New aspects in plant conservation –

Phylogeography, population dynamics, genetics and management of steppe plants in Bavaria

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Summary

Nutrient-poor, dry calcareous grasslands in Central Europe are characterized by their immense regional biodiversity and are of high conservation value. Human beings have transformed these regions for centuries by clearing woods, grazing livestock and cutting grass. Today dry grasslands and many of their related species are greatly endangered by intensification of agricultural practice or abandonment. The establishment of adequate conservation management techniques to preserve the unique floristic composition of dry grasslands demands new scientific investigations on biology, ecology and genetics of endangered species. Therefore, the present study focused on several different aspects in life history of steppe plants and demonstrated the importance of consolidated knowledge on taxonomic, phylogeographic, biological and population genetic conditions to develop new conservation strategies and to set adequate conservation priorities.

Chapter 2 dealt with the early colonization history of *Scorzonera purpurea* during the glacial and postglacial period. We reconstructed the postglacial expansion processes of the steppe plant into Central Europe by using molecular markers (AFLP). A survey of genetic variation among and within populations across the whole distribution range provided insights into potential refugial areas and immigration pathways. The phylogeographic analysis revealed that *S. purpurea* might have survived times of glaciations within at least two separated refugial areas, one located in the southern part of France and one located in the south-eastern part of Europe near the Hungarian Plains. After the retreat of glaciers and the following climate warming, *S. purpurea* immigrated via two main migration routes into Germany, both coming from the eastern part of Europe. One route may have lead along the river Danube into Bavaria and up to Central Germany. The other one have lead via Moravia, Silesia into the river valleys of Oder and Elbe. In Central Germany both genetic lineages have met and formed contact zones. The French populations, which were strongly isolated and genetically differentiated from all other populations, did not contribute any genetic material to the colonization of Central Germany. The detection of a potential contact zone in Germany, where different genetic lineages have met, highlights this region for conservation efforts.

Chapter 3 focused on the same species compiling a copious monograph on plant's life history, habitat requirements, population dynamics and conservation status. We prepared basic

information by literature survey as well as own measurements including field work, laboratory experiments and greenhouse studies to enable the detection of potential life cycle risk factors and to improve existing conservation programs for *S. purpurea*. Furthermore, in chapter 7 we used all gained information on *S. purpurea* to compile a population viability analysis and to elaborate detailed recommendations for future managements.

Chapter 4 dealt with the genetic affiliation of *Stipa bavarica*, an endemic steppe species of Bavaria, to its closely related taxa. We investigated wether molecular analyses support its taxonomic status as a distinct species and its importance as management unit of high conservation value. The analysis comprised individuals from populations of *S. bavarica*, *S. pulcherrima* and *S. pennata*. Genetic differentiation between species was high for *S. pulcherrima* and *S. pennata* as well as *S. pennata* and *S. bavarica*. In contrast, there was no genetic differentiation among populations of *S. bavarica* and *S. pulcherrima* indicating strong effects of still existing or recently interrupted gene flow. From this point of view the taxonomic separation of *S. bavarica* as a distinct species seemed not to be very reasonable. However, genetic variation within population and the content of rare bands emphasized the genetic importance of *S. bavarica* as valuable management unit for the preservation of genetic biodiversity in ex situ conservation programs.

In chapter 5 we elucidated the doubtful taxonomic position of *Tephroseris integrifolia* in Bavaria. High morphological variation within and among populations of *T. integrifolia* complicated the exact taxonomic positioning of individual populations for long time. Especially one population in the north of Bavaria is supposed to be a local endemic subspecies, which would grant stronger legal protection. Using a population genetic approach we analysed individuals of three Bavarian and one Austrian population by AFLP. All studied populations differentiated on a very low level, in which the strongest genetic differentiation could be revealed for the grouping of all Bavarian populations against the Austrian population. Within the Bavarian populations no genetic differentiation could be detected and therefore, the taxonomic separation of the population in the north of Bavaria as a distinct subspecies seemed not to be very reasonable in the view of population genetics.

In chapter 6 we focused on the population dynamics of the strongly endangered perennial grassland herb *Tephroseris integrifolia* subsp. *vindelicorum* by using demographic, site-specific and climatic approaches. Long-term demographic investigations over five years in permanent plots gave valuable insights into magnitude and consequences of spatio-temporal

fluctuations as well as demographic sensitivities associated with environmental conditions. Annual finite rates of increase strongly varied among years and sites, but on average both studied populations showed positive population developments ($\lambda > 1$). Recruitment was usually high and positively correlated with site-specific parameters such as percentage of bare ground, moss layer and Ellenberg indicator value for light. However, mean annual transition probabilities among different age stage categories revealed high mortality rates for recruits and determined this age stage category to be the most critical for population dynamic. Furthermore, individuals of *Tephroseris integrifolia* subsp. *vindelicorum* showed high sensitivities against climatic fluctuations. Reduced flowering rates seemed to result from lacking vernalization processes by low winter and early spring temperatures. Therefore, we concluded that long-term survival of *Tephroseris integrifolia* subsp. *vindelicorum* is well supported by grazing as management regime, which enables the creation of safe sites for germination, but due to its strong climatic sensitivity *Tephroseris integrifolia* subsp. *vindelicorum* is permanently facing the unpredictable threats by environmental stochasticity.

Finally, in chapter 7 we concluded with a comparison of population viability analyses for two steppe species, which demonstrated the importance of different and comprehensive scientific approaches in plant conservation to define best management recommendations. The chapter dealt also with perspectives for future scientific research. Especially the extension of genetic analyses on threatened species is required to increase the understanding of past processes and actual patterns of genetic variation. Large-scale genetic screenings of indigenous plant species are essential to localize hotspots of genetic biodiversity and ecologically important genetic regions. From obtained information, a network of best sites for genetic plant conservation throughout Europe could be identified by molecular markers and used for conserving genetic biodiversity. This may force the improvement of conservation priority settings and the selection of taxa which we focus our conservation activities on.

Zusammenfassung

Halbtrockenrasen gehören zu den artenreichsten Habitaten in Mitteleuropa und sind daher von besonderem Interesse für den Naturschutz. Durch das Abholzen der Wälder, Viehzucht und Mahd schuf der Mensch über Jahrhunderte hinweg diesen beeindruckenden Lebensraum. Heutzutage sind viele Trockenrasen infolge landwirtschaftlicher Intensivierung oder aber Nutzungsaufgabe in ihrer Existenz bedroht und viele ihrer typischen Pflanzenarten gelten als besonders gefährdet. Die Entwicklung adäquater Instrumentarien und Managementmaßnahmen zum Schutze dieser einzigartigen Flora erfordert neue wissenschaftliche Untersuchungen zu Biologie, Ökologie und Genetik vieler gefährdeter Arten. Aus diesem Grunde zielte die hier vorliegende Arbeit darauf ab, möglichst viele verschiedene Aspekte im Leben von Steppenpflanzen zu beleuchten und die immense Bedeutung von fundiertem Wissen zu Taxonomie, Phylogeographie, Biologie und Populationsgenetik für den Schutz dieser Artengruppe zu demonstrieren.

Kapitel 2 beschäftigte sich vor allem mit der frühen Besiedelungsgeschichte von *Scorzonera purpurea* während des Glazials und Postglazials. Mit Hilfe molekularer Marker (AFLP) konnten ehemalige Besiedelungsprozesse dieser Steppenpflanze in Mitteleuropa rekonstruiert werden. Die Erfassung genetischer Variation zwischen und innerhalb von Populationen des gesamten Verbreitungsgebiets ermöglichte Einblicke in potentielle Refugialräume und Wanderrouten. Die phylogeographische Analyse zeigte, dass *S. purpurea* die Zeiten großflächiger Vereisung in (mindestens) zwei getrennten Refugialräume überleben konnte, einerseits im südlichen Teil Frankreichs und zum anderen im südöstlichen Bereich Europas in der Nähe der ungarischen Tiefebene. Nach dem Rückzug der Gletscher und der darauf folgenden Klimaerwärmung wanderte *S. purpurea* über zwei Hauptrouten von Osten her nach Deutschland ein. Eine Wanderroute erstreckte sich entlang der Donau bis nach Bayern und von dort aus bis in die Mitte Deutschlands. Die andere führte über Mähren und Schlesien in die Flusstäler von Oder und Elbe. In Mitteldeutschland trafen beide Einwanderungslinien aufeinander und bildeten eine Kontaktzone. Die französischen Populationen, die stark isoliert und genetisch von allen anderen Populationen differenziert sind, trugen kaum etwas zur Besiedelung Mitteldeutschlands bei. Der Nachweis einer potentiellen Kontaktzone in Deutschland, in der verschiedene genetische Linien aufeinander treffen, betont die Bedeutung dieser Region für den Natur- und Artenschutz.

In Kapitel 3 beschäftigten wir uns ebenfalls mit *Scorzonera purpurea* und erstellten eine umfassende Monographie zu Lebensgeschichte, Habitatanforderungen, Populationsdynamik und naturschutzfachlicher Stellung dieser seltenen Steppenart. Die grundlegenden Informationen wurden von uns durch eine umfassende Literaturstudie sowie durch eigene Erhebungen im Feld, Laborarbeiten und Gewächshausexperimenten erarbeitet. Sie dienen der Feststellung möglicher Risikofaktoren im Lebenszyklus der Art und der Optimierung bestehender Schutzprogramme für *S. purpurea*. Desweiteren wurden diese Ergebnisse zur Erstellung einer Populationsgefährdungsanalyse mit detaillierten Empfehlungen für zukünftige Artenschutzmaßnahmen in Kapitel 7 genutzt.

Kapitel 4 evaluierte die genetische Zugehörigkeit von *Stipa bavarica*, einer endemischen Pflanzenart in Bayern, zu ihren nah verwandten und räumlich benachbarten Sippen. Es wurde untersucht, ob molekulare Analysen die taxonomische Stellung dieser Art als eigenständige Sippe unterstützen und inwiefern diese Population als wichtige Management-Einheit mit hohem naturschutzfachlichem Wert angesehen werden kann. Die Studie umfasste Individuen von 21 Populationen von *S. bavarica*, *S. pulcherrima* und *S. pennata*. Die genetische Differenzierung zwischen den Arten *S. pulcherrima* und *S. pennata* ($\Phi_{PT} = 0.25$) sowie den Arten *S. bavarica* und *S. pennata* ($\Phi_{PT} = 0.24$) war verhältnismäßig hoch. Dagegen konnte keine genetische Differenzierung zwischen den Populationen von *S. pulcherrima* und *S. bavarica* festgestellt werden, was dafür spricht, dass es noch immer Genfluss zwischen den Populationen gibt oder dieser erst seit kurzer Zeit unterbrochen ist. Folglich erscheint die taxonomische Abtrennung von *S. bavarica* als eigenständige Art aus populationsgenetischer Sicht nicht empfehlenswert. Allerdings unterstreichen die hohe genetische Variation und der Anteil seltener und charakteristischer Banden die genetische Bedeutung von *S. bavarica* als naturschutzfachlich wertvolle Management-Einheit für die Sicherung der genetischen Variationsbreite im Rahmen von in situ und ex situ Maßnahmen.

In Kapitel 5 beschäftigten wir uns mit der zweifelhaften taxonomischen Stellung von *Tephroseris integrifolia* in Bayern. Ihre hohe morphologische Variation zwischen und innerhalb der Populationen von *T. integrifolia* erschwerte die genaue taxonomische Zuordnung einzelner Populationen seit langer Zeit. Besonders eine Population im Norden von Bayern könnte morphologisch als neuer lokaler Endemit betrachtet werden, was stärkere gesetzliche Schutzmaßnahmen nach sich ziehen würde. Mit Hilfe eines populationsgenetischen Ansatzes untersuchten wir Individuen aus drei bayerischen und einer

österreichischen Population mit Hilfe genetischer Fingerprints (AFLP). Die untersuchten Populationen zeigten nur eine sehr geringe genetische Differenzierung, wobei die stärkste Differenzierung für die Gruppierung aller bayerischen Populationen gegen die österreichische Population festgestellt werden konnte. Innerhalb der bayerischen Populationen konnte keine nennenswerte genetische Differenzierung ermittelt werden und daher erscheint die taxonomische Abgrenzung der nordbayerischen Population als eigenständige Art aus populationsgenetischer Sicht nicht sinnvoll.

In Kapitel 6 stand die Analyse der Populationsdynamik der stark gefährdeten Kalkmagerrasenart *Tephroseris integrifolia* subsp. *vindelicorum* mit Hilfe demographischer, habitatspezifischer und klimatischer Ansätze im Mittelpunkt. Demographische Langzeit-Untersuchungen über fünf Jahre in 56 Daueruntersuchungsflächen gaben Hinweise über das Ausmaß und die Auswirkungen spatio-temporaler Schwankungen sowie über demographische Empfindlichkeiten gegenüber Umweltbedingungen. Jährliche Zuwachsraten schwankten sehr stark zwischen den Jahren und den Untersuchungsflächen, allerdings zeigten beide untersuchten Populationen im Mittel positive Bestandsentwicklungen ($\lambda > 1$). Die Verjüngungsrate war hoch und stand in positivem Zusammenhang mit habitatspezifischen Parametern, wie prozentualem Offenbodenanteil, Moosdeckung und dem Ellenberg-Indikatorwert für Licht. Allerdings zeigten die mittleren jährlichen Übergangs-wahrscheinlichkeiten zwischen verschiedenen Lebensalter-Kategorien eine hohe Sterblichkeitsrate für Jungpflanzen (44.7 %). Somit muss dieses Altersstadium als das kritischste im Lebenszyklus dieser Art angesehen werden. Desweiteren reagieren Individuen von *Tephroseris integrifolia* subsp. *vindelicorum* sehr empfindlich auf Klimaschwankungen und büßen bei fehlenden Vernalisationsprozessen durch niedrige Winter- oder Frühjahrstemperaturen ihre Blühfähigkeit ein. Letzten Endes können wir schlussfolgern, dass das langfristige Überleben dieser Art durch das gut angepasste Beweidungsregime positiv unterstützt wird, vor allem durch die Schaffung von Offenbodenstellen für die Keimung, allerdings stellt die starke Klimasensitivität von *Tephroseris integrifolia* subsp. *vindelicorum* eine ständige und unvorhersehbare Bedrohung in Form umweltbedingter Stochastizität dar.

Abschließend erfolgte in Kapitel 7 die vergleichende Darstellung von Populationsgefährdungsanalysen für zwei bedrohte Steppenpflanzen, was die Bedeutung umfassender wissenschaftlicher Untersuchungen im Bereich Artenschutz unterstreichen und die Festlegung gezielter Management-Empfehlungen ermöglichen sollte. Dieses Kapitel gibt

weiterhin einen Ausblick auf zukünftige Beschäftigungsfelder im Bereich der Naturschutzforschung. So könnte die Ausweitung molekularer Untersuchungen an gefährdeten Pflanzenarten das Verständnis historischer Prozesse und aktueller Verbreitungsmuster genetischer Variation fördern. Ein groß angelegtes genetisches Screening einheimischer Arten ist essentiell, um Hotspots genetischer Biodiversität und genetisch maßgebliche Regionen auch im kleineren Länder-Kontext zu lokalisieren. Diese Information könnte man nutzen, um Diversitätszentren für den genetischen Artenschutz in ganz Europa ausfindig zu machen. Die Bemessungsgrundlage für die Feststellung von Artenschutz-Prioritäten könnte damit verbessert und die Auswahl von Maßnahmen für bestimmte Taxa zielgerichteter ausgearbeitet werden.

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Chapter 1

Steppe plants in Central Europe – where do they come from and where will they go?

The Eurasian steppe belt

Steppes are dry grasslands of the temperate zone and can be found in areas with continental climate characterized by warm, dry summers and cold winters (Buček *et al.* 2006). Lack of rainfall and frost limit the growth of woody plants and prevent the development of a closed forest cover. Although, steppe ecosystems only represent a small percentage (< 5%) of the total extension of Europe and Asia, they host a large variety of rare and endemic plant species (Pérez-Collazos *et al.* 2008). The typical Eurasian steppe belt is located between 45° and 55° of northern latitude and extends from the Puzsta in Hungary via Kazakhstan to the steppes of Transbaikalia, northwest China, Mongolia and the Amur region (Fig. 1; Franzke *et al.* 2004). The width of this steppe zone varies from 150 km at its western end and up to 1000 km in the region between the Black Sea and the Caspian Sea (Formozov 1966).

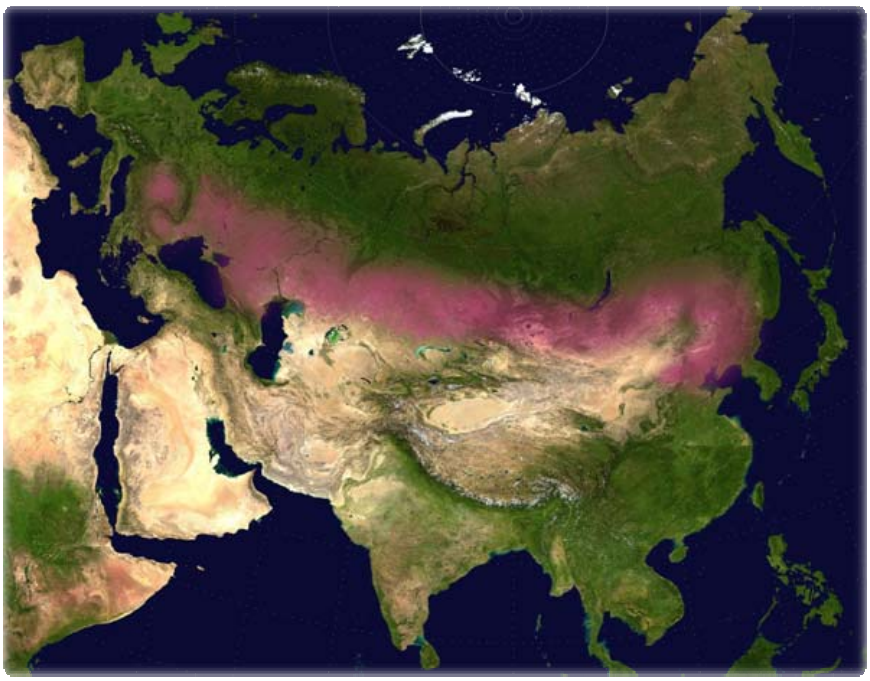


Fig. 1 The Eurasian steppe belt. (http://commons.wikimedia.org/wiki/File:Eurasian_steppe_belt.jpg)

Steppes are dominated by a grassy vegetation cover with predominance of perennial grasses and other species of grass-like appearance, which are able to resist droughts (Formozov 1966; Walter & Straka 1970). Steppe soils are characterized by a dark-colored horizon (chernozems), rich in humus and therefore, most valuable as agricultural soil. Mainly all steppes in Eurasia have been cultivated for long time and are still under intensive agricultural practice. Larger remains of steppe landscapes in Eurasia could only be preserved in few nature reserves (Buček *et al.* 2006). The most important nature reserve for steppes, the Central Cernozem Zapovednik, can be found in the western Russian plateau in the near of Kursk.

Steppes in Central Europe

The vast Eurosiberian steppe landscapes are characterized by far horizons and prevalent flat and undulated reliefs, an 'epitome of unlimited freedom' (Buček *et al.* 2006). In the more central and western part of Europe the continuous distribution range of the Eurasian steppe belt breaks up. Subsequent to the Carpathian basin, only few occurrences of steppe-like vegetation can be found located only on the driest and well-drained places (< 600 mm precipitation), e.g. the Vienna basin or some scattered xerothermic areas in Germany. While the eastern steppe landscapes are characterized by distinctive continental conditions, Central European grasslands are simultaneously influenced by eastern, western and southern climatic effects and are characterized by a mixed floristic composition of pannonian, pontic and submediterranean elements. This transitional floristic character makes Central European grasslands unique (Walter & Straka 1970).

Little is known about the colonization history of European steppe plants and their erratic evolution. However, distribution of steppe species in Eurasia is considered to have experienced several phases of expansion and contraction since the Pleistocene. The origins of the steppe biome are considered to be in the region of Mesopotamia, where Neolithic agriculture originated and enabled the rise of first ancient civilizations (Buček *et al.* 2006). In Central Europe, natural grasslands are restricted to areas in alpine regions, gravel banks along rivers and outcrops. The extent of their distribution was influenced by several strong impacts both of climatic and anthropogenic origin. Nowadays most dry grasslands are of anthropogenic origin. It is still under debate whether the natural landscape before human settlement was dominated by a close forest cover or whether it was a more open landscape

due to the impact of mega-herbivores (see Poschlod *et al.* 2010). Anyway, patches of grasslands may have existed continuously in temperate Europe for millions of years to different extent (Pärtel *et al.* 2005). In early post-glacial times for example, large areas of Central Germany have been covered by steppe vegetation (Lang 1994; Hewitt 1996).

During the more humid times after glaciations, deciduous forests proceeded and caused natural restrictions to grassland expansions. These times have been followed by periods of increasing human activities. Neolithic peasants affected the post-glacial development of vegetation by preventing a more or less closed forest cover due to soil cultivation and livestock rearing. Therefore, human impact facilitated the development of steppe- and forest-steppe-like habitats (Buček *et al.* 2006). Grazing of livestock, grass mowing and rising demand of wood during medieval times had enhanced open vegetation structures and forest free habitats. However, the raising industrial progress of the 20th century has mainly replaced the traditional forms of land use, e.g. sheep herding, and reversed the expansion of dry grasslands in Central Europe (Poschlod & WallisDeVries 2002). Modern land use practices narrowed their extent dramatically. At present, only small and isolated islands can be recognized deserving special conservation protections.

Steppe plants typically occur in plant communities of dry calcareous or sandy grasslands, rocky slopes and xerophilous pine forests. In Germany, less than 1000 ha of steppe-like vegetation are still left. Typical areas with high amounts of steppe-like vegetation in Central Europe are regions of the Upper and Middle Rhine valley, the Danube valley, the Swabian and Franconian Alb, the Main valley, Kyffhäuser in Thuringia, the Weser Hills, terraces along the Oder as well as the dry landscapes of Bohemia and Moravia and Lower Austria .

Threats to steppe plants

In the economically transformed modern landscapes of Central Europe, steppe plants are facing a large variety of threats, which strongly affect population viability. Three main categories of threats can be distinguished: (1) threats imposed by environmental changes, either of natural or anthropogenic origin, (2) threats resulting from disturbances of crucial interactions with other species and (3) genetic threats (Brigham & Schwartz 2003). While threats of the first category involve direct destruction of populations or individuals, independent from population size, the second and third category are generally associated with threats through small population size, low population densities and isolation.

Environmental threats

Habitat destruction, degradation and fragmentation caused by changes in land use practices, eutrophication and suppression of natural dynamic processes are often regarded as the major environmental threats to plant populations (Brigham & Schwartz 2003). All of them directly alter plant habitats in area, stability, connectivity and quality. The breaking-up of a formerly continuous habitat into series of large to small fragments has important demographic consequences for processes on population and metapopulation level. Ecological consequences are increased edge effects, small population sizes, spatial isolation and reduced exchange of individuals and genes among populations (Barrett & Kohn 1991; Kruess & Tscharntke 1994; Young *et al.* 1996; Lande 1998). Habitat fragmentation may also alter plant-pathogen and plant-herbivore interactions (Fischer 1998).

Beside habitat fragmentation, climate change is also regarded to be an increasing environmental threat to the long-term survival of plant populations. Rapid global changes will act as a critical bottleneck for plant populations within our actually highly fragmented landscape. While in an undisturbed natural landscape species would either move their range or adapt under gradual climate change, both processes are severely disturbed within our highly fragmented landscape (Oostermeijer 2003). Effects of climate change on plants can be direct, affecting growth, flowering and phenology, as well as indirect through modifications of interactions with herbivores (Fox *et al.* 1999), pollinators (Stenström *et al.* 1997; Harrison 2000) or effects on microsite availability in the plant community (Sternberg *et al.* 1999).

Threats by disturbed biotic interactions

The most striking negative effects for plants caused by disturbed biotic interactions in relation to habitat fragmentation are pollen and dispersal limitation (Poschlod *et al.* 1996; Bonn & Poschlod 1998). Many plants rely on animal pollinators to transfer pollen onto their stigmas for ovule fertilization. Especially in small and isolated populations, plant density is often less attractive to pollinators as food sources and visitation rates become less frequently (Rathcke & Jules 1993). This phenomenon is known as Allee effect (Allee *et al.* 1949). While in self-compatible species, low densities of individuals lead to increased geitonogamy (de Jong *et al.* 1993), self-incompatible plants suffer from receiving not enough compatible pollen and, in consequence, from reduced seed set (Byers & Meagher 1992). In the long run, this may lead to changes in demographic processes and raise the extinction risk of isolated populations. In

several studies evidence for reduced seed and fruit set as a consequence for pollen limitation has been already found (Kephart *et al.* 1999; Robertson *et al.* 1999; Harris & Johnson 2004).

Indications for disturbed biotic interactions affecting dispersal of seeds are also available for different kind of vectors. Impaired migration of various birds and mammals (especially sheep) in fragmented landscapes reduce the probability of connecting effects between plant populations and limit the chance of reaching new suitable habitats by long distance dispersal (Fischer *et al.* 1996; Poschlod *et al.* 1998; Seifert & Fischer 2010). Especially the development of transhumant shepherding in the 18th century favored the connection of regions, which were more than a hundred kilometers apart, by dispersing seeds of many calcareous grassland plants (Poschlod & WallisDeVries 2002). Nowadays, traditional shepherding became increasingly uneconomical and most sheep flocks were kept stationary in paddocks, a fact which strongly limits the spread of seeds among populations.

Extinction of native plants can also be a consequence of direct interactions with alien species. Low competitive and light-demanding plant communities, such as dry grassland communities, are often affected by the increasing dominance of strongly competitive alien species (Pyšek & Pyšek 1995; Carlsen *et al.* 2000). Herbivory by introduced as well as native mammals, insects or molluscs may also cause raising mortality or reduced fecundity rates and enlarges the extinction risk of rare plants (Traveset & Richardson 2006).

Genetic Threats

Population viability may also be affected by genetic threats caused by ongoing landscape fragmentations. Especially small and isolated populations are prone to genetic deterioration, which strongly influences genetic variation (Barrett & Kohn 1991; Ellstrand & Elam 1993; Young *et al.* 1996). The main effects associated with genetic isolation of populations are loss of genetic variation through genetic drift and increasing inbreeding rates (Fischer & Matthies 1998; Paschke *et al.* 2002). Genetic variation is crucial in maintaining high levels of individual fitness and allowing populations to adapt to changing environmental conditions (Heschel & Paige 1995). Therefore, habitat fragmentation might lead to reduction of adaptive potential (Franklin 1980), inbreeding depression (Menges 1991; Oostermeijer *et al.* 1994b; Fischer & Matthies 1998; Kéry *et al.* 2000; Luijten *et al.* 2000) and accumulation of deleterious mutations (Lynch *et al.* 1995). In self-incompatible plant species, the loss of

alleles at the self-incompatibility locus may further lead to a rapid reduction in fertilization success (Vekemans *et al.* 1998) and strongly promote population extinction.

Thesis outline

Dry calcareous grasslands are one of the most diverse plant community types in Europe and contain a unique mixture of pontic, pannonian and submediterranean plant species (Walter & Straka 1970). The ongoing loss of species, even of steppe species, strongly influences their characteristic floristic composition and lowers their high level of biodiversity. Furthermore, populations at the border of species range seem to be of great interest both from a historical and an evolutionary point of view (Tyler 2002a; Wróblewska 2008). Marginal populations are often under stronger local selection than populations occupying the centre of species range, a fact, which might lead to a number of genetically divergent populations, each one adapted to its own habitat conditions (Holsinger & Gottlieb 1991). In times of global climate change, these populations are getting more and more interesting, because they might be a starting point for future migrations into more western parts of Europe. Consequently, conserving high levels of genetic variation and large numbers of viable populations of steppe species in Central Europe are essential to guarantee evolutionary adaptability and population's long-term survival.

The present study aims at elucidating various aspects in conservation biology of three indigenous steppe plants and at assessing their past, present and future fate within Central Europe. Modern as well as conventional methods have been applied to solve actual difficulties in preserving these critically endangered plant species by focussing on three different conservation levels. Fig. 2 gives an overview about the topics that are addressed in the individual chapters and about how they are linked with basic conservation strategies.

Chapter 2 deals with the post-glacial colonization history of *Scorzonera purpurea*. By means of molecular analyses (AFLP), a survey of genetic variation across the whole species range provides the opportunity for revealing exemplarily potential refugial areas for steppe plants during glaciations and for reconstructing their post-glacial expansion routes into Central Europe. Knowing the pattern of genetic variation of a species is fundamental to set conservation priorities. Populations with different genetic lineages, for example, might demand special conservation and management effort.

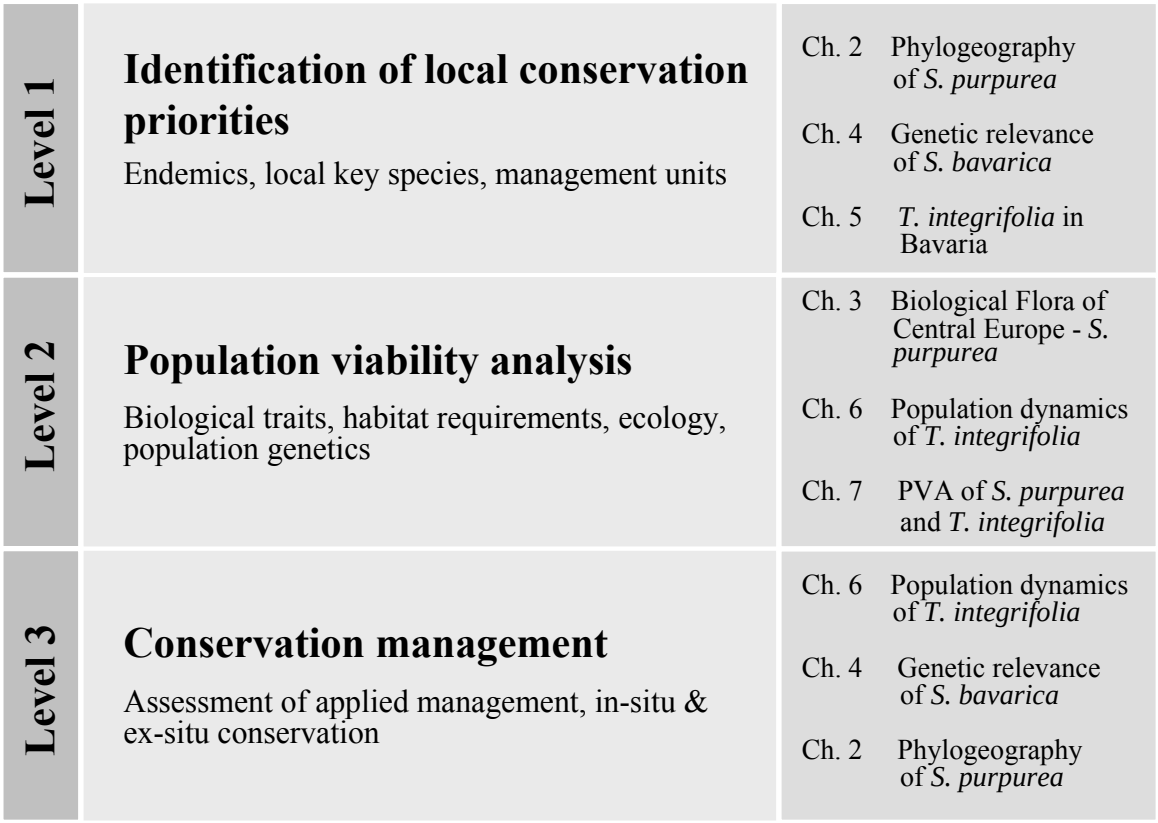


Fig. 2 Levels of local conservation strategies and their interaction with scientific conservation research.

In chapter 3 we focus on the same species compiling a copious monograph on plant's life history, habitat requirements, conservation strategies and population viability. Information are gained by literature survey as well as by own measurements including field work, laboratory experiments and greenhouse studies to complete the sparse knowledge on this highly endangered plant species. All these basic data are crucial to assess population's long-term survival and to improve existing conservation programs for *S. purpurea*.

Chapter 4 deals with the genetic affiliation of an endemic steppe plant in Bavaria to its closely related taxa and the probability of being the result of potential hybridization between them. Taxonomic uncertainties due to low morphological variations posed the question of genetic relevance of the endemic steppe plant *Stipa bavarica* for conservation and its importance as management unit. In most cases, the question of how many and which populations represent significant amounts of species total diversity still remains unsolved (Pérez-Collazos *et al.* 2008). Especially, peripheral populations situated at the edges of species range are more sensitive to genetic drift and/or strong directional selection (Barrett & Husband 1990). They are thought to be of great evolutionary significance and to deserve conservation priority.

Chapter 5 deals also with a taxonomic issue concerning the various species group of *Tephroseris integrifolia*. High morphological variation within and among different populations of *T. integrifolia* in Bavaria questions their taxonomic position to each other. Especially the position of one population in the northern part of Bavaria is still doubtful and demands a more detailed analysis. Solving genetic uncertainties by population genetics may provide means for setting conservation priorities and for preventing erroneous decisions if the taxonomic status of population is not correctly assigned. In the case of revealing a potential endemic subspecies, one population of *T. integrifolia* in the north of Bavaria might receive stronger legal protection and more specific conservation efforts.

In chapter 6 we focus on the analysis of the development of population structure in the endemic steppe plant *T. integrifolia* subsp. *vindelicorum*. Long-term investigations over five years give valuable insights into population processes and may help to assess the applied management practice. In long-lived species, size and number of populations may not be good indicators to provide important implications for management decisions. Detailed demographic studies can reveal critical stages in the life cycle, provide the basis for modeling population dynamics, allow predictions about the future development of populations and enable comparisons among different management methods (Hutchings 1991; Menges & Dolan 1998).

Finally, chapter 7 summarizes the importance of different scientific approaches in plant conservation and illustrates future perspectives within conservation research and conservation practice. Compiling population viability analyses for two comparable steppe species demonstrates the high importance of detailed biological investigations to reveal potential risk factors in plant's life history and to define best management recommendations.

Chapter 2

Survival or recolonization? Glacial history of the steppe plant *Scorzonera purpurea* in Central Europe

Abstract

Climate fluctuations during Pleistocene had a strong impact on geographic distribution of plants and their genetic variation. Grasslands are one of the most diverse plant community types in Europe and contain many threatened species. Although grasslands have existed continuously in temperate Europe for millions of years, they have undergone several processes of large expansions and severe contractions. These fluctuations strongly shaped the floristic composition of European grasslands by migration processes among different biomes, varying driving forces for speciation and species extinctions.

There is still a lack of information about history and postglacial migration routes of herbaceous grassland plants in Central Europe. In the present study we investigated the phylogeography of the steppe plant *Scorzonera purpurea* L. (Asteraceae) and tried to infer its potential migration and colonization pattern in Europe. We explored genetic variation by amplified fragment length polymorphism (AFLP) within and between 37 populations covering large parts of the distribution range with a main focus on Central European populations. Genetic variation in *S. purpurea* analysed by PCoA and cluster analysis revealed some kind of geographic structures. The most distinct geographic structure consisted of a strong separation of the French populations from all other populations. AMOVA also supported the separation into two main groups containing on the one hand all French populations and on the second hand all other populations (Φ_{RT} = 15.3 %). In addition, PCoA revealed an east-west-orientation of all studied populations according to their geographic location. Furthermore, a strong positive correlation of genetic and geographic distances reflected the close relationship between central and eastern populations (R_M = 0.420, p = 0.001). Therefore we can assume that postglacial recolonization of previously climatic unfavourable parts in Central Europe mainly took place from the south-east of Europe.

This conclusion was also supported by the distribution of genetic variation within populations. The most diverse populations were the Hungarian and West-Russian populations

indicating the proximity of potential refugial areas in this region, e.g. the Balkans or the Black Sea. According to STRUCTURE analysis, two main migration routes seem to be likely for the recolonization of Germany via Pannonia. One route may have lead along the river Danube into Bavaria and up to central Germany and one via Moravia, Silesia to the river valleys of Oder and Elbe into the central German dry landscapes. In this region the different genetic lineages may have met and formed a contact zone.

Finally, our results suggest the splitting of a formerly continuous species range into two distinct groups during the cold stages of glaciation, when large parts of Central Europe were covered by tundra and cold steppe. One group of individuals retreated into Southern France from where no major re-colonization took place after climate warming. Another group retreated into the south-east of Europe. After deglaciation all other parts of Europe were colonized by descendants of these south-eastern refugial populations.

Introduction

The history of European dry grasslands is a history of severe climatic changes and anthropogenic impacts (Poschlod *et al.* 2010). During Pleistocene, long periods of cold and dry (glacial) climatic conditions alternated with short interglacial periods, which were characterized by warm and moist conditions (Pärtel *et al.* 2005). The cold periods, especially the LGM (Last Glacial Maximum, ~ 20 000 YBP), were characterized by a large ice sheet covering northern Europe and the alpine mountains. Polar desert, permafrost and steppe-tundra vegetation dominated vast areas of Central Europe improper for thermophilous species not adapted to extremely low temperatures and arid conditions. Many grassland species were largely restricted to warmer refugia in the southern and south-eastern parts of Europe, primarily the peninsula of Iberia, Italy and the Balkans and possibly near the Caucasus and the Caspian Sea (Taberlet *et al.* 1998; Hewitt 1999). However, recent phylogenetic studies demonstrated also evidence for local survival of grassland species within parts of Central Europe (Bylebyl *et al.* 2008).

During interglacial periods and especially during times of ongoing climate warming (around 10 000 YBP), glaciers retreated and many European grassland species migrated rapidly and expanded their ranges north-, east- and westwards from their refugia (Lang 1994; Adams & Faure 1997). These recurring range expansions, isolation processes and recolonization events of plant species created an alternating mixture of grassland communities within European

landscapes (Pärtel *et al.* 2005). Furthermore, the impact of human settlement, pastoralism and different types of arable farming since the Neolithic time period shaped the floristic composition of European grasslands by alternating driving forces for speciation and ecological adaptations (Poschlod *et al.* 2010). Today, temperate grasslands are characterized by high floristic biodiversity and a unique mixture of floristic elements originating from different biomes, such as steppes, temperate forests, alpine grasslands, tundra and Mediterranean plant communities. Beside ecological and physiological factors, these past processes have influenced species present-day distribution pattern and their intraspecific genetic variation.

By uncovering the genetic structure within and between populations of a typical European steppe plant, we can get a more detailed insight into Europe's historical background. Due to a lack of suitable prehistoric pollen deposition possibilities within dry habitats and little pollen production of insect-pollinated herbs, information about exact localization of glacial refugia of steppe plants and their fate during Quaternary glaciations are still scarce (Malm & Prentice 2002; Tyler 2002b; Wroblewska *et al.* 2003; Franzke *et al.* 2004; Hensen & Oberprieler 2005; Wróblewska & Brzosko 2006; Bylebyl *et al.* 2008; Vrancken *et al.* 2009). By using modern DNA techniques population histories and potential colonization routes of plants after glaciation can be reconstructed (Taberlet *et al.* 1998; Schönswetter *et al.* 2003). The present-day geographic structure of genetic variation within and among populations may reflect these past processes of colonization, gene flow and genetic drift conserved over multiple generations (Hewitt 1996). Intraspecific genetic structure may differ, if species have expanded their range gradually from a single source in a southern stable refuge or from several differentiated geographic sources (Hewitt 1996). A strong loss of genetic variation within populations can be the result of founder effects at the migrating front and therefore highest genetic variation within populations should be present in refugial areas and decrease to the more distant and recently recolonized parts (Hewitt 1999). In contrast, high genetic variation within populations in recently deglaciated regions may be an indication for contact zones of different genetic lineages originated from different refugia (Walter & Epperson 2005). Long-term isolated populations or populations founded by long-distance dispersal should be characterized by stronger genetic differentiation than populations expanding their range continuously (Hewitt 1996; Tyler 2002a).

Several phylogeographical studies on animal species in northern Europe (Fedorov & Stenseth 2001; Brunhoff *et al.* 2003), some forest tree species (Bennett *et al.* 1991; Demesure *et al.* 1996; Magri *et al.* 2006) and glacial relicts in the Alps and Fennoscandia (Despres *et al.* 2002; Malm & Prentice 2002; Reisch *et al.* 2003b; Schönswetter *et al.* 2004; Schönswetter & Tribsch 2005) used information about level and distribution of genetic variation to detect splitting events, relationships between populations, historical bottlenecks and potential refugial areas (Schönswetter *et al.* 2003). For arctic-alpine plant species, molecular techniques revealed already detailed evidence for two alternative survival strategies during glaciation: (1) total extinction in glaciated areas and survival in peripheral refugia followed by subsequent re-immigration into vacant areas (tabula rasa hypothesis), or (2) long-term in situ survival on ice-free mountains within glaciated areas (nunatak hypothesis) (Stehlik 2000).

To elucidate the history of steppe plants in Central Europe and to prove postglacial re-colonization scenarios hypothesized by authors like Gradmann (1950), Walter & Straka (1970), Lang (1994), Küster (1995) and Hegi (1998), we analysed population genetic structure and the level of genetic variation among and within 37 populations of the perennial steppe plant *Scorzonera purpurea* across Europe. The above mentioned authors predicted southern survival of steppe plants during glaciation and a rapid postglacial expansion after climate warming. Based on the actual geographic distribution of floristic elements, few typical migration routes for pontian plant species into Central Europe can be assumed. One of the colonization routes seems to have been the river valley of the Danube via Pannonia and Lower Austria. Following the course of the river, steppe plants coming from the east may have reached the plains of the Hungarian Puszta, the Vienna Basin and more far in the west the southern German dry landscapes. Other routes may have lead via Pannonia, Bohemia, Moravia and Silesia to the river valleys of Oder and Elbe and Central Germany.

Colonization history may be conserved within the genetic structure of plants and therefore, postglacial expansion processes could be revealed by using molecular markers (AFLP). The present study is aimed at the identification of existing intraspecific genetic variation among and within populations of the European steppe plant *Scorzonera purpurea* across large parts of its distribution range. We try to reconstruct the Tertiary and Quaternary history of *S. purpurea* in Europe. In particular, we ask if populations have survived the last ice age within Central Europe and if not from which source areas re-immigration of steppe plants into Central Europe took place.

Material & Methods

Species description and sampling strategy

Scorzonera purpurea L. (Asteraceae) is a perennial, diploid (2n = 14) herb with narrow-lineal, grass-like rosette leaves and a robust, central tap-root. Its root-collar is densely covered with bristle-like fibres of dead foliar petioles. One individual can build a 15 to 45 cm high, simple or branched flowering stem with 1 to 5 light-purple or violet-pink heads (Tutin *et al.* 1964). Experiments with exclusion of pollinators showed that the species is obligate outcrossing by insects and produces only small amounts (ca. 25 seeds per capitulum) of achenes with large pappi of pinnate bristles. Despite these appendices, seed dispersal by wind (< 100 m) and animals is largely limited to short distances (see chapter 3). Germination takes place in autumn and spring after a short (two week) flowering period between May and June. Seed-set usually is good and under artificial conditions most seeds germinate readily and without any special treatment (e.g. cold stratification; pers. obs.). The species prefers sunlit sites and open vegetation structure. Typical habitats are steppes, flood plains, steppe meadows, steppe woods, stone debris and limestone slopes (Tutin *et al.* 1964) in the continental regions of Eurasia. Its main distribution range comprises vast areas of the steppe region in South and Central Russia, Western Siberia and Ukraine (Fig. 3). In Central Europe, the species is restricted to highly fragmented sites of dry nutrient-poor grasslands on calcareous soils, e.g. the Pannonian and Vienna Basin, parts of Poland, Germany, Czech Republic and Slovakia. In the Massif Central of southern France, *S. purpurea* reaches its most western part of distribution. In most parts of Europe, the species is strongly endangered by habitat loss (Schnittler & Günther 1999).

To study the phylogeographic pattern of *S. purpurea* in Europe, 37 populations were chosen in order to cover a broad range of species distribution with a main focus on Central Europe (Tab. 1). We collected plant samples from different sites along the European steppe belt. The minimum distance between two locations was 550 m, with the maximum distance being 4 114 km. Population sizes were estimated for some populations and used for size-dependent correlations. For AFLP analysis we sampled leaves of 15 individuals per population with a minimum distance of >1 m between individuals.

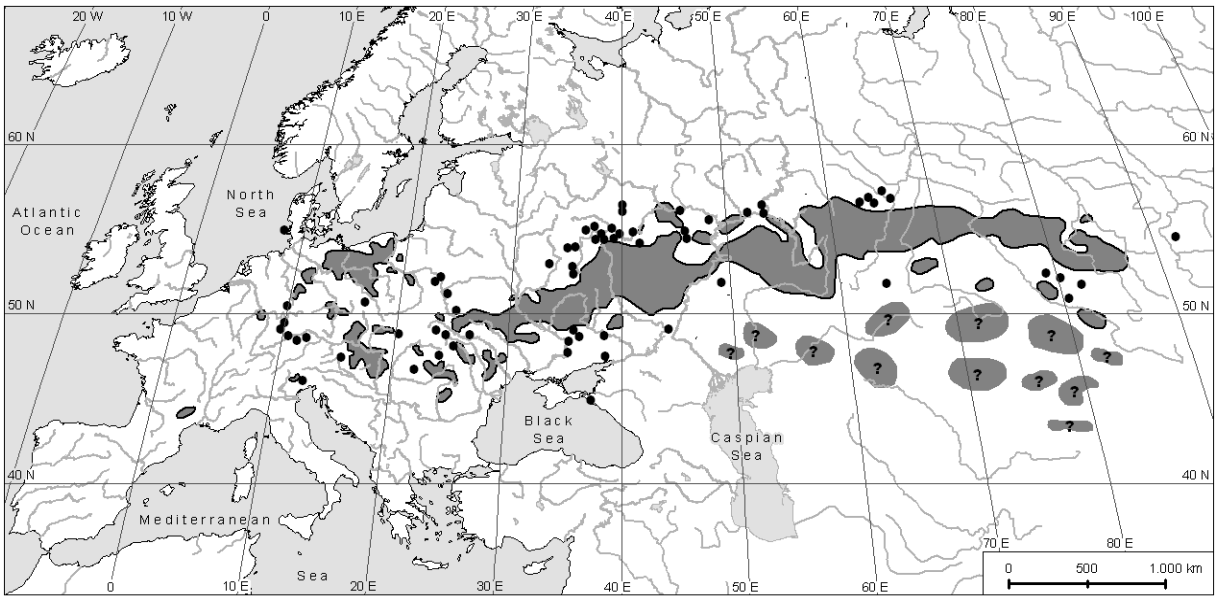


Fig. 3 Distribution range of *S. purpurea* in Eurasia. Map is based on Meusel & Jäger (1992). Question marks symbolize vague geographical information about occurrences. Dots symbolize local scattered occurrences.

DNA extraction and amplified fragment length polymorphism (AFLP) analysis

Fresh plant material was sampled in the field and dried immediately with an adequate amount of silica gel. Genomic DNA was isolated following the CTAB (cetyltrimethylammonium bromide) method (Rogers & Bendich 1994) adapted as previously described by Reisch et al. (2005). For this study, we chose the AFLP marker system to amplify selected fragments from the digestion of total DNA by polymerase chain reaction. The protocol was carried out following the procedure described by Vos et al. (1995). We used non-radioactive fluorescent dye-labelled primers on an automated DNA sequencer (CEQ 8000, Beckman Coulter). Eight randomly selected individuals from different populations throughout the study area were screened with 32 primer pair combinations for clear and reproducible bands. We chose three primer pairs fulfilling these options for analyses of the total sample set (D2: *Mse*I-CTC/*Eco*RI-AAC, D3: *Mse*I-CAC/*Eco*RI-AAG, D4: *Mse*I-CAT/*Eco*RI-ACT). Genomic DNA (approximately 50 ng) was digested with the restriction enzymes *Eco*RI and *Mse*I and ligated with T4 DNA Ligase conducted in a thermal cycler for 2 h at 37 °C. Polymerase chain reactions (PCRs) were run in a reaction volume of 5 ml. Preselective amplifications were performed using primer pairs with a single selective nucleotide, *Mse*I-C and *Eco*RI-A, H_2O, buffer S, dNTPs, and Taq. PCR reaction parameters were: 2 min at 94 °C, 30 cycles of 20 s of denaturing at 94 °C, 30 s of annealing at 56 °C, and 2 min of extension at 72 °C, followed by 2 min at 72 °C and ending with 30 min at 60 °C. Selective amplifications were performed

with the three selected primer pair combinations and H_2O, buffer S, dNTPs, and Taq. PCR reactions were performed with the touch-down profile: 2 min at 94 °C, ten cycles of 20 s of denaturing at 94 °C, 30 s of annealing, which was initiated at 66 °C and then reduced by 1 °C for the next ten cycles, 2 min of elongation at 72 °C, followed by 25 cycles of 20 s of denaturing at 94 °C, 30 s of annealing at 56 °C and 2 min of elongation at 72 °C, ending with a final extension for 30 min at 60 °C. After DNA precipitation, DNA pellets were vacuum dried and dissolved in a mixture of Sample Loading Solution (Beckman Coulter) and CEQ Size Standard 400 (Beckman Coulter). The fluorescence-labelled selective amplification products were separated by capillary gel electrophoresis on an automated sequencer (CEQ 8000, Beckman Coulter). Raw data were collected and analysed with the CEQ Size Standard 400 using the CEQ 8000 software (Beckman Coulter). Data were exported as crv-files, showing synthetic gels with AFLP fragments for each primer combination separately from all studied individuals and analysed in BIONUMERICS, version 3.6 (Applied Maths). Files were examined for strong, clearly defined bands. Each band was scored across all individuals as either present or absent. The genotyping error rate calculated according to Bonin et al. (2004) was 3.1 %.

Data analysis

In the AFLP data matrix, the presence of a band was scored as 1, whereas the absence of a band was coded as 0. The resulting binary (0/1) data matrix represented all scored AFLP markers with sizes between 60 and 460 bp. Bands that were not perfectly reproducible between replicates were eliminated from the matrix.

To quantify genetic variation, we calculated the percentage of within-population polymorphic bands (%PB), Nei's unbiased expected Gene Diversity (GD) assuming Hardy-Weinberg equilibrium and Shannon Index (I) for each population using the programme POPGENE version 1.32 (Yeh *et al.* 1997). Genetic variation within groups was estimated separately for each locus and averaged. In addition, the rarity of markers was evaluated by the frequency-down-weighted (DW) marker value (Schönswetter & Tribsch 2005). DW values were computed for each population and for each group by using the DW function in the R-script AFLPdat (Ehrich 2006). Linkage disequilibrium between AFLPs was tested by using the χ^2 test following Miyashita et al. (1999). We also calculated an AMOVA derived measure of genetic variation by calculating the population-wise AMOVA sums of squares divided by n-1 (Fischer & Matthies 1998) with the program GenAlEx V5 (Peakall & Smouse 2001).

To compare mean values of genetic variation, we separated the whole dataset into six groups according to different AFLP clusters revealed by a cluster analysis and carried out uni-factorial analyses (ANOVA, Post-hoc: LSD) by using SPSS 17.0. In a second approach, we assigned individuals to four geographical regions (Eastern, South-eastern, Central and Western Europe). To assess the relationship of GD and SSWP/n-1 with the geographic position (longitude) of each population, regression coefficients were calculated by SPSS 17.0.

Tab. 1 Geographic localization of 37 studied populations of *Scorzonera purpurea*. Containing information about estimated population sizes (Est. Pop size), sample sizes (n) and genetic variation within population measured as Nei's Gene Diversity (GD), Shannon Index (I), percentage of polymorphic bands per population (%PB), rarity value (DW), linkage disequilibrium (LD) and AMOVA sums of squares divided by n-1 diversity (SSWP/n-1).

No.	AFLP group	Country	Population	Region	Est. Pop size	Longitude (E)	Latitude (N)	n	GD	I	% PB	DW	LD	SSWP /n-1
1	**G6**	France	Aures (Au)	Languedoc-Roussillion	>1000	3°28'22"	44°12'18"	10	0.16	0.23	40.4	6.1	2.2	20.0
2	**G6**		Bondons (Bo)	Languedoc-Roussillion	>1000	3°36'53"	44°20'48"	10	0.17	0.25	43.0	6.3	2.8	21.7
3	**G6**		Barre (Ba)	Languedoc-Roussillion	>1000	2°49'35"	43°45'10"	10	0.14	0.20	35.6	6.2	1.8	17.3
4	**G2**	Italy	San Colombo (Sc)	Abruzzi	250	13°36'29"	42°21'10"	10	0.19	0.29	54.8	7.2	5.1	26.0
5	**G3**	Southern	Lechfeld (Le)	Swabia	100	10°52'18"	48°12'08"	10	0.19	0.28	51.9	6.6	3.8	26.7
6	**G3**	Germany	Garchinger Heide (Gh)	Upper Bavaria	100	11°37'45"	48°15'58"	10	0.16	0.24	43.0	6.3	2.4	22.6
7	**G3**		Rosenau 1 (Ro1)	Lower Bavaria	50	12°34'22"	48°39'31"	10	0.15	0.22	40.0	6.3	2.1	20.4
8	**G3**		Rosenau 2 (Ro2)	Lower Bavaria	50	12°34'42"	48°39'43"	10	0.15	0.22	38.5	6.2	2.5	19.8
9	**G5**		Külsheim (Kü)	Middle Franconia	60	10°24'51"	49°31'28"	10	0.18	0.26	47.0	6.4	3.6	23.7
10	**G5**		Siebenbuckel (Si)	Middle Franconia	10	10°21'27"	49°35'07"	5	0.10	0.15	26.7	6.4	1.3	17.4
11	**G5**	Central	Kyffhäuser 1 (Ky1)	Thuringia		11°05'56"	51°22'01"	10	0.19	0.28	51.5	6.8	3.2	25.2
12	**G5**	Germany	Kyffhäuser 2 (Ky2)	Thuringia		11°02'45"	51°22'30"	10	0.16	0.23	42.6	6.9	2.0	21.9
13	**G5**	Northeastern	Krugberg (Kr)	Oder valley	60	14°22'59"	52°33'29"	10	0.17	0.26	46.7	7.0	2.7	23.4
14	**G5**	Germany	Eichelberg (Ei)	Havelland	220	12°46'27"	52°26'42"	10	0.15	0.22	40.7	6.9	2.4	20.0
15	**G5**		Mallnow (Ma)	Oder valley	2100	14°28'55"	52°27'52"	10	0.18	0.27	48.2	7.1	2.8	23.6
16	**G5**		Priesterschlucht (Pr)	Oder valley	64	14°32'31"	52°28'57"	10	0.17	0.25	45.9	6.8	3.3	23.2
17	**G5**		Krielower Berg (Kb)	Havelland	30	12°49'54"	52°24'37"	10	0.16	0.24	44.4	7.0	2.2	23.4
18	**G5**	Austria	Mödling 1 (Mö1)	Lower Austria		16°17'02"	48°05'18"	10	0.19	0.27	48.9	6.5	4.3	24.8
19	**G5**		Mödling 2 (Mö2)	Lower Austria		16°17'02"	48°05'18"	10	0.17	0.24	45.6	6.3	3.0	22.1
20	**G5**		Hainburg (Ha)	Lower Austria		16°56'50"	48°07'29"	9	0.16	0.24	43.0	6.4	3.1	22.3
21	**G5**	Czech Republic	Srbsko (Sr)	Stredocesky		14°08'26"	49°56'41"	10	0.16	0.23	43.0	6.7	2.2	22.8
22	**G5**	Slovakia	Spisske Podhradie (Sp)	Presov Kraij	100	20°46'10"	48°59'45"	10	0.17	0.25	46.3	6.7	2.9	23.4
23	**G5**		Devinska kobyla (Dk)	Bratislavski Kraij	500-1000	16°59'10"	48°10'50"	10	0.18	0.26	44.8	6.5	3.1	23.4
24	**G4**	Poland	Gasawka (Ga)	Gasawka river valley	< 20	17°42'02"	52°59'11"	4	0.13	0.19	33.0	6.0	2.6	21.3
25	**G4**		Starogrod (St)	Lower Vistula valley	< 20	18°23'02"	53°18'19"	8	0.13	0.19	33.7	6.1	1.1	18.9
26	**G1**	Hungary	Domony-Valley (Dv)	Hajdú-Bihar		19°23'30"	47°37'34"	10	0.20	0.29	54.1	7.0	3.8	27.8
27	**G1**		Vértes-Mountains (Vm)	Pest		18°26'03"	47°23'55"	10	0.21	0.31	56.7	6.7	4.5	29.5
28	**G2**	Romania	Dumbrava (Du)	Salaj		23°13'43"	46°49'28"	10	0.19	0.29	54.1	7.2	3.8	26.7
29	**G2**		Feleacu (Fe)	Cluj		23°36'11"	46°41'51"	10	0.18	0.27	51.1	6.5	3.0	27.2
30	**G2**		Hoia (Ho)	Cluj		23°31'37"	46°46'12"	5	0.15	0.23	40.4	5.8	2.5	27.4
31	**G2**	Ukraine	Zolochiv (Zo)	L´vivs´ka oblast		24°43'40"	49°48'48"	10	0.20	0.30	54.4	6.4	3.9	27.2
32	**G2**		Rohatyn (Rh)	Ivano-Frankivs´ka olast		24°40'26"	49°24'05"	10	0.18	0.27	51.5	6.7	4.4	24.6
33	**G1**	Russia	Voronezh (Vo)	Voronezh		39°11'55"	51°39'38"	10	0.21	0.30	56.3	6.7	3.7	30.0
34	**G1**		Krutoje (Kt)	Lipetskaya Oblast		38°59'18"	52°39'15"	9	0.20	0.29	49.6	6.4	5.2	26.2
35	**G1**		Nugush (Nu)	Bashkortostan		56°22'54"	53°01'25"	10	0.20	0.29	51.5	6.6	3.7	25.9
36	**G1**		Aytuar 1 (Ay1)	Orenburg		57°39'00"	51°09'15"	8	0.17	0.25	43.7	6.6	1.8	23.8
37	**G1**		Aytuar 2 (Ay2)	Orenburg		57°42'11"	51°03'24"	10	0.18	0.27	47.4	6.6	4.1	23.6
mean									**0.17**	**0.25**	**45.7**	**6.6**	**3.1**	**23.7**
SE									**0.00**	**0.01**	**1.14**	**0.1**	**0.2**	**0.51**

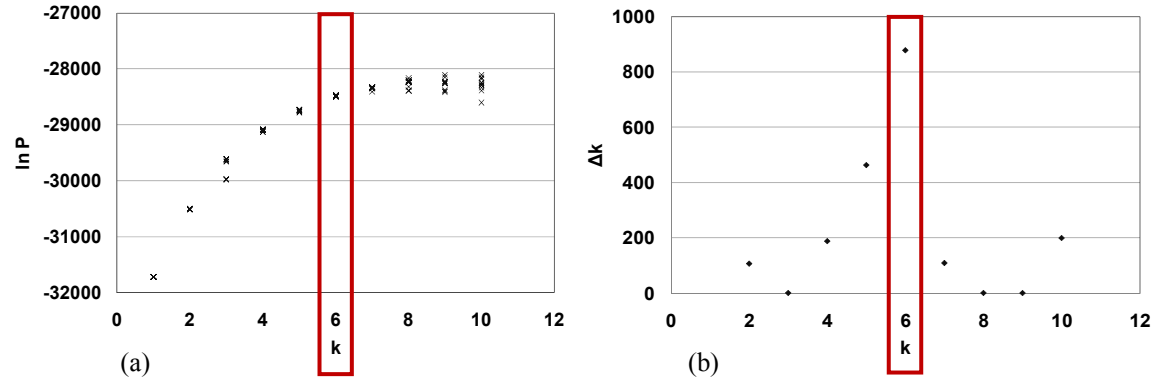


Fig. 4 Results of structure analysis based on amplified fragment length polymorphism data for *S. purpurea*. (a) Likelihood of each k for each run. (b) Calculation of Δk according to Evanno et al. (2005).

Genetic structuring and group assignment were investigated with Bayesian clustering in STRUCTURE, version 2.2 (Pritchard et al. 2007). STRUCTURE performs model-based clustering, based on Bayesian Markov chain Monte Carlo parameters. This program enables the identification of the number of genetic groups (k) and the assignment of individuals to these genetic groups, which is based on allele frequencies at each locus. The following settings were used: no-admixture and uncorrelated allele frequencies models with the parameters k from 2 to 38, ten replicate runs for each k, a burn-in period of 10^4 and 10^4 iterations (Fig. 4). The most likely number of k present in the dataset was calculated by using Δk according to Evanno et al. (2005). To assess the genetic pattern in higher dimensional space, a Principal Coordinates Analysis (PCoA) based on Bray-Curtis similarities was implemented in MVSP version 3.12f (Kovach 1999). To explore genetic relatedness among populations, we constructed a majority rule (50 %) consensus UPGMA tree of 1000 bootstrap replicates using the program FAMD 1.08 (Schlüter & Harris 2006). The UPGMA tree based upon a chord distance matrix (single-locus chord distance; Cavalli-Sforza 1967) calculated from allele frequency data (estimated in a Bayesian framework with a non-uniform prior derived from among-locus information; Zhivotovsky 1999).

Genetic variation between populations was quantified by an analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) using the program GENALEX version 5 (Peakall & Smouse 2001). AMOVA allows the calculation of variance components and their significance level for variation among groups of populations (regions), among populations within groups and within populations. In this study, populations were initially assigned to two different groups based on their geographic location in the most western part (France) and the central

and eastern part (all other populations) of Europe. Furthermore, we calculated an AMOVA for three geographic regions containing the eastern group (Russia, Ukraine, Romania and Hungary), the central group (Slovakia, Czech Republic, Poland, Austria and Germany) and the western group (France). A third AMOVA was calculated for six different UPGMA groups. To assess the migration pattern of *S. purpurea* into Central Europe we analysed the small-scale pattern of differentiation in Germany by using a reduced data-set. Isolation by distance was assessed with a Mantel test, which correlates the matrix of pair-wise genetic distances (Φ_{PT}) taken from the AMOVA among populations and the matrix of geographical distances (km) among populations (Mantel 1967). Significance tests were based on 999 permutations.

Results

Three AFLP primer combinations revealed 268 clear and reproducible fragments in *S. purpurea*. All detected fragments were polymorphic. The length of the analysed fragments ranged from 60 to 460 bp and the number of fragments per population varied between 169.0 (SD = 10.3) and 204.0 (SD = 16.3). The three primer combinations distinguished all individuals as separate genotypes.

Genetic structuring analysis performed with STRUCTURE identified six genetic groups as being most likely. For k = 6 ten replicated runs showed the highest similarity and a high likelihood value (Fig. 4). In Fig 5 locations of studied populations and their characterization by six different genetic lineages are given. Each population and each individual within populations could be classified by one color or a combination of several different colors symbolizing different genetic lineages. The most heterogeneous populations with many different color combinations were the Hungarian and one of the Slovakian populations (No. 22, 26, 27). They contained various frequencies of five different genetic lineages. All other populations were characterized by one main color derived from one of these six genetic lineages and only few or no concomitant colors. The red color was typical for all Russian populations, the dark blue color for Polish, Ukrainian and partly Romanian populations and the turquoise color for Romanian and Italian populations. The largest genetic group revealed by STRUCTURE contained most populations from Central Europe (Slovakia, Czech Republic, Austria, North-Eastern and Central Germany) and was characterized by a yellow group color. The main color for Bavarian populations (No. 5, 6, 7, 8, 9, 10) was green. French

populations were the most homogeneous group and consisted of only one genetic lineage (pink color), which was characteristic for this region and could not be observed in any other population of the studied distribution range.

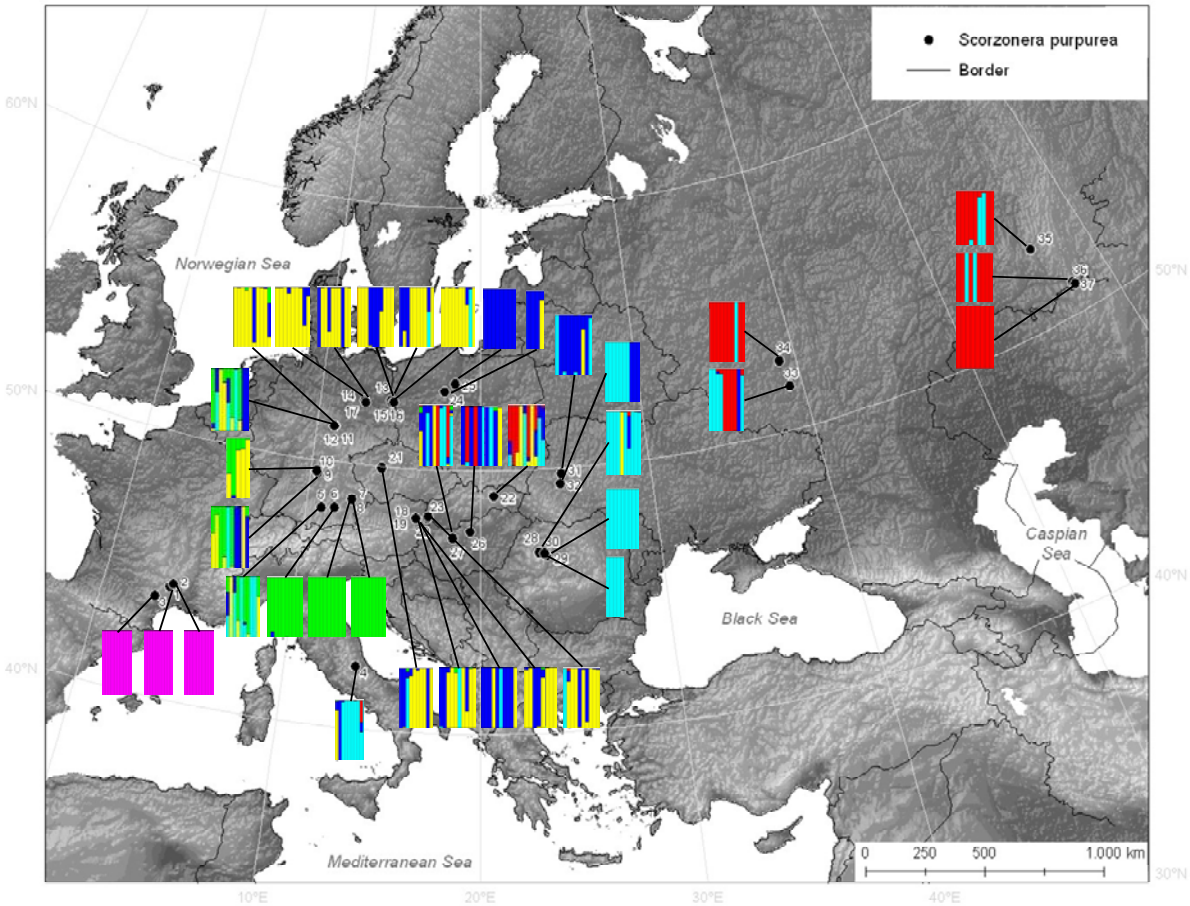


Fig. 5 Geographic distribution and genetic lineages of 37 analysed populations of *Scorzonera purpurea* across Eurasia. Bar plots indicate the proportional assignment of each individual to six different genetic clusters as detected by a STRUCTURE analysis of AFLP data.

The UPGMA dendrogram mostly supported the results derived from STRUCTURE and reflected also certain geographic relationships (Fig. 6). All populations could be assigned to four main geographic groups consisting of the eastern group with all populations from Russia and Hungary (G1), the south-eastern group containing populations from Ukraine and Romania accompanied by the population from Italy (G2), the central group containing populations from Slovakia, Czech Republic, Austria, Poland and Germany, and the western group containing the populations from France (G6). Furthermore, the central part was divided into three smaller subgroups: G3 including samples from Bavaria/Southern Germany, G4

including samples from Poland and finally G5 contains samples from Slovakia, Czech Republic, Austria, Central and North-eastern Germany.

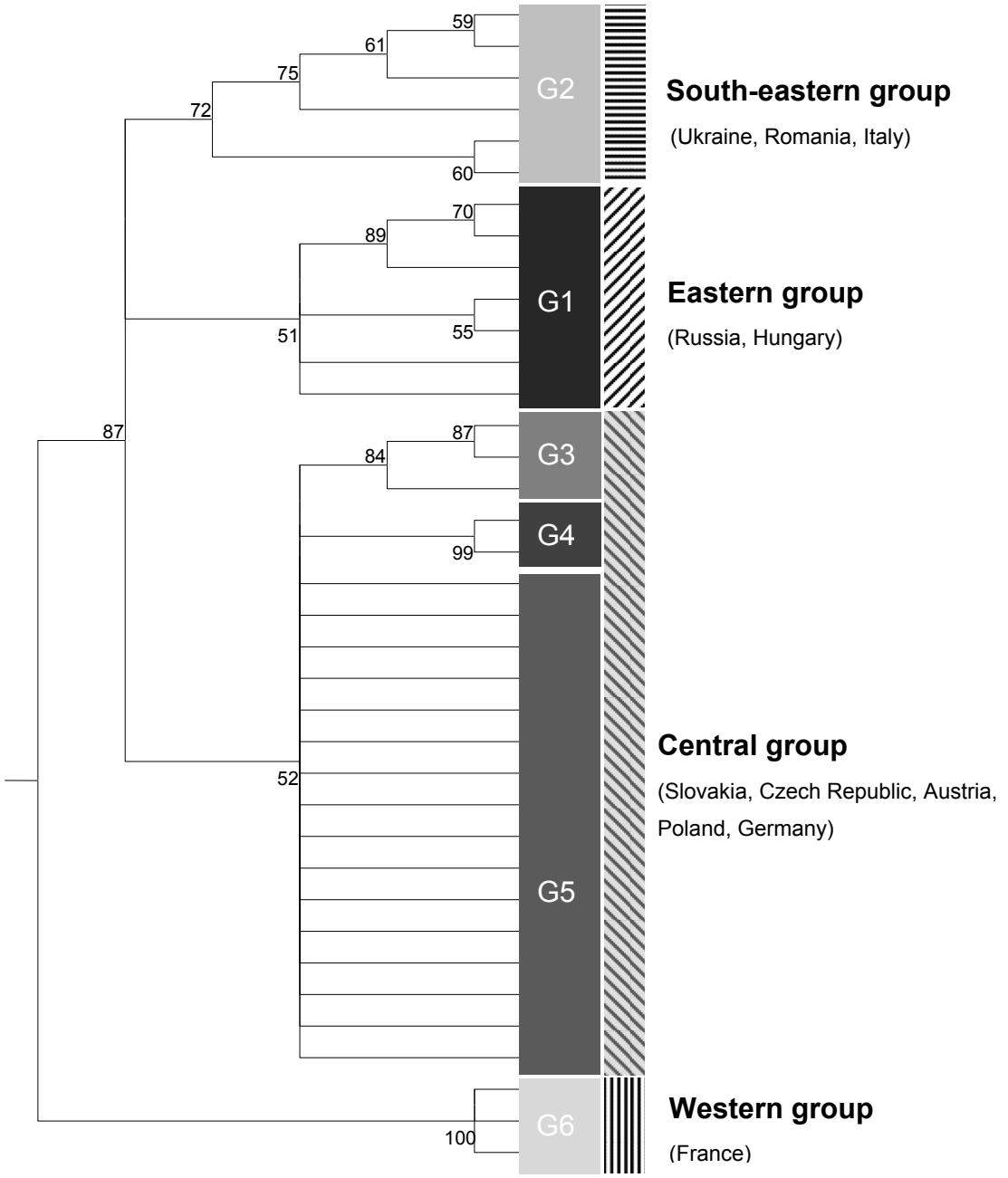


Fig. 6 UPGMA dendrogram of 37 populations of *S. purpurea* based on 268 amplified fragment length polymorphism marker. Population distances were calculated by using single-locus chord distance by Cavalli-Sforza & Edwards (1967). Bootstrap values based on 1000 permutations are indicated at each node.

Ordination of all individuals by using Principle Coordinates Analysis (PCoA) detected 26.6 % of the overall genetic variation between populations explained by the first three ordination axes. A scatter plot of axis two and three (cumulative genetic variance: 14.1 %) revealed a

distinct separation of the French populations (G6) from all Russian and Hungarian populations (G1) along the vertical axis (6.7 %). All other individuals assigned to UPGMA groups (G2, G3, G4, G5) built a continuous cluster located mostly in the centre and right part of the ordination diagram (Fig. 7). Populations from group G1 to group G5 were arranged along the horizontal axis and reflected the geographic east-west orientation of populations.

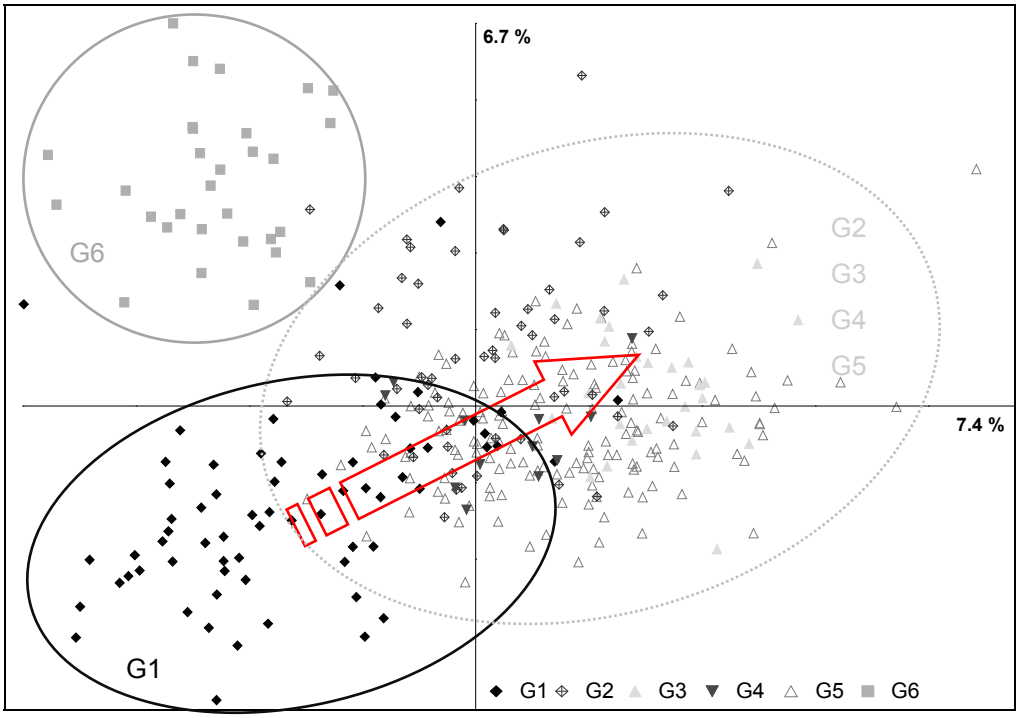


Fig. 7 Principle Coordinates Analysis (PCoA) based on AFLP data of 348 sampled *S. purpurea* individuals. A red arrow reflects an existing geographic east-west gradient. Different symbols represent six genetic groups detected by Neighbour Joining dendrogram. G1 = Russian + Hungarian populations, G2 = Ukrainian, Romanian and Italian populations, G3 = Bavarian populations, G4 = Polish populations, G5 = Central German, Austrian, Czech, Slovakian populations, G6 French populations.

The overall genetic variation among populations revealed by AMOVA was 18.9 %. Best resolution for genetic variation among regions or groups of populations could be revealed by partitioning the populations into two geographical subgroups containing the French populations on the one hand and all other populations on the other hand (Φ_{RT} = 15.3 %). Additional AMOVAs could not enhance the among-region variance (Tab. 2). By separating all populations into six genetic groups according to the UPGMA tree (Fig. 6; G1 to G6), only 12.0 % of variation among regions could be achieved. Using other grouping variants, e.g. according to their geographical location in Eastern, Central or Western Europe, the variation

among regions was less high than between populations within regions (Φ_{RT} = 10.0 % among regions, Φ_{PR} = 12.4 % among populations within regions).

To verify the hypothesis of two different migration routes into Central Europe, we tested genetic variation among groups of populations within Germany. One migration route might have lead from Pannonia via the Moravian Gap into the northern parts of Germany and one route might have followed the river Danube into Southern Germany. According to these hypotheses, we pre-defined three groups of populations and compared genetic variation between them (Tab. 2). One group comprised populations from Bavaria (Le, Gh, Ro1, Ro2, Kü, Si), one group populations from Thuringia (Ky1, Ky2) and one group populations from Brandenburg (Kr, Ei, Ma, Pr, Kb). Genetic variation between populations from Brandenburg and Bavaria was higher (Φ_{RT} = 8.05 %) than between Brandenburg and Thuringia (Φ_{RT} = 5.25 %). Genetic variation between populations of Bavaria and Thuringia was on a low level (Φ_{RT} = 2.15 %).

Tab. 2 Analysis of molecular variance (AMOVA) for AFLP phenotypes in 37 populations of *S. purpurea* across Europe. df: degrees of freedom, SS: Sums of squares, %: percentage of total variance, Φ_{PT}: genetic differentiation.

Source of variation	d.f.	SS	Variance components	%	*Φ_{PT}
Global analysis					
Among populations total	36	2723.65	5.52	18.86%	0.19
Within populations	311	7389.50	23.76	81.14%	
Regional analysis					
France ↔ all other populations					
Among regions	1	352.27	5.14	15.30%	0.29
Among populations within regions	35	2371.38	4.69	13.96%	
Within populations	311	7389.50	23.76	70.74%	
Six NJ groups					
Among regions	5	1166.65	3.64	12.04%	0.21
Among populations within regions	31	1557.00	2.81	9.29%	
Within populations	311	7389.50	23.76	78.67%	
Geographical regions (eastern, central, western)					
Among regions	2	709.01	3.06	9.98%	0.10
Among populations within regions	34	2014.64	3.79	12.37%	
Within populations	311	7389.50	23.76	77.64%	
Local analysis in Germany					
Bavaria + Thuringia + Brandenburg					
Among populations total	12	721.09	3.90	14.71%	0.15
Within populations	112	2533.60	22.62	85.29%	
Bavaria ↔ Brandenburg					
Among regions	1	166.00	2.21	8.05%	0.18
Among populations within regions	9	443.90	2.83	10.30%	
Within populations	94	2110.00	22.45	81.66%	
Bavaria ↔ Thuringia					
Among regions	1	71.10	0.57	2.15%	0.14
Among populations within regions	6	314.38	3.23	12.26%	
Within populations	67	1511.00	22.55	85.59%	
Thuringia ↔ Brandenburg					
Among regions	1	84.79	1.39	5.25%	0.14
Among populations within regions	5	224.72	2.20	8.28%	
Within populations	63	1446.20	22.96	86.46%	

* All p-values were <0.001

A Mantel test calculated on the basis of pair-wise Φ_{PT} values and geographic distances revealed a regression coefficient R_M of 0.419 ($p = 0.002$) for the total data set. Therefore genetic distances increased with geographical distances and isolation by distance could be assumed. Using a reduced data set without the French population, correlation of genetic and geographic distances could also be observed ($R_M = 0.420$; $p = 0.001$).

Genetic variation within populations was expressed by percentage of polymorphic loci (%PB), Nei's Gene Diversity (GD), Shannon Index (I) and AMOVA-SS diversities (Tab. 1). Proportion of polymorphic loci varied between 26.7 % and 56.7 % (mean 45.7 %, SE 1.1). GD ranged from 0.10 and 0.21 (mean 0.17, SE 0.004) and I between 0.15 and 0.31 (mean 0.25, SE 0.01). In all three cases, the most diverse population was Vertès in Hungary. Other diverse populations were Voronezh/Ru, San Colombo/It and Zolochiv/Uk. Among the least diverse populations were Siebenbuckel/Ger, Gasawka/Pl and Starogrod/Pl, which had all very low sample sizes. Population size-corrected SSWP/n-1 values detected Voronezh/Ru (30.0) as the most diverse population, followed by the Hungarian populations Vertes/Hu (29.5) and Domony/Hu (27.8). Among the least diverse populations were Barre/Fr (17.3), Siebenbuckel/Ger (17.4) and Starogrod/Pl (18.9).

Tab. 3 Differences in genetic variation within populations of *Scorzonera purpurea* assigned to main UPGMA regions (eastern, south-eastern, central and western) detected by a one-factor ANOVA. F = test statistic, df = degrees of freedom, p = level of significance.

Genetic variation	F	df	p
SSWP/n-1	14.2	3	0.000
GD	6.9	3	0.001
I	6.8	3	0.001
%PB	6.6	3	0.001
DW	1.5	3	n.s.
LD	5.9	3	0.002

ANOVA detected significant differences in genetic variation between four main UPGMA groups (eastern, south-eastern, central and western group; Tab. 3). Mean percentage of polymorphic loci, Nei's Gene Diversity and Shannon Diversity were significantly higher within the eastern and south-eastern populations than in the central and western populations. Population size-corrected SSWP values differed significantly from each other, even in central and western population (Fig. 8). No significant differences could be detected for DW values.

Linkage disequilibrium as a sign for strong bottlenecks in the past, ranged between 1.3 (Si/Ger) and 5.2 (Kt/Ru) and were highest in the south-eastern and eastern subgroups.

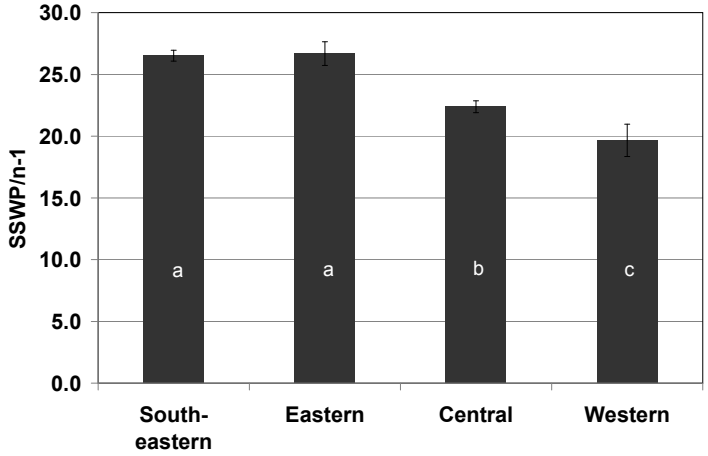


Fig. 8 Mean SSWP/n-1 diversity values and standard error of 37 *Scorzonera purpurea* populations grouped according to four main groups detected by UPGMA dendrogram. One-factor ANOVA tested for differences among groups. Different letters indicate significant differences between groups resulting from pair wise comparisons (LSD-test, $p<0.05$).

The regression between longitude and Nei's Gene Diversity as well as SSWP/n-1 value revealed statistically significant values. A cubic curve estimation showed the highest regression coefficients with $r = 0.497$ for GD and $r = 0.658$ for SSWP/n-1 ($p = 0.024$ and $p = 0.000$). The region with highest genetic variation within populations across Europe lies between the 19th and 40th degree of longitude (Fig. 9).

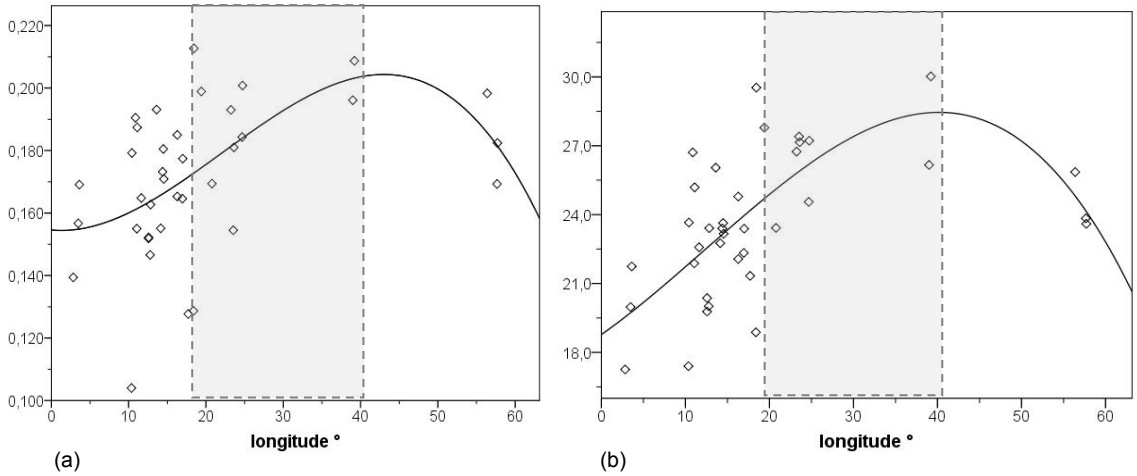


Fig. 9 Regression analysis of degree of longitude and genetic variation of 37 populations of *S. purpurea* across Europe. (a) Nei's Gene Diversity ($r = 0.497$, $p = 0.024$) and (b) SSWP/n-1 ($r = 0.658$, $p = 0.000$). Grey areas symbolize the most divers region between the 19th and 40th degree of longitude regarding genetic variation of *S. purpurea*.

Discussion

Within the present study, phylogeographical analyses gave evidence for a fluctuating colonization history of the steppe plant *Scorzonera purpurea* in Europe. Different genetic parameters indicated a medium level of genetic variation within populations (e.g. %PB: 26.7-56.7 %, mean 45.7 %). Genetic variation among different geographical regions was also on a moderate level ($\Phi_{RT} = 15.3$ % for grouping all French populations against all other populations). The relatively low genetic distances among populations were positively correlated with geographical distances between them supporting the isolation-by-distance model. Level of DW values was comparable all across Europe. PCoA ordination and UPGMA revealed a strong separation of the French populations from all other populations. The most diverse region according to the genetic variation within populations could be detected for the region between 19th and 40th degree of longitude comprising populations in Hungary, Slovakia, Romania, Ukraine and Western Russia.

Distribution of genetic variation within populations and evidence for potential refugia

Distribution pattern of genetic variation within populations across the geographical range of *S. purpurea* gave indication for existence of potential refugial areas during the Last Glacial Maximum (LGM) in Europe. Theory predicts that refugial areas were sources for subsequent recolonization and harbour higher levels of within-population genetic variation than their descendant populations (Hewitt 1996; Comes & Kadereit 1998). With increasing distance from refugial areas a reduction in genetic variation within populations is to be expected due to repeated bottlenecks at the leading edge during postglacial expansion (Hewitt 1996). Several studies with different plant species, especially from the Alps, and different molecular marker affirmed this hypothesis and revealed potential refugial areas in Europe: Van Dijk & Bakx-Schotman for *Plantago media* (cpDNA; 1997), King & Ferris for *Alnus glutinosa* (cpDNA; 1998), Magri et al. for *Fagus sylvatica* (AFLP; 2006), Ronikier *et al.* for *Campanula alpina* (AFLP; 2008) and Bylebyl *et al.* for *Eryngium campestre* (AFLP; 2008). For *S. purpurea* in Europe, highest level of genetic variation within populations could be found for Hungarian and some western Russian populations. Therefore, the region between 19th and 40th degree of longitude could be characterized as the most diverse, a fact which suggests the proximity of glacial refugia in this region. Cubic curve estimations indicated reduced levels of genetic variation both west- and eastwards from this region for correlations of genetic variation

within populations and longitudes. This is in accordance to results of other studies with dry grassland species, which presume southern glacial refugia near the Balkans, the Caucasus and the Caspian Sea (Hewitt 1999; Ronikier *et al.* 2008; Wróblewska 2008). Populations expanded from these regions to northern, western and southern Europe before post-glacial climate warming, which enhanced the rapid advance of tree vegetation throughout Europe (Taberlet *et al.* 1998; Hewitt 1999; Schmitt & Seitz 2001).

For *S. purpurea* analysis of genetic variation within populations revealed medium levels of variation throughout the distribution range in Europe, which covers more than 4000 km between the most distant populations. Medium levels of genetic variation probably result from bottlenecks in the past and *S. purpurea*'s young recolonization history in Europe. Migrations and range expansions may have lead to a loss of genetic variation and low-frequency fragments (Konnert & Bergmann 1995). The fact that all populations are genetically depaupered is confirmed by the low number or lack of unique bands in *S. purpurea* populations, which are characteristic for young (recently established) rather than older populations (Wróblewska 2008). Hence, the vast areas of tundra and cold steppe in Central Europe during quaternary glaciation phases seem to have been improper for the survival of the steppe plant *S. purpurea* and today's populations in Central Europe are the result of post-glacial recolonization events. Several plant species in Central Europe were subjected to strong population size decreases or even total glacial extinctions in that region. Thermophile tree species, for example, have used micro-environmentally suitable habitats in the south for their survival during glaciation, e.g. the Near East and the three southern peninsulas of Europe (Balkan, Italian and Iberian), and started their re-immigration after post-glacial climate warming (Huntely & Birks 1983; Bennett *et al.* 1991). For open grassland species, potential refugial areas may have been located in parts of southern Europe exhibiting suitable steppe-like conditions, especially in south-western France, Italy, the Balkans and in the south of the Black Sea (Adams & Faure 1997).

Another possible reason for low level of genetic variation could be the result of random genetic drift increasing in small and declining populations (Nei *et al.* 1975; Barrett & Kohn 1991). Although *S. purpurea* is a perennial, outcrossing Asteraceae, genetic variation within populations is located on a moderate level, not typical for plant species representing similar life history traits (Hamrick & Godt 1989). Even some endangered plant species with similar geographical range, such as *Dictamnus albus* or *Pulsatilla vulgaris*, both long-lived perennial

and mainly outcrossing plant species (Hensen & Oberprieler 2005; Hensen *et al.* 2005), showed considerable higher amounts of genetic variation at population level than the analysed steppe plant. While in some studies population sizes were positively correlated with levels of genetic variation within population, no significant correlation could be found for 19 populations of *S. purpurea*, for which data of estimated population sizes were available (Spearman's rho: r = 0.217, N = 19, p = 0.373). This is in accordance to other studies with small and fragmented populations, which also could not detect any correlation between population size and genetic variation (Kahmen & Poschlod 2000; Hensen *et al.* 2009). Due to the long and heterogeneous history of most plant species in Europe (including range fragmentation, refugial isolation, molecular divergence, recolonization and range expansions; Comes & Kadereit 1998), generalizations of correlations between current population sizes and genetic variations may hardly be predicted (Lönn & Prentice 1995). Recurrence of population bottlenecks or founder events in the past of presently large populations may have lead to a loss of genetic variation (Dolan 1994). Otherwise, presently small populations may still show high genetic variation due to demographic inertia, that covers effects of recently fragmentation, especially in perennial plants.

Genetic differentiation and separation of *Scorzonera purpurea* during glaciations

Among the studied *S. purpurea* populations throughout Europe, we observed some genetic structuring, but no significant differences regarding the level of DW values. Both, the population based UPGMA tree as well as the PCoA ordination of all individuals showed a slight, but significant grouping of the French populations. All other samples in the ordination diagram largely overlapped and built a central cluster with a certain east-west orientation according to their geographic localization. Similar results could be revealed by the molecular variance analysis detecting the highest among-region differentiation for the separation of French populations and all other populations. No other predefined grouping could enhance the among-region differentiation.

According to these results, two main refugial areas can be assumed: one in south-western France, from where no major expansion occurred after the last glacial maximum, and a larger one within the south-eastern part of Europe, the Balkans or the region around the Black Sea. Populations from this south-eastern refugium seemed to have played a major role for the recolonization of whole Central and Eastern Europe and formed a closely related subgroup. The exposed position of French populations in our study supported by their low levels of

variation can be explained by processes caused during intense cooling phases in Europe during glaciations. The impact of climatic changes have hampered the environmental growing conditions for thermophile plants and forced the separation of primarily connected populations throughout Europe. During migrations through physical barriers, French populations may have suffered from the loss of variation and after the onset of climate warming these populations were not able to re-migrate into Central Europe. Similar colonization patterns could be revealed by Vrancken et al. (2009) for *Rhinanthus angustifolius*, a widespread European annual, that showed a strong geographic structure of its five AFLP groups, each being largely specific to a particular region of Europe.

Furthermore, theory predicts, that in long-term isolated glacial refugia, e.g. nunataks in the Alps, values of DW are expected to be high due to accumulation by mutations, whereas newly established populations are expected to exhibit low values (Schönswetter & Tribsch 2005). For *S. purpurea*, comparable levels of DW values across Europe give no clear indication for long-term survival in small and isolated habitats comparable to nunataks in the Alps. It is rather likely, that there have been suitable peripheral areas in the south-eastern part of Europe, huge enough to maintain a certain level of genetic variation without gaining high amounts of rare fragments. After postglacial climate warming open grasslands largely expanded and steppe plants spread rapidly throughout Europe. Due to this, relatedness of *S. purpurea* populations is extremely strong. The increasing isolation of our presently highly fragmented landscape, which largely restricts gene flow between populations and enhances differentiation between populations, has still not affected the genetic relationship among populations and represents a comparably realistic pattern of historical processes in *S. purpurea*.

Potential European expansion routes during postglacial warming

STRUCTURE analysis revealed six main genetic lineages throughout Europe to be the most likely. The most homogeneous geographical group consisted of the French populations containing only one genetic lineage and which emerged in no other of the studied populations. This can be an indication for early separation of the French populations from all others during Quaternary climate fluctuations. Strong glaciations of the Alps may have disrupted their connection to the eastern populations and due to a lack of gene flow, they genetically diverged. After the onset of post-glacial climate warming, these populations have not been able to leave their isolated refuge in the Massif Central, to cross geophysical barriers and to spread eastwards.

The most heterogeneous populations according to their composition of different genetic lineages were the Hungarian and one of the Slovakian populations. They comprised amounts of all other genotypes (except the French lineage) and seemed to be potential sources for the post-glacial recolonization of Central and Eastern Europe after the onset of warmer interglacial conditions from about 16 000 ^{14}C y. At this time, the ice sheets began to retreat and climate warming suddenly increased (Hewitt 1999). Across most of Europe, there was a change in herbaceous communities. Dry and cold-climate steppe-tundra changed towards steppe vegetation and open grasslands. European steppe plants spread from their glacial refugia near the Balkans, the Caucasus or the Caspian Sea north- and westwards (Pott 1995). This colonization pattern coincides with results of many other studies (Huntely & Birks 1983; Bennett *et al.* 1991; Comes & Kadereit 1998; Taberlet *et al.* 1998; Hewitt 1999). Trees had not enough time to spread back over Europe and therefore, an open steppe vegetation cover pre-dominated, affording suitable conditions for *S. purpurea* and a rapid large-scale expansion throughout European landscapes.

According to the results of STRUCTURE, main immigration routes may have started from a region within or next to the Pannonian Plains. From there, *S. purpurea* spread east- and westwards forming different genetic clusters. Localization of colonization origins is in accordance to results of other studies, which proposed postglacial expansion of steppe plants via Pannonia. Pannonia is a region, which has been associated to one of the most important south-eastern refugial areas during the Last Glacial Maximum, the Balkans. From Pannonia, two main immigration routes into Central Europe were presumed: one along lower Austria via the Danube valley into Southern Germany, the other one via Bohemia and the Elbe valley into central and eastern Germany. Bylebyl *et al.* (2008) also detected the Elbe valley as important migration route into central and western Germany. Geophysical barriers, such as the Carpathian Mountains could be transcended via natural gaps (Wroblewska *et al.* 2003). The most important passage between lower Austria and the Elbe valley is called the Moravian Gap. For *S. purpurea,* similar colonization routes can be assumed by means of different genetic clusters. Northern parts of Central Europe might have been colonized by individuals coming from Hungary via Slovakia, lower Austria, Czech Republic and the Moravian Gap into Silesia. From there, three genetic lineages might have formed the Polish and Eastern German populations along the Oder valley. Two of these genetic lineages could also be found within the Ukrainian populations indicating that immigration to north-western Ukraine probably took also place via the Moravian Gap. Central German regions were also colonized

by these lineages, but furthermore, by a genetic cluster originating from southern German populations. It is likely that two different colonization routes have met in Central Germany and have built contact zones near the region of Kyffhäuser (Fig. 10). The southern genetic lineage probably has immigrated from Hungary via lower Austria and the Danube Valley. From there, individuals spread further to southern Bavaria along few routes throughout the loess and calcareous territories along the rivers Isar and Lech. A second route might have been spreading to the northern Bavarian populations and the Central German populations. STRUCTURE results were also supported by AMOVA, which revealed similar levels of genetic variation between central and north-eastern populations and central and southern populations within Germany. According to this, we can assume a certain contact zone in the centre of Germany. If central populations could be assigned in main parts to the north-eastern or to the southern genetic lineage, AMOVA would have revealed no or only slight genetic variation among one of the studied groups.

Ambiguity consisted about the origin of the Russian genetic cluster. Most of the Russian populations comprised one characteristic lineage, which also can be found within the Hungarian and the Slovakian populations. Accordingly, colonization potentially occurred via the Southern Carpathian Mountains into the most eastern part of Europe. The possibility that the Russian genetic lineage resulted from a second glacial refuge, e.g. from regions near the Caspian Sea, and immigrated after climate warming into the Hungarian Plains, needs to be proved by future investigations with selective samples from that region.

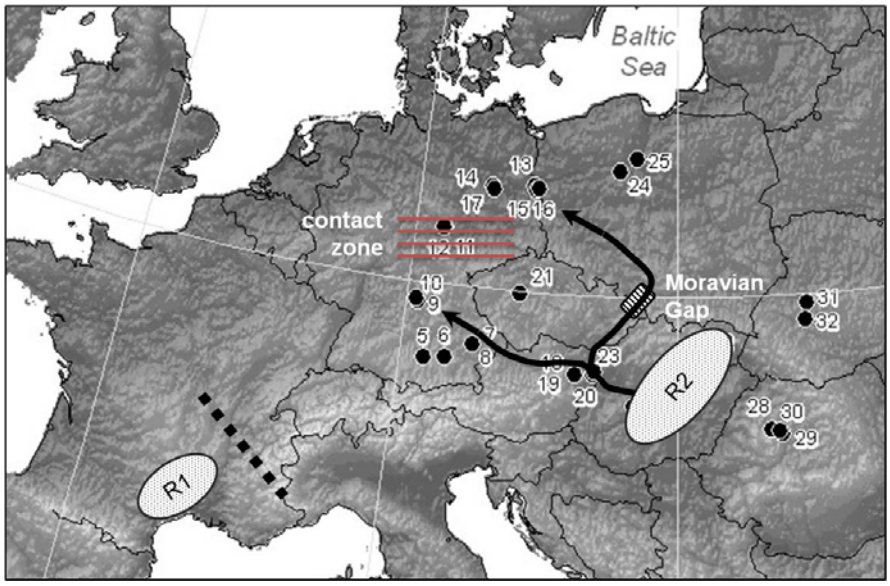


Fig. 10 Tentative location of refuge areas (R) of *S. purpurea* during last glacial maximum and main colonization routes into Germany during post-glacial warming.

Chapter 3

The Biological flora of Central Europe: *Scorzonera purpurea* L. (Asteraceae)

Abstract

Scorzonera purpurea is a perennial steppe plant native to Central Europe. Its distribution range stretches from Southern France via Central Europe up to the Western part of Siberia. In Central Europe, *S. purpurea* occurs in xerothermic plant communities of dry calcareous grasslands. Due to changes in agricultural farming practices and increasing field abandonment its habitats are strongly declining. Due to this, populations of *S. purpurea* become more and more threatened by extinction and deserve strong conservation efforts. This article reviews taxonomy, morphology, ecology and population genetics of *S. purpurea* as well as its conservation status in Germany.

Taxonomy and morphology

Taxonomy

The genus *Scorzonera* L. s.l. (*Asteraceae*, *Lactuceae*) comprises about 160 species and is widely spread in arid regions of Eurasia and Africa (Owen *et al.* 2006). Milky latex and floral structures are characteristic for the tribe Lactuceae and make it easily distinguishable from all other Asteraceae (Krak & Mráz 2008). Due to the high morphological variability taxonomic treatment of the genus is still contentious. According to Lipschitz (1935), *Scorzonera* s.l. consisted of two subgenera: *Euscorzonera* and *Podospermum*. Later he changed this view and differentiated three subgenera: *Podospermum*, *Pseudopodospermum* and *Scorzonera* (Lipschitz 1964 in Mavrodiev *et al.*, 2004). In Flora Europaea the genus *Scorzonera* splits into three sections: *Podospermum*, *Scorzonera* and *Lasiospora*, while more recent analyses emphasized the polyphyletic status of *Scorzonera* s.l. and divided it into several taxa of independent generic status (Nazarova 1990, 1997; Owen *et al.*, 2006).

In the traditional taxonomic system of Flora Europaea (Tutin *et al.* 1964) *Scorzonera purpurea* L. belongs to the section *Scorzonera*, which includes biennial and perennial plant

species with entire to dentate leaves and glabrous achenes. In former times, *S. purpurea* has been placed into the genus *Podospermum* and has been described as *Podospermum purpureum* Koch & Steud. (Tab. 4). Candolle (1838) removed it from the genus *Podospermum* and placed it into the genus *Scorzonera* (Mavrodiev *et al.* 2004). Morphological attributes of *S. purpurea*, such as achenes with stout bases and the strong vanilla or chocolate odor of the flowers, are different to both genera, *Scorzonera* as well as *Podospermum*. Furthermore, the coloration of flowers is also characteristic. The ligules of *S. purpurea* are lilac, rose or purplish on both surfaces, while the ligules of other species of the genera *Podospermum* and *Scorzonera* are yellow inside and yellow, reddish, rose or purplish outside, but never lilac, rose or purplish on both surfaces (Chater 1976 in Mavrodiev *et al.*, 2004).

Tab. 4 Taxonomy, synonyms and common names of *S. purpurea*.

***Scorzonera purpurea* L.**	
Synonyms	
S. purpurea subsp. *eupurpurea* Herrm.	
Podospermum purpureum Koch ex Steud.	
Names in other languages	
German - Violette Schwarzwurzel	Bulgarian - Розовоцветен кокеш
English - Purple Viper´s Grass	Czech - Hadí mord nachový
French - Scorzonère pourpre	Polish - Wężymord stepowy
Italian - Scorzonera porporina	Romanian - Скорцонерэ пурпурие
Albanian - Skorzonerë e purpurt	

Phylogenetic analyses in Scorzonerinae based on ITS sequence data revealed, that *Podospermum purpureum* (*Scorzonera purpurea*) is well removed from other *Scorzonera* species and weakly supported as sister species to *Podospermum* (Mavrodiev *et al.* 2004). Owen et al. (2006) stated in their molecular study of the genera *Scorzonera* L. and *Podospermum* (L.) DC, that *S. purpurea* may require independent treatment from both genera. The current taxonomic name of *S. purpurea* is *Podospermum purpureum* (L.) W. D. J. Koch & Ziz (Euro+Med 2006-).

Taxonomic uncertainties exist also on the level of subspecies. Flora Europaea reported on three subspecies of S. *purpurea*: subsp. *purpurea*, subsp. *rosea* and subsp. *peristerica*. *S. purpurea* subsp. *peristerica* occurs only in Greece and Macedonia (Baltisberger & Widmer

2009). Other taxonomists, such as Pignatti (1982) in the Flora d'Italia, gave *S. rosea* generic status. *S. rosea* differs from *S. purpurea* by broader, flat leaves, larger heads, greater number of involucral bracts and by the achene's ribs. *S. rosea* is a mountainous plant species and its habitats are alpine and subalpine meadows, lucid forests and limestone (Kumarov 2001). It is known from the Apennine (Emilia to Abruzzi), the South-Eastern Alps (Switzerland), the Carpathians and the mountains of the Balkan Peninsula (Hess *et al.* 1972).

Morphology

S. purpurea is a perennial plant species consisting of a vertical, less than 1 cm thick rootstock (Tutin *et al.* 1964). Its root-collar is densely clothed with numerous black-brown fibres of dead foliar petioles. The roots as well as all other parts of the plant contain yellow to orange colored, milky and sticky latex. Basal leaves are narrow-linear, gramineous and 1 to 3 mm wide. Up to 30 of them are arranged in a rosette. Leaves are entire margined, triangular-grooved and more or less erect. Leaves measure 10 to 25 cm of length and are glabrous or weakly arachnoid-pubescent. A single plant can form one to few flowering stalks at the same time. Flowering stem is erect, 15 to 45 cm (maximum up to 70 cm) tall and usually branched in the upper parts. It is endowed with five to seven sessile cauline leaves. Inflorescence consists of two to five solitary terminal capitula. Below the flower heads as well as at the bases of leaves arachnoid hair can be sporadically observed. The flower heads are of medium size (up to 2.5 cm long) and surrounded by 10 to 16 ovate, brown bordered involucral bracts. The outer bracts are oblong-cylindrical, almost glabrous or slightly pubescent. The inner bracts are more lanceolate, obtuse and overtop the outer ones about two times in length. Light purple ligulate florets, up to two times as long as the involucre, are very sensitive to weather conditions and close already in the late morning as well as in times of rain (Oberdorfer 2001). Flowers exude a slight vanilla smell (Hegi 1998). Achenes of *S. purpurea* are relatively large (up to 12 mm long), smooth and ribbed. A large dirty-white pappus consists of pinnate bristles and is as long as the achene.

Distribution and habitat requirements

Geographical Distribution

S. purpurea is a typical plant species of continental steppe regions. Its distribution range stretches from Central Europe, the northern and central parts of Italy as well as the Balkans up

to Western Siberia and Minor Asia (Fig. 11). It occurs in Western Himalaya, the Altay region, Kazakhstan, Southern, Eastern and Central European Russia, Ukraine, Romania, Albania, Hungary, Poland, Slovakia, Czech Republic and Austria. In Germany, last occurrences of *S. purpurea* represent the western border of its mostly continuous distribution range. More far in the west, large, but strongly isolated populations of *S. purpurea* can be found in the mountainous region of Southern France (Cevennes). The exposed position of these populations may be the result of glacial events during Quaternary and of species survival in distinct glacial refugia. Nowadays, populations from the main areal are genetically differentiated from the French populations (see chapter 2).

Throughout large parts of its distribution range population numbers and sizes are strongly declining and *S. purpurea* is listed in Red Data Books of most European countries (Schnittler & Günther 1999). In Germany, *S. purpurea* is known from Brandenburg, Berlin, Rhineland-Palatinate, Saxony-Anhalt, Thuringia and Bavaria. In Hesse, Lower Saxony and Mecklenburg-West Pomerania the species is already extinct. Less than 10 % of all global occurrences are located in Germany and therefore, the country has only a medium responsibility for plant's long-term survival (Welk 2002).

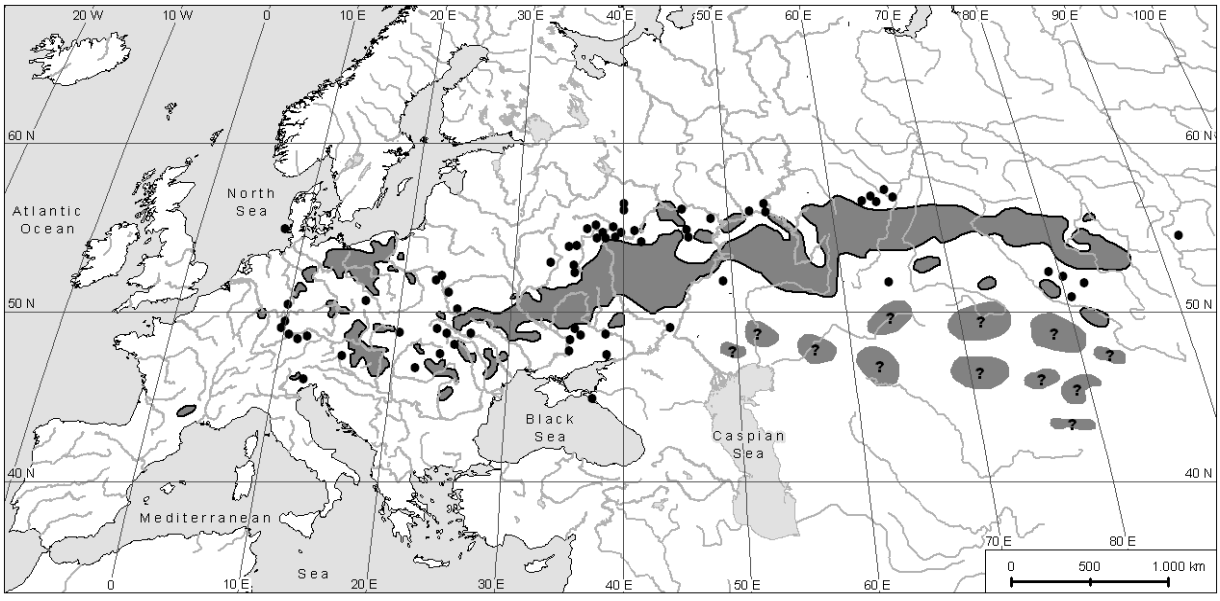


Fig. 11 Global distribution pattern of *S. purpurea*. Map is based on Meusel & Jäger (1992). Question marks symbolize vague geographical information about occurrences.

Habitat

In Central Europe *S. purpurea* typically occurs in open calcareous grasslands, which are characterized by dry and almost steppe like conditions. Most of these habitats are of semi-natural origin and harbour a high variety of plant species from different biomes. Habitats of *S. purpurea* outside Central Europe are steppes, flood plains, steppe meadows, sands and edges of steppe forests (Kumarov 2001). In the European part of the USSR *S. purpurea* is a typical plant species of species-rich steppes, while in *Stipa*-steppes the species occurs seldom (Lipschitz 1935).

In Germany *S. purpurea* occurs on different geological bedrock, primarily on limestone, porphyry and gypsum (Oberdorfer 2001). *S. purpurea* prefers full light sites with a low competitive vegetation structure and open safe sites for germination. In Germany most habitats of *S. purpurea* are of anthropogenic origin and strongly deserve a consequent management regime, such as mowing or grazing by sheep, to keep their traditional open vegetation structure.

Plant communities

S. purpurea is a diagnostic species of continental steppe grasslands (Adonido-Brachypodietum & Festucion valesiacae; Oberdorfer, 2001). In Germany it occurs predominantly in xerothermic and semi-dry grassland communities together with xerophilous species, such as *Anthericum liliago, Alyssum montanum, Biscutella laevigata, Chamaecytisus ratisbonensis, Linum perenne, Pulsatilla vulgaris, Adonis vernalis, Aster linosyris, Inula hirta, Gypsophila fastigiata* and *Jurinea cyanoides*. In *Stipa pennata*-steppes of the Pannonian region, *S. purpurea* is accompanied by *Lathyrus pannonicus, Linum flavum, Dictamnus albus, Tephroseris integrifolia*, etc. (Hegi 1998). In western Siberian steppe woods, *S. purpurea* is rather scarce and occurs together with *Libanotis montana, Delphinium elatum, Centaurea scabiosa, Tragopogon pratensis* and *Dracocephalum ruyschiana* (Hegi 1998).

Life cycle and biology

The following data were mainly obtained by our long-term permanent plot observations within four Bavarian populations as well as by greenhouse and laboratory studies.

Phenology, breeding system and development of seeds

S. purpurea starts to flower in early May with a main flowering period from mid till end of May. In some years, when springs are extremely warm and dry, flowering time can be shortened. *S. purpurea* is polycarpic and, depending on environmental conditions, it is able to flower each year. Due to their obligate xenogamous breeding system, plants are strongly dependent on pollinators, such as *Hymenoptera*, *Lepidoptera*, *Diptera* and *Coleoptera*. Revealed by a hand-pollination experiment, seed set of cross-pollinated plants contained 84.3 % of fertile seeds, while seed set of self-pollinated flower heads failed completely (Fig. 12). In contrast to the dark brown fertile seeds of cross-pollinated plants, infertile seeds were pale and contained no viable embryo. Fertile seeds showed high germination rates of 94.0 %. Individuals, which had the possibility to flower free in the open greenhouse, showed a reduced set of fertile seeds due to pollinator limitation.

Therefore, pollination limitation might be a severe risk factor for *S. purpurea*, especially in Central Europe, where populations are usually small and fragmented. In this case, populations might be less attractive to pollinators and might be visited less frequently (Rathcke & Jules 1993). Moreover, in small populations, the local density of plants is often reduced and pollen transfer between individuals is largely limited (Kunin 1997; Roll *et al.* 1997).

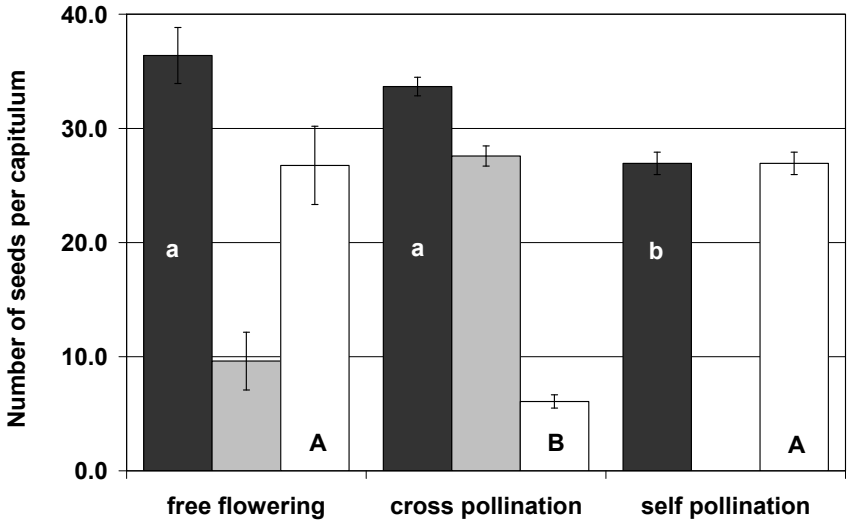


Fig. 12 Pollination experiment of *S. purpurea*. Black bars: total seed number, grey bars: number of fertile seeds, white bars: number of sterile seeds. Different letters show significant differences between bars of the same color. Comparison of means was conducted by non-parametric H- and U-tests.

Dissemination and dispersal

Each genet of *S. purpurea* is able to produce one to several flowering stalks each with one to five flower heads. The mean number of seeds per flower head ranged from 24.0 to 32.5 among the studied Bavarian populations. 67.3 to 77.3 % of all produced seeds per flower head contained a viable embryo, about one third of all seeds were sterile. Seed set is strongly dependent on weather conditions. In periods of persistent rainfall, both pollination as well as ripening of fruits could be limited and affected by the decay of the whole capitulum. Furthermore, inflorescences of *S. purpurea* are very exposed in dry grasslands during flowering time and are strongly affected by damages caused by wild game, such as hares and roe deer.

Dissemination of diaspores starts at mid June. Achenes consist of a large (c. 14.6 mm) bristle-pinnate pappus and a large, oblong seed (length: 11.1 mm, height: 0.9 mm, width: 1.1 mm). Fruits are often dispersed as a group of several. Due to their high seed weight (c. 5.3 mg, including pappus) dispersal is largely limited to the close vicinity of mother plants. The terminal velocity of a falling fruit is quite high and reaches 0.9 m s^{-1}, which largely prevents long-distance dispersal by wind. Only 1.6-3.2 % of the fruit-set might be able to reach a reference distance of 100 m (Tackenberg *et al.* 2003). This means, that an individual consisting of two flower heads à 25 achenes may only contribute 0.8-1.6 achenes to reach a distance of 100 m by using wind as dispersal vector. Long distance dispersal may only take place by the attachment of fruits to the fur of animals. Experiments showed that the epizoochorous dispersal capacity of achenes to sheep fur is at medium level. After 19 h 55.3 % of the applied fruits could still be found on the fur (Fig. 13). However, sheep pasturing is strongly declining in Central Europe and only in few cases large-scale grazing by transhumant sheep herds is applied as conservation management practice, such as in the calcareous grasslands of the lower Lech valley in Southern Germany. Furthermore, time of sheep grazing should match the time of fruit shedding to guarantee successful attachment of seeds to the fur. In most cases, long distance dispersal by sheep seems to be rather scarce and therefore, dispersal distances in *S. purpurea* may be limited to several few meters.

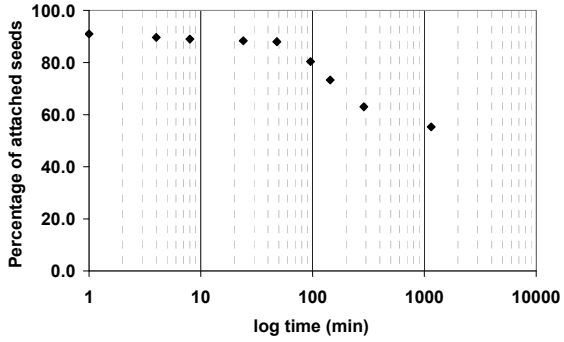


Fig. 13 Ectozoochorous dispersal capacity of *S. purpurea* seeds. Seeds were attached to sheep fur and treated by artificial sheep movements for 19 hours.

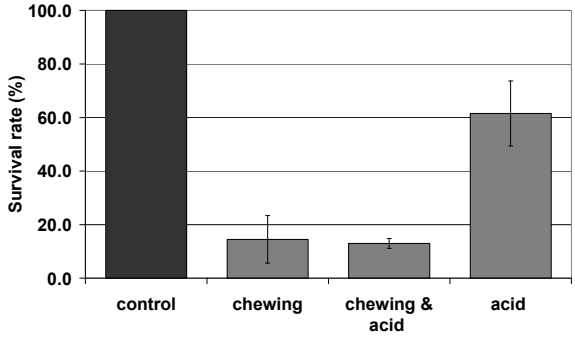


Fig. 14 Endozoochorous dispersal capacity of *S. purpurea* seeds. Chemical digestion was simulated by acid treatment; mechanical influences were simulated by artificial chewing (see Bonn 2005).

Endozoochorous dispersal of *S. purpurea* seeds by sheep seems to be ineffective as well. While in some plant species digestion of seeds by large herbivores enhances germination rates, *S. purpurea* showed extremely low survival rates of seeds after impact of internal processes. Experimentally simulated chewing and digestion (according to Bonn 2005) revealed a high sensitivity of seeds to mechanical damages by chewing (survival rate 14.5 %), while chemical damages by digestion were rather low (survival rate 61.5 %; Fig. 14).

Germination and soil seed bank persistence

Seeds of *S. purpurea* showed no kind of primary dormancy, which has to be broken by prolonged cold temperatures during winter. In some plant species cold stratification is an adaptation to unfavourable conditions in winter preventing high rates of seedling mortality (Schütz 2002). Seeds of *S. purpurea* are able to germinate directly after seed shedding facing several risk factors, such as droughts during summer and frost periods during winter. Germination takes place under both light and dark conditions, which prevents accumulation of seeds in deeper soil layers as a reservoir for unfavourable environmental conditions. This is in accordance to results revealed by analyses of the soil seed bank. Within two Bavarian populations of *S. purpurea* in the Lechfeld, we took 80 soil samples in dry calcareous grasslands. To assess the temporal persistence of seeds in the soil seed bank, we took samples from two depths (0-5 cm and 5-10 cm). According to the key of Thompson *et al*. (1997) three seed bank categories can be distinguished: transient (seeds surviving less than 1 year), short-term (seeds surviving 1-4 years) or long-term persistent (seeds surviving for > 4 years in the soil). No seeds of *S. purpurea* were found in the soil seed bank and therefore *S. purpurea* is

considered to have a transient seed bank. In the case of grassland restoration, the plant is not able to regenerate from soil reservoirs and has to be introduced by hand.

Furthermore, experiments showed comparable germination rates at fluctuating and constant temperature ranges (Fig. 15). Fluctuating temperature regimes represent amplitudes between day and night temperatures within sparse vegetation cover. Especially low competitive plant species, which deserve certain safe sites for germination, would profit by the recognition of gaps in dense vegetation structures (Thompson & Grime 1983). Within gaps temperature fluctuations among day and night are higher than within dense vegetation structures. However, seeds of *S. purpurea* germinate both under constant and fluctuating temperatures at comparable levels. They have no gap detection mechanism and therefore, they are not able to detect safe sites for germination within vegetation structures. Under natural conditions, mortality rate of seedlings is supposed to be high, because germination also takes place in unfavourable habitat structures.

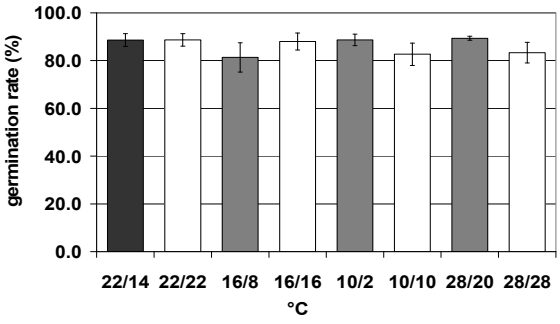


Fig. 15 Germination experiment of *S. purpurea* with different temperature regimes. No significant differences could be observed. Grey bars: fluctuating day/night temperatures, white bars: constant day/night temperatures.

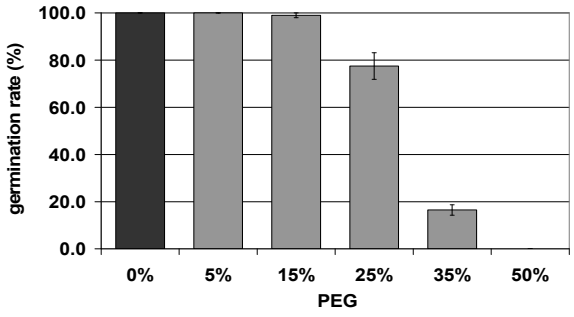


Fig. 16 Germination experiment of *S. purpurea* with different water potentials. PEG = polyethylene glycol; 5% PEG = -0.1 MPa, 15% PEG = -0.25 MPa, 25% PEG = -0.7 MPa, 35% PEG =-1.4 MPa, 50% PEG = -3.1 MPa.

Germination of *S. purpurea* is possible over a large range of temperature. Day temperatures from 10 to 28 °C resulted in comparatively high germination rates of 81.3 to 89.3 % enabling germination events during the whole growing season (Fig. 15). This might be deleterious for *S. purpurea* seedlings, especially when high temperatures in summer are accompanied by periods of insufficient precipitation. Regarding soil conditions, germination rates are highest on wet to humid-fresh soils tested under artificial conditions by using polyethylene glycol (PEG) to simulate different moisture conditions (Fig. 16). Germination usually started three

days after watering and has been completed after ten days. Under drier conditions germination phase was prolonged up to 30 days.

Response to abiotic factors, competition and management

Little is known by literature about habitat requirements of *S. purpurea* and its response to abiotic factors. Population biological analyses of four Bavarian populations revealed that the number of flower heads and the height of flowering stems varied among habitats and years indicating strong dependency on site and climatic specific relations. In habitats with higher contents of phosphorus and potassium, plants were significantly higher and produced significantly more flower heads. In spring hot and dry conditions significantly reduced the fitness of plants regarding the number of flowering heads and the height of flowering stems.

Interspecific competition

S. purpurea is a typical dry grassland plant species and well adapted to habitats, which are frequently managed by mowing or grazing. In case of damage a comparatively vigorous rootstock enables the formation of new shoots and flowering stalks. Its basal leaves are grass-like and arranged in a rosette. Due to this, plants are very sensitive to shading and high competitive stress by tall growing plant species. It prefers xerothermic grassland communities and disappears in communities dominated by tall grasses or in the case of field abandoning.

Analyses of habitat and vegetation characteristics in 63 permanent plots of four Bavarian populations of *S. purpurea* revealed specific requirements for seedling emergence and flowering capacity. We could detect high numbers of flowering individuals in plots with dense bryophyte layers as well as high proportions of plants with great affinity to light and temperature (Fig. 17a). Furthermore, plots with high contents of plant litter contained less flowering individuals of *S. purpurea*. In contrast, seedling emergence and seedling survival increased with percentage of bare ground. Furthermore, *S. purpurea* seedlings showed high sensitivity to moisture conditions and decreased with high proportions of thermophilous plant species (Fig. 17b).

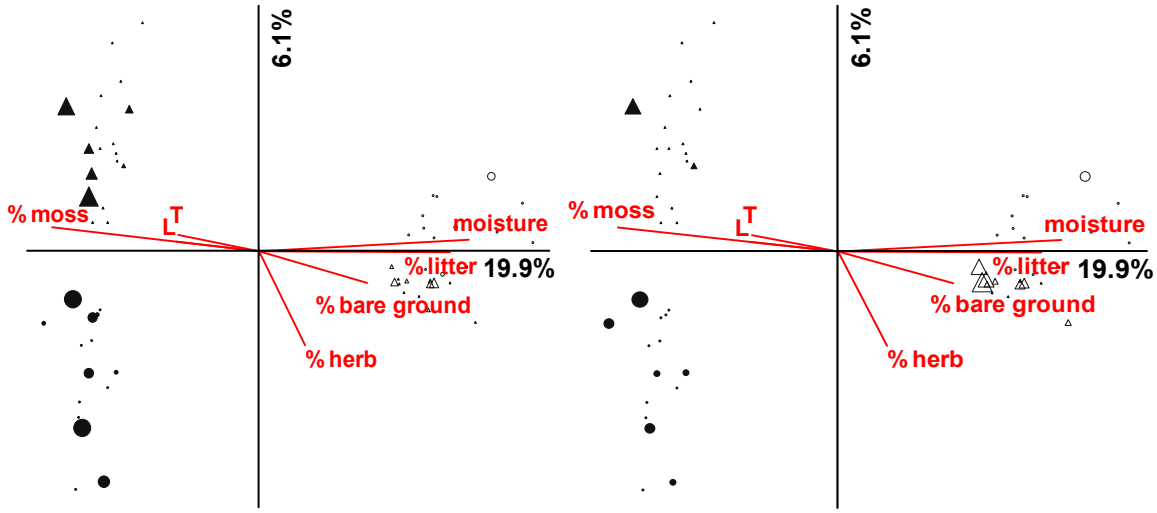


Fig. 17 Principal Components Analysis (PCA) of vegetation composition within 63 permanent plots of four *S. purpurea* populations. Site parameters were correlated with the axes. Species abundances are implemented in the analysis as percentage of species cover. Explained percentage of variance: axis 1: 19.9 %, axis 2: 6.1 %. Black dots: Rosenau I, black triangles: Rosenau II, white dots: Lechfeld West, white triangles: Lechfeld East.

(a) Different sizes of plot symbols resulted from their different content of **flowering individuals**. The bigger the symbols the higher the content of flowering individuals.

(b) Different sizes of plot symbols resulted from their different content of **recruits**. The bigger the symbols the higher the content of recruits of *S. purpurea*.

Historic processes and management

Little is known about the glacial history of grassland species in Central Europe, but recent studies tried to elucidate past processes during times of quaternary glaciation (see chapter 2). *S. purpurea* is supposed to be part of plants, which disappeared during glaciation in Central Europe and re-migrated after climate warming from southern refugia, such as the Balkans. During this post-glacial times expansion of grasslands might have been largest and steppe plants could be found throughout Central Europe (Lang 1994; Hewitt 1996). Afterwards climate was getting more and more humid and the expansion of trees started the replacement of many dry grassland habitats (Hensen *et al.* 2005). Remnant pieces of grasslands were restricted to few exposed and shallow sites, where trees had no chance to grow. Due to human activities, especially in the Medieval times, clearing and grazing by huge sheep flocks enhanced re-expansion of open grasslands and the formation of species-rich communities (Poschlod & WallisDeVries 2002). Nowadays, these habitats are strongly declining in Central Europe. Abandonment, intensification of traditional farming and destructive land use practices caused habitat deterioration and increased the threat of characteristic dry grassland species (Korneck *et al.* 1996).

Most occurrences of *S. purpurea* in Germany are located on traditionally grazed areas, which are actually largely under protection. These habitats and their high biodiversity can only be maintained by consequent conservation management. This is also the case for *S. purpurea.* While in some protected areas mowing is meant to be the best management practice, others focus on the effect of grazing to create safe sites for germination and the enhancement of gene flow among isolated populations by epizoochorous dispersal. Demographic studies in permanent plots within four Bavarian populations were used to assess the applied management regimes. Two populations were located on mown sites, two on grazed sites. Only one population of the mown site was characterized by increasing growth rates ($\lambda > 1$). Densities of plants in all four studied populations hardly reached one individual per square meter in a total study area of 72 m². Flowering rates of *S. purpurea* were usually high (on average 64.9 %) reaching up to 100 %, but are strongly dependent on site and weather specific conditions. While in year 2008 percentage of flowering individuals was high ranging from 49.2 to 100.0 % per population, flowering rates decreased in the following year (18.6 to 59.0), partly about more than 60 %. Only one population, located on a mown habitat, was able to reach comparable flowering ratios in both study years. Flowering two times in series was

rather scarce on the grazed study sites and more frequent on mown sites. Population structure was characterized by high percentage of adult plants (63 to 90 %) and low levels of juveniles (10 to 37 %). *S. purpurea* seemed to prefer mown habitats with dense, but low competitive vegetation structure, while sites with higher degree of disturbance by grazing lead to a more static or regressive population structure.

Herbivores and pathogens

In some regions populations of *S. purpurea* are seriously affected by damages of mammalian herbivores. Especially in spring, fresh leaves and growing flowering stalks were often found to be bitten off. Characteristic feeding traces (flower heads as typical leftovers) suggest that hares or rabbits might be the main herbivores within the studied populations. There are also damages by roe deer, which feed on *S. purpurea,* especially in regions with high game density. Although, *S. purpurea* is able to produce new shoots after damage, flower productivity in small populations might be extremely limited by these damages. To enhance reproductive capacity in small and thoroughly threatened populations, protecting agents might be applied during flowering and fruiting.

According to Hegi (1998) *S. purpurea* can be attacked by two pathogenous fungi, *Ustilago scorzonerae* and *Puccinia scorzonerae,* both specific to the genus *Scorzonera*. *U. scorzonerae* (Ustilaginomycetes, Basidiomycota) is a smut fungus, which infects all rosettes of a genet. Infections by *U. scorzonerae* are systemic. Infected plants produce inflorescences, but these are completely sterile (Colling & Matthies 2004). The flower heads contain enormous amounts of dark teliospores, which are dispersed by wind at the same time or shortly before uninfected plants flower. Therefore, *U. scorzonerae* can strongly reduce the effective population size of its host and might be deleterious especially in small populations. *Ustilago* infections are also reported for other *Scorzonera* species, such as *S. humilis* and *S. hispanica* (Paravicini 1917; Foitzik 1996). *P. scorzonerae* is a rust fungus and produces different kind of spores on both sides of the leaves (Gäumann 1953; Reimers & Scholz 1959). *Scorzonera* plants are strongly weakened by the pathogen resulting in reduced reproductive capacity. In populations of *S. purpurea* from Germany no infections of pathogenous funghi are known.

Mycorrhiza

There is no information available about the mycorrhizal associations between the roots of *S. purpurea* and the hyphes of fungi. Eriksen et al. (2002) could find arbuscular mycorrhiza in *S. humilis*, which could probably also be expected for *S. purpurea*. Several authors emphasize the importance of mycorrhizal fungi for plant biodiversity and for grassland restoration in conservation practice (Francis & Read 1994; Zobel *et al.* 1997; van der Heijden *et al.* 1998). Knowledge about fungus-plant interactions is necessary, especially in the case of introduction and re-introduction of rare and vulnerable plant species to restored habitats. Maybe, some plant life stages might be strongly dependent on the existence of mycorrhizal structures and lacking symbiotic interactions might be a potential risk factor for plant's establishment and persistence (Eriksen *et al.* 2002).

Biochemical data

Although, there are no published records on the phytochemistry of *S. purpurea*, a wide range of biochemical analyses were taken for other species within the genus. Many important secondary compounds could be revealed for different *Scorzonera* species from all over the world. Consequently, it can be assumed that *S. purpurea* may possess also a huge phytochemical potential. Coumarins, flavonoids and guaiane-type sesquiterpenoids have been reported from this genus (Li *et al.* 2004; Jiang *et al.* 2007; Zhu *et al.* 2009). Ethanol and chloroform extracts of the aerial parts of *S. sandrasica* for example, exhibited significant activity against multiresistant strains of the bacterium *Stenotrophomonas maltophilia* (Ugur *et al.* 2010). Analgesic compounds are responsible for the usage of some species of the genus *Scorzonera* in traditional medicines (Jiang *et al.* 2007; Tsevegsuren *et al.* 2007; Wang *et al.* 2009; Zhu *et al.* 2009; BahadIr *et al.* 2010). *Scorzonera austriaca*, for example, has been employed as a Tibetan folk medicine for the treatment of fever, carbuncles, inflammation and mastitis in the People's Republic of China, *S. divaricata* possesses antipyretic and antidote activities, *S. pseudodivaricata* is used for treatment of diarrhea, lung oedema and parasitic diseases, *S. radiata* is used as diuretic agent as well as for its therapeutic activities in treatment of poisonous ulcer and fever accompanying bacterial and viral infections (Wang *et al.* 2009). Mastic is prepared from the latex of *S. latifolia* in Turkish folk medicine as analgesic and as anthelmintic compound. Furthermore, *S. hispanica* has attracted attention by its use as remedy against snakebites (Koehne 1895).

Beside its importance as a widely used medicinal plant, the genus *Scorzonera* is also used as a food plant. In many European countries *S. hispanica* L. (black salsify) is cultivated as a vegetable comparable to asparagus coming from Spain in the 17th century (Koehne 1895).

Genetic data

Karyological data

There are conflicting chromosome counts for *S. purpurea* within its distribution range. Kuzmanov et al. (1993) reported on a diploid chromosome number of 2n = 12, while in most other analyses chromosome number was 2n = 14 (van Loon & Kieft 1980; Dvořák & Dadáková 1984; Nazarova 1997; Owen *et al.* 2006). According to Dvořák & Dadáková (1984) a diploid set of chromosomes consists of eight metacentric and six submetacentric chromosomes (chromosome formula: 8 A^m + 6 B^{sm}). On the 5th pair of homologous chromosomes satellites could be observed. These structures result from secondary constrictions on the chromosome, which comprise the genes of rRNA. In one mitotic metaphase an accessory metacentric chromosome could be observed (chromosome formula: 2n = 14 + 1 = 9 A^m + 6 B^{sm}).

Genetic variation within and among populations

Genetic variation was analysed by amplified fragment length polymorphisms (AFLP) in 37 populations of *S. purpurea* throughout its distribution range from southern France up to the western part of the Ural Mountains. The most diverse populations of *S. purpurea* were located in the Hungarian steppe region. This area is meant to be the designated origin of post-glacial immigration processes into Central Europe (see chapter 2). Climatic conditions in most parts of Central Europe had been extremely harsh during glaciation and prevented largely the survival of thermophilous plant species in Germany. Two immigration routes might have been taken by *S. purpurea* individuals into Germany: one group followed the course of the Danube from Hungary via Slovakia and Austria to the southern and central parts of Germany and one group migrated from Hungary, Slovakia and the Czech Republic via the Moravian gap into Poland and the north-eastern part of Germany (see chapter 2).

Within Germany genetic variation within populations of *S. purpurea* was on a moderate level (mean percentage of polymorphic loci at population level: %PB = 45.7, SE = ±1.14). Medium levels of genetic variation within populations might be the result of population bottlenecks

during times of glaciation and species fast re-immigration after climate warming. This assumption is also supported by the low value of total genetic variation among populations ($\Phi_{PT} = 0.19$). Although, most of the populations in Central Europe are more or less spatially isolated from each other, genetic relations are still high. Typical life history traits, such as self-incompatibility, perennial life strategy and dispersal capacity by sheep herding, might have prevented stronger differentiation between populations. Just one region in France was characterized by high genetic distance to all other populations. These French populations might have been isolated for long time and genetic exchange to the more eastern populations was interrupted since quaternary glaciations. After the retreat of glaciers *S. purpurea* individuals from France were not able to migrate into Central Europe. Thus, two refugia can be assumed for the continental steppe plant *S. purpurea*, one in southern France and one in the region of the Hungarian Plain (see chapter 2).

Conservation status of the species

S. purpurea is an indigenous species of Central Europe. Presently, its main habitats are threatened by abandonment, loss of natural dynamics and intensification of agricultural used sites. Its threat status for whole Central Europe was rated as vulnerable except for the Pannonian region (Schnittler & Günther 1999). In Hungary, *S. purpurea* is widely distributed and not threatened by extinction. In the European countries Czech Republic, Slovakian Republic and Austria, *S. purpurea* is listed as vulnerable (Schnittler & Günther 1999), while in Germany its threat status is assessed as endangered (Korneck *et al.* 1996). Its occurrences in Germany are restricted to the federal states of Saxony-Anhalt, Thuringia, Berlin, Brandenburg, Rhineland-Palatinate and Bavaria. There is no special international responsibility of Germany for the conservation of *S. purpurea* (Ludwig *et al.* 2007). However, populations at the western boarder of a more or less continuous range might be potential sources for future expansions in the face of global warming. Different genetic lineages within Germany developed from different migration routes into Central Europe deserve special conservation efforts at local scale to prevent the loss of characteristic genetic pattern and to preserve the actual level of genetic variation (see chapter 2).

Conservation effort: Habitat restoration and species reintroduction

Populations of *S. purpurea* in Germany, as well as in large parts of Central Europe, are small and strongly isolated in our highly fragmented landscape. There is an urgent need for conservation management programs to save these populations for future. Habitat deterioration, caused by intensification of traditional land-use practices and eutrophication, belongs to the main risk factors for this steppe plant. Therefore, extensification programs have been set up in many regions, which aim to restore these habitats and their species-rich communities (Riegel 2001). Reversion of intensive agricultural farming into traditional land use programs, such as extensive mowing and sheep herding, have been established to prevent local populations for extinction. However, even if habitats can be restored, re-establishment of a diverse plant community takes time and reaching the former grassland constitution is hardly to achieve. One reason might be that especially rare and endangered plants cannot reach new established habitats, because diaspores are severely limited in the surrounding landscape as well as within the soil seed bank (Pywell *et al.* 2002; Ozinga *et al.* 2005). Colonization of restored habitats may be enhanced by deliberate introductions of species and may overcome these limitations (Maunder 1992; Pywell *et al.* 2007). Introduction of seeds by sowing them into vegetation gaps within the restored grassland sward may promote seedling establishment of *S. purpurea*. Safe sites for germination, especially in dense vegetation structures, are necessary to guarantee successful recruitment. Alternatively, the introduction of young plants instead of seeds can be applied as an auxiliary conservation measure.

A transplantation experiment on five grassland sites of different degree of restoration and vegetation composition revealed first promising results. Following sites have been analysed: one site with a long-term established grassland community, a ten-year-old restored grassland with open vegetation structure, a ten-year-old restored grassland with dense vegetation cover, a ten-year-old restored grassland with high disturbances by rodents, and a two-year-old restored ex-arable field created by topsoil remove. Established individuals have been transplanted and tracked over two years. Most introduced individuals survived and showed high flowering ratios in the following year after transplantation. The plants were extremely vigorous and able to form several flowering stalks. However, herbivore pressure was extremely high, especially on sites of sparse or lacking vegetation cover, and barely any individual could reach the reproductive status without damage. Two years after transplantation of plants, survival rates and fitness of introduced individuals strongly varied among the studied habitat types (Fig. 18).

Best conditions for *S. purpurea* survival seemed to offer the 10-year-old restored site with open vegetation structure as well as a two-year-old restored ex-arable field. On sites with established vegetation cover or high disturbances by rodents survival rates of *S. purpurea* were significantly reduced.

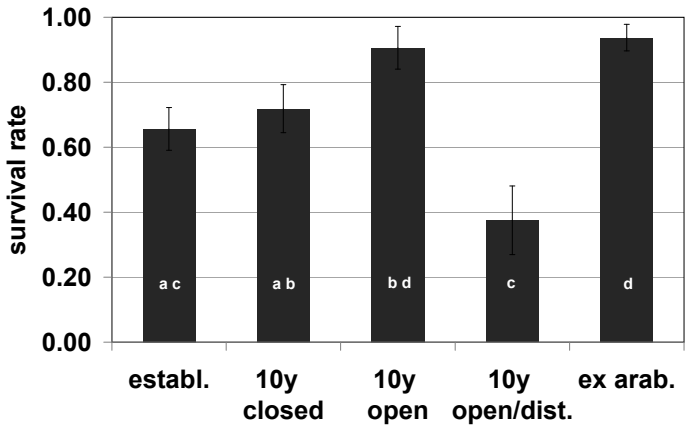


Fig. 18 Survival rates of transplanted individuals of *S. purpurea* in five different grassland types. Establ.: established grassland, 10y: restored grassland (10 years old), ex arab.: ex-arable field (topsoil removed 2 years ago), closed: closed vegetation cover, open: open vegetation cover, dist.: high disturbances by rodents. Comparison of means was conducted by non-parametric H- and U-tests. Different letters symbolize significant differences.

Considering the results of the transplantation experiment, introduction of young plants may allow the creation of new populations at sites, where habitat quality is not sufficiently restored for natural seedling establishment. Introduction of young plants can be used to found new populations on pioneer habitats, where germination would fail due to highly drained habitat conditions. The sensitive seedling stage can be bypassed and this may be a faster and more efficient way to establish new populations of long-lived plants. Introduced plants, especially in the first two years, were more vital (bigger rosettes, higher flowering stalks, more flower heads) and they flowered much earlier than plants within the indigenous habitats. However, time and effort involved in raising plants in the greenhouse and transplanting them is considerably higher than for sowing. Moreover, the introduction of plants from captive breeding prevents local selection during germination. Therefore, the sensitive stage of seedling establishment might be bypassed, but may also result in a reduction of long-term performance of populations. However, assessing the success in restoring populations of perennial plants requires long-term monitoring.

Chapter 4

Loosing an endemic plant species? Genetic relevance of *Stipa bavarica* as management unit

Abstract

Preservation of biological diversity is a fundamental goal in conservation biology and comprises all levels of biodiversity - habitats, species and genes. Especially in conserving genetic variation, detailed analyses are crucial to define valuable management units. The present study aims to answer the question, whether molecular analyses support the taxonomic status of *Stipa bavarica* as an independent, endemic plant species and its genetic relevance for conservation. Morphological characteristics and the strongly restricted occurrence of *S. bavarica* on a single Jurassic rock in Bavaria gave indication for the assumption that *S. bavarica* might originate from hybridization among closely related feather grass species. Due to their close regional proximity to the *S. bavarica* population in Bavaria, *S. pennata* and *S. pulcherrima* s.str. may have been potential hybridization partners.

We analysed genetic variation among populations of *S. pulcherrima, S. bavarica* and *S. pennata* and tried to elucidate the genetic relationship between them. The investigation comprised 92 individuals from 10 populations of *S. pulcherrima*, 90 individuals from 10 populations of *S. pennata* and 13 individuals of *S. bavarica*. Three populations originated from Slovakia, all others from Germany with a main focus on Bavaria. AFLP analysis resulted in 297 fragments of which 95.3 % were polymorphic. Genetic variation within populations varied between 0.127 and 0.256 of Nei's Gene Diversity.

Genetic variation among species was high for *S. pulcherrima* and *S. pennata* ($\Phi_{PT} = 0.25$), as well as for *S. pennata* and *S. bavarica* ($\Phi_{PT} = 0.24$). Between *S. pulcherrima* and *S. bavarica* no genetic variation among populations could be observed and therefore, gene flow between these populations seemed to be high. A Principle Coordinate Analysis (PCoA) showed a quite distinct separation of *S. pennata* and *S. pulcherrima* populations, but no separation of *S. bavarica* and *S. pulcherrima* populations. In a population based cluster analysis, *S. bavarica* was grouped within populations of *S. pulcherrima*.

Although, molecular analysis revealed no fundamental genetic reason to place *S. bavarica* on the taxanomic rank of an independent, endemic species, we could detect specific rare fragments in the population of *S. bavarica*. These considerable amounts of rare fragments as well as morphological characteristics emphasized the genetic importance of *S. bavarica* as valuable management unit and its relevance for preservation of genetic biodiversity in ex situ conservation programs.

Introduction

Preservation of biological diversity is a fundamental goal in conservation biology. The protection of habitats and species are in the main focus of global and regional conservation programs (Convention on Biological Diversity, Fauna Flora Habitat Directive, etc.). Especially rare and geographically restricted species deserve intensive management efforts using both in situ and ex situ conservation strategies. Beside investigations on biological traits, many conservation policies have added the analysis of genetic data to improve the understanding of dynamics in populations and to identify populations, which deserve conservation priority, e.g. due to their high amounts of genetic variation.

Species with low levels of genetic variation are thought to be more vulnerable to environmental changes and to be at greater risk of extinction than species with high genetic variation (Barrett & Kohn 1991; Dolan 1994). The levels of genetic variation and their distribution within and among populations are crucial measures to elucidate historical and present-day impacts on species dynamics. With the use of molecular marker (isozymes, RAPDs and AFLPs) bottlenecks, migration routes, founder effects, gene flow and levels of inbreeding can be determined (Nei *et al.* 1975; Hewitt 1999; Gaudeul *et al.* 2000; Kahmen & Poschlod 2000; Despres *et al.* 2002; Reisch *et al.* 2003b; Schönswetter & Tribsch 2005; Bylebyl *et al.* 2008). Furthermore, molecular data were used for the identification of high priority populations in conservation programs and allow the selection of a minimum number of populations, which should be preserved to mitigate the loss of genetic diversity of a threatened species (Arens *et al.* 1998). Fingerprinting techniques such as AFLPs are high-resolvent and screen nuclear DNA regions throughout the genome. Even in studies dealing with the systematic status of species, AFLPs were used to investigate relationship among closely related taxa (Kardolus *et al.* 1998; Hedren *et al.* 2001; Koopman *et al.* 2001; Zhang *et al.* 2001). Due to their low evolutionary rate, the sequencing of coding regions have only

limited power at the genus or species level (Soltis *et al.* 1993) and therefore AFLP analyses promise to resolve closer relationships among species.

In conservation issues, especially ex situ conservation efforts with limited financial and spatial resources, localization and assessment of genetic variation within species is very important to identify suitable units for conservation. Several approaches for such units were introduced by different conservation biologists: evolutionary significant units (ESU; Ryder 1986) based upon genetic structure and dynamics of populations, management units (MU; Moritz 1994) based upon allelic data within a phylogeographical scenario and relevant genetic units for conservation (RGUC) defined by Caujap et al. (2004) are based upon the common possession of usual and rare alleles. Selected units for conservation should reflect the heterogeneity of genetic composition within a species at best and support potential taxonomic subdivisions into subspecies or classification of independent species.

Stipa bavarica Martinovsky & H. Scholz is a narrow endemic plant species in Bavaria restricted to a single localization on a Jurassic rock in the southern Franconian Alb called Finkenstein. Due to its endemic status, the Bavarian feather grass has become a flagship species in the European Guideline for Conservation. It is listed in European and national conservation policy documents, such as the Annex IV to the European Habitats Directive and in German Red Lists as critical endangered, both at federal and national level (Korneck et al 1996). Endangerment consists mainly in its small population size (approximately 60 individuals) and its occurrence on a single rock surrounded by forests. Its habitats are threatened by succession, mainly by the invasion of shrubs.

The genus *Stipa* is very species-rich and worldwide more than 300 species are known (Hegi 1998). *S. bavarica* belongs to the species group of *S. pennata* agg. (Wisskirchen & Haeupler 1998). Due to high variation within morphological characteristics, its taxonomic status as independent species is highly controversial. Martinovsky & Scholz (1968) differentiated the individuals of Finkenstein from *S. pulcherrima* due to differences in hairlines, sizes of glumes and length of awns and gave it an independent species rank, whereas Conert listed *S. bavarica* as a subspecies of *S. pulcherrima* s.l. (Conert *et al.* 1981).

Individuals of *S. bavarica* not only show morphological similarities to *S. pulcherrima*, but also to S. *dasyphylla*, another species of the *S. pennata* agg. These morphological similarities of two different species suggest, that *S. bavarica* could be the result of potential hybridization between *S. pulcherrima* and *S. dasyphylla* (Martinovsky & Scholz 1968). However, due to the

lack of *S. dasyphylla* populations in spatial or even regional proximity to Finkenstein (even in the past) and no reported hybridization events in regions with sympatric occurrences of *S. pulcherrima* and *S. dasyphylla*, hybridization of theses two species seems to be rather unlikely. However, hybridization might have occurred between two other *Stipa* species growing in close vicinity to *Stipa bavarica*: *S. pulcherrima* and *S. pennata* s. str..

In the present study, a genetic analysis of *S. bavarica* was carried out to answer the following questions: (1) How large is the level of genetic variation within the population of Finkenstein in comparison to other regional populations of *S. pulcherrima*? (2) Is it possible to detect genetic differentiation among populations of *S. bavarica* and *S. pulcherrima* s.str.? (3) Is there any genetic characteristic of *S. bavarica* to treat this population as a significant unit for conservation? (4) Is there molecular evidence for *S. bavarica* of being a hybrid of *S. pulcherrima* and *S. pennata* s. str.?

Material & Methods

Species description and sampling strategy

For the present study, we collected samples from the single population of *Stipa bavarica* at Finkenstein and ten populations each of *Stipa pulcherrima* (Poaceae) subsp. *pulcherrima* and *Stipa pennata* s. str. from Bavaria, Thuringia and Slovakia (Tab. 6). One population of *Stipa eriocaulis* originating from France served as an outgroup population. For the AFLP analysis, we sampled fresh leaf material of 5 to 15 individual tussocks per population and dried it immediately with an adequate amount of silica gel. Minimum distance between sampled tussocks was one meter. Minimum distance between two locations was 40 m, with the maximum distance being 990 km.

Fig. 19 *Stipa pennata* at the peak of fruit ripening in the nature reserve Rosenau in Bavaria.

Stipa is a very diverse genus with several species and most of them are adapted to dry habitats in warm and temperate regions of both hemispheres. Several approaches were undertaken to comprise this huge variety to systematic categories like series, sections and groups. For North-America, hybrids of different species from one section and also from different sections are reported (Hegi 1998). Within this study, all analysed species belong to the *Stipa pennata* group following the systematic classification of Wisskirchen & Haeupler (1998, Tab. 5).

The typus species, *S. pulcherrima* K. Koch s.l., is a wind-pollinated long-lived perennial tussock grass and occurs in dry nutrient-poor grasslands on calcareous soils. Its distribution range extends from the southern Russian steppes to the continental regions of Central Europe and parts of Southern Europe (Spain), where it is restricted to highly fragmented sites (Tutin *et al.* 1964). In Germany, flowering takes place between May and June followed by ripening of the characteristic caryopses (Fig. 19). Long and hygroscopic awns facilitate dispersal by wind and animals.

Tab. 5 The taxonomic group of *Stipa pennata*. Species, which were analysed in the present study, are listed in bold print.

Stipa L. (Poaceae)	
Stipa pennata agg.	
Stipa borsythenica	Klokov ex Prokudin
subsp. borsythenica	
subsp. germanica	(Endtm.) Martinovsky & Rauschert
Stipa dasyphylla	(Cernjaev ex Lindem.) Trautv.
Stipa eriocaulis	Borbás
subsp. austriaca	(Beck) Martinovsky
subsp. lutetiana	H. Scholz
Stipa pennata	L.
Stipa pulcherrima	K. Koch
subsp. bavarica	(Martinovsky & H. Scholz) Conert
subsp. pulcherrima	
Stipa tirsa	Steven

DNA extraction and amplified fragment length polymorphism (AFLP) analysis

Genomic DNA was isolated following the CTAB (cetyltrimethylammonium bromide) method (Rogers & Bendich 1994) adapted as previously described by Reisch et al. (2005). For this study, we chose the AFLP marker system to amplify selected fragments from the digestion of total DNA by polymerase chain reaction. The protocol was carried out following the procedure described by Vos et al. (1995). We used non-radioactive fluorescent dye-labelled

primers on an automated DNA sequencer of Beckman Coulter. Eight randomly selected individuals from different populations throughout the study area were screened with 32 primer pair combinations for clear and reproducible bands. We chose three primer pairs fulfilling these options for analyses of the total sample set (D2: *Mse*I-CAA/*Eco*RI-AAC, D3: *Mse*I-CTC/*Eco*RI-AAG, D4: *Mse*I-CAG/*Eco*RI-ACA). Genomic DNA (approximately 50 ng) was digested with the restriction enzymes *Eco*RI and *Mse*I and ligated with T4 DNA Ligase conducted in a thermal cycler for 2 h at 37 °C. Polymerase chain reactions (PCRs) were run in a reaction volume of 5 ml. Preselective amplifications were performed using primer pairs with a single selective nucleotide, *Mse*I-C and *Eco*RI-A, H_2O, buffer S, dNTPs, and Taq. PCR reaction parameters were: 2 min at 94 °C, 30 cycles of 20 s of denaturing at 94 °C, 30 s of annealing at 56 °C, and 2 min of extension at 72 °C, followed by 2 min at 72 °C and ending with 30 min at 60 °C. Selective amplifications were performed with three primer combinations and H_2O, buffer S, dNTPs, and Taq. PCR reactions were performed with the touch-down profile: 2 min at 94 °C, ten cycles of 20 s of denaturing at 94 °C, 30 s of annealing, which was initiated at 66 °C and then reduced by 1 °C for the next ten cycles, 2 min of elongation at 72 °C, followed by 25 cycles of 20 s of denaturing at 94 °C, 30 s of annealing at 56 °C and 2 min of elongation at 72 °C, ending with a final extension for 30 min at 60 °C. After DNA precipitation, DNA pellets were vacuum dried and dissolved in a mixture of Sample Loading Solution (Beckman Coulter) and CEQ Size Standard 400 (Beckman Coulter). The fluorescence-labelled selective amplification products were separated by capillary gel electrophoresis on an automated sequencer (CEQ 8000, Beckman Coulter). Raw data were collected and analysed with the CEQ Size Standard 400 using the CEQ 8000 software (Beckman Coulter). Data were exported as crv-files, showing synthetic gels with AFLP fragments for each primer combination separately from all studied individuals and analysed in BIONUMERICS, version 3.6 (Applied Maths). Files were examined for strong, clearly defined bands. Each band was scored across all individuals as either present or absent.

Data analysis

In the AFLP data matrix, the presence of a band was scored as 1, whereas the absence of the band was coded as 0. The resulting binary (0/1) data matrix represented all scored AFLP markers with sizes between 60 and 460 bp. Bands, that were not perfectly reproducible between replicates, were eliminated from the matrix.

To quantify genetic variation within populations, we calculated the percentage of polymorphic bands (%PB), Nei's unbiased expected Gene Diversity (GD) assuming Hardy-Weinberg equilibrium and Shannon Index (I) for each population using the program POPGENE version 1.32 (Yeh *et al.* 1997). Genetic variation was estimated separately for each locus and averaged. To avoid population size dependent differences, we randomly chose five individuals per population for calculation of genetic variation. In addition, the rarity of markers was evaluated by the frequency-down-weighted marker (DW) value (Schönswetter & Tribsch 2005). DW values were computed for each population and for each group by using the DW function in the R-script AFLPdat (Ehrich 2006). We also calculated an AMOVA derived measure of genetic diversity by calculating the population-wise AMOVA sums of squares divided by n-1 (AMOVA-SS diversity) (Fischer & Matthies 1998) with the program GenAlEx V5 (Peakall & Smouse 2001).

To identify populations, which contain singular allelic compositions and which should be prioritized for conservation, both the differences in frequencies of the most common alleles (Culley *et al.* 2002) and the presence of rare alleles, which represent newly generated variants (Bengtsson *et al.* 1995), were used. Rare fragments may strengthen the species capability to withstand environmental changes. Bands, which show overall frequency lower than 0.10 and which were present in less than 20 % of the populations, were considered as rare.

Genetic structuring and group assignment were investigated with Bayesian clustering in STRUCTURE, version 2.2 (Pritchard *et al.* 2007). STRUCTURE performs model-based clustering based on Bayesian Markov chain Monte Carlo parameters. The program allows to find the optimal number of groups (K) and to assign individuals to the different groups based on allele frequencies at each locus. The following settings were made: no-admixture and uncorrelated allele frequencies models with the parameters K from 2-22, ten replicate runs for each K, a burn-in period of 10^4 and 10^4 iterations. The most likely number of K present in the dataset was calculated by using ΔK according to Evanno et al. (2005). To assess the genetic pattern in higher dimensional space, a Principal Coordinates Analysis (PCoA) based on Bray-Curtis similarities was implemented in MVSP version 3.12f (Kovach 1999). To explore genetic relatedness among populations, we constructed a majority rule (50 %) consensus UPGMA tree of 1000 bootstrap replicates using the program FAMD 1.08 (Schlüter & Harris 2006). The UPGMA tree based upon a chord distance matrix (single-locus chord distance; Cavalli-Sforza 1967) calculated from allele frequency data (estimated in a Bayesian

framework with a non-uniform prior derived from among-locus information; Zhivotovsky 1999). Furthermore, we constructed a Neighbor Joining tree based on the pairwise population Φ_{PT} distances derived by AMOVA using the program MEGA version 4 (Tamura *et al.* 2007).

Genetic differentiation was quantified by an analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) using the program GENALEX version 5 (Peakall & Smouse 2001). AMOVA allows the calculation of variance components and their significance level for variation among groups of populations (regions), among populations within groups (regions) and within populations. In this study, populations were initially assigned to two different groups based on their taxonomic status as *S. pulcherrima* and *S. bavarica*. Furthermore, we compared groups of *S. pennata* and *S. bavarica*.

Tab. 6 Geographic location of 22 studied populations of *S. pulcherrima*, *S. pennata* and *S. eriocaulis*. Containing description of sample size (n) and genetic variation within populations measured as percentage of polymorphic bands per population (PB%), Nei's Gene Diversity (GD), Shannon Index (I), rarity value (DW) and AMOVA sums of squares divided by n-1 diversity (SSWP/n-1).

Species	Location	Region	No of samples	sample size corrected	Latitude (N)	Longitude (E)	SSWP/n-1	GD	I	%PB	DW
S. pulcherrima subsp. *bavarica*	Finkenstein (1)	Bavaria	**13**	**5**	48°44'00"	11°07'29"	**34.8**	**0.165**	**0.246**	**46.1**	**12.63**
S. pulcherrima subsp. *pulcherrima*	Fellinger Berg (2)	Bavaria	15	5	49°01'46"	12°09'13"	28.2	0.143	0.213	39.4	10.99
	Ebenwies (3)	Bavaria	13	5	49°02'44"	11°59'15"	30.3	0.182	0.271	49.8	12.91
	Schulerloch (4)	Bavaria	7	5	48°55'54"	11°49'13"	24.5	0.173	0.252	43.8	14.58
	Essing (5)	Bavaria	6	5	48°56'06"	11°47'29"	28.3	0.184	0.274	50.5	13.77
	Kyffhäuser (6)	Thuringia	7	5	51°22'35"	11°02'08"	32.5	0.178	0.261	45.5	12.76
	Schafberg 1 (7)	Thuringia	10	5	51°12'58"	11°43'14"	31.1	0.14	0.21	39.7	11.73
	Badraer Lehde (8)	Thuringia	5	5	51°40'33"	11°00'00"	23.2	0.145	0.219	41.8	13.15
	Harslebener Berge (9)	Saxony-Anhalt	9	5	51°52'00"	11°05'54"	35.7	0.185	0.276	50.8	11.58
	Devinska kobyla 1 (10)	Slovakia	10	5	48°52'10"	16°39'11"	31.0	0.204	0.304	55.6	13.36
	Devinska kobyla 2 (11)	Slovakia	10	5	48°52'10"	16°39'11"	33.5	0.176	0.265	49.8	12.98
			∑ 92	**50**		**MW**	**29.83**	**0.171**	**0.255**	**46.67**	**12.78**
						SE	**1.23**	**0.007**	**0.010**	**1.72**	**0.34**
S. pennata (S. joannis)	Harrerberg (12)	Bavaria	10	5	49°02'48,4"	11°58'20"	37.1	0.256	0.376	66.3	13.38
	Mattinger Hänge (13)	Bavaria	13	5	48°58'03,9"	12°00'10"	29.3	0.187	0.272	46.1	13.08
	Rosenau (14)	Bavaria	10	5	48°39'40,5"	12°34'42"	25.5	0.127	0.189	35.4	11.66
	Essing (15)	Bavaria	9	5	48°56'06,3"	11°47'27"	28.1	0.143	0.214	40.0	12.27
	Weltenburg (16)	Bavaria	5	5	48°53'58,4"	11°49'41"	22.4	0.147	0.219	40.7	11.94
	Napptal (17)	Thuringia	7	5	51°21'51"	11°05'53"	25.9	0.156	0.232	42.4	12.89
	Harslebener Berge (18)	Saxony-Anhalt	10	5	51°52'00,6"	11°05'54"	28.7	0.156	0.232	43.4	11.76
	Mittelberg (19)	Saxony-Anhalt	8	5	51°25'44,1"	10°58'07"	29.2	0.166	0.251	48.2	13.01
	Neue Göhle (20)	Saxony-Anhalt	9	5	51°13'29"	11°45'09"	32.1	0.146	0.215	37.4	12.8
	Devinska kobyla (21)	Slovakia	9	5	48°48'27"	16°38'51"	25.9	0.146	0.218	40.4	12.49
			∑ 90	**50**		**MW**	**28.43**	**0.163**	**0.242**	**44.03**	**12.53**
						SE	**1.28**	**0.011**	**0.017**	**2.75**	**0.19**
S. eriocaulis	Provence-Alpes-Côte d´Azur (22)	France	**10**	**5**	43°37'21"	06°14'56"	**30.6**	**0.167**	**0.243**	**41.4**	**17.26**

Results

By using three different primer pairs, AFLP analysis of 205 *Stipa* individuals of 22 populations revealed 297 clear and reproducible bands of which 283 were polymorphic (95.3 %). Number of fragments per population ranged between 105 (35.4 %) to 197 (66.3 %). For three populations, *S. pulcherrima* in Schulerloch and Badraer Lehde and *S. eriocaulis* in

France, private bands could be detected. One private fragment was found in each individual of population Schulerloch and one in population Badraer Lehde, three fragments were restricted to individuals of *S. eriocaulis*. Each examined individual exhibited an individual AFLP pattern.

Population size corrected genetic variation within populations measured as Nei's Gene Diversity (GD) and Shannon Index (I) was on a moderate level and showed no significant differences between the studied *Stipa* species (Tab. 6). GD ranged between 0.127 (Rosenau) to 0.256 (Harrerberg) and I between 0.189 (Rosenau) and 0.376 (Harrerberg). The level of DW values was significantly higher in *S. eriocaulis* (17.26) than in the other studied *Stipa* species (12.53 to 12.78). Within *S. pulcherrima* s. l. the most diverse population was Devinska kobyla 1 from Slovakia; the least diverse were Schafberg from Thuringia and Fellinger Berg from Bavaria. The population of Finkenstein showed comparable levels of genetic variation to other populations of *S. pulcherrima* and was not characterized by remarkable higher or lower values. Six of the 297 bands detected by the AFLP analysis coincided with the rarity criteria of low frequency and restricted presence. Three of the six can be found in the population of Finkenstein.

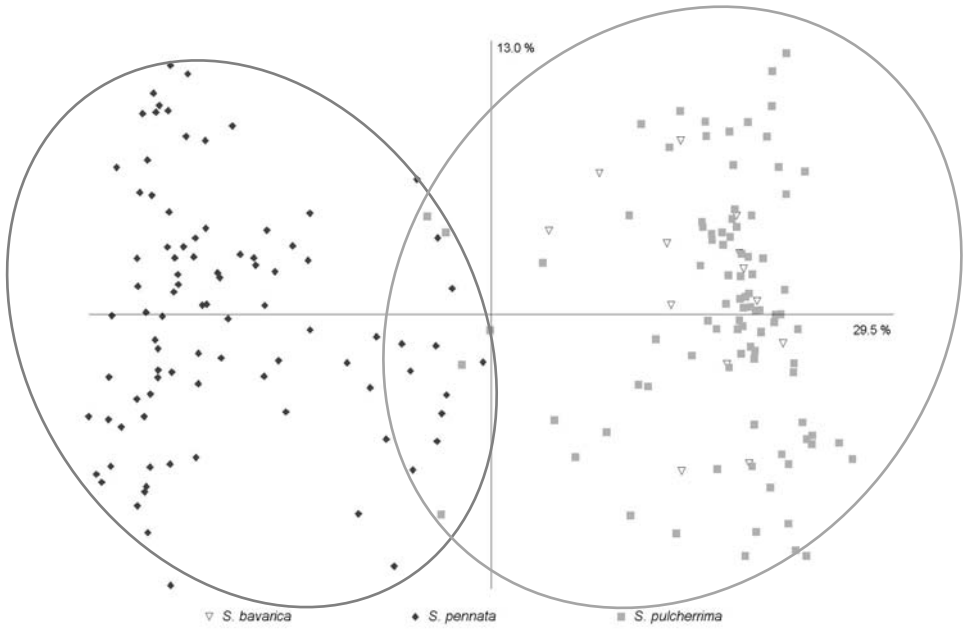


Fig. 20 Principle Coordinates Analysis based on AFLP data of 195 *Stipa* individuals of three different taxa represented by different symbols.

A principal coordinates analysis, based upon Bray-Curtis distance matrix, was calculated and used for assessing genetic similarity among individuals. Ordination of all individuals of *S. bavarica, S. pulcherrima* and *S. pennata* revealed two separated groups (Fig. 20). One group contained individuals of *S. pennata* and one contained all individuals of *S. pulcherrima* together with individuals of *S. bavarica*. In between, some individuals of both groups overlapped. The overall genetic variation among individuals of the studied *Stipa* taxa, explained by the first two axes of the scatter plot, was 42.5 %. A principal coordinate analysis of *S. pulcherrima* and *S. bavarica* was not able to reveal a distinct genetic separation of these two taxa. All individuals were mixed and not partitioned by populations. Explained overall genetic variation by the first three axes was 39.5 %.

Tab. 7 Analyses of molecular variance (AMOVA) of *S. pulcherrima*, *S. bavarica* and *S. pennata*. Level of significance (p) based on 999 iteration steps. d.f.: degrees of freedom, Φ_{PT}: genetic distance.

Source of variation	d.f.	Sum of squares	Variance components	% total variance	Φ_{PT}*
Total					
Among populations total	20	2303.29	8.71	20.10%	0.20
Within populations	174	6025.67	34.63	79.90%	
S. pennata* ↔ *S. pulcherrima* + *S. bavarica					
Among regions	1	1218.09	11.96	24.39%	0.29
Among populations within regions	19	1087.88	2.46	5.01%	
Within populations	174	6022.99	34.62	70.60%	
S. pennata* ↔ *S. pulcherrima					
Among regions	1	1162.69	12.14	24.82%	0.30
Among populations within regions	18	1016.43	2.46	5.02%	
Within populations	162	5558.23	34.31	70.16%	
S. pennata* ↔ *S. bavarica					
Among regions	1	314.06	11.26	23.75%	0.28
Among populations within regions	9	461.87	1.91	4.02%	
Within populations	92	3151.34	34.25	72.23%	
S. pulcherrima* ↔ *S. bavarica					
Among regions	1	71.45	0.00	0.00%	0.07
Among populations within regions	9	554.56	2.87	7.49%	
Within populations	94	3336.36	35.50	92.51%	

* All p-values were <0.001

Molecular variance analysis revealed 20.1 % of total genetic variation among populations of *S. pulcherrima*, *S. bavarica* and *S. pennata* (Tab. 7). Most of variation was found among individuals within populations (79.9 %). Highest resolutions for genetic variation among regions or groups of populations could be reached by partitioning the dataset into two subgroups containing the individuals of *S. pennata* on the one hand and the individuals of *S.*

pulcherrima and *S. bavarica* on the other hand (Φ_{PT} = 0.29). Similar results could be achieved by grouping *S. pennata* against *S. pulcherrima* without regarding *S. bavarica* (Φ_{PT} = 0.30). No variation (0 %) could be detected between the groups *S. pulcherrima* and *S. bavarica*. For this combination, almost 93 % depend on variation within populations. Φ_{PT} of 0.07 between populations of *S. pulcherrima* and *S. bavarica* indicated strong relationship and high gene flow. To represent 99 % of the total genetic diversity among all studied *S. pulcherrima* s.l. populations, only one population (n = 1.01) would be enough. Between the groups *S. pennata* and *S bavarica*, 23.75 % of genetic variation could be found.

Genetic structuring analysis of *S. pennata*, *S. pulcherrima* and *S. bavarica* performed with STRUCTURE revealed two genetic groups as being most likely. For k = 2 ten replicated runs showed highest similarity and a high likelihood value. The first genetic group comprised all individuals of *S. bavarica* and all individuals of 10 *S. pulcherrima* populations. Three individuals, belonging to populations of *S. pennata* (two individuals from Harrerberg and one from Neue Göhle), were assigned to the genetic group of *S. pulcherrima*, whereas all other individuals of *S. pennata* showed a distinct own genetic lineage.

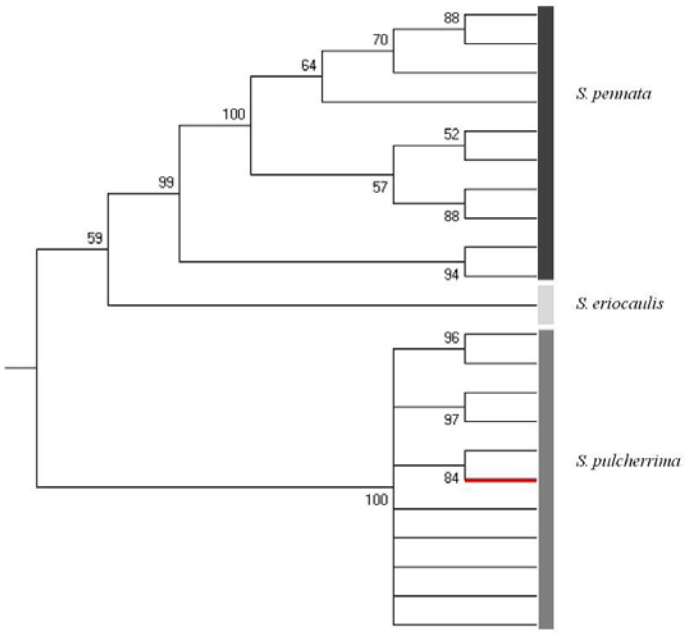


Fig. 21 UPGMA dendrogram of 22 populations of *S. pulcherrima* s.str., *S. bavarica*, *S. pennata* and *S. eriocaulis* based upon 297 amplified fragment length polymorphism marker. Population distances were calculated by using single-locus chord distance by Cavalli-Sforza & Edwards (1967). Bootstrap values upon 50 % based on 1000 permutations are indicated at each node. The branch of population *S. bavarica* is red colored.

A population based UPGMA dendrogram supported the above mentioned results of having two distinct groups containing on the one hand all individuals of *S. pennata* and on the other hand all individuals of *S. pulcherrima* and *S. bavarica* (Fig. 21). The latter one is imbedded in the highly bootstrap-supported group (100 %) of *S. pulcherrima*. *S. eriocaulis*, used as an outgroup population, clustered more closely to *S. pennata* than to *S. pulcherrima*.

Quite the same results could be achieved by a Neighbour Joining dendrogram based on the pair wise population Φ_{PT} distances derived by AMOVA (Fig. 22). In this case, *Stipa bavarica* clustered with all other *S. pulcherrima* populations from Bavaria, Thuringia, Saxony-Anhalt and Slovakia. *S. eriocaulis* is more closely grouped to *S. pennata* than to *S. pulcherrima*.

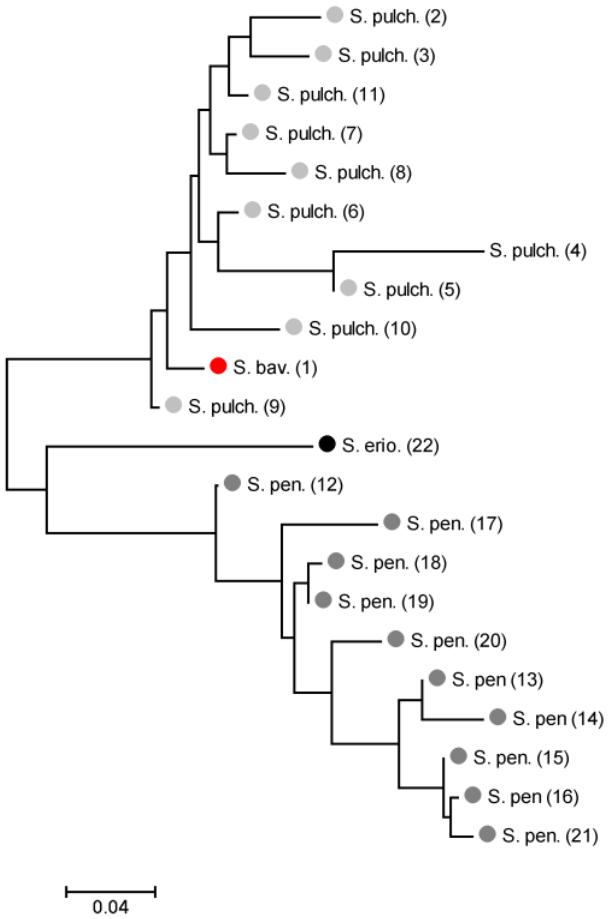


Fig. 22 Neighbour Joining dendrogram of 22 populations of *S. pulcherrima* s.str., *S. bavarica*, *S. pennata* and *S. eriocaulis* based upon 297 amplified fragment length polymorphism marker. Population distances were calculated by using pair wise population Φ_{PT} values derived from AMOVA. Numbers refer to population's localization according to Tab. 6.

Discussion

Taxonomic status of *S. bavarica*

The level of genetic variation within populations is suggested to be strongly dependent on life history traits and geographical range (Hamrick & Godt 1996; Nybom & Bartish 2000). The analysed *Stipa pulcherrima* taxa are perennial, wind-pollinated and allogamous plant species affected by an increasing fragmentation of their distribution range in Central Europe. The population of *S. bavarica* is restricted to a single location and listed as a narrow endemic plant species of Bavaria. While its biological characteristics may contribute to maintain high levels of genetic variation, the isolation of habitats by landscape fragmentation may lead to restricted gene flow and increasing inbreeding. Strong decreases in population sizes in the early and recent history have affected the genetic constitution of several dry grassland species, e.g. *Dictamnus albus* (Hensen & Oberprieler 2005) and *Pulsatilla vulgaris* (Hensen *et al.* 2005). Especially steppe plants, such as *Stipa*, with their main distribution range in the more eastern part of Europe and Russia suffered from habitat loss in Central Europe during times of glaciations and more recently during times of changing agricultural landscape (Hensen *et al.* 2009). Compared to other outcrossing grass species, *S. pulcherrima* showed moderate values of genetic variation. Genetic variation within populations (GD: 0.171, 0.165) was lower than the mean values reported for Poaceae, GD = 0.284, (Hamrick & Godt 1996), but higher than the values for the common Eurasian steppe grass *S. capillata* in Central Europe studied with RAPD, max. GD = 0.111 (Hensen *et al.* 2009). *S. krylovii* from the Inner Mongolia steppe showed comparable values for the Shannon Diversity (Zhao *et al.* 2006) as the analysed *Stipa* taxa in the present study, as well as the alpine and lower mountain grass *Sesleria albicans* in Central Europe (Reisch *et al.* 2003a) for percentage of polymorphic bands.

The level of genetic variation within populations differed not significantly between the endemic *S. bavarica* and its more widespread congener *S. pulcherrima*. Theory predicted by Hamrick & Godt (1996) and other authors, which compared narrow endemics and their widespread congeners (Loveless & Hamrick 1987; Purdy *et al.* 1994; Fréville *et al.* 1998), would suggest strong differences in genetic variation among populations of *S. pulcherrima* s.str. and *S. bavarica*. Sympatric endemics may show reduced levels of genetic polymorphism due to strong directional natural selection in a limited array of environments or changes in allele frequencies caused by genetic drift and/or founder effects (Karron 1987). Especially in

a population with few individuals and potentially non-existing gene flow such as *S. bavarica*, evolutionary constraints may be intense and strongly affect its genetic constitution. Population structure and levels of variation largely depend on these interacting processes of gene flow, natural selection and genetic drift (Slatkin 1987). If gene flow is interrupted, due to habitat fragmentation, differentiation of populations increases. Especially in small and isolated populations, genetic drift is high and enhances local adaptation and fixation of alleles. If isolation will be continued over several generations, this may be the starting point of a new species (Levin 1995). For *S. bavarica*, the analysis of allele frequencies per population revealed three low-frequency fragments, but no private bands could be observed. Furthermore, the location of Finkenstein did not exhibit extraordinary habitat conditions, which obviously would enhance the evolution of a new species. In other plant species, high selection pressure, as high tide fluctuations, and habitats unusual for the progenitor are typical for recent speciation (Crawford *et al.* 1985; Kadereit & Kadereit 2005)

Despite the ongoing fragmentation of their habitats in Central Europe, genetic variation among populations of *S. pulcherrima* was low and grouping populations into subspecies did not result in a higher Φ_{PT} ($\Phi_{PT} = 0.074$) than ignoring them ($\Phi_{PT} = 0.075$). No population was characterized by remarkable high DW values (indicators for past genetic bottlenecks) or strong differences in their genetic constitution. This pattern could be the result of a relatively young colonization history of the steppe plant in Europe having not enough time to evolve clearly different genetic lineages. Starting from one refugium, migration may have taken place rapidly after deglaciation and populations have spread continuously all over the vast open areas of Central Europe (Küster 1995) resulting in a close relationship of populations. However, much stronger may be the influence of typical life history traits on the observed pattern of strong relationship among populations. Wind-pollination and seed dispersal by wind and animals within grass species is very common and strongly support genetic exchange and diminish the effects of fragmentation in younger times (Reisch *et al.* 2003a).

The assumption, that *S. bavarica* might be a hybrid between *S. pulcherrima* and *S. pennata*, both species, which grow in spatial proximity to the location of Finkenstein, could not be confirmed by the present study. Cluster-, PCo- and STRUCTURE analyses, all showed a distinct genetic separation of *S. pulcherrima* s.str. and *S. pennata*. *S. bavarica* as a hybrid would take an intermediate position between them, but in no one of the genetic analyses this pattern could be observed. Rather *S. bavarica* grouped clearly within the cluster of *S.*

pulcherrima s.str. Even the analysis of molecular variance revealed no genetic variation among groups of populations of *S. pulcherrima* and *S. bavarica*, whereas genetic variation between groups of populations of *S. pennata* and *S. bavarica* was 23.75 %.

Although, there is no report about hybrids of *Stipa* species in Central Europe, some natural hybrids are known in Russia and North America (Hegi 1998). The individuals of Finkenstein show some morphological characteristics, which are typical for individuals of *S. dasyphylla* and so this taxon might act as a parent species of the potential hybrid *S. bavarica*. In Central Europe *S. dasyphylla* is extremely rare and not located in the spatial proximity of Finkenstein. In some other regions, *S. dasyphylla* and *S. pulcherrima* exist in sympatric sites, but no hybridization event could be observed until now. The probability for hybridization and following colonization in the region of Finkenstein seems to be very low, but was not tested genetically in the present study.

AFLP data did not separate *S. bavarica* from *S. pulcherrima*. A close relationship between the population of Finkenstein and all other studied populations of *S. pulcherrima* not only in Bavaria, but also in Thuringia, Saxony-Anhalt and Slovakia could be observed. The status of *S. bavarica* as a distinct species proposed by Martinovsky & Scholz (1968), could not be confirmed by molecular methods. Nevertheless some morphological characteristics are typical for individuals of *S. bavarica* (Martinovsky & Scholz 1968) and can be used to define a regional subgroup of *S. pulcherrima*. According to the proposals of Du Rietz (1930) and Rothmaler (1941) about the use of subspecies in botanical taxonomy, subspecies should be more or less separated by a combination of characters without being usually genetically isolated. Following these concepts *S. bavarica* should be treated at subspecific rank of *S. pulcherrima* rather than at specific rank, as it is already used in taxonomic lists such as the taxonomic standard list for Germany from Wisskirchen & Haeupler (1998).

Genetic relevance of *S. bavarica* for conservation

Maintenance of genetic variation is an important goal in conservation efforts, especially in endangered species. Genetic variation present in a given number of populations and the distribution of genetic variation among these populations can be calculated and used to determine notable units for conservation with remarkable high values of genetic variation or to define a sampling strategy for ex situ management practices (Schoen & Brown 1991). Considering other criteria, such as population sizes, would not give an adequate base to assess

conservation priority of populations, because in some cases present population sizes do not reflect genetic variation realistically. Small population sizes usually are attended by reduced levels of genetic variation due to increased genetic drift and inbreeding (Ellstrand & Elam 1993), but effects of past processes, founder events and population bottlenecks influenced intraspecific genetic constitution of populations and are partly independent from population sizes. In our case, the most divers population were *S. pulcherrima* from Devinska kobyla 1 (Slovakia) with GD = 0.204 and Harslebener Berge (Germany) with GD = 0.185. *S. bavarica* was not characterized by remarkable high values of genetic variation.

Beside the stabilization and preservation of populations of threatened species in situ, growing attention is focused on ex situ preservation programs to provide insurance against catastrophic events and to facilitate the possibility of reintroduction in the future when appropriate habitats become available (Holsinger & Gottlieb 1991). Determining the number of populations, which will be needed to maintain a certain proportion of genetic variation, the formula $1 - (\Phi_{PT})^n$ can be used (Ceska *et al.* 1997). Applying this formula to the case of *S. pulcherrima*, samples of a single population would be enough for ex situ protection to obtain more than 99 % of the present genetic variation found in *S. pulcherrima* within the study area. In addition, conservation programs should take into account the arguments of Schoen & Brown (1991), which include empirical data for expected heterozygosity, to detect populations with remarkable high amounts of genetic variation. For *S. pulcherrima* s.l. all populations were on the same level of genetic variation and showed only a small range of standard error ($GD_{ME} = 0.175$ SE = 0.007; $I_{ME} = 0.261$ SE = 0.010). Special adaptations to ecological conditions enhance the enrichment of rare alleles within a population. Therefore, we analysed low-frequency alleles to detect populations with high levels of rare fragments. The population of Finkenstein exhibited three rare fragments, more than any other studied population.

However, rare fragments in a population with few individuals should be carried with caution, because the evolutionary profit of the fragments for preservation, especially for ex situ conservation, is controversy. On the one hand, rare fragments are thought to be necessary for the continued successful evolution of a species and on the other hand they may be potentially deleterious, when genotype frequencies increase in an unnatural way due to genetic drift (Ellstrand & Elam 1993). To preserve genotype frequencies at their natural level and to ensure the capture of rare alleles, Hamrick et al. (1991) recommended, that for ex situ conservation at least 50 individuals from each population should be sampled. For *S. bavarica*, preservation of

population sizes on a minimum level in situ is very important to prevent possible risks such as outcrossing depression by rare fragments and ex situ conservation programs should comprise an adequate minimum sample size of 50 individuals per population.

Chapter 5

Resolving taxonomic uncertainties by conservation genetics - *Tephroseris integrifolia* in Bavaria

Abstract

The present study focused on the doubtful taxonomic position of *Tephroseris integrifolia* in Bavaria. High morphological variability posed the question if local populations can be distinguished by molecular markers. Using a conservation genetic approach, we analysed genetic variation among populations and inferred if there is any genetic evidence to classify new endemic subspecies, especially for the middle Franconian population.

The AFLP analysis comprised 44 individuals of three Bavarian populations and 17 individuals from a reference population in Austria. Despite their strong geographic isolation from each other and small population sizes, we could reveal a high level of genetic variation within populations. Furthermore, there is no indication for any inbreeding depression or any effect of genetic drift. All studied populations are differentiated on a very low level (Φ_{PT} = 0.10). Highest genetic variation could be found between two groups consisting of the Bavarian populations on the one hand and the Austrian population on the other hand. A principal coordinate analysis could also detect a slight grouping of the Austrian individuals, but no genetic separation of the Bavarian populations.

The reason for low differentiation among populations might be located within plant's life history. Typical traits, such as self-incompatibility, high levels of reproduction and a perennial life span, may have largely counteracted genetic deterioration and differentiation among populations. Furthermore, time since fragmentation of their primarily connected habitats has been rather short and deleterious genetic effects might not have emerged yet. As a consequence, there is no population genetic evidence, which supports the assignment of the Bavarian populations to different kinds of subspecies. Hence, in the view of population genetics the taxonomic separation of the middle Franconian population as a distinct, maybe endemic subspecies seems not to be very reasonable. However, due to the high morphological variation of the whole species a common-garden experiment would be essential to clarify the morphological distinctiveness.

Introduction

Due to their high morphological variability, the taxonomic classification of the genus *Tephroseris* (Reichenbach) Reichenbach 1842 is highly complex. Actually more than 60 species, subspecies, varieties and forms are described. One of the central positions within the genus is occupied by the species *Tephroseris integrifolia*, a typical plant species of continental steppe regions and dry grasslands in North America, Asia and Europe (Krach 2001). Occurrences of *T. integrifolia* in Europe extend from the South of England via Norway up to the far eastern part of Russia. The most western part of its range is reached in the dry grasslands of Germany (Conert *et al.* 1981). Eight different subspecies of *T. integrifolia* are distinguished within Europe (Tutin *et al.* 1964; Wisskirchen & Haeupler 1998). Within Germany few occurrences of *T. i.* subsp. *integrifolia* and *T. i.* subsp. *vindelicorum* can be found. The latter one was described by Krach (1988) as an endemic plant species for Bavaria restricted to the lower Lech valley in the south of Augsburg. Both subspecies are listed on the Red Data Book of Germany and are considered to be threatened by extinction due to ongoing deterioration of their habitats (Schnittler & Ludwig 1996). Their last occurrences are restricted to few protected areas within a largely fragmented landscape.

Dry grasslands are relicts of a traditionally used landscape. Sheep herding as well as grazing by cattle and goats are considered to be the driving forces for the creation of these biodiversity hot spots within Central Europe (Pykälä 2000). Actually, intensification of agricultural farming, changes in land use practices and abandonment of traditionally used areas are the main threats for these traditional, semi-natural habitats and their characteristic floristic composition (Hillier *et al.* 1990). The fragmentation of landscapes coincides with increasing isolation of formerly connected populations and genetic exchange by pollen or seeds among these populations becomes more and more interrupted (Slatkin 1987). Depending on population size and genetic constitution impacts resulting from spatial isolation, are more or less severe. Especially in small and isolated populations, repeated crossing events of closely related individuals may cause homogenization of population genetic potential. These inbreeding events often result in decreased vitality rates of singular plants (reduced reproduction rates as well as seedling survival) and weaken population's long-term survival potential (Charlesworth & Charlesworth 1987; Raijmann *et al.* 1994; Heschel & Paige 1995). Furthermore, recurring inbreeding events strongly affect the evolutionary adaptation capacity to changing environmental conditions. However, geographic isolation is

one of the driving forces for the evolution of new species (Turelli *et al.* 2001). In varying ecological niches mutation, selection and recombination might act differently and enhance the formation of new genotypes (Reisch & Poschlod 2009).

In Bavaria *T. integrifolia* is located on few, isolated sites within a largely fragmented landscape. Even within populations, plants are characterized by high morphological variability regarding shape of basal leaves, hairiness and formation of marginal florets. In 1988, morphological and cytotaxonomic investigations by Krach on *T. integrifolia* revealed significant differences in variations of plants located in the south of Augsburg and plants of the species type. Anatomical characteristics (e.g. smaller heights, smaller leaf sizes, longer anthers, and bigger stomata) as well as chromosome numbers supported the separation of the Swabian population and its classification as distinct subspecies called *T. integrifolia* subsp. *vindelicorum*. While *T. integrifolia* subsp. *integrifolia* is characterized by a diploid chromosome set (2n = 48), *T. integrifolia* subsp. *vindelicorum* is tetraploid (2n = 96), similarly to individuals of two further occurrences of *T. integrifolia* in Bavaria. One of these populations is located in lower Franconia near Grettstadt (Fig. 23) and shows morphological similarities to the Pannonian type of *T. integrifolia* subsp. *integrifolia*, typical for individuals originating from the Vienna basin (Krach 1988). The other one is located in middle Franconia near Markt Nordheim and seems to be morphologically different to both known subspecies, *T. i.* subsp. *integrifolia* as well as subsp. *vindelicorum* (Krach & Krach 1991).

In the present study, we tried to infer the doubtful genetic position of the middle Franconian population of *T. integrifolia*. Therefore, we used conservation genetic approaches to compare all populations of *T. integrifolia* from Bavaria characterized by a tetraploid chromosome number (2n = 96) with the Austrian population characterized by a diploid chromosome number (2n = 48). Studying the genetic variation among populations might give indication for taxonomic separation of the middle Franconian population and support its classification as a distinct subspecies. In this case, the taxonomic status of the middle Franconian population would rise to an endemic status and assign high responsibility to Bavaria for its global preservation.

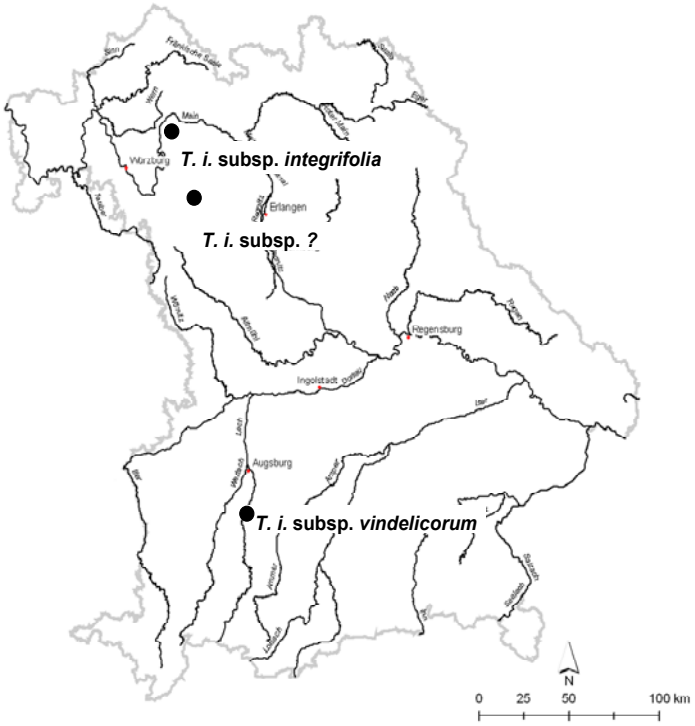


Fig. 23 Geographic location of *T. integrifolia* populations in Bavaria. A question mark symbolizes the doubtful taxonomic position of the middle Franconian population.

Methods

Species description

T. integrifolia (L.) Holub (Syn. *Senecio integrifolius* (L.) Clairv.) is a perennial yellow flowering Asteraceae with a short vertical tap root (Smith 1979). Basal leaves are round to ovate and arranged in a basal rosette. Flowering stem is simple, erect, up to 60 cm high and produces two to five (at maximum 15) flower heads. Within the subspecies *vindelicorum* capitula of some individuals show broad and tongue-shaped marginal flowers, while others contain marginal flowers which resemble large tubular florets. Some flower heads consist of small tubular florets without any marginal flowers (Krach 2001). Furthermore, coloration of flower heads is usually striking yellow, but some individuals vary to a more reddish shade of color (Fig. 24). In spring, basal leaves and buds are covered by a dense coat of trichomes. During vegetation period most of these trichomes drop off and only few can be found at the lower side of basal leaves. Flowering time reaches from mid May to late June.

Fig. 24 Flower heads of *Tephroseris integrifolia* subsp. *vindelicorum* show high morphological variations.

Study design

For the present study, we investigated plant material of four strongly isolated populations of *T. integrifolia*. Three populations were located in the Bavarian districts of lower Franconia, middle Franconia and Swabia and one population originated from Austria next to Vienna (Tab. 8). The Austrian population as well as the population in lower Franconia are allocated to the taxonomic status of *T. i.* subsp. *integrifolia*. The Swabian population slightly differs from the typical morphological characteristics of *T. i.* subsp. *integrifolia* and was classified as regional endemic plant species *T. i.* subsp. *vindelicorum*. Although, individuals of the middle Franconian population are similar to the other Bavarian *T. integrifolia* populations

considering their chromosome numbers (2n = 96), there are some morphological divergences to the taxa subsp. *integrifolia* as well as subsp. *vindelicorum* (Krach & Krach 1991), a fact which raised the question of its taxonomic affiliation.

Plant material was collected in summer 2008 within the Bavarian populations and in 2009 within the Austrian population. In each population, we sampled fresh basal leaves of 13 to 17 individuals with a minimum distance between individuals of one meter.

Tab. 8 Geographic location, taxonomic status and population characteristics of studied populations of *Tephroseris integrifolia*. MF: middle Franconia, LF: lower Franconia, SW: Swabia, VI: Vienna.

No	population	region	species	subspecies	longitude (E)	latitude (N)	population characteristics
Germany							
1	Nature reserve "Sieben Buckel"	MF	*T.i.*	?	10°21'29"	49°35'06"	tetraploid (2n = 96), morphological differences to subsp. *integrifolia* and subsp. *vindelicorum*
2	Nature reserve "Grettstadt"	LF	*T.i.*	*integrifolia*	10°16'14"	49°59'17"	tetraploid (2n = 96), morphological similarity to subsp. *integrifolia*
3	Military area Lechfeld	SW	*T.i.*	*vindelicorum*	10°52'39"	48°11'59"	tetraploid (2n = 96), morphological differences to subsp. *integrifolia*
Austria							
4	Vienna	VI	*T.i.*	*integrifolia*	16°14'52"	48°07'38"	diploid (2n = 48)

AFLP analysis

Analysis of the doubtful taxonomic position of the middle Franconian population was conducted by molecular fingerprinting. Approved by several conservation genetic approaches, AFLP (amplified fragment length polymorphisms) are considered to be powerful and highly reliable in resolving genetic variation within and among closely related populations and in exploring ecogeographic relationships (Mueller *et al.* 1999). Especially in rare or endangered plant species, the use of AFLP affords the investigation of genetic patterns without weakening population fitness. Just small amounts of plant material are necessary to gain sufficient DNA for the creation of illuminative molecular markers (Mueller *et al.* 1999). Though, genetic variation, even between closely related individuals, can be revealed and analysed by various numerical methods.

DNA extraction

Isolation of genomic DNA was conducted by Cetyltrimethylammoniumbromid (CTAB) method according to Rogers & Bendich (1994). Purification of the DNA solution was enlarged by an additive phenol/chloroform/isoamylalcohol step. Per individual, we used 20 mg of dried leave material. We choose the AFLP marker system to amplify selected fragments from the digestion of total DNA by polymerase chain reaction. The protocol was carried out following the procedure described by Vos et al. (1995). We used non-radioactive fluorescent dye-labelled primers on an automated DNA sequencer of Beckman Coulter (D2: *Mse*I-CTT/*Eco*RI-AGC, D3: *Mse*I-CTG/*Eco*RI-AAG, D4: *Mse*I-CTA/*Eco*RI-ACT). Genomic DNA (approximately 50 ng) was digested with the restriction enzymes *Eco*RI and *Mse*I and ligated with T4 DNA Ligase conducted in a thermal cycler for 2 h at 37 °C. Polymerase chain reactions (PCRs) were run in a reaction volume of 5 ml. Preselective amplifications were performed using primer pairs with a single selective nucleotide, *Mse*I-C and *Eco*RI-A, H_2O, buffer S, dNTPs, and Taq. PCR reaction parameters were: 2 min at 94 °C, 30 cycles of 20 s of denaturing at 94 °C, 30 s of annealing at 56 °C, and 2 min of extension at 72 °C, followed by 2 min at 72 °C and ending with 30 min at 60 °C. Selective amplifications were performed with three primer combinations and H_2O, buffer S, dNTPs, and Taq. PCR reactions were performed with the touch-down profile: 2 min at 94 °C, ten cycles of 20 s of denaturing at 94 °C, 30 s of annealing, which was initiated at 66 °C and then reduced by 1 °C for the next ten cycles, 2 min of elongation at 72 °C, followed by 25 cycles of 20 s of denaturing at 94 °C, 30 s of annealing at 56 °C and 2 min of elongation at 72 °C, ending with a final extension for 30 min at 60 °C. After DNA precipitation, DNA pellets were vacuum dried and dissolved in a mixture of Sample Loading Solution (Beckman Coulter) and CEQ Size Standard 400 (Beckman Coulter). The fluorescence-labelled selective amplification products were separated by capillary gel electrophoresis on an automated sequencer (CEQ 8000, Beckman Coulter). Raw data were collected and analysed with the CEQ Size Standard 400 using the CEQ 8000 software (Beckman Coulter). Data were exported as crv-files, showing synthetic gels with AFLP fragments for each primer combination separately from all studied individuals and analysed in BIONUMERICS, version 3.6 (Applied Maths). Files were examined for strong, clearly defined bands. Each band was scored across all individuals as either present or absent.

Data analysis

Within the AFLP data matrix, the presence of a band was scored as 1, whereas the absence of a band was coded as 0. The resulting binary (0/1) data matrix represented all scored AFLP markers with sizes between 60 and 380 bp. Bands that were not perfectly reproducible between replicates were eliminated from the matrix. To quantify genetic variation within populations, we calculated the percentage of polymorphic bands (%PB), Nei's unbiased expected Gene Diversity (GD) assuming Hardy-Weinberg equilibrium and Shannon Diversity (I) for each population using the program POPGENE version 1.32 (Yeh *et al.* 1997). Genetic variation was estimated separately for each locus and averaged.

To explore genetic relatedness among individuals, we used a cluster analysis by constructing a dendrogram based on Dice similarity coefficient and Wards minimum variance method. To assess the genetic pattern in higher dimensional space, a Principal Coordinates Analysis (PCoA) based on Bray-Curtis similarities was implemented in MVSP version 3.12f (Kovach 1999). Genetic variation among population was quantified by an analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) using the program GENALEX version 5 (Peakall & Smouse 2001). AMOVA allows the calculation of variance components and their significance level for variation among groups of populations (regions), among populations within groups (regions) and within populations.

Results

AFLP analysis of 61 *T. integrifolia* individuals generated 234 different fragments ranging in size from 60 to 380 base pairs.

Genetic variation within populations

Genetic variation within populations was very high. Regarding all studied populations, 89.7 % of all generated fragments were polymorph. Private bands, characteristic for only one population, could not be detected. Three bands were typical for the Bavarian populations, but not for the Austrian population. Within populations, proportion of polymorphic bands ranged from 75.6 % to 81.6 %. The lower Franconian population (LF) exhibited the lowest, the Swabian population (SW) the highest genetic variation (Tab. 9). Nei's gene diversity varied from 0.30 (VI, LF) to 0.33 (MF) and Shannon Diversity from 0.43 (VI) to 0.47 (SW, MF), which reflected the high genetic variation within populations.

Tab. 9 Geographic location, taxonomic status and genetic variation of four studied *Tephroseris integrifolia* populations. Chr.number: chromosome number, n: number of analyzed individuals, GD: Nei Gene Diversity, SI: Shannon Index, %PB: percentage of polymorphic bands, MF: middle Franconia, LF: lower Franconia, SW: Swabia, VI: Vienna.

No	population	region	species	subspecies	chr. number	n	GD	SI	%PB
Germany									
1	Nature reserve "Sieben Buckel"	MF	*T.i.*	?	96	13	0.33	0.47	78.2
2	Nature reserve "Grettstadt"	LF	*T.i.*	*integrifolia*	96	16	0.30	0.44	75.6
3	Military area Lechfeld	SW	*T.i.*	*vindelicorum*	96	15	0.32	0.47	81.6
Austria									
4	Vienna	VI	*T.i.*	*integrifolia*	48	17	0.30	0.30	76.9

Genetic variation among populations

Genetic variation among *T. integrifolia* populations in Bavaria and Austria was investigated by using cluster analysis. Within the dendrogram individuals of the Austrian population were clustered as a distinct group, which was low supported by bootstrap analysis. Within the Bavarian populations all individuals were highly mixed and could not be assigned to a specific group (Fig. 25).

Similar results could be revealed by principal coordinate analysis. The ordination showed no population specific differentiation of Bavarian individuals and only a slight separation of the Austrian population along the first axis (Fig. 26).

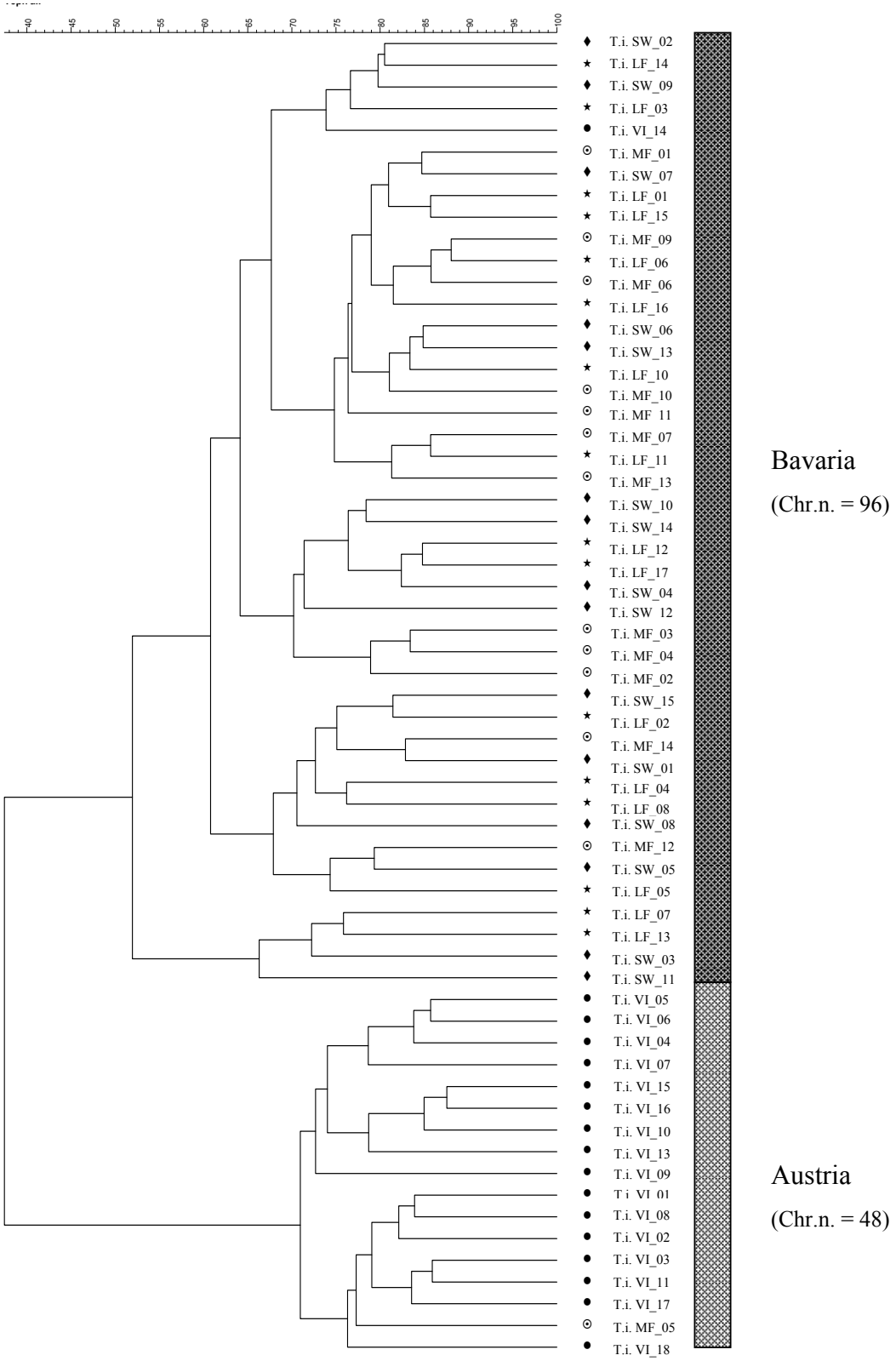


Fig. 25 Cluster analysis of 61 individuals of *Tephroseris integrifolia*. Ti: *Tephroseris integrifolia*, VI: Vienna, MF: middle Franconia, LF: lower Franconia, SW: Swabia, Chr.n: chromosome number.

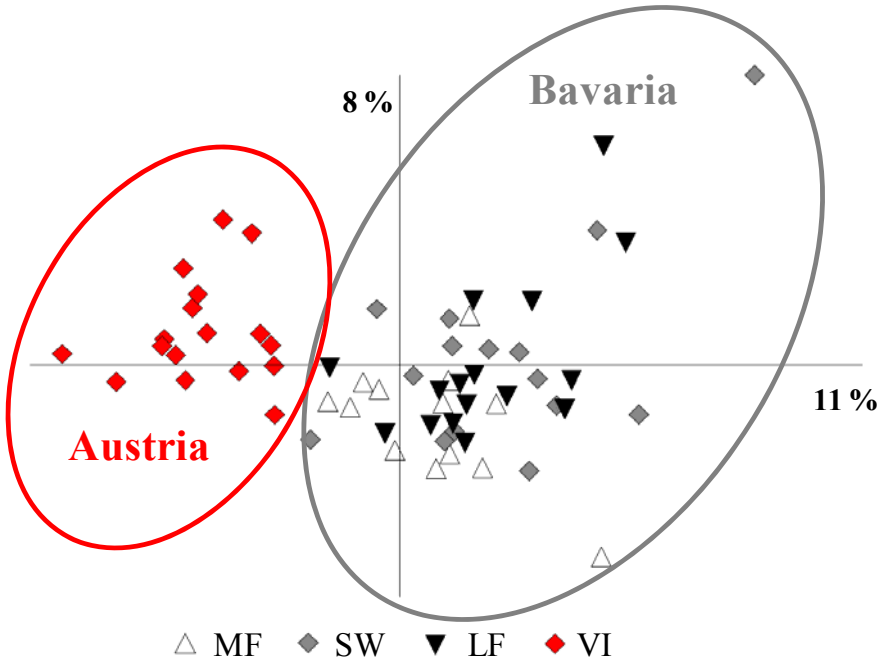


Fig. 26 Principal Coordinates Analysis of 61 *T. integrifolia* individuals from Bavaria and Austria. Ordination is based on 234 AFLP fragments. VI: Vienna, MF: middle Franconia, LF: lower Franconia, SW: Swabia.

Analysis of molecular variance (AMOVA) detected a total variance of 93 % among all studied individuals. Only 7 % of variance could be explained by variation among populations. To localize highest genetic variation among groups, each population was defined as one group and analysed against the others: (2) Vienna against middle Franconia, (3) Vienna against lower Franconia, (4) Vienna against Swabia, (5) middle Franconia against lower Franconia, (6) middle Franconia against Swabia and (7) lower Franconia against Swabia. The highest levels of variation (10 to 11 %) could be revealed for the Austrian population in comparison to each single Bavarian population (Tab. 10). Molecular variance among Bavarian populations was quite low (1 to 4 %). Analysing all Bavarian individuals against all Austrian individuals revealed a slight variation of 7 % among the Bavarian populations and the Austrian population (Fig. 27).

Tab. 10 Summary of analyses of molecular variance (AMOVA) for *Tephroseris integrifolia* within Bavaria and Austria. Level of significance was based on 999 iteration steps. SS: Sums of squares, MS: mean squares, %: proportion of genetic variation, F_{st}: genetic distance, p: level of significance.

Genetic variation	df	SS	MS	%	F_{st} *
Without grouping					
(1) all populations					
Variation among populations	3	220.8	73.6	7	0.07
Variation within populations	57	1978.9	34.7	93	
Grouping according to populations					
(1) Vienna versus middle Franconia					
Variation among populations	1	88.7	88.7	10	0.10
Variation within populations	28	924.6	33.0	90	
(2) Vienna versus lower Franconia					
Variation among populations	1	99.9	99.9	11	0.11
Variation within populations	31	1041.2	33.6	89	
(3) Vienna versus Swabia					
Variation among populations	1	97.1	97.1	10	0.10
Variation within populations	30	1030.3	34.3	90	
(4) Middle Franconia versus lower Franconia					
Variation among populations	1	50.3	50.3	3	0.03
Variation within populations	27	948.6	35.1	97	
(5) Middle Franconia versus Swabia					
Variation among populations	1	55.4	55.4	4	0.04
Variation within populations	26	937.7	36.1	96	
(6) Lower Franconia versus Swabia					
Variation among populations	1	44.9	44.9	1	0.01
Variation within populations	29	1054.4	36.4	99	
Regional grouping					
(7) Bavaria versus Austria					
Variation among Bavaria and Austria	1	120.8	120.8	7	0.10
Variation among populations	2	100.0	50.0	3	
Variation within populations	57	1978.9	34.7	90	

* all values are significant ($p<0.05$)

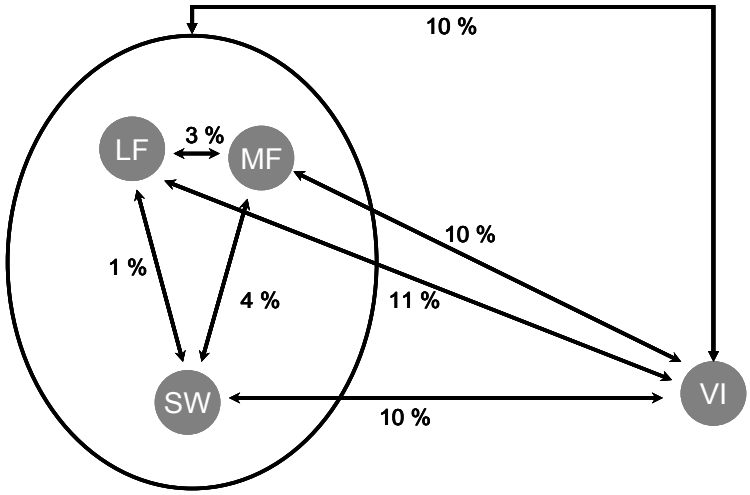


Fig. 27 Distribution pattern of genetic variation among populations of *T. integrifolia* from Bavaria and Austria. VI: Vienna, MF: middle Franconia, LF: lower Franconia, SW: Swabia.

Discussion

Beside high levels of variation within populations, AFLP analysis of four *Tephroseris integrifolia* populations in Bavaria and Austria revealed only a slight genetic variation among populations (Φ_{PT} = 0.10). Within the Bavarian populations no population specific genetic differentiation could be observed. Consequently, there is no molecular evidence for the taxonomic classification of the Bavarian populations into different subspecies. Allocation of the middle Franconian population as an independent subspecies could not be confirmed by population genetic analyses.

In the case of interspecific, or at least inter-subspecific differentiation, genetic variation would be supposed to reach a higher level than it could be observed among the studied *T. integrifolia* populations. Genetic variation among populations could not reach the level of variation typical for other xenogamous angiosperms (ϕ_{PT} = 0.28; Nybom & Bartish 2000). However, there were also some other AFLP studies dealing with rare and isolated plant populations, which showed similar low values for genetic variation among populations. Hensen et al. (2005) revealed also very low ϕ_{PT} values of 0.17 for intraspecific variation in the plant species *Pulsatilla vulgaris*. In the case of interspecific variation significant higher levels of genetic variation among populations would be expected. In spite of quite low morphological differences, Reisch (2004) stated considerably higher values for genetic variation among coexisting species of *Taraxacum* sect. *Erythrosperma* (87.7 % genetic variance among

species). In contrast to the self-incompatible species *T. integrifolia*, these species are known to be apomictic, a reproductive mode, which enhances the genetic differentiation of species. Furthermore, Cole & Kuchenreuther (2001), who investigated the genetic relationship of *Aconitum noveboracense* and *A. columbianum* by RAPD analyses, favoured the treatment as a single species due to the low genetic variation revealed among the studied populations (ϕ_{PT} = 0.24).

The lack of genetic variation among populations might be the effect of two main processes. Consistent gene flow among populations, effected by exchange of pollen or seeds, prevents loss of genetic information by genetic drift or inbreeding and maintains high values of genetic variation within populations (Slatkin 1987; Ellstrand & Elam 1993). Another reason for low differentiation between populations might be rooted in the long-term history of plants. Many plant species, which are presently rare or even endangered by extinction, showed a more different distribution pattern in the past (Hensen & Oberprieler 2005). Especially steppe and dry grassland species underwent a fluctuating history. The increasing demand of firewood in the Middle Ages and the extension of sheep grazing (transhumance) in the 19th and the beginnings of the 20th century, lead to the creation of large open habitats and to the expansion of typical dry grassland species (Poschlod & WallisDeVries 2002). However, in the last 50 to 70 years, most of these anthropogenic influenced sites disappeared due to the ongoing intensification of agricultural practices and landscape changes. Nowadays, many dry grassland species in Central Europe are threatened by extinction (Butaye *et al.* 2005).

T. integrifolia is also affected by these changes. According to several herbarium specimen, at the beginning of the last century, *T. integrifolia* subsp. *vindelicorum* was almost continuously distributed all along the river Lech and occupied a long distance from Thierhaupten in the north to Kaufering in the south (Krach & Krach 1989). Presently, there are only two last records documented on the distribution map of *T. integrifolia* subsp. *vindelicorum*. The distribution pattern of *T. integrifolia* in Bavaria and Austria is restricted to few, suitable grassland sites and populations are getting more and more isolated. Therefore, genetic exchange by pollen or seeds seems to be rather unlikely. Potential pollinators, such as syrphids or apids, are not able to cope with these large distances (Kwak *et al.* 1998) and natural as well as anthropogenic landscape barriers largely prevent the genetic exchange among populations. Even bumblebees, which undertake large foraging flights (up to two kilometers; Walther-Hellwig & Frankl 2000), fail to act as pollinators between the studied

populations. Vectors for long distance dispersal of seeds, such as sheep flocks, are only used within a small scale and are not able to maintain the connecting effects among these strongly isolated populations. Dispersal of seeds by wind is also restricted to only few hundreds of meters (Tackenberg *et al.* 2003; Meindl & Poschlod 2007) and therefore it is not relevant for gene flow between the studied populations.

Despite the strong isolation, genetic variation among populations was low and not affected by differentiation processes. Furthermore, the genetic variation within populations was extremely high and no inbreeding effect could be recognized. We rather supposed that the studied populations are relict populations of a formerly more widespread and more continuous distribution range of the species in Bavaria and Austria. During former times, genetic exchange by pollen or seeds between populations might have been possible without any restriction. Therefore, effects of the ongoing habitat fragmentation and genetic isolation are probably not yet visible due to the short time period, which has passed by.

Furthermore, typical plant history traits of *T. integrifolia*, such as self-incompatibility, high levels of reproduction, overlapping generations and perennial life span, enhance the maintenance of high levels of genetic variation even in small and isolated populations. Regarding other plant species with similar life history traits and distribution patterns, level of genetic variation of *T. integrifolia* in Bavaria was on a similar level. Comparable values have been revealed for *Pulsatilla vulgaris*, a perennial plant species of dry grasslands (Hensen *et al.* 2005), for *Eryngium alpinum*, a long-lived, insect-pollinated plant species of the Alps (Gaudeul *et al.* 2000), and for *Scabiosa columbaria*, a perennial, mostly outcrossing mesobromian plant species (Reisch & Poschlod 2009).

Considering the results of the present study, the Bavarian populations are not distinguishable on the level of population genetics. If there are no significant and genetically fixed morphological characteristics, the treatment of the middle Franconian population as an independent subspecies of *T. integrifolia* seems not to be reasonable. The fact, that all Bavarian populations are more similar to each other on the level of population genetics than the Austrian and the lower Franconian population, both classified as *T. i.* subsp. *integrifolia*, underlines the disputable taxonomic classification within the species. However, to answer this complex issue, the present study was not designed for and much more populations of *T. i.* subsp. *integrifolia* from additional countries should be included.

However, cytological investigations by Krach (1988) revealed already the common characteristic of a tetraploid set of chromosomes (2n = 96) within the Bavarian populations and differentiated them from the Austrian individuals, which had only a simple set of chromosomes (2n = 48). Furthermore, in analysing herbarium specimen and living plants Krach detected certain morphological, phenological and cytological characteristics, which support the classification of the Swabian populations as subspecies *vindelicorum*. Our genetic analyses could not reveal any genetic differentiation of the Bavarian populations and only a slight differentiation of the Austrian population. The middle Franconian population may also bare some population specific traits without showing a differentiated genetic pattern. Especially in plant species with high levels of morphological variation, even within a single population, assessment of environmental adaptations is essential to exclude the possibility of local, ecogeographic species variations. Therefore, classification of populations as distinct subspecies should be based on quantitative morphological, phenological, cytological and genetic analyses to reflect the real pattern of species relationship at best.

To resolve the taxonomic position of the middle Franconian population of *T. integrifolia* even on the level of morphology and phenology, further experiments should be conducted. An extensive common-garden-experiment for example would show, if seed-emerged plants, cultivated under identical conditions, keep their population specific characteristics. Sampling of seeds for a common-garden-experiment should include a representative amount of individuals to cover the whole genetic variation of a given population. In addition, more populations from Austria and several other countries should be included to the analysis to obtain valuable insights into the whole species variation.

Chapter 6

Influence of vegetation structure and climatic variations on population dynamics and fitness of *Tephroseris integrifolia* subsp. *vindelicorum*

Abstract

Due to changing land-use practices and abandonment during the last century many European calcareous grasslands and their characteristic floristic composition are getting more and more threatened. Dry grassland species are dependent on frequent disturbances, such as grazing or mowing. However, effects of the applied management regime on population dynamics can vary strongly among species. Therefore, the understanding of demographic processes is crucial to identify best conservation practices and to improve applied management regimes for endangered plant species.

Our study was aimed to elucidate population dynamics of the perennial, calcareous grassland herb *Tephroseris integrifolia* subsp. *vindelicorum* within two occurrences in southern Germany. We used demographic, site-specific and climatic approaches to assess the magnitude and consequences of spatio-temporal variation and to reveal demographic sensitivities associated with environmental conditions. Demographic data were obtained from 56 permanent plots by a five year (2005-2009) census study. Annual finite rates of population increase strongly fluctuated among years and sites, but on average both populations showed positive developments (growth rates > 1.0) underlining the beneficial influence of sheep grazing to population dynamics. However, the number of flowering individuals strongly decreased during the study period and it could be supposed that inappropriate climatic conditions might be the main reason for reduced flowering rates (lacking vernalization by low winter and early spring temperatures).

Furthermore, *T. integrifolia* subsp. *vindelicorum* could be identified as a rather short-term perennial characterized by a mean time of expiration of 3.7 to 3.9 years. Recruitment varied significantly through space and time (19.4 % to 75 %) and was positively correlated with site-specific parameters, such as percentage of bare ground, moss layer and Ellenberg indicator value for light. Mean annual transition probabilities between different age stage categories

revealed a high mortality rate of recruits (44.7 %) and determined this age stage category to be the most critical to population dynamics. Therefore, creation of safe sites for germination should be one of the main goals in applying an adequate management regime for this rather short-lived plant species. Heterogeneous and periodically changing vegetation structures are considered to enhance the establishment of stable to progressive population structures, because they yield optimal conditions for recruitment (safe sites) as well as for adult plants, which prefer a denser (but low competitive) vegetation cover preventing damages by harsh climatic conditions.

The present study has clearly shown that, in order to preserve the remaining populations of *T. integrifolia* subsp. *vindelicorum*, an adequate management regime is necessary to enable long-term survival of this rather short-lived plant species. Knowledge about mean individual life span, critical life stages and life history traits characterizes *T. integrifolia* subsp. *vindelicorum* as a species well adapted to frequent disturbances generated by management regimes such as sheep grazing. Populations under this management regime might evolve stable to progressive population structures, which diminish the risk of going extinct. However, due to its strong climatic sensitivity, the last populations of *T. integrifolia* subsp. *vindelicorum* are permanently facing the unpredictable threats by environmental stochasticity.

Introduction

Many plant species in Central Europe are affected by habitat loss and habitat deterioration caused by changing modes of land utilization during the last decades (Fischer & Stöcklin 1997; Dolek & Geyer 2002). Especially open, semi-natural habitats, such as dry calcareous grasslands, which have been emerged mainly by human agricultural impact, has declined steeply in numbers and extent coinciding with a high loss of biodiversity (Korneck & Sukopp 1988). Nowadays, in few remnant habitats dry grassland species try to cope with changing environmental conditions (eutrophication, abandonment, competitive stress) and they strongly depend on special conservation efforts. Most of them are highly endangered and listed in the Red Data Books of many European countries (Schnittler & Günther 1999).

In dry calcareous grasslands, mowing and grazing are important management practices to maintain and to restore high levels of species richness by creating different kinds of small-scale habitat structures. Especially grazing is considered to enhance the creation of gaps in the sward, which facilitate seedling recruitment (Watt & Gibson 1988; Bullock *et al.* 1994a), whereas mowing generates homogeneous vegetation structures, which can prevent asymmetric light competition (Lepš 1999). However, habitat requirements of plants strongly differ and introduction of new management practices and modulation of management intensity should be monitored intently, especially for species of high conservation value.

Changes in biotic or abiotic habitat conditions may strongly influence population dynamics and therefore, many populations of plants vary over space and time. Fluctuations in recruitment, survival and mortality rates, determine these variations and result in spatio-temporal varying population dynamics (White 2000). Three sources are mainly responsible for population dynamics: spatial, temporal and individual variation.

Spatial variation mainly depends on the availability of suitable habitats. Habitats of plants contain a combination of resources and environmental conditions, which are necessary for occupancy, persistence and reproduction of individuals (Franklin *et al.* 2000). Beside the overall geographic range of a species, habitat quality plays a fundamental role in spatial variation and strongly affects survival and reproductive performance of individuals (Riba *et al.* 2002; Vergeer *et al.* 2003).

Temporal variation in population dynamics is often represented as environmental stochasticity, which simultaneously affect recruitment and mortality rates of all individuals in

one population (Shaffer 1987; Lande 1993). Even large populations can be strongly influenced by environmental stochasticity and may be threatened by extinction (Goodman 1987; Shaffer 1987), especially in populations, whose long-term growth rate is close to zero (Lande 1993). One source of temporal variation is climatic variation. Weather extremes can function as a catastrophic event and may be associated with sudden large-scale mortality. Annual climatic variations may have remarkable influences on plant's life history and may cause changes in reproductive output or germination success.

Individual variation expressed by varying ability of individuals to cope with environmental conditions influences the persistence of small and endangered populations (Łomnicki 1988). Especially in plants, life history traits, such as pollination mode, dispersal capacity and germination requirements, strongly influence spatio-temporal variations in population dynamics and investigations on biological parameters are crucial to detect potential individual risk factors (Poschlod *et al.* 2000). Population viability analyses try to incorporate all these forms of variation to predict the persistence probability of a given population (Menges 2000).

Demographic studies are fundamental to understand these variations and to develop suitable conservation strategies (Meagher *et al.* 1978; Lande 1988; Schemske *et al.* 1994). In perennial plant species long-term demographic analyses offer the best way to detect beginning regressive population developments, because usually it may take some time before populations reach their new equilibrium after changes in environmental conditions (Fischer & Stöcklin 1997). In several long-term studies, new insights into demography of plant populations have been already provided (Keddy 1981; Menges 1990; Bengtsson 1993; Bullock *et al.* 1994b; Bastrenta *et al.* 1995; Boeken & Canham 1995; Oostermeijer *et al.* 1996). However, few of them have investigated the impact of fine-scale spatial and temporal variation in relation to habitat and climatic characteristics.

In the present study, we focussed on population dynamics of the endemic plant species *Tephroseris integrifolia* subsp. *vindelicorum*, a highly endangered Asteraceae in dry calcareous grasslands. Last remnant populations are situated on a restricted military area in the south of Bavaria. At present, these sites are not threatened by habitat destruction due to agreements of military and conservation agencies. Therefore, the fate of these last populations totally depends on the applied management regime and plant's ability to cope with current environmental conditions.

We present demographic data of *T. integrifolia* subsp. *vindelicorum* gained in a five year permanent plot study. To assess the present risk potential for *Tephroseris integrifolia* subsp. *vindelicorum*, we analysed causes for temporal and spatial variations in population dynamics by examining:

(1) annual growth rates, flowering ratios and the density of plants.

(2) temporal variations of population structure and recruitment.

(3) age stage structure of populations and long-term survival rates.

(4) existing critical stages in species life cycle.

(5) dependency of population dynamics on external factors, such as small-scale habitat structure, plant functional traits of surrounding vegetation and climatic factors.

Material & Methods

Management situation

T. integrifolia subsp. *vindelicorum* is a rare and highly endangered plant species in Bavaria/Germany. Its endemic distribution is restricted to the dry calcareous grasslands of the lower Lech valley in the south of Augsburg. Irregular or even absent land-use and management practices, increasing eutrophication and habitat destruction caused the loss of many populations during the last decades. Today only few occurrences of *T. integrifolia* subsp. *vindelicorum* still exist. The largest population consisting of two subpopulations is located on two sites within the military area "Lechfeld" (denominated as site C and site D). Until the early 1980ies, these sites have been grazed, but afterwards they have been fallowed for long time (Riegel, pers. comm). Immigration of high-competitive plant species, such as tall growing grasses (e.g. *Calamagrostis egigeios*) and shrubs, as well as the accumulation of plant litter has lead to a strong decline in population numbers and sizes. To protect the last remnant populations of *T. integrifolia* subsp. *vindelicorum*, sheep grazing has been applied as management regime since 2001, specifically adapted to species phenology. Annual countings of flowering individuals were used to monitor population dynamics and to assess positive or negative demographic developments. Although, grazing was considered to be an ideal method to improve habitat conditions for *T. integrifolia* subsp. *vindelicorum*, the annual population monitoring by counting flowering individuals could not reveal positive population

developments (Fig. 28). Therefore, permanent plots have been introduced in 2005 to monitor small-scale population developments and to detect potential risk factors for species long-term survival.

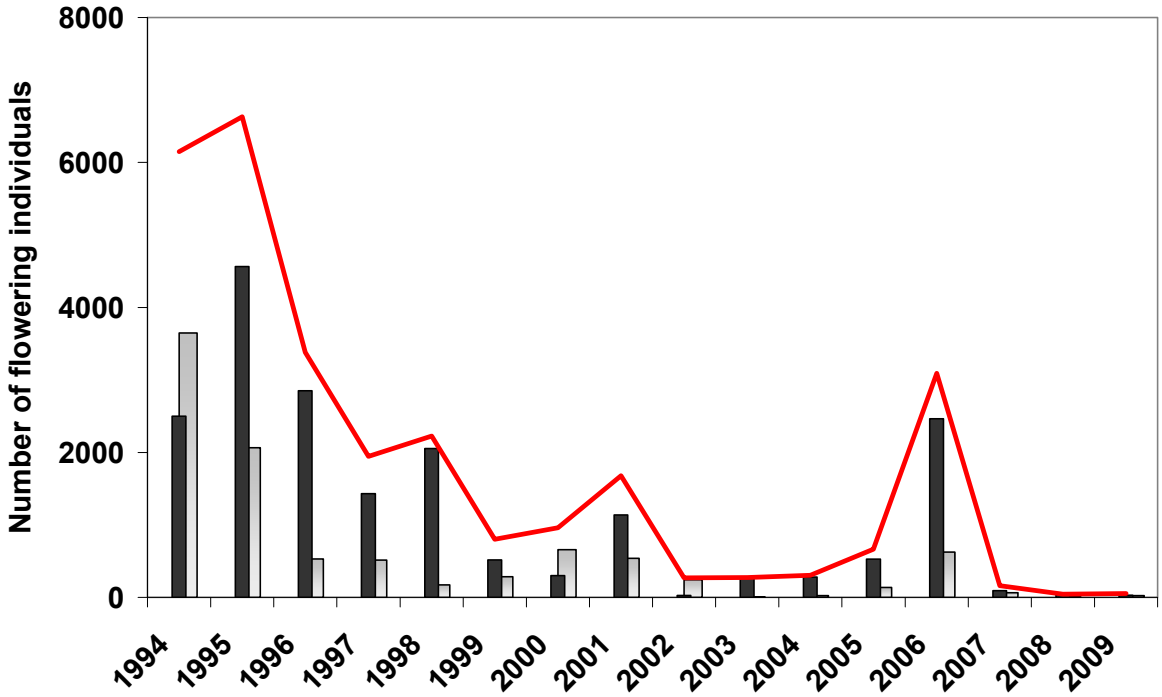


Fig. 28 Population dynamics of *T. integrifolia* ssp. *vindelicorum* at two sites in the military area Lechfeld (Bavaria) based on counts of flowering individuals from 1994 to 2009. Red line: total number of flowering plants on site C and site D, bright bars: number of flowering plants on site C, black bars: number of flowering plants on site D.

Species description

T. integrifolia subsp. *vindelicorum* is a perennial Asteraceae with striking yellow flowers. Its basal leaves are arranged in a rosette, which is densely covered by cottony hairs in spring. A single, erect flowering stem evolves in the center of the basal rosette. Flowering occurs from late May to late June, but not all individuals are able to flower in any given year. The number of flowering stem per population varies strongly among years, presumably due to weather conditions (Smith 1979; Widén 1987). Flowering stems usually develop two to six flower heads arranged in a terminal corymb. Each capitulum contains up to 90 tubular florets, which are self-incompatible (Meindl & Poschlod 2007). Dispersal of achenes by wind starts in July. Most seeds germinate during their first autumn and, due to a missing seed dormancy and germination even in darkness, they are not able to form a persistent soil seed bank (Meindl & Poschlod 2007). After fruit dispersal in summer, basal rosette leaves degenerate. Small lateral

buds may be developed in the axils of basal leaves and in the next spring, these buds may develop into daughter rosettes (Fig. 29). According to Smith (1979) daughter rosettes sometimes may become detached from the parent plant.

Fig. 29 Lateral shoots of *T. integrifolia* subsp. *vindelicorum*. Leaves and flowering stem of the mother plant in the center are degenerated. Two daughter rosettes originated in the axils of basal leaves.

Field methods

Spatio-temporal population dynamics of *T. integrifolia* subsp. *vindelicorum* have been surveyed by demographic studies in 56 permanent plots (4 m² each) within two subpopulations on site C and site D in the military area "Lechfeld" (in the following we used the more convenient term 'population' instead of 'subpopulation'). Individuals were localized through x and y coordinates (in cm) within each plot and tracked individually throughout the study period of five years. This method permits high efficiency even in dense covered plots. Exact position of individuals within the plots was translated into a coordinate system and visualized graphically (App. 1 & 2). At the peak of flowering in June, all individuals were mapped and characterized by reproductive status, height of flowering stem, number of flower heads per flowering stem and rosette diameters. Furthermore, population sizes were determined on the whole location by counts of flowering individuals in each year (Fig. 28).

In 2009, we additionally recorded habitat characteristics of each permanent plot by examining vegetation structure and abiotic factors. To describe species composition, the abundance of all vascular plant species was estimated by using the Braun-Blanquet scale. We estimated total

vegetation cover, the cover of moss, plant litter and the percentage of bare soil surface as indicators of vegetation cover. Light intensity penetrating to the ground was measured using a Sun Scan Canopy Analysis Systems (Delta-T Devices, Cambridge, England). We recorded photosynthetically active radiation (PAR) simultaneously at ground level as well as in full light above the canopy. Leaf Area Index (LAI) was calculated as percentage of ground level light to full light conditions. Light measurements were performed in spring 2009 and repeated two times in each plot.

Demographic analysis

Based on individual mappings, flowering rates as well as annual growth rates ($\lambda = n_{t+1}/n_t$) of flowering, non-flowering and total number of individuals were calculated to compare demographic variations between years and populations. Furthermore, the analysis of population age stage structure after five years shed light upon plant's life history strategy. To estimate mean survival rates of populations with heterogeneous age structure, depletion curves based on linear curve estimations were calculated. We used four initial states per population (2005, 2006, 2007 and 2008) to prove the survival potential of individuals by calculating population half life (HL) and time of expiration (T).

Transition probabilities from different life stages were evaluated by using the formula $n_i(t+1) = A*n_i(t)$, where $n_i(t)$ correspond to the number of individuals in stage *i* at time t and t+1, respectively (Caswell 1989). Following life cycle stages (*i*) were distinguished: (1) generative recruits, (2) vegetative recruits, (3) generative established plants, (4) vegetative established plants and (5) dormant or missing plants.

We used a Detrended Correspondence Analysis (DCA) of vegetation cover data to assess compositional gradients in vegetation structure and habitat characteristics. Furthermore, we calculated cover weighted means of Ellenberg indicator values for moisture, temperature, light and nutrients (Ellenberg *et al.* 1992). Correlations between axes scores and habitat variables were analysed using the relative Euclidean coefficient.

A plant functional trait analysis was performed in order to detect general rules and relationships between vegetation structure as well as habitat attributes and the different life cycle stages of *T. integrifolia* subsp. *vindelicorum*. We selected four plant functional traits, which were supposed to influence directly or indirectly plant performance and persistence in grasslands. The traits were: canopy height, canopy structure (rosettes, semi-rosettes, regularly

foliated), specific leaf area (SLA) and life form. Each plant species in the vegetation cover data was graded for each functional trait according to the attributes. Based on these plant functional traits and habitat characteristics per plot a Principal Components Analysis was performed and site scores were correlated with population structure data. Detrended Correspondence Analysis (DCA) and Principal Components Analysis (PCA) were performed using the software package PC-ORD 5.15. Correlation analysis and ANOVA were carried out with SPSS 17.0.

To test the influence of climatic variables on population dynamics and individual plant performances Pearson correlation analyses were made. Climatic data were provided by a meteorological station on the military base (App. 4). Using annual transition rates, flowering rates and recruitment as dependent variables, predications about weather induced temporal variations were possible. In many plants flower formation is dependent on size and growth of plants, which mostly occur in autumn and spring, as well as on temperature ranges during the winter period. Dependency of reproductive status on rosette sizes reached in the previous growing season was tested by using a non-parametric Whitney-U-test in SPSS 17.0.

Results

Annual growth rates, flowering ratios and density of plants

The demographic study of *T. integrifolia* subsp. *vindelicorum* was based on total mappings of 1,146 individuals encountered in the permanent plots on site C (App. 1) and 580 individuals on site D (App. 2) during five consecutive years. The total number of individuals within the permanent plots strongly varied among years and among populations (Tab. 11). Remarkable high fluctuations among years showed the population on site C. Until 2009, the size of population C has risen almost exponentially and, in comparison to the initial state of 2005, the number of plants increased more than six fold from 123 individuals to 788 individuals. Considerable lower was the population development on site D. In 2005 the studied population consisted of 156 plants. Until 2009, the population increased two fold in number to 314 plants. Plant densities within the studied permanent plots, which comprised a total size of 112 m² on each site, ranged between 1.1 and 7.0 plants per m² on site C and 1.4 and 3.6 on site D (Tab. 11).

Tab. 11 Total number of individuals, number of flowering individuals, number of vegetative (non-flowering) individuals, flowering rates and density of *T. integrifolia* subsp. *vindelicorum* in permanent plots within the study period from 2005 to 2009. n: number of individuals, %: percentage of individuals, n/m²: density.

Year	Population C						Population D					
	total		flowering		vegetative		total		flowering		vegetative	
	n	n/m²	n	%	n	%	n	n/m²	n	%	n	%
2005	123	1.1	27	22.0	96	78.0	156	1.4	62	39.7	94	60.3
2006	221	2.0	84	38.0	137	62.0	216	1.9	145	67.1	71	32.9
2007	235	2.1	49	20.9	186	79.1	213	1.9	18	8.5	195	91.5
2008	683	6.1	3	0.4	680	99.6	399	3.6	38	9.5	361	90.5
2009	788	7.0	20	2.5	768	97.5	314	2.8	9	2.9	305	97.1

On both study sites, the increase in population size from one year to the next was highest for the year 2007 to 2008 (Tab. 12). Within this time period growth rate on site C was 2.9 and on site D 1.9. In total, annual growth rates for population C varied from 1.1 (2006/2007) to 2.9 (2007/2008) and for population D from 0.8 (2008/2009) to 1.9 (2007/2008).

Despite the strong annual population increase, development of total flowering ratios evolved in a contrary way. Both sites showed strong annual fluctuations with maximum flowering rates of 38.0 % (site C) and 67.1 % (site D) in 2006, and minimum flowering rates of 0.4 % (site C) in 2008 and 2.9 % (site D) in 2009. Throughout the study period, the annual flowering rate of population D was higher than the annual flowering rate of population C (Tab. 11).

Tab. 12 Annual, mean and total growth rates of *T. integrifolia* subsp. *vindelicorum* in permanent plots within the study period from 2005 to 2009. N: number of individuals, SE: standard error.

	Population C			Population D		
	total	flowering	vegetative	total	flowering	vegetative
N_{2006}/N_{2005}	1.8	3.1	1.4	1.4	2.3	0.8
N_{2007}/N_{2006}	1.1	0.6	1.4	1.0	0.1	2.7
N_{2008}/N_{2007}	2.9	0.1	3.7	1.9	2.1	1.9
N_{2009}/N_{2008}	1.2	6.7	1.1	0.8	0.2	0.8
Mean λ	**1.8**	**2.6**	**1.9**	**1.3**	**1.2**	**1.6**
SE	0.4	1.5	0.6	0.2	0.6	0.5
Total $\lambda_{N2009/N2005}$	**6.4**	**0.7**	**8.0**	**2.0**	**0.1**	**3.2**

In general, we could observe a strong population increase on both study sites (black line in Fig. 30) coinciding with a strong decrease in flowering ratios (black bars in Fig. 30). In most of the study years, vegetative individuals dominated the population structure on both study sites, but there was also a high number of recruits (grey bars in Fig. 30). Regarding the proportion of generative individuals, flowering ratios declined from more than 50 % to less than 3 %. The observed negative development of flowering individuals within the study period 2005 to 2009 could not only be observed within the permanent plots, but also for the whole study sites (red line in Fig. 30).

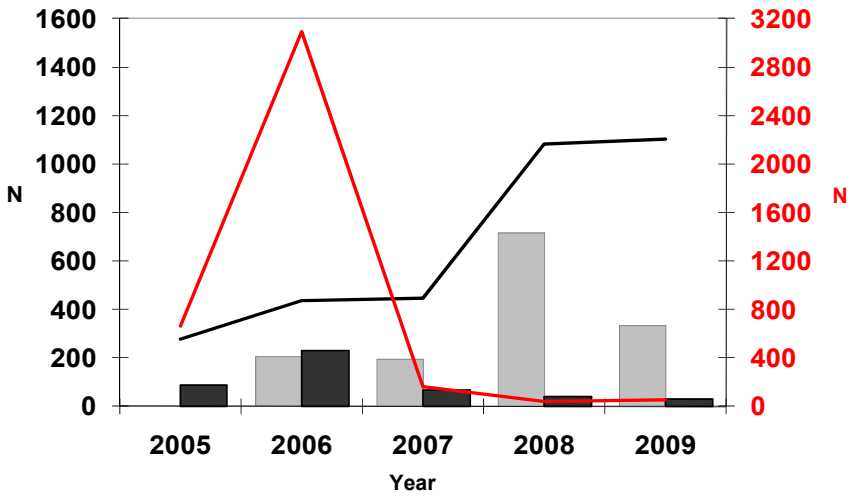


Fig. 30 Population dynamics of *T. integrifolia* subsp. *vindelicorum* within permanent plots on site C and D from 2005 to 2009. *Primary axis (black)* - black line: total number of plants within permanent plots, grey bars: recruits within permanent plots, black bars: flowering individuals within permanent plots. *Secondary axis (red)* - red line: countings of flowering individuals on the whole study sites.

Population structure and recruitment

Population structure of *T. integrifolia* subsp. *vindelicorum* could be classified by the proportion of plants in different life stage categories in each year. We distinguished five categories: flowering recruits (1), vegetative recruits (2), flowering established plants (3), vegetative established plants (4) and dead or missing plants (5).

The balance between established plants and recruits on site C has fluctuated over time (Fig. 31). In the study period from 2006 to 2007, there were as much established plants as recruits. In the following year, remarkable high numbers of recruits could be recorded. More than 75 % of all mapped individuals were plants, which emerged for the first time. In the year 2009, the positive development of recruitment for site C was lower, but still high (34.5 % of

all recorded individuals were recruits). On average, established plants accounted for 46.9 % and recruits on average for 53.1 % on site C.

On site D, we had also annual fluctuations concerning the balance between established plants and recruits (Fig. 32). Throughout the study period, annual recruitment on site D was lower than the proportion of established plants. Mean annual percentage of established plants accounted for 63.5 % and of recruits for 36.5 %.

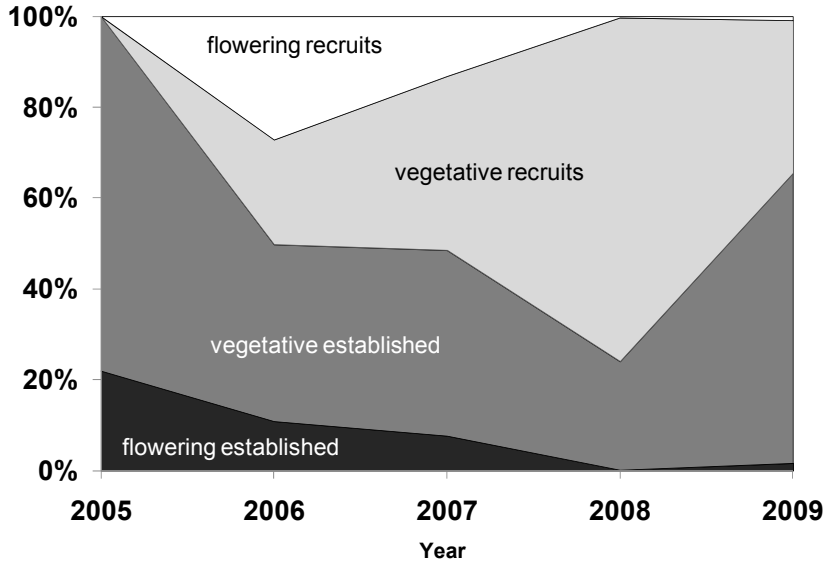


Fig. 31 Temporal variation of *T. integrifolia* subsp. *vindelicorum* within permanent plots from 2005 to 2009 considering percentage of four different life cycle stages on site C.

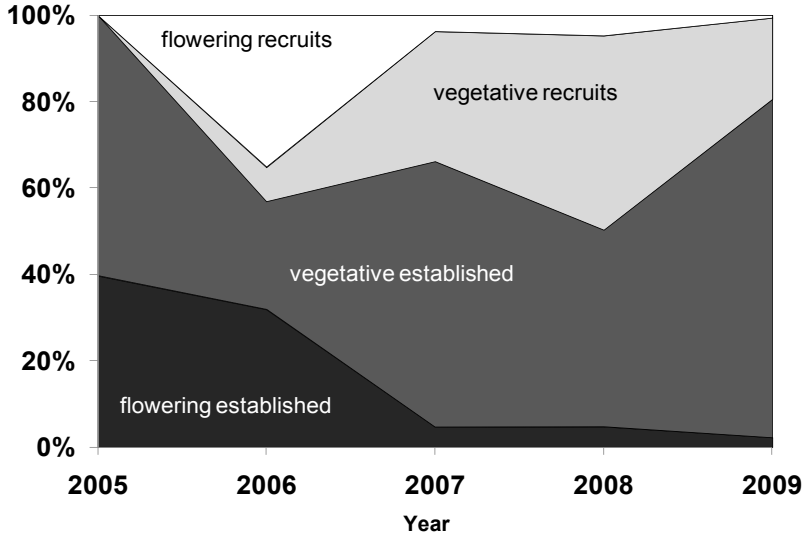


Fig. 32 Temporal variation of *T. integrifolia* subsp. *vindelicorum* within permanent plots from 2005 to 2009 considering percentage of four different life cycle stages on site D.

Age stage structure and long-term survival rates

On both study sites, the observed age stage structure after five years was dominated by recruits and two-year-old plants (Fig. 33). In population C the number of individuals aged one and two years covered more than 80 %. On site D, more than a half of all individuals in 2009 (55 %) belonged to the age classes one and two. Some plants could be recorded in every year of the study period and due to this their minimum age must be five years. Plants, which were absent in only one year of the study period, were regarded to have passed a dormant stage and ranked among the category of five-year-old plants. In population C the proportion of five-year-old plants reached only 5.5 %, whereas in population D 21.0 % belonged to this category. In the age stage category of three- and four-year-old plants, the number of individuals in population D exceeded the number of individuals in population C.

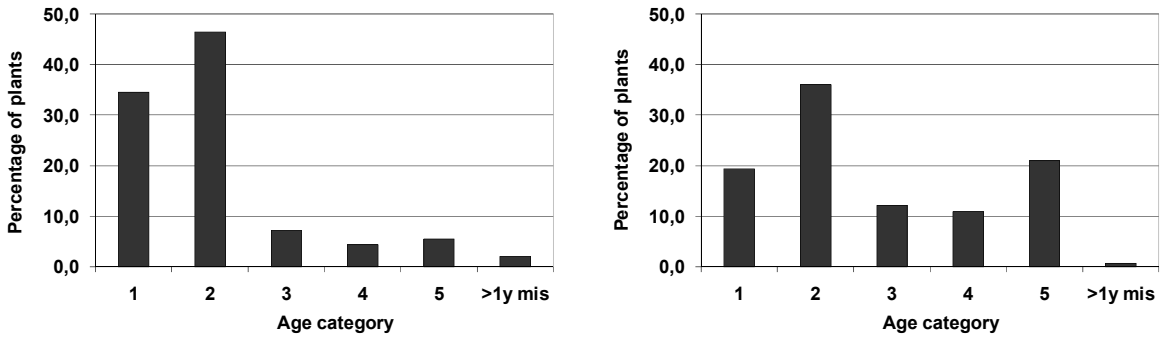
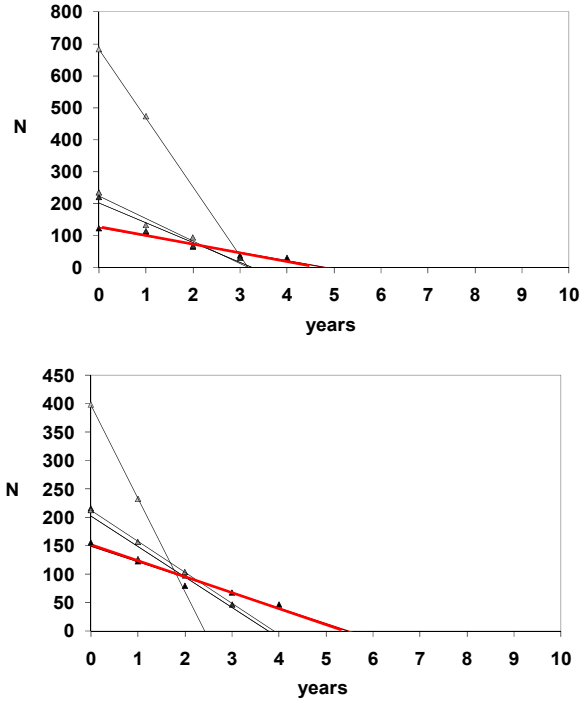


Fig. 33 Population structure of *T. integrifolia* subsp. *vindelicorum* on site C (left) and D (right) considering the final study year 2009. $N_{Pop\ C}$ = 788, $N_{Pop\ D}$ = 314, >1y mis = individuals missing for more than 1 year.

In both studied populations, depletion curves gave significant indication for a rather short survival rate of *T. integrifolia* subsp. *vindelicorum* (Fig. 34). In population C, the time of expiration ranged between 3.2 and 4.8 years, in population D between 2.4 and 5.5 years. After this time no individual of a given population would be alive. Although, the time of expiration in population D fluctuated more strongly among the different initial states than in population C, the mean values of both populations are quite comparable ($T_{mean}C = 3.7$; $T_{mean}D = 3.9$). Half-life time of a population ranged between 1.5 and 2.4 years for population C and 1.2 and 2.6 years for population D.



Depletion curves	Site C	Site D
(a) 2005 until 2009		
Initial state of population	123	156
Function	y = -25.9x + 124.8	y = -27.3x+149.4
R^2	0.948	0.96
p	0.005	0.003
Half life (HL)	2.4	2.6
Time of expiration (T)	4.8	5.5
(b) 2006 until 2009		
Initial state of population	221	216
Function	y = -61.9x + 201.1	y = -53.6x+202.4
R^2	0.935	0.954
p	0.033	0.023
Half life (HL)	1.5	1.8
Time of expiration (T)	3.3	3.8
(c) 2007 until 2009		
Initial state of population	235	213
Function	y = -70.5x + 224.5	y = -54.5x+212.5
R^2	0.938	0.999
p	n.s.	0.01
Half life (HL)	1.5	1.9
Time of expiration (T)	3.2	3.9
(d) 2008 until 2009		
Initial state of population	683	399
Function	y = -209x + 683	y = -166x+399
R^2	1	1
p	-	-
Half life (HL)	1.6	1.2
Time of expiration (T)	3.3	2.4

Fig. 34 Depletion curves of *T. integrifolia* subsp. *vindelicorum* on site C (above) and D (below) regarding four different initial states. Linear curve estimations were used to calculate population half life and time of expiration. Red regression lines symbolize the development of populations from 2005 to 2009. Regression coefficients (R^2) and p-values are given in the table on the right. n.s. = not significant.

Transition rates of life cycle stages and demographic effects

During the study period of five years demographic transition probabilities of different life cycle stages varied among years as well as among populations (Fig. 35 + Fig. 36). Population structure was strongly affected by these variations resulting in three main demographic effects: (1) progressive effects, which enhanced population long-term survival potential especially by high flowering ratios and recruitment, (2) static effects, which focussed on population persistence and (3) regressive effects, which diminished population long-term survival potential. The regressive status was represented by adult plants, which did not appear above ground for one year (dormant stage) and plants which did not appear above ground for two and more years (missing/dead stage). Plants of the dormant stage were regarded to be able to change back into a vegetative status; plants of the missing/dead stage were considered to be missing or dead.

Regressive effects – The most critical pathway in plant's life cycle seemed to be the transition from recruits into the adult status. In most cases (up to 50 %), recruits were not able to appear in the next growing season and fell into a regressive status (missing/dead). Flowering recruits were more often affected by regressive effects than vegetative recruits (on

site C 49.5 % of flowering recruits and 34.5 % of vegetative recruits; on site D 48.5 % of flowering recruits and 38.8 % of vegetative recruits).

Another critical life stage for long-term population survival was the high mortality/dormancy rate of adult plants both of flowering and non-flowering individuals. About one third of all adult plants tend to disappear in the following growing season. Especially in the year $t_{2006} \rightarrow t_{2007}$, remarkable high numbers of flowering adult plants changed into the missing/dead status (Fig. 36). However, some plants of the regressive status seemed to be able to return after a dormant year into a vegetative adult status (up to 25 %).

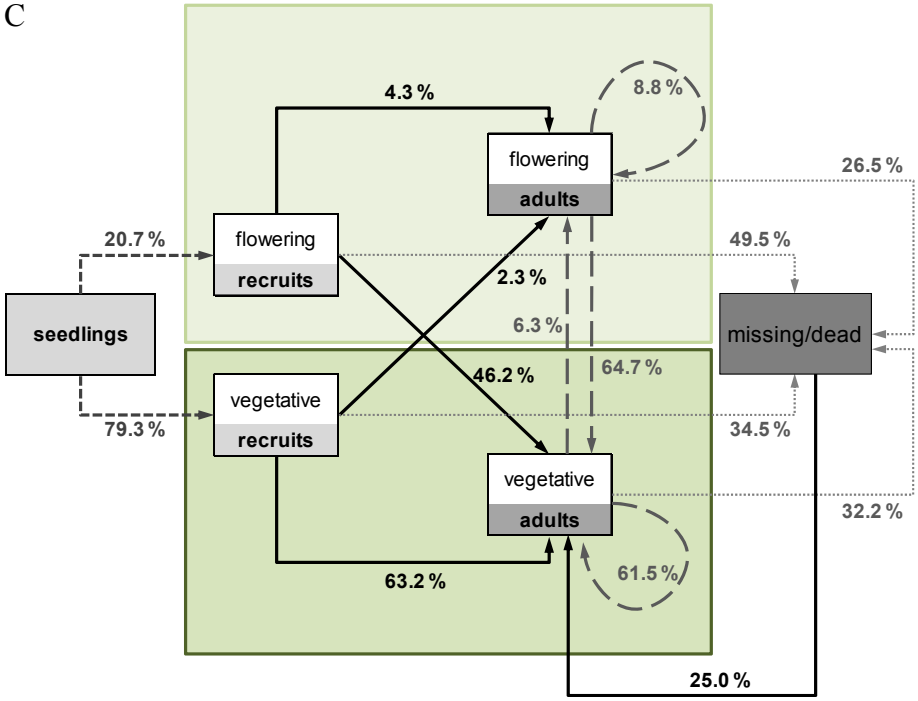
Progressive effects – Contributions to a more progressive population structure consisted mainly of high numbers of seedlings or bud-originated daughter rosettes, which emerged for the first time. On average, 20 to 26 % of all recruits were able to flower in their first year of emergence.

However, the total number of flowering individuals (both recruits as well as adults) was rather low: less than 5 % of the last year's recruits (both flowering as well as vegetative recruits) were able to reach the flowering adult status. For vegetative adult plants, the probability to change into a flowering status was also rather scarce, but on site D the probability was slightly higher than on site C (vegetative plants: 13.4 % on site D and 6.3 % on site C). 14.8 % of all generative adults on site D and 8.8 % of all generative adults on site C were able to flower two times in series. Percentage of flowering recruits, which were able to flower in the following year, was higher on site C than on site D (4.3 % versus 1.0 %).

Most progressive effects differed strongly among years. One of the most striking annual variation could be detected in the transition matrix from t_{2006} to t_{2007} (Fig. 36), where we had the highest percentage of recruits, which flowered in their first year of emergence (54.1 % flowering recruits on site C, 81.7 % flowering recruits on site D).

Static effects – Static effects were regarded to be transitions of recruits as well as adults from both reproductive stages into a vegetative stage. On both sites, most recruits of *T. integrifolia* subsp. *vindelicorum* changed into a non-flowering adult status in the year t+1 (from 46.2 % to 63.2 %). Furthermore, vegetative adult plants showed a high affinity to stay vegetative in year t+1 (61.5 % on site C and 56.4 % on site D). Regarding flowering adult plants in year t, more than 50 % changed into a vegetative stage in year t+1.

Population C



Population D

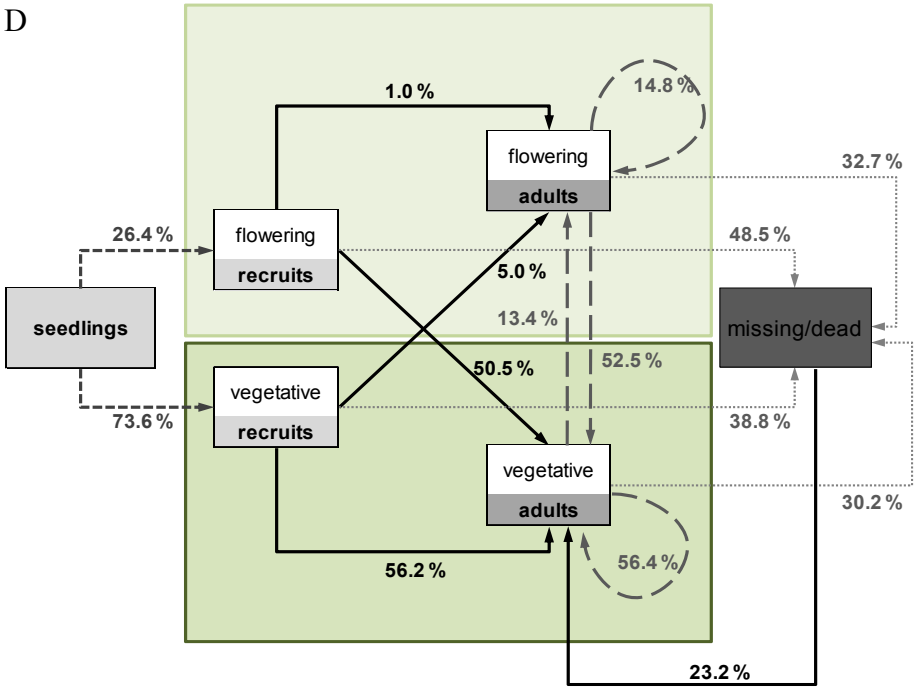
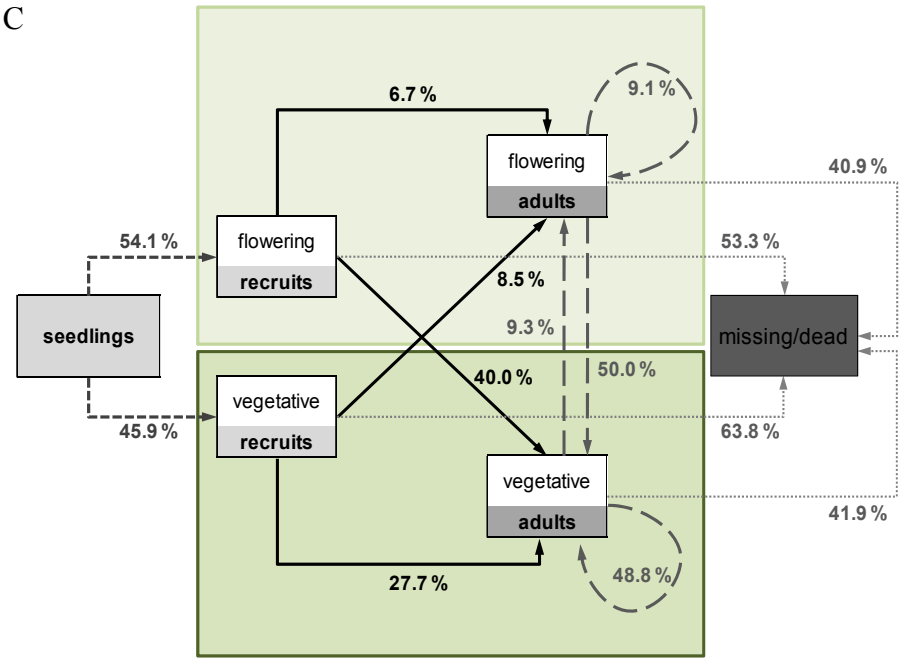


Fig. 35 Mean transition probabilities (%) of different life cycle stages of *T. integrifolia* subsp. *vindelicorum* on site C (above) and D (below). Annual transition probabilities ($t_n \rightarrow t_{n+1}$) were calculated from one year to the following for n = 2005, 2006, 2007 and 2008 and averaged.

Population C



Population D

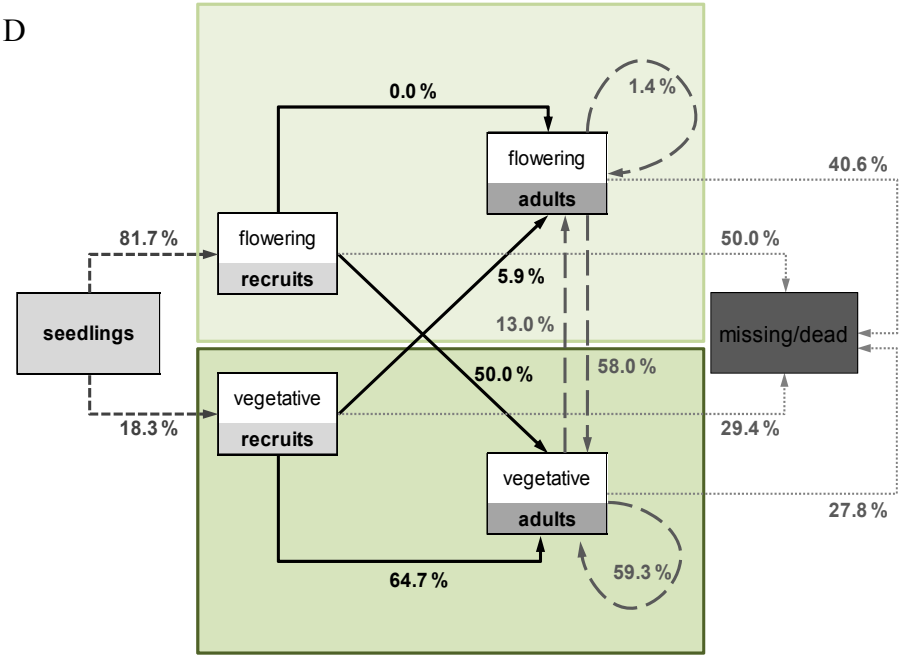


Fig. 36 Annual transition probabilities (%) of different life cycle stages of *T. integrifolia* subsp. *vindelicorum* on site C (above) and site D (below). Annual transition probabilities were calculated from the year 2006 to the year 2007 (t_{2006} → t_{2007}). Within this time period populations showed highest flowering ratios.

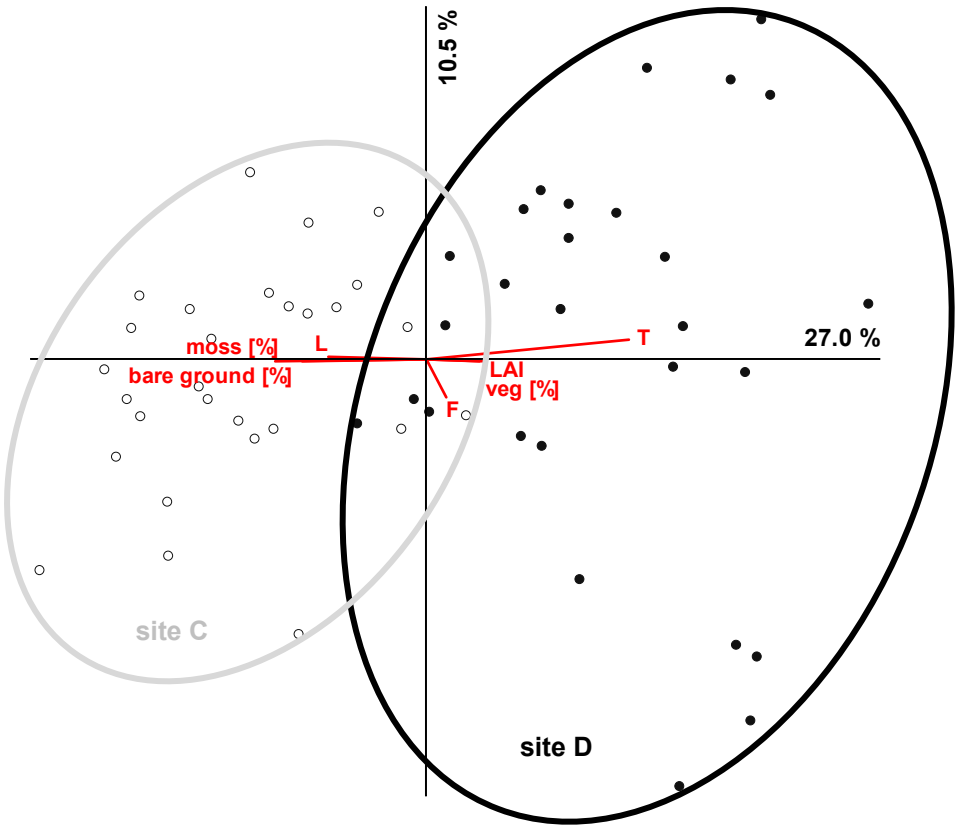


Fig. 37 Characterization of 56 permanent plots on two study sites of *T. integrifolia* subsp. *vindelicorum* by Detrended Correspondence Analysis (DCA). ○ plots on site C, ● plots on site D.
Main matrix: calculation of site scores based upon abundances of 87 species. *Second matrix*: Calculation based upon 10 site specific variables. Diagram was carried out with cut-off R^2 = 0.1. *Vectors: L* = Ellenberg indicator value for light; *F* = Ellenberg indicator value for moisture; *T* = Ellenberg indicator value for temperature; *veg [%]* = percentage cover of vegetation; *moss [%]* = percentage cover of moss; *bare ground [%]* = percentage cover of bare ground; *LAI* = leaf area index.

Abiotic and biotic characterization of habitat structures

A DCA ordination diagram of vegetation relevés revealed a clear floristic gradient among plots on site C and site D along the first axis (Fig. 37). Several habitat parameters showed strong correlations with the axes and could be used to characterize the two different study sites. The distribution pattern of plots indicated a more heterogeneous vegetation and habitat structure on site D than on site C. Plots on site D were strongly scattered along the first (27.0 %) and second axis (10.5 %) caused by a more varying species composition.

The most important site specific differences between plots on site C and plots on site D were the percentage of bare ground, moss cover, vegetation cover and Ellenberg indicator values for light and temperature. Typical habitat characteristics of plots on site C were high percentages of moss (r = -0.603), bare ground (r = -0.548) and a large fraction of species with high Ellenberg indicator values for light (r = -0.486).

Plots on site D were mostly characterized by a high abundance of species with high Ellenberg indicator values for temperature (r = 0.699), percentage of vegetation cover (r = 0.377) and LAI (r = 0.357). Furthermore, site D consisted of plots with dense as well as plots with sparse vegetation cover. In contrast, on site C all plots were more or less uniform consisting of sparse vegetation with low percentages of vegetation cover.

Habitat model - correlation of habitat characteristics and population structure

To detect general correlations between habitat and population characteristics of *T. integrifolia* subsp. *vindelicorum*, we calculated a Principal Coordinate Analysis (PCA) on the basis of plant functional traits and habitat characteristics (Fig. 38). In a second approach, population characteristics of the two studied *T. integrifolia* subsp. *vindelicorum* populations were used to detect correlations between recruitment, flowering behaviour, age stage categories and specific habitat characteristics.

Basically, variables such as percentage of vegetation cover (r = 0.664), bare ground (r = -0.672), 'specific leaf area' (SLA; r = -0.505) and 'evenness of species distribution' (r = -0.563) showed high correlations with the first axis. Ellenberg indicator value for light and percentage of moss were negatively correlated with the first axis (r_L = -0.553; $r_{\%moss}$ = -0.470). The highest positive correlations with the second axis showed Ellenberg indicator values for nutrient (r = 0.611) and moisture (r = 0.705) as well as the plant functional trait 'canopy height' (r = 0.541).

According to population characteristics, the analysis revealed a high negative correlation of young individuals with the first axis (Fig. 38; recruits: r = -0.508; 2-year-old individuals: r = -0.482). Plots with high contents of young plants were also characterized by high percentages of bare ground, moss cover, light availability and evenness of species distribution. Most plots with remarkable high abundances of young individuals belonged to site C. Most plots on site D were characterized by high percentage of vegetation cover and low percentage of bare ground as well as low light availability.

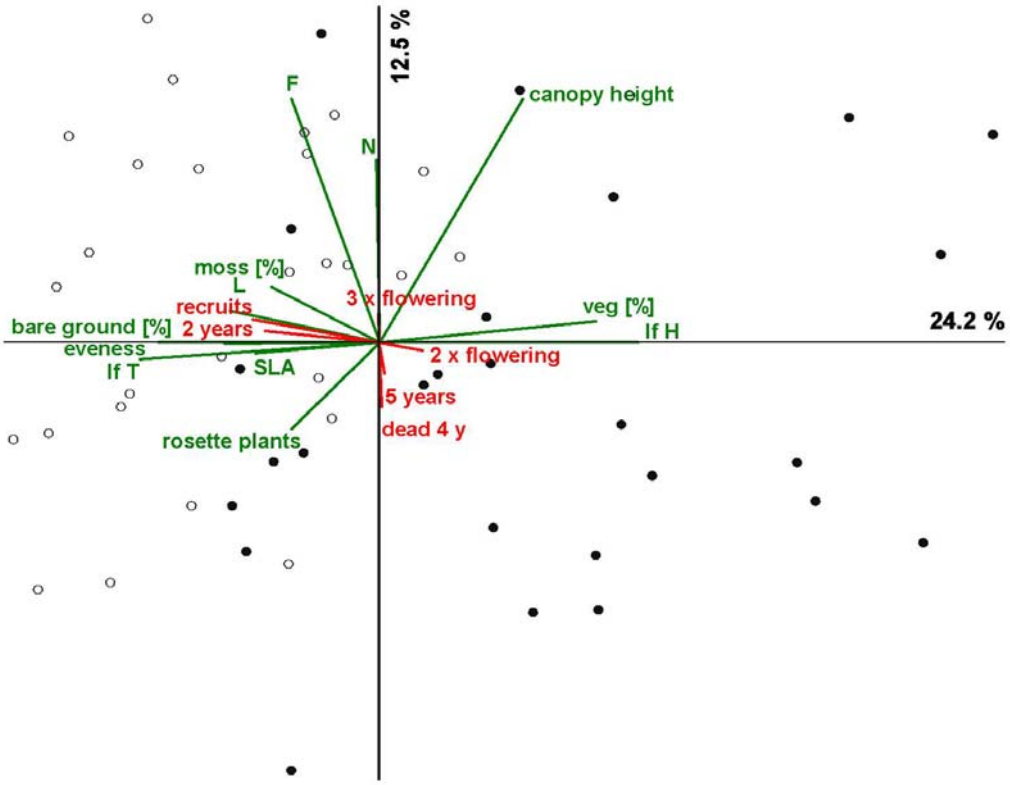


Fig. 38 Principal Component Analysis (PCA) of plant functional traits and site specific parameters within 56 plots of two *T. integrifolia* subsp. *vindelicorum* populations. Analysis was based on 20 plant functional traits and site specific parameters. ○ plots on site C, ● plots on site D.

green vectors - plant functional traits and site specific parameters: SLA = Specific leaf area; lf T = life form therophyt; lf H = life form hemicryptophyt; *L* = Ellenberg indicator value for light; *F* = Ellenberg indicator value for moisture; *N* = Ellenberg indicator value for nutrient; *veg [%]* = percentage cover of vegetation; *moss [%]* = percentage cover of moss; *bare ground [%]* = percentage cover of bare ground;.

red vectors – population characteristics: *5 years* = individuals, which occurred in every year of the study period; *2 years* = individuals, which were mapped in 2008 and 2009; *recruits* = individuals, which were mapped firstly in 2009; *3(2) x flowering* = individuals flowering in three (two) years of the study period; *dead 4y* = individuals, which disappeared after 4 years.

Individuals, which were able to flower two times in series (2 x flowering: r = 0.296), were more often located in plots on the right side of the ordination diagram, which were typical for site D and plots, which were characterized by a medium-dense vegetation cover. Individuals flowering even three times in series were typical for plots located on the positive side of axis 2 (3 x flowering: r = 0.241) and which were characterized by parameters such as high indicator values for nutrient as well as moisture and canopy height. Occurrences of 5-year-old individuals (5 years: r = -0.252) and individuals, which have died after four years of mapping (dead 4 y: r = -0.362), were negatively correlated with the second axis. Plots with high numbers of these ‘old plants’ showed also quite high abundances of rosette plants.

Influences of weather conditions on population dynamics and plant performances

Transition probabilities and plant performances varied strongly among years and were most likely the result of different temperature and rainfall patterns during the growing season (App. 3). Despite the short study period demographic data could give some crucial indications for potential climate dependencies.

Flowering – In terms of flower formation, winter and spring temperatures as well as precipitation in spring seemed to be most influential. Cold temperatures during the winter months of December, January and February, expressed by low minimum values, enhanced the chance for previously flowering individuals to flower again and for vegetative adult plants to change into a generative status in the following growing season (Fig. 39 a+b). Furthermore, the number of flower heads increased with extremely low minimum temperatures in December and spring (Fig. 39 c + d). In contrast soft winters resulted in a lower probability for flower formation in previously flowering recruits (Fig. 39 e). High precipitation values in the spring months increased the probability for flowering adult plants to flower again in the following year (Fig. 39 f).

Lack of rainfall in the spring months (March, April, May) coinciding with high minimum temperatures prevented flowering recruits to flower again in the following year (Fig. 39 g + h). The lack of extremely low minimum temperatures in spring and high values of precipitation were ideal conditions to support flower formation both in non-flowering recruits and established plants. Moderate summer temperatures in the previous growing season were positive for flower formation and resulted in a high transition rate of vegetative adults to flowering adults in the next growing season.

Survival – Both flowering and vegetative recruits seemed to be negatively affected by high summer temperatures resulting in low survival rates (Fig. 40 i + j). Furthermore, there were many recruits, which disappeared after a warm winter characterized by high minimum, maximum and medium values, especially in the months October and December. During the spring months of March, April and May, high temperatures and low precipitation had negative effects on the survival of recruits (Fig. 40 k + l, m + n). Especially in March and April, the combination of high temperature and low rainfall expressed as the proportion of precipitation and temperature, increased the disappearance of recruits as well as adult individuals.

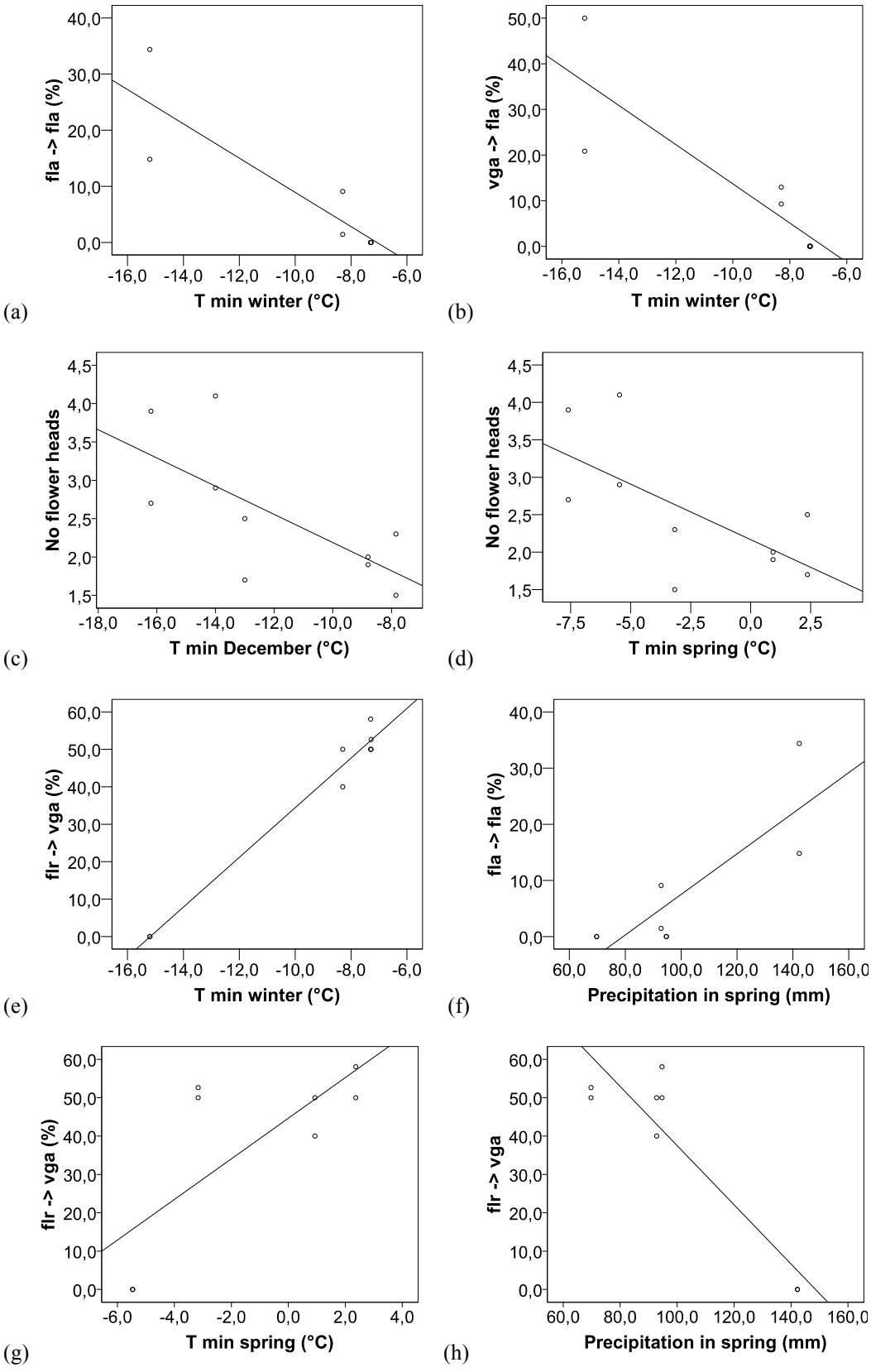
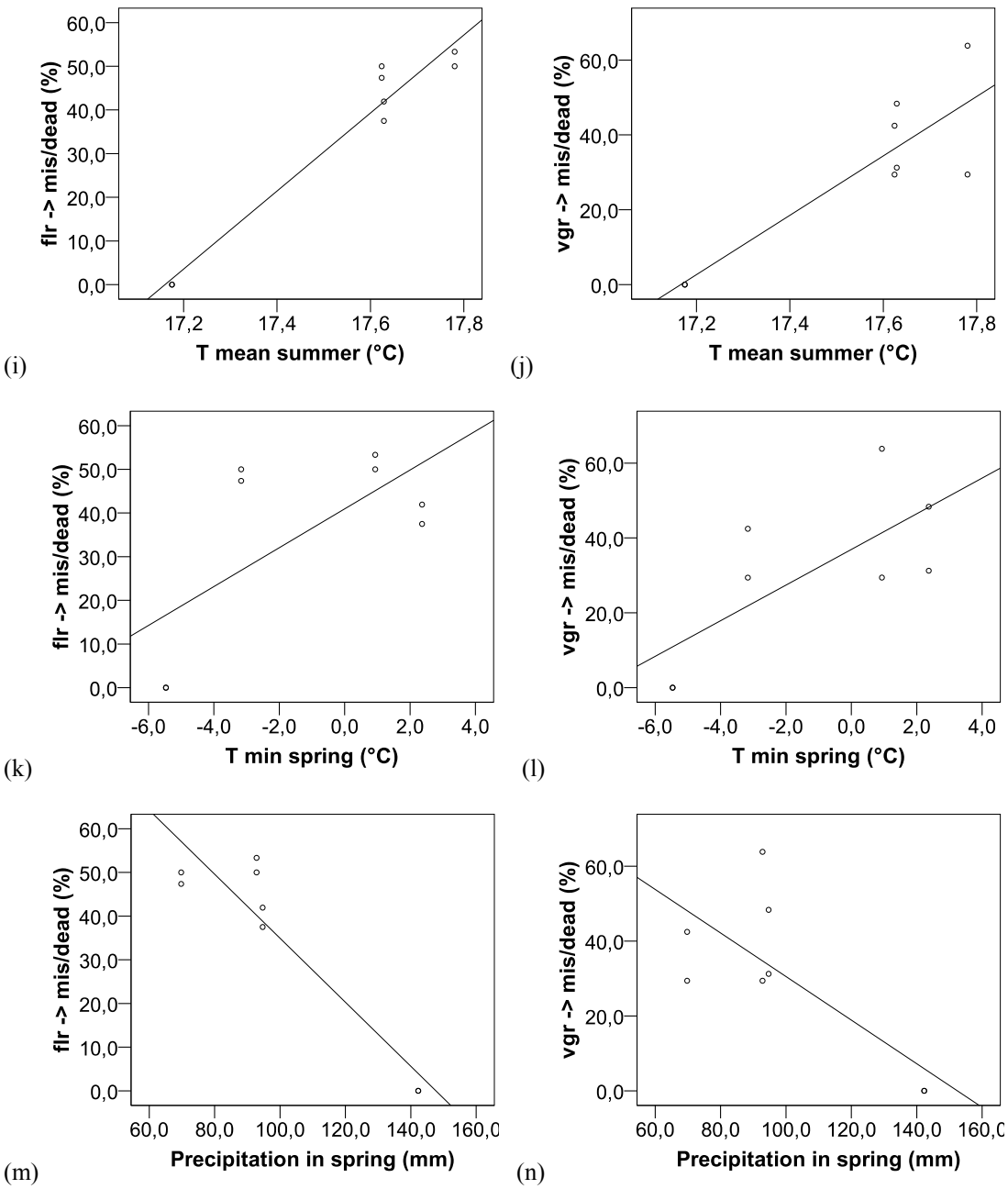


Fig. 39 Pearson correlation of transition probabilities, reproductive parameters and weather variables. fla: flowering adult plant, vga: vegetative adult plant, flr: flowering recruit, vgr: vegetative recruit, T min: minimum temperature.



(i) (j) (k) (l) (m) (n)

Fig. 40 Pearson correlation of transition probabilities and weather variables. flr: flowering recruit, vgr: vegetative recruit, mis/dead: missing or dead individuals, T min: minimum temperature, T mean: mean temperature.

Discussion

Population characteristics and plant life strategy

The present study revealed high spatio-temporal variation in demographic parameters of *T. integrifolia* subsp. *vindelicorum*. Although habitat type and management regime have been on a comparable level on both study sites, small-scale habitat differences seemed to have caused remarkable variations in population structure.

On site C, analysis of population structure during five years revealed a more dynamic population type, while population structure on site D corresponded to a more stable population type. Dynamic populations usually show high densities and a higher proportion of seedlings and juveniles than adult plants, whereas regressive (or senile) populations are characterized by low density or lacking recruitment (Oostermeijer *et al.* 1996). Finite rates of increase ranging about $\lambda = 1$ signify stable population structures comprising as much recruits as adults. On site C, we had about 80 % of recruits and juveniles (two-years-olds), while on site D about 50 % belonged to these two stage categories. Furthermore, the annual finite rate of increase on site D ranged more often about 1.0, while on site C the annual finite rate of increase was consequently higher and reached 2.9 in 2007/2008.

Dynamic population types are often advantageous for unpredictable and frequently disturbed environments, where seed production and recruitment are more important than survival of adults (Schmid & Matthies 1994). In stable habitats with less intensive disturbances, plants more often focus on persistence by long-term survival of established individuals. Life strategy of *T. integrifolia* subsp. *vindelicorum* seemed to correspond to the more dynamic population type profiting from large habitat heterogeneity and annually changing vegetation structures provided by sheep grazing as management regime.

Age stage structure & long-term survival

Life span of *T. integrifolia* subsp. *integrifolia*, sibling species to *T. integrifolia* subsp. *vindelicorum*, is specified by literature as short- to long-lived perennial (Smith 1979; Widén 1993). Smith (1979) suggested by rootstock observations, that *T. integrifolia* might be a rather short-lived plant species. Widén (1987) calculated the expected half-life of *T. integrifolia* in four Swedish populations to between 7.2 and 39.3 years (Isaksson 2009).

Results of the present study gave indication for a rather short life span of *T. integrifolia* subsp. *vindelicorum*. Depletion curves, indicating the time until no individual of a given population would be alive, determined the mean time of expiration to 3.7 (population C) and 3.9 (population D) years. Half-life time ranged between 1.2 and 2.6 years for the studied populations. This is a quite short life span and not comparable to the values of *T. integrifolia* in Sweden. However, differences of more than 30 years within the four Swedish populations might indicate a high life span plasticity of *T. integrifolia*. Varying ecological and environmental factors (e.g. temperature, nutrients, moisture, disturbances, inter- and intraspecific competition) strongly influence the individual life span of species (Schweingruber & Poschlod 2005). Populations of *T. integrifolia* might be able to colonize habitats with varying ecological and climatic conditions as well as different management types. Therefore, life span variations might be possible.

However, the life span of a plant is a very important trait affecting plant's persistence and competitiveness. Populations of long-lived species may persist for extended periods of time, even when recruitment is no longer occurring (Eriksson 1996). Especially in fragmented habitats short-lived species (annuals, biennials) are reported to have a higher extinction risk than long-lived species (Fischer & Stöcklin 1997). Consequently, management programs for *T. integrifolia* subsp. *vindelicorum* should be aimed at the maintenance and improvement of favourable habitat structures, which are crucial for a high level of recruitment. Safe sites for germination might counteract the threat of population decrease and enhance population's long-term survival.

Critical life cycle stages and habitat requirements

The most critical phase in life cycle of *T. integrifolia* subsp. *vindelicorum* seemed to be the establishment of recruits. Almost one half of all recruits failed to evolve into an adult stage in the following year. Results from vegetation and habitat analyses demonstrated that patterns of recruitment were strongly related to vegetation structures.

In particular, the percentage of bare ground had a strong impact on recruitment. The number of recruits increased in plots with high cover percentages of bare ground as well as the presence of a bryophyte layer. On site C, population structure was dominated by 80 % of recruits and juveniles (two-years-olds), while on site D about 50 % belonged to these two stage categories. Most plots on site C were characterized by a quite sparse vegetation cover

interspersed with parts of bare ground and high proportions of cryptogams. Vegetation composition was dominated by small grass species (e.g. *Carex humilis*), rosette plants (e.g. *Plantago media*) and semi-parasitic species (e.g. *Thesium linophyllon*, *Rhinantus minor*) creating favourable conditions for a low-competitive plant species such as *T. integrifolia* subsp. *vindelicorum*.

This is in accordance to several other studies of grassland species. Interspecific competition is denoted to be one of the main risk factors for low-competitive grassland species suffering from succession after field abandonment (Bobbink & Willems 1987; Kahmen *et al.* 2002; Moog *et al.* 2002). Especially the dependency on safe sites (sensu Harper 1977) is reported for many herb species in calcareous grasslands (Gross & Werner 1982; Klinkhamer & Jong 1988; Silvertown & Smith 1989; Rusch & Fernández-Palacios 1995; Bakker & Olff 2003). Plots on site C were characterized by high amounts of safe sites for germination of *T. integrifolia* subsp. *vindelicorum* and this might be the reason for high finite rates of population increase. On site D, sheep grazing intensity was less intense and therefore, impact on habitat structure, especially the creation of safe sites for germination, was reduced. Dense vegetation structures and low percentages of bare ground diminished the rates of recruitment in comparison to site C. In a grazing experiment, Bullock et al. (1994b) could also detect rising finite rates of increase for *Cirsium vulgare* with increasing grazing intensity, because removal of plant litter strongly improved germination conditions.

Another reason for high numbers of recruits might have been the ability of *T. integrifolia* subsp. *vindelicorum* to produce daughter rosettes in the axils of basal leaves. In many plants of unpredictable environments, the formation of axillary shoots is very common, not as a mode of clonal reproduction, but rather as a way to increase seed production (Groenendael & Slim 1988). Stimulated by damages, *T. integrifolia* subsp. *vindelicorum* might have been able to produce side rosettes from axillary buds. Such dormant buds are also known from other plant species in calcareous grasslands. Wildeman & Steeves (1982) reported on a reserve of buds for *Pulsatilla patens*, which can be activated by external factors (e.g. trampling, feeding, cutting) and were able to form new shoots in the event of damage to the growing region. During grazing, many plots on site C were lacking of protecting plant material and were strongly affected by sheep trampling. On site D, a short, but dense vegetation cover may have buffered largely destructive effects on the sensitive growing region of rosettes. This might be the reason for lower recruitment on site D than on site C.

Patterns of recruitment in *T. integrifolia* subsp. *vindelicorum* gave some indication for a reservoir of dormant buds. Although it was not possible during this study to differentiate between seed originated and bud originated recruits without digging the individuals out to examine potential connections between individuals below ground, we presumed a certain amount of bud-based recruits in each year. Especially in the last study years, characterized by only few flowering individuals and extremely high numbers of recruits in plots on site C, the expansion of mother plants by daughter rosettes might have been a possible reason for increasing recruitment numbers. Lacking correlation between emerged recruits and proportion of flowering individuals in the previous year underlined a certain influence of side rosettes to the total number of recruits, especially because seeds were not able to persist in a soil seed bank for long time (Meindl & Poschlod 2007).

To uncover the total extent of vegetative recruitment by axillary buds in comparison to generative recruitment by seeds, further investigations would be promising. Therefore, the systematic mapping of seedlings in autumn or spring would be useful to solve this question. Another possibility would be the analysis of the small-scale genetic structure of local patches of *T. integrifolia* subsp. *vindelicorum* by molecular methods. The detection of individual genetic relationships in combination with long-term demographic analyses would help to elucidate the exact extent of vegetative reproduction to population structure.

Variations in transition rates of life cycle stages between site C and site D indicated that there might have been further habitat specific relations. Higher numbers of recruits flowering two times in series in plots on site C than on site D might be influenced by higher light availability due to the more open vegetation cover. Regarding survival rates of adult plants, the present study revealed a higher percentage of individuals in plots on site D than on site C. Close, but short-growing vegetation structures with rather low percentages of moss cover and bare ground seemed to be favourable to older individuals. Plots with high occurrences of at least five year old individuals were characterized by rather low nutrient availability and rather dry soil conditions, both factors which prevent the growing of high competitive species.

This is in contrast to most recruits of *T. integrifolia* subsp. *vindelicorum*, which preferred open habitat structures with high percentages of bare ground and cryptogam layers. However, findings from other studies could also reveal different habitat requirements for recruits and established plants (Losos 1995; Ehrlen & Eriksson 2000; Gustafsson *et al.* 2002). The survival potential of adult individuals of *T. integrifolia* subsp. *vindelicorum* might have been

associated with buffering capacity of surrounding vegetation against severe climatic variations (Keizer *et al.* 1985). Especially high temperatures and drought in summer and spring could be recognized as main factors for the transition of adult plants into a regressive stadium. In plots with denser vegetation cover, evapotranspiration of rosette leaves might be reduced and protect them against exsiccation. Furthermore, the presence of a dense vegetation cover may have served as protection for the overwintering shoots and thus reduced winter mortality (Ryser 1993).

Flowering effort & climatic variations

Interactions between plant performance and annual climatic fluctuations are complex, because weather variables may differentially affect flowering ratios, recruitment and vegetative growth. Our investigations focussed mainly on relations between climatic variations and flowering capacity of previous and current growing seasons.

Low temperatures in winter and early spring, for example, seemed to be beneficial for flower induction and enhanced the formation of flower heads. Flowering capacity might be reduced, if minimum temperatures in the winter months did not fall long enough below a certain threshold. This might be the result of vernalization processes, which are reported to be essential for certain plant species (Werner & Caswell 1977; Gross 1981). Especially in a seasonally varying environment, flower formation of some plant species is known to be regulated by temperature (Chouard 1960). In biennials as well as winter annuals, the importance of low temperatures for flowering is widely recognized and reported for species as *Thlaspi arvense* (McIntyre & Best 1978), *Arabidopsis thaliana* (Napp-Zinn 1987), *Daucus carota* (Lacey 1988) and *Beta vulgaris* ssp. *maritima* (Van Dijk *et al.* 1997). Even for perennial rosette plants, such as *Saxifraga rotundifolia*, *Draba aizoides*, *Scabiosa canescens* and various species of *Primula*, *Potentilla*, *Bromus* and *Festuca*, the exposure to the prolonged cold of winter and early spring is necessary to acquire the competence to flower in the spring (Chouard 1960).

Beside environmental effects on flower formation, internal effects might be responsible for variations in flowering capacity of *T. integrifolia* subsp. *vindelicorum*. As reported by Werner (1975) for *Dipsacus sylvestris*, especially biennial rosette plants seem to be strongly dependent on rosettes size or individual age for flower formation. Due to high energy costs associated with flowering, rosettes first may reach a critical size in one or several growing

seasons before they may start their reproductive phase (Werner & Caswell 1977; Gross 1981; Meagher & Antonovics 1982; Byers & Meagher 1997). For *T. integrifolia* subsp. *vindelicorum* we could also reveal size dependent flower formation. Rosettes, from which flowering individuals emerged, were significant larger in the previous year than rosettes, which stayed in the vegetative status (Mann-Witney-U-test: N = 449, U = 5876.5, p = 0.000). Mean diameters of vegetative rosettes (in year t), which evolved into reproductive individuals (in year t+1), were 16.2 cm, while mean diameters of rosettes, which stayed vegetative, were 11.1 cm.

However, strong temporal variations of flowering individuals could not be explained sufficiently by these size dependencies. Rather it is likely that environmental variations, which affected both sites simultaneously, were responsible for the demographic pattern of *T. integrifolia* subsp. *vindelicorum.* Furthermore, several other studies provided already clear evidence for relations between climate and individual reproductive capacity in natural populations (Bengtsson 1993; Woodward 1997; Wells *et al.* 1998).

Survival of recruits as well as adult plants was also strongly influenced by climatic variations. Especially survival of recruits was negatively affected by high summer temperatures as well as high temperatures and low precipitation in spring. Therefore, drought stress in summer and spring seemed to be one of the main risk factors for survival. A high sensitivity to drought could be explained by the habit of the root system of *T. integrifolia* subsp. *vindelicorum* (App. 5). A short, low penetrating and low branching root system hampers the plants to reach deeper soil layers. Therefore the acquisition of water might be a large problem during times of low precipitation and might reduce survival capacity of young plants.

Implications for conservation

Intensification of agricultural land use practices as well as field abandonment are the main risk factors for maintenance of high species diversity in calcareous grasslands (Fischer & Stöcklin 1997; Dolek & Geyer 2002). As a consequence of their dependency on frequent disturbances, long-term survival of grassland species is related to traditional management practices (Brys *et al.* 2004). The effect of applied management regimes on population dynamics of individual plant species can vary strongly and need to be monitored over years, especially for endangered plant species.

The aim of the present study was to investigate patterns of spatio-temporal variations of *T. integrifolia* subsp. *vindelicorum*. In accordance to other studies (Bühler & Schmid 2001; Colling *et al.* 2002; Bissels *et al.* 2004), our results confirmed that the analysis of population structure and dynamics of perennial plant species are crucial to understand the meaning of habitat requirements, climatic variations and individual risk factors on populations long-term survival.

T. integrifolia subsp. *vindelicorum* showed high dependency on different kinds of vegetation structures influencing all life cycle stages, such as recruitment, flower induction and survival. Regarding the applied management regime, grazing by sheep seemed to be beneficial for population viability. Disturbances during grazing showed positive effects on recruitment by creating safe sites and, presumably, by stimulating the formation of daughter rosettes, which are able to increase the reproductive capacity. Furthermore, grazing leads to heterogeneous vegetation structures, which are necessary for different life cycle stages of *T. integrifolia* subsp. *vindelicorum*. While intensively disturbed parts are occupied by seedlings and juveniles, closed vegetation structures are preferred by adult individuals, which profit from buffering effects by surrounding vegetation. High light availability due to a short vegetation cover enhances the formation of flowering stems.

On a local scale, metapopulation dynamic with extinction and recolonization events might be essential for the long-term viability of *T. integrifolia* subsp. *vindelicorum*. Therefore, applied management strategies should be aimed to create medium-open vegetation structures, in which germination and establishment remain possible for many years. Due to the short life span of *T. integrifolia* subsp. *vindelicorum*, repeated sequences of unfavourable years would lead to a reduction and ultimately perhaps to the extinction of the whole population. The optimal management strategy would be to keep survival of plants as high as possible (preventing high-competitive stress) as well as to improve conditions for germination and recruitment (creating safe sites for germination). Well balanced population structures demand high flexibility and coordination of the applied management regimes, because variation in disturbance regime may change environmental conditions very fast (Löfgren *et al.* 2000). Especially in grass dominated areas, strong competitors are able to increase rapidly, when grazing intensity will not be high enough or the starting point of grazing is delayed.

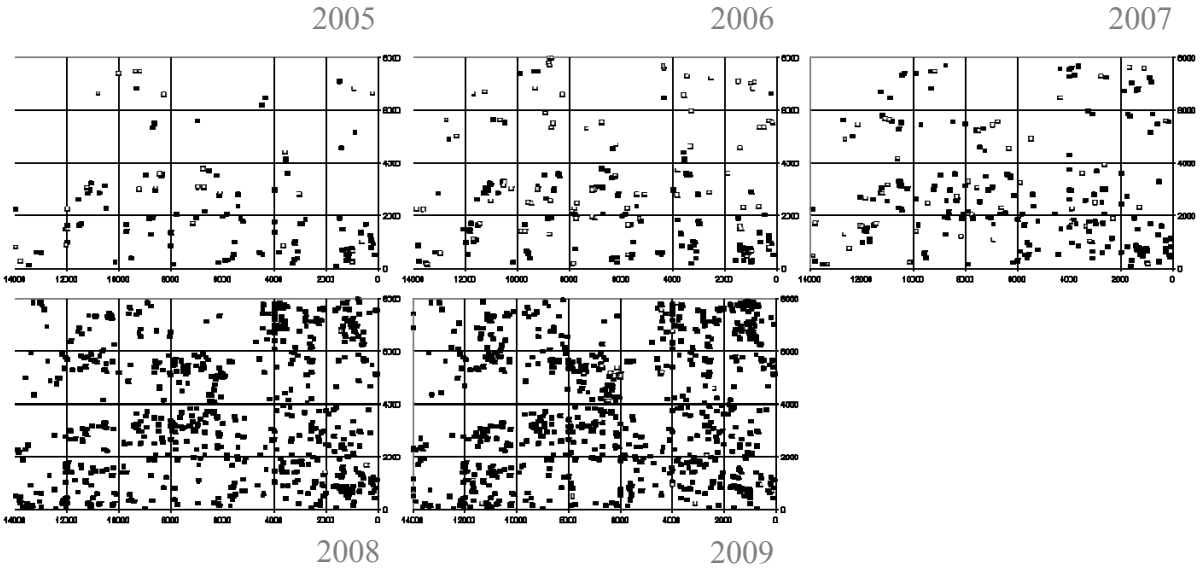
T. integrifolia subsp. *vindelicorum* seems to be well adapted to the site conditions of traditionally managed grasslands. High numbers of viable seeds (> 90 seeds per flower head,

2-5 flower heads per individual) and high levels of recruitment permit the persistence in even frequently disturbed grasslands (Oostermeijer *et al.* 1996).

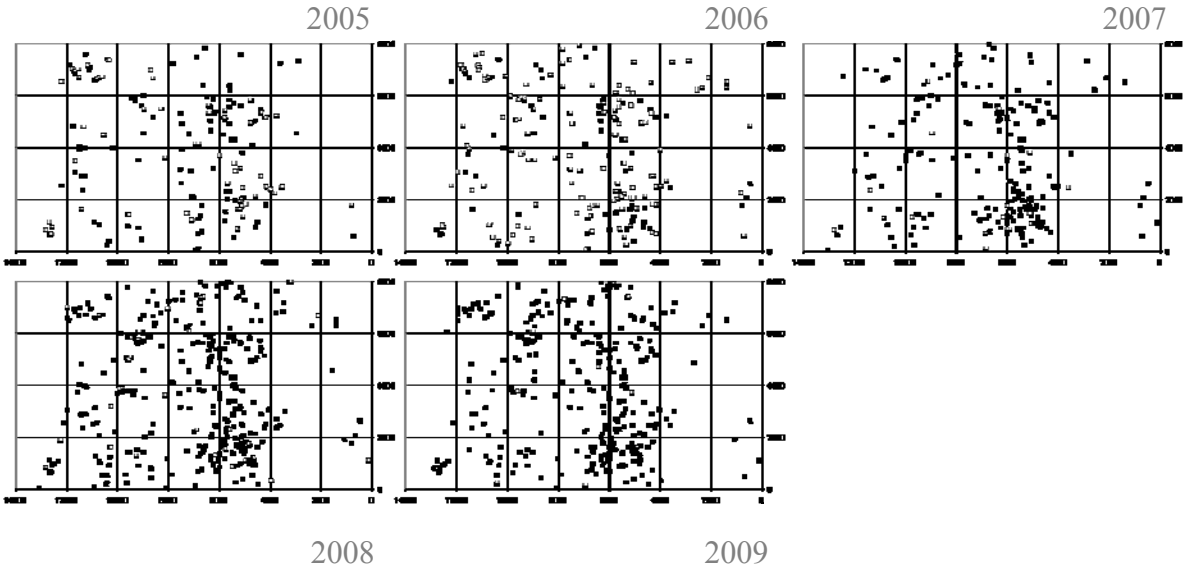
Due to high weather sensitivity of *T. integrifolia* subsp. *vindelicorum*, countings of flowering individuals are highly insufficient to monitor population viability adequately. Dynamic and viability of populations can best be followed by monitoring the fate and performance of individual plants in the population. This was already mentioned for many other small and endangered plant populations (Hutchings 1991; Oostermeijer *et al.* 1992). In contrast to conventional monitoring practices, which are based on counts of flowering individuals, demography analysis yield high information on population viability and its relation to management regime (Hegland *et al.* 2001). High among-year variability in finite rates of population increase highlights the importance of multiple demographic estimates over time (Oostermeijer *et al.* 1994a; Horvitz *et al.* 1995; Kephart & Paladino 1997).

In general, it can be concluded that disturbances of intermediate intensity, such as grazing, will most likely result in populations of the stable or dynamic type, but continuous monitoring is needed to reveal deleterious developments in time.

Appendix



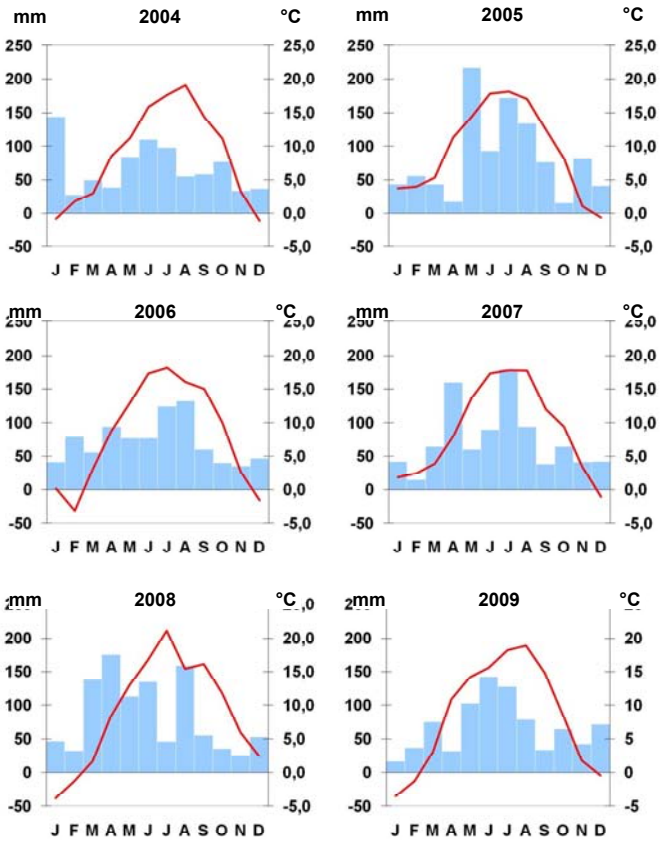
App. 1 Population dynamics of *T. integrifolia* subsp. *vindelicorum* on site C from 2005 to 2009. White squares symbolize flowering individuals, black squares non-flowering individuals. Scale units are millimeter.



App. 2 Population dynamics of *T. integrifolia* subsp. *vindelicorum* on site D from 2005 to 2009. White squares symbolize flowering individuals, black squares non-flowering individuals. Scale units are millimeter.

App. 3 Pearson correlations of demographic parameters and weather variables. p = level of significance (* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$), n = number of cases, No = number, r = Pearson correlation coefficient, T = temperature, min = minimum; PR = precipitation, fla = flowering adult status, flr = flowering recruit status, vga = vegetative adult status, vgr = vegetative recruit status, mis/death = missing or dead status.

Demographic parameters	type of relation	weather variables	n	r	p
Flowering					
fla -> fla	+	Precipitation in spring	8	0.836	**
	-	T min winter	8	-0.884	**
	-	T mean summer	8	-0.781	*
	+	RR/T March	8	0.818	*
vga -> fla	+	Precipitation in spring	8	0.831	*
	-	T min winter	8	-0.875	**
	-	T mean summer	8	-0.735	*
vgr -> fla	+	T min spring	8	0.711	*
No of flower heads	-	T min December	10	-0.697	*
	-	T min spring	10	-0.666	*
flr -> vga	+	T min winter	8	0.988	***
	+	T mean winter	8	0.811	*
	+	T min spring	8	0.747	*
	-	Precipitation in spring	8	-0.916	***
Survival					
flr -> mis/death	+	T mean summer	8	0.976	***
	+	T max winter	8	0.807	*
	+	T min winter	8	0.949	***
	+	T mean winter	8	0.938	***
	+	T min December	8	0.802	*
	+	T mean spring	8	0.864	**
	-	Precipitation spring	8	-0.931	***
	-	PR/T March	8	-0.967	***
	-	PR/T April	8	-0.759	*
vgr -> mis/death	+	T mean summer	8	0.867	**
	+	T max winter	8	0.736	*
	+	T min winter	8	0.824	*
	+	T mean winter	8	0.839	**
	+	T max October	8	0.707	*
	+	T min spring	8	0.720	*
	+	T mean spring	8	0.718	*
	-	Precipitation spring	8	-0.740	*
	-	PR/T March	8	-0.872	**
vga -> mis/death	-	PR/T March	8	-0.764	*



App. 4 Climate diagrams for the military area "Lechfeld" in the years 2004 to 2009. Red line = temperature in °C, blue bars = precipitation in mm.

App. 5 Root system of *T. integrifolia* subsp. *vindelicorum*.

Chapter 7

Conservation biology of steppe plants

In Central Europe dry calcareous grasslands are regional biodiversity hotspots and of high conservation value (Bignal & McCracken 1996; Poschlod & WallisDeVries 2002; WallisDeVries *et al.* 2002; Sanchez-Zapata *et al.* 2003). As human-made habitats they harbor a characteristic composition of xerothermic plants and numerous species, whose primary habitats (outcrops or hilly domes with shallow soils, steep slopes or gravel banks along rivers; Poschlod & WallisDeVries, 2002) have been largely destroyed. However, semi-natural grasslands are fragile because their maintenance depends on traditional farming techniques, which are more and more disappearing. Some European grasslands exhibit a unique mixture of submediterranean and pontic floristic elements (Walter & Straka 1970). The importance of these cenoses for the preservation of biodiversity is demonstrated by their inclusion among biotopes of European significance within the EU program NATURA 2000 (Ssymank *et al.* 1998). Subpannonian steppe grasslands, as well as interrelated calcareous, dry and semi-dry grasslands are under special conservation. In order to meet the obligations of NATURA 2000, scientific understanding of biodiversity has to be broadened and existing knowledge must be used more effectively. New scientific instruments, such as modern population genetic techniques, promise raising possibilities in conserving biological evolution and biodiversity.

The study presented here deals with several different aspects in life history of steppe plants and demonstrates the high importance of detailed investigations in order to assess current management programs, to develop new conservation strategies and to set adequate conservation priorities. It has been shown that colonization history of steppe plants in Central Europe resulted in different genetic lineages within Germany, which deserve a more differentiated point of view in preservation of local genetic biodiversity (Ch 2). In the case of *Scorzonera purpurea*, conservation strategies should aim at the maintenance of all populations within Germany, especially populations, which contain several different genetic lineages, such as the Lechfeld population in the south of Augsburg. As a consequence of this specific historic pattern and due to the critical status of most populations within Germany, in situ as well as ex situ conservation should be reinforced immediately to conserve the current status of genetic variation.

Furthermore, resolving taxonomic uncertainties by molecular markers is not only necessary to recognize potential local endemics (Ch 5), but also to identify the conservational peculiarity of individual outstanding populations (Ch 4). In the case of *Tephroseris integrifolia*, most genetic variation was located within populations and no molecular evidence could be found for considerable genetic differentiation. From this point of view, it would not be recommendable to grant a special endemic status for local populations. For *Stipa bavarica*, we could rule out the possibility of hybridization between two closely related local *Stipa* species and strongly questioned its status as endemic species. Rather it would be advisable for conservation practice to focus on its remarkable genetic structure containing characteristic and rare bands.

The commonly used method of counting flowering individuals to monitor survival potential of endangered plant species as well as to assess the success of applied management practices was demonstrated to be largely insufficient in the case of *T. integrifolia* subsp. *vindelicorum* (Ch 6). Population development is influenced by multiple different abiotic and biotic factors affecting all kinds of demographic processes. Therefore, several different life cycle stages of perennial plant species have to be evaluated over time to monitor population viability. The more time consumption and financial costs of demographic studies in permanent plots are thoroughly counterbalanced by the possibility to establish more accurate management recommendations, even for single critical life stages.

Last but not least, we could summarize a large spectrum of information on *Scorzonera purpurea* to gain more insights into plant's life history and its current threat status (Ch 3). Demographic studies as well as biological, ecological and genetic investigations are the fundamental basis of population viability analyses, which enable the identification of potential risk factors and the evaluation of population's long-term survival potential (Ch 7). On the basis of these results, important conservation recommendation could be formulated.

Population viability analyses

Information about biological traits and demographic parameters in plants is usually limited and hampers the successful preservation of endangered species by adequate management strategies. Population viability analyses (PVA) are one of the most promising tools for assessing potential risk factors in endangered plant species and for developing new conservation programs (Boyce 1992). They are based on past and present distribution patterns

as well as the principles of population biology, involving demography, ecology and population genetics. PVA combines impacts of deterministic and stochastic factors, which may be causes for species extinction, and generates complex models to predict the fate of populations (Menges 1990). Therefore, PVA is a principal component of conservation research and conservation practice (Henle *et al.* 1999). It requires an understanding of threats on plant species and the effect of these threats on population dynamics. The multitude and complexity of threats demand detailed and long-term investigations on population characteristics. For most threatened species only limited information on life history, demographic relations, habitat requirements, interactions with other species, reactions to climate changes and population genetics exists. Although, the final intention of PVA might be the construction of complex quantitative models to predict the future fate of populations, the process of PVA might gain more considerable benefits for species survival, than the quantitative predictions output (Boyce 1992). Some of the most prominent examples for plant PVA studies are the investigations of Werner & Caswell (1977), Groenendael & Slim (1988), Menges (1990), Bullock et al. (1994b), Ehrlén (1995), Menges & Dolan (1998), Valverde & Silvertown (1997) and Oostermeijer (2000).

Population viability analysis for two rare steppe species in Bavaria

Within the present study, we used demographic, biological, ecological and genetic investigations to enlarge the existing knowledge on two rare steppe species in the Lechfeld, *Tephroseris integrifolia* subsp. *vindelicorum* and *Scorzonera purpurea*. By combining all gained information, population viabilities could be assessed and the persistence of their populations as well as the risk of their going extinct could be determined. The two plant species belong to the family of Asteraceae and, according to several plant life history traits (pollination mode, seed bank type, life form) they are very similar to each other. However, their potential extinction risk differs significantly. Fig. 41 summarizes the most striking parameters affecting persistence and long-term survival of the two steppe species.

Species	*Tephroseris integrifolia* subsp. *vindelicorum*	*Scorzonera purpurea*
Occurrences		
- in the study region	one local population (> 500 individuals)	one population (ca. 100 individuals)
- in Bavaria	one local population, endemic	< 10 populations
Characteristics in life cycle & biology	reproduction by **many, well dispersed seeds**	reproduction by **few large, not very well dispersed seeds**
	long-lasting development until reproduction high flowering sensitivity to climatic conditions	long-lasting development until reproduction extremely high predation pressure
Characteristics of population dynamics	no selfpollination mechanism transient seed bank short life span **progressive** population structure high spatial dynamics	no selfpollination mechanism transient seed bank life span unknown **regressive** population structure low spatial dynamics
Threats by		
- genetic factors	low (no inbreeding depression)	high (low genetic variation within population, small population size)
- demographic factors	medium	high
- environmental catastrophes	medium to high	high
Total assessment of endangerment	**medium**	**high**
Management recommondation	- adequate habitat management (grazing by sheep) - establishing new populations by seed sowing or transplantation of young plants into suitable habitats	- adequate habitat management (mowing, grazing by sheep) - enhancing flowering rates - creating safe sites for germination - application of game repellents - introduction of planted individuals into pioneer habitats - ex-situ conservation (gene bank, collections in botanical gardens)

Fig. 41 Risk analysis and management recommendation for two steppe plants in the region of Lechfeld/Augsburg according to Schmid & Matthies (1994).

In *S. purpurea*, low seed production per individual and dispersal limitation lead to a strong threat caused through environmental stochasticity, such as unfavourable climatic conditions, herbivores and interspecific competition. Small populations of *S. purpurea* with only few reproductive individuals are very sensitive to failure in seed set, because there is no seed reservoir in the soil, which could buffer unfavourable times. *S. purpurea* is a perennial, but presumably short-lived plant species with a more or less regressive population structure dominated by old individuals and only few recruits within the study area. It may not withstand a series of improper habitat conditions for long time. Strong isolation of populations, as we can usually find in Central Europe, increases the endangerment of *S. purpurea* due to reduced pollination success and genetic factors. In the study area, high predation pressure additionally

reduces seed set and lowers successful reproduction. Therefore, the analysis of risk factors results in a high level of endangerment for *S. purpurea* in the Lechfeld. Preservation of this endangered plant species within the next years demands a more precise concept in management due to its extremely low competitiveness. Combined management strategies of in situ and ex situ conservation are necessary to save it from extinction. Beside the applied grazing regime, which creates safe sites for germination, it will be strongly necessary to enhance seed production in the field. Therefore, special arrangements should be made to reduce damages by games (fencing off during flowering, repellents or frequent human disturbances). Inclusion of *S. purpurea* into ex situ conservation programs would also enhance future perspectives. Especially, professional cultivation of young plants and reintroduction into suitable habitats might bypass the most critical life stage under natural conditions and increase reproductive success in the following generations. A transplantation experiment of cultivated individuals into restored arable fields revealed already first positive results (see chapter 3).

T. integrifolia subsp. *vindelicorum* is considered to be critically endangered due to ongoing fragmentation and habitat loss. However, in habitats with an adequate grazing regime, as it is applied in the Lechfeld, population viability seems to be rather stable. *T. integrifolia* subsp. *vindelicorum* produces numerous seeds per individual (> 200), which possess high dispersal capacity and show high levels of germination. Long individual flowering times and up to 90 flowers per capitulum enhance the probability of successful pollination and high amounts of viable seeds. Although, it has only a transient seed bank, comparable to *S. purpurea*, intermittent unfavourable environmental conditions can be counterbalanced by few flowering individuals each year. Demographic processes of *T. integrifolia* subsp. *vindelicorum* in the Lechfeld seem to be stable or even progressive resulting from the well adapted grazing regime, which creates several safe sites for germination and open vegetation cover favoured by low competitive plant species. Despite the low flowering rates of the last years, recruitment is still high and population structure is mostly dominated by young individuals. Genetic variation is not affected by inbreeding effects or genetic drift due to increasing isolation. Perspectives of long-term survival of the established *T. integrifolia* subsp. *vindelicorum* population are considered to be good, if the applied management regime will be continued. However, population expansion into adjacent habitats as well as re-establishing of populations does not occur and reinforce the risk of environmental stochasticity. Therefore, future conservation programs should include new management efforts in establishing new

populations of *T. integrifolia* subsp. *vindelicorum* within suitable habitats by seed application or transplantation of recruits from ex situ cultures.

Perspectives in plant conservation

The present study revealed highly interesting and elucidating facts concerning the life history, demography and conservation relevance of steppe plants in Central Europe. It highlights the importance of population genetic investigations for basic taxonomic issues and global phylogeographical principles. The use of codominant DNA markers, such as AFLP, is of tremendous significance to assess survival potential as well as to categorize populations according to their relative extinction risk and to set conservation priorities.

Ex situ conservation belongs to one of the most ambitious projects of current and future plant conservation programs. Ex situ methods imply the collecting of representative genetic samples of species and storing them outside the natural environmental conditions in which the species has evolved (Heywood & Iriondo 2003). According to the Global Strategy for Plant Conservation (GSPC), a resolution decreed from the Convention on Biological Diversity (CBD), more than 60 % of all endangered plant species should be incorporated into special ex situ collections to preserve present genetic biodiversity for future. However, for most endangered plant species little is known about distribution pattern of genetic variation, local genetic characteristics or genetic relevance of populations in the context of species global genetic variation. Differences in levels of genetic variation may be the result of several parameters and processes: historical and current population sizes, population bottlenecks, breeding system, natural selection, different mutation rates, immigration and emigration among populations as well as interactions among the above factors. Furthermore, isolated population, which are located near the outer boundary of species geographic range, are considered to be of high conservation priority (Korneck *et al.* 1996). Geographic outliers, such as the central European steppe species, are likely to occur in ecologically marginal or stressful conditions and are facing divergent natural selection than populations located in the centre of species range (Lesica & Allendorf 1995). To date, most conservation efforts, either in situ or ex situ, have proceeded with little information on genetic variation that was conserved and there is an urgent need to remedy this situation (Ramanatha Rao & Hodgkin 2002).

The extension of population genetic investigations and the molecular examination of additional steppe species (e.g. *Inula hirta, Buphtalmum salicifolia*, *Asperula tinctoria*, *Thesium linophyllon*, *Scabiosa canescens*) as well as threatened plant species would be essential to assess the genetic significance of local populations and to design general conservation management schemes based on population genetics. Large-scale genetic screenings of indigenous plant species might be useful to localize hotspots of genetic biodiversity and ecologically important genetic regions. The European Plant Conservation Strategy (Planta Europa), the European Community Biodiversity Strategy (EPCS), the Convention on Biological Diversity (CBD) and the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO), all stress the need to improve the efficiency of conservation techniques, particularly those related to in situ genetic conservation (Maxted 2003). Comparable to the Important Plant Areas (IPAs) program of International PlantLife (Anderson 2002), a network of best sites for genetic plant conservation throughout Europe should be identified by molecular markers and used for conserving genetic biodiversity. Currently, there is no accurate assessment of where or what proportion of European protected areas are being managed as genetic reserves, where the goal is not only to maintain the local ecosystems but also to conserve genetic variation within the component plant populations. Supported by cartographic techniques (e.g. the geographical information system, GIS) maps of genetic biodiversity hotspots might be compiled and compared to the existing network of European protected areas and ex situ conservation collections. This may help to identify gaps within the network and to determine plant genetic resources for future conservation priorities.

Finally, there continues to be a substantial need for research on many aspects of the extent and distribution of genetic variation (Ramanatha Rao & Hodgkin 2002). We are forced to improve the settings of conservation priorities and the selection of taxa which we focus our conservation activities on.

Danksagung

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