Jpn. J. Environ. Entomol. Zool. 17 (1) : 67－75 (2006)
環動昆　第17巻　第2号：67－75 (2006)

Original Article

Interspecific relationship between an exotic ladybird beetle, *Adalia bipunctata* (Linnaeus) (Coleoptera: Coccinellidae), and native predacious ladybird beetles

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(Received May 1, 2006 ; Accepted June 29, 2006)

Abstract

An exotic ladybird beetle, *Adalia bipunctata*, was found on garden trees, such as *Acer buergerianum* and *Rhaphiolepis umbellate* together with native ladybird beetles, *Harmonia axyridis* and *Menochilus sexmaculatus*, in spring. The breeding season of *A. bipunctata* was earlier than that of native ladybird beetles, and it was occasionally observed that pre-pupae and pupae of *A. bipunctata* were fed on by *H. axyridis* in the field. In small plastic cases in the laboratory, the larvae of *H. axyridis* preyed on larvae and pre-pupae of *A. bipunctata* irrespective of the amount of food supplied, whereas the larvae of *M. sexmaculatus* preyed on larvae and pre-pupae of *A. bipunctata* only in the case of insufficient food. On the other hand, *A. bipunctata* did not prey on native ladybird beetles irrespective of the amount of food supplied. Two species of native ladybird beetle were probably superior to *A. bipunctata* in interspecific competition. However, their asynchronous life history may escape direct competition each other in the field in Japan, unlike in North America.

Key words : Exotic species, *Adalia bipunctata*, Ladybird beetles, Interspecific relationships

Introduction

Once exotic species has invaded, they have often given various and serious effects on native species and ecosystems (e.g., Washiatani and Murakami, 2000 ; Goka *et al.*, 2004). Exotic ladybird beetles have had grand scale effects on native species. For example, *Coccinella septempunctata* (Linnaeus) and *Harmonia axyridis* (Pallas) were introduced as natural enemies in North America. Thereafter, their geographical distribution expanded, resulting in a considerable decrease in native ladybird beetle populations (Elliott *et al.*, 1996 ; Brown, 2003 ; Evans, 2004).

About 180 species of ladybird beetle have been recorded in Japan (Sasaji 1996). Ten species of ladybird beetle were recorded in recent years, most of them may be immigrants (Sasaji, 1992). *Adalia bipunctata* (Linnaeus) is a well-known predacious ladybird beetle distributed in Europe, Central Asia and North America (Hodek and Honěk, 1996). This species, like *C. septempunctata*, is an important predator of aphids in Europe. The biology of *A. bipunctata* has been investigated (Hodek and Honěk, 1996), and many studies on control agents of aphids using this species have been carried out (Obrycki and Tauber, 1981; Kehrli and Wyss, 2001).

An adult of *A. bipunctata* was first discovered in Japan on 16th December 1993, in the Osaka Bay area (Sakuratani, 1994). From the following spring (1994) some active individuals were observed on a garden tree, *Rhaphiolepis umbellate* (Makino), in the above area. *A. bipunctata* is multivoltine (hibernation in adults, without aestivation) in most of Europe, though univoltine in summer in the countries in northern Europe such as Iceland (Hamalainen and Markkula, 1977 ; Hodek and Honěk, 1996). However, univoltism (occurrence in only spring) of this species with estivo-hibernation [Type IA (Hagen, 1962)] was observed in Japan, where the climate differs from that of Europe (Sakuratani *et al.*, 2000).

The main habitats of *A. bipunctata* are two species of garden trees, *Acer buergerianum* (Miq) and *R. umbellate*,

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and native predacious ladybird beetles [*H. axyridis* and *Menochilus sexmaculatus* (Fabricius)] are also found on these trees (Sakuratani *et al.*, 2000). In contrast to *A. bipunctata*, *H. axyridis* and *M. sexmaculatus* are multivoltine in Japan. Interspecific relationships between native ladybird beetle and *A. bipunctata* are observed, because *A. bipunctata* has the same habitat and food as native species, and their active periods in spring overlap somewhat (Sakuratani *et al.*, 2000). Kajita *et al.* (2000) reported that the interspecific relationships between *A. bipunctata* and Japanese native predacious ladybird beetles in the cage. However, the population of *A. bipunctata* used by Kajita *et al.* (2000) was European one and the experiment was carried out in laboratory.

In our study, the interspecific relationships between *A. bipunctata* (Japanese population) and native predacious ladybird beetles were investigated in the field and laboratory, and we discuss with regard to the degrees of interspecific competition between *A. bipunctata* and native predacious ladybird beetles.

Materials and Methods

Field observation

The field observations were carried out at Nanko Central Park (34.6 °N, 135.4 °E, alt. 5 m, area 25 ha) located in Suminoe-Ku, Osaka-City, Central Japan. Census periods were from April 1999 to March 2000 and from April 2001 to December 2001. The census interval was two or three days, during the developmental season (from March to May) of *A. bipunctata*, and three or four times per month between aestivation and hibernation seasons. The main census was done on *A. buergerianum* with the aphid, *Periphyllus californiensis* (Shinji) in spring, on four species of oak tree, *Quercus glauca* (Thunb) and *Q. myrsinaefolia* (Blume) in summer and winter, and on *Q. acutissima* (Carruthers) and *Q. serrata* (Thunb) in autumn. The reason of seasonal change of tree species investigated is the seasonal change of occurrence of aphids and the seasonal change of aestivating and overwintering sites of *A. bipunctata*. The number of individuals of *A. bipunctata* and native ladybird beetles on shoot of those trees within a height of 2 m from the ground was counted. The numbers of investigated shoots of *A. buergerianum* were random 150 shoots of 30 trees, and *Q. acutissima* and *Q. serrata* were random 50 shoots of 15 trees, respectively in active season. As it is difficult to evaluate the real number of aphids in most case, aphid density on plants had been counted at some density levels (Heathcote, 1972; Tanaka, 1976). In our observation, the aphid density level was set as following: level 1; aphids attached on the shoot sparsely, level 2; aphids covered the tip of a sprout, level 3; aphids covered the upper part of a sprout, level 4, aphids covered much of the upper part of a shoot, level 5; aphids covered the entire upper part of a shoot. The adults of *A. bipunctata* aestivating or overwintering were motionless in leaf-shelter or under the dead bark (Sakuratani *et al.*, 2000). These adults on the trees were counted as inactive adults. The numbers of investigated shoots of *Q. glauca* were random 90 shoots of 30 trees and *Q. myrsinaefolia* were random 30 shoots of 20 trees, respectively in inactive season.

Laboratory experiment

Adults of *A. bipunctata*, *H. axyridis* and *M. sexmaculatus*, were collected from Nanko Central Park, Osaka City after hibernation. *Aphis craccivora* (Kochi) was reared on broad bean (*Vicia faba* L.) for use as food of ladybird beetles, and eggs of ladybird beetles laid were put into an incubator controlled at 20℃., R.H.50%, 14L:10D. First instar larvae of ladybird beetles were used for the experiment. A combination of *A. bipunctata* and *H. axyridis* (5 larvae: 5 larvae, 20 sets) and a combination of A. bipunctata and M. sexmaculatus (5 larvae: 5 larvae, 20 sets) were prepared in transparent plastic cases (95 mm in diameter, 62 mm in height). Ten of 20 sets were given surplus number of aphids (*A. craccivora*), the remaining 10 sets were given daily about 50 aphids which were consumed by ladybird beetles within several hours. Single culture of *A. bipunctata*, *H. axyridis*, and *M. sexmaculatus* with sufficient food (each 10 larvae of ladybird beetle, 5 sets) and with insufficient food (each 10 larvae of ladybird beetle, 5 sets; they were given daily about 50 aphids) were set up for control, respectively. The number of live ladybird beetles was counted everyday, until all ladybird beetles died or emerged to adult. Mortality rates and developmental periods of single and mix cultures were compared, and the degrees of competition among both cultures were estimated.

The results were analyzed by using x^2-test with regard to mortality, and multiplex comparison test (Tukey-Kramer) with regard to developmental periods.

Results

Field observations

Adults of *A. bipunctata* were found on *A. buergerianum* together with the native species, *H. axyridis* and *M. sexmaculatus*, in early spring in Nanko, Osaka, Japan in each year. However, density of *M. sexmaculatus* was very low and only two or three adults were found throughout the observation (**Fig. 1**). Although some active adults of *A. bipunctata* feeding on *Cervaphis quercus* (Takahashi) were observed in autumn, reproduction did not occur in autumn in 1999 and 2001 (**Fig. 1**).

The pre-pupae and pupae of *A. bipunctata* were fed by the larvae of *H. axyridis*, occasionally. The predators which preyed on pre-pupae and pupae of *A. bipunctata* were just larvae of *H. axyridis* on *A. buergerianum*. The number of corpse of both pre-pupae and pupae of *A. bipunctata* which might be fed by the larvae of *H. axyridis* was 48 (5.1%) of 950 individuals of *A. bipunctata* counted in this observation. When *H. axyridis* terminated overwintering and the number of aphids began to increase, *A. bipunctata* had already started reproduction. As the total number of larvae of *A.*

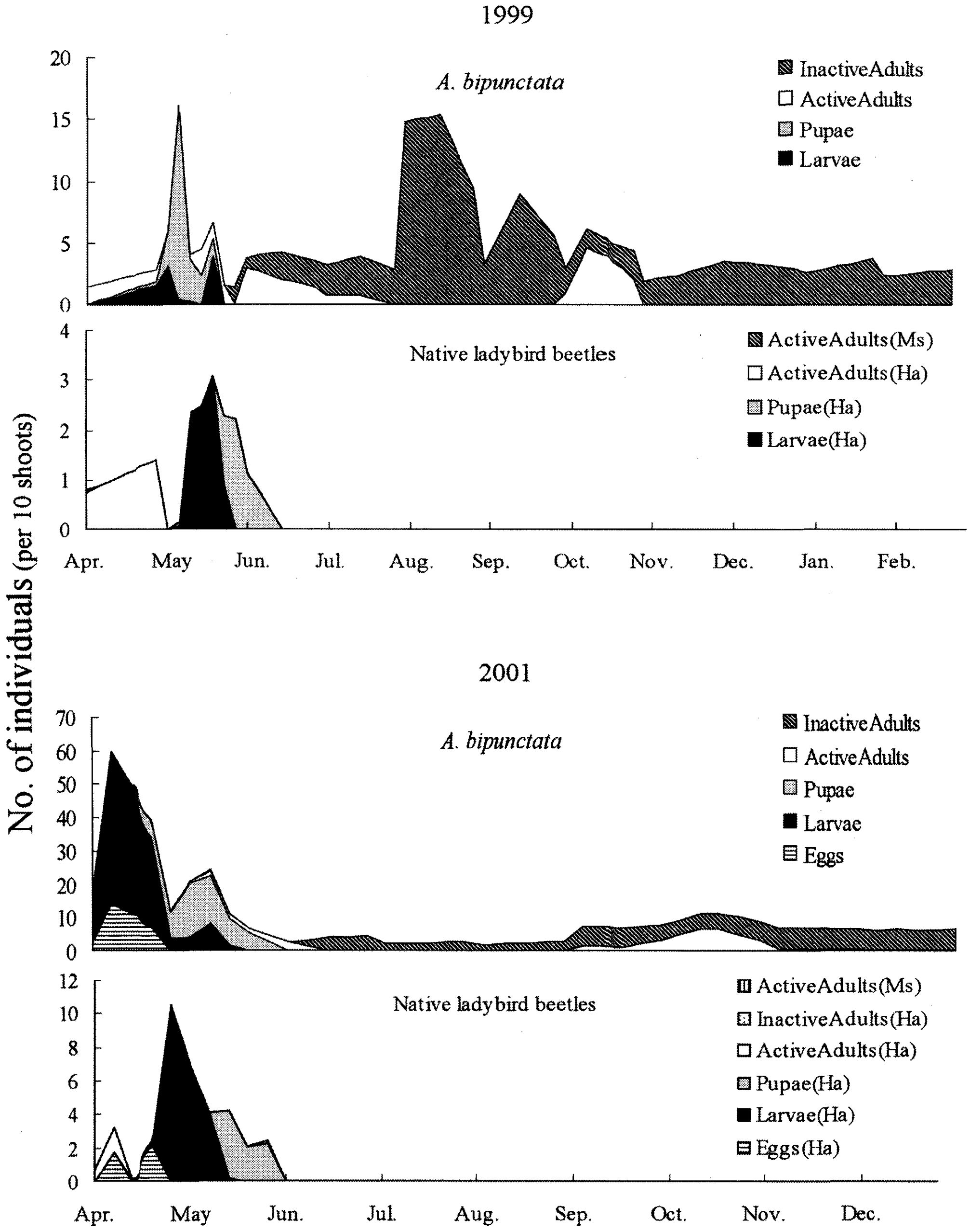

Fig. 1 Seasonal changes in each stage of *A. bipunctata* and native ladybird beetles at Osaka Nanko, Japan in 1999 and 2001. (Number of individuals per 10 shoots) (Ha :*H. axyridis*, Ms :*M. sexmaculatus*)

bipunctata peaked, most individuals of *H. axyridis* were still in the egg stage (**Fig. 2**). When the number of aphids began to decrease, most individuals of *A. bipunctata* had become pupae. However, at that time, most *H. axyridis* were still larvae (**Fig. 2**).

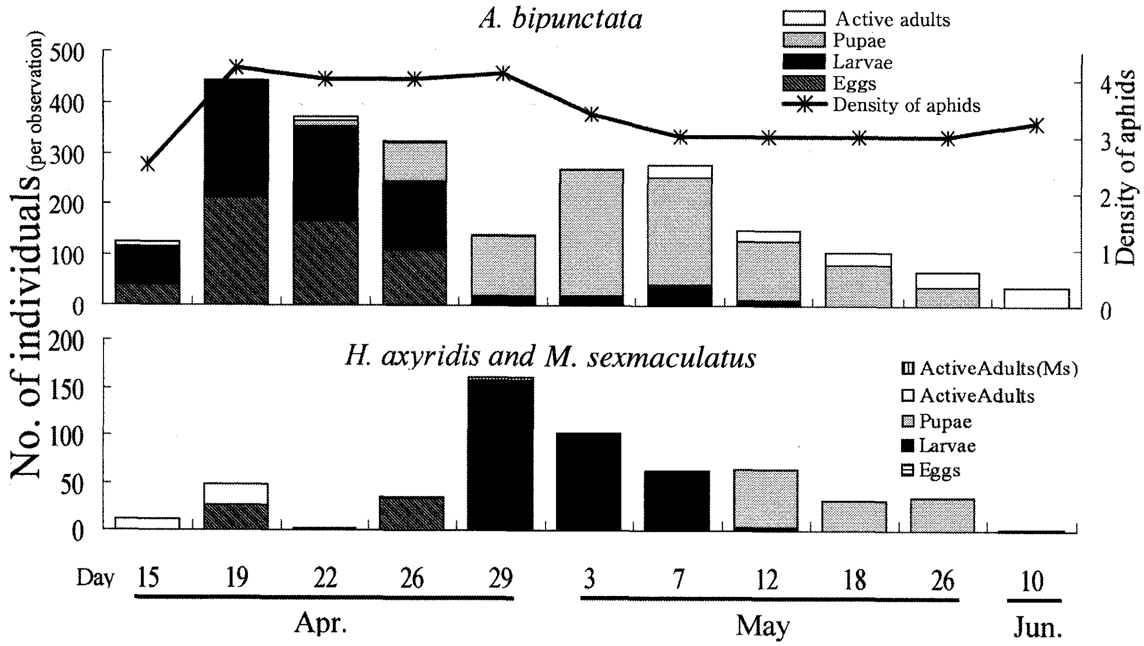

Fig. 2 Seasonal changes in the number of *A. bipunctata, H. axyridis, M. sexmaculatus* and aphids (mean level) throughout the developmental season, 2001 at Osaka Nanko, Japan. (Ha and Ms, see **Fig. 1**)

Laboratory experiment

Mortality

(1) *A. bipunctata* with sufficient food

Mortalities of single culture of *A. bipunctata*, and combination culture with *H. axyridis*, were 42.0% and 88.0%, respectively. The mortality was significantly higher than a single culture (x^2=14.43, d.f.=1, p<0.05). Mortality of *A. bipunctata* in combination with *M. sexmaculatus* was 22.0% and the mortality was not significant between both cultures (x^2=4.60, d.f.=1, p>0.05) (**Fig. 3A**).

(2) *H. axyridis* with sufficient food

Mortalities of single culture of *H. axyridis*, and combination culture were with *A. bipunctata*, were 24.0% and 38.0%, respectively. The mortality was not significant between both cultures (x^2=14.43, d.f.=1, p>0.05) (**Fig. 3B**).

(3) *M. sexmaculatus* with sufficient food

Mortalities of single culture of *M. sexmaculatus*, and combination culture were with *A. bipunctata*, were 8.0% and 12%, respectively. The mortality was not significant between both cultures (x^2=0.44, d.f.=1, p>0.05) (**Fig. 3C**).

(4) *A. bipunctata* with insufficient food

Mortalities of single culture of *A. bipunctata*, and combination culture with *H. axyridis*, were 78.0% and 100%, respectively. The mortality was significantly higher than single culture (x^2= 11.11, d.f.=1, p<0.05). Mortality of *A. bipunctata* in combination with *M. sexmaculatus* was 92.0% and the mortality was not significant between both cultures (x^2=2.99, d.f.=1, p>0.05) (**Fig. 3D**).

(5) *H. axyridis* with insufficient food

Mortalities of single culture of *H. axyridis*, and combination culture with *A. bipunctata*, were 80.0% and 64.0%, respectively. The mortality was not significant between both cultures (x^2=3.17, d.f.=1, p>0.05) (**Fig. 3E**).

(6) *M. sexmacilatus* insufficient food

Mortalities of single culture of *M. sexmaculatus*, and combination culture with *A. bipunctata*, were 68.0% and 60.0%, respectively. The mortality was not significant between both cultures (x^2= 0.69, d.f.=1, p>0.05) (**Fig. 3F**).

The individuals remained whole bodies were assumed to be natural death, and those that only a

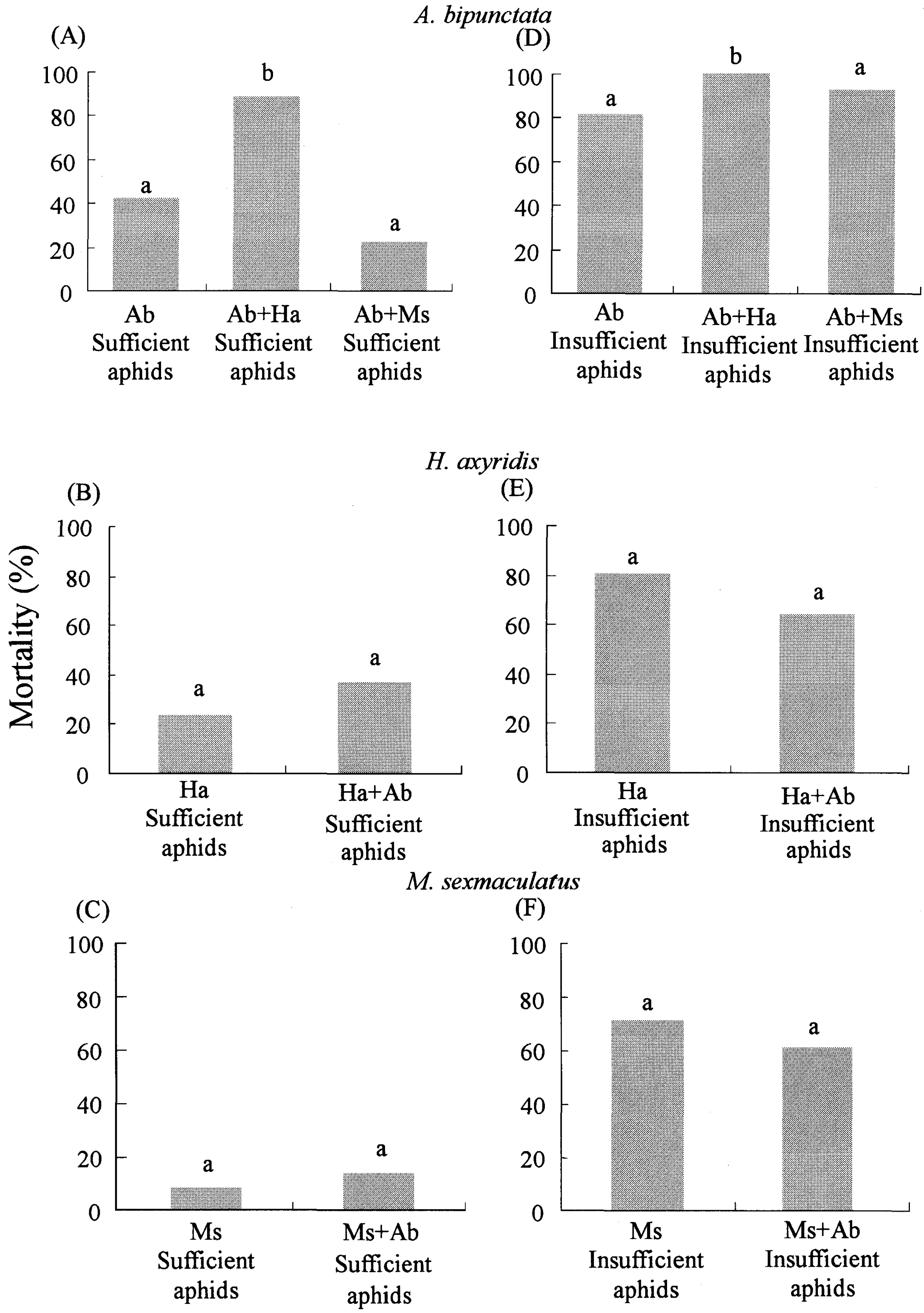

Fig. 3 Mortality of *A. bipunctata* and native ladybird beetles. Bars with the same letter are not significantly different (x2-test, p>0.05) (Ab : *A. bipunctata*, Ha and Ms, see **Fig. 1**)

part such as heads remained were assumed to be death by cannibalism or predation. Moreover, it was observed that *A. bipunctata* was fed by *M. sexmaculatus* in the case of insufficient food, and *H. axyridis* prayed on *A. bipunctata* regardless of whether or not the food supply was sufficient.

Developmental days

(1) *A. bipunctata* with sufficient food

Developmental days of single culture of *A. bipunctata*, combination culture with *H. axyridis*, and combination culture with *M. sexmaculatus* were 24.3 days, 21.7 days and 24.0 days, respectively. Developmental days were not significant between these culture (**Fig. 4A**).

(2) *H. axyridis* with sufficient food

Developmental days of single culture of *H. axyridis*, and combination culture with *A. bipunctata* were 24.9 days and 23.8 days, respectively. Developmental days were not significant between both cultures (**Fig. 4B**).

(3) *M. sexmaculatus* with sufficient food

Developmental days of single culture of *M. sexaculatus*, and combination culture with *A. bipunctata*, were 25.3 days and 25.1 days, respectively. Developmental days were not significant between both cultures (**Fig. 4C**).

(4) *A. bipunctata* with insufficient food

Developmental days of single culture of *A. bipunctata*, and combination culture with *H. axyridis*, were not clear, because all *A. bipunctata* died (most individuals of *A. bipunctata* were fed by *H. axyridis*). Combination culture with *M. sexmacilatus* was 23.0 days and developmental days were significantly less than single culture (**Fig. 4D**).

(5) *H. axyridis* with insufficient food

Developmental days of single culture of *H. axyridis*, and combination with *A. bipunctata*, were 26.5 days and 26.8 days, respectively. Developmental days was not significant between both cultures (**Fig. 4E**).

(6) *M. sexmaculatus* with insufficient food

Developmental days of single culture of *M. sexaculatus*, and combination culture with *A. bipunctata*, were 27.0 days and 21.8 days, respectively.

Developmental days were significantly less than single culture (**Fig. 4F**).

Developmental days of *M. sexmaculatus* with insufficient food were shorter than in case of sufficient food, because they prey on each other and *A. bipunctata*.

Discussion

In our laboratory experiment, native ladybird beetles were not affected by *A. bipunctata*, regardless of whether or not the food supply was sufficient. However, the effects of native ladybird beetles on *A. bipunctata* were serious in the case of insufficient food. The same result was obtained from laboratory experiments on the effects of *H. axyridis* and *C. septempunctata* to *A. bipunctata* (Britain population) (Kajita *et al.* 2000). However, in our study, in contrast to the findings of Kajita *et al.* (2000), larvae of *H. axyridis* preyed on the larvae of *A. bipunctata* despite having sufficient food. It is suggested that the frequency of encounter of each species was high, because our experiment was carried out in a small plastic case. If their occurrences synchronize each other and the aphids are sufficient, two species of native ladybird beetles, *H. axyridis* and *M. sexmaculatus*, may be superior to *A. bipunctata* in interspecific competition, because native ladybird beetles preyed on *A. bipunctata* whereas *A. bipunctata* did not prey on native ladybird beetles in laboratory experiment.

However, developmental stage of *A. bipunctata* and *H. axyridis* did not synchronize, and developmental stage of *A. bipunctata* was earlier than *H. axyridis* in the field (**Fig. 2**). This makes *A. bipunctata* advantageous from native species in the competition over food. When larvae of *H. axyridis* became fourth instar, they suffered from shortage of food because of host alternation of aphids (Osawa, 1992). There is a high possibility that larvae of *H. axyridis* prey on pre-pupae and pupae of *A. bipunctata* when the number of aphids, *P. californiensis*, declined. Indeed, the larvae of *H. axyridis* preyed on larvae, pre-pupae and pupae of *A. bipunctata* even when provided with sufficient food in this experiment. We observed occasionally that larvae of *H. axyridis* preyed on pre-pupa and pupa of *A. bipunctata* in this field investigation. However, there might be few influence of predation by *H. axyridis* in the field, because the number of *H. axyridis* was less than *A. bipunctata*, and *A. bipunctata* could utilize aphids earlier than *H. axyridis* (**Fig. 2**). In the case of *M. sexmaculatus* with sufficient food, there was no influence on the developmental period of *A. bipunctata*. There is no interaction between these two ladybird beetles in the field, because the population density of *M. sexmaculatus* on *A. buergerianum* tree was far lower than that of *A. bipunctata* (**Fig. 1**). The influence on *A. bipunctata* by *H. axyridis* and *M. sexmaculatus* is inferred to depend on the degree of the

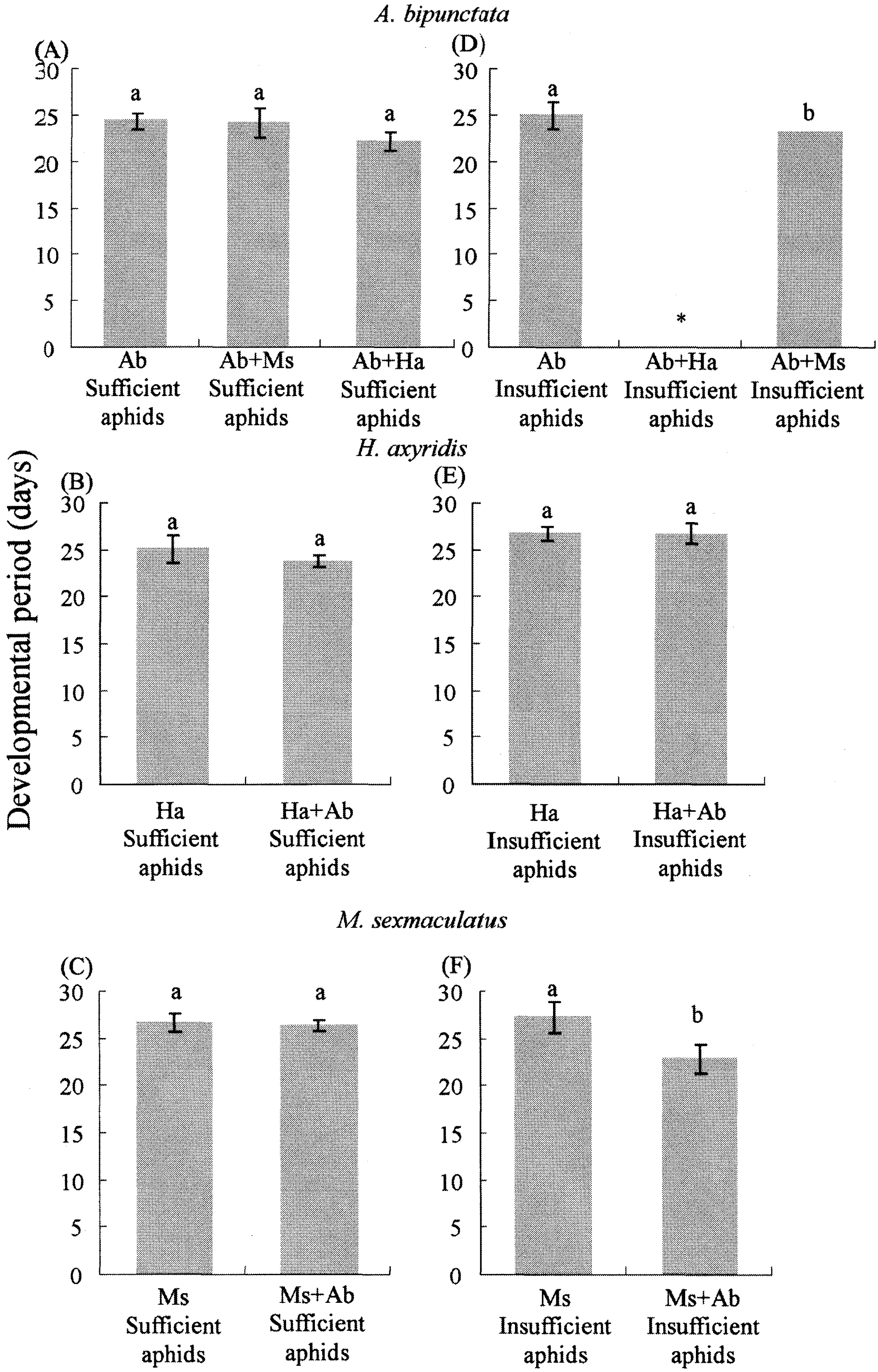

Fig. 4 Developmental period with standard deviation of *A. bipunctata* and native ladybird beetles. Bars with the same letter are not significantly different (Tukey-Kramer's test) (Ab, Ha and Ms, see Fig.3)

aggressiveness of two native ladybird beetles. Intraguild predation (IGP) is affected by body size, aggressiveness and food habit (Lucus *et al.*, 1998). In terms of food habit, *H. axyridis* and *M. sexmaculatus* are polyphagous species (Hodek and Honěk, 1996, 1998). However, body size of *H. axyridis* is larger than *M. sexmaculatus*, and *H. axyridis* is a well known stronger intraguild predator (Agarwala *et al.*, 2003). Competition over aestivating and overwintering sites(e.g. leaf shelter), between *A. bipunctata* and native ladybird beetles, was not observed in this observation.

In North America, two species of exotic ladybird beetles, *H. axyridis* and *C. septempunctata*, gave serious effects on the native ladybird beetles, such as *Coccinella transversoguttata richardsoni* (Brown) (Elliott *et al.*, 1996 ; Evans, 2004). The native and exotic ladybird beetles inhabit on same plant and their life histories synchronize each other in North America (Elliott *et al.*, 1996 ; Evans, 2004). However, in the case of *Rodolia cardinalis* (Mulsant), introduced as a natural enemy for control of the citrus pest, *Icerya purchasi* (Maskell), into Japan and California (Yasumatsu, 1970), no great effect on native species had been observed, because *R. cardinalis* is basically monophagous, and its populations depend solely on the density of *I. purchasi* (Kiritani, 1986). In Japan, these species, *A. bipunctata* and native ladybird beetles had coexisted each other for about ten years, because most individuals of *A. bipunctata* escape from competition by asynchronous life history, though native ladybird beetles are stronger in interspecific competition with *A. bipunctata* in our field observations and laboratory experiments.

Acknowledgements

This works was supported by a grant-in-aid from the Japan Society for the Promotion of Science (No 15380039). We are grateful to Prof. H. Yasuda, Yamagata University, Prof. H. Nakamura, Shinshu University, and Prof. T. Sugimoto, Faculty of Agriculture, Kinki University, for valuable discussions and advice in this study.

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外来種フタモンテントウと在来捕食性テントウムシとの種間関係について

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近年外来種問題が注目され、在来生態系に与える影響が懸念されている。日本ではこの 10 年間で約 10 種の外来テントウムシが記録されており、ヨーロッパ原産で捕食性テントウムシのフタモンテントウは 1993 年に大阪南港で日本で初めて発見された。本種は在来捕食性テントウムシのナミテントウとダンダラテントウと共にトウカエデ等に生息しており、ナミテントウの幼虫がフタモンテントウの前蛹や蛹を捕食するのがしばしば観察されている。室内実験ではナミテントウの幼虫は餌の量に関わらずフタモンテントウの幼虫を捕食し、ダンダラテントウの幼虫は餌が不足した場合のみフタモンテントウの幼虫を捕食した。しかし、フタモンテントウの幼虫は餌が不足した場合でもこれら 2 種の在来種を捕食することはなかった。すなわち、種間競争において在来種はフタモンテントウより優位だが、野外では互いの出現期のずれや生活史の相違により種間競争が緩和され、これら 3 種の捕食性テントウムシの共存が可能になっていることが示唆された。