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RESEARCH ARTICLE

Wind Direction and Strength Determine the Genetic Structure of an Insect-Pollinated Plant Across Heterogeneous Landscape

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ABSTRACT

Aim: Dispersal of plant propagules and their genes is crucial for plant responses to landscape heterogeneities, yet the mechanisms behind this dispersal remain unclear. *Ficus* species depend on wind-borne fig wasps for pollination, but research on airflow effects on *Ficus* genetic structure has produced conflicting results. Our study aims to clarify the role of wind in shaping the genetic structure of such plants with wind-borne insect pollinators by examining how geomorphological complexity interacts with air movements to influence genetic structures.

Location: Southwest China: Sichuan, Yunnan, Guangxi and Guizhou provinces.

Taxon: *Ficus tikoua* Bur., *Ficus*, Moraceae.

Methods: We sampled 56 *F. tikoua* sites across southwest China, characterised by high geomorphologic complexity. River basins and predominant winds were visualised across the sampled regions. Wind connectivity between sampled sites during the main pollination season was modelled based on hourly daily wind data. The maternal and biparental genetic structure of sites were reconstructed using chloroplast DNA (cpDNA) and nuclear SSR (nuSSR) markers. Links between genetic structure, location, and wind parameters were estimated by Mantel or partial Mantel tests.

Results: The plant's maternal genetic structure was defined by river systems, with two distinct cpDNA groups located in the Yangtze and Pearl River basins, respectively. The boundaries for nuclear variation were less clearly delimited geographically. Sites with mixtures of nuSSR groups were concentrated where prevailing winds arrived from several directions. Stronger between-site air flows increased nuSSR geneflow and genetic similarities, while populations receiving more wind flow were also more genetically variable.

Main Conclusions: Our study reveals how plant gene dispersal reflects air and water movements that in turn respond to geomorphologic complexity, thereby directly demonstrating the effects of wind on gene flow of plants with wind-borne insect pollinators. Wind data matching pollinator flight times and large sample sizes are crucial for testing wind effects.

1 | Introduction

Patterns of biodiversity pose long-standing questions in evolutionary and ecological biology (Avise 2000; Fenderson et al. 2019). Variation in diversity is determined by events that took place in the past, as well as contemporary conditions, but fortunately the evolutionary past of populations can to some extent be inferred from contemporary genetic diversity, population genetic structure (Avise 2009) and gene flow (Ricklefs 2004). An understanding of genetic variation across landscapes and how this variation came about can also guide the management of current biodiversity at a landscape scale, as well as helping model responses to future environmental changes (Avise 2009; Schierenbeck 2017; Cruzan and Hendrickson 2020).

The dispersal of plant propagules and their genes is central to how species respond to physical features within their landscapes and can strongly influence their ability to respond successfully to future climate change (Nathan 2006; Gamba and Muchhala 2020; Kling and Ackerly 2020). However, dispersal, especially for plant propagules, remains one of the least understood processes in ecology and evolutionary biology (Favre et al. 2015; Cruzan and Hendrickson 2020). Traditional dispersal studies tended to focus on the geographic distances attained (Cruzan and Hendrickson 2020) but theoretical studies suggest that both average dispersal distances and the whole dispersal kernel are critical to a plant's population genetic structure (Nathan 2006; Gillespie et al. 2012). Plant genes are often dispersed by multiple vectors with varying dispersal abilities and behaviours that do not necessarily correlate with geographical distance. Consequently, the responses of dispersal agents to heterogeneous landscapes have a central role in determining where pollen and seeds are transferred, and whether plant gene flow is restricted or extensive (Bacles et al. 2005; Bacles and Jump 2011). Variation in the responses of vectors to heterogeneous landscapes therefore needs to be taken into account if gene flow patterns are to be understood.

Heterogeneous landscapes often impose barriers to plant gene flow, leading to genetic differentiation among populations (Cruzan and Hendrickson 2020). However, occasional long-distance dispersal events can partially counteract the genetic consequences of restricted gene flow in such environments (Cruzan and Hendrickson 2020). The relationship between air movements and plant population structure is inherently complex (Kling and Ackerly 2021). Wind regimes (their direction and strength) directly influence long-distance dispersal, particularly in wind-dispersed or wind-pollinated plant species (Kling and Ackerly 2021). For instance, predominant wind directions and speeds influence plant population genetic structure (Gillespie et al. 2012), with lower wind speeds facilitating genetic differentiation (Emerson et al. 2020; Scalercio et al. 2020) and the more extensive gene flow associated with stronger winds reducing landscape-scale genetic differentiation (Kling and Ackerly 2021). Additionally, wind can indirectly facilitate gene flow by influencing the movement patterns of insect pollinators (Ahmed et al. 2009). Despite its potential impact on plant genetic structure in heterogeneous landscapes, empirical evidence for landscape effects on wind-mediated pollinator dispersal remains limited.

Ficus species (fig trees, Moraceae) are ecologically significant plants that rely on one or a small number of host-plant-specific fig wasps (Agaonidae) for pollination (Weiblen 2002; Herre et al. 2008). Fig wasps are small (less than 3 mm long) and short-lived insects (adults survive up to 1–3 days after emerging from their natal figs) that depend on air currents for long-distance dispersal between host trees (Ware and Compton 1994; Compton 2002; Ahmed et al. 2009). Molecular studies demonstrated that predominant winds shaped pollen flow direction in an African fig tree (Ahmed et al. 2009), a pattern likely to be typical of many *Ficus* species (Rojas-Cortés et al. 2024). Seed dispersal distances are determined by the behaviour and characteristics of a plant's seed dispersal agents (Shanahan et al. 2001; Lomáscolo et al. 2008, 2010). *Ficus* seeds are usually dispersed by animals (Shanahan et al. 2001) and their seed dispersal is typically much more spatially limited than that of *Ficus* pollen (Yu et al. 2010; Krishnan and Borges 2018). Although a strong link between wind regimes and the genetic structure of wind-influenced genomes is to be expected (Kling and Ackerly 2021), no link or just a modest link has been detected in *Ficus* species by studies directly exploring wind-genetic correlates (Suppoting Information in Kling and Ackerly (2021); Rojas-Cortés et al. (2024)).

Ficus tikoua is pollinated by an undescribed *Ceratosolen* fig wasp species (Cruaud et al. 2012). It is a prostrate dioecious shrub that is widely distributed across the isolated mountain ranges ('sky islands') of Southwest China that are known to be regional centres of plant endemism and diversity (He and Jiang 2014). Gradual uplift of the eastern Qinghai–Tibet Plateau (QTP) created the region's complex topography by forming major mountain chains and reorganising river systems (Clark et al. 2004; Sun et al. 2022). It also generated the Asian monsoon systems (Zhisheng et al. 2001). These long-term developments, overlain by periodic changes in climate associated with glacial cycles, mean that the distributions of plants and their associated animals in the region must inevitably have changed over time. Periods with colder climates will have generated local extinctions and driven *F. tikoua* populations into refugia at lower elevations, whereas warmer periods will have allowed populations to expand and re-colonise higher elevations. Periods when *F. tikoua* populations were smaller and more isolated are believed to have facilitated the observed divergence among geographically distinct populations of its undescribed *Ceratosolen* pollinator (Deng et al. 2020).

Possibly unique within the genus *Ficus*, male *F. tikoua* trees produce two fig phenotypes that differ in male: female floral ratios and distribution patterns (Deng et al. 2016). Strong barriers to the dispersal of its *Ceratosolen* pollinator have been identified around the Hengduan Mountains (Deng et al. 2020), suggesting an influence of these mountain chains on the winds that carry these insects, and a consequent impact on the plant's pollen flow. However, the geographic distribution of floral phenotypes of *F. tikoua* does not closely correspond with the gene flow barriers identified for its pollinator (Deng et al. (2020); Figure S1), implying that the plant's gene flow is also influenced by additional factors.

Long-distance gene flow via seed dispersal by animals would seem unlikely in *F. tikoua* because the figs appear poorly suited to attract the birds or bats that are known to carry fig seeds over long distances (Shanahan et al. 2001). The mature figs of this species are small, light brown in colour, aromatic, and are partly buried in the soil, a fruiting syndrome which suggests that their 'target' vectors are small mammals. Fruit-eating small mammals are often seed predators that generate only short seed dispersal distances for any seeds that survive being eaten (Brunke et al. 2019). Although long-distance seed dispersal in *F. tikoua* appears unlikely, the species may achieve long-distance gene flow through vegetative propagation. This occurs when detached plant fragments, such as root or stem segments, are transported by abiotic factors (e.g., water) and successfully establish in new locations. Although the plant does not float particularly well, viable living stems have been found among water-borne potential propagules collected from tributaries of the Yangtze River (Su et al. 2022). Hydrochory could provide not only down-river transport but also allow rare crossings of major rivers, especially during periods of flooding. Survival after transport by water may be aided by the creeping growth form of *F. tikoua*, which should favour casual rooting by broken stems. Since pollen only contains nuclear DNA, whereas seed and vegetative units contain both nuclear and chloroplast DNA, the patterns of dispersal of *Ficus* chloroplast DNA need not necessarily correspond with those of its nuclear genes, nor with that of its associated fig wasps (Liu et al. 2015). Both maternal and biparental genetic structures can be assessed when both cpDNA and nuclear markers are employed, due to cpDNA markers only being maternally inherited (Greiner et al. 2015).

Based on samples from natural populations of *F. tikoua* across much of the plant's geographic range, we used cpDNA and nuSSR markers to distinguish its maternal and biparental genetic structures and related these to landscape features across the region. Our initial questions were: do the direction and extent of air movements influence its population genetic structure and is the impact of air movements only evident with biparentally inherited genes and not those inherited maternally? We then tested the following: (1) Flow hypothesis: is there more gene flow between populations with greater air flow between them? (2) Isolation hypothesis: when there is more air flow between populations, are they less genetically distinct? (3) Diversity hypothesis: do the populations that receive more inbound air flow exhibit higher genetic diversity than those receiving less air flow?

2 | Materials and Methods

2.1 | The Study Area and Study Species

Southwest China is among the most biodiverse regions in the world, with exceptionally high species richness and many endemic plants and animals (Xing and Ree 2017; Mao et al. 2021). This rich biodiversity is considered to result from the region's topographic and habitat heterogeneity acting in combination with climate changes associated with uplift of the QTP (Favre et al. 2015; Rahbek et al. 2019; Yu et al. 2019; Deng et al. 2020; Mao et al. 2021). Uplift of the QTP created novel environmental conditions and habitats when it formed major mountain chains, re-organised river systems and generated Asia's monsoon rainfall systems (Yuan et al. 2022). Environmental changes are also believed to have impeded the dispersal of species regionally, resulting in strong geographic isolation that facilitated subsequent divergence (Deng et al. 2020; Mao et al. 2021; Yuan et al. 2022). However, gene flow has been detected between some geographically isolated populations, showing that long-range dispersal dynamics in the region are taxon-specific and cannot be ignored (Favre et al. 2015; Ebersbach et al. 2017; Wu et al. 2022).

F. tikoua is distributed across Southwest China into Laos, North India, and North Vietnam (Zheng et al. 1978). In China, its natural populations occur in the 'sky islands' region that includes the Hengduan Mountains (HDM), the Sichuan Basin (SCB) and the Yungui Plateau (YGP) (Deng et al. 2020). Together, these encompass the major Yangtze and Pearl River drainage areas. The release from male figs and diurnal flight of *Ceratosolen* sp. adults occurs mainly in May and June when the female figs are at their receptive stage, ready to be pollinated (Zhao et al. 2014). This is also the time when the Asian Summer Monsoon is dominant in the region, with South Asian summer monsoon winds flowing from southwest to northeast and East Asian summer monsoon winds moving from southeast to northwest (Lim et al. 2002; He et al. 2023).

2.2 | Sampling Protocols

In order to examine the effects of geographical barriers and monsoon winds on genetic structure, we selected *F. tikoua* sites from the Yangtze River (36 sites) and Pearl River Basins (20 sites) that are subject to both South and East Asian summer monsoon winds. The fifty-six natural sites were sampled between May 2013 and May 2015 (Table S1). It is difficult to distinguish individual plants due to their creeping and prostrate life forms. In order to prevent repeated sampling of the same individual, samples of young leaves were only taken from single branches located at least 30 m apart. Sampled leaves were dried and preserved in silica gel.

2.3 | cpDNA Sequencing and nuSSR Genotyping

Total genomic DNA was extracted using a Genomic DNA Extraction kit (Tiangen Biotech (Beijing) CO., LTD.).

The chloroplast fragments of three noncoding regions were sequenced in two directions using the primer pairs psbM–trnD (Shaw et al. 2005), atpB–rbcL (Chiang et al. 1998) and rps12–rpl20 (Shaw et al. 2005). Three to five individuals were sequenced at each site (Table S1). Only sequences with unambiguous chromatograms in both directions were used. The sequences were aligned in MEGA 7 (Kumar et al. 2016). The three sequenced fragments were concatenated for subsequent analyses.

Thirteen species-specific microsatellite markers (T75, T78, T84, T86, T89, T93, T97, T104, T108, T114, T158, T166, T187) specifically developed for *F. tikoua* were used to genotype *F. tikoua* individuals, following the method of Zhang et al. (2016). Seven to 36 individuals had their microsatellites genotyped from each site. Unbalanced sample sizes can bias the estimates of genetic parameters, such as F_{ST} and genetic diversities (Puechmaille 2016). Ten is the commonly accepted threshold for sample size in population genetics studies (Puechmaille 2016). Consequently, we limited site-level nuSSR analyses to sites with no less than ten individuals, reducing the sample size to 1281 individuals from 51 sites (Table S1). However, as unbalanced sample size does not affect group-level analyses, we used all 1304 individuals from 54 sites to assess the genetic groups and their distributions. We checked for linkage disequilibrium between each pair of loci using FSTAT 2.9.3 (Goudet 1995). No linkage disequilibria were detected between any pairs of nuSSR loci and therefore all loci were used in the following analyses.

2.4 | Phylogeographic Structure and Genetic Diversity

Phylogeographic structure was checked using population differentiation measures (G_{ST} and N_{ST}) calculated in Permut 2.0 (Pons and Petit 1996), based on the combined chloroplast sequences. One-tailed tests based on the hypothesis that N_{ST} was greater than G_{ST} were performed using 1000 permutations.

Two methods were employed to define cpDNA groups. Network analysis was performed in TCS 1.21 under a 95% connection limit statistical criterion using combined chloroplast sequences (Clement et al. 2000), and a principal coordinate analysis (PCoA) was carried out in GENALEX 6.5 (Peakall and Smouse 2006) using cpDNA haplotypes.

A Bayesian approach was used to estimate the genetic differentiation of nuSSR genotypes using STRUCTURE 2.3.4 (Pritchard et al. 2000). Twenty runs were conducted for K from 1 to 10, with 100,000 MCMC repetitions and 10,000 burn-in for each run. The optimal K number (Evanno et al. 2005) was determined by ΔK values that were estimated on the website of STRUCTURE HARVESTER (Earl 2012). Another two independent runs were then conducted for optimal K with 1,000,000 MCMC repetitions and a 100,000 burn-in to assign sampled individuals to each group without considering their geographic coordinates. The composition of both cpDNA and nuSSR groups in the sites was counted and mapped in ArcGIS 10.5 (http://www.esri.com/) to visualise the geographic distribution of genetic groups.

Global and pairwise population differentiation indices based on cpDNA sequences (ϕ_{ST}) and nuSSRs (F_{ST}) were estimated in ARLEQUIN 3.5 (Excoffier and Lischer 2010) and FSTAT using 1000 permutations. The mean pairwise distances of cpDNA sequences between all sites were calculated using Mega 7 (Kumar et al. 2016) with Tamura's 3-parameter model, deleting all indels from the sequences. To explore the phylogeographic relationships between pollinators and *F. tikoua*, a regression analysis was conducted using pairwise genetic differentiation indices (F_{ST}) between sampled sites based on nuSSR of the *Ceratosolen* pollinator of *F. tikoua* (data were downloaded from Deng et al. 2020) against the corresponding F_{ST} of the host plant. This analysis was performed in R version 3.3 using the package 'vegan' (Oksanen et al. 2016), with 1000 permutations to assess statistical significance across the sampled region, as well as within the Yangtze and Pearl River basins.

Parameters of genetic diversity were estimated based on both cpDNA and nuSSR data. For cpDNA, the number of haplotypes (h), average numbers of nucleotide diversity (k), haplotype diversity (Hd) and nucleotide diversity (π) were assessed using DNASP 5.10 (Librado and Rozas 2009). N_A (number of alleles), SII (Shannon information index), D (diversity) and UD (unbiased diversity) were estimated using GENALEX 6.5 (Peakall and Smouse 2006).

For nuSSR data, the Hardy–Weinberg equilibrium (HWE) in each site was examined by the multi-loci exact test in GENEPOP 4.0 (Rousset 2008). Then, the number of alleles (N_A), observed (H_O) and unbiased expected heterozygosity (H_E) were calculated in FSTAT (Goudet 1995). Inbreeding coefficients (F_{IS}) for each site were calculated in FSTAT with 1000 permutations. Allelic richness (A_R) and average numbers of private alleles per locus (P_A) were estimated using HP-RARE (Kalinowski 2005).

The demographic dynamics of the Yangtze and Pearl basin populations were checked using neutral statistical tests of cpDNA variation (Tajiama's D and Fu's F_s). Mismatch distributions for each group under both sudden and spatial expansion models were analysed using ARLEQUIN (Excoffier and Lischer 2010). We used two methods to test whether *F. tikoua* has undergone latitudinal expansion. We examined the relationships between genetic diversity (A_R, H_O and H_E) and the latitude of sampled sites. In addition, we calculated genetic differentiation (F_{ST}) and the geographic distances of each site to the most southern site (YMZ), then tested their relationships using linear regression. All the tests were performed in R version 3.3.

2.5 | Gene Flow and Its Barriers

To get a $\text{Flow}_{\text{nuSSR}}$ matrix based on nuSSR data, biparental gene flows between all sampled sites were estimated using the divMigrate command in the diveRsity package in R (Keenan et al. 2013).

Genetic discontinuities of cpDNA components between site pairs were explored with Monmonier's maximum-difference algorithm using BARRIER 2.2 (Manni et al. 2004) to reveal the location of barriers for maternal gene flow. The robustness of the gene-flow barriers was estimated with 1000 Nei's genetic distance matrices. *Nei*'s Genetic distance matrices were created in MICROSATELLITE ANALYSER 4.05 (MSA) (Dieringer and Schlötterer 2003) by bootstrapping on cpDNA reconstructed haplotypes (only keeping variable sites). One thousand cpDNA distance matrices were produced.

To assess possible fracturing effects on gene flow by big rivers, the drainage areas of the Yangtze and Pearl Rivers in the region were visualised based on DEM data in ArcGIS with SRTM DEM 90 data downloaded from the Geospatial data cloud (http://www.gscloud.cn/). The sites located within the Yangtze drainage area were pooled separately from those within the Pearl watershed. Genetic differentiation of cpDNA haplotypes and nuSSR genotypes within and between the two basins was then estimated using an analysis of molecular variance (AMOVA) performed in ARLEQUIN with 1000 permutations (Excoffier and Lischer 2010).

We analysed the spatial genetic structure for the Yangtze and Pearl basin sites using SPAGeDi version 1.5 (Hardy and Vekemans 2002). The pairwise kinship coefficient (Moran's I) was estimated between individuals for each locus and then averaged over loci. For the Yangtze and Pearl sites, nine and ten distance intervals were defined, respectively, with even intervals of 100 km. The fine-scale spatial genetic structure was visualised using a spatial autocorrelogram, plotting the kinship coefficient against geographic distance (Vekemans and Hardy 2004). A permutation test with 1000 iterations was performed to determine the significance and 95% confidence intervals (CI) for the index at each distance. Standard errors for each comparison were estimated by jackknifing over loci.

2.6 | The Influence of Winds

Wind-related variables covering the May–June diurnal flight period of *Ceratosolen* sp. were downloaded for the 06:00 a.m. to 18:00 p.m. diurnal pollinator flight period across the region from the dataset MERRA-2 (https://disc.gsfc.nasa.gov/datasets?project=MERRA-2) in regular 0.625° × 0.5° longitude-by-latitude grids (data averaged for thirty years, 1990–2019). Regional daily wind speeds and directions were imported into ArcGIS to visualise prevailing wind directions across the sampled region.

Hourly daytime wind (07:00 a.m.–19:00 p.m.) directions and speeds at 10 m above ground in May and June were also downloaded for each grid cell across the sampled region from Climate System Forecast Reanalysis (CFSR) (thirty-one years, 1980–2010) (http://cfs.ncep.noaa.gov, (Saha et al. 2014)). All individual hourly wind data were used in the model. Wind connectivity among sites was modelled using the R package 'windscape' (Kling 2021), following the method of Kling and Ackerly (2021). An analysis with $n = 25{,}366$ time steps was used to construct a wind connectivity graph. Then the wind hours between every site pair (the average time that wind will take to travel between sites) in both directions were estimated. Wind hours were then converted to their reciprocals to give an asymmetric wind flow matrix ($\text{Wind}_F[i, j] = 1/(\text{wind hours from } i \text{ to } j)$). To assess net source and sink sites, we calculated the ratio of inbound to outbound wind flow to create another asymmetric matrix ($\text{Wind}_R[i, j] = (\text{Wind}_F[i, j]/\text{Wind}_F[i, j]$). Sink sites were defined based on a value larger than 1 in Wind_R. To assess the wind connectivity between site pairs, a symmetric matrix was generated by averaging the wind flow in the two directions ($\text{Wind}_C[ij] = (\text{Wind}_F[i, j] + \text{Wind}_F[j, i])/2$)).

2.7 | The Effects of Wind Flow on Genetic Structure and Gene Flow

Matrices of air flow, gene flow, and genetic diversities for each site were calculated based on the formula listed in Table 1. A geographic distance matrix (Dis) was calculated based on the sites' GPS coordinates. The catchment matrix (Cat) was coded with 0 for site pairs located in the same river basin and 1 for site pairs in different river basins. Following Kling and Ackerly (2021), three hypotheses were tested to determine the interplay between air flows and river basin location for the genetic structure of *F. tikoua* sites, using Mantel tests or partial Mantel tests to calculate the Pearson's correlation coefficients of the matrices. Three specific hypotheses were tested: (1) Flow hypothesis: that there is more gene flow between sites with more air flow between them—tested by the partial Mantel correlation between the nuSSR gene flow ($\text{Flow}_{\text{nuSSR}}$) and the air flow matrices (Wind_F); (2) Isolation hypothesis: that sites with more air flow between them will be less isolated from one another—tested by the partial Mantel correlation between the wind connectivity (Wind_c) and the genetic differentiation matrices ($\text{Dif}_{\text{nuSSR}}$, Dif_{cp}); (3) Diversity hypothesis: that for all pairs of sites, the site that receives more wind should have higher genetic diversity than those with less air flow—tested using Mantel correlations of the wind flow ratio matrix (Wind_R) and three nuclear diversity ratio matrices ($\text{Div}_{\text{nuSSR1}} - \text{Div}_{\text{nuSSR3}}$) and five cpDNA diversity ratio matrices ($\text{Div}_{\text{cp1}} - \text{Div}_{\text{cp5}}$). In addition, partial Mantel tests were used to assign relative importance for shaping genetic variation to geographic distance, wind flow, and basin (Yangtze or Pearl). In some analyses, variables were log (natural) transformed to reduce the effect of very large values.

TABLE 1 | Formulae calculating wind flow, gene flow, genetic differentiation and the genetic diversity matrix for *Ficus tikoua* sites.

Matrices	Genetic data	Parameters	Formula
$\text{Flow}_{\text{nuSSR}}$	nuSSR	Pairwise gene flow	—
$\text{Dif}_{\text{nuSSR}}$	nuSSR	Genetic differentiation	$1/F_{\text{ST}}$
Dif_{cp}	cpDNA	Mean evolutionary pairwise distances	—
Wind_F	—	Wind flow	$\text{Wind}_F\,[i, j] = 1/(\text{wind hours from } i \text{ to } j)$
Wind_C	—	Wind connectivity	$\text{Wind}_C\,[ij] = (\text{Wind}_F\,[i, j] + \text{Wind}_F\,[j, i])/2$
Wind_R	—	Wind flow ratio	$\text{Wind}_R\,[i, j] = (\text{Wind}_F\,[j, i]/\text{Wind}_F\,[i, j])$
$\text{Div}_{\text{nuSSR1}}$	nuSSR	NA (number of alleles)	$\text{Div}_{\text{nuSSR1}}\,[i, j] = \text{NA}[i]/\text{NA}[j]$
$\text{Div}_{\text{nuSSR2}}$	nuSSR	NP (number of private alleles)	$\text{Div}_{\text{nuSSR2}}\,[i, j] = \text{NP}[i]/\text{NP}[j]$
$\text{Div}_{\text{nuSSR3}}$	nuSSR	AR (allelic richness)	$\text{Div}_{\text{nuSSR3}}\,[i, j] = \text{AR}[i]/\text{AR}[j]$
Div_{cp1}	cpDNA	NA (number of alleles)	$\text{Div}_{\text{cp1}}\,[i, j] = \text{NA}[i]/\text{NA}[j]$
Div_{cp2}	cpDNA	ENA (effective number of alleles)	$\text{Div}_{\text{cp2}}\,[i, j] = \text{ENA}[i]/\text{ENA}[j]$
Div_{cp3}	cpDNA	SII (Shannon information index)	$\text{Div}_{\text{cp3}}\,[i, j] = \text{SII}[i]/\text{SII}[j]$
Div_{cp4}	cpDNA	*D* (diversity)	$\text{Div}_{\text{cp4}}\,[i, j] = D[i]/D[j]$
Div_{cp5}	cpDNA	UD (unbiased diversity)	$\text{Div}_{\text{cp5}}\,[i, j] = \text{UD}[i]/\text{UD}[j]$

3 | Results

Three chloroplast genes were sequenced from 266 *F. tikoua* individuals representing 56 sites (Table S1), resulting in 24 distinct sequences (GenBank accessions: OR888963-OR888986). Three gene sequences were concatenated for each individual. The combined cpDNA sequences were 2237 bp long. Nineteen haplotypes were distinguished (Table S2). In addition, the nuSSR genotypes of 1304 individuals from 54 sites were obtained (Table S1).

3.1 | Genetic Divergence and Genetic Groups

Analysis of the cpDNA detected a significant phylogeographic signal ($N_{\mathrm{ST}} = 0.85 > G_{\mathrm{ST}} = 0.74$, $p < 0.05$), with highly significant differences between sites ($\phi_{\mathrm{ST}} = 0.90$, $p < 0.001$). Even though all nineteen haplotypes were linked into a single network (Figure 1a), a principal coordinate analysis (PCoA) of cpDNA variation distinguished two genetic groups along the PCoA1 axis, which explained 60.9% of the total variance (Figure 1c). The two groups were parapatric and located within the Yangtze and Pearl River drainage basins (Figure 1d). AMOVA indicated that 62.7% of the cpDNA variation was attributable to river catchment ($p < 0.001$, Table 2), suggesting limited dispersal between the two basins (Figure 1d). Six haplotypes were detected in the Yangtze basin, with H08 being the dominant and most widely distributed haplotype, present in about 82% of the total of 34 sites (Figure 1b). In contrast, none of the thirteen haplotypes in the southerly Pearl basin were as dominant, and there was more variation between sites (Figure 1a,b). The Pearl basin's most common haplotypes were H18 and H09, whereas the dominant Yangtze haplotype H08 was only present in a boundary site (YXW) (Figure 1b; Table S1). YXW, which physically straddles the watershed, contained haplotypes common to both basins (H08, H09) (Table S1). The boundary between the two cpDNA groups was almost exactly along the watershed separating the two major river systems (Figure 1b).

Clear evidence for population expansion in the Yangtze group was provided by three cues: (1) the star-like network with all the cpDNA haplotypes connecting to the most common haplotype (H08) by no more than three mutations; (2) the significantly negative Tajiama's D ($D = -1.50$, $p < 0.05$); (3) the mismatch distribution simulation (spatial, p (SSD) = 0.54; sudden, p (SSD) = 0.26). In contrast, the same analyses for the Pearl group suggested it was relatively stable. Neutral tests did not detected expansion (Fu's $F_s = 1.55$, $p = 0.74$; Tajiama's $D = 0.07$, $p = 0.59$), although mismatch distribution analysis suggested some recent spatial expansion (spatial, p (SSD) = 0.45; sudden, p (SSD) = 0.11). The different demographic history between the northern Yangtze group and the southern Pearl group indicated a northward direction of expansion, which was further verified by nuSSR genetic structure. Correlation analysis revealed that the genetic diversities (A_R, H_O and H_E) in the sample sites significantly declined with their latitudes (Figure 2a), again indicating a northward expansion. Linear regression also revealed a significant positive correlation between genetic and geographic distances between each sample site and the southernmost site (YMZ) (Figure 2b), further suggesting a northward expansion by this species.

Significant differentiation between sites was also detected using nuSSRs ($F_{\mathrm{ST}} = 0.21$, $p < 0.001$), with ΔK values again indicating the presence of two genetic groups (Figure S2b). Most individuals were assigned to one of the two groups. but some had mixed components (Figure S2c). The geographic distributions of the two nuSSR groups were again centered on the Yangtze or Pearl basins and were mostly concordant with those of the cpDNA groups (Figures 1d and 3). Spatial autocorrelation analyses revealed the possibility of long-distance dispersal and successful plant establishment within each basin. Both Yangtze and Pearl sites exhibited significant positive kinship coefficients (Moran's I) within 300 km (Figure S4), indicating significant genetic relatedness within this distance. However, the nuclear gene flow was not restricted to within each basin, with nuclear genes from the Pearl basin expanding into the Yangtze basin, resulting in mixed genetic components in several Yangtze sites (YKM, YHZ, YZX, SGL, GKY, Figure 3). Nuclear gene flow between the two river basins was also indicated by the genetic composition of 87 individuals that could not be assigned to either basin group (Figures 3 and S2c).

Mantel analysis indicated an overall positive correlation between the genetic differentiation of pollinators and that of *F. tikoua*, with a significant result across the whole sampled region, Yangtze and Pearl basins (Figure S3), suggesting the role of pollinators in shaping the genetic structures of *F. tikoua*.

3.2 | The Effects of Wind Source on nuSSR Variation

The complex topography within and between the Hengduan Mountains, Sichuan Basin and Yungui Plateau resulted in complex wind patterns during the season and time of day when the tree's pollinator is flying, producing prevailing wind directions that vary greatly across the region (Figure 3). Fifteen Yangtze and 10 Pearl sites were defined on the basis of nuSSR components assigned by STRUCTURE, with all their individuals assigned to one nuSSR group (either Yangtze or Pearl group). The remaining 29 sites, defined as mixed nuSSR sites, either contained individuals from both groups or included individuals that could not be assigned to either group (Figures 3 and S2c). The mixed nuSSR sites were found to be usually located in contact zones where winds are converging from different directions (Figure 3).

3.3 | The Contributions of Wind Connectivity on Genetic Structure

All three hypotheses for wind effects were demonstrated in the biparental genetic structures of *F. tikoua* using Mantel or partial Mantel tests. (1) Flow hypothesis: the wind flow between site pairs had a significantly positive correlation with nuSSR gene flow ($p = 0.026$; Table 3, model 1). (2) Isolation hypothesis: a significant positive relationship was present between wind connectivity and site similarity as assessed with nuSSRs ($\mathrm{Dif}_{\mathrm{nuSSR}}$) ($p = 0.046$; Table 3, model 4). (3) Diversity hypothesis: the net sink sites (where the ratio of inbound to outbound wind flows was larger than one) had comparatively more nuSSR alleles ($p = 0.049$; Table 3, model 10) and more nuSSR private alleles ($p = 0.029$; Table 3, model 11). However,

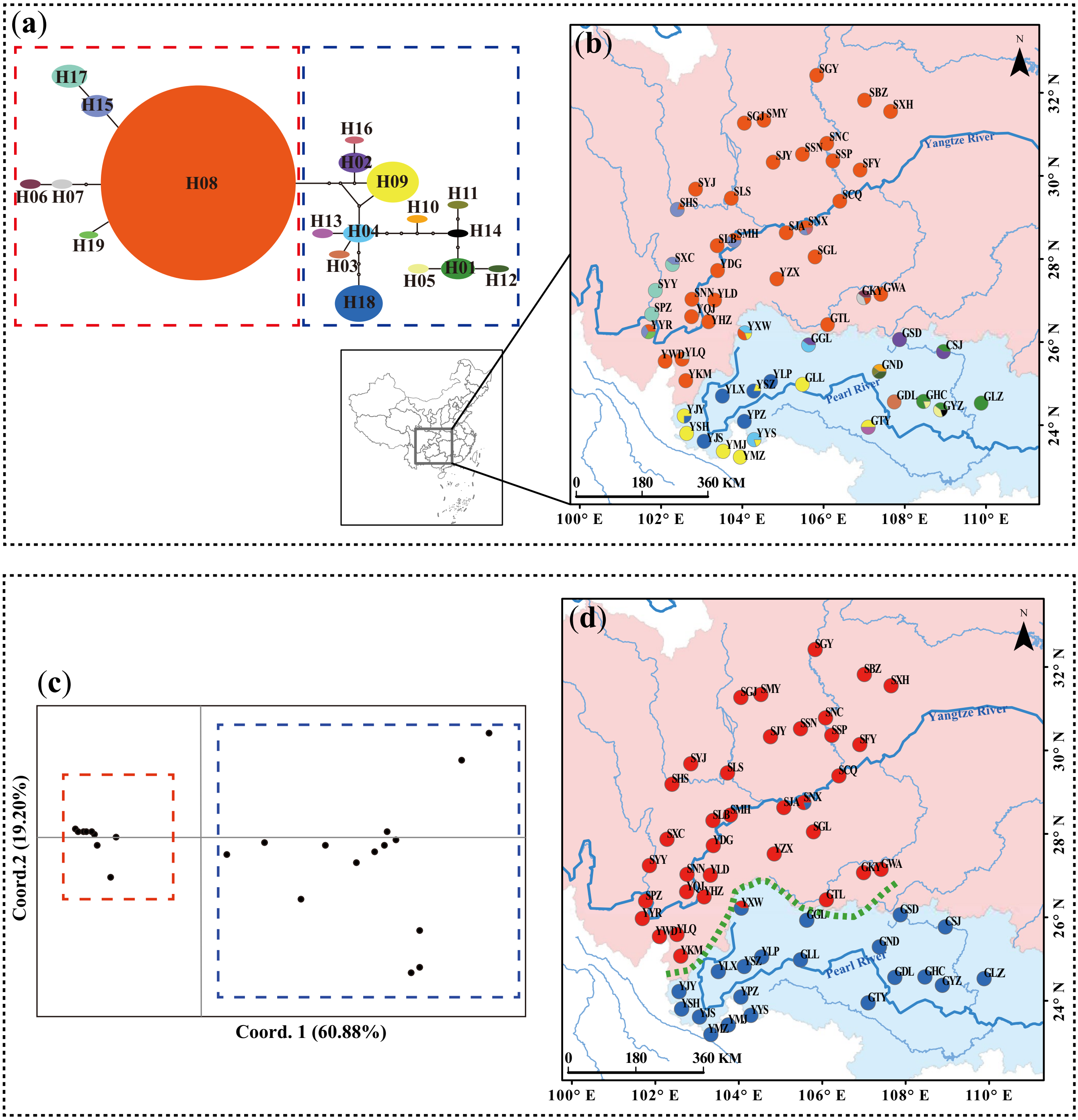

FIGURE 1 | The relationships and geographic distributions of cpDNA haplotypes of *Ficus tikoua*. (a) The network constructed by TCS, with red and blue dashed squares distinguishing two genetic groups identified by PCoA. Each haplotype is represented by an ellipse with size indicating its frequency. (b) The drainage basins of the Yangtze and Pearl Rivers (pink and light blue patches respectively) with the locations and frequency of each cpDNA haplotype. Colours correspond to those in (a). (c) PCoA of cpDNA haplotypes with the genetic groups indicated by dashed squares. (d) The geographic distributions of the two cpDNA groups distinguished by the PCoA. Pie chart colours correspond to that of dashed squares in (c). The green dashed lines highlight barriers to gene flow identified by BARRIER. Three letter codes indicate site identities (Table S1).

allelic richness did not differ significantly between net sink and source sites ($p = 0.331$; Table 3, model 12).

Wind flow characteristics had no significant effects on the maternal genetic structures of *F. tikoua*. No correlation was detected between wind connectivity and site similarity when assessed with cpDNA (Dif_{cp}) ($p = 0.259$; Table 3, model 5), and inbound–outbound wind flow ratios did not influence any of the measures of cpDNA diversity (Table 3, models 13–17). Rather, genetic differentiation among *F. tikoua* sites was influenced by geographic distance and river basin catchment location. However, the effects on site nuSSRs were again different from their cpDNA, with geographic distance affecting only nuSSRs ($p = 0.045$ for nuSSRs, $p = 0.363$ for cpDNA; Table 3, models 6 and 7), while catchment location had a significant effect on cpDNA, but not nuSSRs ($p = 0.298$ for nuSSR and < 0.001 for cpDNA; Table 3, models 8 and 9).

4 | Discussion

Ficus species rely on wind-borne fig wasps for pollen transfer, but there have been conflicting results from previous studies of the relationship between air flows and *Ficus* genetic structure. A prevailing wind direction at the time of its pollinator's nocturnal flights resulted in corresponding directional pollen flow in the African *F. sycomorus* (Ahmed et al. 2009), but other studies testing the wind effects found no or only small wind-genetic correlation (Kling and Ackerly 2021; Rojas-Cortés et al. 2024). The complex topography of Southwest China's mountains generates different local wind regimes (both in direction and strength) in different parts of the range of *F. tikoua*. We used the maternal and biparental genetic structures of *F. tikoua* to assess relationships with their local wind regimes and heterogeneous landscapes.

TABLE 2 | Analysis of molecular variance (AMOVA) for *Ficus tikoua* based on cpDNA and nuSSR data. The two groups were defined by the Yangtze and Pearl drainage basins.

Variation sources	D.F.	Sum of squares	Percentage of variation
*cp*DNA			
Between basins	1	1096.63	62.71 ($p < 0.001$)
Among sites	54	1045.57	27.60 ($p < 0.001$)
Within sites	210	279.97	9.69 ($p < 0.001$)
Total	265	2422.17	
nuSSRs			
Between basins	1	399.18	6.60 ($p < 0.001$)
Among sites	52	1846.56	15.44 ($p < 0.001$)
Within sites	2554	8528.95	77.97 ($p < 0.001$)
Total	2607	10774.68	

4.1 | Wind Patterns Shape Biparental but Not Maternal Genetic Structures

Maternally-inherited genetic structure was associated with river drainage systems in *F. tikoua*. Two parapatric maternal (cpDNA) groups were present, distributed in the Yangtze and Pearl River catchments. The geographic distribution of nuSSR groups broadly matched that of the plant's cpDNA groups, but the two nuSSR groups were not restricted to a single river basin and some sites harboured components of both groups (Figure 2). The presence of these mixed nuSSR groups suggests that there has been dispersal of *F. tikoua* pollen across the uplands separating the watersheds. *Ficus* species rely on movements by their specific pollinating fig wasps for pollen transfer and pollination. Fig wasps are slow-flying and short-lived insects that can only achieve long-distance dispersal by being carried by the wind (Compton 2002; Chen et al. 2008; Ahmed et al. 2009; Liu et al. 2013; Wang et al. 2018; Tong et al. 2021). Correlates between *F. tikoua* genetic structure and local wind regimes show that wind flow patterns, including both their direction and strength, can have a significant impact on the genetic structure of this *Ficus* species.

The Asian Summer Monsoon dominates the weather during the main pollination season of *F. tikoua* (May and June), with either South or East Asian Summer Monsoon conditions prevailing in different regions occupied by *F. tikoua*. The complex topography of the region results in sites generally experiencing predominant winds from a single direction that vary from site to site. The few sites that experience winds arriving from different directions

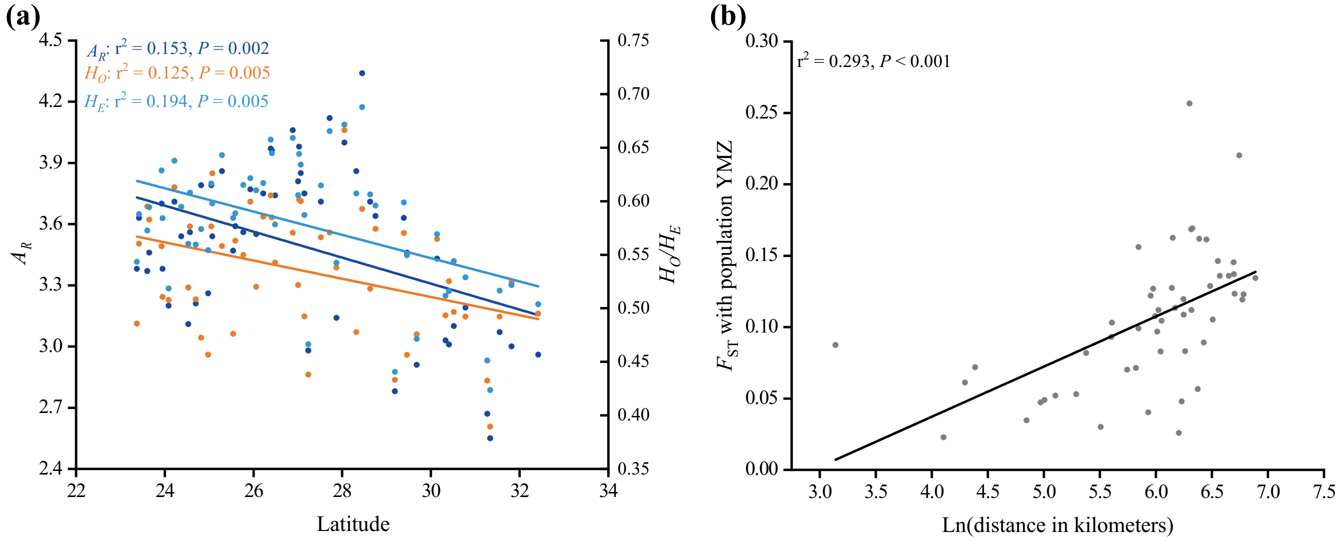

FIGURE 2 | Relationship between genetic diversity (A_R, H_O and H_E) and latitude (a), a linear regression of relationships between genetic distance and geographical distance of other populations to the most southern population YMZ (b).

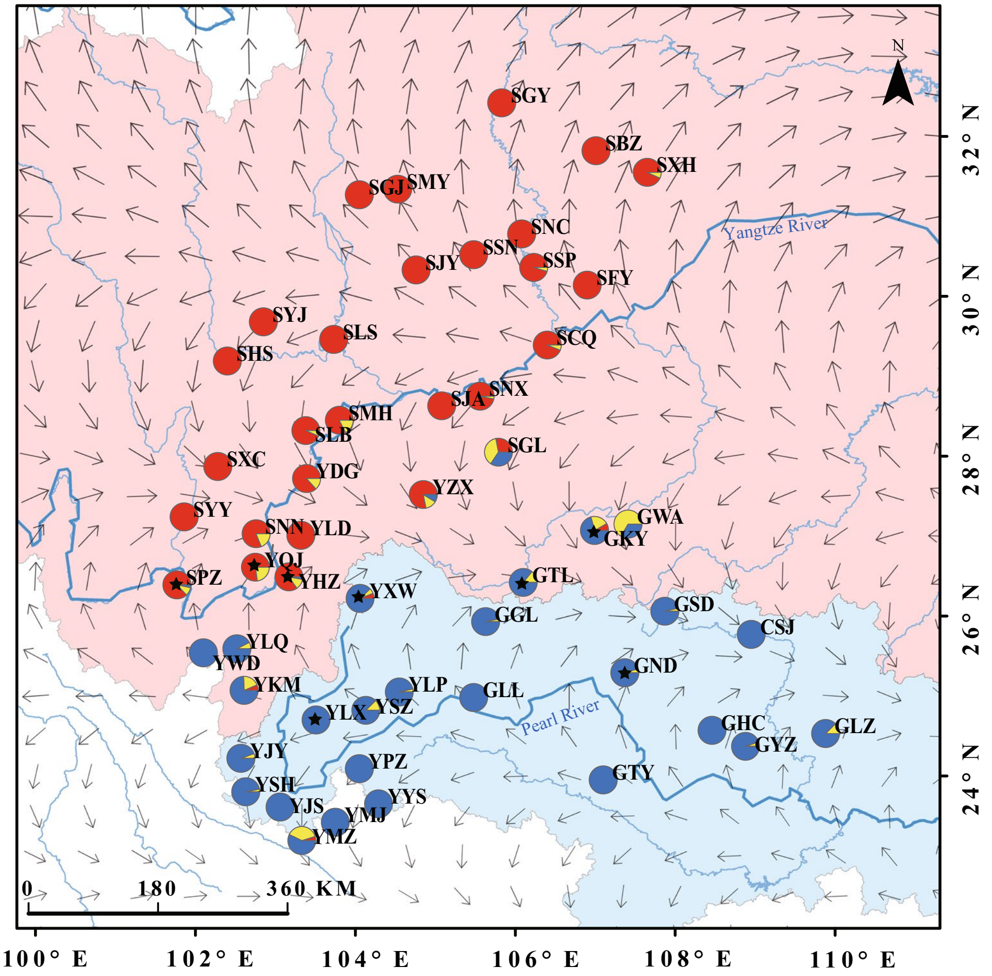

FIGURE 3 | Prevalent wind directions during the months and times when the *Ceratosolen* pollinator of *F. tikoua* is flying, in relation to the plant's nuSSR components. The prevailing winds (arrows) during the main pollination season and *F. tikoua* nuSSR composition (pies). Yangtze and Pearl Basins as in Figure 1. Red and blue in pies indicates proportions of each nuSSR group; yellow indicates the frequencies of individuals that could not be assigned to either group. Populations that included multiple pollinator groups were indicated with black stars.

have mixed nuSSR genetic structures, consistent with increased gene flow and genetic mixing facilitated by the diverse wind patterns.

The significant effects of wind patterns on nuclear but not cytoplasmic genetic structures of *F. tikoua* sites were verified by model tests. In line with previous studies (Kling and Ackerly 2021), *F. tikoua* sites with more air flow between them experienced more nuSSR gene flow and accordingly were more genetically similar to each other than to other sites with less mutual air flow. Also, sites estimated to have received more wind flow were more genetically variable than those that received less. In contrast, cpDNA differentiation was found to be solely influenced by river catchment, because sites within the same basin were more genetically similar, independent of geographic distance and wind flow patterns.

River systems appear to have defined the boundaries for seed and/or vegetative dispersal in *F. tikoua*, whereas wind movements have transported its pollinators across the basins' boundaries, thereby enhancing pollen exchange among sites. This interaction is particularly notable in contact zones where genetically distinct pollinator groups coexist. Deng et al. (2020) detected three genetic clusters in the *Ceratosolen* pollinator sites, that they called the Hengduan Mountains (HDM), Sichuan Basin (SCB) and Yungui Plateau (YGP) groups. The distribution of nuSSR variation in their host *F. tikoua* roughly corresponded to those of these pollinator groups, with Yangtze Basin hosts being mostly associated with HDM and SCB pollinator groups and the Pearl River plants associated with the YGP pollinator group. Mixed nuSSR host sites were commonly associated with two or three pollinator groups (Figures 2 and S1), suggesting that mixed fig wasp-mediated pollen flow is contributing to the plant's nuclear genetic structures. The contribution of pollinators to the plant biparental genetic structure was verified by the positive relationship between the genetic differentiation of pollinators and that of *F. tikoua* (Figure S3). Elsewhere in China, no equivalent air flow-related effects on nuSSR structure were detected among13 sites of *F. pumila* (Kling and Ackerly 2021). A recent study also excluded wind as one of the main factors determining nuclear SNP (single nucleotide polymorphisms) structure among 17 sites of *F. pringlei* in Mexico (Rojas-Cortés et al. 2024). However, the flight times (season, day or night) of the trees' species-specific pollinating fig wasps were not taken into account in either study (Kling and Ackerly 2021; Rojas-Cortés et al. 2024). The absent or modest wind effects they

TABLE 3 | Mantel correlation and partial Mantel correlation details and results.

Hypotheses and models	Tested variables	Control variables	Correlation	p
Flow hypothesis				
Model 1	$\text{Flow}_{\text{nuSSR}}$, $\ln(\text{Wind}_F)$	$\ln(\text{Dis})$, Cat	0.093	**0.026**
Model 2	$\text{Flow}_{\text{nuSSR}}$, $\ln(\text{Dis})$	$\ln(\text{Wind}_F)$, Cat	−0.092	**0.028**
Model 3	$\text{Flow}_{\text{nuSSR}}$, Cat	$\ln(\text{Wind}_F)$, $\ln(\text{Dis})$	−0.084	**0.018**
Isolation hypothesis				
Wind				
Model 4	$\text{Dif}_{\text{nuSSR}}$, $\ln(\text{Wind}_C)$	$\ln(\text{Dis})$, Cat	0.107	**0.046**
Model 5	Dif_{cp}, $\ln(\text{Wind}_C)$	$\ln(\text{Dis})$, Cat	−0.051	0.259
Distance				
Model6	$\text{Dif}_{\text{nuSSR}}$, $\ln(\text{Dis})$	$\ln(\text{Wind}_C)$, Cat	−0.109	**0.045**
Model 7	Dif_{cp}, $\ln(\text{Dis})$	$\ln(\text{Wind}_C)$, Cat	−0.029	0.363
Catchment				
Model 8	$\text{Dif}_{\text{nuSSR}}$, Cat	$\ln(\text{Wind}_C)$, $\ln(\text{Dis})$	0.029	0.298
Model 9	Dif_{cp}, Cat	$\ln(\text{Wind}_C)$, $\ln(\text{Dis})$	0.375	**<0.001**
Diversity hypothesis				
Model 10	Wind_R, $\text{Div}_{\text{nuSSR1}}$		0.157	**0.049**
Model 11	Wind_R, $\text{Div}_{\text{nuSSR2}}$		0.123	**0.027**
Model 12	Wind_R, $\text{Div}_{\text{nuSSR3}}$		0.042	0.331
Model 13	Wind_R, Div_{cp1}		−0.103	0.146
Model 14	Wind_R, Div_{cp2}		−0.093	0.169
Model 15	Wind_R, Div_{cp3}		−0.084	0.130
Model 16	Wind_R, Div_{cp4}		−0.082	0.135
Model 17	Wind_R, Div_{cp5}		−0.083	0.132

Note: Dis and Cat indicate the geographic distances and river basin catchments for site pairs respectively. Other variables were the same as in Table 1. Significant p values are in bold.

detected may reflect a mismatch between their pollinators' flight times and the wind data they utilised. In addition, the sample sizes were rather small. We were able to detect significant air flow effects by incorporating a larger number of sample sites and using wind measurements relevant to the main flight season and flight times of *F. tikoua*'s pollinator.

4.2 | River System Location Defines the Maternal Genetic Structure of *F. tikoua*

Two distinct cpDNA groups were identified, and AMOVA and partial Mantel tests showed that these genetic groups defined sites in either the Yangtze or the Pearl river basins. Cytoplasmic genetic structure in *F. tikoua* will be shaped by the dispersal of its seeds and possibly through movements of vegetative propagules by streams and rivers. The mountain ranges separating the two catchments appear to be effective barriers to movements of the plant's seed dispersers. This would be typical of the small terrestrial mammals that are the plant's suspected seed dispersers (Brunke et al. 2019; He et al. 2019). Interestingly, though, there was no evidence that the river systems were also acting as barriers for maternal genes because sites located on different sides of the two major rivers were not distinct (Figure 1a). One possibility is the transport by water of figs or vegetative parts from one bank to the other during flood events. The heterogeneous habitats of river catchments provide diverse micro-environments that support the establishment of both dispersed seeds and vegetative fragments of *F. tikoua*. The possibility of long-distance dispersal events and subsequent plant establishments within river basins was supported by the positive Moran's I within 300 km in both Yangtze and Pearl River basins. *Ficus tikoua* is not a strictly riparian species but it can be abundant along riversides (Su et al. 2022). Hydrochory is important for the dispersal of some plants (Nilsson et al. 2010; de Jager et al. 2019) and although mature figs and seeds of *F. tikoua* are not very buoyant, its stems do float and can be spread by water (Su et al. 2022). The close association between *F. tikoua*'s chloroplast genetic structure and river catchments is consistent with possible transport of cytoplasmic genes by water, and it is noteworthy that tributaries of both rivers run through the one site that contained components of both cytoplasmic groups (YXW) (Zhao et al. 1995). Another possibility is the long-distance secondary dispersal by big animals preying on the small mammals (Thornton et al. 1996). Among them, various local fox species may be efficient dispersers (Chen et al. 2017). They are omnivorous and may also eat the figs directly as well as any seeds in rodent prey. Foxes can travel a long distance each night and have a relatively slow digestive tract transit. When they travel along a river valley, they could therefore facilitate the dispersal of *F. tikoua* seeds.

The demographic history of *F. tikoua* exhibited a rapid expansion from south to north. The recent expansion in the Yangtze Basin, which is located in the northern parts of this species' natural distribution, was supported by the star-like cpDNA haplotype network, neutral tests and mismatch analysis. The expansion direction was indicated by a significant decline in genetic diversities along latitudinal gradients and the significant correlation between genetic differentiation and geographic distance of sites relative to the southernmost site. Notably, the most abundant haplotype (H08) in the Yangtze River Basin differs by only four mutations from several prevalent haplotypes in the Pearl River Basin, implying that the (presumed) recolonization of the Yangtze River Basin may have originated from populations in the Pearl River Basin. The monsoonal wind regimes may have facilitated long-distance pollen dispersal, promoting the northward expansion of *F. tikoua* from the Pearl to Yangtze basins, which was consistent with the modelling results for SCB group in Deng et al. (2020). However, their modelling suggested the potential distribution of *F. tikoua* contracted in the Pearl River Basin during LGM, while our results suggested *F. tikoua* populations were relatively stable in the Pearl River Basin. This discrepancy may arise from the limitations of paleoclimate reconstructions used in ecological modelling, particularly their inability to account for fine-scale environmental conditions or microrefugia that could have shaped the distributions of species. In contrast, genetic data offer direct insights into historical demographic processes. These findings underscore the critical need to integrate genetic evidence with ecological models to achieve a more nuanced understanding of species' historical dynamics.

4.3 | Conclusion

The dispersal of individual plants and their genes is central to how species respond to geomorphologic complexity and changing climatic conditions. We focused on *F. tikoua*, a common species occupying heterogeneous landscapes across mountainous Southwest China, to explore the forces shaping its genetic structures. The drainage areas of the Yangtze and Pearl Rivers defined its cytoplasmic genetic structures, whereas wind regimes shaped its biparental gene flow, genetic differentiation, and the genetic diversities of its sites. Our study strongly suggests that wind flow patterns, including both their direction and strength, are likely to have a significant impact on the genetic structure of *Ficus* species in general. However, wind data matching the flight times of pollinating fig wasps and big sample sizes are essential for testing wind effects.

Author Contributions

Y.C. and J.M.G. conceived the ideas; J.-Y.D., R.-H.F., Y.C., M.M.K., K.J. and M.L. collected and analysed the data; J.-Y.D., J.M.G., S.G.C. and Y.C. interpreted analyses and wrote the manuscript. X.-Y.C., Y.-B.S. and M.M.K. provided critical feedback on manuscript. All authors contributed to the writing and gave final approval for publication.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

cpDNA haplotypes are available on GenBank, cpDNA sequences for individuals at each site, microsatellite genotypes, and wind data are available on Dryad, which reviewers can access via the following link https://datadryad.org/stash/share/advJkIbmghjJSm8eq_YmtQSeFOGHfZhiQDY2-lrvZA.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.