

# 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 (Bruehlheide *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 (Bruehlheide *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 (Bruehlheide *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' (Bruehlheide *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, Bruehlheide *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'-TCCAAAACGGAATAAGGTTG-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 Oosterhout 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  $\sigma^2$  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  $\sigma$  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  $\sigma$  to  $20\sigma$  (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  $\sigma$ . The program iteratively adjusts the spatial range of the autocorrelation analysis until  $\sigma$  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  $\sigma$  from (1) and (2) were computed as  $\pm 2$  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)	<i>Ardisia</i> population density (individuals/plot <sup>a</sup> )	Elevation (m a.s.l.)	Successional stage <sup>b</sup>	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

<sup>a</sup> Plot size was 30 × 30 m.

<sup>b</sup> 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  $\sigma$  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  $\sigma$  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,  $\sigma$  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

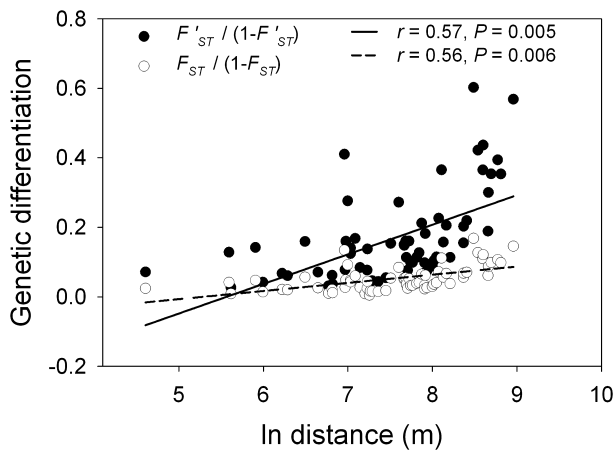
After model simplification, only population density and species diversity remained with a significant effect on SGS among plots. The final model explained 53% of the variation in  $Sp$  values ( $t = -2.547$ ,  $P = 0.03$  and  $t = 2.699$ ,  $P = 0.02$ , for population density and species diversity, 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.

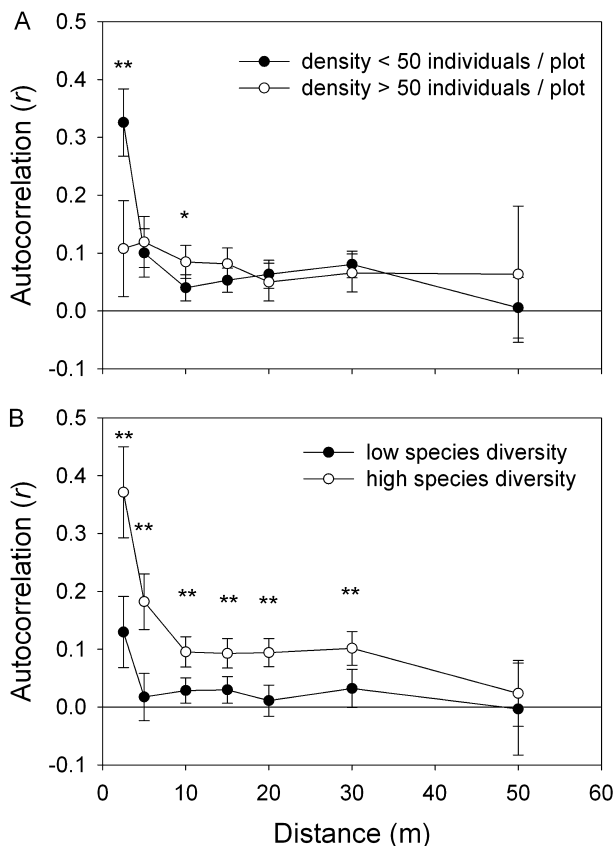
## DISCUSSION

### 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;  $\omega$ -test indicates overall significance (A:  $\omega = 31.84$ ,  $P = 0.004$ ; B:  $\omega = 82.43$ ,  $P = 0.001$ ). \* $P < 0.05$  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 (Nybohm 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  $\sigma = 5.4$ – $10.6$  m,  $N = 25$ , table S1) and shrubs ( $\sigma = 1.4$ – $7.4$  m,  $N = 3$ ) than to those of trees ( $\sigma = 390$ – $685$  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 be

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 (Bruehlheide *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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