

KARYOTYPIC VARIATIONS IN *LILIUM* SPP.

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Abstract

Karyotypic variations in five lily taxa (*Lilium candidum*, *L. martagon*, *L. ciliatum*, *L. carniolicum* ssp. *ponticum* and *L. carniolicum* var. *ponticum*) have been investigated by means of numerical methods (Cluster and Principal Component Analyses). By the analysis of this species from six chromosome related characters, it has been determined that relative length is the best character which represents the variations in them. It was also found that the results from numerical analysis of the karyologic characters provide distinct evidences, which are corresponding to the morphology for recognition of the taxa.

Introduction

The genus *Lilium* L. (Liliaceae) includes up to 100 species widely distributed across Temperate Asia, Europe and North America (Zeybek and Zeybek 1994). According to the records in the Flora of Turkey, the genus is represented by five species in Turkey and most of these species are distributed in North Anatolia (Davis 1984). Early sub-divisions of the genus were clearly artificial as they were based solely on flower shape and aspect (Smyth 1994). A more detailed classification was proposed by Comber (1949) who defined seven sections based on a combination of 15 traits. Particularly importance was placed on seed weight and germination patterns, leaf arrangement, and the habit of the bulb and bulb scales in addition to the fact that floral characters were also considered (Smyth 1994). In recent years the genus *Lilium* has been the subject of karyological studies; among the well-known are Stewart (1947) and Smyth *et al.* (1989). Although numerical taxonomy has a relatively long history, it must be considered essentially a development of this decade. In many studies the karyotypic charactes was interpreted in terms of numerical analysis (Azkue and Martinez 1988, Koopman and Joung 1996). But no studies in this aspect have previously been reported in lily species distributed in Turkey. Thus, the object of this study is to determine the importance of chromosomal variability in the taxonomy of lily species by means of numerical methods.

Materials and Methods

Lilium candidum L., *L. martagon* L., *L. ciliatum* P.H. Davis, *L. carniolicum* ssp. *ponticum* (C. Koch) Davis & Henderson and *L. carniolicum* var. *ponticum* (C. Koch) Davis & Henderson were used as operational taxonomic units (OTUs). They were collected from the mountains of N.E. Anatolia in 1997 and identified by using Flora of Turkey and the East Aegean Islands (Davis 1984). The vouchers of the species were kept in the Herbarium of Karadeniz Technical University, Department of Biology (KTUB).

For karyotype analysis, bulbs or bulbils were grown in vermiculate for several weeks at 20 - 25°C and roots excised and treated in 0.02% (w/v) colchicine for 4 - 5 h. They were fixed in alcohol-acetic acid (3 : 1) at 4°C and hydrolyzed in 1 N HCl at 60°C for 10 - 12 min (Smyth *et al.* 1989). Karyotypic characters were determined and scored from permanent slide (Lavan *et al.* 1965, Hayirlioglu and Beyazoglu 1997) for numerical analysis. Characters were neither standardized nor arranged before analysis. The data matrix seen in Table 1 was treated by two

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types of numerical analyses: cluster analysis (CA) and principal component analysis (PCA). For PCA, a correlation matrix was used for extracting eigenvalues and eigenvectors. CA was calculated in order to group species of similar composition. A phonogram was drawn for similarity matrix using the unweighted pair group method with arithmetic average (UPGMA). Cophenetic correlation coefficient (R), cluster separation power (r_i), % contributions to distance, the ratio of cluster (V_1) are computed from the results of CA. Cophenetic correlation coefficient was calculated to measure the fit of a dendrogram to the originating distance matrix. Cluster separation power, % contribution to distance, the ratio of cluster were calculated in order to determine the importance of variable in clustering. Principal component analysis was performed in order to find combination of karyotypic parameters that accounted for most of variations. All these analyses were carried out by using SynTax-pc (Version 5.0) computer programs for Multivariate Data Analysis in Ecology and Systematics (Podani 1993).

Results and Discussion

The raw data matrix subjected to UPGMA from distance computed by Euclidean distance and the phonogram resulting from UPGMA are presented in Fig. 1 and Table 1, respectively. According to the phonogram, the amalgamated order of five lily taxa in terms of taxonomic distance computed from chromosome - related variables are as follows: *L. carniolicum* var. *ponticum*, *L. carniolicum* ssp. *ponticum*, *L. martagon*, *L. ciliatum*, *L. candidum*. As seen in Fig. 1, *L. carniolicum* var. *ponticum* and *L. carniolicum* ssp. *ponticum* are the nearest species while *L. carniolicum* var. *ponticum* and *L. candidum* are the farthest in respect to taxonomic distance. As previously described (Smyth *et al.* 1989, Stewart 1947), the most different karyotypic characters were also observed in *L. candidum* with this study.

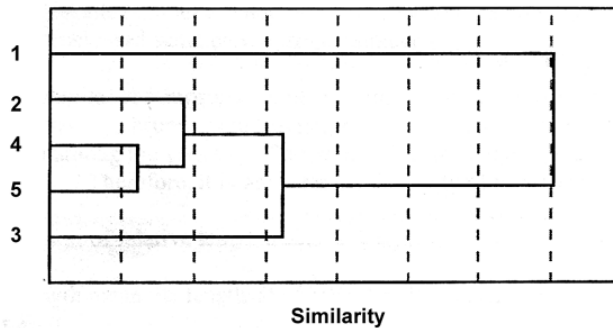


Fig. 1. UPGMA phonogram based on taxonomic distance among karyotypic characters. For species number explanation see Table 1.

Of the four main clustering methods, UPGMA is the most frequently used (Rosembug 1984) and also appears to produce the best results (Radford 1986). Cophenetic correlation is especially useful to select the clustering algorithm that produce the best recovery of a given data matrix. The simplicity of the Cophenetic correlation coefficient has led to its extensive application (Sneath and Sokal 1973). It has generally been found to vary from 0.6 - 0.95, depending on the method producing the phonogram (UPGMA in this study) and the natural structure of species classified; our phonogram had a Cophenetic correlation coefficient of 0.59. The higher correlation coefficient is found in UPGMA clustering algorithm and this correlation coefficient is actually close to the lower bound of range of most frequently occurring Cophenetic correlation coefficient reported by Sneath and Sokal (1973) and Padoni (1994).

In numerical classification studies, it is always a relevant problem to evaluate how the individual variables explain the resulting cluster. The variables contribute differently to the basic aspect of classification. In this study, cluster separation power (r_i), ratio (V_i) and % contributions are computed from distance matrix to determine the importance of variables in clustering. The perfect value of r_i is 1 (Podani 1994). As it can be seen in Table 2, the variables 1 and 2 take the higher values, the variable 6 takes the lower value. In contrast to r_i , the lower ratio of variable better the discriminatory power of variable (Padoni 1994). According to this information, the variables 1 and 2 take the important value of ratio. At the same time, the higher % contribution value becomes the variable 1 (77.30), and the lower becomes the variable 6. In conclusion, among the karyological characters used in this study, the relative length is the most important and the coefficients of variability are less important characters in clustering of lily species which has the same chromosome number (Davis 1984).

Table 1. Karyotypic data matrix of five *Lilium* taxa (X₁: relative length, X₂: total chromosome length, X₃: SAT-chromosome, X₄: centromeric index, X₅: arm ratio (long arm length/short arm length, X₆: coefficients of variability).

No.	Species	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆
1.	<i>L. candidum</i>	13.58	9.47	3	18.27	6.32	8.32
2.	<i>L. martagon</i>	7.03	13.01	0	15.91	7.34	8.32
3.	<i>L. ciliatum</i>	6.76	9.44	2	18.71	5.73	8.32
4.	<i>L. carnolicum</i> subsp. <i>ponticum</i>	5.23	10.76	0	16.05	7.63	8.33
5.	<i>L. carnolicum</i> var. <i>ponticum</i>	7.09	10.43	0	17.11	7.23	8.32

Table 2. The importance of variable in clustering five *Lilium* spp.

Rank	Characters	Ratio of cluster	% contrib. to distance	Sep. power (ri)
1	X1	0.04	77.30	1.00
2	X3	0.42	10.75	0.75
3	X5	2.21	1.51	-0.16
4	X4	1.66	4.62	-0.25
5	X2	1.80	5.80	-0.33
6	X6	3.00	0.0	-1.91

For characters number explanation see Table 1.

PCA results using six chromosome related characters are as follows: the Fig. 2 shows variable and species on the first two components, which explain most of the variations in the examined species. As it can be seen in Fig. 2, the five lily species are easily separated from each other on the first two PCs. Table 3 gives the results of principal analysis showing eigenvalues, percentages of explained variance, and percentage of cumulative variance of the first four PCs. Only the first three components were taken into account because of their eigenvalues. As shown in Table 3, the three components that, combined, account for 99.23% variations. The first components alone account for 78.85 % of variations. The second component accounts for 17.47% so that together they account for 96.32 % of variations. PCA also shows that first component was defined by relative length and SAT-chromosome and the second component was defined by total chromosome length. The third component was defined by coefficients of variability. Table 3 also

shows variable weights as percentage on the first four PCs. Most characters weight almost equally on the first component, the exceptions are the relative length and SAT-chromosome which determine the first PC. The second component was determined by total chromosome length and the third component is determined by most of the characters examined. All these PCA results show that the differences in karyotypic characters in *Lilium* are mainly due to a variation in relative length and total chromosome length.

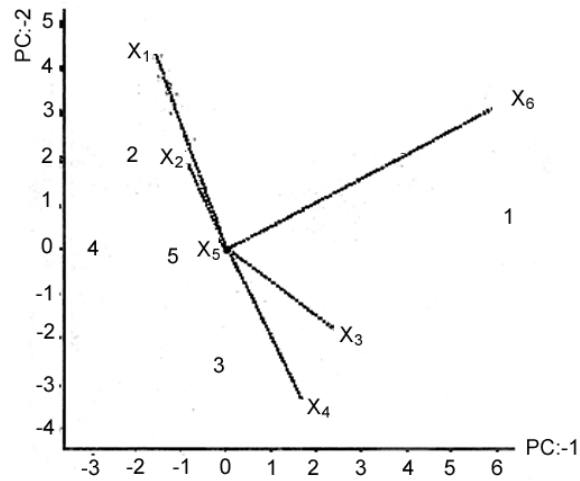


Fig. 2. PCA plots based on components 1 and 2. For characters and species number explanation see Table 1.

Table 3. Principal component analysis of karyotypic characters in *Lilium* (eigenvalues, variance, cumulative variance of the first six components and charactrs weight (%) on the these components).

Characters	PC-1	PC-2	PC-3	PC-4
1	93.89	6.00	0.07	0.02
2	34.31	53.98	11.60	0.10
3	82.52	10.17	3.75	3.54
4	49.91	44.64	2.15	3.28
5	39.91	40.12	20.35	0.16
6	22.54	0.09	28.24	49.11
Eigenvalues	0.13	0.29	0.49	0.12
% of explained variance	78.85	17.47	2.91	0.72
% of cumulative variance	78.85	96.32	99.23	100.0

For characters number explanation see Table 1.

With this present study which is a preliminary steps in the analysis of karyological characters by means of numerical analysis, it was found that the relative length and total chromosome length is the best chromosome-related characters to assess the variations among lily species. In addition it was also determined that the five lily taxa which have the same chromosome number could be easily distinguished with chromosome-related characters used in this study.

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