Ecology of Meimuna mongolica (Hemiptera: Cicadidae) Nymphs: Instars, Morphological Variation, Vertical Distribution and Population Density, Host-Plant Selection, and Emergence Phenology

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RESEARCH

Ecology of *Meimuna mongolica* (Hemiptera: Cicadidae) Nymphs: Instars, Morphological Variation, Vertical Distribution and Population Density, Host-Plant Selection, and Emergence Phenology

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ABSTRACT. The cicada *Meimuna mongolica* (Distant) (Hemiptera: Cicadidae) is one of the most important pests of economic forest in Guanzhong Plain of Shaanxi Province, China. Information about ecological characteristics and some sustainable control measures of this species is urgently required for its control. In this study, nymphal instars, morphological variation, vertical distribution, and population density in soil, and emergence phenology of nymphs of *M. mongolica* on three main host plants (*Pinus tabuliformis* Carr., *Populus tomentosa* Carr., and *Pyrus xerophila* Yü) were studied, based on combined morphological and molecular identification, investigation of the first-instar nymphs hatched from eggs and others excavated from soil, and investigation of exuviae in the adult emergence period. Five nymphal instars of *M. mongolica* were redetermined according to the distribution plots of the head capsule widths of the nymphs. Nymphs of third and fourth instars showed morphological variation, which is closely related to host-plant association. The mean densities of nymphs in soil under the three host plants were significantly different, indicating a distinct host preference. The nymphs could extend their distribution from the 0–10 cm soil layer to the 51–60 cm soil layer underground but not beyond 60 cm soil layer under all the three host plants. The 21–30 cm soil layer under all the three host plants has the highest nymphal population density. The sex ratio of the entire population was nearly 50:50, but males dominated in the early half of the duration of the emergence. These ecological characteristics of *M. mongolica* could provide important information for sustainable control measures.

Key Words: Hemiptera, Cicadidae, molecular identification, immature stage, morphological variation

Adaptations of insects to their specific hosts are the consequences resulting from continuous environmental stress over evolutionary time. Host specificity allows insects to find nutrition and suitable habitat, which can well satisfy their needs of enough nutrition and adaptable habitat (Scriber 2002). When phytophagous insects lay their eggs on various host species, adult females do not select all hosts equally. Host preferences show a strong heritable component and are thought to express the suitability of hosts for larval survival (Singer 1983, Courtney et al. 1989, Singer et al. 1989, Thompson 1998). Suitability can depend upon a number of factors such as nutritional quality, host-plant defensive materials, and prevalence of natural enemies or microenvironment (Thompson and Pellmyr 1991).

Adult cicadas feed exclusively on the xylem fluid from branches of their host plants (Lloyd and White 1987). Further injuries caused by the feeding of cicada usually go undetected as their nymphs are long lived underground and feed exclusively on the xylem sap from roots of their host plants. Cicadas cause great harm including twig dieback in host plants when large numbers of certain cicada species insert eggs into the stems of trees and shrubs (Lloyd and White 1987). Although nymphal cicadas feed on xylem roots and cause damages to their host plants (White and Strehl 1978, Chou et al. 1997), Speer et al. (2010) concluded that some specific host-plant species exhibited longer term growth increase following the emergence event of periodical cicadas (*Magicicada* spp.) consistent with the nutrient pulse hypothesis. The cicada *Meimuna mongolica* (Distant) is widely distributed in South Palaearctic Region and North Oriental Region (e.g., North Korea, South Korea, Mongolia, and China). This species is one of the dominant pest species of the Cicadidae feeding on several host plants in Guanzhong Plain of Shaanxi Province, China, which lies north of Qinling Mountains, the convergence zone of the Palaearctic and the Oriental Regions and the natural boundary between northern China and southern China with various vegetations and complex environments (Chou et al. 1997).

As cicadas are exclusively subterranean in nymphal stage, it is especially difficult to study the biology, morphology, ecology, and host-plant associations of nymphs. Studies have shown that a few cicada species have a five-instar nymphal stage, e.g., *Mogannia minuta* Matsumura, *Magicicada septendecim* (L.), and *Diceroprocta apache* (Davis) (Hayashi 1976, Maier 1980, Ellingson et al. 2002), while a few other species have a four-instar nymph stage, e.g., *Cryptotympana atrata* (F.) and *Leptopsalta yamashitai* (Esaki and Ishihara) (Hu et al. 1990, Kang et al. 2005). Regarding the nymphal instars of *M. mongolica*, four instars were identified recently by Li and Wei (2013) based on investigations of the head width of nymphs. Other previous studies on this species were mainly focused on adult morphology and taxonomy (Chou et al. 1997), anatomy of the alimentary canal and Malpighian tubules (Zhong et al. 2011), ultrastructure of the antennal sensilla (Li and Wei 2013), morphology of the salivary glands (Zhong et al. 2013), and morphometrics of the final-instar nymphs and exuviae (Lee et al. 2012, Hou et al. 2014). However, the vertical distribution and population density in soil, morphological variation and host-plant selection of nymphs, and adult emergence phenology of *M. mongolica* are unclear.

We collected a large number of nymphs of different size from soil and obtained the first-instar nymphs hatched from eggs of *M. mongolica* and investigated the nymphs beneath three different host plants of this species in Guanzhong Plain, Shaanxi, China, to redetermine the nymphal instars and study the morphological variation, vertical distribution and population density in soil, and host-plant selection. We also collected exuviae of *M. mongolica* to investigate its adult emergence phenology and sex ratio.

Materials and Methods

Study Site. Field work was carried out on the campus of Northwest A&F University, Yangling, Shaanxi Province, China (34° 16′ 56.24″ N, 108° 4′ 27.95″ E). The climate is semiarid and prone-to-drought. Average annual temperature is 11.0–14.0°C; average annual rainfall is 650 mm with peaks in July and September; and average annual evaporation is 800 mm.

Sampling Methods. In total, 26 pits (1 m by 1 m by 0.7 m) were excavated to investigate the population of nymphs of *M. mongolica* under the three main host plants [i.e., *Pinus tabuliformis* Carr. (Pinaceae), *Populus tomentosa* Carr. (Salicaceae), and *Pyrus xerophila* Yü (Rosaceae)] in July 2013, with 12, 6, and 8 pits under *P. tabuliformis*, *Po. tomentosa,* and *Py. xerophila*, respectively. The different host-plant forests are in the same vicinity. All pits were randomly selected under the host plants crown. Vertical distribution of nymphs in soil was ascertained by counting the number of nymphs distributed in each 10 cm layer of soil.

To investigate the adult emergence phenology and sex ratio of *M. mongolica*, exuviae were collected in the woods of *P. tabuliformis* and *Po. tomentosa* at 9:00 am every day during the adult emergence period in 2013 and 2014.

First-Instar Nymphs. Adults of *M. mongolica* were collected on the campus of Northwest A&F University between late July and late August 2013. To obtain eggs, some adults were raised on branches (with no eggs) of *Py. xerophila*, which were shrouded by an insect net for several days. The females inserted their ovipositors into dead twigs and laid eggs. The eggs were collected and placed in plastic Petri dishes (50 mm in diameter, 10 mm in depth) in sealed plastic pots (150 mm in diameter, 60 mm in depth) on September 26, 2013. The air in these pots was saturated with water vapor released from wet cotton. The eggs were kept at 8°C under a photoperiod of 16:8 (L:D) h for 2 mo. Then the eggs were removed to room temperature (modified from Moriyama and Numata 2009). On May 19, 2014, we obtained the first-instar nymphs which were used for morphology study and molecular identification.

Morphology Study. All captured nymphs were transferred alive to a beaker and anesthetized by chilling in a 4°C refrigerator. Species were identified by morphology. Observations of morphological features were carried out using a SMZ168 Stereoscopic Zoom Microscope (Motic, Xianmen, China). Photographs of nymphs were taken for morphological measurements using a scientific digital micrography system equipped with an Auto-montage imaging system and a Retiga 2000R digital camera (CCD) (Qimaging, Surrey, BC, Canada). Measurements of head capsule width (HW) and abdominal width (AW) were taken from photos of nymphs using Image Lab version 2.2.4.0 software (MCM Design, Hillerød, Denmark).

Statistical Analyses. A total of 394 nymphs were collected, including 330 nymphs dug from soil quadrats and 64 nymphs hatched from the eggs. The heads of three nymphs were damaged during excavation, and the vertical soil positions of six nymphs remained obscure; they were excluded from the statistical analyses.

Data were processed using SPSS 17.0. Linear regression analysis, based on the Dyar's rule (Dyar 1890), was used to analyze the fit between instar number (indicated by the width of head capsule) and the natural log of mean head capsule measurements (millimeter converted to micrometer). t tests were used to compare the widths of abdomen. The population density (square-root translation) was assessed by analysis of variance, with a significance level of 5% ($\alpha = 0.05$). A χ^2 test with one degree of freedom was used to compare the observed sex ratios (males/females) in adult emergence to the expected sex ratio of 50:50. The number of exuviae was added up for every 7-d period, and the line describing emergence phenology was drawn.

Molecular Identification. The mitochondrial *COI* barcode was employed to further ensure that all the captured nymphs belong to *M. mongolica*. A total of 14 *COI* sequences (591 bp) from 14

representatives were obtained, i.e., two, two, three (including all the three morphs of the third-instar nymphs), three (including all the three morphs of the fourth-instar nymphs), two nymphs of the first- to fifth-instars, respectively, and two adults. Nymphal instar was determined using head capsule measurements. All sequences obtained in this study were submitted to GenBank (accession numbers: KM576904–KM576917).

Results

Determination of Species and Nymphal Instars of *M. mongolica*. Molecular identification showed that the *COI* sequences of all individuals (including the variants) had little divergence, with the corrected distances from 0.0 to 0.2%. This indicates that the nymphs of different instars examined based on morphological characters all belong to *M. mongolica*.

In total, 394 nymphs were collected in this study, including 64 nymphs hatched from eggs and 330 nymphs dug from soil quadrats. The distribution plots of the HWs of the 391 undamaged individuals show five distinct clusters (Table 1). Therefore, five instars were clearly identified, because no overlapping was detected between the HWs of successive instars (Table 1). These results were also supported by Dyar's rule (Dyar 1890), which hypothesizes a geometric head capsule growth. In addition, a regression analysis of instar versus head width to verify the determination of the instar was conducted, and the linear regression equation was highly significant ($P < 0.01$, $r^2 = 0.9985$) (Fig. 1).

Morphological Variation of third-, fourth-Instar Nymphs and Their Association With the Host Plants. Nymphs of third- and fourth-instars exist morphological variation (Fig. 2). Two types were observed according to the widths of abdomen, e.g., swollen abdomen (Type I) and unswollen abdomen (Type II) (Table 2). The mean widths of abdomen of the third-instar nymphs of the two types were highly significant ($t = -34.39$, df $= 11$, $P < 0.001$), i.e., 3.44 ± 0.14 mm (Type I) and 1.80 ± 0.04 mm (Type II), respectively. The mean widths of abdomen of the fourth-instar nymphs of the two types were also highly significant ($t = -54.40$, df $= 32$, $P < 0.001$), i.e., 6.03 ± 0.14 mm (Type I) and 3.29 ± 0.16 mm (Type II).

The morphological variation is generally related to host-plant association. Abdomens of nymphs feeding on *Po. tomentosa* were usually swollen (Type I) with body color mainly white or greenish white (Fig. 2D–G); abdomens of nymphs feeding on *Py. xerophila* were usually swollen (Type I) with body color mainly white abdomens (Fig. 2D, G); abdomen of nymphs feeding on *P. tabuliformis* were either swollen (Type I) or unswollen (Type II), and the former with body color mainly white (Fig. 2D, G) and the latter mainly pale brown (Fig. 2C, H).

Vertical Distribution and Population Density of Nymphs Under Different Host Plants. We collected 42 nymphs in 12 quadrats under *P. tabuliformis*, 76 nymphs in 6 quadrats under *Po. tomentosa*, and 212 nymphs in 8 quadrats under *Py. xerophila*. The mean densities of nymphs in soil under the three host plants were significantly different ($F = 53.52$, df $= 2$, 23, $P < 0.001$), i.e., 3.50 nymphs/m^2, 12.67 nymphs/m^2, and 26.5 nymphs/m^2 under *P. tabuliformis*, *Po. tomentosa*, and *Py. xerophila*, respectively.

The nymphs could extend their distribution from the 0–10 cm soil layer to the 51–60 cm soil layer underground but not beyond 60 cm soil layer under all the three host plants (Fig. 3). No nymphs were observed in 0–10 soil layer below *P. tabuliformis*. The 21–30 cm soil layer under all the three host plants has the highest population density.

Adult Emergence Phenology and Sex Ratio. Adult emergence of *M. mongolica* commenced on mid-July and ended in late August. Emergence of *M. mongolica* feeding on *Po. tomentosa* showed two peaks in 2013 but one peak in 2014 (Fig. 4A), whereas emergence feeding on *P. tabuliformis* showed one peak in both 2013 and 2014 (Fig. 4B). Except nymphs on *Po. tomentosa* in 2013, data suggest that

Table 1. HW means and ranges for first- to fifth-instar nymphs of *M. mongolica*

Nymphal instar	Sample size	Mean ± SD (mm)	Size range (mm)	Dyar's constant
1	64	0.40 ± 0.02	0.36–0.43	
2	5	0.88 ± 0.06	0.84–0.99	2.20
3	51	1.65 ± 0.13	1.38–1.92	1.88
4	90	3.25 ± 0.21	2.82–3.86	1.97
5	181	7.10 ± 0.28	6.10–7.68	2.18

$$\ln y = 0.7059x + 3.0065$$
$$r^2 = 0.9985$$
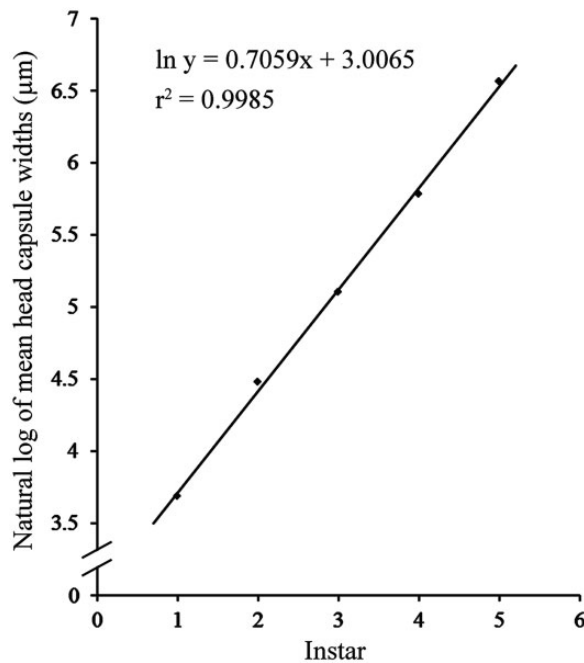
Fig. 1. Regression relationship between the natural logarithm of the mean nymphal HWs and the instars.

males may slightly outnumber females from the beginning to the 17th day, whereas later in the season, the reverse is true, indicating a somewhat protandrous emergence pattern.

In the woods of *Po. tomentosa*, 803 exuviae were collected in 2013, with sex ratio of the entire population nearly 50:50; and 542 exuviae were collected in 2014, with sex ratio of the entire population also nearly 50:50 (Table 3).

In the woods of *P. tabuliformis*, 365 exuviae were collected in 2013, with sex ratio of the entire population nearly 50:50; and 344 exuviae were collected in 2014, with the sex ratio of the entire population also nearly 50:50 (Table 3).

Discussion

Nymphal Instars of *M. mongolica*. Li and Wei (2013) collected 118 nymphs of *M. mongolica* by digging in soil beneath the host plants, and their measurements of head width of all the nymphs were distributed into four clusters. In this study, the measurements of head width of 391 investigated nymphs were distributed into five clusters, i.e., 64 first-instar nymphs hatched from eggs in the first cluster and 330 nymphs dug from soil quadrats in the last four clusters. This result indicates that all the individuals dug from soil belonged to the second- to fifth-instar nymphs and that the first- and second-instar nymphs were difficult to discover in soil due to their small size (with head width ranged 0.36–0.43 mm and 0.84–0.99 mm, respectively). The success of obtaining the first-instar nymphs through cultivating the eggs under appropriate conditions and redetermination of nymphal instars of *M. mongolica* in our study provide useful information for studies of other cicadas.

Morphological Variation of Nymphs and Host-Plant Preference. We found significant differences in the width of abdomen and body color in the nymphs of *M. mongolica* of third- and fourth-instars, which were feeding on different host plants. Body color could be affected by abdominal contents due to different food intake and accumulation of metabolin and pigments, which is closely related to chemical makeup of the host plants. AW of cicada nymphs was not significantly affected by their quantity of feeding because of the rigid exoskeleton. The two types of abdominal condition (swollen or not) in third- and fourth-instar nymphs may be due to the age (time) after every emergence. Molecular identification confirmed that all the individuals investigated in our study belong to *M. mongolica*. White and Lloyd (1975) observed that nymphs of the periodical cicada *Magicicada cassini* (Fisher) of the same age in the same forest or orchard were variable in size and suggested that place-to-place differences in availability of food may greatly affect their growth rate. Niche variation can profoundly affect the population's stability, the amount of intraspecific competition, the fitness-function shapes, and the population's capacity to diversify rapidly. We found that nymphs of *M. mongolica* showed, in addition to the morphological variation, a distinct preference to *Py. xerophila* among the three host plants. Whether morphological variation of nymphs is closely related to host plants merits further investigation.

In addition, we revealed that the lowest population density of *M. mongolica* nymphs in soil was under *P. tabuliformis* among the three host plants. Cook et al. (2001) found that ovipositing females of *Ma. cassini* displayed clear preferences for host species; Yang (2006) suggested that cicadas use light for oviposition site selection in woods of the same host plant. However, host-plant selection for oviposition of *M. mongolica* and also other cicadas among different host plants needs further investigation, as is closely related to the population density of cicada nymphs in soil and their effects on the growth of host plants.

Vertical Distribution of Nymphs. Uematsu and Onogi (1980) observed that nymphs of *Platypleura kaempferi* (F.) were mainly distributed in the 10–30 cm soil layer. Maier (1980) found that nymphs of *Ma. septendecim* occurred between 7.6 and 45.0 cm deep in an apple orchard, and most nymphs were discovered between 7.6 and 22.5 cm soil layer. In this study, we found that nymphs of *M. mongolica* could extend to 60 cm deep but were mainly distributed in the 21–30 cm soil layer. This result could be related to the root system of host plants; the soil layer where cicada nymphs mainly distributed could have most small roots with more food source available for cicada nymphs (Maier 1980). Nymphs of *M. mongolica* were observed in the 0–10 soil layer below both *Po. tomentosa* and *Py. xerophila*, but none were observed in the 0–10 soil layer below *P. tabuliformis*, which may be related to the differences in composition and content in the litter layer between pine forest and broad-leaved forest. Nymphs were not discovered beyond the 60 cm soil layer under all the three host plants may be due to the compaction of soil, which may be another factor that prevents nymphs from reaching roots at greater depths.

Adult Emergence Phenology and Protandrous Emergence Pattern. In general, there is only one peak in the adult emergence in cicadas (Callaham et al. 2000, Whiles et al. 2001). In this study, we found that only one peak existed in the adult emergence of *M. mongolica* on the host *P. tabuliformis* in 2013 and 2014 and on the host *Po. tomentosa* in 2014. Surprisingly, we found two peaks in the adult
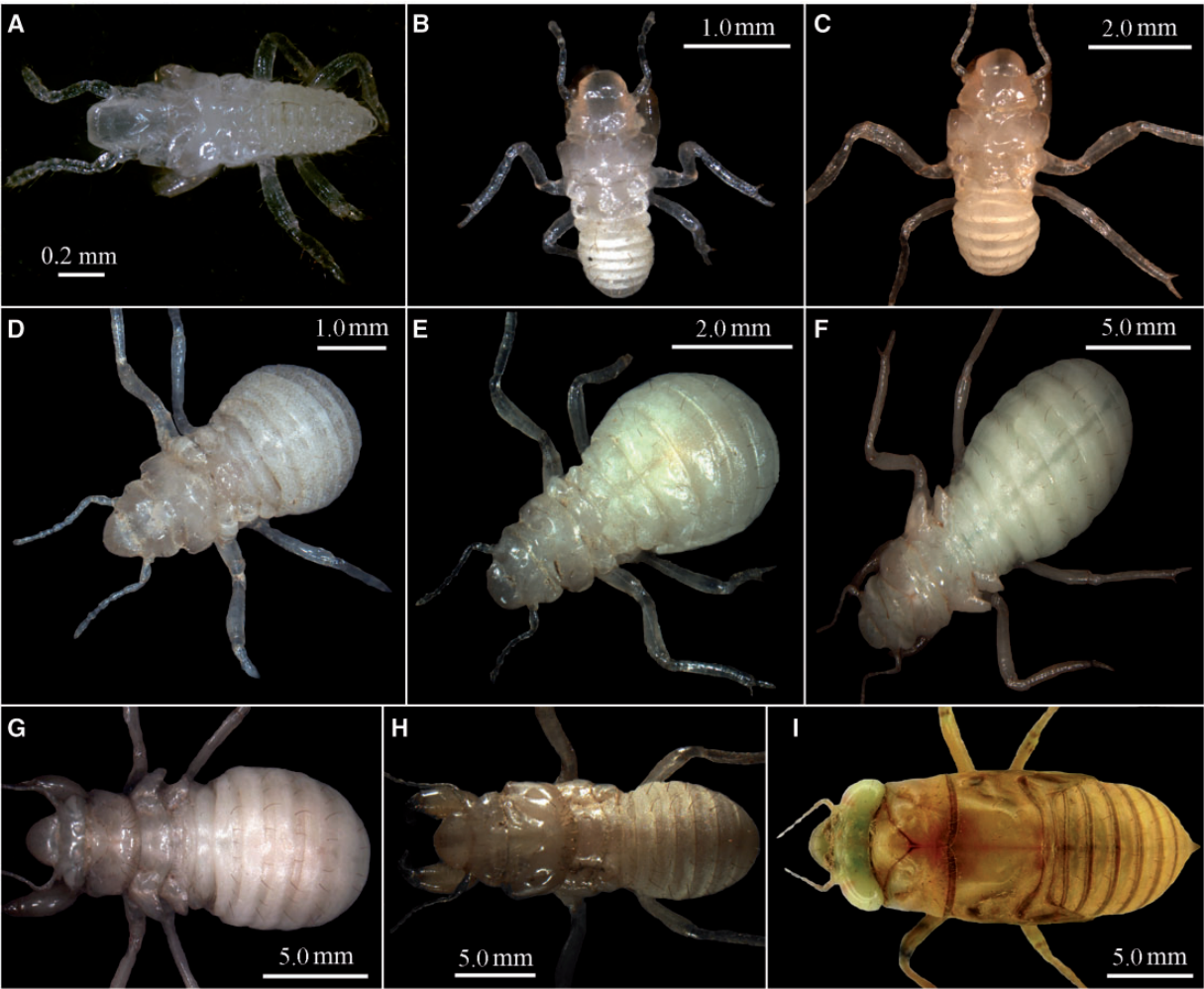
Fig. 2. Nymphs of *M. mongolica* of different instars. (A) First-instar nymph; (B) second-instar nymph; (C) third-instar nymph (Type II, pale brown); (D) third-instar nymph (Type I, white); (E) third-instar nymph (Type I, greenish white); (F) fourth-instar nymph (Type I, greenish white); (G) fourth-instar nymph (Type I, white); (H) fourth-instar nymph (Type II, pale brown); and (I) fifth-instar nymph.

Table 2. AW and HW means and ranges for third- and fourth-instar nymphs of *M. mongolica*

Type of nymphs	Sample size	AW mean ± SD (mm)	AW range (mm)	HW mean ± SD (mm)	HW range (mm)
Type I (3rd)	10	3.44 ± 0.14	3.10–3.59	1.66 ± 0.15	1.42–1.88
Type II (3rd)	10	1.80 ± 0.04	1.75–1.90	1.63 ± 0.14	1.38–1.81
Type I (4th)	17	6.03 ± 0.14	5.79–6.31	3.26 ± 0.18	2.93–3.69
Type II (4th)	17	3.29 ± 0.16	3.01–3.65	3.23 ± 0.18	2.86–3.64

emergence of *M. mongolica* on the host *Po. tomentosa* in 2013. This unusual emergence pattern needs to be investigated further.

Darwin (1871) hypothesized that protandry, males emerging before females, is likely to provide males an advantage in competition for mates. Protandry is a common emergence pattern among butterflies (Wiklund and Fagerstrom 1977) and solitary Hymenoptera (Evans and West-Eberhard 1970, Gwynne 1980) as well as some other groups of insects (Richards 1927, Nielsen and Nielsen 1953). A somewhat protandrous emergence pattern was evident in *M. mongolica*, which is consistent with some other cicadas (Graham and Cochran 1954, Whiles et al. 2001, Logan et al. 2014). In this study, the males of *M. mongolica* that emerge before females and remain alive until females emerge were found to have the highest mating success. Protandry may be a useful strategy in attracting virgin females (Logan et al. 2014), enhancing the mating success of individual males in *M. mongolica*. Whether protandrous emergence pattern occurs commonly in the Cicadoidea merits further study.
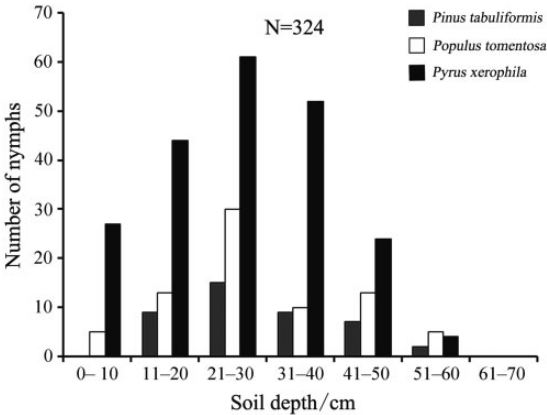

Fig. 3. Density of nymphal *M. mongolica* in different soil layers under different host plants.
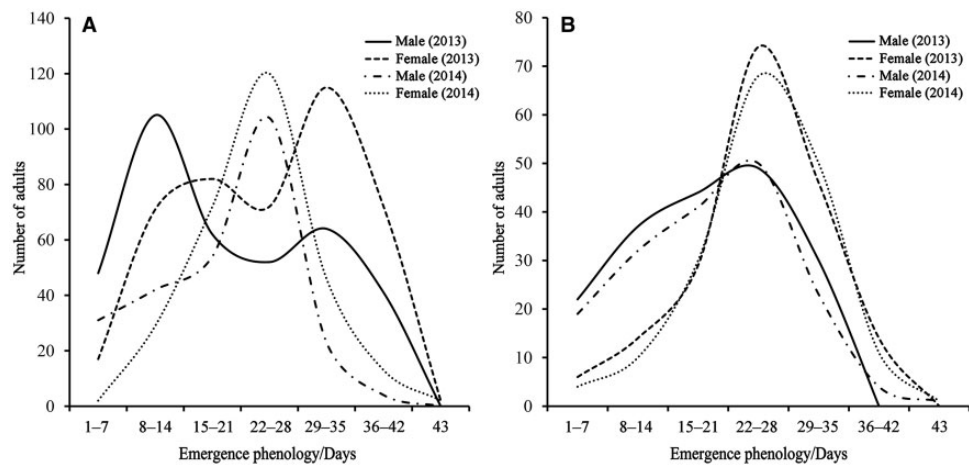
Fig. 4. Emergence phenology for *M. mongolica* in woods of (A) *Po. tomentosa* and (B) *P. tabuliformis*.

Table 3. Sex ratio of *M. mongolica* in the adult emergence

Host plant	Year	Male	Female	Sex ratio = male/female	χ^2 test with one degree of freedom
Po. tomentosa	2013	372	431	46.3:53.7	$\chi^2 = 4.33, P = 0.077$
	2014	258	284	47.6:52.4	$\chi^2 = 1.25, P = 0.233$
P. tabuliformis	2013	182	183	49.9:50.1	$\chi^2 = 0.0027, P = 0.515$
	2014	170	174	49.4:50.6	$\chi^2 = 0.0465, P = 0.470$

Conclusions

The results from this study suggest that the cicada *M. mongolica* has five nymphal instars. Nymphs of third- and fourth-instars showed morphological variation, which is closely related to host plants. The mean densities of nymphs in soil under the three host plants were significantly different, indicating a distinct host preference. The nymphs could extend their distribution from the 0–10 cm soil layer to the 51–60 cm soil layer underground but not beyond 60 cm soil layer under all the three host plants. The 21–30 cm soil layer under all the three host plants has the highest nymphal population density. The sex ratio of the entire population was nearly 50:50, but males dominated in the early half of the duration of the emergence and a protandrous emergence pattern was evident.

Acknowledgments

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