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DIPLOMARBEIT

Titel der Diplomarbeit

Genetic patterns within and among populations of *Gypsophila fastigiata* subsp. *arenaria* (Caryophyllaceae) in the Western Pannonian region

angestrebter akademischer Grad

Magistra der Naturwissenschaften (Mag[a]. rer.nat.)

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Wien, im November 2008

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1 Abstract

Increasing agriculture lead to a major decease of formerly widespread dry grasslands and steppe areas in the Pannonian region. The highly endangered Pannonian *Gypsophila fastigiata* subsp. *arenaria* reaches its westernmost distribution range in eastern Austria, where the species was present only in one original population until two filial populations were founded in the course of a restoration project. The main objectives of the present study were to compare genetic diversity patterns among the original population and the newly established populations. Furthermore the diversity in adjacent populations and occurrences in the main distribution area were investigated as well as differentiation patterns among all populations. In addition the breeding system and demography were investigated in the Austrian populations. No loss of genetic diversity was found in newly established populations compared to the original population. However, a decrease of genetic diversity from the main distribution area of the species in the central Pannonian region towards the isolated populations in the west was observed. The investigated populations were clearly genetically separated according to their geographical distribution. Pollination experiments and pollen/ovule ratios revealed a mixed mating system in the species. Demographic observations showed a correlation between vegetation cover and seedling mortality. The study attests that restoration activities could preserve the original genetic variation in the newly founded Austrian populations.

2 Kurzfassung

Die Intensivierung der Landwirtschaft führte zu einer massiven Abnahme der früher weit verbreiteten Trockenrasen und Steppengebiete in der Pannonischen Region. Die stark gefährdete pannonische *Gypsophila fastigiata* subsp. *arenaria* erreicht in Ostösterreich mit einer einzigen Population ihre westlichste Verbreitungsgrenze. Im Zuge eines Rekultivierungsprogramms wurden in der Nähe der Originalpopulation zwei Filialpopulationen gegründet. Die Hauptziele der vorliegenden Arbeit waren, die genetischen Diversitätsmuster zwischen der Originalpopulation und den neu etablierten Populationen zu vergleichen und darüber hinaus die Diversität der benachbarten Populationen und der Vorkommen im Hauptverbreitungsgebiet, sowie die Differenzierungsmuster zwischen allen Populationen zu untersuchen. Zusätzlich wurden Fortpflanzungssysteme und Demographie der österreichischen Populationen untersucht. Verglichen mit der Originalpopulation konnte in den Filialpopulationen kein genetischer Diversitätsverlust festgestellt werden. Hingegen wurde eine Abnahme der genetischen Diversität von den Populationen der zentralpannonischen Region im Hauptverbreitungsgebiet in Richtung der isolierten Populationen im Westen beobachtet. Die untersuchten Populationen waren genetisch klar entsprechend ihrer geographischen Verteilung voneinander getrennt. Bestäubungs-Experimente und Pollen/Ovule Ratios ergaben ein Mixed Mating System der untersuchten Art. Demographische Beobachtungen zeigten eine Korrelation zwischen Vegetationsdecke und Keimlingssterblichkeit. Die Studie zeigt, dass die Erhaltungsmaßnahmen die ursprüngliche genetische Variation in den neu gegründeten österreichischen Populationen bewahren konnten.

3 Introduction

Dry grasslands and steppe areas of the Pannonian region are characterised by amazing species diversity. The fact that the Pannonian plain was recognised as an independent biogeographical region by the European Union in 2000, demonstrates its outstanding importance in Europe (Illyés et al. 2007). In Austria the westernmost occurrences of Pannonian steppe habitats can be found. The Pannonian flora province belongs to a European landscape that has been affected by man for the longest time and also most intensively (Buček et al. 2006). This anthropogenic impact on the one hand and the characteristics of steppe areas on the other hand form a very fragmented habitat. Especially plants requiring open habitats depend on traditionally managed grasslands or pastures. These habitats were previously widespread but have decreased dramatically over the last centuries. Thus, populations of many grassland species became disjunct and small, because the remaining semi-natural grasslands are becoming increasingly fragmented. Genetic structure and population dynamics of fragmented and small population systems, caused by anthropogenic disturbance is of high interest for evolutionary biology and conservation biology (Lönn & Prentice 2002; Manel et al. 2005).

The western border of the Pannonian region is found in Lower Austria. Due to this marginal position populations of many Pannonian species are rather isolated towards the west. These populations are affected by this margin effect and additionally by habitat fragmentation leading to isolation effects in comparison to the populations of the main distribution area. These effects find expression in decreasing population sizes, increasing spatial disjunction and genetic drift in the separated populations. Ecological genetics often deal with such interactions between large-scale geographic patterns and genetic dynamics among small, partially isolated and potentially locally adapted populations (Lowe et al. 2004). Genetic drift in small and isolated populations is expected to lead to a decrease in gene diversity (Nei et al. 1975), to a reduction of allelic richness within populations (Rich et al. 1979) and to an increase of the between-population component of diversity, i.e. to greater differentiation (Brakefield 1989).

The present study centres on *Gypsophila fastigiata* subsp. *arenaria* (Waldst. et Kit.) Domin, a perennial species bound to sandy, open habitats. Wether a rare species

becomes endangered depends on various attributes, such as mode of reproduction, dispersal ability, habitat specificity and structure of the intervening landscape (Wiens 1997). Due to habitat specification the species occupies a very disjunct area and is highly endangered in Europe. The taxon thus represents several Pannonian species reaching their westernmost distribution in Austria (e.g. *Dianthus serotinus*, *Pulsatilla pratensis*) that are affected by anthropogenic influence leading to habitat fragmentation and loss to various degrees. In Austria *Gypsophila fastigiata* subsp. *arenaria* originally occurred only in one population in a small nature reserve in Lassee, near the River March in Lower Austria. Quite recently two filial populations were founded near Lassee, one filial population in Erdpresshöhe in 2000, and another one in Windmühle in 2003 (details in Introduction 1.2).

Based on the fact that Amplified fragment length polymorphisms (AFLP; Vos et al. 1995) have become a standard method in investigations on population level, this technique was used to assess the genetic structure of the investigated populations. The greatest advantage of AFLPs is their high polymorphism due to dispersion of the loci over the whole genome requiring no prior sequence knowledge (Ridout & Donini 1999; Meudt & Clarke 2007), which gives this technique benefits over other techniques on population level. These obtained multi-locus fingerprints are highly reproducible, reliable and robust (Vos et al. 1995; Jones et al. 1997). The majority of AFLP applications have been used regarding genome mapping and breeding studies of intra-specific studies, although the application of AFLPs in ecological genetics is becoming widespread especially for studies of gene diversity, population structure and clonality (Robinson and Harris 2000).

Mating systems are a central element of the floral diversity of higher plants (Barrett 2003). The way of pollen transfer from the male to the female reproductive structures of the flowers is an integral part of mating systems. Determining whether a species is cross-pollinated or self-pollinated. Mixed mating systems, the ability of a plant for cross- and self-pollination, are common in many species to assure reproductive success, e.g. if pollinators are limited. In many species mating is regulated prezygotical, after pollination but before fertilization has taken place. The most important aspect of the mating system is the degree of genetic relatedness among mates, which determines the level of inbreeding.

The pollen-ovule (P/O) ratio developed by Cruden (1976) is one of several indicators, for the breeding system in flowering plants besides flower size and morphology. It reflects the likelihood of a pollen grain reaching the stigma. P/O ratios can be divided into five classes: xenogamy, facultative xenogamy, facultative autogamy, obligate autogamy and cleistogamy. The evolutionary shift from class to class is accompanied by a significant decrease in the mean P/O ratio. This pattern is found in anemophilous plants as well as in zoophilous plants. The more efficient the transfer of pollen the lower is the P/O ratio, thus cleistogamous flowers have the lowest P/O ratio whereas xenogamous flowers have the highest. An evolutionary shift from xenogamy to autogamy has been observed in several studies (Grant & Grant 1965; Erhardt & Jäggi 1995) and also has been accompanied by a decrease of P/O ratio (Jürgens et al. 2002). Autogamy is often indicated by decreased flower size and alterations in floral morphology, e.g. the absence of nectar, which reduces the energetic cost per flower. In general it is assumed that autogamy is an adaptation to disturbed habitats, xenogamy occurs in advanced successional stages and intermediate habitats lead to a balance between autogamy and xenogamy (Baker 1955; Cruden 1976, 2000). The “reproductive assurance” hypothesis states that the assurance of seed production lies in the selective advantage for self-pollination when pollinators are limited. This results in stable mixed mating systems where selfing rates depend on the level of pollen limitation (Baker 1955). Thus most systems employ several morphological features for pollinator attraction, genetic and physiological mechanisms to prevent self-pollen germination and/or pollen tube growth, e.g. herkogamy, dichogamy or self-incompatibility.

Inbreeding depression is the loss of fitness due to self- or within-family fertilization (Charlesworth & Charlesworth 1990) compared to out-crossed individuals. Self-compatibility can secondarily evolve, if the inbreeding depression is small enough, through the breakdown of a self-incompatible system. In large populations the self-incompatible-systems should not break down, in small populations the breakdown of self-incompatibility is more likely, especially after repeated bottlenecks of founder effects. Two subjects of fundamental importance to many areas in evolutionary biology are the effects of inbreeding within populations and outcrossing between different populations (Lynch 1991). Inbreeding may be a factor affecting the Austrian populations of *Gypsophila fastigiata* subsp. *arenaria* due to their spatial structure and

small size. As a result of the intense agricultural utilisation of the area around the small nature reserves, with great monocultures and a very low percentage of green corridors between the crop fields, the populations may suffer from reduced pollinator service.

Demographic processes, such as the change in population size and the transitions among age or stage classes and also habitat fragmentation clearly have an impact on genetic variation and thus genetic drift (Lawton-Rauh 2008). Oostermeijer et al. (2003) approached an integration of demography and genetics for understanding the important processes regarding population viability. A focus on genetics, reproductive ecology and on demographic vital rates is needed. Information on demographic processes provides an identification of critical stages in plant life cycles and this allows conclusions for optimizing conservation measurements (Bengtsson 2000; Lienert 2004).

The principle objectives of the study were to investigate in *Gypsophila fastigiata* subsp. *arenaria*:

(I) The impact of restoration measures on genetic diversity patterns in the original and the newly established populations and genetic differentiation among them.

(II) The diversity and differentiation between the Austrian populations, adjacent populations and populations in the main distribution area.

(III) Information on the pollination syndrome and the breeding system.

(IV) The key demographic processes in the Austrian populations with respect to different landscape characters.

3.1 The study site

The Pannonian Plains, named after the ancient Roman province Pannonia, are part of the Carpathian Basin that remained after the Pannonian Sea dried out in Central Europe. It is delimited by the Carpathians, the Alps, the Dinarides and the Balkan mountains. The core of the Pannonian biogeographic region is the great Hungarian Plain (Alföld).

The Pannonian plains in Eastern Austria belong to a special landscape with very warm and xeric climate that is significantly influenced by continental effects from the east and partly also by the sub-mediterranean climate from the south in the summer, and with rainfall maxima in cold winters (Buček et al. 2006). Due to these influences, markedly thermophilic plant and animal species occur here. Man in many ways has affected this landscape and thus the vegetation since the Neolithic ages. In accordance to geographic, macro- and microclimatic conditions thermophilic oak forests would form the natural vegetation, but due to human influence (i.e. agriculture, cattle grazing and hey harvesting) a secondary or tertiary steppe with dry grasslands and shrub lands could develop. Especially dry grasslands were kept open by cattle grazing, which created reminiscence of central Ukrainian and South Russian continental steppes in the vicinity of Vienna. Through many centuries or even millennia these grasslands were used for husbandry.

On a geological time scale, the dunes of Lower Austria are quite young. While wind-borne sand from the “Gänserndorfer Terrace” has been deposited during the last ice age, the sediments along the March river valley and the “Prater Terrace” developed post-glacially (Wiesbauer 2002). Over millennia, the sand layers were bound by vegetation, but clearing of the forests from the Middle Ages on enhanced eolian erosion and thus the formation and dynamics of the sand dunes. The formerly copious soil (Chernozems) that developed over several layers of loess, was eroded and the sandy ground exposed. Afforestations started under the control of empress Maria Theresia in the 18th century to curb the eolian sands. In the 20th century the beginning of the industrialisation of agriculture, led to a major decease of nutrient-poor grasslands in this region. Traditional husbandry was abandoned; the grasslands were drained, plugged and turned into crop-fields or used for settlements and

infrastructure purposes (Wiesbauer 2002). The March river valley in Austria has been used since the early Neolithic ages as agricultural land with increasing intensity towards the present. Only very few former characteristic landscape elements such as dunes, dry grasslands, marsh areas or saline habitats have remained.

In Middle-Europe sand dunes and wind-borne sand habitats are among the most endangered habitat types. According to the EU- Fauna-Flora-Habitat (FFH) guidelines, they are a “priority habitat” and are thus under special protection (Wiesbauer 2002). The wind-borne areas and sand dunes are among the most arid locations of the Austrian cultural landscapes, due to soil characteristics and the Pannonian influence on the climate. Predominantly drought-resistant plants, which survive extreme heat, strong radiation and the dynamic substrate, thrive in these areas. Many plants are capable of adapting to the arid habitat. Xerophytes are able to survive on small amounts of water by specific morphological and physiological features. Adaptations of xerophytes include reduced evaporation due to wax covering, thick covering of hair or cuticles, rolling up the leaves or even closing the stomata during the dry hot days. These adaptations protect the plants also from immense radiation, which in sandy areas comes from all directions, since open sand areas are reflectors (Albert 1997). Also the presence of thick and flat stems to store water, deep taproots or widespread fibrous roots prevents the plants from loss of water. Another strategy for survival is to shift the vegetative or generative phase to time periods in which higher soil moisture prevails.

Migrating dunes contain only small amounts of organic substance as a result of continuous shifting in the upper-most sediment zone, whereas in stabilised dunes soil can develop more or less. As organic matter mixes into the sandy soil, fixed dunes are colonised by more or less closed vegetation (Molnár et al. 2003). With rising fertility of the substrate and rising capacity of water-storage characteristic species of bare sandy grounds lose their habitats (Wiesbauer & Mazzucco 1999).

These western Pannonian grasslands form smaller patches than those of the central steppe zone in the east (Illyés et al. 2007). Open sand areas in the Pannonian region have decreased dramatically in the past decades, the characteristic grasslands of the sand areas are among the scarcest and most endangered types of vegetation in Central Europe. Often only small and poorly numbered populations of characteristic

species can be found (Wiesbauer & Mazzucco 1999). In 1927 the 'Weikendorfer Remise', a sand dune area in Lower Austria was the very first habitat under protection in Austria. The declaration of the nature reserve in Lassee followed in 1942 (Wiesbauer & Mazzucco 1997). Lassee covers a comparatively small area (1.38 ha) but in spite of the dimension it is a floristically very impressive location, first of all because here is the only natural occurrence of *Gypsophila fastigiata* subsp. *arenaria* in Austria.

3.2 The study species – taxonomy and distribution

The genus *Gypsophila* L. is a mainly Eurasian taxon and comprises 126 species. It occurs in the northern temperate part of the Old World, it is present from the Atlantic to the Pacific and is found mainly between the latitudes of 30° and 60°. There are some northern and southern outposts e.g. *Gypsophila fastigiata* reaches in Scandinavia latitudes of 69°. *Gypsophila* is a typical steppe element and more than a half of the species has a very limited geographic distribution. Most likely the genus *Gypsophila* originated in its recent main variation centre, the area of the Caucasus, the Transcaucasian region and East Turkey (Barkoudah 1962).

Gypsophila fastigiata s.l. is a diploid perennial plant with cushionlike rosettes and a thick, central taproot (Lönn & Prentice 2002). The species has linear to linear-spathulate shaped, greyish leaves and one to several inflorescences, forming thyrses, in the upper part of the stem. *Gypsophila fastigiata* s.l. is hermaphroditic and self-compatible, but predominantly outcrossing, it does not spread vegetatively (Bengtsson 2000). The number of inflorescences varies greatly from one up to 200 between individuals. Flowering starts in June and continues to September with a peak in July. Most seed capsules are ripe in late August.

Taxonomy distinguishes between two subspecies according to the different shape of the seed papillae, flower size and different hairiness in the type specimens. After Scheffer (1926), however, a clear differentiation can only be found in seed characters. According to this the seed testa sculpture in subsp. *fastigiata* has short and stump papillae, whereas in subsp. *arenaria* the papillae are long and cone-shaped. *Gypsophila fastigiata* s.l. is a characteristic element of the western Sarmatic

flora (Meusel & Mühlberg 1979) and reminds in this respect on *Dianthus arenarius*, *Pulsatilla pratenis*, *Sempervivum soboliferum* and *Astragalus arenarius*. According to Meusel & Mühlberg (1979), the taxonomic classification into two subspecies correlates with the chorological division of the distribution area (Fig 1). The main distribution area in northeastern Europe is occupied by *Gypsophila fastigiata* subsp. *fastigiata*, whereas subsp. *arenaria* (Fig 2 & 3) is found only in the Pannonian part of the area. However, the two subspecies are not generally accepted. In Stroh's 'Die Gattung *Gypsophila*' (1937), *Gypsophila arenaria,* as a synonym, is mentioned but

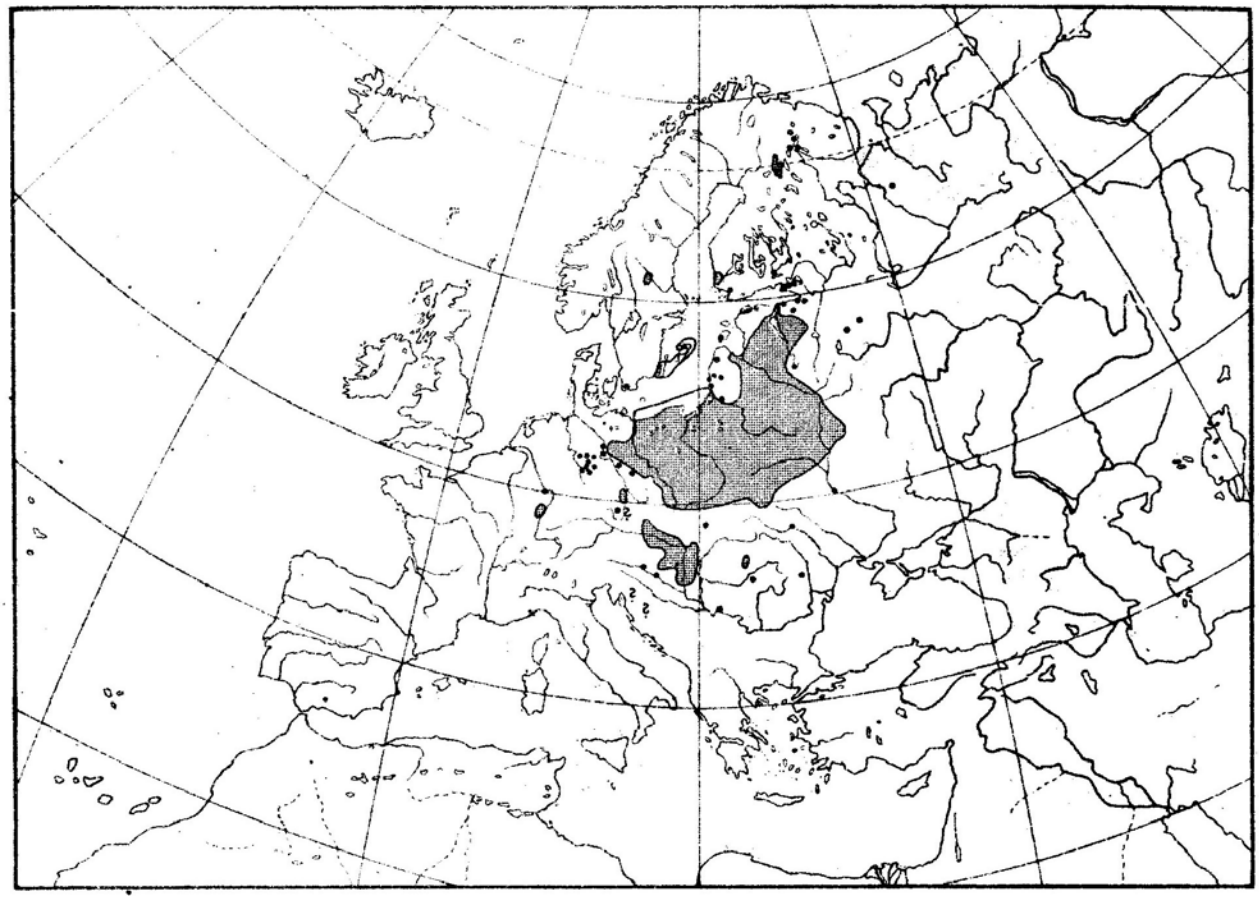

Fig 1: Distribution map (ex Jäger 1972), the two main occurrences describe the distribution of *Gypsophila fastigiata* subsp. *fastigiata* in the northern area and the distribution of *Gypsophila fastigiata* subsp. *arenaria* in the southern, Pannonian area.

integrated in *Gypsophila fastigiata*. In Barkoudah´s revision of the genus *Gypsophila* (1962) the subspecies are mentioned but "the differences are not pronounced and the two forms pass into each other in the central part of the area". In the Flora Europaea (Barkoudah et al. 1993) *Gypsophila fastigiata* subsp. *arenaria* is not accepted, as "the difference in size is the only character that remains stable in cultivation". The seed character is first mentioned in Scheffer (1926). Holub et al. (1971) supposed that the seed character is constant in the Pannonian region, but not

Fig 2: *Gypsophila fastigiata* subsp. *arenaria* in Erdpresshöhe, Lassee.

Fig 3: Flower and capsules of *Gypsophila fastigiata* subsp. *arenaria*.

in several German populations. The seed type of *Gypsophila fastigiata* subsp. *fastigiata* seems to occur constantly in more eastern and northern populations (Eastern Germany, Poland, Scandinavia). In the “Oesterreichische Exkursionsflora” (Fischer et al. 1994) *Gypsophila arenaria* is included in *Gypsophila fastigiata*, in the following issue (Fischer et al. 2005) *Gypsophila fastigiata* subsp. *arenaria* is mentioned as the taxon occurring in Austria.

In Austria *Gypsophila fastigiata* subsp. *arenaria* was originally present only in one small population in an extremely small nature reserve in Lassee in the Marchfeld, Lower Austria. The next populations are located in the Pannonian regions of the Czech Republic, Slovak Republic and in Hungary. The first Austrian herbarium records document one specimen from Angern an der March 1852 (Herbar des Schottenstifts) and another specimen from 1891 (‘bei Lassee’) collected by Mehart in herbarium W and several later records in herbarium WU published by Beck (1893) from Lassee ‘Obere Haide’ collected by A. Ginzberger and A. Teyber. The available information could indicate that the plant was more widespread around Lassee in former times; however, the notoriously imprecise information on old herbarium labels does not allow any final conclusion in this respect. The plant was even classified as probably extinct (“ausgerottet, ausgestorben oder verschollen”) by Niklfeld & Schratt-Ehrendorfer (1999), and found again by H. Wiesbauer in 1996 and independently by K. Mazzucco in 2001 (details in Greimler & Tremetsberger 2001), thus the species has been rediscovered for the scientific public.

Given the very small nature reserve and the low number of individuals of the original *Gypsophila* population both *in situ* (mowing, removing invaders) and *ex situ* measures (growing them in the Botanical Garden of the University Vienna, HBV) were initiated to preserve this population and to found a new population in a similar habitat in close vicinity in the year 2000 (Greimler & Tremetsberger 2001). These measures were continued by Wiesbauer (pers. comm.), who founded a second filial population in the framework of a restoration program in Windmühle near Lassee in 2003. The plants for both new populations were first grown in the HBV from seeds collected from the original population and transplanted in the following spring to their new sites. Additionally a proportion of seeds of the source population were sown out on one of the new sites. The germination success, however, was poor (Greimler & Tremetsberger 2001; Tremetsberger & Schönswetter 2002). The first filial population was founded on a sandy hill surrounded by an abandoned field about 1.5 km SE from

Lassee in Erdpresshöhe. This site represents an old stabilized sand dune where sand does not shift any more due to effective windbreaking vegetation surrounding the location. 850 seeds were sown in autumn 2000 and 43 juvenile plants transplanted in April 2001 of which 32 survived until November 2001. Another 43 individuals, from the same original seed collection were transplanted in 2002 (Tremetsberger & Schönswetter 2002). In 2001 invading *Solidago gigantea* was removed. Characteristic sand vegetation occurred only marginally before the conservation measurements have been launched. The second filial population was founded in the location Windmühle on sandy soil in a small nature reserve surrounded by arable fields about 2.5 km W of the original population. 106 plants of *Gypsophila fastigiata* subsp. *arenaria* were transplanted from the HBV in 2003, in 2004 only 29 individuals could be counted (F. Tod, pers. obs). In the meantime the two filial populations have developed well and produce many seedlings and juvenile plants. The plants grow widespread around the defined areas, where the transplants developed. In both newly founded sites the measurements obviously were successful: two populations could establish. A one-year demographic survey on the original site of Lassee showed that the selection of the locality in regards to vegetation cover was the most important factor for the success: No seedlings survived on sites with vegetation cover of 95%, whereas the seedling mortality declined to 94% on areas with vegetation cover of 80 %, and even to 88% on sites with 50% vegetation cover (Tremetsberger & Schönswetter 2002). On the newly founded sites open vegetation allows seedling survival and thus a regeneration of the population, whereas the original population in Lassee is strongly affected by the dense vegetation cover. In 2001 only about 30 individuals could be counted there (Greimler & Tremetsberger 2001). After initiation of conservation measurements such as mowing and partial opening of the area some seedlings survived. In 2007 about 64 flowering individuals were counted (pers. obs.).

The present study also attempts to gain insight in the effects of the conservation measurements in 2001 and 2002. For further conservation purposes the knowledge of more details, especially about the mating system (degree of outcrossing or selfing and data on pollinators), phenology and reproduction, genetic diversity and structure within the populations is needed. This is also true for the adjacent populations, to get insight in the edge effects.

4 Methods

4.1 AFLP

4.1.1 Sampling

For AFLP analysis, leaf material of *Gypsophila fastigiata* subsp. *arenaria* was collected and immediately dried in silica gel. Eleven populations were sampled (Tab 1, Fig 1) in total. Three Austrian populations and populations from the Czech Republic and Slovakia were sampled in the year 2007. Populations from Hungary were sampled in 2003. Seed samples from German *Gypsophila fastigiata* subsp. *fastigiata* were collected in 1999 by Josef Greimler and the plants raised in the botanical garden of the University of Vienna (HBV). Peter Schönswetter and Gerald Schneeweiss collected seeds from the original population in Lassee in 1999 for ex-situ cultivation in the HBV. Those juvenile plants were then transplanted to the Lassee sites (see details in 1.2). The silica gel dried material from the HBV material was collected in 2003. The sampled populations are representative for the distributional range of the species in the westernmost part of the Pannonian region. In the western part of Slovakia at seven locations near the River March, and at another location in the Czech Republic no individuals were found. The sample size, varied from nine to thirty-three individuals per population depending on the number of individuals found on those sites. From each sampled population one voucher was taken and deposited in the Herbarium of the Faculty Centre of Botany, University of Vienna (WU).

4.1.2 DNA isolation

DNA isolation followed the Cetyl-trimethylammonium bromide (CTAB) protocol by Doyle & Doyle (1987) with minor modifications. Total genomic DNA was extracted from comparable amounts (one and a half leaf) of grinded silica gel dried leaf material in 700 µl CTAB buffer (recipe for 1000 ml: 12.114g Tris ultrapure, 5.844 g EDTA-Ethylendiaminetetraacetic acid, 81.9 g NaCl, 20 g CTAB-N-Cetyl-N,N,N-trimethylammonium bromide, 40 g PVP-Polyvinylpyrrolidone, dissolved in 1000 ml with Aqua dest., dissolve at 60°C, before use 2% Mercaptoethanol were added, pH 8.0) for 30 min at 65°C. Then 500 µl chloroform/isoamylalcohol (24:1) were added and then put at 4 °C for 5 min. After centrifugation at 8.000 rpm for 2 min, the upper

clear phase was transferred to another tube and 350 µl isopropanol (2-propanole) were added. The cups were gently shaken and stored for 30 min at 4°C. The cups were spinned at 13.000 rpm for 2 min, and the supernatant removed. The DNA pellet was washed three times in 1 ml 70% ethanol, air-dried and resuspended in 100 µl TE-buffer with 4 µl RNAse (recipe for 500 ml TE-buffer: 605.7 mg Tris ultrapure, 146.1 mg EDTA, in 500 ml with Aqua dest, ph 8.0). The quality of the extractions was checked on a 1% TAE-agarose gel (1 µl Ethidiumbromide/50 ml TAE-agarose) in an electrophoresis chamber at 90V for 20 min and extractions were stored at -18°C.

4.1.3 AFLP fingerprinting

The AFLP procedure followed Vos et al. (1995) with some modifications according to Schönswetter et al. (2002). Genomic DNA was digested with two restriction endonucleases MseI (New England BioLabs) and EcoRI (Promega) and ligated to double-stranded EcoRI and MseI adapters in one step for 2.5 hours at 37°C and for half an hour at 17 C°. A master mix for the restriction-ligation step with 6 µl genomic DNA contained per sample: 1.2 µl 10x T4 buffer, 1.2 µl NaCl (0.5M), 0.6 µl BSA (1 mg/ml), 1 µl Mse-Adaptors (50µM), 1 µl Eco-Adaptors (5µM) (adaptor pairs were denatured at 95°C for 5 min before use), 0.02 µl MseI (50 Units/µl), 0.625 µl EcoRI (20 Units/µl), 0.2 µl Ligase (3 Units/µl) and 0.7175 µl ddH_2O. The restriction-ligation product was checked on a 1.0% TBE-agarose gel and then diluted 10-fold with ddH_2O.

The Master mix for the preselective amplification of 2 µl restriction-ligation product contained: 5.86 µl ddH_2O, 1.14 µl 10x Taq-Buffer, 0.20 µl RedTaq DNA Polymerase (Sigma-Aldrich), 0.22 µl dNTPs (10mM), 0.50 µl of each preselective primer C (MseI; 5 pmol/µl) and A (EcoRI; 5pmol/µl), The PCR program started with 72°C for 2 min followed by 25 cycles of 94°C for 1 sec, 56°C for 30 sec and 72°C for 2 min. The final step was an incubation at 60°C for 30 min. The preselective product was checked on a 1.0 TBE-agarose gel and diluted 10 times with ddH_2O.

Initial primer trials using twelve selective primer combinations were performed with eight individuals from three populations. Three primer combinations that gave clear and reproducible bands and showed variation within and between populations were chosen for selective amplification: EcoRI–ACA (FAM-blue)/ MseI–CA, EcoRI–AGG

(VIC-green)/ MseI–CT and EcoRI–AAC (NED-yellow)/ MseI–CT. The master mix for the selective amplification comprised: 5.5 µl ddH_2O, 1.0 µl 10x Taq-Buffer, 0.2 µl RedTaq, 0.22 µl dNTPs (10mM), 0.54 µl MseI-primer (5µM) and EcoRI-primer (1µM). The master mix was added to 2 µl diluted preselective product. Each plate of a given colour (primer combination) was always run on the same PCR-machine on the same position to avoid differences in the results induced by slight changes in temperature, due to differences between the PCR-machines.

The selective amplification started with a denaturation step at 94°C for 2min, followed by 65°C for 30 sec for annealing, followed by an extension temperature of 72°C for 2 min, repeated eight times where the denaturation step was only held for 1 sec and the annealing temperature decreased for 1°C at every cycle. During the remaining 23 cycles the annealing temperature was 56°C. The final step was at 60°C for 30 min. The incubation of the restriction-ligation as well as the polymerase chain reactions (PCRs) were performed on a GeneAmp® PCR System 9700 thermal cycler (AB Applied Biosystems, Foster City, California, USA). The fluorescence-labelled selective amplification products were then prepared for the detection in the capillary sequencer. The purifying procedure for the sequencer was performed according to following protocol: 200µl Sephadex were pipetted in each slot of a filter plate and centrifuged at 600 g for 1 min, this been repeated twice, then after the third addition of Sephadex centrifuged at 600 g for 5 min. Then the Sephadex columns were provided with 5µl of each colour of the selective product centrifuged at 600 g for 5 min. A master mix with an internal size standard for the detection was prepared with 10 µl GeneScan-500 ROX Size Standard (AB Applied Biosystems) and 980 µl HiDi (formamide) for one plate. 1.5 µl of the filtered and combined selective product were added to 10 µl master mix and spinned down at 1000 g. Afterwards a septum was applied and the samples were denatured for 2 min at 95°C and immediately cooled in ice water. The fragments were then separated on a capillary sequencer (AB 3130 xl, Applied Biosystems).

Raw data were collected and aligned with the internal size standard using GENESCAN Analysis software (PE Applied Biosystems). Subsequently the GENESCAN-files were analysed with GENOGRAPHER (version 12.0.0, Montana State University 2001; http://hordeum.msu.montana.edu/genographer/) for scoring the fragments. Each AFLP fragment was scored as present or absent using the ‘thumbnail’ option, which

allows a comparison of all signals over all samples. Fragments were scored between a range of 70 to 495 bp. Criteria for evaluating AFLP bands were visual clarity, straightforward interpretability, similar and total fluorescence intensity and reproducibility between independent replicates. Fragments that showed ambiguous peaks were excluded from the analysis; peaks of low intensity were included into the analysis if unambiguous scoring was possible (Terrab et al. 2008). Eight individuals were replicated on each plate to exclude non-repetitive bands. Four individuals respectively were additionally replicated within each plate as internal control to detect mistakes in the procedure. One blind sample as a negative control (no DNA) was included in every plate (Bonin et al. 2004) and showed no amplification. The reproducibility was generally high and the few non-reproducible fragments were omitted from further analyses. The scoring results were exported as a binary (1/0) matrix and used for further analyses.

4.1.4 AFLP data analysis

For each population the total number of fragments present (F_{pop}), the number and percentage of polymorphic fragments (F_{poly}) and the number of the average gene diversity (H_D) were calculated with ARLEQUIN 3.01 (Excoffier et al. 2005) and FAMD 1.08 (Schlüter & Harris 2006). Two additional diversity estimates, the number of fixed fragments (F_{fix}) and the number of private fragments (F_{priv}) were calculated. Analyses of molecular variance (AMOVA), which partitions the overall genetic variation into proportions of each level (within populations, among populations and among groups of populations) was performed with ARLEQUIN 3.01 as well. F_{ST} estimates were derived from the AMOVA. A neighbour-joining tree of all individuals based on the distance measure among all AFLP phenotypes by Nei & Li (1979) was constructed with PAUP (Swofford 2003), robustness of nodes was tested by using the bootstrap option with 1000 replicates. The tree was rooted with the next related taxon *Gypsophila fastigiata* subsp. *fastigiata*. The outgroup was then omitted from further analyses. A neighbour-net was conducted with SPLITTSTREE 4.8 (Hudson & Bryant 2006). A Principal Co-ordinate Analysis (PCoA) of populations based on the same matrix was calculated with the program NTSYS 2.0 (Rohlf 1997) and plotted with SIGMAPLOT 10.0. The overall population structure was analysed using BAPS 4.13 (Corander et al. 2003, 2004). BAPS was run with a different number of groups (K) set between 1-20. Each run was replicated 10 times. Admixture analysis was conducted

with the following settings: minimal size of clusters three individuals, 10000 iterations to estimate the admixture coefficients for the individuals, 50 simulated reference individuals from each population and 10 iterations to estimate the admixture coefficients for the reference individuals. As an alternative approach for genetic admixture analysis STRUCTURE 2.1 (Pritchard et al. 2000) was used. STRUCTURE performs model-based clustering based on a Bayesian Markov chain Monte Carlo (MCMC) approach, and estimates the likelihood of the data for user-defined K values (Ehrich et al. 2007, Latch et al. 2006). Ten runs for each K value ranging from one to ten and one million MCMC replicates (100000 additional replicates as burn-in) were performed. All STRUCTURE runs were carried out on the freely available Bioportal of the University of Oslo (www.bioportal.uio.no). Similarity among different runs was calculated using an R-script AFLPDAT (Ehrich 2006), to assess whether the clusters obtained from each K value were consistent. The K value with the highest likelihood was chosen. Because two numbers of groups appeared with the same likelihood, an additional similarity test for these groups was performed to exclude one of them. To assess the similarity of the outcomes of individual runs of the STRUCTURE analysis CLUMPP 1.1 (Cluster matching and Permutation Program) was used (Appendix I). The pairwise similarity was computed with ten runs and 1000 replicates with the algorithm termed 'Greedy' (Jakobsson et al. 2007).

4.2 Pollen/ovule (P/O) ratios

For the determination of pollen/ovule ratios (= P/O ratios; Cruden 1976) mature buds just before anthesis with still closed anthers were used. Buds were collected from three populations of *Gypsophila fastigiata* subsp. *arenaria* the original population in Lassee, one filial population (Erdpresshöhe) and one population from Slovakia (Partizanske) and preserved in 70% ethanol. One bud each from fifteen different individuals per population was collected for the estimation of P/O ratios. For measurement of the ovule number the ovaries were placed in a drop of water on a microscope slide, opened and ovules were counted under the dissecting microscope. From the same flower all pollen grains in one anther were counted and then multiplied by the number of anthers (flowers of *Gypsophila fastigiata* contain ten anthers). The anther was transferred on a slide as well and counted under a microscope. It was assumed that all ovules would develop into a seed if fertilization

occurred, thus no differentiation in ovule size was made. The bud was discarded, if it has been destroyed by grazers, which often especially in the Slovak samples occurred. Then the P/O ratios were calculated by dividing the estimated number of pollen grains per flower by the number of ovules per flower. A one-way analysis of variance (ANOVA) and an LSD-test (least significant difference) were performed for the P/O ratios of the three samples sites. All statistical calculations were performed with SPSS 10.

4.3 Pollination experiments

Selfing ability by autodeposition of pollen was tested, by bagging closed flowers at late budding stage, to exclude pollinator access. Bridal veil was used as bagging fabric, as it is said to have the least effect on the microclimate of the bagged flowers (Wyatt et al. 1992). The bags were tied with sewing thread. After the bagged flowers had withered the bags were removed.

Four treatments were used: (i) Unbagged and unmanipulated flowers were marked and served as a control group. (ii) Selfing ability by autodeposition was tested. (iii) Flowers were emasculated (the undehisced anthers were removed from the flowers) to prevent selfing and pollen from different individuals was manually applied to the recipient stigma. (iv) Artificially enhanced outcrossing was tested with non-emasculated flowers that where pollinated by hand before anther dehiscence. Except for the control group all flowers were bagged to exclude pollinators and grazers.

4.4 Demography

The demographic survey started in autumn 2007 after establishment of five plots (1.0 m^2) on two different sites of the filial population in Lassee Windmühle. (The installation of a site at the original population in Lassee was impossible due to early mowing in this year.) The sites were chosen to represent two different mowing regimes; two plots were located on a very open site with no mowing, three plots on a vegetationally denser but regularly mowed site. At each census a woody frame covered by a 10 cm grid of thin wire was fitted over the marked plot corners and a map of all sites was drawn. Individuals were classified according to size. The six stage-categories were: stage 0: seedlings, stage 1: unbranched juveniles, stage 2:

branched juveniles, stage 3: adults, leaf rosette smaller than 5 cm, stage 4: adults, leaf rosette larger than 5 cm, stage 5: adults, leaf rosette larger than 10 cm.

At each census the number of inflorescences and infructescences per individual were counted. In order to investigate the relations between microhabitat and demographic parameters three habitat parameters were recorded. The parameters were: vascular plant cover (%), bryophyte and lichen cover (%) and cover of dead leaves and litter (%). To be able to identify the seedlings of *Gypsophila fastigiata* subsp. *arenaria* in the field, seeds were germinated in Petri dishes. On this occasion it was observed that the germination was enhanced when the seeds were covered with sand. In general, germination of all seeds was high. Nearly 90 % of all seeds germinated.

4.5 Seed weight

In order to compare another metric character seed weights of two different Austrian populations (original population Lassee and filial population Erdpresshöhe) were measured. Fifty seeds per population were weighted with a Mettler AE 260 Delta range balance. Seeds from other populations could not be included as those sites were visited for collection of DNA samples during flowering season to facilitate the search. A significance test and a non-parametric statistic analysis were applied to the seed weight results using SPSS 10.

Tab 1: The investigated populations with sampling details, genetic data and estimates.

ID	Pop	Location Name	Country	Year	N	Coordinates (E/N)	$Frag_{pop}$	$Frag_{poly}$ (%)	$Frag_{fix}$	$Frag_{priv}$	$H_D \pm SD$
L	1	Lassee	A	2007	33	16.855°/48.216°	268	170 (35.49)	179	1	0.126 ± 0.07
LA	2	Lassee HBV	A	2003	12	16.855°/48.216°	272	238 (49.69)	154	0	0.145 ± 0.07
E	3	Erdpresshöhe	A	2007	25	16.870°/48.210°	265	213 (44.47)	158	0	0.136 ± 0.07
W	4	Windmühle	A	2007	25	16.821°/48.211°	264	196 (40.92)	166	0	0.128 ± 0.06
TA	5	Tatarszentgyörgy	H	2003	17	19.319°/47.048°	266	252 (52.61)	146	3	0.180 ± 0.09
TB	6	Tatarszentgyörgy	H	2003	18	19.325°/47.049°	271	293 (61.17)	132	3	0.202 ± 0.10
N	7	Nagyszentjanos	H	2003	10	17.865°/47.704°	275	260 (54.28)	149	6	0.206 ± 0.11
C	8	Cenkov	Sk	2007	9	18.522°/47.768°	274	229 (47.81)	158	1	0.188 ± 0.10
P	9	Partiszánske	Sk	2007	28	18.451°/48.643°	269	248 (50.77)	136	2	0.152 ± 0.07
S	10	Sokolnice	Cz	2007	21	16.769°/49.113°	262	230 (48.02)	147	2	0.142 ± 0,07
K	11	Kyffhäuser	Ger	2003	10	11.103°/51.365°	250	213 (44.47)	143	6	0.171± 0.09

Pop: population number; $Frag_{pop}$: mean number of AFLP fragments per population; $Frag_{poly}$: number of polymorphic AFLP fragments per population; $Frag_{fix}$: number of fixed fragments per population; $Frag_{priv}$: number of private AFLP fragments per population; $H_D \pm$ SD: average gene diversity ± standard deviation; N: number of individuals analysed.

5 Results

5.1 AFLP analysis

Analysis of AFLP (Tab 1) fragments of all three primer combinations of 208 individuals of all populations of *Gypsophila fastigiata* subsp. *arenaria* (Fig 4) yielded a total of 479 unambiguously scorable fragments, of which 405 (84.5%) were polymorphic. The length of the fragments ranged from 71 to 495 bp. The primer combinations EcoRI(Fam)-ACA/MseI-CA yielded 147 fragments, EcoRI(Vic)-AGG/MseI-CT 149 fragments and EcoRI(Ned)-AAC/MseI-CT 183 fragments. The mean number of fragments per population ranged from 250 in population Kyffhäuser to 275 in population Nagyszentjanos. (mean $Frag_{pop}$ = 267; SD = 11.40; Tab 1). The number of polymorphic fragments ranged from 170 (35.49%) in the original Austrian population to 293 (61.17%) in a Hungarian population from Tatarszentgyörgy (mean $Frag_{poly}$ = 250; SD = 20.50; Tab 1).

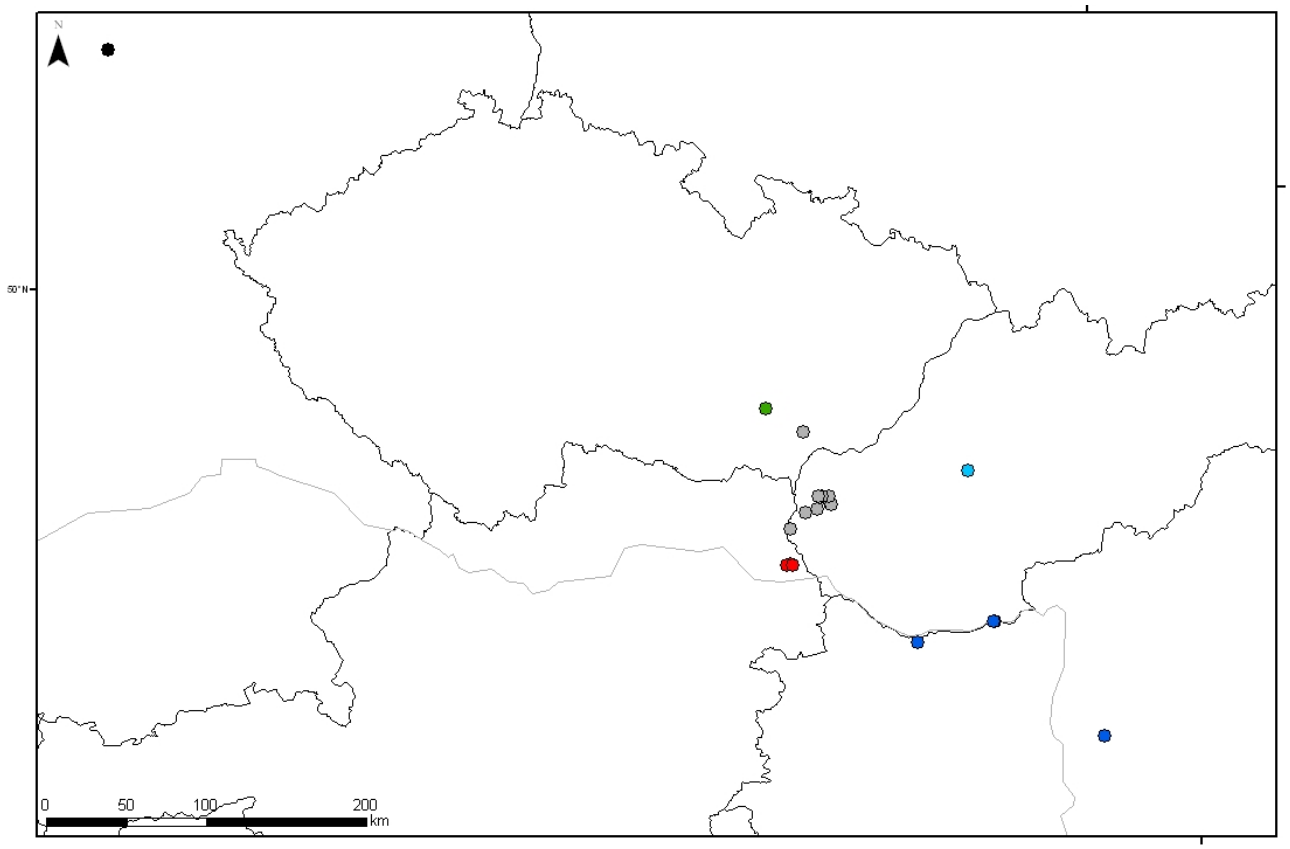

Fig 4: Sampled populations of *Gypsophila fastigiata* subsp. *arenaria*. Red dots indicate Austrian, green dots the Czech, blue dots the Slovak and Hungarian populations (pale blue the central Slovak, dark blue the southern Slovak and the Hungarian populations); the black dot illustrates the studied German population of *Gypsophila fastigiata* subsp. *fastigiata*. Grey dots show sites where no individuals were found (see Appendix II). The colouring refers to the PCoA results (see below).

5.1.1 Similarity patterns

The unrooted neighbour-joining tree of all populations (Fig 5) revealed six clusters essentially separating the Austrian populations, the population from the Czech Republic, the central Slovak population and the southern Slovak population forming two clusters, with the Hungarian populations in between and the German population on a longer branch. In the neighbour-joining tree, rooted with *Gypsophila fastigiata* subsp. *fastigiata* as outgroup, (Fig 6) the hierarchical structure became somewhat clearer, however, in the bootstrap consensus tree (Fig 7) only the outgroup was clearly separated with 100% bootstrap support from all other samples. The ingroup formed a huge polytomy consisting of the Hungarian samples mixed with the southern Slovak samples and three groups that are well separated by bootstrap supports over 80 %. Being part of the polytomy these groups are: the Austrian populations (84%), the central Slovak population (85%) and the Czech population (98%).

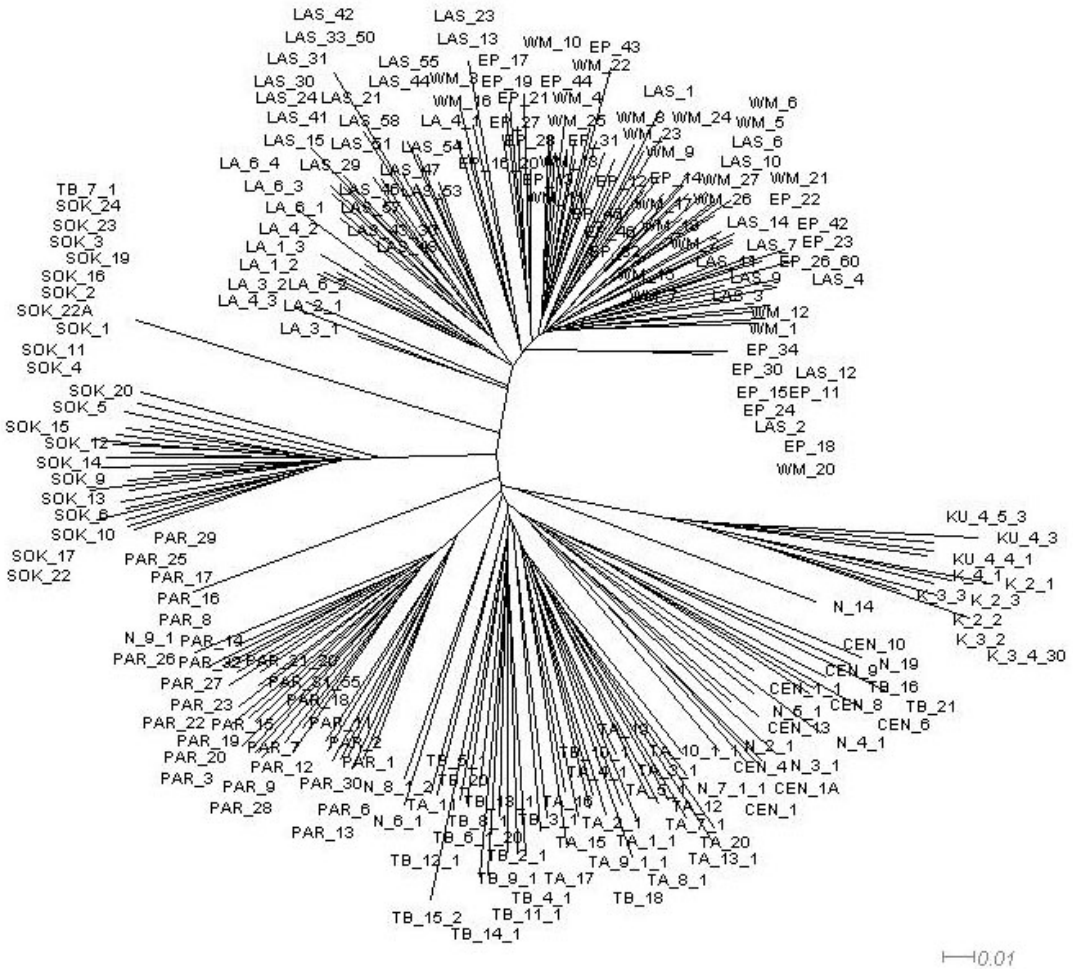

Fig 5: Unrooted phylogram of neighbour-joining analysis based on Nei & Li distances.

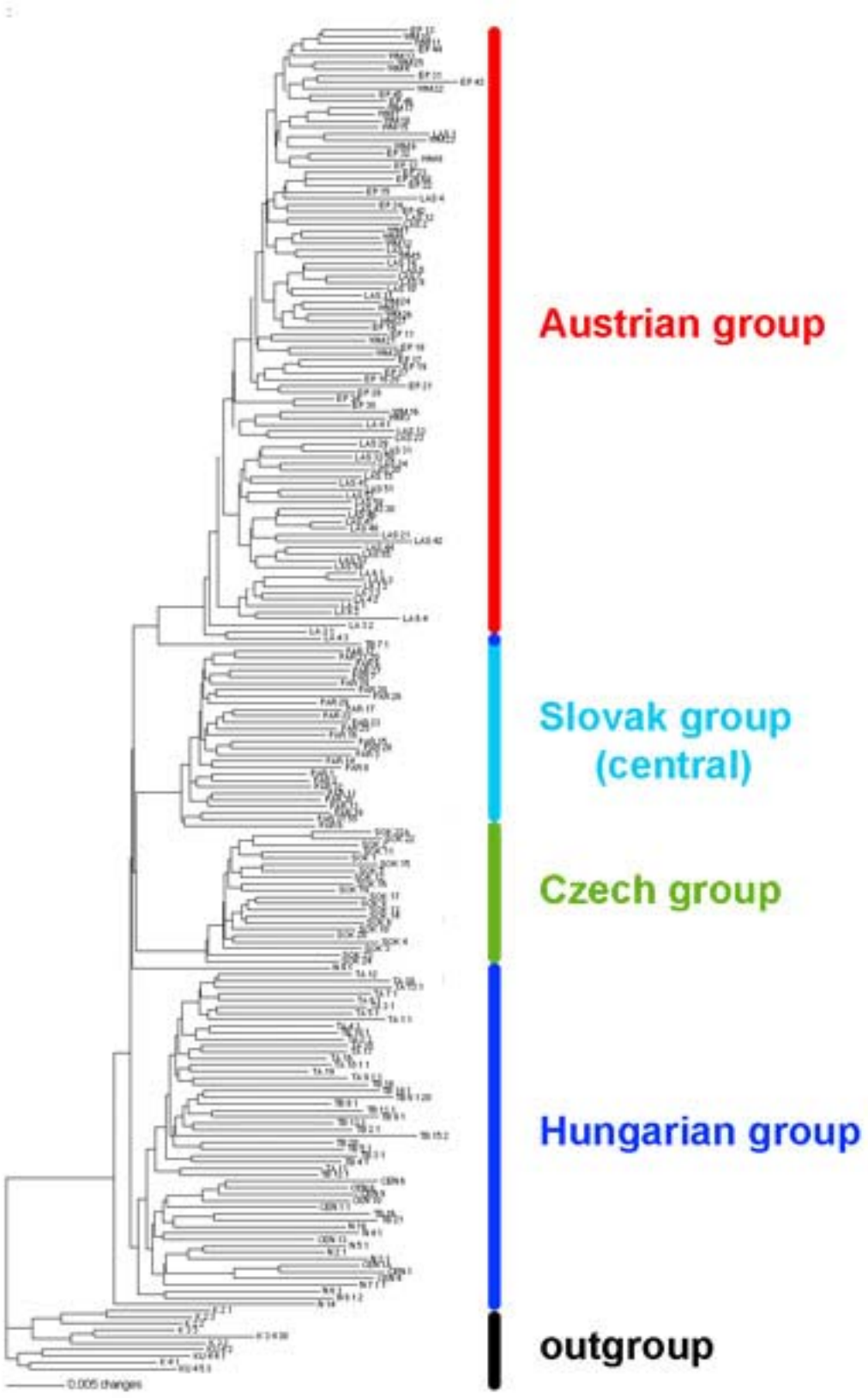

Fig 6: Rooted neighbour-joining tree, without bootstrap values.

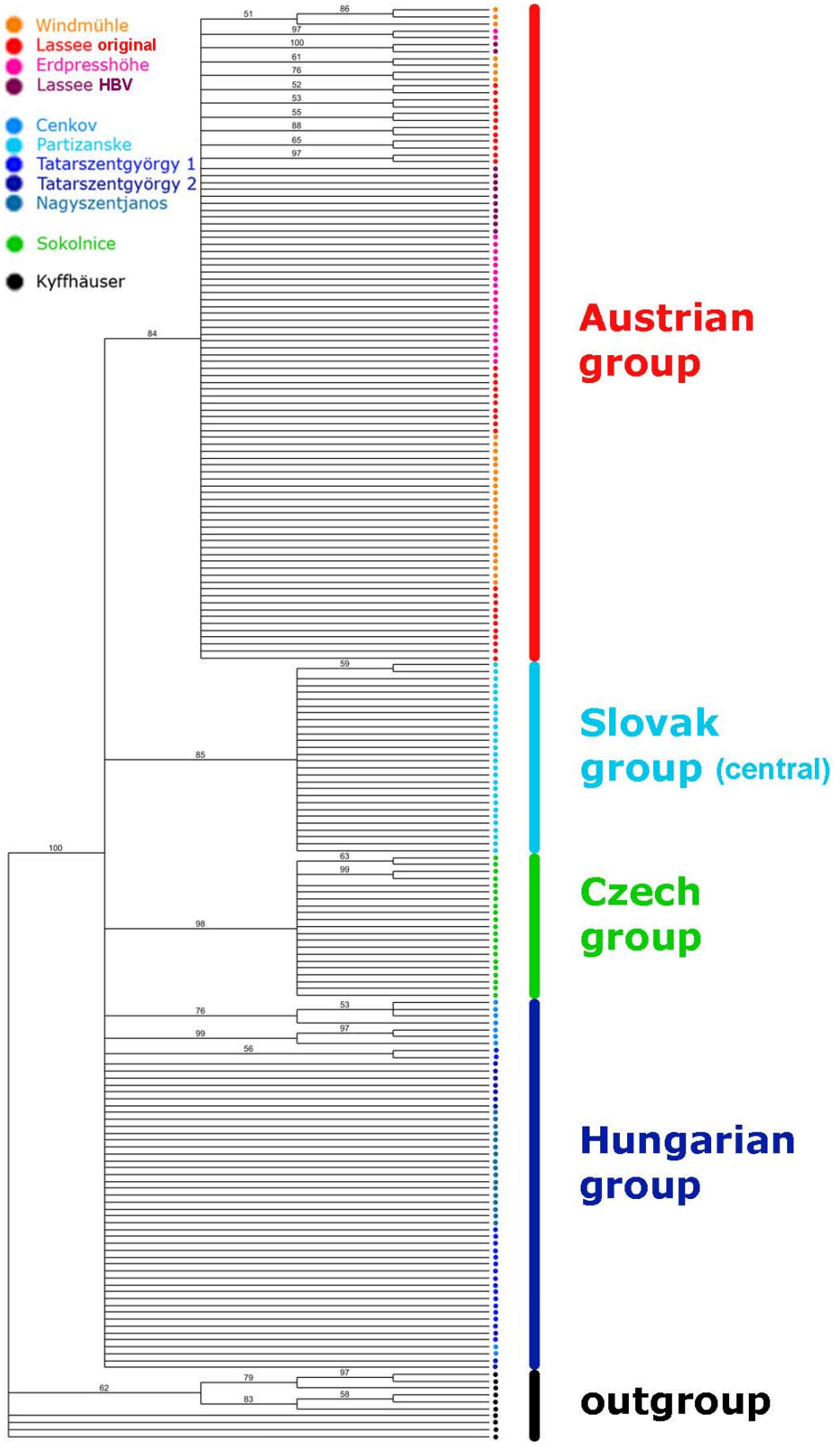

Fig 7: Neighbour-joining tree rooted with *Gypsophila fastigiata* subsp. *fastigiata*. Bootstrap support is indicated above the nodes.

The neighbour-net analysis (shown in Appendix III) revealed the same patterns as the neighbour-joining analysis.

The Principal Coordinate Analysis (PCoA) (Fig 8) of the AFLP data set gave a mostly similar pattern. It reflected the genetic distance according to the geographical distribution (Fig 4). The first two axes explained only 5.53% and 2.14% of the total variation respectively, but separated the three geographically distinct groups clearly, where the Slovak and the Hungarian populations fell together in one cloud, separated from the Austrian and the Czech groups.

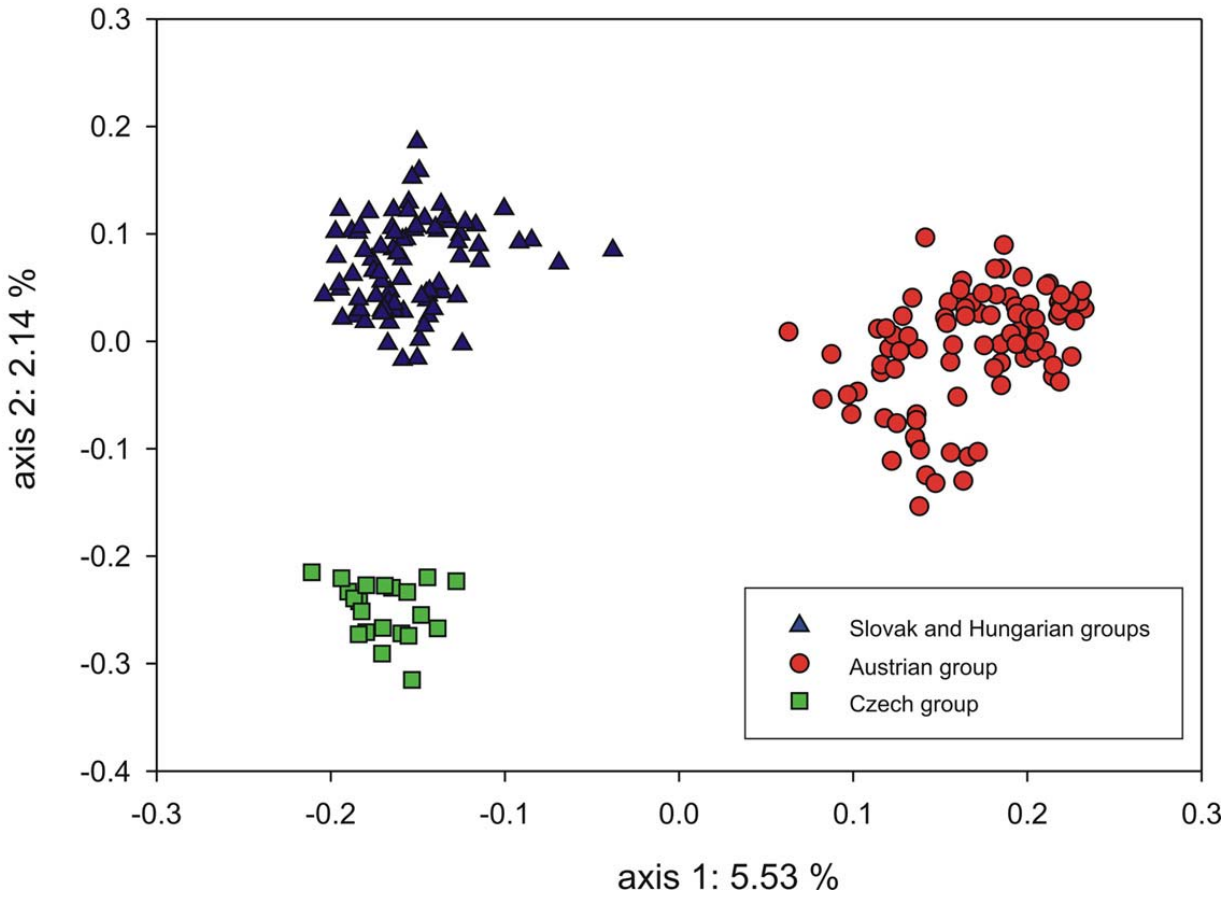

Fig 8: Plot of the first two axes from the Principal Coordinate Analyses (PCoA) of the 198 individuals of *Gypsophila fastigiata* subsp. *arenaria*. The percentage of variation explained by each axis is indicated**.**

5.1.2 Genetic mixture and admixture

Bayesian clustering with a data set of 198 individuals (Fig 9) conducted with BAPS resulted in a best partitioning into four clusters with log marginal likelihood of -27189 at P=1. All individuals sampled in one region were assigned to the same cluster. The following clusters were detected in a mixture analysis: Cluster I: all four Austrian populations (1-4); Cluster II: the three Hungarian populations (5-7) and the population from southern Slovakia (8); Cluster III: the central Slovak population (9); and Cluster IV: the Czech population. The admixture analysis detected few cases of admixture. Only four individuals throughout the whole set of populations show admixture from different genetic clusters; all populations are more or less homogeneous within themselves. The four individuals showing admixture are sample 22 from the original population in Lassee, Austria (p-values: cluster I: 0.8, cluster II: 0.05, cluster III: 0.07, cluster IV: 0.08); sample 41 from HBV, Austria (p-values: cluster I: 0.8, cluster II: 0.20); sample 138 from Nagyszentjanos, Hungary (p-values: cluster I: 0.20, cluster II: 0.62, cluster III: 0.17, cluster IV: 0.01) and sample 141 from Cenkov, southern Slovakia (p-values: cluster I: 0.08, cluster II: 0.52, cluster III: 0.34, cluster IV: 0.06).

Genetic mixture analysis conducted with STRUCTURE (Fig 10) confirmed the regional structure as found by BAPS. All STRUCTURE runs performed with K = 4 showed consistent results. Runs with K = 5 showed the same likelihood, but the average similarity between runs differed and thus these results were not considered (Appendix I). A permutation analysis performed with CLUMPP showed the highest value of H (average pairwise similarity) at 0.999. The STRUCTURE results show more cases of admixture than the BAPS results. The Austrian group from the original Lassee population (samples 1-33) and the ex-situ samples from HBV (samples 34-45) showed genetic admixture of all other clusters, the populations from Erdpresshöhe and Windmühle showed little admixture. The Hungarian group contained the greatest proportion of different gene pools, whereas the populations from central Slovakia and the Czech Republic hardly show any admixture. Individual proportions of admixture are shown in Appendix IV.

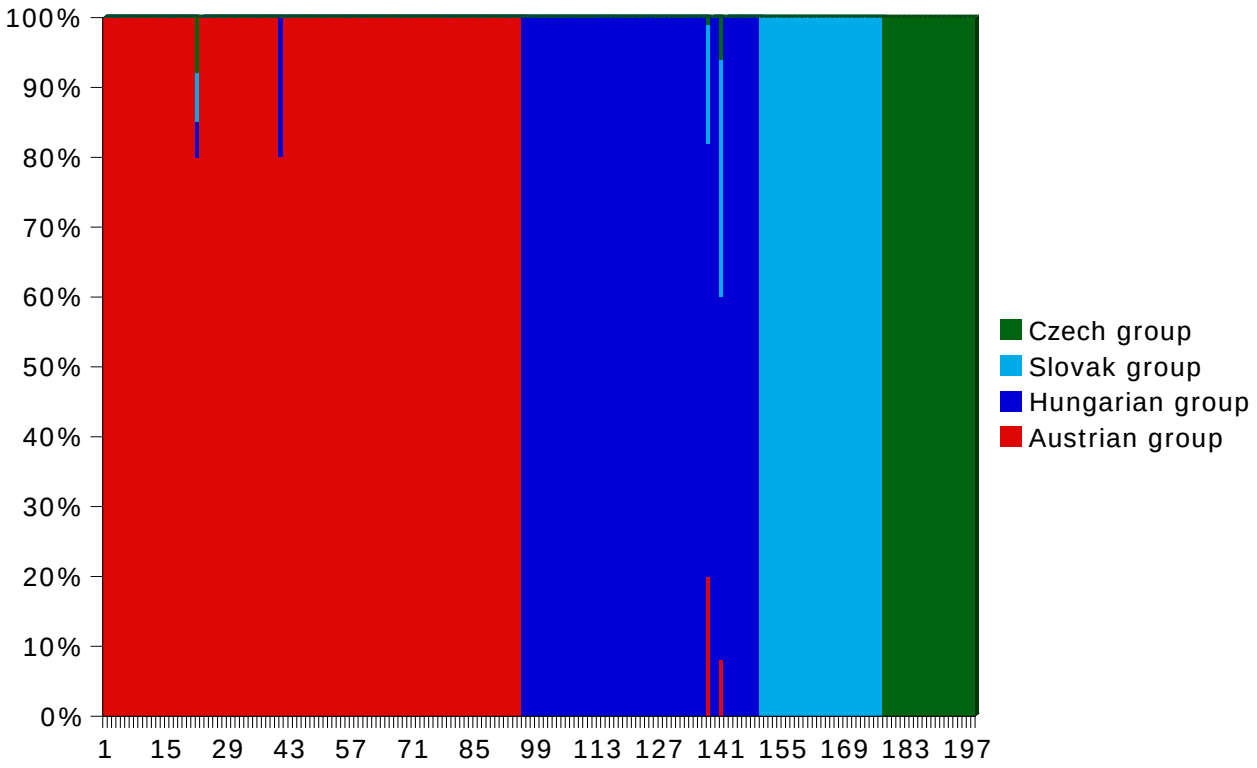

Fig 9: BAPS based clusters with admixture patterns, colours correspond to the regional clusters I-IV from bottom up as explained in the legend.

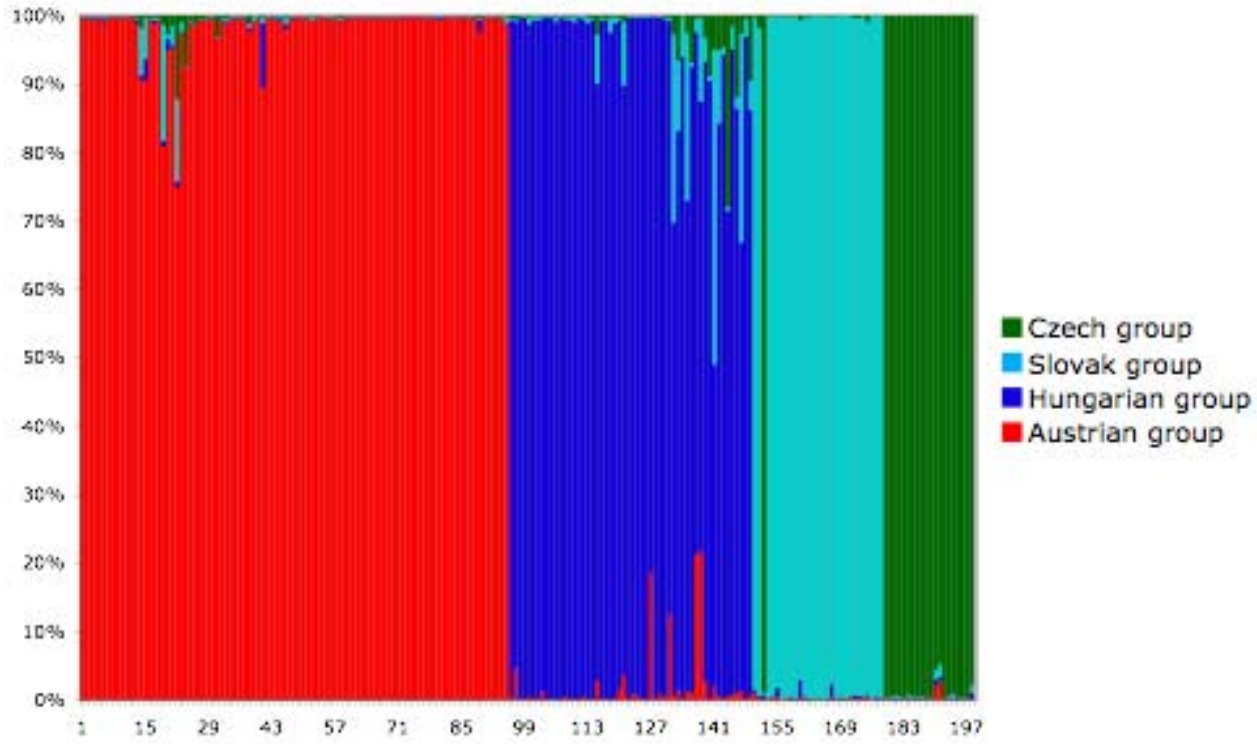

Fig 10: STRUCTURE based clusters with admixture patterns, colours correspond to the clusters I-IV from bottom up as explained in the legend.

5.1.3 Molecular variance and within population diversity

A non-hierarchical analysis of molecular variance (AMOVA) revealed that most of the overall genetic variation among all ten populations was explained by the component of variation within populations (72.57%) (Tab 2). The remaining component of variation among populations provided an F_{ST} estimate of 0.27, which indicates a rather high differentiation among populations. This F_{ST} changes slightly to 0.26 when only the original population from Austria is included. A hierarchical AMOVA according to the geographically distinct groups as revealed by the PCoA explained 24.75% of the variation among groups and the variation within populations of 68.09%. If only one Austrian population (Lassee) is included in the data set for the AMOVA of the geographical groups the variation among groups shifts to 18.20%; the variation within the populations to 71.64%. If only the Austrian populations are taken into account the variance within populations was 91.65%, and provided an F_{ST}- estimate of 0.08.

The average gene diversity over loci (H_D) for each population shown in Tab 1 varied between 0.126 (± 0.07) in the original Lassee population and 0.206 (± 0.11) in the Hungarian population from Nagyszentjanos. The Hungarian populations in general showed the highest gene diversity, followed by the Slovak ones, whereas the Austrian populations showed the lowest gene diversity values. The decrease of gene diversity from the Southeast to the Northwest is shown in Fig 11 and Fig 12.

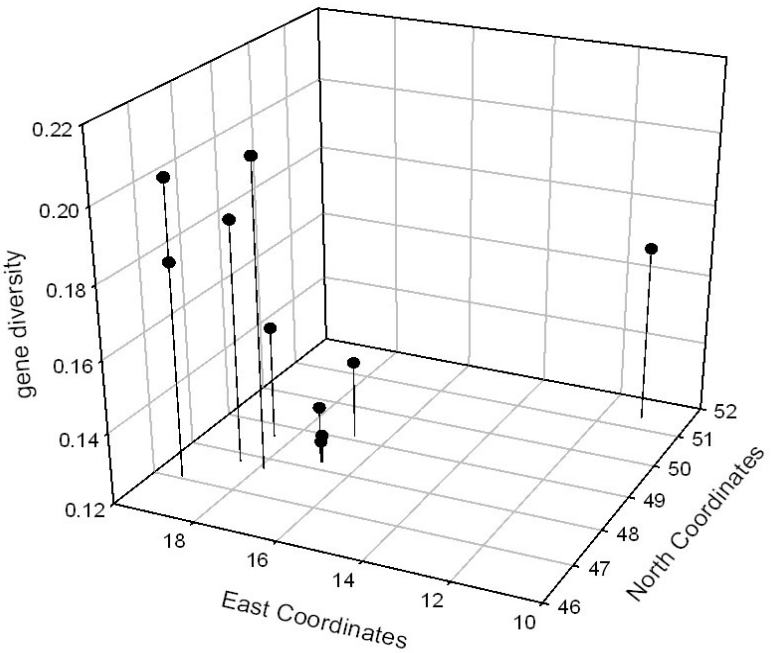

Fig 11: Average gene diversity over loci plotted against the geographical distribution.

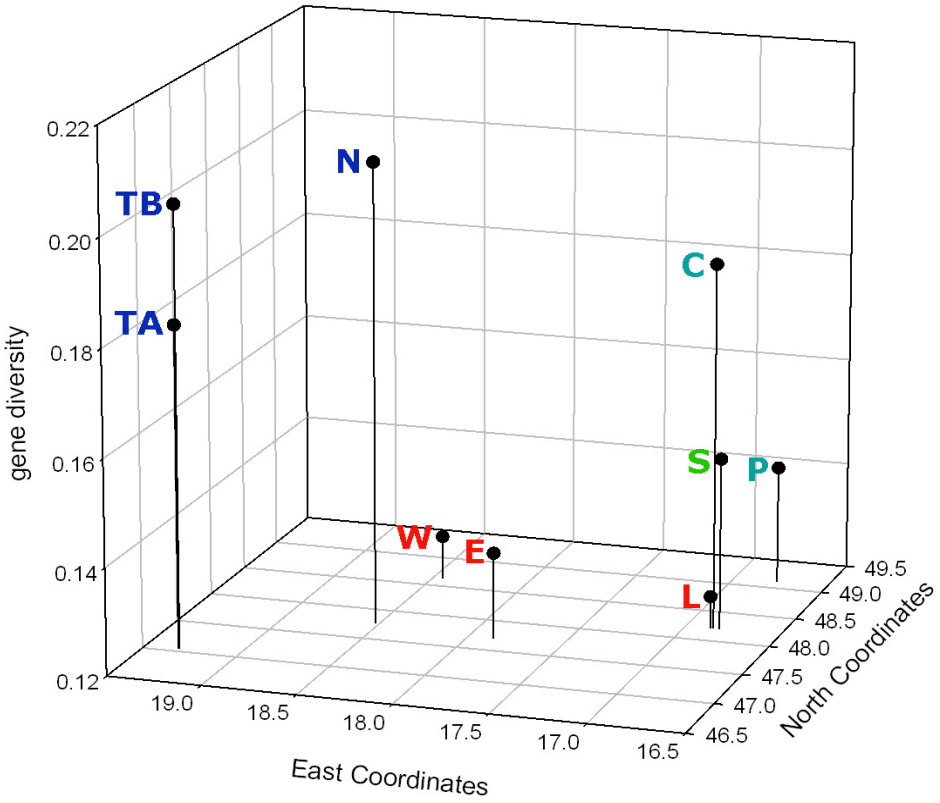

Fig 12: Average gene diversity over loci plotted against the geographical distribution, population from Kyffhäuser excluded.

Tab 2: Analyses of molecular variance (AMOVA) of 10 investigated populations of *Gypsophila fastigiata* subsp. *arenaria* (Population Kyffhäuser excluded). ** all P-values were < 0.001.

	Source of variation	d.f.	Sum of squares	Variance components	% Total variance **
All populations without regional groups	Among populations	9	2779.86	13.96	27.43
	Within population	188	6945.55	36.94	72.57
Four groups according to PCoA	Among groups	3	2137.87	13.43	24.75
	Among populations within groups	6	661.98	3.88	7.16
	Within populations	196	6945.55	36.94	68.09
One Austrian population and all others	Among populations non-hierarchical	6	1849.02	14.25	26.51
	Within populations non-hierarchical	129	5094.76	39.49	73.49
Four groups according to PCoA (only one Austrian population)	Among groups	3	1510.55	10.03	18.20
	Among populations within groups	3	338.48	5.60	10.16
	Within populations	129	5094.76	39.49	71.64
only Lassee populations	Among populations	3	303.51	2.99	8.40
	Within populations	91	2964.43	32.58	91.60

5.2 Reproductive Features

5.2.1 Pollen/Ovule (P/O) ratios

The P/O ratios of the two Austrian populations (the original Lassee population and the filial population Erdpresshöhe) result in 438 and 436 (Tab 3). According to Cruden's classification of breeding systems (1976) (Tab 7), the values lie between facultative autogamy and facultative xenogamy. The P/O ratio of the Slovak population of 494 is higher than that of the Austrian ones but lies still within the two breeding systems (Tab 3). Statistical results of the P/O ratio analyses are shown in Tab 4.

Tab 3: Results of the P/O ratios of the three measured populations.

	Lassee NSG (A)		**Erdpresshöhe (A)**		**Partizanske (Sk)**	
buds	ovules	pollen	ovules	pollen	ovules	pollen
1	14	5950	12	5640	13	6490
2	7	5770	7	5410	12	7010
3	13	6460	14	6090	12	7390
4	16	5750	15	5930	13	7900
5	14	5250	16	7350	13	6850
6	15	6120	15	5680	12	5290
7	16	5950	16	6520	14	5370
8	13	4860	16	7130	12	6560
9	14	5590	13	4100	14	7090
10	16	4520	13	6610	15	9640
11	14	6670	15	6730	16	6640
12	15	6690	15	4700	12	5030
13	15	5360	17	5640	14	5820
14	10	5620	11	5920	15	4810
15	14	5950	16	5290	13	6310
mean	13.73	5767.33	14,07	5916.00	13.33	6546.67
SD	± 2.32	± 590.85	± 2.49	± 844.68	± 1.25	± 1199.71
P/O ratios	**438.86**		**436.72**		**494.00**	
SD	**± 125.83**		**± 116.01**		**± 94.55**	

Tab 4: Statistics of the P/O ratios.

	N	mean	Std.Dev.	Std.Err.	95%-confidence interval for mean		min.	max.
					lower bound	upper bound		
Partisanzke (Sk)	15	494.00	94.55	24.41	441.64	546.36	320.67	642.67
Lassee (A)	15	438.86	125.83	32.49	369.18	508.54	282.50	824.29
Erdpressh.(A)	15	436.72	116.01	29.95	372.47	500.96	313.33	772.86
total	45	456.52	113.50	16.92	422.42	490.62	282.50	824.29

Tab 5: One-way ANOVA (Analysis of variance).

ANOVA	sum of squares	df	mean square	F	significance
among groups	31628.54	2	15814.27	1.24	0.30
within groups	535238.24	42	12743.77		
total	566866.78	44			

Tab 6: Pairwise comparisons, LSD-test (Least significant difference).

(I) pop No.	(J) pop No.	mean difference (I-J)	Std.Err.	significance	95%-confidence interval	
					lower bound	upper bound
1	2	55,14	41,22	0,19	-28,05	138,33
	3	57,28	41,22	0,17	-25,91	140,47
2	1	-55,14	41,22	0,19	-138,33	28,05
	3	2,14	41,22	0,96	-81,05	85,33
3	1	-57,28	41,22	0,17	-140,47	25,91
	2	-2,14	41,22	0,96	-85,33	81,05

Tab 7: P/O ratios according to breeding system after Cruden (1976).

breeding system	pollen/ovule ratio
cleistogamy	4.7 ± 0.7
obligate autogamy	27.7 ± 3.1
facultative autogamy	168.5 ± 22.1
facultative xenogamy	796.6 ± 87.7
xenogamy	5859.2 ± 936.5

The one-way ANOVA (Tab 5) did not show significant differences in the P/O ratios between the Austrian and the Slovak populations. Also the LSD-test (Least significant difference) (Tab 6), an equivalent to t-test, showed no significant difference between the P/O ratios.

5.2.2 Pollination-Experiments

Due to the extreme harsh weather conditions in 2007 (a very hot summer with several days over 36°C, heavy rain showers and thunderstorms with hail), most experiments failed. The bride veil made the bagged flowers more vulnerable because

of larger surface and less flexibility, which destroyed the treated buds or broke them off during the hail. Only the control group with no bags stayed completely unharmed by the weather. A small amount of the treated flowers survived, which makes conclusions for this experiments very difficult.

After counting out the remaining flowers some seeds were found in every treatment (Tab 8, Tab 9) In some cases it was impossible to distinguish between unfertilized ovules and fertilized ovules that have stopped their development due to the above-mentioned reasons. Therefore the data set of ovules may contain cases of such not fully developed ovules; thus the number of ovules may also contain some seeds that were stopped in their development, when the bud broke off. I assume that some of these ovules that were found in all groups may represent undeveloped seeds.

Tab 8: Results of the pollination experiments in the original population of Lassee. Dashes indicate cases with no distinguishable structures.

Lassee (original population)			
CONTROL 0			
individuals	flowers	seeds	ovules
1	1	0	11
2	1	0	14
3	0	0	-
4	1	0	-
5	1	0	13
6	1	2	-
EMASCULATED E			
bags	flowers	seeds	ovules
1	1	0	12
2	2	0	14
3	0	0	-
4	1	2	-
5	1	0	14
6	1	0	-
7	1	0	14
8	1	0	13
9	1	0	-
SELF-POLLINATED S			
bags	flowers	seeds	ovules
1	1	0	-
2	1	0	9
3	0	0	-
4	1	0	-
5	1	0	-

Tab 9: Results of the pollination experiments from site Erdpresshöhe (filial population).

Lassee Erdpresshöhe			
CONTROL 0			
individuals	flowers	seeds	ovules
1	3	15	-
2	1	3	-
3	1	4	-
4	0	0	-
5	12	1,2,3,1,3	7
6	7	0	7
7	1	0	14
8	1	4	-
9	0	0	-
EMASCULATED E			
bags	flowers	seeds	ovules
1	1	0	14
2	1	0	13
3	1	0	9
4	1	0	15
5	1	0	14
6	2	0	13
7	1	0	8
8	0	0	-
SELF-POLLINATED S			
bags	flowers	seeds	ovules
1	1	0	13
2	1	0	16
3	0	0	-
4	1	0	15
5	7	2	-
6	1	0	14
7	1	0	16
ENHANCED OUTCROSSING F			
bags	flowers	seeds	ovules
1	1	12	0
2	0	0	0
3	1	14	0
4	1	4	0
5	3	12,13,13	0

5.2.3 Seed weight

The seed weight measurement of 50 mature seeds per population showed a significant difference (significance conducted with t-test: 0.0067) between the populations Lassee (0.524 ± 0.102 mg) and filial population Erdpresshöhe (0.458 ± 0.134 mg). However, because of non-normal data distribution, non-parametric statistics have been applied additionally. This analysis provided the same median value of 0.5 for both populations (Details are shown in Tab 10). The seed weight range of population Erdpresshöhe is higher than that of population Lassee, which is also expressed in the percentiles of the values and the interquartile ranges in Fig 13.

Tab 10: Non-parametric statistics of seed weights (mg).

	Lassee	**Erdpresshöhe**
Median	0.5	0.5
minimum	0.3	0.2
maximum	0.7	0.8
range	0.4	0.6
interquartil range	0.1	0.2
Percentiles		
25	0.5	0.4
75	0.6	0.6

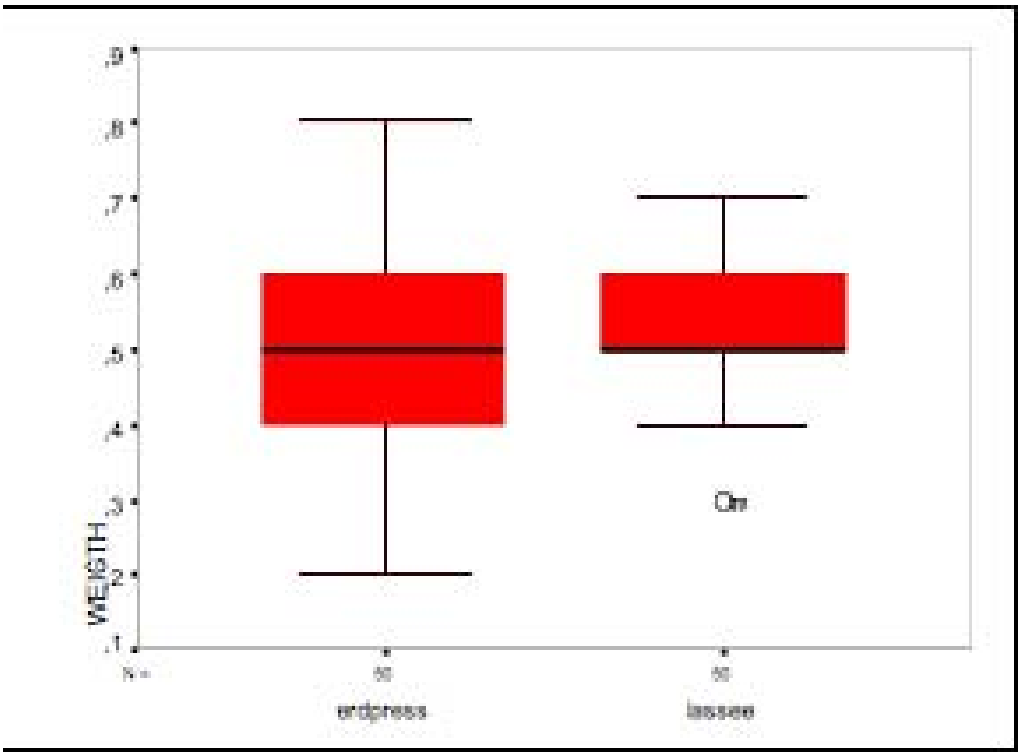

Fig 13: Distribution of the seed weight values (mg) in the two Austrian populations. Vertical bars show the range (circle indicates outliner), boxes include observations between 25 and 75 percentiles, and horizontal bold bars indicate medians.

5.3 Demographic investigations

The demographic survey conducted over 1.5 years on the site Erdpresshöhe provided little interpretable results due to the short observation during the study. But some results according to the different mowing regimes could be revealed. The vegetation cover varied between the two different categories of sites (mowed and un-mowed plots). The mowed plots (1-3) showed a higher amount of different species and an overall higher percentage of covering by lichens, bryophytes and vascular plants than un-mowed plots (4-5). In mowed plots more seedlings were detected and the highest number of vegetative plants was found (plot 3). The highest number of individuals with inflorescences from the last year was also found in the mowed plots. The highest number of individuals with inflorescences from this year appeared in plot 2 and 4. Seedling mortality was independent from the mowing regime. For details see Appendix V.

6 Discussion

6.1 Genetic variation and genetic structure

The principle objective of this study was to investigate the genetic constitution of the Austrian original population of *Gypsophila fastigiata* subsp. *arenaria* in Lassee and the two newly founded filial populations that have been established in context of a restoration project (Greimler & Tremetsberger 2001; Wiesbauer, personal communication). The emphasis was on assessing the consequences of the applied methods of establishment on possible loss of genetic diversity in the two new populations. A further focus of the present study was on the differentiation between the Austrian populations and adjacent populations, especially from the main distribution area in Hungary and Slovakia.

The diversity values of the three Austrian populations were on a similar level, however, the filial populations showed somewhat higher values and possessed a higher amount of polymorphic fragments. The expected diversity loss in the filial populations was surprisingly not confirmed. This indicates obviously, that the restoration efforts by founding the two new populations, i.e. transplanting juvenile plants gained from seeds and additionally sowing seeds of the original population, provided a sufficiently large sample of the gene pool. The slightly higher diversity values of the new populations may indicate a better mixture of the local gene pool due to seed collection from several individuals. The new generations established from seeds and probably their progeny provide further novel genotypes due to recombination. On the site of the original Lassee population basically old individuals occur, whereas in both filial populations individuals of all development stages are numerous. The obsolescence of the original populations could have lead to strong directional selection. The Lassee original population showed the lowest percentage of polymorphic fragments and the lowest genetic diversity. Genetic depauperation decreases the adaptive potential, which is critical for small populations if environmental changes occur. In the filial populations the sexual component is probably higher due to higher plant densities and thus a higher proportion of pollinator visits. In general the conclusion can be made that slightly higher diversity values of the quite young filial populations are due to the higher fitness (i.e. more

offspring) of these populations in comparison to the original population. The genetic diversity of the original population has been preserved in the filial populations.

Between the Austrian populations hardly any structure could be found, which is not surprising after only five to eight years since their establishment. The original population from Lassee and the other filial populations from Erdpresshöhe and Windmühle do not form distinct groups. Part of the individuals from the original Lassee population clustered somewhat together in the neighbour-joining analysis, however, part of them appeared mixed with the individuals from the filial populations. The low differentiation among them in terms of F_{ST} (0.08) confirms this pattern. Whether the three Austrian populations are in actual contact via gene flow cannot sufficiently be answered. Gene flow may be mostly due to pollinators, because gene flow by seeds is extremely restricted. Seed dispersal occurs mostly within a radius of 20 cm from maternal individuals (Bengtsson 2000). The moderate distance could allow pollen transfer among the populations, but on the other hand the present landscape structure makes gene flow unlikely (Holderegger & Wagner 2008).

The Hungarian populations showed the highest average gene diversity over loci, most polymorphic bands and also most private bands. The peripheral Austrian populations, on the contrary showed the lowest gene diversity. Since the Austrian populations are the westernmost populations of the whole geographic distribution range of the species, the genetic depauperation in this marginal population is not particularly surprising. Disjunct populations that occupy a peripheral position show a greater degree of genetic isolation than more central populations (Lönn & Prentice 2002). So a genetic depauperation from eastern sites to the western and northern marginal populations was observed (Fig 12). Central populations are open to gene flow from all directions, whereas gene flow in peripheral populations is directionally constrained (Prober & Brown 1994). Only one private fragment occurred in the original Austrian population, the filial populations did not show private fragments, whereas all the other populations had at least one but mainly three or six.

The neighbour-joining analysis as well as the PCoA showed a genetic separation of the geographically distinct groups. The computed trees (Fig 5, 6 and 7) revealed a clear differentiation between the Austrian and the other investigated populations by a bootstrap support above 80%. The Czech population from Sokolnice and the central

Slovak population from Partizanske as well as the Hungarian populations including the southern Slovak population from Cenkov were clearly separated from the Austrian populations. The southern Slovak population from Cenkov clearly fell together with the Hungarian populations. The fact that the southern most Slovak population clusters within the Hungarian group is not surprising, since the distance to the next Hungarian site is much smaller than to the next observed central Slovak population (Fig 4). However, the Cenkov population showed the lowest genetic distance to the population from Nagyszentjanos, although it is geographically closer to the populations from Tatarszentgyörgy. The Austrian populations showed highest geographical and genetic distances to the Hungarian populations. The geographical distances between the investigated populations are relatively high, at least 50 kilometres, which makes actual gene flow between them unlikely. AMOVA derived F_{ST} values (0.27 between all populations, 0.26 if only one Austrian population is considered in the analysis) are relatively high, suggesting a high differentiation between the groups and thus infrequent gene flow among the populations.

Genetic drift, the change in allelic frequencies due to random sampling from generation to generation (Hartl & Clark 1997), can be enhanced at the margins of the distribution range. Small as well as large populations are affected by genetic drift, but the effects are more severe in small populations due to neutral genetic diversity loss (Linert 2004). The original Austrian population as well as the Czech and the central Slovak population are obviously isolated. Generally the low genetic diversity values favour this hypothesis. AFLPs as dominant markers, however, do not provide information on heterozygosity. The original Austrian population with rather old plants and presumably no sufficient regeneration in comparison to the two filial populations may be strongly affected by genetic drift or selection. The filial populations showing slightly higher genetic diversity values are as well affected but due to increased sexual reproduction and larger population size more heterozygous gene pools are likely.

The admixture analysis conducted with both programs STRUCTURE and BAPS resulted in the same non-hierarchical clustering, however, in different admixture patterns. The greatest confidence in results is obtained when results are achieved independently by two different methods to search for the most likely number of clusters, especially if differentiation among groups is small (Latch et al. 2006). Both programs computed a

best likelihood for partitioning of four clusters, but BAPS (ver. 4.13) revealed only four individuals with admixture from different genetic clusters, whereas STRUCTURE showed some admixture in nearly every individual. The four individuals appearing in the BAPS analysis occurred also in the STRUCTURE analysis but not with the same p-values (Appendix IV). STRUCTURE showed additional individuals with even more admixture, though the four individuals were present. BAPS obviously identified admixture only in highly significant cases (over 20%), whereas STRUCTURE displayed all significant and non-significant admixture proportions. This, however, does not explain the different outcome of the four individuals revealed by BAPS compared to the STRUCTURE results for these individuals. STRUCTURE revealed an admixture proportion over 20 % in eight individuals, six of them belonging to the Hungarian cluster, which is congruent with the results from the genetic diversity analysis, where the Hungarian populations showed the highest diversity in comparison to the other populations. The Austrian populations, where the other two individuals with significant admixture belong to, showed proportions of all other gene pools. The highest admixture percentages came from the Slovak cluster, followed by the Czech cluster; both populations are very isolated and show thus low admixture values within their clusters. Admixture from the Hungarian cluster was also found in the Austrian populations but is comparatively low, which can be explained by the geographical distance to these populations. Notably admixture in the Austrian populations occurred mainly in the original Lassee population and the ex-situ samples from HBV, whereas the filial populations showed barely any admixture, possibly due to the genotypes in better agreement with Hardy-Weinberg equilibrium.

6.2 Reproductive features

The P/O ratios provided clear evidence for a facultative xenogamy. Autogamy is common in Caryophyllaceae (Jürgens et al. 2001), but *Gypsophila fastigiata* s.l. has protandrous flowers, a feature to avoid self-pollination. The flowers offer nectar and the anthers are coloured pink which also leads to the conclusion that the species invests in attraction of pollinators. Previous studies in *Gypsophila fastigiata* subsp. *fastigiata* showed that the species is insect pollinated and primarily outcrossing (Lönn et al. 1995). The pollination experiments showed seed set in every treatment, which points to a mixed mating system. Most seeds were found in the control group, due to the partly massive intervention in the differently treated flowers this was no surprise.

The emasculated flowers showed seed sets though the treatment was very invasive. During the removal of the anthers of the small buds it was difficult not to hurt the remaining organs. The results of the emasculated flowers are for sure also affected by the method. Self-pollinated flowers on the other hand were not harshly treated but due to the protandry of the species the pollination of the stigma with the pollen from the same flower could not be ensured totally. Although a lot of self-pollen was brought on the stigma wind could have blown away the pollen from there even if the flowers were caged. The same is true for the enhanced outcrossing (without emasculation), where pollen from other individuals was brought on the stigma. It is unclear whether the pollination took place from foreign pollen or from the later distributed self-pollen.

Considering that some of the counted ovules would have developed to a seed, if not stopped by braking off due to weather conditions leads to following conclusion: The pollination experiments in connection with morphology and results of the P/O ratios indicate a mixed mating in the species that is able to produce seeds by selfing but tends to favour outcrossing. According to Cruden (1976) this would be facultative xenogamy. The P/O-ratios of the three analysed populations did not show significant differences in any pairwise comparison between the Austrian and the Slovak populations, although the Slovak population showed a slightly higher P/O-ratio value. Caryophyllaceae with two styles (*Gypsophila* species) have in general less pollen grains and ovules than species with five styles (Jürgens et al. 2001). It has been demonstrated that pollination is directly affected by habitat fragmentation due to reduction of species diversity and abundance of pollinators (Steffan & Tscharntke 1997). Fragmented sites are visited more than 50% less frequently by pollinators than larger homogenous sites. Pollinators also change their behaviour in more fragmented localities, which reduces pollen dispersal among flowers and thus increases inbreeding and hence reduction of genetic variability (Goverde et al. 2002). In *Silene* (Caryophyllaceae) species it was observed that selfing occurs in older protandrous flowers, if pollinator frequency is low, because the anthers still bearing pollen move downwards and come in contact with the mature style (Jürgens et al. 2001).

Whether inbreeding is a factor in the Austrian populations is difficult to attest with AFLP data, that give no information about heterozygosity (Falush et al. 2007), but the

original Austrian population from Lassee is a very small one and it has obviously been isolated for a long time (Barkoudah 1962; Janchen 1977; Meusel & Mühlberg 1971). In small populations inbreeding depression is higher than in large and panmictic ones (Glemin et al. 2001). Isolated and small populations are prone to inbreeding and genetic drift and due to low levels of heterozygosity may loose adaptive potential (Ellstrand & Elam 1993; Lienert 2004). In *Schiedia menziessi* (Caryophyllaceae) it was found that the expression of inbreeding depression varies dramatically between habitats (Rankin et al. 2002). A study of the self-compatible *Schiedea membranacea* showed that outcrossing is stable in this species and maintained by notable levels of inbreeding depression (Culley et al. 1999). Genetic drift, resulting either in fixation of alleles or neutral genetic diversity loss, may have stronger effects on isolated populations than inbreeding depression. Observed facultative xenogamy leads to the conclusion that considerable heterozygosity may exist in the isolated populations.

Several studies show that seed weight has significant effects on seedling growth and survival (Navarro 2002). The seed weight analysis gave a significant mean difference between the two examined populations, but the medians were the same. It was also notable that the population from Erdpresshöhe showed a higher range of weight-value distribution. Unfortunately the demographic survey on the site of the original population in Lassee was impossible; therefore no conclusions for different seedling growth and survival rate compared with seed weights can be made.

6.3 Demographic survey

A demographic survey was included in the study to reveal differences according to fitness parameters between the original population in Lassee and one of the filial populations in Windmühle. This intention failed, however, due to the very early mowing on the original population site. Thus a strict comparison with the original population is currently impossible. However, without any detailed analysis the strong difference between the two habitats is obvious. Seedling germination in the original population was hardly detected, due to the dense grass cover. Adult individuals showed less inflorescences and less flowers than individuals in the open habitats of the filial populations. A study in Öland showed a sharp decrease in population size

with increasing vegetation cover. The most dynamic populations were found in species-poor alvar habitats with low vegetation cover (Bengtsson 2000).

The arrangement of the plots on the Windmühle site was chosen according to vegetation cover, but no site in Windmühle showed such strong vegetation cover as the site of the original population. The demographic survey in Windmühle showed only 55 % of the counted seedlings reaching the stage of a class 1 plant (classes in Methods 2.1). These results compare well with demographic studies of *Gypsophila fastigiata* in Öland showing high levels of mortality in the pre-reproductive stages (seedling mortality 65-100%) and rather low adult mortality (Bengtsson 2000). The number of inflorescences and flowers per individual showed no clear difference between the plots, and the values were in a middle range compared to the Öland study, where no significant associations between number of inflorescences and vegetation characteristics had been detected. The number of individuals (64) did not change in the Lassee original population during the two observation years (in comparison to the count in 2001 were only 30 individuals had been found by J. Greimler & K. Tremetsberger). Yet the mowing, very short to the ground, strongly affected the old individuals of *Gypsophila fastigiata* subsp. *arenaria*, Every individual has been marked with plastic rings, but during spring only three individuals could be found, since the stems of the plants had been mowed off and the dense hey cover provided a very mouldy milieu. Although there are no precise data, personal observations show clear evidence that the original population in Lassee suffers from the dense vegetation cover. It is also obvious that the culture of sod lawn on the field next to the nature reserve affects the vegetation composition in a negative direction.

6.4 Conclusions

In practical conservation of the Lassee populations it is obviously difficult to maintain sort of a traditional grassland management. For these Austrian populations the conclusions of Bengtsson (2000) can be applied. She stated after a demographic survey over eight years: ‘For the survival of the Öland populations of *Gypsophila fastigiata*, a habitat which is open and disturbed enough to enable seedling establishment and at the same time stable enough to allow established plants to become reproductive is needed. This can probably best be archived by keeping moderate grazing pressure, which also is adjusted to the weather cycles: in other

words, in the way these grasslands have been managed traditionally for thousands of years'.

The restoration activities by founding the filial populations near Lassee attempted to increase the number of populations. This study leads to the conclusion that the filial populations seem to develop quite beneficial, since no loss of genetic diversity compared to the original population could be found. 'Restored populations should reflect the extant variation found in remnants' (Dolan et al. 2008). A better demographic performance, due to better habitat conditions on the new sites is obvious and allows the conclusion that the site of the original population needs advanced nature reserve management such as partial opening of the soil and maybe even a better buffering towards the adjacent fields. To summarise, the filial populations have successfully developed, but conservation of the only original population of *Gypsophila fastigiata* subsp. *arenaria* in Austria needs to be optimised.

7 Deutsche Kurzfassung

7.1 Einleitung

Pannonische Trockenrasen und Steppengebiete besitzen eine außergewöhnliche Artenvielfalt. Die Pannonische Tiefebene wurde im Jahr 2000 zu einer eigenständigen biogeographischen Region erklärt, was den unschätzbaren Wert dieser Region unterstreicht (Illyés et al. 2007). In Österreich befindet sich die westlichste Ausdehnungsgrenze der pannonischen Steppengebiete. Die pannonische Florenprovinz gehört zu den europäischen Landschaften, die am intensivsten durch menschliche Einflüsse geprägt wurden (Buček et al. 2006). Der anthropogene Einfluss einerseits und die Charakteristik der Steppengebiete andererseits formen ein sehr fragmentiertes Habitat. Die Populationen typischer Pflanzen der Trockenrasen- und Steppengebiete sind disjunkt, klein und oftmals stark gefährdet, da sie durch den menschlichen Einfluss zunehmend reduziert werden. Genetische Struktur und Populationsdynamik kleiner, fragmentierter Populationen steht im Mittelpunkt der Evolutionsbiologie und der Naturschutzforschung (Lönn & Prentice 2002; Manel et al. 2005). Die österreichischen Populationen solcher Arten des Pannonikums sind durch die westliche Arealgrenze, im Gegensatz zu den Populationen des Hauptverbreitungsgebiets, isoliert, was zur Reduktion der Populationsgrößen, größerer räumlicher Trennung und genetischer Drift führt. Genetische Drift kann ein Auslöser für geringere genetische Diversität (Nei et al. 1975), Reduktion von Allelvielfalt innerhalb der Populationen (Rich et al. 1979) und Verstärkung der Diversität zwischen den Populationen sein (Brakefield 1989).

Die vorliegende Studie konzentriert sich auf *Gypsophila fastigiata* subsp. *arenaria* (Waldst. et Kit.) Domin, eine ausdauernde Art, die an sandige, offene Habitate gebunden ist. Durch die Lebensraumspezialisierung und das disjunkte Verbreitungsgebiet ist die Art in Europa stark gefährdet. In Österreich kam *Gypsophila fastigiata* subsp. *arenaria* ausschließlich in einem kleinen Naturschutzgebiet in der Nähe von Lassee, im Marchfeld in Niederösterreich vor. Vor kurzem wurden zwei Filialpopulationen in der Näheren Umgebung von Lassee

gegründet, eine Filialpopulation in Erdpresshöhe im Jahr 2000 (Greimler & Tremetsberger 2001), die zweite am Standort Windmühle im Jahr 2003 (Wiesbauer pers. Komm.).

Die grundsätzlichen Ziele der Studie waren, folgendes festzustellen:
(I) Die Auswirkung der Erhaltungsmaßnahmen auf die genetische Diversität in der Originalpopulation und in den neu gegründeten Filialpopulationen, sowie die genetische Differenzierung zwischen den drei österreichischen Populationen.
(II) Die Diversität und Differenzierung zwischen den österreichischen, den benachbarten und den Populationen des Hauptverbreitungsgebiets.
(III) Das Fortpflanzungssystem und insbesondere das Bestäubungssystem.
(IV) Die wichtigsten demographischen Prozesse in den österreichischen Populationen unter besonderer Berücksichtigung der unterschiedlichen Landschaftsmerkmale.

7.1.1 Der untersuchte Standort

Die Pannonische Ebene wurde nach der römischen Provinz Pannonia benannt und wird durch das Karpathische Becken, die Alpen, die Dinariden und die Berge des Balkans begrenzt. Das warme und trockene Klima ist einerseits durch kontinentale Effekte aus dem Osten und andererseits durch mediterrane Verhältnisse aus dem Süden geprägt (Buček et al. 2006). Die Dünen Niederösterreichs sind geologisch gesehen relativ jung, sie sind während beziehungsweise nach der letzen Eiszeit entstanden (Wiesbauer 2002). Obwohl diese Gebiete jahrtausendelang landwirtschaftlich genutzt wurden, zeigt erst die Industrialisierung ab Beginn des 20. Jahrhunderts verheerende Auswirkungen. Die ehemals nährstoffarmen Gebiete wurden in landwirtschaftliche Flächen umgewandelt, die Dünen und damit Erosionsflächen befestigt und Aufforstungen durchgeführt. Diese Maßnahmen führten zu einem dramatischen Rückgang der Pionierstandorte. Offene Sandflächen kommen heute nur noch in Bereichen von Feldwegen, Kahlschlägen oder Extremstandorten vor. Die Sandstandorte des Pannonikums zählen zu den trockensten Gebieten Österreichs, wodurch in diesem Bereich hauptsächlich thermophile Arten vorkommen. Durch Trockenheit, hohe Einstrahlung, geringen Nährstoffgehalt und Sedimentumlagerungen verfügen Arten sandiger Standorte über

spezifische morphologische und physiologische Anpassungen wie die Ausbildung sklerotisierter oder kutinisierter Oberflächen, dichte Behaarung, lange Pfahlwurzeln, das Schließen der Stomata oder eine Verlagerung der vegetativen oder der generativen Phase in günstigere Jahreszeiten (Wiesbauer & Mazzucco 1999).

7.1.2 Die untersuchte Art

Die Gattung *Gypsophila* ist ein hauptsächlich eurasisches Taxon, das 126 Arten umfasst und großteils Steppen der nördlichen, temperaten Alten Welt besiedelt. *Gypsophila fastigiata* ist eine diploide perennierende Pflanze mit linealischen, grauen Blättern, einer Pfahlwurzel und einer bis mehreren Infloreszenzen, die einem Thyrsus bilden. Die Art ist hermaphroditisch und selbst-kompatibel, aber vorwiegend auskreuzend, sie verbreitet sich nicht vegetativ (Bengtsonn 2000). Aufgrund unterschiedlicher Samenpapillen als differentialdiagnostisches Merkmal (Scheffer 1927), unterscheidet die Taxonomie zwei Unterarten. Die taxonomische Gliederung in zwei Unterarten entspricht der chorologischen Differenzierung. Im pannonischen Teilareal kommt subsp. *arenaria* mit langen, konischen Papillen, im Hauptverbreitungsgebiet subsp. *fastigiata* mit kurzen, stumpfen Papillen vor. In der Flora Europaea (Barkoudah et al. 1993) wird subsp. *arenaria* nicht anerkannt, in der ersten Ausgabe der österreichischen Exkursionsflora (Fischer et al. 1994) wurde sie in *Gypsophila fastigiata* inkludiert, in der folgenden Ausgabe von 2005 allerdings als pannonische Unterart angeführt.

In Österreich war *Gypsophila fastigiata* subsp. *arenaria* nur an einem Standort bekannt, einem sehr kleinen Naturschutzgebiet in Lassee im Marchfeld. Die nächsten Populationen befinden sich in den pannonischen Gebieten Tschechiens, der Slowakei und Ungarns. Um das österreichische Vorkommen der Art zu erhalten wurden im Jahr 2000 in-situ und ex-situ Maßnahmen gesetzt (Greimler & Tremetsberger 2001). Am Originalstandort wurde gemäht, der dichte Grasfilz entfernt und der sandige Boden teilweise wieder freigelegt um das Aufkommen von Jungpflanzen zu erleichtern. Zusätzlich wurden aus Samen Pflanzen im Botanischen Garten der Universität Wien (HBV) herangezogen und die Jungpflanzen an einem geeigneten Standort in Erdpresshöhe, nahe Lassee, ausgepflanzt. Erdpresshöhe stellt eine alte Sanddüne dar, die aber nicht mehr mobil ist, wo sich aber zahlreiche offene Sandstandorte finden lassen. Neben dem Ausbringen von Jungpflanzen

wurden auch Samen angesät um eine neue Population aufbauen zu können. Gleiche Maßnahmen wurden 2003 von H. Wiesbauer an einem weiteren Standort, Windmühle, durchgeführt. Die zwei gegründeten Filialpopulationen haben sich mittlerweile gut etabliert. Die vorliegende Arbeit hat unter anderem zum Ziel Einblicke über die Effekte der Konservierungsmaßnahmen zu gewinnen, da für weitere Erhaltungsmaßnahmen Wissen über Bestäubungssysteme, Phenologie, Reproduktion, genetische Diversität und Struktur nötig ist. Darüber hinaus sind auch die benachbarten Populationen von Bedeutung um Informationen über eventuelle Randeffekte in den österreichischen Populationen zu bekommen.

7.2 Methoden

7.2.1 AFLP

Für die AFLP Analyse wurde Blattmaterial von elf Populationen gesammelt und sofort in Silicagel getrocknet. Die drei österreichischen und darüber hinaus slowakische, tschechische und ungarische Populationen wurden besammelt (Tab 1, Fig 4). Teilweise wurden Proben von älteren Aufsammlungen verwendet. Als ‚outgroup' wurde *Gypsophila fastigiata* subsp. *fastigiata* aus Deutschland verwendet. Zusätzlich wurden auch Aufsammlungen der ex-situ im botanischen Garten der Universität Wien (HBV) aus Samen gezüchteten Pflanzen der Originalpopulation untersucht.

Die DNS Isolation folgte dem Protokoll von Doyle & Doyle (1987) mit einigen kleinen Veränderungen. Die AFLP Analyse wurde nach dem Protokoll von Vos et al. (1995) durchgeführt. Folgende Primerkombinationen wurden nach vorangegangenen Primertests verwendet: EcoRI–ACA (FAM-blau)/ MseI–CA, EcoRI–AGG (VIC-grün)/ MseI–CT and EcoRI–AAC (NED-gelb)/ MseI–CT. Alle PCRs wurden mit einem GeneAmp® PCR System 9700 (AB Applied Biosystems, Foster City, California, USA) durchgeführt. Die Fragmente wurden in einem Kapillar-Sequenzer (AB 3130xl, Applied Biosystems) aufgetrennt.

Die Daten wurden anschließend mit GENESCAN (PE Applied Biosystems) nach einem Größenstandard ausgerichtet und mit GENOGRAPHER (version 12.0.0, Montana State University 2001; http://hordeum.msu.montana.edu/genographer/) analysiert. Fragmente zwischen 70 und 495 bp wurden ausgewertet. Für weitere Analysen des

Datensatzes wurden folgende Programme verwendet: ARLEQUIN 3.01 (Excoffier et al. 2005) und FAMD 1.08 (Schlüter & Harris 2006) um Anzahl der Fragmente, Anzahl der polymorphen Fragmente und die genetische Variation festzustellen. PAUP (Swofford 2003) wurde herangezogen um ein Neighbour-Joining Phylogramm zu erstellen, NTSYS 2.0 (Rohlf 1997) um eine Principle Coordinate Analyse zu errechnen und SIGMAPLOT 10.0. um diese darzustellen. Die Programme BAPS 4.13 (Corander et al. 2003, 2004) und STRUCTURE 2.1 (Pritchard et al. 2000) wurden verwendet um genetische Strukturen innerhalb und zwischen den Populationen zu analysieren.

7.2.2 Pollen/ovule (P/O)-Ratios

Um P/O-Ratios (Cruden 1976) zu errechnen wurden Knospen kurz vor der Anthese verwendet. Drei Populationen (Originalpopulation, Filialpopulation Erdpresshöhe und eine Population aus der Slowakei: Partizanske) wurden besammelt. 15 Blüten von unterschiedlichen Individuen pro Populationen wurden in Alkohol eingelegt und anschließend ausgezählt. Um die P/O-Ratio zu bestimmen wurde die Anzahl der Pollenkörner durch die Anzahl der Samenanlagen dividiert. Eine ANOVA und ein LSD-Test wurden für die P/O-Ratios mit SPSS 10 durchgeführt.

7.2.3 Bestäubungsversuche

Noch geschlossene Blüten wurden mit Brautschleier von Bestäubern abgeschirmt um Selbstbestäubung zu testen. Vier unterschiedliche Ansätze wurden verwendet. (i) Selbstbestäubungstest durch Abschirmen; (ii) Emaskulation, die Antheren der Blüten wurden entfernt und Fremdpollen auf die Narbe aufgebracht, diese Blüten wurden anschließend auch abgeschirmt; (iii) geförderte Fremdbestäubung, Fremdpollen wurde auf die Narbe gebracht, die Antheren wurden jedoch nicht entfernt, die Blüten wurden abgeschirmt; (iv) eine unbehandelte Kontrollgruppe wurde markiert.

7.2.4 Demographische Erhebungen

Im Herbst 2007 wurden 5 Erhebungsflächen an zwei unterschiedlichen Lagen in der Filialpopulation Windmühle angelegt. Die Erhebungsflächen wurden so gewählt, dass sie zwei unterschiedliche Mahd-Anwendungen repräsentieren. Drei Flächen wurden

in einem gemähten Gebiet installiert, zwei Flächen in einem ungemähten. Die Erhebungsflächen waren 1x1 m groß. Die Individuen wurden nach Größe klassifiziert: Klasse 0: Keimlinge, Klasse 1: unverzweigte Jungpflanzen, Klasse 2: verzweigte Jungpflanzen, Klasse 3: Pflanzen mit Blattrosette kleiner als 5 cm, Klasse 4: Pflanzen mit Blattrosette größer als 5 cm und Klasse 5: Pflanzen mit Blattrosette größer als 10 cm. Bei jeder Erhebung wurden die Zahlen der Infloreszenzen und Blüten pro Individuum erhoben, weiters die Vegetationsdichte und die Deckung durch Moose, Flechten und totes Blattmaterial.

7.2.5 Samengewichte

Samengewichte von einer Filialpopulation und der Originalpopulation wurden erhoben. Leider konnten keine Samen von Populationen aus dem Hauptverbreitungsgebiet gesammelt werden, da die Aufsammlungen des Blattmaterials für die genetischen Analysen zum Zeitpunkt der Blüte erfolgte um ein besseres Auffinden der Individuen zu gewährleisten.

7.3 Resultate

7.3.1 AFLP

In 208 analysierten Individuen konnten 479 eindeutig auswertbare Fragmente bestimmt werden (Tab 1). Die nicht hierarchische Neighbour-Joining Analyse (Fig 5) ergab sechs Gruppen: die der österreichischen Populationen, die tschechischen, die Population der zentralen Slowakei, der südlichen Slowakei, die ungarischen Populationen und die deutsche Population. In der hierarchischen Neighbour-Joining Analyse mit *Gypsophila fastigiata* subsp. *fastigiata* (Fig 6) zeigten sich die Strukturen besser und ergaben ähnliche Gruppen wie in der PCoA (Fig 8), allerdings bildete die zentralslowakische Population eine eigene Gruppe. Die vier resultierenden Gruppen waren die österreichische, die tschechische, die ungarische zusammenfallend mit der südslowakischen Population und die zentralslowakische Population, was der geographischen Verteilung der Populationen entspricht. Die AMOVA (Tab 2) zeigte, dass die größte genetische Variation innerhalb der Populationen liegt. Der F_{ST} von 0,27 deutet eine hohe Differenzierung zwischen den einzelnen Populationen an. Eine

AMOVA nur mit den österreichischen Populationen zeigte einen F_{ST} von 0,08 und eine Variation von 91,65% innerhalb der Populationen. Die Bayesian-Clustermethoden lieferten vier Gruppen, die auch der geographischen Verteilung entsprechen. Die vier Cluster waren die österreichischen Populationen, die tschechische Population, die zentralslowakische und die ungarischen Populationen zu welchen auch die südslowakische gehört. Die anschließende Admixture Analyse der Genfluss-Ereignisse zeigte mit BAPS in nur wenigen Individuen eine Beimischung aus einem anderen genetischen Cluster, mit STRUCTURE mehr Beimischung. Die größte Durchmischung ließ sich im ungarischen Cluster feststellen, aber auch in der österreichischen Originalpopulation waren Anteile anderer genetischer Cluster feststellbar. Die Analyse der durchschnittlichen Gendiversität ergab eine klare Abnahme vom Südosten in Richtung Nordwesten, zum Arealrand hin.

7.3.2 P/O-Ratios

Die mittlere Anzahl der Samenanlagen pro Blüte lag zwischen 13 und 14 über alle drei Populationen ohne signifikante Unterschiede (Tab 3). Die mittlere Anzahl der Pollenkörner pro Blüte liegt bei 5767 (Lassee original), 5961 (Erdpresshöhe) und 6547 (Partizanske). Daraus ergeben sich P/O-Ratios zwischen 438 und 494. Die P/O-Ratios, welche Rückschlüsse auf das Bestäubungssystem erlauben, ergeben in diesem Fall ein Mixed Mating System (zwischen fakultativer Autogamie und fakultativer Xenogamie).

7.3.3 Bestäubungsversuche

Durch extreme Wetterbedingungen im Sommer 2007 wurden die meisten Versuchsansätze von einem Hagelsturm abgebrochen. In jedem Versuchsansatz konnten Samen gefunden werden (Tab 8, Tab 9), jedoch lassen die wenigen unbeschädigten Ansätze nur vage Schlussfolgerungen zu.

7.3.4 Samengewichte

Die gemessenen Samengewichte von 50 Samen pro Population zeigten keinen Unterschied in den Medianen zwischen der Originalpopulation und der

Filialpopulation aus Erdpresshöhe (Tab 10). Die Spannweite der Samengewichte in der Filialpopulation war größer als in der Originalpopulation (Fig 13).

7.3.5 Demographie

Durch den kurze Beobachtungszeitraum der demographischen Erhebungen und das Fehlen der Aufnahmen der Originalpopulation, verursacht durch das frühzeitige Mähen im Jahr 2007, ließen sich nur wenige Ergebnisse festhalten. Jedoch ließ sich zeigen, dass Keimlinge unter zu dichtem Vegetationsdruck leiden. Auch Unterschiede an gemähten und ungemähten Erhebungsflächen waren zu beobachten (Appendix V).

7.4 Diskussion

Der Fokus der Arbeit lag auf Untersuchungen der genetischen Konstitution der österreichischen Originalpopulation und der beiden, im Zuge der Erhaltungsmaßnahmen, neu gegründeten Filialpopulationen von *Gypsophila fastigiata* subsp. *arenaria*. Ein Schwerpunkt war der mögliche genetische Diversitätsverlust der Filialpopulationen. Darüber hinaus war es ein Ziel, das Ausmaß der Differenzierung zwischen den österreichischen und benachbarten Randpopulationen sowie den Populationen des Hauptverbreitungsareals zu untersuchen.

Die Diversitätswerte der drei österreichischen Populationen waren auf einem ähnlichen Niveau, die der Filialpopulationen sogar wenig höher, was den erwarteten Diversitätsverlust überraschenderweise nicht bestätigte. Die Erhaltungsmaßnahmen der Neugründung der Filialpopulationen lieferten durch ein gut angelegtes Besammeln der Originalpopulation den Grundstein für einen ausreichend durchmischten Genpool. Die Überalterung der Originalpopulation hätte zu einer starken Selektion in eine Richtung führen können, da diese Individuen auch die geringste Anzahl polymorpher Fragmente und die niedrigste genetische Diversität zeigten. Allerdings ist die sexuelle Komponente der Filialpopulationen, durch die höhere Anzahl der Pflanzen und der daraus resultierenden höheren Bestäubungswahrscheinlichkeit, vermutlich bedeutender. Generell lassen die Diversitätswerte der österreichischen Populationen die Schlussfolgerung einer etwas

besseren Fitness der Filialpopulationen zu. Ob die österreichischen Populationen durch Genfluss in Kontakt stehen lässt sich nicht nachweisen, die geringe Differenzierung, die sich in der Neighbour-Joining wie auch in der PCoA Analyse zeigt, lässt sich auf die erst vor kurzem durchgeführte Neugründung der Filialpopulationen zurückführen, was auch durch den F_{ST} von 0,08 bestätigt wird. Genfluss durch Samen kann weitgehend ausgeschlossen werden, da Samenverbreitung hauptsächlich in einem Radius 20 cm um die Mutterpflanze stattfindet (Bengtsson 2000). Die geringe Distanz zwischen den Populationen würde Genfluss durch Pollen zulassen, aber die Landschaftsstrukturen machen dies eher unwahrscheinlich (Holderegger & Wagner 2008).

Die ungarischen Populationen zeigten die größte Diversität und auch die meisten polymorphen Fragmente im Gegensatz zu den österreichischen Populationen. Da die österreichischen Vorkommen die westlichste Verbreitungsgrenze der Art darstellen ist eine genetische Verarmung der Randpopulationen nicht unbedingt überraschend. Während in zentralen Populationen Genfluss aus allen Richtungen möglich ist, wird Genfluss in Randpopulationen durch die Lage begrenzt (Prober & Brown 1994). In Randvorkommen werden Isolationseffekte wirksam, was in der genetischen Verarmung vom östlichen Hauptareal in Richtung der Randpopulationen im Westen (Fig 12) und auch in der Admixture-Analyse (Fig 9 und 10) verdeutlicht wird. Isolationseffekte können zu genetischer Drift und Inzucht-Depression führen. Ob Inzucht in den Randpopulationen eine Rolle spielt ist schwer zu beantworten, da AFLPs als dominante Marker keine Aussage über Heterozygotie zulassen. Die Bestäubungsversuche und die P/O-Ratios jedoch ergaben fakultative Xenogamie in *Gypsophila fastigiata* subsp. *arenaria*, was darauf hinweisen könnte, dass in den Populationen, trotz Isolation, noch genug Heterozygotie vorhanden sein kann. Diese Ergebnisse weisen Genetischer Drift möglicherweise eine größere Rolle als der Inzucht-Depression zu.

Die demographischen Erhebungen an der Filialpopulation in Windmühle und persönliche Beobachtungen am Originalstandort Lassee, unterstützt durch frühere demographische Erhebungen (Tremetsberger & Schönswetter 2002), zeigen dass die Art sehr an offene Standorte gebunden ist. Die Zunahme der Vegetationsdichte geht einher mit zunehmender Keimlingssterblichkeit. Je offener der Standort, desto

besser können sich Jungpflanzen entwickeln. Die Filialpopulationen profitieren von der Standortswahl und konnten sich bis jetzt sehr gut etablieren. In der Originalpopulation, wo zwar gemäht wird, das Mähgut aber liegen bleibt, lässt sich beobachten dass die Pflanzen unter der dichten Vegetationsdecke leiden. Um die Art an ihrem einzigen natürlichen Standort in Österreich zu erhalten ist es deshalb von großer Bedeutung, die Erhaltungsmaßnahmen auszubauen und zu verbessern.

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9 Appendix I

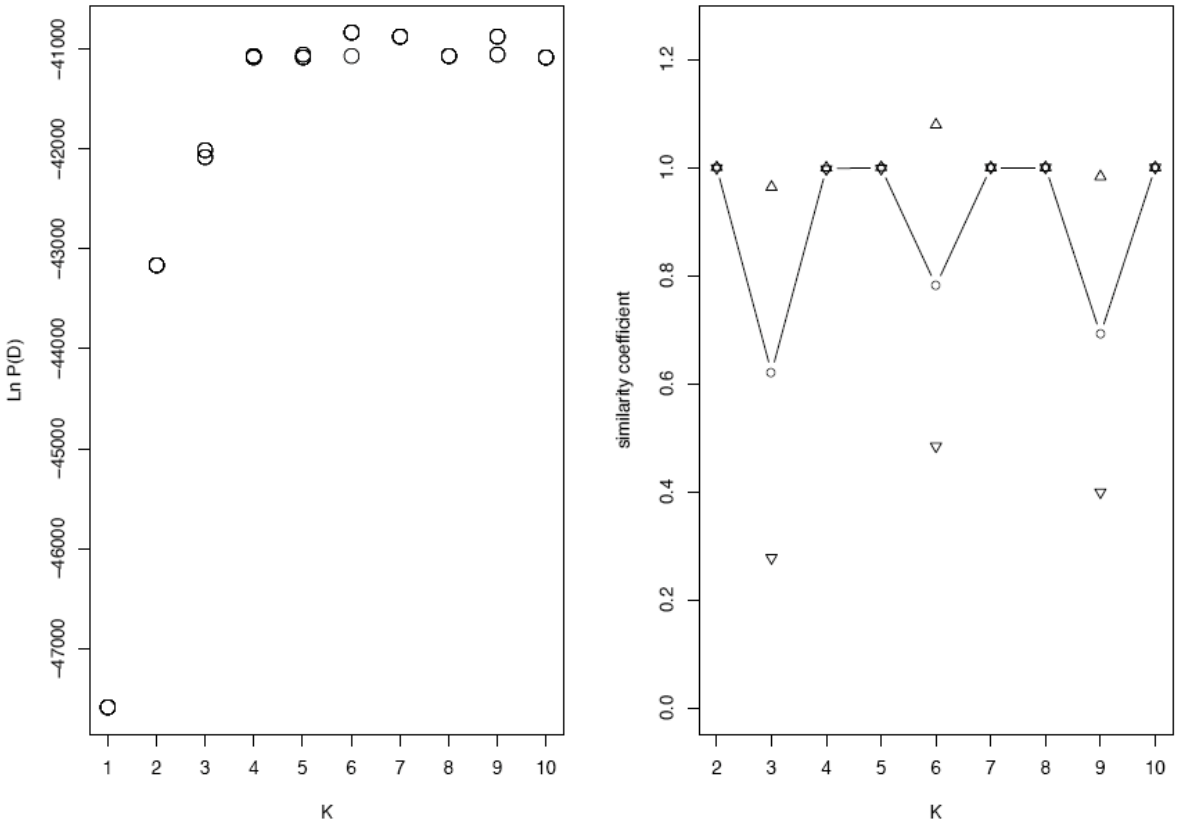

Fig 14: STRUCTURE results for K 4. On the left, the likelihood of each number of groups (K) for each of ten runs plotted against the K values; on the right side the average similarity coefficients among each pair of structure runs for each K with standard deviations.

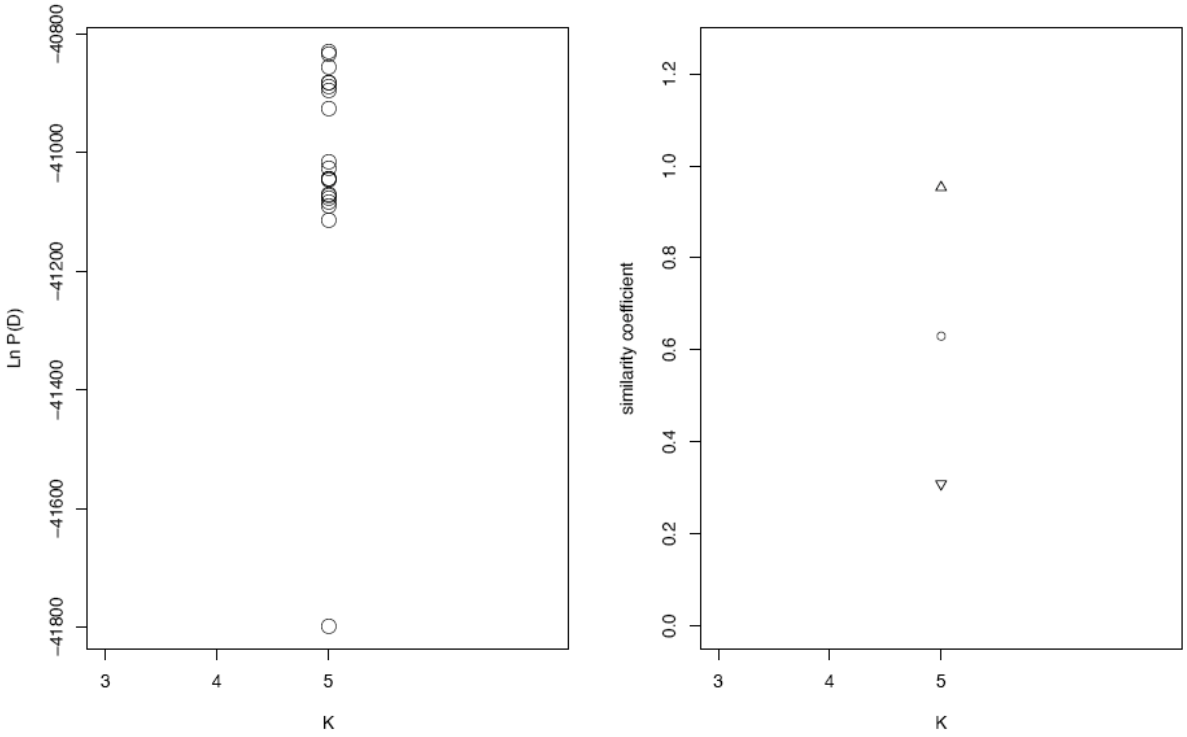

Fig 15: STRUCTURE test for K 5.

10 Appendix II

Tab 11: Localities, where no individuals could be found.

Location	Country	Coordinates (E/N)
Mistrin	Cz	17.074°/48.958°
Vel'ké Leváre	Sk	17.022°/48.499°
Zahorie 1	Sk	17.242°/48.544°
Zahorie 2	Sk	17.250°/48.533°
Studienka	Sk	17.126°/48.516°
Mikulasov	Sk	17.237°/48.579°
Laksárska Nova Ves	Sk	17.183°/48.583°
Laksárska Nova Ves	Sk	17.146°/48.587°
Suchohrad	Sk	16.882°/48.417°

11 Appendix III

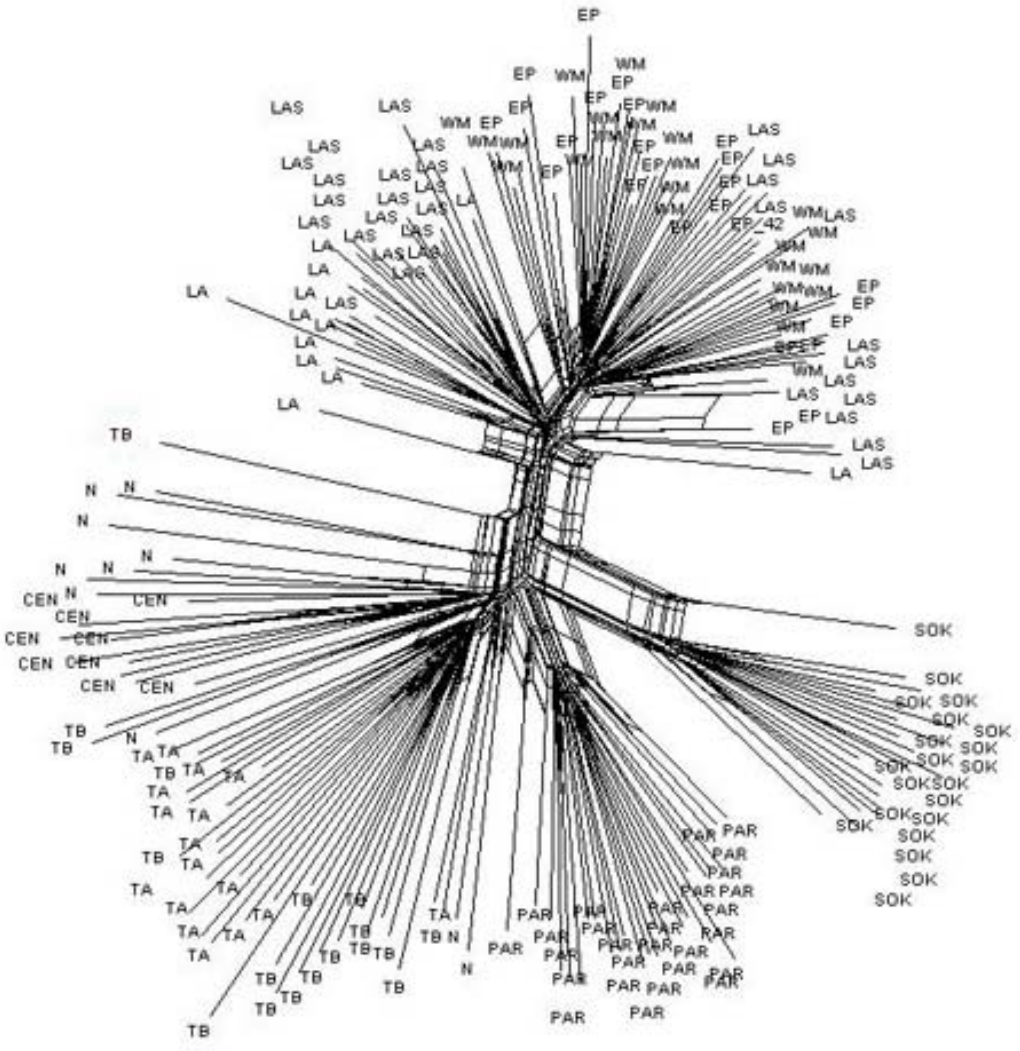

Fig 16: Neighbour-net of all investigated *Gypsophila fastigiata* subsp. *arenaria* populations.

12 Appendix IV

Tab 12: Proportion of admixture revealed by STRUCTURE.

sample	Hungarian	Slovak	Austrian	Czech		sample	Hungarian	Slovak	Austrian	Czech
1	0,0010	0,0010	0,9970	0,0010		51	0,0010	0,0010	0,9970	0,0010
2	0,0010	0,0010	0,9970	0,0010		52	0,0020	0,0050	0,9910	0,0020
3	0,0020	0,0020	0,9940	0,0020		53	0,0010	0,0010	0,9970	0,0010
4	0,0030	0,0020	0,9940	0,0010		54	0,0010	0,0010	0,9970	0,0010
5	0,0010	0,0010	0,9970	0,0010		55	0,0010	0,0010	0,9970	0,0010
6	0,0050	0,0030	0,9900	0,0020		56	0,0010	0,0030	0,9950	0,0010
7	0,0010	0,0010	0,9970	0,0010		57	0,0010	0,0010	0,9970	0,0010
8	0,0010	0,0010	0,9980	0,0000		58	0,0030	0,0030	0,9900	0,0040
9	0,0020	0,0010	0,9950	0,0020		59	0,0010	0,0010	0,9970	0,0010
10	0,0010	0,0010	0,9970	0,0010		60	0,0010	0,0010	0,9970	0,0010
11	0,0010	0,0010	0,9970	0,0010		61	0,0010	0,0010	0,9970	0,0010
12	0,0010	0,0007	0,9973	0,0010		62	0,0010	0,0010	0,9970	0,0010
13	0,0072	0,0020	0,9871	0,0037		63	0,0010	0,0010	0,9970	0,0010
14	0,0085	0,0681	0,9049	0,0185		64	0,0010	0,0010	0,9970	0,0010
15	0,0322	0,0594	0,9043	0,0040		65	0,0010	0,0010	0,9980	0,0000
16	0,0020	0,0010	0,9950	0,0020		66	0,0020	0,0010	0,9960	0,0010
17	0,0060	0,0040	0,9865	0,0035		67	0,0020	0,0020	0,9950	0,0010
18	0,0020	0,0020	0,9907	0,0053		68	0,0010	0,0010	0,9970	0,0010
19	0,0080	0,1699	0,8084	0,0137		69	0,0010	0,0010	0,9970	0,0010
20	0,0155	0,0060	0,9500	0,0285		70	0,0010	0,0010	0,9970	0,0010
21	0,0048	0,0277	0,9520	0,0155		71	0,0010	0,0010	0,9970	0,0010
22	0,0102	0,1202	0,7477	0,1219		72	0,0010	0,0005	0,9975	0,0010
23	0,0010	0,0140	0,9750	0,0100		73	0,0020	0,0010	0,9960	0,0010
24	0,0010	0,0020	0,9228	0,0742		74	0,0010	0,0010	0,9980	0,0000
25	0,0010	0,0020	0,9900	0,0070		75	0,0010	0,0010	0,9978	0,0002
26	0,0010	0,0010	0,9850	0,0130		76	0,0010	0,0010	0,9970	0,0010
27	0,0012	0,0010	0,9918	0,0060		77	0,0010	0,0010	0,9970	0,0010
28	0,0010	0,0020	0,9910	0,0060		78	0,0010	0,0010	0,9970	0,0010
29	0,0010	0,0010	0,9960	0,0020		79	0,0012	0,0010	0,9968	0,0010
30	0,0020	0,0020	0,9935	0,0025		80	0,0040	0,0022	0,9928	0,0010
31	0,0015	0,0020	0,9643	0,0322		81	0,0020	0,0010	0,9960	0,0010
32	0,0010	0,0020	0,9938	0,0032		82	0,0010	0,0010	0,9970	0,0010
33	0,0042	0,0040	0,9868	0,0050		83	0,0010	0,0010	0,9980	0,0000
34	0,0020	0,0020	0,9950	0,0010		84	0,0010	0,0010	0,9970	0,0010
35	0,0020	0,0020	0,9940	0,0020		85	0,0010	0,0010	0,9970	0,0010
36	0,0010	0,0010	0,9967	0,0013		86	0,0010	0,0010	0,9970	0,0010
37	0,0020	0,0020	0,9945	0,0015		87	0,0010	0,0010	0,9970	0,0010
38	0,0065	0,0050	0,9755	0,0130		88	0,0020	0,0010	0,9960	0,0010
39	0,0020	0,0080	0,9890	0,0010		89	0,0177	0,0027	0,9746	0,0050
40	0,0020	0,0018	0,9952	0,0010		90	0,0010	0,0010	0,9980	0,0000
41	0,0951	0,0080	0,8939	0,0030		91	0,0010	0,0010	0,9973	0,0007
42	0,0010	0,0020	0,9960	0,0010		92	0,0010	0,0010	0,9970	0,0010
43	0,0012	0,0010	0,9958	0,0020		93	0,0010	0,0010	0,9970	0,0010
44	0,0035	0,0030	0,9915	0,0020		94	0,0010	0,0010	0,9970	0,0010
45	0,0030	0,0030	0,9920	0,0020		95	0,0033	0,0092	0,9855	0,0020
46	0,0077	0,0100	0,9793	0,0030		96	0,9900	0,0020	0,0040	0,0040
47	0,0020	0,0030	0,9940	0,0010		97	0,9440	0,0030	0,0470	0,0060
48	0,0010	0,0010	0,9980	0,0000		98	0,9960	0,0010	0,0010	0,0020
49	0,0010	0,0010	0,9970	0,0010		99	0,9950	0,0020	0,0010	0,0020
50	0,0010	0,0010	0,9970	0,0010		100	0,9830	0,0100	0,0040	0,0030

Tab 12 continued.

sample	Hungarian	Slovak	Austrian	Czech		sample	Hungarian	Slovak	Austrian	Czech
101	0,9910	0,0050	0,0030	0,0010		150	0,0040	0,9810	0,0120	0,0030
102	0,9908	0,0052	0,0020	0,0020		151	0,0030	0,9763	0,0030	0,0177
103	0,9820	0,0025	0,0135	0,0020		152	0,0055	0,0037	0,0020	0,9888
104	0,9970	0,0010	0,0010	0,0010		153	0,0010	0,9960	0,0020	0,0010
105	0,9960	0,0020	0,0010	0,0010		154	0,0025	0,9935	0,0030	0,0010
106	0,9900	0,0060	0,0010	0,0030		155	0,0118	0,9802	0,0060	0,0020
107	0,9960	0,0020	0,0010	0,0010		156	0,0010	0,9970	0,0010	0,0010
108	0,9884	0,0033	0,0058	0,0025		157	0,0010	0,9955	0,0020	0,0015
109	0,9920	0,0040	0,0020	0,0020		158	0,0020	0,9930	0,0030	0,0020
110	0,9885	0,0075	0,0020	0,0020		159	0,0020	0,9960	0,0010	0,0010
111	0,9952	0,0020	0,0018	0,0010		160	0,0254	0,9636	0,0040	0,0070
112	0,9880	0,0050	0,0060	0,0010		161	0,0020	0,9920	0,0030	0,0030
113	0,9860	0,0090	0,0020	0,0030		162	0,0017	0,9953	0,0010	0,0020
114	0,9920	0,0020	0,0020	0,0040		163	0,0010	0,9970	0,0010	0,0010
115	0,8708	0,0724	0,0292	0,0276		164	0,0010	0,9970	0,0010	0,0010
116	0,9910	0,0040	0,0020	0,0030		165	0,0010	0,9970	0,0010	0,0010
117	0,9900	0,0020	0,0020	0,0060		166	0,0010	0,9960	0,0010	0,0020
118	0,9738	0,0222	0,0010	0,0030		167	0,0203	0,9740	0,0037	0,0020
119	0,9855	0,0095	0,0030	0,0020		168	0,0020	0,9955	0,0015	0,0010
120	0,9770	0,0030	0,0160	0,0040		169	0,0020	0,9950	0,0020	0,0010
121	0,8609	0,0921	0,0372	0,0098		170	0,0010	0,9960	0,0018	0,0012
122	0,9930	0,0020	0,0030	0,0020		171	0,0020	0,9950	0,0020	0,0010
123	0,9872	0,0020	0,0088	0,0020		172	0,0048	0,9890	0,0022	0,0040
124	0,9888	0,0032	0,0070	0,0010		173	0,0030	0,9900	0,0030	0,0040
125	0,9950	0,0020	0,0020	0,0010		174	0,0030	0,9930	0,0020	0,0020
126	0,9950	0,0020	0,0020	0,0010		175	0,0020	0,9817	0,0060	0,0103
127	0,8113	0,0010	0,1867	0,0010		176	0,0010	0,9970	0,0010	0,0010
128	0,9960	0,0010	0,0020	0,0010		177	0,0040	0,9940	0,0010	0,0010
129	0,9885	0,0020	0,0085	0,0010		178	0,0010	0,9960	0,0020	0,0010
130	0,9910	0,0020	0,0040	0,0030		179	0,0010	0,0010	0,0010	0,9970
131	0,8654	0,0047	0,1259	0,0040		180	0,0028	0,0020	0,0010	0,9942
132	0,6913	0,2754	0,0060	0,0274		181	0,0020	0,0030	0,0020	0,9930
133	0,8157	0,1031	0,0153	0,0659		182	0,0020	0,0030	0,0010	0,9940
134	0,9378	0,0582	0,0020	0,0020		183	0,0010	0,0010	0,0010	0,9970
135	0,7154	0,2456	0,0140	0,0250		184	0,0023	0,0050	0,0018	0,9909
136	0,9147	0,0052	0,0105	0,0696		185	0,0020	0,0030	0,0010	0,9940
137	0,7622	0,0190	0,2135	0,0053		186	0,0012	0,0010	0,0020	0,9958
138	0,6563	0,1223	0,2173	0,0040		187	0,0030	0,0020	0,0010	0,9940
139	0,9054	0,0343	0,0271	0,0332		188	0,0010	0,0010	0,0020	0,9960
140	0,9012	0,0070	0,0030	0,0888		189	0,0010	0,0010	0,0010	0,9970
141	0,4710	0,4600	0,0195	0,0495		190	0,0108	0,0140	0,0197	0,9555
142	0,8362	0,1066	0,0060	0,0512		191	0,0057	0,0185	0,0283	0,9475
143	0,9403	0,0125	0,0020	0,0452		192	0,0010	0,0010	0,0010	0,9970
144	0,7089	0,0050	0,0063	0,2798		193	0,0010	0,0030	0,0030	0,9930
145	0,9427	0,0343	0,0080	0,0150		194	0,0020	0,0060	0,0010	0,9910
146	0,8533	0,0162	0,0108	0,1197		195	0,0020	0,0010	0,0020	0,9950
147	0,6546	0,3048	0,0140	0,0266		196	0,0010	0,0020	0,0010	0,9960
148	0,9670	0,0260	0,0020	0,0050		197	0,0010	0,0010	0,0010	0,9970
149	0,8548	0,0427	0,0070	0,0955		198	0,0110	0,0107	0,0010	0,9773

13 Appendix V

Tab 13: Results of the demographic survey of plot 1 Windmühle.

Gypsophila fastigiata Plot W1 2007	Subplot (0.5x0.5m)	cover (%)	Cover vascular plants (%)	cover bryophytes and lichens (%)	cover dead leaves/ mulch (%)	cover soil & rock (%)	seedling	veg 1, unbranched	veg 2, branched	veg 3, branched, smaller than 5 cm	veg 4, branched, bigger than 5 cm	veg 5, branched, bigger than 10 cm	veg total/without seedling	ind. w. current inflorescences class 1	ind. w. current inflorescences class 2	ind. w. current inflorescences class 3	ind. w. current inflorescences class 4	ind. w. current inflorescences class 5	ind. w. current inflorescence total	ind. w. old inflorescences class 1	ind. w. old inflorescences class 2	ind. w. old inflorescences class 3	ind. w. old inflorescences class 4	ind. w. old inflorescences class 5	ind. w. old inflorescneces total	No. inflores./Ind old mean	No. inflores/Ind current mean	No. flowers	No. flowers / Ind	No. flowers / stalk
10.09.2007	total						0	2	16	12	10	5	45	3			9	4	5				3	9	4	16					
	1		20	5	10				2	1			3													6					
	2		20	10	5			1	2	1	3	2	9			1			1				2	4		6	9.5	8	558	185.6	15.5
	3		15	5	10				2	3	2	3	10				1		1				2	2		4	2.8	1	74	74	74
	4		40	5	5			1	10	7	5		23						1				2			6			10	10	10
11.10.2007	total						318																								
	1						44																								
	2						77																								
	3						92																								
	4						105																								
31.05.2008	total							53	8	13	9	5	88																		
	1		12	5	2			4	2	2			8																		
	2		15	5	2			12	4	2	2	2	22																		
	3		15	5	2			13	1	3	1	3	21																		
	4		30	5	2			24	1	6	6		37																		

Tab 14: Results of the demographic survey of plot 2, Windmühle.

Gypsophila fastigiata Plot W2 2007	Subplot (0.5x0.5m)	cover (%)	Cover vascular plants (%)	cover bryophytes and lichens (%)	cover dead leaves/ mulch (%)	cover soil & rock (%)	seedlings	veg 1, unbranched	veg 2, branched	veg 3, branched, smaller than 5 cm	veg 4, branched, bigger than 5 cm	veg 5, branched, bigger than 10 cm	veg total/without seedling	ind. w. current inflorescences class 1	ind. w. current inflorescences class 2	ind. w. current inflorescences class 3	ind. w. current inflorescences class 4	ind. w. current inflorescences class 5	ind. w. current inflorescence total	ind. w. old inflorescences class 1	ind. w. old inflorescences class 2	ind. w. old inflorescences class 3	ind. w. old inflorescences class 4	ind. w. old inflorescences class 5	ind. w. old inflorescences total	No. inflores./Ind old mean	No. inflores/Ind current mean	No. flowers	No. flowers / Ind	No. flowers / stalk
10.09.2007	total							14	3	13	6	5	41	1			1	1	2	1			1	2	5	8	6	1.5	22	7.3	7.3
	1		15	5	2			1	2	3	1	1	8													3	6	1.5	22	7.3	7.3
	2		10	5	2			2		5	3	1	11			1							1	1	2	2	5	3	42	22	7
	3		10	5	5			5	1	2	1	2	11										1	1	1	2	6	1	37	18.5	18.5
	4		15	5	10			6		3	1	1	11					1	1	1					1	1	12	9	153	153	17
11.10.2007	total						228																							
	1						39																							
	2						47																							
	3						46																							
	4						96																							
31.05.2008	total							84	12	14	8	5	123																	
	1		20	5	5			17	4	5	1	1	28																	
	2		20	5	5			12	4	4	5	1	26																	
	3		5	5	10			36	2	2	1	2	43																	
	4		10	5	10			19	2	3	1	1	26																	

Tab 15: Results of the demographic survey of plot 3, Windmühle.

Gypsophila fastigiata Plot W3 2007	Subplot (0.5x0.5m)	cover (%)	Cover vascular plants (%)	cover bryophytes and lichens (%)	cover dead leaves/ mulch (%)	cover soil & rock (%)	seedlings	veg 1, unbranched	veg 2, branched	veg 3, branched, smaller than 5 cm	veg 4, branched, bigger than 5 cm	veg 5, branched, bigger than 10 cm	veg total/without seedling	ind. w. current inflorescence class 1	ind. w. current inflorescences class 2	ind. w. current inflorescences class 3	ind. w. current inflorescences class 4	ind. w. current inflorescences class 5	ind. w. current inflorescence total	ind. w. old inflorescences class 1	ind. w. old inflorescences class 2	ind. w. old inflorescences class 3	ind. w. old inflorescences class 4	ind. w. old inflorescences class 5	ind. w. old inflorescences total	No. inflores./Ind old mean	No. inflores/Ind current mean	No. flowers	No. flowers / Ind	No. flowers / stalk
10.09.2007	total							1	17	26	12	3	59				2		2			6	6	2	14					
	1		40	5	5				1	12	3	1	17				1		1			3	1	1	5	1.8	1	133	133	7
	2		30	5	2			1	9	7	3	1	21				1		1			1	3		4	2.25	2	14	14	0
	3		30	5	5				1	5	3		9									2	1		3	1,3	0	0	0	0
	4		20	5	10				6	2	3	1	12										1	1	2	3	0	0	0	0
11.10.2007	total						240																							
	1						96																							
	2						62																							
	3						37																							
	4						45																							
31.05.2008	total							137	14	27	13	3	194																	
	1		50	10	5			36	2	10	3	1	52																	
	2		60	10	5			35	6	8	3	1	53																	
	3		60	10	10			25	5	4	5		39																	
	4		40	10	10			41	1	5	2	1	50																	

Tab 16: Results of the demographic survey of plot 4, Windmühle.

Gypsophila fastigiata Plot W4 2007	Subplot (0.5x0.5m)	cover (%)	Cover vascular plants (%)	cover bryophytes and lichens (%)	cover dead leaves/ mulch (%)	cover soill & rock (%)	seedlings	veg 1, unbranched	veg 2, branched	veg 3, branched, smaller than 5 cm	veg 4, branched, bigger than 5 cm	veg 5, branched, bigger than 10 cm	veg total/without seedling	ind. w. current inflorescences class 1	ind. w. current inflorescences class 2	ind. w. current inflorescences class 3	ind. w. current inflorescences class 4	ind. w. current inflorescences class 5	ind. w. current inflorescence total	ind. w. old inflorescences class 1	ind. w. old inflorescences class 2	ind. w. old inflorescences class 3	ind. w. old inflorescences class 4	ind. w. old inflorescences class 5	ind. w. old inflorescneces total	No. inflores./Ind old mean	No. inflores/Ind current mean	No. flowers	No. flowers / Ind	No. flowers / stalk
10.09.2007	total							5	3	4	2	5	19			1		4	5				2	5	7					
	1		25	5	5			1	2		1		4										1		1	5	0	0	0	0
	2		70	10	5					1		2	3			1		2	3					2	2	17	5.5	431	143.7	13
	3		10	3	5			4	1	1	1	1	8					1	1				1	1	2	9,5	11	372	372	33.8
	4		70	10	5					2		2	4					1	1					2	2	30,5	6	163	163	27.2
11.10.2007	total						150																							
	1						21																							
	2						20																							
	3						33																							
	4						76																							
31.05.2008	total						1	73	4	2	1	4	84																	
	1		40	55	2				2	1			3																	
	2		50	45	2			1		1		2	4																	
	3		10	60	2		1	51	2		1		54																	
	4		50	45	2			21				2	23																	

Tab 17: Results of the demographic survey of plot 5, Windmühle.

Gypsophila fastigiata Plot W5 2007	Subplot (0.5x0.5m)	cover (%)	Cover vascular plants (%)	cover bryophytes and lichens (%)	cover dead leaves/ mulch (%)	cover soill & rock (%)	seedlings	veg 1, unbranched	veg 2, branched	veg 3, branched, smaller than 5 cm	veg 4, branched, bigger than 5 cm	veg 5, branched, bigger than 10 cm	veg total/without seedling	ind. w. current inflorescences class 1	ind. w. current inflorescences class 2	ind. w. current inflorescences class 3	ind. w. current inflorescences class 4	ind. w. current inflorescences class 5	ind. w. current inflorescence total	ind. w. old inflorescences class 1	ind. w. old inflorescences class 2	ind. w. old inflorescences class 3	ind. w. old inflorescences class 4	ind. w. old inflorescences class 5	ind. w. old inflorescneces total	No. inflores./Ind old mean	No. inflores/Ind current mean	No. flowers	No. flowers / Ind	No. flowers / stalk
10.09.2007	total							4		2	1	2	6					2	2			1	1	2	4					
	1		5	2	2			2		1	1		4									1	1		2	2	0	0	0	0
	2		10	2	2							1	1					1	1					1	1	16	8	449	449	56.12
	3		5	2	2			2		1		1	4					1	1					1	1	25	8	244	244	30.5
	4		2	2	2			0	0	0	0	0	0																	
11.10.2007	total						107																							
	1						39																							
	2						10																							
	3						19																							
	4						23																							
31.05.2008	total						9	124	2	2	1	2	131																	
	1		5	5	2		1	51	1	1	1		54																	
	2		30	5	2		2	23				1	24																	
	3		30	10	2		5	22	1	1		1	25																	
	4		10	5	2		1	43					43																	

14 Lebenslauf

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Ausbildung

1987-1991:	Volkschule in Wien
1991-1999:	AHS Amerlingstraße
1999:	Matura
1999:	Beginn des Studiums Biologie an der Universität Wien
seit 2004:	Studium der Botanik - Schwerpunkte: Populationsbiologie, molekulare Phylogenie, Biogeographie, Ultrastrukturforschung - Teilnahme an den den Aufnahmearbeiten zur floristischen Kartierung Österreichs, sowie Exkursionen im In- und Ausland mit floristischem und biogeographischem Schwerpunkt
2007:	Beginn der Diplomarbeit: „Genetic patterns within and among populations of *Gypsophila fastigiata* subsp. *arenaria* in the Western Pannonian region", im Rahmen eines KIÖS-Projekts.

Sonstige Tätigkeiten

seit 2008:	Projektmitarbeit am ENSCONET (European Native Seed COnservation Network)
seit 2008:	Anstellung am Naturhistorischen Museum Wien für Aufnahme- und Datenbankarbeiten im Herbarium der Universität Wien
seit 2007:	Digitalisieren des chinesischen Herbarmaterials von Handel-Mazzetti im Herbarium der Universität Wien
seit 2006:	Tutorium bei den „Biologischen Einführungsübungen"
seit 2004:	Vermittlungsarbeit im Heilkräutergarten der Apotheke „Zum Löwen von Aspern" – Denkraum Donaustadt
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Publikation

Schönswetter P., Lachmayer M., Lettner C., Prehsler D., Rechnitzer S., Reich D.S., Sonnleitner M., Wagner I., Hülber K., Schneeweiss G.M., Trávnícek P. & Suda J. (2007) Sympatric diploid and hexapoid cytotypes of *Senecio carniolicus* (Asteraceae) in the Eastern Alps are separated along an altitudinal gradient. Journal of Plant Research 120: 721-725.

15 Danksagung

Zuallererst möchte ich mich bei Josef Greimler bedanken, mit dem ich alle Sammelreisen unternommen habe und der die Arbeit, vor allem in der Schlussphase, mit großem Einsatz betreut hat. Pavol Elias von der Universität Nitra und sein Team haben uns beim Suchen der slowakischen Populationen sehr geholfen. Weiters bedanken möchte ich mich bei Thorsten Englisch und Peter Schönswetter für wertvolle Tipps auf ihrem Gebiet. Ganz besonderer Dank gilt Carolin Anna Rebernig und Katharina Bardy, die mich bei der Laborarbeit und bei den Analysen unterstützt haben. Ich bedanke mich auch bei David Prehsler für die Unterstützung bei der Laborarbeit und seine kritischen Bemerkungen zur Arbeit. Vera Lachmayer möchte ich für die Hilfe bei den Bestäubungsversuchen und den demographischen Erhebungen in Lassee danken. Mein Dank gilt auch Lea Atanasova und Gudrun Kohl. Darüber hinaus danke ich Markus Schlüter und Johanna Zechner für ihre Unterstützung in jeder Hinsicht.

Abschließend möchte ich mich bei meinen Eltern bedanken; ihnen möchte ich diese Arbeit widmen.

Der Kommission für Interdisziplinäre Ökologische Studien (KIÖS) der Akademie der Wissenschaften danke ich für die finanzielle Unterstützung dieses Projekts.