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## RESEARCH ARTICLE - BEES

### Melittopalynology and Trophic Niche Analysis of *Apis cerana* and *Apis mellifera* in Yunnan Province of Southwest China

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#### Abstract

Qualitative melittopalynological data of 34 honey samples obtained from *Apis cerana* and *Apis mellifera* between 2011 and 2012 in Mengzi (Yunnan, China) are analyzed. And trophic niche of the two bee species on pollen food resources are also investigated in the present study. Results show that a diverse spectrum of 17 pollen types was identified into 14 families, and the Asteraceae and Myrtaceae are the most frequent at non-nectar flow and the Lythraceae are most frequent at main nectar flow of pomegranate in our study area. As for trophic niche analysis of *Apis cerana* and *Apis mellifera*, the niche breadth value is 0.65 and 0.57 at non-nectar flow and 0.41 and 0.24 at nectar flow, respectively. Meanwhile, the value of trophic niche overlap index at nectar flow (0.68) is greater than that of non-nectar flow (0.61). And the value of interspecific competition index at nectar flow (0.92) is greater than non-nectar flow (0.77), too. These results may promote the development of local beekeeping and provide some reference for scholars to assess the influence of introducing exotic bees on Chinese native bees.

#### Introduction

Honey can be produced from the nectar of plants (nectar honey), from secretions of living parts of plants or from the excretion of plant sucking insects (honeydew honey). However, honey analysis was focus on nectar honeys and identified the nectar plants in this region through the analysis of pollen of nectar honeys (Caccavari, M. et al. 2010). This research method is generally recognized (Behm et al., 1996).

Bees are generally regarded as beneficial insects for their role in pollination (Goulson, 2003). So the species of honeybee *Apis mellifera* Linnaeus was introduced into China 100 years ago for their good production of honey. *Apis cerana* Fabricius is the most widely distributed native bee species before *Apis mellifera* been introduced. However, its colonies become less and less in China now. *Apis cerana* and *Apis mellifera* are two different species; they have many differences in their body structure and habits. The average length of worker bees body of *Apis mellifera* is 12.0 ~ 14.0 mm, and

that of *Apis cerana* is 9.5 ~ 13.0 mm. They normally display different strategies in cooling hive temperature (Yang et al., 2010) and guarding against invading bee viruses (Sharm & Dharam, 2005; Ai et al., 2012) or parasitic mites (Peng et al., 1987). Different bee species show their preference for different plants (Ramírez-Arriaga & Navarro-Calvo, 2011), resulting in a variety of pollen types and special proportions of them in honeys.

Bees play a very important role in balancing the local ecosystem, especially for the pollination and reproduction of many plant groups (Larkin et al., 2008). Pollen analysis can get an effective assessment on ecological impacts of invasive bee species on native bees (Stout & Morales, 2009). The use of trophic niche analysis methods can effectively assess the impact of bees on the local ecological environment (Santos & Absy, 2010).

In this research, we study the pollen types and proportion in samples of honey from two bee species, *Apis cerana* and *Apis mellifera*, at non-nectar flow and nectar flow two



periods, to assess the impact of introduced bees on native bees and compare the pollen date and analyze their trophic niche as well.

## Materials and methods

### Study area

Honey samples were collected from different colonies of *Apis cerana* (n=17) and *Apis mellifera* (n=17) between 2011 and 2012. Different colonies were placed at two different apiaries (~300 m apart) around the city of Mengzi (23°31'N; 103°25'E). The region is located in the Southeast of Yunnan Province, China. The climate is Subtropical plateau monsoon, with an annual rainfall of 857 mm and an annual temperatures of 18.6. The altitude of apiary is about 1288 m and the sunshine is sufficient with an annual 2234 hours.

### Honey preparation and melissopalynological analysis

Honey samples produced by bees in different months and different main nectar flow were provided by beekeepers and centrifuged at first. The collecting time of honey samples is shown in Table 1.

Qualitative melissopalynological analysis were conducted by the methods reported by Louveaux *et al.* (1978) with slightly modification. In brief, 20 g of honey samples were dissolved in 40 mL distilled water and centrifuged (10 minutes, 4000 r/min). The supernatant was disposed and the residue washed again with 20 mL water. Pollen sediment was mounted in glycerine-gelatine and sealed with paraffin to determine frequency classes by microscope. Pollen types were

Table 1 - Serial number of honey samples from *Apis cerana* and *Apis mellifera* at different main nectar flow.

	Jul/2011 (non-nectar flow)	Apr/2012 (nectar flow)
<i>A. cerana</i>	No.1-9	No.19-26
<i>A. mellifera</i>	No.10-18	No.27-34

identified by comparing them with a reference collection that was obtained from the plants grown in the area surrounding the beehives. Pollen atlases of China Woody Plants Pollen Photo using Scanning Electron Microscopy (2011) and Nectariferous Plant of China (1992) were also consulted.

The Pollen types were classified, according to frequency, into four categories: predominant pollen ( $\geq 45\%$ ), secondary pollen (16–45%), important minor pollen (3–15%) and minor pollen ( $\leq 3\%$ ) (Louveaux *et al.*, 1978). The frequency occurrence of pollen, expressed as a percentage, was calculated per melliferous area.

### Trophic niche analysis

Niche is a functional relationship between a species and other population at the same time and space in the ecosystem. In a biome, niche overlap is a phenomenon of multiple species to feed on the same food, and the resulting competition between different species and increasing competition while having scarce food.

In this paper, set up a framework to think about and estimate the difference between *Apis cerana* and *Apis mellifera* in terms of ecological niche. The ecological parameters were calculated using a niche breadth, trophic niche overlap index and interspecific competition index.

Niche Breadth ( $B$ ) were calculated by the equation (Levins, 1968):

$$B = \frac{1}{S \sum_{i=1}^S P_i^2} \quad (1)$$

Where  $B$  is niche breadth,  $S$  is the level of resource,  $P_i$  is the proportion of the species take advantage of  $i$ -th level resources accounted in total resources.

Trophic niche overlap index ( $C$ ) were calculated by the equation (Colwell & Futuyma, 1971):

$$C_{ij} = 1 - \frac{1}{2} \sum_{h=1}^S |P_{ih} - P_{jh}| \quad (2)$$

Where  $C_{ij}$  is the trophic niche overlap index and  $C_{ij} = C_{ji}$ ,  $P_{ih}$  and  $P_{jh}$  are the proportion of the species  $i$  and species  $j$  take advantage of  $h$  resource sequence accounted in total resources,  $S$  is the grade of the resource sequence.

Interspecific competition index ( $\alpha$ ) were calculated by the equation (Southwood, 1978):

$$\alpha = \frac{\sum P_i P_j}{[(\sum P_i^2)(\sum P_j^2)]^{1/2}} \quad (3)$$

Where  $\alpha$  is the interspecific competition index of species  $i$  and species  $j$  in a same resource.  $P_{ih}$  and  $P_{jh}$  are the proportion of the species  $i$  and species  $j$  in each resource sequence. Interspecific competition is not fierce in the resource utilization when  $\alpha \geq 1$  and competition is fierce when  $\alpha < 1$ .

### Statistical analysis

For descriptive purposes, simple statistical analysis, was applied into our study using SPSS 17.0. The arithmetic means and standard deviations were obtained for variables measured in the present study. The pollen types number of honey samples from two kinds of bee species were compared by one-way analysis of variance (ANOVA) and LSD for multiple comparison tests ( $p > 0.05$ ). Data were assessed, such that statistical significance was based on  $p < 0.05$ .

**Results**

Honeys were sampled on July 2011(n=18) and April 2012(n=16). At July, there is not any nectar flow of plant in study area. April is nectar flow of *Punica granatum* and bee activity for honey production. Thus two different periods, covering the periods of non-nectar flow and main nectar flow of one plant, were representative of the two different species in trophic niche analysis of the study area.

Honey produced by *Apis cerana* and *Apis mellifera* in non-nectar flow showed no significant difference ( $p>0.05$ ). Honey from *Apis cerana* and *Apis mellifera* showed a significant difference in nectar flow of pomegranate flowers ( $p<0.05$ ), and contained  $8.13\pm 1.46$  and  $6.00\pm 1.77$  (mean value  $\pm$  standard deviation) pollen types, respectively. The number of pollen types are show in Table 2.

**Table 2** - The number of pollen types in honey samples by *Apis cerana* and *Apis mellifera* in different main nectar flow.

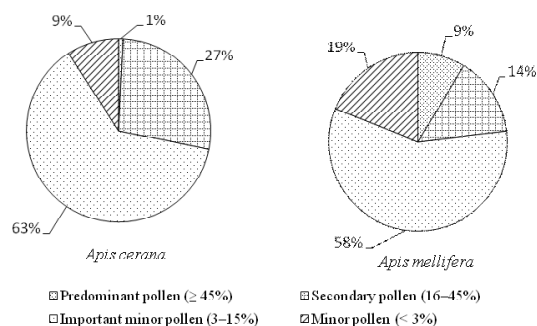
	non-nectar flow			nectar flow		
	Mean	Range	S.D.	Mean	Range	S.D.
<i>A. cerana</i>	7.44 <sup>a</sup>	5-10	1.42	8.13 <sup>a</sup>	6-11	1.46
<i>A.s mellifera</i>	7.56 <sup>a</sup>	4-8	1.42	6 <sup>b</sup>	4-9	1.77

Note: S.D.-standard deviation. Different letters in the same line indicates significant difference ( $p<0.05$ )

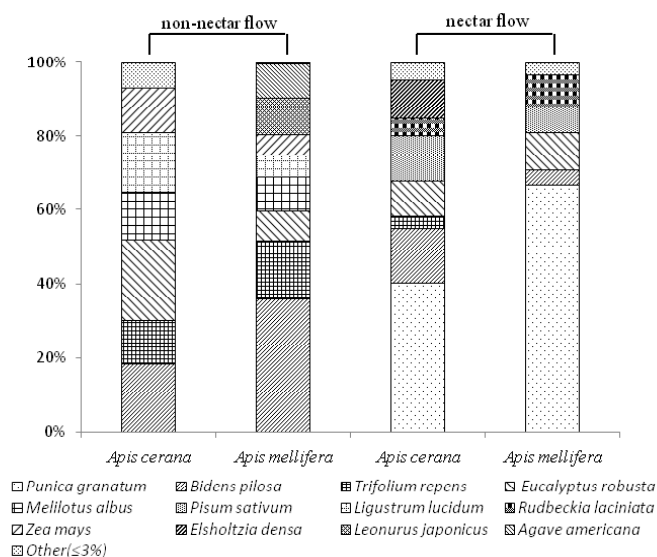
A total of 17 pollen types from 34 honey samples were identified to belong to 14 families (Table 3). It is apparent that none of the pollen types in all samples. The most important families of pollen were Lythraceae, Asteraceae, Fabaceae and Myrtaceae according to their percentage frequency. In non-nectar flow, the *Bidens pilosa* of Asteraceae is the most represented in the pollen of honey by *Apis mellifera*, the next is *Trifolium repens* of Fabaceae. And the *Eucalyptus robus-*

*ta* of Myrtaceae is the most represented by *Apis cerana*, the next is *Bidens pilosa*. In nectar flow, the *Punica granatum* of Lythraceae is the main pollen in all samples from *Apis mellifera* and *Apis cerana*, the next is *Bidens pilosa* (*Apis cerana*) and *Eucalyptus robusta* (*Apis mellifera*).

As for the percentage of honey samples produced by *Apis cerana* and *Apis mellifera* in different months (Fig 1), the distribution of various pollen types percentage is balanced at non-nectar flow, the range of percentage (except minor pollen) is from 11.83% to 18.34% by *Apis cerana* and from 5.61% to 35.91% by *Apis mellifera*. In study areas, as soon as the pomegranate began flowering at the end of March, bees started to visit pomegranate flowers, thereby producing the pomegranate honey. At the nectar flow of *Punica granatum*, the percentage of *Punica granatum* pollen becomes important. However, there is still some other pollen types, like *Bidens pilosa* (14.59%) and *Pisum sativum* (12.43%) of Fabaceae sampled by *Apis cerana*, like *Eucalyptus robusta* (10.08%) and *Rudbeckia laciniata* (8.77%) of Asteraceae sampled by *Apis mellifera*. The range of percentage are from 3.24% to 40.32% by *Apis cerana* and from 4.05% to 66.82% by *Apis mellifera*. The distribution of pollen percentage is polarized (Fig. 2).



**Fig 2.** Frequency of appearance of pollen types in samples by *Apis cerana* and *Apis mellifera*.



**Fig 1.** Pollen type spectra in honey samples by *Apis cerana* and *Apis mellifera* in different main nectar flow.

The pollen types were classified into four categories. The percentage of one same category is different between *Apis cerana* and *Apis mellifera* through data analysis of all honey samples. Important minor pollen is the largest proportion in honey by *Apis cerana* (63%) and *Apis mellifera* (58%). Comparing the honey produced by *Apis cerana* and *Apis mellifera* in other three pollen categories, the percentage is a wide difference. The percentages of four categories are show in Fig 2.

Through statistical analysis of all pollen information and trophic niche analysis, the Niche Breadth, Trophic niche overlap index and Interspecific competition index were calculated to show the relationship of the two kinds of bees in ecology. The proportion of each resource sequence and calculated value are show in Table 4. The Niche breadth compared the different

Table 3. Pollen types identified in 34 honeys analysed and their frequency classes—referring to Table 1.

Family	Pollen type	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34			
Agavaceae	<i>Agave Americana</i> L.	I	M																																			
Asteraceae	<i>Bidens pilosa</i> L.	I	I	I	S	S	S	S	S	S	P	S	S	S	S	S	S	S	S	S																		
	<i>Rudbeckia laciniata</i> L.																																					
	<i>Taraxacum mongolicum</i> Hand.-Mazz.																																					
	<i>Trifolium repens</i> L.																																					
Fabaceae	<i>Pisum sativum</i> L.																																					
	<i>Trifolium repens</i> L.																																					
Geraniaceae	<i>Pelargonium hortorum</i> Bailey																																					
Labiatae	<i>Eisholtzia densa</i> Benth.																																					
	<i>Leonurus artemisia</i> (Laur.) S. Y. Hu																																					
Lamiaceae	<i>Ginnamomum glanduliferum</i> (Wall.) Nees																																					
Leguminosae	<i>Melilotus alba</i> Medic.																																					
Lythraceae	<i>Punica granatum</i> L.																																					
Malvaceae	<i>Malva sinensis</i> Cav.																																					
Myrtaceae	<i>Eucalyptus robusta</i> Smith																																					
Oleaceae	<i>Ligustrum lucidum</i> Ait.																																					
Poaceae	<i>Zea mays</i> L.																																					
Verbenaceae	<i>Verbena officinalis</i> L.																																					

bee species within the same period. At non-nectar flow, the Niche breadth is 0.65 and 0.57, respectively. At nectar flow, the Niche breadth is 0.41 and 0.24, respectively. The Niche breadth of *Apis cerana* is greater than *Apis mellifera* at two different periods. The trophic niche overlap index and Interspecific competition index compared the bees within two periods of non-nectar flow and nectar flow. The value of Trophic niche overlap index at nectar flow (0.68) is greater than that of non-nectar flow (0.61) and the value of Interspecific competition index at nectar flow (0.92) is also greater than non-nectar flow (0.77). Through the two values described above, the two species show stronger interspecific competition at nectar flow.

Table 4. Frequency of pollen types from samples of *Apis cerana* and *Apis mellifera* and trophic niche analysis.

Family	P <sub>i</sub> (non-nectar flow)		P <sub>i</sub> (nectar flow)		
	<i>Apis cerana</i>	<i>Apis mellifera</i>	<i>Apis cerana</i>	<i>Apis mellifera</i>	
Agavaceae	<i>Agave americana</i> L.	1.92	9.60	—	—
Asteraceae	<i>Bidens pilosa</i> L.	18.34	35.91	14.59	4.05
	<i>Rudbeckia laciniata</i> L.	—	—	4.65	8.77
	<i>Taraxacum mongolicum</i> Hand.-Mazz.	—	—	—	0.28
Fabaceae	<i>Trifolium repens</i> L.	11.83	15.71	3.24	1.60
	<i>Pisum sativum</i> L.	—	—	12.43	6.97
Geraniaceae	<i>Pelargonium hortorum</i> Bailey	0.89	—	—	—
Labiatae	<i>Eisholtzia densa</i> Benth.	—	—	10.38	—
Lamiaceae	<i>Leonurus artemisia</i> (Laur.) S. Y. Hu	—	9.60	—	—
Lauraceae	<i>Cinnamomum glanduliferum</i> (Wall.) Nees	2.37	0.25	—	—
Leguminosae	<i>Melilotus alba</i> Medic.	12.87	9.10	1.19	—
Lythraceae	<i>Punica granatum</i> L.	—	—	40.32	66.82
Malvaceae	<i>Malva sinensis</i> Cav.	—	—	0.43	0.75
Myrtaceae	<i>Eucalyptus robusta</i> Smith	21.89	8.10	9.62	10.08
Oleaceae	<i>Ligustrum lucidum</i> Ait.	16.12	6.11	—	—
Poaceae	<i>Zea mays</i> L.	11.83	5.61	2.92	0.66
Verbenaceae	<i>Verbena officinalis</i> L.	1.92	—	0.22	—
Niche Breadth		0.65	0.57	0.41	0.24
Trophic niche overlap index			0.61		0.68
Interspecific competition index			0.77		0.92

Discussion

The present study provides new insights of the ecological relationship between two bee species by pollen composition analysis. The honey samples used in this study were collected for drawing pollen atlases and knowing the main nectar plants at the study area. These studies will help the local beekeeping.

The honey from the different bee species of non-nectar flow and nectar flow has their own characteristic. At non-nectar flow, they show 8 pollen types, but *Eucalyptus robusta* is the maximum frequency in samples from *Apis cerana*, followed by *Bidens pilosa*, *Ligustrum lucidum*, *Trifolium repens* and *Zea mays*. *Bidens pilosa* is the maximum frequency in samples from *Apis mellifera*, followed by *Trifolium repens*, *Agave Americana* and *Leonurus japonicas*. At nectar flow, they also show 8 pollen types and they have *Punica granatum* pollen as predominant pollen. The next is *Bidens pilosa* (*Apis cerana*) and *Eucalyptus robusta* (*Apis mellifera*), respectively. Different bee species show their preference of plants, the reasons for which may be that pheromone attract produced by plant to two bee species is different (Jennifer et al., 2009).

As for the varying degrees, the number of pollen types in honey and the niche breadth of *Apis cerana* is greater than *Apis mellifera*. Whether nectar flow or not, the Trophic niche overlap index and Interspecific competition index all are sho-



wed that two bee species exist in strong interspecific competition from each other. Bees pollinate most of the world's wild plant species and provide economically valuable pollination services to crops. The western honeybee, *Apis mellifera*, has been introduced to many parts of the world and sometimes purported to be detrimental to native bees because it reduces their food base (Forup & Memmott, 2005). Except the competition in food, there are some more direct competition, for example, *Apis mellifera* may stolen the honey from hives of *Apis cerana* sometimes, and *Apis mellifera* drone chase and attempt to mate with *Apis cerana* virgin queen caused mating interferes to drone and virgin queen of *Apis cerana* (Wang et al., 2003). Certainly, we have to face the fact that some plants which mainly depend on *Apis cerana* for pollination is becoming less with the reduction of *Apis cerana*.

Some researchers have started to pay attention to assess the ecological impact of alien bee species on native bee by building various niche models (Villanueva and Roubik, 2004; Franco et al., 2009; Villemant et al., 2011). The alien invasive species, some are not the hopes of the people but some are introduced because of its high production capacity. *Apis mellifera*, which is really good at production of honey, have been introduced to China. However, consideration must be given to the potential impact that expanding populations of introduced bees could have on native flora and fauna (Howlett & Donovan, 2010). Little is known of the potential co-evolution of flowers and bees in changing, biodiverse environments (Roubik & Villanueva, 2009).

Therefore, these studies assist us to understand the pollen types in honey and main nectar plants at the study area and could better help in evaluating the effects of the presence of *Apis mellifera* on the foraging of *Apis cerana* in this habitat.

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