Universität für Bodenkultur Wien
University of Natural Resources
and Life Sciences, Vienna

# Master Thesis

# Morphological and genetical analysis of pollen collected by honeybees *Apis mellifera* in urban and park-like surroundings in Vienna, Austria

Submitted by

**Lars PUSEWEY, BSc**

in the framework of the Master programme

Umwelt- und Bioressourcenmanagement

in partial fulfilment of the requirements for the academic degree

Diplom-Ingenieur

Vienna, March 2022

Supervisor:

Univ.Prof. Dipl.-Biol. Dr.rer.nat Harald Meimberg
Institute of Integrative Conservation Research
Department for Integrative Biology and Biodiversity Research

Co-Supervisor:

MSc. Julia Lanner
Institute of Integrative Conservation Research
Department for Integrative Biology and Biodiversity Research

Acknowledgment:

Ich möchte herzlich meiner Familie und meinen Freunden und Freundinnen danken, die mir während der einjährigen Erstellung dieser Arbeit immer mit motivierenden Worten und Gesten beiseite standen. Besonders möchte ich meiner Freundin danken, die bei allen Höhen und Tiefen für mich da war. Ebenso möchte ich Harry, Julia, Sonja, Eva und Christina herzlich danken.

Eidesstattliche Erklärung:

Ich erkläre eidesstattlich, dass ich die Arbeit selbständig angefertigt habe. Es wurden keine anderen als die angegebenen Hilfsmittel benutzt. Die aus fremden Quellen direkt oder indirekt übernommenen Formulierungen und Gedanken sind als solche kenntlich gemacht. Diese schriftliche Arbeit wurde noch an keiner Stelle vorgelegt.

*Abstract:*

Pollen, the male gametes of plants, play a crucial role in fertilization biology. Molecular biology research on pollen has already been elaborated on PCR settings, pollination networks and public health concerning pollen allergies. However other characteristics of pollen, namely its color and homogeneity, have gained less attention. In this study, pollen was collected from 8 honeybee colonies on 7 collection days from two sites in Vienna and analyzed both morphologically and genetically. The collected pollen were weighed, counted and divided into 12 color categories. Most samples most samples were in the weight range of 0-5 grams and between 15-20 grams. 85% of all collected pollen were yellow. Rare colors such as pink, purple, red, green and others accounted only for 4% of all pollen loads. The genetic analysis using blasts against a local database shows that the plant composition differs little between the days and locations of the honeybees, but that there are clear differences between the colors and colonies. The species *Koelreuteria paniculata* was most often detected. A threshold value of 85% shows that a large proportion of the pollen loads are homogeneous (n=192, f=70). Almost all analyzed pollen loads (n=192, f=160) consisted of 50% of one plant species. Studies with multiple sites and more colonies are recommended.

*Kurzzusammenfassung:*

Pollen, die männlichen Keimzellen von Pflanzen, spielen eine entscheidende Rolle in der Befruchtungsbiologie. Die molekularbiologische Forschung zu Pollen hat sich bereits mit PCR-Einstellungen, Bestäubernetzwerken und der öffentlichen Gesundheit in Bezug auf Pollenallergien befasst. Anderen Merkmalen von Pollen, nämlich ihrer Farbe und Homogenität, wurde jedoch bisher weniger Aufmerksamkeit geschenkt. In dieser Studie wurde Pollen von 8 Honigbienenvölkern an 7 Sammeltagen an zwei Standorten in Wien gesammelt und sowohl morphologisch als auch genetisch analysiert. Die gesammelten Pollen wurden gewogen, gezählt und in 12 Farbkategorien eingeteilt. Die meisten Proben lagen in einem Gewichtsbereich von 0-5 Gramm und 15-20 Gramm. 85 % aller gesammelten Pollen waren gelb. Seltene Farben wie rosa, lila, rot, grün und andere machten nur 4 % aller Pollen aus. Die genetische Analyse mit Hilfe eines Abgleichs mit einer lokalen Datenbank zeigt, dass sich die Pflanzenzusammensetzung zwischen den Tagen und Standorten der Honigbienen kaum unterscheidet, dass es aber deutliche Unterschiede zwischen den Farben und den Völkern gibt. Die Art *Koelreuteria paniculata* wurde am häufigsten nachgewiesen. Bei einem Kontaminationsschwellenwert von 85 % zeigt sich, dass ein großer Teil der Pollenladungen homogen ist (n=192, f=70). Fast alle analysierten Pollenladungen (n=192, f=160) bestanden zu 50% aus einer Pflanzenart. Studien mit mehreren Standorten und mehr Kolonien werden empfohlen, um die Ergebnisse dieser Studie zu untermauern.

## Table of Contents

# 1. Introduction

The polymerase chain reaction (PCR) is better known than ever. But few are aware that it is a method that primarily has nothing to do with the coronavirus per se. Rather, it generally helps to amplify DNA fragments from a wide variety of organisms such as tigers, grasses, birds, coronaviruses, and pollen to make them verifiable (Solanki, 2012).

Pollen, the male gametes of plants, play a crucial role in fertilization biology. Without pollen, there would be no generative reproduction of plants, no offspring, much less fruits, vegetables, nuts and fruits in general. Many insects such as honeybees serve as vectors, carry pollen from one plant to the stigma of another for pollination (Lack and Real, 1985).

Pollen has been studied from many different angles in science. They play a role in pollination biology, public health because of pollen allergies(Glick, Gehrig and Eeftens, 2021), beekeeping, human nutrition, basic research on pollinators (Bogdanov, 2016), and thus also in molecular biology and the laboratory. Molecular biology research on pollen has already been elaborated on primers, PCR settings and chemicals for DNA isolation (Longhi *et al.*, 2009). However, one characteristic of pollen, namely its color, has gained less attention.

How homogeneous are pollen loads? Does a pollen load come from one plant? From many different ones? Can a color serve as an indicator of how homogeneous or heterogeneous the pollen load is? Does it depend on the location of the bees? How much pollen do bees collect per day? Does it also depend on the colony and its location?

In this research gap this case study acts and tries to clarify corresponding questions.

# 2. Literature Review

## 2.1. Honeybees, their nutrition and foraging behavior

Honeybees *Apis mellifera* (Linnaeus, 1758) are kept by beekeepers to harvest honey, pollen, propolis, wax, to sell queens and colonies and profit from pollination services for other agronomists. The honey bee plays a central role for the cultural landscape and the pollination of agricultural crops (Kuchling *et al.*, 2018). In this context, pollination not only increases the quantity of fruits, but also their quality, such as fruit size (Mandl and Sukopp, 2011). In Germany 130 000 beekeeper exist and around 500 directly depend on a full-time basis economically on the health of their bees (Deutscher-Imkerbund, 2021). For various reasons, there has been an increased decline in honey bee populations over the last

decade (Genersch *et al.*, 2010). These reasons include the biodiversity decline of the agricultural landscape, loss of diverse structures such as hedgerows, increasing pesticide residues in agriculture, and the industrialization of agriculture in general (Brodschneider and Crailsheim, 2013). The decline in populations is also strongly related to the spread of the Varroa mite *Varroa destructor*. This mite parasitizes the honeybee, feeds on its brood and transmits various diseases such as the "Acute bee paralysis virus" (APBV) and the "Deformed wing virus" (DWV). Not only diseases, parasites, pesticides and beekeeping practices are crucial for the health of honey bees (Genersch *et al.*, 2010), but also their nutrition (Alaux, Ducloz, *et al.*, 2010; Omar *et al.*, 2017).

The nutrition of honeybees and their colony depends on the surrounding flora. On their collecting flights, bees collect water, nectar and pollen. The collected nectar serves as a source of carbohydrates for the colony and the collected pollen as a source of carbohydrates, protein, fat and minerals (Brodschneider and Crailsheim, 2010). Not only the quantity, i.e. the appropriate amount of pollen, but also the quality, i.e. the plant origin and diversity are decisive for a healthy colony development. A monofloral diet, i.e. a diet consisting mainly of pollen of one species, is unfavorable for the health of a honey bee colony (e.g. rapeseed field) (Brodschneider *et al.*, 2019). Honeybees are polylectic, meaning that they collect pollen from multiple plant species. A polyfloral diet contains complementary amino acid profiles, which are crucial for brood development, body composition and general health, growth, general performance and thus for the number of individuals in a colony (Brodschneider *et al.*, 2018). The main reason as said are the amino acid profiles which complement each other and help bees to synthesize appropriate proteins, e.g. enzymes (Brodschneider and Crailsheim, 2010). Longevity of single individuals, rearing of young brood, and honey production are all reduced when protein availability and diversity in the colony environment is low (Crailsheim, 1990; Brodschneider and Crailsheim, 2010; Di Pasquale et al., 2016). Pollen of different plant taxa differ significantly in their protein content (Radev, 2018). The percentage of average protein content in 50 studied species examined) varies from 11.5% in *Chondrilla juncea* to 27.4% in *Cucumis melo* (Radev, 2018). Interestingly, the average protein content also depends on whether the plant is a wind-pollinating or insect-pollinating species. The average protein content in insect-pollinated taxa is 20.9% and significantly higher than the average protein content in wind-pollinated taxa, 14.5% (Radev, 2018). Honey bees tend to collect pollen with high protein content (Crailsheim, 1990).

Bee colonies function according to a division of labor called polyethism. Depending on the age of the bees, they have different tasks. Thus, young bees primarily take care of the brood and the care of the hive, while older bees are responsible for defense and especially for collecting. Pollen enters the colony via two routes. Nectar collectors, who gather nectar and pollen during their collection flights, and specially trained pollen collectors gather solely pollen. They collect the individual pollen grains in their

pollen basket (*corbicula)* and mix it with some saliva to form a stable pollen load. Back in the hive, they pass this pollen load on to other bees. They store the pollen loads in the combs, so that with a little honey, bacteria and time they ferment into bee bread *(perga).* The bee bread is an easily digestible substance for the bees and serves as food for times of lower pollen availability, such as in winter. Sufficient pollen and therefore protein availability, is essential to maintain all metabolic functions of the bees, the brood with a specific jelly and the queen with royal jelly (Crailsheim, 1990).

Bees fly to their foraging plants with a certain flower constancy. This phenomenon describes individual bees of one colony constantly fly to a certain floral type or plant species at certain times to meet their energy needs with nectar and pollen. This behavior leads to pollination, because the male gametes made their way from one plant individual to another individual of the same species. If the bees would rather gather very randomly and with no flower constancy, pollination would be less effective. (Amaya-Márquez, 2009).

## 2.2. Environmental aspects of honeybee nutrition

Honeybee colonies and their nutrition are subject to various environmental factors. These are primarily given by their locations and the influences of the beekeeper. On the one hand, agricultural side effects have a direct impact on honeybee colonies. These are mainly pesticide residues, monocultures and the decline of biodiversity in the agricultural landscape. On the other hand, bees are increasingly struggling with parasites and viruses (Genersch *et al.*, 2010; Brodschneider and Crailsheim, 2013).

Bees very depend on the environment of their nest. Different authors describe their flight radius between 1km and 3km (Visscher and Seeley, 1982; Beekman *et al.*, 2004; Barron *et al.*, 2005). The further away floral resources are from the nest, the more energy is required for their flight, i.e. less of the collected energy remains at the nest and thus at the colony. Therefore, a flight radius of 1km is more likely to be assumed if food is available (Visscher and Seeley, 1982; Beekman *et al.*, 2004; Barron *et al.*, 2005).

It is important to describe, that in the modern cultural landscape, which is very much characterized by industrial agriculture, hardly any wild honeybee colonies live. Occasionally, feral colonies can be found in forests, remote areas and urban areas (Bila Dubaić *et al.*, 2021). Often these are descended from domesticated colonies and have settled in these wild places after swarming. Most colonies are kept by beekeepers in special wooden, plastic or polystyrene hives. The corresponding locations must be registered with the responsible veterinary office, as some diseases are notifiable and their spread can be stopped early.

In a special form of beekeeping, migratory beekeeping, the hives are transferred between different sites. In this way, different flowering landscapes can be harvested. This often involves, for example, starting canola, cherry, sunflower, apple and buckwheat monocultures. Bee colonies are therefore mainly located near agricultural fields (Simone-Finstrom *et al.*, 2016).

Pesticides from industrial agriculture affect the health of bees. In recent years, the focus has primarily been on the so-called neonicotinoids. They are often used as seed dressings for sugar beet seed. Residues of the agents are found in wastewater and on plants (Hauer *et al.*, 2017) Bees collect the water and then suffer health impairments to lethal effects due to the toxins (Krupke *et al.*, 2012).

A large German monitoring study, which analyzed about 1200 bee colonies over 4 years, showed that the winter mortality of bees has four primary causes: heavy infestation with Varroa destructor, infection with the Deformed wing virus, infection with the Acute bee paralysis virus, age of the queen and weakening of the colonies in autumn due to e.g. insufficient food stocks (Genersch *et al.*, 2010). Pesticides, however, are considered within this study as a negligible factor for the winter mortality of bees. Various sources (Brückmann, 2004; Truckenbrodt, 2014) criticize this study precisely because of this factor, since it was heavily financed by the agricultural lobby and pesticide-producing companies and therefore the suspicion of influence exists. Because many other studies provide strong evidence that pesticides have a major impact on the general health (Di Prisco *et al.*, 2013), navigation (Fischer *et al.*, 2014), winter mortality of bees (Alaux, Brunet, *et al.*, 2010), weakens bees so that viruses can establish (Di Prisco *et al.*, 2013).

Some studies show the strong influence of landscape composition on winter mortality of bees. For example, a multi-year study showed that winter mortality of honey bees was the lowest in "semi-natural areas, coniferous forests and pastures" (Kuchling *et al.*, 2018). Compared to this the highest winter mortality can be found in regions with artificial surfaces (Kuchling *et al.*, 2018).

## 2.3. Research on pollen (collected by honeybees)

Pollen is being researched from various directions and perspectives. In the following, some of these research directions, which are rather marginal for this work, will be summarized and for those that are of great importance, a detailed overview of the state of research will be given.

Some studies investigate the influence of different pollen diets on the formation of certain physiological traits in bees. A study investigating the effects of different monofloral pollen diets and a polyfloral pollen diet on the development of hypopharyngeal glands concluded that pollen quality is a crucial factor for the development of hypopharyngeal glands. With the help of these glands, the young

nurse bees produce a jelly with which they feed young larvae. Functionality depends directly on the quality, quantity and diversity of the collected pollen. A shortage of pollen can therefore lead to a decrease in the population of a certain colony (Di Pasquale *et al.*, 2013). Another frequently studied physiological parameter is vitellogenin. Vitellogenin is a protein associated with longevity and lower winter losses. The influence of certain factors on vitellogenin gene expression has therefore been investigated in various studies (Di Pasquale *et al.*, 2013, 2016; Alaux *et al.*, 2017). Interestingly, gene expression is not highest at the highest diversity of pollen intakes, but is highest for specific monofloral pollen types such as from the plants of *Rubus* or *Erica* (Di Pasquale *et al.*, 2013). Furthermore, the influence of different pollen diets on the health and immunocompetence of honeybees is investigated. The following parameters in particular are examined: Hemocyte concentration, fat body content, phenol oxidase activity and glucose oxidase concentration. Haemocyte concentration is a crucial parameter for the activity of phagocytosis, i.e. for rendering foreign bodies harmless. Fat body content is crucial for the longevity of the winter bees and thus for the survival of the colony over winter. Phenoloxidase is an important parameter because it is involved in the synthesis of antimicrobial peptides. Glucose oxidase is an enzyme, which helps the bees in two ways: to sterilize the food the colony and their brood. These four parameters are therefore important to measure the immunocompetence of certain individuals and colonies. Interestingly, the availability of pollen changes the concentration of these parameters to a certain level. Once reached, a more of pollen in quantitative matters, does not change the expression of these enzymes and processes. Nevertheless, results show, that the diversity of pollen feed and therefore the diversity of the different amino acid profiles can enlarge their concentrations even more (Alaux, Ducloz, *et al.*, 2010).

Bees are hardly ever lack pollen resources completely. However, the composition and amount of available pollen loads changes over the course of a year. The study of pollen availability is fundamental, to guarantee an appropriate amount and quality of pollen either naturally or the beekeeper can supplement proteins so that the health of the bees is guaranteed (Brodschneider *et al.*, 2018).

Feeding bees traditionally actually refers to substituting the extracted honey with sugar solutions, which then serve as winter food for the bees. Some beekeepers also extract less honey from the bees and leave the bees their own stores without adding sugar. Generally, however, supplemental feeding happens in almost all beekeeping operations, including those certified organic. Feeding bees with pollen is also a common practice. It is mainly used when the pollen supply in nature is still low in spring and the beekeeper wants to initiate increased growth. Some beekeepers do this with pollen collected by bees; however, this is expensive. For this reason, another field of research has emerged: substituting proteins, fats and minerals from non-bee products. Thus, bees are fed with paddies made

from soy, microalgae or even milk proteins and their acceptance and effectiveness are tested (Brodschneider and Crailsheim, 2010; Ricigliano, 2020).

Paddies from microalgae have significant advantages over other protein sources. They contain all essential amino acids and antioxidants like natural pollen paddies and can be produced very energy efficient without pesticide use. Furthermore, compared to soybeans or milk-producing cows, they do not require arable land, but can be produced in large basins in a wide variety of already sealed locations (Lamminen *et al.*, 2019; Ricigliano, 2020).

Research on pollen is of central importance for beekeepers. Honeys are often sold as mixed honeys or as varietal honeys. Varietal honeys, i.e. honeys that derive more than 50% from nectar of one plant species, may be marketed accordingly. In addition to a smell and taste test and the analysis of the sugar ratio contained, a pollen analysis is necessary for the exact determination of which plants a certain batch of honey comes from. For this purpose, microscopy is traditionally and currently very often used. Per sample 500 pollen grains are determined and counted and their relative frequency is calculated. This then determines whether a particular batch of honey may be called for example lime, cherry, buckwheat or chestnut honey (Brodschneider *et al.*, 2018).

Pollen metabarcoding also plays a role in public health. The identification of taxa is crucial for a movement map for pollen allergy sufferers. High exposure to pollen in the air can lead to a significant reduction in personal quality of life. Therefore, allergy sufferers often try to avoid corresponding areas with a high load of grass, early blossom plants or birch pollen. This requires appropriate public information. Metabarcoding can achieve usable results here quickly and efficiently (Pollenwarndienst, 2021).

## 2.4. Genetical analysis and metabarcoding of pollen

Metabarcoding has been used to address a variety of ecological questions and studies that require accurate characterization of the organismal structures contained in a sample (Creer et al., 2016). Metabarcoding can, for example, help to better understand species composition or conduct biodiversity monitoring. The starting material for metabarcoding studies is usually either individuals, biomass, or directly DNA/RNA, which is then analyzed with a 454 or an Illumina sequencer. The extracted DNA is amplified using PCR, sequenced using the tools just discussed, and then analyzed using bioinformatics methods (Lamb et al., 2019).

Metabarcoding is also used in several pollen studies: to reveal and quantify plant-pollinator interactions (Pornon *et al.*, 2016), to experimentally quantify pollen with different primers like ITS1 and trnL (Baksay *et al.*, 2020), to test the effectiveness of exine rupture in different DNA extraction

methods (Swenson and Gemeinholzer, 2021) or to detect the origin and abundance of different pollen in agroecosystems and thus check the food availability for different insects (Richardson *et al.*, 2015). Metabarcoding is also successfully used in the identification of airborne pollen (Leontidou *et al.*, 2021) and seems to be more efficient in gathering more diversity data with less sampling effort in comparison to a traditional community plant survey (Johnson *et al.*, 2021).

In the following, I use palynological approaches, which for example helps with the analysis of beekeeping products, in which especially the authenticity and marketing of honeys play a major role.

Palynology has several areas of expertise. These include forensic palynology, which uses pollen samples from crime suspects to help convict the perpetrator or exclude other suspects (Bell et al., 2016). Similarly, there exists a form of archeological palynology that pursues, among other things, the task of examining and classifying prehistoric and fossil pollen (BRYANT and HOLLOWAY, 1983). Another field of expertise is that of melissopalynology, that is, the analysis of honey samples. Here, the examination of the origin of honeys and their authenticity plays a particularly important role.

Traditionally, honey samples are examined in many state and private analytical laboratories with the help of microscopy. For this purpose, a number of pollen, usually 500, of a honey sample is determined, counted and thus their relative share in the total sample is calculated.

Richardson et al. (2015) point out the disadvantages and therefore in comparison the advantages of metabarcoding and molecular methods in the analysis of pollen:

*"These methods can provide reliable data when properly executed, but they suffer from being (1) highly dependent on human expertise and vulnerable to human error, (2) limited in taxonomic precision, as many taxa are only identifiable to family, and (3) prohibitively time-consuming for large-scale studies.* (Richardson *et al.*, 2015)*".*

## 2.5. Focus of this study

This paper has two main focuses. Firstly, it focuses on morphological aspects of pollen collected by honeybees, such as color allocation and weight. Secondly, the study has the aim to conduct genetical analysis on the homogeneity/heterogeneity of pollen loads with special consideration of the pollen load color.

To the best of my knowledge there has been no research conducted on the homogeneity, heterogeneity of pollen loads in terms of their color that used NGS. This research work tries to get first insights into this research gap. The aim of this work is to answer the following questions:

- *To what extent does the amount of collected pollen loads differ between honeybee colonies, hive locations and the day of collecting?*

- *How homogenous or heterogenous are pollen loads collected by Apis mellifera in an urban environment and a park-like surrounding?*
- *Are certain pollen colors more homogenous?*
- *Does a multiple doubling of the loaded pollen loads per well during the DNA isolation, also lead to a multiple doubling of the detected sequences after Sequencing?*

# 3. Methods and Materials

## 3.1. Sample collection

The samples were collected in Vienna, Austria on the following seven days: 28.6.21-1.7.21 and 23.7.21-25.7.21. Pollen loads were collected between 10-12 o´clock on the roof of the NH-Hotels in Lindengasse (site 1), which is in the seventh Viennese district and between 13-15 o´clock on the roof of the economic university of Vienna (site 2) which is in the second Viennese district. In site 1 there are many multistory buildings, streets, urban infrastructure, stores, but little greenery and parks. By contrast, site 2 is situated next to the Viennese Prater, which is one of the largest park-like green areas with many different tree species, small water bodies and meadows.

The pollen loads were collected by using pollen traps attached to Zander beehives. Pollen traps have narrowed tubes at the entrance, which the forager bees must pass through to get inside the hive. In the process, a large part loses their pollen loads, which then fall into a wooden frame with a fine grid. This frame can then be pulled out from behind the hive and the pollen harvested. A total of eight colonies were harvested. Three colonies at site 1 and five colonies at site 2. The colonies belong to the "Vienna District Honey Farm", which is a private honey farm that owns apiaries in each district of Vienna.

*Figure 1: Site 1. Beehives on the NH hotel in the seventh Viennese district. Format of beehives is Zander. Pollen traps are installed on the bottom board. Own recording.*

*Figure 2: Site 1 is located within a highly urban environment. Location is added with a buffer zone of 1 km marking the expected collection range following. Source: www.homecrosing.de*

*Figure 3: Beehives on the Economic University of Vienna. Format of beehives is Zander. Pollen traps are installed on the bottom board. Hives have different numbers of boxes due to different amounts honey storage. Source: Own recording.*

*Figure 4: Study area site 1 is located within a park-like surrounding with lots of green spaces. Location is added with a buffer zone of 1 km marking the expected collection range. Source: www.homecrossing.de*

*Figure 5: Pollen trap at site 1 on the left and pollen trap at site 2 on the right. The amount, colour and distribution of pollen on the trap differ from day to day and from colony to colony. Source: Own recording.*

After the pollen traps were opened, clinging bees were first carefully removed, the pollen trap was photographed, and the total amount of collected pollen from the previous day was weighed. Then Eppendorf tubes were filled with pollen. On the first three days, 1-hour samples were taken in addition to the all-day samples. For this purpose, a stopwatch was started after the respective pollen trap was emptied and after exactly one hour the pollen trap was photographed again, the pollen weighed and all pollen loads filled in Eppendorf tubes.

The collected samples were then transported to the laboratory at the University of Natural Resources and Applied Life Sciences Vienna at Linneplatz, where they were directly cooled and stored at -20 degrees Celsius.

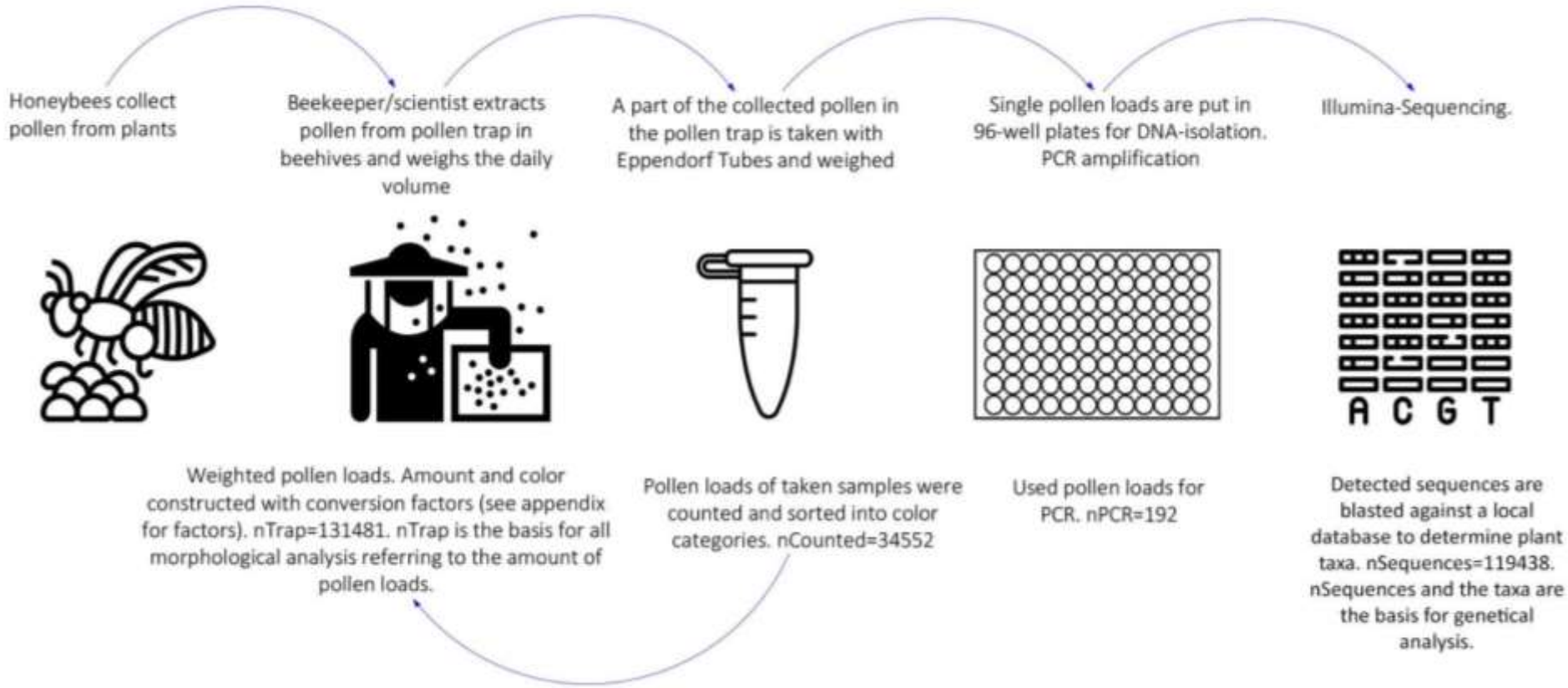


*Figure 6: Overview of the various work steps of this study. Different pool sizes: nTrap, nCounted, nPCR and nSequences. Conceptual legend.*

## 3.2. Morphological analysis

Collected samples were assigned to color categories. The color categories were determined inductively from the drawn samples by inspecting all samples, in order to be able to cover all color tones when assigning the pollen grains to the categories. The following color categories were created: *Light Yellow, Dark Yellow, Green, Orange, Cream White, Gray, Pink, Purple/Blue, Red, Light Brown, Dark Brown, Black.* All pollen grains collected from the pollen traps in Eppendorf tubes were spread out on a piece of kitchen roll in the laboratory and photographed using an SLR camera. The decisive reason for not assigning the pollen grains to color categories directly in the laboratory was that increased DNAses can be formed at room temperature, which could degrade the DNA. For the photographs, the pollen grains were exposed to room temperature for only a few seconds, as they were placed on ice from the -20-degree freezer and then photographed directly. They were then returned to the -20-degree freezer.

All photographs were then analyzed using a grid and the counting tool of the software "Photoshop" (Adobe, 2020). Each count was documented accordingly and a screenshot of the count was taken (see Figure 6).

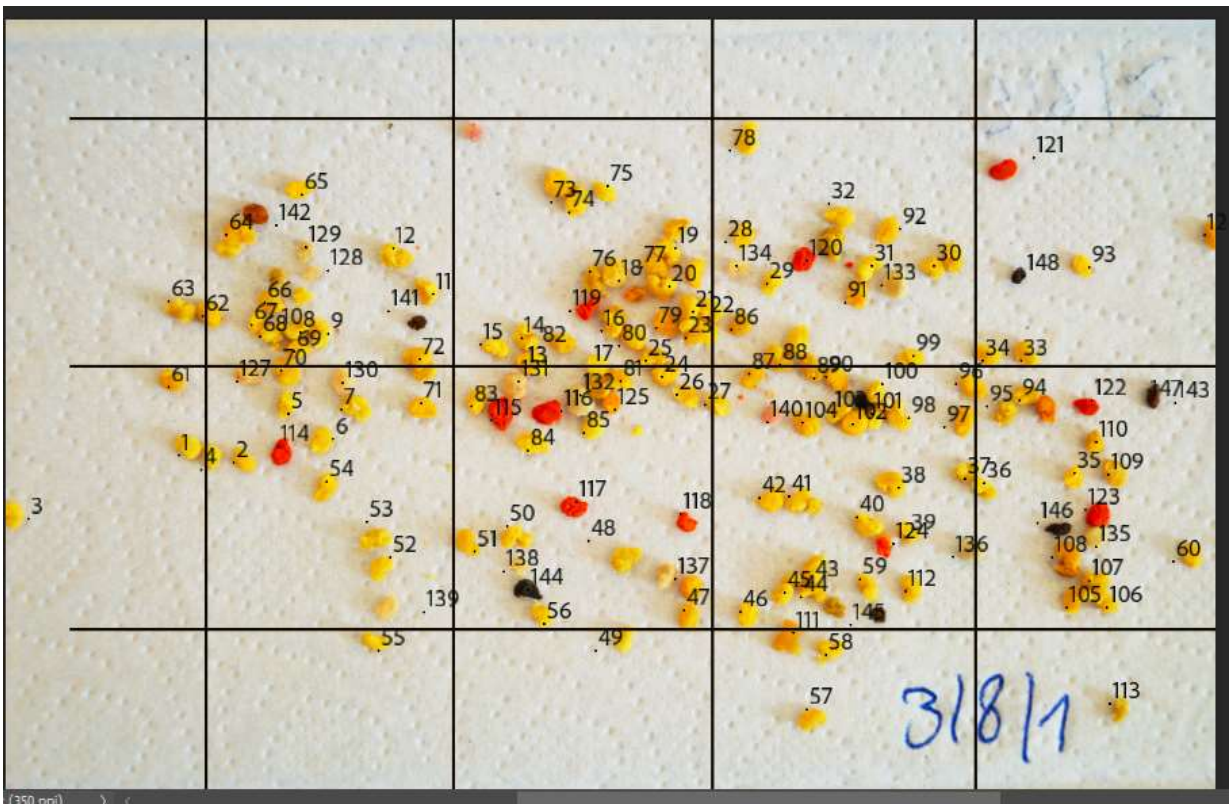


*Figure 7: Each pollen load of each sample was counted manually and assigned to a color category. This was done with a grid and counting tool. Own recording.*

The color distribution of all pollen loads, that the specific bees gathered during one day, was extrapolated using the color distribution of the samples, the number of pollen loads of each colony and the weight of the samples. For this purpose, a multiplication factor was used, which represents the ratio of the sample pollen load number to the total amount of pollen loads that were harvested. The resulting numbers were then rounded down or up so that integral numbers were obtained.

The morphological analysis was conducted in R (R-Core-Team, 2021) and figures were produced using the R-package ggplot2 (Wickham, 2008).

A Shapiro-Wilk normality test was performed to determine whether the data for the "weight" category were normally distributed. The Shapiro-Wilk normality test indicates for $p>0.05$ that the data are normally distributed and for $p<0.05$ that they are not (Hatzinger, Hornik and Nagel, 2011).

To determine the strength of the linear relationship between the two variables "weight" and "pollen loads", a correlation analysis was performed. The result is the correlation coefficient R. If this coefficient is greater than 0.80, it is possible to speak of a strong correlation of the two variables studied (Hatzinger, Hornik and Nagel, 2011). The application of this correlation analysis was based on the question of whether the number of pollen loads is statistically in a linear relationship with the weight. That is, whether the weight of a sample increases with the number of pollen loads. To test if the colony has an influence on the diversity of the colors and to test the influence of the factor “day” on the weight of the pollen samples two ANOVAs were performed. A tukey-test was performed to analyze the influence of each colony to each other in more detail and focus on their interaction individually (Hatzinger, Hornik and Nagel, 2011).

To possibly verify the results of the Illumina-sequencing and to receive another perspective of the collected samples microscopic images were created. Firstly, one pollen load of each color category was mixed in an Eppendorf tube together with 80% ethanol. This mixture was stored for one week so that the pollen loads separated into individual pollen grains and mix with the alcoholic solution. Meanwhile a mixture of 0,5g fuchsine, 50ml of 50% ethanol and 10ml of glycerin was prepared. Finally, two drops of the pollen preparation and two drops of the fuchsine mixture were put on each of the twelve microscopic slides and covered with a coverslip. As a last point of different regions with characteristic pollen grains were photographed.

## 3.3. Genetical analysis

### 3.3.1. DNA isolation

The genetical analysis took place in the molecular biodiversity research lab at the integrative nature conservation research institute of the University of Natural Resources and Life Sciences Vienna. I have isolated DNA in a total of three 96-well plates. In plate 1, one pollen load per well was given. For this,

pollen loads of 12 different colors (see above) were prepared with 8 repetitions each. Plate 2 was filled in the same way. So in total, each color was repeated 16 times. The 3rd plate was a mixed plate. On this plate the four most common colors "orange"; "light-yellow"; "dark-yellow" and "white" were analyzed with 3,6,12 and 24 pollen loads respectively. With this I wanted to find out if an increase in pollen load number is linearly related to the DNA extracted from it. That is, whether more pollen loads also means more DNA. Furthermore, on the third plate, the samples that the bees collected within one hour and different color mixtures were evaluated.

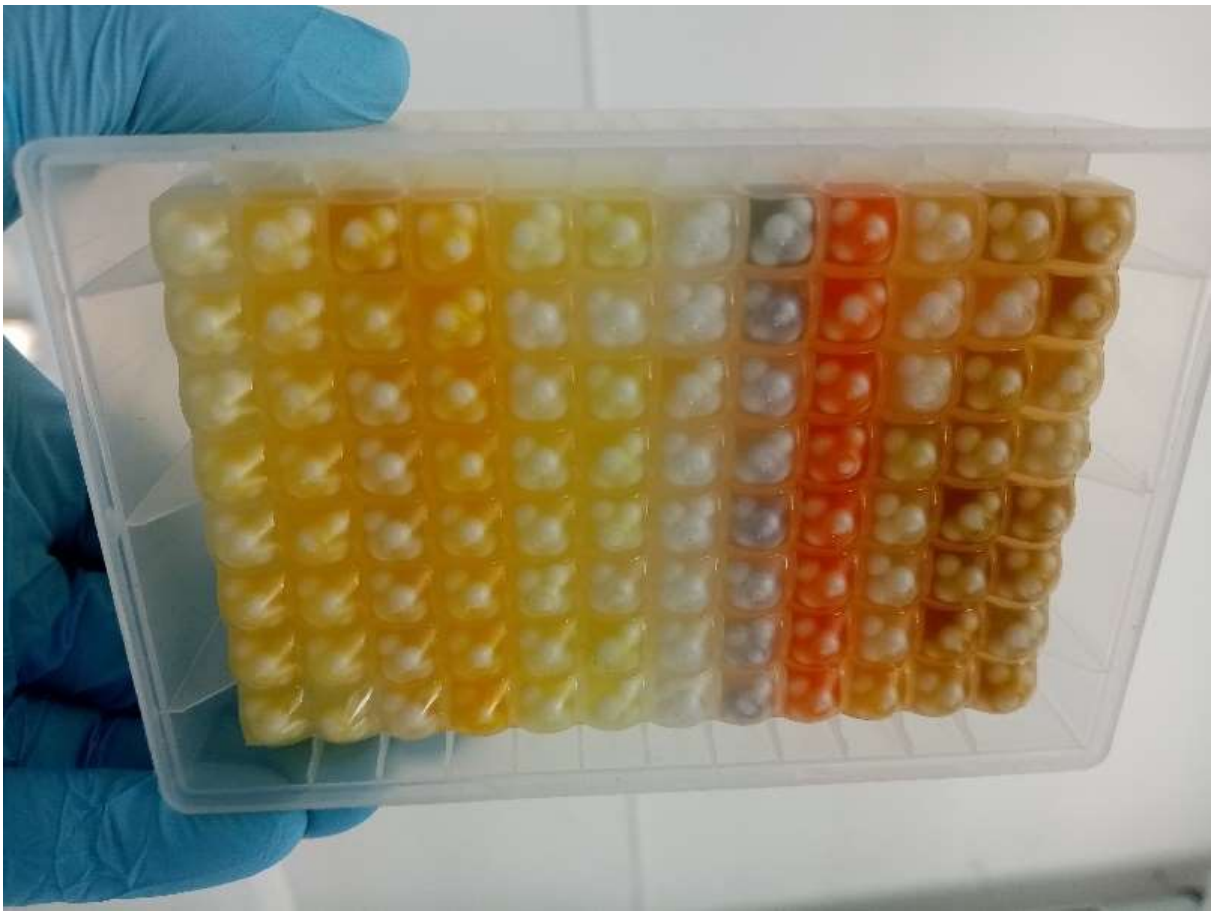

*Figure 8: Pollen loads of 12 different colors in 96-well plate before DNA-isolation. At the bottom of the well the Zirkonium dioxid can be seen. Own recording.*

To isolate the DNA 450µL lysis buffer and 4 Zirkonium dioxid beads were put into each well. After that single or multiple pollen loads were added to the liquid. To shatter the strong wall of the pollen grains the plates were then mixed by a collomix shaker for 5 minutes. The walls break due to the combination with the Zirkonium dioxid beads in each well. Hereafter, the plates were loaded into a centrifuge and centrifuged at 1000 rpm for 1 minute to achieve phase separation so that the solid components are at the bottom of the well and the liquid components are at the top. 15 µL of Proteinase K [1mg/ml] were then added to each well and incubated on a thermo block for one hour at 56°C and 300 rpm. The Proteinase K enzyme helps to digests proteins like nucleases, that would otherwise degrade the pollen DNA. Moreover, it helps to reduce contamination. Thereafter 15µL of Ribonuclease [10 mg/ml] was

added and another incubation for 30 minutes 37°C at 300 rpm took place to ensure the operating temperature for the Ribonuclease, which catalyzes the decomposition of RNA. After this second incubation 113 µL of Potassium Acetat, which functions as a neutralizing buffer, was added. To ensure the proper reaction with Potassium Acetat, the samples were frozen at -20°C for one night.

The next day, samples were then slowly thawed on crushed ice and subjected to the following centrifugation steps: *1000 rpm 1min; 2000 rpm 1min; 4000 rpm 1min; 8000 rpm 1min; 1100 rpm 7min*. Solid components were positioned at the bottom of each well and 500 µL supernatant could be mixed in a prepared plate with 500 µL binding buffer by pulling the multi-pipette up and down for ten times. After mixing, the solution was then transferred to an econospin plate sitting on a 96-well plate and centrifugated for 15 seconds at a speed of 2000 rpm and for 1 minute at a speed of 4000 rpm. The econospin plate is a plate containing a filter with a silica membrane to which around 20-30 µL DNA can bind. After centrifugation, supernatant that has collected at the bottom of the 96 wells plate was disposed. To remove the solvation shell around the DNA and to aggregate the DNA, the samples were washed twice with 600 µL 80% ethanol, which which both times was followed by centrifugation at 4000 rpm for 1 minute. and at 6000 rpm for 2 minutes. At both ethanol washes, the supernatant, that has collected at the bottom of the 96 wells plate was disposed. To remove the remaining ethanol components from the econospin plate, it is placed on an elution plate and dried on air for one hour. After air drying, 50 µL of elution buffer are added per well so that the DNA detaches from the silica membrane again. The first elution is obtained by centrifugation at 6000 rpm for 5 minutes, which is then sealed airtight with an adhesive film and stored in the freezer. The second elution is obtained by adding 80 µL of elution buffer and centrifuging at 6000 rpm for 5 minutes, which is then also sealed with an adhesive film and stored in a freezer.

*Table 1: All steps of the lysis and isolation protocol used to isolate pollen DNA from samples.*

| ***Ingredients, steps*** | ***Amount, details*** |
|---|---|
| Lysis buffer | 500 µL |
| Zirkonium dioxid beads | 4 beads |
| Centrifugation | 1000 rpm; 1min |
| Collomix | 5 min |
| Proteinase K [1mg/ml] | 15 µL |
| Thermo Block Incubation | 300 rpm; 1h; 56°C |
| Ribonuclease [10mg/ml] | 15 µL |
| Thermo Block Incubation | 300 rpm; 0,5h; 37°C |
| Potassium Acetat | 113 µL |
| Centrifugation steps | 1000 rpm; 1min<br>2000 rpm; 1min<br>4000 rpm; 1min<br>8000 rpm; 1min<br>1100 rpm; 7min |
| Supernatant | 500 µL |
| Binding buffer | 500 µL |
| Centrifugation | 2000 rpm; 15sec |

| | |
|---|---|
| | 4000 rpm; 1min |
| Supernatant | discarded |
| 1. Ethanol[80%] wash | 600 µL |
| Centrifugation | 4000 rpm; 1min<br>6000 rpm; 2min |
| 2. Ethanol[80%] wash | 600 µL |
| Air drying | 1h |
| Centrifugation | 4000 rpm; 1min<br>6000 rpm; 2min |
| 1. Elutionbuffer | 50 µL |
| Centrifugation | 6000 rpm; 5min |
| 2. Elutionbuffer | 80 µL |
| centrifugation | 6000 rpm; 5min |

### 3.3.2. Amplification of isolated DNA with PCR

The following three primer pairs were used in the genetic analyses of this study, have already been used in many different studies before and have been recommended to identify plant species with genetic analysis:

*TrnL-F_f, TrnL-F_e* (Chen *et al.*, 2013; Kamo *et al.*, 2018; Leontidou *et al.*, 2018)*; rbcLr506, rbcLa-F* (Little, 2014; Tanaka *et al.*, 2020; Swenson and Gemeinholzer, 2021)*; ITS2 Pollen F and ITS2 Pollen R* (Mishra *et al.*, 2016; Kamo *et al.*, 2018; Bell *et al.*, 2019). A detailed description of their sequences, number of base pairs and targeting genome regions can be seen in table 2.

*Table 2: Used primer, sequences and their target area.*

| ***Oligo name*** | ***Sequence (5' → 3')*** | ***Direction*** | ***Number of base pairs*** | ***Target*** | ***Reference*** |
|---|---|---|---|---|---|
| TrnL-F_*f* | ATTTGAACTGGTGACACGAG | Forward | 20 | cpDNA | *Eurofins MWG Synthesis GmbH/ INF* |
| TrnL-F_*e* | GGTTCAAGTCCCTCTATCCC | Reverse | 20 | cpDNA | *Eurofins MWG Synthesis GmbH/ INF* |
| rbcLr506 | AGGGGACGACCATACTTGTTCA | Reverse | 22 | cpDNA | (Williams *et al.*, 2021) |
| rbcLa-F | ATGTCACCACAAACAGAGACTAAAGC | Forward | 26 | cpDNA | (Williams *et al.*, 2021) |
| ITS2_Pollen_F | ATGCGATACTTGGTGTGAAT | Forward | 20 | rDNA | (Yao *et al.*, 2010) |
| ITS2_Pollen_R | GACGCTTCTCCAGACTACAAT | Reverse | 21 | rDNA | (Yao *et al.*, 2010) |

Before PCR could be performed, three master mixes had to be prepared first. For this purpose, the corresponding reagents (see table 3) were mixed in an Eppendorf tube and placed in the liquid handling station.

The Multiplex-Mix contained the Hot-Star Taq Polymerase and was provided by *QIAGEN* as the *Multiplex PCR Kit.* The primer pairs were used in a concentration of 10µM [dilution 1:10] and were provided by *Eurofins MWG Synthesis GmbH.*

First, samples were removed from the freezer in 96-well plates, thawed slowly and gently on crushed ice, and then centrifuged at 1000 rpm for 1 minute. The pipetting of samples and reagents was performed with the aid of the pipetting robot "liquid handling station". This robot ran with the “Brand Liquid Handling Station" software and the program duration of each full pipetting step was 16:08 minutes. To perform the pipetting, the robot was filled in advance with a 384 plate, a waste bucket, pipette tips, the 96-well plate and the 3 master mixes. The composition of the reagents pipetted into each well of the 384-well plate is shown in table 3 and is derived from the respective master mixes and the DNA sample from the 96-well plate. In each case, the composition of the master mixes differed only in the primer pairs.

*Table 3: Composition of PCR reagents per well used for all three PCR-runs. ddH2O is water with a very high degree of sterility.*

| Plate size | Final volume | Solution | Amount (µL) |
|---|---|---|---|
| 384 | 5µL | Multiplex-Mix | 2,5 |
| | | $ddH_2O$ | 0,1 |
| | | Reverse Primer (1:10) | 0,2 |
| | | Forward Primer (1:10) | 0,2 |
| | | DNA | 2,0 µL |

After pipetting was complete, the 384 plates were placed in the PCR thermal cycler to amplify the DNA segments using PCR. The PCR thermal cycler used was the *C1000 Touch* by the company *Bio-Rad* and the program used was the Bee52 with 35 cycles. The Bee52-35 program entails the steps shown in table 4.

*Table 4: Bee52-35 PCR program steps for amplifying Pollen-DNA-segments.*

| Step | Timespan (min) | Temperature (°C) | Number of cycles |
|---|---|---|---|
| Initial Denaturation | 15:00 | 95 | 1 |
| Denaturation | 00:30 | 95 | 35 |
| Annealing | 01:00 | 52 | |
| Extension | 01:00 | 72 | |
| Final Extension | 10:00 | 72 | 1 |
| Hold | 10:00 | 10 | 1 |

After the program of the Thermo-Cycler was finished, the 384-well plates were frozen again at -20°C.

### 3.3.3. Purification of PCR-Products

After PCR using the thermo cycler, the 384 plates were each transferred back to a 96 plate. Each well then contained a mixture of the same sample amplified with the three different primers. The volume that resulted per well was 4,5 µL of PCR-product, including 1,5 µL per primer pair. Purification was then performed using the *Promega ProNex*®-System to purify the solutions from short primers and short PCR products, enzymes and salts. First, 5,9 µL of the Promega *ProNex* magnetic beeds were presented to a new 96-well purification plate. Then, the described 4,5 µL of PCR-Product were added and mixed by pipetting 10 times and incubated for 5 minutes. To bind the DNA to the magnetic beeds a magnetic separator was used with careful circular movements for 2 minutes. The magnetic separator was then transferred one after another to two 96-well plates, with 200 µL of 80% ethanol. On these plates careful circular movements were again applied to wash the PCR-Products for 45 seconds each. To remove ethanol residues the magnetic separator was placed upside down and air-dried for 5 minutes. To elute the DNA again, two steps were necessary. First, the magnetic separator was transferred to a new plate, carefully removed from the attached sample plate and rotated by hand in slow circular movements for two minutes. The DNA was dissolved in the following elution buffer: 20 µL 50mM Tris-HCl [pH=8,3], which was heated to 65°C beforehand. Second, the sample plate was again attached to the magnetic separator and again moved in circular motions to bind the magnetic beeds back on magnet. This movement was performed for about 2 minutes until the solution was unambiguously transparent. The purified PCR-product was then again stored at -20°C.

### 3.3.4. Indexing of purified Samples

A second PCR, the so-called Index-PCR was performed, to give each sample an individual index, an individual combination of base pairs, which bind to the amplicons. Each sample then has its own index with which it can be traced after Illumina-Sequencing. All three purified 96-well PCR-product plates were pooled together in one 384-well plate with the *Liquid Handling Station.* 1 µL of the pooled purified PCR-product with the DNA of the samples, 2,75 µL Multi-Mix with the Index-Forward-Primer and 1,25 µL of the Index-Reverse-Primer were added to each well.

*Table 5: Constituents of each well for Index-PCR.*

| Plate size | Final volume (µL) | Solution | Amount (µL) |
|---|---|---|---|
| 384 wells | 5 | Multi Mix + Forward Primer [Stock: 100 µM; Dilution 1:121] | 2,75 |
| | | Reverse Primer [Stock:100µM; Dilution: 1:125] | 1,25 |

DNA (Pooled purified PCR Product) 1

This 384-well plate was then placed into the thermal cycler and the Index-PCR program started.

*Table 6: Steps of Index-PCR*

| Step | Timespan (min) | Temperature (°C) | Number of cycles |
|---|---|---|---|
| Initial Denaturation | 15:00 | 95 | 1 |
| Denaturation | 00:30 | 95 | 9 |
| Annealing | 01:00 | 58 | |
| Extension | 01:00 | 72 | |
| Final Extension | 05:00 | 72 | 1 |
| Hold | 10:00 | 12 | 1 |

After the Index-PCR, we pooled all the content of each well into one Eppendorf-tube, which was then frozen at at -20°C and was ready for transport to the TUM in Munich for Illumina-Sequencing.

### 3.3.5. Quality control with gel electrophoresis

To test the quality of DNA-products, PCR-Products and indexing PCR-Products 6 gels were poured and analysed. For DNA the following solutions were used to perform the quality control: *1,5% Agarose Gel (1x TAE, HDgreen+), Lambda DNA/EcoRI+Hind III Marker, 3 (Ladder) and Loading Buffer*.

For PCR-products the following solutions were used to perform the quality control: *1,8% Agarose Gel, 100bp peqGOLD (Ladder) and Loading Buffer.*

The equipment I used for the gel electrophoresis was the *ENDURO Power Supplies, 300V (Labnet International. Inc.)* and for Imaging the *INTAS Science Imaging (Program: Intas GDS Windows).*

The DNA-gels were ranked from 0-4. If there were no bands at all for one sample the measurement 0 was given. 1 was slightly visible, 2 good visible and the measurement 3 was given to broad bands with optimal visibility. The categorization shows that the most samples were slightly visible (f=102). Almost the same number of samples had optimal bands (f=92) or weren´t visible at all (f=87). The category with the lowest counts was category 2 (f=57).

### 3.3.6. Sequencing and Metabarcoding

Illumina-Sequencing was performed by lab personal at the Technical University Munich. Results were sent back in form of a *FASTQ* file. These results were “purified” from the indexes using a pipeline (Curto *et al.*, 2019) to create consensus sequences. These were then BLASTed with *Bio-Edit* against a local TrnL-database, given by the institute. Unclear sequences were added to the library with individual ncbi-blasts (Sayers *et al.*, 2020). Every sample was BLASTed on its own and the results saved in an *Excel*

file. All files have then been merged using the “countif”-function of Excel. (Microsoft Corporation, 2018). The sequences were only analyzed for TrnL.

The results from the blast were analyzed with Excel (Microsoft Corporation, 2018). All detected sequences along with their characterizations such as Collection Date, Site, Colony and Color were compiled into one large numerical table. This was used to generate classical descriptive statistics such as mean, minimum and maximum values and graphical analysis. Some of the graphs were post-processed with Photoshop so that the graphs fit on one page, the axes are easy to see, and the relationships presented are easily discernible.

# 4. Results

## 4.1. Morphological analysis

### 4.1.1. Colour allocation of collected pollen loads

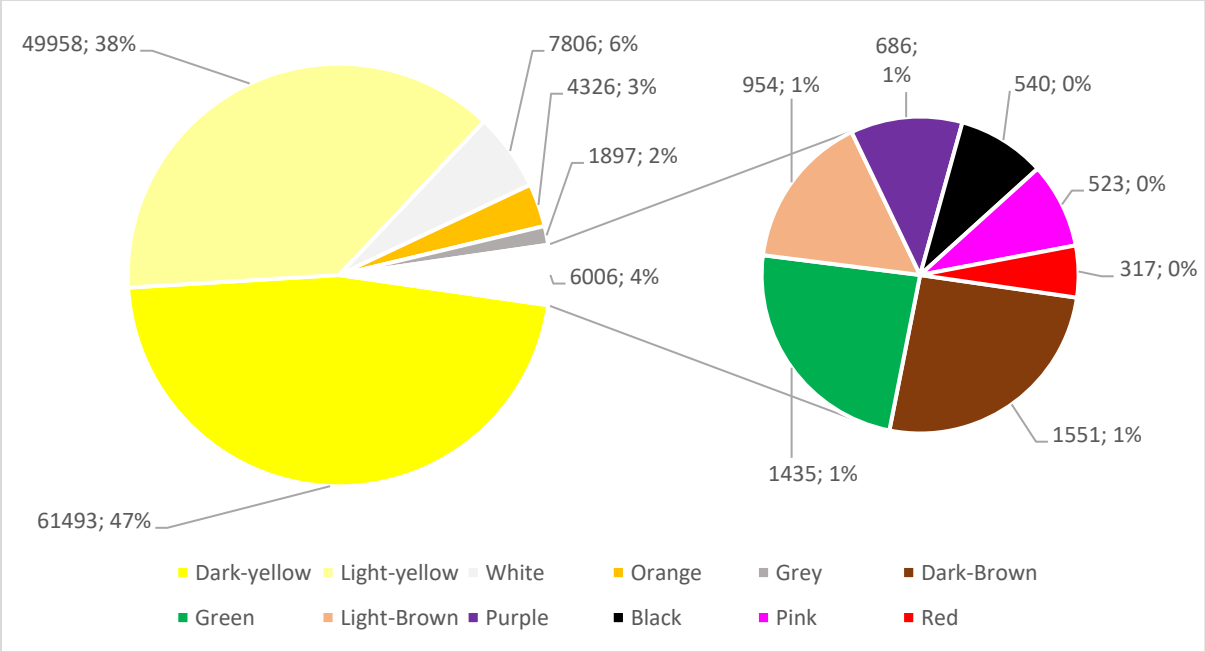


*Figure 9: Color allocation of all pollen in pollen traps. Extracted pollen extrapolated with the help of the total weight (nTrap=131481). Dominant colors are in the right chart and more rare pollen loads color in the right sub-pie chart.*

The honeybees collected mainly yellow pollen during the collection period. Approximately 85% of all collected pollen loads were yellow. Dark-yellow pollen (n=131481; f=61493) was more frequent than light-yellow pollen (n=131481; f=49958). The remaining 15% of all pollen loads fall largely on the colors white (n=131481; f= 7806), orange (n=131481; f=4326) and grey (n=131481; f=1897). Rare pollen load colors can be seen on the right side of the pie chart graphic. They only represent around 4% of all pollen loads. Green is the most frequent rare pollen color (n=131481; f=1435) and red (n=131481; f=317) the least frequent one.

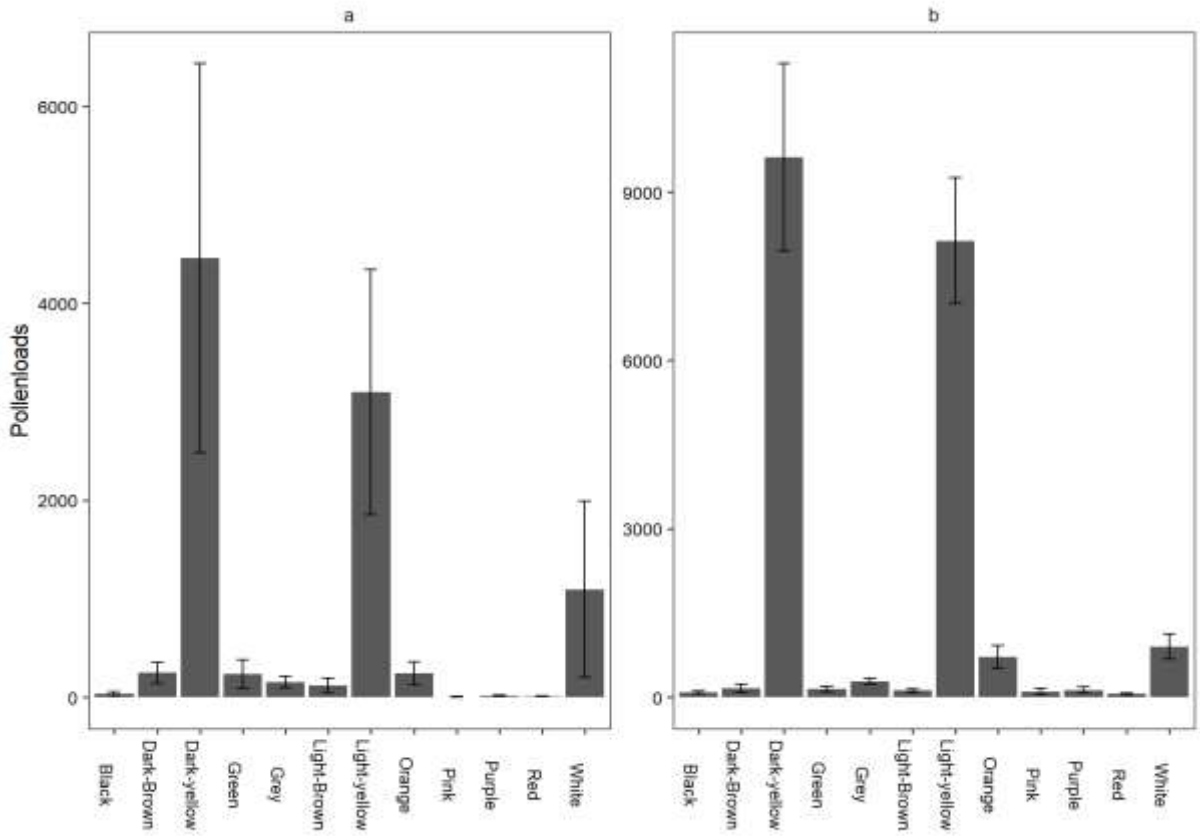


*Figure 10: The graph shows the average number of pollen loads of a color. The average of 56 samples was extrapolated using weight percentages. Differences of the pollen loads in terms of their color distribution sorted by the site of the beehives (nTrap=131481). Site a: urban environment. Site b: park-like surrounding.*

Site a consists of three and site b of five colonies and the colonies of site a, especially colony 1, collected much less pollen than the colonies of site b (see 4.1.2). Respecting these facts, similar numbers of pollen loads of the colors light-yellow and dark-yellow were found at both sites. However, the colonies from site a have collected relatively large amounts of white (n=7806, f=3285), dark brown (n=1551, f=741) and green (n=1436, f=709) pollen loads.

*Table 7: Measured values of all pollen loads of different colors and colonies (nTrap=131481). Site a: urban environment. Site b: park-like surrounding.*

| | ***Site*** | ***Colony*** | ***White*** | ***Grey*** | ***Dark-yellow*** | ***Light-yellow*** | ***Orange*** | ***Green*** | ***Pink*** | ***Red*** | ***Purple*** | ***Light-Brown*** | ***Dark-Brown*** | ***Black*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***a*** | ***1*** | 41 | 47 | 1271 | 1051 | 65 | 20 | 7 | 4 | 11 | 20 | 78 | 9 |
| | ***a*** | ***2*** | 372 | 178 | 4028 | 2894 | 204 | 514 | 0 | 16 | 23 | 272 | 449 | 79 |
| | ***a*** | ***3*** | 2872 | 240 | 8076 | 5344 | 465 | 175 | 0 | 10 | 11 | 65 | 214 | 16 |
| ***Total a*** | | | 3285 | 465 | 13375 | 9289 | 734 | 709 | 7 | 30 | 45 | 357 | 741 | 104 |
| | ***b*** | ***4*** | 1427 | 456 | 15316 | 11757 | 727 | 217 | 14 | 39 | 170 | 166 | 128 | 179 |
| | ***b*** | ***5*** | 1384 | 294 | 9410 | 7596 | 627 | 54 | 237 | 29 | 326 | 80 | 49 | 51 |
| | ***b*** | ***6*** | 856 | 312 | 6609 | 5474 | 312 | 121 | 48 | 30 | 7 | 123 | 105 | 65 |
| | ***b*** | ***7*** | 296 | 224 | 10741 | 9438 | 441 | 35 | 0 | 121 | 4 | 30 | 76 | 18 |
| | ***b*** | ***8*** | 558 | 143 | 6042 | 6402 | 1484 | 300 | 217 | 71 | 131 | 197 | 452 | 124 |
| ***Total b*** | | | 4521 | 1429 | 48118 | 40667 | 3591 | 727 | 516 | 290 | 638 | 596 | 810 | 437 |
| ***Grand Total*** | ***a+b*** | | 7806 | 1894 | 61493 | 49956 | 4325 | 1436 | 523 | 320 | 683 | 953 | 1551 | 541 |

All colonies foraged mainly dark-yellow and light-yellow pollen loads. It is noticeable that some colonies have also collected relatively large quantities of other colors. White pollen e.g., was strongly collected by colonies 3 (n=7806, f=2872) 4 (n=7806, f=1427), 5 (n=7806, f=1384) and 6 (n=7806, f=856), but hardly by colonies 1 and 7. It is also interesting to note that over 34% of all orange pollen loads were collected by colony 8 (n=4325; f=1484). Two of the rare colors were collected almost half exclusively from colony 5: pink (n=523, f=237; 45,3%) and purple (n=683, f=326; 47,8%). The result of the ANOVA also fits these specific perspectives, because the ANOVA calculations show a strong gnificance between the colony number and the diversity of colors (p=5.32e-08).

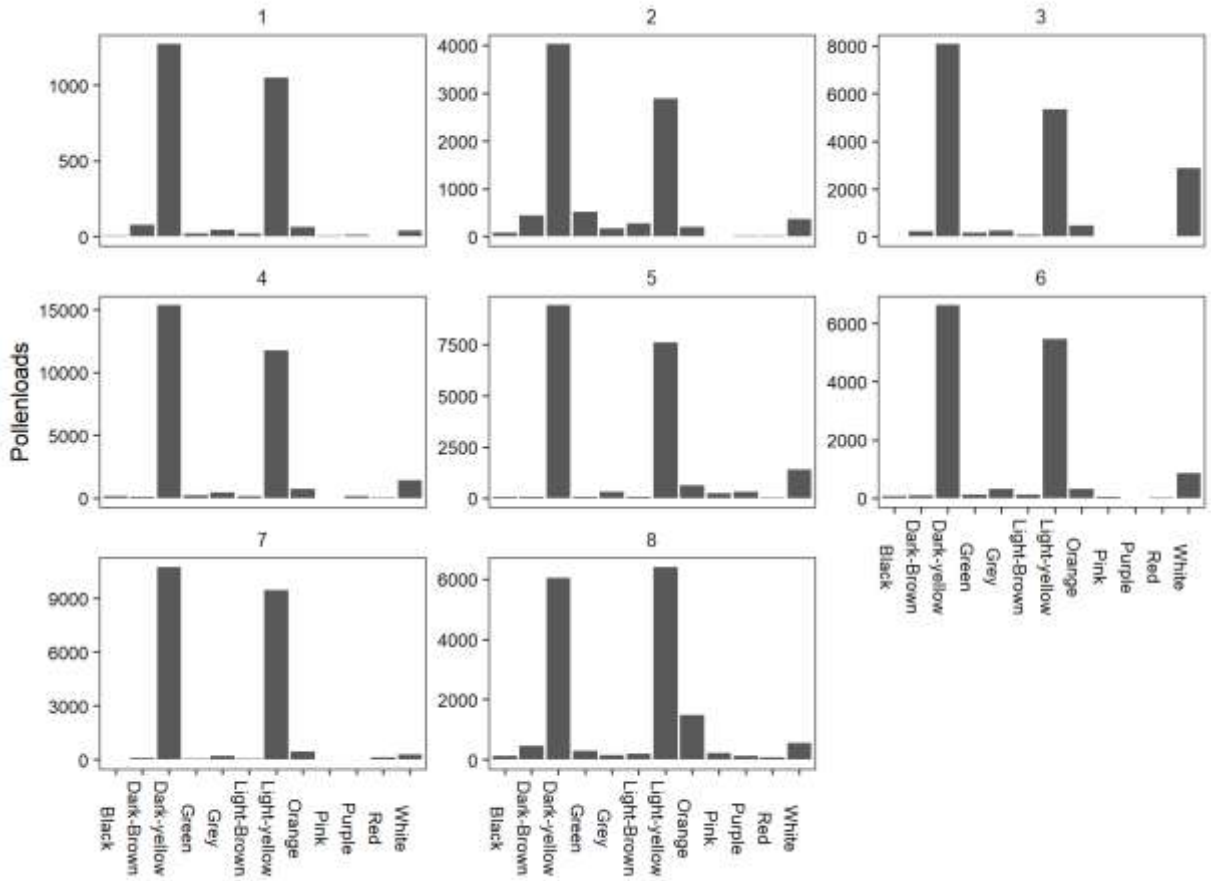


*Figure 11: The graphs show the average number of pollen loads of a color. The average of 56 samples was extrapolated using weight percentages. Differences of the pollen loads in terms of their color distribution sorted by colonies. (nTrap=131481).*

### 4.1.2. Weight of collected pollen loads

The result of the Shapiro-wilk normality test is: W = 0.9361, p-value = 2.285e-16. Hence, the data for the category “weight” is not normally distributed. The honeybees collected a total of 892,31 gram of pollen with an average of 15,34 gram per sample (n=56). The average weight of 1000 pollen loads are 6,79 grams. And the average amount of pollen loads per gram is approximately 147.

*Table 8: All weight measurements of different dates and colonies. In total 892,31 gram of pollen were collected by eight colonies in seven days.*

| Date | Colony 1 | Colony 2 | Colony 3 | Colony 4 | Colony 5 | Colony 6 | Colony 7 | Colony 8 | Total |
|---|---|---|---|---|---|---|---|---|---|
| 28.06.2021 | 2,75 | 10 | 26,75 | 45,16 | 30,47 | 12,34 | 29,95 | 14,43 | 171,85 |
| 29.06.2021 | 4,54 | 12,11 | 25,8 | 35,45 | 16,44 | 7,09 | 25,47 | 7,32 | 134,22 |
| 30.06.2021 | 1,27 | 3,64 | 11,47 | 16,53 | 11,01 | 3,45 | 3,6 | 3,91 | 54,88 |
| 01.07.2021 | 1,02 | 2,54 | 33,02 | 29,42 | 11,04 | 2,63 | 19,31 | 4,87 | 103,85 |
| 23.07.2021 | 3,74 | 6,17 | 6,33 | 21,3 | 20,51 | 19,85 | 9,46 | 18,1 | 105,46 |
| 24.07.2021 | 2,87 | 15,35 | 18,42 | 33,85 | 45,02 | 21,06 | 13,93 | 23,99 | 174,49 |
| 25.07.2021 | 5,93 | 18,14 | 17,81 | 32,24 | 17,29 | 17,19 | 13,44 | 25,52 | 147,56 |
| Total | 22,12 | 67,95 | 139,6 | 213,95 | 151,78 | 83,61 | 115,16 | 98,14 | 892,31 |

Figure 11 shows the sample frequency in relation to their weight. It shows a right-skewed distribution. Most of the samples *(n= 56; f=12)* are in the weight group between 0-5 grams. The second highest weight group is between 15-20 grams *(n=56; f=11).* Very few samples are part of the weight group between 35-50 gram *(n=56; f=3)*.

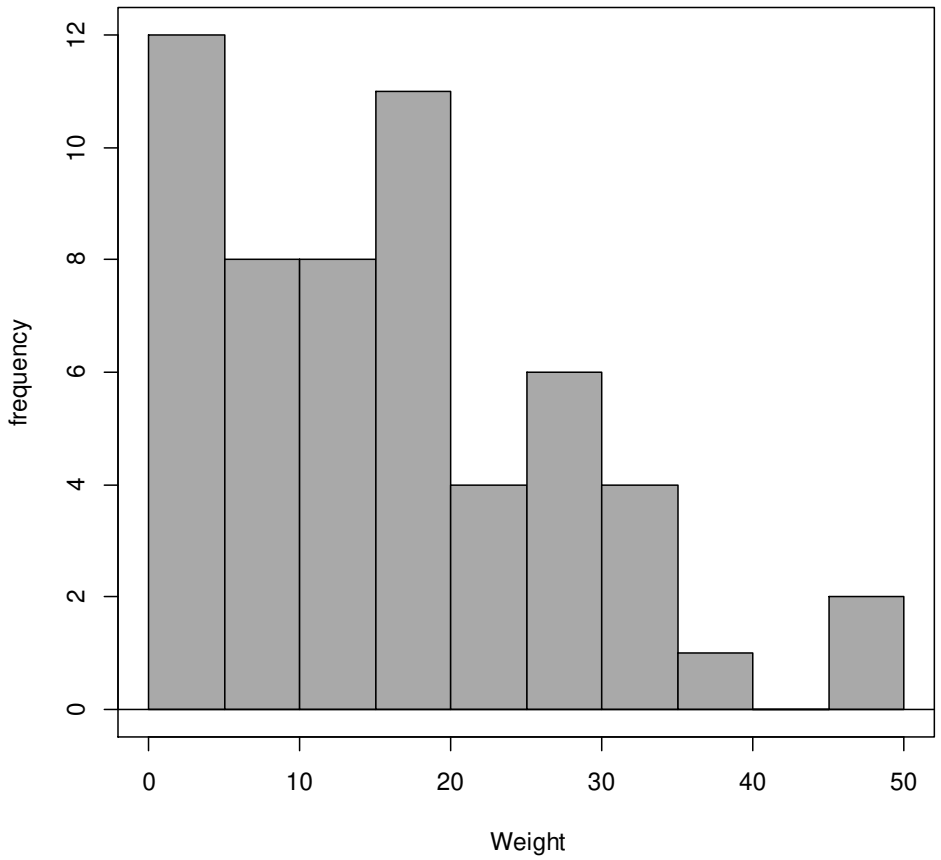


*Figure 12: Weight of the complete content of pollen traps of all days and hives (n=56.) Weight groups in steps of 5 grams.*

The correlation analysis shows a strong linear relationship between the two variables "pollen load" and "weight" [R=0.84; p<0.05].

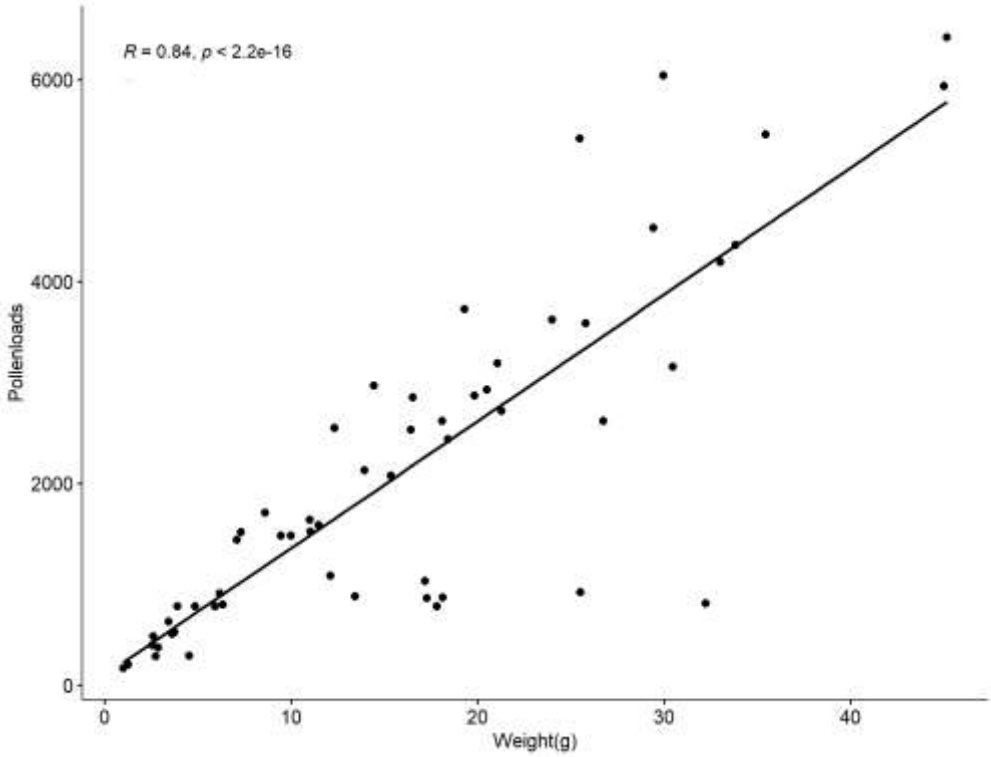


*Figure 13: Correlation analysis. X-axis: Weight (g). Y-axis: Pollenloads. Measuring points oscillate around correlation line. The weight of 56 samples were analysed.*

The correlation analysis also shows a similar result for the individual sites. Both sites were studied independently, and both show a strong linear correlation between the "weight" and the number of

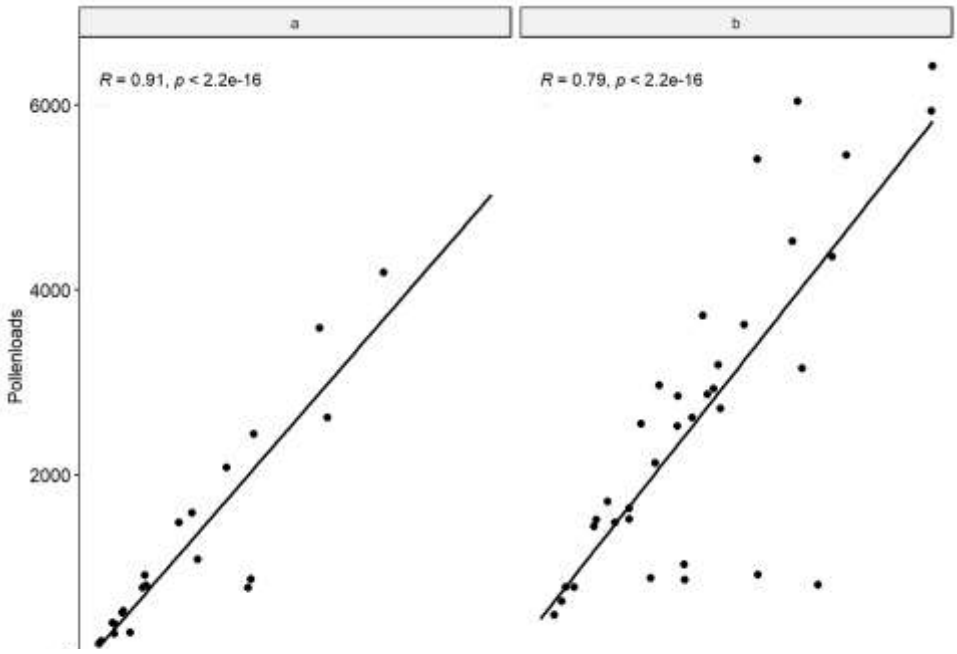


*Figure 14: Correlation analysis. X-axis: Weight (g). Y-axis: Pollenloads. Measuring points oscillate around correlation line. Both sites were analyzed indepently.*

"pollen loads". Thereby, the correlation is strong at site a [ R=0.91; p<0.05] and slightly lower at site b [site b: R=0.79; p<0.05].

The graph 14 shows the distribution of the weight measurement values. The two sites a and b each have their own frame. Each boxplot represents all weight measurements of the respective colony. The ANOVA results show that the colony factor has a significant effect on the weight of the samples: F-value= 27.63; P-value: *1.98e-07.* Hence, a strong correlation is given and the factor colony seems to be an important influence factor for the weight of the pollen loads. The tukey-test showed that only between the colonies 3-1 (p=0.0003341) and between 8-4 (p=0.0202107) there is a significant difference. All other combinations are not significant.

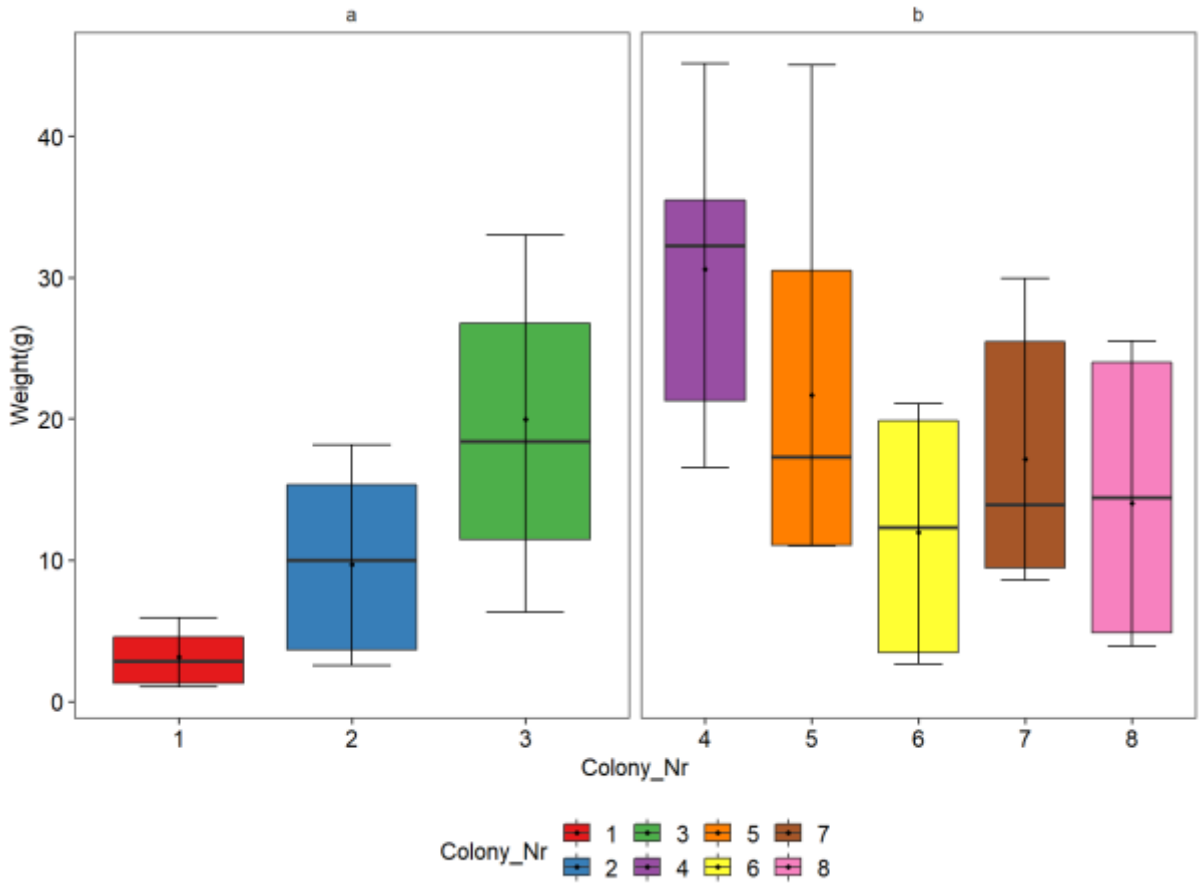


*Figure 15: Boxplots of each colony and the weight of the pollen in the traps. The mean of all sample is shown. On the left side are the colonies of site a (7. Viennese district) and on the right side are the colonies of the site b (2. Viennese district).*

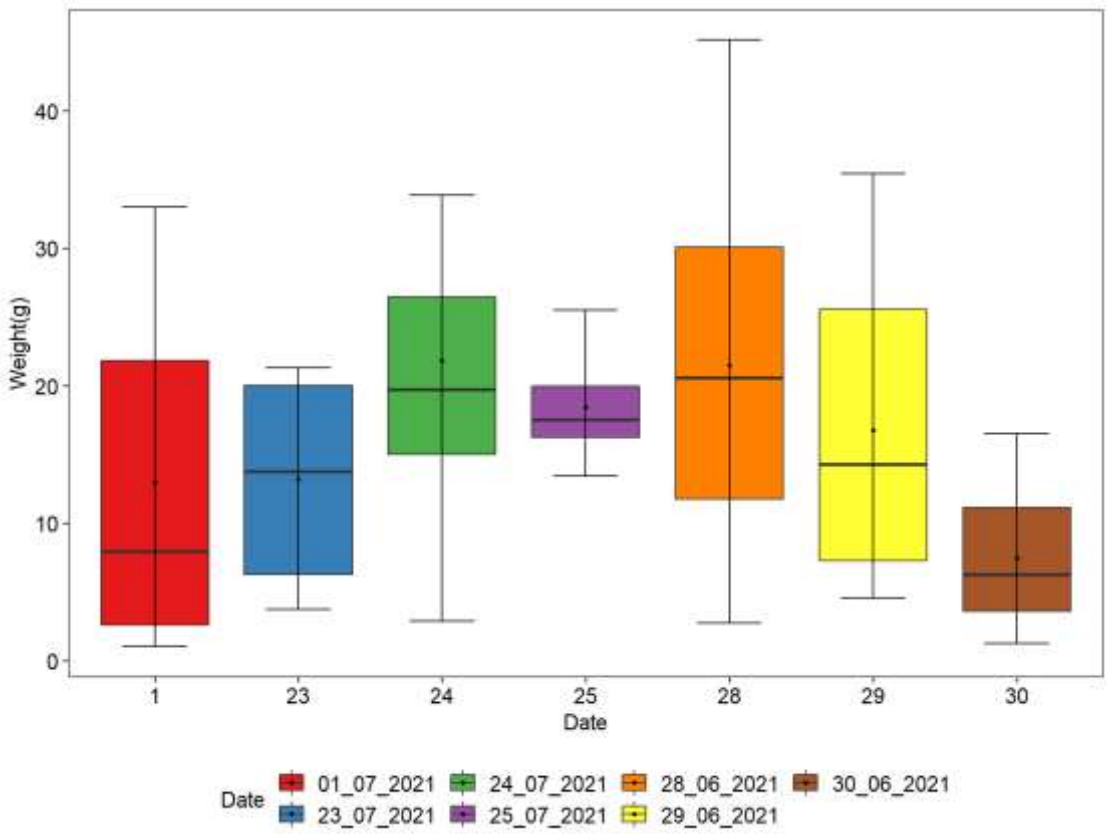


*Figure 16: Weight of the pollen samples according to the collection date. The mean of No significant difference between the collection days.*

The ANOVA for the factor *site* and *weight* of the samples shows a very strong significance (p=< 2e-16). The bees on the site b, in the prater, foraged more pollen than the bees on the urban site.

Another factor is the *day*. The results show that the day has no significant influence on the weight of the pollen (p= 0.0718). *Figure 15* shows the distribution of the pollen samples weight according to the different days.

### 4.1.3. Microscopic images of pollen loads from different color categories

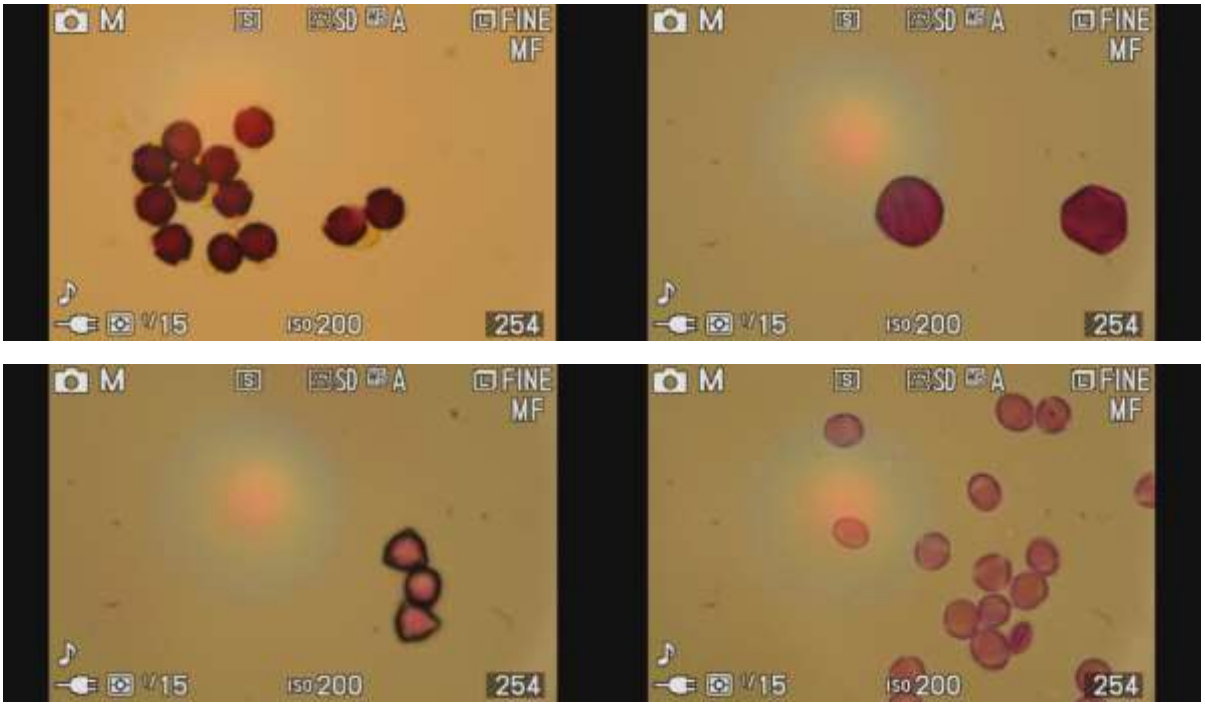


*Figure 17: Pollen grains in a dark-brown pollen load. Unknown taxa. Picture on the top left. Figure 18: Pollen grains in a dark-yellow pollen load. Suspicion: Parthenocissus. Picture on the top right. Figure 19: Pollen grains in green pollen loads. Unknown taxa. Picture on the bottom left. Figure 20: Pollen grains in a grey pollen load. Unknown taxa. Picture on the bottom right.*

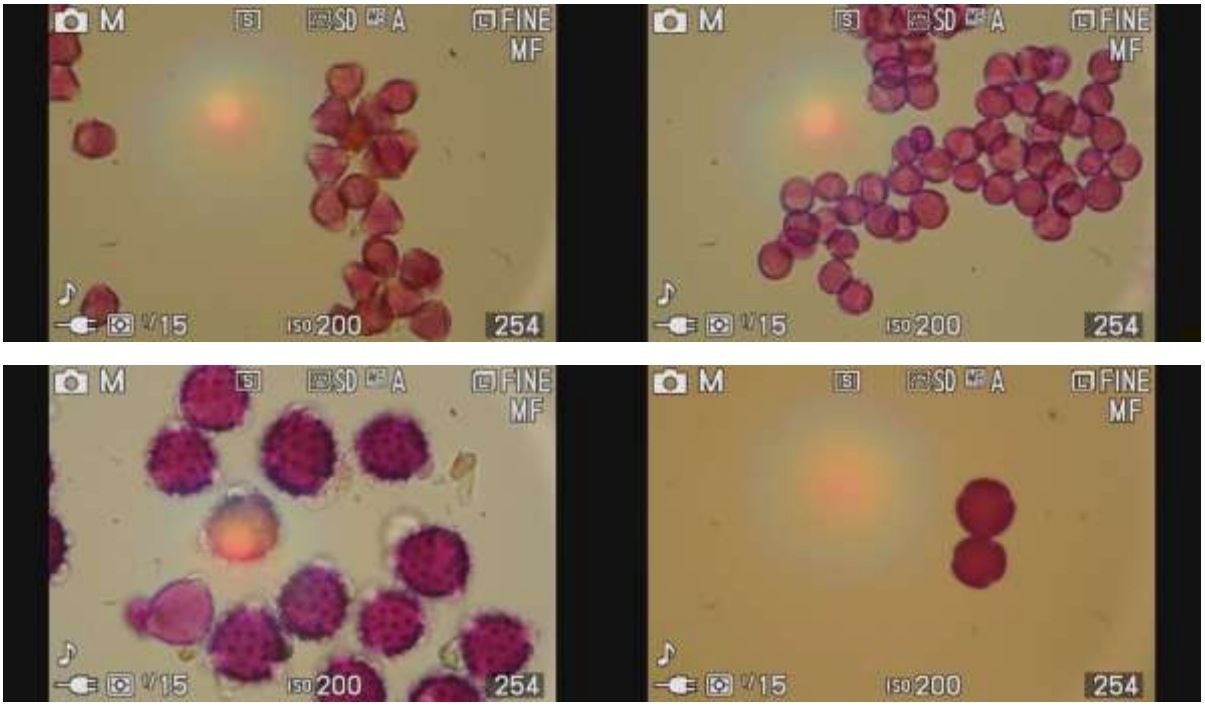


*Figure 21: Pollen grains in a light-brown pollen load. Unknown taxa. The yellow substance is pollenkitt/viscin, an oily adhesive on pollen helping to stick to stigmas of flowers. Picture on the left. Figure 22: Pollen grains in a white pollen load. Unknown taxa. Picture on the top right. Figure 23: Pollen grains in purple pollen loads. Family: Asteraceae. Suspicion: Echinacea. Picture on the bottom left. Figure 24: Pollen grains in a light-yellow pollen load. Unknown taxa. Picture on the bottom right.*

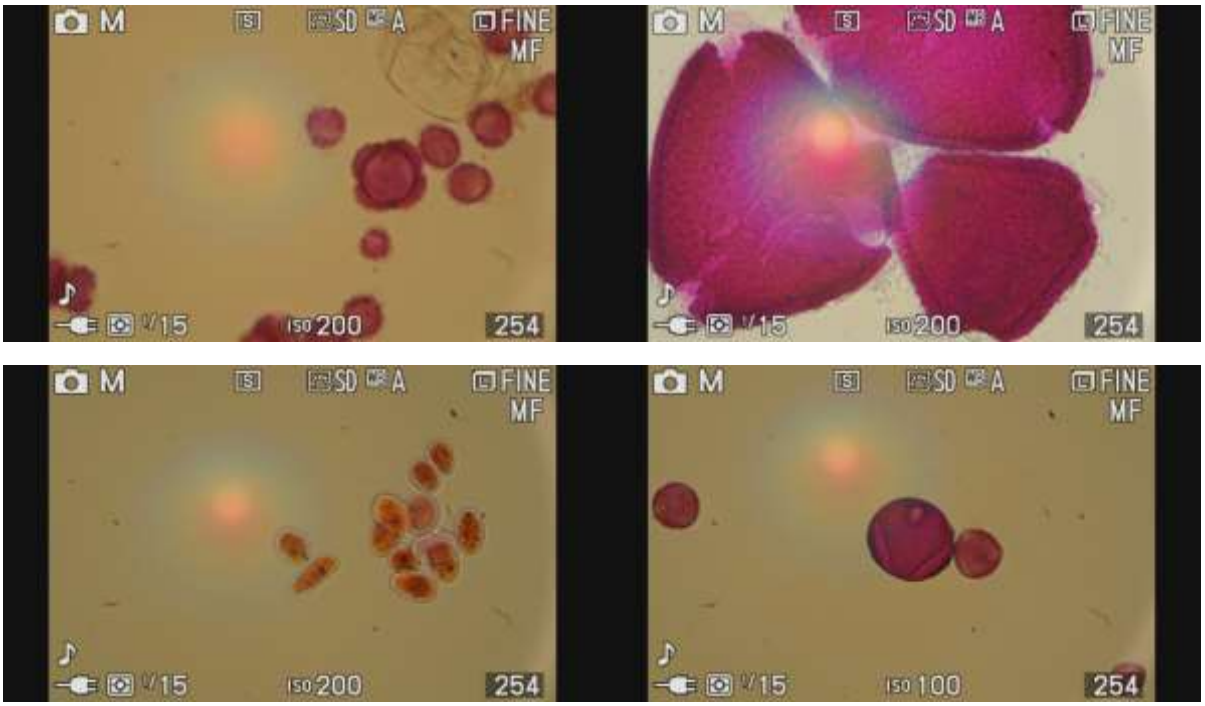


*Figure 25: Pollen grains in an orange pollen load. Family: Asteraceae. Suspicion: Achillea-Type, Tanacetum. Picture on the top left. Figure 26: Pollen grains in a pink pollen load. Family: Caprifoliaceae. Suspicion: Scabiosa. Picture on the top right. Figure 27: Spores in a red pollen load. Unknown taxa. Picture on the bottom left. Figure 28: Pollen grains in a black pollen load. The pollen load in the middle is of Tilia. Family: Malvaceae. Picture on the bottom right.*

## 4.2. Genetical analysis

### 4.2.1. DNA-isolation and general description of the detected sequences

Most samples showed clear bands in gel electrophoresis *(see Appendix).* Gel electrophoresis after the first PCR run and after index PCR also showed clear bands (*see Appendix).*

The blasting resulted in different forms of taxa. Sometimes the plant family is given, often the genus and sometimes the actual species. In the 192 samples 130 taxa with 119438 sequences have been detected. The plant which showed the most sequences was *Koelreuteria paniculata* (n=119438, f=25378). It was detected in 82,3% of all samples (n=192, f=158). It is also known as the *goldenrain tree* or as *Blasenesche* in german. Its bright yellow flowers in the form of panicles appear in July. This fits the collection period. Only two other species were detected in more samples: *Parthenocissus quinquefolia* (n=192, f=174; 90,6%) and *Ligustrum lucidum* (n=192, f=168; 87,5%). *Parthenocissus quinquefolia* is also known as *Virginia creeper* or as *Wilder Wein/Selbstkletternde Jungfernrebe.* It blooms from June to July and is very common in public places, inner courtyards and parks. *Ligustrum lucidum,* which trivial name is *broad-leaf privet* blooms between May and July. In Europe its mainly planted in hedges as an ornamental plant. *Ligustrum lucidum* and *Parthenocissus quinquefolia* also showed the second and third most frequent sequences overall (see graphic x). Another very frequent taxa is *Firmiana,* which could be detected in 69,2% of all samples (n=192, f=133). *Firmiana* is a genus of *Malvaceae.* One frequent species of this genus in European parks is *Firmiana simplex* which is also

known as the *Chinese parasol tree* or as *Chinesischer Sonnenschirmbaun/Wutong-Baum*. It blooms in July, which makes its finding plausible. Of all 130 detected taxa only 13 taxa represent more than 80% of all detected sequences. Interestingly, many of the taxa found show relatively low sequence counts. All found taxa (n=130, f=98) with less than 500 sequences were subsumed in the graphic as "others" to ensure readability.

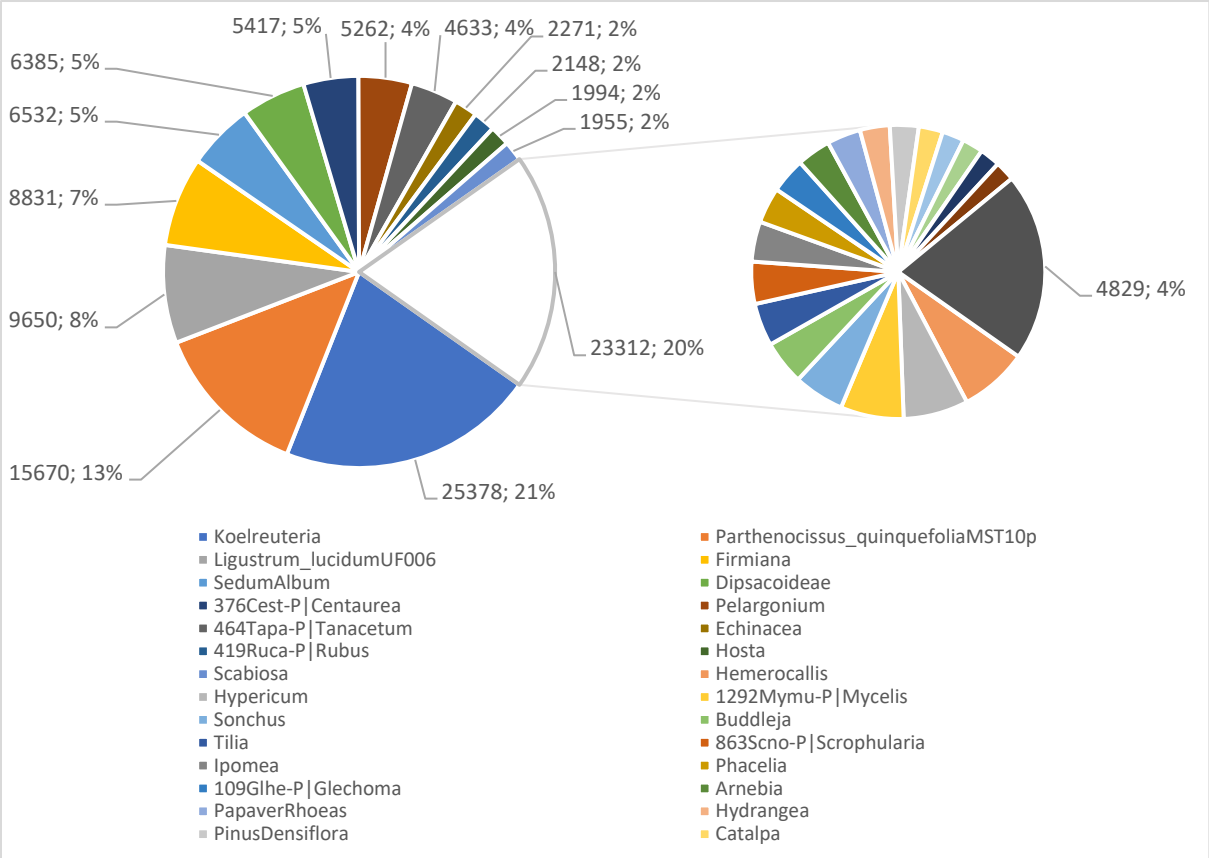


*Figure 29: Distribution of detected sequences. Dominant species are part of the left in the main pie chart and more rare sequences which represent 1% or less of all sequences are subsumed in the sub-pie chart (nSequences=119438).*

### 4.2.2. Detected sequences in relation to the color of the analyzed pollen loads

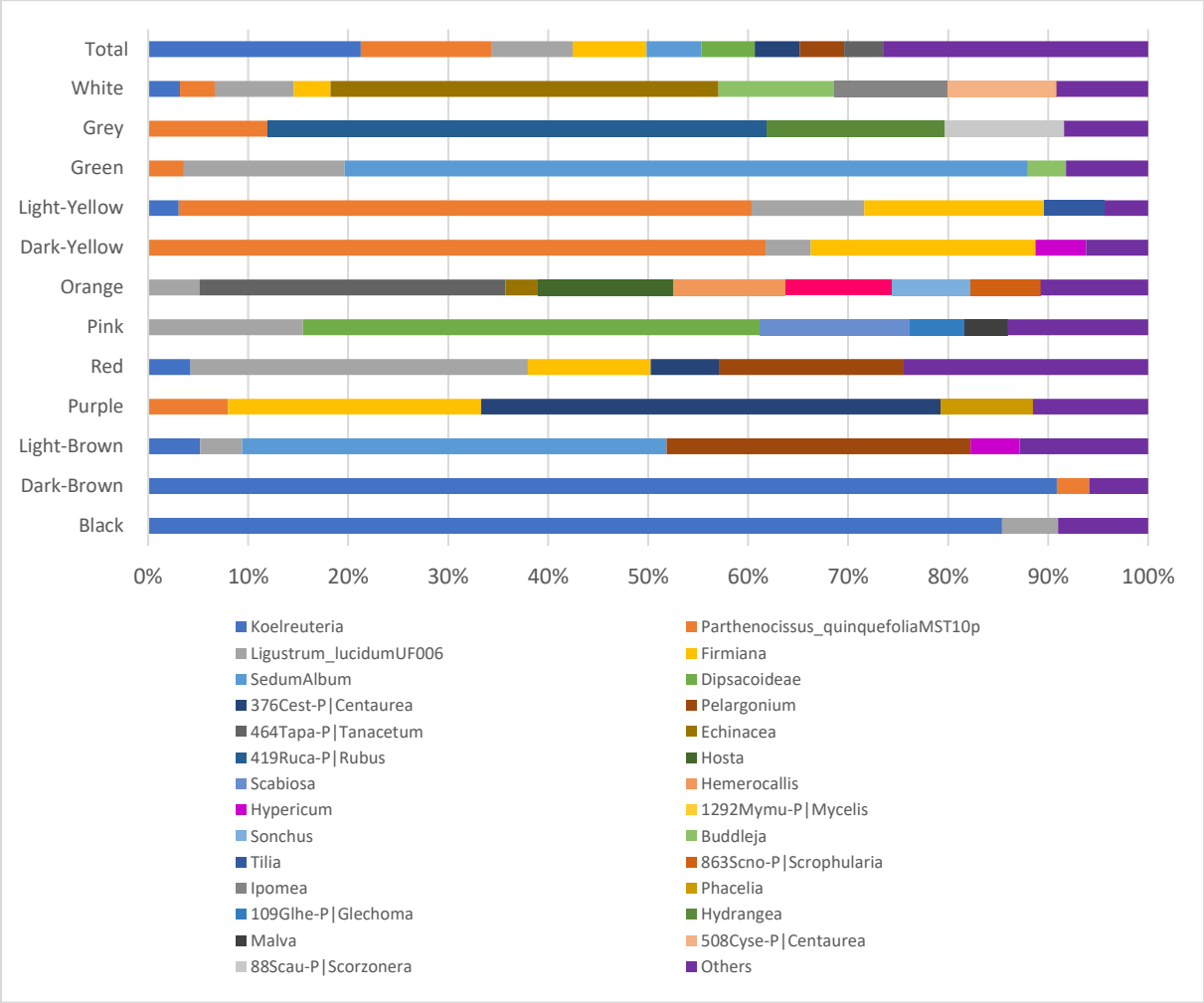


*Figure 30: Detected taxa in pollen loads with different color (nSequences=119438). Species/taxa with less than 3% of the specific sequences of one pollen load color have been subsumed as "others". A list with the exact value of all "others" is attached in the appendix.*

Pollen loads of different colors are composed of pollen from different plant species. Eight of the twelve color categories have very different species compositions, which are not similar to another color. Four of the color categories show very similar species compositions. Light-yellow and dark-yellow seem to be almost identical, as well as dark-brown and black pollen loads. They are described in the following.

The analyzed light-yellow pollen loads (n=11362) consisted of very similar sequences to the dark-yellow pollen loads (n=10274): *Parthenocissus quinquefolia* (light-yellow: f=6509; dark-yellow: f=6138), *Firmiana* (light-yellow: f=2045; dark-yellow: f=2234), *Ligustrum lucidum* (light-yellow: f=1280; dark-yellow: f=449). These three taxa made up more than 80% of the analyzed light-yellow and dark-yellow pollen loads. Their main difference is that the analyzed light-yellow pollen loads also consisted of *Tilia* (f=684) and *Koelreuteria paniculata* (f=347) and the dark-yellow pollen loads also entailed pollen of the taxa *Hypericum* (f=502).

The analyzed dark-brown pollen loads (n=15340) and the black pollen loads (n=13316) were almost identical. Both consisted mainly of the taxa *Koelreuteria paniculata* (dark-brown: f=13475; black: f=9834). A single plant taxon made up more than 85% of the black pollen loads and even over 90% of the analyzed dark-brown pollen loads. The main difference is that dark-brown loads also entailed quantities of *Parthenocissus quinquefolia* (f=483) and the black loads quantities of *Ligustrum lucidum* (f=646).

The other pollen load colors seem to be very different from each other, but still interesting overlaps can be found. A big part of the light-brown pollen loads (n=11887) was *Sedum album* (f=4858; 40,8%), which was also very present in green pollen loads (n=2426; f=1580; 65,1 %). Although never dominant, *Ligustrum lucidum* occurred in 8 out of 12 pollen colors: white (n=3377*,* f=266), green (n=2426, f=372), orange (n=14792, f=759), pink (n=13065, f=2022), red (n=8753, f=2952), light-brown (n=11887; f=480) and the above described quantities in dark-yellow, light yellow and black.

There are several taxa which only occurred in big quantities in one specific pollen color, such as *Hydrangea* (f=736) in grey pollen loads (n=4137), *Dipsacoideae* (f=5974) in pink pollen loads (n= 13065), *Tanacetum* (f=4527) and *Mycelis* (f=1576) in orange pollen loads (n=14792) and *Echinacea* (f=1310) in white pollen loads (n=3377). Likewise, there are two taxa that occur only in two pollen colors: *Pelargonium* in light-brown (n=11887, f=3479) and red loads (n=8753, f=1617) and *Centaurea* in purple (n=10709, f=4609) and red loads. (n=8753, f=597).

In total, all samples (n=119438) consisted mostly of nine different taxa: *Koelreuteria paniculata* (f=25378), *Parthenocissus quinquefolia* (f=15670), *Ligustrum lucidum* (f=9650), *Firmiana* (f=8831), *Sedum album* (f=6532), *Dipsacoidea*e (f=6385), *Centaurea* (f=5417), *Pelargonium* (f=5262) and *Tanacetum* (f=4633).

To illustrate the variation of the pollen loads in one specific color, between colors, but also inside of pollen loads themselves the graphic 32 is given. The difference in homogeneity or heterogeneity of the different pollen loads becomes visible. So black and dark brown pollen loads are very uniform in their entirety and very identical. And, the individual samples show few outliers. With other colors like purple it looks like half of the 16 samples are dominated by one taxon and the other half by another. The plot allows the dominance of certain taxa across colors to be perceived as blocks. Thus, the presence of dominant taxa such as *Parthenocissus quinquefolia* (graphic color: orange), *Koelreuteria paniculata* (graphic color: light-blue)*, Firmiana* (graphic color: yellow)*, Dipsacoideae* (graphic color: grass green) and *Pelargonium* (graphic color: brown) can be easily recognized. Also, the heterogeneity of white and green pollen loads becomes visible. The species with low occurrence, which are either rare or

represent contamination, are found at the right edge of the graphic. More about the homogeneity of pollen loads in chapter 4.2.7.

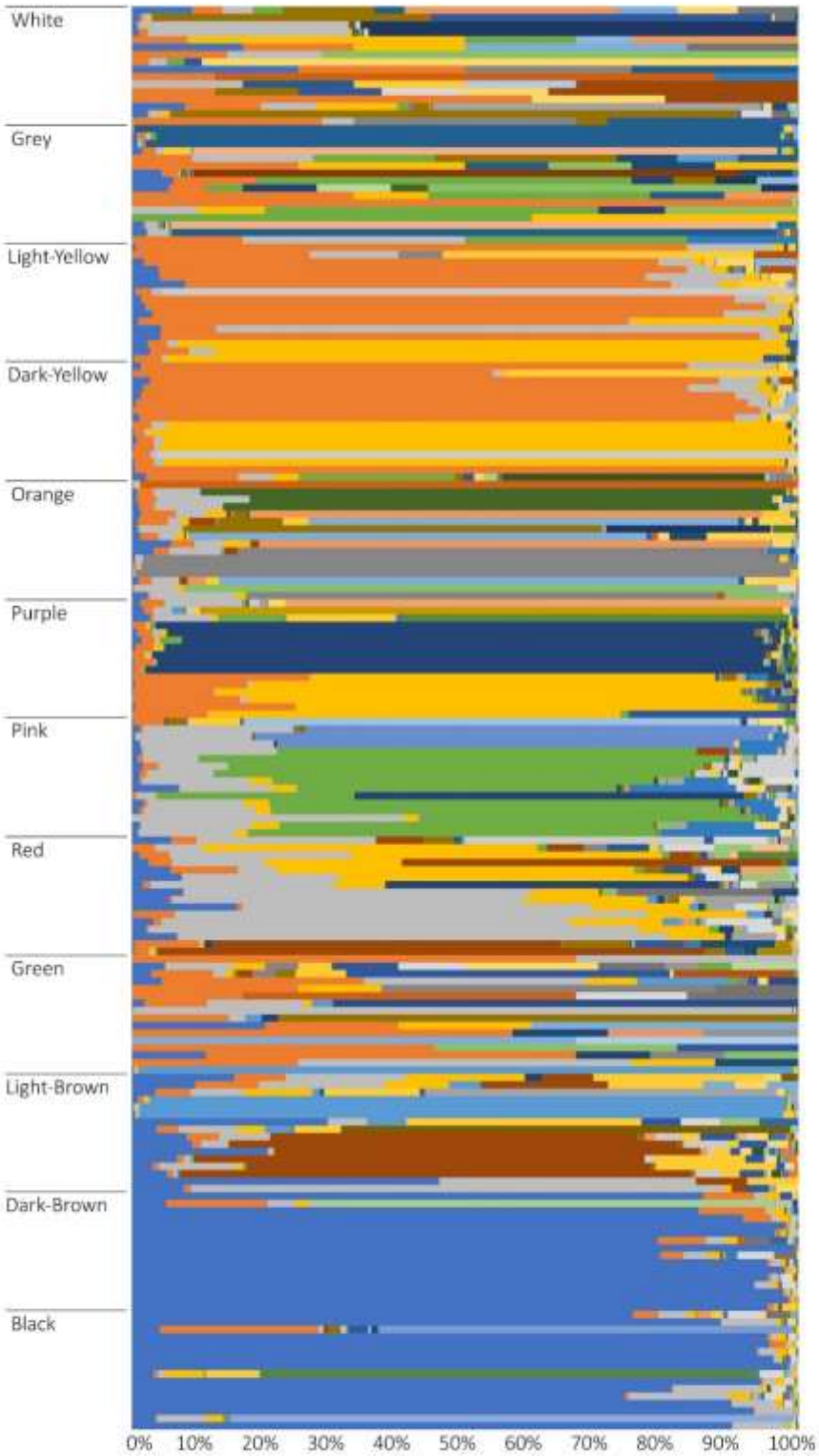


*Figure 31: Pollen loads sorted by their color. Y-axis shows the end and beginning of the specific colony pollen loads. X-axis shows the percentage of each taxa contributing to the specific pollen load. Every color category was analyzed with 16 samples (n=192).*

### 4.2.3. Detected sequences in relation to the specific honeybee colony

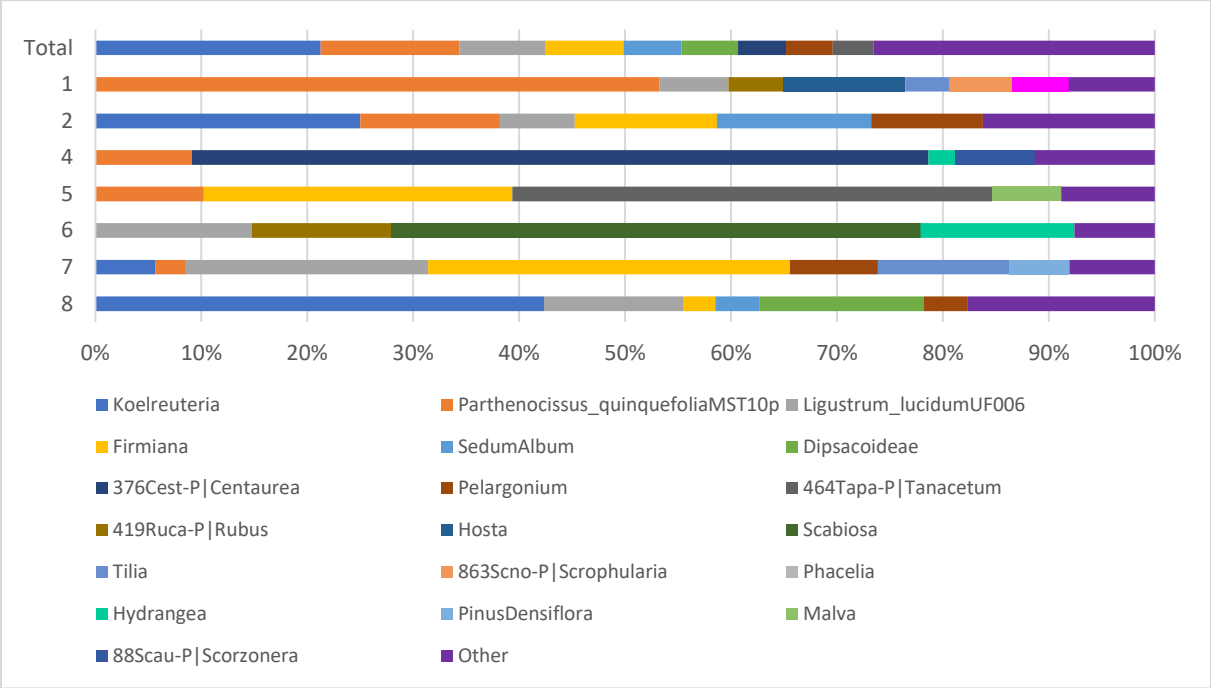


*Figure 32: Detected plant species collected by different honeybee colonies (n=119438).*

The analyzed pollen from different honeybee colonies shows, that none of the colonies collected pollen from only one species. The detected sequences in the analyzed pollen loads differs between the colonies. Nevertheless, some taxa are more dominant in the pollen loads of one colony than in another. Over 50% of all detected sequences of the loads, which were gathered by colony 1 (n=17948) were from *Parthenocissus quinquefolia* (f=9206). This taxon is also present in big quantities in the pollen loads of colony 2 (n=37891, f= 4411), colony 4 (n=6969, f=604), colony 5 (n=9222, f=880) and in colony 7 (n=1394, f=38). *Koelreuteria paniculata* is the taxa with the most sequences. This is mainly due to pollen loads of the colonies 8 (n=42109, f=16409) and 2 (n=37891, f=8373). Other very dominant taxa are more equally distributed over all colonies. So are the sequences of *Ligustrum lucidum* entailed in the pollen loads of five colonies: colony 1 (n=17984, f=1131), colony 2 (n=37891, f=2362), colony 6 (n=3905, f=576), colony 7 (n=1394, f=300) and colony 8 (n=42109, f=5072). Also, the taxa *Firmiana* is present in the pollen loads of four colonies, but mainly in the colonies 5 (n=9222, f=2508) and 7 (n=1394, f=448). Interestingly, there are many taxa that only appear in the pollen loads of a single colony. For example, *Scrophularia* (f=1027), *Hosta* (f=1994) and *Phacelia* (f=918) in colony 1 (n=17948). Other examples are *Scabiosa* in colony 6 (n=3905, f=1951), *Tanacetum* in colony 5 (n=9222, f=3900) and *Dipsacoideae* in colony 8 (n=42109, f=6002). Moreover, there are also taxa exclusively occurring in the pollen loads of two colonies: *Tilia* in loads of colony 1 (n=17984, f=722) and 7 (n=1394, f=163). Rubus in loads of colony 1 (n=17984, f=893) and 6 (n=3905, f=514). *Tilia* and *Rubus* occurred on both

beehive locations. Other taxa, especially those who could only be found in the pollen of one specific colony, could be found at one particular site. More about the detected sequences related to the specific site of the honeybee hives in chapter 4.2.5. To illustrate the variation of pollen loads from different colonies the graphic 34 is given. The plot allows the dominance of certain taxa across certain colonies to be perceived as blocks. Thus, the presence of dominant taxa such as *Parthenocissus quinquefolia* (graphic color: orange), *Koelreuteria paniculata* (graphic color: light-blue), *Firmiana* (graphic color: yellow), *Dipsacoideae* (graphic color: grass green) and *Pelargonium* (graphic color: brown) can be easily recognized. For example, *Parthenocissus quinquefolia* is very dominant in the pollen loads of the colony 1 and *Koelreuteria paniculata* in the pollen loads of colony 8.

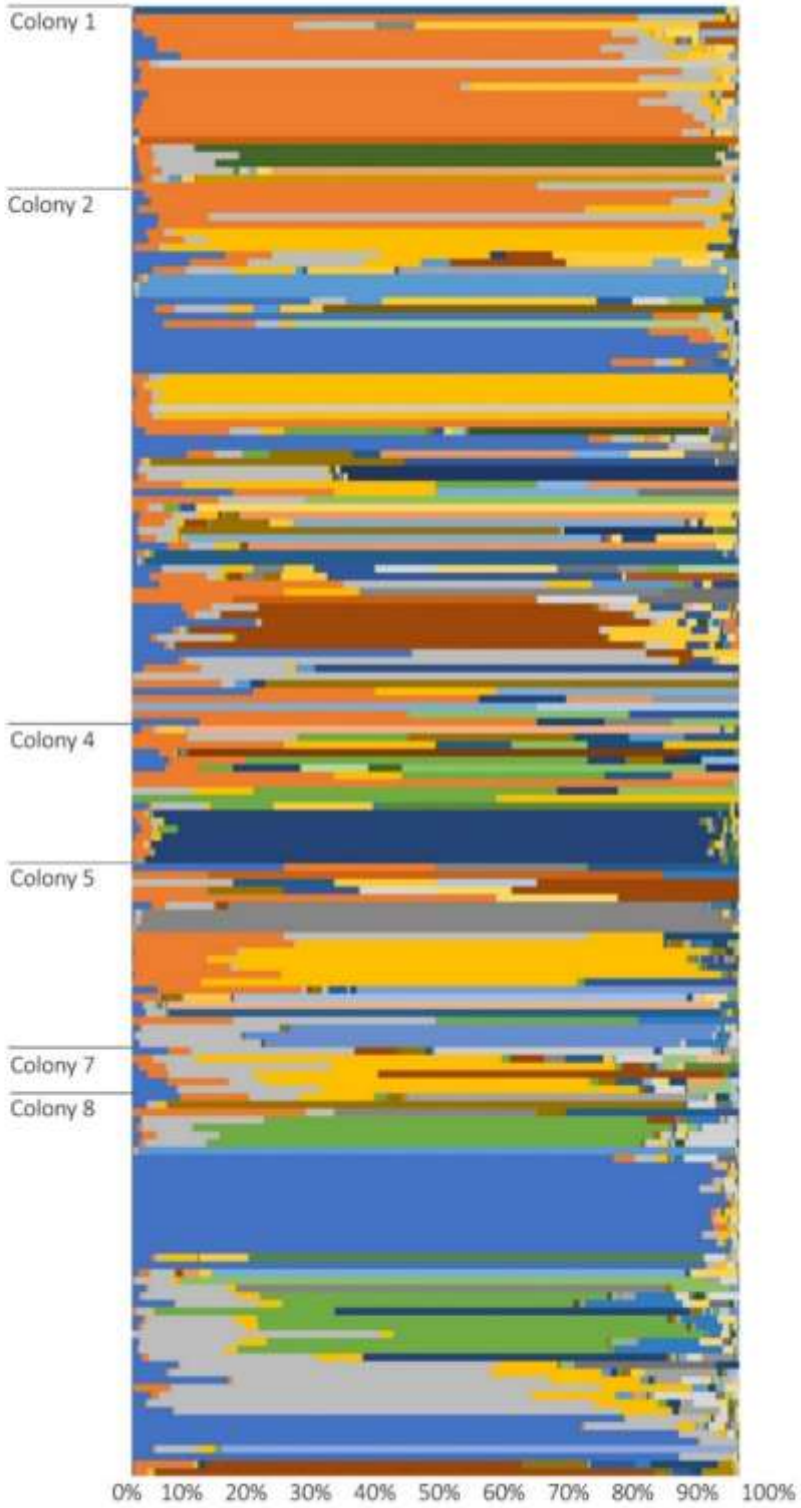


*Figure 33: Pollen loads sorted by the colonies. Y-axis shows the end and beginning of the specific colony pollen loads. X-axis shows the percentage of each Taxa contributing to the specific pollen load. A full legend for all colors is given in the appendix.*

### 4.2.4. Detected sequences in relation to the collection date of the analyzed pollen

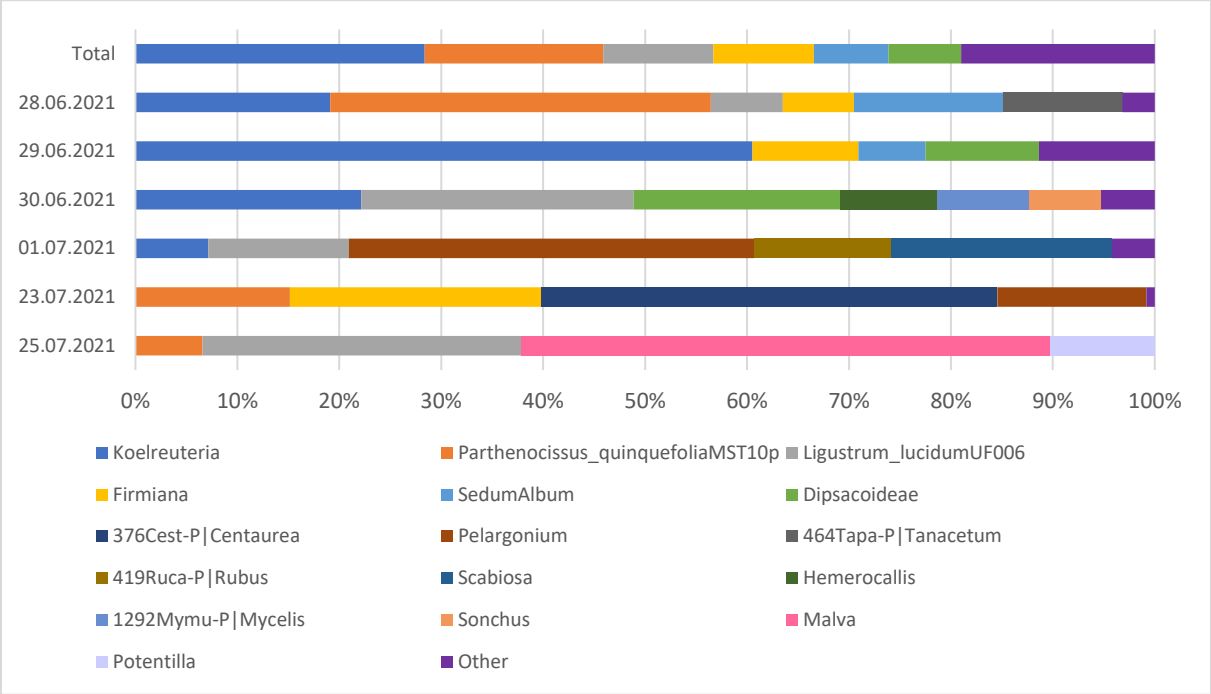


*Figure 34: Detected plant species of pollen loads collected on different collecting dates (nSequences=119438).*

The composition of the collected pollen also differs between collection days. It is noticeable that bees collected relatively large proportions of *Koelreuteria paniculata* pollen (28.6.21, n=41449, f=6375; 29.6.21, n=26721, f=14238; 30.6.21, n=24628; f=3904; 1.7.21, n=11806, f=645) especially in the first collection period from 28.6.-1.7. In contrast, this species hardly appears in the second collection period. This is the same for the taxa *Dipsacoideae*, which appeared only on two collection days of the first collection period (29.6.21, n=26721, f=2631; 30.6.21, n=24628, f=3574). This phenomenon also appears for the taxa *Malva* (25.7.21, n=1304, f=560), *Potentilla* (25.7.21, n=1304, f= 111), and *Centaurea* (23.7.21, n= 13530, f=4630), which appear only on the collection days one month later, but not on the earlier ones.

All other taxa cannot be assigned to either collection period. In contrast, they occur throughout the entire collection period: *Parthenocissus quinquefolia Parthenocissus quinquefolia* (28.6.21, n=41449, f=12442; 23.7.21, n=13530, f=1570; 25.7.21, n=1304, f=71), *Ligustrum lucidum* (28.6.21, n=41449, f=2360; 30.6.21, n=24628, f=4711; 01.7.21, n=11806; 25.7.21, n=1304, f=337), *Firmiana* (28.6.21, n=41449, f=2325; 29.6.21, n=26721, f=2456; 23.7., n=13530, f=2554) and *Pelargonium* (01.7.21, n=11806, f=3585; 23.7., n=13530; f=1513). The presence of dominant taxa such as *Parthenocissus quinquefolia* (graphic color: orange), *Koelreuteria paniculata* (graphic color: light-blue), *Firmiana* (graphic color: yellow), *Dipsacoideae* (graphic color: grass green) and *Pelargonium* (graphic color: brown) for each collecting day can be seen in figure 33. For example, *Parthenocissus quinquefolia* is

very dominant in the pollen loads of the 28.06., as well as *Koelreuteria paniculata* in the pollen loads of 29.06.

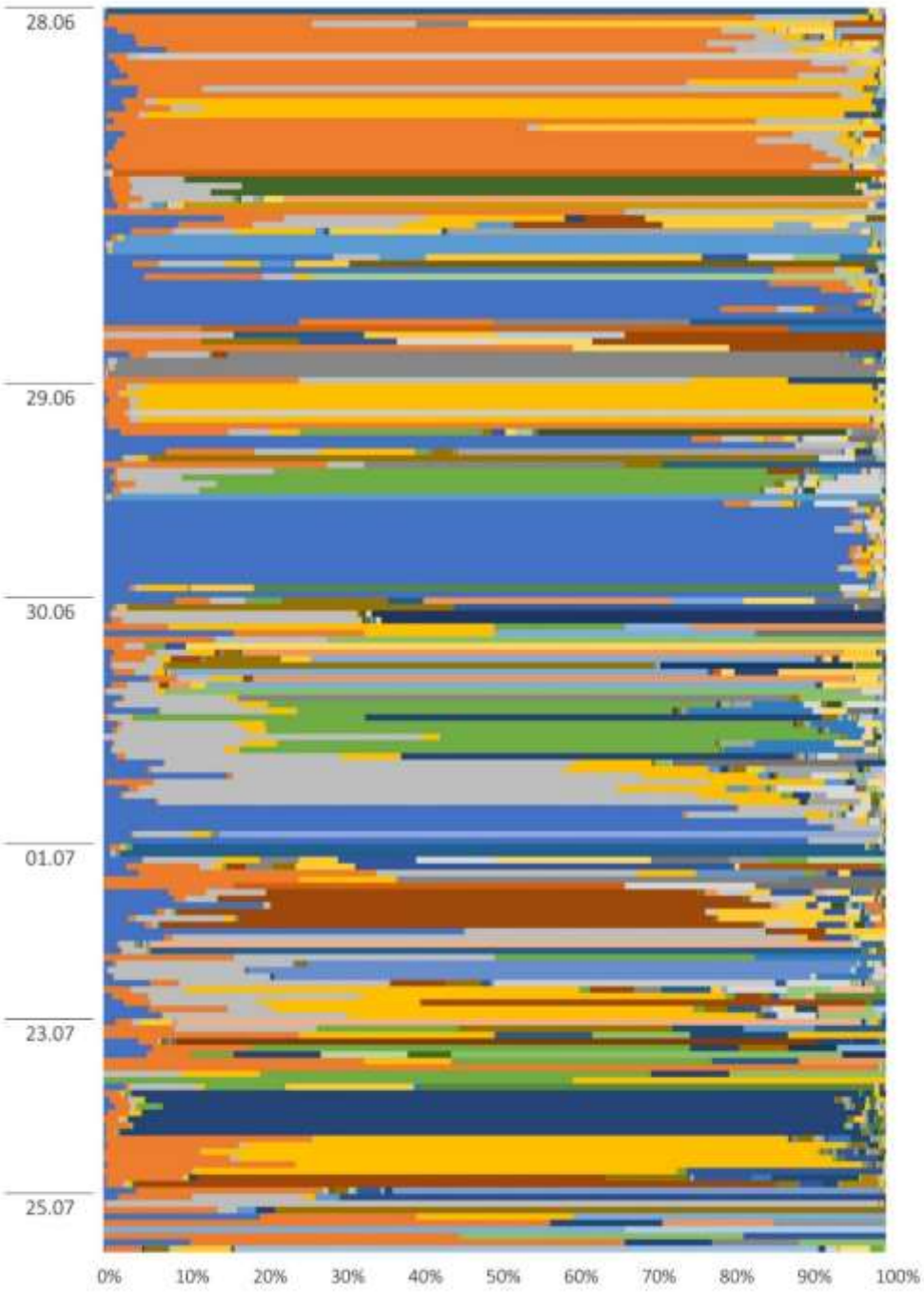


*Figure 35: Pollen loads sorted by the collecting date. Y-axis shows the end and beginning of the specific colony pollen loads. X-axis shows the percentage of each taxa contributing to the specific pollen load. A full legend for all colors is given in the appendix*

### 4.2.5. Detected sequences in relation to the actual location of the honeybee colonies

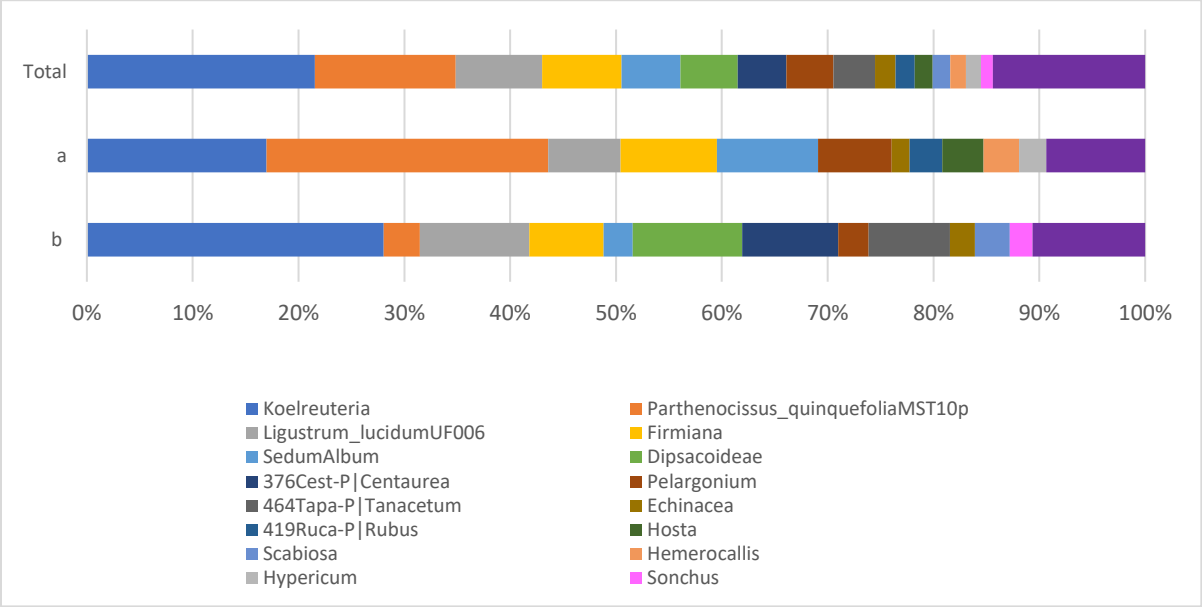


*Figure 36: Detected plant species of pollen loads from two different sites. Site a: Urban area in the seventh viennese district. Site b: park-like surrounding near Prater (nSequences=119438).*

Furthermore, the collected and analyzed pollen samples can also be characterized according to the locations of the bee colonies. A relatively similar composition of species is shown for both locations. At both sites, six taxa dominate over half of all samples*: Koelreuteria paniculata* (site a, n=55839, f=8684; site b, n=63599, f= 16694)*, Parthenocissus quinquefolia* (site a, n=55839, f=13617; site b, n=63599, f= 2053), *Ligustrum lucidum* (site a, n=55839, f=3493 ; site b, n=63599, f= 6157), *Firmiana* (site a, n=55839, f=4654; site b, n=63599, f= 4177), *Sedum album* (site a, n=55839, f= 4893; site b, n=63599, f= 1639) and *Pelargonium* (site a, n=55839, f= 3543; site b, n=63599, f= 1719). In the samples from location a sequences of *Parthenocissus quinquefolia* (site a, n=55839, f=13617) and in location b, sequences of *Koelreuteria paniculata* (site b, n=63599, f=16694) are found most frequently. Similarly, there are taxa that occur exclusively at one site (this only applies if one includes the simplification of grouping species together that are less than 1% to the category "others"). At site a, the rather urban site in the seventh district of Vienna, the following taxa occur exclusively: *Hemerocallis* (site a, n=55839, f=1712)*, Hosta* (site a, n=55839, f=1994) *and* Hypericum (site a, n=55839, f=1322). In contrast, the following taxa occur exclusively at site b, i.e. the park-like site in the second district of Vienna near the Vienna Prater: *Dipsacoidea* (site b, n=63599, f=6152)*,* Centaurea (site b, n=63599, f=5406), *Tanacetum* (site b, n=63599, f=4579) and *Sonchus* (site b, n=63599, f=1281). To illustrate the variation of pollen loads from different the two different sites the *figure 35* is given. The plot allows the dominance of certain taxa across the two sites to be perceived as blocks. Thus, the presence of dominant taxa such as *Parthenocissus quinquefolia* (graphic color: orange), *Koelreuteria paniculata* (graphic color: light-blue)*, Firmiana* (graphic color: yellow)*, Dipsacoideae* (graphic color: grass green) and *Pelargonium* (graphic color: brown) can be easily recognized. For example, *Parthenocissus*

*quinquefolia* is very dominant is very dominant at site a. As well as *Pelargonium.* Site b shows different results: *Koelreuteria paniculata* and *Dipsacoideae* are more frequent.

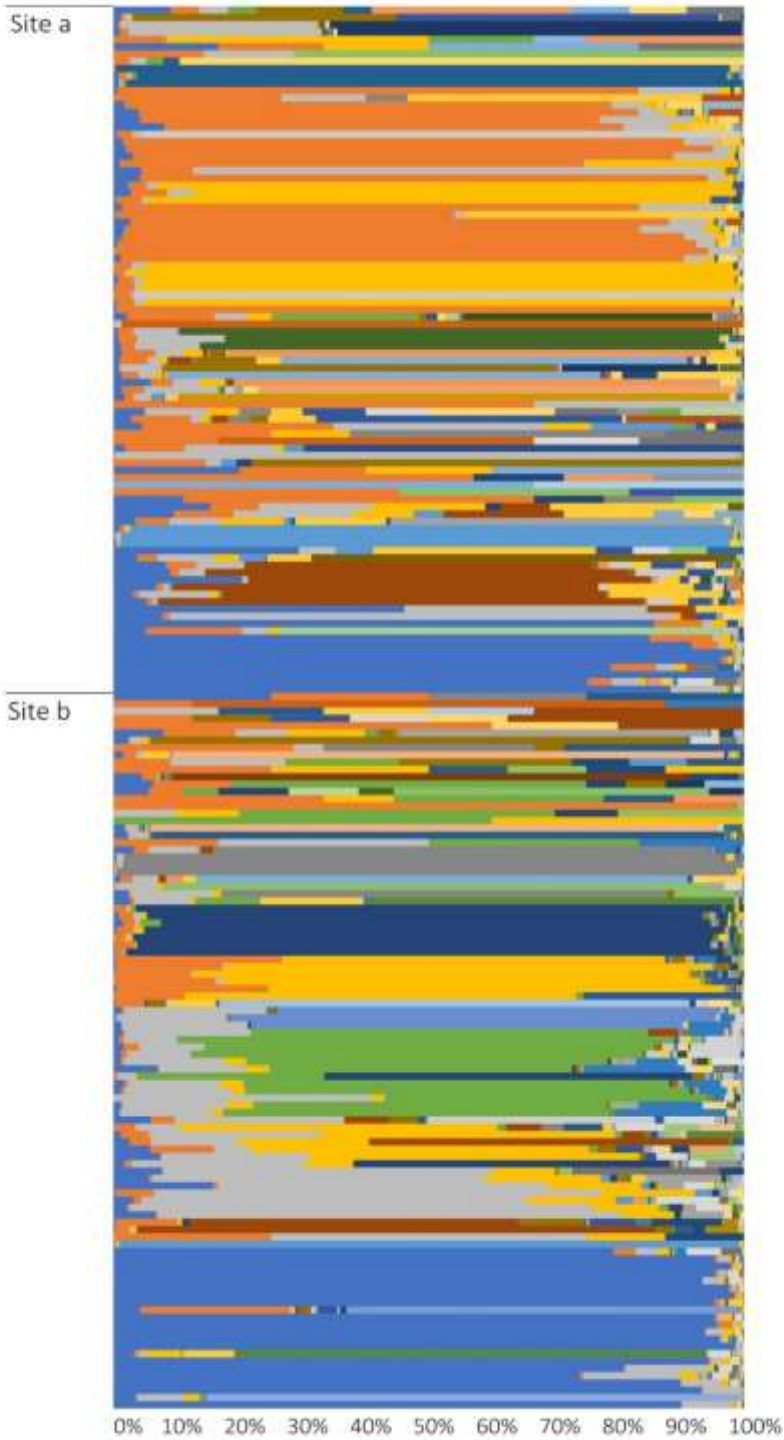


*Figure 37: Pollen loads sorted by the location of the hives. Y-axis shows the end and beginning of the specific colony pollen loads. X-axis shows the percentage of each taxa contributing to the specific pollen load. A full legend for all colors is given in the appendix. Dominant colors will be explained in the running text.*

### 4.2.6. Homogeneity of pollen loads

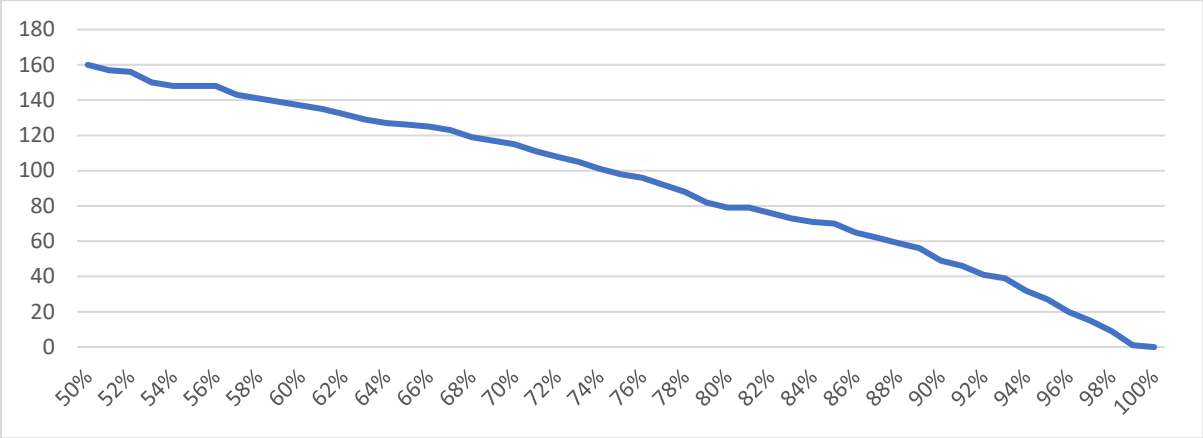


*Figure 38: The x-axis shows different homogeneity-criteria/thresholds for homogeneity as percentages. The y-axis shows the actual number of homogenous pollen loads. The number of homogenous pollen loads is directly proportional to the assumed homogeneity threshold.*

This graph shows the extent to which the number of homogeneous samples changes depending on which threshold is assumed to be the homogeneity criterion. For example, if it is assumed that a pollen load is homogeneous exactly when it consists of 50% of one taxon, then 83.3% (n=192, f=160) of the analyzed pollen loads would be homogeneous. This number decreases with increasing the homogeneity criterion. For example, 115 pollen loads consist of 70% (n=192, f=115) of one plant species/taxa. Assuming a homogeneity criterion of 85%, 70 pollen loads would be homogeneous. This is 36.4% (n=192, f=70) of all samples. I will continue with the threshold of 85% in the following. See the Appendix for all exact values of the homogeneity criteria between 50-100%.

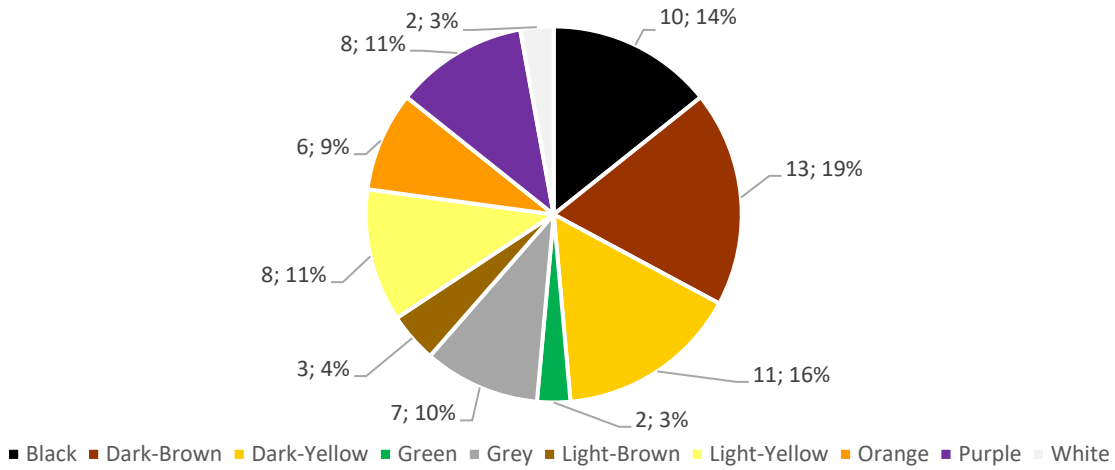


*Figure 39: Color of homogenous pollen loads. Absolute and relative frequency (n=70).*

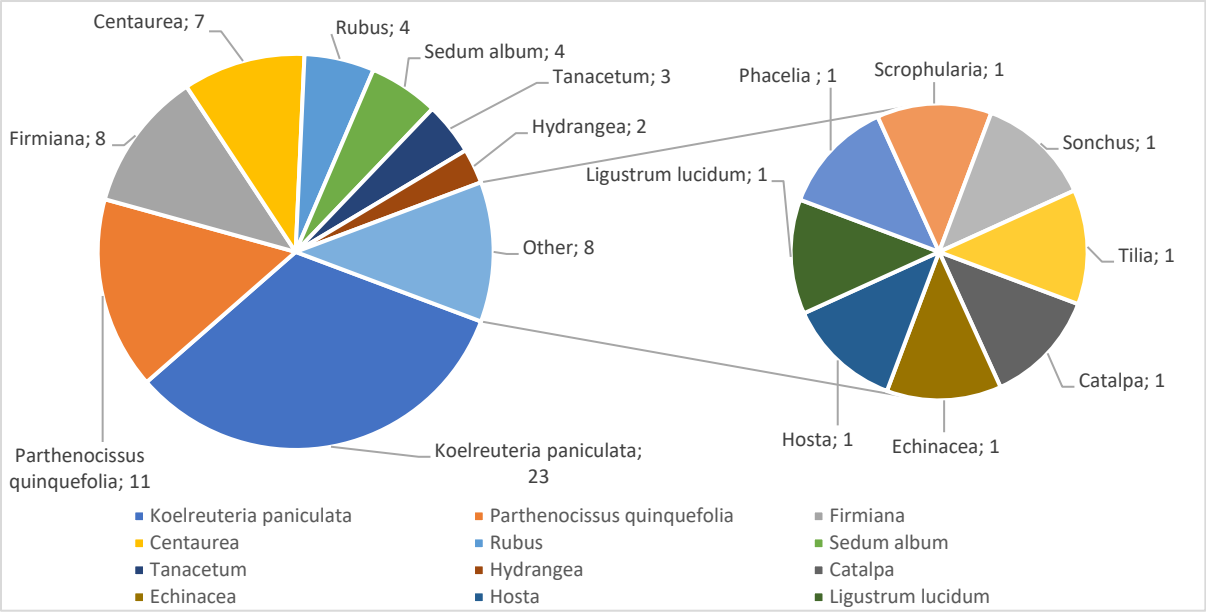


*Figure 40: Dominant Taxa of homogenous pollen loads (n=70).*

The homogeneity of the pollen loads differs between the colors. Relatively many of the dark brown (n=16, f=13), dark yellow (n=16, f=11) and black pollen loads (n=16, f=10) are homogeneous. Somewhat less homogeneous pollen loads could be found in the colors purple (n=16, 8), gray (n=16, f=7), light-yellow (n=16, f=8) and orange (n=16, 6). At the lower end, however, there are also colors that can rather be described as heterogeneous, since only relatively few of their pollen loads are homogeneous: light-brown (n=16, f=3), green (n=16, f=2) and white (n=16, f=2). None of the pollen loads of pink and red were homogeneous. They can be considered as very heterogeneous.

The homogeneous pollen loads have different taxa as their dominant species. Most homogeneous pollen loads are from *Koelreuteria paniculata* (n=70, f=23). The second most abundant homogeneous pollen loads are from *Parthenocissus quinquefolia* (n=70, f=11). The Taxa *Centaurea* (n=70, f=7), Rubus (n=70, f=4), *Sedum album* (n=70, f=4), *Tanacetum* (n=70, f=3) and *Hydrangea* (n=70, f=2) also occur relatively frequently. All taxa that have only a single homogeneous pollen load are shown in the graph on the right side.

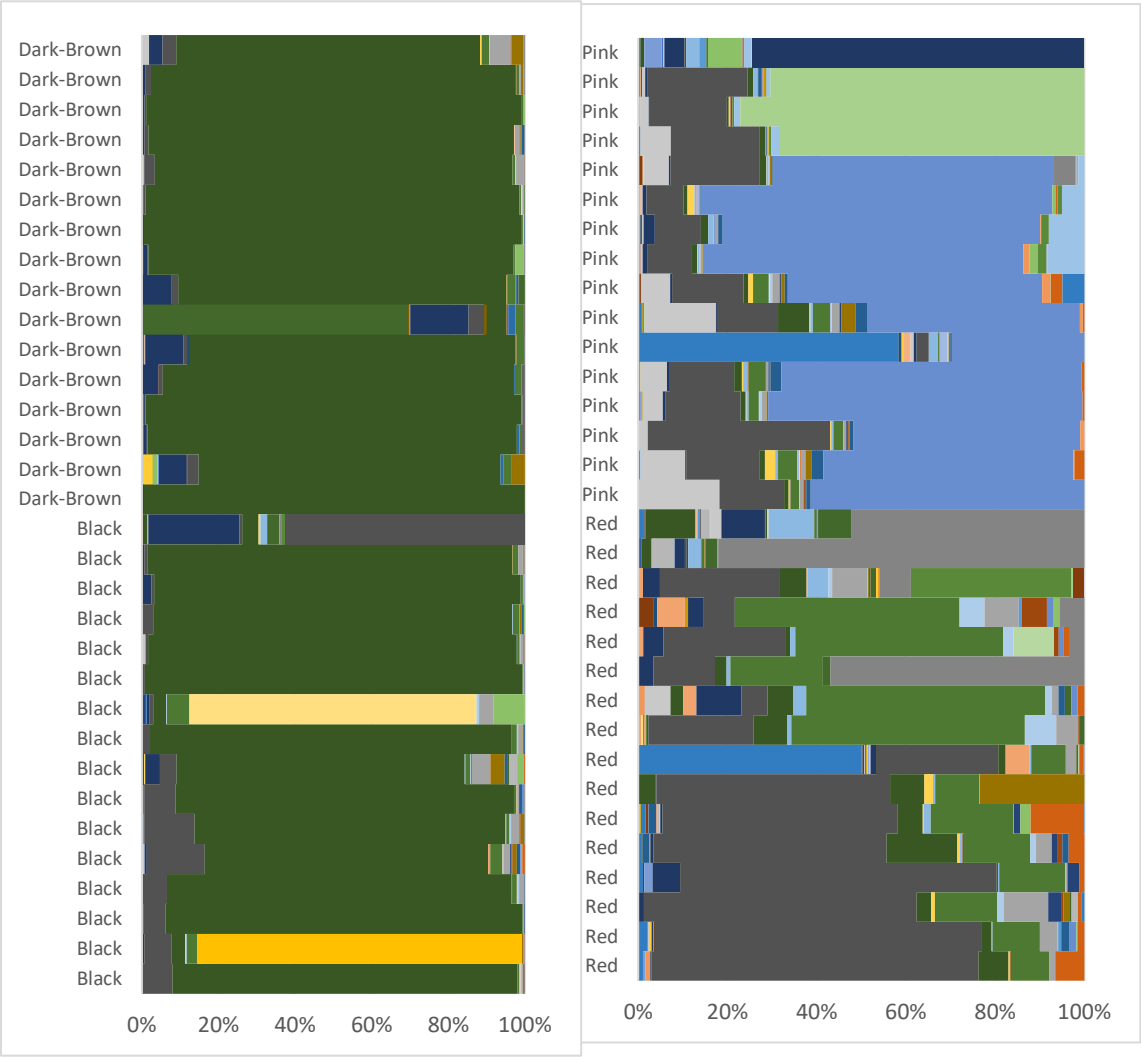


*Figure 41: Examples of homogenous and heterogeneous pollen loads as stack bars. Y-axis shows the color of the specific pollen load. X-axis shows the percentage of each taxa contributing to the specific pollen loads. Dominant taxa: Koelreuteria paniculata (darkgreen, left), Ligustrum lucidum (dark grey; right), Dipsacoideae (light-blue; right) and Scabiosa (turquoise; right).*

Dark-Brown and Black pollen loads have not only been found to be very homogeneous, but also to be nearly identical in their constitution. Pink and red pollen loads are found to be very heterogenous pollen loads. In fact, none of the 32 pollen loads of pink and red can be considered as homogeneous according to a specific homogeneity criterion of 85%

### 4.2.7. Results of the multiple pollen load samples

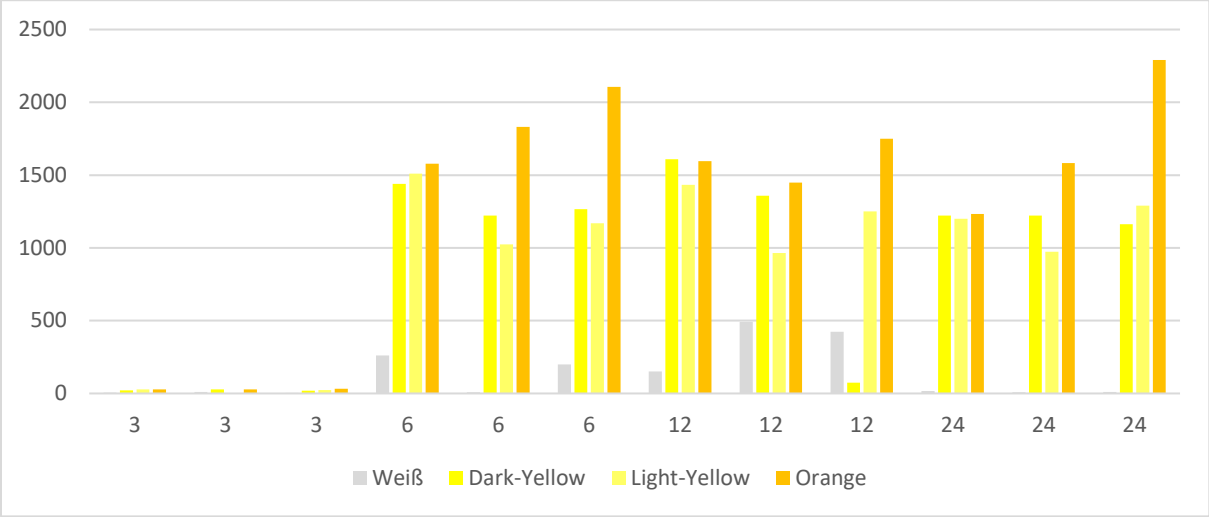


*Figure 42: Samples with multiple pollen loads with four different colors. Number of detected sequences depending on the amount of pollen and the color of the pollen load. The x-axis shows the number of pollen loads per well and the y-axis shows the number of detected sequences (n=38607).*

The graphic shows the results of the analysis with multiple pollen loads. The influence of different amounts of pollen on the number of detected sequences after Illumina sequencing was surveyed. For this purpose, the four most frequent color categories resulting from the morphological analysis were taken and 3, 6, 12 or 24 pollen loads were loaded per well. This was done per color and number of pollen loads with threefold repetition.

In a total 38607 sequences were detected in 48 samples. This results in an average of 804,3 sequences per sample. The distribution shows that there were hardly any reads for the samples that were loaded with 3 pollen loads (n=38607; f=229). This phenomenon is independent of the color. Furthermore, relatively few sequences could be detected for all pollen loads with the color white (n=38607; f=1590). The two colors light yellow (f=10865) and dark yellow (f=10646) resulted in almost identical numbers of reads. Most sequences were detected in pollen of orange color (f=15506).

The distribution clearly shows that there is no linear relationship between the pollen load number and the detected sequences. The doubling of pollen loads per sample does not lead to a doubling of sequences. Rather, from 6 pollen loads per sample onwards, a kind of plateau develops in which the sequence numbers oscillate between approximately 1200 and 2000 sequences. A numeric table with all samples, their color and measured values is given in the Appendix of this work.

# 5. Discussion

When discussing the homogeneity of pollen loads one topic plays a crucial role. The threshold value or homogeneity criterion. It is crucial for deciding whether a pollen load can be described as monofloral/homogenous or polyfloral/heterogenous. The results of this paper showed that there is a linear relationship between the threshold criterion and the amount of homogenous pollen loads. For this paper a threshold value of 15% was set, another study set it at 10% (Brodschneider *et al.*, 2018). The question behind this consideration was the share of contaminants (un)detected in a sample. Contamination can not only happen in the lab, but more often during the process of collecting pollen from pollen traps. The pollen lay very close to each other in the pollen trap and little contamination is relatively probable, because of the softness of the pollen loads. Still, they are entities themselves, but some pollen grains of microscopic levels can mix with other pollen loads. This changes after freezing because the pollen gets hard. To prevent contamination of the pollen, it would be best to remove the pollen loads directly from the bees' corbicula before they enter the hive. The authors of another study observed bees as they collected on *Eremochla ophiuroides*. They found that the pollen were 100% of this species and therefore absolutely homogenous (Jones, 2014). If this result is included in the analysis of the homogeneity of the pollen in this study, it seems likely that even more pollen loads would be homogeneous and the obtained result of 70 homogeneous pollen loads (n=192) should be considered high despite probable contamination.

The morphological results of this study showed that dark-yellow loads represent 47% and light-yellow 38% of the collected pollen. Together, these represented 85% of all pollen loads. The genetic analysis showed that dark-yellow and light-yellow pollen loads contained very similar plant species and were both mostly homogenous and can therefore be considered as identical. The color classification between dark-yellow and light-yellow is therefore redundant and unrewarding. Instead, I recommend characterizing them simply as yellow pollen and to neglect the brightness of the yellow pollen.

Black and brown pollen are also very identical and homogeneous. Of all pollen loads that the honeybees collected during the study period only 2091 loads (n=131481; black: f=540; dark-brown: f=1551) were black and dark-brown, which are approximately 1,6% of all pollen loads. Black and dark-brown pollen loads are mainly of *Koelreuteria paniculata.* The majority of the detected *Koelreuteria* sequences have been found in dark-brown and black pollen loads (n=25378, f=23309; 91,85%). In conclusion, this indicates that the dominance of the detected sequences of *Koelreuteria paniculata* has little to do with the actual reality of the collected plant species. K. paniculatea is overrepresented in the pollen color black and brown. But this does not reflect plant taxa diversity used by honey bees during the study.

In comparison to another study on the color of pollen loads(Brodschneider *et al.*, 2021), few rare colors were found in urban area, but more frequently in park-like surrounding. Rare colors, such as pink, red, purple and black were 5 to 10 times more frequent in the Prater than in the urban site of seventh district.

Furthermore, the same study points out that 2000 pollen weigh approximately 20 grams (Brodschneider *et al.*, 2021). This is different to the findings of my research. The average weight of 2000 pollen loads for the samples I collected is approximately 13,57 grams. This is a difference of 6,43 grams. The results of another paper, which deals specifically with the variation of the weight of pollen loads, shows similar results with 2000 pollen loads and 15,78 grams on average (García-García, Ortiz and Díez Dapena, 2004). The authors pointed out that the weight of the pollen loads varies significantly because of several factors. The pollen themselves differ in weight depending on the time of the day and the plant species, which emitted the pollen. Moreover the size of the pollen loads depends heavily on the foraging intensity of the honeybee (García-García, Ortiz and Díez Dapena, 2004).

The comparison of both ANOVAs shows that, the influence of the factor “site” is bigger than the influence of the factor “colony”. Nevertheless, the colony is a strong influence factor on the weight of the samples. Moreover, in this particular study only two sites were compared to each other. Therefore, to substantiate the suspicion that the site has a huge impact on the weight of the pollen samples other studies with bigger repetitions in sites are needed. It is similar with the results on the collection days. Unfortunately, the data for the survey were not evenly distributed. There are tendencies that the collected species are different depending on the collection day and especially also depending on the month. However, these results should always be thought with the background that for some days only a relatively small number of pollen loads were examined.

There are various reasons possible for the plateau in the numbers of reads in the analysis of multiple pollen loads. It was already noticeable during the DNA isolation that the pollen in the wells might clumped together with an increased number of pollen loads. The amount of 24 pollen grains overloaded the wells, so that the DNA could not adhere well to the Econospin Columns. Another possible reason is that the washing step with ethanol or the final elution step did not work sufficiently. Another possible explanation could be that we saw a saturation and that increased number of pollen did not result in an increased number of reads. More specific studies with different amounts of pollen are requested.

# 6. Conclusion

The weight of pollen loads is related to the number of pollen grains and this differs significantly depending on the collection performance of the specific colony. There are also signs that the location of the colonies plays an important role. Pollen loads were relatively often homogeneous. Their homogeneity also depended on their color. It became also clear that a color definition is not purposeful for all colors. Colors such as light yellow and dark yellow as well as dark brown and black are to be regarded as identical.

In order to study the homogeneity and the contents of pollen loads in more detail, further studies are needed, which in the best case also work with a high number of locations.

# 7. List of tables/Appendix

*Table 9: Weight of the pollen collected by the honeybee colonies per day, weight of the taken samples and the resulting conversion factor. 56 samples were taken. The total weight of all collected pollen is 892,31 g.*

| | *Weight* | *Colony 1* | *Colony 2* | *Colony 3* | *Colony 4* | *Colony 5* | *Colony 6* | *Colony 7* | *Colony 8* | *Total (day)* |
|---|---|---|---|---|---|---|---|---|---|---|
| *28. Jun* | *Total* | 2,75 | 10 | 26,75 | 45,16 | 30,47 | 12,34 | 29,95 | 14,43 | **171,85** |
| | *Sample* | 2,75 | 3,11 | 2,74 | 2,16 | 2,97 | 1,84 | 1,95 | 1,43 | |
| | *Factor* | 1 | 3,22 | 9,76 | 20,91 | 10,26 | 6,71 | 15,36 | 10,09 | |
| *29. Jun* | *Total* | 4,54 | 12,11 | 25,8 | 35,45 | 16,44 | 7,09 | 25,47 | 7,32 | **134,22** |
| | *Sample* | 4,54 | 3,52 | 2,8 | 2,45 | 2,44 | 2,09 | 2,47 | 2,32 | |
| | *Factor* | 1 | 3,44 | 9,21 | 14,47 | 6,74 | 3,39 | 10,31 | 3,16 | |
| *30. Jun* | *Total* | 1,27 | 3,64 | 11,47 | 16,53 | 11,01 | 3,45 | 3,6 | 3,91 | **54,88** |
| | *Sample* | 1,27 | 3,64 | 3,47 | 3,53 | 3,01 | 3,45 | 3,6 | 3,91 | |
| | *Factor* | 1 | 1 | 3,31 | 4,68 | 3,66 | 1 | 1 | 1 | |
| *01. Jul* | *Total* | 1,02 | 2,54 | 33,02 | 29,42 | 11,04 | 2,63 | 19,31 | 4,87 | **103,85** |
| | *Sample* | 1,02 | 2,54 | 4,02 | 4,42 | 3,04 | 2,63 | 4,31 | 4,87 | |
| | *Factor* | 1 | 1 | 8,21 | 6,66 | 3,63 | 1 | 4,48 | 1 | |
| *23. Jul* | *Total* | 3,74 | 6,17 | 6,33 | 21,3 | 20,51 | 19,85 | 9,46 | 18,1 | **105,46** |
| | *Sample* | 3,74 | 6,17 | 6,33 | 6,24 | 5,41 | 6,48 | 6,18 | 6,48 | |
| | *Factor* | 1 | 1 | 1 | 3,41 | 3,79 | 3,06 | 1,53 | 2,79 | |
| *24. Jul* | *Total* | 2,87 | 15,35 | 18,42 | 33,85 | 45,02 | 21,06 | 13,93 | 23,99 | **174,49** |
| | *Sample* | 2,87 | 5,92 | 6,08 | 6,27 | 5,98 | 6,69 | 6,07 | 6,38 | |
| | *Factor* | 1 | 2,59 | 3,03 | 5,4 | 7,53 | 3,15 | 2,29 | 3,76 | |
| *25. Jul* | *Total* | 5,93 | 18,14 | 17,81 | 32,24 | 17,29 | 17,19 | 13,44 | 25,52 | **147,56** |
| | *Sample* | 5,93 | 6,11 | 6,1 | 6,1 | 6,1 | 6,1 | 6,1 | 6,1 | |
| | *Factor* | 1 | 2,97 | 2,92 | 5,29 | 2,83 | 2,82 | 2,2 | 4,18 | |
| *Total (colony)* | | **22,12** | **67,95** | **139,6** | **213,95** | **151,78** | **83,61** | **115,16** | **98,14** | **892,31** |

- Koelreuteria
- Dipsacoideae
- 419Ruca-P|Rubus
- Hypericum
- Ipomea
- Hydrangea
- 508Cyse-P|Centaurea
- Gaura
- Ailanthus
- 1104Atbe-P|Atropa
- Acer
- Dahlia
- Cucurbita
- Quercus
- 136Aseu-P|Asarum
- 1275Arto-P|Arctium
- 1375Cazo-P|Campanula
- 1298Altu-P|Allium
- 337Gesa-P|Geranium
- 954Puof-P|Pulmonaria
- 802Gali-P|Aster
- 265Relu-P|Reseda
- 147Pore-P|Potentilla
- 851Plme-P|Plantago
- 1160Vear-P|Veronica
- 312Ansy-P|Anthriscus
- Firmiana
- 464Tapa-P|Tanacetum
- Hemerocallis
- Buddleja
- Arnebia
- Malva
- 1055Rosa-P|Rosa
- Nepeta
- SjaponicaPollen
- 494Sani-P|Sambucus
- Sedum
- 1367Orvu-P|Origanum
- OcimumTenuiflorum
- 1391Acal-P|Acinos
- Magnolia
- Lilium
- 324Dica-P|Dianthus
- Lagersoemia_indicaUF006
- 572Ropa-P|Rorippa
- 1017Scpu-P|Scorzonera
- Mespilus
- 282Vech-P|Veronica
- Robinia
- 1386Powu-P|Potentilla
- 1395Veor-P|Veronica
- Portulaca
- Parthenocissus_quinquefoliaMST10p
- 376Cest-P|Centaurea
- Hosta
- 1292Mymu-P|Mycelis
- Phacelia
- PinusDensiflora
- 88Scau-P|Scorzonera
- Althaea
- PinusYunnanensisM00874:404:000000(
- 1110Alsc-P|Allium
- 139Chma-P|Chelidonium
- Duranta
- 64Gani-P|Galanthus
- 1285Seti-P|Serratula
- 394Saeb-P|Sambucus
- Brassica
- 771Chst-P|Chenopodium
- UFL006SpecM00874:404:000000000-JP
- 536Stsy-P|Stachys
- 675Scum-P|Scrophularia
- 1319Hivi-P|Hieracium
- 1132Dafu-P|Dactylorhiza
- 1015Phor-P|Phyteuma
- 673Dece-P|Avenella
- 1306Gesy-P|Geranium
- 479Saal-P|Salix
- SedumAlbum
- Echinacea
- Tilia
- 863Scno-P|Scrophularia
- PapaverRhoeas
- 498Gero-P|Geranium
- 280Plla-P|Plantago
- 1270Hisp-P|Hieracium
- Dryopteris
- PinusSylvestris
- 1035Schu-P|Scorzonera
- TaraxacumOfficinale
- 542Cara-P|Campanula
- SecurigeraVaria
- Jasminum
- 278Alpr-P|Alopecurus
- 1164Crbi-P|Crepis
- 1236Sara-P|Sambucus
- 1290Adal-P|Adenostyles
- 662Ruid-P|Rubus
- HumulusLupulus
- 684Plma-P|Plantago
- Malus
- 1136Cyal-P|Cystopteris
- 1030Vilu-P|Vicia
- Wisteria
- Ligustrum_lucidumUF006
- Pelargonium
- Scabiosa
- Sonchus
- 109Glhe-P|Glechoma
- Catalpa
- Pinus
- 446Gesy-P|Geranium
- Potentilla
- 515Cytr-P|Centaurea
- Dipsacus
- 519Brsy-P|Brachypodium
- 208Vatr-P|Valeriana
- 389Coma-P|Convallaria
- Cepalanthus_occidentalisOrGlabratusUF006
- AesculusWangii
- 822Coda-P|Cotoneaster
- 1302Lomu-P|Lolium
- 1111Prla-P|Prunella
- 1207Doge-P|Dorycnium
- Daucus
- 845Sagl-P|Salvia
- 450Gaer-P|Galium
- 1401Catr-P|Campanula
- 1051Chfi-P|Chenopodium
- Vitex_specMST10p

*Figure 43: Legend of figure 31, 33 , 35 and 37.*

*Table 10: Detected plant species and their abundance in the twelve color categories. Plate 1 and Plate 2. To maintain readability and to actual being able to show the data in this work I had to split up the "mastertable" into different tables with subtotals.*

| | ***White*** | ***Grey*** | ***Green*** | ***Light-Yellow*** | ***Dark-Yellow*** | ***Orange*** | ***Pink*** | ***Red*** | ***Purple*** | ***Light-Brown*** | ***Dark-Brown*** | ***Black*** | ***Total*** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Koelreuteria* | 107 | 63 | 18 | 347 | 110 | 198 | 188 | 370 | 76 | 592 | 13475 | 9834 | 25378 |
| *Parthenocissus_quinquefolia* | 118 | 496 | 82 | 6509 | 6138 | 269 | 99 | 254 | 799 | 170 | 483 | 253 | 15670 |
| *Ligustrum lucidum* | 266 | 43 | 372 | 1280 | 449 | 759 | 2022 | 2952 | 166 | 480 | 215 | 646 | 9650 |
| *Firmiana* | 125 | 6 | 19 | 2045 | 2234 | 131 | 187 | 1077 | 2534 | 130 | 154 | 189 | 8831 |
| *SedumAlbum* | 2 | 0 | 1580 | 0 | 2 | 6 | 12 | 48 | 17 | 4858 | 1 | 6 | 6532 |
| *Dipsacoideae* | 16 | 36 | 2 | 5 | 200 | 1 | 5974 | 13 | 119 | 6 | 1 | 12 | 6385 |
| *Centaurea* | 1 | 5 | 3 | 1 | 2 | 1 | 193 | 597 | 4609 | 3 | 0 | 2 | 5417 |
| *Pelargonium* | 0 | 0 | 4 | 4 | 3 | 82 | 47 | 1617 | 1 | 3479 | 15 | 10 | 5262 |
| *Tanacetum* | 16 | 6 | 13 | 13 | 5 | 4527 | 13 | 13 | 19 | 1 | 5 | 2 | 4633 |
| *Echinacea* | 1310 | 7 | 37 | 0 | 10 | 484 | 88 | 226 | 57 | 30 | 0 | 22 | 2271 |
| *Rubus* | 12 | 2062 | 1 | 7 | 9 | 7 | 10 | 26 | 3 | 9 | 2 | 0 | 2148 |
| *Hosta* | 0 | 0 | 0 | 0 | 0 | 1993 | 0 | 0 | 1 | 0 | 0 | 0 | 1994 |
| *Scabiosa* | 0 | 0 | 0 | 0 | 0 | 0 | 1953 | 2 | 0 | 0 | 0 | 0 | 1955 |
| *Hemerocallis* | 7 | 1 | 1 | 1 | 0 | 1664 | 0 | 9 | 5 | 36 | 0 | 27 | 1751 |
| *Hypericum* | 1 | 21 | 23 | 115 | 502 | 114 | 82 | 22 | 117 | 560 | 27 | 82 | 1666 |
| *Mycelis* | 5 | 1 | 4 | 1 | 0 | 1576 | 5 | 12 | 9 | 2 | 0 | 4 | 1619 |
| *Sonchus* | 7 | 5 | 5 | 2 | 1 | 1166 | 53 | 30 | 18 | 0 | 7 | 6 | 1300 |
| *Buddleja* | 391 | 4 | 88 | 41 | 22 | 26 | 8 | 149 | 175 | 181 | 21 | 25 | 1131 |
| *Tilia* | 0 | 1 | 0 | 684 | 0 | 19 | 54 | 163 | 19 | 153 | 0 | 4 | 1097 |
| *Scrophularia* | 9 | 2 | 3 | 3 | 1 | 1034 | 5 | 0 | 4 | 3 | 17 | 0 | 1081 |
| *Ipomea* | 383 | 4 | 0 | 3 | 1 | 46 | 59 | 168 | 13 | 336 | 2 | 14 | 1029 |
| *Phacelia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 918 | 0 | 0 | 0 | 918 |
| *Glechoma* | 7 | 5 | 0 | 1 | 1 | 50 | 718 | 37 | 37 | 2 | 30 | 15 | 903 |
| *Arnebia* | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 339 | 0 | 1 | 526 | 868 |
| *Papaver rhoeas* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 0 | 0 | 861 | 866 |
| *Hydrangea* | 0 | 736 | 0 | 0 | 0 | 1 | 6 | 0 | 1 | 20 | 0 | 0 | 764 |
| *Pinus densiflora* | 54 | 4 | 3 | 5 | 11 | 42 | 77 | 208 | 3 | 41 | 116 | 176 | 740 |

| | White | Grey | Green | Light-Yellow | Dark-Yellow | Orange | Pink | Red | Purple | Light-Brown | Dark-Brown | Black | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Catalpa* | 54 | 1 | 7 | 107 | 68 | 86 | 109 | 21 | 22 | 92 | 15 | 37 | 619 |
| *Malva* | 0 | 0 | 0 | 0 | 0 | 0 | 560 | 0 | 0 | 0 | 0 | 2 | 562 |
| *Centaurea* | 367 | 1 | 0 | 0 | 0 | 149 | 0 | 4 | 2 | 0 | 1 | 0 | 524 |
| *Scorzonera* | 0 | 495 | 0 | 0 | 0 | 1 | 4 | 1 | 7 | 1 | 0 | 1 | 510 |
| *Rosa* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 447 | 2 | 0 | 458 |
| *Pinus* | 55 | 2 | 5 | 14 | 7 | 0 | 62 | 154 | 0 | 13 | 93 | 73 | 478 |
| *Plantago* | 20 | 28 | 1 | 2 | 0 | 65 | 8 | 205 | 88 | 1 | 3 | 10 | 431 |
| *Gaura* | 0 | 0 | 0 | 4 | 333 | 4 | 45 | 0 | 0 | 40 | 0 | 0 | 426 |
| *Althaea* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 412 | 412 |
| *Geranium* | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 4 | 358 | 0 | 0 | 0 | 366 |
| *Geranium* | 0 | 0 | 0 | 8 | 18 | 0 | 0 | 0 | 0 | 0 | 509 | 0 | 535 |
| *Nepeta* | 0 | 0 | 0 | 0 | 0 | 0 | 238 | 0 | 0 | 0 | 0 | 0 | 238 |
| *Hieracium* | 0 | 0 | 0 | 0 | 0 | 192 | 1 | 7 | 6 | 2 | 0 | 0 | 208 |
| *Ailanthus* | 3 | 8 | 0 | 71 | 39 | 11 | 6 | 3 | 1 | 7 | 9 | 3 | 161 |
| *Pinus yunnanensis* | 9 | 4 | 0 | 2 | 5 | 3 | 13 | 72 | 0 | 18 | 4 | 19 | 149 |
| *Potentilla* | 0 | 0 | 114 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 114 |
| *Styphnolobium japonicum* | 0 | 0 | 32 | 45 | 30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 107 |
| *Dryopteris* | 0 | 3 | 2 | 11 | 0 | 1 | 0 | 58 | 14 | 9 | 0 | 5 | 103 |
| *Atropa* | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 79 | 4 | 1 | 0 | 2 | 89 |
| *Centaurea* | 0 | 1 | 0 | 0 | 0 | 19 | 2 | 7 | 40 | 0 | 0 | 0 | 69 |
| *Allium* | 0 | 17 | 0 | 0 | 0 | 18 | 0 | 0 | 0 | 14 | 29 | 0 | 78 |
| *Sambucus* | 6 | 0 | 0 | 2 | 12 | 2 | 5 | 27 | 0 | 1 | 2 | 1 | 58 |
| *Pinus sylvestris* | 1 | 2 | 0 | 2 | 6 | 1 | 6 | 26 | 0 | 5 | 2 | 6 | 57 |
| *Acer* | 0 | 0 | 0 | 0 | 0 | 0 | 43 | 5 | 0 | 0 | 0 | 0 | 48 |
| *Chelidonium* | 0 | 2 | 0 | 0 | 0 | 0 | 32 | 9 | 1 | 0 | 2 | 0 | 46 |
| *Dipsacus* | 8 | 0 | 1 | 0 | 0 | 1 | 23 | 1 | 9 | 0 | 0 | 0 | 43 |
| *Scorzonera* | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 4 | 31 | 0 | 0 | 39 |
| *Dahlia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 38 | 0 | 0 | 38 |
| *Brachypodium* | 1 | 5 | 0 | 0 | 0 | 0 | 0 | 4 | 21 | 0 | 0 | 3 | 34 |
| *Sedum* | 0 | 2 | 0 | 2 | 5 | 0 | 0 | 0 | 0 | 8 | 25 | 0 | 42 |
| *Origanum* | 0 | 3 | 0 | 0 | 9 | 2 | 7 | 0 | 6 | 4 | 0 | 0 | 31 |
| *Taraxacum officinale* | 0 | 1 | 1 | 0 | 0 | 3 | 12 | 5 | 4 | 5 | 0 | 0 | 31 |
| *Cucurbita* | 0 | 21 | 0 | 0 | 0 | 0 | 0 | 2 | 5 | 0 | 0 | 0 | 28 |

| | White | Grey | Green | Light-Yellow | Dark-Yellow | Orange | Pink | Red | Purple | Light-Brown | Dark-Brown | Black | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Duranta* | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 3 | 1 | 0 | 29 | 0 | 35 |
| *Galanthus* | 6 | 1 | 1 | 3 | 2 | 3 | 0 | 0 | 0 | 7 | 1 | 0 | 24 |
| *Valeriana* | 0 | 0 | 0 | 0 | 22 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 23 |
| *Ocimum tenuiflorum* | 0 | 6 | 0 | 4 | 0 | 1 | 2 | 2 | 3 | 1 | 0 | 2 | 21 |
| *Campanula* | 5 | 1 | 0 | 0 | 5 | 2 | 5 | 0 | 1 | 0 | 1 | 0 | 20 |
| *Quercus* | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 4 | 0 | 2 | 7 | 16 |
| *Serratula* | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 3 | 6 | 0 | 0 | 15 |
| *Acinos* | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 4 | 0 | 4 | 0 | 1 | 13 |
| *Securigera Varia* | 0 | 0 | 0 | 1 | 0 | 9 | 0 | 1 | 0 | 2 | 0 | 0 | 13 |
| *Sambucus* | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 12 |
| *Asarum* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 9 | 2 | 0 | 12 |
| *Convallaria* | 3 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 13 |
| *Magnolia* | 0 | 0 | 0 | 0 | 0 | 3 | 8 | 0 | 0 | 0 | 0 | 0 | 11 |
| *Jasminum* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 11 |
| *Arctium* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 8 | 0 | 0 | 0 | 10 |
| *Brassica* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 10 |
| *Lilium* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 10 |
| *Aesculus wangii* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 8 | 0 | 0 | 1 | 0 | 10 |
| *Cepalanthus occidentalis* | 0 | 2 | 0 | 2 | 1 | 0 | 0 | 0 | 1 | 0 | 5 | 0 | 11 |
| *Campanula* | 0 | 1 | 0 | 4 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| *Alopecurus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 7 | 8 |
| *Chenopodium* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 1 | 1 | 8 |
| *Cotoneaster* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 2 | 0 | 1 | 7 |
| *Dianthus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 | 0 | 2 | 7 |
| *Crepis* | 0 | 1 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 1 | 7 |
| *Allium* | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 1 | 1 | 0 | 6 |
| *Lolium* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 2 | 1 | 0 | 0 | 5 |
| *UFL006Spec* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 4 | 0 | 6 |
| *Sambucus* | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 4 |
| *Geranium* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 4 |
| *Prunella* | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 4 |
| *Adenostyles* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 4 |
| *Pulmonaria* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 0 | 4 |

| | *White* | *Grey* | *Green* | *Light-Yellow* | *Dark-Yellow* | *Orange* | *Pink* | *Red* | *Purple* | *Light-Brown* | *Dark-Brown* | *Black* | *Total* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Rorippa* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 4 |
| *Stachys* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 4 |
| *Lagersoemia indica* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 5 |
| *Dorycnium* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 |
| *Scorzonera* | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 3 |
| *Rubus* | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| *Aster* | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 3 |
| *Hieracium* | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| *Daucus* | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 3 |
| *Humulus lupulus* | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 3 |
| *Scrophularia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 0 | 3 |
| *Reseda* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| *Dactylorhiza* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 |
| *Salvia* | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Veronica* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| *Plantago* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 |
| *Potentilla* | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Galium* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| *Robinia* | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Mespilus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 3 |
| *Phyteuma* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 |
| *Malus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 |
| *Plantago* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *Avenella* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Campanula* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Cystopteris* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| *Veronica* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| *Chenopodium* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *Veronica* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Vicia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *Anthriscus* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Salix* | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Vitex spec* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |

| | *White* | *Grey* | *Green* | *Light-Yellow* | *Dark-Yellow* | *Orange* | *Pink* | *Red* | *Purple* | *Light-Brown* | *Dark-Brown* | *Black* | *Total* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Portulaca* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| *Wisteria* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| *Potentilla* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| *Geranium* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |
| Sum | **3377** | **4137** | **2426** | **11362** | **10274** | **14792** | **13065** | **8753** | **10709** | **11887** | **15340** | **13316** | **119438** |

*Table 11: Detected plant species and their abundance in the eight honeybee colonies. Plate 1 and Plate 2.*

| | **1** | **2** | **4** | **5** | **6** | **7** | **8** | **Total** |
|---|---|---|---|---|---|---|---|---|
| *Koelreuteria* | 311 | 8373 | 75 | 85 | 51 | 74 | 16409 | 25378 |
| *Parthenocissus_quinquefoliaMST10p* | 9206 | 4411 | 604 | 880 | 9 | 38 | 522 | 15670 |
| *Ligustrum_lucidumUF006* | 1131 | 2362 | 77 | 132 | 576 | 300 | 5072 | 9650 |
| *Firmiana* | 165 | 4489 | 35 | 2508 | 0 | 448 | 1186 | 8831 |
| *SedumAlbum* | 18 | 4875 | 1 | 5 | 2 | 0 | 1631 | 6532 |
| *Dipsacoideae* | 16 | 217 | 122 | 18 | 6 | 4 | 6002 | 6385 |
| *376Cest-P\|Centaurea* | 0 | 11 | 4610 | 6 | 4 | 0 | 786 | 5417 |
| *Pelargonium* | 8 | 3535 | 0 | 30 | 0 | 109 | 1580 | 5262 |
| *464Tapa-P\|Tanacetum* | 20 | 34 | 4 | 3900 | 8 | 5 | 662 | 4633 |
| *Echinacea* | 2 | 868 | 26 | 76 | 13 | 30 | 1256 | 2271 |
| *419Ruca-P\|Rubus* | 893 | 690 | 3 | 6 | 514 | 21 | 21 | 2148 |
| *Hosta* | 1994 | 0 | 0 | 0 | 0 | 0 | 0 | 1994 |
| *Scabiosa* | 0 | 0 | 0 | 2 | 1951 | 2 | 0 | 1955 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Hemerocallis* | 0 | 1712 | 1 | 9 | 0 | 0 | 29 | 1751 |
| *Hypericum* | 650 | 672 | 112 | 72 | 4 | 2 | 154 | 1666 |
| *1292Mymu-P\|Mycelis* | 4 | 1078 | 4 | 6 | 1 | 1 | 525 | 1619 |
| *Sonchus* | 0 | 19 | 16 | 9 | 2 | 0 | 1254 | 1300 |
| *Buddleja* | 3 | 760 | 2 | 200 | 6 | 14 | 146 | 1131 |
| *Tilia* | 722 | 153 | 0 | 6 | 12 | 163 | 41 | 1097 |
| *863Scno-P\|Scrophularia* | 1027 | 33 | 2 | 11 | 0 | 0 | 8 | 1081 |
| *Ipomea* | 9 | 344 | 9 | 8 | 5 | 3 | 651 | 1029 |
| *Phacelia* | 918 | 0 | 0 | 0 | 0 | 0 | 0 | 918 |
| *109Glhe-P\|Glechoma* | 4 | 6 | 11 | 73 | 77 | 0 | 732 | 903 |
| *Arnebia* | 0 | 0 | 339 | 0 | 0 | 0 | 529 | 868 |
| *PapaverRhoeas* | 0 | 0 | 3 | 0 | 0 | 0 | 863 | 866 |
| *Hydrangea* | 2 | 20 | 168 | 1 | 568 | 0 | 5 | 764 |
| *PinusDensiflora* | 16 | 112 | 3 | 3 | 4 | 74 | 528 | 740 |
| *Catalpa* | 180 | 252 | 1 | 3 | 9 | 1 | 173 | 619 |
| *Malva* | 0 | 0 | 0 | 562 | 0 | 0 | 0 | 562 |
| *498Gero-P\|Geranium* | 0 | 535 | 0 | 0 | 0 | 0 | 0 | 535 |
| *508Cyse-P\|Centaurea* | 0 | 517 | 3 | 0 | 0 | 0 | 4 | 524 |
| *88Scau-P\|Scorzonera* | 0 | 4 | 497 | 3 | 2 | 0 | 4 | 510 |
| *Pinus* | 0 | 149 | 0 | 0 | 2 | 0 | 327 | 478 |
| *1055Rosa-P\|Rosa* | 0 | 449 | 0 | 0 | 1 | 2 | 6 | 458 |
| *280Plla-P\|Plantago* | 2 | 55 | 76 | 66 | 1 | 2 | 229 | 431 |
| *Gaura* | 3 | 378 | 0 | 0 | 0 | 0 | 45 | 426 |
| *Althaea* | 0 | 0 | 0 | 412 | 0 | 0 | 0 | 412 |
| *446Gesy-P\|Geranium* | 356 | 0 | 1 | 2 | 0 | 4 | 3 | 366 |
| *Nepeta* | 0 | 0 | 0 | 11 | 34 | 0 | 193 | 238 |
| *1270Hisp-P\|Hieracium* | 0 | 156 | 6 | 2 | 0 | 0 | 44 | 208 |
| *Ailanthus* | 104 | 39 | 2 | 3 | 1 | 1 | 11 | 161 |
| *PinusYunnanensisM00874:404:000000000-JP357:1:2113:10871:13369* | 6 | 22 | 0 | 0 | 4 | 52 | 65 | 149 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Potentilla* | 0 | 114 | 0 | 0 | 0 | 0 | 0 | 114 |
| *SjaponicaPollen* | 74 | 33 | 0 | 0 | 0 | 0 | 0 | 107 |
| *Dryopteris* | 13 | 12 | 11 | 3 | 1 | 1 | 62 | 103 |
| *1104Atbe-P\|Atropa* | 2 | 1 | 0 | 7 | 0 | 0 | 79 | 89 |
| *1110Alsc-P\|Allium* | 18 | 43 | 0 | 0 | 17 | 0 | 0 | 78 |
| *515Cytr-P\|Centaurea* | 0 | 19 | 40 | 0 | 1 | 0 | 9 | 69 |
| *494Sani-P\|Sambucus* | 12 | 7 | 0 | 0 | 2 | 1 | 36 | 58 |
| *PinusSylvestris* | 8 | 7 | 2 | 0 | 0 | 13 | 27 | 57 |
| *Acer* | 0 | 0 | 0 | 0 | 0 | 0 | 48 | 48 |
| *139Chma-P\|Chelidonium* | 0 | 2 | 0 | 31 | 2 | 0 | 11 | 46 |
| *Dipsacus* | 1 | 1 | 7 | 3 | 0 | 0 | 31 | 43 |
| *Sedum* | 0 | 40 | 2 | 0 | 0 | 0 | 0 | 42 |
| *1035Schu-P\|Scorzonera* | 0 | 32 | 5 | 0 | 0 | 0 | 2 | 39 |
| *Dahlia* | 0 | 38 | 0 | 0 | 0 | 0 | 0 | 38 |
| *Duranta* | 0 | 30 | 0 | 1 | 0 | 0 | 4 | 35 |
| *519Brsy-P\|Brachypodium* | 0 | 0 | 21 | 7 | 0 | 0 | 6 | 34 |
| *1367Orvu-P\|Origanum* | 1 | 14 | 1 | 6 | 0 | 0 | 9 | 31 |
| *TaraxacumOfficinale* | 0 | 6 | 5 | 14 | 0 | 1 | 5 | 31 |
| *Cucurbita* | 0 | 0 | 26 | 0 | 0 | 2 | 0 | 28 |
| *64Gani-P\|Galanthus* | 5 | 8 | 0 | 6 | 1 | 0 | 4 | 24 |
| *208Vatr-P\|Valeriana* | 1 | 22 | 0 | 0 | 0 | 0 | 0 | 23 |
| *OcimumTenuiflorum* | 4 | 4 | 0 | 3 | 6 | 0 | 4 | 21 |
| *542Cara-P\|Campanula* | 6 | 8 | 1 | 0 | 0 | 0 | 5 | 20 |
| *Quercus* | 2 | 1 | 5 | 0 | 0 | 0 | 8 | 16 |
| *1285Seti-P\|Serratula* | 0 | 6 | 3 | 0 | 0 | 0 | 6 | 15 |
| *389Coma-P\|Convallaria* | 0 | 13 | 0 | 0 | 0 | 0 | 0 | 13 |
| *1391Acal-P\|Acinos* | 0 | 8 | 0 | 0 | 0 | 0 | 5 | 13 |
| *SecurigeraVaria* | 1 | 2 | 0 | 9 | 0 | 1 | 0 | 13 |
| *136Aseu-P\|Asarum* | 0 | 12 | 0 | 0 | 0 | 0 | 0 | 12 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *394Saeb-P\|Sambucus* | 0 | 0 | 9 | 2 | 0 | 1 | 0 | 12 |
| *Cepalanthus_occidentalisOrGlabratusUF006* | 0 | 8 | 2 | 1 | 0 | 0 | 0 | 11 |
| *Magnolia* | 0 | 3 | 0 | 0 | 5 | 0 | 3 | 11 |
| *Jasminum* | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 11 |
| *1275Arto-P\|Arctium* | 0 | 0 | 2 | 6 | 0 | 0 | 2 | 10 |
| *Brassica* | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| *AesculusWangii* | 0 | 0 | 0 | 0 | 0 | 8 | 2 | 10 |
| *Lilium* | 0 | 10 | 0 | 0 | 0 | 0 | 0 | 10 |
| *278Alpr-P\|Alopecurus* | 0 | 1 | 0 | 0 | 0 | 0 | 7 | 8 |
| *1375Cazo-P\|Campanula* | 6 | 2 | 0 | 0 | 0 | 0 | 0 | 8 |
| *771Chst-P\|Chenopodium* | 0 | 0 | 0 | 1 | 0 | 0 | 7 | 8 |
| *822Coda-P\|Cotoneaster* | 0 | 3 | 0 | 1 | 0 | 0 | 3 | 7 |
| *324Dica-P\|Dianthus* | 0 | 5 | 0 | 2 | 0 | 0 | 0 | 7 |
| *1164Crbi-P\|Crepis* | 0 | 5 | 1 | 0 | 0 | 0 | 1 | 7 |
| *1298Altu-P\|Allium* | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 6 |
| *UFL006SpecM00874:404:000000000-JP357:1:1106:6465:5591* | 1 | 4 | 0 | 0 | 0 | 0 | 1 | 6 |
| *1302Lomu-P\|Lolium* | 2 | 2 | 0 | 0 | 0 | 0 | 1 | 5 |
| *Lagersoemia_indicaUF006* | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 5 |
| *1236Sara-P\|Sambucus* | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 4 |
| *337Gesa-P\|Geranium* | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| *536Stsy-P\|Stachys* | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 4 |
| *1111Prla-P\|Prunella* | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 |
| *572Ropa-P\|Rorippa* | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 4 |
| *1290Adal-P\|Adenostyles* | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 4 |
| *954Puof-P\|Pulmonaria* | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 4 |
| *675Scum-P\|Scrophularia* | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 3 |
| *1207Doge-P\|Dorycnium* | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 3 |
| *1017Scpu-P\|Scorzonera* | 0 | 0 | 1 | 2 | 0 | 0 | 0 | 3 |
| *662Ruid-P\|Rubus* | 0 | 2 | 0 | 1 | 0 | 0 | 0 | 3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *802Gali-P\|Aster* | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 3 |
| *1319Hivi-P\|Hieracium* | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| *Daucus* | 0 | 2 | 1 | 0 | 0 | 0 | 0 | 3 |
| *Mespilus* | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 3 |
| *HumulusLupulus* | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 3 |
| *265Relu-P\|Reseda* | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| *1132Dafu-P\|Dactylorhiza* | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 2 |
| *845Sagl-P\|Salvia* | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| *282Vech-P\|Veronica* | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 |
| *684Plma-P\|Plantago* | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| *147Pore-P\|Potentilla* | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *1015Phor-P\|Phyteuma* | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2 |
| *450Gaer-P\|Galium* | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Robinia* | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Malus* | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| *851Plme-P\|Plantago* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| *673Dece-P\|Avenella* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *1401Catr-P\|Campanula* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *1386Powu-P\|Potentilla* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *1136Cyal-P\|Cystopteris* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *1160Vear-P\|Veronica* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *1306Gesy-P\|Geranium* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| *1051Chfi-P\|Chenopodium* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| *1395Veor-P\|Veronica* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| *1030Vilu-P\|Vicia* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| *312Ansy-P\|Anthriscus* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *479Saal-P\|Salix* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| *Vitex_specMST10p* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| *Portulaca* | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Wisteria* | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| | **17948** | **37891** | **6969** | **9222** | **3905** | **1394** | **42109** | **119438** |

*Table 12:Detected plant species and their abundance on six collecting dates. Plate 1 and Plate 2.*

| ***Species*** | **28.06.2021** | **29.06.2021** | **30.06.2021** | **01.07.2021** | **23.07.2021** | **25.07.2021** | **Total** |
|---|---|---|---|---|---|---|---|
| *Koelreuteria* | 6375 | 14328 | 3904 | 645 | 119 | 7 | **25378** |
| *Parthenocissus quinquefolia* | 12442 | 1125 | 276 | 186 | 1570 | 71 | **15670** |
| *Ligustrum_lucidumUF006* | 2360 | 902 | 4711 | 1241 | 99 | 337 | **9650** |
| *Firmiana* | 2325 | 2456 | 1019 | 474 | 2554 | 3 | **8831** |
| *SedumAlbum* | 4875 | 1568 | 64 | 17 | 1 | 7 | **6532** |
| *Dipsacoideae* | 17 | 2631 | 3574 | 21 | 142 | 0 | **6385** |
| *376Cest-P\|Centaurea* | 4 | 2 | 772 | 4 | 4630 | 5 | **5417** |
| *Pelargonium* | 38 | 65 | 60 | 3585 | 1513 | 1 | **5262** |
| *464Tapa-P\|Tanacetum* | 3908 | 28 | 648 | 27 | 21 | 1 | **4633** |
| *Echinacea* | 14 | 1024 | 862 | 72 | 245 | 54 | **2271** |
| *419Ruca-P\|Rubus* | 901 | 18 | 7 | 1214 | 8 | 0 | **2148** |
| *Hosta* | 1994 | 0 | 0 | 0 | 0 | 0 | **1994** |
| *Scabiosa* | 0 | 0 | 0 | 1953 | 0 | 2 | **1955** |
| *Hemerocallis* | 5 | 27 | 1676 | 36 | 6 | 1 | **1751** |
| *Hypericum* | 854 | 137 | 114 | 391 | 113 | 57 | **1666** |
| *1292Mymu-P\|Mycelis* | 7 | 5 | 1588 | 3 | 12 | 4 | **1619** |
| *Sonchus* | 3 | 23 | 1237 | 3 | 29 | 5 | **1300** |
| *Buddleja* | 69 | 28 | 422 | 281 | 326 | 5 | **1131** |
| *Tilia* | 722 | 41 | 0 | 328 | 4 | 2 | **1097** |
| *863Scno-P\|Scrophularia* | 1058 | 7 | 9 | 4 | 3 | 0 | **1081** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Ipomea* | 340 | 393 | 266 | 17 | 12 | 1 | **1029** |
| *Phacelia* | 918 | 0 | 0 | 0 | 0 | 0 | **918** |
| *109Glhe-P\|Glechoma* | 54 | 107 | 590 | 82 | 70 | 0 | **903** |
| *Arnebia* | 0 | 527 | 2 | 0 | 339 | 0 | **868** |
| *PapaverRhoeas* | 0 | 2 | 861 | 0 | 3 | 0 | **866** |
| *Hydrangea* | 2 | 5 | 0 | 588 | 168 | 1 | **764** |
| *PinusDensiflora* | 25 | 308 | 288 | 116 | 3 | 0 | **740** |
| *Catalpa* | 207 | 79 | 233 | 98 | 1 | 1 | **619** |
| *Malva* | 0 | 0 | 0 | 0 | 2 | 560 | **562** |
| *498Gero-P\|Geranium* | 517 | 18 | 0 | 0 | 0 | 0 | **535** |
| *508Cyse-P\|Centaurea* | 1 | 0 | 520 | 0 | 3 | 0 | **524** |
| *88Scau-P\|Scorzonera* | 0 | 0 | 4 | 5 | 500 | 1 | **510** |
| *Pinus* | 60 | 152 | 250 | 14 | 2 | 0 | **478** |
| *1055Rosa-P\|Rosa* | 449 | 0 | 6 | 3 | 0 | 0 | **458** |
| *280Plla-P\|Plantago* | 15 | 24 | 81 | 4 | 301 | 6 | **431** |
| *Gaura* | 15 | 346 | 33 | 32 | 0 | 0 | **426** |
| *Althaea* | 0 | 0 | 0 | 0 | 412 | 0 | **412** |
| *446Gesy-P\|Geranium* | 356 | 1 | 2 | 4 | 3 | 0 | **366** |
| *Nepeta* | 0 | 193 | 0 | 34 | 0 | 11 | **238** |
| *1270Hisp-P\|Hieracium* | 1 | 0 | 195 | 2 | 9 | 1 | **208** |
| *Ailanthus* | 122 | 10 | 18 | 8 | 3 | 0 | **161** |
| *Pinus yunnanensis* | 16 | 24 | 44 | 65 | 0 | 0 | **149** |
| *Potentilla* | 0 | 0 | 0 | 3 | 0 | 111 | **114** |
| *SjaponicaPollen* | 75 | 0 | 0 | 31 | 0 | 1 | **107** |
| *Dryopteris* | 15 | 1 | 62 | 10 | 14 | 1 | **103** |
| *1104Atbe-P\|Atropa* | 2 | 0 | 4 | 1 | 81 | 1 | **89** |
| *1110Alsc-P\|Allium* | 48 | 0 | 0 | 30 | 0 | 0 | **78** |
| *515Cytr-P\|Centaurea* | 0 | 1 | 27 | 1 | 40 | 0 | **69** |
| *494Sani-P\|Sambucus* | 16 | 9 | 29 | 4 | 0 | 0 | **58** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *PinusSylvestris* | 11 | 4 | 24 | 15 | 3 | 0 | **57** |
| *Acer* | 0 | 0 | 48 | 0 | 0 | 0 | **48** |
| *139Chma-P\|Chelidonium* | 2 | 0 | 11 | 2 | 1 | 30 | **46** |
| *Dipsacus* | 3 | 16 | 15 | 0 | 8 | 1 | **43** |
| *Sedum* | 35 | 5 | 0 | 0 | 2 | 0 | **42** |
| *1035Schu-P\|Scorzonera* | 0 | 0 | 2 | 31 | 6 | 0 | **39** |
| *Dahlia* | 0 | 0 | 0 | 38 | 0 | 0 | **38** |
| *Duranta* | 29 | 0 | 4 | 0 | 2 | 0 | **35** |
| *519Brsy-P\|Brachypodium* | 0 | 1 | 1 | 0 | 32 | 0 | **34** |
| *1367Orvu-P\|Origanum* | 1 | 9 | 9 | 5 | 7 | 0 | **31** |
| *TaraxacumOfficinale* | 3 | 0 | 4 | 6 | 6 | 12 | **31** |
| *Cucurbita* | 0 | 0 | 0 | 2 | 26 | 0 | **28** |
| *64Gani-P\|Galanthus* | 11 | 2 | 3 | 8 | 0 | 0 | **24** |
| *208Vatr-P\|Valeriana* | 1 | 21 | 0 | 1 | 0 | 0 | **23** |
| *OcimumTenuiflorum* | 4 | 4 | 1 | 7 | 5 | 0 | **21** |
| *542Cara-P\|Campanula* | 7 | 5 | 7 | 0 | 1 | 0 | **20** |
| *Quercus* | 2 | 3 | 6 | 0 | 5 | 0 | **16** |
| *1285Seti-P\|Serratula* | 6 | 0 | 6 | 0 | 3 | 0 | **15** |
| *389Coma-P\|Convallaria* | 6 | 4 | 3 | 0 | 0 | 0 | **13** |
| *1391Acal-P\|Acinos* | 4 | 1 | 0 | 4 | 4 | 0 | **13** |
| *SecurigeraVaria* | 10 | 0 | 0 | 3 | 0 | 0 | **13** |
| *136Aseu-P\|Asarum* | 12 | 0 | 0 | 0 | 0 | 0 | **12** |
| *394Saeb-P\|Sambucus* | 0 | 0 | 0 | 1 | 11 | 0 | **12** |
| *Cepalanthus occidentalis* | 7 | 1 | 0 | 0 | 3 | 0 | **11** |
| *Magnolia* | 0 | 3 | 3 | 5 | 0 | 0 | **11** |
| *Jasminum* | 0 | 0 | 0 | 11 | 0 | 0 | **11** |
| *1275Arto-P\|Arctium* | 0 | 0 | 0 | 0 | 10 | 0 | **10** |
| *Brassica* | 10 | 0 | 0 | 0 | 0 | 0 | **10** |
| *AesculusWangii* | 0 | 1 | 1 | 8 | 0 | 0 | **10** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Lilium* | 10 | 0 | 0 | 0 | 0 | 0 | **10** |
| *278Alpr-P\|Alopecurus* | 0 | 8 | 0 | 0 | 0 | 0 | **8** |
| *1375Cazo-P\|Campanula* | 8 | 0 | 0 | 0 | 0 | 0 | **8** |
| *771Chst-P\|Chenopodium* | 0 | 1 | 1 | 0 | 6 | 0 | **8** |
| *822Coda-P\|Cotoneaster* | 2 | 1 | 0 | 0 | 4 | 0 | **7** |
| *324Dica-P\|Dianthus* | 3 | 2 | 0 | 0 | 2 | 0 | **7** |
| *1164Crbi-P\|Crepis* | 0 | 1 | 5 | 0 | 1 | 0 | **7** |
| *1298Altu-P\|Allium* | 5 | 0 | 0 | 1 | 0 | 0 | **6** |
| *UFL006Spec* | 5 | 0 | 1 | 0 | 0 | 0 | **6** |
| *1302Lomu-P\|Lolium* | 3 | 0 | 2 | 0 | 0 | 0 | **5** |
| *Lagersoemia_indicaUF006* | 5 | 0 | 0 | 0 | 0 | 0 | **5** |
| *1236Sara-P\|Sambucus* | 0 | 1 | 0 | 3 | 0 | 0 | **4** |
| *337Gesa-P\|Geranium* | 4 | 0 | 0 | 0 | 0 | 0 | **4** |
| *536Stsy-P\|Stachys* | 0 | 2 | 0 | 0 | 1 | 1 | **4** |
| *1111Prla-P\|Prunella* | 0 | 0 | 4 | 0 | 0 | 0 | **4** |
| *572Ropa-P\|Rorippa* | 1 | 0 | 0 | 3 | 0 | 0 | **4** |
| *1290Adal-P\|Adenostyles* | 0 | 0 | 4 | 0 | 0 | 0 | **4** |
| *954Puof-P\|Pulmonaria* | 0 | 0 | 0 | 2 | 2 | 0 | **4** |
| *675Scum-P\|Scrophularia* | 2 | 0 | 0 | 0 | 1 | 0 | **3** |
| *1207Doge-P\|Dorycnium* | 0 | 0 | 0 | 3 | 0 | 0 | **3** |
| *1017Scpu-P\|Scorzonera* | 0 | 0 | 0 | 0 | 2 | 1 | **3** |
| *662Ruid-P\|Rubus* | 1 | 2 | 0 | 0 | 0 | 0 | **3** |
| *802Gali-P\|Aster* | 0 | 0 | 2 | 1 | 0 | 0 | **3** |
| *1319Hivi-P\|Hieracium* | 0 | 0 | 0 | 0 | 3 | 0 | **3** |
| *Daucus* | 1 | 0 | 0 | 1 | 1 | 0 | **3** |
| *Mespilus* | 0 | 3 | 0 | 0 | 0 | 0 | **3** |
| *HumulusLupulus* | 0 | 0 | 1 | 1 | 1 | 0 | **3** |
| *265Relu-P\|Reseda* | 0 | 1 | 1 | 0 | 0 | 0 | **2** |
| *1132Dafu-P\|Dactylorhiza* | 0 | 0 | 0 | 1 | 1 | 0 | **2** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *845Sagl-P\|Salvia* | 0 | 0 | 0 | 2 | 0 | 0 | **2** |
| *282Vech-P\|Veronica* | 0 | 0 | 0 | 2 | 0 | 0 | **2** |
| *684Plma-P\|Plantago* | 0 | 0 | 0 | 0 | 2 | 0 | **2** |
| *147Pore-P\|Potentilla* | 2 | 0 | 0 | 0 | 0 | 0 | **2** |
| *1015Phor-P\|Phyteuma* | 1 | 1 | 0 | 0 | 0 | 0 | **2** |
| *450Gaer-P\|Galium* | 0 | 2 | 0 | 0 | 0 | 0 | **2** |
| *Robinia* | 1 | 0 | 0 | 1 | 0 | 0 | **2** |
| *Malus* | 2 | 0 | 0 | 0 | 0 | 0 | **2** |
| *851Plme-P\|Plantago* | 0 | 0 | 0 | 0 | 1 | 0 | **1** |
| *673Dece-P\|Avenella* | 0 | 0 | 0 | 0 | 1 | 0 | **1** |
| *1401Catr-P\|Campanula* | 1 | 0 | 0 | 0 | 0 | 0 | **1** |
| *1386Powu-P\|Potentilla* | 1 | 0 | 0 | 0 | 0 | 0 | **1** |
| *1136Cyal-P\|Cystopteris* | 0 | 0 | 0 | 0 | 1 | 0 | **1** |
| *1160Vear-P\|Veronica* | 1 | 0 | 0 | 0 | 0 | 0 | **1** |
| *1306Gesy-P\|Geranium* | 1 | 0 | 0 | 0 | 0 | 0 | **1** |
| *1051Chfi-P\|Chenopodium* | 0 | 0 | 1 | 0 | 0 | 0 | **1** |
| *1395Veor-P\|Veronica* | 0 | 0 | 0 | 0 | 0 | 1 | **1** |
| *1030Vilu-P\|Vicia* | 0 | 0 | 0 | 0 | 1 | 0 | **1** |
| *312Ansy-P\|Anthriscus* | 0 | 0 | 0 | 0 | 1 | 0 | **1** |
| *479Saal-P\|Salix* | 0 | 1 | 0 | 0 | 0 | 0 | **1** |
| *Vitex_specMST10p* | 0 | 0 | 0 | 0 | 1 | 0 | **1** |
| *Portulaca* | 0 | 0 | 0 | 0 | 1 | 0 | **1** |
| *Wisteria* | 0 | 0 | 0 | 1 | 0 | 0 | **1** |
| *297Sane-P\|Salvia* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *369Stre-P\|Stachys* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *546Crbi-P\|Crepis* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *352Heca-P\|Helianthemum* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *782Saof-P\|Salvia* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *179Agre-P\|Elymus* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *1172Ermu-P\|Eragrostis* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *872Anma-P\|Antirrhinum* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *500Anof-P\|Anchusa* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *606Dide-P\|Dianthus* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *825Pemi-P\|Persicaria* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *Salvia* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *Syringa* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *Gypsophila* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *Rudbeckia* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *Picris* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| *Olea* | 0 | 0 | 0 | 0 | 0 | 0 | **0** |
| | **41449** | **26721** | **24628** | **11806** | **13530** | **1304** | **119438** |

*Table 13: Detected plant species and their abundance on the two different beehive sites. Plate 1 and Plate 2.*

| *Table 12:* ***Species*** | **a** | **b** | **Total** |
|---|---|---|---|
| *Koelreuteria* | 8684 | 16694 | **25378** |
| *Parthenocissus quinquefolia* | 13617 | 2053 | **15670** |
| *Ligustrum_lucidumUF006* | 3493 | 6157 | **9650** |
| *Firmiana* | 4654 | 4177 | **8831** |
| *SedumAlbum* | 4893 | 1639 | **6532** |
| *Dipsacoideae* | 233 | 6152 | **6385** |
| *376Cest-P\|Centaurea* | 11 | 5406 | **5417** |
| *Pelargonium* | 3543 | 1719 | **5262** |
| *464Tapa-P\|Tanacetum* | 54 | 4579 | **4633** |
| *Echinacea* | 870 | 1401 | **2271** |
| *419Ruca-P\|Rubus* | 1583 | 565 | **2148** |
| *Hosta* | 1994 | 0 | **1994** |
| *Scabiosa* | 0 | 1955 | **1955** |
| *Hemerocallis* | 1712 | 39 | **1751** |
| *Hypericum* | 1322 | 344 | **1666** |
| *1292Mymu-P\|Mycelis* | 1082 | 537 | **1619** |
| *Sonchus* | 19 | 1281 | **1300** |
| *Buddleja* | 763 | 368 | **1131** |
| *Tilia* | 875 | 222 | **1097** |
| *863Scno-P\|Scrophularia* | 1060 | 21 | **1081** |
| *Ipomea* | 353 | 676 | **1029** |
| *Phacelia* | 918 | 0 | **918** |
| *109Glhe-P\|Glechoma* | 10 | 893 | **903** |
| *Arnebia* | 0 | 868 | **868** |
| *PapaverRhoeas* | 0 | 866 | **866** |
| *Hydrangea* | 22 | 742 | **764** |
| *PinusDensiflora* | 128 | 612 | **740** |
| *Catalpa* | 432 | 187 | **619** |
| *Malva* | 0 | 562 | **562** |
| *498Gero-P\|Geranium* | 535 | 0 | **535** |
| *508Cyse-P\|Centaurea* | 517 | 7 | **524** |
| *88Scau-P\|Scorzonera* | 4 | 506 | **510** |
| *Pinus* | 149 | 329 | **478** |
| *1055Rosa-P\|Rosa* | 449 | 9 | **458** |
| *280Plla-P\|Plantago* | 57 | 374 | **431** |
| *Gaura* | 381 | 45 | **426** |
| *Althaea* | 0 | 412 | **412** |
| *446Gesy-P\|Geranium* | 356 | 10 | **366** |
| *Nepeta* | 0 | 238 | **238** |
| *1270Hisp-P\|Hieracium* | 156 | 52 | **208** |
| *Ailanthus* | 143 | 18 | **161** |

| | | | |
|---|---|---|---|
| *Pinus yunnanensis* | 28 | 121 | **149** |
| *Potentilla* | 114 | 0 | **114** |
| *SjaponicaPollen* | 107 | 0 | **107** |
| *Dryopteris* | 25 | 78 | **103** |
| *1104Atbe-P\|Atropa* | 3 | 86 | **89** |
| *1110Alsc-P\|Allium* | 61 | 17 | **78** |
| *515Cytr-P\|Centaurea* | 19 | 50 | **69** |
| *494Sani-P\|Sambucus* | 19 | 39 | **58** |
| *PinusSylvestris* | 15 | 42 | **57** |
| *Acer* | 0 | 48 | **48** |
| *139Chma-P\|Chelidonium* | 2 | 44 | **46** |
| *Dipsacus* | 2 | 41 | **43** |
| *Sedum* | 40 | 2 | **42** |
| *1035Schu-P\|Scorzonera* | 32 | 7 | **39** |
| *Dahlia* | 38 | 0 | **38** |
| *Duranta* | 30 | 5 | **35** |
| *519Brsy-P\|Brachypodium* | 0 | 34 | **34** |
| *1367Orvu-P\|Origanum* | 15 | 16 | **31** |
| *TaraxacumOfficinale* | 6 | 25 | **31** |
| *Cucurbita* | 0 | 28 | **28** |
| *64Gani-P\|Galanthus* | 13 | 11 | **24** |
| *208Vatr-P\|Valeriana* | 23 | 0 | **23** |
| *OcimumTenuiflorum* | 8 | 13 | **21** |
| *542Cara-P\|Campanula* | 14 | 6 | **20** |
| *Quercus* | 3 | 13 | **16** |
| *1285Seti-P\|Serratula* | 6 | 9 | **15** |
| *389Coma-P\|Convallaria* | 13 | 0 | **13** |
| *1391Acal-P\|Acinos* | 8 | 5 | **13** |
| *SecurigeraVaria* | 3 | 10 | **13** |
| *136Aseu-P\|Asarum* | 12 | 0 | **12** |
| *394Saeb-P\|Sambucus* | 0 | 12 | **12** |
| *Cepalanthus occidentalis* | 8 | 3 | **11** |
| *Magnolia* | 3 | 8 | **11** |
| *Jasminum* | 0 | 11 | **11** |
| *1275Arto-P\|Arctium* | 0 | 10 | **10** |
| *Brassica* | 10 | 0 | **10** |
| *AesculusWangii* | 0 | 10 | **10** |
| *Lilium* | 10 | 0 | **10** |
| *278Alpr-P\|Alopecurus* | 1 | 7 | **8** |
| *1375Cazo-P\|Campanula* | 8 | 0 | **8** |
| *771Chst-P\|Chenopodium* | 0 | 8 | **8** |
| *822Coda-P\|Cotoneaster* | 3 | 4 | **7** |
| *324Dica-P\|Dianthus* | 5 | 2 | **7** |
| *1164Crbi-P\|Crepis* | 5 | 2 | **7** |
| *1298Altu-P\|Allium* | 6 | 0 | **6** |

| | | | |
|---|---|---|---|
| *UFL006Spec* | 5 | 1 | **6** |
| *1302Lomu-P\|Lolium* | 4 | 1 | **5** |
| *Lagersoemia_indicaUF006* | 5 | 0 | **5** |
| *1236Sara-P\|Sambucus* | 2 | 2 | **4** |
| *337Gesa-P\|Geranium* | 4 | 0 | **4** |
| *536Stsy-P\|Stachys* | 0 | 4 | **4** |
| *1111Prla-P\|Prunella* | 0 | 4 | **4** |
| *572Ropa-P\|Rorippa* | 4 | 0 | **4** |
| *1290Adal-P\|Adenostyles* | 0 | 4 | **4** |
| *954Puof-P\|Pulmonaria* | 0 | 4 | **4** |
| *675Scum-P\|Scrophularia* | 2 | 1 | **3** |
| *1207Doge-P\|Dorycnium* | 3 | 0 | **3** |
| *1017Scpu-P\|Scorzonera* | 0 | 3 | **3** |
| *662Ruid-P\|Rubus* | 2 | 1 | **3** |
| *802Gali-P\|Aster* | 0 | 3 | **3** |
| *1319Hivi-P\|Hieracium* | 0 | 3 | **3** |
| *Daucus* | 2 | 1 | **3** |
| *Mespilus* | 0 | 3 | **3** |
| *HumulusLupulus* | 0 | 3 | **3** |
| *265Relu-P\|Reseda* | 0 | 2 | **2** |
| *1132Dafu-P\|Dactylorhiza* | 0 | 2 | **2** |
| *845Sagl-P\|Salvia* | 2 | 0 | **2** |
| *282Vech-P\|Veronica* | 0 | 2 | **2** |
| *684Plma-P\|Plantago* | 0 | 2 | **2** |
| *147Pore-P\|Potentilla* | 2 | 0 | **2** |
| *1015Phor-P\|Phyteuma* | 1 | 1 | **2** |
| *450Gaer-P\|Galium* | 2 | 0 | **2** |
| *Robinia* | 2 | 0 | **2** |
| *Malus* | 2 | 0 | **2** |
| *851Plme-P\|Plantago* | 0 | 1 | **1** |
| *673Dece-P\|Avenella* | 0 | 1 | **1** |
| *1401Catr-P\|Campanula* | 1 | 0 | **1** |
| *1386Powu-P\|Potentilla* | 1 | 0 | **1** |
| *1136Cyal-P\|Cystopteris* | 0 | 1 | **1** |
| *1160Vear-P\|Veronica* | 1 | 0 | **1** |
| *1306Gesy-P\|Geranium* | 1 | 0 | **1** |
| *1051Chfi-P\|Chenopodium* | 0 | 1 | **1** |
| *1395Veor-P\|Veronica* | 0 | 1 | **1** |
| *1030Vilu-P\|Vicia* | 0 | 1 | **1** |
| *312Ansy-P\|Anthriscus* | 0 | 1 | **1** |
| *479Saal-P\|Salix* | 0 | 1 | **1** |
| *Vitex_specMST10p* | 0 | 1 | **1** |
| *Portulaca* | 0 | 1 | **1** |
| *Wisteria* | 1 | 0 | **1** |
| | **55839** | **63599** | **119438** |

*Table 14: Number of homogeneous pollen loads depending on specific threshold values.*

| Percentage (Homogeneity-Criteria) | Homogeneous Pollen loads |
|---|---|
| 50% | 160 |
| 51% | 157 |
| 52% | 156 |
| 53% | 150 |
| 54% | 148 |
| 55% | 148 |
| 56% | 148 |
| 57% | 143 |
| 58% | 141 |
| 59% | 139 |
| 60% | 137 |
| 61% | 135 |
| 62% | 132 |
| 63% | 129 |
| 64% | 127 |
| 65% | 126 |
| 66% | 125 |
| 67% | 123 |
| 68% | 119 |
| 69% | 117 |
| 70% | 115 |
| 71% | 111 |
| 72% | 108 |
| 73% | 105 |
| 74% | 101 |
| 75% | 98 |
| 76% | 96 |
| 77% | 92 |
| 78% | 88 |
| 79% | 82 |
| 80% | 79 |
| 81% | 79 |
| 82% | 76 |
| 83% | 73 |
| 84% | 71 |
| 85% | 70 |
| 86% | 65 |
| 87% | 62 |
| 88% | 59 |
| 89% | 56 |
| 90% | 49 |
| 91% | 46 |

| | |
|---|---|
| 92% | 41 |
| 93% | 39 |
| 94% | 32 |
| 95% | 27 |
| 96% | 20 |
| 97% | 15 |
| 98% | 9 |
| 99% | 1 |
| 100% | 0 |

# 8. Bibliography/List of references


Alaux, C., Ducloz, F., *et al.* (2010) 'Diet effects on honeybee immunocompetence', *Biology Letters*, 6(4), pp. 562–565. doi: 10.1098/rsbl.2009.0986.

Alaux, C., Brunet, fr J. L., *et al.* (2010) 'Interactions between Nosema microspores and a neonicotinoid weaken honeybees (Apis mellifera)', *Environmental Microbiology*, 12(3), pp. 774–782. doi: 10.1111/j.1462-2920.2009.02123.x.

Alaux, C. *et al.* (2017) 'A â €'Landscape physiology' approach for assessing bee health highlights the benefits of floral landscape enrichment and semi-natural habitats', *Scientific Reports*, 7(January). doi: 10.1038/srep40568.

Amaya-Márquez, M. (2009) 'Floral constancy in bees: A revision of theories and a comparison with other pollinators', *Revista Colombiana de Entomologia*, 35(2), pp. 206–216.

Baksay, S. *et al.* (2020) 'Experimental quantification of pollen with DNA metabarcoding using ITS1 and trnL', *Scientific Reports*, 10(1), pp. 1–9. doi: 10.1038/s41598-020-61198-6.

Barron, A. B. *et al.* (2005) 'Influence of flight time and flight environment on distance communication by dancing honey bees', *Insectes Sociaux*, 52(4), pp. 402–407. doi: 10.1007/s00040-005-0827-8.

Beekman, M. *et al.* (2004) 'Comparing foraging behaviour of small and large honey-bee colonies by decoding waggle dances made by foragers', *Functional Ecology*, 18(6), pp. 829–835. doi: 10.1111/j.0269-8463.2004.00924.x.

Bell, K. L. *et al.* (2019) 'Quantitative and qualitative assessment of pollen DNA metabarcoding using constructed species mixtures', *Molecular Ecology*, 28(2), pp. 431–455. doi: 10.1111/mec.14840.

Bila Dubaić, J. *et al.* (2021) 'Unprecedented Density and Persistence of Feral Honey Bees in Urban Environments of a Large SE-European City (Belgrade, Serbia)', *Insects*, 12(12), p. 1127. doi: 10.3390/insects12121127.

Bogdanov, S. (2016) 'The Bee Pollen Book Wild Thyme and Sav ' ry set around their cell', (April), pp. 1–11.

Brodschneider, R. *et al.* (2018) 'What We Can (or Cannot) Learn from Multifloral Pollen Pellets', *Bee World*, 95(3), pp. 78–80. doi: 10.1080/0005772x.2018.1483057.

Brodschneider, R. *et al.* (2019) 'A citizen science supported study on seasonal diversity and monoflorality of pollen collected by honey bees in Austria', *Scientific Reports*, 9(1), pp. 1–12. doi: 10.1038/s41598-019-53016-5.

Brodschneider, R. *et al.* (2021) 'Csi pollen: Diversity of honey bee collected pollen studied by citizen scientists', *Insects*, 12(11), pp. 1–23. doi: 10.3390/insects12110987.

Brodschneider, R. and Crailsheim, K. (2010) 'Nutrition and health in honey bees', *Apidologie*, 41(3), pp. 278–294. doi: 10.1051/apido/2010012.

Brodschneider, R. and Crailsheim, K. (2013) 'Fünf Jahre Untersuchung des Bienensterbens in Österreich', *österr. Ent. Ges.*, 20, pp. 195–205.

Brückmann, V. T. (2004) 'Methodische Mängel'.

Chen, C. W. *et al.* (2013) 'TrnL-F is a powerful marker for DNA identification of field vittarioid gametophytes (Pteridaceae)', *Annals of Botany*, 111(4), pp. 663–673. doi: 10.1093/aob/mct004.

Crailsheim, K. (1990) 'The protein balance of the honey bee worker', *Apidologie*, 21(5), pp. 417–429. doi: 10.1051/apido:19900504.

Curto, M. *et al.* (2019) 'Application of a SSR-GBS marker system on investigation of European Hedgehog species and their hybrid zone dynamics', *Ecology and Evolution*, 9(5), pp. 2814–2832. doi: 10.1002/ece3.4960.

Deutscher-Imkerbund (2021) 'Imkerei_in_Deutschland_Zahlen_Daten_Fakten @ deutscherimkerbund.de'. Available at: https://deutscherimkerbund.de/161-Imkerei_in_Deutschland_Zahlen_Daten_Fakten.

Fischer, J. *et al.* (2014) 'Neonicotinoids interfere with specific components of navigation in honeybees', *PLoS ONE*, 9(3), pp. 1–10. doi: 10.1371/journal.pone.0091364.

García-García, M. C., Ortiz, P. L. and Díez Dapena, M. J. (2004) 'Variations in the weights of pollen loads collected by Apis mellifera L.', *Grana*, 43(3), pp. 183–192. doi: 10.1080/00173130410020350.

Genersch, E. *et al.* (2010) 'The German bee monitoring project: A long term study to understand periodically high winter losses of honey bee colonies', *Apidologie*, 41(3), pp. 332–352. doi:

10.1051/apido/2010014.

Glick, S., Gehrig, R. and Eeftens, M. (2021) 'Multi-decade changes in pollen season onset, duration, and intensity: A concern for public health?', *Science of the Total Environment*, 781, p. 146382. doi: 10.1016/j.scitotenv.2021.146382.

Hatzinger, R., Hornik, K. and Nagel, H. (2011) *R: Einführung durch angewandte Statistik*. Available at: https://books.google.com/books?id=jmCuEY07s7UC&pgis=1.

Hauer, M. *et al.* (2017) 'Neonicotinoids in sugar beet cultivation in Central and Northern Europe: Efficacy and environmental impact of neonicotinoid seed treatments and alternative measures', *Crop Protection*, 93, pp. 132–142. doi: 10.1016/j.cropro.2016.11.034.

Johnson, M. D. *et al.* (2021) 'Airborne environmental DNA metabarcoding detects more diversity, with less sampling effort, than a traditional plant community survey', *BMC Ecology and Evolution*, 21(1), pp. 1–15. doi: 10.1186/s12862-021-01947-x.

Jones, T. M. (2014) 'Why is the lawn buzzing?', *Biodiversity Data Journal*, 2(1). doi: 10.3897/BDJ.2.e1101.

Kamo, T. *et al.* (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), pp. 353–361. doi: 10.1007/s13355-018-0565-9.

Krupke, C. H. *et al.* (2012) 'Multiple routes of pesticide exposure for honey bees living near agricultural fields', *PLoS ONE*, 7(1). doi: 10.1371/journal.pone.0029268.

Kuchling, S. *et al.* (2018) 'Investigating the role of landscape composition on honey bee colony winter mortality: A long-term analysis', *Scientific Reports*, 8(1), pp. 1–10. doi: 10.1038/s41598-018-30891-y.

Lack, A. J. and Real, L. (1985) 'Pollination Biology.', *The Journal of Ecology*, 73(2), p. 720. doi: 10.2307/2260521.

Lamminen, M. *et al.* (2019) 'Different microalgae species as a substitutive protein feed for soya bean meal in grass silage based dairy cow diets', *Animal Feed Science and Technology*, 247(January), pp. 112–126. doi: 10.1016/j.anifeedsci.2018.11.005.

Leontidou, K. *et al.* (2018) 'DNA metabarcoding of airborne pollen: new protocols for improved taxonomic identification of environmental samples', *Aerobiologia*, 34(1), pp. 63–74. doi: 10.1007/s10453-017-9497-z.

Leontidou, K. *et al.* (2021) 'Plant biodiversity assessment through pollen DNA metabarcoding in

Natura 2000 habitats (Italian Alps)', *Scientific Reports*, 11(1), pp. 1–12. doi: 10.1038/s41598-021-97619-3.

Little, D. P. (2014) 'A DNA mini-barcode for land plants', *Molecular Ecology Resources*, 14(3), pp. 437–446. doi: 10.1111/1755-0998.12194.

Longhi, S. *et al.* (2009) 'Biomolecular identification of allergenic pollen: A new perspective for aerobiological monitoring?', *Annals of Allergy, Asthma and Immunology*, 103(6), pp. 508–514. doi: 10.1016/S1081-1206(10)60268-2.

Mandl, S. and Sukopp (2011) 'No Title:Bestäubungshandbuch', *Sammlung eigener Untersuchungen und Zusammenfassung der Fachliteratur*, pp. 138–155.

Microsoft Corporation (2018) 'Microsoft Excel'. Available at: https://office.microsoft.com/excel.

Mishra, P. *et al.* (2016) 'Feasibility of nuclear ribosomal region ITS1 over ITS2 in barcoding taxonomically challenging genera of subtribe Cassiinae (Fabaceae)', *PeerJ*, 2016(12), pp. 1–21. doi: 10.7717/peerj.2638.

Omar, E. *et al.* (2017) 'Influence of different pollen diets on the development of hypopharyngeal glands and size of acid gland sacs in caged honey bees (Apis mellifera)', *Apidologie*, 48(4), pp. 425–436. doi: 10.1007/s13592-016-0487-x.

Di Pasquale, G. *et al.* (2013) 'Influence of Pollen Nutrition on Honey Bee Health: Do Pollen Quality and Diversity Matter?', *PLoS ONE*, 8(8), pp. 1–13. doi: 10.1371/journal.pone.0072016.

Di Pasquale, G. *et al.* (2016) 'Variations in the availability of pollen resources affect honey bee health', *PLoS ONE*, 11(9). doi: 10.1371/journal.pone.0162818.

Pollenwarndienst (2021) 'www.pollenwarndienst.at'. Available at: https://www.pollenwarndienst.at/.

Pornon, A. *et al.* (2016) 'Using metabarcoding to reveal and quantify plant-pollinator interactions', *Scientific Reports*, 6(May), pp. 1–12. doi: 10.1038/srep27282.

Di Prisco, G. *et al.* (2013) 'Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees', *Proceedings of the National Academy of Sciences of the United States of America*, 110(46), pp. 18466–18471. doi: 10.1073/pnas.1314923110.

R-Core-Team (2021) 'R: A language and environment for statistical computing. R Foundation for Statistical'. Available at: http://www.r-project.org/.

Radev, Z. (2018) 'Variety in Protein Content of Pollen from 50 Plants from Bulgaria', *Bee World*, 95(3),

pp. 81–83. doi: 10.1080/0005772x.2018.1486276.

Richardson, R. T. *et al.* (2015) 'Application of ITS2 Metabarcoding to Determine the Provenance of Pollen Collected by Honey Bees in an Agroecosystem', *Applications in Plant Sciences*, 3(1), p. 1400066. doi: 10.3732/apps.1400066.

Ricigliano, V. A. (2020) 'Microalgae as a promising and sustainable nutrition source for managed honey bees', *Archives of Insect Biochemistry and Physiology*, 104(1), pp. 1–8. doi: 10.1002/arch.21658.

Sayers, E. W. *et al.* (2020) 'Database resources of the National Center for Biotechnology Information', *Nucleic Acids Research*, 48(D1), pp. D9–D16. doi: 10.1093/nar/gkz899.

Simone-Finstrom, M. *et al.* (2016) 'Migratory management and environmental conditions affect lifespan and oxidative stress in honey bees', *Scientific Reports*, 6(August), pp. 1–10. doi: 10.1038/srep32023.

Solanki, G. (2012) 'POLYMERASE CHAIN REACTION', 2(2), pp. 81–82.

Swenson, S. J. and Gemeinholzer, B. (2021) 'Testing the effect of pollen exine rupture on metabarcoding with Illumina sequencing', *PLoS ONE*, 16(2 February), pp. 1–16. doi: 10.1371/journal.pone.0245611.

Tanaka, K. *et al.* (2020) 'Using pollen DNA metabarcoding to profile nectar sources of urban beekeeping in Kōtō-ku, Tokyo', *BMC Research Notes*, 13(1), pp. 1–6. doi: 10.1186/s13104-020-05361-2.

Truckenbrodt, H. (2014) 'Das Deutsche', *Das mehrsprachige Klassenzimmer*, (April), pp. 35–65. doi: 10.1007/978-3-642-34315-5_3.

Visscher, P. K. and Seeley, T. D. (1982) 'Foraging Strategy of Honeybee Colonies in a Temperate Deciduous Forest Author ( s ): P . Kirk Visscher and Thomas D . Seeley Published by : Wiley Stable URL : http://www.jstor.org/stable/1940121 REFERENCES Linked references are available on JSTOR for this', *Ecology*, 63(6), pp. 1790–1801. Available at: http://www.jstor.org/stable/1940121 Accessed:

Wickham, H. (2008) *Elegant Graphics for Data Analysis: ggplot2*, *Applied Spatial Data Analysis with R*.

Williams, K. E. *et al.* (2021) 'Using next generation sequencing of alpine plants to improve fecal metabarcoding diet analysis for Dall's sheep', *BMC Research Notes*, 14(1), pp. 1–4. doi: 10.1186/s13104-021-05590-z.

Yao, H. *et al.* (2010) 'Use of ITS2 region as the universal DNA barcode for plants and animals', *PLoS*

*ONE*, 5(10). doi: 10.1371/journal.pone.0013102.