

Ant-Mediated Seed Dispersal Contributes to the Local Spatial Pattern and Genetic Structure of *Globba lancangensis* (Zingiberaceae)

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Abstract

Globba lancangensis is a typical myrmecochorous perennial herb found in southwest China. In 2002 and 2003, seed dispersal by ants was examined. Twelve ant species were recorded and found to move seeds 0.01–3.35 m. This removal promoted seed dispersal. In 2003, ant exclusion was made in 4 of 8 study plots for 1 year to practically detect the effects of ant dispersal. As a result, nearest neighbor analyses indicated that although ant dispersal could not significantly change the mean nearest neighbor distance, it obviously reduced the clustering degree of seedlings. Spatial genetic structure (SGS) of mature plants in 2003 and sequent seedlings in 2004 were investigated with intersimple sequence repeats. The whole correlogram of all individuals from 2003 showed significant positive autocorrelations between genetic and geographical distances within 4 m, suggesting a patchy structure at such short distances in the studied population. This pattern is likely associated with limited seed dispersal. The comparative analysis of fine-scale correlograms (<4 m) for ant-excluded treatment indicated lacking ants as the primary dispersers could have a similar pattern to ant dispersal presence, which is likely due to the restriction of ant dispersal distance. In conclusion, ant-mediated dispersal contributes to reduce seedling clustering degree and plays a minor role in developing and maintaining the local SGS in *G. lancangensis*.

Seed dispersal patterns and their genetic properties produce initial population templates, which define the context of future ecological, demographic, and genetic interactions of emerging seedling cohorts (Houle 1992; Hamrick et al. 1993; Kalisz et al. 1999). However, the demographic and evolutionary consequences of seed dispersal began to receive attention only a few years ago (Muller-Landau and Hardesty 2005; Bacles et al. 2006; Nathan 2006). As a result, information available on the causes and effects of dispersal patterns from seeds to new adults remains scarce, creating a major gap in our knowledge on the ecology of seed dispersal.

As a short-distance dispersal model (the mean dispersal distance is about 0.96 m, $n = 2524$, Gómez and Espadaler 1998), ant-mediated seed migration is quite common in higher plants, especially in tropical forests (Horvitz and Schemske 1986; Gorb EV and Gorb SN 1995; Beattie and Hughes 2002). Dispersal by ants may provide various benefits to plants such as escape from rodent predation or pathogen infection (Howe and Smallwood 1982; Gibson

1993a), movement of seeds to fertile microsite (Gibson 1993b), and avoiding fire (Beattie 1985).

A key ecological result from such dispersal is that ants can alter both the spatial arrangement of seeds and the establishment of seedlings. Beattie and Culver (1979) showed that dispersal by ants promoted the aggregation of seedlings, while other studies showed that seedlings became more scattered (Kjesson 1985; Horvitz and LeCorff 1993). These differences could also be due to differences in ant behavior (Hughes and Westoby 1992). However, most of these studies are based on direct observations of seeds transported by ants immediately after they drop to the ground and the actual origin of seedlings is difficult to identify (Horvitz and Schemske 1994). Therefore, further studies are required to better understand the impact of dispersal by ants on seedling spatial patterns.

The main genetic effect associated with seed dispersal by animals appears to change the degree of relatedness between conspecific seeds aggregated in space by altering the arrangement of seeds. This would then affect the spatial genetic

structure (SGS) of sequent seedlings (Hamrick et al. 1993). For myrmecochorous plants, a small-scale local genetic structure is traditionally hypothesized as being particularly common because gene movement via seed dispersal by ants is thought to be spatially restricted (Peakall and Beattie 1995; Pfeiffer et al. 2004). Kalisz et al. (1999) proposed that dispersal by ants could reduce the probability of aggregations of sibling seeds and might further affect the genetic relatedness of neighboring seedlings. Peakall and Beattie (1995) undertook a multisite study on the myrmecochore *Sclerolaena diacantha* using an enzyme technique. Their study showed that ants played an important role in generating the local SGS. However, the SGS in mature plants represents a culmination of ecological and evolutionary processes that act in both the past and present. Other than seed and pollen dispersal, many other factors such as mating system, local selection, and history events, etc. could also act on the formation and maintenance of the SGS within populations (Hedrick 1986; Kalisz et al. 2001; Vekemans and Hardy 2004). To what extent seed dispersal by ants can influence the genetic structure of a population is still poorly understood.

Genetic markers have been used to test the genetic consequences of seed dispersal (Schnabel et al. 1998; Degen et al. 2001; Vekemans and Hardy 2004; Hardesty et al. 2005). Of them, dominant markers with sufficient polymorphism are considered to be ideal when codominant markers are unavailable (Degen et al. 2001; Torres et al. 2003). Intersimple sequence repeats (ISSR) can produce a large number of polymorphic loci. They are also more stable and easy to produce than those with other dominant markers (Zietkiewicz et al. 1994; Godwin et al. 1997; Camacho and Liston 2001). Thus, we employed ISSR to detect the genetic structures related to seed dispersal in this study.

We investigated the sequential progress from seed dispersal by ants to the results of such dispersal on the local spatial pattern and SGS of seedlings. A representative myrmecochore *Globba lancangensis* was employed as a model system. Our detailed investigation included 1) intensive field observation on dispersal by ant species and the distance transported, 2) comparative studies of seedling spatial patterns between plots accessible to ants and plots in which ants were artificially excluded for 1 year, 3) investigations on the SGS of mature plants, and 4) explorations of the effects of ant-mediated seed dispersal on the SGS. The general aim of the study was to determine to what extent ant-mediated dispersal can affect the local spatial pattern and SGS in the studied population of *G. lancangensis*.

Materials and Methods

Description of Study Species and Site

Globba lancangensis Y. Y. Qian (Zingiberaceae) is a representative myrmecochorous species. It is a small perennial herb that only grows in the understory of evergreen broad-leaved deciduous trees in the Yunnan Province of southwest China (21°95'N–22°55'N, 99°55'E–100°5'E) (Qian 1994). The plant consists of a single stem with a height of about 60

cm and flowers from August to September. A previous experiment indicated it is a predominantly outcrossing species and the effective pollinator is the honeybee *Megapis dorsata* (Liu et al. 2004a). *G. lancangensis* propagates mainly by seeds. Each plant can produce 1–5 fruits, and each fruit usually contains about 30 olivary or spherical diaspores (2–3 mm in diameter). When a fruit ripens, the capsule opens and the diaspores quickly release and fall to the ground beneath the parent plant. The diaspores perform typical myrmecochory, bearing a white-color elaiosome on the seed top that can attract ants. The fallen diaspores are usually found in 1 min to 4 h by ants and moved away. The plants may also bear a small number of bulbils (mean \pm standard error [SE] = 2.24 ± 0.1 per plant, $n = 184$) (Liu et al. 2004b).

The study was conducted in a secondary forest in the Menghai County of the Xishuangbanna Prefecture, Yunnan Province, China (21°59'N, 100°16'E; altitude: 1180 m; annual average temperature: 18.3 °C; annual average rainfall: 1339 mm). The forest is 20–30 years old and covers an area of more than 20 ha. Several Fagaceae species, such as *Lithocarpus fobaiensis* and *Quercus acutissima*, are the dominant plants. In this forest, *G. lancangensis* is scatteredly distributed in open areas. Field observation indicates that the seedlings of *G. lancangensis* are not obviously subjective to herbivory by animals in this area.

Observations of Seed Dispersal

Fieldwork was conducted from 25 October to 1 November 2002 and from 20 October to 3 November 2003 in the study site. We observed seed handling by ants under natural conditions and experimentally studied removal of seeds. Ants approach toward the fallen seeds was observed and followed to determine how far they carry the seeds and where they drop them. Ant characteristic, forage strategy (single/work together to transport seeds) and distances transported were also recorded. Seed transport distances of different ant species were noted as beeline over ground. Each fruit was observed for 1–5 h. In only a few observed cases were seeds dropped to the ground before ants reached their nests; however, in some cases, we lost the foragers after several meters. If a seed was discarded by ants midway, we marked the seed and the location and rechecked whether the seed was at this location 24 h later. Visiting ants were collected with forceps and stored in vials with 75% alcohol for species identification.

Ant Exclusion and Sample Collection

In early October 2003, a woodland area of 70 \times 100 m was targeted and within this area eight 4 \times 4 m plots were selected for our study. These plots were good representatives of the density of study plants in this forest. Distances between plots varied from 6 to 100 m (Figure 1). Ant exclusion treatment was then conducted in 4 of the 8 plots. First, ant pesticides were used to kill all ants within the plots. Steel nets (100 eyelets/cm²) were then set up to prevent any ants from entering the plots (0.6 m underground and 0.4 m above-ground). A petroleum butter line was painted along the upper

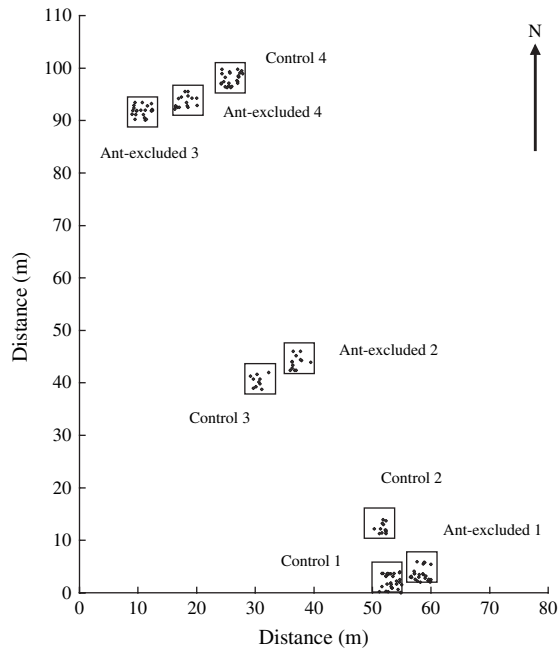


Figure 1. Distribution of the 8 studied plots for control and ant-excluded treatments. The black dots in each plot are portrayed based on the locations of 155 sampled individuals of *Globba lancangensis* before ant exclusion in 2003.

edge of the net to prevent ants from climbing into the plots. Continuous rechecking over the next 8 months was conducted to ensure no live ants inside the plots.

For the samples used for spatial pattern analysis and ISSR, all mature individuals in the 8 plots in 2003 were collected before ant exclusion treatment, and a total of 155 samples were obtained. In 2004, a total of 2410 seedlings were recorded in the 8 plots and from each plot 20 seedlings were randomly sampled (Table 1). Fresh leaves of all 155 samples from 2003 and 160 samples from 2004 were collected and dried with silica gel and stored in ziplocked plastic bags until required for DNA isolation.

ISSR Analyses

Genomic DNA was extracted using a modified cetyltrimethylammonium bromide method of Doyle JJ and Doyle JH (1990). After comparing the effects of concentrations of magnesium, the template DNA, and the annealing temperature, 14 ISSR primers (UBC primer set No. 9, Biotechnology Laboratory, University of British Columbia, British Columbia, Canada) that produced bright and reproducible bands were used to further amplify all samples (Table 2). Polymerase chain reaction (PCR) amplification was undertaken in a total reaction volume of 20 µl containing 2 µl of 10× PCR buffer (100 mM Tris-HCl [pH 8.3], 500 mM KCl, 15 mM MgCl₂), 100 µM dNTP, 0.375 µM primer, 50 ng DNA template, and 1.5 U Taq polymerase (TaKaRa Biotech Inc., Dalian, China). The reaction was performed in an Eppendorf master cyclor and commenced with an initial denaturation

Table 1. Total number of all mature individuals collected in 2003 and all seedlings collected in 2004 from the 4 control plots (ant accessible) and 4 ant-excluded plots

Plot	Year 2003	Year 2004
Control 1	31	514
Control 2	12	241
Control 3	24	294
Control 4	10	171
Ant excluded 1	26	317
Ant excluded 2	13	246
Ant excluded 3	23	316
Ant excluded 4	16	311

step at 95 °C for 3 min, followed by 35 cycles of 25 s at 94 °C, 30 s at annealing temperature (50 or 52 °C, Table 2), 1.5 min at 72 °C, and a final 7 min extension at 72 °C. Amplification products were resolved on 1.5% agarose gels stained with ethidium bromide. After electrophoresis, the gels were visualized and photographed using the GeneSnap Bio Imaging System (Syngene, Frederick, MD). Molecular weights were estimated using a 100-bp DNA ladder (Sangon Inc., Shanghai, China). ISSR bands were scored as present (1) or absent (0) for each DNA sample and presented in a binary data matrix.

Data Analyses

Data on dispersal by ants from field observations were collected and analyzed, and patterns of seed dispersal were shown as computer-generated graphs.

Nearest neighbor analysis was made under Point Pattern Analysis program (Chen and Getis 1998) to analyze spatial patterns of seedlings and mature plants in the end of 2004 when all field and laboratory experiments were finished.

The mean nearest neighbor distance was calculated by averaging the distances between each point to its closest point:

$$\bar{d} = \left(\sum_{i=1}^N d_i \right) / N,$$

where *N* is the number of points and *d_i* is the nearest neighbor distance for point *i*. The expected value of the nearest neighbor distance in a random pattern:

$$E(d_i) = 0.5\sqrt{(A/N)} + \left(0.0514 + \frac{0.041}{\sqrt{N}} \right) \times B/N,$$

where *A* is the area and *B* is the length of the perimeter of the study area. The variance:

$$\text{Var}(d) = 0.070A/N^2 + 0.037B\sqrt{(A/N^5)}.$$

Z-statistic (standard normal variate):

$$Z = (d - E(d)) / \text{Var}(d).$$

Z-statistic can be used to reflect the tendencies of spatial pattern toward either clustering or regularity. A negative *Z* score indicates clustering, and a positive score means dispersion or evenness. The higher the absolute *Z*-value is, the more clustering or dispersion the seedling pattern is.

Table 2. Fourteen ISSR primers used for amplification of all collected samples in 2003 and 2004. Those primers were screened from all the 96 primers of UBC primer set No. 9 after preliminary amplification

Primer no.	Sequence	Annealing temperature (°C)
807	(AG) ₈ T	50
808	(AG) ₈ C	52
810	(GA) ₈ T	50
824	(TC) ₈ G	50
827	(AC) ₈ G	50
835	(AG) ₈ YC	50
840	(GA) ₈ YT	50
855	(AC) ₈ YT	52
857	(AC) ₈ YG	52
886	V DV (TC) ₇	52
887	D VD (TC) ₇	52
888	B DB (CA) ₇	52
889	B DB (AC) ₇	52
890	V HV (GT) ₇	52

B = C/G/T, D = A/G/T, H = A/C/T, V = A/C/G, Y = C/T.

In order to avoid possible bias due to differences in sampling methods over 2 years as described in sample collection section, we did not make comparison between years for each type of treatments. Instead, we compared the nearest neighbor distances and the *Z* scores between different treatments in 2003 and 2004, respectively, by 2-tailed *t*-test with SPSS11.5 software.

We used the analysis of molecular variance software (Excoffier 1993) to examine the genetic variation of ISSR bands obtained. On the basis of the binary data matrix, a matrix of Euclidean genetic distance was produced using the DCFA program (Zhang and Ge 2002).

A matrix of pairwise geographical distances of all samples was calculated from their geographical coordinates and then grouped according to distance class. For the distance class matrix, we divided the pairwise geographical distance of all samples into 15 distance classes as <0.1, 0.1–0.5, 0.5–1, 1–2, 2–3, 3–4, 4–8, 8–12, 12–20, 20–30, 30–40, 40–50, 50–60, 60–80, and 80–105 m.

Spatial autocorrelation analysis was undertaken with Mantel correlogram analyses from the PASSAGE program (Rosenberg 2001) in the end of 2004 when all field and laboratory experiments were finished. The variate Mantel correlogram is a modification of the Mantel test (Oden and Sokal 1986). The decay in genetic similarity associated with increasing spatial distance between individuals was illustrated by testing and plotting spatial autocorrelation at above distance intervals. In this case, the autocorrelation coefficient (Mantel's r) is similar to the commonly used Moran's *I*, and has a mean of 0 when there is no correlation, and is bounded by -1 and $+1$ (Smouse and Peakall 1999). For each analysis, we used 1000 permutations to estimate the 2-tailed 95% confidence intervals around zero and the bootstrap test was used to provide the 95% confidence intervals for the autocorrelation estimate for each distance class. Thus, the extent of SGS can be defined by a decrease in the correlation coefficient until a distance where it stabilizes and shows no further trend (Hardy et al. 2004; Jones et al. 2007).

Results

Seed Dispersal by Ants

Totally, the ant dispersal of 216 fallen seeds were observed and recorded in 2002 and 254 seeds in 2003, respectively. The data from the 2 observation years showed that 93% of the fallen seeds were transported into ant nests and the rest were discarded or lost midway. Some seeds without an elaiosome did not attract ants, which together with those seeds discarded by ants were usually eaten by rodents or herbivores.

A total of 14 ant species from 12 genera and 4 subfamilies visited the seeds of *G. lancangensis* at the study site in 2002 and 2003. Of these, 12 species were effective dispersers. No obvious differences in visiting frequency were found over the 2 years (2-tailed *t*-test, $P \geq 0.05$). Of the 12 ant species, *Odontoponera transversa* (Smith), *Pachycondyla luteipes* (Mayr), *Pheidole watsoni* (Forel), and *Pheidole capellini* (Emery) were the most abundant species and dispersed the majority of the fallen seeds. They contributed 71.8 and 90.2% of dispersed seeds in 2002 and 2003, respectively.

Different ant species showed different forage behavior. For the 4 most important species, *O. transversa* is large in body size (approximately 1 cm in body length), and they usually transport a seed solely. *Pheidole watsoni* is tiny (about 2 mm in body length), and for them often 2–3 individuals work together to transport a diaspore, and need much time to finish transportation. *Pheidole luteipe* and *P. capellini* have medium body size (3–4 mm in body length), and they work solely and move seeds fast.

The mean distance of seeds transported by ants was 0.47 ± 0.03 m (mean \pm SE, range 0.01–3.35 m, $n = 470$). Most seeds (22%) were moved 0.1–0.2 m by the ants. Nevertheless, some seeds were dispersed more than 1 m away from their parent plants, and consequently a long tail was found (Figure 2A,B).

Results of Spatial Pattern Analysis

The mean nearest neighbor distance of ant-excluded plots was not significantly different from that of control plots (2-tailed *t*-test, $P \geq 0.05$) (Figure 3A). There was also no significant difference between the 2 types of treatments, although 4 plots were ant excluded for 1 year ($P \geq 0.05$). This indicated that ant exclusion did not greatly affect the distances of neighboring individuals. In the first year, the mean *Z*-values were positive in the 4 control plots and the 4 plots excluding ants, and they were not significantly different (2-tailed *t*-test, $P \geq 0.05$, Figure 3B). This indicated a dispersion or evenness pattern in those plots in natural conditions. In the second year, the mean *Z*-value was also positive for the control plots, while for the plots excluding ants for 1 year, the mean *Z*-value was greatly changed to negative (2-tailed *t*-test, $P \leq 0.05$). This suggested that the seedlings were more clustered when there was no ant dispersal.

Results of Spatial Genetic Structure Analyses

From the 155 mature plants sampled in 2003, 14 ISSR primers (Table 2) completely produced 86 reproducible and

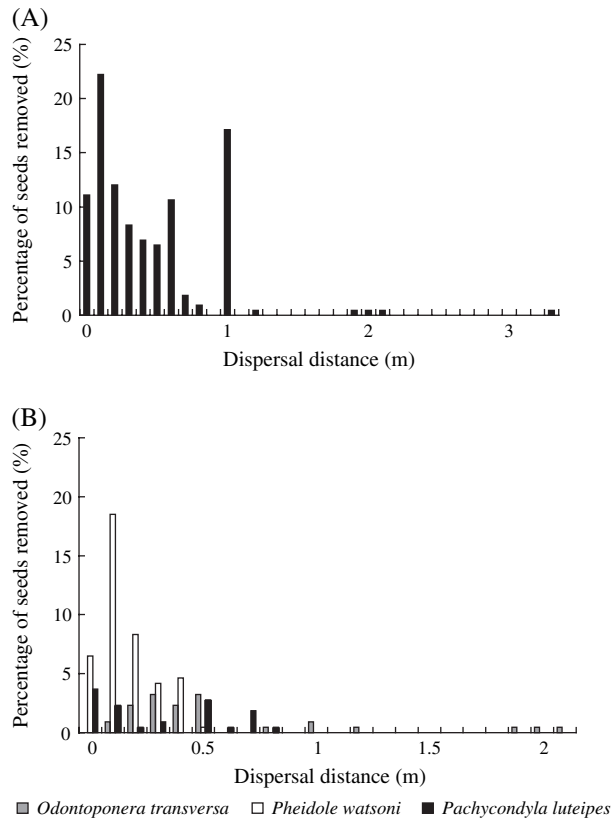


Figure 2. Percentage and dispersal distance of seeds removed by (A) all ants and (B) 3 most important species.

clear amplification fragments ranging in size from 290 to 2300 bp. Of the 86 bands, 57 were polymorphic (Percentage of polymorphic bands (PPB) = 66.28%). We obtained 89 polymorphic bands from 108 loci from 160 seedlings selected in 2004 (PPB = 82.41%). The data showed that a lot of genetic variation existed in the studied population.

For the detection of SGS of the whole population, the correlogram of all 155 mature individuals collected from 2003 showed significant positive values of Mantel's r ($P \leq 0.01$) over all 6 distance classes within 4 m (Figure 4), indicating there were significant positive correlations between genetic and geographic distances at those scales. The highest r value was 0.099 at 1.0–2.0 m distance class. Another significant positive correlation (Mantel's $r = 0.059$, $P \leq 0.01$) occurred at 8–12 m class. Beyond this, values of Mantel's r were not different from 0 to negative values, indicating the neighboring individuals were more genetically distant than random individuals.

Because ant dispersal is very limited (<4 m) and could only have an effect at such a local scale, the comparative analysis of mantel correlograms for different treatments just focuses on the 6 distance classes <4 m. As a result, for the control plots, the correlograms were not obviously different between the 2 years, and significant positive correlations between genetic and geographic distances were found for most distance classes within 4 m in both years (Figure 5). For ant-excluded plots before treatment, Mantel's r values tended to

be negative within 4 m although the r values were not significantly different from 0 at class 1 and class 2. This suggested the neighboring individuals were more genetically distant than random individuals. However, this pattern was greatly changed after ant-exclusion treatment for 1 year. Positive values of Mantel's r appeared at all distance classes within 4 m. This indicated the neighboring individuals were genetically closer than random individuals (Figure 5).

Discussion

In our study, seed dispersal distance by ants was short, with an average of 0.47 m ($n = 470$, range 0.01–3.5 m). This dispersal distance was a bit shorter than that of the *Globba* spp. (1.12 m, $n = 132$, range 0.04–8 m) reported by Pfeiffer et al. (2004) and the global mean distance of myrmecochorous dispersal (0.96 m, $n = 2524$, range = 0.01–77 m) (Gómez and Espadaler 1998). Although the largest proportion of seeds were transported only a very short distance (0.1–0.2 m), it was obvious that seeds were moved away from the parent plants and that the degree of seed aggregation was reduced. This result supports the conclusions of some studies (Kjesson 1985; Horvitz and LeCorff 1993).

As previous studies indicated, effects of seed dispersal were mainly ascribed to the size and behavior of the main transporting ant species (Hughes and Westoby 1992; Ness et al. 2004). For *G. lancangensis*, the large Ponerinae species *O. transversa* and *P. luteipes* are the 2 most important dispersers. They usually search for food individually and take seeds directly to their nests. Often they transport seeds a long distance from the parent plants (>2 m) than other ant dispersers and seem to significantly promote seed dispersal (Figure 2B). However, for some small ant species (e.g., *P. watsoni*), often several individuals work together to carry one seed. The distances that seeds were transported by these small ants were usually short (<0.6 m) (Figure 2B). In this case, the several main large ant species play a major role in producing postdispersal seed patterns and further influence the distribution of seedlings.

After 1 year treatment, the comparative analysis showed that ants could not significantly change the nearest neighbor distances of seedlings, but affected the clustering degrees. This result is consistent with many previous studies showing that ants promoted a decrease in seedling aggregation (Kjesson 1985; Horvitz and LeCorff 1993; Horvitz and Schemske 1994). However, the changes of seedling spatial pattern were limited due to the reality of restricted dispersal distance.

The overall spatial pattern of genetic diversity is not random, and there is a local SGS in this population. In the correlogram for all mature plants from 2003, the significant positive correlations between genetic and spatial distances at short distance classes (<4 m) probably reflect the occurrence of patches of genetically similar individuals (Sokal and Oden 1978; Torres et al. 2003). The average patch size can be estimated by the distance at which the values of Mantel's r becomes insignificant (Sokal 1979). Thus, for *G. lancangensis*, the patch size is about 4 m that is consistent with the distribution pattern of *G. lancangensis* found in field. This is likely

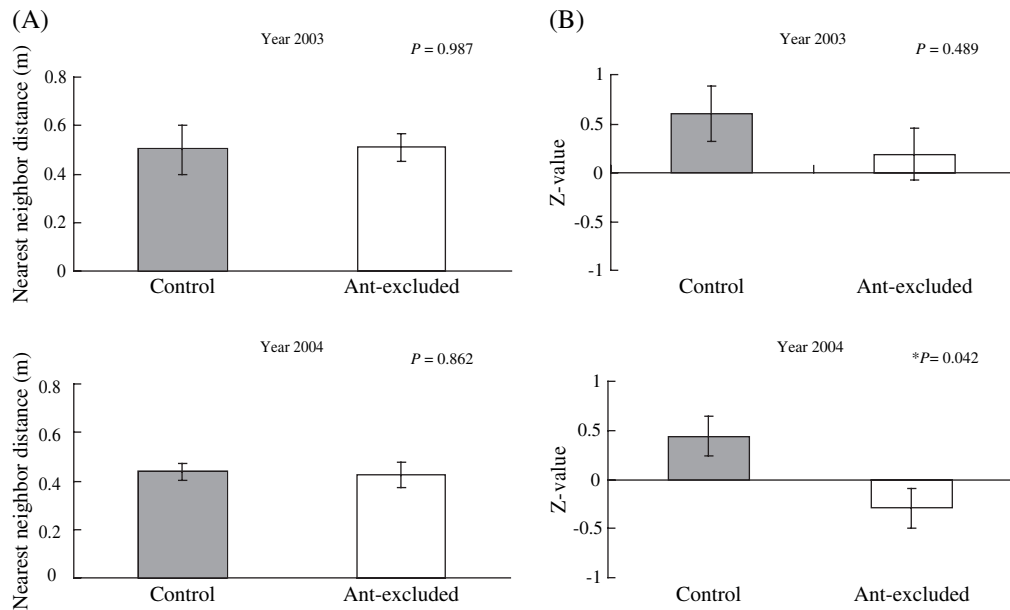


Figure 3. Comparative study of nearest neighbor analysis between control (ant accessible) and ant-excluded treatment before (year 2003) and after ant exclusion (year 2004). **(A)** Comparison of nearest neighbor distances (mean \pm SE, $n = 4$). **(B)** Comparison of Z-values (mean \pm SE, $n = 4$). Two-tailed t -test was used, and * P represents significant difference.

associated with limited seed dispersal distance. At distance interval 4–12 m, significant positive correlation was often found, which could result from the pollen flow by honeybees. Beyond this, the insignificant or significant negative autocorrelation suggested there was overlap of seedling shadows (due to occasional long-distance seed dispersal by rodents or rain) or other “families” were encountered.

The comparative analysis focused on the distance classes <4 m to check the effects of ant dispersal and found ant dispersal played an important role in maintaining the SGS within 4 m. For controls, significant correlation between genetic and geographical distances occurred within 4 m in both years, and there was no distinctive change between the 2 years. The conclusion is supported by our field observations that their seeds were intensively transported by ants over distances of less than 3.35 m. For ant-excluded treatments in the first year, the negative correlation in most distance classes may suggest a serious aggregation of different single plant progenies. It likely results from the collective transportation of ant and proves that ant can reduce the relatedness of neighboring individuals (Kalisz et al. 1999). After ant exclusion for 1 year, positive correlation between genetic and geographical distances appeared at all short distance classes. The change is a result of lacking ants as the primary dispersers and uncertain dispersal. However, the pattern after ant exclusion is similar to that of controls. This suggests that ant-mediated seed dispersal may contribute to the developing and maintaining the fine-scale SGS, but it cannot act as a determinant but just a minor factor.

Breeding systems and pollinator behavior can also affect the magnitude and scale of genetic structures (Kevin et al. 2004). *Globba lancangensis* is a highly outcrossing species,

and the effective pollinator is *M. dorsata* (Liu et al. 2004a). Pollinators often successively visit most flowers of several nearby individuals and then fly to another cluster of individuals about 10 m apart (Zhou H, Chen J, Chen F, personal observation). This type of pollination in the outcrossing *G. lancangensis* could result in the development of local genetic structures and increased homozygosity (Turner et al. 1982). In the SGS analysis for mature plants, significant positive r values were found at 8–12 m distance class. This pattern could be a reflection of gene movement via pollen migration by the pollinators. As such, dissection of the relative

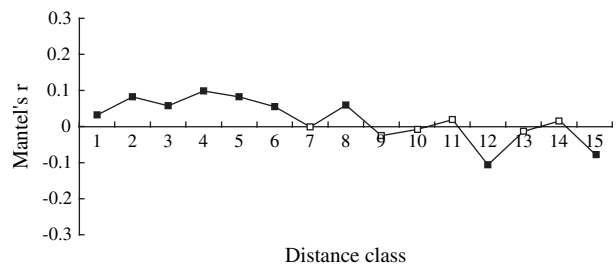


Figure 4. SGS of the whole studied *Globba lancangensis* population reflected from Mantel correlogram of all samples from 2003. Autocorrelation analysis between genetic and geographical distances was made at 15 distance classes: <0.1, 0.1–0.5, 0.5–1, 1–2, 2–3, 3–4, 4–8, 8–12, 12–20, 20–30, 30–40, 40–50, 50–60, 60–80, and 80–105 m. Black and blank squares, respectively, represent significant ($P \leq 0.05$) and insignificant ($P \geq 0.05$) correlation coefficient (Mantel's r). Each r was tested with a 1000 permutation test.

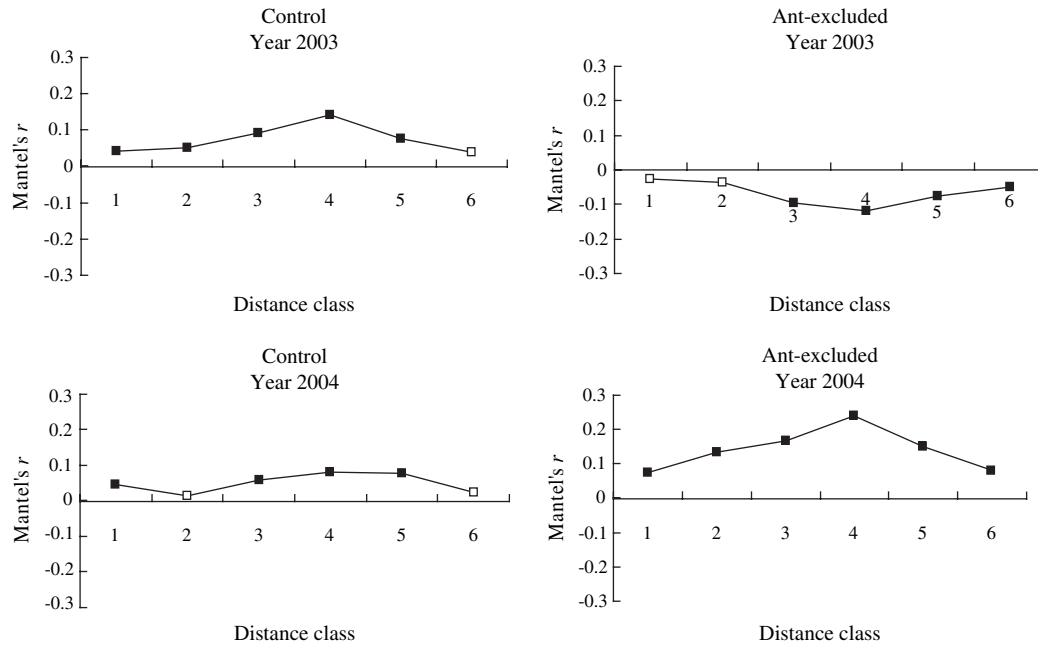


Figure 5. Comparative analysis of fine-scale Mantel correlograms of control and ant-excluded treatment between year 2003 (before ant exclusion) and year 2004 (after ant exclusion). The fine-scale Mantel correlograms were produced at 6 short distance classes: <0.1, 0.1–0.5, 0.5–1, 1–2, 2–3, and 3–4 m. Black and blank squares, respectively, represent significant ($P \leq 0.05$) and insignificant ($P \geq 0.05$) correlation coefficient (Mantel's r). Each r was tested with a 1000 permutation test.

contribution of seed and pollen dispersal to the SGS in future studies may aid in understanding the generative mechanism of the current genetic structure.

Globba lancangensis is propagated predominately by seeds and infrequently by bulbils. Stehlik and Holderegger (2000) indicated that clonal reproduction may influence the SGS. According to field observations, the mean number of bulbils produced per inflorescence is quite small (mean \pm SE = 2.24 ± 0.1 , $n = 184$) (Liu et al. 2004b). Moreover, of the 155 mature individuals sampled in 2003, our ISSR data indicated that only 2 shared the same genotype. This suggested that asexual propagation was not common in the natural population. Thus, the small number of bulbils was considered unable to greatly impact the SGS.

The accuracy of SGS assessment also depends on genetic markers and sample design. In this study, the sufficient number of polymorphic bands obtained demonstrated that ISSR is effective in detecting genetic structure. We also maximized the range of spatial scales encompassed by the samples to best characterize the SGS. Although the study in 2003 was based on a relatively modest sample size (155 samples), the sampling scheme was designed to maximize the range of spatial scales within one larger plot (70×100 m). In this fashion, the samples ensured relatively detailed accounts at the fine scale while not missing data at a larger scale. Although some gaps could not be avoided (approximately at distances around 30 m and 65–85 m; Figure 1), they did not affect study conclusions because they appeared well beyond the extent of the statistically significant SGS.

Globba lancangensis is a relatively rare and narrowly distributed species (Qian 1994, and also authors' field observation).

Studies on its seed dispersal and the SGS may help to enhance our knowledge that is suggestive to any conservation strategy development on this plant species.

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References

- Bacles CFE, Lowe AJ, Ennos RA. 2006. Effective seed dispersal across a fragmented landscape. *Science*. 311:628.
- Beattie AJ. 1985. The evolutionary ecology of ant-plant mutualisms. Cambridge (MA): Cambridge University Press.
- Beattie AJ, Culver DC. 1979. Neighborhood size in *Viola*. *Evolution*. 33:1226–1229.
- Beattie AJ, Hughes L. 2002. Ant-plant interactions. In: Herrera CM, Pellmyr O, editors. *Plant-animal interactions*. London: Blackwell Science Ltd. p. 223–235.

- Camacho FJ, Liston A. 2001. Population structure and genetic diversity of *Botrychium pumicola* (Ophioglossaceae) based on inter-simple sequence repeat (ISSR). *Am J Bot.* 88:1065–1070.
- Chen DM, Getis A. 1998. Point Pattern Analysis (PPA), Software package and documentation. Department of Geography, San Diego State University.
- Degen B, Caron H, Bandou E, Maggia L, Chevallier MH, Leveau A, Kremer A. 2001. Fine-scale spatial genetic structure of eight tropical tree species as analysed by RAPDs. *Heredity.* 87:497–507.
- Doyle JJ, Doyle JH. 1990. Isolation of plant DNA from fresh tissue. *Focus.* 12:13–15.
- Excoffier L. 1993. Analysis of molecular variance (AMOVA) version 1.55. Geneva (Switzerland): Genetics and Biometry Laboratory, University of Geneva.
- Gibson W. 1993a. Selective advantages to hemi-parasitic annuals, genus *Melampyrum*, of a seed-dispersal mutualism involving ants: II. Seed-predator avoidance. *Oikos.* 67:345–350.
- Gibson W. 1993b. Selective advantages to hemi-parasitic annuals, genus *Melampyrum*, of a seed-dispersal mutualism involving ants: I. Favorable nest sites. *Oikos.* 67:334–344.
- Godwin ID, Aitken EAB, Smith LW. 1997. Application of inter-simple sequence repeat (ISSR) markers to plant genetics. *Electrophoresis.* 18:1524–1528.
- Gómez C, Espadaler X. 1998. Myrmecochorous dispersal distance: a world survey. *J Biogeogr.* 25:573–580.
- Gorb SN, Gorb EV. 1995. Removal rates of seeds of five myrmecochorous plants by the ant *Formica polyctena* (Hymenoptera: Formicidae). *Oikos.* 73:367–374.
- Hamrick JL, Murawski DA, Nason JD. 1993. The influence of dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetatio.* 107/108:281–297.
- Hardesty BD, Dick CW, Kremer A, Hubbell S, Bermingham E. 2005. Spatial genetic structure of *Simarouba amara* Aubl. (Simaroubaceae), a dioecious, animal-dispersed Neotropical tree, on Barro Colorado Island, Panama. *Heredity.* 95:290–297.
- Hardy OJ, Gonzalez-Martinez SC, Colas B, Freville H, Mignot A, Olivieri I. 2004. Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae). II. Correlated paternity within and among sibships. *Genetics.* 168:1601–1614.
- Hedrick PW. 1986. Genetic polymorphism in heterogeneous environments: a decade later. *Annu Rev Ecol Syst.* 17:535–566.
- Horvitz CC, LeCorff J. 1993. Spatial scale and dispersion pattern of ant- and bird-dispersal herbs in two tropical lowland rain forests. *Vegetatio.* 107/108:351–362.
- Horvitz CC, Schemske DW. 1986. Seed dispersal of a neotropical myrmecochore: variation in removal rates and seed dispersal distance. *Biotropica.* 18:319–323.
- Horvitz CC, Schemske DW. 1994. Effects of dispersers, gaps, and predators on dormancy and seedling emergence in a tropical herb. *Ecology.* 75:1949–1958.
- Houle G. 1992. Spatial relationship between seed and seedling abundance and mortality in a deciduous forest of north-eastern North America. *J Ecol.* 80:99–108.
- Howe HF, Smallwood J. 1982. Ecology of seed dispersal. *Annu Rev Ecol Syst.* 13:201–228.
- Hughes L, Westoby M. 1992. Fates of seeds adapted for dispersal by ants in *Australian sclerophyll* vegetation. *Ecology.* 73:1300–1312.
- Jones TH, Vaillancourt RE, Potts BM. 2007. Detection and visualization of spatial genetic structure in continuous *Eucalyptus globulus* forest. *Mol Ecol.* 16:697–707.
- Kalisz S, Hanzawa FM, Tonsol SJ, Thiede DA, Voigt S. 1999. Ant-mediated seed dispersal alters pattern of relatedness in a population of *Trillium grandiflorum*. *Ecology.* 80:2620–2634.
- Kalisz S, Nason JD, Hanzawa FM, Tonsol SJ. 2001. Spatial population genetic structure in *Trillium grandiflorum*: the roles of dispersal, mating history and selection. *Evolution.* 55:1560–1568.
- Kjesson G. 1985. Seed fates in a population of *Carex piluifera* L. I. Seed dispersal and ant-seed mutualism. *Oecologia.* 67:416–423.
- Liu ZQ, Chen J, Bai ZL. 2004a. Reproductive characteristics of *Globba lancangensis* and their evolutionary implications. *Acta Phytoecol Sin.* 28:1–8.
- Liu ZQ, Chen J, Bai ZL. 2004b. Comparative studies on reproductive mechanisms of three species in *Globba* (Zingiberaceae). *J Wuhan Bot Res.* 22:145–152.
- Muller-Landau HC, Hardesty BD. 2005. Seed dispersal of woody plants in tropical forests: concepts, examples and future directions. In: Burslem DFRP, Pinard MA, Hartley SE, editors. *Biotic interactions in the tropics: their role in the maintenance of species diversity.* Cambridge (MA): Cambridge University Press. p. 267–309.
- Nathan R. 2006. Long-distance dispersal of plant. *Science.* 313:786.
- Ness JH, Bronstein JL, Andersen AN, Holland JN. 2004. Ant body size predicts dispersal distance of ant-adapted seeds: implications of small-ant invasions. *Ecology.* 85:1244–1250.
- Ng KKS, Lee SL, Koh CL. 2004. Spatial structure and genetic diversity of two tropical tree species with contrasting breeding systems and different ploidy levels. *Mol Ecol.* 13:657–669.
- Oden NL, Sokal RR. 1986. Directional autocorrelation: an extension of spatial correlograms to two dimensions. *Syst Zool.* 35:608–617.
- Peakall R, Beattie AJ. 1995. Does ant dispersal in *Scelerolaena diacantha* (Chenopodiaceae) generate local spatial genetic structure? *Heredity.* 75:351–361.
- Pfeiffer M, Nais J, Linsenmair KE. 2004. Myrmecochory in the Zingiberaceae: seed removal of *Globba franciscii* and *G. propinqua* by ants (Hymenoptera: Formicidae) in rainforests on Borneo. *J Trop Ecol.* 20:705–708.
- Qian YY. 1994. A new species of *Globba*. *Acta Bot Sin.* 9:51–52.
- Rosenberg MS. 2001. PASSAGE. Pattern analysis, spatial statistics, and geographic exegesis. Tempe (AZ): Arizona State University.
- Schnabel A, Nason JD, Hamrick JL. 1998. Understanding the population genetic structure of *Gleditsia triacanthos* L.: seed dispersal and variation in female reproductive success. *Mol Ecol.* 7:819–832.
- Smouse PE, Peakall R. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity.* 82:561–573.
- Sokal RR. 1979. Ecological parameters inferred from spatial correlograms. In: Patil GP, Rosenzweig ML, editors. *Contemporary quantitative ecology and related ecometrics.* Fairland (MD): International Cooperative Publishing House. p. 167–196.
- Sokal RR, Oden NL. 1978. Spatial autocorrelation in biology. 1. Methodology. *Biol J Linn Soc Lond.* 10:199–249.
- Stehlik I, Holderegger R. 2000. Spatial genetic structure and clonal diversity of *Anemone nemorosa* in late successional deciduous woodlands of Center Europe. *J Ecol.* 88:424–435.
- Torres E, Iriondo JM, Escudero A, Perez C. 2003. Analysis of within-population spatial genetic structure in *Antirrhinum microphyllum* (Scrophulariaceae). *Am J Bot.* 90:1688–1695.
- Turner ME, Stephens JC, Anderson WW. 1982. Homozygosity and patch structure in plant populations as a result of nearest-neighbor pollination. *Proc Natl Acad Sci USA.* 79:203–207.
- Vekemans X, Hardy OJ. 2004. New insights from fine-scale spatial genetic structure analysis in plant populations. *Mol Ecol.* 13:921–935.
- Zhang FM, Ge S. 2002. Data analysis in population genetics. I. Analysis of RAPD data with amova. *Biodivers Sci.* 10:438–444.
- Zietkiewicz E, Rafalski A, Labuda D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics.* 20:176–183.

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