35: 1-7, 2017
**Nordic Journal of Botany** ~~**000: 001–007, 2016**~~
doi: 10.1111/njb.01311, ISSN 1756-1051

Subject Editor: Stefan Andersson. Editor-in-Chief: Torbjörn Tyler. Accepted 16 August 2016

# Gene flow in, and mating system of, *Rhododendron simsii* in a nature reserve in subtropical China

**Christoph Z. Hahn, Stefan G. Michalski and Walter Durka**

*C. Z. Hahn (http://orcid.org/0000-0002-8399-4682) (christoph.hahn@ufz.de), S. G. Michalski, W. Durka, Helmholtz Centre for Environmental Research - UFZ, Dept of Community Ecology (BZF), Halle, Germany. WD also at: German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany.*

Mating system and gene dispersal distances are two important characteristics that govern the distribution of genetic variation within species. Genetic variation is an important resource for adaptation, but also allows insight into a species reproductive biology. As the reproductive biology is species specific, general inferences across species may be inaccurate and for many species not much is known about the details of gene flow and mating, especially in species-rich ecosystems such as the subtropics.

We characterised the mating system and determined historical and current gene flow distances in *Rhododendron simsii* using microsatellite markers. Adult individuals and seeds were sampled in a near-natural nature reserve in southeast China. We examined the fine-scale spatial genetic structure (SGS), kinship coefficients, outcrossing rates and biparental inbreeding coefficients. Furthermore, we estimated pollen dispersal distances using paternity analysis.

We found high outcrossing rates and significant biparental inbreeding. Population differentiation was low while observed heterozygosity and allelic richness were high. Estimates of historical and current gene flow were similar, indicating that genes are on average dispersed over distances of between 10 and 20 m. Paternity analyses suggest frequent mating among neighbouring individuals.

We conclude that *R. simsii* is predominantly, but not obligately outcrossing. Moderate amounts of biparental inbreeding and overall low fine-scale SGS indicate that mating among related individuals is common, but does neither lead to pronounced population differentiation nor to strong aggregation of related individuals. Most likely, gene flow distances in this species are affected by its flowering phenology. Mass-flowering, pollen/pollinator limitation and gravity/wind dispersal of seeds in concert cause short gene dispersal distances. Lastly, population genetic descriptors suggest that *R. simsii* in the study area represents a large, well connected population in which large amounts of genetic variability are maintained.

Genetic diversity in natural plant populations is commonly distributed non-randomly (Wright 1946, Vekemans and Hardy 2004) resulting in a more or less pronounced spatial genetic structure (SGS) (Takahashi et al. 2008, Zeng et al. 2012). Such non-random spatial distribution of genotypes is a direct consequence of the sessile nature of plants and spatially restricted gene flow. Disregarding clonal propagation, genotypes are stationary until gene movement via pollen or seeds allows for further dispersal. Hence, determinants of gene dispersal capabilities, e.g. mating system and type of seed dispersal, shape SGS (Vekemans and Hardy 2004, Auld and de Casas 2013). In addition to present gene dispersal, past incidents such as bottlenecks or founder events are strong determinants of genetic diversity (Nowak et al. 2014). As the above processes are species-specific, the extent of gene flow and spatial genetic structure remains obscure for most plant species and require continued empirical research.

The spatial genetic structure within species is the result of mutation, selection, drift and gene flow, with the latter reflecting the strength and direction of genetic exchange between individuals and populations. Due to limited gene dispersal, genetic differentiation among individuals and populations increases with pairwise spatial distance (Wright 1943). Commonly, historic gene flow is inferred by regressing pairwise population differentiation ($F_{ST}$) on geographic distance (Rousset 1997) or based on the regression slope of pairwise kinship coefficients with geographical distance (Vekemans and Hardy 2004). In contrast, current gene flow can be quantified directly by following individual genes across the landscape (Sork et al. 1999). Paternity analyses allow calculating physical parent–offspring distances and outcrossing rates if non-parents are reliably excluded. Both indirect and direct measures of historic and current gene flow allow identifying the strength and spatial scale at which evolutionary forces might act on populations and thus may aid species management and policy makers in the face of anthropogenic habitat alterations.

Gene flow in plants is realized via the movement of pollen and seeds, with the former resulting in mating and the latter in dispersal away from the maternal plant. Thus, a species ability to disperse genes is often reflected by its propagule mobility and dispersal syndrome (Chybicki and Burczyk 2013). Long-distance gene flow has the potential to genetically homogenize populations and reduce local SGS (Dick et al. 2008). In contrast, low dispersal distances, e.g. for gravity-dispersed seeds, can cause spatial clumping of related individuals which might increase inbreeding at the population level (Loveless and Hamrick 1984, Griffin and Eckert 2003) and local SGS. The species-specific sexual behaviour, i.e. the mating system, affects population inbreeding (Ellstrand and Elam 1993). In its extremes, the mating system ranges between obligate selfing (e.g. cleistogamy) and obligate outcrossing (e.g. self-incompatibility), with mixed-mating being a comparably common syndrome where species exhibit both selfing and outcrossing.

The mating system was found to correlate with the life form of plant species (Duminil et al. 2009). Most trees are outcrossing (Ward et al. 2005) and maintain high genetic diversity (Petit and Hampe 2006) while population differentiation remains comparably low (Hamrick and Godt 1996). Rather successful gene dispersal in the face of often low population densities has been attributed to life-history traits of trees, in particular high propagule production combined with a high release height (Petit and Hampe 2006). As abundant as the literature is regarding trees, tropical ones in particular (Hamrick et al. 1993, Doligez and Joly 1997, Degen et al. 2004), as scarce it is concerning shrubs. Although shrubs and trees share common features, they can differ substantially in other traits, most noticeably in height. As a consequence, shrubs are often confined to a shadowy existence in the understory, but surprisingly little is known about how understory shrubs compare to trees in terms of mating systems and gene flow. This lack of knowledge could severely impede our understanding of what governs the distribution of genetic diversity in forests.

The mountains of east China host an enormous amount of biodiversity and constitute the third most diverse area in China (Tang et al. 2006). Yet, subtropical broad-leaved evergreen forests in this biodiversity hotspot only recently received scientific attention. In particular, patterns of gene flow and SGS in understory shrubs are poorly understood. A recent study hypothesized that SGS of shrubs in highly diverse subtropical communities more likely resembles patterns found in herbs than those of trees (Zeng et al. 2012). Here, we present a study aiming at alleviating this lack of knowledge, conducted in a near-natural nature reserve in subtropical China. We chose *Rhododendron simsii* Planchon (Ericaceae), a common shrub of the local understory. We genotyped adult individuals with their offspring to determine paternity and calculate population genetic descriptors. Specifically, we aimed at 1) resolving the fine-scale spatial genetic structure, 2) characterising the mating system, and 3) quantifying the magnitude of both historic and current gene flow.

## Material and methods

### Study species and sampling

*Rhododendron simsii* is a small shrub (2–5 m) that occurs naturally in forests and forest margins at altitudes between 500–2700 m a.s.l. (eFloras) and is distributed from subtropical China to Japan, Laos, Myanmar and Thailand. It is described as shade-intolerant and self-sterile, with bee-pollinated flowers and small seeds (Ng and Corlett 2000b). Previous studies in Hong Kong found low genetic differentiation and little geographical structure in this species (Ng and Corlett 2000a). *Rhododendron simsii* has some economic value as it is the horticulturally-used wild ancestor of the common pot azalea. Sample collection was carried out in the Gutianshan National Nature Reserve (GNNR), located in Zhejiang province, southeast China (29°8′–29°17′N, 118°2′–118°11′E). This nature reserve occupies an area of approximately 81 km$^2$ and is categorized as subtropical evergreen broad-leaved forest. Over 1400 reported vascular plant species make this area a major diversity hotspot of subtropical China (Lou and Jin 2000). With its establishment in 1975, silvicultural management ceased, now providing opportunity to investigate biodiversity and ecosystems functioning in a regenerating near-natural forest.

In 2009, the Biodiversity Ecosystem Functioning project China (BEF China) established 27 comparative study plots (CSPs) in the GNNR (Bruelheide et al. 2011). The plots have a projected area of 30 × 30 m and are scattered randomly throughout the mountainous reserve to resemble a successional gradient. The maximum distance between individuals was 8.7 km. In the study area, *R. simsii* constitutes one of the common understory shrubs; however, it occurred only in 19 plots. To increase sample size, we sampled an additional, larger population in the GNNR near Hong Yuan (plot size approx. 50 × 20 m). Sampling was restricted to plots and we marked and georeferenced all flowering and non-flowering individuals therein in spring 2012. Leaves and fully ripened seed capsules were collected from 182 and 83 plants, respectively in late September–early October. Leaves were stored in a freezer at –4°C and later lyophilized for 72 h, whereas seed capsules were air-dried.

### Genotyping

Between 5–15 mg of dried leaves were used to extract total genomic DNA using the QIAGEN DNeasy 96 Plant Kit following the factory provided protocol, using 2 × 25 µl instead of 1 × 50 µl in the last elution step. Prior to offspring genotyping, seeds were germinated in Petri dishes on filter paper with water and 100 nM gibberellic acid to speed up germination. Petri dishes were placed in plant growing chambers set to a 14 h/10 h day-night cycle with 20°C and 16°C, respectively. After seedlings had grown to sufficient size (~ 5–10 mg fresh weight) they were lyophilized for 60 h and later DNA was extracted using the QIAGEN 96 Plant Kit. We aimed at genotyping 24 seedlings per mother and raised a total of 1813 seedlings from 83 mothers.

A total of 30 published microsatellite primers (Tan et al. 2009, Wang et al. 2009, 2010) were tested for suitability in *R. simsii*. A total of 6 of these performed well enough (RDW43, RDW1, N16, RDW35, R-432 and N25) to be used for our study (Supplementary material Appendix 1, Table A1). With these markers, a three primer touch-down PCR with a total volume of 5 µl was run on all samples following Schuelke (2000). The master mix contained 2.5 µl QIAGEN Multiplex Mastermix, 1 µl DNA, 1.5 µl primer mix (2.5 µM), 1.5 µl fluorescently labelled primer (2.5 µM). Multiplex PCRs were run in 384-well plates on an Eppendorf Thermal cycler with a touchdown protocol: one cycle of 95°C for 15 min, followed by 20 cycles with 30 s of denaturing at 94°C, 30 s of annealing at 60°C (reduced by 0.5°C per cycle) and 90 s of extension at 72°C, respectively, followed by another 20 cycles with annealing at 50°C and a final extension step of 10 min at 72°C. Fragment analysis was carried out with GENESCAN LIZ 500 size standard on an ABI 3130xl genetic analyser. We used Genemapper 5 for allele binning and scoring.

### Spatial genetic structure and historic gene flow

We calculated descriptors of spatial genetic structure on all adult individuals using SPAGeDi v1.5a (Hardy and Vekemans 2002). We used predefined distance classes at 7 m, 15 m, 25 m, 35 m, 50 m, 100 m, 1000 m, 5000 m and >5000 m and calculated individual pairwise kinship coefficients (Loiselle et al. 1995) with 1000 bootstraps and jack-knifing over loci. To infer spatial genetic structuring we calculated the Sp-statistic as $Sp = -\hat{b}_F \big/ \left(1 - \hat{F}_{(1)}\right)$ (Vekemans and Hardy 2004) with $\hat{b}_F$ being the slope of the regression of individual pairwise kinship coefficients over log(distance) and $\hat{F}_{(1)}$ the mean kinship coefficient in the first distance class assuming isotropic dispersal. Lastly, we calculated gene dispersal sigma ($\sigma$), half the mean squared parent–offspring distance. Among plots, census population densities (D) varied between 45–555 ind. ha$^{-1}$. Then, we estimated the effective population density to range from $D_e = 5$–277 individuals ha$^{-1}$ (with $D/D_e \sim 0.1$–0.5) (Frankham 1995). As population densities varied almost two orders of magnitude, we calculated $\sigma$ for a wide range ($D_e = 5$–600) of assumed effective population densities (Table 1).

### Mating system

To assess the mating system we calculated multi-locus outcrossing rates ($t_m$) and biparental inbreeding ($t_m - t_s$) using MLTR v3.2 (Ritland 2002). A total of 1649 genotyped seedlings from 83 mothers were included in our analyses. Analyses were carried out on the population level and at the level of mothers with 1000 bootstraps and the default settings. We tested whether individual-level multi-locus outcrossing rates were related to plot successional stage, plot elevation and within-plot mean neighbour distance in separate linear mixed-effects models (Bates et al. 2015) using R v3.2.1 (R Core Team 2015). Plot successional stage describes plot age (sensu Bruelheide et al. 2011), plot elevation is the elevation above sea level and within-plot mean neighbour distance is the mean distance of each individual to its within-plot neighbours. In these models, plots were set to be random factors with random intercepts. Factor significance was assessed by performing analysis of variance tests between two models, one containing the environmental factor in question and a second model where it was removed (null-model). Two plots were removed from statistical analyses due to low sample size.

Table 1. Estimates of gene dispersal ($\sigma$) over a range of effective population densities ($D_e$, individuals ha$^{-1}$). Est. $N_b$ = estimated neighbourhood size, SE = standard error, $\sigma$ = gene dispersal distance (m), n.c. = non-convergence on these parameter values.

| $D_e$ | Est. $N_b$ | SE | $\sigma$ | SE |
|---|---|---|---|---|
| 5 | 522.21 | n.c. | 288.3 | n.c. |
| 10 | 622.51 | n.c. | 219.3 | n.c. |
| 20–250 | n.c. | n.c. | n.c. | n.c. |
| 300 | 89.15 | n.c. | 15.38 | n.c. |
| 400 | 86.91 | n.c. | 13.15 | n.c. |
| 500 | 92.00 | 38.43 | 12.10 | 2.56 |
| 600 | 79.97 | 47.32 | 10.30 | 2.81 |

### Population level paternity analysis and recent gene flow

We used CERVUS v3.0.3 (Kalinowski et al. 2007) on maternal and filial genotypes to determine the most likely sire in order to assess parent–offspring distances and recent gene flow. We first simulated 10 000 offspring with 10 candidate fathers each, typed at a minimum of four loci with a genotyping error of 1%. The proportion of sampled candidate fathers was set to 40% as an approximation of our sampling extent considering the overall abundance of our target species within plots. The default confidence levels for relaxed and strict paternity assignment were 80% and 95%, respectively. In a second step we used the simulated parameters to assign paternity on actual offspring genotypes. We considered mating to be restricted within plots as most of our plots are well distanced from another. After assigning paternity we calculated GPS-based Euclidean parent–offspring distances between parent pairs and mating events were assigned to one of ten mating-distance classes (range: 5–100 m, interval: 5–10 m). We tested whether the number of observed mating events deviates from a random-mating scenario by randomly assigning a new within-plot sire to each observed mating event. This was repeated 100 times and the number of randomized mating events per distance class was counted to calculate the mean and confidence intervals per distance class. In a last step, we compared the frequency of observed mating events against the random-mating distribution with its 95% CI. Points falling outside this expected distribution of random matings indicate deviations from a random-mating null hypothesis.

## Results

### Spatial genetic structure and historic gene flow

Kinship coefficients significantly decreased with spatial distances (Fig. 1A). The slope of the regression with distance was negative with $\hat{b}_F$ –0.00308 ($p < 0.001$). The kinship coefficient in the first distance class, $\hat{F}_{(1)}$ was 0.0311.

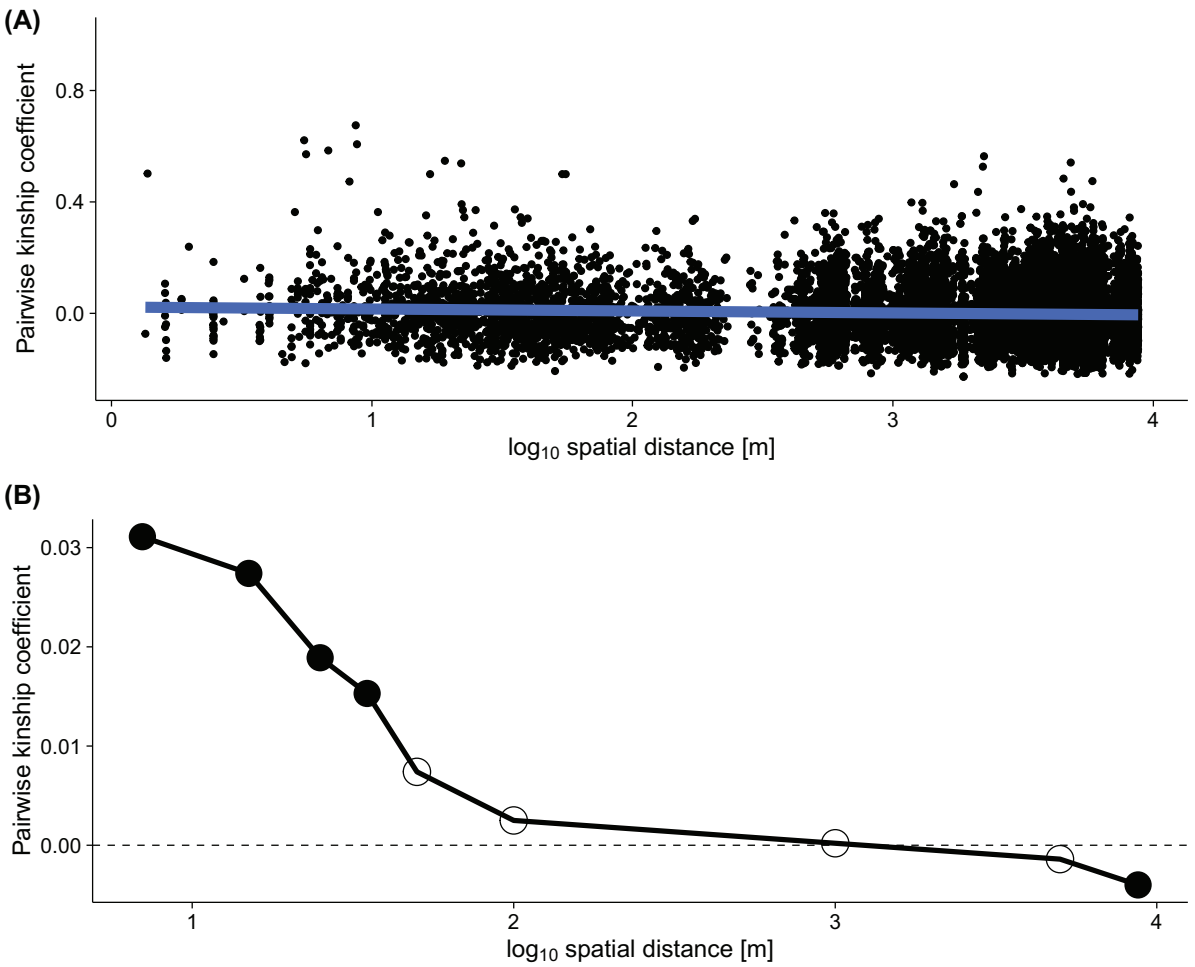


Figure 1. (A) correlation between individual pairwise kinship coefficient and pairwise distance. The solid line represents the linear regression slope ($R^2_{adj} = 0.0014$, $p < 0.001$), (B) mean pairwise kinship coefficients within predefined distance classes. Kinship coefficients that differ significantly from zero are represented by filled circles.

Consequently, Sp equated to 0.00318. Distance class based kinship coefficients were low and decreased over the full range of observations (Fig. 1B). Gene dispersal σ was estimated for effective population densities between 5–600 individuals ha$^{-1}$ (Table 1). For high $D_e$ (300–600 individuals ha$^{-1}$), σ ranged between 15.4–10.3 m, respectively, whereas low estimated $D_e$ (5–10 individuals ha$^{-1}$) resulted in a σ of 288.3–219.3 m (Table 1).

### Mating system

Overall mean outcrossing rate $t_m$ estimated from progeny arrays was 0.919. At plot level, the highest multi-locus outcrossing rate ($t_m = 0.996$, SD = 0.131) was found in CSP22, a young plot, whereas the lowest outcrossing rate ($t_m = 0.788$, SD = 0.178) was found in CSP15, a plot categorized as successionally old. The overall mean biparental inbreeding ($t_m - t_s$) was 0.175. Biparental inbreeding was highest in CSP25 ($t_m - t_s = 0.224$, SD = 0.055), and lowest in CSP6 ($t_m - t_s = 0.137$, SD = 0.063), both plots being successionally intermediate. None of the tested plot environmental properties were significantly correlated to the observed multi-locus outcrossing rate (data not shown).

### Paternity analysis and recent gene flow

A total of 1579 seedlings could be genotyped at four or more loci and were taken into account during paternity analysis. The mean number of alleles per locus was 20.3 and expected heterozygosity was 0.8633. The combined non-exclusion probability of the first and second parent were 0.0001838 and 0.00000261, respectively, with a combined non-exclusion probability of the parent pair of $2.12^{-10}$. In total, we could assign 423 sires (26.8%) by allowing within-plot mating. Mean and maximum observed pollen dispersal distance were 24.3 and 95.9 m, respectively. Pollen dispersal distance largely followed the expectation of random mating within plots although we detected a significantly larger number of mating events than expected at 10 m and fewer than expected in the 40 m distance class (Fig. 2).

## Discussion

### Mating system

According to our findings, *R. simsii* is predominantly outcrossing (mean $t_m = 0.919$). Considering the evidence

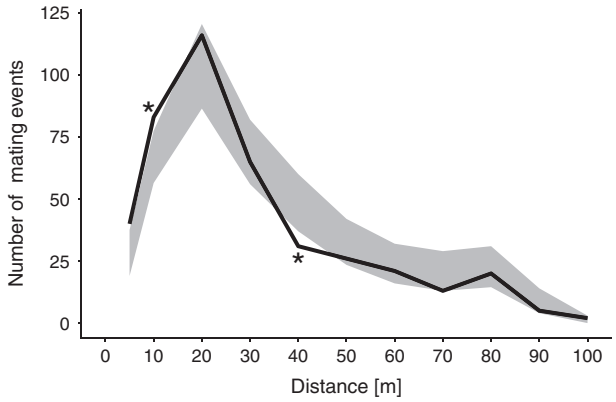


Figure 2. Frequency distribution of observed and expected mating distances within plots. The black line depicts the number of observed mating events, the grey envelope represent the 95% confidence interval obtained from randomizing all mating events. Significant departures from random mating are indicated by asterisks.

of some selfed progeny, *R. simsii* appears to efficiently, but not perfectly avoid the production of selfed seeds. As outcrossing rates were generally high and not affected by plot environmental properties, the rate of outcrossing appears to be similar across the populations sampled in this study.

The above is in concord with the observation of Ng and Corlett (2000a) that in *R. simsii* seeds from selfing are rare, and their conclusion that the species is mostly self-sterile, although the underlying mechanisms are unknown. High outcrossing rates may foster gene movement, reducing the spatial genetic autocorrelation between individuals. Consequently, spatial genetic structure would be low in highly outcrossing species (Vekemans and Hardy 2004), an assumption we confirm for *R. simsii*. However, despite high levels of outcrossing we detected a significant amount of biparental inbreeding. On average, about 17% of all mating events involved related individuals. The presence of inbred offspring may lead to significant SGS which, however, was very low in our study. A plausible cause to this may be a strong expression of inbreeding depression. Deleterious recessive alleles might not be subjected to purging in self-incompatible species and may accumulate over time (Charlesworth and Charlesworth 1987, Brennan et al. 2005). Inbred offspring could then suffer from deleterious recessives and be selected against. Failure of inbred offspring to establish could in part explain the overall low strength of local SGS. Indeed, it is thought that obligately outcrossing species should suffer strongest from biparental inbreeding depression as they lack 'natural' inbreeding when compared to selfing species (Lande and Schemske 1985, Heywood 1993). Significant inbreeding depression was also reported in other *Rhododendron* species (Hirao 2010, Delmas et al. 2014) and might similarly affect inbred offspring in *R. simsii*. Nevertheless, the seeds that were genotyped were not yet subjected to selection under natural conditions and any detrimental effect of inbreeding remains speculative until offspring fitness is quantified.

## Historical gene flow and spatial genetic structure

We found weak population structure in *R. simsii* with individual kinship coefficients following an isolation-by-distance pattern over the whole range of pairwise comparisons, demonstrating that *R. simsii* is dispersal-limited at the spatial scale examined. The extent of fine-scale genetic structure in this species is very low (Sp = 0.00318) and similar to outcrossing, animal-pollinated trees (Vekemans and Hardy 2004). Gene dispersal σ, an estimate of historical dispersal, was low for high effective population densities (σ = 15.4–10.3 m, $D_e$ = 300–600 ind. ha$^{-1}$) and similar to values found in other tropical and subtropical shrubs (Zeng et al. 2012, Theim et al. 2014). Gene dispersal distances might be even shorter, considering that these estimates greatly depend on estimates of the effective population density. However, these low values for σ are surprising considering this species is highly outcrossing and largely self-sterile. Dispersal limitation could occur at two different stages. Firstly, seeds could be limiting gene dispersal if they are not dispersed away from the maternal plant. In contrast to other *Rhododendron* species, seeds of *R. simsii* do not exhibit pronounced winged structures (Ng and Corlett 2000a) that would allow efficient dispersal by wind, suggesting a reliance on gravity for seed dispersal. The same authors also estimated that less than 0.01% of the seeds in *R. simsii* are dispersed more than 80 m away from the maternal plant. Furthermore, it appears unlikely that animals are involved in seed dispersal as seeds are tiny (average 1000 seed weight = 0.07 g, Royal Botanic Gardens Kew 2016), offering little rewards for potential dispersers. Secondly, gene dispersal could be limited by pollen, either as a consequence of insufficient floral pollen production or limited pollen transport by pollinators. Indeed, *R. simsii* was shown to have low pollen/ovule ratios, similar to those of facultative autogamous species (Cruden 1977, Michalski and Durka 2009), low floral nectar production, very low pollinator visitation rates and strong pollen limitation of fruit set (Ng and Corlett 2000a). These reproductive traits of *R. simsii* may result in short gene dispersal distances by reducing opportunities for long-distance gene flow via pollen or seeds. Nevertheless, we found high levels of heterozygosity, indicating that populations are genetically diverse and, although genes only move short distances, homogenizing effects of gene flow appear to dominate over the differentiating effects of drift.

## Current gene flow via pollen

Pollen dispersal distances derived from paternity analyses essentially coincide with estimates of historical gene flow based only on genetic structure of adults. Whereas we observed a maximum mating-distance of 95 m, we found most mating events to be confined to distances of 10–20 m, close to estimates of gene dispersal distance based on genetic structure. These findings confirm that in *R. simsii* pollen is commonly transported over short distances while long-distance (> 100 m) pollen transport appears to be rare. This could be a consequence of *R. simsiis* flowering phenology where single individuals produce flowers en masse. Pollinators may spend more time foraging on each individual due to large floral

display sizes (Makino and Sakai 2007) which in turn could reduce the amount of outcross pollen that is deposited on distant individuals (Karron and Mitchell 2012). Additionally, biparental inbreeding may increase if related individuals are clumped together and pollinators move between them. Nevertheless, by increasing pairwise kinship coefficients of neighbouring individuals, localized kin mating and seed dispersal should increase spatial genetic structure, but this was not the case in our study. Following, we argue that mating of related individuals is common, but less so at the spatial scale examined in the first distance class of our Sp-statistic (0–7 m). We conclude that biparentally inbred individuals should be rather well separated. Alternatively, high local abundances of unrelated individuals could dilute the effects of kin mating. Considering that only ~ 25% of the mating events could be assigned to pollen donors from within our plots and considering relatively low pollen dispersal distances, large and well connected (effective) populations are not only realistic but could also be important in assuring population viability in this species. As our sampling was restricted within plots, our maximum pollen dispersal distance is likely biased downwards as potential long-distance mating events between plots are disregarded. Nevertheless, as short-distance mating makes up the majority of mating events, few undetected long-distance matings are unlikely to strongly affect the overall picture. It appears that short-distance gene flow in *R. simsii* in the GNNR is sufficient to counteract population differentiation and maintain large amounts of genetic variability, despite low observed gene dispersal distances. Considering that our study area comprises one of the glacial refugia to which *R. simsii* retreated during the last glacial maximum (Li et al. 2012), large numbers of genetic variants could have been maintained in the Gutianshan forests. The near-natural state of the Gutianshan Nature Reserve could be essential in maintaining large amounts of genetic diversity, considering that clear cutting is a common practice in conventional forest management. In the face of increasing anthropogenic habitat fragmentation and alterations, nature reserves are crucial refugia for inter- and intraspecific diversity alike.

In summary, we could show that *R. simsii* is highly outcrossing with moderate amounts of biparental inbreeding. Past and present gene flow is largely restricted to short distances but does not lead to strong fine-scale spatial genetic structure. Instead, populations maintained high amounts of genetic diversity with very weak fine-scale SGS.

*Acknowledgements* – We would like to thank the members of the BEF China project and the staff of the Gutianshan National Nature Reserve for their assistance during our field campaigns. German Science Foundation (DFG FOR 891/2; Du 404/3-2 to WD) is highly appreciated.

## References

Auld, J. R. and de Casas, R. R. 2013. The correlated evolution of dispersal and mating-system traits. – Evol. Biol. 40: 185–193.

Bates, D. et al. 2015. Fitting linear mixed-effects models using lme4. – J. Stat. Softw. 67: 1–48.

Brennan, A. C. et al. 2005. Modes and rates of selfing and associated inbreeding depression in the self-incompatible plant *Senecio squalidus* (Asteraceae): a successful colonizing species in the British Isles. – New Phytol. 168: 475–486.

Bruelheide, H. et al. 2011. Community assembly during secondary forest succession in a Chinese subtropical forest. – Ecol. Monogr. 81: 25–41.

Charlesworth, D. and Charlesworth, B. 1987. Inbreeding depression and its evolutionary consequences. – Annu. Rev. Ecol. Syst. 18: 237–268.

Chybicki, I. J. and Burczyk, J. 2013. Seeing the forest through the trees: comprehensive inference on individual mating patterns in a mixed stand of *Quercus robur* and *Q. petraea*. – Ann. Bot. Lond. 112: 561–574.

Cruden, R. W. 1977. Pollen–ovule ratios – conservative indicator of breeding systems in flowering plants. – Evolution 31: 32–46.

Degen, B. et al. 2004. Limited pollen dispersal and biparental inbreeding in *Symphonia globulifera* in French Guiana. – Heredity 93: 585–591.

Delmas, C. E. L. et al. 2014. High lifetime inbreeding depression counteracts the reproductive assurance benefit of selfing in a mass-flowering shrub. – BMC Evol. Biol. 14.

Dick, C. W. et al. 2008. Spatial scales of pollen and seed-mediated gene flow in tropical rain forest trees. – Trop. Plant Biol. 1: 20–33.

Doligez, A. and Joly, H. I. 1997. Genetic diversity and spatial structure within a natural stand of a tropical forest tree species, *Carapa procera* (Meliaceae), in French Guiana. – Heredity 79: 72–82.

Duminil, J. et al. 2009. Plant traits correlated with generation time directly affect inbreeding depression and mating system and indirectly genetic structure. – BMC Evol. Biol. 9: 177.

eFloras. – <www.efloras.org/flora_page.aspx?flora_id=2>, accessed 28 Apr 2016.

Ellstrand, N. C. and Elam, D. R. 1993. Population genetic consequences of small population-size – implications for plant conservation. – Annu. Rev. Ecol. Syst. 24: 217–242.

Frankham, R. 1995. Effective population size–adult population size ratios in wildlife – a review. – Genet. Res. 66: 95–107.

Griffin, C. A. M. and Eckert, C. G. 2003. Experimental analysis of biparental inbreeding in a self-fertilizing plant. – Evolution 57: 1513–1519.

Hamrick, J. L. and Godt, M. J. W. 1996. Effects of life history traits on genetic diversity in plant species. – Phil. Trans. R. Soc. B 351: 1291–1298.

Hamrick, J. L. et al. 1993. The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. – Vegetatio 108: 281–297.

Hardy, O. J. and Vekemans, X. 2002. SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. – Mol. Ecol. Notes 2: 618–620.

Heywood, J. S. 1993. Biparental inbreeding depression in the self-incompatible annual plant *Gaillardia pulchella* (Asteraceae). – Am. J. Bot. 80: 545–550.

Hirao, A. S. 2010. Kinship between parents reduces offspring fitness in a natural population of *Rhododendron brachycarpum*. – Ann. Bot. Lond. 105: 637–646.

Kalinowski, S. T. et al. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. – Mol. Ecol. 16: 1099–1106.

Karron, J. D. and Mitchell, R. J. 2012. Effects of floral display size on male and female reproductive success in *Mimulus ringens*. – Ann. Bot. Lond. 109: 563–570.

Lande, R. and Schemske, D. W. 1985. The evolution of self-fertilization and inbreeding depression in plants. 1. Genetic models. – Evolution 39: 24–40.

Li, Y. et al. 2012. Phylogeographic analysis and environmental niche modeling of widespread shrub *Rhododendron simsii* in China reveals multiple glacial refugia during the last glacial maximum. – J. Syst. Evol. 50: 362–373.
Loiselle, B. A. et al. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). – Am. J. Bot. 82: 1420–1425.
Lou, L. H. and Jin, S. H. 2000. Spermatophyta flora of Gutianshan Nature Reserve in Zhejiang. – J. Beijing For. Univ. pp. 33–39.
Loveless, M. D. and Hamrick, J. L. 1984. Ecological determinants of genetic structure in plant populations. – Annu. Rev. Ecol. Syst. 15: 65–95.
Makino, T. T. and Sakai, S. 2007. Experience changes pollinator responses to floral display size: from size-based to reward-based foraging. – Funct. Ecol. 21: 854–863.
Michalski, S. G. and Durka, W. 2009. Pollination mode and life form strongly affect the relation between mating system and pollen to ovule ratios. – New Phytol. 183: 470–479.
Ng, S. C. and Corlett, R. T. 2000a. Comparative reproductive biology of the six species of *Rhododendron* (Ericaceae) in Hong Kong, south China. – Can. J. Bot. 78: 221–229.
Ng, S. C. and Corlett, R. T. 2000b. Genetic variation and structure in six *Rhododendron* species (Ericaceae) with contrasting local distribution patterns in Hong Kong, China. – Mol. Ecol. 9: 959–969.
Nowak, M. D. et al. 2014. The founding of Mauritian endemic coffee trees by a synchronous long-distance dispersal event. – J. Evol. Biol. 27: 1229–1239.
Petit, R. J. and Hampe, A. 2006. Some evolutionary consequences of being a tree. – Annu. Rev. Ecol. Evol. Syst. 37: 187–214.
R Core Team 2015. – <www.R-project.org/>, accessed 15 Jun 2016.
Ritland, K. 2002. Extensions of models for the estimation of mating systems using n independent loci. – Heredity 88: 221–228.
Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. – Genetics 145: 1219–1228.
Royal Botanic Gardens Kew 2016. – <http://data.kew.org/sid/>, accessed 14 Jun 2016.
Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. – Nat. Biotechnol. 18: 233–234.
Sork, V. L. et al. 1999. Landscape approaches to historical and contemporary gene flow in plants. – Trends Ecol. Evol. 14: 219–224.
Takahashi, T. et al. 2008. Genetic succession and spatial genetic structure in a natural old growth *Cryptomeria japonica* forest revealed by nuclear and chloroplast microsatellite markers. – Forest Ecol. Manage. 255: 2820–2828.
Tan, X. X. et al. 2009. Development and characterization of eight polymorphic microsatellites for *Rhododendron simsii* Planch (Ericaceae). – Conserv. Genet. 10: 1553–1555.
Tang, Z. Y. et al. 2006. Biodiversity in China's mountains. – Front Ecol. Environ. 4: 347–352.
Theim, T. J. et al. 2014. Spatial genetic structure in four understory *Psychotria* species (Rubiaceae) and implications for tropical forest diversity. – Am. J. Bot. 101: 1189–1199.
Vekemans, X. and Hardy, O. J. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. – Mol. Ecol. 13: 921–935.
Wang, X. Q. et al. 2009. Isolation and characterization of twenty-four microsatellite loci for *Rhododendron decorum* Franch. (Ericaceae). – Hortscience 44: 2028–2030.
Wang, X. Q. et al. 2010. Cross-amplification and characterization of microsatellite loci for the genus *Rhododendron*. – Hortscience 45: 1394–1397.
Ward, M. et al. 2005. To self, or not to self... A review of outcrossing and pollen-mediated gene flow in neotropical trees. – Heredity 95: 246–254.
Wright, S. 1943. Isolation by distance. – Genetics 28: 114–138.
Wright, S. 1946. Isolation by distance under diverse systems of mating. – Genetics 31: 39–59.
Zeng, X. Q. et al. 2012. Species diversity and population density affect genetic structure and gene dispersal in a subtropical understory shrub. – J. Plant Ecol. 5: 270–278.

Supplementary material Appendix NJB-01311 at <www.nordicjbotany.org/appendix/njb-01311>. Appendix 1.