Biochemical Systematics and Ecology 39 (2011) 441–448

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Genetic diversity and differentiation of the extremely dwarf *Ficus tikoua* in Southwestern China

Yan Chen [a,b], Zhu-Xi Jiang [a], Stephen G. Compton [c], Min Liu [a], Xiao-Yong Chen [a,*]

[a] Department of Environmental Sciences, Tiantong National Field Observation Station for Forest Ecosystems, Shanghai Key Laboratory of Ecological Processes and Restoration in Urban Areas, East China Normal University, Shanghai 200062, China
[b] Ecological Security and Protection Key Laboratory of Sichuan Province, Mianyang Normal University, Mianyang, Sichuan 621000, China
[c] Faculty of Biology Sciences, University of Leeds, Leeds LS2 9JT, United Kingdom

ARTICLE INFO

Article history:
Received 5 April 2011
Accepted 3 June 2011
Available online 1 July 2011

Keywords:
Genetic differentiation
Dispersal
Growth form
Isolation-by-distance
Microsatellites
Ficus tikoua

ABSTRACT

Fig wasps are short-lived, weak fliers, and their long-distance dispersal depends on the ability to enter fast-flowing air above the canopy. Therefore, growth form of fig species may affect fig wasps' dispersal. We employed six microsatellite markers to examine gene flow in Chinese populations of the dioecious *Ficus tikoua*, a prostrate shrub with figs partially buried in the soil. Moderate genetic diversity was found within populations of *F. tikoua*. Differentiation among six *F. tikoua* populations ($F_{ST} = 0.196$, $p < 0.001$) was higher than those of other dioecious figs, and significant differentiation was found between each pair of populations, indicating potential restricted gene flow. This was further demonstrated by significant isolation-by-distance pattern ($p = 0.039$), because low gene flow among population was needed to balance the minor effect of genetic drift, given *F. tikoua* was locally common. Restricted gene flow suggests that growth form may determine differences in gene flow between fig species.

1. Introduction

The genetic structure of natural populations is a result of the interplay of gene flow, selection and genetic drift (Loveless and Hamrick, 1984). The dispersal distances of pollen and seeds largely determine the spatial scale at which populations respond to selection (Lenormand, 2002; Chen et al., 2008a; Ahmed et al., 2009; Gandon and Nuismer, 2009) and the extent of effects of drift on genetic composition (Kuehn et al., 2003; Jordan and Snell, 2008). With restricted gene flow, high genetic differentiation among populations and low genetic diversity within populations are expected (Loveless and Hamrick, 1984; Hastings and Harrison, 1994; Lu et al., 2006). Gene flow also determines the extent and boundaries of effective breeding units (Nason et al., 1998), which is a critical question for population persistence, especially in fragmented landscapes (Ghazoul, 2005; Zhao et al., 2006).

With about 750 species distributed across the tropics and sub-tropics, fig trees (*Ficus*, Moraceae) are keystone plant resources in many tropical ecosystems (Herre et al., 2008), providing food for a highly diverse assemblage of mammals, birds and other fig-eating animals which also disperse their seeds. *Ficus* species rely on their host specific pollinating wasps (Hymenoptera, Agaonidae) for pollen dispersal. Therefore, genetic structure of fig populations, especially those of locally dispersed seed, heavily depends on the dispersal of fig wasps. Using molecular markers, effective breeding units and gene flow distances for monoecious fig trees were found to be among the highest known (Herre et al., 2008) with long-distance

* Corresponding author. Tel./fax: +86 21 6223 3303.
E-mail address: xychens@hotmail.com (X.-Y. Chen).

0305-1978/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bse.2011.06.006

pollen flow well documented (Nason et al., 1998; Zavodna et al., 2005a), and pollen dispersal can exceed 160 km, the most extensive known for any plant (Ahmed et al., 2009). These exceptional pollen dispersal distances have mainly been recorded for monoecious fig trees and are far shorter in functionally dioecious species (Yokoyama, 2003; Harrison and Rasplus, 2006; Wang et al., 2009). A recent study on *Ficus hirta*, a small dioecious tree in Southern Asia, found an exception, suggesting a high pollen-to-seed migration ratio and relatively long-distance dispersal by its pollinators (Yu et al., 2010). These results suggested breeding system may not be responsible for the contrasting pollen flow between monoecious and dioecious species. The recorded difference in pollen flow of *Ficus* species reflects variation in the flight behavior of their pollinators (Compton et al., 2000; Harrison and Rasplus, 2006) and may reflect underlying variation in the density, fruiting patterns and growth forms of their host plants, rather than breeding system *per se* (Harrison, 2003; Ghazoul, 2005).

Fig wasps are short-lived, weak-flying insects. The observed long-distance dispersal in fig wasps was because some wasps could use the fast-flowing air, which was above the canopy (Compton et al., 2000). Therefore, distance from the host fig to the fast-flowing air may affect wasps' dispersal. Fig wasps from figs that grow higher are more close to the fast-flowing air, and are expected to have a long-distance dispersal. Fig species vary greatly in growth form and include creepers, epiphytes, hemi-epiphytes (stranglers), free standing trees and shrubs (Janzen, 1979). Larger plants in general are more 'apparent' to herbivores, including pollinators, making them easier to find (Feeny, 1976; Theis et al., 2007). However, most studies of fig population genetic structure have focused on large monoecious trees that produce synchronized crops of figs (see Nason et al., 1998; Ahmed et al., 2009). This is in contrast to the dioecious shrubby species that have been examined, which are shrubs or small trees (Yokoyama, 2003; Zavodna et al., 2005a). If growth form is responsible for the reduced pollen dispersal distances recorded for most dioecious fig trees, extremely dwarf species should have extremely reduced gene flow.

Here we examine the relationship using *Ficus tikoua* Bur., known as 'di-guo' in Chinese, which means 'fruits from the soil'. *F. tikoua* is a functionally dioecious prostrate shrub that can extend for several meters along the ground, but rarely grows more than 30 cm in height. Its figs are small, reaching 10–20 mm in diameter at maturity and produced at the leaf axils, where they are often partially buried in the soil. It is distributed in South China, Northeastern India, Laos, and Vietnam, and is typically found in wastelands, grassy banks, rock crevices and open woodland (Wu and Raven, 1994). Based on molecular evidence, and confirmed by its pollinator (an un-described species of *Ceratosolen*), *F. tikoua* belongs to subgenus *Sycomorus* (F Kjellberg and YQ Peng, pers. comm.). Subgenus *Sycomorus* also includes the large monoecious fig trees that have the longest documented pollen dispersal distances of any fig trees (Ahmed et al., 2009).

In this study, we employed microsatellite markers to genotype *F. tikoua* individuals of six populations, with the following aims: (1) to analyze genetic diversity within population, (2) to analyze genetic differentiation among populations, and (3) to infer the role of growth form in genetic structure of fig populations.

2. Materials and methods

2.1. Plant samples

Six *F. tikoua* populations were sampled in Southwest China, five in Sichuan Province and one in Yunnan Province (Table 1, Fig. 1). The mean geographic distance between our sampled populations was 333 km (ranging from 31 to 690 km). Four populations (YT, ST, ZJ, SH) were located in the Sichuan Basin (a lowland region with elevations ranging from 400 to 800 m), and two (XC, LQ) were located in the Hengduan Mountains (the largest north-to-south mountain chain in China) at elevations ranging from 1300 to 4000 m. Between 22 and 35 individuals were sampled in each population (Table 1), with the samples taken at least 30 m apart to avoid sampling the same individual. Young, healthy leaves were collected and dried with silica gel in sealable bags.

Table 1
Locations and genetic diversities of *Ficus tikoua* populations in Southwest China. *A*, average number of alleles per locus; A_R, average allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; *, $p < 0.05$.

Populations	Abbr.	Location	Sample size	A	A_R	H_O	H_E	F_{IS}	Loci with null alleles
Yanting, Sichuan	YT	N31°18′ E 105°32′	32	3.80	3.728	0.388	0.507	0.238*	*Fsyc06* *Fs3-31*
Shantai, Sichuan	ST	N31°11 E105°14′	30	3.80	3.701	0.443	0.563	0.216*	none
Zhongjiang, Sichuan	ZJ	N31°02′ E104°41′	32	3.60	3.458	0.375	0.489	0.236*	none
Shehong, Sichuan	SH	N30°55′ E105°20′	35	3.60	3.479	0.361	0.544	0.340*	*Fsyc06*
Xichang, Sichuan	XC	N 27°54′ E102°16′	33	2.80	2.629	0.327	0.364	0.102	none
Luquan, Yunan	LQ	N25°49′ E102°36′	22	4.00	3.991	0.278	0.501	0.451*	*Fsyc04* *Fsyc06*
Mean				3.60	3.500	0.362	0.495	0.264	

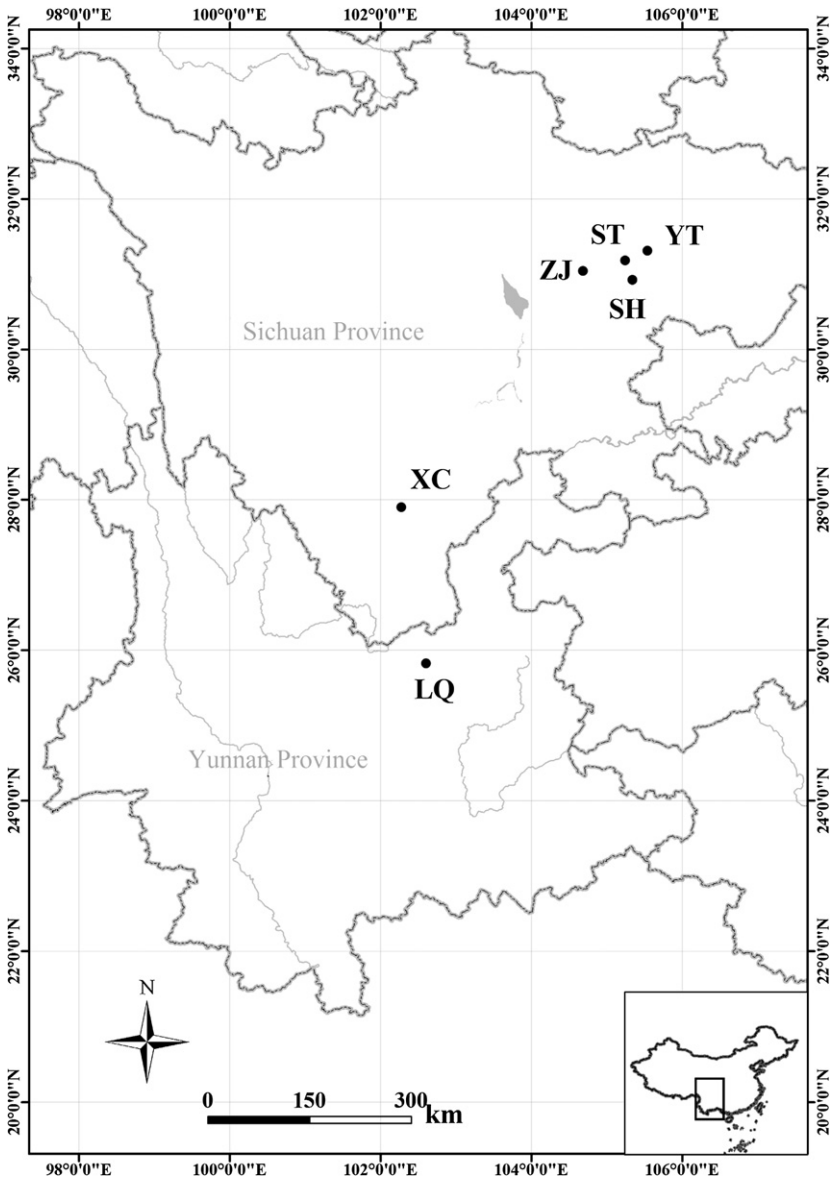

Fig. 1. *Ficus tikoua* sample sites in Southwest China. Population abbreviations are listed in Table 1.

2.2. Microsatellite analysis

Genomic DNA was extracted from 0.05 g of dried leaves using a modified CTAB method (Fan et al., 2004). Six microsatellite loci developed from other *Ficus* species (*Fsyc04* and *Fsyc06* from Ahmed et al. (2007), *Fs4-11*, *Fs3-31* and *Fm4-70* from Zavodna et al. (2005b), *finsN1* from Vignes et al. (2006)) were screened. The microsatellite PCR amplification was performed on a PTC-220 DNA engine DYAD™ thermal cycler (MJ Research, USA) in a 20 μL reaction mixture containing approximately 50 ng of genomic DNA, 0.4 mM each dNTP, 0.2 μM of each primer, 2 μL 1× PCR buffer (100 mM KCl, 80 mM $(NH_4)_2SO_4$, 100 mM Tris–HCl, NP-40, pH 9.0), 1.875 mM $MgCl_2$ and 1 U of DNA Taq polymerase (Sangon). The amplification program used an initial denaturation of 4 min at 94 °C, followed by 30 cycles of 94 °C for 30 s, annealing for 45 s at 53–65 °C (depending on primers used), 72 °C for 1 min, and a final extension of 72 °C for 2 min. Amplification products were separated on 8% polyacrylamide denaturing gel and visualized by silver staining with pUC19 DNA/MapI (HpaII) (Fermentas) as the ladder. The alleles were interpreted manually and scored by Quantity One software using Gel Doc2000™ (BIO-RAD, USA) as well.

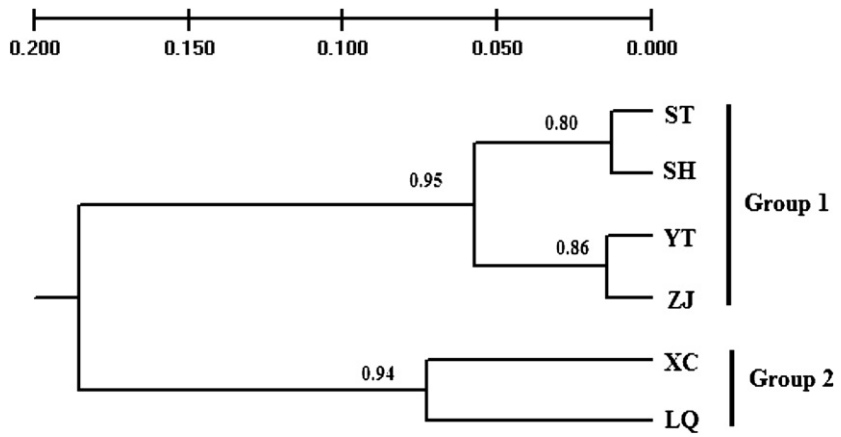

Fig. 2. An UPGMA tree of *Ficus tikoua* populations, showing bootstrap percentages on the branches.

2.3. Statistical analyses

Genotypic disequilibrium between all pairs of loci in each and all populations was tested by both GENEPOP 4.0.10 (Rousset, 2008) and FSTAT 2.9.3.2 (Goudet, 1995, 2001) to make sure all linked loci were revealed. Only independent loci were chosen for subsequent analyses. Genotyping errors for microsatellite markers (null alleles, stuttering and large allele dropout) were checked using the software Micro-Checker (Van Oosterhout et al., 2004).

Deviation from Hardy–Weinberg equilibrium (HWE) was examined in each population with the multi-loci exact test in GENEPOP 4.0.10 (Rousset, 2008) using the Markov chain method and heterozygote deficit as the alternative hypothesis. The number of alleles (A), allelic richness (A_R) were calculated by FSTAT 2.9.3.2, observed (H_O) and unbiased expected heterozygosities (H_E) (Nei, 1978) were assessed using TFPGA 1.3 (Miller, 1997). Genetic differentiation between populations was assessed by standardized F_{ST} using FSTAT 2.9.3.2 and RecodeData v. 0.1 (Meirmans, 2006), which has been described as appropriate for quantifying population differences (Hedrick, 2005). Significance of population differentiation was tested without the assumption of Hardy–Weinberg equilibrium with 1000 iterations using FSTAT 2.9.3.2.

The genetic groups were determined using two approaches. First, the UPGMA method in TFPGA 1.3 was used to cluster populations using Nei's (1978) unbiased minimum distances, and 1000 permutations were generated to get the bootstrap percentages of each group. Second, the optimal number of groups (K) was explored with STRUCTURE 2.2 (Pritchard et al., 2000) using the following scheme: 20 runs were performed for each K value from 1 to 6 (the original population number) with a burn-in of 10,000, and MCMC repetitions of 10,000. ΔK as described by Evanno et al. (2005) was calculated as the criterion for the most reliable K value. Individuals were then assigned to each group while ignoring their original population

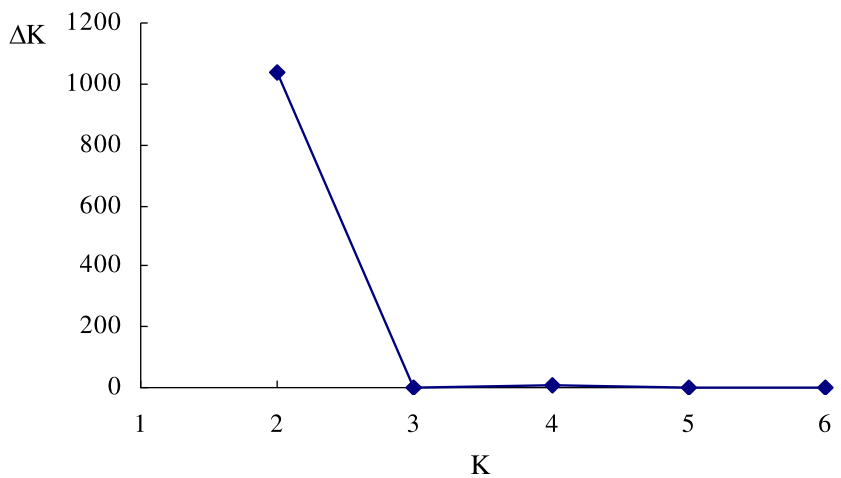

Fig. 3. Magnitude of ΔK as a function of assigned genetic groups (K) simulated by STRUCTURE.

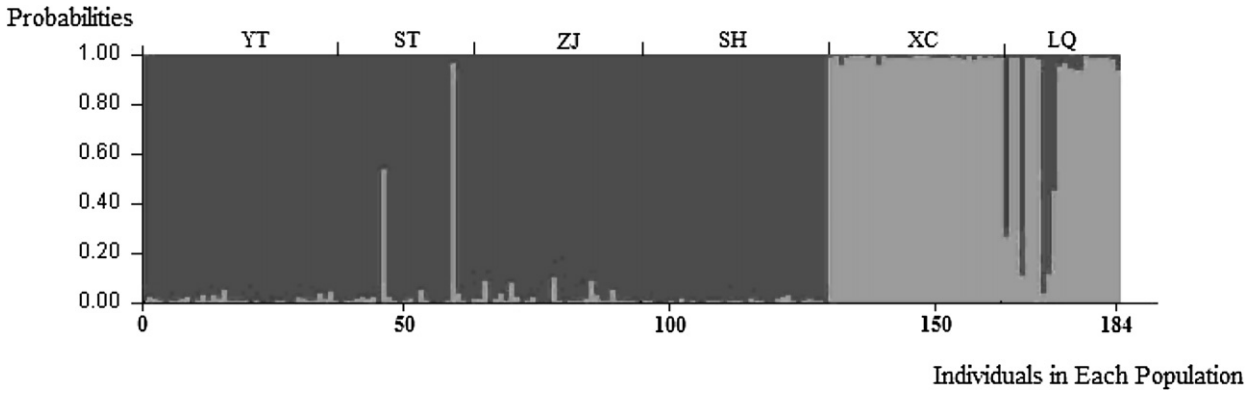

Fig. 4. The group membership probabilities of 184 *Ficus tikoua* individuals assigned by STRUCTURE ($K = 2$), arranged by sample population. Group 1 membership (black), group 2 (gray).

information, setting burn-in length as 300,000 and MCMC repetitions as 1,000,000. To assess the differentiation significance between the forgoing determined groups, locus by locus AMOVA (Excoffier et al., 1992), implemented in ARLEQUIN 3.01 (Excoffier et al., 2005), was performed to evaluate the contribution of each covariance component (among groups, among populations within groups, within populations). Significance was tested based on 10,000 permutations.

The relationship between genetic and geographic distances was estimated to detect the spatial scale of genetic structure. Mantel tests (Mantel, 1967) were used to evaluate the correlations between pair-wise genetic and geographic distances. Taking account of arguments on the appropriate parameters for estimating genetic differences, we used various indexes (F_{ST}, F_{ST}/1-F_{ST} and standardized F_{ST}) in the tests.

3. Results

Genotypic disequilibrium tests detected significant linkage overall between loci fsyc06 and fm4-70 ($p = 0.003$). Locus *fm4-70* was therefore excluded from subsequent analyses. No evidence for stuttering and large allele dropout was detected in either populations, but null alleles were found in one or two loci in YT, SH, LQ populations (Table 1).

Five populations ($p < 0.05$), the exception being XC ($F_{IS} = 0.102$, $p = 0.12$), deviated from HWE when assessed by multi-locus tests, with significant heterozygote deficits. This result was also supported by their significant inbreeding coefficient ($p < 0.05$, Table 1).

Number of alleles and allelic richness per locus ranged from 2 to 10 and 1 to 7 across all populations, with means of 5.6 and 4.9, respectively. The mean number of alleles per population ranged from 2.8 (XC) to 4.0 (LQ) with an average of 3.6. The mean allelic richness was 3.5, ranging from 2.6 (XC) to 4.0 (LQ) (Table 1). Average observed and expected within-population heterozygosities of *F. tikoua* were 0.362 and 0.495, respectively. The lowest observed and expected heterozygosities were found in populations LQ (0.278) and XC (0.364), respectively, and the highest in population ST ($H_O = 0.443$, $H_E = 0.563$) (Table 1).

The UPGMA tree divided the populations into two groups with bootstrap percentages of over 90% (Fig. 2). Populations YT, ST, ZJ and SH clustered together, and XC plus LQ formed the second group. STRUCTURE also divided all individuals into 2 groups as indicated by ΔK values ($\Delta K_2 = 1034.192$, $\Delta K_{3-5} \leq 5.205$, Fig. 3). The population distribution was the same as in the UPGMA tree, with group 1 including populations YT, ST, ZJ and SH, group 2 embracing XC and LQ. However, two individuals from ST fell into group 2, and five individuals from LQ fell into group 1 with likelihoods of over 50% when they were assigned into genetic groups independent of their source populations (Fig. 4). AMOVA showed significant differentiation among the two groups ($p < 0.001$, Table 2).

Global F_{ST} was 0.196 ± 0.056 (SE) ($p < 0.001$). Standardized pair-wise F_{ST} values ranged from 0.042 (between ST and SH) to 0.627 (between XC and YT), with a mean value of 0.354 (Table 3). Significant pair-wise differentiation was demonstrated in every pair of populations ($p < 0.05$, Table 3), even between the two nearest populations, separated only 31 km. AMOVA

Table 2
AMOVA of *Ficus tikoua* populations. Two groups were determined based on the UPGMA tree and the simulation result of STRUCTURE software.

Source of variation	Sum of squares	Variance components	Percentage variation (%)	*p* value
Among groups	63.445	0.356	20.763	<0.001
Among populations within groups	35.538	0.125	7.311	<0.001
Within populations	444.679	1.233	71.926	<0.001
Total	543.662	1.714		

Table 3
Pair-wise standard F_{ST} among *Ficus tikoua* populations. F_{ST} were shown in lower left, p values in upper right.

	YT	ST	ZJ	SH	XC	LQ
YT		<0.001	0.025	<0.001	<0.001	<0.001
ST	0.118		<0.001	0.034	<0.001	<0.001
ZJ	0.049	0.196		<0.001	<0.001	<0.001
SH	0.170	0.042	0.307		<0.001	<0.001
XC	0.627	0.607	0.626	0.626		<0.001
LQ	0.434	0.393	0.457	0.399	0.253	

analysis also demonstrated significant divergence among populations ($p < 0.001$), although they only contributed 7.31% of overall variation (Table 2). Mantel tests indicated that genetic distances (F_{ST}, $F_{ST}/1\text{-}F_{ST}$ and standardized F_{ST}) were significantly correlated with geographic distances, irrespective of genetic distance parameter employed ($r = 0.664$, $p = 0.039$, Fig. 5).

4. Discussion

Differences in dispersal distances among *Ficus* species have been attributed to breeding system, reflecting the shorter pollen dispersals recorded in dioecious species (reviewed by Herre et al., 2008). *F. tikoua* is pollinated by a *Ceratosolen* species. Although no data on pollinators of *F. tikoua*, deep divergences were found in congener pollinators of two dioecious *Ficus* species that separated by 200–500 km, indicating potentially restricted pollen dispersal in their host populations (Moe and Weiblen, 2010). In contrast, Zavodna et al. (2005a) showed that dioecious *Ficus* species can have long-distance pollen dispersal as monoecious species and that the link with breeding system is not universal. In *F. tikoua*, the particularly high genetic divergence we recorded between populations is consistent with the hypothesis that growth form, rather than breeding system may contribute difference in pollinator movements and gene flow among fig tree species.

F. tikoua is a common, obligate outcrossing species, which might be expected to display low genetic differentiation. However, *F. tikoua* populations are highly differentiated. The value of F_{ST} (0.196) was much higher than those of reported dioecious fig species, where F_{ST} is generally smaller than 0.07 with similar spatial scale (Saddoud et al., 2007; Chen et al., 2008b; Yu et al., 2010). This pattern was further demonstrated by the fine spatial scale of population divergence in *F. tikoua*. Significant difference was also found between two *F. tikoua* populations only 31 km apart, a distance shorter that that reported by Moe and Weiblen (2010). Restricted gene flow was also supported by the isolation-by-distance pattern, which indicated a balance between gene flow and genetic drift. Given large population sizes in the present study, impact of genetic drift was small, and thus only limited gene flow was enough to counter it. Moderate or high gene flow would have resulted in low genetic differentiation between populations across a large range of distances, and resulted in violation of the observed isolation-by-distance pattern (Hutchison and Templeton, 1999).

Difference in flowering phenology and physical barriers of pollinator dispersal are common factors leading to high differentiation (Hendry and Day, 2005). Dioecious fig species generally have large synchronous crops, and un-oviposited and less-oviposited synconia may last two or three weeks (Khadari et al., 1995; Tzeng et al., 2006), some even over 4 weeks (personal observation), waiting for pollinators. Thus flowering phenology did not play a major role in the observed differentiation among *F. tikoua* populations. Four studied populations were located in Sichuan Basin with very similar environmental conditions, and the other two populations were highland populations. Although fine-scale genetic structure can be

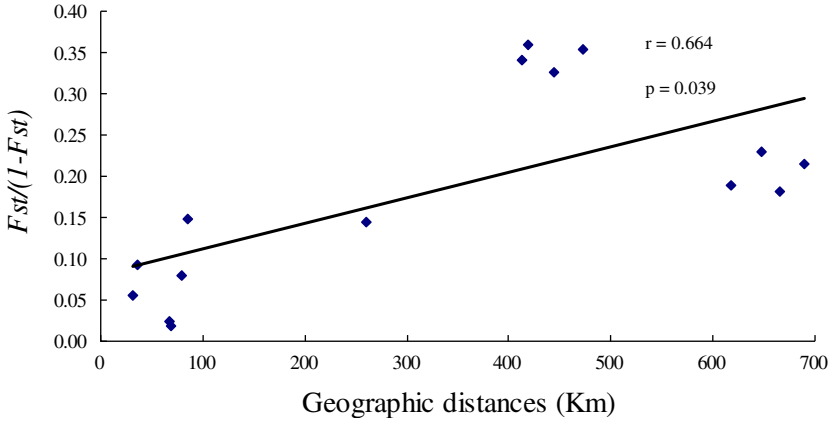

Fig. 5. The relationship between genetic distances ($F_{ST}/(1\text{-}F_{ST})$) and geographic distances among six populations of *Ficus tikoua*.

affected by physical geography, among-population differentiation was less affected by it in the present study, because the studied region is dominated by southwestern Monsoon and among-population pollen dispersal via pollinating wasps is affected by the similar factor. Furthermore, if physical geography plays a critical role, violation of equilibrium between dispersal and drift will be expected, and isolation-by-distance pattern will be violated due to higher or lower dispersal among some populations.

The high genetic differentiation in *F. tikoua* was likely linked with its extreme prostrate habit. Such an effect of plant size can nonetheless be seen in the pollinators of more typical dioecious fig trees. Zavodna et al. (2005a) analyzed population genetic structure of the pollinators of a shrubby and a tree species growing together: genetic differentiation was lower in the pollinator of the tree. In other tree species, lower differentiation or higher gene flow were also frequently observed, which was attributed to their tallness and appearance to herbivores (Petit and Hampe, 2006).

Pollinating fig wasps are delicate, short-lived and weak-flying, but their ability to disperse long distances is well documented (Zavodna et al., 2005a; Ahmed et al., 2009). Such long-distance dispersal is heavily dependent on uncontrolled downwind dispersal in air currents (Ware and Compton, 1994; Compton et al., 2000; Harrison and Rasplus, 2006), with most rainforest fig wasps dispersing high above the canopy, in both primary and secondary forest (Harrison and Rasplus, 2006). The sprawling growth form and partially buried synconia of *F. tikoua* mean that long-distance dispersal of its pollinators is poorly suited to finding the figs of this species, where the volatile attractants for pollinators are likely to be highly localized and close to the ground (Harrison and Rasplus, 2006). Instead, the pollinators may stay close to the ground where the potential receptive figs are to be found and where they can often maintain control of their direction of flight.

Gene flow via seed dispersal is often even more limited than pollen flow in dioecious figs (Yu et al., 2010). Cattle, water buffalo, sheep and small rodents are potential mammalian dispersers of *F. tikoua* at our study sites, along with ground-foraging birds, though these are rare. This suite of potential fig-feeding species is consistent with short seed dispersal distances in *F. tikoua* and is very different from that seen in fig tree species with figs presented above the ground (Shanahan et al., 2001).

Our results support the idea that growth form has a significant impact on pollen flow (and seed dispersal) in fig species, but the extent of this impact can only be judged once comparative studies are available across the whole range of growth forms in *Ficus*.

Acknowledgments

We thank Yan-Qiong Peng for help in collecting samples; Da-wei Gao for help in digitizing the sampling map. This work was supported by the National Natural Science Foundation of China (30870361) and “211 Project” of ECNU to X.-Y. Chen and open foundation from Shanghai Key Laboratory of Ecological Processes and Restoration in Urban Areas to Y. Chen.

References

Ahmed, S., Compton, S.G., Butlin, R.K., Gilmartin, P.M., 2009. Wind-borne insects mediate directional pollen transfer between desert fig trees 160 kilometers apart. Proc. Natl. Acad. Sci. USA 106, 20342–20347.
Ahmed, S., Dawson, D.A., Compton, S.G., Gilmartin, P.M., 2007. Characterization of microsatellite loci in the African fig *Ficus sycomorus* L. (Moraceae). Mol. Ecol. Notes 7, 1175–1177.
Chen, X.Y., Fan, X.X., Hu, X.S., 2008a. Roles of seed and pollen dispersal in natural regeneration of *Castanopsis fargesii*. For. Ecol. Manag. 256, 1143–1150.
Chen, Y., Shi, M.M., Ai, B., Gu, J.M., Chen, X.Y., 2008b. Genetic variation in island and mainland populations of *Ficus pumila* (Moraceae) in eastern Zhejiang of China. Symbiosis 45, 37–44.
Compton, S.G., Ellwood, M.D.F., Davis, A.J., Welch, K., 2000. The flight heights of chalcid wasps (Hymenoptera, Chalcidoidea) in a lowland Bornean rain forest: fig wasps are the high fliers. Biotropica 32, 515–522.
Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.
Excoffier, L., Laval, G., Schneider, S., 2005. Arlequin version 30: an integrated software package for population genetics data analysis. Evol. Bioinform. 1, 47–50.
Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.
Fan, X.X., Shen, L., Zhang, X., Chen, X.Y., Fu, C.X., 2004. Assessing genetic diversity of *Ginkgo biloba* L. (Ginkgoaceae) populations from China by RAPD markers. Biochem. Genet. 42, 269–278.
Feeny, P.P., 1976. Plant apparency and chemical defense. In: Wallace, J.W., Mansell, R.L. (Eds.), Recent Advances in Phytochemistry. Bichemical Interaction between Plants and Insects, vol. 10. Plenum Press, New York, pp. 1–40.
Gandon, S., Nuismer, S.L., 2009. Interactions between genetic drift, gene flow, and selection mosaics drive parasite local adaptation. Am. Nat. 173, 212–224.
Ghazoul, J., 2005. Pollen and seed dispersal among dispersed plants. Biol. Rev. 80, 413–443.
Goudet, J., 1995. FSTAT version 12: a computer program to calculate F-statistics. J. Hered. 86, 485–486.
Goudet, J., 2001. A Program to Estimate and Test Gene Diversities and Fixation Indices, Version 2.9.3. Available from. http://www2.unil.ch/popgen/softwares/fstat.htm.
Hastings, A., Harrison, S., 1994. Metapopulation dynamics and genetics. Annu. Rev. Ecol. Syst. 25, 167–188.
Harrison, R.D., 2003. Fig wasp dispersal and the stability of a keystone plant resource in Borneo. Proc. Roy. Soc. Lond. B. Biol. Sci. 270, 76–79.
Harrison, R.D., Rasplus, J.Y., 2006. Dispersal of fig pollinators in Asian tropical rain forests. J. Trop. Ecol. 22, 631–639.
Hedrick, P.W., 2005. A standardized genetic differentiation measure. Evolution 59, 1633–1638.
Hendry, A.P., Day, T., 2005. Population structure attributable to reproductive time: isolation by time and adaptation by time. Mol. Ecol. 14, 901–916.
Herre, E.A., Jander, K.C., Machado, C.A., 2008. Evolutionary ecology of figs and their associates: recent progress and outstanding puzzles. Annu. Rev. Ecol. Evol. Syst. 39, 439–458.
Hutchison, D.W., Templeton, A.R., 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53, 1898–1914.
Janzen, D.H., 1979. How to be a fig. Annu. Rev. Ecol. Syst. 10, 13–51.

Jordan, M.A., Snell, H.L., 2008. Historical fragmentation of islands and genetic drift in populations of Galapagos lava lizards *Microlophus albemarlensis* complex. Mol. Ecol. 17, 1224–1237.
Khadari, B., Gibernau, M., Anstett, M.C., Kjellberg, F., Hossaertmckey, M., 1995. When figs wait for pollinators - the length of fig receptivity. Am. J. Bot. 82, 992–999.
Kuehn, R., Schroeder, W., Pirchner, F., Rottmann, O., 2003. Genetic diversity, gene flow and drift in Bavarian red deer populations *Cervus elaphus*. Conserv. Genet. 4, 157–166.
Lenormand, T., 2002. Gene flow and the limits to natural selection. Trends Ecol. Evol. 17, 183–189.
Loveless, M.D., Hamrick, J.L., 1984. Ecological determinants of genetic structure in plant populations. Annu. Rev. Ecol. Syst. 15, 65–95.
Lu, H.P., Cai, Y.W., Chen, X.Y., Zhang, X., Gu, Y.J., Zhang, G.F., 2006. High RAPD but no cpDNA sequence variation in the endemic and endangered plant, *Heptacodium miconioides* Rehd. (Caprifoliaceae). Genetica 128, 409–417.
Mantel, N., 1967. Detection of disease clustering and a generalized regression approach. Cancer Res. 27, 209–220.
Meirmans, P.G., 2006. Using the AMOVA framework to estimate a standardised genetic differentiation measure. Evolution 60, 2399–2402.
Miller, M.P., 1997. Tools for Population Genetic Analyses TFPGA V13: A Windows Program for the Analysis of Allozyme and Molecular Genetic Data. Department of Biological Sciences, Northern Arizona University, Flagstaff.
Moe, A.M., Weiblen, G.D., 2010. Molecular divergence in allopatric *Ceratosolen* (Agaonidae) pollinators of geographically widespread *Ficus* (Moraceae) species. Ann. Entomol. Soc. Am. 103, 1025–1037.
Nason, J.D., Herre, E.A., Hamrick, J.L., 1998. The breeding structure of a tropical keystone plant resource. Nature 391, 685–687.
Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.
Petit, R.J., Hampe, A., 2006. Some evolutionary consequences of being a tree. Annu. Rev. Ecol. Evol. Syst. 37, 187–214.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.
Rousset, F., 2008. GENEPOP'007, a complete re-implementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Resour. 8, 103–106.
Saddoud, O., Chatti, K., Salhi-Hannachi, A., Mars, M., Rhouma, A., Marrakchi, M., Trifi, M., 2007. Genetic diversity of Tunisian figs *Ficus carica* L. as revealed by nuclear microsatellites. Hereditas 144, 149–157.
Shanahan, M., So, S., Compton, S.G., Corlett, R., 2001. Fig-eating by vertebrate frugivores, a global review. Biol. Rev. 76, 529–572.
Theis, N., Lerdau, M., Raguso, R.A., 2007. The challenge of attracting pollinators while evading floral herbivores: patterns of fragrance emission in *Cirsium arvense* and *Cirsium repandum* Asteraceae. Int. J. Plant Sci. 168, 587–601.
Tzeng, H.Y., Lu, F.Y., Ou, C.H., Lu, K.C., Tseng, L.J., 2006. Pollinational-mutualism strategy of *Ficus erecta* var. *beecheyana* and *Blastophaga nipponica* in seasonal Guandaushi Forest Ecosystem. Taiwan. Bot. Stud. 47, 307–318.
Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538.
Vignes, H., Hossaert-Mckey, M., Beaune, D., Fevre, D., Anstett, M.C., Borges, R.M., Kjellberg, F., Chevallier, M.H., 2006. Development and characterization of microsatellite markers for a monoecious *Ficus* species, *Ficus insipida*, and cross-species amplification among different sections of *Ficus*. Mol. Ecol. Notes 6, 792–795.
Wang, R., Ai, B., Gao, B.Q., Yu, S., Li, Y.Y., Chen, X.Y., 2009. Spatial genetic structure and restricted gene flow in a functionally dioecious fig, *Ficus pumila* L. var *pumila* (Moraceae). Popul. Ecol. 51, 307–315.
Ware, A.B., Compton, S.G., 1994. Dispersal of adult female fig wasps, 2. Movements between trees. Entomol. Exp. Appl. 73, 231–238.
Wu, Z.Y., Raven, P.H., 1994. Floral of China. Science Press, Beijing. http://www.efloras.org/ Missouri Botanical Garden Press, St Loui. Available from.
Yokoyama, J., 2003. Cospeciation of figs and fig wasps, a case study of endemic species pairs in the Ogasawara Islands. Popul. Ecol. 45, 249–256.
Yu, H., Nason, J.D., Ge, X., Zeng, J., 2010. Slatkin's paradox, when direct observation and realized gene flow disagree a case study in *Ficus*. Mol. Ecol. 19, 4441–4453.
Zavodna, M., Arens, P., van Dijk, P.J., Partomihardjo, T., Vosman, B., van Damme, J.M.M., 2005a. Pollinating fig wasps, genetic consequences of island recolonization. J. Evol. Biol. 18, 1234–1243.
Zavodna, M., Arens, P., van Dijk, J., Vosman, B., 2005b. Development and characterization of microsatellite markers for two dioecious *Ficus* species. Mol. Ecol. Notes 5, 355–357.
Zhao, A.L., Chen, X.Y., Zhang, X., Zhang, D., 2006. Effects of fragmentation of ever-green broad-leaved forests on genetic diversity of *Ardisia crenata* var. *bicolor* (Myrsinaceae). Biodivers. Conserv. 15, 1339–1351.