Wild Bee Visitors of Squash (*Cucurbita Maxima* D.) and Tomato (*Solanum Lycopersicum* L.) Flowers In Two Types of Satoyama Habitats

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Abstract. The purpose of this study is to compare the diversity and abundance of flower-visiting bees in squash (*Cucurbita maxima*) and tomato (*Solanum lycopersicum*) flowers between three small valleys surrounded by forest and three open grasslands outside forest. Both type habitats intermingled as mosaic of satoyama, which is a socio-ecological production landscape managed by agriculture and forestry located in rural Japan. Both squash and tomato were planted separately in different pots, where each set were placed at particular site. Number of flowers and fruits were recorded and the flowers-visiting bees were collected using sweep net from June to August 2013. A total 96 individuals from 9 species (2 families) of bees were collected from squash and tomato. The number of bees visited squash (91 individuals and 9 species) outnumbered those visited tomato (5 individuals and 2 species). Tomato flowers in the open grasslands were only visited by *Ceratina*. Meanwhile, *Bombus diversus* and *Ceratina japonica* were the most abundant bees visited squash in small valleys and in open grasslands, respectively. The successful number of squash fruits was significantly higher presented in small valleys than in open grasslands, although no significant differences were found in number of flowers between both habitats.

Keywords: bee, diversity, tomato, squash, satoyama

1. Introduction

Cultivated fruit plants, which suppose to have good fruit crop qualities, somehow highly depend on pollination from animals especially insects [1-4]. Squash and tomatoes were belonged to cultivated fruit plants that were visited by various bee species [2-5]. Squash is a monoecious plant where stamen and pistil were separately in different flower [4,5]. Therefore, squash pollination by wild bees surely need in enormous number in order to ensure the fertilization and the successful of set fruits. Meanwhile, pollination of tomato would be more effective with the anther vibration by bumble bees to release the pollen [3,6].

Some manage and wild bees were known to pollinate squash and tomatoes flowers. There have been several reports of honeybees as effective pollinator on squash and tomatoes flowers and increases in yield [2,3,5,7]. Native bees were noticed also important value as well as honeybees or other managed bees. *Peponapis* and *Xenoglossa* are two genera of bees that commonly associated
with squash flowers [8]. Bumblebees (*Bombus vosnesenskii*) are the primary pollinator in greenhouse tomatoes production even though tomato is self-compatible [7], while *Apis* and *Trigona* could be the alternative pollinator of tomato in tropical region [3].

The role of native bees on wild and crop plants pollination is important, which is depending to land covers and habitat condition [7,8]. Satoyama landscapes consisted of different habitat types provide native bees habitats [9]. Priawandiputra [10] showed that the different habitats of satoyama (small valleys and open grasslands) clearly form the different wild bee species composition, depend on wild flowering plant species composition. Those habitats might also play important roles not only in sustaining wild pollination services but also in crops pollination. In this present study, the effect of the different habitats of satoyama on wild bees contribution to squash and tomatoes pollination was examined. We also discussed what kind of bees that giving role to the successful of fruit and how different place could affect those results.

2. Study sites and methods

2.1. Plant preparation
Firstly, seedling the cultivars of squash and tomatoes were planted in one site with full sun and protection. The sprouts of squash and tomatoes were directly filled into the containers from May 2013. Twelve containers were prepared where 6 containers for squash and another 6 containers for tomatoes. Each container has a standard type plastic pot, with the same long, wide, and high. The container was filled with the soil mix and put 2 and 3 sprouts of squash and tomatoes, respectively. After the plants were growth, one set pots (1 pot tomatoes and 1 pot squash) were moved to each study site.

2.2. Study sites
The study sites were located near Kakuma Campus Kanazawa University, Kanazawa, Japan (figure 1). The sites were in 3 small valley surrounded forests and 3 open grassland outside of forest. The three small valleys were selected such as Kitadan valley, Minamidan valley, and Zontan valley. Kitadan and Zontan valleys are used as small terraced paddies and small pond, which contribute as environment education sites for students, while Minamidan valley is abandoned valley with dense grasslands and some ponds. Grasslands were selected in Shizenken, Kakuma no Sato, and Kakuma Guchi where there are wide-open lands without any kind of canopy from trees.

![Figure 1. Pairs of squash and tomato plants in each study site](image)
2.3. **Sampling methods**

Bees that visited tomatoes and squash flowers were collected during 8:00 to 13:00 from June-August 2013, using sweep net. In each site, approximately 20 minutes were allocated to captured visited bees during same day. On the census day, male and female flowers of squash and flower of tomatoes were counted. The successful number of fruits was also counted. Total 27 sampling times (2 times in June, 16 in July, 8 in August and 1 in September) were conducted in this study. Environment data were also measured such as temperatures, moistures, and sunlight. Ranges of temperature, moistures and sunlight were 26-40°C, 30-74% and 105-1410 lux, respectively.

2.4. **Identification and data analysis**

Each bee specimen was placed individually in tube. The specimens were identified to species level using Michener [11] and Yamane et al. [12]. Specimens were kept in Laboratory of Ecology, Faculty of Science, Kanazawa University.

Wilcoxon test and Kruskal-Wallis test were used to examine the differences in 1) number of flowers and fruits of squash and tomato and 2) abundance and species richness of flower-visiting bee between open grasslands and small valleys. Degree of the similarity of bee assemblages between open grasslands and small valleys was analysed using non-metric multidimensional scaling (NMDS) based on Bray-Curtis. The correlation between number of bee individuals in each bee species and success of fruit set was calculated using Person correlation. These analyses were performed using PAST software version 1.95 [13]. Meanwhile, the linkages between bees and flowers of squash and tomato was constructed using R, version 3.4, bipartite package [14,15].

### 3. Results

#### 3.1 Number of flowers and fruit-set: squash and tomatoes

Number of flower and fruits of squash and tomatoes were compared between open grasslands and small valleys (table 1). No significant differences were found in flowers of squash and tomato and fruits of tomato between open grasslands and small valleys (Wilcoxon test, P>0.05). In other results, the successful number of squash fruits was significantly higher in small valleys than in open grasslands (Wilcoxon test, P<0.05).

|        | Open grasslands | Small valleys |
|--------|-----------------|---------------|
| **Flowers** |                 |               |
| Squash  | 3.4 ± 1.1 flowers/day | 2.2 ± 0.4 flowers/day |
| Tomatoes| 4.7 ± 1.7        | 5.5 ± 0.6     |
| **Fruits** |                 |               |
| Squash  | 1 ± 1 fruits     | 2.6 ± 0.5 fruits |
| Tomatoes| 8.3 ± 7          | 9 ± 2.6       |

#### 3.2 Abundance and species richness of bees

A total 96 individuals from 9 species (2 families) of bees were visited squash and tomato flowers in both habitats (table 2). Bees preferred squash flowers to tomato flowers whether in open grassland or small valleys. Most bees were barely visited tomato flowers where in total only 5 individuals were directly observed to visit tomato flowers. Number of individuals and species of visited bees were higher in open grasslands (64 individuals and 8 species) than in small valleys (32 individuals and 7 species). However, we did not find significant difference in abundance and species richness of bees, which visited squash, tomato, and both plants between open grasslands and small valleys (table 3, Wilcoxon test, P> 0.05).

In our studies of squash, 15.3% of 91-recorded visits were made by Bombus diversus in small valleys and 26.3% by Ceratina japonica in open grasslands (table 2). B. diversus and C. japonica
were the most abundant bees visiting squash in 3 valleys and in 3 open grasslands, respectively (table 2). However, only *B. diversus* was significantly higher for visiting squash flowers in small valleys than open grasslands (Kruskal-Wallis test, P<0.05). Meanwhile, tomato was only visited by two species of *Ceratina* in open grasslands, although no significant difference was found.

### 3.3 Species composition of bees

There were separately different of bee species composition that visited squash and tomato between small valleys and open grasslands (figure 2). Six bee species were found overlap in open grassland and small valleys (table 2). *Amegilla florea* and *Halictus aerarius* were collected only in open grasslands, while *B. diversus* was only caught in small valleys. Sex flowers of squash affected the composition of bees where most bees preferred male flowers to female flowers (figure 3). Male flowers in open grasslands were visited by 8 bee species, while seven bee species visited male flowers in small valleys. *C. japonica* and *B. diversus* were the most visited bee species to the male flowers in open grasslands and small valleys, respectively. *C. flavipes* and *C. japonica* did not only visit male flowers of squash but also flowers of female squash and tomato in open grasslands. Meanwhile, only *Lassioglosum occidens* visited male and female flowers of squash in small valleys.

**Table 2.** The list of bees and their numbers collected from squash and tomatoes flowers in open grasslands and small valleys.

| Families | Species                          | Open grasslands | Small valleys | Grand Total |
|----------|----------------------------------|-----------------|---------------|-------------|
|          |                                  | Squash | Tomato | Total | Squash | Tomato | Total |          |
| Apidae   | *Amegilla florea* (Smith)        | 1      | 0      | 1     | 0      | 0      | 0     | 1         |
|          | *Bombus diversus* (Smith)        | 0      | 0      | 0     | 14     | 0      | 14    | 14        |
|          | *Ceratina esakii* (Yasumatsu et Hirashima) | 5      | 0      | 5     | 2      | 0      | 2     | 7         |
|          | *C. flaviceps* (Smith)           | 17     | 2      | 19    | 2      | 0      | 2     | 21        |
|          | *C. iwatai* (Yasumatsu)          | 4      | 0      | 4     | 4      | 0      | 4     | 8         |
|          | *C. japonica* (Cockerell)        | 24     | 3      | 27    | 1      | 0      | 1     | 28        |
| Halictidae| *Halictus aerarius* (Smith)      | 3      | 0      | 3     | 0      | 0      | 0     | 3         |
|          | *Lassioglosum japonicum* (Dalla torre) | 4      | 0      | 4     | 7      | 0      | 7     | 11        |
|          | *L. occidentis* (Smith)          | 1      | 0      | 1     | 2      | 0      | 2     | 3         |
| Total    |                                  | 59     | 5      | 64    | 32     | 0      | 32    | 96        |

**Table 3.** Comparison of diversity parameters for flowering plants and bee assemblages between open grasslands and small valleys (parentheses)

|                        | Open grasslands | Small valleys |
|------------------------|-----------------|---------------|
| Bees visit squash      | Number of individuals | 59 (19.6 ± 17.6) | 32 (10.6 ± 5.8) |
|                        | Number of species     | 8 (5 ± 2)       | 7 (5 ± 2)       |
| Bees visit tomato      | Number of individuals | 5 (1.6 ± 2.08)  | 0 (0)           |
|                        | Number of species     | 2 (1 ± 1)       | 0 (0)           |
| Bees visit squash and tomato | Number of individuals | 64 (21.3 ± 19.7) | 32 (10.6 ± 5.8) |
|                        | Number of species     | 8 (5 ± 2)       | 7 (4 ± 1)       |

**3.4 Correlation between bee abundance and fruits-set**

We analyse the correlation between visitation of bees and number of fruits with two assumptions: 1) bees that visit male flowers of squash also visit female flowers and 2) bees that visit squash flower also visit tomatoes. Success of squash fruits set only depended on visitation of *B. diversus* (figure 4,
R = 0.87; P < 0.04). Meanwhile, success of tomato fruits were affected by the visitation from total abundance of all bee species (figure 4, R = 0.88; P < 0.03).

4. Discussion

4.1. The land cover will change the visitor of squash
The cultivated plants that were placed in different cover land habitats (open grasslands or small valleys) could be visited by different wild bee species. In this case, squash were more visited by bees either in open grassland or in small valleys. Squash flowers attract more bee species and individuals than tomatoes due to high on nectar, pollen and attractive flower appearances. Squash flowers have a nectar sugar concentration of about 14-16%, so honeybees and bumblebees, which visit flowers with a sugar concentration range of 10-74% [16], could be visit squash flowers. In our study, honeybees were not collected in squash and tomatoes plants. The dominant bee species, which visited squash in small valleys, was B. diversus while in open grasslands, it did not appeared. In spite of small patch of flower resources, it could attract bumblebees because the location surrounded by forest. B. diversus was highly collected in satoyama forests [17]. The result probably would be different if the number of squash flower were increased where “the best resources patch” would be a resources track for bumblebees.

4.2. Effect of all bees and dominant bee species on fruits sets
Squash flowers that were visited by B. diversus showed the successful in fruit set production where at least 1 fruit was growth. It means flower that were placed in small valley indirectly influence to the successful of squash fruit set. B. diversus with wide range flight move from one forest through other forests [17]. Not only honey bee, wild and managed bumble bees also has been utilized for crop pollination [1,4] Another dominant species was also C. japonica that utilized squash flower as food resources even though no effect of these visitations on successful fruit production. Although C. japonica does not give significant effect for crop pollination, it has important role in pollination service for other wild flowers. Priawandiputra [10] and Priawandiputra et al. [18] found that C. japonica highly visited wild flowers in abandoned satoyama habitats.

![Figure 2. NMDS ordination by Bray-Curtis index showed the variation in bee species composition between open grasslands and small valleys. Blue line represents open grasslands and green line represents small valleys. The stress for two-dimensional solution was 0.06.](image-url)
Figure 3. The comparison in linkages of bees and flowers (squash and tomato) between a) open grasslands and b) small valleys. Red, green and blue colours represented the subfamily Apinae, Xylocopinae and Halictinae, respectively. Meanwhile, orange and yellow colours described the flower of squash and tomato, respectively. Note: Ameflo-Amegilla florea, Ceresa-Ceratina esakii, Cerflav-Ceratina flaviceps, Ceriwa-Ceratina iwatai, Cerjap-Ceratina japonica, Halaer-Halictus aerarius, Lasjap-Lassioglossum japonicum, Lasoc-Lassioglossum occidens, Bomdiv-Bombus diversus.

Figure 4. The correlation between abundance of bees (all species, C. japonica and B. diversus) and number of fruits (squash and tomato).

4.3. Satoyama can be a suitable landscape for cultivated plant visitor
Some research showed that satoyama landscape was important habitats for bee assemblages [10, 17-21]. Satoyama landscapes give positive effect on bee communities where bee could utilize outside and inside forests in satoyama area (17,18). Therefore, cultivated plants within satoyama landscape would be high on fruits production.

The number of species that visited squash and tomatoes in small valleys and open grasslands was compared with the study from Priawandiputra [10] (figure 5). Priawandiputra [10] observed species richness of bee that visited wild flowering plants in same area. It described that bee species that
visited wild flowering plants were also visited cultivated plants in small valleys and open grasslands. Most number of species that visited squash and tomatoes shared between small valleys and open grasslands (6 species). Few number of species that visited squash and tomatoes only captured in small valleys (1 species) and open grassland (2 species).

Priawandiputra [10] also found that *B. diversus* dominantly visited wild flowering plants in June, October and November in small valleys and few numbers in June and November in open grassland (figure 6). In present data, *B. diversus* visited squash flower in July. It suggested that when wild flowers decrease in summer, squash flowers could be alternative flower resources for *B. diversus*.

*Ceratina* has many options of resources such as wild flowers or cultivated flowers in summer season. *C. flavipes* and *C. japonica* were highly visited in July and decrease in August in open grasslands but only in July in small valleys (figure 6). However it depended on number of flowers. *C. flavipes* and *C. japonica* suddenly increase from April to May and August to October but decreased from May to July found high in small valleys and open grasslands.
5. Conclusion
Bee species composition, which visited squash flowers were different between open grasslands and small valleys, while tomato flowers was barely visited by bees. *B. diversus* that highly visited squash in small valleys could be a factor that affects the successful fruit production of squash. All bees that visited two cultivated plants were also found visiting wild flowering plants in both habitats.

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