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Genetic variation in island and mainland populations of *Ficus pumila* (Moraceae) in eastern Zhejiang of China

Yan Chen[1], Miao-Miao Shi[1], Bin Ai[1], Jin-Min Gu[1], and Xiao-Yong Chen[1,2*]

[1]Department of Environmental Sciences, East China Normal University, Zhongshan R. (N.) 3663, Shanghai, 200062, China, Tel. +86-21-6223-7895, Email. xychen@des.ecnu.edu.cn;
[2]Shanghai Key Laboratory for Ecological Processes and Restoration, Shanghai 200062, China

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Abstract

Genetic variation, which is a prerequisite for the evolutionary response to environmental changes, may influence the long-term persistence of populations. Using six microsatellite markers, we investigated the genetic structure of island and mainland populations of *Ficus pumila* from the Zhoushan archipelago and the nearby eastern China mainland. The number of alleles per locus ranged from 2 to 4 with an average of 3.2. Several populations showed a significant bias from the Hardy-Weinberg equilibrium, mostly due to heterozygote deficit as indicated by positive inbreeding coefficients. The Mean of the number of alleles per locus, the allelic richness, the observed and the expected heterozygosities were 2.98, 2.94, 0.34 and 0.43, respectively. The overall genetic differentiation was 0.075. The island populations had comparable within-population diversity to the mainland populations. Genetic differentiation among the mainland populations (0.047) was about one half of that of the island populations (0.081), which was attributed to one island population. When this population was excluded, the island populations had the same F_{ST} as the mainland populations. High gene flow in *Ficus pumila* may explain the similar genetic variation found in the island and mainland populations.

Keywords: *Ficus pumila*, Zhoushan archipelago, microsatellites, genetic diversity, genetic differentiation

1. Introduction

Given their discrete geographical nature and a diversity of habitats and species, islands have provided opportunities for studying evolution (Emerson, 2002). Since the era of Darwin, island systems have received attention from many evolutionary biologists. The islands are spatially isolated from the mainland or other islands, and water provides a barrier for gene flow between populations. Although isolation and limited geographical range increase their vulnerability (Frankham, 1998), the relative simplicity of island populations makes them ideal for study. Due to small population sizes and decreased gene flow, there is often a lower genetic diversity in island populations than in their mainland counterparts (Frankham, 1997), However some studies failed to observe reduced genetic diversities in island populations (for examples see Frankham, 1997), and this is usually where species have an extremely high dispersal ability (Zavodna et al., 2005a), a multi-colonization from different and diverse mainland sources, or were the source of mainland recolonization after the ice age (Tsumura and Ohba, 1993).

Species interaction can also influence the fate of populations in closely associated species. During the last two hundred years, many island populations have gone extinct due to changes in species interactions (Primack, 1998). Comparisons between fragmented and continuous populations have indicated that a high degree of specificity in pollination systems may increase extinction risk, since no effective alternative partners are available as the pollinator (Bond, 1994; Harris and Johnson, 2004). Figs (genus *Ficus*, Moraceae) and their pollinating wasps (family Agaonidae) provide an extreme example of plant-pollinator interaction. Figs can only be pollinated by the female wasps, and the wasps can only lay their eggs within *Ficus* inflorescences (syconia), where their offspring feed on some of the developing seeds (Cook and Rasplus, 2003).

*The author to whom correspondence should be sent.

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Obviously, in island habitats, it is more difficult for such species to maintain sufficient genetic variation, to adapt to a changing environment. Only a few studies have focused on the genetic variation of island populations of fig-fig wasp interaction (Yokoyama, 2003; Khadari et al., 2005; Zavodna et al., 2005a).

The Zhoushan archipelago was originally an extended part of the Tiantai Mountains in eastern Zhejiang Province, China. These islands were separated from the mainland about 7000–9000 years ago because of the rising sea level (Wang et al., 2005). During its formation, the Zhoushan archipelago underwent several marine invasions, and two of the three largest ones were exposed to higher sea levels than at present. The Zhoushan archipelago is the largest archipelago in China and consists of 1339 islands with a total land area of 1371 km^2. Zhoushan Island is the largest (467.8 km^2), and only 58 islands are larger than one km^2 (Wang et al., 2005). There is a long history (about 5000 years) of human activity on the islands, and currently about twenty of the larger islands are inhabited by humans (Wang et al., 2005).

Ficus pumila L. (Moraceae) is a dioecious, rootclimbing evergreen perennial, which is native to Vietnam, Japan, and China, where it is widely distributed in subtropical areas. This species grows in sun to moderate shade, and is usually found on old walls, rocks or trees. *Ficus pumila* is a functionally dioecious species, consists of female individuals whose synconia include only long-style female flowers and "male" individuals whose synconia include male flowers and short-style flowers. Ma and Wu (1989) reported that *F. pumila* has a single flowering period, i.e., late April, on Tianmu Mountain in Zhejiang Province which has a similar latitude to the present study sites. However, Luo et al. (2000) found that "male" individuals have another flowering period, i.e., mid July, in Ningde, Fujian Province, about three degree of latitude south to the present study sites. *Ficus pumila* is pollinated by *Wiebesia pumilae* (Agaonidae). The female wasps are about 2.7 mm in length and the males are about 2.5 mm in length. During the flowering period, female wasps fly out of synconia of "male" individuals and enter new synconia either to oviposit or pollinate (Ma and Wu, 1989). The wasps have less than a day to find new synconia after emerging from the figs (personal communications with Yong Chen of Ningde Teachers College). Seeds of *F. pumila* mature in fall and can be dispersed by birds (Chen et al., 2002). There is a long history of local human inhabitants making a jelly using the dried achenes of *F. pumila* (Wu and Fang, 1999), but there is no tradition of cultivating *F. pumila*.

In population genetic studies, microsatellites have emerged as one of most popular techniques (Selkoe and Toonen, 2006), due to their advantages (e.g. codominance, high polymorphism) over dominant markers, such as RAPDs, ISSRs, or less polymorphic allozymes. The present study had three objectives: (1) to evaluate the genetic variation of *Ficus pumila* in eastern China, (2) to estimate the degree of genetic differentiation among populations, and (3) to compare genetic diversity of island and mainland populations.

2. Materials and Methods

Sampling

In spring 2006, healthy, young leaves of *F. pumila* were sampled in ten populations from two different habitats (Fig. 1) in the eastern Zhejiang Province. We sampled seven island populations (DX, DH, MH, SJ, PT, JT, and TH) of the Zhoushan archipelago and three mainland populations (BF, ZH, and TT) nearest to the Zhoushan archipelago (Fig. 1, Table 1). In each population, 26 to 33 individuals with at least 30 m distance between each other were collected (Table 1). Leaves were cleaned using a napkin to avoid contamination from microorganisms, and dried over silica gel.

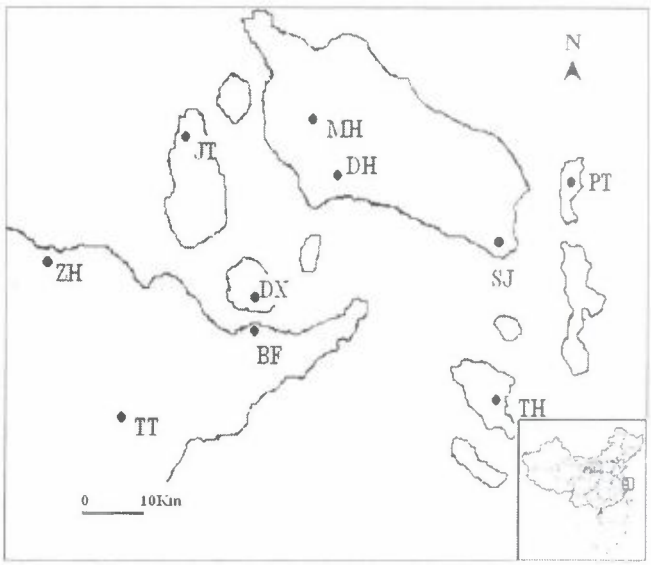

Figure 1. Sampling sites (filled spots) of *Ficus pumila* on the Zhoushan archipelago and nearby mainland.

Microsatellite protocols

Genomic DNA was extracted from dried leaves (0.05 g) according to a modified CTAB protocol (Fan et al., 2004). Six previously published microsatellite primers designed for other species of *Ficus* were screened for applicability in *F. pumila* (Giraldo et al., 2005; Zavodna et al., 2005b). The microsatellite PCR reactions were performed in a 20 µl

Table 1. Sampling sites and sizes of *Ficus pumila* populations.

Locality	Abbreviation	Latitude	Longitude	Estimated population size	Sample size
Island population:					
Daxie Island	DX	29°55′N	121°57′E	150	29
Dinghai of Zhoushan Island	DH	30°02′N	122°07′E	350	38
Mahuangshan of Zhoushan Island	MH	30°03′N	122°01′E	250	27
Shenjiamen of Zhoushan Island	SJ	29°57′N	122°18′E	600	29
Putuoshan Island	PT	30°00′N	122°23′E	750	30
Jintang Island	JT	30°04′N	121°51′E	300	26
Taohua Island	TH	29°49′N	122°17′E	800	30
Mainland population:					
Baifeng	BF	29°52′N	121°57′E	250	30
Zhenhai	ZH	29°58′N	121°39′E	100	32
Tiantong	TT	29°48′N	121°47′E	500	30

reaction mixture containing 60 ng genomic DNA, 0.2 μM of each primer, 2 μl of Taq DNA polymerase buffer (100 mMKCl, 80 mM$(NH_4)_2SO_4$, 100 mM Tris-HCl, NP-40, pH 9.0), 0.75 unit of Taq DNA polymerase, 0.4 mM of each dNTP and 1.875 mM $MgCl_2$. PCR reactions consisted of 28 cycles each of 15s at 94°C, 45 s at annealing temperature of 48.5–65 degrees (depending on the primer used) and 30 s at 72°C, preceded by an initial melting step of 3 min at 94°C, and followed by 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). Amplification products were separated by electrophoresis on 6% denaturing polyacrylamide gels and detected by staining with silver nitrate. pUC19 DNA/MspI(HpaII) marker 23 (Fermentas) was used as the reference of products' length.

Analysis of genetic variation

Data were checked for misprint, scoring errors and deviations from Hardy-Weinberg equilibrium (HWE) due to the presence of null alleles using MICRO-CHECKER (Van Oosterhout et al., 2004). Deviations from Hardy-Weinberg equilibrium at each locus in every population were examined by an exact test for HWE with a Markov Chain algorithm as implemented in the program TFPGA 1.3 (Miller, 1997).

Within-populations genetic variation was estimated for each population using descriptive population genetic parameters such as number of alleles (A), allelic richness (A_R, an estimate of the number of alleles per population standardized using the rarefaction method) (El Mousadik and Petit, 1996), and observed (H_O) and expected heterozygosity (H_E) (Nei, 1978). These parameters were estimated using population genetic analysis software FSTAT 2.9.3.2 (Goudet, 1995) and TFPGA 1.3 (Miller, 1997).

Tests for linkage disequilibria (LD) between loci for each population were performed with the help of FSTAT version 2.9.3.2 (Goudet, 1995). Fixation indices, F_{IS} (inbreeding within individuals in population; inbreeding coefficient) and F_{ST} (an indicator of the degree of differentiation among populations) were calculated based on Weir and Cockerham (1984) estimators f and θ, respectively, using FSTAT 2.9.3.2 (Goudet, 1995). Permuting over loci was performed using 1000 replicates to generate 95% C.I. for F_{ST}. The theoretical number of migrants among populations per generation (Nm) was estimated using the formula $Nm = (1 - F_{ST}) / 4F_{ST}$ (Wright, 1951). Nei's (1987) unbiased genetic distance between populations was also calculated, and relationship between genetic and geographic distances was assessed using the Mantel test (Mantel, 1967) as implemented in TFPGA 1.3 (Miller, 1997).

The software BOTTLENECK (Cornuet and Luikart, 1996) was used to test for a recent reduction of effective population size under the assumption that alleles are generally lost faster than heterozygosity, and recently bottlenecked populations will therefore display an excess of heterozygosity relative to that expected on the number of alleles (Nei et al., 1975).

3. Results

Initial observations

Ficus pumila occurs on a few of the islands in the Zhoushan archipelago. The number of mature individuals ranged from about twenty to more than one hundred per island (personal observation). We observed that *F. pumila* individuals flower in late April to early May in the Zhoushan archipelago. However, whether they flower again in July needs to be confirmed. We found that some synconia remain un-pollinated and no seeds were found during our two-week survey in 2006. Aborted synconia were also observed, indicating that some island populations

Table 2. Genetic diversity at microsatellite loci within sampling sites.

Locus	DX	DH	MH	SJ	PT	JT	TH	BF	ZH	TT
FS3-31										
A	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
A_R	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
H_E	0.13	0.32	0.10	0.26	0.32	0.20	0.15	0.12	0.22	0.42
H_O	0.14	0.39	0.11	0.31	0.40	0.23	0.17	0.13	0.25	0.60*
F_{IS}	–0.057	–0.233	–0.040	–0.167	–0.234	–0.111	–0.074	–0.055	–0.127	–0.415
FM4-18										
A	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
A_R	2.86	2.66	3.00	2.98	3.00	3.00	3.00	2.83	2.98	3.00
H_E	0.19	0.29	0.63	0.19	0.57	0.47	0.39	0.28	0.17	0.51
H_O	0.10*	0.03*	0.33*	0.07*	0.17*	0.31*	0.03*	0.07*	0.13	0.30*
F_{IS}	0.465	0.911	0.488	0.648	0.713	0.367	0.916	0.771	0.297	0.429
FS4-11										
A	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
A_R	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	2.00	3.00
H_E	0.60	0.55	0.59	0.64	0.62	0.65	0.63	0.56	0.44	0.53
H_O	0.45*	0.37*	0.56	0.38*	0.73	0.42*	0.43*	0.20*	0.41	0.43
F_{IS}	0.269	0.347	0.078	0.421	–0.166	0.371	0.323	0.651	0.094	0.198
FM3-64										
A	4.00	3.00	2.00	3.00	4.00	3.00	4.00	4.00	3.00	3.00
A_R	3.85	2.89	2.00	3.00	3.83	3.00	4.00	3.83	3.00	2.98
H_E	0.52	0.50	0.28	0.49	0.47	0.53	0.63	0.49	0.49	0.21
H_O	0.52	0.47	0.33	0.48	0.43	0.46	0.60	0.40	0.53	0.17
F_{IS}	0.014	0.061	–0.182	0.032	0.090	0.154	0.063	0.207	–0.063	0.229
FM4-15										
A	3.00	3.00	3.00	3.00	3.00	2.00	3.00	3.00	3.00	3.00
A_R	3.00	3.00	3.00	3.00	3.00	2.00	3.00	3.00	3.00	3.00
H_E	0.45	0.51	0.46	0.46	0.65	0.50	0.53	0.51	0.59	0.63
H_O	0.48	0.25*	0.44	0.39	0.55	0.64	0.60	0.43	0.31*	0.30*
F_{IS}	–0.047	0.523	0.059	0.173	0.167	–0.263	–0.109	0.182	0.481	0.533
LMFC25										
A	4.00	4.00	4.00	4.00	4.00	3.00	4.00	3.00	3.00	4.00
A_R	3.98	3.99	3.85	3.98	3.67	2.96	3.99	3.00	2.97	4.00
H_E	0.55	0.36	0.23	0.40	0.33	0.14	0.30	0.54	0.21	0.56
H_O	0.48	0.26*	0.11*	0.34	0.40	0.15	0.27	0.57	0.10*	0.38*
F_{IS}	0.142	0.285	0.537	0.159	–0.190	–0.047	0.116	–0.034	0.541	0.335
Mean										
A	3.17	3.00	2.83	3.00	3.17	2.67	3.17	3.00	2.83	3.00
A_R	3.12	2.92	2.81	2.99	3.08	2.66	3.17	2.94	2.66	3.00
H_E	0.41	0.42	0.38	0.41	0.49	0.42	0.44	0.42	0.35	0.48
H_O	0.36	0.30	0.31	0.33	0.45	0.37	0.35	0.30	0.29	0.36
F_{IS}	0.126	0.310	0.198	0.209	0.108	0.136	0.215	0.301	0.203	0.253

A: mean number of alleles; A_R: mean allelic richness; H_E: unbiased expected heterozygosity; H_O: observed heterozygosity; F_{IS}: inbreeding coefficient.* Deviation from Hardy-Weinberg expectations ($p<0.05$).

suffered from pollinator limitations. Thus on the islands, this plant-insect relationship may be vulnerable to habitat fragmentation and disturbance (Zavodna et al., 2005a).

Genetic variation

The number of alleles per locus ranged from two (FS3-31) to four (FM3-64, LMFC25). The total number of alleles across loci was nineteen. Locus FM4-18 showed consistent heterozygote deficit, most likely due to the presence of null allele(s) across eight populations, indicated by MICRO-CHECKER. Nine populations showed a heterozygote deficit at locus FS4-11, and among them six were significantly deviated from HWE. MICRO-CHECKER revealed that five of them were due to null allele(s). Several populations showed a significant bias from the Hardy-

Table 3. Nei's distances (lower triangle) and F_{ST} (upper triangle) of population pair-wise of *Ficus pumila*.

	DX	DH	MH	SJ	PT	JT	TH	BF	ZH	TT
DX	–	0.193*	0.116*	–0.005	0.079*	0.053*	0.015	–0.010	0.040*	0.061*
DH	0.206	–	0.052	0.176*	0.101*	0.148*	0.148*	0.157*	0.204*	0.148*
MH	0.106	0.053	–	0.095*	0.055*	0.076	0.066*	0.087*	0.116*	0.071*
SJ	0.011	0.185	0.088	–	0.057*	0.033	0.003	0.004	0.027	0.045
PT	0.088	0.115	0.060	0.067	–	0.035*	0.031	0.056*	0.068*	0.052*
JT	0.056	0.154	0.074	0.041	0.047	–	0.006	0.052*	0.041*	0.081*
TH	0.026	0.159	0.067	0.018	0.045	0.021	–	0.013	0.018	0.055*
BF	0.008	0.165	0.084	0.018	0.068	0.059	0.027	–	0.033	0.048
ZH	0.038	0.196	0.094	0.029	0.063	0.040	0.023	0.034	–	0.058*
TT	0.069	0.173	0.075	0.055	0.074	0.094	0.069	0.060	0.055	–

*$p<0.05$.

Weinberg equilibrium (HWE) at some of remaining four loci using an exact test with a Markov Chain algorithm: DH (FM4-15, LMFC25), MH (LMFC25), ZH (FM4-15), TT (FM3-31, FM4-15, LMFC25). Most of them were due to heterozygote deficit as indicated by the positive inbreeding coefficients (Table 2). No significant linkage disequilibrium was detected in any locus pair at the population level.

The mean number of alleles per locus averaged 2.98 (range 2.83–3.17), and the mean allelic richness varied between 2.66 (population ZH) to 3.12 (population DX) (Table 2). Observed and expected heterozygosities averaged 0.34 (range 0.29–0.45) and 0.43 (range 0.36–0.49), respectively. No significant difference was found in measures of genetic diversity between island and mainland populations ($p>0.2$). However, there was a significant, positive relationship between expected heterozygosity and estimated population size ($p=0.0195$), while observed heterozygosity ($p=0.0749$) and allelic richness ($p=0.0545$) were marginally related to population size.

Nei's genetic distance ranged from 0.008 to 0.206 (Table 3). Pair-wise F_{ST} values were relatively low (range –0.0102 to 0.2044) (Table 3), indicating substantial historical gene flow. The overall among population differentiation was 0.075 ($p<0.01$), and the calculated gene flow (Nm) was high (3.08). F_{ST} of island populations (0.081, $p<0.01$) was approximately twice as high as the mainland populations (0.047, $p<0.01$). However, large F_{ST}s were usually linked with population DH (Table 3). When DH was excluded, the F_{ST} ($p<0.01$) of the island populations was same to that of the mainland populations. No significant correlation was observed between genetic differentiation and geographic distance (Mantel test, $r = -0.1192$, $p = 0.7670$) among populations.

The heterozygosity excess test revealed that three populations had higher observed gene diversity, but only in the mainland population TT was the excess significant ($p=0.0303$) from mutation-drift equilibrium under the infinite allele model.

4. Discussion

Ficus pumila is a widespread, long-lived, dioecious species in southern China. Allozymes and RAPDs have revealed high genetic diversity in other plant species with a similar life history characteristics (Hamrick and Godt, 1989; Nybom et al., 2004). However, the present study revealed relatively low microsatellite genetic diversity in both island and mainland populations (Table 2). Measures of genetic diversity were lower than those of many species, including threatened and endemic species. For instance, in the endangered Japanese endemic tree *Magnolia stellate* (Ueno et al., 2005), average number of alleles per locus (5) and heterozygosity (0.58) were larger than corresponding measures of genetic diversity in *F. pumila* (Table 2). Indeed, low genetic diversity seems to be general in figs. In *F. carica*, 26 polymorphic SSRs revealed a total of 79 alleles, ranging from two to eight alleles per SSR, with an average of three alleles per SSR (Giraldo et al., 2005). Although more alleles per loci have been found in some *Ficus* species (Khadari et al., 2001; Zavodna et al., 2005b; Vignes et al., 2006), these value are also in the mid to low range among reported SSR data detected in similar sized samples.

An explanation for the relative low genetic diversity in *Ficus* species could be their obligate mutualism relationship with fig wasps. In obligate systems, tight reciprocal adaptation mediates the specificity, and stabilizes polymorphism of involved species (Kawakita and Kato, 2006; Tellier and Brown, 2007). An increase in variation may violate this obligate relationship, and the new mutant may have a lower fitness due to a decrease in the success of fig fertilization or fig wasp oviposition. Although SSR markers are usually regarded as neutral, they may play functional roles (Li et al., 2002). In humans, several heritable diseases, such as Huntington's disease, are caused by mutations in SSR loci (Ranum and Day, 2002).

Given the limited population size and increased

isolation, genetic drift and inbreeding leads to a low genetic diversity in fragmented populations, as has been observed in a variety of plant species (Young et al., 1996; Chen, 2000). However, some studies have failed to observe a decrease in genetic variation and one explanation relates to a low number of generation cycles following a recent fragmentation (Young et al., 1996). The formation of islands provides the opportunity for studying the genetic consequences of long-term fragmentation. Generally, a lower genetic diversity is observed in island populations than in their mainland counterparts (Frankham, 1997). However studies have often involved only one or two populations, leaving open the question of statistical validity (Hinten et al., 2003). In addition, some studies failed to reveal declined diversity in island populations (Tomaru et al., 1994; Frankham, 1997; Skotnicki et al., 2004; Zavodna et al., 2005a).

Our study involving seven island and three mainland populations revealed that *F. pumila* populations on the Zhoushan archipelago had a slightly higher or a similar genetic diversity to those on the nearby mainland. This can be explained by a high gene flow among island and mainland populations, as indicated by the mall F_{ST} and high calculated gene flow. High dispersal ability can explain similar or even an increased genetic diversity in island versus mainland populations (Frankham, 1997). For example, in *Alnus rubra*, an early successional species with light, small and winged seed, the mean number of alleles per locus of island populations was slightly higher than that of mainland populations (Xie et al., 2002).

In *F. pumila* the F_{ST} of island populations was about twice that of mainland populations, which seems reflect a decline in gene flow among the island populations. However, differentiation analysis of pairwise populations indicated that large F_{ST}s were linked with population DH (Table 3). Population DH was located on the largest island and not at the margins of the study range. Why this population is so specific is unknown. When it was excluded, the F_{ST} of the island populations was the same as that of the mainland populations.

F. pumila is pollinated by fig wasps and can be dispersed by birds. A number of studies have described remarkably long flights (5–15 km) by fig wasps (Nason et al., 1998; Compton et al., 2000; Zavodna et al., 2005a) and by seed dispersers, such as bats (Shilton et al., 1999) and birds (Compton et al., 1996). Long dispersal distance of pollinators and seed dispersers may overcome isolation by water. In this way, high gene flow leads to low differentiation between populations on the islands and the mainland.

The Zhoushan archipelago lies on the northern edge of the *F. pumila* range in China. During the last glacial period, *F. pumila* may have been absent from the studied sites, because temperature was about 6 to 12°C lower than present. About 8000 years ago, Zhoushan archipelago was formed by the rising sea level, and *F. pumila* recolonized. The program BOTTLENECK did not reveal any evidence for a population bottleneck in any of the island populations. In contrast, the mainland population, TT, located in a national forest park and adjacent areas, revealed a significant difference between observed and expected heterozygosities. This suggests that a bottleneck has occurred in the recent past. When visiting local villagers, during the sample collecting, we were told that *F. pumila* was very common in the area some years ago. However, construction of new buildings and roads had destroyed many fig habitats. The effect of this recent fragmentation on genetic variation may account for our results and those reported for the understory shrub *Ardisia crenata* var. *bicolor* (Zhao et al., 2006). It is interesting that two other mainland populations (BF and ZH) of *F. pumila* with smaller population sizes than TT, showed no sign of a recent bottleneck. While there is a long history of human disturbance around these two small populations, which may have caused a bottleneck in the more distant past, our data do not reveal a recent impact.

Obligately coevolved interspecific systems are very sensitive to environmental changes (Koh et al., 2004). Within the fig-fig wasp system, wasps may be more vulnerable to isolated conditions than *Ficus*. Theoretical analyses showed that the extinction of the affiliate fig wasps are linearly related to the extinction of the host *Ficus* (Koh et al., 2004). Observations also showed that *Ficus* species usually (re-)colonized a new habitat much earlier than fig wasps (Zavodna et al., 2005a). Such populations can sustain themselves for a long time, given their long life history and high gene flow. Although no estimation of pollinating wasps' dispersal was available for *F. pumila*, pollinator limitations in some island populations indicated that the wasps are only capable of short-distance dispersal as found in other dioecious figs (Harrison, 2003).

The present study reveals that the populations of *F. pumila* on the Zhoushan archipelago have a comparable genetic variation to mainland populations and that high seed dispersal may facilitate the maintenance of local fig populations on islands. However, further studies, including demography and population genetics, of pollinating fig wasp(s) are warranted to improve our understanding of the maintenance of fig-fig wasp relationship in the island populations.

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