Interpretation of morphometric data using microsatellite DNA markers

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INTRODUCTION

Features of the forewing of honeybee workers are used for the determination of the three subspecies of bees bred in Poland (*A. m. mellifera*, *A. m. carnica* and *A. m. caucasica*). The wing vein junctions are treated as coordinates and then subjected to canonical analysis; determination of 19 vein junctions and calculation procedures are automated with a computer software [1].

Based on canonical analysis, models for each subspecies have been elaborated. Most of the colonies of a given subspecies lay within a circle of radius 3 the around the mean canonical scores of this subspecies. It could be assumed that if a colony is within the circle it belongs to the subspecies.

Nevertheless, it was shown that the range of metric variability of the forewings in *A. m. carnica* and *A. m. caucasica* strongly overlap [1, 2]. During the survey we found colonies that did not fit the model of the declared subspecies as well as any other developed models (later called: outside the model).

Here, we aimed to verify identity of these bees on the basis of microsatellite DNA analysis.

MATERIALS AND METHODS

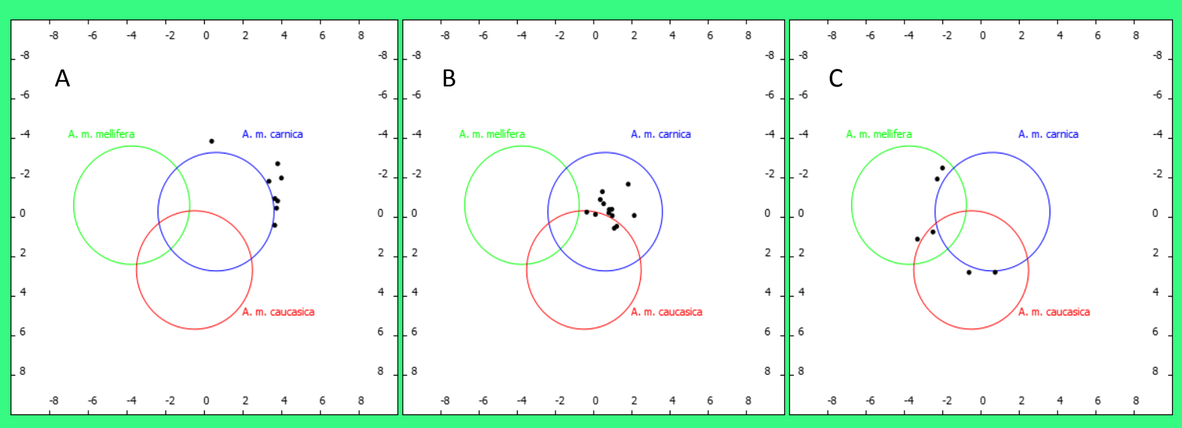
In this study we included samples from commercial Carniolan and Caucassian bee breeds, which were previoulsy examined on the basis of forewing venation. For the DNA analysis we selected samples which were outside the morphometric model (Fig. 1 A) or disqualified due to crossbreeding (Fig. 1 B,C). Altogether, we analysed 34 colonies of Carniolan bees and 24 colonies of Caucasian bees.

From each colony, a sample of 16 worker bees were analyzed. Insects were taken directly from hive frames and conserved in 90% ethanol.

DNA was extracted from thoraces with Insect Easy DNA Kit (Omega Bio-Tek) according to the manufacturer instructions. In the study we used 17 nuclear microsatellite loci amplified with fluorescent labeling of primers in two multiplex reactions: M1- A113, A24, A7, A88, Ap28, Ap43, Ap55, Ap66; M2- A025, Ac011, Ap090, Ap103, Ap226, Ap238, Ap243, Ap249, Ap256 (primer sequences in [3]). PCR reaction was done with Multiplex PCR Kit (Qiagen) following the kit instructions. The separation of fragments was carried out on automated sequencer ABI PRISM 3130xl (Applied Biosystems) using the internal size standard (LIZ 600, Applied Biosystems). Resulting electropherograms were scored using GeneScan ver. 3.7 and Genotyper ver. 3.7 software (Applied Biosystems).

In order to assign genotypes to specific subspecies, we used a Bayesian statistical method implemented in the STRUCTURE ver. 3.2.1 [4]. As input data we used queen genotypes inferred from their offspring with MSF [5, 6]. Reference samples of the three subspecies from their original geographical range were also included (*A. m. mellifera*-north Poland, *A. m. carnica*-Hungary, *A. m. caucasica*-Georgia). Mean individual assignment probabilities, q_i, and their 90 % credible limits were estimated for each individual.

Figure 1. Graphical presentation of results of subspecies affinity analysis for Carniolan bees. A- samples outside the model, B- positively verified, C- disqualified due crossbreeding. The horizontal and vertical axes correspond to the first and second canonical variables, CV1 and CV2, respectively.



RESULTS AND DISCUSSION

There was a strong relationship between assignments to subspecies based on wing venation and microsatellites (r=0.7, p<0.0001) (Fig.2).

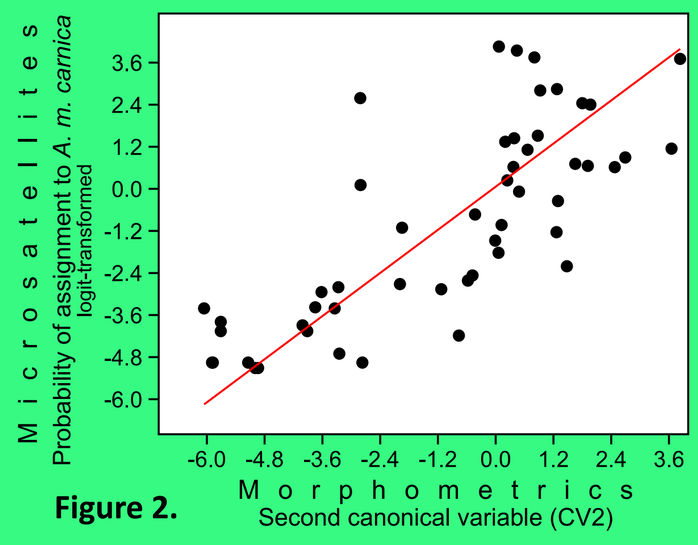


Figure 2.

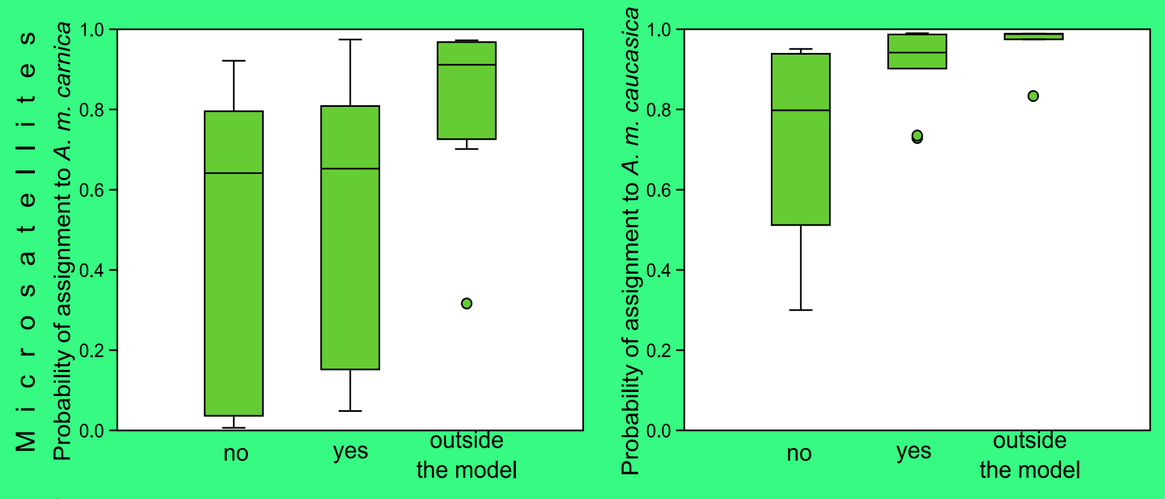
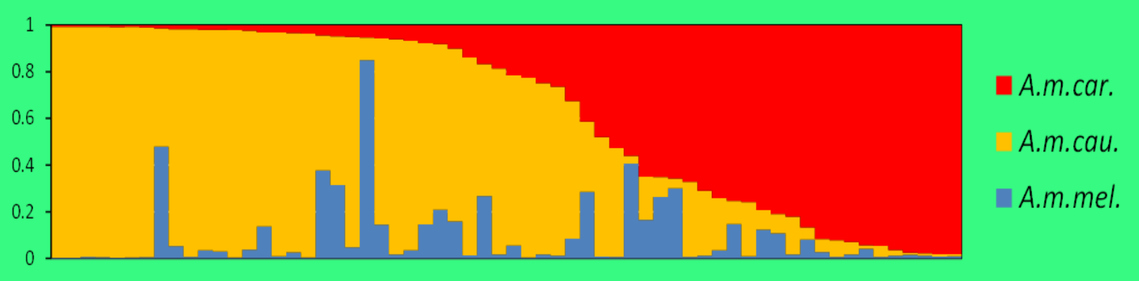


Figure 3. Compliance with the morphometric model

The probability of belonging to the declared subspecies was lower in samples disqualified based on wing venation. In contrast, the highest probability of assignmet to the right subspecies was estimated for samples outside the morphometric model (Fig 3).

STRUCTURE plot assuming K = 3 populations



Our results indicated that studied breeding lines show signs of hybridization involving several subspecies, which cannot be precisely detected by examining the wing morphometry.

CONCLUSION

Morphometric models that are currently used for discrimination of honeybee subspecies should be updated based on samples with the identity verified by DNA analysis.

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