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Species diversity and population density affect genetic structure and gene dispersal in a subtropical understory shrub

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Abstract

Aims

The dispersal of pollen and seeds is spatially restricted and may vary among plant populations because of varying biotic interactions, population histories or abiotic conditions. Because gene dispersal is spatially restricted, it will eventually result in the development of spatial genetic structure (SGS), which in turn can allow insights into gene dispersal processes. Here, we assessed the effect of habitat characteristics like population density and community structure on small-scale SGS and estimate historical gene dispersal at different spatial scales.

Methods

In a set of 12 populations of the subtropical understory shrub *Ardisia crenata*, we assessed genetic variation at 7 microsatellite loci within and among populations. We investigated small-scale genetic structure with spatial genetic autocorrelation statistics and heterogeneity tests and estimated gene dispersal distances based on population differentiation and on within-population SGS. SGS was related to habitat characteristics by multiple regression.

Important Findings

The populations showed high genetic diversity (H_e = 0.64) within populations and rather strong genetic differentiation (F'_{ST} = 0.208) among populations, following an isolation-by-distance pattern, which suggests that populations are in gene flow–drift equilibrium. Significant SGS was present within populations (mean Sp = 0.027). Population density and species diversity had a joint effect on SGS with low population density and high species diversity leading to stronger small-scale SGS. Estimates of historical gene dispersal from between-population differentiation and from within-population SGS resulted in similar values between 4.8 and 22.9 m. The results indicate that local-ranged pollen dispersal and inefficient long-distance seed dispersal, both affected by population density and species diversity, contributed to the genetic population structure of the species. We suggest that SGS in shrubs is more similar to that of herbs than to trees and that in communities with high species diversity gene flow is more restricted than at low species diversity. This may represent a process that retards the development of a positive species diversity–genetic diversity relationship.

Keywords: *Ardisia crenata* • BEF China • gene flow • habitat effects • spatial genetic structure

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INTRODUCTION

The spatial distribution of genetic variability within natural plant populations is related to both, evolutionary and ecological processes (Epperson 1989; Lewontin 1974; Wright 1978). Within homogeneous plant populations, spatial genetic structure (SGS) develops due to spatially restricted gene dispersal and is mainly determined by the interplay between gene flow by seeds and pollen (Heywood 1991; Van Rossum and Triest 2006). Therefore, species traits like the mating system (Van

Rossum and Triest 2007), pollination syndrome, and propagule dispersal mechanisms (Hardy *et al.* 2006; Heuertz *et al.* 2003) are expected to strongly affect SGS. Consequently, plant species with discrete seed shadows and/or low pollen dispersal distance, e.g. because of high selfing rates, should develop a pronounced SGS while plant species with overlapping seed shadows and/or high pollen dispersal distance should show a more homogenous distribution of genetic diversity within populations (Hamrick *et al.* 1993; Hamrick and Nason 1996).

For a given plant species, SGS may be affected by processes like biotic interactions, population history or abiotic conditions. Among biotic interactions, the density of co-occurring individuals may indirectly affect SGS by its effect on outcrossing rates and patterns of pollen dispersal (Born *et al.* 2008; Hanson *et al.* 2008; Ward *et al.* 2005). However, density might shape SGS differently for species depending on their pollen vectors. In wind-pollinated species, pollen flow among individuals in low-density populations is likely to be reduced, and hence, SGS should be more pronounced compared to high-density populations (see e.g. Friedman and Barrett 2008). This pattern is not necessarily expected in species with a more directed pollen transport. For SGS in insect pollinated species, the number of co-flowering species might be more important than population density as it can lead to either competition for pollinators or facilitation with contrasting effects on pollen flow within species (Mitchell *et al.* 2009). Furthermore, in forests, tree density in general and canopy gaps can affect the strength of SGS as they affect light and microclimatic conditions (Bizoux *et al.* 2009) that determine maternal reproductive success and the clustering of siblings (Born *et al.* 2008). Also the behaviour of seed dispersal vectors may depend on tree density or on the abundance of fruits available leading to effects on efficiency and distance of seed dispersal (Hardesty *et al.* 2006).

In addition, population history can be imprinted in SGS. Along successional series, the balance between local population extinction and recolonization is expected to influence small-scale genetic structure (Troupin *et al.* 2006). Simulation studies predict that SGS will develop quickly under isolation-by-distance and that populations from late-successional stages have stronger SGS than recently established populations (Epperson 2005; Sokal and Wartenberg 1983). Moreover, an increase in generation overlap in late-successional stage may also increase the magnitude of SGS (Doligez *et al.* 1998). However, also a number of processes change during succession that may lead to a decrease of SGS over time, like increasing overlap of seed shadows (Hamrick and Nason 1996) through reduced spatial aggregation of reproductive individuals (Doligez *et al.* 1998).

Furthermore, abiotic conditions may indirectly affect genetic structure through demographic effects. Elevation gradients are supposed to affect genetic variation because environmental conditions and population density can change rapidly with altitude (Ohsawa and Ide 2008). Variations in the drift–gene flow balance along the altitudinal dimension of a species' distribution are expected to lead to altitudinal variation in genetic structuring and diversity of populations (Herrera and Bazaga 2008; Shi *et al.* 2011).

The scale at which SGS develops depends on the interaction between gene flow, genetic drift and selection (Ennos 2001; Gehring and Delph 1999). Genetic diversity can appear spatially structured at different scales, such as landscape, population or among neighbouring individuals due to different ecological and genetic factors operating in natural populations. Hence, the degree of genetic structure detected depends on the observed spatial scale but also on the methods used to find the spatial structure (Berg and Hamrick 1995; Chung *et al.* 2002; Clauss and Mitchell-Olds 2006). Therefore, genetic structure may be present but undetected at spatial scales smaller or larger than those investigated. Because a direct observation of current gene dispersal at the different scales is often more difficult than the assessment of an SGS, it has been used to estimate historical dispersal distances, i.e. dispersal distances that have led to current spatial genetic patterns (Rousset 1997; Vekemans and Hardy 2004). An independent assessment of gene flow based on the SGS at different spatial scales, e.g. within and among populations, may allow insight into scale-dependent processes (Fenster *et al.* 2003). Until now, little is known about how SGS varies among populations or at larger landscape-level spatial scales (Chung *et al.* 2002). Therefore, studies of SGS in multiple populations and at multiple spatial scales are in demand to assess the different drivers involved in shaping the spatial distribution of genetic diversity within single species.

With respect to growth forms, SGS has often been studied in trees and herbaceous species, while shrubs from the forest understory are less well studied. However, compared to trees and herbs, shrubs may have a different accessibility for pollinators and seed dispersers and may also show intermediate generation times, both with impact on SGS.

Subtropical evergreen broad-leaved forest is one of the most species rich forest types and also harbours many shrub species (Wu 1980). At the community level, it has been shown that in such forests the species composition is dispersal limited and driven by continuous immigration (Bruelheide *et al.* 2011). Thus, also for individual component species, the genetic population structure is likely to be affected by dispersal limitation and continuous immigration. *Ardisia crenata* (Myrsinaceae) is a widely distributed shrub in subtropical evergreen forest but found mostly in later successional stages (Bruelheide *et al.* 2011), which may be due to limited seed dispersal. We investigated genetic variation and structure of *A. crenata* at different scales and across different habitat characteristics and estimated gene flow distances. We investigated the relationship between biotic and abiotic habitat factors and small-scale genetic structure. We hypothesize that *A. crenata* has a strong SGS within and among populations because of limited seed dispersal; however, we expect differing within-population SGS because of varying habitat characteristics. Specifically we ask: (1) What is the level of genetic variation within and among populations?

(2) What is the level of gene dispersal assessed at the within-population and among-population scale? (3) Do environmental variables and community structure affect the level of small-scale genetic structure in different populations?

MATERIALS AND METHODS

Study species

Ardisia crenata Sims (Myrsinaceae) is a small evergreen shrub native in a range from Japan to India occurring in the understory of tropical and subtropical forests (Bailey 1925; Roh *et al.* 2006). The species is insect-pollinated and self-compatible like many congeners (Cheon *et al.* 2000; Pascarella 1997; Zhao *et al.* 2006). However, there is conflicting evidence with respect to the mating system (Chen *et al.* 2001; Cheon *et al.* 2000; Mu *et al.* 2010; Zhao *et al.* 2006). After ripening, its bright red berries remain on the plant throughout the year, providing a food source for seed dispersing birds and other wildlife. In the study region, seed eating birds like pheasants (*Lophura nycthemera*) are relatively rare, mainly ground active and have small ranges and thus may not provide efficient long distance dispersal. *Ardisia crenata* was introduced to the USA for ornamental purposes (Kitajima *et al.* 2006) and is regionally regarded as an invasive pest plant (Langeland and Craddock Burks 1998).

Study area and populations

The study was conducted in Gutianshan National Nature Reserve (GNNR), Kaihua county, Zhejiang province, China (29°10′19″N, 118°03′50″E). The reserve was set up in 1975 to preserve species-rich natural old-growth subtropical evergreen broad-leaved forest (Yu *et al.* 2001). GNNR covers a total area of 8 107 ha and is characterized by steeply sloping mountains up to 1 258 m a.s.l. Natural fires have been recorded in the Gutianshan region historically. Before 1975, the area was partly used by local farmers for agriculture, charcoal production and tapping for resin (Editorial Group of Forestry Chorography in Kaihua County 1988). Therefore, GNNR currently consists of a range of successional forest stages and density levels. Forests are particularly species rich in shrub and tree species but the herb layer is relatively sparse and species poor (Bruelheide *et al.* 2011).

Ardisia crenata is widely distributed in the study region below 1 000 m a.s.l. covering a wide range of environmental conditions ranging from dense intermediate forest to open late-successional forest, being very rare in young forest. We established 12 plots of 30 × 30 m (Table 1), which correspond to comparative study plots in the project 'Biodiversity and Ecosystem Functioning of China, BEF China' (Bruelheide *et al.* 2011). The plots covered the full altitudinal range of the species and different forest types. For each plot, we recorded successional stage and elevation, tree density (number of woody stems, >1 m height) as well as species diversity of all woody species (based on rarefaction analysis, Bruelheide *et al.* 2011). We mapped all *A. crenata* individuals inside the plots and determined the number of *A. crenata* individuals per plot as population density, which ranged from 29 to 136 individuals per plot. Leaf samples were randomly taken from 30 individuals per plot, dried at 40°C for 48 h and then kept dry in silica gel. For the analysis of outcrossing rate, a total of 181 seeds were collected from 24 maternal plants and were kept frozen prior to genetic analysis.

Genotyping

Genomic DNA was extracted from leaf samples using the DNeasy Plant minikit (QIAGEN). Seven highly polymorphic microsatellite loci were used for genotyping (Hong *et al.* 2008). To allow multiplexing, new primers were designed to adjust fragment length for the following loci: Ac52: F 5′-CCCCATCATAATTTCTGCATC-3′ (fragment length: 162–176 bp), Ac63: R 5′-TCCAAAAACGGAATAAGGTTG-3′ (209–285 bp) and Ac07: R 5′-TTGTGGGGAAGAGAGCAAAG-3′ (271–307 bp). We performed two multiplex polymerase chain reactions (PCRs) with primers for loci Ac27, Ac07, Ac49 and Ac53 in one reaction and Ac26, Ac52 and Ac63 in a second one. Multiplex PCR was performed using the Multiplex PCR Kit (QIAGEN) in a reaction volume of 10 µl following supplier instructions. Fragment analysis was performed on an ABI PRISM 3130 with GS600-LIZ (Applied Biosystems) as internal size standard and using GeneMapper v.3.7 software for genotyping.

Population genetic analysis

Preliminary analyses showed that the loci had different fixation indices, suggesting the presence of null alleles. Based on the high outcrossing rate observed (see below), we assumed populations to be in Hardy–Weinberg equilibrium and thus were able to test for the presence of null alleles with the Van Oosterout null allele estimator using Micro-Checker 2.2.3 (Van Oosterhout *et al.* 2004). At three loci (Ac53, Ac52, Ac63), null alleles were indicated and single-locus genotypes were manually revised by introducing an additional allele as suggested by MicroChecker. If not otherwise indicated, we used all loci for the regional-scale analyses and only the four loci without null alleles to calculate fixation indices and for the analyses of within-population SGS. We estimated the outcrossing rate based on the genotypes of maternal plants and their seeds. We regarded individual seeds as outcrossed when they had at least one allele not present in the maternal genotype. The minimum outcrossing rate was calculated as the proportion of outcrossed seeds per maternal plant. Genetic diversity within populations was characterized by allelic richness (A_r, i.e. number of alleles estimated with a rarefaction approach using a minimum sample size of 29 individuals), expected heterozygosity (H_e), number of rare alleles (total abundance < 5%, A_{r5}) and fixation index (F_{IS}). Genetic differentiation among all populations was estimated as F_{ST} (Weir and Cockerham 1984) and F'_{ST} (Hedrick 2005, equation 4b), a standardized measure of genetic differentiation. We used RecodeData (Meirmans 2006) to estimate F_{ST_max} necessary for standardization. To test for an isolation-by-distance pattern,

among-population, matrices of pairwise log geographical and genetic distances $(F_{ST}/(1-F_{ST}), F'_{ST}/(1-F'_{ST}))$ were correlated and the significance of the relationships was tested using a Mantel test with 1 000 randomizations. All these analyses were performed with Fstat v. 2.9.3.2 (Goudet 2001).

Within-population SGS was assessed by spatial autocorrelation methods using SPAGeDi v. 1.3 (Hardy and Vekemans 2002). We computed pairwise kinship coefficients (F_{ij}) (Loiselle *et al.* 1995) to construct spatial autocorrelograms. We used distance class limits of 2.5, 5, 10, 15, 20, 30 and 50 m in order to obtain a high spatial resolution and to assure a sufficient number of individual pairs per distance class. Significance of mean F_{ij} per class was tested with 1 000 permutations of multilocus genotypes. We used the *Sp* statistic (Vekemans and Hardy 2004) to quantify SGS for individual plots as $Sp = -b_{log}/(1 - F_{(1)})$, where b_{log} is the slope of the regression of kinship coefficient on log geographic distance and $F_{(1)}$ is the mean kinship coefficient between individuals of the first distance class.

Estimation of gene dispersal

Indirect estimates of gene dispersal were estimated from SGS both at regional scale and at population level by two closely related approaches.

(1) Rousset (1997) showed that for a stepping-stone model of population structuring in 2D space, the slope (b_{log}) of the regression of pairwise $F_{ST}/(1 - F_{ST})$ values between populations on log geographical distances is proportional to the quantity $4\pi D\sigma^2$, where σ^2 is the square of half the average parent–offspring distance, D the effective population density and b_{log} the regression slope (Rousset 1997). Hence, using the formula $4\pi D\sigma^2 = 1/b_{log}$ and a known effective population density, an indirect estimate of the gene dispersal σ can be obtained.
(2) For each plot and assuming a continuous distribution of genotypes, gene dispersal parameters can be similarly estimated from the *Sp* statistic, which is expected to equal $1/4\pi D\sigma^2$ in two-dimensional space in a restricted range of σ to 20σ (Heuertz *et al.* 2003; Vekemans and Hardy 2004).

Both approaches assume that the observed SGS is representative for an isolation-by-distance process at equilibrium and require an estimate of the effective population density D. Demographic studies suggest that D typically ranges between 0.1 and 0.5 of the census population density (Frankham 1995). For (2), we used SPAGeDi to calculate a lower and an upper range of σ. The program iteratively adjusts the spatial range of the autocorrelation analysis until σ converges using an estimate of D (Hardy *et al.* 2006). We manually adjusted levels of D at low (from 0.05 to 0.15 of census density) and high (0.45 to 0.6) density levels until convergence was achieved at values of 0.15 and 0.6, respectively.

Approximate confidence intervals (CIs) for estimates of σ from (1) and (2) were computed as ± 2 SE, where SE is the standard error of b_{log} estimated by jackknifing over loci (Fenster *et al.* 2003). For consistency, we used the four loci without null alleles for both analyses; however, analyses with the full data set provided very similar results.

Effects of habitat conditions on SGS

The effect of habitat conditions (population density, successional stage, woody density, species diversity and elevation) on within-population SGS, quantified by the *Sp* value, was assessed by a multiple linear regression approach. Population density was log10 transformed to obtain normally distributed errors. The full model included all interaction terms and was reduced stepwise by excluding the least significant terms until only significant terms remained. The analysis was performed within R v.2.9.2 (R Development Core Team 2009).

To compare SGS between different types of populations and to allow a more detailed analysis of possible differences, we pooled populations according to habitat conditions that remained significant in the model: low versus high species diversity (6 low populations/6 high populations) and low versus high population density (<50 or >50 individuals/plot). The pooled data sets were used to perform heterogeneity tests (Smouse *et al.* 2008) to determine whether the different types of populations exhibited heterogeneous autocorrelation patterns using the same distance classes as above. These tests were performed using GenAlEx v.6.3 (Peakall and Smouse 2006).

RESULTS

Genetic variation within populations

Across 359 samples and using 7 loci, we detected 82 alleles in total with 5–16 alleles among loci. The populations showed high gene diversity (H_e) that ranged from 0.59 to 0.67 with a mean of 0.64 (SD = 0.03) (Table 2). Allelic richness (A_r) ranged from 4.98 to 6.80 with a mean of 5.90 (SD = 0.63). Variation in allelic richness was largely due to the effect of rare alleles, as indicated by a strong positive correlation between number of rare alleles (A_{r5}) and A_r ($r = 0.963$, $P < 0.001$). Fixation indices (F_{IS}) obtained from loci without null alleles ranged from -0.11 to 0.21 and 6 out of 12 populations showed significant departure from Hardy–Weinberg equilibrium, mostly positive indicating lack of heterozygotes (Table 2). All individuals had unique multilocus genotypes, indicating that clonal propagation did not affect our results. Paternity analysis revealed a high minimum outcrossing rate (mean t_{min} = 0.93), indicating that the species is predominantly outcrossed.

Population differentiation

Overall, populations were significantly differentiated from each other (overall $F_{ST} = 0.046$, $\theta = 0.050$, $P < 0.01$). The standardized measure of differentiation after Hedrick (2005) was $F'_{ST} = 0.208$, indicating rather strong genetic differentiation. Population differentiation followed an isolation-by-distance model as we found a significantly positive relationship

Table 1: habitat parameters of study sites of *Ardisia crenata* in Gutianshan area

Population code	Latitude (°N)	Longitude (°E)	*Ardisia* population density (individuals/plot[a])	Elevation (m a.s.l.)	Successional stage[b]	Tree density (individuals/plot)	Woody species richness	Woody species diversity
CSP1	29.23957	118.11587	38	522	3	353	44	27.1
CSP2	29.24926	118.13484	47	590	5	476	69	35.1
CSP4	29.24963	118.12015	36	542	5	296	44	29.7
CSP6	29.25497	118.14747	29	880	3	656	39	22.3
CSP7	29.25184	118.14373	41	903	4	740	46	24.5
CSP10	29.25188	118.15791	53	670	4	358	41	27.9
CSP11	29.27383	118.13647	33	647	4	646	35	18.1
CSP14	29.24944	118.13518	104	639	5	385	38	24.2
CSP15	29.24917	118.13106	67	618	5	309	39	26.3
CSP18	29.24516	118.12461	32	569	3	543	52	28.6
CSP21	29.27059	118.08084	136	566	5	537	56	32.7
CSP27	29.24709	118.13605	47	665	5	414	46	25.5

[a] Plot size was 30 × 30 m.
[b] Successional stage: 3 = intermediate forest (<60 years); 4 = mature forest (<80 years); 5 = old forest (⩾80 years) from Bruelheide *et al.* (2011).

Table 2: genetic variation and small-scale genetic structure at 7 microsatellite loci in 12 populations of *Ardisia crenata*

Population	H_e	A_r	A_{r5}	F_{IS}	$F_{(1)}$	b_{log}	*Sp*
CSP1	0.59	5.97	16	0.12*	0.044	−0.014	0.014
CSP2	0.63	5.82	15	0.10	0.120**	−0.036**	0.040
CSP4	0.62	4.98	9	0.16**	0.201**	−0.042**	0.052
CSP6	0.67	5.29	12	0.09	0.032	−0.005	0.005
CSP7	0.67	6.40	19	0.03	0.114**	−0.023*	0.026
CSP10	0.65	6.26	20	0.10*	0.028	−0.033**	0.034
CSP11	0.62	5.39	13	−0.11*	0.057*	−0.019*	0.020
CSP14	0.66	6.80	22	0.10*	−0.044	0.014	−0.014
CSP15	0.64	6.67	20	0.21**	0.048	−0.016	0.017
CSP18	0.60	4.98	10	0.06	0.245**	−0.066**	0.087
CSP21	0.64	6.39	20	0.03	0.074	−0.022*	0.024
CSP27	0.67	5.82	15	0.02	0.070*	−0.019*	0.020
Average	0.64	5.90	16	0.07	0.082	−0.023	0.027

H_e, gene diversity; A_r, allelic richness; A_{r5}, number of rare alleles; F_{IS}, fixation index; $F_{(1)}$, kinship coefficient in the first distance class; b_{log}, regression slope of spatial genetic autocorrelation; *Sp*, statistic.
*$P < 0.05$, **$P < 0.01$.

between genetic and geographic distance over the 8-km study area (Fig. 1). This suggests that populations are in gene flow–drift equilibrium. Eight out of 12 study plots showed a significant SGS, with *Sp* values ranging from 0.020 to 0.087, with a mean *Sp* = 0.027 (Table 2).

Indirect estimates of gene flow

At the regional scale and based on pairwise $F_{ST}/(1 - F_{ST})$ values between populations, gene dispersal distance σ ranged from 10.3 m (CI: 8.7−13.3) to 20.6 m (CI: 17.4−26.7) using values of 0.15 and 0.6 for the ratio of effective to census population density, respectively. Based on standardized measures of differentiation, $F'_{ST}/(1 - F'_{ST})$, gene dispersal was smaller and σ ranged from 4.8 m (CI: 4.3–5.5) to 9.6 m (CI: 8.7–11.0). At the plot scale, based on the within-population SGS, σ was 12.8 m (CI: 10.1–13.3) and 22.9 m (CI: 20.1–26.6) for the two effective density estimates, respectively.

Effects of habitat conditions on SGS

After model simplification, only population density and species diversity remained with a significant effect on SGS among plots. The final model explained 53% of the variation in *Sp* values ($t = -2.547$, $P = 0.03$ and $t = 2.699$, $P = 0.02$, for population density and species diversity, respectively). Population density and species diversity were not correlated with each other ($P = 0.31$) and showed no significant effect on *Sp* in univariate analyses ($P > 0.15$).

Similar to the multiple regression analysis, the heterogeneity tests revealed that plots with low population density had significantly stronger SGS than plots with high population density, and plots with high species diversity had significantly stronger SGS than plots with low species diversity (Fig. 2). This was mainly due to an effect of the first distance class in which populations with low population density or high species diversity had a much higher mean kinship coefficient.

DISCUSSION

Genetic diversity and population structure

Ardisia crenata is a species wide spread in Asia, and genetic variation has been studied previously. However, previous results on variation within and among populations and on the mating system revealed largely contrasting results (Chen *et al.* 2001; Cheon *et al.* 2000; Mu *et al.* 2010; Zhao *et al.* 2006) leading

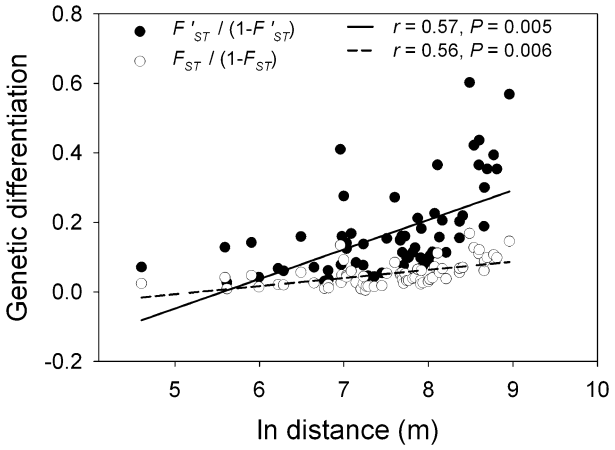

Figure 1: correlation between genetic differentiation and log geographical distance of 12 populations of *Ardisia crenata* (Mantel test).

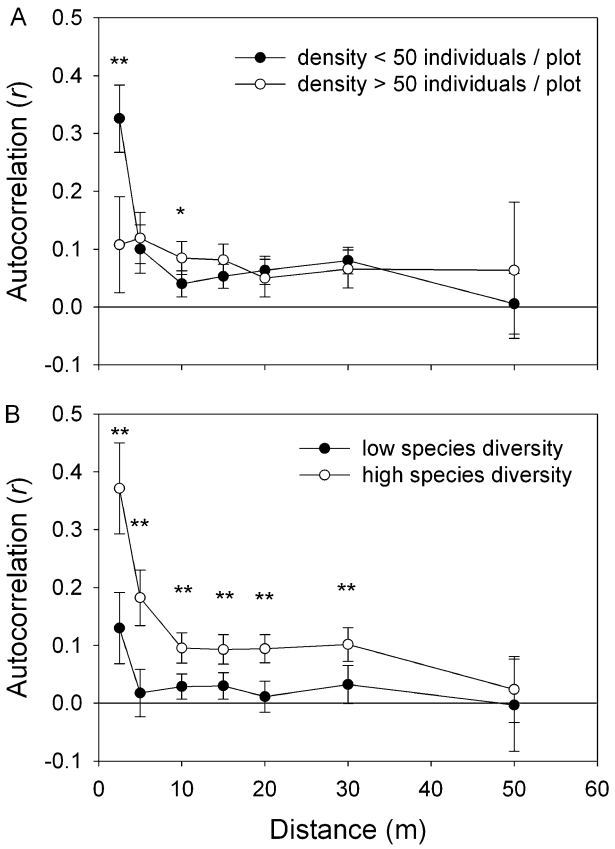

Figure 2: correlograms of genetic autocorrelation of *Ardisia crenata* and comparison of correlogram homogeneity for populations grouped according to (**A)** population density or (**B)** species diversity; ω-test indicates overall significance (A: ω = 31.84, $P = 0.004$; B: ω = 82.43, $P = 0.001$). *$P < 0.05$ and **$P < 0.01$ indicate significant differences for single classes.

to conflicting expectations concerning small-scale genetic structure. Both, pronounced clonality (Cheon *et al.* 2000) and high genetic variation within populations (Mu *et al.* 2010; this study) and both strong (e.g. Mu *et al.* 2010; Zhao *et al.* 2006) and weak (Cheon *et al.* 2000) population differentiation were found. In these studies, different marker systems were used including allozymes, Random Amplification of Polymorphic DNA (RAPD) and microsatellites, the latter analysed with either gel or capillary electrophoresis. These methodological differences may partly account for the observed differences. A high outcrossing rate was independently found in two studies (Chen *et al.* 2001; this study), suggesting a general pattern. In contrast, low outcrossing rates were discussed as a potential reason for high-observed fixation indices (Mu *et al.* 2010), which however, could be also a result of Wahlund effects or of null alleles, as observed in our study. Furthermore, the outcrossing rate in *A. crenata* seems to be variable among populations and depends on population density and population size (Chen *et al.* 2001). The large differences among studies in estimates of genetic variation within populations of *A. crenata* are best explained by different mutation rates of the marker systems, which affect the number of alleles and all descriptors of genetic variability (Nybom 2004). However, in contrast to genetic diversity within populations, estimates of population differentiation should be less affected by marker type. Here, the spatial scale studied may predetermine the ability to detect differentiation. In fact, considerable genetic differentiation has been found at the biogeographical scale (Mu *et al.* 2010), landscape scale (Zhao *et al.* 2006; this study) and also at the within-population scale which is consistent with restricted gene flow at all spatial scales. Isolation-by-distance was found both at the main distribution scale (Mu *et al.* 2010) and landscape scale (this study) indicating an equilibrium between gene flow and genetic drift. In contrast, isolation-by-distance at the landscape scale was not found by Zhao *et al.* (2006). This indicates that local conditions, like population size, which varied strongly within the study of Zhao *et al.* (2006), or the landscape context, which included successional forests in our study, may affect regional population structure.

Spatial genetic autocorrelation within populations was driven by high kinship coefficients in the first distance class (mean $F_{(1)} = 0.082$). Although this value is less than the relatedness expected among half sibs ($F = 0.125$, Loiselle *et al.* 1995), it indicates spatial clustering of closely related plants. The small-scale SGS of *A. crenata* (mean $Sp = 0.027$) is comparable to species with similar reproductive ecology and life form, which is between the value for outcrossing species (mean $Sp = 0.013$) and the value for mixed mating species ($Sp = 0.037$) and close to the value for small trees and shrubs ($Sp = 0.026$) reported by Vekemans and Hardy (2004). Moreover, the mean *Sp* value of *A. crenata* is close to the value reported for gravity-dispersed species ($Sp = 0.028$, Vekemans and Hardy 2004). Hence, our findings on within-population SGS are in concordance with relatively localized gene dispersal, most likely because of a limited seed dispersal capability.

Estimates of gene flow

We indirectly estimated distances of historical gene flow from both, among-population differentiation and

within-population SGS. The estimate based on pairwise $F_{ST}/(1 - F_{ST})$ values between populations and that based on the within-population SGS revealed similar values of 10.3–22.9 m, depending on estimates of effective population density. However, the value based on Hedrick's (2005) standardized measure of differentiation $F'_{ST}/(1 - F'_{ST})$ was smaller (4.8–9.6 m), caused by the higher estimates of differentiation between populations. Thus, although the study species is outcrossing and fruits provide food for larger bird species, historical gene dispersal has been rather limited. This may be due to limited foraging distances of pollinating insects within the forest and due to overall low fruit production and rarity of seed dispersers. Our estimates of gene dispersal were much closer to literature records for understory herbs (mean σ = 5.4–10.6 m, N = 25, table S1) and shrubs (σ = 1.4–7.4 m, N = 3) than to those of trees (σ = 390–685 m, N = 22). Thus, although these literature values are from species with various breeding systems and modes of pollen and seed dispersal, it may be hypothesized that effective historical gene dispersal for small statured or understory species seems to be generally restricted relative to trees.

Environmental factors and community structure influencing within-population SGS

The structuring of genetic variation of neutral markers within and among populations is affected by neutral processes of genetic drift and gene flow. Environmental factors are likely to influence gene flow mechanisms. Indeed, two factors of community structure included in our study were found to be significant when they were jointly used to explain variation in within-population SGS in a multiple regression. In populations where high density of individuals was accompanied by low species diversity, we found little genetic structuring likely because gene flow by seed and pollen is large enough to counter the formation of a more pronounced isolation-by-distance pattern. In turn, in populations with lower individual density and higher species diversity, the movement of gene dispersal vectors, and most likely especially the movement of pollinators among individuals might be less effective, and hence, SGS is more pronounced.

The effect of species diversity on SGS might be explained by a positive correlation between species diversity and the number of co-flowering individuals in the community. The resulting increased competition for pollinator services among species might affect gene flow within species through changes in visit number and quality (Mitchell *et al.* 2009). Hence, in particular for a predominantly outcrossing species like *A. crenata*, it can be hypothesized that increased species diversity can enhance SGS both by an increase in selfing because of pollinator sharing (e.g. Bell *et al.* 2005) and by the reduction in mate diversity and gene dispersal distances (Campbell 1985). The latter is likely to increase SGS also in low-density populations, which prevents a clear separation of the effects of pollinator competition and population density. In our study, a relationship between genetic diversity and species diversity could not been shown. We suggest that in communities with high species diversity, gene flow is more restricted than at low species diversity, which ultimately may affect the level of genetic diversity. This may represent a process that retards the development of the proposed positive species diversity–genetic diversity relationship (Vellend and Geber 2005).

In conclusion, our study showed that gene flow in the shrub *A. crenata* is restricted to a few metres indicating limited dispersal. This pattern is consistent with the analysis of the species at community level in the very same species-rich ecosystem (Bruelheide *et al* 2011). In the future, the exact mechanisms that mediate the effects of community structure on dispersal processes and thus on small-scale genetic structure need to be explored in more detail. This calls for studies of seed dispersal and pollen movement and their biotic vectors in communities differing in species diversity and population density.

SUPPLEMENTARY MATERIAL

Supplementary material is available at *Journal of Plant Ecology* online.

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REFERENCES

Bailey LH (1925) The Standard Cyclopedia of Horticulture. London: McMillan Co.

Bell JM, Karron JD, Mitchell RJ (2005) Interspecific competition for pollination lowers seed production and outcrossing in Mimulus ringens. *Ecology* **86**:762–71.

Berg EE, Hamrick JL (1995) Fine-scale genetic structure of a Turkey oak forest. *Evolution* **49**:110–20.

Bizoux JP, Dainou K, Bourland N, *et al.* (2009) Spatial genetic structure in *Milicia excelsa* (Moraceae) indicates extensive gene dispersal in a low-density wind-pollinated tropical tree. *Mol Ecol* **18**:4398–408.

Born C, Hardy OJ, Chevallier MH, *et al.* (2008) Small-scale spatial genetic structure in the Central African rainforest tree species *Aucoumea klaineana*: a stepwise approach to infer the impact of limited gene dispersal, population history and habitat fragmentation. *Mol Ecol* **17**:2041–50.

Bruelheide H, Böhnke M, Both S, *et al.* (2011) Community assembly during secondary forest succession in a Chinese subtropical forest. *Ecol Monogr* **81**:25–41.

Campbell DR (1985) Pollen and gene dispersal: the influences of competition for pollination. *Evolution* **39**:418–31.

Chen XY, Li N, Shen L (2001) The mating system of *Ardisia crenata* var. *bicolor* (Myrsinaceae), a subtropical understory shrub, in Tiantong National Forest Park, Zhejiang Province. *Acta Phytoecol Sinica* **25**:161–65.

Cheon CP, Chung MY, Chung MG (2000) Allozyme and clonal diversity in Korean populations of Ardisia japonica and Ardisia crenata (Myrsinaceae). *Israel J Plant Sci* **48**:239–45.

Chung MY, Nason J, Chung MG, *et al.* (2002) Landscape-level spatial genetic structure in Quercus acutissima (Fagaceae). *Am J Bot* **89**:1229–36.

Clauss MJ, Mitchell-Olds T (2006) Population genetic structure of *Arabidopsis lyrata* in Europe. *Mol Ecol* **15**:2753–66.

Doligez A, Baril C, Joly HI (1998) Fine-scale spatial genetic structure with nonuniform distribution of individuals. *Genetics* **148**:905–19.

Editorial Group of Forestry Chorography in Kaihua County (1988) Forestry Chorography in Kaihua County. Hangzhou, China: Zhejiang People's Publishing House.

Ennos RA (2001) Inferences about spatial processes in plant populations from the analysis of molecular markers. In: Silvertown J, Antonovics J (eds). *Integrating Ecology and Evolution in a Spatial Context, 14th Special Symposium of the British Ecological Society, Royal Holloway College, University of London*. : Blackwell Science, 45–71.

Epperson BK (1989) Spatial patterns of genetic variation within plant populations. In: Brown AHD, Clegg MT, Kahler AL, Weir BS (eds). *Plant Population Genetics, Breeding and Genetic Resources*. Sunderland, MA: Sinauer Associates, 229–53.

Epperson BK (2005) Estimating dispersal from short distance spatial autocorrelation. *Heredity* **95**:7–15.

Fenster CB, Vekemans X, Hardy OJ (2003) Quantifying gene flow from spatial genetic structure data in a metapopulation of Chamaecrista fasciculata (Leguminosae). *Evolution* **57**:995–1007.

Frankham R (1995) Effective population-size adult-population size ratios in wildlife—a review. *Genet Res* **66**:95–107.

Friedman J, Barrett SCH (2008) High outcrossing in the annual colonizing species Ambrosia artemisiifolia (Asteraceae). *Ann Bot* **101**:1303–9.

Gehring JL, Delph LF (1999) Fine-scale genetic structure and clinal variation in Silene acaulis despite high gene flow. *Heredity* **82**:628–37.

Goudet J (2001) *FSTAT, A Program to Estimate and Test Gene Diversities and Fixation Indices (version 2.9.3)*. Available from http://www2unil.ch/popgen/softwares/fstat.htm.

Hamrick JL, Murawski DA, Nason JD (1993) The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetatio* **108**:281–97.

Hamrick JL, Nason JD (1996) Consequences of dispersal in plants. In: Rhodes OE, Chesser RK, Smith MH (eds). *Population Dynamics in Ecological Space and Time*. Chicago, IL: University of Chicago Press, 203–46.

Hanson TR, Brunsfeld SJ, Finegan B, *et al.* (2008) Pollen dispersal and genetic structure of the tropical tree Dipteryx panamensis in a fragmented Costa Rican landscape. *Mol Ecol* **17**:2060–73.

Hardesty BD, Hubbell SP, Bermingham E (2006) Genetic evidence of frequent long-distance recruitment in a vertebrate-dispersed tree. *Ecol Lett* **9**:516–25.

Hardy OJ, Maggia L, Bandou E, *et al.* (2006) Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Mol Ecol* **15**:559–71.

Hardy OJ, Vekemans X (2002) SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol Ecol Notes* **2**:618–20.

Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution* **59**:1633–8.

Herrera CM, Bazaga P (2008) Adding a third dimension to the edge of a species' range: altitude and genetic structuring in mountainous landscapes. *Heredity* **100**:275–85.

Heuertz M, Vekemans X, Hausman JF, *et al.* (2003) Estimating seed vs. pollen dispersal from spatial genetic structure in the common ash. *Mol Ecol* **12**:2483–495.

Heywood JS (1991) Spatial analysis of genetic variation in plant populations. *Annu Rev Ecol Syst* **22**:335–55.

Hong L, Niu HG, Shen H, *et al.* (2008) Isolation and characterization of polymorphic microsatellite loci in Ardisia crenata (Myrsinaceae). *Conserv Genet* **9**:491–4.

Kitajima K, Fox AM, Sato T, *et al.* (2006) Cultivar selection prior to introduction may increase invasiveness: evidence from Ardisia crenata. *Biol Invasions* **8**:1471–82.

Langeland KA, Craddock Burks K (1998) *Identification and Biology of Non-Native Plants in Florida's Natural Areas. IFAS Publication SP 257*. Gainesville, FL: University of Florida.

Lewontin R (1974) *The Genetic Basis of Evolution*. New York: Columbia University Press.

Loiselle BA, Sork VL, Nason J, *et al.* (1995) Spatial genetic structure of a tropical understory shrub, Psychotria officinalis (Rubiaceae). *Am J Bot* **82**:1420–5.

Meirmans PG (2006) Using the AMOVA framework to estimate a standardized genetic differentiation measure. *Evolution* **60**:2399–402.

Mitchell RJ, Flanagan RJ, Brown BJ, *et al.* (2009) New frontiers in competition for pollination. *Ann Bot* **103**:1403–13.

Mu HP, Hong L, Cao HL, *et al.* (2010) Genetic variation of Ardisia crenata in south China revealed by nuclear microsatellite. *J Syst Evol* **48**:279–85.

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Mol Ecol* **13**:1143–55.

Ohsawa T, Ide Y (2008) Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. *Global Ecol Biogeogr* **17**:152–63.

Pascarella JB (1997) Breeding systems of *Ardisia* Sw. (Myrsinaceae). *Brittonia* **49**:45–53.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol Ecol Notes* **6**:288–95.

R Development Core Team (2009) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Roh MS, Lee AK, Suh JK, *et al.* (2006) Confirmation of cross-pollination of *Ardisia crenata* by sequence-characterized amplified region (SCAR) markers. *Scientia Horticult* **109**:361–7.

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* **145**:1219–28.

Shi M, Michalski SG, Chen XY, *et al.* (2011) Isolation by elevation: genetic structure at neutral and putatively non-neutral loci in a dominant tree of subtropical forest, *Castanopsis eyrei*. *PLoS ONE* **6**:e21302.

Smouse PE, Peakall R, Gonzales E (2008) A heterogeneity test for fine-scale genetic structure. *Mol Ecol* **17**:3389–400.

Sokal RR, Wartenberg DE (1983) A test of spatial auto-correlation analysis using an isolation-by-distance model. *Genetics* **105**:219–37.

Troupin D, Nathan R, Vendramin GG (2006) Analysis of spatial genetic structure in an expanding *Pinus halepensis* population reveals development of fine-scale genetic clustering over time. *Mol Ecol* **15**:3617–30.

Van Oosterhout C, Hutchinson WF, Wills DPM, *et al.* (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* **4**:535–8.

Van Rossum F, Triest L (2006) Fine-scale genetic structure of the common *Primula elatior* (Primulaceae) at an early stage of population fragmentation. *Am J Bot* **93**:1281–8.

Van Rossum F, Triest L (2007) Fine-scale spatial genetic structure of the distylous *Primula veris* in fragmented habitats. *Plant Biol* **9**:374–82.

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Mol Ecol* **13**:921–35.

Vellend M, Geber MA (2005) Connections between species diversity and genetic diversity. *Ecol Lett* **8**:767–81.

Ward M, Dick CW, Gribel R, *et al.* (2005) To self, or not to self. A review of outcrossing and pollen-mediated gene flow in neotropical trees. *Heredity* **95**:246–54.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population-structure. *Evolution* **38**:1358–70.

Wright S (1978) Evolution and the Genetics of Populations Vol. 4, Variability within and among Natural Populations. Chicago, IL: University of Chicago Press.

Wu ZY (1980) *Vegetation of China*. Beijing, China: Science Press.

Yu MJ, Hu ZH, Yu JP, *et al.* (2001) Forest vegetation types in Gutianshan Natural Reserve in Zhejiang. *J Zhejiang Univ (Agriculture & Life Science Edition):* **27**:375–80.

Zhao AL, Chen XY, Zhang X, Zhang D (2006) Effects of fragmentation of evergreen broad-leaved forests on genetic diversity of Ardisia crenata var. bicolor (Myrsinaceae). *Biodiversity Conserv* **15**:1339–51.

Table S1. Indirect estimates of gene dispersal σ within populations based on small scale genetic structure. Gene dispersal is usually given for a lower and upper estimate of effective density, both of which are listed.

Species	Breeding system	Marker type	Gene dispersal σ (m)	Effective density	Pollen vector	Seed vector	Reference	Comments
herbaceous								
Alcantarea *Alcantara geniculata*	mixed mating	SSR	17.1-38.3	0.1-0.5D	bees and sphingid moths	wind	Barbara *et al.* 2008	
A. glaziouana	predominantly selfing	SSR	3.9-8.7	0.1-0.5D	bats	wind	Barbara *et al.* 2008	site LPP
A. glaziouana	predominantly selfing	SSR	7.5-16.7	0.1-0.5D	bats	wind	Barbara *et al.* 2008	site LNI
A. glaziouana	predominantly selfing	SSR	1.7-3.7	0.1-0.5D	bats	wind	Barbara *et al.* 2008	site LPA west
A. glaziouana	predominantly selfing	SSR	4.9-11.1	0.1-0.5D	bats	wind	Barbara *et al.* 2008	site LPA east
A. imperialis	predominantly outcrossing	SSR	7-15.6	0.1-0.5D	bats	wind	Barbara *et al.* 2008	site IMCgreen
A. imperialis	predominantly outcrossing	SSR	14.7-32.9	0.1-0.5D	bats	wind	Barbara *et al.* 2008	site IMC
A. regina	predominantly selfing	SSR	17.2-38.5	0.1-0.5D	bats	wind	Barbara *et al.* 2008	
Cryptotaenia	predominantly	allozymes	0.58	D	insect	gravity	Vekemans and Hardy 2004	site HD

canadensis	selfing							
C. canadensis	predominantly selfing	allozymes	1.6	D	insect	gravity	Vekemans and Hardy 2004	site LD
Daucus carota ssp. carota	predominantly outcrossing	SSR	4-12	0.1-0.5D	insect	wind and animals	Rong *et al.* 2010	site WM
D. carota ssp. carota	predominantly outcrossing	SSR	10-31	0.17-0.5D	insect	wind and animals	Rong *et al.* 2010	site WAL
Geranium pratense	predominantly outcrossing	SSR	3.2-6.8	0.1-0.5D	insect	gravity and animal	Michalski unpublished	
Hordeum spontaneum	predominantly selfing	SSR	0.29-0.64	0.1-0.5D	wind	wind and animals	Volis *et al.* 2010	site SB, mean value for 3 years for $\sigma_{(400)}$
H. spontaneum	predominantly selfing	SSR	0.22-0.49	0.1-0.5D	wind	wind and animals	Volis *et al.* 2010	site BG, mean value for 4 years for $\sigma_{(400)}$
Medicago trunculata	predominantly selfing	SSR	1.14	D	insect	animal	Vekemans and Hardy 2004	site aude1
M. trunculata	predominantly selfing	SSR	1.33	D	insect	animal	Vekemans and Hardy 2004	site aude3
Primula sieboldii	predominantly outcrossing	SSR	15.7	genet density	bumblebee	gravity	Ishihama *et al.* 2005	
Sanicula odorata	mixed mating	allozymes	0.86	D	insect	animal	Vekemans and Hardy 2004	site HD
S. odorata	mixed mating	allozymes	3.81	D	insect	animal	Vekemans and Hardy 2004	site LD
Silene acaulis	predominantly outcrossing	allozymes	1.48	D	insect	gravity	Vekemans and Hardy 2004	site slope2

S. acaulis	predominantly outcrossing	allozymes	4.73	D	insect	gravity	Vekemans and Hardy 2004	site Krummholz
S. acaulis	predominantly outcrossing	allozymes	2.92	D	insect	gravity	Vekemans and Hardy 2004	site Summit
S. acaulis	predominantly outcrossing	allozymes	4.98	D	insect	gravity	Vekemans and Hardy 2004	site Flat1
Silene latifolia	self-incompatible	SSR	3.8-22.7m	0.007-0.25ind./ m^2	insect	gravity	Barluenga *et al.* 2011	autosomal markers
mean (SD)			5.4 (5.4)-10.6 (12.5)					
woody -trees								
Araucaria angustifolia	self-incompatible	SSR	68	0.25D	wind	gravity	Stefenon *et al.* 2008	mean of 4 populations
A. angustifolia	self-incompatible	AFLP	42.75	0.25D	wind	gravity	Stefenon *et al.* 2008	mean of 4 populations
Aucoumea klaineana	predominantly outcrossing	SSR	210-570	0.1-0.5D	insect	wind	Born *et al.* 2008	
Copaifera langsdorffii	mixed mating	SSR	44	0.5D	insect	animal	Sebbenn *et al.* 2011	
Distemonanthus benthamianus	Predominantly outcrossing	SSR	993-1155	0.1-0.25D		wind	Debout *et al.* 2011	site Ebolowa
D. benthamianu	Predominantly outcrossing	SSR	438-746	0.1-0.5D		wind	Debout *et al.* 2011	site Sangmelima

s								
D. benthamianus	Predominantly outcrossing	SSR	396-426	0.25-0.5D		wind	Debout *et al.* 2011	site CEB
Fagus crenata	predominantly outcrossing	SSR	36.84-82.38	0.1-0.5D	wind	gravity and animal	Oddou-Muratorio *et al.* 2010	adults, site FC
F. sylvatica	predominantly outcrossing	SSR	31.36-70.12	0.1-0.5D	wind	gravity and animal	Oddou-Muratorio *et al.* 2010	adults, site FS1
F. sylvatica	predominantly outcrossing	SSR	64.69-144.64	0.1-0.5D	wind	gravity and animal	Oddou-Muratorio *et al.* 2010	adults, site FS2
F. sylvatica	predominantly outcrossing	SSR	65.97-147.52	0.1-0.5D	wind	gravity and animal	Oddou-Muratorio *et al.* 2010	adults, site FS3
Milicia excelsa	predominantly outcrossing	SSR	3700-7100	0.1-0.5D	wind	animal	Bizoux *et al.* 2009	site Mindourou
M. excelsa	predominantly outcrossing	SSR	1000-2600	0.1-0.5D	wind	animal	Bizoux *et al.* 2009	site Djoum
Prunus avium	self-incompatible	SSR	105.6	D	insect	animal	Schueler *et al.* 2006	
P. avium	self-incompatible	SSR	75.7-180.3	0.1-0.5D	insect	animal	Vaughan *et al.* 2007	site A
P. avium	self-incompatible	SSR	63.3-93.7	0.22-0.5D	insect	animal	Vaughan *et al.* 2007	site B
Quercus ilex	self-incompatible	SSR	55-80	10-100trees/ha	wind	animal	Soto *et al.* 2007	extracted from figure 3
Q. suber	self-incompatible	SSR	20-115	5-100trees/h	wind	animal	Soto *et al.* 2007	extracted from figure 3

				a				
Quercus petraea	self-incompatible	SSR	117.04 – 145.17	0.5–0.9D	wind	animal	Valbuena-Carabana *et al.* 2007	
Q. pyrenaica	self-incompatible	SSR	168.11	0.9D	wind	animal	Valbuena-Carabana *et al.* 2007	
Sorbus torminalis	mixed mating	SSR	417-506	0.5D-D	insect	animal	Oddou-Muratorio and and Klein 2008	seedlings
S. torminalis	mixed mating	SSR	472	D	insect	animal	Oddou-Muratorio and Klein 2008	adults
mean (SD)			390 (793) - 685 (1542)					
woody - shrubs								
Helicteres brevispira	predominantly outcrossing	allozymes	7.35		humming bird	gravity	Franceschinelli and Kesseli 1999	
Hibiscus moscheutos	predominantly outcrossing	allozymes	2.09	D	insect	gravity	Vekemans and Hardy 2004	site2
H. moscheutos	predominantly outcrossing	allozymes	1.4	D	insect	gravity	Vekemans and Hardy 2004	site8
mean (SD)			3.6 (3.3)					

References

Barbara T, Lexer C, Martinelli G, Mayo S, Fay MF, Heuertz M (2008) Within-population spatial genetic structure in four naturally fragmented species of a neotropical inselberg radiation, *Alcantarea imperialis*, *A.geniculata*, *A.glaziouana* and *A.regina* (Bromeliaceae). *Heredity* **101**:285-296.

Barluenga M, Austerlitz F, Elzinga JA, Teixeira S, Goudet J, Bernasconi G (2011) Fine-scale spatial genetic structure and gene dispersal in Silene latifolia. *Heredity* **106**:13-24.

Bizoux JP, Dainou K, Bourland N, Hardy OJ, Heuertz M, Mahy G, Doucet JL (2009) Spatial genetic structure in *Milicia excelsa* (Moraceae) indicates extensive gene dispersal in a low-density wind-pollinated tropical tree. *Molecular Ecology* **18**:4398-4408.

Born C, Hardy OJ, Chevallier MH, Ossari S, Atteke C, Wickings J, Hossaert-Mckey M (2008) Small-scale spatial genetic structure in the Central African rainforest tree species *Aucoumea klaineana*: a stepwise approach to infer the impact of limited gene dispersal, population history and habitat fragmentation. *Molecular Ecology* **17**:2041-2050.

Byrne M, Elliott CP, Yates C, Coates DJ (2007) Extensive pollen dispersal in a bird-pollinated shrub, *Calothamnus quadrifidus*, in a fragmented landscape. *Molecular Ecology* **16**:1303-1314.

Debout GDG, Doucet JL, Hardy OJ (2011) Population history and gene dispersal inferred from spatial genetic structure of a Central African timber tree, Distemonanthus benthamianus (Caesalpinioideae). *Heredity* **106**:88-99.

Franceschinelli EV, Kesseli R (1999) Population structure and gene flow of the Brazilian shrub *Helicteres brevispira*. *Heredity* **82**:355-363.

Heuertz M, Vekemans X, Hausman JF, Palada M, Hardy OJ (2003) Estimating seed vs. pollen dispersal from spatial genetic structure in the common ash. *Molecular Ecology* **12**:2483-2495.

Ishihama F, Ueno S, Tsumura Y, Washitani I (2005) Gene flow and inbreeding depression inferred from fine-scale genetic structure in an endangered heterostylous perennial, *Primula sieboldii*. *Molecular Ecology* **14**:983-990.

Oddou-Muratorio S, Bontemps A, Klein EK, Chybicki I, Vendramin GG, Suyama Y (2010) Comparison of direct and indirect genetic methods for estimating seed and pollen dispersal in *Fagus sylvatica* and *Fagus crenata*. *Forest Ecology and Management* **259**:2151-2159.

Oddou-Muratorio S, Klein EK (2008) Comparing direct vs. indirect estimates of gene flow within a population of a scattered tree species. *Molecular Ecology* **17**:2743-2754.

Rong J, Janson S, Umehara M, Ono M, Vrieling K (2010) Historical and contemporary gene dispersal in wild carrot (*Daucus carota* ssp *carota*) populations. *Annals of Botany* **106**:285-296.

Schueler S, Tusch A, Scholz F (2006) Comparative analysis of the within-population genetic structure in wild cherry (*Prunus avium* L.) at the self-incompatibility locus and nuclear microsatellites. *Molecular Ecology* **15**:3231-3243.

Sebbenn AM, Carvalho ACM, Freitas MLM, Moraes SMB, Gaino A, da Silva JM, Jolivet C, Moraes MLT (2011) Low levels of realized seed and pollen gene flow and strong spatial genetic structure in a small, isolated and fragmented population of the tropical tree *Copaifera langsdorffii* Desf. *Heredity* **106**:134-145.

Soto A, Lorenzo Z, Gil L (2007) Differences in fine-scale genetic structure and dispersal in *Quercus ilex* L. and *Q. suber* L.: consequences for regeneration of mediterranean open woods. *Heredity* **99**:601-607.

Stefenon VM, Gailing O, Finkeldey R (2008) The role of gene flow in shaping genetic structures of the subtropical conifer species *Araucaria angustifolia*. *Plant Biology* **10**:356-364.

Valbuena-Carabana M, Gonzalez-Martinez SC, Hardy OJ, Gil L (2007) Fine-scale spatial genetic structure in mixed oak stands with different levels of hybridization. *Molecular Ecology* **16**:1207-1219.

Vaughan SP, Cottrell JE, Moodley DJ, Connolly T, Russell K (2007) Distribution and fine-scale spatial-genetic structure in British wild cherry (*Prunus avium* L.). *Heredity* **98**:274-283.

Vekemans X, Hardy OJ (2004) New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* **13**:921-935.

Volis S, Zaretsky M, Shulgina I (2010) Fine-scale spatial genetic structure in a predominantly selfing plant: role of seed and pollen dispersal. *Heredity* **105**:384-393.

Wang R, Ai B, Gao BQ, Yu S, Li YY, Chen XY (2009) Spatial genetic structure and restricted gene flow in a functionally dioecious fig, *Ficus pumila* L. var. *pumila* (Moraceae). *Population Ecology* **51**:307-315.