論文：

Daily changes in egg size of the field *Emma* cricket *Teleogryllus emma* (Orthoptera: Gryllidae) inhabiting the slope of Oishi Dam

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Abstract

The egg period, egg size, and daily changes in egg size were investigated in the field cricket, *Teleogryllus emma*. *T. emma* inhabits the slope of the Oishi Dam (OD) (38.03°N, 139.57°E) and Arakawa riverside (AR) (38.09°N, 139.57°E) in Sekikawa Village, Niigata Prefecture, Japan. We studied OD, AR, and the F1 hybrids, viz, OdAr (OD females × AR males), and ArOd (AR females × OD males) and determined whether daily changes in egg size are related to shortening of the egg period of T. *emma* on the dam slope.

The egg periods in OD, AR, OdAr, and ArOd were shorter at higher temperatures ($p \leq 0.05$). Furthermore, the egg period in OD was shorter than that in AR and OdAr ($p \leq 0.05$). After oviposition, the eggs enlarged due to water absorption and entered diapause at the embryonic stage of the array. The major axes of eggs in OD, AR, OdAr, and ArOd expanded daily and were influenced by temperature whereby a higher temperature resulted in an increase in the major axes. At 15°C, the major axis gradually increased after oviposition, reaching its maximum at 130 days. The eggs grew rapidly at temperatures ≥ 20°C and reached their maximum size at 10－14 days, 5－7 days, and 4－6 days after oviposition at 20, 25, and 30°C, respectively. The expansion of the major axis up to 7 days after oviposition showed that the egg size in AR increased faster than that in OD at 15°C, while that in OD increased faster than AR at 20, 25, and 30°C, indicating that the expansion rate in OD and AR eggs differed with temperatures. Temperature-dependent changes in the expansion rates in OD and AR up to 7 days after oviposition suggest that they may be associated with shortened egg period.

"Key words: egg expansion rate, egg period, egg size, evolution, hatchability, speciation"

INTRODUCTION

The slope of the Oishi Dam in Sekikawa Village, Niigata Prefecture, Japan (38.03°N, 139.57°E), is exposed from early June to mid-October due to water discharge from the lake, forming a green field. Several species of crickets including *Teleogryllus emma* inhabit green fields. It begins to hatch in mid-June, emerges as an adult, and lays eggs by mid-October when the dam slope is submerged. The eggs overwinter in the soil of the lake and hatch the following year (Yamaura *et al*., 1999).

In the process of isolation on the dam slope and adapting to the new environment, the egg size and head width of T. *emma* inhabiting the dam slope became smaller than those found on the Arakawa riverside (38.09°N, 139.57°E). The head width of T. *emma* on the dam slope appeared smaller than that in the surrounding field and the period from egg hatching to adult emergence appeared shorter (Yamaura *et al*., 1999).

In this paper, we considered whether variations in egg characteristics, such as egg period, hatchability, egg size, and daily changes in egg size, contributed to the shortening of the egg period in T. *emma*.

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MATERIALS AND METHODS

Insects

T. *emma* nymphs were collected in July 2003 at two locations; the slope of the Oishi Dam (38.03°N, 139.57°E, average sea level, 160–170 m) and the Arakawa riverside (38.09°N, 139.57°E, average sea level, 29 m) in Sekikawa Village, Niigata Prefecture, Japan. The two collection sites were approximately 7 km apart at a straight distance. The crickets were reared in cases (20 × 40 × 27 cm) a 50 crickets per case. Cases were kept under continuous light (LL) in a thermostatic room at 28 ± 1°C in the Yamaguchi Prefectural University laboratory. Humidity in the thermostatic room was 50–60%. The crickets were fed insect pellets (Oriental Kobo Kogyo K.K., Osaka, Japan) and water during the experiments. Cabbage and carrot slices were fed to the crickets during the breeding of successive generations. T. *emma* collected from the field on the slope of the Oishi Dam and the Arakawa riverside were abbreviated as "OD" and "AR," respectively. The hybrids, which were made by mating unmated females and males collected in the field of OD and AR, that is, OD-females × AR-males and AR-females × OD-males, were abbreviated as "OdAr" and "ArOd," respectively.

Egg period

Eggs were kept on wet filter papers within 24 h after being laid and incubated at 20, 25, and 30°C. The number of hatched eggs was counted every 24 h.

Egg size

Eggs within 24 h of laying were kept on wet filter papers. The egg size was measured within 24 h of oviposition. The major and minor axes diameters of the eggs were measured with a micrometer using a stereo microscope (Nikon Corporation, Tokyo, Japan). The temperature was controlled using incubators (Nihon Ikakikai K.K. Osaka, Japan). In measuring daily changes in egg size, eggs were kept in incubators 15, 20, 25, and 30°C within 24 h of oviposition, and the major axis was measured every 24 h, and only surviving eggs were used to show the expansion rate.

Statistical analysis

All statistical analyses were performed using EZR–Version 2.7-1 (Kanda 2013). Three or more factors were analyzed using Tukey's test; hatchability data were analyzed using Fisher's exact test and Bonferroni test.

RESULTS

Egg period

T. *emma* egg periods for OD, AR, OdAr, and ArOd shortened with increasing temperature (Figure 1). The egg period of OD was the shortest at any temperature, followed by ArOd, OdAr, and AR. There was no significant difference in the egg period between OD and ArOd at either of the three incubation temperatures (20, 25, and 30ºC), OdAr and ArOd at 25ºC, and ArOd and OdAr at 30ºC. However, a significant difference ($p < 0.05$) was observed among all other comparisons. The cumulative hatching rate curves for OD and ArOd at each temperature almost overlapped, with OdAr between OD and AR at 20ºC, almost overlapping at 30ºC, and close to OD at 25ºC (Figure 2).

Hatchability

Hatchability was lower at higher temperatures. The OD showed the highest hatchability at 20, 25, and 30ºC

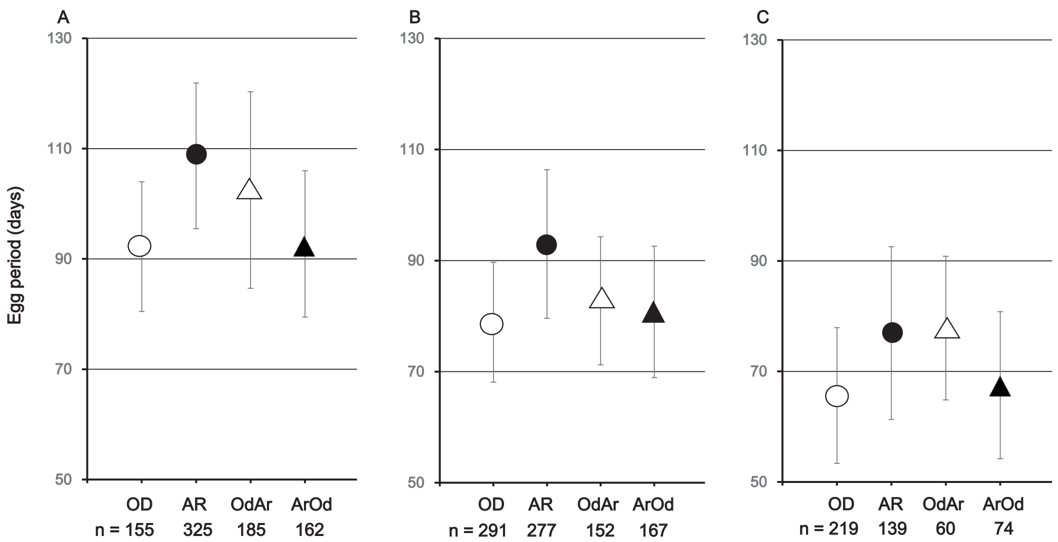


Figure 1. Egg period in *Teleogryllus emma* under different temperatures. A: 20°C. B: 25°C. C: 30°C. n = number of eggs.

(Figure 3A); although significant differences ($p \leq 0.05$) were observed, there was no significant difference between AR, OdAr, and ArOd at 20ºC, OD and AR at 25ºC, OD and OdAr at 30ºC, and AR and ArOd at 30ºC (Figure 3B).

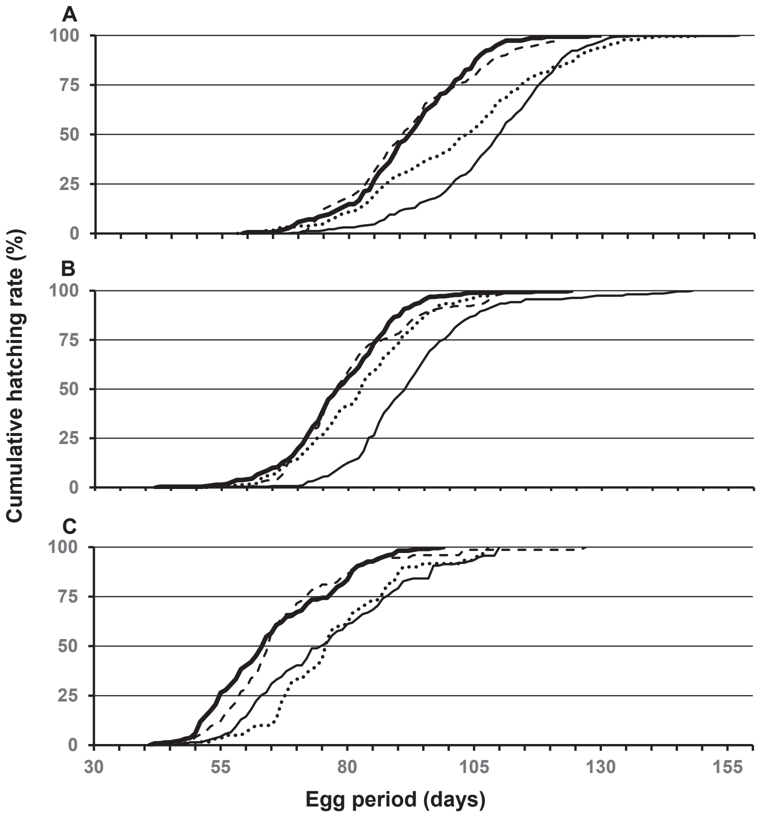
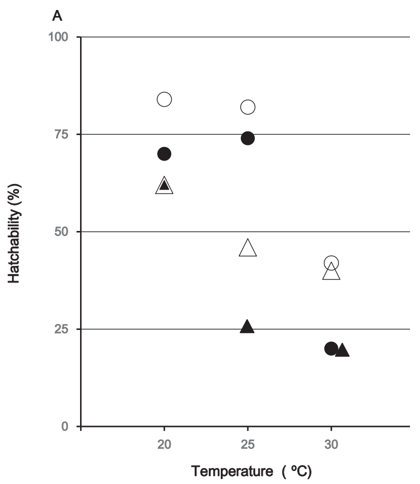


Figure 2. Cumulative hatching rate in *Teleogryllus emma* under different temperatures. A: 20ºC. B: 25ºC. C: 30ºC. Thick solid line: OD (the slope of the Oishi Dam). Thin solid line: AR (Arakawa riverside). Dotted line: OdAr (OD-female × AR-male. Dashed line: ArOd (AR-female × OD-male).



B

20ºC	OD	AR	OdAr	ArOd
OD	–	$p \leq 0.05$	$p \leq 0.05$	$p \leq 0.05$
AR		–	NS	NS
OdAr			–	NS
ArOd				–

25ºC	OD	AR	OdAr	ArOd
OD	–	NS	$p \leq 0.05$	$p \leq 0.05$
AR		–	$p \leq 0.05$	$p \leq 0.05$
OdAr			–	$p \leq 0.05$
ArOd				–

30ºC	OD	AR	OdAr	ArOd
OD	–	$p \leq 0.05$	NS	$p \leq 0.05$
AR		–	$p \leq 0.05$	NS
OdAr			–	$p \leq 0.05$
ArOd				–

NS : no significant difference

Figure 3. Hatchability in *Teleogryllus emma* eggs under different temperatures. A: Percenage hatchability. B: Significant test results. White circle: OD (the slope of the Oishi Dam). Black circle: AR (Arakawa riverside). White triangle: OdAr (OD-female × AR-male. Black triangle: ArOd (AR-female × OD-male). Significance difference test: Fisher's exact test.

Egg size

The major and minor axes of eggs laid within 24 h were measured, as well as those of only the hatched eggs among them. The major axis of eggs within 24 h after oviposition was the shortest in OD, and longer in the order AR > OdAr > ArOd (Figure 4A). Significant differences were observed for all except between OD ($p < 0.05$) (Figure 4C). The major axis of only hatched eggs was the shortest in OD, and longer in the order AR > OdAr > ArOd (Figure 4B). The difference in the major axis of only hatched eggs was significant excluding the difference between OD and AR, OdAr and ArOd ($p < 0.05$) (Figure 4D). The minor axis of eggs laid within 24 h after oviposition and hatched eggs among them was only significantly different ($p < 0.05$) between OD and OdAr (Figure 5).

Daily changes in egg size

Eggs of crickets begin to absorb water after oviposition, which enables the elongation of both the major and minor axes. The higher the temperature, the earlier the start of water absorption, and the shorter the period it took for the egg to reach its maximum size (Figure 6). The time taken to reach maximum egg size at 15ºC gradually increased until approximately 90 days after oviposition (Figure 7A), whereas it increased rapidly between 10–14 days at 20ºC (Figure 7B), 5–9 days at 25ºC (Figure 7C), and 4–6 days at 30ºC (Figure 7D).

Egg expansion magnification

The major axis of eggs within 24 h after oviposition was set to 1. Eggs expansion magnification was examined after stabilization of the egg size (day 160 at 15ºC, day 50 at 20 and 25ºC, and day 45 at 30ºC). The egg expansion magnifications for OD, OdAr, and ArOd were higher at higher temperatures (Figure 8), although a similar pattern was observed in AR

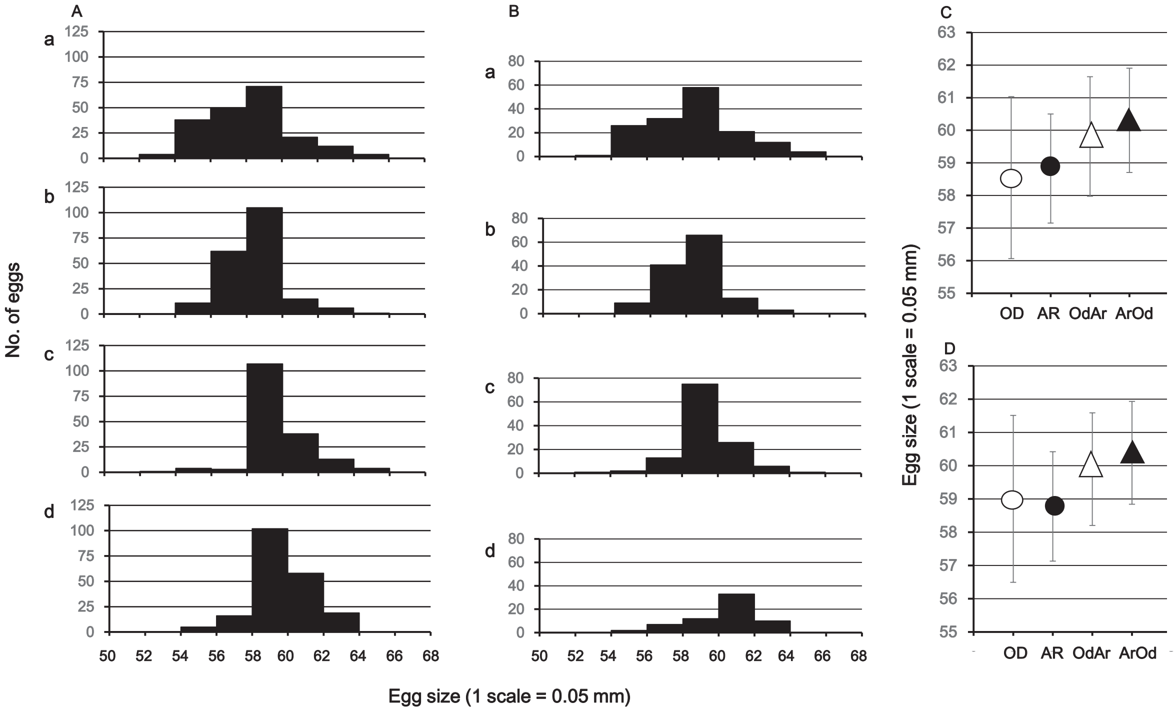


Figure 4. Major axis of eggs in *Teleogryllus emma*. A: Frequency distribution within 24 h after oviposition. B: Frequency distribution of hatched eggs. C: Mean and standard deviation of A. D: Mean and standard deviation of B. a: OD (the slope of the Oishi Dam). b: AR (Arakawa riverside). c: OdAr (OD-female × AR-male). d: ArOd (AR-female × OD-male).

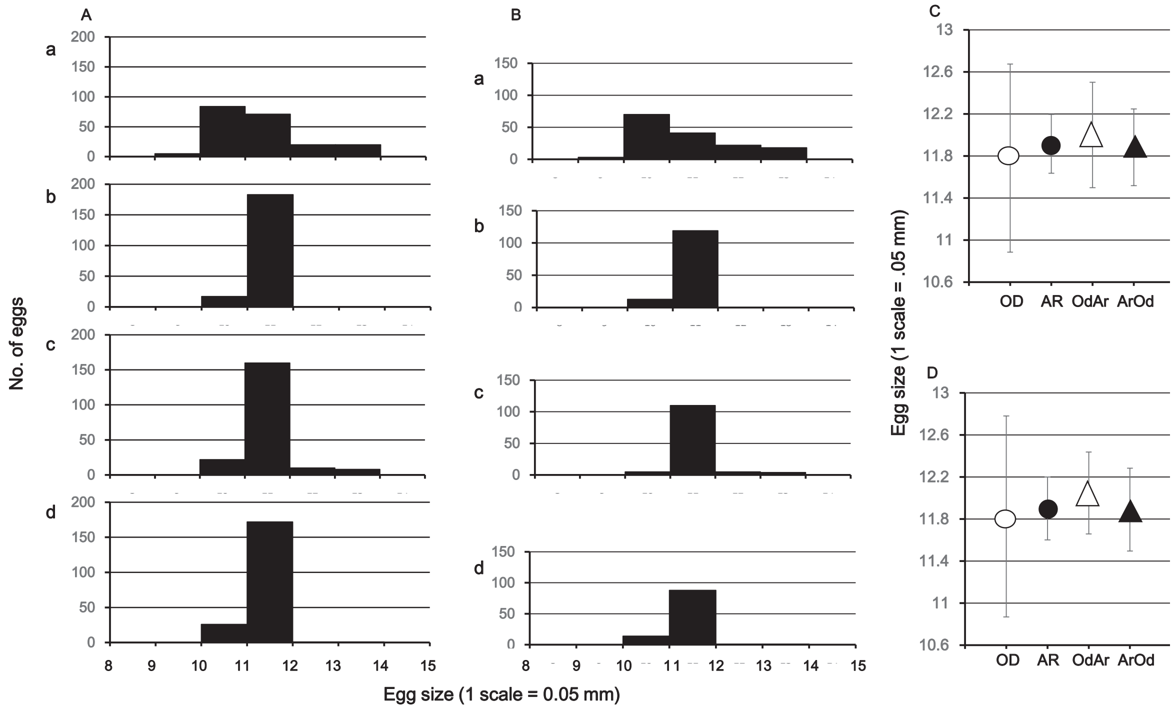


Figure 5. Minor axis of eggs in *Teleogryllus emma*. A: Frequency distribution within 24 h after oviposition. B: Frequency distribution of hatched eggs. C: Mean and standard deviation of A. D: Mean and standard deviation of B. a: OD (the slope of the Oishi Dam). b: AR (Arakawa riverside). c: OdAr (OD-female × AR-male). d: ArOd (AR-female × OD-male).

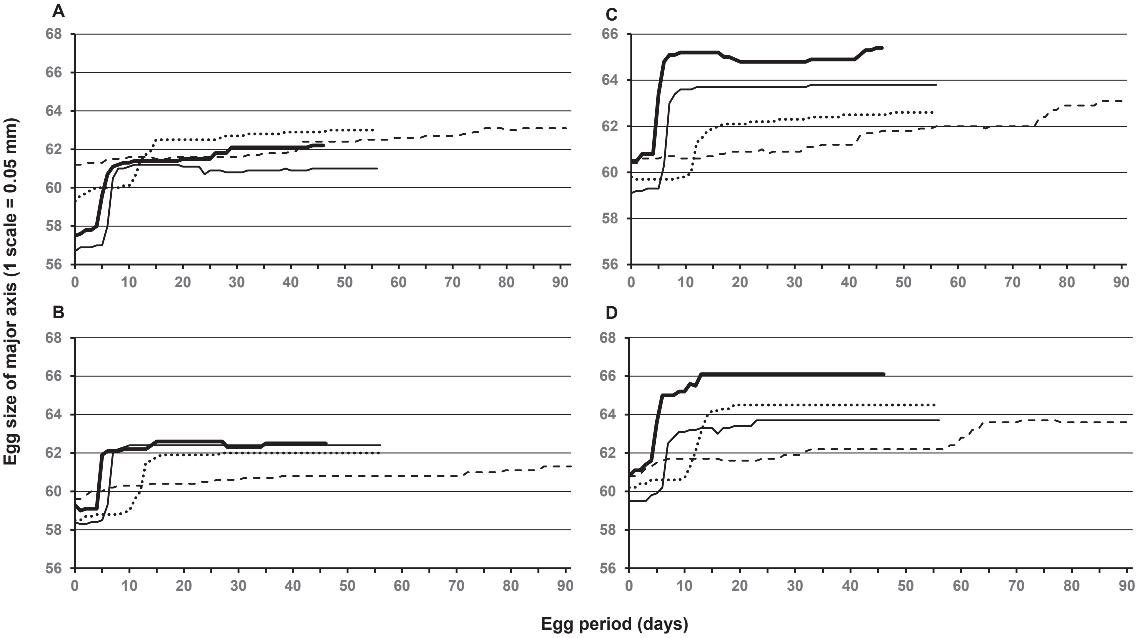


Figure 6. Daily changes in major axis of eggs over time in *Teleogryllus emma*. A: OD (the slope of the Oishi Dam). B: AR (Arakawa riverside). C: OdAr (OD-female × AR-male). D: ArOd (AR-female × OD-male). Dashed line: 15°C. Dotted line: 20°C. Thin solid line: 25°C. Thick solid line: 30°C.

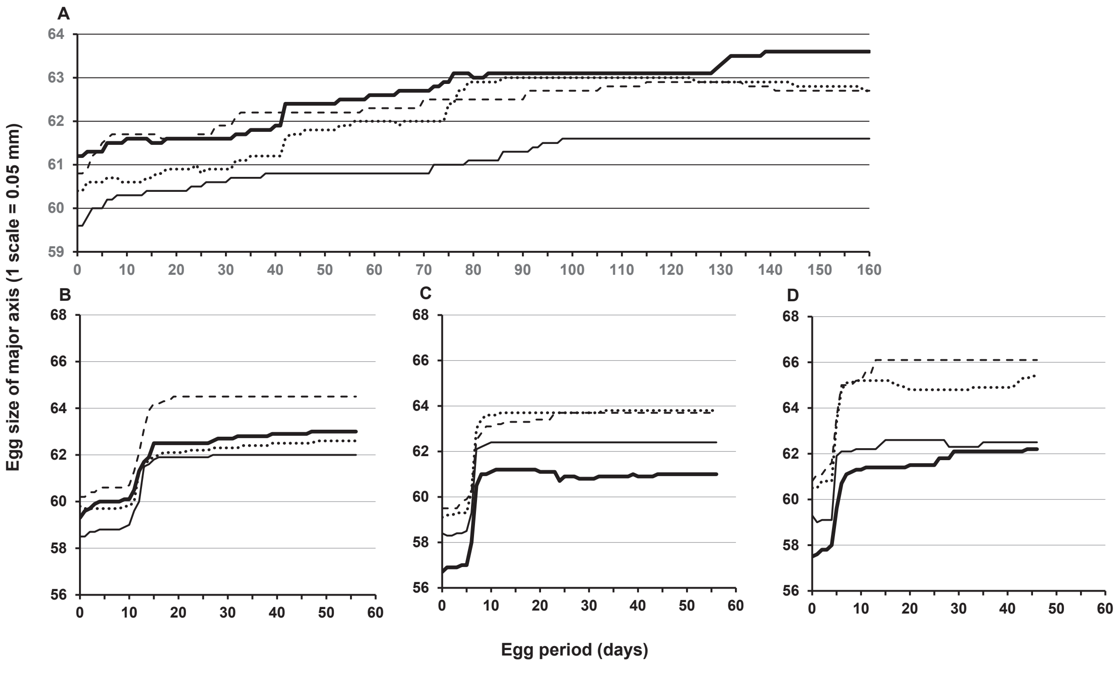


Figure 7. Daily changes in major axis of eggs over time in *Teleogryllus emma*. A: 15°C. B: 20°C. C: 25°C. D: 30°C. Thick solid line: OD (the slope of the Oishi Dam). Thin solid line: AR (Arakawa riverside). Dotted line: OdAr (OD-female × AR-male. Dashed line: ArOd (AR-female × OD-male).

in that the egg expansion magnification increased with increasing temperatures (15ºC > 20ºC > 25ºC), with a slight decrease at 30ºC (Figure 8). The egg expansion magnification was similar for OD, OdAr, and ArOd at 15ºC and 25ºC; OD and AR at 20ºC; OD, OdAr, and ArOd at 30ºC.

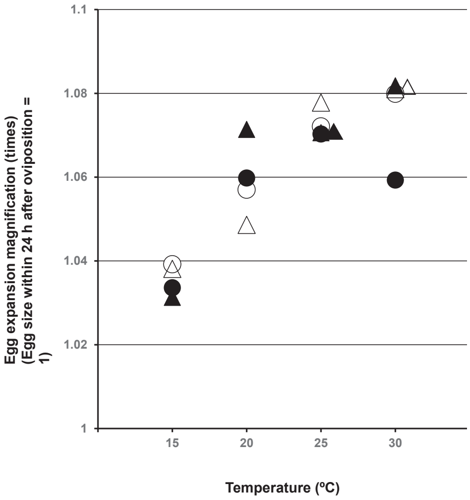


Figure 8. Egg expansion magnification in *Teleogryllus emma*. The egg size within 24 h after oviposition is taken as 1, and the egg expansion magnification at 160 days at 15°C, 50 days at 20 and 25°C, and 45 days at 30°C. White circle: OD (the slope of the Oishi Dam). Black circle: AR (Arakawa riverside). White triangle: OdAr (OD-female × AR-male. Black triangle: ArOd (AR-female × OD-male).

Egg expansion rate

Daily changes in egg expansion rate were examined whereby the measurements at the time of egg size stabilization (day 160 at 15ºC, day 50 at 20 and 25ºC, and day 45 at 30ºC) were considered as 100%. Eggs of OD, AR, OdAr, and ArOd gradually expanded until approximately 100 days after oviposition at 15ºC (Figure 9, broken line). The egg expansion rates of AR and ArOd at approximately 30 days after oviposition were approximately twice those of OD and OdAr (Figure 10A). The egg expansion rate increased rapidly at 20ºC (Figure 9, dotted line; Figure 10B), 25ºC (Figure 9, thin solid line; Figure 10C), and 30ºC (Figure 9, thick solid line; Figure 10D), between 10 and 14 days, 5 and 9 days, and 4 and 6 days, respectively. The changes in the egg expansion rates of OD, AR, OdAr, and ArOd at 15, 20, 25, and 30ºC were shown schematically in Figure 11. A detailed examination of the daily changes in the egg expansion rate for up to 7 days revealed that the patterns of OD, AR, OdAr, and ArOd varied with temperature. The egg expansion rate of AR increased faster than that of OD at 15ºC. However, at 20, 25, and 30ºC, the expansion rate of OD increased faster than that of AR with the difference in the degree of expansion being smaller at higher temperatures (Figure 12). The egg expansion rate for OdAr or ArOd did not show a constant trend between them and OD or AR.

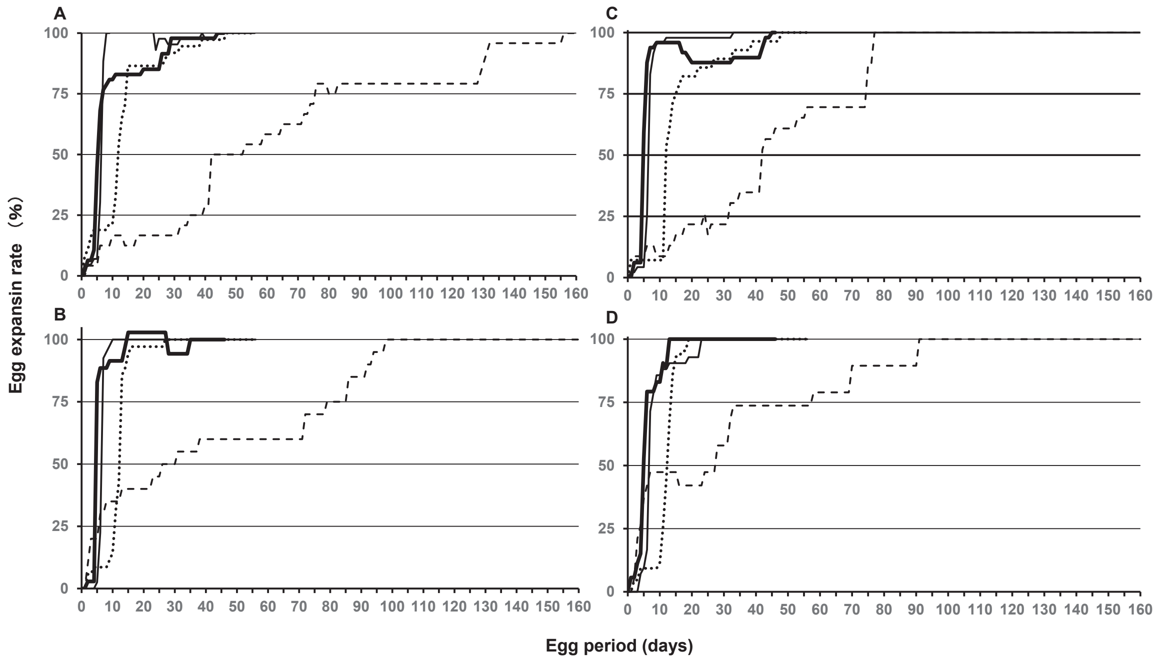


Figure 9. Daily changes over time in the expansion rate of major axis of eggs in *Teleogryllus emma*. Based on the egg size within 24 h after oviposition, indicated by egg expansion rate at 160 days at 15°C, 50 days at 20°C and 25°C, and 45 days at 30°C. A: OD (the slope of the Oishi Dam). B: AR (Arakawa riverside). C: OdAr (OD-female × AR-male. D: ArOd (AR-female × OD-male). Dashed line: 15°C. Dotted line: 20°C. Thin solid line: 25°C. Thick solid line: 30°C.

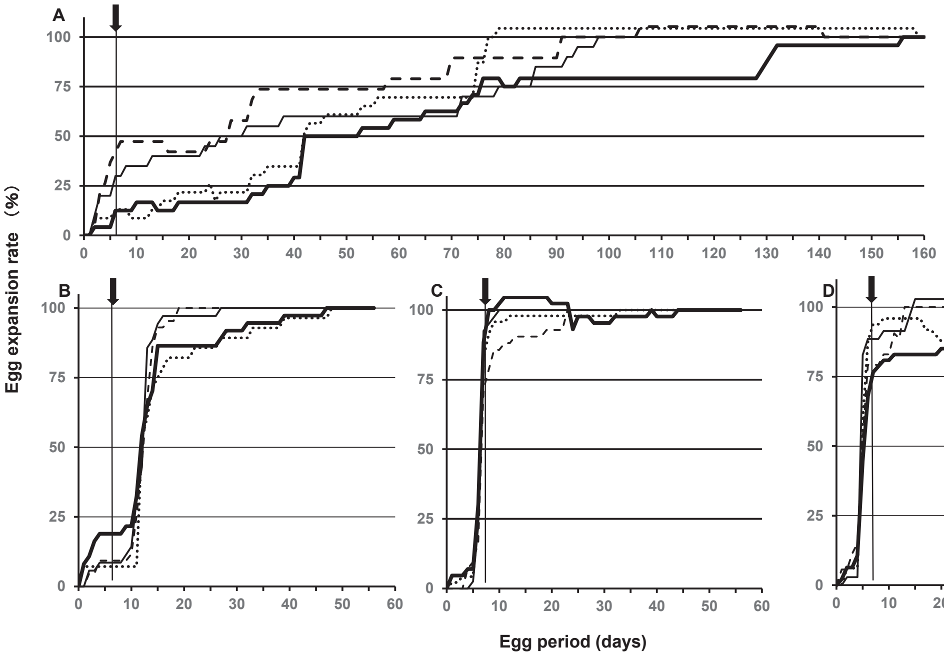


Figure 10. Daily changes over time in the expansion rate of major axis of eggs in *Teleogryllus emma*. Based on the egg size within 24 h after oviposition, and the egg expansion rate was expressed as 100% at 160 days at 15°C, 50 days at 20°C and 25°C, and 45 days at 30°C. A: 15°C. B: 20°C. C: 25°C. D: 30°C. Thick solid line: OD (the slope of the Oishi Dam). Thin solid line: AR (Arakawa riverside). Dotted line: OdAr (OD-female × AR-male. Dashed line: ArOd (AR-female × OD-male). Black arrows indicated 7 days after oviposition.

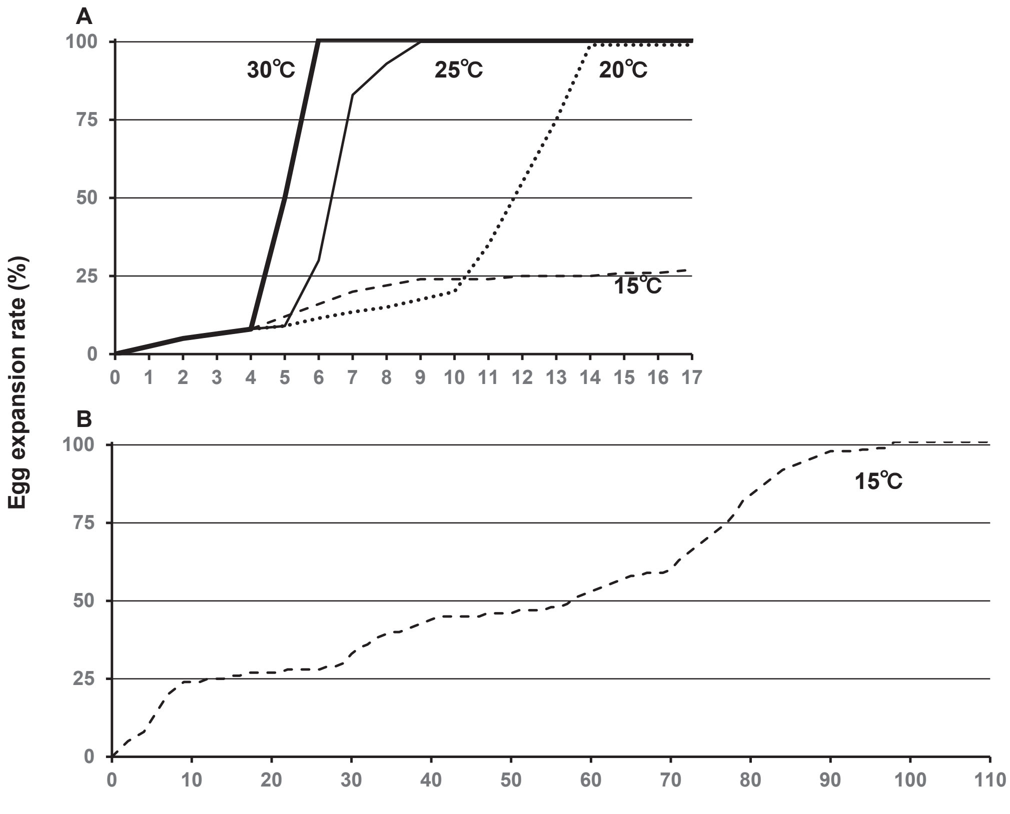


Figure 11. Schematic diagram daily changes in the expansion rate of major axis in *Teleogryllus emma* eggs at different temperatures. A: Expansion rate up to 17 days at 15, 20, 25, and 30°C. B: Expansion rate up to 110 days at 15°C.

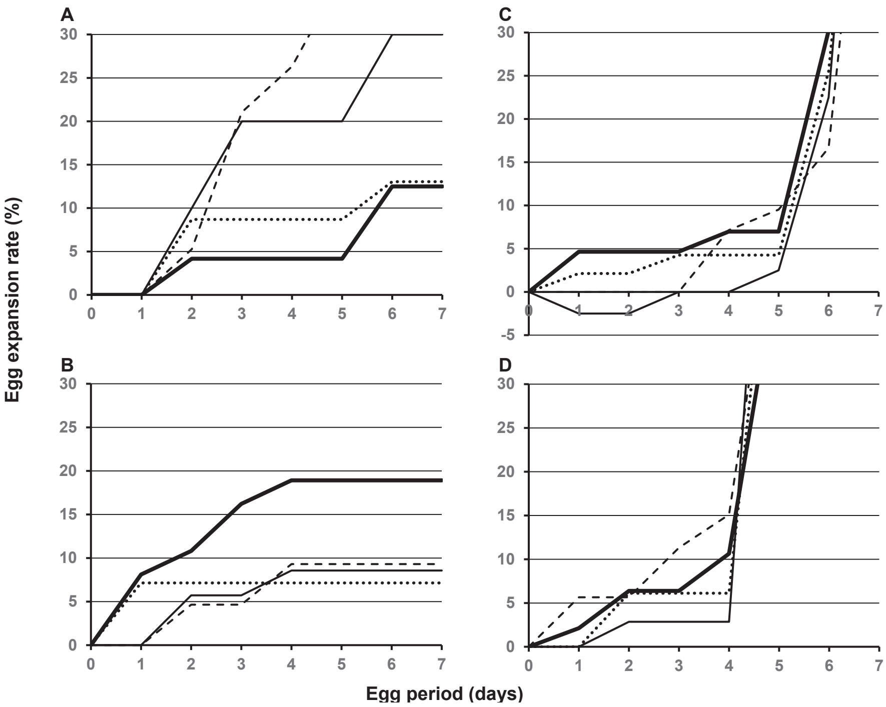


Figure 12. Daily changes over time up to 7 days in the expansion rate of major axis of eggs in *Teleogryllus emma*. A: 15°C. B: 20°C. C: 25°C. D: 30°C. Thick solid line: OD (the slope of the Oishi Dam). Thin solid line: AR (Arakawa riverside). Dotted line: OdAr (OD-female × AR-male. Dashed line: ArOd (AR-female × OD-male).

Low temperature sensitivity of embryo and daily change of egg size

The major axis of eggs in OD at 25℃ increased rapidly from the 5th day after oviposition and was almost at its maximum on the 9th day (Figure 11). When the eggs were transferred from 25℃ to 7℃ on the 3rd day after oviposition, when water absorption was not very advanced, and then returned to 25℃ after they were maintained at 7℃ for 2, 6, and 14 weeks, hatchability was 49, 14, and 0%, respectively (Figure 13) (Arai, in printing). The hatchability was 52, 64, and 73%, respectively, when the eggs were transferred on the 7th day after oviposition when water absorption was advanced (Figure 13) (Arai, in printing). This indicated that the eggs could possibly survive the winter by absorbing enough water and growing to the diapause stage.

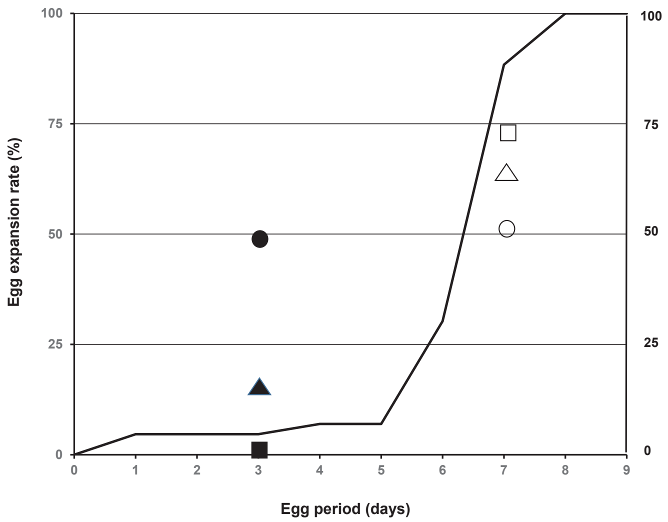


Figure 13. Daily changes over time in the expansion rate of major axis of eggs at 25°C in OD (the slope of the Oishi Dam) of *Teleogryllus emma* and the hatchability of OD when eggs were kept at 25°C for 3 (black mark) or 7 days (white mark) and then transferred to 7°C. Circle: Maintained at 7°C for 2 weeks. Triangle: Maintained at 7°C for 6 weeks. Square: Maintained at 7°C for 14 weeks. The hatchability was quoted from Arai (inpress).

DISCUSSION

The Oishi Dam was constructed in 1967 as a multipurpose dam for both flood control and power generation to prevent damage caused by the Uetsu Flood. Construction began in 1972, upstream of the Oishi River, which flows into the Arakawa River, and was completed in 1978. The water level of the Oishi Dam drops by approximately 30 m from its full capacity between June and September, exposing the dam slope and forming greenbelts. Surveys of terrestrial insects on the slope of the Oishi Dam have been conducted since 1994, and it has been reported that insects such as *T. emma* have inhabited the greenbelts (Ministry of Land, Infrastructure, Transport and Tourism: MLITT, 1999). It is unknown when and where *T. emma* invaded the slope of the Oishi Dam. However, it has been recorded that *T. emma* completed its life history on the dam lake slope, sporting a smaller body size and spending a shorter time from egg hatching to adult emergence than those in the surrounding areas (Yamaura *et al*., 1999). The variations found in the isolated *T. emma* on the dam slope were very small. However, they were in the early stages of speciation, suggesting that they may serve as valuable models for examining the process of microevolution. In this study, we examined whether daily changes in egg size were involved in shortening the egg period in *T. emma*.

Teleogryllus emma overwinters when in egg form. Eggs are diapause in the array stage (Umeya, 1953) and develop to the array stage in approximately 9 days at 25ºC. When eggs were kept at 25ºC for 3 days after oviposition, transferred to 7ºC, and then returned to 25ºC, many eggs died, indicating that the embryos did not develop till the diapause stage. However, when the eggs were kept at 25ºC for 7 days, the hatchability was high, indicating that the embryos developed till the diapause stage (Figure 13). The water surface of the lake begins to rise in early October and becomes full by late October, after which the slope becomes submerged. Therefore, oviposition on the dam slope is possible until mid-late October; however, embryos must enter the diapause stage until submerging. The mean temperature in Sekikawa Village, Niigata Prefecture, from late September to early October is approximately 19.6ºC. At 20ºC, the embryos developed to the diapause stage 14 days after oviposition (Figure 10). Therefore, oviposition must occur by late September at the latest. This suggests that egg development is influenced by the temperature after oviposition and the timing of the rise in water surface of the lake. Furthermore, the shortening of the time between egg hatching and oviposition may be attributed to this selection pressure. As eggs on the dam slope must develop till the diapause stage by early October to overwinter, it is likely that selection pressure for development was exerted at temperatures > 20ºC. After mid-October, eggs were submerged in water below 15ºC, and the embryos were expected to die before reaching the diapause stage (Figure 13), suggesting that at 15ºC there was no selection pressure on embryo development. Eggs on the Arakawa riverside were not in danger of submersion, and no selective pressure was exerted on egg development at temperatures above 20ºC. However, at 15ºC, selective pressure was exerted to increase the possibility of overwintering, which may have promoted egg development (Figure 12). The difference in egg expansion rate within 7 days after oviposition between the dam slope and the Arakawa riverside was very small and did not affect the egg period at that moment (Figure 12). However, it is suggested to be the beginning of adaptation to a new environment.

Teleogryllus emma distributed in the Japanese archipelago between 30 and 43ºN is univoltine with overwintering eggs. The head width of adults was smaller in more northern populations and varied geographically according to the “Inverse Bergmann’s rule” (Masaki, 1967, 1978). The nymphal period was also shorter in the northern populations (Masaki, 1978). The shorter the photoperiod, the smaller the head width of *T. emma*, and the shorter the nymphal period (Masaki, 1965, 1978; Arai, in printed). These results indicate a close relationship between adult size and nymphal period, and between them and photoperiods. Although there may be no direct relationship between the egg period and adult size or nymphal period, the egg period was shorter in northern populations and geographic gradients (Masaki, 1978). Eggs of *T. emma* diapause for most of the egg period, and the egg period itself corresponded to the depth of diapause, which was shallower in the northern populations. Even though the dam slope and the Arakawa riverside were located at approximately the same latitude, the egg period on the dam slope was shorter, and the depth of diapause was shallower than that of the Arakawa riverside, which may be indicative of T. emma displaying characteristics closer to that of northern population. Geographic gradients in head width, nymphal period, and egg period (diapause depth) may be associated with life-history variation.

Adaptation to the new environment in isolation and transmission of the mutated trait to the next generation are thought to initiate divergence into a new species. It is, therefore, important to verify the sprouting of speciation even when such mutations are small.

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