

Sympatric coexistence of sibling species *Harmonia yedoensis* and *H. axyridis* (Coleoptera: Coccinellidae) and the roles of maternal investment through egg and sibling cannibalism

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Abstract. The sibling species *H. yedoensis* Takizawa coexists sympatrically and simultaneously with *H. axyridis* only on pine trees in Japan. To elucidate the mechanisms enabling coexistence of these two sympatric sibling species, a laboratory experiment was performed that focused on differences in their maternal investment through eggs and the role of sibling cannibalism. The egg size (volume) of *H. yedoensis* was 24.91% larger than that of *H. axyridis*. Cluster size in *H. axyridis* was significantly larger than that in *H. yedoensis*; however, the total number of eggs and oviposition cost (by volume) per female in *H. yedoensis* were not significantly different from those in *H. axyridis*, although total number of clusters tended to be slightly higher in *H. yedoensis* than in *H. axyridis*. The percentage of undeveloped eggs per cluster in *H. yedoensis* was not significantly different from that in *H. axyridis*, whereas the percentage of developed eggs with delayed hatching per cluster was significantly larger in *H. yedoensis* than in *H. axyridis*. Moreover, the cost of sibling cannibalism per hatched larval cluster in *H. yedoensis* (worth 4.43 sibling eggs) was 3.36 times larger than that in *H. axyridis*. Therefore, maternal investment through egg and sibling cannibalism in developed eggs with delayed hatching are more intense in *H. yedoensis* than in *H. axyridis*, implying a higher larval survival rate through higher ability of prey capturing at the first instar. The results in this study suggest that the higher survival rate and accelerated development in *H. yedoensis* by the two maternal investments, i.e., a large egg and intense sibling cannibalism of developed eggs with delayed hatching, may play an important role in sympatric coexistence with the aggressive aphidophagous ladybird beetle *H. axyridis*.

INTRODUCTION

Species can be defined as sets of organisms that can mate with each other and produce viable grandchildren, as sets of organisms resembling each other morphologically, or as DNA sequences that act as evolutionarily independent units (e.g., Sterns & Hoekstra, 2000). Generally, it is known that three closely related species concepts exist: biological, ecological, and phenetic species (e.g., Ridley, 2004). Some biologists suggest that the phenetic species concept, which defines species in general by shaped phenetic attributes, has serious theoretical defects rendering it ambiguous and typological theories of species are also rejected (e.g., Ridley, 2004). The phenetic species concept is based on the premise that phenetic differences among species largely contribute to their reproductive isolation. However, sibling species, as pairs of species that differ reproductively but not morphologically, illustrate that phenetic and reproductive units do not necessarily coincide (Ridley, 2004).

The two ladybird beetles *Harmonia axyridis* Pallas and *H. yedoensis* Takizawa (Coleoptera: Coccinellidae) are good examples of sibling species. *H. axyridis* is distributed in the northeast part of Asia and its biology has been studied extensively (e.g., ecological genetics, ecology,

systematics, and applied entomology; e.g., Hodek & Honěk, 1996). *H. axyridis* has recently been imported to North America, Canada, and Europe, mainly for biological control (e.g., Snyder & Evans, 2006), where it has had a large impact on the native aphidophagous guilds: Some native ladybird species have disappeared and biodiversity of some aphidophagous community has decreased following invasion by *H. axyridis*, which is an aggressive species. Intense intra-guild predation by *H. axyridis* has been inferred by several authors (e.g., Colunga-García & Gage, 1998; Yasuda & Ohnuma, 1999; Adriaens et al., 2003; Brown, 2003; Koch, 2003; Yasuda et al., 2004; Snyder & Evans, 2006).

Adults of *H. axyridis* are very mobile with high prey searching ability, resulting in concentrated adult arrivals and oviposition in suitable habitats (Osawa, 2000). Larval mortality is a key-factor in *H. axyridis* population dynamics (Osawa, 1993) and the survival rates of early instars are low (Osawa, 1992) mainly because of their low ability to capture aphids (Kawai, 1978). Sibling cannibalism occurs in more than 90% of egg batches and increases the survival rate of cannibals through unequal resource allocation (Osawa, 1989). Both undeveloped eggs and developed eggs with delayed hatching play an important role in maternal investment, resulting in a high

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survival rate and accelerated development of first instars (Osawa, 2002, 2003). Therefore, sibling cannibalism at hatching plays an important role in the life history of *H. axyridis* in natural populations (Osawa, 1993). Moreover, strong density-dependence has been observed in *H. axyridis* populations, caused partly by cannibalism. Egg and pupal cannibalism by larvae have been observed but not egg parasitism, and adults, and maybe eggs as well as larvae, are not eaten heavily by other predators (Osawa, 1993). These stabilizing and persistent self-regulatory mechanisms in *H. axyridis* populations, based on density-dependent mortality and sibling cannibalism at hatching, promote survival in temporally and patchily distributed habitats (Osawa, 1993, 2000), and may be important in enabling *H. axyridis* to dominate in a large number of habitats in invaded countries. In Japan, where *H. axyridis* is native, it is also a dominant predator of several species of aphid, but it coexists with various other aphidophagous predators with the guild displaying temporal and spatial niche differentiation (Osawa, 1991).

H. yedoensis had been regarded as a synonym of *H. axyridis* (e.g., Sasaji, 1971a), but was resurrected as a distinct species by Sasaji (1971b, 1977, 1981). Reproductive isolation between *H. axyridis* and *H. yedoensis* was found to be complete (100%) when the two species mated under laboratory conditions (Okada et al., 1978; Sasaji, 1981). However, the ecology of *H. yedoensis* is poorly understood.

Harmonia axyridis and *H. yedoensis* are difficult to distinguish in the adult stage because of their morphological similarity. Adults of *H. axyridis* and *H. yedoensis* have four types of multi-colored elytra, and morphological differences between adults are observed only in the male genitalia (Sasaji, 1981; Nakagawa & Sasaji, 1988). Furthermore, the ridge at the tip of the elytra (not all *H. axyridis* adults have the ridge on the elytra) is observed only in *H. axyridis* (Sasaji, 1981). Morphological differences at the larval stage, especially for third and fourth instars, are distinct (Sasaji, 1977).

H. axyridis is regarded as a polyphagous predator, whereas *H. yedoensis* is thought to be an oligophagous aphid predator. The giant pine aphid *Cinara pini* Linne and Thunberg's pine aphid *Eulachmus thunbergii* Wilson are the only reported prey of *H. yedoensis* in field habitats (Tanigishi, 1975). The habitat of *H. axyridis* varies greatly (e.g., Osawa, 1993, 2000), whereas that of *H. yedoensis* is limited to pine trees (Tanigishi, 1975), where it can be found co-occurring with the more aggressive *H. axyridis*.

The habitats of *H. axyridis* are categorized into those suitable for survival and reproduction and those providing temporal refuge, according to density and quality of aphids in the habitats (Osawa, 2000). However, habitat segregation in *H. yedoensis* and *H. axyridis* is not based on strict difference in suitable food; at a laboratory condition, *H. yedoensis* develops normally, pupates, emerges, and oviposits when *Cryptosiphum artemisiae* Buckton and *Macrosiphoniella* sp. occur on *Artemisia princeps* (Sasaji, 1981) and *Aphis spiraeicola* (Osawa, pers.

observ.), which are prey for *H. axyridis*, but not for *H. yedoensis* in nature. These results suggest that ecological mechanisms may promote coexistence of the two sibling species on a pine tree, which is an interesting evolutionary issue. Furthermore, clarification of such mechanisms may aid in predicting how aphidophagous predators may interact with invasive populations of the aggressive *H. axyridis* in other parts of the world. In areas invaded by *H. axyridis*, aphidophagous predators are forced to cope with it, but potential mechanisms of coexistence remain to be determined.

We investigate possible mechanisms of coexistence of the sympatric sibling species, *H. axyridis* and *H. yedoensis*. We especially focus on the differences in their maternal investment through eggs and the role of sibling cannibalism at laboratory experiments.

MATERIAL AND METHODS

Laboratory experiments

The laboratory experiment was conducted from 27 May to 22 November, 2005. More than 100 *H. axyridis* pupae on *Prunus persica* infested by *Myzus varians* Davidson and on *Salix koriyanagi* infested by *Chaitophorus horii* Takahashi and eight adults of *H. yedoensis* (five females and three males) on *Pinus densiflora* (forma *umbraculifera*) infested by *Cinara pini* Linne were collected at the Botanical Garden of Kyoto University (35°02'N 135°47'W) in mid May 2005. Forty newly-emerged adults of *H. axyridis* (20 females and 20 males) were randomly chosen before mating (ca. within a week after emergence) and used in the experiment. The eight *H. yedoensis* adults were held together as a stock culture with random mating. From them, we obtained many eggs and reared these offspring at each stage in plastic cups (13 cm wide, 10 cm high) to the adult stage to obtain more than 100 newly-emerged adults. We provided the *H. yedoensis* larvae with a surplus of frozen *Ephestia kuehniella* Zeller eggs (Beneficial Insectary®), which was changed daily and reared them at 25°C, 16L : 8D, and ca. 70% relative humidity. Of this second generation adults, 40 newly-emerged and unmated individuals (20 females and 20 males) were randomly chosen for the experiment. Newly-emerged adults of this second generation of *H. yedoensis* were used because (1) there were not enough unmated adults in the original field collection for the statistical analysis and (2) we could confirm the identification of *H. yedoensis* through larval morphology at the fourth instar.

We provided *H. axyridis* and *H. yedoensis* adults with the surplus of frozen *E. kuehniella* eggs, which was changed daily. To evaluate oviposition ability of females of *H. axyridis* and *H. yedoensis* without sperm shortage, the 20 experimental females of each species, each with a conspecific male, were individually held in plastic Petri dishes (7 cm wide, 2 cm high) at 25°C, 16L : 8D, and ca. 70% relative humidity. They were provided with a surplus of frozen eggs, which was changed daily. Each day, we checked the Petri dishes, counted all eggs that were laid, and recorded the number of eggs per cluster. After removing the beetles, each egg cluster was individually kept in a labelled Petri dish at the same laboratory conditions and checked once or twice daily. All the females of *H. axyridis* and *H. yedoensis* were reared until they died.

In cases of sibling cannibalism in aphidophagous ladybird beetles, cannibals eat two types of sibling eggs in cluster form, undeveloped eggs and developed eggs with delayed hatching (Kawai, 1978; Osawa, 1992), although infertilization of the undeveloped eggs has not been confirmed. A cannibalized egg containing an embryo (the color is normally dark yellow when

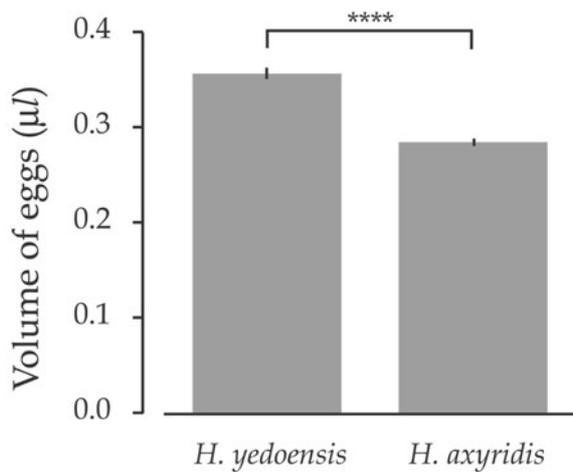


Fig. 1. Egg size in *Harmonia yedoensis* and *H. axyridis*. Vertical lines indicate S.E.

other eggs in the cluster hatch) is regarded as a developed egg with delayed hatching; that without an embryo (the color is normally light yellow when other eggs in the cluster hatch) is regarded as an undeveloped egg (Brown, 1972; Osawa, 1992). We distinguished the two types of cannibalized eggs under a stereo microscope (Carl Zeiss® SV 11 Apo) before, during, and after hatching. Hatching time of the cluster was regarded as the period from oviposition to the time of occurrence of the first larval hatching in a cluster.

We recorded the number of larvae 24 h after the eggs hatched. Thereafter, missing eggs were considered to have been cannibalized. In total, 103 egg clusters derived from five adults of *H. yedoensis* (i.e., no oviposition for 15 females) and 131 egg clusters derived from ten adults of *H. axyridis* (i.e., no oviposition for 10 females) were obtained until the females died and were used in the following analysis.

Measurement of egg volume and cost

To measure egg volume, another sets of 20 females and 23 males of *H. axyridis* at the first generation derived from the field population and 10 females and 13 males of *H. yedoensis* at the second generation derived from the eight field collected adults were placed together at the each stock cultures. They were reared with a surplus of frozen eggs in plastic cups (13 cm wide, 10 cm high) at 25°C, 16L : 8D, and ca. 70% relative humidity. Ten eggs of *H. yedoensis* and 20 eggs of *H. axyridis* were randomly chosen from different egg batches of each stock culture, and egg length (L) and width (W) were measured to the nearest 0.025 mm using a micrometer with the stereo microscope.

Egg volume (V , μl), which is regarded as the cost of egg production to the female, was calculated as: $V = LW^2\pi/6$ (see Takakura, 2004). Using this value, oviposition cost was calculated as: Oviposition cost (μl) = Egg volume (V , μl) \times Egg number. From this equation, the following were calculated: total oviposition cost per female throughout her lifetime, the cost of sibling cannibalism per cluster, the cost of undeveloped eggs per cluster, the cost of developed eggs with delayed hatching per cluster, and sibling cannibalism cost/larva per cluster.

Statistical analysis

Egg sizes of *H. yedoensis* and *H. axyridis* were compared by t -test. Similarly, t -tests were performed to compare *H. yedoensis* and *H. axyridis* for the following: cluster size, hatching time, number of clusters per female, total oviposition cost per female, percentage of hatching, number of undeveloped and developed

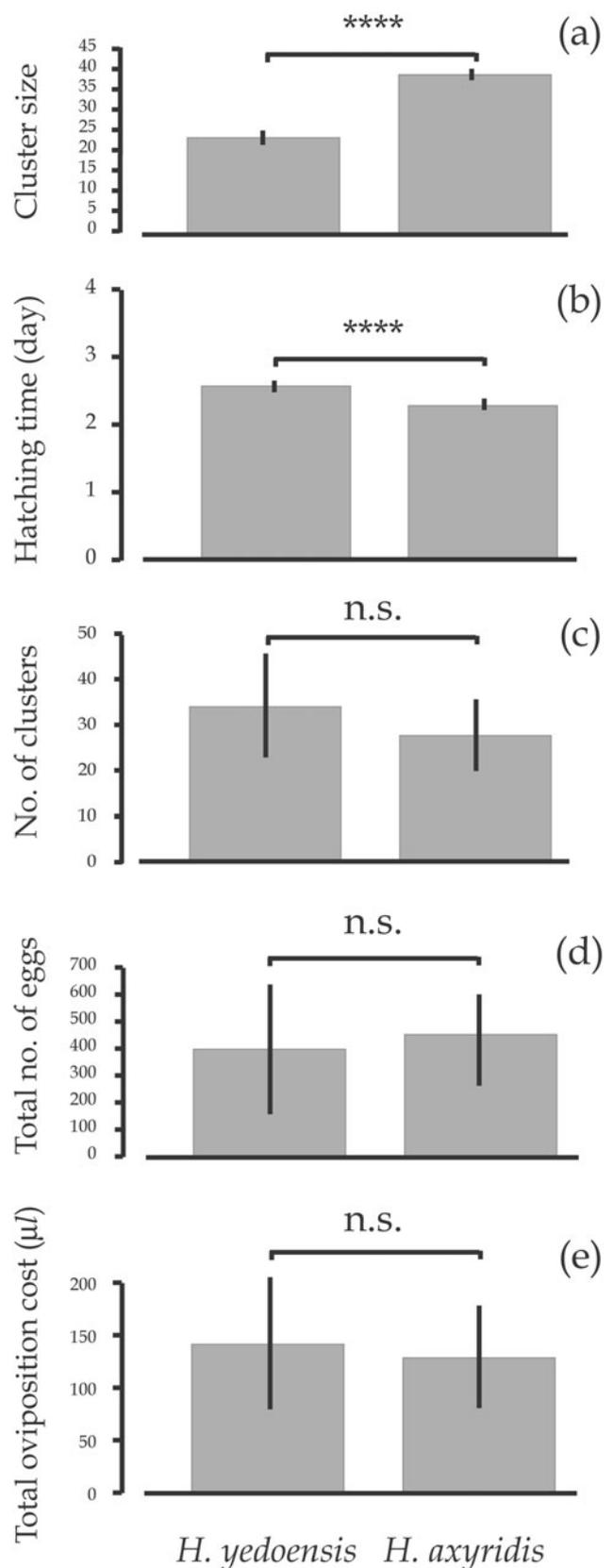


Fig. 2. Cluster size (a), hatching time (days) (b), number of clusters (c), total number of eggs (d), and total oviposition cost (μl) (e) in *Harmonia yedoensis* and *H. axyridis*. Vertical lines indicate S.E.

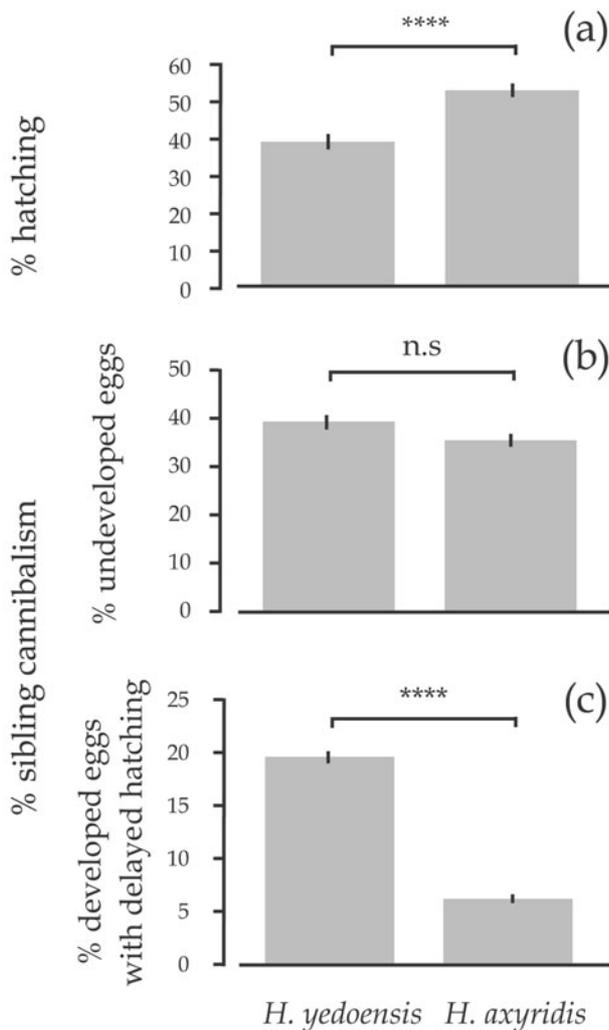


Fig. 3. The percentage of hatching (a), of undeveloped eggs (b), and of developed eggs with delayed hatching (c) per cluster in *Harmonia yedoensis* and *H. axyridis*. Vertical lines indicate S.E.

eggs with delayed hatching per cluster, and costs per cluster of oviposition, undeveloped eggs, developed eggs with delayed hatching per cluster, and sibling cannibalism/larva. We used a two-level nested ANOVA to analyze the relationship between the percentage of hatching, undeveloped eggs, and developed eggs with delayed hatching within cluster and (a) the size of that cluster, or (b) female age, for *H. yedoensis* and *H. axyridis*, respectively. We eliminated one cluster from this analysis because one female produced only that cluster and thereafter we could not evaluate the female effect of this cluster. In total, 103 clusters from five females of *H. yedoensis* and 130 clusters from nine females of *H. axyridis* were used in this analysis. All of the percentage data were arcsine transformed. We used JMP Discovery Software (SAS, 2000) for all statistical analyses.

RESULTS

Characteristics of eggs and sibling cannibalism

Female size in *H. yedoensis* (7.0985 ± 0.0729 mm, $n = 20$) was not significantly different from that in *H. axyridis* (7.2555 ± 0.0726 mm, $n = 20$) (t -test, $t = 1.5238$, $p = 0.1358$). The volume of *Harmonia yedoensis* eggs (0.3564 ± 0.0094 μ l, mean \pm S.E.) was 24.91% larger

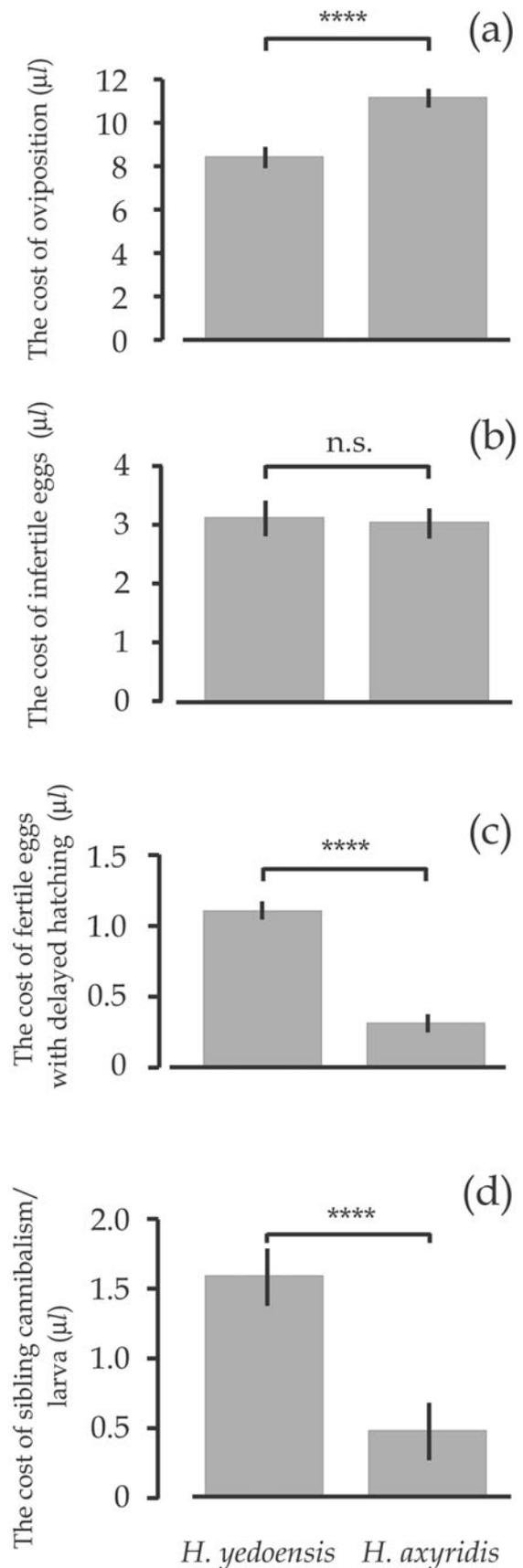


Fig. 4. The cost of oviposition (μ l) (a), of undeveloped eggs (μ l) (b), of developed eggs with delayed hatching (μ l) (c), and of sibling cannibalism/larva (d) per cluster in *Harmonia yedoensis* and *H. axyridis*. Vertical lines indicate S.E.

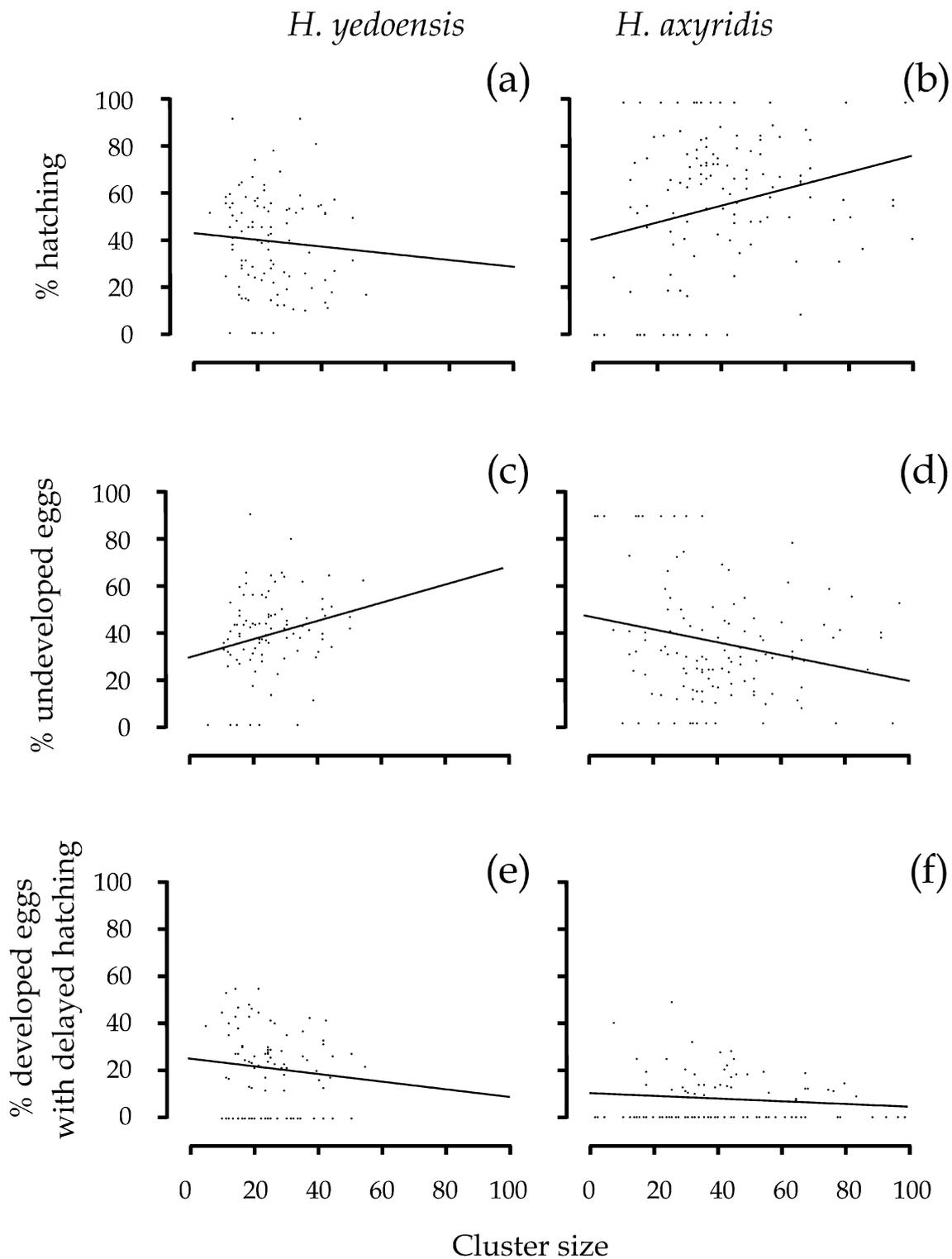


Fig. 5. The relationship between cluster size and percentage hatching (a, b), that between cluster size and percentage of undeveloped eggs (c, d), and that between cluster size and percentage of developed eggs with delayed hatching (e, f) per cluster in *Harmonia yedoensis* and *H. axyridis*, respectively.

than that of *H. axyridis* (0.2848 ± 0.0067) (t -test: $t = 6.2054$, $p < 0.0001$; Fig. 1). Cluster size of *H. axyridis* (39.1069 ± 1.4742 , mean \pm S.E.) was significantly larger than that of *H. yedoensis* (23.5631 ± 1.6626) (t -test: $t = 6.9952$, $p < 0.0001$; Fig. 2a). Hatching time of *H. yedoensis* (3.0106 ± 0.0584 day) was significantly longer

than that of *H. axyridis* (2.6754 ± 0.0530) (t -test: $t = 4.2512$, $p < 0.0001$; Fig. 2b). The number of clusters (20.6000 ± 6.4903), total number of eggs (401.2000 ± 231.1600), and total oviposition cost per female ($142.9700 \pm 68.3360 \mu\text{l}$) in *H. yedoensis* were not significantly different from those in *H. axyridis* ($13.1000 \pm$

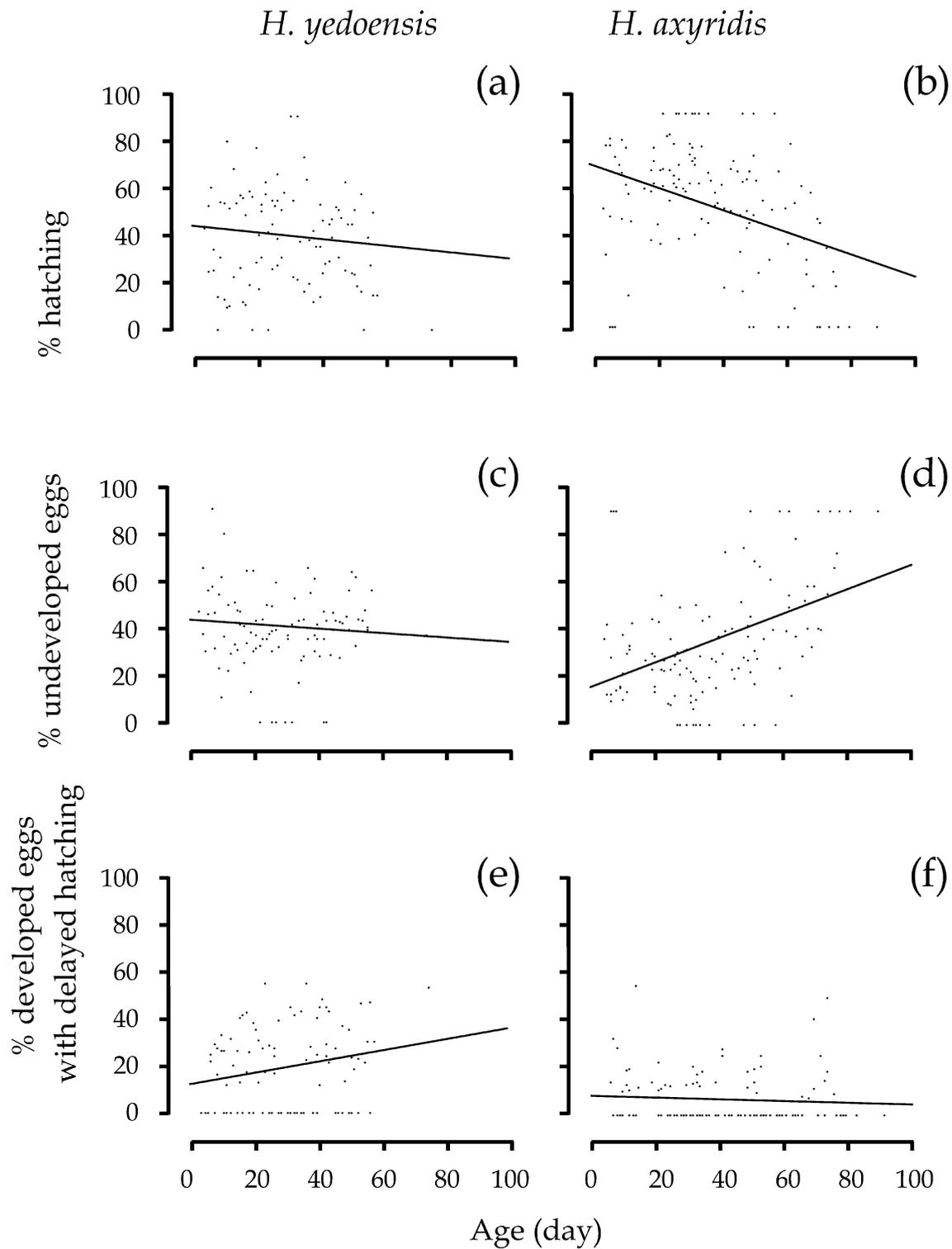


Fig. 6. The relationship between female age and percentage hatching (a, b), that between female age and percentage of undeveloped eggs (c, d), and that between female age and percentage of developed eggs with delayed hatching (e, f) per cluster in *Harmonia yedoensis* and *H. axyridis*, respectively.

4.5894, 455.4000 ± 163.4600 , and 129.6960 ± 48.3210 , respectively) (t -test: $t = 0.9435$, $p = 0.3626$; $t = 0.1914$, $p = 0.8511$; and $t = 0.1586$, $p = 0.8764$, respectively), although total number of clusters tended to be slightly greater in *H. yedoensis* than in *H. axyridis* (Fig. 2c–e).

The percentage of hatching per cluster in *H. axyridis* ($51.9395 \pm 2.1220\%$, mean \pm S.E.) was significantly higher than that in *H. yedoensis* ($38.2994 \pm 2.4034\%$) (t -test: $t = 4.2544$, $p < 0.0001$; Fig. 3a). In sibling cannibalism, the percentage of undeveloped eggs per cluster in *H. yedoensis* ($39.1838 \pm 2.2220\%$) was not significantly

different from that in *H. axyridis* ($35.4255 \pm 1.9596\%$) (t -test: $t = 1.2685$, $p = 0.2059$; Fig. 3b), whereas the percentage of developed eggs per cluster with delayed hatching in *H. yedoensis* ($19.5105 \pm 1.3735\%$) was significantly greater than that in *H. axyridis* ($6.0382 \pm 1.2127\%$) (t -test: $t = 7.3531$, $p < 0.0001$; Fig. 3c).

The cost of oviposition per cluster in *H. axyridis* ($11.1374 \pm 0.4400 \mu\text{l}$, mean \pm S.E.) was 32.64% larger than that in *H. yedoensis* (8.3968 ± 0.4962), a significant difference (t -test: $t = 4.1328$, $p < 0.001$; Fig. 4a). In cases of sibling cannibalism, the cost of undeveloped eggs per cluster in *H. yedoensis* ($3.1449 \pm 0.3167 \mu\text{l}$) was not significantly different from that in *H. axyridis* ($3.0654 \pm 0.2808 \mu\text{l}$) (t -test: $t = 3.9186$, $p = 0.8510$; Fig. 4b), whereas the cost per cluster of developed eggs with delayed hatching in *H. yedoensis* ($1.1071 \pm 0.0976 \mu\text{l}$) was 3.49 times greater than that in *H. axyridis* ($0.3174 \pm 0.0865 \mu\text{l}$) (t -test: $t = 6.0541$, $p < 0.0001$; Fig. 4c). Furthermore, the cost of sibling cannibalism per hatched larva/cluster in *H. yedoensis* ($1.5781 \pm 0.2094 \mu\text{l}$) was 3.36 times greater than that in *H. axyridis* ($0.4696 \pm 0.1902 \mu\text{l}$), and the difference was statistically significant (t -test: $t = 3.9186$, $p < 0.0001$; Fig. 4d).

Factors affecting sibling cannibalism

Cluster size was not a significant factor affecting the percentage of hatching per cluster in *H. yedoensis* (Two-level nested ANOVA: female code, $F = 21.3869$, $df = 4$, $p < 0.0001$; cluster size [female code]; $F = 0.7306$, $df = 5$, $p = 0.6023$) (whole model: $R^2 = 0.5534$, $F_{9,89} = 12.2541$, $p < 0.0001$), but it was a significant factor in *H. axyridis* (female code, $F = 2.1696$, $df = 8$, $p = 0.0353$; cluster size [female code]; $F = 2.0558$, $df = 9$, $p = 0.0398$) (whole model: $R^2 = 0.2793$, $F_{17,109} = 2.4851$, $p = 0.0024$; Fig. 5a, b). In sibling cannibalism, cluster size was a marginally significant factor affecting the percentage of undeveloped eggs per cluster in *H. yedoensis* (female code, $F = 6.0952$, $df = 4$, $p = 0.0002$; cluster size [female code]; $F = 2.0733$, $df = 5$, $p = 0.0763$) (whole model: $R^2 = 0.3600$, $F_{9,88} = 5.4992$, $p < 0.0001$), and a significant factor in *H. axyridis* (female code, $df = 8$, $F = 1.9284$, $p = 0.0629$; cluster size [female code]; $F = 1.9730$, $df = 9$, $p = 0.0493$) (whole model: $R^2 = 0.2677$, $F_{17,108} = 2.3229$, $p = 0.0047$; Fig. 5c, d). However, cluster size did not significantly affect the percentage of developed eggs with delayed hatching per cluster in either *H. yedoensis* (female code, $F = 20.1566$, $df = 4$, $p < 0.0001$; cluster size [female code]; $F = 1.2607$, $df = 5$, $p = 0.2880$) (whole model: $R^2 = 0.5573$, $F_{9,89} = 12.4473$, $p < 0.0001$) or *H. axyridis* (female code, $F = 4.5698$, $df = 8$, $p < 0.0001$; cluster size [female code]; $F = 1.0529$, $df = 9$, $p = 0.4035$) (whole model: $R^2 = 0.3305$, $F_{17,109} = 3.1654$, $p < 0.0001$; Fig. 5e, f).

Female age significantly influenced the hatching percentage per cluster in both *H. yedoensis* (two-level nested ANOVA: female code, $F = 8.6974$, $df = 4$, $p < 0.0001$; cluster size [female code]; $F = 2.6354$, $df = 5$, $p = 0.0286$) (whole model: $R^2 = 0.5950$, $F_{9,89} = 14.5302$, $p < 0.0001$) and *H. axyridis* (female code, $F = 5.2359$, $df = 8$, $p < 0.0001$; cluster size [female code]; $F = 8.6565$, $df = 9$, $p <$

0.0001) (whole model: $R^2 = 0.5084$, $F_{17,109} = 6.6303$, $p < 0.0001$; Fig. 6a, b). In sibling cannibalism, female age was not a significant factor affecting the percentage of undeveloped eggs per cluster in *H. yedoensis* (female code, $F = 2.0823$, $df = 4$, $p = 0.0899$; cluster size [female code]; $F = 0.7725$, $df = 5$, $p = 0.5721$) (whole model: $R^2 = 0.3147$, $F_{9,88} = 4.4891$, $p < 0.0001$), but it was a significant factor in *H. axyridis* (female code, $F = 5.1211$, $df = 8$, $p < 0.0001$; cluster size [female code]; $F = 7.9024$, $df = 9$, $p < 0.0001$) (whole model: $R^2 = 0.4859$, $F_{17,108} = 6.0045$, $p < 0.0001$; Fig. 6c, d). Female age did not significantly affect the percentage of developed eggs with delayed hatching per cluster in either *H. yedoensis* (female code, $F = 15.9044$, $df = 4$, $p < 0.0001$; cluster size [female code]; $F = 1.8479$, $df = 5$, $p = 0.1116$) (whole model: $R^2 = 0.5705$, $F_{9,89} = 13.1354$, $p < 0.0001$) or *H. axyridis* (female code, $F = 1.1796$, $df = 8$, $p = 0.3179$; cluster size [female code]; $F = 1.2298$, $df = 9$, $p = 0.2844$) (whole model: $R^2 = 0.3394$, $F_{17,109} = 3.2941$, $p < 0.0001$; Fig. 6e, f).

DISCUSSION

Egg size can affect progeny fitness; larger offspring often mature earlier, to have improved ability to avoid or withstand predation and competition, or to have greater survival in stressful environments in comparison with smaller offspring (e.g., Azevedo et al., 1997; Fox & Czesak, 2000; Roff, 2002; Czesak & Fox, 2003; Fischer et al., 2003, 2006, 2007). Conversely, a female cannot increase offspring number without decreasing the size of individuals because of the presumed trade-off between number and size of offspring, which is a fundamental principle of life history theory in many taxa (e.g., Vance, 1973; Smith & Fretwell, 1974; Brockelman, 1975; McGinley et al., 1987; Sinervo 1990; Sterns, 1992; Roff, 2002). This study showed that: (1) cluster size in *Harmonia axyridis* was significantly larger but egg size was smaller than that in *H. yedoensis*, (2) the number of *H. yedoensis* clusters tended to be slightly higher than that of *H. axyridis*, and (3) total number of eggs and oviposition cost per female in *H. yedoensis* over her life time were not significantly different from those in *H. axyridis*. The maternal investment of the two species differed; *H. yedoensis* females lay larger eggs in fewer numbers, whereas *H. axyridis* females lay smaller eggs in larger numbers. The end result is almost equal reproductive effort between *H. yedoensis* and *H. axyridis*. Furthermore, female size is generally thought to determine the number of progeny (e.g., Thornhill & Alcock, 1983; Birkhead et al., 1998). Female size in *H. yedoensis* was not significantly different from that in *H. axyridis* in this study, implying further support to the equality of reproductive effort between *H. yedoensis* and *H. axyridis*.

Egg size reflects maternal reproductive investment and the initial amount of resources available to progeny (e.g., Sinervo, 1990). In *H. yedoensis* and *H. axyridis*, mothers do not care for offspring; thus, egg size has an especially important role in determining maternal investment in these species. Several characteristics of these two species,

including morphology in adult and larval stages, are difficult to distinguish and they overlap in habitat on pine trees (e.g., Sasaji, 1977). However, this study showed that maternal investment in offspring, as measured the volume of eggs, was 24.91% greater in *H. yedoensis* than in *H. axyridis* (Fig. 1). These results suggest that larval survival rate, especially in the first instar, may be higher in *H. yedoensis* than in *H. axyridis* under natural conditions because of higher ability of prey capturing based on larger maternal investment in eggs.

A strong positive relationship exists between reproductive biomass and adult weight, and egg size is a function of adult weight and ovariole number in coccinellids (Stewart et al., 1991). Furthermore, Dixon & Guo (1993) predicted that egg size is the least variable reproductive trait and that cluster size is the most variable. These results suggest that maternal investment in eggs is allometrically determined by adult weight and ovariole number. In general, egg size is constrained by the minimum size at which first instar larvae can capture active prey and complete their development before prey become scarce (Stewart et al., 1991). However, food conditions around newly hatched first instar larvae are critical, mainly because of the difficulties larvae experience in capturing prey (Dixon, 1959; Kawai, 1978) and the fact that the oviposition site is set apart from aphid colonies to avoid non-sibling cannibalism in *H. axyridis* (Osawa, 1989). Thus, maternal investment through egg size alone may be insufficient for newly hatched *H. yedoensis* and *H. axyridis* larvae to develop into adults. The cost of sibling cannibalism per hatched larva/cluster in *H. yedoensis* was 3.36 times greater than that in *H. axyridis* (Fig. 4d), suggesting that the realized maternal investment for offspring in *H. yedoensis* is actually more than 3.36 times that in *H. axyridis* when combined with investment through an egg.

The maternal investment in each *H. yedoensis* larva ($1.5781 \pm 0.2094 \mu\text{l}$ in Fig. 4d) amounts to 4.43 eggs (egg volume in *H. yedoensis*: $0.3564 \pm 0.0094 \mu\text{l}$ in Fig. 1), when egg cannibalism is included. In *H. axyridis*, the survival rate of first instar larvae increases 6.27 times when larvae cannibalize three eggs at low aphid densities and 2.58 times at high aphid densities (Osawa, 1992). Furthermore, newly hatched *H. axyridis* larvae successfully molt to the second instar when they feed on three eggs (Kawai, 1978; Osawa, 1991). Assuming that the beneficial effect of sibling cannibalism on the first instar is similar in *H. yedoensis*, the greater initial larval size of *H. yedoensis*, combined with the maternal investment through sibling cannibalism at hatching, may further improve the survival of the first instar *H. yedoensis* relative to the first instar *H. axyridis*. Thus, the actual maternal investment per offspring is $4.197[1.249$ (in egg size) $\times 3.36$ (in sibling cannibalism)] times greater in *H. yedoensis* than in *H. axyridis* when the maternal investments in egg size and sibling cannibalism are combined.

Sibling cannibalism promotes more rapid development and larger adult size in *H. axyridis*, suggesting that faster development may be adaptive for resource tracking, and

large adult size increases fecundity in females and mating success in males through female mate choice, both resulting in an increase in the fitness of cannibals (Osawa, 2002). Furthermore, this study showed that hatching time of egg clusters was longer in *H. yedoensis* than in *H. axyridis* (Fig. 2b). A positive relationship between egg size and hatching time is evident in Diptera and Coleoptera (Azevedo et al., 1997; Fox, 1997), suggesting that eggs of *H. yedoensis* develop more slowly than those of *H. axyridis*. Therefore, the effectiveness of maternal investment through sibling cannibalism in increasing adult body size and shortening developmental period may be superior to that through eggs, which may be an ultimate factor favoring maternal investment through sibling cannibalism.

In sibling cannibalism in *H. axyridis*, a cannibal can choose from two types of eggs: undeveloped eggs or developed eggs with delayed hatching (Osawa, 1992). These two types of eggs exist in both *H. axyridis* and *H. yedoensis*. Undeveloped/trophic eggs are regarded as a direct maternal investment (i.e., the mother can directly control their production) in offspring (Osawa, 2003), although the infertility of the undeveloped egg and the control mechanisms for the production of the undeveloped eggs have yet to be clarified. The undeveloped eggs are regarded to be a maternal investment in *H. axyridis*; the proportion of undeveloped/trophic eggs in a cluster is manipulated based on information from prey encounters (Perry & Roitberg, 2005). The present study showed that cluster size as well as the identity of the mother significantly affected the percentage of undeveloped eggs per cluster in *H. axyridis*, and cluster size was marginally significant in *H. yedoensis* (Fig. 5). Furthermore, age of mothers was a significant factor affecting the percentage of undeveloped eggs per cluster in *H. axyridis*, the reverse was true in *H. yedoensis* (Fig. 6). These results suggest that physiological mechanisms for the production of eggs may be combined with maternal manipulation in the production of undeveloped eggs in *H. yedoensis* and *H. axyridis*.

In *H. axyridis*, Osawa (1992) explained sibling cannibalism of developed eggs with delayed hatching through kin selection with three potential beneficiaries, i.e., the cannibal, the victim, and the mother. The altruistic behavior of being a victim was beneficial to the victim only when the victim was cannibalized by full siblings at low aphid densities. At the same time, cannibalism was not maladaptive for the mother; mothers attained almost equal fitness regardless of the proportion of developed eggs with delayed hatching. Therefore, intense kin relatedness between the cannibal and the victim, and low survival rate of the siblings in cluster favor the occurrence of sibling cannibalism.

The proximate factor for occurrence of sibling cannibalism was hatching asynchrony in clusters (Osawa, 1992). Male-killing *Spiroplasma* plays a role in the production of developed eggs with delayed hatching (Nakamura et al., 2006); however, the precise mechanism is not fully understood. In birds, asynchronous hatching is gen-

erally believed to result from the asynchronous timing of egg development (e.g., Ricklefs, 1993). Cluster size therefore may be involved in the occurrence of sibling cannibalism, if variation in developmental time arises from eggs being laid over an extended period of time within a cluster. However, our study showed that cluster size-related mechanisms are not involved in the production of developed eggs with delayed hatching in *H. yedoensis* and *H. axyridis*, implying that independently of cluster size, the female of *H. yedoensis* and *H. axyridis* manipulates the hatching time of offspring in a cluster. Parental strategies exist to alleviate the effects of asynchronous hatching in clusters (e.g., in birds: Wiebe & Bortolotti, 1994; Much & Nager, 2006; in insects: Frechette & Coderre, 2000). The implication of this study, i.e., mothers of *H. yedoensis* and *H. axyridis* may manipulate the hatching time of their offspring, suggests that the production of developed eggs with delayed hatching is a secondary mechanism for manipulating sibling cannibalism opportunities.

This study showed that the percentage and cost of developed eggs with delayed hatching per cluster was greater for *H. yedoensis* than for *H. axyridis*, whereas the percentage and cost of undeveloped eggs per cluster was not significantly different between the two species. These results suggest that sibling cannibalism of developed eggs with delayed hatching is most important in determining the contrasting intensity of cannibalism between *H. yedoensis* and *H. axyridis*. One notes that the intensity of sibling cannibalism of developed eggs with delayed hatching does not affect the mother's fitness regardless of food conditions for offspring, but the intensity of sibling cannibalism of undeveloped eggs reduces the mother's fitness at high aphid densities for the offspring in *H. axyridis* (Osawa, 1992). Thus, maternal investment in sibling cannibalism of developed eggs with delayed hatching may be an especially adaptive reproductive strategy that does not reduce the mother's fitness also in *H. yedoensis*.

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