



Comparison of Pollination Activity among *Apis mellifera* L., *Apis cerana* Fab., and *Bombus terrestris* L. in Apple Blossom

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Abstract

Apple trees rely primarily on insects as pollinators, and in Korea, *Apis mellifera* and *Bombus terrestris* are commonly used. However, due to the recent decline in *A. mellifera* populations, research into alternative pollinators is being conducted. In China, *Osmia excavata* has been suggested as a substitute, but its limited availability in Korea makes it difficult for farmers to use. In other Asian countries, *Apis cerana* is used as a pollinator due to its resistance to disease and cold weather. This study aimed to compare the pollination efficiency of *A. cerana* with *A. mellifera* and *B. terrestris*. The experiment was conducted from April 20 to May 7, 2023, in two enclosed net houses located in Chungju, South Korea. Four camcorders were installed in each net house to observe bee activity. The results indicated no significant difference in the flower visit frequency between *A. mellifera* and *A. cerana*, but *B. terrestris* had the fewest visits. *A. cerana* spent the shortest time per flower, while *B. terrestris* stayed the longest. Although there was no significant difference in total activity time among the species, *A. cerana* began its activities earlier than *A. mellifera*. When examining the number of bees entering and exiting the hives, *A. cerana* exhibited the most active behavior, while *B. terrestris* was the least active. These results suggest that *A. cerana* has potential as a pollinator, though economic challenges remain. It is recommended that *A. cerana* be used as a supplementary pollinator. As this study was conducted in a limited location and closed environment, further research across different regions is needed to validate these findings.

Keywords

Honeybee, Bumble bee, Apple blossom, Foraging behavior

INTRODUCTION

Apples are an important fruit crop in South Korea, with a cultivation area of 34,603 hectares (Statistics Korea, 2023). Apple trees rely primarily on insects for pollination (Ollerton *et al.*, 2015; Ouyang *et al.*, 2019), and farmers typically use the honeybee, *Apis mellifera* Linnaeus (1758), for this purpose (Yoon *et al.*, 2021). However, in recent years, there has been a global decline in pollinator populations, particularly *A. mellifera* (Lee *et al.*, 2023). Although various studies have been conducted to identify the causes of this decline and to find ways to address it, the resolution of this issue remains uncertain (Lee *et al.*, 2023; Oh *et al.*, 2024; Son *et al.*, 2024).

In China, research on alternative pollinators for apple trees has been conducted, and the mason bee *Osmia excavata* Alfken (1903) was evaluated as the most efficient species for apple cultivation, being less affected by weather conditions (Lyu *et al.*, 2023). However, due to insufficient supply caused by the difficulty of outdoor propagation, *O. excavata* is not widely used in Korea (NIAS, 2016; Yoon *et al.*, 2021). Currently, *A. mellifera* and the bumble bee *Bombus terrestris* (Linnaeus, 1758) are commonly used for apple cultivation in Korea (Yoon *et al.*, 2021), and considerable research has been conducted on their use (Lee *et al.*, 2010, 2013; Kwack *et al.*, 2012; Lee *et al.*, 2016; Park *et al.*, 2016).

In other Asian countries, there is also growing interest

in the Eastern honeybee, *Apis cerana* Fabricius (1793). *A. cerana* is a species widely distributed across East Asia and Southeast Asia, and it is known to be more tolerant to cold compared to *A. mellifera* (Qin *et al.*, 2017). It is also noted for its agility and strong resistance to pests and diseases, particularly its high resistance to the varroa mite (Peng *et al.*, 1987; Grindrod and Martin, 2023). As a result, comparative research on *A. mellifera* and *A. cerana*, along with studies examining *A. cerana*'s pollination role is more common internationally (Suryanarayana *et al.*, 1992; Rana *et al.*, 1997; Tatsuno and Osawa, 2016; Kumar *et al.*, 2020). Although there have been some studies in Korea on the pollination of *A. cerana* (Chang *et al.*, 2000), they are limited compared to the research on *A. mellifera* and *B. terrestris*. Therefore, this study aims to compare the pollination activities of *A. mellifera*, *B. terrestris*, and *A. cerana* on apple trees and assess the potential of *A. cerana* as a pollinator.

MATERIALS AND METHODS

1. Study area and study species

The study was conducted from April 20 to May 7, 2023, in two net houses (each 500 m²) located in Chungju, North Chungcheong Province. The 'Fuji' apple variety was cultivated in both net houses. To compare the three species of pollinating insects, two experiments were conducted simultaneously, using two species at a time and comparing each species against a common one. From April 20 to April 28, *A. mellifera* and *A. cerana* were introduced, while from April 28 to May 7, *A. mellifera* and *B. terrestris* were introduced. A total of 7 frames (approximately 15,000 workers) of *A. mellifera* were used from a bee colony, while 5 frames (approximately 12,000 workers) of *A. cerana* were used. For *B. terrestris*, a commercially purchased colony (approximately 80 workers) was used (Sallimbee, Sallim Agricultural Corporation, Bonghwang-ri, Miryang, Gyeongnam).

2. Entry activity assessment and foraging behavior observation

The experiment began on April 20, 2023, coinciding with the start of apple blossom, and concluded on May 7, 2023, in Chungju. Cameras (AT-Q61CR, AUSEK LIMITED, China) were installed in front of each hive to

monitor the daily activity of the bees. Additionally, a temperature and humidity data logger (S500-EX, HUATO, Guangdong, China) was used to record real-time temperature and humidity data during the trial period. The collected data were utilized to understand the weather conditions when the bees visited flowers and their daily activity patterns, including the temperature at which the bees first started their activities, the temperature at which they returned last, as well as daily working hours and entry frequency by time. The starting and ending temperatures were defined as the temperatures at which the first and last bees began and ended their activities, respectively. Observations of *A. mellifera* and *A. cerana* were conducted from April 20 to 24, and from April 28 to May 7, *A. cerana* was replaced with *B. terrestris* for observations of *A. mellifera* and *B. terrestris*.

Between 10 AM and 5 PM, the number of individuals visiting flowers was measured every hour for 10 minutes. To facilitate this observation, three cameras were installed in each net house to capture the trees. Additionally, the time spent on each flower was measured by recording the duration when a bee landed on a flower for more than one second before taking off. During this observation, over 100 individuals from each species were monitored to compare the time spent on flowers for each species. *A. mellifera* and *A. cerana* were observed on the same day, as were *A. mellifera* and *Bombus terrestris*. Cameras were installed at the hive entrances to observe the entry and exit activities of the three species. Between 6 AM and 7 PM, the number of entries was measured every hour for 10 minutes to compare the average number of entries per minute per day. This data was divided by date and then by species. Since the aim was to observe the number of active individuals, measurements were conducted in terms of the number of individuals rather than ratios.

Observations were primarily conducted on days without rain due to issues such as camera obstruction and reduced visibility of bee behavior during rainy conditions.

To assess pollination efficiency and fruit quality, we compared the seed amount and weight of apples from two distinct enclosures. While a comprehensive comparison among all three species was not feasible, we aimed to examine the differences between *A. mellifera* and the other two species by comparing these two enclosures. Each enclosure was labeled with a letter, and trees within

each enclosure were assigned numbers to facilitate apple collection. Subsequently, the seed amount and weight of apples from each enclosure were analyzed to compare the effects. Additionally, we examined any differences based on proximity to the hive, categorizing apples based on whether they were collected from trees closer to or farther from the hive.

3. Statistical analysis

The number of flower visits per minute (flowers/min) is calculated by dividing the number of honeybees observed over a 10-minute period by 10. When comparing the number of flower visits and time spent on flowers, *A. mellifera* was used as the reference species. The duration of time spent by *A. mellifera* on different days was compared using Student's t-test or Mann-Whitney test based on normality tests, and if no significant difference was found, *A. cerana* and *B. terrestris* were compared accordingly. The comparison of apple seed counts and weights between enclosures was also conducted using either Student's t-test or the Mann-Whitney test, depending on the results of the normality test ($P < 0.05$). The analysis of the first and last activity times of bees for each species was conducted by species rather than by date. The average number of entries per minute per day was calculated by averaging the number of entries for each species divided by 10. Statistical analyses were performed using SigmaPlot 12.5 software (Crafiti LLC, Palo Alto, CA, USA). The Shapiro-Wilk test was used to assess normality. Depending on the results of the normality tests, either Student's t-test or the non-parametric Mann-Whitney test was used for comparisons of flower visits, time spent on flowers, and average entries per minute per day. One-way analysis of variance (ANOVA) was used for comparing activity times, followed by Tukey's HSD test for post-hoc analysis ($P < 0.05$). If the data did not pass the normality test, the non-parametric Kruskal-Wallis test was used.

RESULTS

1. Comparison of foraging activities between *A. mellifera*, *A. cerana*, and *B. terrestris*

The foraging activity patterns of *A. mellifera*, *A. cerana*, and *B. terrestris* differed (Figs. 1 and 2). No significant

difference was found in the number of flower visits per minute between *A. mellifera* and *A. cerana* (6.56 and 5.83 flowers/min, respectively; $t = 0.746$, $P = 0.322$). In contrast, *B. terrestris* visited flowers less frequently than *A. mellifera* (1.64 and 3.96 flowers/min, respectively; $t = 5.892$, $P < 0.05$). Since a difference was found in the flower visit rates of *A. mellifera*, comparisons of the visit rates of *A. cerana* and *B. terrestris* were not conducted (6.56 and 3.96 flowers/min, respectively; $t = 4.538$, $P <$

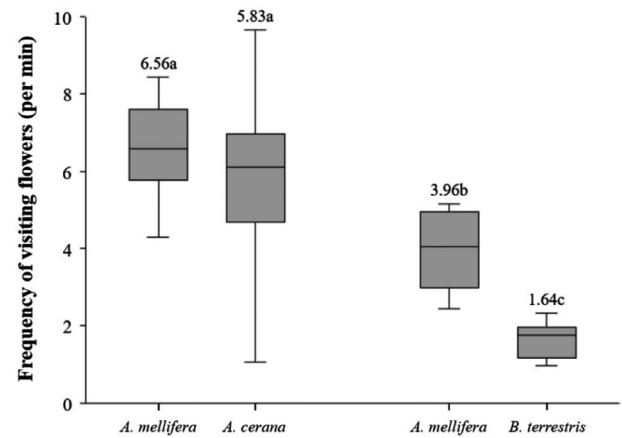


Fig. 1. Frequency of visiting flowers (per min) for *A. mellifera*, *A. cerana*, and *B. terrestris*. Observations of *A. mellifera* and *A. cerana* were conducted from April 21–23, while observations of *A. mellifera* and *B. terrestris* were carried out from May 2–4. The numbers above the box plot represent the mean values, and the letters indicate significant differences (based on the results of either Student's t-test or Mann-Whitney test according to the normality test, $P < 0.05$).

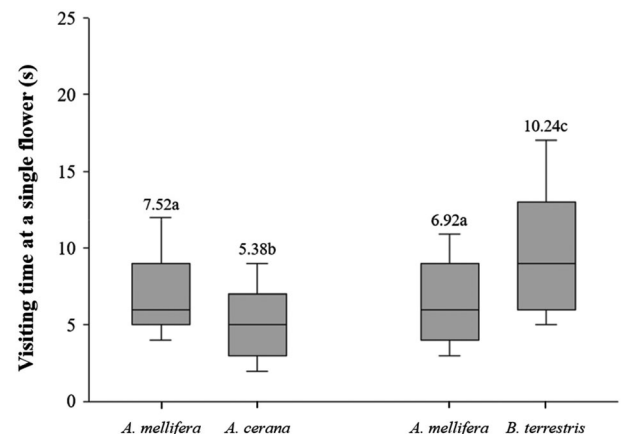


Fig. 2. Visiting time at a single flower(s) for *A. mellifera*, *A. cerana*, and *B. terrestris*. Observations of *A. mellifera* and *A. cerana* were conducted from April 21–23, while observations of *A. mellifera* and *B. terrestris* were carried out from May 2–4. The numbers above the box plot represent the mean values, and the letters indicate significant differences (based on the results of either Student's t-test or Mann-Whitney test according to the normality test, $P < 0.05$).

0.001; Fig. 1). When comparing the time spent on flowers by *A. mellifera*, no significant difference was detected between the two groups (7.52 and 6.92 s, respectively; $U=4748.5$, $P=0.537$). Based on this, a comparison between *A. cerana* and *B. terrestris* was made, revealing that *B. terrestris* spent significantly more time on flowers than *A. cerana* (10.24 and 5.38 s, respectively; $U=1695$, $P<0.001$). Comparisons among *A. mellifera*, *A. cerana*, and *B. terrestris* indicated that *A. mellifera* spent more time on flowers than *A. cerana* (7.52 and 5.38 s, respectively; $U=3073$, $P<0.001$), but spent less time than *B. terrestris* (6.92 and 10.24 s, respectively; $U=2984$, $P<0.001$; Fig. 2). The temperatures during the observations from April 21–23 and May 2–4 showed no significant difference ($P=0.454$).

2. Comparison of daily activity levels of *A. mellifera*, *A. cerana*, and *B. terrestris*

The emergence time of the first bee and the entrance time of the last bee were measured for *A. mellifera*, *A. cerana*, and *B. terrestris* to compare the total activity duration of each species. *A. mellifera* and *A. cerana* showed a significant difference in the time of the first bee emergence (6.60 and 5.63 h, respectively, $F=4.314$; $df=2$; $P<0.05$), while *B. terrestris* did not differ from either species (6.03 h, $F=4.314$; $df=2$; $P>0.05$). The

entrance times of the last bee showed no significant differences among *A. mellifera*, *A. cerana*, and *B. terrestris* (18.78, 18.97, and 19.03 h, respectively, $H=0.368$; $P>0.05$). The total daily activity duration was approximately 12 hours for *A. mellifera*, 13 hours for *A. cerana*, and 13 hours for *B. terrestris*, with no significant differences among them (12.20, 13.34, and 13.00 h, respectively, $F=2.677$; $df=2$; $P=0.122$).

The comparison of the average number of individuals entering and exiting per minute revealed that there were no significant differences in the entrance rates of *A. mellifera* on different dates (4.41 and 4.22, respectively, $U=94.50$; $P>0.05$; 4.19 and 4.42, respectively, $U=87.50$; $P>0.05$). Based on this, we compared the entrance rates of *A. cerana* and *B. terrestris*. For both exiting and entering individuals, *A. cerana* showed significantly higher values than *B. terrestris* (10.16 and 0.28, respectively, $U=8.50$; $P\leq 0.001$; 11.00 and 0.30, respectively, $U=14.00$; $P\leq 0.001$). *A. cerana* also exhibited significant differences compared to *A. mellifera* observed on the same day (4.41 and 10.16, respectively, $t=-2.28$; $P<0.05$; 4.19 and 11.00, respectively, $U=50.0$; $P<0.05$). Additionally, *B. terrestris* showed significant differences compared to *A. mellifera* observed during the same period (4.22 and 0.28, respectively, $U=39.0$; $P<0.05$; 4.42 and 0.30, respectively, $U=29.5$; $P<0.05$).

Table 1. Comparison of activity duration among *A. mellifera*, *A. cerana*, and *B. terrestris*

Bee species	Earliest hive exist time (h)	Latest hive return time (h)	Daily working hours (h)
<i>Apis mellifera</i>	6.60 ± 0.17a	18.78 ± 0.23a	12.20 ± 1.48a
<i>Apis cerana</i>	5.63 ± 0.05b	18.97 ± 0.04a	13.34 ± 0.04a
<i>Bombus terrestris</i>	6.03 ± 0.34ab	19.03 ± 0.06a	13.00 ± 0.39a

The values are expressed as mean ± standard error. Different letters in the same column indicate significant differences (one-way analysis of variance followed by Tukey's HSD test, $P<0.05$).

Table 2. Comparison of the number of individuals entering and exiting per minute for *A. mellifera*, *A. cerana*, and *B. terrestris*

Day	Bee species	Numbers of bees exiting the hive (per min)	Numbers of bees entering the hive (per min)
April 21–23	<i>Apis mellifera</i>	4.41 ± 1.06a	4.19 ± 1.02a
	<i>Apis cerana</i>	10.16 ± 2.28b	11.00 ± 2.59b
May 2–4	<i>Apis mellifera</i>	4.22 ± 0.95a	4.42 ± 0.95a
	<i>Bombus terrestris</i>	0.28 ± 0.04c	0.30 ± 0.04c

Values are presented as mean ± standard error. Different letters following the values in the same column indicate significant differences (based on the results of Student's t-test or Mann-Whitney test according to normality testing, $P<0.05$).

Table 3. Comparison of seed amount and weight between *A. mellifera* enclosure and *A. cerana* and *B. terrestris* enclosure

Bee species	Seed amount	Seed weight (mg)
<i>Apis mellifera</i>	4.667 ± 0.432a	0.371 ± 0.035a
<i>Apis cerana</i> and <i>Bombus terrestris</i>	1.900 ± 0.268b	0.144 ± 0.019b

Values are presented as mean ± standard error. Different letters following the values in the same column indicate significant differences (based on the results of Student's t-test or Mann-Whitney test according to normality testing, $P < 0.05$).

3. Comparison of apple seed amount and weight

The seed amount and weight of apples from enclosures using only *A. mellifera* were compared with those from enclosures using *A. cerana* and *B. terrestris*. Apples in the *A. mellifera* enclosure contained approximately 2.4 times more seeds than those in the *A. cerana* and *B. terrestris* enclosures (4.667 and 1.9, respectively, $U = 118.5$; $P \leq 0.001$). The weight of the apples was also approximately 2.5 times heavier in the *A. mellifera* enclosure (0.371 mg and 0.144 mg, respectively, $U = 109.5$; $P \leq 0.001$). Additionally, when comparing the seed amount and weight of apples based on their distance from the hive (near vs. far trees) in each enclosure, no significant differences were observed in either enclosure.

DISCUSSION

This study was conducted to compare the pollination activities of *A. mellifera*, *B. terrestris*, and *A. cerana* based on the frequency of flower visits, duration of stay on flowers, total activity time, and activity levels. The experimental results indicated that there was no significant difference in the frequency of flower visits between *A. mellifera* and *A. cerana*; however, *A. cerana* exhibited higher activity levels than *A. mellifera* and also demonstrated an earlier onset of activity duration. Conversely, *B. terrestris* recorded the lowest values in both frequency of flower visits and activity levels, yet it had the longest duration of stay on flowers, which was approximately twice as long as that of *A. cerana*, which had the shortest duration. No significant difference was observed in total activity time among the three species. In terms of fruit quality, a clear difference was observed

between the enclosures with *A. cerana* and *B. terrestris* and the enclosure with *A. mellifera*. The *A. mellifera* enclosure showed more than double the number of seeds and seed weight, indicating higher pollination efficiency compared to the other two species. Since *A. cerana* and *B. terrestris* were observed in the same enclosure, a comparison between these two species was not possible. These findings suggest that *A. cerana* may serve as a complementary alternative pollinator to *A. mellifera*.

In this study, both *A. cerana* and *A. mellifera* were found to be highly effective pollinators for apple trees. These results are consistent with findings from a study conducted in India, which also evaluated both species as effective pollinators for apple trees. However, some differences exist between the two studies. While our research found no significant difference in total activity time between *A. cerana* and *A. mellifera*, the Indian study reported that *A. cerana* was active for a longer duration. The Indian study was conducted at three different locations and indicated that variations in activity between *A. cerana* and *A. mellifera* were influenced by altitude (Ahmad *et al.*, 2017). Additionally, research conducted in Nepal demonstrated that when comparing pollination activities in open and enclosed environments, both species exhibited greater activity in open environments (Devkota and Thapa, 2005). This suggests that the pollination capabilities of *A. cerana* and *A. mellifera* may vary depending on their surrounding environment and geographical region.

A. cerana demonstrated comparable activity levels as a pollinator to *A. mellifera*. Additionally, its resilience to cold temperatures and high resistance to diseases represent significant practical advantages for agricultural applications (Tan *et al.*, 2012; Oh *et al.*, 2016). However, *A. cerana* has the disadvantage of smaller colony sizes compared to *A. mellifera*, and from an economic perspective, it tends to be more expensive. Therefore, it is proposed that *A. cerana* could be utilized as a supplementary pollinator, effectively addressing some of the challenges associated with *A. mellifera*.

Chungju, located inland in North Chungcheong Province, may exhibit climatic differences when compared to coastal and mountainous regions. Chungju experiences significant temperature fluctuations, with notable differences between summer and winter temperatures. In contrast, coastal areas tend to have a marine climate, result-

ing in relatively minor annual temperature variations. Additionally, mountainous regions display climatic variations depending on altitude, often characterized by harsh cold conditions during winter (KMA, 2024). Due to the significant climate variations between coastal, inland, and mountainous areas, caution is needed when generalizing these results to other regions of Korea. Additionally, since this study compared three species in only two enclosures, a complete comparison of fruiting across all species was not possible. This is an important aspect for the comparison of pollination efficiency, and further research is needed to address this limitation.

In conclusion, this study demonstrates that *A. cerana* can also serve as an effective pollinator for apple blossoms. These findings suggest the potential for introducing *A. cerana* as a novel pollinator for apple orchards. However, since this research was conducted in a single location, there are limitations in generalizing the results to other regions. Additionally, because the study was performed in a closed environment, its applicability to open agricultural settings is also restricted. Therefore, further experiments across various regions and over extended periods are necessary to assess the activity of *A. cerana* under diverse conditions. Furthermore, to optimize the use of *A. cerana*, detailed research on the efficient placement and number of beehives, as well as management intervals, will be essential.

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