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Leaf Phenotypic Variation of Chuanqian Crape Myrtle in Natural Populations

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***Abstract.* Leaves are the primary tissue for photosynthesis and transpiration, playing an irreplaceable role in the ecosystem. Understanding variations in leaf morphology across different populations is crucial for better insight into plant classification, evolution, and cultivation practices. From 10 populations of *Lagerstroemia excelsa* and 80 individual specimens, we measured key leaf traits, including leaf length (LL), leaf width (LW), leaf area (LA), leaf circumference (LC), leaf basal angle (LBA), and leaf vein angle (LVA), to determine the geographic variation trends in leaf morphology across natural populations. Our results revealed several important findings: 1) significant differences in leaf morphology were observed among the populations; 2) temperature appears to be a primary factor influencing the potential geographic distribution of *L. excelsa*; 3) the 10 populations were grouped into two main categories, indicating that similar germplasm tends to be distributed contiguously. These insights are valuable for future efforts in the introduction, breeding, selection, and cultivation of *L. excelsa*.**

Leaves play a crucial role in photosynthesis and transpiration (Tian et al. 2016), making them indispensable components of the ecosystem. Studying leaf morphology offers valuable insights into how plants absorb water and nutrients, release photosynthetic products, and facilitate gas exchange and light absorption (Nobel 2009). Leaf characteristics are influenced by the interaction between plant genetics and environmental conditions (Falconer and Mackay 1960). Variations in leaf characteristics among plant populations (Wang et al. 2021) reflect their adaptive mechanisms to environmental conditions and serve as indicators of genetic diversity (Cao et al. 2014). Therefore, understanding changes in leaf morphological traits across various plant populations in diverse ecological environments is essential for insights into plant classification, evolution, and the selection of new varieties.

Chuanquan crape myrtle [*Lagerstroemia excelsa* (Dode) Chun ex S.K. Lee & L.F. Lau], a tall tree species in *Lythraceae*, is native to China and widely distributed across Sichuan, Guizhou, Hunan, Hubei, and Chongqing (Xiang et al. 2017). This species is popular as an ornamental plant and is notable for its strong disease and insect resistance, long flowering period, and adaptability to stone and pebble soils, making it an essential species for controlling rocky desertification (Zhou et al. 2018). Additionally, its graceful trunk has made it a favored choice for bonsai production. Despite its broad ecological range and potential applications, research on the geographic variation in the leaf morphology of *L. excelsa* remains limited. Investigating the differences in leaf characteristics among various populations and exploring the response of leaf phenotypic traits to environmental changes is imperative.

Methods such as dispersion degree analysis, principal component analysis (PCA), and cluster analysis of leaf circumference are commonly employed to identify leaf morphological characteristics (Fawad et al. 2023). These methods help researchers understand the variations and differences in leaf traits among plant populations, significantly contributing to leaf morphology classification (Pegiou et al. 2023). However, it is important to note that these analyses primarily focus on comparing plant populations and may not adequately capture the correlation between leaf characteristics and environmental factors. Therefore, incorporating environmental attributes into morphological studies of diverse plant populations is both necessary and valuable.

This study aims to investigate the geographic variation in leaf morphology among ten populations of *L. excelsa* through statistical analysis and correlation analysis between leaf morphology and environmental factors. Specifically, the research seeks to answer the following questions: (1) Are there significant differences in leaf morphology among different populations of *L. excelsa*? (2) If such differences exist, are they significantly related to environmental characteristics?

Materials and Methods

Population sites. We first gathered distribution data for *L. excelsa* populations using resources such as the Global Biodiversity Information Facility (http://www.gbif.org), the Chinese Virtual Herbarium (http://www.cvh.org.cn), Plant Plus of China (http://www.iplant.cn), and the China National Knowledge Infrastructure (https://www.cnki.net). Subsequently, 10 populations from five provinces, each characterized by distinct climatic conditions and representing the primary distribution areas, were selected as the research subjects (Fig. 1, Table 1).

Environmental attributes. Environmental data were collected from global climate and weather sources (WorldClim, http://www.worldclim.org), which included 19 environmental variables (bio1–bio19) (Table 2) and one terrain variable (Elevation, Ele).

Plant materials. From each population, eight healthy plants with similar growth stages were selected, ensuring more than 30 m between each plant. Twelve leaves were collected from different orientations in the middle section of the branches of each tree. To ensure data consistency, only fully mature leaves were collected during the flowering period, specifically excluding leaves proximal to buds. This strict sampling protocol guaranteed that all analyzed leaves had reached

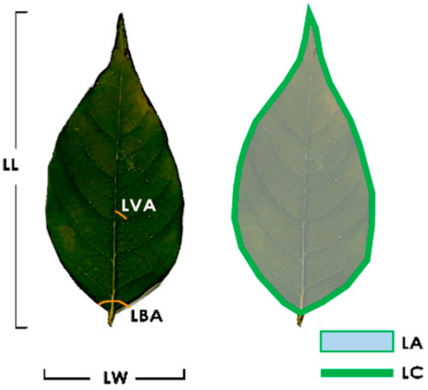

Fig. 1. Measurement of morphological factors. LA = leaf area (cm^2), LBA = leaf base angle, LC = leaf circumference (cm), LL = leaf length (cm), LW = leaf width (cm), LVA = leaf vein angle.

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Table 1. Locations of 10 *Lagerstroemia excelsa* populations.

Population	Geographic region	Longitude (°E)	Latitude (°N)
ES	Enshi, Hubei, China	109.5	30.4
WL	Wulong, Chongqin, China	107.8	29.3
CN	Changning, Sichuan, China	104.9	28.6
GZ	Guzhang, Hunan, China	109.9	28.6
TZ	Tongzi, Guizhou, China	106.6	28.3
MT	Meitan, Guizhou, China	107.5	27.7
CB	Chengbu, Hunan, China	110.3	26.4
TR	Tongren, Guizhou, China	108.6	28
BL	Bolin, Chongqin, China	106.5	28.7
SZ	Sangzhi, Hunan, China	110.2	29.4

Table 2. Environmental attributes collected from global climate and weather data sources.

Attributes	Summary
bio1	Annual mean temperature (°C)
bio2	Mean diurnal range (°C)
bio3	Isothermality
bio4	Temperature seasonality
bio5	Max temperature of warmest month (°C)
bio6	Min temperature of coldest month (°C)
bio7	Temperature annual range (°C)
bio8	Mean temperature of wettest quarter (°C)
bio9	Mean temperature of driest quarter (°C)
bio10	Mean temperature of warmest quarter (°C)
bio11	Mean temperature of coldest quarter (°C)
bio12	Annual precipitation (mm)
bio13	Precipitation of wettest month (mm)
bio14	Precipitation of driest month (mm)
bio15	Precipitation seasonality coefficient of variation
bio16	Precipitation of wettest quarter (mm)
bio17	Precipitation of driest quarter (mm)
bio18	Precipitation of warmest quarter (mm)
bio19	Precipitation of coldest quarter (mm)
Ele	Elevation (m)

their complete developmental stage. The leaves were then flattened and scanned to measure the leaf vein angle (LVA), leaf area (LA, cm^2), leaf base angle (LBA), leaf length (LL, cm), leaf circumference (LC, cm), and leaf width (LW, cm) using AutoCAD (Fig. 1). The aspect ratio (AR) is defined as the ratio of LL to LW. The morphometric factor (MF) is calculated using the formula $4\pi \times LA/LC^2$, Both AR and MF were subsequently calculated using IBM SPSS 26 (IBM Corp., Armonk, NY, USA).

Data analysis. Four types of analyses were conducted to identify the geographic variation trends in *L. excelsa* leaf morphology: 1) analysis of intrapopulation leaf trait variation, 2) correlation analysis of leaf traits, 3) correlation analysis between leaf traits and environmental factors, 4) PCA of leaf trait variation among different populations, and 5) cluster analysis and genetic classification of leaf morphological characteristics. All data analyzes were performed using IBM SPSS 26 (IBM Corp.).

Results

Analysis of intrapopulation leaf morphological characteristics variation. The statistical analysis results of phenotypic traits for 10 *L. excelsa* populations are presented in Table 3. Significant variations were observed among the eight analyzed leaf phenotypic traits, with coefficient of variation (*CV*) values ranging from 6.94% to 35.37%. The *CV* values for each trait, ranked from highest to lowest, are as follows: LVA, 35.37%; LA, 24.32%; LBA, 21.79%; LL, 14.62%; LC, 13.57%; LW, 11.00%; AR, 10.02%; and MF, 6.94%.

Table 3. Mean and coefficient of leaf variation of 10 *Lagerstroemia excelsa* populations.

Population	LL	LW	LA	LC	AR	LBA	LVA	MF
ES	97.33	46.07	2580.27	217.07	2.11	27.67	32.67	0.69
WL	106.8	50.30	3156.17	249.17	2.12	26.70	20.30	0.64
CN	92.92	40.50	2256.00	214.83	2.29	27.50	25.42	0.61
GZ	98.07	38.2	2281.33	222.47	2.57	24.67	29.00	0.58
TZ	111.44	46.56	3284.25	258.94	2.39	25.42	29.58	0.62
MT	96.33	41.25	2328.25	222.50	2.34	19.17	25.42	0.59
CB	90.4	37.73	2150.40	207.87	2.40	24.67	31.00	0.63
TR	71.33	38.00	1532.00	173.00	1.88	31.67	16.67	0.64
BL	70.33	36.33	1554.33	170.00	1.94	13.33	20.00	0.68
SZ	104.33	43.00	2628.5	245.5	2.43	20.00	52.5	0.55
Maximum	111.44	50.30	3284.35	258.94	2.57	31.67	52.50	0.69
Minimum	70.33	36.33	1532.00	170.00	1.88	13.33	16.67	0.55
Average	93.93	41.79	2375.15	218.14	2.25	24.08	28.26	0.62
CV	14.62	11.00	24.32	13.57	10.02	21.79	35.37	6.94

AR = aspect ratio; *CV* = coefficient of variation; LA = leaf area (cm^2); LBA = leaf base angle; LC = leaf circumference (cm); LL = leaf length (cm); LVA = leaf vein angle; LW = leaf width (cm); MF = morphometric factor. See Table 1 for population definitions.

These results indicate that the MF exhibits the lowest variability, with a *CV* of only 6.94%, suggesting that the leaf morphology of *L. excelsa* is relatively stable. In contrast, traits related to leaf area (LVA, LA, LBA) showed significant variations, all exceeding 15%. This suggested that as latitude, longitude, and environmental conditions change, *L. excelsa* adjusted its leaf size to adapt to its environment. These traits demonstrated high sensitivity to environmental changes, resulting in substantial variations in their characteristics.

Correlation analysis of leaf traits. The results of the correlation analysis of leaf traits are presented in Table 4. LL showed significant positive correlations with LW, LA, LC, and AR. LW was significantly positively correlated with LL, LA, and LC. LA exhibited significant positive correlations with LL, LW, and LC. LC had significant positive correlations with LL, LW, LA, and AR. AR was significantly positively correlated with LL and LC but showed a significant negative correlation with MF. Notably, the correlation between LL and LC had the highest correlation coefficient of 0.990.

Correlation analysis of leaf traits with environmental factors. LL is significantly positively correlated with bio5 (maximum temperature of the warmest month), with a correlation coefficient of 0.688, suggesting that LL increases as the maximum temperature of the warmest month rises. Additionally, LL is significantly negatively correlated with elevation, with a coefficient of −0.659, indicating that LL decreases as elevation increases. Similarly, the leaf AR is significantly negatively correlated with elevation, with a coefficient of −0.701, implying that the length-to-width ratio of the leaf also decreases with increasing elevation (Table 5).

PCA of leaf trait variation among different populations. The PCA analysis identified two principal components, with contribution rates of 56.3% and 23.8%, respectively. In the analysis of the first principal component, LL and LC had high eigenvalues of 0.987, suggesting that this component can be considered the "leaf size factor." In the analysis of the second principal component, LW and MF had significant eigenvalues of 0.657 and 0.667, respectively, indicating that the second component can be termed the "leaf shape factor" (Table 6). The cumulative contribution rate of the first two principal components is 80%, and the rapid increase in the cumulative contribution rate of each shape indicator suggests that the genetic diversity of leaf morphology in *L. excelsa* is relatively limited across multiple dimensions.

Table 4. Correlation analysis of leaf morphology of 10 *Lagerstroemia excelsa* populations.

	LL	LW	LA	LC	AR	LBA	LVA	MF
LL	1.000	0.764*	0.955**	0.990**	0.677*	0.145	0.514	−0.443
LW	0.764*	1.000	0.889**	0.774**	0.045	0.289	0.162	0.064
LA	0.955**	0.889**	1.000	0.960**	0.458	0.184	0.354	−0.221
LC	0.990**	0.774**	0.960**	1.000	0.642*	0.114	0.51	−0.481
AR	0.677*	0.045	0.458	0.642*	1.000	−0.084	0.597	−0.746*
LBA	0.145	0.289	0.184	0.114	−0.084	1.000	−0.201	0.094
LVA	0.514	0.162	0.354	0.51	0.597	−0.201	1.000	−0.556
MF	−0.443	0.064	−0.221	−0.481	−0.746*	0.094	−0.556	1.000

AR = aspect ratio; LA = leaf area (cm^2); LBA = leaf base angle; LC = leaf circumference (cm); LL = leaf length (cm); LVA = leaf vein angle; LW = leaf width (cm); MF = morphometric factor. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 5. Correlation analysis of leaf traits with environmental factors.

	LL	LW	LA	LC	AR	LBA	LVA	MF
Longitude	0.108	−0.07	−0.015	0.068	0.283	0.104	0.505	−0.201
Latitude	0.263	0.546	0.323	0.245	−0.212	0.042	0.248	0.202
Elevation	−0.659*	−0.31	−0.479	−0.599	−0.701*	0.050	−0.547	0.486
Bio 1	0.553	0.381	0.508	0.508	0.452	0.151	0.135	−0.138
Bio 2	0.101	−0.118	−0.046	0.056	0.311	0.054	0.449	−0.0.275
Bio 3	−0.038	−0.282	−0.155	−0.064	0.253	0.08	0.269	−0.242
Bio 4	0.191	0.009	0.050	0.148	0.323	0.018	0.524	−0.276
Bio 5	0.688*	0.522	0.622	0.625	0.512	0.136	0.268	−0.163
Bio 6	0.391	0.381	0.440	0.379	0.178	0.084	−0.145	0.051
Bio 7	0.177	0.018	0.041	0.129	0.289	0.026	0.481	−0.242
Bio 8	0.487	0.424	0.440	0.461	0.284	0.152	0.106	−0.220
Bio 9	0.470	0.357	0.463	0.439	0.341	0.146	−0.002	−0.065
Bio 10	0.624	0.407	0.539	0.566	0.543	0.157	0.293	−0.218
Bio 11	0.470	0.357	0.463	0.439	0.341	0.146	−0.002	−0.065
Bio 12	0.078	−0.083	−0.054	0.017	0.260	0.261	0.529	−0.127
Bio 13	0.136	−0.167	−0.052	0.068	0.443	0.253	0.586	−0.293
Bio 14	−0.174	−0.482	−0.334	−0.227	0.315	0.210	0.317	−0.183
Bio 15	0.116	0.228	0.191	0.131	−0.111	−0.062	−0.247	0.100
Bio 16	0.125	−0.137	−0.043	0.056	0.392	0.282	0.570	−0.220
Bio 17	−0.089	−0.404	−0.233	−0.140	0.358	0.164	0.338	−0.162
Bio 18	0.214	0.167	0.104	0.155	0.161	0.259	0.597	−0.109
Bio 19	−0.089	−0.404	−0.233	−0.140	0.358	0.164	0.338	−0.162

AR = aspect ratio; LA = leaf area (cm^2); LBA = leaf base angle; LC = leaf circumference (cm); LL = leaf length (cm); LVA = leaf vein angle; LW = leaf width (cm); MF = morphometric factor. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Cluster analysis and genetic classification of leaf morphological characteristics. The Euclidean distance hierarchical clustering method was used to analyze the leaf characteristics of different Chuanqian crape myrtle populations, with the *CV* of four leaf traits, LVA, LA, LBA, and LL, as parameters. When the Euclidean distance was set to 25, the 10 populations were initially grouped into two main categories. TR and BL formed one category, whereas WL, TZ, ES, SZ, CB, MT, CN, and GZ formed the other (see Table 1 for population definitions). Notably, ES and SZ exhibited a close relationship, as did CB, MT, CN, and GZ (Fig. 2).

Discussion

Geographic leaf variation of natural L. excelsa *populations.* Studying the phenotypic traits of plant leaves can enhance our understanding of the variation and diversity of *L. excelsa*, which is beneficial for its introduction, breeding, selection, and cultivation (Henry and Nevo 2014). Through an analysis of phenotypic diversity in 10 populations of *L. excelsa*, we found that the traits MF and AR changed in response to variations in environmental and geographic factors. Leaf size–related traits such as LVA, LA, and LBA exhibited significant changes, whereas morphological traits such as MF and AR showed minor variations. This suggests that the leaf size of *L. excelsa* was greatly influenced by environmental conditions, whereas its overall leaf morphology remained relatively stable. The findings also indicate that *L. excelsa* possessed strong adaptability to the environment, highlighting its breeding potential.

Correlation analysis revealed that LL and AR were significantly negatively correlated with elevation, indicating that the leaf length and aspect ratio of *L. excelsa* decreased as elevation increases. Previous research had shown that plant leaf size often varied with elevation (Liu et al. 2020; Milla and Reich 2011). As elevation increases, leaf size typically decreases due to significant changes in environmental conditions, such as lower temperatures, reduced air pressure, and diminished light intensity. These changes affect plant growth and survival, prompting adaptive responses. Leaf size is a crucial indicator of a plant's adaptation to its environment (Wang et al. 2019). At high altitudes with lower light intensity, plants may reduce leaf area to minimize water loss through transpiration and photosynthetic inefficiency, enhancing leaf functional efficiency. This adaptive strategy helped plants survive and reproduce successfully in varied elevation conditions (Gonzalo-Turpin and Hazard 2009), optimizing leaf function under ecological pressures and limited resources (Niklas et al. 2023).

Table 6. Principal component (PC) analysis of leaf trait variation among different *Lagerstroemia excelsa* populations.

	LL	LW	LA	LC	AR	LBA	LVA	MF
PC_1	0.987	0.703	0.987	0.987	0.721	0.112	0.618	−0.554
PC_2	0.119	0.657	0.987	0.112	−0.553	0.551	−0.509	0.667

AR = aspect ratio; LA = leaf area (cm^2); LBA = leaf base angle; LC = leaf circumference (cm); LL = leaf length (cm); LVA = leaf vein angle; LW = leaf width (cm); MF = morphometric factor.

The results also showed that the leaf length of *L. excelsa* increased with the rise in the maximum temperature of the warmest month. This finding aligned with the distribution patterns of tree species in forests. In warmer, more humid lower-latitude regions, broadleaf forests with larger leaves dominated, whereas colder, drier higher-latitude regions were characterized by coniferous forests with smaller leaves (Kira 1991). The significant increase in leaf length with higher maximum temperatures indicates that *L. excelsa* develops longer leaves in warmer environments. In lower-latitude regions with higher temperatures, abundant rainfall, and high humidity, the vapor pressure gradient on leaf surfaces was low, which hindered transpiration. A narrower leaf shape, characterized by increased leaf length and minimal width changes, facilitates transpiration and reduces leaf temperature, reflecting an adaptation to high-temperature conditions.

PCA of *L. excelsa* leaf traits identified the leaf size factor and leaf shape factor as two major principal components influencing leaf morphology, with a cumulative contribution rate of 80%. PCA is a dimensionality reduction technique that transforms multiple correlated variables into a smaller set of uncorrelated variables, known as principal components (Salem and Hussein 2019). These components are arranged by the variance they explain, and the contribution rate shows their explanatory power for the overall data variability (Dunteman 1989). The high contribution rates of the first two principal components suggested that the leaf size and shape factors effectively captured leaf morphological variation, indicating that most variability was concentrated along these dimensions.

Cluster analysis grouped the 10 *L. excelsa* populations into two main clusters: TR and BL formed one category, and WL, TZ, ES, SZ, CB, MT, CN, and GZ comprised the other. This demonstrated a degree of phenotypic

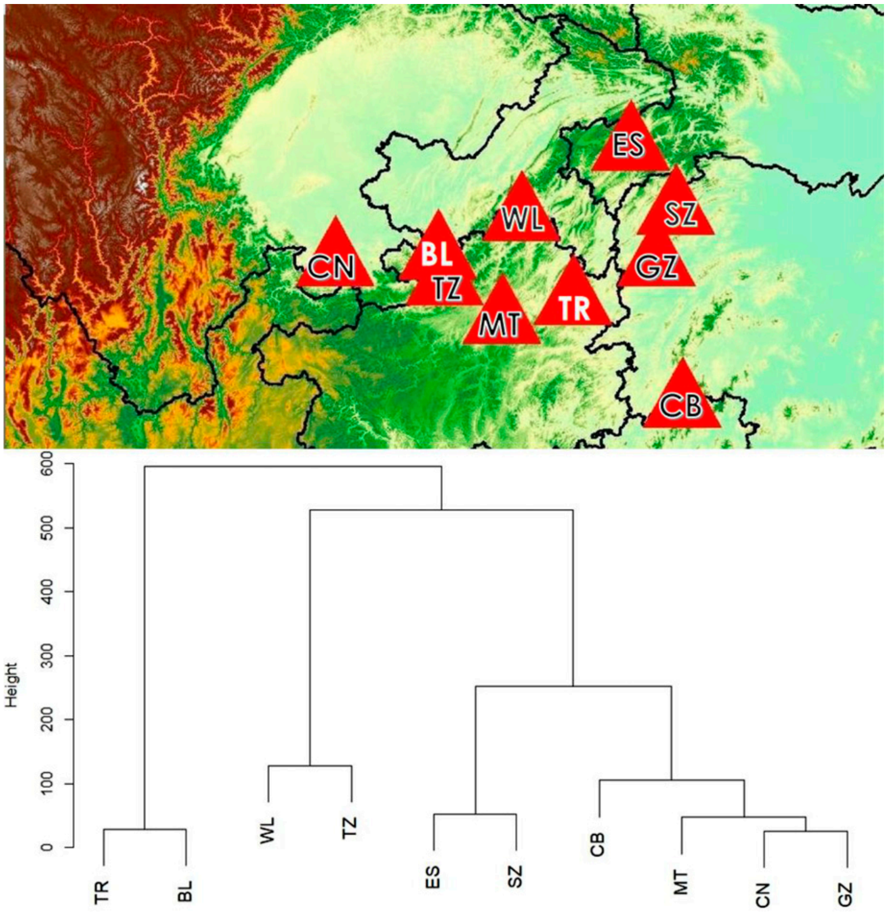

Fig. 2. Cluster analysis of leaf morphological characteristics. Using the coefficient of variation of four leaf traits—leaf vein angle (LVA), leaf area (LA, cm^2), leaf base angle (LBA), and leaf length (LL, cm)—the 10 populations were initially grouped into two main categories.

diversity among *L. excelsa* populations. On the basis of their geographic distribution, most similar germplasm sources are located in contiguous regions. This suggests that over long-term evolution, different populations have undergone directional differentiation under various natural selection pressures, resulting in distinct variations in leaf morphological traits. This study classifies *L. excelsa* populations based on the morphological traits of leaves from different geographic regions, shedding light on the geographic variation trends in leaf morphology.

Conclusion

This study examined the leaf morphological diversity of *L. excelsa* from 10 populations across 5 provinces in China, analyzing 960 leaf samples and 8 phenotypic traits using statistical methods. The results revealed significant variation and rich diversity in leaf phenotypic traits, with various degrees of correlation among the 8 traits. Leaf size–related traits of *L. excelsa* were notably influenced by environmental changes, whereas overall leaf morphology remained relatively stable. The rapid increase in cumulative contribution rates of all traits suggested relatively low genetic diversity in multiple directions of *L. excelsa* leaf morphology.

Cluster analysis grouped the 10 populations into two main categories, with similar germplasm found to be distributed contiguously. This highlights the distinct geographic characteristics of leaf morphology in *L. excelsa* and provides valuable insights for the geographic classification of its germplasm. These findings are essential for future efforts in the introduction, breeding, selection, and cultivation of *L. excelsa*.

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