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Variability of morphological characteristics of middle-European bees of the 'Northern M' line

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Summary

The aim of the study was to evaluate the variability of morphological characteristics of native middle-European bees (Apis m. mellifera) of the 'Northern M' line. The research covered characteristics of breed (the length of proboscis, the cubital index), body size (the width of tergite 4 and the sum of widths of tergites 3 and 4) and wing size (length and width). The study compared bees harvested from a leading apiary and from collaborating apiaries participating in a program for the protection of genetic resources of bees of this line. The material for the study was harvested in 10 consecutive years. The samples were collected by the "cluster drawing" method (the multi-stage method of clustering described by Zee et al. in 2013). Each sample consisted of 25 to 30 bees. The frames were loaded in an instrument for the morphological measurement of bees (Apimeter). Seven measurements were taken on prepared body parts of each bee. The length and width of the wing and the length of the cubital vein were measured on the right front wing (hereinafter referred to as the "wing"). In addition, the width of abdominal tergites 3 and 4 and the length of proboscis were measured in each instance. In total, 4291 bees were harvested and 30 037 measurements were taken. The conclusion is that the program for the protection of genetic resources of bees of the 'Northern M' line can be implemented in Poland based on the leading apiary and on the collaborating apiaries, and bees of this line display characteristics of middle-European bees. Moreover, the study demonstrated a consistency of values of the studied characteristics of the 'Northern M' line with the applicable references of morphological characteristics for Apis m. mellifera. In addition, based on a review of results of the author's research and based on collected literature originating from the 1960s, the study proves that a dwarfing trend has emerged among middle-European bees.

Keywords: native middle-European bee, morphological characteristics, variability characteristics

Detailed characteristics of bee populations inhabiting Europe were unknown until the 1920's. Alpatov (1-4) and Goetze (16) were the first contributors to the description of these bees. The research (4) enabled identification of changes in bee body dimensions depending on latitude. A similar dependency was described (17) for the length of proboscis while (43) dealing with the length of bee legs of the third pair. Subsequent researchers (10, 14, 41, 42) described subspecies/breeds of the honey bee species and identified ranges of their occurrence based on complex statistical analyses of morphological characteristics. Most of Poland was inhabited by *Apis m. mellifera* (6, 40-42). Based on morphometric research (6), four areas were identified that used to be inhabited by different populations of middle-European bees. Unfavorable changes caused by environmental degradation, parasites, pathogens and genetic isolation deteriorating viability as a result of inbreeding (7, 54) have led to a drastic drop in the number of honey bees, including middle-European bees. An additional drop in the number of *Apis m. mellifera* was caused by mass importation of Caucasian and Carniolan queens considered more profitable by bee keepers.

Currently, middle-European bees represent a subspecies at a high risk of extinction. Protection of middle-European bees in areas where they evolved is becoming increasingly important. The purpose of each program for the protection of genetic resources of *Apis m. mellifera* is to retain features typical for middle-European bees in a possibly unchanged state while preserving maximum genetic diversity. Efforts to protect native bees were first taken in Poland approximately 40 years ago. Experience gained over this period made it possible to identify threats and launch effective protection programs. The necessity of preserving native bees as a general environmental requirement that should be met by establishment of sanctuaries, i.e. areas taking account of the reproductive biology of bees, was realized as early as in the 1960s and 1970s (21). Recent COLOSS action studies (2014-2016) suggest that bees from local populations, such as *Apis m. mellifera*, are the most adapted to environmental conditions and are better at fighting diseases, parasites and other pathogens, and one of the ways to achieve this, could be to establishing of protected areas of endangered populations (8, 25, 32, 53). Today, implementation of updated programs for the protection of genetic resources of middle-European bees (four lines: 'Augustów M', 'Kampinos M', 'Northern M' and 'Asta M') is monitored by the Ministry of Agriculture and Rural Development acting through the Animal Husbandry Institute of the State Research Institute and through the National Center for Animal Breeding. The Olecko Apiary owned by the KRIR Breeding Apiary Ltd. based in Parzniew (former Animal Breeding and Insemination Institute Ltd. in Bydgoszcz) has been one of the direct contractors for such programs since January 1, 2014. The Olecko Apiary breeds two protected lines: 'Northern M' and 'Augustów M'. The implementation of programs for individual lines is based on the sharing of work between leading apiaries and collaborating apiaries (26, 27, 36, 46, 47). The protection of middle-European bees in Poland covers two lines ('Northern M' and 'Asta M') under a system of leading apiaries and collaborating apiaries without any administrative districts. The lack of a genetic reserve for these lines is an additional factor that makes their protection more difficult.

Two native bee populations that evolved in the natural climatic and environmental conditions of the northern Poland were the progenitors of the Northern line. Dr. W. Ostrowska and, later, A. Ejsmont bred a population of native bees of the 'Mazuria' line in an apiary of the Animal Husbandry Experimental Institute of the Animal Husbandry Institute in Siejnik, in the north-eastern part of Poland, in the 1970s while the 'Pomeranian' line was selectively bred in West Pomerania in the 1990s (26). Although the current 'Northern M' line has no administrative protection areas, the organizational scheme of the program is similar to that of the 'Augustów M' and 'Kampinos M' lines, based on closed conservation districts established in 1976 (23, 26). Likewise, as in the case of all the programs for protection of genetic resources of middle-European bees, there is a two-directional exchange of breeding material for the 'Northern M' line between the leading apiary and the collaborating apiaries. The leading swarm of this line is maintained in Olecko and the collaborating apiaries are situated near Olecko, Kętrzyn and Lidzbark Warmiński. This line, among all the protected populations, is most exposed to adverse consequences of increased inbreeding because of the lack of a natural population that could be drawn from in the event of narrowing of the genetic diversity (36, 38, 47).

Tab. 1. Range and mean values of the cubital index for individual lines of middle-European bees determined by the Goetze method (G), converted into the Alpatov method (A) (38)

Lines	Cubital index by method	Range	Mean	Modal value
Northern M	G	1.11-3.62	1.86	1.81
	A	27.62-90.09	53.76	55.25
Augustów M	G	0.99-3.81	1.85	1.81
	A	26.25-101.01	54.05	55.25
Kampinos M	G	0.79-3.34	1.76	1.73
	A	29.94-126.58	56.82	57.80
Asta M	G	1.04-4.14	1.96	1.90
	A	24.15-96.15	51.02	52.63

The programs for protection of genetic resources of middle-European bees of the 'Northern M' line have the following goals:

– to maintain sufficient populations of preservation swarms and retain and preserve features typical for the populations,

– to improve breeding characteristics, such as melliferous capacity, non-swarming and mildness while preserving the phenotypic and biological characteristic features of the protected population.

Preservation of phenotypic features defined in references for each line is one of the requirements of the protraction programs. The cubital index (13) is an important morphometric indicator because it is particularly useful as a taxonomic feature. Rostecki (38) measured values of this parameters of the Polish protected lines (Tab. 1). Because protected populations can become contaminated with foreign genes (from drones of foreign breeds, migrations of swarms, etc.), the breeding material of the 'Northern M' line is being carefully selected. Body color and values of morphometric features decisive for classification of bees of a given family as middle-European bees including the 'Northern M' line – such as the length of proboscis, value of the cubital index, the width of tergite 4 or the sum of widths of abdominal tergites 3 and 4 (in addition to behavioral and productive features) – are the main selection criteria (20). Various methods were used to capture morphological data and the methods improved in time. Measuring bees under the microscope or displaying wing images on a special screen and measuring wings with dedicated rules represented the early methods (6, 24, 50). The evolution of computer-aided techniques opened new opportunities (11, 12, 15, 29, 30, 38, 39, 45, 49). The perspective of automating such measurements was an important factor driving the development of the computer-aided techniques (48, 50). The "standard" morphometry based on venation of the front wing addressed distances and angles between selected vein intersections while contemporary geometric morphometry measures coordinates of all vein intersections. On the other hand, differences between sub-species can be presented as vectors that deform the grid of the diagram. This method facilitates interpretation of measurements vs. the comparison of multiple distances and angles (28, 51). A comparison of the two methods showed that geometric morphometry can differentiate sub-species slightly better than the standard method. However, the standard method is sufficient for differentiating bee populations (51). The same author points at a need for comparing results of this method to the results obtained by (40). The DNA analysis is used increasingly more frequently for the elimination of hybrid specimens from protected populations (34, 35, 51). But Oleksa and Burczyk (34) caution against selecting bees based on markers only because the process can lead to loss of important genes critical for the phenotype in a situation where diagnostic alleles for middle-European bees and alleles responsible for characteristic phenotypic features of native bees including the 'Northern M' are not combined.

The use of advanced methods does not preclude application of the traditional morphometric evaluation which is, indeed, more time-consuming but cheaper and, most importantly, sufficient for determination of membership in a breed. Moreover, it measures such features as the bee size or the length of proboscis and allows for tracking changes in these parameters, which is very important for the performance of the programs for protection of genetic resources of middle-European bees, including the 'Northern M' line. In addition, the generally accepted and valid references and models for *Apis m. mellifera*, under development since the 1960s, are still used in the determination of breed membership of middle-European bees including the 'Northern M' line (13, 38, 52).

The purpose of the study was to evaluate the analysis of variability of morphological characteristics of native middle-European bees of the 'Northern M' line. The study investigated features indicative of breed membership (the length of proboscis, the cubital index), bee body size (the width of tergite 4 and the sum of tergites 3 and 4) and the size of wings (length and width). Also, the study focused on verifying whether guidelines for the program for the protection of genetic resources of the 'Northern M' line based on the sharing of work between leading apiaries and collaborating apiaries enable preservation features of middle-European bees and whether the features are consistent with the references valid for *Apis m. mellifera*.

Material and methods

Middle-European bees (*Apis m. mellifera*) of the 'Northern M' line, covered by the program for the protection of genetic resources of bees in Poland represented the research material. The material for the study was harvested in 10 consecutive years at the Breeding Apiary in Parzniew owned by the Animal Breeding and Insemination Institute in Bydgoszcz that, in turn, is currently owned by the National Council of Agricultural Boards. The bees were harvested from the leading apiary and from the collaborating apiaries covered by the programs for protection of middle-European bees.

Samples of bees of the 'Northern M' line were harvested from five locations: the leading apiary (located in Markowskie) and collaborating apiaries (located in Gradowo, Skierki near Kętrzyn, Kobiela near Lidzbark Warmiński and Dojliwa near Olecko).

The samples were collected by the "cluster drawing" method – a multi-stage method of clustering described in (55). Bees representing samples of formerly randomly selected families were harvested in each year. The collection of experimental material was placed in Fotie boxes. The array was ill-balanced because of the difference in the numbers of bees between individual years. Each sample consisted of 25 to 30 bees. Seven measurements were taken on prepared body parts of each bee. In total 4291 bees were harvested and 30 037 measurements were taken.

The bees were thrown into boiling water to straighten their probosces for measurement, drained and preserved in glass vessels with 70% ethyl alcohol. Next, preparations were made from such preserved samples. The proboscis, the wing, and abdominal tergites 3 and 4 were removed from each bee. These body parts harvested from each bee were placed on 3 transparency frames with microscopic cover glasses glued on the inner side. The wing was placed on the first frame, tergites 3 and 4 on the second frame and the proboscis on the third frame.

The frames were loaded in an instrument for morphological measurement of bees (apimeter). The device displayed images of the prepared bee body parts magnified by 20 times. The body parts were then measured with a dedicated slide caliper with plexiglass jaws. Readouts were recorded as actual dimensions in a computer database.

The results were elaborated statistically in the PASW Statistics 23 (2017) software suite using the unifactorial analysis by the mean smallest squares method. The description of the study takes account of only those interactions between factors the effect of which was statistically significant ($p < 0.01$ or $p < 0.05$), which was determined after initial statistical analyses. The Pearson correlation coefficient was calculated to establish dependencies between the tested features, dependencies between features of middle-European bees of the 'Northern M' line harvested from the leading apiary and from the collaborating apiaries.

Results and discussion

Wing parameters and the length of proboscis in middle-European bees of the 'Northern M' line featured the smallest variability compared to other tested features (Tab. 2). The value of the coefficient of variability for the wing length was smaller by 49% than the value of the parameter for the width of tergite 4 and smaller by 26% than the sum of tergites 3 and 4. On the other hand, variability for the wing width, wing aspect ratio and the length of proboscis varied slightly and differences ranged from 7 to 11 per cent. The largest variability expressed by the coefficient of variability for bees of this line was noted for veins that are decisive

Tab. 2. Morphological characteristics of middle-European bees of the 'Northern M' line

Line	Feature	Number	Range	Mean ± se	Standard deviation	Coefficient of variability
Northern M	Wing length (mm)	4,291	8.57-9.92	9.387 ± 0.003	0.170	1.8
	Wing width (mm)	4,291	2.86-3.60	3.208 ± 0.001	0.091	2.8
	Wing aspect ratio	4,291	2.70-3.31	2.928 ± 0.001	0.076	2.6
	Cubital index (Goetze)	4,291	1.02-2.73	1.690 ± 0.004	0.251	14.9
	Width of tergite 4: T4 (mm)	4,291	2.08-2.85	2.292 ± 0.001	0.080	3.5
	Sum of tergites 3 & 4: T3 + T4 (mm)	4,291	4.08-5.40	4.777 ± 0.002	0.148	3.1
	Length of proboscis (mm)	4,291	5.60-6.60	6.120 ± 0.002	0.156	2.5
Morphological feature reference*						
	Cubital index (Goetze)		1.25-2.00	1.63		
	Width of tergite 4: T4 (mm)		2.04-2.60	2.32		
	Length of proboscis (mm)		5.75-6.50	5.90		

Explanation: *morphological feature reference for the middle-European breed valid for the Northern line in Poland (52)

Tab. 3. Morphological characteristics of middle-European bees of the 'Northern M' line with distinction between the leading apiary and the collaborating apiaries, conforming to organizational schemes of programs for protection of middle-European bees

Area	Feature	Number	Range	Mean ± se	Standard deviation	Coefficient of variability
Leading apiary	Wing length (mm)	1,967	8.57-9.90	9.401 ± 0.004	0.173	1.8
	Wing width(mm)	1,967	2.86-3.60	3.177 ± 0.002	0.096	3.0
	Cubital index (Goetze)	1,967	1.06-2.52	1.639 ± 0.005	0.241	14.7
	Width of tergite 4: T4 (mm)	1,967	1.90-2.56	2.266 ± 0.002	0.089	3.9
	Sum of tergites 3 & 4: T3 + T4 (mm)	1,967	4.08-5.21	4.731 ± 0.004	0.16	3.4
	Length of proboscis (mm)	1,967	5.60-6.55	6.076 ± 0.004	0.161	2.7
Collaborating apiaries	Wing length (mm)	2,324	8.84-9.92	9.376 ± 0.004	0.167	1.8
	Wing width (mm)	2,324	2.91-3.54	3.235 ± 0.002	0.078	2.4
	Cubital index (Goetze)	2,324	1.04-2.73	1.733 ± 0.005	0.252	14.5
	Width of tergite 4: T5 (mm)	2,324	2.10-2.57	2.314 ± 0.001	0.062	2.0
	Sum of tergites 3 & 5: T3+T5 (mm)	2,324	4.44-5.40	4.816 ± 0.003	0.125	2.6
	Length of proboscis (mm)	2,324	5.71-6.60	6.158 ± 0.003	0.141	2.3
Morphological feature reference*						
	Cubital index (Goetze)		1.25-2.00	1.63		
	Width of tergite 4: T4 (mm)		2.04-2.60	2.32		
	Length of proboscis (mm)		5.75-6.50	5.90		

Explanation: as in Tab. 2.

for values of the cubital index. The coefficient for the cubital index was more than 4 times larger than values noted for bee sizes described by the width of tergite 4 and by the sum of widths of tergites 3 and 4. Values of the cubital index, the width of tergite 4 and the length of proboscis were compared to the references of morphological characteristics applicable to the 'Northern M' line. The conclusion was that mean values of the features determined by the authors' research were contained within the range set by the morphological feature reference (Tab. 2). Mean values of the width of tergite 4 and the length of proboscis were larger than the corresponding values of the reference by 3.7% and only the mean width of tergite 4 was smaller by 1.2%.

The program for the protection of genetic resources of middle-European bees of the 'Northern M' line is being implemented based on the breeding material maintained by the leading apiary and by the collaborating apiaries. Accordingly, this division was addressed in the elaboration of the results (Tab. 3). By comparing wing parameters of bees harvested from both types of apiaries, the study demonstrated that only the mean length of wings of bees from the leading apiary was larger by 0.3% than the mean length of wings of bees from the collaborating apiaries. On the other hand, bees harvested from the collaborating apiaries had wings wider by 1.8% and the cubital index larger by 5.7% than bees from the leading apiary. The remaining

Tab. 4. Mean values of morphological characteristics of bees of the middle-European breed of the 'Northern M' line

Apiaries	Wing length (mm)	Wing width (mm)	Cubital index (Goetze)	Width of tergite 4: T4 (mm)	Sum of tergites 3 & 4: T3 + T4 (mm)	Length of proboscis (mm)
	Mean	Mean	Mean	Mean	Mean	Mean
Leading	9.409^{A}	3.191^{a}	1.646^{A}	2.281^{a}	4.761^{a}	6.079^{A}
Collabor.	9.366^{B}	3.185^{b}	1.689^{B}	2.277^{b}	4.757^{b}	6.094^{B}

Explanation: means with different superscript letters differ significantly a, b – at $p \leq 0.05$; A, B – at $p \leq 0.01$

Tab. 5. Dependencies between features of middle-European bees of the 'Northern M' line harvested from the leading apiary and from the collaborating apiaries

	Wing length	Wing width	Wing aspect ratio	Cubital index	Width of tergite 4: T4	Sum of tergites 3 & 4: T3 + T4	Proboscis length
Wing length	1						
Wing width	0.458	1					
	< .0001						
Wing aspect ratio	0.195	–0.782	1				
	< .0001	< .0001					
Cubital index	0.085	–0.108	0.178	1			
	< .0001	< .0001	< .0001				
Width of tergite 4: T4	0.224	0.322	–0.200	–0.045	1		
	< .0001	< .0001	< .0001	0.0035			
Sum of tergites 3 & 4: T3 + T4	0.284	0.358	–0.196	–0.044	0.856	1	
	< .0001	< .0001	< .0001	0.0039	< .0001		
Proboscis length	0.205	0.362	–0.256	–0.131	0.186	0.178	1
	< .0001	< .0001	< .0001	<.0001	< .0001	< .0001	

parameters related to the bee size – the mean width of tergite 4, the mean sum of widths of tergites 3 and 4 and the mean length of proboscis – were also larger for collaborating apiaries: by 2.1, 1.8 and 1.35%, respectively.

Mean values of morphological characteristics of bees harvested from the leading apiary and from the collaborating apiaries separately, and in general for the whole the 'Northern M' line, were contained within the range of the applicable reference of morphological characteristics. The mean value of the cubital index of bees from the leading apiary was larger by 0.01 and almost identical with the mean from the reference. Likewise, the mean value of the cubital index for bees from the collaborating apiaries was also larger than the reference, by just 0.1. The mean width of tergite 4 for bees harvested from the collaborating apiaries was very similar: the difference was 0.01 mm while the difference for the leading apiary was 0.05 mm. Mean values of the length of proboscis for bees harvested from both the leading and the collaborating apiaries were larger than the mean from the reference by 3 and 4.4 per cent, respectively.

Differences between mean values of morphological characteristics between bees harvested from the leading apiary and from the collaborating apiaries were very small and ranged from 0.006 mm for the width of wings to 0.015 mm for the length of proboscis. Differences between the tested features were found to be significant or highly significant (Tab. 4). Bees harvested from the leading apiary had significantly longer wings (by 0.043 mm) than bees from the collaborating apiaries. In addition, bees from the collaborating apiaries also featured a significantly larger cubital index (by 0.044), significantly wider tergite T4 (by 0.004 mm) and respectively larger sum of widths of tergites T3 and T4 (by 0.004 mm).

The Pearson correlation coefficient was calculated to establish dependencies between the tested features (Tab. 5). Correlations for middle-European bees of the 'Northern M' line were estimated for the same number of degrees of freedom, df = 4290. All the dependencies turned out to be significant or highly significant. Correlation coefficients, r, took on similar values for the dependency between the sum of widths of tergites 3 and 4 on the one hand and the width of tergite 4 on the other hand ($r = 0.856$, $p < 0.01$). A negative correlation was found between the wing aspect ratio and the wing width ($r = –0.782$, $p < 0.01$). On the other hand, the dependency between the wing length and width was $r = 0.458$ ($p = 0.01$). Note the low correlation of the cubital index with the wing length and width, as well as the relatively low, but statistically important, correlation between both indexes, as of $r = 0.178$, p = 0.0001. It is interesting that there were low, but negative, dependencies between the wing aspect ratio and bee size expressed by the width of tergite 4 or the sum of widths of tergites 3 and 4.

The study investigated the variability of morphological characteristics of middle-European bees of the 'Northern M' line covered by the program for the protection of genetic resources of bees.

The cubital index is the particularly important morphological feature that is used as a basis for evaluation of membership of a population in a specific sub-species/breed of bees. According to the author's research, the values of the cubital index for middle-European bees of the 'Northern M' line, both with and without the split into the categories of apiaries (leading and collaborating), were 1.690, 1.639 and 1.733, respectively, comparable to results obtained by other researchers. Rutter et al. (44) measured values of the parameters for the Danish population of bees 1.56-1.88 and (17) identified the values for Germany 1.69. By comparing the Russian population 1.54 (2) and the Lithuanian one 1.81-1.90 (33) to the author's own results, it can be seen that the two populations had higher values of the cubital index within the range of 6% to 12%, respectively, and, at the same time, lower values within the range of 7% to 16%. The values of the cubital index for bees of the 'Northern M' line from the collaborating apiaries were very close to the values for bees from France: 1.76 (31) and 1.78 (10) for Brittany and 1.70-1.88 (9) for Cavennes. An almost identical mean value of the cubital index was measured in Austria: 1.721; the value was just 0.7% smaller than the corresponding value obtained as part of the author's research for bees of the 'Northern M' line harvested from the collaborating apiaries. The populations described (42) showed a higher mean cubital index: 1.84, i.e. 12% larger than that for bees from the leading apiary, 6% larger than for bees from the collaborating apiaries, and 9% larger than for all bee families of the 'Northern M' line.

Mean values of the length of proboscis for all the examined bees of the 'Northern M' line, taken together and separately (taking account of the division in connection with the protection program) for bees from the leading apiary and for bees from the collaborating apiaries, are 6.120, 6.076 and 6.158 mm, respectively. Results of the author's research confirm the values determined (41), 5.8-6.4 mm, and (44), 5.95-6.19 mm. However, the mean values of the length of proboscis given in the study are smaller than the results obtained by (14) by 0.22 mm for bees from the collaborating apiaries, by 0.14 mm for bees from the leading apiary and by 0.18 mm for all bees of the 'Northern M' line. Likewise, the differences for bees described by (42), 6.44 mm, are 0.36, 0.28 and 0.320 mm, respectively.

The mean sums of widths of abdominal tergites 3 and 4 for bees from the leading apiary, bees from the collaborating apiaries and for the 'Northern M' line (considered without organizational splits) obtained as part of the author's research were 4.731, 4.816 and 4.777 mm, respectively. The results are consistent with those obtained by other researchers: 4.798 mm for (2), 4.66-4.84 mm for (33) and 4.522-4.676 mm for (44). Consistency of these results was validated by comparing sizes of bees originating from Poland, defined by

Tab. 6. Mean values of the cubital index obtained by various authors for middle-European bees including those of the 'Northern M' line covered by the program for protection of genetic resources in Poland

Author	Cubital index	
	Goetze method	Alpatov method [%]
Gromisz and Bornus 1971	1.658*	60.30
Gromisz 1972	1.626*	61.50
Gromisz 1981	1.628*	61.40
Gromisz and Płatek 1999	1.600*	62.50
Rostecki 2009		
– Line 'Northern M'	1.86	53.76
Author's research		
– Line 'Northern M'	1.69	59.17**

Explanations: *index values determined by the Alpatov method converted into the Goetze method using (38), **index values determined by the Goetze method converted into the Alpatov method using (38)

Tab. 7. Mathematical and morphological models of bee populations for selected Polish breeds (12)

Feature	Breed of bees		
	Middle-European	Carniolan	Caucasian
Width of tergite 4	z = 24.2718x – 57.1845	z = 24.2718x – 55.8252	z = 24.2718x – 54.4175
Length of proboscis	z = 10.2042x – 62.3980	z = 10.2042x – 65.9184	z = 10.2042x – 71.3878
Cubital index	z = 0.311x – 19.082	z = 0.311x – 15.925	z = 0.311x – 16.983

Tab. 8. Values of morphological characteristics covered by the reference for the 'Northern M' line of bees computed in accordance with mathematical and morphological models for the middle-European population

Line	Area	Feature			$\bar{y}$
		Width of tergite 4: T4 z	Length of proboscis z	Cubital index z	
Northern M	Leading apiary	–2.1846	–0.3973	–0.1070	0.8963
	Collab. apiaries	–1.0196	0.4395	–1.1362	0.8651

Explanations: $z = (x - \bar{x})/s$; $y = |z|$; $\bar{y} = 1/n = |(x_{\downarrow}1 - \bar{x}_{\downarrow}1)/s| + ... + |(x_{\downarrow}n - \bar{x}_{\downarrow}n)/s|$ (6)

Tab. 9. Comparison of consistency of ranges and mean values of morphological characteristics for the 'Northern M' line of bees to references applicable in Poland

Line	Area	Feature/Consistency with the reference											
		Cubital index				Width of tergite 4				Length of proboscis			
		Range	+/–	Mean	+/–	Range	+/–	Mean	+/–	Range	+/–	Mean	+/–
Northern M	Leading apiary	1.06-2.52	–	1.639	+	1.90-2.56	–	2.266	+	5.60-6.55	–	6.076	+
	Collab. apiaries	1.02-2.73	–	1.733	+	2.10-2.57	+	2.314	+	5.71-6.60	–	6.158	+

Explanation: + consistency with the reference; – inconsistency with the reference

the sum of widths of tergites 3 and 4, established by (19), 4.73-4.90 mm, (37), 4.78 mm, and by the author.

According to research carried out in the 1960s and 1970s, middle-European bees including those of the 'Northern M' line were larger. The mean sum of tergites 3 and 4 was 4.86 mm according to (5) and 4.85 mm according to (22). Considering widths of tergite 4 of bees in more recent years – 2.356 mm according to (19) and 2.292 mm according to the authors' research on the 'Northern M' line – values of the parameters were getting smaller, which is indicative of the existence of a dwarfing trend in middle-European bees. Mean values of the cubital index obtained in years 1971-1999 by (18, 22, 23) are consistent with values obtained as part of the authors' research for bees of the 'Northern M' line (Tab. 6). The value of the mean cubital index found by (38) was larger than that obtained as part of the authors' research (Tab. 6). A comparison of the mean length of proboscis from the authors' research for the 'Northern M' line, 6.120 mm, to results obtained by (5), 6.238 mm, (19), 6.120-6.230 mm, (18), 6.115 mm, (22), 6.149 mm, and (23) supports a claim that the parameter has remained fixed since the 1960s. Polish bee breeding programs use mathematical and morphological models tailored for individual breeds (Tab. 7). Models for middle-European bees are also used for determining the consistency of morphometric parameters of bee lines covered by programs for protection, such as the 'Northern M' line.

Based on the results shown in Table 8, the conclusion is that the 'Northern M' line bees harvested from the leading apiary show sufficient similarity to the model, although the "z" values are smaller than –2.1. The negative "z" values for all the categories of apiaries show a deviation towards "small" bees, so they corroborate the existence of the dwarfing trend in middle-European bees in Poland. By comparing values of the mean probability factor $\overline{y}$ ($0 < \overline{y} < 3$) for the three features (the width of tergite 4, the length of proboscis and the cubital index), the study shows that bees of the 'Northern M' line, harvested both from the leading apiary and from the collaborating apiaries) are very similar to the reference population, which is demonstrated by the value of $\overline{y} < 1$.

Based on Table 9, the conclusion is that mean values of the cubital index, the width of tergite 4 and the length of proboscis for the 'Northern M' line, both for the leading apiary and for the collaborating apiaries, are consistent with the reference for middle-European bees. Moreover, the study shows that except the width of tergite 4 for the collaborating apiaries the ranges of these features are inconsistent with the reference.

The study compared correlation coefficients between bee sizes expressed by the sum of widths of abdominal tergites 3 and 4 and by the remaining parameters: the length and width of the wing, the cubital index and the length of proboscis obtained by the authors and by Bornus (5). According to the authors' research, the coefficient of correlation between the wing width and the bee size is highly significant: r = 0.358 ($p < 0.01$). It is higher than that determined by (5): r = 0.242 for F0.01. Likewise, the "r" ratio of the wing length and the bee size is different from that determined by (5). Bornus (5) obtained a low but significant coefficient for this relationship, r = 0.063 (F0.05), while in the authors' research it was: r = 0.284 ($p < 0.01$). The dependency between the cubital index and the bee size was very low and negative, though highly relevant: r = –0.044 ($p < 0.01$). On the other hand, the value found by (5) was higher, also negative and highly relevant: r = –0,230 (F0.01). In turn, dependencies between the bee size and the proboscis length, both in the authors' research and in the finding of (5), were very small: r = 0.178 ($p < 0.01$) and r = 0.013 (F0.05), respectively, though significant and highly significant.

Conclusions:

1. The bees of the 'Northern M' line display characteristics of middle-European bees.
2. The program for protection of genetic resources of middle-European bees of the 'Northern M' line was implemented in accordance with the assumptions.
3. The morphological characteristics of the 'Northern M' line were consistent with the references applicable to *Apis m. mellifera*.

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