Honey DNA Metabarcoding method reveals the floral diversity and abundance by Apis cerana japonica and Apis mellifera in Japan, link with landscape variables

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Abstract

The decline of pollinator species is a global disaster for food production and biodiversity. According to numerous studies, the reasons for this phenomenon are mainly anthropogenic. In particular, the introduction of exotic species in new environments negatively impacts native species.
Therefore, the evaluation of the diet of *Apis cerana japonica*, native to Japan, and *Apis mellifera*, introduced, is essential to understand the influence of the latter on the Japanese environment.

In order to answer this, this study analyzes the floral diversity presents in the honey of 36 Japanese and Western honey bee apiaries from different regions of Japan from August 2022 to November 2022. DNA is analyzed by metabarcoding method and DNA sequences are associated with taxa following a bioinformatics work. Each environment surrounding the apiaries is analyzed to extract landscape metrics. These data were used to explain the taxonomic richness present in honey samples.

To do so, a statistical GLMM model was implemented to determine the variables that best explain floral diversity. At the same time, the floral richness was described by a community analysis for each honey bee species in order to highlight: the plant species most present, a possible food overlap between honey bees and their food preferences.

Finally, the results indicate that landscape variables such as Shannon diversity index, Integral Index of Connectivity and total patch density best explain the taxonomic richness detected in honey samples. Then, more abundant floral species present only in one or the other honey bee species were noticed. *Apis cerana japonica* mainly counts woody and tree species such as *Ulmus parvifolia* and *Ulmus* sp., *Hedera nepalensis* and *Chengiopanax sciadophylloides*, *Apis mellifera*, while preferring herbaceous species: *Physalis angulata*, *Triticum aestivum* and *Cosmos bipinnatus*. Following a Venn diagramm, few plant species appear to be shared between honey bees. However, a G-test of the food choice of *Apis cerana japonica* for woody or herbaceous species according to the cohabitation with the Western bee shows an influence of the latter on the feeding behavior of the Japanese honey bee. Thus, the different results are contradicted and seem to be explained only by the limited number of samples which does not allow to draw conclusions as to the presence of competition or not between the two species of honey bee in Japan.

Keywords : *Apis cerana japonica* - *Apis mellifera* - Honey metabarcoding - Bioinformatics - Taxa diversity - Food preference - Japan

Résumé

Le déclin des espèces pollinisatrices est une catastrophe mondiale pour la production alimentaire et la biodiversité. D'après de nombreuses études, les raisons de ce phénomène sont principalement anthropiques. Notamment, l'introduction d'espèces exotiques dans de nouveaux environnements impacte négativement les espèces natives.
C'est pourquoi, l'évaluation du régime alimentaire de *Apis cerana japonica*, espèce native du Japon, et de *Apis mellifera*, espèce introduite, est primordiale pour comprendre l'influence de cette dernière sur l'environnement japonais.

Afin de répondre à cela, cette étude analyse la diversité florale présente dans le miel de 36 ruchers d'abeilles japonaises et occidentales de différentes régions du Japon de août 2022 à novembre 2022. L'ADN est analysé par la méthode du métabarcoding et les séquences ADN sont associées à des taxons suite à un travail de bioinformatique. Chaque environnement entourant les ruchers est analysé pour extraire des données paysagères. Ces données ont servi à expliquer la richesse taxonomique présente dans les échantillons de miel.

Pour se faire, un modèle statistique GLMM a été mis en place afin de déterminer les variables qui expliquent au mieux la diversité florale. Parallèlement, cette dernière a été decrite par une analyse de communautés pour chaque espèce d'abeille afin de mettre en évidence : les espèces végétales les plus présentes, un possible chevauchement alimentraire entre les abeilles mellifères et leurs préférences alimentaires.

Au final, les résultats indiquent que les variables paysagères: indice de diversité de Shannon, *Integral Index of Connectivity* et densité totale de parcelles, sont celles qui expliquent le mieux la richesse taxonomique détectée dans les échantillons de miel. Ensuite, des espèces florales plus abondantes et présentes uniquement chez l'une ou l'autre espèce d'abeille ont été remarquées. *Apis cerana japonica* comptabilise principalement des espèces ligneuses et des essences telles que *Ulmus parvifolia* and *Ulmus* sp., *Hedera nepalensis* and *Chengiopanax sciadophylloides*, *Apis mellifera*, quant à elle, préfère les espèces herbacées: *Physalis angulata*, *Triticum aestivum* and *Cosmos bipinnatus*. Suite à l'observation d'un diagramme de Venn, peu des espèces végétales semblent être partagées entre les abeilles mellifères. Cependant, un G-test du choix alimentaire de *Apis cerana japonica* pour des espèces ligneuses ou herbacées en fonction de la cohabitation avec l'abeille occidentale montre une influence de cette dernière sur le comportement alimentaire de l'abeille japonaise. Ainsi, les différents résultats se contredisent et ne semblent s'expliquer que par le nombe limité d'échantillons qui ne permet pas de tirer des conclusions quant à la présence de compétition ou non ente les deux espèces d'abeille mellifères au Japon.

Mots clés : *Apis cerana japonica* - *Apis mellifera* - Metabarcoding sur miel - Bioinformatique - Variables paysagères - Diversité taxonomique - Préférence alimentaire - Japon

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Glossary

A. cerana japonica *Apis cerana japonica.*
A. cerana *Apis cerana.*
A. mellifera *Apis mellifera.*
AICc Akaike Information Corrected Criterion.
BCL Binary Base Call.
DBF Deciduous Broad-leaf Forest.
DHARMa residual Residual Diagnostics for Hierarchical Regression Model.
DNF Deciduous Needle-leaf Forest.
EBF Evergreen Broad-leaf Forest.
ENF Evergreen Needle-leaf Forest.
GLM Generalized Linear Model.
GLMM Generalized Linear Mixed Model.
IIC Integral Index Connectivity.
LULC Land Use and Land Cover.
MPM-seq Multiplexed microsatellite-PCR sequencing.
NGS Next Generation Sequence.
PCoA Principal Coordinate Analysis.
PCR Polymerase Chain Reaction.
PD Patch Density.
SHDI Shannon's Diversity Index.

I Introduction

1 Bees

i Pollinator and insect decline

Nowadays, populations of pollinating species, such as bees, flies wasps, beetles and butterflies, are globally declining (Ballantyne et al., 2017). These insects have a major importance for food production (Lamontagne-Drolet et al., 2019; Requier et al., 2015) as 88% of angiosperm species rely on animals to pollinate (Ollerton et al., 2011). The abundance and diversity of bee species are particularly affected (Park & Nieh, 2017). Anthropogenic activities are the main causes of this decline (Potter et al., 2019; Soorangkattan et al., 2021; Theisen-Jones & Bienefeld, 2016).

First, urbanisation is one of the main causes of this phenomenon (Potter et al., 2019). By reducing natural spaces, replaced by built surfaces and hard coverings, some insect families have not been able to adapt to changes in the environment. Added to this is the emergence of exotic plant species that are not appropriate to their diet (Fernandes et al., 2022).

Secondly, agricultural intensification also has a large responsibility in the decline of insect populations (Requier et al., 2015). This translates into reduced food resources (Haubruge et al., 2006; Horn et al., 2021), increased exposure to pesticides (Lamontagne-Drolet et al., 2019) and a loss of diversity of wild plant species on which insects depend (Haubruge et al., 2006; Rotheray et al., 2017).

In addition, changes brought by humans on its environment significantly disrupt services and functions of our ecosystems (Soorangkattan et al., 2021). And without bees, the pollination of flowers and crops around is compromised (Requier et al., 2015).

Eventually, many other factors are also responsible for the decline of pollinators: exotic parasites, diseases (Haubruge et al., 2006; Lamontagne-Drolet et al., 2019), climate change (Haubruge et al., 2006), the destruction of their habitats (FAO, 2018) and the importation of *Apis mellifera* into alien zones leading to the decline of other bees (Theisen-Jones & Bienefeld, 2016).

Finally, it is still important to note the ability of some species to continue to evolve in a deeply disturbed environment. For example, C. Chen et al. (2018) describes *Apis cerana japonica* as having the potential to respond positively to climate change. Or that several native bee species have adapted to urbanisation (Fernandes et al., 2022). And, the introduction of *A.mellifera* has helped to maintain native plant species (Park & Nieh, 2017).

ii *Apis mellifera* Linnaeus, 1758

Apis mellifera L., the Western honey bee has an enormous importance for our agriculture (FAO, 2018) to carry out pollination (González-Varo et al., 2013) and for the production of honey and other beehive products (Webster, 2019).

Classification and taxonomy

- KINGDOM Animalia
- PHYLUM Arthropoda
- CLASS Insecta
- ORDER Hymenoptera
- FAMILY Apidae
- GENUS *Apis*
- SPECIES *A.mellifera*

Biology and life cycle

A honey bee colony is characterised by a social structure in which each individual has a role and interacts with others (Devillers & Pham-Delegue, 2002). First there is the queen bee, which is unique in the colony and has the function of laying, then the workers, who embodies several tasks throughout their life, and finally, the males, so-called drones that serve just for the reproduction with the virgin queen (gyne) (Boguslavsky & Zakharov, 2021; Webster, 2019). Within the colony, 20,000 to 40,000 workers and zero to a few thousand drones are counted and there is only one queen (Page & Peng, 2001). Although bees, as individuals, are poikilothermic organisms, the colony is homeothermic. The workers will constantly regulate the temperature and humidity in the nest (Boguslavsky & Zakharov, 2021).

The life cycle of the bee is defined by four different stages: egg, larva, pupa and adult (Winston, 1987). Their development cycle lasts: 16 days for the queen (Devillers & Pham-Delegue, 2002), 21 days for the workers (Suwannapong et al., 2012) and 24 days for the males (Devillers & Pham-Delegue, 2002); depending on the season.

The queen is not able to feed herself or feed her larvae, care for the hive or protect it, her only functions are to lay eggs (Boguslavsky & Zakharov, 2021), mate and secrete hormones (Devillers & Pham-Delegue, 2002). Her life expectancy is much longer than workers and drones (Boguslavsky & Zakharov, 2021). On average, she lives at least up to 2 years (Devillers & Pham-Delegue, 2002; Page & Peng, 2001; Remolina & Hughes, 2008) and at most up to 4 years (Bodenheimer, 1937). Queens come from fertilised eggs which, at first, are not so different from workers' eggs. However, these eggs are laid in larger cells and fed differently than other females. Its larval development period is also shorter. Adult, the queen is twice as large and has a long abdomen, characteristic of a specialised anatomy for laying function (Page & Peng, 2001). In order to lay fertilised eggs, gynes must take her nuptial flight. Once fertilised, the queen returns to her colony where workers take care of the brood (Suwannapong

et al., 2012).

Workers have a much lower life expectancy than their mother, they live about one month for summer bees (Boguslavsky & Zakharov, 2021) and four months for those born in autumn (Page & Peng, 2001). Biotic and abiotic conditions can have a direct impact on survival rate. In fact, it decreases with the presence of parasites, pests or because of poor internal ventilation (Boguslavsky & Zakharov, 2021), high external heat (Tomlinson et al., 2015) or high humidity (Li et al., 2019).
Like queens, workers come from fertilised eggs (Boguslavsky & Zakharov, 2021). In the course of their life they evolve in their tasks, such as taking care of the larvae, cleaning the hive, collecting nectar, propolis and pollen, building the cells and defending the hive (Devillers & Pham-Delegue, 2002), and these age-related changes in individuals are induced by intrinsic and extrinsic factors. For example, workers become foragers around the age of two or three weeks (Page & Peng, 2001).

Drones are formed from unfertilised eggs, haploid gametes formed from a parthenogenesis (Tucker, 1958). These are laid in cells larger than those of workers, allowing the queen to know what she must lay (fertilised eggs or not) (Boguslavsky & Zakharov, 2021). Males do not live longer than few months (Boguslavsky & Zakharov, 2021) or even few weeks (Devillers & Pham-Delegue, 2002), either they die directly after copulation or they are rejected from the colony in the early fall (Boguslavsky & Zakharov, 2021; Winston, 1987).

Competition with native pollinator species

From a general point of view, honey bees are considered essential to our biodiversity and economy (Abrol, 2011; Goulson, 2003). Its role as a pollinating insect and honey producer has led many people to introduce it to foreign countries (Goulson, 2003). In addition, due to urban sprawl and intensive agriculture, *Apis mellifera* have been widely distributed throughout the world and thanks, among other things, to their ability to adapt in these anthropized environments (Donkersley et al., 2021). Or has been displaced by beekeepers in protected areas, welcoming already wild bee species (Ropars et al., 2022). Unfortunately, sometimes, the population density of introduced bees is so high that the deleterious effects of their presence are equally so (Abrol, 2011). The presence of the Western honey bees is not always positive for our environment and can affect native pollinator species.

First, *A.mellifera* compromises access to the food resources of other pollinators in the environment in which it is introduced. According to Abrol (2011), the different species overlap in terms of the period of the year in which they are active and, although this does not represent evidence of competition between species, it seems likely that the reduction of food resources at the same time of year by introducing bees is negative for native bee species. This hypothesis is confirmed by the study by Ropars et al. (2022) which indicates that competition for floral resources is present in early spring and decreases over the season.

Next, it was observed that the pressure induced by the presence of *Apis mellifera* is increased in areas that have been heavily disturbed by humans. This makes competition for nectar and pollen from native flowers more intense and promotes the pollination of exotic vegetation (Kato et al., 1999), which is just as problematic as the pressure placed on native bee species.

It is important to note that honey bees are often advantaged compared to wild species because they are fed with syrup during a shortage of floral resources (Abrol, 2011). Conversely, wild bees have to adapt and sometimes change their feeding behaviour. They will, for example, avoid plant species and review their floral preferences with the presence of *Apis mellifera* (Ropars et al., 2022).

Then, the reduction of food resources is not the only pressure put by the introduced honey bee on native bees; competition for nesting, the transmission of parasites and exotic diseases (Goulson, 2003).

There is also a competition between *A.cerana japonica* and *A.mellifera* but this part will be developed in later section.

Natural habitat

Apis mellifera and *Apis cerana* are the only species of *Apis* that can be domesticated by humans. Having a hidden natural nesting behaviour, unlike the species of dwarf or giant bees that nest in the open (Suwannapong et al., 2012), it makes it easy to introduce bees into hives and manipulate them to benefit from their productions (Yadav et al., 2017). And main reasons that will push beekeepers to adopt the practice are: the source of income and the leisure that this represents (Dzierżon, 1882).

Apis mellifera is originally widely scattered throughout the world: in Africa, Europe and the Middle East. From these different regions stand out five main clades of *A.mellifera* due to genetic variations between them (Webster, 2019). Currently, *A.mellifera* is also found in Asia where it was introduced in 1877 for its high yield in honey production (Wang & Tan, 2014).

iii *Apis cerana japonica* Radoszkowski, 1877

Classification and taxonomy

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- ORDER Hymenoptera
- FAMILY Apidae
- GENUS *Apis*
- SPECIES *A.cerana*

- SUBSPECIES *A.cerana japonica*

Biology and life cycle

Apis cerana japonica is very similar to *Apis mellifera* in terms of colony structure and biology (Suwannapong et al., 2012; Theisen-Jones & Bienefeld, 2016). For example, they have in common the construction of their nest which is done in cavities with parallel rays (Suwannapong et al., 2012). However, the Asian honey bee is smaller in size and number in a colony, from 2,000 to 20,000 bees compared to 50,000 for *A.mellifera* (Koetz, 2013). In addition, it has developed a more advanced hygiene behaviour against the parasitic mites *Varroa jacobsoni* and *Varroa destructor* than its Western counterpart (Büchler et al., 1992; Theisen-Jones & Bienefeld, 2016). *Apis cerana japonica* has grooming behaviour resulting from exogenous stimuli and genetically endogenous processes that activate this behaviour (Diao et al., 2018).

The life cycle is similar to that seen above, all bees go through a complete metamorphosis in four stages (Suwannapong et al., 2012; Wilson & Jamieson, 2019). Concerning the structure within the colony, like *A.mellifera*, *A.cerana japonica* possesses three castes: one queen, a hundred drones and several thousand workers (Suwannapong et al., 2012). The queen is the only fertile female, her daughters are unfertilized and can only lay males (Devillers & Pham-Delegue, 2002).

Unlike the Western bee, the Asian swarms at longer periods in the year. Swarms were observed for five months, from April to August, and several times by the same hives (Sugahara, 2000). The reasons that will drive bees to swarm are mainly the shortage of food resources, especially pollen (Hepburn & Radloff, 2011), which differs from *A.mellifera* (Table 1).

Table 1: Comparison table of biological characteristics differentiating between both species, *A.cerana japonica* and *A.mellifera*.

	Apis cerana japonica	***Apis mellifera***	**References**
Number of individuals in one colony	2,000 to 20,000 bees (1)	30,000 (2) to 50,000 bees (1)	(1)(Koetz, 2013) (2)(Bodenheimer, 1937)
Time of the year swarm occurs	From April to August (1)	From late spring to early summer (2)	(1)(Sugahara, 2000) (2)(Seeley et al., 1999; Ramsey et al., 2020)
Reasons of swarming	Mainly the shortage of food resources (1)	Mainly because the colony outgrows (2)	(1)(Hepburn et al., 2011) (2)(Seeley et al., 1999)

Competition between *A.cerana japonica* and *A.mellifera*

Honey bees, *A.mellifera* and *A.cerana japonica*, can mutually impact each other's habits through their presence. It seems that there is no real competition for food resources between both honey bees (Mohamadzade Namin et al., 2022; Nagamitsu & Inoue, 1999) (Table 2). Floral preferences do not overlap interspecies, competition is more intraspecific at the beginning and end of the season. *A.cerana japonica* promotes pollen from tall trees, where *A.mellifera* prefers short grasses, demonstrating the difference in location of resources (Nagamitsu & Inoue, 1999). In addition, *A.cerana japonica* goes foraging earlier in the day

than *A.mellifera*, when temperatures are lower. Indeed, the Western honey bee needs a higher chest temperature to be able to feed (Tan et al., 2012).

However, Theisen-Jones and Bienefeld (2016) notes that there are tensions between the two species. They steal each other's honey between hives when they are close physically, with *A.mellifera* more aggressive and effective than *A.cerana japonica*. Moreover, even if they are remote, they can be harmful to each other by a transmission of pathogens: fungi like *Nosema ceranae* and parasites like varroa (Theisen-Jones & Bienefeld, 2016).

Table 2: Summary table of food preferences between *A.cerana japonica* and *A.mellifera*.

	Apis cerana japonica	***Apis mellifera***	**References**
Floral preferences	Tall trees	Short grasses	((Nagamitsu et al., 1999)
Peak of foraging activity at different temperatures [°C]	(1) 10:00 at 10°C (2) Between 09:00 and 11:3 at 15.5 to 21°C	(1) 11:30 at 20°C (2) Between 11:00 and 13:30 at 21 and 25°C	(1) (Tan et al., 2012) (2) (Verma et al., 1986)
Chest temperature needed at an ambient temperature of 6°C [°C]	21.8±0.23	23.6±0.22	(Tan et al., 2012)

Natural habitat

Physical demographic factors, such as mountain ranges and channels, are responsible for genetic differentiation in *A.cerana*. This is considerable and is at the level of the subspecies of the Asian bee (Hepburn & Radloff, 2011; Radloff et al., 2010). Six morphoclusters are presented in the book of Hepburn and Radloff (2011) and Radloff et al. (2010), differentiating the subspecies of *Apis cerana* according to their region of origin. In addition, genes linked to environmental adaptation (Radloff et al., 2010), especially between tropical and temperate subspecies (Koetz, 2013), have been identified (C. Chen et al., 2018). The different genetic strains differ in their ecology and behaviour (Koetz, 2013).

Beekeeping has been practised in Asia for centuries to take advantage of the products of the hive and pollination delivered by bees (Stanley et al., 2017; Tanaka et al., 2020). That is why beekeeping companies are widely found there. These can be done on a small scale or more intensively; stationary or migratory. This is why there are multiple structures of hives, variable between them, unlike the conformed hives of the West (Theisen-Jones & Bienefeld, 2016).

Apis cerana japonica is native to climatic zones such as rainforests, savannahs, steppes, meadows and taigas (Radloff et al., 2010). Currently, it is almost exclusively present in its natural distribution zones (Donkersley et al., 2021). *A.cerana japonica* populations are increasing in urban areas (Sugahara, 2000). Bees are extremely important for the pollination of Asian ecosystems (Tanaka et al., 2020; Yadav et al., 2017), thanks in particular to their wide distribution in various climates (C. Chen et al., 2018).

iv Foraging

Foraging behaviour

As presented above, the floral preferences between *A.mellifera* and *A.cerana japonica* may differ, although there is a food overlap. Western honey bees appear to prefer exotic plant species in a context where the insect has been introduced to a foreign country, and Japanese honey bees focus primarily on native plants (Tatsuno & Osawa, 2016). This observation changes with the seasons, when resources begin to become scarce, native plants can be replaced by horticultural and naturalized plants (Lowe et al., 2022; Park & Nieh, 2017).

Certain physiological characteristics of pollinators may be responsible for the floral choice. The length of their tongue is shorter in Japanese bees, it is 6.36 mm for *A.mellifera* and 5.19 mm for *A.cerana japonica*. However, the presence of a food overlap is explained by the almost identical body size of the two honey bee species (Tatsuno & Osawa, 2016) and for both Apis species, there are more nectar-collecting foragers than pollen (Verma & Dulta, 1986). Moreover, both honey bees do not share the same preferences in the harvested floral products; colour and nectariferous flowers preference play a role in their selection of plant species for foraging (Tatsuno & Osawa, 2016).

The patch that will be visited by all the foragers of the same colony is chosen according to the plant species present. Both honey bees prefer to focus their research efforts on healthy, nutritious and abundant plant species (Lau et al., 2019). Time also plays on the quality of the patches visited. In early spring, bees choose small flower beds that are close by, while during drought and floral scarcity, they are more dispersed and remote (Park & Nieh, 2017). Thus, bees select flower patches. They prefer to focus on rich sources when the season is good and visit a wide range of resources in case of food shortages (Seeley, 1986).

To assess the quality of nectar and pollen harvested, foragers use the rate of uptake of the resource by their colony as a benchmark. Thus, when the quality of a flower bed decreases, the foragers will reduce the number of visits and start looking for other patches (Seeley, 1986).

This is the reward that the forager will get from it that guides the floral choice. The quantity and quality of nectar or pollen harvested is a factor in the decision whether or not to forage this plant patch (Tatsuno & Osawa, 2016). In fact, foragers go on tour only when they can get the greatest food reward. The activity of the bees is stronger in the afternoon than in the morning, when the flowering of the flowers is the most optimal. Temperature plays an important role here, as it has been observed that foragers emerge more when the mercury rises to 20 and 28°C. Conversely, the opposite phenomenon is observed when temperatures are below 15°C and above 30°C (Ghosh et al., 2020).

Honey bees share the location of interesting food sources and water points with other foragers through the dance language (Dyer & Seeley, 1991; Webster, 2019). The time of the

dance, the vibrations and ripples, the orientation of the curves, etc. serve as indications to other bees as to the location of interesting plant species (von Frisch & Lindauer, 1956). It has even been observed that different dialects in dance exist between the various subspecies of *Apis cerana* in Asia (Dyer & Seeley, 1991).

Nutritional requirements

Honey bees collect pollen, which is necessary for its proteins and lipids, and nectar, which is rich in carbohydrates. It is the micronutrient ratio that will guide the selection of bees for a particular plant species (Ghosh et al., 2020). They will have to cope with the interspecies variability in essential nutrients of these plants (Ghosh et al., 2020; Vanderplanck et al., 2014).

However, amino acid content remains the primary driver of choice (Cook et al., 2003; Ghosh et al., 2020; Vanderplanck et al., 2014), as proteins represent a range from 2.5% to 61% of pollen content (Roulston et al., 2000). Protein and amino acid content are essential for growth (Ghosh et al., 2020; Noël et al., 2023), somatic maintenance (Cook et al., 2003; Ghosh et al., 2020), health (Lamontagne-Drolet et al., 2019; Noël et al., 2023) and reproduction of individuals (Ghosh et al., 2020; Vanderplanck et al., 2014). Early season nutritional deficiency can have an irremediable impact on the size of individuals and the reproductive capacity of the queen (Rotheray et al., 2017).

Both honey bees, *A.cerana japonica* and *A.mellifera*, belong to the group of polylectic species, meaning that they need to collect pollen from many plant families, as opposed to oligolectic bee species (Vanderplanck et al., 2014). And although considered supergeneralists in their food choices, some species and plant groups are indispensable (Hawkins et al., 2015). Pollinators will prefer pollen collection from one plant species to another species because of the quality of the amino acids contained in (Cook et al., 2003; Hanley et al., 2008; Roulston et al., 2000). Thus, pollen diversity and quantity play an indispensable role in the diet of honey bees (Hanley et al., 2008; Noël et al., 2023) to meet their high energy needs (Abrol, 2011).

Foraging plant choice: in urban conditions

Regarding the decline of pollinators and their foraging needs, it will be interesting to analyse the reactions of bees.

Several studies indicate that honey bees and other pollinators have a more diverse diet in urbanised areas (Ayers & Rehan, 2021; Fox et al., 2022; Richardson et al., 2021; Wilson & Jamieson, 2019). Urban and peri-urban sites have the greatest pollen diversity and higher temporal renewal (Richardson et al., 2021). Through landscapes composed of more diverse and highly heterogeneous plant communities, urban areas provide a greater number and variety of pollinator habitats and niches (Fox et al., 2022). Bees can obtain pollen and nectar from patches of wildflowers(Potter et al., 2019) or gardens, which are a primary food resource and nesting sites for pollinators (de Vere et al., 2017; Lowe et al., 2022) and exotic plants (Wilson & Jamieson, 2019). However, it appears that different urban, suburban or

rural areas do not explain the richness of plant taxa visited by honey bees (Noël et al., 2023; Wilson & Jamieson, 2019), nor the total abundance of plant species (Wilson & Jamieson, 2019).

Nevertheless, it is important to remember that urbanisation remains a reason for the decline of pollinators (Ayers & Rehan, 2021; Potter et al., 2019). This is why creating and making available flowering strips, patches of wildflowers (Potter et al., 2019) or aboveground resources (Wilson & Jamieson, 2019) is essential and can provide the necessary resources for pollinators.

Moreover, it remains clear that urbanisation significantly changes resource availability and pollinator diversity (Lau et al., 2019). Insects are filtered through interaction with local characteristics (Ayers & Rehan, 2021; Wilson & Jamieson, 2019). In particular, specialised species, large insects (Ayers & Rehan, 2021), soil-nesting pollinators (Ayers & Rehan, 2021; Wilson & Jamieson, 2019), eusocial and native bees (Wilson & Jamieson, 2019) are disadvantaged or even erased from the urban landscape.

Foraging plant choice: in rural conditions

Access to food sources is a growing problem in agricultural settings (Horn et al., 2021). Pollinator declines threaten pollination of field crops and wildflowers (Requier et al., 2015). In addition to urban areas, it is important to assess pollinator responses to environmental change.

Monocultures and rotations of field crops such as cereals, colza, maize and sunflower have a detrimental impact on honey bee colonies. Through gaps and lack of synchronisation in the food supply, pollinators are weakened and gradually lose their resilience. In contrast, rotations such as rye, dandelion, clover, sunflower and phacelia or colza, buckwheat and phacelia have been found to provide a continuous supply of nectar and pollen and to ensure colony viability (Horn et al., 2021).

In addition, despite the presence of improved grasslands with rich floral diversity, bees do not appear to benefit from a broad floral diversity due to the general loss of diversity by intensive farming (Fox et al., 2022). In rural areas, nectar resources come mainly from crops, while pollen comes from a wide variety of herbaceous and woody species such as trees and weeds. These constitute a large part of the diet of bees between periods of massive flowering and a source of floral diversity dependent on the composition of the local landscape (Requier et al., 2015). Forests and national parks also provide support for the food needs of honey bees (Potter et al., 2019).

Across season

The timing of the choice or nutritional needs of honey bees has already been noted several. Availability and nutritional requirements depend on the seasons and influence the diet of bees at times (Khan et al., 2021; Leponiemi et al., 2023; Park & Nieh, 2017; Seeley, 1986).

As presented earlier, honey bees have different needs between summer and winter for ingested micronutrients (Bonoan et al., 2017; Khan et al., 2021). The annual development cycle of the colony depends on nutritional resources and adapts accordingly. This development cycle will not take place in the same way between temperate and tropical regions. In cold climates, the colony reduces breeding in autumn in preparation for wintering and starts again in spring, in parallel with the floral resources. While in warm climates, brood is active throughout the year (DeGrandi-Hoffman et al., 2021).

The reasons are not always related to bees demand but also to seasonal availability and plant phenology (Donkersley et al., 2021; Lowe et al., 2022; Tanaka et al., 2020). In particular, the mass of pollen or nectar harvested varies throughout the year (Requier et al., 2015). Floral diversity also depends on time; in spring, greater plant richness and woody taxa are observed (Lau et al., 2019; Noël et al., 2023), while in summer and autumn, they are mostly species of the herbaceous taxa (Noël et al., 2023).

2 Pollen analysis

The identification of pollen grains in honey can be used for a variety of purposes, including determining the geographical origin of honey, preferences, diet (Milla et al., 2021) and feeding behaviour of honey bees (Mohamadzade Namin et al., 2022). Many approaches can be used but, as far as this work is concerned, metabarcoding will be explained. Indeed, this technique provides useful information in determining the most abundant floral components visited by both honey bee species in a given area (Hawkins et al., 2015; Kamo et al., 2018; Mohamadzade Namin et al., 2022; Potter et al., 2019).

Barcoding and metabarcoding are robust and effective methods for identifying honey pollen components and nectar (Leponiemi et al., 2023; Milla et al., 2021; Mohamadzade Namin et al., 2022; Tanaka et al., 2020). Both are based on DNA identification of species contained in a sample (de Vere et al., 2017). The stages of analysis of its techniques are: DNA extraction; DNA amplification through PCR and high-throughput sequencing only for metabarcoding method; and analysis of the results via a suitable database and a bioinformatics work (Bell et al., 2016). The characteristic of barcoding is that it allows the identification of a particular species by a standard genetic marker during the amplification stage (Coissac et al., 2012).

Conversely, the amplification of metabarcoding is done via universal genetic markers with high-throughput sequencing (Next generation sequencing technology) (de Vere et al., 2017; Mohamadzade Namin et al., 2022). This allows DNA analysis of several different species contained in a sample (de Vere et al., 2017). The most commonly used universal markers for plant identification are: *rbcL, matK, trnL* and *trnH-psbA* from the chloroplast genome (Bell et al., 2016; de Vere et al., 2017) and the nuclear ribosomal ITS2 region (Bell et al., 2016).

This method allows for better taxonomic discrimination, reduces analysis time, increases

sample size, and does not require palynological experts (de Vere et al., 2017; Hawkins et al., 2015). In addition, Milla et al. (2021) indicates that its results between metabarcoding and melissopalynology are similar in relation to the number of families and genera detected in the samples but with better resolution at the specific level with the DNA metabarcoding method.

On the other hand, there are also disadvantages to this method such as: the excessive amplification of certain species due to the choice of genetic marker; the degeneration of DNA by time and conservation methods (Coissac et al., 2012); false positive results in taxa assignment (Fox et al., 2022); inability to identify taxa due to under-representation of some taxa in reference databases (Saravanan et al., 2019; Tanaka et al., 2020); the lack of detection of low-level DNA density in the sample (Hawkins et al., 2015); and unreliable in terms of quantification (Hawkins et al., 2015; Richardson et al., 2015). For these reasons, it is important to avoid all sources of sample deterioration and to handle them with care (Kamo et al., 2018; Liu et al., 2020).

3 Objectives

Human influence has a disastrous impact on many species around the world. In particular, the introduction of the exotic species *Apis mellifera* in Japan to benefit from their productions had an impact on *Apis cerana japonica* (Abrol, 2011). Understanding the influence of its introduction is essential to be able to respond correctly to the challenges it generates. As explained above, the behavior of the Western bee affects native species such as *Apis cerana japonica* by the use of their food resources (Ropars et al., 2022), by the grabbing of their habitat (Abrol, 2011) and by the installation of pressure (aggressiveness, diseases, etc.)(Goulson, 2003; Theisen-Jones & Bienefeld, 2016).

In matter of fact, the Western honey bee is also one of the most studied bee species and can easily be used for research (Wood et al., 2020). Conversely, *Apis cerana japonica* remains relatively poorly handled compared to its Western colleague (Hepburn & Radloff, 2011). As well as honey metabarcoding, working on *Apis cerana japonica*'s honey is an innovative aspect.

The environment around apiaries is a factor that can significantly impact the taxonomic richness foraged by honey bees (Fox et al., 2022; Potter et al., 2019; Requier et al., 2015). This is why it is necessary to integrate landscape variables into the analysis. The different types of habitat, but also landscape metrics are potentially sources of change and decision in the foraging choice of honey bees.

The hypotheses of this work are that *Apis mellifera*, although having a very wide floral range, does not compete with *Apis cerana japonica* since both honey bee species do not share the same food preferences. In addition, landscape variables impact the floral composition found in honey

This study aims to assess the taxonomic richness of the flora visited by *Apis cerana japonica* and *Apis mellifera* in Japan. Floral diversity will be linked to landscape variables and a community analysis will be conducted to determine if there is competition between both honey bee species. The goal of the approach is to observe a possible food overlap between both honey bee species depending on their foraged plant taxa diversity and a potentially change in the dietary behaviour of *Apis cerana japonica* because of the cohabitation or not with *Apis mellifera* colony.

II Materials and methods

1 Study area

The aim of this study is to understand the flora visited by *Apis cerana japonica* in comparison with that foraged by *Apis mellifera* and to assess a likely competition between them. Japan is an island state of 377,972 km² consisting of over 10,000 islands, the four largest islands are Hokkaidō, Honshū, Shikoku and Kyūshū. The country is located in East Asia, between the Pacific Ocean and the Sea of Japan (GSI, 1990). Its territory comprises almost 75% of mountains, hills and volcanoes, at the foot of which are inhabited valleys, cultivated or composed of forests (GSI, 1990; OECD, 2002).

Bimodal temperatures are observed throughout the country, with annual averages varying from 27.5°C to Hokkaidō and 17.5°C to Kyūshū. Most of its territory is in a temperate climate but some areas are in a subtropical climate. High annual relative humidity, between 54% and 88%, is observed throughout the territory, especially between June, July and August (GSI, 1990).

The Japanese land use ratio from 1987 indicates that forests and cropland are the two largest occupations in the territory, followed by water, rivers and streams by far (GSI, 1990).

i Experimental design

We followed 36 apiaries which provided 46 honey samples, each from a different region in Japan. Thirty of them gave honey samples from *Apis cerana japonica* colonies only and 16 provided honey from both species, *A. mellifera* and *A. cerana japonica* (Table 3). Samples were collected from August 2022 to November 2022. Samples from *A. mellifera* honey were collected once a year, mostly in September. Concerning *A. cerana japonica*, the collection can be done three or five times a year from August till November.

Table 3: Honey collection from apiaries of *Apis cerana japonica* and *Apis mellifera*. Sources : Chiba University, Japan.

Japanese honey samples	**Japanese collection dates**	**Western honey samples**	**Western collection dates**
A-1	28-09-22	B-1	21-09-22
A-2	04-09-22		
A-3	26-09-22		
A-4	28-08-22	B-2	04-09-22
A-5	10-08-22		
A-6			
A-7	04-11-22		
A-8			
A-9	30-09-22		
A-10	25-09-22		
A-11			
A-12	17-10-22		
A-13	29-09-22		
A-14			
A-15	14-09-22	B-3	14-09-22
A-16	14-10-22		
A-17			
A-18	26-10-22		
A-19	10-09-22		
A-20			
A-21	03-11-22		
A-22	10-09-22		
A-23	22-10-22		
A-24	28-09-22		
A-25	15-09-22		
A-26			
A-27	01-10-22		
A-28	02-09-22		
A-29	07-11-22		
A-30	22-09-22	B-4	20-09-22
A-31			
A-32	20-09-22	B-5	22-09-22
A-33		B-6	
A-34			
A-35	30-09-22	B-7	25-05-23
		B-8	25-08-23
		B-9	14-09-23
		B-10	02-10-23
A-36			

Honey samples were from apiaries located all over Japan, from the South in Kumamoto prefecture to the North in Iwate prefecture. In total, the samples are spread in 12 different zones with nine of the samples were from Tokyo and four from Chiba prefecture. Collection was done independently of the urban to rural gradient (Figure 1).

250 ml of honey was requested from beekeepers and 15 ml was used for analyses. Fresh honey collection is an important step to secure a good pollen analysis (Layek et al., 2020). Therefore, the beekeepers were asked to send honey immediately after harvest to Chiba University, Japan. Once the honey was collected, it was stored in a 4°C fridge at Chiba University, Japan.

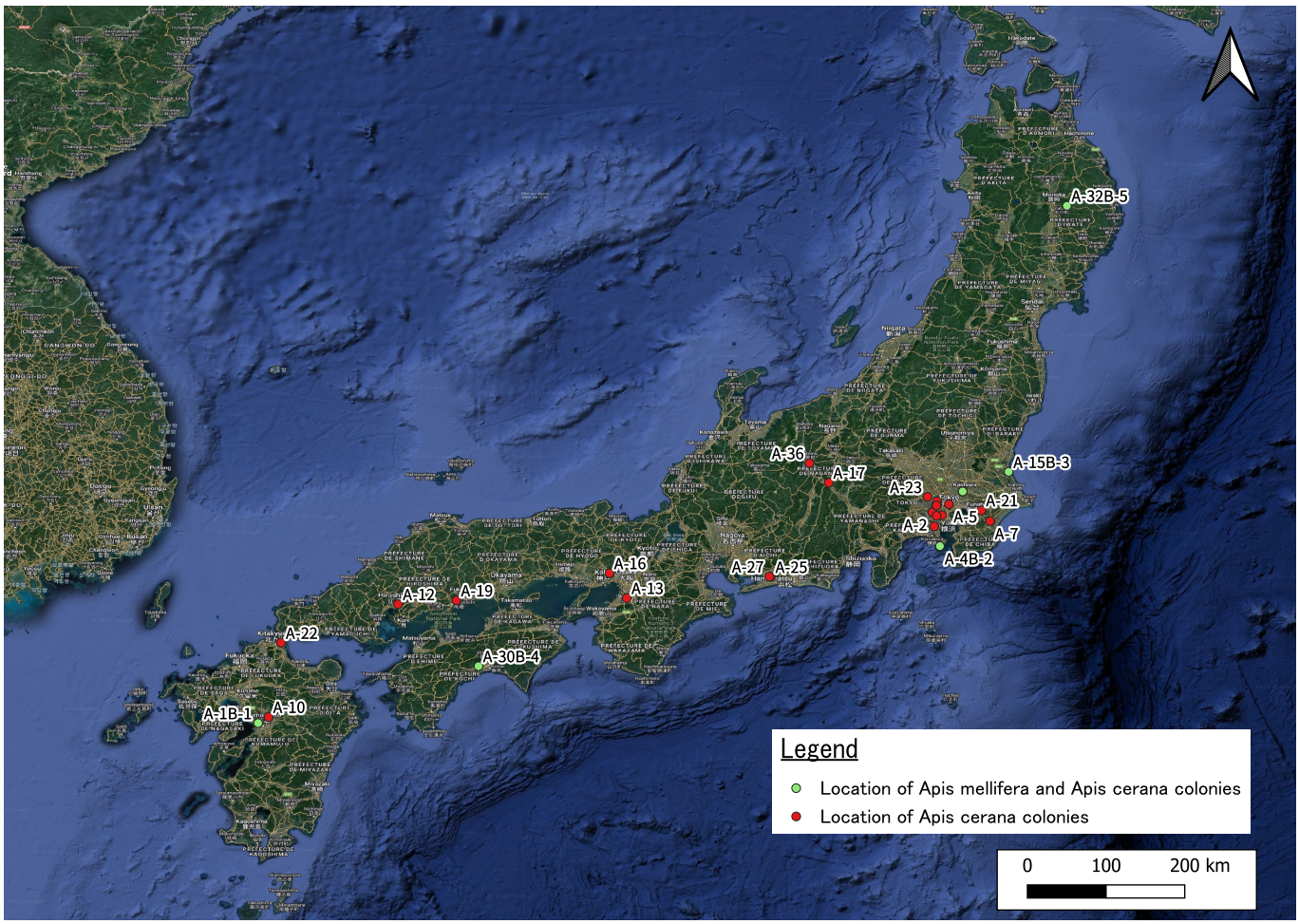


Figure 1: Distribution of apiaries in Japan. In legend, green dots represent apiaries of *A. cerana japonica* and *Apis mellifera* in cohabitation and red dots are the location of *A. cerana japonica* apiaries. Sources: Chiba University, Japan; and Google Hybrid. Date: December 2023.

2 Cartography analysis

Each apiary was located on a map covering all of Japan. One layer Land Use & Land Cover (LULC) with 10 m resolution (2018-2020) (Earth Observation Research Center (EORC), Japan Aerospace Exploration Agency (JAXA)) was analysed to define the different landscape areas around apiaries (Figure 2), such as: (1) water bodies, (2) built-up areas, (3) paddy fields, (4) cropland, (5) grassland, (6) deciduous broad-leaf forest (DBF), (7) deciduous needle-leaf forest (DNF), (8) evergreen broad-leaf forest (EBF), (9) evergreen needle-leaf forest (ENF), (10) bare areas, (11) bamboo forest and (12) solar panel areas.

To do so, a 500-metre perimeter of action has been defined to analyse the structure of the landscape around the apiaries via RStudio software with *"sf"* package. The value of buffers radius was defined as the foraging distance flight distance of *Apis cerana japonica* (Dyer and Seeley, 1991; Koetz, 2013). The result of this first step of the landscape analysis gave 32 raster files containing all the information related to the LULC of each 500-metres buffer around the apiaries.

These raster files were then exploited on RStudio with the *"lconnect"* (Mestre and Silva, 2023) and *"landscapemetrics"* (Hesselbarth et al., 2019) packages in order to derive landscape variables such as the Integral Index Connectivity (IIC) or the Patch Density (PD).

Two tables containing the values of each landscape metrics were generated. Each LULC of each 500-metres buffer around the apiaries was analysed, giving a table as a first column: the identifier of the apiary; second column: the number of the LULC layer (from 1 to 12), and the following column corresponds to the landscape variables : SLC – Area of the largest group of interconnected patches (Pascual-Hortal and Saura, 2006), CCP – Class coincidence probability (Pascual-Hortal and Saura, 2006), LCP – Landscape coincidence probability (Pascual-Hortal and Saura, 2006), CPL – Characteristic path length (Minor and Urban, 2008), ECS – Expected component (or cluster) size (Fall et al., 2007; O'Brien et al., 2006) and IIC – Integral index of connectivity (Pascual-Hortal and Saura, 2006). It is important to specify that each of the 500-metres buffer zones around the apiaries does not necessarily have the 12 LULC present on the layer.

The second table includes the values of landscape metrics, such as : the mean of patch area, the effective mesh size, the number of patch, the patch density, the total area, Shannon's diversity index, Simpson's diversity index, Simpson's evenness index and Shannon's evenness index (Hesselbarth et al., 2019). Therefore, every line represents an apiary following with all landscape metric values.

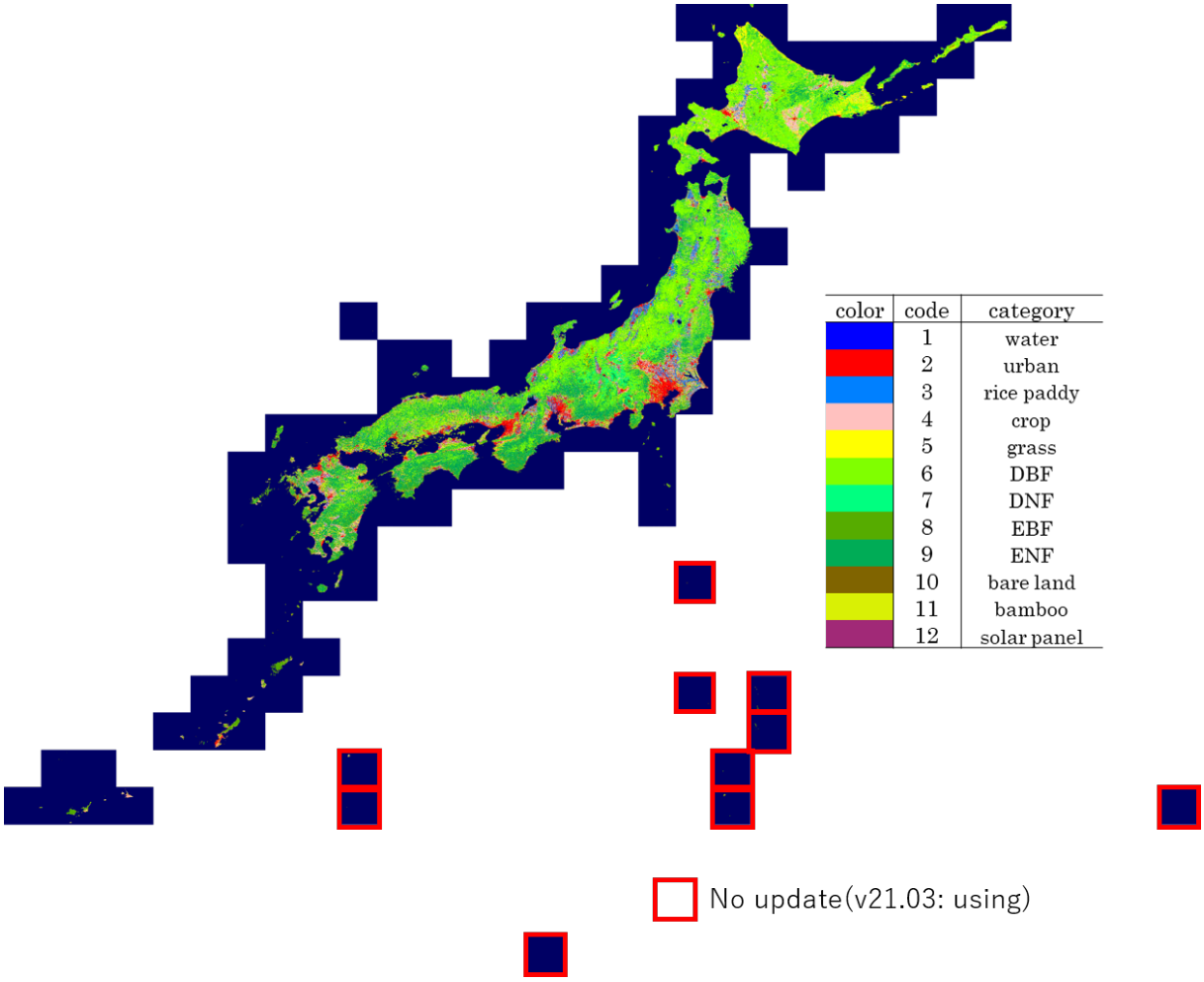


Figure 2: Land Use and Land Cover Map for Japanese territory. Sources : Earth Observation Research Center (EORC) and Japan Aerospace Exploration Agency (JAXA). Legend: (1) water bodies, (2) built-up areas, (3) paddy fields, (4) cropland, (5) grassland, (6) deciduous broad-leaf forest (DBF), (7) deciduous needle-leaf forest (DNF), (8) evergreen broad-leaf forest (EBF), (9) evergreen needle-leaf forest (ENF), (10) bare areas, (11) bamboo forest and (12) solar panel.

3 DNA analysis

Concerning DNA extraction, it is important to notify that the analyses were done by professional external laboratories: (i) BioInsight Co., Ltd, Analysis Department, Japan for the DNA extraction and; (ii) Tohoku University, Japan for library preparation and sequencing. The procedure, described below, follows that presented by Suyama et al. (2022) in its article "Complementary combination of multiplex high-throughput DNA sequencing for molecular phylogeny".

i DNA extraction

About 15 ml of honey (20-25 g when solidified) was added to a 50 ml Falcon tube with sterile ultrapure water. This mixture was then heated to 50°C for 30 minutes until the honey was completely dissolved. The dissolved honey was centrifuged to remove impurities, and the pellet containing the pollen was collected. Since the properties of the pollen cell wall vary between different plant species, the collected pellet has undergone pre-treatment steps such as crushing to efficiently extract DNA. Then, a complete DNA extraction kit (NucleoSpin Food) was used to extract the whole genomic DNA.

ii Library preparation and sequencing

Multiplexed microsatellite-PCR sequencing (MPM-seq) Five genetic markers were used to create the MPM library. Three of the genetic markers are located on the chloroplastic genomic regions: *psbAtrnH, rbcLa and trnL*; and two of the internal nuclear transcribed spaces: ITS1 and ITS2. They were amplified simultaneously using the MPM-seq primer set for plants.

A first PCR is performed using the Multiplex PCR Assay kit Ver. 2 (Takara Bio, Kusatsu, Japan) and primers with tail sequences, various lengths of N-bases, and locus-specific sequences (Cheng et al., 2016; Kress et al., 2009; Sang et al., 1997; Taberlet et al., 1991). This was carried out under specific conditions : Initial activation at 94°C for 1 min; 27 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 1 min, and extension at 72°C for 1 min; followed by final incubation at 72 °C for 10 min. Following the first PCR, its products were purified and balanced, and the short fragments (approximately <250 bp) were removed using AMPure XP (Beckman Coulter, Brea, CA, USA).

A second PCR is made from the purified products of the first PCR following a protocol similar to Multiplexed ISSR-PCR and microsatellite-PCR sequencing (MIG-seq). However, it comprised a double indexing system with forward and reverse primers, with five and nine bases, respectively.

Thirdly, the products of the second PCR were mixed and purified, and the short fragments (about <250 bp) were removed using AMPure XP (Beckman Coulter).

Then a next generation sequence (NGS) was made from an Illumina MiSeq platform and MiSeq Reagent Nano Kit v2 (500 cycles; Illumina, San Diego, CA, USA). Sequences were read using the peer-end sequencing method (Reads 1 and 2) for fragment ends and the index method (Index-1 and -2 reads) for indexes. Finally, the first three bases of the anchoring of the second PCR primers were excluded in readings 1 and 2 thanks to the "DarkCycle" option of the MiSeq Control Software (Illumina).

4 Bioinformatics works

The research method follows the BioInsight Co., Ltd, Analysis Department, Japan procedure. Following the creation of a library, the DNA was analysed via bioinformatic work on the software Claident pipeline 0.2.2019.05.10 (Tanabe and Toju, 2013).

Firstly, Binary Base Call (BCL) data is converted into FASTQ data format via the *BCL2FASTQ* program provided by Illumina. It allows a clearer reading of the DNA sequences.

Secondly, samples of raw FASTQ reads were demultiplexed. They were divided following the index sequences and primers used for the identification of individual samples, using the *clsplitseq* command (Tanabe and Toju, 2013).

Thirdly, quality filtering and sequence concatenation were carried out. The command *clconcatpais* was used to concatenate both pair-end (Reads 1 and 2) which were overlapping in the ITS2 region. Then, sequences with a low-quality score, with 10% of low-quality positions (Phred quality score, Q, <27), were deleted. Artificial padding sequence (ACGTACGTACGTACGT) was added between quality-filtered Reads 1 and 2 to concatenate the amplified regions in the cpDNA region, because it was too long to be merged. Low-quality Reads (containing > 10% of <Q27 bases) or shorter than 210 bp were filtered. Moreover, an elimination of noisy or chimeric sequences was applied thanks to the *clcleanseqv* program, using recommended parameters. The remaining reads are grouped into operational taxonomic units (OTUs) based on 99% sequence similarity. A representative Operational Taxonomic Unit (OTU) is chosen for each individual.

Fourthly, sequences are aligned using MAFFT 7.313 software (Katoh and Standley, 2013). Alignment columns with gaps are removed using a heuristic selection method based on similarity statistics of trimAl 1.4.rev15 (Capella-Gutiérrez et al., 2009).

Finally, a suitable nucleotide substitution model is determined using Kakusan4 (Tanabe, 2011). Phylogenetic trees based on the maximum likelihood method are constructed using RAxML 8.2.10 (Stamatakis, 2014), with 1000 parallelized bootstrap search repetitions to evaluate the robustness of phylogenetic groups.

i Quality control

The data was exported to an Excel file (.csv). The OTU data was classified into three columns; samplename (the sample name), nb_hit (the sample occurrence number) and seq_dna (the DNA sequence). Taxonomic data contains the classification of each sample down to variety. This data file is completed with the missing values in the species column with the name of the genus plus "sp.". Or no identification was possible, in this case it is indicated "Undetermined".
Once the files were completed, the "OTU" data and the "Taxonomy" data were joined by the RStudio software. The *"tidyverse"* package was used, as well as its function *left_join()* to merge the files by "samplename" column (Wickham, 2014). Indeed, both files had the same first column : the sample name.
Following the merger, the "samplename" column was separated into three: obs_name (the observation name), sample and primer.

To create a matrix of data, every files were merged into one file for data based on 95% of certainty and based on 97% of certainty, that is, it has been affiliated to each OTU to a taxa with a clustering threshold of 95% and 97% certainty from a reference database (Ammon et al., 2018; Falentin et al., 2019; Klymus et al., 2017; O'Rourke et al., 2020). From this general matrix, different tables could be created to match the needs of the analyses.

5 Community analysis

The columns of the different datasets used for community analysis contain: percentages of certainty, sample names, bee species, honey collection dates, the cohabitation or not of different bee species hives and the name of plants at different taxonomic levels (species, genus and family).

First, four pie charts are generated in order to visualise the main floral species present : one comprising the plant species present in the 97% certitude dataset, one for the 95% certainty dataset, and one for each bee species dataset, *Apis cerana japonica* and *Apis mellifera*, percentages of certainty combined. All species with a presence of less than 1% proportional to all species appearing for each group are placed in the categories "Other taxa" (Suyama et al., 2022). To generate the graphs, the *"ggplot2"* package on RStudio is used (Wickham, 2009).

In order to assess the food overlap between the two bee species, a Venn diagram was generated. For the three taxonomic levels: species, genus and family, graphs allow to visualise the lists of taxa and the proportion of taxa present in both bee species and the lists of taxa found only in one or the other (Jia et al., 2021). As well as the number of taxa in common or individual to each honey bee species. This is achieved through the *"VennDiagram"* package (H. Chen and Boutros, 2011) and *"ggVenn"* package (Wickham, 2009). Then, different specific richness are observed. Two boxplots are generated including the number of species in a community, either for one of the two bee species, or for the percentage of certitude. Finally,

two other boxplots are generated representing the floral abundance according to either the two bee species or the percentage of certainty.

In order to fully assess the alpha diversity, an accumulation curve is generated for different factors and generates corresponding plots using the *"vegan"* package (Oksanen et al., 2020) and *"ggplot2"* package (Wickham, 2009). In order to achieve this, the nonparametric estimator, Chao index (Chao and Jost, 2012), is calculated for the species richness in the dataset, the total floral community. The Chao index calculates a measure of species richness that estimates the total number of species present in a community and those that have not been observed (Chao, 1984). In the end, it computes a global accumulation curve of the number of observations according to the specific richness for the total species pool. Thanks to this curve, it is possible to observe the evolution of observed species when other samples are added to the dataset, and helps to estimate potential species richness in a biological community (Colwell et al., 1997; Gotelli and Colwell, 2001). In other words, it allows us to observe the floral taxonomic richness visited by *Apis cerana japonica* and *Apis mellifera* depending on the sampling effort (Colwell et al., 2004; Dove and Cribb, 2006).

After that, different accumulation curves are generated for specific factors: bee species and percentage of certitude. This operation was carried out thanks to the *"BiodiversityR"* package (Kindt, 2020). Each graphic shows the species richness depending on the observation number for both variables in each specific factor : 95% and 97% for the percentage of certitude and *Apis cerana japonica* and *Apis mellifera* for bee species. This makes it possible to evaluate the estimation of the specific richness, as well as the sampling effort.

After the analysis of alpha diversity, beta diversity is assessed. Initially, a Bray-Curtis distance matrix was calculated after Hellinger transformation for the entire dataset (Rao, 1995) via the *"vegan"* package on RStudio (Oksanen et al., 2020). This transformation allows standardisation of the floral abundance dataset and avoids overrepresentation of dominant floral species during the analysis (Beals, 1984). In this way, it is possible to measure the dissimilarity between samples. The higher the Bray-Curtis dissimilarity is, the more different is the foraged floral communities from honey samples.

From the Bray-Curtis dissimilarity matrix, PCoA (Principal Coordinate Analysis) is calculated (Gower, 2015), also via the *"vegan"* package on RStudio (Oksanen et al., 2020). Then, a PCoA graph is generated for each specific factor, percent of certitude and bee species and ordination ellipses with a confidence limit of 0,8 are added to better understand the distribution of points in the graph space. This step allows us to observe the dissimilarity and to visualise the structure of distances for both factors.

Finally, each plant species was identified as either herbaceous or woody. To do so, so-called herbaceous species have been identified as having no woody organs above the ground, and bushes, shrubs and trees are considered as woody plants. To achieve this, data from

"efloras index" and the "catalogueoflife" ("eFloras.org Home", n.d.; "World Plants: Plant List", n.d.) lists was used to complete the dataset. Then, the proportion of abundance between woody and herbaceous species according to the variable cohabitation (yes or no) was carried out. In order to evaluate the independence between the two categorical variables for *A. cerana japonica* data (cohabitation and trait plants) in a contingency table, a G-test of independence or the "likelihood ratio test" is made thanks to the RStudio package *"RVAideMemoire"* (Hervé, 2018). The null hypothesis indicates both variables are independents (Woolf, 1957). Therefore, a statistic significant result leads to a significant association between variables as conclusion. Thus, a potential change in the foraging behaviour of *Apis cerana japonica* depending on the presence or not of *Apis mellifera* is observable. After that step, graphics are made representing the proportion of plant traits for *A. cerana japonica* and *A. mellifera*.

6 GLMM model

There are 144 covariates, including 142 of landscape variables, that can explain the taxonomic richness. From these landscape variables, a selection is made in order to keep the variables useful for the whole taxonomic richness and exclude irrelevant terms (McCullagh, 2019). Moreover, since correlation makes it possible to observe the relationship between two variables, it makes it possible to remove strongly correlated variables that may bring redundancy in statistical analysis (Figure 3) (Senthilnathan, 2019). Then, the correlation calculation is made on the selected 22 landscape variables and the most correlated variables (> 0.7) are removed from the analysis, such as: the total number of patches (tot_np), Shannon's evenness index (shei), Simpson's diversity index (sidi) and Simpson's evenness index (siei). In addition, all IIC variables from each LULC were removed so that all landscape metrics correspond to the entire buffer zone around an apiary. Among the four indexes, the Shannon's Diversity Index (SHDI) was kept because it corresponds to the search question and can be considered as the most significant factor to evaluate the diversity value when the number of species is not too high (DeJong, 1975).

This step allows you to switch to the Generalized linear mixed model (GLMM) with : the total class area; the total proportion of build area; the total effective mesh size (Spanowicz and Jaeger, 2019); the total patch density (McGarigal et al., 2002); Shannon's diversity index (Shannon, 2001); and the Integral Index of Connectivity (Pascual-Hortal and Saura, 2006).
This statistical model is an extension of Generalized Linear Models (GLM) that allows both fixed and random effects to be considered. Like GLM, a GLMM takes into account datasets that do not meet the hypothesis of normality and homoscedasticity, therefore does not fit for a classical linear model. But the difference is that it includes a component for random variables (Bolker et al., 2009). Its general form is:

$$y = X\beta + Zb + \epsilon \tag{1}$$

Where y is the dependant variable to explain with N×1 column vector; X is N×p with p predictor variables and β is fixed effects regression coefficients with a p×1 vector; Z is N×q

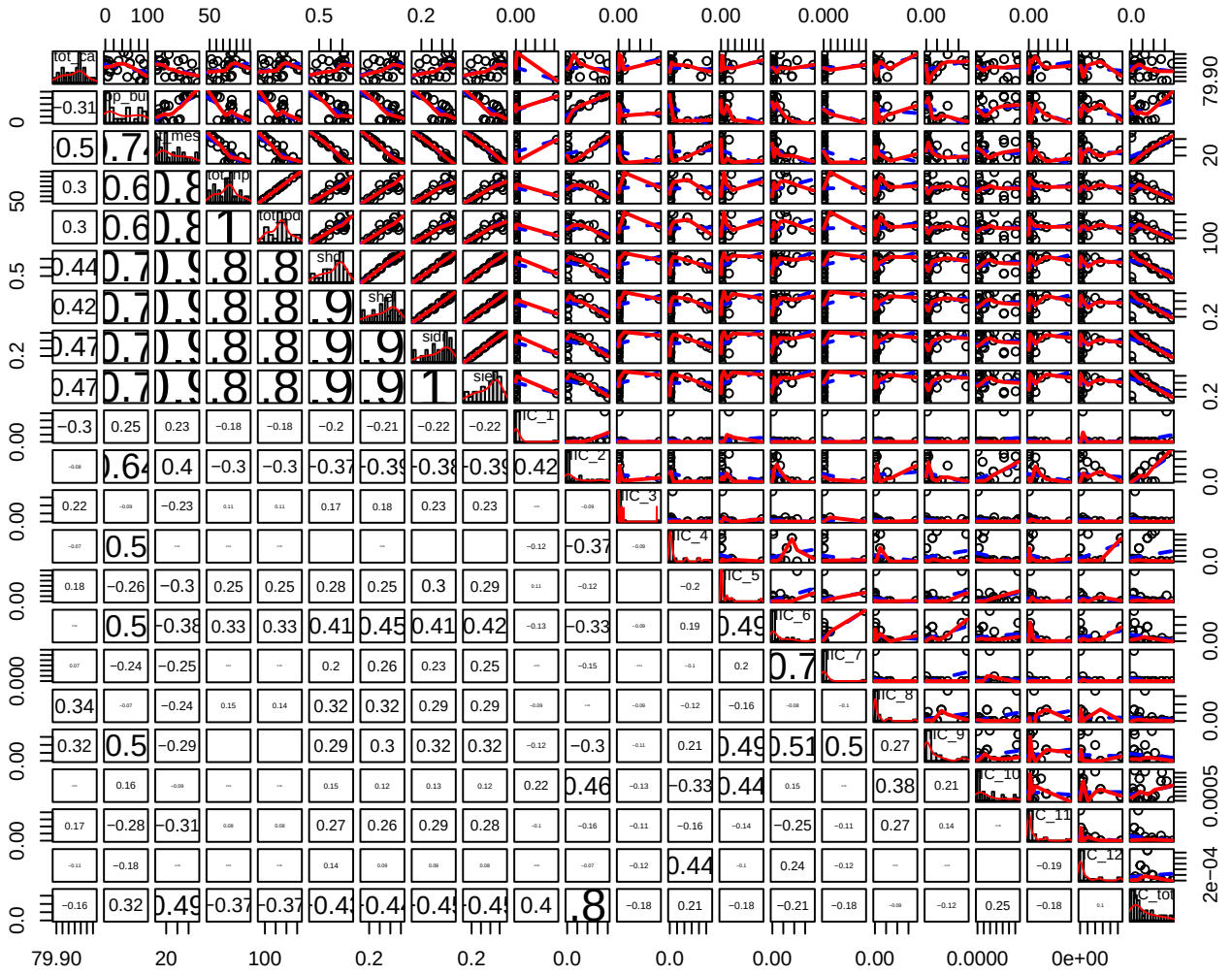


Figure 3: Correlation graph of landscape variables.

matrix with q random effects and b is a vector q×1 of the random effects; and ϵ is a N×1 column vector of residuals (McCullagh, 2019).
Here, the variable to be explained is the taxonomic richness; the fixed effects are bee species, cohabitation or not between both bee species, and selected landscape variables; the random effect is the site location (expressed as factor data in R).

From that statement, a first model is made and its significant statistic is analysed. After that, a model selection based on a backward method is carried out (Jamil et al., 2013). Each new model is made from the previous one, except for the variable with the highest *p-value*. Model selection goes on until all variables are statistically significant.

Then, a residual diagnostics for hierarchical regression model is made via *"DHARMa"* package in order to assess the quality of model estimation (Loy and Hofmann, 2013). It is based on a simulation-based approach to create readily interpretable scaled (quantile) residuals.

In an effort to select the right correspondent model between the six models created, a Akaike Information Corrected Criterion (AICc) is proceeding (Akaike, 1998). It compared the quality of different models taking into account the sample size and the number of parameters in the model whereas it provided a penalty to models in order to avoid an overfitting (Brewer et al., 2016). An AICc comparative table is generated from the *"AICcmodavg"* package (Mazerolle, 2020) and provides different estimators : AICc, Delta_AICc, Weight of AICc, Cumulative weight and Log-Likelihood. The model with the lower value for AICc or with the heaviest weight is the most suitable model to explain the taxonomic richness.

It appears that the fitter model for the GLMM is composed of : IIC, SHDI and PD. IIC assesses the ecological connectivity of a landscape. Ranging from 0 to 1, the more it increases, the greater the connectivity. The entire landscape is considered occupied by habitat when it reaches unit value. It takes into account the surface of each patch as well as the distance between the points of the habitats between these points (Pascual-Hortal and Saura, 2006).

Concerning Shannon's diversity index, it is a measure of biological diversity in a particular environment. This index includes the total number of taxa (species richness) as well as the relative distribution of taxa abundances. A near-zero index indicates low diversity, while a higher Shannon's index suggests higher diversity as well (Ramezani et al., 2010; Shannon, 2001).

Thirdly, PD is an aggregate metric in landscape variables. It is calculated by the total number of habitat patches, or in this case LULC, divided by total area of study area and it describes the fragmentation of the landscape. Its unit is number per 100 hectares and the PD metric increases when the study area gets more patches (Hesselbarth et al., 2019; McGarigal et al., 2002).

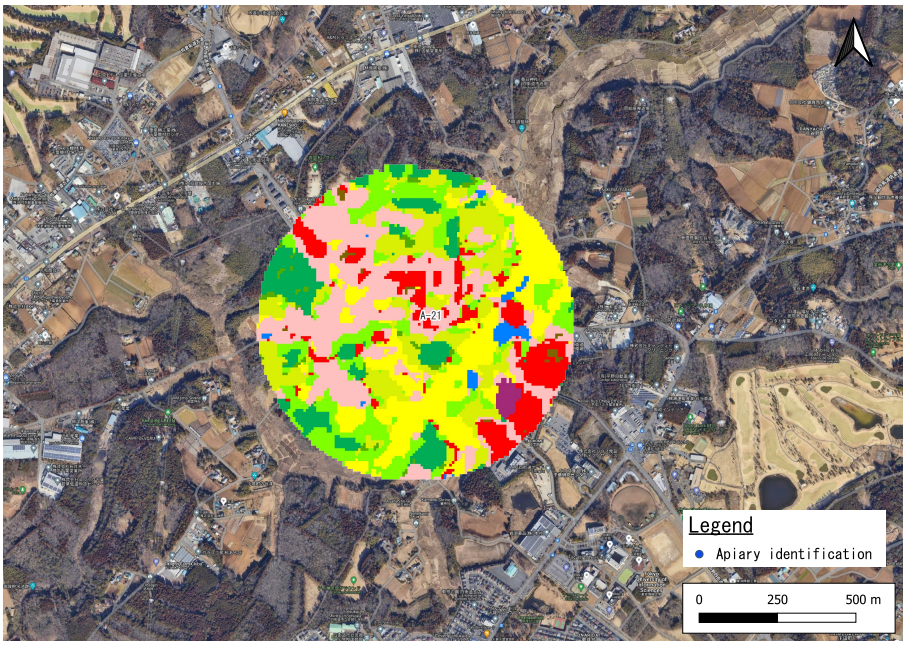


Figure 4: Zoom on an apiary of the map of Japan with its 500-metres of buffer. Sources: Chiba University, Japan; Earth Observation Research Center (EORC); Japan Aerospace Exploration Agency (JAXA); and Google Hybrid.

III Results

1 Landscape analysis and metrics

i Buffers area and different zones of habitat

The landscape analysis allows to generate 32 buffer areas around apiaries. Of the 36 apiaries at the beginning, four had not been located precisely. Buffer areas show different types of habitat surrounding bee colonies (Figure 4) and according to the legend presented earlier in this text, a maximum of 12 different LULC can be present. Evergreen needle-leaf forest (ENF), grassland, cropland and urban area are the most common type of habitat; it appears in 27 of the 32 apiaries. Unlike water that is present only in the 500-metres buffer zone of 5 apiaries. In addition, the urban surfaces and cropland count, on all the surfaces of the buffers around the apiaries, the largest surfaces with, respectively, 897.04 hectares and 505.11 hectares (Table 4).

Table 4: Frequency and total area of the twelve LULC present in the area of 500-metres around apiaries. Sources: Earth Observation Research Center (EORC) and Japan Aerospace Exploration Agency (JAXA).

	1.water	**2.urban**	**3.rice paddy**	**4.crop**	**5.grass**	**6.DBF**
Number of frequency	5	27	19	27	27	26
Total surface [ha]	1.85	897.04	48.55	505.11	116.01	171.09
Proportion [%]	0.09	41.51	2.25	23.37	5.37	7.91
Mean and standard error [ha]	0.07 (±0.04)	33.22 (±5.45)	1.79 (±0.97)	18.71 (±3.69)	4.3 (±1.13)	6.34 (±1.39)
	7.DNF	**8.EBF**	**9.ENF**	**10.bare land**	**11.bamboo**	**12.solar panel**
Number of frequency	7	24	27	26	22	19
Total surface [ha]	22.49	74.53	91.05	33.57	192.04	7.95
Proportion [%]	1.04	3.45	4.21	1.55	8.89	0.37S
Mean and standard error [ha]	0.83 (±0.65)	2.76 (±0.95)	3.37 (±0.92)	1.24 (±0.24)	7.11 (±2.1)	0.29 (±0.09)

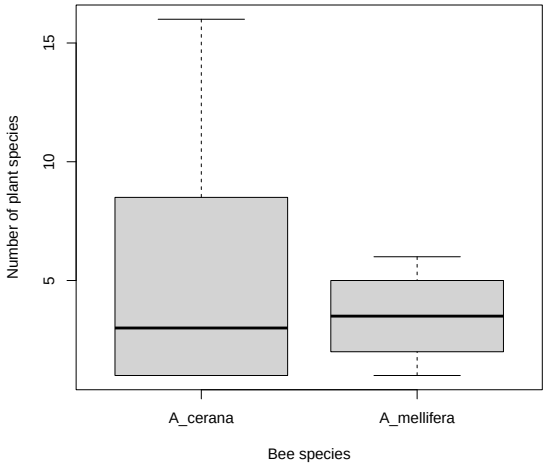
ii Landscape metrics

Fifteen landscape variables gave values for each LULC, as well as the total value of the landscape metrics for each sample. These last values are kept, with exception for the IIC, in order to check the correlation between the variables. At the end of the analyses, the landscape variables kept to explain the taxa richness in the GLMM model are: Patch Density (PD), Shannon's Diversity Index (SHDI) and Integral Index of Connectivity (IIC).

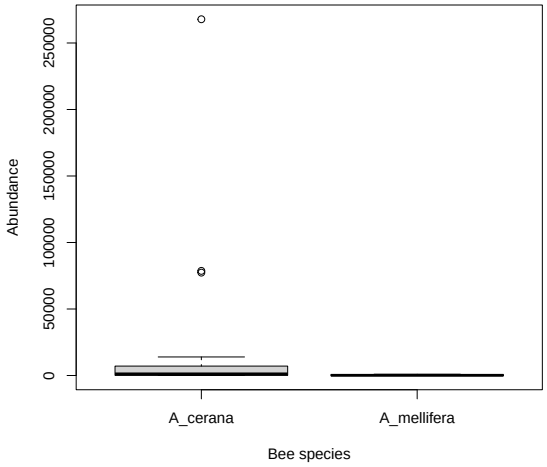
2 Taxonomic richness and diversity

The total number of reads in the 97% of certitude dataset is 479,319. On average, there are 3,033.67 reads per plant species detected with a standard deviation of 11,685.97 of reads. In the same way, the mean value of the number of reads per sample is 16,528.24 with a standard deviation of 52,250.98. Concerning the taxonomic richness, 142 floral species are detected in all samples. Per sample, there are 4.9 floral species detected on average, with a standard deviation of 4.46 plant species.

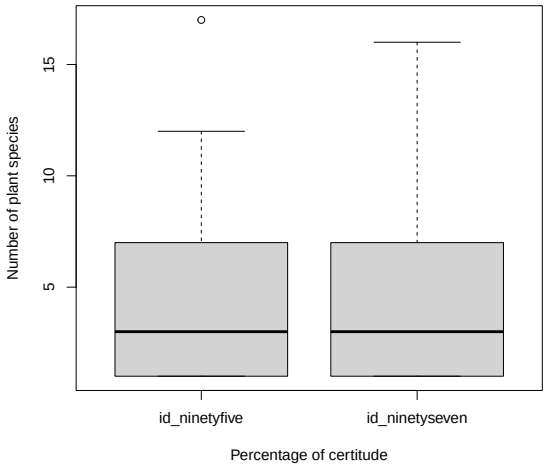
By observing the distribution of the number of taxa and the abundance of reads according to the groups: bee species and percentage of certitude, it shows that *Apis cerana japonica* has an interquartile ranging from 1 to 8.50 for the species number and from 96 to 7,084 for the abundance with a median of, respectively, 3 and 1,203. As for *Apis mellifera*, it is ranging from 2.25 to 4.75 for the species number with a median of 3.5 and from 47 to 500.2 for the abundance with 134.5 as median (Figure 5a-5b) (Table 5). While for the group parameters "percent of certitude", they have a similar distribution (Figure 5c-5d) (Table 5).



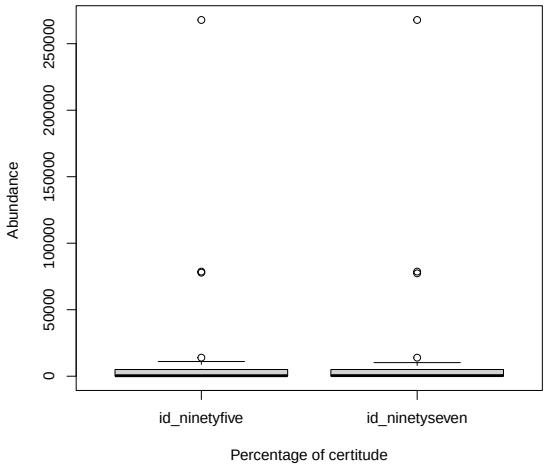
(a) Number of plant species per bee species



(b) Abundance per bee species



(c) Number of plant species per percentage of certitude



(d) Abundance per percentage of certitude

Figure 5: Box plot of *Apis cerana japonica* and *Apis mellifera* and 95% and 97% of certitude with (a)(c) the number of plant species and (b)(d) the abundance of plant species.

Table 5: Summary table of plant taxa distribution.

	1st quartile	**Median**	**Mean and standard error**	**3rd quartile**
Species number for Apis cerana japonica	1.00	3.00	5.26 (±1.02)	8.50
Species number for Apis mellifera	2.25	3.50	3.50 (±0.76)	4.75
Abundance of Apis cerana japonica	96	1,203	20,758 (±1,2130.29)	7,084
Abundance of Apis mellifera	47.00	134.5	313.3 (±156.46)	500.2
Species number for 95% of certitude	1.00	3.00	4.77 (±0.81)	6.75
Species number for 97% of certitude	1.00	3.00	4.9 (±0.83)	7
Abundance of 95% of certitude	58.25	371.00	16,025.60 (±9,393.11)	4,558.75
Abundance of 97% of certitude	68	454	16,528 (±9,702.76)	5,048

Specific taxa richness is also observed for both different groups. It can be observed that the families of plants, genus and their species are very similar between parameters

95% and 97% certitude (Figures 6a-6b-7a-7b-8a-8b). As well as the families, genera and species of plants visited by *Apis cerana japonica* which are identical to those of percentage of certitude (Figures 6c-7c-8c). As for *Apis mellifera*, it differs from the list of plant taxa (Figures 6d-7d-8d). It is important to note that only the proportion of taxa above 1% are represented and taxa proportion with $< 1\%$ of reads are grouped as "Other taxa" in the graph.

Looking at species level, for *Apis cerana japonica* (Figure 8c), the most abundant plants are: *Aralia elata* (Araliaceae); *Aralia* sp. (Araliaceae); *Bidens Pilosa* (Asteraceae); *Causonis japonica* (Vitaceae); *Chengiopanax sciadophylloides* (Araliaceae); *Hedera nepalensis* (Araliaceae); *Humulus scandens* (Cannabaceae); *Kalopanax septemlobus* (Araliaceae); *Lagerstroemia* sp. (Lythraceae); *Paederia foetida* (Rubiaceae); *Paederia* sp. (Rubiaceae); *Rhus chinensis* (Anacardiaceae); *Solidago* sp. (Asteraceae); *Ulmus parvifolia* (Ulmaceae); *Ulmus* sp. (Ulmaceae). Concerning *Apis mellifera* (Figure 8d), there are: *Cosmos bipinnatus* (Asteraceae); *Daphniphyllum oldhamii* (Daphniphyllaceae); *Perilla frutescens* (Lamiaceae); *Physalis angulata* (Solanaceae); *Styrax hemsleyanus* (Styracaceae); *Triticum aestivum* (Poaceae).

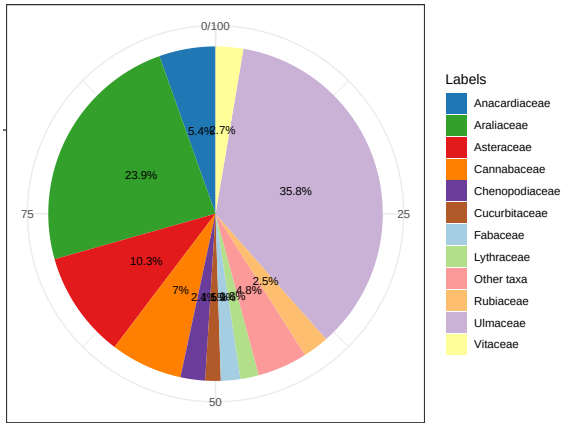
As it appears, the proportion of shared taxa is equal to 20.0% for family level, 8.2% for genus level and 3.3% for species level (Figure 9). At species level, shared taxa are: *Ambrosia trifida*, *Perilla frutescens*, *Rhus chinensis* and the fourth is "Undetermined" taxa (Annex A). These three common species represent 5.99% of the total abundance of the all dataset and undetermined taxa are 9.43% of the total abundance. Then, the proportion of taxa visited by *Apis cerana japonica* is 71.1% for family level, 78.8% for genus level and 83.6% for species level. In the same way, the proportions of taxa abundance for *Apis mellifera* are 8.9%, 12.9% and 13.1%.

The accumulation curve generated presents the cumulative number of taxon richness reported in the samples with 97% certitude (Figure 10). It shows an increasing line with the cumulative number of floral taxa. Concerning indexes such as : Chao (Chao, 1984), Jackknife and Bootstrap (Shao & Tu, 2012), their values are, respectively, 643.42 ($\pm$187.66), 227.24 ($\pm$27.92) and 162.82 ($\pm$12.24) (Table 6). When looking separately at the pattern of parameters 95% and 97% of certitude on the accumulation curve, the two lines are increasing (Figure 11a). As for the two bee species, *Apis cerana japonica* also has an increasing line and the number of observations of *Apis mellifera* is very low (Figure 11b).

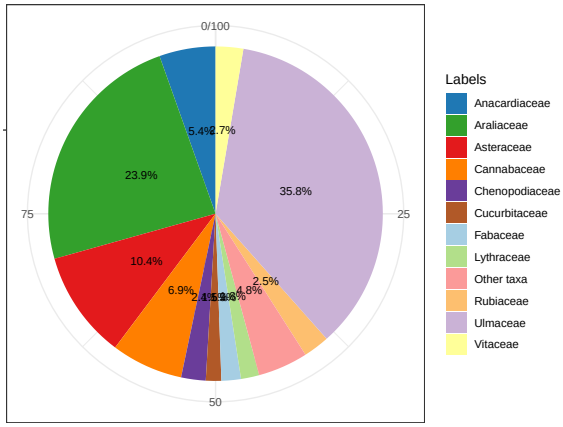
Table 6: Chao, Jackknife and Bootstrap estimates and standard errors.

	Species	**Chao**	**chao.se**	**Jack1**	**jack1.se**	**jack2**	**Boot**	**boot.se**	**n**
All	122	643.42	187.66	227.24	27.92	318.85	162.82	12.24	29

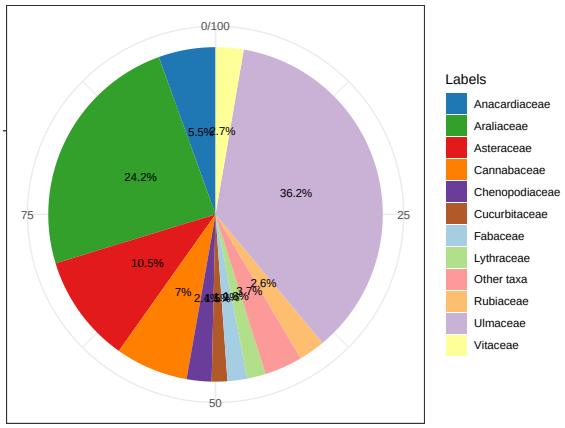
The calculation to determine the Principal Coordinates (PCoA) indicates that the cumulative sum of the first two axes explains 13.97% of the structure of the observations for all the samples (Figure 12a) and 13.32% for the samples analysed with 97% of certitude



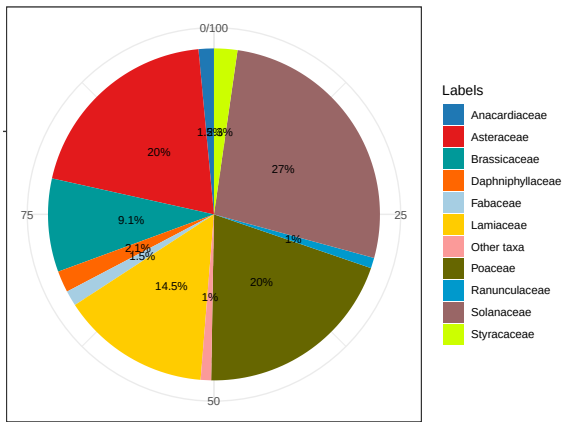
(a) 95% of certitude



(b) 97% of certitude

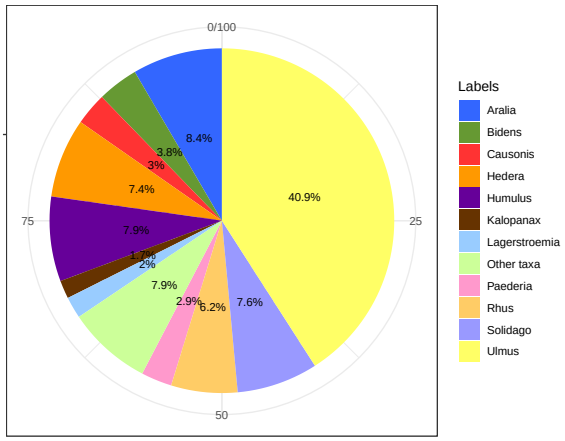


(c) *Apis cerana japonica*

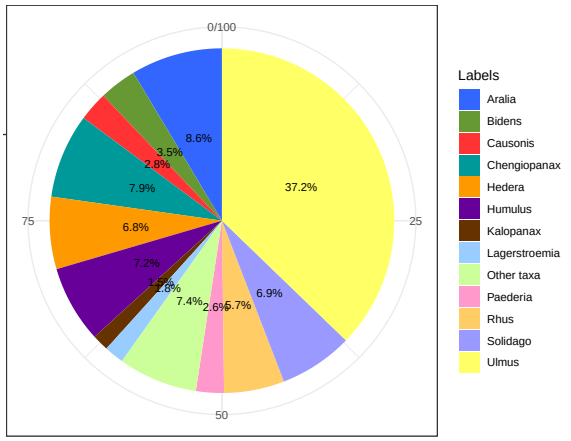


(d) *Apis mellifera*

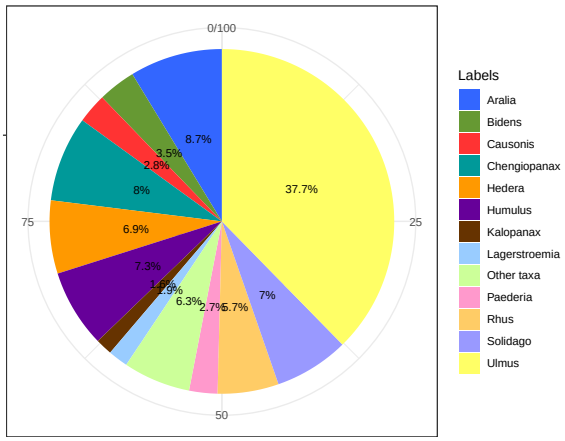
Figure 6: Abundance of each floral familiy of *Apis cerana japonica* and *Apis mellifera* and 95% and 97% of certitude, in percentage.



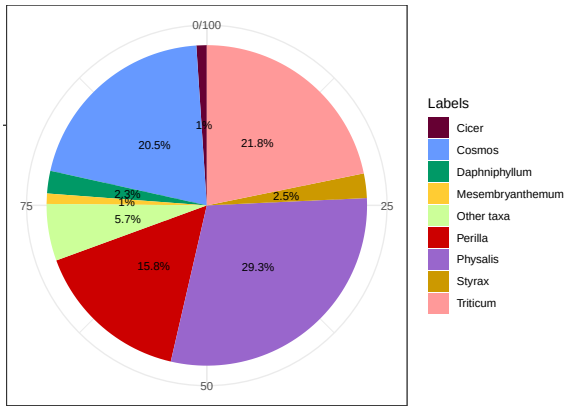
(a) 95% of certitude



(b) 97% of certitude

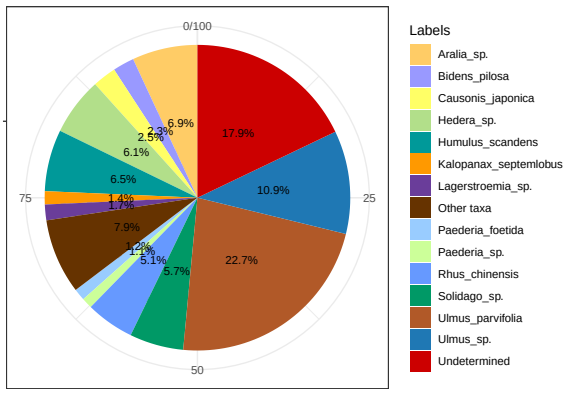


(c) *Apis cerana japonica*

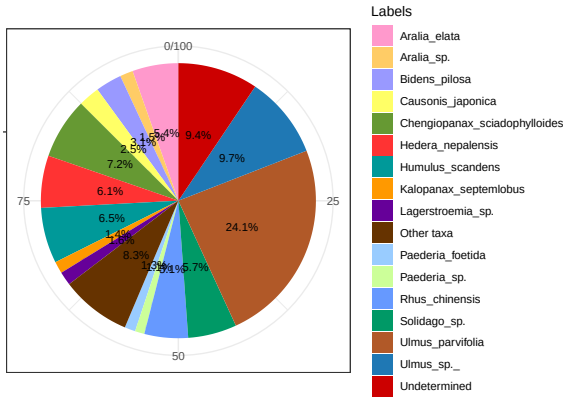


(d) *Apis mellifera*

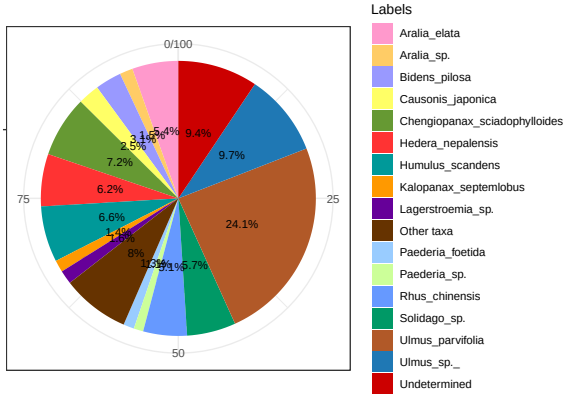
Figure 7: Abundance of each floral genus of *Apis cerana japonica* and *Apis mellifera* and 95% and 97% of certitude, in percentage.



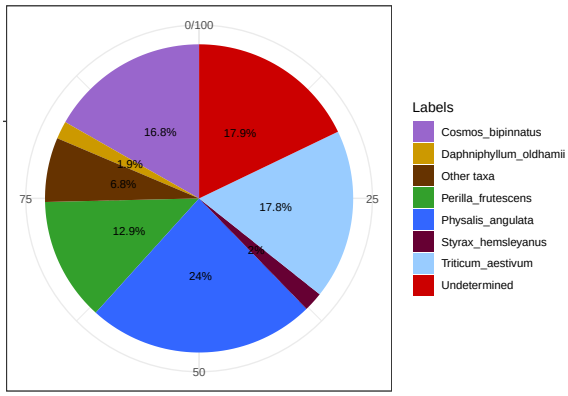
(a) 95% of certitude



(b) 97% of certitude

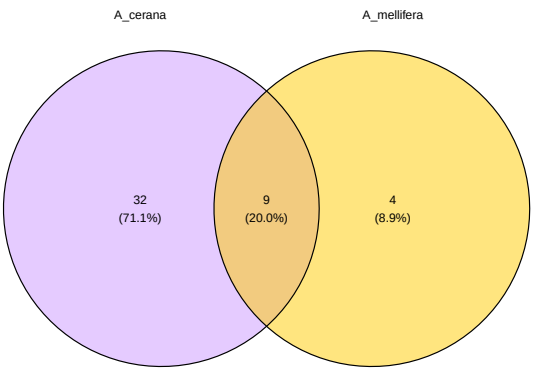


(c) *Apis cerana japonica*

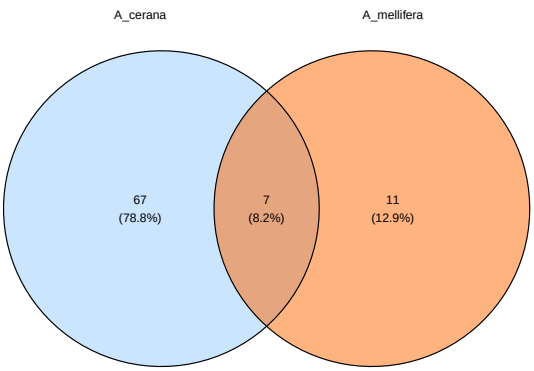


(d) *Apis mellifera*

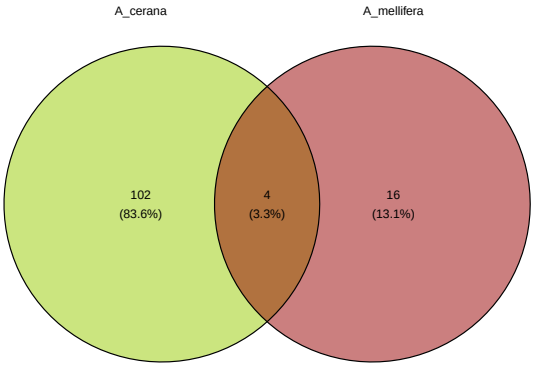
Figure 8: Abundance of each floral species of *Apis cerana japonica* and *Apis mellifera* and 95% and 97% of certitude, in percentage.



(a) Families



(b) Genus



(c) Species

Figure 9: Venn diagram of the number and the proportion of plant taxa of *Apis cerana japonica* and *Apis mellifera* at different taxa level: (a) Families, (b) Genus, (c) Species.

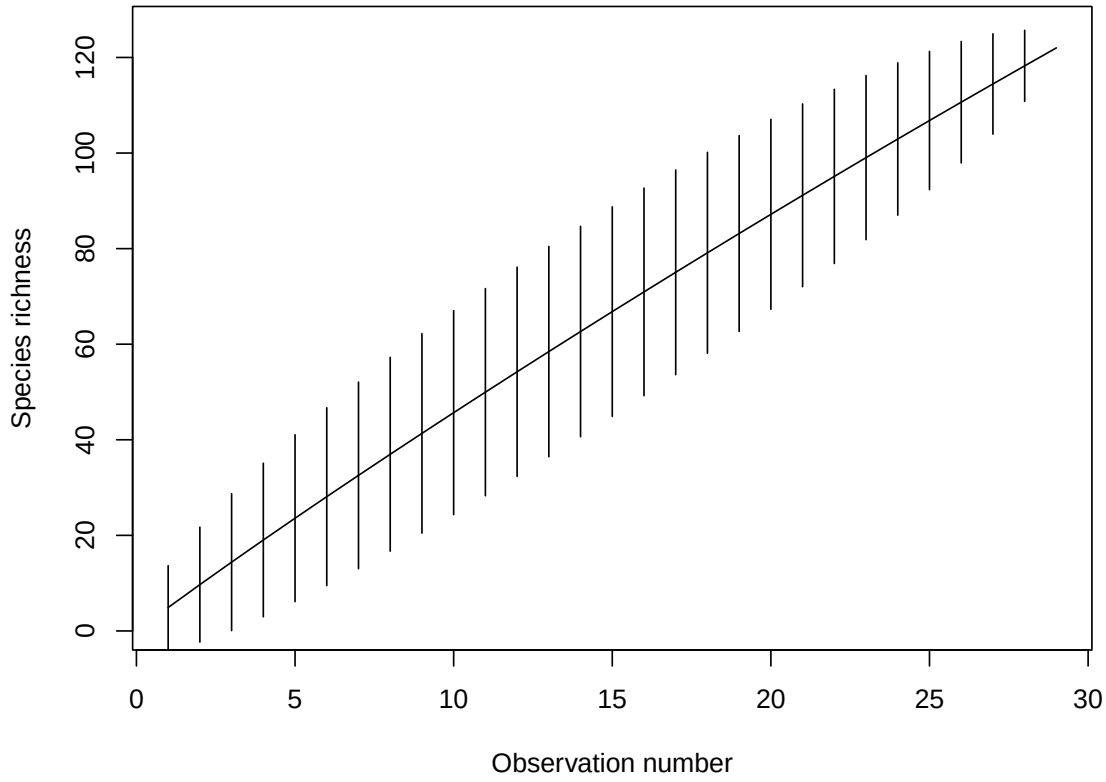
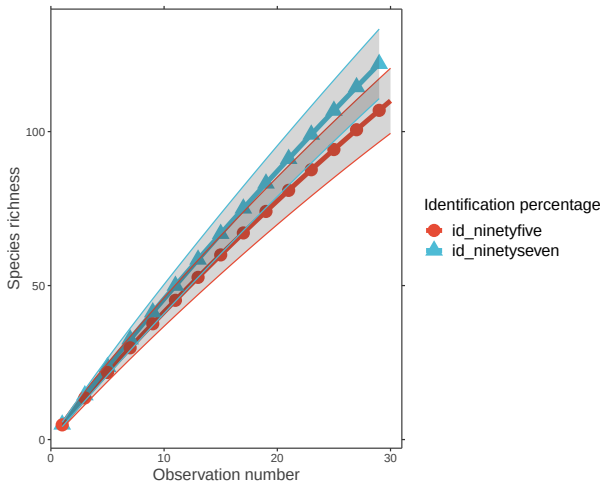
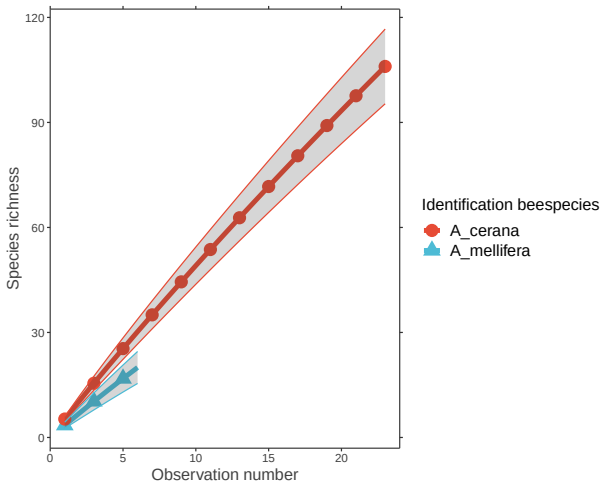


Figure 10: Accumulation curve of the dataset containing 97% of certitude information.

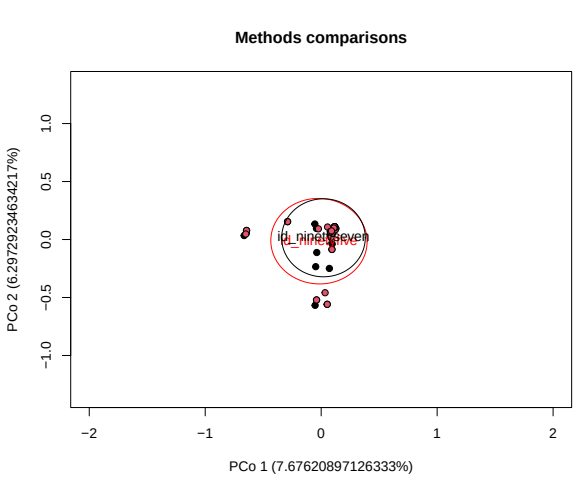


(a) Percentage of certitude with 95% of certitude in red and 97% of certitude in blue

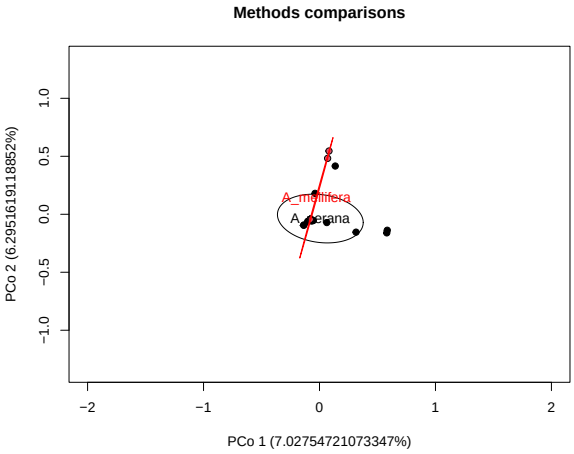


(b) Bee species with *Apis cerana japonica* in red and *Apis mellifera* in blue

Figure 11: Comparison of accumulation curve of (a) 95% and 97% of certitude dataset and (b) both bee species dataset.



(a) Percentage of certitude with 95% in red and 97% in black



(b) Bee species with *Apis cerana japonica* in black and *Apis mellifera* in red

Figure 12: Principal Coordinates Analysis between two groups : (a) percentage of certitude and (b) bee species.

(Figure 12b). Different patterns of group structure are drawn in the two graphs. At first, groups composed of percentage of certitude parameters are shown and the second represents the diet of both bee species.

Once the landscape variables are selected, a GLMM is made to explain the taxonomic richness by the variables. At first, all landscape variables selected following the correlation, as well as the variables "bee species" and "cohabitation" are analysed with the random variable ("localisation"). From the sixth model, all variables are significant with < 2e-16 p-value (Table 7). Then, the AICc table presents the different values such as : AICc value or AICc weight for all models (Table 8). The first model gets 147.17 as AICc value and 6.67e-04 as AICc weight, while the sixth model gets, respectively, 133.27 and 0.7.

Table 7: Summary table of the sixth GLMM model.

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
(Intercept)	1.34	0.01	225.88	<2e-16 ***
tot_pd	-1.16	0.01	-190.76	<2e-16 ***
shdi	0.57	0.01	92.70	<2e-16 ***
IIC_tot	-0.38	0.01	-64.26	<2e-16 ***

Table 8: AICc table of GLMM analysis of the taxonomic richness.

	Equation	K	AICc	AICcWt	Cum.Wt
6	Taxa richness= tot_pd + shdi + IIC_tot + (1|localisation)	5	133.27	0.7	0.7
5	Taxa richness=cohabitation + tot_pd + shdi + IIC_tot +(1|localisation)	6	135.33	0.25	0.94
4	Taxa richness=cohabitation + prop_build + tot_pd + shdi + IIC_tot + (1|localisation)	7	138.59	0.05	0.99
3	Taxa richness=cohabitation + prop_build + tot_mesh + tot_pd + shdi + IIC_tot + (1|localisation)	8	142.60	0.01	9.98e-01
7	Taxa richness= (1|localisation)	2	146.10	1.14e-03	9.99e-01
2	Taxa richness=cohabitation + tot_ca + prop_build + tot_mesh + tot_pd + shdi + IIC_tot + (1|localisation)	9	147.17	6.67e-04	99.99e-02
1	Taxa richness=bee_species + cohabitation + tot_ca + prop_build + tot_mesh + tot_pd + shdi + IIC_tot + (1|localisation)	10	152.33	5.05e-05	1

The chosen model is the sixth one and it is composed of the three explanatory variables: IIC, SHDI and total patch density. The graphs of the three explanatory variables show decreasing lines of taxonomic richness as a function of variables (Figure 13).

Finally, the analysis of the foraging preferences of *Apis cerana japonica*, as for the traits of the taxa visited, shows that of the 122 observations, 56 taxa are woody and 56 are herbaceous. Of the 56 herbaceous taxa, 51 observations are not co-located with colonies of *Apis mellifera*. While regarding woody taxa, 38 of the observations are not in cohabitation.

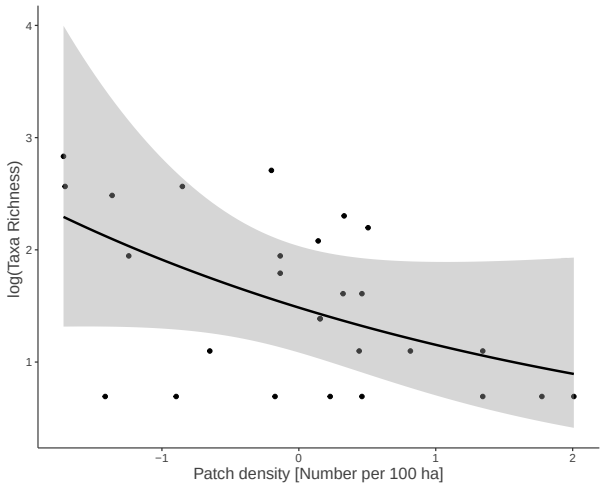
The G-test of the abundance of the traits of the floral taxa according to the cohabitation or not of the two bee species is highly significant (Table 9-10). Thus, the null hypothesis, that the distribution of woody and herbaceous species visited by *Apis cerana japonica* is the same, whether *Apis mellifera* is present or not, is rejected. Moreover, it can be observed the Japanese honey bee food preferences in cohabitation or not (Figure 14a) and that the Western honey bee prefers herbaceous plants in cohabitation (Figure 14b).

Table 9: Contingency table of two categorical variables (cohabitation and trait plants).

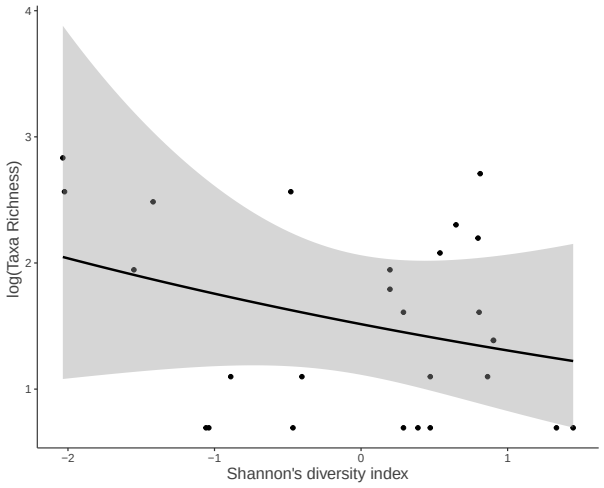
Cohabitation	**Herbaceous**	**Woody**
No	94,682	326,659
Yes	1,066	10,049

Table 10: G-test of independence table.

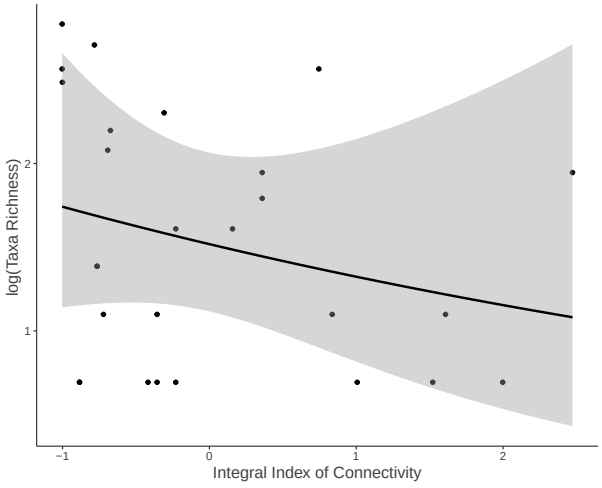
G	**Df**	**p-value**
1,246.5	1	2,2 e-16



(a) Plot of Patch density

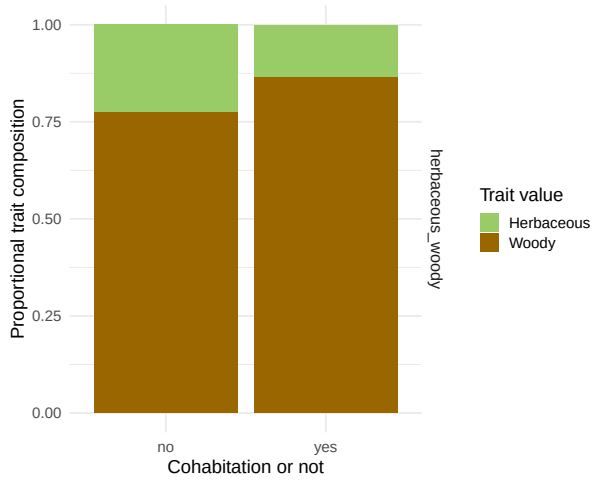


(b) Plot of Shannon's diversity index

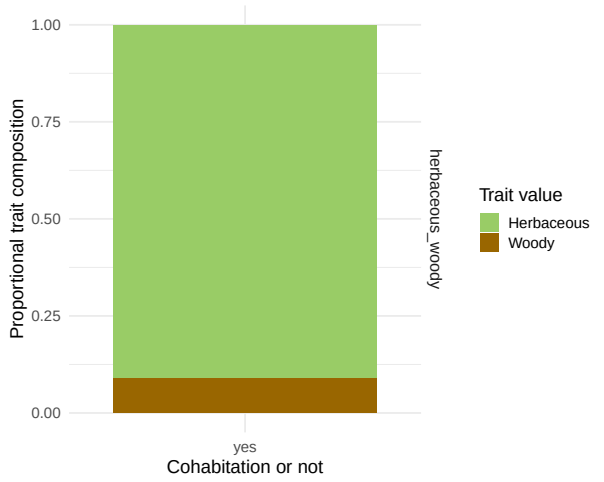


(c) Plot of Integral Index of Connectivity

Figure 13: Plot of (a) Patch Density, (b) Shannon's Diversity Index and (c)Integral Index of Connectivity depending on the logarithm of the taxa richness.



(a) *Apis cerana japonica*'s diet preferences



(b) *Apis mellifera*'s diet preferences

Figure 14: Proportional trait composition of plant taxa foraged by (a)*Apis cerana japonica* depending on cohabitation parameters and (b) *Apis mellifera*.

IV Discussion

The results showed, first of all, the preference of *Apis cerana japonica* for woody plant species and the western bee for herbaceous floral species in autumn. A link can also be made with the highest proportions of habitat areas such as urban and cropland areas and the most abundant plant species visited by the two bee species. However, a lack of data concerning *Apis mellifera* due to the difficulty to find beekeepers having both, Japanese and Western honey bee colonies, was a limitation in the research. This does not allow us to draw conclusions on certain points such as the presence or absence of food overlap between both honey bee species. Finally, the taxonomic richness is explained by three landscape metrics that are the Shannon's Diversity Index, the Integral Index of Connectivity and the Patch Density.

1 Honey-based metabarcoding analysis and identification

This study analysed the DNA contained in honey from *Apis cerana japonica* and *Apis mellifera*, in Japan. Forty-six apiaries provided the honey used in the study throughout August and November 2022. The appeal of this study is the use of metabarcoding, with five different primers used, as a method of DNA detection of plants visited by Japanese and Western honey bees. In the end, of the 167 samples, there were 426 taxa identification in total of which 345 correspond to plant taxa including 95% and 97% of certitude, the rest are from the Kingdom of Fungi.

The accumulation curve allowed to show a likeness between both parameters : 95% and 97% of certitude, at one point that 97% of certitude got a higher specific richness, probably due to its stricter threshold for the identification of OTU to reference taxa (Ammon et al., 2018; Falentin et al., 2019). That is the reason why analysis comparing *Apis cerana japonica* and *Apis mellifera* were done with that dataset. However, looking at the high proportion of undetermined species, this method choice can be discussed, as well as the efficacy of metabarcoding techniques.

First, the initial quality of DNA samples is essential. The collection method is a crucial step to avoid contamination and get a good preservation for the DNA(Bell et al., 2016; Liu et al., 2020).

Then, the DNA extraction process is also a delicate step to reach good quality (Bell et al., 2016; Hawkins et al., 2015; Liu et al., 2020). A low quantity or a degradation of DNA in a sample can lead to difficulties to recover fragments during PCR or to create errors during the sequencing step (Coissac et al., 2012; Liu et al., 2020). This can generate bias or no identification in results.

Secondly, the choice of genetic markers influences taxonomic identification capacity, its discriminatory power and sequencing step (Bell et al., 2016). Since the goal is to be able to identify several organisms at the same time from an unique sample, the choice of genetic

markers is crucial in order to correctly distinguish taxa, as well as, the availability of reference sequences for taxonomic attribution (Liu et al., 2020). And as it was observed in our results, primers ITS1 and ITS2 provided together 77.84% of all samples comparable to *psbAtrnH* and *trnL* that detected 22.16% of all samples. Therefore, genetic markers located in internal nuclear transcribed spaces identified more plant taxa in this study.

As ITS1 and ITS2 are the genetic markers that have generated the most results when identifying taxa, it is important to note that these two primers are commonly used for the detection of plants and fungi. Indeed, internal nuclear transcribed spaces primers are used as official DNA analysis marker for species-level identification of fungi (Blaalid et al., 2013; Raja et al., 2017) and can partly explain the identification of so many fungi, compared with *psbAtrnH* and *trnL* are traditionally used for plant species identification (Cheng et al., 2016; Wallinger et al., 2012).

Thirdly, during the PCR step, especially when the sample contains several species, some of them may be amplified unevenly due to differences in the target sequences. This process introduces distortions in the relative representation of plants and leads to imprecision in the quantification of detected taxa (Hawkins et al., 2015). These biases in the quantification of relative abundance is the major disadvantage of metabarcoding although it is recognized for its quality in identification (Liu et al., 2020).

These three points are possible causes for misidentification or bad quantification of taxa presented in other studies. This may partly explain the presence of a large proportion of "Undetermined" and fungi in our samples.

Moreover, pollen would yield more results compared with honey, as suggested in the study of Martins et al. (2023) where 46.3 plant species per bee species in honey samples are detected against 53.67 in pollen samples. This can be explained by the complexity of honey composed of H_2O_2 added with other components and an acid pH which accelerates the degradation of DNA in honey (Mohamadzade Namin et al., 2023). Nevertheless, metabarcoding from honey as a matrix has potential and can be a valuable technique for identifying the floral composition of samples (de Vere et al., 2017; Hawkins et al., 2015; Mohamadzade Namin et al., 2022). It is more more true for *A. cerana japonica* with which it is not possible to use the traditional pollen trap because the size of its hives are different than those of *A. mellifera* and the Japanese bee can easily escape.

2 Landscape analysis

For this study, many landscape metrics were calculated to determine those explaining the taxonomic richness visited by both species of honey bees. However, several of them were strongly correlated. Indeed, on the 500-metres of buffer around the apiaries, many landscape metrics bring the same information such as : between the Shannon's and Simpson's indices, or

between the number of patches and the PD.

Each landscape variable was generated from the entire study area, that is, the twelve LULC composing the landscape (buffer of 500-metres around the apiaries). Thus, no difference between the twelve habitat zones has been assessed but the floral richness is analysed by the composition of the landscape as a whole. In addition, the layer of LULC has a resolution of 10 m which does not allow it to detect precisely the type of habitat and flower beds that the landscape gets. It is an approximation of the most representative habitat areas of the territory.

However, the more abundant and frequent presence of certain habitat areas can be noticed such as urban areas and cropland. In addition to being present in 27 of the 32 apiaries, these are the areas with the largest total areas with 897.04 hectares and 505.11 hectares, respectively. Recall that as observed in the introduction, urban areas appear to have a higher floral diversity and a valuable resources for wild and honey bees (Fox et al., 2022; Richardson et al., 2021). But be careful that these urban areas could be depleted of abundant floral resources necessary for the growth of bee colonies (Richardson et al., 2021). On the other hand, it has been shown that there is lower floral diversity in rural environments as cropland (Fox et al., 2022). This environment can be problematic for the survival of bee populations unless specific crop rotations (rye, dandelion, clover, sunflower, colza, etc.) are set up to provide them with pollen and nectar constantly (Horn et al., 2021).

3 Taxonomic richness and diversity

Concerning alpha diversity, floral taxonomic richness and taxon abundance are greater for the Japanese bee than the Western honey bee as it is represented on boxplots where *Apis cerana japonica* has greater interquartile range and mean than the other honey bee. This contradicts what has been shown by Sakai et al. (1991) that noticed a greater diversity of plant species for *Apis mellifera* than *Apis cerana japonica*.
Moreover, specific richness does not allow to know if the relative abundance is equally distributed, nor does it demonstrate a sufficient sampling to represent the floral diversity visited by both bee species. The first point will focus further, while the second, the different accumulation curves have shown an insufficient number of taxa discovered to represent the total species pool expected. Indeed, the graph represents an increasing line, this means that other species can still be observed in the samples because no plateau is reached (Figure 10). This is more true looking at the accumulation curves paralleling *Apis cerana japonica* and *Apis mellifera*: one remains straight and does not curve; the other very short demonstrates an insufficient number of samples (Figure 11b). Thus, more observations can be made in order to have an almost complete sample of the flora visited by honey bees.

This is corroborated by indexes of Chao (Chao, 1984), Jackknife and Bootstrap (Shao & Tu, 2012). First, the high number of the Chao index suggests that the total species richness could be greater and that more extensive sampling would be required. However, since Chao's

standard error is very important, the estimate is not completely true. Then, the Jackknife and Bootstrap indices also have higher values than the number of species observed in the samples. This shows that there might be other floral species, not sampled, that can be visited by *Apis cerana japonica* and *Apis mellifera*. Moreover, as their standard error is lower than that of the Chao index, their estimate is more likely possible. Therefore, at least 40 other taxa could be on the list of plant species foraged by Japanese honey bees and Western honey bees.

In addition, looking at the results of PCoA for bee species, we can see that it is difficult to interpret the structure of the observations in *A. mellifera* (Figure 12b). Indeed, the Japanese honey bee gets a greater range compared to the western honey bee which is limited in its number of observations. This is due to a lack of data concerning *Apis mellifera* diet.

Then, we noticed that the direction of the SHDI estimates is positive in the summary table of the sixth GLMM model but is a decreasing line when the taxonomic richness is set according to the Shannon's diversity index, alone. Since the addition of an explanatory variable in the model changes the contribution of the other variables already present, these interaction effects can influence the direction of the estimates of the explanatory variables (Bolker et al., 2009; Pinheiro & Chao, 2006). In addition, when variables are strongly correlated and there is collinearity, the effect of each of them on the variance of the variable to be explained merges with one of the others (Senthilnathan, 2019). In our case, a strong correlation (=0.8) was observed between the explanatory variables SHDI and PD. This would explain SHDI's conflicting composure with the direction it is taking when evaluated alone in the model.

However, despite the disadvantage of collinearity between variables, the model presented remains the best model that can explain taxonomic richness since its AICc has the smaller value with a weight near 70% (Table 8) and with a Residual Diagnostics for Hierarchical Regression Model (DHARMa residual) fitting well (Annex B). Indeed, the DHARMa residual indicates that the variables of the sixth model are adapted to explain the taxonomic richness. It appears that there is no deviation from the expected distribution, there is no overfitting, nor misfit of the model. Other models, based on other highly correlated variables, were tried but none gave such good results in residual DHARMa and AICc tests.

It is therefore these three variables, SHDI, IIC and PD, that best explain the taxonomic richness of plants foraged by *Apis cerana japonica* and *Apis mellifera*. Their behaviour and the direction their estimates are taking are not what we can expect. Indeed, based on other studies, the increase in these landscape variables is expected to lead to an increase in plant taxonomic richness (Aubert, 2016; Paterson et al., 2019).

Nevertheless, looking more precisely at SHDI, we understand that with the increase in the index, the specific richness decreases. Knowing that the Shannon's index formula takes into account the species richness and relative abundance of taxa, when it increases, it means that the number of species increases and is at its maximum when they are equally

represented (Omayio & Mzungu, 2019; Shannon, 2001). Except that this last point is not applicable in our case. Indeed, looking at the boxplots and Pie charts, we notice that the abundance of plant taxa is not well distributed and some species are more dominant than others.

In addition, boxplots, pie charts, accumulation curves and PCoA demonstrate a very similar distribution between 95% and 97% of certitude parameters. Moreover, it highlights a likeness between these parameters and *Apis cerana japonica* distribution. It means that globally the dataset is more representative of the Japanese honeybee diet than *Apis mellifera*'s and that the proportion between both bee species is unbalanced.

The pattern of taxa richness decreasing with SHDI has also been observed in another study which suggests that urban areas should bring a more diversified flora to bees. Since urban areas are considered highly heterogeneous, they can host more diverse plant communities than a wider and more heterogeneous landscape (Fox et al., 2022). Thus, a more heterogeneous environment and diversification in its habitats does not increase taxonomic richness because urban areas alone would offer more floral diversity than a all landscape. However, this argument can be questioned in light of the results of other studies, showing that urban and rural areas do not explain the taxonomic richness (Noël et al., 2023; Wilson & Jamieson, 2019).

Regarding Patch Density and Integral Index of Connectivity, these two landscape metrics also have an unexpected pattern compared to what can be found in the scientific literature (Aubert, 2016; Fujiwara & Washitani, 2017; Scariot, 1999). However, other studies highlight certain interesting points to understand this.
First, several studies showed the influence of the environment on different bee species (Paterson et al., 2019; Wojcik & McBride, 2012). For example, small bees, such as honey bees, will easily forage from urban environments unlike large bees that need wild landscapes (Wojcik & McBride, 2012). This suggests that depending on LULC around apiaries, honey bees do not necessarily visit more different species of flowers with a bigger PD or with a higher connectivity.

In addition, looking at the most abundant plant species foraged by both honey bees, diet preferences can be seen and links with the natural distributions of these plants in different habitats can be analysed. Pie charts show different abundant floral species visited by *A. cerana japonica* and *A. mellifera* and each are mostly distributed in the same type of habitat areas. This suggests that bees forage in some of the 12 LULC and increasing the diversity of habitats does not contribute to their diet.

Then, patches containing a high density of flowers are preferred because it reduces the flight times between flowers (Barley et al., 2022). Honey bees preferably choose plots containing the same plant species when they are assured of the quality of the flower bed, that is, if it contains plant species nutritionally good and healthy (Lau et al., 2019). Thus, it is saving the energy of pollinators, which is especially true for bee species that have a small

capacity for research and dispersal (Paterson et al., 2019). Although *Apis cerana japonica* and *Apis mellifera* are not considered to have a small foraging capacity (Marzinzig et al., 2018), the point is that for energy saving, bees will not disperse inconsistently to forage.

Finally, Aubert (2016) points out in his study that patch augmentation only improves pollination services when the study area is less than 15 ha, knowing that our surface area is about 80 ha.

Although few studies corroborate the results of this work, they provide us with some statements or information that can explain the pattern of explanatory variables in the face of taxonomic richness.

4 Foraging preferences and cohabitation between *Apis cerana japonica* and *Apis mellifera*

Thanks to the pie chart (Figure 8c-8d), it was observed that some plant species appeared more abundantly than others such as *Ulmus parvifolia* and *Ulmus* sp., *Hedera nepalensis* and *Chengiopanax sciadophylloides* for *Apis cerana japonica* and *Physalis angulata*, *Triticum aestivum* and *Cosmos bipinnatus* for *Apis mellifera*.

Ulmus parvifolia is a tree, to 25 metres tall, with deciduous leaves and blooms late in summer and in early autumn and it is pollinated by honey bees (Kim et al., 2012). This species can be found in Japan, in woods and disturbed sites.
As for *Humulus scandens*, it is located in Japan, in woodland areas at the borders of the forests, in meadows, on river banks, in rural habitats or in wastelands. This plant is annual and blooms in spring and summer. Therefore, pollen detected may have been stored by honey bees and thus be found in honey. Moreover, Wu et al. (2023) already observed that *A. cerana* used to visit this plant species.
Concerning *Chengiopanax sciadophylloides*, it is also a deciduous tree distributed in Japan and found in sub-canopy tree species, widely distributed in its temperate forests (Torimaru et al., 2014). It blooms from September to December, which corresponds to the dates of the honey collection ("eFloras.org Home", n.d.; "World Plants: Plant List", n.d.).

As for the plant species most visited by *Apis mellifera, Physalis angulata* is an annual herb of 30-50 cm tall insect-pollinated such as honey bees (Figueiredo et al., 2020), located in forests, villages, roadsides, wetland and fields (Balah & Balah, 2022), all around Asia. It blooms and produces fruits throughout the year.
The second most abundant species is *Triticum aestivum*, which is a cultivated species. To 60–130 cm tall, this plant is found in worldwide fields. The problem with this plant species is that it is recognized as self-pollinating and it also depends heavily on the wind for its pollination (Willenborg & Van Acker, 2008). It is therefore abnormal to find such an abundance of DNA in the honey of *A.mellifera*. But let's highlight that the honey bee is

considered as an environmental bioindicator species such for pollution (Singh et al., 2023) due to its body that it is covered with hairs (Balayiannis & Balayiannis, 2008). Thus, it has been observed that components in the surrounding air can be found in the honey of some hives of *Apis mellifera* (Singh et al., 2023). What is possible in view of the proportion of cropland area around the apiaries, pollen of *Triticum aestivum* carried by the wind may have ended up in the surrounding environment when the Western bee was foraging. Then, concerning the high abundance of wheat compared to other plant species found in honey, it is considered that biases can occur during PCR and the amplification state of the DNA strands (Liu et al., 2020). Finally, *Cosmos bipinnatus* is an annual insect-pollinated flower plant, widely distributed in gardens all around the world and it is a season-long flowering ("eFloras.org Home", n.d.; Malerba & Nattero, 2012; "World Plants: Plant List", n.d.).

The notable difference between the two bee species is that the first will have more woody plant species mostly distributed in forest areas, while the second has more visited herbaceous species located in different habitats. This can be compared with the most represented proportion of LULC in a buffer of 500-metres around apiaries. Indeed, most common types of habitat are cropland and urban areas, which is partly consistent with plant species distributions. That is more true for *Apis mellifera* that mainly got plant species found in cropland. Concerning *Apis cerana japonica*, these are plant species found in the forest and two of the plant species are deciduous broad-leaf trees. Since DBF represents 7.91% of the total surface area and it presents 26 times on 32 apiaries, it corroborates with the distribution of the top three of detected plant species of the Japanese honey bee and the statement of which forests provide shelter and food support to *A. cerana japonica* (Potter et al., 2019).

In addition, as it has been explained earlier, croplands are very important for honey bees' diet to provide their needs in nectar. Concerning pollen supply, both herbaceous and woody species are necessary (Requier et al., 2015). Although, *A. cerana japonica* and *A. mellifera* get their plant trait preferences, both floral traits and the distribution of habitat areas in 500-metres buffers meet their nutritional needs for protein and amino acid.

This last observation is verified by figures 14a and 14b where we see that, apart from cohabitation, each bee species will have opposite behaviour as regards of diet choice. Indeed, looking at the ten plant species most foraged by *A. cerana japonica*, 60% of them are woody. While in *A. mellifera*, only two out of seven floral species are woody and are not abundant (3.9%)(Annex A). As it has been observed in previous studies, the Japanese honey bee forages on tall trees and the Western bee prefers short herbs (Nagamitsu & Inoue, 1999).

The preference of *A. cerana japonica* for woody plant species has also been observed by other studies. In Japan, Fujiwara and Washitani (2017) emphasises the importance of landscape areas such as "Satoyama" which serve as nesting and foraging areas for *Apis cerana japonica*. Within these areas, deciduous forests are essential for the Japanese bee to forage, especially in spring when the trees bloom.

As for the specific richness, the two bee species have 50-50 woody and herbaceous plants. Indeed, concerning *A. cerana japonica*, although woody plant species are crucial, the bee still needs herbaceous species during autumn when trees stop flowering (Fujiwara & Washitani, 2017; Lau et al., 2019; Noël et al., 2023). Nevertheless, Venn diagrams show a very small proportion of plant species in common (Figure 9c). So, although there is an equal number between woody and herbaceous species for both bee species, they do not share much between them.

Therefore, they each have their own foraging preferences and, as it has been observed, it suggests a low level of competition between both bee species (Mohamadzade Namin et al., 2022). Moreover at this time of the year, Nagamitsu and Inoue (1999) suggests that cooler temperatures in autumn and plant traits lead to two different reactions of both bee species. The Western bee will quicker forage herbaceous plants whereas the Japanese bee will prefer to feed a bit longer on trees, such as *Aralia* spp. This is also verified in other studies, where a seasonal change in food choice effect on bees is observed. Indeed, woody species are generally more visited in spring and summer (Lau et al., 2019; Noël et al., 2023).

A last point analysed during this work is the change of diet of *A. cerana japonica* in cohabitation with *A. mellifera*. Indeed, the dependence of the food choice of the Japanese bee according to the presence of the other honey bee species was found significant in this analysis, so there is an association between both (Figure 14a). It can be observed with the proportion of plant species visited by *Apis cerana japonica*, on 122 floral species, 56 are woody plant and 56 herbaceous and 38 of 56 woody plant species are not in cohabitation against 51 for herbaceous plants. It demonstrates that in cohabitation, Japanese honey bees will forage more on woody plants.

This suggests some form of competition between the two bee species. As a confirmation, the aggressiveness of *Apis mellifera* against *Apis cerana japonica* in direct competition has been identified several times in different studies (Koetz, 2013; Theisen-Jones & Bienefeld, 2016).

Nevertheless, it should be noted that this result seems to be contradictory to what is discussed above. Indeed, despite the obvious difference in the choice of plant species foraged by the two bee species, *Apis mellifera* interferes in the foraging behaviour of *Apis cerana japonica*. However, this can easily be explained by the imbalance in the number of samples and abundance between *Apis cerana japonica* in apiaries without and with *Apis mellifera*. Since the number of observations of the Japanese bee represents 85% of the whole dataset and 99% of the total abundance, it seems normal to observe two distinct diet groups between the two bee species.

5 Limitations and perspectives

Many aspects of this study have represented barriers or limitations in carrying out the work and some perspectives must be provided to improve this work.

First, the lack of data was an obstacle to the analysis of foraging overlap as it was not possible to compare the diet of *Apis cerana japonica* and *Apis mellifera* in view of the imbalance in the number of samples between both. Only a list could be established in order to visualise the taxa visited by the two bee species, but it was not possible to quantify the overlapping. Furthermore, it is difficult to draw conclusions about honey bee dietary preferences when the dataset is not complete, compared to Chao's estimates and the accumulation curve.

However, what remains interesting is to compare the diets of *Apis cerana japonica* in cohabitation with *Apis mellifera* and without cohabitation. This would allow us to better understand the foraging behaviour of the Japanese bee with or without competition.

Secondly, in order to better explain the taxonomic richness from the landscape variables, it will be interesting to work with a two kilometres buffer around the apiaries. This would continue to evaluate the metric patterns over a larger area, with more diversity in the patches. A comparison between the two databases, between 500-metres and two-kilometres of buffer, could show differences in the behaviour of taxonomic richness according to landscape metrics. As well as links with the taxa visited by bees and the most represented habitat areas.

V Conclusion

This study allows the analysis of the flora visited by honey bees in Japan, *Apis cerana japonica* and *Apis mellifera*. The aim was to evaluate the floral composition of the honey of the Japanese bee and the Western bee in Japan with metabarcoding technique. By using honey as an analysis matrix, it allows to have an overview of the surrounding plants in these different areas and to know their diversity. In addition to being useful to study of the floral ecology of these regions (Milla et al., 2021; Mohamadzade Namin et al., 2022), it allows to compare the diets of each of both honey bee species.

Thirty-six apiaries from different regions of Japan provided honey samples for laboratory analysis using the metabarcoding method. The use of honey in metabarcoding and the study of flora foraged by *Apis cerana japonica*, among others, are innovative aspects that were developed in this study.

The study of the floral diversity foraged by the two species of honey bees was made in connection with landscape variables. This revealed that three main variables can explained the taxonomic richness of plants detected in honey: Integral Index of Connectivity, Shannon's Diversity Index and total patch density. However, their pattern does not seem to follow what has already been observed in other studies, taxa richness does not rise with the increase of landscape metrics. But this can be explained by a lack of data, a too small study landscape area and an imbalance in the abundance of taxa.

The lack of data has been a real obstacle to the study of floral diversity and food overlap between both honey bee species. In addition to preventing the evaluation of the overlap index, no conclusions regarding a possible competition between *Apis cerana japonica* and *Apis mellifera* are possible. Then, this lack of samples was demonstrated by several tests and indices (accumulation curve, Chao index and PCoA) and indicate that a fully pool plant species was not obtained.

However, the abundant presence of certain plant species in one or the other honey bee species has been highlighted. As well as the food preferences of the Japanese bee for woody species and the Western bee for herbaceous species. The most represented plant species were described and links between different habitat types were observed. Woody species, visited by *Apis cerana japonica*, correspond in time (honey collection in autumn) and places. Indeed, of the different LULC, deciduous broad-leaf forests are present abundantly around the apiaries. The same observation can be made with *Apis mellifera* despite the lack of data. The flowering of the herbaceous plants and their natural habitat correspond to the period of honey collection and the strong presence of croplands around the apiaries.

Finally, honey bee preferences for woody or herbaceous species align with results from other studies. At the same time, the change of the behaviour of *Apis cerana japonica* in the

presence of *Apis mellifera* highlights that it has an impact on the diet of the Japanese bee, in Japan. To conclude, since the plant species overlapping is low, no competition between the two honey bee species could be found but it is observed that the presence of the Western honey bee influences the foraging behaviour of the Japanese honey bee and the lack of data prevents conclusions from being drawn.

VI Personal contribution

This Master thesis follows five years of collaboration with Chiba University, Japan. The data were taken on site and laboratory and bioinformatics analyses were done by Japanese laboratories.
My contribution was to make community and landscape analysis, as well as statistical analysis to respond to the project's objectives.

This work allowed me to invest in a field that I was not familiar with and to learn about different soft-wares. From the work of landscape analysis on QGIS and RStudio, it taught me to adapt to different programs and learn from different scientific communities to achieve my goals. Eventually, I had the opportunity to discover community analyses thanks to the theory presented and the help provided by the supervising team.

References

Abrol, D. P. (2011). Foraging. In H. R. Hepburn & S. E. Radloff (Eds.), *Honeybees of asia* (pp. 257–292). Springer. https://doi.org/10.1007/978-3-642-16422-4_12

Akaike, H. (1998). Information theory and an extension of the maximum likelihood principle. In E. Parzen, K. Tanabe, & G. Kitagawa (Eds.), *Selected papers of hirotugu akaike* (pp. 199–213). Springer. https://doi.org/10.1007/978-1-4612-1694-0_15

Ammon, U. v., Wood, S. A., Laroche, O., Zaiko, A., Tait, L., Lavery, S., Inglis, G. J., & Pochon, X. (2018). Combining morpho-taxonomy and metabarcoding enhances the detection of non-indigenous marine pests in biofouling communities [Number: 1 Publisher: Nature Publishing Group]. *Scientific Reports*, *8*(1), 16290. https://doi.org/10.1038/s41598-018-34541-1

Aubert, M. (2016). Effects of forest fragmentation on plant-bird mutualisms in new zealand lowland forests [Publisher: University of Canterbury]. Retrieved December 10, 2023, from http://hdl.handle.net/10092/14396

Ayers, A. C., & Rehan, S. M. (2021). Supporting bees in cities: How bees are influenced by local and landscape features [Number: 2 Publisher: Multidisciplinary Digital Publishing Institute]. *Insects*, *12*(2), 128. https://doi.org/10.3390/insects12020128

Balah, M. A., & Balah, A. M. (2022). Growth and ecological characteristics of physalis angulata invasive weed species in several invaded communities. *Biologia*, *77*(2), 325–338. https://doi.org/10.1007/s11756-021-00944-1

Balayiannis, G., & Balayiannis, P. (2008). Bee honey as an environmental bioindicator of pesticides' occurrence in six agricultural areas of greece. *Archives of Environmental Contamination and Toxicology*, *55*(3), 462–470. https://doi.org/10.1007/s00244-007-9126-x

Ballantyne, G., Baldock, K. C. R., Rendell, L., & Willmer, P. G. (2017). Pollinator importance networks illustrate the crucial value of bees in a highly speciose plant community [Number: 1 Publisher: Nature Publishing Group]. *Scientific Reports*, *7*(1), 8389. https://doi.org/10.1038/s41598-017-08798-x

Barley, T. A., Martinez Algarin, M. G., & Bauer, J. T. (2022). The effects of flower patch density on pollinator visitation. *Environmental Entomology*, *51*(2), 482–491. https://doi.org/10.1093/ee/nvab143

Beals, E. W. (1984, January 1). Bray-curtis ordination: An effective strategy for analysis of multivariate ecological data. In A. MacFadyen & E. D. Ford (Eds.), *Advances in ecological research* (pp. 1–55, Vol. 14). Academic Press. https://doi.org/10.1016/S0065-2504(08)60168-3

Bell, K. L., de Vere, N., Keller, A., Richardson, R. T., Gous, A., Burgess, K. S., & Brosi, B. J. (2016). Pollen DNA barcoding: Current applications and future prospects [Publisher: NRC Research Press]. *Genome*, *59*(9), 629–640. https://doi.org/10.1139/gen-2015-0200

Blaalid, R., Kumar, S., Nilsson, R. H., Abarenkov, K., Kirk, P. M., & Kauserud, H. (2013). ITS1 versus ITS2 as DNA metabarcodes for fungi [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/1755-0998.12065]. *Molecular Ecology Resources*, *13*(2), 218–224. https://doi.org/10.1111/1755-0998.12065

Bodenheimer, F. S. (1937). Studies in animal populations. II. seasonal population-trends of the honey-bee [Publisher: The University of Chicago Press]. *The Quarterly Review of Biology*, *12*(4), 406–425. https://doi.org/10.1086/394540

Boguslavsky, D. V., & Zakharov, I. S. (2021). Role of external factors in embryogenesis of apis mellifera. *Russian Journal of Developmental Biology*, *52*(6), 422–429. https://doi.org/10.1134/S1062360421060023

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.-S. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution [Publisher: Elsevier]. *Trends in Ecology & Evolution*, *24*(3), 127–135. https://doi.org/10.1016/j.tree.2008.10.008

Bonoan, R. E., Tai, T. M., Tagle Rodriguez, M., Feller, L., Daddario, S. R., Czaja, R. A., O'connor, L. D., Burruss, G., & Starks, P. T. (2017). Seasonality of salt foraging in honey bees (apis mellifera) [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/een.12375]. *Ecological Entomology*, *42*(2), 195–201. https://doi.org/10.1111/een.12375

Büchler, R., Drescher, W., & Tornier, I. (1992). Grooming behaviour ofApis cerana, apis mellifera andApis dorsata and its effect on the parasitic mitesVarroa jacobsoni andTropilaelaps clareae. *Experimental & Applied Acarology*, *16*(4), 313–319. https://doi.org/10.1007/BF01218573

Capella-Gutiérrez, S., Silla-Martínez, J. M., & Gabaldón, T. (2009). trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*, *25*(15), 1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Chao, A. (1984). Nonparametric estimation of the number of classes in a population [Publisher: [Board of the Foundation of the Scandinavian Journal of Statistics, Wiley]]. *Scandinavian Journal of Statistics*, *11*(4), 265–270. Retrieved December 4, 2023, from https://www.jstor.org/stable/4615964

Chao, A., & Jost, L. (2012). Coverage-based rarefaction and extrapolation: Standardizing samples by completeness rather than size [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1890/11-1952.1]. *Ecology*, *93*(12), 2533–2547. https://doi.org/10.1890/11-1952.1

Chen, C., Wang, H., Liu, Z., Chen, X., Tang, J., Meng, F., & Shi, W. (2018). Population genomics provide insights into the evolution and adaptation of the eastern honey bee (apis cerana). *Molecular Biology and Evolution*, *35*(9), 2260–2271. https://doi.org/10.1093/molbev/msy130

Chen, H., & Boutros, P. C. (2011). VennDiagram: A package for the generation of highly-customizable venn and euler diagrams in r. *BMC Bioinformatics*, *12*(1), 35. https://doi.org/10.1186/1471-2105-12-35

Cheng, T., Xu, C., Lei, L., Li, C., Zhang, Y., & Zhou, S. (2016). Barcoding the kingdom plantae: New PCR primers for ITS regions of plants with improved universality and specificity [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/1755-0998.12438]. *Molecular Ecology Resources*, *16*(1), 138–149. https://doi.org/10.1111/1755-0998.12438

Coissac, E., Riaz, T., & Puillandre, N. (2012). Bioinformatic challenges for DNA metabarcoding of plants and animals [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1365-294X.2012.05550.x]. *Molecular Ecology*, *21*(8), 1834–1847. https://doi.org/10.1111/j.1365-294X.2012.05550.x

Colwell, R. K., Coddington, J. A., & Hawksworth, D. L. (1997). Estimating terrestrial biodiversity through extrapolation [Publisher: Royal Society]. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *345*(1311), 101–118. https://doi.org/10.1098/rstb.1994.0091

Colwell, R. K., Mao, C. X., & Chang, J. (2004). Interpolating, extrapolating, and comparing incidence-based species accumulation curves [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1890/03-0557]. *Ecology*, *85*(10), 2717–2727. https://doi.org/10.1890/03-0557

Cook, S. M., Awmack, C. S., Murray, D. A., & Williams, I. H. (2003). Are honey bees' foraging preferences affected by pollen amino acid composition? [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1365-2311.2003.00548.x]. *Ecological Entomology*, *28*(5), 622–627. https://doi.org/10.1046/j.1365-2311.2003.00548.x

DeGrandi-Hoffman, G., Corby-Harris, V., Carroll, M., Toth, A. L., Gage, S., Watkins deJong, E., Graham, H., Chambers, M., Meador, C., & Obernesser, B. (2021). The importance of time and place: Nutrient composition and utilization of seasonal pollens by european honey bees (apis mellifera l.) [Number: 3 Publisher: Multidisciplinary Digital Publishing Institute]. *Insects*, *12*(3), 235. https://doi.org/10.3390/insects12030235

DeJong, T. M. (1975). A comparison of three diversity indices based on their components of richness and evenness [Publisher: [Nordic Society Oikos, Wiley]]. *Oikos*, *26*(2), 222–227. https://doi.org/10.2307/3543712

de Vere, N., Jones, L. E., Gilmore, T., Moscrop, J., Lowe, A., Smith, D., Hegarty, M. J., Creer, S., & Ford, C. R. (2017). Using DNA metabarcoding to investigate honey bee foraging reveals limited flower use despite high floral availability [Number: 1 Publisher: Nature Publishing Group]. *Scientific Reports*, *7*(1), 42838. https://doi.org/10.1038/srep42838

Devillers, J., & Pham-Delegue, M.-H. (2002, February 21). *Honey bees: Estimating the environmental impact of chemicals* [Google-Books-ID: yov_MkEt6EwC]. CRC Press.

Donkersley, P., Covell, L., & Ota, T. (2021). Japanese honeybees (apis cerana japonica radoszkowski, 1877) may be resilient to land use change [Number: 8 Publisher: Multidisciplinary Digital Publishing Institute]. *Insects*, *12*(8), 685. https://doi.org/10.3390/insects12080685

Dove, A. D. M., & Cribb, T. H. (2006). Species accumulation curves and their applications in parasite ecology. *Trends in Parasitology*, *22*(12), 568–574. https://doi.org/10.1016/j.pt.2006.09.008

Dyer, F. C., & Seeley, T. D. (1991). Dance dialects and foraging range in three asian honey bee species. *Behavioral Ecology and Sociobiology*, *28*(4), 227–233. https://doi.org/10.1007/BF00175094

Dzierżon, J. (1882). *Dzierzon's rational bee-keeping: Or, the theory and practice of dr. dzierzon ...* [Google-Books-ID: e1ADAAAAQAAJ]. Houlston & sons.

eFloras.org home. (n.d.). Retrieved December 11, 2023, from http://www.efloras.org/index.aspx

Falentin, H., Auer, L., Mariadassou, M., Pascal, G., Rué, O., Dugat-Bony, E., Delbes, C., Nicolas, A., Rifa, E., Mondy, S., Le Boulch, M., Cauquil, L., Hernandez Raquet, G., Terrat, S., & Abraham, A.-L. (2019). Guide pratique à destination des biologistes, bioinformaticiens et statisticiens qui souhaitent s'initier aux analyses métabarcoding [Publisher: INRA]. *Le Cahier des Techniques de l'INRA*, *2019*(97), 1–23. Retrieved December 17, 2023, from https://hal.science/hal-02311421

Fall, A., Fortin, M.-J., Manseau, M., & O'Brien, D. (2007). Spatial graphs: Principles and applications for habitat connectivity. *Ecosystems*, *10*(3), 448–461. https://doi.org/10.1007/s10021-007-9038-7

FAO. (2018). *The pollination of cultivated plants: A compendium for practitioners: Volume 1*. Retrieved July 6, 2023, from https://www.fao.org/documents/card/en/c/I9201EN

Fernandes, K., Prendergast, K., Bateman, P. W., Saunders, B. J., Gibberd, M., Bunce, M., & Nevill, P. (2022). DNA metabarcoding identifies urban foraging patterns of oligolectic and polylectic cavity-nesting bees. *Oecologia*, *200*(3), 323–337. https://doi.org/10.1007/s00442-022-05254-0

Figueiredo, M. C. C., Passos, A. R., Hughes, F. M., Santos, K. S. d., Silva, A. L. d., & Soares, T. L. (2020). Reproductive biology of physalis angulata l. (solanaceae). *Scientia Horticulturae*, *267*, 109307. https://doi.org/10.1016/j.scienta.2020.109307

Fox, G., Vellaniparambil, L. R., Ros, L., Sammy, J., Preziosi, R. F., & Rowntree, J. K. (2022). Complex urban environments provide apis mellifera with a richer plant forage than suburban and more rural landscapes [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/ece3.9490]. *Ecology and Evolution*, *12*(11), e9490. https://doi.org/10.1002/ece3.9490

Fujiwara, A., & Washitani, I. (2017). Dependence of asian honeybee on deciduous woody plants for pollen resource during spring to mid-summer in northern japan [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/ens.12228]. *Entomological Science*, *20*(1), 96–99. https://doi.org/10.1111/ens.12228

Ghosh, S., Jeon, H., & Jung, C. (2020). Foraging behaviour and preference of pollen sources by honey bee (apis mellifera) relative to protein contents [Number: 1 Publisher: BioMed Central]. *Journal of Ecology and Environment*, *44*(1), 1–7. https://doi.org/10.1186/s41610-020-0149-9

González-Varo, J. P., Biesmeijer, J. C., Bommarco, R., Potts, S. G., Schweiger, O., Smith, H. G., Steffan-Dewenter, I., Szentgyörgyi, H., Woyciechowski, M., & Vilà, M. (2013). Combined effects of global change pressures on animal-mediated pollination. *Trends in Ecology & Evolution*, *28*(9), 524–530. https://doi.org/10.1016/j.tree.2013.05.008

Gotelli, N. J., & Colwell, R. K. (2001). Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1461-0248.2001.00230.x]. *Ecology Letters*, *4*(4), 379–391. https://doi.org/10.1046/j.1461-0248.2001.00230.x

Goulson, D. (2003). Effects of introduced bees on native ecosystems [_eprint: https://doi.org/10.1146/annurev.ecolsys.34.011802.132355]. *Annual Review of Ecology,*

Evolution, and Systematics, *34*(1), 1–26. https://doi.org/10.1146/annurev.ecolsys.34.011802.132355

Gower, J. C. (2015). Principal coordinates analysis [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/9781118445112.stat05670.pub2]. In *Wiley StatsRef: Statistics reference online* (pp. 1–7). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118445112.stat05670.pub2

GSI. (1990). *The national atlas of japan | GSI* [Geospatial information authority of japan, ministry of land, infrastructure, transport and tourism]. Retrieved July 13, 2023, from https://www.gsi.go.jp/atlas/atlas-e-etsuran.html

Hanley, M. E., Franco, M., Pichon, S., Darvill, B., & Goulson, D. (2008). Breeding system, pollinator choice and variation in pollen quality in british herbaceous plants [Publisher: [British Ecological Society, Wiley]]. *Functional Ecology*, *22*(4), 592–598. Retrieved September 29, 2023, from https://www.jstor.org/stable/20142845

Haubruge, E., Nguyen, B. K., Widart, J., Thomé, J.-P., Fickers, P., & De Pauw, E. (2006). Le dépérissement de l'abeille domestique, apis mellifera l., 1758 (hymenoptera : Apidae) : Faits et causes probables. [Publisher: Faculté des Sciences Agronomiques de Gembloux. Zoologie Générale et Appliquée, Gembloux, Belgium]. *Notes Fauniques de Gembloux*, *59*(1). Retrieved February 16, 2023, from https://orbi.uliege.be/handle/2268/28284

Hawkins, J., Vere, N. d., Griffith, A., Ford, C. R., Allainguillaume, J., Hegarty, M. J., Baillie, L., & Adams-Groom, B. (2015). Using DNA metabarcoding to identify the floral composition of honey: A new tool for investigating honey bee foraging preferences [Publisher: Public Library of Science]. *PLOS ONE*, *10*(8), e0134735. https://doi.org/10.1371/journal.pone.0134735

Hepburn, H. R., & Radloff, S. E. (2011, January 4). *Honeybees of asia* [Google-Books-ID: zNuwxBP8MTUC]. Springer Science & Business Media.

Hervé, M. (2018). RVAideMemoire: Testing and plotting procedures for biostatistics. *R package version 0.9-69*, *3*. Retrieved December 4, 2023, from http://cran.opencpu.org/web/packages/RVAideMemoire/RVAideMemoire.pdf

Hesselbarth, M. H. K., Sciaini, M., With, K. A., Wiegand, K., & Nowosad, J. (2019). Landscapemetrics: An open-source r tool to calculate landscape metrics [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/ecog.04617]. *Ecography*, *42*(10), 1648–1657. https://doi.org/10.1111/ecog.04617

Horn, J., Becher, M. A., Johst, K., Kennedy, P. J., Osborne, J. L., Radchuk, V., & Grimm, V. (2021). Honey bee colony performance affected by crop diversity and farmland structure: A modeling framework [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/eap.2216]. *Ecological Applications*, *31*(1), e02216. https://doi.org/10.1002/eap.2216

Jamil, T., Ozinga, W. A., Kleyer, M., & ter Braak, C. J. (2013). Selecting traits that explain species–environment relationships: A generalized linear mixed model approach [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1654-1103.2012.12036.x]. *Journal of Vegetation Science*, *24*(6), 988–1000. https://doi.org/10.1111/j.1654-1103.2012.12036.x

Jia, A., Xu, L., & Wang, Y. (2021). Venn diagrams in bioinformatics. *Briefings in Bioinformatics*, *22*(5), bbab108. https://doi.org/10.1093/bib/bbab108

Kamo, T., Kusumoto, Y., Tokuoka, Y., Okubo, S., Hayakawa, H., Yoshiyama, M., Kimura, K., & Konuma, A. (2018). A DNA barcoding method for identifying and quantifying the composition of pollen species collected by european honeybees, apis mellifera (hymenoptera: Apidae). *Applied Entomology and Zoology*, *53*(3), 353–361. https://doi.org/10.1007/s13355-018-0565-9

Kato, M., Shibata, A., Yasui, T., & Nagamasu, H. (1999). Impact of introduced honeybees, apis mellifera, upon native bee communities in the bonin (ogasawara) islands [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1007/s101440050025]. *Population Ecology*, *41*(2), 217–228. https://doi.org/10.1007/s101440050025

Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772–780. https://doi.org/10.1093/molbev/mst010

Khan, K. A., Ghramh, H. A., Ahmad, Z., El-Niweiri, M. A. A., & Mohammed, M. E. A. (2021). Honey bee (apis mellifera) preference towards micronutrients and their impact on bee colonies. *Saudi Journal of Biological Sciences*, *28*(6), 3362–3366. https://doi.org/10.1016/j.sjbs.2021.02.084

Kim, G.-T., Kim, H.-J., & Choo, G.-C. (2012). Study on flowering, pollination and samara characteristics of chinese elm, ulmus parvifolia in wonju, korea [Publisher: Korean Society of Environment and Ecology]. *Korean Journal of Environment and Ecology*, *26*(4), 588–592. Retrieved January 1, 2024, from https://koreascience.kr/article/JAKO201227933000828.page

Kindt, R. (2020). BiodiversityR: Package for community ecology and suitability analysis [Accepted: 2021-04-20T05:05:18Z]. Retrieved December 4, 2023, from https://cgspace.cgiar.org/handle/10568/113431

Klymus, K. E., Marshall, N. T., & Stepien, C. A. (2017). Environmental DNA (eDNA) metabarcoding assays to detect invasive invertebrate species in the great lakes [Publisher: Public Library of Science]. *PLOS ONE*, *12*(5), e0177643. https://doi.org/10.1371/journal.pone.0177643

Koetz, A. H. (2013). Ecology, behaviour and control of apis cerana with a focus on relevance to the australian incursion [Number: 4 Publisher: Multidisciplinary Digital Publishing Institute]. *Insects*, *4*(4), 558–592. https://doi.org/10.3390/insects4040558

Kress, W. J., Erickson, D. L., Jones, F. A., Swenson, N. G., Perez, R., Sanjur, O., & Bermingham, E. (2009). Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in panama [Publisher: Proceedings of the National Academy of Sciences]. *Proceedings of the National Academy of Sciences*, *106*(44), 18621–18626. https://doi.org/10.1073/pnas.0909820106

Lamontagne-Drolet, M., Samson-Robert, O., Giovenazzo, P., & Fournier, V. (2019). The impacts of two protein supplements on commercial honey bee (apis mellifera l.) colonies [Publisher: Taylor & Francis _eprint: https://doi.org/10.1080/00218839.2019.1644938].

Journal of Apicultural Research, *58*(5), 800–813. https://doi.org/10.1080/00218839.2019.1644938

Lau, P., Bryant, V., Ellis, J. D., Huang, Z. Y., Sullivan, J., Schmehl, D. R., Cabrera, A. R., & Rangel, J. (2019). Seasonal variation of pollen collected by honey bees (apis mellifera) in developed areas across four regions in the united states [Publisher: Public Library of Science]. *PLOS ONE*, *14*(6), e0217294. https://doi.org/10.1371/journal.pone.0217294

Layek, U., Mondal, R., & Karmakar, P. (2020). Honey sample collection methods influence pollen composition in determining true nectar-foraging bee plants [Publisher: Sociedade Botânica do Brasil]. *Acta Botanica Brasilica*, *34*, 478–486. https://doi.org/10.1590/0102-33062020abb0086

Leponiemi, M., Freitak, D., Moreno-Torres, M., Pferschy-Wenzig, E.-M., Becker-Scarpitta, A., Tiusanen, M., Vesterinen, E. J., & Wirta, H. (2023). Honeybees' foraging choices for nectar and pollen revealed by DNA metabarcoding [Number: 1 Publisher: Nature Publishing Group]. *Scientific Reports*, *13*(1), 14753. https://doi.org/10.1038/s41598-023-42102-4

Li, X., Ma, W., Shen, J., Long, D., Feng, Y., Su, W., Xu, K., Du, Y., & Jiang, Y. (2019). Tolerance and response of two honeybee species apis cerana and apis mellifera to high temperature and relative humidity [Publisher: Public Library of Science]. *PLOS ONE*, *14*(6), e0217921. https://doi.org/10.1371/journal.pone.0217921

Liu, M., Clarke, L. J., Baker, S. C., Jordan, G. J., & Burridge, C. P. (2020). A practical guide to DNA metabarcoding for entomological ecologists [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/een.12831]. *Ecological Entomology*, *45*(3), 373–385. https://doi.org/10.1111/een.12831

Lowe, A., Jones, L., Brennan, G., Creer, S., & de Vere, N. (2022). Seasonal progression and differences in major floral resource use by bees and hoverflies in a diverse horticultural and agricultural landscape revealed by DNA metabarcoding [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/1365-2664.14144]. *Journal of Applied Ecology*, *59*(6), 1484–1495. https://doi.org/10.1111/1365-2664.14144

Loy, A., & Hofmann, H. (2013). Diagnostic tools for hierarchical linear models [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/wics.1238]. *WIREs Computational Statistics*, *5*(1), 48–61. https://doi.org/10.1002/wics.1238

Malerba, R., & Nattero, J. (2012). Pollinator response to flower color polymorphism and floral display in a plant with a single-locus floral color polymorphism: Consequences for plant reproduction. *Ecological Research*, *27*(2), 377–385. https://doi.org/10.1007/s11284-011-0908-2

Martins, A. C., Proença, C. E. B., Vasconcelos, T. N. C., Aguiar, A. J. C., Farinasso, H. C., de Lima, A. T. F., Faria, J. E. Q., Norrana, K., Costa, M. B. R., Carvalho, M. M., Dias, R. L., Bustamante, M. M. C., Carvalho, F. A., & Keller, A. (2023). Contrasting patterns of foraging behavior in neotropical stingless bees using pollen and honey metabarcoding [Number: 1 Publisher: Nature Publishing Group]. *Scientific Reports*, *13*(1), 14474. https://doi.org/10.1038/s41598-023-41304-0

Marzinzig, B., Brünjes, L., Biagioni, S., Behling, H., Link, W., & Westphal, C. (2018). Bee pollinators of faba bean (vicia faba l.) differ in their foraging behaviour and pollination

efficiency. *Agriculture, Ecosystems & Environment*, *264*, 24–33. https://doi.org/10.1016/j.agee.2018.05.003

Mazerolle, M. (2020). Model selection and multimodel inference using the AICcmodavg package. Retrieved December 5, 2023, from https://www.semanticscholar.org/paper/Model-selection-and-multimodel-inference-using-the-Mazerolle/de05d9426bf81799e1059ff2df32397f38f651da

McCullagh, P. (2019, January 22). *Generalized linear models* [Google-Books-ID: UzmDDwAAQBAJ]. Routledge.

McGarigal, K., Cushman, S., Neel, M., & Ene, E. (2002). FRAGSTATS: Spatial pattern analysis program for categorical maps.

Mestre, F., & Silva, B. (2023). Lconnect r package: A versatile tool for evaluating landscape connectivity and prioritizing habitat patches in conservation research. *Ecological Modelling*, *484*, 110489. https://doi.org/10.1016/j.ecolmodel.2023.110489

Milla, L., Sniderman, K., Lines, R., Mousavi-Derazmahalleh, M., & Encinas-Viso, F. (2021). Pollen DNA metabarcoding identifies regional provenance and high plant diversity in australian honey [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/ece3.7679]. *Ecology and Evolution*, *11*(13), 8683–8698. https://doi.org/10.1002/ece3.7679

Minor, E. S., & Urban, D. L. (2008). A graph-theory framework for evaluating landscape connectivity and conservation planning. *Conservation Biology: The Journal of the Society for Conservation Biology*, *22*(2), 297–307. https://doi.org/10.1111/j.1523-1739.2007.00871.x

Mohamadzade Namin, S., Ghosh, S., & Jung, C. (2023). Honey quality control: Review of methodologies for determining entomological origin [Number: 10 Publisher: Multidisciplinary Digital Publishing Institute]. *Molecules*, *28*(10), 4232. https://doi.org/10.3390/molecules28104232

Mohamadzade Namin, S., Kim, M.-J., Son, M., & Jung, C. (2022). Honey DNA metabarcoding revealed foraging resource partitioning between korean native and introduced honey bees (hymenoptera: Apidae). *Scientific Reports*, *12*. https://doi.org/10.1038/s41598-022-18465-5

Nagamitsu, T., & Inoue, T. (1999). Differences in pollen sources of apis cerana and apis mellifera at a primary beech forest in central japan [Publisher: Taylor & Francis _eprint: https://doi.org/10.1080/00218839.1999.11100997]. *Journal of Apicultural Research*, *38*(1), 71–78. https://doi.org/10.1080/00218839.1999.11100997

Noël, G., Mestrez, A., Lejeune, P., Francis, F., Kawai, J., Miwa, M., Uehara, K., & Nagase, A. (2023). Pollen meta-barcoding reveals different community structures of foraged plants by honeybees (apis mellifera l.) along space-time gradient in japan [Publisher: Elsevier GmbH]. *Urban Forestry and Urban Greening*, *79*. https://doi.org/10.1016/j.ufug.2022.127794

O'Brien, D., Manseau, M., Fall, A., & Fortin, M.-J. (2006). Testing the importance of spatial configuration of winter habitat for woodland caribou: An application of graph theory. *Biological Conservation*, *130*(1), 70–83. https://doi.org/10.1016/j.biocon.2005.12.014

OECD. (2002, January 16). *Environmental performance review of japan: Conclusions and recommendations*. https://doi.org/10.1787/9789264033931-en

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., Stevens, M., Szöcs, E., & Wagner, H. (2020, December 3). *Vegan community ecology package version 2.5-7 november 2020*.

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1600-0706.2010.18644.x]. *Oikos*, *120*(3), 321–326. https://doi.org/10.1111/j.1600-0706.2010.18644.x

Omayio, D., & Mzungu, E. (2019). Modification of shannon-wiener diversity index towards quantitative estimation of environmental wellness and biodiversity levels under a non-comparative scenario. https://doi.org/10.7176/JEES/9-9-06

O'Rourke, D. R., Bokulich, N. A., Jusino, M. A., MacManes, M. D., & Foster, J. T. (2020). A total crapshoot? evaluating bioinformatic decisions in animal diet metabarcoding analyses [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/ece3.6594]. *Ecology and Evolution*, *10*(18), 9721–9739. https://doi.org/10.1002/ece3.6594

Page, R. E., & Peng, C. Y. .-. (2001). Aging and development in social insects with emphasis on the honey bee, apis mellifera l. *Experimental Gerontology*, *36*(4), 695–711. https://doi.org/10.1016/S0531-5565(00)00236-9

Park, B., & Nieh, J. C. (2017). Seasonal trends in honey bee pollen foraging revealed through DNA barcoding of bee-collected pollen. *Insectes Sociaux*, *64*(3), 425–437. https://doi.org/10.1007/s00040-017-0565-8

Pascual-Hortal, L., & Saura, S. (2006). Comparison and development of new graph-based landscape connectivity indices: Towards the priorization of habitat patches and corridors for conservation. *Landscape Ecology*, *21*(7), 959–967. https://doi.org/10.1007/s10980-006-0013-z

Paterson, G. B., Smart, G., McKenzie, P., & Cook, S. (2019). Prioritising sites for pollinators in a fragmented coastal nectar habitat network in western europe. *Landscape Ecology*, *34*(12), 2791–2805. https://doi.org/10.1007/s10980-019-00884-x

Pinheiro, J. C., & Chao, E. C. (2006). Efficient laplacian and adaptive gaussian quadrature algorithms for multilevel generalized linear mixed models. *Journal of Computational and Graphical Statistics*, *15*(1), 58–81. https://doi.org/10.1198/106186006X96962

Potter, C., Vere, N. d., Jones, L. E., Ford, C. R., Hegarty, M. J., Hodder, K. H., Diaz, A., & Franklin, E. L. (2019). Pollen metabarcoding reveals broad and species-specific resource use by urban bees [Publisher: PeerJ Inc.]. *PeerJ*, *7*, e5999. https://doi.org/10.7717/peerj.5999

Radloff, S. E., Hepburn, C., Hepburn, H. R., Fuchs, S., Hadisoesilo, S., Tan, K., Engel, M. S., & Kuznetsov, V. (2010). Population structure and classification of apis cerana [Number: 6 Publisher: EDP Sciences]. *Apidologie*, *41*(6), 589–601. https://doi.org/10.1051/apido/2010008

Raja, H. A., Miller, A. N., Pearce, C. J., & Oberlies, N. H. (2017). Fungal identification using molecular tools: A primer for the natural products research community [Publisher:

American Chemical Society]. *Journal of Natural Products*, *80*(3), 756–770. https://doi.org/10.1021/acs.jnatprod.6b01085

Ramezani, H., Holm, S., Allard, A., & Ståhl, G. (2010). Monitoring landscape metrics by point sampling: Accuracy in estimating shannon's diversity and edge density. *Environmental Monitoring and Assessment*, *164*(1), 403–421. https://doi.org/10.1007/s10661-009-0902-0

Rao, C. R. (1995). A review of canonical coordinates and an alternative to correspondence analysis using hellinger distance [Accepted: 2007-12-07T10:26:32Z Publisher: Institut d'Estadística de Catalunya]. Retrieved December 4, 2023, from https://upcommons.upc.edu/handle/2099/4059

Remolina, S. C., & Hughes, K. A. (2008). Evolution and mechanisms of long life and high fertility in queen honey bees. *AGE*, *30*(2), 177–185. https://doi.org/10.1007/s11357-008-9061-4

Requier, F., Odoux, J.-F., Tamic, T., Moreau, N., Henry, M., Decourtye, A., & Bretagnolle, V. (2015). Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds [Publisher: [Wiley, Ecological Society of America]]. *Ecological Applications*, *25*(4), 881–890. Retrieved February 9, 2023, from https://www.jstor.org/stable/24432096

Richardson, R. T., Eaton, T. D., Lin, C.-H., Cherry, G., Johnson, R. M., & Sponsler, D. B. (2021). Application of plant metabarcoding to identify diverse honeybee pollen forage along an urban–agricultural gradient [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/mec.15704]. *Molecular Ecology*, *30*(1), 310–323. https://doi.org/10.1111/mec.15704

Richardson, R. T., Lin, C.-H., Sponsler, D. B., Quijia, J. O., Goodell, K., & Johnson, R. M. (2015). Application of ITS2 metabarcoding to determine the provenance of pollen collected by honey bees in an agroecosystem [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.3732/apps.1400066]. *Applications in Plant Sciences*, *3*(1), 1400066. https://doi.org/10.3732/apps.1400066

Ropars, L., Affre, L., Thébault, É., & Geslin, B. (2022). Seasonal dynamics of competition between honey bees and wild bees in a protected mediterranean scrubland [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/oik.08915]. *Oikos*, *2022*(4), e08915. https://doi.org/10.1111/oik.08915

Rotheray, E. L., Osborne, J. L., & Goulson, D. (2017). Quantifying the food requirements and effects of food stress on bumble bee colony development [Publisher: Taylor & Francis _eprint: https://doi.org/10.1080/00218839.2017.1307712]. *Journal of Apicultural Research*, *56*(3), 288–299. https://doi.org/10.1080/00218839.2017.1307712

Roulston, T. H., Cane, J. H., & Buchmann, S. L. (2000). What governs protein content of pollen: Pollinator preferences, pollen–pistil interactions, or phylogeny? [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1890/0012-9615%282000%29070%5B0617%3AWGPCOP%5D2.0.CO%3B2]. *Ecological Monographs*, *70*(4), 617–643. https://doi.org/10.1890/0012-9615(2000)070[0617:WGPCOP]2.0.CO;2

Sakai, T., Ono, M., Sakai, T., & Ono, M. (1991). Comparative studies on bionomics of apis mellifera l. and apis cerana japonica radoszkowski hymenoptera apidae ii. pollen gathering and foraging preferences. *Bulletin of the Faculty of Agriculture, Tamagawa University*, *31*, 169–178. Retrieved December 18, 2023, from https://eurekamag.com/research/007/136/007136360.php

Sang, T., Crawford, D. J., & Stuessy, T. F. (1997). Chloroplast DNA phylogeny, reticulate evolution, and biogeography of paeonia (paeoniaceae) [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.2307/2446155]. *American Journal of Botany*, *84*(8), 1120–1136. https://doi.org/10.2307/2446155

Saravanan, M., Mohanapriya, G., Laha, R., Sathishkumar, R., & Boatwright, J. (2019). DNA barcoding detects floral origin of indian honey samples [Publisher: Canadian Science Publishing]. *Genome*, *62*(5), 341–348. https://doi.org/10.1139/gen-2018-0058

Scariot, A. (1999). Forest fragmentation effects on palm diversity in central amazonia [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.1365-2745.1999.00332.x]. *Journal of Ecology*, *87*(1), 66–76. https://doi.org/10.1046/j.1365-2745.1999.00332.x

Seeley, T. D. (1986). Social foraging by honeybees: How colonies allocate foragers among patches of flowers. *Behavioral Ecology and Sociobiology*, *19*(5), 343–354. https://doi.org/10.1007/BF00295707

Senthilnathan, S. (2019, July 9). Usefulness of correlation analysis. https://doi.org/10.2139/ssrn.3416918

Shannon, C. E. (2001). A mathematical theory of communication. *ACM SIGMOBILE Mobile Computing and Communications Review*, *5*(1), 3–55. https://doi.org/10.1145/584091.584093

Shao, J., & Tu, D. (2012, December 6). *The jackknife and bootstrap* [Google-Books-ID: VO3SBwAAQBAJ]. Springer Science & Business Media.

Singh, A., Sohal, S. K., Singh, N., Arora, S., & Fnu, S. (2023). Cosmopolitan honey bee, apis mellifera, as quick and efficient marker of pesticide pollution in environment through RP-HPLC. *Environmental Science and Pollution Research*, *30*(35), 83452–83462. https://doi.org/10.1007/s11356-023-28051-5

Soorangkattan, S., Nalluchamy, K. D., Arumugam, S., Sivagnanam, C., Thulasinathan, B., Ramu, S. M., Alagarsamy, A., & Muthuramalingam, J. B. (2021). Studies on the influence of natural resource utilization by humans on foraging behavior of honey bees at rural ecosystems. *Environmental Science and Pollution Research*, *28*(26), 33942–33956. https://doi.org/10.1007/s11356-021-13192-2

Spanowicz, A. G., & Jaeger, J. A. G. (2019). Measuring landscape connectivity: On the importance of within-patch connectivity. *Landscape Ecology*, *34*(10), 2261–2278. https://doi.org/10.1007/s10980-019-00881-0

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stanley, J., Sah, K., Subbanna, A. R., Preetha, G., & Gupta, J. (2017). How efficient is apis cerana (hymenoptera: Apidae) in pollinating cabbage, brassica oleraceavar. capitata ?

pollination behavior, pollinator effectiveness, pollinator requirement, and impact of pollination [Publisher: Entomological Society of America]. *Journal of Economic Entomology*, *110*(3), 826–834. https://doi.org/10.1093/jee/tox115

Sugahara, M. (2000). Feral colonies of japanese honeybees, apis cerana japonica and their life history. 2. natural nests and swarming. *Honeybee Science*, *21*(1), 35–39. Retrieved July 4, 2023, from https://www.cabdirect.org/cabdirect/abstract/20000507667

Suwannapong, G., Benbow, M. E., & Nieh, J. C. (2012, December 1). *Biology of thai honeybees: Natural history and threats*. Retrieved July 4, 2023, from https://escholarship.org/uc/item/3w1448mh

Suyama, Y., Hirota, S. K., Matsuo, A., Tsunamoto, Y., Mitsuyuki, C., Shimura, A., & Okano, K. (2022). Complementary combination of multiplex high-throughput DNA sequencing for molecular phylogeny [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/1440-1703.12270]. *Ecological Research*, *37*(1), 171–181. https://doi.org/10.1111/1440-1703.12270

Taberlet, P., Gielly, L., Pautou, G., & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, *17*(5), 1105–1109. https://doi.org/10.1007/BF00037152

Tan, K., Yang, S., Wang, Z.-W., Radloff, S. E., & Oldroyd, B. P. (2012). Differences in foraging and broodnest temperature in the honey bees apis cerana and a. mellifera. *Apidologie*, *43*(6), 618–623. https://doi.org/10.1007/s13592-012-0136-y

Tanabe, A. S. (2011). Kakusan4 and aminosan: Two programs for comparing nonpartitioned, proportional and separate models for combined molecular phylogenetic analyses of multilocus sequence data [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1755-0998.2011.03021.x]. *Molecular Ecology Resources*, *11*(5), 914–921. https://doi.org/10.1111/j.1755-0998.2011.03021.x

Tanabe, A. S., & Toju, H. (2013). Two new computational methods for universal DNA barcoding: A benchmark using barcode sequences of bacteria, archaea, animals, fungi, and land plants [Publisher: Public Library of Science]. *PLOS ONE*, *8*(10), e76910. https://doi.org/10.1371/journal.pone.0076910

Tanaka, K., Nozaki, A., Nakadai, H., Shiwa, Y., & Shimizu-Kadota, M. (2020). Using pollen DNA metabarcoding to profile nectar sources of urban beekeeping in kōtō-ku, tokyo. *BMC Research Notes*, *13*(1), 515. https://doi.org/10.1186/s13104-020-05361-2

Tatsuno, M., & Osawa, N. (2016). Flower visitation patterns of the coexisting honey bees apis cerana japonica and apis mellifera (hymenoptera: Apidae) [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/ens.12206]. *Entomological Science*, *19*(3), 255–267. https://doi.org/10.1111/ens.12206

Theisen-Jones, H., & Bienefeld, K. (2016). The asian honey bee (apis cerana) is significantly in decline [Publisher: Taylor & Francis _eprint: https://doi.org/10.1080/0005772X.2017.1284973]. *Bee World*, *93*(4), 90–97. https://doi.org/10.1080/0005772X.2017.1284973

Tomlinson, S., Dixon, K. W., Didham, R. K., & Bradshaw, S. D. (2015). Physiological plasticity of metabolic rates in the invasive honey bee and an endemic australian bee species [Num

Pages: 835-844 Place: Heidelberg, Netherlands Publisher: Springer Nature B.V.], *185*(8), 835–844. https://doi.org/10.1007/s00360-015-0930-8

Torimaru, T., Okayama, T., Kumagai, Y., & Akada, S. (2014). Development and characterization of microsatellite loci in the sub-canopy tree chengiopanax sciadophylloides (araliaceae) using next-generation sequencing technology. *Conservation Genetics Resources*, *6*(3), 799–801. https://doi.org/10.1007/s12686-014-0223-7

Tucker, K. W. (1958). Automictic parthenogenesis in the honey bee. *Genetics*, *43*(3), 299–316. Retrieved September 28, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1209882/

Vanderplanck, M., Moerman, R., Rasmont, P., Lognay, G., Wathelet, B., Wattiez, R., & Michez, D. (2014). How does pollen chemistry impact development and feeding behaviour of polylectic bees? [Publisher: Public Library of Science, San Franscisco, United States - California]. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0086209

Verma, L. R., & Dulta, P. C. (1986). Foraging behaviour of apis cerana indica and apis mellifera in pollinating apple flowers [Publisher: Taylor & Francis _eprint: https://doi.org/10.1080/00218839.1986.11100717]. *Journal of Apicultural Research*, *25*(4), 197–201. https://doi.org/10.1080/00218839.1986.11100717

von Frisch, K., & Lindauer, M. (1956). The "language" and orientation of the honey bee [_eprint: https://doi.org/10.1146/annurev.en.01.010156.000401]. *Annual Review of Entomology*, *1*(1), 45–58. https://doi.org/10.1146/annurev.en.01.010156.000401

Wallinger, C., Juen, A., Staudacher, K., Schallhart, N., Mitterrutzner, E., Steiner, E.-M., Thalinger, B., & Traugott, M. (2012). Rapid plant identification using species- and group-specific primers targeting chloroplast DNA [Publisher: Public Library of Science]. *PLOS ONE*, *7*(1), e29473. https://doi.org/10.1371/journal.pone.0029473

Wang, Z., & Tan, K. (2014). Comparative analysis of olfactory learning of apis cerana and apis mellifera. *Apidologie*, *45*(1), 45–52. https://doi.org/10.1007/s13592-013-0228-3

Webster, M. T. (2019). Apis mellifera. *Trends in Genetics*, *35*(11), 880–881. https://doi.org/10.1016/j.tig.2019.08.003

Wickham, H. (2009). *Ggplot2: Elegant graphics for data analysis*. Springer. https://doi.org/10.1007/978-0-387-98141-3

Wickham, H. (2014). Tidy data. *Journal of Statistical Software*, *59*, 1–23. https://doi.org/10.18637/jss.v059.i10

Willenborg, C. J., & Van Acker, R. C. (2008). The biology and ecology of hexaploid wheat (triticum aestivum l.) and its implications for trait confinement [Publisher: NRC Research Press]. *Canadian Journal of Plant Science*, *88*(5), 997–1013. https://doi.org/10.4141/CJPS07144

Wilson, C. J., & Jamieson, M. A. (2019). The effects of urbanization on bee communities depends on floral resource availability and bee functional traits [Publisher: Public Library of Science]. *PLOS ONE*, *14*(12), e0225852. https://doi.org/10.1371/journal.pone.0225852

Winston, M. L. (1987). *The biology of the honey bee*. Harvard University Press.

Wojcik, V. A., & McBride, J. R. (2012). Common factors influence bee foraging in urban and wildland landscapes. *Urban Ecosystems*, *15*(3), 581–598. https://doi.org/10.1007/s11252-011-0211-6

Wood, T. J., Michez, D., Paxton, R. J., Drossart, M., Neumann, P., Gérard, M., Vanderplanck, M., Barraud, A., Martinet, B., Leclercq, N., & Vereecken, N. J. (2020). Managed honey bees as a radar for wild bee decline? *Apidologie*, *51*(6), 1100–1116. https://doi.org/10.1007/s13592-020-00788-9

Woolf, B. (1957). The log likelihood ratio test (the g-test) [_eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1469-1809.1972.tb00293.x]. *Annals of Human Genetics*, *21*(4), 397–409. https://doi.org/10.1111/j.1469-1809.1972.tb00293.x

World plants: Plant list. (n.d.). Retrieved December 11, 2023, from https://www.worldplants.de/world-plants-complete-list/complete-plant-list

Wu, F., Liu, S., Jiang, H., Wang, Q., Chen, K., & Li, H. (2023). Pollination difference between apis cerana cerana and apis mellifera ligustica during the late autumn and winter. *Biodiversity Science*, *31*(5), 22528. https://doi.org/10.17520/biods.2022528

Yadav, S., Kumar, Y., & Jat, B. L. (2017). Honeybee: Diversity, castes and life cycle. In Omkar (Ed.), *Industrial entomology* (pp. 5–34). Springer. https://doi.org/10.1007/978-981-10-3304-9_2

Appendices

A Plant species overlap between *Apis cerana japonica* and *Apis mellifera*

Table 11: Plant species overlap between *Apis cerana japonica* and *Apis mellifera*.

Apis cerana japonica	**Overlap**	***Apis mellifera***
Abelmoschus esculentus	Ambrosia trifida	Actinidia chinensis
Actinidia arguta	Perilla frutescens	Ambrosia trifida
Actinidia polygama	Rhus chinensis	Chrysanthemum lavandulifolium
Actinidia sp.	Undetermined	Cicer arietinum
Aeschynomene indica		Clematis terniflora
Allium cepa		Commelina communis
Allium sp.		Cosmos bipinnatus
Amaranthus palmeri		Daphniphyllum oldhamii
Amaranthus sp.		Erythrina sp.
Amaranthus viridis		Koelreuteria sp. Harder 5724
Ambrosia trifida		Lagerstroemia tomentosa
Ampelopsis bodinieri		Mesembryanthemum cordifolium
Anemone hupehensis		Perilla frutescens
Angelica gigas		Physalis angulata
Angelica keiskei		Rhus chinensis
Aralia californica		Styrax hemsleyanus
Aralia elata		Thalictrum sp.
Aralia sp.		Toxicodendron sp.
Artemisia sp.		Triticum aestivum
Benincasa hispida		Undetermined
Berberis sp.		
Berchemia racemosa		
Berchemia sp.		
Bidens andicola		
Bidens aurea		
Bidens pilosa		
Brassica sp.		
Callicarpa nudiflora		
Camellia japonica		
Camellia sinensis		
Camellia tachangensis		

Castanea crenata Castanea dentata Causonis japonica Cenchrus compressus Chengiopanax sciadophylloides Chenopodium album Chenopodium sp. Citrullus sp. Clematis apiifolia Corchoropsis tomentosa Cosmos sulphureus Cucurbita maxima Dendropanax morbifer Dieteria canescens Eleusine indica Euphorbia maculata Fagopyrum esculentum Fatoua villosa Gamblea ciliata Gentiana scabra Glycine max Hedera nepalensis Helianthus annuus Helianthus sp. Hovenia dulcis Humulus scandens Hydrangea hydrangeoides Hydrangea petiolaris Juniperus sp. Justicia procumbens Kalopanax septemlobus Lagerstroemia indica Lagerstroemia sp. Lagerstroemia subcostata Luffa aegyptiaca Mallotus sp. Nelumbo nucifera Nymphaea mexicana Oryza sp. Osmanthus fragrans Paederia foetida		

Paederia sp. Paspalum dilatatum Patrinia sp. Perilla frutescens Phytolacca sp. Pittosporum sp. Plantago asiatica Plantago sp. Prunus spinulosa Prunus zippeliana Pueraria montana Pyrrosia hastata Raphanus sativus Rhaphiolepis bibas Rhus chinensis Rudbeckia laciniata Sanguisorba sp. Scilla scilloides Sicyos sp. Solanum lycopersicum Solanum sp. Solidago canadensis Solidago sp. Spiraea prunifolia Styphnolobium japonicum Triadica sebifera Trichosanthes sp. Triticum monococcum Ulmus parvifolia Ulmus sp. Undetermined Zanthoxylum ailanthoides Zelkova sp.		

B Residual Diagnostics for Hierarchical Regression Model (DHARMa residual)

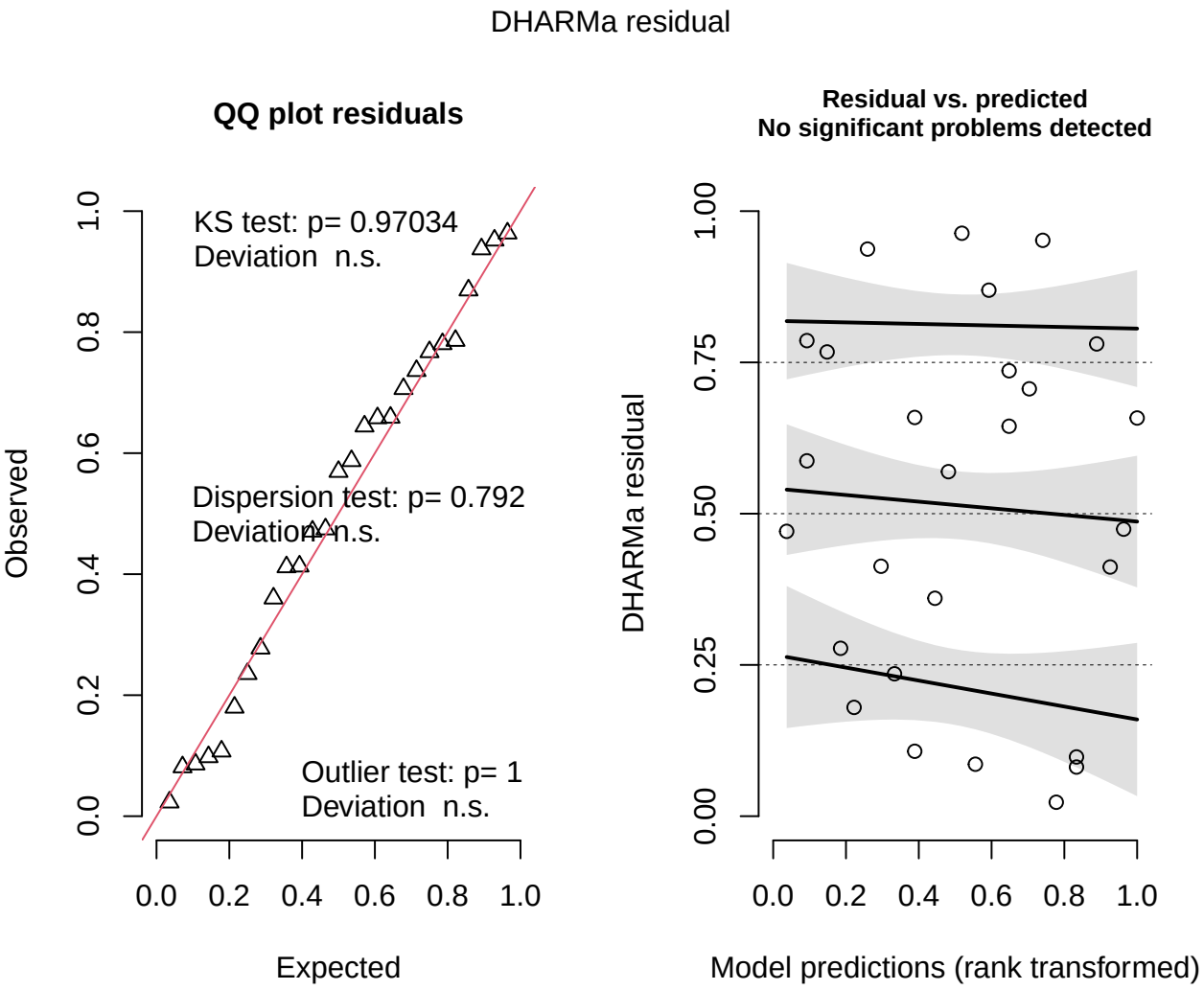


Figure 15: Residual Diagnostics for Hierarchical Regression Model: on the left, QQ plot of residuals; and on the right, DHARMa residual graph of the three landscape variables.