

VEČLETNO UGOTAVLJANJE STOPNJE HIBRIDIZACIJE POPULACIJE KRANJSKE ČEBELE (*Apis mellifera carnica* Pollmann, 1879) V SLOVENIJI NA PODLAGI OBARVANOSTI OBROČKOV NA ZADKU

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Povzetek

V Sloveniji je kranjska čebela (*Apis mellifera carnica* Pollman 1879) avtohtona in zaščitena podvrsta medonosne čebele. Na podlagi morfoloških značilnosti je uvrščena v C filogenetsko linijo medonosnih čebel in je druga najbolj razširjena podvrsta medonosne čebele na svetu. *A. m. carnica* je v Sloveniji avtohtona in njeno varstvo je opredeljeno z zakonom. V zadnjih desetletjih je bilo v populaciji *A. m. carnica* v Sloveniji opaženih več čebel z rumenimi obročki na zadku. Te značilnosti so posledica križanja z drugimi podvrstami. Danes na populacijo *A. m. carnica* močno vplivajo čebelarke dejavnosti (trgovina s čebeljimi družinami in maticami, premiki čebel na pašo), pa tudi naravne razmere in hibridizacija na območjih naravne hibridizacije. Da bi določili odstotek križancev čebel v populaciji *A. m. carnica* v Sloveniji, smo opravili morfološki pregled na podlagi obarvanosti obročkov na zadkih čebel. Ugotovili smo, da je najvišji odstotek čebel z rumenimi obročki na zadku še vedno prisoten v zahodnem delu Slovenije, ob meji z Italijo (Primorska, Obalno-kraška in Goriška regija). V povprečju je v populaciji *A. m. carnica* v Sloveniji 1,13 odstotka čebel z rumenimi obročki na zadku. Ta rezultat potrjuje uspešnost različnih ukrepov, ki so bili v zadnjih letih izvedeni za zaščito kranjske čebele v Sloveniji.

Ključne besede: čebele, hibridizacija, carnica

MULTIANNUAL DETERMINATION OF HYBRIDIZATION IN THE CARNIOLAN BEE POPULATION (*Apis mellifera carnica* Pollmann, 1879) IN SLOVENIA BASED ON COLOUR OF ABDOMEN

Abstract

The Carniolan honey bee (*Apis mellifera carnica* Pollmann 1879) is an autochthonous and protective honey bee subspecies in Slovenia. Based on morphology characteristics, *A. m. carnica* is classified into the C evolutionary lineages of honey bees and is the second most widely spread honey bee subspecies in the world. Slovenia is the origin of *A. m. carnica* and its protection is also defined in law. In the last decades, more honey bees with yellow bands on the abdomen have been seen in the population of *A. m. carnica* in Slovenia. These characteristics are the result of the hybridization with other honey bee subspecies. *A. m. carnica* is nowadays much influenced by beekeeping activities (trade with bee colonies and queens, migratory beekeeping), natural conditions, and hybridization in the zones of natural hybridization. To find out the percentage of hybrid honey bees in the population of *A. m. carnica* in Slovenia, a morphological investigation based on the colour of workers' bands at the abdomen was made. We found out that the biggest percentages of honey bees with yellow bands are still present in the western part of Slovenia near the border with Italy (Primorska, Obalno-kraška and Goriška region).

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On average, 1,13 % of honey bees in the population of *A. m. carnica* in Slovenia have yellow bands on the abdomen. This result confirms the success of various measures taken in recent years to protect the Carniolan honey bee in Slovenia.

Keywords: honey bees, hybridization, carnica

1 INTRODUCTION

European honey bee subspecies evolved and developed after the last Ice Age, approximately 8,000 to 10,000 years BC (Wallberg et al. (2012)). The northern dark bee (*A. m. mellifera*) spread northward from the Iberian Peninsula, the Italian bee (*A. m. ligustica*) formed on the Apennine Peninsula, the Carniolan bee (*A. m. carnica*) developed on the Balkan Peninsula, and the Macedonian bee (*A. m. macedonica*) expanded towards Europe from the Near East. In addition to these, the Caucasian bee (*A. m. caucasica*) also developed relatively independently (Ruttner, 1988).

Based on morphological research, Ruttner et al. (1978) and Ruttner (1988) created a map outlining the boundaries between the different subspecies. They identified the distribution area of the Carniolan bee as southern Austria, Slovenia, Croatia, Bosnia and Herzegovina, Serbia, Montenegro, North Macedonia, Kosovo, Romania, Hungary, the Czech Republic, and the southern part of Poland. In their description of the morphological characteristics of the Carniolan bee, they noted that it is a dark bee, while the Italian bee is described as the only subspecies with yellow pigmentation on the abdomen.

In the last 150 years there has been very intensive trade in bee colonies in Europe, and more recently, especially with queen bees, which has led to the disappearance of local bee populations (Garnery et al., 1998; Jensen et al., 2005; Soland et al., 2009). The disappearance of European *A. mellifera* subspecies due to hybridization has been documented in many western and northern European countries (Franck et al., 2000; De la Rúa et al., 1998, 2001a, b, 2003; Ivanova et al., 2007). Hybridization was particularly intense in Germany, where the native dark bee no longer exists. Bees in Germany are now hybrids between subspecies: *A. m. mellifera*, *A. m. carnica*, *A. m. ligustica*, and *A. m. caucasica* (Kauhausen-Keller and Keller, 1994). In Denmark, the native bee population was lost due to the introduction of the Italian bee (Jensen et al., 2005), while in France, the presence of both Carniolan and Italian bees was observed (Garnery et al., 1998). In Poland and the Czech Republic, this type of subspecies hybridisation began somewhat later than in Western Europe, but beekeepers there also could not resist the pressures of foreign bee subspecies (Gromisz, 1997; Poklukar, 1999). As a result, *A. m. mellifera* is considered an endangered bee subspecies in Europe, and to preserve the dark bee, an organization (Societas Internationalis pro Conservazione Apis Melliferae Melliferae (SICAMM) was established to conserve the dark bee in areas where local populations still exist (Jensen et al., 2005).

1.1 Carniolan honey bee

The Carniolan honey bee, or Carniolan grey bee, is classified within the Southeast European group of honey bees. Its native range is defined as both north and south of the Karawanks, on both sides of the border between Austria and Slovenia. Based on morphological characteristics, the entire population of the Carniolan bee is divided into three larger groups: Alpine (Slovenia, Austria, Slovakia), Pannonian (Hungary, Romania) and Mediterranean (Croatia, Bosnia and Herzegovina, Serbia, Montenegro), with the area near the Italian border

marked as undefined in terms of subspecies due to the mixing of Carniolan and Italian bees (Ruttner, 1988).

To more precisely define the population of Carniolan bees in Slovenia and to characterize potential ecotypes, Sušnik et al. (2004) studied the entire population using genetic markers but found no genetic differences within the Carniolan bee population in Slovenia. Likewise, Kozmus (2008), through an analysis of wing venation, did not identify differences between potential ecotypes within the bee population in Slovenia. Both studies suggest that the Carniolan bee population in Slovenia is homogeneous. The Carniolan bee is an indigenous bee breed in Slovenia and is particularly important from the standpoint of conserving honey bee subspecies and local variants, which have adapted to specific conditions over a long period and thus represent valuable genetic diversity in nature. On the other hand, the disappearance of local bee variants leads to the loss of valuable trait combinations that have developed through selection processes over an extended time.

1.1.1 Color of the Abdominal Bands

Evaluating bees based on the color of the abdominal bands became common during the period of bee trading. Later studies (Ruttner, 1988) also confirmed that the Carniolan bee can be reliably distinguished from the Italian bee by the color of the bands on the abdomen. The dorsal bands can either be uniformly dark or may have spots and markings on the sides, or the entire band may be brownish-reddish-yellow. The Italian bee has 1-3 yellow bands, whereas the Carniolan bee has spots and bands of a reddish-brown color. Verbič (1947) described that the Carniolan bee gained worldwide fame without yellow bands. According to the regulations of the German Beekeepers' Association from 1969, the Carniolan bee should not have yellow bands. If bees with yellow bands on the abdomen are found in a colony, it is known that they are either imported Italian bees or their hybrids, or possibly hybrids with Buckfast bees.

In 2011, 5.6% of the Carniolan honey bee population was found to have yellow bands on the abdomen, Kozmus (2011). As a result, a queen exchange measure was set up under which beekeepers received free queens. To determine whether the measure has achieved its objective and whether the percentage of hybrids in Slovenia has decreased, a morphological study of the Carniolan bee population was carried out, in which the percentage of bees with yellow bands was determined in different areas of Slovenia in three different years.

2 MATERIALS AND METHODS

2.1 Sampling

The worker bees used in the analysis were collected in Slovenia in three different years. In 2015 and 2022, we collected approximately three samples of worker bees in each municipality, and one sample in 2019. One sample consisted of at least 25 worker bees, which we collected directly from the plants on which they collected nectar and/or pollen. Each sample thus represented the situation from several apiaries around the sampling site. In total, we collected 1345 samples containing 33,611 bees (Table 1). The bees were kept in a -20°C freezer until analysed.

Table 1. Number of honey bees sampled per year

Year	2015	2019	2022
Number of samples	602	201	538
Number of sampled honey bees	15.069	5.054	13.488
Total of sampled honey bees	33.611		

2.2 Morphological Analysis of Abdominal Band Colour

In the morphological analysis of abdominal band colour, we examined each individual bee separately. The bees were visually inspected under good lighting and on a white background. All the bees examined were categorized into two groups:

- workers with a grey abdomen (characteristic of Carniolan worker bees),
- workers with two yellow bands on the abdomen (Figure 1).

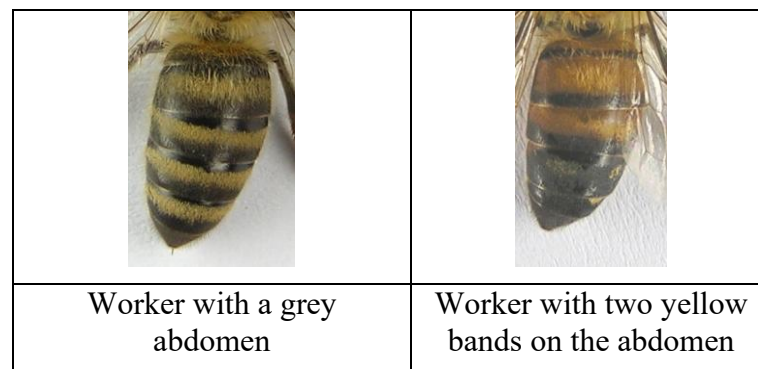


Figure 1. Two different colour patterns of the honey bee worker's abdomen.

3 RESULTS

Results were grouped according to regions. For each region, we calculated the percentage of bees that had two yellow bands on the abdomen. Results are shown in the graph (Figure 2.).

The highest percentage of bees with yellow rings on the rump was found in the western part of the country (Goriška, Primorska and Obalno-Kraška regions) and the lowest in the eastern part of the country.

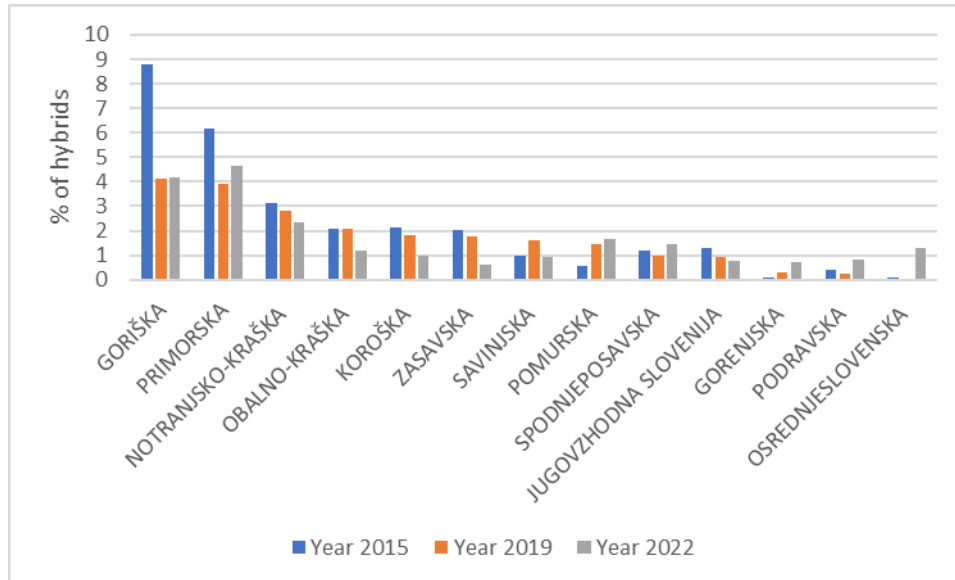


Figure 2. Percentage of hybrids in different regions of Slovenia in 2015, 2019 and 2022.

4 DISCUSSION AND CONCLUSIONS

The results of our study clearly indicate that the highest percentage of hybrids is still present in the western part of Slovenia. This confirms previous findings that mixing occurs in this region, along with Furlanija-Julijska Krajina, between the Carniolan bee (*A. m. carnica*) and the Italian bee (*A. m. ligustica*) (Comparini and Biasiolo, 1991; Ruttner, 1988).

Based on the morphological analysis of bees in Slovenia, we found that an average of 1.13% of bees exhibit two yellow bands on the abdomen. This result is lower than that reported by Kozmus (2011) in 2011, supporting the hypothesis of a decreasing trend in the percentage of hybrids in recent years. The most significant decline was recorded in the Obalno-kraška region, where the percentage of hybrids was 14% in 2011, while our study found it to be only 4.2%.

The analysis results indicate that the process of hybridization is slowing down, suggesting that the measures implemented to protect the Carniolan bee are yielding results. It appears that we have, at least for now, managed to limit hybridization and prevent its increase. Nevertheless, it can be seen that the sale and traffic with bee colonies is ongoing and that the percentage of hybrids is rising in some regions (Osrednjeslovenska), but this is within the permitted range (below 2%).

These findings are encouraging, as they confirm that we can preserve the Carniolan bee in Slovenia for the future, which is crucial for the stability of local beekeeping. Our research thus contributes to the understanding of the dynamics of bee populations and the importance of preserving indigenous breeds.

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