

doi: 10.1111/tpj.70165

Intraspecific character displacement in oaks

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SUMMARY

Character displacement refers to the process by which species diverge more in sympatry due to competition for resources. This competition-driven speciation can also occur within populations, known as intraspecific character displacement (ICD). ICD can promote divergence within species by influencing intraspecific competition or encouraging the evolution of alternative phenotypes. Despite its significance, ICD remains understudied and requires further exploration. In this study, we investigate how competition influences genetic and morphological differentiation within species in sympatric and allopatric populations. We focused on Quercus serrata (in China and Japan) and Q. serrata var. brevipetiolata (found only in China), which belong to a small monophyletic group of oak species nested within Section Quercus (white oaks). Using genetic markers, we detected divergence between Chinese and Japanese populations and further diversification within China, with asymmetric historical gene flow primarily from Q. serrata (the earlier diverged species) to Q. serrata var. brevipetiolata (the later variety). Although genetic differentiation did not differ between sympatric and allopatric populations, leaf morphological variation, analyzed through the geometric morphometric method (GMM) and traditional morphological method, revealed greater trait variation in sympatry. In addition, we found an allometric growth relationship between leaf size and leaf mass of *Q. serrata* and *Q.* serrata var. brevipetiolata, with the leaf area of Q. serrata var. brevipetiolata decreasing more disproportionately to leaf mass. This suggests a resource trade-off, where Q. serrata var. brevipetiolata, the later diverged variety, adopts more resource-conservative traits in sympatry. Further analysis of trait variation with environmental factors supports these findings, while genetic variation along climate gradients showed significant responses primarily in Q. serrata, regardless of sympatric or allopatric conditions. Although neutral genetic markers are insufficient to capture selection-driven adaptive differentiation, we inferred that Q. serrata var. brevipetiolata is progressing towards ecological divergence from Q. serrata. Overall, our results highlight the role of ICD in driving morphological diversification and resource-use strategies within species in response to competitive pressures.

Keywords: *Q. serrata*, *Q. serrata* var. *brevipetiolata*, genetic assignment, leaf morphology, intraspecific variation, sympatry, allopatry.

BACKGROUND

Trait divergence due to natural selection, aimed at reducing competition in sympatry between species, was first noted by Darwin (1859) as "divergence of character" and then coined as "character displacement" (CD) by Brown and Wilson (1956). CD is sometimes considered to have a major influence on the origin, maintenance, and distribution of biodiversity (Pfennig & Pfennig, 2020). However, few examples have been documented, with some classical empirical evidence, e.g., finches on the Galápagos Islands (Grant & Grant, 2006). In addition, competitiondriven divergence may also occur within species (Dayan & Simberloff, 2005; Pfennig & Pfennig, 2009). Actually, intraspecific competition may be more frequent than

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interspecific competition (Gurevitch et al., 1992) because individuals are more likely to encounter conspecifics. Moreover, competition for resources becomes more intense when differentiation is recent and reproductive isolation is incomplete (Pfennig & Pfennig, 2010). This intensified competition drives disruptive selection, leading to rapid divergence of traits by favoring extreme phenotypes with adaptive advantages over intermediate ones, thereby maintaining or even increasing variation in natural populations (Doebeli & Dieckmann, 2003; Rueffler et al., 2006). This ultimately leads to the presence of alternative phenotypes in resource use within species, promoting the evolution of phenotypic polymorphism (Day & Young, 2004; Smith & Skúlason, 1996). Consequently, natural selection reduces resource competition by promoting divergence within species—a process known as intraspecific character displacement (ICD)-which may be more common than previously recognized (Dayan & Simberloff, 2005; West-Eberhard, 2003). Despite its significance, ICD remains understudied and warrants greater attention.

Theoretically, the character involved in CD can be morphological, ecological, behavioral, reproductive, or physiological, with the phenotype being the most intuitive, as Brown and Wilson (1956) stated "...divergence of this nature inevitably entails phenotypic 'characters' of the type employed in ordinary taxonomic work. Character displacement therefore may be considered as merely the aspects of such divergence that are recognizable to the taxonomist and some other favored organism". These phenotypic characters are often associated with feeding patterns in animals, such as the break size and shape of finches on Galápagos Island (Grant & Grant, 2006) and the head shape of salamanders in North America (Adams & Rohlf, 2000). However, the phenotypic characters involved in plants are complicated, encompassing a wide range of characteristics. For example, flower structure is related to plant-pollinator interactions and is likely to be displaced to prevent reproductive interference and hybridization. Seed size, on the other hand, might affect the outcome of seedling competition (Dayan & Simberloff, 2005: Grant, 1994; Veech et al., 2000).

Leaves are the primary photosynthetic organs of plant species, and their shape variation generally reflects natural selection acting on their functional efficiency (Nicotra et al., 2011; Wright et al., 2004). Leaf morphological traits are traditionally estimated by quantitative and qualitative variables (Henderson, 2006; Marcus, 1990). However, these methods have been criticized due to the lack of consistent criteria (Mitteroecker & Gunz, 2009). The geometric morphometric method (GMM), which digitizes the original geometry of the leaf shape by Cartesian coordinates of landmarks (Klingenberg, 2011; Mitteroecker & Gunz, 2009; Zelditch et al., 2004), provides an intuitive and efficient tool for leaf shape analysis. Notably, GMM is especially useful for leaf shape analysis within species, as it allows the capture of all aspects of leaf shape variation (Qi et al., 2024; Rellstab et al., 2016; Viscosi et al., 2012).

Another important factor to consider when studying ICD is leaf functional traits, which reflect the adaptation strategies of plants to their environment. For example, leaf functional traits such as leaf area, leaf mass, and specific leaf area (SLA, the ratio of leaf area to leaf dry mass) are directly involved in resource acquisition for water, nutrients, light, etc. (Arenas-Navarro et al., 2020; Milla & Reich, 2007; Onoda et al., 2017; Wright et al., 2004). Therefore, these functional traits are critical when examining ICD. In particular, SLA is a key indicator of a plant's investment strategy. However, interpreting SLA can be complicated because it might not always represent a simple trade-off between leaf mass investment and leaf size (Sancho-Knapik et al., 2020). For example, previous studies have shown that increases in leaf area might fail to keep pace with increases in leaf dry mass (Niklas et al., 2007, 2009). As more resources are spent on leaf mass, this leads to thicker and/or denser leaves, especially under harsh environmental conditions, an adaptation for resource conservation (Stotz et al., 2022). This must be taken into account when interpreting the SLA results.

The oak genus (Quercus spp.) is one of the most diverse and ecologically important tree genera, occurring throughout the Northern Hemisphere (Denk et al., 2018). White oaks (section Quercus), which are mainly distributed in North America and Eurasia, have been well studied in North America and Europe. In Europe, detailed phylogeographic studies using putatively neutral genetic markers have been conducted extensively (Dumolin-Lapègue et al., 1997; Petit et al., 2002), with comparative studies also conducted in North America (Grivet et al., 2006). Moreover, white oak leaves are highly complex, and great progress has been made in understanding how leaf morphology variation adapts to environmental conditions (e.g., Cavender-Bares, 2019; Hipp et al., 2018; Li, Wei, et al., 2021; Li, Zhang, et al., 2021; Skelton et al., 2021). While Asia is a major center of species diversity for white oaks (Oldfield & Eastwood, 2007), studies on range-wide genetic variation in this region are still lacking.

A total of seven white oaks are found in East Asia (Hipp et al., 2020; Huang et al., 1999), with *Quercus aliena* Blume, *Q. dentata* Thunberg, *Q. serrata* Thunb., and *Q. mongolica* Fisch. ex Ledeb. co-occurring in China, Japan, and Korea. Notably, there is a variant of *Q. serrata*, known as *Q. serrata* var. *brevipetiolata* (A. de Candolle) Nakai, which is found only in China (Huang et al., 1999). *Q. serrata* and *Q. serrata* var. *brevipetiolata* are morphologically similar, but they differ in the smaller leaves and shorter petioles of *Q. serrata* var. *brevipetiolata* (Fujiwara & Harada, 2015; Huang et al., 1999). The shorter petioles may influence water use efficiency or structural stability under

specific environmental conditions, such as dry or wind-exposed environments, as reported in other oak species (Bruschi et al., 2003). Both taxa have overlapping distributions along the Qinling Mountains, where local geographic and climatic factors may play an important role in maintaining species diversity, promoting divergent selection and adaptation. Thus, *Q. serrata* and *Q. serrata* var. *brevipetiolata* offer an ideal model for testing genetic and morphological divergence within species, particularly in the context of ICD.

In this study, we first conducted a range-wide genetic analysis to investigate the genetic divergence, extent, direction of gene flow, and morphological divergence of *Q. serrata* between China and Japan. Next, we aimed to test for genetic and morphological divergence, indicative of ICD, by comparing *Q. serrata* and *Q. serrata* var. *brevipetiolata* in both sympatric and allopatric populations. If divergence was detected, we further sought to explore how genetic and morphological variations respond to environmental factors.

RESULTS

Genetic diversity and differentiation

All 12 nrSSR loci were polymorphic, with 4-28 alleles per locus. Estimates of genetic diversity varied across populations (Table S1). The genetic diversity of *Q. serrata* was higher in Japan ($H_0 = 0.61$, $H_E = 0.69$) than in China $(H_{\rm O} = 0.56, H_{\rm E} = 0.64)$ (P < 0.05) (Table S4). The inbreeding coefficient (F) for Q. serrata var. brevipetiolata was 0.01, while the inbreeding coefficients for the Q. serrata-China and Q. serrata-Japan populations were 0.08 (Table S4). In addition, most of the genetic variation resided within populations, ranging from 84% (Q. serrata) to 96% (Q. serrata in Japan only) (Table S5). The fixation index (F_{ST} , a measure of population differentiation due to genetic structuring) values between species were higher for populations of *Q. serrata* in China ($F_{ST} = 0.08$) than for *Q. serrata* in Japan ($F_{ST} = 0.04$) (P < 0.001). When focusing on the sympatric and allopatric populations in China, we found that the genetic diversity of *Q. serrata* was significantly lower in sympatry ($H_0 = 0.53$, $H_E = 0.63$) than in allopatry $(H_{\rm O} = 0.58, H_{\rm E} = 0.65)$ (P < 0.05). In contrast, the genetic diversity of *Q. serrata* var. brevipetiolata was significantly higher in sympatry ($H_0 = 0.68$, $H_E = 0.69$) than in allopatry $(H_{O} = 0.59, H_{E} = 0.65)$ (P < 0.05). F_{ST} values of Q. serrata and Q. serrata var. brevipetiolata did not differ in sympatric and allopatric populations (0.10 vs. 0.11) (Table S5).

Population admixture

Across 20 replicate STRUCTURE runs for each K, maximum-likelihood scores were highest for K = 2, suggesting the existence of two clusters corresponding to Q. *serrata* in China (blue) and Japan (purple) (Figure S2;

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Figure 1b). Additionally, the statistics also gave support for K = 3 (Figure S2). When K = 3, individuals of *Q. serrata* in China were further subdivided into two groups: O. serrata (blue) and *Q. serrata* var. brevipetiolata (red) (Figure 1b). A total of 77 genetically admixed and 181 genetically purebred individuals were identified with a strict threshold value of 0.9. The PCoA results based on the genetic distance matrix at the individual level were largely concordant with the STRUCTURE analysis; the three groups can be well separated along the first two PC axes (Figure S3). When we focused on the sympatric and allopatric populations, we found a higher percentage of admixed individuals in sympatry (43%) than in allopatry (30%), with the threshold Q value of 0.9 (Table S1). The PCoA results also showed that purebreds and admixed individuals were intermingled in both sympatry (Figure 2a) and allopatry (Figure 2b). In addition, we found that the genetic variation of Q. serrata and Q. serrata var. brevipetiolata along the climatic gradient did not differ in both sympatry and allopatry (Figure 2c,d).

Demographic history

MIGRATE-N analysis indicated that asymmetric historical gene flow had occurred mainly from *Q. serrata*-China to *Q. serrata* var. *brevipetiolata* and *Q. serrata*-Japan with N_em values 42.0 and 14.1, respectively. In contrast, the reverse gene flow was lower, with values of 32.5 and 5.1 from *Q. serrata* var. *brevipetiolata* and from *Q. serrata*-Japan to *Q. serrata*-China, respectively (Table S6).

In the DIYABC analysis, the most strongly supported scenario was scenario 1 with a posterior probability of 0.8 (95% CI: 0.79-0.81) and low RMAE values for most parameters (Table S8; Figure S4). The best demographic scenario indicated that *Q. serrata*-China and *Q. serrata*-Japan populations initially split from their most common ancestor, and then Q. serrata var. brevipetiolata split from Q. serrata-China (Figure 1c). In addition, the median values of the effective population sizes of Q. serrata-China, Q. serrata var. brevipetiolata, and Q. serrata-Japan were 6.96×10^3 , 8.35×10^3 and 8.28×10^3 , respectively. The effective population size of the ancestral population was 5.09×10^2 , suggesting these three groups had experienced expansion after differentiation (Table S8; Figure 1c). The estimated median times of divergence between Q. serrata-China and Q. serrata-Japan (t2) were 4390 (95% Cl = 1640-8820) generations ago; Q. serrata var. brevipetiolata divergence from Q. serrata-China (t1) was 429 (95% CI = 105-1380) generations ago (Table S8). If we use a generation time of 80 years, based on a previous study on Quercus species (Yang et al., 2016), the divergence times for t2 and t1 would correspond to 0.35 Mya (95% CI = 0.13-0.71 Mya) and 0.034 Mya (95% CI = 0.008-0.11 Mya), respectively (Table S8; Figure 1c).

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Figure 1. Sampling sites, genetic assignment, and demographic history of *Q. serrata* and *Q. serrata* var. *brevipetiolata*. (a) Sampling location of *Q. serrata* (filled black circles) and *Q. serrata* var. *brevipetiolata* (open circles) in China and Japan. (b) Genetic assignment of *Q. serrata* and *Q. serrata* var. *brevipetiolata* at K = 2 and K = 3.

(c) Optimal demographic history scenarios of *O. serrata* and *O. serrata* var. *brevipetiolata* from four hypothetical scenarios using approximate Bayesian computation (ABC). Four demographic history scenarios and parameter settings are shown in Figure S4 and Table S7. t# represents time scale in terms of the number of generations and N# represents the effective population size.

Leaf morphological variation

We used GMM and the traditional morphological method to estimate leaf traits. CVA revealed that *Q. serrata*-China, *Q. serrata* var. *brevipetiolata*, and *Q. serrata*-Japan formed three groups along CV1 (89% of the total variance), with a relatively small overlap among *Q. serrata*, and *Q. serrata* var. *brevipetiolata* (Figure S5a). DA showed significant morphological differences among *Q. serrata*-China, *Q. serrata* var. *brevipetiolata*, and *Q. serrata*-China, *Q. serrata* var. *brevipetiolata*, and *Q. serrata*-China, *Q. serrata* var. *brevipetiolata*, and *Q. serrata*-Japan (Figure S5; P < 0.001). We detected significant differences in six traditional traits and two functional traits—leaf area and leaf mass (Figure S6). However, *Q. serrata*-China and *Q.* *serrata*-Japan exhibited significant differences only in petiole length (Figure S6b).

When we focused on the sympatric and allopatric populations of *Q. serrata* and *Q. serrata* var. *brevipetiolata* in China, we found that leaf shape CV1 and traditional leaf trait PC1 accounted for 83% and 77% of the total leaf variance, respectively. These metrics were used as a proxy for leaf variation in GMM and the traditional morphological methods. We detected greater leaf trait differentiation between *Q. serrata* and *Q. serrata* var. *brevipetiolata* in China in sympatry than in allopatry using both GMM (Figure 3a,b) and traditional morphological methods



Figure 2. Genetic differentiation between Q. serrata and Q. serrata var. brevipetiolata in China.

Principal component analysis (PCA) plot at individual level in sympatry (a) and allopatry (b).

Relationship between genetic and climatic PC for *Q. serrata* and *Q. serrata* var. brevipetiolata in China in sympatry (c) and allopatry (d) using genetically purebred individuals. The gray shading represents the 95% confidence interval (CI) of the regression line.

(Figure 3c,d; Figure S7). DA results also revealed greater morphological differences in sympatry (Figure S8a) than in allopatry (Figure S8b). Furthermore, leaf length and petiole length, as well as leaf area and leaf mass, showed greater morphological differentiation in sympatry than in allopatry (Figure S9). Additionally, we found positive $D_{\text{sym-allo}}$ values in all traits but SLA; controlling for genetically purebred individuals did not alter these findings (Table 1; Table S9).

Leaf area versus dry mass scaling relationships

Statistically significant scaling relationships between leaf area and mass were observed for *Q. serrata* and *Q. serrata* var. *brevipetiolata* (Table 1). Leaf area and mass were correlated ($0.5 < R^2 < 0.9$), with leaf area generally scaling less than a one-to-one ratio with increasing leaf mass. This suggests that increases in leaf area do not keep pace with

increases in leaf mass. We found statistically significant differences in the scaling of area and mass, as reflected by numerical values of α and log β for *Q. serrata* and *Q. serrata* var. *brevipetiolata* in sympatry. However, in allopatry, *Q. serrata* and *Q. serrata* var. *brevipetiolata* exhibited the same α and log β value (Table 2).

Morphological variation along climate

PCA analysis indicated an overlap in climate conditions between *Q. serrata* and *Q. serrata* var. *brevipetiolata* (Figure S10). We found greater morphological variation along the climatic gradient in sympatry than in allopatry, particularly in leaf length, petiole length, leaf area, and leaf mass (Figure 4; Figure S11). Additionally, leaf morphological variation responds differently to climatic factors in sympatry and in allopatry. In sympatry, leaf



Figure 3. Leaf trait difference for *Q. serrata* and *Q. serrata* var. *brevipetiolata* in China based on the genetically purebred individuals. Canonical variate analysis (CVA) plot of leaf shape measured by the geometric morphometric method (GMM) in sympatry (a) and allopatry (b) at the individual level with 90% confidence ellipses.

Principal component analysis (PCA) of six multivariate traits measured by the traditional method in sympatry (c) and allopatry (d) at the individual level with 90% confidence ellipses.

morphological variation in *Q. serrata* var. *brevipetiolata* was more strongly related to climate than in allopatry, particularly in lamina length, petiole length, lobe width, sinus width, WP, number of lobes, leaf area, and leaf mass (Figure S11). However, leaf trait variation of *Q. serrata* appears to be quite stable along this climatic gradient (Figure S11).

DISCUSSION

We conducted a comprehensive range-wide study to explore, for the first time, the genetic divergence, gene flow, and morphological variation of Q. serrata between China and Japan. Our findings revealed evidence of asymmetric gene flow, with genetic material primarily moving from China to Japan. The gene flow likely occurred during the glacial periods of the Quaternary, when lower sea levels formed a land bridge across the East China Sea, facilitating migration between these regions. This historical gene flow was followed by diversification within Chinese populations, suggesting that Q. serrata underwent significant evolutionary differentiation after the migration event. We then extended our investigation to test for character displacement (CD) by comparing the genetic and morphological divergence of *Q. serrata* and *Q. serrata* var. *brevipetiolata* in both sympatric and allopatric populations. Evidence of leaf morphological variation was detected and further analyzed with environmental factors, providing insights into how these variations interact with ecological pressures.

Demographic history

Our comparative study suggests that *Q. serrata* populations in China and Japan diverged from a common ancestor (approximately 4390 generations ago), followed by the separation of *Q. serrata* var. *brevipetiolata* from the Chinese populations (Figure 1c). However, divergence time estimates based on microsatellite data may be less reliable due to their high mutation rate and complex mutation model, which can lead to homoplasy and potential underestimation, particularly in scenarios of

Sympatry Allopatry Q. serrata var. Q. serrata var. Trait Q. serrata brevipetiolata Q. serrata brevipetiolata Dallo D_{sym} D_{sym - allo} Leaf shape CV1 -1.9 1.4 -21 2.0 -3.4 _4 1 0.7 Area (cm²) 35.2 18.7 31.0 19.9 16.6 11.0 5.6 Mass (mg) 254.8 130.9 214.5 148.2 123.8 66.2 57.6 SLA (cm^2/g) 143.8 142.1 145.6 131.6 1.6 14.1 -12.5 Lamina length (cm) 11.1 8.1 10.6 8.5 3.0 2.1 0.9 Petiole length (cm) 08 0.3 0.7 0.3 06 04 02 Lobe width (cm) 2.6 1.8 2.6 1.9 0.9 0.7 0.2 Sinus width (cm) 2.3 1.5 2.2 1.6 0.8 0.5 03 WP (cm) 6.1 4.4 6.0 4.8 0.5 1.7 1.2 Number of lobes 11.9 9.8 11.5 11.1 2.1 0.4 1.7 Traditional trait PC1 1.5 -1.61.3 -1.03.2 2.3 0.9

 Table 1 Direct tests of leaf traits divergence for sympatric and allopatric populations of *Q. serrata* and *Q. serrata* var. brevipetiolata based on the genetically purebred individuals

Positive $D_{sym - allo}$ values are indicative of patterns associated with divergence character displacement. Bold values indicate statistical significance at P < 0.05.

et al., 2022).

Table 2 Standardized major axis regression slopes and elevations (α and log β , respectively) for log-log linear relationships between leaf area and leaf mass for *Q. serrata* and *Q. serrata* var. *brevipetiolata* based on the genetically purebred individuals

	Sympatry		Allopatry	
	Q.	Q. serrata var.	Q.	Q. serrata var.
	serrata	brevipetiolata	serrata	brevipetiolata
α	0.9	0.5	0.7	0.7
logβ	2.1	1.8	1.9	1.9
95% CI	(0.8, 1.0)	(0.3, 0.7)	(0.6, 0.9)	(0.6, 0.8)
<i>R</i> ²	0.8	0.5	0.7	0.8
<i>P</i>	<0.001	<0.001	<0.001	<0.001

95% Cl indicates 95% confidence interval, estimating the precision of the α value. R^2 is the coefficient of determination, a number between 0 and 1 that measures how well a statistical model predicts an outcome. *P* value indicates statistical significance at P < 0.05.

population expansion or high gene flow (Lepais et al., 2022; Zhivotovsky, 2001). During the Quaternary glaciations, the East China Sea acted as a land bridge between China and Japan, facilitating gene flow among plant populations as sea levels dropped (Qiu et al., 2011). However, during warmer interglacial periods of the late Pleistocene (~40–20 ka), rising sea levels separated these regions again, leaving an imprint of secondary contact and contributing to the geographic separation we observed today. This pattern may explain the phylogenetic history of many plant species in the Sino-Japanese Floristic Region, including white oaks such as *Q. mongolica, Q. aliena*, and *Q. dentata* (T. Nagamitsu, personal communication).

Within China, we observed asymmetric historical gene flow, primarily from *Q. serrata* to *Q. serrata* var.

brevipetiolata (Table S6). Simulation studies indicates that introgression tends to occur predominantly from the local (early-colonizing) species to the invading (later-colonizing) species (Currat et al., 2008), which is a somewhat counterintuitive finding. However, this pattern is consistent across various empirical studies in both animals and plants, as seen in the Neanderthal genome, where gene flow occurred from the local Neanderthals to invading modern humans (Green et al., 2010). In this context, *Q. serrata* acts as the early-colonizing species, while *Q. serrata* var. *brevipetiolata* represents the later-colonizing taxon. The direction of introgression follows a pattern observed both in conifers (Du et al., 2009, 2011) and in deciduous oaks (Du

Character displacement along environmental gradient

Despite the rigorous framework for the study of CD proposed by Schluter and McPhail (1992), a direct test of divergence between two species in sympatry, but not allopatry, is sufficient to describe the pattern (Adams, 2004; Kooyers et al., 2017; Stuart et al., 2017; Stuart & Losos, 2013). Using different trait measurements, we found that both methods revealed greater trait variation in sympatry than in allopatry (Figure 3; Table 1). Geometric mormethod (GMM) phometric better capture leaf configuration, while traditional morphological methods focus on leaf dimensions and numbers. The complementary use of the two methods allowed us to detect divergence patterns that might be missed by relying on a single method. Statistical tests demonstrated that these trait differences are likely not caused by random phenotypic plasticity; however, it is important to note that phenotypic plasticity itself can contribute to CD (Pfennig & Murphy, 2002; Pfennig & Pfennig, 2009; Wilson, 1992).

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Figure 4. Relationship between leaf morphology and climatic PC for *Q. serrata* and *Q. serrata* var. brevipetiolata in China based on the genetically purebred individuals.

Variation in leaf shape using the geometric morphometric method in sympatric (a) and allopatric (b) populations as a function of climatic PC. Variation in leaf traits using traditional morphological methods in sympatric (c) and allopatric (d) populations as a function of climatic PC.

In addition, we observed that *Q. serrata* var. *brevipe-tiolata* exhibits traits such as smaller leaves, shorter petiole length, fewer lobes, and lower SLA (Table 1; Table 2), indicating a resource-conservative strategy. Species with more lobed leaves are commonly associated with environments such as cold or dry climates, as increased leaf lobation may help reduce water loss and surface area, improving water use regulation (Cavender-Bares, 2019; Royer et al., 2008; Wright et al., 2017). Moreover, shorter petioles can increase leaf overlap, potentially reducing light interception over the canopy but conserving moisture and mitigating heat stress (Bruschi et al., 2003; Kusi & Karsai, 2020). These characteristics correspond to a

resource-conservative strategy, which tends to be advantageous in more competitive or resource-limited environments (Goud et al., 2023; Wright et al., 2004). This suggests that *Q. serrata* var. *brevipetiolata*, having diverged later, has evolved these conservative traits in response to stronger competition for resources in sympatric environments.

The analysis of morphological variation along climate gradients revealed that *Q. serrata* var. *brevipetiolata*, the later-colonizing species which diverged more recently, shows greater responsiveness to environmental variations than *Q. serrata* (Figure 4). The different variation pattern in leaf traits along environmental gradients suggests that *Q.*

serrata var. brevipetiolata faces increased competition for resources in sympatric environments. Considering the role of demographic history in shaping population evolutionary potential, we infer that the imbalance in demographic history (i.e., effective population sizes) has contributed to the difference in leaf traits during the colonization of the later-colonizing species (Du et al., 2022; Lagache et al., 2014).

In contrast to the significantly greater differentiation in leaf morphology in sympatry than in allopatry, we detected no such trend using genetic markers (Figure 2). A first possible explanation for this discrepancy is that the observed morphological differences are due to phenotypic plasticity, i.e., the ability of a genotype to produce different phenotypes. However, as noted earlier, while phenotypic plasticity itself can contribute to CD, it is unlikely to fully explain the consistent patterns of divergence observed across multiple populations. A second explanation is that the putatively neutral genetic markers we used are insufficient for detecting adaptive divergence and are limited to identifying neutral genetic differentiation (Chung et al., 2023). Some genomic regions, or "genomic islands," might be associated with specific adaptive divergence, which could contribute to the observed morphological differences (Martin et al., 2023). To better understand this, further RAD sequencing, whole-genome resequencing, or even epigenetic studies are needed to identify such regions and clarify the role of adaptive divergence in shaping the traits we examined.

Resource_conservative vs. acquisitive strategy

We detected greater differentiation in leaf area and leaf mass in sympatric populations than in allopatric populations for both *Q. serrata*, the earlier-colonizing species, and *Q. serrata* var. *brevipetiolata*, the later-colonizing taxon (Table 1). It is interesting that the alpha value (regression slopes between leaf area and leaf mass) of *Q. serrata* increased from 0.7 in allopatry to 0.9 in sympatry, whereas the variety decreased from 0.7 to 0.5 (Table 2). This reveals that in sympatry, the variety is resource conservative, i.e., a higher investment in leaf mass components relative to leaf area and *Q. serrata* is resource-acquisitive (Onoda et al., 2017).

The trade-off between competition for resources and colonization ability was previously demonstrated in white oaks between species (Du et al., 2022). Although more evidence based on fitness assessment is required, this is, to our knowledge the first time such a trade-off has been detected within species. In this case, *Q. serrata* var. *brevipetiolata*, the later diverged variety, adopts a more resource-conservative strategy, investing more in leaf mass per unit area. Its morphological differentiation in sympatry could represent the early stages of ecological speciation or incipient speciation, where trait divergence

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reduces direct competition. *Q. serrata*, the earlier diverged species, follows a faster life history strategy with a greater focus on resource acquisition. If sympatric divergence continues, these morphological differences may become genetically fixed, leading to further differentiation between the two taxa over time. This possibility warrants further investigation using RAD sequencing and whole-genome resequencing data.

MATERIALS AND METHODS

Sampling

Since range-wide genetic data for *Q. serrata* in China are lacking (see Liu et al., 2021; Li, Wei, et al. (2021); Li, Zhang, et al. (2021) for studies covering part of its distribution) but chloroplast DNA (cpDNA) analysis has been conducted for populations in Japan (Okaura et al., 2007), we employed different sampling strategies in China and Japan. For populations in China, a comprehensive database of *Q. serrata* and *Q. serrata* var. brevipetiolata populations, with geographical information, was constructed using data from the Chinese Virtual Herbarium (CVH, https://www.cvh.ac.cn/) and Global Biodiversity Information Facility (GBIF, https://www.gbif. org/). Based on this database, we sampled 208 trees from 27 natural populations across the full species distribution range in mainland China. Initial species identification was performed in the field using morphological traits. In China, Q. serrata and Q. serrata var. brevipetiolata co-occur in the Qinling-Daba Mountain, which are considered as sympatric populations. Consequently, our sampling in China included eight populations (67 individuals) from the Qinling-Daba Mountain, categorized as sympatric, and 19 allopatric populations (141 individuals) from other regions (Figure 1a). To avoid sampling clonal individuals, we ensured that the study populations were at least 30 km apart and that sampled individuals within populations were spaced at least 10 meters apart. All trees we sampled were reproductively mature with a minimum diameter at breast height of 30 cm. For each tree, we collected one young leaf or new branch for DNA isolation, as well as four to five mature leaves from the four cardinal directions in the middle layer (mostly shade leaves) of the canopy for leaf morphological analysis. All leaf materials were rapidly dried in silica gel. Geographic information for each sampled population was recorded using a 621sc global positioning system (GPS) device (Garmin, Beijing, China).

For populations in Japan, we used 50 individuals from three populations representing the main lineages of *Q. serrata* as identified by cpDNA (Okaura et al., 2007) (Figure 1a; Table S1).

DNA extraction and SSR genotyping

We isolated total genomic DNA from leaf or the fresh cambium of branches for all 258 individuals using a Plant Genomic DNA Extraction Kit (Tiangen, Beijing, China) according to the manufacturer's recommendations. We selected 12 nuclear microsatellite loci from 25 SSR loci that were previously developed from *Q. robur, Q. petraea* (Durand et al., 2010), *Q. mongolica* (Ueno et al., 2008; Ueno & Tsumura, 2008), and effectively differentiate other white oak species in China (Lyu et al., 2018; Qi et al., 2021). These loci are located on eight chromosomes (Table S2) and have been demonstrated to have no null alleles using Micro-Checker v.2.2. In addition, we used coalescent simulations of the distribution of F_{ST} conditioned on heterozygosity to detect loci violating assumptions of neutrality implemented in FDIST2 (Beaumont & Nichols, 1996). We found no

loci linked to adaptive genetic variation (Figure S1). Microsatellite amplification and genotyping were performed as described in Lyu et al. (2018). We analyzed the amplified products using an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and the allele sizes and genotypes of individuals were determined by GeneMarker v.2.2 (Softgenetics LLC, State College, PA, USA). Microsatellite data are available in the figshare (doi: 10.6084/m9.figshare.27058843).

Genetic diversity and differentiation

Mean observed heterozygosity (H_0), mean expected heterozygosity (H_E), number of alleles (N_A), number of effective alleles (N_E), and Shannon index (I) were calculated by GenAlEx 6.5 (Peakall & Smouse, 2012). The significance of diversity differences between the three groups (*Q. serrata*-China, *Q. serrata* var. *brevipetiolata*, and *Q. serrata*-Japan) was tested using a Mann–Whitney *U* test implemented in SPSS v.22 (SPSS Inc., Chicago, IL, USA).

We estimated the genetic differentiation among populations using hierarchical analysis of molecular variance (AMOVA) implemented in Arlequin v.3.5 (Excoffier & Lischer, 2010). The significance of genetic differentiation was tested using 1000 permutations in Arlequin v.3.5. Pairwise genetic differentiation (F_{ST}) was calculated among all pairwise combinations of populations. We inferred the genetic clusters without consideration of sampling information using a model-based clustering program implemented in STRUCTURE v.2.3 (Pritchard et al., 2000). The program was run with the number of clusters (K) varied from 1 to 10 with 20 independent replicates for each K-value, and each run included 100,000 Markov Chain Monte Carlo (MCMC) repetitions and a burn-in of 100,000 steps. We determined the best K using △K statistics performed in the program STRUCTURE HARVESTER (Earl & VonHoldt, 2012; Evanno et al., 2005). We used admixture coefficient (Q) values to distinguish whether the sampled individuals were purebreds or admixtures with a strict threshold value of 0.9, as suggested in other oak population studies (Lepais et al., 2009; Peñaloza-Ramírez et al., 2010). Then, we visualized the genetic clusters using DISTRUCT v.1.1 (Rosenberg, 2004). In addition, we conducted a principal coordinate analysis (PCoA) based on genetic covariance between Q. serrata and Q. serrata var. brevipetiolata and plotted the first two eigenvectors to visualize genetic relatedness in GenAlEx 6.5 (Peakall & Smouse, 2012). We also performed a principal component analysis (PCA) to visualize the genetic relatedness among individuals of *Q. serrata* and *Q.* serrata var. brevipetiolata in sympatry and allopatry using the "ggpubr" R package (Kassambara, 2023).

Gene flow and demographic history

We estimated the historical migration rates using the continuous Brownian motion model implemented in MIGRATE-N v.4.4.3 (Beerli, 2006; Beerli & Felsenstein, 2001). We estimated the parameters θ (four times effective population size multiplied by mutation rate per site per generation) and M (migration rate divided by the mutation rate) with slice sampling and uniform prior distribution. The three long chains were performed with 10,000 steps at a sampling interval of 20 increments after discarding the first 10,000 records as a burn-in for each chain. This was followed by a static heating scheme at four temperatures (1.0, 1.5, 3.0, 1 000 000) to efficiently search the genealogy space. We repeated this procedure three times to ensure consistency of estimates and assessed average maximum-likelihood estimates of θ and M along with 95% confidence intervals (CIs). We selected the best model by comparing the marginal likelihoods using the Bezier approximation and harmonic mean method, and we calculated log Bayes

factors to determine which model was the best fit for the data (Beerli & Palczewski, 2010).

To further elucidate the population demographic history, we performed an approximate Bayesian computation (ABC) analysis in DIYABC v.2.1 (Cornuet et al., 2014). To simplify the scenarios, we defined three groups based on the results of STRUCTURE: Q. serrata-China, Q. serrata var. brevipetiolata, and Q. serrata-Japan (Figure 1b). Four historical population divergence scenarios: scenarios 1, 2, and 3 differed in that the first diverging group was Q. serrata-China, Q. serrata var. brevipetiolata, or Q. serrata-Japan, respectively, whereas under scenario 4, the three groups diverged simultaneously (Figure S4a). To identify the most likely divergence scenario, we calculated the posterior probability with 95% CIs for each scenario by performing a logistic regression on the 1% of simulated data that was closest to the observed data (following Cornuet et al., 2008). To assess the precision of parameter estimation, we computed the median of the relative median of absolute errors (RMAE) on 500 pseudo-observed data sets simulated under the best-fit scenario. In addition, we estimated parameter posterior distributions to check the model and visualize the fit between simulated and observed data by the option "model checking" in DIYABC v.2.1 (Cornuet et al., 2014).

Leaf morphology

We used the geometric morphometric method (GMM) and traditional morphological method to estimate the leaf morphological variation for 1224 leaves from 248 individuals (four or five leaves per individual) (Table S1). We flattened, dried, and scanned these leaves at 300 dpi resolution using a CanoScan 5600 F scanner (Canon, Tokyo, Japan). For GMM, we characterized scanned images by 13 landmarks (Figure 5a) as suggested for other white oak species using Image J v.1.5 (Abràmoff et al., 2005; Viscosi, Lepais, Gerber, & Fortini, 2009; Liu et al., 2018; Du et al., 2022). Three of these landmarks, LM1-LM3, were distributed along the middle axis of leaves, while the nine others (LM4-LM13) were in pairs and symmetrically distributed on both sides of the leaves (Savriama & Klingenberg, 2011) (Table S3). Then these landmarks were converted to a configuration of 13 pairs of Cartesian coordinates (x, y) for each leaf. For traditional morphological methods, we measured six traditional traits: lamina length, petiole length, lobe width, sinus width, length of lamina at largest width (WP), and lobe number, and three functional traits: leaf mass, leaf area, and specific leaf area (SLA, leaf area per unit of dry leaf weight; $cm^2 g^{-1}$) (Figure 5b). The trait value for each tree was calculated by averaging the values of four to five selected leaves from the same tree.

Multivariate analyses of leaf morphology

For GMM, Cartesian coordinates (x, y) of the landmarks were used for the following analysis using the MorphoJ program (Klingenberg, 2011). We first performed a generalized procrustes analysis (GPA) (Rohlf & Slice, 1990) to minimize differences between landmark configurations by translation, scaling, and rotation to maximize the coincidence of leaf coordinate data (Klingenberg, 2011). Next, we took the mean of the landmarks across four to five leaves of the same tree to create mean configurations at the tree level. Using mean landmark values per tree rather than individual leaf landmark data does not result in a significant loss of information in subsequent analyses (Viscosi & Cardini, 2011). Finally, we generated a covariance matrix of the average configuration for the leaf shape variation. To visualize the differences in leaf shape between Q. serrata and Q. serrata var. brevipetiolata in sympatry and allopatry, we conducted discriminant analysis (DA) and canonical variate analysis (CVA) using pure individuals identified



Figure 5. Morphological measurements for leaf traits.

Landmark configuration of leaves (a), showing the locations of the 13 landmarks described in Table S3, and description of the traditional leaf morphological traits in *Q. serrata* and *Q. serrata* var. *brevipetiolata* (b).

by genetic delimitation as grouping variables. CVA maximizes the separation of specified groups to test group differences and produces ordination plots based on Mahalanobis distances with the statistical significance of pairwise differences in mean shapes, which were assessed by permutation tests (T^2 statistics; 10 000 permutations per test). DA provides reliable information on groups through cross-validation scores classification with T^2 statistics (*P* value for tests with 1000 permutations <0.0001) (Klingenberg, 2011; Viscosi, 2015).

For traditional traits, we compared the differences in lamina length, petiole length, lobe width, sinus width, WP, lobe number, leaf mass, leaf area, and SLA between *Q. serrata* and *Q. serrata var. brevipetiolata* in sympatry and allopatry based on the genetically purebred individuals using the "ggplot2" and "ggpubr" R packages (Kassambara, 2023; Wickham, 2016). We also performed a PCA of six traditional traits (lamina length, petiole length, lobe width, sinus width, WP, and lobe number) for pure *Q. serrata* and *Q. serrata var. brevipetiolata* individuals identified by genetic delimitation using the "vegan" and "ggplot2" packages in R (Oksanen et al., 2022; Wickham, 2016).

Scaling relationships of leaf functional traits

To detect if the leaf area and leaf mass of studied species follow a quantifiable scaling relationship, we checked if the leaf area is independent of intraspecies differences in leaf mass as suggested by Niklas et al. (2007). We calculated the standardized major axis slopes and intercepts of leaf area and leaf mass for *Q. serrata* and *Q. serrata var. brevipetiolata* based on the genetically purebred individuals. Preliminary regression analyses showed that all bivariate relationships were log–log linear; therefore, we used all subsequent statistical analyses as log₁₀-transformed data. We computed these parameters and their respective 95% Cls using R (version 4.2.0) (R Core Team, 2022).

Character displacement

To test for intraspecific character displacement, where greater divergence occurs in sympatry, we calculated the mean leaf trait difference of the *Q. serrata* and *Q. serrata* var. *brevipetiolata* in the sympatric (D_{sym}) and allopatric (D_{allo}) populations, along with the difference between these values ($D_{sym-allo}$), as described by Adams (2004). To enhance the comparability of results between

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sympatric and allopatric populations, we minimized the influence of admixed individuals by focusing on genetically purebred individuals, applying a strict Q value threshold of 0.9 based on genetic structure analysis (Du et al., 2022). A positive $D_{\text{sym-allo}}$ value indicates greater divergence in sympatry than in allopatry.

Genetic and morphological variation in response to climate

We extracted 19 bioclimatic variables of the studied populations from WorldClim v.2 raster layers at 30 s (~ 1 km²) resolution (Fick & Hijmans, 2017). These variables represent the average temperature and precipitation values recorded at meteorological stations globally between 1970 and 2000. Then, these variables were transformed into principal components (PCs) using PCA in the "vegan" R package (Oksanen et al., 2022). We selected six principal components (PCs) that cumulatively explained more than 99% of the total variance. A weighted combination of these PCs was used to create a comprehensive environmental axis, where the weights were based on the variance contribution of each PC. This new variable represents an integrated environmental index for each site and was used for subsequent association analysis for Q. serrata and Q. serrata var. brevipetiolata in sympatry and allopatry. We evaluated traits' response to climate in sympatry and allopatry, considering population as random variables. The statistical significance of fixed effects was determined using an ANOVA test implemented in the "car" R package (Fox et al., 2013). In addition, we estimated genetic variation response to climate in sympatry and allopatry using genetically purebred individuals. Although SSR markers are neutral, they can reflect underlying demographic processes influenced by environmental factors. This assessment was conducted by analyzing the relationships between genetic and climate variables, followed by linear regression modeling to test for associations performed in R. Correlation coefficients were calculated to quantify the strength of these relationships.

AUTHOR CONTRIBUTIONS

FD designed the research; MQ, JW, and RW performed the experiments; MQ performed the analyses; MQ, FD, YL, SU and YS wrote the manuscript; and all authors contributed to its revision.

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ACKNOWLEDGEMENTS

We thank Yixin Kang, Yang Xu, and Yuejuan Li from Beijing Forestry University (BFU), P.R. China; Ayako Kanazashi, Keiko Kitamura, and Teruyoshi Nagamitsu from Forestry and Forest Products Research Institute (FFPRI) for leaf sampling; and Asako Matsumoto (FFPRI) for DNA preparation of the two populations in Japan. We also thank comments from Dr. Qingmin Han (FFPRI) for the explanation of leaf functional traits; Dr. Teruyoshi Nagamitsu (FFPRI) about the demographic history of Japanese oaks; and Dr. Rémy J. Petit from French National Institute for Agriculture, Food, and Environment (INRAE) for discussion on character displacement and comments on the whole manuscript. Finally, we thank Prof. Wenting Wang from Northwest University for Nationalities (XBMU), P.R. China, for guidance in data analysis and Prof. Myong Gi Chung from Gyeongsang National University, South Korea for finally editing when proofreading the draft. This research was supported by the National Key Programme of Research and Development, the Ministry of Science and Technology (2022YFF1301401) to YBL, National Science Foundation of China (No. 42071060) to FD. The sampling in Japan was partly supported by "Japan Science and Technology Agency (JST) SAKURA SCIENCE Exchange Program (Sakura Science Plan), Japan" to SU and FD.

CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Photographs of sampling sites can be obtained at https://www.oakofchina.org/photo-of-sampling/. Genotyping data can be found at https://doi.org/10.6084/m9. figshare.27058843, and leaf morphological data can be obtained at https://doi.org/10.6084/m9.figshare.27058927.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1. Distribution of F_{ST} values from the 12 SSR loci in function of the heterozygosity using FDIST2 with the 95% confidence level.

Figure S2. Variation of ΔK as a function of K, suggesting the existence of two and three clusters as the likely scenario.

Figure S3. Genetic differentiation between *Q. serrata* and *Q. serrata* var. *brevipetiolata* by principal coordinate analysis (PCoA) among populations (a) and individuals (b) implemented in GenAIEx 6.5.

Figure S4. Results from approximate Bayesian computation (ABC). (a) The four scenarios for the population history of the three groups (*Q. serrata*-China, *Q. serrata* var. *brevipetiolata*, and *Q. serrata*-Japan) in DIYABC, with 95% confidence intervals (CI) of each scenario based on the logistic regression approach. (b) Comparison of posterior probabilities for four simulated scenarios of species differentiation obtained by logistic and direct regression. Scenario 1 was found to be the most probable in both the linear direct and logistic regression tests. (c) Prior and posterior values estimated for scenario 1 were close to the observed dataset, supporting the certainty of the chosen scenario.

Figure S5. Leaf geometric morphometric analysis for *Q. serrata* var. brevipetiolata, *Q. serrata*-China, and *Q. serrata*-Japan based on the genetically purebred individuals. (a) Scatter plot of the canonical variate analysis (CVA) at the individual level with 90%

confidence ellipses. (b) Discriminant analysis (DA) for the leaf shape differentiation of *Q. serrata* var. *brevipetiolata* vs. *Q. serrata*-China, (c) *Q. serrata*-China vs. *Q. serrata*-Japan, and (d) *Q. serrata* var. *brevipetiolata* vs. *Q. serrata*-Japan.

Figure S6. Leaf trait difference estimated by the traditional method for *Q. serrata var. brevipetiolata*, *Q. serrata*-China, and *Q. serrata*-Japan based on the genetically purebred individuals. (a) lamina length; (b) petiole length; (c) lobe width; (d) sinus width; (e) WP; (f) number of lobes; (g) leaf area; (h) leaf mass; (i) SLA. The box in each boxplot shows the lower, median, and upper quartile values, and whiskers indicate the ranges of the variation in each species. ****P* < 0.001; ***P* < 0.015.

Figure S7. Plot of six traditional leaf trait PC1 of *Q. serrata* and *Q. serrata* var. *brevipetiolata* in sympatry vs. allopatry based on the genetically purebred individuals. The box in each boxplot shows the lower, median, and upper quartile values, and whiskers indicate the ranges of the variation in each species. ***P < 0.001.

Figure S8. Discriminant analysis (DA) of leaf shape in sympatry (a) and allopatry (b) at the individual level for *Q. serrata* and *Q. serrata* var. *brevipetiolata* based on the genetically purebred individuals.

Figure S9. Leaf trait difference of *Q. serrata* and *Q. serrata* var. *brevipetiolata* in sympatry vs. allopatry based on the genetically purebred individuals. (a) lamina length; (b) petiole length; (c) lobe width; (d) sinus width; (e) WP; (f) number of lobes; (g) leaf area; (h) leaf mass; (i) SLA. Error bars are estimations of 95% confidence intervals. The blue and red lines represent the difference in leaf traits between sympatry and allopatry for *Q. serrata* and *Q. serrata* var. *brevipetiolata*, respectively. ****P* < 0.001; **P* < 0.05.

Figure S10. Biplot obtained by principal component analysis (PCA) according to the 19 climate variables for *Q. serrata* and *Q. serrata* var. *brevipetiolata* in China.

Figure S11. Relationship between leaf morphology and climatic PC for *Q. serrata* and *Q. serrata* var. *brevipetiolata* in China based on the genetically purebred individuals. (a) lamina length; (b) petiole length; (c) lobe width; (d) sinus width; (e) WP; (f) number of lobes; (g) leaf area; (h) leaf mass; (i) SLA.

Table S1. Details of the 30 natural populations of *Q. serrata* and *Q. serrata* var. *brevipetiolata* (see separate file).

Table S2. Detailed information of 12 pairs of SSR primers.

Table S3. The description of 13 landmarks.

Table S4. Estimation of genetic diversity of *Q. serrata* and *Q. serrata* var. *brevipetiolata* populations implemented in GenAlEx 6.5.

 Table S5. Analysis of molecular variance (AMOVA) for *Q. serrata* and *Q. serrata* var. brevipetiolata using Arlequin v. 3.5.

Table S6. Historical gene flow as estimated by MIGRATE-N between *Q. serrata* and *Q. serrata* var. *brevipetiolata* populations. **Table S7.** Prior distributions of the parameters used in DIYABC.

Table S8. Posterior parameter estimations for the best-supported scenario 1 in ABC. Distribution of posterior probabilities at 2.5% and 97.5% percentiles are indicated. Relative median of the absolute error (RMAE) of parameters of scenario 1 are indicated.

Table S9. Direct tests of leaf traits divergence for sympatric and allopatric populations of *Q. serrata* and *Q. serrata* var. *brevipetio-lata* using all individuals.

Table S10. Loading values of climate PCA.

REFERENCES

Abràmoff, M.D., Magalhães, P.J., Paulo, J. & Ram, S.J. (2005) Image processing with image J. Biophotonics International, 11, 36–42.

Adams, D.C. (2004) Character displacement via aggressive interference in Appalachian salamanders. *Ecology*, **85**, 2664–2670.

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- Adams, D.C. & Rohlf, F.J. (2000) Ecological character displacement in *Plethodon*: biomechanical differences found from a geometric morphometric study. *Proceedings of the National Academy of Sciences*, 97, 4106–4111.
- Arenas-Navarro, M., García-Oliva, F., Terrazas, T., Torres-Miranda, A. & Oyama, K. (2020) Leaf habit and stem hydraulic traits determine functional segregation of multiple oak species along a water availability gradient. *Forests*, **11**, 894.
- Beaumont, M.A. & Nichols, R.A. (1996) Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 263, 1619–1626.
- Beerli, P. (2006) Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. *Bioinformatics*, 22, 341–345.
- Beerli, P. & Felsenstein, J. (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. Proceedings of the National Academy of Sciences, 98, 4563–4568.
- Beerli, P. & Palczewski, M. (2010) Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics*, 185, 313–326.
- Brown, W.L. & Wilson, E.O. (1956) Character displacement. Systematic Zoology, 2, 49–64.
- Bruschi, P., Grossoni, P. & Bussotti, F. (2003) Within-and among-tree variation in leaf morphology of *Quercus petraea* (Matt.) Liebl. Natural populations. *Trees*, **17**, 164–172.
- Cavender-Bares, J. (2019) Diversification, adaptation, and community assembly of the American oaks (*Quercus*), a model clade for integrating ecology and evolution. *New Phytologist*, 221, 669–692.
- Chung, M.Y., Merilä, J., Kim, Y., Mao, K., López-Pujol, J. & Chung, M.G. (2023) A review on Q ST-F ST comparisons of seed plants: insights for conservation. *Ecology and Evolution*, **13**(3), e9926.
- Cornuet, J.M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R. et al. (2014) DIYABC v2. 0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. *Bioinformatics*, 30 (8), 1187–1189. Available from: https://doi.org/10.1093/bioinformatics/btt763
- Cornuet, J.M., Santos, F., Beaumont, M.A., Robert, C.P., Marin, J.M., Balding, D.J. et al. (2008) Inferring population history with DIY ABC: a userfriendly approach to approximate Bayesian computation. *Bioinformatics*, 24, 2713–2719.
- Currat, M., Ruedi, M., Petit, R.J. & Excoffier, L. (2008) The hidden side of invasion: massive introgression by local genes. *Evolution*, 62, 1908–1920.
 Darwin, C. (1859) On the origins of species by means of natural selection.
- London: Murray, p. 247. Day, T. & Young, K.A. (2004) Competitive and facilitative evolutionary diver-
- Day, I. & Young, K.A. (2004) Competitive and facilitative evolutionary diversification. Bioscience, 54, 101–109.
- Dayan, T. & Simberloff, D. (2005) Ecological and community-wide character displacement: the next generation. *Ecology Letters*, 8, 875–894.
- Denk, T., Grimm, G.W., Manos, P.S., Deng, M. & Hipp, A.L. (2018) An updated infrageneric classification of the oaks: review of previous taxonomic schemes and synthesis of evolutionary patterns. In: Gil-Pelegrín, E., Peguero-Pina, J. & Sancho-Knapik, D. (Eds.) Oaks physiological ecology. Exploring the functional diversity of genus Quercus L.Cham, Switzerland: Springer, pp. 13–38.
- Doebeli, M. & Dieckmann, U. (2003) Speciation along environmental gradients. Nature, 421, 259–264.
- Du, F., Peng, X.L., Liu, J.Q., Lascoux, M., Hu, F.S. & Petit, R.J. (2011) Direction and extent of organelle DNA introgression between two spruce species in the Qinghai-Tibetan plateau. *New Phytologist*, **192**(4), 1024–1033. Available from: https://doi.org/10.1111/j.1469-8137.2011.03853.x
- Du, F., Qi, M., Zhang, Y. & Petit, R.J. (2022) Asymmetric character displacement in mixed oak stands. New Phytologist, 236, 1212–1224.
- Du, F.K., Petit, R.J. & Liu, J.Q. (2009) More introgression with less gene flow: chloroplast vs. mitochondrial DNA in the *Picea asperata* complex in China, and comparison with other conifers. *Molecular Ecology*, **18**, 1396– 1407.
- Dumolin-Lapègue, S., Demesure, B., Le Corre, V., Fineschi, S. & Petit, R.J. (1997) Phylogeographic structure of white oaks throughout the European continent. *Genetics*, **146**, 1475–1487.
- Durand, J., Bodénès, C., Chancerel, E., Frigerio1, J., Vendramin, G., Sebastiani, F. et al. (2010) A fast and cost-effective approach to develop and

map EST-SSR markers: oak as a case study. *BMC Genomics*, **11**, 570, 570. Available from: https://doi.org/10.1186/1471-2164-11-570

- Earl, D.A. & VonHoldt, B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, 4, 359–361.
- Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**(8), 2611–2620. Available from: https://doi.org/10. 1111/j.1365-294X.2005.02553.x
- Excoffier, L. & Lischer, H.E.L. (2010) ARLEQUIN suite v.3.5: a new series of programs to perform population genetics analyses under Linux and windows. *Molecular Ecology Resources*, **10**, 564–567.
- Fick, S.E. & Hijmans, R.J. (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatol*ogy, 37, 4302–4315.
- Fox, J., Friendly, M. & Weisberg, S. (2013) Hypothesis tests for multivariate linear models using the car package. *R Journal*, 5, 39–52.
- Fujiwara, K. & Harada, A. (2015) Warm-temperate deciduous forests around the northern hemisphere. Cham, Switzerland: Springer.
- Goud, E.M., Agrawal, A.A. & Sparks, J.P. (2023) A direct comparison of ecological theories for predicting the relationship between plant traits and growth. *Ecology*, **104**, e3986.
- Grant, P.R. & Grant, B.R. (2006) Evolution of character displacement in Darwin's finches. *Science*, 313, 224–226.
- Grant, V. (1994) Modes and origins of mechanical and ethological isolation in angiosperms. *Proceedings of the National Academy of Sciences*, 91, 3–10.
- Green, R.E., Krause, J., Briggs, A.W., Maricic, T., Stenzel, U., Kircher, M. et al. (2010) A draft sequence of the Neandertal genome. *Science*, 328, 710–722.
- Grivet, D., Deuilloux, M.F., Petit, R.J. & Sork, V.L. (2006) Contrasting patterns of historical colonization in white oaks (*Quercus* spp.) in California and Europe. *Molecular Ecology*, **15**, 4085–4093.
- Gurevitch, J., Morrow, L.L., Wallace, A. & Walsh, J.S. (1992) A metaanalysis of competition in field experiments. *American Naturalist*, 140, 539–572.
- Henderson, A. (2006) Traditional morphometrics in plant systematics and its role in palm systematics. *Botanical Journal of the Linnean Society*, 151, 103–111. Available from: https://doi.org/10.1111/j.1095-8339.2006.00526.x
- Hipp, A.L., Manos, P.S., González-Rodríguez, A., Hahn, M., Kaproth, M., McVay, J.D. et al. (2018) Sympatric parallel diversification of major oak clades in the Americas and the origins of Mexican species diversity. New Phytologist, 217, 439–452.
- Hipp, A.L., Manos, P.S., Hahn, M., Avishai, M., Bodenes, C., Cavender-Bares, J. et al. (2020) Genomic landscape of the global oak phylogeny. New Phytologist, 226, 1198–1212.
- Huang, C.J., Zhang, Y.T. & Bartholomew, B. (1999) Fagaceae. In: Wu, Z.Y. & Raven, P.H. (Eds.) *Flora of China*, Vol. 4. Beijing: Science Press, pp. 370– 380.
- Kassambara, A. (2023) ggpubr: 'ggplot2' based publication ready plots. R package v.0.6.0. Available from: https://CRAN.R-project.org/ package=ggpubr
- Klingenberg, C.P. (2011) MorphoJ: an integrated software package for geometric morphometrics. *Molecular Ecology Resources*, **11**, 353–357.
- Kooyers, N.J., James, B. & Blackman, B.K. (2017) Competition drives trait evolution and character displacement between *Mimulus* species along an environmental gradient. *Evolution*, **71**, 1205–1221.
- Kusi, J. & Karsai, I. (2020) Plastic leaf morphology in three species of *Quercus*: the more exposed leaves are smaller, more lobated and denser. *Plant Species Biology*, **35**, 24–37.
- Lagache, L., Klein, E.K., Ducousso, A. & Petit, R.J. (2014) Distinct male reproductive strategies in two closely related oak species. *Molecular Ecology*, 23, 4331–4343.
- Lepais, O., Aissi, A., Véla, E. & Beghami, Y. (2022) Joint analysis of microsatellites and flanking sequences enlightens complex demographic history of interspecific gene flow and vicariance in rear-edge oak populations. *Heredity*, **129**, 169–182.
- Lepais, O., Petit, R.G., Guichoux, E., Lavabre, J.E., Alberto, F., Kremer, A. et al. (2009) Species relative abundance and direction of introgression in oaks. *Molecular Ecology*, 18(10), 2228–2242. Available from: https://doi. org/10.1111/j.1365-294X.2009.04137.x

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- Li, X., Wei, G., El-Kassaby, Y.A. & Fang, Y. (2021) Hybridization and introgression in sympatric and allopatric populations of four oak species. *BMC Plant Biology*, 21, 266.
- Li, Y.J., Zhang, Y.Y., Liao, P.C., Wang, T.R., Wang, X.Y., Ueno, S. et al. (2021) Genetic, geographic and climatic factors jointly shape leaf morphology of an alpine oak, *Quercus aquifolioides* Rehder & E.H. Wilson. *Annals of Forest Science*, 78(3), 64, 64. Available from: https://doi.org/10. 1007/s13595-021-01077-w
- Liu, X., Chang, E., Liu, J.F. & Jiang, Z.P. (2021) Comparative analysis of the complete chloroplast genomes of six white oaks with high ecological amplitude in China. *Journal of Forestry Research*, **32**, 2203–2218.
- Liu, Y., Li, Y.J., Song, J.L., Zhang, R.P., Yan, Y., Wang, Y.Y. et al. (2018) Geometric morphometric analyses of leaf shapes in two sympatric Chinese oaks: *Quercus dentata* Thunberg and *Quercus aliena* Blume (Fagaceae). *Annals of Forest Science*, **75**(4), 1, 90–12. Available from: https://doi. org/10.1007/s13595-018-0770-2
- Lyu, J., Song, J., Liu, Y., Wang, Y.Y., Li, J.Q. & Du, F.K. (2018) Species boundaries between three sympatric oak species: *Quercus aliena, Q. Dentata,* and *Q. Variabilis* at the northern edge of their distribution in China. Frontiers in Plant Science, 9, 414.
- Marcus, L.F. (1990) Traditional morphometrics. In: Rohlf, F.J. & Bookstein, F.L. (Eds.) Proceedings of the Michigan morphometrics workshop. Michigan: University of Michigan Museum of Zoology, pp. 77–122.
- Martin, C.A., Sheppard, E.C., Ali, H.A.A., Illera, J.C., Suh, A., Spurgin, L.G. et al. (2024) Genomic landscapes of divergence among Island bird populations: evidence of parallel adaptation but at different loci? *Molecular Ecology*, 33(12), e17365. Available from: https://doi.org/10.1111/mec. 17365
- Milla, R. & Reich, P.B. (2007) The scaling of leaf area and mass: the cost of light interception increases with leaf size. *Proceedings of the Royal Soci*ety B: Biological Sciences, 274, 2109–2115.
- Mitteroecker, P. & Gunz, P. (2009) Advances in geometric morphometrics. Evolutionary Biology, 36, 235–247.
- Nicotra, A.B., Leigh, A., Boyce, C.K., Jones, C.S., Niklas, K.J., Royer, D.L. et al. (2011) The evolution and functional significance of leaf shape in the angiosperms. *Functional Plant Biology*, 38, 535–552.
- Niklas, K.J., Cobb, E.D., Niinemets, Ü., Reich, P.B., Sellin, A., Shipley, B. et al. (2007) "Diminishing returns" in the scaling of functional leaf traits across and within species groups. *Proceedings of the National Academy* of Sciences, 104, 8891–8896.
- Niklas, K.J., Cobb, E.D. & Spatz, H.C. (2009) Predicting the allometry of leaf surface area and dry mass. *American Journal of Botany*, 96, 531–536.
- Okaura, T., Quang, N.D., Ubukata, M. & Harada, K. (2007) Phylogeographic structure and late quaternary population history of the Japanese oak *Quercus mongolica* var. *crispula* and related species revealed by chloroplast DNA variation. *Genes & Genetic Systems*, 82, 465–477.
- Oksanen, J., Simpson, F.G., Simpson, F.G., Kindt, R., Legendre, P. & McGlinn, D. (2022) VEGAN: Community ecology package. R package v.2.5-7. https://CRAN.R-project.org/package=vegan
- Oldfield, S.E. & Eastwood, A. (2007) The Red List of Oaks. Cambridge, UK: Fauna and Flora International.
- Onoda, Y., Wright, I.J., Evans, J.R., Hikosaka, K., Kitajima, K., Niinemets, Ü. et al. (2017) Physiological and structural tradeoffs underlying the leaf economics spectrum. New Phytologist, 214, 1447–1463.
- Peakall, R. & Smouse, P.E. (2012) GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research-an update. *Bioin-formatics*, 28, 2537–2539.
- Peñaloza-Ramírez, J.M., González-Rodríguez, A., Mendoza-Cuenca, L., Caron, H., Kremer, A. & Oyama, K. (2010) Interspecific gene flow in a multispecies oak hybrid zone in the sierra Tarahumara of Mexico. Annals of Botany, 105, 389–399.
- Petit, R.J., Brewer, S., Bordács, S., Burg, K., Cheddadi, R., Coart, E. et al. (2002) Identification of refugia and post-glacial colonisation routes of European white oaks based on chloroplast DNA and fossil pollen evidence. Forest Ecology and Management, 156, 49–74.
- Pfennig, D.W. & Murphy, P.J. (2002) How fluctuating competition and phenotypic plasticity mediate species divergence. *Evolution*, 56, 1217– 1228.
- Pfennig, D.W. & Pfennig, K.S. (2010) Character displacement and the origins of diversity. The American Naturalist, 176, S26–S44.

- Pfennig, D.W. & Pfennig, K.S. (2020) Character displacement. Current Biology, 30, R1023–R1024.
- Pfennig, K.S. & Pfennig, D.W. (2009) Character displacement: ecological and reproductive responses to a common evolutionary problem. *The Quarterly Review of Biology*, 84, 253–276.
- Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, 155, 945–959.
- Qi, M., Du, F., Guo, F., Yin, K.Q. & Tang, J.J. (2024) Species identification through deep learning and geometrical morphology in oaks (*Quercus* spp.): pros and cons. *Ecology and Evolution*, 14, e11032.
- Qi, M., Zhang, Y.Y., Li, J.W. & Du, F. (2021) Genetic diversity and differentiation study of *Quercus dentata* with regards to its distribution in China and Japan. *Journal of Lanzhou University (Natural Sciences)*, **57**, 720– 726 (in Chinese with English Abstract).
- Qiu, Y.X., Fu, C.X. & Comes, H.P. (2011) Plant molecular phylogeography in China and adjacent regions: tracing the genetic imprints of quaternary climate and environmental change in the world's most diverse temperate flora. *Molecular Phylogenetics and Evolution*, **59**, 225–244.
- R Core Team. (2022) R: A language and environment for statistical computing. Vienna: R Foundation for statistical computing. Accessed 1 July 2022. https://www.r-project.org/.
- Rellstab, C., Bühler, A., Graf, R., Folly, C. & Gügerli, F. (2016) Using joint multivariate analyses of leaf morphology and molecular-genetic markers for taxon identification in three hybridizing European white oak species (*Quercus*, spp.). Annals for Science, **73**, 1–11.
- Rohlf, F.J. & Slice, D.E. (1990) Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology*, **39**(1), 40–59. Available from: https://doi.org/10.2307/2992207
- Rosenberg, N.A. (2004) Distruct: a program for the graphical display of population structure. *Molecular Ecology Notes*, **4**, 137–138.
- Royer, D.L., McElwain, J.C., Adams, J.M. & Wilf, P. (2008) Sensitivity of leaf size and shape to climate within Acer rubrum and Quercus kelloggii. New Phytologist, 179, 808–817.
- Rueffler, C., Van Dooren, T.J.M., Leimar, O. & Abrams, P.A. (2006) Disruptive selection and then what? *Trends in Ecology & Evolution*, 21, 238– 245.
- Sancho-Knapik, D., Escudero, A., Mediavilla, S., Scoffoni, C., Zailaa, J., Cavender-Bares, J. et al. (2020) Deciduous and evergreen oaks show contrasting adaptive responses in leaf mass per area across environments. *New Phytologist*, 230, 521–534.
- Savriama, Y. & Klingenberg, C.P. (2011) Beyond bilateral symmetry: geometric morphometric methods for any type of symmetry. BMC Evolutionary Biology, 11, 280–280.
- Schluter, D. & McPhail, J.D. (1992) Ecological character displacement and speciation in sticklebacks. *The American Naturalist*, 140, 85–108.
- Skelton, R.P., Anderegg, L.D.L., Diaz, J., Klinga, M.M., Pappera, P., Lamarqued, L.J. et al. (2021) Evolutionary relationships between drought-related traits and climate shape large hydraulic safety margins in western north American oaks. Proceedings of the National Academy of Science, 118(10), e2008987118. Available from: https://doi.org/10. 1073/pnas.2008987118
- Smith, T.B. & Skúlason, S. (1996) Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annual Review of Ecology and Systematics, 27, 111–133.
- Stotz, G.C., Salgado-Luarte, C., Escobedo, V.M., Valladares, F. & Gianoli, E. (2022) Phenotypic plasticity and the leaf economics spectrum: plasticity is positively associated with specific leaf area. *Oikos*, 2022, e09342.
- Stuart, Y.E., Inkpen, S.A., Hopkins, R. & Bolnick, D.I. (2017) Character displacement is a pattern: so, what causes it? *Biological Journal of the Linnean Society*, **121**, 711–715.
- Stuart, Y.E. & Losos, J.B. (2013) Ecological character displacement: glass half full or half empty? *Trends in Ecology & Evolution*, 28, 402–408.
- Ueno, S., Taguchi, Y. & Tsumura, Y. (2008) Microsatellite markers derived from *Quercus mongolica* var. *crispula (Fagaceae)* inner bark expressed sequence tags. *Genes & Genetic Systems*, 83, 179–187.
- Ueno, S. & Tsumura, Y. (2008) Development of ten microsatellite markers for *Quercus mongolica* var. *crispula* by database mining. *Conservation Genetics*, 9, 1083–1085.
- Veech, J.A., Charlet, D.A. & Jenkins, S.H. (2000) Interspecific variation in seed mass and the coexistence of conifer species: a null model test. *Evolutionary Ecology Research*, 2, 353–363.

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- Viscosi, V. (2015) Geometric morphometrics and leaf phenotypic plasticity: assessing fluctuating asymmetry and allometry in European white oaks (*Quercus*). *Botanical Journal of the Linnean Society*, **179**(2), 335–348.
- Viscosi, V., Antonecchia, G., Lepais, O., Fortini, P., Gerber, S. & Loy, A. (2012) Leaf shape and size differentiation in white oaks: assessment of allometric relationships among three sympatric species and their hybrids. *International Journal of Plant Sciences*, **173**, 875–884.
- Viscosi, V. & Cardini, A. (2011) Leaf morphology, taxonomy and geometric morphometrics: a simplified protocol for beginners. *PLoS One*, 6, e25630.
- Viscosi, V., Lepais, O., Gerber, S. & Fortini, P. (2009) Leaf morphological analyses in four European oak species (*Quercus*) and their hybrids: a comparison of traditional and geometric morphometric methods. *Plant Biosystems*, 143, 564–574.
- West-Eberhard, M.J. (2003) Developmental plasticity and evolution. New York: Oxford University Press.

- Wickham, H. (2016) gplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag. Available from: https://ggplot2.tidyverse.org
- Wilson, E.O. (1992) The Diversity of Life. Cambridge (MA): Harvard University Press.
- Wright, I.J., Dong, N., Maire, V., Prentice, I.C., Westoby, M., Díaz, S. et al. (2017) Global climatic drivers of leaf size. *Science*, 357, 917–921.
 Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F.
- et al. (2004) The worldwide leaf economics spectrum. Nature, 428, 821–827.
- Yang, J., Di, X., Meng, X., Feng, L., Liu, Z. & Zhao, G. (2016) Phylogeography and evolution of two closely related oak species (*Quercus*) from north and northeast China. *Tree Genetics & Genomes*, 12, 89.
- Zelditch, M.L., Swiderski, D.L., Sheets, H.D. & Fink, W.L. (2004) Geometric morphometrics for biologists: A Primer. San Diego: Elsevier.
- Zhivotovsky, L.A. (2001) Estimating divergence time with the use of microsatellite genetic distances: impacts of population growth and gene flow. *Molecular Biology and Evolution*, 18(5), 700–709.