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Pollen analysis of honey from the Baltic region, Estonia

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Abstract

The present study reports the results of pollen analyses of Estonian honey collected from 2000 to 2011. Altogether, the pollen content of 325 honey samples was analysed with an average of 400 pollen grains counted in a sample. The main focus was on the floristic spectrum of plants and on the identification of the most common and important plant sources for honey. More than 120 pollen types were identified in the examined honey samples. The results showed that the pollen types of Rosaceae, Brassicaceae, *Salix* and *Trifolium* were the most abundant among the samples. The pollen of Apiaceae, Fabaceae, Asteraceae, Poaceae, *Fagopyrum esculentum*, *Frangula alnus* and *Calluna* were present in more than 25% of samples. Typical Estonian honey is polyfloral, the average number of species is 13 taxa per sample. During the study period, changes observed were that the percentage of the Rosaceae and *Calluna* pollen types had decreased, while the proportions of the Brassicaceae and *Salix* pollen grains showed an increase. The concentration of pollen grains per gram of honey varies from 100 to 700 000. The current information provides new insights into the pollen composition of Estonian honey and could be used to develop analytical standards for the pollen content of Estonian honey.

Keywords: *pollen analysis, honey, melissopalynology, Estonia*

The botanical origin of honey can characterise differences between colour, taste, flavour and content of physiologically active ingredients. There are several methods for determining the origin of honey. However, the information on sugar composition, electrical conductivity and amino acid analysis of honey is not always a reliable determination of the botanical origin of honey (Kaškonienė et al., 2010). The most common method used to determine the botanical origin of honey is pollen analysis. The identification of plant species whose pollen occurs in honey samples allows a characterisation of the vegetation surrounding the beehives and also the geographical region, from which the nectar was collected (Louveaux et al., 1978; von der Ohe et al., 2004). Thus, melissopalynological analysis is still the most reliable test, and data could provide a large amount of information that is generally disregarded.

There are many reasons why it is important to determine the botanical and geographical origin of honeys. For example, legislation requires the correct descriptions on honey labels, so pollen analysis is used as the

quality control of marketing to determine the botanical origin of honey. The analysis of the pollen spectrum of honey is also used to detect nectar sources over the apicultural period. Long-term analytical studies of pollen can also reveal some trends in pollen composition connected with human activities. For example, over the last 50 years, the increase in the cultivation of oil-seed rape has increased the proportions of the Brassicaceae pollen in Finnish honeys (Salonen et al., 2009). In addition, we cannot exclude climatic changes that can influence the overall process of pollination (Schweiger et al., 2010). We also have to consider the increasing loss of pollinators (Kearns et al., 1998; Steffan-Dewenter et al., 2005) and that the richness of bee species and insect-pollinated plants has decreased (Biesmeijer et al., 2006). These factors may also have an effect on the pollen content of honey.

Although there are more than 7000 beekeepers in Estonia, most of them do not sell honey. Honey production is the source of subsistence for around 300 beekeepers that maintain about 27 000 bee colonies. The total honey yield is ~500–700 tons annually

(Kilk, 2007; Statistikaamet, 2012). The number of beekeepers in Estonia is about the same as those in other Baltic States (Dirina & Bugina, 2012). Most Estonian beekeepers collect honey from their colonies only once a year. Estonian commercial honey is typically polyfloral honey (which means that honey contains bee-collected nectar from several plant species) and products are labelled as: Estonian Honey, Meadow Flower Honey or Wildflower Honey. In some years, under favourable weather conditions, clover, fireweed, heather, lime, rape, stonecrop, buckwheat, willow and raspberry could be used to produce unifloral honey (the presence of a single pollen type in quantities greater than 45% of the total pollen content of the honey).

There have been profound changes in agriculture and silviculture in Estonia since the 1990s after the collapse of the Soviet agricultural system. The changes include the decrease of fields of cereal grains and the increase of oilseed rape cultivation. The more intense deforestation all over Estonia has led to the overgrowth of shrubs. These changes have affected floristic diversity, which in turn should be reflected in the floral composition of honey.

Melissopalynological studies of Estonian honey are rare, based on a low number of analysed samples (14), collected in one year and connected with the physicochemical characterisation of honeys (Kirs et al., 2011; Laos et al., 2011). There are some studies about the amino acid content of Estonian honeys and about the development of that method (Rebane & Herodes, 2008, 2010). The same is true for honey of Lithuania (Čeksterytė et al., 2006; Kaškonienė et al., 2010). There are studies on Latvian honey examining the toxic metals in their honey. However, the conclusions about the botanical origin were based on the information provided by honey producing companies on the product trade labels or by the beekeepers themselves (Vincevica-Gaile et al., 2012). Pollen analyses of honey from Finland included earlier studies and compared the properties and melissopalynological results of Finnish honey from the 1960s until today, or honeys from different regions of Finland (Salonen et al., 2009; Salonen, 2011; Salonen & Julkunen-Tiito,



Figure 1. Location of Estonia.

2012). These studies are regionally the closest and the most thorough and may be relevant to the present study.

The goal of the present study is to report the results of pollen analyses from honey samples collected from 2000 to 2011. The changes in the plant compositions and pollen concentrations of honey are considered during the investigation period. The data obtained can be compared with data from other northern European areas and the Baltic region.

Material and methods

Estonia (57° 30'–59° 49' N, 21° 46'–28° 13' E; Figure 1) is situated in the boreo-nemoral forest zone. The vegetation is characterised by a mixture of boreal coniferous and deciduous tree species (*Picea abies* (L.) H. Karst, *Pinus sylvestris* L., *Betula pendula* Roth and *B. pubescens* Ehrh.). Nemoral deciduous trees (e.g. *Corylus avellana* L., *Tilia cordata* Mill., *Ulmus glabra* Huds., *Quercus robur* L.) occur widely but are less common than the boreal taxa. Around 1000 nectariferous plants are known in Estonia. However, the main honey production originates from less than 100 plant species (Table I; Tammet, 2007). Climatically, Estonia lies in the northern part of the temperate climate zone, at the transition

Table I. The most common honeyplants in Estonia (Tammet, 2007).

From early spring to June	From June to July	From August to late autumn
<i>Acer platanoides</i> , <i>Alnus</i> spp., <i>Caragana arborescens</i> , <i>Corylus avellana</i> , <i>Lamium album</i> , <i>Pulmonaria officinalis</i> , <i>Salix</i> spp., <i>Sinapis arvensis</i> , <i>Sorbus aucuparia</i> , <i>Taraxacum</i> spp., <i>Tussilago farfara</i> , different fruit trees and berry bushes	<i>Brassica napus</i> subsp. <i>napus</i> , <i>Cirsium arvense</i> , <i>Echium vulgare</i> , <i>Epilobium angustifolium</i> , <i>Fagopyrum esculentum</i> , <i>Galega orientalis</i> , <i>Geranium pratense</i> , <i>Lathyrus pratensis</i> , <i>Melilotus alba</i> , <i>Origanum vulgare</i> , <i>Phacelia tanacetifolia</i> , <i>Rubus idaeus</i> , <i>Thymus serpyllum</i> , <i>Tilia cordata</i> , <i>Trifolium</i> spp., <i>Vicia cracca</i>	<i>Arctium tomentosum</i> , <i>Calluna vulgaris</i> , <i>Echinops sphaerocephalus</i> , <i>Leonurus cardiaca</i> subsp. <i>villosus</i> , <i>Melissa officinalis</i> , <i>Solidago virgaurea</i>

Table II. Number of samples, recorded pollen types, the most frequent pollen types (occurred in more than 20% samples) (in alphabetic order), percentages of unifloral samples (samples, in which one pollen type represented more than 45%) per year.

Year	Number of samples	Number of recorded pollen types	Most frequent pollen types	Percentages of unifloral samples and examples
2000–2002	35	72	Apiaceae , Asteraceae, Brassicaceae , <i>Calluna</i> , <i>Centaurea cyanus</i> , Fabaceae, <i>Lotus</i> , Melilotus , Rosaceae , Salix , Taraxacum , Trifolium , <i>Vicia</i>	68%; <i>Apiaceae</i> , <i>Brassicaceae</i> , <i>Calluna</i> , Fabaceae, <i>Fagopyrum esculentum</i> , <i>Lotus</i> , <i>Rhamnus</i> , <i>Rosaceae</i> , <i>Salix</i> , <i>Scrophularia</i>
2005	27	52	<i>Alnus</i> , Apiaceae , Brassicaceae , <i>Calluna</i> , Fabaceae, <i>Fagopyrum esculentum</i> , <i>Frangula alnus</i> , <i>Lotus</i> , Melilotus , Poaceae, <i>Prunus/Pyrus</i> , <i>Ranunculus</i> , Rosaceae , Salix , Taraxacum , Trifolium , <i>Vicia</i>	33%; <i>Apiaceae</i> , <i>Fagopyrum esculentum</i> , <i>Rosaceae</i> , <i>Salix</i>
2006	51	64	<i>Acer</i> , <i>Alnus</i> , Apiaceae , Asteraceae, Brassicaceae , <i>Fagopyrum esculentum</i> , <i>Frangula alnus</i> , <i>Lotus</i> , Melilotus , Poaceae, <i>Ranunculus</i> , Rosaceae , Salix , Trifolium	47%; <i>Brassicaceae</i> , Fabaceae, <i>Fagopyrum esculentum</i> , <i>Rosaceae</i> , <i>Salix</i> , <i>Sedum</i>
2007	60	57	<i>Alnus</i> , Apiaceae , Asteraceae, <i>Betula</i> , Brassicaceae , <i>Calluna</i> , <i>Centaurea cyanus</i> , Chenopodiaceae, <i>Frangula alnus</i> , <i>Hypericum</i> , Melilotus , <i>Pinus sylvestris</i> , Poaceae, <i>Ranunculus</i> , Rosaceae , Salix , <i>Rumex</i> , Taraxacum , Trifolium , <i>Vicia</i>	61%; <i>Brassicaceae</i> , <i>Rosaceae</i> , <i>Salix</i>
2008	30	46	<i>Acer</i> , <i>Alnus</i> , Apiaceae , <i>Betula</i> , Brassicaceae , <i>Calluna</i> , Caryophyllaceae, <i>Fagopyrum esculentum</i> , <i>Frangula alnus</i> , <i>Gentiana/Gentianella</i> , <i>Lotus</i> , Melilotus , <i>Prunus/Pyrus</i> , <i>Ranunculus</i> , Rosaceae , Salix , Taraxacum , Trifolium	53%; <i>Brassicaceae</i> , <i>Lotus</i> , <i>Melilotus</i> , <i>Rosaceae</i> , <i>Salix</i> , <i>Trifolium</i>
2009	60	59	<i>Acer</i> , <i>Alnus</i> , Apiaceae , <i>Betula</i> , Brassicaceae , <i>Calluna</i> , <i>Frangula alnus</i> , <i>Lotus</i> , Melilotus , Rosaceae , Salix , Taraxacum , Trifolium	46%; <i>Brassicaceae</i> , <i>Lotus</i> , <i>Rosaceae</i> , <i>Salix</i> , <i>Sedum</i>
2010	60	49	<i>Acer</i> , Apiaceae , Brassicaceae , <i>Calluna</i> , <i>Centaurea cyanus</i> , <i>Fagopyrum esculentum</i> , <i>Frangula alnus</i> , <i>Lotus</i> , Melilotus , Poaceae, <i>Ranunculus</i> , Rosaceae , Salix , Taraxacum , Trifolium	63%; <i>Brassicaceae</i> , <i>Calluna</i> , <i>Lotus</i> , <i>Rosaceae</i> , <i>Salix</i> , <i>Tilia</i>
2011	2	21	<i>Apiaceae</i> , <i>Brassicaceae</i> , <i>Centaurea cyanus</i> , <i>Fagopyrum esculentum</i> , <i>Gentiana/Gentianella</i> , <i>Melilotus</i> , Poaceae, <i>Prunus/Pyrus</i> , <i>Rosaceae</i> , <i>Salix</i> , <i>Trifolium</i> , <i>Viola</i>	–

Note: Taxa that occurred in more than 20% samples in all studied years (2000–2010) are given in bold (except for 2011 when only two samples were analysed).

between moderately continental (in the east) and slightly more maritime climate (in the west). From 2000 to 2011, the mean temperature in July was 18.6 °C and in February –4.7 °C, annual precipitation is 705 mm (Estonian Meteorological and Hydrological Institute, Tallinn).

For the current characterisation work of Estonian honey, 325 honey samples were obtained during 2000–2011 (except for the years 2003 and 2004, when we did not have any samples). The samples of 2000 to 2002 were grouped together as one period, because they were not marked by individual years. Most were collected by the Estonian Beekeeping Association from different honey-selling enterprises, or assigned for quality control analyses by beekeepers or the Estonian Chamber of

Agriculture and Commerce. The number of samples per year varied from 27 to 60 (Table II). Only two samples were analysed in 2011. Honey samples came from different hives (mostly unknown for the authors) and different locations each year and labelled as of Estonian origin.

Melissopalynological analysis of honey samples followed the standard acetolysis method (Moore & Webb, 1978). To determine the number of pollen grains per gram of honey sample, tablets containing a known number of *Lycopodium clavatum*-spores were added to each sample (Stockmarr, 1971). Five classes were established according to the number of pollen grains (Maurizio, 1939), which established content of one gram of honey (I <2000; II 2000–10 000; III 10 000–50 000; IV 50 000–100 000;

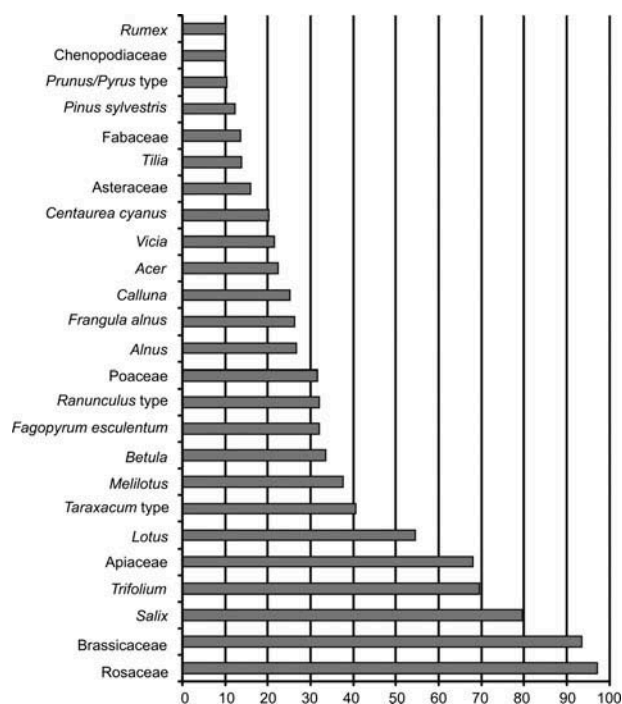


Figure 2. Frequency of occurrence of pollen types (>10% frequency) in the years 2000–2011.

V >100 000 pollen grains). The pollen count was based on a minimum of 400 grains. Pollen identification was made using a compound light microscope (400× magnification). The pollen grains were identified to species rank whenever possible, otherwise to genus, family or pollen type. Pollen type includes species and/or genera present in the sample, which have the same, or barely different, pollen morphology. Pollen grains were identified by comparison to the reference pollen collection prepared at the Institute of Ecology at Tallinn University and from the relevant literature (Moore & Webb, 1978; Moore et al., 1991; Reille, 1992). The frequency of occurrence was calculated as the percentage of samples, in which one pollen type was observed. The samples, in which one pollen type represented >45%, were classified as unifloral or monofloral and the honey was classified according to the pollen type. Honey, in which no pollen type was >45% of the total number of grains counted, was classified as multifloral or polyfloral (Louveaux et al., 1978; von der Ohe et al., 2004; White, 2005).

Results

Altogether, 127 different pollen types were identified from the 325 honey samples. The frequency of occurrence denoted the number of samples, in which a certain pollen type was encountered (Figure 2). The highest pollen frequency was noted for pollen grains of

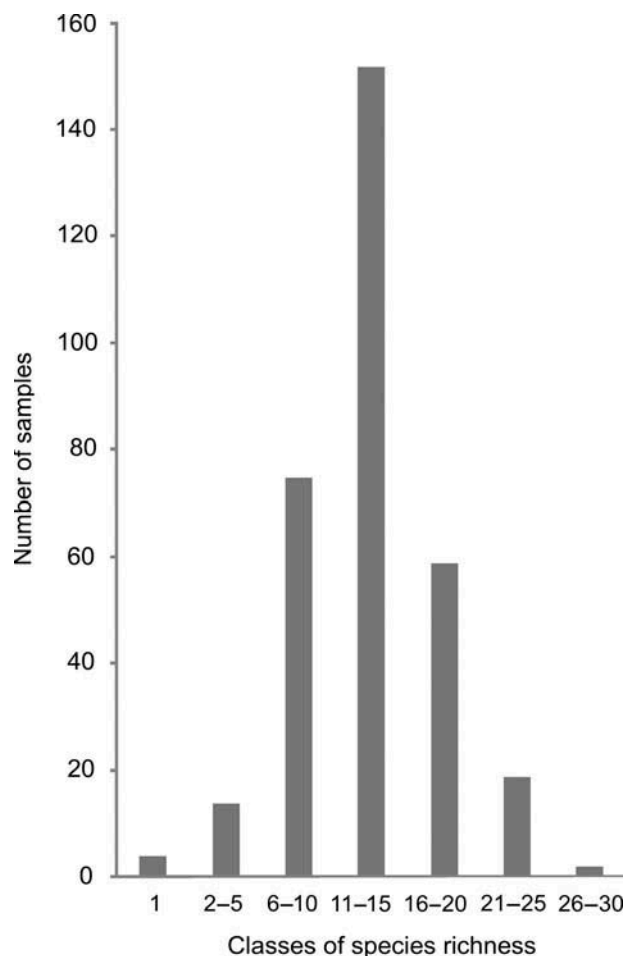


Figure 3. Classes of species richness in the years 2000–2011.

Rosaceae (97%), Brassicaceae (93%) and *Salix* (80%). In turn, a pollen frequency of over 55% was observed in *Trifolium*, *Apiaceae* and *Lotus*. Occurring types (>25%) were also those of *Calluna*, *Fagopyrum esculentum* Moench, *Frangula alnus* Mill., *Melilotus*, *Ranunculus* and *Taraxacum*-type. Pollen of *Campanula*, *Caryophyllaceae*, *Cirsium*, *Epilobium angustifolium* L., *Galium*, *Hypericum*, *Saxifraga*, *Sedum* and *Senecio* were found in less than 10% of the samples. Pollen spectra of honey samples revealed a variety of not only nectariferous but also nectarless sources available to bees, like *Alnus*, *Betula*, *Pinus*, *Picea*, *Plantago*, *Poaceae*, *Quercus* and *Corylus*. A few exotic pollen grains were also found (e.g. *Helianthus* spp., *Zea mays* L.); some unidentified pollen grains occurred only in a few samples.

The average number of pollen types was 13 per sample (maximum 28; Figure 3). However, monofloral samples were described through the study period: honey of Brassicaceae (mostly *Brassica napus* L. and *Sinapis arvensis* L.), Rosaceae (*Malus*, *Sorbus*, *Rosa* and *Rubus*), *Calluna*, *Salix*, *Trifolium*, *Sedum*, *Lotus* and *Tilia* (Figure 4; Table II). Some

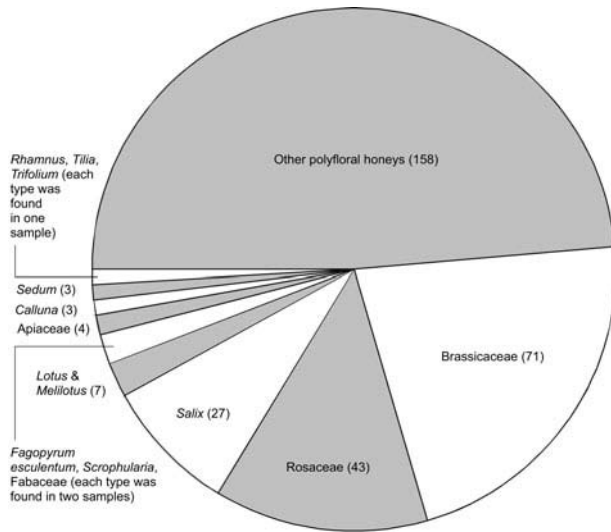


Figure 4. The distribution of honey samples through the study period 2000–2011, in which a single pollen type represented >45% of the pollen grains counted. Numbers behind the names correspond to the number of samples.

monofloral honeys contained only one pollen type (*Calluna*, Brassicaceae (*Brassica napus*), Rosaceae and Apiaceae). Polyfloral honeys were characterised by a high percentage of pollen of *Salix*, Brassicaceae and Rosaceae.

The results indicate that the proportion of Rosaceae pollen type and *Calluna* has decreased during the study years 2000–2010 (from 30% to 16% and 6% to 2%, respectively) and has been replaced

by Brassicaceae and *Salix* pollen (from 19% to 40% and 6% to 20%, respectively).

The concentration of pollen grains per gram of honey varies markedly from 10 to 700 000 (average 10 000; Figure 5A) and was not homogeneous through the study period (Figure 5B; Table III). Quantitative analysis shows that some analysed honey samples were highly rich in pollen, four of them were included in class V of the Maurizio classification and four in class IV. Two-hundred and twenty samples showed a moderate amount of pollen grains per gram of sample (classes II and III), and 97 samples had a low pollen concentration (class I; Figure 5B). Class I contains 21 samples, which had very low pollen concentration (<500 pollen grains per gram of honey). Although there was no clear correlation between concentrations and the pollen composition of honey, we can highlight a few characteristics. Apiaceae and Fabaceae were present in the first class (<2000 pollen grains per gram of honey) relatively more than in the other groups. The samples, which belong to the second and third classes of the Maurizio classification, contain mostly pollen of Rosaceae, Brassicaceae and *Salix*. Honey samples characterised by the domination of the *Calluna* and Rosaceae pollen belong to the fourth and fifth classes.

Discussion

Our results suggest that Estonian honey is mostly mixed and contains more than ten different plant

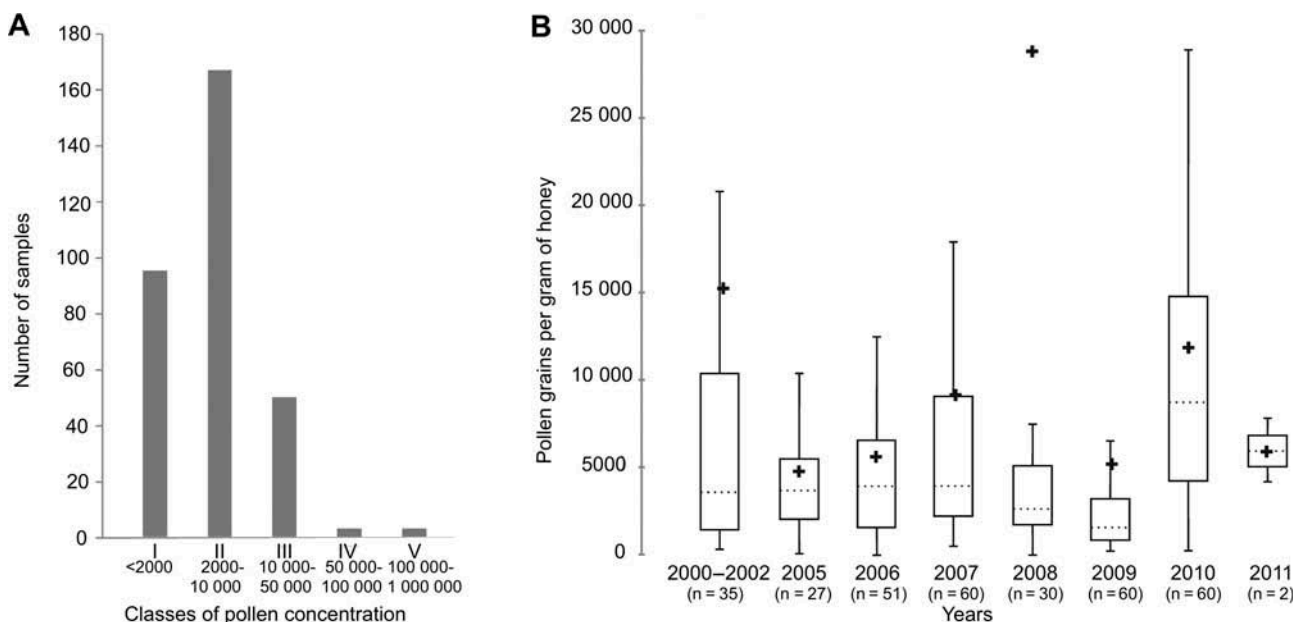


Figure 5. Distribution of honey samples according to Maurizio's classes (A) and changes in pollen concentration values per gram of honey through the study period expressed as median (dotted line) and mean value (cross) and interquartile range (Q1–Q3) (B). The number of samples analysed per year (*n*) is added.

Table III. Pollen concentration data of studied Estonian honey samples in the years 2000–2011.

Year	Range of concentrations (pollen grains per gram)	Number of samples according to Maurizio's classes
2000–2002	242–154 185 (average 15 258)	I – 10; II – 16; III – 6; IV – 1; V – 2
2005	56–27 014 (average 4779)	I – 6; II – 19; III – 2
2006	10–61 261 (average 5612)	I – 15; II – 32; III – 3; IV – 1
2007	465–159 132 (average 9118)	I – 15; II – 32; III – 12; V – 1
2008	19–755 734 (average 28 821)	I – 10; II – 17; III – 2; IV – 1
2009	146–91 022 (average 5167)	I – 34; II – 23; III – 1; IV – 2
2010	247–47 646 (average 11 867)	I – 7; II – 28; III – 25
2011	4074–7727 (average 5900)	II – 2

species; there is also no dominant pollen type, which could give a great variability for characteristics of honey. There are several reasons for the domination of honeys originated from different plants. There are not so many and huge monocultural fields around hives and diversity of honey plants is rather high (Tammets, 2007). The results suggest that pollen in honey originates from several sources: wild plants, cultivated crops and garden plants. Most Estonian beekeepers collect honey from their colonies only once a year. Therefore, Estonian honey is a typically polyfloral nectar honey and nectar originates from several plants flowering from spring to autumn. However, there could be climatically favourable years to harvest honey twice and produce honeys, which would be described by less than five plant species or can even be unifloral.

Although it is said, that there is no absolutely pure unifloral honey, because bees always collect nectar from several plant species, we can highlight four samples of the analysed 325, which consist of 100% of one pollen type (*Calluna*, *Brassica napus*, Apiaceae and Rosaceae). However, herein many samples were identified as monofloral honeys, in which one pollen type represented >45% and, therefore, are considered unifloral by the standard measures. Pollen grains of plant species may be normally represented (percentage of pollen grains correlates one-to-one with the amount of nectar, from which the honey is derived), over-represented (percentage of pollen grains is higher than the amount of nectar, from which the honey is derived), or under-represented (the percentage of pollen grains is lower than the amount of nectar from which the honey is derived) in honey samples (Bryant & Jones, 2001; Salonen, 2011). In the case of Estonian honey, pollen grains of some plant species (e.g. *Tilia cordata*, *Epilobium angustifolium*, *Fagopyrum esculentum* and *Taraxacum* spp.), which are described as very good nectariferous plants (Tammets, 2007), are probably under-represented in the honey spectra, and 10–30% of these pollen types should be considered sufficient to indicate a honey as unifloral (Sawyer, 1988; Jones & Bryant, 1992; Bryant & Jones, 2001; Salonen, 2011). For over-represented

pollen types (mainly *Salix* and several species of Brassicaceae), a honey is suggested to be regarded as unifloral only when the predominant pollen type represents more than suggested 45% of the total.

There can be seen some changes in the pollen content of honey from 2000 to 2011. The decrease in the proportion of Rosaceae pollen type and *Calluna* has been observed (from 30% to 16% and 6% to 2%, respectively) and the proportion of Brassicaceae and *Salix* pollen increased (from 19% to 40% and 6% to 20%, respectively). One explanation for this observation is the increase in area of fields of oilseed rape. Oilseed rape (*Brassica napus*) is an important crop whose cultivation area has increased significantly in Estonia: in 2001, rape was grown on an area of 27 600 ha (Statistikaamet, 2002) and in 2010 on 98 000 ha (Statistikaamet, 2012); this is an increase of 255%. The same tendency has been noted in Finland. In the southern regions of Finland, pollen of Brassicaceae was found to occur more frequently, which is linked with the regionalisation of rape crops (Varis et al., 1982; Salonen et al., 2009). The increase of *Salix* pollen can be explained by changes in agriculture and silviculture in Estonia since the 1990s. These are, for example, the decrease of arable land and the more intense clear cut of forests all over Estonia, which has enhanced the overgrowth of bushes, among them willow (*Salix*).

Pollen spectra of analysed honeys revealed a variety of pollen types, which can be recognised as typical Estonian nectariferous plants. In addition, nectarless sources were recorded and the presence of these plants also indicated the Estonian origin of analysed samples. Furthermore, a few exotic pollen grains were found (e.g. *Helianthus* spp., *Zea mays*) within the samples analysed. Although there are no official statistics about the area of crops occupied by these plants, our personal observations indicate that sunflower and corn are popularly cultivated in gardens, and pollen of these species are likely to be incorporated into honey as well. Alternatively, the few occurrences of *Helianthus* and *Zea* pollen types could be interpreted as due to the mixing of Estonian honey with exotic honey, or the feeding of bees over

the winter months with honey from other regions (Aleksander Kilk, pers. comm. August 2010).

Kirs et al. (2011) described 14 samples of Estonian honeys, and the results of the melissopalynological analyses were similar to those observed in the present study. The most dominant pollen types in both studies were Brassicaceae (mostly *Brassica napus*), Rosaceae (*Rubus*), *Salix* and *Trifolium*. In addition, different species of Apiaceae and Fabaceae (*Melilotus*) as well as *Taraxacum* and *Frangula alnus* were present. Therefore, the previous research (14 samples) and the present one (325 samples) give a good overview of the pollen composition of Estonian honey.

The melissopalynological data from the nearest and ecologically similar areas in the neighbourhood of Estonia, where similar studies have been conducted, also show some similarities, and the most frequently represented pollen types are the same (e.g. Finland, Lithuania, Poland; Wróblewska & Stawiarz, 2004; Čeksterytė et al., 2006; Salonen et al., 2009; Kaškonienė et al., 2010; Stawiarz & Wróblewska, 2010). These studies also described the representation of Brassicaceae, Rosaceae, Apiaceae, *Salix*, *Trifolium* and *Taraxacum*. However, *Calluna*, *Epilobium*, *Rumex* and *Pinus* are more frequent in Estonia and Finland than in Lithuania and Poland, while *Centaurea cyanus* L. and *Aesculus* are more common in Central Europe.

Although there are some similar studies done in our neighbouring countries, we do not have nearby studies, where pollen concentration data are discussed. Therefore, we need to compare our results with studies, which have been done in Mediterranean countries, and where concentration data are available (La-Serna Ramos et al., 1999, 2002; de Sá-Otero et al., 2006; Silici & Gökceoglu, 2007). According to Maurizio's (1939) classification most of the samples belonged to class II (52%) and class I (30%), which means that honeys produced in Estonia are rather moderate in pollen concentration but not rich. In contrast, honeys from the Mediterranean region can be described rich or moderately rich in pollen and concentrations are mostly (~90% of cases) more than 10 000 pollen grains per gram of honey (La-Serna Ramos et al., 1999, 2002; Silici & Gökceoglu, 2007). However, our results of Estonian honeys revealed also some very rich samples. One reason for extremely high pollen concentrations (>100 000) of some samples beside natural characteristic, could be the amount of pollen added by beekeepers after collecting honey.

As mentioned earlier, there were no clear trends between concentrations and the pollen composition of honey. Differences in pollen production of different plant species, vegetation types around the hives and also variations in the climatic conditions from year to year (sunshine hours, temperature,

precipitation, etc.) means that there is considerable variation in concentration and composition. This variation is probably more so for Estonian honey, where summers are shorter than further south, and the flowering season varies in both duration and timing as a result. The preponderance of polyfloral honeys in Estonia is a reflection of this and makes it difficult to provide a generalisation about the content and composition of the honey.

The results show changes in the pollen concentration through the study period. We do not have a clear explanation for the differences, for example, we did not see any relationships between climatic variables and pollen concentrations during the study period. Many variations between the samples through the research period can be interpreted by the selection of samples. Unfortunately, most of the analysed samples lacked additional information about the vegetation around the hives, harvesting times and other variables likely to influence pollen composition and content of the honey. However, the abundant number of the samples gives an adequate picture of the pollen content of Estonian honey.

Conclusion

Herein, the first step was done by describing pollen content of Estonian honey and 325 honey samples were analysed. On the basis of the data obtained from the study, the following conclusions can be drawn. Pollen analysis of Estonian honey samples showed a wide variety of botanical sources. The pollen types of wild growing and cultivated plants are derived from typical Estonian flora. The results showed that the pollen types of Brassicaceae and Rosaceae were the most abundant among the samples. The pollen of *Salix*, *Trifolium*, *Calluna*, Apiaceae, Fabaceae, Asteraceae, Poaceae, *Fagopyrum esculentum* and *Frangula alnus* were also present. Several monofloral samples were described through the study period: honey of Brassicaceae, Rosaceae, *Calluna*, *Rubus*, *Salix*, *Trifolium*, *Sedum*, *Lotus* and *Tilia*. The pollen concentration values of Estonian honeys ranges from high to extremely low; the average values were 10 000 or below.

There have been profound changes in agriculture in Estonia since the 1990s. The increase of *Brassica napus* cultivation and more intensive clearcutting of forests all over Estonia have affected floristic diversity, which is in turn reflected also in the pollen composition of honey. The analytical data obtained in this research may be useful in future studies concerning the characterisation and properties of Estonian honeys. The present study supplements previous melissopalynological studies from our neighbouring regions and gives new information about regional plant sources for honey pollen.

The weakness of the current study was that most of the involved samples were 'blind' and not obtained directly from beekeepers. Therefore, we have not evaluated the floral origin of the honey by means of hive location, season and available floral source. Further studies should also consider this aspect.

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