Apidologie 38 (2007) 191–197
© INRA/DIB-AGIB/ EDP Sciences, 2007
DOI: 10.1051/apido:2006068

Original article

Apis mellifera mellifera in eastern Europe – morphometric variation and determination of its range limits*

Marina D. MEIXNER[a], Miroslaw WOROBIK[b,c], Jerzy WILDE[c], Stefan FUCHS[b], Nikolaus KOENIGER[b]

[a] Department of Entomology, Washington State University, Pullman WA 99164-6382, USA
[b] Institut für Bienenkunde, Fachbereich Biowissenschaften, Universität Frankfurt, Karl-von Frisch-Weg 2, 61440 Oberursel, Germany
[c] Apiculture Division, Warmia and Mazury University, Sloneczna 48, 10-710 Olsztyn, Poland

Received 7 February 2006 – Accepted 30 August 2006

Abstract – The variability of *Apis mellifera mellifera* in Eastern Europe was investigated with a morphometric analysis of 136 samples from Poland, Belarus and the Ukraine. Samples from the northern part of this area were unambiguously classified as *A. m. mellifera*, but the proportion of uncertain allocations increased towards the south, where some samples were classified as hybrids between *A. m. mellifera* and subspecies of the lineages C and O. In the Ukraine, one third of the samples were classified as *A. m. mellifera,* one third as *A. m. macedonica,* and one third as hybrids. Our results confirm earlier reports of a large hybrid zone in Poland and the Ukraine, but they unexpectedly also show a strong influence of the morphological O lineage. However, the true extension of this hybrid zone currently remains unknown. The bees of northeastern Belarus showed an extreme position on the border of the *A. m. mellifera* cluster, potentially indicating ecotypic variation within *A. m. mellifera* in the northeast of its range.

***Apis mellifera mellifera* / morphometry / hybrid zone / Eastern Europe / range limit**

1. INTRODUCTION

The subspecies *Apis mellifera mellifera* L. is known from all of northern Europe between France in the west and Russia in the east, reaching as far north as 60°N in Scandinavia. It is considered to extend to the Ural mountains (Ruttner, 1988, 1992), where its eastern limit also falls together with the eastern border of *Apis mellifera* as a species. Morphologically, it can be characterized by its large size, dark pigmentation and long cover hair, and some characters of wing venation such as the low cubital index. Molecular evidence suggests that *A. m. mellifera* spread east and north from a glacial refuge, probably located in France or Spain (Franck et al., 1998; Garnery et al., 1998a, b). In spite of this vast range, only the western part of its distribution has been thoroughly studied. Most of the morphometric and molecular data available for this subspecies to date have been obtained from populations close to its Pleistocene refuge or regions that were colonized early during its eastward movement, while the vast area closer to its eastern periphery has received little scientific scrutiny. Apart from early publications on morphometric variation of single characters in Russia (Michailov, 1924; Alpatov, 1925, 1929), very little is known about its variability in eastern Europe and its relations to adjacent subspecies. In this paper, we present the results of the first comprehensive morphometric analysis of honey bee populations from Poland, Belarus and the Ukraine in eastern Europe. We report new information regarding the range

---

Corresponding author: M.D. Meixner, mmeixner@wsu.edu
* Manuscript editor: Walter S. Sheppard

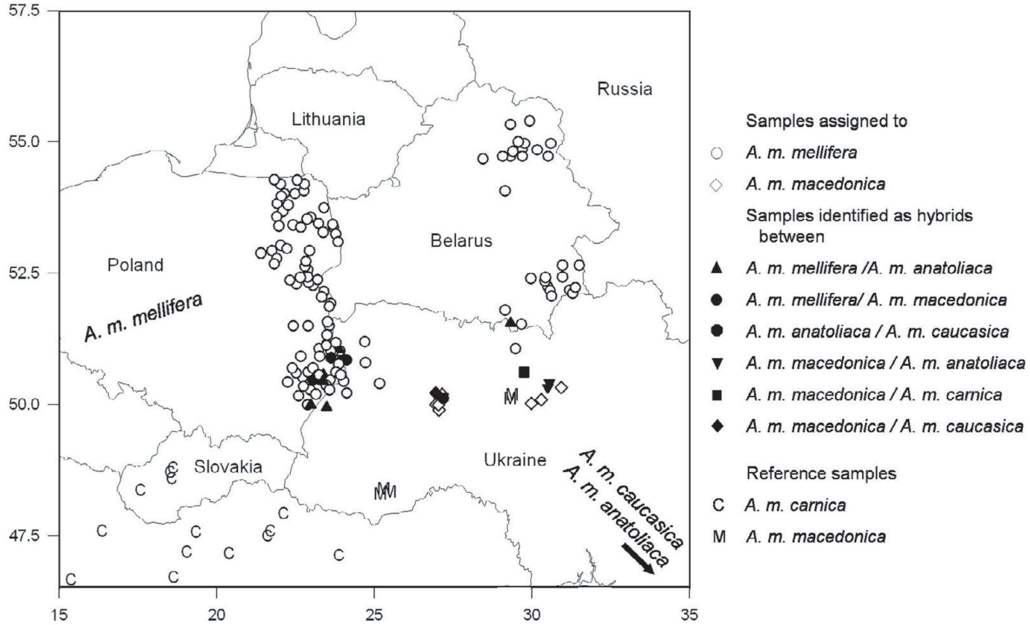


Figure 1. Geographic position and subspecific classification of the samples in study and some of the reference samples. The reference samples of *A. m. mellifera, A. m. caucasica* and *A. m. anatoliaca* are located outside of the range of this map.

limit of *A. m. mellifera* in the southeast of our sampling area and investigate its relationship to neighboring subspecies of the morphological lineages C and O.

2. MATERIALS AND METHODS

2.1. Collection of bee samples and morphometric analysis

A total of 136 honey bee samples were collected from 103 locations in northeastern Poland (35 samples), central eastern Poland (53), northeastern Belarus (15), southeastern Belarus (16), and Ukraine (17) (Fig. 1, see table as online material). Each sample contained more than 30 worker bees of one colony. Bees were preserved in 75% ethanol.

Fifteen worker bees of each sample were dissected and 38 morphometric characters were measured according to methods described by Ruttner (1988). Measurements of size and wing venation were performed using a stereomicroscope and a computer-aided measuring system based on a video system and measuring program. For measurements of pilosity and color scaling a stereomicroscope was used.

2.2. Statistical analysis of the data

Colony sample means, standard deviation, and standard error were computed for each character of each sample and represented estimates for the colony. Reference samples of the following subspecies from the morphometric data base in Oberursel were included: *A. m. mellifera* (37), *A. m. carnica* (173), *A. m. macedonica* (25), *A. m. ligustica* (40), *A. m. caucasica* (30), *A. m. anatoliaca* (40).

The data were submitted to factor analysis and sample scores were plotted on principal component (PC) co-ordinates for visualization. Discriminant analysis was used to perform reallocation of samples to their respective groups, based on the results of the factor analysis. Subsequently, morphometric similarities were investigated by cluster analysis, based on group means of the characters in each collecting region and including the reference samples. Calculations were performed using the SPSS for Windows 12.00 and Systat 9.00 statistical packages.

Table I. Multiple F values between group centroids from discriminant analysis. All group differences were significant with $P < 0.001$ (**).

	NE Poland	SE Poland	NE Belarus	SE Belarus
SE Poland	13.95**			
NE Belarus	22.70**	40.10**		
SE Belarus	10.95**	11.91**	19.57**	
Ukraine	19.77**	11.60**	38.45**	12.82**

3. RESULTS

In an initial factor analysis the samples from all five groups fell into discernible, but partly overlapping groups according to their respective region of origin. In this analysis, the bees of Poland and Belarus were closely associated with the plot area occupied by the *A. m. mellifera* reference samples. This local distinctiveness of the eastern European sampling area was confirmed by a discriminant analysis where all samples from the study area were reassigned to their groups of origin with significant differences between group centroids (Tab. I).

The allocation of the samples to the reference groups (*A. m. mellifera, A. m. carnica, A. m. macedonica, A. m. ligustica, A. m. anatoliaca,* and *A. m. caucasica*) was performed with a forced discriminant analysis based on all characters measured (except length of proboscis and pigmentation of tergum 2). In the plot of this analysis (Fig. 2), the samples were arranged in three major groups, corresponding to the regions of northern and western Europe (reference samples of *A. mellifera*), southeastern Europe (*A. m. carnica, A. m. ligustica* and *A. m. macedonica*) and western Asia (*A. m. caucasica* and *A. m. anatoliaca*). Honey bees from northeast Belarus took an extreme position on the margin of the *A. m. mellifera* cluster, while samples from the Ukraine appeared in the center of the figure and connected the three branches. The other groups (NE Poland, SE Poland, SE Belarus) appeared scattered between these two extremes.

All of the samples collected in northeastern Poland and northeastern Belarus were classified as *A. m. mellifera* with probability scores of 1.0. However, the proportion of samples with uncertain classification increased in southeastern Belarus and southeastern Poland. Of the 57 samples from southeastern Poland, two were classified as hybrids with *A. m. macedonica*, and six as hybrids with subspecies of the O lineage, *A. m. caucasica* or *A. m. anatoliaca*. The collection from southeast Belarus showed influence of *A. m. macedonica* in one sample and *A. m. caucasica* in a second one. One third of the samples from the Ukraine were classified as *A. m. mellifera* and one third as *A. m. macedonica*. The remaining six of our seventeen samples from the Ukraine were classified as hybrids: three between *A. m. macedonica* and *A. m. anatoliaca* and one each between *A. m. macedonica and A. m. carnica, A. m. caucasica*, and *A. m. mellifera*. The geographic position of the samples together with

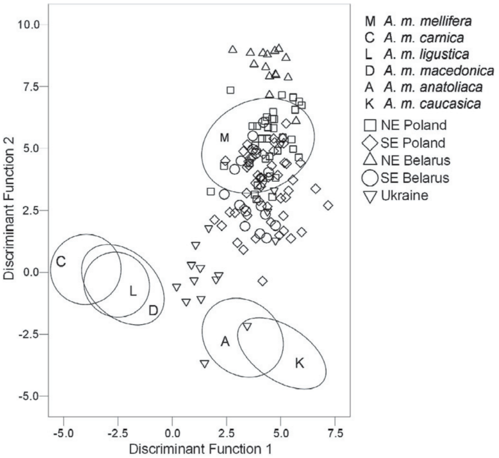
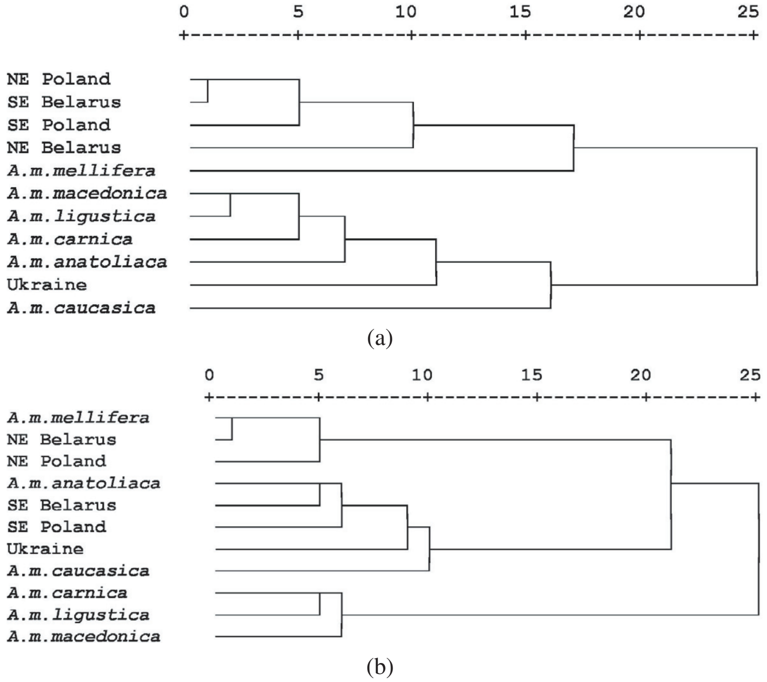


Figure 2. Positions of the samples studied in a discriminant analysis. Abscissa: discriminant function 1, ordinate: discriminant function 2. The ellipses of confidence (75%) calculated from classified reference samples are given. To improve the readability of the figure, positions of individual reference samples are omitted.



(a)

(b)

Figure 3. (a) Dendrogram of a cluster analysis based on all characters measured and the mean values of the samples from each region, including the reference samples. (b) Dendrogram of a cluster analysis based only on the wing venation characters and the mean values of the samples from each region, including the reference samples.

their morphometric classification is presented in Figure 1.

A cluster analysis based on the means of all characters resulted in two main groups. All samples collected from Poland and Belarus were combined with the *A. m. mellifera* reference group (Fig. 3a), whereas the samples from the Ukraine were incorporated into the second cluster formed by *A. m. macedonica, A. m. ligustica*, and *A. m. carnica* (lineage C) that also contained *A. m. anatoliaca* and *A. m. caucasica* (lineage O). However, if the cluster analysis was based instead only on the characters of wing venation, three major groups resulted (Fig. 3b). The first group contained the subspecies of the C lineage (*A. m. carnica, A. m. macedonica* and *A. m. ligustica*), the second cluster was formed by the subspecies of the O lineage (*A. m. caucasica* and *A. m. anatoliaca*) together with the samples from Ukraine, SE Belarus and SE Poland; the third cluster contained *A. m. mellifera* and also incorporated the samples from NE Poland and NE Belarus.

4. DISCUSSION

Our study presents the first comprehensive morphometric analysis of eastern European *Apis mellifera mellifera*, confirming the presence of this subspecies in Poland and Belarus and contributing new information towards the extent of its natural range and its relations to adjacent subspecies in this region.

The work of Alpatov (1925) gave a first impression of the morphological variability of *Apis mellifera* in Eastern Europe, describing a gradual increase of the proboscis length of honey bees between St. Petersburg in the north to Kiev in the south. The same trend was also observed for the size of the hind legs, while the body size of the bees showed the opposite tendency to decrease from north to south (Alpatov, 1929). Alpatov (1929) discussed these observations as clinal variation in relation to climate, apparently confirming the validity of the biogeographic rules of Bergmann and Allen for honey bees. However, Ruttner (1952) and Goetze (1964) later showed that this gradual variation of characters from north to south could be readily interpreted as the consequence of a broad hybrid zone in eastern Europe. In this region, *A. m. mellifera*, presumably having spread east from France to Russia, meets the southeastern European subspecies *A. m. carnica* and *A. m. macedonica* who, in turn, had expanded north from Pleistocene refuges on the Balkan peninsula (summarized in Ruttner, 1988). Studies based on molecular markers corroborated the hypothesis of *A. m. mellifera* spreading north and east, probably from refuges in Spain or southern France after the last ice age (Franck et al., 1998; Garnery et al., 1998a,b). The movement of *A. m. mellifera* from a refuge close to the Pyrenees and the northward range expansion of populations representing the C lineage from the Balkan peninsula appear to follow pathways of postglacial re-colonization that are well documented for a number of organisms (Hewitt, 1999).

Our results confirm the existence of a large hybrid zone in southern Poland and the Ukraine, but they unexpectedly also show a strong influence of the morphological O lineage in the south of the sampling region. However, there is no obvious explanation for the occurrence of bees showing affinities to the O lineage. The subspecies of this lineage are found in western Asia, ranging from the east coast of the Mediterranean to eastern Iran (Ruttner, 1988) and Kazakhstan (Sheppard and Meixner, 2003). The reference samples of *A. m. caucasica* and *A. m. anatoliaca* in our analysis were the geographically closest representatives of the O lineage available to us; however, the current knowledge about the existence and character of honey bee populations south of our sampling area towards the Caucasus or in regions further to the east is very scarce. According to Skorikov (1929), bees were only brought to the Ukraine by humans about 500 years ago. On the other hand, Skorikov does not provide data on the number and origin of bee colonies introduced or the locations to where they were transported. Based on our results three morphological lineages, M, C and O (Ruttner, 1988) come together and form a hybrid population in southern Poland and the Ukraine. Thus, our morphometric data do not support Skorikov's suggestion, rather they point to southern Poland and the Ukraine as a genuine "melting pot" of honey bee strains from western Europe, the Balkans and western Asia. However, the true character and extension of this region of hybridization remains largely unknown, as honey bees from European regions south and east of our sampling areas remain unstudied. The existence of eastern glacial refugia in the regions of the Black Sea and the Caspian has been suggested (Hewitt, 1999), and may be useful in future characterization of honey bees from these regions.

The influence of the O lineage extends far north into southern Poland and Belarus, as demonstrated in the cluster analysis based on characters of wing venation only. These characters are little influenced by environmental conditions and, thus, they represent good indicators of genetic relationships between populations. However, if characters influenced by environmental conditions – such as body size and pilosity – are included into the same analysis, the transition zone between samples classified as *A. m. mellifera* and the lineages O and C shifts to the south into the Ukraine, blurring the differences between lineages C and O. Thus, this pattern reflects the phylogeographic history of post-glacial northward expansion of the O lineage, but is overlaid by the variation of characters influenced by environment from north to south, corresponding to patterns of climate and vegetation in this region. Between southeast Poland and the Ukraine, a major shift of climate and vegetation occurs from the forest vegetation of north and west Europe to the steppe vegetation systems of southeast Europe, where patches of meadow-steppe within predominant forest alternate with forest islands in steppe regions (Walter and Breckle, 1986).

While the samples from the southern range of the collecting region demonstrate a transition from *A. m. mellifera* to the lineages C and O, bees from the northeast of Belarus were noticeably set apart on the far side of the *A. m. mellifera* cluster in Figure 2. Based on the available data, it is not possible to decide whether this result suggests ecotypic variation within our sampling area or represents an indication of another separate subspecies of honey bees in the northeastern part of the range of *A. mellifera*. The bees of middle Russia were named *A. m. silvarum* (forest bee) by Alpatov (1929), indicative of their adaptation to a densely forested environment. The bees of northern Belarus may correspond to the westernmost representatives of the Russian forest bee, but further analyses will be required to reveal the character of honey bee populations east of the current study area.

***Apis mellifera mellifera* en Europe de l'Est: variation de la morphométrie et détermination des limites de son aire de répartition.**

***Apis mellifera mellifera* / morphométrie / zone d'hybridation / Europe de l'Est / aire de répartition**

Zusammenfassung – ***Apis mellifera mellifera* in Osteuropa – morphometrische Variabilität und Bestimmung ihrer Verbreitungsgrenzen.** Das Verbreitungsgebiet von *Apis mellifera mellifera* umfasst ganz Nordeuropa, von Frankreich im Westen, nach Skandinavien im Norden und Russland im Osten. Es reicht vermutlich bis zum Uralgebirge, wo seine Ostgrenze mit der Ostgrenze von *Apis mellifera* als Art zusammenfällt. Von früheren Publikationen über die morphometrische Variation von einzelnen Merkmalen in Russland abgesehen, ist sehr wenig über die Variabilität dieser Unterart in Osteuropa und ihre Beziehungen zu umgebenden Bienenpopulationen bekannt. In dieser Arbeit stellen wir die erste umfassende morphometrische Analyse von Populationen der Honigbiene in Osteuropa vor.

Wir untersuchten die Variabilität von *A. m. mellifera* im Osten ihres Verbreitungsgebiets mit einer morphometrischen Analyse von 136 Proben, die in Nordost- und Südostpolen, sowie in Nordost- und Südost-Weißrussland und der Ukraine gesammelt wurden. In die Analyse wurden Referenzproben von umgebenden Unterarten einbezogen. In einer Diskriminanzanalyse (Abb. 2) wurden die Proben in drei Hauptgruppen angeordnet, die den geographischen Regionen Nord- und Westeuropa (Referenzproben von *A. m. mellifera*), Südosteuropa (*A. m. carnica, A. m. ligustica* and *A. m. macedonica*) und dem westlichen Asien (*A. m. caucasica* and *A. m. anatoliaca*) entsprachen. Bienen aus dem nördlichen Weißrussland nahmen eine extreme Position am Rand des *A. m. mellifera* Clusters ein, während Proben aus der Ukraine die drei Äste im Zentrum der Abbildung verbanden. Zu den anderen Gruppen (Nordost- und Südostpolen, Südost-Weißrussland) gehörende Proben erschienen zerstreut zwischen diesen Extremen. Proben aus Nordostpolen und Nordost-Weißrussland wurden eindeutig als *A. m. mellifera* klassifiziert, aber die Anzahl der unsicheren Zuordnungen nahm in Südostpolen und Südost-Weißrussland zu, wo einige Proben als Hybriden zwischen *A. m. mellifera* und *A. m. carnica, A. m. macedonica, A. m. caucasica* oder *A. m. anatoliaca* eingeordnet wurden. Die Zuordnung der Proben aus der Ukraine war noch mehr gemischt; hier wurde je ein Drittel der Proben als *A. m. mellifera*, *A. m. macedonica* und als Hybriden klassifiziert.

Unsere Ergebnisse bestätigen damit ältere Berichte über eine ausgedehnte Hybridzone in Südpolen und der Ukraine, aber sie demonstrieren auch einen unerwarteten und starken Einfluss der morphologischen O-Linie im südlichen Teil des Sammelgebiets. Der Charakter und die tatsächliche Ausdehnung dieser Hybridzone ist jedoch unbekannt, da keine Daten aus Regionen östlich und südlich unseres Sammelgebiets vorhanden sind. In allen Analysen nahmen die Bienen aus Nordost-Weißrussland extreme Positionen am Rand des *A. m. mellifera* Clusters ein. Möglicherweise ist dies ein Hinweise auf ökotypische Variation von *A. m. mellifera* im nordöstlichen Teil ihres Verbreitungsgebiets.

***Apis mellifera mellifera* / Morphometrie / Hybridzone / Osteuropa / Verbreitungsgrenze**

REFERENCES

Alpatov W.W. (1925) Über die Verkleinerung der Rüssellänge der Honigbiene vom Süden nach dem Norden hin, Zool. Anz. Bd. LXV Heft 3/4, 102–111.

Alpatov W.W. (1929) Biometrical studies on variation and races of the honeybee *Apis mellifera* L., Rev. Biol. 4, 1–57.

Franck P., Garnery L., Solignac M., Cornuet J.-M. (1998) The origin of west European subspecies of honeybees (*Apis mellifera*): new insights from microsatellite and mitochondrial data, Evolution 52, 1119–1134.

Garnery L., Franck P., Baudry E., Vautrin D., Cornuet J.-M., Solignac M. (1998a) Genetic diversity of the west European honey bee (*Apis mellifera mellifera* and *A. m. iberica*). I. Mitochondrial DNA, Genet. Sel. Evol. 30 (Suppl. 1), S31–S47.

Garnery L., Franck P., Baudry E., Vautrin D., Cornuet J.-M., Solignac M. (1998b) Genetic diversity of the west European honey bee (*Apis mellifera mellifera* and *A. m. iberica*). II. Microsatellite loci, Genet. Sel. Evol. 30 (Suppl. 1), S49–S74.

Goetze G. (1964) Die Honigbiene in natürlicher und künstlicher Zuchtauslese, Parey, Hamburg.

Hewitt G. (1999) Post-glacial re-colonization of European biota, Biol. J. Linn. Soc. 68, 87–112.

Michailov A.S. (1924) On the tongue length of the bees from the Tscherepovetz district in connection with the problems of local differences in tongue length, Pchelowodnoje Djela, Febr. [in Russian].

Ruttner F. (1952) Alter und Herkunft der Bienenrassen Europas, Österr. Imker. 2, 8–10.

Ruttner F. (1988) Biogeography and taxonomy of honey bees, Springer, Berlin.

Ruttner F. (1992) Naturgeschichte der Honigbienen, Ehrenwirth Verlag, München.

Sheppard W.S., Meixner M.D. (2003) *Apis mellifera pomonella*, a new honey bee subspecies from the Tien Shan mountains of Central Asia, Apidologie 34, 367–375.

Skorikov A.S. (1929) Beiträge zur Kenntnis der kaukasischen, Honigbienenrassen, Rep. Appl. Entomol. 4, 1–59 [in Russian with German summary].

Walter H., Breckle S.W. (1986) Ökologie der Erde. Bd 3. Spezielle Ökologie der gemä*β*igten und arktischen Zonen Euro-Nordasiens, Fischer UTB, Stuttgart.

To access this journal online:
www.edpsciences.org