**ЕКОЛОГІЯ** 

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Conservation of wild western honey bees *Apis Mellifera* in the Polissia natural zone of Ukraine: history, sources of nectar and pollen

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Preservation of the diversity of wild honey bees is complicated by insufficient knowledge about their distribution and status in individual territories. The wild population of the western honey bee A. Mellifera, which exists in the territory of Polissia of Ukraine, is an aboriginal subspecies and a natural component of the fauna of these places. The purpose of the research is to assess the current state of this population, which has been preserved thanks to the development of the ancient craft of local residents for extracting honey - «bortnitsvo». Such beekeeping is based on the capture of wild swarms and does not involve selective breeding. Wild honey bees are a valuable genetic resource for biodiversity conservation, as they are an important reservoir of local adaptations that determine their survival in the wild. The results of the physical and chemical analysis of honey obtained from the apiaries of local beekeepers of the Polissia Nature Reserve: diastase – 29.73DN, pH-4.9, F/G-1.41, HMF-6.33mg/kg, proline-608.87mg/kg, and melissopalynological analysis of pollen in honey, % (Callúna vulgáris - 35, Potentilla erecta – 19, Frangula alnus – 10, Lamium purpureum

- 8, Vaccinium myrtillus L - 5, Sisymbrium officinale L - 5, Quercus robur L - 4, etc.) confirmed its botanical origin and value as a source of nutrients for bees.

The assessment of the frequency of pollen grains showed the absence of pollen of one species in more than 45%. Only two species are defined as secondary. This is the pollen of the *Callúna vulgáris L family (Ericaceae)* – 35 % and *Potentilla erecta L (Rosaceae)* – 19 %. Pollen of such species as *Frangula alnus L (Rhamnaceae), Lamium purpureum L (Lamiaceae), Vaccinium myrtillus L (Ericaceae), Sisymbrium officinale L (Brassicaceae), Quercus robur L (Fagaceae), Artemisia vulgáris L (Asteraceae),* although they occupy the studied honey together 35 % of the total volume of pollen, however, according to the classification, each of these species is defined as important secondary.

Pollen of *Potentilla erecta L, Frangula alnus L, Lamium purpureum L,* as species with the longest flowering season, remain available almost throughout the honey collection season, and the anemophilic pollen-producing tree of the Fagaceae family *Quercus robur L* is a common source of pollen for honey bees in Polissia forests.

Key words: aboriginal subspecies, beekeeping, pollen, fresh honey, wild honey bees.

Problem statement and analysis of recent research. Bees (Apidae) are the most important group of pollinators, represented by 20,000 species, but most of them are wild species (Ollerton, Winfree, & Tarrant, 2011). Wild and managed pollinators support biodiversity to maintain ecosystem stability and provide human food (Klein et al, 2007, Potts et al., 2016). Pollinator depletion is one of the most pressing global environmental challenges of the 21st century (Gilbert, 2014, Goulson, Nicholls, Botias, & Rotheray, 2015, Alaux, Le Conte, & Decourtye, 2019). In fact, the situation is complex and controversial, both regarding pollinator coexistence and pollinator - flower interactions (Potts, et al., 2010, Prado, et al., 2020, Elliott et al., 2021).

Conservation of wild bees is complicated by insufficient knowledge about the distribution and status of different species in individual territories, due to their great diversity and variations in life histories (Pirk et al., 2017, Wood et al., 2020). In particular, there is debate over the threat posed by both managed and wild honey bees to native bees, and whether managed honey bees should be excluded from protected areas to minimize their impact on native bees (Goulson, 2003, Henry & Rodet, 2018, Requier et al., 2019).

However, studying the quality of life, the state of wild honey bee populations at the national level and individual territories will help identify threatened regions, as well as take the necessary measures for their conservation on a continent-wide scale. (Requier et al., 2019, Requier, 2019a, Parreño, et al., 2022).

The western honey bee, Apis mellifera, is the most widespread and best-studied species of agricultural bee worldwide. This species exhibits a multifaceted nature, both native and exotic, managed and "wild" in many regions. (Requier et al., 2019, Klein et al., 2007). In Europe, the local area of A. mellifera is limited to 60°N. (Ruttner, 1988), its colonies, as a commercially important species, are controlled by beekeepers. However, recent studies have highlighted the role of managed populations of the western honey bee Apis mellifera as a potential threat to wild pollinators, so their number in natural areas should be regulated (Moritz, Hartel, & Neumann, 2005, Henry & Rodet, 2018, Wood et al., 2020).

In addition, wild honey bees are associated with forests around the world, as the flowers provide them with nutrients and the trees provide shelter for the swarms. Therefore, such natural areas are critically important for the conservation of local subspecies and genotypes (Alaux, Ducloz, Crauser, & Conte, 2010, Alaux et al., 2017, Parreño, et al. 2022).

Therefore, forest ecology and beekeeping in forested or agroforestry areas must include an understanding of both managed and wild populations, including native or introduced subspecies, to encourage integrated conservation planning for all wild bees (Hill, & Webster, 1995, Moritz, Hartel, & Neumann, 2005, Cannizzaro, Keller, Wilson, & Elliott, 2022). Interactions between populations of different pollinator species (Amaya-Márquez, 2009, Alaux et al., 2017, Requier, & Leonhardt, 2020) and flowering plants, the available food landscape, and the health status of bees are crucial in determining how floral communities and appropriate conservation measures can support these populations (De la Rúa, Jaffé, Dall'Olio, Munoz, & Serrano, 2009, Di Pasquale, et al., 2013, Frias, Barbosa, & Laurenco, 2016). At the moment, there is no scientifically based information about the number of wild honey bees A. mellifera in Ukraine, there is not enough information about the genetic relationship and the degree of genetic isolation of individual populations, the level of anthropogenic influence on them. In particular, information on their forage preferences and pollen diet is needed to study and conserve ecologically and genetically valuable wild honey bees.

Purpose and tasks. We consider the western honey bee *A. mellifera*, which has long existed in the territory of Polissia of Ukraine, as an aboriginal subspecies and a natural component of the fauna, and confirm its current status as a wild population in these territories. We also define as "wild" all honey bee colonies that live here without human intervention, regardless of potential past human-assisted hybridization.

**The purpose of our research** is to assess the threats and pay attention to the current state of the wild population of *A. mellifera* in the territory of Ukrainian Polissia, to present a picture of the range of this subspecies of honey bees, which has been preserved thanks to the development of the ancient craft of the local population of honey extraction in this region – "bortnitsvo".

Therefore, our task was to collect data on today's distribution of "bortnitsvo", to investigate and evaluate the physicochemical characteristics of honey samples produced by wild colonies of *Apis mellifera*, to determine the botanical sources of nectar and pollen in the surrounding landscapes, which key flower species and groups plants are visited by honey bees living in a wild/ semi-wild state on the territory of the Polissia Nature Reserve.

This study has a methodological weakness related to the fact that we collected samples for the study once in early autumn at the end of the honey collection period. Bees collect and use pollen intensively in the spring when they need protein, so we were unable to determine exactly how much pollen is collected from spring honeydew, as it is used for mass rearing of brood in the early spring period. We did not take into account the phenology of plant flowering and pollen formation, weather conditions, but determined periods when wild honey bees may experience a shortage of pollen and nectar. In our region, this is the beginning of spring, and also, the second half of summer, before the flowering of Callúna vulgáris L.

Location and short history. Ukrainian Polissia is a part of Polissia, which covers the zone of mixed forests and the Polissia lowland within the borders of Ukraine. In the extreme northwest of the territory of the Zhytomyr region, in the Polissia lowland, the Polissia nature reserve is located. It occupies an area of 20104 hectares with geographical coordinates of 51°32'05" N. sh. 28°06'20"E, online address: http://polesyereserve.in.ua/museum-drs/borti/borti.html. The landscape of this area is swamps and forestswamp complexes with pine forests and sandy hills, and low-lying areas are covered with honey-bearing plants. Free access to the reserve, complete deforestation, reclamation works, hunting, fishing, mushroom and berry picking are prohibited here. Such natural conditions, as well as the consequences of the accident at the Chernobyl nuclear power plant in 1986, ensured weak urbanization and isolation of settlements here and contributed to the preservation of the traditional occupation of the local population forest beekeeping, which has long been called " bortnitsvo ". The small village of Selezivka is located on the protected area, the center of the reserve, where most of the residents are engaged in collecting forest honey.

Forest beekeeping in Polissia of Ukraine nowadays is the keeping of bees in logs made of pine wood, which in Polissia have received the local name "bort". Thus, in addition to traditional beekeeping with the breeding of bees in hives, which are fully cared for by beekeepers, in the north-western part of Ukraine, there is a unique form of it – "bortnitsvo", which has been preserved in a slightly modified form since the times of Kyivan Rus (IX- XIII century).

For centuries, wild honey bees have settled among Polissia forests in hollow, most often pine trees, in which a gap has appeared as a result of a lightning strike, or for other reasons the middle part has rotted and a hole has formed. Such openings are ideal for the habitat of wild bees. They firmly close the holes with propolis, protecting themselves from other insects, and thus create additional insulation for the winter. Such "settlement" has its advantages for the forest and tree – in particular, there is no rotting and damage to the wood, because the wax and propolis produced by bees have a disinfecting effect on the environment. The next period of development, most of which has survived to our time, is to put pine logs on trees with holes hollowed out for bees. Forest logs can serve up to 100 years and be passed down in the family to several generations of boarders as heirlooms.

The method of keeping bees in the "bort". Nowadays, local beekeepers use such beehives made of logs, which are fixed on trees. They call these logs "bort". The place for installing such a "bort" is carefully chosen in the forest. Usually they are placed on strong branches and on wooden spokes dug into the tree, at a height of 10-20 meters. The tree where the bees settled is highly valued, because not always the bee colony will settle in the place chosen by the beekeeper, and therefore, sometimes it is necessary to move logs from one tree to another for years. To protect against rain, the bort is covered with boards. Bort are made from old pines with a porous core with a diameter of 50 cm or more. In the middle of the log, a chamber with a height of 1.5-1.8 meters is hollowed out, with the calculation that the thickness of the walls is at least 10 cm. A wooden bar and a longitudinal lath on pegs close the hole in size (15 cm wide, 50 cm high) into a vertically hollow ovoid chamber where bees build their nest with honeycombs. On the opposite side of the log there is an opening for flying bees. In a thick, dry hive weighing more than 100 kg, bees can easily withstand frosts and do not overheat in summer. Photo: Vyshgorod Historical and https://yizhakultura.com/ Cultural Reserve. material/20200727 0034

In the forests of Polissia until our time, extracting honey from a log inhabited by bees is considered the most convenient method of beekeeping, even after the invention of the frame beehive. According to the employees of the Polissia Nature Reserve, about 1800 borts are concentrated in this territory and in a 15-kilometer zone around it – this is more than 70% of all borts in Polissia. Today's Polissia beekeeper – "bortnyk" has an average of 20-30 hives suitable for keeping bees, of which about half are inhabited by bee families. The intervention of beekeepers in the life of such a bee family is reduced only to the collection of honey at the end of summer. They open the log and use a beekeeper's knife to cut out the honey along with the combs, leaving the necessary amount of food for the wintering of the family, because such bees do not receive the usual sugar feeding. Honey is taken from the bort only once a year, usually in autumn, and not much – up to 10 kg at most, maybe not every year. A part has to be left for the bees for successful wintering.

With this method of keeping, there is no influence of the beekeeper on the selection and breeding of bees. The genetics of such populations of honey bees in Ukraine has not been sufficiently studied, but this beekeeping is based on the capture of wild swarms and does not involve selective breeding. In our opinion, wild populations of the western honey bee in Ukrainian Polissia represent a very valuable genetic resource for the preservation of biological diversity. the nesting of wild swarms, is also particularly important for the preservation of local subspecies and genotypes of bees. However, in the last decade, the area of the buffer zone around the reserve has undergone large-scale changes in land use (Kryvyi, Yushchenko, Dikhtiar, Lisohurska, & Stepanenko, 2021) due to increased planting of agricultural crops, in particular Heliánthus ánnuus and the production of sunflower honey by managed colonies of Apis mellifera. It is possible that many native and endemic bee species found there will be threatened by habitat loss and hybridization (Moritz, Härtel, & Neumann, 2005, Requier et al., 2019). Wild honey bees, pollinating plants, play their role as an integral element in the life of the forest ecosystems of the Ukrainian Polissia, but the interaction between managed and wild honeybees in the Polissia landscapes needs research.



Fig. 1. Wild western honey bees in the natural zone of Ukrainian Polissya.

Threats and problems for the conservation of wild populations. The entire northern part of Ukrainian Polissia, including the territory around the nature reserve, has a rich species composition of plant taxa (Sichenko, Kryvyi, & Dikhtiar, 2021). They bring a diversity of flowers to the diet of pollinators, which is important for them (Hendriksma, & Shafir, 2016, Requier, & Leonhardt, 2020, Jachuła, Denisow, Wrzesień, & Ziółkowska, 2022) and is generally absent in the surrounding farmland. The presence here of old trees with cavities, which are necessary for As the experience of various European countries shows, migratory beekeeping and queen trade combined with a system of promiscuous mating exposes the growing introgressive hybridization of native European honey bees with managed non-native subspecies, which leads to the loss of valuable combinations of traits formed by natural selection. Scientists suggest that a large part of the *A. mellifera* population across Europe is now artificially hybridized (De la Rúa, Jaffé, Dall'Olio, Munoz, & Serrano, 2009, Requier et al., 2019). There are several

endemic subspecies of *Apis mellifera* in Europe, but the distribution of these subspecies today is largely influenced by managed beekeeping (Moritz, Härtel, & Neumann, 2005). This raises concerns about the loss of biological diversity and the possible disappearance of subspecies from their former ranges (Alaux, Le Conte, & Decourtye, 2019, De la Rúa, Jaffé, Dall'Olio, Munoz, & Serrano, 2009).

Modern beekeeping throughout the territory of Ukraine is developed in managed apiaries using standard hives and under the year-round intensive control of beekeepers. They move hives long distances to agricultural land to collect nectar and pollinate. Beekeepers treat bees against pests and pathogens, and control reproduction, such as swarming, queen rearing. And queen selection and displacement lead to human-mediated hybridization. In Ukraine, at the moment, there is a complete lack of data to assess the level of introgression, but it is known that beekeepers, at their discretion, without any control from state authorities, massively replace queens with A. mellifera Carnica, or Buckfast in managed honeybee populations bees to increase the productivity of bee colonies. Because of that, locally adapted wild populations of A. Mellifera in Ukraine are also under threat, because such introgressive hybridization of managed colonies can negatively affect the wild and, in particular, lead to the loss of traits related to endurance and adaptation to the environment (De la Rúa, Jaffé, Dall'Olio, Munoz, & Serrano, 2009, Meixner, Kryger, & Costa, 2015, Pirk, Crewe, & Moritz, 2017).

Additionally, coexistence with managed apiaries exposes wild honey bee populations to bee pests and pathogens. For example, treatments against Varroa mites can interfere with the natural development of parasite resistance/tolerance in managed colonies (Pirk, Crewe, & Moritz, 2017). Their hybridization with the wild is likely to result in the transfer of susceptible phenotypes to wild populations, thereby increasing the risk of extinction in the wild. Conversely, the presence of wild honey bees undergoing natural selection can have a positive effect on the resistance and persistence of managed introduced populations through the transmission of adaptive traits. Wild populations are an important reservoir of local adaptations that determine the survival of honey bees in the wild. For example, in Africa and North America, it appears that wild populations actually moderate the effects of Varroa mites, allowing colonies to develop resistance (De la Rúa, Jaffé, Dall 'Olio, Munoz, & Serrano, 2009, Fürst, McMahon,

Osborne, Paxton, & Brown, 2014). This resilience is likely based on interactions between wild and managed bees, as a large proportion of the total honey bee population in these regions is wild and not exposed to human influence. Of course, a better ratio of wild to managed colonies can also ensure that beneficial adaptations in wild colonies will trickle down to managed ones. Therefore, to maintain local ecosystem services. it is important to maintain healthy populations of pollinators that are regionally endemic and not to transport them across ecological boundaries or continents (Pirk, Crewe, & Moritz, 2017). Under this condition, regionally adapted hybridized populations can also be a source of variability from an evolutionary perspective (Requier et al., 2019).

Materials and methods of research. Through personal communication with beekeepers working in different places on the territory of the Polissia Nature Reserve, we selected 25 samples of fresh honey in honeycombs. The obtained honey samples were stored at a temperature not higher than 20°C without access to sunlight. In the combs that were filled with honey and sealed by bees, the wax caps were cut with a bee knife and the combs with honey were filtered through a sieve with holes of 0,5 mm in diameter to separate the honey from the comb. Laboratory samples were homogenized by careful thorough mixing for at least three minutes, so that as little air as possible entered the honey. Raw honey samples without heating were used in all analyses.

The samples were analyzed according to the following indicators: moisture content (%) using a manual digital refractometer to determine the humidity of honey PAL22S; diastase activity according to the Schade method using a ULAB 102 spectrophotometer at 660 nm; the color of honey samples according to Pfund's color grader (comparator) and classifier; hydroxymethylfurfural (HMF) mg/kg based on UV adsorption at 550nm (spectrophotometer ULAB 102); absorption of proline (mg/kg) was titrometrically read by a spectrophotometer at 520nm (ULAB 102); fructose, glucose by HPLC on a spectrophotometer ULAB102; pH, free acidity potentiometrically at 20°C using a Gryf 209L pH meter (Gryf HP); electrical conductivity at 20°C in solutions of honey samples in deionized water with a CDM210 conductometer (Radiometer Analytical SAS).

Extraction of pollen from honey was carried out by standard methods of melissopalinological analysis using equipment: a medical centrifuge ELMI CM 6M with a rotation speed of 3500 RPM. OHAUS PA 214C analytical scales with a resolution of 0,0001 g from the base level. Pollen was examined under a Leica MD 500 LED binocular microscope with a magnification of 400-1000 times. Identification was carried out using the electronic online database of pollen grains PalDat (https://www.paldat.org/).

The pollen frequency of each honey-bearing plant was estimated according to the method proposed by (Louveaux, Maurizio, & Vorwohl, 1978). The result was expressed as a percentage of the total amount of pollen. Pollen types that had no proven botanical affinity remained "indeterminate", but they accounted for less than 0,4% of the total pollen mass. Laboratory tests were performed in triplicate on each sample, and the results were determined as the mean value of all samples with standard deviation (SD). The methods used for the analysis were based on the methods of the Association of Official Analytical Chemists (AOAC, 1990), and/or the Harmonized Methods of the European Honey Commission and the International Honey Commission (Bogdanov et al., 1999, EC, 2002). All chemicals used were analytical or general purpose reagents. Statistical analysis was performed using the "Data Analysis" software module in Microsoft Excel.

We personally interviewed beekeepers in the village Selezivka to collect material on the distribution of wild populations of the western honey bee *Apis mellifera*. In addition, we used the results of observations of bees by a leading specialist in ecological and educational work of the Polissky Nature Reserve Zhyla S.M.

Results of physico-chemical and melissopalinological analysis of honey table 1 shows the average values, standard deviations and ranges of various physicochemical parameters of honey.

All physico-chemical parameters of the honey that underwent research correspond to Codex Alimentarius norms (FAO, 2001, EC, 2002). Honey contained moisture within normal limits (<20%). Hydroxymethyliurfural (HMF) of all honeys analyzed was below 8,42 mg/kg, and the mean value of diastase was 29,73 (DN). These values are below the upper limit of 40 mg/ kg for HMF and above 8 DN for diastase. The color of honey is defined as amber, the average value on the Pfund scale is 104 mm.

The predominant sugar in the studied honey is fructose with an average value of 34,17g/100g. The glucose level is lower with an average value of 24,18g/100g. The minimum and maximum content of fructose in g/100g ranged from 29,91 to 38,26 in different samples, glucose from 20,49 to 31,27 respectively. The determined total average content of glucose and fructose is 58,35g/100g, which is below the minimum limit recommended by the Codex Alimentarius for flower honey (60g/100g), but, at the same time, it is much more than the standard for honey dew– over 45g/100g of honey. The average fructose/ glucose ratio is 1,41.

| Parameter                       | Mean   | SD    | Min    | Max    | Codex<br>Alimentarius |
|---------------------------------|--------|-------|--------|--------|-----------------------|
| Moisture (%)                    | 17.5   | 1.1   | 16.0   | 18.2   | 20                    |
| рН                              | 4.9    | 0.1   | 4.5    | 5.0    | 3.2-4.95              |
| Free Acidity (meq/kg)           | 32     | 1.2   | 28     | 36     | 50                    |
| Electrical conductivity (mS/cm) | 0.638  | 0.135 | 0.613  | 0.721  | 0.8                   |
| Color (mm Pfund)                | 104    | 12    | 95     | 113    | 0–150                 |
| HMF (mg/kg)                     | 6.33   | 0.87  | 5.17   | 8.42   | 40                    |
| Diastase activity (DN)          | 29.73  | 1.33  | 24.23  | 31.21  | 8                     |
| Fructose г/100г                 | 34.17  | 1.01  | 29.91  | 38.26  | -                     |
| Glucose г/100г                  | 24.18  | 0.99  | 20.49  | 31.27  | -                     |
| Fructose + Glucose г/100г       | 58.35  | 2     | 50.4   | 69.53  | 60                    |
| Fructose/Glucose                | 1.41   | 0.24  | 1.47   | 1.22   | 1                     |
| Proline, мg/кg                  | 608.87 | 13.37 | 578.34 | 634.29 | 180                   |

Table 1 – Mean values of physicochemical parameters

All tested samples were acidic (free acidity at the level of 32 mEq/kg, pH was estimated at 4,90), but the indicator is within the limit limits. The average content of proline in honey samples was 608,87 mg/kg, which is more than 3 times higher than the recommended minimum norm of 180 mg/kg. According to the value of electrical conductivity, the studied honey can be classified as flower honey. All samples had an electrical conductivity of less than 0,8 mS/cm. The results of the analysis showed that the pollen profile of the studied honeys is represented by 10 morphotypes of pollen grains of different plant species with a low percentage. Honey contained a small amount of natural impurities.

The diversity of pollen obtained from honey varied from 10 to 15 pollen morphotypes, but, as shown in table2 main ones are 10.

Bees visited different types of plants such as trees, shrubs and herbaceous flowering plants. The content of pollen in honey reflects the diversity of plant life in the territory of Polissia of Ukraine, which contributes to the production of honey with different properties, and shows how much honey bees strive for a variety of pollen diet. The estimation of the frequency of pollen grains, according to the applied method of melissopalynological analysis, showed the absence of pollen of one species in more than 45%. Only two species are defined as secondary. This is the pollen of the *Callúna vulgáris*  L family (Ericaceae) – 35% and Potentilla erecta L (Rosaceae) - 19%. Pollen of such species as Frangula alnus L (Rhamnaceae), Lamium purpureum L (Lamiaceae), Vaccinium myrtillus L (Ericaceae), Sisymbrium officinale L (Brassicaceae), Quercus robur L (Fagaceae), Artemisia vulgáris L (Asteraceae), although they occupy the studied honey together 35% of the total volume of pollen, but according to the classification, each of these species is defined as important secondary. So, together, all these plants were the main attractive sources for bees to obtain nectar or pollen.

The analysis helped to outline the pollen of *Potentilla erecta L, Frangula alnus L, Lamium purpureum L* as species with the longest flowering season, they remain available almost throughout the honey collection season from the end of spring to the beginning of autumn. The anemophilic pollen-producing tree of the Fagaceae family, *Quercus robur L*, is also a common pollen source for honeybees in Polissia forests.

Our research showed that bees can collect only pollen from some plants, such as *Quercus robur L*, *Genista tinctoria L*, and pollen and nectar from other plants. Another aspect is that bees often collect pollen from plants that they have easy access to from their hives, and most choose plants that can provide both nectar and pollen.

| Pollen type               | Percentage (%)<br>of pollen | Families     | Flowering period in Ukrainian<br>Polissia |
|---------------------------|-----------------------------|--------------|---|
| Callúna vulgáris L,       | 35                          | Ericaceae    | August – September                        |
| Potentilla erecta L       | 19                          | Rosaceae     | June – September                          |
| Frangula alnus L          | 10                          | Rhamnaceae,  | May – June, August                        |
| Lamium purpureum L        | 8                           | Lamiaceae    | May – September                           |
| Vaccinium myrtillus L     | 5                           | Ericaceae    | April – May                               |
| Sisymbrium officinale L   | 5                           | Brassicaceae | June – July                               |
| Quercus robur L           | 4                           | Fagaceae     | May                                       |
| Artemisia vulgáris L      | 3                           | Asteraceae   | July – September                          |
| Astragalus glycyphyllos L | 2                           | Fabaceae     | June – August                             |
| Genista tinctoria L       | 1                           | Fabaceae     | June – July                               |
| Other pollen grains       | 0.4                         | _            | _   |

 Table 2 – Melisopalynological analysis of honey from natural areas

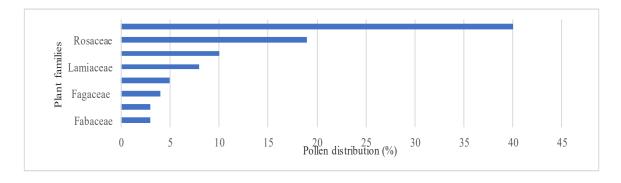


Fig. 2. Distribution of pollen by plant families.

Figure 2 shows the distribution of pollen in all honey samples based on plant families. This information shows the availability of desirable plant sources for honeybees within the foraging range of the hives. Two families are represented in honey by the pollen of two plant taxa. These are Callúna vulgáris L, Vaccinium myrtillus L from Ericaceae (together 40%), and Astragalus glycyphyllos L, Genista tinctoria L from Fabaceae (together 3%). And, if the largest amount of Ericaceae pollen in honey confirms the importance of this family for the production of beekeeping products by honey bees in the natural landscapes of Polissia, then the pollen content of Astragalus glycyphyllos L and Genista tinctoria L (Fabaceae), Artemisia vulgáris L (Asteraceae) can also be explained by the phenology of their flowering, which coincides with a period of scarcity when bees can select these types of pollen to balance their diet for different essential nutrients.

**Discussion.** The results of the physical and chemical analysis are consistent with public data from various scientific sources. Thus, the moisture content within the standard limit indicates the maturity of honey, the ability to resist fermentation and granulation, contribute to a long shelf life (Bayram, & Demir, 2018, Amariei, Norocel, & Scripcă, 2020, Beykaya, 2021). Diastase activity indicators and HMF parameters can be used to assess honey freshness and/or overheating (El Sohaimy, Masry, & Shehata, 2015), but HMF content can range from 2,5 mg/kg to 12,3 mg/kg depending on the species honey (Beikaya, 2021).

In our case, the color intensity cannot depend on the beekeeper's handling of the combs, so high Pfund values may indicate a higher content of phenolic compounds and flavonoids (El Sohaimy, Masry, & Shehata, 2015). The sum of free acids within the normal range shows the presence of organic acids in equilibrium with the lactone, internal complex esters and inorganic ions and indicates the freshness of all the tested samples. The acidity of honey affects its characteristic taste and resistance to microbial attack (Kirs, Palla, Martverka, & Laos, 2011, Beykaya, 2021).

Honey is constantly analyzed for sugar content. According to (Kaskoniene, Venskutonisa, & Ceksteryte, 2010), fructose content in various honeys from Southern European countries can range from 314 to 431 mg/g, glucose from 237 to 407 mg/g. In non-European honeys, these fluctuations are higher. The amount of fructose and glucose in all studied samples of Lithuanian honey varied from 329,2 to 426,3 mg/g. But factor, fructose/ glucose was 0.78 - 1.16. In comparison, other honey studies found a factor of 1,766 for acacia (Robinia pseudoacacia) honey, which has the lowest tendency to crystallize (Scripcă, Norocel, & Amariei, 2019 Amariei, Norocel, & Scripcă, 2020). According to these sources, the fructose content of such honey can be at the levels of 44,523 - 45,98 g/100 g, and the fructose/glucose factors can be used to predict the ability of honey to crystallize. Crystallization of honey occurs faster if F/G is below 1.0 and slows down when it is greater than 1. Glucose is less soluble in water than fructose.

Proline makes up more than half of the total amount of amino acids in honey. It is added by bees during the conversion of nectar into honey, but proline is present in various plants in quantities. The content of proline in different polyfloral honey samples ranged from 503,46 mg/kg to 696,09 mg/kg according to data (Bayram, & Demir, 2018) and ranged from 404,2 to 881,7 mg/kg according to research results (Beykaya, 2021). Therefore, this indicator shows the level of maturity of honey, and is used as a criterion for determining fake honey with sugar syrup.

The stability of honey bee populations in the natural landscapes of Ukrainian Polissia depends on the diversity of flowering plant communities (Sichenko, Kryvyi, & Dikhtiar, 2021), which provide a sufficient amount of various nutrients. These substances must be found in nectar and pollen resources available to bees (Wilson, et al., 2021 Parreño, et al., 2022). Nectar provides them with carbohydrates to support energy and metabolic processes. Pollen is the main source of proteins, fats, mineral elements, vitamins, etc., for tissue homeostasis, development and growth of larvae (Roulston, & Cane, 2000, Hanley, Franco, Pichon, Darvill, & Goulson, 2008, Frias, Barbosa, & Laurenco, 2016).

Callúna vulgaris is one of the main food sources at the end of the season because it affects the wintering of honey bees. It has an open, small (<6 mm) corolla that allows bees easy access and offers abundant pollen and nectar (Descamps, Moquet, Migon, & Jacquemart, 2015). Early spring resources of Vaccinium myrtillus are known to have a critical effect on the development of honey bee colonies after winter, despite the fact that its flowering period is short and lasts no more than a few weeks. During the flowering period of different plant species, which coincide in time, bees may visit V. myrtillus mainly for nectar resources. (Moquet, Mayer, Michez, Wathelet, & Jacquemart, 2015). According to our results, Vaccinium myrtillus and Quercus robur also belong to the main early spring pollen sources. Pollen from Potentilla erecta plays a significant role in supporting populations of various bee species at their location in the Swiss Alps (Müller, & Richter, 2018, Müller, 2018a). According to melisopalynological studies of honey in Estonia and Lithuania, Frangula alnus constitutes a significant proportion of pollen collected by bees (Čeksteryte, Kurtinaitienė, & Balžekas, 2013, Kirs, Palla, Martverka, & Laos, 2011). Pollen and nectar composition influence colony foraging behavior (Ghosh, Jeon, & Jung, 2020), but floral resource availability also matters to bees if the source of pollen is less nutritious. Scientific studies in different climatic conditions (Wilson, et al., 2021, Parreño, et al., 2022) support the assumption that bees choose pollen to balance their diet in terms of protein, lipids, essential fatty acids to maintain homeostasis on colony levels. The protein content of pollen from different plants varies greatly, ranging from 2,5 % to 61 %, but most average 25–45 % (Roulston, & Cane, 2000, Hanley, Franco, Pichon, Darvill, & Goulson, 2008).

According to the results of pollen studies, the average relative protein content in % was:

(Genista tinctoria – 22,8, Potentilla erecta – 16,3 Calluna vulgaris - 13,9 (Hanley, Franco, Pichon, Darvill, & Goulson, 2008), Sisymbrium officinale - 22,2 (Somerville, 2005), Lamium purpureum from 21,82 - 24,90 % in different periods (Cınbırtoğlu, & Güney, 2021). Furthermore, Somerville notes that a significant nutritional value of Sisymbrium officinale pollen may be the high content lipids with an average value of 5,8 %, not the protein level. Palynology of our studies showed that Genista tinctoria, with the highest protein level, accounted for only 1% of the total pollen mass, while Calluna vulgaris, with the lowest protein content, accounted for 35%. Although well honey bees are known to show loyalty to certain flowers (Sedivy, Müller, & Dorn, 2011), further study of the effect of pollen quality and nutrient content on bee visitation is needed. For example, according to (Tellería et al., 2019) protein content in the pollen of the Asteraceae family is variable, and research (Radev, & Zheko, 2018) did not find a connection between the amount of pollen collected by bees and its protein content. The amount of pollen collected by bees also depends on the availability to the bee population of certain plants they visit and the type of flowers, direct access and distance to hives (Hill, & Webster, 1995).

Lamium purpureum, Potentilla erecta in the conditions of Polissia of Ukraine bloom for 3-4 months, so bees collect this pollen all season. Entomophilous weeds provide relatively little pollen on a landscape scale, but they increase resource diversity and balance bee diets throughout the season (Jachuła, Denisow, Wrzesień, & Ziółkowska, 2022). The content of pollen grains of Potentilla erecta, Lamium purpureum, Sisymbrium officinale, Artemisia vulgáris, Astragalus glycyphyllos, Genista tinctoria in our studies confirms this conclusion. In addition, wind-pollinated plants can be common sources of pollen in both gardens and forests, including in the tropics (Alaux, et al., 2017, Cannizzaro, Keller, Wilson, & Elliott, 2022). In our experiments, it is *Quercus robur*. Honey bees prefer this pollen in the spring (Persson, et al., 2018). Overall, our results confirm that the main nutritional value of pollen for honey bees is provided by 7-16 plant species, depending on the habitat. Species that each produce <2% of the total pollen mass are also important for food supply (Alaux, Ducloz, Crauser, & Conte, 2010, Wilson et al., 2021).

Bees need spatial and temporal diversity of natural habitats, physiological adaptations to cope with the unfavorable chemical composition of certain types of pollen, and, as suggested by different various sources, the mechanisms underlying this process differ for different types of pollen (Menzelm, et al., 2005, Amaya-Márquez, M.(2009). Natural flora taxa provide about 80% of the pollen diversity, so it is necessary to preserve and protect this native flora in the bee habitat (Alaux, et.al., 2017).

Various floral sources of nectar and pollen allow bees to dilute various toxic compounds or pesticides, thus exposing themselves to less exposure to harmful substances. In particular, the palynology of pollen reserves in *Osmia* bee nests (Ruddle, et al., 2018) showed a maximum average of 31% canola (*Brassica napus L*) pollen in any plot, with *Quercus robur* pollen accounting for up to 86%. Furthermore, they do not simply incorporate new sources, but specifically target those that supplement specific colony nutritional deficits by navigating with spatial memory (Menzelm et al., 2005, Hendriksma, & Shafir, 2016).

Bees in an environment with significant biodiversity can receive more different pathogens, parasites, infections, including from managed populations (Flores, et al., 2021). However, access to diverse nutritional resources makes them more resilient to such risks through better immunity (Goulson, Nicholls, Botias, & Rotheray 2015, Jack, Uppala, Lucas, Sagili, 2016, Parreño, et al., 2022). They may also adjust their diet to fight infection, for example with antimicrobial secondary plant metabolites. According to (Koch, et al., 2019), the most bioactive species was *Calluna vulgaris*, the best producing nectar plant in our studies. Pollen analysis of honey confirmed that wild honey bees of Ukrainian Polissia survive in local natural conditions thanks to their adaptation, visit the same taxa of plants to collect nectar and pollen, as well as different species of wild bees (Müller, & Richter, 2018, Müller, 2018a, Descamps, Moquet, Migon, & Jacquemart, 2015).

However, modern research shows that the availability of nutrients in the habitat does not always and everywhere meet the needs of local bee colonies (Amaya-Márquez, 2009, Requier, & Leonhardt, 2020). To study these complex and contradictory relationships between plants and pollinators, monitoring the availability and flow of nutrients for honey bee colonies in the natural landscapes of Ukraine will also help.

**Conclusion.** Wild honeybees are native to Ukrainian Polissia, but work is needed to preserve the network of interactions between all wild bee populations in the region, including honeybees, including through the regulation of managed beekeeping, to mitigate the risks of managed apiaries impacting wildlife. To implement such tasks, it is necessary to study the experience, encourage and support local beekeeping – "bortnitsvo", which uses specific methods of beekeeping management without threatening the local ecology, which contributes to the preservation of biodiversity.

Ukraine needs a program for conducting scientific research at the national level in various aspects of biology, genetics, control of bee diseases and protection of valuable ecotypes to ensure the conservation of different species of local honey bees. There is a need for monitoring programs to assess the density of feral colonies, the level of reproduction and the factors that determine the dynamics of the survival of their population throughout the country. Increasing the level of professionalism of beekeepers will help their awareness of the risks associated with the decrease in the population of wild honey bees and the loss of their adaptive characteristics. The results of this study may be useful for obtaining information about plants as potential food sources for managed beekeeping.

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Збереження диких західних медоносних бджіл *Apis Mellifera* у Поліській природній зоні України: історія, джерела нектару та пилку

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Збереження різноманіття диких медоносних бджіл ускладнюється недостатніми знаннями про їх поширення та статус на окремих територіях. Дика популяція західної медоносної бджоли A. mellifera, яка існує на території Полісся України, є аборигенним підвидом і природним компонентом фауни цих місць. Мета дослідження – оцінити сучасний стан цієї популяції, яка збереглася завдяки розвитку давнього ремесла місцевих жителів із добування меду – «бортництва». Таке бджільництво засноване на відлові диких роїв і не передбачає селекційного розведення. Дикі медоносні бджоли є цінним генетичним ресурсом для збереження біорізноманіття, оскільки вони є важливим резервуаром місцевих адаптацій, які визначають їхнє виживання в дикій природі. Результати фізико-хімічного аналізу меду, отриманого з пасік місцевих бджолярів Поліського природного заповідника: активність діастази – 29,73, pH – 4,9, фруктоза / глюкоза – 1,41, гідроксиметилурфурол – 6,33 мг / кг, пролін - 608,87 мг / кг, а мелісопалінологічний аналіз пилку в меду, % (Callúna vulgáris-35, Potentilla erecta – 19, Frangula alnus – 10, Lamium purpureu -8, Vaccinium myrtillus L-5, Sisymbrium officinale L-5, Quercus robur L-4 та ін.) підтвердили його ботанічне походження та цінність як джерела поживних речовин для бджіл.

Оцінка частоти пилкових зерен показала відсутність пилку одного виду понад 45 %. Тільки два види визначені як вторинні. Це пилок *Callúna* vulgáris L podunu (Ericaceae) – 35 % та Potentilla erecta L( Rosaceae) – 19 %. Пилок таких видів як Frangula alnus L (Rhamnaceae), Lamium purpureum L (Lamiaceae), Vaccinium myrtillus L (Ericaceae) Sisymbrium officinale L (Brassicaceae), Quercus robur L (Fagaceae), Artemisia vulgáris L (Asteraceae) хоча і займає в досліджуваному меду разом 35 % від усього обсягу, проте за класифікацією кожен з цих видів визначається як важливий другорядний.

Пилок Potentilla erecta L, Frangula alnus L, Lamium purpureum L як видів з найбільшим сезоном цвітіння залишається доступним практично упродовж усього сезону медозбору, а анемофільне пилкоутворювальне дерево родини Fagaceae Quercus robur L є звичайним джерелом пилку для медоносних бджіл у лісах Полісся.

Ключові слова: аборигенний підвид, бджільництво, пилок, свіжий мед, дикі медоносні бджоли.



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