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DIPLOMARBEIT

Regional dynamics of *Calamagrostis pseudophragmites* on riverbanks in the NP Gesäuse inferred from AFLPs

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Verfasserin	Lea Atanasova
Matrikel-Nummer	0504094
Studienrichtung	Ökologie
Betreuerin / Betreuer:	Ao. Univ.-Prof. Dr. Josef Greimler

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Abstract

Calamagrostis pseudophragmites (Poaceae) is a pioneer reedgrass that grows on riverbanks of alpine rivers, and is one of Austria's endangered species because of the increasing river regulations and increasing number of hydropower plants. The population system of *C. pseudophragmites* in national park Gesäuse was investigated to assess the population clonal structure and role of clonal migration along the river Enns, as well as to determine the genetic structure, differentiation and mode of regional dynamics within the population.

The present survey suggests that the sexual reproduction is predominant in this *C. pseudophragmites* population and that vegetative propagation is important on the subpopulation level. The overall genetic differentiation between subpopulations is moderate, yet both Haslau subpopulations differentiate very well from other eastern subpopulations. So there is notable gene flow in the population probably mostly due to pollen flow. Seed (or ramet) dispersal occurs between all subpopulations. The population of *C. pseudophragmites* in Gesäuse provides evidence for metapopulation dynamics as extinction and (re)colonization processes were notable in the few years since the study of Kammerer (2003).

As long as the regeneration of the population is possible and the population does not suffer larger size reduction no special genetic threat of the survival of this species in Gesäuse is recognized. Yet further constant monitoring and higher protection of the sensible habitat of *C. pseudophragmites* is proposed.

1 Introduction

1.1 Riverbanks as changing habitats

The riverbanks represent a small part of the floodplain, yet, because they are the lowest part, they are most affected by water management (Roberts 1998). Consequently, the riverbanks often show biological differences as a response to water level oscillations (Roberts 1998). The variation in water level, the alternate states of being wet and dry, determines the structure of the riverbank plant communities (Roberts 1998). These fluctuations are therefore necessary for the existence of this habitat type, though they must be caused naturally (river dynamics), because they only assure reversibility in the habitat. Human interference such as river regulation, excavation of gravel, mass tourism, (rafting, swimming and other recreation activities) can lead to irreversible effects in this fragile habitat. This habitat type was once frequent in Austria, but nowadays the quality in some regions is decreasing. Furthermore, the riverbanks in nature protection areas are difficult to conserve while the river dynamics outside the areas is disturbed.

In hydrology, hydropower is defined as the power of water on the riverbed and the riverbanks. The force of water causes the relocation (erosion, allocation) of sediment and other materials along the river. These are natural processes which occur in the riparian zone. The riverbank habitats and the organisms living in this area are adapted to the fluctuation of the water level and many of them do not survive without these dynamics. Hydro power stations and river regulations have greatly affected these habitats and their plant communities. Channelization and construction of hydropower plants lead to a truncated fluvial system (Hohensinner et al. 2004) and, respectively, to loss of floodplain habitats.

Plant communities of the order Epilobietalia fleischeri (class Thlaspietea rotundifolii) form the pioneer vegetation of gravel and sandy alluvium habitats along mountain streams and rivers in the alpine forelands and on the front ends of the glaciers, including communities of Myricario-Chondrilletum chondrilloidis, Epilobio dodonei-Scrophularietum caninae, Calamagrostietum pseudophragmitis and Epilobietum fleischeri (Pott 1995). Characteristic species of the order Epilobietalia fleischeri are *Epilobium fleischeri*, *Epilobium dodonaei*, *Hieracium staticifolium* and *Hiracium piloselloides*, as well as some differential species such as *Myricaria germanica*, *Calamagrostis stricta* and *Scrophularia canina* (Pott 1995). These alluvial gravel plant communities are dependent upon periodical habitat disturbance.

1.2 Local and regional population dynamics

River corridors serve as important pathways for plant dispersal (Johannson et al. 1996). Plants that grow in the water or at the riverside are likely to utilize water to disperse their seeds. Water movements connect all riverbanks transporting the majority of diaspores downstream (Fér & Pfosser, unpublished). Riverbank habitats are often fragmented along the rivers, and provide patchy habitats for organisms. Natural disturbance events are important factors that assure occupancy mostly by the pioneer species and not by species of later successions. Water action provides both the connection between several patches and the suitable habitats for occupation. Habitat patches with local extinction and colonization are often typical for these dynamic river systems (Jacquemyn et al. 2006). The alternating colonization and extinction suite the definition of metapopulation dynamics, yet the stability of such a system depends on the existence of a certain amount of suitable but presently unoccupied habitat (Freckleton & Watkinson 2003). These authors and Watkinson et al. (2000) assert that the "different types of populations differ fundamentally and qualitatively in the nature of their regional dynamics" and proposed that true metapopulation dynamics are not common in plants although the individuals are distributed in a patchy manner across the landscape. Also Hanski (2002) stressed that the spatially patchiness is not a synonym for metapopulation structure and that the patterns are not of such importance as the processes behind the definition of metapopulation.

THE METAPOPULATION CONCEPT: Dispersal of different kinds (by seeds, spores or vegetative) enables the immigration of suitable sites by new individuals. Every population shows particular dynamics, which is not only influenced by the local processes (birth, death, intra- and interspecific competition, self-thinning, regulation and limitation etc.) but more so by the regional processes. Regional processes, such as immigration and emigration, occur in a system of populations and are meaningful just in a spatial context of several populations (Nentwig et al. 2004). Distance has a great impact on reciprocal exchange of individuals between populations, so the more distant populations are from each other the smaller is the chance of exchange. If each individual not dependent on population size has the same probability d to emigrate by the time t, the number of the emigrated individuals is $dN_t(t)$. Population i is not just a source but also a recipient of the individuals from the neighbor populations ($i-1$ and $i+1$). The emigration of the individuals from $i+1$ to i leads to $0.5\ dN_{t+1}(t)$, which means that half of the individuals get to one of

the two neighbor populations (no mortality during emigration assumed). Therefore, the population size by the time *t+1* and population growth λ (Nentwig et al. 2004) is:

$N_t(t+1) = \lambda_i N_i(t) - dN_i(t) + 0.5\, dN_{i-1}(t) + 0.5\, dN_{i+1}(t)$.

The dynamics of a population is given as the ratio N_{t+1}/N_t called the annual or the finite rate of increase (λ) (Silvertown & Charlesworth 2001). Population growth is dependent upon the rate at which a population or species reproduces itself (reproduction rate or R_0). R_0 can be calculated for simple population models if the average number of offspring per individual in time (the birth rate *b*) is multiplied by the average lifetime of an individual (value reciprocal of the death rate) providing $R_0 = b / d$. The reproduction rate is mean number of born individuals and describes population growth from generation to generation (Nentwig et al. 2004). Habitat quality may affect the population growth, yet the presence of a population is not an indicator for habitat (patch) suitability for the species – the population might be present because immigration highly influence the local growth rate (Hanski 2002). Immigration and emigration are important characteristics of population systems, because the local populations can go extinct, but can also be colonized any time. If this is the case the metapopulation concept can be applied, where populations persist because of the flow of the migrants. These kinds of populations are not influenced only by local dynamics, but also by underlying regional processes. Silvertown & Charlesworth (2001) distinguish three kinds of regional dynamics:

- A **metapopulation** as a "network of local populations connected by dispersal, whose persistence depends upon reciprocal but unsynchronized migrations between local populations".
- **Source-sink** dynamics dominate if migration occurs uniformly in one-way, from source populations, where R_0 of population > 1, to recipient population where $R_0 < 1$.
- **Remnant populations** prevails if the population is "able to persist without immigration through periods that are unfavorable for recruitment" as long-lived adults or seeds in the soil.

Source populations disperse a net number of individuals to the less viable sink populations (Howe et al. 1991). If the emigration rate of a source population exceeds the rate of the sink population, then it is possible that the sink population overgrows the source population regarding size, yet this may be improbable in nature (Hanski 2002). The **source-sink concept** assumes that the migration between source and sink should be greater than the migrations among source or among sink habitats and that the number of migrants among source habitats should be higher then those among sink habitats (Dias et al. 1996).

The term **metapopulation** was established by Levins (1970) to describe a "population consisting of many local populations, in the same sense in which a local population consists of individuals" (Hanski 2002). This classical metapopulation model assumes that the equal-sized patches are equally connected to each other by migration, yet the model is rarely applicable in nature (Silvertown & Charlesworth 2001). Allowing two sizes of patches (large and small) Hanski (1985) created a simple extension of the Levins` model and found that the proportion of populated sites can be greater than that predicted by Levins (Silvertown & Charlesworth 2001). Though some patches may go extinct, the regional persistence of the metapopulation depends on the ability of patches to be re-colonized. According to Silvertown & Charlesworth (2001) the following features are required for a metapopulation: "(i) natural populations do have turnover (i.e. go locally extinct), (ii) local population dynamics are not synchronized across populations, and (iii) seed dispersal between populations, rather than a seed bank, is the main source of colonists (otherwise the remnant population model would apply)". Freckleton & Watkinson (2002) stressed that a threshold number of density of suitable patches are the main requirements for large-scale metapopulation persistence. Freckleton & Watkinson (2003) and Watkinson et al. (2000) thus proposed a different classification on the regional scale: (i) *metapopulations* in the classic sense, which persistence depend on patch colonization, extinction and recolonization; (ii) the *regional ensembles*, "systems of essentially unconnected local populations persisting in an ill-defined mosaic of suitable and unsuitable habitat"; and (iii) *spatially extended populations*, "essentially a single extended population occupying large tracts of suitable habitat, but whose regional dynamics are essentially a simple extension of local dynamics".

The dynamic river systems can serve as excellent models for observation of metapopulation turnover, genetic diversity and differentiation (Jacquemyn et al. 2006). Out of the genetic distances between populations one can indirectly reveal the amount of migrating diaspores (gene flow) among populations (Fér & Pfosser, unpublished), or the distribution of genetic variation among (sub)populations can serve as an estimate of gene flow (Ouborg et al. 1999).

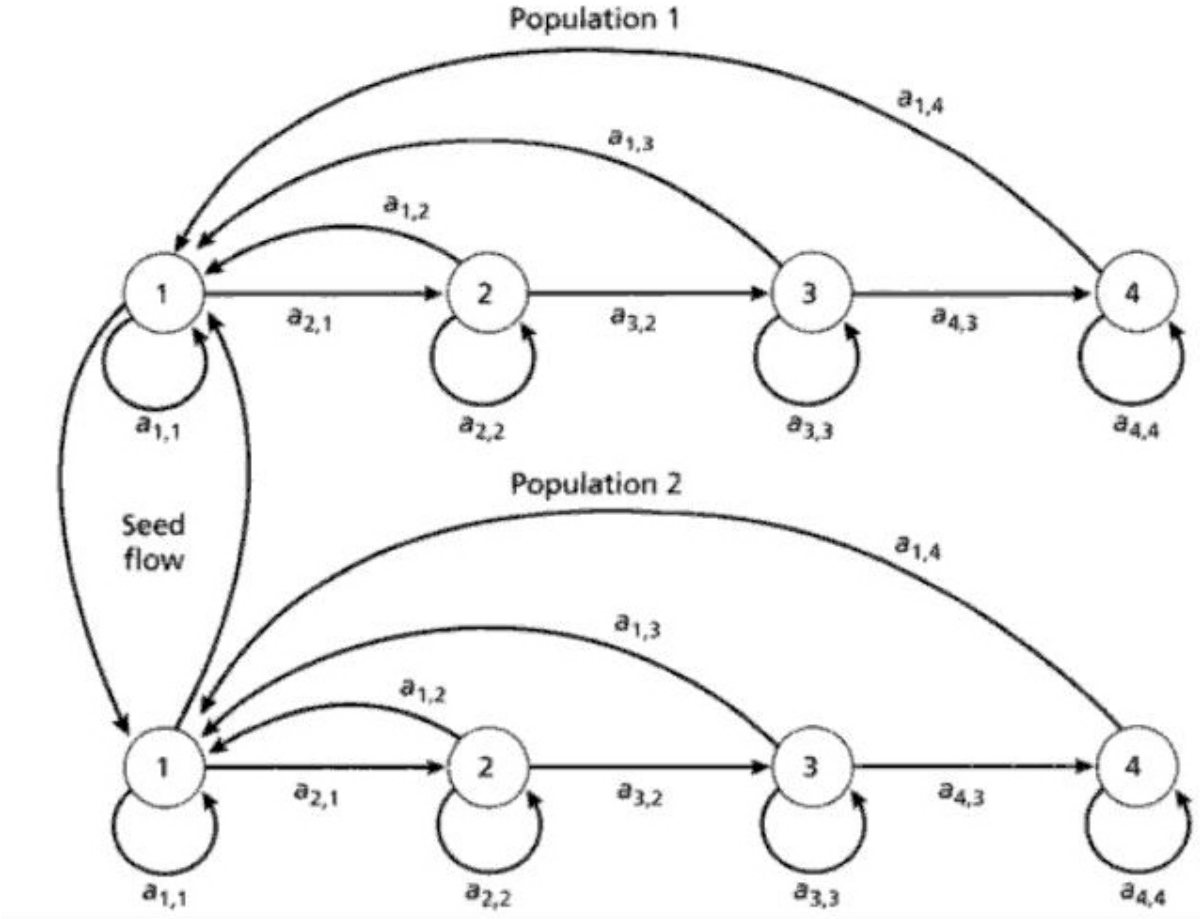

Figure 1: Example of metapopulation dynamics, where life cycle graphs of two local populations are linked by seed dispersal. The figure shows four stage-classes of *Primula vulgaris*, where class 1 are seeds. Values $a_{1,x}$ are fecundities, values $a_{x,x}$ are the rates of survival for plants that do not change stage class and values $a_{x+1,x}$ are the rates at which plants move up a stage class. The illustration is taken from Silvertown & Charlesworth (2001).

Many river plants are able to disperse vegetative diaspores beside seed dispersal, e.g. detached portions of rhizomes or even unrooted whole plants (Fér & Pfosser, unpublished). In extreme environments, more than 90% of species reproduce vegetatively e.g. using propagules such as rhizomes, stolons, or bulbils (Escaravage et al. 1998). The plants in clonal populations can be recognized at two different organizational levels: genets and ramets and thus represent special problems for population analyses (Escaravage et al. 1998). A genet corresponds to an individual that originated from sexual reproduction and can extend asexually by producing ramets. The ramet is an identical member or modular unit of a clone that may or may not be separated from the parent organism (Pornon et al. 2000). High degree of genetic monomorphism is often a consequence of high asexual reproduction (Pornon et al. 2000).

EXTINCTION OF POPULATIONS: At regional scales, seed dispersal is important to maintain the colonization of new sites, and decrease the possibility of local populations' extinction (Ouborg et al. 1999). The Levins model describes a species as rare, when the extinction rate is high and the colonization rate is low, yet not so low that the metapopulation would go extinct (Hanski 2002). Rare species have generally restricted distributions, but the individuals in populations are abundant (Silvertown & Charlesworth 2001). This suggests that the local dynamics that affect the abundance within the populations are not the cause for such rarity, but the insufficient regional dynamics are decreasing the population number (Silvertown & Charlesworth 2001). Extreme long-distance migration and random disturbance lead to the case, where most of the larger sites are occupied, however, with low abundance (Hanski 2002). Quite contrary are species with short-range migration and high disturbance (Hanski 2002). These species occupy only a small part of the sites, yet with high abundance and vagility. The distribution and abundance of the rare species is anyhow mostly determined by the level of disturbance and less by the range of migration (Hanski 2002). The most actual risk of population extinction is due to a small population size (Hanski 2002). Yet the populations that grow on large habitat patches might have lower extinction rates because the mosaics of habitats inside the patch are unlikely to become unfavorable at the same time (Hanski 2002).

The species and the specific riverbank plant communities are not endangered only because of river regulations, hydro power stations, aforestation etc. Together with increasing human activities alien plants have become one of the most important cause of global biodiversity loss after direct habitat destruction. In general, shoreline river habitats offer great conditions for the establishment of neophyte populations (Bertrand 2004). Less vegetated habitats and unutilized resources increase the invasion rate (Fridley et al. 2007) on the riverbanks. In addition, sunlight and eutrophication of the river enable integration of alien species. Fridley et al. (2007) emphasized that there is some evidence that a single species cannot utilize all available resources or space in seasonal or yearly fluctuating environments. Furthermore, communities with only a few species are less resistant to invasions as more diverse communities, which more likely include particular invasion-resistant species (Fridley et al. 2007).

1.3 The population genetics approach

A population as defined by Silvertown & Charlesworth (2001) is "a collection of individuals belonging to the same species, living in the same area". This definition has

genetic (belonging to the same species) and a spatial component (individual living in the same area), "but populations are neither genetically nor spatially homogenous" (Silvertown & Charlesworth 2001). Populations have four kinds of processes occurring simultaneously inducing a flux of individuals. Birth (*b*) and death (*d*) of individuals in a time interval Δt together with the number of immigrated (*i*) and emigrated (*e*) individuals are four primary population processes that determine a population size in time $t+\Delta t$ (Nentwig et al. 2004). Hence, the change of population size from time t to the moment $t + \Delta t$ results in the fundamental equation (Nentwig et al. 2004):

$N(t + \Delta t) = N(t) + b - d + i - e.$

If the population size is decreasing, then mortality and emigrations have outweighed natality and immigration (Begon et al. 1996). The following equation describes exponential increase of a population size after x years for a population that starts with N_t individuals (Silvertown & Charlesworth 2001):

$N_{t+x} = N_t e^{rx}$,

where *e* is the base of natural logarithms (not rate of emigration) and *r* the intrinsic rate of increase. The balance between parameters determines whether the population remains stable ($N_{t+1}=N_t$ and $\lambda=1$), increases ($N_{t+1}>N_t$ and $\lambda>1$) or decreases ($N_{t+1}<N_t$ and $\lambda<1$) (Silvertown & Charlesworth 2001).

Some populations contain smaller groups, called subpopulations or demes, which may be partially isolated from each other (Page & Holmes 2000). A genetic subdivision or genetic structure in plant population occurs often, as the limited mating between neighbors is very common in sessile organisms (Silvertown & Charlesworth 2001). Sexual reproduction assures genetic diversity by recombination and gives sexual populations a number of evolutionary advantages, as for instance the removal of deleterious mutations (Page & Holmes 2000). The accumulation of deleterious mutations may generate declining fitness in asexual organisms as individuals without deleterious mutations in the population become rare (Page & Holmes 2000).

An important measure of genetic diversity is the proportion of loci at which polymorphism is found (Silvertown & Charlesworth 2001). A locus is usually classified as polymorphic when the most common allele occurs at a frequency of less then 99% (sometimes 95%) (Silvertown & Charlesworth 2001). Another measure is gene diversity H_e that reveals the chance for two randomly chosen alleles to be different and can be calculated from the allele frequencies as (Silvertown & Charlesworth 2001):

$H_e = 1 - \Sigma p^2_i$,

where p_i is the frequency of the *i*th allele type. The p^2_i is the chance that the two selected alleles will be of the same type, so the result reveals the chance that two alleles in the sample are not the same (Silvertown & Charlesworth 2001). Bonin et al. (2007) listed several parameters which are in common usage for calculation of genetic variation based on AFLPs. They include various coefficients of similarity (Jaccard, Dice, or Simple-matching coefficient), the Shannon index (Shannon 1948) and the nucleotide diversity (Clark et al. 1993).

Although population subdivision leads to local non-random mating, gene flow via pollen flow or seed dispersal may induce gene migration between subpopulations (Page & Holmes 2000) New genetic variation in (sub)populations increases heterozygosity within them (Page & Holmes 2000). Therefore "the rate of gene flow is inferred from the amount of genetic differentiation among populations" (Ouborg et al. 1999). Ouborg et al. (1999) distinguish between dispersal and gene flow, although they are clearly related. They define dispersal as movement of seeds or other propagules able to establish themselves, while gene flow as movement of genes occurs with both seed and pollen migration. The level of genetic differentiation is calculated with the parameter F_{ST} (Wright 1978), which increases with increasing isolation between (sub)populations (Ouborg et al. 1999). Assuming a large number of demes (subpopulations), the relationship is (Silvertown & Charlesworth 2001):
$F_{ST} = 1/\ 1+4N_em$,

including a constant migration rate m (Slatkin 1981) and effective population size N_e. The F_{ST} is a reasonable approximation to the true mode of gene flow estimated among populations and can compare population structure of different plants, even if it is calculated with different genetic markers (Silvertown & Charlesworth 2001). Using dominant markers, like RAPD and AFLP, estimation of F_{ST} values is difficult because they do not allow estimation of allele frequencies (Ouborg et al. 1999). Another approach for estimating F_{ST} in dominant markers is known as AMOVA (Analysis of MOlecular VAriance) (Excoffier et al. 1992) and based on comparisons between multilocus profiles of different individuals (Ouborg et al. 1999). Corander et al. (2003) introduced Bayesian Analysis for estimation of the degree of population differentiation using multilocus molecular data and the geographical information provided by the sampling design. The statistical method based upon the Hardy-Weinberg equilibrium (HWE) and linkage equilibrium between loci within each observed population (Corander et al. 2003). Inside of the populations the unlinked marker loci will provide information about population

substructure assuming that genetic markers within each population are in HWE (Corander et al. 2003).

1.3.1 Population genetic analysis

Numerous highly variable markers were developed since 1966 for population genetic analysis. Nowadays many types of molecular markers are used for genetic analyses, among others minisatellite fingerprints and microsatellites or SSR (simple sequence repeats), AFLP (amplified fragment length polymorphism), RFLP (restriction fragment length polymorphism) and RAPD (random amplified polymorphic DNA) (Ouborg et al. 1999). These molecular markers have different properties, displaying different amounts of variation (Ouborg et al. 1999).

The amplified length polymorphism (AFLP, Vos et al. 1995) technique is a PCR- based approach. This technique involves a digestion of genomic DNA and PCR amplification of gained fragments (Ritland & Ritland 2000). First DNA has to be cut with a pair of restriction enzymes: with a frequent and the rare cutter. Frequent cutter recognizes a sequence of 4 base pairs, while the rare cutter recognizes 6 base pairs. Restriction fragments are amplified with the help of adapter and restriction site sequence as target sites for primers. Ligated adaptors generated template DNA for preselective amplification. Selective amplification of DNA with additional nucleotides at the ends of the AFLP primers follows to reduce the number of bands. Only those restriction fragments, in which the nucleotides on the restriction site match the selective nucleotides, will be amplified. The amplified fragments are then separated and visualized on acrylamide gels. The number of fragments obtained depends on the genome size. AFLP markers can be useful to tool detect variability and can provide data for measures of genetic diversity (i.g. proportion of bands that individuals share) comparing different populations (Silvertown & Charlesworth 2001).

1.4 Gesäuse, the study area

In 1958 Gesäuse was declared the first nature protection area in Styria. Since then this wild and fascinating part of the Enns valley is known as one of the last undamaged and rather natural river sections (Kerschbaumer & Marek 2005). Gesäuse was declared national park in 2002 and since then it is the third largest national park in Austria.

River Enns is the backbone of the Gesäuse, so its hydrology has a great impact on the life inside the national park (Kammerer 2003). Temperate nival regime with marginal changes

within a seasonal cycle is typical for Enns. Characteristic is a drainage minimum in winter and a maximum in May (Kammerer 2003). Absence of glacial areas in its catchment area is a reason for low drain data in July and August (Kammerer 2003). Since the early 20th century, as the hydropower was put to profitable use (Tamerl 2006), the water capacity of river Enns changed. Nowadays in total eight hydropower plants (Tamerl 2006) are build on the whole length of the river Enns up to the border of Upper Austria. As already mentioned neophytic plants invade more and more natural habitats and are a threat for populations of native species and plant communities. Relevant for Gesäuse are five neophytes that can be found along the river Enns: *Fallopia japonica*, *Impatiens glandulifera*, *Impatiens parviflora*, *Solidago canadensis* and *Solidago gigantea* (Kammerer 2003).

In the vegetation-ecological study of Gesäuse riverbanks Kammerer (2003) pointed out that the fine-sand riverbanks of Enns are the most sensitive habitats in the national park, yet the most popular resting places after recreation. These fine-sand aggradations represent a natural habitat for the reedgrass *Calamagrostis pseudophragmites*. Moreover, some of the habitat locations are assessed to be potentially suitable for *Myricaria germanica*, which became extinct in native locations of Styria. Hence, this specific habitat in Gesäuse needs specific protection (Kammerer 2003).

1.5 The study plant

Calamagrostis pseudophragmites (Haller f.) Koeller (Poaceae) is a perennial herb with erect 20-150 (-200) cm long culms and elongated rhizomes for clonal dispersal (Conert et al. 1989). The ligule is a 4-10 mm long eciliate membrane (Clayton et al. 2006). Leaf-blades are flat, or conduplicate, 15–35 cm long (Clayton et al. 2006) and 3-8 (-10) mm wide (Conert et al. 1989). Leaf-blade surface is glabrous (Conert et al. 1989). Glumes are disproportional in length; the awn arises below the two-dentate incision of the lemmas; panicle 10-40cm long; spikelets solitary, anthers with well developed pollen, fruit 2-2.3 mm long, glabrous (Figure 2) (Conert et al. 1989, Jäger & Werner 2002, Fischer et al. 2005, Clayton et al. 2006). *C. pseudophragmites* is a tetraploid species, the chromosome number is 2n=28 (Conert et al. 1989). In all *Calamagrostis* tetraploids no disruption in meiosis was found. All of the tetraploids are allogamous (Conert et al. 1989).

Figure 2: *Calamagrostis pseudophragmites a*- spikelet; *b*- seed; *c*- inflorescences. Data source: figure *a* and *b*: *seeds.eldoc.ub.rug.nl/root/Poaceae/Calamagrostis/*; figure c: Photo J. Greimler 2006.

Calamagrostis pseudophragmites forms almost no varieties (forms) in Europe. A differentiation on the base of the color of spikelets is possible, yet the distinguishing of *C. pseudophragmites* f. *rubens* (purpure spikelets) and f. *pallens* (yellow-green spikelets) is trivial (Conert et al. 1989). In Asia more subspecies are distinguished beside *C. pseudophragmites* ssp. *pseudophragmites* that grows in Europe (Conert et al. 1989).
Calamagrostis pseudophragmites occurs mainly in temperate regions of the Northern hemisphere. It is distributed in central, southwestern, southeastern, and eastern Europe; in Siberia, Russian Far East and Middle Asia, Caucasus, western Asia, China, Mongolia, and eastern Asia. The most tropical areal of this species is in India (Clayton et al. 2006).

DISTRIBUTION IN AUSTRIA: Conert (1989) reports that *C. pseudophragmites* was widespread, but rare and scattered in Austria (with a lack in Burgenland), however, declining where the rivers have become eutrophic and regulated. According to Fischer et al. (2005) *C. pseudophragmites* is scattered or rare in the colline-montane zone of Austria, lacking in Burgenland. The data from Niklfeld & Schratt-Ehrendorfer (unpublished data for the grid map of Austrian Flora) show the distribution of *C. pseudophragmites* from observations since 1945 (Figure 3).

The nearest sites to Gesäuse were searched for *C. pseudophragmites* populations. On the riverbanks along the Enns west of Hieflau and north of Hieflau (Greimler, pers. comm.) as well as along Große Fölzgraben in Eisenerz no ramet was found. *C. pseudophragmites* is

probably extinct in the Murtal locations (Scharfetter, pers. comm.) and declining along Salzachtal (Wittmann, pers. comm.). In 1991 few groups of C. *pseudophragmites* were observed in Almtal between Peham and Auinger south Grünau, on west hillside of Bramberg southwest from Lake Almsee, in Sulzgraben and in Hetzau (mapping data: Niklfeld & Schratt-Ehrendorfer, unpublished). These probably next populations could not be sampled.

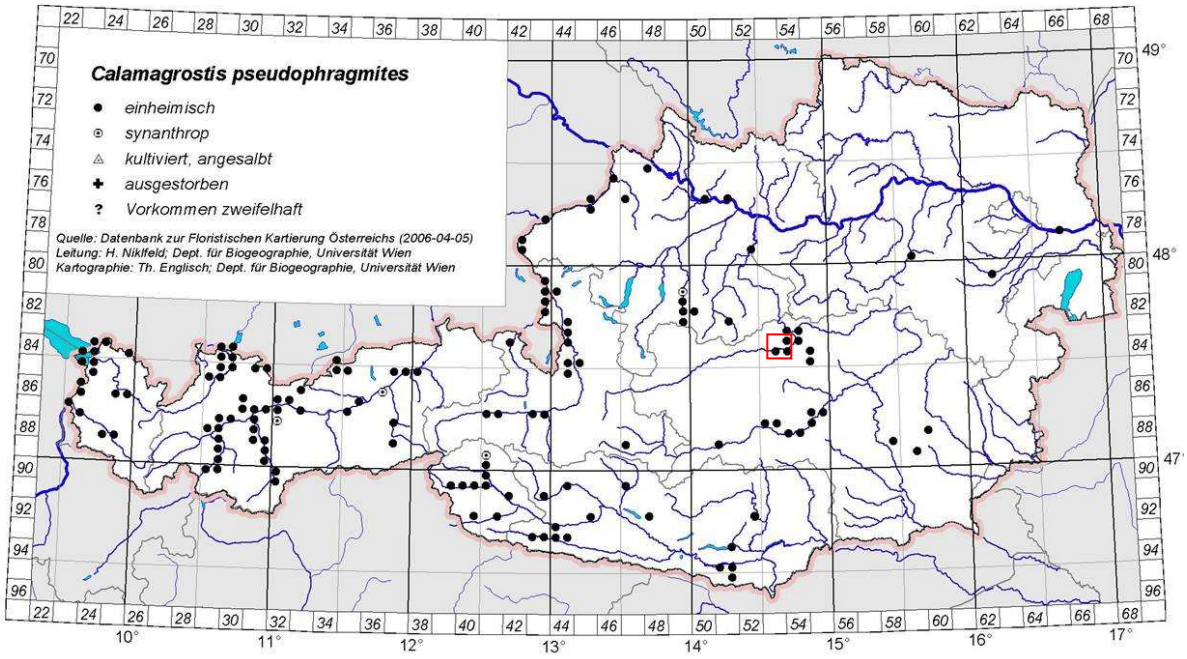

Figure 3: Grid map showing the distribution of *Calamagrostis pseudophragmites* in Austria (Niklfeld & Schratt-Ehrendorfer, unpublished data for the grid map of Austrian flora, without literature evaluation). Population of *C. pseudophragmites* in national park Gesäuse is marked with a frame. Note: the dots indicate observations since 1945!

SYNTAXONOMY: *Calamagrostis pseudophragmites* is the characteristic species of the association Calamagrostietum pseudophragmitis. One can find this pioneer community on fine sand or silty sediments of the riverbanks of unregulated rivers in the Alps (Pott 1995). *C. pseudophragmites* prefers empty banks, where sand and slit have accumulated due to slow water motion (Pott 1995). Because of the ability to grow with rhizomes this reedgrass species can spread very rapidly after flooding and thus dominate the community. Pott (1995) assigned the Calamagrostietum pseudophragmitis to the order Epilobietala fleischeri, while Mucina et al. (1993) incorporated this community to the order of Phragmitetalia under Phalaridion arundinaceae alliance.

In Austria the Calamagrostietum pseudophragmitis was found along the river Lech in Tyrol (Müller & Bürger 1990), along river Ill in Voralberg (Grabherr & Polatschek 1986),

along Saalach and Salzach in Salzburg (Wittmann& Strobl 1990). Yet, in the regions Voralberg and Salzburg this community was considered highly endangered and close to extinction (Mucina et al. 1993).

CONSERVATION: Extinction of *C. pseudophragmites* is already noted in the Netherlands (Conert 1989); in Slovenia this grass was not found since the second half of last century (Martinčič et al. 2004). Rennwald (2000) classified the Calamagrostietum pseudophragmitis in Germany as "highly endangered"; the same categorization used Wittmann & Strobl (1990) for province Salzburg in Austria. Kammerer (2003) assumed that this may also be the case in province Styria. Many countries have added C. *pseudophragmites* to Red Lists of Threatened Species. In Switzerland this plant is classified as near threatened[1] (NT). Niklfeld & Schratt-Ehrendorfer (1999) put this grass on the Austrian Red List as endangered species in Austria. In Germany as well *C. pseudophragmites* is on the Red List (Bundesamt für Naturschutz Deutschland 2008) of endangered species. Holub et al. (1979) included this species to the list of highly endangered species of Czech Republic (Conert 1989). *C. pseudophragmites* was not found in the data base of IUCN Red List of Threatened Species (International Union for Conservation of Nature and Natural Resources 2008), however, the plant community Calamagrostietum pseudophragmitis is protected under FFH-Habitat type 3220 (Table 1; *Alpine rivers and the herbaceous vegetation along their riverbanks*) included in Natura 2000 (Kammerer 2003).

Table 1: Habitat directive annex I (and Bern convention resolution N° 4 (1996)). Habitat type 3220/24.222 Calamagrostietum pseudophragmitis is included in FFH- Habitat Directive under NATURA 2000.

CODE	NAME	COMMENTS
3220 24.221. 24.222	ALPINE RIVERS AND THE HERBACEOUS VEGETATION ALONG THEIR BANKS 3222/24.222 Calamagrostietum pseudophragmitis: Alluvions along the Enns on the west side, pioneer formations on the riverbanks with *C. pseudophramites*, *Salix eleagnos*, *S. purpurea*, dealpine species; with *Salix – Alnus* shrubs.	Important, yet rare spawn biotope with disturbance through sport and gravel removal. A habitat of pioneer species *Calamagrostis pseudophragmites.*

[1]A species is near threatened when it does not currently qualify for critically Endangered **(**CR), endangered (EN) or vulnerable (VU) status, but is likely to qualify for a "threatened" category in the near future (Swiss Federal Office for the Environment 2007).

1.6 Specific aims of the research

In this study the structure of genetic variation among and between subpopulations of the pioneer plant species *Calamagrostis pseudophragmites* will be investigated using a dominant DNA marker. For this purpose AFLP (Amplified Fragment Length Polymorphism (Vos et al. 1995) will be used.

In detail the investigations aim to:

(1) Assess the clonal structure within local subpopulations and the role of clonal migration between them along the river.

(2) Determine genetic differentiation among local subpopulations and assess the amount of gene flow among them.

(3) Determine the genetic structure and diversity within local subpopulations.

(4) Infer the most likely mode of regional dynamics (metapopulation or source sink or other dynamics) among the local subpopulations.

(5) Extract conclusions on the viability of the Gesäuse population system and propose specific conservation measures if necessary.

2 The population system in NP Gesäuse

Figure 4 shows (marked) locations of *Calamagrostis pseudophragmites* subpopulations on the six sand and gravel river banks in national park Gesäuse identified by Kammerer (2003). Further details on the six sites are given in Table 2.

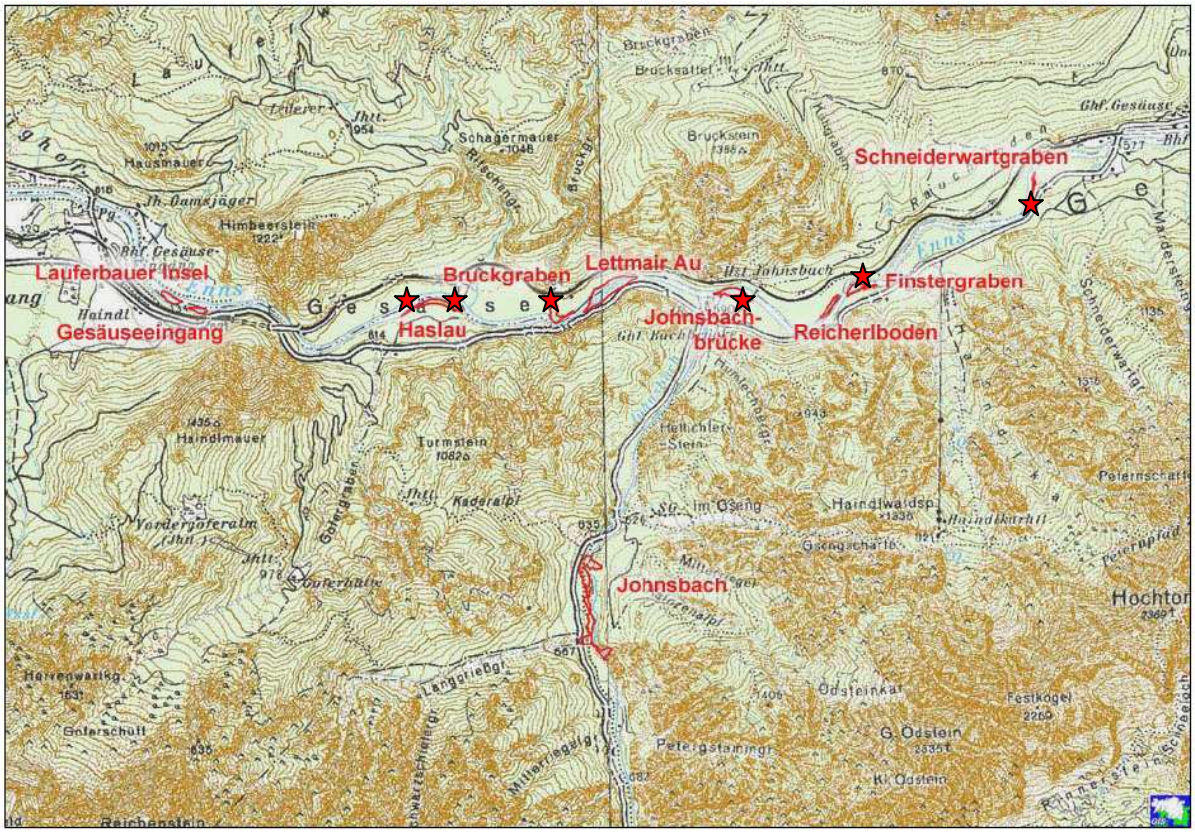

Figure 4: Map showing riverbanks of national park Gesäuse. Riverbanks where *Calamagrostis pseudophragmites* was found are marked with a star. Data source: Kammerer (2003).

On the six sites (riverbanks) Haslau "island" (west and east), Bruckgraben, Johnsbachbrücke, Finstergraben and Schneiderwartgraben *Calamagrostis pseudophragmites* was sampled for the present study.

2.1 Locations and population status in 2003

Haslau is a long ephemeral island that is separated from the riverside depending on the water level (Figure 5). On the west side there is one more or less vegetation-poor gravel bank with moderate accumulation dynamics. Gravel is interspersed with sand and often overgrown by mosses (Kammerer 2003). In the middle of the "island" a *Salix* grove is spreading out, ending on the west side in an alluvium of deadwood. On slightly broader sites on the west and east head of the "island" that are (or were) more exposed to the river

dynamics one can find the subpopulations of *Calamagrostis pseudophragmites* with the largest on the west side (Kammerer 2003; pers. obs.) in Gesäuse. The largest subpopulation is distributed in smaller patches all over the west side of the island and includes about thousand ramets in contrast to the eastern subpopulation, where there may be a hundred (Kammerer 2003). On the west site *Calamagrostis pseudophragmites* is dominant together with *Deschampsia cespitosa*, *Festuca arundinacea*, *Petasites hybridus*, and *Agrostis stolonifera*. In-between some juvenile *Salix eleagnos* and other *Salix* species occur sporadically. This constitutes a variant of the rare plant community Calamagrostietum pseudophragmitis. The sand and gravel bank on the east side is densely covered by tall herbs and grasses including *Calamagrostis pseudophragmites*.

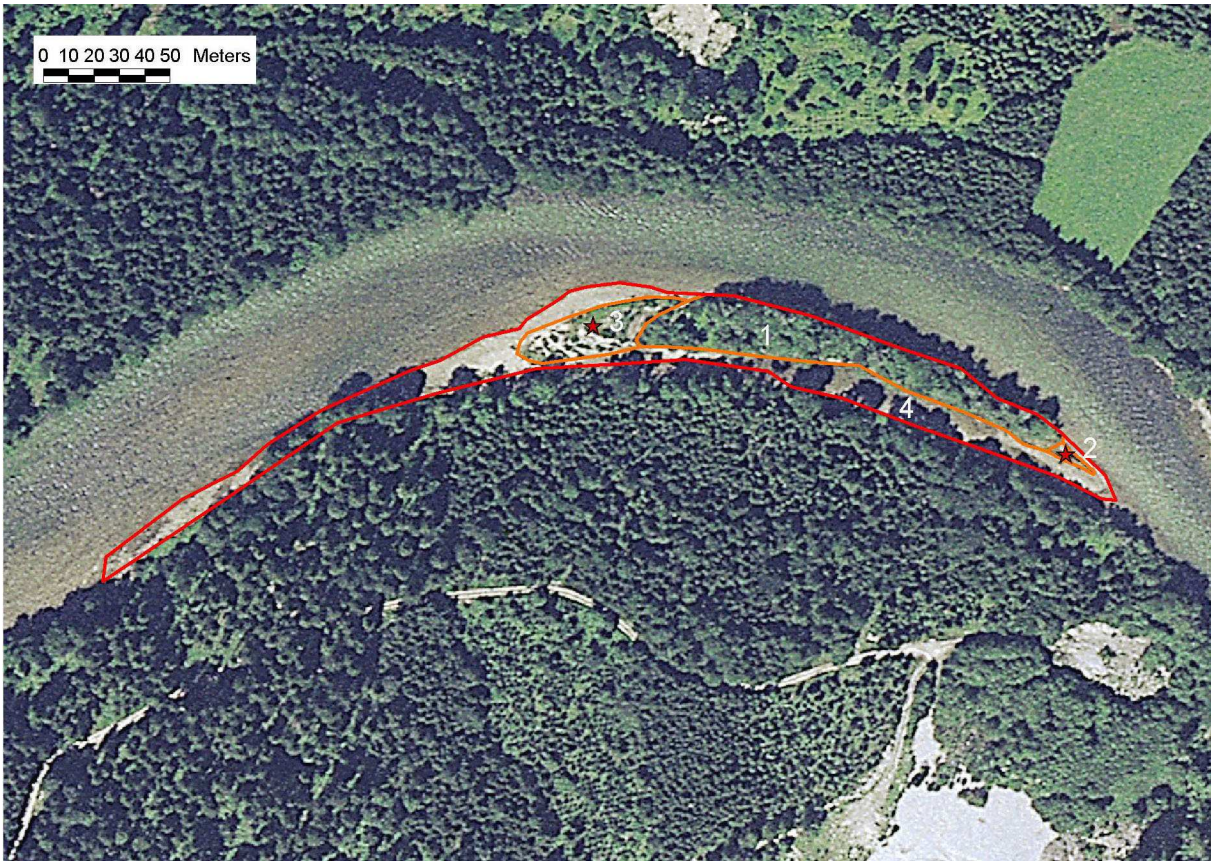

Figure 5: Haslau "island" east of Gofergraben with the west and east sites where sand and gravel accumulated. The colored lines show study area and boundaries of biotopes investigated by Kammerer (2003). The stars mark the presence of *Calamagrostis pseudophragmites* in 2003. Data source: Kammerer (2003).

Bruckgraben site is an impressive alluvial cone on the orthographic left bank of Enns (Figure 6), showing the patterns of high disturbance by the brook coming from the north (Kammerer 2003). In spring-time the snow water transports the mass of coarse gravel down the Buchstein massif and deposits the material in Bruckgraben and Enns respectively. So the pioneer vegetation in this area is regularly damaged. In the alluvial cone two small "forest

islands" are formed with *Picea abies*, *Salix* sp. and *Alnus* sp. with the understory of a Carici albae-Fagetum. This area is mainly affected by the dynamics of spring snow melting water from the mountains and not as usual by the dynamics of river Enns. Kammerer (2003) found two small patches (1 + 0.25 m^2) of *Calamagrostis pseudophragmites* together with few *Salix eleagnos*. These plants did not grow on the gravel bank, but on the very edge to the forest. The third patch was found 50 m eastern directly on the forest margin along the Enns.

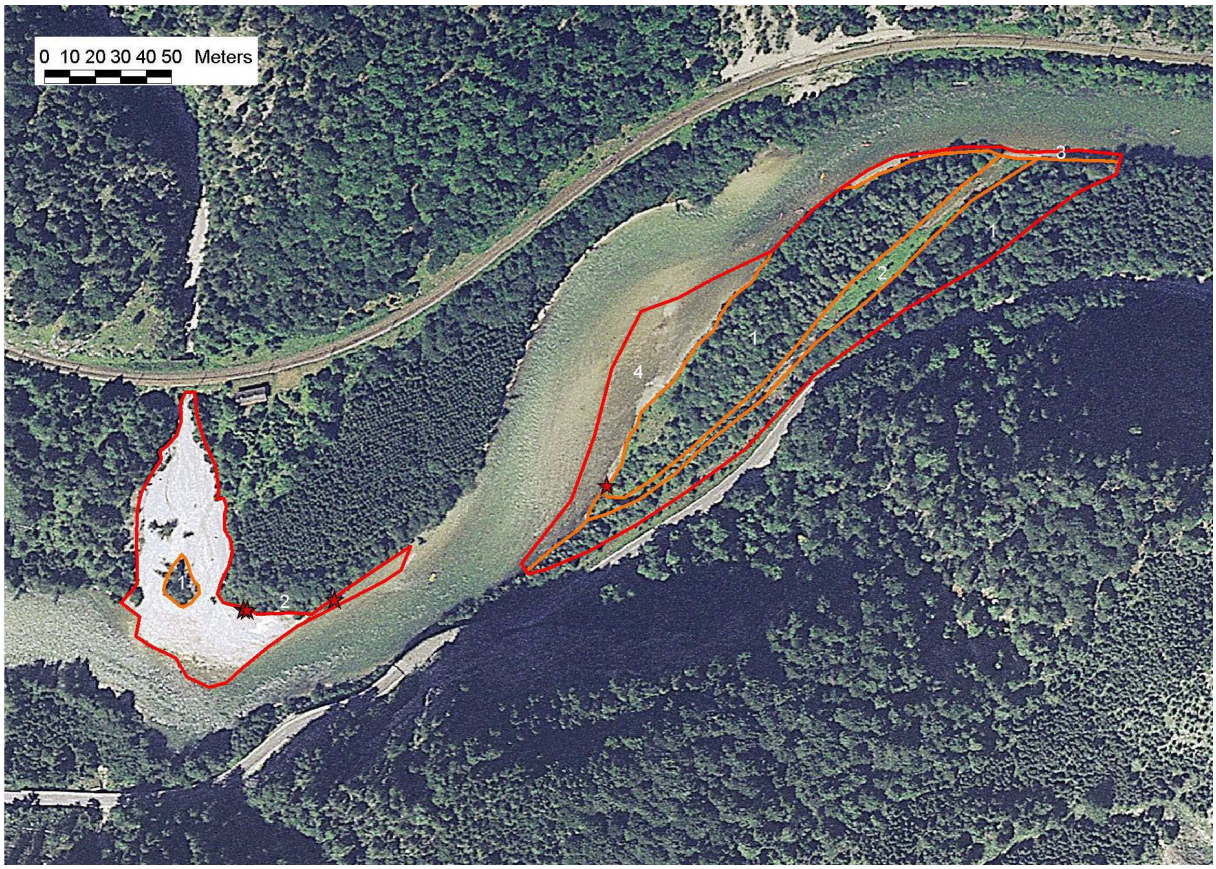

Figure 6: Large area of Bruckgraben alluvial cone on the left and Lettmair-Au (floodplain) on the right. The colored lines show study area and boundaries of biotopes investigated by Kammerer (2003). The stars mark the presence of *Calamagrostis pseudophragmites* in 2003. Data source: Kammerer (2003).

Lettmair-Au is a floodplain with primary Salicion albae vegetation. *Alnus incana* and *Salix eleagnos* are dominant in the elevated area, while in the flood area one can find rather *Alnus incana* with *Salix alba*. Jungwirth et al. (1996) found the "beautiful *Calamagrostis pseudophragmites* population" in Lettmair-Au in 1992. But in the year 2003 only one small patch (1 m^2) of *C. pseudophragmites*, surrounded by the vegetation of Petasition officinalis with *Mentha longofolia*, was documented by Kammerer (2003) at the entrance of the sidearm (Figure 6). Kammerer (2003) also reported an absence of water dynamics and early succession on empty sites as well as a large increase of neophyte species since the year 1992.

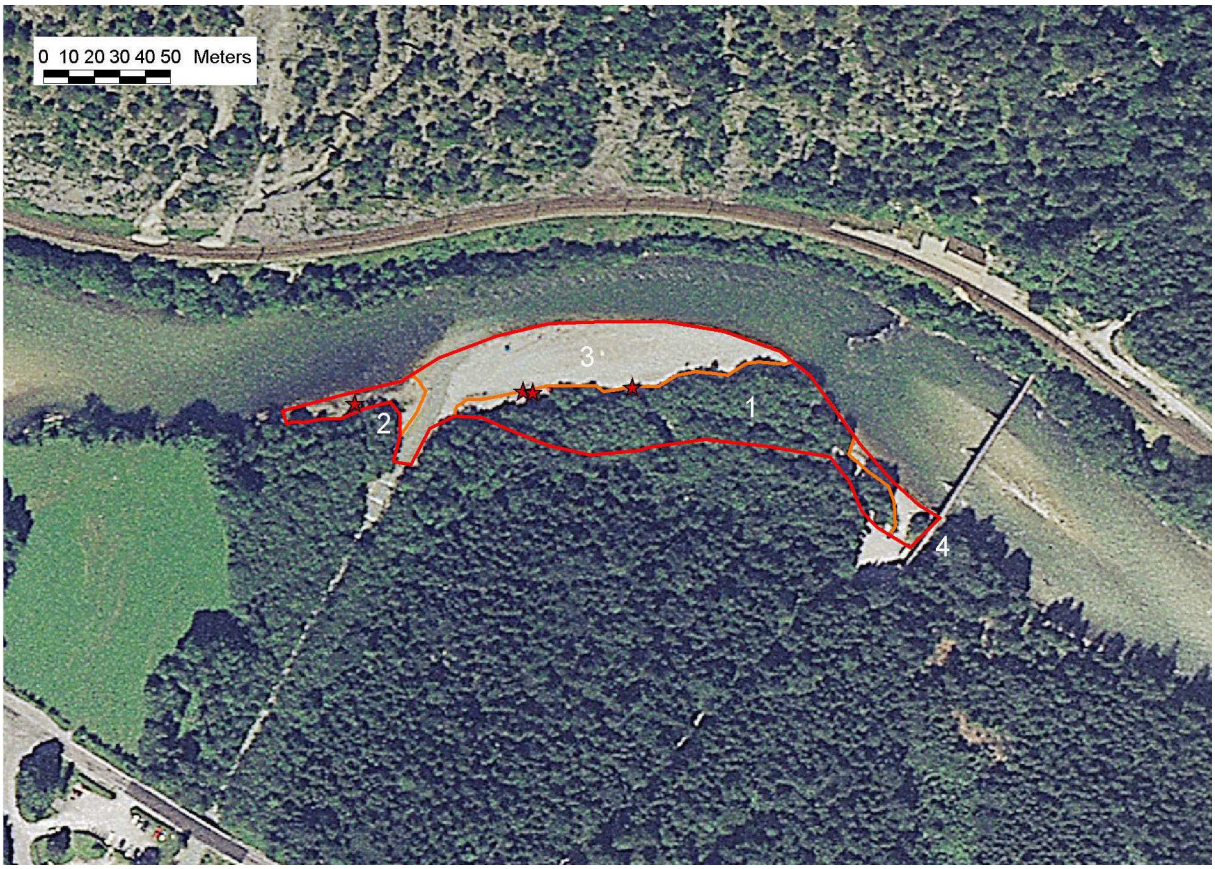

Figure 7: Johnsbachbrücke riverbank on the right side of the estuary of stream Johnsbach and left from the bridge over Enns called "Johnsbachbrücke". The colored lines show study area and boundaries of biotopes investigated by Kammerer (2003). The stars mark the presence of *Calamagrostis pseudophragmites* in 2003. Data source: Kammerer (2003).

Johnsbachbrücke (Figure 7) is a riverbank on the right side of Enns that is stretched from the confluence (estuary) of stream Johnsbach into Enns to the bridge over the Enns called "Johnsbachbrücke". Immediately on the west side of the bridge lies a much utilized entry and exit site for many water sports, that is also entered by other visitors (Kammerer 2003). The riverbank consists of coarse gravel and it is mostly free of vegetation. On the west side of the Johnsbach estuary one can find a Salicetum incano-purpureae forest. Four small patches of C. *pseudophragmites* were noted by Kammerer in 2003; a 4 m^2 large patch was found where the furcating canal enters the riparian forest, another two smaller (1-2 m^2) were located on the edge of the west part, which was also disturbed by wild campers at the time of Kammerer`s mapping in 2003. All three patches are placed on the border of the riparian forest without being affected by normal river dynamics. The fourth location mapped in 2003 was placed on the riverbank west of the Johnsbach estuary with a size of 1 m^2.

Just before the new tunnel, crossing the local road that runs through the Gesäuse, lays **Finstergraben** brook (Figure 8). On the west side of the cone that is used for disembarking,

a large plain with coarse gravels on the very sandy background is offering the potential habitat for *C. pseudophragmites*. Groups of Salicetum incano-purpureae are situated in the middle of the riverbank. In the year 2006 and 2007 a large area of the river bank was flooded, dry spots were covered with dead wood. On the east side of the riverbank, on a long sandy bank, Kammerer (2003) mapped a vital *Calamagrostis pseudophragmites* patch and noted that there is a high potential for its expansion because of the surrounding habitat. The group of plants was located on the edge of the *Salix eleagnos* forest.

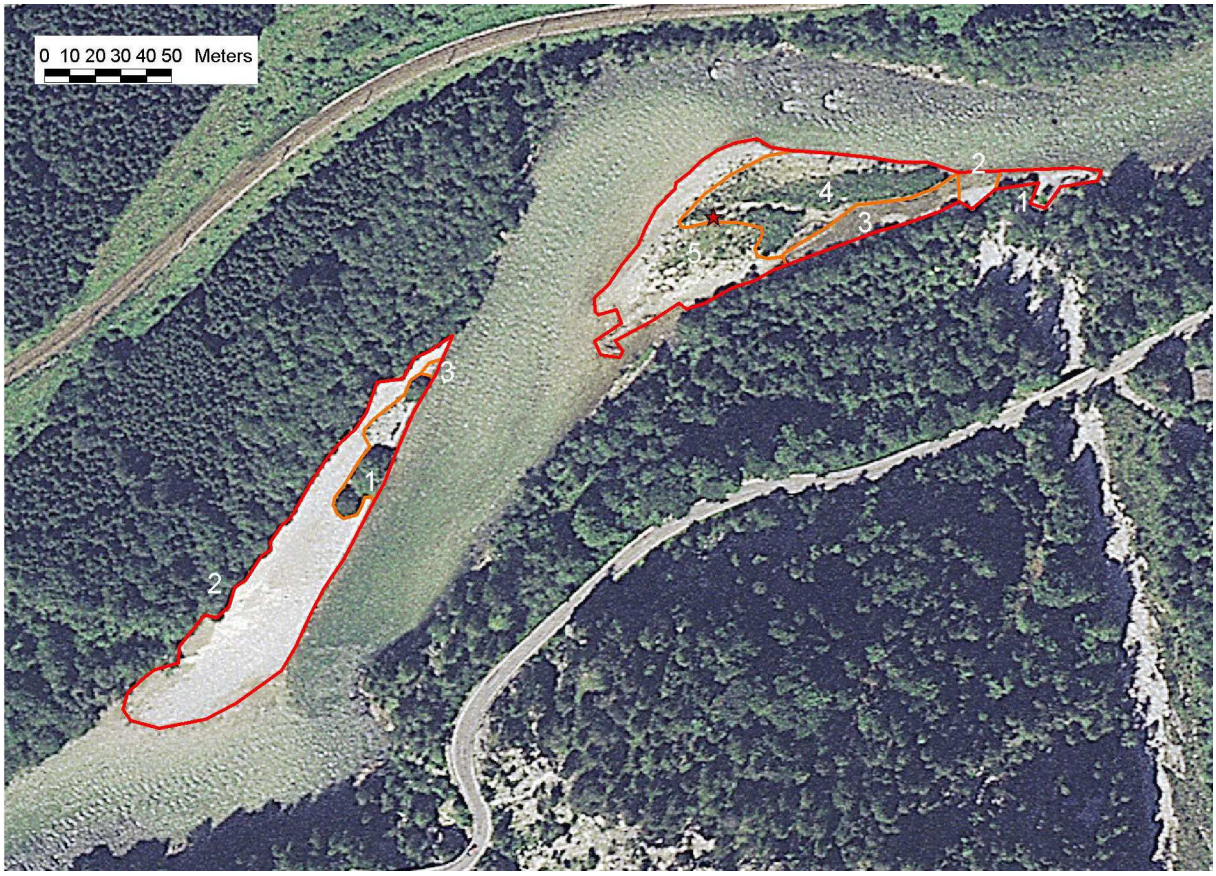

Figure 8: Finstergraben brook which crosses the road and ends in the Enns (on the right) and Reicherlboden (left). The colored lines show study area and boundaries of biotopes investigated by Kammerer (2003). The stars mark the presence of *Calamagrostis pseudophragmites* in 2003. Data source: Kammerer (2003).

The last riverbank lies on the orographically right shoreline of Enns just before the bridge at Gstatterboden, north of the brook **Schneiderwartgraben**. It is a long and narrow riverbank stretching along the Enns (Figure 9) north and south of the brook estuary. Along the river different types of riverbanks can be observed; on the scree many *Salix* seedlings grow; the following sand riverbank is highly disturbed as is it used for parties and camping, sport and other human activities. This riverbank is therefore mainly vegetation free. On the north side of the debris zone follows a less vegetated, rather sandy alluvium with some indication of

anthropogenic disturbance. Kammerer (2003) mapped a small *C. pseudophragmites* group with three ramets, partly disturbed by humans.

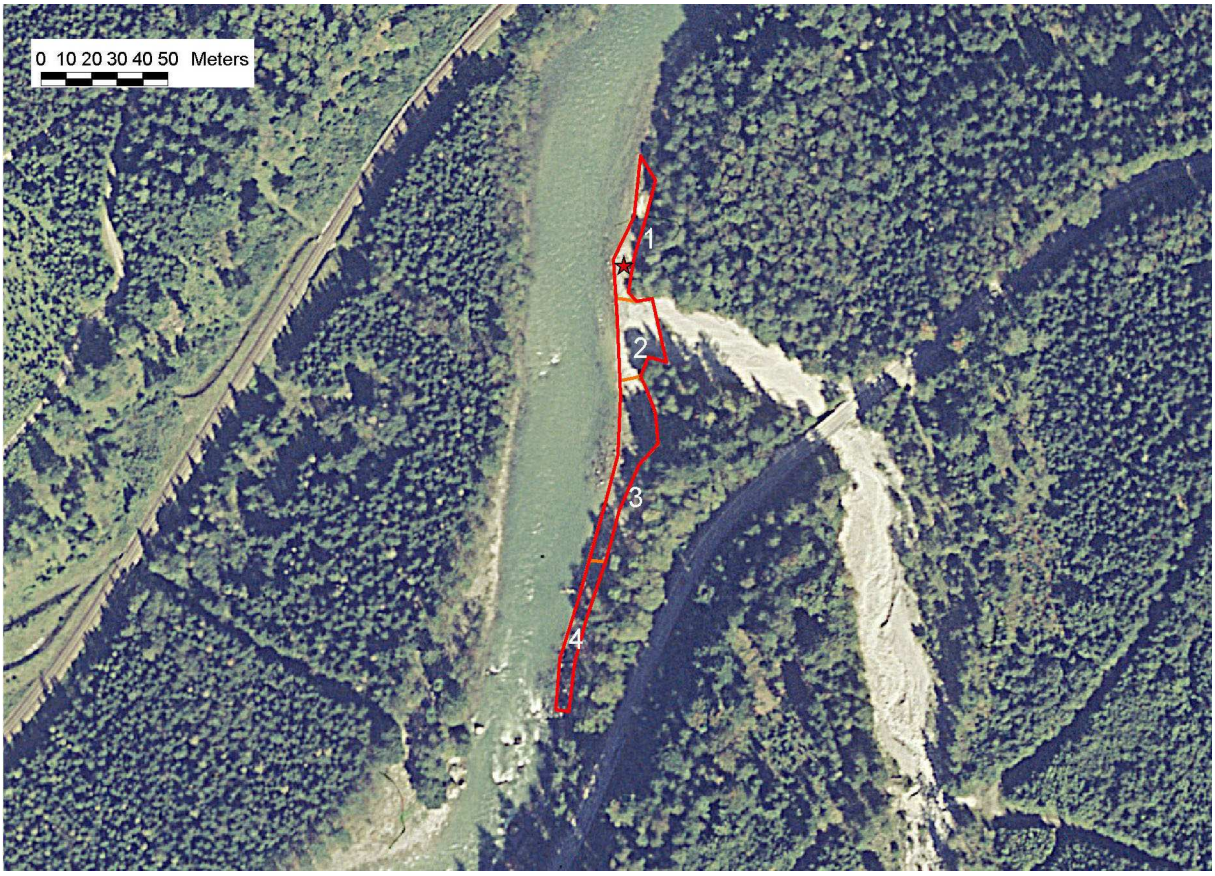

Figure 9: The estuary of Schneiderwartgraben into Enns with the riverbanks. The colored lines show study area and boundaries of biotopes investigated by Kammerer (2003). The stars mark the presence of *Calamagrostis pseudophragmites* in 2003. Data source: Kammerer (2003).

2.2 Population status in 2006 and 2007

All locations mentioned in the report of Kammerer (2003) in Gesäuse were searched for *Calamagrostis pseudophragmites* in 2006. All subpopulations reported by Kammerer (2003) were found except for subpopulation Lettmair-Au. Additionally sites outside Gesäuse mentioned in old reports (Wagner & Mecenovic 1973) were explored for *C. pseudophragmites* (Figure 2; Niklfeld & Schratt-Ehrendorfer, unpublished). Thus riverbanks along the Enns in Hieflau and north of this village as well as possible sites along Große Fölz Graben, Eisenerz were searched.

Table 2: Locations of the sampled subpopulations with patch numbers, coordinates, elevation and year of collection. All populations except Große Fölz are located in Gesäuse national park.

Id.	Location	Patches	Coordinates	Year of collection
w	Haslau "island" west, Gesäuse	1-14	N 47°34′54″ E 14°34′01″	2006
o	Haslau "island" east, Gesäuse	15	N 47°34′53″ E 14°34′08″	2006
j	Johnsbachbrücke, Gesäuse	16-19; 30-31	N 47°34′55″ E 14°35′39″	2006, 2007
f	Finstergraben, Gesäuse	20; 26-29	N 47°34′58″ E 14°36′22″	2006, 2007
s	Scneiderwartgraben, Gesäuse	21; 24-25	N 47°35′20″ E 14°37′17″	2006, 2007
b	Bruckgraben, Gesäuse	22	N 47°34′50″ E 14°34′43″	2006
	Große Fölz Graben, Eisenerz	*Calamagrostis varia*	N 47°33′16″ E 14°51′24″	2007

In July and September 2006 *Calamagrostis pseudophragmites* was found in five of six described locations (Kammerer 2003) in Gesäuse. The largest subpopulation grows on the **west** side of **Haslau** "island" (Figure 11). This site with more open vegetation was divided into 14 patches (Figure 10), whereas on the **east** side a large and more homogenous association of Calamagrostietum pseudophragmitis was found. From each patch on the west side 2-3 ramets were sampled, patches number 4, 5, 7, 8 and 9 were sampled in more detail (Figure 10) for the purpose of identifying clonal structure. In total 73 ramets were included in the analysis from west side of Haslau. Subpopulation Haslau east (patch number 15) lays 150 m stream up where *C. pseudophragmites* is mixed with *Festuca arundinacea* and *Deschampsia cespitosa*. In total 9 ramets were sampled there. Between 2006 and 2007 Haslau west subpopulation did not change, on the east side of the island spreading of C. *pseudophragmites* subpopulation was observed.

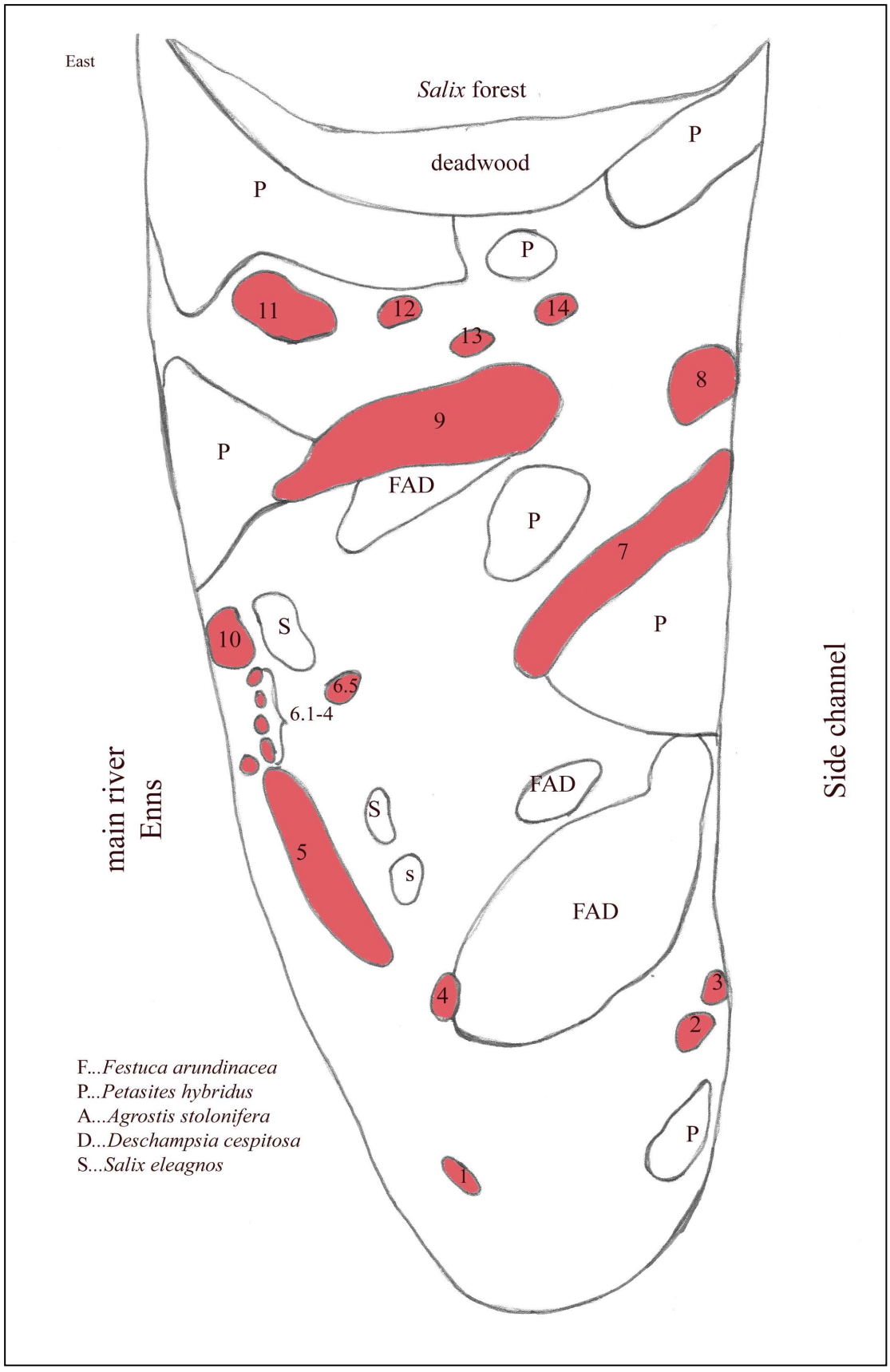

Figure 10: Patches of *Calamagrostis pseudophragmites* (red) in the west side of Haslau "island" with additional notation of other vegetation.

Figure 11: Marked patches of *Calamagrostis pseudophragmites* in Haslau west (left) and Haslau east (right) in 2007. Orthophoto: National Park Gesäuse.

Figure 12: *Calamagrostis pseudophragmites* patches in Haslau west side of the island in July 2006. Photos: J. Greimler.

At **Johnsbachbrücke** four patches of *C. pseudophragmites* were found in 2006 and from each, five samples were stored in silica gel. First patch (number 16), surrounded with *Salix eleagnos*, *Alnus incana*, *Acer pseudoplatanus*, *Picea abies*, *Salix appendiculata*, *Petasites paradoxus*, *Eupatorium canabinum*, *Salix purpurea* etc., is located on the higher river terrace next to Johnsbach estuary. Approximately 15 m east of patch 16, also on the terrace just behind its eroded edge, patch number 17 was found, and 15 m from there patch 18 (terrace not eroded, but in succession). At the front end of the parking place right at the beginnings of the woody vegetation one large patch of *C. pseudophragmites* was found and sampled as patch number 19 (Figure 13). This patch was not mentioned by Kammerer in 2003. Patch 19 propagated much in year 2007 although it was obviously not affected by

normal water dynamics. Another new patch was found by the estuary just along the shoreline on a 4 x 1 m area with ca. 50 ramets, thereof 30 juvenile. Four samples were taken from this patch (number 30). Western from the estuary one small linear patch along the shoreline with ca. 40 ramets was found. This patch was already mentioned by Kammerer (2003). Five samples from the patch 31 were analyzed. In total, 26 ramets were used for further analysis.

Figure 13: Patches of *Calamagrostis pseudophragmites* sampled in 2007 in Johnsbachbrücke subpopulation. Few ramets in the forest were not included in the analysis. Orthophoto: National Park Gesäuse.

Near **Bruckgraben** brook three patches were mapped by Kammerer in 2003. Only one patch was found also in 2006 (J. Greimler, pers. observ.). The other two patches obviously had disappeared because of erosion dynamics (Figure 14). Four ramets were sampled and three of them were included in the analysis (Patch 22).

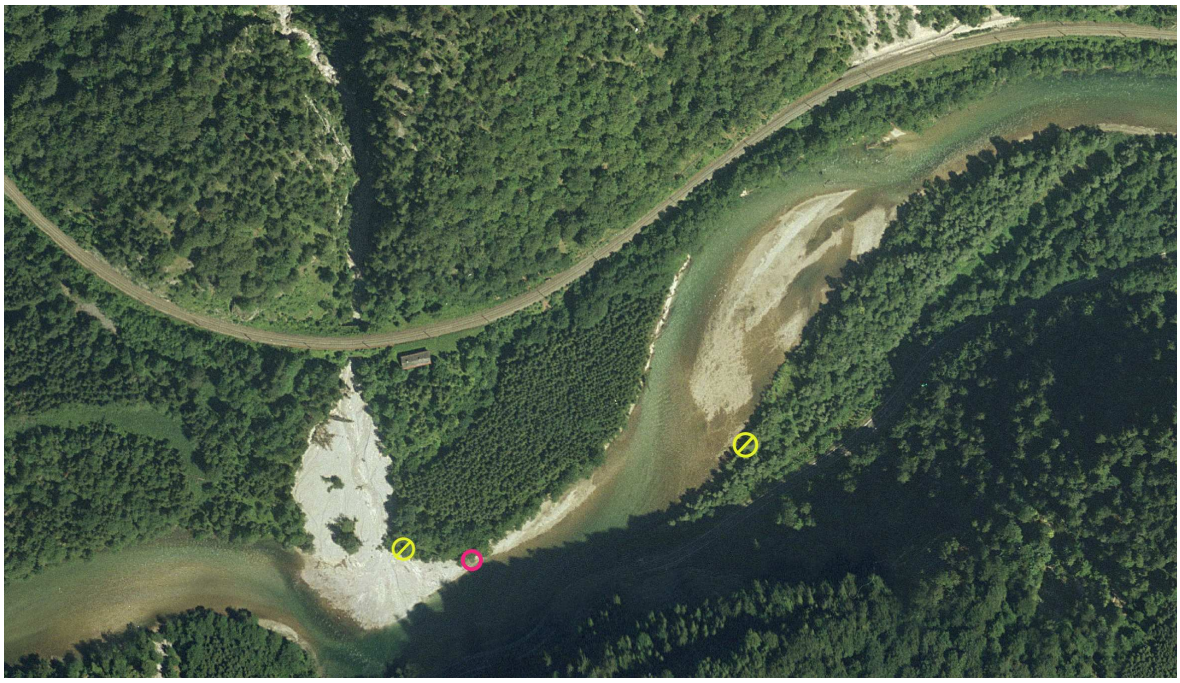

Figure 14: In the Bruckgraben alluvial cone (left) one patch that probably merged with its neighbor patch was found. The other patch, found by Kammerer (2003) was not present any more. In the Lettmair-Au no ramets of *Calamagrostis pseudophragmites* were found (marked with yellow). Orthophoto: National Park Gesäuse.

A channel that divides the river bank from the land on the **Finstergraben** alluvial fan was flooded in year 2006. Vast damage of vegetation with large amounts of deadwood, owing to the last strong floodwater, was observed on the riverbank. One patch (number 20) of C. *pseudophragmites* in a size of 40 x 40 cm with ca. 20 ramets was found in 2006. The patch reported by Kammerer (2003) was obviously covered with accumulated deadwood. Two samples were taken within a distance of 30 cm. On the presumable site of the patch (patch 26) observed by Kammerer (2003) deadwood was removed in summer 2007 due to another flooding. On this site two additional patches (patches 27 and 28) were found (Figure 15). Another new patch 29 arose near the main river 30 m western from the patch 20. Additional samples from patches 26, 27, 28 and 29 were taken in 2007. Patch 20 spread greatly in one year and was not sampled again.

Figure 15: Finstergraben subpopulation with five patches found on the riverbank in 2007. Orthophoto: National Park Gesäuse.

Figure 16: Finstergraben riverbank covered with deadwood, September 2006. Photo: J. Greimler.

On the riverbank of **Schneiderwartgraben** three small patches were located (Figure 18) at a linear distance of 5.5 m. The first one with 10 ramets was 1.5 m distant from the next patch with 6 ramets, the next one 4 m away had only 2 ramet. From each patch one ramet

was sampled (all three patches counted as patch number 21). In the year 2007 the patches were found much larger than the year before. Two new patches with few ramets (ca. 10) were detected on the east (patch 24) and west side (patch 25) of the old ones. New samples were taken (from each patch two). On the left (west) side of Schneiderwartgraben large accumulations of fine sand were detected in year 2007. Although the habitat conditions seem optimal for growth of C. *pseudophragmites* no ramet was found.

Figure 17: Small patch of *Calamagrostis pseudophragmites* in the sand of Schneiderwartgraben riverbank. July 2007. Photo: J. Greimer.

In the **Lettmair-Au** location no ramet of *C. pseudophragmites* was found in 2006. In 2007 this location could not be investigated due to high water levels (Figure 14).

Figure 18: In Schneiderwartgraben subpopulation three patches were found in 2007. Orthophoto: National Park Gesäuse.

We searched for Gesäuse`s nearest populations of *C. pseudophragmites* based on the data from Niklfeld & Schratt-Ehrendorfer (unpublished, Figure 3) and Wagner & Mecenovic (1973). On the riverbanks along the Enns in Hieflau and north of this village as well as along Große Fölz Graben in Eisenerz no ramet was found, but three individuals of C. *varia* were sampled there to be used as an outgroup.

3 Material and Methods

3.1 Laboratory work

3.1.1 DNA extraction

Leaf samples of *Calamagrostis pseudophragmites* from the six locations in national park Gesäuse and three individuals of *C. varia* as outgroup (Table 2) were collected. All material was immediately stored in silica gel. The DNA extraction protocol followed Doyle & Doyle (1987) with the modification of Samuel and Tremetsberger (unpubl. protocol, 2005). Leave material was ground 2x 5 min before the extraction. Total genomic DNA was extracted from the silica gel-dried material in 700 µl pre-warmed (60°C) CTAB buffer (2% CTAB, 100 mM Tris, 1.4 M NaCl, 20mM EDTA, 0.2% mercaptoethanol, pH 8.0) for 30 min at 60°C. After incubation 500 µl chloroform: isoamylalcohol (24:1) was added and mixed gently. The mixture was kept at 4°C for 20 min. After centrifugation (5 min, 10.000 rpm) 600 µl of clear aqueous phase was transferred into another tube and 200 µl isopropanol (cooled) was added to precipitate the nucleic acid. The mixture was left at -20°C for 45 min. After centrifugation (5 min, 14.000 rpm) the very loose DNA pellet is precipitated on the bottom of the tube. The supernatant was carefully poured off. The remaining pellet was washed twice (with 70% ethanol) afterwards. Dried DNA was finally re-suspended in 50 µl TE buffer.

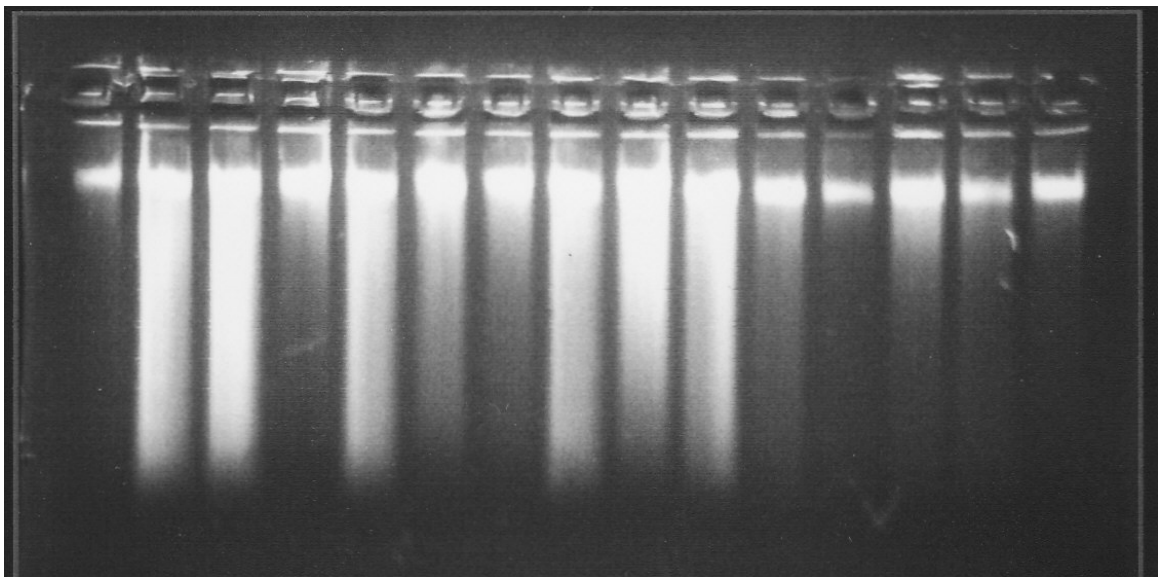

Figure 19: DNA extracts (from left to right): ramets 28.1-3, 29.1-3, 30.1-4 and 31.1-5, checked on a 1% TAE agarose gel.

In each DNA sample 2 µl of RNAse (Fermentas, 200 u) was added and kept for 1 hour at 37 min. All extracts were checked on an ethidium bromide dyed (1%) TAE agarose gel (Figure 19).

3.1.2 AFLP analysis

AFLP method starts with digestion of genomic DNA. Two restriction enzymes (endonucleases), EcoRI and MseI, cut on different sites and with different frequency because of selection based on their recognition sequence and cleavage sites ("four-" and "six-base cutter"). First the MseI and EcoRI adaptor pair were heated to 95°C to be annealed. A total volume of 11 µl per sample was digested with following components: 5.5 µl of Genomic DNA; 1.1 µl 10xT4 Buffer; 1.1 µl NaCl (0.5 M); 0.55 µl BSA (1 mg/ ml); 0.33 µl T4 Ligase (3U/ µl); 1 µl MseI adaptor pair (50 µM); 1 µl EcoRI adaptor pair (5 µM); 0.02 µl MseI endonuclease (50U/ µl); 0.25 µl (20u/ µl); 0.65 µl ddH_2O. The total volume (11 µl) was incubated for 2 hours at 37°C in a PCR cycler. The restriction-ligation reaction was loaded on a 1% TBE gel for checking. For preselective amplification the restriction-ligation result was diluted 16x with $TE_{0.1}$ buffer (20mM Tris-HCl, 0.1 mM EDTA, pH 8.0). An amount of 2 µl of diluted restriction-ligation DNA was mixed with total aliquot of 8 µl Master Mix: 5.86 µl ddH_2O; 0.22 µl ABI dNTPs; 1.14 µl RedTaq buffer (Sigma); 0.2 µl RedTaq polymerase (Sigma, 250 U); each 0.29 µl preselective primer MseI and EcoRI (each 5 mM). The total volume of 10 µl was put in the PCR for 2 hours. AFLP reactions with primers having none or a single selective nucleotide were performed for 20 cycles with following cycle profile: the program started with 72°C for 2 min, followed by 20 cycles of 94°C for 1 sec (denaturation), 56°C for 30 sec (annealing) and 72°C (extension) for 2 min. A final step of 60°C for 30 min ended the program and the reaction was cooled down to 4°C. Reaction product (3 µl) was checked on the 1% TBE agarose gel, the rest was diluted 16 x with the $TE_{0.1}$ buffer and 2 µl thereof was used for each of the three selective runs. Three different selective primer combinations were used: Eco-AGC (NED-Yellow)/ Mse-CAA, Eco-ACG (HEX-Green)/ Mse-CAA, Eco-ACT (FAM-Blue)/ Mse-CAA. These tree combinations showed to be appropriate after a primer trail with 36 primer combinations. 2 µl preselective amplification product were prepared for selective amplification together with: 5.5 µl ddH2O; 1 µl 10xRedTaq buffer (Sigma); 0.22 µl ABI dNTPs (10 mM); 0.2 µl RedTaq (Sigma, 250 U); 0.54 µl Mse-Primer (5 pmol/µl) and 0.54 µl Eco-Primer (1 pmol/µl). Reaction mixes of a total volume of 10 µl for each primer combination (yellow, green and blue) were put into the PCR machine for 3 hours. The cycling program started with initial cycle of 94°C for 2 min, 65°C for 30 sec and 72°C for 2

min. Thirty-one cycles followed at 94°C for 1 sec, 64°C for 30 sec and 72°C for 2 min. In the first 8 cycles (at 64°C) the annealing temperature decreased by 1°C in each cycle from 64°C to 57°C. During the remaining 23 cycles, the annealing temperature was held on 56°C. The program stopped at 60°C for 30 min and then cooled down to 4°C. For each PCR a GenAmp® PCR System 9700 (AB Applied Biosystems) has been used. To purify the samples for the sequencer Sephadex purification gel was applied. The membrane multi-plate was filled with 200 µl of Sephadex gel. The plate was centrifuged at 600 G, the filtrate was removed (the procedure was repeated 3 x) and 2 µl of each primer combination mix were pipeted on the gel. Probes were centrifuged (600 G) and the purified DNA was captured in filtrate. A Mix of 0.10 µl of the Genescan^{TM} 500 Rox Size Standard (AB Applied Biosystems) and 7.9 µl High-Dye Formamid was added to the 2 µl of the DNA eluate. Before the probes were put into the sequencer DNA was denaturised at 95°C for 2 min.
Fragments were separated in an ABI 3130 Automated Capillary DNA Sequencer (AB Applied Biosystems) at the department for Systematic and Evolution of Higher Plants (University of Vienna, Faculty Center Botany) and gained as raw GenScan® files. Accordingly the data were aligned with the internal size standard using ABI Prism® GeneScan® version 3.7. (© 1989 – 2001 Applied Biosystems) and subsequently imported into the Genographer version 1.6.0. (Benham 2001) for scoring the fragments.

Fragments (i.e. peaks) were scored as present or absent in a size range from 50 to 500 bp and were extracted as binary (1/0) matrix. Samples of *Calamagrostis varia* from Eisenerz were scored simultaneously as outgroup. To assess reproducibility of the AFLP procedure and also to determine the distance between clones, samples were replicated within the plates. Although reproducibility was high in general, occasional irreproducible fragments were excluded from further analysis.

3.2 Data analysis

The presence/absence matrix was imported into Microsoft® Excell® 2002 (© Microsoft Corp. 1985-2001) and SPSS (13.0.1 for Windows, SPSS Inc. 1989-2004) for preparing the data sets for further analysis. Input files for further analysis were also prepared with FAMD 1.104 beta (Fingerprint Analysis with Missing Data, Schlüter & Harris 2006).
Neighbor joining Analysis and Nei-Li (1979) genetic distance implemented in TREECON 1.3b (Van de Peer & De Wachter 1997) were used to investigate similarities. Branch support was estimated with 1000 bootstrap replicates. The trees were rooted with AFLP profiles from three individuals of *Calamagrostis varia*. A UPGMA dendrogram was

constructed with FAMD (Schlüter & Harris 2006) using Squared Euclidian distances. Jaccard´s coefficient of similarity (Jaccard 1908) and the program FAMD (Schlüter & Harris 2006) were used for principal coordinates analyses (PCA).

Genetic diversity measures such as number of polymorphic sites per population and mean number of pairwise differences, as well as Analysis of Molecular variance (AMOVA) were computed with ARLEQUIN 3.11 (Excoffier et al. 2006). For AMOVA algorithm the number of permutations was set to 1000.

Non-hierarchical clustering was performed with BAPS 3.2. (Bayesian analysis of population structure, Corander & Marttinen 2006). The number of clusters was examined using the individual mixture analysis. Afterwards the clusters identified by the mixture analysis were checked for admixture. K vector was set to 4-9 with several replicates. Results are based on 100 simulations from posterior allele frequencies and 100 iterations inside of the set.

A triangular matrix of distances between the pairs of ramets using Squared Euclidian distances was applied to track the clones. According to the level of reproducibility and frequency analysis of distance intervals the threshold for recognizing clones was determined.

To determine whether genetic distances correlate with geographical distances between genotypes a Mantel test was performed using GENEPOP 3.1 (Raymond & Rousset 1995). The Mantel test estimates association between two independent matrices (e.g. genetic distance and geographical distance) (Escaravage et al. 1998). The geographical distance matrix was assembled from distances between each subpopulation measured from their midpoint in meters. Squared Euclidian distance matrix calculated in FAMD (Schlüter & Harris 2006) provided the genetic distance matrix. Graphical editing was done in Adobe® Photoshop® CS2 and Microsoft® Powerpoint® 2003.

4 Results

4.1 Analysis of genetic diversity

The three primer combinations applied on 129 samples of *Calamagrostis pseudophragmites* and additionally on 3 individuals of *C. varia* generated a total of 399 scorable and reproducible fragments ranging from 50 to 500 bp. Overall 356 (89.2%) of the AFLP markers were polymorphic. For each primer combination the following percentages of bands were polymorphic: Mse-CAA/EcoRI-AGC 88.3%; Mse-CAA/EcoRI-ACG 93.6%, and Mse-CAA/EcoRI-ACT 87.4%. On the subpopulation level an unexpectedly high level of polymorphic sites were found (Table 3). High amounts of polymorphic markers were obtained in Haslau west (65.4%) as well as in Johnsbachbrücke (53.9%) and Haslau east (48.2%). The number of polymorphic sites was highly correlated with the number of sampled ramets per subpopulation. However, a very dense sampling of ramets was only applied in Haslau west for the purpose of detecting clones. The mean numbers of pairwise differences show the high diversity of Haslau east subpopulation, where only 9 ramets were sampled. The nucleotide diversity estimate (Table 3): mean number of pairwise differences (π) did not show any clear diversity pattern among the subpopulations. After removal of one ramet from Bruckgraben subpopulation the π value increased very much, yet also with very high standard deviation. The Bruckgraben dataset was definitely too small for any meaningful estimation. The very low level of mean number of pairwise differences in Finstergraben increased after removal of multiple clones from the analysis, but the estimate is not meaningful because of the sample size.

Table 3: Investigated subpopulation characteristics (subpopulation name, subpopulation abbreviation and number of samples) with genetic diversity measures gained from AFLP: number of fixed fragments (f_f), number of private fragments (f_p), number of polymorphic sites per population (S) and mean number of pairwise differences (π) with standard deviation (sd).

Abbr.	Subpopulation	Nr. of samples	f_f	f_p	S	Π	Sd
w	Haslau west	73	85	0	261	68,87	30,03
o	Haslau east	9	124	0	194	77,50	36,94
f	Finstergraben	11	198	0	116	40,22	18,95
s	Schneiderwartgraben	7	189	0	117	50,86	25,15
j	Johnsbachbrücke	26	127	1	215	63,31	28,23
b	Bruckgraben	3	220	0	83	55,33	33,43

Table 4: Molecular diversity indices gained from dataset without multiple clones: number of polymorphic sites per population (S) and mean number of pairwise differences (π) with standard deviation (sd).

Abbr.	Subopulation	Nr. of samples	S	Π	Sd
w	Haslau west	44	258	72,98	32,05
o	Haslau east	8	189	79,14	38,25
f	Finstergraben	3	101	67,33	40,39
s	Schneiderwartgraben	5	113	55,00	28,84
j	Johnsbachbrücke	24	215	63,93	28,58
b	Bruckgraben	2	81	81,00	57,63

Table 5: Spearman correlation (R_S) between number of samples, number of polymorphic sites and number of pairwise differences.

Spearman-Rho	Nr. of samples	Nr. of polymorphic sites	Nr. of pairwise differences
Nr. of samples Correlation coefficient Significance	 1,0 0,0	 0,829 0.045	 0,314 0,544
Nr. of polymorphic sites Correlation coefficient Significance	 0,829 0,042	 1,0 0,0	 0,657 0,154
Nr. of pairwise differences Correlation coefficient Significance	 0,314 0,544	 0,657 0,154	 1,0 0,0

A bivariate correlation test was applied to check the correlation between the number of samples, polymorphic sites and pairwise differences. Spearman-Rho correlation was chosen because the data were not normally distributed. A significant correlation was obtained between the number of samples, as surrogate for population size and number of polymorphic sites (R_S = 0.829, P = 0.042), while the correlation between the number of samples and the standardized measure pairwise differences was not significant. The mean number of pairwise differences does not correlate with number of polymorphic sites.

4.2 Similarity Analyses

A UPGMA dendrogram of the whole data set (Figure 20) based on Squared Euclidian distances revealed two clear major clusters apart from the outgroup (*Calamagrostis varia*). Subpopulations Haslau west and east form one group clearly distant to other subpopulations. Two ramets sampled in Haslau west are genetically nested in Haslau east. The second cluster includes subpopulations Johnsbachbrücke, Bruckgraben, Finstergraben and Schneiderwartgraben. Ramets of Johnsbachbrücke, however, occur in four subclusters that mostly do not correspond to the spatial pattern. Interestingly Finstergraben and part of Schneiderwartgraben are genetically nested in the subpopulation Johnsbachbrücke.

Figure 20: UPGMA dendrogram based on Squared Euclidian distance reveals two major clusters in the Gesäuse population system.

The Neighbor joining analysis with TREECON 1.3b (Van de Peer & De Wachter 1997) (Figure 21) provided a tree with a very weak "backbone". The Bootstrap analysis supported a few small groups, yet not the overall spatial subpopulation structure. In the phylogram the subpopulations Johnsbachbrücke, Finstergraben, Schneiderwartgraben were

mostly resolved. The Haslau west and east subpopulations are not separated. Furthermore the basic divisions in the tree separate a few groups of Haslau samples from the rest. Overall a few samples are not found within their subpopulation cluster, e.g. Finstergraben ramet 29.1 is found in the Johnsbachbrücke cluster and ramets 20.1 and 20.2 of Finstergraben sampled in 2006 are found in the Schneiderwartgraben cluster. Ramet 18.5 of Johnsbachbrücke is found in the Finstergraben cluster and ramet 31.5 of Johnsbachbrücke is found in a Haslau cluster.

No clear separation was found between the two Haslau subpopulations. Ramets of the smaller east subpopulation are scattered over the whole diffuse Haslau group. Inside the Haslau subpopulations several groups were revealed. Most obvious is the agglomeration of ramets that are clonally connected (note the short branch lengths in the clusters) together with related ramets. Four samples from patch 9 (9.1, 9.7, 9.10 and 9.11) and one from patch 15 (15.9) are forming one group of related ramets. Relation between ramets 6.5, 10.1 and 13.1 was also obtained. The Ramets 5.1.23-25 shows connection with patch 4 and 1 and also with ramets 7.2 and 7.7. In a large cluster close relations between ramets 6.2, 7.3, 7.8-11, 8.2-4, 9.2-6, 9.8-9, 11.1-2 and 12 can be recognized. Haslau east ramets (all except mentioned 15.9) are namely nested in this group thereby showing a relation with patches 6, 7, 8, 9, 11 and 12. Another cluster resolves relationships between patch 2, some ramets from patch 7 (7.1, 7.4-6) and ramets 31.5 (from Johnsbachbrücke!) 3.1, 8.1, and 10.2. Two clusters were revealed sharing ramets from patch 5. One cluster includes 5.1.9, 5.1.11, 5.1.12, 3.2 and 6.3. In the other group 6.1 is related to weakly supported cluster distinguishing two highly supported groups 5.1.27/5.2/5.3 and 5.1-19.

Subpopulation Johnsbachbrücke group forms three small clusters. Samples from the year 2007 (patches 30 and 31) are linked to samples from the year before. Finstergraben without 29.1 is a highly supported subpopulation (bootstrap 97%). The ramets from Bruckgraben are not building a single group. Two of three ramets are linked with Johnsbachbrücke cluster and are closely related to ramets 31.1 and 31.2 sampled in 2007.

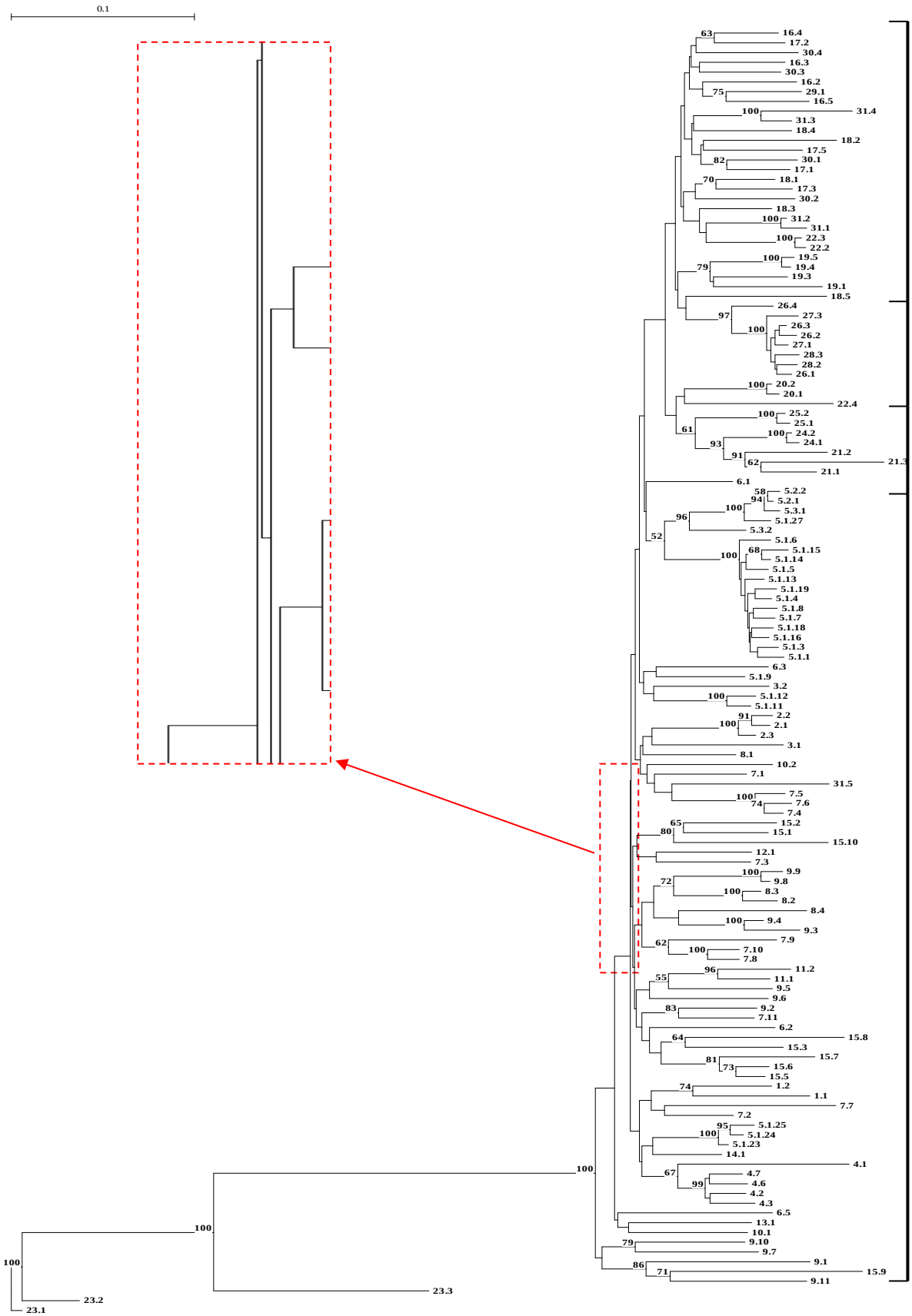

Figure 21: Neighbor joining tree based on Nei-Li distance with samples of all six *Calamagrostis pseudophragmites* subpopulations, rooted with *Calamagrostis varia*. Bootstrap values greater than 50% are marked above branches. On the left side is the enlargement of the branching in the red rectangle.

Four groups are resolved in the PCA analysis (Figure 22) with the first three axes explaining approximately 28% of the total variance (13.9%, 8.9% and 4.9% respectively). Ramets of Haslau west and east (with two exceptions) are found in group I, yet without revealing separate east and west subgroups. Ramets of patch 5.1 (group II) are distant to other Haslau west ramets. Samples of Schneiderwartgraben show up in group III, whereas Johnsbachbrücke, Finstergraben and Bruckgraben are forming group IV.

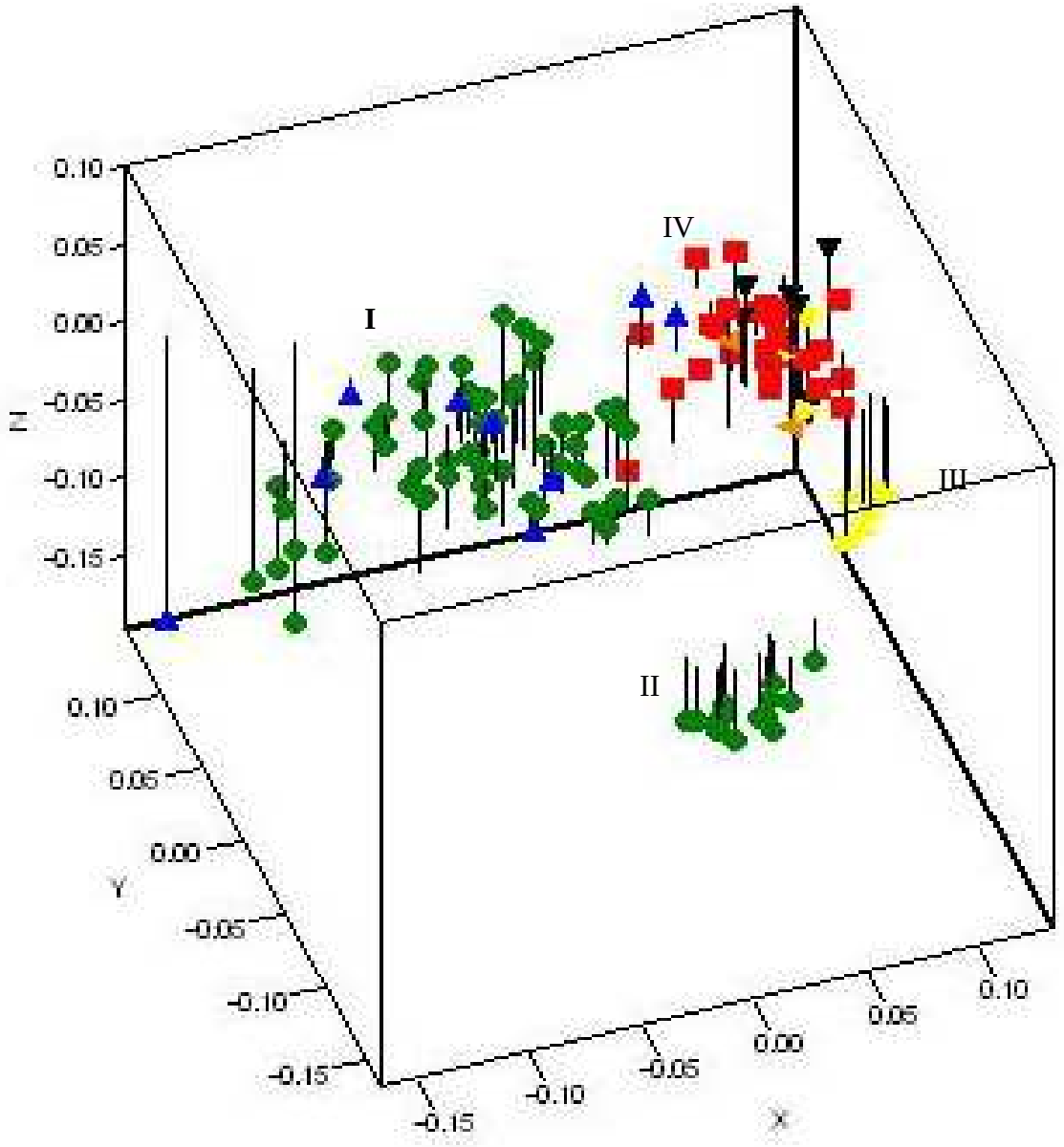

Figure 22: PCA of the whole data set based on Jaccard similarity coefficient. ● Haslau west, ■ Johnsbachbrücke, ▲ Haslau east, ►Finstergraben, ▼ Schneierwartgraben, ◄ Bruckgraben.

4.3 Clonal patterns

For genetic tracking of clones in the *Calamagrostis pseudophragmites* population system in Gesäuse a Squared Euclidian distance matrix was used. Using 8 replicates a reproducibility level of 95% among samples of *C. pseudophragmites* was estimated. Those 5% irreproducible fragments have been removed from the data set. However, to account for further irreproducible fragments due e.g. to technical errors, a threshold for the definition of a genet was set at 3% genetic distance (Table 6). A frequency analysis was applied to detect discontinuities that may indicate autocorrelations among ramets due to clonality. The distribution of the distance intervals among pairs of ramets (Figure 23) shows that there is a clear outlier position of the lower distance classes between 1.5 and 5.3% corresponding to an interval of 6 to 21 mutational steps. Part of these differences could still be technical artifacts inherent in the method that could not be detected by the small set of replicates. However, the outlier position of these low distance classes together with the fact that the distance intervals among ramet pairs between 10 and 30% show a nearly Gaussian distribution provide good arguments for the detection of clonal patterns amongst the lowest distance classes. Therefore the threshold for identifying clones was set to 5% (5.3% exactly). All pairs of ramets that revealed a similarity of at least 95% are counted as belonging to the same genet.

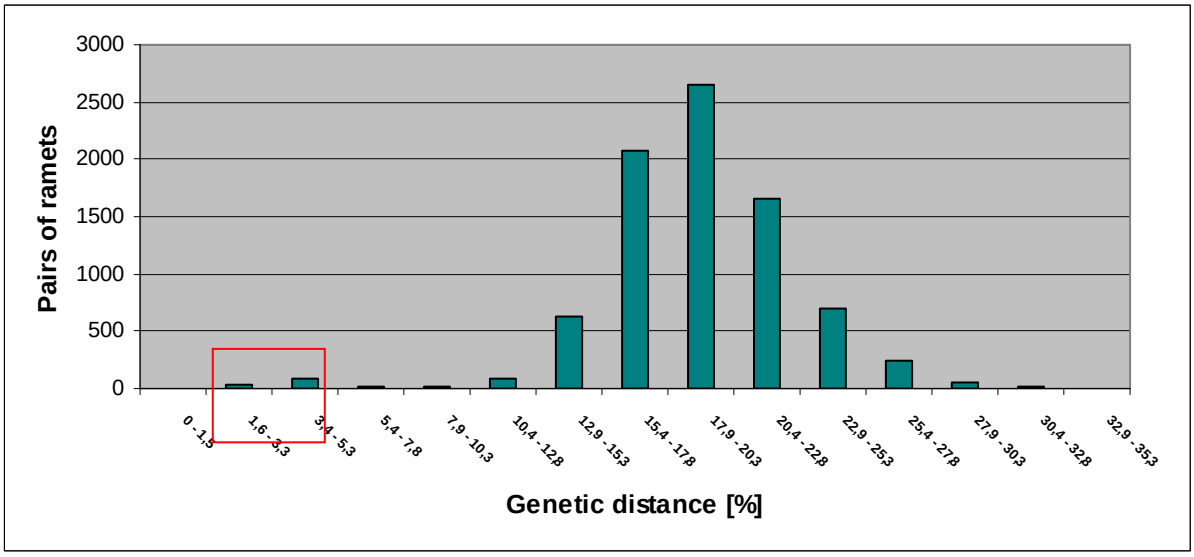

Figure 23: Histogram of distance classes based on Squared Euclidian distances that appeared between all possible pairs of ramets among the *Calamagrostis pseudophragmites* inferred by AFLP.

Applying the above criteria clones can be found in each subpopulation (Table 6), yet a very high amount of clonal pairs of ramets and multiple clonal ramets was detected in the densely sampled group indicated by number 5.1 of the Haslau west subpopulation.

Table 6: Clones identified by the Squared Euclidian distances with a limit up to 3% (red) and 5% (yellow) mutational changes.

HW	2.3	2.1												
HW	2.2	2.1												
HW	4.2	4.3	4.7	4.6										
HW	4.7	4.6												
HW	5.1.3	5.1.1												
HW	5.1.4	5.1.1	5.1.3											
HW	5.1.5	5.1.1	5.1.3	5.1.4										
HW	5.1.6	5.1.1	5.1.4	5.1.5										
HW	5.1.7	5.1.1	5.1.3	5.1.4	5.1.5	5.1.6								
HW	5.1.8	5.1.1	5.1.3	5.1.4	5.1.5	5.1.6	5.1.7							
HW	5.1.12	5.1.11												
HW	5.1.13	5.1.1	5.1.3	5.1.4	5.1.5	5.1.6	5.1.7	5.1.8						
HW	5.1.14	5.1.1	5.1.3	5.1.4	5.1.5	5.1.6	5.1.7	5.1.8						
HW	5.1.15	5.1.1	5.1.3	5.1.5	5.1.8	5.1.14								
HW	5.1.16	5.1.1	5.1.3	5.1.4	5.1.5	5.1.6	5.1.7	5.1.8	5.1.13	5.1.14	5.1.15			
HW	5.1.18	5.1.1	5.1.3	5.1.4	5.1.6	5.1.7	5.1.8	5.1.13	5.1.14	5.1.15	5.1.16			
HW	5.1.19	5.1.1	5.1.3	5.1.4	5.1.5	5.1.6	5.1.7	5.1.8	5.1.13	5.1.14	5.1.15	5.1.16	5.1.18	
HW	5.1.24	5.1.23												
HW	5.1.25	5.1.23	5.1.24											
HW	5.2.1	5.1.27												
HW	5.2.2	5.1.27	5.2.1											
HW	5.3.1	5.1.27	5.2.1	5.2.2										
HW	7.5	7.4												
HW	7.6	7.4	7.5											
HW	7.10	7.8												
HW	8.3	8.2												
HW	9.4	9.3												
HW	9.9	9.8												
HO	15.6	15.5												
John	19.5	19.4												
Finst	20.2	20.1												
Bruck	22.3	22.2												
Schnei	24.2*	24.1*												
Schnei	25.2*	25.1*												
Finst	26.2*	26.1*												
Finst	26.3*	26.1*	26.2*											
Finst	27.1*	26.1*	26.2*	26.3*										
Finst	27.3*	26.1*	26.2*	27.1*										
Finst	28.2*	26.1*	26.2*	26.3*	27.1*	27.3*								
Finst	28.3*	26.1*	26.2*	26.3*	27.1*	27.3*	28.2*							
John	31.2*	31.1*												

*sampled in 2007

On the east side of the island (patch 15) only two ramets out of nine were detected as clones. In Johnsbachbrücke subpopulation (patches 16-19 and 30-31) only four ramets, two pairs of clones (19.4-5 and 31.1-2), sampled from different patches were found, although

four to five samples were taken from each of the six patches. In Schneiderwartgraben the four sampled ramets from patches 24 and 25 made two pairs of clones. Patch 21 collected in 2006 does not show any clonal relationships. Inside the Finstergraben patches 26-29 from 2007 a large interpatch clone was tracked. Seven of nine ramets (26.1-3, 27.1, 27.3, and 28.2-3) are part of this clone (Figure 28), which connects more patches in the subpopulation. In patch 20 two ramets also were recognized as clones.

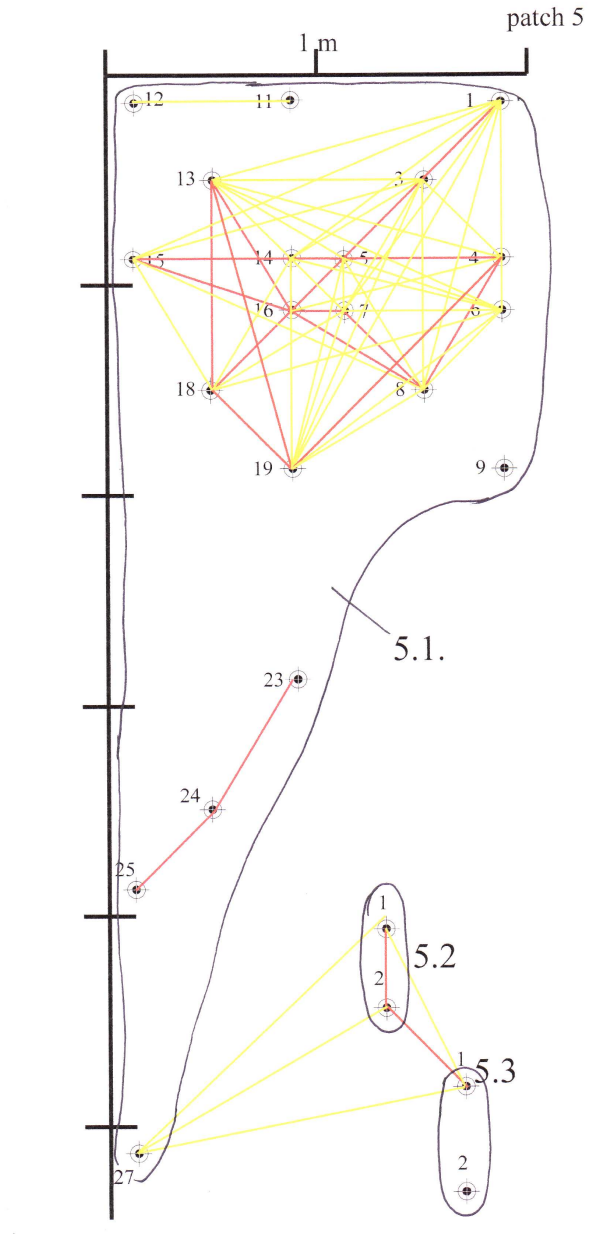

Figure 24: Haslau west patch number 5 with sampled ramets and clonal connections between them. Red lines are marking clonal connections based on 3% and the yellow ones on 5% threshold.

The detailed sampled patch 5 in Haslau west reveals a complex clonal pattern (Figure 24). Patch 5 was separated into three groups of ramets: 5.1, 5.2 and 5.3. Ramets 5.1.11 and 5.1.12 belongs to a different genet separated from the other clonal group. The ramets 5.1.23, 5.1.24 and 5.1.25 form a linear clone. Ramet 9 from group 5.1 does not show any clonal connections. Ramets of the groups 5.2 and 5.3 are clonally connected with ramet 5.1.27.

Marking clones in the Neighbor joining tree (Figure 29) revealed that clone 5.1 is closely related to clone 5.2/5.3. The clone 5.1.23/24/25 is related to a clone in patch 4.
However, without the highly sampled patch 5, a clonal connection could only be identified in 15% of the ramets in the Haslau west subpopulation. All three ramets from patch 2 as well as four of five ramets from patch 4 (Figure 25) resulted in one clone per patch. In patch number 3 no clone was found, but remarkably ramet 3.1 seems to be genetically closer to patch 2 than to ramet 3.2, which is more related to clone 5.1.11/12.

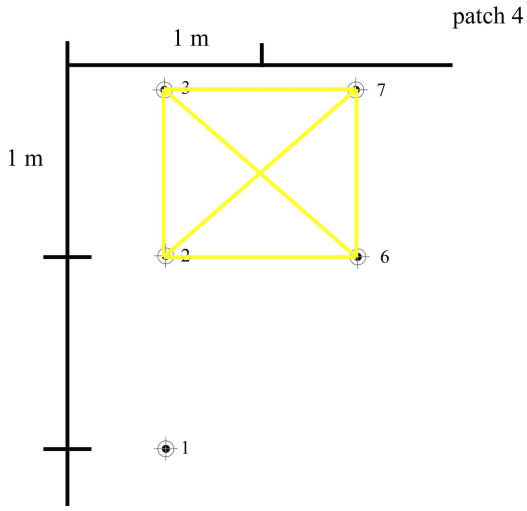

Figure 25: Patch number 4 on Haslau west with five ramets, with four of them clonally connected (marked with yellow line). Sampling distance was ca. 1m.

In patch number 7 (Figure 26) a pair and a group of three ramets in a clonal connection were found. It is remarkable that the ramets of the clone were sampled 2 m apart, where the other ramets growing closer together seem to be autonomous genets. Neighbor joining analysis did not reveal any specific relationships between different clones and other ramets from this patch (Figure 26). Clone 7.4/5/6 and ramet 7.1 are related to patch 2 and 3, while clone 7.8/10 and ramet 7.9 are linked with patches 8 and 9.

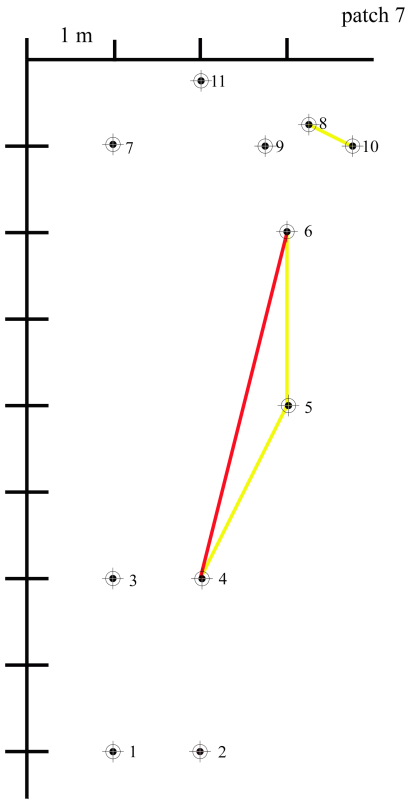

Figure 26: Patch number 7 on Haslau west with a pair and a clonal group of three ramets. Red lines are marking clonal connections based on 3% and the yellow ones on 5% threshold.

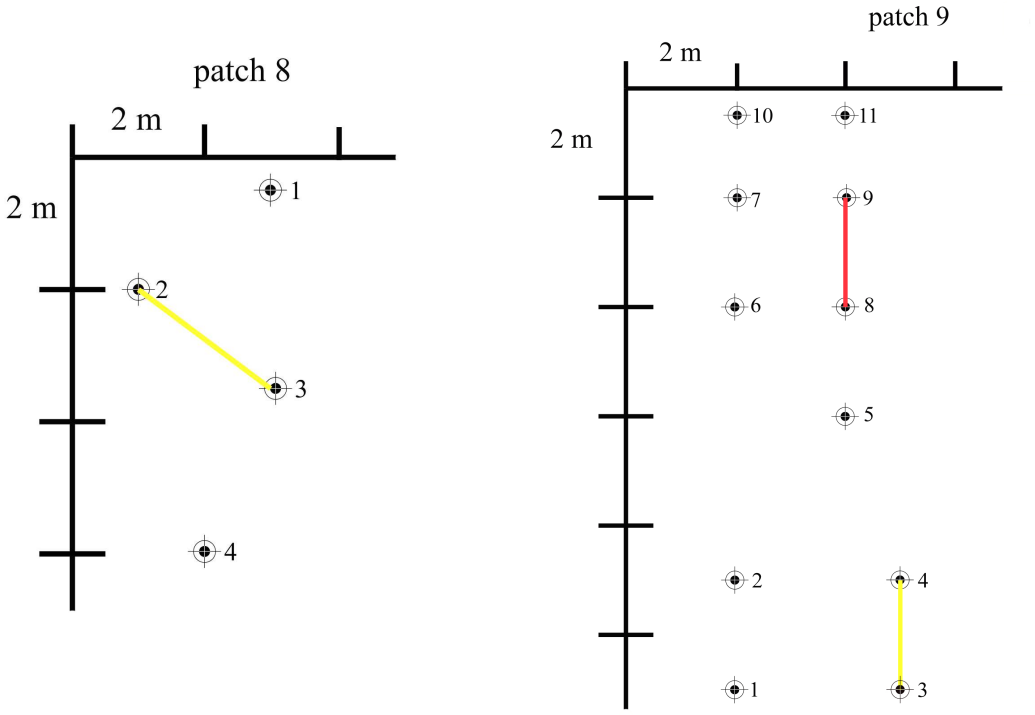

Figure 27: Left patch number 8, right patch 9 from Haslau west. Red lines are marking clonal connections based on 3% and the yellow ones on 5% threshold. Sampling distance was 2m.

Two out of four ramets in patch 8 (Figure 27, left) and four out of eleven ramets in patch 9 (Figure 27, right) are recognized as clones. The Neighbor joining tree (Figure 29) shows that the clone 8.2/3 is linked with a clone 9.8/9 and ramet 8.4 with clone 9.3/4. Ramet 8.1 is related to patch 2. The sampling distance in patch 8 and 9 was 2 m. Samples of patches 1, 3, 6, 10, 11, 12, 13 and 14 in the Haslau west subpopulation did not reveal any clonality. However, in these patches (except patch 6 with 4 ramets) only one or two ramets per patch were sampled.

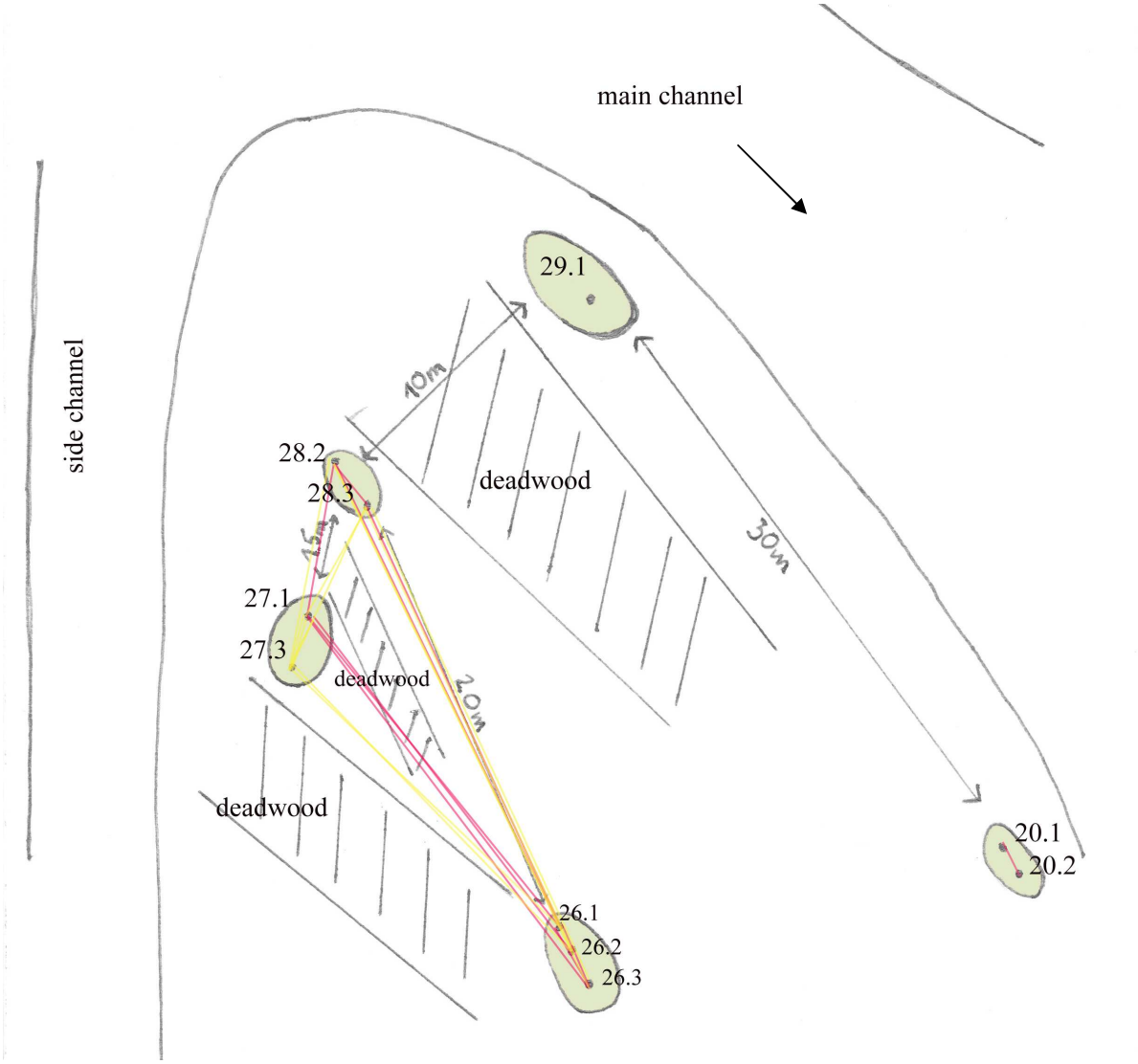

Figure 28: Subpopulation Finstergraben in 2007 with five patches and its clonal connections. Red lines are marking clonal connections based on 3% and the yellow ones on 5% threshold.


0.1
JOHNSBACHBRÜCKE
FINSTERGRABEN
SCHNEIDERWARTGRABEN
HASLAU
PATCH 5
PATCH 2
PATCH 4

Figure 29: Neighbor joining tree based on Nei-Li distance with samples of all six *Calamagrostis pseudophragmites* subpopulations, rooted with *Calamagrostis varia*. Bootstrap values greater than 50% are marked above branches. Red lines are marking clonal connections based on 3% and the yellow ones on 5% threshold.

Figure 29 can be found on the previous page.

4.4 Detailed analyses of Haslau subpopulations

Haslau west (patch 1 to 14) and east (patch 15) subpopulations are separated by only 150 m. The Neighbor joining analysis did not show any separation between these two subpopulations (Figure 29). This pattern can be also seen in the PCA analysis. In the PCA analysis the first three axes revealed approximately 32% of variation of the Haslau data set (16.9, 7.8 and 7.2% respectively). Clonal groups 5.1 (without 5.1.11-12 and 5.1.23-25) and 7.4/5/6 (indicated by I and VI, respectively; Figure 30) as well as three ramets 9.1, 9.11 and 15.9 are found very distant from the main cluster. Tight grouping of the 5.2, 5.3 and 5.1.27 indicates the clonal relationships among them (Figure 30; group IV) as in the NJ tree. Ramets from patch 4 are connected with the mentioned clone. Patches 7, 8 and 9 (Figure 30; II), are slightly separated and also form a group in the Neighbor joining tree (Figure 29). Clones from patch 2 and 5.23/24/25 can be seen as condensed group V. Group III includes ramets 8.2-3 that were identified as clones together with 8.1, 9.5, 9.2 and 7.2 that did not form a cluster in NJ dendrogram.

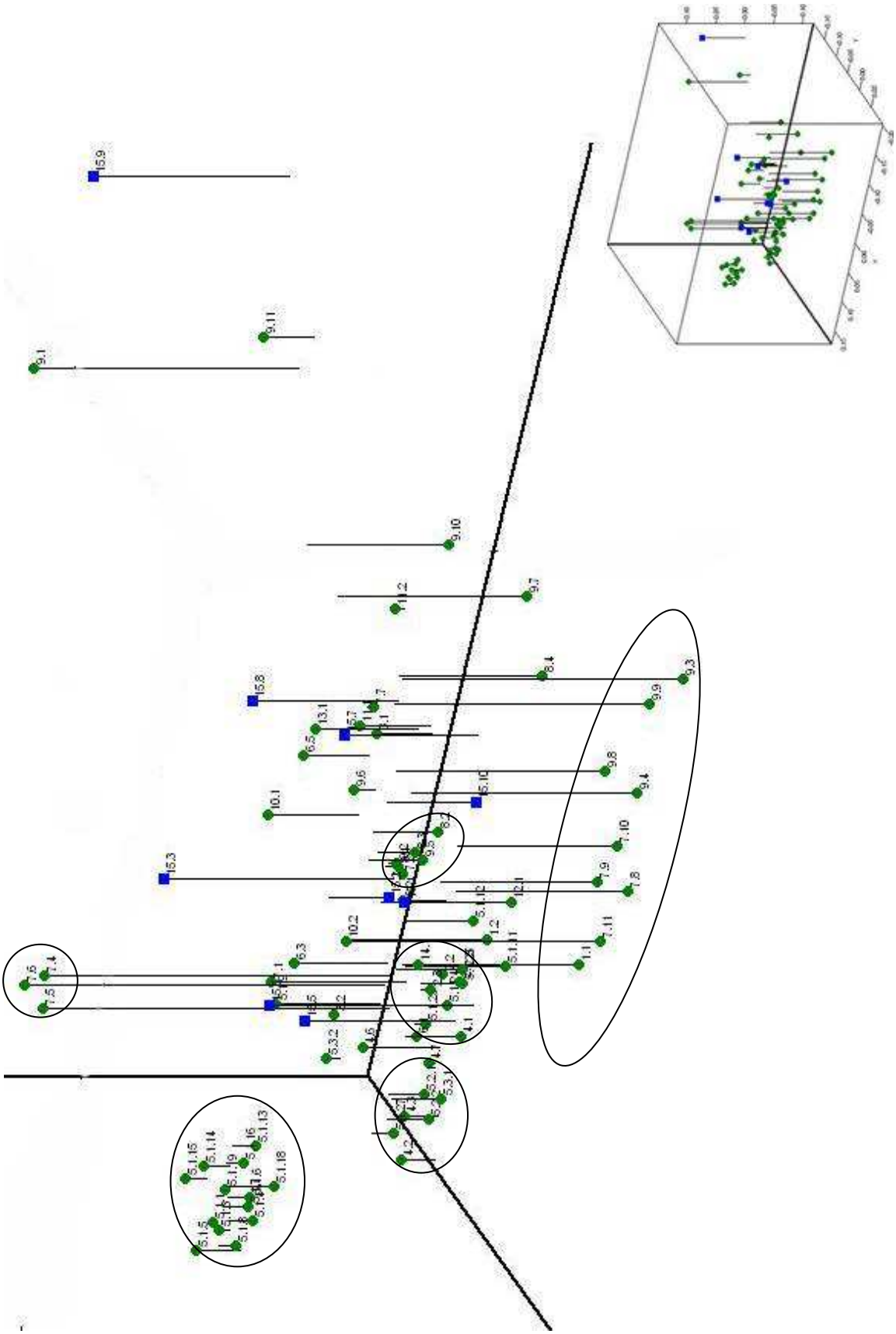

Figure 30: PCA with data from Haslau west and east subpopulations. ● Haslau west, ■ Haslau east. Groups I-IV are in accordance with the clusters revealed by the Neighbor joining analysis (Nei-Li distance) and clonal tracking (Squared Euclidian distance) inside the subpopulations Haslau east and west. The insert shows PCA scores.

4.5 Genetic structure

4.5.1 AMOVA

Analysis of Molecular Variance (AMOVA, Table 7) shows a high component of variation within subpopulations in Gesäuse (80.09%), the remaining 19.91% are due to variation among subpopulations. This proportion of variation among subpopulations provides an F_{ST} estimate (0.20) that indicates a moderate gene flow between subpopulations. AMOVA without multiple clones generates a lower F_{ST} value of 0.16.

Table 7: Analysis of molecular variance (AMOVA) including all subpopulations.

Source of variaton	d.f.	Sums of squares	Variance components *	Percentage of variation
Among subpopulations	5	810.91	8.07 Va	19.91
Within subpopulations	123	3989.85	32.44 Vb	80.09
Total	128	4800.76	40.50	

* for all variance components: $p < 0.000001$

Fixation Index F_{ST} : 0.199

Table 8: Analysis of molecular variance (AMOVA) of the four groups of patches in Haslau west subpopulation.

Source of variaton	d.f.	Sums of squares	Variance components *	Percentage of variation
Among groups of patches	3	325.85	4.80 Va	13.33
Within groups of patches	69	2153.66	31.21 Vb	86.67
Total	72	2479.51	36.01	

* for all variance components: $p < 0.000001$

Fixation Index F_{ST} : 0.133

AMOVA (Table 8) within the Haslau west subpopulation shows lower differentiation between groups of patches (13.33%) providing an F_{ST} estimate of 0.13. Four groups of patches that are spatially close together were formed to check the variation among those groups; (i) patches 2 and 3, (ii) patches 7 and 8, (iii) patches 1, 4, 5 and 6, (iv) patches 9, 10, 11, 12, 13 and 14. Other groupings revealed a slightly lower but still very similar differentiation ($F_{ST2} = 0.130$ and $F_{ST3} = 0.111$).

4.5.2 Bayesian Analysis of Population Structure (BAPS)

The individual level mixture analysis using BAPS identified 6 clusters with log(ml) -14390.8 for optimal partition (Figure 31). However, the six genetic clusters are not fully in line with the subpopulations. Four of the partitions are found inside of subpopulation Haslau west with most samples from Haslau forming the largest cluster (yellow) together with seven ramets from Haslau east and one from Johnsbachbrücke. There is obviously some relationship between Haslau west and east as they share gene pool IV. Three small gene pools (clusters); the clone 5.1.27/5.2.1/5.2.2/5.3.1 (pink), the clonal group 5.1 (green) and ramets 9.1, 9.11 (all from Haslau west) and 15.9 (Haslau east) found on Haslau island seem to be distant to the other Haslau west patches. The second largest gene pool V (red) includes ramets from Johnsbachbrücke, Finstergraben, Bruckgraben and Haslau east. Thus these subpopulations are probably related to each other. Schneiderwartgraben is related to Finstergraben (to the samples of Finstergraben in 2006) and Bruckgraben via gene pool VI (magenta).

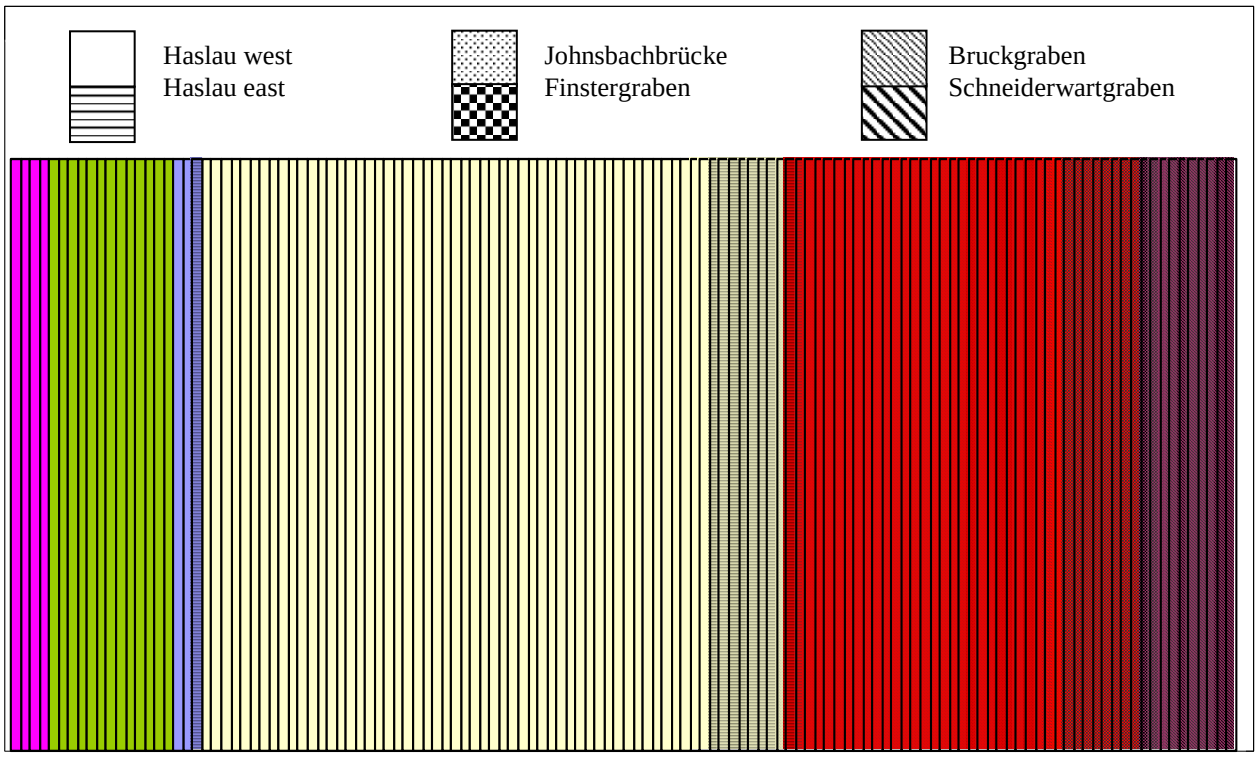

Figure 31: Mixture clusters (colored) produced with BAPS plotted together with subpopulations pattern; cluster 1 with 4 ramets (pink), cluster 2 with 61 ramets (yellow), cluster 3 with 13 ramets (green), cluster 4 with 38 ramets (red), cluster 5 with 10 ramets (magenta), cluster 6 with 3 ramets (blue).

In the admixture analysis based on mixture clustering of the whole data significant ($P < 0.05$) or marginally significant ($P < 0.075$) admixture was found in 15% of the ramets

(Figure 32). A great amount of significant admixture was found in patch 4 and 6 (Haslau west), in patch 15 (Haslau east), and in ramets 3.2, 5.3.2, 7.3, 9.7, 9.10, 10.1 (Haslau west), 18.5, 19.1 and 31.5 (Johnsbachbrücke) (Figure 32). Haslau west mainly contains the gene pool II (Figure 32; red), while gene pool IV (Figure 32; green) includes most ramets from subpopulations Johnsbachbrücke, Finstergraben and Bruckgraben. The clonal group 5.1 forms a homogenous cluster III nearly without any other admixture (Figure 33; yellow). The ramet 5.1.9, the clone 5.1.11-12 and the linear clone 5.1.23-25 belong to gene pool II with (low) admixture from gene pool III and IV and V. The homogenous clonal group 5.2/ 5.3/ 5.1.27 is found in an own gene pool I (Figure 33; blue) that is found in low admixture (<10%) in other ramets. Ramets 4.2, 9.1 and 9.7 (Haslau west) share the gene pool VI (Figure 33; orange). Higher admixture of this gene pool is mostly found in Haslau subpopulations, predominantly in patches 9, 10, 11, 13, 14, 15 and also in patch 16 (Johnsbachbrücke). Haslau west ramets 9.9-11 and 4.1 are included in gene pool IV that is mostly significant for Johnsbachbrücke and Finstergraben. Haslau east subpopulation contains mostly gene pool II, yet the ramets show high admixture with gene pool VI and occasionally with gene pools III, IV and V. Johnsbachbrücke subpopulation patch 16, with the exception of ramet 16.5, is part of the gene pool II slightly mixed with gene pool VI. The rest of the patches sampled in both years (17, 18, 19, 30 and 31) with the exception of 31.5 (with very significant admixture) belong to gene pool IV. Admixture from all gene pools can be observed in Johnsbachbrücke, although patches sampled in 2006 (17-19) have more admixture from gene pool II, III and VI, while additional patches from 2007 (30 and 31) show more admixture from gene pool I and V. Schneiderwartgraben ramets from 2006 and 2007 appear in a unique gene pool (cluster V) which includes two ramets from Finstergraben sampled in 2007. The rest of Finstergraben subpopulation is mainly a part of gene pool IV, as well as two out of three ramets from Bruckgraben. The third ramet shows a highly significant admixture containing all six gene pools.

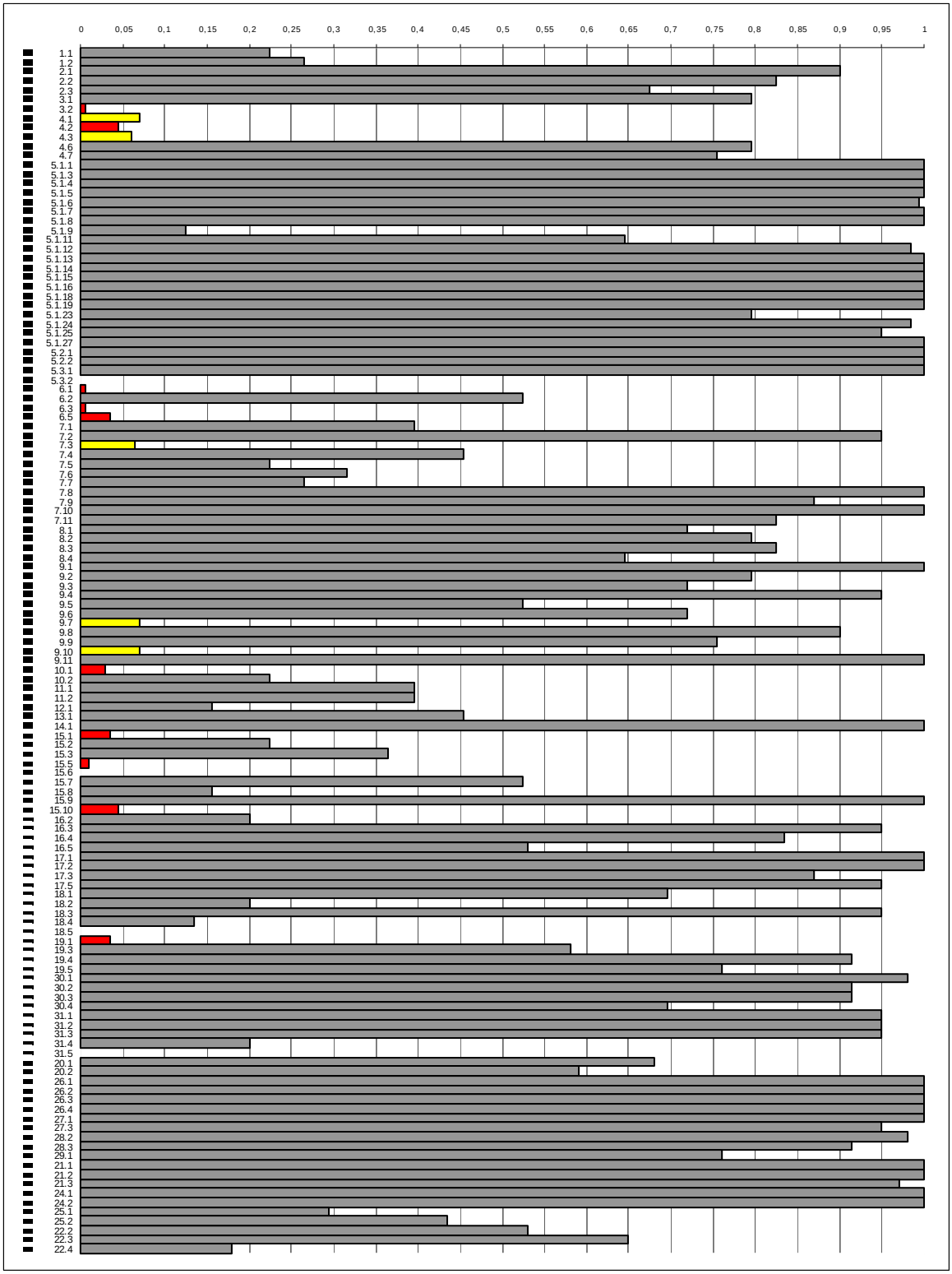

Figure 32: The probability profile shows the proportion of admixture in all ramets if the minimum population size is set to 3. P-values of 0.05 or below indicate significant admixture (red), whereas those with marginal significant admixture (below 0.075) are marked with yellow. The subpopulations are marked with their capitals (Haslau west – w, Hasalu east – o, Johnsbachbrücke – j, Finstergraben – f, Schneiderwartgraben – s, and Bruckgraben – b).

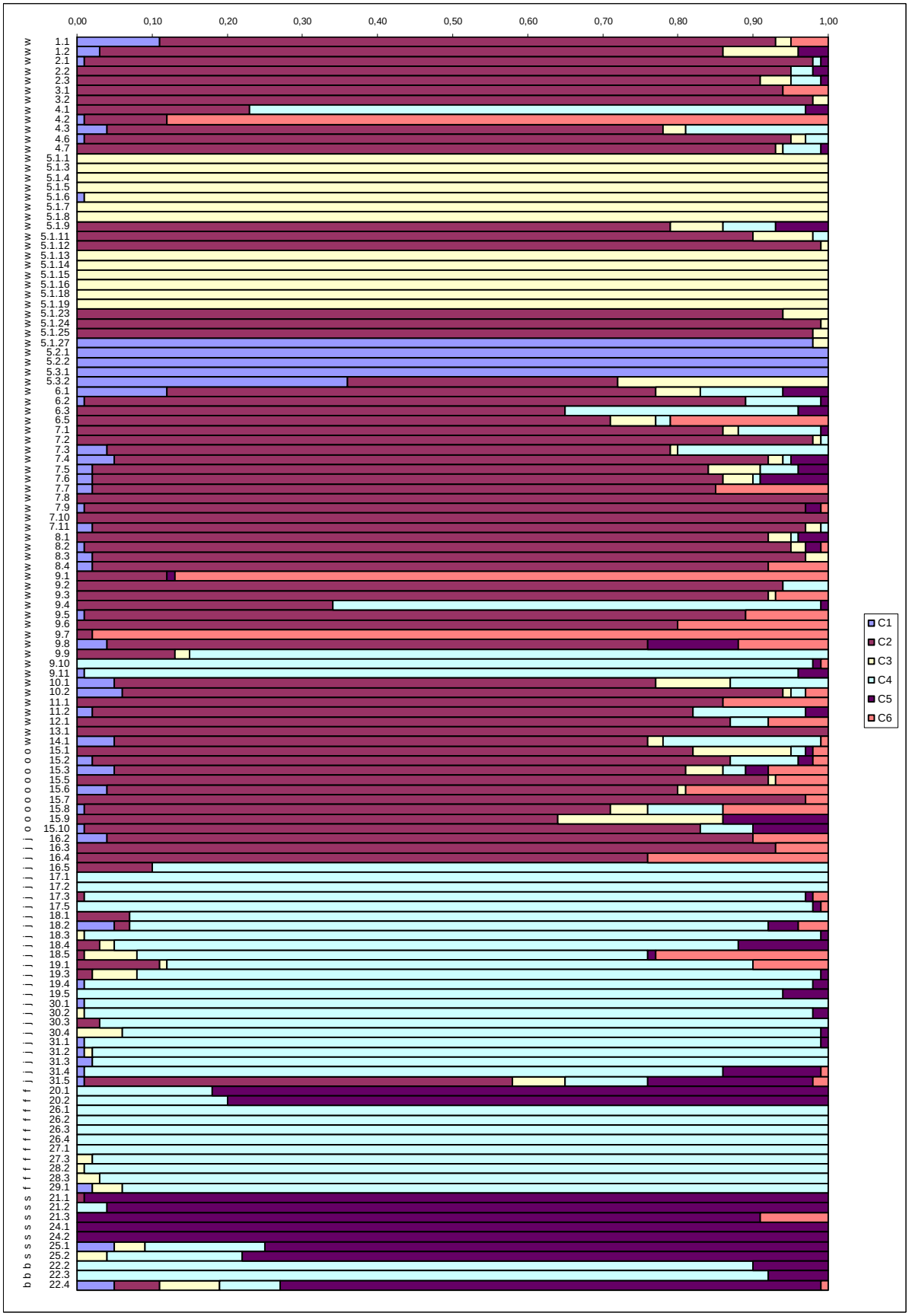

Figure 33: Results of admixture analysis based on mixture clustering of 129 ramets. The subpopulations are marked with their capitals (Haslau west – w, Haslau east – o, Johnsbachbrücke – j, Finstergraben – f, Schneiderwartgraben – s, and Bruckgraben – b).

For further analysis multiple clones were removed from the data set i.e. only one ramet representing a clonal group (genet) was used (86 individuals). The individual level mixture analysis using BAPS identified 3 clusters with log(ml) -10748.7 for optimal partition. The existence for 3 clusters was associated with a 0.89 probability, while the partition in 4 clusters had a probability of 0.11. In this case the partitioning is mostly in line with the spatial pattern, as Haslau west and east subpopulations (Figure 34; yellow) can be distinguished from Bruckgraben, Johnsbachbrücke, Finstergraben and Schneiderwartgraben (Figure 34; green). Only ramet 15.6 from Haslau east subpopulation is found in another large cluster. Three ramets 9.1, 9.11 and 15.9 that already showed distance to the whole population in the Neighbor joining tree (Figure 29) again formed a small cluster (Figure 34; blue).

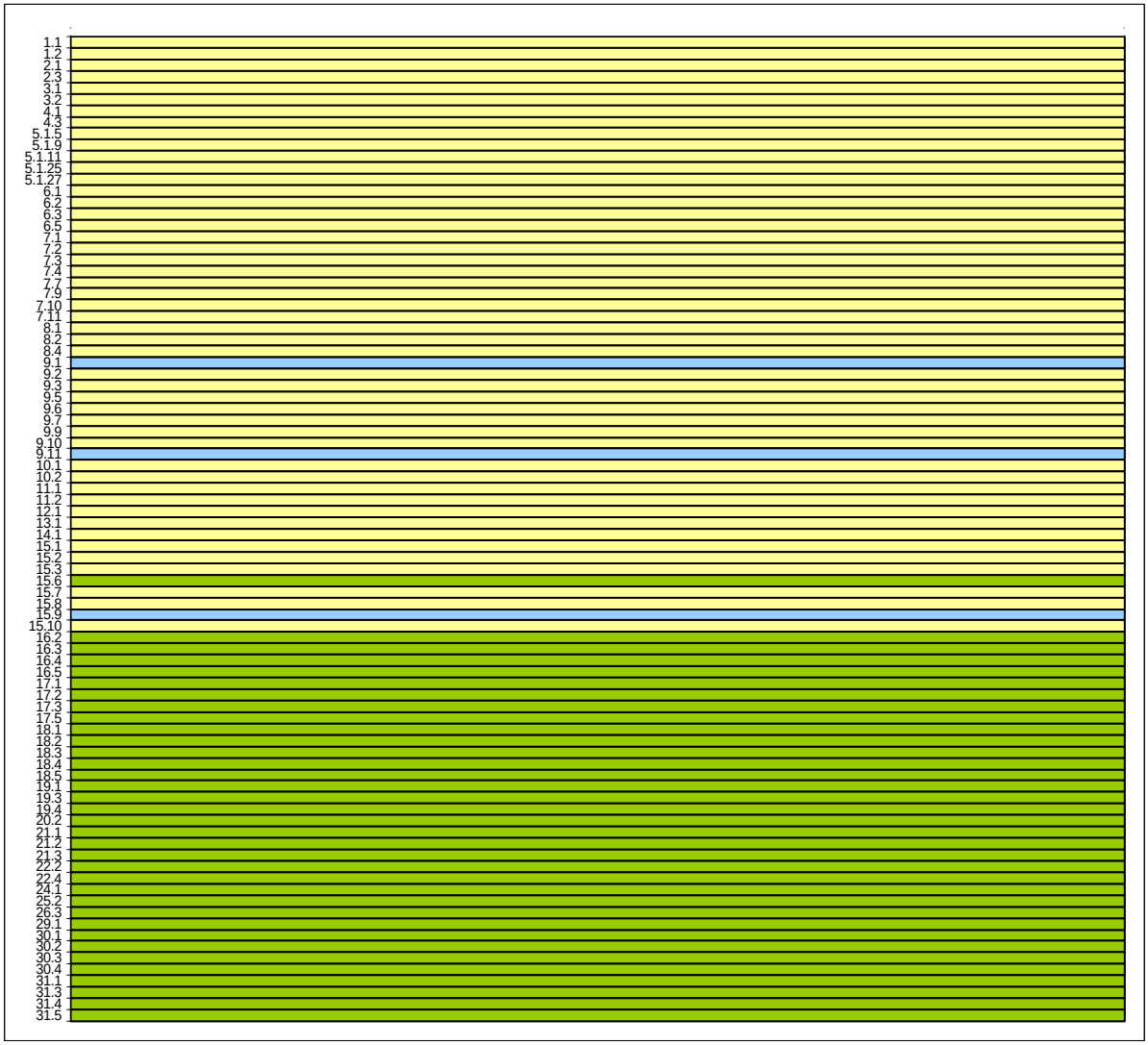

Figure 34: Mixture clusters produced with BAPS using only genets; cluster 1 – yellow; cluster 2 – green; cluster 3 – blue.

The probability profile (Figure 35) shows several highly significant or marginally significant cases of admixture (21%). Ramet 5.1.5 from Haslau west that represents a large clone in patch 5.1 interestingly showed significant admixture. Significant admixture was also found in patch 6, ramets 7.3, 9.7, 9.10, 10.1, 12.1, 15.3, 15.5, 15.8, 18.5, 19.1, 31.5, and 21.3. The admixture proportions are shown in Figure 36. Half of the ramets from Haslau east (15.7-15.10), all ramets from Haslau west patches 10, 11, 12, 14 and 9 (with an exception of 9.2 with admixture from gene pool II) and ramets 6.5, 7.7, 7.9, 8.4 and 8.2 from Haslau west subpopulation have high admixture from this gene pool. In the subpopulations Bruckgraben, Johnsbachbrücke, Finstergraben and Schneiderwartgraben the admixture from gene pool III is more sporadic; the genets 18.5, 19.1 and 31.5 (Johnsbachbrücke) and 21.3 (Schneiderwartgraben) have higher admixture from gene pool III, while genets 16.3, 17.3, 17.5, 18.2 31.4 (Johnsbachbrücke) and 26.3 (Finstergraben) show admixture from this gene pool with less then 3%. The admixture of gene pool I is also present in these subpopulations. Low admixture with gene pool I can be found in whole Schneiderwartgraben, in genets 26.3 and 29.1 from Finstergraben, in genets 31.1, 19.3, 19.1, 16.2, 16.5, and in the patch 18 (without 18.3) from Johnsbachbrücke. Genet 31.5 from Johnsbachbrücke (2007) belongs to the gene pool Haslau with very high admixture from its neighbors. Haslau subpopulations include admixture from gene pool II in patch 2, 4, 5 (except 5.1.25), 6, 7 (except 7.2 and 7.7), 10, 12, 13 (Haslau west) and in the other half of Haslau east genets (15.1-3). Genet 15.6 from Haslau east belongs to gene poll II.

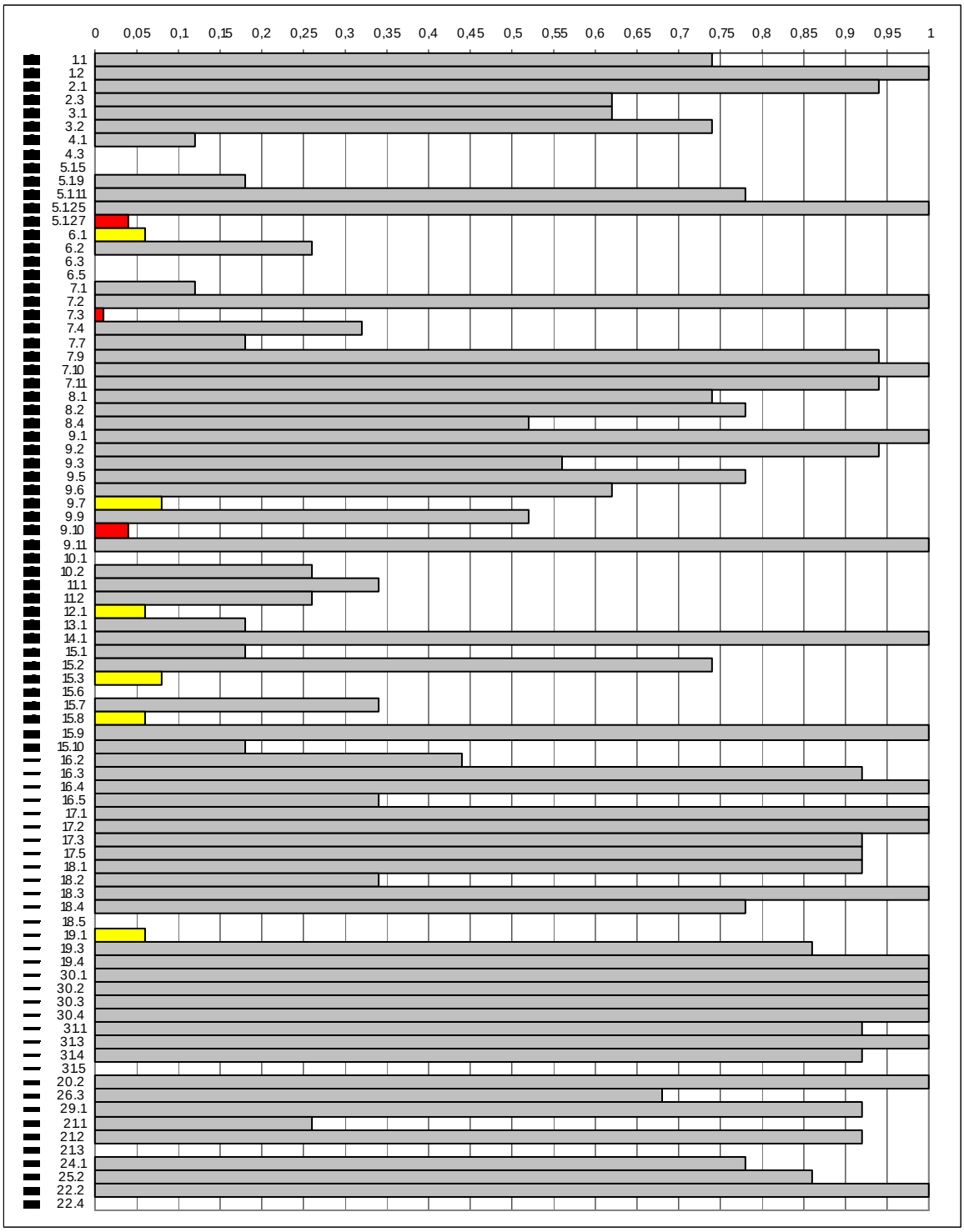

Figure 35: The probability profile shows genets with high admixture. P-values of 0.05 or below indicate significant admixture (red), whereas those with marginal significant admixture (below 0.075) are marked with yellow. The subpopulations are marked with their capitals (Haslau west – w, Hasalu east – o, Johnsbachbrücke – j, Finstergraben – f, Schneiderwartgraben – s, and Bruckgraben – b).

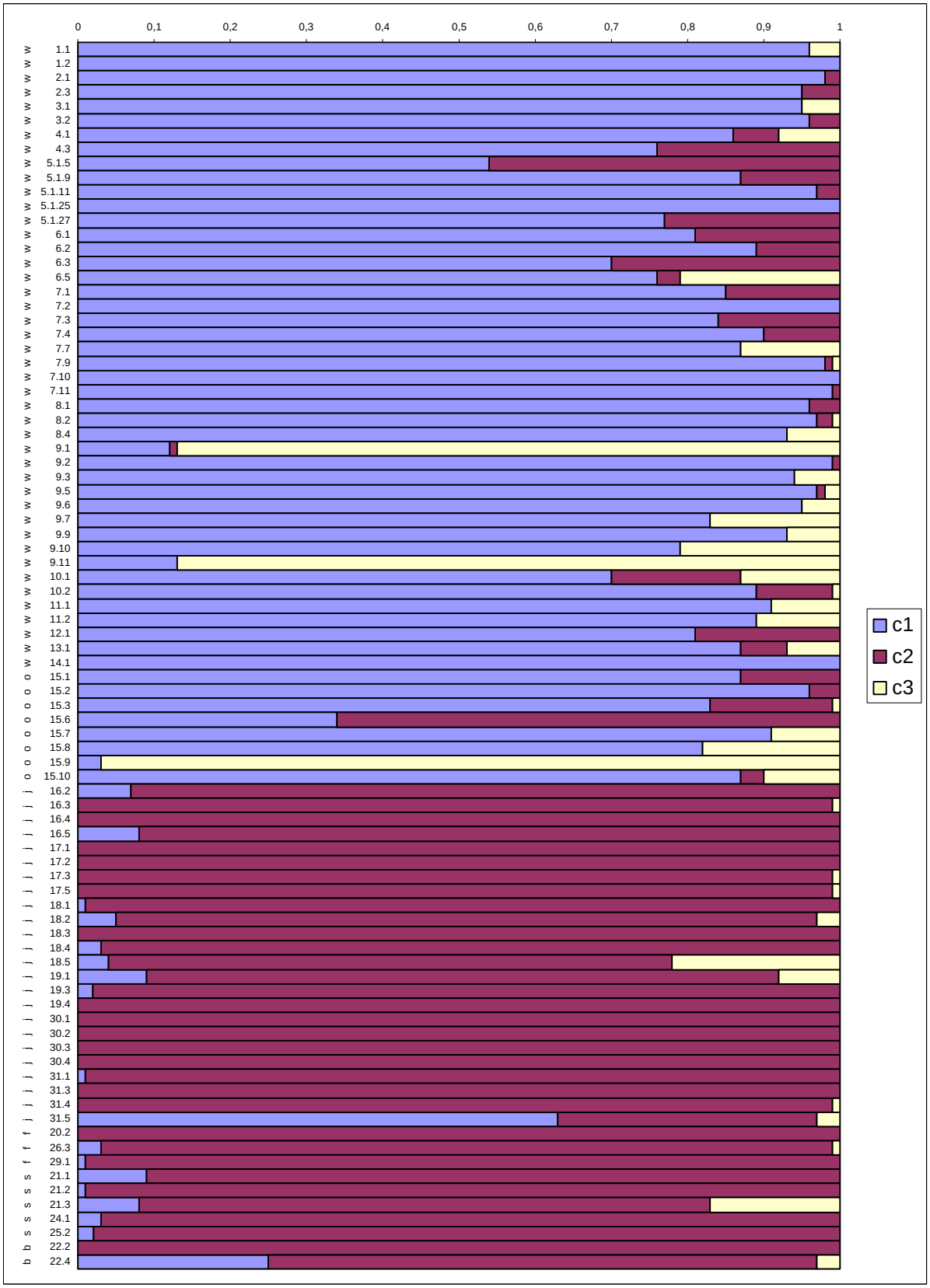

Figure 36: Results of admixture analysis without clones based on mixture clustering of 86 genets. The subpopulations are marked with their capitals (Haslau west – w, Hasalu east – o, Johnsbachbrücke – j, Finstergraben – f, Schneiderwartgraben – s, and Bruckgraben – b).

4.5.3 Mantel test

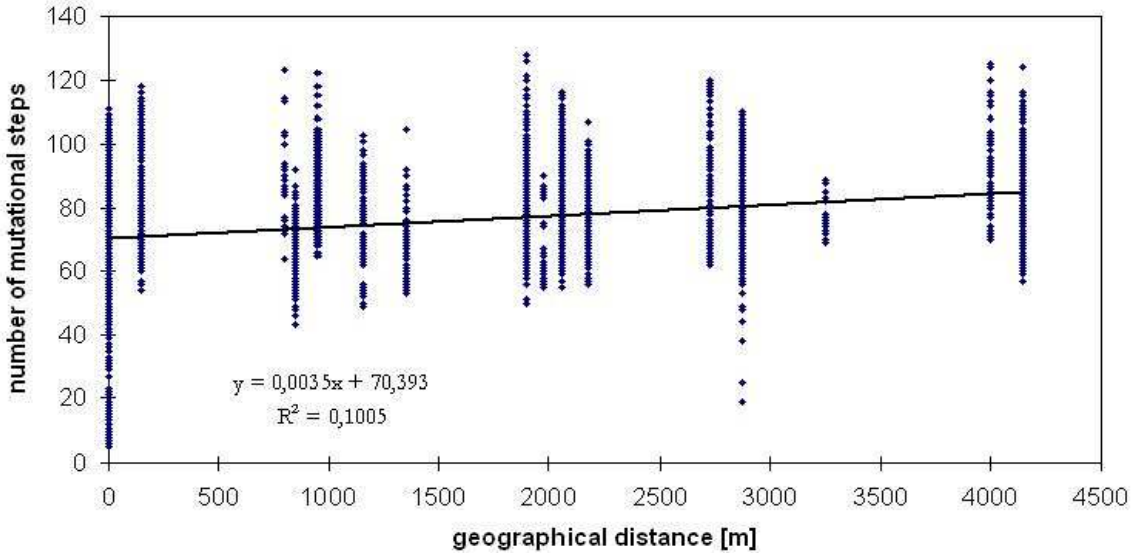

Figure 37: Mantel test between geographical distances among subpopulations and genetic distances between all possible pairs of ramets.

A mantel test revealed only a low isolation by distance effect (Rm = 0.317, P= 0.001). About 10% of the genetic variation among subpopulations is explained by geographic distance.

Discussion

5.1 Clonal patterns

Clonal patterns in the population system of *Calamagrostis pseudophragmites* were identified in each subpopulation. In the data set of 129 ramets in the Gesäuse population 62 of them showed a clonal connection with at least one other ramet applying a 5% genetic distance threshold. However, detecting clones strongly depends on the sampling design. In patch 5, where a dense sampling was applied, 22 ramets with clonal connections were found. In total, 19 groups of clones were found, of witch 18 were identified within patches. Overall 86 genets were identified inside the sampling dataset. Despite the often small number of samples in small subpopulations, e.g. in Bruckgraben, at least one clone in each subpopulation was found, which indicates that vegetative dispersal within patches is common. Clonal growth is especially important within patches. The plants spread faster, as no mating is necessary and the patches become dense. Large numbers of ramets were detected belonging to one genet when the ramets were sampled around 20-30 cm apart, but not all ramets in the neighborhood were necessarily part of the identified clones. A clonal pattern was also tracked between ramets of 2 m distance (patches 7, 8, 9), yet relatively low number of clones were tracked in large patches. The overall sampling design was namely applied to track the number of clonal groups, rather than the number of identical ramets. Also multiclonal patches were found. However, in multiclonal populations, the spatial distribution of clones depends on the clonal growth strategy (Escaravage et al. 1998). The clonal groups probably include more ramets than can be shown in this study. However, the spatial extension of the clonal groups may be limited, since the present analysis resolved only few of them in large patches. Generally, the shifting from sexual to vegetative reproduction varies along environmental gradients (Gaudeul et al. 2007). The importance of vegetative propagation increases where the availability of mates is low, i.e. in small and sparse populations with scarce resources and also where soil conditions allow rhizomes to spread easily (Gaudeul et al. 2007). In summary the present survey suggests that sexual reproduction is predominant in *C. pseudophragmites* population and that vegetative propagation is important on the subpopulation level.

Clones were also found among three patches within one subpopulation. Two of the patches are 1.5 m apart, yet deadwood lies between them. The third patch is approximately 20 m apart from those. This group of patches was covered with deadwood a year before. It is

possible that the plants existed as underground rhizomes and regenerated, as the plants found in 2007 were adult, sterile or the spikelets infertile.

No clonal patterns were identified among subpopulations. Obviously vegetative propagation plays an important role only in a local context. Fragmentation and migration of ramets are obviously rare events. The rhizomes may be too thick to be separated easily. Yet, the water dynamics could cause larger perturbations on the sandy riverbanks, thus part of the rhizomes could probably be fragmented. The distance between subpopulations (150 m to 4.2 km) is perhaps too large for ramets or fragments of rhizomes to become established after longer transport. It is also possible that this kind of dispersal is much rarer than assumed; the sampling design would have to be modified to reveal such events. Some ramets belonging to different subpopulations appear in the same genetic clusters. The similarity analyses did not reveal them as clones, yet they are still very closely related, so it is possible that they once migrated as clones to another subpopulation and then somatic mutations accumulated through time or that they mated with their neighbors, thus receiving a genetic admixture from them. Another scenario would be that ramets did establish from seed dispersal between subpopulations. In general, clonal dynamics seems to be important within subpopulations, as clear evidence for clonal migration between subpopulations was not revealed.

Calamagrostis pseudophragmites is a perennial plant that elongates itself with underground rhizomes (Clayton et al. 2002). Little information was found about the vegetative extension of this species or about the importance of clonal reproduction. Fragmentation of rhizomes in *C. pseudophragmites* was not reported in literature (e.g. Conert 1989). Reproduction is partially sexual. The seeds are 2-2.3 mm small wind-adapted caryopses (Conert 1989). This reedgrass grows in open habitats as a pioneer species, so high *r*-strategy seedling recruitment is expected. Yet the plants are often found with infertile spikelets (Greimler, pers. comm.).

The present study suggests that clonal propagation on the small scale may contribute to high estimates of regional population differentiation in *C. pseudophragmites*. In the population, the mean F_{ST} was 0.20 when the multiple clones were included, and 0.16 when the multiple identical genotypes were excluded from the analysis. The same patterns were found also by Gaudeul et al. (2007) for Swedish and Norwegian populations of *Arabidopsis lyrata*. In this recent study (Gaudeul et al. 2007) identical genotypes were

detected (with microsattelite markers) in many populations, suggesting that they are a result of clonal propagation. Clones were found with one exception (one multilocus genotype was found in two different populations) within the same populations, and the clonal connections were always found among the closest neighbors (Gaudeul et al. 2007). The proportion of identical genotypes was inversely related to the population size of *Arabidopsis lyrata*, which is capable of vegetative propagation through the production of subterranean runners (Gaudeul et al. 2007). This correlation may be because the number of potential mates correlates negatively with the level of vegetative propagation (Gaudeul et al. 2007). However, this is not the case with *C. pseudophragmites* because the subpopulation sizes do not correlate with the number of identified clones.

In general, low recruitment rates from seeds have been observed in clonal populations even if seed production is sufficient (Pornon et al. 2000). Hence, the clonal propagation in *C. pseudophragmites* was assumed to be high and the actually high degree of genetic diversity was unexpected. Literature surveys on molecular markers variation in clonal plants found that clonal populations can have a high genetic diversity (Escaravage et al. 1998, Ellstrand & Roose 1987).

Using AFLP markers, investigation of *Rhododendron ferrugineum* revealed a multiclonal population with 32 intermingling genotypes on a 200-m^2 site (Escaravage et al. 1998). Lamote et al. (2002) found clonal patterns only within *Iris pseudacorus* populations, whereas in the study of *Typha* (Lamote et al. 2005) not only samples from the same locations, but also those from different river basins were revealed as clones. In this study Lamote et al. (2005) however suggest that high similarities shown in the study are artifacts because *Typha latifolia* is highly homozygous (predominant self-fertilization) and shows low genetic diversity among genets.

5.2 Technical aspects of identifying clones

The AFLP technique (Vos et al. 1995) was applied for this study, as this method has been used in several applications to assess population structure as well as clonal patterns within and among the populations. AFLP markers are considered a successful tool for identifying individuals in clonal populations (Escaravage et al. 1998, Lamote et al. 2002, Albert et al. 2003, Douhovnikoff & Dodd 2003, Gaudeul et al. 2007). Studies are increasingly using AFLPs for detecting plant clones, because AFLP can reveal large numbers of fragments with a high degree of reproducibility, and so efficiently replace the isoenzyme and RAPD

methods (Douhovnikoff & Dodd 2003). A study of clonal diversity of *Rhododendron ferrugineum* (Escaravage et al. 1998) assessed that the genotypic distribution gained with AFLP suits the spatial distribution of clones and that the AFLP technique efficiently identify genotypes, but the detection of somatic mutations between identical genotypes is not possible. Lamote at al. (2005) concluded that the extent of a species` diversity determines the efficiency of the methodology applied for detecting clones. They found that for clonal species with a high degree of genetic diversity (e.g. Lamote et al. 2002), the AFLP method is suitable for detecting clonal propagation. Alternatively, in species with low level of genetic diversity other techniques, such as SSRs, could be useful because loci with high mutation rates would be investigated (Lamote et al. 2005).

A 95% rate of reproducibility of AFLP patterns was detected in the *C. pseudophragmites* population. The 5% of irreproducible markers were removed form the dataset. However in the histogram a clear outlier position of distance classes between 1.5 and 5% was identified, corresponding to an interval of 6 to 20 mutational steps. Therefore the clones still show genetic differences up to 5%. Furthermore, the frequency of the distance classes among ramet pairs between 10% and 30% follows a nearly Gaussian distribution, so the outlier position of the lowest distance classes can be assigned to clonality. According to this the threshold was set to 5%.

In the study of genetic variation in *Iris pseudacorus* (Lamote et al. 2002) the degree of reproducibility ranges from 97.9 to 98.5%. Therefore the samples with differences of 2% or less were considered as identical genotypes (Lamote et al. 2002). Allowing no (additional) intraclonal variation they considered only three ramets as a clone. Additionally they found two pairs of clones applying a relaxed threshold of 4%. In a study on the genetic diversity of *Typha* (Lamote et al. 2005) the lowest genetic similarity observed between replicates (0.94) was used as the clonality threshold. Ramets sampled in the same location together with some from different river basins revealed AFLP genetic similarities higher then 0.94 (Lamote et al. 2005). In the clonal population of *Rhododendron ferrugineum* (Escaravage et al. 1998), however, a similarity index of 0.85 was applied to discriminate one genotype from another. Somatic mutations, which are common in tree species and are inherited to the following generations, were inferred to explain high genetic diversity among identical genotypes (Escaravage et al. 1998).

Previous reproducibility tests in plants (Jones et al. 1997, Hansen et al. 1999, Bonin et al. 2004) reported AFLP error rates below 5%. Only 6% of replicates from the whole data set were included in present analysis, yet current estimations of genotyping errors (Bonin et al. 2004) recommended that 5-10% of the samples should be repeated.

A high clonal variation in the (probably long-lived) *C. pseudophragmites* could occur because of somatic mutations, as found by Escaravage et al. (1998) in another long-lived species. In general, in clonal populations a low level of sexual reproduction has been observed, but a high level of genetic diversity was often reported for such populations (Ellstrand & Roose 1987, Esselman et al. 1999, Kreher et al. 2000, Stehlik & Holderegger 2000, Lamote et al. 2002). Somatic mutations could be the reason for high genetic variation present in clonal populations, yet Lamote et al. (2002) assume that the genetic variation detected in the population of *Iris pseudacorus* was maintained from an initial diversity of the populations and high sexual reproduction. In the present study, a much larger number of replicates might have reduced the intraclonal variation.

5.3 Differentiation patterns among and within subpopulations

The subpopulations are genetically moderately differentiated. A great differentiation was found only between both Halsau subpopulations and other subpopulations. The Neighbor joining analysis revealed a very weak backbone typical for AFLP analysis within single species. Besides the Haslau subpopulation a group of ramets with Johnsbachbrücke, Schneiderwartgraben and Finstergraben was formed. Also the UPGMA dendrogram and the PCA (explaining about 28% of total variation) show the separation of Haslau west and east from other subpopulations. The Schneiderwartgraben subpopulation forms a cluster together with two samples from Finstergraben in a BAPS individual level mixture analysis with all clones included, as well as in PCA and in the UPGMA. BAPS mixture clustering of all data showed a pattern partly in accordance with spatial structure. A differentiation between (i) Haslau west together with Haslau east and (ii) Schneiderwartgraben, and (iii) Johnsbachbrücke together with Finstergraben, was revealed. Population differentiation is not high regarding the F_{ST} value (0.20). The reason for low differentiation between Haslau west and east could be the close distance of 150 m between the subpopulations. Johnsbachbrücke and the Finstergraben are ca. 850 m apart. Schneiderwartgraben, which is partly related to Finstergraben, lays ca. 1350 m to the east. These distances are obviously close enough to allow seed dispersal among subpopulations, which leads to low differentiation among those subpopulations.

Gene flow occurring between Haslau subpopulations and the other subpopulations may be predominantly due to pollen flow. A few ramets found in genetic clusters to which they do not belong spatially, however, may have dispersed by seeds or clonal migration. Yet, no clonal connection was found between these ramets and those in the cluster to which they belong. Perhaps the sister ramet in another subpopulation was not collected by the applied sampling design. This could only be determined when all ramets in the population were sampled. The isolation by distance was low. Thus the close subpopulations do not have much greater similarities than the further ones. The low isolation by distance indicates that seed recruitment also occurs over longer distances and not just few meters from the "mother" plant (Escaravage et al. 1998). Seed dispersal can be inferred between Haslau west, Johnsbachbrücke and Schneiderwartgraben. The (minor) admixture inside of the clusters (e.g. Haslau west, east and Johnsbachbrücke) occurs mostly due to pollen flow.

Successful reproduction by a plant occurs when its seeds are dispersed to sites where they can germinate and establish seedlings (Fenner 1987). The transport often involves external agents such as wind, water, birds, other animals or the plants on their own can disperse itself by an exploding pod (Fenner 1987). Within any plant community there are often few different dispersal mechanisms to be found, though the used agencies vary with the vegetation type (Fenner 1987). In early successional stages, where seeds are the first to arrive at the new site, species tend to disperse mostly by wind, while further increasing vegetation complexity attracts birds or other animals (Fenner 1987). Any structure of the wind-dispersed seeds that may reduce the speed with which they fall to the ground will increase its chances of being transported far away (Fenner 1987). The velocity of a falling seed is determined by the air resistance offered by its surface, hence, in the small seeds it is relatively high (high surface/ volume ratio) and increases by possible appendages such as hairs, wings or plumes (Fenner 1987). The wind dispersal functions optimally in open habitats, where the surrounding vegetation does not reduce the wind speed (Fenner 1987). Dispersal of seed by water (hydrochory) is a very important way of dispersal in wetland ecosystems (Shimamura et al. 2007). Hydrochory has an impact on short- and long-distance dispersal and hydrochorous seeds can often be buoyant for long periods (Shimamura et al. 2007). The species living in the river corridors often use river currents for the dispersal of their diaspores that occurs predominantly uniform downstream (Fér & Pfosser unpublished). Pollen transfer can be mediated by the wind or by various pollinators. Anemophilous plants produce very light pollen grains that can easily be

dispersed, whereas entomophilous plants have rather heavy, nutritious pollen adapted for dispersal by insect pollinators (sometimes by other animals).

In the Haslau west, Johnsbachbrücke and Finstergraben subpopulation a spatial structuring of the subpopulations was found. However, the genetic analyses revealed that in Johnsbachbrücke no genetic structure is present. The similarity analysis and BAPS admixture analysis indicate genetic structure in Finstergraben. This population is mainly related to Johnsbachbrücke and partly to Schneiderwartgraben and so belongs to two genetic clusters. Genetic structure regarding F-statistics was analyzed in the largest subpopulation of Haslau west. The F_{ST} value of 0.13 indicates lower differentiation, i.e. higher gene flow than detected in the whole population (F_{ST} = 0.20), although the differentiation within the subpopulation is notable as additional gene pools were identified by the Bayesian analysis. Two of the gene pools revealed by BAPS were identified with two clone groups. BAPS analysis without multiple clonal ramets still revealed two gene pools in the Haslau subpopulations.

5.4 Regional dynamics

Subpopulation size, neighborhood, and migration between subpopulations are fundamental factors that impact the survival of endangered species (Kawata 2001, Tero et al. 2005). In 2006 the population of *Calamagrostis pseudophragmites* appeared to be as large as in 2003 based on earlier observations (Kammerer 2003). However, in only six of seven riverbank locations in Gesäuse the species was found. Kammerer (2003) reported *C. pseudophragmites* to be present as rudimental and sporadic in Lettmair-Au, Bruckgraben and Schneiderwartgraben. In 2006 no ramets were found in Lettmair-Au and in 2007 sampling was not possible because the riverbank was under water. In 2007 new ramets were found in Johnsbachbrücke, Finstergraben and Schneiderwartgraben. The last two subpopulations are rather small compared to the Haslau west subpopulation.

In recent decades the metapopulation approach has become important for understanding "the large-scale population dynamics, the spatial distribution of species and the effects of habitat fragmentation on biodiversity" (Hanski & Gaggiotti 2004). Levins (1970) defined a metapopulation as an assemblage of spatially delimited local populations that are connected by some degree of migration. Because of continuing habitat loss and fragmentation the metapopulation approach becomes more important (Hanski & Gaggiotti 2004). Is the population of *Calamagrostis pseudophragmites* in Gesäuse, fragmented by

the nature of its habitat, an example for metapopulation dynamics? The local subpopulations of *C. pseudophragmites* are namely discrete entities in space that interact via migration and gene flow. Freckleton and Watkinson (2002) argue that the metapopulation model does not suit for many plant populations and that the true metapopulation systems may be limited to relatively few cases. They suggested that other concepts of plant regional dynamics such as "spatially extended populations" and "regional ensembles" should be used.

The population of *C. pseudophragmites* in Gesäuse provides evidence for metapopulation dynamics as the extinction and colonization of patches (i.e. subpopulations) of ramets are essential processes in a metapopulation system. The Lettmair-Au subpopulation was, according to Kammerer (2003) rudimentarily/sporadically present. In 2006 no ramets of *C. pseudophragmites* were found on this riverbank. A temporal extinction of most patches in Finstergraben occurred in 2006 with a re-appearance in 2007. The temporal "extinction" of patches (leaving only one visible patch in the subpopulation) was due to covering by deadwood, this may also be an example of a remnant population (Silvertown & Charlesworth 2001). The Lettmair-Au and Finstergraben sites suggest that extinction of the subpopulations is an active process in the population system of *C. pseudophragmites*. One patch from Bruckgraben, where the situation was similar to Lettmair-Au in 2003, disappeared in 2006. A new patch was found in Johnsbachbrücke in 2006 (patch 19). The tendency for extinction and colonization is indeed increased in single patches within the population, yet still there is evidence for one whole subpopulation extinction and probably in future more turn over will have happen again. There is also evidence for a migration between subpopulations, yet from the present data, distinguishing between ramet or seed migration is not possible. The following four conditions introduced by Hanski (1997) hold for the *C. pseudophragmites* population system, and thus this can be treated as a metapopulation: i) suitable habitats are discrete and may be occupied by local reproducing subpopulations, ii) the largest subpopulation (Haslau west) has a measurable risk of extinction, iii) subpopulations are not too isolated to inhibit the recolonization, and iv) the dynamics of subpopulations are not completely synchronous.

The differences in the local production of seeds in a source-sink population result in an asymmetrical gene flow from rich (source) to the poor habitats (sink), preventing local adaptation in the latter (Dias et al. 1996). Source and sink populations are generally not genetically differentiated due to the high gene flow among them (Dias et al. 1996). The

Gesäuse population system of *C. pseudophragmites* gave a first impression that the Haslau west subpopulation could be a source for other subpopulations due to its large size, the proportion of patches and upstream location. Yet this hypothesis was abandoned because the differentiation of Haslau west and east from other subpopulations is distinct and the gene flow via seeds between subpopulations occurs obviously in both directions; upstream and downstream.

Besides the *metapopulations* in the classic sense, where persistence depends on patch colonization, extinction and recolonization, Freckleton and Watkinson (2002) defined at the regional scale also the *regional ensembles*: "systems of essentially unconnected local populations persisting in an ill-defined mosaic of suitable and unsuitable habitat"; and *spatially extended populations*, that are "essentially single extended populations occupying large tracts of suitable habitat, but whose regional dynamics are essentially a simple extension of local dynamics". *C. pseudophragmites* does not form a spatially extended population, because the overall dynamics are not determined by local density-dependence (competition for resources or microsites). Dispersal of seeds in the Gesäuse population occurs from suitable and not fully occupied sites (e.g. Schneiderwartgraben) also towards other suitable habitat sites (e.g. Finstergraben). The regional ensembles model also does not fit the present population system because the subpopulations are linked by dispersal and they occur in discrete habitats.

The population of *C. pseudophragmites* showed colonization/extinction dynamics in the few years covered by the study including the report of Kammerer (2003). The *C. pseudophragmites* population suits the metapopulation that is "spatially structured into assemblages of local breeding (sub)populations and where migration among local populations has some effect on local dynamics, including the possibility of local population reestablishment following extinction" (Hanski & Simberloff 1997). Yet on the larger time scale more evidence and maybe a detailed mode of regional dynamics could be assessed because the population turnover indeed is based on the water dynamics and habitats depending on these dynamics.

5.5 Management, conservation

Calamagrostis pseudophragmites is one of Austria's endangered species (Kammerer 2003) and is part of the vegetation of the protected FFH-Habitat type 3220 (*Alpine rivers and the herbaceous vegetation along their riverbanks*) under NATURA 2000. *C.*

pseudophragmites is growing in unstable habitats as a pioneer species and builds the association Calamagrostietum pseudophragmitis. This is classified as highly endangered in the province of Salzburg (Wittmann & Strobl, 1990) and is also likely to be highly endangered in Styria (Kammerer, 2003).

Sandy riverbanks along the river Enns in Gesäuse are very sensitive habitats and at the same time they are favored places for tourism and sports (Kammerer 2003, pers. observ.). The vegetation of sandy habitats, including Calamagrostietum pseudophragmitis, is under pressure from tourism and sports. The observations in this study support all of Kammerer's (2003) management proposals. Again it is stressed, that three years later the riverbanks still show human utilization and impact, especially in Johnsbachbrücke. Rests of campfires were found also on other sandy riverbanks.

Due to the large size, upstream location and higher genetic variability of Haslau west and also due to the great genetic variability of the Haslau east subpopulation no special genetic threat of survival of this species in Gesäuse is recognized in the present survey. The low isolation by distance indicates that seed (or ramet) dispersal ranges will set the possibilities for the colonization of new sites (Ouborg et al. 1999) and will influence the probability of extinction of the Gesäuse population system. As long as the regeneration of the population is possible, no reinforcing or supplementation is necessary. In the long term, however, it is to be expected that the high isolation from other populations one day may cause genetic drift in this population when the population suffers size reduction. Gene flow currently acts to homogenize allele frequencies among subpopulations. The variability of the Haslau subpopulations will prevent the dominance of one or few genotypes.

At present a high sexual component was detected, as a high number of genets and thus a high number of genotypes were identified. A high variation despite a strong clonal component may concur with the periodic "healthy" river dynamics of river Enns providing the special *C. pseudophragmites´* habitat. Inside the subpopulations the amount of clonally produced ramets will probably increase if no habitat perturbation from river dynamics occurs. Clonal propagation decreases the level of variability in a population and should not become the main reproduction pattern. For proper assurance that colonization of empty sites will occur, a high rate of seed dispersal is necessary. Habitat disturbance can lead to massive destruction of subpopulations, which cannot be regenerated without a proper seed source, if all subpopulations are disrupted. It is thus important for this population to

maintain the dispersal of ripe seeds. Also the importance of rhizome survival in soils in disruptive situations can be a subject of further analysis.

Anyhow, further constant monitoring is proposed. The fertility or sterility of the spikelets should be yearly observed in all subpopulations, to be sure that proper seed dispersal occurs at least occasionally. Also, monitoring of the colonization and extinction of patches and subpopulations is required to assure its further viability. The regeneration of the patches and subpopulations as well as the establishment of seedlings should also be kept under surveillance, in particular on riverbanks with high human impact, sports and tourism, as for instance in Johnsbachbrücke. This study highly recommends prohibiting the access to the riverbanks and in this manner putting up prohibiting signs.

6 Conclusions

The study of *Calamagrostis pseudophragmites* in Gesäuse shows that the clonal propagation in this population system occurs predominantly inside of the patches. Clonal connections of patches were also detected, yet only in one subpopulation. Thus the present survey suggests that sexual reproduction is predominant in the overall *C. pseudophragmites* population and that vegetative propagation is important on the subpopulation level.

Genetic differentiation between subpopulations is moderate, indicating some gene flow in the population. Differentiation is notable between the Haslau subpopulations and other eastern subpopulations. Furthermore, Schneiderwartgraben is differentiated from Johnsbachbrücke and Finstergraben. Seed (or ramet) dispersal as well as pollen flow occurs between subpopulations and contributes to low isolation by distance. Minor admixture patterns may be mostly due to pollen flow. In Haslau west, Johnsbachbrücke and Finstergraben spatial structuring inside of subpopulation was found. In Johnsbachbrücke no genetic structure is present, while in Haslau west genetic structure can be observed mainly due to the patchy arrangement and the major clone groups. Finstergraben belongs to two genetic clusters that are also spatially discrete.

The population of *C. pseudophragmites* in Gesäuse provides evidence for metapopulation dynamics as extinction and (re)colonization processes occur in this population system on a reasonable time scale. The following four conditions introduced by Hanski (1997) hold for the *C. pseudophragmites* population system, and thus this can be treated as a

metapopulation: i) suitable habitats are discrete and may be occupied by local reproducing subpopulations, ii) the largest subpopulation (Haslau west) has a measurable risk of extinction, iii) subpopulations are not too isolated to inhibit the recolonization, and iv) the dynamics of subpopulations are not completely synchronous.

Due to the large size, upstream location and higher genetic variability of the Haslau west and also due to the great genetic variability of Haslau east subpopulation no special genetic threat of survival of this species in Gesäuse is recognized in the present survey. As long as the regeneration of the population is possible, no reinforcing or supplementation is necessary. Further constant monitoring of the population is proposed. The sterility of the spikelets should be yearly observed in all subpopulations, to be sure that sexual reproduction occurs in reasonable intervals. Regeneration of the patches and subpopulations as well as the establishment of seedlings should also be kept under surveillance, in particular on riverbanks with high human impact, sports and tourism, as for instance near Johnsbachbrücke.

7 Deutsche Kurzfassung

7.1 Einleitung

Schotterbänke sind Teile des Überschwemmungsgebietes in größeren Bächen und Flüssen, sie sind jedoch wegen der niedrigen Lage am meisten von Wasserdynamik geprägt. Die Wasserschwankungen sind für das Schotterbankbiotop notwendig. Dieses war einmal sehr häufig in Österreich, die Zahl ist jedoch stark rückläufig. Die Organismen in diesen Habitaten haben sich an die Flussdynamik angepasst und können ohne gelegentlichen Wasserniveauwechsel nicht überleben. Wasserkraftwerke und Flussregulationen beeinträchtigten die Schotterbankhabitate und ihre Pflanzengesellschaften. Die Gesellschaften Myricario -Chondrilletum chondrilloidis, Epilobio dodonei-Scrophularietum caninae, Calamagrostietum pseudophragmitis und Epilobietum fleischeri der Ordnung Epilobietalia fleischeri (Klasse Thlaspietea rotundifolii) formieren entlang der Alpenflüsse die Pioniervegetation auf diesen Kies- und Feinsandstandorten (Pott 1995).

Flussfluren wurden als wichtige Pflanzenverbreitungswege betrachtet (Johannson et al. 1996). Die Wasserbewegungen verbinden das ganze Strom- und Augebiet und transportieren die Mehrheit der Diasporen flussabwärts (Fér & Pfosser, unveröff.). Die „kurzlebigen“ Patches und das periodische, lokale Aussterben und Kolonisieren der Arten sind charakteristisch für solche dynamische Flusssysteme (Jacquemyn et al. 2006). Der Ausgleich zwischen Kolonisation und Extinktion entspricht der Definition von Metapopulationsdynamik. Die Existenz von solchen Systemen ist von der Menge verfügbarer und geeigneter Habitate abhängig (Freckleton & Watkinson 2003). Jede Population ist nicht nur von lokalen Prozessen (intra- und interspezifische Konkurrenz, Self-thinnig, Regulation, Limitation usw.), sonder auch von regionalen Prozessen geprägt (Nentwig et al. 2004). Diese Dynamik tritt in einem System von mehreren Populationen auf und ist nur in einem räumlichen Zusammenhang dieser Populationen und Immigration sowie Emigration sinnvoll zu betrachten (Nentwig et al. 2004).

Levins (1970) führte den Begriff Metapopulation ein und beschrieb damit eine „Population“, die aus vielen lokalen Populationen besteht. Dieses klassische Model nimmt unrealistischer Weise an, dass alle Patches die gleiche Größe haben und dass sie gleichmäßig durch Migration verbunden sind (Silvertown & Charlesworth 2001). Regionale Persistenz einer Metapopulation hängt primär von der Fähigkeit ab, die Patches zu rekolonisieren. Laut Silvertown & Charlesworth (2001) sind folgende Eigenschaften für

eine Metapopulation spezifisch: (i) naturnahe Populationen haben ein Turnover (z.B. sterben lokal aus), (ii) lokale Populationsdynamik ist nicht bei allen Populationen synchronisiert und (iii) nicht die Samenbank sondern die Samenausbreitung zwischen den Populationen ist der Hauptgrund der Besiedlung. Silvertown & Charlesworth (2001) unterscheiden zwischen drei Typen von regionaler Dynamik: (i) eine Metapopulation als vernetztes System von lokalen Populationen, das von gegenseitigen aber unsynchronen Migrationen zwischen lokalen Populationen abhängig ist; (ii) Source-sink Dynamik überwiegt, wenn die Migration konstant einseitig von der Spender- („Source“) zur Empfängerpopulation („Sink“) stattfindet; (iii) Remnant Populationen können ohne Immigration lange ungünstige Zeiten überleben, da die Adulten langlebig sind oder über langlebige Samen im Boden verfügen. Dynamische Flusssysteme gelten für die Untersuchung der Metapopulation und ihre genetsche Diversität und Differenzierung als außerordentliche Modelsysteme (Jacquemyn et al. 2006). Die Menge der verbreiteten Diasporen (Genfluss) kann man indirekt aus den genetischen Distanzen zwischen Populationen (Fér & Pfosser, unveröff.) beziehungsweise aus der Verteilung der genetischen Variation zwischen Populationen ableiten (Ouborg et al. 1999).

Pflanzen, die sich clonal verbreiten, sind problematisch für eine Populationsanalyse, weil sich Individuen auf zwei verschiedenen Ebenen erkennen lassen; als Genet und Ramet (Escaravage et al. 1998). Ein Genet entspricht einem Individuum, das aus Samen entstanden ist und sich weiter mit den Rameten ausdehnen kann, während ein Ramet ein Mitglied oder eine modulare Einheit eines Clons ist und sich durch Trennen als unabhängige Einheit etablieren kann (Pornon et al. 2000).

7.1.1 Populationsgenetische Analyse mit AFLP Technik

Die Amplified lenght polymorphism (AFLP) Technik ist ein PCR basiertes Verfahren, das Vos et al. (1995) einführten. Diese Technik schließt die Verdauung der genomischen DNS, PCR Amplifizieung der Fragmentmenge und deren Analyse ein (Ritland & Ritland 2000). Zuerst wird die DNS mit zwei unterschiedlich oft schneidenden Restriktionsenzymen geschnitten (Vos et al. 1995). PCR Amplifizieung von Restriktionsfragmenten erfolgt mittels Primer, der zur Adapter-Sequenz komplementär ist. Danach findet die selektive Amplifizieung mit zusätzlichen Nukleotiden an Primer statt, wo ein Primer fluoreszenzmarkiert ist. Amplifizieung mit zusätzlichen Nukleotiden ist erforderlich, um die Menge an Fragmenten zu reduzieren (Vos et al. 1995). Die amplifizierten Fragmente werden dann auf einem Acrylamid Gel getrennt und sichtbar gemacht.

Das AFLP-Protokoll folgte dem von Vos et al. (1995) beschriebenen Verfahren mit Modifikation nach Samuel & Tremetsberger (2006, unveröff. Praktikumsanweisungen). Die genomische DNS wurde mit den Restriktionsendonukleasen *EcoRI* und *MseI* verdaut. Die verwendeten Enzymkonzentrationen, Primer, Zusatzstoffe, Inkubationszeiten und Temperaturen sind im Kapitel Material und Methoden beschrieben.

7.1.2 Gesäuse, das Untersuchungsgebiet

Bereits 1958, als das Gesäuse als erstes Naturschutzgebiet der Steiermark ausgewiesen wurde, zeichnete es sich durch einen der letzten naturnahen Wildflussabschnitte aus (Kerschbaumer & Marek 2005). Im Jahr 2002 wurde das Gesäuse zum Nationalpark erklärt. Die Eigendynamik an Schutt- und Schotterablagerungen der Enns und ihrer vielen Zubringer und Seitengräben ist der Grund für das vielfältige Gesäuserelief. Entlang der Enns ab Gstatterboden bis zur oberösterreichischen Grenze befinden sich acht Ausleitungskraftwerke (Tamerl 2006), die zu Wasserkapazitätsänderungen führen. In der Vegetationsökologischen Studie von Schotterbänken im Gesäuse bezeichnete Kammerer (2003) die sandigen Anlandungen als die empfindlichsten Standorte im Nationalpark. Zusätzlich werden sie als Einstiegsstellen von Wassersportlern und zum Zelten benutzt (Kammerer 2003). Sandakkumulationen auf Fein- und auch Grobschutt bilden den Lebensraum der gefährdeten Art Ufer-Reitgras, *Calamagrostis pseudophragmites* (Kammerer 2003).

7.1.3 Die Studienpflanze: *Calamagrostis pseudophragmites*

Calamagrostis pseudophragmites (Haller f.) Koeller (Poaceae) ist ein ausdauerndes Gras, das etwas bläulichgrüne, lockere Horsten bildet. Die Halme sind 20-150 (-200) cm hoch, aufrecht und meist unverzweigt (Conert 1989). Die Blattscheiden sind stark gerieft, kahl und glatt, die Blattspreiten bis 30 cm lang und 3-8(10) mm breit, flach ausgebreitet, auf den Rippen beiderseits aufgrund von kurzen Stachelhaaren rauh. Die Ährchen sind 1-blütig, 5-7(8) mm lang, die Hüllspelzen untereinander sehr ungleich (Conert 1989).

Das Ufer-Reitgras gehört in Mitteleuropa zu den Arten, deren Bestand gefährdet ist (Conert 1989). Diese Art ist in ganz Österreich, mit Ausnahme Burgenland, vorhanden, jedoch nur selten und sehr zerstreut (Fischer et al. 2005). Man findet sie in Pionierrasen auf feuchten Sandbänken und Kiesbändern, im oberen und mittleren Flussbereich und größeren Gebirgsbächen auf Sand und trockenem Geröll, auf feuchten, periodisch überfluteten, kalkreichen, basischen, doch nährstoffarmen Boden (Conert 1989). Durch

Flussverbauung und Eutrophierung der Gewässer ist *Calamagrostis pseudophragmites* zurückgegangen (Conert 1989). Die Art ist österreichweit gefährdet (Niklfeld & Schratt-Ehrendorfer 1999).

C. pseudophragmites ist eine Charakterart des Calamagrostietum pseudophragmitis, der Ufer-Reitgras-Flur (Verband Epilobion fleischeri) und trat oft mit der weitgehend ausgestorbenen *Myricaria germanica* zusammen auf (Conert 1989). In Deutschland ist das Calamagrostietum pseudophragmitis stark gefährdet (Rennwald 2000), was ebenso für das Bundesland Salzburg gilt (Wittmann & Strobl 1990). *C. pseudophragmites* ist mit großer Wahrscheinlichkeit im Murtal ausgestorben (Scharfetter, pers. Komm.). Auch im Salzachtal ist die Population sehr rückläufig (Wittmann pers. Komm.). Es gibt keine aktualisierten Angaben für das Almtal, dennoch bestätigen die Kartierungsdaten aus dem Jahr 1991 (Niklfeld et al., Schratt-Ehrendorfer et al., Sinn, Justin et al., 1991) weniger Bestände. Für die Studie wurden jedoch diese Standorte nicht kontrolliert. Es wurde erfolglos nach den nächsten Populationen von *Calamagrostis pseudophragmites* rund um das Gesäuse anhand der Angaben von Niklfeld & Schratt-Ehrendorfer (1945, unveröff. Kartierungsmaterial für den Atlas der Flora von Österreich) und Wagner & Mecenovic (1973) gesucht.

7.1.4 Ziele der Untersuchung

In vorliegender Studie wurde folgendes untersucht:

(1) Die clonale Struktur innerhalb der Subpopulationen von *C. pseudophragmites* im Gesäuse und die Rolle der clonalen Verbreitung entlang der Enns
(2) Die genetische Differenzierung zwischen den Subpopulationen und das Ausmaß des Genflusses zwischen diesen
(3) Die genetische Struktur und Diversität innerhalb der lokalen Subpopulationen
(4) Die herrschende regionale Dynamik zwischen den Subpopulationen (Metapopulation, Source-sink oder andere)
(5) Die für eine Viabilität der Population im Gesäuse anhand der genetischen Analyse weiter erforderlichen Naturschutzmaßnahmen

7.2 Material und Methoden

7.2.1 Standorte

Im Jahr 2003 verzeichnete Kammerer in Gesäuse *Calamagrostis pseudophragmites* Bestände auf sechs Schotterbänken: Haslau, Bruckgraben, Lettmair-Au, Johnsbachbrücke,

Finstergraben und Schneiderwartgraben. Schotterbänke wurden für die vorliegende Studie erforscht. Weiters wurde auch rund um das Gesäuse nach Ufer-Reitgras Standorten gesucht. Im Juli und September 2006 wurde *Calamagrostis pseudophragmites* erneut auf fünf der sechs bekannten Schotterbänke gefunden, und jeder Standort als eigene Subpopulation bezeichnet.

Die größte Subpopulation wächst auf der Schotterbank Haslau. Auf der westlichen Seite der Schotterbank gibt es den größten Bestand im ganzen Nationalpark, der im Folgenden als Haslau West Subpopulation benannt wird. Dort befinden sich 14 Patches. Insgesamt 73 Rameten wurden gesammelt und in die Analyse inkludiert. In Patch 4, 5, 7, 8, 9 wurde zur Identifizierung der Clone detailliert gesammelt. Auf der östlichen Seite der Schotterbank, 150 m von Haslau West entfernt, wurde ein homogener *C. pseudophragmites* Bestand (Patch 15) gefunden. Insgesamt 9 Rameten von der Haslau East Subpopulation wurden analysiert. Durch die Teilung der Schotterbank Haslau auf zwei Subpopulationen, sind somit insgesamt sechs Subpopulationen in die Studie inbegriffen.

In der Subpopulation Johnsbachbrücke wurden 2006 vier *C. pseudophragmites* Patches gefunden und von jedem fünf Samples genommen. Patch 16, 17 und 18 befinden sich auf einer erodierten Terrasse jeweils 15 m auseinander. Am Waldrand im Westen des Parkplatzes wurde ein großer *C. pseudophragmites* Bestand (Patch 19) neu entdeckt und es wurde festgestellt, dass es in diesem Bereich seltener eine Hochwasserdynamik gibt. Trotzt dieses Umstandes hat sich Patch 19 im Jahr 2007 weiter ausgebreitet. Im Jahr 2007 wurde ein neuer Patch (Patch 30) neben der Johnsbachmündung entdeckt.

In der Nähe des Bruckgrabens kartierte Kammerer (2003) drei Patches. In 2006 wurde festgestellt, dass zwei in einen länglichen Patch fusionierten (Patch 22) und dass der Dritte verschwunden war. Insgesamt drei Rameten wurden in die Analyse einbezogen. Auf der Finstergraben Schotterbank war 2006 zu erkennen, dass eine Überflutung einen großen Vegetationsschaden hinterlassen hat. Große Mengen von Totholz führten dazu, dass die von Kammerer im Jahr 2003 beschriebenen Ufer-Reitgras Bestände nicht mehr zu erkennen waren. Nur ein Patch wurde 2006 gefunden und auch gesammelt (Patch 20). Im darauf folgenden Jahr wurde das Totholz durch eine andere Überflutung weggeschwemmt und die Schotterbank wieder für die Vegetation frei. Die Patches Nummer 26, 27, 28 wurden dadurch freigelegt und 29 wurde zusätzlich gefunden. Insgesamt wurden elf Rameten von fünf Patches analysiert.

Auf der Schotterbank Schneiderwartgraben wuchsen im Jahr 2006 drei kleine lineare Bestände auf 5.5 m Distanz. Aus jedem wurde ein Ramet gesammelt und analysiert (alle als Patch 21 bezeichnet). Im Jahr 2007 erweiterte sich dieser Patch auf ca. 100 Ramets. Auch zwei neue Patches wurden in der Nähe nachgewiesen und von jedem zwei Samples genommen. Insgesamt sieben Rameten wurden in die Studie eingeschlossen. Westlich vom Schneiderwartgraben wurde eine längliche Feinsandaufschüttung gefunden, die sich als sehr günstiges Lebensraum für das Ufer-Reitgras anbietet. Auf der Schotterbank Lettmair-Au wurden in 2006 keine Ufer-Reitgras Bestände gefunden. In 2007 war der Betritt wegen Hochwassers nicht möglich.

Es wurden keine *C. pseudophragmites* entlang Enns im Westen von Hieflau (eigene Beobachtung und persönliche Mitteilung J. Greimler) und im Norden von Hieflau (persönliche Mitteilung J. Greimler) als auch entlang des Großen Fölzgrabens in Eisenerz (eigene Beobachtung) gefunden. Im Großen Fölzgraben wurden drei Proben von *C. varia* gesammelt und in die Analyse als Out-Group eingeschlossen.

7.2.2 DNS Extraktion und AFLP Analyse

Das Blattmaterial von *Calamagrostis pseudophragmites* aus den sechs Subpopulationen und von drei Individuen von *C. varia* wurde in Silicagel getrocknet. Die DNS Extraktion folgte nach dem Protokoll von Doyle & Doyle (1987) mit weiteren Modifikationen nach Samuel und Tremetsberger (2005, unveröff.). Das Blattmaterial wurde gemahlen und die ganze genomische DNS wurde mit dem 700 µl warmen CTAB Puffer (2% CTAB, 100 mM Tris, 1.4 M NaCl, 20mM EDTA, 0.2% Mercaptoethanol, pH 8.0) extrahiert. Nach der Inkubation wurde 500 µl Chloroform: Isoamylalcohol (24:1) dazugegeben, zentrifugiert und 600 µl von der wässrigen Phase wurden aufgehoben. Mit dem dazugegebenen 200 µl kalten Isopropanol fiel die Nukleinsäure aus. Beim Zentrifugieren sammelte sich DNS auf dem Boden. Nachdem der Überstand entfernt und die DNS mit Ethanol gewaschen wurde, trocknete die Nukleinsäure aus. Die DNS wurde am Schluss in 50 µl TE Puffer resuspendiert und 2 µl RNAse (Fermetnas, 200 u) wurde hinzugefügt.

Die Verdauung (Restriction-Ligation) erfolgte mit folgenden Komponenten: 5.5 µl genomische DNS; 1.1 µl 10xT4 Puffer; 1.1 µl NaCl (0.5 M); 0.55 µl BSA (1 mg/ ml); 0.33 µl T4 Ligase (3U/ µl); 1 µl MseI Adaptorpaar (50 µM); 1 µl EcoRI Adaptorpaar (5 µM); 0.02 µl MseI Endonuklease (50U/ µl); 0.25 µl (20u/ µl); 0.65 µl ddH_2O. Die Mischung wurde in PCR cycler 2 Stunden inkubiert. Vor der Preselective Amplifikation wurde die Restriction-Ligation 16x mit $TE_{0.1}$ Puffer (20mM Tris-HCl, 0.1 mM EDTA, pH 8.0)

verdünnt. Zur 2 µl Restriction-Ligation wurde die folgende 8 µl Mischung dazugegeben (je Probe): 5.86 µl ddH_2O; 0.22 µl ABI dNTPs; 1.14 µl RedTaq Puffer (Sigma); 0.2 µl RedTaq Polymerase (Sigma, 250 U); jeweils 0.29 µl preselective Primer MseI und EcoRI (jeweils 5 mM). Ingesamt 10 µl pro Probe wurden 2 Stunden in der PCR Maschine inkubiert. Das Ergebnis wurde 16x mit $TE_{0.1}$ verdünnt und 2 µl davon wurden für jede der drei selektiven Amplifikationen eingesetzt. Drei verschiedene Primerkombinationen wurden verwendet: Eco-AGC (NED- Gelb)/ Mse-CAA, Eco-ACG (HEX-Grün)/ Mse-CAA, Eco-ACT (FAM-Blau)/ Mse-CAA. Die Mischung für jede Kombination schließt (je Probe) folgendes ein: 5.5 µl ddH2O; 1 µl 10xRedTaq Puffer (Sigma); 0.22 µl ABI dNTPs (10 mM); 0.2 µl RedTaq (Sigma, 250 U); 0.54 µl Mse-Primer (5 pmol/µl) und 0.54 µl Eco-Primer (1 pmol/µl). Für jede Primerkombination wurde das Volumen von 10 µl in der PCR Maschine 3 Stunden inkubiert. Bevor die Proben in den Seqencer gegeben wurden, folgte noch die Sephadex Reinigung und Mischung mit Genescan™ 500 Rox Size Standard und High-Dye Formamid.

7.2.3 Die Datenanalyse

Die 1/0 Matrix wurde in Microsoft® Excell® 2002 (© Microsoft Corp. 1985-2001) and SPSS (13.0.1 für Windows, SPSS Inc. 1989-2004) für weitere Analysen vorbereitet. Neighbor Joining Analyse wurde mit einer Nei-Li (1979) genetischen Distanzmatrix in TREECON 1.3b (Van de Peer & De Wachter 1997) durchgeführt. Das UPGMA Dendrogramm wurde im FAMD 1.104 beta (Fingerprint Analysis with Missing Data, Schlüter & Harris 2006) mittels Squared Euclidian Distanzmatrix erstellt. FAMD wurde auch für die Durchführung und die graphische Darstellung der Principal Coordinates Analyse (PCA; Gower, 1966) mit Jaccard Symilarity Koeffizient verwendet. Nicht-hierarchisches Clustering wurde mit BAPS 3.2. (Bayesian analysis of population structure, Corander & Marttinen 2006) ausgeführt. Es wurden 4-9 mögliche Cluster mit mehreren Replikaten untersucht. Genetische Diversitätsmessungen, Anzahl der polymorphen Fragmente pro Population, „mean number of pairwise differences“ und auch AMOVA wurden mit ARLEQUIN 3.11 (Excoffier et al. 2006) berechnet. Eine Matrix genetischer Distanzen zwischen allen möglichen Paaren von Rameten wurde mit Squered Euclidian Distanzes erstellt. Der Reproduzierbarkeit und der Frequenzanalyse von Distanzintervallen entsprechend, wurden die Rametenpaare unter dem 5% Grenzbereich als Clone bestimmt.
Um die „isolation by distance“ zu bestimmen wurde ein Mantel Test mittels GENEPOP 3.1 (Raymond & Rousset 1995) ausgeführt. Die Graphische Bearbeitung wurde mit Adobe® Photoshop® CS2 and Microsoft® Powerpoint® 2003 gemacht.

7.3 Resultate

7.3.1 Analyse der genetischen Diversität

Ein allgemein hoher Level an polymorphen Loci ist im ganzen Datensatz vorhanden. Die Anzahl von polymorphen Fragmenten korreliert mit der Populationsgröße, aber nicht mit der Anzahl der „pairwise differences“. Aus dem UPGMA Dendrogramm ist ersichtlich, dass sich die Haslau west und Haslau East Subpopulationen von anderen Subpopulationen trennen und selber ein eigenes Cluster bilden. Die Neighbor Joining Analyse zeigt Gruppierungen der Subpopulationen Johnsbachbrücke, Schneiderwartgraben und Finstergraben die schwach von Haslau getrennt sind. Die Bootstrap Analyse zeigte kleine gut unterstützte Gruppen, aber keine Unterstützung der Cluster und keine entsprechende räumliche Subpopulationsstruktur.

Die Haslau West und East Subpopulationen lassen sich nicht voneinander unterscheiden. Zusammen sind sie in mehreren Gruppen unterteilt, jedoch ist Haslau East nur in einer zu finden. Die Gruppen unterliegen keiner räumlichen Struktur. In der Gruppe Johnsbachbrücke gibt es keine klare genetische Aufteilung auf Patches. Nur Patch 19 ist von den anderen klar zu unterscheiden und schließt sich an die Gruppe Finstergraben an. Die Rameten von 2007 sind nahe verwandt mit jenen von 2006 und mischen sich miteinander. Die Rameten der Subpopulation Finstergraben aus 2007 bilden eine gut unterstütze Gruppe. Der Ramet 22.4 von Bruckgraben ist mit dem Finstergraben Patch 20 verbunden. In der Subpopulation Schneiderwartgraben sind der Patch 21 (von 2006) 24 und 25 (von 2007) nahe miteinander verwandt. Der Ramet 29.1 (Johnsbachbrücke) taucht in der Gruppe Finstergraben auf.

In PCA Analyse, wo die ersten drei Achsen etwa 28% der totalen Varianz (13.9%, 8.9% and 4.9%) erklären, lassen sich sechs Subpopulationen in vier Gruppen erkennen; Haslau West und East sind zusammengefasst in Gruppe I, die Rameten vom Cluster 5.1 bilden die Gruppe II, Schneiderwartgraben bildet Gruppe III und Johnsbachbrücke, Finstergraben und Bruckgraben die Gruppe IV.

Das Nachweisen der Clones im *Calamagrostis pseudophragmites* Populationssystem erfolgte mittels Squared Euclidian Distanzes. Anhand von acht Replikaten wurde ein Reproduzierbarkeitslevel auf 95% berechnet. Die unreproduzierbaren Fragmente wurden aus dem Datensatz entfernt. Eine Frequenzanalyse der paarweisen genetischen Distanzen (Mutationsschritte) wurde durchgeführt um eine Autokorrelation zwischen Rameten zu

erkennen und die daraus folgende Clonalität zu detektieren. Die Verteilung von Distanzintervallen zwischen Rametenpaaren zeigte dass zwischen 1.5 und 5% Distanz, ein Ausreißerprozentsatz vorkommt, was genau einem Intervall von 6 bis 20 Mutationsschritten entspricht. Dieser Ausreißerprozentsatz und die Tatsache, dass die Distanzintervalle zwischen 10 und 30% eine Gauß'sche Verteilung zeigen, sind gute Argumente dafür, dass die Werte, die unter 5% liegen, auf eine clonale Verbreitung hinweisen.

Mit den oben beschriebenen Kriterien wurde in jeder Subpopulation zumindest ein Paar Clone gefunden. In Haslau East wurden von neun Rameten nur zwei und in der Subpopulation Johnsbachbrücke nur vier Rameten bzw. zwei Paare als Clone erkannt. In der Subpopulation Schneiderwartgraben wurden zwei Clonpaare von verschiedenen Patches gefunden (24 and 25). Alle gesammelte Rameten der Patches 26, 27 und 28 der Subpopulation Finstergraben aus dem Jahr 2007 wurden als ein Clon indetifiziert. Patch 20 aus dem Jahr 2006 zeigte auch eine Clonale Verbindung zwischen den zwei gesammelten Rameten. Patch 5 von Haslau West zeigte ein komplexes clonales Muster. Dieser Patch wurde beim Sammeln in drei Gruppen geteilt, 5.1, 5.2 and 5.3. Die Rameten 5.1.11 und 5.1.12 sowie die Rameten 5.1.23, 24 and 25 bilden jeweils einen eigenen Clon. Auch die Gruppen 5.2, 5.3 und der Ramet 5.1.27 formten einen Clon. Alle drei Rameten von Patch 2, sowie vier der fünf Rameten von Patch 4 kamen als separate Clone vor. In Patch 7 bildeten drei bzw. zwei Rameten jeweils einen Clon. Die Neighbor Joining Analyse sagte aus, dass die zwei Clone nicht miteinander verwandt sind. Zwei von vier Rameten aus dem Patch 8 und vier der elf Ramets aus Patch 9 wurden auch als Clone erkannt. Samples der Patches 1, 3, 6, 10, 11, 12, 13 und 14 der Haslau West Subpopulation brachten keine Clone hervor.

7.3.2 Genetische Struktur

7.3.2.1 AMOVA

Analysis of Molecular Variance (AMOVA) zeigte eine hohe Variation innerhalb der Subpopulationen im Gesäuse (80.09%). Die restlichen 19.91% betrug der Variationsanteil zwischen den Subpopulationen. Dieser Anteil lieferte einen F_{ST} Wert von 0.20, der auf einen mäßigen Genfluss hinweist. Bei AMOVA ohne multiple Clone betrug der F_{ST} Wert nur 0.16. AMOVA innerhalb der Haslau West Subpopulation zeigte eine niedrigere Differenzierung zwischen Gruppen von Patches (13.33%) mit einen F_{ST} Wert von 0.13.

7.3.2.2 Bayesian Analyse (BAPS)

In der mit BAPS durchgeführten Individual Level Mixture Analyse wurden 6 Cluster mit log(ml)-14390.8 für optimale Partition erkannt. Diese Cluster entsprechen nicht den räumlichen Mustern der geographischen Subpopulationen. Die auf Mixture Clustering basierende Admixture Analyse vom gesamten Datensatz zeigte, dass die Rameten der Haslau West Subpopulation hauptsächlich gene pool II angehören, während die meisten Rameten von Johnsbachbrücke, Finstergraben und Bruckgraben zum gene pool IV gehören. Neben gene pool II schließt Haslau West noch kleinere gene pools ein. Die clonale Gruppe 5.1 gehört zum gene pool III, Ramet 5.1.9 und die Clone 5.1.11-12 und 5.1.23-25 gehören zum gene pool II, die Clone 5.2/5.3/5.1.27 zum gene pool I. Rameten 4.2, 9.1 und 9.7 teilen sich gene pool VI. Alle diese kleine gene pools haben fast keine Admixture, während sonst die Admixture der Haslau Subpopulationen, überwiegend in Patch 9, 10, 11, 13, 14, 15 und auch 16 (Johnsbachbrücke) sehr hoch ist. Rameten 9.9-11 und 4.1 gehören zum gene pool IV, der signifikant für Johnsbachbrücke und Finstergraben ist. Die Rameten von Haslau East enthalten am meisten von gene pool II mit hoher Admixture von gene pool VI und auch III, IV und V. Patch 16 der Subpopulation Johnsbachbrücke (mit der Ausnahme 16.5) gehört zum gene pool II, der für Haslau West und East bezeichnend ist. Die Rameten aus den Patches 17, 18, 19, 30 und 31 (mit der Ausnahme 31.5) stellen zusammen mit den Rameten der Subpopulation Finstergraben, die in 2007 gesammelt wurden, gene pool IV dar. Die Subpopulation Johnsbachbrücke enthält eine niedrigere Admixture aller gene pools, während die Rameten der Subpopulation Finstergraben fast keine beinhalten. Finstergraben Patch 20 aus dem Jahr 2006 gehört zum gene pool V, der signifikant für die gesamte Subpopulation von Schneiderwartgraben ist. Zwei Bruckgraben Rameten beinhalten gene pool IV, während der Ramet 22.4 eine sehr hoch signifikante Admixture mit allen gene pools zeigt.

Für die weitere Analyse wurde nur ein Ramet pro Gruppe von Clonen unter anderen Rameten, die keine Clonalität aufwiesen, verwendet. Noch eine Individual Level Mixture Analyse wurde ausgeführt und deutete eine Aufteilung auf drei Clusters mit log(ml)-10748.7 für optimale Partition an. Eine klare Aufteilung auf zwei Gruppen wurde beobachtet, da sich Haslau West und East mit einem anderem gene pool von östlichen Subpopulationen unterscheiden. Drei Rameten 9.1, 9.11 und 15.9, die auch im NJ Dendrogramm eine Distanz zur anderen Rameten zeigten, formieren einen eigenen gene pool. Das Admixture-Diagram mit 86 Rameten zeigt eine hohe Admixture von diesen gene

pools in anderen Rameten, überwiegend in einer Hälfte von Haslau East Rameten (15.7-10) und in östlichen Haslau West Patches 10, 11, 12, 14 und 9. Die westlichen Haslau West Patches beinhalten im Gegensatz zu den östlichen einen höhere Admixture Anteil der östlichen Subpopulationen. Bemerkenswert ist auch, dass die Hälfte der Subpopulation Haslau East eine hohe Admixture der östlichen Subpopulationen enthält. Ansonsten gibt es keine Unterscheidung zwischen Subpopulationen Bruckgraben, Johnsbachbrücke, Finstergraben und Schneiderwartgraben. Ramet 31.5 aus Johnsbachbrücke gehört zum gene pool I von den Haslau Subpopulationen, wovon auch Ramet 22.4 von Bruckgraben hohe Admixture zeigt. Weiterhin zeigen die Rameten 18.5 und 19.1 aus Johnsbachbrücke und 21.3 aus Schneiderwartgraben eine hohe Admixture von gene pool III. Die Rameten von Johnsbachbrücke gesammelt in 2007 (Patch 30 und 31) zeigen überhaupt keine Admixture.

7.3.2.3 Mantel Test

Der Mantel Test deutet nur auf einen niedrigeren „isolation by distance“ Effekt (Rm = 0.317, P = 0.001). Etwa 10% der genetischen Variation zwischen Subpopulationen klärt sich mit geographischer Distanz auf.

7.4 Diskussion

Die Studie von *Calamagrostis pseudophragmites* in Gesäuse zeigt, dass die clonale Verbreitung in diese Populationssystem hauptsächlich innerhalb der Patches erfolgt. Die clonale Verbindung zwischen Patches wurde auch detektiert, jedoch nur in einer Subpopulation. Daher wurde vermutet, dass die sexuelle Reproduktion in der C. *pseudophragmites* Population überwiegend ist und dass die vegetative Verbreitung eine wichtige Rolle auf der Subpopulations-Ebene spielt.

Die genetische Differentiation zwischen den Subpopulationen ist nicht sehr hoch. Eine Differenzierung ist zwischen Haslau West und Haslau East bemerkenswert. Weiterhin trennt sich Subpopulation Schneiderwartgraben von Johnsbachbrücke und Finstergraben. Die genetische Differenzierung zwischen den Subpopulationen deutet auf einen mäßigen Genefluss in der gesamt Population. Sowohl Samen Ausbreitung als auch Pollenfluss erfolgen zwischen Subpopulationen. Eine niedrigere „isolation by distance“ weist darauf, dass die Samen Ausbreitung auch auf lange Distanzen stattfinden kann. In Subpopulationen Haslau West, Johnsbachbrücke und Finstergraben wurde in 2006 und 2007 eine räumliche Struktur beobachtet. Keine genetische Struktur wird jedoch bei

Johnsbachbrücke nachgewiesen. Die genetische Struktur in Haslau West ist anhand von vielen clonalen Gruppen zu beobachten, während Subpopulation Finstergraben zu zwei genetischen Clustern, die auch räumlich getrennt sind, gehört.

In der *C. pseudophragmites* Population im Gesäuse gibt es Hinweise dafür, dass eine Metapopulationsdynamik mit Extinktion und (Re)Kolonisation im Populationssystem stattfindet. Die folgenden Bedingungen, vorgestellt von Hanski (1997), treffen für *C. pseudophragmites* im Gesäuse zu und deswegen kann die Population als Metapopulation betrachtet werden: i) geeignete Habitate sind diskontinuierlich und können jeder Zeit von lokalen reproduzierenden Subpopulationen bewohnt werden; ii) auch für die größte Subpopulation (Haslau West) besteht eine Extinktionsgefahr; iii) die Subpopulationen sind nicht zu isoliert um eine lokale (Re)Kolonisation zu verhindern; und iv) die Subpopulationen haben keine synchrone Dynamik.

Wegen der Größe, oberwasserseitigen Lage und höheren genetischen Variabilität der Subpopulation Haslau West und aufgrund der sehr hohen genetischen Variabilität der Subpopulation Haslau East, besteht derzeit genetisch gesehen keine große Überlebensgefahr für diese Art in Gesäuse. So lange eine Regeneration der Population möglich ist, ist keine Verstärkung oder Ergänzung der Population notwendig. Es wird weiteres konstantes Monitoring der Population vorgeschlagen. Jährlich sollte die Fruchtbarkeit der Ährchen ermittelt werden um sicher zu sein, dass eine sexuelle Reproduktion in angemessenen Intervallen geschieht. Die Regeneration der Patches und Subpopulationen, sowie die Etablierung der Keimlinge sollten regelmäßig kontrolliert werden, insbesondere auf den Schotterbänken mit einer sehr hohen menschlichen Belastung, i.e. Sport und Tourismus.

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9 Appendix:

Table 9: Admixture results of whole data set using minimal population size 3 as they are represented in Admixture diagram (Figure 33). For each ramet percentage of each cluster and probability value are listed.

Ramet	C1	C2	C3	C4	C5	C6	Probability
1.1	0,11	0,82	0,02	0,00	0,00	0,05	0,225
1.2	0,03	0,83	0,10	0,00	0,04	0,00	0,265
2.1	0,01	0,97	0,00	0,01	0,01	0,00	0,9
2.2	0,00	0,95	0,00	0,03	0,02	0,00	0,825
2.3	0,00	0,91	0,04	0,04	0,01	0,00	0,675
3.1	0,00	0,94	0,00	0,00	0,00	0,06	0,795
3.2	0,00	0,98	0,02	0,00	0,00	0,00	0,005
4.1	0,00	0,23	0,00	0,74	0,03	0,00	0,07
4.2	0,01	0,11	0,00	0,00	0,00	0,88	0,045
4.3	0,04	0,74	0,03	0,19	0,00	0,00	0,06
4.6	0,01	0,94	0,02	0,03	0,00	0,00	0,795
4.7	0,00	0,93	0,01	0,05	0,01	0,00	0,755
5.1.1	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.3	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.4	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.5	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.6	0,01	0,00	0,99	0,00	0,00	0,00	0,995
5.1.7	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.8	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.9	0,00	0,79	0,07	0,07	0,07	0,00	0,125
5.1.11	0,00	0,90	0,08	0,02	0,00	0,00	0,645
5.1.12	0,00	0,99	0,01	0,00	0,00	0,00	0,985
5.1.13	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.14	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.15	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.16	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.18	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.19	0,00	0,00	1,00	0,00	0,00	0,00	1
5.1.23	0,00	0,94	0,06	0,00	0,00	0,00	0,795
5.1.24	0,00	0,99	0,01	0,00	0,00	0,00	0,985
5.1.25	0,00	0,98	0,02	0,00	0,00	0,00	0,95
5.1.27	0,98	0,00	0,02	0,00	0,00	0,00	1
5.2.1	1,00	0,00	0,00	0,00	0,00	0,00	1
5.2.2	1,00	0,00	0,00	0,00	0,00	0,00	1
5.3.1	1,00	0,00	0,00	0,00	0,00	0,00	1
5.3.2	0,36	0,36	0,28	0,00	0,00	0,00	0
6.1	0,12	0,65	0,06	0,11	0,06	0,00	0,005
6.2	0,01	0,88	0,00	0,10	0,01	0,00	0,525
6.3	0,00	0,65	0,00	0,31	0,04	0,00	0,005
6.5	0,00	0,71	0,06	0,02	0,00	0,21	0,035
7.1	0,00	0,86	0,02	0,11	0,01	0,00	0,395
7.2	0,00	0,98	0,01	0,01	0,00	0,00	0,95
7.3	0,04	0,75	0,01	0,20	0,00	0,00	0,065
7.4	0,05	0,87	0,02	0,01	0,05	0,00	0,455
7.5	0,02	0,82	0,07	0,05	0,04	0,00	0,225
7.6	0,02	0,84	0,04	0,01	0,09	0,00	0,315
7.7	0,02	0,83	0,00	0,00	0,00	0,15	0,265
7.8	0,00	1,00	0,00	0,00	0,00	0,00	1
7.9	0,01	0,96	0,00	0,00	0,02	0,01	0,87
7.10	0,00	1,00	0,00	0,00	0,00	0,00	1
7.11	0,02	0,95	0,02	0,01	0,00	0,00	0,825
8.1	0,00	0,92	0,03	0,01	0,04	0,00	0,72
8.2	0,01	0,94	0,02	0,00	0,02	0,01	0,795
8.3	0,02	0,95	0,03	0,00	0,00	0,00	0,825
8.4	0,02	0,90	0,00	0,00	0,00	0,08	0,645

9.1	0,00	0,12	0,00	0,00	0,01	0,87	1
9.2	0,00	0,94	0,00	0,06	0,00	0,00	0,795
9.3	0,00	0,92	0,01	0,00	0,00	0,07	0,72
9.4	0,00	0,34	0,00	0,65	0,01	0,00	0,95
9.5	0,01	0,88	0,00	0,00	0,00	0,11	0,525
9.6	0,00	0,80	0,00	0,00	0,00	0,20	0,72
9.7	0,00	0,02	0,00	0,00	0,00	0,98	0,07
9.8	0,04	0,72	0,00	0,00	0,12	0,12	0,9
9.9	0,00	0,13	0,02	0,85	0,00	0,00	0,755
9.10	0,00	0,00	0,00	0,98	0,01	0,01	0,07
9.11	0,01	0,00	0,00	0,95	0,04	0,00	1
10.1	0,05	0,72	0,10	0,13	0,00	0,00	0,03
10.2	0,06	0,88	0,01	0,02	0,00	0,03	0,225
11.1	0,00	0,86	0,00	0,00	0,00	0,14	0,395
11.2	0,02	0,80	0,00	0,15	0,03	0,00	0,395
12.1	0,00	0,87	0,00	0,05	0,00	0,08	0,155
13.1	0,00	1,00	0,00	0,00	0,00	0,00	0,455
14.1	0,05	0,71	0,02	0,21	0,00	0,01	1
15.1	0,00	0,82	0,13	0,02	0,01	0,02	0,035
15.2	0,02	0,85	0,00	0,09	0,02	0,02	0,225
15.3	0,05	0,76	0,05	0,03	0,03	0,08	0,365
15.5	0,00	0,92	0,01	0,00	0,00	0,07	0,01
15.6	0,04	0,76	0,01	0,00	0,00	0,19	0
15.7	0,00	0,97	0,00	0,00	0,00	0,03	0,525
15.8	0,01	0,70	0,05	0,10	0,00	0,14	0,155
15.9	0,00	0,64	0,22	0,00	0,14	0,00	1
15.10	0,01	0,82	0,00	0,07	0,10	0,00	0,045
16.2	0,04	0,86	0,00	0,00	0,00	0,10	0,2
16.3	0,00	0,93	0,00	0,00	0,00	0,07	0,95
16.4	0,00	0,76	0,00	0,00	0,00	0,24	0,835
16.5	0,00	0,10	0,00	0,90	0,00	0,00	0,53
17.1	0,00	0,00	0,00	1,00	0,00	0,00	1
17.2	0,00	0,00	0,00	1,00	0,00	0,00	1
17.3	0,00	0,01	0,00	0,96	0,01	0,02	0,87
17.5	0,00	0,00	0,00	0,98	0,01	0,01	0,95
18.1	0,00	0,07	0,00	0,93	0,00	0,00	0,695
18.2	0,05	0,02	0,00	0,85	0,04	0,04	0,2
18.3	0,00	0,00	0,01	0,98	0,01	0,00	0,95
18.4	0,00	0,03	0,02	0,83	0,12	0,00	0,135
18.5	0,00	0,01	0,07	0,68	0,01	0,23	0
19.1	0,00	0,11	0,01	0,78	0,00	0,10	0,035
19.3	0,00	0,02	0,06	0,91	0,01	0,00	0,58
19.4	0,01	0,00	0,00	0,97	0,02	0,00	0,915
19.5	0,00	0,00	0,00	0,94	0,06	0,00	0,76
30.1	0,01	0,00	0,00	0,99	0,00	0,00	0,98
30.2	0,00	0,00	0,01	0,97	0,02	0,00	0,915
30.3	0,00	0,03	0,00	0,97	0,00	0,00	0,915
30.4	0,00	0,00	0,06	0,93	0,01	0,00	0,695
31.1	0,01	0,00	0,00	0,98	0,01	0,00	0,95
31.2	0,01	0,00	0,01	0,98	0,00	0,00	0,95
31.3	0,02	0,00	0,00	0,98	0,00	0,00	0,95
31.4	0,01	0,00	0,00	0,85	0,13	0,01	0,2
31.5	0,01	0,57	0,07	0,11	0,22	0,02	0
20.1	0,00	0,00	0,00	0,18	0,82	0,00	0,68
20.2	0,00	0,00	0,00	0,20	0,80	0,00	0,59
26.1	0,00	0,00	0,00	1,00	0,00	0,00	1
26.2	0,00	0,00	0,00	1,00	0,00	0,00	1
26.3	0,00	0,00	0,00	1,00	0,00	0,00	1
26.4	0,00	0,00	0,00	1,00	0,00	0,00	1
27.1	0,00	0,00	0,00	1,00	0,00	0,00	1
27.3	0,00	0,00	0,02	0,98	0,00	0,00	0,95
28.2	0,00	0,00	0,01	0,99	0,00	0,00	0,98

28.3	0,00	0,00	0,03	0,97	0,00	0,00	0,915
29.1	0,02	0,00	0,04	0,94	0,00	0,00	0,76
21.1	0,00	0,01	0,00	0,00	0,99	0,00	1
21.2	0,00	0,00	0,00	0,04	0,96	0,00	1
21.3	0,00	0,00	0,00	0,00	0,91	0,09	0,97
24.1	0,00	0,00	0,00	0,00	1,00	0,00	1
24.2	0,00	0,00	0,00	0,00	1,00	0,00	1
25.1	0,05	0,00	0,04	0,16	0,75	0,00	0,295
25.2	0,00	0,00	0,04	0,18	0,78	0,00	0,435
22.2	0,00	0,00	0,00	0,90	0,10	0,00	0,53
22.3	0,00	0,00	0,00	0,92	0,08	0,00	0,65
22.4	0,05	0,06	0,08	0,08	0,72	0,01	0,18

Table 10: Admixture results of 86 genets set using minimal population size 3 (Figure 36). For each ramet percentage of each cluster and probability value are listed.

ramet	c1	c2	c3	probability
1.1	0,96	0	0,04	0,74
1.2	1	0	0	1
2.1	0,98	0,02	0	0,94
2.3	0,95	0,05	0	0,62
3.1	0,95	0	0,05	0,62
3.2	0,96	0,04	0	0,74
4.1	0,86	0,06	0,08	0,12
4.3	0,76	0,24	0	0
5.1.5	0,54	0,46	0	0
5.1.9	0,87	0,13	0	0,18
5.1.11	0,97	0,03	0	0,78
5.1.25	1	0	0	1
5.1.27	0,77	0,23	0	0,04
6.1	0,81	0,19	0	0,06
6.2	0,89	0,11	0	0,26
6.3	0,7	0,3	0	0
6.5	0,76	0,03	0,21	0
7.1	0,85	0,15	0	0,12
7.2	1	0	0	1
7.3	0,84	0,16	0	0,01
7.4	0,9	0,1	0	0,32
7.7	0,87	0	0,13	0,18
7.9	0,98	0,01	0,01	0,94
7.10	1	0	0	1
7.11	0,99	0,01	0	0,94
8.1	0,96	0,04	0	0,74
8.2	0,97	0,02	0,01	0,78
8.4	0,93	0	0,07	0,52
9.1	0,12	0,01	0,87	1
9.2	0,99	0,01	0	0,94
9.3	0,94	0	0,06	0,56
9.5	0,97	0,01	0,02	0,78
9.6	0,95	0	0,05	0,62
9.7	0,83	0	0,17	0,08
9.9	0,93	0	0,07	0,52
9.10	0,79	0	0,21	0,04
9.11	0,13	0	0,87	1
10.1	0,7	0,17	0,13	0
10.2	0,89	0,1	0,01	0,26

11.1	0,91	0	0,09	0,34
11.2	0,89	0	0,11	0,26
12.1	0,81	0,19	0	0,06
13.1	0,87	0,06	0,07	0,18
14.1	1	0	0	1
15.1	0,87	0,13	0	0,18
15.2	0,96	0,04	0	0,74
15.3	0,83	0,16	0,01	0,08
15.6	0,34	0,66	0	0
15.7	0,91	0	0,09	0,34
15.8	0,82	0	0,18	0,06
15.9	0,03	0	0,97	1
15.10	0,87	0,03	0,1	0,18
16.2	0,07	0,93	0	0,44
16.3	0	0,99	0,01	0,92
16.4	0	1	0	1
16.5	0,08	0,92	0	0,34
17.1	0	1	0	1
17.2	0	1	0	1
17.3	0	0,99	0,01	0,92
17.5	0	0,99	0,01	0,92
18.1	0,01	0,99	0	0,92
18.2	0,05	0,92	0,03	0,34
18.3	0	1	0	1
18.4	0,03	0,97	0	0,78
18.5	0,04	0,74	0,22	0
19.1	0,09	0,83	0,08	0,06
19.3	0,02	0,98	0	0,86
19.4	0	1	0	1
30.1	0	1	0	1
30.2	0	1	0	1
30.3	0	1	0	1
30.4	0	1	0	1
31.1	0,01	0,99	0	0,92
31.3	0	1	0	1
31.4	0	0,99	0,01	0,92
31.5	0,63	0,34	0,03	0
20.2	0	1	0	1
26.3	0,03	0,96	0,01	0,68
29.1	0,01	0,99	0	0,92
21.1	0,09	0,91	0	0,26
21.2	0,01	0,99	0	0,92
21.3	0,08	0,75	0,17	0
24.1	0,03	0,97	0	0,78
25.2	0,02	0,97	0	0,86
22.2	0	1	0	1
22.4	0,25	0,72	0,03	0

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Lebenslauf

Name:	Lea Atanasova
Adresse:	Vegagasse 20/Pf.14
	1190 Wien
	Telefon: 0676 5837215
	lea.atanasova@gmail.com
Geburtsdatum und -ort:	15.07.1984 in Maribor, Slowenien
Staatsangehörigkeit:	Slowenien

Schulische Ausbildung

1991 bis 1999	Grundschule Vižmarje–Brod in Ljubljana, Slowenien
1999 bis 2003	Gymnasium Šentvid in Ljubljana,
	Abschluss: Matura (Abitur)

Studium

Oktober 2003 bis September 2006	**Univerza v Ljubljani,** Biotehniška fakulteta, Studium Biologie Vertiefungsrichtung Systematik und Forschung
September 2005 bis September 2006	Auslandsjahr in Wien (Universität Wien, Fakultät für Lebenswissenschaften)
Oktober 2006	**Universität Wien,** Fakultät für Lebenswissenschaften, Weiterführung des Studiums Biologie, Studienrichtung Ökologie
Seit Juni 2006	**Diplomarbeit**: "Regional dynamics of *Calamagrostis pseudophragmites* on riverbanks in the NP Gesäuse inferred from AFLPs", Betreuer: Ao. Univ.-Prof. Dr. Josef Greimler, Facultly center Botany, Universität Wien
Oktober 2008	**Fertigstellung der Diplomarbeit**

Sprachkenntnisse

Slowenisch, Deutsch, Englisch, Mazedonisch, Serbisch und Kroatisch (in Wort und Schrift)

Praktika bzw. Exkursionen

August 2001	Jungforschercamp Mislinja 2001, Slowenien, Floristische Aufnahmen
August 2002	Jungforschercamp Mislinja 2002, Slowenien, Floristische Aufnahmen
Mai 2004	Forschungscamp Ecosystem of Dalmatia, Insel Korčula, Kroatien
Juli 2004	Forschungscamp –RTŠB Dekani 2004, Slowenien
Juli 2005	Forschungscamp –RTŠB Lovrenc na Pohorju 2005, Slowenien
April 2007	Botanische Mittelmeerexkursion, Andalusien, Spanien (Prof. Schönswetter und Prof. Schneeweiß)

Referenzen

Auszeichnung für die beste Forschungsarbeit beim 36. Nationalen Jungforschertreffen Sloweniens 2002 (**Subspecies of *Cornus sanguinea* L. in Slovenia**).

Artikel : Atanasova L., Jogan N., Strgulc-Krajšek S (2002). **Subspecies of *Origanum vulgare* L. in Slovenia**. Natura Sloveniae (Jahrgang 4, Nr. 1).

Teilnahme am internationalen Symposium "Flora and Vegetation of Slovenia and neighbourhood Countries 2005": "**Morphometric study of *Gymnocarpium* in Slovenia**."

Internationale naturwissenschaftliche Konferenz für Studenten in Vilnius, Litauen 2005; Vortrag: „**Subspecies of *Cornus sanguinea* L. in Slovenia**".

Sonstige Tätigkeiten

2003 bis 2005	Freiwillige aktive Mitarbeit im Studentenverein der Biologen in Ljubljana (*Societas studiosorum Biologiae Universitatis Labacensis*)

Wien, Oktober 2008