forests

MDPI

Article

Inter- and Intraspecific Variation Patterns of Leaf Phenotypic Traits in Two Sympatric Pine Species

Yuan Yang [1,†], Meng Hu [1,†], Mingyang Fan [1], Lu Wang [1], Ting Lv [1], Huanchao Zhang [2], Kewang Xu [1], Yifan Duan [1] and Yanming Fang [1,*]

[1] Co-Innovation Center for Sustainable Forestry in Southern China, Key Laboratory of State Forestry and Grassland Administration on Subtropical Forest Biodiversity Conservation, College of Biology and the Environment, Nanjing Forestry University, Nanjing 210037, China
[2] College of Forestry, Nanjing Forestry University, Nanjing 210037, China
* Correspondence: jwu4@njfu.edu.cn; Tel.: +86-25-8542-7428
† These authors contributed equally to this work.

Abstract: Individual persistence under changing climate conditions can be aided by phenotypic plasticity. Needle morphology reflects pine species' adaptation to their habitats, and adaptive plasticity is beneficial to pine survival. As two closely related pines, *Pinus massoniana* Lamb. and *Pinus hwangshanensis* W. Y. Hsia sympatrically occur in the subtropical region of China, forming hilly forests (i.e., subtropical evergreen needleleaf forests) at lower elevations and montane forests (i.e., temperate evergreen needleleaf forests) at medium and high elevations. However, little is known about the intraspecific phenotypic variation patterns of the two pine species and their relationship with environmental factors. Here, we investigated the interspecific and intraspecific variation patterns of needle traits in the two sympatric pine species, focusing on six traits—i.e., leaf length, leaf thickness, leaf width, leaf area, specific leaf area, and leaf dry matter content—as well as the relationship between needle traits and environmental factors. We found significant phenotypic trait differences among populations of each species. Except for leaf length and leaf width, variation within species was greater than variation between species in the needle traits measured. Even more intriguingly, the leaf size traits (i.e., length, width, thickness) were more conservative than the leaf economic traits (i.e., specific leaf area and leaf dry matter content). In other words, the intraspecific variability of the former was weaker than that of the latter. The nature of *P. massoniana* needle traits was mainly shaped by latitude, while the needle traits of *P. hwangshanensis* were significantly affected by annual precipitation. Therefore, phenotypic plasticity may be an essential mechanism for the two pine species to better cope with changing external conditions. The intraspecific variation patterns found in the two pine species and the relationships between traits and environmental factors can provide substantial scientific data for large-scale exploration of intraspecific phenotypic variation in pine species and their breeding practice.

Keywords: phenotypic variation; needle traits; plasticity; interspecific and intraspecific; *Pinus massoniana* Lamb.; *Pinus hwangshanensis* W. Y. Hsia

Citation: Yang, Y.; Hu, M.; Fan, M.; Wang, L.; Lv, T.; Zhang, H.; Xu, K.; Duan, Y.; Fang, Y. Inter- and Intraspecific Variation Patterns of Leaf Phenotypic Traits in Two Sympatric Pine Species. *Forests* **2022**, *13*, 2014. https://doi.org/10.3390/f13122014

Academic Editor: Stéphane Maury

Received: 18 October 2022
Accepted: 24 November 2022
Published: 29 November 2022

1. Introduction

Understanding phenotypic variation (i.e., the measurable or observable variation in a trait) or phenotypic plasticity (i.e., the ability of a given genotype to express different phenotypes in different environmental circumstances) is necessary to investigate a trait's function and fitness, as well as its ecology and evolution [1,2]. Among other hypotheses, phenotypic plasticity is assumed to facilitate evolution via 'buying time', 'plasticity-led evolution', and 'non-genetic evolution' [3]. Environmental phenotypic change precedes evolutionary adaptation, according to the 'Plasticity-first' hypothesis, which uses a mechanism similar to 'buying time', [4]. However, a thorough review of related literature reveals that plasticity can both support and impede evolutionary change, and that it is not always adaptive but can also be neutral or even maladaptive [5].

Conversely, several eco-evolutionary mechanisms—such as dispersal, selection, plasticity, and (epi)genetic mutations—can influence how phenotypic variation is expressed in unexpected contexts or under environmental stress [2]. Empirical studies, reciprocal transplanting strategies, and standard garden experiments have demonstrated that genetic or epigenetic characteristics [6] and environmental factors (e.g., geography, climate, and soil) [7] affect plants' phenotypic variation. Despite this, empirical studies on phenotypic variation in plants (i.e., incredible natural populations) are greatly limited.

Pines are a dominant species in forests of the Northern Hemisphere [8] and form large-scale coniferous forests in Asia, Europe, and North America. With 111 species divided into 2 subgenera, 4 sections, and 11 subsections, the genus *Pinus* enjoys a rich species diversity and an intricate evolutionary history [9]. With the deepening of global changes in climate and biodiversity, pines are facing many challenges, including death from drought stress [10] and disease attacks [11]. Undoubtedly, observing phenotypic variation is critical for delving deeply into pine trees' strategies and mechanisms for coping with global changes.

Among the pine species, *P. massoniana* and *P. hwangshanensis* are two genetically closely related taxa that belong to the subgenus *Pinus*, section *Pinus*, and subsection *Pinus*. Here, *P. hwangshanensis* is treated as an independent species after being merged with *P. taiwanensis*—a species found in Taiwan [12]. The evolutionary events of these pines are related to climate and geography. On the other hand, these two pine species are considered to have distinct ecological characteristics and form coniferous forests with different properties. *P. massoniana* is a pioneer species that forms a subtropical evergreen needleleaf forest [13], whereas *P. hwangshanensis* is a pioneer or climax species that forms a temperate evergreen needleleaf forest [14]. More interestingly, the horizontal ranges of *P. massoniana* (27°55′~32°30′ N, 102°40′~109°55′ E) and *P. hwangshanensis* (22°48′~31°48′ N, 112°30′~122°30′ E) are highly overlapped. However, there are noticeable differences in their elevation distribution. Generally, the boundary is 700 m, with *P. hwangshanensis* at the medium and high elevations and *P. massoniana* at the low elevations [15]. It is known that plants' functional traits are expected to change as altitude changes, with plants at higher altitudes having smaller and thicker leaves than plants at lower altitudes [16].

Phenotypic variation in physiology and growth has been observed in pine species populations for needle traits such as leaf length, leaf width, leaf thickness [17], epidermis thickness with cuticle, resin duct diameter [18], stomatal row density, specific leaf area [19], and leaf area [20]. Meanwhile, existing research has reported that needle length, needle length–width ratio, the biomass of needles, and needle longevity follow a precise longitude and latitude pattern [21,22], and that hydraulic efficiency traits exist in a pattern along the altitudinal gradient [23]. However, the results demonstrated that in garden experiments, conifer traits associated with cold and drought resistance may be affected by a combination of genotype and environment [24]. Moreover, it has been emphasized that multiple analytical approaches have led to the conclusion that the environment has an overwhelming impact on survival and growth [25]. Since the two viewpoints are still inconclusive, we focused on the effects of the environment on the needle traits of *P. hwangshanensis* and *P. massoniana*.

When studying phenotypic variation, it is important to note that plants frequently exhibit substantial intraspecific variation in leaf traits—especially in species with heterogeneous environments, along environmental gradients, and with wide ranges [26]. Relevant data show that up to 40% of total variation in some plant traits occurs within species [27]. With increasing altitude, needle length decreases, and leaf dry matter content decreases predictably [28]. Indeed, intraspecific variation arises from heritable differences and plasticity [29], as in the case of *Pinus pinaster* Ait., which has evolved to adapt to its local environment, resulting in high genetic differentiation among populations, along with geographically highly structured intraspecific variation in quantitative traits and neutral genetic variation [30]. Based on previous studies, we explored the sources of variation in *P. hwangshanensis* and *P. massoniana* through empirical studies.

Two species of pine trees from different elevation gradients on five mountains were selected for the present study, and a total of six leaf morphological traits—leaf length (LL), leaf thickness (TL), leaf width (DL), leaf area (LA), specific leaf area (SLA), and leaf dry matter content (LDMC)—were determined for various groups of pine needles. LL, TL, DL, and LA are traits that are related to needle size and are intuitive trait indicators; SLA is indicative of leaf biomass, and LDMC reflects the efficiency of resource utilization and the environmental adaptation strategy [31]. By using a broad survey of needle structure, we tested the following hypotheses: (1) the six needleleaf traits of the two pine species have a wide range of variation, with LDMC plasticity being significantly higher in *P. hwangshanensis* than in *P. massoniana*; (2) the total variation in most needle leaf traits of the two pine species has higher intraspecies variation than interspecies variation; and (3) the needle leaf morphological traits of the two pine species are influenced by different environmental factors, among which latitude and annual precipitation are the most significant.

2. Materials and Methods

2.1. Study Area and Sampling

The study areas were located in Jiangxi, Anhui, and Zhejiang, China; these provinces are among the core ranges of *P. hwangshanensis* and *P. massoniana*. In order to investigate the impact of elevation on the leaf phenotypic traits of two closely related pines, we identified five mountains as the objects of investigation. *P. massoniana* and *P. hwangshanensis* are located at low and high elevations, respectively. Five different populations for each pine—namely, Mt. Sanqingshan (SQS, 1820 m above sea level), Mt. Lushan (LS, 1474 m), Mt. Jinggangshan (JGS, 1779 m), Mt. Huangshan (HS, 1864 m), and Mt. Tianmushan (TMS, 1506 m)—were involved in our investigation (Figure 1). All five mountains are located in the central subtropical zone, belonging to the subtropical monsoon climate; the climate varies significantly vertically due to the influence of elevation, and the soils are yellow loam, red loam, yellow–brown loam, and other types, mostly related to elevation changes [32–34].

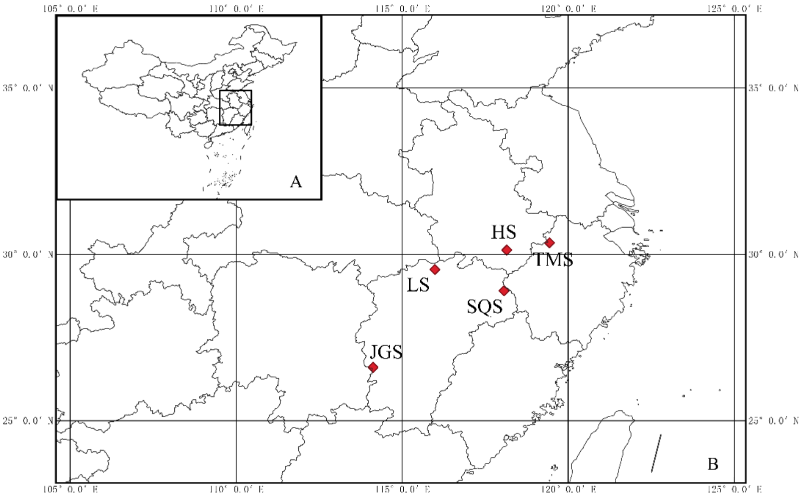


Figure 1. (**A**) The core distribution of *P. massoniana* and *P. hwangshanensis* in Jiangxi, Anhui, and Zhejiang, China. (**B**) The population locations of the two pine species. The codes and corresponding populations are SQS (Mt. Sanqingshan, Jiangxi), LS (Mt. Lushan, Jiangxi), JGS (Mt. Jinggangshan, Jiangxi), HS (Mt. Huangshan, Anhui), and TMS (Mt. Tianmushan, Zhejiang).

The geographic locations for each population are shown in Table 1. According to the elevation gradients of the two pine species, two or three sites (i.e., elevations) were selected for each population of the two pine species. A 20 × 50 m quadrat was set up for each site.

The number of sites was 20 and 15 for *P. massoniana* and *P. hwangshanensis*, respectively (Tables 1 and S1).

Table 1. The geographic locations and main meteorological factors of the sample populations of *P. massoniana* and *P. hwangshanensis*.

Population	Abbreviation	Longitude (°E)	Latitude (°N)	Annual Average Temperature (°C)	Annual Average Precipitation (mm)
Mt. Sanqingshan, Jiangxi	SQS	118°00′~118°06′	28°52′~28°57′	11	1860
Mt. Lushan, Jiangxi	LS	115°50′~116°10′	29°28′~29°45′	12	2024
Mt. Jinggangshan, Jiangxi	JGS	113°39′~114°23′	26°27′~26°40′	17	1890
Mt. Huangshan, Anhui	HS	116°49′~118°59′	29°13′~31°05′	14	2583
Mt. Tianmushan, Zhejiang	TMS	118°36′~120°06′	29°52′~30°55′	14	1870

Needle samples were collected from 60 *P. massoniana* individuals and 44 *P. hwangshanensis* individuals. Three mature individuals of *P. massoniana* and *P. hwangshanensis* with a similar diameter at breast height (DBH) were selected for each site, and fresh needles were randomly sampled from different directions around the tree crowns. The needles were mixed and sealed in a zip-lock bag and then returned to the laboratory for determination of their morphological characteristics.

2.2. Measurements

Ten fascicles of needles were selected randomly from each sampled tree for the measurement of LL, DL, and TL. LL was measured with a metric ruler with an accuracy of 0.1 cm; DL and TL were measured using an outside micrometer with an accuracy of 0.01 mm. An electronic balance was used to measure the saturated fresh weight and dry weight of the leaves, with an accuracy of 0.0001 g. The term 'saturated fresh weight of leaves' refers to the mass of fresh needles that have been immersed in distilled water for 36 h and then extracted after being dried on the surface. Following the aforementioned determination, the needles were dried in an oven at 70 °C until the mass remained constant, and the dry weight of the leaves was then measured. Then, the leaf area (LA), specific leaf area (SLA), and leaf dry matter content (LDMC) were calculated separately.

Referring to the method of Zhang et al. [35], the needle trait indices were calculated as follows:

$$\text{Area of single cambered surface of a needle } (S_{SCS}):\ S_{SCS} = \frac{\pi \times \text{DL} \times \text{LL}}{2} \quad (1)$$

$$\text{Leaf area } (S_{LA}):\ S_{LA} = \frac{\pi \times \text{DL} \times \text{LL}}{2} + \text{DL} \times \text{LL} \quad (2)$$

$$\text{Specific leaf area } (S_{SLA}):\ S_{SLA} = \frac{\text{leaf area}}{\text{leaf dry mass}} \quad (3)$$

$$\text{Leaf dry matter content } (C_{LDMC}):\ C_{LDMC} = \frac{\text{leaf dry mass}}{\text{saturated fresh mass}} \times 100\% \quad (4)$$

2.3. Data Analysis

Means, coefficients of variation (CVs), and standard deviations for each trait among the populations were estimated. Data from each single needle measurement were used to conduct analysis of variance.

We log-transformed the data for all needle traits and performed a nested ANOVA using the lme [36] and varcomp [37] functions in R [38], in the following increasing order: individuals, elevations, populations, and species. The code was as follows (e.g., LL):

LL < - lme (log10 (LL) ~1, random = ~1 | species/population/elevation, data = E, method = "REML")

mLL < - varcomp (LL, TRUE, cum = FALSE)

The same model was applied to TL, DL, LA, SLA, and LDMC, only replacing LL with these traits in the R code. REML refers to a restricted maximum likelihood method in the 'lme' function of R (version 4.2.1).

Clustering analysis among populations of *P. massoniana* and *P. hwangshanensis* was performed using the average method in R by the unweighted pair group method with arithmetic mean (UPGMA). The average values of each needle trait were used for cluster analysis. In order to investigate the effects of external factors (geographic and environmental) on the needle traits of the two pine species, redundancy (RDA) analysis was used. The environmental and geographic data for each sampling site were collected by searching WorldClim (http://www.worldclim.org/, accessed on 20 September 2022), using three geographic factors (latitude, longitude, and elevation) and two environmental factors (annual average temperature and annual precipitation). The statistical analysis of this study was completed in SPSS 25.0 and R 4.2.1 [39].

3. Results

3.1. Variation of Needle Traits in the Two Pine Species

The mean values of seven needle morphological traits of *P. massoniana* and *P. hwangshanensis* were significantly different between the populations (Tables 2 and 3). The variation coefficients of morphological traits of *P. massoniana* ranged from 4.11% to 25.85%, and the average variation coefficients of LL, LA, and LDMC were relatively small, while TL, DL, and SLA showed a great range of variation. Additionally, the fluctuation in needle traits of *P. hwangshanensis* was marked with variation coefficients of 4.94~94.80%. LDMC and TL showed significant variation, while the average variation of the other traits was slight. Interestingly, according to the boxplot, seven needle traits within a mountain were markedly different between the *P. massoniana* population and the *P. hwangshanensis* population, and such patterns occurred on all five mountains (Table S1, Figure 2).

Table 2. Mean values, coefficients of variance (CV%), and analysis of variance of needle traits in *P. massoniana*. The population abbreviations are the same as in Table 1. Numbers in bold symbolize statistical significance.

Traits	**SQS**		**LS**		**JGS**		**HS**		**TMS**		**ANOVA**	
	Mean	**CV%**	**Mean**	**CV%**	**Mean**	**CV%**	**Mean**	**CV%**	**Mean**	**CV%**	***F***	***p* (>*F*)**
LL (cm)	11.305	13.48	13.574	17.89	15.638	11.05	13.658	15.98	14.189	11.09	140.600	<0.01
TL (mm)	0.404	4.11	0.423	9.04	0.416	9.62	0.466	15.07	0.403	15.39	62.402	<0.01
DL (cm)	0.079	7.10	0.088	14.15	0.081	11.95	0.080	8.48	0.075	18.98	56.719	<0.01
LA (cm^2)	2.288	16.00	3.075	20.98	3.255	20.11	2.798	19.33	2.731	22.72	87.540	<0.01
SLA (cm^2/g)	149.348	20.46	140.607	19.39	164.363	17.06	137.186	15.37	167.568	25.85	50.105	<0.01
LDMC (g/g)	0.443	16.85	0.455	18.91	0.439	18.12	0.532	14.09	0.402	17.52	83.717	<0.01

Abbreviations: SQS, Mt. Sanqingshan; LS, Mt. Lushan; JGS, Mt. Jinggangshan; HS, Mt. Huangshan; TMS, Mt. Tianmushan.

3.2. Sources of Needle Traits' Variation in the Two Pine Species

The four levels of species, populations, elevations, and individuals had different effects on the variation of the seven functional leaf traits (Figure 3). Except for LDMC, the sources of variation for the needle leaf traits consisted mainly of interspecific and intraspecific contributions. Most of the variation between populations was smaller than the variation within populations for most of the measured needle traits. The variation in LL and DL was mainly derived from interspecific variation, with contributions of 61.48% and 55.73%, respectively. Compared with the other four traits, intraspecies variation contributed more to LA and SLA. The variation in TL was mainly due to intraspecific variation (35.84%), followed by interspecific variation (29.50%). Elevation contributed the most to LDMC (63.79%).

Table 3. Mean values, coefficients of variance (CV%), and analysis of variance of needle traits in *P. hwangshanensis*. The population abbreviations are the same as in Table 1. Numbers in bold symbolize statistical significance.

Traits	SQS		LS		JGS		HS		TMS		ANOVA	
	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	*F*	*p* (>*F*)
LL (cm)	9.477	20.84	8.623	19.52	11.438	10.31	9.763	17.96	9.671	14.14	58.209	<0.01
TL (mm)	0.427	4.94	0.477	10.36	0.498	9.13	0.466	19.51	0.540	8.89	97.383	<0.01
DL (cm)	0.094	8.66	0.099	10.64	0.104	11.29	0.094	13.63	0.106	9.94	50.904	<0.01
LA (cm^2)	2.289	22.94	2.190	20.17	3.066	16.20	2.370	26.23	2.662	20.86	61.234	<0.01
SLA (cm^2/g)	142.461	17.61	121.316	20.69	129.870	15.83	120.501	19.83	117.189	16.31	27.646	<0.01
LDMC (g/g)	0.474	21.74	0.485	21.18	0.249	94.80	0.515	16.30	0.461	84.71	22.928	<0.01

Abbreviations: SQS, Mt. Sanqingshan; LS, Mt. Lushan; JGS, Mt. Jinggangshan; HS, Mt. Huangshan; TMS, Mt. Tianmushan.

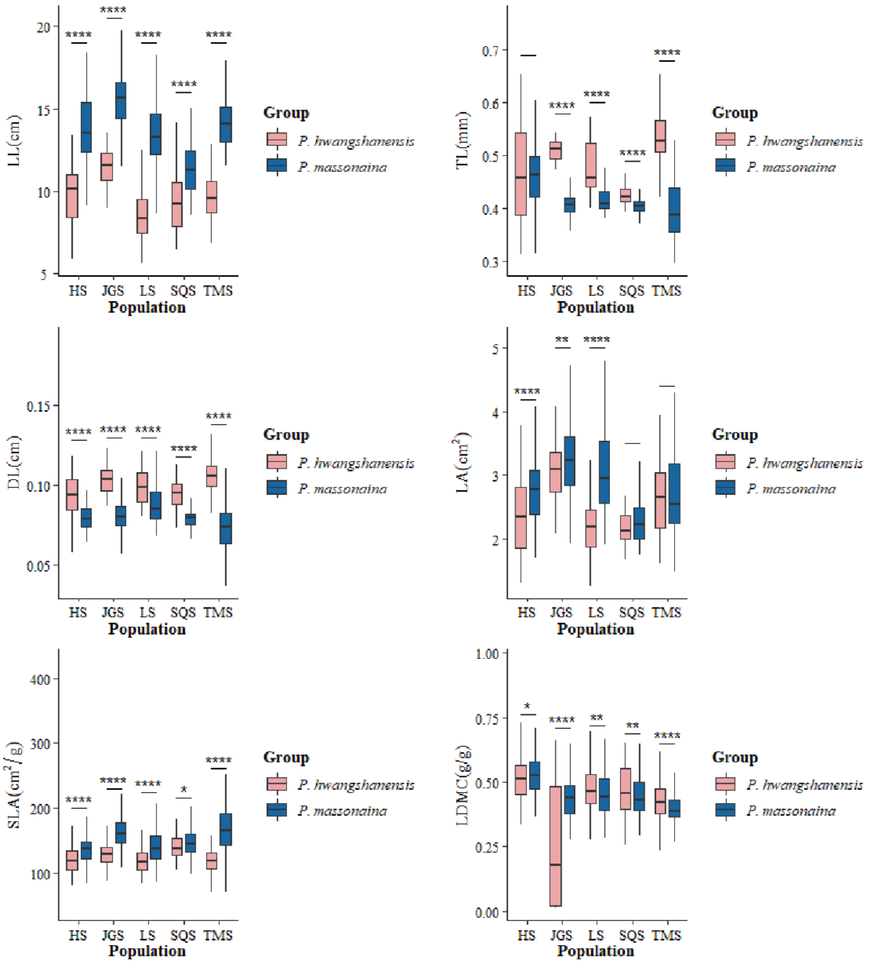


Figure 2. Boxplots of the needle traits in each population between two pine species. The population abbreviations are the same as in Table 1. The three upper, middle, and lower lines represent the 75th, 50th, and 25th percentile values of the variables, respectively. * Significant differences ($p < 0.05$) exist in the populations between *P. massoniana* and *P. hwangshanensis* (** $p < 0.01$, **** $p < 0.0001$). Abbreviations: SQS, Mt. Sanqingshan; LS, Mt. Lushan; JGS, Mt. Jinggangshan; HS, Mt. Huangshan; TMS, Mt. Tianmushan.

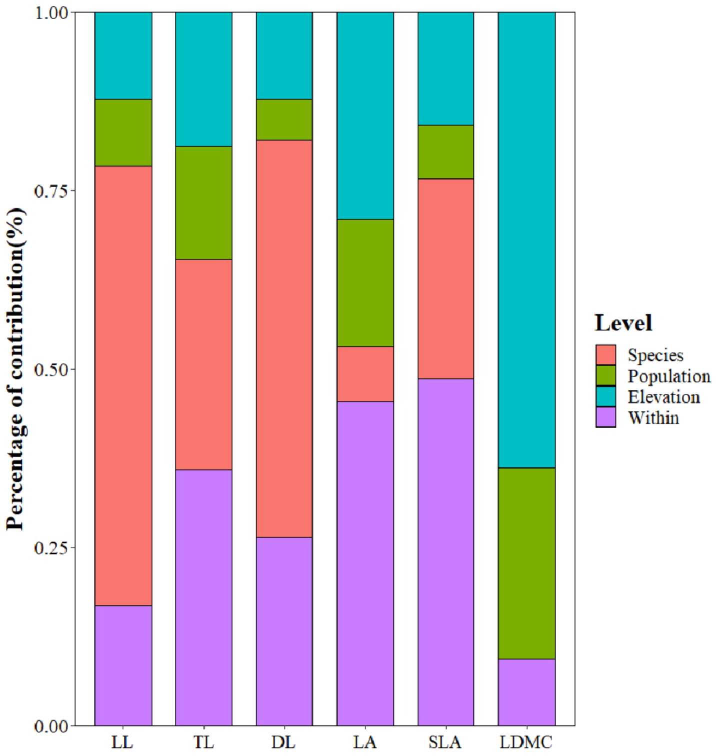


Figure 3. Variance partitioning of needle traits at different levels and between species across geographic populations.

3.3. Cluster Analysis

The two species were divided into two groups by cluster analysis (Figure 4), except for JGS clustered into another group. For *P. massoniana*, the populations in LS and HS were clustered into one group, and the populations in TMS and JGS were clustered into another group. Similar results occurred in *P. hwangshanensis*, where the populations in LS and HS (i.e., the high-latitude populations) were clustered into one group.

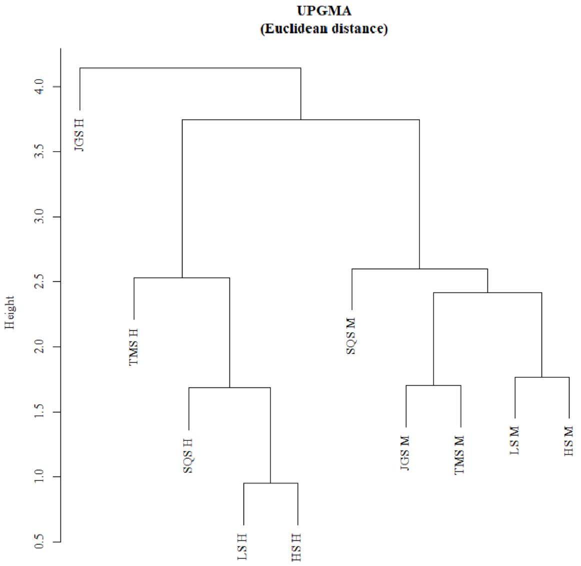
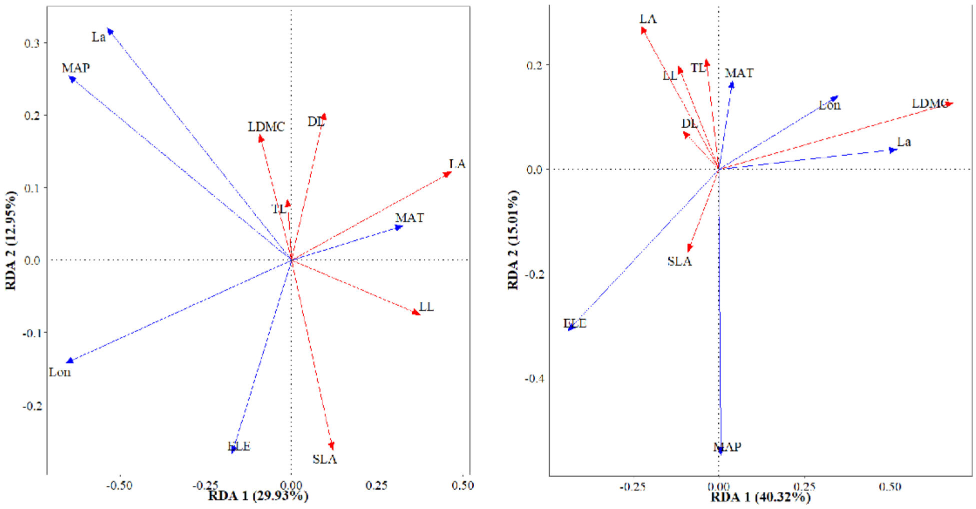


Figure 4. Cluster analysis based on needle traits in five populations of *P. massoniana* and *P. hwangshanensis*. Abbreviations: SQS, Mt. Sanqingshan; LS, Mt. Lushan; JGS, Mt. Jinggangshan; HS, Mt. Huangshan; TMS, Mt. Tianmushan. M, *P. massoniana*; H, *P. hwangshanensis*.

3.4. Redundancy Analysis

The results of the RDA analysis showed that the first three RDA ranking axes included 44.65% of the information on needle traits and their corresponding geographic variables for *P. massoniana*, and latitude (La) had more influence on the distribution of needle traits than other factors (Figure 5a). On the other hand, the first three ordination axes in Figure 5b explain 62.01% of the information of *P. hwangshanensis* needle traits and their corresponding environmental variables. Annual precipitation (MAP) had the greatest influence on the distribution of needle traits.



(**a**) (**b**)

Figure 5. Redundancy analysis based on needle traits of (**a**) *P. massoniana* and (**b**) *P. hwangshanensis*. Abbreviations: Lon: longitude, La: latitude, ELE: elevation, MAT: annual average temperature, MAP: annual precipitation.

4. Discussion

4.1. Phenotypic Variation and Plasticity

The study of needle traits in *P. massoniana* and *P. hwangshanensis* revealed a wide range of phenotypic variation (Tables 2 and 3). Furthermore, *P. massoniana* had less variation than *P. hwangshanensis*—for example, the variation in the leaf dry matter content of *P. hwangshanensis* was pronounced (up to 98.4%). The latest histological studies also proved that *P. hwangshanensis* differed significantly from *P. massoniana* in the formulation phase [40]. Stapley et al. [41] proposed that phenotypic variability was one of the foundations in determining the intrinsic factors that define species and their biogeography, and that adaptation in any species requires phenotypic variation in traits [1,42]. In fact, nearly all traits will be influenced by plasticity [5]. Physiological changes, on the other hand, are thought to be central to phenotypic variation, and because of physiology's systemic nature and sensitivity to environmental changes, the division of labor between traits could be regulated by these changes—particularly the endocrine system [43]. That is, divergence in phenotypic plasticity could result from physiological mechanisms with a genetic basis. This allows us to have a more in-depth discussion of phenotypic variation and phenotypic plasticity.

In the present study, the coefficient of variation of needle traits was used to characterize the ability of plasticity; the average coefficients of variation of LL, TL, and DL were lower than those of LA, SLA, and LDMC, indicating the low plasticity in LL, DL, and TL. Despite the fact that our study did not calculate the phenotypic plasticity index (PPI) [44], the coefficient of variation produced results that were consistent with it [45]. Based on 20 morphological and anatomical needle traits and 10 geographic populations of *Pinus tabuliformis*, Zhang et al. [46] reported that the inter-population heritability of LL, TL, and DL was greater than 0.8, indicating good genetic stability. Meanwhile, they had low levels of plasticity and small coefficients of variation when compared to other traits.

4.2. Phenotypic Variation Patterns of Leaf Traits among and within Species

Conifers are widely assumed to be typical wind-pollinated species, with chloroplast DNA that is primarily inherited paternally via pollen [47]. However, evidence has suggested that geographical barriers may result in low levels of differentiation between populations by limiting the pollen dispersal [48]. A similar conclusion was reached by Meng et al. [49], who reported that the genetic diversity of most needle traits was found to be most abundant within the population of *Pinus tabuliformis* in 18 needle traits. Xie et al. [50] used SRAP markers to study five endemic *Pinus* taxa from China (*Pinus tabulaeformis*, *P. tabulaeformis* var. *mukdensis*, *P. tabulaeformis* f. *shekanensis*, *Pinus massoniana*, and *Pinus henryi*), which showed more genetic diversity within taxa, rather than between taxa.

When investigating genetic variability and adaptive differentiation in conifers, it is well known that variation in needle variability traits is a useful visual indicator [51,52]. Within populations of *Pinus yunnanensis*, the proportion of phenotypic variation in nearly all needle traits was greater than 50%. In other words, trees within populations accounted for the vast majority of total variation [53]. Based on the phenotypic variation of needle traits in *Pinus tabuliformis*, Zhang et al. [46] found that more variation was expressed within populations. Similar results were obtained in our study (Figure 3), which demonstrated that the intraspecific components were generally higher than interspecific components in most of the measured needle traits. However, different needle traits showed different levels of variation within species; intraspecific trait variation (ITV) was relatively high for morphological traits linked to the leaf economics spectrum (e.g., leaf N and P, SLA, LDMC) [54], but low for traits related to leaf size (i.e., LL, TL, DL), which are typically considered to be independent of the leaf economics spectrum but have been linked with adaptation to broad climatic gradients [55].

Additionally, it has been demonstrated that intraspecific variation could have consequences that are comparable to or even greater than those of interspecific variation [27], and that genetics and habitat divergence are the primary forces behind phenotypic variation within species [56].

4.3. Adaptive Phenotypes in the Context of Changing External Factors

Researchers began to understand as early as the middle of the 20th century that environmental changes in a variety of morphological and physiological traits could precede and facilitate genetic changes [57]. Levis and Pfennig [58] underlined the dual function of environmental change as a generator and then selector of phenotypic variation, which reoriented how we think of the ‘plasticity-led evolution hypothesis’. In other words, by enabling organisms to react appropriately and immediately to environmental variation, plasticity may confer a special evolutionary benefit [3]. As a result, environmental variation is essential in phenotypic plasticity as well as evolutionary adaptation.

Phenotypic trait variation and genetic architecture are highly influenced by geographic and environmental variations—particularly those linked to photoperiod and temperature (e.g., latitude, longitude, and elevation) [6]. Our study’s RDA analysis showed that the two pine species’ needle traits were affected by various environmental factors to various degrees (Figure 5). Elevation had a significant impact on needle traits, and the results of earlier investigations [53,59] were consistent in that LL and LDMC showed a negative correlation with altitude. Since *P. hwangshanensis* populations are distributed at higher elevations, they are subjected to more severe winter stress than *P. massoniana* populations, which are found at lower elevations. Thus, their needles are thicker and shorter to withstand the stress of strong winds and snow [60]. In addition, trees that are located at high altitudes face a lack of available water, leading to drought stress [61], and the lower leaf area minimizes stomatal transpirational water loss. Moreover, due to the short growing season at high altitudes and the tendency for soil moisture to freeze in winter—resulting in root uptake—MAP is extremely crucial for this species. *P. hwangshanensis*, on the other hand, had a higher level of plasticity in LDMC than *P. massoniana*, which can be attributed to nutrient deficiencies and low-temperature stresses caused by increased elevation. Cluster analysis could support the peculiar latitude patterns that SLA and LDMC show in *P. massoniana* and *P. hwangshanensis* (Figure 4). SLA decreased as latitude increased, while LDMC increased as latitude increased. Due to the fact that SLA is associated with plants' growth rates and ecological strategies, it was discovered that *Pinus koraiensis* has smaller LDMC and larger SLA at low latitudes [61]. The greater the SLA, the more effectively leaves photosynthesize in order to adapt to changing habitats [62]. The investment in defense against low temperatures increases with latitude [63]. Based on 'leaf economic spectrum theory' [54], we could conclude that both *P. massoniana* and *P. hwangshanensis* were typical 'slow investment-return'-type species. However, our findings indicated that the resource trade-off strategies of the two pine species differed markedly.

P. massoniana and *P. hwangshanensis* share a common geological history across the mainland, which accounts for their similar levels of genetic diversity [64]. Due to the presence of refugees during the ice ages, climatic changes might have low impacts on these populations at mid-latitudes. Therefore, *P*. *massoniana* was able to achieve population expansion, while population decline has only recently started, indicating that its population was dealing with serious survival issues [48]. In cold biomes, where the climate is changing most rapidly, the ability of a species to adjust its phenotypes under new circumstances is vital [7].

5. Conclusions

In sum, the needle traits of *P*. *massoniana* and *P. hwangshanensis* displayed significant heterogeneity, whereas the leaf size traits displayed more consistent heritability between populations. Likewise, the variation of all needle traits within populations was greater than that between populations, suggesting that both genetics and environment played a role in the intraspecific phenotypic variation of the needles. The variation of needle traits was affected by different environmental factors (i.e., longitude, latitude, elevation, temperature, and precipitation), reflecting the adaptability of the traits to the heterogeneous environment. Limited by knowledge, this study is the first to systematically report the intraspecific and interspecific variation patterns of needle traits in multiple populations of *P. hwangshanensis*. Our findings might provide a theoretical reference for the adaptive evolution and breeding practice of Pinaceae.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13122014/s1, ID 502, Dataset name: "Table S1", Table S1: Needle trait indicators of different populations of *P. massoniana* and *P. hwangshanensis*.

Author Contributions: Conceptualization, Y.Y., M.H., K.X. and Y.F.; data curation, L.W. and T.L.; formal analysis, T.L., H.Z. and Y.D.; funding acquisition, Y.D., K.X. and Y.F.; investigation, Y.Y., M.H., L.W., M.F. and Y.F.; methodology, Y.Y., M.H., H.Z. and Y.F.; project administration, Y.D., K.X. and H.Z.; resources, M.F. and M.H.; software, T.L.; validation, L.W.; writing—original draft, Y.Y.; writing—review and editing, L.W., T.L. and Y.F. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Special Foundation for National Science and Technology Basic Resources Investigation of China (2019FY202300 to Y.F.).

Data Availability Statement: All data used in this study are available in the Supplementary Materials.

Acknowledgments: We thank all reviewers who provided valuable comments for this paper. We are grateful to Huang Kexin for help with sample collection.

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

References

1. Sultan, S. Phenotypic Plasticity as An Intrinsic Property of Organism. In *Phenotypic Plasticity & Evolution*; Pfennig, D.W., Ed.; CRC Press: London, UK; New York, NY, USA, 2021; pp. 1–24.
2. Thompson, M.J.; Capilla-Lasheras, P.; Dominoni, D.M.; Reale, D.; Charmantier, A. Phenotypic variation in urban environments: Mechanisms and implications. *Trends Ecol. Evol.* **2022**, *37*, 171–182. [CrossRef]

3. Pfennig, D.W. Key Questions about Phenotypic Plasticity. In *Phenotypic Plasticity & Evolution*; Pfennig, D.W., Ed.; CRC Press: London, UK; New York, NY, USA, 2021; pp. 55–88.
4. Levis, N.A.; Pfennig, D.W. Evaluating 'Plasticity-First' Evolution in Nature: Key Criteria and Empirical Approaches. *Trends Ecol. Evol.* **2016**, *31*, 563–574. [CrossRef] [PubMed]
5. Hendry, A.P. Key Questions on the Role of Phenotypic Plasticity in Eco-Evolutionary Dynamics. *J. Hered.* **2016**, *107*, 25–41. [CrossRef] [PubMed]
6. Diouf, I.; Derivot, L.; Koussevitzky, S.; Carretero, Y.; Bitton, F.; Moreau, L.; Causse, M. Genetic basis of phenotypic plasticity and genotype x environment interactions in a multi-parental tomato population. *J. Exp. Bot.* **2020**, *71*, 5365–5376. [CrossRef] [PubMed]
7. Henn, J.J.; Buzzard, V.; Enquist, B.J.; Halbritter, A.H.; Klanderuds, K.; Maitner, B.S.; Michaletz, S.T.; Potschs, C.; Seltzer, L.; Telford, R.J.; et al. Intraspecific Trait Variation and Phenotypic Plasticity Mediate Alpine Plant Species Response to Climate Change. *Front. Plant Sci.* **2018**, *9*, 1548. [CrossRef]
8. Richardson, D.M.; Rundel, P.W.; Jackson, S.T.; Teskey, R.O.; Aronson, J.; Bytnerowicz, A.; Wingfield, M.J.; Proches, S. Human impacts in pine forests: Past, present, and future. *Annu. Rev. Ecol. Evol. Syst.* **2007**, *38*, 275–297. [CrossRef]
9. Gernandt, D.S.; Lopez, G.G.; Garcia, S.O.; Liston, A. Phylogeny and classification of Pinus. *Taxon* **2005**, *54*, 29–42. [CrossRef]
10. Ferriz, M.; Martin-Benito, D.; Canellas, I.; Gea-Izquierdo, G. Sensitivity to water stress drives differential decline and mortality dynamics of three co-occurring conifers with different drought tolerance. *For. Ecol. Manag.* **2021**, *486*, 118964. [CrossRef]
11. Drenkhan, R.; Ganley, B.; Martin-Garcia, J.; Vahalik, P.; Adamson, K.; Adamcikova, K.; Ahumada, R.; Blank, L.; Braganca, H.; Capretti, P.; et al. Global Geographic Distribution and Host Range of *Fusarium circinatum*, the Causal Agent of Pine Pitch Canker. *Forests* **2020**, *11*, 724. [CrossRef]
12. Zheng, W.J. *Sylva Sinica*; Chien, S.S., Chen, H.Y., Eds.; China Forestry Publishing House: Beijing, China, 1983; pp. 297–299.
13. Yu, S.X.; Li, Y.; Wang, Y.F.; Zhou, C.F. The vegetation classification and its digitized map of Heishiding Nature Reserve, Guangdong. I.The distribution of the vegetation type and formation. *Act Sci. Nat. Univ. Sunyasen* **2000**, *39*, 61–66.
14. Wang, L.P.; Zhou, Z.D. On the Positional Problem of the *Pinus Taiwanensis* Forest in the Vegetation Altitude Belts of Mt. Lushan. *Chin. J. Plant Ecol.* **1989**, *13*, 28–35.
15. Tong, Z.K.; Fan, Y.R. Dividing the distribution area of *Pinus taiwanensis* based on climatic and ecologic factors. *J. Cent. South For. Univ.* **1993**, *13*, 81–87.
16. Urbaniak, L.; Chudzinska, E.; Faferek, S. Differentiation of *Pinus sylvestris* populations of the Tatra Mts and the Tuchola forest expressed in the needle anatomical traits. *Dendrobiology* **2008**, *60*, 35–43.
17. Bozkurt, A.E.; Coskuncelebi, K.; Terzioglu, S. Population variability of scots pine (*Pinus sylvestris* L.) in Turkey according to the needle morphology. *Sumar. List* **2021**, *145*, 347–354. [CrossRef]
18. Popovic, V.; Nikolic, B.; Lucic, A.; Rakonjac, L.; Jovanovic, D.S.; Miljkovic, D. Morpho-anatomical trait variability of the Norway spruce (*Picea abies* (L.) Karst.) needles in natural populations along elevational diversity gradient. *Trees-Struct. Funct.* **2022**, *36*, 1131–1147. [CrossRef]
19. Flores, A.; Climent, J.; Pando, V.; Lopez-Upton, J.; Alia, R. Intraspecific Variation in Pines from the Trans-Mexican Volcanic Belt Grown under Two Watering Regimes: Implications for Management of Genetic Resources. *Forests* **2018**, *9*, 71. [CrossRef]
20. Jankowski, A.; Wyka, T.P.; Zytkowiak, R.; Nihlgard, B.; Reich, P.B.; Oleksyn, J. Cold adaptation drives variability in needle structure and anatomy in *Pinus sylvestris* L. along a 1900 km temperate-boreal transect. *Funct. Ecol.* **2017**, *31*, 2212–2223. [CrossRef]
21. Nobis, M.P.; Traiser, C.; Roth-Nebelsick, A. Latitudinal variation in morphological traits of the genus Pinus and its relation to environmental and phylogenetic signals. *Plant Ecol. Divers.* **2012**, *5*, 1–11. [CrossRef]
22. Viveros-Viveros, H.; Camarillo-Luna, A.R.; Saenz-Romero, C.; Aparicio-Renteria, A. Altitudinal variation in morphological traits of *Pinus patula* populations from Oaxaca state, Mexico, and its use in the zoning. *Bosque* **2013**, *34*, 173–179.
23. Taneda, H.; Funayama-Noguchi, S.; Mayr, S.; Goto, S. Elevational adaptation of morphological and anatomical traits by Sakhalin fir (*Abies sachalinensis*). *Trees-Struct. Funct.* **2020**, *34*, 507–520. [CrossRef]
24. Donnelly, K.; Cavers, S.; Cottrell, J.E.; Ennos, R.A. Genetic variation for needle traits in Scots pine (*Pinus sylvestris* L.). *Tree Genet. Genomes* **2016**, *12*, 40. [CrossRef]
25. Taibi, K.; del Campo, A.D.; Aguado, A.; Mulet, J.M. The effect of genotype by environment interaction, phenotypic plasticity and adaptation on *Pinus halepensis* reforestation establishment under expected climate drifts. *Ecol. Eng.* **2015**, *84*, 218–228. [CrossRef]
26. Bussotti, F.; Pollastrini, M.; Holland, V.; Brüggemann, W. Functional traits and adaptive capacity of European forests to climate change. *Environ. Exp. Bot.* **2015**, *111*, 91–113. [CrossRef]
27. Fraser, L.H. TRY-A plant trait database of databases. *Glob. Chang. Biol.* **2020**, *26*, 189–190. [CrossRef] [PubMed]
28. O'Sullivan, K.S.W.; Vila-Cabrera, A.; Chen, J.C.; Greenwood, S.; Chang, C.H.; Jump, A.S. High intraspecific trait variation results in a resource allocation spectrum of a subtropical pine across an elevational gradient. *J. Biogeogr.* **2022**, *49*, 668–681. [CrossRef]
29. Matesanz, S.; Horgan-Kobelski, T.; Sultan, S.E. Phenotypic Plasticity and Population Differentiation in an Ongoing Species Invasion. *PLoS ONE* **2012**, *7*, e44955. [CrossRef]
30. Bucci, G.; Gonzalez-Martinez, S.C.; Le Provost, G.; Plomion, C.; Ribeiro, M.M.; Sebastiani, F.; Alia, R.; Vendramin, G.G. Range-wide phylogeography and gene zones in *Pinus pinaster* Ait. revealed by chloroplast microsatellite markers. *Mol. Ecol.* **2007**, *16*, 2137–2153. [CrossRef]
31. Wilson, P.; Thompson, K.; Hodgson, J. Specific leaf area and leaf dry matter content as alternative predictors of plant strategies. *New Phytol.* **1999**, *143*, 155–162. [CrossRef]

32. Gong, J.; Lu, L.; Jin, X.L.; Nan, W.; Liu, F. Impacts of tourist disturbance on plant communities and soil properties in Huangshan Mountain scenic area. *Acta Ecol. Sin.* **2009**, *29*, 2239–2251.
33. Li, X.H.; Song, M.Z.; Liang, T.J. Characteristics of wild vine resources in Lushan Mountain and their landscape application. *J. South. Agric.* **2013**, *44*, 994–998.
34. Ren, R.F.; Xie, H.S.; Jian, Z.H.; Xu, Y.K.; Zhang, J. Soil Geoligy and Geochemistry Characteristics in the Tianmu Mountain Area Zhejiang Province. *J. Earth Sci. Environ.* **2008**, *30*, 49–53.
35. Zhang, K.; Hou, J.H.; He, N.P. Leaf functional trait distribution and controlling factors of *Pinus tabuliformis*. *Acta Ecol. Sin.* **2017**, *37*, 736–749.
36. Bates, D.; Machler, M.; Bolker, B.M.; Walker, S.C. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [CrossRef]
37. Qu, L.; Guennel, T.; Marshall, S.L. Linear Score Tests for Variance Components in Linear Mixed Models and Applications to Genetic Association Studies. *Biometrics* **2013**, *69*, 883–892. [CrossRef] [PubMed]
38. Messier, J.; McGill, B.J.; Lechowicz, M.J. How do traits vary across ecological scales? A case for trait-based ecology. *Ecol. Lett.* **2010**, *13*, 838–848. [CrossRef]
39. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing: Vienna, Austria. Available online: http://www.r-project.org/index.html (accessed on 13 October 2022).
40. Jin, W.T.; Gernandt, D.S.; Wehenkel, C.; Xia, X.M.; Wei, X.X.; Wang, X.Q. Phylogenomic and ecological analyses reveal the spatiotemporal evolution of global pines. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2022302118. [CrossRef]
41. Stapley, J.; Reger, J.; Feulner, P.G.D.; Smadja, C.; Galindo, J.; Ekblom, R.; Bennison, C.; Ball, A.D.; Beckerman, A.P.; Slate, J. Adaptation genomics: The next generation. *Trends Ecol. Evol.* **2010**, *25*, 705–712. [CrossRef]
42. Savolainen, O.; Pyhäjärvi, T.; Knürr, T. Gene flow and local adaptation in trees. *Annu. Rev. Ecol. Evol. Syst.* **2007**, *38*, 595–619. [CrossRef]
43. Ledón-Rettig, C.C.; Ragsdale, E.J. Physiological Mechanisms and the Evolution of Plasticity. In *Phenotypic Plasticity & Evolution*; Pfennig, D.W., Ed.; CRC Press: London, UK; New York, NY, USA, 2021; pp. 113–137.
44. Bohnke, M.; Bruelheide, H. How do evergreen and deciduous species respond to shade?—Tolerance and plasticity of subtropical tree and shrub species of South-East China. *Environ. Exp. Bot.* **2013**, *87*, 179–190. [CrossRef]
45. Chen, J.; Zhang, X.J.; Li, Q.Y.; Tao, J.P. Relationships between competition intensity and leaf phenotypic plasticity of woody plants in subalpine forests on different slope. *Acta Ecol. Sin.* **2022**, *42*, 1788–1797.
46. Zhang, M.; Meng, J.; Zhang, Z.; Zhu, S.; Li, Y. Genetic Analysis of Needle Morphological and Anatomical Traits among Nature Populations of *Pinus Tabuliformis*. *J. Plant Stud.* **2017**, *6*, 62. [CrossRef]
47. Robledo-Arnuncio, J.J. Wind pollination over mesoscale distances: An investigation with Scots pine. *New Phytol.* **2011**, *190*, 222–233. [CrossRef] [PubMed]
48. Ge, X.J.; Hsu, T.W.; Hung, K.H.; Lin, C.J.; Huang, C.C.; Huang, C.C.; Chiang, Y.C.; Chiang, T.Y. Inferring Multiple Refugia and Phylogeographical Patterns in *Pinus massoniana* Based on Nucleotide Sequence Variation and DNA Fingerprinting. *PLoS ONE* **2012**, *7*, e43717. [CrossRef]
49. Meng, J.X.; Chen, X.Y.; Huang, Y.J.; Wang, L.M.; Xing, F.Q.; Li, Y. Environmental contribution to needle variation among natural populations of *Pinus tabuliformis*. *J. For. Res.* **2019**, *30*, 1311–1322. [CrossRef]
50. Xie, Q.; Liu, Z.H.; Wang, S.H.; Li, Z.Q. Genetic diversity and phylogenetic relationships among five endemic *Pinus* taxa (Pinaceae) of China as revealed by SRAP markers. *Biochem. Syst. Ecol.* **2015**, *62*, 115–120. [CrossRef]
51. Lexer, C.; Fay, M.F. Adaptation to environmental stress: A rare or frequent driver of speciation? *J. Evol. Biol.* **2005**, *18*, 893–900. [CrossRef]
52. Tobler, M.; Palacios, M.; Chapman, L.J.; Mitrofanov, I.; Bierbach, D.; Plath, M.; Arias-Rodriguez, L.; de Leon, F.J.G.; Mateos, M. Evolution in extreme environments: Replicated phenotypic differentiation in livebearing fish inhabiting sulfidic springs. *Evolution* **2011**, *65*, 2213–2228. [CrossRef] [PubMed]
53. Xu, Y.L.; Woeste, K.; Cai, N.H.; Kang, X.Y.; Li, G.Q.; Chen, S.; Duan, A. Variation in needle and cone traits in natural populations of *Pinus yunnanensis*. *J. For. Res.* **2016**, *27*, 41–49. [CrossRef]
54. Wright, I.J.; Reich, P.B.; Westoby, M.; Ackerly, D.D.; Baruch, Z.; Bongers, F.; Cavender-Bares, J.; Chapin, T.; Cornelissen, J.H.C.; Diemer, M.; et al. The worldwide leaf economics spectrum. *Nature* **2004**, *428*, 821–827. [CrossRef]
55. Craine, J.M.; Ocheltree, T.W.; Nippert, J.B.; Towne, E.G.; Skibbe, A.M.; Kembel, S.W.; Fargione, J.E. Global diversity of drought tolerance and grassland climate-change resilience. *Nat. Clim. Chang.* **2013**, *3*, 63–67. [CrossRef]
56. Jordan, L.; He, R.C.; Hall, D.B.; Clark, A.; Daniels, R.F. Variation in loblolly pine ring microfibril angle in the Southeastern United States. *Wood Fiber Sci.* **2007**, *39*, 352–363.
57. Bateman, K.G. The genetic assimilation of four venation phenocopies (Reprinted). *J. Genet.* **2005**, *84*, 227–257. [CrossRef] [PubMed]
58. Levis, N.A.; Pfennig, D.W. Innovation and Diversification Via Plasticity-Led Evolution. In *Phenotypic Plasticity & Evolution*; Pfennig, D.W., Ed.; CRC Press: London, UK; New York, NY, USA, 2021; pp. 211–240.
59. Lopez, R.; Climent, J.; Gil, L. Intraspecific variation and plasticity in growth and foliar morphology along a climate gradient in the Canary Island pine. *Trees-Struct. Funct.* **2010**, *24*, 343–350. [CrossRef]

60. Groover, A. Gravitropisms and reaction woods of forest trees—evolution, functions and mechanisms. *New Phytol.* **2016**, *211*, 790–802. [CrossRef] [PubMed]
61. Poorter, H.; Niinemets, U.; Poorter, L.; Wright, I.J.; Villar, R. Causes and consequences of variation in leaf mass per area (LMA): A meta-analysis. *New Phytol.* **2009**, *182*, 565–588. [CrossRef]
62. Marshall, J.D.; Monserud, R.A. Erratum: Foliage height influences specific leaf area of three conifer species. *Can. J. For. Res.-Rev. Can. Rech. For.* **2003**, *33*, 1591. [CrossRef]
63. Dong, Y.C.; Liu, Y.H. Response of Korean pine's functional traits to geography and climate. *PLoS ONE* **2017**, *12*, e0184051. [CrossRef]
64. Chiang, T.Y.; Schaal, B.A. Phylogeography of plants in Taiwan and the Ryukyu archipelago. *Taxon* **2006**, *55*, 31–41. [CrossRef]