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Spatial genetic structure in an understorey dioecious fig species: the roles of seed rain, seed and pollen-mediated gene flow, and local selection

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Summary

1. The spatial genetic structure (SGS) of a plant population is mainly determined by gene flow via seed and pollen, various natural selection pressures and spatial patterns of existing plants. However, the role of those factors in shaping SGS requires further investigation.

2. We studied the relative importance of those factors in shaping the SGS of an understorey dioecious fig species (*Ficus cyrtophylla*). Spatial patterns of existing individuals and microhabitat were surveyed, patterns of seed rain were investigated, seed dispersers were observed in the field, and the gene flow and SGS of a focal population were determined.

3. Three mid-sized bulbul (*Pycnonotus* spp.) species were the primary dispersers of *Ficus cyrtophylla* fruits. All the age cohorts exhibited clumped patterns with a decreasing trend from seeds to seedlings, saplings and adults. Seed rain occurred in a non-random pattern with high clumping in moderately lit microhabitats. The observed pattern suggests disperser preferences for those microhabitats resulting in higher seed deposition.

4. Seeds and pollen of *F. cyrtophylla* were widely dispersed (ranging from 9 m to 2.75 km and 10 m to 3 km, respectively). About two-thirds of seeds and half of the pollen grains were locally dispersed (≤ 250 m) within the focal population.

5. Significant positive autocorrelations occurred at a local scale (≤10 m) in seeds and seedlings but not in saplings and adults. The SGS pattern disappeared when we reduced the sample size of seedlings to that for the saplings, which suggests that demographic thinning effects (e.g. density-dependent predation and competition) on spatial distribution may lead to the loss of SGS through transitions from seedlings to saplings and adults.

6. *Synthesis*. Although *F. cyrtophylla* seeds and pollen were widely dispersed, the significant SGS present in seedlings probably resulted from clumped seed dispersal due to dispersers' behaviour and high seedfall beneath parent trees. The loss of SGS in sapling and adult life stages is probably caused by demographic selection effects during recruitment. Our study emphasizes the different roles of dispersal and local selection in shaping SGS of plant populations.

Key-words: dioecious, *Ficus cyrtophylla*, gene flow, local selection, pollen dispersal, seed dispersal, seed rain, spatial genetic structure

Introduction

Disentangling the factors that influence spatial genetic structure (SGS) and their interactions is crucial in order to better understand the ecological and evolutionary dynamics of plant populations (Loveless & Hamrick 1984). Theoretically, SGS is determined by gene dispersal, natural selection, genetic drift and the spatial arrangement of existing individuals. How those factors actually shape SGS requires further investigation (Epperson 1993; Hamrick, Murawski & Nason 1993; Boshier, Chase & Bawa 1995; Doligez, Baril & Joly 1998).

Spatial genetic structure of plant populations is fundamentally determined by gene flow (Sokal, Jacquez & Wooten 1989; Epperson 1990; Vekemans & Hardy 2004). In the tropics, up to 90% of tree species rely on frugivorous animals to disperse their seeds (Howe & Smallwood 1982). Seed dispersal largely determines plant recruitment and spatial distribution patterns, which influence future seed dispersal directly by producing

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seedfall and indirectly by influencing frugivore behaviour (Harms 1997; Bustamante & Simonetti 2000; Bullock *et al.* 2003). As an agent and consequence of gene flow, seed dispersal could greatly influence the formation of SGS (Hamrick, Murawski & Nason 1993; Nathan & Muller-Landau 2000; Jordano & Godoy 2002). Similarly, gene dispersal via pollen may depend on pollinator movements and the spatial arrangement of existing plants.

Since seed and pollen are the two major agents of gene flow, measurements of seed and pollen dispersal are essential for understanding SGS (Schnabel & Hamrick 1995; Heuertz *et al.* 2003). Theoretically, maternally inherited seed may contribute two-thirds of total gene flow, while paternally inherited pollen contributes one-third – as an offspring results from the combination of a haploid spermatozoon and a diploid oocyte (Hamilton 1999). However, in natural populations, the relative contributions of seed and pollen to total gene dispersal cannot be estimated so simply. Seed and pollen flow generally happen at different spatial scales and magnitude and may make very different contributions to gene dispersal (Schnabel & Hamrick 1995; McCauley 1997). Kalisz *et al.* (2001) pointed out four possible alternative scenarios with these dispersal features: (i) When both pollen and seed dispersal are highly localized around the maternal parent and have similar variances, then inbreeding and genetic substructuring of a population will evolve as described by the isolation-by-distance model (Wright 1946); (ii) Conversely, when both pollen and seeds are widely dispersed within a population, then neither inbreeding nor spatial genetic structure will develop; (iii) When pollen dispersal is highly localized but seed is widely dispersed, then neither inbreeding nor genetic structure will develop (if selfing is prohibited); and (iv) When pollen is widely dispersed but seed dispersal is highly localized, there will be no inbreeding but spatial aggregations of siblings will result in significant fine-scale genetic structure. Thus, the magnitude and scale of seed and pollen dispersal needs to be measured to estimate their consequences for gene movement and SGS.

The patterns of SGS may also be affected during recruitment by various kinds of natural selection and intrinsic plant characteristics (e.g. self-incompatibility). Demographic selection can strongly shape the early distribution pattern of genetic variation established by seed and pollen dispersal (Herrera 1995; Hamrick & Nason 1996; Garrido, Rey & Herrera 2005). Hamrick, Murawski & Nason (1993) suggested that spatial distribution of individuals controls the levels of SGS through the overlap of seed shadows, and a demographic thinning effect caused by density-dependent predation and competition for resources can greatly weaken SGS across life stages. Also, spatial aggregation of seedlings resulting from adaptation to specific microhabitats may produce a slight increase of local SGS (Doligez, Baril & Joly 1998; Lan *et al.* 2009). There are few empirical studies that illustrate demographic effects on SGS (but see Epperson 2000; Kalisz *et al.* 2001; Chung, Epperson & Chung 2003). Based on existing knowledge, we hypothesize that the following two scenarios might occur from the effects of local selection on SGS: (i) Density-dependent predation and competition for resources will greatly reduce the clumping of seeds or seedlings, which will weaken local SGS; and (ii) Individuals will tend to clump in some places resulting from adaptation to specific microhabitat conditions, leading to the enhancement of local SGS.

Since different factors may operate on SGS at various recruitment stages, the degree of SGS may also differ across life stages, particularly in long-lived trees. Comparing patterns across different life stages is considered to be an effective approach to determine the relative impacts of various factors and selection forces (Aguinagalde *et al.* 2005; Duminil *et al.* 2007; Benard & McCauley 2008). However, the cohort approach to studying the factors that shape SGS has been used in only a few studies, and SGS patterns differ among them (Hamrick, Murawski & Nason 1993; Tonsor *et al.* 1993; Epperson & Alvarez-Buylla 1997; Chung, Epperson & Chung 2003; Hardesty *et al.* 2005). Most studies found a decreasing trend of SGS from seedling to adult stages, indicating that post-dispersal juvenile cohorts were more structured than adult cohorts, emphasizing the role of mating and dispersal in the development of SGS (Hamrick, Murawski & Nason 1993; Epperson & Alvarez-Buylla 1997; Chung, Epperson & Chung 2003). Contrary results were obtained by Tonsor *et al.* (1993); in their study less structure appeared in seedlings relative to adults, suggesting significant effects of post-dispersal selection on adult SGS. The inconsistency among studies is possibly due to different degrees of demographic selection relative to dispersal. Therefore, studies that incorporate and document gene flow and local selection using the cohort approach are necessary for a better understanding of local selection on SGS.

In this study, we used a focal population of the dioecious fig species *Ficus cyrtophylla* (a bird-dispersed shrub or small tree species) to evaluate the following factors which drive gene flow and SGS: (i) the patterns of seed rain and existing plant distribution; (ii) the effects of dispersers and microhabitat on seed rain; (iii) the effective gene dispersal distance via seed and pollen and their relative contributions to the total gene flow; and (iv) the roles of gene flow and local selection in determining SGS across different life stages. The major aim of this study was to determine how the SGS of *F. cyrtophylla* populations is shaped by seed and pollen dispersal and local selection during recruitment.

Materials and methods

STUDY SITE AND SPECIES

The study was conducted in a limestone mountain area (21°54′07′–21°55′20′ N, 101°16′05′–101°17′04′ E) of south-east Xishuangbanna, Yunnan province, China. The region has a typical tropical monsoon climate. Mean annual temperature is 22 °C, and annual temperature accumulation is about 8000 °C (the sum of daily temperature > 10 °C); monthly mean temperature is 15.9 °C during the coldest month (December) and 25.7 °C during the warmest month (June). Annual precipitation ranges from 1200 to 1556 mm, of which more than 80% falls during the rainy season from May to the end of October. The vegetation is typical limestone seasonal rain forest dominated by *Cleistanthus sumatranus*, *Sumbaviopsis albicans*, *Lasiococca comberi* and *Cleidion brevipetiolatum* (Zhu, Wang & Li 1998; Zhu *et al.* 2003). *Ficus cyrtophylla* plants are common and found in clusters in moist valleys of the limestone forest.

Ficus cyrtophylla is an evergreen shrub or small tree, 3–6 m high, occurring in the forest understorey or mid-canopy layer. It is pollinated by a species-specific wasp of genus *Kradibia* (Agaonidae). It is functionally dioecious. *Ficus* are characterized by the production of closed inflorescences called figs. Fig pollinating wasps enter figs and develop within, in galled female flowers. Before leaving their natal fig (the fig where the wasp was born) they become loaded with pollen. They then leave in search for a receptive fig of the same species. If they enter a fig on a female tree, they pollinate, but they cannot oviposit. Thus female trees produce seeds but no wasps in their figs. If the wasp enters a fig on a functionally male tree, it will oviposit in ovules. Functionally male trees produce wasps and pollen, but no seeds. Figs are produced in crops, and a crop remains receptive (attractive to pollinators) for 7–19 days. A single wasp is sufficient to ensure pollination of a fig. *Ficus cyrtophylla* usually produces each year 2–3 crops of oval figs (8–10 mm in diameter) clumped at the leaf axils or on the branches (Shi & Yang 2006). According to the observations of Sanitjan & Chen (2009), several frugivorous bulbul species (red-whiskered bulbul *Pycnonotus jocosus*, black-crested bulbul *P. melanicterus* and sooty-headed bulbul *P. aurigaster*) are common visitors to fruiting *F. cyrtophylla* plants, indicating that seeds are likely bird-dispersed.

PLANT CENSUS AND SAMPLE COLLECTION

Within the study area, the limestone forests have been fragmented by rubber plantations and other crops for many years, producing extensive gaps between forest patches. In this study, groups of *Ficus cyrtophylla* individuals within distinct forest patches are defined *a priori* as 'populations'. The *F. cyrtophylla* population in the Lüshilin Limestone Mountain forest (21°54′35′ N, 101°16′54′ E) was selected as the focal population. In December 2006, all *F. cyrtophylla* individuals (total 289) in this population were mapped and categorized by life stage: seedlings (d.b.h. < 2 cm, 167 individuals), saplings (d.b.h. 2–8 cm, 24 individuals) and adults (d.b.h. > 8 cm, 98 individuals). The d.b.h. and location of each plant were measured and recorded. One to two fresh leaves from each plant were collected and stored in zip-locked plastic bags with silica gel until DNA isolation. Adult trees of two extended populations (located in separate forest fragments 2–3 km away from the focal population) were also mapped, recorded and sampled as candidate parent trees (36 and 57 adult plants, respectively). The sex identification of adults was continuously made during the study year until all of the 191 mapped adults were successfully distinguished.

Seed traps were installed throughout the study area. Thirty-six seed traps were put in the centre of the study site at 20 m-intervals, and 10 traps were situated around the perimeter of the study area. A seed trap consisted of a 1.0 × 1.0 m polyester mesh suspended 1.0 m above the ground with a bamboo framework. Rain could easily pass through the seed traps, while fig seeds and other debris were collected. *Ficus cyrtophylla* fruits and seeds, and other debris were collected from each trap weekly for 1 year (from January to December 2007). Seeds were germinated and leaves were collected for microsatellite genotyping. In the traps, seeds contained in fig debris were assumed to have come from seedfall of overhanging trees while seeds within faeces were assumed to have come from bird dispersal. Additional observations indicated that seeds passing through a bird did not have significantly enhanced germination. For the traps containing > 5 seeds, five seeds were randomly selected as samples, while for the traps containing ≤5 seeds, all seeds were sampled. In total, 136 seeds were sampled and further analysed.

DISPERSERS AND MICROHABITAT

In August 2007, we focused on three fruiting trees to observe bird behaviour using a telescope and camera. Survey time was from 6h30 to 18h30 (12 h per day, 3 days for each tree, for a total of 108 h of observation). Data recorded included the species of visiting frugivores, their visiting number, visit length and frequency, feeding behaviour (singly or in flocks), preferred position to drop seeds, and the proportion of fruits removed during the day (from 6h30 to 18h30) and night (from 18h30 to next 6h30). Three mist nets were also hung around each focal tree to capture foraging birds. A preliminary feeding experiment was then performed on captured frugivores to assist in their identification as seed predators or dispersers.

To determine the effects of microhabitat on seed rain and seed dispersers, microhabitat parameters (light density, canopy index and temperature) above seed traps were measured using a Quantitherm light meter (Hansatech Instruments, Norfolk, UK) and LAI-2000 plant canopy analyzer (Li-cor, Inc., Lincoln, NE, USA).

GENE FLOW AND SGS ANALYSES WITH MICROSATELLITE MARKERS

Microsatellite markers were used to detect gene flow and SGS. Genomic DNA was extracted from all 382 plant samples and 136 seed samples using a modified cetylmethylammonium bromide (CTAB) protocol by Doyle & Doyle (1990). Seven microsatellite primers (Table S1in Supporting Information) were screened for *F. cyrtophylla* analyses from published *Ficus* primers (Khadari *et al.* 2001; Zavodna *et al.* 2005a; Vignes *et al.* 2006; Crozier *et al.* 2007). Polymerase Chain Reaction (PCR) was carried out for extracted DNA materials of all 518 samples.

DATA ANALYSES

Location data of seedlings, saplings and adults were used to assess plant recruitment patterns, and seed trap data were used to determine seed rain and dispersal patterns. Nearest-neighbour analysis was performed with a Point Pattern Analysis (PPA) program to assess the spatial patterns of individuals (Chen & Getis 1998). Point Pattern Analysis was also used to compare the spatial patterns of seed rain, seedlings, saplings and adult trees to determine the spatial concordance of different age cohorts. The mean nearest-neighbour distance was calculated by averaging the distances between each point to its closest point; the Z statistic is used to reflect the tendencies of spatial patterns towards clustering or regularity. A negative Z score indicates clustering and a positive score indicates dispersion or evenness. Higher absolute Z-values indicate more clustered or dispersed spatial patterns.

Spatial genetic structure of each age cohort was detected using the GenALEx6 program (Peakall & Smouse 2006), and comparison of SGS across different life stages was done to analyse SGS concordance. A genetic distance matrix summed over all loci and matching geographic distance matrix for the same set of samples was calculated, and the autocorrelation results (R) at certain distance class sizes were presented as a correlogram. To understand whether the change of SGS between differently aged individuals was caused by intrinsic genetic properties or natural selection, we randomly sampled 24 individuals (equivalent sample size of saplings) from the pool of 167 seedlings and conducted the analyses of SGS (50 iterations), thereby testing the effects of sample size on the SGS of seedlings. Given the patchiness of seed and seedlings observed in the field, we further examined the fine-scale genetic structure of seed, seedlings and saplings using GenALEx6's 2D LSA (two-dimensional local spatial autocorrelation analyses) function.

For gene flow analyses, seeds and seedlings were treated as two recruitment events allowing two measurements of gene flow, since they came from different years. Parentage analyses for offspring (seed or seedling) were conducted against the entire pool of potential parents. If no mother or father could be identified for an offspring, they were considered to come from outside our sample area. Maternity assignment was done by simple exclusion, comparing multi-locus genotypes of offspring with candidate females. Potential mothers were rejected if alleles at any of the seven loci were incompatible with those of the offspring in question, and hence, they were not an exact multi-locus match (Hardesty, Hubbell & Bermingham 2006). For cases in which multi-locus matching resulted in 2 + suitable candidate mothers for an offspring, we assigned the nearest female as the mother, thereby conservatively estimating the maternal–offspring recruitment distance. We combined the exact multi-locus matching for maternity with likelihood estimates of parentage for paternity assignment. We used CERVUS 3.0 (Marshall *et al.* 1998) to perform paternity analyses. We limited our analyses and discussion to offspring for which we have high confidence (80%, per CERVUS) assigning the paternal parent. The effective pollen dispersal distance was estimated by measuring the distance between the mother and father of an offspring. By using this approach, the relative contributions of seed and pollen to gene dispersal could be successfully evaluated.

Results

CLUMPING OF SEED RAIN AND DIFFERENTLY AGED INDIVIDUALS AND ITS RELATION TO DISPERSERS AND MICROHABITAT

From January to December 2007, a total of 6013 *F. cyrtophylla* seeds were collected (ranging from 0 to 1387 seeds per trap). Seeds were obtained in each month of the study year with the largest numbers of seeds collected from July to September.

The differences in seed quantity collected from each trap indicated a non-random spatial distribution of seed rain (Kolmogorov–Smimov test, $P < 0.01$, $n = 6013$). Over the whole year, four seed traps (8.7%) never received seeds, 24 traps (52.2%) received 10–300 seeds and four traps (8.7%) received an extremely large number of seeds (in total ≥ 1000 seeds). Two of the four traps that received > 1000 seeds were under female fig trees while the other two were not. Correlation analyses between the number of seeds collected and microhabitat above those traps indicated that the total seed number was significantly parabolically correlated with the leaf area ($r = 0.429$, $P < 0.01$, $n = 46$) and light density ($r = 0.362$, $P < 0.01$, $n = 46$) above traps (Fig. 1).

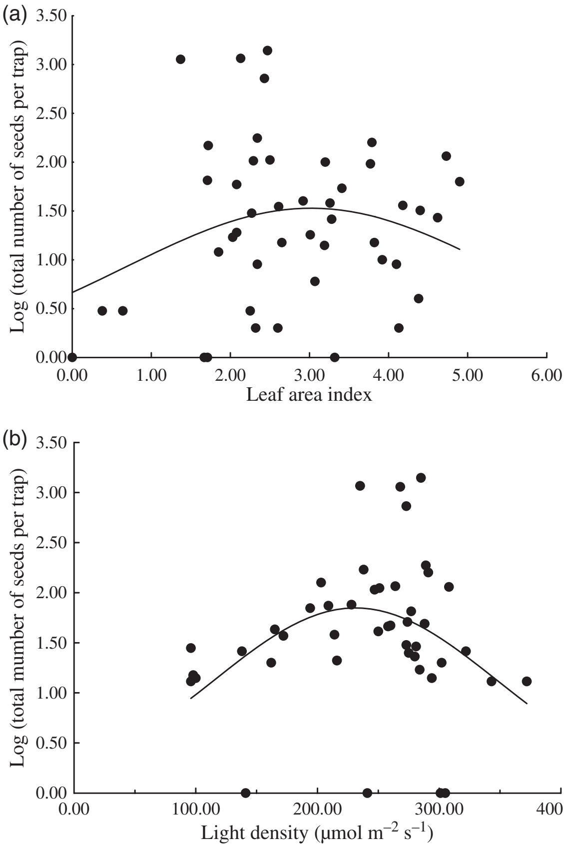

Fig. 1. The relationship of seed number collected from seed traps with light density and canopy conditions: (a) Leaf area index ($r = 0.429$, $n = 46$), (b) Light density ($r = 0.362$, $n = 46$).

In total, four frugivorous bird species were recorded as visitors: *Pycnonotus jocosus*, *P. melanicterus*, *P. aurigaster* and *Alophoixus pallidus*. *Pycnonotus jocosus* had the largest visiting number (117), followed by *P. melanicterus* (4) and *P. aurigaster* (6). *Alophoixus pallidus* was only recorded once. Additionally, three bat individuals (*Cynopterus sphinx*) were also captured. Our feeding experiment further demonstrated that the three bulbul species (*P. jocosus*, *P. melanicterus* and *P. aurigaster*) were indeed the major dispersers of *F. cyrtophylla* fruits and the seeds collected from the birds' faeces germinated well.

Point Pattern Analysis showed that the Z-values of all age cohorts were < 0, and the absolute Z-values increased from seedling to sapling cohorts. This result indicated that individuals were distributed in clumps across all age categories, but the degree of clumping and numbers of individuals decreased with increasing life stage. The average nearest-neighbour distance of seedlings was far smaller than that of adults and saplings (Table 1).

Table 1. Results of spatial pattern analysis by Point Pattern Analyses (PPA) program for different age cohorts of *Ficus cyrtophylla*. The Z score indicates the clustering degree: a negative Z score indicates clustering, and a positive score means dispersion or evenness; higher absolute Z-values indicate a more clustered or dispersed spatial distribution pattern. *NND1, 2, 3* indicates the distance to the first-, second- and third-nearest neighbour, respectively

Age cohort	Mean Z	Mean *NND1* (m)	Mean *NND2* (m)	*Mean NND3* (m)
Seedling	−17.3	2.7	5.4	8.1
Sapling	−1.6	10.631	20.8	28.5
Adult	−4.5	6.334	10.0	13.1
All plant samples	−18.3	3.331	5.9	8.1

Table 2. The pattern of gene flow in *Ficus cyrtophylla* detected from maternally assigned seeds and seedlings

	Seed dispersal or recruitment distance	Pollen dispersal distance
Seed (n = 50)	Range 9.0–2673.0 m Mean 510.3 ± 127.4 m Median 86.4 m Proportion of immigrants 22%	Range 12.0–3495.3 m Mean 1114.8 ± 160.3 m Median 1004.8 m Proportion of immigrants 52%
Seedling (n = 64)	Range 10.4–2713.4 m Mean 647.4 ± 112.5 m Median 131.8 m Proportion of immigrants 31.3%	Range 10.8–3120.0 m Mean 1095.5 ± 134.5 m Median 877.9 m Proportion of immigrants 51.6%

GENE FLOW DETECTED FROM SEEDS AND SEEDLINGS

A total of 136 seeds were genotype analysed, and 62 seeds were successfully maternally assigned. Among the 62 assigned seeds, 50 seeds were assigned to their only possible mother. The mean distances of seed and pollen dispersal detected from the 50 seeds are shown in Table 2. For the other 12 seeds that matched 2+ suitable candidate mothers, we assigned the nearest female to be the mother. Thus, a conservative dispersal distance for the 12 seeds was obtained (mean = 33.1 m, median = 25.2 m, range = 8.5–80.1 m, SE = 6.8, n = 12).

A total of 167 seedlings were genotype analysed and 76 seedlings were successfully maternally assigned. Among the 76 assigned seedlings, 64 were assigned to their only possible mother. The mean distances of seed and pollen dispersal detected from the 64 seedlings are presented in Table 2. For the 12 seedlings that matched 2+ suitable candidate mothers, we again assigned the nearest female to be the mother. The mean dispersal distance for the 12 seedlings was 119.9 m (median = 118.0 m, range = 12.2–233.0 m, SE = 20.6, n = 12).

For the maternally assigned seeds and seedlings, about 70% came from within the focal population, and 30% from outside. Pollen dispersal exhibited a nearly equal proportion between within and outside the focal population (Table 2). Within the study range (focal population and adult trees from the two nearest forest patches 2–3 km away), distance distribution analyses showed that about 70% seeds were dispersed ≤ 250 m, with some gaps at longer distance and a tail at long distance reaching 2750 m. About 40% of pollen genes were transported ≤ 250 m, over 40% were dispersed longer distances (1000–2750 m) and a small portion (≤4%) to the longest distance (> 3000 m; Fig. 2). Distances of seed and pollen dispersal within the focal population were not significantly different (two-tailed t test, $P > 0.05$), while pollen dispersed

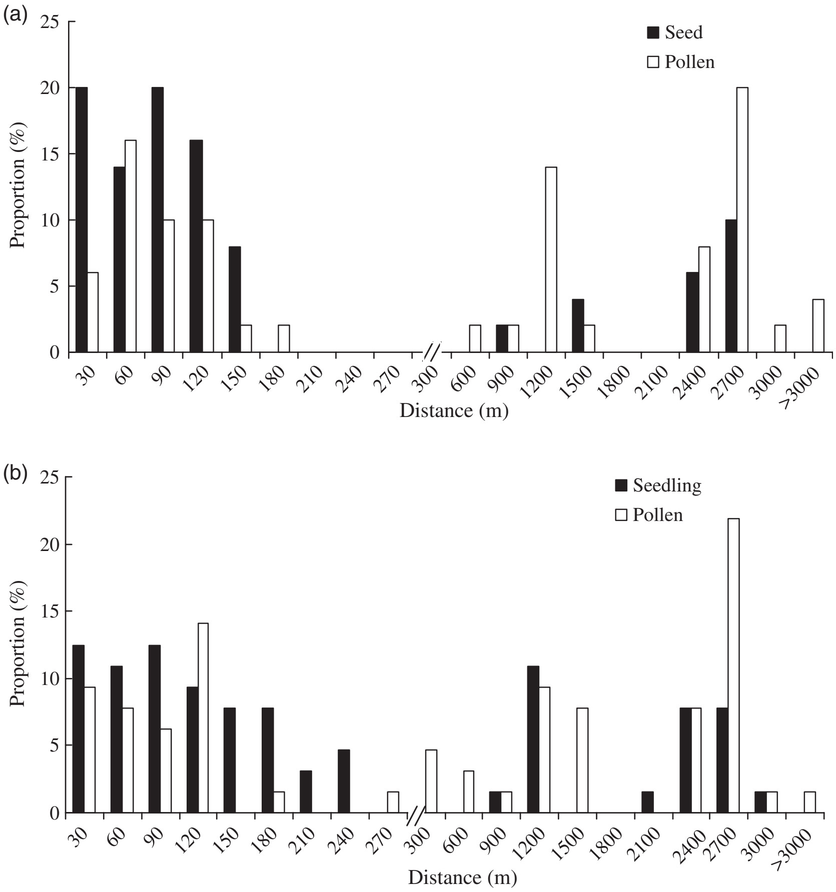

Fig. 2. Distance distribution of effective seed dispersal (black bars) and pollen movement (white bars) detected from maternally assigned seeds (a) and seedlings (b). The scale of distance is 30 m per unit before 300 m and 300 m per unit after 300 m.

significantly farther than seeds within the study range ($P < 0.05$).

SGS ACROSS DIFFERENT LIFE STAGES

The analysis of molecular variance (AMOVA) from GenALEx6 program indicated a relatively high genetic heterozygosity within the focal population: the mean effective number of alleles per locus (*Ae*) was 4.71, the mean polymorphism information content (PIC) was 0.60, and the expected heterozygosity per locus (*He*) was 0.66. For seeds and seedlings, spatial autocorrelation analyses indicated significant positive autocorrelations at distance class ≤10 m (r = 0.054 and 0.033, $P < 0.01$) and some larger distance classes

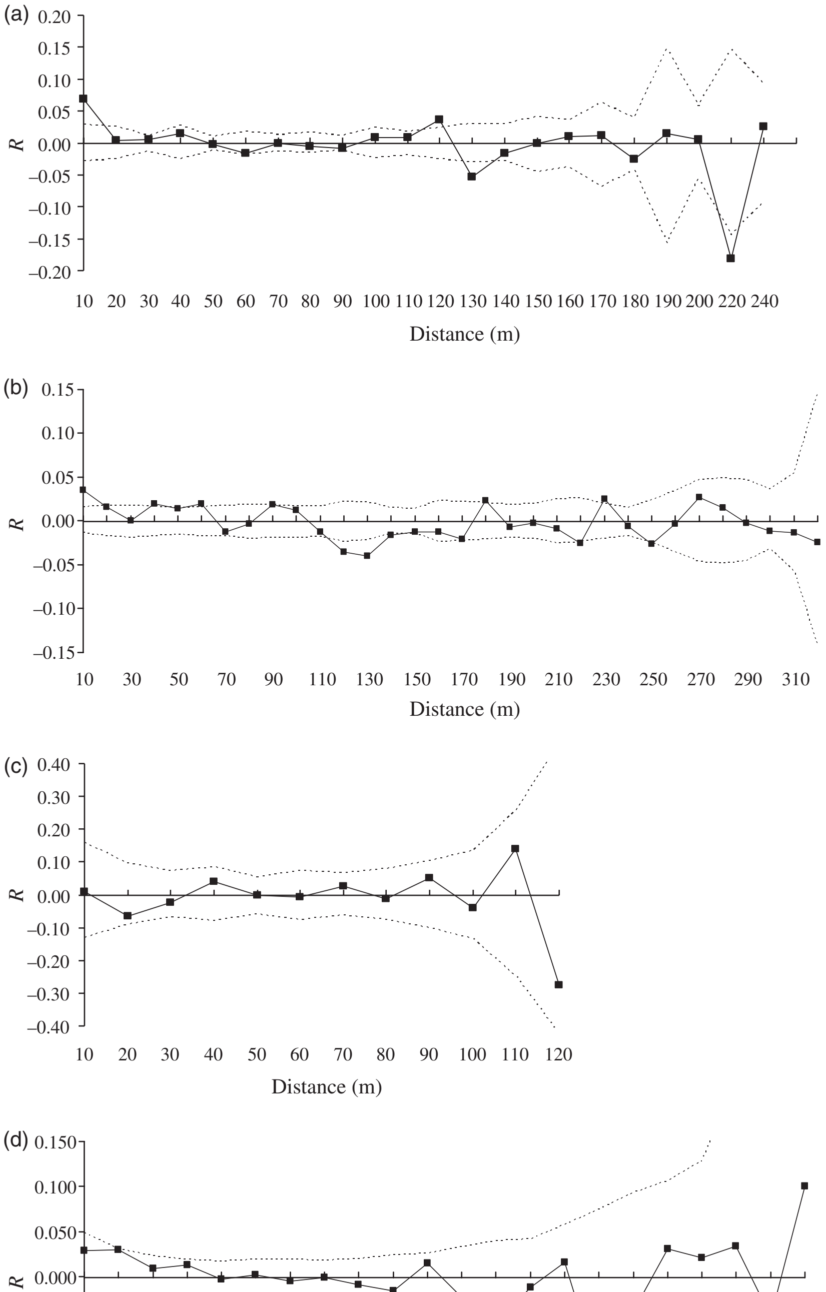

Fig. 3. The correlograms of spatial genetic structure for (a) seeds, (b) seedlings, (c) saplings, and (d) adult trees in the focal *Ficus cyrtophylla* population. The distance class was partitioned by an interval of 10 m; the black squares represent *R* value at each distance class.

(Fig. 3a,b). There was a pattern of alternating positive and negative *r* values over all the distance classes suggesting a patchy distribution pattern for seeds and seedlings. However, in the SGS correlograms of saplings and adults, no significant positive autocorrelation was found (Fig. 3c,d). For the randomly sampled 24 seedlings (the sample size of saplings) from the total seedling pool, the SGS was not detected in most of the 50 repeats ($P < 0.05$). The results of 2D LSA also showed the appearance of significant positive autocorrelations in seed and seedling cohorts but not in the sapling cohort (Fig. 4).

Discussion

CLUMPING SEED RAIN AND PLANTS OF *F. CYRTOPHYLLA*

Results from this study showed that seeds, seedlings and trees of *F. cyrtophylla* all exhibit clumped distribution patterns. The spatial pattern within a plant population is primarily determined by seed dispersal, microhabitat conditions and the spatial arrangement of existing plants (Dalling, Hubbell & Silvera 1998; Russell & Schupp 1998; Willson & Traveset 2000). Patterns of seed rain or seed shadow are the direct result of dispersal and seedfall, which can affect subsequent recruitment patterns (Jordano & Godoy 2002) and local population genetic structure (Hamrick, Murawski & Nason 1993). The clumping patterns of *F. cyrtophylla* seeds and seedlings are likely related to the spatial arrangement of existing individuals, and also to the behaviour of their seed dispersers.

Bulbuls live in overdispersed pairs in the breeding season and semi-nomadic flocks in the non-breeding season (Weir & Collett 2007). They tend to make short feeding visits, and the gut passage times have a wide range (5–47 min; Lambert 1989; Graham *et al.* 1995; So 1999; Shanahan 2000). In our study site, *F. cyrtophylla* is primarily dispersed by three mid-sized bulbul species. Field observation found that those bulbuls were particularly active around *F. cyrtophylla* trees during the peak fruiting season. Bulbuls visited fruiting plants to feed, and discarded or released seeds when perching in the *F. cyrtophylla* canopy and other lower canopies that generally provided mid-light and roosting habitat. The spatial arrangement of existing plants and microhabitat thus indirectly affected seed dispersal by influencing disperser behaviour. Laman (1996) reported that a large number of seeds will fall directly to the ground beneath the fig tree canopy. Our data from the seed collection experiment also showed that the traps under female *F. cyrtophylla* trees caught the largest number of *F. cyrtophylla* seeds, usually with some decaying figs. Deposition of a large number of seeds by dispersers at roosting sites and direct seedfall under the canopy of fruiting trees are the two major factors that led to seed clumping.

Seed deposition is crucial for seedling recruitment since the patterns of where seeds are deposited can determine seedling establishment and survival (Nathan & Muller-Landau 2000). Local selection may also have marked effects on recruitment patterns (Schupp 1995; Nathan & Casagrandi 2004). In *F. cyrtophylla*, we found a conflict in the microhabitat of seedling establishment with seed deposition: seedlings were often found in open areas (roadside, canopy gaps and forest edge), while seeds were usually deposited beneath the closed canopy. Furthermore, the total number of individuals and the degree of aggregation sharply decreased from seeds to seedlings, and to saplings, indicating a strong thinning effect during the recruitment process. Thus, the clumping pattern in seedlings does not result from simple persistence of seed clumps, but is the result of local selection (microhabitat requirements of seed germination and seedling establishment).

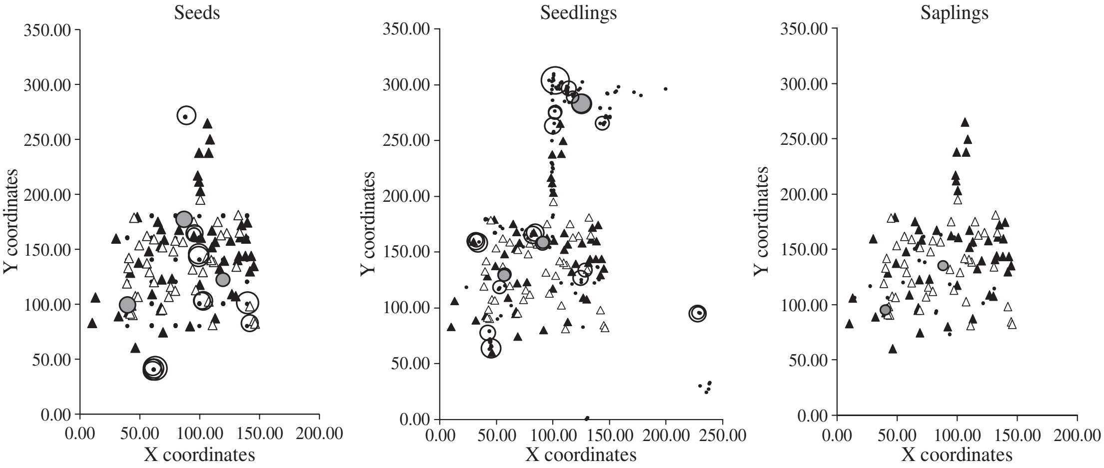

Fig. 4. Bubble plots of two-dimensional local spatial autocorrelation analyses (2D LSA) of seeds, seedlings and saplings. Bubbles surround territories with positive (open circles) or negative (shaded circles) *R* values that fell within the ± 5% tails of the permuted distribution. Bubble size is proportional to the magnitude of *R*; significant positive *R* values in those plots range from 0.15 to 0.32. Calculations of *R* values were based on sampling ten nearest neighbours. The black triangles represent female trees and white triangles represent male trees.

GENE DISPERSAL VIA SEED AND POLLEN AND THEIR INFLUENCE TO SGS

Gene dispersal via seeds in this study ranged from 9 to 2.75 km. About 78% of seeds were locally dispersed (within 250 m), while some seeds were dispersed up to 2.75 km. This seed dispersal distance (2.75 km) is generally consistent with what Weir & Collett (2007) estimated from radio-telemetry, and is much longer than the dispersal distance by bats (989 m) found by Wang *et al.* (2009). However, this maximum dispersal distance is a rather conservative estimate since the maternal parent of many seeds was not found within the total area sampled (within the focal population or adult trees in the nearest forest patches). Thus, bulbul dispersers produced a stratified pattern of *F. cyrtophylla* gene dispersal: 78% localized gene dispersal, and 22% gene dispersal associated with long-distance movements (900–2750 m). Our findings provide strong evidence that bulbuls indeed play a crucial role in dispersing *F. cyrtophylla* seeds at different scales, which may have great importance for colonization of new sites and reducing inbreeding.

This study demonstrated that fig wasps, the only possible pollinators, can provide long-distance gene dispersal, even in an understorey habitat in which wind assistance is not common. In general, long-distance flights can be achieved for wasps in canopy fig trees with the assistance of wind (Nason, Herre & Hamrick 1996; Compton *et al.* 2000; Zavodna *et al.* 2005b), but it is not common for understorey fig species without wind assistance (Harrison 2003). The dispersal range in this study was even wider than the 775 m estimated for the dioecious climbing fig *F. pumila* (Wang *et al.* 2009) and was closer to the dispersal distance of 3.6 km in monoecious hemi-epiphytic figs studied by Harrison (2008). The actual capacity of pollen dispersal mediated by fig wasps may be influenced by multiple factors, such as tree height and density (Harrison 2003), fig phenology (Shi & Yang 2006; Herre, Jander & Machado 2008), wasp behaviour (Kjellberg *et al.* 2001), etc., that need to be addressed in future studies.

Within our study range, about two-thirds of the seeds came from the focal population and the remaining one-third immigrated from extended populations. Pollen dispersal exhibited a nearly equal proportion within and outside the focal population. The higher proportion of gene flow among populations from pollen than seeds may be partly explained by the phenology of this dioecious fig species. At the population level, *F. cyrtophylla* produced figs year-round, synchronously within an individual tree but asynchronously among trees. Fruiting was much more asynchronous within male trees than females. For individual fig trees, the female phase in which figs are receptive to wasps only persists for 7–19 days (Shi & Yang 2006). Pollinating fig wasps develop in the closed receptacle (syconium) with a short lifespan for the free-living adult (Kjellberg, Doumesche & Bronstein 1988). When a female pollinator leaves the fig where it was born to find a receptive fig, the nearest neighbouring fig trees may not be receptive. Fig wasps must then fly longer distances to find suitable figs (usually assisted by wind currents) achieving long-distance pollen dispersal if they are able to locate and enter a syconium on a female tree. Combining the dispersal distances with proportions within and outside the focal population, we found that gene dispersal via seed and pollen was predominantly local (< 300 m), but with a long tail to the distribution that may reach 2–3 km.

As mentioned above, Kalisz *et al.* (2001) proposed four scenarios predicting SGS in response to the dispersal of both seeds and pollen. None of the cases proposed matches the results of this study. Both dispersers (i.e. bulbuls) and pollinators (fig wasps) provided stratified gene dispersal (local and long-distance). In *F. cyrtophylla*, a significant SGS appeared within 10 m for seed and seedling stages. We believe that the result was due to the strong clumped distribution of seed rain and seedlings caused by the spatial patterns of maternal trees and disperser behaviour as discussed above.

EFFECTS OF LOCAL SELECTION ON SPATIAL PATTERN AND SGS

Similar to previous studies (Hamrick, Murawski & Nason 1993; Epperson & Alvarez-Buylla 1997; Chung, Epperson & Chung 2003), we detected SGS discordance across different life stages. Significant positive autocorrelations (< 10 m) were detected in seeds and seedlings (Figs 3 and 4), but not in saplings and adults.

The significant local SGS in seeds was certainly due to localized direct seedfall from reproductive trees and limited gene dispersal via dispersers and pollinators. The local SGS in seedlings may be due to the persistence of seed SGS and the results of seedling clumping due to local adaptation to microhabitat. The elimination of SGS in saplings and adults could be the effects of density-dependent predation and resource competition on clustered seedlings. Additionally, we found that sample size greatly affected SGS emergence in seeds and seedlings, illustrating the effects of demographic selection on SGS that results from the decrease in individuals from seeds to seedlings to saplings. The pattern found here is similar to the study of Hardesty *et al.* (2005), which suggested that demographic thinning (e.g. density-dependent predation and competition) occurs between seed deposition, seedling establishment and recruitment to later life stages.

Fig plants are best known for their species-specific interactions with pollinating wasps (Wiebes 1979), and they are also recognized as the key food resources for tropical frugivores (Janzen 1979; Yang, Li & Yang 1997; Shanahan *et al.* 2001; Corlett 2006). For such a special group, understanding the contributions of seed dispersers and wasp pollinators to gene flow and genetic structure is always a central question.

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Supporting Information

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

Table S1. Seven pairs of microsatellite primers for the amplification of all samples. F, forward primer; R, reverse primer.

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