Chapter

Application of Environmental DNA: Honey Bee behavior and Ecosystems for Sustainable Beekeeping

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Abstract

Honey prices can vary widely depending on the production areas and/or the nectar plants, and quality control, therefore, is of great significance. Also, the identification of the nectar plants is one of the major concerns regardless of the purposes of beekeeping, namely, commercial, recreational, or for environmental education. In recent years, the scope for the application of eDNA technology has been expanding. We conducted an eDNA analysis of the 14 types of honey sold in supermarkets. The result showed that all of the honey samples contained DNA of several plants and revealed that there was no monofloral honey. In addition, there were cases where there was a discrepancy between the plants listed on the labels and the species whose DNA was the most prominent in the sample. DNA analysis of honey is considered to have the potential to enhance exponentially the understanding of the plant species that honeybees used as nectar plants and their proportions.

Keywords: environmental DNA, ecosystem services, sustainable beekeeping, multifloral honey, monofloral honey, honey-source plant, urban beekeeping, beekeeping in Japan

1. Introduction

Honey is a relatively expensive food product, but the prices vary largely. The differences in popularity and prices of honey are largely determined by factors such as country of origin and nectar plants, as well as the stories associated with the honey – who made it, when they made it, and where they made it. Hence, it is important to understand these stories behind each honey product.

There are various types of honey in the world, but information that we can obtain is limited. So, in this chapter, we start with a brief explanation of the status of beekeeping in the world, followed by an introduction to the history of Japanese beekeeping that is rarely told in western literatures written in English. We then share an overview of urban beekeeping that has gained popularity in recent years and discuss the risks and regulations surrounding the movement. In the last chapter, we show the results of our eDNA analysis of honey and indicate the validity of these results.

The history of beekeeping is showing that beekeeping is a part of culture of human society [1]. The beekeeping culture is historically included in regional food culture [2]. Urban beekeeping became one of the main ways of beekeeping in different regions of the world [3]. Urban environment can contribute to maintenance of diversity of honeybee species including native species [4]. Alternatively, the issues of pesticides are becoming serious in some countries [5], although the honeybee colony declines can be caused by complex factors including pesticides and global honey trade [6]. Trends of beekeeping and honey production in Japan and South Korea are analyzed in the previous study [7]. The transmission of the knowledge of beekeeping is the one of the research topics in the field of environmental studies [8].

As the IPBES assessment report suggested, management pollination services are an urgent global task. In this circumstance, urban beekeeping is gaining attention in terms of various aspects including ecosystem diversity, genetic diversity of honeybee, educational practices, and so on. The size of urban areas is relatively small as compared with farmland and other habitats of honeybee. However, the roles of urban areas to maintain the genetic diversity of organisms and to enhance the environmental awareness of citizens are suggested by the existing studies [4, 7], and urban beekeeping is expected as a main way of beekeeping. In the promotion of urban beekeeping, rack of scientific evidence of behavior of urban honeybee is a serious issue. To provide the scientific evidence, environmental DNA analysis can be utilized to detect the details of nectar sources. In this regard, this chapter reviews the status and trend of urban beekeeping and discusses the results of the application of environmental DNA analysis. The methods provided in this chapter can be applied for other cases and contribute to accumulating the scientific evidence for making relevant policies of urban beekeeping.

2. Materials and methods

This paper consists of two analytical frameworks. First, we reviewed the historical documents, articles, and relevant policy documents in order to capture status and trends of issues of honey production from bees.

As second phase of analysis, we conducted e-DNA analysis to identify the nectar source of honey produced by the authors and compared them with those products sold in the supermarket.

We extracted the DNA according to the method of Hawkins et al. [9] and amplified the DNA using the ITS-p3/ITS-u4 primer pair [10]. Then, the DNA metabarcoding of the plant contained in honey was implemented using the next-generation sequencer MiSeq.

3. Results and discussion

3.1 The status of beekeeping

3.1.1 The status of beekeeping around the world

Honeybees are important not only as honey producers but also as pollinators for agricultural crops [11]. Their importance has been raised as an easily comprehended example of the ecosystem service brought by nature in the context of conservation of biodiversity. In the engagement of the report of The Economics of Ecosystem and Biodiversity (TEEB), attempts have been made to quantify (and if possible, monetize) various services of the ecosystem, and as such, the importance of the pollinating function is described as "five times value of the production of honey" in the report [12]. Also, at the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), the phrase "Nature's Contribution to People

(NCP) has been advocated instead of the services of the ecosystem. In a report published by IPBES, too, the economic value of the pollinating service of honeybees is estimated to be up to 577 billion dollars, highlighting its importance [13].

The decline of the pollination function has been a major concern socially and economically, as well as scientifically. In the report, which points out the decrease of pollinators and their related pollinating services, some examples of research into the decline of pollinators and related vegetation are showcased as a global concern [11].

In the case of honeybees, examples of Colony Collapse Disorder (CCD) have been observed involving a serious shortage of honeybees in the United States and Europe. In the United States, roughly one third of the honeybees kept for pollination was lost from 2007 to 2008, causing a major concern [14]. In Europe, too, similar phenomena were reported in Germany, Belgium, France, Holland, Poland, Spain, and so on. Further, similar cases were reported in other countries elsewhere including Brazil, India, Taiwan, and Japan.

While a number of theories have been put forward to address the causes of CCD, including agrichemicals, infections, malnutrition, electromagnetic waves, and genetically modified crops, the mechanism of CCD has not been fully understood. However, neonicotinoid agrochemicals are one of the possible candidates as a cause of the disorder [15], and they are thus restricted in EU countries [16].

For beekeeping around the world, *Apis mellifera* is predominantly used. There are said to be approximately 10 species of honeybees, and *Apis mellifera* is considered superior to others in terms of amount of honey production and the ease of keeping. Other species have hardly been domesticated.

3.1.2 Beekeeping in Japan

In Japan, the two species, namely, *Apis mellifera* and *Apis cerana*, are used for beekeeping. Until *Apis mellifera* was introduced in the nineteenth century, only *Apis cerana* was used. In some of the old literature, sketches of beekeeping using *Apis cerana* can be found [17, 18].

In “the Chronicles of Japan (*Nihon Shoki*)”, there is a description that in 643, some Koreans attempted beekeeping on Mt. Miwa using four sheets of honeycomb but failed [19].

There is a record from 739 of honey being listed as one of the offerings from Korea, along with other products such as those made from panthers and ginseng, which implies that honey was treated as a precious imported item [18].

Entering the 900s, a record was found that honey and comb honey were presented to the Imperial Court from various countries. Considering the amount of the honey presented from each prefecture was around 2–4 L, it is thought that it was an extremely valuable commodity [18]. “*The Tale of Genji (Genji monogatari),*” the oldest novel in the world written in 1008, describes how honey was used as one of the ingredients to make incense.

Arriving at the Edo-era (after 1600), with the advance in the research into honey production, educational books explaining the beekeeping technology accompanied by illustrations began to be published. As the amount of honey production increased, it came to be used mainly as medicine by the general public (**Figure 1**).

The person who succeeded in the most sophisticated beekeeping in Japan, before the time any modern beekeeping technology was introduced there, was Ichiemon Sada in Wakayama prefecture. He standardized the hive boxes and kept several hundreds of bee colonies. The honey yielded from one of these colonies was 4.7 kg, which is a considerable amount for beekeeping using *Apis cerana* [17–19].

The illustration above is a part of “The Honey Catalogue (*Hachimitsu Ichiran*),” compiled to be exhibited at the World Expo held in 1873 in Austria. It is evident

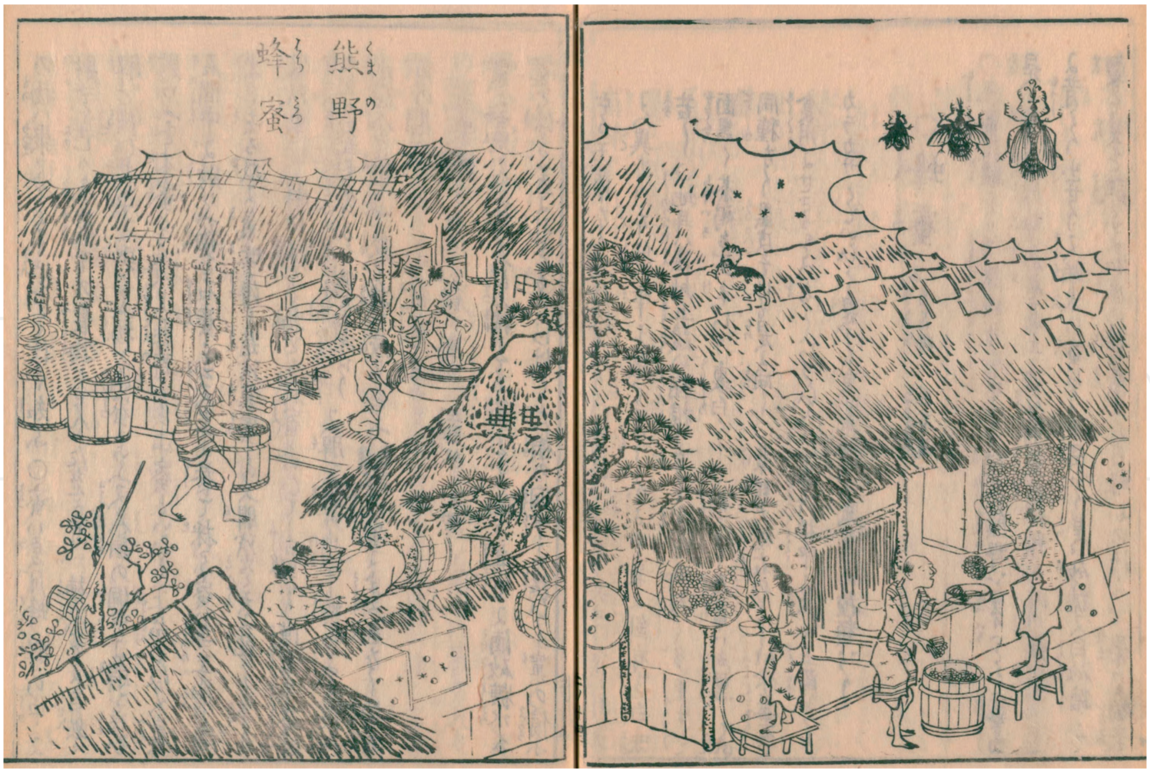

Figure 1.
*Beekeeping in the Edo period using Japanese honeybees (*Apis cerana japonica*). From "Noted Products from Land and Sea of Japan in Pictures (*Nihon sankai meisan zue*)," 1799.*

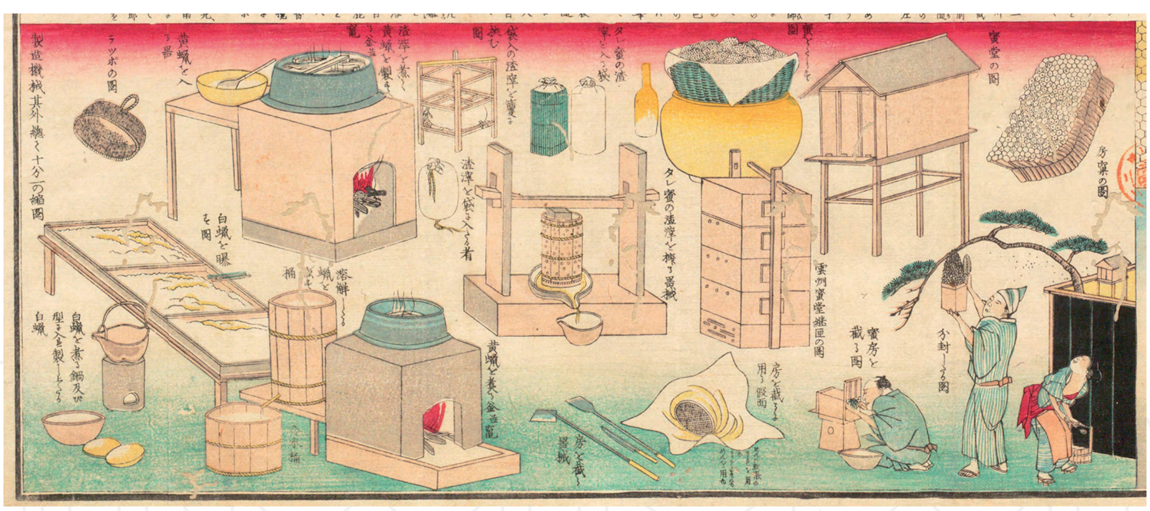

Figure 2.
Illustrations of beekeeping before introduction of Apis mellifera*. This figure shows the process of beekeeping and production of beehoney using native Japanese species in Edo period. The words in the figures are written in ancient languages describing the names and process of production of the individual tools. From "The Honey Catalogue (*Hachimitsu Ichiran*)," 1873.*

from this that a kind of technology very similar to the level of the modern beekeeping was already established (**Figure 2**).

In 1877, *Apis mellifera* was imported from the United States for the first time. From around 1905, the number of domestic beekeepers started to increase rapidly and so did the domestic production of honey. In Japan, honey production reached its peak around 1965 [20, 21].

Advancement of Japanese industrialization accelerated the development of agricultural land and the accompanying reduction of land suitable for beekeeping. The area planted with astragalus, which was a major source plant for honey, decreased to roughly 11% compared with the level of around 1970, while for rapeseed, it dropped to about 5% over the same period. Meanwhile, relatively the ratio of cheap imported honey increased in the Japanese market, and the aging of domestic beekeepers was accelerated. Furthermore, the number of domestic beekeepers became approximately half compared with before that period [18]. Currently, domestic honey production has been hovering around 2800 tons with the domestic self-sufficiency ratio of approximately 5–7% [22]. Of all the imported honey, 70% comes from China and 10% from Argentina [22]. Domestic honey production was on a declining trend up to around 2010 but has been moving sideways since then.

In Japan, beekeeping using domestic species has been gaining popularity, and some organizations have been set up to share beekeeping know-how [23]. Japanese honeybees (*Apis cerana japonica*) have a mild temperament and are less dangerous but produce less honey, so they are relatively more suited for hobby beekeeping [24]. A common method of beekeeping is to guide wild honeybees into a tree hollow or some artificial hive box with a cavity.

In Japanese honey bees (*Apis cerana japonica*), some aggregation pheromone [a mixture of 3-hydroxyoctanoic acid (3-HOAA) and 10-hydroxy-(E)-2-decenoic acid (10-HAD)], which is found in orchidaceous plants, has been identified [25]. Therefore, beekeepers use a type of orchid *Cymbidium floribundum*, which secretes this aggregation pheromone or the composite thereof in order to induce honeybees into their artificial hive boxes. Also, some of the other beekeepers have been attempting to establish other methods using Langstroth hives or flow hive boxes [26–28].

3.1.3 Urban beekeeping

There are records of beekeeping in urban areas dating from ancient time, but it is only recently when it gained prominence. Since 2005, urban beekeeping began to expand in various European countries, before spreading into North America, Asia, Latin America, and Africa.

In Palais Garnier of the Paris Opera, beekeeping has been going on for the last 30 years, and now, it is seen in various landmark locations in the city such as Orsay Museum and Grand Palais. In the United Kingdom, it has increased by 200% between 1999 and 2006, while in New York, the number of beehives kept has gone up to 10 times since 2010. In Paris, over 700 bee colonies are in existence [29].

In South Korea, there are business entities specializing in urban beekeeping, while in Japan, one of the beekeeping traders with the longest history is engaged in commercial beekeeping in the surrounding areas of the Imperial Palace.

In addition to honey production, urban beekeeping is thought to be contributing to the conservation of biodiversity by compensating for the function of the indigenous pollinators such as *Apis cerana* that had decreased due to the development of the natural environment over the years. In fact, in the surrounding areas of the Imperial Palace in Tokyo, known for the cherry blossoms, more cherry fruits have been observed after the blossoms. This suggests that the increase of urban beekeeping near the Imperial Palace may be a factor.

In the United Kingdom, community groups play an important role in the development of urban beekeeping, but there is also a support from local government to promote it [30].

It is generally understood that urban beekeeping has a greater role in improving quality of life as it provides a form of hobby and a communication tool, in addition to the function of honey production and pollinating. In fact, commercial beekeeping is rare in urban areas, and for the most part, the number of bee colonies is usually only up to a few per area. In Japan, where NGOs, private companies, as well as local government are involved in urban beekeeping, the primary objective there is to revitalize civic activities through encouraging collaboration among the residents and enhance their understanding of the environment and ecosystem services [31].

Beekeeping at the Paris Opera and the White House is widely known, and the main purpose of much of these examples of urban beekeeping is awareness of the environmental issues and improvement of quality of life. In the Kyodo district in the Setagaya Ward in Tokyo, local residents began to keep the swarming of honeybees in the green area on the rooftop of an apartment block. As a result of this, this area, which was hardly in use before, became a focal point for the local residents where they would gather collectively mainly on weekends for potluck parties using the honey collected. This led to strengthening of the bond between the residents as well as voluntary planting and cleanup activities in the area. In Tokyo, where greenery is scares in residential areas, the value of properties with well-managed green spaces tends to be high, and beekeeping can be a contributing factor for raising property values.

Ginza on the eastside of the Tokyo railway station is a commercial district known for its land prices that are the highest in Japan. An NGO called "Ginza Honeybee Project" began urban beekeeping in 2006 using the rooftop of a building in Ginza [32]. It was initially started as a means for environmental and dietary education, but through the years, its achievements such as greening of the urban areas, the large amount of honey collected (producing around one ton per year), and successful sale of other agricultural and processed products began to be publicized nationwide as best practice examples of community revitalization. Today, similar activities have grown and expanded into over 30 cities throughout Japan [31, 33].

3.1.4 Governance issues around urban beekeeping

On the other hand, beekeeping in urban areas also caused negative results – some of them were predictable by urban dwellers, but others were not. The problem most easily predicted would be stings. Improper access to beehives could lead to stings. However, in reality, there are not so many cases of damage caused by stings in urban areas with a concentrated population. As anybody involved in beekeeping would know, it is very rare for a person to be stung by honeybees away from beehives. Nonetheless, sight and buzzing of a large number of honeybees kept on a balcony of an apartment would probably be enough to scare the neighbors, and they may find it dangerous for their children to play freely outside.

Further, damage caused by the feces of honeybees often poses as important issues of concern. Honeybees flying out into the first rays of the spring sun after enduring the long winter make a large amount of feces. Unfortunately, they love white and yellow colors and pure white sheets, and your favorite yellow T-shirt would appear to them as the mark of "enticing toilets." The stains and smell from the honeybee feces dropped on laundry, and cars are a practical threat to city dwellers.

Although not being given too much attention until recently, urban beekeeping can be a cause for ecological competition. In other words, alien species that were not present in the unique biodiversity of a particular region could bring about adverse impact on the indigenous species [34].

Apis mellifera is an indigenous species in Europe, Africa, and the Middle East but not in the most parts of the Americas, Australia, and Asia. However, as the modern beekeeping with *Apis mellifera* spread to various parts of the world, these bees began to be kept in the fields worldwide [35].

As honeybees are herbivorous, they would not cause such direct adverse impact like attacking other creatures for consumption, but it is conceivable that other indirect damage may result. There are essentially two possibilities of negative impact: one is the competition with other species over limited honey sources [36] and the other, the spread of diseases and parasites [34, 37, 38].

Generally speaking, in the natural environment, the amounts of flowers available as the sources of nectar change dramatically. In the times when there are flowers in abundance, there can be nectar in excess of what the users can consume, but at other times, competition over limited nectar sources usually prevails. The potential competitors for honeybees include other types of honeybees, bumble bees, insects such as butterflies, bird species such as hummingbirds, and mammals like bats [39–41].

These competitors may not regard the honeybees brought into urban areas by humans as their desirable neighbors. It is quite possible that the nectar reserve available to them may have been reduced because of these urban honeybees. Such examples have rarely been verified at the level of scientific research, but there is a case study reported from some countries [42, 43].

Also, urban honeybees may bring with them more serious, undesirable guests. At the moment, one of the most worrying factors troubling beekeepers worldwide is honeybee mites. The two particularly well-known species, Varroa mite/Varroa destructor and honeybee tracheal mite/*Acarapis woodi*, can often cause fatal damage to honeybees.

In addition, there are infectious diseases such as Nosema and Sacbrood, which are caused by parasites, microbes, and viruses. They have been traveling with the honeybees by beekeepers and have now spread to most parts of the world. In their new habitats, these diseases can pose as new threats of infectious diseases to the indigenous species.

There has been a report of a rare case of “sugar theft” by honeybees. This problem came into light when mysteriously colored red and green honey was produced in the apiary adjacent to a candy factory in Manhattan (the colors were the same as those of the syrups used for candy production) [44].

Due to the large number of benefits that honeybees bring to humans, these negative impacts are rarely discussed where they should be sufficiently. Especially in urban areas, where the number of species making up the biodiversity is usually small, a loss of a species with specific functions could lead to serious consequences [45].

Therefore, there should be sufficient discussions on urban beekeeping both in the social and economic context of securing safe living for people and in the ecological context of conserving biodiversity.

In the United States, where honeybees have been regarded as a dangerous species, beekeeping in urban areas has been restricted. However, there has been relaxation of these restrictions as the case for urban beekeeping gained momentum [46]. London in the United Kingdom is one of the most active cities in terms of beekeeping [42]. In Japan, however, there are no regulations that restrict beekeeping, and beekeeping can be conducted anywhere in principle [47].

Act on the Promotion of Beekeeping was amended in 2012, and even small-scale hobby beekeepers are now required to notify to the government. However, there is still practically no regulation as to the restrictions on the locations of beehives and guidelines to follow. The width and depth of governance for urban beekeeping vary greatly between countries and regions. Now is the time to establish an adequate governance structure, where measures can be taken depending on the need of a particular country or a region.

3.2 Approaching with the latest technology (eDNA)

3.2.1 Adding value to honey with DNA analysis

3.2.1.1 Research on honey-source plants with environmental DNAs (eDNA)

In the paddy fields in Fukushima Prefecture, beekeeping has been conducted with hairy vetches after their decontamination, with the aim to produce a local honey specialty for sale. Thorough analysis on radioactivity is in place for food safety and has been verified with the eDNA analysis technique that the product is honey from hairy vetches.

Identification of honey-source plants with eDNA analysis technique has been tried since around 2010 (**Figure 3**). It has some advantage over the conventional pollen analysis, but it is not fully verified that it can demonstrate a level of contribution of each honey-source plant accurately [9, 48].

Although DNA is an effective indicator of honey-source plants, most honey products do not show the results of their DNA analysis. In Gifu Prefecture where beekeeping is active, it is indicated on the label on some of the honey products that they are from cherries and ilexes by their DNA analysis, but this is one of the few examples.

3.2.1.2 Is it honey from acacia trees?

The eDNA analyses were conducted on 14 honey products purchased at high-end supermarkets in Tokyo. None of the products sold as single-flower honey/monofloral was actually from a single source. The DNA of false acacia was detected in all of the seven well-known acacia brands, but it was dominant in only four of them and the second-dominant following other plants in the other three products. In some of those sold as honey from a single source such as astragalus, ilex, amur cork, buckwheat, or manuka, these nominal source plants were not the dominant sources, or their DNA was not detected at all. None of the products analyzed was actually from a single source. Since honeybees visit various flowers, this is not surprising.

Acacia single-flower honey is much sought after in Japan, and it is traded at a high price. Among the seven acacia monofloral/single-flower honey products, however, acacia was found to be a dominant source for honey only in four of them, and the other three products contained honey only from false acacia as its second-dominant source. A product sold as monofloral honey may actually have been from several source plants/multifloral honey. Astragalus honey made in Tokyo is from

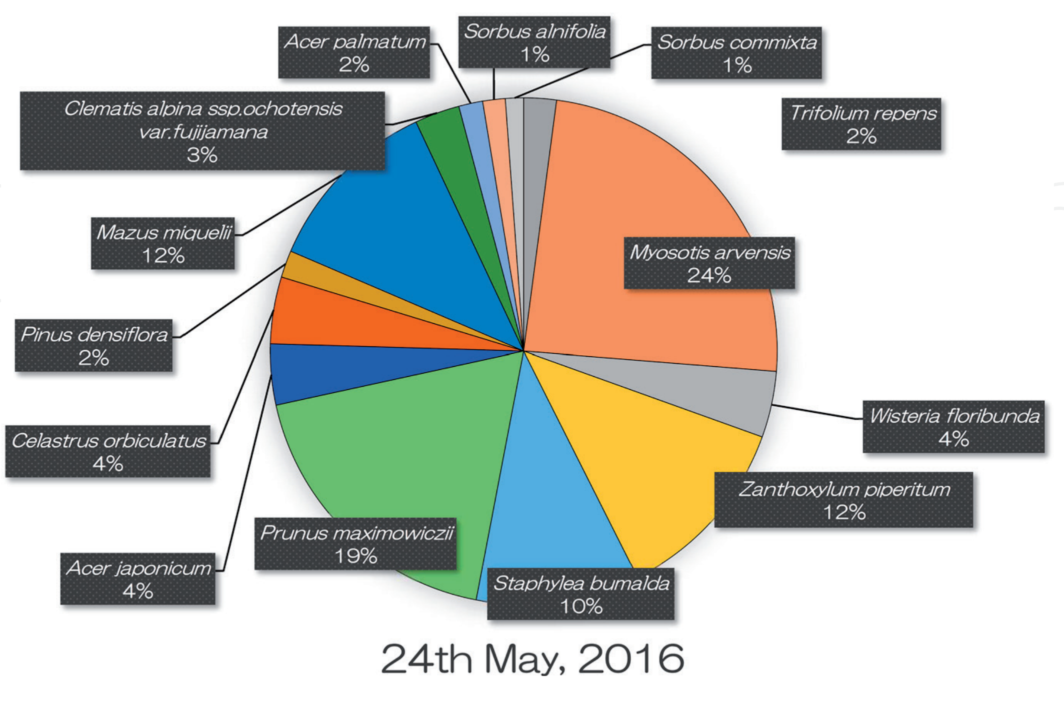

Figure 3.
An example of analysis results of honey we produce. More than 20 kinds of plants were confirmed. With the exception of Prunus maximowiczii, Wisteria floribunda, *and* Trifolium repens, *most species are not famous as honey source plants. Based on data from IDEA Consultants, Inc.*

three sources, while 18 source plants were detected in another astragalus honey made in Fukuoka. Some of the honey brands tested had plants containing toxic alkaloid as their source plants.

3.2.1.3 eDNA would reveal the authenticity of honey

Verifying the quality of high value-added honey is important for the development of a healthy beekeeping industry. Thus, DNA analysis can be a powerful tool to identify origins. If the list on the label contains a plant species that does not grow in the local area or does not contain the DNA that should be detectable as a species on the product label, it may be misrepresenting the honey and its origin.

We analyzed 14 honeys purchased at a luxury supermarket (**Table 1**). More than half of the products contained plant DNA on the label, but some products were not detected at all. Manuka DNA was not detected in the Manuka honey analyzed this time. Manuka honey may have been shipped after aging for several years after harvest, so it is possible that DNA degraded during this aging period. DNA is generally unstable and fragile. Since it breaks down over time, there is a possibility that the labeled honey source cannot be detected by DNA analysis, even if it is correct. However, this sample remains suspicious because it has confirmed several plant DNAs normally growing in New Zealand. Following the accusation that their Manuka honey was fake, New Zealand authority has mandated DNA analysis for their Manuka honey since 2018 [49].

3.3 eDNA analyses can open the door for new scientific findings

3.3.1 Discovering the flora and phenology in surrounding areas

With eDNA analysis, we can better understand the flora of the areas around the hives. This method could only identify plant DNAs that were visited by bees. Furthermore, this method may not show a correlation between the amount of DNA and the volume of existing plants. Despite these disadvantages, honey eDNA analysis is a effective tool to verify the general trends of honey origins.

3.3.2 Honeybees are experts at flower hunting

Bees are much better at finding flowers than humans. Occasionally, plant species that humans are incapable of identifying may be found in the DNA of honey. In the honey produced near Mt. Fuji, the DNA of several types of plants which were not identified in and around the production area were detected. One of them, *Gaultheria pyroloides,* distributes in the alpine area of Mt. Fuji, which was more than 8 km away in terms of horizontal distance and 1500 m in vertical distance from the beehives. This simple result provides two possibilities that this plant grows at low attitudes or the bees fly over a distance of more than 8 km. It is a new scientific discovery in any case.

3.3.3 Importance of woody plants as a source for honey

It is typically thought that honeybees mainly use grassland plants. This may be because we usually observe only house flowers near the ground. The honey made by our bees contains a lot of woody plants, and we can also see such examples in previous studies showing that woody plants are more prominent as nectar plants than previously thought [50].

Type	**Monofloral**												**Mixed**	
Labeling name and Country Specific name	Manuka (*Leptospermum scoparium*)	*Phellodendron amurense*	*Fagopyrum esculentum*	*Robinia pseudoacacia*							*Aesculus turbinata*	—	Forest	Alpine plant
	New Zealand	Tokyo, Japan	Tokyo, Japan	India	Romania	Switzerland	Okayama, Japan	Saitama, Japan	Kyoto, Japan	Tokyo, Japan	Okayama, Japan	Mexico	Switzerland	Switzerland
Asteraceae spp.1	34,767	66,624	54,573	1	596	877	3971	273	497	371	7853	38,068	2642	15
Robinia pseudoacacia	3			13,640	63,643	40,622	18,815	18,583	20,915	14,749				49
Quercus sp.	2413		1	8	890	1223	3558	2784	2121	2809	35,956	208	1600	3
Toxicodendron sp.			187	54		9	3753	28,996	9758	2059	1439		2	21
Rosaceae spp.1	840	589		2014	2422	865	4634	3321	1725	2803	28,530		324	1
Actinidia sp.	1560	18,107	387			6	4070	577	28,172	106	467		1	72
Persoonia spp.	27,832													
Wisteria floribunda				15		12	3438	5863	3205	23,550	8569		21	9
Fabaceae spp.3	790		3	2	1	2	301	1759	1499	2	246		2342	4
Prunus sp.1			1	278	3279	1846	4491	15,956	5396	8330	7245		648	3
Lauraceae spp.	1236												14,884	
Aesculus turbinata						2	490		122	645	14,680		27	
Dalbergia sp.				13,117										
Cryptocarya sp.												1	12,754	
Prosopis sp.				10,885								1	57	

Type						Monofloral							Mixed	
Pterospermum heterophyllum												10,169		4
Prunus sp.4			1		3	5	11	20	16	13	18		1	
Rosa sp.	2		1	1396	9395	6972	2786	2013	6905	4859	392		5092	14
Rosaceae spp.2	1082					1		110	460	583	1275		6039	3
Myrtaceae spp.1		2		1107									7856	
Asteraceae spp.2		1						1				7202		
Weinmannia spp.	6997													
Acer sp.				2	2	1	373	890	285	400	6540		22	
Picrasma quassioides				13		8	1971	6440	339		675		1	
Anacardiaceae spp.1				6403										
Fabaceae spp.1	457				6199	4678	688		7				35	
Prunus sp.2				3			461	1174	2483	6184	4733		10	5
Prunus sp.3				3			461	1174	2483	6184	4733		10	5
Populus sp.	3147			5511		89								
Lysiloma sabicu												5237		
Monimiaceae spp.	5039													
Melicytus spp.	4766													
Asteraceae spp.3			1	1468		13	4763	2920	145	183		1818	1310	

Type	Monofloral											Mixed		
Lopezia langmaniae												4747	318	
Argemone mexicana												4092		
Filipendula vulgaris					3850									1
Cornus controversa				11		2	254	3680	549	824	1561			
Kerria japonica								92	63	6	3583		11	
Podocarpus sp.	3430													
Leucaena leucocephala				3387								159		
Asparagus sp.			1676			100	1346		1926					2
Phellodendron amurense			48			4	1549	379	1692	46	614		1	4
Asteraceae spp.4	20	1158	80									1		
Number of detected species	31	11	16	31	21	36	36	38	42	31	30	23	45	25
Presence of labeling species	N.D.	N.D.	N.D.	1st	1st	1st	1st	2nd	2nd	2nd	3rd	—	—	N.D.

Table 1.
A result of eDNA analysis of honey sold at Supermarkets. All honey contained DNA from multiple plant species. Of the 11 samples sold as single flower honey, 4 actually had the highest amount of DNA. Based on data from IDEA Consultants, Inc.

3.3.4 What does the DNA of animal origin in honey indicate?

We found the DNA of the Varroa mite *Varroa destructor* in our honey throughout the seasons. Most of the time during the study period, we could not find the Varroa mite by visual inspection. In other words, the presence of parasites that humans cannot find can be examined by the eDNA analysis.

In general, the amount of DNA in a sample is correlated with the abundance of organisms. Therefore, by monitoring the amount of the DNA of the Varroa mite in honey, we can predict the level of parasite damage in advance and help as early countermeasures.

The presence of the DNA of aphids and scale insects in honey suggests that the honey is honeydew honey. Honeydew honey is made not from nectar, but honeydew refined by parasite insects on plants. It is widely produced as a specialty product in Germany and New Zealand. In Japan, honeydew honey was not detected until 2019. The bees we keep in the forest make dark honey in August when there are few flowers. We assumed that the honey would contain honeydew. Analysis of whole eukaryotes using the eDNA analysis revealed the presence of aphid DNA. This result suggests that honeydew honey is a constituent in our honey (**Table 2**).

3.4 Beekeeping using the eDNA technique can revitalize communities

3.4.1 Urban beekeeping is spreading

In the 2000s, urban beekeeping began to appear in various cities around the world [7, 51, 52]. In many cases, it does not aim to produce honey but is used as a means to revitalize the community and improve the quality of life. For example, beekeeping at the White House and the Paris Opera has a greater effect on appealing to the environmental friendliness of public venues than its value as a place for honey production.

3.4.2 Honeybees as an indicator of urban biodiversity

On the other hand, it may be used as a simple method for monitoring the quality of the urban environment. In general, it is difficult for civilians to measure and monitor the quality of the living environment by professional and scientific methods. However, eDNA analysis can be the "litmus paper" or "canary" to assess the quality of the environment. It is possible to grasp the flora, phenology, and the safety of the surrounding environment by combining with the analysis of substances that affect health such as pesticides. Such activities can be expected to improve citizens' environmental literacy.

In an apartment house in Tokyo, beekeeping began on the green space on the roof. Residents gathered every week to enjoy harvested honey in a rooftop green area that was rarely used before. In urban areas of Japan, the lack of connections between residents has become a major social concern nowadays, but in this house, a sense of cooperation was born, thanks to the opportunities brought about by beekeeping. Residents planted seasonal flowers in the green spaces managed by each house, cooperated to repel hornets, and voluntarily cleaned the communal space. As a result, residents' autonomy and governance improved. It is also said to have increased the real estate value.

Kasumigaseki in Tokyo's Chiyoda Ward is the administrative center of Japan, where central government ministries are concentrated. An NGO has been beekeeping for several years on the library rooftop in Hibiya Park adjacent to the Ministry of the Environment [53]. Here, government officials, corporate employees, and

Kingdom	Phylum	Class	Order	Family	Genus	Scientific name
Animalia	Chordata	Mammalia	Primates	Hominidae	*Homo*	*Homo sapiens*
	Mollusca	Bivalvia	Venerida	Veneridae	*Ruditapes*	*Ruditapes philippinarum*
	Arthropoda	Insecta	Hemiptera	Aphididae		Aphididae spp.
		Arachnida	Acari	Varroidae	*Varroa*	*Varroa destructor*
			Mesostigmata	Phytoseiidae	*Neoseiulus*	*Neoseiulus womersleyi*
			Opiliones	Phalangiidae		Phalangiidae spp.
			Prostigmata	Eriophyidae		Eriophyidae spp.
						Eriophyidae spp.
Fungi	Ascomycota	Dothideomycetes	Dothideales	Dothioraceae	*Aureobasidium*	*Aureobasidium pullulans*
			Capnodiales	Cladosporiaceae	*Cladosporium*	*Cladosporium cladosporioides*
		Eurotiomycetes	Chaetothyriales			Chaetothyriales sp.
		Saccharomycetes	Saccharomycetales	Phaffomycetaceae	*Wickerhamomyces*	*Wickerhamomyces anomalus*
				Saccharomycetaceae	*Debaryomyces*	*Debaryomyces nepalensis*
					Saccharomycetales	*Saccharomycetales* sp.
					Zygosaccharomyces	*Zygosaccharomyces rouxii*
				Incertae sedis	*Kodamaea*	*Kodamaea ohmeri*
					Starmerella	*Starmerella bombicola*
	Basidiomycota	Basidiomycota	Microbotryomycetes		*Curvibasidium*	*Curvibasidium pallidicorallinum*
	Incertae sedis		Mucorales			Mucorales spp.
Apicomplexa		Conoidasida	Neogregarinorida	Lipotrophidae	*Apicystis*	*Apicystis bombi*

Table 2.
Eukaryotic DNA contained in honey. A list of eukaryotes other than honeybee in DNA. These data are derived from honey that we produce ourselves. There were several types of mites, such as Varroa, aphids, and multiple molds. Clam species, does not live near our apiary, was also detected. Based on data from IDEA Consultants, Inc.

local residents cooperate to improve environmental literacy through beekeeping. They conducted eDNA analysis as one of the methods to assess the biodiversity of the city.

The analysis showed that urban areas also have diverse sources of honey, and some bees flew to the imperial palace, where public access is prohibited. These characteristic results have enough momentum to stimulate the curiosity of the participating citizens and enhance future activities.

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