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ORIGINAL RESEARCH PAPER

Establishment and yearly/seasonal occurrence of the exotic coccidophagous ladybird *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae) in citrus groves in Shizuoka City, central Japan: a 5-year survey on adult numbers

Shuji Kaneko[1,2]

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Abstract *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) is a ladybird native to Australia, preying on mealybugs and soft scales, and has been utilized worldwide as a biological control agent. It has long been recognized that *C. montrouzieri* that was introduced into the main island of Japan had failed to become established. The present study monitored yearly and seasonal occurrence of *C. montrouzieri* adults in citrus groves at Shizuoka Prefectural Fruit Tree Research Center in Shizuoka City, central Japan in 2008–2012 by using sticky traps and beating citrus trees. Adults of *C. montrouzieri* were continuously captured for 5 and 4 years in a pesticide-free citrus grove and a neighboring reduced-pesticide grove, respectively. Larvae of *C. montrouzieri* were observed consuming a cottony scale, *Pulvinaria aurantii* Cockerell, on citrus trees. These results provide unequivocal evidence for the ladybird's establishment in central Japan. The number of trapped ladybird adults exhibited four peaks a year: in mid-April, early to late June, mid-August, and late September to early October. Adult numbers in each grove varied largely across years, showing a great increase followed by a rapid decline during a period of 4 years. Factors affecting the seasonal/yearly occurrence of *C. montrouzieri* adults in citrus groves are discussed.

✉ Shuji Kaneko
kaneshu@ja2.so-net.ne.jp

[1] Shizuoka Prefectural Research Institute of Agriculture and Forestry, Fruit Tree Research Center, Shizuoka 424-0905, Japan

[2] Present Address: Research Institute of Environment, Agriculture and Fisheries, Osaka Prefecture, Osaka 583-0862, Japan

Keywords *Cryptolaemus montrouzieri* · Exotic coccidophagous coccinellid · Seasonal prevalence · Citrus · Sticky trap

Introduction

Cryptolaemus montrouzieri Mulsant (Coleoptera: Coccinellidae) that is indigenous to eastern Australia (Booth and Pope 1986) has been introduced around the world to control mealybugs and soft scale insects on various crops since its first release into citrus groves in California, USA, in 1891 (DeBach and Hagen 1964; Kairo et al. 2013). The exotic ladybird has become established in natural environments in many countries, exclusively in tropical and subtropical regions (see a review by Kairo et al. 2013).

In Japan, *C. montrouzieri* was introduced in 1934 from Saipan into the Ogasawara Islands (also known as the Bonin Islands) which are located in a subtropical region (Sakimura 1935), and has become established on the islands (Kurosawa et al. 1985). On the other hand, into Honshu Island (the main island of Japan), located in a temperate zone, the ladybird had been introduced twice so far (Furuhashi and Nishino 1980; Ishii 1955). First, the ladybird imported from Hawaii in 1931 was released into a citrus grove in Okayama Prefecture, located in the western portion of the main island of Japan, to suppress mealybugs, but was unable to overwinter (Ishii 1955), possibly due to low temperature in winter (Bartlett 1974; Maes et al. 2015). Then, *C. montrouzieri* sent from California in 1979 was introduced into a pesticide-free citrus grove in Shizuoka City, the central portion of the main island in 1980, but afterwards the ladybird had not been detected in the grove (Furuhashi and Fushimi 2001). Therefore,

it has long been recognized that *C. montrouzieri* had failed to become established on the main island of Japan (Murakami 1997).

Recently, however, *C. montrouzieri* has been recorded at many sites in the central portion of the main island of Japan (Furuhashi and Fushimi 2001; Ishikawa 2011; Kawashita and Matsubara 2002; Ohashi 2001; Tanaka and Saito 2001; Yahiro 2008). In the citrus grove in Shizuoka City where *C. montrouzieri* was released in 1980, adults and larvae of the ladybird were found in 2001 (Furuhashi and Fushimi 2001). In addition, *C. montrouzieri* adults and larvae have been noted since 1999 in several non-agricultural fields in central Japan, including the prefectures of Osaka (Ohashi 2001), Hyogo (Tanaka and Saito 2001), Shiga (Yahiro 2008), and Kanagawa (Kawashita and Matsubara 2002). However, all these studies were conducted for only 1 or 2 years, and hence could not completely prove the ladybird's establishment on the main island of Japan. Long-term studies on the occurrence of *C. montrouzieri* at particular sites on the main island of Japan therefore need to be carried out to provide unequivocal evidence for the ladybird's permanent establishment on the island. In addition, long-term monitoring would also document yearly change and seasonal prevalence of the abundance of *C. montrouzieri* in fields in Japan. This information would help elucidate factors determining population dynamics of the ladybird and relationships between the ladybird and its prey insects on the main island of Japan.

The present study monitored yearly and seasonal occurrence of adults of *C. montrouzieri* during the 5 years of 2008–2012 in experimental citrus groves, including the one where the ladybird was introduced in 1980 and rerecorded in 2001 (Furuhashi and Fushimi 2001), at Shizuoka Prefectural Fruit Tree Research Center in Shizuoka City by using sticky traps set inside citrus tree canopies and beating citrus leaves. Furuhashi and Fushimi (2001) implied the establishment of the ladybird in the citrus grove based on a single-month survey. Multiple-year monitoring is necessary to supply more convincing evidence for the ladybird's establishment in the grove. In addition, insect species that *C. montrouzieri* larvae were feeding on was recorded in the citrus groves. Monitoring of *C. montrouzieri* adults was also conducted in nine commercial citrus groves in Shizuoka City during the 5 years using sticky traps to detect its occurrence in the city. Based on the results obtained, this study discusses the possibility of the ladybird's reproduction and establishment in the citrus groves on the main island of Japan, the ladybird's possible prey insects on citrus, and factors influencing its yearly/seasonal population changes in citrus groves in central Japan.

Materials and methods

Ladybird

The body of the *C. montrouzieri* adult is 3.8–4.2 mm in length (Kurosawa et al. 1985). The head and pronotum are light red–orange in color. The elytra are dark brown–black (ground color), with a light red–orange hind apex. The pronotum and elytra are densely covered with short silver hairs.

Yearly and seasonal occurrence at Shizuoka Fruit Tree Research Center

Occurrence of *C. montrouzieri* adults was monitored in experimental groves (each more than 0.05 ha in area) cultivating Satsuma mandarin, *Citrus unshiu* Marc, at Shizuoka Prefectural Fruit Tree Research Center in Komagoe, located in the southern, coastal area of Shimizu-ku, Shizuoka City (Fig. 1, No. 7); the research center was moved to a different site called Mobata in the city in 2015. Figure 2 shows the surveyed groves (sites A–E), including one where no pesticide had been applied for over 30 years [hereafter referred to as pesticide-free grove: KO-A(f)], ones where petroleum oil was only sprayed in June [hereafter referred to as organic grove: KO-B(o), KO-E(o)], one where pesticide application was reduced approximately 50% less than that in conventionally managed groves [hereafter referred to as reduced-pesticide grove: KO-C(r)], and one that was conventionally managed [hereafter referred to as conventional grove: KO-D(c)].

Monitoring using sticky traps was conducted in four and five groves in 2008 (from May through December) and 2009–2012 (each from January through December), respectively (Table 1). Three (in 2008) or five (in 2009–2012) citrus trees, which were approximately 5 m apart from one another, were selected at the center of each grove. Inside each tree canopy, a single piece of double-sided, yellow sticky card trap (a yellow adhesive sheet ('IT-sheet', Sankei Chemical CO., LTD) of 100 × 200 mm attached on each side of a yellow acrylic plate of 200 × 200 mm) was set in a vertical position 1.3–1.5 m above the ground using a wooden pole and metal fittings. These traps were replaced at an interval of 6–8 days (generally 7 days). The number of *C. montrouzieri* adults caught on the collected traps was counted under a binocular microscope (OLYMPUS SZX12); identification of the ladybird was based on the external morphology described above.

Survey by beating citrus leaves was carried out in four and five groves in 2008 (from May through October) and 2009–2012 (each from April through November), respectively (Table 2). This was done twice a month: early (on

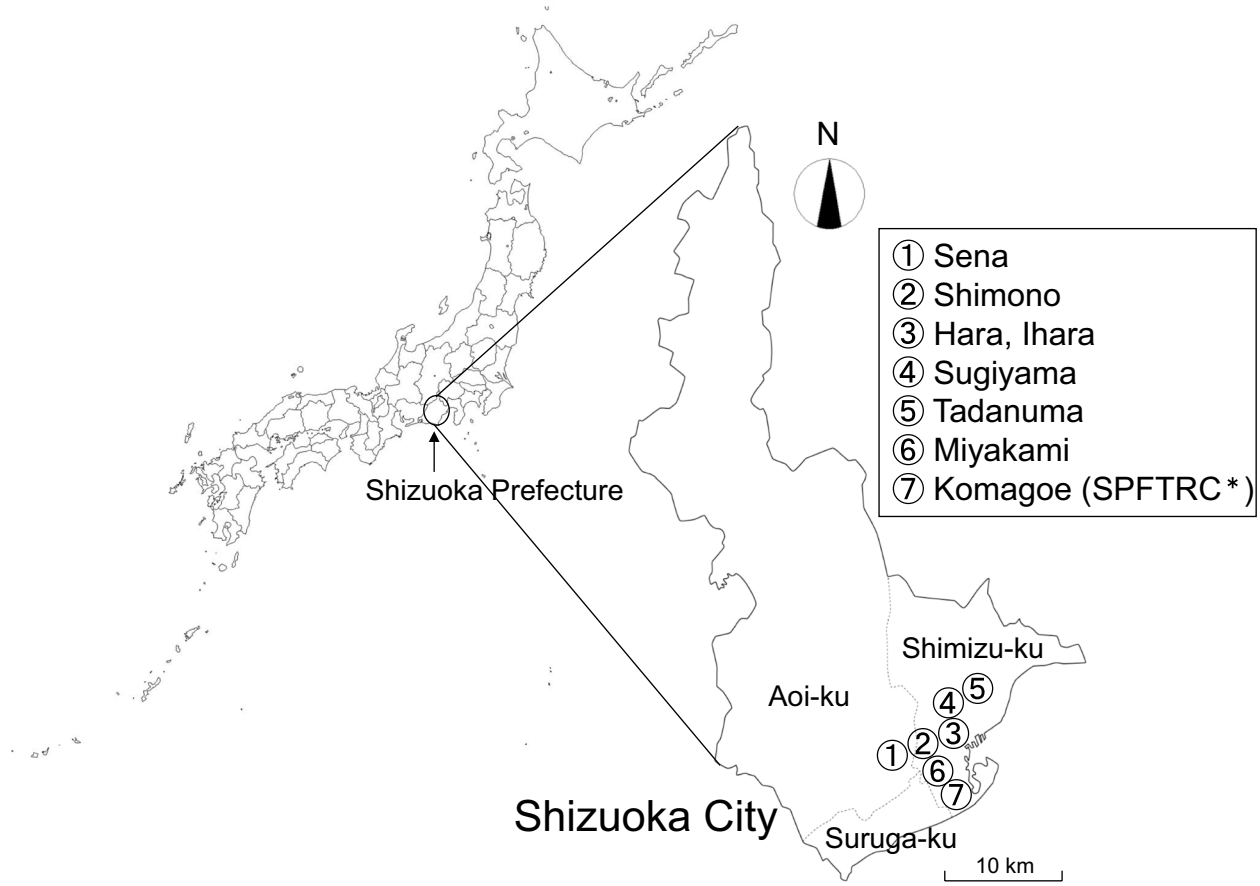

Fig. 1 Map showing citrus groves where *Cryptolaemus montrouzieri* adults were surveyed using sticky traps in Shizuoka City, central Japan in 2008–2012. *Shizuoka Prefectural Fruit Tree Research Center

6–8th, generally 7th) and late (on 22–24th, generally 23rd) in each month. Ten citrus trees were chosen randomly at the center of each grove; citrus trees inside which sticky traps were set were not included. Then, two portions (each involving approximately 100–200 leaves) on opposite sides (180° apart) were selected on each tree. Citrus leaves and twigs of each portion were beaten 10 times using a wooden pole (1.5 cm in diameter, 1 m in length) and insects falling from the leaves were caught using an insect net (20 cm in diameter) held under each portion. Thus, a total of 200 times of beating were done in each grove. Adults of *C. montrouzieri* captured were counted under the binocular microscope.

Identification of prey insects

To find *C. montrouzieri* larvae and its prey insects, five citrus trees were chosen randomly at the center of each citrus grove when replacing sticky traps, and then the leaves and twigs of each tree were roughly observed for about 2 min. Insects that many *C. montrouzieri* larvae were consuming were collected from citrus trees only in KO-C(r) in mid-September 2011 and then were identified in the laboratory.

Occurrence in Shizuoka City

Monitoring of *C. montrouzieri* adults using sticky traps was performed in commercial *C. unshiu* groves (each more than 0.3 ha in area), located in the southern part of Shizuoka City, mostly in Shimizu-ku, one of the major citrus-producing areas in Shizuoka Prefecture (Fig. 1, nos. 1–6); a total of two, five, six, nine, and nine groves in 2008 (from July through November), 2009, 2010 (both from May through November), 2011, and 2012 (both from April through November), respectively (Table 3). The groves included ones in Sena-1, Shimono, and Sugiyama that were organically managed [hereafter referred to as SE-1(o), SH(o), and SU(o)], one in Tadanuma-1 that was a reduced-pesticide

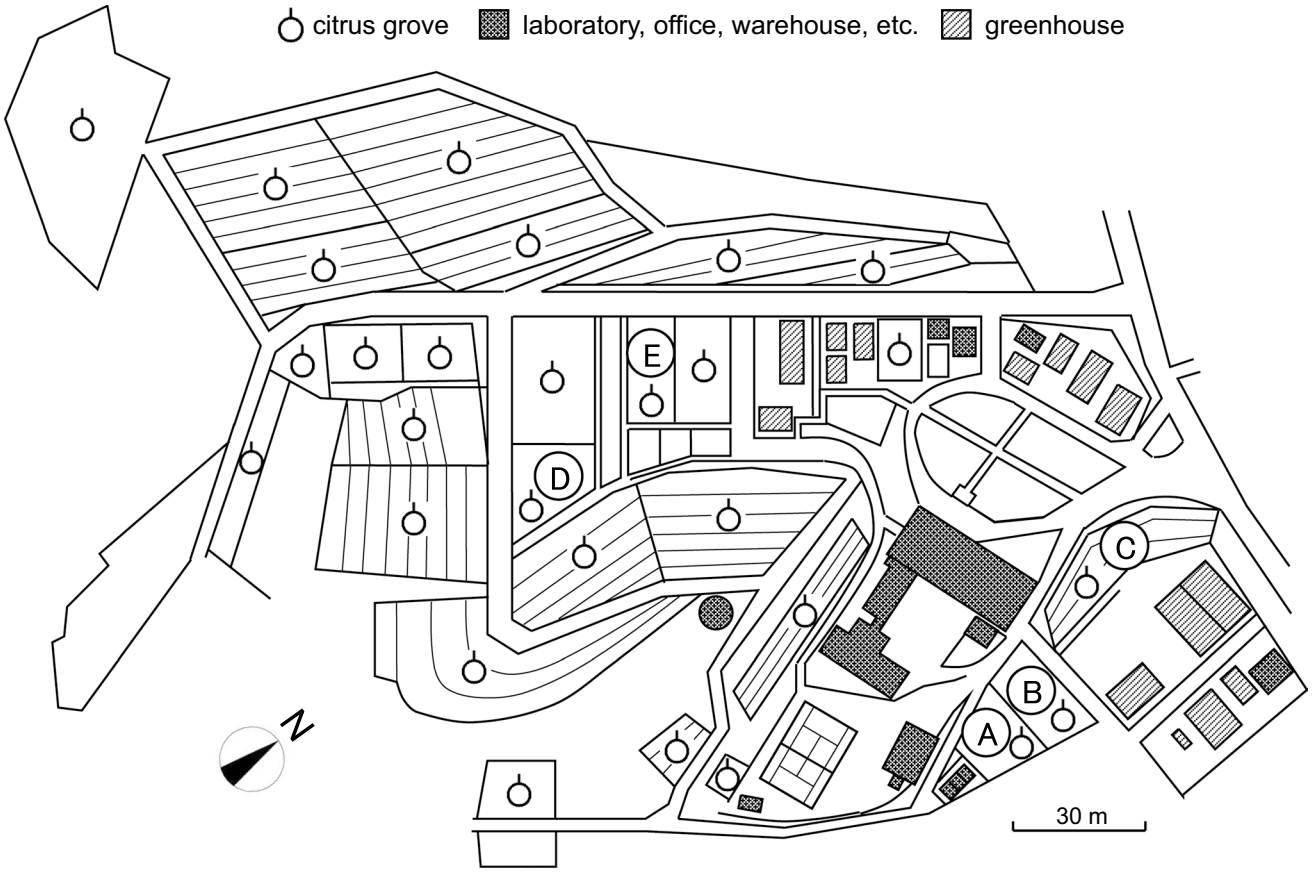

Fig. 2 Map showing experimental citrus groves where *Cryptolaemus montrouzieri* adults were monitored using sticky traps and beating method in Komagoe (Shizuoka Prefectural Fruit Tree Research Center) in Shizuoka City in 2008–2012. *A* pesticide-free, *B* organic, *C* reduced-pesticide, *D* conventional, *E* organic grove

Table 1 The total number of *Cryptolaemus montrouzieri* adults captured on sticky traps set inside citrus trees in each experimental citrus grove in Komagoe (Shizuoka Prefectural Fruit Tree Research Center) in Shizuoka City in 2008–2012. Numbers per trap are given

Year	No. traps	Citrus grove				
		KO-A(f)[c]	KO-B(o)	KO-C(r)	KO-D(c)	KO-E(o)
2008[a]	3	0.3	0	0	0	–[d]
2009[b]	5	3.0	0.8	0.6	0.2	0
2010[b]	5	4.6	0.2	1.0	0	0
2011[b]	5	0.8	0	0.6	0	0
2012[b]	5	0.2	0	0	0	0

Traps were replaced at an interval of ca. 7 days

[a] Monitoring was conducted from May through December

[b] Monitoring was conducted from January through December

[c] Letter in parentheses means: *f* pesticide-free, *o* organic, *r* reduced-pesticide, *c* conventional

[d] Monitoring was not conducted

grove [TA-1(r)], and ones in Sena-2, Hara, Ihara, Tadanuma-2, and Miyakami that were conventionally managed [SE-2(c), HA(c), IH(c), TA-2(c), and MI(c)]. These groves were selected because different levels of pesticide application might affect the occurrence of *C. montrouzieri* directly and/or indirectly through reducing abundance of its prey. In 2008, adults were surveyed in one reduced-pesticide, and one conventional grove. In 2009, adults were monitored in one reduced-pesticide, and four conventional groves. In 2010, monitoring was performed in one organic, one

Table 2 The total number of *Cryptolaemus montrouzieri* adults caught by beating citrus leaves in each experimental citrus grove in Komagoe (Shizuoka Prefectural Fruit Tree Research Center) in Shizuoka City in 2008–2012

Year	No. trees	Citrus grove				
		KO-A(f)[c]	KO-B(o)	KO-C(r)	KO-D(c)	KO-E(o)
2008[a]	10	0	0	0	0	–[d]
2009[b]	10	21	6	1	0	0
2010[b]	10	31	13	9	0	0
2011[b]	10	2	0	16	0	0
2012[b]	10	1	0	3	0	0

200 times of beating were done early and late in each month

[a] Monitoring was conducted from May through October

[b] Monitoring was conducted from April through November

[c] Letter in parentheses means: *f* pesticide-free, *o* organic, *r* reduced-pesticide, *c* conventional

[d] Monitoring was not conducted

Table 3 The total number of *Cryptolaemus montrouzieri* adults captured on sticky traps set inside citrus trees in each commercial citrus grove in Shizuoka City in 2008–2012. Numbers per trap are given

Year	No. traps	Citrus grove								
		Organic			Reduced-pesticide	Conventional				
		SE-1(o)[d]	SH(o)	SU(o)	TA-1(r)	SE-2(c)	HA(c)	IH(c)	TA-2(c)	MI(c)
2008[a]	3	–[e]	–	–	0	–	–	–	0	–
2009[b]	3	–	–	–	0	–	0	0	0	0
2010[b]	3	0	–	–	0	–	0	0	0	0
2011[c]	3	0	0	0	0	0	0	0	0	0
2012[c]	3	0.3	0	0	0	0	0	0	0	0

Traps were replaced at an interval of ca. 14 days

SE-1 Sena-1, *SH* Shimono, *SU* Sugiyama, *TA-1* Tadanuma-1, *SE-2* Sena-2, *HA* Hara, *IH* Ihara, *TA-2* Tadanuma-2, *MI* Miyakami

[a] Monitoring was conducted from July through November

[b] Monitoring was conducted from May through November

[c] Monitoring was conducted from April through November

[d] Letter in parentheses means: *o* organic, *r* reduced-pesticide, *c* conventional

[e] Monitoring was not conducted

reduced-pesticide, and four conventional groves. In 2011 and 2012, this was done in three organic, one reduced-pesticide, and five conventional groves.

Three citrus trees, approximately 5 m apart from one another, were chosen at the center of each grove. A piece of double-sided, yellow sticky card trap was set inside each tree canopy, as stated above. These traps were replaced at an interval of 12–16 days (generally 14 days), and the number of *C. montrouzieri* adults captured on the collected traps was counted under a binocular microscope (OLYMPUS SZX12).

Results

Yearly adult occurrence

In a pesticide-free citrus grove [KO-A(f)], the number of *C. montrouzieri* adults caught on sticky traps was greatest among the five groves surveyed at Shizuoka Fruit Tree Research Center (Table 1). The ladybird adults were continuously trapped for the 5 years examined, but the numbers varied largely across the years, greatly increasing from 2008 to 2009, reaching a peak in 2010, and then decreasing rapidly. In a reduced-pesticide grove [KO-C(r)], *C. montrouzieri* adults were captured for three consecutive years (2009–2011), with the numbers exhibiting a small peak in 2010. In an organic grove [KO-B(o)] that was adjacent to KO-A(f), some adults were recorded in 2009 and 2010. In a conventional grove [KO-D(c)] and another organic grove [KO-E(o)], adults were rarely detected.

In KO-A(f), *C. montrouzieri* adults collected by beating citrus leaves were also most abundant among the five groves, showing the same yearly change in numbers as the adults caught on sticky traps (Table 2). In KO-C(r), adults were noted for 4 years straight (2009–2012), and the

numbers increased largely from 2009 to 2010, reaching a peak in 2011.

No clear tendency was found for the effect of pesticide application levels on the ladybird abundance.

Seasonal adult occurrence

Adults of *C. montrouzieri* were captured on sticky traps at the research center mainly from early April through June and from mid-August through October, with the largest numbers in late September to early October (Fig. 3). In each of 2009 and 2010 when many adults were detected, adult numbers showed four peaks: in mid-April, early to late June, mid-August, and late September to early October.

Many adults were caught by beating citrus leaves from early April to early June and from late August through November, with more adults in October (Fig. 4). In both monitoring methods, adults were more abundant in autumn. Beating citrus leaves collected many adults in November, but sticky traps did not.

Prey insects

Insects that many larvae of *C. montrouzieri* were consuming on citrus trees in KO-C(r) in mid-September 2011 were identified as larvae of a cottony scale, *Pulvinaria aurantii* Cockerell (Hemiptera: Coccidae).

Occurrence in Shizuoka City

Among nine commercial citrus groves in Shizuoka City, a single *C. montrouzieri* adult was captured on sticky traps only in an organic grove [Sena-1(o)] in 2012 (Table 3).

Discussion

Adults of the exotic ladybird *C. montrouzieri* were caught for five and four consecutive years in a pesticide-free citrus grove and a neighboring reduced-pesticide citrus grove, respectively, in Shizuoka City, located in the central part of the main island of Japan (Tables 1, 2). Larvae of the ladybird were also observed in the latter grove, preying on *P. aurantii* on citrus trees. These results indicate that *C. montrouzieri* had continuously passed many generations through reproduction in the citrus groves during the 5 or 4 years; this means that the ladybird has become established in the groves. Several studies have detected so far the occurrence of the ladybird in central Japan (Furuhashi and Fushimi 2001; Ishikawa 2011; Kawashita and Matsubara 2002; Ohashi 2001; Tanaka and Saito 2001; Yahiro 2008), but they all carried out 1- or 2-year surveys. Therefore, the present long-term (5-year) study provides the first unequivocal evidence for the establishment of *C. montrouzieri* on the main island of Japan.

The geographical distribution of *C. montrouzieri* in Japan had been limited to the Ogasawara Islands, located in a subtropical region, before the 1990s (Kurosawa et al. 1985). The present study proves the establishment of the ladybird on the main island of Japan, and other studies (Ishikawa 2011; Kawashita and Matsubara 2002; Ohashi 2001; Tanaka and Saito 2001; Yahiro 2008) suggest its wide distribution in central Japan. The present study also detected its presence at one site in Shizuoka City that was ca. 10 km away from the citrus groves where the ladybird has become established (Table 3). Several species of exotic predacious ladybirds that had intruded into Japan, such as *Adalia bipunctata* (Linnaeus) and *Platynaspidius maculosus* (Weise), are also now expanding their distributions on the main island (Kaneko 2013; Toda and Sakuratani 2006). Some alien aphidophagous ladybird species have caused declines in the abundance of indigenous ladybird species (e.g., Brown et al. 2011; Elliott et al. 1996; Evans 2004). Increased abundance of *C. montrouzieri* on the main island of Japan might negatively affect abundance of native ladybirds, such as *Hyperaspis japonica* (Crotch) and *Nephus phosphorus* (Lewis) that attack mealybugs and soft scales. Therefore, we need to carefully monitor the spread of distributions and abundance of exotic ladybirds, including *C. montrouzieri*, in Japan. Setting sticky traps inside tree canopies would be a useful method for surveying ladybirds, as shown by the present study and others (Kaneko et al. 2006; Kaneko 2013).

The present study documented by beating citrus leaves that many *C. montrouzieri* adults existed on citrus trees in late November and early April at Shizuoka Fruit Tree Research Center that is located in the southern, coastal area of central Japan (Figs. 1, 4). Sticky traps set inside citrus trees, on the other hand, collected few *C. montrouzieri* adults in those seasons (Fig. 3), which might be due to lower foraging (especially flying) activities of the ladybird adults at lower temperatures. The result of beating citrus trees implies that the ladybird overwintered in the adult stage in the citrus groves or neighborhood. The ladybird is unable to survive cold winters in temperate zones (Bartlett 1974; Maes et al. 2015). Bartlett (1974) experimentally showed that half of the *C. montrouzieri* adults died when exposed to an air temperature of −3.9 °C for about 7 h. Meteorological data at an AMeDAS point named 'Shimizu' (the nearest AMeDAS point from Shizuoka Fruit Tree Research Center) indicate that the lowest air temperature was never lower than −3.9 °C during 2008–2012 (the lowest temperature during the period was −3.5 °C on 3 February 2012; Japan Meteorological Agency 2016a). This suggests that the relatively warmer winter might allow *C. montrouzieri* adults to overwinter at the research center

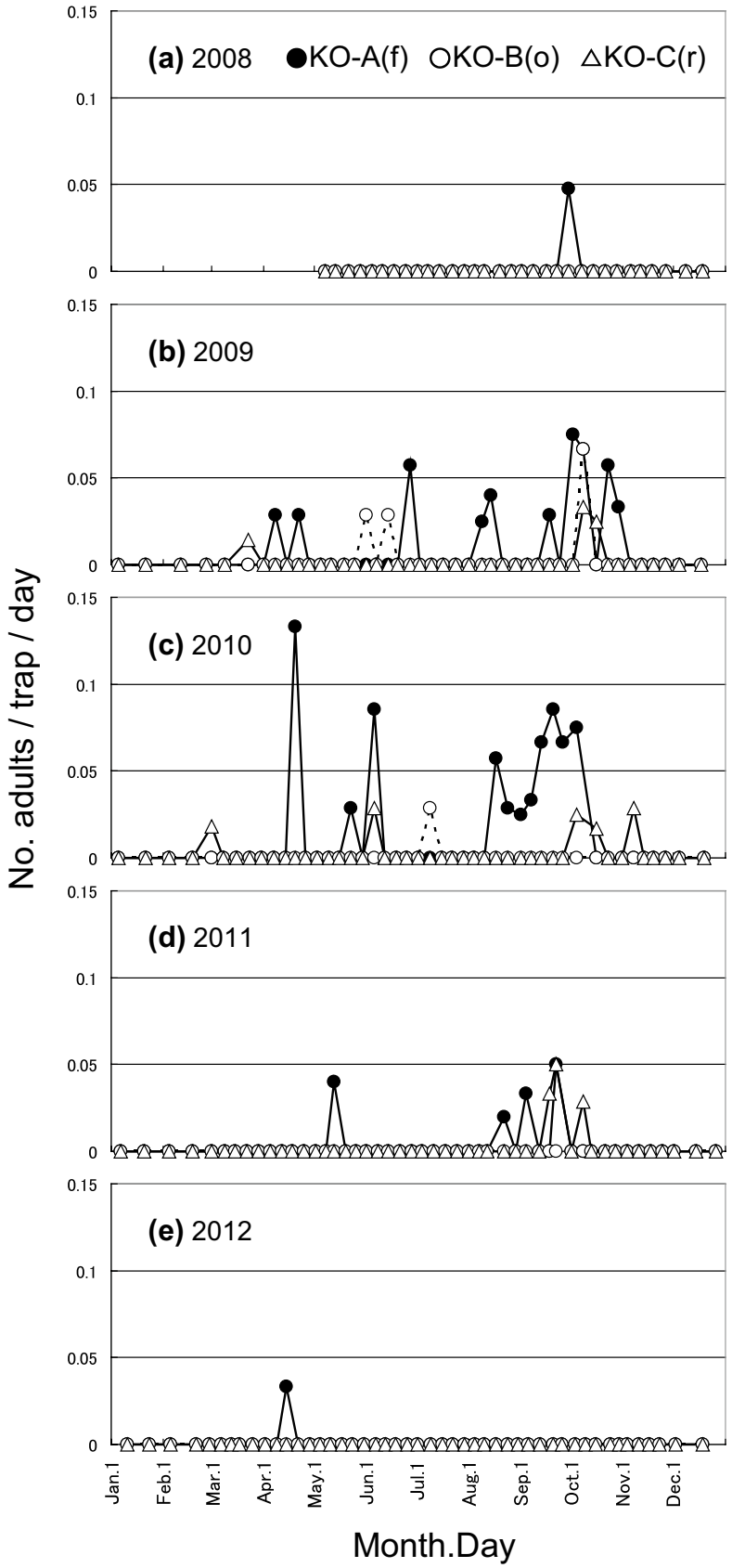

Fig. 3 Seasonal changes in the number of *Cryptolaemus montrouzieri* adults captured on sticky traps set inside citrus trees in each experimental citrus grove in Komagoe (Shizuoka Prefectural Fruit Tree Research Center) in Shizuoka City in 2008–2012

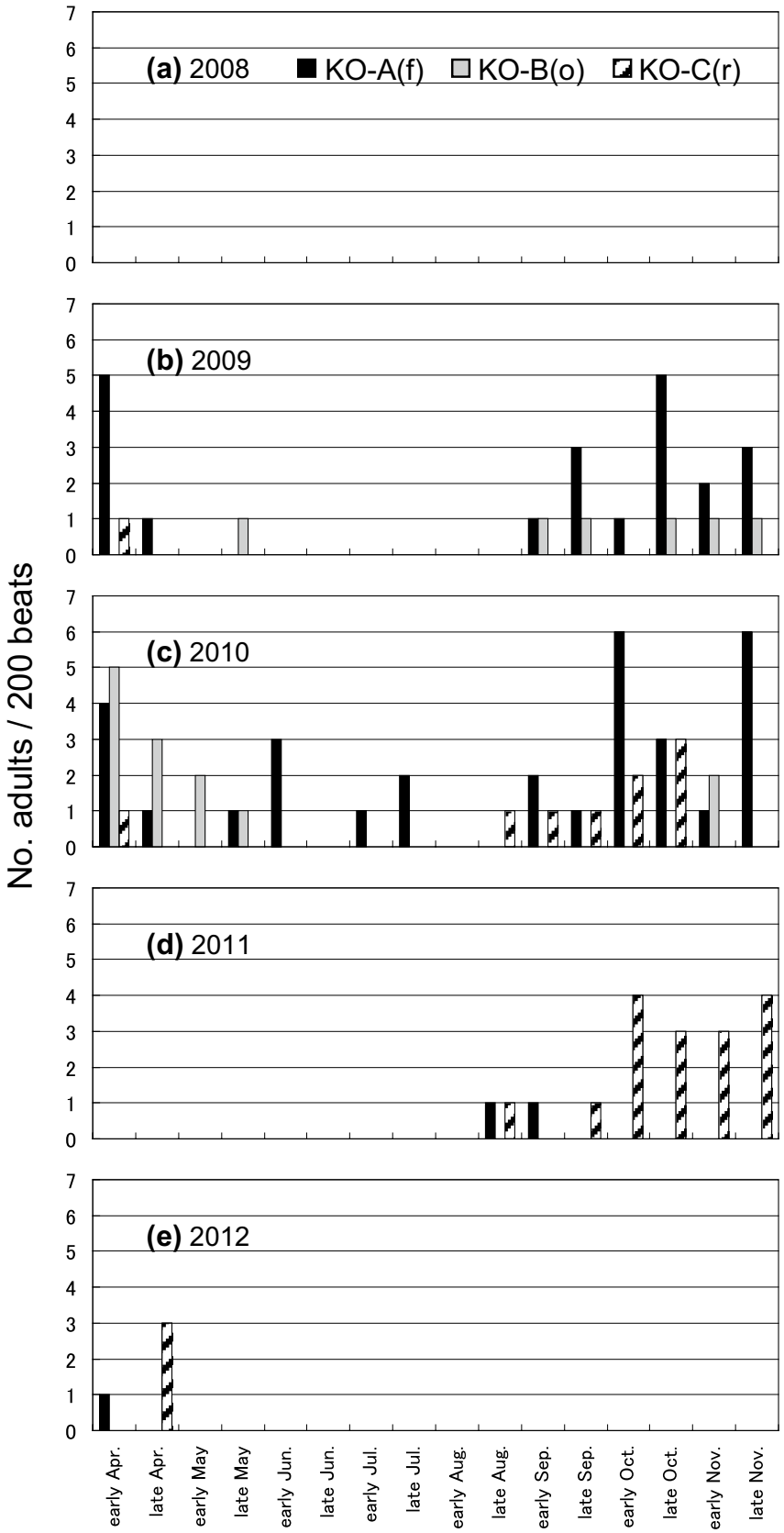

Fig. 4 Seasonal changes in the number of *Cryptolaemus montrouzieri* adults caught by beating citrus leaves in each experimental citrus grove in Komagoe (Shizuoka Prefectural Fruit Tree Research Center) in Shizuoka City in 2008–2012. No ladybird was captured in 2008 (see Table 2)

even though it is located in a temperate zone. Microhabitats where the ladybird adults stay in winter (e.g., on citrus/other trees, beneath leaf litter, or under rocks) are unclear at the site, thus need to be determined.

Cryptolaemus montrouzieri attacks a wide range of hemipteran species (Kairo et al. 2013). The present study found *C. montrouzieri* larvae feeding on a cottony scale *P. aurantii* on citrus trees. Predation by the ladybird on *P. aurantii* on citrus was also shown by Bozorg-Amirkalaee et al. (2015) in Iran. Ohashi (2001) observed that *C. montrouzieri* adults and larvae consumed *P. aurantii* on oleander, *Nerium oleander* L., in Japan; this is the only study that reported the prey insect of *C. montrouzieri* in the country. Thus, *P. aurantii* would be one of important prey insects for the exotic ladybird in Japan.

Seasonal occurrence of adults of coccidophagous ladybirds is likely to be associated with that of their prey insects in the case that ladybird larvae preferentially feed on particular developmental stages of prey (Kaneko et al. 2006). Adult females of *C. montrouzieri* lay eggs into the egg sacs of mealybugs and soft scales, and then hatched ladybird larvae consume eggs, crawlers, and young larvae of the prey insects (Shylesha and Mani 2016). Adult females of *P. aurantii* form cottony egg sacs in mid-May and mid- to late August in Japan, producing crawlers of the following generation in late May to early June and early to mid-September (Kawai 1980). The present study indicated that the number of *C. montrouzieri* adults trapped in the citrus groves in late summer to autumn exhibited two peaks: in mid-August and late September to early October (Fig. 3). It might be expected that in the groves *C. montrouzieri* females search for and oviposit into egg sacs of *P. aurantii* females in mid-August and then hatched larvae grow by preying on *P. aurantii* eggs and larvae, finally becoming adults in late September to early October. Average air temperature over 1981–2010 at an AMeDAS point named 'Shimizu' (the nearest AMeDAS point from the citrus groves) was approximately 25 °C in mid-August to late September (Japan Meteorological Agency 2016b). *Cryptolaemus montrouzieri* requires ca. 47 days from deposited egg to adult emergence under laboratory conditions of 25 °C (Babu and Azam 1987; Kairo et al. 2013). These facts may explain the duration between the two peaks of the ladybird adult numbers detected in the citrus groves in late summer to autumn. On the other hand, two peaks of the ladybird adult numbers were also noted in mid-April and early to late June (Fig. 3). However, egg sac formation by *P. aurantii* females in spring occurs in mid-May (Kawai 1980), which cannot account for abundant ladybird adults in mid-April; this might be because the ladybird exploited prey insects other than *P. aurantii* in the citrus groves in spring to early summer. Seasonal changes in the occurrence of *C. montrouzieri* larvae and *P. aurantii* larvae and their interactions on citrus trees therefore need to be documented to assess influence of *P. aurantii* on the seasonal occurrence of *C. montrouzieri* adults in citrus groves.

Abundance of *C. montrouzieri* adults in each citrus grove varied largely across the 5 years examined, greatly increasing and then decreasing rapidly during a period of 3 to 4 years (Tables 1, 2). In the citrus grove KO-C(r), abundance of *P. aurantii* on citrus trees showed a similar pattern to that of the ladybird, being much higher in 2010–2011 and extremely low in 2012 (S. Kaneko, personal observation). This implies that the drastic change in *C. montrouzieri* adult abundance in the citrus groves might be connected with the change in *P. aurantii* density. On the other hand, mealybugs and soft scales also occurred in the citrus groves, and hence there remains a possibility that the ladybird exploited insects other than *P. aurantii* there. Composition of insect species that *C. montrouzieri* attacks on citrus trees and its seasonal difference in Japan need to be investigated in order to determine factors affecting yearly/seasonal population dynamics of the ladybird and to evaluate its role in controlling populations of insect pests in citrus groves.

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