

*Full Length Research Paper*

# Genome size, morphological and palynological variations, and heterostyly in some species of the genus *Linum* L. (Linaceae) in Iran

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Accepted 24 January, 2012

Heterostyly is the occurrence of flowers with different sexual organ arrangements in different plants of the same species. This floral polymorphism occurs in four sections of genus *Linum*. The present study compares the morphological, palynological and genome size (C-value content) characteristics in the long-styled and short-styled plants in three *Linum* species, that is, *Linum austriacum* L., *Linum album* Ky.ex Boiss. and *L. glaucum* Boiss. & Nöe. 15 qualitative and quantitative morphological characters from both vegetative and reproductive organs of these plants were studied. A higher mean value of the plant height, size of the basal leaves width, flower leaves width, calyx length, sepal length and petal length occurred in the long-styled plants, while the mean value of branch number, basal leaves length, flower leaves length, calyx width, pedicel length and sepal length was higher in the short-styled plant populations. T-test analysis of morphological characters showed significant difference ( $p < 0.05$ ) for some of the characters studied. Principal coordinate analysis (PCoA) plot of long-styled and short-styled plant populations based on all morphological characters also separated these two kinds of plants in the three species studied. The pollen obtained from the mature buds was prepared for light microscopy (LM) and scanning electron microscopy (SEM). The polar and equatorial views of the pollen grains were similar in the long-styled (Ls) and short-styled (Ss) plants but the aperture shape differed in these populations. C-values obtained by flow cytometry, differed in the long-styled and short-styled plants of the species studied. The analysis of variance (ANOVA) test performed among the three *Linum* species showed a significant difference in 2C-value content. Positive significant correlation was observed between 2C-value and northern distribution of the *Linum* species studied, while a negative significant correlation occurred with eastern distribution.

**Key words:** Heterostyly, *Linum*, morphology, palynology, genome size.

## INTRODUCTION

The occurrence of flowers with different sexual organ arrangements in different plants of a single species is called heterostyly (Darwin, 1888). It is a sexual

polymorphism in which populations are composed of two (distyly) or three (tristyly) floral morphs with reciprocal arrangements of anthers and stigmas (reciprocal herkogamy) (Ganders, 1979). Plants in distylous species produce either all long-styled (LS or Pin) or all short-styled (SS or Thrum) flowers. Flowers with the LS morphology have stigma(s) positioned above anthers,

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whereas flowers with the SS morphology have anthers above stigma(s). Heterostyly has been documented in 28 angiosperm families (Barrett et al., 2000).

Family Linaceae is geographically widespread with about 300 species worldwide distributed (Hickey, 1988). Several species are shrubs and occur in tropical areas, while perennial and annual species are found in temperate areas. About 22 species, subspecies or varieties are reported from Iran (Sharifnia and Assadi, 2001) which are classified into five sections (Rechinger, 1974). Due to wide range of diversity within the genus *Linum*, it has received considerable attention from botanists (Diederichsen and Richards, 2003). Distyly is widespread and very common in *Linum* and about 40% of these species are distylous occurring in four out of five sections of the genus, namely *Linum*, *Syllinum*, *Dasylinum* and *Linastrum* (Rogers, 1979; Sharifnia and Assadi, 2001).

Various differences have been reported in the long-styled and short-styled plants in different species, for example, Armbruster et al. (2006) found variation in distyly of *Linum suffruticosum* L., with bent styles and stamens, achieving a three-dimensional arrangement. In heterostylous species, some morphological and micro-morphological characters were different in long-styled and short-styled plants including the number and size of pollen grains, stamens shape, shape and color of stigma and its surface papillae (Richards and Barrett, 1992). In some heterostylous species of *Linum* such as; *Linum perenne*, *Linum grandiflorum* and *Linum alpinum* exine sculpturing structure differed in the long-styled and short-styled plants (Dulberger, 1981).

C-value (genome size = size of the monoploid chromosome set) data is considered as useful characteristics for infrageneric classification, species delimitation or hybrid identification (Keller et al., 1996; Buitendijk et al., 1997; Bare et al., 1998, 2004). Moreover, correlation between DNA content and plant life-histories, plant phenology, environmental factors, climatic variation and geographical plant distribution has been determined in various studies performed on plant species (Bennett, 1976; Poggio et al., 1998; Baranyi and Greilhuber, 1995; Bureš et al., 2004). Evans (1968) found that varieties of *Linum usitatissimum* which grew in different ecological condition, such as; high nitrogen concentrations and high temperatures increased 10% in DNA content. In the present study, difference in morphological and palynological characters as well as genome size (C-value content) was investigated in the long-styled and short-styled plants of three *Linum* species namely *Linum austriacum* L., *Linum album* Ky.ex Boiss, and *Linum glaucum* Boiss., for the first time.

## MATERIALS AND METHODS

### Plant samples

Three heterostylous species including *L. austriacum*, *L. album* and

*L. glaucum* Boiss. & Nöe were studied. Plant specimens were collected from both short-styled and long-styled population of these species during the growing season in spring 2010 and 2011 (Table 1). From each species three populations were studied and in each population four plant specimens were used for detailed investigation.

### Morphology

15 qualitative and quantitative morphological characters from both vegetative and reproductive organs of these plants were studied. Morphological characters used include: the length and diameter of stems, number and status of stem branches, size, shape and diameter of the basal leaves and inflorescence leaves, dimensions of calyx and corolla and pedicle length. T-test analysis was performed to show morphological differences between long-styled and short-styled plants, while unweighted paired group using average method (UPGMA) and neighbor joining (NJ) trees as well as ordination plot based on principal coordinate analyses (PCoA) were used for grouping the species and also long-styled and short-styled plants in each species.

For multivariate analyses the mean of quantitative characters were used, while qualitative characters were coded as binary/multistate characters. Standardized variables (mean = 0, variance = 1) were used for statistical analyses. The average taxonomic distance and Manhattan distance were used as dissimilarity coefficient in cluster analysis of morphological data (Podani, 2000). UPGMA and NJ trees as well as ordination plot based on PCoA were used for grouping the species and also long-styled and short-styled plants in each species.

### Palynology

The pollen was obtained from the mature buds and used for light microscopy (LM) and scanning electron microscopy (SEM) by the prolonged acetolysis method of Erdtman (1960). For LM, the pollen was mounted in glycerin jelly and sealed with paraffin. The polar (P), equatorial (E) and colpus lengths, and sizes of pollen grains from six populations in three species were measured under the light microscope and P/E ratios were calculated. For SEM, the pollen grains were transferred directly to double-sided tape affixed stubs and were vacuum-coated with gold in Biorad E5200 auto sputter coater and photographed with a Camscan MV2300 scanning electron microscope. The terminology in this paper is based on Moore et al. (1991) work. T-test analysis was performed to show pollen characteristic difference between long-styled and short-styled plants.

### Flow cytometry

Three species of the genus *Linum* were analyzed with flow cytometer. For each taxon, three to five populations were collected and their 2C-value DNA content was determined. The nuclei suspensions were prepared from small amount of mature fresh leaf tissue together with an equal weight of mature leaf tissue of the external standard. The external standard used in for *L. austriacum* was Parsley (*Petroselinum crispum* cv. Champion Moss Curled) which had a 2C DNA value of 4.46 pg (Yokoya et al., 2000), for *L. album*, *Rosa wichurana* Crép was used with 2C value of 1.13 pg (Yokoya et al., 2000) and for *L. glaucum* Boiss. & Nöe was *Allium cepa* which had a 2C DNA value of 33.5 pg (Greilhuber and Ebert, 1994). One-step protocol was used for preparation of the nuclear suspension. The leaves were chopped with a single-used sharp

**Table 1.** The species and populations locality and their vouchers.

Species	Locality	Collector	Voucher number
<b>Sect. <i>Linum</i></b>			
<i>Linum austriacum</i> L. long-styled	Kurdistan, Sanandaj, Abidar mountain, 1645 m,	Talebi	HSBU 2011112
<i>Linum austriacum</i> L. long-styled	Rasht,Roudbar,Darestan Jungle, 544 m,	Talebi	HSBU 2011126
<i>Linum austriacum</i> L. long-styled	Hamedan to Tehran, Avaj, Razan 1898 m,	Talebi	HSBU 2011118
<i>Linum austriacum</i> L. short-styled	Saveh to Hamedan,Bahar ,1685 m	Talebi	HSBU 2011104
<i>Linum austriacum</i> L. short-styled	Saveh to Hamedan, Ghargh abad,1464 m,	Talebi	HSBU 2011100
<i>Linum austriacum</i> L. short-styled	Kurdistan, Sanandaj, Abidar mountain, 1645 m,	Talebi	HSBU 2011167
<i>L. glaucum</i> Boiss. & Nöe. long-styled	Kurdistan, Sanandaj,1585 m,	Talebi	HSBU 2011158
<i>L. glaucum</i> Boiss. & Nöe. long-styled	Kurdistan, Sanandaj,Saghez,1546 m,	Talebi	HSBU 2011160
<i>L. glaucum</i> Boiss. & Nöe. long-styled.	Kurdistan, 25 Km Baneh to Saghez, 1623 m,	Talebi	HSBU 2011161
<i>L. glaucum</i> Boiss. & Nöe. short-styled	Kurdistan,140 km Sanandaj to Saghez,1620 m	Talebi	HSBU 2011159
<i>L. glaucum</i> Boiss. & Nöe. short-styled	Kurdistan, Sanandaj,Kilaneh,1471 m,	Talebi	HSBU 2011156
<i>L. glaucum</i> Boiss. & Nöe. short-styled	Kurdistan, Saghez to Divandareh, 1617 m,	Talebi	HSBU 2011163
<b>Sect. <i>Syllinum</i> Griseb.</b>			
<i>Linum album</i> Ky.ex boiss. short-styled	Markazi, Saveh, Ghargh Abad ,1464 m,	Talebi	HSBU 2011101
<i>Linum album</i> Ky.ex boiss. short-styled	Hamedan to Kermanshah, Salavat Abad ,1929 m,	Talebi	HSBU 2011110
<i>Linum album</i> Ky.ex boiss. short-styled	Kurdistan, Sanandaj, Abidar mountain, 1645 m,	Talebi	HSBU 2011166
<i>Linum album</i> Ky.ex boiss. long-styled	Kurdistan, Sanandaj,1476 m,	Talebi	HSBU 2011113
<i>Linum album</i> Ky.ex boiss. long-styled	Kurdistan, Sanandaj to kamyaran, 1329 m,	Talebi	HSBU 2011114
<i>Linum album</i> Ky.Ex boiss. long-styled	Markazi, Saveh, Ghargh Abad, 2237 m,	Talebi	HSBU 2011146

scalpel with adding 400 µl nuclei isolation buffer in the plastic Petri dish at room temperature. To staining the nuclei, 1600 µl DNA fluorochrome or 2, 6-diamidino-2-phenylindole (DAPI) was added and suspensions was filtered through a 50 µm nylon mesh into a labeled sample tube. Stained nuclei suspensions were analyzed with a Partec flow cytometer (Partec Germany). The flow cytometric statistics such as; coefficient of variation (CV), mode, mean and the no. of cells counted in each sample, are showed in the histograms obtained.

DNA amounts were measured in picograms (pg) and the status of nuclei described in terms of 'C' values (Doležel et al., 2007). 1 pg of DNA represents 978 mega base pairs (Mbp). The amount of nuclear DNA of each sample was calculated based on the values of the G1 peak means (Doležel and Bartoš, 2005) as follows:

$$\frac{\text{Sample 2C peak mean position}}{\text{Standard 2C peak mean position}} \times \text{Standard 2C DNA amount} = \text{Sample 2C DNA (pg) amount}$$

T-test analysis was performed to show C-value (genome size) difference between long-styled and short-styled plant populations, while analysis of variance (ANOVA) test was performed to showed difference in 2C-value content among the three *Linum* species studied. Pearson coefficient of correlation was determined between C-value and geographical distribution of plant populations studied. SPSS ver. 9 (1988) and NTSYS ver.2 (1988) softwares were used for statistical analyses.

## RESULTS

### Plant morphology

Morphological comparison (Table 2) between long-styled

and short-styled plant populations in *L. glaucum*, showed a higher mean value of the plant height, size of the basal leaves width, flower leaves width, calyx length, sepal length and petal length in the long- styled plants, while the mean value of branch number, basal leaves length, flower leaves length, calyx width, pedicel length and sepal length was higher in the short- styled plant populations. T-test analysis of morphological characters showed significant difference ( $p < 0.05$ ) for basal leaf length, calyx width and length.

Similar analysis in *L. austriacum* showed a higher mean value of the plant height and branch number in the long- styled plants, while the mean value of the other characters was higher in the short- styled plant populations. In *L. album*, a higher mean value of the basal leaves length, flower leaves length, flower leaves width, calyx width and sepal width occurred in the long-styled plants, while, the short- styled plant populations had a higher mean value for the other characters studied. T-test analysis of morpho-logical characters showed significant difference ( $p < 0.05$ ) for some of the characters studied. PCoA plot of long- styled and short- styled plant populations based on all morphological characters also separated these two kinds of plants in the three species studied (Figures 1 and 2).

The three species studied also differed in morphological characters studied and were separated in

**Table 2.** Morphology characteristics in long styled and short styled plant populations studied.

Plant population	Statistical parameter	Stem length	Branch length	Base length	Base width	Flower length	Flower width	Calyx length	Calyx width	Pediceal length	Sepal length	Sepal width	Petal length	Petal width
<i>Linum album</i> long-styled plants	Mean	19.87	5.00	1.35	0.37	1.32	0.27	0.72	0.36	0.08	0.42	0.17	2.12	1.75
	Std. deviation	5.53	5.09	0.31	0.15	0.40	0.09	0.09	0.04	0.01	0.09	0.02	0.09	0.37
<i>L. album</i> short-styled plants	Mean	28.47	5.25	1.60	0.31	1.30	0.20	0.82	0.35	0.09	0.5	0.15	2.47	2.30
	Std. deviation	7.23	3.59	0.42	0.08	0.33	0.01	0.12	0.05	0.02	0.08	0.05	0.49	0.53
<i>L. austriacum</i> long-styled plant	Mean	58.87	8.75	1.37	0.15	0.87	0.10	0.42	0.35	0.10	0.20	0.10	1.75	1.37
	Std. deviation	3.72	0.95	0.29	0.04	0.15	0.00	0.05	0.05	0.00	0.00	0.00	0.26	0.18
<i>L. austriacum</i> short-styled plant	Mean	49.37	4.50	1.67	0.18	1.12	0.12	0.46	0.4775	0.10	0.20	0.13	2.15	1.15
	Std. deviation	5.02	1.29	0.30	0.08	0.35	0.04	0.04	0.045	0.00	0.01	0.04	0.12	0.05
<i>L. glaucum</i> Boiss. & Nöe long-styled plants	Mean	59.00	4.50	1.60	0.23	1.02	0.24	0.52	0.36	0.10	0.23	0.10	5.00	5.35
	Std. deviation	8.30	0.57	0.14	0.02	0.12	0.24	0.05	0.04	0.03	0.04	0.01	0.50	0.50
<i>L. glaucum</i> Boiss. & Nöe short-styled plants	Mean	53.875	15	1.875	0.2	1.375	0.1775	0.475	0.45	0.1	0.225	0.125	1.975	1.82
	Std. deviation	7.375	6.782	0.263	0	0.126	0.052	0.05	0.058	0	0.029	0.05	0.171	0.17

All values are in cm.

to distinct clusters/groups in UPGMA tree and PCoA plot, irrespective of long styled/short styled (Figure 3).

### Palynology

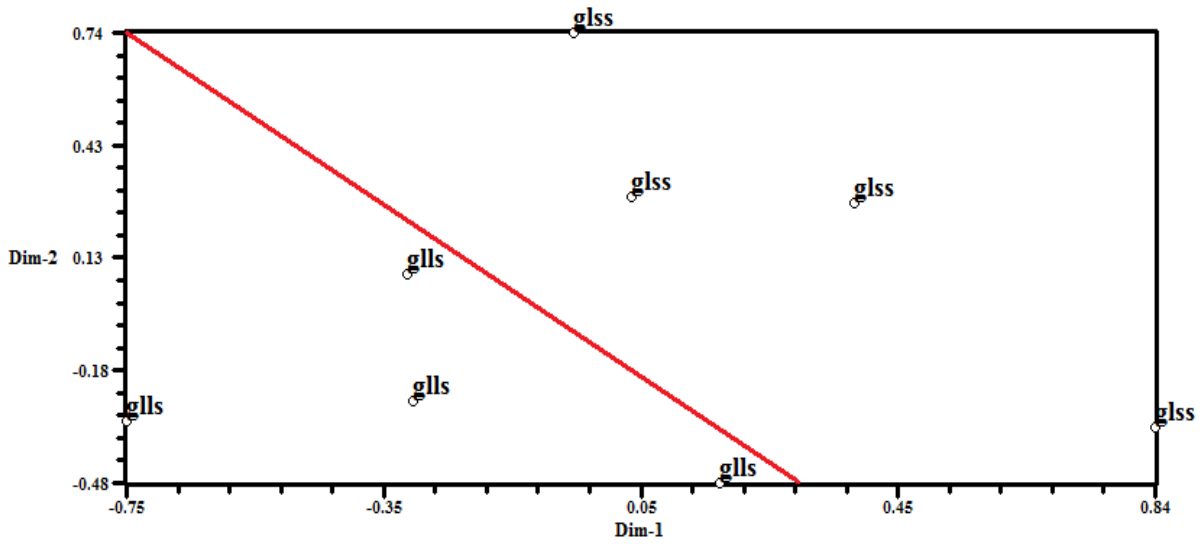
In general, the pollen shape was circular in the three species studied, but its shape varied in the equatorial view in these species that is, circular in

*L. austriacum* but elliptic in the other two species. Moreover, it was elliptic-obtuse and elliptic-truncate in *L. album* (Table 3).

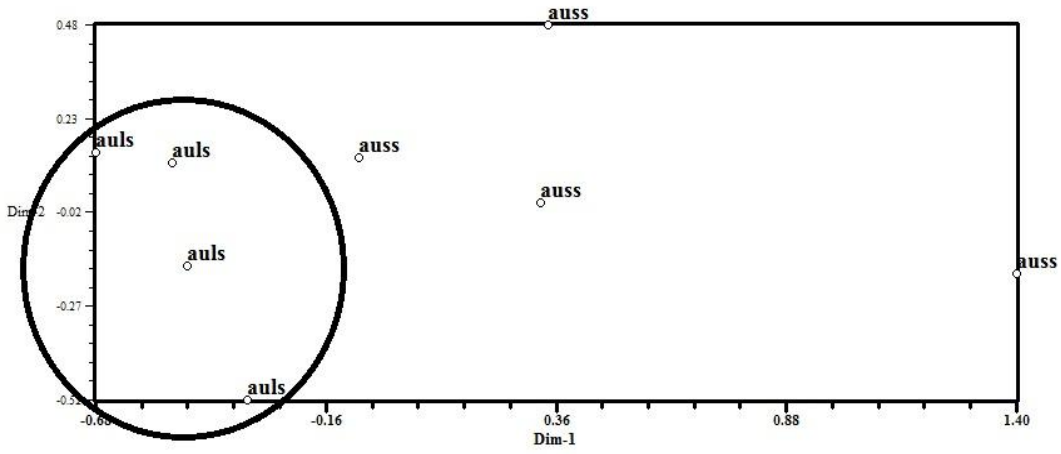
The polar and equatorial views of the pollen grains were similar in Ls and Ss plants of the species studied, while the aperture shape varied in these populations. For example, it was polygonal gemmate in *L. austriacum* long-styled plants but gemmate shape in the short-styled plants of this species. The aperture shape was

pilate and baculate in the long-styled plant populations of *L. album* while, it was pilate shape in the short-styled plant populations. Similarly in the long-styled plant populations of *L. glaucum* the aperture shape was gemmate and pilate to clavate but it was clavate in the short-styled plant populations. (Figure 4).

Comparison of pollen characteristics (Table 3) between long-styled and short-styled plant populations in *L. glaucum*, showed a higher mean



**Figure 1.** PCoA plot of long- styled and short- styled plant populations in *L. glaucum* Boiss. & Nöe based on morphological characters. glls, long- styled plants; glss, short- styled plants.



**Figure 2.** PCoA plot of long- styled and short- styled plant populations in *L. austriacum* based on morphological characters. auls, long- styled plants; auss, short- styled plants.

value of the aperture width and length, distance between apertures, polar length and colpi width as well as colpi length, in the long-styled plants, while the mean value of mesocolpi and equatorial length was higher in the short-styled plant populations. T-test analysis of morphological characters showed significant difference ( $P < 0.05$ ) for distance between apertures, aperture width and length.

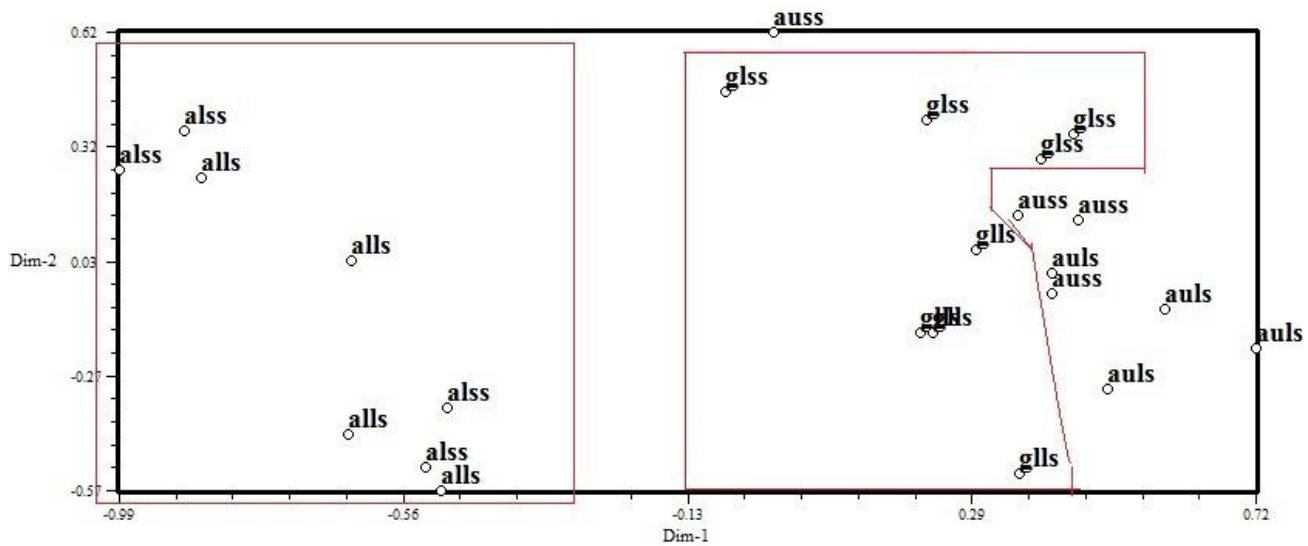
Similar analysis in *L. austriacum* showed a higher mean value of almost all pollen characteristics higher in the short-styled plant populations compared to those in the long-styled plant populations. In *L. album*, a higher mean value of the aperture length and distance between apertures were observed in the long- styled plants, while,

the short-styled plant populations had a higher mean value for the other characters studied. T-test analysis of morphological characters showed significant difference ( $P < 0.05$ ) for these characters.

PCoA plot of long- styled and short- styled plant populations based on all palynological characters also separated these two kinds of plants in all studied three species (Figures 5 and 6).

**Flow cytometry**

In order to compare C-value (genome size), difference



**Figure 3.** PCoA plot of *Linum* species studied based on morphological characters. alls, *L. album* long- styled plants; alss, *L. album* short-styled plants; glls, *L. glaucum* Boiss. & Nöe long- styled plants; glss, *L. glaucum* Boiss. & Nöe short- styled plants; auls, *L. austriacum* long- styled plants; auss, *L. austriacum* short- styled plants.

between long-styled and short-styled plants, three to five populations of each type were studied with flow cytometer in *L. austriacum*, *L. album* and *L. glaucum* Boiss. & Nöe (Table 4 and Figure 7). Each histogram has tree peaks. The right peak refers to the known reference standards (the external standards for *L. album* and *L. austriacum* and *L. glaucum* Boiss. & Nöe were Parsley and *Rosa wichurana* and *Allium cepa* respectively) and the left peak belongs to the *Linum* species studied.

C-values obtained differed between long-styled and short-styled plants of the species studied. For example, in *L. austriacum*, the mean 2C-value of long- styled plants was  $2.65 \pm 0.30$  pg but in the short-styled plants it was  $1.99 \pm 0.12$  pg. However, this was not a significant difference ( $P = 0.11$ ). In case of *L. album*, long-styled plants had the mean 2C-value of  $4.61 \pm 0.71$  while and short- styled plants had the mean 2C-value of  $3.61 \pm 0.07$ . Difference of 2C-values between long-styled and short-styled plants in this species was also not significant ( $P = 0.23$ ). The same holds true ( $P = 0.17$ ) for *L. glaucum* Boiss. & Nöe with 2C-value of  $1.98 \pm 0.01$  in Ls plants and  $1.78 \pm 0.11$  in Ss plants. However, the ANOVA test performed among the three *Linum* species showed a significant difference in 2C-value content ( $F = 11.88$ ,  $P < 0.001$ ).

A negative significant correlation ( $r = -0.85$ ,  $P < 0.05$ ), was observed between 2C-value and the stem length, but a positive significant correlation was observed between 2C-value and sepal length ( $r = 0.83$ ,  $P < 0.05$ ). Similarly, a positive significant correlation was observed between 2C-value and northern distribution of the *Linum* species studied ( $r = 0.54$ ,  $P < 0.05$ ), while a negative significant

correlation ( $r = -0.57$ ,  $P < 0.05$ ) occurred with eastern distribution. No correlation was noticed between 2C-value and altitude of the species studied ( $r = 0.2$ ,  $P > 0.05$ ).

## DISCUSSION

Heterostylous plants have been characterized by the presence of two or three discrete morphs that differ in their sex organ position within populations. This polymorphism is widely distributed among the angiosperms, but detailed studies are limited to few taxonomic groups and it is suggested that, when precise measurements of the sexual whorls are reported, an evolutionary meaningful variations of the heterostylous syndrome will be understood (Sánchez et al., 2010).

The evolution of heterostyly appears to occur within some general constrains on floral morphology (Ganders, 1979). Heterostylous flowers are generally moderate sized and have a floral tube, a limited number of stamens, and a syncarpous ovary with few carpels. For example, in the genus *Cordia* (Boraginaceae), smallest-flowered species showed dioecy but heterostyly occurred in medium to large size flowers (Opler et al., 1975), while in the genus *Melochia* (Sterculiaceae), the smallest-flowered plant lacks distyly (Martin, 1966). In *Hypericum aegypticum* and *Cratogeomys formosum* (Guttiferae) the heterostylous plants which have many stamens, are often grouped into three to five bundles with 30 to 125 stamens (Ornduff, 1975; Lewis, 1982).

Detailed comparison of flower characters between the long-styled and short-styled plants showed that in *L. glaucum*, the long-styled plants have significantly a

**Table 3.** Pollen characteristics in the *Linum* species studied.

Plant population	Statistical parameter	Sculpture width	Sculpture length	Sculpture distance	Equator length	Polar length	Colpi width	Colpi length	P/E	Mesocolpi
Alls	Mean	0.74	1.29	0.51	37.67	49.33	3.27	38.92	1.31	32.30
	Std. Dev.	0.21	0.73	0.37	3.90	3.21	0.32	7.13		2.06
Alss	Mean	1.10	1.17	0.35	45.30	37.41	7.53	68.42	0.83	37.83
	Std. Dev.	0.21	0.18	0.13	3.10	0.50	1.50	5.79		3.01
Auls	Mean	1.20	1.36	0.52	55.94	51.49	9.36	35.33	0.92	44.26
	Std. Dev.	0.69	0.57	0.14	3.82	0.81	1.36	55.97		2.25
Auss	Mean	1.38	1.49	0.53	68.22	60.1	14.28	68.97	0.88	54.11
	Std. Dev.	0.25	0.30	0.11	3.24	3.73	1.81	54.91		4.96
Glls	Mean	1.38	1.54	0.51	53.96	65.67	7.22	51.77	1.22	43.59
	Std. Dev.	0.32	0.45	0.26	3.82	2.79	1.90	3.99		3.01
Glss	Mean	1.04	1.13	0.31	55.49	53.04	6.03	48.74	0.96	47.29
	Std. Dev.	0.16	0.14	0.07	3.18	3.55	4.26	3.05		0.56

Alls, *Linum album* long-styled plants; Alss, *L. album* short-styled plants; Auls, *L. austriacum* long-styled plants; Auss, *L. austriacum* short-styled plants; Glls, *L. glaucum* Boiss. & Nöe long-styled plants; Glss, *L. glaucum* Boiss. & Nöe short-styled plants (values in  $\mu\text{m}$ ).

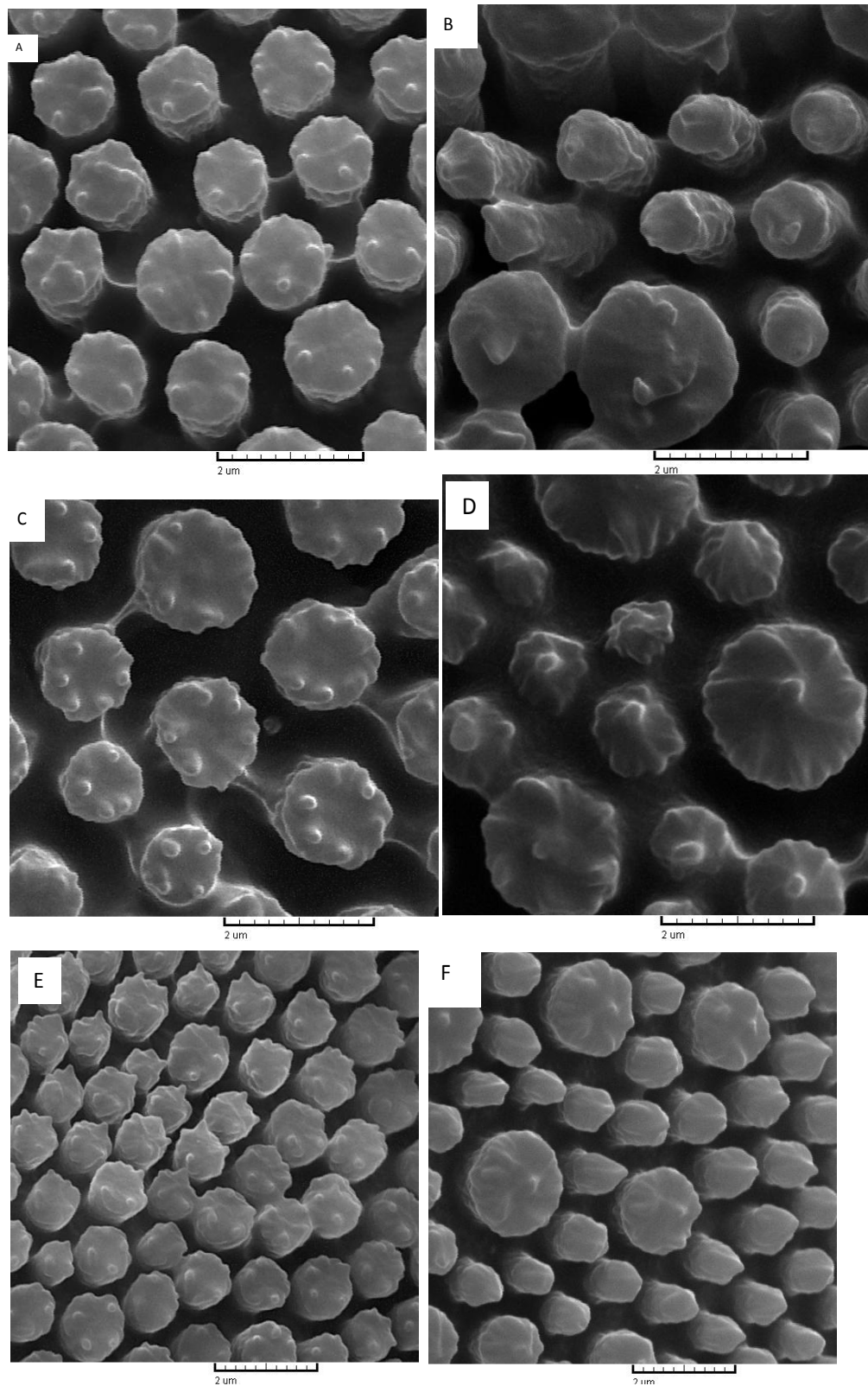
Higher mean value of the flower leaves length, and calyx width compared to those of the short-styled plants, while in *L. album*, the long-styled plants show a significant higher mean values in the calyx width, petal length and petal width. However, no significant difference in flower characters was observed in *L. austriacum* between the short-styled and long-styled plant populations. However these species showed distinct range of morphological variations as they were separated from each other in the UPGMA tree. The reproductive features such as; petal and sepal dimensions, pedicle length and some of vegetative characters such as basal and flower

leaves dimensions were bigger in Ss samples rather than Ls samples.

Study of different aspects of floral morphology in heterostylous species has been widely used as a tool to understand the pollination process, as it significantly influences pollen transfer and reproduction (Turketti, 2010). Research shows that many of heterostylous species are self-compatible (Barrett and Cruzan, 1994) *Linum* species have distinct hermaphrodite flowers with attractive petals and nectars which absorbed different pollinator's insect. Heterostyly facilitated cross-pollination in *Linum* and increases the insect mediated pollination (Darwin, 1864).

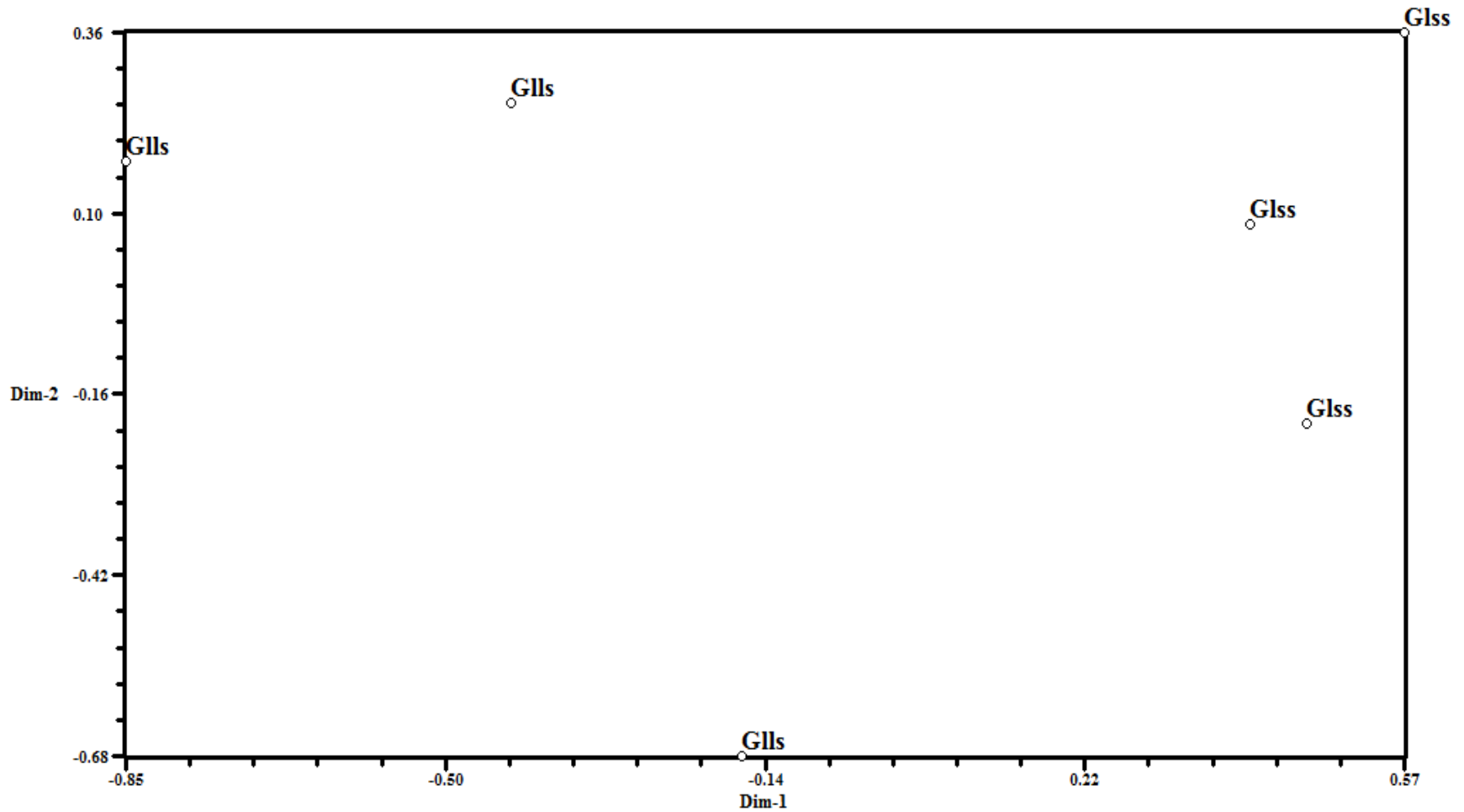
Rogers (1979) described *Linum suffruticosum* as heterostylous and intramorph-incompatible in eastern Spain showing that the pollen size is the same in the two morphs, but that the exine sculpturing differs. Three-dimensional heterostyly observed in *L. suffruticosum* is very effective in pollen distribution. When a pollinator insect visits this heterostylous species, the pollens of long-styled plants are placed on the underside of pollinator while, the pollens of short-styled plants are placed on the top of thorax and abdomen. The stigmas of long-styled plants contact the flies on the dorsum and pick up predominantly short-styled plants pollens.





**Figure 4.** Electronic micrograph of pollen exine sculpturing. (A) *L. album* Ss; B) *L. album* Ls; C) *L. austriacum* Ss; D) *L. austriacum* Ls; E) *L. glaucum* Boiss. & Nöe Ss; F) *L. glaucum* Boiss. & Nöe Ls.



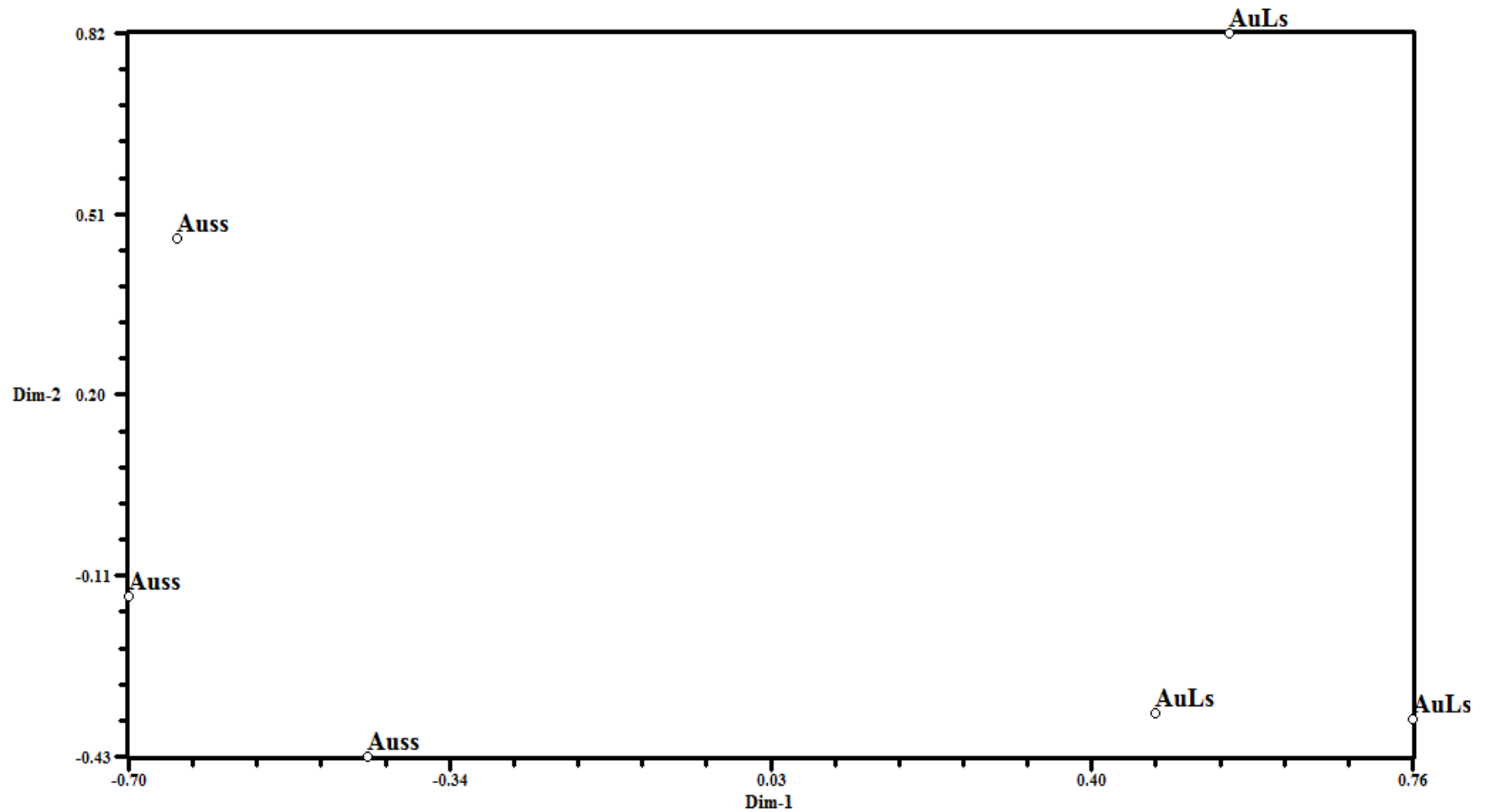


**Figure 5.** PCoA plot of long- styled and short- styled plant populations in *L. glaucum* Boiss. & Nöe based on palynological characters. Gls, long- styled plants; Glss, short- styled plants.

Moreover, the stigmas of the short-styled plants contact the flies on the ventral surface, picking up predominantly long-styled pollens (Armbruster et

al., 2006). Armbruster et al. (2006) also reported morphological variations in distylous *L.*

*suffruticosum* L., with bent styles and stamens, achieving a three-dimensional arrangement. Pin (L) and thrum (S) morphs were found to be of



**Figure 6.** PCoA plot of long- styled and short- styled plant populations in *L. austriacum* based on pollen characters. AuLs, long- styled plants; Auss, short- styled plants.

nearly identical appearance, except for the length and orientation of the sexual parts. Style and stamen lengths differed significantly; however, the differences were small compared with those in

many other species of *Linum* and other heterostylous species. There was no detectable difference in pollen size. However, stigma width differed significantly between morphs. The

stamens differed between pin and thrum morphs. Pin stamens spread from near the base, appraised to the corolla wall, and extending one-third of the way up the petals; the openings of

**Table 4.** Nuclear DNA amount and genome size in studied taxa of the genus *Linum*.

Plant name	2C DNA amount (pg)	Genome size (Mbp)
<i>L. austriacum</i> long-styled	2.65	2591.7
<i>L. austriacum</i> short-styled	1.99	1946.22
<i>L. album</i> short-styled	3.61	3530.58
<i>L. album</i> long-styled	5.00	4890.0
<i>L. glaucum</i> Boiss. & Nöe long-styled	1.98	1936.44
<i>L. glaucum</i> Boiss. & Nöe short-styled	1.78	1740.84

the dehiscent anthers face inwards. The thrum stamens were erect, forming a column in the center of the flower, and the anthers are rotated so that the openings of the dehiscent anthers face outwards.

Phylogenetic analysis and MACCLADE reconstruction of character states performed by Armbruster et al. (2006) also showed that heterostyly has originated several times, not only within *Linum*, but also within sect. *Linastrum*, including one to three reversions to monomorphism. They suggested that heterostyly has evolved at least twice in the two *Linastrum* clades, although equivocal character transitions and the limited sample of taxa preclude inference of the exact number of shifts. The least restrictive optimization of heterostyly is consistent with at least three independent origins of heterostyly in *Linum*.

Sánchez et al. (2010) also reported stigma height dimorphism, as opposed to distyly in the genus *Nivenia* as the only genus within the Iridaceae containing heterostylous species. The presence of different types of polymorphism within the genus is consistent with hypotheses of the evolution of heterostyly. The role of the pollinators as the leading force of the transition seems to be apparent, since floral integration is related to reciprocity. Variation of sexual whorls was observed in different *Nivenia* species with *Nivenia fruticosa* as the only monomorphic species.

For the species with two floral morphs, stigma height was significantly different in all of them and the length of the stamens was statistically different between morphs in all cases, except for the population of *Nivenia argentea* at Aasvoëlkrans. Thus, those were considered as distylous, and *N. argentea* at Aasvoëlkrans as stigma-height dimorphic since two style-length morphs were present but anther heights remained indistinguishable between morphs.

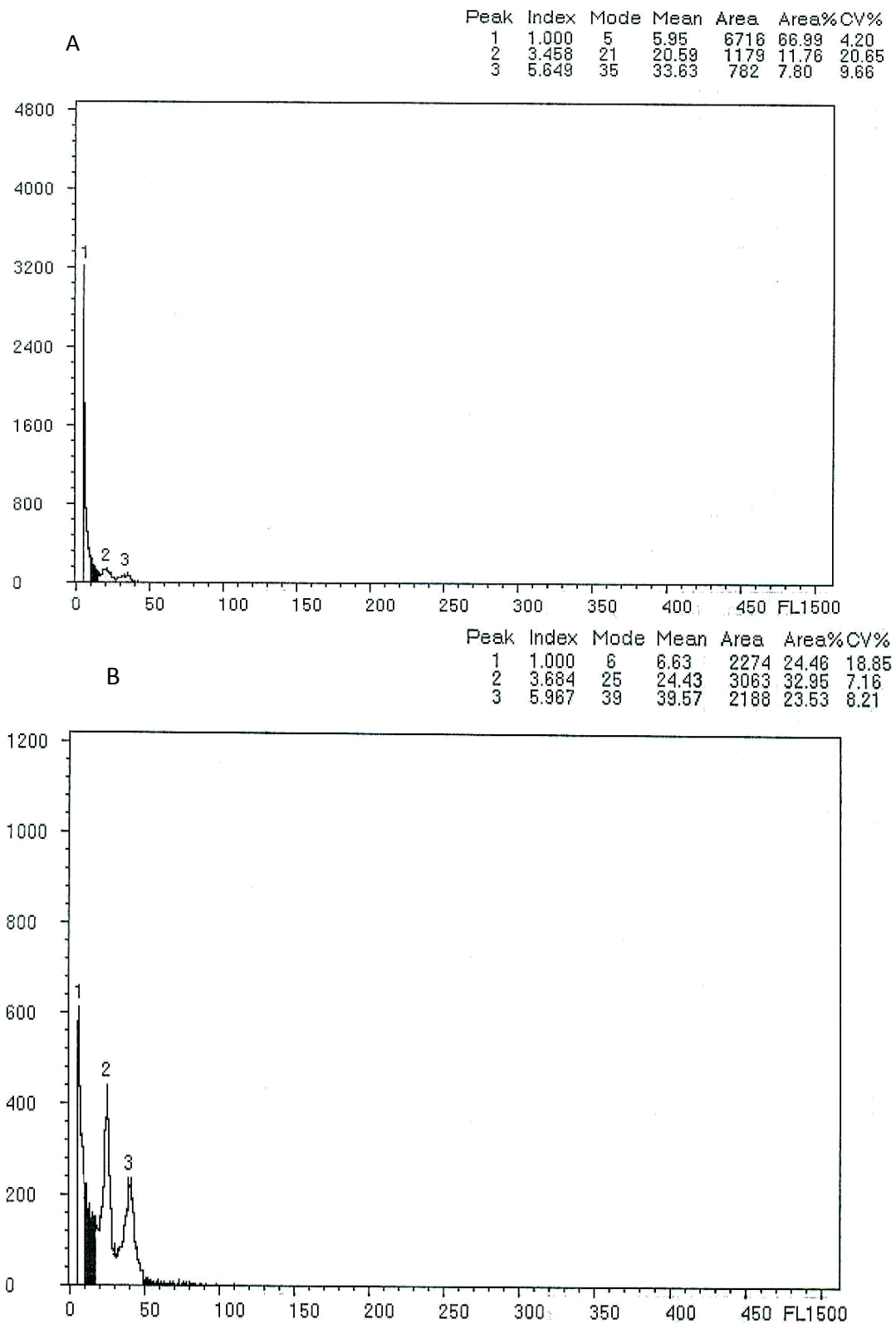
Our palynological study revealed that the shape of pollen grains in the equatorial and polar views are uniform between Ss and Ls plant populations in the *Linum* species studied, but other pollen characteristics like the aperture shape and dimensions, polar and equatorial axis length differed between long-styled and short-styled plant populations. Previous reports indicate

that in some heterostyled *Linum* species, the pollen surface in short-styled plants is composed of homo-sized apertures but the exine surface in long-styled plants contains small and big aperture with variable papillae shape and size (Dulberger, 1981). Nicholls (1986) in *L. perenne* found that short-styled plant was a better pollen-donor and the long-styled plant was a better pollen-receiver. The short-styled samples produced more pollen, but the long-styled samples matured more seeds. These observations suggest a degree of sexual dimorphism in *L. perenne*, which the short-styled plants behaving functionally more male and the long-styled plant functionally more female.

Richards and Barrett (1992) reported differences in the number and size of pollen grains (for example *Pontederia cordata*), dimorphic in stamens position (*Primula vulgaris*), (Darwin, 1877), pollen grain color (*Linum pubescens*), (Wolfe, 2001), stylar bending (in *Linum grandiflorum*), shape and colors of stigma and its surface papillae (*Linum hirsutum*, Unal and Yildirim-Fazla, 2007). In some heterostylous species of *Linum* such as; *L. perenne*, *L. grandiflorum* and *L. alpinum*, exine sculpturing structure were differed between long-styled. Heterostyly is usually associated with polymorphisms of pollen between samples (Barrett, 1992). Exine pattern of pollen interacts with biotic and abiotic pollination vectors and affected the surface area of the stigma interface and mediated stigma adhesions.

Aperture size, number and its complexity affected environmental vulnerability to desiccation, fungal invasion and mechanical stress, and serve as portals for pollen tube exit during germination (Edlund et al., 2004). Wang et al. (2009) found that in *Pedicularis* (Orobanchaceae) there was a significant association between pollen aperture types and corolla types, as well as between pollination syndromes and corolla. There was a distinct correlation between exine ornamentation, floral morphology and pollination in *Bauhinia*. (Ferguson and Pearce, 1986).

Nuclear DNA C-value varies in different taxonomic taxa (Yokoya et al., 2000) and is considered as a mean for adaptation, and is affected key parameters of plant growth such as; the duration of the cell size, cell cycle,



**Figure 7.** Flow cytometric histograms showing the difference in 2C DNA content in studied heterostylous species of the genus *Linum*. (A) *L. album* short-styled; (B) *L. album* long-styled; (C) *L. glaucum* Boiss. & Nöe short-styled; (D) *L. glaucum* Boiss. & Nöe long-styled.

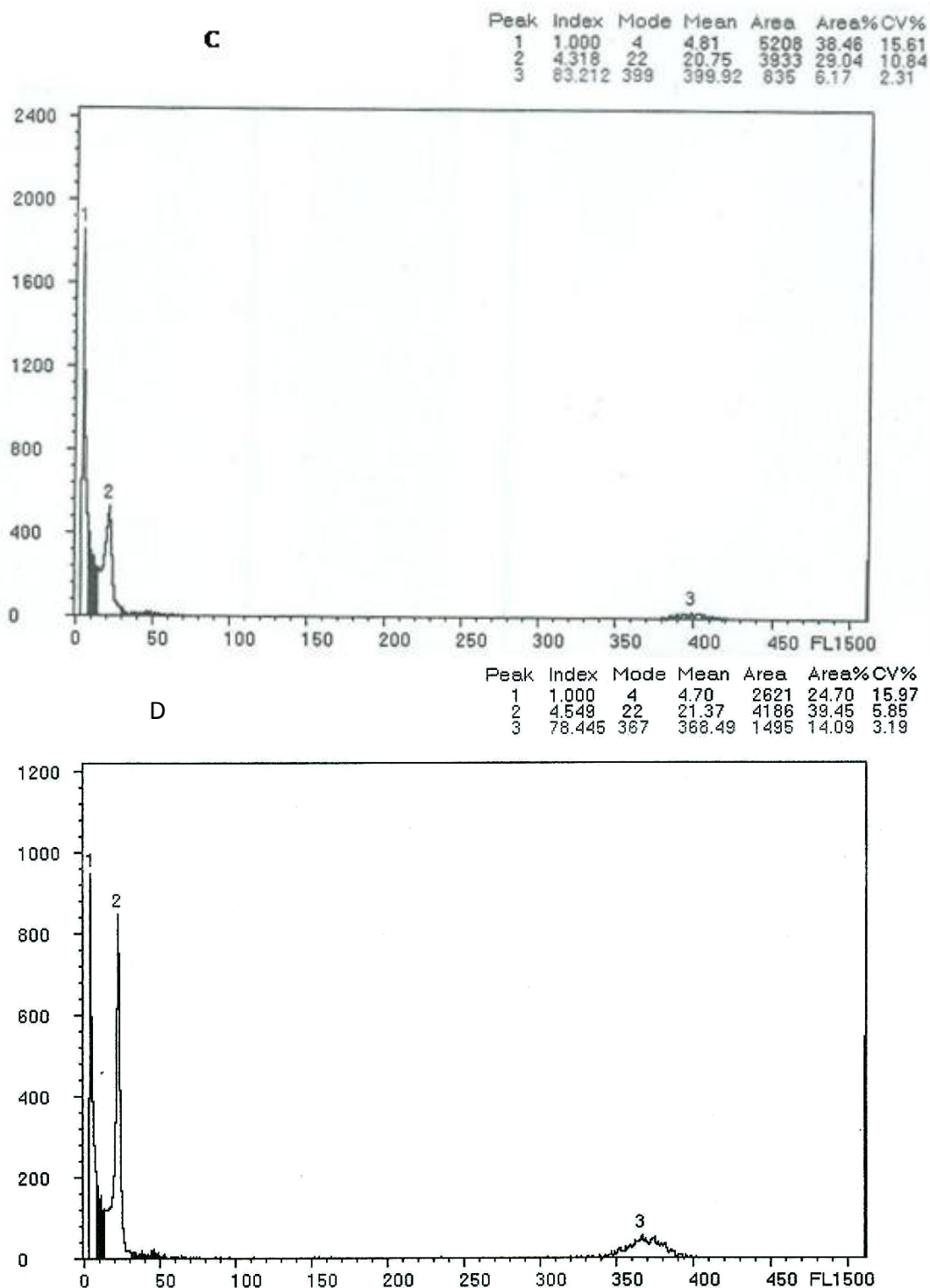


Figure 7. Continued.

rate of cell division, sensitivity to radiation, ecological behavior in plant communities and life forms (Bennett et al., 2000). The 2C-values obtained here indicate genomic

content difference between short-styled and long-styled plant populations and the possible role of quantitative genetic changes in heterostyly. Significant difference in

2C-values of the three *Linum* species studied indicates the possible role of DNA content change during species diversification. This may also suggest the use of 2C-value as supplementary data for species grouping in the genus *Linum*. Although not significant, negative relation between 2C-values and some morphological and palynological characters occurred in *L. austriacum* and *L. album*. The short-styled plants of *L. austriacum* and *L. album* have smaller genome size and nuclear DNA 2C-values compared to the long-styled plants. In the short-styled plants, the petal and sepal dimensions, pedicle length, the mean size of basal and flower leaves and polar and equatorial axis of pollen are relatively bigger than the long-styled plants.

Significant correlation obtained between 2C-values and some of the quantitative morphological characters may indicate the effect of genome size on this character and the possible adaptive nature of these characters (although not apparent to us by now). Such correlation has been reported in other plant species including *Cirsium* species (Nouroozi et al., 2011). Significant positive correlation occurred between 2C-values and ratio of pappus length/seed size and involucre length.

Positive significant correlation was observed between 2C-value and northern distribution of the *Linum* species studied, while a negative significant correlation occurred with eastern distribution. No correlation was noticed between 2C-value and altitude of the species studied. This may indicate some relation with ecological and population differentiations which should be investigated to be confirmed. Studies performed on *Cirsium* species (Nouroozi et al., 2011) showed significant negative correlation between 2C-value and latitude, and positive significant correlation between 2C-value and the mean annual rain fall, while Bureš et al. (2004) reported that the species with larger genomes prefer dry habitats in areas with more oceanic climates, and species with smaller genomes grow in more humid habitats (wetlands) in continental areas.

## Abbreviations

**PCoA**, Principal coordinate analysis; **LM**, light microscopy; **SEM**, scanning electron microscopy; **Ls**, long-styled; **Ss**, short-styled.

## ACKNOWLEDGEMENT

This project was supported by Iran National Science Foundation (INSF), with project No. 90007661.

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