

DISCRIMINATION OF THREE HONEYBEE SUBSPECIES BASED ON FOREWING VENATION

Dariusz Gerula¹, Paweł Węgrzynowicz¹, Adam Tofilski², Beata Panasiuk¹, Małgorzata Bieńkowska¹
¹Institute of Pomology and Floriculture, Apiculture Division, Kazimierska 2, 24-100 Puławy, Poland,
 e-mail: dariusz.gerula@man.pulawy.pl
²Bee Research Department, Agricultural University, 29 Listopada 52, 31-425 Kraków, Poland
 e-mail: rotofilski@cyf-kr.edu.pl



INTRODUCTION AND AIM OF THE WORK

The identification of honeybee (*Apis mellifera*) subspecies can be based on measurements of various body parts. Usually many traits are measured simultaneously (Ruttner et al. 1978). Gromisz (1967) found that length of proboscis, sum of the width of the third and fourth tergites and cubital index allow discrimination of subspecies bred in Poland. Finally, in accordance with requirements of National Breeding Program of honeybees, measurements of three features were accepted: length of proboscis, width of the fourth tergite and cubital index (Gromisz 1981). The measurements are time consuming, therefore, limited number of colonies were controlled using this method. The identification of honeybee subspecies can be based on forewing venation (DuPraw 1965). There were attempts to automate the measurements (Daly et al. 1982, Batra 1988). It is possible to automatically determine characteristic points on honeybee wing (Tofilski 2004). The points can be used to measure distances and angles (Tofilski 2008). Automatic measurements of wings are quick and more precise than manual measurements (Tofilski 2007). The aim of this study was to elaborate an automatic method of discrimination three honeybee subspecies bred in Poland. The discrimination was based on forewing venation.

MATERIALS AND METHODS

In the study we used honeybee workers from Polish breeding apiaries. The workers represented 1042 colonies, 45 breeding lines and three subspecies: *A. m. carnica*, *A. m. caucasica*, *A. m. mellifera*. From every colony 20 workers were collected from the center of the nest and stored in alcohol. First method of Gromisz (1981) was used to determine if the colonies belong to the subspecies declared by breeders. The verification allowed to choose 970 colonies for further analysis. The right forewing of each worker was dissected, mounted in glass photographic frames and scanned with a Nikon Coolscan 5000 ED scanner equipped with an SF-210 slide feeder. For every wing image the coordinates of 19 vein junctions (Fig. 1) were determined automatically using DrawWing software (Tofilski, 2004). The vein junctions were used as landmarks. The landmarks were aligned according to generalized orthogonal least-squares procedures (Rohlf and Slice, 1990) using tspSuper software (Rohlf, 2004). Forward stepwise discriminant function analysis (tolerance = 0.01; F to enter = 1.0) (StatSoft, 2001) was used to determine classification functions. The discriminant analysis was followed by canonical analysis. The discrimination was based on the 19 aligned landmark coordinates.

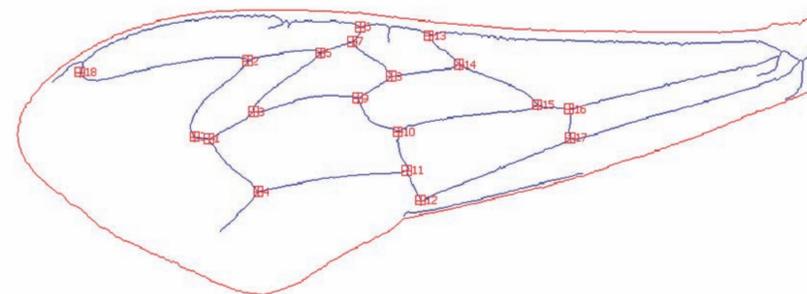


Fig. 1. Vein junctions determined automatically by DrawWing software.

Table 1. Identifications of honeybee subspecies based on discriminant functions.

Subspecies	Correctly classified (%)	Number of colonies classified as		
		<i>A. m. carnica</i>	<i>A. m. caucasica</i>	<i>A. m. mellifera</i>
<i>A. m. carnica</i>	97,5	739	11	8
<i>A. m. caucasica</i>	71,4	28	70	0
<i>A. m. mellifera</i>	93,8	5	2	107
Total	94,4	772	83	115

Table 2. Mean canonical variate of three honeybee subspecies.

Subspecies	1 st canonical variate	2 nd canonical variate
<i>A. m. carnica</i>	0.62453	0.25920
<i>A. m. caucasica</i>	-0.48842	-2.69262
<i>A. m. mellifera</i>	-3.73271	0.59123

RESULTS

Using stepwise discriminant function analysis, 29 of 38 variables were incorporated into the classification model. The classification function obtained from the discriminant analysis allowed correct classification 94.4% of colonies. The highest proportion of correctly classified colonies was in *A. m. carnica* and the lowest in *A. m. caucasica* (Tab. 1).

Canonical variate analysis separated the subspecies into three clouds of points (Fig. 2). *A. m. mellifera* was well separated from the two other subspecies but *A. m. carnica* and *A. m. caucasica* overlapped to some degree (Fig. 2). The first canonical variable discriminated mainly between *A. m. mellifera* and the other two subspecies. The second canonical variable mainly discriminated between *A. m. carnica* and *A. m. caucasica*.

In order to avoid classification of large proportion of colonies believed to be *A. m. caucasica* as *A. m. carnica* the classification was based on mean canonical variates (Tab. 2). Around the means circles of radius three were drawn (Fig. 2). If the colony is within this circle it is assumed not to differ from particular subspecies.

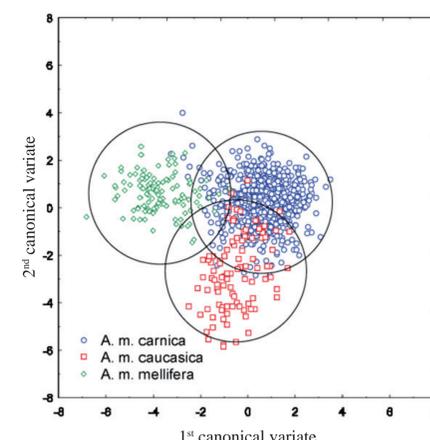


Fig. 2. Discrimination of three honeybee subspecies based on canonical variates analysis.

CONCLUSIONS

Discrimination of *A. m. carnica*, *A. m. caucasica*, *A. m. mellifera* based on forewing venation allows to correctly classify 94.4% of colonies.
If the discrimination is based on classification functions large proportion of colonies believed to be *A. m. caucasica* is classified as *A. m. carnica*.
Discrimination based on mean canonical values classifies almost all colonies as expected; however, it can lead to introgression between subspecies.
The discrimination of honeybee subspecies can be automated by using scanner with slide feeder and image analysis.

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