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The difference in pollen harvest between *Apis mellifera* and *Apis cerana* in a Tibetan alpine meadow

HU Lei https://orcid.org/0000-0003-1897-2425; e-mail: hl007873@163.com

WU Xin-wei* https://orcid.org/0000-0001-5577-5637; e-mail: wuxw@nju.edu.cn

* *Corresponding author*

Department of Ecology, School of Life Sciences, Nanjing University, Nanjing 210023, China

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Abstract: To explore the difference in pollen harvest between the western honey bee *Apis mellifera* and a native eastern honey bee *A. cerana* in the Zoige alpine meadows in the northeastern part of the Qinghai-Tibetan Plateau, we investigated species diversity of the harvested pollen and the amount of harvested pollen per bee for both species, and calculated the niche overlap (in terms of similarity in harvested pollen) between the two bee species during the flowering season from June to August 2016. Results showed that the species diversity of the harvested pollen was indistinguishable between the two bee species. Nevertheless, *A. mellifera* carried more (although not significant) pollens per bee than *A. cerana*. Moreover, pollen composition differed between the two bee species: *A. mellifera* mainly foraged on *Anemone rivularis*, *Saussurea nigrescens* and *Anemone trullifolia*, while *A. cerana* foraged on *Anemone rivularis*, *Stellera chamaejasme*, and *Pedicularis longiflora*. Consistently, the niche overlap between the two honeybee species was particularly small in several observations. Our results indicate a niche separation in pollen resource between the two honeybee species in a Tibetan alpine meadow.

Keywords: Apiculture; Introduced bees; Niche separation; Pollen richness; Qinghai-Tibet Plateau; Alpine meadow

Introduction

Since honeybees were domesticated about 4000 years ago (Crane 1990), apiculture has become one of the most productive agricultural industries in the world (Moritz et al. 2005). For example, the estimated production of the world natural honey was about 1.2 million tons in 2015 (Kaur et al. 2016). Although the large numbers of honeybee colonies are beneficial to the productive success of both agricultural crops and wild plants that depend on insect pollination, introduced honeybees also result in a series of ecological threats on native ecosystems (Goras et al. 2016).

Numerous studies have shown that introduced honeybees can reduce survival, growth, reproduction, and feeding behavior of native pollinators (Cross & Mackay 1998; Kato et al. 1999; Thorp et al. 2000; Cross 2001; Singh et al. 2015; Liu et al. 2016) because of their competitive superiority (Miller & Aplet 1992). For example, the introduced *Apis mellifera* reduced the foraging efficiency of the native *Bombus occidentalis* in coastal California (Thomson 2004), and it also suppressed feeding activities of two endemic nectarivorous bird species on the island of Mauritius, Indian Ocean (Hansen 2002). Moreover, introduced honeybees do not always confer fitness benefits to native wild plants because they sometimes decrease the quantity and/or quality of pollen transferred among plants (Vaughton 1996; Cross & Mackay 1998; England et al. 2001; Hansen et al. 2002; Dick et al. 2003; Dupont & Hansen 2004; Celebrezze & Paton 2004). For example, in tropical north Queensland of Australia, the introduced honeybee *A. mellifera* reduced fruit-set

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and seed-set of *Melastoma affine* because they deposited less pollen on stigmas than native bees while they actively removed pollen from stigmas (Cross & Mackay 1998). In the Amazonian rainforest, Dick et al. (2003) showed that introduced honeybees (*A. mellifera*) reduced the pollen dispersal distance of *Dinizia excelsa*, thereby decreasing the outcrossing rate in the plant species.

The Western honeybee *A. mellifera* were introduced to China in the 1920s (Yu et al. 2008). This species and the Eastern honeybee species *A. cerana* are the two most common domestic species in China, approximating 8 and 4 millions of honeybee colonies in 2012, respectively. Compared with *A. cerana*, *A. mellifera* have larger body size, longer flight range and stronger defensiveness (Badano & Vergara 2011). *A. mellifera* thus show advantages on nectar robbing (Huang et al. 1963), nuptial flights and matings (Wang et al. 2003), and disease transmitting (Yang 2005). However, because of a larger body size that requires a higher thoracic temperature, *A. mellife*ra start foraging later than *A. cerana* (Tan et al. 2012), and *A. cerana* are thus more adaptable to extreme fluctuations in ambient temperature and long periods of rainfall (Feng et al. 2014). In addition, *A. cerana* are also better on distinguishing color and grating patterns than *A. mellifera* because of their stronger learning and memory capabilities (Qin et al. 2012). These biological differences raise a question about to what extent these two species differ in their resource exploitation, which have attracted the attention of Chinese ecologists (e.g., Yang et al. 2016; Duan et al. 2003). For example, Yu et al. (2008) and Liu et al. (2013) observed that *A. cerana* foraged on more plant species than *A. mellifera* in Wannan mountain areas of Anhui Province and Kunming and Mengzi Cities of Yunnan Province. In contrast, Hu et al. (2016) recently observed that *A. mellifera* visited more plants than *A. cerana* in Xiangyun Basin in Yunnan Province, China. These contrasting results may result from site-specific effects on honeybee foraging behavior, which asks for more case studies at different sites.

Apiculture has been practiced continuously in the Zoige alpine meadows in the northeastern part of the Qinghai-Tibetan Plateau since 1981 (Mu et al. 2014). Since the 1990s, when *A. mellifera* was introduced to the Zoige Plateau, *A. cerana* and *A. mellifera* have being simultaneously cultured in this area. In the present study, we adopted the method of palynology to explore the ecological differences between *A. mellifera* and *A. cerana* in an alpine meadow. Specifically, we investigated the variations in pollen richness, amount and pollen niche width and overlap between *A. mellifera* and *A. cerana* during a growing season.

1 Study Area

This study was conducted in Hongyuan County, Sichuan Province, China, in the eastern Qinghai-Tibetan Plateau (N: 32°50′-33°22′, E: 102°01′-103°23′). The study site has an average altitude of 3500 m, with a mean annual temperature of 1.1 °C, mean annual precipitation of 650 mm-730 mm (80% of which occurs during May to August), and the annual mean duration of snow cover is 76 days (Li et al. 2011). The meadow is of the continental cold type characterized by a short spring and autumn, a long winter, and a mild to cool summer, and has a growing season of 120-140 days from early May to late September (Wu et al. 2011). The pasture is typically dominated by sedges (*Kobresia setchwanensis*, *Blysmus sinocompressus*), grasses (*Agrostis hugoniana, Elymus nutans, Deschampsia caespitosa*), and forb species such as *Potentilla anserina, Saussurea nigrescens, Taraxacum lugubre, Anemone trullifolia* var. *linearis*, *Anemone rivularis*, *Euphrasis hirtella*, *Trollius farreri*, *Pedicularis* spp and *Thalictrum alpinum*. The plant coverage is higher than 80% and plant height is about 45-60 cm for the tallest grasses. The study pasture has been under intensive grazing in winter only for decades since 1980 when grasslands were primarily classified as summer and winter pastures by the local government (Wu & Sun 2010).

Located in the grasslands used as a winter or summer pasture, the study site is grassland by livestock during the winter only. The apiculture has been practiced continuously since 1981 by employing the honey bees. It is estimated that there are currently about 300 beekeepers and 80 million honey bees every summer on the winter-grazing pasture that covers about 1500 km^2 of the Hongyuan County; the estimated annual honey production ranges between 40 and 60 Mg (Mu et al. 2014).

Based on our filed observation, the honey bees usually began their foraging activity at about 9:00 am and ended it at 3:00-5:00 pm dependent on the weather condition.

2 Materials and Methods

2.1 Methods

We collected bee individuals from hives belonging to a beekeeper. The beekeeper had ten hives for both of two species, each hive having about 6 000 individuals. The beekeeper arranged the *A. mellifera* hives and *A. cerana* hives to two separate sites, with an interval of 10 m. Each hive in one site was about 1 m apart. In addition, there were no other beekeepers nearby (10 km^2). The total density of the two species could be deemed to be similar.

In a fine day at 3:00-5:00 pm, 10 middle size individuals of each of *A. mellifera* (~15.0 mm) and *A. cerana* (~12.0 mm) were randomly caught from the entrance of the ten hives using a nylon net with a mesh size of 2 mm, respectively. After sampling, we immediately scraped the pollen grains from the corbiculate legs of each bee individual, and then the pollen grains were bottled (ten ml). Although both the head and abdomen of bees also carried pollens, the bee corbiculate legs carried more pollens. The field samplings lasted from early June to Mid-August of 2016 when the main nectariferous plants were flowering. There were eight sampling times, i.e. 7 June, 12 June, 21 June, 5 July, 19 July, 27 July, 8 August and 17 August. In laboratory, the pollen grains were identified using acetolysis method, and the pollen grains were compared with the pollens collected from the local following plants over the growing season. The identification features mainly including pollen morphology, color and luster properties, size and volume, location and number of bourgeon-hole channels, and exinous markings, and the pollen was identified to species level (see pollen morphology of the main plants in Appendix 1).

An acetolyzed technique was used to quantity the sampled pollens from the bee species according to Erdtman (1960 and 1966). The pollen grains were equably vibrated in acetic anhydride-sulfuric acid (9:1) liquids (2 ml). 100 μL liquids were dropped in blood counting chamber and chilled for about five minutes under the microscope (×400). Then, we carefully identified and counted the pollens within the 100 μL liquids. Each sampled pollen grains from one bee body were counted for 6 times.

2.2 Response variables

The pollen niche width was calculated as follows:

$$B = \frac{1}{S\sum_{i=1}^{S} P_i^2} \tag{1}$$

where, P_i was the proportion of pollen of species *i*, *S* was the number of the whole pollen species (Yu et al. 2008; Levins 1968).

The pollen niche overlap was calculated as follows:

$$\alpha_{AMAC} = n_{AMAC_t}/(n_{AM_t} \times n_{AC_t})^{1/2} \tag{2}$$

where, n_{AMAC} was the number of common plants in the diets of the species *A. mellifera* and *A. cerana* during the period *t*, and n_{AM_t} or n_{AC_t} is the total number of plants visited by the species in question over the same period. This index might vary from 1 to 0, i.e. from total overlap to complete segregation in terms of resource utilization (Cortopassi-Laurino 1982; Cody 1974).

If both the honey bees did not share the food resources equally, the overlapping extent of their trophic niches depended on the intensity of which common resource they visited. The percentage similarity (*PS*) could be expressed by Schoener index:

$$PS = 1 - \frac{1}{2}\sum_{i=1}^{n} |Pi_{AM} - Pi_{AC}| \tag{3}$$

where, Pi_{AM} and Pi_{AC} were the proportions of pollen from species *i* collected by species *A. mellifera* and *A. cerana*, respectively (Cortopassi-Laurino 1982; Schoener 1968).

2.3 Data analyses

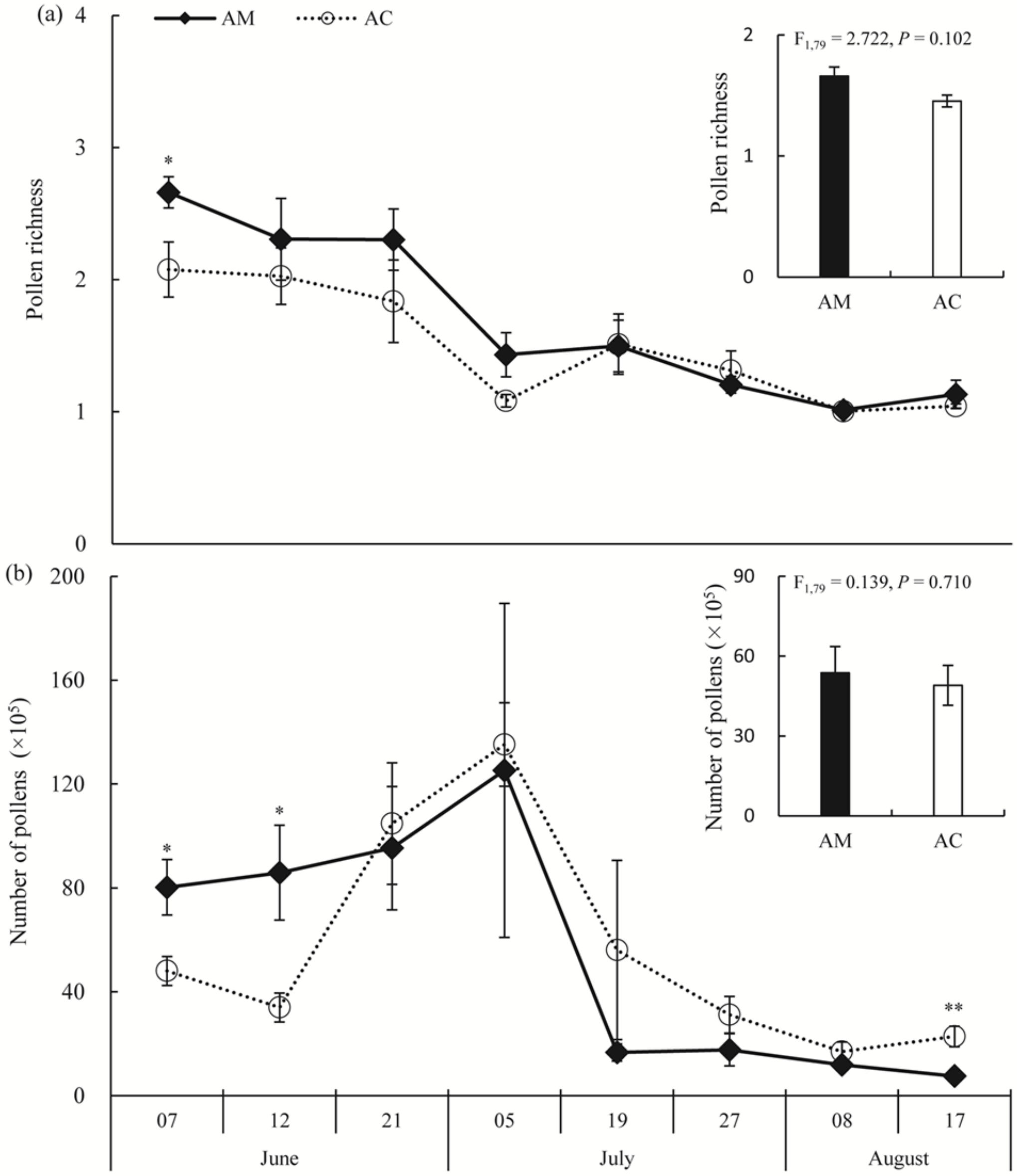
All data were log-transformed to achieve normality. Two-way analysis of variance (ANOVA) was used to examine the effects of species, sampling date and their interaction on pollen richness, the number of pollens and pollen niche over the experimental period. Once a significance was detected, post-hoc Tukey's HSD tests were used to determine the differences for each sampling date. All statistical analyses were performed in R 3.3.1 (R Core Team 2016).

3 Results

3.1 Pollen richness and amount

The total pollen richness was indistinguishable between *A. mellifera* (22) and *A. cerana* (21) over the experiment. However, *A. mellifera* foraged on more plant species than *A. cerana* on June 7. Pollen richness of both *A. mellifera* and *A. cerana* decreased with sampling dates (Table 1 and Figure 1a).

There was also non-significant difference in the total number of pollen grains between *A. mellifera* and *A. cerana* over the experiment. The number of pollen grains of *A. mellifera* was significantly higher than that of *A. cerana* on June 7 and 12. However, the number of pollen grains of *A. mellifera* became significantly lower than that of *A. cerana* on August 17 (Table 1 and Figure 1b).



AM: *Apis mellifera* AC: *A. cerana*.

Figure 1 The pollen richness (a) and pollen amount (b) foraged by the two honey bees over the experimental period. Insets represent the mean values over the experimental period. Error bars are ±1 SE. $^{*}P < 0.05$; $^{**}P < 0.01$.

Table 1 Results of two-way ANOVAs showing the effects of sampling date, bee species, and their interaction on pollen richness, the number of pollens and niche width. *df*=difference of freedom.

Items		Pollen richness		Pollen amounts		Pollen niche width	
	df	F	*P*	F	*P*	F	*P*
Date	7	10.040	< 0.001	6.953	< 0.001	7.259	< 0.001
Species	1	2.245	0.137	0.009	0.923	1.912	0.170
Date×Species	7	0.694	0.677	0.959	0.465	1.444	0.195

Sampling date also significantly affected the number of pollen grains of the two species (Table 1). Specifically, the number of pollen grains of both species increased and reached the peak on July 5, and then sharply declined (Figure 1b).

3.2 Pollen composition

Pollen composition differed evidently between *A. mellifera* and *A. cerana* during the experiment. Specifically, in June *A. mellifera* foraged on *Anemone rivularis* (> 50%) and *Carduus nutans* (16.14% on June 21 only) (Figure 2a). In July, *Anemone trullifolia* was always the main foraged species, occupying 28.56%, 50.20%, and 31.66%, respectively. In addition, *A. mellifera* also foraged on *Carduus nutans* (24.41% on July 5), *Saussurea hieracioides* (28.44% on July 27) and *Taraxacum lugubre* (52.60% on July 27) (Figure 2a). In August, *Saussurea nigrescens* was the only dominant pollen species (Figure 2a).

The main pollen species harvested from *A. cerana* were *Anemone rivularis* (> 85%) in June 7 and 12, while in June 21 both the *Stellera chamaejasme* (62.95%) and *Anemone rivularis* (31.39%) dominated the pollen grains, which continued to July 5 (79.95%). The pollens of *Anemone rivularis* again held on the leading position in July 19 (59.03%) and July 27 (54.33%) until the *Pedicularis longiflora* (54.27%) and *Cremanthodium lineare* (45.73%) dominated in August 8. On August 17 *Pedicularis longiflora* took full advantage on the pollens and contributed to 92.41% (Figure 2b**)**.

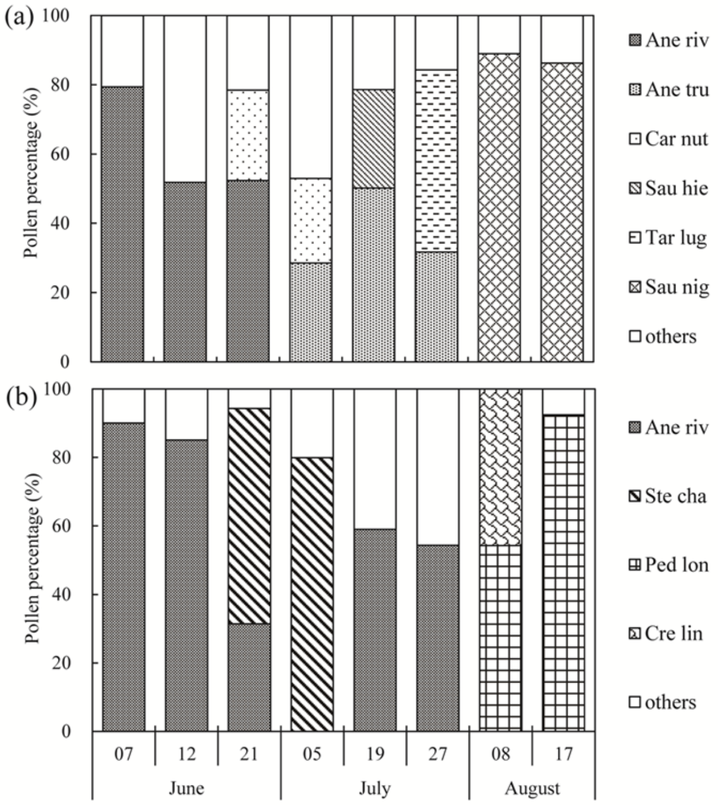
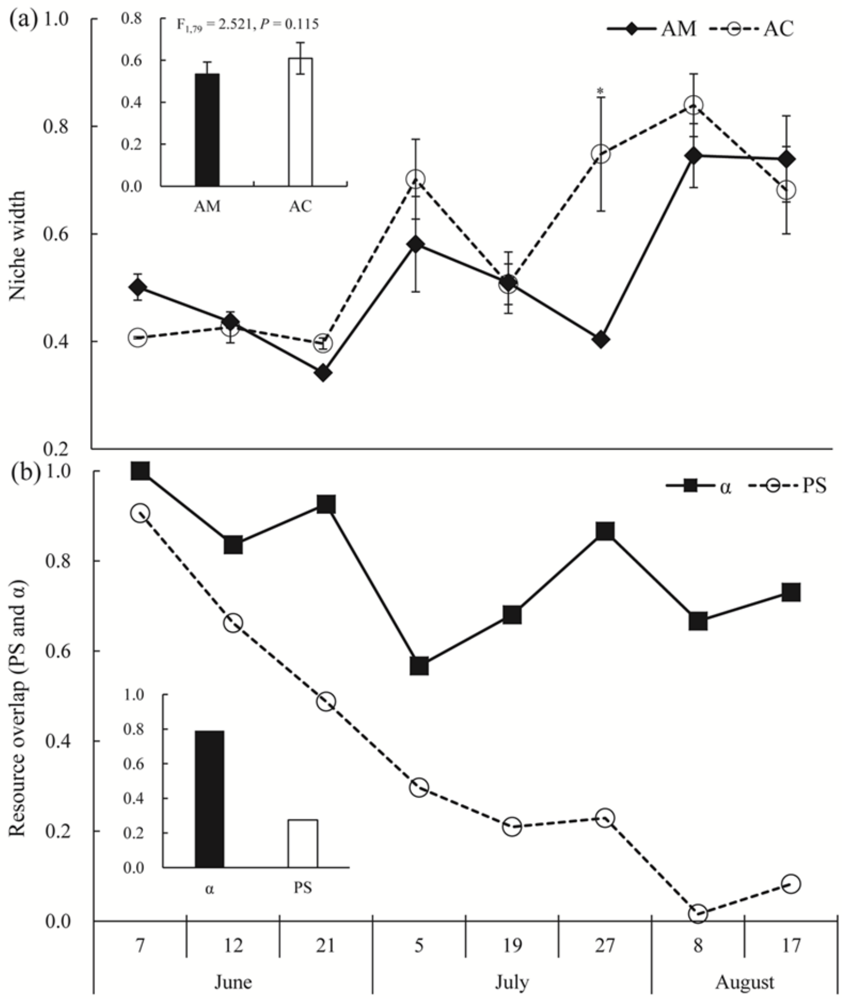


Figure 2 The pollen percentage from bees *Apis mellifera* (a) and *A. cerana* (b) over the experimental period. The dominant pollen species with percentage over 20% were shown.
Ane riv: *Anemone rivularis*; Ane tru: *Anemone trullifolia;*Car nut: *Carduus nutans;* Sau hie: *Saussurea hieracioides;*Tar lug: *Taraxacum lugubre*; Sau nig: *Saussurea nigrescens;*Ste cha: *Stellera chamaejasme*; Ped lon: *Pedicularis longiflora*; Cre lin: *Cremanthodium lineare.*

3.3 Pollen niche width and overlap

Both the honey bees foraged on pollens from diverse plant sources. In total, the pollen niche width of (*B*) *A. cerana* was larger than *A. mellifera* although not statistically significantly. From June 12 to August 8, the *B* index of *A. cerana* was always higher than *A. mellifera*, and significant on July 27 (Table 1 and Figure 3a). The *B* index of *A. mellifera* and *A. cerana* both reached the peak in July 5 and August 8, and then decreased (Figure 3a).

In general, there was high pollen niche overlap (α = 0.79) between the two honey bees, while they did not shared the same pollen resource (PS = 0.27) equally.



AM: *Apis mellifera*, AC: *A. cerana*.

Figure 3 The pollen niche width (a) and resource overlap (b) over the experimental period. Insets represent the mean values over the experimental period. Error bars are ±1 SE. $^{*}P < 0.05$.

The percentage similarity (PS) in gathering remained below the qualitative overlap (α), although there were fluctuations in magnitude of the two parameters. From June 7 to July 19 high proportions of common pollen species occurred in the samples (higher α), but these pollens were not highly shared (lower PS) and declined with sampling date (Figure 3b).

4 Discussion

4.1 Pollen richness and amount

Pollen characteristics (pollen richness and amount) of the *A. mellifera* and *A. cerana* varied with changes in main flowering plants. This result was inconsistent with previous studies (Yu et al. 2008; Liu et al. 2013; Hu et al. 2016). More pollen richness of *A. mellifera* than *A. cerana* indicated that *A. mellifera* could forage on more resource types (Kajobe 2006).

More pollen species were visited by *A. mellifera* than *A. cerana* from June 7 to July 5 because *A. mellifera* has larger body size and longer flying distance to visit more plant species and carry more pollens (Kajobe 2006). However, from July 19 on, *A. cerana* foraged on more pollen species than *A. mellifera*. This might be due to the change in 1) the quality and quantity of pollen and, 2) plant community composition, i.e., the Compositae species become dominant. The change in plant community composition had limited effect on the generalist *A. cerana* but significantly changed the performance of *A. mellifera* with strong selectivity to nectar plants (Liu et al. 2013). The fact that the pollen richness of the two honey bees declined with sampling dates was possibly because of the bee foraging behavior. They may collect more pollens to rear the young larvae during the early stage of the growing season, and during the late stage of the growing seasons they may collect more nectar as food for the winter.

Pollen amount was also different between *A. mellifera* and *A. cerana*. The western honey bee *A. mellifera* carried more pollens than *A. cerana* on June 7 and 12 presumably because of their larger body size and longer flight range (Kajobe 2006). It was notable that the pollen amount of both the two species declined and maintained at a low level from July 19 to August 17, during which the honey bees might mainly forage nectar other than pollen. In addition, the special pollen spines of Compositae plants may also partly prevent bees from foraging on pollens (Bernard et al. 1994).

4.2 Pollen composition

Both the two bees can visit various plant species but they prefer to only a few ones, namely the important food resources because of their no-polylectic habits (Cortopassi-Laurino and Ramalho 1988). In the study site, *A. mellifera* mainly foraged on the Compositae (*Taraxacum lugubre* and *Saussurea nigrescens*) and Ranunculaceae (*Anemone rivularis* and *Anemone trullifolia*) plants, and *A. cerana* foraged on *Anemone rivularis*, *Stellera chamaejasme*, *Pedicularis longiflora* and *Cremanthodium lineare* in decreasing order. This difference may be ascribed to the differences in both plants and bees. On one hand, in addition to high coverage and nectar concentration (Mu et al. 2014), these plant species also have special functional traits. For example, *Anemone rivularis* have a high sexual shoot (reaches to about 25 cm). Plant height was often under pollinator-mediated selection, and pollinators preferred plants with tall selection lines (Zu & Schiestl 2017). *Anemone trullifolia* and *Stellera chamaejasme* distribute in cluster or patch, which was also propitious to attract pollinators (Trapnell & Hamrick 2006). On the other hand, *A. cerana* with small body size can easily enter into the pollen tubes of *Pedicularis longiflora*.

4.3 Pollen niche width and overlap

Although there were some fluctuations in pollen niche width of the two honey bees with sampling date, *A. cerana* had a larger niche width than *A. mellifera* from June 12 to August 8. This may be attributed to the non-preference to nectar plants of *A. cerana*. They can forage on nectar plants with low coverage and low height while *A. mellifera* preferred to high nectar plants (Liu et al. 2013). Before July 19, *A. mellifera* foraged on the dominant and higher flowering plants such as *Anemone rivularis* and *Taraxacum lugubre*. However, many low and rear species such as *Cynoglossum zeylanicum* and *Leontopodium nanum* with small pollen size flowered after July 19. *A. cerana* foraged on these plants, as indicated by the higher *B* diversity index. From the performance of pollen overlap between *A. mellifera* and *A. cerana* we may speculated that when there were more same available food resources (higher α value), these resources were not highly shared (lower PS value).

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