

**On the relationship between biotic and abiotic habitat diversity and
genetic diversity of *Ranunculus acris* L. (Ranunculaceae), *Plantago
lanceolata* L. (Plantaginaceae), and *Anthoxanthum odoratum* L. (Poaceae)
within and between grassland sites**

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CHAPTER ONE
General Introduction

Biodiversity

"Biodiversity" can be defined as the variety of all forms of life, from genes to species, through to the broad scale of ecosystems (Gaston 1996). Biodiversity is typically studied at three levels - genetic diversity, species diversity and ecosystem diversity. Genetic diversity is the variety of genes within a species. Each species is made up of individuals that have their own particular genetic composition. This means a species may have different populations, each having different genetic compositions, in terms of both allele type and frequency. Species diversity is the number of different species (species richness) in a given community weighted by some measure of abundance such as number of individuals or biomass, e.g. species evenness, (Smith & Wilson 1997). Ecosystem diversity can be defined as the variety of communities of organisms in a given landscape, their interaction with each others and with their physical environment.

In recent years ecology and population genetics have contributed greatly to the advances in biodiversity research. Ecology seeks to understand the patterns of variation in habitats and their importance in maintaining ecosystem functions and process, while population genetics especially seeks to understand the forces that generate genetic variation, particularly the intraspecific variation, which can be regarded as the ultimate source of species and ecosystem diversity.

Biodiversity is researched for various reasons. It can be studied for its sake in order to better understand how different organisms live together and operate to perform certain functions. Importantly, biodiversity can be studied because of conservation reasons: by studying diversity at various levels (genes, species, and ecosystems), we can better understand which type of diversity level is likely to decline and could lead to extinction under particular conditions, thus we can know the best strategy to protect and save variability in ecosystems (Frankham 1995).

Levels of Biodiversity

Although most studies on biodiversity have been mainly focused on species diversity in communities, particularly because it is easy to assess and measure, biodiversity is composed of three fundamental levels: genetic, species, and ecosystem. Although these three levels are fundamentally different and can be studied separately, they interact to regulate ecosystem functions and processes.

Genetic Diversity

Genetic diversity provides the basis for all other levels of biodiversity, mainly species and ecosystem diversity. Genetic diversity can be defined as the range of genes within a species and can be studied at the individual, population and species level. (e.g. Nei 1987; Lowe et al. 2004). Genetic diversity can be characterised by the set of possible alleles (different variants of the same gene) and their frequencies, by entire genes, or by even units larger than genes such as structures on chromosomes.

Populations of all organisms in their habitat contain an abundant variation in morphology, physiology, and behaviour. Much of this abundant variation may be reflected in the genetic diversity of organism, which often interact with habitat variation and thus produces the phenotypic variation of organism (e.g. Lowe 2004).

Genetic diversity is typically measured by estimating the allelic diversity which involves measuring the number of alleles per locus or the number of polymorphic loci (e.g. Nei 1987). Genetic diversity of a species, the clay of evolution, is constantly created by mutation and at the same time eroded by selection and genetic drift (e.g. Hedrick 2000).

Genetic diversity of a species is often influenced by environmental variability and stress conditions (e.g. Mitton 1997; Nevo 2001). If the environment changes, different alleles will have an advantage at different times or places. In this situation genetic diversity remains high because many alleles are in the population at any given time. If the environment does not change, then the small number of genes that have an advantage in that unchanging environment spread at the cost of the others, causing a decrease in genetic diversity.

Genetic diversity of a species may also increase with the increase in diversity of species (e.g. Mitton 1997; chapter 4 of this thesis). How much it increases depends not only on the number of species, but perhaps also on how closely related the species are and also on the environmental conditions at sites. For example, species that are closely related to each other may have similar genetic structure and makeup and therefore do not contribute much additional genetic diversity. An increase in species diversity can also affect the genetic diversity, and can do so differently at different levels. If there are many species in a given community, the genetic diversity at that level might be larger than when there are fewer species. On the other hand, genetic diversity within a species can decrease with the increase in species diversity of communities. This can happen if higher species diversity results in more complete niche filling, and thus decreased genetic diversity in the local constituent populations. However, studies linking either species diversity or genetic diversity within a species to niche characteristics are scarce in the literature.

Genetic diversity within and between local populations species may also be related to selection pressures imposed via variation in habitat characteristics. The majority of genetic markers used to investigate the influence of habitat variation to infer the role selection affecting genetic diversity of a species can be considered as selectively neutral (e.g. Nevo 2001). However, some supposedly neutral markers (e.g. allozyme, RAPD-PCR, AFLP) have been shown to have adaptive significance or be closely linked to genes under selection (Lowe 2004). For example, adaptive characteristics have been demonstrated for the allozymes variation (e.g. Watt 1977; Koehn & Hilbish 1987). Additionally, comparisons of genetic variation derived from coding versus non-coding genomic regions of *Crassostrea virginica* (Karl & Avis 1992) and *Passerella iliaca* (Zink 1986; 1994) have revealed different levels of population genetics differentiation. Significant population heterogeneity in DNA markers was not reflected in allozyme variation, implying that markers located within the coding regions may not be subjected to selection (Lowe 2004). Moreover, interaction of marker loci with other regions of the genome that are subject to selection can also influence genetic diversity of neutral markers (Charlesworth et al. 1997).

Furthermore, genetic diversity in plant species may be influenced by other processes which affect species' population, such as size and historical events like habitat modification by agricultural practices (Gray 1996; Young et al. 1998). Random changes in genetic diversity within a species may be related to the population size: the smaller the

population size, the more likely chance events are to change allele frequency of populations (e.g. Lowe et al. 2004). This random change in genetic diversity in small populations is called genetic drift, and it is a result of random sampling of gametes (e.g. Nei 1987). At its most extreme case, genetic drift can lead to the extinction of alleles and the loss of genetic polymorphism such that a locus becomes fixed for a single allele (e.g. Hedrick 2000; Lowe et al. 2004). However, genetic drift is believed to be independent of natural selection and thought to be of less importance in large populations (e.g. Mitton 1997). The relationship between genetic diversity and population size has been studied in various organisms. For example, Nevo et al. (1984) found that in a sample of 717 species of plants and animals, genetic diversity increased with the number of individual in the species. Moreover, Soulé (1976) estimated the population size of a wide diversity of lizards, fish, mammals, marine invertebrates, and *Drosophila* and found increase in genetic diversity with the increase in population size. However, in some case genetic diversity is not always increases with the increase in population size (Mitton 1997).

Species Diversity

Species diversity can be defined as the number of different species (species richness) weighted by some measure of abundance such as number of individuals or biomass (species evenness) (e.g. Smith & Wilson 1996).

Species have very important roles in communities. Sustainable efforts from several biodiversity projects have indicated the role of species diversity in maintaining ecosystem function and processes (e.g. Loreau et al. 2001). Some biodiversity studies have focused on altering and manipulating experimentally species diversity across several trophic levels (Naeem et al. 1994, Roscher et al. 2004); others on the effects of plant taxonomic diversity and plant functional-group diversity on primary production in grassland ecosystems (e.g. Tilman et al. 1996). Several of these biodiversity studies have shown that primary production exhibits a positive relationship with plant species and functional-group diversity (Loreau et al. 2001). DIVA, a biodiversity project described below, used a different approach in which natural grasslands with a gradient of species diversity was used (Perner et al. submitted) to study various level of diversity in relation to grassland productivity and ecological processes.

Ecosystem Diversity

In recent years our knowledge about the role of species diversity in ecosystems has increased dramatically. Yet less is known about the role of intraspecific genetic diversity and even less is known about ecosystem diversity and its consequences on function and processes in ecological systems. Ecosystem diversity focuses on species abundance and distributions, community patterns, the role of different functional groups in communities, and the interaction between various organisms and their habitats. In other words, ecosystem diversity deals with all diversity levels higher than the species. This includes different communities and their interaction with the physical environment.

Estimating Diversity

To detect variation in biodiversity we need ways to measure and estimate diversity at various levels. Estimating and quantifying biodiversity is not a straightforward task and it depends on the viewpoint and the aim of the study. Often it is less informative to express biodiversity only as a single measure or number. Several measures of biodiversity have been suggested over the past years, these include:

A) Simple measures such as numbers:

The use of simple measures of diversity is perhaps the oldest approach and the simplest developed. To measure species diversity one might use only the number of different species present in a given area (also can be expressed in unit of mass, or other units), while measuring genetic diversity can be also done simply by measuring the number of different alleles for a single locus. For ecosystem diversity one can simply quantify the number of taxonomic groups (which are higher than species) present in a community. Numbers are obviously simple measure of diversity and easy to measure, but over different scales one has to standardise such measures.

B) Diversity measures and indices

In some cases simple numbers are not good representation of diversity. For example, a community with many individuals of the same species will have a low diversity although it might have many species present. To take into account the relative abundance of various

species in a community, evenness measures have been developed to measure to what extent individuals are evenly distributed among species in a community (Smith & Wilson 1996).

Species number and species evenness are the most common measures used to characterise species diversity in a community. In recent years a large number of different abundance measures and evenness indices have been suggested (Magurran 1988; Smith & Wilson 1996). Some of these measures include:

1. Shannon Diversity Index

$$H' = -\sum_{i=1}^S P_i \ln P_i$$

where P_i is the relative abundance of species i in a community, S is the number of species, and \ln is the natural logarithm.

2. Simpson Diversity Index

$$D = 1 / \sum_{i=1}^S P_i^2$$

Species diversity in a community can also be expressed as species evenness; which is based on the variance in species abundance and is independent of species richness. Several evenness indices have been suggested, amongst the most common and robust index according to Smith & Wilson (1996) is:

$$E_{\text{var}} = 1 - (2/\pi) \arctan \left\{ \frac{\sum_{j=1}^S \left(\ln(X_j) - \sum_{k=1}^S \ln(X_k) / S \right)^2 / S}{S} \right\}$$

where S is the number of species in a community and X_k is the abundance of the k^{th} species.

Other different measures of diversity have been suggested recently such as the one suggested by Ganeshiah et al. (1997), which is called “Avalanche index”. This index uses not only species numbers and frequencies but also the biological and ecological differences among species comprising a community. The index attempts to integrate over all possible

species combinations, the biological differences among the species in proportion to their frequencies in the community.

DIVA- A BIODIVERSITY PROJECT IN JENA

“The relationship between Biodiversity and Ecosystem Functioning in Grassland Ecosystems”

General overview of the project

With the daunting threat of humans to natural ecosystem properties and function, biodiversity research has received a high profile among ecologists and in the public media. In Central Europe, where in recent years human impact on natural ecosystems is accelerating through for example habitat fragmentation and changes in agriculture use, government and funding agencies recognize the importance of biodiversity loss. Therefore, a number of biodiversity projects have been approved and funded by various funding agencies. In Germany, the Federal Ministry of Research and Education (Bundesministerium für Bildung und Forschung, BMBF), is funding a number of projects that dealing with biodiversity and ecosystem function. DIVA is a BMBF funded project in Jena which is part of BIOLOG-Europe (<http://www.biolog-europe.de>) which aims to promote research in the context of global change and increasing the loss of biodiversity. DIVA is a collaborative research project conducted by scientists from Friedrich-Schiller-University and Max-Planck-Institute for Biogeochemistry in Jena, and the Bureau for Ecological Studies in Bayreuth. The research sites of the project are the grassland systems located in Thüringer Schiefergebirge/Frankenwald in Central Germany (Figure 1). The project is conducted in two phases, the first phase from 2001 to 2003 and the second phase continuing in the period 2004 to 2006. The aim of DIVA project is to investigate the relationship between biodiversity and ecosystem processes such as carbon and nitrogen fluxes. Various research groups in the areas of plant physiology, biogeochemistry, entomology, mathematical modelling, plant taxonomy, mycorrhizal biology, and landscape ecology are cooperating to use experimental and theoretical approaches to investigate the importance of biodiversity for the stability and functioning of ecosystems. The project consisted in the first phase of four main subprojects:

(1) **STOFF**, which is designed to (i) determine causal relationships between biodiversity and selected ecosystem functions and (ii) to assess the effect of disturbance on these relationships. Therefore, the carbon and nitrogen fluxes are quantified in relation to plant diversity within the experimental plots. By manipulating the water regime and the abundance of insects, resistance and resilience of the studied grasslands system is investigated.

(2) **ENTO**, which aims to manipulate the presence of herbivorous insects in grassland plots in order to study the relationships between plant diversity, insect herbivory and ecosystem processes. The manipulation of insect herbivory can also help to determine the role of insects in ecosystem responses to stress (e.g. drought). ENTO also aimed to assess insect diversity in the experimental plots to study the relationship between various level of diversity and compositions, i.e., the relationship between the compositional structure of the insect, plant diversity and composition, and plant productivity.

(3) **GENMOD** is the topic of my thesis and it has been designed to investigate the hidden component of biodiversity, i.e. genetic diversity within a species. The major aims of GENMOD are to determine and quantify the genetic diversity among and between grassland populations at different spatial scales and different levels of diversity. Moreover, to study the relationship between genetics diversity between these selected plant species and other level of biodiversity particularly species diversity, and in relation to other features such as habitat type and nutrient characteristics.

(4) **RAUM** subproject aims to estimate the structural and taxonomic diversity in grassland ecosystems expected to be varied in management regimes, and to develop an indicator system for ecosystem function using easily estimated structural and taxonomic parameters of plant biodiversity. In the first phase of the project, in 2001, 78 different grasslands sites (Figure 1A) of differential plant species diversity, were surveyed in a collaborative effort of the members of the subprojects. The results of this survey were used to select 19 grassland sites (Figure 1B) of different levels of diversity in which experimental manipulations were carried out (Kahmen et al. submitted).

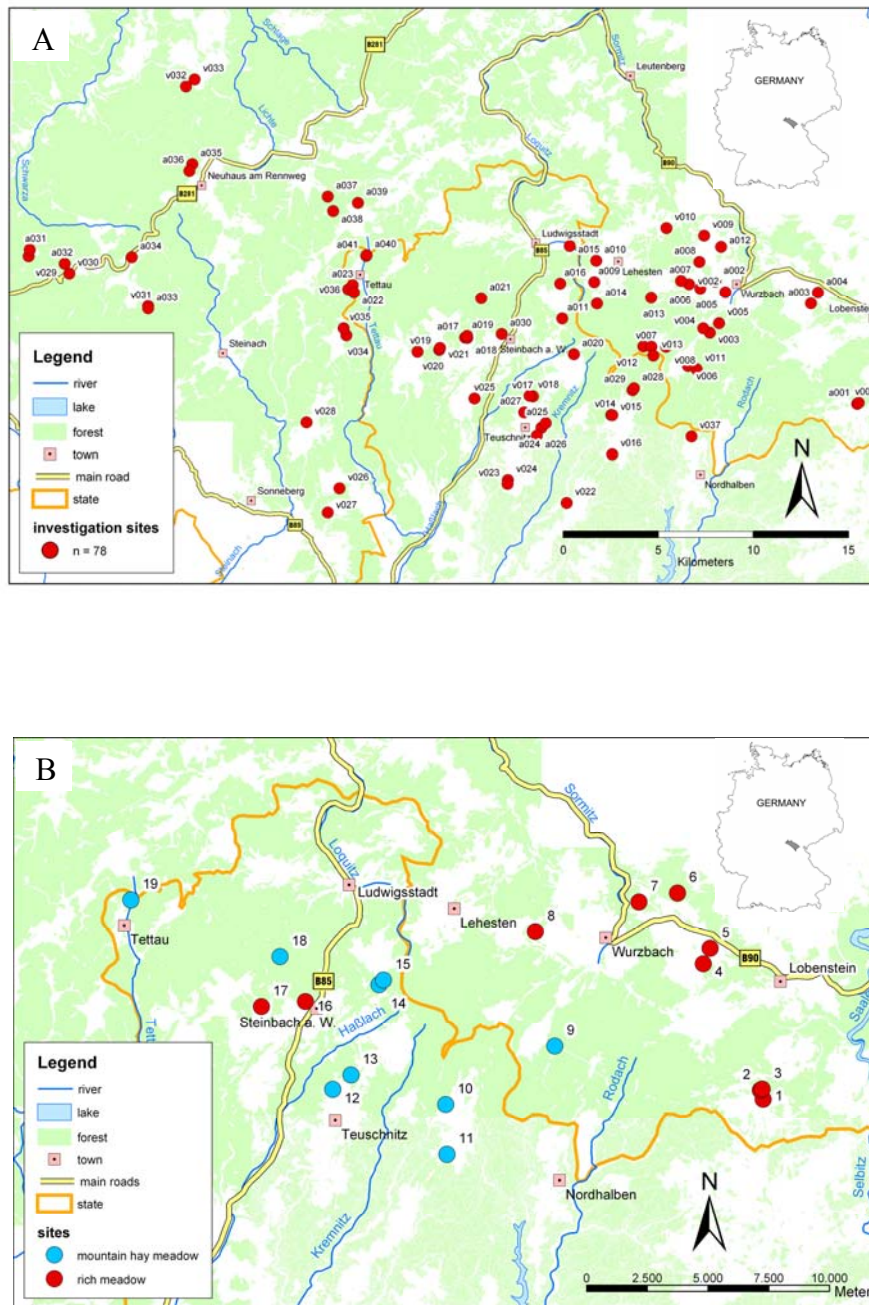


Figure 1 A) The 78 study sites in Central Germany surveyed in 2001 and B) the 19 sites that have been selected based on the gradient in species diversity for the manipulation experiments for the subsequent years (2002-2003).

Characteristics of the study sites

The study was conducted in the Thüringer Schiefergebirge/Frankenwald, a plateau-like mountain range at the Thuringian/Bavarian border in central Germany which reaches a near maximum height of 870 m (Perner et al. subm.). The bedrock material in the investigated area produces a carbonate-poor, nutrient-poor soil. Average annual precipitation is above 1000 mm with a slight summer maximum (Perner et al. subm.). Annual average temperature is 5 °C. Sites were a minimum of one hectare in size, and only sites with comparable elevation, edaphic and climatic factors were selected (Perner et al. subm.). Grasslands were uncut by the time of the survey, with the exception of very intensively managed grasslands with more than three cuts per year. A more detailed description of the studied plant communities can be found in Kahmen et al. (subm.). The selected grassland sites studied can be classified into two types: “semi-natural; Bergwiese” and “agriculturally improved; Fettwiese” (Figure 2). Semi-natural sites are typically dominated by *Trisetum flavescens*, while agriculturally improved sites are dominated *Arrhenatherum elatius*. All grassland sites are of good quality and not fragmented or under human impact.

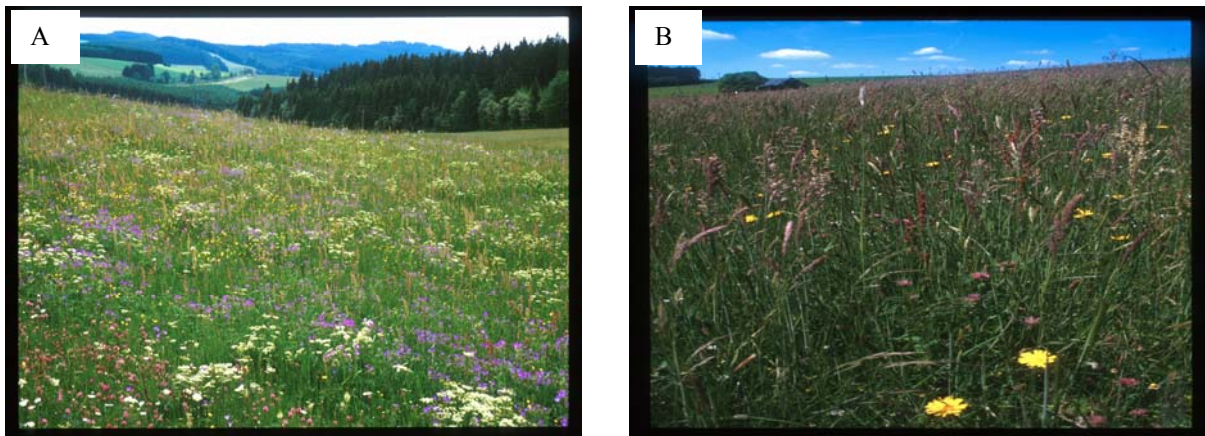


Figure 2 The studied grasslands of Thüringer Schiefergebirge/Frankenwald in Central Germany: (A) “Bergwiese” site and (B) “Fettwiese” site. (Photos by A. Kahmen).

In the period between 28th May and 9th June 2001, 78 sites were visited and all abiotic and vegetation structure parameters were sampled within a 2 m x 2 m quadrat. At each site edaphic parameters were determined: soil moisture, topsoil pH, total concentrations of soil nitrogen (N_{total}), soil carbon (C_{total}), extractable phosphorus (P_{total}), and extractable ion concentrations of potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}),

and sulphate (SO_4^2), as well as the amount of mineralized nitrogen (N_{min}) and the carbon-nitrogen ratio (C:N) (Perner et al. subm.).

Objectives and structure of the thesis

With the increasing threat from human activities on biodiversity, most biodiversity research is focused mainly on the species diversity in communities and the role species play in maintaining ecosystem function. This is partly because it is easy to work with and to manipulate in controlled experiments. Also species diversity is relatively inexpensive to measure, requiring less complex methodology than that used to estimate and study genetic and ecosystem diversity. Estimating genetic diversity requires special technical instruments and chemical reagents and it is generally time demanding and costly. On the other hand, estimating ecosystem diversity requires special devices that enable researchers to study not only the number and the abundance of various species in a community but also the interactions between organisms and their physical environment.

The central issue of the thesis is to study the pattern and distribution of genetic diversity within and between three selected plant species in relation to plant community diversity (e.g. species number and species evenness, relative abundance of species) in grassland systems in central Germany. The three selected plant species (*Ranunculus acris*, *Plantago lanceolata* and *Anthoxanthum odoratum*) were chosen because of their difference in a number of characteristics such as breeding system and dispersal mechanism. In addition, the three plants species studied are common and typical of central Germany. Specifically, the thesis aimed to assess the following hypotheses:

- (1) Genetic diversity (AFLP; Vos et al. 1995) of these species varies within and between grasslands sites.
- (2) This variation in genetic diversity is correlated with the diversity of plant species in grassland communities, and with variation in the abiotic characteristics of sites.
- (3) Genetic diversity within the three species is correlated with the relative abundance of these species in grassland communities.

(4) Genetic diversity across local populations is influenced by the geographical proximity of sites.

The results of the thesis are important for both biodiversity research and conservation purposes. For example, it is important to know whether conservation efforts implemented to positively affect one level of diversity are likely to also positively affect the other levels, or whether there are conservation conflicts between both of these diversity levels.

The thesis is structured in six chapters. Chapter 1 gives a general introduction about the theme of the thesis. Mainly, I have briefly given an introduction about the definition and overview about biodiversity levels, some of the ways by which diversity at various level (genetic, species, and ecosystem) can be measured and quantified. Chapter 2 contains information about the methodology of amplified fragment length polymorphisms (AFLP) that I have used for measuring genetic diversity in the three plant species studied.

In Chapter 3 I use AFLPs to assess the genetic diversity within and between ten populations of *Ranunculus acris* in relation to species diversity (richness and evenness) of grassland communities of two different habitat types, 'semi-natural' and 'agriculturally-improved'. Correlations between genetic diversity, at the levels of within and between populations of *Ranunculus acris* and the plant species richness and species evenness are tested. Moreover, I test whether populations from the two habitats are genetically different.

Chapter 4 aimed to investigate the genetic diversity of a particular plant species, *Plantago lanceolata*, which is wind pollinated, in relation to grassland species diversity and environmental variation within and between sites. Specifically, I addressed the following questions: (i) is genetic diversity within and between populations of *P. lanceolata* correlated with grassland species diversity? (ii) are the genetic diversity of populations of *P. lanceolata* and species diversity within and between grasslands correlated with environmental variables? and (iii) if so, is the relationship between genetic and species diversity mediated by environmental variation rather than being causal via ecological niche diversity?.

Chapter 5 is aimed to examine the influence of biotic and abiotic habitat variability on the genetic diversity of the two plants *Plantago lanceolata* and *Anthoxanthum*

odoratum. Particularly the chapter aimed to study the relative importance of habitat heterogeneity, both biotic and abiotic on genetic diversity of *P. lanceolata* and *A. odoratum*. The following questions were asked: (1) what is the level of genetic diversity within and between local populations of *P. lanceolata*, (2) is this genetic diversity correlated with biotic and abiotic characteristics of sites, (3) does habitat heterogeneity influence the local abundances of *P. lanceolata*, and therefore, (4) is genetic diversity influenced indirectly by population size or directly by habitat characteristics.

Chapter 6 presents general discussions of the thesis and presents the conclusions that I draw from the study and I finally give recommendations and prospective for future investigations.

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CHAPTER TWO: ARTICLE 1

The use of amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD-PCR) markers in studying genetic diversity

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ABSTRACT

Genetic diversity is a fundamental component of biodiversity as it forms the basis of species and ecosystem diversity. Studying genetic diversity within and between local populations of a species is important not only to improve our scientific knowledge in population genetics and evolution but also it necessary to manage and conserve populations which experience large anthropogenic disturbances. In recent years various molecular methods have been developed and used for studying the genetic variations of diverse taxa of organisms. These methods differ in the type of marker they generate, discrimination power, reproducibility, the ease of generation, and cost. Amongst the molecular methods that are used extensively to study genetic diversity in plants are RAPD and AFLP. After the introduction of these methods a wealth body of information about the genetic variation of different organisms have become available and thus more insights about the spatial distribution of genetic variation have been amassed. This minireview describes the principles, advantages and disadvantages, and the mode of inheritance of AFLP and RAPD. Moreover, estimates of population genetic diversity parameters and statistics based on AFLP and RAPD data are also presented. Information about some common computer packages that used to analyse AFLP and RAPD genetic data are given.

Keywords: AFLP, RAPD-PCR, genetic diversity, genetic distance, advantages, software packages

INTRODUCTION

Genetic diversity is a fundamental component of biodiversity as it is the basis of species diversity and ecosystem diversity. Studying genetic diversity within and between local populations of a species is important not only to improve our scientific knowledge in population genetics and evolution but also it is necessary to manage and conserve populations which experience some anthropogenic disturbances (e.g. Frankham 1995).

Moreover, studying the genetic diversity using molecular markers is important to assess ecological conditions that influence important population parameters (Parker et al. 1998). For example, characterization of the geographic structure or connectivity of populations is critical to ecological assessments and can be done effectively with molecular markers (e.g. Haig 1998). Because habitat fragmentation, which is common nowadays, reduces genetic diversity primarily through the effects of selection and genetic drift, a reduction in genetic diversity is indicative of deteriorating environmental conditions (Hoffmann et al. 1995).

In the past scientists surveyed the population genetics of various species using the variation in proteins, allozymes, as an estimate of the variation in the DNA sequence that determines the amino acid sequence of these proteins (Lewontin & Hubby 1966; Harris 1966). However, after the introduction of Polymerase Chain Reaction (PCR; Saiki et al. 1985; 1988) various molecular methods have been developed and used for studying the genetic variations of diverse taxa of organisms. These molecular methods differ in the type of marker they generate, the discrimination power, reproducibility, the ease of the procedure, cost and the time of generation of data. Amongst the molecular methods that are commonly used to study genetic diversity in plant species are AFLP (Vos et al. 1992) and RAPD (Williams et al. 1990). After the introduction of these methods a wealth body of information about the genetic variation of different organism have become available and thus more insights about the spatial distribution of genetic variation of different plant species have been amassed.

This minireview describes the principles, advantages and disadvantages, and the mode of inheritance of AFLP and RAPD. Moreover, description of some population genetic diversity parameters is also presented with some common computer packages that used to analyse genetic data obtained by AFLP and RAPD. Detailed descriptions of other techniques and type of data yielded by each marker type are not presented here but can be found elsewhere (Bruford et al. 1992; Avise 1994; Olmstead & Palmer 1994; Weising et al. 1995; Jarne & Lagoda 1996).

PRINCIPLE OF AFLP AND RAPD

AFLP

Amplified fragment length polymorphism-polymerase chain reaction (AFLP-PCR) is a relatively new method (Vos et al. 1995). AFLP is regarded as a relatively cheap, easy, fast and of high reproducibility (e.g. Powell et al. 1996; Jones et al. 1997). AFLP markers can be generated for DNA of diverse taxa of organisms, and no initial investment in primer/probe development or sequence analysis is required (Vos et al. 1995). Partially degraded DNA can be used, but DNA typically should be highly purified and free of polymerase chain reaction (PCR) inhibitors. A very small amount of DNA (about 200ng or even less) are digested with combinations of restriction enzymes such as EcoRI, PstI, HindIII, ApaI with MseI or TaqI (Vos et al. 1995; Savelkoul et al. 1999) and the AFLP adaptors are joined (ligation) to the digested ends (Figure 1). Adaptor ligations are performed in the presence of restriction enzymes so that any fragment-to-fragment ligations are immediately resealed by the restriction enzyme. The adaptor is designed so that ligation of a fragment to an adaptor does not reconstitute the restriction site (Figure 1). The end sequences of each adapted fragment consist of the adaptor sequence and the remaining part of the restriction sequence. These known ends serve as priming sites in the subsequent AFLP-PCR. Depending on genome size, restriction-ligation generates thousands of adaptors of adapted fragments. For visualization after electrophoresis, only a subset of these fragments is amplified. To achieve selective amplification of a subset of these fragments, primers are extended into the unknown part of the fragments, usually one to three arbitrarily chosen bases beyond the restriction site (Baker & Parkin 2000). By using combinations of primers with different extensions, a series of AFLP amplifications can thus screen a representative fragments dispersed throughout the whole genome (Vos et al. 1995).

AFLP-PCR products can be separated using simple agarose or polyacrylamide gel electrophoresis. Polyacrylamide gel electrophoresis provides maximum resolution of AFLP banding patterns to the level of single-nucleotide length differences (Jones et al. 1997), whereas fragment length differences of less than ten nucleotides are difficult to score on agarose gels (Jones et al. 1997; Vos et al. 1995).

The main disadvantage of AFLP is the difficulty in identifying homologous markers (alleles), rendering this method less useful for studies that require precise assignment of allelic states. Nevertheless, because of the rapidity and the ease with which reliable, high-resolution markers can be generated (Vos et al. 1995), AFLPs are established as a powerful method for studying genetic diversity of many organisms (Vos et al. 1995; Mueller & Wolfenbarger 1999). For example, AFLP has been used in genome mapping applications and ecological genetics (e.g. Mueller & Wolfenbarger 1999), conservation biology (e.g. Zhu et al. 1998; De Riek et al. 1999), parentage analysis (Gerber et al. 2000), and population genetics (e.g. Powell et al. 1996). Figure 1 shows the major steps of AFLP method.

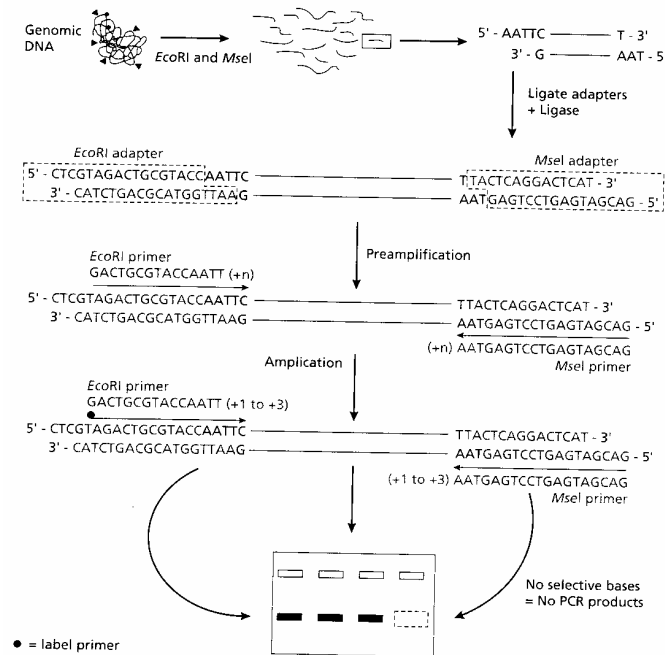


Figure 1 Schematic diagram shows the steps of amplified fragment length polymorphism AFLP (after Baker & Parkin 2000)

RAPD

RAPDs are random segments of DNA that amplified by the PCR using arbitrary oligonucleotide primers (see Figure 2). PCR products, which are scored for presence or absence in each individual, represent genomic polymorphisms that appear to be inherited in

a dominant Mendelian fashion (Williams et al. 1990). Absence of an amplified product of DNA in an individual or population can be caused by loss of priming site due either to point mutations or deletion or insertion of DNA (Jones et al. 1997). When introduced, the acquisition of RAPDs markers was considered as a quick, simple process because the technique requires no nucleotide-sequence information for the production of primers. Although a number of researchers have used RAPDs as their methods of investigating genetic diversity, there are some disadvantages that limit its use. Reliable amplification of specific DNA segments from the same template may be difficult to achieve due to extreme sensitivity to reaction conditions (PCR temperature profile, concentration of ions etc.), and reproducibility may be a problem (Jones et al. 1997). However, it was reported that if condition, reagent, purified degree of template DNA were strictly controlled, results could be reproducible. Moreover, the dominant mode of inheritance of RAPD markers requires the assumption of random mating and Hardy-Weinberg equilibrium in order to calculate allele frequencies and population genetic statistics.

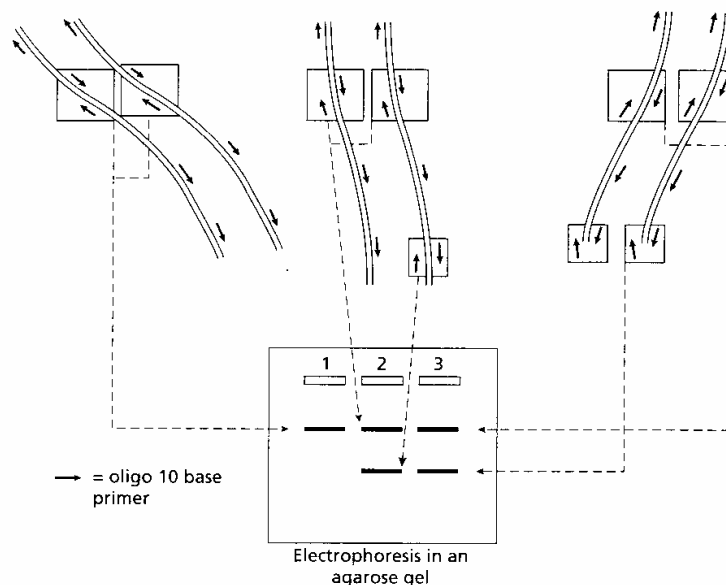


Figure 2 Schematic diagram of randomly amplified polymorphic DNA (RAPD) which demonstrates the presence and absence of polymerase chain (PCR) products. For extract 1 there is one PCR product, for extracts 2 & 3 there are two products (after Baker & Parkin 2000).

ANALYSIS OF RAPD AND AFLP MARKERS AND INTERPRETATION

To study the pattern of genetic variation within and between local populations of a species and the influence of evolutionary processes such as, selection, inbreeding, genetic drift, gene flow, and mutation acting on species we need ways to quantify the genetic diversity

in populations. Genetic diversity carried by local populations can be characterised in two levels (i) genetic diversity within populations, i.e. between different individuals of a population and (ii) genetic diversity (genetic differentiation) between populations.

(I) Genetic diversity within population

Several measures have been used to describe the AFLP and RAPD genetic variation at a single locus and on a number of loci, these include:

1. Heterozygosity

The amount of heterozygosity is the most widespread and most biological informative measure of genetic variation in a population because individuals in diploid organisms are either heterozygous or homozygous at a particular locus (Hedrick 2000; Nei 1987). Because AFLPs and RAPDs are dominant genetics markers heterozygosity can not be calculated. Nonetheless, two measures are commonly used to estimate genetic diversity within a population using dominant markers:

- 1) Nei gene diversity index (H_E) Nei (1987):

$$H_E = 1 - \sum P_i^2,$$

where P_i is the population frequency of each allele (1 and 0) at locus i . The average genetic diversity is then calculated as the average of this quantity across all loci studied.

Nei (1973; 1987) called this measure gene diversity and recommended it for organisms with different reproductive systems and ploidy levels. More information about this measure of genetic diversity and its applications for different organism is detailed by Nei (1987).

- 2) Shannon's index:

$$H = -\sum P_i \ln(P_i),$$

where P_i is the proportion of the i th allele in the population.

2. The proportion of polymorphic loci

Genetic polymorphism is a term used often to describe the amount of genetic diversity present within a population. According to Ford (1940) genetic polymorphism can be defined as “the occurrence together in the same habitat of two or more discontinuous forms in such proportion that the rarest of them cannot be maintained by recurrent mutation.” Although this definition is not precise, it has been commonly used because it based on population genetic theory (Hedrick 2000). Moreover, with the introduction of the neutrality theory, which assumes that mutation and genetic drift are major forces that affect the population genetic diversity, this definition is not generally appropriate (Hedrick 2000). Another definition and more useful one of the genetic polymorphism is introduced by Cavalli-Sforza and Bodmer (1971) which is “genetic polymorphism is the occurrence in the same population of two or more alleles at one locus, each with appreciable frequency. “

To estimate the proportion of polymorphic loci (P) for a population where a number of loci have been collected, the following equation is used:

$$P = \frac{x}{m},$$

where x is the number of polymorphic loci in a sample of m loci. A locus is considered to be polymorphic if there are at least to individuals differ at this locus. However, some suggest that this measure is more appropriate for allozyme loci but not for highly variable loci in which a high proportion of the loci are polymorphic in most populations (Hedrick 2000; Nei 1987).

(ii) Genetic diversity (genetic differentiation) between populations

When genetic data is collected from a number of populations or different species by using a number of genetic markers, one may therefore wish to estimate the extent of genetic diversity between the local populations of a species or the degree of genetic differentiation between populations. Estimating the level of genetic diversity between local populations of a species may be used in order to test hypothesis about the effect of some factors such as gene flow, isolation by distance and the influence of habitat variability that might influence the genetic structure of populations of a given species. Estimating the genetic

differentiation between populations can be achieved using standard similarity and/or dissimilarity (distance) measures. A number of genetic similarity and distance measures have been introduced and used to evaluate the amount of genetic variation shared between populations (for detail see Nei 1987).

Similarities or dissimilarities in the genetic diversity between populations can be the result of one or several forces (Hedrick 2000). For example if two populations said to be genetically similar, this may indicate that (1) they recently separated into two populations, or (2) gene flow occurred between them, or (3) similar selection pressures affected loci similarly in both populations. Similarly, if two populations are different, then this could indicate (1) they have been isolated for a long time and there has been no gene flow between them, or (2) genetic drift has generated large differences between them.

Several measures of genetic distances have been introduced in recent years. Ideally, different measures suppose to be highly correlated with each other (Hedrick 2000). Here, three most widely used genetic distances are described:

1. The standard genetic distance (D_S) of Nei (1972, 1978)

The standard genetic distance of Nei (1972, 1978) is one of the most commonly used genetic distances (Kalinowski 2002). For populations X and Y with r loci and m alleles per locus, the standard genetic distance is defined as:

$$D_S = -\ln\left(J_{XY} / \sqrt{J_{XX} J_{YY}}\right),$$

where

$$J_{XY} = \sum_{i=1}^m \sum_{j=1}^r (x_{ij} \cdot y_{ij}) / r, \quad J_{XX} = \sum_{i=1}^m \sum_{j=1}^r x_{ij}^2 / r, \quad J_{YY} = \sum_{i=1}^m \sum_{j=1}^r y_{ij}^2 / r$$

x_{ij} is the frequency of the i^{th} allele at the j^{th} locus in population X, and y_{ij} is the frequency of the i^{th} allele at the j^{th} locus in population Y.

2. The D_A distance of Nei (Nei et al. 1983)

In many studies of genetic diversity of natural populations a relatively small number of individuals are collected as a sample. This mainly because of the limitation of money to survey a large number of individuals and the time needed to study large numbers of samples. Nei et al. (1983) has introduced a formula to calculate unbiased estimate of the genetic distance D by a correction for the homozygosity estimates of J_x and J_y , in which unbiased estimate of D_A is calculated as:

$$D_A = 1 - \sum_{i=1}^m \sum_{j=1}^r \frac{\sqrt{x_{ij} \cdot y_{ij}}}{r},$$

where m and r are the numbers of loci examined in populations X and Y respectively. This distance is a modification of the original Cavalli-Sforza distance (1967). D_A takes a maximum value of 1.0 which occurs when two populations share no alleles at any locus. The D_A distance has proven to be useful for reconstructing phylogenetic trees (Takezaki & Nei 1996).

Wright's F_{ST} is also another common genetic distance used to measure the population genetic structure (Kalinowski 2002). Analogous distance measures (e.g. θ , β , and G_{ST}) have also been developed to describe the genetic distance (differentiation) between local populations of a species (Excoffier 2001). These measures have different statistical characteristics (Nei & Kumar 2000; Excoffier 2001) and represent distinct but related concepts (Kalinowski 2002). For more details about these distance measures see Weir 1996 and Nei 1987.

Generally, the genetic distances and similarities between the studied populations are visualised using phenetic trees and thus making the relationships between the studied populations better to understand. Moreover, the effect of some factors, e.g. environmental characteristics (Cooper 1998; Odat et al. 2004), on the pattern of the relationship between populations can be assessed. Presumably, populations that have many similar alleles are closely related, and those have different alleles are distantly related (Hedrick 2000). Several methods of constructing phylogenetic trees from genetic data such as those of RAPD and AFLP have been introduced. For good reviews about various methods and

explanatory examples see (Felsenstein, 1988; Nei 1996; Nei & Kumar 2000). Amongst the most common methods are: (1) the unweighted-Pair-Group Method (UPGMA) which was developed originally by Sokal and Sneath (1963) and was used first to analyse morphological data. UPGMA is an easy method to understand but it assumes a constant evolutionary rate of time for all lineages (Hedrick 2000), (2) the neighbor-joining method (NJ), developed by Saitou and Nei (1987) which based on the minimum evolution principle (Hedrick 2000; Nei 1987). These two methods are called distance matrix methods because they depend on the measure of the genetic distance between all the pairs of populations under investigations (Nei 1987).

SOFTWARE PACKAGES USED IN DATA ANALYSIS OF AFLP AND RAPD

In recent years with the advance in computer technologies several software packages have been developed for analysis of genetic diversity within and between local populations of a species (e.g. Labate 2000). These softwares have increased the efficiency for calculating various parameters of population genetics. Within few hours one can achieve a large number of genetic data statistics using appropriate software. Fundamentally, most of these available software packages are offer the same genetic parameters and do the same analysis (Labate 2000). However, it seems that the choice of particular software depends on the ease and friendly of using particular software (Mohammadi & Prasanna 2003). Most of the softwares available are provided free of charge through the World Wide Web. Table 1 gives some of the most common software packages that are used for estimating genetic diversity parameters.

Table 1. Computer packages and various population genetic parameters analysis of AFLP, RAPD and other genetic markers (modified from Labate 2000).

Statistics	Software					
	TFPGA	Arlequin	GDA	GENEPOP	GeneStrut	POPGENE
Diversity						
Observed heterozygosity	Yes	Yes	Yes	No	Yes	Yes
Expected heterozygosity	Yes	Yes	Yes	No	Yes	Yes
No. alleles/locus	No	Yes	Yes	No	Yes	Yes
Effective no. alleles	No	Yes	No	No	Yes	Yes
% polymorphic loci	Yes	Yes	Yes	No	Yes	Yes
Shannon-Weaver	No	No	No	No	No	Yes
Population structure						
F-statistics	Yes	Yes	Yes	Yes	Yes	Yes
G-statistics	No	No	No	No	Yes	Yes
AMOVA	No	Yes	Yes	No	No	No
Isolation-by-distance	Yes	Yes	No	Yes	No	No
Genetic distances						
Nei's	Yes	Yes	Yes	No	Yes	Yes
Rogers'	Yes	Yes	Yes	No	No	No
Pairwise F_{ST}	Yes	Yes	Yes	No	No	No
Clustering methods						
Neighbor-Joining	No	No	Yes	No	No	No
UPGMA	Yes	No	Yes	No	Yes	Yes
Neutrality tests	No	Yes	No	No	No	Yes

The following software packages are amongst the most common used for data analysis of both AFLP and RAPD markers:

1. POPGENE

POPGENE (Yeh et al. 1997) is a widely used package which can be used to analyse data from haploid or diploid genomes and is useful for both dominant and codominant markers. Amongst the statistics that are implemented by the software and can be used to analyse

RAPD and AFLP data are: allele frequency, number of alleles, and effective number of alleles, percent polymorphic loci, expected heterozygosity, and Shannon index. The software can give estimate for the population structure using F-statistics and G-statistics (for formulas see Nei 1987). Two genetic distances and two genetic similarities those of Nei (1972, 1978) are implemented in the software. These genetic distance and genetic identities can be visualised as tree using a UPGMA dendrogram. Test for neutrality can be done using the statistics of Ewens-Watterson (Ewens, 1972; Watterson, 1978). The software is a user-friendly and can be downloaded from (<http://www.ualberta.ca/~fyeh>).

2. TFPGA

TFPGA (Miller et al. 1997) can be used to estimate genetic diversity statistics for haploid and diploid data and can be used to give statistics for dominant and codominant genetics markers. The software is easy to work with and data files can be prepared using simple editor files. The software is provided with a pdf help file that describes the analysis implemented by the program and some general introduction about the genetic statistics given. For RAPD and AFLP genetic data the analysis include allele frequency that is calculated based on the square root of the frequency of recessive genotype or by Taylor expansion approach (Lynch and Milligan 1994). The software also gives estimates of the observed and expected heterozygosity (Nei 1987), percentage of polymorphic loci and Shannon index. These statistics are given under a subheading in the analysis menu as a descriptive statistics. The statistics can be done for the entire data set or for each population or for a group of populations. F-statistics can be also estimated for each allele, each locus, and over all loci studies following (Weir and Cockerham 1984). Several genetic distances and genetic identities are implemented in the software. These include: Nei's minimum (original and unbiased, Nei 1972, 1978), Rogers's (1972) and modified Rogers' (Wright 1978) and coancestry (Reynolds et al. 1983). A UPGMA dendrogram with bootstrap values is provided. Others statistics such as Mantel test (Mantel 1967) and exact tests for population differentiation (Raymond & Rousset 1995) can be done. TFPGA is very easy to work with and the input file can be prepared very easily. The software can be downloaded free of charge under <http://herb.bio.nau.edu/~miller>.

3. ARLEQUIN

Arlequin (Schneider et al. 2000) is a very comprehensive software package that can be used to analyse haploid and diploid data. The software can be used to calculate diversity indices, disequilibrium test, neutrality tests, and population structure. Diversity indices include allele frequency, observed and expected heterozygosity, observed homozygosity, number of polymorphic loci, and number of alleles per locus. Various methods to estimate genetic distances between populations and individuals are implemented. Population structure by Analysis of Molecular Variance (AMOVA; Excoffier et al. 1992) which based on an analysis of variance of gene frequencies is provided by Arlequin (see the manual for more description about the AMOVA). AMOVA analysis can be conducted at various levels such as diversity within and between populations and within and between groups of populations. Pairwise F_{ST} values can be estimated and these can be given in the form of pairwise data matrix. The significance of the pairwise genetic distances between populations can be obtained by permutation. Several neutrality tests are also provided by Arlequin. The software utilise specific format for the input file (project file) that can be prepared using any text editor or use the program's (project outline wizard). It is also possible to convert by the software various data files to and from other genetic softwares including GENEPOP, PHYLIP, MEGA and WinAmova. The manual given with Arlequin describe in details the software features.

4. NTSYSpc

NTSYSpc (Numerical Taxonomy System; Rolf 1993) is a software that originally developed to analyse multivariate statistics in the context of ecology and other areas of biology and systematic. Various multivariate statistics are implemented in this system. It appears that the use of this software for genetic data is mainly to conduct cluster analysis and some ordination statistics. Clustering by UPGMA and NJ methods using various methods of distance and similarity coefficients are implemented in this software. Among the ordination methods that are possible in this software are Principle Components Analysis (PCA) and Principal Coordinates Analysis (PCORDA). Nonmetric multidimensional scaling and matrix comparison using Mantel test are also possible with this software. The input file can be an Excel file which can be saved and imported as normal text editor file. Unfortunately, NTSYSpc is not available free of charge for users.

Other software commonly used for data analysis of dominant markers such as AFLP and RAPD include: GDA, GENEPOP and GeneStrut (Labate 2000).

COMPARISONS OF AFLP AND RAPD WITH OTHER TECHNIQUES

Although they are dominant markers by which less information can be obtained about the level of heterozygosity level, AFLP and RAPD are comparable and in some case preferable over other genetic methods that are currently available. Table 2 gives a summary of comparison of AFLP and RAPD with other genetic markers available to study genetic diversity within a species with respect to reproducibility and robustness, discrimination power, operational aspects and the cost of development.

Table 2 Comparison of techniques commonly used for measuring population genetic diversity (modified from Engel et al., 1996; Storfer 1996; Mallet, 1996. Yang et al. 1994; Jones et al. 1997; Powell et al. 1996).

Feature	Isozymes	RFLP	SSR	RAPD	AFLP
Data gathered	Protein variation	Genotype fragment variation	Genotype fragment variation	Dominant multilocus genotypes fragment variation	Dominant multilocus genotypes fragment variation
Reproducibility	Very high	High to very high	Medium to high	Low to medium	Medium to high
Amount of sample required per sample	Several mg of tissue	2-10 mg DNA	10-20 ng DNA	2-10 ng DNA	200 ng DNA
Ease of development	Moderate	Difficult	Difficult	Easy	Moderate
Ease of assay	Easy to moderate	Difficult	Easy to moderate	Easy to moderate	Moderate to difficult
Equipment needed	Inexpensive	Moderate	Moderate to expensive	Moderate	Moderate to expensive
Resolution	Moderate	Moderate	Very high	High	Very high

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CHAPTER THREE: ARTICLE 2

Genetic diversity of *Ranunculus acris* L. (Ranunculaceae) populations in relation to species diversity and habitat type in grassland communities

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ABSTRACT

Correlates between genetic diversity at intra- and interpopulation levels and the species diversity in plant communities are rarely investigated. Such correlates may give insights into the effect of local selective forces across different communities on the genetic diversity of local plant populations. This study has employed amplified fragment length polymorphism (AFLP) to assess the genetic diversity within and between ten populations of *Ranunculus acris* in relation to species diversity (richness and evenness) of grassland communities of two different habitat types, ‘semi-natural’ and ‘agriculturally-improved’, located in central Germany. Within-population genetic diversity estimated by Nei’s unbiased gene diversity (H_e) was high (0.258 – 0.334), and was not correlated with species richness (Pearson’s $r = -0.17$; $P = 0.64$) or species evenness (Pearson’s $r = 0.15$; $P = 0.68$) of the plant communities. However, the genetic differentiation between *R. acris* populations was significantly correlated with the difference in species evenness (Mantel’s $r = 0.62$, $P = 0.02$), but not with difference in species richness of plant communities ($r = -0.17$, $P = 0.22$). Moreover, we also found that populations of *R. acris* from ‘semi-natural’ habitat were genetically different (AMOVA, $P < 0.05$) from those in ‘agriculturally-improved’ habitats, suggesting that gene flow between these habitat types is limited. The results reported in this study may indicate that habitat characteristics influence the genetic diversity of plant species.

Keywords: genetic diversity, species diversity, species evenness, localized selection, genetic differentiation, AFLP

INTRODUCTION

Correlates between genetic diversity at intra- and interpopulation levels and the species diversity in plant communities are rarely investigated. Such correlates may give insights into the effect of local selective forces across different communities on the genetic diversity of local plant populations (Nevo 1988). Several studies have provided evidence

that environmental, and mainly habitat, variability has the potential to influence the genetic differentiation between local populations (Antonovics 1971; Linhart and Grant 1996; Mitton 1997; Gray & Sork 2001). Among the processes through which habitat variability can influence genetic diversity are localized selection processes (Hamrick & Allard 1972; Zangerl & Bazzaz 1984; Owuor et al. 1999), and differential gene flow by seed or pollen dispersal (Schaal 1975; Waser 1987).

In addition to habitat variability, genetic diversity in plant is influenced by other processes which affect species' population, such as size and contemporary events like habitat modification by agricultural practices (Gray 1996; Young et al. 1998). In central Europe human interference of plant communities and their natural habitats through, for instance, the use of intensive management practices for agricultural purposes has led to the isolation of many plant populations and to the reduction in their sizes. This eventually can result in a decrease in genetic diversity and in an increase in the genetic divergence between local plant populations (Frankham 1996).

As part of an interdisciplinary project focussing on the relationship between biodiversity and ecosystem function of grassland communities in central Germany, we aimed to study the genetic diversity of several plant species in relation to the species diversity of plant communities. For this purpose we used amplified fragment length polymorphism (AFLP; Vos et al. 1995) to assess the genetic diversity of the plant populations (Powell et al. 1996; Kölliker et al. 1998, Mueller & Wolfenbarger 1999). Based on a large survey of 19 investigation areas, where we investigated *R. acris* populations, we found that the grassland communities can be classified into two types based on differences in plant species composition (see Results). These differences seem to originate from difference in habitat properties mainly the intensity of management and the history of land use in the past (Ellenberg 1988). Therefore, habitats less subjected to fertilisation and mowing were called 'semi-natural; Bergwiese', whereas intensively fertilised and mown habitats in the past were called 'agriculturally-improved; Fettwiese'.

In this study, we particularly aimed to investigate the genetic diversity within and between ten populations of *Ranunculus acris* (Ranunculaceae) in relation to the species diversity of these grassland communities. Additionally, we aimed to see whether populations of *R. acris* of 'semi-natural' and 'agriculturally-improved' habitats are

genetically different. Species diversity of a community consists of two components: species richness – the number of plant species in a given community, and species evenness – a measure of how relative abundances are distributed between species. Previous studies showed that species evenness explains a larger proportion of the variance (53%) of diversity in plant communities than does species richness (6%) (Stirling & Wilsey 2001), and determines the intensity of plant–plant interaction in a community (Polley et al. 2003). We correlate within-population genetic diversity of the studied populations of *R. acris* with both species richness and species evenness. We also correlated, using a Mantel test (Mantel 1967), the genetic differentiation (genetic distances) between *R. acris* populations with the pairwise difference in species diversity (richness and evenness) between sites. This enabled us to test whether the diversity in plant species across sites, through possibly different selection forces, influence the genetic differentiation of *R. acris* populations. For this study, we chose *R. acris* because it is abundant in all our sites and it grows frequently in mown and managed areas (Grime et al. 1988). This allowed us to see whether there is a substantial gene flow in *R. acris* between the two habitat types.

MATERIAL AND METHODS

Species and population description

The meadow buttercup *Ranunculus acris* L. (Ranunculaceae) is a perennial herbaceous plant with a wide distribution throughout Europe and Western Siberia (Hegi 1982). The species grows in dry and wet meadows, and frequently inhabits grazed and mown habitats (Grime et al. 1988). Flowering time is between May and September and seeds are set from June onwards (Hegi 1982). The five-petaled, yellow flowers are self-incompatible and insects of the families Muscidae and Anthomyiidae are the main pollinators (Totland 1993, 1994b).

The study sites were located in central Germany with altitudes ranging from 580 to 730 m. Ten populations of *R. acris* from both habitat types (semi-natural and agriculturally improved) were chosen (Table 1). A preliminary study of 19 sites in our investigation areas showed that the two habitats are different in plant species composition (Figure 1). The populations of *R. acris* were separated by forests and agricultural fields and the geographical distances between populations ranged from 1 to 16.5 km. At each of the study

sites from which *R. acris* individuals were sampled, we established four 3x3 m² plots and recorded the number of plant species and their relative abundance (percentage cover). We then randomly sampled leaves of *R. acris* from seven to twelve individuals per population, and the leaves were immediately placed in drying silica gel prior to DNA extraction.

Table 1 Characteristics of the studied populations of *Ranunculus acris*. ‘A’ denotes populations from agriculturally improved habitat, ‘S’ denotes populations from semi-natural habitat. Species richness is measured as number of species and species evenness as E_{var} index in four 3x3 m² plots, see Materials and Methods. Sample size is the number of individuals use in AFLP. Refer to Fig. 1 for the classification of the two habitats.

Habitat types	Population	Longitude (east)	Latitude (north)	Species richness	Species evenness	Sample size	Gene diversity (H_c)
Agriculturally-improved	A1	20°35'41"	11°27'08"	31	0.278	9	0.327
	A2	20°35'56"	11°27'28"	33	0.234	9	0.281
	A3	20°34'47"	11°28'42"	41	0.282	9	0.333
	A4	20°33'27"	11°28'30"	31	0.205	12	0.297
	A5	20°29'51"	11°27'49"	21	0.195	10	0.334
Semi-natural	S1	20°26'47"	11°23'57"	33	0.397	7	0.310
	S2	20°22'52"	11°24'16"	36	0.228	7	0.271
	S3	20°23'29"	11°24'36"	37	0.269	8	0.258
	S4	20°24'26"	11°26'37"	36	0.259	7	0.318
	S5	20°24'35"	11°26'43"	47	0.314	8	0.313

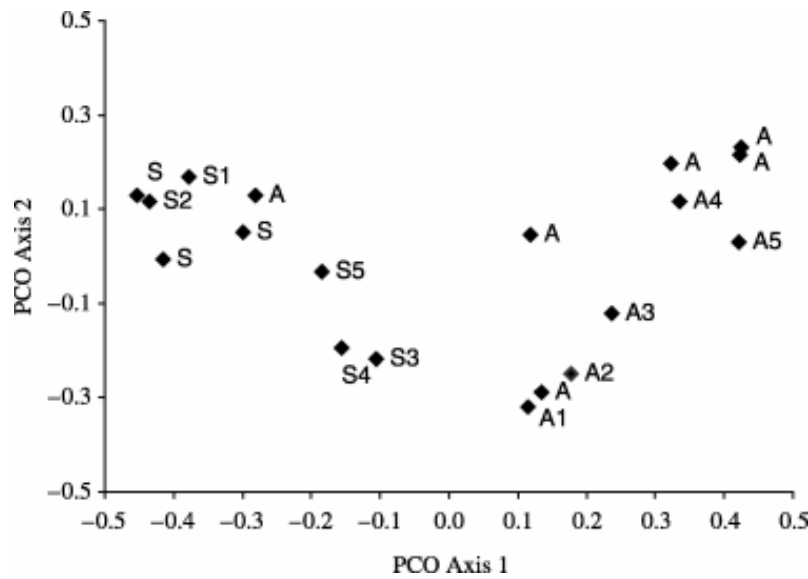


Figure 1 Ordination of the relative abundances of plant species based on a preliminary survey of 19 sites of our investigation areas by Principal Coordinates Analysis using Bray-Curtis distance matrix. 'A' denotes agriculturally-improved habitat, 'S' denotes semi-natural habitat, and numbers indicate the ten populations of *Ranunculus acris* used in this study (see Table 1).

DNA extraction and AFLP protocol

Fifty milligrams of dried leaf material from each individual plant was used for DNA extraction using a rapid method (Hellwig et al. 1999), which is described in detail in Krüger et al. (2002). The AFLP procedure was performed according to Shiemann et al. (1999), using the AFLP Core Reagent Kit (Life Technologies, Inc.). Genomic DNA (25 ng/ μ l) was digested with a pair of restriction enzymes (*EcoRI/MseI*) and then ligated to doubled stranded *EcoRI/MseI* adapters. The ligate was preamplified with the nonselective primers (*EcoRI* E00, E-A and *MseI* M00, and M-C). For the final selective AFLP amplifications five selective primer pairs (E-AAC/M-CCT, E-AGA/M-CCG, E-AGA/M-CTA, E-AAC/M-CTG, E-AGC/M-CTC) were used. Products from the final selective amplification were separated on a 8% polyacrylamide gel and the amplified AFLP bands were visualized and collected using GeneReader 4200 (version 3.52, LICOR).

Data Analysis

A data matrix of presence/absence (i.e. 1/0) of amplified loci was produced for each individual from each population and for all selective primers with the help of RFLPscanTM version 2.0 (Scanalytics). We used gene diversity, H_e , as a measure of within-population genetic variability following Nei (1987). The genetic differentiation between populations of *R. acris* based on all AFLP loci was calculated according to Nei's (1978) genetic distance. A cluster analysis using an unweighted pair-group method with arithmetic averaging (UPGMA; Sneath & Sokal 1973) was performed using the software POPGENE 1.32 (Yeh et al. 1997). The cluster generated by UPGMA was evaluated by a bootstrap analysis with 1000 iterations (Felsenstein 1985) using the software TFPGA (Miller 1997). We used the analysis of molecular variance (AMOVA; Excoffier et al. 1992) to partition the total genetic variation among individuals within populations, between populations within a habitat, and between habitat types (agriculturally-improved vs. semi-natural). AMOVA was made using WINAMOVA version 1.55 (Excoffier 1992), with the input files prepared by AMOVA-PREP (Miller 1998). We quantified species diversity by calculating species richness (the number of species present at each site), and species evenness using the E_{var} index, which is based on the variance in abundance of species and is independent of species richness (Smith & Wilson 1996):

$$E_{var} = 1 - (2/\pi) \arctan \left\{ \frac{\sum_{j=1}^s \left(\ln(X_j) - \sum_{k=1}^s \ln(X_k) / S \right)^2 / S}{S} \right\}$$

where S is the number of species in a community and X_k is the abundance of the k^{th} species. Correlations between within-population gene diversity (H_e) of *R. acris*, species richness, and species evenness at each site were tested by Pearson correlation coefficient using SPSS statistical package version 11.0 (SPSS Inc. 1989–99). Pairwise differences in species diversity (richness and evenness) between sites were calculated using Euclidean distance and they were compared, using a simple Mantel test, with genetic distances between *R. acris* populations. We also tested whether genetic distances between pairs of populations were significantly correlated with corresponding

geographical distances. Analyses of Mantel tests were achieved using the software zt (Bonnet & Yves 2002).

RESULTS

Genetic diversity estimated by AFLP

Large numbers of AFLP loci were obtained by each of the five primer combinations; however, for the final analysis a total of 258 AFLP loci that were unambiguous and easily scored were used. Of all loci analysed, 79.5% were polymorphic and 20.5% monomorphic within or between populations. Table 1 gives the values of within-population genetic diversity estimated by Nei's gene diversity H_e (1987). The gene diversity averaged over all loci ranged from 0.258 to 0.334. Table 2 shows the genetic differentiation (Nei's 1978 genetic distance) between *R. acris* populations. The genetic distances did not correlate significantly with the corresponding geographical distances (Mantel test; $r = 0.11$, $P = 0.18$).

The partitioning of total genetic variation of *R. acris* by AMOVA is shown in Table 3. Most genetic variation occurred between individuals within populations (89.03%) rather than between populations (10.97%).

Within-population genetic diversity of *R. acris* in relation to species diversity

Within-population genetic diversity of *R. acris* (Nei's gene diversity; H_e) was neither significantly correlated with species richness (Pearson's $r = -0.17$; $P = 0.64$), nor with species evenness of the plant communities (Pearson's $r = 0.15$; $P = 0.68$).

Table 2 Pairwise genetic distances (below diagonal) and geographical distances (in km, above diagonal) between the populations of *Ranunculus acris* studied.

Pop	A1	A2	A3	A4	A5	S1	S2	S3	S4	S5
A1	–	1.0	3.0	3.5	7.0	12.0	16.0	15.0	13.5	13.0
A2	0.129	–	2.5	3.5	7.5	13.0	16.5	15.5	14.0	13.5
A3	0.042	0.145	–	1.5	6.0	13.0	16.5	15.3	13.0	12.5
A4	0.064	0.134	0.099	–	4.5	11.5	15.0	14.0	11.5	11.0
A5	0.043	0.116	0.065	0.034	–	8.0	10.5	10.0	7.0	6.5
S1	0.147	0.221	0.092	0.233	0.174	–	4.5	4.0	5.5	5.5
S2	0.121	0.034	0.146	0.131	0.108	0.210	–	1.0	5.0	5.0
S3	0.145	0.036	0.177	0.124	0.117	0.249	0.038	–	4.0	4.0
S4	0.088	0.059	0.103	0.118	0.083	0.165	0.038	0.066	–	1.0
S5	0.111	0.084	0.089	0.123	0.091	0.161	0.080	0.089	0.087	–

Table 3 Analysis of molecular variance (AMOVA) for 86 individuals of 10 *Ranunculus acris* populations. Nested analysis was done on the two groups of populations based on their habitat type (agriculturally-improved vs. semi-natural).

Source of variation	d.f.	Sum of Squares	Variance	% Total	Significance
Between populations	9	817.56	5.442	10.97%	$P < 0.001$
Within populations	76	3358.48	44.190	89.03%	
Total	85	4176.05			
Nested analysis					
Between habitat types	1	158.84	1.821	3.56	$P = 0.02$
Between populations/ habitat	8	658.73	4.447	8.81	$P < 0.001$
Within populations	76	3358.48	44.191	87.58	$P < 0.001$
Total	85	4176.05			

Between-population genetic differentiation of *Ranunculus acris* in relation to difference in species diversity

Between-population genetic differentiation (genetic distances) of *R. acris* was significantly correlated with the differences in grasslands species evenness (Mantel's $r = 0.62$, $P < 0.02$; Fig. 2), but was not correlated with differences in species richness (Mantel's $r = -0.17$, $P = 0.22$).

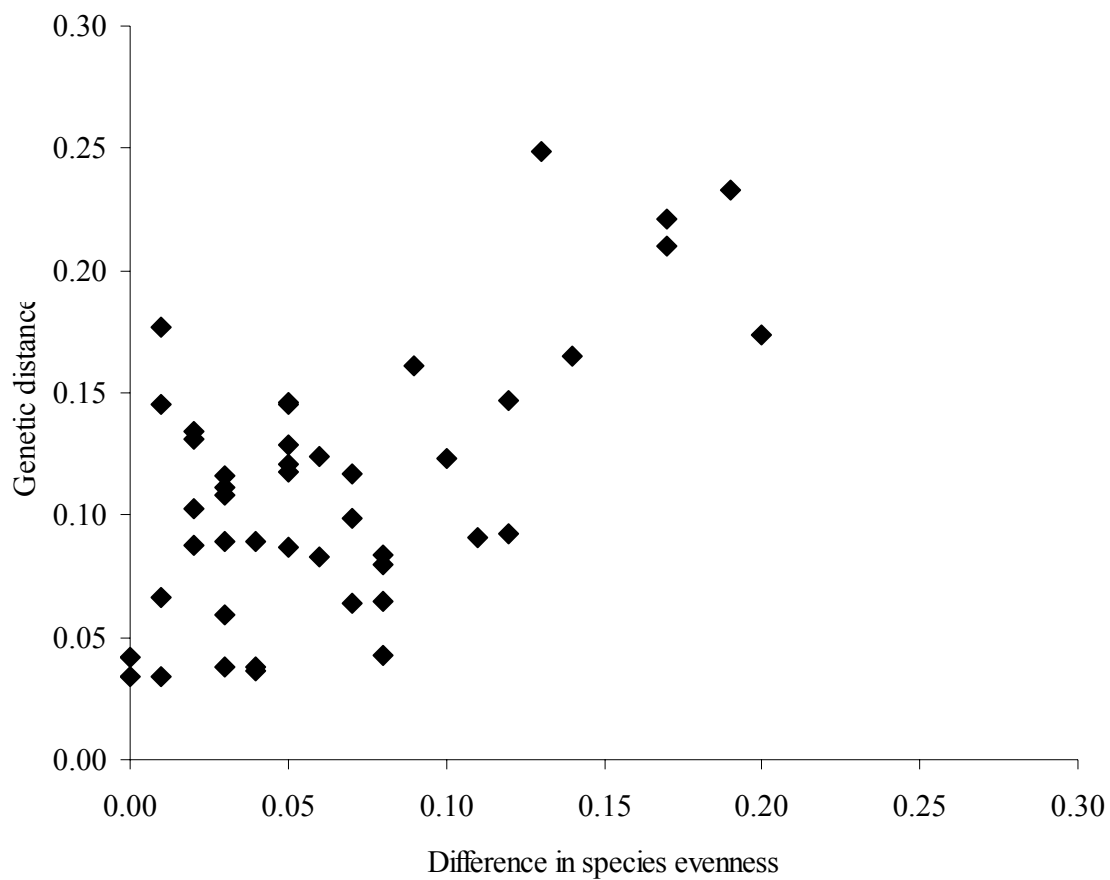


Figure 2 Association between the genetic differentiation (pairwise Nei's genetic distances) and difference in species evenness (measured as E_{var} index, see Materials and Methods) among the studied populations of *Ranunculus acris*. Mantel's $r = 0.62$; $P < 0.02$.

Genetic diversity in relation to habitat type

Within-population genetic diversity of *R. acris* was higher in agriculturally-improved populations (mean 0.314, SD 0.024) compared with populations from semi-natural habitats (mean 0.294, SD 0.027), although the difference was not significant (pooled *t*-test: $P > 0.2$). However, the UPGMA dendrogram based on the genetic distances between populations showed two main clusters of populations that may be related to habitat type (Fig. 3). Moreover, nested AMOVA analysis of the two groups of populations (i.e. agriculturally-improved vs. semi-natural habitats) showed significant genetic differentiation between these habitat types (AMOVA, $P < 0.05$; Table 3).

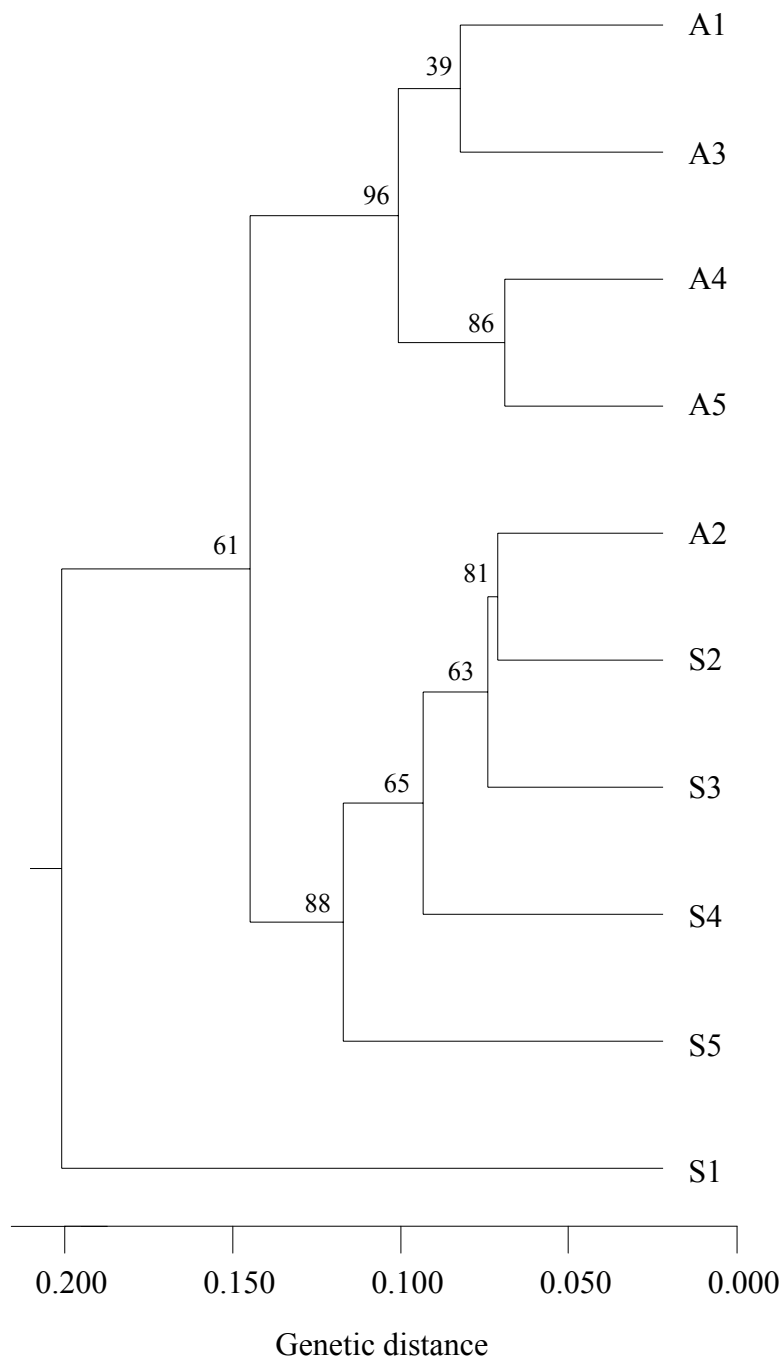


Figure 3 UPGMA dendrogram based on Nei's (1978) genetic distance of *Ranunculus acris* populations of agriculturally-improved (A1-A5) and semi-natural (S1-S5) habitats. Bootstrap percentages are given on each node (1000 replications).

DISCUSSION

Genetic diversity

To our knowledge this is the first study attempting to assess the genetic diversity of a particular plant species in relation to the species diversity (richness and evenness) of the local plant communities. Initially, random amplified polymorphic DNA markers (RAPD; Williams et al. 1990) were tested for estimating genetic diversity of *R. acris* populations. However, we were unable to obtain a sufficient number of unambiguous, polymorphic and reproducible RAPD loci. In contrast, AFLP proved to be an excellent molecular technique for the analysis of the genetic diversity of *R. acris* populations in this study. We obtained many polymorphic loci per primer combination that enabled us to describe the genetic diversity within and between populations of *R. acris*. Within-population genetic diversity values were high (Table 1), suggesting that individuals within populations display a large proportion of genetic loci of high allelic variation. The characteristics of *R. acris* as a geographically wide-spread, outbreeding, long-lived perennial plant species (Loveless & Hamrick 1984; Karron 1987; Hamrick & Godt 1990; Richter et al. 1994) may contribute to the high within-population genetic diversity we observed. Moreover, the populations of *R. acris* we studied are very large and their habitats are stable and not fragmented; such characteristics may also contribute to high within-population genetic diversity (e.g. Young et al. 1996).

Species diversity effects

Our results demonstrate a significant correlation between the genetic differentiation of *R. acris* populations and the differences in species evenness of plant communities (Fig. 2). The higher the difference in species evenness of plant communities between two given sites, the higher the genetic differences of *R. acris* populations at these sites. As species evenness explains a larger amount of the variance in communities (Stirling & Wilsey 2001) and defines the identity of species (Polley et al. 2003), we propose that this aspect of a community is a good measure to study the effect of community diversity on the genetic diversity of plant species. According to Linhart and Grant (1996) association between genetic differentiation, as those we found in *R. acris* populations, and differences in species evenness may be explained by two processes: firstly, different communities produce different selective forces, and these, in turn, shape the genetic heterogeneity

between local populations. Secondly, habitat variation often generates ecological barriers against gene flow and thus enhances genetic differentiation between local populations. If migrants move from their source population to an area with different habitat characteristics, they will be perhaps poorly established to the new habitat and thus may be less likely than residents to pass their genes on to the next generation (Cooper 1998). As the localized selective forces and gene flow are operating synergistically on genetic diversity in natural populations (Linhart & Grant 1996), it is difficult to conclude, however, to what extent community diversity represents a barrier against gene flow in *R. acris* populations. Studies linking seed or pollen movements with the diversity of communities are lacking in the literature. It is known, however, that diverse communities are less invaded by other species (Stirling & Wilsey 2001).

Finally, our results did not detect a significant correlation between the within-population genetic diversity of *R. acris* and species diversity (species evenness species richness), or between the genetic differentiation of *R. acris* populations and the difference in the species richness. This could be either due to reduced statistical power, or it could be that such associations are absent in *R. acris* populations. Other plant species in our plant communities however might show such associations.

Habitat type effect

The meadow buttercup *R. acris* grows in different meadow types and inhabits grazed and mown habitats (Grime et al. 1988). We aimed also to test whether populations of *R. acris* that grown in agriculturally improved habitats are genetically different from those grown in semi-natural habitats. The observed difference in the genetic differentiation between these habitats (Table 3, Fig. 3) may suggest that there is no substantial gene flow between these habitat types. As we pointed out, these two habitat types differ in both abiotic (e.g. the intensity of management and the land use in the past) and biotic factors such as plant species composition (see Fig. 1). These factors may act as ecological forces against a success exchange of seed or pollen (gene flow) between these habitat types. Management regimes and frequent mowing have been shown to influence the genetic variability of some plant species (Snaydon & Davies 1982; Snaydon 1987; Kölliker et al. 1998). Moreover a study of genetic variation among little bluestem (*Schizachyrium scroparium*) populations

suggested that local site differences in soil characteristics and ecological history can promote genetic differentiation (Huff et al. 1998).

We conclude that local selection processes have influenced the genetic diversity of *R. acris* populations. Habitat variability is correlated with apparent genetic differentiation. The factors that influence population genetic diversity will become more evident when the use of genetic markers are used in parallel with studies that entail the physiological characterisation of each genetically distinct types. In turn, causation and the impact of distinct traits of individuals must be assessed using experiments that manipulate and measure genetic succession in model plant communities.

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CHAPTER FOUR: ARTICLE 3

On the relationship between plant species diversity and genetic diversity of *Plantago lanceolata* (Plantaginaceae) within and between 15 grasslands sites

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ABSTRACT

The relationship between genetic diversity within a species and species diversity of ecological communities is of fundamental importance for biodiversity research and conservation biology. We studied the relationship between plant species richness at a site and AFLP genetic diversity of populations of *Plantago lanceolata* in 15 grasslands in Central Germany. Within-population AFLP diversity was positively correlated with both plant species richness and abundance of *P. lanceolata*. However, all three variables were higher at nutrient-poor sites, and partial correlation analyses indicated, that the relationship between plant species richness and genetic diversity of *P. lanceolata* was indirect through ecological site characteristics rather than causal via niche width. Moreover, because higher species richness automatically reduces mean abundance per species, such a positive relationship may be the exception rather than the rule. In addition, genetic diversity between *P. lanceolata* populations, estimated as Φ_{ST} genetic distances, was positively correlated with the pairwise differences in plant species composition between sites. This relationship was best explained by the pairwise differences in ecological conditions across sites, which may affect gene flow of *P. lanceolata* and migration by other grassland species in similar ways. We conclude that positive relationships between species diversity of ecological communities and genetic diversity of a species can exist both within and between sites, and that such relationships do not necessarily indicate causal effects of species diversity on ecological niche diversity.

Keywords: AFLP genetic diversity, genetic differentiation, plant species diversity, environmental variables, conservation, *Plantago lanceolata*

INTRODUCTION

The relationship between genetic diversity within a species and species diversity, e.g. species richness, of ecological communities is of fundamental importance for biodiversity

research and conservation biology. So far, biodiversity at the species level and at the genetic level were studied separately. However, diversity at one level may feedback on diversity at the other level. For example, if genetic diversity within a population is reduced, the population is likely to be at high risk of extinction (Newman and Pilson 1997), which in turn may lead to reduction in species diversity of ecological communities. On the other hand, higher species diversity at a site may affect the niche space available for a constituent population (Whelan 2001), which in turn may influence its genetic diversity. For conservation biologists it is important to know whether diversity effects of human impacts on habitat quality, e.g. via land use changes, and on habitat quantity, e.g. via habitat fragmentation, affect the genetic and species levels of diversity in the same or in opposite directions. Moreover, it is important to know whether conservation efforts implemented to positively affect one level of diversity are likely to also positively affect the other level, or whether there are conservation conflicts between both of these diversity levels.

The niche-variation hypothesis suggests that higher habitat diversity results in wider ecological niches and therefore may allow genetic diversity within species to increase (Dobzhansky 1970). In plants, the geographical range of species has been used as indirect measure for ecological niche width (Mitton 1997). Across hundreds of plant species, genetic diversity was found to increase with broader geographic and habitat ranges (e.g. Hamrick & Godt 1990), which seems to support the niche-variation hypothesis. If higher species diversity brings with it higher habitat diversity for constituent populations (Whelan 2001), this would suggest that genetic diversity should be higher in sites with higher plant species diversity.

Nevertheless, the relationship between genetic diversity and habitat diversity is not always found to be positive and related to niche width. Johnson (1973) found a negative relationship between allozyme variation and the abundance of sympatric species in 48 species of *Drosophila*. Similarly, a negative relationship between species diversity and genetic diversity was reported in a study of populations of Plethodontid salamanders, *Desmognathus fuscus* (Karlin et al. 1984). Perhaps higher numbers of closely related sympatric species might indicate that the niche is more completely filled, leaving less niche space available for genetically variants of a species. Because of the lack of studies directly relating plant species diversity to measures of niche diversity or genetic diversity, the

relevance of niche variation in the context of the species diversity–genetic diversity relationship is not clear.

Moreover, correlation between species and genetic diversity could also be driven indirectly via ecological conditions of habitats. Habitats of low productivity may cause an increase in species richness and abundance of specialised plants, which may lead to an increase in the genetic diversity of specialist populations. On the other hand, an increase in species richness may also decrease genetic diversity within a species, because higher species diversity is confounded with lower average abundance of a species.

Also between sites, genetic diversity between local populations of a species, i.e. population genetic differentiation, may be related to species diversity, i.e. differentiation in species composition. Such a relationship can come about if ecological differences similarly affect the composition of plant communities and genetic diversity of local populations (e.g. Linhart & Grant 1996; Mitton 1997). Moreover, it may come about if ecological differences between sites affect both gene flow within species (e.g. Cooper 1998; Lugon-Moulin et al. 1999) and the rates of extinction and recolonisation of species in similar ways.

Here, we studied the relationship between the genetic diversity of *Plantago lanceolata* and plant species diversity within and between 15 grasslands in Central Germany. The grasslands all belonged to the phytosociological taxon of the Arrhenatherum, but nevertheless varied in species richness and other ecological conditions. We selected *Plantago lanceolata* for this study because it is a typical species of such grassland habitats (e.g. Grime 1988; Kuiper & Bos 1992). We measured genetic diversity by using amplified fragment length polymorphism (AFLP; Vos et al. 1995), which offers a high resolution (Mueller & Wolfenbarger 1999). Specifically, we addressed the following questions: (i) is genetic diversity within and between populations of *P. lanceolata* correlated with grassland plant species diversity? (ii) are genetic diversity of populations of *P. lanceolata* and species diversity within and between grasslands correlated with environmental variables? and (iii) if so, is the relationship between genetic and species diversity mediated by environmental variation rather than being causal via ecological niche diversity?

MATERIALS AND METHODS

Study species, study sites, and plant material

Plantago lanceolata L. (Plantaginaceae) is a common rosette perennial herb that commonly inhabits base-rich meadows and waysides. Its distribution covers most of Europe and Northwestern Asia (Rothmaler 1996). *Plantago lanceolata* is self-incompatible, wind-pollinated, and flowers from May through early September (Grime 1988).

Our study populations of *P. lanceolata* were randomly selected in a plateau-like montane range of the Thuringer Schiefergebirge/Frankenwald in Central Germany. Pairwise geographical distances between the populations ranged from 1 to 28 km. The vegetation of the semi-natural grasslands varied in plant species composition and richness (Odat et al. 2004).

At each of the 15 study sites with *P. lanceolata* populations, we recorded the presence and relative abundance of all higher plant species in an area of 6 x 6 m² composed of four randomly placed separate quadrats of 3 x 3 m². In some of our study sites the abundance of *P. lanceolata* was so low that it was not present in the recording plots. In these cases we scored the abundance of *P. lanceolata* as zero, although it was of course present at the site (see Table 1).

At each site we randomly sampled leaves of 5 to 27 (mean 9) flowering *P. lanceolata* plants and immediately placed them in drying silica gel for transportation to the DNA extraction lab.

Table 1 Plant community diversity, abundance and gene diversity of *Plantago lanceolata* and means of the Ellenberg indicator values for nutrients and soil reaction for the 15 studied grassland sites. Species richness and evenness of higher plants were obtained from records of plant species presence and abundance in four randomly selected 3x3 m² plots per site. Genetic diversity of *Plantago lanceolata* was measured by using AFLP (see Methods for details).

Site	Plant species richness	Plant species evenness	Abundance of <i>Plantago lanceolata</i>	Gene diversity of <i>Plantago lanceolata</i> (SD)	Nutrients - N	Soil reaction - R
1	26	0.238	0.00	0.191 (0.203)	6.265	5.880
2	24	0.215	0.00	0.190 (0.211)	6.406	6.566
3	33	0.234	9.50	0.221 (0.209)	6.348	5.418
4	31	0.205	0.00	0.156 (0.206)	6.869	6.449
5	21	0.195	0.00	0.188 (0.212)	7.139	6.091
6	38	0.292	13.00	0.221 (0.206)	3.946	4.343
7	33	0.397	11.25	0.208 (0.205)	4.251	4.829
8	38	0.249	14.25	0.230 (0.205)	4.498	4.910
9	36	0.228	2.25	0.312 (0.167)	3.642	4.143
10	37	0.269	17.50	0.256 (0.197)	5.165	4.736
11	36	0.260	11.75	0.214 (0.207)	4.912	4.459
12	24	0.220	0.00	0.235 (0.203)	6.176	5.393
13	33	0.210	0.00	0.207 (0.208)	5.971	4.876
14	31	0.240	21.25	0.245 (0.207)	3.859	4.439
15	28	0.227	5.50	0.218 (0.206)	3.566	4.289

AFLP diversity

Template genomic DNA from individual plants was prepared as described in detail by Krüger et al. (2002). The AFLP procedure was performed according to Vos et al. (1995) using the AFLP[®] Core Reagent Kit (Invitrogen Life Technologies, Karlsruhe, Germany), with a few modifications as outlined in Odat et al. (2004). After a survey of 40 plants from five populations with 26 primer pairs we used four selective primer pairs (EcoRI-AAC/MseI-CCT, EcoRI-AAG/ MseI-CCG, EcoRI-AGG/ MseI-CAA, EcoRI-AAG/ MseI-CGA) (MWG Biotech AG, Ebersberg, Germany) to screen all 142 plants.

Statistical analysis

Genetic diversity of *Plantago lanceolata* within and between populations

We established the presence-absence (1/0) matrix of amplified AFLP bands for each of the 142 plants with the help of RFLPscanTM version 2.1 (Scanalytics, Inc.). We estimated within-population genetic diversity of *P. lanceolata* as gene diversity H_E after Nei (1973) with the software POPGENE (Yeh et al. 1997).

To estimate genetic differentiation between populations of *P. lanceolata* we calculated pairwise genetic distances Φ_{ST} (an analogue of F_{ST}) with analysis of molecular variance (AMOVA; Excoffier et al. 1992).

Plant community diversity within and between study sites

We quantified species diversity at each site as species richness and species evenness. We estimated evenness (E_{var}), which is based on the variance in species abundance and is independent of species richness, according to Smith & Wilson (1996) as

$$E_{var} = 1 - (2/\pi) \arctan \left\{ \frac{\sum_{j=1}^s \left(\ln(X_j) - \sum_{k=1}^s \ln(X_k) / S \right)^2 / S}{S} \right\}$$

where S is the number of species in a community and X_k is the abundance of the k^{th} species. With the software PC-ORD, we calculated a Bray-Curtis coefficient used as distance measure in a pairwise 15 x 15 matrix of grassland communities based on the difference in relative abundances of all species across sites (Faith et al. 1987).

Ecological conditions at sites and differences between sites

To characterise ecological conditions at each study site, we calculated means of Ellenberg's indicator values for light, temperature, continentality, moisture, soil reaction and nutrient levels (Ellenberg et al. 1992). To describe ecological conditions between pairs of sites we calculated a 15 x 15 Euclidian distance matrix based on the six-dimensional space spanned by the Ellenberg coordinates.

Relationship between genetic diversity of *Plantago lanceolata* and plant community diversity

To test for a relationship between genetic diversity of *P. lanceolata* within populations and plant community diversity (species richness and species evenness) within sites we used Spearman's rank coefficient (Table 1). To test whether such a relationship is likely to be causal, we also tested whether gene diversity was related to the abundance of *P. lanceolata*, and whether it was related to any of Ellenberg's indicator values. We used partial correlations to test how the observed relationship between AFLP diversity and species diversity changed after correction for these potential determinants of AFLP genetic diversity.

To test for a relationship between genetic diversity of *P. lanceolata* between populations and plant community diversity between sites we did Mantel tests (Mantel 1967) with the software ZT (Bonnet & Van de Peer 2002). First, we tested the relationship between the 15 x 15 matrix of pairwise genetic distances (Φ_{ST}) between *P. lanceolata* populations and the 15 x 15 matrix of pairwise distances in plant community composition (Bray-Curtis coefficient) with a simple Mantel test (Mantel 1967). To test whether this relationship could have been due to geographical distances or to ecological differences, we also tested the relationships of pairwise genetic and community distances with geographic distances and with ecological distances with Mantel tests. Finally, to test the relationship between genetic and plant community distances independent of possibly confounding effects of geographic or ecological distances, we performed partial Mantel tests (Manly 1997).

RESULTS

Genetic diversity of *Plantago lanceolata* within and between populations

The four selective primer pairs enabled us to score 259 AFLP loci, of which 59.79% were polymorphic. Gene diversity H_E within the studied populations of *P. lanceolata* ranged from 0.156 to 0.312 (Table 1).

Analysis of molecular variance AMOVA revealed significant genetic differentiation among populations (9.70% of variance between populations, global $\Phi_{ST} = 0.097$, $P < 0.0001$), although within-population genetic variation (90.30%) was very high. Pairwise genetic distances Φ_{ST} between populations of *P. lanceolata* ranged from 0.004 to 0.208. Of the 105 pairwise genetic distances between populations 101 were statistically significant (Table 2).

Table 2 Pairwise distances in genetic diversity of *Plantago lanceolata* (Φ_{ST} , measured with AFLP, below diagonal) and in plants community composition (measured as Bray-Curtis coefficient, above diagonal) between the 15 studied grassland sites. Significant pairwise genetic distances are indicated in bold

Site	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	—	0.181	0.701	0.607	0.589	0.872	0.886	0.824	0.952	0.754	0.763	0.739	0.564	0.916	0.946
2	0.140	—	0.711	0.573	0.614	0.884	0.893	0.824	0.958	0.758	0.769	0.759	0.573	0.922	0.955
3	0.054	0.092	—	0.700	0.706	0.817	0.796	0.711	0.849	0.501	0.675	0.710	0.631	0.809	0.817
4	0.084	0.158	0.100	—	0.627	0.752	0.857	0.784	0.935	0.764	0.793	0.784	0.719	0.883	0.933
5	0.116	0.155	0.114	0.087	—	0.875	0.933	0.868	0.963	0.792	0.786	0.741	0.685	0.946	0.971
6	0.135	0.199	0.144	0.167	0.136	—	0.409	0.402	0.569	0.615	0.567	0.772	0.707	0.413	0.476
7	0.110	0.186	0.108	0.146	0.132	0.034	—	0.282	0.437	0.595	0.614	0.811	0.646	0.387	0.368
8	0.131	0.208	0.147	0.156	0.126	0.069	0.032	—	0.491	0.530	0.502	0.705	0.581	0.332	0.386
9	0.084	0.118	0.075	0.096	0.082	0.064	0.050	0.053	—	0.600	0.586	0.891	0.744	0.414	0.289
10	0.133	0.151	0.099	0.157	0.127	0.099	0.098	0.115	0.017	—	0.380	0.626	0.550	0.445	0.635
11	0.150	0.169	0.112	0.170	0.159	0.110	0.120	0.135	0.029	0.004	—	0.551	0.674	0.403	0.575
12	0.131	0.175	0.124	0.155	0.130	0.097	0.099	0.084	0.051	0.059	0.098	—	0.560	0.788	0.856
13	0.134	0.208	0.148	0.149	0.133	0.110	0.132	0.102	0.062	0.097	0.113	0.063	—	0.684	0.736
14	0.134	0.146	0.124	0.144	0.103	0.086	0.113	0.094	0.037	0.030	0.060	0.021	0.048	—	0.321
15	0.153	0.193	0.138	0.175	0.156	0.092	0.125	0.132	0.059	0.066	0.084	0.032	0.066	0.039	—

Relationship between genetic diversity of *Plantago lanceolata* and plant community diversity within sites

Within-population gene diversity H_E of *P. lanceolata* was significantly positively correlated with plant species richness ($N = 15$, Spearman's $r = 0.516$, $P = 0.049$; Fig. 1a, Table 1) and marginally positively significantly with species evenness at a site ($N = 15$, Spearman's $r = 0.486$, $P = 0.066$). H_E was significantly higher at sites with lower Ellenberg reaction value, with lower Ellenberg nutrient values, and with higher abundance of *P. lanceolata*, and these three measures were highly significantly correlated with each other (Table 3).

In partial correlations, where we corrected for variation in Ellenberg reaction values, Ellenberg nutrient values, or abundance of *P. lanceolata* the relationship between AFLP genetic diversity and species diversity was weak and non-significant.

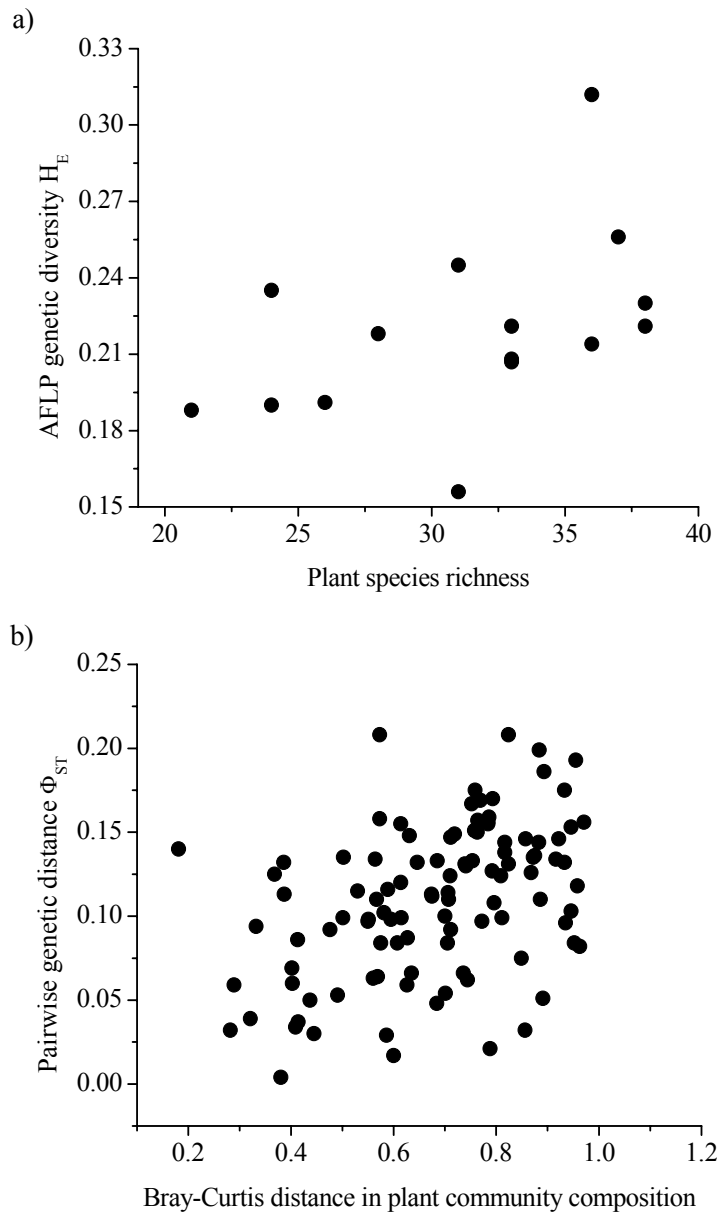


Figure 1 Relationship between plant community diversity and AFLP genetic diversity of *Plantago lanceolata* within and between 15 grassland sites. (a) Relationship between plant species richness and gene diversity (H_E) of *Plantago lanceolata* within 15 grassland sites (Spearman's $r = 0.516$, $P = 0.049$). (b) Relationship between pairwise distances in plant community composition (Bray-Curtis coefficient) and pairwise genetic differentiation Φ_{ST} (Mantel $r_M = 0.433$ $P = 0.0009$) between study sites. For details on measures of diversity and distances see methods.

Table 3 Spearman's rank correlations between gene diversity of *Plantago lanceolata*, species diversity (number and evenness), abundance of *Plantago lanceolata*, and site means of six ecological indicator values (Ellenberg 1992) for the 15 study sites in Central Germany. The values are correlation coefficients; *P*-values are given in parentheses. * < 0.05; ** < 0.01; *** < 0.001

	Light (L)	Temperatu re (T)	Continentali ty (K)	Moistu re (F)	Reactio n (R)	Nutrie nt (N)
Gene diversity of <i>Plantago lanceolata</i>	0.300 (0.277)	-0.483 (0.068)	-0.298 (0.280)	0.166 (0.554)	-0.683 (0.005)**	-0.651 (0.009)**
Plant species number	0.460 (0.084)	-0.290 (0.295)	0.036 (0.899)	0.239 (0.391)	-0.556 (0.031)*	-0.509 (0.053)*
Plant species evenness	0.286 (0.302)	-0.418 (0.121)	0.204 (0.467)	0.107 (0.704)	-0.521 (0.046)*	-0.564 (0.028)*
Abundance of <i>Plantago lanceolata</i>	0.431 (0.125)	-0.559 (0.030)*	0.140 (0.618)	0.275 (0.322)	-0.605 (0.017)*	-0.620 (0.014)*

Relationship between genetic diversity of *Plantago lanceolata* and plant community diversity between sites

The matrix of pairwise genetic distances Φ_{ST} between *P. lanceolata* populations was significantly positively correlated with the matrix of pairwise distances of plant community composition (Simple Mantel's $r_M = 0.433$, $P = 0.0009$).

Both genetic (Simple Mantel's $r_M = 0.641$, $P = 0.0001$) and plant community distances (Simple Mantel's $r_M = 0.438$, $P = 0.0029$) between sites were significantly positively correlated with the matrix of pairwise geographical distances between sites. Moreover, matrices both of genetic (Simple Mantel's $r_M = 0.470$, $P = 0.0019$) and of community distances (Simple Mantel's $r_M = 0.846$, $P = 0.0009$) between sites were significantly positively correlated with the matrix of pairwise ecological distances between sites. When we controlled for the effect of geographic distances using a partial Mantel test, the positive relationship between the distance matrices of pairwise genetic Φ_{ST} and plant community (Bray-Curtis coefficient) was maintained (Partial Mantel's $r_M = 0.220$, $P = 0.031$). However, when we controlled for the effect of ecological distance the positive relationship between the distance matrices of pairwise genetic Φ_{ST} and plant community was weak and non-significant (Partial Mantel's $r_M = 0.074$, $P = 0.304$).

DISCUSSION

Relationship between genetic diversity of *Plantago lanceolata* and plant community diversity within sites

The high proportion of 90.30% of the AFLP genetic variation in *P. lanceolata* within rather than between populations corresponds well with the outcrossing mating system of this common and widespread wind-pollinated perennial plant species (Loveless & Hamrick 1984; Hamrick & Godt 1990; Richter et al. 1994).

Within-site AFLP genetic diversity of *P. lanceolata* was positively correlated with species richness (Fig. 1a), a pattern that has been not reported for plants before. This finding contradicts the prediction that in highly diverse communities, ecological niche space is filled and does not allow for genetically diverse populations. At first sight, it is consistent with the idea that higher species diversity brings with it more variable niche space, which in turn might lead to higher genetic population diversity, as suggested earlier for higher habitat diversity (Hedrick et al. 1976; Linhart & Grant 1996).

However, we also tested, whether species diversity was confounded with habitat quality, and whether habitat quality was likely to affect genetic population diversity. Species richness turned out to be higher at more nutrient poor and less acidic sites, as is commonly observed in grasslands (Ellenberg 1996). The abundance of *P. lanceolata* was also higher at such sites. Corresponding to the situation in many other species (e.g. Frankham 1996), population genetic variation was higher at sites with higher abundance of *P. lanceolata*. This suggests that the correlation between species richness and genetic diversity observed in our study appears to be indirect rather than causal. Indeed, when we took habitat quality into account in partial correlations, the correlation between species richness and genetic diversity disappeared.

In our study, the positive relationship between species diversity and genetic diversity of *P. lanceolata* turned out to be mediated by abundance of *P. lanceolata*. However, only few species may have increased abundance at sites with higher species diversity because higher species richness automatically reduces mean abundance per species. Thus, in other species also negative relationships may be found.

Relationship between genetic diversity of *Plantago lanceolata* and plant community diversity between sites

Between sites, pairwise genetic distances between *Plantago* populations were positively correlated with pairwise differences between sites in species composition (Fig. 1b). Moreover, both distance measures were also positively correlated with geographic distances between sites and with ecological distances between sites. Partial Mantel tests showed, that the positive relationship between genetic distance and community distance was best explained by pairwise ecological differences between sites.

As AFLP markers are considered to be selectively neutral it is unlikely that this came about because plant community diversity was shaped by forces that simultaneously exerted selection on the genetic diversity of *P. lanceolata*. However, it would be interesting to test this interesting hypothesis with quantitative genetic methods rather than with selectively neutral markers such as AFLPs (Storfer 1996). Alternatively, ecological differences between sites may affect both gene flow, which shapes genetic population differentiation (Linhart & Grant 1996; Cooper 1998; Lugon-Moulin et al. 1999), and the pattern of local extinctions and colonisations, which shape community differences between sites, in similar ways.

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CHAPTER FIVE: ARTICLE 4

The effects of biotic and abiotic habitat heterogeneity of grassland sites on the genetic diversity of *Plantago lanceolata* L. (Plantaginaceae) and *Anthoxanthum odoratum* L. (Poaceae)

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ABSTRACT

Habitat heterogeneity has the potential to affect genetic diversity within and between local populations of a species. In this study, the effects of biotic and abiotic habitat heterogeneity on genetic diversity of *Plantago lanceolata* and *Anthoxanthum odoratum* were investigated. We particularly asked the following questions: (1) what is the level of genetic diversity within and between the local populations of these two species, (2) is genetic diversity correlated with biotic and abiotic characteristics of grassland sites, (3) is habitat heterogeneity influence the local abundances, and therefore, (4) is genetic diversity influenced directly by habitat characteristics or indirectly through local abundance of the two species. In both species abundant genetic diversity was present at the level of within and between the local populations, reflecting their characteristics such as mating system and their ecological distribution. The results also showed that both biotic and abiotic habitat variability influence the abundance and the genetic diversity within and between the local populations of *P. lanceolata* but not *A. odoratum*.

Keywords: genetic diversity, AFLP, habitat heterogeneity, grasslands diversity, *Plantago lanceolata*, *Anthoxanthum odoratum*

INTRODUCTION

In central Europe human impact on ecosystems nowadays has largely influenced biodiversity through changes in land use and habitat fragmentation. Consequently, many species become restricted in distribution with small and isolated populations which eventually increase the chance of extinction (e.g. Menges 1992; Vitousek 1994).

Such disturbance of natural habitats and the influence of landscape diversity have the potential to influence species diversity and eventually the genetic diversity within a

species. The loss of genetic diversity and fixation of deleterious alleles by genetic drift is most likely to increase by decreasing the population size of a species as a result of habitat fragmentation (Barrett & Kohn 1991; Boyce 1992; Ellstrand & Elam 1993; Young et al. 1996).

Biotic and abiotic habitat heterogeneity often influences the distribution and the diversity of species. According to “habitat heterogeneity” hypothesis (e.g. MacArthur & Wilson 1967; Lack 1969), complex diverse habitats may provide more niches and diverse ways of exploiting environmental resources and thus increase species diversity (Bazzaz 1997).

The genetic diversity within a species seems to be related to the variability and the quality of habitats (e.g. Linhart & Grant 1996; Hedrick et al. 1976). According to the “niche-variation” hypothesis increasing habitat variability results in an increase in the width of ecological niches and therefore leads to increase in the genetic diversity within a species (Dobzhansky 1970). In plants, the geographical range of a species could be used as indirect measure for the width of ecological niches (Mitton 1997). Data from hundreds of plant species indicated that genetic diversity increases with broader geographic and habitat range (e.g. Hamrick & Godt 1990). However, negative correlation between genetic diversity and species diversity has been also reported. For example, Johnson (1973) found a negative relationship between allozyme variation and the abundance of sympatric species in 48 species of *Drosophila*. A similar negative relationship between species diversity and genetic diversity was reported in a study of local populations of Plethodontid salamanders, *Desmognathus fuscus* (Karlin et al. 1984). The number of closely related sympatric species here could be considering as one measure of the niche breadth of a species (Mitton 1997).

Although, the relationship between habitat diversity and species diversity is well documented (e.g. Tews et al 2004), less attention has paid to the influence of habitat diversity on the genetic diversity within a species. In the present study, the relative effects of biotic and abiotic habitat heterogeneity on genetic diversity of *Plantago lanceolata* and *Anthoxanthum odoratum* were investigated. The two species were chosen as they differ in life form and other characteristics. Also these two species are common and typical plants of grassland systems of Germany and of Europe in general (Grime 1988). We particularly asked the following questions: (1) what is the level of genetic diversity within and between

the local populations of the two species, (2) is genetic diversity in these two species correlated with biotic and abiotic characteristics of grassland sites, (3) is habitat heterogeneity influence the local abundances, and therefore, (4) is genetic diversity influenced directly by habitat characteristics or indirectly through local abundance of these species.

MATERIALS AND METHODS

Study species and sites

We studied the genetic diversity of fifteen local populations of *Plantago lanceolata* L. (Plantaginaceae) and nine populations of *Anthoxanthum odoratum* L. (Poaceae). *Plantago lanceolata* is a perennial herb that commonly inhabits meadows and roadsides. *P. lanceolata* is self-incompatible, wind-pollinated and flowers from May through early September (Hegi 1982). *A. odoratum* is a small perennial bunchgrass and is dispersed by wind (Hegi 1982). Both species have a distribution nearly all over Europe and Northwestern Asia (Rothmaler 1996). At each study site we randomly sampled leaves of 5 to 27 (mean 9) flowering plants and immediately placed them in drying silica gel for transportation to the DNA extraction lab in Jena. To study genetic diversity we used amplified fragment length polymorphisms AFLP (Vos et al. 1995). Information about DNA extraction, AFLP protocol, primers and the chemical reagents used are given in detail in Odat et al. (2004).

Data analysis

To estimate the AFLP genetic diversity within populations we used gene diversity (H_E) according to Nei (1973):

$$H_E = 1 - \sum P_i^2,$$

where P_i is the population frequency of each allele (1 and 0) at locus i . The average genetic diversity is then calculated as the average of this quantity across all loci studied. To estimate genetic differentiation between populations of *P. lanceolata* we calculated pairwise genetic distances and calculated analysis of molecular variance (AMOVA; Excoffier et al. 1992) for partitioning the total genetic diversity into within and between populations levels.

At each site we determined species richness, species evenness, and the difference in relative abundances of all species at sites using a Non-Metric Multidimensional Scaling (NMDS) ordination technique (Perner et al. submitted), see Table 1. NMDS is an iterative search for a ranking and placement of n entities (samples) in k dimensions (ordination axes) that minimizes the k -dimensional configuration. NMDS ordination was based on square-root-transformed cover data. For the calculation we used the program PC-ORD (McCune & Mefford 1997). As a distance measure, the Bray-Curtis coefficient was used (also known as Sørensen or Czekanowski coefficient), which is one of the most robust measures for this purpose (Perner et al. submitted).

Table 1 The grassland study sites at which *Plantago lanceolata* was collected. Values of species richness, species evenness and abundance of *Plantago lanceolata* were obtained from records of plant species presence and abundance in four randomly selected 3x3 m² plots per site. NMDS1 and NMDS2 are the ordinations that derived from the Non-Metric Multidimensional Scaling method to characterise the variation in plant compositions at sites (see Methods).

Site	NMDS1	NMDS2	Species richness	Species evenness	Abundance of <i>P. lanceolata</i>	Genetic diversity of <i>P. lanceolata</i>
1	1.280	0.327	26	0.238	0.00	0.191
2	1.123	0.230	24	0.215	0.00	0.190
3	0.683	-0.498	33	0.234	9.50	0.221
4	1.291	-0.182	31	0.205	0.00	0.156
5	1.075	1.126	21	0.195	0.00	0.188
6	-0.781	-0.294	38	0.292	13.00	0.221
7	-0.947	-0.354	33	0.397	11.25	0.208
8	-0.669	-0.195	38	0.249	14.25	0.230
9	-1.586	-0.442	36	0.228	2.25	0.312
10	-0.384	-0.156	37	0.269	17.50	0.256
11	-0.299	-0.242	36	0.260	11.75	0.214
12	-0.117	0.997	24	0.220	0.00	0.235
13	-0.175	0.642	33	0.210	0.00	0.207
14	-0.970	-0.558	31	0.240	21.25	0.245
15	-1.065	-0.554	28	0.227	5.50	0.218

For abiotic factors at each site, the following soil parameters were determined (Table 2): soil pH (pH; mean: 5.5 ± 0.55 SE), total concentrations of soil nitrogen (N_{total} ; mean: 4.1 ± 0.65 SE mg/g), soil carbon (C_{total} ; mean: 49.5 ± 10.84 SE mg/g), extractable phosphorus (P_{total} ; mean: 0.04 ± 0.07 SE mg/g), extractable ion concentrations of potassium (K_+ ; mean: 0.05 ± 0.05 mg/g), calcium (Ca_{2+} ; mean: 1351.2 ± 0.69 SE mg/g), magnesium

(Mg²⁺; mean: 0.23±0.14 SE mg/g), and sulfate (SO₄²⁻; mean: 0.03±0.01 SE g/mg), as well as the amount of mineralized nitrogen (N_{min}; mean: 3.0±2.93 SE g/mg) and the carbon-nitrogen ratio (C:N; mean: 14.0±1.84 SE g/mg). A detailed description of the data sampling and the analytical methods is found in Kahmen et al. (subm.). We used Principal component analysis (PCA) based on a correlation matrix of all abiotic environmental variables to separate and condense variables into orthogonal components. To achieve more interpretable results from initial components we used a quartimax rotation to maximise separation. Two components (PCA1 and PCA2) were further included as independent variables in order to test the best predictor of genetic diversity of the two species (Table 3).

Table 2 Means abiotic soil characteristics of the study sites. These measures were obtained based on four sub samples of soils collected in 20 cm X 50 cm.

site	NO ₃	NH ₄	N _{min}	K	Mg	Na	P	S	Ca	C	N	C/N	pH
1	2.64	4.44	7.08	254.45	310.41	133.15	34.88	13.72	1.51	44.45	4.15	12.48	6.00
2	0.89	5.20	6.09	127.85	291.62	123.28	56.43	13.24	1.57	43.46	4.08	12.42	6.11
3	0.55	4.28	4.83	47.54	256.07	220.81	16.08	17.18	1.10	39.80	3.62	12.80	6.01
4	0.84	5.02	5.86	45.64	326.19	136.57	20.52	18.00	1.14	34.68	3.67	11.00	5.72
5	2.81	4.62	7.43	58.19	495.67	136.39	66.56	18.79	1.58	53.68	5.16	12.11	6.28
6	0.07	4.92	4.99	38.78	316.27	149.71	11.07	13.00	1.31	52.70	4.47	13.75	5.79
7	0.34	3.04	3.38	50.45	89.59	171.11	16.81	23.52	0.81	54.38	4.94	12.83	5.03
8	0.14	3.94	4.08	49.81	81.65	158.17	12.87	15.55	1.35	59.93	5.30	13.19	5.55
9	0.17	4.43	4.60	47.99	75.51	177.27	28.41	34.33	0.43	71.62	6.59	12.68	4.46
10	0.15	5.95	6.09	57.99	142.64	168.49	22.66	33.37	0.78	67.43	6.25	12.59	4.80
11	0.16	3.54	3.70	154.44	113.60	143.18	18.89	23.46	0.66	44.22	4.18	12.31	5.11
12	0.54	3.89	4.43	68.17	111.92	146.95	38.47	25.41	0.61	41.78	4.06	11.98	5.01
13	0.56	3.29	3.85	378.97	94.96	126.13	50.36	23.23	0.81	45.04	3.93	13.32	5.27
14	0.15	3.89	4.04	42.86	88.02	150.50	26.03	21.60	1.12	55.91	4.56	14.29	5.40
15	0.05	2.95	3.01	48.25	66.88	178.56	18.95	33.01	0.64	70.97	5.45	15.19	5.01

Table 3 Factor loadings of a Principle Components Analysis (PCA) used to separate and condense explanatory abiotic variables into orthogonal components. A quartimax rotation method was used to maximise separation of variables.

	PCA-1	PCA-2
NO₃	0.789	0.178
NH₄	0.456	0.673
N_{MIN}	0.804	0.540
K	0.265	-0.553
Mg	0.877	0.308
Na	-0.543	0.217
P	0.560	0.063
S	-0.787	0.296
Ca	0.840	0.109
C	-0.670	0.573
N	-0.558	0.733
C/N	-0.536	-0.133
pH	0.886	-0.067

RESULTS

Genetic diversity of *Plantago lanceolata* and *Anthoxanthum odoratum* within and between grassland sites

For *P. lanceolata* the four selective primer pairs enabled us to score 259 AFLP loci, of which 59.79 % found to be polymorphic. Gene diversity H_E within the studied populations of *P. lanceolata* ranged from 0.156 to 0.312 (Table 1). Within population genetic diversity of *P. lanceolata* and its abundance at a site were positively correlated ($r = 0.642$, $P = 0.01$). For *A. odoratum* we scored 249 loci using three primer pairs, and the genetic diversity within populations ranged from 0.262 to 0.384 (Table 4).

Table 4 The grassland study sites at which *Anthoxanthum odoratum* was collected. Values of species richness, species evenness and abundance of *Anthoxanthum odoratum* were obtained from records of plant species presence and abundance in four randomly selected 3x3 m² plots per site. NMDS1 and NMDS2 are the axes from Non-Metric Multidimensional Scaling (NMDS) ordination technique which explain the difference in relative abundances of all plant species at sites

site	Species richness	Species evenness	Genetic diversity	abundance	NMDS1	NMDS 2
1	31	0.2787	0.384	58.750	0.368	-0.226
2	33	0.234	0.281	62.500	0.683	-0.498
3	33	0.397	0.262	21.250	-0.947	-0.354
4	36	0.228	0.356	27.500	-1.586	-0.442
5	37	0.269	0.319	20.000	-0.384	-0.156
6	36	0.260	0.330	32.500	-0.299	-0.242
7	47	0.3146	0.382	22.500	-0.264	-0.092
8	24	0.220	0.370	10.500	-0.117	0.997
9	33	0.210	0.333	19.250	-0.175	0.642

The analysis of molecular variance AMOVA revealed significant genetic differentiation among populations of both *P. lanceolata* (9.70 % of variance between populations, global $\Phi_{ST} = 0.097$, $P < 0.0001$), and *A. odoratum* (5.9 %, $\Phi_{ST} = 0.059$; $P < 0.001$). Pairwise genetic distances between populations of *P. lanceolata* ranged from 0.004 to 0.208, and from 0.022 to 0.119 for *A. odoratum*. Table 5 gives the distribution and diversity of AFLP bands in both plant species.

Table 5 Distribution of population genetic parameters within and between populations of *Plantago lanceolata* and *Anthoxanthum odoratum*.

	<i>Plantago lanceolata</i>	<i>Anthoxanthum odoratum</i>
Number of populations	15	9
Number of individuals	142	121
Number of bands	259	249
% of Polymorphic bands	59.79	86.64
% of Monomorphic bands	40.21	13.54
Genetic variation within population	90.30	94.1
Genetic variation between populations	9.70	5.9

Effects of biotic and abiotic habitat heterogeneity on genetic diversity within populations of *Plantago lanceolata* and *Anthoxanthum odoratum*

Within-population gene diversity H_E of *P. lanceolata* was significantly positively correlated with plant species richness ($N = 15$, Spearman's $r = 0.516$, $P = 0.049$) and marginally positively significantly with species evenness at a site (Spearman's $r = 0.486$, $P = 0.066$). For *Plantago lanceolata* NMDS1 and NMDS2, which explain differences in the relative abundances of plant species between sites were negatively correlated with both genetic diversity within populations and with the abundance of *P.lanceolata* at a site (Table 6). Moreover, higher genetic diversity and abundance of *P. lanceolata* was present on the sites with lower NO_3 , Mg, Na, Ca, C_{total} , N_{total} , and pH (Table 7).

The first two axes of PCA of abiotic variables (Table 2; Figure 2) accounted for more than 50% of the total variance. The first axis (46.8%) was negatively correlated with both the genetic diversity and the abundance of *P. lanceolata* (Table 8). Axis 2 was neither correlated with genetic diversity nor with the abundance.

Table 6 Correlation between genetic diversity and abundance of *Plantago lanceolata* and the biotic conditions at sites. Values are correlation coefficients and P values are between parentheses, boldface indicates significance P values.

	<i>NMDS1</i>	<i>NMDS2</i>	<i>Species richness</i>	<i>Species evenness</i>
Genetic diversity of <i>Plantago lanceolata</i>	-0.707	-0.443	0.516	0.486
	(0.003)	(0.098)	(0.049)	(0.066)
Abundance of <i>Plantago lanceolata</i>	-0.640	-0.634	0.703	0.804
	(0.010)	(0.011)	(0.003)	(0.003)

Table 7 Correlation between genetic diversity and abundance of *Plantago lanceolata* and the abiotic conditions at sites. Values are correlation coefficients and P values are given in parentheses.

	NO_3	NH_4	N_{MIN}	K	Mg	Na	P	S	Ca	C	N	C/N	pH
Genetic diversity of <i>P. lanceolata</i>	-0.6	-0.1	-0.2	-0.3	-0.6	0.6	-0.3	0.47	-0.58	0.5	0.5	0.4	-0.6
	(0.001)	(0.72)	(0.38)	(0.18)	(0.02)	(0.01)	(0.28)	(0.08)	(0.02)	(0.03)	(0.06)	(0.14)	(0.009)
Abundance of <i>P. lanceolata</i>	-0.8	-0.1	-0.3	-0.5	-0.4	0.6	-0.7	0.1	-0.1	0.5	0.5	0.5	-0.3
	(0.001)	(0.77)	(0.24)	(0.06)	(0.16)	(0.02)	(0.001)	(0.69)	(0.60)	(0.06)	(0.08)	(0.03)	(0.30)

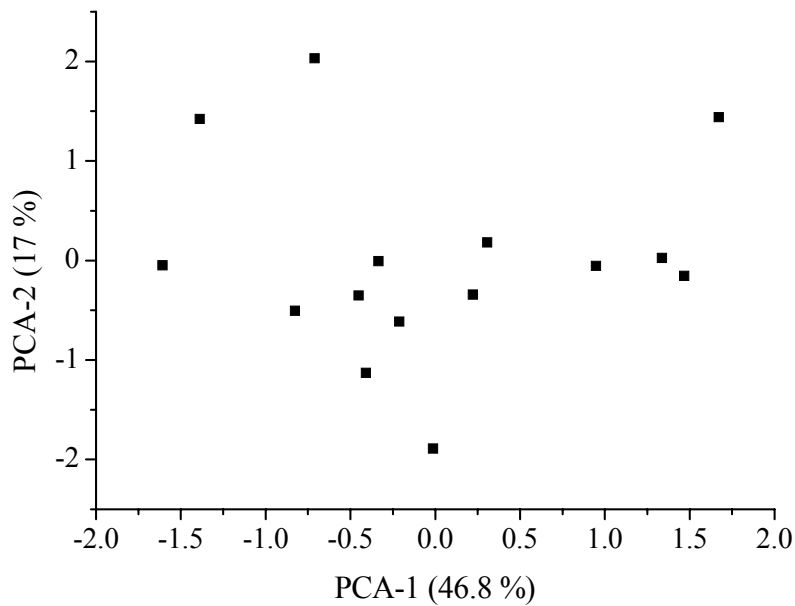


Figure 2 Ordination of the grassland sites (dots) along the two axes extracted by a PCA analysis based on 13 abiotic habitat variables measured at each site.

The within-population genetic diversity of *A. odoratum* was not correlated with the species richness ($r = -0.119$; $P = 0.760$) and the species evenness ($r = -0.100$; $P = 0.798$) of plant communities (Figure 3). Additionally, genetic diversity was not significantly correlated with the relative abundance of *A. odoratum* at site ($r = -0.017$; $P = 0.966$; Figure 4) Moreover, variation in the relative abundances of plants at sites as estimated based on NMDS1 and NMDS2 did not explain the variation in genetic diversity and the abundance of *A. odoratum*. The genetic diversity of *A. odoratum* also was not influenced by the variation in abiotic characteristics of sites.

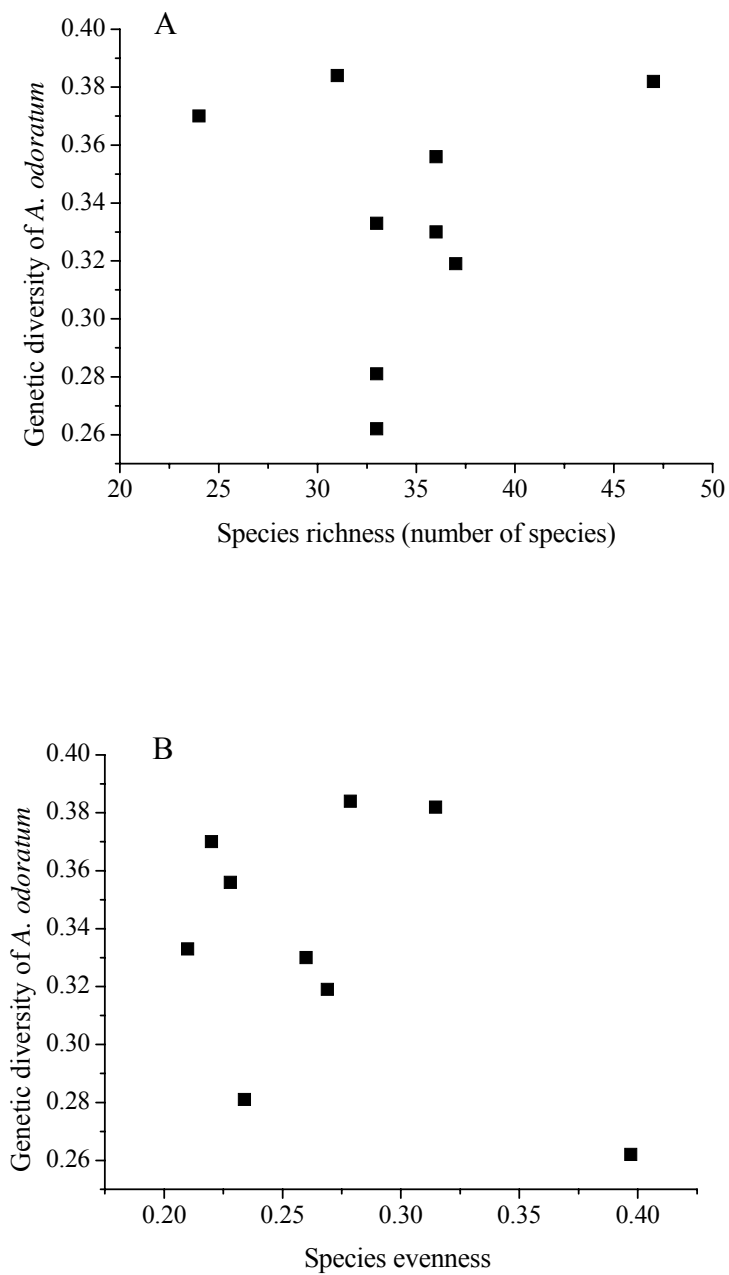


Figure 3 correlations between the genetic diversity of *Anthoxanthum odoratum* and (A) species richness and (B) species evenness of plant communities.

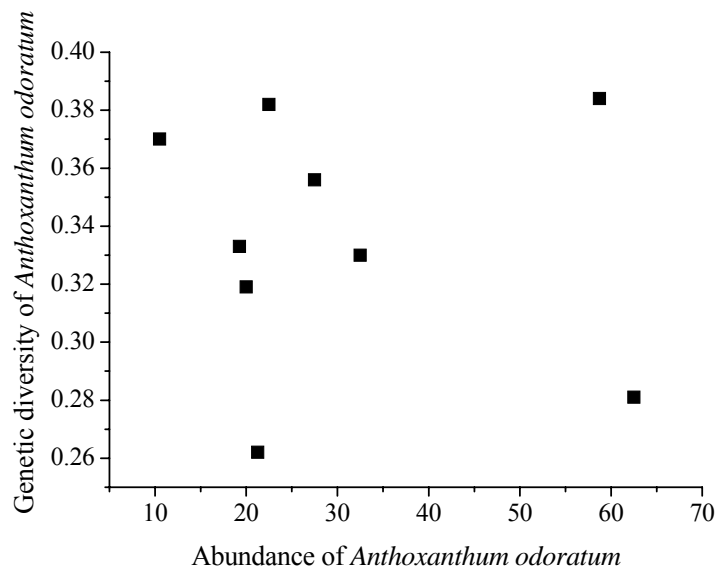


Figure 4 Correlation between the genetic diversity and abundance of *Anthoxanthum odoratum*.

Table 8 Correlation between the genetic diversity and abundance of *Plantago lanceolata* and the two principle component axes derived based on abiotic factors of study sites. Values are correlation coefficient and *P* value is given in parenthesis. Significant *P* value is indicated by boldface.

	PCA-1	PCA-2
Genetic diversity of <i>Plantago lanceolata</i>	-0.664	0.150
	(0.007)	(0.594)
Abundance of <i>Plantago lanceolata</i>	-0.551	0.118
	(0.033)	(0.675)

Relationship between genetic diversity of *Plantago lanceolata* and *Anthoxanthum odoratum* and plant community diversity between sites

The matrix of pairwise genetic distances between *P. lanceolata* populations was significantly positively correlated with the matrix of pairwise distances of plants community composition (Simple Mantel's $r_M = 0.433$, $P = 0.0009$) but not with *A. odoratum* ($r_M = 0.176$; $P > 0.05$). For *P. lanceolata* both genetic (Simple Mantel's $r_M = 0.641$, $P = 0.0001$) and plant community distances (Simple Mantel's $r_M = 0.438$, $P = 0.0029$) between sites were significantly positively correlated with the matrix of pairwise

geographical distances between sites, and with the matrix of pairwise ecological distances based on the abiotic factors between sites. For *A. odoratum* the matrix of genetic distance however was correlated with the matrix of geographical distances between site ($r_M = 0.342$; $P = 0.023$). For *Plantago* when the effect of geographic distances is controlled by using a partial Mantel test, the positive relationship between the distance matrices of pairwise genetic and plant community (Bray-Curtis coefficient) was maintained (Partial Mantel's $r_M = 0.220$, $P = 0.031$). Nevertheless, when the effect of ecological distance was controlled the positive relationship between the distance matrices of pairwise genetic and plant community was weak and non-significant (Partial Mantel's $r_M = 0.074$, $P = 0.304$).

DISCUSSION

In plants genetic diversity and its spatial distribution within and between populations are varied from one species to another and are influenced by several characteristics such as life history traits, breeding system, dispersal mechanism and historical events (Hamrick & Godt 1990). In chapter 3, it is shown that the variation in plant communities across sites affected the population genetic structure of the buttercup plant *Ranunculus acris*, an insect pollinated plant (Odat et al. 2004). However, the relationship between the AFLP variation within populations and the species diversity at a site was not found in this particular species.

In the present study we tested this relationship using a different plant species, *P. lanceolata*, and show for the first time to our knowledge, at least in plants, that both within and between populations AFLP genetic diversity is significantly correlated with the plant community diversity. Such positive correlation between the gene diversity within *P. lanceolata* populations and species diversity at a site is may be due to the localised selective forces imposed by different plant communities. This finding seems to be in agreement with theoretical and empirical studies suggesting a positive relationship between genetic diversity within a species and habitat variability (Hedrick et al. 1976; Linhart & Grant 1996; Gram & Sork 2001; Nevo 2001). However, our study is different from previous ones in that the diversity of habitats here is measured directly in the field (by characterising biotic and abiotic characteristics of sites) at small local scales, rather than inferred from the degree of the geographic distribution, successional status, or the degree of specialisation (e.g. Hamrick & Godt 1990; Mitton 1997).

It is shown also in this paper that the population genetic structure of *P. lanceolata* is significantly correlated with pairwise differences in grassland communities and geographical distances across sites. However and interestingly, when the effect of geographical isolation is disentangled, the correlation between diversity in plant communities between sites and the genetic differentiation of *P. lanceolata* populations remains significant. Two processes may account for the effect of community diversity on the genetic structuring of *P. lanceolata* populations we observed in this study. First, different communities may produce different selective forces, and these, in turn may shape the genetic heterogeneity between local populations of a species (e.g. Linhart & Grant 1996). Second, ecological variation often creates barriers that probably influence gene flow, thereby shaping patterns of population-genetic structuring (Cooper 1998; Lugon-Moulin et al. 1999). Direct measure of gene flow in relation to habitat diversity is lacking from this debate and merit further investigation.

While we are aware that correlations between genetic diversity within and between population and the community diversity such as those we presented in this study do not demonstrate cause and effect relationship (Linhart & Grant 1996), our results represent a preliminary steps toward investigating the role of biotic and abiotic habitat variation in shaping the genetic diversity within plant species.

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CHAPTER SIX General Discussion

Discussion

Although most studies on biodiversity have been mainly focused on species diversity in communities, biodiversity is composed of three fundamental levels: genetic, species, and ecosystem. Thus far, biodiversity at various levels was studied separately and typically at the species level. However, diversity at one level may feedback on diversity at the other level. For example, if genetic diversity within a population is reduced, the population is likely to be at high risk of extinction (Newman and Pilson 1997), which in turn can lead to reduction in species diversity of ecological communities. On the other hand, higher species diversity at a site may affect the niche space available for a constituent population (Whelan 2001), which in turn may influence its genetic diversity.

The three plant species investigated in this thesis differ in several aspects. *R. acris* is a perennial herbaceous plant and insect pollinated (Totland 1993, 1994b), while on the other hand, *P. lanceolata* is a rosette perennial herb and is wind pollinated. The third species studied in this thesis is *A. odoratum* which is a small, perennial bunchgrass and wind pollinated (Grime 1988). Ecologically, the three species have a distribution nearly all over Europe and Northwestern Asia (e.g. Rothmaler 1996) and they are typical species of central Germany (Hegi 1985).

The results of the thesis have shown that the population genetic diversity of the three plant species studied within grasslands sites was relatively high. This indicates that diverse communities tend to have more genetically diverse local population of a species. Such high diversity may suggest that individuals of the three species display a large proportion of genetic loci of high allelic variation, which corresponds well with the outcrossing mating system of these species. This finding seems to be in agreement with other previous studies. For example, it is known that in general outcrossing species maintain higher genetic diversity between individuals of local populations compared with self-fertilizing species but exceptions are also present (Richter et al. 1994). Moreover, the high within population genetic diversity found for the three species may attributed to the fact that these three plant species are geographically wide-spread, long-lived perennial

which thought to maintain higher genetic diversity compared to the less common and narrowly distributed plant species (Loveless & Hamrick 1984; Karron 1987; Hamrick & Godt 1990; Richter et al. 1994).

Genetic diversity within local populations of a species is often influenced by the population size and the habitat characteristics, gene flow, genetic drift and historical events (e.g. Gray 1996; Young et al. 1998). The populations of the three species studied in this thesis are relatively large and their habitats are stable and not fragmented (personal observation). Such characteristics may therefore contribute to the high genetic diversity of the three species studied in this thesis. The neutral theory of evolution predicts that higher population size maintains higher genetic diversity within a species (e.g. Mitton 1997). Several empirical studies, however, showed that some species agree with this prediction but other do not agree. In the present thesis the correlation between population size (measured as population density at a site) and the within-population genetic diversity was present in *P. lanceolata*, but not found in *R. acris* and *A. odoratum*.

The genetic diversity of the three species was found to be structured into within- and between-populations variability. Such pattern of population genetic structure of a species is believed to determine the evolutionary potential of a species (e.g. Loveless & Hamrick 1984). The genetic structure of a species is largely thought to be controlled by several evolutionary forces such as gene flow and local selection pressures of sites which can lead to either local population differentiation or genetic homogeneity (Slatkin 1987). The spatial genetic structure found in the three species may reflect habitat selection, but may also reflect non-selective process such as population history and restricted gene flow (Hamrick 1987; Levin 1988). Indeed, the genetic distances between *P. lanceolata* and *A. odoratum* plants were significantly correlated with geographical distances between sites, indicating that geographical distances between sites allow gene flow between populations, but limit a complete one.

The relationship between genetic diversity of a species and the species diversity (e.g. species number) of plant communities might be influenced directly through characteristics of ecological niches available at a site or indirectly through the population size of a species (see Figure 1).

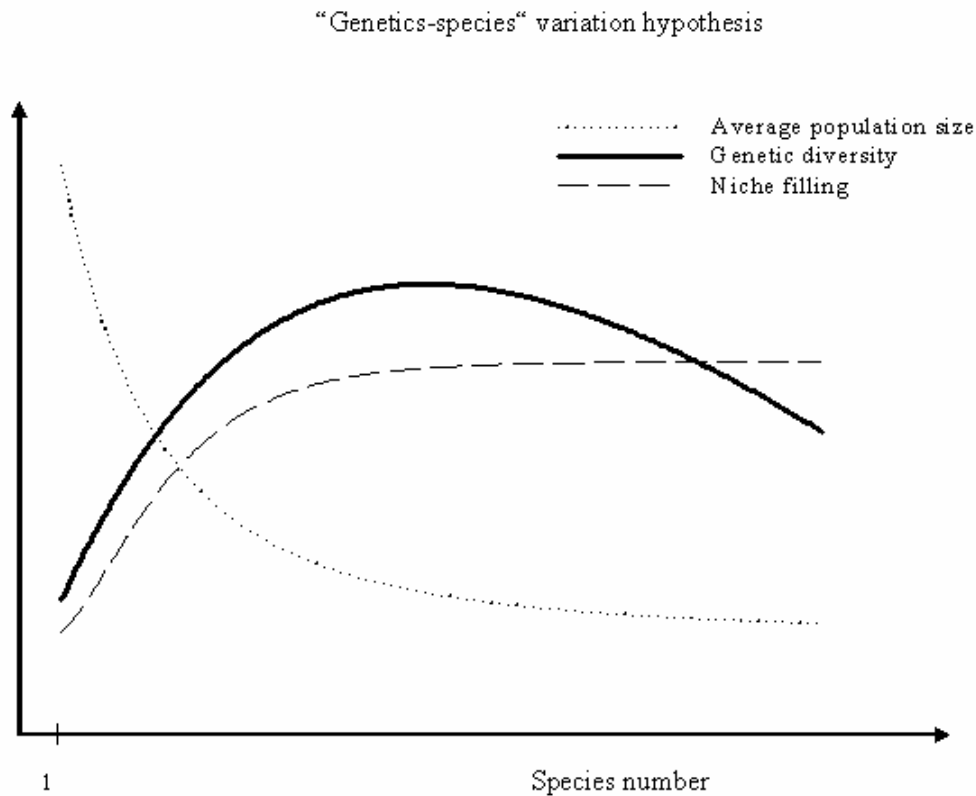


Figure 1 The expected relationship between genetic diversity of a species and species diversity (species number) which may arise through ecological niche differentiation and population size.

Higher species diversity at a site may affect the niche space available for a constituent population (e.g. Whelan 2001), which in turn may influence its genetic diversity. According to the niche-variation hypothesis, higher habitat diversity offers wider niches and therefore may allow genetic diversity within species to increase (Dobzhansky 1970). In the present thesis a positive correlation between genetic diversity of *R. acris* and *P. lanceolata* and aspect of habitat diversity of grassland sites was found. This positive correlation may reflect the effect of niche on the genetic diversity of the studied species. According to some authors the first step to demonstrate whether niche influence the level of genetic diversity in local populations is to study associations between genetic diversity and habitat diversity (e.g. Hedrick et al. 1976; Ennos 1983; Endler 1986). If there is a genetic-habitat correlation, the genetic variation that is due to among habitat variation within population can be interpreted in terms of habitat diversity and this seems to support the niche-variation hypothesis (Prentice et al. 1995).

On the other hand, the relationship between genetic diversity of a species and species diversity may also be negative. Such negative correlation may arise if the increase in species diversity results in a decrease in average population size of a species (e.g. McGrady-Stead & Morin 2000; Valone & Hoffman 2003). In highly diverse communities, the size of a typical local population of a species often will be small and therefore genetic diversity is likely to be reduced through some processes such as genetic drift and increased inbreeding (Figure 1).

Therefore, it seems that genetic diversity of a species and species diversity of communities may be related to each other via the effects of species diversity on niche and on population size. Based on this I propose a new hypothesis called “genetics-species” variation hypothesis to explain the relationship between the genetics diversity within a target species and overall species diversity in ecological communities (see Figure 1). This hypothesis predicts that the within-population genetic diversity of a species will increase with the increase in species number of the community. This will occur if higher species diversity in a community leads to more filling of the ecological niches which in turn may lead to a wider variety in selective pressures. Additionally, my proposed hypothesis predicts that the within-population genetic diversity of a species may decrease if the effect of reduced population size of the target species with increased species number of the community will become dominant (Figure 1).

It is also expected that the genetic differentiation between local populations of a species and variation in species number or alternative measures of community diversity, such as species evenness, species compositions, between sites may show a similar relationship. In this case, however, other factors in addition to population size and niche like gene flow, geographical isolation, and abiotic habitat characteristics may shape this relationship. It is found in this thesis that differences in habitat properties, mainly mediated through differences in species composition, clearly shaped the observed differences in the genetic composition of constituting species. Therefore, species diversity alone (such as species number or any diversity index) might be a poor predictor of genetic diversity of a member species, if not combined with the species compositional information about the community.

Conclusions

Based on the results presented in this thesis it may be concluded that positive correlations between species diversity (species number) of ecological communities and genetic diversity of a species may exist at the levels of within and between sites. From the conservation viewpoint this appears relieving, because it does not suggest a conflict between the promotion of high species diversity and high genetic diversity of a species.

The results of the present thesis indicate that the two levels of biodiversity, genetic and species diversity are largely influenced by habitat characteristics. Given the current trend for deterioration of the quality of natural and semi-natural habitats and increased similarity of habitats over whole landscapes (e.g. Vitousek 1994; Vergeer et al. 2003) this may suggest that diversity will decrease both at the genetic and species diversity level.

It also may be concluded that local selection processes have influenced the genetic diversity of *P. lanceolata* and *R. acris* populations. Habitat variability is correlated with apparent genetic differentiation between the local populations of the two species. This may suggest that highly diverse habitats offer more microhabitats in which different genotypes might be favored.

Future investigations

Much scientific work in how diversity regulates ecosystem function has been conducted in the last years (e.g. Tilman et al. 1996, McGrady-Steed *et al.* 1997, Naeem and Li 1997, Tilman et al. 1997, Hector et al. 1999, Loreau et al. 2002). Obviously, this appears to be due to the concern over the increasingly loss of biota because of the intensive land use and habitat fragmentation (e.g. Loreau et al. 2001) and their potential influence on ecosystem processes and functions (McGrady-Steed et al. 1997). However, much more studies are needed at other diversity levels such as genetic diversity and the effects on ecosystems processes.

In natural populations of a species many factors might influence the pattern of genetic diversity within and between populations. Therefore, to study the relationship between species diversity and genetic diversity independently of environmental variation,

this thesis suggests studying it in experiments with controlled manipulations of species diversity in otherwise common environments (e.g. Diemer et al. 1997; Roscher et al. 2004) with both molecular and quantitative genetic methods.

In the present study AFLP is used to study the genetic diversity of the three plants species. Despite the fact that this method is very powerful and offers high resolution compared with other genetic markers, it is considered to be selectively neutral thus making it difficult to conclude about to what degree selection pressures imposed by the variation in plant communities across sites is influencing the pattern of genetic diversity within plant species. Therefore, it would be interesting to test this interesting hypothesis with quantitative genetic methods (Storfer 1996) rather than with selectively neutral molecular markers.

The approach used in this thesis to estimate the genetic diversity and its relation to other diversity levels such as species diversity of plant communities is rather general and can be applied to any organism and possibly to other ecosystems once the genetic diversity is quantified.

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Summary

The thesis aimed to research the genetic diversity of three selected plant species (*Ranunculus acris*, *Plantago lanceolata* and *Anthoxanthum odoratum*). The genetic diversity of these species was quantified at the levels of within and between populations, and this variation was tested for correlation with the variations in plant communities and abiotic characteristics of grassland sites in central Germany. The results presented in this thesis are important to increase our knowledge in biodiversity research and also they are of importance for conservation biology.

In the three plant species studied, a high genetic diversity was found within and between local populations across different grassland sites. This indicates that diverse plants communities tend to contain more genetically diverse local populations of constituting species. This high genetic diversity within and between grassland sites may be attributed to the intrinsic characteristics of the studied plants (e.g. mating system and dispersal mechanism) and/or to the extrinsic characteristics of the habitats (e.g. large and not fragmented) at which the three species typically settled.

Moreover, the results indicate that the higher the species richness (number of species) of grassland communities, the higher the genetic diversity of *P. lanceolata* is, but no significant relationship was found between the genetic diversity of *R. acris* or *A. odoratum* and the grasslands species richness. This positive correlation between genetic diversity of *P. lanceolata* and species richness of grasslands seems to support the niche-variation hypothesis which suggest that high diverse habitats offer more niches and this in turn may influence the genetic diversity within a species which constitute communities. The absence of correlations between species richness and genetic diversity of both *R. acris* and *A. odoratum* might be due to the reduced statistical power, i.e. small number of populations studied, or it could be that such relationship does not exist in these two species.

It is also found in this thesis that abiotic characteristics of grassland sites affect the genetic diversity of *P. lanceolata*, but this was less evident in *R. acris* and *A. odoratum*. However, statistical analyses have shown that this influence of abiotic conditions on the genetic diversity is indirect through the effects on population size rather direct through niches. These results may be explained by the differences in selective forces associated with the variation in grassland communities.

Additionally, the genetic diversity, i.e. genetic differentiation, between the local populations of the three species was found to be small but significant. This significant genetic differentiation between populations was found to be correlated with variation in plant communities across sites and with the abiotic characteristics such as nutrient contents in the soil. The correlation was statistically significant in both *R. acris* and *P. lanceolata* but was not present in *A. odoratum*. These results could be explained by two processes: firstly, the variations in plant communities and abiotic factors may cause variation in selective forces and thus influence the pattern of genetic diversity between local populations of a species, and secondly, such ecological variation between sites might form a barrier against complete exchange of seed and pollen (gene flow) thus might lead to subdivision of the genetic diversity between sites.

Based on the results of the thesis it may be concluded that the association between habitat diversity and genetic diversity within a particular species clearly support a genetic-environment relationship. Both levels of diversity (genetic and species) seem to be influenced similarly by the habitat conditions. Given the current modification of habitat characteristics, through for instance the shift in land use and habitat fragmentation, this might suggest that both diversity levels will be influenced in the same manner.

Studies using experiments with controlled manipulations of species diversity in otherwise common environments, accompany with the use of other genetic markers such as quantitative genetic methods are recommended.

Zusammenfassung

Die Arbeit hat die Untersuchung der genetischen Diversität von drei ausgewählten Pflanzenarten (*Ranunculus acris*, *Plantago lanceolata* and *Anthoxanthum odoratum*) mittels molekularbiologischer Methoden (AFLP) und deren Zusammenhang zur phänotypischen Biodiversität (Artenkomposition) von Bergwiesen zum Ziel. Die genetische Diversität der drei jeweiligen Arten wurde innerhalb und zwischen verschiedenen Populationen quantifiziert. Weiterhin wurde die genetische Diversität dieser drei Arten auf Korrelationen mit der Variation in der Zusammensetzung der Pflanzengesellschaften und den abiotischen Faktoren der Wiesenflächen Mitteldeutschlands untersucht. Die Ergebnisse dieser Arbeit vermitteln ein tieferes Verständnis über die Rolle von Biodiversität und sind deshalb bedeutsam für den Schutz der biologischen Vielfalt.

In den drei untersuchten Pflanzenarten zeigt sich eine relativ hohe genetische Diversität zwischen und innerhalb lokaler Populationen beim Vergleich verschiedener Wiesenflächen. Dies deutet darauf hin, dass verschiedene Pflanzengesellschaften zur Aufrechterhaltung genetisch diverser lokaler Populationen von konstituierenden Arten tendieren. Die hohe genetische Diversität innerhalb und zwischen Wiesengesellschaften könnte auf die spezifischen Charakteristiken der untersuchten Pflanzen (z.B. Fortpflanzungs- und Verbreitungsmechanismen) und/oder die äusseren Standortfaktoren der Habitate, auf welchen die Pflanzen siedeln, zurückzuführen sein.

Die Ergebnisse zeigen, dass mit steigender Artenvielfalt in den Wiesengesellschaften sich die genetische Diversität von *P. lanceolata* erhöht. Andererseits ist das Verhältnis zwischen der Artenanzahl an Pflanzen in Wiesengesellschaften und der genetischen Diversität von *R. acris* und *A. odoratum* relativ schwach und auf dem 5%-Niveau nicht signifikant. Die positive Korrelation zwischen genetischer Diversität von *P. lanceolata* und der Artenvielfalt von Wiesengesellschaften scheint die Hypothese der Nischenvariation zu stützen, dass in hochdiversen Habitaten mehr Nischen zu Verfügung stehen, welche wiederum die genetische Diversität innerhalb der Anzahl von Arten, die diese Gesellschaft bilden, beeinflussen. Eine negative Beziehung könnte jedoch auch indirekt durch die bei höheren Artenzahlen tendenziell geringere Populationsgrösse entstehen. Andererseits könnte die geringe Anzahl an untersuchten Populationen die Ursache dafür sein, dass eine mögliche schwache Korrelation zwischen der Artenvielfalt und der genetischen Diversität von *R. acris* und *A. odoratum* noch nicht sicher nachgewiesen werden konnte (oder eine derartige Beziehung existiert für beide Arten überhaupt nicht).

Desweiteren hat sich in dieser Arbeit gezeigt, dass die abiotischen Bedingungen der untersuchten Flächen die genetische Diversität von *P. lanceolata* deutlich beeinflussen, jedoch die von *R. acris* und *A. odoratum* nur schwach. Statistische Analysen haben ergeben, dass der Einfluss der abiotischen Faktoren auf die genetische Diversität einzelner Arten eher indirekt über die Populationsgrösse wirkt. Diese Ergebnisse lassen sich durch die unterschiedlichen selektiven Kräfte erklären, die durch die Variation der Artenkomposition der Wiesengesellschaften entstehen.

Zusätzlich zeigt sich, dass die genetische Differenzierung zwischen den lokalen Populationen der Arten jeweils klein, aber signifikant ist. Das Muster der genetischen Differenzierung ist sowohl mit der Variation der Pflanzengesellschaften auf den

untersuchten Flächen als auch mit abiotischen Eigenschaften, wie der Verfügbarkeit von Bodennährstoffen, korreliert. Die Korrelation ist statistisch signifikant bei *R. acris* und *P. lanceolata*, jedoch nicht bei *A. odoratum*. Diese Ergebnisse können durch zwei Prozesse erklärt werden. Erstens, die Variationen in den Pflanzengesellschaften und abiotischen Faktoren könnten Variationen in der selektiven Kraft hervorrufen und somit das Muster der genetischen Diversität zwischen lokalen Populationen einer Art beeinflussen. Zweitens, eine derartige ökologische Variation zwischen den Flächen könnte eine Barriere gegen erfolgreichen Austausch von Samen und Pollen (Genfluss) bilden. Diese würde zur Unterteilung der genetischen Diversität zwischen den Flächen führen.

Basierend auf den Ergebnissen in meiner Arbeit kann man schliessen, dass die Assoziation zwischen Habitatdiversität und genetischer Diversität innerhalb ausgewählter Arten auf eine klare Genetik-Umwelt-Beziehung deuten. Dabei ist die Beziehung zu Habitatfaktoren und zur Artenzusammensetzung deutlich enger als zur Biodiversität schlechthin (d.h. zur Artenzahl oder einem Diversitätsindex). Beide Diversitätsebenen (genetisch und artspezifisch) scheinen in ähnlicher Weise durch die spezifischen Habitatbedingungen (Qualität und Quantität) beeinflusst zu werden. Gegenwärtige anthropogen bedingte Modifikationen der Habitatsmerkmale, wie zum Beispiel Veränderungen in der Landnutzung oder Habitatfragmentierung, würden - nach den Ergebnissen dieser Arbeit - beide Diversitätsebenen in gleicher Weise beeinflussen.

Künftige Studien, die Experimente mit einer gesteuerten Manipulation der Artendiversität in ansonsten gleichen Umweltbedingungen und auch quantitative genetische Methoden, wie genetische Marker, einsetzen, sind notwendig, um die Zusammenhänge besser zu klären.

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STATEMENT

I verify that the work presented in this thesis is all my own. Collaborations with others are mentioned below.

In all chapters I performed the field work and the genetic analyses, discussed the results with G Jetschke and FH Hellwig and wrote the chapters.

In chapter four I collected the samples, conducted the lab work, performed the analysis, discussed the results with G Jetschke, FH Hellwig, and M Fischer and wrote the paper.

In chapter five I collected the samples, conducted the lab work, performed the analysis, discussed the results with G Jetschke, FH Hellwig, and wrote the manuscript. Cooperation with A. Kahmen has been done by which he provided the data about the soil nutrients for this chapter.

September 27, 2004, Jena

Nidal Odat

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July 2004-present: Postdoctoral researcher, Department of Genetics and Evolution, MPI Jena, Max-Planck-Institute for Chemical Ecology in Jena.

April 2001–present: PhD Research, Institute of Ecology, Friedrich-Schiller-University, Jena, Germany. Within the framework of a scientific programme BIOLOG-Europe (Project for Biodiversity and Ecosystem Function of Grasslands). The programme is funded and established by the Federal Ministry of Education and Research (BMBF). <http://www2.uni-jena.de/biologie/ecology/biolog/diva.htm>. Duties: Determining and quantifying the genetic diversity among and between grassland populations at different spatial scales and different levels of biodiversity. Additionally, investigating the influence of grassland manipulation (artificial drought, insect removal) on genetic diversity in the field. Part of my work is to use Randomly Amplified Polymorphic DNA (RAPD-PCR, AFLP) as molecular techniques in order to achieve these objectives.

1999-2000: Volunteer Research and Diving Assistant, Marine Science Station, Iribid, Jordan. Duties: Oceanographic studies to study density currents including bathymetric chart, water sampling using nekton bottles, CTD measurements, small boat operation with outboard engine. Sediment sample and seawater collection for nutrient analysis, including silica, carbon, nitrogen analysis, seawater filtration. Zooplankton and phytoplankton

collection with nets. Underwater visual census of fish populations and transect laying. Aquaculture of tilapia and aquarium maintenance. Dissections of the sandbar shark, *Carcharhinus plumbeus* for removal of gonads, stomach contents and vertebrae for aging. Collection of scientific samples of corals and sponges. Fish larvae collection using light traps, fish trap collection.

1997-1999: Teaching and Laboratory assistant, Yarmouk University, Irbid, Jordan

Duties: Assisted the “Introduction to Biology” course as a teaching and laboratory assistant, preparation of teaching specimens including dissections, grading of student exams and laboratory assignments, invigilator for laboratory examinations.

1998-1999: Research assistant, Biology Dept., Biochemistry laboratory, Yarmouk University, Irbid, Jordan Duties: Isolation and Characterization of proteins from snakes using gas chromatography, ion-exchange chromatography, investigation of antibody development.

1996-1997: Biology Science Teacher, Hiteen Secondary School, Irbid, Jordan.

Duties: Teaching biological sciences curriculum to secondary school children.

1995-1996: Medical laboratory research assistant, Irbid Public Hospital, Irbid, Jordan

Duties: Trainee in medical laboratory analysis, Analysis of urine and blood samples, pregnancy testing, microbial analysis; food quality analysis.

PUBLICATIONS

Odat N (2003) Length-Weight Relationship of Fishes from Coral Reefs of the Coastline of Jordan, Gulf of Aqaba. *Naga* 26: 9-10.

Odat N, Jetschke G, Hellwig FH (2004) Genetic diversity in *Ranunculus acris* L. (Ranunculaceae) population in relation to species diversity and habitat type. *Molecular Ecology* 13: 1251–1257.

Odat N, Jetschke G, Fischer M, Hellwig FH (Submitted) On the relationship between plant species diversity and genetic diversity of *Plantago lanceolata* (Plantaginaceae) within and between 15 grasslands sites. *Molecular Ecology*.

Perner J, Wytrykush C, Kahmen A, Buchmann N, Egerer I, Creutzburg S, **Odat N**, Audorff V, Weisser W (In press) Effects of plant diversity, plant productivity and habitat parameters on arthropod abundance in montane European grasslands. *Ecography*.