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ORIGINAL ARTICLE

Spatial genetic structure and restricted gene flow in a functionally dioecious fig, *Ficus pumila* L. var. *pumila* (Moraceae)

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Abstract The mutualism between fig plants and fig wasps has been recognized as one of the most specialized systems of symbiosis. Figs are pollinated by their highly specific pollinating fig wasps, and the pollinating fig wasps are raised within the syconia of figs. Recent studies indicated a difference between monoecious and dioecious figs in the dispersal range of pollinating wasps, which has potential consequences for gene flow. In this study, we detected the gene-flow pattern of the dioecious climbing fig, *Ficus pumila* L. var. *pumila*, at both local and regional scales. At the local scale, spatial autocorrelation analysis indicated strong genetic structure at short distances, a pattern of limited gene flow. This result was also supported by a high inbreeding coefficient ($F_{\mathrm{IS}} = 0.287$) and significant substructuring ($F_{\mathrm{ST}} = 0.060$; $P < 0.001$). Further analysis indicated that the effective gene dispersal range was 1,211 m, and the relative contribution of seed dispersal was smaller than that of pollen dispersal. The inferred effective range of pollen dispersal ranged from 989 to 1,712 m, while the effective seed dispersal range was less than 989 m. Lack of long-distance dispersal agents may explain the limited seed dispersal. The high density of receptive fig trees was the most likely explanation for limited pollen dispersal, and the position of syconia and relatively low wind speed beneath the canopy may contribute to this phenomenon. At the regional scale, significant negative correlations (kinship coefficient F_{ij} ranging from −0.038 to −0.071) existed in all comparisons between the studied population and other populations, and the assignment test grouped almost all individuals of the studied population into a distinct cluster. Asynchronous flowering on the regional scale, which provides a barrier for the pollinating wasps to fly from the studied population to the other populations, is probably responsible for the limited gene flow on the regional scale.

Keywords Assignment test · *Ficus pumila* L. var. *pumila* · Microsatellites · Pollen and seed dispersal · Spatial genetic structure

Introduction

The mutualism between fig plants (*Ficus*, Moraceae) and pollinating fig wasps (Hymenoptera, Chalcidoidea, Agaonidae) has been recognized as one of the most ancient and specialized systems of symbiosis (Janzen 1979; Herre et al. 1999; Machado et al. 2001). Figs develop closed, urn-shaped inflorescences or syconia with tiny uniovulate flowers. The pollinating fig wasp larvae develop inside the syconium. To reproduce, the short-lived adult female wasps disperse, carrying pollen from their natal plant, to a tree with receptive syconia (Cook and Rasplus 2003; Harrison 2003). In monoecious figs, the pollinating female wasps enter the syconium, attempt to oviposit and achieve pollination during the process. Ovules that receive a wasp egg form a gall on which the wasp larva feeds, while others develop into a seed if they are pollinated. In dioecious figs, wasps can reproduce only in the functionally male syconia. When entering a female syconium, the wasp pollinates

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during the process of searching for suitable oviposit sites (Harrison et al. 2000).

A difference in the dispersal range of pollinating wasps was found between monoecious and dioecious figs. In general, pollinators of monoecious figs fly over a larger range than pollinators of dioecious figs, as determined by the observation of different recovery rates after local extinction (Harrison 2000, 2003), and population genetic and paternity studies (Nason et al. 1998; Yokoyama 2003), although long-range dispersal was observed in some cases (e.g., Zavodna et al. 2005a). One possible explanation is related to the density of host plants. The short-range dispersal of the pollinators of dioecious figs was generally linked to a high density of receptive trees, therefore, nullifying the need for long-distance dispersal (Harrison 2003). Another possible explanation is linked to the differential behavior of pollinating wasps, which is correlated to the position of the host plant in the canopy. In general, dioecious figs grow beneath the canopy, while monoecious figs are canopy species (Harrison 1996). Pollinating wasps are weak fliers depending on wind speeds. Wind speeds are higher above the canopy than beneath, which may be associated with dispersal over a long distance (Kameyama et al. 1999). Whatever the cause, the difference in dispersal ranges of the pollinators of monoecious and dioecious figs may lead to differences in pollen dispersal, and consequently differences in gene flow.

Seed dispersal is an important component of gene flow. Seeds of fig trees are dispersed by animals, some of which, such as bats and birds, have the ability to fly long distance (Lambert and Marshall 1991; Compton et al. 1996; Tan et al. 1998; Shilton et al. 1999). Rapid colonization in newly emerged volcanic islands shows that seeds of many figs are dispersed over long distances (Thornton et al. 1996; Shanahan et al. 2001a). However, no difference in seed dispersal between monoecious and dioecious figs has been reported.

Studies in recent decades have shown that the spatial genetic structure (SGS) provides an important indicator of gene flow (Hardy and Vekemans 1999; Fenster et al. 2003; Heuertz et al. 2003; Peakall et al. 2003; Valbuena-Carabana et al. 2007). Although diverse evolutionary drivers have roles in shaping SGS, gene flow and selection are recognized as the most important (Sokal et al. 1989; Epperson 1990). However, if we use neutral markers, such as microsatellites, the revealed SGS is affected mainly by isolation by distance (Escudero et al. 2003). Given such a premise, SGS has proved useful to infer gene flow in plant populations (Heuertz et al. 2003; Epperson 2007). Spatial autocorrelation analysis, a method that treats individuals as basic units, is efficient and precise in deciphering SGS (Sokal and Oden 1978; Epperson and Li 1996; Manel et al. 2003; Epperson 2004). Gene flow and the relative contribution of pollen and seed dispersal can be evaluated when this method is combined with newly developed statistical measures (Vekemans and Hardy 2004).

In this study, we used neutral microsatellite markers and spatial autocorrelation analysis to examine the relative contribution of pollen dispersal and seed dispersal in *Ficus pumila* L., a dioecious evergreen perennial root-climber. Two varieties, *F. pumila* var. *pumila* and *F. pumila* var. *awkeotsang*, have been identified in this species. We focused mainly on a *F. pumila* var. *pumila* population in Zhejiang Province, China. Mature *F. pumila* var. *pumila* individuals are known to flower in late April to early May (Ma and Wu 1989). More recent observations found that male individuals also flower in the autumn in Fujian Province, but the female individuals do not (Chen et al. 2002). Both male and female *F. pumila* var. *awkeotsang* flower in the spring and in the autumn in Fujian Province, China (Chen et al. 2002, 2008a).

The wasp *Wiebesia pumilae* (Agaonidae) has been identified as a fig pollinator. During the flowering period, the female pollinating wasps emerge from male syconia and seek receptive fig trees to oviposit or pollinate the flowers depending on the gender of the figs that they entered (Ma and Wu 1989; Chen et al. 2002). Female *F. pumila* var. *pumila* bear syconia with long- and pin-styled uniovulate flowers. The pollinating wasps cannot lay their eggs in these figs because there is no oviposition platform on the style, and their ovipositor is shorter than the styles and thus cannot reach the ovule. Functionally male trees bear syconia with male flowers and short-styled female flowers in which the pollinating wasps can lay their eggs.

The fruits of *F. pumila* var. *pumila* mature in autumn. Bats have been reported to consume *F. pumila* seed in Hong Kong (Corlett 2006); however, there are no fruit bats north of the Tropic of Cancer in China (S.-Y. Zhang, personal communication). According to villagers, rats and ants may be seed predators. In a previous study, significant differentiation was found among island and mainland populations of figs, suggesting that limited gene flow may exist in this species (Chen et al. 2008b). This limitation was supported by the failure to find pollinating wasps on some islands or in areas of a large island.

This study was focused mainly on a mainland population of figs, and was designed to answer the following two questions. (1) Does the significant genetic structure occur on a fine scale? (2) How large are the dispersal ranges of pollen and seed?

Methods

Sampling

This study was conducted in Tiantong National Forest Park and three adjacent villages in eastern Zhejiang

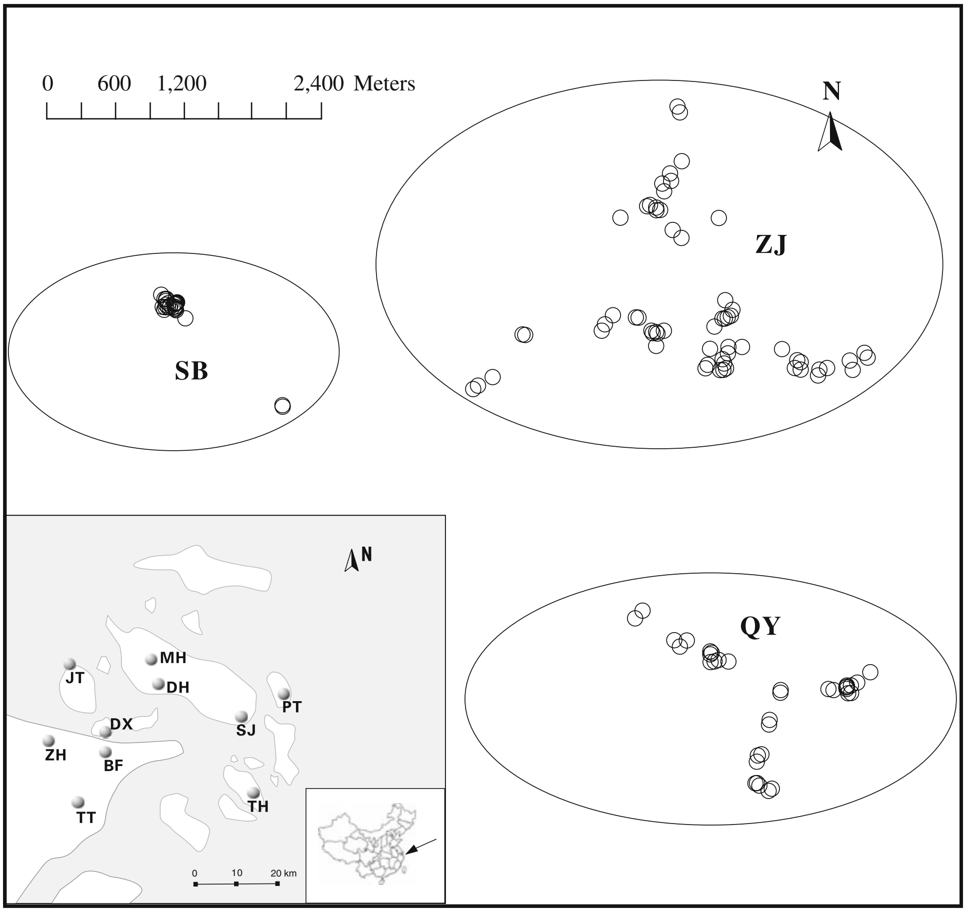

Fig. 1 Positions of each sampled individual in Tiantong (population TT). Individuals sampled in the Tiantong area are represented by *open circles* and the subpopulations are indicated by *ellipses*. The sampling sites including the present sampled site (TT) in Chen et al. (2008b) are shown in the small map

Province, China (Fig. 1). Zonal vegetation is evergreen broadleaf forest dominated by *Castanopsis fargesii* Franch., *Castanopsis carlesii* (Hemsl.) Hayata., and *Schima superba* Gardn. et Champ. (Song and Wang 1995). This *F. pumila* var. *pumila* population (population TT) is surrounded by mountains and therefore relatively isolated from other populations. *Ficus pumila* var. *pumila* individuals are scattered in the forest park and the villages, and can be grouped into three subpopulations: ZJ, SB, and QY (Fig. 1). After extensive surveys, 129 *F. pumila* var. *pumila* individuals were found and sampled in the summer of 2005 and the spring of 2006. The position of each individual was determined with a portable GPS. Several fresh healthy leaves were taken from each individual and dried in a sealable bag with about 50 g of silica gel. No *F. pumila* var. *awkeotsang* individuals were found in this area.

DNA extraction and microsatellite genotyping

Total genomic DNA was extracted according to a modified CTAB method (Fan et al. 2004). The extracted DNA were dissolved in 150 μl of TE buffer and stored at −70°C. After searching the microsatellite primers of congeners, we used 10 polymorphic microsatellites, in which five loci (fs4-11, fm4-18, fs3-31, fm4-15, and fm3-64) were from Zavodna et al. (2005b) and the rest (lmfc17, lmfc20, lmfc25, lmfc32, and lmfc37) were from Griraldo et al. (2005). Each PCR reaction was performed in a 20-μl reaction mixture composed of 2 μl of Taq DNA polymerase buffer [100 mM KCl, 80 mM $(NH_4)_2SO_4$, 100 mM Tris–HCl, NP-40, pH 9.0], 0.75 unit of Taq DNA polymerase (Shanghai Sangon Biological Engineering Technology & Services), 60 ng genomic DNA, 0.2 μM of each primer, 0.4 mM of each dNTP, and 1.875 mM $MgCl_2$. PCR reaction process included an initial melting step of 3 min at 94°C; 28 cycles of 15 s at 94°C, 45 s at annealing temperature of 40.5–65°C (depending on the primer used) and 30 s at 72°C; and a final extension step of 7 min at 72°C. All reactions were run on a PTC-220 DNA Dyad thermal cycler (MJ Research, Waltham, MA, USA). The amplification products were separated by electrophoresis in 6% denaturing polyacrylamide gels and stained with silver nitrate. pUC19 DNA/MspI(HpaII) marker 23 (Fermentas) was used to be the reference of the products' lengths.

Analysis of genetic diversity and genetic differentiation

At the subpopulation and population levels, the number of alleles per locus (*A*), the allele richness per locus (A_R)

(El Mousadik and Petit 1996), and the inbreeding coefficients (F_{IS}) were calculated with FSTAT version 2.9.3.2 (Goudet 1995). The observed and unbiased expected heterozygosities (Nei 1978) were assessed using TFPGA1.3 (Miller 1997). The Hardy–Weinberg equilibrium was tested with Fisher's exact test using the software TFPGA1.3 (Miller 1997). Genetic differentiation among subpopulations was evaluated by F_{ST} (Weir and Cockerham 1984) using FSTAT (Goudet 1995).

Spatial autocorrelation analysis

The spatial genetic structure of *F. pumila* var. *pumila* in Tiantong (population TT, Fig. 1) was analyzed using SPAGeDi version 1.2 (Hardy and Vekemans 2002). Kinship coefficients were chosen, since these have many advantages, such as not taking the Hardy–Weinberg equilibrium as a basic assumption (Vekemans and Hardy 2004). The *F. pumila* var. *pumila* population had a low level of polymorphism with the greatest number of alleles per locus being four. We used the kinship coefficient as defined by Loiselle et al. (1995) to analyze the genotype dataset because this index is the best choice when the level of polymorphism is low (Vekemans and Hardy 2004). We divided 8,256 pair-wise comparisons into 10 intervals of equal distance (630 m for each). A total of 9,999 permutations of locations and genes were made to determine the 95% CI for the index in each distance class.

Spatial genetic structure was analyzed at the regional scale by including the data for nine populations studied by Chen et al. (2008b). Six microsatellite loci (fs4-11, fm4-18, fs3-31, fm4-15, fm3-64, and lmfc25) were used in both studies, and thus SGS on the regional scale was applied to these loci. We used the index described by Loiselle et al. (1995) to extend the detection of SGS to the regional scale. Each population described by Chen et al. (2008b) was defined as a particular group. The standard errors were estimated by jackknifing over loci for each comparison.

Estimation of effective ranges of pollen and seed dispersal

We used SPAGeDi (Hardy and Vekemans 2002) to estimate the neighborhood size (*Nb*) and then estimated the dispersal distance indirectly by inferring σ from *Nb* (Hardy et al. 2006), where σ^2 is the square of half the average axial parent–offspring distance. In Wright's isolation-by-distance model, the pairwise kinship coefficients between individuals in the two-dimensional space are expected to vary linearly with the logarithm of geographic distance within a distance range of σ and 20σ considering a mutation rate for microsatellites of $\mu = 10^{-3}$ (Hardy and Vekemans 1999; Heuertz et al. 2003). At this spatial scale, the slope (*blog*) of regressing pairwise kinship coefficient on ln(distance) (σ to 20σ) may be adopted to estimate *Nb* using the formula:

$$Nb = -\frac{1-F}{blog}$$

where F is an estimation of Wright's inbreeding coefficient (Fenster et al. 2003; Heuertz et al. 2003). Then σ can be obtained easily from the effective population density (D_{e}). In practice, an iterative procedure is applied to determine *Nb* and σ (Vekemans and Hardy 2004), if σ converges, the range of gene dispersal will be determined. In woody species, *D*/4 can be considered as the approximate value of D_{e} (Hardy et al. 2006), where D is the census population density. In the present study, since we had searched the sampling area extensively and sampled almost all individuals that can be distinguished, the sample size was considered as an estimate of population size. Then, D was estimated as 4.384 individuals km^{-2} (sampling area $\sim$29.43 km^2), and D_{e} was 1.096 individuals km^{-2}. The initial distance range for regression was set to 300–6,000 m.

According to Vekemans and Hardy (2004), the coefficients of the cubic regression between kinship coefficients and the natural logarithm of distances can be used to estimate the relative contributions of pollen and seed dispersal to total gene dispersal (Heuertz et al. 2003). Concavity ($k > 0$) and convexity ($k < 0$) in the regression curve indicate $\sigma_{\mathrm{s}} < \sigma_{\mathrm{p}}$ and $\sigma_{\mathrm{s}} > \sigma_{\mathrm{p}}$, respectively (see Vekemans and Hardy 2004 for details), where σ_{s} and σ_{p} are the effective seed dispersal and pollen dispersal, respectively. Therefore, on the basis of the information of relative contribution of pollen and seed dispersal, we can estimate the range of effective dispersal of pollen or seed using the relationship:

$$\sigma^2 = \sigma_{\mathrm{s}}^2 + \frac{1}{2}\sigma_{\mathrm{p}}^2$$

If effective dispersal of pollen is larger than that of seed dispersal, setting $\sigma_{\mathrm{p}} = \sigma_{\mathrm{s}}$, the minimum value of σ_{p} is $\sqrt{2/3}\sigma$, while the maximum value of σ_{p} is $\sqrt{2}\sigma$, given $\sigma_{\mathrm{s}} = 0$, and the maximum value of σ_{s} is $\sqrt{2/3}\sigma$, when $\sigma_{\mathrm{p}} = \sigma_{\mathrm{s}}$.

Assignment test

A Bayesian cluster analysis was conducted using the method implemented in STRUCTURE version 2.2 (Pritchard et al. 2000). The admixture of individuals regardless of the geographic locations of the samples was used for clustering all individuals from the study population and the nine populations studied by Chen et al. (2008b). We first explored which value of K (number of clusters) maximized the likelihood of the data. Simulations were performed by replicating 20 runs for each value of *K* from 1 to 10, with the following settings: admixture model, correlated allele frequencies, burn-in length of 10,000, and MCMC repetitions of 10,000 (Pritchard et al. 2000). All other parameters were set at their default values. The approach described by Evanno et al. (2005) was adopted to estimate the most reasonable *K* by using ΔK as the criterion. Once the most reliable value of *K* was defined, all individuals were assigned to the *K* populations probabilistically by using a burn-in of 300,000 and 1,000,000 MCMC repetitions.

Results

Each individual *Ficus pumila* var. *pumila* had a unique genotype over the 10 microsatellite loci. No significant linkage disequilibrium was found in any pair of loci. The number of alleles per locus ranged from 2 to 4, with a mean of 3.3. The average H_O and H_E of the population were 0.38 ± 0.15 and 0.53 ± 0.11, respectively. Fisher's exact test indicated that all 10 loci deviated significantly from Hardy–Weinberg equilibrium in population TT except for fm3-64. Eight of 10 values of F_{IS} were positive, indicating that heterozygosity deficiency had a critical role in the deviation.

The mean number of alleles per locus, the mean allelic richness, and the heterozygosity showed that the subpopulations had similar genetic variation (Table 1). Seventeen of 30 F_{IS} values deviated significantly from zero and among them, 13 were caused by heterozygosity deficiency. Significant difference were observed among the subpopulations of *F. pumila* var. *pumila*, with an F_{ST} value of 0.060 ($P < 0.01$). Pair-wise F_{ST} between subpopulations was also significantly larger than zero ($P < 0.01$ in all comparisons).

Kinship coefficient analysis showed a strong spatial structure in the first distance class (within 630 m) with an F_{ij} value of 0.0614 (Fig. 2). In the second distance class (630–1,260 m), F_{ij} was negative (−0.0094) but not significantly different from the expected value. Values of F_{ij} fluctuated in the following eight distance classes. A significantly positive value was found in the third distance class while significantly negative values were found in the eighth and the tenth distance classes (Fig. 2). However, at the regional scale, values of F_{ij} were significantly negative and all populations exhibited similar levels of differentiation with population TT (F_{ij} ranged from −0.038 to −0.071) (Fig. 3).

Table 1 Genetic diversity over 10 microsatellite loci in population TT and three subpopulations

Subpopulation	Number of samples	A	A_R	H_e	H_o	F_{IS}
ZJ	66	3.20	3.15	0.51	0.37	0.275
SB	27	3.20	3.18	0.51	0.39	0.236
QY	36	3.10	3.07	0.50	0.38	0.246
Total	129	3.30	3.30	0.53	0.38	0.287

A Mean number of alleles, A_R mean allelic richness, H_E unbiased expected heterozygosity, H_O oberved heterozygosity, F_{IS} inbreeding coefficient

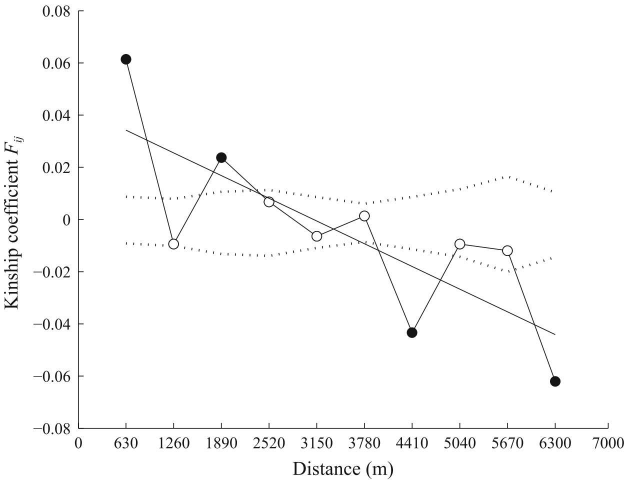

Fig. 2 Plot of the curve of kinship coefficient versus the geographic distance within population TT. The *filled circles* are significant values of F_{ij}. The *dotted lines* are 95% confidence interval of no association from 9,999 permutations. The kinship coefficient was significantly related to spatial distance

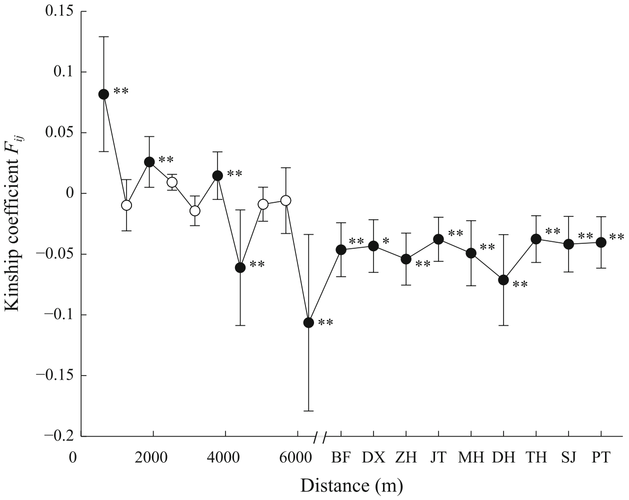

Fig. 3 Comparison of the kinship coefficients (F_{ij}) within population TT and samples in the study by Chen et al. (2008b) based on six shared microsatellite loci. The *error bar* for each index value is the standard error obtained from jackknifing over six loci. The results of permutation tests are shown: *$P < 0.05$, **$P < 0.01$

After the iterative procedure, σ converged and was estimated to be 1,211 m, and the deduced neighborhood

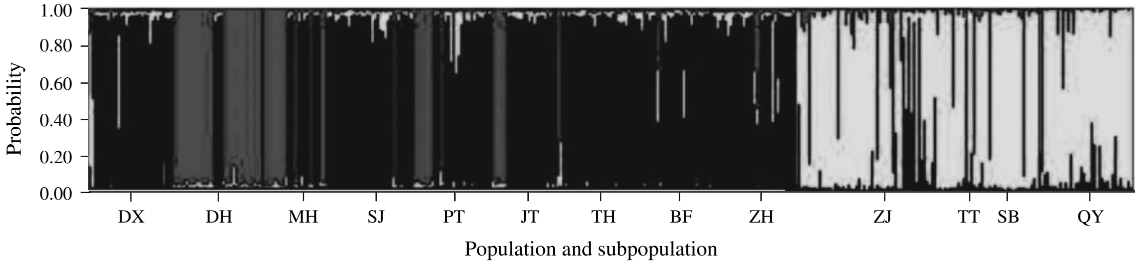

Fig. 4 Genetic structure of populations as defined by STRUCTURE (Pritchard et al. 2000). Each *thin vertical line* represents a particular individual. The membership for each individual is indicated by partitioning the corresponding line into several parts with different colors. Individuals are grouped by the original populations or subpopulations (in population TT)

size (*Nb*) was 18.47 individuals. Using a cubic regression ($R^2 = 0.7441$, $P < 0.01$), the coefficients of the second and the third power terms were 0.873 and −0.0385, respectively. The calculated k was 0.426, indicating that the contribution of seed dispersal was smaller than that of pollen dispersal. The effective pollen dispersal distance was estimated to range from 989 to 1,712 m, while the effective seed dispersal range was estimated to be less than 989 m.

Combined with data from Chen et al. (2008b), there were 10 *F. pumila* var. *pumila* populations that had been analyzed with six common microsatellite loci. After 20 repeated runs for K numbered 1–10, there was a peak of ΔK at $K = 3$, indicating that the most logical number of clusters was three. Using the STRUCTURE software, individuals from the study population (TT) were almost all assigned to a distinct cluster, which seldom included individuals of the other nine populations. Individuals of populations DH and MH were assigned mainly to another cluster, and the third cluster consisted mainly of individuals of the other natural populations (see Fig. 4 for details).

Discussion

Using 10 neutral microsatellite loci, autocorrelation analysis indicated that the *F. pumila* var. *pumila* population exhibited a strong spatial genetic structure at short distances, a pattern of restricted gene flow (e.g., Sokal et al. 1997; Epperson et al. 1999). The estimated effective pollen dispersal was 989-1,712 m, much shorter than the distances estimated for monoecious figs (e.g., Nason et al. 1998; Harrison 2008), and shorter than those of two dioecious fig plants reported by Zavodna et al. (2005a). Seed dispersal was limited compared to pollen dispersal. F_{ST} among subpopulations also indicated significant genetic structure at a fine scale. The estimated value of F_{ST} (0.06) among subpopulations of population TT with a range of about 29.43 km^2 is slightly larger than $F_{ST} = 0.05$ reported by Saddoud et al. (2007) for analysis with a dataset of 72 *F. carica* ecotypes collected across Tunisia. Restricted gene flow was supported also by the high degree of inbreeding within population TT. Theoretically, dioecious species are obligate outcrossers and a low level of inbreeding is expected (Hamrick and Godt 1989). In functionally dioecious *F. carica*, F_{IS} has been reported to range from 0.011 to 0.053 (Saddoud et al. 2007), much smaller than the value in this study ($F_{IS} = 0.287$).

Limited seed dispersal in the study population is consistent with the conclusion that climbing figs are poor colonizers (Shanahan et al. 2001a). Lack of a long-distance seed disperser might explain the results for the study population. Animals, including birds and bats with long-distance dispersal ability, are seed dispersers of fig species (Shanahan et al. 2001b). Bats have been observed eating the fruit of *F. pumila* var. *pumila* in Hong Kong (Corlett 2006), but no fruit bat has been found in areas north of the Tropic of Cancer in China (S.-Y. Zhang, personal communication). For fig fruits, inconspicuous colors are less attractive to birds (Yokoyama 2003). The mature syconia of *F. pumila* var. *pumila* are dark green, with no great difference from the color of its leaves. Furthermore, *F. pumila* var. *pumila* usually grows in open areas associated with human activities, such as banks of creeks and abandoned walls, and rarely climbs to the forest canopy. Therefore, there seem to be very few birds that will eat the syconia of *F. pumila* var. *pumila*. Villagers told us that syconia might be consumed by rodents and ants. In the field, we observed that most ripe fruits of *F. pumila* var. *pumila* in the Tiantong area fell to the ground and were not removed or consumed for several days.

At a local scale, the effective range of pollen dispersal was greater than that of seed dispersal, and this was a common phenomenon found in other plants over a large area (Petit et al. 2005). However, the pollen dispersal range was less than that reported for other fig species (e.g., Nason et al. 1998; Zavodna et al. 2005a). The limited pollen dispersal could be explained by the high density of mature *F. pumila* var. *pumila* individuals. Density might affect insect forage behavior, and therefore lead to differences in the mating systems of plants (Barret and Eckert 1990). The distance (ranging from 8 to 775 m with a mean of 108 m) between the closest mature *F. pumila* var. *pumila* individuals in population TT was much smaller than the minimum flight distance for pollinating fig wasps from the natal tree to a receptive individual in monoecious fig species (>3.6 km, Harrison 2008). However, with a high density of receptive fig trees, *W. pumilae* might not have to fly very far to reach a receptive fig. A high density of receptive host figs has been used to explain the reduced dispersal range of pollinating wasps in other dioecious fig species (Harrison 2003). As suggested by Harrison (2003), restricted pollen dispersal might be linked to low canopy positions of *F. pumila* var. *pumila*. The low wind speed in the understory is not favorable for the flight of pollinating fig wasps (Kameyama et al. 1999), which use wind-assisted dispersal (Compton et al. 2000). Furthermore, because the study population is surrounded by mountains, decreased wind speeds are expected, leading to reduced pollinating wasp dispersal.

Autocorrelation analysis at a regional scale indicated that the *F. pumila* study population was negatively related to other populations (Fig. 3). Assignment analysis assigned almost all individuals of the study population to a distinct cluster while individuals of the other nine populations were assigned to two other clusters (Fig. 4). These results indicated restricted gene flow between the study population and other populations, with substantial gene flow among the other nine populations, most of which were growing on oceanic islands. A high level of gene flow among island populations can be explained by the strong air currents over the sea, which may promote the flight of pollinating fig wasps (Zavodna et al. 2005a).

Asynchronous flowering might also explain the restricted gene flow observed between the study population and other populations. In the 2007 survey, most *W. pumilae* had emerged from ripe male figs in population TT in late April. However, no mature fig wasps were observed in the island populations of the Zhoushan archipelago in the same period. In 2008, although the flight of *W. pumilae* was delayed by about 10 days in the study population, the difference between the studied and island populations was still observed. Given the short lifespan of adult female pollinating fig wasps (usually less than 1 day) (Kjellberg et al. 1988; Harrison 2000, 2003), asynchronous flowering at a regional scale provides a barrier for the female pollinating wasps to fly from the study population to the other populations. The asynchronous flowering might be due to differential local climate. However, whether the asynchronous flowering is fixed or casual needs further investigation.

Conclusion

From spatial analysis on both local and regional scales, we inferred restricted gene flow in a *F. pumila* var. *pumila* population. The effective gene dispersal range was 1,211 m, and the relative contribution of seed dispersal was less than that of pollen dispersal. The inferred effective range of pollen dispersal was 989–1,712 m, while the effective seed dispersal range was less than 989 m. A lack of long-distance dispersers was the probable cause of the limited seed dispersal. A high density of mature *F. pumila* var. *pumila* individuals was the most probable reason for the limited pollen dispersal. The position of syconia and relatively low wind speed below the canopy may contribute to the limited pollen dispersal in the Tiantong area. Asynchronous flowering on the regional scale may provide a barrier for the pollinating female wasps to fly from the study population to other populations. Further studies on seed dispersal, phenology, and local climates may aid better understanding the gene flow in *F. pumila* var. *pumila*.

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