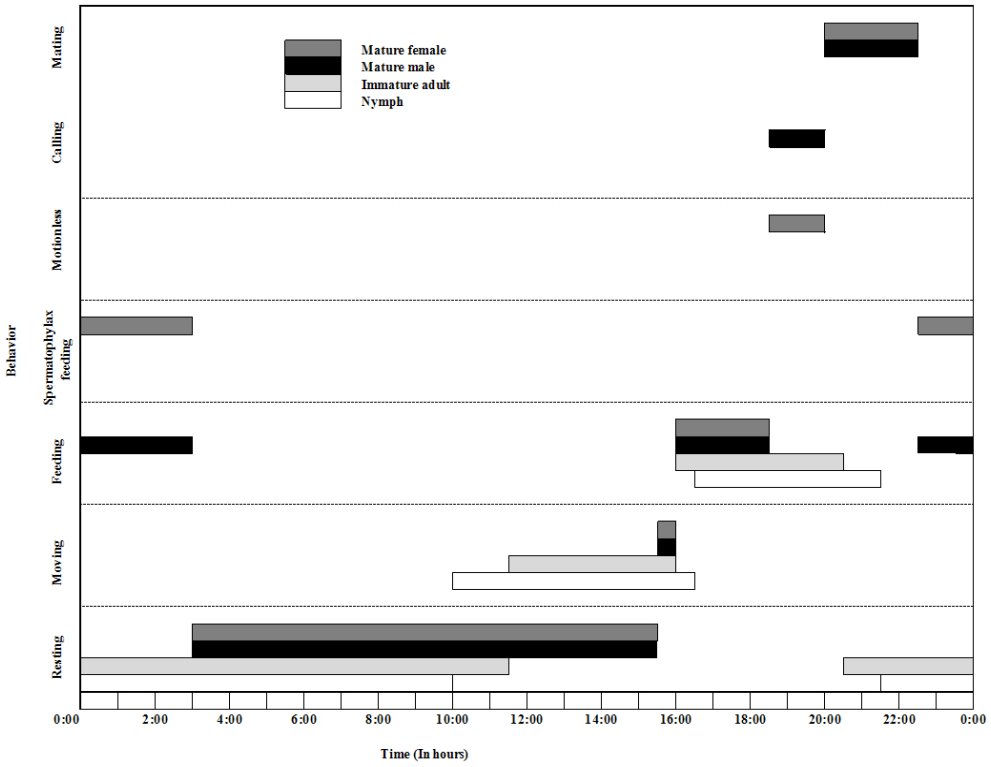

Fig. 1. General daily and mating behavioral rhythm of *P. ussuriensis* over 24 h. The daily behavior was divided into moving, feeding and resting. Mating behavior includes calling, motionlessness and spermatophylax feeding by the female.

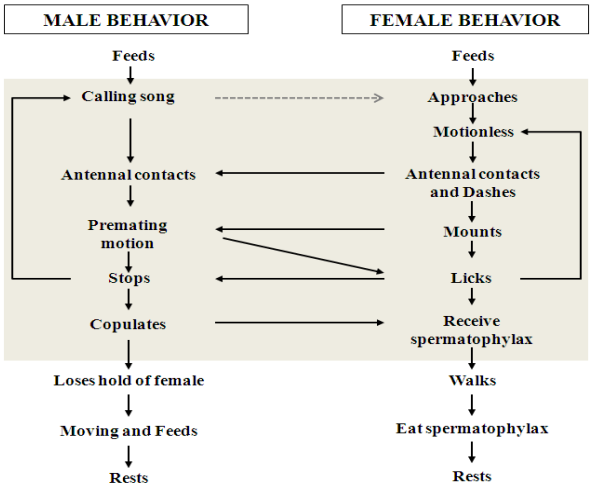

Fig. 2. Mating behavior sequence of ussur brown katydid, specifically in *P. ussuriensis* male and female adults.

Each spermatophore consisted of a spermatophylax, which is a white, highly nutritious protein material, and a sperm ampulla, which was transferred from the genitalia of male to the genitalia of female. Females then took 270 min to 360 min to eat the spermatophylax.

Discussion

The daily rhythms of *P. ussuriensis* were compared for 3 different stages. Generally, the behavioral patterns of nymphs and the adult stages of mites, aphids, and thrip are similar. For longer-life cycle insects, such as Lepidoptera and Coleoptera, heavy feeding takes place at the larval or nymph stages, while adults usually exhibit reduced feeding. Adults need sufficient energy for mating and oviposition during their relatively short life cycle (Danks, 2006). Nevertheless, adults of *P. ussuriensis* fed for a relatively long time. Newly emerged immature adults showed behavior times similar to those of the last nymph stage (Table 1, 2). Moving time and feeding time were reduced according to the developmental progression. The feeding range of *P. ussuriensis* was found to include weeds, oak and fruit tree leaves or fruit (unpublished data). The feeding preference of *P. ussuriensis* is based on nitrogen content, as reported by Kim et al (2009). However, the reasons for sudden surges in the population of *P. ussuriensis* are not clear. Surges in the population of grasshoppers cause limited food and cannibalism, which

results in the migration of swarms of grasshoppers. (Bazazi et al., 2008). The development and survival of *P. ussuriensis* were determined to be affected by temperature in a study of seasonal occurrence and growth characteristics (Moon et al., 2009). Embryonic development and the termination of egg diapause has been found to be associated with temperature in *P. ussuriensis* (Bang et al., 2009; 2011). The experiments reported here show varying differences in the behavior of each stage according to the temperature and humidity. The behavioral patterns were similar between the last instar nymphs and immature adults, but those of mature adults were different (Table 3). However, the temperatures of the initial time of moving showed a different pattern for nymphs compared both immature and mature adults. The humidity caused differences between the time of moving and the time of resting, and different patterns were observed among the 3 different stages of *P. ussuriensis*. However, these results suggest that there is no correlation between the humidity and behavior.

Generally, mating behavior occurred after sunset, at which stage nymphs and immature adults were resting (Fig. 1). The mating behavior and sequence of *P. ussuriensis* were similar to those of other katydids. The mating behavioral sequence of katydids generally progress begins with the calling song of males and the formation of the male and female pair. Courtship behavior follows, and then, copulation and transfer of the spermatophylax occurs followed by the after-mating behavior. Several studies of other katydid species have observed that females are attracted to the courtship songs of males, and the calling song is undoubtedly an important factor in mating behavior (Morris, 1980; Gwynne, 1982; Berg and Greenfield, 2005; Franklin et al., 2009).

Once a male produces his calling song, several females aggregate around the male, but only one female is allowed to make antennal contact. Heavy competition is involved, and both males and females exhibit high activity at this time. Therefore, it was difficult to determine which females actually copulated because copulation does not occur unless the spermatophylax is transferred. Females that had finished mating fed on the spermatophylax, including the spermatic sac, which hung at the end of the female abdomen. The size of the spermatophylax affects both the egg size and the number of eggs laid. For example, female katydids of the species *Requena verticalis* may eat the spermatic sac when the spermatophylax is too small (Gwynne, 1984). Male size is also important enough to mating that female katydids of the species *Conocephalus nigropleurum* (Tettigoniidae) always choose larger males because the larger male produces larger spermatophores (Gwynne, 1982). The spermatophylax is an important source of nutrition, and this ultimately influences mating patterns through sexual selection. Approximately 270 min to 360 min was necessary to completely consume the spermatophylax in *P. ussuriensis* (Table 3). Aggressive behavior and calling songs were more frequently observed under communal conditions compared to isolation. In the present study, a total of five released males and five released females resulted in communal conditions. Therefore, the experimental conditions likely affect the results.

Over the last several years, the habitat of *P. ussuriensis* has become broader, resulting in damage to fruit orchards and/or other economically important crops. Thus, it is necessary to determine their occurrence, as well as their specific ecology, so that further insight might be gained into methods of controlling *P. ussuriensis* populations.

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