Hindawi Publishing Corporation
Psyche
Volume 2010, Article ID 384371, 6 pages
doi:10.1155/2010/384371

Research Article

Several New Aspects of the Foraging Behavior of *Osmia cornifrons* in an Apple Orchard

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Received 3 August 2009; Revised 13 November 2009; Accepted 15 December 2009

Academic Editor: Claus Rasmussen

We investigated the foraging behavior of *Osmia cornifrons* Radoszkowski, which is a useful pollinator in apple orchards consisting of only one kind of commercial cultivars such as "Fuji", and of different types of pollinizers, such as the red petal type, "Maypole" or "Makamik". It was confirmed that, in terms of the number of foraging flowers per day, visiting flowers during low temperatures, strong wind, and reduced sunshine in an apple orchard, *O. cornifrons* were superior to honeybees. We indicated that *O. cornifrons* seemed to use both petals and anthers as foraging indicator, and that not only female, but also males contributed to apple pollination and fertilization by the pollen grains attached to them from visiting flowers, including those at the balloon stage. It was confirmed that *O. cornifrons* acts as a useful pollinator in an apple orchard consisting of one kind of cultivar with pollinizers planted not more than 10 m from commercial cultivars.

1. Introduction

Osmia cornifrons Radoszkowski is one of the more useful pollinators of Rosaceae fruit production including apples. Although honeybees (*Apis mellifera* Linnaeus) are the most important natural carriers of pollen in an apple orchard, the use of *O. cornifrons* is on the increase in Japan due to its superior characteristics over honeybees, such as its higher pollination rates produced by not moving along the rows, its superior safety from being stingless, and the fact that it flies and pollinates apples in cooler and damper weather [1, 2]. Moreover, since the fertilization area it covers while collecting pollen in an apple orchard is smaller than that of honeybees, *O. cornifrons* are especially useful in relatively small and densely planted apple orchards in Japan consisting of one kind of cultivar with Crab-apples as pollinizers that are planted not more than 10 m from the cultivar [3].

Previously, we investigated the usefulness of the foraging behavior of *O. cornifrons* in an apple orchard consisting of a pollinizer and a commercial cultivar, "Fuji" [4]. We demonstrated that *O. cornifrons* showed strong flower constancy during one pollen-nectar foraging trip of 4–8 minutes, though the bees seemed to forage different types of flowers, for example, from pollinizers with red petals to commercial cultivars with white petals during their 16–22 pollen-nectar foraging trips [4]. From the results showing that the pollen from pollinizers not brushed off from the pollinator's body could be used for the fertilization of commercial cultivars visited on their next foraging trip, *O. cornifrons* seemed to be a useful pollinator in apple orchards consisting of a single cultivar, such as "Fuji" and of pollinizers of different types, such as the red petal types, "Maypole" or "Makamik" [4]. Moreover, *O. cornifrons* seemed to be a useful pollinizer for "Delicious," which is difficult for honeybees to access as a pollinator due to its sideways approach [4, 5].

In this paper, we investigated the foraging behavior of *O. cornifrons* and elucidated their daily foraging time, foraging indicators, and the likelihood of male contributions to fertilization.

2. Materials and Methods

2.1. Experimental Area. Our research was conducted from 2006 to 2009 at a 9.0-ha apple orchard at the Nagano Fruit

Tree Experiment Station, in Nagano, Japan, as well as in sectors of experimental farms at both Gifu University and Nagoya University [3, 4].

At Gifu University, the area occupied by one nesting shelter of *O. cornifrons* consisted of ca. 400 females together with four "Seirin Spur" (a "Fuji" sport) and three "Maypole" trees. We planted four-year-old apple trees in March, 2006, and established 1 to 8 rows composed of either "Maypole" or "Seirin Spur" and "Maypole", with the distance from the nesting shelter to rows 1 to 8 being 1.8 m, 3.7 m, 7.6 m, 13.4 m, 20.3 m, 21.2 m, 30.8 m, and 33 m, respectively [3]. In March, 2009, we cut down 14 "Maypole" trees located in rows 2 to 7.

2.2. Foraging Time of O. cornifrons. The apple orchard at Nagano was partitioned into 49 blocks (most were 2000 m^2) with 9 nesting shelters of *O. cornifrons* comprised of at least 1000 females, which means that a sufficient number of *O. cornifrons* were present everywhere in the orchard [3]. *Osmia cornifrons* uses a reed tube as its nest, and the *O. cornifrons* we used were originally captured by Mr. Takazawa in 1966 on the thatched roof of his house located close to the Nagano Fruit Tree Experimantal Station. The number of bees has increased two to three times in one year without any troubles, such as an attack of natural enemies. To observe the daily foraging period of *O. cornifrons*, we counted the average number of flying individuals in front of the nesting site in block no. 14 at 5-minute intervals on an hourly basis from 8:00 to 18:00. We recorded 20 when more than 20 individuals were counted since counting their exact number was difficult. We measured the temperature, solar radiation, and wind speed using the FreeSlot-68KD system (M. C. S Co., Sapporo, Hokkaido, Japan) settled at block no. 19 at the Nagano Fruit tree experimental station [3]. All of the data are recorded automatically every minute from morning till night.

2.3. Indicators for Foraging and Pollination by Males. In Nagano, we randomly selected 80 or 83 "Fuji" flowers in one tree (2 total of 160 or 163 flowers in two trees) in block no. 17, then removed the petals (20 or 21 flowers), anthers (20 or 21 flowers), and both petals and anthers (20 flowers) to determine what part of the flower was most attractive to visiting *O. cornifrons*. From 2006 through 2008, the percentage of "Fuji" fruit set was determined to be 18 to 23 days after full bloom. We confirmed that the flowers with standing sepals and a tendency toward swollen ovaries and surrounding receptacle tissues had succeeded in fertilizing and setting fruit. We also manipulated some flowers in "Maypole" and "Dolgo" and observed that *O. cornifrons* were visiting them.

At Gifu (2008) and Nagoya (2009), we used nets to cover three "Alps-Otome" planted at one-meter intervals with three "Maypole" planted along the same line at one meter intervals. Again using nets, we covered one "Alps-Otome" and one "Maypole" planted at one-meter intervals at Gifu (2008) and Nagoya (2009) for the control experiment. All of the four-year-old "Alps-Otome" and "Maypole" plants were grown in individual pots until February of 2009, then planted in soil at the experimental farm of Nagoya University. Between the 13th to 22nd of April, a total of 40 and 30 males of *O. cornifrons* were introduced to the planting area at Gifu (2008) and Nagoya (2009), respectively. The percentage of "Alps-Otome" fruit set was determined as mentioned. We investigated the fruit set of 100 flowers located at the top of each tree (2008) as well as that of all the flowers (2009). The data of each year from 100 to 575 flowers were averaged out (mean ± SD).

3. Results and Discussion

3.1. Foraging Time and Distance of Osmia cornifrons. We observed the activity of *O. cornifrons* at the nesting side for 16 days (5 days in 2006, 4 days in 2007, and 7 days in 2008) and discovered several characteristics of their foraging behavior. First, they started foraging from 9:00 AM (29th or 30th of April 2007 and 2008, Figures 1(a) and 1(b)) or 8:00 AM (2nd of May 2007, Figure 1(c)) or 7:00 AM (1st of May 2008, Figure 1(d)), and the temperature seemed to be the critical point from which they began foraging. As we found that some individuals started foraging at 10.7°C and 10.9°C on April 28th and May 4th 2006, respectively, the temperature at which they began daily activity appeared similar to that of *O. cornuta* (10°C to 12°C), but lower than that of *A. mellifera* (12°C to 14°C) [6]. In addition, *O. cornifrons* showed longer periods of activity in fine weather (from 7:00 A.M. to 6:00 PM, Figure 1(d)) than might be expected by Mr. Kitamura (from 8:00 A.M. to 5:00 PM, unpublished results). We found that *O. cornifrons* were already in flight at an apple orchard at 6:10 A.M. on May 2nd 2008 and confirmed that they were collecting pollen from "Fuji" flowers at 6:30 A.M. Since the pollen of flowers under bright sunlight had thoroughly dried by 6:15 A.M., they seemed to have started foraging for pollen at 6:30 A.M. As *O. cornifrons* visit ca. 15 flowers per minute, which is a rate higher than that of honeybees (6 flowers per minute) (Kitamura, personal communication), the rate of apple flowers pollinated by *O. cornifrons* must be higher than that by honeybees. Although they could not forage under very strong winds (3.9–5.4 m/s) and reduced sunshine (0.61–1.66 MJ/m^2) (Figure 1(c), Kitamura, unpublished results) [6], in 2008, they were able in fact to fly under relatively severe conditions (strong winds of 2.5–4.2 m/s and reduced sunshine of 0.48–0.79 MJ/m^2) (3:00 to 4:00 PM in Figure 1(d)). Weather conditions in 2008 were hurriedly for *O. cornifrons* since fine weather with no trace of rain continued from April 27th to May 2nd, so that the flowering period of "Fuji" (10 days) was the shortest in the last 3 years. This might explain why they flew in spite of such severe conditions.

Previously, we showed that the pollination of apple trees by *O. cornifrons* maintained high levels at a 33 m location from the nesting side [4]. For that reason, both the pollinizers (Maypole) and commercial cultivars (Seirin Spur) were planted at 3.7 m, 7.6 m, 13.4 m, and 33 m from the nesting side. We cut down all "Maypole" except for three plants at the closest point (1.8 m) to the nesting side, thus reducing the number of pollinizers. In that rearranged area, fruit set was

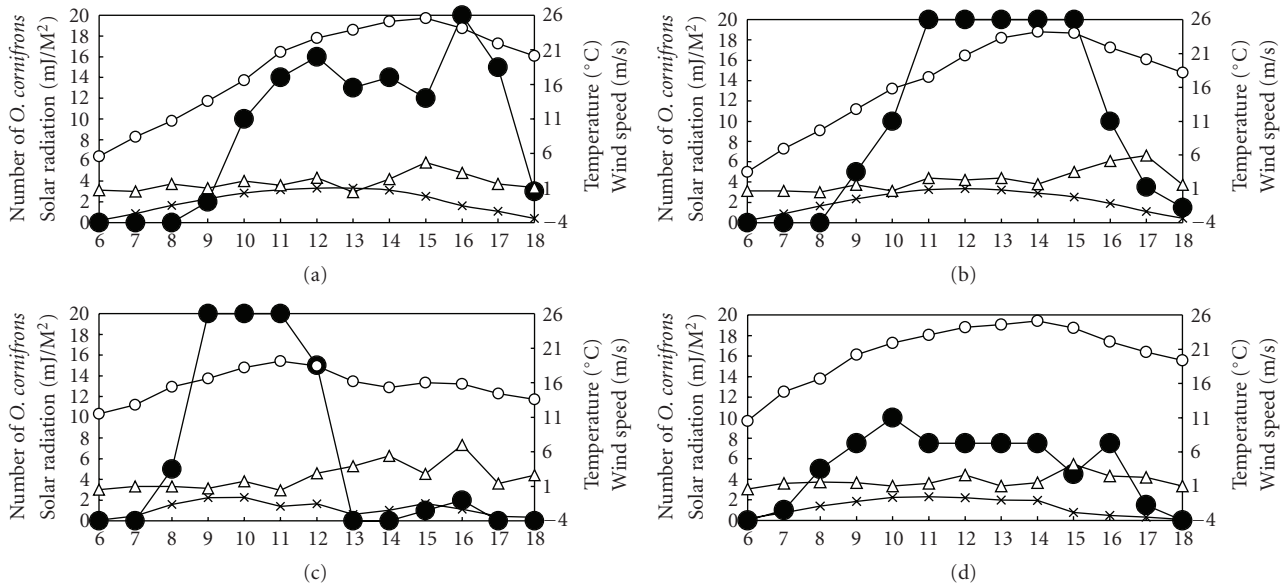

Figure 1: Activity of *O. cornifrons* (black circles) during 4 days at a nesting site in the Nagano orchard. Ambient temperature (°C, white circles), solar radiation (MJ/m^2, cross), and wind speed (m/s, triangles) are also shown. (a) 30th of April, 2007, (b) 29th of April, 2008, (c) 2nd of May, 2007, and (d) 1st of May, 2008.

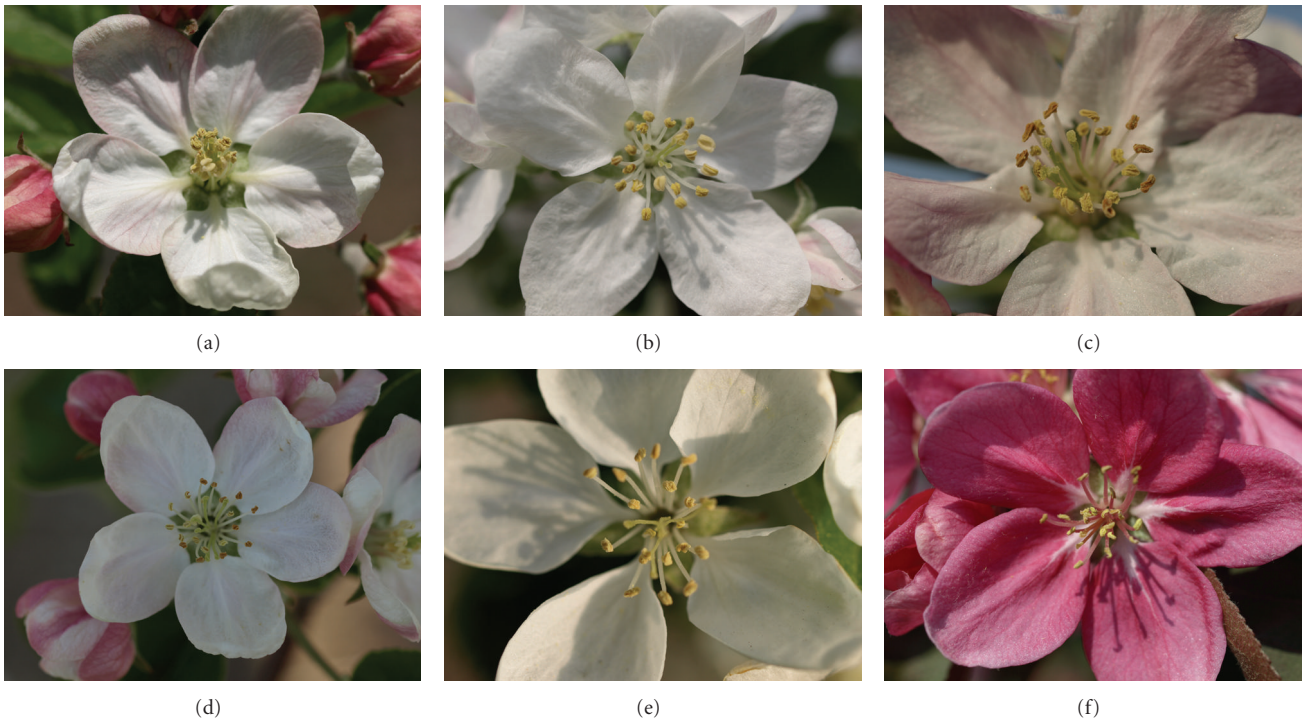

Figure 2: Stamens and a pistil morphology of apple flowers. (a) "Starking Delicious", (b) "Fuji", (c) "Shinano Gold", (d) "Tsugaru", (e) "Dolgo", and (f) "Maypole".

still maintained at high levels up to 13.4 m from the nesting side (rate of fruit set (%) of 3.7 m, 7.6 m, and 13.4 m were 87.0%, 84.3%, and 72.6%, resp. (No. of fruits/No. of flowers of 3.7 m, 7.6 m, and 13.4 m were 849/976, 848/1006, and 863/1188, resp.)) but was significantly reduced at the 33 m point (rate of fruit set (%) was 43.9% (No. of fruits/No. of flowers was 220/501)). Although we did not count the exact number of *O. cornifrons* visiting the 33 m point, they seemed to be scanty compared to those from 2006 to 2008. Moreover, since we cut down 82% of the "Maypole" pollinizers (14/17), pollen amounts available to the pollinizers might have been insufficient. These findings suggested that our previous

Table 1: Fruit set of "Fuji" fruit formed by open-pollination.

Flower morphology	Year	No. of flowers	No. of fruits	Rate of fruit set (mean ± S.E.%)	No. of seeds/fruit (mean ± S.E.%)
Anthers	2007	21	18	85.7	8.2
removed	2007	20	15	75.0	9.7
	2008	20	15	75.0	8.5
	2008	20	15	75.0	8.9
	2007-2008			77.7 ± 2.7	8.8 ± 0.7
Petals	2007	21	14	66.7	7.7
removed	2007	20	9	45.0	8.7
	2008	20	10	50.0	6.3
	2008	20	13	65.0	5.5
	2007-2008			56.7 ± 5.4	7.1 ± 0.7
Anthers and	2007	20	13	65.0	7.1
petals	2007	20	5	25.0	5.8
removed	2008	20	4	20.0	5.0
	2008	20	4	20.0	3.8
	2007-2008			32.5 ± 10.9	5.4 ± 0.7
Untreated	2007	21	21	100.0	8.2
	2007	20	18	90.0	8.9
	2008	20	17	85.0	10.2
	2008	20	20	100.0	9.0
	2007-2008			93.8 ± 3.8	9.1 ± 0.4

Table 2: Rate of dehiscere anthers within a balloon-stage "Maypole".

No. of flowers	Year	No. of flowers having at least 1 dehiscere anther	Rate of flowers having at least 1 dehiscere anther (mean ± S.E.%)	No. of anthers/ flower	No. of dehiscere anthers/ flower	Rate of dehiscere anthers/flower (mean ± S.E.%)
10	2006	7	70.0	17.5	2.0	11.4
10	2007	4	40.0	19.4	1.1	5.7
10	2007	5	50.0	19.6	2.9	14.8
10	2007	4	40.0	19.1	1.6	8.4
10	2007	5	50.0	19.2	1.7	8.9
10	2007	3	30.0	18.1	0.3	0.02
			46.7 ± 5.6			8.2 ± 2.1

Table 3: Fruit set of "Alps-Otome" fruit formed by "Maypole" pollen carried by *Osmia cornifrons* male.

Distance from "Maypole"	Year	No. of flowers	No. of fruits	Rate of fruit set (mean ± S.E.%)
1	2008	100	18	18.0
1	2009	365	129	35.3
	2008-2009			26.7 ± 8.7
2	2008	100	19	19.0
2	2008	575	128	22.3
	2008-2009			20.7 ± 1.7
3	2008	100	26	26.0
3	2009	480	140	29.2
	2008-2009			27.6 ± 1.6
1*	2008	100	1	1.0
1*	2009	121	1	0.83
	2008-2009			0.9 ± 0.1

* Control, no male.

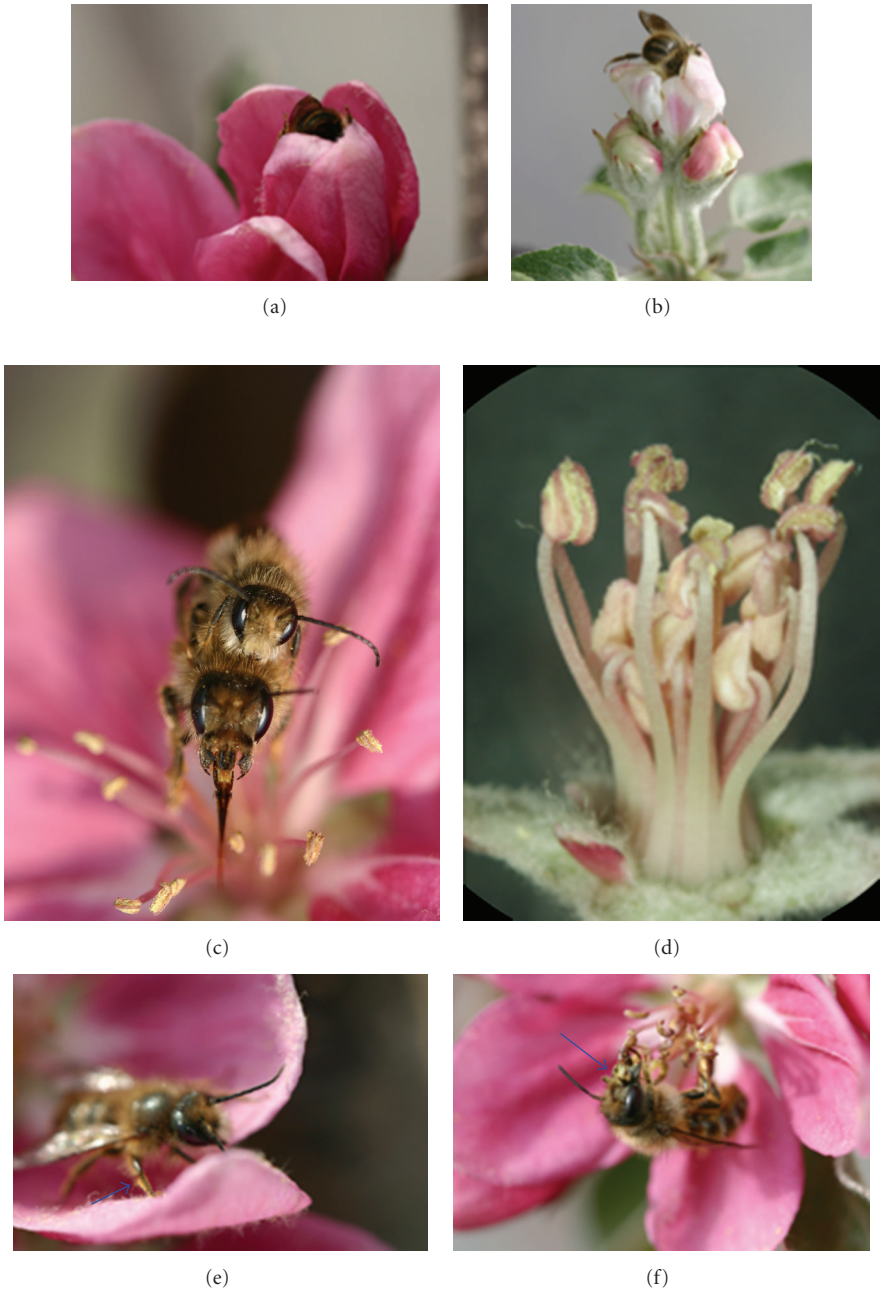

Figure 3: *Osmia cornifrons* visiting balloon stage flowers. (a) *O. cornifrons* visiting "Maypole", (b) *O. cornifrons* visiting "Fuji", (c) *O. cornifrons* male mounted on female drinking "Maypole" nectar, (d) Dehiscere anthers within a balloon-stage "Maypole" (Petals removed.), and (e) and (f) Pollen grains adhered to male's leg (e) and female's mouth (f).

pollinizers should have been planted not more than 10 m from commercial cultivars in an apple orchard.

3.2. Foraging Indicators of Osmia cornifrons. Previously, we found that *O. cornifrons* preferred pollen from "Delicious" flowers in spite of their considerable distance from the nesting side. As shown in Figure 2, the stamens of "Starking Delicious" (Sport of "Delicious") flowers were arranged in an upright position compared to those of other pollinizers and cultivars [7]. The upright stamens of "Delicious" flowers might be suitable for pollen collecting bees, such as *O. cornifrons*, since that made it easier to attach an abundance of pollen to their abdomen at one time. Since collecting pollen is the most important task for *O. cornifrons* females seeking to make pollen loaves for their larvae, it is also recognized that anthers, in addition to petals, also must be seen as important indicators for their visiting flowers. As shown in Table 1, fruit set levels (%) of "Fuji" (93.8%) were reduced by the removal of flower petals or anthers (56.7% and 77.7%, resp., in Table 1) and extremely reduced by the removal of

both anthers and petals (32.5% in Table 1). We observed *O. cornifrons* visiting flowers lacking either petals or anthers, but rarely approaching flowers lacking both petals and anthers. Bees could navigated by U.V. light, and in the case of apple flowers, their anthers, pollen, and petals showed high levels of U.V. absorption (data not shown). As a result, *O. cornifrons* seemed to visit petals or stamens of flowers such as visible guidance.

3.3. Visiting Balloon Stage Flowers. We kept *O. cornifrons* at 4°C for a few weeks to adapt their foraging behavior to apple flowers. *Osmia cornifrons* males and females began to emerge at the apple orchard of the Nagano Fruit Tree Experiment Station ca. two weeks and one week earlier, respectively, than the flowering time of "Fuji". They visited *Veronica persica* Poir., *Vicia angustifolia* L., *Taraxacum officinale* Weber, *Prunus avium* L., and *Pyrus commusis* L. in an effort to survive until apple flowering and then turned to visiting apple flowers once they bloomed.

We found that males and females visited "Maypole" and "Fuji" flowers at the balloon stage (Figures 3(a) and 3(b)). Some individuals visited "Maypole" flowers for 6 to 8 seconds. Females were mainly concerned with collecting pollen and drinking nectar, while the main purpose for males was randomly to search for females and drink nectar (Figure 3(c)). We investigated the conditions of anthers at the balloon stage of flowers. As shown in Table 2 and Figure 3(d), 46.7% of the stamens of balloon stage "Maypole" flowers had at least one dehiscent anther, and 8.2% dehisced anthers at the balloon stage, suggesting that *O. cornifrons* would accumulate pollen on their bodies while visiting balloon stage flowers for 6 to 8 seconds. We found that both males and females picked up pollen grains around their mouth and legs (Figures 3(e) and 3(f)).

3.4. Male Contributions to Pollination. As already mentioned, we found that some males picked up pollen around their mouth and/or legs (Figure 3(e), unpublished results). Although as has been suggested, such accumulations made no contribution to pollination, since their foraging trips were mainly concerned with finding nectar and/or females, we considered that the pollen attached to a male's body could incidentally result in the pollination and fertilization of apples. We investigated whether or not the pollination and fertilization of "Alps-Otome" only occurred by the intervention of *O. cornifrons* males. The experiment was carried out using three "Maypole" and three "Alps-Otome" covered by nets. As shown in Table 3, all "Alps-Otome" trees were fertilized by "Maypole" pollen carried by *O. cornifrons* males (20.7%–27.6% fruit set in Table 3), suggesting that males could serve as apple pollinators.

Acknowledgment

This research was supported by the Research Project for Utilizing Advanced Technologies in Agriculture, Forestry and Fisheries, Japan.

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