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作品名稱 **The influence of lanscape on nest preferences and behavior of twig nesting Hymenoptera**

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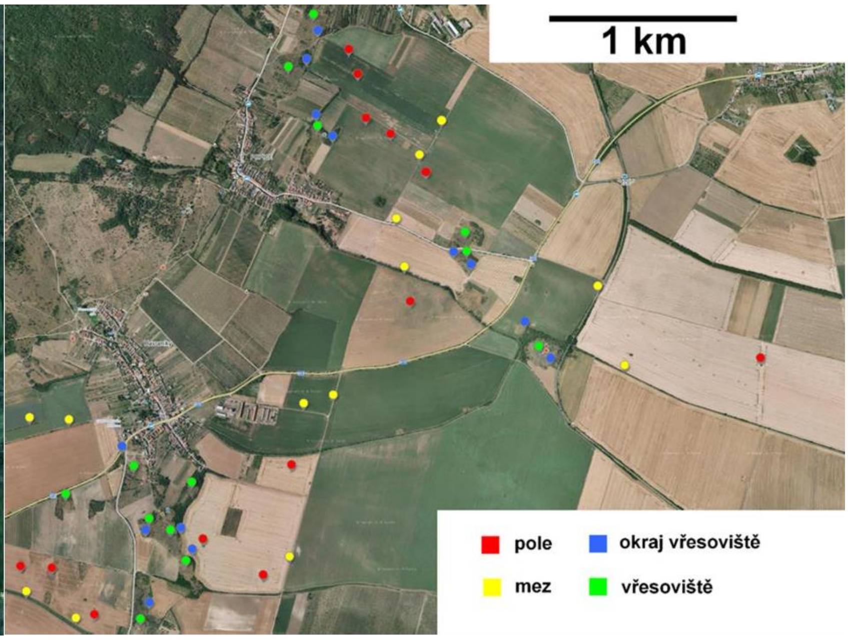
1. Introduction

In this thesis I am focusing on the influence of landscape on nesting behavior of insects. Nowadays, we can often hear about the extinction of insects and the impact of agriculture on this phenomenon. But there is little scientific research devoted to the changes in insect behavior and ecology that the modern landscape can cause, and almost all researches have taken place in Amerika. I chose bees in dry stems as model insects, as I have been working with this group for several years already. I dealt with the nesting preferences of bees depending on the structure of landscape. I chose for this comparison bees nesting in dry stems, for which I have placed nesting opportunities in four different habitats - heathlands, heath edges, field boundaries and fields. The studied locality was located south of Znojmo near the Podyjí National Park in Czech Republic. I focused mainly on solitary bees Ceratina, whose behavior I have been intensively researching for a long time. Ceratina are especially interesting for their nesting behavior, care for offspring and simple social behavior. The species Ceratina nigrolabiata, for example, is the only bee species in the world that has been found to have biparental care for its offspring.

***The small carpenter bees of genus Ceratina**. On the right: Ceratina chalybea – most abundant Ceratina bee on our localita, On the left: Ceratina chalybea – the only known bee with biparental care for offsprongs in the world.*

2. Methodology

Dry twigs of plants that contain pith are an excelent nesting oportunity for wide spectrum of organisms. The pit his tissue with big, dead cells with thin cell walls. It is soft and is placed in the middle of the twig. For this particular experiment I have made sheafs from twigs of 4 species of plants.



Pic. 1: Handmade nesting oportunity (sheaf) for twig nesting Hymenoptera Pic. 2: Map of places, where the nesting oportunities were put: red – filds, blue – heath edges, green – heaths, yellow – field boundaries

The nesting oportunities were placed in 4 biotopes – heathlands, heath boundaries, field edges and fields. There were 13 places of each biotope, where the nesting oportunities were placed – overal 52 places (pic. 2). On every place, I have put 5 sheafs of twigs, which contained 16 twigs each. The places had to be at least 50 meters far from each other.

In summer, I had always randomly chosen a sheaf from each place and all twigs in the sheaf were covered with tape to prevent the insects from escaping. I have then dissected all nests from the sheaf. This way there were dissected nests from cca 3 sheafs in each place. The dissection was made by using gardening scissors. With them we carefully vertically opened the nest, we jotted down the nest structure and adult Hymenoptera from the nests were put in 96% ethanol for genetical analysis or species determination. During the nest dissection were noted down data like nest length, number of offsprings, pith width and a presence of parents in the nest. Young offsprings were then fostered until adulthood and then preserved in ethanol.

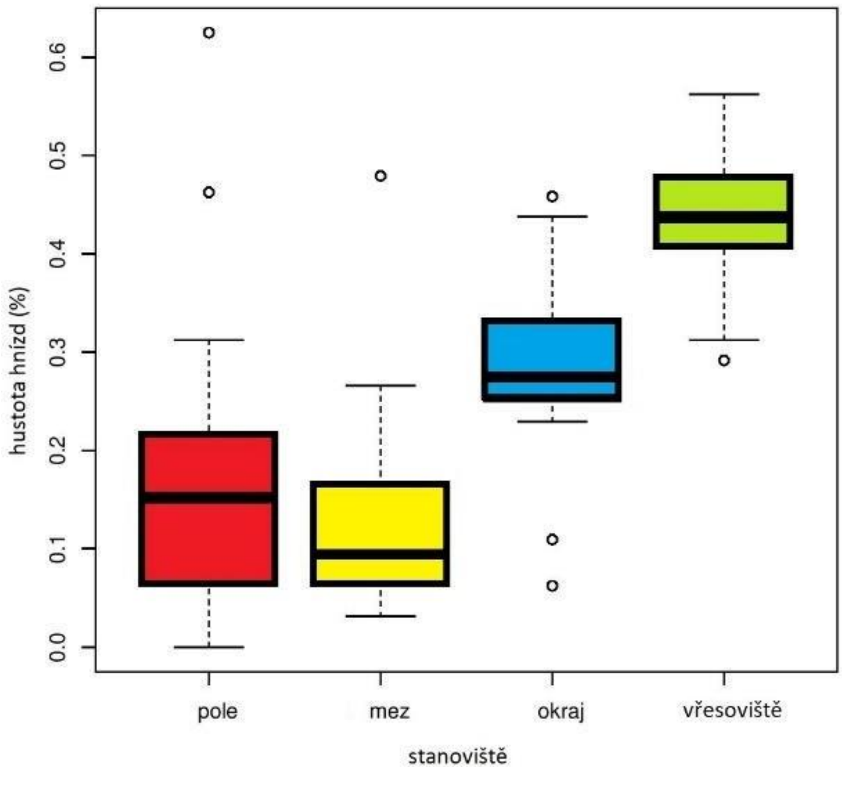
3. Goals

My goal was to find out and evaluate the differences in the nesting ecology of bees in the dry plant stems between the agrarian habitat (field) and the usually sought habitat for nesting (heathlands), then the habitat that forms a kind of transition between these two habitats. I especially wanted to focus on small carpenter bees Ceratina and their behavior. The aim was to find out which species prefer which habitats, their nesting structure, sex of offspring, maternal investment rate and other factors, and then to assess how these phenomena differ between habitats. Using the analysis of microsatellite loci, I also wanted to find out whether the number of mates in Ceratina nigrolabiata differs between habitats, as i tis known that mother has offsprings with many differents fathers

4. Results and discussion

1.1 Proportion of occupied twigs

In total, more than a third of the offered twigs were occupied. This number is relatively high and thanks to it we can consider the produced sheaves and their location to be generally suitable for research of twig-nesting Hymenoptera. However, twig occupancy varied greatly between biotopes (Pic. 3). The density of nests is probably strongly dependent on the usability of the habitat. The most nutritious habitat with the largest amount of food in this case is the heathland habitat. The most abundant inhabitants of the sheaves were small carpenter bees Ceratina. Their short ontogenetic development can contribute to this effect as an advantage, which allows parents to nest several times a year. The other advantage of Ceratina bees is their care for the offspring by a mother (or father) and relatively low mortality of the offspring as a result. The most abundant Ceratina species was the Ceratina chalybea, whose nests accounted for a third of all nests analyzed. In addition to other stems, there were also nests of biparental Ceratina nigrolabiata, Ceratina cyanea and, rarely, Ceratina cucurbitina. Other species of nesting bees were also Pemphredon lethifer or Hoplitis tridentata. It is necessary to také in consideration that the stems that we prepared for nesting contained pith, so other bees nested in them than if we offered hollow stems (eg. reed).



Pic. 3: Proportion of occupied sheaves between habitats by all bee species

1.1.1 Field habitats

The lowest density of used twigs was recorded in the field habitats, which is probably due to their remoteness from the heaths, where the main bee population is located. It can also be caused by a lack of food, as habitats in the middle of fields were usually far away from blooming plants. Ceratina bees pollinate a very wide range of plants. The fact that some fields were sown with sunflowers, so there should be no shortage of food, rather suggests that the nesting opportunities in the field were too far from heaths to be populated from faraway heaths.

Sunflowers bloom from June to August, which is the time of the highest activity of supplying of the brood chambers of Ceratina by mothers with pollen, so they should have had enough pollen to supply the chambers. Tschrantke 1998 et al. but states that the frequency of solitary bees increases not depending on the abundance of flowering plants, but on the diverzity of plant species, which was low in these monocultural fields. It is therefore possible that we are seeing a similar trend here.

1.1.2 Field boundaries

Field boundaries are an important landscape element that is essential in todays monotonous agrarian landscape and can have a significant effect on migration of organisms in the landscape. It is assumed that these stripes of vegetation between fields can serve as a refuge and a migration corridor for movement between isolated islands of natural habitats. At the field boundaries, the density of occupied twigs was slightly higher than in the field, but the edge of heaths and heathlands was still relatively small compared to the habitat. There were fewer nests than in the field biotope, which is the least of all habitats. Importantly, our research does not show that the boundaries are significantly more suitable for nesting than isolated islands of heaths in the fields. This is probably due to good flight ability of bees. However, one habitat was located more than a kilometer from the nearest heathland, and we did not notice a single nesting of bees. So this distance is too high. There were usually significantly more blooming plants in the vicinity of the sheaves than in the field, and the difference in the density of the used twigs is relatively marginal. So we did not succeed to confirm the role of field boundaries in the migration of insects between habitats, which may be due to the relatively disparate types of boundaries on which we have placed sheaves (both boundaries formed by shrubs and herbs). In some places, the dense vegetation has outgrown the sheaves we placed and therefore made it impossible for bees to nest there. This phenomenon could therefore reduce the total number of recorded nests at the field boundaries.

1.1.3 Heath edges

The heath edges form the boundary of various habitats. Unlike the boundaries that form the boundary between the same habitat, there are two completely different habitats, the poorest in insect fields and the richest in biodiverzity heaths. The aim was to find out how the effects of both of these habitats manifest themselves here. It is known that the boundaries of ecosystems are sometimes the richest in species, as species typical for both ecosystems can be found there. We recorded the highest number of nesting species in this habitat together with heathland. It was significantly higher in these two habitats than in the field boundaries and fields, where biodiverzity was again comparable. Thus, we did not notice the heth edge habitat being richer in species than heathlands, which, according to the theory that ecotones are the most species-rich, could be perceived belief. The density of nests here was very high in comparison with the habitats of the field and the field boundaries. The influence of the heathland, where the density of nests was the highest, is evident here, but at the same time we see that the density is significantly lower than in heathlands. Most species that prefer the habitat of heathlands is therefore looking for its center, not the border with a potentially inhospitable habitat being near. In the heath edges was a higher presence of various field weeds that can serve as food for bees. However, these plants bloom in spring rather than summer and usually do not bloom during the activity of twig nesting bees, so they did not contribute on the effect, we detcted.

1.1.4 Heathlands

Xerothermic heaths are an ideal habitat for most local twig-nesting bees, as there are plenty of nesting opportunities and food there. It is therefore no surprise that we found the highest concentration of nests per habitat here. The local heathlands had the character of small islands in agricultural landscape of this natural habitat. In a small island of the original ecosystem, there may be

higher competitive pressures on individuals and increased intra-species and inter-species competition. In the same way, we can encounter a higher density of nests here, as individuals are forced to fit into a small area. E.g. The study by Howell et al. 2017 states that small fragments of

ecosystem has higher nest density than large ones, also here females complete their nests faster and there is higher parasitism (probably due to higher host population density). We believe that the number of nesting opportunities in the heathlands is probably more limiting factor than the amount of food here.

Pic. 4 -7: The four chosen biotopes in our study: heathlands, fields, heath edges and field boundaries

1.2 Biotope preferences of species

Ceratina chalybea and Ceratina nigrolabiata strongly preferred heath habitats and heathland edges between field boundaries and fields. Thus, they prefer the natural environment to the agrarian landscape. Ceratina chalybea, however, preferred the heathland habitat significantly more strictly than Ceratina nigrolabiata. Of the total number of analyzed nests of Ceratina chalybea, 11% were from field habitats or field boundaries, for Ceratina nigrolabiata this value was one third. The reason for their high abundance in heaths is probably their ability to compete with other species that nest here in a significantly lower proportion. Ceratina chalybea is significantly larger in size than other Ceratina bees. This fact gives her an important competitive advantage. Another reason may be the greater requirements for the amount of food for feeding the offspring of Ceratina chalybea due to its larger size and heathlands are richer in food resources than other biotopes.

In heathlands, were also more frequent nests of different species than Ceratina such as Hoplitis tridentata, Hylaeus kahri, Ceratina cucurbitina or Trypxylon sp. However, not enough of these nests were present to allow the preference of habitat to be tested statistically. For most species,

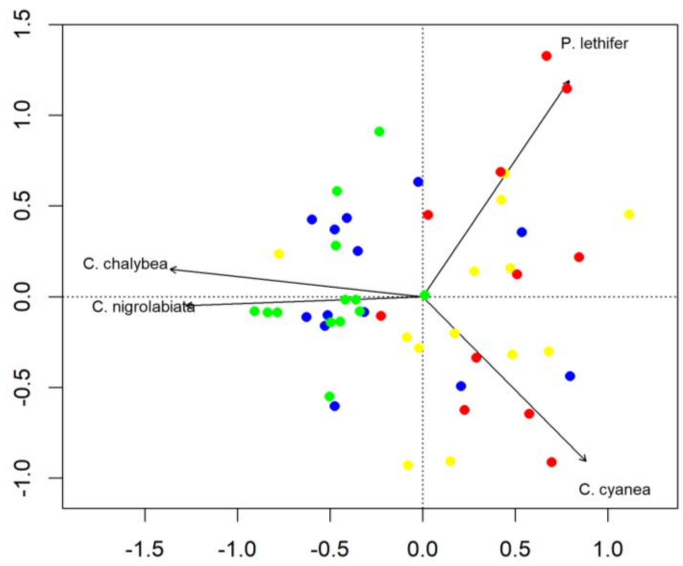
heath is the most suitable habitat.

Pemphredon lethifer and Ceratina cyanea, on the other hand, preferred field habitats and field boundaries. Three-quarters of Pemphredon lethifer's nests that we dissected were from fields or foeld boundaries. Their phenomenon may be due to their food preferences. Unlike Ceratinas, Pemphredons supply their nests with aphids. There may be many more of these in the fields than in the heaths, as the fields are one crop monocultures it is easier for aphids to multiply here. Pemphredons can také advantage out of this because they are offered a large reservoir of food. We also found that Pemphredon nests were significantly more abundant in habitats located in corn fields than sunflower fields. It is therefore possible that aphids have multiplied here on corn.

Three-quarters of Ceratina cyanea's nests dissected were from field or field boundary habitats. This value may be skewed by the relatively low number of Ceratina cyanea nests that we collected. However, we can see a clear preference here for agrarian type of habitats. There is an explanation for this effect that Ceratina cyanea is pushed to nest in field habitats due to less parasitism and less competitive pressure. Out of the four species of Ceratina bees that live in the Czech Republic, Ceratina cyanea is competitevly weakest due to its small size and absence of biparental care for offsprings, which makes Ceratina nigrolabiata competitevly stronger species. However, Ceratina cyanea nests did not suffer from food shortages in the field habitat, because Ceratina cyanea does not produce a large number of offsprings, which requires a large amount of pollen. Another possible explanation for the preferrence of field habitats by this species may be the effect of excessive competitive and parasitic pressures, which take place heathlands, where the population density of Ceratina bees is very high. The competitively weaker Ceratina cyanea may be compiled and subsequently forced to look for another place to nest.

Ceratina cyanea may be the weakest in competition because i tis together with Ceratina nigrolabiata is the smallest Ceratina. All other local Ceratina bees also have defense machinsms against parasites – biparental care in Ceratina nigrolabiata, cleaning of offspring at Ceratina cucurbitina and creating empty brood chambers in nests in Ceratina Chalybea. It is therefore the most vulnerable to parasites and for this reason may prefer to choose habitats to nest in where the parasites are less common.

These results suggest that there are species that clearly prefer heathlands habitats (Ceratina chalybea and Ceratina nigrolabiata) and species that prefer field habitats (Ceratina cyanea, Pemphredon lethifer). The habitats of heaths and the edges of heaths form a kind of transition between these two, but the edge of the heath is closer to the species composition of heaths and the field boundaries.



Pic. 8: The preferences of species of Ceratina genus for habitat they nest in. Red: fields, yellow: field boundaries, blue: heath edges, green: heathlands

1.3 Offspring mortality

Ceratina bees are strong K-strategists, as evidenced by the mother's guarding of the offspring, biparental care in the Ceratina nigrolabiata or cleaning of offspring that we encounter in Ceratina cucurbitina. Ceratina bees also make up an overall smaller number of supplied chambers per nest compared to other bees. The mortality of their offsprings is relatively low due to their K-strategy compared to other twig-nesting bees.

Offspring mortality in Ceratina was around 15-20%. In all Ceratina species we can see a clear relationship between population density and offspring mortality, with higher mortality that we encounter in habitats with more nests. In the case of Ceratina chalybea these habitats are heathlands and edges of the heathlands. This dependence has also been found in other studies, such as Falcon-Brindis et al. 2020.

Even in Ceratina cyanea, we can find higher mortality in habitats where it´s nests were more frequent (fields and field boundaries), even though the mortality test was insignificant. However, we can see slight dependence here and the insignificance is probably due to the small number of analyzed nests.

This phenomenon may be explained by the higher density of predators and parasites in places with a higher population density of their hosts. By nesting in a non-preferred habitat, Ceratina can avoid dangers in the form of parasites and predators. The frequency of diseases and infections works the same way in the population - individuals isolated from the main population are less likely to become infected.

The overall mortality of Hoplitis tridentata was on average similar to mortality of Ceratina bees (Ceratina - 20.60%, Hoplitis - 20.03%). Here, however, we encounter twice as high mortality in the habitats that Hoplitis preferred (heathlands and heath edges), where mortality reached 40-50%. Unlike Ceratina, Hoplitis tridentata does not guard the nest until adulthood, and after the construction of the brood chambers is completed, it only clogs it with a plug of chewed leaves. But it makes a higher number of brood chambers than Ceratina. It therefore ranks to the R-strategists among the bees. For this reason, Hoplitis bees are susceptible to parasites, which are found in large numbers in the heathland habitats and the edges of heathlands. So if Hoplitis nests in a non-preferred habitat and manages to get enough food to supply its brood chambers, most of her offspring will reach adulthood. But Hoplitis tridentata needs much more pollen to form brood chambers than Ceratina, as it is larger in size. This may be the reason why it prefers natural habitats, even if it is exposed to higher parasite pressure.

1.4 Nest guarding

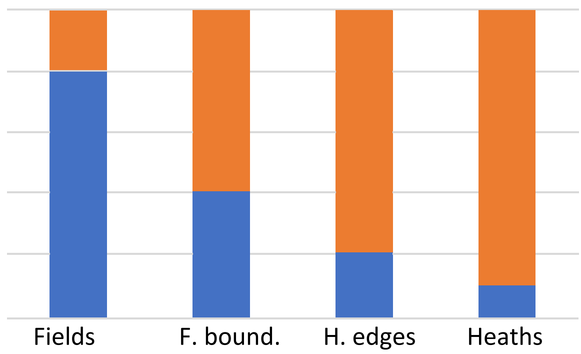
Ceratina bees are known mainly for their intensive care for their offspring and guarding of nests, which in some species lasts until adulthood of the offspring. This parental care significantly reduces the mortality of the offsprings thanks to protection against parasites and predators who would like to penetrate the nest through the entrance corridor.

In our experiment in Ceratina chalybea nests, the mother guards the nest until adulthood, leaving the nest unguarded in about a third of the cases. The presence of mother did not differ significantly between the habitats, as is the case with the nests of Ceratina nigrolabiata.

Ceratina nigrolabiata is unique in its care for its offspring, as the male is also involved in guarding the nest. In our experiment, however, we encountered a significantly different ratio of biparental and maternal guarded nests between habitats (Pic. 5). It is clear from our results that we can see a clear gradient that with the growing agricultural character of the landscape, nests guarded by both parents are decreasing and nests guarded only by the mother are increasing. This fact is very interesting, because Ceratina nigrolabiata has so far been considered as strictly biparental and the situation when the male was not present was considered an orphaned nest, not an alternative strategy. The explanation for this gradient may be the low population density of Ceratina bees in field habitats, where it can be difficult for males to find a nest with a female. Due to the low population density, males do not stay in the field (even if they hatch here, they do

emigrate to a place where it is easier to find a mating partner). The female therefore lays unfertilized eggs and guards the nest herself due to the absence of her father. This mother would then have only male offsprings. However, there were also frequent nests in the field, which were guarded only by the mother, but also had female offspring, so it had to be fertilized. The female may have been mated a long time ago (thanks to her ability to store sperm in the spermatozoa). The fact that a female offspring also appears in a nest where males predominate means that the female has been mated recently. After mating, the male can leave the nest and be replaced with another male, but this may not always happen. However, there is a shortage of males in the field habitat, so females often have to guard the nest themselves most of the time.

This finding may indicate that Ceratina nigrolabiata is only an facultativly biparental species and that father-care is not necessary for successful offspring breeding.



Obr. 4: Proportion of biparentally guarded nests to maternally guarded nests depending on the habitat (%). Orange – biparental nests, blue – maternally guarded nests

1.4.1 Number of fathers of offsprings in Ceratina nigrolabiata nests

It is typical for Ceratina nigrolabiata that the offspring have different fathers in one nest. Thus, the currently guarding male in the entrance hall is usually not the father of any of the offspring or only small number of them. The daughters of individual fathers are not lined up in the nest and they can be mixed up with other father´s daughters or males, which do not have fathers. But it is more common for daughters from one father to be in chambers close to each other. The alternation of the daughters of different fathers in the nest is made possible by the mother's ability to store sperm in the semen. We also encountered this phenomenon in the nests we analyzed. The highest number of descendants of different fathers in one nest was 8. We compared whether the number of offspring´s fathers differed between habitats and we recorded the highest number of fathers per number of offspring in field habitats. The lowest number of fathers was in the heathlands. There may have been more fathers in the nests infields because the mating of mothers with males took place before the nest was established and this semen was not surplused by the semen of a new male, which would mate the female while laying eggs, as might have happened in the heathlands. Sperm stored in spermatozoa lasts a very long time, in Japanese Ceratina flavipes even over winter.

1.5 Sex proportion of offsprings

In most Hymenoptera species, we encounter the ability to consciously decide the which sex will have a laid egg. The female is able to decide whether or not to let the semen into the egg. It is known that males need less food for their development than females, so in hostile areas it is favourable to have male offsprings, as i tis not so hard for mothers to provision them.

In the case of Ceratina chalybea, we found a significant gender deviation in favor of male offspring in field habitats. This finding may indicate that the mother did not find the males to mate, as a result of which she laid only the unfertilized eggs from which the males originate. It also could bet he case, where the female is already mated but voluntarily chooses to produce males because she is aware that she will not have enough food to supply the same number of chambers of the female offspring. Both of these influences probably work here at the same time. However, these mechanisms would lead to an excess of males in the next generation in the field. However, we do not encounter this, on the contrary, we believe that there is a shortage of males in the field. The explanation may be that due to the low population density, males are unable to find females for mating and fly to search for females in places with higher population density (heaths).

A much more slight deviation in the sex ratio of the offspring was found in Ceratina nigrolabiata in favor of males in field and heath habitats. The reason why the deviation is milder here may be the less strict habitat preference than Ceratina chalybea, and therefore a slightly higher population density of individuals. Thus, Ceratina nigrolabiata females are more likely to find males for mating and there is a lower frequency of nests with unfertilized offsprings, which would be exclusively male. Another argument is the fact that Ceratina nigrolabiata is pushed into thinner twigs on the heath. Because males are physically smaller than females, the mother may be forced to lay males

offspring because the females would not fit into such a thin twig. This phenomenon was studied, for example, on the American species Osmia lungaria, where females were significantly more often produced in wider twigs.

5. Conclusion

In total, we analyzed 693 nests of 13 species of twig-nesting bees. I recorded the highest nest density and species diversity in the heathland. Most species were most common on this habitat. This habitat was preferred, for example, by Ceratina chalybea, Ceratina nigrolabiata or Hoplitis tridentata. On the contrary, the lowest nesting and lowest biodiversity was in field habitats. However, this habitat was preferred by Pemphredon lethifer or Ceratina cyanea. The habitats edge of the heathland and the field boundary formed a transition between the two extremes. I have not been able to prove that they function as a kind of migration corridor.

I did not find any differences in the number of brood chambers or the length of the nest between habitats. In field habitats, the rate of parasite nest infestation was significantly lower than in heathlands, and offspring mortality was very low there. This is probably due to the low population density of bees and the consequent reduction in the risk of disease transmission and parasite infestation.

Ceratina bees nests from field habitats had more male offspring than heath nests. This may be due to a lack of suitable males for mating and the subsequent laying of a higher number of unfertilized eggs. I did not notice a significant difference between the size of the offspring in the field and the heath, so the individuals in the field did not suffer from food shortages. In the biparental Ceratina nigrolabiata, I noticed a difference in nest guarding between habitats. In the field, most nests of this species were guarded only by the mother, on the heathland, on the contrary, biparentally. This phenomenon is in Ceratina nigrolabiata is very unusual and may suggest that this species is only facultatively biparental, not strictly as it has been considered so far.

This study helps to understand the impact of intensive agricultural landscapes on diversity and behavior of bees. The results of this study can be helpful in finding a sustainable way of farming that takes these groups of insects into account. Unlike other works that focus only on the overall biodiversity or abundance of individuals in individual habitats, this work also describes the impact of agricultural landscape on the behavior of these species.

【評語】050018

This study surveyed the solitary bees in the south of Znojmo near the Podyji National Park in Czech Republic. The main purpose of this study is to investigate the nesting preference to four different habitats, including heathland, edge heathland, field and field boundary. The author has conducted this study for three years and has well knowledge about the solitary bee species. The methods for collecting the samples are proper and the results are quite conclusive to hypothesis of this study. One intriguing finding of this study is the biparental care behavior is strongly affected by the habitats, i.e. the proportion of biparentally guarded nests to maternally guarded nests depending on the habitats. This interesting result is worthy for future research to explore the possible physical and biological factors.