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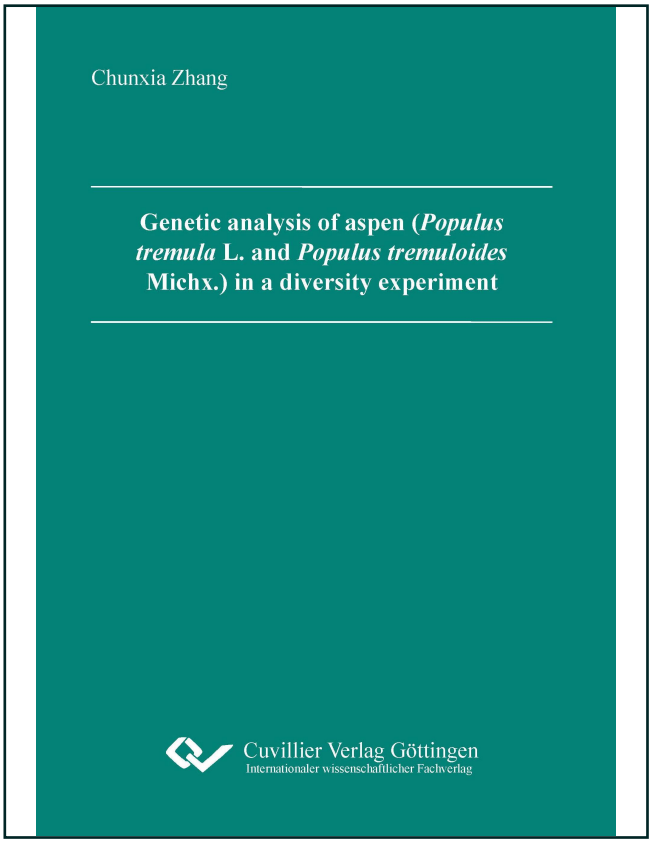
Genetic analysis of aspen (Populus tremula L. and Populus tremuloides Michx.) in a diversity experiment



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1 INTRODUCTION

1.1 Ecological importance of genetic diversity

Biodiversity is the full range of variety and variability within and among living organisms and the ecological complexes in which they occur, including diversity at the levels of individual, species and community (Jensen et al. 1993). It is driven fundamentally by diversity at the individual level. Thus, genetic diversity is the foundation of all biodiversity. Genetic diversity is the variety of alleles and genotypes present among individuals within a population of a single species, which provides raw material for natural selection, playing an important role in maximizing species' potential to withstand and adapt to biotic and abiotic environmental changes (Jump et al. 2009). Species diversity is the variety and abundance of different types of organisms that inhabit a region, which is one of key components of biodiversity**.** Both species diversity and genetic diversity are connected and interactively influence community properties and ecosystem processes (Vellend and Geber 2005; Lankau and Strauss 2007). Therefore, maintaining high levels of biodiversity is important for maximizing its ecosystem functioning.

However, in recent years, biodiversity is decreasing rapidly driven by human activities and climate change (Butchart et al. 2010). The ecological consequences of biodiversity loss have become a prominent public and scientific issue (Loreau et al. 2001; Hooper et al. 2005). There is much concern about the effect of species diversity on ecosystem functioning in the past decades. Numerous studies provide evidence that reduced species diversity has negative effects on ecosystem processes such as productivity, community stability and trophic interactions (Tilman and Downing 1994; Tilman et al. 2001; Cardinale et al. 2006; Haddad et al. 2009; Scherber et al. 2010; Zavaleta et al. 2010; Haddad et al. 2011). Whereas relatively less evidences exist regarding the effect of genetic diversity within species on ecosystem functioning. The effect of genetic diversity on ecosystem functioning should capture much more attention since biodiversity loss actually begins with the decrease of genetic diversity within species. Genetic diversity may have important ecological effects on ecosystem functioning such as community structure, resistance to disturbance, productivity, and fluxes of energy and nutrients (Hughes et al. 2008) which are described in detail below.

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1.1.1 Genetic diversity and ecosystem functioning

1.1.1.1 Genetic diversity and associated organisms

Genetic diversity at individual level (genotypic diversity) is essential for the conservation of associated species in a community. Plant genotypic diversity is an important determinant to diversity of associated organisms. An experimental study in *Solidago altissima* showed that the number of arthropod species in 12-genotype plots was on average 27% greater than that in single-genotype plots and the richness of herbivore species and predator also increased with increasing genotypic diversity (Crutsinger et al. 2006). Similar results were found in *Oenothera biennis* that patches with eight genotypes had 18% more arthropod species and a greater abundance of omnivorous and predacious arthropods compared with monocultures (Johnson et al. 2006). These results highlight the importance of plant genotypic diversity in determining the number of associated organisms.

Genetic variation at the population level is also important to conserve variable communities. The heritable tree-trunk bark variation of *Eucalyptus globulus* among geographic races significantly influenced the associated communities of microorganisms, invertebrates, and vertebrates in the aspect of species richness and abundance (Barbour et al. 2009). Genetic variation of a tropical tree species was found to influence the associated epiphytic plant and invertebrate communities in a complex tropical forest ecosystem, and there was a positive relationship between genetic distance of trees and community difference of the associated species (Zytynska et al. 2011), which means that genetically more similar trees are host to more similar epiphyte and invertebrate communities. Moreover, genetic diversity of cottonwood hybrids across 11 natural stands was significantly correlated with arthropod diversity (Wimp et al. 2004).

1.1.1.2 Genetic diversity and ecosystem stability

Genetic diversity facilitates the ecosystem stability which refers to resistance to disturbance, resilience, persistence and variability. High levels of genetic diversity increased ecosystem stability disturbed by grazing geese and shortened their recovery time (Hughes and Stachowicz 2004) and increasing genotypic diversity of seagrass also buffered against thermal stress (Reusch et al. 2005). Furthermore, genetic diversity

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within species is one remedy to resist the devastating pathogen and pest invasion such as leaf blight in corn (Strange and Scott 2005) and bark beetle in elm (Lee et al. 2011). Therefore, high levels of genetic diversity can enhance resistance or resilience to perturbation and act as an insurance against perturbations and environmental fluctuations although the underlying mechanism is still unclear (Hughes and Stachowicz 2011).

1.1.1.3 Genetic diversity and productivity

The effect of species diversity on productivity has aroused much attention over the last decades with the increasing demand for wood and renewable energy sources nowadays. In grassland species, the positive relationship has been disentangled between species diversity and productivity (Loreau and Yachi 1999; Tilman et al. 2001; Catovsky et al. 2002; Schmid et al. 2008). Also, for forest tree species, plantations with different tree species achieved greater productivity than monocultures in the humid tropics (Erskine et al. 2006). Conversely, only a few work was carried out on the effect of plant genotypic diversity on productivity in grassland species (Kotowska et al. 2010). In addition, the effect of genetic diversity on productivity in forest trees is still unknown because forest trees have a long life span and the productivity may vary in different years (Munzbergova et al. 2009). However, forest trees species play an important role in providing raw materials for wood products and biofuel production, which highlight the urgent need to study the productivities on trees, especially for fast growing trees such as poplars and eucalyptus. For forest tree species, most genetic variation is present within populations due to their predominantly outcrossing mating system; hence, it is necessary to further study the genetic diversity within forest tree species on the productivity.

1.1.1.4 Genetic diversity and nutrient cycling

Emerging evidence has shown that plant genetic variation may have ecosystem-level consequences by affecting carbon and nitrogen cycling and resource quality for litter decomposers and foliar herbivores (Classen et al. 2007). For instance, reductions in the genetically based phenotypic diversity of *Quercus laevis* leaf litter significantly affected carbon and nitrogen cycling during decomposition (Madritch and Hunter 2002; Madritch and Hunter 2005). Similarly, small genetic differentiation among populations of *Metrosideros polymorpha* caused significant differences in nutrient cycling through

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litter quality (Treseder and Vitousek 2001). Furthermore, different aspen genotypes were reported to influence below ground carbon and nitrogen contents and microbial activities (Madritch et al. 2009). In addition, genetic identity of aspen may also influence the leaf litter decomposition and release of carbon, nitrogen and sulfur from the leaf litter to the soil (Madritch et al. 2006).

1.1.2 Study approaches

1.1.2.1 Observational approach

The studies on the relationship between genetic variation and ecosystem functioning described above in forest trees mainly focus on observation method in natural or managed forest ecosystems. For instance, this method has been used to compare the productivity in mixtures of eucalypts and rainforest species and their monoplanting (Erskine et al. 2006) and to monitor the growth rate of mixed tree species plantations and their monoculture plantations (Piotto 2008). However, with this approach, it is difficult to know exactly the diversity effect on ecosystem functions (e.g. productivity) for example due to the influence of climatic conditions. In consequence, there is still no clear evidence about the effect of tree species diversity on some ecosystem functions (e.g. productivity) because they are mainly influenced by the heterogeneous environment such as soil properties and climate conditions (Vila et al. 2003). Thus, it is important and necessary to control such potentially environmental factors in a diversity experiment.

1.1.2.2 Experimental approach

Most previous biodiversity and ecosystem functioning studies have been conducted in controlled experiments using grassland species because they are easy and fast to establish such as the Jena experiment (http://www.the-jena-experiment.de/) and European BIODEPTH network of plant-diversity manipulation experiments (Spehn et al. 2005). The advantages of controlled experiments are obvious. First, it is easy to simplify the complex natural ecosystem and manipulate species diversity or genetic diversity. Second, the effects of heterogenous environments on ecosystem functioning can be controlled, which allow direct link the phenotype to genotype. Last but not least, it is easy to conduct treatments in a controlled experiment.

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To date, most previous controlled experiments have been established with limited numbers of genotypes or clones (maximum 18 genotypes) in grassland species (Crutsinger et al. 2006; Whitlock et al. 2007; Munzbergova et al. 2009; Fridley and Grime 2010). These studies are not representative for forest tree species which are different from grassland species in many characters such as size and life span. Most woody plant species reproduce predominantly or exclusively sexually, and the evolution of higher organisms critically depends on the creation of variation in each generation by sexual processes, notably the recombination of genes. Experimental studies based on the observation of different clonal diversity patterns in plant populations rarely reflect the intraspecific diversity present in natural ecosystems dominated by woody species and do not allow to link ecological processes to the evolution of populations. Hence, it is necessary to establish an experiment using forest tree species with sexual reproductive material to explore the effects of genetic diversity on ecosystem functioning and to reveal the underlying mechanisms. Accordingly, there is an urgent need to better understand ecological effects of different intraspecific diversity patterns in experimental populations comprising sexually produced progenies (Hughes et al. 2008).

1.2 Poplars as model tree species

Poplars (*Populus* spp.) have several advantages as model tree systems including their wide distribution, relatively small genome size and rapid growth. They are widely distributed across the Northern Hemisphere from the tropics to beyond the Arctic Circle (Dickmann and Kuzovkina 2008). Due to their continuous geographic distribution as well as sexual reproduction, poplars exhibit abundant genetic variation within populations revealed by molecular markers (Smulders et al. 2008; De Woody et al. 2009). Large genetic variation of poplars facilitates the study in the field of ecology and genetics.

Poplars are preferred model tree species in plant biology (Bradshaw et al. 2000). Rapid growth makes this species attractive in the study of biomass productivity which is one of key aspects of ecosystem functioning. Morphological growth traits have a genetic basis. The abundant genetic variation in natural poplar populations determines their large morphological variation that provides possibilities to select highly productive clones (Bradshaw et al. 2000). The large genetic variation of poplars living in

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contrasting environments offers possibilities to study of other aspects of ecology, such as adaptation (Hall et al. 2007; De Carvalho et al. 2010).

Meanwhile, poplars also act as model in the field of genetics and genomics (Tuskan et al. 2006; Jansson and Douglas 2007). It is the first tree species (*Populus trichocarpa*) with a full sequenced genome (Tuskan et al. 2006). Although the introduction of new sequence technologies and next generation sequencing makes it easier to obtain the complete genome sequence of other tree species, poplars are still preferred tree species due to sound understanding of genetics, genomics and physiology over the last decades. The construction of molecular genetic linkage maps and the development of other genomic resources in poplars help in finding candidate genes that control targeted quantitative traits, and therefore provide an important insight in the fields of forest evolution and forest tree breeding.

As foundation tree species, together with their importance in genetics and ecology, poplars are ideal tree species in the field of community and ecosystem genetics (Whitham et al. 2006; Whitham et al. 2008). Genetic analysis of foundation species reveals much information about the structure and function of a diverse community or ecosystem. Poplars facilitate studying species interaction as favorite hosts of many microbial species (Bahram et al. 2011). In addition, the development of various genetic, genomic and biochemical tools facilitate the study of the ecological services including carbon sequestration, bioremediation, nutrient cycling, biofiltration and diverse habitats (Madritch et al. 2009). Therefore, poplars are preferred trees in the study of complex ecosystem functioning.

1.3 The Göttingen Poplar Diversity Experiment (POPDIV)

The Göttingen poplar diversity experiment (POPDIV) was established to examine whether intraspecific genetic diversity in forest tree species affects ecosystem functioning (http://www.uni-goettingen.de/de/76731.html). The overall hypothesis of the experiment is that reduced intraspecific genetic diversity has negative effects on ecosystem functions and services. This project is associated with the Göttingen cluster of excellence “Functional Biodiversity Research”. The research cluster was established in 2008 at the University of Göttingen with the overall topic of biodiversity and

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ecosystem functioning, including experiments in grasslands and in the soil as well as historical studies in terms of biodiversity alterations.

The POPDIV project involved several working groups to study different aspects of ecosystem functioning. Plant-insect and plant-fungi interactions affecting growth performance were investigated by the Department of Agroecology and the Department of Forest Botany and Tree Physiology. The growth performances from a pool of phenological, morphological and physiological traits were investigated by the Department of Plant Biology. This research, from the Department of Forest Genetics and Forest Tree Breeding, conducted genetic analysis of the experiment and the results will provide important genetic background information for the parallel analyses of associated organisms and productivity investigations conducted by other research groups.

The experiment has been established with seven *Populus tremula* demes from a south-north gradient across Europe and one *Populus tremuloides* deme from the USA. The term “deme” was used in this investigation in its wide definition as originally proposed by Gilmour and Gregor (1939) as “any assemblage of taxonomically closely related individuals” to identify and distinguish different progeny arrays. A deme can denote any group of organisms of a specified taxon. It is used as a description of a group of plants from a single location irrespective of pedigree and family relations. Thus, the demes can be clone mixtures, single tree progenies after open pollination, progenies from presumably few seed parents and population samples.

1.4 *Populus tremula* L. & *Populus tremuloides* Michx.

1.4.1 Ecology and distribution

Aspen (*P. tremula* L. & *P. tremuloides* Michx.) belong to the genus *Populus* in the Salicaceae family. *P. tremula* is native to Europe while *P. tremuloides* is native to North America. They are dioecious and accordingly, outcrossing species; pollen and seeds are dispersed by wind and can travel over long distances (Geburek 2006; Tamm 2006). They propagate asexually through sucker shoots that arise from horizontal roots leading to clonal stands that may cover several hectares (Barnes 1966; Dickmann and Kuzovkina 2008). Clonal structures can be formed beside dead trees or in burned sites

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and can be precisely identified by molecular markers (Suvanto and Latva-Karjanmaa 2005). Sexual reproduction in nature is rare in *P. tremula* (Worrell 1995) and *P. tremuloides* due to the low seed germination and survival rate of seedlings (Latva-Karjanmaa et al. 2003). Therefore, sexual reproduction of aspen is mainly done in nurseries under controlled conditions.

P. tremuloides is the most geographically widespread native tree species in North America occurring from Mexico to North-western Alaska and from the Atlantic to the Pacific Ocean, while the distribution of *P. tremula* ranges from the British Isles to the east tip of Asia, and from Northern Africa to Northern Scandinavia (Fig. 1). Due to their wide distribution, aspens can tolerate a wide range of climate conditions, soil fertility and water supply and they are not sensitive to drought and wind. Thus, they are important pioneer species on poor soils and have the lowest demand regarding habitat conditions (Worrell 1995).

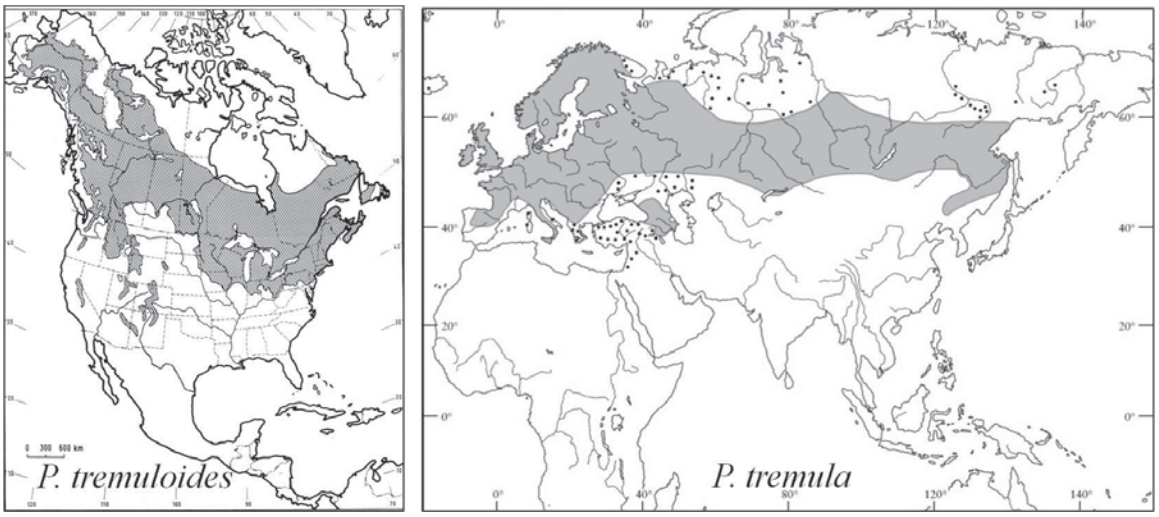

Fig. 1 Geographical distribution of *P. tremula* and *P. tremuloides*
(after Geburek 2006 and Tamm 2006, respectively)

1.4.2 Ecologically important tree species

Aspens have been considered as keystone species for biodiversity in temperate forests due to their importance of maintaining biodiversity in the landscape (Campbell and Bartos 2001). They are principal host for many animal and fungal species and more than 150 species are known to be exclusively associated with European aspen in the boreal forests of Finland (Kouki et al. 2004). Compared to surrounding conifer forests, aspen communities contain a higher diversity of mammals (Oaten and Larsen 2008). Due to

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the high levels of plant and animal diversity within aspen communities compared to surrounding conifer forests, they have been considered as oases for plant and animal diversity (Shepperd et al. 2006). The ecological importance of aspens makes them preferred model tree species for ecosystem functioning studies (Campbell and Bartos 2001; Shepperd et al. 2006; Kleemann et al. 2011).

In recent years, aspen stands are suddenly declining (Worrall et al. 2008) because of climate changes (Rehfeldt et al. 2009) or pathogens, insect defoliation, drought and thaw-freeze (Frey et al. 2004). This phenomenon highlights the importance of exploring the effect of reduced intraspecific genetic diversity on ecosystem functioning.

1.4.3 Genetic variation

As an outcrossing species, aspen is expected to have high genetic variation within populations (Hamrick et al. 1992). *P. tremula* exhibits a large amount of natural phenological variation such as bud set and leaf abscission which facilitate the study on adaptation (Luquez et al. 2008). The leaf size and height of aspen and its resistance to frost differs among different locations (Worrell 1995; Kleemann 2010). Bud burst, leave numbers, growth performance, photosynthetic and leaf water status parameters (photosynthetic capacity, leaf water potential and others) related to productivity and physiological traits of aspen from different origins are also different (Müller 2011).

Genetic variation is one of the underlying reasons for the morphological variation. The application of molecular markers such as microsatellites shows high levels of genetic diversity in *P. tremuloides* populations (Cole 2005; De Woody et al. 2009). The genetic diversity of *P. tremula* has mainly been estimated as nucleotide diversity in candidate genes related to the growth cessation and bud set (Ingvarsson 2005; Ingvarsson et al. 2006), while the genetic diversity at neutral markers which may reflect the evolutionary history of a species is very limited. It is necessary to understand the neutral genetic diversity of aspen within and among populations which is fundamental for studying the effect of genetic diversity on ecosystem functions.

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1.5 Assessment of genetic diversity

Molecular markers are powerful tools to assess genetic diversity. There are many different molecular markers, including restriction fragment length polymorphism (RFLP), random amplified fragment length polymorphism (RAPD), simple sequence repeats or microsatellite (SSR), amplified fragment length polymorphism (AFLP) and single nucleotide polymorphism (SNP). The most commonly used molecular markers to investigate neutral genetic diversity are SSR and AFLP markers (Schlotterer 2004). Also, the neutral genetic diversity may relate to some ecosystem functions which are not influenced by specific genes or loci.

1.5.1 Nuclear microsatellites

Nuclear microsatellites or simple sequence repeats (SSRs) are short tandem repeats (1-6bp) in the nuclear DNA, which are co-dominant markers and highly variable with regard to the number of repeat motifs. They can be used either in single primer or in multiplex reactions to save money and time (Ciofi et al. 2011). Microsatellite primers are species-specific but can be transferred to closely related species (Faria et al. 2010).

The disadvantages of this marker are the occurrences of null alleles and size homoplasy (Estoup et al. 2002). Null alleles occur in the case of mutations on one or both primer binding sites which might result in the primers failure to bind the flanking region, and thus, no fragment amplification can occur. It is better to exclude the locus with partial null alleles present only in some specific populations. Size homoplasy means that microsatellite markers (alleles) have similar lengths but they are different in nucleotide sequences which becomes most pronounced when comparing genealogically distant individuals or groups or phylogenetic relationship analysis between or within species.

There are different mutation models for this marker (Estoup et al. 2002). The infinite allele model means that any new allele can appear, and the repeat motif does not matter for the statistics analysis (Kimura and Crow 1964). In this model F_{ST} is used for the analysis of differentiation. Another model is the stepwise mutation model, meaning that one repeat motif loss or gain occurring one time (Kimura and Ohta 1978). In this model Slatkin's R_{ST} is used for the analysis of differentiation. No statistic is best under a strict stepwise mutation model. While R_{ST} better reflects population differentiation in

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populations characterized by very low gene exchange, F_{ST} gives better estimates in cases of high levels of gene flow (Balloux and Lugon-Moulin 2002).

Microsatellites are widely used in the study of genetic variation (Wyman et al. 2003; Cole 2005; De Woody et al. 2009), genetic linkage map construction (Cervera et al. 2001; Woolbright et al. 2008; Pakull et al. 2009), parentage identification (Pospiskova and Salkova 2006; Goryslavets et al. 2010), gene flow (Rathmacher et al. 2010), introgressive hybridization (Fossati et al. 2003) and clone identification (Suvanto and Latva-Karjanmaa 2005).

1.5.2 Amplified fragment length polymorphism

The amplified fragment length polymorphisms (AFLPs) technique was first described by Vos et al. (1995). This technique is based on the selective PCR amplification of restriction fragments from a digestion of total genomic DNA. It includes the restriction of the total genomic DNA with enzymes (e.g., *Eco*R1, a 6-base or rare cutter and *Mse*1, a 4-base or frequent cutter) and ligation of oligonucleotide adaptors, selective amplification of sets of restriction fragments and visualization of restriction fragments. It is regarded as a combination of RFLP with the PCR technique.

AFLPs are high-throughput, dominant markers that can be used without prior sequence information of a species. It is a powerful tool in forest tree genetics studies (Cervera et al. 2000) and widely used in studies of genetic variation (Winfield et al. 1998; Cao et al. 2006), population genetic structure (Smulders et al. 2008; Foll et al. 2010), phylogenetic relationships analyses (Cervera et al. 2005), disease resistance analyses (Cervera et al. 1996) and molecular linkage map construction (Cervera et al. 2001; Hanley et al. 2002).

1.6 Parentage analysis

The genetic diversity is influenced by evolutionary forces such as mutation, gene flow, mating system, genetic drift and selection (Finkeldey and Hattemer 2007). In addition, the genetic diversity may relate to population size, especially to the effective population size. Usually, a large population is expected to have higher level of genetic diversity than the smaller one (Amos and Harwood 1998), because genetic drift may happen in a small population resulting in the loss of rare alleles. Similarly, the genetic diversity of

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demes collected from seeds of one generation or seedlings is mainly influenced by the effective population size, which is determined by the number of contributed seed trees or pollen donors. Parentage analysis can provide family numbers information related to the effective population size which directly determines the level of genetic diversity.

With the advent of highly polymorphic and codominant microsatellite markers following the rules of Mendelian segregation and independence, parentage analysis is becoming a booming science over last decades in addressing some ecological questions. For instance, parentage analysis is widely used to study species hybridization (Curtu et al. 2009), assess parent numbers (Sefc and Koblmuller 2009), infer breeding values (Blonk et al. 2010), mating systems (Kanno et al. 2011) and reconstruct sibling relationships (Liu and Ely 2009).

Different techniques have also been developed in the study of parentage analysis. Jones et al. (2010) categorized parentage analysis techniques into six: exclusion, categorical allocation, fractional allocation, full probability parentage analysis, parental reconstruction and sibship reconstruction. The exclusion method is based on the principle that each parent shares at least one allele per locus with each of its offspring in a diploid species. The candidate parents are excluded if they fail to share at least one allele with the offspring at each locus. Categorical allocation, also known as parentage assignment, is to assign the entire offspring to the candidate parent with the highest likelihood or posterior probability of being the true parent. In fractional allocation method, each offspring is assigned partially to each of the nonexcluded candidates according to their likelihoods of parentage which is the same in the categorical allocation method. The full probability parentage analysis is a model based method which has the potential to simultaneously estimate the parentage patterns and other population level variables of interest. These four methods are only feasible when the candidate parents are available.

Without parental information, sibling reconstruction is possible in the field of parentage analysis which use genotypic data of the progenies to partition them into groups of full siblings or groups of full siblings and half siblings (Jones et al. 2010). Several methods have been developed to reconstruct sibling groups without requiring parental information (Jones et al. 2010) and these methods are falling into two main categories. One is a statistical likelihood, in which the algorithms attempt to partition the

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individuals into maximum likelihood sibling groups. Numerous computer programs have been developed based on this method to reconstruct the sibling groups. For example, the open source java program Kingroup uses the maximum likelihood approach to partition the individuals into different kin groups (Konovalov et al. 2004). The online program Pedigree (http://herbinger.biology.dal.ca:5080/Pedigree/) uses the Markov chain Monte Carlo (MCMC) approach to find the maximum likelihood of sibship reconstruction and also allow generating the genotypes of the unknown parents (Smith et al. 2001; Butler et al. 2004). The program Colony implements the full-pedigree likelihood method to simultaneously infer sibship and parentage based on multilocus genotype data from dominant or codominant markers (Jones and Wang 2010).

The other categories are combinatorial approaches which use Mendelian laws of inheritance to reconstruct sibling groups (Almudevar and Field 1999; Berger-Wolf et al. 2007). These approaches are based on a simple allele inheritance rule in diploid organisms: an offspring inherits alleles from both parents at each locus. Also, they do not need the prior knowledge of the population which is considered to be particularly appropriate for reconstructing sibling groups in wild populations (Berger-Wolf et al. 2007). Based on this method, the website program Kinalyzer has been developed to reconstruct full sib groups (Ashley et al. 2009). The two-allele algorithm with parsimony assumption implemented in this program is expected to perform well even when few loci are sampled or the allelic diversity is low. However, to date there is no single specific method which is absolutely correct to reconstruct the sibling groups. Usually different algorithms are used together to reduce their mistakes.

1.7 Research questions and hypotheses

The POPDIV experiment was established using one North American *P. tremuloides* deme and seven European *P. tremula* demes, and no genetic background information is available for the demes before the establishment of this experiment. The estimation of genetic diversity of demes and the POPDIV experiment is a basic study for understanding the effect of intraspecific genetic diversity on ecosystem functions and services. Thus, in this investigation, the following research questions regarding the demes and the experiment were posed:

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- Is there any clonal structure in the experiment?
- What are the levels of genetic diversity of the eight demes used in the experiment?
- How high is genetic differentiation between demes?
- Are the genotypes of the individuals of all demes homogenously distributed in the experiment?
- What are the levels of genetic diversity of all plots in the experiment? Is it possible to predict the level of genetic diversity for plots according to the number of demes?
- How many full sibling groups are present within demes? Is there any relationship between the number of full sibling groups and the level of genetic diversity?

In order to address above research questions, the following hypothesis were formulated:

- There is no clonal structure in the experiment;
- The levels of genetic diversity within the eight demes are not different;
- There is no significant differentiation among demes;
- The genotypes of all individuals for a given deme are homogenously distributed in the experiment;
- Genetic diversity of plots comprising a given number of demes (one, two, four and eight) is homogeneous;
- The plots with the maximum number of demes have the highest level of genetic diversity;
- The demes are propagated from few seed trees;
- The numbers of full sibling groups is related to the level of genetic diversity across the eight demes.

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