Journal of
Plant Ecology

VOLUME 10, NUMBER 1,
PAGES 213–221

FEBRUARY 2017

doi:10.1093/jpe/rtw054

Advance Access publication
31 May 2016

available online at
www.jpe.oxfordjournals.org

Genetic diversity and differentiation follow secondary succession in a multi-species study on woody plants from subtropical China

Christoph Z. Hahn[1,*], *Stefan G. Michalski*[1], *Markus Fischer*[2] *and Walter Durka*[1,3]

[1] *Department of Community Ecology (BZF), Helmholtz Centre for Environmental Research—UFZ, Theodor-Lieser-Straße 4, D-06120 Halle, Germany*
[2] *Institute of Plant Sciences, University of Bern, Altenbergrain 21, CH-3013 Bern, Switzerland*
[3] *German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Deutscher Pl. 5E, 04103 Leipzig, Germany*
*Correspondence address. Department of Community Ecology (BZF), Helmholtz Centre for Environmental Research—UFZ, Theodor-Lieser-Straße 4, D-06120 Halle, Germany. Tel: +49-345-5585315; Fax: +49-345-5585329; E-mail: christoph.hahn@ufz.de

Abstract

Aims
Species diversity and genetic diversity may be affected in parallel by similar environmental drivers. However, genetic diversity may also be affected independently by habitat characteristics. We aim at disentangling relationships between genetic diversity, species diversity and habitat characteristics of woody species in subtropical forest.

Methods
We studied 11 dominant tree and shrub species in 27 plots in Gutianshan, China, and assessed their genetic diversity (A_r) and population differentiation (F'_{ST}) with microsatellite markers. We tested if A_r and population specific F'_{ST} were correlated to local species diversity and plot characteristics. Multi-model inference and model averaging were used to determine the relative importance of each predictor. Additionally, we tested for isolation-by-distance (IBD) and isolation-by-elevation by regressing pairwise F'_{ST} against pairwise spatial and elevational distances.

Important Findings
Genetic diversity was not related to species diversity for any of the study species. Thus, our results do not support joint effects of habitat characteristics on these two levels of biodiversity. Instead, genetic diversity in two understory shrubs, *Rhododendron simsii* and *Vaccinium carlesii*, was affected by plot age with decreasing genetic diversity in successionally older plots. Population differentiation increased with plot age in *R. simsii* and *Lithocarpus glaber*. This shows that succession can reduce genetic diversity within, and increase genetic diversity between populations. Furthermore, we found four cases of IBD and two cases of isolation-by-elevation. The former indicates inefficient pollen and seed dispersal by animals whereas the latter might be due to phenological asynchronies. These patterns indicate that succession can affect genetic diversity without parallel effects on species diversity and that gene flow in a continuous subtropical forest can be restricted even at a local scale.

Keywords: Allelic richness, population differentiation, habitat characteristics, gene flow, species–genetic diversity correlation, SGDC

Received: 7 October 2015, Revised: 22 April 2016, Accepted: 23 May 2016

INTRODUCTION

Genetic and species diversity have largely been studied separately, but over the last decades increasing evidence suggests that both might be controlled by the same natural forces (Antonovics 1976). Genetic variation is ultimately caused by mutation and can be partitioned into selectively neutral and adaptive variation (Holderegger *et al.* 2006), different levels of the former being a result of inbreeding, genetic drift and gene flow whereas the latter is also governed by natural

selection. Analogous processes can be found at the level of species. Speciation is caused by mutation, selection, drift and migration (Gavrilets 2003) and equivalently, selection, drift or migration can create communities containing different levels of species diversity. Recognizing these similarities, a functional connection between both levels of diversity has been proposed (Antonovics 1976). A positive correlation of species with genetic diversity (positive species–genetic diversity correlation, SGDC) is expected if the above mentioned processes act in parallel on both diversity levels (Vellend 2004; Vellend and Geber 2005). Empirical data confirming this hypothesis has increased over the last decade, reporting positive SGDCs in various habitats and species (Cleary *et al.* 2006; He *et al.* 2008; Odat *et al.* 2010; Papadopoulou *et al.* 2011; Wehenkel *et al.* 2006). Nevertheless, studies concerned with SGDCs often focus on single species while comparative studies incorporating multiple species remain scarce (but see Taberlet *et al.* 2012). Consequently, reports of non-existing or negative SGDCs observed in single species question the general validity of SGDCs (Odat *et al.* 2004; Puscas *et al.* 2008). Furthermore, environmental factors are often reported to influence both species and genetic diversity (Lamy *et al.* 2013; Odat *et al.* 2010). Thus, simultaneously testing for SGDCs and environmental influences can disentangle mutual interactions of species diversity, genetic diversity and environmental factors (Yang *et al.* 2017) and provide new insights in which factors determine the evolutionary potential of species and populations.

Studying genetic diversity along environmental gradients yielded valuable information on drivers of genetic structure. Especially elevational clines encompass gradients of many environmental factors, such as temperature or moisture which not only determine the selective regime but may also affect phenological traits such as the time of flowering (Blionis *et al.* 2001; Qian *et al.* 2014; Scheepens *et al.* 2012) or pollination success (Alonso 2005). Consequently, by creating ecological gradients, elevational clines can have a strong impact on neutral genetic processes such as drift and gene flow (Loveless and Hamrick 1984; Ohsawa and Ide 2008; Shi *et al.* 2011). As drift is strongly determined by population size, a gradual change in population density, as it is often seen along altitudinal clines, will result in genetic drift if not countered by gene flow (Lande 1988; Lynch *et al.* 1995).

Similar to elevational clines, secondary succession constitutes strong environmental gradients. The gradual transition from pioneer to climax communities is accompanied by a gradual change in the availability of natural resources such as light and space (Wang *et al.* 2017). As species compete for resources in late successional stages, this has direct effects on species composition and on population structure and density of individual species which in turn can affect genetic diversity (Takahashi *et al.* 2008). Changing population densities will affect neutral processes such as drift or gene flow. This can be direct as in the case of extensive canopies in late successional stages acting as pollen traps (McKibbin 2006) or stochastically via demographic processes (Chung *et al.* 2007). As environmental predictability increases with increasing forest age, locally adapted genotypes might be favoured, reducing genetic diversity (Mulcahy 1975). Thus, secondary succession can affect the distribution of genetic diversity by both selection regimes and population demography (Wehenkel *et al.* 2011).

The determinants of within-population genetic diversity also affect among-population differentiation. If genetic drift is at an equilibrium with the homogenizing effect of gene flow, a pattern of isolation-by-distance (IBD) is expected due to distance limitations of gene flow (Hutchison and Templeton 1999; Wright 1945). Gene flow itself can be restricted either by physical barriers such as mountains or decrease with geographic distance. Selection among habitats with strong environmental clines will select against maladapted migrants and result in populations with similar genetic structuring as under IBD (Sexton *et al.* 2014; Slatkin 1985, 1987). Thus, with increasing ecological distance between populations the isolation may increase as well resulting in isolation-by-environment. Gradients that potentially lead to local adaptation include climatic conditions (Franks and Weis 2009), habitat types (McNeilly 1968; Nosil *et al.* 2005) or elevation (Shi *et al.* 2011). Isolation-by-environment patterns are expected to evolve for adaptive genetic variation. However, also for neutral genetic variation they may become apparent due to genetic linkage or when environmental distance coincides with partial reproductive isolation.

To elucidate the relationship between genetic diversity, species diversity and environmental conditions we conducted a comparative genetic study in subtropical mixed evergreen broad-leaved forest, one of the global biodiversity hotspots. Because the effects of gene flow, drift and selection in a given community are species specific, a multi-species approach is necessary to distinguish common from species-specific patterns. We therefore assessed species diversity of communities and genetic diversity and differentiation between populations of eleven abundant woody species. Using data on plot-level environmental conditions we also tested for the effect of the environment on genetic diversity and differentiation. Specifically we asked: (i) Is genetic diversity within species correlated to species diversity of the local community (SGDC), local environmental conditions or a combination thereof? Furthermore, we tested whether (ii) population differentiation increases with geographic, elevational or environmental distance. Working in a multi-species framework allows us to test whether responses follow a general pattern or if responses vary among species.

MATERIALS AND METHODS

Study area, species and sampling

Our study was conducted in subtropical South-Eastern China, in the Gutianshan National Nature Reserve (GNNR), Zhejiang Province (29°14′34.81″N–118° 6′43.89″E). This nature reserve has been established in 1975 and harbours old growth, mixed

evergreen broad-leafed forests of advanced successional stages on a steeply sloped, mountainous terrain. With over 1400 species of vascular plants (Lou 2000) it is one of the biodiversity hotspots of China and the subtropics. In 2008, 27 plots, stratified for successional stage, were randomly chosen in the GNNR (for details see Bruelheide *et al.* 2011). These so-called comparative study plots (CSP) have a projected area of 30 m × 30 m, range in woody species diversity between 25 and 69, and range in elevation from 251 to 903 meters above sea level (m a.s.l.). We selected 11 target species according to high individual species abundances across the CSPs and the availability of molecular markers. Among these species were two shrubs (*Ardisia crenata* Sims, *Syzygium buxifolium* Hooker & Arnott), two shrub-small trees (*Rhododendron simsii* Planchon, *Vaccinium carlesii* Dunn) and seven large trees (*Castanopsis eyrei* (Champion ex Bentham) Tutcher, *Castanopsis fargesii* Franchet, *Cyclobalanopsis glauca* (Thunberg) Oersted, *Daphniphyllum oldhamii* (Hemsley) K. Rosenthal in Engler, *Lithocarpus glaber* (Thunberg) Nakai, *Quercus serrata* Murray, *Schima superba* Gardner & Champion) (Table 1). Most of the species had a certain preference to certain successional stages, whereas for four species no such preference was observed (Table 1). Up to 30 leaf samples were collected for each species, if present, from each CSP, or in the direct vicinity (*R. simsii* in CSP 21; Table 1). Samples were oven-dried or lyophilized for 48 h after collection. Our study includes previously published data on *C. eyrei* (Shi *et al.* 2011, data from CSPs, excluding populations A–D for tests of plot characteristics) and *A. crenata* (Zeng *et al.* 2012) collected in the same plots. We considered the following plot characteristics as assessed by Bruelheide *et al.* (2011): elevation, species diversity (number of woody species taller than 1 m) and plot age, i.e. the age in years of the fifth-largest individual tree per plot, as a measure of successional stage. When we used successional stage classes (1–5) rather than plot age, we arrived at very similar results and therefore only present the ones with plot age.

DNA extraction and genotyping

Genomic DNA was extracted from leaf samples using the QIAGEN DNEasy Plant 96 kit (QIAGEN Hilden, Germany) for all species except for *R. simsii*, which produced large amounts of mucilage during extraction. For these samples we used the peqGOLD Tissue DNA kit (peqlab GmbH Erlangen, Germany). PCR primers were either adapted from recent publications or specifically designed after whole-genome shot-gun sequencing in the case of *D. oldhamii* (online supplementary Table S1). All newly designed primers were developed using the Primer3 web tool (Untergasser *et al.* 2012). PCR was then run as a multiplexed three primer touchdown PCR with the following protocol: 15 min at 95°C followed by 20 cycles of 30 s at 94°C, 30 s of annealing at 60°C decreasing by 0.5°C per cycle and 90 s at 72°C, followed by another 20 cycles with a constant annealing temperature of 50°C and a final extension step at 72°C for 10 min. PCR products were analysed on an ABI3130 capillary sequencer using LIZ500 size standard. Individual genotyping was carried out using the GeneMapper software v5.0 (Life Technologies GmbH, Darmstadt, Germany).

Data editing

Because we also included primers not originally designed for our target species we assumed the existence of null alleles which would bias the results. Therefore, we performed the following procedure on all but already published data: first we excluded all individuals with less than three successfully genotyped loci. Second, assuming that lack of amplification was due to presence of homozygous null alleles, we replaced missing genotype data with a hypothetical additional allele in homozygous genotype. We used Microchecker v2.2 (Van Oosterhout *et al.* 2004) to assess the frequency of null alleles and excluded loci which showed >20% null alleles in >33% of the analysed populations. Genotypes were then adjusted using the Oosterhout estimation method implemented in Microchecker by introducing the hypothetical null allele in heterozygous state. All following computations were carried out using the adjusted genotypes. Since microsatellites are found in non-coding areas in the genome they are assumed to be selectively neutral. To test if this holds true, we performed a test for selective neutrality using the lositan selection workbench (Beaumont and Nichols 1996). We used the default settings and loci outside the 95% confidence interval were assumed to be under diversifying selection and excluded from further analyses. Analyses with Microchecker resulted in the exclusion of between one and four loci in 8 out of 11 species and the lositan analysis in the exclusion of 1–4 loci in 5 out of 11 species (online supplementary Table S2). After this procedure the number of loci analysed ranged between three and nine per species. The final data set consisted of 2439 individuals of 11 species with between 6 and 25 populations per species (Table 1).

Genetic diversity and differentiation

We calculated descriptors of genetic diversity and genetic differentiation as allelic richness A_r and F'_{ST} values, respectively, using the Fstat software v2.9.3.2 (Goudet 1995). Genetic differentiation, both overall and population specific, was calculated as the standardized F'_{ST}, because in highly diverse marker systems such as microsatellites, F_{ST} can remain low even though differentiation between populations might be high (Hedrick 2005). This was done as $F'_{ST} = F_{ST}/F_{ST\_max}$ with F_{ST_max} being the maximum possible F_{ST} given our data. F_{ST_max} was calculated with *F*stat after re-coding genotype data with the software RecodeData v0.1 (Meirmans 2006). Population specific F'_{ST} values were calculated as the mean of all pairwise F'_{ST} values between a population and the remaining populations.

Model averaging and linear regressions

To test whether genetic diversity and population differentiation are influenced by species diversity and local plot properties we used a stepwise model selection and multi-model inference procedure (Burnham and Anderson 2002, 2004)

Table 1: life history traits of the 11 species chosen in the present study

Species	Family	LF[a]	LH[a]	Poll.	Sdisp	FruiSeedPr, 1k SeedW[b]	SuccPref[c]	PopDens Ind./ha[a]
Ardisia crenata	Myrsinaceae	S	E	I[d]	M, B[b,d]	Bright red berries, 221g[d]	Late, 80+ years	0.1
Castanopsis eyrei	Fagaceae	T	E	I[e,f]	G, R[e]	Nut[e]	No preference	516.0
Castanopsis fargesii	Fagaceae	T	E	I[f,g]	G, R, B[g,h]	Nut	Mid, <60 years	51.3
Cyclobalanopsis glauca	Fagaceae	T	E	W[f]	A[i]	Nut[i]	Mid-early, <40 years	67.5
Daphniphyllum oldhamii	Daphniphyllaceae	S/T	E	I in *Daphniphyllum teijsmanni*[j]	M, B[i]	Drupe[i]	Late, 80+ years	113.3
Lithocarpus glaber	Fagaceae	T	E	I[k]	A[i]	Nut, 1162g[i]	Late, 80+ years	46.4
Quercus serrata	Fagaceae	T	D	W[f]	A[i]	Nut, 1934g[i]	Early, <20 years	146.2
Rhododendron simsii	Ericaceae	S	D	I[l]	W, G[l]	Capsule, 0.07g[l]	Early, <20 years	196.7
Schima superba	Theaceae	T	E	I[k]	W, G[i]	Capsule, 4.2g[i]	No preference	354.8
Syzygium buxifolium	Myrtaceae	S	E	I[m]	M, C, B[b,m]	Berry	No preference	142.8
Vaccinium carlesii	Ericaceae	S	E	I	B[b,i]	Berry[i]	No preference	75.1

Abbreviations: LF = life form (S = shrub, T = tree), LH = leaf habit (D = deciduous, E = evergreen), Poll = pollinator (I = insect, W = wind), Sdisp = seed disperser (A = animals, B = birds, C = civet, G = gravity, M = mammals, R = rodents, W = wind), FruiSeedPr = fruit and seed properties, 1k SeedW = average 1000 seed weight, SuccPref = successional preference, PopDens = population density.
References given in table header apply to the whole column. Information without references had to be inferred from congeneric species or personal observations.
[a]Ma *et al.* (2009), [b]Royal Botanic Gardens Kew (2016), [c]Bruelheide *et al.* (2011), [d]Zeng *et al.* (2012), [e]Shi *et al.* (2011), [f]Manos *et al.* (2001), [g]Chen *et al.* (2008), [h]Xiao *et al.* (2005), [i]Du *et al.* (2009), [j]Yumoto (1987), [k]Corlett (2001), [l]Ng *et al.* (2000a), [m]Lughadha and Proenca (1996), [n]Zhang *et al.* (2006).

on linear models containing A_r and F'_{ST} as response variable and plot characteristics as linear predictors. We used the function dredge() from the 'MuMIn' R-package (Barton 2015) for model selection and to evaluate all possible combinations of predictors starting from a full global model of the form: lm(A_r/F'_{ST} ~ species diversity × plot age × elevation × species).

Model fits were assessed by the AIC_c, a sample size corrected version of the Akaike information criterion which penalizes the number of model parameters more heavily than the AIC does. We calculated ΔAIC_c for each model, which is the difference in AIC_c of each model to the model with the lowest AIC_c (the best-fitting model). Critical ΔAIC_c was set to be <4 as suggested in Barton (2015). Models with a ΔAIC_c <4 were used to calculate averaged and weighted coefficients that allowed to assess the significance of model terms. Secondly, we performed separate linear regression analyses for each species with both A_r and F'_{ST}. We used a model averaging approach to test for general effects across species whereas linear regressions allowed us to explore species-specific responses.

Testing for isolation patterns

We tested for IBD, isolation-by-elevation (IBE), isolation-by-community divergence (IBC) and isolation-by-succession (IBS) patterns. We used Mantel and partial Mantel tests using the function mantel() and mantel.partial() implemented in the R- package 'vegan' (Oksanen *et al.* 2015). Preliminary tests revealed a significant correlation between log(distance) and log(elevation) over all CSPs ($r = 0.204$, $P = 0.004$). Consequently, we tested for IBD and IBE using partial Mantel-tests, correlating F'_{ST} with log(distance) and log(elevation) while correcting for log(elevation) and log(distance), respectively. Pairwise community divergence was measured as Bray–Curtis dissimilarity based on individual densities of taxa. Mantel tests were carried out to assess the correlation between F'_{ST} and community divergence. Lastly, we also performed Mantel tests on F'_{ST} and plot age to test for IBS. All the above computations were carried out in the R statistical software v3.2.1 (R Core Team 2015).

RESULTS

Effect of species diversity and environment on genetic diversity and differentiation

Mean allelic richness ranged between A_r = 2.433 in *C. glauca* populations and A_r = 6.522 in *C. eyrei* populations. Standardized F'_{ST} ranged from 0.062 to 0.243 in *D. oldhamii* and *S. buxifolium*, respectively (Table 2). According to multi-model inference species identity had the highest impact on allelic richness followed by plot age, elevation and species diversity (Table 3). Population differentiation was strongly affected by both species identity and plot age whereas elevation and species diversity had relatively low importance (Table 3). Linear regressions revealed significant correlations of A_r with plot age in *R. simsii* ($P = 0.0002$) and *V. carlesii* ($P = 0.0207$) (Fig. 1). Similarly, F'_{ST} was significantly correlated with plot age in *L. glaber* ($P = 0.0136$) and *R. simsii* ($P = 0.0249$).

Isolation patterns

In total, we found isolation patterns in four species (online supplementary Table S4). *Ardisia crenata* and *S. superba* showed significant IBD. Furthermore, we found significant IBE patterns in *C. eyrei* and *D. oldhamii*. We did not detect any significant IBC and although successional stage and altitude were significantly correlated we did not find any significant IBS pattern either.

Table 2: general overview on total, mean and minimum sample size, number of populations and loci and global levels of within population diversity (A_r) and among population differentiation (F'_{ST}) for each of 11 woody species of subtropical forest in China

Species	Total sample size	Mean sample size/population	Minimum sample size	No. of population	Loci used	A_r	F'_{ST}
Ardisia crenata	359	29.9	29	12	5	6.198	0.154
Castanopsis eyrei	583	23.4	12	20	7	6.522	0.151
Castanopsis fargesii	109	18.2	13	6	7	5.210	0.147
Cyclobalanopsis glauca	90	12.9	6	7	7	2.433	0.117
Daphniphyllum oldhamii	77	10.9	4	8	9	3.486	0.062
Lithocarpus glaber	251	17.9	8	14	3	3.552	0.122
Quercus serrata	115	12.2	5	9	4	3.887	0.138
Rhododendron simsii	147	7.4	4	18	4	4.903	0.239
Schima superba	432	17.3	7	24	7	4.684	0.145
Syzygium buxifolium	105	17.5	8	6	4	5.750	0.243
Vaccinium carlesii	171	19.0	11	9	5	5.721	0.086

Table 3: variable importance per predictor after model averaging

	Measure of genetic diversity	
Model term	A_r	F'_{ST}
Species	1	1
Plot age	0.40	1
Elevation	0.23	0.29
Species diversity	0.19	0.29
Plot age × species diversity	—	0.07
Plot age × elevation	—	—
Species diversity × plot age	—	—
Model with highest Akaike weight	A_r ~ species	F'_{ST} ~ plot age + species

Higher values indicate that factors appear more frequently in significant models.

DISCUSSION

Genetic diversity and species diversity

Our study does not support parallel effects on species and genetic diversity as proposed by Vellend and Geber (2005). Studies showing connections between species and genetic diversity often considered isolated or island populations (Cleary *et al.* 2006; Fady and Conord 2010; Lamy *et al.* 2013; Struebig *et al.* 2011). Under such conditions locality characteristics such as area or heterogeneity potentially affect both levels of diversity (Vellend and Geber 2005). In contrast, our study system is a continuous forest where only the mountain topography represents a potential physical barrier. That we found IBD and IBE indicates that dispersal limitation occurs at this spatial scale for some species. However, the majority of species did not show limitation of gene flow. Thus, the lack of SGDC likely is a consequence of high gene flow and negligible drift across the study system for many of the species. This is in line with earlier findings at the community level, where only weak community differentiation between successional stages was reported for these plots (Bruelheide *et al.* 2011).

Genetic diversity, environment and succession

Plot age was the only plot property of significant impact on genetic diversity. Allelic richness continuously declined with plot age, in particular for two common shrub species, *R. simsii* and *V. carlesii*. We also found increasing population differentiation with succession in *R. simsii* and *L. glaber.* As we omitted putatively selected loci from the analysis, these effects should be caused by neutral processes. Therefore, we suggest the following scenario. When a community undergoes secondary succession, initially induced by natural or anthropogenic disturbance, the available open space is filled by individuals from the regional pool. Since resources are not limiting at this successional stage, stochastic sampling from the available gene pool allows for a large number of genotypes per species to establish, sampling the genetic diversity of the whole regional gene pool. With progressing succession, competition for resources such as light and space reduces individual species abundance according to competitive strength (Tilman 1994), leading to reduced population sizes during succession, especially for weak competitors. Since genetic diversity, especially allelic richness, is strongly correlated with population size, a decrease in neutral genetic diversity is expected with succession, in agreement with our findings for the two shrub species *R. simsii* and *V. carlesii*.

Simultaneously, a reduction of population sizes enhances population differentiation if not countered by among population gene flow as seen in *R. simsii* and the tree *L. glaber*. *Rhododendron simsii* has been shown to be shade intolerant (Ng and Corlett 2000b) which is corroborated by the observed reduction of abundance in late successional stages (online supplementary Table S3). Also, we rarely found *R. simsii* flowering in late successional plots (C. Z. Hahn, CZH, personal observation), which reduces the opportunity for pollen-mediated gene flow. That not all three species showed both a reduction of diversity and

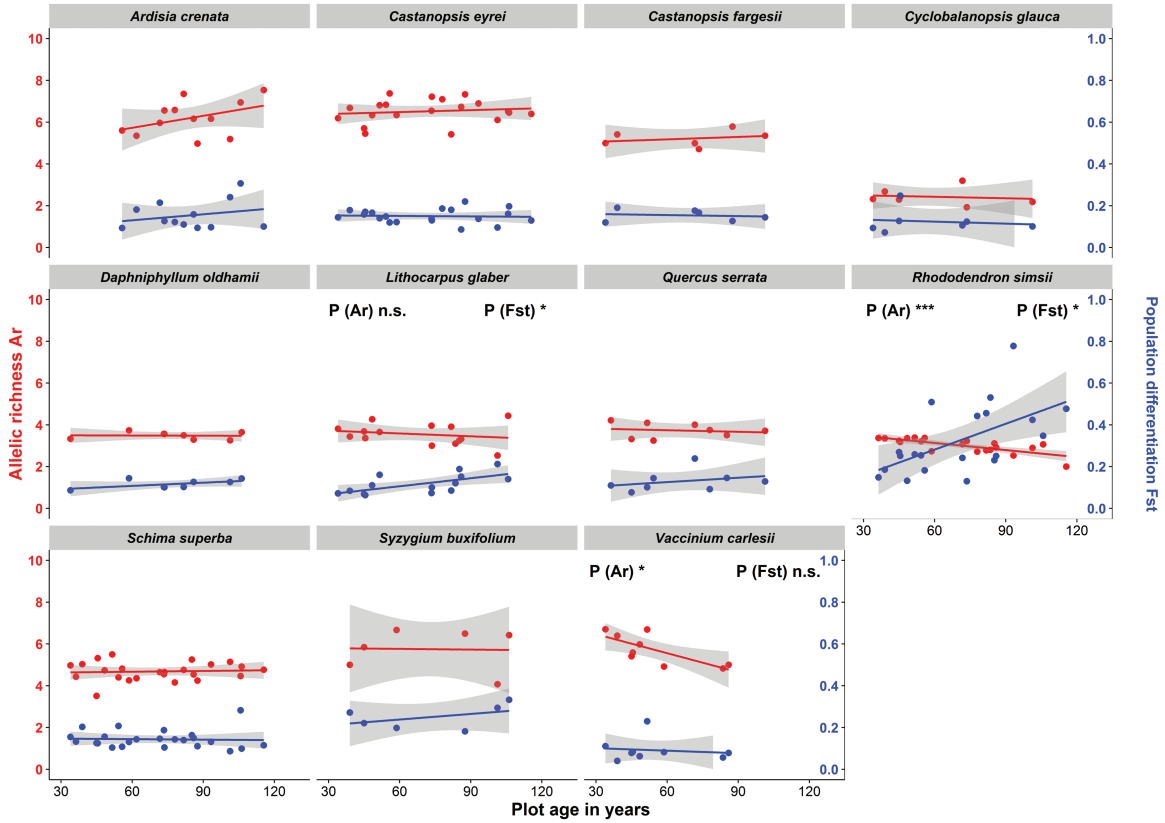

Figure 1: population differentiation and allelic richness for 11 woody species in subtropical forest in China in relation to plot age. Points and lines in blue refer to population differentiation, red represents allelic richness. Grey envelopes show the 0.95 confidence interval. *$P < 0.05$; ***$P < 0.001$.

an increase of differentiation is likely due to different allele frequency distributions between species and to the stochasticity of genetic drift. Especially, the frequency of the most common allele has been shown to strongly affect F_{ST} (Jakobsson *et al.* 2013), but is less relevant for allelic richness.

In summary, we suggest that forest dynamics and secondary succession play an important role for maintaining high levels of genetic diversity through disturbance, especially in early successional understory species. The species in which genetic diversity or differentiation was affected by succession were not characterized by a particular set of life history traits. Thus, demographic changes through succession rather than life history traits appear to be suited explaining the observed patterns.

Patterns of genetic differentiation

In two species, one shrub and one tree, we found evidence of IBD indicating dispersal limitation at a spatial scale of <5 km. Gene flow can be limited for pollen or seeds. For both, gene flow depends on efficient dispersal agents and a lack of dispersal facilitates reproductive isolation (Hamrick *et al.* 1993; Slatkin 1985), especially in obligate outcrossers or self- incompatible species (Ghazoul 2005). Low levels of gene flow were already reported in our study area for *A. crenata* (Zeng *et al.* 2012), a small insect-pollinated shrub. The authors argued that short foraging distances of pollinating insects and seed dispersing mammals were causing high population differentiation. As *S. superba* is also insect-pollinated (Table 1), limited pollinator availability could similarly cause IBD in this species. As mentioned above, the study area is also characterized by low abundances of seed dispersing mammals and birds (Zeng *et al.* 2012). Furthermore, small population sizes have been shown to decrease pollination effectiveness (e.g. Wilcock and Neiland 2002). This could further foster population differentiation in *A. crenata* as this species often maintains small populations. Thus, the observed patterns of IBD in two species indicate inefficient gene flow *via* pollen or seeds in these species.

In addition to IBD we detected IBE in *C. eyrei* and *D. oldhamii*. IBE is observed along elevational clines that impose barriers to gene flow *via* shifts in abiotic habitat qualities among which temperature appears to be the main driver (Normand *et al.* 2002). These shifts in turn affect traits such as time and duration of flowering (Blionis *et al.* 2001; Gomez-Garcia *et al.* 2009; Singh *et al.* 2015) or bud burst (Normand *et al.* 2002; Rusch 1993) potentially leading to phenological asynchrony reducing gene flow between different elevations. Additionally, IBE could also indicate drift due to small effective population sizes at elevational extremes as shown by Herrera and Bazaga (2008), which may be particularly relevant for *D. oldhamii* which had low population densities in the study plots. Small effective population sizes and phenological shifts along elevational clines offer valid explanations for the observed pattern of IBE, both of which are not mutually exclusive.

Finally, more than half of our species did not exhibit any isolation pattern. As mean levels of population differentiation were low to moderate, this indicates that gene flow is more influential than drift (Hutchison and Templeton 1999). Thus, the majority of our species is not dispersal limited and maintains sufficient levels of gene flow at the spatial scale investigated to prevent genetic drift. Determining whether this is through efficient dispersal or adaptive phenological strategies such as synchronous flowering (Zhang *et al.* 2010) offers interesting research avenues for future studies.

CONCLUSIONS

This observational multi-species study showed clearly that genetic diversity was not related to species diversity. Instead, successional stage of populations and spatial and elevational distances between populations affected genetic diversity in several species. This adds to the increased knowledge about the various effects of succession on tree growth (Chi *et al.* 2017), litterfall (Huang *et al.* 2017) and fungal communities (Zhang *et al.* 2017) in this forest ecosystem. Furthermore, idiosyncratic and species-specific processes were observed similar to other studies (Zeng *et al.* 2017b) in this multispecies community. We conclude that our multi-species approach was decisive to distinguish common and species-specific patterns (see also Zeng *et al.* 2017a) and we advocate further multi-species studies of genetic diversity and its drivers.

SUPPLEMENTARY MATERIAL

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

FUNDING

German Science Foundation (DFG FOR 891/2; Du 404/3-2 to W.D.).

ACKNOWLEDGEMENTS

We thank the staff of the Gutianshan National Nature Reserve and the BEF China team for their assistance in every aspect. Additionally, we would like to thank Xueqin Zeng for her help in sampling and Jennifer Born, Valeska Vees and Leonard Schulte for their help in genotyping. *Conflict of interest statement*. None declared.

REFERENCES

Alonso C (2005) Pollination success across an elevation and sex ratio gradient in gynodioecious *Daphne laureola*. *Am J Bot* **92**:1264–9.

Antonovics J (1976) The input from population genetics: "the new ecological genetics". *Syst Bot* **1**:233–45.

Barton K (2015) *MuMIn: Multi-Model Inference*. R package version 1.14.0. https://cran.r-project.org/web/packages/MuMIn/MuMIn.pdf.

Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. *Proc R Soc B* **263**:1619–26.

Blionis GJ, Halley JM, Vokou D (2001) Flowering phenology of Campanula on Mt Olympos, Greece. *Ecography* **24**:696–706.

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.

Burnham KP, Anderson DR (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. New York: Springer-Verlag New York.

Burnham KP, Anderson DR (2004) Multimodel inference—understanding AIC and BIC in model selection. *Sociol Method Res* **33**:261–304.

Chen XY, Fan XX, Hu XS (2008) Roles of seed and pollen dispersal in natural regeneration of *Castanopsis fargesii* (Fagaceae): implications for forest management. *Forest Ecol Manag* **256**:1143–50.

Chi XL, Guo Q, Fang JY, *et al*. (2017) Seasonal characteristics and determinants of tree growth in a Chinese subtropical forest. *J Plant Ecol* **10**:4–12.

Chung MY, Nason JD, Chung MG (2007) Effects of population succession on demographic and genetic processes: predictions and tests in the daylily *Hemerocallis thunbergii* (Liliaceae). *Mol Ecol* **16**:2816–29.

Cleary DFR, Fauvelot C, Genner MJ, *et al*. (2006) Parallel responses of species and genetic diversity to El Nino Southern Oscillation-induced environmental destruction. *Ecol Lett* **9**:301–7.

Corlett RT (2001) Pollination in a degraded tropical landscape: a Hong Kong case study. *J Trop Ecol* **17**:155–61.

Du YJ, Mi XC, Liu XJ, *et al*. (2009) Seed dispersal phenology and dispersal syndromes in a subtropical broad-leaved forest of China. *Forest Ecol Manag* **258**:1147–52.

Fady B, Conord C (2010) Macroecological patterns of species and genetic diversity in vascular plants of the Mediterranean basin. *Divers Distrib* **16**:53–64.

Franks SJ, Weis AE (2009) Climate change alters reproductive isolation and potential gene flow in an annual plant. *Evol Appl* **2**:481–8.

Gavrilets S (2003) Perspective: models of speciation: what have we learned in 40 years? *Evolution* **57**:2197–215.

Ghazoul J (2005) Pollen and seed dispersal among dispersed plants. *Biol Rev* **80**:413–43.

Gomez-Garcia D, Azorin J, Aguirre AJ (2009) Effects of small-scale disturbances and elevation on the morphology, phenology and reproduction of a successful geophyte. *J Plant Ecol* **2**:13–20.

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. *J Hered* **86**:485–6.

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.

He TH, Lamont BB, Krauss SL, *et al*. (2008) Covariation between intraspecific genetic diversity and species diversity within a plant functional group. *J Ecol* **96**:956–61.

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 (Edinb)* **100**:275–85.

Holderegger R, Kamm U, Gugerli F (2006) Adaptive vs. neutral genetic diversity: implications for landscape genetics. *Landscape Ecol* **21**:797–807.

Huang YY, Ma YL, Zhao K, *et al*. (2017) Positive effects of tree species diversity on litterfall quantity and quality along a secondary successional chronosequence in a subtropical forest. *J Plant Ecol* **10**:28–35.

Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influence of gene flow and drift on the distribution if genetic variability. *Evolution* **53**:1898–914.

Jakobsson M, Edge MD, Rosenberg NA (2013) The relationship between F_{ST} and the frequency of the most frequent allele. *Genetics* **193**:515–28.

Lamy T, Jarne P, Laroche F, *et al*. (2013) Variation in habitat connectivity generates positive correlations between species and genetic diversity in a metacommunity. *Mol Ecol* **22**:4445–56.

Lande R (1988) Genetics and demography in biological conservation. *Science* **241**:1455–60.

Lou J (2000) Spermatophyta flora of Gutianshan Nature Reserve in Zhejiang. *J Beijing For Univ* **22**:33–9.

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. *Annu Rev Ecol Syst* **15**:65–95.

Lughadha EN, Proenca C (1996) A survey of the reproductive biology of the Myrtoideae (Myrtaceae). *Ann Mo Bot Gard* **83**:480–503.

Lynch M, Conery J, Burger R (1995) Mutation accumulation and the extinction of small populations. *Am Nat* **146**:489–518.

Ma KP, Chen B, Mi XC (2009) *Gutianshan Forest Dynamic Plot Tree Species and Their Distribution Patterns.* Beijing: China Forestry Publishing House.

Manos PS, Zhou ZK, Cannon CH (2001) Systematics of Fagaceae: phylogenetic tests of reproductive trait evolution. *Int J Plant Sci* **162**:1361–79.

McKibbin R (2006) Modelling pollen distribution by wind through a forest canopy. *Jsme Int J B-Fluid T* **49**:583–9.

McNeilly T (1968) Evolution in closely adjacent plant populations. 3. *Agrostis tenuis* on a small copper mine. *Heredity* **23**:99–108.

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

Mulcahy DL (1975) Differential mortality among cohorts in a population of *Acer saccharum* (Aceraceae) seedlings. *Am J Bot* **62**:422–6.

Ng SC, Corlett RT (2000a) Comparative reproductive biology of the six species of Rhododendron (Ericaceae) in Hong Kong, South China. *Can J Bot* **78**:221–9.

Ng SC, Corlett RT (2000b) Genetic variation and structure in six *Rhododendron* species (Ericaceae) with contrasting local distribution patterns in Hong Kong, China. *Mol Ecol* **9**:959–69.

Normand F, Habib R, Chadoeuf J (2002) A stochastic flowering model describing an asynchronically flowering set of trees. *Ann Bot-London* **90**:405–15.

Nosil P, Vines TH, Funk DJ (2005) Perspective: reproductive isolation caused by natural selection against immigrants from divergent habitats. *Evolution* **59**:705–19.

Odat N, Hellwig FH, Jetschke G, *et al*. (2010) On the relationship between plant species diversity and genetic diversity of *Plantago lanceolata* (Plantaginaceae) within and between grassland communities. *J Plant Ecol-UK* **3**:41–8.

Odat N, Jetschke G, Hellwig FH (2004) Genetic diversity of *Ranunculus acris* L. (Ranunculaceae) populations in relation to species diversity and habitat type in grassland communities. *Mol Ecol* **13**:1251–7.

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.

Oksanen J, Guillaume Blanchet F, Kindt R, *et al*. (2015) *vegan: Community Ecology Package*. R package version 2.2-1. http://CRAN.R-project.org/package=vegan.

Papadopoulou A, Anastasiou I, Spagopoulou F, *et al*. (2011) Testing the species-genetic diversity correlation in the *Aegean archipelago*: toward a haplotype-based macroecology? *Am Nat* **178**:241–55.

Puscas M, Taberlet P, Choler P (2008) No positive correlation between species and genetic diversity in European alpine grasslands dominated by *Carex curvula*. *Divers Distrib* **14**:852–61.

Qian SH, Saito W, Mimura M, *et al*. (2014) Asymmetric gene flow and the distribution of genetic diversity in morphologically distinct *Abies mariesii* populations in contrasting eco-habitats. *Plant Ecol* **215**:1385–97.

R Core Team (2015) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Royal Botanic Gardens Kew (2016) *Seed Information Database (SID)*. Version 7.1. http://data.kew.org/sid/ (6 April 2016, date last accessed).

Rusch VE (1993) Altitudinal variation in the phenology of *Nothofagus pumilio* in Argentina. *Rev Chil Hist Nat* **66**:131–41.

Scheepens JF, Frei ES, Armbruster GFJ, *et al*. (2012) Pollen dispersal and gene flow within and into a population of the alpine monocarpic plant *Campanula thyrsoides*. *Ann Bot* **110**:1479–88.

Sexton JP, Hangartner SB, Hoffmann AA (2014) Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution* **68**:1–15.

Shi MM, 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 forests, *Castanopsis eyrei*. *PLOS ONE* **6**:e21302.

Singh N, Ram J, Tewari A, *et al*. (2015) Phenological events along the elevation gradient and effect of climate change on *Rhododendron arboreum* Sm. in Kumaun Himalaya. *Curr Sci India* **108**:106–10.

Slatkin M (1985) Gene flow in natural populations. *Annu Rev Ecol Syst* **16**:393–430.

Slatkin M (1987) Gene flow and the geographic structure of natural populations. *Science* **236**:787–92.

Struebig MJ, Kingston T, Petit EJ, *et al*. (2011) Parallel declines in species and genetic diversity in tropical forest fragments. *Ecol Lett* **14**:582–90.

Taberlet P, Zimmermann NE, Englisch T, *et al*. (2012) Genetic diversity in widespread species is not congruent with species richness in alpine plant communities. *Ecol Lett* **15**:1439–48.

Takahashi T, Tani N, Niiyama K, *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 Manag* **255**:2820–8.

Tilman D (1994) Competition and biodiversity in spatially structured habitats. *Ecology* **75**:2–16.

Untergasser A, Cutcutache I, Koressaar T, *et al*. (2012) Primer3—new capabilities and interfaces. *Nucleic Acids Res* **40**:1–12.

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.

Vellend M (2004) Parallel effects of land-use history on species diversity and genetic diversity of forest herbs. *Ecology* **85**:3043–55.

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

Wang C, Ma YL, Trogisch S, *et al*. (2017) Soil respiration is driven by fine root biomass along a forest chronosequence in subtropical China. *J Plant Ecol* **10**:36–46.

Wehenkel C, Bergmann F, Gregorius HR (2006) Is there a trade-off between species diversity and genetic diversity in forest tree communities? *Plant Ecol* **185**:151–61.

Wehenkel C, Corral-Rivas JJ, Hernandez-Diaz JC (2011) Genetic diversity in relation to secondary succession of forest tree communities. *Pol J Ecol* **59**:45–60.

Wilcock C, Neiland R (2002) Pollination failure in plants: why it happens and when it matters. *Trends Plant Sci* **7**:270–7.

Wright S (1945) Isolation by distance under diverse systems of mating. *Genetics* **30**:571–2.

Xiao ZS, Zhang ZB, Wang YS (2005) The effects of seed abundance on seed predation and dispersal by rodents in *Castanopsis fargesii* (Fagaceae). *Plant Ecol* **177**:249–57.

Yang B, Li Y, Ding BY, *et al*. (2017) Impact of tree diversity and environmental conditions on the survival of shrub species in a forest biodiversity experiment in subtropical China. *J Plant Ecol* **10**:179–89.

Yumoto T (1987) Pollination systems in a warm temperate evergreen broad-leaved forest on Yaku Island. *Ecol Res* **2**:133–45.

Zeng XQ, Durka W, Fischer M (2017a) Species-specific effects of genetic diversity and species diversity of experimental communities on early tree performance. *J Plant Ecol* **10**:252–8.

Zeng XQ, Michalski SG, Fischer M, *et al*. (2012) Species diversity and population density affect genetic structure and gene dispersal in a subtropical understory shrub. *J Plant Ecol* **5**:270–8.

Zeng XQ, Durka W, Welk E, *et al*. (2017b) Heritability of early growth traits and their plasticity in 14 woody species of Chinese subtropical forest. *J Plant Ecol* **10**:222–31.

Zhang LY, Turkington R, Tang Y (2010) Flowering and fruiting phenology of 24 plant species on the north slope of Mt. Qomolangma (Mt. Everest). *J Mt Sci Engl* **7**:45–54.

Zhang X, Chen XY, Zhang D (2006) Effect of regeneration method on RAPD-based genetic variation of *Cyclobalanopsis glauca* (Fagaceae). *New Forest* **32**:347–56.

Zhang YH, Ni J, Tang FP, *et al*. (2017) Diversity of root-associated fungi of *Vaccinium mandarinorum* along a human disturbance gradient in subtropical forests, China. *J Plant Ecol* **10**:56–66.