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Morphometric Characterization of Honey Bee, *Apis mellifera* Linnaeus, Inbred Lines in Korea

Olga Frunze, Yong Soo Choi, Dong Won Kim, Bo Sun Park, Hee Geun Park and Eun Jin Kang*
Department of Agricultural Biology, National Institute of Agricultural Science, RDA. Wanju 55365, Korea

국내 서양종꿀벌 순계의 형태적 특징

올가프런제 · 최용수 · 김동원 · 박보선 · 박희근 · 강은진*
농촌진흥청 국립농업과학원 농업생물부

ABSTRACT: The A, C, F colonies of *Apis mellifera ligustica* Spin. and D, V colony of *Apis mellifera caucasia* Gorb. bees were collected from 2005-2007. Consequently, inbred lines were derived from the bees of original colonies by matting in the isolated island with due regard for pure breeding. This project helps in the selection of colonies with higher production capacity, aiming to improve honey and royal jelly production and breeding programs. Twenty-three standard morphological traits of honeybee were evaluated, and samples were compared with the data of the two original subspecies. The result suggested that 8 traits partly preserved in bees of inbred lines, and the bees from *A. m. ligustica* preserved more traits than bees from *A. m. caucasia*. Among the studied inbred lines, the F line is distinguished by an increase in leg parameters, considered as a favorable phenotypic trait of inbred lines. Importantly, bred of beelines in the same area can be classified as remote and isolated areas. Therefore, we observed differences of inbred lines with the origin subspecies in description acquired with morphometric characteristics as a result of adaptation, breeding, purebred individual lines used as an important resource for breeding novel cross-breeding colonies.

Key words: *Apis mellifera* L., Morphometry, Inbred line, Bee breeding

초 록: 이탈리안벌인 A, C, F계통과 코카시안벌인 D, V계통을 2005년부터 2007년까지 국내에서 수집하였다. 수집한 계통은 육종을 위해 격리된 섬에서 근친교배를 통해 순계로 분리하였다. 이 연구는 꿀, 로열젤리 다수확계통 선발에 있어 개체군 선발과 육종 효율을 높이기 위해 수행되었다. 23 개의 형태학적 특성을 평가하고 두 아종의 기존 데이터와 비교한 결과, 이탈리안벌 순계계통은 코카시안벌 순계 계통과 달리8개의 특성이 기존의 이탈리안벌과 유사해 더 많은 특성이 보존되고 있음을 알 수 있었다. 또한 국내에서 유지되고 있는 순계들은 타 지역의 동일 계통과 차이를 보여 분리된 순계의 형태적인 특징이 확인되었다.

검색어: 꿀벌, 형태적 특징, 순계, 육종

Previously, several studies have been identified 27 subspecies of *A. mellifera* L. based on morphometric characteristics (Garnery et al., 1993; Ilyasov and Kwon, 2019), but this subspecies of bees is not present in South Korea's nature. The honey bee has been reared in Korea since the early 1900's, and today the number of beehives reached approximately 2,000,000 (Kim et al., 2015). Honey bees produce honey, royal jelly, propolis, pollen, and also mediate pollination of plants. The role of honeybees to the nature are tremendous. Further, the increase in production of beekeeping was performed when beekeepers began to breed not only the local species *Apis cerana* F. but to the introduced *A. mellifera* L. too (Jung and Cho, 2015; Lee, 2019). However, *A. mellifera* problems include a lack of genetic diversity, resulting in reduced populations, and regional limitations. In addition, parasites, pathogens, and pesticides have contributed to their decline. However, the genetic resources of honey bees

***Corresponding author:** kangeunjin1@korea.kr
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are very limited in Korea. National Institute of Agricultural Science (NIAS) Rural Development Administration (RDA) established in 1995 in order to apiary breed lines of *A. mellifera* from purebred colonies . The A, C, F colonies of *Apis mellifera ligustica* Spin. and D, V colony of *Apis mellifera caucasia* Gorb. bees were collect from 2005 to 2007 (Kim et al*.,* 2015*)*. Consequently, inbred lines A, C, F and D, V were derived from the bees of original colonies by mating in island with due regard the pure breeding. Lee et al. (2014) evaluated the triple crossed hybrid honeybees from six inbred lines on the honey collection, hibernation, and hygienic behavior. Kim et al. (2015) examined hygienic behaviors in six inbred lines A, C, D, E, F, G of *A. mellifera* and have stressed issues related to the value of hygienic behavior to colony health. Lee et al. (2017) selected a crossbreed for the high yield of royal jelly during the period of nectar flow and non-nectar flow. Lee et al. (2019) reported the morphological characteristics of high royal jelly producing crossbred bees, chemical compositions of royal jelly, development of hypopharyngeal gland.

Despite extensive work on the breeding and study of crossbred bee colonies, breeding lines, control of the breed morphometric traits of bees in introduction conditions was not carried out.

Ruttner (1998) used the multivariate statistical analysis of standard morphometry data and defined some ecotypes within a geographical region. So Alattal et al. (2014) studied the morphometric affiliation of the honey bees of Saudi Arabia, and related them to reference samples of 7 subspecies in the Oberursel Data Bank. Recent studies of bees compared the accuracy of both morphometric and geometric methods and showed different results (Tofilski, 2008; Kandemir et al., 2011; Alattal et al., 2014; Bustamante et al., 2020). The one study concludes that the success of the geometric method found the best accuracy of the results of standard morphometric data. So, there are no recommendations for choosing a method for the morphometric study. However, Kekeçoğlu et al. (2020) showed the problem of hybridization Anatolian honeybee in Turkey using geometric and standard morphometric techniques. In addition, Kirpik et al. (2010) showed the differences between two subspecies, *Apis mellifera caucasia, Apis mellifera remipes,* and a hybrid form of *A. mellifera* L. using morphometric characterization and gel electrophoresis of total protein. Consequently, extended studies have used the microsatellites and mitochondrial DNA restriction site polymorphisms to investigate which subspecies have contributed to mixed populations (Clarke et al., 2001; Suppasat, 2007; Kirpik et al., 2010; Brandorf et al., 2012; Ilyasov et al., 2019). Furthermore, many surveys based on allozyme and mt DNA variation support the findings of Ruttner (1988), Kandemir et al. (2000, 2006). Therefore, the standard morphometric method of bees and evaluating the degree of hybridization and promiscuous mating among the different honeybee races are important subject (Kandemir et al., 2000, 2006; Ruttner, 1988; Kekeçoğlu et al., 2020) and remains to be studied.

In this study, we examined twenty-three standard morphological characters in five inbred lines of *A. mellifera* at the NIAS in South Korea and first compared with the standard data of the two original subspecies, *A. m. ligustica* and *A. m. caucasia*. Moreover, it is worth mentioning that our inbred lines have been imported long ago to Korea and have been bred in the same area as can be classified as remote and isolated areas. Therefore, we observed inbred lines with the base subspecies in description acquired morphometric characteristics as a result of adaptation, breeding, purebred individual lines used as an important resource for breeding novel cross-breeding colonies.

Materials and Methods

Honey Bee Breeding Lines

The A, C, F colonies of *Apis mellifera ligustica* Spin. and D, V colony of *Apis mellifera caucasia* Gorb. bees were collected in 2005–2007 (Table 1). Consequently, inbred lines A, C, F and D, V were derived from the bees of original colonies by artificial selection with due regard for the introduction and pure breeding (Ruttner, 2006). The honey bee breeding lines have been controlled through the production of the queen from

Table 1. The origin of honeybee colony *A.mellifera* L. in Korea

Colony	Subspecies	Color	Trait
A	*A.m.ligustica*	Dark-brown	Gentless
C	*A.m.ligustica*	Light-brown	High fertility
D	*A.m.caucasia*	Dark-brown	High fertility
F	*A.m ligustica*	Brown	High fertility
V	*A.m.caucasia*	Dark-brown	High fertility

a selected colony and matting using by mating in isolated apiary in island Wido.

Collection of Honey Bee Samples

Samples of adult worker bees were collected from five *A. mellifera* L. in November 2019 at the apiary in Korea placed in NIAS, and preserved in 70% ethanol (Alpatov, 1948a; Ruttner, 1988). 50–65 worker bees of each sample were morphometrically analyzed.

Morphometric Analysis

Each bee was studied by 23 external morphometric characters according to the classical method (Ruttner, 1988): length of the body, width of abdomen, length of abdomen, length of tergite 2, width of tergite 2, length of tergite 3, width of tergite 3, distance between apodeme tergite 3, length of tergite 4, width of tergite 4, distance between apodeme tergite 4, length of leg, length of head, width of head, length of the forewing, width of the forewing, cubital index Ci Alpatov %, cubital index Ci Goetze, length of the antenna, length of proboscis, tarsal index %, hamuli of the wing, color. The study provides the value of two variants of the cubital index for the possibility of comparison with the data of researchers. The leg length was taken as the sum of the length of tarsus, coxa, femur, tibia, metatarsus.

In total, more than 1,250 measurements were obtained for each colony, a total of more than 6,250 for the population. The bee was dissected, the measurements were carried out using a Leica MZ16 A stereomicroscope and the TCapture computer program.

Statistical Analysis

All data were expressed as Means ± Standard Deviations and analyzed using the SPSS statistics software (ver.25), MS Excel with the XLSTAT application, program R, WinClada program with the Nona application.

The assessment of data on the discrimination model of bees by the method of multidimensional scaling (MDS) and method Soft k-means in the R program was carried out. The most significant characteristics of each beeline were identified when constructing phylogenetic trees in the WinClada program with the Nona application. All data were evaluated by descriptive statistics for normal distribution to obtain a numerical description of the morphometric traits of bees. The method of one-way analysis of variance (ANOVA) and posthoc Tukey's test was used to compare parameters 3 more colony bees. Student's t-test was used to compare the 2 groups. Differences were considered significant at P-values < 0.05. All techniques allow us to reliably distinguish the lines of bees from each other. The classification of inbred lines *A. mellifera* L. and origin colonies *A. m. ligustica*, *A. m. caucasia* carry out used the hierarchical cluster analysis Ward's method (Legendre and Legendre, 2012).

Results and Discussion

Inbred lines A, C, F from the bees of original colonies of *A. m. ligustica* and D, V lines from *A. m. caucasia* evaluated by using the morphometric method.

Morphometric Differences in Inbred Lines of Honeybees

The method principal component analyses used to study morphometric characters (Bustamante et al., 2020). But the results from PCA included only 2 - 3 main characters from twenty-two traits in this study and PCA rejected. For this reason, the discrimination of five inbred lines was carried out by Multidimensional scaling. The goal of its classical MDS-given pairwise dissimilarities, reconstruct a map that preserves distances. Each point is the coordinate of the distance, calculated from 22 morphometric parameters.

One line of bees was extracted from the two-dimensional map (Fig. 1). This scatter plot revealed that line F had formed distant clusters. So, line V located on the other side totals the cloud from line F. Method Soft k-means correctly identified 100% of inbred line F and partly line V from a total cloud of line A, C, D. The MDS data grouping model is confirmed by clustering the method Soft k-means (Fig. 2) (Dunn, 1973; Bezdec, 1976). This result (cMDS, method Soft k-means) confirmed in other methods of analyses using the construct the phylogenetic tree in other programs WinClada (NONA application) (Fig. 3).

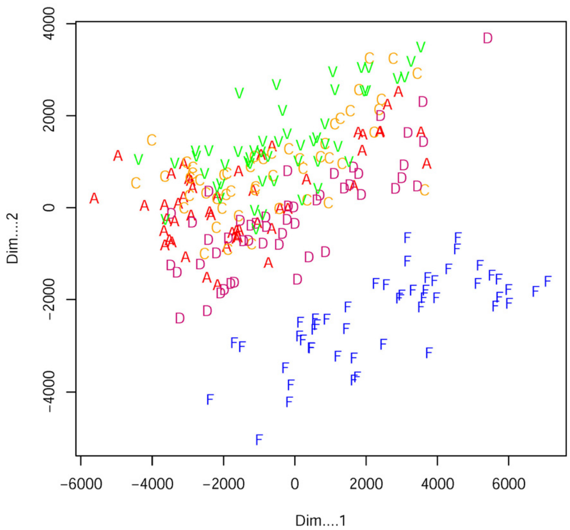

Fig. 1. Two-dimensional map of inbred honey bee lines using the classical scaling algorithm on twenty-two morphometric characters (classical MDS). Bees of line F are observed in separate cloud.

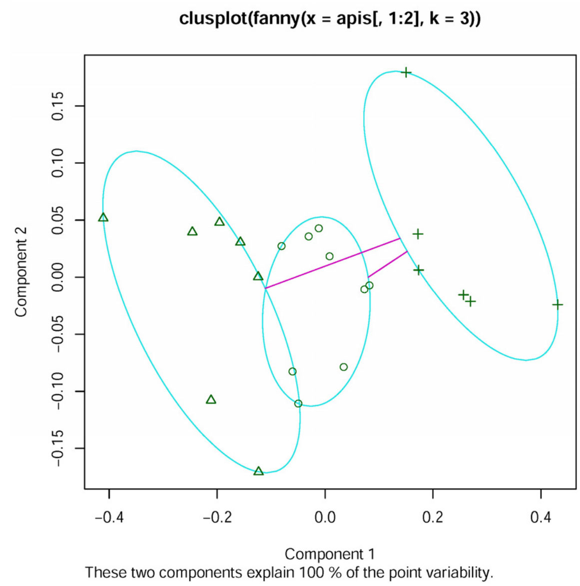

Fig. 2. The discrimination model the dataset of honey bee lines on clouds using methods Soft k-means (Dunn, 1973; Bezdek, 1981). + △ ○ designations of samples distributed in different clouds based on the results of calculations methods Soft k-means. The MDS data grouping model is confirmed by clustering.

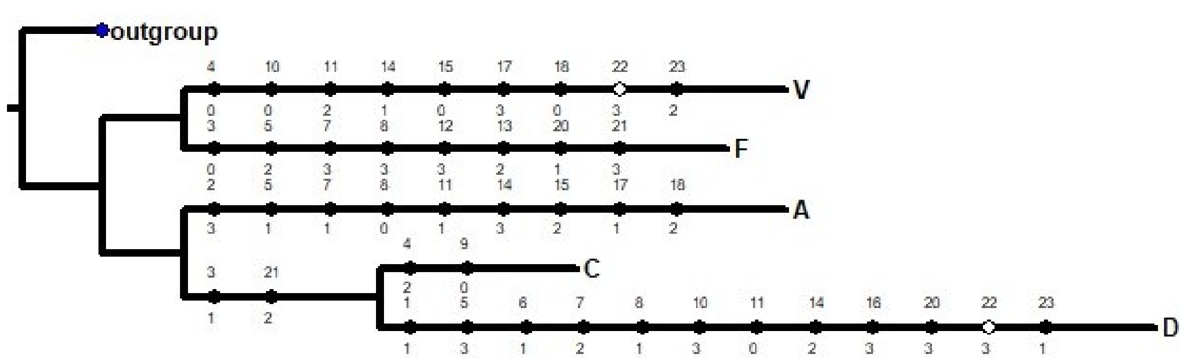

Fig. 3. Phylogenetic tree based on the morphometric characteristics of breeding lines of bees *A.mellifera*, software WinClada with NONA. Outgroup is a group with no severity of characters. Above the line is the number of the characters (Table 2, 3), under the line is the degree of expression of different characters from 0 to 3. Characters 23 shows the color of the individual: 0 - yellow, 1 - there are individuals with both black and yellow, 2 - black. The color is divided into 2 groups: the first includes bees of lines V and F, the second - A, C, D.

Characteristics of the Inbred Beelines

Honeybee lines were evaluated together by the phylogenetic program WinClada (NONA application). Lines F and V were distinguished from lines A, C, D (Fig. 3). After this separating morphometric characters of each beeline were verified statistical methods.

The lines of bees A, C, D have large body sizes (Table 2). At the same time, line A bees are characterized by a wide width of head and abdomen, which increases the volume of the abdomen, which is necessary for honey productivity (one-way ANOVA, posthoc Tukey's test, p=0.0001).

Line C bees are characterized by the minimum length of tergite 2, the physiological significance of which may be insignificant.

Bees of line D stand out with an increased length of tergite 2, which provides an increased volume of the abdomen, also have a wider forewing and hamulies that ensure flight stability in conditions strong winds and have the longest proboscis. Researchers showed that in the mountain regions of Transylvania

Table 2. Morphometric characters of the studied lines of bees A, C, D

	Line	A		C		D		Significant
	Characters	Mean	SD	Mean	SD	Mean	SD	
	n	65		63		62		
1	Length of body, mm	14,57	0,89	14,45	0,73	13,99	1,01	NS
2	Width of abdomen, mm	**4,66****	0,18	4,50	0,18	4,57	0,22	*
3	length of abdomen, mm	8,63	0,77	8,34	0,62	8,29	0,84	NS
4	Length of tergite 2, mm	2,33	0,11	**2,29****	0,13	2,32	0,10	*
5	Width of tergite 2, mm	7,82	0,23	7,73	0,31	**8,02****	0,22	*
6	Length of tergite 3, mm	2,15	0,09	2,15	0,09	2,12	0,09	NS
7	Width of tergite 3, mm	8,74	0,19	8,72	0,24	8,83	0,21	NS
8	Distance between apodeme tergite 3, mm	**4,50****	0,17	4,59	0,14	4,56	0,15	*
9	Length of tergite 4, mm***	2,08	0,10	2,03	0,11	2,07	0,09	NS
10	Width of tergite 4, mm	8,22	0,20	8,25	0,23	8,32	0,20	NS
11	Distance between apodeme tergite 4, mm	4,39	0,12	4,44	0,15	**4,37****	0,16	*
12	Length of leg, mm	11,13	0,31	10,98	0,27	11,31	0,28	NS
13	Length of head, mm	3,01	0,20	2,94	0,24	3,16	0,14	NS
14	Width of head, mm	3,92	0,09	3,87	0,08	3,90	0,06	NS
15	Length of forewing, mm	9,26	0,22	9,29	0,15	9,34	0,18	NS
16	Width of forewing, mm	3,09	0,07	3,12	0,07	**3,17****	0,08	*
17	Cubital index (Ci) Alpatov %	41,79	6,71	41,01	7,22	41,24	8,00	NS
18	Ci Goetze	2,46	0,41	2,53	0,54	2,51	0,47	NS
19	Length of antenna, mm	4,42	0,07	4,44	0,08	4,48	0,11	NS
20	Length of proboscis, mm	6,10	0,24	6,09	0,19	**6,34****	0,20	*
21	Tarsal index %	56,59	3,53	58,40	5,26	57,83	3,99	NS
22	Hamuli of wing	20,72	1,49	21,14	1,75	**22,42****	1,83	*

SD - Standard Deviation from the mean.
23th character "body color" is not shown in the table and is used only in dendrogram.
Table 2 shows (*) the significant characteristics of bee lines within one group, isolated on a phylogenetic dendrogram (Fig. 3):
**a trait that significant distinguishes the bee lines A, C, D. Used one-way ANOVA, posthoc Tukey's Test, α=0.05.
***a trait that common to bees of all the A, C, D, F, V.
4,66 - the morphometric traits identified as a result of phylogenetic analysis, which have a statistically reliable difference in the allocated group.

worker proboscis was longer 6.21 mm than that in lower regions 5.99 mm (Marghitas and Paniti-Teleky, 2008). Importantly, that proboscis length may predict which bee species undergo population declines due to global change (Harder, 1985; Gerard et al., 2020).

The lines of bees V and F had 5 different characteristics with maximum or minimum traits and significantly different from each other in 9 listed parameters out of 23 (t-test, 95%). Bees of line V have shortened wings and tergites, which shows a change in flight characteristics compared to larger bees (Table 3). Bees of the F line have increased parameters of the legs and tergites. Like this the morphological modifications can also occur as a result of global warming (Gerard et al., 2020). Important, the fundamental interaction in ecosystems is the mutualism between pollinators and their plants (Ockendon et al., 2014).

The structural features of line F bees noted on the MDS plot, are expressed in an increased tarsal index (respectively) by 5.6 - 2.6% compared to bees of lines A and C, an elongated proboscis by 0.5%, increased by 2.3% of the length of tergites

Table 3. Morphometric characters of the studied lines of bees F, V

	Line	F		V		Significant
	Characters	Mean	SD	Mean	SD	
	n	50		65		
1	Length of body, mm	13,52	0,80	13,71	0,69	NS
2	Width of abdomen, mm	4,53	0,20	4,57	0,21	NS
3	length of abdomen, mm	**8,09**	0,73	8,51	0,21	**
4	Length of tergite 2, mm	2,33	0,12	**2,22**	0,11	**
5	Width of tergite 2, mm	7,88	0,25	7,77	0,23	NS
6	Length of tergite 3, mm	2,20	0,09	2,08	0,10	NS
7	Width of tergite 3, mm	**8,91**	0,20	8,67	0,25	**
8	Distance between apodeme tergite 3, mm	4,63	0,14	4,57	0,18	NS
9	Length of tergite 4, mm***	2,07	0,11	2,06	0,12	NS
10	Width of tergite 4, mm	8,26	0,21	**8,08**	0,25	**
11	Distance between apodeme tergite 4, mm	4,45	0,10	4,42	0,17	NS
12	Length of leg, mm	**12,76**	0,36	10,85	0,29	**
13	Length of head, mm	3,06	0,15	3,04	0,14	NS
14	Width of head, mm	3,86	0,06	3,88	0,06	NS
15	Length of forewing, mm	9,28	0,17	**9,06**	0,15	**
16	Width of forewing, mm	3,09	0,07	3,01	0,07	NS
17	Cubital index (Ci) Alpatov %	40,70	6,95	**44,28**	7,66	**
18	Ci Goetze	2,53	0,44	2,33	0,43	NS
19	Length of antenna, mm	4,40	0,09	4,38	0,08	NS
20	Length of proboscis, mm	6,13	0,50	6,02	0,40	NS
21	Tarsal index %	**59,96**	4,31	55,47	5,14	**
22	Hamuli of wing	20,97	1,52	**22,46**	1,89	**

SD - Standard Deviation from the mean
23 character "body color" is not shown in the table and is used only in dendrogram.
Table 3 shows (**) the significant characteristics of bee lines within one group, isolated on a phylogenetic dendrogram (Fig. 3):
**a trait that significant distinguishes the bee line F, V. Used unpaired t-test, 95%.
***a trait that common to bees of all the A, C, D, F, V.
8,91 -the morphometric traits identified as a result of phylogenetic analysis, which have a statistically reliable difference in the allocated group.

3 while maintaining the minimum body size.

Comparison of Honeybee Lines

Comparison of data of bees of lines A, C, F and D, V by 8 morphometric characters for bee breeds *A. m. ligustica, A. m. caucasia.* The data of origin bees taken from publications (Alpatov, 1948b; Ruttner, 1988). Used in the discussion of the terms 'stable' and 'unstable'. Phenotype traits are called stable if they are resistant to environmental factors (Kamshilov, 1972). The unstable (labile) characteristics are not resistant to environmental factors.

Stable Subspecies Traits in Inbred Lines of Bees

Researchers believe that the survival of a species through climate change, habitat loss, and ecosystem changes is due to their physiological tolerance limits, ecological characteristics (Williams, 2008; Yancan et al., 2019). Changing these characteristics makes it possible to survive and be fixed at the level of genotype, the phenotype of bees (Kekeçoğlu et al., 2020). Morphological characters that allow distinguishing the subspecies of bees from each other must be resistant to external environmental conditions (Kamshilov, 1972; Ruttner, 2006). Usually, 8 parameters are used (Figs. 4, 5). They have not previously been tested for stability under conditions of introduction.

Stable subspecies traits for inbred lines of bees (A, C, F) and original bees of *A. m. ligustica* were 4 traits: length of the forewing, length of proboscis, cubital index to Alpatov, to Goetze. These traits confirm the origin of the bees from the

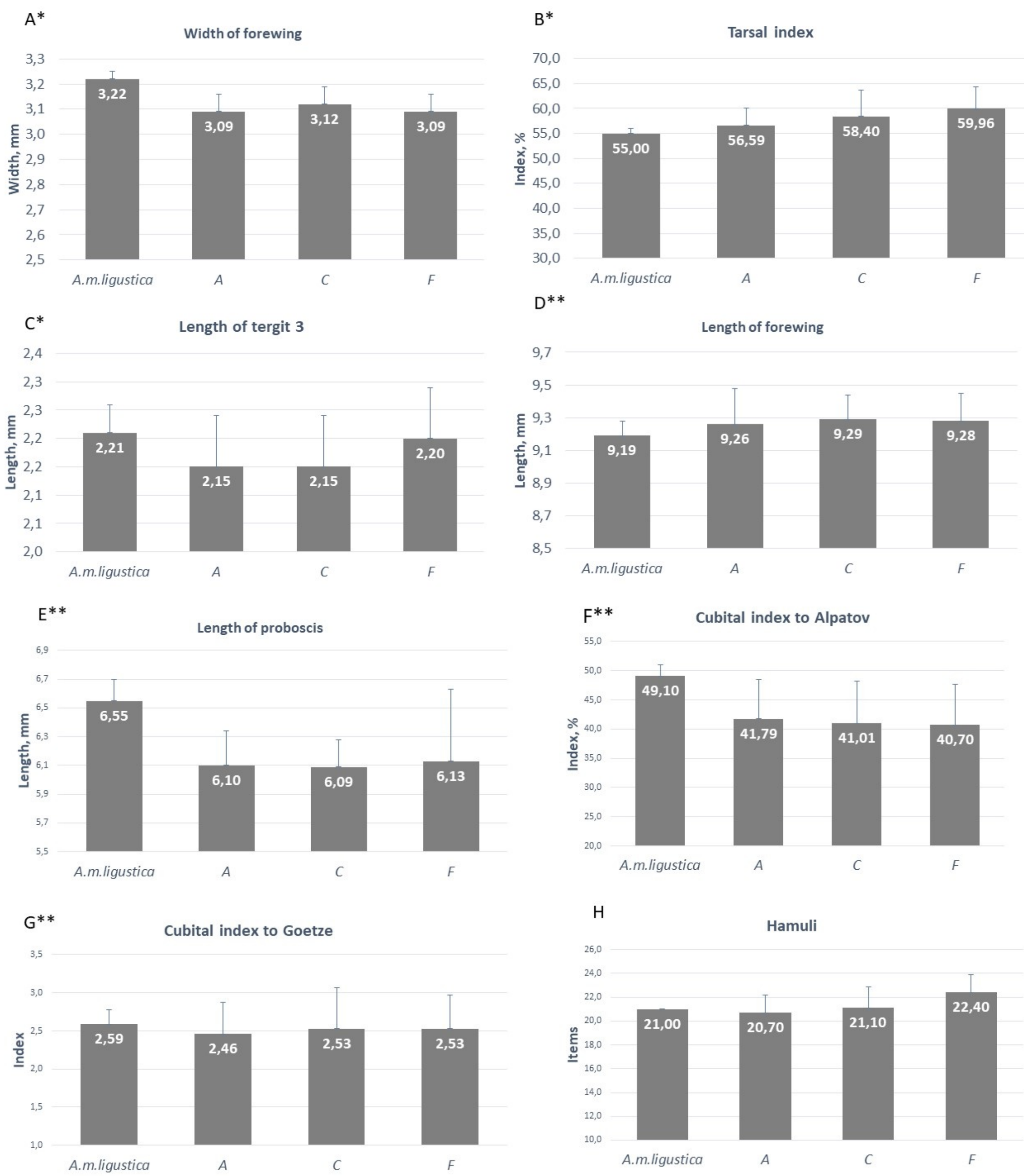

Fig. 4. Mean and Standard Deviation of 8 morphometric characters of the inbred bee lines A, C, F in comparison with the standard data of the initial bees *A. m. ligustica* (Shiabi et al., 2007; Ruttner, 1988; Lien, 2018). Used one-way ANOVA, posthoc Tukey's Test, α=0.05.*Unstable characters indicate significant differences: A* (F=7.20, df=231, P=0.0001); B* (bees lines A=bees *A. m. ligustica*, F=7.0551; df=182; P=0.0002), C* (F=4.0215; df=183; P=0.0084). **Stable characters don't indicate significant differences: D**, E**, F**, G**. H: standard data for *A. m. ligustica* are represented by average values and therefore was no made statistical comparison.

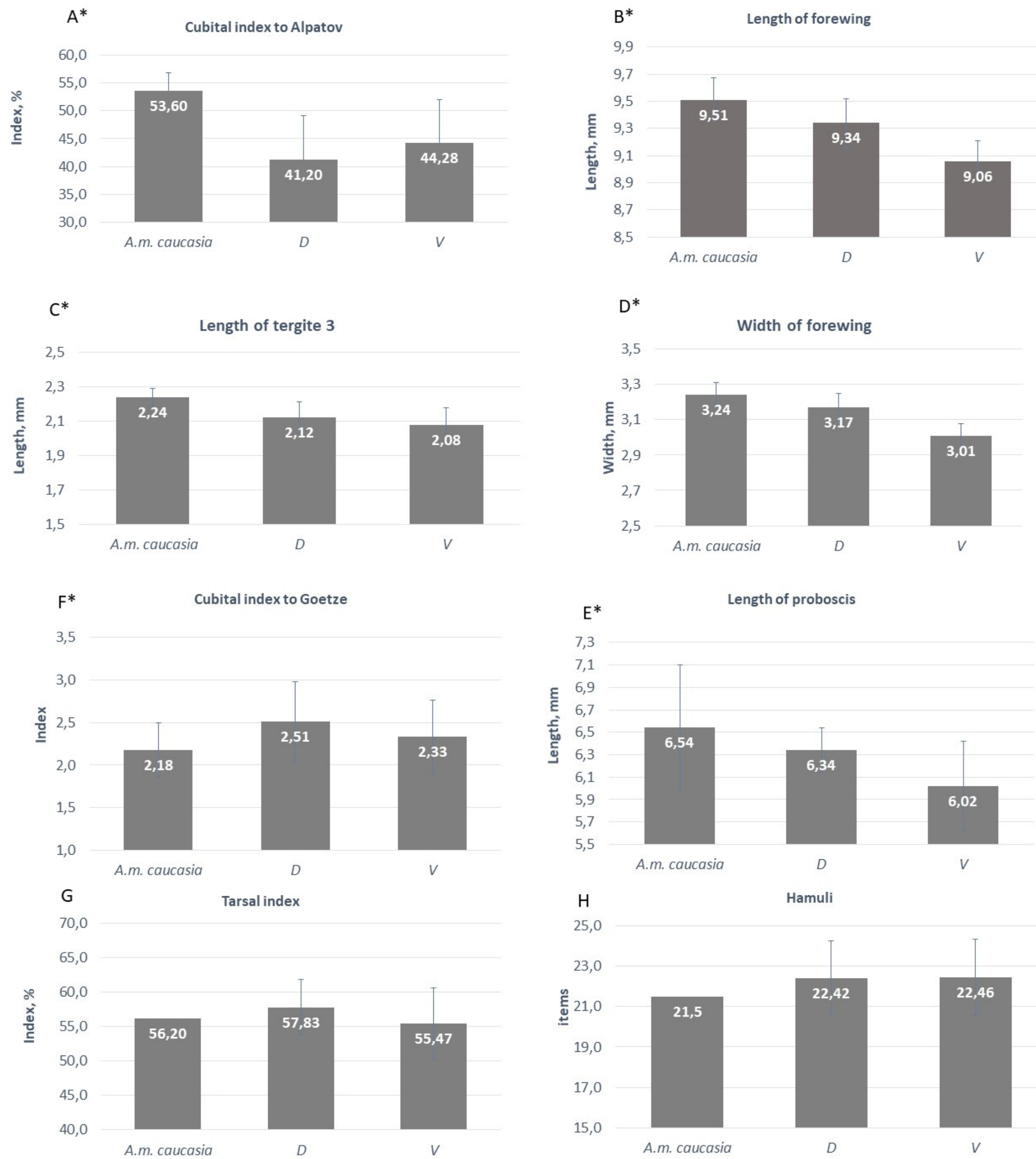

Fig. 5. Mean and Standard Deviation of 8 morphometric characters of the inbred bee line D, V in comparison with the standard data of the initial bees *A. m. caucasia* Gorb. Used one-way ANOVA, posthoc Tukey's Test, α=0.05. *Unstable characters indicate significant differences: A* (F=7.011177, df=170, P=0.00119); B* (F=5963.6; df=170; P=0.0000001), C* (F=895,9881; df=132; P=0.0009), D* (F=3358.6, df=170, P=0.0001), F* (F=4.903456, df=162, P=0.008574), E* (F=7.707876, df=120, P=0.00071) G, H: standard data for *A. m. caucasia* are represented by average values and therefore was no made statistical comparison.

corresponding subspecies. For bees of line D stable subspecies traits with bees of the original breed *A. m. caucasia* are not shown.

Cubital index to Goetze was stable for beelines A, C, F and original bees of *A. m. ligustica*. However, the instability of the cubital index Goetze is shown for *A. m. carnica* bees (Kekeçoğlu et al., 2020).

Bees lines A, C, F with initial genotype *A. m. ligustica* under conditions of introduction are more resistant to traits (4 out of 7, 57%) than bees with the *A. m. caucasia* genotype. Stable subspecies characteristics persisted for all years of breeding after the introduction.

Our study showed that the stability of subspecies characteristics of bees under the conditions of the introduction of *A. m. ligustica* is greater than that of *A. m. caucasia*. The study showed partial retention of subspecies traits in the studies introduced lines A, C, F *A. m. ligustica*.

The stability and instability of subspecies morphological characteristics of bees under conditions of introduction make it possible to assess the characteristics of the traits used for identification. The instability of morphometric traits is the result of hybridization and geographical variability and is studied by many scientists (Ken et al., 2003; Villa et al., 2009).

Unstable Subspecies Traits in Inbred Lines of Bees

The problem of preserving the genotype of bees is uncontrolled mating. After this bee has the unstable traits in the genotype or phenotype traits to compare to the control group in the study of local populations (Bouga et al., 2005; Chambo, 2016). In our study, there is no problem with mating, because *A. mellifera* L. bees are absent in nature and the import of subspecies of the bee into the country is limited. In addition, the use of mating in the isolated apiary in island allows controlling the quality of beelines. Therefore, unstable subspecies can be mainly the result of phenotypic variability.

Find three unstable characters out of 8 standard morphometric traits for each subspecies traits. Bees of colonies A, C, F differ from bees *A. m. ligustica* in subspecies characteristics: width of the forewing, tarsal index, length of the 3rd tergite. The least resistant all characters of bees in line D when compared with bees of subspecies *A. m. caucasia*. Kekeçoğlu et al. (2020) was found morphological deformation in the wings of worker bees *A. m. carnica*. Shown is a trait of common to both bred bees, the length of the 3rd tergite. However, this feature significantly distinguishes bees from the original subspecies.

Introducing *A. m. ligustica* bees, the parameter is the width of the forewing reduced, while the subspecies level of the length of the forewing and cubital index Goetze are preserved. Thus, a narrowing of the wing of the introduced bees of colonies A, C, F is observed. For the introduced *A. m. caucasia* bees, the length of the forewing traits is reduced, while the subspecies level of the width of the forewing and the cubital index Goetze are preserved. Thus, a shortening of the wing of the introduced bees of colonies D and V is observed. In previous studies, Kirpik et al. (2010) reported similar results for *A. m. caucasia* bees.

Subspecies characters unstable showed phenotypic variability not typical for bees in natural habitat (3 for *A. m. ligustica* bees, all for *A. m. caucasia* subspecies bees). Based on this, we observe the example changes of subspecies traits of inbred beelines based on introduced bees. Their study confirmed the previous studies on honey bees that environmental factors have a major impact on morphological characteristics (Eischen et al., 1982; Milne and Pries, 1984; Stanimirovic et al., 2019).

The Classification of Inbred Lines and Origin Colonies

The classification of inbred lines *A. mellifera* L. and origin colonies *A. m. ligustica*, *A. m. caucasia* based on hierarchical cluster analysis Ward's method (Legendre and Legendre, 2012). It was shown that according to 8 subspecies morphometric characters, bees of the studied lines were allocated into a separate group (Fig. 6). This cluster distinguishes the lines of bees C and D, V and F as well as on Fig. 3. The configuration differentiation between the phylogenetic tree (Fig. 3) and cluster

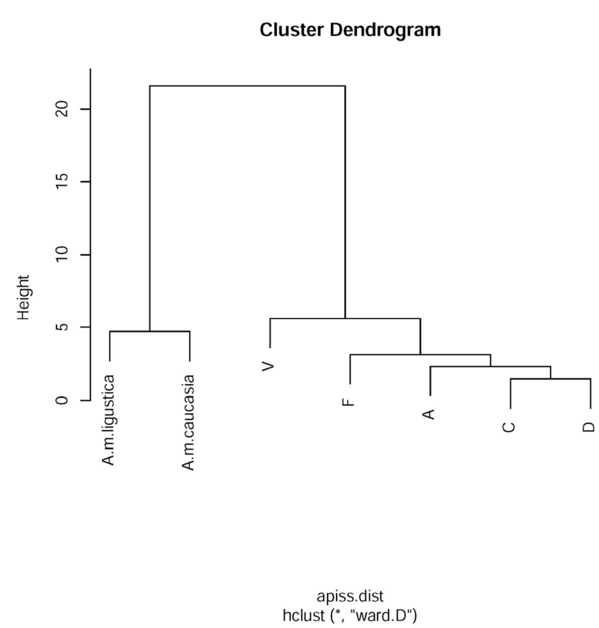

Fig. 6. The classification of inbred lines *A. mellifera* L. and origin colonies *A. m. ligustica*, *A. m. caucasia* based on hierarchical cluster analysis *Ward's* method (Legendre and Legendre, 2012). It was shown that according to 8 subspecies morphometric characters, bees of the studied lines were allocated into a separate group.

analyses (Fig. 6) showed alteration of lines of bees due to inbreeding in terms of introduction in comparison with the origin subspecies.

Morphometric Traits of Bees Associated with Productivity

Many researchers note the correlation of body measurements, bigger legs, and wings to honey yield (Milne and Pries, 1984; Szabo, 1988; Waddington, 1989; Kolmes and Sam, 1991; Mostajeran et al., 2006). Mostajeran et al. (2006) found that honey production was related to tongue length, fore wing length and width, hind wing length, leg length, femur length, tibia length, and metatarsus width. Edriss et al. (2002) indicated that honey production can be improved through the selection of the forewing width.

Lines of bees with high productivity are used in our study. At the same time, the traits indicated in the literature as productive characters partially coincide with subspecies, for example, wing parameters (length, width, indices). For bees, the wing parameters (length, width, cubital index Goetze) were the same in comparison with bees of the corresponding subspecies, and the tarsal index was increased. Since the high productivity of lines has been studied earlier, there are reasons to consider these signs as a general parameter of high productivity of bee lines with both honey and royal jelly productivity. Bees of lines with high honey productivity had large body sizes from (14.57 ± 0.89 mm) in line A to (14.45 ± 0.73 mm) in line C, which contributes to great opportunities for filling the honey crop of forage bees and as a result of the increased honey productivity of the line. So, among the morphometric traits associated with productivity, beelines A, C are characterized by increased body size, leg length, and tarsal index. The parameters of the wing (length, width, cubital index Goetze) are shown as general signs of high productivity of bees in all lines.

Moreover, it is worth mentioning that our inbred lines have been imported long ago to Korea and have been bred in the same area as can be classified as remote and isolated areas. Therefore, we observed an incomplete correspondence of inbred lines with the base subspecies in description acquired with morphometric characteristics as a result of adaptation, breeding, purebred individual lines used as an important resource for breeding novel cross-breeding colonies.

Bees, as the main pollinators of plants, represent a natural resource that needs to be carefully managed (Kekeçoğlu et al., 2020). The present study has emphasized the effect introduction on the morphometric subspecies traits of bees. Studies on the morphometric changes of lines of bees through the loss of their original traits have been showed. Hence, the findings of this study provide an evaluation of bee breeding resources for contribution to the current project and breed of new line Korean honeybee gen resources.

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저자 직책 & 역할

올가프런제: 국립농업과학원, 박사후연구원; 형태 분석 및 논문작성
최용수: 국립농업과학원, 연구사; 실험설계
김동원: 국립농업과학원, 연구사; 시료 채취 및 형태분석
박보선: 국립농업과학원, 박사후연구원; 실험수행 및 형태적 분석
박희근: 국립농업과학원, 연구사; 시료 채취 등 실험수행
강은진: 국립농업과학원, 연구사; 실험설계 및 논문작성

모든 저자는 원고를 읽고 투고에 동의하였음.

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