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Morphometric Characterization of Newly Defined Subspecies *Apis cerana koreana* (Hymenoptera: Apidae) in the Republic of Korea

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국내 토종벌(*Apis cerana koreana*) 아종의 형태적 특성 분석

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ABSTRACT: There has been much debate on the morphometric divergence between the recently identified *Apis cerana koreana* and *Apis cerana* honey bees. The aim of this study was to obtain phenotypic information that can be used to compare *A. c. koreana* data with other *A. cerana* subspecies data from open resources and determine breeding results on the basis of morphometric traits. To differentiate *A. c. koreana*, we investigated 22 classic morphological characteristics; royal jelly secretion; and the weight of workers, queens, and drones of *A. c. koreana* bred in Korea. To define the selection results, we used the geometric morphometric method. The artificially selected *A. c. koreana* secreted significantly more royal jelly (1.18 times) than the naturally selected *A. c. koreana*, which positively influenced the health of the colonies. These honey bees were identified more clearly with the geometric morphometric method than with the classic morphometric method, which is traditionally used to determine the subspecies. Large trends were noted for *A. c. koreana* on the basis of our results and literature from the 1980s regarding *A. cerana* sizes in Korea (tarsal index, length of forewing, and cubital index were measured). The cluster analysis revealed the proximity of *A. c. koreana*, *A. cerana* in China, and *A. c. indica* on the basis of eight classic characters, which, perhaps, relay the origin of the honey bees. The results of this study defined the morphometric responses of *A. c. koreana* honey bees to geographic isolation, climate change, and selection, which are important to identify, protect, and preserve honey bee stock in Korea.

Key words: *Apis cerana*, Classic morphometry, Geometric morphometrics, Subspecies discrimination, Honey bee

초 록: 새롭게 육성된 낭충봉아부패병 저항성 신품종 토종벌(*Apis cerana koreana*) 과 기존 농가에서 관행적으로 사육되는 토종벌 사이의 형태학적 차이를 육안으로 확연하게 구분하는 것은 어렵지만 본 연구에서는 신품종 토종벌(*A. c. koreana*) 을 기존 토종벌(*A. c. koreana*) 품종 및 계통 간 형태학적 비교를 통해 신품종 만의 특성을 결정할 수 있는 표현형 정보를 제공하였다. 신품종 토종벌(*A. c. koreana*)의 외부형질을 이용한 품종 특성은 22가지의 형태학적 특성을 기하학적, 형태학적 분석 방법을 적용하고 토종벌(*A. c. koreana*)의 로얄젤리 생산량, 일벌, 여왕벌, 수벌의 특성을 비교 분석하였다. 본 연구 결과, 신품종 토종벌(*A. c. koreana*)은 기존 토종벌과 앞날개의 길이에 차이를 보였으며, 중국의 동양종꿀벌(*A. cerana*)과 비교한 결과, 일벌은 몸무게, 혀의 길이, 앞날개의 길이 등의 값이 높았다. 또한, 신품종 토종벌(*A. c. koreana*)은 *A. cerana indica* 보다 두 가지 부위에서 형태학적인 차이를 보였다. 그리고 신품종 토종벌(*A. c. koreana*)은 로얄젤리를 다른 품종과 비교하여 많이 분비하여 봉군의 발육에 긍정적인 영향을 끼쳤다. 따라서 본 연구결과는 신규 육성 토종벌(*A. cerana*)에 대한 형태학적 분석 방법을 이용하여 품종을 분류하는데 도움이 될 것으로 기대한다.

검색어: 동양종꿀벌, 전통적 형태분석법, 기하학적 형태분석법, 아종

Apis cerana is known as an excellent pollinator of various economic crops with a wide foraging range (Wang et al., 2021). The native habitat of *A. cerana*, the Republic of Korea, is a peninsula that is separated by the ocean from other countries in

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the south, west, and east, having only a border with North Korea in the north region that restricts any random introduction of honey bees outside. The Republic of Korea is located on the east coast of the Eurasian Continent and adjacent to the Western Pacific. It shows climate characteristics with an annual mean temperature from 10 to 16°C that provided a successful environment for the vegetation of honey plants from April to November (https://www.weather.go.kr/eng/biz/climate_01.jsp).

The subspecies determination of honey bees in the Republic of Korea had a complicated path. In this location, Lew (1992) reported that the *A. c. indica* honey bee was kept in the first part 17^{th} century, but their subspecies traits were not described. Later in the 20^{th} century, the population of *A. cerana* species in Korea was not be identified to the subspecies level, because the general scientific recommendation was not accepted to estimate the *A. cerana* subspecies neither by morphometric or genetic methods (Lee and Choi, 1986; Kwon and Huh, 1992, Park et al., 2015). The majority of *A. cerana* subspecies have no clear taxonomic status by morphometric characters yet (Sarah et al., 2005; Radloff et al., 2010; Ilyasov et al., 2019, 2021; Proshchalykin et al., 2020). However, the differences between *A. cerana* honey bees from plains and hills regions were found more than in the north and south regions which were studied by morphological and biochemical characteristics (Lee, 1993). Moreover, the *A. cerana* honey bee in Korea (Seoul) had a distinct morphocluster out from *A. cerana* in Japan by the 12 morphometric characters (Radloff et al., 2005; 2010).

Unfortunately, in the first part of the 20th century in Korea the number and distribution of *A. cerana* colonies was decreased, because a number of *A. mellifera* colonies were introduced to increase the beekeeping resources (Ai et al., 2012).

Additionally, in Korea, the sacbrood virus (SBV) disease was the reason for more than 90% of eastern honey bees' mortality in 2008 (Choi et al., 2010; Vung et al., 2017). However, the *A. cerana* honey bee resistant to SBV was selected from the remaining honey bees and bred in Korea (Vung et al., 2020). Researchers utilized the hygienic behavior in honey bees to confer colony-level resistance against brood disease for artificial selection of *A. cerana* colonies (R, H) and checked the brood survival rate after colonies were SBV-inoculated (Vung et al., 2020). Also, some Korean beekeepers manage the *A. cerana* honey bees which survived by natural selection after extensive eastern honey bees' mortality.

These honey bees were identified for the first time by not morphometry, but by the genetic method. Ilyasov et al. (2018) used modern genomic technologies to discover and prove the taxonomic uniqueness of Korean honey bee subspecies. So, novel subspecies *A. c. koreana* resistant to SBV virus in Korea (Ilyasov et al., 2018), has recently been identified and it was uploaded to the DDBJ/Genbank database (AP018431). Scientists performed a comparative analysis of the complete mtDNA sequence of the Korean, Japanese, Chinese, and Taiwan *A. cerana* population and performed phylogenetic analysis (Ilyasov et al., 2018). So, report above confirmed the differences on genetic level of the *A. c. koreana* breed (Vung et al., 2020) out from other *A. cerana*. Although, the differences on the colony level between *A. cerana* honey bees was not studied in Korea, it was found between *A. mellifera* honey bees after breeding in closed population for 4 years by Szabo and Lefkovitch (1988).

Similar achievements were studied by morphometric methods in our research. To differentiate the *A. c. koreana*, we investigated the 22 classic morphological characteristics, the royal jelly secretion, the weight of workers, queen, and drone bees of *A. c. koreana* bred in the Republic of Korea. To define the result of selection, we additionally applied the geometric morphometric analysis. Specifically, the aim of this research is to provide phenotypic information that can compare *A. c. koreana* with other *A. cerana* subspecies from open resources and determine breeding effect by morphometric traits. This study provides the understanding of the *A. cerana* honey bees' morphometric response to the geographic isolation and selection. The characteristic of honey bees is important to identify, protect, and preserve of honey bee stock surviving in the new hazards arising in climate change cases.

Materials and Methods

Honey bees

A. c. koreana honey bees (R, H, RH) against sacbrood virus disease were bred by artificial selection on the breeding station using a method of pure breeding from 2015 year (Vung et al., 2020). The honey bees (L) of natural selection were keep in the breeding station without any treatments. The honey bee breeding lines have been controlled through the production of the queens from a selected colony and mate using the artificial insemination and later natural mating method by isolating station on the island.

The names of honey bee colonies and lines L, R, H, RH are at random. A total of 15 *A. c. koreana* colonies were sampled at the same time in July 2020 from the breeding station in National Institute of Agricultural Science (Wanju-gun) in the south-west of the Republic of Korea.

Classic morphometric method

Thirty-five worker bees were collected from each assigned colony and killed immediately with hot water (higher than 80°C) to extend their proboscises and were then preserved in 75% ethanol until use. 24-32 worker bees from each line were dissected and quantified for morphological analysis using the methods described by Ruttner (1988) and Carreck et al. (2013). A total of 22 characteristics associated with production performance were quantified from 107 sampled honey bees using a Leica MZ16 A stereomicroscope and the TCapture computer program.

For classic morphometric method, the twenty-two characters are the Length of body, Length of abdomen, Width of abdomen, Length of tergite 2, Length of tergite 3, Length of tergite 4, Length of head, Width of head, Length of proboscis, Length of tongue, Length of antenna, Tarsal index, Length of femur, Length of tibia, Length of metatarsus, Width of metatarsus, Length of forewing, Width of forewing, Length of hind wing, Width of hind wing, Ci Goetze, and the Hamuli of hind wing. Means of colony sample, standard deviation, and Coefficient of Variance (CV) were computed for each character from the samples. Eight morphological characters were common to the databases of *A. cerana* from Korea, China, India, Sri Lanka, Philippines, and Japan. This eight characters were the Cubital index, Length of proboscis, Length of femur, Length and Width of metatarsus, Length and Width of forewing, and the Length of tibia. Three morphological characters were common to the database of *A. cerana* honey bee studied earlier in Korea by Lee and Choi (1986). This three characters were the Tarsal index, Length of forewing, and the Cubital index. Multivariate statistical analysis of the classic morphometric data included Principal Components Analysis (PCA) to identify possible splitting of *A. c. koreana*. The hierarchical cluster analysis was done to identify the colonies within *A. c. koreana* and with data of *A. cerana* honey bees. The linear discriminant analysis carried out to determine the characters which had high linear discriminant function as result of highest contribution in determination of colonies, and defined the percentage of correct classification of colonies in *A. c. koreana* honey bees.

Geometric morphometric method

For the geometric morphometric method, the 50 right forewing images for every *A. c. koreana* R, H, L honey bee lines were photographed and stored in the computer. A total of 19 landmarks on the forewings were identified (Fig. 1). Tps files were prepared using tpsUtil 32, and landmarks were digitized

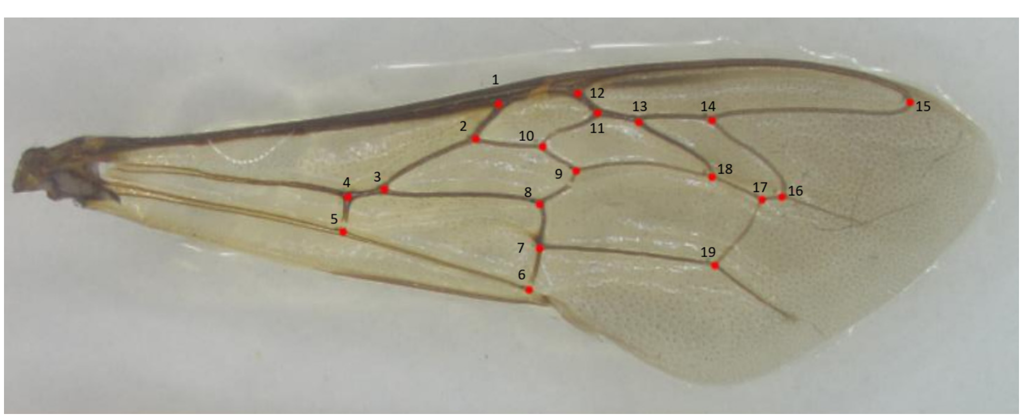

Fig. 1. Location of the nineteen landmarks on the right forewing of *Apis cerana koreana* considered in the geometric morphometric analysis.

into images using tpsDig232 (Ferdous and Armbruster, 2012). Later, landmarks were superimposed using a generalized least-square algorithm in MorphoJ (Klinberger, 2011; Berezin, 2019). This landmark-based morphometric method removes all non shape variation that can be attributed to differences in the location, orientation, and scale of the specimens (Koca and Kandemir, 2013). A multivariate analysis of variance (MANOVA) and pairwise tests were carried out on the landmark data by using MorphoJ in order to compare *A. c. koreana* honey bees. Superimposed x, y coordinate data were than used as the data set for multivariate statistical analysis of *A. c. koreana* honey bees. To assess the variation between the studied honey bees, multivariate statistical analysis (Canonical Variate Analysis, PCA) were carried out on geometrics morphometric data collected from the same set of samples taken for classic morphometric analysis.

Weights of *Apis cerana koreana* workers, queens, and drones and royal jelly secretion by worker bees

To study the weight, the live worker and drone bees were collected from *A. c. koreana* colonies in totals of 470 worker bees, 78 drones from queenright colonies, and 151 drones from laying worker colonies. The virgin queens were reared from *A. c. koreana* colonies accordingly to recommendations for *A. cerana* honey bees using queen cells on the frame (Vung et al., 2017). The queen pupae in cells (11 days from grafting larvae) were put in the queen cage individually and were placed in an incubator at 35^{o}. The next day the weight of the live virgin queens were measured by the scales. Fertile queens were replaced from queenright honey bees' colonies and measured immediately.

To study the royal jelly secretion, the standard methods for *A. mellifera* L. royal jelly research were used (Hu et al., 2017) with modification for *A. c. koreana* honey bees by Vung et al. (2017) from June to September in 2020. Frames with grafted larvae were removed from rearing colonies after 48 hours and taken to the bee breeding laboratory for royal jelly collection and measurements on the electric scale balance.

Statistical analysis

Data were analyzed by using the MS Excel with the XLSTAT (https://www.xlstat.com/en/), AtteStat application (http://offext.ru/library/science/development/87.aspx), program R, SAS Enterprise Guide 7.1 program. The one-way ANOVA and Tukey post-hoc test (in case for three and more groups) or t-test (in case for two comparing groups) provided the testing of the significant differences between the multiply means of the morphometric characters of honey bees. In all stages, the significant levels were set at $\alpha = 0.05$.

Results

Classic morphometric method

The group means, standard deviation, and CV of colony means for a total of 22 morphological characteristics were presented in Table 1. CV were calculated and shown the inconsistent variations among the different morphological characteristics of *A. c. koreana* honey bees (Table 1). The CV of Cubital index the Length of abdomen was the highest among all morphological characteristics, and the average CV was 13.165 and 10.370% respectively. By contrast, the CVs of other morphological characteristics did not exceed 9% and the Length of forewing had the lowest CV (1.719%). It should be noted that low CV demonstrates the extent of variability of morphometric characters in relation to the mean of the population, we suppose that morphometric characters with the low CV is the candidate to assess the breeding treatments as the most accurate characters.

To check breeding clusters, a linear discriminant analysis was used. Lastly, four groups were entered for the following 22 characters with definition of their discriminatory powers (Table 2). The results classified 100% of data for both *A. c. koreana* R (n = 25) and *A. c. koreana* H (n = 24), and 96.88% *A. c. koreana* RH (n = 32) with one data misclassifying into artificially selected *A. c. koreana* H group of honey bees; 92.31% of the naturally selected *A. c. koreana* L honey bees group (n = 26) but two data misclassified into *A. c. koreana* H group.

To test the equality of the groups means for the characters used in the discrimination function, Wilks lambda approximated by the F-statistic was determined. A significant difference between the means of the four groups was detected ($\lambda = 0.008$, $F_{66.00} = 14.80$, $P < 0.0001$). Next, the eight characters with scores more than 300 were extracted which could allow a comprehensive assessment of *A. c. koreana:* the Length of tergite 4, Length of antenna, Length of metatarsus, Length of hind wing, Length of tergite 3, Length of forewing, Width of head, and the Width of forewing (in decreasing order of the linear discriminant function) (bolded characters in Table 2).

Additionally, the hierarchical cluster analysis by these eight characters with high score of linear discriminate function (Fig. 2A) indicated that these can slightly discriminate *A. c. koreana* honey bees on branch of artificial (*A. c. koreana* R, RH, H) and natural (*A. c. koreana* L) selection. Selected *A. c. koreana* honey bees confirmed significant differences (one-way ANOVA, Tukey post-hoc test, λ=0.05) only in three morphological characteristics with high score of linear discriminant function. These characters were the Length and Width of forewing, and the Length of hind wing (Table S1). Although the breeding results of *A. c. koreana* honey bees were identified by these three traits, we could not differentiate the lines *A. c. koreana* by general 22 classic morphometric characters.

Besides, we compared the morphometric characters of *A. cerana* honey bees from different countries to define the peculiarities of *A. c. koreana* honey bees in Korea. *A. c. koreana* is distinct from other *A. cerana* (Fig. 2B) by the hierarchical cluster analysis based on our research and literature data of eight total morphometric characters (Table S2). *A. c. koreana* have been included in branch MI and did not match to MII and

Table 1. Means, standard deviation, and coefficient of variation of 22 morphological characteristics of *Apis cerana koreana* artificial (Ack R, H, RH) and natural (Ack L) selection in the Republic of Korea. Size measurements are presented in mm; the tarsal index is presented in percentage (%)

Characters	Ack L		Ack R		Ack H		Ack RH		CV, %	average Ack	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		Mean	SD
Length of body	12.926	0.892	13.786	0.562	13.746	0.511	13.501	0.609	4.768	13.490	0.643
Length of abdomen	6.538	0.910	7.510	0.532	6.921	0.702	7.066	0.763	10.370	7.009	0.727
Width of abdomen	4.031	0.222	3.944	0.176	3.971	0.267	3.993	0.156	5.148	3.985	0.205
Length of tergite 2	2.053	0.120	1.947	0.124	1.925	0.211	2.043	0.071	6.590	1.992	0.131
Length of tergite 3	1.879	0.100	1.840	0.066	1.916	0.085	1.960	0.090	4.491	1.899	0.085
Length of tergite 4	1.827	0.092	1.810	0.056	1.917	0.063	1.858	0.077	3.881	1.853	0.072
Length of head	2.793	0.219	2.820	0.152	2.901	0.181	2.679	0.212	6.826	2.798	0.191
Width of head	3.566	0.080	3.569	0.057	3.698	0.109	3.580	0.067	2.174	3.603	0.078
Length of proboscis	4.820	0.310	4.848	0.097	4.894	0.122	4.908	0.112	3.291	4.868	0.160
Length of tongue	3.085	0.299	3.169	0.065	3.161	0.105	3.186	0.068	4.260	3.150	0.134
Length of antenna	4.196	0.090	4.100	0.061	4.073	0.072	4.152	0.083	1.852	4.130	0.076
Tarsal index, %	55.398	4.755	57.016	3.810	56.797	3.419	56.936	4.751	7.400	56.537	4.184
Length of femur	2.304	0.134	2.329	0.083	2.368	0.063	2.375	0.107	4.119	2.344	0.097
Length of tibia	2.857	0.128	2.977	0.109	2.933	0.082	3.063	0.091	3.467	2.958	0.103
Length of metatarsus	1.909	0.097	1.814	0.061	1.870	0.062	1.909	0.133	4.684	1.876	0.088
Width of metatarsus	1.054	0.071	1.032	0.068	1.062	0.075	1.084	0.085	7.067	1.058	0.075
Length of forewing	8.387	0.212	8.691	0.134	8.682	0.123	8.739	0.123	1.719	8.625	0.148
Width of forewing	2.647	0.142	2.905	0.065	2.824	0.070	2.900	0.067	3.063	2.819	0.086
Length of hind wing	5.955	0.189	6.021	0.134	6.113	0.117	6.159	0.096	2.210	6.062	0.134
Width of hind wing	1.561	0.063	1.653	0.059	1.621	0.079	1.658	0.059	4.006	1.623	0.065
Ci Goetze	4.350	0.613	2.557	0.383	3.349	0.465	2.942	0.277	13.165	3.300	0.434
Hamuli of hind wing	17.731	1.282	18.320	1.249	18.417	1.840	16.844	1.629	8.413	17.828	1.500
n	26		25		24		32			107	

SD, standard deviation; Ci, cubital index; n, number of dissected honey bees; CV, coefficient of variation
Five characters of *A. c. koreana* honey bees in bold indicate no statistically significant differences (one-way ANOVA, P > 0.05).

Table 2. The component score of the linear discriminant function to define the morphometric characters which high and low contributions to the division of *Apis cerana koreana* honey bees

Variable	Ack H	Ack L	Ack R	Ack RH
Constant	-13864.000	-13632.000	-13374.000	-13848.000
Length of body	47.530	45.154	47.078	45.911
Length of abdomen	-28.365	-29.645	-27.297	-29.889
Width of abdomen	374.236	377.513	367.087	373.191
Length of tergite 2	89.608	91.519	88.815	100.069
Length of tergite 3	769.070	758.757	726.258	766.620
Length of tergite 4	1159.000	1141.000	1105.000	1127.000
Length of head	14.639	11.246	16.663	1.052
Width of head	403.924	377.726	369.101	362.339
Length of proboscis	698.855	707.413	676.976	706.714
Length of tongue	-420.525	-435.155	-397.275	-426.392
Length of antenna	1092.000	1123.000	1090.000	1103.000
Tarsal index, %	8.238	8.176	8.154	8.151
Length of femur	89.515	74.189	92.481	88.972
Length of tibia	-46.119	-45.536	-28.228	-24.354
Length of metatarsus	986.256	1001.000	960.962	986.218
Width of metatarsus	-562.663	-560.475	-554.860	-541.340
Length of forewing	518.006	505.144	516.140	525.215
Width of forewing	365.686	349.865	379.274	377.402
Length of hind wing	924.514	915.426	898.238	926.143
Width of hind wing	-331.814	-323.972	-287.108	-294.584
Ci Goetze	167.860	175.099	158.680	163.202
Hamuli of hind wing	45.811	45.852	43.365	43.444

Eight characters with high scores for the linear discriminate function in bold in the row variable.

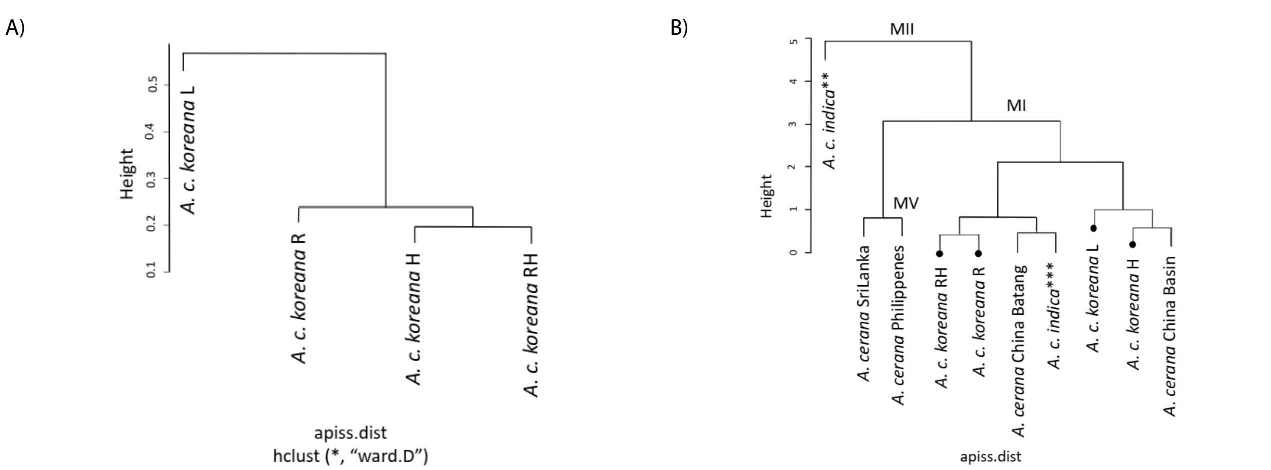

Fig. 2. Hierarchical clustering dendrogram of the morphology data (A) for eight traits with high scores of linear discriminant function (LDF): Length of tergite 4, Length of antenna, Length of metatarsus, Length of hind wing, Length of tergite 3, Length of forewing, Width of head, and Width of forewing of *Apis cerana koreana* honey bees; (B) eight traits of *Apis cerana* F. honey bees: Cubital index, Length of proboscis, Length of femur, Length of tibia, **Length** and Width of **metatarsus, Length and Width of forewing.** Characters in bold have high LDF. MI, "Northern cerana;" MII, "Himalayan cerana;" MV, "Philippine cerana" (Radloff et al., 2010). The data of *Apis cerana* Sri Lanka (Szabo, 1990), *Apis cerana indica* (Baskaran, 2016; ***Ibrahim and Chandel, 2019; **Rajkum et al., 2020); *Apis cerana* Philippines (Tilde et al., 2000), *Apis cerana* Basin and *Apis cerana* Batang (Zhou et al., 2016; Zhu et al., 2017), and *Apis cerana japonica* (Ruttner, 1988) were used.

MV morphoclusters, according to the classification of *A. cerana* honey bees (Radloff et al., 2010). So, we found the proximity *A. c. koreana, A. cerana* in China, and *A. c. indica* honey bees in one brunch by the eight total classic characters which, perhaps, relay the origin of the *A. cerana* honey bees. In this study, means of the Length of proboscis, Length of forewing *A. c. koreana* were higher than *A. c. indica* (Rajkumari et al., 2020), and *A. cerana* Sri Lanka (Szabo, 1990), but lower than *A. cerana* in China (Zhu et al., 2017). Moreover, the mean Cubital index of artificially selected *A. c. koreana* (RH, R) honey bees was lower than those of other *A. cerana* honey bees in the research. Hence, *A. c. koreana* honey bees differed from *A. cerana* subspecies by honey bee morphology due to geographic isolation and adaptation to breeding environment in the Korean peninsula.

Additionally, to test a result of selection by the differences of the group *A. c. koreana* with *A. cerana* studied by Lee and Choi (1986) in the same place in Korea, we studied and found that there were increase in the Tarsal index, Length of forewing, and the stability in the Cubital index at 56.537 and 53.530%, 8.625 and 8.274 mm, 3.30 and 3.31 respectively.

Geometric morphometric method

Shape coordinates were superimposed to successfully eliminate the size effect, which was apparent from Procrustes analysis. The deformed wireframe was drawn on the shape among three lines of honey bees (*A. c. koreana* R, H, L) to interpret shape changes that support the Relative Warp (RW) analysis. This illustrated deformation in shape from the control group *A. c. koreana* L that corresponds to selected positions in the ordination. Canonical Variate Analysis (CVA) extracted Mahalanobis and Procrustes distances among groups found to be highly significant ($p < 0.0001$) (Tables 3, 4). Canonical variate 1 and canonical variate 2 explained 50.710% and 36.014% of the total variance, respectively, while canonical variate 3 explained only 13.276% of the total variance. Classification results of CVA indicated that all the specimens of each group were allotted to their respective groups. So, wing vein geometric morphometrics were able to clearly identify differentiation between samples of *A. c. koreana* honey bees R, H, L by CVA (Fig. 3).

Table 3. Mahalanobis distances based on the geometric morphometric method

Lines and colony	R	H	L
R		< 0.0001	< 0.0001
H	5.340		< 0.0001
L	5.453	5.630	

Pair-wise matrix of Mahalanobis distances among groups (lower diagonal) and *p*-value (upper diagonal) of canonical variate analysis.

Table 4. Procrustes distances based on geometric morphometric method

Lines and colony	R	H	L
R		< 0.0001	< 0.0001
H	0.0213		< 0.0001
L	0.0210	0.0177	

Pair-wise matrix of Procrustes distances among groups (lower diagonal) and *p*-value (upper diagonal) of canonical variate analysis.

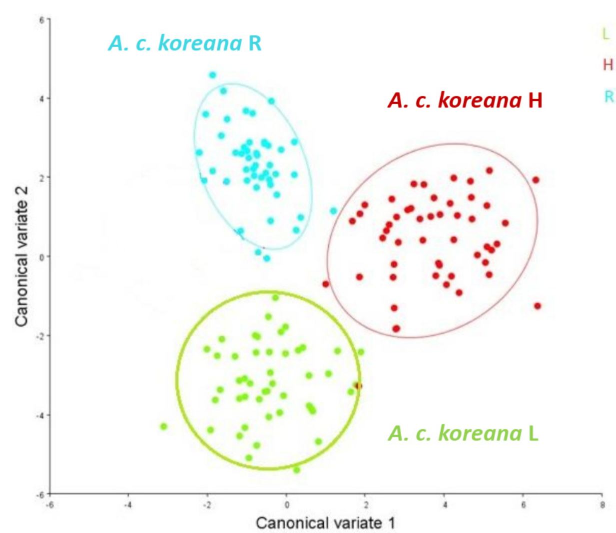

Fig. 3. Differentiation of artificial *Apis cerana koreana* R and H and natural *A. c. koreana* L selected honey bee colonies on the basis of the geometric morphometry of 19 landmarks using canonical variate analysis.

Body weights of *Apis cerana koreana* workers, queens, and drones and royal jelly secretion by worker bees

The differences in Body weight of honey bees can be caused by many factors such as temperature and species of plants. The

Table 5. Means weights of *Apis cerana koreana* workers, queens, and drones and amount of royal jelly secretion by the worker bees

	m(Wb), mg	m(RJ), mg	m(vQ), mg	m(fQ), mg	m(D), mg	m(wbD), mg
mean	69.29	123.41	150.42	185.33	118.17	91.56
SD	15.32	25.91	15.02	20.64	12.58	11.69
min	46.00	65.00	121.00	166.00	79.00	60.00
max	116.00	171.00	178.00	213.00	139.00	121.00
CV, %	22.11	20.19	11.13	9.98	10.65	12.76
n	470	46	24	6	78	151

SD - standard deviation, CV - coefficient of variation, Wb - worker bee, RJ - average weight of royal jelly, vQ - virgin queen, fQ - fertile queen, D - drone from queenright colony, wbD - drone from laying worker colony

mean weight of worker bees *A. c. koreana* was lower than *A. cerana* in China but higher than *A. cerana* in Indonesia, showing 69.29 ± 15.32 mg in Korea, 73.95 ± 0.55 mg in China, and 46.060 ± 7.015 mg in Indonesia, respectively (Yue et al., 2017; Widiawati et al., 2020).

The means weight of drones from the queenright colony were higher than that of the drones from laying worker colony *A. c. koreana,* drones *A. cerana* in China (Phokasem et al., 2021), providing 118.17 ± 12.58, 91.56 ± 11.69, and 103.56 ± 12.51 mg, respectively. The means weight of virgin and fertile queen *A. c. koreana* were 150.42 ± 15.02 and 185.33 ± 20.64 mg, respectively. The weight of fertile queen *A. c. koreana* in this research was higher than that reported for fertile queen bee *A. c. koreana* (169.00 ± 0.16 mg) at the same location in Korea (Vung et al., 2018) (Table 5).

Despite that the *A. cerana* honey bee is not used as royal jelly producers, the amount of secreted royal jelly by worker bees influences the honey bee colony development such as the size of the honey bees (Szabo and Lefkovitch, 1988). The weight of body of the artificially and naturally selected *A. c. koreana* honey bees did not have significant differences (t-test, $P > 0.01$), but the royal jelly secretion of the artificially selected honey bees was higher (t-test, $P < 0.01$) than naturally selected honey bees, providing 132.96 ± 20.70 (n = 24) and 113.00 ± 25.35 mg (n = 22), respectively.

Discussion

The results presented here show that geometric morphometric yielded marginally better discrimination of the artificial and naturally selected *Apis cerana* honey bees than classic morphometry, which also was marked by Tofilski to differentiate *Apis mellifera* honey bee subspecies (2008). Better discrimination on geometric morphometric traits compared to classic morphometry is not surprising, as the former used 19 variables as opposed to the 8 variables from 22 classic morphometry. Last 8 characters were selected by discriminant analysis for the classification model only in the variables that contribute to the discrimination. This solved the problem, when a large number of variables are used, although some of them become redundant because of the correlations between them (Tofilski, 2008). However, from eight extracted characters confirmed three with significant differences (one-way ANOVA, Tukey post-hoc test, $\alpha = 0.05$) were the Length of hind wing, Length of forewing, and the Width of forewing. It can indicate the low identification of artificial and naturally bred *A. cerana koreana* honey bees by the classic morphometric method which was survived bred after mortality in 2008. Also, the significantly intensive royal jelly secretion were found at artificially bred against naturally survived and bred *A. c. koreana* honey bees in Korea, indicating the high potential development of artificially bred honey bees.

The aim of this research is to provide phenotypic information that can compare *A. c. koreana* with other *A. cerana* subspecies and determine breeding results by morphometric traits. Increase trends were noted for the *A. c. koreana* honey bee based on results of current research and on literature of the 1980s concerning *A. cerana* sizes in Korea measured by the Tarsal index, Length of forewing, and the Cubital index. The cluster analysis revealed the proximity of *A. c. koreana, A. cerana* in China, and *A. c. indica* honey bees by the eight total classic characters which, perhaps, relay the origin of the *A. cerana* honey bees. If the *A. cerana* worker bees in China having higher values of the Length of proboscis, and Length of forewing than *A. c. koreana* honey bees, but last one had higher these two morphometric characters, than *A. c. indica* honey bees. The differences between artificially and naturally selected and bred *A. c. koreana* honey bees were found by 19 landmarks of the geometric morphometric method, but not by 22 characters of the classic morphometric method. However, the artificially selected honey bees secreted significantly more royal jelly than naturally selected *A. c. koreana* honey bees, which positively influence the development of the honey bee colony through enhanced nutrition of the larva. The results of this study are helpful to elucidate the phenotypic evolution of *A. cerana* honey bees and provide the information for identification, protection, and preservation of honey bee stocks in Korea by analyzing the morphometric characteristic of newly defined subspecies *A. c. koreana* honey bees to. Also, this study expands our understanding of how honey bees will respond to natural and artificial selection in response to the new climate condition that we are faced with.

Supplementary Information

Supplementary data are available at Korean Journal of Applied Entomology online (http://www.entomology2.or.kr).

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