Embryo-to-embryo communication facilitates synchronous hatching in grasshoppers

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Abstract

Synchronous hatching within single egg clutches is moderately common in locusts and other insects and can be mediated by vibrational stimuli generated by adjacent embryos. However, in non-locust grasshoppers, there has been little research on the patterns of egg hatching and the mechanisms controlling the time of hatching. In this study, the hatching patterns of six grasshoppers (*Atractomorpha lata*, *Oxya yezoensis*, *Acrida cinerea*, *Chorthippus biguttulus*, *Gastrimargus marmoratus*, and *Oedaleus infernalis*) were observed under various laboratory treatments. Under continuous illumination and a 25/30°C thermocycle, the eggs of these grasshoppers tended to hatch during the first half of the daily warm period. Eggs removed from egg pods and cultured at 30°C tended to hatch significantly earlier and more synchronously when kept in groups vs. singly. In general, eggs hatched earlier when egg group size was increased. Egg hatching was stimulated by hatched nymphs in some species, but not in others. In all species, two eggs separated by several millimeters on sand hatched less synchronously than those kept in contact with one another, but the hatching synchrony of similarly separated eggs was restored if they were connected by a piece of wire, suggesting that a physical signal transmitted through the wire facilitated synchronized hatching. In contrast, hatching times in the Emma field cricket, *Teleogryllus emma*, which lays single, isolated eggs, were not influenced by artificial clumping in laboratory experiments. These results are discussed and compared with the characteristics of other insects.

Keywords

egg hatching, egg pod, hatching synchrony, Orthoptera, vibration

Introduction

Most grasshopper species deposit their eggs a few centimeters underground in a foamy egg pod that can contain as many as 200 clumped eggs, depending on species (Uvarov 1977, Stauffer and Whitman 1997). In temperate zones, the eggs typically overwinter and then hatch in the spring. Field observations suggest that different species tend to hatch at different times of the day, and some species exhibit hatching synchrony such that the majority of eggs in a single egg pod hatch more or less simultaneously (Uvarov 1977, Smith et al. 2013).

The daily hatching time in grasshoppers is thought to be controlled by environmental factors such as daily photoperiod and temperature cycles, as observed in other insects (Tauber et al. 1986, Danks 1987, Saunders 2002). For example, eggs of the desert locust *Schistocerca gregaria* (Forskål, 1775) hatched around dawn in the field (Ellis and Ashall 1957) and during the low-temperature period of a thermocycle or during the dark phase of a photoperiod in the laboratory (Padgham 1981, Nishide et al. 2015a, b). In contrast, eggs of the migratory locust *Locusta migratoria* (Linnaeus, 1758) and the lubber grasshopper *Romalea microptera* (Beauvois, 1817) (Chen 1999, Smith et al. 2013, Nishide et al. 2017a) hatched during the day or during warm periods of thermocycles. Because the eggs of most grasshopper species are laid underground where light might not penetrate, it is possible that the eggs use changes in soil temperature rather than photoperiod to control hatching time. For example, *S. gregaria* eggs removed from a pod and exposed to light-dark cycles under constant temperature hatched mainly during the dark period (Padgham 1981, Nishide et al. 2015a, b). However, they hatched during the light and dark periods at similar frequencies when they were covered with a layer of sand or kept in naturally laid pods deposited underground (Nishide et al. 2015b), suggesting that the light had not reached the eggs buried in the sand. Species-specific hatching times may have evolved to maximize the survival of the fragile fresh hatchlings, which are susceptible to predation and environmental extremes (Uvarov 1977, Smith et al. 2013). Overall, however, the timing of hatching and the degree of synchronous hatching are relatively understudied in grasshoppers.

Synchronous hatching within a single egg pod was originally hypothesized to be triggered by a thermal threshold mechanism, whereby the eggs are ready to hatch but require a certain temperature to do so. In this scenario, rising temperatures in the spring heat the soil, and synchronous hatching is induced on the day when the soil at the level of the buried eggs finally exceeds the species-specific threshold temperature (Smith et al. 2013). However, this proposed mechanism cannot explain how synchronous hatching can occur in grasshoppers with 5- to 11-cm-long egg pods buried vertically, because the threshold temperature would reach only the top eggs. Likewise, it does not explain what triggers

synchronous hatching in warm climate grasshoppers where soil temperatures typically remain above proposed hatching-threshold temperatures.

Recently, a new mechanism controlling synchronized hatching was discovered. In *S. gregaria*, *L. migratoria*, and the Bombay locust *Nomadacris* (also known as *Patanga*) *succincta* (Johannson, 1763), eggs kept in contact with one another hatched synchronously, while those kept separately hatched asynchronously (Nishide and Tanaka 2016, Tanaka 2017, 2021, Tanaka et al. 2018, Sakamoto et al. 2019). However, the latter also hatched synchronously when connected by a piece of wire, suggesting that a physical stimulus transmitted through the wire was involved in the synchronized hatching. In *L. migratoria*, sound recordings of the vibrations emitted by an embryo influenced the hatching time of other eggs, again, suggesting that vibrations from hatching eggs can stimulate hatching in nearby eggs. Communication by vibration is reasonable, considering that grasshopper eggs typically touch one another in the tightly packed egg pod. How many other grasshopper and insect species employ this mechanism is unknown.

In the present study, I document the hatching behavior of six grasshopper species in response to thermocycles, number of eggs in the group, presence of early hatched nymphs, and vibrations transferred through a wire. To explore the taxonomic breadth of the vibration response, I also tested to see if the eggs of a cricket that lays eggs singly would hatch synchronously if artificially placed in a group. This paper describes the results of these observations and compares them with those previously reported for other insects.

Materials and methods

Insects.—Five species of grasshopper – the longheaded grasshopper *Atractomorpha lata* (Motschilsky, 1866), the Oriental longheaded grasshopper *Acrida cinerea* (Thunberg, 1815) the bow-winged grasshopper *Chorthippus biguttulus* (Linaeus, 1758), the band winged grasshopper *Gastrimargus marmoratus* (Thunberg, 1815) and *Oedaleus infernalis* Saussure, 1884 – were collected in Tsukuba, Ibaraki, Japan from August to October of 2017 and 2018. Egg pods of the rice grasshopper *Oxya yezoensis* Shiraki, 1910 were collected in Tsukuba in September 2017 and in paddy fields in Kuroishi, Aomori, Japan in May 2018 and sent to Tsukuba, where experiments were performed. All species are of the family Acrididae, except for *A. lata*, which is of the family Pyrgomorphidae. Adults of each species were reared under outdoor conditions on various host plants, such as *Bromus catharticus*, *Artemisia indica* var. *maximowiczii*, and *Miscanthus sinensis* in nylon-screened cages (22 × 39 × 43 cm) in which a plastic cup (volume: 340 ml) filled with moist sand (10–15% water by wt) was placed as the oviposition substrate. *L. migratoria* and *C. biguttulus* are bivoltine and produce non-diapause eggs in early summer but diapausing eggs in the fall. For the five species, I used overwintering, diapausing eggs, which, in nature, remain in the egg stage for several months. Laid egg pods were kept outdoors until December and then stored in a refrigerator (7°C) for 2–5 months until used. The eggs of all five species appeared to have entered diapause at the end of the anatrepsis stage, by the arrival of winter, and were ready to hatch upon transfer to warm conditions in late January. In contrast, eggs of *A. lata* are known to have no diapause and overwinter in a state of quiescence (Y. Ando, pers. comm.), but were maintained as above. All of these species occur in grasslands in Japan and hatch in the spring when semimonthly mean soil temperatures measured every 60 min at a depth of approximately 3 cm at an exposed site in

Tsukuba ranged from 12.8 to 28.8°C from early April to late July in 2020 (Tanaka, S. pers. obs.).

For the experiments, eggs of all species were handled in the same way: each egg pod was washed with chlorinated tap water; the eggs were separated from the pod and individually placed on wet tissue paper in a 9-cm plastic Petri dish until used. They were maintained at 30 ± 1°C under continuous illumination in incubators. The compound eyes could be seen through the chorion several days before hatching. The number of eggs per pod varied from ~ 10 in *C. biguttulus* to more than 100 in *A. cinerea*.

Hatching under thermocycles.—Eggs of each species were kept either singly or in a group in pits on moist non-sterilized white sand (~ 15% moisture content by wt; Brisbane White Sand, Hario Co. Ltd., Japan) in a 9-cm Petri dish with a transparent lid and exposed to a thermocycle of 25/30°C under continuous illumination at least 5 days before hatching, unless otherwise mentioned. The eggs were incompletely covered with sand. The time required for the hatching rhythm of each species to be entrained by the thermocycle is unknown, but it was assumed that 5 days was sufficient based on previous studies with other grasshopper species (Nishide et al. 2015a, b; Tanaka 2021). The dishes were photographed from above with a digital camera every half-hour until no more hatching was observed. Hatching times were later recorded. The number of eggs that hatched every half-hour was recorded each day and then pooled for the 2–5 days of the experiment.

Effect of egg group size on hatching time.—Eggs from each pod were divided into treatments that differed in the number of grouped eggs: 1 vs. 2, 2 vs. 4, or 4 vs. 10, except for *C. biguttulus*, in which only two treatments (1 vs. 15 eggs) were prepared because fewer eggs were available for this species. The eggs in a group were held in a sand pit in a plastic Petri dish, and singly kept eggs were held in sand pits in another dish, as described earlier. The hatching time of eggs was recorded under continuous illumination and temperature (30 ± 1°C). Mean hatching times of the various treatment groups were calculated and compared. For each species, a value of 5 h was assigned to the mean hatching time of the largest group to standardize comparisons between different group sizes.

Effect of hatched nymphs on the hatching times of late-hatching eggs.—Whether the hatching time of an egg was influenced by the presence of an early-hatching nymph was determined under 30 ± 1°C and continuous illumination by treating pairs of eggs from the same pod in three different ways: 1) two eggs placed horizontally and in contact with one another on moist sand in a well of plastic 24-well plates (Thermo Fisher Scientific KK, Tokyo, Japan), 2) eggs separated by 2–3 mm on sand, and 3) eggs separated by a stainless steel wire screen that kept hatchlings from physically touching unhatched eggs. The hatching times were determined as described earlier, and the hatching intervals of eggs in pairs were calculated. Because photographs were taken every half-hour, 0.5 h was added to the hatching interval of two eggs and, thus, the minimum hatching interval was 0.5 h.

Stimuli inducing synchronized hatching.—To determine the stimuli responsible for synchronized hatching when two eggs are kept in contact, pairs of eggs from the same pod in each species were treated in three different ways: (1) eggs horizontally placed in contact with one another on sand in the same well, (2) those separated
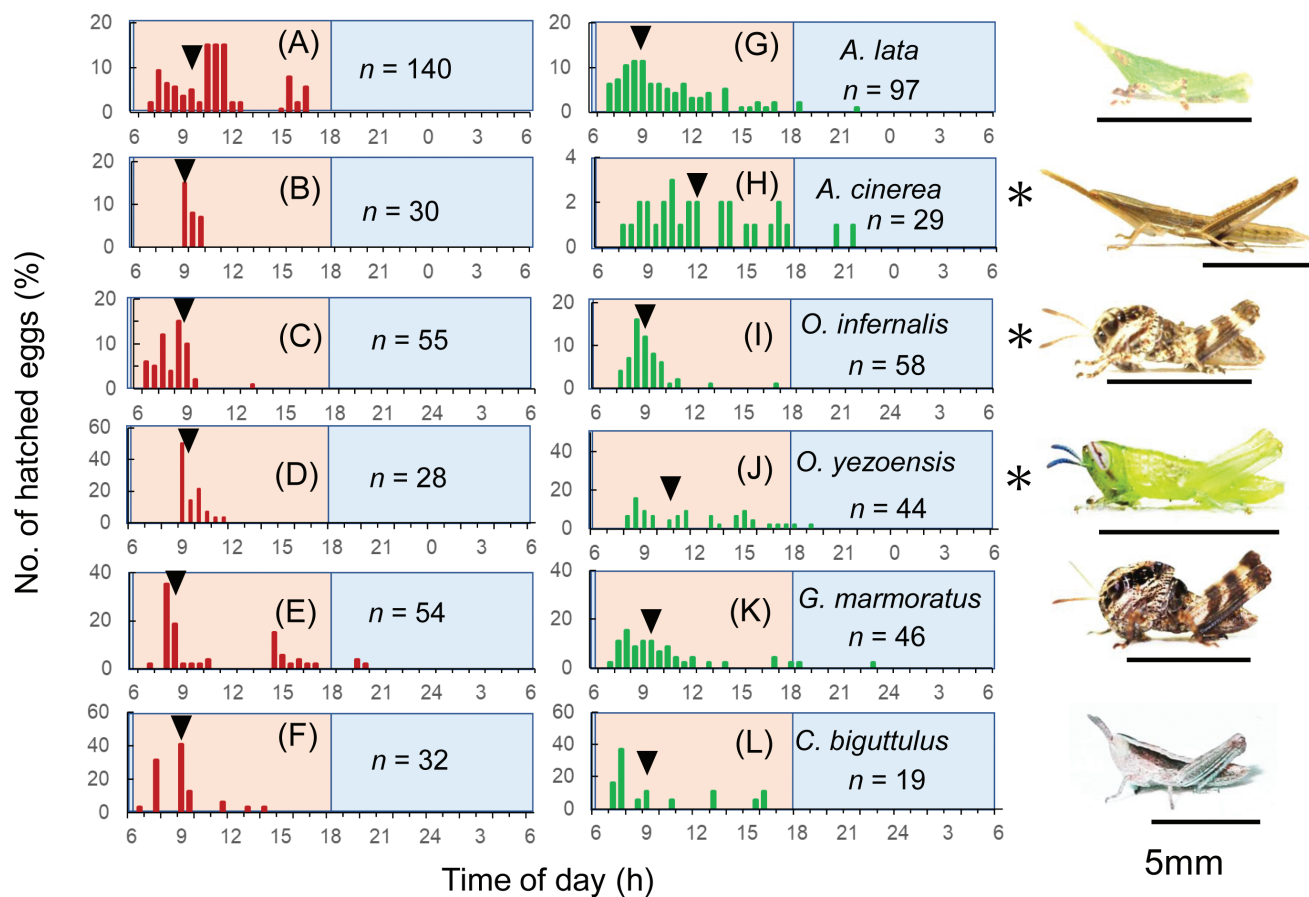
Fig. 1. Hatching activity of eggs kept in a group (**A–F.**) and those kept singly (**G–L.**) in a thermocycle of 30 (orange) and 25 °C (blue) under continuous illumination in the six indicated grasshopper species. The numbers of eggs that hatched over 2–5 days were pooled and plotted against the time of day. Black arrows indicate the medians. Asterisks indicate a significant difference between the two treatments by the Mann Whitney *U*-test at the 5% level. The photographs on the right show hatchlings of respective species. Scale bars: 5 mm.

by 2–5 mm, and (3) those similarly separated but connected by a piece of stainless steel wire (diameter, 0.1 mm; length, 0.7 cm). All treatments were done for all grasshoppers except for the two species in which the separation of eggs did not show a marked effect on the hatching intervals. In the last two species, the eggs in (2) were separated by a wire screen, and those in (3) were separated by a screen but connected by a piece of stainless steel wire placed through the screen separator. Connecting wires were laid on top of the two eggs (Fig. 4). The hatching times were determined as described above, and the hatching intervals of eggs in pairs were determined.

Effect of clumping of cricket eggs on the hatching time.—More than 20 adults of the Emma field cricket, *Teleogryllus emma* (Ohmachi & Matsuura, 1951), were collected in Tsukuba in August and September 2018, and allowed to lay eggs in moist sand in plastic cups at room temperature. The cups containing the eggs were then kept outdoors until February, when the eggs were ready to hatch when transferred to warm conditions (Tanaka, S. pers. obs.). The eggs were separated from the sand by washing with cold tap water and divided into two batches; 5 groups of 10 eggs were placed either as groups or singly in sand pits in 9-cm plastic Petri dishes. The dishes were then incubated at 30 ± 1°C under continuous illumination with 10 days required for the eggs to hatch. The hatching times of the eggs were recorded.

Statistical analyses.—The hatching times were compared using ANOVA, Tukey's multiple comparison test, or *t*-test. The proportions of eggs that hatched synchronously were compared with the χ^2-test. The comparisons of hatching intervals were made with the Steel-Dwass test or the Mann-Whitney's *U*-test. These analyses were performed using a statistics service available at http://www. gen-info.osaka-u.ac.jp/MEPHAS/kaiseki.html. Descriptive Statistics were presented in Excel (Microsoft Office 365) or StatView (SAS Institute Inc., NC, USA). Differences were judged as significant when $p < 0.05$.

Results

Hatching under thermocycles.—The hatchlings of each species had a characteristic body shape, size, and color (Fig. 1). Most eggs of all tested species hatched during the high-temperature phase of the thermocycle regardless of whether they were grouped or singly, and the majority hatched during the first half of the thermophase (Fig. 1). However, the variance of hatching times was significantly smaller in treatments with grouped eggs vs. single eggs in all species (*F*-test; $p < 0.05$ each) except for *G. marmoratus* (*F*-test; $p = 0.22$). The Mann Whitney *U*-test indicated a significant difference in hatching time of day between the two treatments in *A. cinerea*, *O. infernalis*, and *O. yezoensis* (asterisks in Fig. 1) but not in *A. lata*, *G. marmoratus*, or *C. biguttulus*.
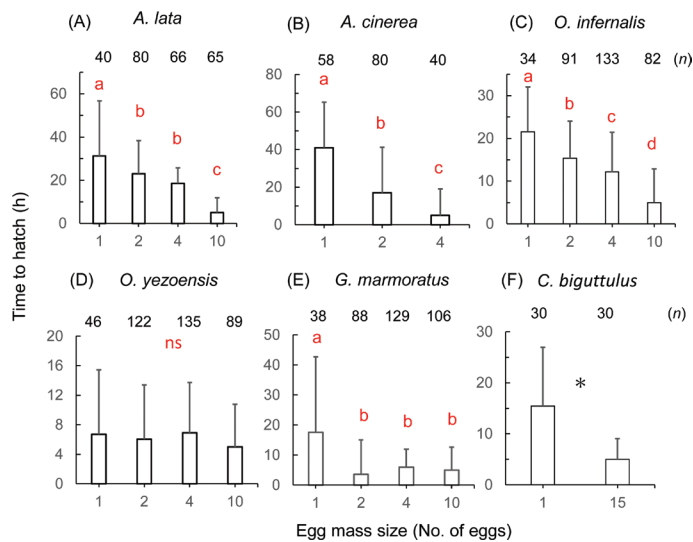
Fig. 2. Relationship between number of eggs in a group and mean hatching times in six grasshopper species under continuous illumination and 30°C temperature. For each species, hatching times were normalized by assigning a value of 5 h to the mean hatching time of the largest group. n (number of eggs in each treatment) is given above each histogram. Different letters indicate significant differences in mean values at the 5% level using the Tukey's multiple test (**A–E.**) or the t- test (**F.**). ns indicates no significant difference.

Effect of egg group sizes on hatching time.—The relationship between number of eggs in a treatment and hatching time varied depending on the species. Eggs hatched earlier as the number of eggs in the group increased from 1 to 4 or 10 in *A. lata*, *A. cinerea*, and *O. infernalis* (Fig. 2A–C), but not in *O. yezoensis*, where group size did not influence hatching time (Fig. 2D). In *G. marmoratus*, hatching time was significantly longer in the eggs kept singly than those kept in groups (Fig. 2E; Tukey's multiple test; $p < 0.05$); however, no significant difference was observed among the grouped (2, 4, or 8 eggs) treatments ($p > 0.05$). In *C. biguttulus*, the hatching time was significantly longer in the eggs kept singly than those kept in a group of 15 eggs (Fig. 2F; Tukey's multiple test; $p < 0.05$).

Effect of hatched nymphs on the hatching times of later-hatching eggs.—The mean hatching interval of two eggs was significantly larger in eggs separated by a few millimeters than those kept in contact with one another, but it was further increased when the eggs were separated by a screen in *A. lata*, *A. cinerea*, *O. infernalis*, and *O. yezoensis* (Fig. 3A–D; Steel-Dwass test; $p < 0.05$ each), suggesting that the early-hatched nymph stimulated the hatching of the later-hatching egg. In contrast, in *G. marmoratus* and *C. biguttulus*, no significant difference was observed in the mean hatching interval between the eggs kept in contact with one another and those kept separated, although the hatching interval for those kept in contact with one another was significantly shorter than those kept with a screen separator (Fig. 3E, F; Steel-Dwass test; $p < 0.05$ each).
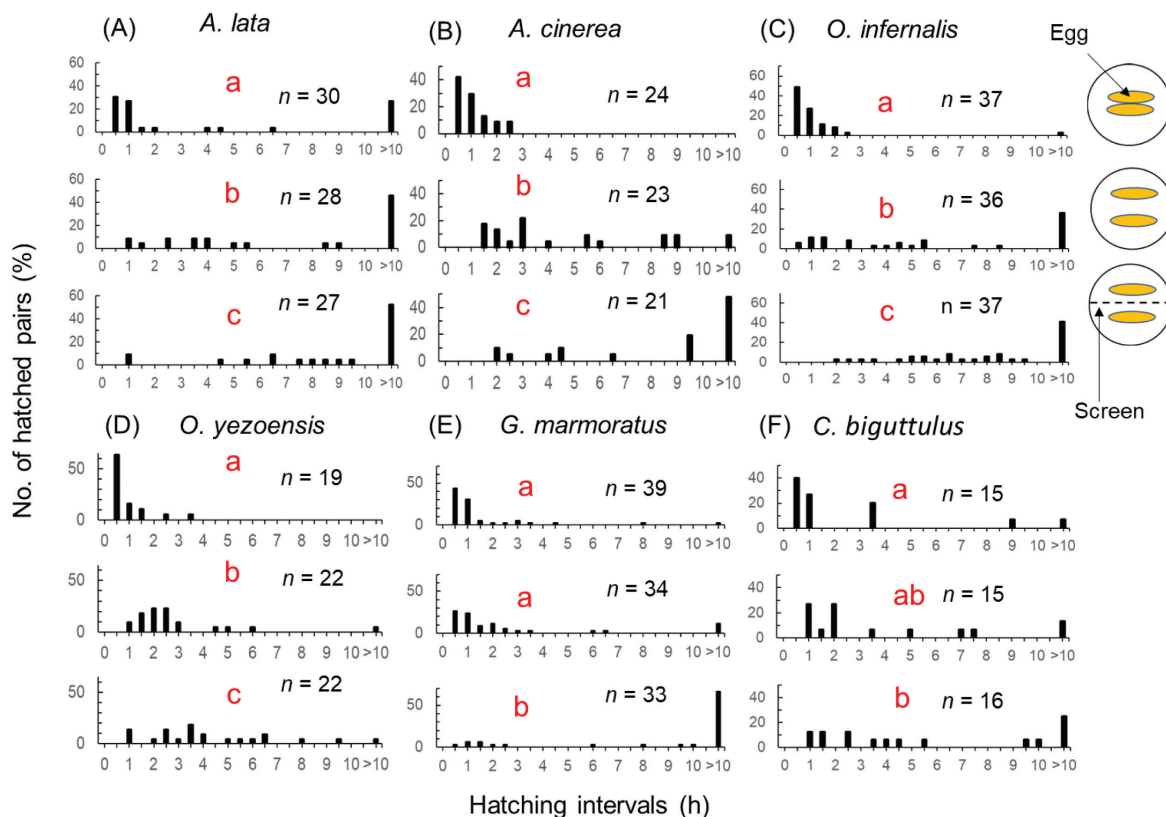

Fig. 3. Hatching intervals of two eggs kept in contact with one another (top panel), separated by 3–5 mm (middle panel), or separated by a screen (bottom panel) in the six indicated grasshopper species. Eggs were maintained under continuous illumination and 30°C temperature (**A–F.**). Different letters indicate significant differences in mean values at the 5% level using the Steel-Dwass test. Diagram on the right shows how the eggs were arranged in wells.
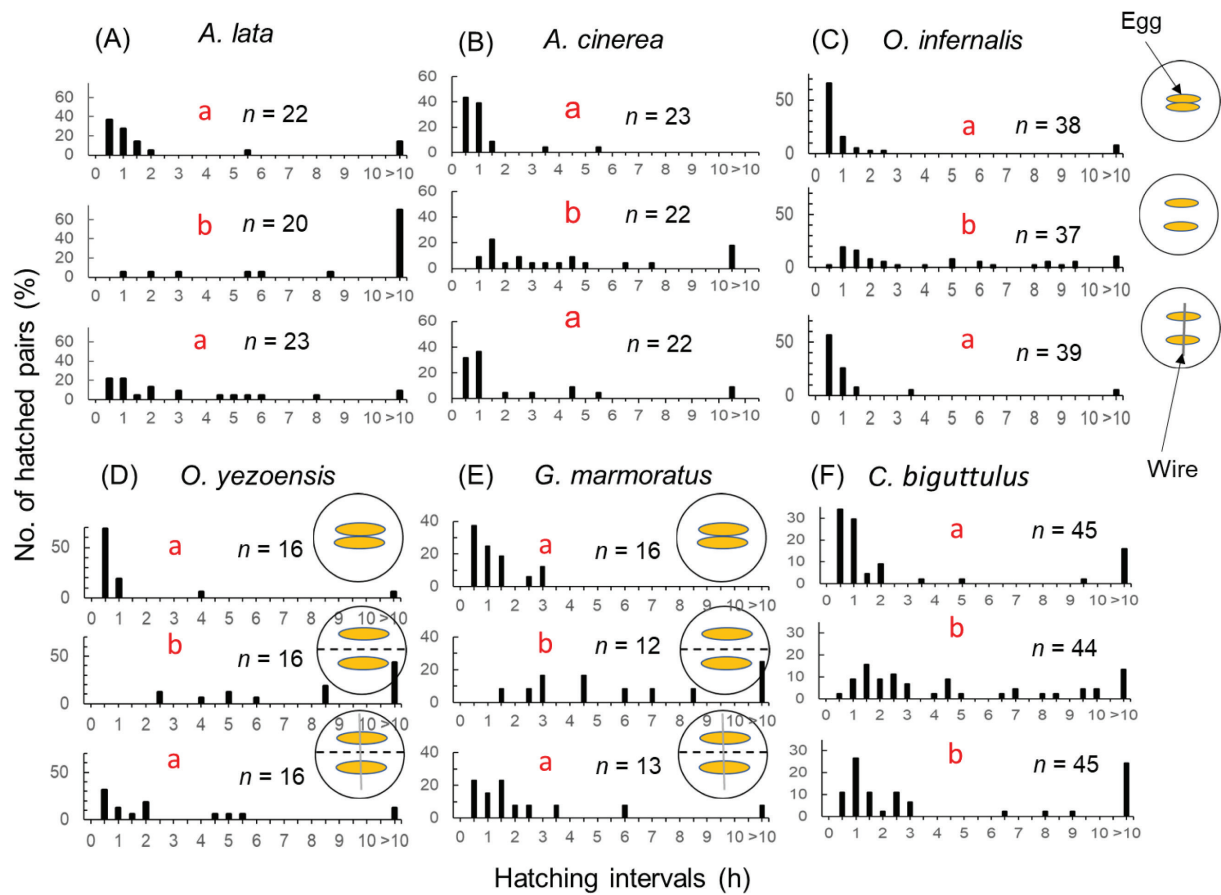
Fig. 4. Hatching intervals of two eggs kept in contact with one another (top panel), separated by ~5 mm (middle panel) with or without a screen, or connected by a piece of wire (bottom panel) at 30°C under continuous illumination in the six indicated grasshopper species (**A–F.**). Different letters indicate significant differences in mean values at the 5% level by the Steel-Dwass test. Diagrams in panels show how the eggs were arranged in wells.

Stimuli inducing synchronized hatching.—The hatching interval of two eggs that were kept in contact with one another was significantly shorter than those that were separated by a few millimeters (Fig. 4A–C; Steel-Dwass test; $p < 0.05$) but similar to those separated but connected by a wire in *A. lata, A. cinerea,* and *O. infernalis* ($p > 0.05$). In *O. yezoensis* and *G. marmoratus,* the effect of egg separation on the hatching interval was small (Fig. 3D, E). Thus, the eggs in these species were separated by a screen (middle panels in Fig. 4 D, E) or connected by a piece of wire through the screen separator (bottom panels). The results were similar to those obtained in the above three species. In *C. biguttulus,* the hatching mean interval for the eggs separated without a separator was significantly larger than for those kept in contact with one another (top and middle panels in Fig. 4F; Steel-Deass test; $p < 0.05$), but the connection of eggs by a piece of wire did not significantly reduce the mean hatching interval (bottom panel in Fig. 4F; $p > 0.05$). The proportion of pairs that hatched with a < 1-h hatching interval in the eggs connected by a piece of wire was 37.8%, which was significantly smaller than the value for the eggs kept in contact with one another (61.4%; $\chi^2 = 4.95$; $p < 0.05$). However, the value was significantly larger than that in the separated eggs (11.1%, $\chi^2 = 8.66$; $p < 0.05$). This result suggested that physical signals transmitted through the wires stimulated other eggs to hatch synchronously, as observed in the other species tested.

Effect of clumping of cricket eggs on their hatching time.—The Emma field cricket showed no significant difference in the mean hatching time (*t*-test; $p = 0.12$) and its variance (*F*-test; $p = 0.07$) between the eggs kept in a group of 10 eggs and those kept singly (Fig. 5). The mean hatching intervals were 37.6 and 35.3 h in those kept in a group and those kept singly, respectively. This difference was insignificant (Mann-Whitney *U*-test; $p > 0.05$; $n = 5$ each). These results imply that the clumping of eggs did not induce synchronized hatching in this cricket.

Discussion

Although the egg pods of many grasshopper species hatch more or less synchronously (Uvarov 1977), the mechanism controlling such behavior has only recently been discovered (Tanaka 2017, 2021, Tanaka et al. 2018, Sakamoto et al. 2019). The present research demonstrates that synchronous hatching is strongly influenced by the number of eggs in a group and provides evidence that vibrational stimuli from hatching eggs induce hatching in adjacent eggs. These results imply egg-to-egg communication. Below, I first discuss the time of day of hatching, followed by synchronicity. These two factors are interrelated, but separate. The former is a property of the population, whereas the latter is a property of individual egg pods.
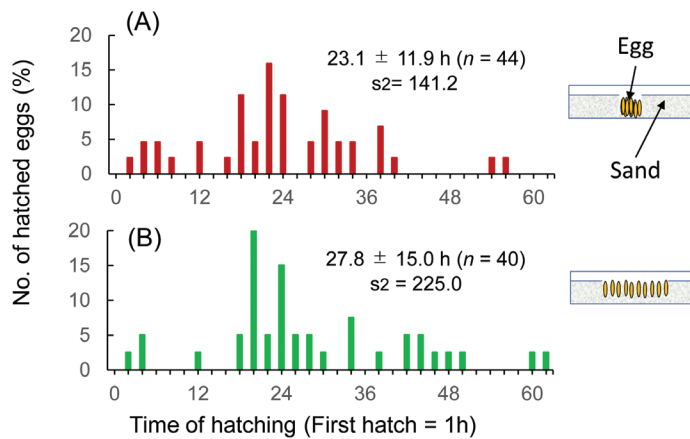
Fig. 5. Hatching activity of the eggs of *Teleogryllus emma* kept in a group (**A.**) and those kept singly (a distance of approximately 5 mm) (**B.**) at 30 °C under continuous illumination. The hatching times for 5 groups of 10 eggs were pooled and calculated by designating the time of the first hatching egg as 1 h. The mean hatching time ± SD (sample size) is given in each panel. s^2 indicates the variance. Diagrams on the right show the experimental setup.

In nature, each grasshopper species tends to hatch at a specific time of day, depending in part on local habitat and current weather (Uvarov 1977, Smith et al. 2013, Nishide et al. 2017a). Many species hatch in the morning as temperatures rise (Nishide et al. 2017a), whereas other species tend to hatch before or at dawn, at midday, at night, throughout the day, or throughout a 24-h period (Ellis and Ashall 1957, Smith et al. 2013). Of the 10 grasshopper species listed in Table 1, eight hatched during the warm period of thermocycles in the laboratory under continuous illumination, whereas *S. gregaria* eggs hatched during the cool period (Nishide et al. 2015a, Tanaka 2021). These results strongly suggest that the thermoperiod controls the time of day of hatching in grasshoppers. In contrast, *N. succincta* eggs hatched during either period (Tanaka 2021) (Table 1).

Hatching time is thought to have evolved to maximize hatchling survival against predators and weather extremes. Hatching at the wrong time of day can be lethal. For example, mid-day hatching would be lethal for grasshoppers living in hot deserts because desert soil temperatures can exceed 65 °C (Whitman 1987), which would instantly kill tiny hatchlings. This may be why some hot-desert grasshoppers hatch at night or in the early morning. For example, desert *S. gregaria* hatch around dawn, the coolest, most humid time of day (Ellis and Ashall 1957, Nishide et al. 2017b, Tanaka 2021). Conversely, for cold-climate grasshoppers, hatching in the late afternoon or evening could be dangerous when falling night temperatures incapacitate hatchlings. This may be why temperate-zone grasshoppers tend to hatch during the day, especially during mid- to late morning (Smith et al. 2013, Tanaka 2021). Indeed, all six of the temperate-zone grasshoppers tested in this paper tended to hatch during the first half of the warm phase in the laboratory (Fig. 1). These six species inhabit grasslands in Japan and hatch in the spring as daily temperatures rise.

Table 1. Summary of hatching behavior and responses to external stimuli in grasshopper species and some other insects.

Species	Hatching time under thermocycles	More eggs		Stimuli from hatched nymph	Vibration from wire	References
		Increased synchrony	Shorter hatching time	Shorter hatching time	Increased synchrony	
Orthoptera: Acrididae						
Locusta migratoria L. 1758	Warm period	+	+	+	+	(Nishide et al. 2015a, Sakamoto et al. 2019, Tanaka 2021)
Schistocerca gregaria Forskål, 1775	Cool period	+	+	+	+	(Nishide et al. 2015a, Tanaka 2021)
Nomadacris succincta Johannson, 1763	Both periods	+	+	+	+	(Tanaka 2021)
Atractomorpha lata Mochulsky, 1866	Warm period	+	+	+	+	This study
Oxya yezoensis Shiraki, 1910	Warm period	+	-	+	+	This study
Acrida cinerea Thunberg, 1815	Warm period	+	+	+	+	This study
Oedaleus infernalis Saussure, 1884	Warm period	+	+	+	+	This study
Gastrimargus marmoratus Thunberg, 1815	Warm period	+	△	-	+	This study
Chorthippus biguttulus L. 1758	Warm period	+	+*	+	+	This study
Orthoptera Romaleidae						
Romalea microptera Palisot de Beauvois, 1817	Warm period	n.d.	n.d.	n.d.	n.d.	(Smith et al. 2013)
Orthoptera: Gryllidae						
Teleogryllus emma Ohmachi & Matsuura, 1951	n.d.	-	-	n.d.	n.d.	This study
Hemiptera: Pentatomidae						
Nezara viridula L. 1758	n.d.	+	+	n.d.	n.d.	(Kiritani 1964)
Halyomorpha halys Stål, 1855	n.d.	+	+	-	+	(Endo et al. 2019, Tanaka and Kotaki 2020)
Lepidoptera: Crambidae						
Chilo suppressalis Walker, 1863	n.d.	+	+	-	n.d.	(Morimoto and Sato 1962)

*Comparison between a group of 15 and singly kept eggs only.

+, present; -, not present; △, single vs. group; n.d., not determined.

Cuticle physiology may have also influenced grasshopper hatching times. This is because hatchlings require time for their integument to harden before they can actively move or feed (Harano et al. 2009). Morning hatching may be advantageous for spring-hatching species because it allows their exocuticle to harden rapidly during the warming day, allowing them time to seek night roosts and to forage before the cold nightfall prohibits movement or feeding. This is seen in *Romalea microptera*, where morning-hatchlings fed that afternoon, but afternoon-hatchlings could not feed until the next day, ~ 20 h after hatching (Rackauskas et al. 2006). In the present study, *N. succincta* hatched during both the warm and cool thermoperiods (Table 1), possibly because this species evolved in a warm humid subtropical environment where moisture and temperature are usually suitable for hatching throughout the 24-h cycle (Japan Meteorological Agency). However, the hatching patterns of these species need to be confirmed in the field.

Although synchronous hatching in grasshoppers has long been known (Uvarov 1977), the mechanisms controlling such behavior have only recently been discovered (Tanaka 2017, Tanaka et al. 2018, Sakamoto et al. 2019, Tanaka 2021). The present research confirms that the number of eggs in a group can strongly influence synchronous hatching, which implies that vibrational stimuli from hatching eggs can induce hatching in adjacent eggs.

Table 1 summarizes the hatching characteristics of various insects. Hatching occurred earlier in eggs kept in a group than in eggs kept separately in all grasshopper species tested, in two true bugs, and one moth (Table 1). The way in which eggs responded to different egg group sizes varied with species. Most grasshoppers (7 species) hatched earlier as the egg group size increased, whereas the eggs of *O. yezoensis* kept in different group sizes showed no significant variation in the hatching time. Other insect species, including the rice stem borer *Chilo suppressalis* Walker, 1863 (Morimoto and Sato 1962), the southern green stink bug *Nezara viridula* (Linnaeus, 1758) (Kiritani 1964), and the brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) (Tanaka and Kotaki 2020), also showed a tendency to hatch earlier with increasing egg group size.

In addition to those mentioned earlier, other insects, such as a cockroach (Provine 1976, 1977) and a mantis fly (Dorey and Merritt 2017), also hatch simultaneously from the egg case or egg group. Overall, synchronized hatching appears to be widespread in insects. However, little is known about the mechanisms controlling synchronized hatching and how they evolved. In nine true bug species tested, five species hatched less synchronously when the eggs were removed from the group and kept separated, whereas four species did not show such a difference, although they all laid eggs as groups (Kiritani 1964, Endo and Numata 2017). In contrast, all nine grasshopper species tested to date hatch more synchronously when kept in a group than when kept singly (Table 1). The difference in the response to egg condition between grasshoppers and true bugs may relate to differences in the duration of their egg stages. Because the incubation period in the true bugs is only 4–7 days at 25°C, their hatching is completed in 3–9 h irrespective of whether the eggs are kept in a group or separated. In other words, because of rapid and uniform physiological development, they hatch more or less synchronously whether or not the eggs are grouped. In contrast, the incubation period (excluding the diapause period) in the grasshopper species lasted 15 –> 50 days at 30°C in the laboratory, and the eggs from the same pod required 2–5 days to complete hatching when removed from the pod and kept separated individually. This indicates a much larger individual variation in the incubation period in the grasshoppers than in the bugs and, thus, it is necessary for the grasshopper species to develop a controlling mechanism if synchronized hatching is important.

This paper confirms previous studies suggesting that vibrational signals from siblings can induce synchronous hatching in some insects (Tanaka 2017, Tanaka et al. 2018, Sakamoto et al. 2019, Tanaka 2021). In the migratory locust, signals from nearby hatching eggs can induce hatching in adjacent embryos that differ in age by more than a day, suggesting that developing eggs reach a stage where they "wait" for hatching signals from podmates (Tanaka 2017, Tanaka et al. 2018). A similar mechanism is known for the desert and Bombay locusts, although the differences in age range that allow eggs to hatch synchronously depend on the species (Tanaka 2021). In migratory locusts, vibrations are emitted by movement of the embryonic abdomen (Sakamoto et al. 2019, http://www.eje.cz/attachments/000076.avi). In the present study, in six grasshopper species, eggs separated by several millimeters hatched sporadically compared with those kept in contact with one another. However, some of those similarly separated hatched synchronously (< 1 h) when connected by a piece of wire (Fig. 4), as observed in the above-mentioned three locust species (Tanaka 2021). Together, these varied results strongly suggest that grasshopper species can use vibrational signals to control the hatching time for synchronized hatching.

The specific time at which the signals are produced by the six grasshoppers tested in this study is currently unknown. In addition to the vibrational signals generated by an embryo, other physical signals from hatching eggs, egg shell cracking, vermiform nymphs wiggling through the egg mass to reach the surface, or new hatchlings walking on the surface could also be involved.

In the present study, the eggs of the Emma field cricket failed to hatch synchronously when artificially kept in a group. This cricket requires a total of ~15 days of incubation at 30°C (excluding diapause) and does not lay eggs as a group. This result is reasonable in view of the fact that in nature, the eggs of this cricket are laid individually in soil and do not hatch synchronously, and neither nymphs nor adults aggregate. Perhaps vibration-induced hatching synchrony has been selected for only in species that lay grouped eggs and benefit from synchronous hatching. Two lady beetles, *Epilachna sparsa orientalis* and *E. vigintioctomaculata*, lay eggs as groups. The former lays eggs so they touch and the latter lays eggs that do not touch one another. Morimoto (1965) observed that egg hatching from groups was completed in 1.8–2.8 h in the former and 4.5–10.5 h in the latter. Although the mechanism responsible for synchronized hatching is unknown in these two species, it is possible that the eggs of *E. s. orientalis* achieve synchronized hatching through a physical stimulus such as vibration transmitted through contact with one another. Morimoto noted that the well-synchronized hatching pattern observed in *E. s. orientalis* might be related to their strong tendency to aggregate as hatchlings compared to the other beetles.

Many grasshoppers and other insects form tight aggregations in the 1st instar (Fig. 6), which may or may not continue into later stages (Uvarov 1977, Costa 2006, Hatle and Whitman 2001). In the desert locust, both solitarious and gregarious hatchlings show similar degrees of aggregative behavior (Guershon and Ayali 2012), although the aggregating tendency in solitarious hatchlings usually weakens in later instars (Uvarov 1977). The advantages and disadvantages of aggregative behavior have been extensively studied and may include diluting individual predation risk, group defense, overcoming plant defenses via group efforts, increased
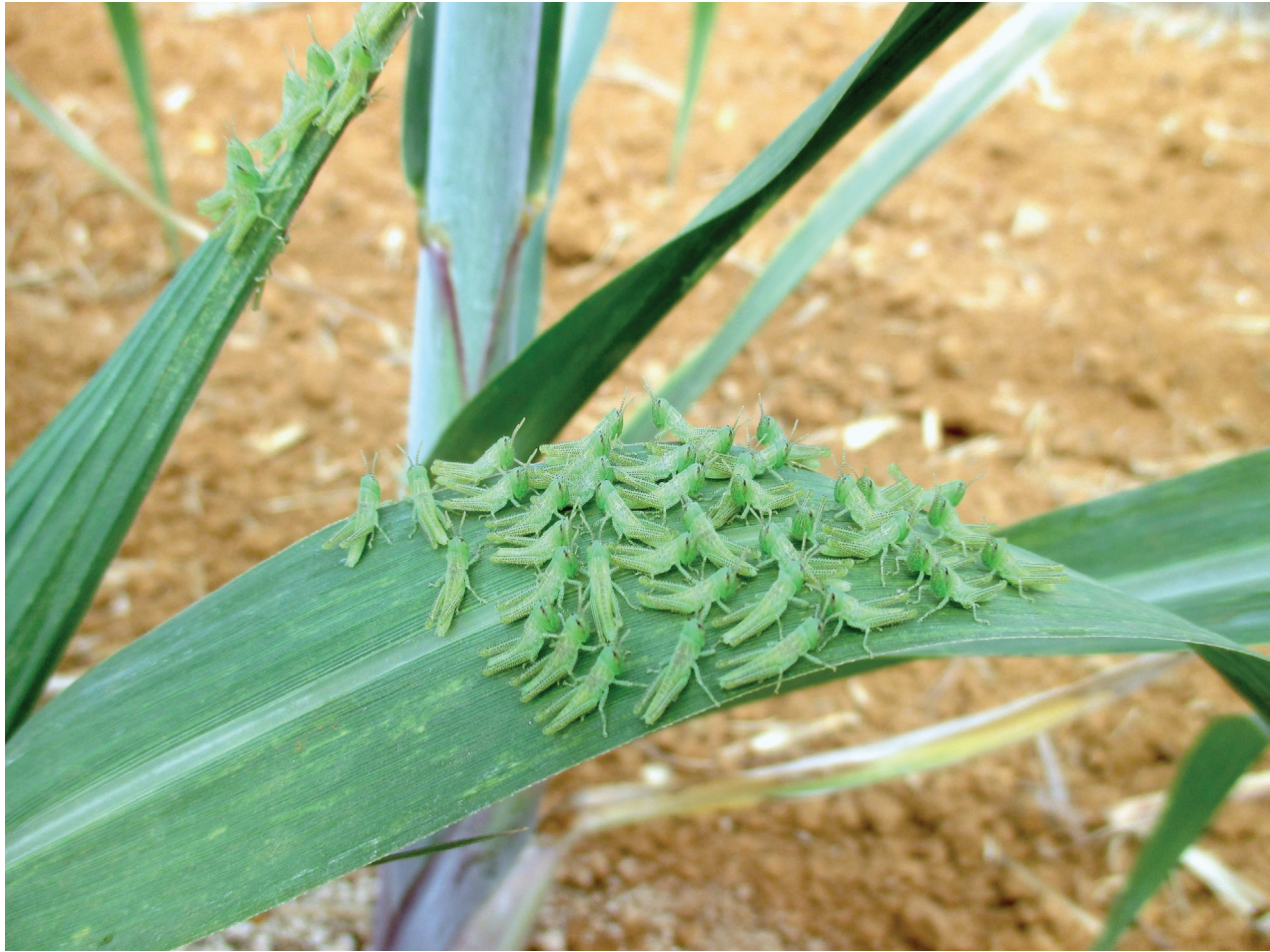
Fig. 6. Aggregations of *Nomadacris succincta* hatchlings at 09:46 on May 25, 2018 on Minami-Daito Island, Japan (Photographed by Masanari Aizawa). This grasshopper aggregates tightly as 1st instars only during the nymphal stage.

thermoregulation or desiccation resistance, shelter building, etc. (Edmunds 1974, Vulinec 1990, Ruxton et al. 2004, Costa 2006). As such, synchronized hatching may have evolved in part to facilitate immediate aggregation in newly hatched insects.

To understand the mechanism underlying synchronized hatching and its evolution, more species of insects that produce eggs in a group with different lengths of embryonic stage should be examined. Grasshopper species would be ideal insects to use to explore this subject.

Acknowledgements

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