Genetic diversity in natural and experimental, subtropical tree communities

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1. Summary

Evolution, the process of heritable change over time is driven by four major processes: Gene flow, random genetic drift, selection and mutation, all of which affect genetic diversity. The former three are central to this thesis and, among others, govern the migration and random loss of genetic variants, respectively, and lead, depending on their relative strength, to a non-random spatial distribution of genetic diversity. Genetic diversity, gene flow distances and population differentiation were quantified to tests for potential links between evolutionary processes and environmental conditions. Furthermore, growth and herbivory have been monitored for three year in experimental tree communities in subtropical China to test for effects of intra- and interspecific diversity on ecosystem functions and properties.

We quantified historic and recent gene flow distances in *Rhododendron simsii*, a common understory shrub of subtropical China. Contrary to our expectations, both historic gene flow inferred from spatial genetic structure and recent gene flow, measured as pollen flow distance, were low and ranged between 10-20 m. These distances were surprisingly low, considering very low spatial genetic structure, an outcrossed mating system and insect pollination. It is hypothesized that the populations under investigation are part of a much larger and dense population where a large effective population sizes ensures the maintenance of genetic diversity. Next, we found genetic diversity and population differentiation to follow a successional gradient in several tree and shrub species occurring in a near-natural nature reserve. However, in most studied species, population genetic descriptors were not affected by environmental conditions, exhibiting a rather idiosyncratic and species-specific behaviour. Lastly, species diversity in artificial tree communities correlated positively with growth, whereas genetic diversity was positively correlated to herbivory but negatively to growth. However, effects of genetic diversity on community performance were only evident in species mixtures.

Taken together, the present thesis provides new insights regarding genetic diversity in subtropical long-lived trees and shrubs. The mating system of *R. simsii* has been described molecularly, secondary succession was identified as a potential driver of population differentiation and genetic diversity and it was shown that both, intra- and interspecific variation are important resources for ecosystem functioning.

2. Zusammenfassung

Evolution, der Prozess erblicher Veränderung über Zeit, wird hauptsächlich von vier Kräften getrieben: Genfluss, genetische Drift, Selektion und Mutation, welche alle auf genetische Diversität einwirken. Die ersten drei Prozesse sind zentraler Bestandteil dieser Dissertation und steuern, u.a., die Migration sowie den zufälligen Verlust genetischer Variation. Dies wiederum kann, abhängig von der Stärke beider Prozesse in Relation zueinander, zu einer räumlich strukturierten Verteilung genetischer Variation führen. Genetische Diversität, Genflussdistanzen und Populationsdifferenzierung wurden quantifiziert, um zu testen, welchen Einfluss Umweltvariablen auf evolutionäre Prozesse ausüben. Des Weiteren wurden Wachstum und Herbivorie über drei Jahre in experimentellen Baumgemeinschaften im subtropischen China gemessen. Damit wurde getestet, ob intra- und interspezifische Diversität Ökosystemfunktionen und -eigenschaften beeinflussen.

In der ersten Studie wurden historische wie gegenwärtige Genflussdistanzen in *Rhododendron simsii*, einem im subtropischen China häufig vorkommenden Unterholzstrauch, ermittelt. Entgegen unserer Erwartungen waren historische wie gegenwärtige Genflussdistanzen verhältnismäßig niedrig mit Werten zwischen 10-20 m. Diese niedrigen Genflussdistanzen sind überraschend, zieht man schwach ausgeprägte räumlich-genetische Struktur, ein auskreuzendes Paarungssystem und Insektenbestäubung in Betracht. Es wird vermutet, dass die untersuchten Populationen Teil einer sehr viel größeren Population sind und dass hohe effektive Populationsdichten zum Erhalt genetischer Diversität trotz niedriger Genflussdistanzen beitragen. In mehreren Baum- und Straucharten konnte in einer zweiten Studie gezeigt werden, dass genetische Diversität und Populationsdifferenzierung einem Sukzessionsgradienten folgen. In dem Großteil der untersuchten Arten wurden populationsgenetische Parameter jedoch nicht von Umweltvariablen beeinflusst sondern folgten stattdessen artspezifischen Mustern. In der letzten hier präsentierten Studie konnte

gezeigt werden, dass Artenvielfalt und Wachstum experimenteller Baumgemeinschaften positiv miteinander korreliert sind. Weiterhin zeigte sich, dass genetische Diversität positiv mit Herbivorie, jedoch negativ mit Wachstum korreliert sind. Dieser Effekt wurden allerdings nur in Artmischungen beobachtet.

In der vorliegenden Arbeit konnten neue Einblicke gewonnen werden, welche die Effekte genetischer Diversität in subtropischen, langlebigen Bäumen und Sträuchern betrifft. Das Paarungssystem von *Rhododendron simsii* konnte molekular charakterisiert werden, sekundäre Sukzession wurde als potentiell treibender Faktor genetischer Diversität und Populationsdifferenzierung identifiziert und es konnte gezeigt werden, dass intra- und interspezifische Diversität wichtige Ressourcen für das Funktionieren von Ökosystemen darstellen.

3. Introduction

Biodiversity is a concept encompassing the entirety of living variety. The term includes variation on all organizational levels of biological diversity; from the molecular variability of genes via the diversity of species to the variety of ecosystems and each of these integral parts of biodiversity provides valuable goods and services (de Groot *et al.* 2002). Genetic diversity is the raw material on which evolution can act upon and invaluable for crop improvement or adaptation to changing environments. Species and the diversity thereof, provide goods like food or fuel. Finally, ecosystems, incorporating all biotic and abiotic diversity in a given area, are invaluable due to their recreational, regulatory and storage properties. The aforementioned are just some of the ways in which biodiversity affects humans. However, the relationship between the two is circular: Biodiversity affects humans who in turn affect biodiversity. Sadly, human activities could cause the sixth mass extinction (Barnosky *et al.* 2011), reducing diversity at the levels of genes and species, even threatening the persistence of ecosystems. Although man-made change is progressively altering diversity at any hierarchical level, the effects that these changes may exert on the goods and services humans require are not yet fully understood. Especially the long-term implications of losing genes and species are in need of continued scientific evaluation. However, evidence from two decades of biodiversity-ecosystem functioning (BEF) research shows that diversity at any biological level can have strong effects on ecosystem properties and processes (Cardinale *et al.* 2012). Therefore, understanding properties of and interactions between levels of biodiversity is of paramount interest for humans as our well-being in the foreseeable future is undoubtedly linked to goods and services provided by biodiversity (Diaz *et al.* 2006).

3. 1 Basic principles and assumptions in population genetics

In 1908, Godfrey Harold Hardy (Hardy 1908) and Wilhelm Weinberg (Weinberg 1908) predicted that the frequency of Mendelian characters in an ideal, panmictic population will quickly reach a stable distribution. After random mating, the frequency of an allele only depends on initial allele frequencies in the parental generation (Hardy–Weinberg–Equilibrium, HWE). However, this only applies to “idealized populations” under a number of underlying assumptions and Hardy already pointed out that, “Hypotheses other than that of purely random mating will give different results, and, […] if […] the character is not independent of that of sex, or has an influence on fertility, the whole question may be greatly complicated (Hardy 1908).” Organisms are subjected to four evolutionary forces: selection, mutation, random genetic drift and gene flow, all of which allow allele frequencies to deviate from idealized expectations. Thus, biological diversity at the genetic level is tightly linked to the evolutionary processes jointly affecting it. Mutations can give rise to novel gene variants via nucleotide alterations and mutation rates are a measure of nucleotide changes per locus per generation. As these rates appear to be relatively constant within species (Barrick *et al.* 2009; Thorpe 1982), they can be used as molecular clocks, allowing inferences about speciation events. Selection affects the ability of different phenotypes to produce viable offspring under specific external conditions (Antonovics 1976b). Phenotypes that are strongly selected against may not contribute alleles to the next generation, thus affecting allele frequencies in subsequent generations (Nielsen 2005). In contrast to selection, which is quantified in terms of fitness, genetic drift causes shifts in allele frequencies due to stochastic sampling of alleles during reproduction and the finite nature of populations. Over time, random shifts will lead to alleles either being lost or fixed in a population if not countered (Slatkin 1987). To counteract the effects of genetic drift, new alleles can be introduced to a population either by mutation or, probably more commonly, by gene migration between populations (Slatkin 1985). Termed “gene flow”, the exchange of genes among populations can lead to genetic homogenization. All of the above processes occur simultaneously and affect the abundance and distribution of genetic variants. At this point it is important distinguishing adaptive from neutral genetic diversity (Holderegger *et al.* 2006). Adaptive genetic diversity affects individual reproductive success (fitness), hence can be selected upon. This type of genetic diversity allows assessing the adaptive and evolutionary potential of populations and species. In contrast, neutral genetic variation has no or negligible effects on fitness and hence is considered to be selectively neutral. Thus, neutral genetic diversity permits studying evolutionarily neutral processes, such as gene flow or drift. However, selection may also affect neutral genetic diversity if it occurs closely located to neutral loci (background selection, e.g. Charlesworth *et al.* 1993). Mutation increases genetic diversity as the ultimate source of new gene variants (e.g. Lynch *et al.* 1995). Selection can both, increase or decrease genetic diversity, depending on the direction of selective pressures (Nielsen 2005). Genetic drift tends to reduces genetic diversity as stochastic sampling of alleles in populations with finite offspring size may result in the fixation of one allele at some point in time if not counteracted. This strongly applies to small populations as well as rare alleles (Ellstrand & Elam 1993). Lastly, gene flow tends to increase local genetic diversity. Strictly speaking, gene flow only allows the migration of existing alleles. In contrast to mutation, gene flow does not ‘create’ genetic variation but rather governs the spatial distribution thereof (Ellstrand 2014). Gene flow and genetic drift may exert opposing effects on genetic diversity and given enough time, this should lead to local gene flow–drift–equilibria (Hutchison & Templeton 1999). Taken together, the above forms a basic, conceptual framework of how biodiversity at the molecular level is affected by natural forces. From these basic principles, population-genetic models such as the aforementioned HWE or Wrights ‘Island Model’

(Wright 1931) were derived. However, it is not uncommon to find that the application of theoretical models to natural phenomena is to some extent accompanied by a violation of any simplifying assumption the model makes. Thus, models should constantly be subjected to refinement in order to remain applicable. In an attempt to refine our current population-genetic understanding, additional empirical and theoretical considerations will be presented in the remainder of this introduction and secondly, research committed to the aforementioned refinement will be presented throughout the body of this dissertational thesis.

3. 2 Population-genetic principles in natural systems

Contrasting Hardy's statement from the beginning of the previous section, mating in natural populations is largely non-random and has a tremendous impact on genetic diversity (Hamrick & Godt 1996). More precisely, the evolutionary history of uniting gametes determines levels of genetic diversity in the resulting generation. The more closely related mating individuals are the more likely they share alleles that are identical–by–descent, resulting in an increased probability of producing homozygous (inbred) offspring while the opposite applies to mating of distantly related individuals. This species-specific sexual behaviour has been formulated as the mating system and can be seen as a measure of population inbreeding (Ellstrand & Elam 1993). In plants, the mating system ranges between obligate selfing (e.g. via cleistogamy) and obligate outcrossing (e.g. via self-incompatibility), with mixed-mating being a comparably common syndrome where species exhibit both, selfing and outcrossing (Goodwillie *et al.* 2005). Although the mating system is considered to be species-specific, it may also vary considerably within species, for example along environmental clines or due to variation in floral traits (e.g. Cheptou *et al.* 2002; Schoen 1982, respectively). Furthermore, mating among related individuals, known as biparental inbreeding (Uyenoyama 1986) may distort mating system parameters and bias estimates of population genetic structure (Ritland 2002). The study of mating and related parameters is particularly interesting from an evolutionary standpoint as it marks the transition between distinct generations. It can be regarded as the point where all genetic and environmental influences, summed over the ontogeny of an organism, translate into allele frequency change of subsequent generations. Following, mating is a process of evolutionary significance.

The absence of gene flow, similar to non-random mating, is another simplistic assumption (of the HWE that is) not met under most natural conditions. Further complicating the matter, any barrier to gene flow, such as rivers (e.g. Kudoh & Whigham 1997) or anthropogenic structures (e.g. Su *et al.* 2003) can increase the genetic dissimilarity between individuals or populations by diminishing the homogenising effect of gene flow relative to that of genetic drift. Gene flow and its limitations have been studied extensively, particularly well so in plants (e.g. Barluenga *et al.* 2011; Debout *et al.* 2011; Dick *et al.* 2008; Zeng *et al.* 2012). Disregarding clonal propagation, gene flow in plants is realized through seeds and pollen and even in the absence of any barrier, gene flow via pollen and seeds may simply decline with geographical distance due to dispersal limitations. This leads to a non-random distribution of alleles and genetic differentiation of remote populations, or spatial genetic structure (SGS). The correlation between genetic and geographic distance was formulated as “Isolation–by–Distance” (IBD, Wright 1943) and the specific extent of this correlation allows inferring local SGS as well as the relative strength of evolutionary processes, that is gene flow and drift (Hutchison & Templeton 1999). In addition to geographical distance, other factors have been found to cause similar patterns of population differentiation as under IBD. For example, populations located at different elevations might not exchange genes freely as the result of local adaptation to varying environmental regimes at different altitudes (Isolation–by–Elevation (IBE), e.g. Herrera & Bazaga 2008; Ohsawa & Ide 2008; Shi *et al.* 2011). This may lead to differential pollination success (Alonso 2005) or shifts in phenological traits such as the time of flowering (Blionis *et al.* 2001; Qian *et al.* 2014; Scheepens *et al.* 2012), all of which may affect the distribution of genetic diversity. Identifying barriers to gene flow provides insight into if and how environmental conditions affect the evolutionary forces determining the distribution of genetic diversity (Linhart & Grant 1996). Furthermore, the assessment of SGS yields important information about population subdivision and the distribution of genetic diversity. For instance, Wrights fixation index F_{ST} (Wright 1951), one of the most widely used population-genetic descriptors, partitions genetic variation into within and among-subpopulation components, providing a measure of population differentiation due to genetic structuring. High values for F_{ST} indicate strong genetic differentiation between distinct populations, providing information that is important to researcher and conservationists alike. The former then may ask which factors can cause such genetic structuring (Loveless & Hamrick 1984) whereas the latter may identify populations vulnerable to extinction (e.g. through genetic isolation or inbreeding, see Edmands 2007; Ellstrand & Elam 1993). To summarize the above, population genetic descriptors have wide applications in ecology and evolutionary biology. Applying mathematical models to genetic data from natural populations permits studying those processes that affect genetic variation over time spans of many generations and represents one of the core interests of population genetics.

3. 3 Different levels, equivalent processes?

Interestingly, processes governing genetic diversity similarly affect species diversity. Analogous to the above, species can be subjected to mutation, migration, selection and stochastic loss, all of which may affect local species diversity. Recognizing these similarities, species and genetic diversity have been proposed to co-vary in nature (Antonovics 1976a) and if the above processes affect species and genetic diversity in a parallel manner, a positive correlation between the two levels of biodiversity (species-genetic diversity correlation, SGDC) is expected (Vellend 2004; Vellend & Geber 2005). Alternatively to parallel effects on species and genetic diversity, either level of biodiversity might be linked to the other through causal relationships (Vellend 2005). Positive SGDCs have been reported from various species and habitats such as butterfly communities in lowland rainforests (Cleary *et al.* 2006), tree and shrub communities in Australian sandplain shrublands (He *et al.* 2008), grassland communities in central Germany (Odat *et al.* 2010), beetle communities of the Aegean archipelago (Papadopoulou *et al.* 2011) or forest tree communities in Germany (Wehenkel *et al.* 2006). Nevertheless, reports of non-existing or negative SGDCs (Odat *et al.* 2004; Puscas *et al.* 2008) demonstrate the need for ongoing research and refinement regarding the underlying theoretical assumptions of SGDCs. Although empirical evidence questions the universality of SGDCs, the proposed relationship between fundamental parts of biodiversity has led to a shift towards an interdisciplinary approach, combining theories from population genetics and ecology.

3. 4 Biodiversity and ecosystem functioning

In addition to species and genetic diversity responding to similar processes, they may also evoke similar effects. The relationship between biodiversity (e.g. species diversity) and ecosystem functioning (e.g. biomass production) has been investigated on extensively (e.g. Baruffol *et al.* 2013; Cardinale *et al.* 2007; Tilman *et al.* 2012). It was observed that diverse communities may produce more biomass than what would be expected from monoculture performance of each component species (Cardinale *et al.* 2007). Proposedly via three mechanisms, namely complementarity, facilitation and sampling effects, species diversity may affect ecosystem properties (Hooper *et al.* 2005). Complementarity effects arise from niche partitioning among species, reducing interspecific competition through the differential use of resources (Reich *et al.* 2004). Facilitation comprises biotic and/or abiotic stress relief (Bruno *et al.* 2003), where one species positively affects another through a mediating agent (often a resource). Prominent examples are nurse plants (e.g. Gomez-Aparicio *et al.* 2004) or Nitrogen-fixers (e.g. Hulvey *et al.* 2013), which can reduce stress in co-occurring plant species. Sampling effects may be observed when communities are dominated by a single species due to inherently high productivity or competitive abilities (Tilman *et al.* 1997). Thus, increasing species richness also increases the probability of encountering such a species which affects ecosystem functions directly rather than through interactive effects of multiple species (Hector *et al.* 1999). Because the above effects are ultimately due to trait variation, both interspecific and intraspecific trait variation may similarly evoke such effects. In fact, genotypes have been shown to cause intraspecific facilitation (Ellers *et al.* 2011) as well as intraspecific complementarity (Reusch *et al.* 2005) whereas genotype identity can induce sampling effects, e.g. on herbivory (Castagneyrol *et al.* 2012). Following, species diversity and genetic diversity share striking similarities in respect to how they are governed as well as how they affect communities. However, there is severe lack of studies manipulating both levels of biodiversity simultaneously to jointly test for effects of biodiversity at different hierarchical levels (but see Cook-Patton *et al.* 2011), especially in long-lived tree and shrub species. Thus, unless this lack is remedied, interactive effects of different levels of biodiversity on ecosystem functioning will remain obscure.

3. 5 Scope of the present thesis

Embedded in the present thesis are three research articles, each dedicated to research questions emerging from the preceding section. Generally, this thesis aims at providing new insights regarding diversity at the molecular level (Fig. 3.1). Specifically, gene flow, mating system parameter and the spatial distribution of genetic diversity are examined. Furthermore, the effects of intra- and interspecific diversity on ecosystem functioning are being investigated. Research has been carried out in a biodiversity hotspot in subtropical China, with a focus on long-lived shrub and tree species. The content and aim of each chapter are briefly presented in the following.

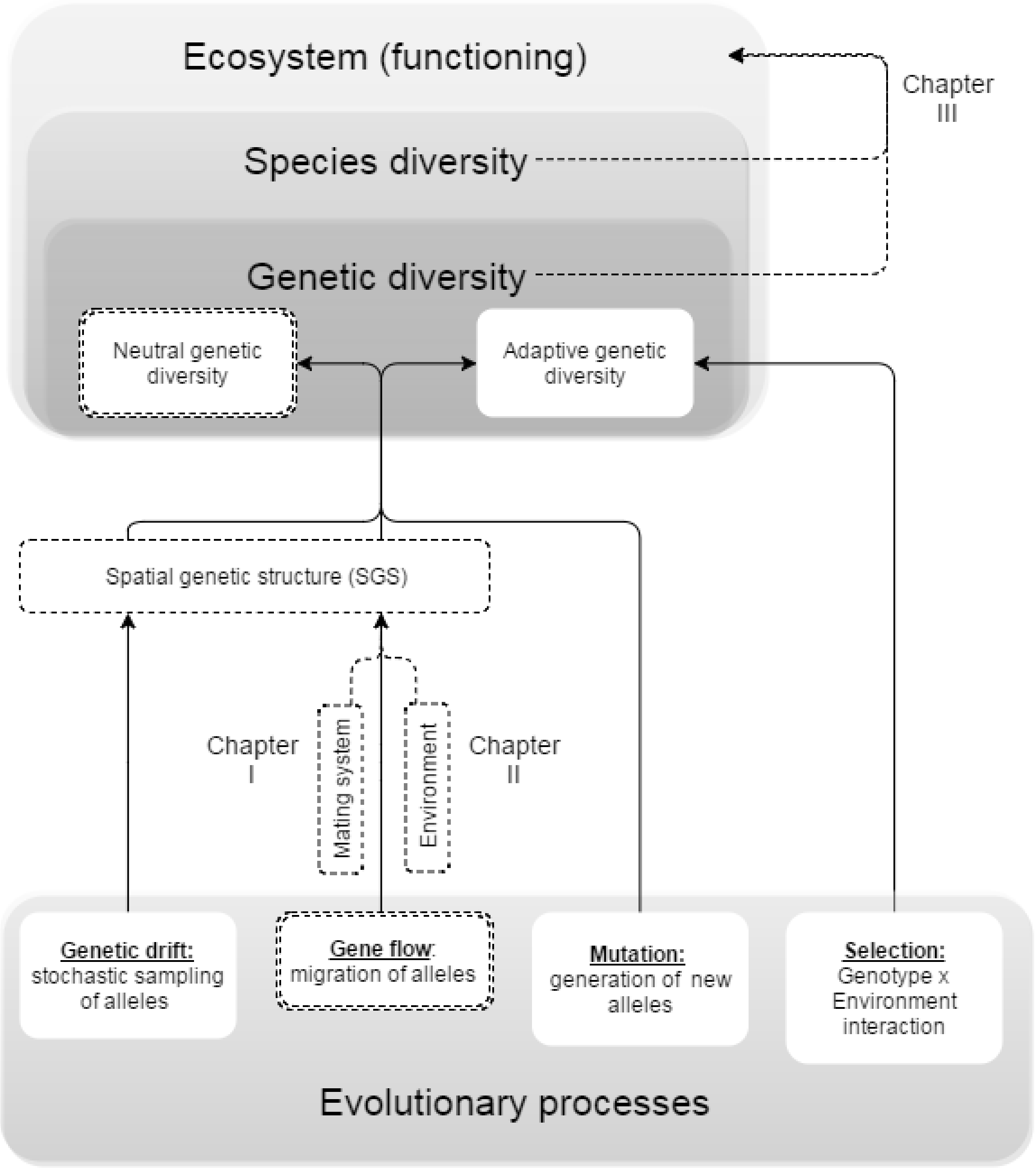
Chapter I: This chapter comprises an observational, single-species study on a common understory shrub in subtropical China, *Rhododendron simsii*. Population genetic descriptors, outcrossing rates as well as paternity were determined through parent-offspring genotyping. Furthermore, gene flow distances were calculated using complementary approaches to compare historic and recent gene flow. The reproductive and evolutionary implications are discussed.
Question: Molecularly, how can mating be characterized in *Rhododendron simsii* and at what spatial distance is gene flow limited?

Chapter II: Here, a multi-species, observational study with eleven naturally co-occurring tree and shrub species is presented. Genetic diversity and population differentiation across species were related to various plot environmental conditions. The inclusion of multiple species allowed distinguishing general from more idiosyncratic patterns, with as focus on generalizations across species.
Question: Do abiotic and/or biotic environmental conditions affect genetic diversity and differentiation?

Chapter III: In the last chapter, intra- and interspecific diversity were manipulated simultaneously in experimental communities harbouring four tree species. Growth and herbivory were monitored over three consecutive years on a large number of trees. It was tested whether intra- or interspecific diversity (or both) produce significant effects on ecosystem functioning.

Question: Do intra- and interspecific diversity additively or interactively affect tree growth and/or plant-herbivore interactions?

Figure 3.1 – Simplified representation of processes relevant to this thesis. The upper part of the figure represents the hierarchical organization of biodiversity; the lower part depicts the evolutionary forces affecting diversity at the genetic level. Arrows indicate causal relationships. Dashed structures show processes and properties assessed in this thesis.



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4. Chapter I – Gene flow in, and mating system of *Rhododendron simsii* in a nature reserve in subtropical China

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4. 1 Abstract

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 not much is known about the details of gene flow and mating in many species, 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 South-East 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.

4. 2 Introduction

Genetic diversity in natural plant populations is commonly distributed non-randomly (Vekemans and Hardy 2004; Wright 1946) 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 (Auld and de Casas 2013; Vekemans and Hardy 2004). In addition to present gene dispersal, past incidents such as bottlenecks or founder events are strong determinants of genetic diversity (e.g. Nowak, Haller and Yoder 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 (Griffin and Eckert 2003; Loveless and Hamrick 1984) 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, Hardy and Petit 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 (e.g. Degen, Bandou and Caron 2004; Doligez and Joly 1997; Hamrick, Murawski and Nason 1993), 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 (i) resolving the fine-scale spatial genetic structure, (ii) characterising the mating system and (iii) quantifying the magnitude of both historic and current gene flow.

4. 3 Material and methods

Study species and sampling

Rhododendron simsii PLANCHON (Ericaceae) is a small shrub (2-5 m) that occurs naturally in forests and forest margins of altitudes between 500 and 2700 m (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, South-East 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). Plots have a projected area of 30 × 30 m and were 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. Furthermore, we sampled an additional, larger population in the GNNR near Hong Yuan to increase sample size (plot size approx. 50 m x 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 (QIAGEN, Venlo; Netherlands) 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, Li and Ge 2009; Wang, Huang and Long 2009; 2010) were tested for suitability in *Rhododendron simsii* after which 6 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 (Applied Biosystems) 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 $Sp = -\hat{b}_F/(1 - \hat{F}_{(1)})$ (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 (σ), half the mean squared parent-offspring distance. Among plots, census population densities (*D*) varied between 45-555 ind./ha. Following, we estimated the effective population density to range from D_e = 5-277 individuals per ha (with D/D_e ~ 0.1-0.5, Frankham 1995). As population densities varied almost two orders of magnitude, we calculated σ for a wide range (D_e = 5-600) of assumed effective population densities (Table 4.1).

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.

Population level paternity analysis and recent gene flow

We used CERVUS v3.0.3 (Kalinowski, Taper and Marshall 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 10000 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.

4. 4 Results

Spatial genetic structure and historic gene flow

Kinship coefficients significantly decreased with spatial distances (Fig. 4.1A). 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. Consequently, Sp equated to 0.00318. Distance class based kinship coefficients were low and decreased over the full range of observations (Fig. 4.1B). Gene dispersal σ was estimated for effective population densities between 5 and 600 individuals per ha (Table 4.1). For high D_e (300-600 individuals per ha) σ ranged between 15.4 and 10.3 m, respectively, whereas low estimated D_e (5-10 individuals per ha) resulted in a σ of 288.3-219.3 m (Table 4.1).

Mating system

Overall mean outcrossing rate t_m estimated from progeny arrays was 0.919. At plot level, the highest multilocus 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 multilocus 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. 4.2).

4. 5 Discussion

Mating system

According to our findings *R. simsii* is predominantly outcrossing (mean t_m= 0.919). Considering the evidence 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 (Brennan, Harris and Hiscock 2005; Charlesworth and Charlesworth 1987). 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 (Heywood 1993; Lande and Schemske 1985). Significant inbreeding depression was also reported in other *Rhododendron* species (Delmas *et al.* 2014; Hirao 2010) 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) and similar to values found in other tropical and subtropical shrubs (Theim, Shirk and Givnish 2014; Zeng *et al.* 2012). 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 (avg. 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 between 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 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 counter 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, Yan and Ge 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.

Conclusion

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.

4. 6 Tables and figures

Table 4.1 – Estimates of gene dispersal (σ) over a range of effective population densities (D_e, individuals/ha). Est. N_b- Estimated neighbourhood size, SE- Standard error, σ- Gene dispersal distance (m), n.c.- Non-convergence on these parameter values.

D_e	Est. N_b	SE	σ	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

Figure 4.1 – 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.

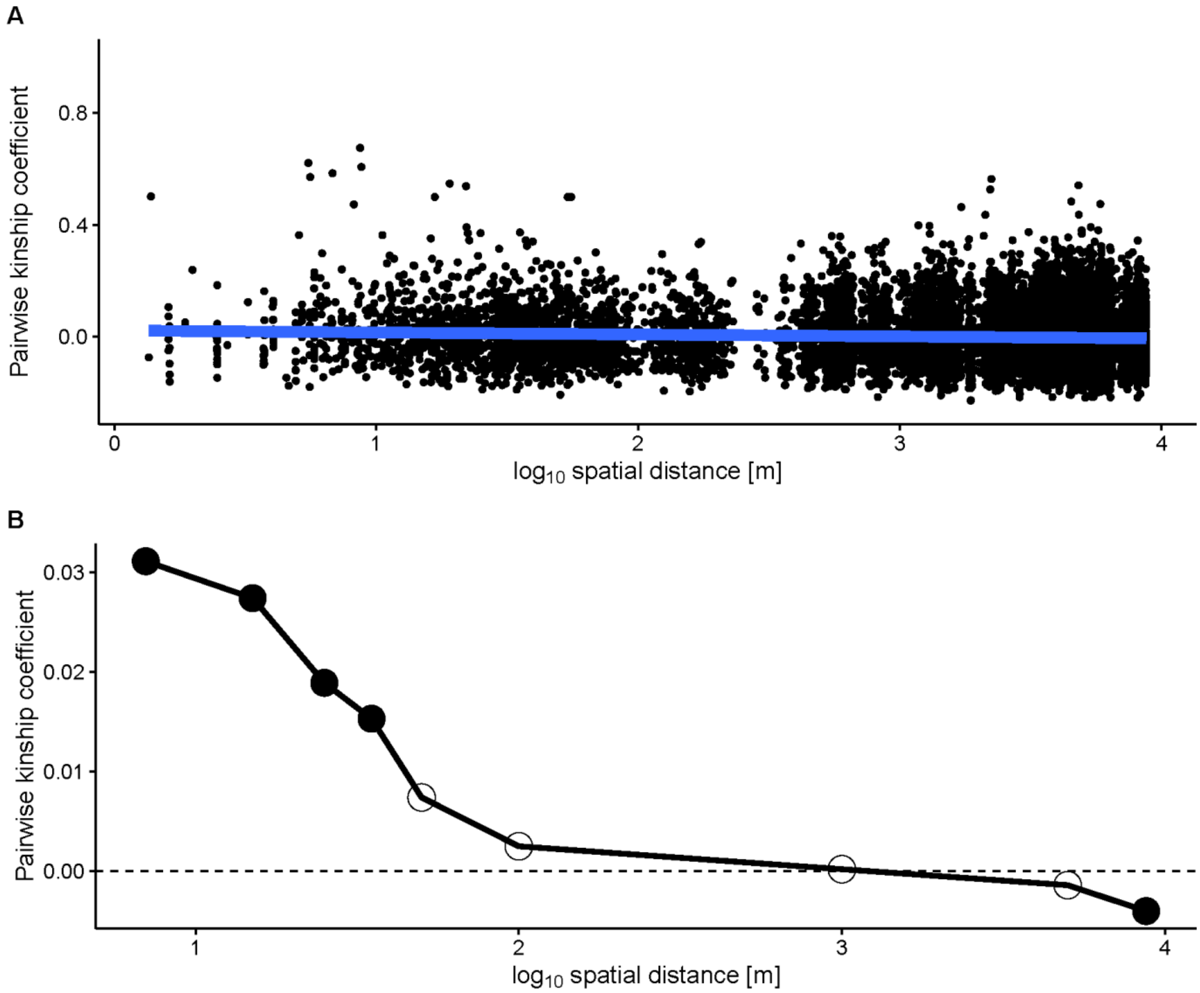
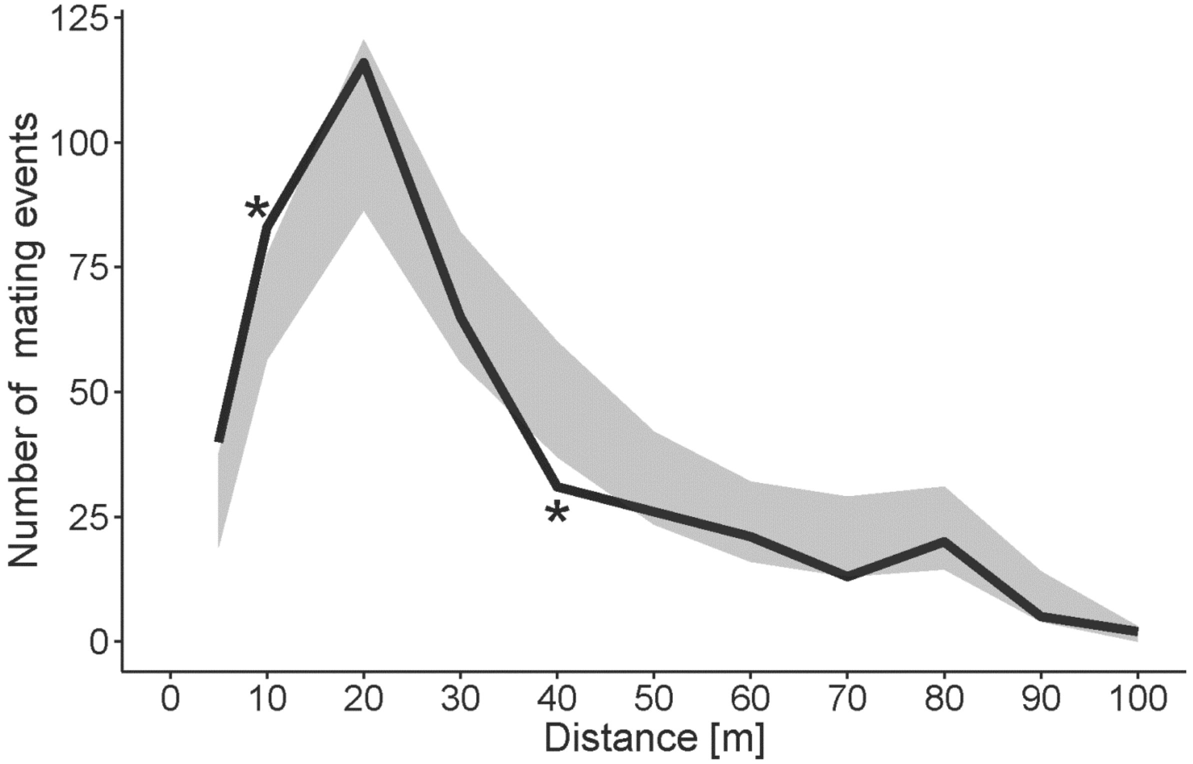


Figure 4.2 – Frequency distribution of observed and expected mating distances within plots. The black line depicts the number of observed mating events, the grey envelope represent the 95 % confidence interval obtained from randomizing all mating events. Significant departures from random mating are indicated by asterisks.



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4. 8 Supplementary material and acknowledgements

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

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5. 1 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 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 *Rhododendron 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 isolation-by-distance 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.

5. 2 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 *et al.* 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 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 *et al.* 1984; Ohsawa *et al.* 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. 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 *et al.* 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; Slatkin 1987). Thus, with increasing ecological distance between populations the isolation may increase as well resulting in isolation-by-environment (IBE). Gradients that potentially lead to local adaptation include climatic conditions (Franks *et al.* 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.

5. 3 Material 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 *et al.* 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 x 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 5.1). Most of the species had a certain preference to certain successional stages, whereas for four species no such preference was observed (Table 5.1). Up to 30 leaf samples were collected for each species, if present, from each CSP, or in the direct vicinity (*Rhododendron simsii* in CSP 21; Table 5.1). Samples were oven-dried or lyophilized for 48h after collection. Our study includes previously published data on *Castanopsis eyrei* (Shi *et al.* 2011, data from CSPs, excluding populations A-D for tests of plot characteristics) and *Ardisia* *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 *Rhododendron 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 *Daphniphyllum oldhamii* (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 minutes at 95°C followed by 20 cycles of 30s at 94°C, 30s of annealing at 60°C decreasing by 0.5°C per cycle and 90s 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 minutes. 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 3 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 more than 20% null alleles in more than 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 *et al.* 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 eight out of 11 species and the lositan analysis in the exclusion of 1-4 loci in five out of 11 species (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 5.1)

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 $F'_{\mathrm{ST}}= F_{\mathrm{ST}}/ F_{\mathrm{ST\_max}}$ with $F_{\mathrm{ST\_max}}$ being the maximum possible F_{ST} given our data. $F_{\mathrm{ST\_max}}$ was calculated with Fstat 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 *et al.* 2002; Burnham *et al.* 2004) 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 isolation-by-distance (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 isolation-by-succession. All the above computations were carried out in the R statistical software v3.2.1 (R Core Team 2015).

5. 4 Results

Effect of species diversity and environment on genetic diversity and differentiation

Mean allelic richness ranged between A_r = 2.433 in *Cyclobalanopsis glauca* populations and A_r=6.522 in *Castanopsis eyrei* populations. Standardized F'_{ST} ranged from 0.062 to 0.243 in *Daphniphyllum oldhamii* and *Syzygium buxifolium*, respectively (Table 5.2). According to multi-model inference species identity had the highest impact on allelic richness followed by plot age, elevation and species diversity (Table 5.3). Population differentiation was strongly affected by both species identity and plot age whereas elevation and species diversity had relatively low importance (Table 5.3). Linear regressions revealed significant correlations of A_r with plot age in *Rhododendron simsii* (p = 0.0002) and *Vaccinium carlesii* (p = 0.0207) (Fig. 5.1). Similarly, F'_{ST} was significantly correlated with plot age in *Lithocarpus glaber* (p = 0.0136) and *Rhododendron simsii* (p= 0.0249).

Isolation patterns

In total, we found isolation patterns in four species (Table S4). *Ardisia crenata* and *Schima superba* showed significant IBD. Furthermore, we found significant isolation-by-elevation patterns in *Castanopsis eyrei* and *Daphniphyllum 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.

5. 5 Discussion

Genetic diversity and species diversity

Our study does not support parallel effects on species and genetic diversity as proposed by Vellend & Geber (2005). Studies showing connections between species and genetic diversity often considered isolated or island populations (Cleary *et al.* 2006; Fady *et al.* 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 *et al.* 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, *Rhododendron simsii* and *Vaccinium carlesii*. We also found increasing population differentiation with succession in *R. simsii* and *Lithocarpus 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 *Rhododendron simsii* and *Vaccinium carlesii*.

Simultaneously, a reduction of population sizes enhances population differentiation if not countered by among- population gene flow as seen in *Rhododendron simsii* and the tree *Lithocarpus glaber*. *R. simsii* has been shown to be shade intolerant (Ng *et al.* 2000b) which is corroborated by the observed reduction of abundance in late successional stages (Table S3). Also, we rarely found *R. simsii* flowering in late successional plots (pers. obs.), which reduces the opportunity for pollen-mediated gene flow. That not all three species showed both a reduction of diversity and 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 isolation-by-distance indicating dispersal limitation at a spatial scale of less than 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 *Ardisia 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 *Schima superba* is also insect-pollinated (Table 1), limited pollinator availability could similarly cause isolation-by-distance 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 & Neiland (2002). This could further foster population differentiation in *Ardisia crenata* as this species often maintains small populations. Thus, the observed patterns of isolation-by-distance in two species indicate inefficient gene flow via pollen or seeds in these species.

In addition to isolation-by-distance we detected isolation-by-elevation in *Castanopsis eyrei* and *Daphniphyllum 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 & Bazaga

(2008), which may be particularly relevant for *Daphniphyllum 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 & 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

Our 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 several species, which was further modulated by more idiosyncratic and species-specific processes. We conclude that our multi-species approach was decisive to distinguish common and species-specific patterns and we advocate further multi-species studies of genetic diversity and its drivers.

5. 6 Tables and figures

Table 5.1 – Life history traits of the eleven species chosen in the present study. 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.

Species	Family	LF [2]	LH[2]	Poll.	Sdisp	FruiSeedPr, 1k SeedW[14]	SuccPref[1]	PopDens Ind./ha[2]
Ardisia crenata	Myrsinaceae	S	E	I[3]	M, B[3][14]	bright red berries, 221g[3]	late, 80+ years	0.1
Castanopsis eyrei	Fagaceae	T	E	I[4][13]	G, R[4]	nut[4]	no preference	516.0
Castanopsis fargesii	Fagaceae	T	E	I[6][13]	G, R, B[5], [6]	nut	mid, <60 years	51.3
Cyclobalanopsis glauca	Fagaceae	T	E	W[13]	A[8]	nut[8]	mid-early, <40 years	67.5
Daphniphyllum oldhamii	Daphniphyllaceae	S/T	E	I in *D. teijsmanni*[9]	M, B[8]	drupe[8]	late, 80+ years	113.3
Lithocarpus glaber	Fagaceae	T	E	I[10]	A[8]	nut, 1162g[8]	late, 80+ years	46.4
Quercus serrata	Fagaceae	T	D	W[13]	A[8]	nut, 1934g[8]	early, <20 years	146.2
Rhododendron simsii	Ericaceae	S	D	I[11]	W, G[11]	capsule, 0.07g[11]	early, <20 years	196.7
Schima superba	Theaceae	T	E	I[10]	W, G[8]	capsule, 4.2g[8]	no preference	354.8
Syzygium buxifolium	Myrtaceae	S	E	I[12]	M, C, B[12][14]	berry	no preference	142.8
Vaccinium carlesii	Ericaceae	S	E	I	B[8][14]	berry[8]	no preference	75.1

[1] (Bruelheide *et al.* 2011), [2] (Ma *et al.* 2009), [3] (Zeng *et al.* 2012), [4] (Shi *et al.* 2011), [5] (Xiao *et al.* 2005), [6] (Chen *et al.* 2008), [7] (Zhang *et al.* 2006), [8] (Du *et al.* 2009), [9] (Yumoto 1987), [10] (Corlett 2001), [11] (Ng *et al.* 2000a), [12] (Lughadha *et al.* 1996), [13] (Manos *et al.* 2001), [14] (Royal Botanic Gardens Kew 2016)

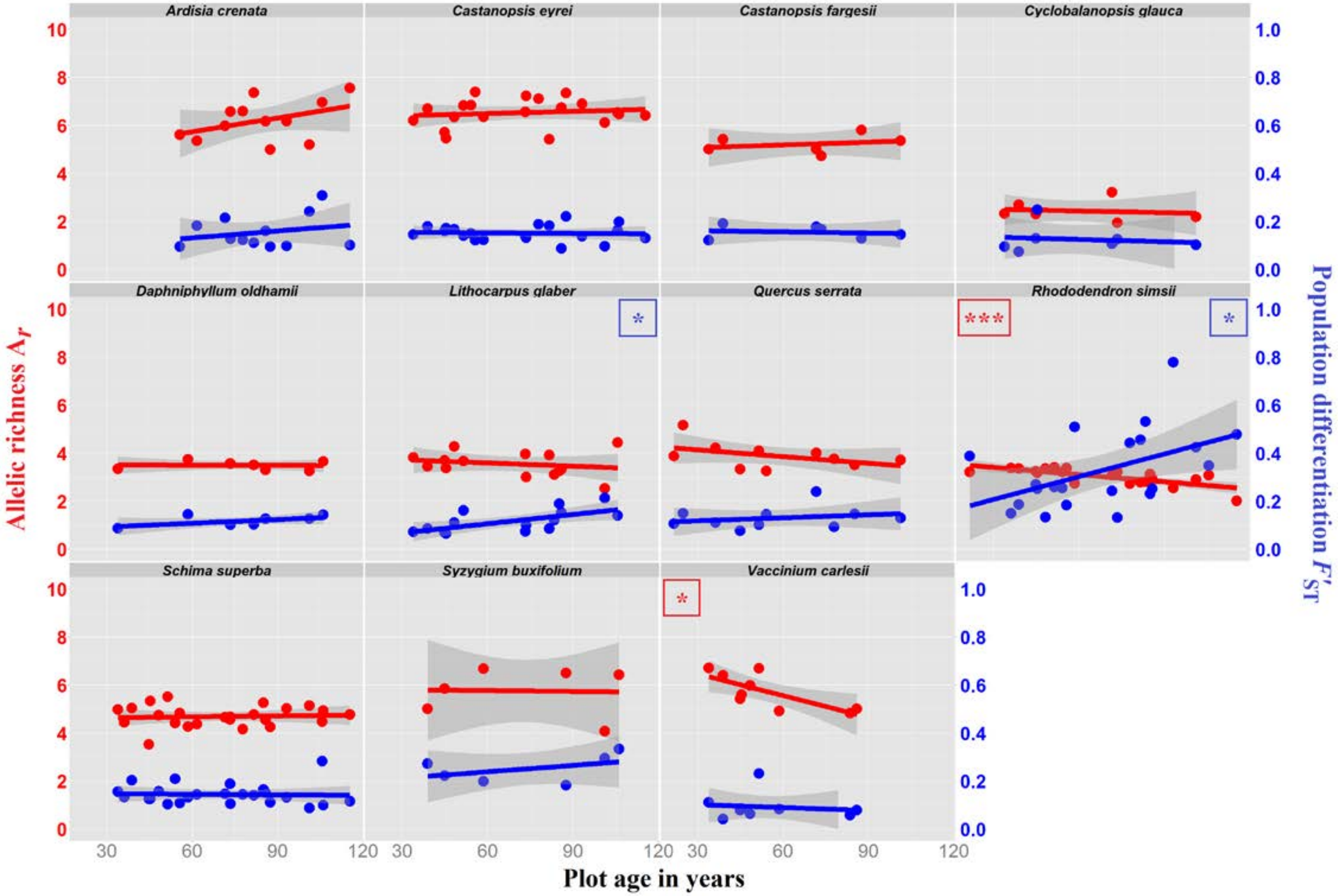
Table 5.2 – General overview on total, mean and minimum sample size, number of populations and loci and global levels of within population diversity (A_r) and among population differentiation (F'_{ST}) for each of 11 woody species of subtropical forest in China

Species	Total sample size	Mean sample size/ pop	Min. sample size	# Pop.	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 5.3 – Variable importance per predictor after model averaging. Higher values indicate that factors appear more frequently in significant models.

Model term	Measure of genetic diversity	
	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 x species diversity	-	0.07
Plot age x elevation	-	-
Species diversity x plot age	-	-
Model with highest Akaike weight	A_r ~ Species	F'_{ST} ~ Plot age + Species

Figure 5.1 – 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.



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5. 8 Supplementary material and acknowledgements

Table S1- Primer references including: locus name in original publication, species the primer was amplified in, new locus name if primer sequence was changed, full forward/backward primer sequence, Genbank accession number and original publication

Table S2- Results of the locus selection procedure giving the number of amplified, omitted and remaining loci with their names.

Table S3- Species-specific abundances and sample sizes per CSP. Additionally, the plot age of each CSP is given in years of the fifth largest individual per plot.

Table S4- Correlation coefficients (*r*) derived from partial Mantel (IBD, IBE) and Mantel-tests (IBC, IBS).

Figure S1- Isolation-by-distance broken down by species; shows the correlation between log (pairwise spatial distance) and F'_{ST}

Figure S2- Isolation-by-elevation broken down by species; shows the correlation between log (pairwise elevational distance) and F'_{ST}

Supplementary material available online after publication

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6. Opposing intra vs. interspecific diversity effects on herbivory and growth in subtropical experimental tree assemblages (Chapter III)

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6. 1 Abstract

Aims: Positive plant diversity-ecosystem function relations are ultimately driven by variation in functional traits among individuals that form a community. To date, research has largely focused on the role of species diversity for ecosystem functioning. However, substantial intraspecific trait variation is common and a significant part of this variation caused by genetic differences among individuals. Here, we studied the relative importance of species diversity and seed family diversity within species for growth and herbivory in experimental subtropical tree assemblages.

Methods: In 2010, we set up a field experiment in subtropical China, using four species from the local species pool. Trees were raised from seeds, with seeds from the same mother tree forming a seed family. We established 23 plots containing one or four species (species diversity treatment) and one or four seed families per species (seed family diversity treatment). Tree growth (stem diameter, plant height and crown expansion) and herbivory (percentage leaf loss due to leaf chewers) were monitored annually from 2011 to 2013.

Important findings: Tree species richness promoted growth but had no effect on herbivory. In contrast, seed family diversity reduced growth and increased herbivory, but only so in species mixtures. Most of the observed effects were time-dependent, with the largest effect found in 2013. Our results suggest that biodiversity can affect plant performance directly via tree species-species interactions, or, context-dependent, via potential effects on inter-trophic interactions. Two important conclusions should be drawn from our findings. Firstly, in future studies regarding BEF relationships, intraspecific genetic diversity should be given similar weight as species diversity as it has often been neglected and its effects are not well understood. Secondly, we demonstrate opposite effects of biodiversity among and within species, stressing the importance to consider the effects of multiple levels of biodiversity simultaneously.

6. 2 Introduction

Exploring potential links between biodiversity and ecosystem functioning (BEF) is a major challenge in contemporary ecology. As a consequence of anthropogenic ecosystem degradation, local species diversity is changing, a process that has the potential to alter ecosystem functions such as nutrient cycling (Cardinale *et al.* 2002) or biomass production (Tilman *et al.* 1996). The biodiversity component of BEF experiments is strongly focussed on the effects of species diversity (Balvanera *et al.* 2006). Such effects of species diversity on ecosystem functions are caused by functional trait diversity among species (Hooper *et al.* 2005) and are of central interest in biodiversity research. However, considerable proportions of trait variation are found within species (Siefert *et al.* 2015). Consequently, both inter- and intraspecific trait variation may evoke biodiversity effects on ecosystem functioning.

Effects of species diversity on ecosystem functions are proposedly caused via complementarity and sampling effects (Hooper *et al.* 2005). Firstly, complementarity effects can comprise biotic or abiotic stress relief (e.g. by nurse plants), resource enhancement (Bruno *et al.* 2003; Fowler 1986) and niche differentiation among species (Tilman *et al.* 1997a). For example, an increase in total water and nitrogen uptake was observed in communities of species with differing rooting depths or between N- fixing and non-fixing species, respectively (Hooper 1998; van Ruijven and Berendse 2005). Similarly, an increase in resource use efficiency was demonstrated by Sun *et al.* (2016) who reported a higher fine-root production in mixtures than in monocultures. Secondly, the sampling effect states that some species affect ecosystems more strongly than others, due to inherently high productivity or competitive ability (Tilman *et al.* 1997b). Thus, increasing species richness also increases the probability of encountering such a species which affects ecosystem functions directly rather than by interactive effects of multiple species (Hector *et al.* 1999). Because the above processes are ultimately due to trait variation, both interspecific and intraspecific trait variation may similarly cause such effects. In fact, genotypes have been shown to cause intraspecific facilitation (Ellers *et al.* 2011) or intraspecific complementarity (Reusch *et al.* 2005) and genotype identity can induce sampling effects, e.g. on herbivory (Castagneyrol *et al.* 2012). Thus, processes that drive genotype diversity-ecosystem functioning relationships are equivalent to those outlined for species diversity.

Intraspecific diversity can have strong effects on ecosystem functioning (Cook-Patton *et al.* 2011; Crutsinger *et al.* 2006; Whitham *et al.* 2003). Intraspecific genetic diversity rather than phenotypic plasticity is relevant here because many functional plant traits are highly heritable (Johnson *et al.* 2009). The identity of plant genotypes in plant mixtures have been shown to affect nutrient cycling, e.g. nitrogen mineralization or nutrient immobilization (Schweitzer *et al.* 2005; Schweitzer *et al.* 2004). Higher genetic diversity may lead to increased community level plant biomass (Drummond and Vellend 2012), elevated levels of chemical defences (Moreira *et al.* 2014) or increased plant survival (Parker *et al.* 2010). However, despite its importance for ecosystem functioning, intraspecific genetic diversity has received comparably little attention in BEF research, particularly in forest BEF experiments. Other causes of positive biodiversity-productivity relationships might be mediated through biotic interactions, for example between plants and herbivores. Genotypic diversity may be more (Crawford and Rudgers 2013), equally (Cook-Patton *et al.* 2011) or not important (Abdala-Roberts *et al.* 2015) relative to species diversity in structuring arthropod communities. With respect to herbivory, both reduced (Parker *et al.* 2010) and increased herbivory (Castagneyrol *et al.* 2012) have been found with increasing genetic diversity.

Biodiversity–ecosystem functioning research has often focussed on primary production. Nevertheless, species diversity can also affect trophic interactions. Regarding herbivory, two general processes have been observed. Firstly, herbivore damage may depend on neighbour identity such that herbivory is reduced when focal plants grow in proximity of highly defended or unpalatable neighbours, or, conversely, that herbivory is increased if focal plants have highly palatable neighbours. Such neighbourhood-dependant effects are known as associational resistance and associational susceptibility (Agrawal *et al.* 2006) and play important roles in tree communities (Erickson *et al.* 2012; Plath *et al.* 2012; Stastny and Agrawal 2014). Secondly, herbivory depends on the density of host plants, affecting the likelihood of an oligophagous herbivore to find a suitable host. This can then either lead to increased (resource concentration, Root 1973) or decreased herbivory (resource dilution, Otway *et al.* 2005). In contrast, herbivory by polyphagous herbivores may increase due to a broad dietary mix provided by high plant diversity (e.g. Schuldt *et al.* 2010).

The present study focuses on effects of both species and intraspecific genetic diversity on tree growth and herbivory in young experimental tree stands. We established a factorial species diversity – genetic diversity experiment in a subtropical biodiversity hotspot in Southeast China and conducted serial measurements of individual tree growth and herbivory over the course of three years to address the following questions: (i) Do tree growth and herbivory depend on species diversity? (ii) Are growth and herbivory driven by genetic diversity? (iii) Do effects of species and genetic diversity interact, and (iv) are these effects time-dependent?

6. 3 Material and methods

Experimental setup

In 2009, a forest BEF experiment was established by the Biodiversity and Ecosystem Functioning China project (BEF- China) at Xingangshan Township, Dexing County of Jiangxi Province, China (see Bruelheide *et al.* 2014 for details). Randomly scattered within site B we used 23 plots, 25.8 by 25.8m in size (1 mu, traditional Chinese unit), each harbouring 400 evenly spaced trees arranged in a 20 × 20 rectangular grid. From the local species pool we selected two deciduous species, *Alniphyllum fortunei* (HEMSLEY) MAKINO (Styracaceae) and *Idesia polycarpa* MAXIMOWICZ (Salicaceae) and two evergreen species*, Cinnamomum camphora* (L.) PRESL. (Lauraceae) and *Daphniphyllum oldhamii* (HEMSLEY) ROSENTHAL (Daphniphyllaceae). All of our target species are trees (*D. oldhamii* classified as tree/shrub) growing up to 30 m in height. Economically, *C. camphora* and *D. oldhamii* are used for timber production, the former species also being used for the extraction of camphor (Flora of China). Plants originated from seeds that were collected in the nearby Gutianshan National Nature Reserve. Seeds were collected from different mother trees, with seeds from the same mother tree forming a seed family (SF) which genetically is considered to consist of half-sibs. The use of seed families allowed us to manipulate the level of genetic diversity as members of a single SF are on average genetically more closely related, and thus, genetically less diverse than members of different seed families. This is supported by preliminary analyses based on microsatellite data in two of the species showing larger kinship coefficients within seed families than among seed families (unpublished data). We manipulated species diversity (SD) and genetic diversity (in terms of SF richness) in a crossed design. Species diversity was either one species (species monocultures) or four species (species mixtures) and genetic diversity was either one seed family (SF monoculture) or four seed families (SF mixture). All seedlings were raised in two local nurseries and transplanted into the field in autumn 2010 at the approximate age of 16 months. We aimed at using eight seed families per species, however, owing to the different availability of seeds, the number of seed families per species varied between eight in *Idesia polycarpa* and 15 in *Daphniphyllum oldhamii* (Table S1). However, the additional seed families made up only 3.7% of the final data set. All analyses were performed on the full and a reduced dataset which only contained seed families that were present in all diversity levels.

Qualitatively, results of the full and reduced dataset did not differ, hence we present the findings based on the latter. In total we planted 40 different species monocultures (8 different SF monocultures and 2 different SF mixtures in each of 4 species) and 13 species mixtures (7 SF monocultures and 6 SF mixtures, Table 6.1) Due to the large number of monocultures of species and seed families, we used ¼ plots of 10 × 10 individuals for species monocultures, but full plots for species mixtures. In each treatment, species and seed families were planted randomly and in equal quantities. Out of 9200 individuals planted in 2010, 58% established in 2011 and survived until 2013. Mean survival among species ranged between 37% in *I. polycarpa* and 84% in *C. camphora* (Table S1).

Plant growth and herbivory

Annually between 2011 and 2013, we measured growth and herbivory between September and October. Measurements included stem diameter at ground height (dgh), plant height, crown diameter and herbivory. Stem diameter was measured as the diameter of the root collar both in north-south and east-west direction and averaged for data analyses. Similarly, crown diameter was measured as the maximal crown expansion in both north-south and east-west direction and also averaged. We further measured tree height as the vertical distance from the soil surface to the highest green leaf. Herbivory was estimated visually as percentage missing leaf area due to insect feeding on seven randomly chosen leaves per tree and later averaged and square root transformed per individual. Lepidopterans are the most, some beetle families the second most common herbivores according to field observation in the natural forest from the same region (Schuldt *et al.* 2010)

Statistical analyses

All data were analysed by analysis of variance (aov function of R 3.2, R Core Team 2015), with group-wise means weighted by underlying sample size and factors fitted sequentially corresponding to the hierarchy of our experimental design (Table 6.2). In biodiversity studies, species diversity effects are generally tested using different species mixtures as replicates (Schmid *et al.* 2002). In our study, there was only a single four-species mixture so that community composition effectively was equal to the variation among species in monoculture. We further tested species richness effects using plot as replicate, i.e. whether particular monocultures and 4-species mixtures were different. With the same rationale, effects of genetic diversity were first tested against plot, later against species × plot as replicate. Species effects were separated into two components. First, we tested for species identity effects in monocultures (using plots as replicates); second, we tested species identity effects in multispecies mixtures (using species × plot as replicate). To test if effects of biodiversity depended on time, we repeated the above tests as interactions with time after testing time against time × plot variances. As we assumed potential effects and interactions to be strongest when species and individuals physically interact, we separately examined data from the last year of measurements. To test whether our measurements were affected by tree mortality we correlated growth and herbivory of monoculture plots with within-plot tree density for each year and species separately. However, out of the 48 performed correlations, only four were significant ($p < 0.05$), one in each species, and in different years (data not shown). Thus, we considered these as spurious effects and did not further take into account mortality or tree density in our analyses.

6. 4 Results

All measures of growth and herbivory depended on species identity, both in species monocultures and species mixtures (Table 6.2, Fig. 6.1). *A. fortunei* was overall the tallest species whereas *I. polycarpa* exhibited the highest levels of herbivory. Species effects interacted with time (Table 6.2) and revealed strong temporal variation, most noticeably seen in *I. polycarpa* which suffered more than twice as much herbivory in 2013 when compared to other species in the same year (Fig. 6.1B, D, F and H).

Species diversity had a significant positive effect on stem diameter ($F_{1,18}$= 7.94, P= 0.012, Fig. 6.2A) and height ($F_{1,18}$= 4.85, P= 0.041, Fig. 6.2C). Mean stem diameter and height were 9.2% and 4.9% larger in species polycultures, respectively. Species diversity effects on stem diameter also varied over time, indicated by a marginally significant species diversity × time interaction ($F_{2,36}$= 2.9, P= 0.069, Fig. 6.2B). Analysing data from 2013 also yielded significant positive species diversity effects on both stem diameter and height ($F_{1,18}$= 8.02, P= 0.012 and $F_{1,18}$= 5.02, P= 0.038, respectively) which was not the case in 2012.

We did not find a statistically significant overall effect of SF richness on growth and herbivory, nor did we find a significant interaction of species and SF diversity. However, statistically significant SF richness × species identity interactions were found for polycultures (Table 5.2), indicating that species were affected differently by genetic diversity, but only when grown in species mixtures. Higher genetic diversity resulted in a reduction of growth when compared to genetic monocultures (Fig. 6.3A, left panel). In contrast, herbivory increased with higher genetic diversity (Fig. 6.3D, left panel). The effects of genetic diversity on growth were independent of time, with the exception of stem diameter ($F_{6,279}$= 2.34, P= 0.033, Table 6.2).

Lastly, SF composition consistently explained a statistically significant fraction of variance in growth measures (Table 6.2). Effects were also found as significant interactions with time for all growth variables (Table 6.2). Surprisingly, herbivory was not affected by SF composition, neither overall, nor over time.

6. 5 Discussion

We found that species and genetic diversity had contrasting effects on growth. Generally, growth increased with species diversity while in species mixtures, growth decreased with genetic diversity. In contrast, herbivory was not affected by species diversity but rather increased with genetic diversity in species mixtures. These effects were significant after accounting for prevalent species identity effects. Our findings not only stress the importance of functional differences between species for ecosystem-functioning but also demonstrate that intraspecific genetic diversity can exert strong effects on community performance at the same time, even contrasting the effects of species diversity. This study is one of the few that directly assessed the influence of both the genetic and species levels of biodiversity simultaneously in experimental forest communities.

Genetic diversity effects

Genetic diversity affected growth and herbivory, but only in species mixtures. Growth was reduced whereas herbivory increased in plots with four seed families. In other words: in species polycultures, genetically rich communities grew slower while suffering higher levels of herbivory than genetic monocultures. Increasing herbivory with increasing SF diversity might be due to dietary mixing (e.g. Bernays *et al.* 1994) or associational susceptibility (reviewed in Barbosa *et al.* 2009) and was also observed when increasing plant genetic diversity (Castagneyrol *et al.* 2012; Kotowska *et al.* 2010). However, the negative effect of genetic diversity on tree growth to be negative was surprising as other studies reported opposite patterns (e.g. Schweitzer *et al.* 2005) and considering the overall positive effect of species diversity on growth. How would growth increase with species diversity while it decreases with genetic diversity in species rich communities? Although growth and herbivory have been found to be positively correlated (Cook-Patton *et al.* 2011; Crutsinger *et al.* 2006) also the opposite is possible as Zvereva *et al.* (2012) showed that low to moderate amounts of simulated herbivory severely reduced growth in *Betula pubescens* subsp. czerepanovii saplings. Thus we hypothesise that high genetic diversity in polycultures favours herbivory, possibly via dietary mixing (Bernays *et al.* 1994) or increased generalist herbivore load (associational susceptibility, Barbosa *et al.* 2009; Plath *et al.* 2012). As herbivory increases, it negatively affects growth by altering assimilate distribution or reducing photosynthetic leaf area. This interpretation is further supported by a recent study conducted on the experimental sites of the BEF China project, reporting a negative correlation between herbivory and growth (Schuldt *et al.* 2015). The authors found herbivory to increase with species richness and most of the herbivore damage was caused by grasshoppers and lepidopteran caterpillars, both of which are assumed to be generalists (Bernays and Chapman 2000; Schuldt *et al.* 2014). Similarly, in the BEF-China experiment, the most abundant herbivore taxa were Lepidoptera, Curculionidae and Auchenorrhyncha, which showed a high degree of generalism in host use (Zhang *et al.* 2016). Such generalism in feeding behaviour may lead to associational susceptibility of target plants, if more palatable species in a plot attract more herbivores who then also attack a less palatable target species. An alternative hypothesis arises from findings of Crawford & Rudgers (2013), who observed effects of genetic diversity in a dominant species on arthropod community composition and on community biomass production (Crawford and Rudgers 2012). They argued that herbivores may be sensitive to emergent community properties, which can be strongly affected by dominant species. Accordingly, in our study growth and herbivory in species polycultures could be affected by genetic diversity in a single rather than all species. For example, genetic diversity in *I. polycarpa*, which exhibited the highest level of herbivory, could be linked to traits that affect herbivory (Eichenberg *et al.* 2015). Among the four species studied, *I. polycarpa* ranked lowest in leaf concentration of total phenolics (Eichenberg & Bruelheide, unpublished), and thus, invested the least in structural defence. In contrast, genetic diversity in *A. fortunei*, the tallest species, may mainly have affected growth. This would not necessitate a causal relationship between growth and herbivory. Whether or not causally related in our case, we demonstrated that plant-herbivore interactions as well as plant growth in interacting tree species are differently affected by intraspecific diversity in different species

Species diversity effects

We found a positive effect of species richness on growth among subtropical tree species. Similar results were found previously (e.g. Vila *et al.* 2013 for temperate forests) and could be due to complementary resource use of different species in mixtures, allowing a more complete utilization of available resources, the alleviation of abiotic and/or biotic stress via interspecific facilitation or sampling effects of especially impactful species. Specifically, resources such as light or nutrients might be acquired and/or used more efficiently in heterogeneous plant assemblies due to complementary crown or root architectures, respectively. Following Ishii & Asano (2010), species' spatiotemporal differentiation in light acquisition could allow complementarity among species. As the species in the present study differ substantially in height (e.g. *A. fortunei* is about twice as tall as *C. camphora*) we cannot rule out a sampling effect of *A. fortunei* potentially driving the overall pattern, but it is also plausible that these differences lead to a multi-layered weave of canopies increasing the light-capture efficiency of the community as a whole. Similarly, belowground niche partitioning, e.g. due to differing rooting depth in species mixtures, was shown to affect biomass productivity (Berendse 1982; Hooper 1998) and could affect mixed communities in a similar manner as their aboveground counterpart by allowing for a more complete use of belowground resources such as water or nutrients. Essentially, heterogeneity is expected to allow species to fill available “space” more completely, both physically and/or ecologically. This heterogeneity could not only lead to complementarity effects (as e.g. in Wacker *et al.* 2008), but also to the alleviation of stress. Similarly to the above, complementary crown and/or root architectures in mixed species communities could reduce the effects of drought or heat. Shade cast by larger species might reduce heat stress in smaller species or lower the rate of soil water evaporation. Drought and heat stress could be important drivers and negatively affect tree growth considering that our experimental site was clear-cut prior to planting and soil surface temperatures exceeded 70°C in exposed locations (personal observation).

Our species pool was comprised of two evergreen and two deciduous species and thus species mixtures always contained both functional groups. The importance of functional group diversity for ecosystem functioning has been pointed out previously (e.g. Loreau *et al.* 2001; Reich *et al.* 2004; Tilman *et al.* 1997a). The combination of evergreen and deciduous species might not only lead to spatio-temporal niche differentiation as described above, but also affect resource dynamics via, for example litter quality and decomposition rates. Leaf chemical properties and timing of senescence can affect resource availability and retention times (see Richards *et al.* 2010 and references therein) and might be more favourable in mixtures of evergreen and deciduous species than in their respective monocultures. Irrespective of the underlying mechanisms, our findings provide evidence that growth in forest communities is positively correlated to species richness (Baruffol *et al.* 2013; Piotto 2008).

Our results suggest that species and genetic diversity might operate context dependant. Growth was positively affected by the number of species present (i.e. complementarity, facilitation or sampling effects), whereas growth was negatively affected by the number of genotypes in polycultures (i.e. associational susceptibility or dietary mixing resulting in reduced growth). Thus, the influence as well as the mode-of-operation of genetic diversity might need further empirical evaluation as species and genetic diversity are rarely manipulated simultaneously and only manipulating one level of biodiversity might give an incomplete picture.

Temporal dynamics

We observed that within only three years after establishment, growth and herbivory were either affected by species richness or, in a special case, genetic diversity within species, showing that community responses to biodiversity establish quickly in subtropical forests, a conclusion also made by Schuldt *et al.* (2015). Evidence gathered from 20 years of BEF research also suggests that effects of biodiversity on ecosystem functioning are likely to strengthen over time (Cardinale *et al.* 2012). We found multiple interactions of time with growth and herbivory. Effects of diversity treatments were most pronounced in the last year and would have remained undetected in earlier years. This also suggests a time lag in the community response to diversity treatments. At the beginning of our measurements, tree canopies were not closed and only started to do so in the last year, indicating that competition may increase in the future. Thus, although we could not confirm an overall effect of intraspecific genetic diversity it will be interesting to follow the development in the future. If genetic diversity is expressed in traits that require physical contact among individuals to produce a community response, effects of genetic variation are expected to become stronger in the future.

Conclusion

Subtropical forests harbour substantial amounts of diversity, but studies regarding biodiversity- ecosystem functioning processes in this ecosystem remain scarce. We showed that species diversity affects tree growth at early developmental stages and that there is considerable within species genetic variation among seed families. Furthermore, genetic diversity might indirectly affect growth through interactions at higher trophic levels. This underlines the importance of tree species and genetic diversity as drivers of ecosystem functioning. In the context of ongoing habitat fragmentation and species loss, our study also suggests that changes in both species and genetic diversity can affect ecosystem functioning and services, even in opposing ways, findings with far-reaching consequences for forest management and conservation.

6. 6 Tables and figures

Table 6.1 – Number of replicates per level of species and genetic diversity. Plot size is given in mu, a traditional Chinese unit of area (25.8 × 25.8m) with 1 mu being the size of a full plot with 400 individuals. SD- Species diversity; GD- Genetic diversity

Species	SD	GD	# of different seed family compositions	Plot size (mu)
Alniphyllum fortunei	1	1	8	0.25
	1	4	2	0.25
Cinnamomum camphora	1	1	8	0.25
	1	4	2	0.25
Daphniphyllum oldhamii	1	1	8	0.25
	1	4	2	0.25
Idesia polycarpa	1	1	8	0.25
	1	4	2	0.25
All 4 species	4	1	7	1
	4	4	6	1

Table 6.2 – Model structure and results of the linear model analyses. Numerator (Term) and denominator (Error) are given with their respective degrees of freedom (df). For growth and herbivory, *F*- values and significances are given to the right.

Term	df	Error	*F* and significance			
			Stem diam.	Height	Crown diam.	Herbivory
Species diversity	1	Sp.mono	0.7	0.22	0.13	0.06
Species diversity	1	Plot	7.94*	0.041*	2.77	1.33
Species in species monocultures (Sp.mono)	3	Plot	11.31***	21.67***	21.42***	23.81***
Genetic diversity	1	Plot	0.53	0.13	1.21	0.1
Species diversity × Genetic diversity	1	Plot	0.23	<0.01	<0.01	0.64
Plot	18					
Seed family composition	34	Subplot	4.21***	3.78***	6.23***	1.2
Species in species mixtures (Sp.mix)	3	Subplot × Species	192.71***	307.02***	297.78***	84.72***
Sp.mix × Genetic diversity	3	Subplot × Species	6.14***	4.54**	5.32**	3.07*
Subplot	33					
Subplot × Species	141					
Time	2	Time × Plot	534.79***	690.03***	434.48***	44.74***
Time × Species div.	2	Time × Sp.mono	0.9	0.2	0.13	0.22
Time × Species div.	2	Time × Plot	2.9.	1.95	0.77	1.37
Time × Species in species monocultures	6	Time × Plot	3.21*	9.76***	5.91***	6.13***
Time × Genetic diversity	2	Time × Plot	0.06	0.13	0.38	1.9
Time × Species diversity × Genetic diversity	2	Time × Plot	0.71	1.37	0.78	1.04
Time × Plot	36					
Time × Seed family composition	68	Time × Subplot	3.18***	2.53***	5.33***	1.22
Time × Species in species mixtures	6	Time × Subplot × Species	44.97***	45.1***	21.44***	46.37***
Time × Sp.mix × Genetic diversity	6	Time × Subplot × Species	2.34*	1.49	1.46	0.95
Time × Subplot	66					
Time × Subplot × Species	279					

Figure 6.1 – Species- specific growth (A, B Stem diameter; C, D Height; E, F Crown diameter) and herbivory (G, H). Effect is shown as overall (A, C, E, G) and annual mean (B, D, F, H) in both, species monocultures and species mixtures. Means are shown as rectangles within boxes. Al- *Alniphyllum fortunei*, Ci- *Cinnamomum camphora*, Da- *Daphniphyllum oldhamii*, Id- *Idesia polycarpa*

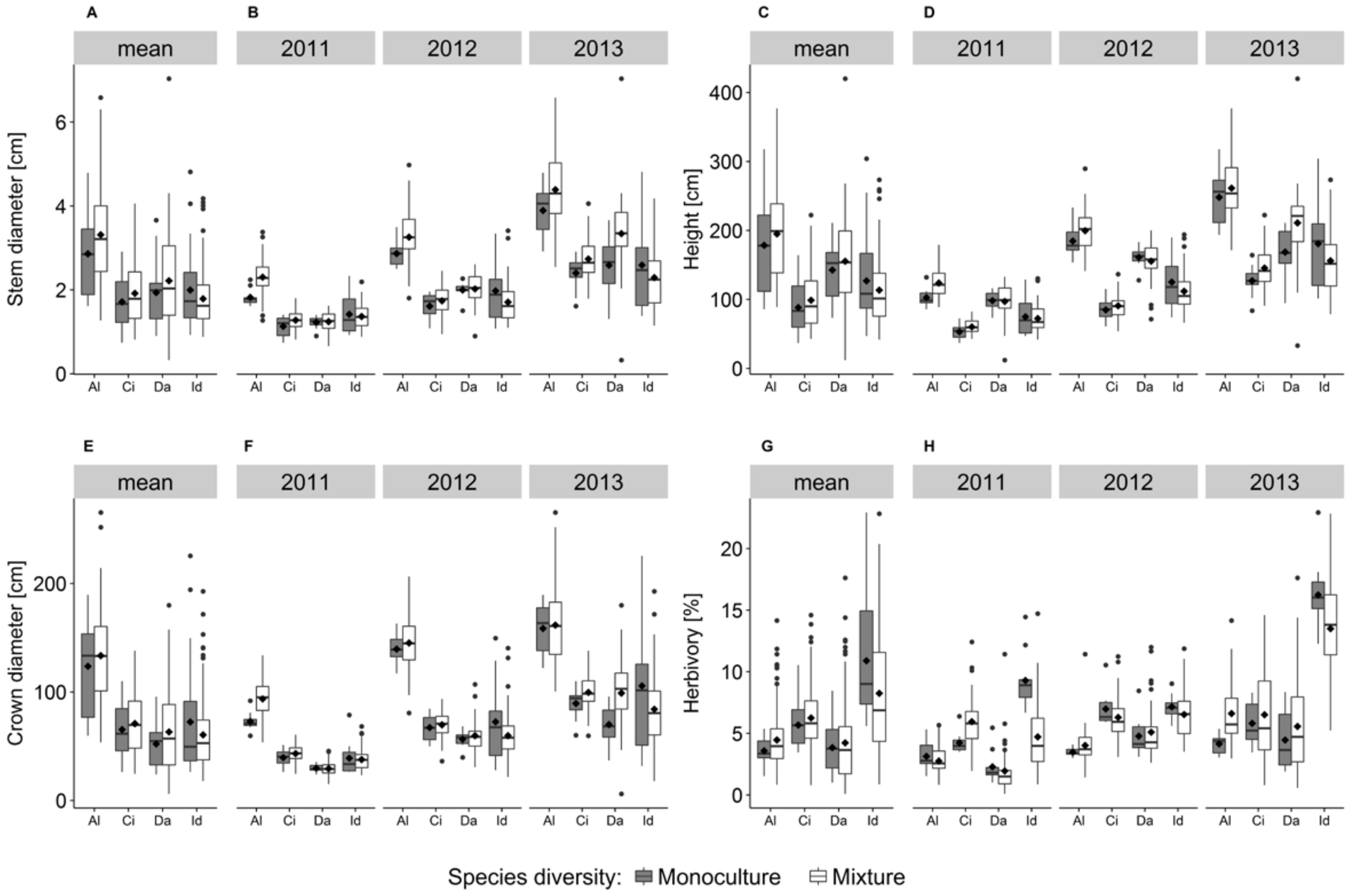


Figure 6.2 – Effect of species diversity on growth (A, B Stem diameter; C, D Height; E, F Crown diameter) and herbivory (G, H). Effect is shown as overall (A, C, E, G) and annual mean (B, D, F, H). (n.s.) - not.significant, (.) - marginally significant, (*) $p < 0.05$

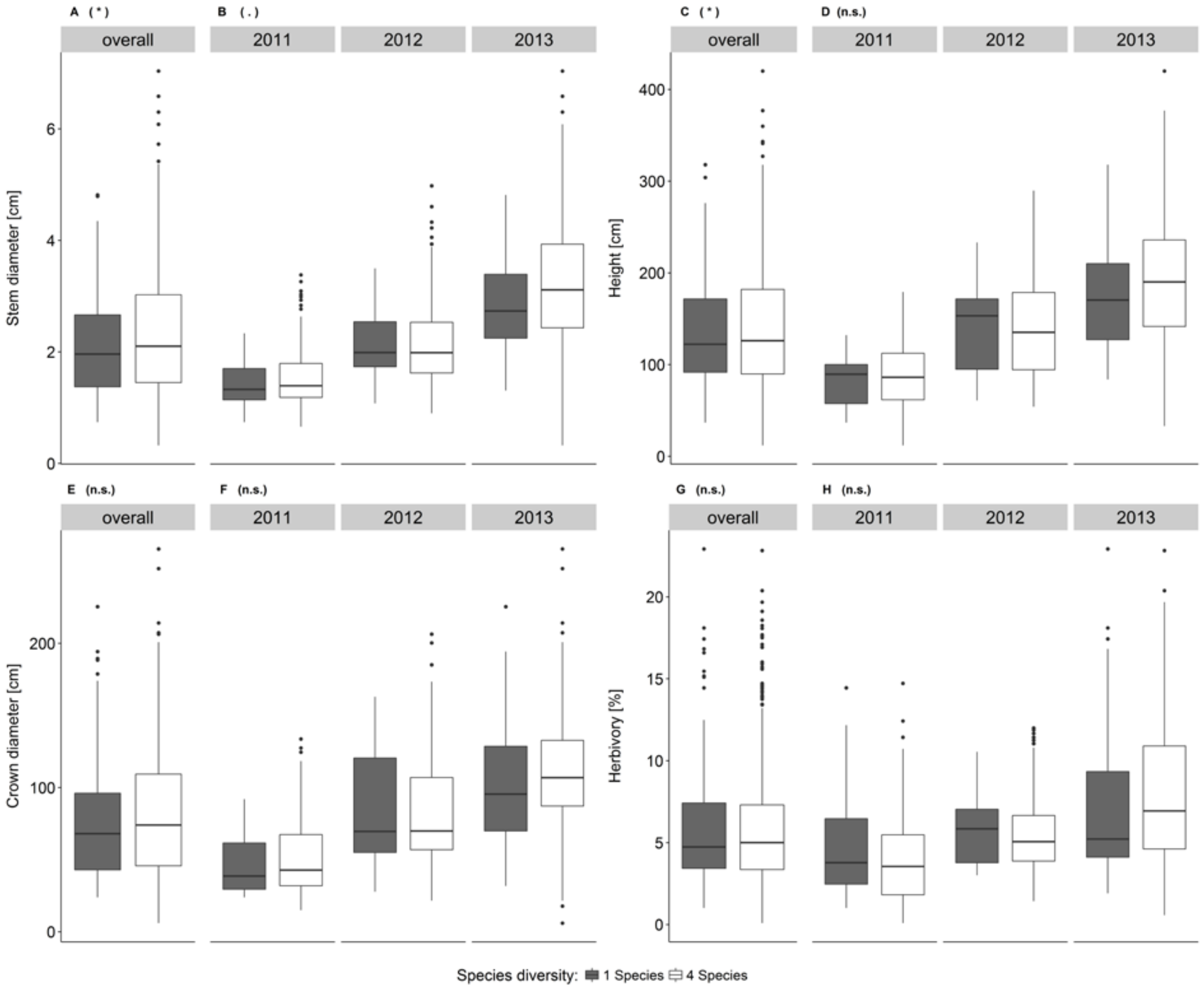
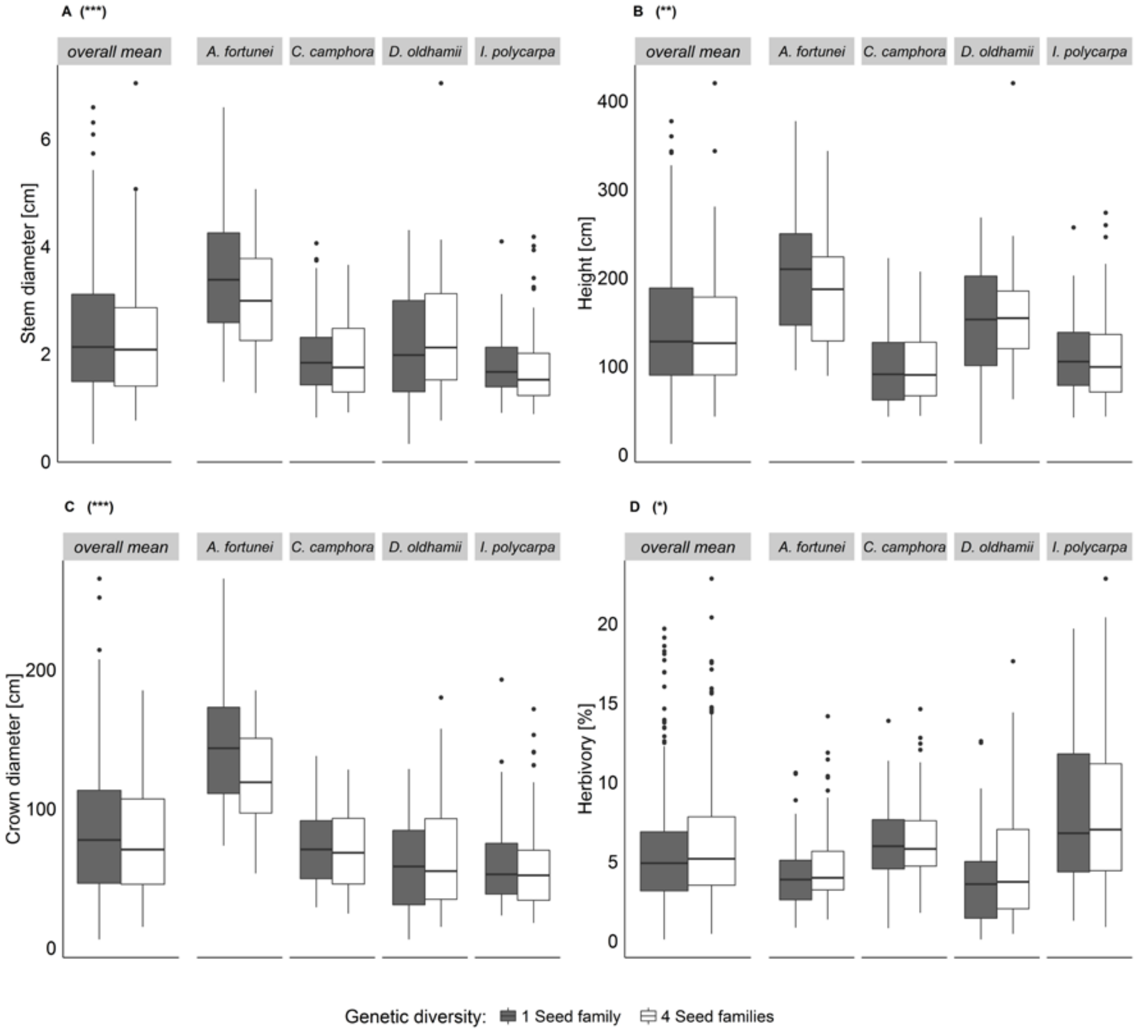


Figure 6.3 – Effect of genetic diversity on growth (A- Stem diameter; B- Height; C- Crown diameter) and herbivory (D) in species mixtures. Overall and species- specific means are shown. (*) - $p < 0.05$, (**) - $p < 0.01$, (***) – $p < 0.001$



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6. 8 Supplementary material and acknowledgements

Table S1: Seed family identity, number of individuals planted in 2010 and number of individuals that survived until 2013 in each species. Seed families in *italic* font have been omitted from analyses due to low number of observations.

Figure S1: Growth (A- Stem diameter; B- Height; C- Crown diameter) and herbivory (D) in 2011 per species and seed family.

Figure S2: Growth (A- Stem diameter; B- Height; C- Crown diameter) and herbivory (D) in 2012 per species and seed family.

Figure S3: Growth (A- Stem diameter; B- Height; C- Crown diameter) and herbivory (D) in 2013 per species and seed family.

Supplementary material available online after publication

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7. Synthesis

The objective of this thesis is the assessment of molecular variation within species, across species as well as testing for effects of intra- and interspecific variation on ecosystem functioning. The scientific literature comprises many examples of genetic diversity producing diverse effects on, for example, herbivory (Barton *et al.* 2015; Castagneyrol *et al.* 2012), arthropod communities (Crutsinger *et al.* 2006) or biomass production (Cook-Patton *et al.* 2011; Drummond and Vellend 2012; Kotowska *et al.* 2010). Likewise, it was shown that genetic diversity can be affected by various factors, such as chemical contaminants (Bickham *et al.* 2000), life-history traits (Hamrick and Godt 1996) or altitudinal gradients (Herrera and Bazaga 2008). However, comparably little is known about how these findings may apply to long-lived trees and shrubs, especially from the Subtropics as for example much of the available literature is concerned with grasslands (e.g. ~ 56% of all studies in Balvanera *et al.* 2006). This may result in inaccurate inferences across live forms and habitats, representing a gap in our understanding of biodiversity-ecosystem functioning, particularly regarding the genetic aspect of biodiversity. The present thesis provides new insights as well as potential research avenues for future investigations, synthesized in the following passage after the main results of each chapter are briefly recapitulated.

(I)- The aim of the first study presented in this thesis was to characterize gene flow and mating system parameters in *Rhododendron simsii*, a subtropical understory shrub. Historic gene flow, estimated from pairwise kinship coefficients, ranged between 10-15 m over a wide range of possible effective population densities. Recent gene flow via pollen, estimated from paternity analysis, could be traced up to ~100 m but most mating events occurred between 10-20 m. Taken together, our results show that *R. simsii* is predominantly, but not obligately outcrossing with short gene dispersal distances. High (effective) population densities may compensate for low dispersal distances and allow for the maintenance of large amounts of genetic diversity.

(II)- The second study of this thesis focussed on parallel processes affecting intra- and interspecific variation. It was found that neither species richness nor most environmental properties affected genetic diversity across species. However, genetic diversity and population differentiation were significantly correlated to plot successional stage in several species. This suggests that forest demographic dynamics can affect the distribution of genetic diversity in tree and shrub species, presumably mediated through changing environmental conditions over the course of secondary succession.

(III)- The effects of species richness and genetic diversity on plant growth and herbivory were assessed simultaneously in a third study. Growth was positively correlated to species richness whereas herbivory was not. However, genetic diversity and growth were negatively correlated while herbivory increased with genetic diversity, but only so in species mixtures. Thus, we confirm that intra- and interspecific diversity are important determinants of plant growth and plant-herbivore interactions. So far, the effects of intra- and interspecific diversity have not been assessed simultaneously in subtropical forests and the results presented throughout the third chapter of this thesis provide novel insights regarding biodiversity-ecosystem functioning relationships.

7. 1 Effects on genetic diversity

In the first two chapters of this thesis it was tested whether genetic diversity and the processes governing it are affected by external conditions. In the first study, *R. simsii* was characterized using molecular techniques, refining the picture described in other studies (Ng and Corlett 2000a; Ng and Corlett 2000b). Interestingly, the strength of genetic structuring in *R. simsii* differed among chapters. Whereas we found very weak fine-scale SGS ($Sp = 0.00318$) in chapter I, population differentiation (F_{ST} = 0.239) was comparably strong in chapter II. If SGS is caused by gene flow limitation, the former suggests little limitation due to the absence of local pedigree structures (Vekemans and Hardy 2004). Conversely, a high F_{ST} shows that a fair amount of genetic variation is maintained among distinct subpopulations (Holsinger and Weir 2009), which may be the result of restricted gene flow among populations (e.g. Theim *et al.* 2014). Thus, we obtain different interpretations from chapters I and II, the former advocating for little and the latter for substantial gene flow limitation in *R. simsii*. Admittedly, both descriptors are quite different from one another as one is a measure of among-population heterozygote deficit (F_{ST}, Holsinger and Weir 2009) whereas the other describes the decrease of pairwise relatedness with distance (*Sp*, Vekemans and Hardy 2004). In this vein it is important to consider the spatial scale at which each estimator is calculated. Fixation indices are based on all available subpopulations, regardless of the distance between them whereas the *Sp*-statistic is strongly affected by what constitutes the first distance class. Thus, the former is a measure of overall SGS whereas the latter describes the fine-scale SGS. This emphasises not only that population genetic descriptors need to be interpreted with care but also shows that direct measures of gene flow may aid in interpreting measures of genetic structure, as was done in chapter I. Calculating historic and recent gene flow distances and estimating effective population sizes allowed detailed conclusions about genetic structuring, but certain aspects regarding mating in *R. simsii* remain obscure. For example, the precise nature of the self-sterility in this species is still unknown. Although *R. simsii* is largely outcrossing, evidence of selfed progeny was reported, both in this thesis as well as by Ng and Corlett (2000a). Thus, the system preventing selfing in this species can be described as largely efficient, but leaky. It will be subject of future studies to determine whether *Rhododendron* species employ a gametophytic/ sporophytic SI (Charlesworth *et al.* 2005; Silva and Goring 2001) or whether other mechanisms such as early seed abortion prevent selfing. In this context it would certainly be of interest to include co-occurring, congeneric *Rhododendron* species from the GNNR, allowing interspecific comparisons. Further investigations may also include observations on pollinator movement or flowering phenology (Augspurger 1980). This could be especially relevant to mass flowering, highly outcrossing species such as *R. simsii*. Abundant floral rewards increase intraspecific competition for pollinators (Delmas *et al.* 2014; Johnson *et al.* 2012). Especially in dense patches of mass-flowering species, where floral rewards are likely to be highest, this should be relevant. Alternatively, pollinator visitation may be correlated to population size instead of floral attractiveness and small populations could suffer from pollen limitation (Ashman *et al.* 2004). Thus, correlating outcrossing rates or gene flow distances to pollinator counts or movement may provide a more detailed picture of gene flow in woody, long-lived species of the Subtropics. Ideally, the above would be complemented by phenological observations on the onset, duration and synchrony of flowering (e.g. Blionis *et al.* 2001; Zhang *et al.* 2010). Asynchronous flowering of congeneric mates may cause reproductive isolation or geitonogamous pollination (Murawski and Hamrick 1992) and investigating on specific flowering patterns is necessary to reveal such dependencies. Furthermore, pollinator and flowering parameters may be correlated to environmental characteristics. Ultimately, it may then be possible to quantify the contributions of evolutionary forces and the biotic and abiotic environment to the genetic structure within and among species.

Methodologically, chapter I and II can be regarded as a demonstration of different scopes of inferences that are based on population genetic descriptors, but also highlight a potential application of these descriptors in conservation efforts. The latter is reflected in the observed match between historical and recent gene flow, suggesting that the populations under investigation are in gene flow-drift equilibrium and have not been subjected to recent changes in population demographic properties. Furthermore, very weak fine-scale SGS indicates that our target species is not dispersal limited at the spatial scale we applied. Large effective population size may help prevent strong effects of random genetic drift (Balloux and Lugon-Moulin 2002), reducing the risk of extinction through the fixation of deleterious mutations (Higgins and Lynch 2001). Although the investigated populations of *R. simsii* appear not to be in need of conservational efforts, the findings and methods presented here have clear application in species conservation.

Although suitable for studying neutral evolutionary processes, a neutral marker system, as employed in chapter I and II, rarely allows inferences about selection (but see Shi *et al.* 2011). It would, however, be interesting to quantify adaptively non-neutral genetic diversity. This could be achieved, for example, through QTL mapping or association mapping approaches (Gailing *et al.* 2009; Schoville *et al.* 2012). Investigating on adaptive genetic diversity is a complementary follow-up to the research presented so far. It could be tested whether adaptive genetic diversity similarly follows secondary succession as observed for neutral genetic variation. As environmental conditions change with ongoing succession, selection for well-adapted genotypes in different successional stages may occur (Pickett 1976). If gene flow among successionally differentiated populations is sufficiently hindered through selection, a pattern of isolation-by-adaptation (Orsini *et al.* 2013) may ensue. Correlation of genetic features with environmental properties (e.g. along successional gradients) may indicate the presence of selective pressures causing deviations from HWE. It then may further be tested whether specific genes/traits are under selection by relating their expressional patterns to environmental conditions. Dillon and colleagues (2014) already demonstrated that variation at several SNP loci correlated with local climate in *Eucalyptus camaldulensis* Dehnh., most notably with variables associated to water stress. Similarly, resources such as light or water may be exerting different selective pressures in our study system. Adaptive genetic variation plays an important role for population viability, especially important under changing environmental conditions (Lande and Shannon 1996). However, the relationship between secondary succession and adaptive genetic diversity has rarely been studied so far (but see Wehenkel *et al.* 2011). It was suggested that ecological disturbance is a strong determinant of both neutral and adaptive genetic diversity (Banks *et al.* 2013). The question now is whether adaptive genetic diversity is maintained in late successional stages or if it is similarly reduced over the course of secondary succession as was observed in some instances regarding neutral genetic diversity. If so, secondary succession should be a central concern for the maintenance of forest genetic resources, the conservation and restoration of which may, in part, depend on the successional status of forests. However, the aforementioned requires explicit testing before being applicable in nature conservation and restoration.

7. 2 Effects of genetic diversity

The third chapter of this thesis considered genetic diversity to exert effects on plant performance and manipulating intra- and interspecific diversity simultaneously proved to be a powerful approach for assessing their combined effects on ecosystem functioning. Commonly, biodiversity in BEF research is manipulated at the species level while the variability contained within species is largely neglected. Thus, much of our current understanding regarding the relationship between biodiversity and ecosystem functioning is derived from generalizations across diversity levels. Potential interactions between intra- and interspecific biodiversity remain undetected unless both levels are assessed simultaneously. Particularly relevant to BEF research is any mechanism through which biodiversity at any given level affects other diversity measures or ecosystem functioning. In this context, chemical compounds are strong candidates for future investigations, as these may vary between genotypes and species. It was shown in *Pinus sylvestris* that chemical diversity of monoterpenes correlates with species richness of associated, individual ground vegetation

(Iason *et al.* 2005). Similarly, herbivore diversity in *Brassica oleracea* differed significantly between cultivars exhibiting glucosinolate profiles dominated by compounds varying in the length of side-chains (Poelman *et al.* 2009). Furthermore, biodiversity may be linked to ecosystem functioning via interactions between above- and belowground biota (Wardle *et al.* 2004). Intra- and interspecific diversity of plants may affect belowground organisms, for example through the chemistry of the litter they return to the soil (Hector *et al.* 2000; Schweitzer *et al.* 2005a; Schweitzer *et al.* 2005b). In turn, leaf litter quality can strongly affect plant growth by imposing effects on nutrient cycling (Schweitzer *et al.* 2004). Although we could confirm the existence of biodiversity effects on growth and herbivory, the precise nature of this relationship remains unknown (discussed in chapter III) and awaits further examination. For example, genetic diversity was manipulated in terms of seed families where the amount of genetic variation actually contained within seed families remains unknown. On the other hand, herbivory was assessed in terms of missing leaf area with no information regarding herbivore identity or abundance. Increasing the accuracy of predictor and response variable would allow more precise inferences about their relationship, for example by genotyping members within seed families and relating their genetic diversity to the abundance of different herbivore guilds. However, the question of how biodiversity affects plant growth or herbivory remains unanswered. Here, plant chemical compounds may offer a mechanistic link in a similar fashion as described in Maldonado-Lopez *et al.* (2015). The authors found significant correlations between genetic distance and chemical similitude as well as between chemical similitude and damage caused by leaf miners in *Quercus castanea*. Equivalently, future investigations may be less concerned with whether there are effects of biodiversity on ecosystem functioning but rather how exactly these are imposed, as the former has been demonstrated numerous times but the latter still largely represents a ‘black box’. In this context, the extended phenotype (Whitham *et al.* 2006; Whitham *et al.* 2003) offers a conceptual framework that aims at unravelling the effects of specific genes which are coding specific products which in turn have predictable effects on whole communities and ecosystems. Applied to chapter III of this thesis, this translates into identifying functional genes coding for important chemical compounds relevant to growth and plant-herbivore interactions, such as condensed tannins (Schweitzer *et al.* 2008), glucosinolates (Poelman *et al.* 2009) or phytohormones (Ponzio *et al.* 2013). Then, variation at those genes could be quantified and correlated to plant growth or plant-herbivore interactions. A common-garden experiment with individuals of known pedigree allows partitioning observed variances into genetic and environmental components. Investigations with the above objective could provide novel insights necessary to create a mechanistic framework linking genetic variation to community and ecosystem responses.

7. 3 Outlook

Potential follow-up research has been suggested at several points throughout the previous discussion. The focus of this last paragraph is put on the importance of long-term experiments and their role in ecological and evolutionary research. As proposed (Cardinale *et al.* 2007; Drummond and Vellend 2012; Tilman *et al.* 2012), biodiversity effects on ecosystem functioning may increase with time. It was also found in the third chapter of this thesis that the effects of biodiversity on ecosystem functioning may be undetectable at early stages of tree development, similarly to findings from two long-term experiments in which biomass production increased non-linearly and more diverse mixtures accumulated increasing amounts of biomass over time (Reich *et al.* 2012). In the same vein, temporally variant gene flow was reported in *Gleditsia triacanthos* (Schnabel and Hamrick 1995), which may be especially common where pollen and seed disperser abundances or activities vary significantly among years. When run over extended periods of time, long-term experiments have the benefit of allowing long-lived shrub and tree species to reach reproductive maturity. In turn, this allows assessing selection and fitness-related parameters which are commonly inaccessible in controlled experiments as it takes years to decades for long-lived species to reach reproductive maturity under natural conditions. If not accounted for, variation in any measure may be affected by temporal heterogeneity, making temporal replication (i.e. long-term observations) critical for accurate inferences. The above contains only few examples of ecologically and evolutionarily important processes, all of which may not only vary over time but may also feed back on each other. Traditionally, these processes are assumed to operate on largely different time-scales but it was suggested that their relationship may be closer than expected (Carroll *et al.* 2007). Much like genetic diversity affects and is affected by the environment, the evolution and ecology of species may be interrelated by feedback-loops. The study of any such relationship requires long-term observations to capture gradual changes in community responses, especially relevant in a context of accelerating global change. This shall be exemplified by circling back to the context of this thesis. The research presented in chapter III was carried out in experimental tree communities of known genotypic origin. With time, these trees will develop, reproduce and progressively interact with community members such as plants, pollinator, herbivores, soil organisms or pathogens, many of which have already been monitored for years. Applying methods employed in chapters I and II, population differentiation, gene flow distances and mating system parameter may be calculated, preferably supplemented by large genotyping efforts. Once reaching maturity, reproductive output, offspring performance and offspring genotypes can be determined. At this point, a generational cycle is completed and it may be possible to quantify and relate all genetic and environmental factors that lead to gene frequency changes from one generation to the next. This is nothing less than a glimpse at evolution and long-term experiments provide ideal conditions for studying the relationship of evolution and ecology through high temporal resolution. In this vein, the present thesis will be concluded by a quote which perfectly reflects the above:

“We see nothing of these slow changes in progress, until the hand of times has marked the long lapses of ages [...].”

– Charles R. Darwin- On the origin of species by means of natural selection

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8. Appendix

8. 1 Curriculum vitae

Informationen zur Person

Name	Christoph Zacharias Hahn
Geschlecht	männlich
Akadem. Titel	Dipl. Biol.
Anschrift	Mühlberg 3, 06108 Halle/S.
Geburtsdatum	31.01.1985
Geburtsort	Rochlitz
Nationalität	Deutsch

Bildungsgang

Mai 2011 – Gegenwart	Doktorand am Helmholtz Zentrum f. Umweltforschung – Halle
April 2011	Erwerb des Diploms, Titel der Arbeit : “Plastic response of growth and leaf traits to topography and site conditions in subtropical forest species”
Oktober 2005 – April 2011	Studium der Biologie an der ‘Martin-Luther Universität Halle-Wittenberg‘ Hauptfach in Geobotanik, Nebenfach in Pflanzenphysiologie, Pflanzenzüchtung and ökol. Umweltbiochemie
August 2004 – August 2005	Freiw. ökologisches Jahr (FÖJ) am Walderlebniszentrum Schernfeld
Juni 2004	Abitur am Einstein Gymnasium Angermünde

Halle (Saale), den 13.03.2017

Christoph Z. Hahn

8. 2 Publikationsliste

Hahn CZ, Michalski SG and Durka W (2016). Gene flow in and mating system of *Rhododendron simsii* in a nature reserve in subtropical China. *Nordic Journal of Botany* (in press) doi: 10.1111/njb.01311

Hahn CZ, Michalski SG, Fischer M and Durka W (2016). Genetic diversity and differentiation follow secondary succession in a multi-species study on woody plants from subtropical China. *Journal of Plant Ecology*- Special Issue (accepted)

Hahn CZ, Niklaus PA, Bruelheide H, Michalski SG, Shi MM, Yang X, Zeng X, Fischer M and Durka W (2016).Opposing intra vs. interspecific diversity effects on herbivory and growth in subtropical experimental tree assemblages. *Journal of Plant Ecology*- Special Issue (accepted)

8. 3 Erklärung über den persönlichen Anteil an den Publikationen

1. Publikation (Chapter I)

Hahn CZ, Michalski SG and Durka W (2016). Gene flow in and mating system of *Rhododendron simsii* in a nature reserve in subtropical China. *Nordic Journal of Botany* (in press) doi: 10.1111/njb.01311

Feldarbeit	C. Z. Hahn (100%)
Laborarbeit	C. Z. Hahn (80%), A. Voigt (HiWi, 20%)
Statistische Auswertung	C. Z. Hahn (80%), W. Durka (10%), S.G. Michalski (10%)
Wiss. Schreiben	C. Z. Hahn (90%), W. Durka (5%), S.G. Michalski (5%)
Kommentare/ Korrekturen	W. Durka, S. G. Michalski

2. Publikation (Chapter II)

Hahn CZ, Michalski SG, Fischer M and Durka W (2016). Genetic diversity and differentiation follow secondary succession in a multi-species study on woody plants from subtropical China. *Journal of Plant Ecology*- Special Issue (accepted)

Feldarbeit	C. Z. Hahn (100%)
Laborarbeit	C. Z. Hahn (60%), S. G. Michalski (40%)
Statistische Auswertung	C. Z. Hahn (80%), W. Durka (10%), S.G. Michalski (10%)
Wiss. Schreiben	C. Z. Hahn (85%), W. Durka (10%), S.G. Michalski (5%)
Kommentare/ Korrekturen	M. Fischer

3. Publikation (Chapter III)

Hahn CZ, Niklaus PA, Bruelheide H, Michalski SG, Shi MM, Yang X, Zeng X, Fischer M and Durka W (2016).Opposing intra vs. interspecific diversity effects on herbivory and growth in subtropical experimental tree assemblages. *Journal of Plant Ecology*- Special Issue (accepted)

Feldarbeit	C. Z. Hahn (100%)
Laborarbeit	nicht zutreffend
Statistische Auswertung	C. Z. Hahn (50%), P. A. Niklaus (50%)
Wiss. Schreiben	C. Z. Hahn (90%), W. Durka (5%), H. Bruelheide (5%)
Kommentare/ Korrekturen	P. A. Niklaus, H. Bruelheide, X. Yang, X. Zeng

Christoph Z. Hahn

Walter Durka

Halle (Saale), den 13.03.2017

8. 4 Eigenständigkeitserklärung

Hiermit erkläre ich, dass die Arbeit mit dem Titel “ **Genetic diversity in natural and experimental, subtropical tree communities**“ bisher weder der Naturwissenschaftlichen Fakultät I der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt wurde.

Ferner erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst sowie keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen wurden als solche von mir kenntlich gemacht.

Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe.

Halle (Saale), den 13.03.2017 ___________________________

Christoph Z. Hahn

8. 5 Liste der Gutachter und Datum der Verteidigung

Erstes Gutachten:	Prof. Dr. H. Bruelheide
Zweites Gutachten:	Dr. W. Durka
Drittes Gutachten:	Prof. Dr. I. Leyer
Datum der Verteidigung	7. März 2017