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Influence of Geographical and Climatic Factors on *Quercus variabilis* Blume Fruit Phenotypic Diversity

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Abstract: *Quercus variabilis* Blume is one of the most ecologically valuable tree species in China and is known to have adaptive mechanisms to climate change. Our objective was to quantify the variation pattern in the fruit morphology of *Q. variabilis*. Fruit samples were collected from 43 natural populations in autumn of 2019. Our results indicated that the coefficient of variation (CV) of the fruit length (FL) and fruit width (FW) were 10.08% and 11.21%, respectively. There were significant differences in the FL, FW, and fruit length-to-width ratios (FL/FW) among the studied populations. Also, there was a significant positive correlation between the FW and FL. The FL decreased with increasing precipitation in the wettest quarter (PWQ). A concave trend was observed in the variations in FL with the equivalent latitude (ELAT), longitude (LON), annual mean air temperature (MAT), and annual precipitation (AP). A similar concave trend was observed for the FL/FW with LON, MAT, and AP. A positive correlation was observed between the FW, FL and FL/FW, and the ELAT. The cluster analysis revealed five groups of the 43 natural populations. Our study findings suggests that *Q. variabilis* has high levels of phenotypic plasticity for geographical and climatic factors.

Keywords: climate factor; equivalent latitude; longitude; fruit size; oak

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Citation: Gao, S.; Ren, Y.; Masabni, J.; Zou, F.; Xiong, H.; Zhu, J. Influence of Geographical and Climatic Factors on *Quercus variabilis* Blume Fruit Phenotypic Diversity. *Diversity* **2021**, *13*, 329. https://doi.org/10.3390/d13070329

Academic Editor: Jorge Capelo

Received: 7 June 2021
Accepted: 15 July 2021
Published: 18 July 2021

1. Introduction

Phenotypic variation among a population of the same species usually reflects the plant's response to different environmental pressures [1,2]. The phenotypic diversity-commonly refers to leaf, flower, fruit, and seed morphology [3–8]. In general, seed size is considered a component of a co-evolving complex of variables, including plant biomass, dispersal, niche specialization, seed dormancy, and competitiveness [9]. Seed size also affects plant dissemination, settlement, and individual development [10,11]. Seed phenotype is malleable to changes in the environment that allow the plant to be better fitted to environmental conditions [1].

Studying the variation pattern of seed morphology and its relationship with environmental factors helps in the understanding of plant plasticity in response to changes in environmental factors and reveals the role of genetics and the environment in plant ecological adaptation [12]. The diversity of fruit morphology often indicates adaptation to different dispersal modes [13]. Recent research has focused on fruit size differences [14,15] and the relationship between the environment and fruit characteristics [16–18]. Maranz and Wiesman [19] found that temperature and rainfall had significant effects on fruit size. Wu et al. [20] indicated that fruit size (length and width) decreased with longitude from west to east. Also, Liu et al. [21] noted an increase in fruit width and fruit weight with longitude, and a gradual decrease with increasing latitude. Leal-Sáenz et al. [22] suggested that populations found in warmer and wetter climates had larger fruits. Soil properties can influence fruit size as well. For instance, the sweet chestnut populations from acidophilic and thermophilic forests were characterized with smaller fruits. In contrast, populations from forests of mesophilic character were characterized with larger fruits [23].

More than 400 species of the genus *Quercus* L. (Fagaceae) are distributed in the northern hemisphere, and there are 51 species of *Quercus* in China [24]. *Quercus variabilis* Blume is an important deciduous broadleaved oak of benefit to the ecology, economy, and culture in China [25,26]. At present, research on *Q. variabilis* has focused mainly on biological characteristics [27,28], population ecosystems [25], and physiological characteristics [29,30]. In this study, we analyzed the morphological characteristics of fruit from 43 natural populations of *Q. variabilis*. To understand the morphological variation of *Q. variabilis* fruits due to environmental influences, we asked: what is the degree of fruit phenotypic diversity within and among populations of *Q. variabilis*? What are the factors playing key roles in the fruit morphological variation of the *Q. variabilis* population? Thus, the aim of this study was to quantify the phenotypic diversity of *Q. variabilis* fruits and the response to climate factors.

2. Materials and Methods

2.1. Study Area

In the geographical distribution area of *Q. variabilis* in China [31], the climate may be temperate, warm temperate, north subtropical, and south subtropical. The soil types in these regions may be dark brown, cinnamon, yellow, red, and other main zonal soil types [12]. Forty-three typical natural secondary forests of *Q. variabilis* were selected in seventeen Chinese provinces (Figure S1; Table 1). The geographical span of the selected region ranged between 23.33° N (Honghe Prefecture, Yunnan) and 43.83° N (Urumqi city, Xinjiang), and between 87.62° E (Urumqi city, Xinjiang) and 123.30° E (Liaoyang city, Liaoning). The annual precipitation ranged between 231 mm (Urumqi city, Xinjiang) and 1556 mm (Nanchang city, Jiangxi). The wettest season precipitation ranged from 87 mm in Urumqi, Xinjiang, to 761 mm in Honghe Prefecture, Yunnan. The average temperature was 7.06 °C in Urumqi, Xinjiang, and 17.78 °C in Changsha city, Hunan. The highest monthly temperature ranged from 20.16 °C in Linzhi city, Tibet, to 33.42 °C in Nanchang city, Jiangxi (Table 1).

2.2. Sample Collection

In each forest stand, we selected a study area with healthy *Q. variabilis* trees and with little to no human interference. We established a 20 m × 20 m plot and recorded the latitude, longitude, and altitude of the test plot [12]. At the peak of fruit maturity in autumn 2019, at least 100 fully developed, disease-free fruits were collected from each plot. Fruits were sterilized by soaking in 5% chlorine bleach for ten minutes [32]. The samples were placed in separate nylon bags and air dried, then stored at 2 °C until the measurements were done [33].

Table 1. Geographical locations and climatic conditions of the 43 study sites of *Q. variabilis* in China.

Site Number	Site Location	Code	LON (° E)	LAT (° N)	ALT (m)	MAT (°C)	MTW (°C)	AP (mm)	PWQ (mm)	ELAT (°)
1	Chuzhou City, Anhui Province	CZA	117.97	32.35	80	15.28	30.89	939	448	31.25
2	Huainan County, Anhui Province	HNA	117.00	32.63	49	15.64	31.64	897	431	31.37
3	Mentougou District, Beijing	MTB	116.09	39.96	213	11.70	30.25	552	422	39.53
4	Miyun County, Beijing	MYB	117.07	40.50	357	9.75	28.76	514	382	40.90
5	Pinggu District, Beijing	PGB	117.13	40.28	353	9.90	28.59	530	399	40.66
6	Tianshui City, Gansu Province	TSG	106.55	34.47	1189	10.81	26.91	642	341	40.82
7	Baise City, Guangxi Province	BSG	106.55	24.77	142	16.55	27.41	1270	682	23.98
8	Anlong county, Guizhou Province	ALG	104.70	24.85	1697	14.66	24.19	1183	636	34.82
9	Xingren County, Guizhou Province	XGG	104.95	25.25	1298	15.94	26.16	1265	676	32.38
10	Dengfeng City, Henan Province	DFH	113.05	34.45	371	13.46	29.76	673	365	34.96
11	Jiyuan City, Henan Province	JYH	112.60	35.07	155	14.60	31.95	567	326	34.34
12	Lushi County, Henan Province	LSH	111.05	34.05	880	13.33	30.58	671	335	38.20
13	Nanzhao County, Henan Province	NZH	112.43	33.46	251	15.11	31.24	804	382	33.21
14	Tongbai County, Henan Province	TBH	113.68	32.53	170	15.13	30.86	974	444	31.88
15	Badong County, Hubei Province	BDH	110.34	31.04	598	15.14	30.07	1216	540	33.17
16	Baokang County, Hubei Province	BKH	111.26	31.88	680	13.72	29.35	1058	472	34.59
17	Enshi City, Hubei Province	ESH	109.49	30.28	491	16.26	31.48	1468	652	31.65
18	Jianshi County, Hubei Province	JSH	109.73	30.60	730	14.84	29.81	1383	600	33.67
19	Jingshan City, Hubei Province	JSA	113.12	31.02	103	16.08	31.17	1071	467	30.03
20	Suixian County, Hubei Province	SXH	112.98	31.53	287	15.05	30.20	1033	447	31.46
21	Jiangui County, Hubei Province	JGH	110.98	30.83	610	15.62	30.49	1167	536	33.04
22	Wuhan City, Hubei Province	WHH	114.31	30.59	27	17.26	33.00	1265	561	29.23
23	Zhushan County, Hubei Province	ZSH	110.23	32.22	418	15.28	31.21	1004	454	33.07
24	Xiangxi Prefecture, Hunan Province	XPH	109.74	28.31	277	17.33	32.57	1339	604	28.20
25	Changsha City, Hunan Province	CSH	112.94	28.23	61	17.78	33.33	1403	597	27.03
26	Nanchang City, Jiangxi Province	NCJ	115.83	28.76	37	17.70	33.42	1556	726	27.44
27	Dalian City, Liaoning Province	DLL	121.79	39.10	137	10.22	26.69	646	402	38.28
28	Liaoyang City, Liaoning Province	LYL	123.30	41.08	171	8.04	27.25	742	473	40.43
29	Yantai City, Shandong Province	YTS	121.74	37.26	222	11.13	26.39	721	434	36.87
30	Xia County, Shanxi Province	XCS	111.37	35.01	1185	9.95	26.49	611	333	41.33
31	Baoji City, Shaanxi Province	BJS	107.14	34.37	680	13.11	30.25	681	358	37.08
32	Shanyang County, Shaanxi Province	SYS	109.88	33.53	726	12.93	29.03	778	375	36.58

Table 1. *Cont.*

Site Number	Site Location	Code	LON (° E)	LAT (° N)	ALT (m)	MAT (°C)	MTW (°C)	AP (mm)	PWQ (mm)	ELAT (°)
33	Shangnan County, Shaanxi Province	SNS	110.88	33.53	826	14.69	31.05	774	373	37.29
34	Weinan County, Shaanxi Province	WNS	109.50	34.50	536	13.16	30.94	606	297	36.19
35	Xianyang City, Shaanxi Province	XYS	108.08	34.27	486	12.76	29.78	648	326	35.60
36	Linzhi City, Tibet	LZT	94.36	29.65	3164	7.90	20.16	651	359	50.11
37	Urumqi, Xinjiang	UQX	87.62	43.83	899	7.06	30.22	231	87	48.10
38	Anning City, Yunnan Province	ANY	102.45	24.99	1852	15.25	24.81	898	496	36.07
39	Honghe Prefecture, Yunnan Province	HHY	103.61	23.33	1655	14.71	22.98	1367	761	33.01
40	Kunming, Yunnan Province	KMY	102.75	25.14	2051	14.30	23.70	921	509	37.65
41	Zhanyi County, Yunnan Province	ZYY	103.55	25.59	2214	13.84	23.23	938	515	39.27
42	Lin'an District, Zhejiang Province	LAZ	119.44	30.33	320	14.78	30.13	1399	554	30.47
43	Yuhang District, Zhejiang Province	YHZ	120.30	30.42	9	16.51	32.29	1262	472	28.96

ELAT, equivalent latitude; LON, longitude; LAT, latitude; ALT, altitude; MAT, annual mean air temperature; MTW, maximum temperature of warmest month; AP, annual precipitation; and PWQ, precipitation of wettest quarter. The climate data for all sites were obtained from the Global Climate and Weather Data website (http://www.worldclim.org (accessed on 15 December 2020)).

2.3. Geographic Information and Climate Data of the Sample Plot

The climate data for all sites, including the annual mean air temperature (MAT), annual precipitation (AP), maximum temperature of warmest month (MTW), and precipitation of wettest quarter (PWQ), were obtained from the Global Climate and Weather Data website (http://www.worldclim.org (accessed on 15 December 2020)) [34] and are presented in Table 1. We used ArcMap10.8 to extract climate data from the WorldClim data website according to Slave [35]. Due to the large differences in altitude among sites, we converted all latitudes into equivalent latitudes (ELAT), as proposed by Alena et al. [36], in order to determine the true effect of latitude and eliminate the influence of altitude factors. The conversion formulas used are as follows:

$$\text{equivalent latitude} = \text{latitude} + (\text{altitude} - 300)/200 \text{ (for altitude lower than 300 m)}$$

$$\text{equivalent latitude} = \text{latitude} + (\text{altitude} - 300)/140 \text{ (for altitude greater than 300 m)}$$

2.4. Determination of Fruit Morphological Characters

The fruit width and fruit length of at least 20 fruits in each population were measured by vernier caliper with an accuracy of 0.01 mm [30]. Fruit width (FW) refers to the distance between the widest points on the left and right side of the fruit, and fruit length (FL) refers to the distance from the bottom to the top of the fruit. All measurements were accurate to two decimal places. Each fruit was measured three times and the data was averaged. The fruit length-to-width ratio (FL/FW) of each fruit was also calculated [17].

2.5. Statistical Analysis

First, the main statistics (arithmetic means, maximum value, minimum value, standard deviation, and coefficient of variation) were calculated for the studied characteristics and populations. Second, a oneway analysis of variance (ANOVA), as implemented in SPSS 25.0 software (IBM company, New York, NY, USA), was used to test the differences between the studied populations [37]. Significant differences among means were assessed using Duncan's multiple comparison at $p \leq 0.05$. Origin 2019b software (Origin Lab company, Northampton, MA, USA) was used to quantify the variability in phenotypic traits as influenced by regional climatic factors [38]. To eliminate dimension and the order of magnitude, the original data were standardized, converted into dimensionless data with a mean of 0 and a variance of 1, and then principal component analysis (PC) was performed. Cluster analysis was carried out by the between groups linkage method and measured according to squared Euclidean distance. Canoco5 (Microcomputer Power, New York, NY, USA) was used in the principal component analysis (PCA) of *Q. variabilis* fruits morphology [39]. These statistical analyses were performed using SPSS 25.0 [1]. Correlation between phenotypic traits and environmental factors were analyzed using Pearson correlation analysis by SPSS 25.0 [40]. In addition, we computed and tested the correlations between: (1) the matrix of geographical distances between pairs of populations and the matrix of morphological differences among populations—the isolation-by-distance pattern [41], and between (2) the matrix of environmental distances and the matrix of morphological differences among populations—the isolation-by-environmental distance [42]. The significance level was assessed after 9,999 permutations, and the simple Mantel test was performed with the R package "Vegan" [43].

3. Results

3.1. Fruit Morphological Characters and Variation Characteristics

The width, length, and length-to-width ratio of fruits were significantly different among the various populations (Tables 2 and 3). The variation in fruit width among groups ranged between 7.96 mm and 27.17 mm, with an average of 17.35 mm. Fruits from the SXH site had the largest width (20.93 mm), while the smallest fruit width was observed in fruits from ESH (11.70 mm). Fruit length ranged between 9.96 mm and 35.04 mm, with an average of 19.97 mm. The average fruit length was largest in UQX (31.69 mm), and shortest in fruits came from BDH (17.17 mm). Fruit length-to-width ratio of fruits ranged between 0.70 and 2.31, with an average value of 1.17. The mean variation coefficient of fruit width, fruit length, and fruit length-to-width ratio among the different populations were 11.21%, 10.08%, and 14.48%, respectively (Table 2). In addition, we revealed that there was a significant correlation between *Q. variabilis* fruit width and fruit length of *Q. variabilis* ($R^2 = 0.17$, $p \leq 0.0001$) (Figure S2).

3.2. The Relationship in the Variation Pattern between Fruit Morphology and Geographical and Environmental Factors

Results of the correlation analysis between the various morphological indicators of *Q. variabilis* fruits and the geographical and ecological factors are presented in Figure 1. Fruit length and ELAT had a concave variation trend ($R^2 = 0.12$; $p = 0.029$; $y = 0.016x^2 - 1.1x + 37.94$), with a minimum value at 33°–35° N (Figure 1A). Fruit length also had a concave variation trend with longitude ($R^2 = 0.43$; $p < 0.0001$; $y = 0.014x^2 - 3.19x + 198.26$), with a minimum value at 111°–113° E (Figure 1B). The fruit length-to-width ratio did not correlate with ELAT, but showed a concave variation trend with longitude ($R^2 = 0.12$; $p = 0.027$; $y = 0.0001x^2 - 0.15x + 9.55$), with a minimum value at 111°–113° E (Figure 1B).

Fruit length and annual mean air temperature showed a concave variation trend ($R^2 = 0.16$; $p = 0.013$; $y = 0.096x^2 - 2.65x + 37.58$) and reached a minimum value at 13.46–14.65 °C (Figure 1C). Fruit length-to-width ratio and annual mean air temperature also showed a concave variation trend ($R^2 = 0.17$; $p = 0.01$; $y = 0.009x^2 - 0.24x + 2.69$) and reached a minimum value at 12.93–13.72 °C (Figure 1C). However, fruit length and length-to-width ratio did not correlate with the maximum temperature of the warmest month (Figure 1D).

Fruit length and annual precipitation showed a concave variation trend ($R^2 = 0.36$; $p < 0.0001$; $y = 0.0001x^2 - 0.024x + 31.29$) and reached a minimum value at 974–1071 mm (Figure 1E). The fruit length-to-width ratio and annual precipitation showed a concave variation trend ($R^2 = 0.34$; $p < 0.0001$; $y = 0.0001x^2 - 0.002x + 2.02$) and reached a minimum value at 939–1004 mm (Figure 1C). Fruit length was negatively correlated with precipitation in the wettest quarter ($R^2 = 0.10$; $p = 0.029$; $y = -0.005x + 22.53$) (Figure 1F). But there was no correlation between the fruit length-to-width ratio and precipitation in the wettest quarter.

Fruit width, fruit length, and fruit length-to-width ratio were positively correlated with the equivalent latitude (ELAT) (Table 4). However, fruit length was significantly negatively correlated with precipitation of the wettest quarter and longitude.

The Simple Mantel test identified significant correlations between the morphological, geographic, and climatic distance matrices (Table 5). Correlations were higher between morphological and climatic matrices ($r = 0.988$, $p < 0.001$), and weaker, but significant, correlations were observed between the morphological and geographical matrices ($r = 0.633$, $p < 0.001$).

Table 2. Statistical data of fruit length (FL), fruit width (FW), and FL/FW of 43 populations of *Q. variabilis*.

Site	FL					FW					FL/FW					Sample Size
	Mean	Max	Min	SD	CV (%)	Mean	Max	Min	SD	CV (%)	Mean	Max	Min	SD	CV (%)	
1	19.51 ^{defghijk}	23.56	15.02	1.71	8.74	18.21 ^{ijk}	26.78	13.13	1.58	8.70	1.07 ^{bcde}	1.58	0.70	0.10	9.08	100
2	20.42 ^{hijkl}	24.22	15.88	1.13	5.53	19.99 ^{lmn}	27.17	13.82	1.50	7.51	1.04 ^{abc}	1.32	0.76	0.06	6.21	120
3	19.20 ^{cdefghij}	22.94	15.49	1.49	7.79	14.82 ^{bc}	18.46	10.87	1.26	8.50	1.30 ^{i}	1.60	1.05	0.07	5.56	80
4	20.52 ^{ijkl}	22.75	18.68	0.97	4.74	18.88 ^{klm}	21.90	16.33	1.43	7.56	1.09 ^{bcde}	1.36	0.94	0.10	9.26	20
5	20.40 ^{hijkl}	24.27	16.69	1.89	9.26	17.42 ^{fghijk}	20.97	15.00	1.41	8.09	1.18 ^{efgh}	1.50	0.86	0.14	11.55	20
6	17.81 ^{abc}	21.52	13.36	2.12	11.88	15.75 ^{cde}	19.52	12.44	1.77	11.26	1.14 ^{cdefg}	1.40	0.94	0.12	10.55	20
7	20.31 ^{hijkl}	24.49	14.14	2.13	10.48	16.53 ^{defghi}	20.69	13.75	1.57	9.52	1.23 ^{fghi}	1.52	1.00	0.13	10.46	30
8	19.53 ^{defghijk}	22.80	13.45	1.92	9.82	18.82 ^{klm}	24.56	14.26	2.07	10.99	1.04 ^{abc}	1.24	0.85	0.10	9.37	30
9	21.15 ^{klmn}	23.84	18.20	1.42	6.71	20.26 ^{mn}	22.89	17.97	1.02	5.03	1.04 ^{abc}	1.23	0.90	0.07	6.89	30
10	19.49 ^{defghijk}	26.80	13.97	1.60	8.23	17.42 ^{fghijk}	22.08	12.57	1.48	8.48	1.12 ^{bcdef}	1.61	0.82	0.10	8.72	438
11	21.69 ^{lmn}	26.10	17.08	1.61	7.44	17.68 ^{fghijk}	21.57	13.97	1.23	6.94	1.23 ^{fghi}	1.50	0.98	0.09	6.94	60
12	19.89 ^{efghijk}	20.74	18.92	0.46	2.29	17.07 ^{efghij}	18.83	14.47	1.27	7.41	1.17 ^{defgh}	1.36	1.00	0.09	7.85	20
13	18.65 ^{abcdefg}	23.00	13.90	1.63	8.76	16.20 ^{cdef}	21.50	10.45	1.53	9.43	1.16 ^{cdefgh}	1.76	0.85	0.11	9.25	80
14	20.36 ^{hijkl}	24.45	15.70	1.29	6.35	20.20 ^{mn}	23.42	16.6	1.49	7.35	1.01 ^{ab}	1.20	0.86	0.05	5.15	60
15	17.17 ^{a}	24.35	9.96	1.43	8.33	14.95 ^{bc}	20.08	9.78	1.26	8.44	1.14 ^{cdefg}	1.44	0.80	0.12	10.82	60
16	19.71 ^{fghijkl}	22.82	14.62	1.32	6.70	17.82 ^{ghijk}	20.40	14.26	1.05	5.89	1.11 ^{cdefg}	1.41	0.90	0.09	8.55	40
17	17.22 ^{a}	20.79	11.56	1.64	9.54	11.70 ^{a}	13.79	9.28	0.91	7.74	1.48 ^{j}	1.99	1.03	0.16	10.62	60
18	20.27 ^{ghijkl}	23.89	17.58	1.91	9.41	18.19 ^{ijk}	21.99	15.31	1.66	9.14	1.12 ^{bcde}	1.41	0.99	0.10	8.87	30
19	20.05 ^{fghijkl}	23.58	17.02	1.05	5.25	17.29 ^{efghijk}	21.63	13.55	1.16	6.69	1.17 ^{defgh}	1.62	0.94	0.09	7.31	60
20	20.08 ^{fghijkl}	22.02	16.65	1.21	6.04	20.93 ^{n}	23.13	18.49	1.12	5.36	0.96 ^{a}	1.12	0.82	0.07	7.25	30
21	18.90 ^{bcdefghi}	22.82	14.62	1.95	10.34	17.72 ^{fghijk}	19.88	15.30	1.19	6.70	1.07 ^{abcde}	1.36	0.90	0.13	12.22	20
22	19.51 ^{cdefghijk}	24.49	13.79	1.28	6.57	17.73 ^{fghijk}	23.33	12.72	1.20	6.75	1.10 ^{bcde}	1.45	0.81	0.07	6.78	99
23	20.95 ^{jklm}	22.73	19.19	0.90	4.30	19.70 ^{lmn}	21.72	17.22	1.06	5.38	1.06 ^{abcde}	1.13	0.96	0.05	4.49	20
24	19.99 ^{efghijk}	23.93	15.12	1.50	7.52	13.58 ^{b}	18.20	9.46	1.34	9.84	1.50 ^{j}	2.01	1.08	0.13	8.47	49
25	19.58 ^{defghijk}	26.79	12.87	1.35	6.90	11.89 ^{a}	20.17	7.96	0.85	7.11	1.68 ^{k}	2.25	1.01	0.14	8.26	180
26	21.12 ^{klmn}	23.61	17.46	1.51	7.14	19.99 ^{lmn}	22.34	16.79	1.54	7.68	1.06 ^{abcde}	1.22	0.95	0.06	5.95	20
27	19.93 ^{efghijk}	22.45	16.91	1.49	7.49	16.16 ^{cdefg}	18.21	12.74	1.34	8.27	1.24 ^{ghi}	1.44	0.96	0.11	8.62	20
28	19.00 ^{bcdefghi}	24.73	14.30	2.06	10.85	16.96 ^{defghij}	21.32	13.19	2.08	12.27	1.13 ^{bcdefg}	1.42	0.93	0.12	10.85	30
29	19.79 ^{efghijk}	26.69	15.66	1.69	8.55	15.73 ^{cde}	19.03	12.22	1.27	8.08	1.26 ^{hi}	1.86	1.04	0.11	8.82	80
30	18.42 ^{abcdef}	20.94	15.68	1.42	7.71	17.34 ^{efghijk}	21.42	14.32	1.53	8.81	1.07 ^{abcde}	1.22	0.91	0.07	6.65	20
31	22.46 ^{n}	24.86	20.48	1.29	5.74	15.45 ^{cd}	17.23	13.68	1.06	6.86	1.46 ^{j}	1.58	1.34	0.06	4.38	20
32	18.84 ^{abcde}	21.78	14.62	1.43	7.56	17.54 ^{efghijk}	21.42	12.07	1.44	8.19	1.08 ^{abcde}	1.35	0.84	0.08	7.69	60
33	19.83 ^{efghijk}	21.78	17.02	1.01	5.12	17.88 ^{ghijk}	21.42	14.16	1.58	8.81	1.11 ^{bcde}	1.30	0.91	0.08	7.55	20

Table 2. *Cont.*

Site	FL					FW					FL/FW					Sample Size
	Mean	Max	Min	SD	CV (%)	Mean	Max	Min	SD	CV (%)	Mean	Max	Min	SD	CV (%)	
34	18.04 ^{abcd}	22.62	14.08	1.78	9.89	17.06 ^{efghij}	21.37	14.09	1.46	8.54	1.06 ^{abcde}	1.28	0.81	0.11	10.09	60
35	20.55 ^{ijkl}	24.04	16.24	1.91	9.30	17.93 ^{hijk}	20.88	13.72	1.53	8.54	1.16 ^{cdefg}	1.44	0.86	0.15	13.25	30
36	18.63 ^{abcdefgh}	22.50	13.84	1.92	10.32	17.78 ^{fghijk}	21.34	13.45	1.87	10.51	1.05 ^{abcd}	1.25	0.86	0.09	8.76	30
37	31.69 ^{o}	35.04	24.65	2.43	7.67	17.86 ^{ghijk}	20.97	14.53	1.76	9.86	1.78 ^{l}	2.31	1.56	0.16	8.97	30
38	18.46 ^{a}	20.64	15.27	1.36	7.38	17.29 ^{defgh}	19.48	15.52	1.04	6.02	1.07 ^{abc}	1.25	0.89	0.06	6.02	50
39	20.67 ^{ijkl}	23.33	18.00	0.93	4.52	18.69 ^{jkl}	21.09	15.77	1.40	7.50	1.11 ^{bcde}	1.34	0.96	0.10	9.16	30
40	17.54 ^{ab}	20.42	11.78	1.80	10.29	16.65 ^{defghi}	20.91	12.28	1.51	9.09	1.06 ^{abcde}	1.24	0.81	0.09	8.93	47
41	22.16 ^{mn}	25.48	17.78	1.50	6.77	18.95 ^{klm}	20.83	15.67	1.16	6.14	1.17 ^{defgh}	1.42	1.03	0.09	7.85	30
42	19.62 ^{defghijk}	24.70	14.76	2.02	10.29	17.69 ^{fghijk}	21.61	15.53	1.44	8.15	1.11 ^{bcde}	1.26	0.91	0.08	7.54	30
43	19.48 ^{defghijk}	21.79	16.01	1.30	6.68	18.14 ^{hijk}	21.46	13.25	1.74	9.60	1.08 ^{bcde}	1.42	0.91	0.11	10.16	30
Total	19.97	23.65	15.77	1.53	7.72	17.35	21.09	13.77	1.40	8.11	1.17	1.46	0.94	0.10	8.41	-
CV between Populations (%)			10.80					11.21					14.48			

Different letters in a column indicate significant differences at $p \leq 0.05$. Refer to Table 1 for details of site locations.

Table 3. ANOVA of fruit width, fruit length, and fruit length-to-width ratio of 43 populations of *Q. variabilis*.

Variance Source		df	SS	MS	F	*p*
Fruit Length	Inter-group	7183	42	171.015	39.28	<0.001
	Intra-group	10,232	2350	4.354		
	Total	17,415	2392			
Fruit Width	Inter-group	11,452	42	272.667	67.70	<0.001
	Intra-group	9464	2350	4.027		
	Total	20,916	2392			
Fruit Length-to-Width Ratio	Inter-group	84	42	2.009	105.42	<0.001
	Intra-group	45	2350	0.019		
	Total	129	2392			

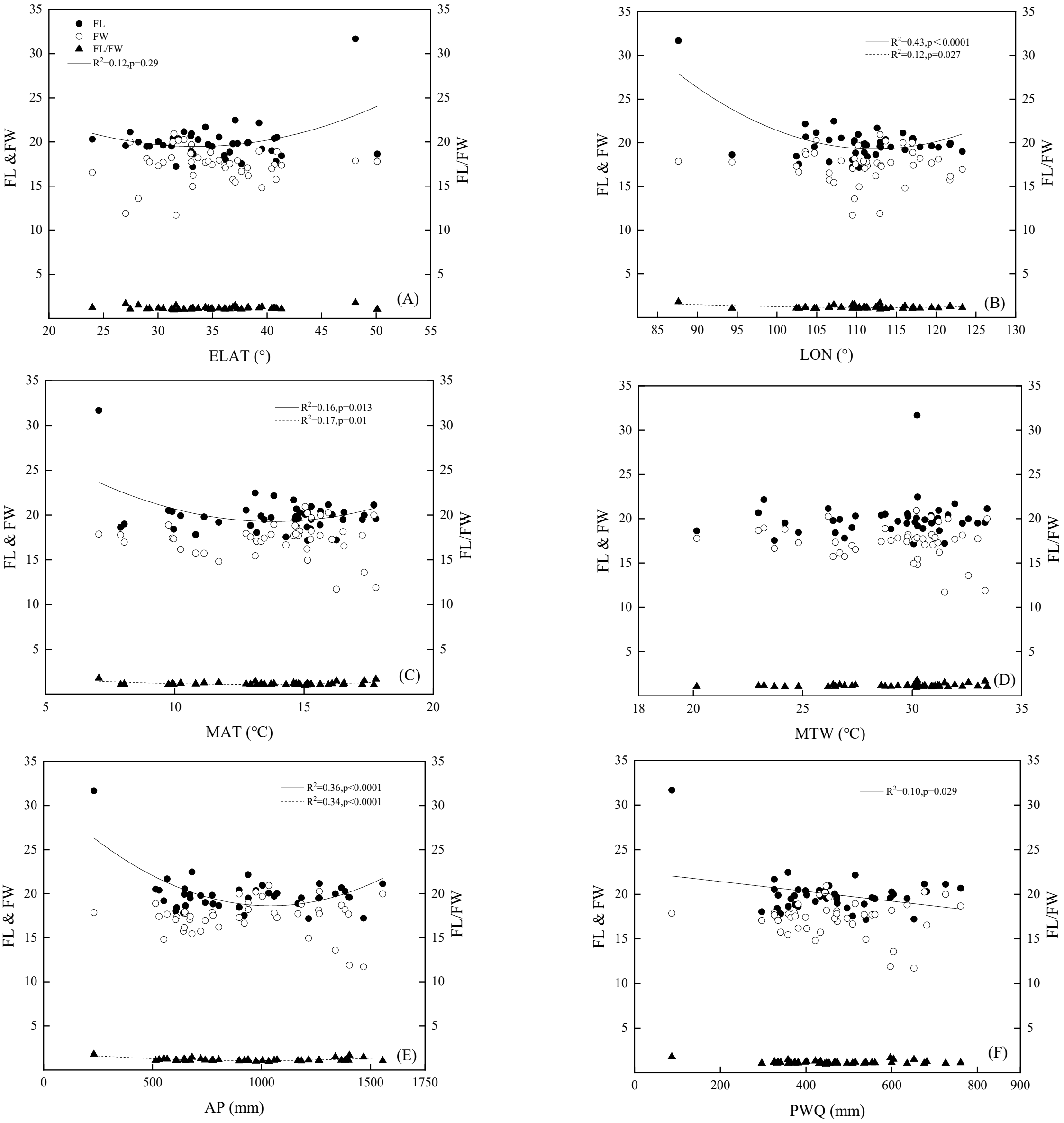

Figure 1. Relationship between fruit width (FW), fruit length (FL), and FL/FW with geographic and climatic factors in 43 populations of *Q. variabilis*. (**A**) relationship between morphological factors with ELAT; (**B**) relationship between morphological factors with LON; (**C**) relationship between morphological factors with MAT; (**D**) relationship between morphological factors with MTW; (**E**) relationship between morphological factors with AP; (**F**) relationship between morphological factors with PWQ. AP, annual precipitation; ELAT, equivalent latitude; LON, longitude; MAT, annual mean air temperature; MTW, maximum temperature of warmest month; and PWQ, precipitation of wettest quarter.

Table 4. Correlation between geoclimatic factors and phenotypic traits of *Q. variabilis* populations.

	LON (°)	ELAT (°)	ALT (m)	MAT (°C)	MTW (°C)	AP (mm)	PWQ (mm)
FL	−0.402 **	0.243	−0.040	−0.261	0.103	−0.282	−0.333 *
FW	−0.038	0.042	0.126	−0.019	−0.155	−0.055	−0.042
FL/FW	−0.244	0.071	−0.174	−0.103	0.263	−0.088	−0.150

AP, annual precipitation; ELAT, equivalent latitude; LON, longitude; MAT, annual mean air temperature; MTW, maximum temperature of warmest month; PWQ, precipitation of wettest quarter; and ALT, altitude. ** $p \leq 0.01$, * $p \leq 0.05$.

Table 5. Correlations between morphological (FL, FW), climatic (MAT, MTW, AP, and PWQ) and geographic (LON, LAT, ALT, and ELAT) distance matrices.

Comparison	r	*p*-Value
Morphological, Geographic	0.633	<0.001
Morphological, Climate	0.988	<0.001

3.3. Principal Component Analysis and Cluster Analysis

Based on the results of principal component analysis (Figure 2, Table 6), 99.60% of the total variance was explained by the first two principal components. The principal component score, associated to each variable on the three principal components, identifies the variables that mostly defined them (Table 6). The PC1, showing 57.57% of total variability, was positively correlated with following variables: FW and FL/FW. The PC2 indicated 42.03% of cumulative variance was positively correlated with FL and FW.

The 43 natural populations of *Q. variabilis* were clustered into five groups with a Euclidean distance of five as the threshold (Figure 3). TBH, SXH, HNA, ZSH, BKH, JGH, JSH, CZA, JSA, WHH, YHZ, LAZ, BDH, NCJ, BSG, MYB, PGB, DLL, YTS, LYL, MTB, TSG, XCS, LSH, SNS, DFH, SYS, XYS, WNS, NZH, JYH, and BJS populations were clustered into the first group, ALG, HHY, XGG, ANY, KMY, and ZYY populations into the second, XPH, CSH, and ESH into the third, LZT was in the fourth, and UQX was in the fifth.

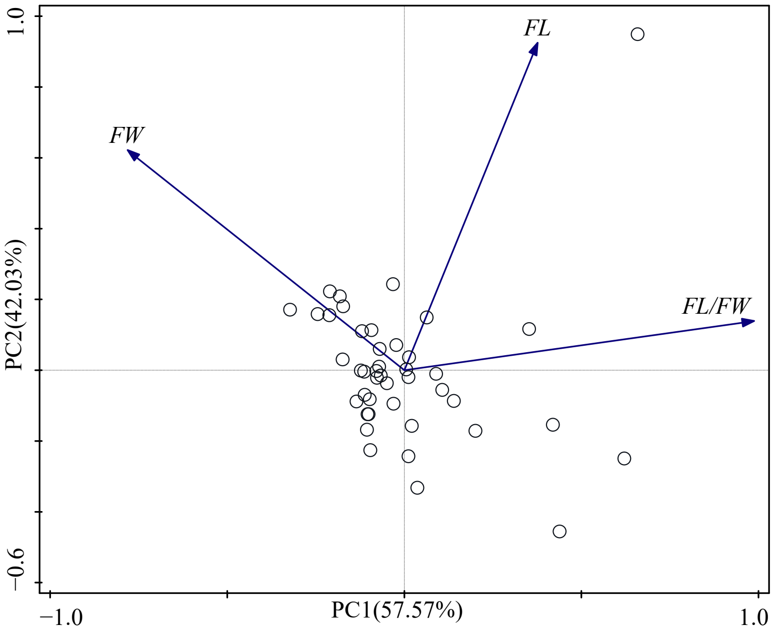

Figure 2. Principal components analysis (PCA) ordination diagram based on morphological variables.

Table 6. Principal component analysis loadings and percentage variance contributions by principal components.

Principal Component	PC1	PC2
FL	0.38	0.93
FW	−0.78	0.62
FL/FW	0.99	0.14
Eigenvalue	1.73	1.26
Variance (%)	57.57	42.03
% Total Variance	57.57	99.60

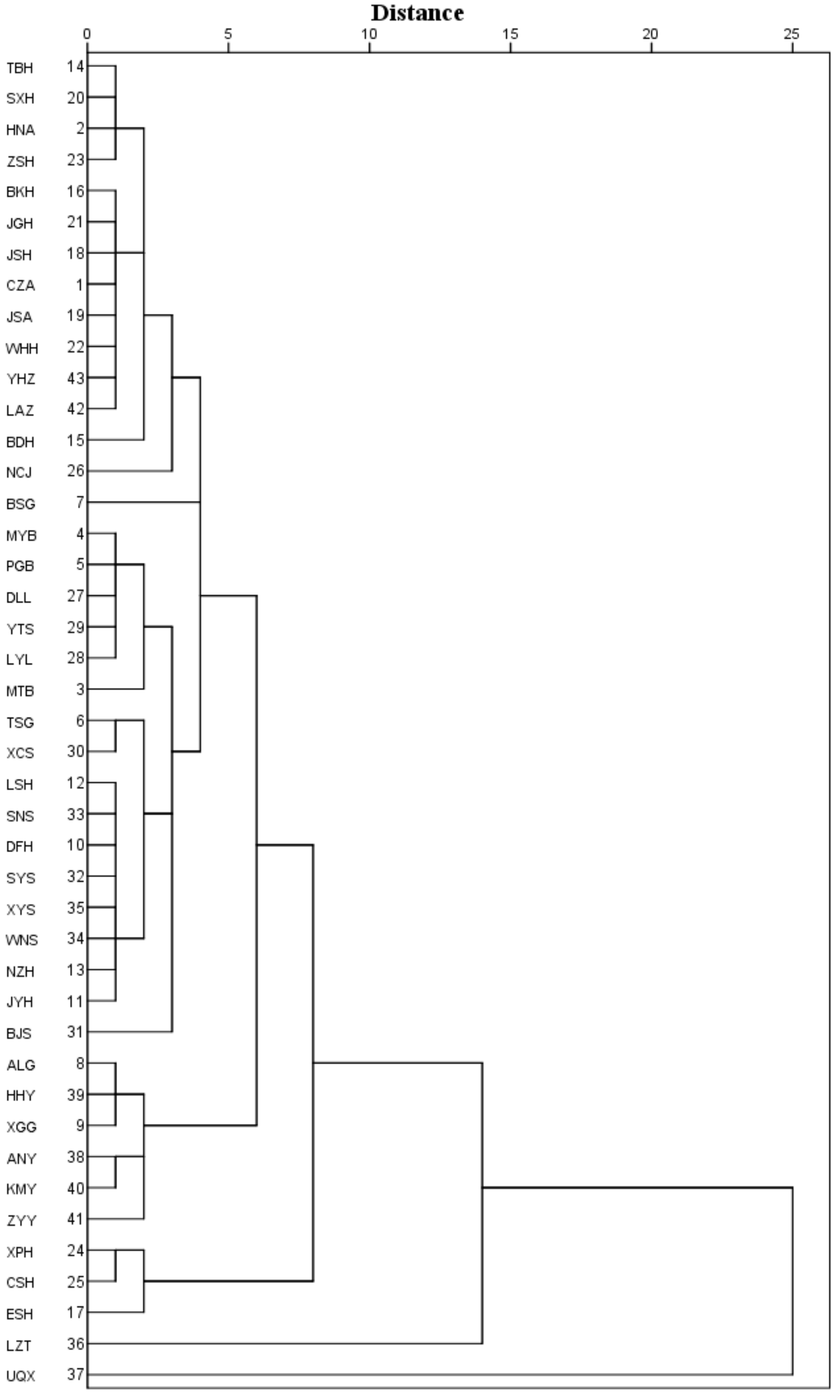

Figure 3. Hierarchical tree dendrogram of 43 populations of *Q. variabilis* based on fruit morphological characters and environmental factors.

4. Discussion

Indeed, fruit morphology can vary significantly among locations, but plants of the same genus can maintain similar fruit size. In this paper, the variation in the average fruit length-to-width ratios of *Q. variabilis* ranged between 0.96 and 1.78. Chen et al. [44] reported that the variation range of the fruit length-to-width ratio of *Q. virginiana* Mill. was between 1.19 and 1.93. Li et al. [45] indicated that the fruit length-to-width ratio of *Q. mongolica* Fisch. ex Ledeb. was between 1.21 and 1.49. Chang et al. [46] observed that the fruit length-to-width ratio of *Q. cocciferoides* Hand.-Mazz. ranged between 0.86 and 1.48. These results are similar with our data. Therefore, the variation in the fruit length-to-width ratios in the *Quercus* genus is relatively moderate. In addition, in the present study, the fruit width and fruit length of *Q. variabilis* showed significant differences among the various populations, with a relatively low intrapopulation variability. These results are consistent with the results published by Zhou et al. [12].

The variation of fruit phenotypic diversity is closely associated with geographical area [1,47]. The geographic isolation from other regions leads to fruit phenotypic variation [20]. Fu et al. [48] showed that the fruit morphological characteristics of *Cornus officinalis* Sieb. have obvious geographical characteristics. Similar trends were observed in our study. Fruit morphological traits of *Q. variabilis* exhibited geographic patterns. The hierarchical tree dendrogram of the 43 populations of *Q. variabilis* showed that populations with close geographic distance, such as the SYS, SNS, WNS, and XYS populations, and the XPH and CSH populations are clustered together. This indicates the regional and continuous pattern of geographic variation. However, a few distant populations were found to be clustered together, such as MYB and LAZ populations, demonstrating random variation patterns. These results indicated that the fruit phenotypic diversity of *Q. variabilis* represented three geographical variation patterns: continuous variation, regional variation, and random variation. In addition, the populations of *Q. variabilis* were characterized by significant phylogeographic structure. The results of previous studies showed morphological variability of seeds along the latitudinal gradient [21,49]. Rewicz et al. [49] found that there was a trend of an increase in seed morphology towards the higher latitude areas. Equally, the results of our study showed that the fruit length, fruit width, and fruit length-to-width ratio of *Q. variabilis* had a positive correlation with the equivalent latitude. This correlation may reflect the adaptability and high phenotypic plasticity of *Q. variabilis*.

In addition to geographical factors, climatic factors also affected the fruit morphology. Fruit phenotypic diversity provides useful results for understanding the adaptation mechanism of plants under different climatic conditions [1,49]. The phenotypic variation was the synthetic effect of multiple climatic factors [50]. The variation trend of *Q. variabilis* fruit width and fruit length, as affected by climatic factors, showed regular variations in fruit morphological characteristics (the fruit width, fruit length, and fruit length-to-width ratio). The fruit length-to-width ratio showed a concave variation trend as affected by annual mean air temperature and annual precipitation, and was similar to fruit length. This showed that the fruit length-to-width ratio of *Q. variabilis* had a synergistic variation in different climatic conditions. Moreover, our results indicated that the fruit width of *Q. variabilis* was negatively correlated with temperature and precipitation, similar to the responses of *Pinus tabuliformis* Carr [50].

Early research on *Quercus* showed that phenotypic diversity was tightly related to local climatic factors with respect to temperature or rainfall [30,51,52]. Thus, the phenotypic characters of *Q. variabilis* should be variable in response to the changes of climatic factors because of its large distribution area. Generally, in a climate of low temperature and low annual precipitation, fruit length is negatively correlated with temperature and rainfall. On the contrary, in a climate of high temperature and high annual precipitation, fruit length is positively correlated with temperature and rainfall [12,53–55]. Similarly, we found various correlation patterns between those phenotypic traits and the climatic factors. In our study, the fruit length of *Q. variabilis* was negatively correlated with precipitation in the wettest quarter. During the development period of fruits between July and September, precipitation in the wettest quarter significantly affected the morphology of *Q. variabilis* fruits. In addition, when the annual precipitation was below 1000 mm, the fruit length was negatively correlated with the annual precipitation. Besides, we found that when the annual precipitation was greater than 1000 mm, the fruit length increased with increasing annual precipitation, and when the temperature was above 13 °C, the fruit length increased with the annual mean air temperature. In addition, we observed that when the temperature was less than 13 °C, the fruit length was negatively correlated with annual mean air temperature. Those various correlation patterns between phenotypic traits and the climatic factors have resulted in a strong adaptability to climate changes.

According to the results of the simple Mantel tests, it was determined that both geographical and climate variables affect the structure of *Q. variabilis* populations [56,57]. Similarly, Poljak et al. [43] suggested that phenotypic divergence of *Alnus incana* (L.) Moench subsp. incana populations was the result of a significant level of isolation both by distance and by the environment. Furthermore, DeWoody et al. [42] revealed that adaptive differentiation and persistent isolation by colonization (IBC) acted in combination to produce the genetic and morphological patterns observed in *Populus nigra* L. populations. In our study, we found that acorn morphological variation was significantly correlated with both climatic heterogeneity and geographic distance.

5. Conclusions

Our study revealed that the phenotypic diversity of *Q. variabilis* fruit is related to both geographical and climatic factors. In terms of geographical factors, the acorn morphology of *Q. variabilis* was significantly different among and within the 43 natural populations, with gradations in different geographical distribution areas. Moreover, the hierarchical tree dendrogram of the five major groups reflects diversified geographic variation patterns of *Q. variabilis* fruit phenotype, namely, random variation, continuous variation, and regional variation. In terms of climatic factors, *Q. variabilis* fruit phenotypic diversity was tightly related to local climate factors with respect to temperature and rainfall. In summary, the fruits of *Q. variabilis* have high phenotypic plasticity, which is conducive to the expansion of its adaptation range.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d13070329/s1, Figure S1: Location of the 43 study sites, covering the geographical distribution of *Quercus variabilis* in China. Figure S2: Correlation analysis between fruit width (FW) and fruit length (FL) of 43 populations of *Quercus variabilis*.

Author Contributions: S.G. contributed the data analysis and wrote the manuscript. H.X., F.Z., Y.R., and J.Z. collected the samples. J.M., H.X., J.Z., and F.Z. revised the manuscript. J.Z. and H.X. secured funding for the research. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the basic scientific research business expenses of the central public welfare scientific research institutes of the Chinese Academy of Forestry (CAFYBB2018ZB001-11), the key research and development program of Hunan Province (2020NK2017), and Innovation and Entrepreneurship training program of Central South University of Forestry and Technology ([2021] no.34).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data in this study are available in presented Tables.

Conflicts of Interest: The authors have no conflict of interest.

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