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Scientific Report from DCE – Danish Centre for Environment and Energy nr. 517 2022

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Abstract: Pollen is a source of protein, fats, vitamins and minerals for honey bees, and pollen nutrition is important for bee health and winter survival. In this study, we investigated the pollen supply of honey bees in Danish landscapes with a view to elucidate the significance of the seasonal development and land use in the surrounding landscape. The report reviews the most important pollen sources (top 10) in each of 10 periods throughout the growing season (April-October) as well as in landscapes with larger shares of agricultural land, urban areas, open nature, forest and water.

Keywords: *Apis mellifera*, bee bread, citizen science, foraging, GIS, honey bee, landscape, pollen diversity, pollen types

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Content

1 Preface

This report was prepared in connection with the project "Hvad er sammenhængen mellem biernes pollenforsyning, varroavækst og vinterdødelighed i danske landskaber?" (in English:" What is the relationship between bee pollen supply, varroa growth and winter mortality in Danish landscapes?") undertaken from 2019-2022. The project was funded by the Danish Agricultural Agency's Beekeeping Program (project number 19-2222-000001).

The project investigated the relationship between bee pollen nutrition and bee health as well as the significance of the surrounding landscape for the pollen supply of a bee colony. Specifically, the project aimed to identify the main pollen sources throughout the flowering season in different regions and landscapes in Denmark and to clarify the relationship between the landscape composition and the bee pollen supply. Furthermore, the relationship between varroa growth and winter mortality in landscapes with different pollen supplies was investigated. This report elucidates honey bee pollen sources throughout the season and in different landscape types in Denmark, while results regarding bee health are reported elsewhere. The naming of plants follows, as far as possible, Atlas Flora Danica (Hartvig, 2015), and the botanical name is followed by the scientific name in parentheses. Appendix 1 provides a comprehensive overview of identified pollen types, including plant families.

A big thank you to the beekeepers across the country who participated in the project by collecting bee bread throughout the season. Without them, the project could not have been carried out! Anna Karin la Cour (Department of Agroecology, AU) was invaluable in assisting with the administration and processing of the submitted samples. We are grateful to Luna Kondrup Marcussen (Department of Ecoscience, AU) and Charlotte Knudsen (Department of Agroecology, AU) for practical assistance with data management and communication. Ghalia Kassem and Sabine Frasch, Quality Services International (QSI), Bremen, performed the botanical determination of pollen samples through microscopy and kindly contributed with images of pollen grains (Figure 1). Thanks to Peter Wind (Department of Ecoscience, AU) for sharing his extensive botanical knowledge and for providing thorough comments on the initial draft of the report. Finally, thanks to Anne Mette Poulsen for translating the report into English.

2 Dansk sammendrag

Pollen fra karplanter indeholder protein, fedtstoffer, vitaminer og bioaktive stoffer og er en vigtig næringskilde for bier. Honningbier, som lever i store, sociale kolonier, samler og opbevarer pollen som bibrød i vokstavler i boet, hvor det bruges primært som larvefoder. Pollen varierer i næringsindhold, og en bifamilies pollenforsyning, særligt proteinindholdet, er påvirket af det omgivende landskabs sammensætning. Honningbiernes pollenernæring har stor betydning for bifamiliens sundhed og vinteroverlevelse. Formålet med nærværende undersøgelse er at identificere de vigtigste kilder til pollen for honningbier. Specifikt undersøges variationen i pollenkilder gennem blomstringssæsonen i forskellige regioner og landskaber i Danmark, og sammenhængen mellem sæson, landskabssammensætning og honningbiernes pollenforsyning klarlægges.

I forbindelse med undersøgelsen er pollenprøver fra bifamilier blevet samlet af frivillige biavlere fra forskellige landsdele og landskabstyper. Biavlerne blev instrueret i at levere prøver af ny pollen fra vokstavlerne hver tredje uge, fra starten af sæsonen (ultimo marts/primo april 2020 henholdsvis ultimo april/primo maj 2021), og indtil primo oktober 2020/2021. I alt blev der leveret 258 bibrødsprøver fra 31 bigårde i 2020 og 53 prøver fra ni bigårde i 2021. Pollen i prøverne blev bestemt ved morfologisk bestemmelse ved lysmikroskopi af pollenlaboratoriet, Quality Services International (QSI), ved brug af standardprotokollen DIN-Norm 10760. Arealanvendelsen af omgivelserne af hver forsøgsbigård blev analyseret ved brug af GIS. En bigårds fødesøgningsområde blev defineret som en tre km cirkel omkring bigården, som omfatter 75% af honningbiers fourageringsafstande (Couvillon et al., 2014). Arealanvendelsen (i ha) blev kategoriseret efter basemap-kategorierne landbrug, natur, byområde, skov, vand og andet (vej, jernbane, grusgrav, ikkekategoriseret).

Der blev i undersøgelsen identificeret i alt 105 pollentyper, bestemt til karplantefamilie, -slægt eller -art. Pollentyperne omfattede både afgrøder og vilde planter. Diversiteten af pollen, det vil sige antal pollentyper, var lav indtil ultimo maj, stigende indtil slutningen af august, hvorefter den igen aftog. I alt 20 pollentyper udgjorde alle hver især mere end 1 % af en given prøve. Disse var: raps (*Brassica*), pil (*Salix*), hvidkløver (*Trifolium repens*), frugttræer (*Pirus/Prunus*), hindbær/brombær (*Rubus*), ahorn (*Acer*), mælkebøtte (*Taraxacum*), vedbend (*Hedera*), honningurt (*Phacelia*), ranunkelfamilien (eventuelt skovranke (*Clematis*)), valmue (*Papaver*), ægte kastanje (*Castanea sativa*), røllike (*Achillea*), rødkløver (*T. pratense*), mjødurt (*Filipendula*), hestekastanje (*Aesculus*), hestebønne (*Vicia faba*), sennep (*Sinapis*), hedelyng (*Calluna vulgaris*) og sommerfuglebusk (*Buddleja*). Der var en stor sæsonmæssig variation i biernes pollenforsyning, som fulgte de forskellige karplanters skiftende blomstringsperioder. Endvidere var biernes pollenforsyning i nogen grad påvirket af det omgivende landskabs sammensætning. Der var dog lav variation i top 10 pollentyper imellem forskellige landskabstyper (landskaber domineret af landbrugsland, byer, lysåben natur, skov og øer). Landskaber med byområder havde dog generelt flere forskellige pollentyper i prøverne.

3 Summary

Pollen from flowering plants contains protein, fatty acids, vitamins, and bioactive substances and is an important source of nutrition for bees. Honey bees live in large, social colonies and store pollen as bee bread in wax combs in their hive, where it is used primarily as larval feed. Pollen is highly variable in quality, in particular in protein content, and in availability, and hence the pollen source of a honey bee colony, is affected by the landscape surrounding the honey bee hive. The quality of the pollen source influences colony health and winter survival. The aim of the current study is to identify important sources of pollen for honey bees. Specifically, we investigate variation in honey bee pollen sources through the flowering season in different regions and landscape types in Denmark to assess the influence of the flowering season and landscape composition on honey bee pollen collection.

Pollen samples from honey bee colonies were collected by volunteer beekeepers from apiaries placed in different landscape types across Denmark. Bee keepers were instructed in collecting bee bread recently produced by honey bees. Bee bread was collected from the combs from the onset of the season (late March/early April in 2020 and late April/early May in 2021) and until early October 2020/2021. In total, 258 bee bread samples were collected from 31 apiaries in 2020 and 53 samples from nine apiaries in 2021. Samples were sent to the pollen laboratory Quality Services International (QSI) and morphologically identified by light microscopy following the standard protocol DIN-Norm 10760. Land use of the landscapes surrounding each experimental apiary was analysed using GIS. The surrounding landscape of an apiary was defined as a three kilometre circle around the apiary, which includes 75% of the honey bee foraging trips (Couvillon et al., 2014). Land use (in ha) was categorised using the basemap classes agriculture, nature, urban, forest, water and other (road, railroad, resource extraction areas, non-categorised).

A total of 105 pollen types were identified to angiosperm family, genus or species level. Pollen types included both crops and wild plants. The diversity of pollen, i.e. the number of pollen types, was low until late May, increasing until late August, after which it decreased. Only 20 pollen types were represented by more than 1% of a pollen sample. These were: oilseed rape (*Brassica*), willow (*Salix*), white clover (*Trifolium repens*), fruit trees (*Pirus/Prunus*), raspberry/bramble (*Rubus*), maple (*Acer*), dandelion (*Taraxacum*), ivy (*Hedera*), lacy phacelia (*Phacelia*), buttercup family (possibly *Clematis*), poppy (*Papaver*), Spanish chestnut (*Castanea sativa*), yarrow (*Achillea*), red clover (*T. pratense*), meadowsweet (*Filipendula*), horse chestnut (*Aesculus*), faba bean (*Vicia faba*), mustard (*Sinapis*), common heather (*Calluna vulgaris*) and butterfly bush (*Buddleja*). A high seasonal variation following the seasonal progression of flowering plants was found in the pollen sources of honey bees. Furthermore, the pollen source was to some extent affected by the composition of the surrounding landscape. However, variation was low in the top 10 pollen types among different landscape types (landscapes dominated by farmland, urban areas, open nature, forest, and islands), In general, pollen samples from landscapes with a high coverage of urban areas had a higher diversity of pollen types.

4 Introduction

4.1 Why do bees collect pollen?

Bees collect nectar and pollen from flowers. Nectar serves as a source of carbohydrates or sugars, used for energy and as a nutrition storage in the form of honey. Pollen is rich in protein, fats, vitamins, and minerals (Herbert and Shimanuki, 1978), see Box 1. Pollen contains essential substances that bees cannot produce themselves, and these substances are crucial for the health of adult bees and the development of larvae (Haydak, 1970). Without this supply, bees would only be able to produce food sap to rear offspring for about a week. After that, their amino acid reserves would be depleted, and their brood care would cease (Haydak, 1970; Keller et al., 2005b, a)

Box 1: What is pollen, and why do plants produce pollen?

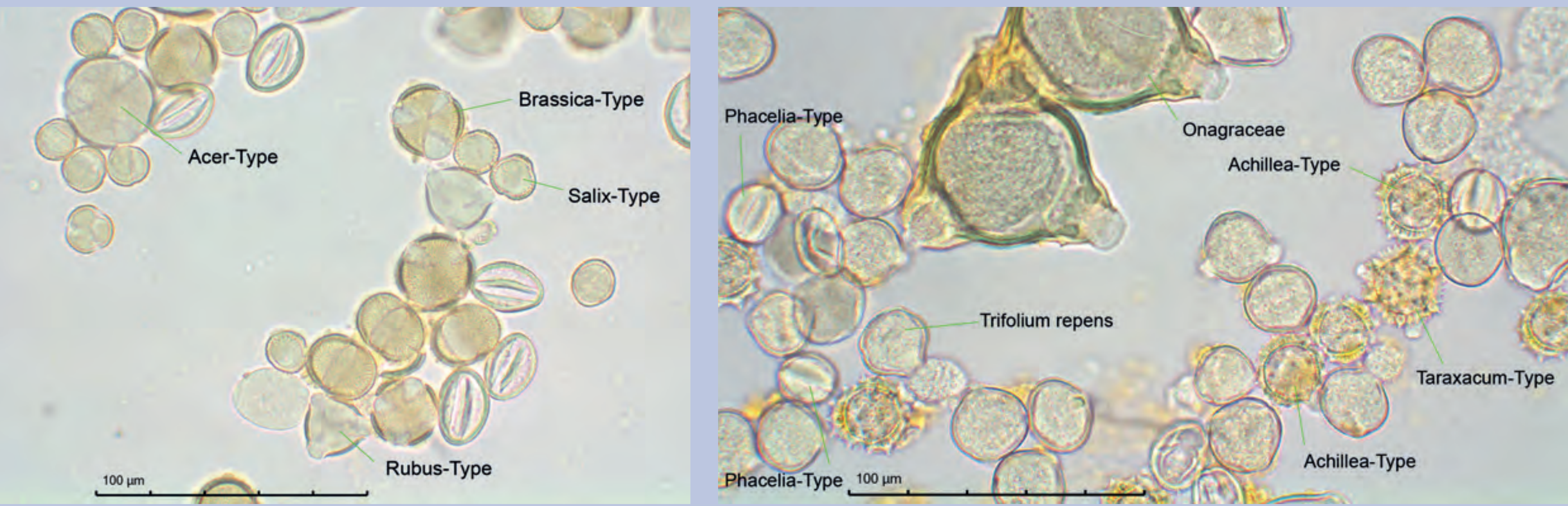


Figure 1. Pollen grains seen under a light microscope. Here is a selection of pollen types: willow (*Salix*), maple (*Acer*), oilseed rape (*Brassica*), raspberry/blackberry (*Rubus*), dandelion (*Taraxacum*), yarrow (*Achillea*), lacy phacelia (*Phacelia*), white clover (*Trifolium repens*), evening primrose family (*Onagraceae*). Photos: Ghalia Kassem.

Pollen, or flower dust, is the male reproductive units of plants and is the plant equivalent of animal sperm. Pollen is cells formed in the male reproductive organs of flowers, the stamens. Pollen is transported by wind or insects (including bees) to the female reproductive organs of other flowers, the pistil, which holds the ovary containing the egg cells.

Insect-pollinated plants attract insects to transport pollen from one flower to another, aiming for cross-pollination and thus cross-fertilisation. As a reward, many plants use nutrient-rich pollen and/or nectar.

Flowers secrete nectar from glands, for example in the flower base, a ring-shaped structure, as in the umbel family (Apiaceae), or at the end of an elongated, backward-facing spur, as found in the flowers of species like toadflax (*Linaria*) and violet (*Viola*). Although bees consume some of the pollen they collect in a flower, some pollen grains will be transferred to another flower's pistil, allowing the egg cells to be fertilised through a complex pollination process, enabling the plant to produce viable seeds.

Pollen grains are often variable with family, genus or species characteristics in appearance, shape, surface structure and size (10-100 µm in diameter) as well as the number of pore openings and the location and shape of the pores (Faegri and Iversen, 1964; Muller, 1979; Hao et al., 2020) (Figure 1). They may even have different colours, see Figure 2.

Figure 2. Pollen from different plants has different colours. Circle: Two honey bees with pollen in the pollen basket on the hind leg. Bees usually collect pollen from a single plant species, and the colour of the bees' pollen load therefore corresponds to the plant from which it was collected. Here, two bees with orange (likely dandelion - *Taraxacum*) and light yellow (likely oilseed rape – *Brassica napus)* pollen in their pollen basket on the hind leg. Photo: Yoko L. Dupont. Pollen loads collected by honey bees. Note the different colours of pollen from various plants. Photo: Per Kryger.

The pollen of various vascular plant species has a species-specific nutritional content. This applies to both the total protein content of the pollen and the composition of various proteins and other substances (Roulston and Cane, 2000). Pollen from leguminous plants is particularly protein-rich because the plants form nodules with bacteria that fix atmospheric nitrogen, which is absorbed and transferred to all parts of the plant (Somerville, 2001). On the other hand, maize pollen has a low protein content, and bee colonies that rely on maize pollen grow slowly (Keller et al., 2005a; Höcherl et al., 2012). Maize pollen is also difficult for honey bees to digest, and some pollen grains pass through the bees' digestive tract without releasing nutrients (Human and Nicolson, 2003).

Pollen often contains small amounts of bioactive substances that can be either toxic or beneficial to bees (Mao et al., 2013). Some plant species naturally produce bitter substances (alkaloids), which are also present in pollen. These include, for example, composites such as sunflower (*Helianthus*) and dandelion (*Taraxacum*), whose bitter substances honey bees cannot tolerate in large quantities (Nicolson and Human, 2013; Vanderplanck et al., 2020). Quercetin is a substance found in almost all plant pollen, which likely helps bees to metabolise pesticides more quickly (Ardalani et al., 2021), see Box 2. In addition, pollen can also absorb pesticides, including the controversial insecticides called neonicotinoids (Wood et al., 2019).

The composition and quality of the pollen collected by bees are crucial for the colony's resistance to diseases, especially varroa, and for the winter survival of the bee colony (Annoscia et al., 2017; Requier et al., 2017; DeGrandi-Hoffman et al., 2018).

Box 2: Secondary metabolites

Photosynthesizing plants, with the help of the sun's energy, produce the food that all animals, including honey bees and humans, consume. Nutrients such as sugars, amino acids, fatty acids, and vitamins that nourish animals are called primary metabolites. They are essential elements in almost all biological metabolism on Earth. Plants produce a multitude of other substances that are collectively called secondary metabolites.

Some secondary metabolites are well-known: caffeine from coffee (*Coffea arabica*), nicotine from tobacco (*Nicotiana tabacum*) or salicylic acid from willow (*Salix*). These substances affect humans and animals in various ways. Caffeine is energising, nicotine is a stimulant, and salicylic acid has pain-relieving properties, known from aspirin. Many of these substances can be lethal in high doses. The list of secondary metabolites is very long. For many of them, the biological effects are unknown.

Honey bees are exposed to various secondary metabolites while collecting pollen and nectar from flowers. Several of these substances are known to be toxic to insects, but unlike insects that feed on plant sap extracted from leaves, honey bees must be able to tolerate these substances. This tolerance might be due to lower concentration of these substances in nectar or pollen compared to other parts of the plant, or because honey bees possess a detoxification system that breaks down the secondary metabolites.

Imidacloprid, a pesticide of the neonicotinoid type, has received much attention due to its association with bee death (Barron, 2015). The substance somewhat resembles the secondary metabolite nicotine. Neonicotinoids are an imitation of the well-known nicotine, but these substances are more toxic to insects and less toxic to humans than nicotine. It turns out that honey bees can convert imidacloprid relatively quickly. Honey bees that receive a high dose of imidacloprid collapse shortly after taking in the substance, often when they return to the hive. However, one or two days later, when the bees' detoxification system has broken down the imidacloprid, the "dead" bees can wake up again (Schott et al., 2017).

A secondary metabolite of interest in this context is quercetin, found in pollen from many different plant species. In plants, the function of imidacloprid is related to the germination of seeds in the soil, the growth of pollen tubes in the pistil and overall plant growth. Experiments have shown that honey bees that ingest quercetin along with sugar water have a faster metabolism of imidacloprid than bees that only receive sugar water (Ardalani et al., 2021). When bees feed on nectar and pollen, as they do naturally, they are unlikely to lack quercetin. However, when bees are fed sugar as a substitute for honey, their detoxification system is not stimulated to the same extent. Quercetin has received attention in connection with COVID-19. It is one of the natural substances, i.e., secondary metabolites, that inhibits virus growth. Whether the substance has the same effect on viruses in honey bees is not clarified, but it illustrates why secondary metabolites are interesting subjects for research.

4.2 How do bees collect pollen?

Bees collect pollen from flowering plants in the landscape surrounding their hive. It is not an easy task as flowering constantly changes from place to place and from week to week. However, honey bees have a special system to monitor and efficiently collect nectar and pollen from abundant flower sources in the surrounding landscape.

In the honey bee colony, a group of bees called foragers is responsible for collecting nectar and pollen from flowers. Among the foragers, there is a smaller number of scouts that fly around the landscape in search of good flower sources. When they find resource-rich flowers, they return to the hive and communicate the location to other foragers, called recruits, by performing a dance (von Frisch, 1965). The dance provides information on the direction and distance that the recruits need to fly to find the flower source. This communication system enables honey bees to harvest rich flower resources in the landscape quickly and efficiently, even when the flowering period is short (Seeley, 1987).

Honey bees are strong fliers and forage over a large area. When collecting nectar, bees can gather 29 kcal with an energy expenditure of 1 kcal, making them highly energy efficient (Southwick and Pimentel, 1981). The distance that they fly depends on the location of flowers, and the foraging distance also varies with the season (Couvillon et al., 2015). In a typical Northern European agricultural landscape, the majority (75%) of flights occurred within 3 km of the hive (Couvillon et al., 2014). However, bees may fly 10-12 km to a rich flower source (Beekman and Ratnieks, 2000).

In this study, the surrounding landscape of a honey bee colony is defined as the area within 3 km of the beehive where the bees typically forage. The same distance is also used as the definition of an apiary's foraging landscape in the EU certification of organic honey (https://lbst.dk/tvaergaaende/oekologi/jordbrugsbedrifter/oekologisk-biavl/).

4.3 What do bees collect?

A bee colony uses 17-34 kg of pollen per year, depending on the colony's size (Keller et al., 2005a). Therefore, honey bees typically collect nectar and pollen from larger flower sources in the landscape. These can be flowering crops such as oilseed rape (*Brassica napus*) and clover (*Trifolium*), flowering trees like cherry plum (*Prunus cerasifera*), maple (*Acer pseudoplatanus*), apple (*Malus*) or larger occurrences of wild flowering plants like dandelion (*Taraxacum*) or heather (*Calluna vulgaris*). The specific flowers that bees collect from depend on the landscape and change throughout the season (Odoux et al., 2012; Requier et al., 2015; Dupont and Søgaard Jørgensen, 2017). Therefore, the pollen nutrition of a bee colony, particularly its protein content, is influenced by the types of landscapes present around the hive (Donkersley et al., 2014).

Whether a particular plant species is a good food source depends on both the number of flowers and what else is blooming at a given time, as well as how far the bees need to fly. They can adjust their collection if the colony, for example, lacks a particular nutrient (Hendriksma and Shafir, 2016; Zarchin et al., 2017). But in general, they prefer to collect from large rather than small flower resources and from flowers nearby rather than far from the hive.

4.4 The European CSI Pollen project

The seasonal dynamics of bee pollen diets have been studied in specific landscapes, including agricultural areas (e.g., Couvillon et al., 2014; Requier et al., 2015) and urban areas (Garbuzov et al., 2015).

In 2014 and 2015, the honey bee research network COLOSS (https://coloss.org/) conducted the European project CSI Pollen ("Citizen Scientist Investigation on pollen diversity forage available to honey bees") (Brodschneider et al., 2019; Brodschneider et al., 2021). The project involved 750 beekeepers from 24 European countries. Beekeepers collected pollen using pollen traps, a device placed at the hive entrance that removes some of the pollen loads of the returning bees. These pollen samples were sorted by colour, and the number of different colours was used as a simple measure of pollen diversity (Brodschneider et al., 2021). The study revealed that pollen diversity was influenced by the surrounding landscape, with urban areas in particular contributing to a high diversity of the pollen collected by honey bees. The importance of a species-rich, natural flora in the surrounding landscape for the winter survival of honey bees has recently been demonstrated in a study of honey bees living wild in power lines in Spain (Rutschmann et al., 2022).

As part of the CSI Pollen project, pollen samples from Austria (Brodschneider et al., 2019) and Denmark (Søgaard Jørgensen et al., 2016), among other countries, were analysed by microscopy and identified as pollen types corresponding to a plant family or genus or, in rare cases, a plant species. These studies showed a greater diversity of pollen types than colour determination alone. In other words, different pollen types can have colours that cannot be visually distinguished. However, the detailed botanical identification supported the main conclusions of the CSI Pollen project regarding seasonal variation and the significance of the surrounding landscape in the pollen supply of honey bees.

4.5 The "Bee Pollen Supply" project

To gain more knowledge about the pollen supply of honey bees in Danish landscapes and the importance of pollen nutrition for bee health, we initiated the research project "What is the connection between bee pollen supply, varroa growth and winter mortality in Danish landscapes?" (hereafter "Bee Pollen Supply") in 2019. The project's objectives were:

1. To identify important pollen sources throughout the flowering season in different regions and landscapes in Denmark and clarify the relationship between landscape composition and pollen supply.
2. To investigate varroa mite growth in landscapes with different pollen supplies.
3. To investigate winter losses in landscapes with different pollen supplies.

Results regarding disease and winter survival are reported elsewhere. This report focuses on the honey bee pollen supply with the aim to explore the seasonal development and the significance of the landscape for the pollen supply of bees in Danish landscapes.

5 Materials and methods

5.1 Recruitment of voluntary sample collectors

Similar to the CSI Pollen project, the Bee Pollen Supply project was a citizen science project, where volunteer beekeepers across Denmark contributed to sample collection. To ensure broad geographical coverage, we recruited volunteers during a workshop at the Danish Beekeeping Conference in March 2020. The conference is an annual national meeting, organised by the Danish Beekeepers Association, that covers over 90% of Danish beekeepers from the entire country. A total of 110 beekeepers participated in the workshop, with 59 subsequently signing cooperation agreements for sample collection. Due to the impact of COVID-19, 30 beekeepers conducted sample collection in the 2020 season. To cover the country and include various Danish landscape types, we specifically contacted beekeepers in 2021, aiming to recruit them from all regions so that as many landscape types as possible would be represented. In 2021, we recruited 14 new participants, nine of whom collected samples in 2021. In total, 39 volunteer beekeepers participated in the pollen sample collection in the project (Figure 3).

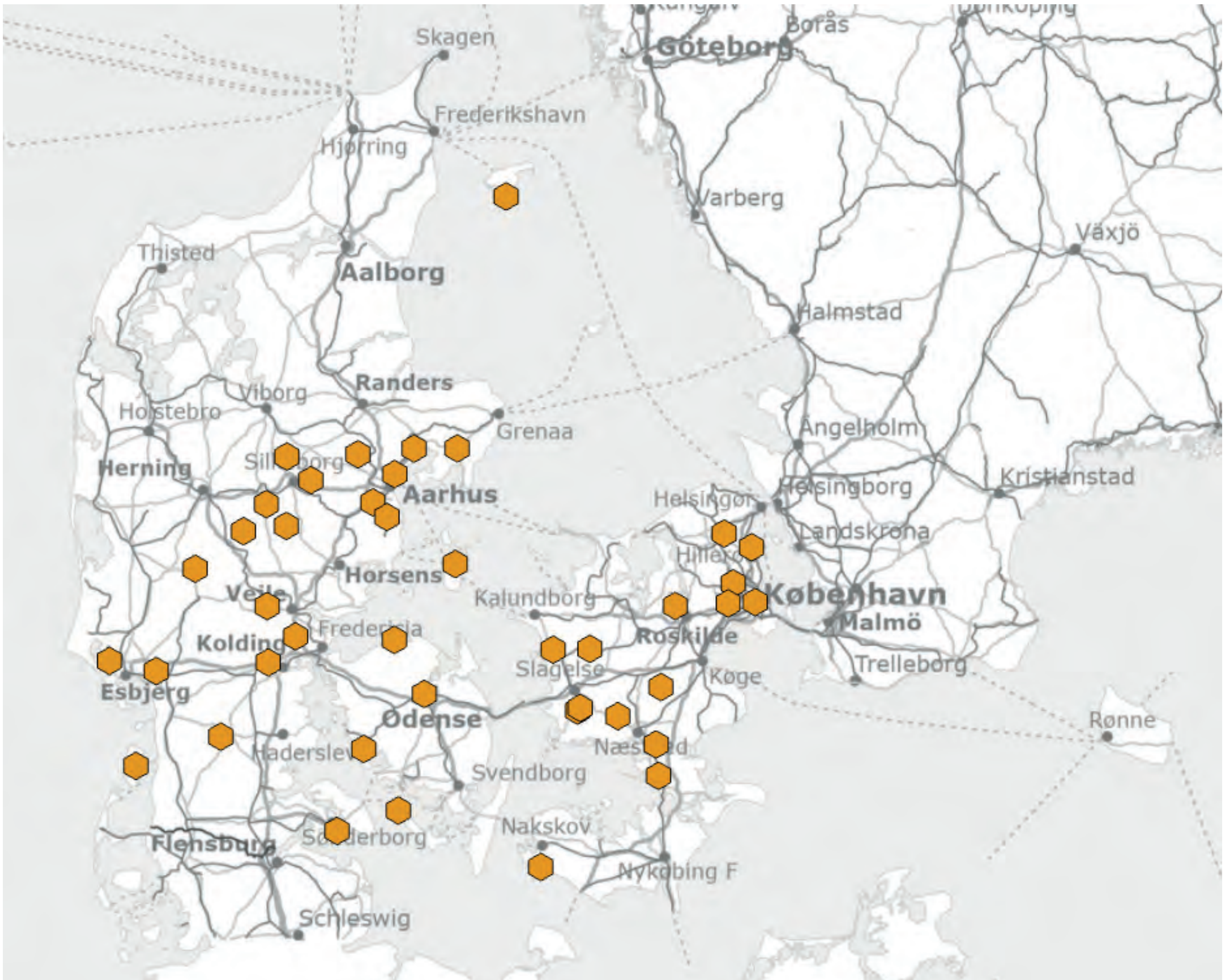


Figure 3. Geographical distribution of observation apiaries where pollen samples were collected during the season 2020 or 2021.

5.2 Collection of pollen samples

As the pollen supply can vary even among bee colonies in apiaries located in the same landscape, each experimental host collected pollen samples from three bee colonies per apiary, if possible. A few apiaries only had two bee colonies. The bee colonies were not used for commercial pollen harvesting during the experiment as removing large amounts of pollen can affect bee health. Sampling was synchronised nationwide by instructing the beekeepers to collect pollen samples during 10 periods, each lasting one week, with three-week intervals throughout the season. Each of the 10 sampling periods is defined as the recommended sampling week, plus six days before and seven days after to ensure that all pollen samples belonged to one sampling period (Table 1). Before each period, beekeepers received an email reminder and a detailed sampling protocol. Pollen samples were collected from late March/early April in 2020 and late April/early May in 2021 to early October in both study years. Beekeepers were advised to adapt sampling to the weather to avoid cooling of the brood, which could have a negative impact on the colony. Due to local climate differences, the first and last sampling could not be conducted simultaneously at all locations. Period 1 is absent from 2021 due to a cold spring.

Table 1. Pollen sampling periods in 2020 and 2021.

	2020		2021	
Period	**Start**	**End**	**Start**	**End**
1	24 March	13 April	-	-
2	14 April	4 May	13 April	3 May
3	5 May	25 May	4 May	24 May
4	26 May	15 June	25 May	14 June
5	16 June	6 July	15 June	5 July
6	7 July	27 July	6 July	26 July
7	28 July	17 August	27 July	16 August
8	18 August	7 September	17 August	6 September
9	8 September	28 September	7 September	27 September
10	29 September	19 October	28 September	18 October

Pollen for samples was collected directly from honey bee wax combs using a bee bread collector, a metal tube with the same diameter as a wax cell, and a piston to eject the bee bread. Alternatively, pollen was scraped using tweezers (Figure 4). Beekeepers were instructed to collect approximately two grams of pollen from cells in the innermost ring around the brood area, closest to unsealed brood (Figure 5). This is assumed to be the newest pollen, reflecting what the bees were collecting at the time of sampling. Pollen samples from different beehives in the same apiary and on the same sampling day were pooled in one sample. All samples were carefully labelled and stored in a freezer until samples could be transferred to the laboratory for botanical analysis.

5.3 Determination of pollen types by microscopy

The samples of bee bread were thoroughly dried in an oven before being sent for botanical analysis. Pollen was identified using microscopy at the QSI Laboratory (Quality Services International, QSI, Germany) following a standard protocol for determination, DIN-Norm 10760 (DIN-Norm-10760, 2002). In the analysis, the collected bee bread was mixed thoroughly with water, and a drop of this mixture was examined under a microscope. The analysis in-

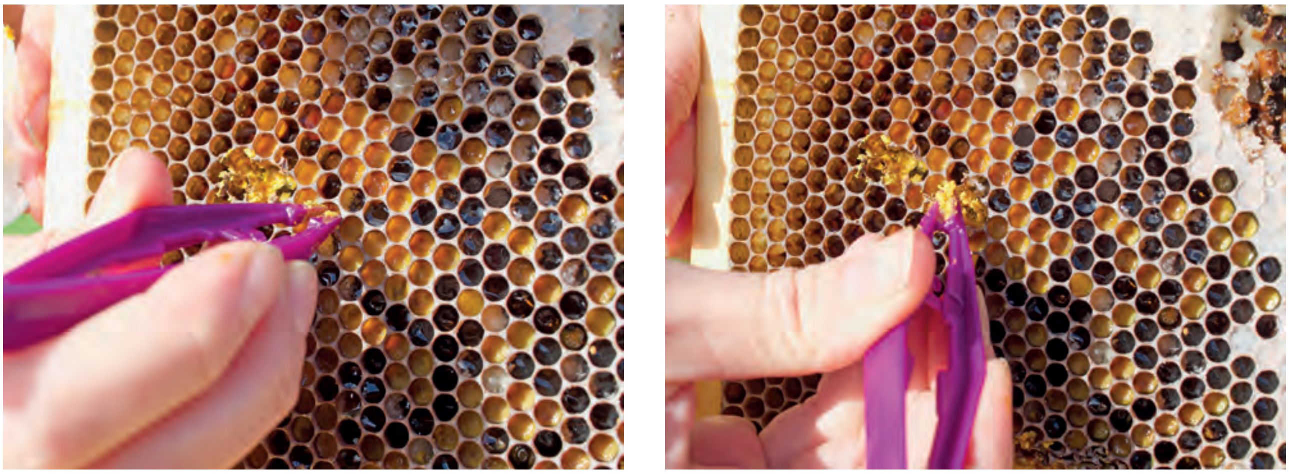


Figure 4. Collection from frame with bee bread, the bees' pollen storage, using tweezers. Photos: Per Kryger and Anna la Cour).

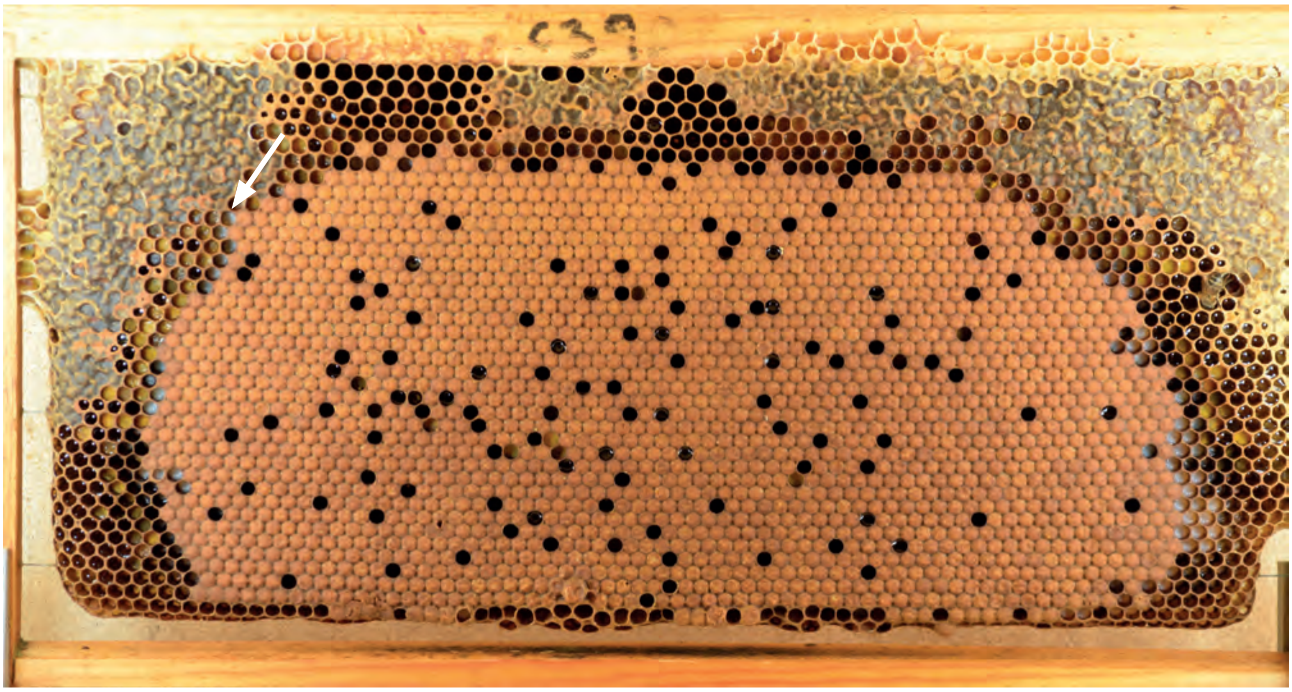


Figure 5. Bee comb from a beehive where the bee bread recently produced by the bees (willow) can be seen as a ring around the sealed brood. Photo: Nuno Capela.

volved counting a maximum of seven of the most prevalent pollen types in a sample of approximately 300 pollen grains. Additionally, the microscopic sample was examined for rarer pollen types. Pollen was identified using reference collections of pollen types (von der Ohe and Von der Ohe, 2007; Halbritter et al., 2018) as well as online databases (paldat.org, pollenatlas.net; globalpollenproject.org; pollenwarndienst.at; pollen.tstebler.ch). Pollen was generally determined to genus or family level and, in a few cases, to species level. Pollen types can include a wide diversity of pollen (number of plant species), and morphological determination of pollen grains did not allow distinction between closely related plant species (G. Kassim, pers. com.).

5.4 Analysis of experimental landscapes

In this study, an apiary's landscape was defined as a circular area with a radius of 3 km around the apiary (see section 4.2 page 10).

In each landscape, we assessed the total area of major landscape types using GIS basemap (Levin, 2019 Table 3.6, p. 36), to obtain the following categories:

- Agriculture (intensively cultivated, fallow land, permanent crops, extensively cultivated areas and unclassified agricultural areas)
- Nature (wet and dry nature)
- Grey city (built-up area, high-density housing, city centre, other housing, industry, airport) and green city (low-density housing, recreational area)
- Forest
- Water (lake, stream, sea)
- Other (road, railway, gravel pit, not categorised)

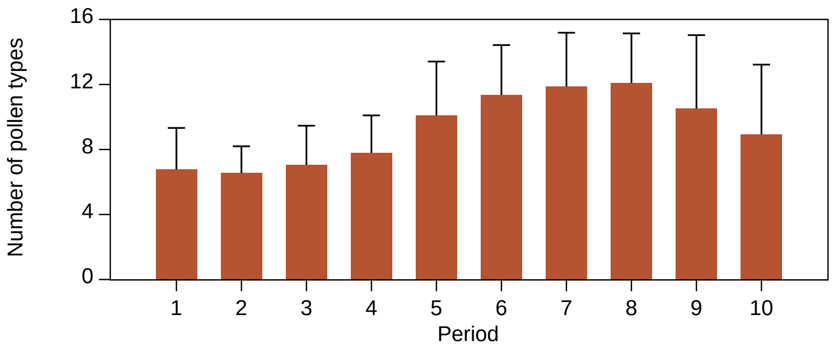
Additionally, these basemap categories were assessed for the whole of Denmark, including a 3 km buffer of water around land areas. All basemap areas were calculated for 2018.

6 Results and discussion

6.1 Diversity of pollen types

In total, 258 bee bread samples were collected from 31 apiaries in 2020 and 53 samples from nine apiaries in 2021. The samples included 264-394 pollen grains per sample (average ± SD: 334 ± 31), and in total 104,321 pollen grains were identified in the study. Morphological determination of pollen through microscopy revealed a total of 105 different pollen types in the bee bread samples. With few exceptions, pollen types were determined to genus or family level of vascular plants. The identified pollen types originated from both crops and wild plants as well as trees, bushes, and herbs. Some forage plants are widely distributed and found throughout the country, while others have limited distribution (Hartvig, 2015).

Figure 6. Seasonal development in pollen diversity. Columns represent the average numbers of detected pollen types per sample (the bars indicate standard deviation between samples). The periods follow Table 1, from late March to mid-October 2020 and 2021.



Bees collect pollen from a wide range of plants, but only a few types were dominant. A total of 20 pollen types occurred with more than 1% in a sample, while the remaining 86 occurred more sporadically.

The honey bees' pollen supply changed throughout the season as various vascular plant species come into bloom. The number of different pollen types per sample was relatively low in late March and early April when relatively few vascular plants bloomed, until period 4, which starts in late May (Figure 6). In this period, the number of flowering insect-pollinated plant species increased accordingly. Pollen diversity then increased until period 8, starting in late August, after which the number of pollen types decreased in autumn when the flowering of plant species declined (Figure 6). On average, 9.42 ± 3.61 pollen types were found per sample. The least varied sample contained only two different pollen types, while the most diverse sample contained 27 pollen types.

6.2 Dominant pollen types

In general, a small number of pollen types constituted most of the samples, while many pollen types were present in relatively small quantities (Figure 7). This shows that honey bees in the study areas specialised in collecting pollen from a smaller number of pollen types at a given time, from which they gather the majority of their pollen reserves. Furthermore, the pollen supply changes throughout the flowering season depending on which vascular plant species are in bloom.

Oilseed rape (*Brassica napus*), willow (*Salix*), white clover (*Trifolium repens*) and fruit trees (*Malus/Pyrus/Prunus*) constituted over 10% of all pollen grains in the samples. The 20 next most abundant pollen types accounted for over 1% of the total number of pollen grains identified in the study. In total, 65

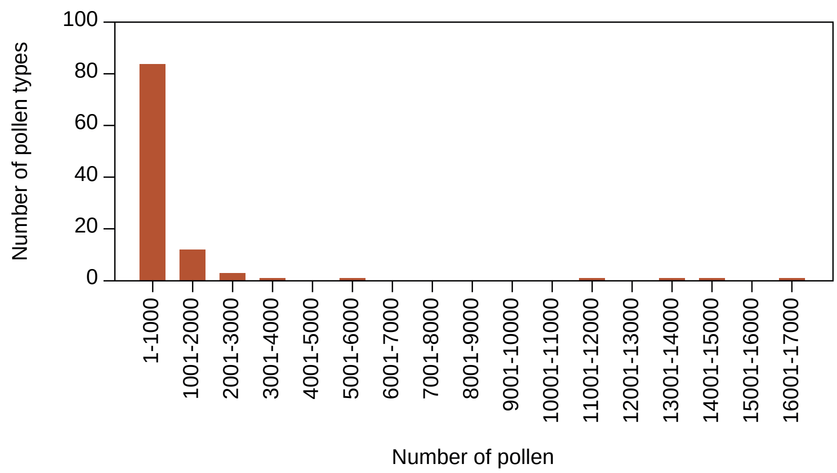


Figure 7. Occurrence of pollen types in bee bread samples. Each bar represents a quantity category determined by the number of pollen found in the overall material (i.e., all identified pollen grains in the bee bread samples: 1-1,000 pollen grains, 1,001-2,000 pollen grains, 2,001-3,000 pollen grains etc.). Most pollen types were represented with fewer than 1,000 pollen grains in the bee bread samples, while a few pollen types were found in larger proportions.

pollen types were represented with more than 50 pollen grains, corresponding to at least 0.05% of the material, see Appendix 1. Pollen types occurring sporadically are not expected to have any nutritional significance for the bees; this may be due to rare visits to the flowers or contamination, for example by pollen deposited on flowers by other flower-visiting insects.

6.3 Period 1 (early April)

The earliest collection of bee bread samples was only conducted in 2020, and the samples were collected from 31 March to 11 April 2020. In this period, a total of 20 different samples were collected, with an overall pollen count of 6,872. A total of 23 different pollen types were identified during this period.

Willow was the most frequently occurring pollen type, occurring in many samples as well as in quantity, counted as number of pollen grains. Willow, the genus *Salix*, encompasses a wide range of species. Their flower buds, known as "catkins", can be observed in late winter, and in early spring staminate and pistillate parts emerge, giving the catkins a yellow and greenish-yellow appearance. Willows are dioecious, meaning that the individual shrubs or trees bear exclusively male or female catkins. Both male and female catkins produce nectar, serving as an essential energy source for many insects in early spring. Additionally, male catkins yield abundant yellow pollen that is collected by honey bees and various wild bee species, with some specialising exclusively in willow pollen collection.

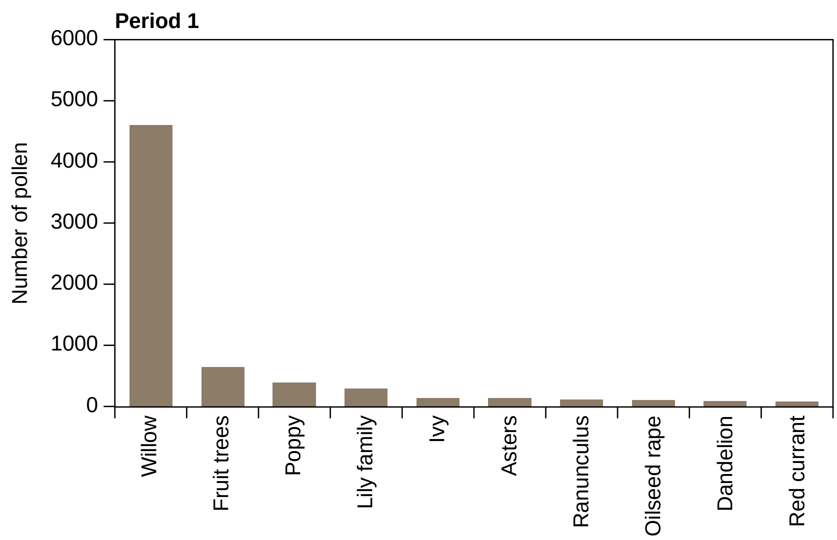


Figure 8. Top 10 of the most numerous pollen types in Period 1.

Figure 9. Top: Flowering bushes of cherry plum (back) and willow (front). Second row: Catkins of male willow (left) and female willow (right). Third row: Honey bee visiting catkins of male willow (left) and female willow (middle and right). Photos: Yoko L. Dupont.

Figure 10. Cherry plum flowering in early spring in urban, rural and nature areas.

The second most prevalent pollen type in Period 1 originated from fruit trees (Figure 8). Cherry plum (*Prunus cerasifera*) is one of the earliest flowering fruit trees, and pollen from fruit trees during this season likely comes from this species (Figure 10). The flowering period for cherry plum is April-May (Frederiksen et al., 2019) but may commence earlier in warm years (pers. obs.). Blackthorn (*Prunus spinosa*) blooms immediately after cherry plum (Frederiksen et al., 2019) and is similarly sought after by honey bees as a source of nectar and pollen.

In the bee bread samples, ivy and asters were also found (Figure 8), which bloom in late autumn. This pollen is likely remnants from the previous season. Pollen identified as buttercups may come from the early-blooming winter aconite (*Eranthis hyemalis*), common in gardens and parks in early spring. Other plants from the buttercup family, from which pollen may originate, include lesser celandine (*Ficaria verna*), growing on forest floors, in parks,


a
b
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i

Figure 11. On warm, sunny spring days, bees eagerly visit the first spring flowers. Garden plants like winter aconite (*Eranthis hyemalis*), crocus (*Crocus* sp.), squill (here Boissier´s glory-of-the-snow, *Scilla luciliae*) and snowdrop (*Galanthus* nivalis) attract bees under the right conditions. However, early flowering wild plants like lesser celandine (*Ficaria verna*) and possibly yellow star-of-Bethlehem (*Gagea lutea*, lily family) may also serve as pollen sources. Photos: Yoko L. Dupont.

gardens and other open areas (Figure 11). Additionally, species of anemone (white anemone, *Anemone nemorosa*, native to Denmark, or, for example, Balkan anemone, *A. blanda*, cultivated in many gardens) may occur (Figure 12). Pollen identified as "lily family" may come from spring-flowering plants related to lilies, including crocus (*Crocus* spp., iris family), snowdrop (*Galanthus nivalis*, amaryllis family) or squill (*Scilla* spp., asparagus family) (Figure 11).

Figure 12. Honey bees gather pollen from wild and planted anemones. Here white anemone (*Anemone nemorosa*, right) and Balkan anemone (*Anemone blanda*, left). Photos. Yoko L. Dupont.

The re-development of the honey bee colony after the winter, when worker bees have been unable to forage, depends on the first flowering plants for the year. The new supply of pollen from spring flowers significantly boosts the production of brood.

6.4 Period 2 (late April)

The second sampling of the year took place from 16 April to 3 May 2020 and from 30 April to 1 May 2021. In this period, 35 samples were collected, totalling 11,879 pollen grains, representing 33 different pollen types.

Pollen from willow (*Salix* spp.) was still the most abundant pollen type, but oilseed rape (*Brassica napus*) and fruit trees (*Malus*/ *Pyrus*/*Prunus*) constituted a significant part of the bees' pollen supply (Figure 13). In Denmark, the majority of cultivated oilseed rape is winter oilseed rape (Axelsen et al., 2011), which blooms from late April to late May. The flowering of oilseed rape is influenced by climatic conditions (d'Andrimont et al., 2020), and various cultivars exist that bloom relatively early or late. Therefore, the flowering of oilseed rape covers an extended period, and different oilseed rape fields in the landscape may bloom at different times.

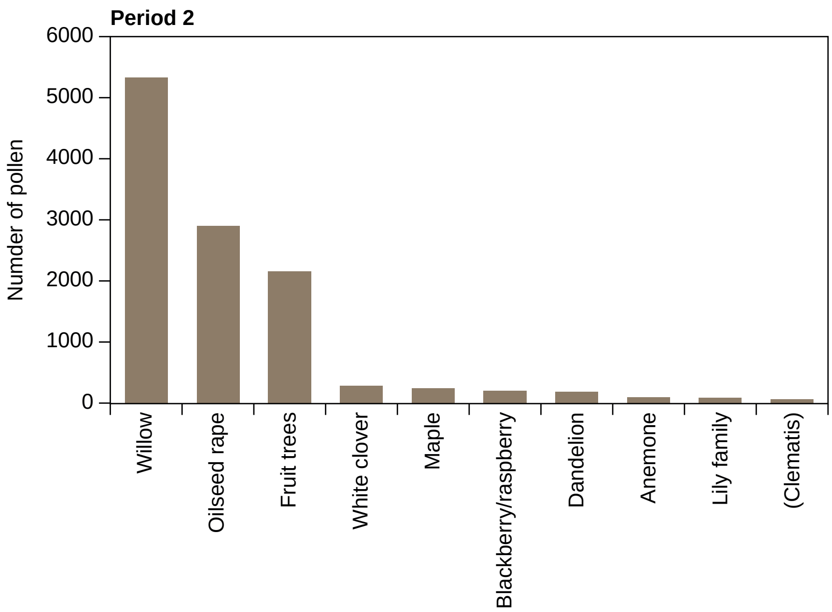


Figure 13. Top 10 of the most numerous pollen types in Period 2.

Winter oilseed rape (Figure 14) is the flowering crop covering the largest area in Denmark (Axelsen et al., 2011), making oilseed rape a rich pollen source available to bees and other insects over a relatively long period in spring. Oilseed rape is a particularly important source of pollen for honey bees, especially when the development of the bee colony has gained momentum, and it has a workforce of a certain size that can bring home abundant pollen and nectar resources from the blooming oilseed rape fields (Svend Sejr, pers. com.).

The large quantities of nectar that honey bees can collect in oilseed rape in a short time also allow the pollen collecting worker bees to search for pollen in other plants than oilseed rape. In spring, various species of fruit trees bloom abundantly, including species of pome fruit (*Malus/Pyrus* spp.) and stone fruit (*Prunus* spp.) in hedges, gardens, and green areas, as well as dandelion, anemone, and other spring-flowering herbs. Poor weather conditions in early spring may prevent bees from flying frequently or far from the hive.

Figure 14. Winter oilseed rape is a widespread crop visited by, among others, honey bees. Photos: Yoko L. Dupont.

Prolonged periods of cold weather or precipitation can significantly delay the development of bees in spring.

Another pollen type that is becoming more common in the pollen supply of honey bees is pollen from fruit trees (Figure 13, 16). Cherries, plums, apples, and pears all bloom in spring and have various cultivars with different flowering times (Jensen et al., 2019). Furthermore, flowering time is influenced by climate conditions (Drepper et al., 2020; Kunz and Blanke, 2022). The flowering period of each fruit tree is usually short, often a week or two. Fruit trees are found in gardens and orchards but also frequently grow wild in hedges and forest edges.

6.5 Period 3 (mid-May)

The third sampling took place from 10 May to 21 May 2020 and from 9 May to 18 May 2021. A total of 40 samples were collected, with a total pollen count of 13,584 distributed among 39 different pollen types.

Figure 15. Top 10 of the most abundant pollen types in period 3.

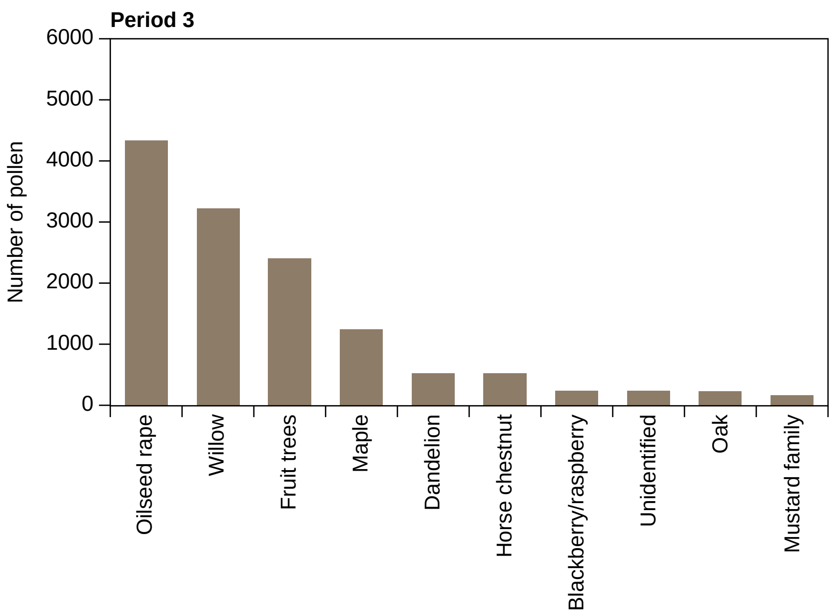


Figure 16. In spring, a wide range of white-flowered fruit trees bloom, including cultivated and wild species: a) apple orchard, b) flowers of sloe, c) flowers of pear, d+e) apple blossoms visited by mining bee (*Andrena haemorrhoa*), f) bird cherry visited by honey bee, g) cherry blossoms, h) flowering tree of cherry plumand i) honey bee with pollen in its pollen basket in crab apple flower. Photos: Yoko L. Dupont.

Figure 17. Field maple (top), sycamore (middle) and Norway maple (bottom) all have greenish/yellowish nectar-rich flowers that bloom in May and are frequently visited by honey bees.

In this period, oilseed rape was the most numerous pollen in the honey bees' pollen supply, followed by willow, fruit trees and maple (Figure 15). In Denmark, the maple genus *Acer* includes the native species sycamore (*Acer pseudoplatanus*), Norway maple (*Acer platanoides*) and field maple (*Acer campestre*), all of which bloom in May (Frederiksen et al., 2019) (Figure 17).

Additionally, several species are cultivated as ornamental plants in gardens. Sycamore is a common tree in deciduous forests, gardens, and parks, where the greenish-yellow inflorescences hang below the newly unfolded light green leaves. The closely related native Norway maple is also a common tree in Danish deciduous forests and is additionally a common sight in urban areas, where it is used as an avenue tree (Hartvig, 2015). The flowers are greenish-yellow, borne in semi-spherical inflorescences and flower before the leaves emerge. Nectar is secreted from a disc in the flower base, making its flowers a good nectar source with up to 800 kg of honey/ha (Janssens et al., 2006), col-

Figure 18. In addition to honeybees (top), dandelions attract a wide variety of insects, including hoverflies (bottom left) and wild bees. Photos: Yoko L. Dupont.

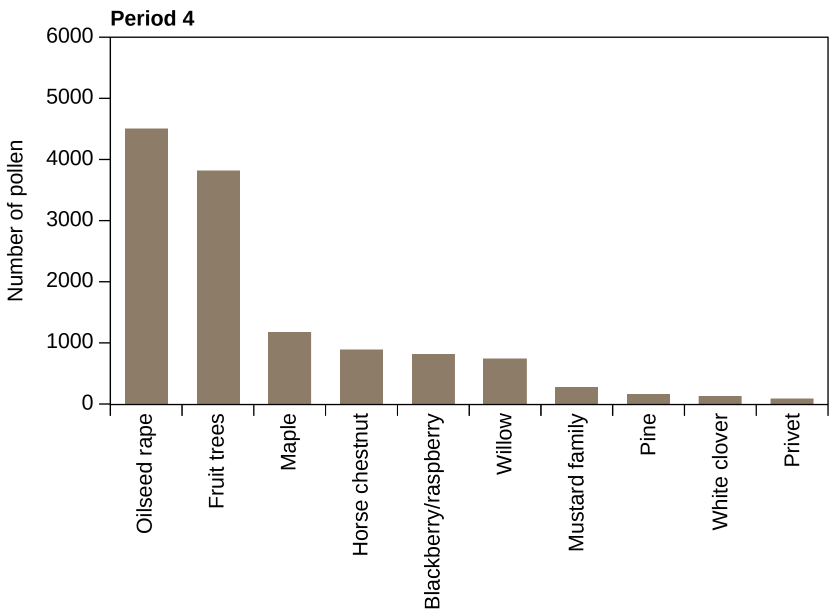
lected by both honey bees and bumble bees as well as other insects. Field maple grows in, among other places, forest edges, hedges, and urban areas. Its flowers resemble those of sycamore and Norway maple.

Dandelion and horse chestnut also constituted a smaller part of the bees' pollen supply in period 3 (Figure 15). Dandelion flowers along roadsides and in gardens and grassy fields from April. In early May grassy areas can turn yellow with dandelions (Figure 18). In addition to honey bees, dandelions attract a wide range of wild bees, flies, and other insects. Pollen identified as dandelion is found in the honey bees' pollen supply throughout the season, although its occurrence peaks during the main flowering of dandelions in May (Figure 15). However, this can also cover other related composite flowers such as hawkweed (*Hieracium* spp.) and catsweed (*Hypochaeris radicata*), which bloom later in the season after the flowering of dandelions (G. Kassem, pers. com.). The fact that pollen from dandelion and related composite flowers does not make up a significant proportion of the honey bees' pollen supply, despite the species' widespread distribution and abundance of flowers, is probably related to the fact that brood development does not occur on a pure dandelion diet (Loper and Berdel, 1980). This could be due to poor nutritional composition or secondary metabolites in composite flower pollen (see box 2), and possibly because the pollen is spiky and thus difficult to digest (Vanderplanck et al., 2020).

6.6 Period 4 (early June)

The fourth sampling took place from 1 June to 15 June 2020 and from 28 May to 6 June 2021. The volunteers collected 40 samples with a total of 13,313 pollen grains distributed among 42 different pollen types.

Figure 19. Top 10 of the most numerous pollen types in Period 4.



In early June, oilseed rape (*Brassica napus*) and fruit trees continued to dominate the pollen types in the samples of bee bread (Figure 19). However, since the flowering of both oilseed rape and fruit trees is usually declining at this time of the year, the pollen supply may reflect the honey bees' pollen stores in a period when flowering in the landscape is sparse.

Pollen identified as fruit tree pollen at this time of year may also include hawthorn (*Crataegus* spp.), rowan (*Sorbus* spp.) (Figure 20), quince (*Cydonia oblonga*) and cotoneaster (*Cotoneaster* spp.) (Figure 21), which are related to the stone fruit genus (*Prunus*) and the pome fruit genus (*Malus/Pyrus*). The native common bird cherry (*Prunus padus* subsp. *padus*) (Figure 20) is common throughout the country

Figure 20. Common rowan (*Sorbus aucuparia*) (top), common hawthorn (*Crataegus monogyna*) (middle) and bird cherry (here common bird cherry, *Prunus padus*, bottom). Hawthorn (*Crataegus* spp.) blooms in hedges throughout Denmark in May-June (Hartvig, 2015) and is often eagerly visited by foraging honey bees. Photos: Yoko L. Dupont.

Figure 21. When cotoneaster is flowering, it attracts a lot of insects. Flowers of the open, upright and white form (here creeping cotoneaster, *Cotoneaster dammeri*) are visited by many different insects, including honey bees, wild bees, butterflies and others. Flowers of the urn-shaped, nodding pink form (here wrinkled cotoneaster, *Cotoneaster bullatus*) attract especially bumblebees (upper panel) (*Bombus* spp.) and wasps (*Vespula* spp. and *Dolichovespula* spp.) (lower panel).

(Hartvig, 2015), while the North American black cherry (*P. serotina*), now classified as invasive (https://mst.dk/natur-vand/natur/artsleksikon/froeplanter/glansbladet-haeg/), is naturalised especially in Jutland (Hartvig, 2015). The former blooms in May and the latter in June (Hartvig, 2015) (Figure 20).

Cotoneaster is a shrub, mostly grown as an ornamental in gardens, but it can also grow wild in Denmark. In addition to the two native species, red- and black-fruited cotoneaster (*Cotoneaster scandinavicus* and *C. niger*), 12 species and a hybrid have been observed in Denmark (Hartvig, 2015). The bushes bear many small flowers that are visited frequently by insects. Cotoneaster has two types of flowers (Proctor et al., 1996). Some species have open, upright white flowers, which superficially resemble the flowers of, for example, hawthorn (Figure 21). They attract many honey bees as well as wild bees, butterflies, hoverflies, and other insects. Other species of cotoneaster have pendant, jar-shaped pink flowers (Figure 21). These are visited especially by bumblebees and wasps, but also honey bees.

Figure 22. In urban areas, privet hedges bloom with sweet-scented white inflorescences if left un-trimmed. Honey bees are fond of privet flowers. Photos: Yoko L. Dupont.

Pollen from maple (*Acer*) and willow (*Salix*) (Figure 19) may also be left over from the spring pollen supply of bees, although some willow species flower in June, and maple pollen may come from the garden plant Japanese maple (*Acer palmatum*), which flowers in June. (Norrie et al., 2003).

White clover (*Trifolium repens*), raspberry (*Rubus idaeus*) and privet (*Ligustrum vulgare*) (Figure 22) are starting to flower in early June (Figure 19). Blackberry and raspberry both belong to the genus *Rubus*, which is why their pollen is easily confused.

Figure 23. Horse chestnut with "candlesticks" of large inflorescenes. The pollen has a characteristic red colour (bottom image: the arrow points to the stamens with red pollen). Photos: Yoko L. Dupont and Per Kryger

Figure 24. Pine belongs to conifers, which bear the male reproductive organs (microsporophylls) in raceme-like structures. The female reproductive organs (macrosporophylls) are carried in cones and grow significantly when pollinated. Left: Pine tree, middle: shoot with male cones, right: cones (fertilised female flowers) at the top and male cones at the bottom of the shoot.

Horse chestnut trees bloom in May (Frederiksen et al., 2019). The trees can grow over 30 m tall (Staun and Møller, 2015) and bear large white or pink inflorescences that are rich in both nectar and pollen. Horse chestnut pollen can be recognised by its red colour, which is otherwise a rare pollen colour (Figure 24).

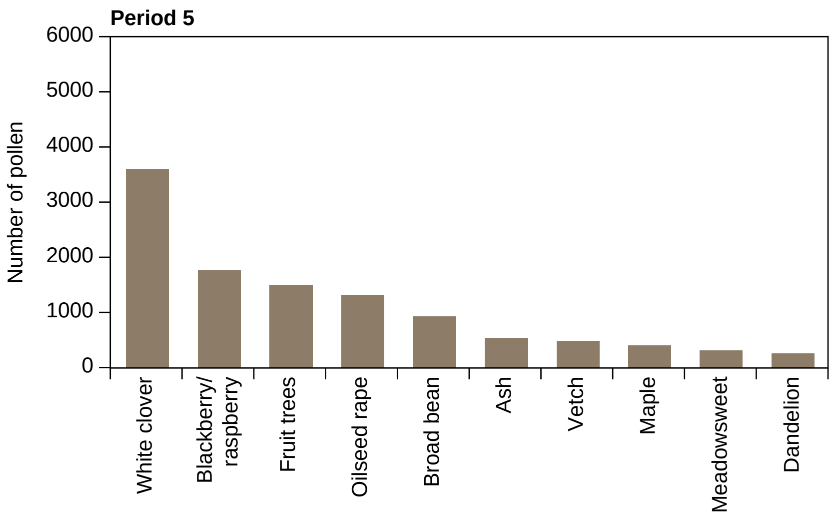
Pine (*Pinus* spp), which blooms in late May, is adapted to wind pollination (Proctor et al., 1996) (Figure 24). Pollen from wind-pollinated plants is generally dry and powdery, and the pollen grains are small and smooth compared to pollen from insect-pollinated plants, which is often larger and sticky (Proctor et al., 1996). Therefore, even small amounts of pine pollen contain many pollen grains, which is why we estimate that pine pollen is not generally a significant part of the honey bee diet. However, the presence of pine pollen in 6 out of 42 samples in this period suggests that bees may, in some cases, collect pollen from wind-pollinated plants, including pine.

6.7 Period 5 (late June)

The fifth sampling was conducted from 16 June to 2 July 2020 and from 19 June to 28 June 2021. Volunteers collected 40 samples with a total of 13,183 pollen grains distributed among 57 different pollen types.

In addition to white clover (*Trifolium repens*), blackberry/raspberry (*Rubus* sect. *Rubus*/*R. idaeus*), fruit trees, and oilseed rape are among the most dominant pollen types in this period (Figure 25). White clover, which blooms from June to September (Frederiksen et al., 2019), becomes the preferred pollen source from

Figure 25. Top 10 of the most abundant pollen types in Period 5.



Period 5 onwards (Figure 25). This species is a crucial pollen source for honey bees and remains the most dominant in bee bread samples up to and including Period 8 (August) (Figure 26). Out of a total of 311 samples, white clover pollen was found in 151 samples with a total pollen count of 13,385 pollen grains, corresponding to 13% of the total number of identified pollen in the study

Oilseed rape pollen may be remnants of pollen from the bees' pollen supply during the flowering of winter oilseed rape in May. However, oilseed rape pollen from this period (from late June) may also originate from spring oilseed rape, which blooms later than winter oilseed rape. However, spring oilseed rape covers only a modest area in Denmark compared to winter oilseed rape (Axelsen et al., 2011). Pollen determined as oilseed rape pollen may also come from the introduced wild mustard (*Brassica rapa* subsp. *campestris*), which grows in fields, roadside verges and other soils where disturbances are frequent (Hartvig, 2015). Other possible pollen sources closely related to oilseed rape include field mustard (*B. rapa* subsp. *oleifera*), turnips (*B. rapa* subsp. *rapa*), wild cabbage (*B. oleracea*) as well as other crops such as brown mustard (*B. juncea*) and black mustard (*B. nigra*), which bloom from June to August (Rostrup and Jørgensen, 1973; Frederiksen et al., 2019) (Figure 27).

Figure 26. White clover is common in lawns in urban areas, grasslands in agricultural areas and as a seed crop in Denmark.

Figure 27. Flowering wild cabbage (*Brassica oleracea*) is visited by many insects, including honey bees. Here, kale (left) and rapini (right). Photos: Mette B. Greve and Yoko L. Dupont.

Pollen determined as fruit tree pollen (Figure 25) may also originate from bee bread collected in spring or from hawthorn, cotoneaster and other summer-flowering shrubs of the rose family. Pollen from maple trees in period 5, covering the last half of June, likely comes from the garden plant Japanese maple (*Acer palmatum*).

Another common pollen type in midsummer samples is pollen from the genus *Rubus*. In June, it is primarily from raspberries (*Rubus idaeus*) (Figure 28), but pollen can also come from other *Rubus* species, including dewberry (*R. caesius*) and early flowering species of blackberries (*Rubus* sect. Rubus) such as common blackberry (*R. plicatus*) or hazel blackberry (*Rubus* sect. *Corylifolii*) (Pedersen and Schou, 1989; Frederiksen et al., 2019).

Figure 28. Raspberries are cultivated as a crop in many gardens (left). Raspberries also grow wild in forest edges and thickets (middle and right). Photos: Yoko L. Dupont and Erik Traun.

At the end of June, pollen from flowering crops such as broad beans (*Vicia faba*), vetch (*Vicia* spp.) and lacy phacelia (*Phacelia tanacetifolia*) is also among the 10 most common pollen types (for a description of lacy phacelia, see period 6). Broad beans are a crop that has seen significant growth in Denmark in recent years (Statistics Denmark) due to an increasing need for local plant protein production. There are different cultivars that vary in flower colour (white, pink to dark violet) as well as seed protein composition and content. The flower can, to some extent, set seeds without insect pollination, but several cultivars have increased yields with visits from bees, while others may give lower yields (Bishop et al., 2020). Bee hives are often placed for pollination in broad bean fields, and broad bean flowers attract both honey bees and bumblebees (Figure 29).

Figure 29. Broad bean fields are increasingly common in Danish farmland. Broad bean flowers are visited by both bumblebees (top left) and honey bees (top right) that collect both nectar and pollen. Photos: Yoko L. Dupont.

The genus vetch (*Vicia*) also includes the species complex *Vicia sativa*, which blooms from May to July (Frederiksen et al., 2019) (Figure 30). This species group consists of the closely related common vetch (*V. sativa* subsp. *sativa*), spring vetch (*V. sativa* subsp. *segetalis*) and narrow-leaved vetch (*V. sativa* subsp. *nigra*), as well as a range of hybrids, especially between spring vetch and narrow-leaved vetch. Common vetch occurs in the wild but is also cultivated for fodder (Hartvig, 2015) and in flower strips (Wind and Berthelsen, 2013). Another widespread species is tufted vetch (*V. cracca*), which grows with blue-violet inflorescences along roadsides, in field edges, scrub and mead-

Figure 30. Tufted vetch (*V. cracca*) often blooms along roadsides in mid-summer (left pictures – note the large earth bumblebee (*Bombus terrestris*) in one of the pictures). Narrow-leaved vetch (*V. sativa* subsp. *nigra*) is seen here in bloom along a road-side (right picture). Photos: Yoko L. Dupont.

ows and can be found in dense stands (Figure 30). Many vetch species bloom in the summer period, from June to August (Frederiksen et al., 2019). Pollen analysis does not distinguish between wild and cultivated vetch species as a source of pollen for honey bees.

Finally, a surprising result of the pollen analysis is that ash (*Fraxinus excelsior*) pollen is the 6th most abundant pollen type in bee bread from this period. Like pine, ash is a wind-pollinated plant (Proctor et al., 1996; Wallander, 2008). The trees are often unisexual male or female but can be bisexual (Frederiksen et al., 2019). The flowers are inconspicuous, and the pollen grains are small and easily carried by the wind (Proctor et al., 1996) (Figure 31). Generally, ash flowers do not attract insects, but under certain circumstances honey bees collect ash pollen. Out of 47 samples from this period, only 7 samples contained ash, and the pollen type represented between 2% and 75% of the bee bread sample.

Figure 31. Ash flowers just before the leaves emerge, and the male or bisexual flowers with stamens hang in small clusters from the branches, allowing the pollen to be easily carried by the wind. Left and middle: blooming ash. Right: ash with leaves. Photos: Yoko L. Dupont.

6.8 Period 6 (mid-July)

During the 6th sampling period, samples of bee bread were collected from 10 July to 27 July 2020 and from 12 July to 24 July 2021. A total of 36 samples were collected, with a combined pollen count of 12,091, distributed across 56 different pollen types.

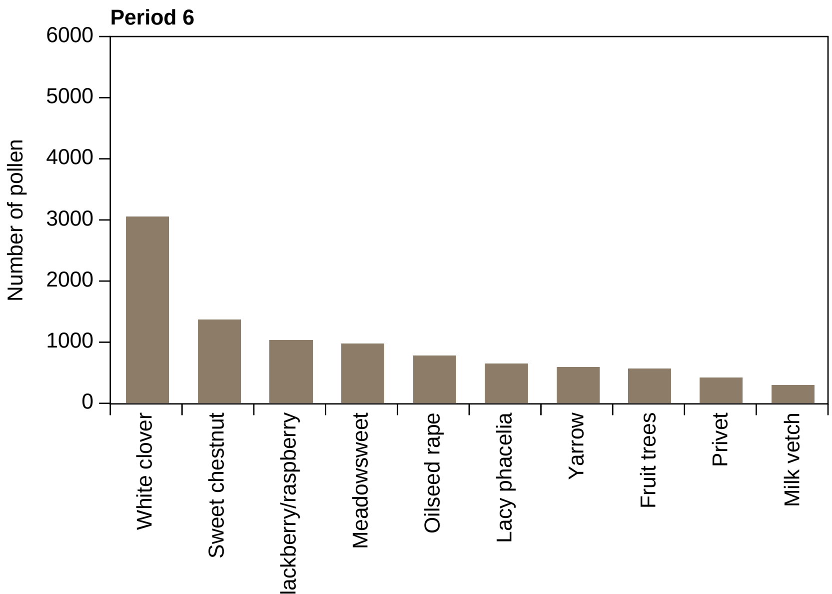


Figure 32. Top 10 of the most numerous pollen types in period 6.

In July, white clover remains by far the most important pollen source, but honey bees also collect a wide range of other pollen types (Figure 32). Pollen from the genus *Rubus* constitutes a good pollen source in period 6, but whereas *Rubus* pollen in the preceding period was likely collected from raspberries, it is probably pollen from blackberries (*Rubus* sect. *Rubus*) in July and later (Figure 33). Blackberry bushes are common in hedges, forest edges, thickets, and meadows throughout the country (Hartvig, 2015) as well as in many gardens.

Pollen from the sweet chestnut (*Castanea sativa*) is the second most numerous pollen type in the samples from this period (Figure 32). It is well known that honey bees visit the flowers of the sweet chestnut (Grygorieva et al., 2016). Sweet chestnut carries its flowers in upright inflorescences, where the upper

Figure 33. Blackberries are actually a group of many closely related apomictic micro-species that bear fruit without fertilisation and are widespread and adapted to local areas (Frederiksen et al., 2019). Blackberries can, therefore, have different growth forms and appearances of flowers and leaves. The flowers are visited by both honey bees and bumblebees as well as other insects. Photos: Yoko L. Dupont.

flowers are typically male and the lower ones female (Figure 34). The flowers can be pollinated by insects, but wind pollination can also occur if the pollen has not been collected by insects and, therefore, dries out (Proctor et al., 1996). Sweet chestnut has small, elongated pollen grains compared to, for example, oilseed rape, which has medium-sized round pollen grains with a volume of up to 10 times the pollen of sweet chestnut (Grygorieva et al. 2016, paldat.org). Thus, the high pollen count of sweet chestnut covers a smaller volume of pollen in the bees' pollen supply compared to pollen types with larger pollen grains.

Figure 34. The sweet chestnut blooms in Denmark in June-July and carries long catkins with many yellowish-white male flowers and at the base a few greenish female flowers (Hansen, 1981). Photos: Per Kryger.

Another pollen type that occurs abundantly in July is meadowsweet (*Filipendula*) (Figure 32, Figure 35). Meadowsweet is a perennial herb. In Denmark, there are two native species, dropwort (*F. vulgaris*) and meadowsweet (*F. ulmaria),* both of which have small white flowers in sweetly aromatic inflorescences. There are also cultivated forms with white or pink flowers. Meadowsweet is widespread and common throughout the country and can grow in dense stands on damp and wet soils.

Figure 35. In July, meadowsweet blooms along roadsides, streams and in moist meadows. The white, sweetly scented flowers are often visited by foraging honey bees. Photos: Yoko L. Dupont.

Pollen classified as oilseed rape (*Brassica* spp.) is among the 10 most abundant pollen types in bee bread samples in both period 6 (mid-July) and period 7 (early August). This pollen may be left over from winter or spring oilseed rape, but it could also come from other species within the *Brassica* genus, including cultivated forms of wild cabbage (*Brassica oleracea*) and turnip (*Brassica rapa* subsp. *rapa*), or wild species such as wild mustard (*Brassica rapa* subsp. *campestris*), brown mustard (*B. juncea*) or black mustard (*B. nigra*), which bloom from June to September (Frederiksen et al., 2019) (Figure 27).

Figure 36. Lacy phacelia is often cultivated in fallow fields or flower strips where it attracts many bees and other insects, especially bumblebees and honey bees. In the image of the honey bee (right), the blue pollen from lacy phacelia is visible in the pollen basket on the bee’s leg. Photos: Yoko L. Dupont and Per Kryger.

Lacy phacelia (*Phacelia tanacetifolia*) pollen is among the top 10 most abundant pollen types from mid-July throughout the remaining season (Figure 32). The flowers are rich in nectar and have long stamens with dark blue pollen. They are often visited by bumblebees and honey bees (Figure 36). Lacy phacelia is cultivated in Denmark as a companion plant in flower strips on fallow fields or as a cover crop and can also grow wild along, for example, roadsides, but it is originally from North America (Hartvig, 2015).

6.9 Period 7 (early August)

The seventh sampling of bee bread took place from 29 July to 16 August 2020 and from 3 to 15 August 2021. Period 7 included 35 samples with a total pollen count of 11,600, distributed among 63 different pollen types.

At the beginning of August, white clover pollen is the most dominant pollen type in the bee bread samples, accounting for more than a quarter of the pollen in the period (more than 3000 pollen grains) (Figure 37). During the same period, several different pollen types occur with smaller proportions, ranging from 300 to 800 pollen grains. Pollen diversity, i.e. the total number of different pollen types per sample, peaks in August (Figure 6), making late summer the most diverse time of the season for bee pollen supply. This may be partly due to bees collecting pollen from many different flowering plant species at this point in the flowering season (Couvillon et al., 2014). Another or complementary explanation is that residues of bee bread from earlier in the season accumulate in the samples.

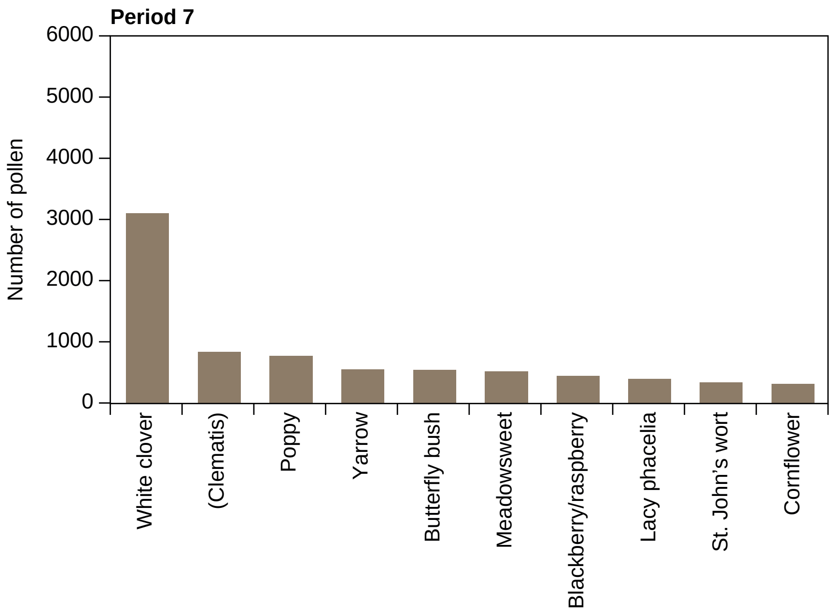


Figure 37. Top 10 of the most abundant pollen types in period 7.

A new pollen type identified as pollen from the buttercup family, likely clematis, is the second most abundant pollen type (Figure 37, Figure 38). Clematis (*Clematis* spp.) is a large genus of climbing plants with colourful flowers, many of which are cultivated as ornamental plants. The introduced late summer-flowering (July-August) old man's beard (*C. vitalba*) is widespread in Denmark, except for the northwestern part of Jutland, west and south of the ice marginal line (Hartvig, 2015). It grows in urban areas and in forests, where it has become naturalised, especially in forest edges and clearings (Hartvig, 2015). The creamy-white, fragrant flowers have numerous stamens with pollen (Frederiksen et al., 2019) and are often visited by honey bees (pers. obs). Old man's beard pollen is one of the most abundant pollen types in August and September (periods 7-9), which may partly reflect the fact that old man's beard is particularly abundant in the areas where the experimental hives were located (Figure 3).

Figure 38. Clematis is a good pollen source for honey bees in late summer. Photos: Yoko L. Dupont.

Another commonly occurring pollen type in late summer is poppy (*Papaver* spp) (period 6+7) (Figure 37, Figure 39). Three red-flowered wild species of poppy are common in Denmark: corn poppy (*Papaver rhoeas*), long-headed poppy (*P. dubium* subsp. *dubium*) and prickly poppy (*P. argemone*), all of which grow in agricultural fields and along roadsides (Hartvig, 2015). The first two bloom from late June to August, while the latter especially flowers in spring (Frederiksen et al., 2019). Moreover, several exotic species of poppy are planted in gardens and parks. Poppy flowers do not contain nectar, but the numerous stamens produce abundant black pollen.

Figure 39. Corn poppy grows in cereal fields, along roads and on fallow land. Poppies are often included in seed mixtures in the flower strips of agricultural areas. Photos: Yoko L. Dupont.

Figure 40. Common yarrow is a perennial native herb often found along roadsides. It has small flower heads with white ray florets. The species is common and has a long flowering period. Photos: Yoko L. Dupont.

Yarrow (*Achillea* spp) are perennial herbs belonging to the Asteraceae family with white flower heads in umbrella-shaped inflorescences (Figure 40). Common yarrow (*Achillea millefolium*) is widespread throughout Denmark and is common along roadsides, on fallow land, meadows and grasslands (Hartvig, 2015). It blooms from July to October (Frederiksen et al., 2019). Sneezewort yarrow (*Achillea ptarmica*) is another species of yarrow occasionally found in moist soil in meadows and ditches, especially in Jutland and northeastern Zealand (Hartvig, 2015). Pollen from yarrow is among the 10 most numerous pollen types in period 6 (mid-July) and in period 7 (early August).

Figure 41. In Danish gardens, many different cultivars of butterfly bush are grown. The flowers contain nectar that attracts butterflies, hoverflies and other insects. The flowers are also a rich pollen source to honey bees. Photos: Yoko L. Dupont.

Butterfly bush (*Buddleja davidii*) produces long clusters of purple, pink or white nectar-rich and sweetly fragrant flowers (Figure 41). The inflorescences attract both butterflies and bees as well as other flower-visiting insects. The plant is a deciduous shrub native to western China (Hartvig, 2015) and is a popular ornamental shrub in gardens. In Denmark, it blooms in August-September (Norrie et al., 2003). The butterfly bush is an important source of pollen for honey bees in period 7 and 8 (August).

6.10 Period 8 (late August)

The eighth sampling took place from 23 August to 6 September 2020 and from 25 August to 3 September 2021. During this period, 32 samples were collected. The total pollen count was 10,702, distributed among 61 different pollen types.

White clover still makes up a significant proportion of the bee bread samples, but this probably reflects in part the earlier pollen supply, as white clover flowering declines in late summer (Birte Boelt, pers. com.) (Figure 42). However, the second most abundant pollen type in Period 8, heather (*Calluna vulgaris*), has its flowering period in August to September (Frederiksen et al., 2019). The flowering usually peaks in late August (Enkegaard et al., 2016). Heather is a dwarf shrub that grows on nutrient-poor soil, in open light or in moderate shade on forest floors (Hartvig, 2015). On heathlands, heather can grow as a dense, purple carpet, and its flowers are frequently visited by bees. The nectar production by heather is highly dependent on the weather during the season (Enkegaard et al., 2016). Although honey bees collect large quantities of nectar from heather, the amount of heather pollen in European heather honey varies from 10 to 76% (Søgaard Jørgensen and Theuerkauf, 2016).

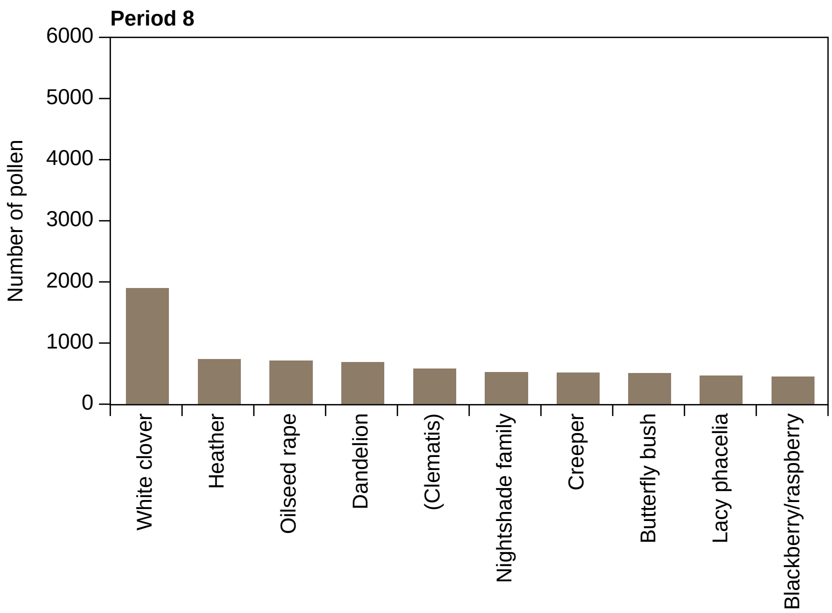


Figure 42. Top 10 of the most abundant pollen types in Period 8.

Surprisingly, pollen identified as dandelion is one of the most common pollen types. Dandelion flowers may appear in late summer, but they are usually scarce. In contrast, the related catsear (*Hypochaeris radicata*) blooms abundantly in grassy fields and roadside ditches, as do other yellow composite flowers such as hawksbeard (*Crepis* spp.), hawkweeds (*Hieracium* spp.), hawkbits (*Leontodon*) and sow thistle (*Sonchus* spp.) (Figure 43). The pollen from these related composite flowers are very similar to each other and are challenging to distinguish under a microscope (G. Kassem, pers. com.). Composite flower pollen occurs in 15 out of 32 samples, usually in a smaller proportion of the sample, possibly due to the content of secondary metabolites in composite flower pollen (see box 2).

Figure 43. Catsear (the top two pictures) and various species of hawkweeds (the bottom two pictures) are common along roadsides and in meadows and other grass-dominated areas. Photos: Yoko L. Dupont and Per Kryger.

In some samples (five samples from period 8), pollen from the nightshade family constitutes a significant proportion. The nightshade family likely includes potatoes (Figure 44), which are cultivated in open fields and therefore found in high densities in agricultural areas. Flowers of the nightshade family have sack-shaped anthers that release pollen through a pore when the flower is vibrated (buzz pollination, Buchmann, 1985). Honey bees are not believed to apply this vibration (Buchmann, 1985), but they have been observed visiting potato flowers.

Another pollen source that honey bees exploit in late summer and early autumn in urban areas is Virginia creeper (*Ampelopsis* spp) and wild grapes (*Parthenocissus* spp) (Figure 42, Figure 45). These decorative climbing plants are probably mainly noticed for their beautiful foliage and colourful berries, but late in the flowering season they bear greenish, pollen-rich flowers.

Figure 44. Potato flowers have sac-shaped anthers (left). Flowering potato fields can be a pollen resource for bees (right). Photo: Yoko L. Dupont.

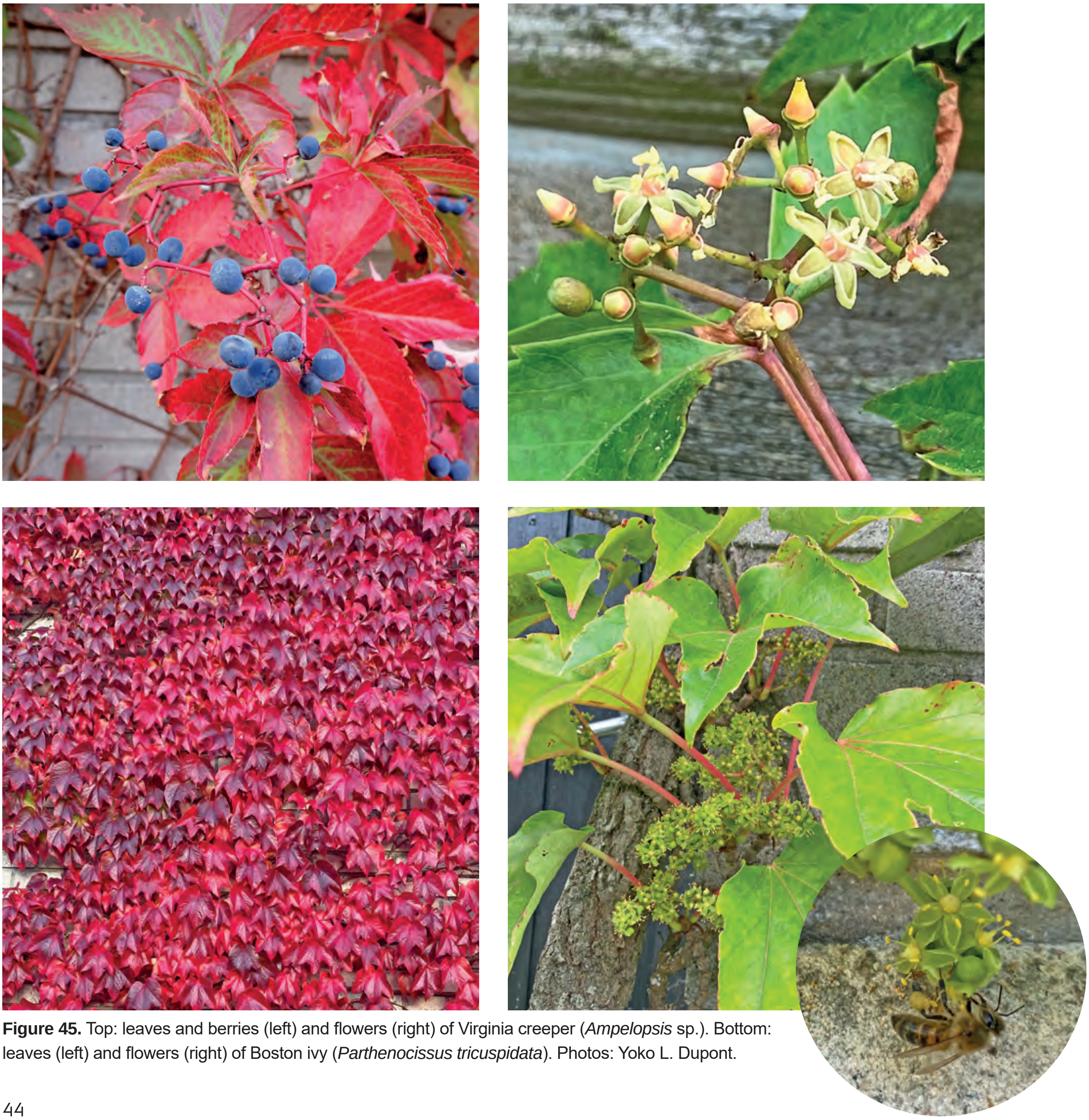
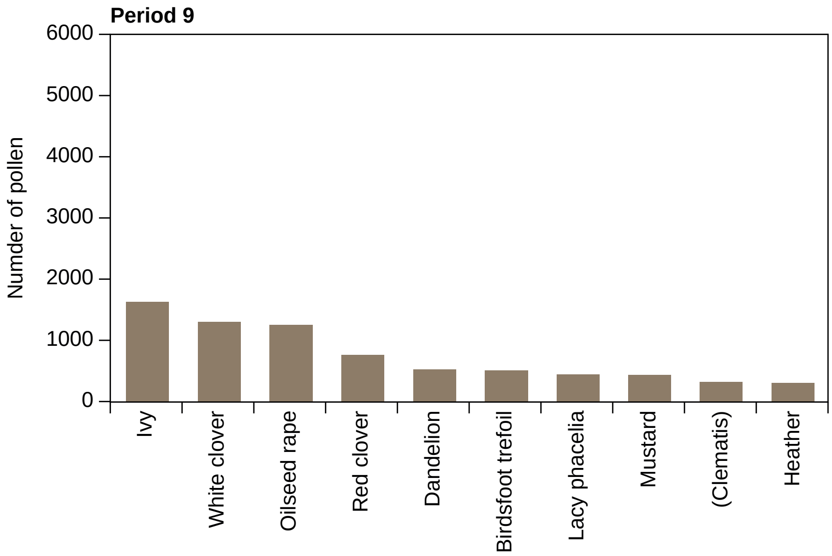


Figure 45. Top: leaves and berries (left) and flowers (right) of Virginia creeper (*Ampelopsis* sp.). Bottom: leaves (left) and flowers (right) of Boston ivy (*Parthenocissus tricuspidata*). Photos: Yoko L. Dupont.

6.11 Period 9 (mid-September) and Period 10 (early October)

The ninth period covered 9-22 September 2020 and 13-14 September 2021, comprising 29 samples. In these samples, 9,727 pollen grains were identified belonging to 57 different pollen types. Finally, two samples were collected on 4 and 10 October 2020 and two samples on 7 and 10 October 2021. These four samples from period 10 contained 1,370 pollen grains distributed among 15 pollen types.

In autumn, a new dominant pollen type emerged, namely English ivy (*Hedera helix*) (Figure 46). The brown, creeping stems with 3-5 lobed, palmate leaves can cover large areas of the ground, or they can climb walls and tree trunks using their adhesive roots. English ivy grows in broadleaf and mixed forests, shrubs, groves, forest edges, forest banks, hedges, coastal cliffs, rock walls and rocky meadows (Hartvig, 2015) and is also common in gardens and parks in urban areas. The flowering shoots are erect without adhesive roots and have elliptical, egg-shaped or rhombic, feather-veined leaves (Frederiksen et al., 2019). On a warm autumn day it can be busy with wasps, bees and flies coming to feed on the flowers. English ivy typically flowers in September-November. Therefore, English ivy is a good source of pollen for insects that are active in the autumn (Figure 47).



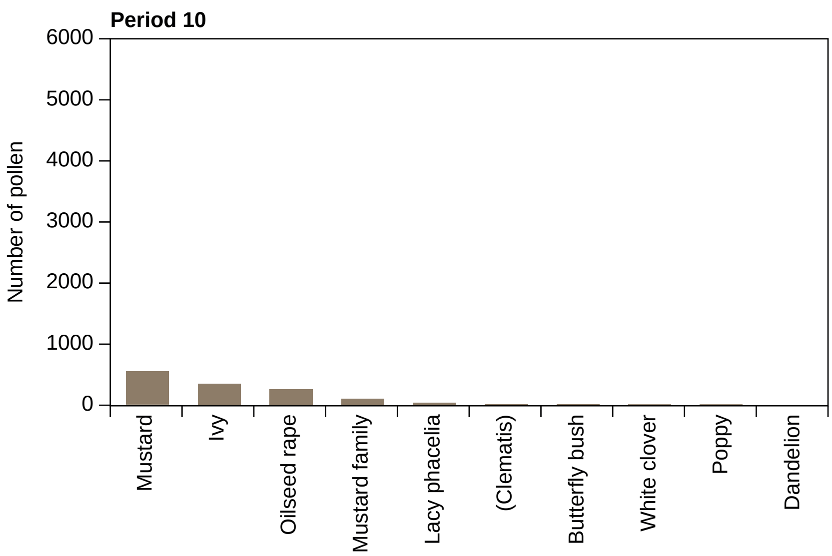


Figure 46. Top 10 pollen types in Period 9 (top) and Period 10 (bottom).

Figure 47. Ivy blooms late in the year in September-November. The flowers are visited by honey bees as well as hornets, hover-flies and other insects that are active in autumn. Left: flowering ivy in a forest, right: honey bee (*Apis mellifera*) on ivy flower. Photos: Yoko L. Dupont.

Red clover (*Trifolium pratense*) is also a commonly occurring pollen type in late summer and autumn (Figure 46, Figure 48) and is found in 9 out of the 32 samples from Period 8 and in 12 out of the 29 samples from Period 9. Red clover is cultivated for seeds and as a forage plant (Brødsgaard and Hansen, 2002), and it also occurs naturally in meadows, beach meadows, dune hollows, green dunes, roadsides, and disturbed land (Hartvig, 2015). The pink flower heads consist of many small 12-15 mm long flowers (Frederiksen et al., 2019). The corolla tube is very long and narrow so that only insects with long tongues, especially bumblebees, can reach the nectar at the bottom. It has been debated whether honey bees are good pollinators of red clover (Brødsgaard and Hansen, 2002). Honey bees actively collect pollen in the flowers (Skovgaard, 1952), and a recent study has shown that honey bees alone can adequately pollinate red clover in cultivation tunnels (Jing et al., 2021).

Figure 48. Red clover is cultivated for production of seeds used in grass-clover mixtures. The flowers are visited especially by large bees, both bumblebees and honey bees (right) but occasionally also by butterflies. Note the dark brown pollen in the honey bee pollen basket (indicated by an arrow). Photos: Yoko L. Dupont.

Figure 49. Mustard (top) and oilseed radish (bottom) are widespread cover crops, flowering in the autumn. Photos: Yoko L. Dupont.

Towards the end of the growing season, pollen from crops grown as cover crops can be found in the pollen samples. Mustard (*Sinapis* spp.) pollen is one of the most abundant pollen types in September, and mustard is the most abundant pollen type in October. Bees are likely to collect pollen from yellow-flowered fields with the cover crop yellow mustard (*Sinapis alba*) (Figure 49). Another widespread cover crop is oilseed radish (*Raphanus sativus* var. *oleiformis*) whose pinkish-white flowers cover large areas of farmland late in the growing season (Figure 49). Oilseed radish is closely related to oilseed rape (*Brassica napus*), and since the pollen of the two species is very similar, oilseed radish pollen is usually classified as *Brassica* (G. Kassem, pers. com.). Lacy phacelia (*Phacelia tanacetifolium*) is also used as a cover crop, and its pollen appears in pollen samples well into the autumn.

6.12 The significance of landscape context for the bee pollen supply

The pollen supply of bees is influenced by the availability of plants within their foraging range and, thus, by the land cover types in the foraging area. The landscape analysis revealed that all experimental landscapes were comprised of different land cover types (Figure 50). However, some landscapes tend to be dominated by certain land cover types compared to others. The distribution of overall land cover types in the experimental landscapes does not differ significantly from the distribution of land cover types in Denmark as a whole (Figure 51). In the following, we will examine the honey bee pollen supply in landscapes that are particularly dominated by specific land cover types.

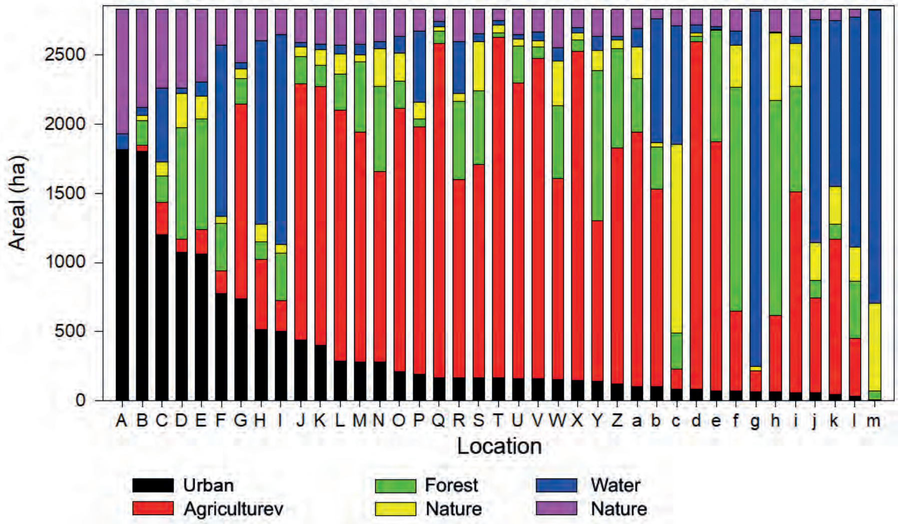


Figure 50. Distribution of land cover types in the landscapes (3 km) around each apiary. Each bar indicates the land cover distribution for an experimental landscape. Landscapes are sorted by the proportion of urban areas and then the proportion of agricultural land.

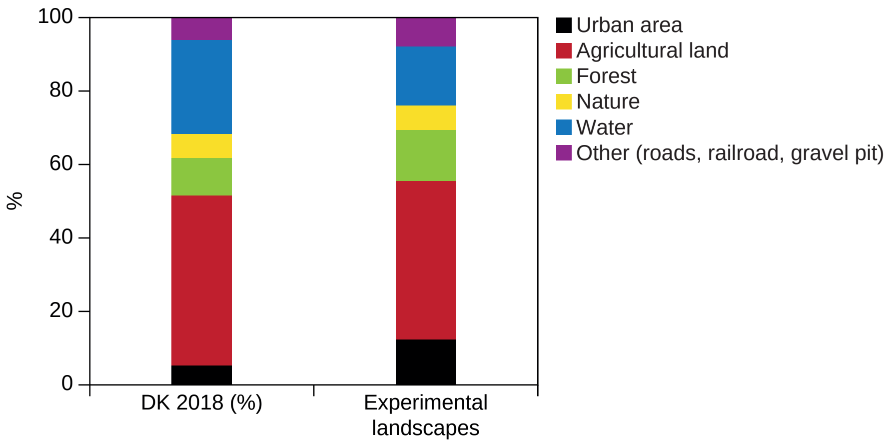


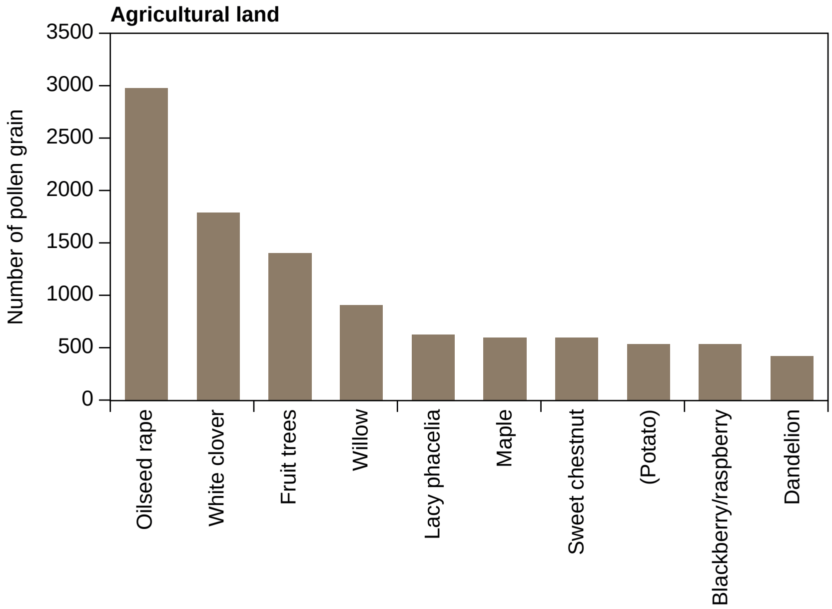
Figure 51. Distribution of land cover types in the experimental landscapes (3 km around each apiary) in % compared to the distribution of the same land cover types in the whole area of Denmark, including a 3 km water zone around the land areas. The land cover distribution is from 2018.

Compared to the distinct seasonal development of dominant pollen types across different sampling periods, differences between landscapes are not as pronounced. In addition to the fact that experimental landscapes are rarely characterised by one or a few landscape types, important pollen plants are often found in different landscape types, as mentioned under each specific forage plant above. Although the pollen composition of the honey bee diet depends, to some extent, on the landscape, there are several pollen types that are common in most Danish landscapes.

6.13 Agricultural land

Six apiaries were surrounded by more than 2,000 hectares of agricultural land, implying that more than 70% of the area within a 3 km radius was covered by agricultural land. The pollen from these apiaries was dominated by flowering

Figure 52. Top 10 pollen types in samples from six apiaries primarily surrounded by agricultural land.



crops (especially oilseed rape and white clover), dandelion from flowering grasslands as well as trees and bushes in hedges (fruit trees, willow, maple, blackberry, and sweet chestnut) (Figure 52).

The experimental landscapes in the study had a slightly lower proportion of broad beans and oilseed radish and a slightly higher proportion of fruit trees and clover than Denmark as a whole, while the coverage of oilseed rape was like the national average (Table 2).

Table 2. Land cover distribution. Area of insect-pollinated crops in hectares and % of the total area of insect-pollinated crops for experimental landscapes (landscape within 3 km of the experimental apiary) and nationally in 2020 and 2021.

	Experimental landscape 2020+2021		Denmark 2020		Denmark 2021	
Crop	**ha**	**%**	**ha**	**%**	**ha**	**%**
Mixed oilseeds	3	0.1	325	0.2	306	0.1
Fruit trees	110	2.8	2,382	1.3	2,428	1.1
Yellow mustard	5	0.1	180	0.1	233	0.1
Broad beans	302	7.8	19,376	10.2	22,221	10.3
Clover	321	8.3	8,026	4.2	8,560	4.0
Oilseed radish	209	5.4	14,312	7.5	20,114	9.3
Rape seed	2,920	75.4	145,803	76.6	162,554	75.1

6.14 Urban areas

Six apiaries had more than 1,000 hectares of urban areas within a 3 km radius, and the bees therefore had access to suburban gardens and/or parks. This is reflected in the presence of typical ornamental plants such as climbing hydrangea (*Hydrangea anomala* subsp. *petiolaris*), butterfly bush (*Buddleja davidii*), asters (*Aster* spp.), clematis (*Clematis vitalba*) and Virginia creeper (*Ampelopsis* spp.). However, these were not among the 10 most common pollen types, which primarily included white clover (*Trifolium repens*) from lawns and roadside areas, as well as fruit trees, raspberries/blackberries, and willow (Figure 53), likely from suburban gardens and green spaces. Surprisingly, crops such as oilseed rape, broad beans and red clover were also in the top 10 pollen types, perhaps because urban areas are rarely so large that agricultural land is beyond the foraging range of honey bees. Honey bees can fly 10-12 km after a rich food source (Beekman and Ratnieks, 2000).

Figure 53. Top 10 pollen types in samples from six apiaries with a high proportion of urban areas in the surrounding landscape.

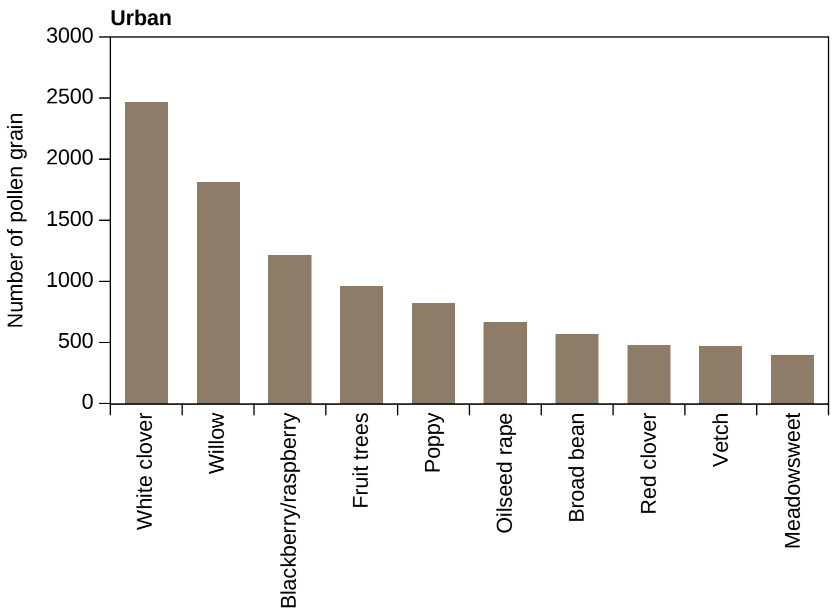
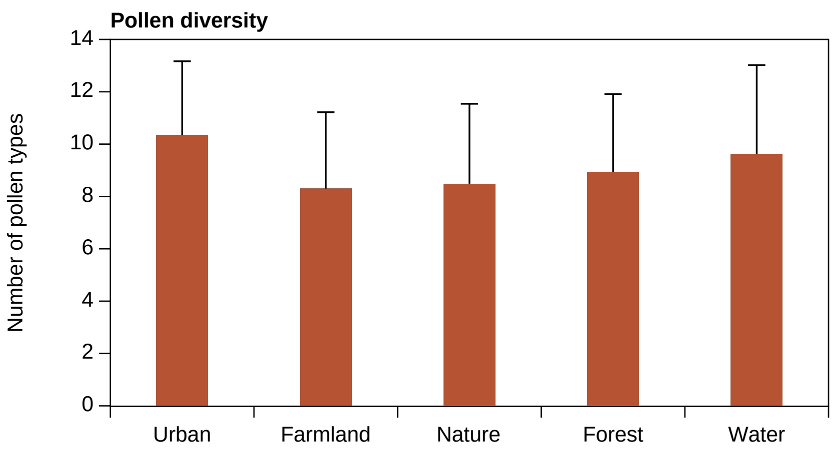
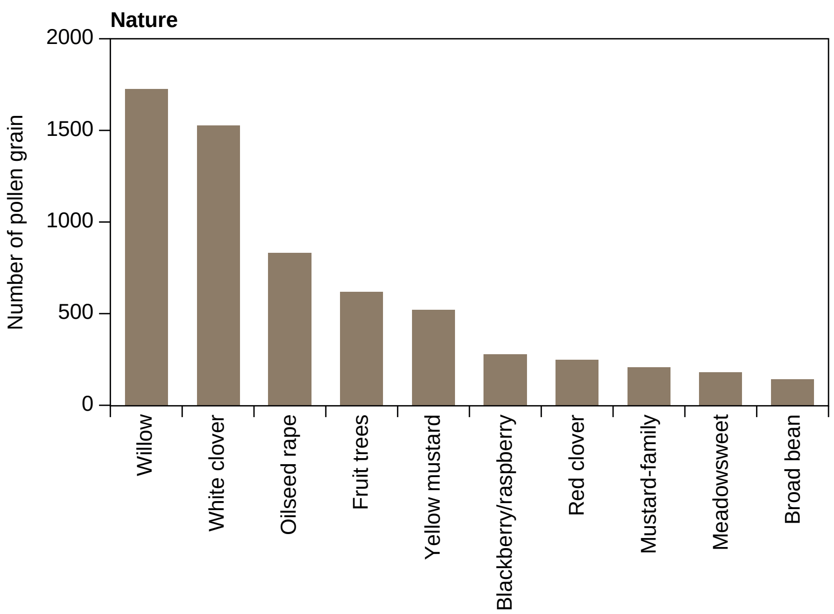


Figure 54. The columns show the average and the bars the variation (standard deviation) of pollen diversity, i.e. the number of different pollen types per samples in landscapes dominated by urban areas, farmland, nature, forest and water.



Samples from landscapes with a large proportion of urban areas are also characterised by a relatively high pollen diversity, and there is thus a high number of different pollen types in the samples (Figure 54).

Figure 55. Top ten pollen types in samples from three apiaries with a high proportion (>400 ha) open nature in the surrounding landscape.



6.15 Open nature

Although natural areas were present in many landscapes, only a few apiaries had a larger proportion of open nature in the surrounding landscape. In three apiaries, the share of open nature exceeded 400 hectares within 3 km, corresponding to more than 14% of the area. Pollen samples from these apiaries therefore reflect mixed landscapes, also including other land use types. In these three apiaries, especially willow (1,725 pollen) and white clover (1,529) were the most numerous pollen types, followed by oilseed rape (832), fruit trees (619) and yellow mustard (523), probably from nearby agricultural land or gardens (Figure 55).

6.16 Forest

Similarly, only a few apiaries had access to larger areas of forest. Only three apiaries had more than 1,000 hectares of forest within 3 km, representing more than 30% of the area. Pollen samples from these apiaries contained especially willow (1,276 pollen), ivy (959), oilseed rape (905) as well as fruit trees (550), dandelion (457) and clematis (413) (Figure 56). While pollen from willow, ivy, fruit trees and clematis may come from forest areas or nearby gardens, oilseed rape and dandelion pollen is likely collected by bees in agricultural areas in the landscape.

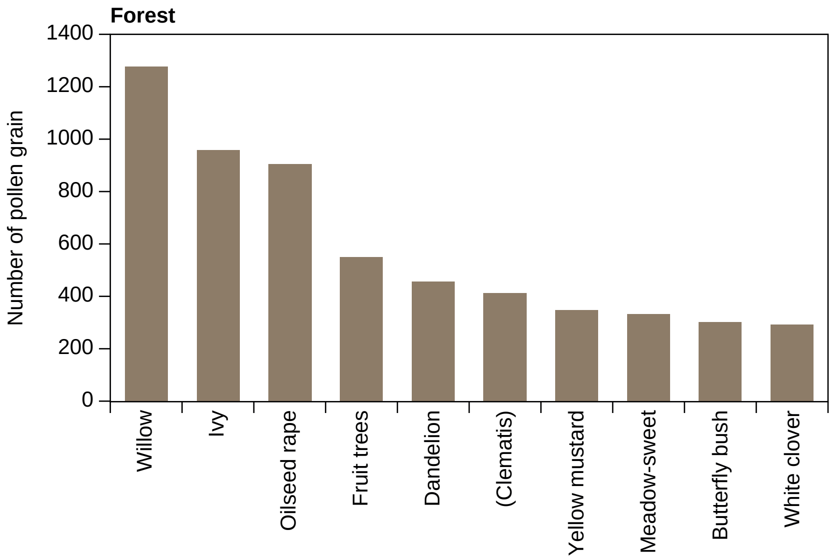


Figure 56. Top ten pollen types in samples from three apiaries with a high proportion of forest in the surrounding landscape.

6.17 Islands

In total, five experimental apiaries were located on islands or along the coast, so that a larger proportion (more than 1,500 hectares) of the landscape within 3 km consisted of water. Bees can to some degree fly over water to collect food (Kryger, 2018). However, bees generally do not use open water for foraging, except in the presence of water lilies. Therefore, the foraging area for these apiaries is assumed to be limited compared to other apiaries. Pollen types in the pollen supply of honey bees in apiaries with a large proportion of water in the surroundings do not differ significantly from pollen types in apiaries with access to larger land areas. As for agricultural land, urban areas, and open nature areas, especially willow, oilseed rape, fruit trees, blackberries and white clover are the dominant pollen types (Figure 57). However, there is a tendency for slightly higher pollen diversity (number of pollen types per sample) compared to agriculture, open natural areas and forests (Figure 54).

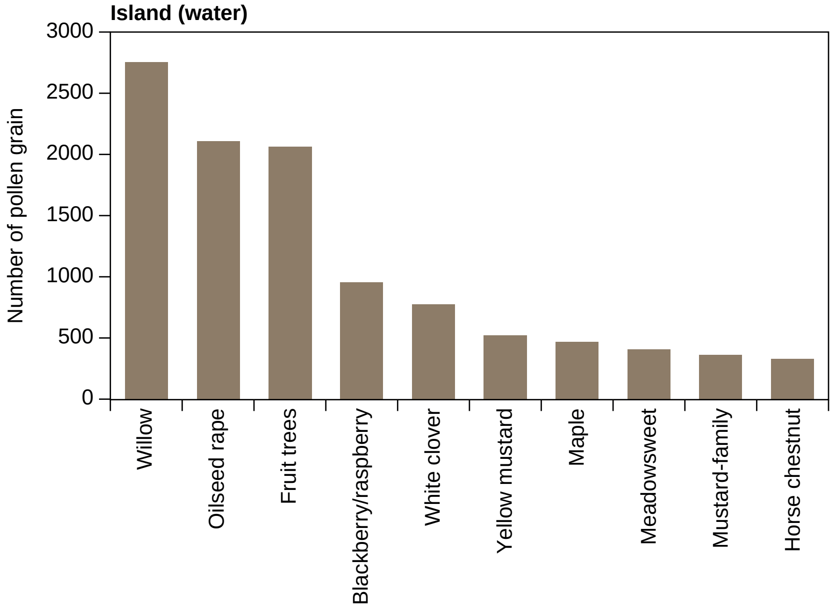


Figure 57. Top 10 pollen types in five apiaries where the proportion of water in the surrounding landscape (3 km) is > 1,500 ha (> 50 % of the area).

7 Discussion

- The pollen analysis of bee bread from a total of 311 samples, collected by sampling 40 apiaries in Danish landscapes in 2020 and 2021, showed that the honey bee pollen supply is mainly influenced by season but also, to some extent, by the surrounding landscape. The seasonal development of pollen sources is due to the fact that bees collect pollen during the flowering period of the plants, which is often limited to a few weeks, although there are local differences in flowering periods (Frederiksen et al., 2019).
- A total of 105 different pollen types were identified in the bee bread samples. Since the pollen of closely related plant species is difficult to separate morphologically in a light microscope, it must be expected that a pollen type in some cases includes several plant species. Pollen diversity, i.e. the number of pollen types per sample, increased throughout the season with a peak in August, after which it declined in autumn. Pollen diversity varied only slightly between different landscape types. The highest diversity was found in urban areas, and to a slightly lesser extent where bee foraging was restricted to a limited area, such as on islands. However, it should be borne in mind that all landscapes contained several land use types within the typical foraging distance of bees, i.e. within 3 km of the apiary.
- Although honey bees collect pollen to a greater or lesser extent from a wide range of plants, the samples were dominated by a few pollen types. The main sources of pollen for bees were oilseed rape, willow, white clover and fruit trees. Other pollen types also occurred in large quantities in some periods and/or certain landscapes.
- Mass-flowering agricultural crops such as oilseed rape, clover, and broad beans, as well as catch crops such as yellow mustard, lacy phacelia and oil radish, are rich sources of pollen for bees to collect.
- Likewise, wild-growing and planted, flowering trees in gardens, hedges and forests are a good source of pollen, especially in spring and early summer (willow, white-flowered fruit trees, maple, horse chestnut, sweet chestnut, hawthorn). Finally, weeds in arable fields and wild plants in nearby fields and natural areas are important for honey bee nutrition. (Odoux et al., 2021). These are usually naturally mass-flowering plants whose pollen occurs in larger proportions in the honey bee pollen supply (dandelion, meadowsweet, clematis, heather).
- Climate is important for both honey bee colony development and vascular plant flowering patterns. It is therefore expected that there will be some annual variation in the pollen supply collected by bees, which we did not investigate in this project.

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Appendix 1

Types of pollen found in samples of bee bread in the seasons 2020 and 2021. The amount of pollen is indicated in the categories based on the number of pollen grains in the total material:

* less than 10 pollen grains

** between 10 and 99 pollen grains

*** between 100 and 999 pollen grains

**** over 1000 pollen grains

Plant family	Pollen type	Common name	Pollen abundance
Maple family	*Acer*	Maple	****
Sambucus family	*cf Sambucus*	Elderberry	***
	Viburnum	Black haw	**
Amaranth family	*Chenopodium*	Goosefoot	**
Amaryllis family	*Allium*	Onion	**
Cashew family	Anacardiaceae type	Sumac family	***
	Apiaceae type	Carrot family	***
	Foeniculum	Fennel	**
	Heracleum	Giant hogweed	**
Holly family	Aquifoliaceae type	Hollies	*
		Holly	**
Ivy family	*Ilex Hedera*	Ivy	****
Asparagus family	*Asparagus*	Asparagus	**
Sunflower family	*Achillea*	Yarrows	****
	Ambrosia	Ambrosia	*
	Arctium	Greater burdock	*
	Artemisia	Common mugwort	**
	Aster	Asters	***
	Asteraceae type	Sunflower family	*
	Carduus	Thistle	***
	Centaurea cyanus	Cornflower	***
	Centaurea jacea	Brown knapweed	**
	Helianthus annuus	Sunflower	*
	Taraxacum	Dandelion	****
Balsom family	*Impatiens*	Jewelweed	***
Birch family	*Betula*	Birch	**
Borage family	*Borago*	Starflower	**
	Echium	Tree echium	**
	Phacelia	Phacelia	****
	Symphytum	Comfrey	*
Cornflower family	*Brassica*	Rapesed	****
	Brassicaceae type	Mustards	***
	Sinapis	Mustard	****

Plant family	Pollen type	Danish name	Pollen abundance
Bellflower family	Campanulaceae type	Bellflower family	**
Honeysuckle family	*Lonicera*	Honeysuckle	**
	Symphoricarpos albus	Snow berry	**
Carnation family	Caryophyllaceae type	Carnation family	**
Bindweed family	*Calystegia*	Bellbind	*
Dogwood family	*Cornus*	Dogwoods	**
Stonecrop family	*Sedum*	Stone crop	***
Graminoid family	Cyperaceae type	Sedges	**
Heather family	*Calluna vulgaris*	Heather	****
	Ericaceae type	Heather family	**
	Vaccinium	Cowberry	**
Legume family	*Astragalus*	Milkvetch	***
	Fabaceae type	Pea family	**
	Genista	Broom	**
	Gleditsia	Honey locust	**
	Lotus	Bird's-foot trefoil	***
	Robinia pseudoacacia	Black locust	*
	Trifolium repens	White clover	****
	Trifolium pratensis	Red clover	****
	Vicia	Vetches	***
	Vicia faba	Broad bean	****
Beech family	*Castanea sativa*	Sweet chestnut	****
	Quercus	Oak	***
Cranesbil family	Geraniaceae type	Geranium family	*
Gooseberry family	*Ribes*	Redcurrant	**
Mock-orange family	*Philadelphus*	Sweet mock orange	***
St. John's wort family	*Hypericum*	St. John's wort	***
Mint family	Lamiaceae type	Mint family	**
	Thymus	Thyme	***
Lily family	*(Narthecium)*	(Bog asphodel)	***
	Liliaceae type	Lily family	**
Mallow family	Malvaceae type	Hibiscus family	*
	Tilia	Lime	**
Olive family	*Fraxinus*	Ash	***
	Ligustrum	Privet	***
Willowherb family	Onagraceae type	Willowherb family	**
Broom-rape family	*Rhinanthus*	Yellow rattle	**
Poppy family	*Papaver*	Poppy	****
Pine family	*Pinus*	Pine	***
Plantain family	Plantaginaceae type	Plantain family	***
Leadwort family	cf *Limonium*	Sea lavender	***
Grass family	*Poaceae*	True grasses	**
Knotweed family	*Persicaria*	Knotweed	**
	Polygonum aviculare	Common knotgrass	*
	Rumex	Bitter dock	**

Plant family	**Pollen type**	**Danish name**	**Pollen abundance**
Clematis family	*(Clematis)*	Clematis	****
	Anemone nemorosa	Anemone	***
	Ranunculus	Buttercup	***
Buckthorn family	*Frangula alnus*	Alder buckthorn	***
Rose family	*(Potentilla, Malus,* unknown)	(Cinquefoil, apple, unknown)	***
	Agrimonia	Common agrimony	*
	Filipendula	Meadowsweet	****
	Fragaria	Strawberry	**
	Pirus/Prunus	Fruit trees	****
	Rubus	Raspberry/Blackberry	****
Goose frass family	*Galium*	Cleaver	**
Willow family	*Salix*	Willow	****
Soapberry family	*Aesculus*	Horse chestnut	****
Butterfly bush family	*Buddleja*	Butterfly bush	****
	Verbascum	Mullein	*
Quassia family	*Ailanthus*	Tree of heaven	*
Nightshade family	Solanaceae type	Nightshade family	***
Tea family	*Camellia*	Tea shrub	*
Nasturtium family	*Tropaeolum*	Nasturtium	**
Cattail family/Pondweed family	*Sparganium (Typhaceae)/Potamogeton (Potamogetonaceae)*	Bur-reed/pondweed	**
Unknown	type 1	Unidentified type 1	***
	type 2	Unidentified type 2	**
	type 4	Unidentified type 4	*
Vervain family	Verbenaceae type	Vervain family	**
Violet family	Violaceae type	Violet family	***
Vine family	*Ampelopsis*	Porcelain berry	****

HONEY BEE POLLEN SUPPLY IN DANISH LANDSCAPES

Pollen is a source of protein, fats, vitamins and minerals for honey bees, and pollen nutrition is important for bee health and winter survival. In this study, we investigated the pollen supply of honey bees in Danish landscapes with a view to elucidate the significance of the seasonal development and land use in the surrounding landscape. The report reviews the most important pollen sources (top 10) in each of 10 periods throughout the growing season (April-October) as well as in landscapes with larger shares of agricultural land, urban areas, open nature, forest and water.

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