

# Plant communities foraged by the western honeybee (*Apis mellifera* L.) and their occurrence along urban road networks in Tokyo and Chiba, Japan

A. Nagase<sup>1</sup> L. Pouilloux<sup>2</sup>, F. Francis<sup>2</sup> and G. Noël<sup>2</sup>

<sup>1</sup> Graduate school of Global and Transdisciplinary Studies, Chiba University, Chiba, Japan;

<sup>2</sup> Functional and Evolutionary Entomology – Gembloux Agro-Bio Tech (University of Liège), Gembloux, Belgium.

## ABSTRACT

Urbanisation is a primary cause of pollinator biodiversity loss because it depletes floral and nesting resources. Therefore, it is crucial to promote urban greening to provide pollen and nectar sources for pollinators. To investigate the floral resources of the urban environment, we used an efficient floral bio-sampler, *Apis mellifera* L. Pollen was collected from three beehives using pollen traps every 2 weeks from May to September 2018 at four different sites in Tokyo (Yaesu and Kiba) and Chiba prefecture (Kashiwanoha and Nishichiba), Japan. DNA metabarcoding of pollen samples was used to identify the taxonomic composition of the floral resources. The results showed that 168 species belonging to 57 taxonomic families were used as pollen sources in total, with a large number of honey-plant species in the Fabaceae (15 spp.), Asteraceae (14 spp.), or Rosaceae (8 spp.) families. Fifty-two percent of the floral resources were alien species, 20% were cultivar species, 25% were native to Japan, and the remaining 3% were not identified. We observed that spontaneously colonising plant species and street planting were important sources of foraging for urban honeybees. We then studied the spatial occurrences of the most foraged species by honeybees around hives. Sixty floral species on urban roads were targeted, and after 60 transects in May 2019, 24 were identified. Urban greening programmes based on our findings could enhance biodiversity by creating green spaces suitable for pollinators in urban areas and along urban road network.

**Keywords:** Biodiversity, pollinators, urban greening, pollen analysis, DNA metabarcoding

## INTRODUCTION

During pollination process, insect pollinators enable fruit growth and seed development in natural and anthropised ecosystems. They participate in ecosystem services and have a significant impact on people's lifestyles (Potts et al., 2016). Given the growing international popularity of urban honey beekeeping, many studies have been conducted to understand the network of plant-pollinator interactions, a factor essential in evaluating the stability of pollination systems (Bosch et al., 2009; Ferreira et al., 2013; Sponsler et al., 2020). However, the flora foraged by western honeybees (*Apis mellifera* L.) is poorly studied in urban areas, especially in Japan. Although the importance of ecological networks in urban areas has been addressed (Ignatieva et al., 2011), little research has been carried out on the contribution of urban road networks to pollen and nectar sources for pollinators. Information on the richness and location of available flora for honeybees is vital and can help stakeholders promote green spaces hosting pollinating insect populations, which in turn contributes to the preservation of biodiversity. The aims of this study were (1) to identify the foraged plant communities and their functional structure in Tokyo and Chiba prefectures (Japan) through DNA metabarcoding analysis of four apiaries in 2018, and (2) to assess the potential of urban road networks in providing the plants most foraged by bees.

## MATERIALS AND METHODS

Pollen was collected every 2 weeks from the end of March to mid-October in 2018 at four different sites in Tokyo (Yaesu and Kiba) and Chiba prefectures (Kashiwanoha and Nishichiba), Japan (Figure 1). At each site, pollen was collected from three hives. Pollen

traps, immediately accepted by honeybee foragers, were placed at the entrance of each beehive for a duration of 2 h. After field collection, all pollen samples were stored in a freezer at  $-18^{\circ}\text{C}$ . Under laboratory conditions, the pollen collected from the three beehives was mixed with the respective pollen collected from the same site on the same day.

We followed the protocol described by Noël et al., (2021) for pollen DNA extraction, DNA library preparation, and DNA sequencing. Pollen identification results were tabulated in an OTU table. Implausible identification data can be attributed to several factors: spurious false-positive BLAST alignments, bees regurgitating stomach honey contents, contact between pollen foragers, and stored bee bread within the hive before foraging (Richardson et al., 2015a). Therefore, a clean-up before the analysis is necessary. A cut-off of 97% identity and number of reads less than 10 was determined (Richardson et al., 2015b). This enabled us to remove 58% of unreliable data. We conducted alpha diversity metrics (i.e. Shannon and Chao1) on DNA metabarcoding data with R package phyloseq (McMurdie and Holmes, 2013) in R environment (R Core Team, 2020).

Alpha diversity was assessed in order to define the species richness by site. Two other alpha diversity indexes were estimated: Chao1 and Shannon indexes. Chao 1 estimates the number of unobserved OTUs from those observed 1 or 2 times given by the following formula derived from Chao (1984):

$$S_1 = S_{obs} + \frac{F_1^2}{2F_2}$$

where  $S_{obs}$  is the number of species in the sample,  $F_1$  is the number of singletons (i.e. the number of species with only a single occurrence in the sample) and  $F_2$  is the number of doubletons (i.e. the number of species with exactly two occurrences in the sample) (Varek, 2011). Shannon index estimates the equitability of the OTU distribution. Shannon index is given by the formula : with  $H$ , Shannon's diversity index,  $S$ , the total number of species in the community (richness) and  $p_i$ , the proportion of  $S$  made up of the  $i^{\text{th}}$  species (Shannon, 1948).

$$H = - \sum_{i=1}^S p_i \ln p_i$$

Additionally, ANOVA analysis was performed ( $p > 0.05$ ). To define the plant traits of the foraged plants, each identified OTU in the samples was characterised by its stratum origin, including whether they were herbaceous (no woody stems above ground) or woody taxon (tree, shrub, liana); and its native status, including native, alien, or cultivar taxa. The plant trait database was built using information from Ylist (Yonekura and Kajita, 2007) and ©Species2000 (Roskov et al., 2019) for the Japanese plant dataset.

To survey the foraged plants in the urban road network, we defined a list of the top 10 foraged plant species per site. For trees and herbaceous plants, the list was created based on (1) the sum of the number of reads per species in all the samples and (2) the frequency of the plant species. Some plant species overlapped because they were found in both (1) and (2) (Table 1). To evaluate the spatial occurrences (presence or absence) of the listed species, we designed transects in the areas most foraged by the honeybees, which covered a 500 m perimeter around the location of the four apiaries (Seeley, 1995). Per site, 50 potential transects of 100 m each were previously mapped using Google Earth Pro software and hand-drawn on practicable roads that did not require traffic permits. Thereafter, 15 transects were randomly selected and recorded using the QGIS software (QGIS Core Team, 2015). A buffer zone of 10 m was created from the selected transects, and the survey areas of the floral resources were marked (Figure 1). To determine the complementarity of DNA metabarcoding and transect techniques, we conducted a Principal Coordinate Analysis (PCoA) using the vegan R package (Oksanen et al., 2019) based on the presence/absence matrix of 12 samples of the DNA metabarcoding technique from May 2018 (all sampling sites) with the data of the transect method in May 2019, merging all the occurrence data per site ( $n \text{ sample} = 4$ ).



Figure 1: Beehives (green points) and transects (red lines) of Nishichiba (latitude 35.627061, longitude 140.104275), Kiba (latitude 35.894376, longitude 139.948703), Kashiwanoha (latitude 35.640849, longitude 139.837398) and Yaesu (latitude 35.678161, longitude 139.768182).

Table 1: Summary of plant species occurrence along urban road networks. Top species classified by families, with pollen or nectar source information, as herbaceous or tree species, and their state of native, non-native, or cultivar species in Japan. Plant species in empty cell were not studied at the site. Species in bold are species found in the field. For the nectar/pollen column, nectar source (N), both nectar and pollen provided (NP), pollen source (P), mainly pollen but nectar also (P(n)), mainly nectar but pollen also (N(p)). NA indicates that information of Nectar/Pollen sources were not available. The second part expresses their ability to exploit the species: excellent, good (well exploited), temporary (temporarily exploited), and rarely (rarely exploited) (Sasaki 2010). Values in the sites columns (i.e., Nishichiba, Kashiwanoha, Kiba and Yaesu) correspond to the number of transect whose plant species were identified.

Taxonomy	Species	Nectar/Pollen sources	Herbaceous or Tree species	Native state	Nishichiba	Kashiwanoha	Kiba	Yaesu
Actinidiaceae	<i>Actinidia chinensis</i>	P good	Tree	Cultivar		2		
	<i>Actinidia deliciosa</i>	P good	Tree	Cultivar		2		
Amaryllidaceae	<i>Allium fistulosum</i>	N(P) good	Herbaceous	Cultivar	0			
Anacardiaceae	<b><i>Rhus chinensis</i></b>	NP good	Tree	Native			1	
Araliaceae	<i>Aralia elata</i>	NP temporary	Tree	Native		0		0
	<b><i>Dendropanax morbifer</i></b>	N(p) good	Tree	Non-native		0	2	
	<i>Schefflera heptaphylla</i>	NA	Tree	Native				0
Asteraceae	<i>Artemisia argyi</i>	P temporary	Herbaceous	Non-native				0
	<i>Bidens pilosa</i>	NP excellent	Herbaceous	Non-native			0	
	<i>Centaurea cyanus</i>	P(n) temporary or good	Herbaceous	Cultivar	0	0		
	<i>Cirsium setidens</i>	NP good	Herbaceous	Non-native		0		
	<i>Cosmos sulphureus</i>	NP good	Herbaceous	Non-native				0
	<i>Helianthus annuus</i>	NP excellent	Herbaceous	Cultivar	0			0
	<b><i>Solidago canadensis</i></b>	NA	Herbaceous	Non-native				10
<i>Taraxacum obtusifrons</i>	NP good	Herbaceous	Non-native			2		

Brassicaceae	<i>Brassica carinata</i>	NP excellent	Herbaceous	Non-native					0
	<i>Brassica napus</i>	NA	Herbaceous	Non-native	0	0			
Euphorbiaceae	<i>Mallotus barbatus</i>	NA	Tree	Non-native	0	1	0	0	
	<i>Triadica sebifera</i>	P(n) temporary	Tree	Non-native	1	0	1		
Fabaceae	<i>Cicer arietinum</i>	NA	Herbaceous	Non-native					0
	<i>Erythrina crista-galli</i>	NP temporary	Tree	Non-native	0		0	0	
	<i>Melilotus officinalis</i>	NA	Herbaceous	Non-native			0		
	<i>Styphnolobium japonicum</i>	N(p) temporary	Tree	Cultivar					0
	<i>Trifolium incarnatum</i>	NP excellent	Herbaceous	Non-native	0				
	<i>Trifolium pratense</i>	NP temporary	Herbaceous	Non-native	6	3	3	0	
	<i>Trifolium repens</i>	NP excellent	Herbaceous	Non-native	2	6	0	0	
	<i>Trifolium tomentosum</i>	NA	Herbaceous	Non-native	0				
	<i>Vicia villosa</i>	NP excellent	Herbaceous	Non-native	7	1			
Fagaceae	<i>Castanopsis fargesii</i>	NA	Tree	Non-native					0
Hydrangeaceae	<i>Dichroa febrifuga</i>	NA	Tree	Cultivar	6	1			
Hypericaceae	<i>Hypericum lancasteri</i>	NA	Tree	Non-native	0				5
Lythraceae	<i>Lagerstroemia indica</i>	P excellent	Tree	Cultivar	0	0	7	0	
	<i>Punica granatum</i>	P temporary	Tree	Cultivar	0	0			
Myrtaceae	<i>Callistemon comboynensis</i>	NP good	Tree	Non-native		0	0		
	<i>Eucalyptus grandis</i>	NP good	Tree	Non-native			0		
	<i>Eucalyptus vicina</i>	NP good	Tree	Non-native			0		
Nelumbonaceae	<i>Nelumbo nucifera</i>	P(n) good	Herbaceous	Non-native					0
Oleaceae	<i>Ligustrum lucidum</i>	NP good	Tree	Cultivar	0	2			0
Onagraceae	<i>Oenothera rosea</i>	NP temporary	Herbaceous	Non-native	4				0
Orobanchaceae	<i>Orobanche panceicii</i>	NA	Herbaceous	Non-native			4		
Pentaphragmaceae	<i>Ternstroemia gymnanthera</i>	P(n) rarely	Tree	Native	0				2
Pittosporaceae	<i>Pittosporum glabratum</i>	N(p) temporary	Tree	Non-native			2		
Plantaginaceae	<i>Plantago asiatica</i>	NA	Herbaceous	Non-native					0
	<i>Plantago lanceolata</i>	P(n) rarely	Herbaceous	Non-native	5	2	4	0	
Poaceae	<i>Lolium perenne</i>	NA	Herbaceous	Non-native	1	6			0
Ranunculaceae	<i>Clematis terniflora</i>	P incidentally	Herbaceous	Native	4				
Rosaceae	<i>Prunus grayana</i>	N(p) good	Tree	Native		0			
	<i>Prunus pseudocerasus</i>	NA	Tree	Cultivar	0				
	<i>Prunus szechuanica</i>	P(n) temporary	Tree	Non-native	0				
	<i>Rhaphiolepis indica</i>	NP temporary	Tree	Native	0				
	<i>Rosa hybrida</i>	P(n) temporary	Herbaceous	Non-native	4	0	2		
	<i>Rubus columellaris</i>	NP good	Herbaceous	Non-native	0				
Rutaceae	<i>Citrus sinensis</i>	N(p) excellent	Tree	Cultivar		0			
Sapindaceae	<i>Acer buergerianum</i>	N(P) good	Tree	Cultivar	0				0
Styracaceae	<i>Styrax grandiflorus</i>	NP excellent	Tree	Non-native					0
Theaceae	<i>Stewartia sinensis</i>	P(n) good	Tree	Native					0
Verbenaceae	<i>Phyla canescens</i>	NP temporary or good	Herbaceous	Native	0	0			
	<i>Verbena hispida</i>	NA	Herbaceous	Non-native			0	0	
	<i>Verbena incompta</i>	NA	Herbaceous	Non-native			0		
Vitaceae	<i>Ampelopsis japonica</i>	NA	Herbaceous	Cultivar					0

## RESULTS AND DISCUSSION

### Listing and overall characteristics of floral species foraged by honeybees

The results showed that 168 species belonging to 57 taxonomic families acted as pollen sources. The identified plant dataset was categorised as 54% herbaceous species, 40% tree species, and 6% representing the non-floral species and floral species with no information (Table 2). Overall, the number of herbaceous species was higher than the number of tree species foraged by honeybees throughout the year. However, the number of

tree species was higher at the end of April, in May, and at the end of the activity season (Figure 2A). Of the floral resources, 52% were non-native species, 20% were cultivar species, 25% were native to Japan, and the remaining 3% were not identified (Table 2). During the entire year, the number of non-native species foraged by honeybees was higher than that of the native species. Moreover, the proportion of non-native species increased after August (Figure 2B).

Previous studies have shown that bee populations (wild and domesticated) prefer to forage native plants compared to non-native ones (Memmott and Waser, 2002; Morandin and Kremen, 2013; Salisbury, et al. 2015; De Vere, et al. 2017). However, the present study showed that the majority of plants foraged were non-native species. Salisbury et al. (2015) affirmed that the planting of both native species and near-native species in gardens would increase the resources available to bees, thereby extending the flowering season and potentially providing resources for specialist pollinator groups. Indeed, only native species from a single region are not optimal pollinator resources. This study showed that *A. mellifera* used more non-native species, especially at the end of the year (Seitz et al., 2020). Some species such as *Eucalyptus vicina* in Kiba and *Solidago canadensis* or *Cicer arietinum* in Yaesu are used by bee populations only from the end of August. In this study, a large number of honey-plant species in the Fabaceae (15 spp.), Asteraceae (14 spp.), and Rosaceae (8 spp.) families were identified. A similar trend was shown in previous studies, in which the largest number of species identified by the metabarcoding method was recorded in Fabaceae in north eastern Nigeria by Dukku (2014), and northern Egypt by Taha et al. (2017). According to a previous report by Sasaki (2010), *Erythrina crista-galli*, and three *Trifolium* species are great sources of nectar and pollen for honeybees. *Trifolium pratense* and *Trifolium repens* are found in great abundance and very frequently in the four different sites according to the metabarcoding method. Both species are sources of nectar and pollen for honeybees and can be found in spontaneous vegetation in urban areas as gaps or street verges in the field.

Table 2: Number of floral species used by honeybees by site (Yaesu, Kiba, Nishichiba and Kashiwanoha). NA corresponds to plant species which were not identified.

Sites	Number of floral species	Ratio of native/non-native, cultivar plant species				Ratio of tree/herbaceous plant species		
		Native	Non-native	Cultivar	NA	Tree	Herbaceous	NA
Nishichiba	72	21 %	56 %	21 %	3 %	43%	51%	6%
Kashiwanoha	83	23 %	52 %	23 %	2 %	46%	51%	3%
Kiba	43	16 %	64 %	16 %	4 %	44%	49%	7%
Yaesu	76	23 %	52 %	22 %	4 %	46 %	47 %	7%
All four sites	162	25 %	52 %	20 %	3 %	40 %	54 %	6%

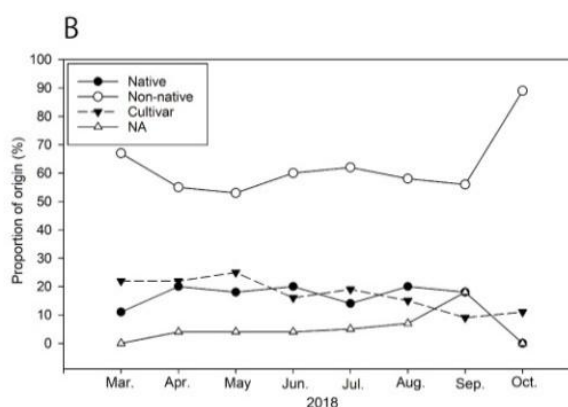
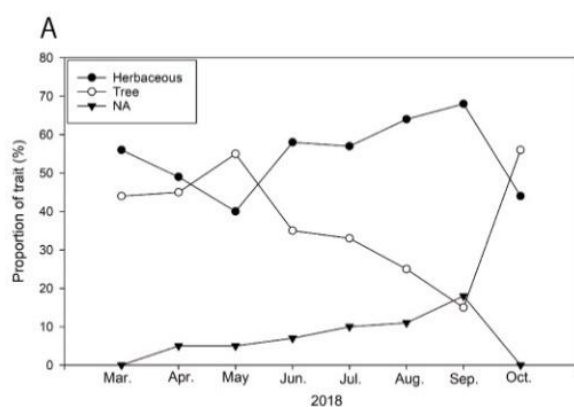


Figure 2 Change of proportion of trait (A) and native status (B) over the sampling period

### Difference of floral species used by honeybees at each four sites

The largest number of floral species used by honeybees is in Kashiwanoha, followed by Yaesu, Nishichiba, and Kiba (Table 2). The alpha diversity metrics comparisons depending on the four sites showed the highest value for the Kashiwanoha site, followed by Yaesu and Nishichiba sites and Kiba site. There was a significant effect of the site on richness (p-value highly significant for Chao 1). Moreover, the effect of beehive localisation on Shannon diversity was also highly significant (FStat = 5.99; df = 3; p-value < 0,001). Thus, effective diversities were not similar from one place to another. For example, Kashiwanoha is dominated by a large number of abundant taxa and, in contrast, Kiba was dominated by a few abundant taxa. This may be due to the location of beehives and plant communities near beehives. Moreover, the richness was higher for the two sites located in the peri-urbanisation area of Tokyo City, Nishichiba, and Kashiwanoha than for the two sites in the downtown area of the capital.

Figure 2 Change of proportion of trait (A) and native status (B) over the sampling period

Sixty floral species on urban roads were targeted, and after 60 transects in May 2019 (Table 1), 24 were identified. Our results suggested that the urban road network could be a refuge for a majority of non-native plant species (63%), but also of a large proportion of cultivar species (25%) and a minority of native species (12%). At Nishichiba and Kashiwanoha campuses, herbaceous species were observed in street verges, microsites, and urban gap designs. Of the 10 transects, *Trifolium pratense*, *Trifolium repens*, *Lolium perenne*, *Vicia villosa*, *Clematis terniflora*, *Plantago lanceolata*, and *Oenothera rosea*, and only one individual of *Taraxacum obtusifrons* has been detected in the streets. We also found *Plantago lanceolata* on microsites (1 transect) and *Vicia villosa* on gaps (1 transect). *Triadica sebifera* and *Dichroa febrifuga* were recorded as ornamental plants (three transects). Furthermore, one transect crossed *Actinidia deliciosa* and *Actinidia chinensis* plantings (agricultural plots) which are pollinated by the honeybee foragers (Blanchet et al., 1991). An example of a more industrialised site is the Kiba site. Tree species were foraged more often than herbaceous species by honeybees, which could be explained by the great resources of the *Eucalyptus* species as already mentioned, but also by the non-presence of private gardens or parks in close proximity. Moreover, in several transects (24 and 36), the green spaces were arranged on large surfaces but did not contain any species useful to bees. One solution to diversify the pollen resources of domestic and wild bees would, therefore, be to reflect these areas in a more ecological dynamic and more attractive way to pollinating species. This is explained by the highly industrialised context and the presence of a very high abundance of the species *Lagerstroemia indica*, especially in August. The two herbaceous clover species (*T. pratense* and *T. repens*) are also significant sources of nectar and are always present in the species most commonly used by honeybees from April to August included for the four sites. In the case of the most urbanised site, Yaesu, it appears that the urban context does not allow bees to benefit from a great source of pollen in traffic lanes. Casanelles-Abella and Morettie (2022) showed that available resources are insufficient to maintain present densities of beehives in urban areas in Switzerland.

The availability of floral resources needs to be sufficient to host both domesticated honeybees and local wild pollinators. Indeed, the plants foraged by the honeybee can also be beneficial to local pollinators due to the great asymmetry in plant-pollinator relationships: the most generalist pollinators would interact with the most specialist flowering plants and vice versa (Bascompte 2003). Therefore, in the first instance, considering the visited plants by honeybee would also benefit the floral requirements for other generalist or specialist pollinators such as *Bombus* species.

In this context, it would be preferable to plant certain plants, specifically herbaceous species. Indeed, many flower beds are initiated in cities, but are not sources of pollen. Therefore, some greening projects should consider the needs of pollinators.

### Comparison between DNA metabarcoding and transect study



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