Original Paper

Wing morphometry of Slovak lines of *Apis mellifera carnica* workers and drones population

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Samples of forewings from 16 hives belonging to various lines of Slovak Carniolan bee kept by queen breeders in Slovakia were taken in year 2013 to perform wing morphometry measurements. The Dawino, complex wing morphometry method, has been applied for workers samples and measurements of Cubital index for drones samples. Worker bees samples showed similarity to Carniolan bee standard from 50 to 84% in 15 cases, on sample was out of standard. Cubital index of drones samples comply with a carnica bee standard in 14 cases, 2 samples were out of the range. The future of use wing morphometry for detection of hybridization in *Apis mellifera carnica* populations is discussed.

Keywords: *Apis mellifera carnica*, workers, drones, wing morphometry, cubital index, Dawino

1 Introduction

Ten of the currently 27 described subspecies of *Apis mellifera* can be found in Europe, but considerable variation can be observed within many of them and several can be further subdivided into a diversity of "ecotypes". Several subspecies and ecotypes can be considered as endangered (De la Rúa et al., 2009). A multitude of reasons lead to a loss of both genetic diversity and specific adaptations to local conditions (Meixner et al., 2013).

On the territory of Slovakia, around 250,000 honey bees colonies of the Carniolan subspecies *Apis mellifera carnica* (sometimes recognized as Carpathian Carnica sub-population or "Slovakian" Carnica) are kept by more than 15,500 beekeepers. The import of other honey bee races has been illegal for a number of decades, but in the last two decades we expect some uncontrolled bee queens' importations from abroad.

Traditionally, the intraspecific taxonomy of the honey bee *Apis mellifera* has been based on morphology. Morphometric methods are used also in Slovakia to verify sub-species purity in all registered stations (i.e. breeding, reproductive and testing stations), mainly based on measurements of cubital index, length of the proboscis; length and width of bees wings and colouring of first three abdominal tergites (which are sometimes yellowish). Sometimes also other morphometric characters are applied including number of hamuli, length and width of III and IV tergits, length and width of hind leg metatarsus and wax mirrors area. Parameters measured are then compared with limits appointed in the Standard for morphometric discrimination of "Slovakian" Carniolan bees. Average values for the length of the proboscis are 6.68 mm and for the workers cubital index are 2.66 (Kopernicky and Chlebo, 2004).

Many studies on bees morphological characteristics has been done (Garnery et al., 2004), including bee wings venation characteristics. A core set of 36 characters selected for discriminative power is described in Ruttner (1988), containing the recognised measurements referred to as "classical morphometry". At present, three different main approaches are in use: classical wing morphometry as defined by Ruttner (1988), the Dawino (Discriminant Analysis With Numerical Output) method (www.beedol.cz), and geometric wing shape analysis (Bookstein, 1991). Classical wing morphometry captures variation in wing shape by calculating 11 angles between 18 junctions in the wing venation, which constitute a subset of a suite of 17 angles first introduced into bee morphometry by DuPraw (1965). The Dawino method consists of the full set of DuPraw's angles, supplemented by 7 linear measurements, 5 indices, and one area.

2 Material and methods

In cooperation with the Slovak Carniolan Bee Breeders Association 16 samples of workers and drones from 9 queen breeders in various regions of Slovakia were

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taken in year 2013. Samples covered local lines of Carniolan bee subspecies: Sklenar, Vucko, Singer, Carnica Sokol, Slovinka, Kranka, Mosovcanka and Sitnan.

Each sample consisted of 20 young worker bees and 20 young drones catch inside a hive to assure the parentage from the colony. Sampled drones and workers were subsequently killed by freezing for several hours and after that dried out. Right-sided forewing from each dead bee was torn off as close as possible to the bee body in order to maintain all wings venations. The wings from one colony sample were mounted on transparent foil to prevent wings deformations and labelled separately for worker bees and drones.

Samples of worker bee wings were scanned and XY co-ordinates of the 19 points are measured on each wing as shown in Figure 1 and subsequently 30 wing characters are calculated on the basis of co-ordinates, including angles, lengths, indexes, shifts and area of 6 fields. Dawino (Discriminant Analysis With Numerical Output) results were computed on the basis of the above defined characters in the Bee Research Institute at Dol, Czech Republic.

The computation runs similarly as by discriminant analysis. Centroids of each of races in standard were calculated from all 30 wing characters. Then the Mahalanobis distances were computed for tested sample between it and each race centroid. The sample is defined with greatest probability to that race which is most closed to, i.e. has shortest Mahalanobis distance to it and vice versa. Posterior Probabilities are computed on the basis of Mahalanobis Distances in the range 0 to 100 per cent. The sum of Posterior probabilities for all races in standard is 100. The result of classification is numeric data expressing the similarity (probability, in %) of the sample to a race in standard. High value for a race denotes that the sample belongs to that race, and vice versa. The probabilities of belonging to other races are summed and labelled "Other".

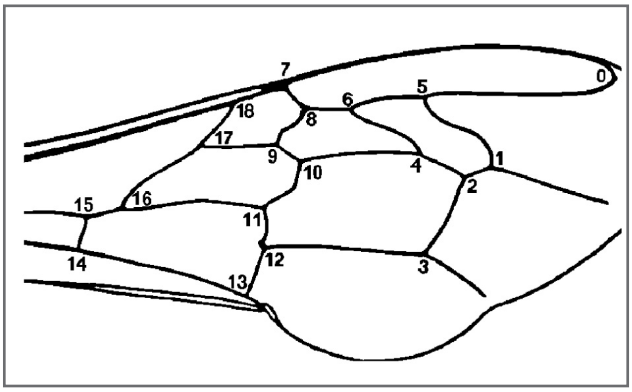

Figure 1 Worker forewing with 19 measured points according the Dawino method

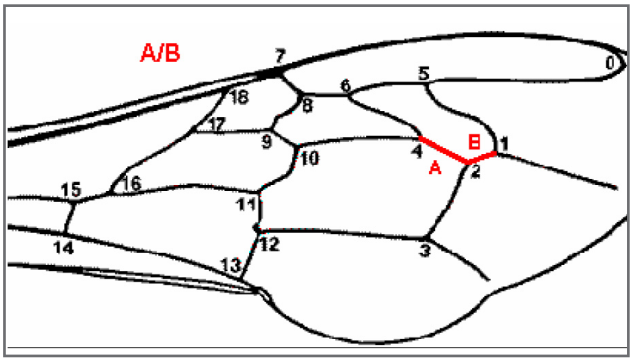

Figure 2 Cubital index of drone forewing – A / B length ratio

Dawino method, as well as other complex wing morphometry methods are designed for worker bees wings only. For drone wings characteristic exists only very few data from the most common morphometry parameter called Cubital index (Ci). For the comparability reason only this index was measured in drone wings samples using the QuickPHOTO MICRO (v 2.3 Czech Republic) software with an accuracy of 0.01 mm. Cubital index is the ratio of two of the wing vein segments as shown in Figure 2. Statistical analysis was performed and the values were compared using T-test.

3 Results and discussion

The result of classification by DAWINO method is numeric data expressing the probability (in %) of the sample to a honeybee subspecies standard summarised in Table 1. Our samples of worker bees from 15 bee colonies showed variability in similarity to Carniolan bee standard from 50 to 84% and in the case of the sample S0162 results shows similarity with other honeybee subspecies – Italian bee *Apis mellifera ligustica*. Five samples showed similarity with the standard just between 50 to 60%. Only two samples (S0155 and S0165) were ranked within more than 80% probability to the Carniolan race of bees. Previous data obtained from Slovakia using the same DAWINO method showed variability from 79 to 90% (Chlebo and Kopernicky, 2004). Comparing with these data some tendency to hybridisation of Slovak Carniolan bee population by others races (most probably Italian race A.m. ligustica) is visible by uncontrolled queen importation.

Cubital index of drone forewings comply with *A. m. carnica* standard in 14 cases as summarised in Table 1. Average drone cubital index according to standard proposed with Ruttner (1988) and Fert (1997) is 2.0 with a variation between 1.8 to 2.3. In two cases of samples T0162 and T0167 is above the maximum range. Comparable data on drone cubital index absents, as wing morphometry is mostly focused on worker bees. German beekeeper association "Deutscher Imkerbund (DIB)" set up for drone

Table 1 Wing morphometry results of 16 honeybee samples from Slovakia – Dawino method for workers (left) and Cubital index for drones (right)

Similarity of the worker samples to a *A. m. carnica* standard by DAWINO method				Cubital index of drones and its compliance with *A. m. carnica* standard		
Code	Declared Carniolan line	Race	%	Code	*Apis mellifera carnica*	Non-compliant with *A. m. carnica* standard
S0154	Sklenar	CARNICA	73	T0154	1.97	
		Other	27			
S0155	Vucko	CARNICA	81	T0155	1.90	
		Other	19			
S0156	Singer	CARNICA	63	T0156	1.96	
		Other	37			
S0157	Sokol	CARNICA	57	T0157	2.02	
		Other	43			
S0158	Slovinka	CARNICA	60	T0158	1.98	
		Other	40			
S0159	Sklenar	CARNICA	50	T0159	1.60	
		Other	50			
S0160	Kranka	CARNICA	66	T0160	1.63	
		Other	34			
S0161	Kranka	CARNICA	60	T0161	2.30	
		Other	40			
S0162	Kranka	LIGUSTICA	44	T0162		2.54
		Other	56			
S0163	Sokol	CARNICA	72	T0163	1.97	
		Other	28			
S0164	Singer	CARNICA	66	T0164	2.00	
		Other	34			
S0165	Singer	CARNICA	84	T0165	1.85	
		Other	16			
S0166	Mosovcanka	CARNICA	76	T0166	2.08	
		Other	24			
S0167	Vucko	CARNICA	60	T0167		2.36
		Other	40			
S0168	Slovinka	CARNICA	72	T0168	1.75	
		Other	28			
S0169	Sitnan	CARNICA	68	T0169	1.95	
		Other	32			

cubital index of Carnica population average values above 1.8 and 0% should be below 1.4. (in Bouga et al., 2011). If we apply German standard with no upper limits all samples are within a range, so the question arises, if the upper limit proposed by Ruttner (1988) is properly set up, because cubital index is out of maximum range even in two other common bee races of the Central European region – *A. m. mellifera* (1.0–1.5) and *A. m. ligustica* (1.6–2.0) and drift of genes of other subspecies is rather unlike due to climatic adaptations. When comparing wing morphometry data, question of compatibility arises. In Norway, two software programmes are in use:

CBeeWing and the Dawino, while samples of bees from the same colony can be judged quite different in the two analyses. Whereas the CBeeWing gives the percentage of bees that are within the morphological limits of the sub-species in question, the Dawino analysis (which uses more wing measurements than the CBeeWing) gives a statistical probability that the samples are taken from the respective sub-species. The proportion of wings within the range defined for *A. m. carnica* in the CBeeWing was not significantly related to the probability that the sample came from *A. m. carnica* colony (in Bouga et al., 2011). The variation can be attributed to improper sampling (solely young bees should be collected, otherwise bees from neighbouring colonies can be presented) or measurement errors. Although morphometric methods are quite suitable to distinguish between morphologically distinctly differentiated honey bee populations, the overlapping of morphometric traits makes them unsuitable to reliably estimate admixture proportions. According to Swiss experiences (in Bouga et al., 2011) during several decades of ongoing admixture and high selection pressure on several morphometric characters with a high heritability the wing measurements methods has lost its informative power to detect hybridisation.

In Slovakian breeding programme for *A. m. carnica* the values of cubital index for workers must be 2.4–3.0 and 1.8–2.3 for drones. The colour of abdomen must be grey and not yellow. In the western part of the country our *A. m. carnica* is probably mixing with *A. m. ligustica*. These bees are easily recognised because of yellow abdomen. If there are more than 2% workers with yellow abdomen (in each colony) queen breeders must replace the queen. The queen rearing practice and transportations of honey bee colonies over the country would not have significant effect on changes the genetic variability and allow us to perform the programme for conserving the native honey bee race. Importing queens and colonies of different genetic structure should not be allowed in order to reduce potential influx of foreign alleles and to allow us to conserve desired genetic diversity.

4 Conclusions

Modern molecular and population genetic methods have been developed to identify hybrids on an individual basis. The future of breeding programmes is making molecular testing freely available to bee breeders, which can greatly improve the confidence in their respective breeding stock. Revaluation of priorities is needed as in most pure breeding communities the cubital index was of highest importance to breeders. Breeders should focus much more on the performance of their colonies as for the purity testing by traditional morphometric methods, because standards between races are overlapping and measurements errors when comparing various approaches are quite often. However, the case of workers and drones samples from one hive (S0162 and T0162) in this study, which are both out of *Carnica* standards, shows some tendency to hybridisation of Slovak Carniolan bee population by others races.

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