Two-Level Factor Analysis of Morphometric Characters of Honeybees Population Sampled (*Apis mellifera* L.) in Turkey

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Classical factor analysis assumes that sampling is independent observations. In morphometric researches, however, the data belonged to honeybee usually have hierarchical structure in which individuals are grouped within colonies within different localities and regions. The assumption of independence among observations is not realistic, because sampling units not share common environment, experiences and interactions. Multilevel factor analysis model is an appropriate methodological tool which has been proposed as an extension to confirmatory factor analysis models for analyzing data with hierarchical structure. In this study, we provide a didactic step-by-step guide to exploratory multilevel factor analysis of morphometric characters of honeybees. The results illustrated that the within and between level factor structure of morphometric characters conformed to expectation which is factor solution with three factors of wing, leg and vacular.

Key words: Multilevel factor analysis, honeybee, morphometry, intraclass correlation

Türkiye Bal Arıları Populasyonu Morfometrik Karekterleri Örnekleminde

(*Apis mellifera* L.) İki Seviyeli Faktör Analizi

Klasik factor analizi örneklemenin bağımsız gözlemlerden oluştuğunu varsayar, oysa örnek verileri genellikle hiyerrarşik yapıdadır. Bu yapı içerisinde bal arıları farklı bölgelerde ve farklı lokasyonlarda koloniler halinde yaşarlar. Bu nedenle gözlemler arasında bağımsızlığın düşünülmesi gerçekçi değildir. Çünkü örneklenen birimler aynı bakım koşullarını ve aynı çevresel etkileri paylaşmazlar. Bu tür hiyerarşik yapıdaki verilerin analizi için çok seviyeli faktör analiz modeli önerilebilir. Bu araştırmada modeli bal arılarının morfometrik ölçüm sonuçlarına uygulayarak çok seviyeli faktör analizini basamak basamak anlatan bir model sunulmuştur. Beklenildiği gibi kanat, bacak, ve damar karakterlerinden oluşan üç factor yapısı oluşmuştur. Sonuçlar, koloniler arası ve koloni içi morfometrik faktör yapısının beklenen ile uyumlu olduğunu göstermektedir.

Key words: Çok seviyeli faktör analizi, bal arısı, morfometri, grup içi korelasyon.

Introduction

Classical factor analysis is applied multivariate statistical technique that is used to explore or confirm the underlying structure among variables. Factor analysis is, however, frequently applied to observational data for which the standard assumption of independence of the vectors of observations, simple random samples; that is not appropriate (Long, 1983). Because members within colonies are grouped or nested in larger organizational or geographical groups. Repeated measures data are also inherently multilevel, with repeated measurements on an outcome measure nested within each individual. As a consequence the data can be regarded as a multistage or cluster sample from different hierarchical levels (Hox, 1993). It is often reasonable to assume that the observations within a group are more similar, because the subjects share common environment, experiences, interactions within group homogeneity, or between-group variation, can be modeled by a regional and group-level correlation structure; at the same time an individual-level correlation structure was considered (Muthén, 1991, 1994). Such a development runs parallel with the extension of

the ordinary regression to random coefficient (mixed) models for clustered observations, since factor analysis models can be formally regarded as ordinary regression models with unknown regressors (Longford and Muthén, 1992). Estimation theory (Muthén, 1989, 1990, 1991; Longford and Muthén, 1992; Muthén, 1994) as well as factor score estimation (Lee and Poon, 1995) that are developed for Multilevel Confirmatory Factor Analysis (MCFA), a special case of Multilevel Structural Equation Modeling (MSEM), can be extended to Multilevel Exploratory Factor Analysis (MEFA) (Reise et al., 2005; Cheung and Au, 2005). Honeybees in a colony may be regarded as family members of a huge family and so they share same environment as well as similar electrophoretic properties. This sampling design and hierarchical structure of honeybees cause dependency among sampling units and hence produce correlated observations. Ignoring dependencies in the data, traditional factor analysis may be lead to unreliable morphometric factor structure for the honeybees because the hierarchical data can bias parameter estimates such as factor loadings. As a consequence, applying multilevel factor analysis to this kind of data is more convenient way to explore morphometric structure of honeybees.

Multi-level analyses at colony, locality (population) orsubspecies level have different interpretations in terms of the microevolutionary processes acting at these levels. For example, partitioning among and between-colony components of covariance is related to quantitative genetics, so it can be observed which amount of variation in traits and in their covariance is determined by genes or environment.

In this study, we investigate morphometric variation in different honeybee (*Apis mellifera* L.) populations in Turkey as an illustration of multilevel factor analysis. Sampled honeybees are nested in hives (colonies) and localities.

Material and Method

Data and Sampling

Honeybee samples were collected from 180 hives in 55 different locations from different geographic regions of Turkey. Turkey is divided into seven geographic regions differing both in climatic conditions and in geological structure. In first stage, 55 different locations from seven geographic regions were selected. In the second stage, 180 colonies were randomly selected from these locations. The last step consisted of randomly selecting 5 honeybees from each of 180 colonies. Total of 900 honeybees were collected in this manner, however, group level sample size was 180 while individual level sample size was 888, since measurements couldn't provided for 12 honeybees.

Eleven morphometric characters were measured. Four for the forewings [Forewing Length (FW), Forewing Width (FW), Cubital A (a), and Cubital B (b)] two for the hind wings [Hind Wing Length (HWL) and Hind Wing Width (HWW)], four for the leg [Femur Length (FL), Tibia Length (TL), Metatarsus Length (ML), and Metatarsus Width (MW)] and an additional one for a proboscis character [Proboscis Length (PL)]. Using these variables, the morphometric structure of honeybees is investigated.

Generally, there are three approaches to analyzing data with nested structures: Disaggregation, aggregation, and multilevel models (Hofmann, 1997; Stapleton, 2006; Hox, 1993; Lee, 1990; Raudenbush and Bryk, 2002; Cheung and Au, 2005; Hofmann, 1997; Klein and Kozlowski, 2000; Cheung and Au, 2005; Hox, 2002; Raudenbush and Bryk, 2002).

Statistical Analysis (The Four-Step Process)

Muthén (1991, 1994) elaborated an explicit set of procedures to follow when conducting conventional MSEM (Confirmatory Multilevel Factor Analysis (CMFA)). Translated into the context of Exploratory Multilevel Factor Analysis (EMFA), these steps are as follows. First, conduct an ordinary exploratory factor analysis of the total covariance (or correlation) matrix, S_T. This "incorrect" analysis is based on treating all the observations as independent. The objective of the first step is to obtain a rough sense of the underlying factor structure. The second step is to estimate the Intra Class Correlation (ICC) for each item. This step establishes whether MFA is necessary. The third and fourth steps, respectively, are to estimate a within and between group covariance (or correlation) matrices, S_{PW} and S_B, and

Table 1. Total, within and between correlation matrices

Tablo 1. Toplam, grup içi ve grup arası korelasyon matrisleri

	FWL	FWW	a	b	HWL	HWW	FL	TL	ML	MW	PL
Total correlation matrix											
FWL	1.000										
FWW	.300	1.000									
a	.041	.046	1.000								
b	.085	.110	-.072	1.000							
HWL	.216	.252	.026	.105	1.000						
HWW	.171	.302	.011	.049	.388	1.000					
FL	.097	.115	-.024	.047	.196	.120	1.000				
TL	.107	.187	-.004	.115	.154	.139	.340	1.000			
ML	.074	.094	.087	.025	.096	.095	.182	.162	1.000		
MW	.104	.104	-.044	.043	.123	.076	.251	.292	.209	1.000	
PL	.028	.084	-.027	.048	.126	.052	.062	.079	-.002	.059	1.000
Variance	.102	.013	.003	.001	.042	.005	.015	.027	.012	.009	.523
Pooled within-sample correlation matrix											
FWL	1.000										
FWW	.205	1.000									
a	.055	.089	1.000								
b	.060	-.014	-.166	1.000							
HWL	.024	.056	.061	-.014	1.000						
HWW	-.005	.104	.008	-.058	.201	1.000					
FL	-.015	-.023	-.016	.012	.068	.016	1.000				
TL	-.024	.010	-.069	.052	.014	.046	.269	1.000			
ML	.023	.025	.088	-.028	.046	.027	.151	.085	1.000		
MW	.022	.030	-.020	.029	.060	.033	.145	.227	.160	1.000	
PL	.003	.018	-.041	-.068	.022	.028	.015	.047	.006	.640	1.000
Variance	.086	.006	.003	.001	.024	.003	.010	.018	.008	.006	.305
Estimated between-sample correlation matrix											
FWL	1.000										
FWW	.598	1.000									
a	-.030	-.040	1.000								
b	.176	.290	.227	1.000							
HWL	.778	.482	-.063	.307	1.000						
HWW	.762	.588	.023	.262	.700	1.000					
FL	.466	.308	-.052	.117	.404	.320	1.000				
TL	.547	.441	.200	.245	.389	.325	.479	1.000			
ML	.255	.199	.088	.136	.182	.234	.243	.322	1.000		
MW	.367	.203	-.121	.067	.222	.156	.445	.416	.306	1.000	
PL	.101	.163	.007	.245	.270	.092	.138	.133	-.014	.052	1.000
Variance	.016	.006	.001	.0005	.018	.002	.005	.009	.004	.003	.219
ICC	.156	.506	.167	.328	.424	.340	.347	.335	.320	.364	.419

Note: ICC = intraclass correlation computed from Equation (2).

conduct factor analysis for each matrix separately. In the following, we illustrate each of these four steps with real honeybee (*Apis mellifera* L.) data set.

Results and Discussion

Step 1: Factor analysis of the total correlation matrix

The data matrix consists of 888 individual honeybees drawn from the 180 colonies. For

this total analysis, the data are treated as independent, and therefore, 888 data vectors (of eleven variables each) are used to the total covariance (S_T) and, correlation matrix (R_T) which is found by dividing each term in S_T given (1) by the appropriate standard deviations

$$S_T = (N-1)^{-1} \sum_{g=1}^{G} \sum_{i=1}^{N_g} (y_{gi} - \bar{y})(y_{gi} - \bar{y})' \quad (1)$$

R_T , the conventional correlation matrix based on individual data consisting of 888 honeybees, is shown at the top in Table 1. Conventional factor analysis was conducted two times, the first for the total correlation matrix based on the individual data and, the second for the total correlation matrix based on the group (colony) means data

$$\rho_I = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_w^2} \quad (2)$$

The total correlation matrix based on individual data was submitted to exploratory maximum likelihood factor analysis using standard software. The first six eigenvalues were 2.319, 1.307, 1.120, 0.980, 0.935, and 0.872. From the resulting screen plot indicated three factors, and thus, three factors were extracted and rotated using Promax. A rotation that allowed the factors to be correlated was selected to avoid the distortions that can occur by forcing orthogonal rotation on the data. The resulting factor loadings are displayed in the first (individual) part of Table 2.

Table 2. Factor loadings for total analyses based on individual honeybees and colony means data

Tablo 2. Bireysel bal arısı ve koloni ortalama verilerine dayandırılarak toplam analizler için faktör yükleri.

item	Individual (N=888)			Colony Means (N=180)		
	1	2	3	1	2	3
FWL	.09	.05	.32	.56	.13	-.01
FWW	-.03	-.02	.84	.52	.03	.20
a	.02	-.05	.07	-.06	-.09	.33
b	.05	.08	.09	.17	-.05	.33
HWL	1.00	-.01	.01	.77	.03	-.09
HWW	.28	.04	.27	.81	-.11	-.02
FL	.06	.55	-.05	.08	.63	-.21
TL	-.03	.59	.05	.01	.55	.32
ML	.00	.32	.02	.01	.32	.11
MW	-.01	.51	-.03	-.07	.70	-.14
PL	.09	.07	.05	.14	-.01	.13

Note. Underlined figures represent loadings greater than .30.

The total correlation matrix based on colony means data was submitted as in individual data. The first six eigenvalues were 3.175, 1.350, 1.117, 0.996, 0.869, and 0.782. From the resulting plot indicated three factors, and thus, three factors were extracted and rotated using Promax. The resulting factor loadings are displayed in the second part of Table 2.

Three factors were assumed for both individual and colony means data. Factor structure for the colony means (aggregate modeling), for which the first dimension is a wing factor (i.e., forewing and hind wing

characters), the second is the leg factor and the last one is the cubital factor (cubital A, and cubital B), was more proper than the individual-level factor structure, except proboscis length (PL). Individual-level factor structure (disaggregated modeling) showed rather distorted factor loadings for morphometric characters. The only interpretable factors were on the second and third factors which can be called the factors of leg and the forewing respectively. However, a, b, HWW, and PL characters don't have high (or moderate) loadings on any dimensions. As a result, if one conducts the disaggregate modeling, no proper

factor solution is reached, and if one conducts the aggregate modeling an explicit solution may be accomplished. At the same time, the differences between factor solutions motivate us to apply multilevel modeling in order to ascertain more accurate structure. Note that, we replied the factor analyses above excluding the variable PL which doesn't load on any dimension, we decided to include it for following steps, since factor solutions got worse when it excluded.

Step 2: Estimation of between-group variance

The preceding analyses are technically incorrect and potentially substantively misleading in that they assume that the between correlation matrix is zero. That is, the analysis of the total correlation matrix assumes that no reliable between-individual differences in elevation are present in the data. To explore the extent to which this is true or false, we computed the ICCs for each of the eleven morphometric characters. The ICC results are shown in the bottom of Table 1, the ICC values for these eleven variables, ranged from .156 to .506, with an average ICC of .337. Given our relatively high ICC values that nearly one third of the variance on average, we concluded that there was sufficient between-group variation in colonies to statistically warrant the use of multilevel analysis, since an ICC of .05 might be considered a very significant number that could seriously impact the power of study. In addition to colonies, ICCs based on 55 localities were also computed, they ranged from .001 to .045 indicating inessentiality of three-level analysis (i.e. individual level, colony level and locality level). Therefore, a two-level factor analysis was applied as the most appropriate number of levels is two (individual level and colony level).

Step 3: Within-group factor structure:

In the third step, a conventional single level exploratory factor analysis is conducted, this time using pooled within correlation matrix (R_{PW}), for which computed pooled within group covariance matrix (S_{PW}) from the Equation is transformed into a pooled within correlation matrix (R_{PW}) by dividing each element by appropriate standard deviations.

Note that the sample pooled within group correlation matrix, R_{PW} which is shown in the middle of Table 1, is a consistent estimator of the population within correlation matrix. Consequently, conventional factor analysis can proceed directly on this matrix.

The within correlation matrix, R_{PW}, was submitted to exploratory, maximum likelihood, factor analysis using standard software. The first six eigenvalues were 1.578, 1.357, 1.170, 1.013, .981, and .897. As with the analysis of total correlation matrix, from the scree plot and detailed investigation the other solutions, three factors were extracted and rotated using Promax. The factor loadings are displayed in first part (within) of Table 3 and visually depicted on the bottom of Figure 1 (loadings <.30 not shown). As seen from the factor loadings for within analysis, it appears that the first dimension is forewing factor with high loadings from only two variables: Forewing length and forewing width.

The second factor is marked by two variables: a and b which are cubital characters of honeybees. And the last factor with high loadings from four variables: FL, TL, ML, and MW which are leg characters. The correlations were found -.252, -.125, and .162 for Factor 1 with Factor 2, Factor 1 with Factor 3, and Factor 2 with Factor 3, respectively. Forewing factor is negatively correlated with factors of cubital and leg.

In this stage, it will be useful to compare factor loadings for within analysis to total analysis based on individual honeybees in the first part of Table 2. The improvement in factor solution for within analysis is rather straightforward by comparison with the total (disaggregated) analysis. For within analysis three factors were found as conceptually meaningful, while for the total analysis only two factors are considered to be interpretable. On the other hand, the variables a, b, HWH, and PL for total analysis, and the variables HWL, HWW, and PL (smaller number of variables respect to total analysis) for within analysis, don't have significant loadings on any of the factors.
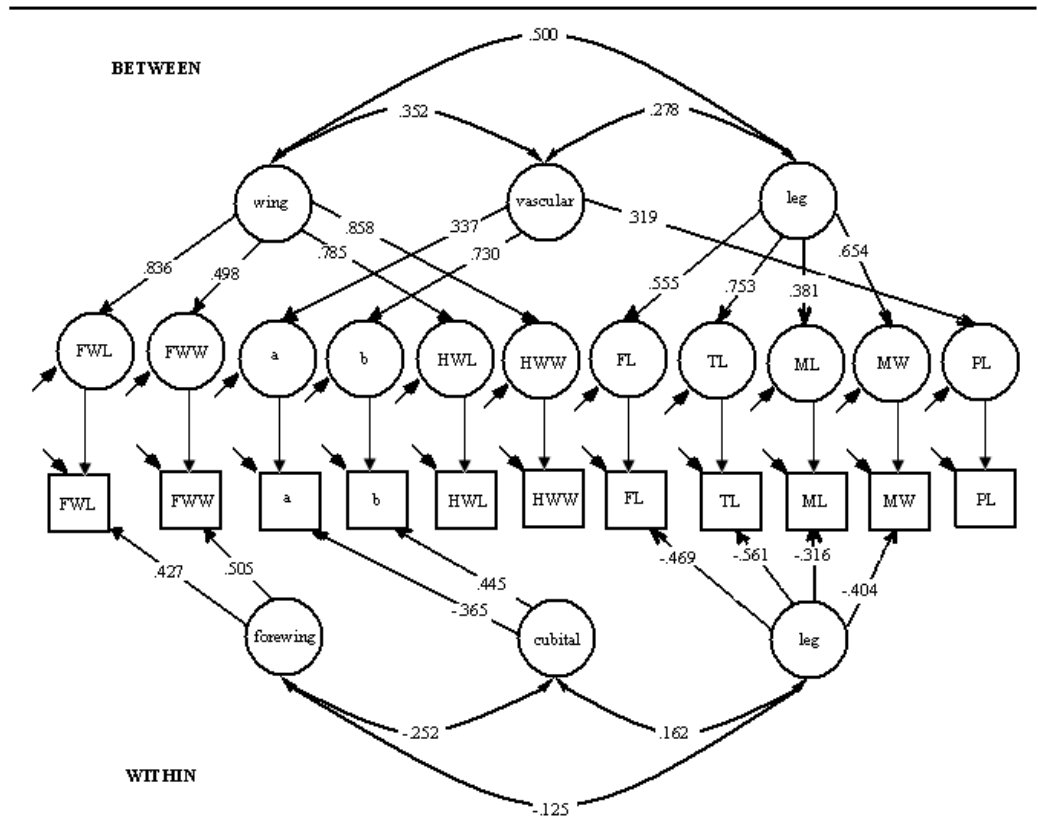
Figure 1. Path diagram of multilevel factor model from within and between analyses
Şekil 1. Grup içi ve grup arası analizlerden çok seviyeli faktör modelinin path diyagramı

Step 4: Between-group factor structure

The factor structure obtained from the within-group level analysis cannot be assumed to also hold at the between-group level analysis (Muthén, 1994). At this step, the appropriateness of the between-group structure is examined. In some research contexts, the data analysis may stop at this point. Either an estimate of the population between-group covariance matrix is used, or if there are practical problems in the analysis when using this matrix (e.g., not positive definite, lack of convergence), the sample between-group covariance matrix, S_B, may be used. However, these problems weren't observed. To obtain corresponding sample between-group correlation matrix, R_B, each element of S_B (or $\hat{\Sigma}_B$ if possible) is divided by the appropriate standard deviations. The estimated between-

group correlation matrix transformed from $\hat{\Sigma}_B$ is shown in the bottom panel of Table 1. This matrix was submitted to exploratory, maximum likelihood, factor analysis using standard software. The first six eigenvalues were 4.073, 1.347, 1.260, 1.006, .764, and .657. Both of the scree plot and detailed investigation of the other factor solutions clearly indicated three factors, and thus, three factors were extracted and rotated using Promax. The factor loadings are displayed in the second (between) part of Table 3 and visually depicted on the top portion of Figure 2 (loadings <.30 not shown). All factor correlations were found significantly positive as .352, .500, and .278 for Factor 1 with Factor 2, Factor 1 with Factor 3, and Factor 2 with Factor 3, respectively.

For illustration purpose, a one-factor model diagram with eight indicator variables for both between and within levels is given in Figure 2 for comparing multilevel factor model diagram
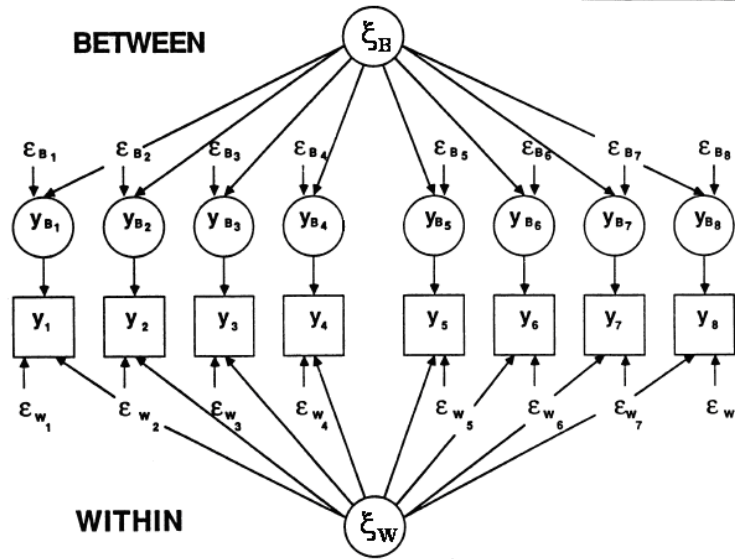
Figure 2. One- Factor Model
Şekil 2 Bir - Faktör Modeli

Comparing with the other analyses based on individual total, aggregated total (colony means), pooled within, the between-groups factor solution was achieved to more proper, conceptually meaningful, and simpler structure. Substantially, all variables, including PL, have high or moderate loadings on a single factor while the other loadings of these variables are relatively small.   The three factors in the between part of Table 3 may be named as wing, vascular and, leg respectively. The wing factor with relatively high loadings from FWL, FWW, HWL, and HWW is composed of forewing and hind wing characters. These variables have dispersed rather for analyses of total and within

correlations. However, in the factor solution from the colony means, the first factor was wing as well, with relatively smaller loadings. However, the variable TL among the leg characters has also moderate loading (.32) on cubital factor in addition the loading (.55) on leg factor, which leads to factor complexity violating the simple structure. Furthermore, for the factor solution based on colony means, the variable PL doesn't have significant loadings on any of the factors, while this variable has been together with the variables a and b, providing the vascular factor (column 2 in the between part of Table 3) in between-group factor solution.

Table 3. Factor loadings for within and between analyses

Tablo 3. Grup içi ve grup arası analizler için faktör yükleri

	Within (N-G=708)			*Between (G=180)*		
item	*1*	*2*	*3*	*1*	*2*	*3*
FWL	.427	.105	.043	.836	-.152	.286
FWW	.505	-.025	.025	.498	.155	.172
a	.082	-.365	.100	-.193	.337	.091
b	.122	.445	-.084	.025	.730	-.011
HWL	.101	-.153	-.095	.785	.086	.042
HWW	.111	-.146	-.076	.858	.060	-.066
FL	-.070	-.004	-.469	.131	-.033	.555
TL	-.024	.128	-.561	.010	.142	.753
ML	.023	-.142	-.316	-.003	.076	.381
MW	.052	.029	-.404	-.039	-.132	.654
PL	-.002	-.047	-.080	.042	.319	.009

Note. Underlined figures represent loadings greater than .30.

Conclusion

In morphometrical researches, the data usually have hierarchical structure in which individual honeybees are grouped within colonies. For this kind of multilevel data, the assumption of independence among observations is not realistic, because units share common environment, experiences and interactions. The traditional factor analysis approach uses all the data, however by ignoring the dependencies in the data, factor loadings may be biased, and the standard errors for parameter estimates and model fit statistics may be misleading. As a result, the classical theory in factor analysis can not be applied to these situations and researchers need to consider analytic procedures that properly account for within and between units variance. Multilevel Factor Analysis (MFA) models is an appropriate methodological tool which has been proposed as an extension to factor analysis models for analyzing data with hierarchical structure. In this paper, we illustrate these concepts using honeybee (*Apis mellifera* L.) data from Turkey. One initial study, discussed in Mok (1995), indicated that MCFA works reasonably well when the multilevel dataset consists of a total of 800 or more observations.

In this paper, we extended Muthén's (1994) five-step Multilevel Confirmatory Factor Analysis (MCFA) procedure to Multilevel Exploratory Factor Analysis (MEFA). We attempted to illustrate its usefulness for morphometric research. This procedure progressively allows researchers to assess factor structure at multiple levels of analysis. Our results illustrated that the within and between level factor structure of morphometric characters conformed to expectations, which is a factor solution with three factors of wing, leg and vascular. On the other hand, no proper solution could be arisen from the conventional factor analysis of individual total correlation matrix. Factor solution of within correlation matrix rather improved respect to analysis of individual total correlation matrix. Also, we found that the factor loadings of the variables were stronger at the between (i.e., colony level) than the within (i.e., within colony) levels of analysis, because the variables in within levels are affected more from measurement errors than

between level variables. As seen from the Figure 2, between level variables are hypothetical latent variables which don't contain measurement errors. Furthermore, It is possible to visualize honeybee data using between level factor scores, because conceptually meaningful three factors were extracted, which visualable data is desired in all fields as well as morphometric researches. In our application, we improved the factor solutions for individual and aggregated data using multilevel modeling approach.

Our results illustrated that the within and between level factor structure of morphometric characters conformed to expectations, which is a factor solution with three factors of wing, leg and vascular. On the other hand, no proper solution could be arisen from the conventional factor analysis of individual total correlation matrix. Factor solution of within correlation matrix rather improved respect to analysis of individual total correlation matrix. In our application, we improved the factor solutions for individual and aggregated data using multilevel modeling approach (Longford and Muthén, 1992; Lee, 1990; Muthén, 1989, 1990, 1994; Lee and Poon, 1995).

Finally, while MCFA which is special case of Multilevel Structural Equation Modeling (MSEM), MEFA as an extension of MCFA, and multilevel regression have substantial potentials for dealing with multilevel or hierarchical data structures, it is important to recognize that this research is still relatively new. It should be noted here that multilevel regression models as well as multilevel factor models may be useful tools for morphometrical researches.

MSEM is just now becoming more commonly accessible with software packages such as Mplus (Muthén and Muthén, 2004), EQS (Bentler and Wu, 2002) and the other conventional SEM software. With the emergence of software packages that can now handle a multitude of multilevel analyses, we need to expand our knowledge and understanding. It is our hope that this paper will lead to a more widespread use and understanding of MEFA as well as MCFA, and in the natural sciences as well as in the social sciences.

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