Genetic diversity and population structure of arable plants

*in situ* and *ex situ* –

How sustainable is long term cultivation in botanical gardens compared to *in situ* conditions?

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Chapters 2 to 5 have been published in international journals or are in preparation for publication. Due to copyright reasons, the main part of this thesis thus gives the abstracts only.
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Summary

The global decline of species and habitats makes *ex situ* cultivation in botanical gardens becoming increasingly important. Up to now approximately 2500 botanical gardens in more than 150 countries cultivate around one quarter of all vascular plants in the world. Most of the cultivated taxa are, however, held in a small number of individuals which may cause genetic problems including losses of genetic diversity by inbreeding and genetic drift. So far, measures to counteract these genetic problems such as artificial cross-pollination are poorly tested with respect to *ex situ* cultivation in plants.

In my PhD project I analyzed these aspects for arable plant species which are among the most threatened species groups in Central Europe. As arable plants are mostly annual and self compatible, they should be sensitive to effects of increasing habitat fragmentation and decreasing population sizes both. Up to now, studies on the genetic structure of arable plants have been relatively sparse. Therefore, genetic structure and diversity of 67 *in situ* and 20 *ex situ* populations of *Adonis aestivalis*, *Anagallis arvensis*, *Anagallis foemina*, *Bupleurum rotundifolium*, *Consolida regalis* and *Nigella arvensis* were analyzed and correlated with population sizes (*in situ* and *ex situ*) and the duration of cultivation (*ex situ*). A pollination experiment with *Bupleurum rotundifolium* was conducted to analyze whether cross-pollination leads to higher genetic diversity than self-pollination.

Genetic diversity was low and genetic structures were pronounced in *in situ* populations of the rare and threatened arable plant species in Germany. This was also evident in a larger scale survey of *Bupleurum rotundifolium* across Central Europe, which revealed different provenance groups of populations that also differed in their genetic diversity. In several species, genetic diversity and structure was negatively affected by *ex situ* cultivation. Contrary to expectations genetic diversity was correlated to population size only in *Nigella arvensis* *in situ* and *ex situ* and was not affected by the duration of *ex situ* cultivation. The majority of *ex situ* populations in botanical gardens was poorly documented with respect to source-populations and was often even founded from other garden material. Artificial cross-pollination did not lead to higher genetic diversity compared with self-pollinated individuals.

Practical considerations: *Ex situ* and *in situ* conservation should be regarded as complementary approaches. The establishment of field margin strips or field flora
reserves could mitigate fragmentation effects and support the stabilization of arable plants *in situ*. For restoration programmes, potentially different provenance groups of plant species should be taken into account. For *ex situ* collections seeds should be collected from many different individuals within a provenance groups, and *ex situ* population should be refreshed every few years with new *in situ* material.

I found less clear evidence for effects of population size and the duration of cultivation. Arable plants may be less sensitive to typical trends in small populations, possibly reflecting adaptations to their usually instable habitats. Nonetheless, the extension of generation time by dry storage of seeds at cold temperatures still offers a cheap and recommended alternative to annual cultivation, and would also be suitable for my species that form large seed banks even in the wild. Most of the analyzed *ex situ* populations should be tested for fitness and similarity to wild populations before they are brought to the field. Facilitating pollination can so far not recommended as a promising tool for *ex situ* conservation: The effort is relatively high compared to the usual practice of simply relying on “natural” pollination, and effects are unclear with respect to the limited available data.
Zusammenfassung

Das globale Artensterben und der Verlust von Habitaten führen zu einer immer größeren Bedeutung des Ex situ-Schutzes in Botanischen Gärten. Derzeit kultivieren etwa 2500 Gärten in mehr als 150 Ländern ein Viertel aller weltweit vorkommenden Gefäßpflanzen. Die meisten Taxa werden aber nur in einer geringen Individuenzahl gehalten, was genetische Probleme wie den Verlust genetischer Diversität durch Inzucht und Drift zur Folge haben kann. Bislang gibt es wenig Versuche, diesen genetischen Problemen bei der Ex situ-Kultivierung, etwa mit gezielter Kreuzbestäubung, zu begegnen.


Insgesamt war in den mitteldeutschen Freilandpopulationen der selteneren und gefährdeten Ackerwildkräuter die genetische Diversität niedrig und die genetische Struktur ausgeprägt. Dies war ebenso ersichtlich in einer Übersichtsarbeiten zu Bupleurum rotundifolium, die in einem größeren Maßstab (Mitteleuropa) stattfand. Hier zeigten sich zudem Unterschiede in der genetischen Diversität in Abhängigkeit von der Herkunft der Populationen. Die Ex situ Kultivierung beeinflusst die genetische Diversität und Struktur mehrerer Arten negativ. Im Gegensatz zu meiner Erwartung konnte eine Korrelation zwischen genetischer Diversität und Populationsgröße aber nur für Nigella arvensis in situ und ex situ gefunden werden. Ein Zusammenhang zwischen Kultivierungsduer und genetischer Diversität fand sich nicht. Die meisten Ex situ-Populationen in Botanischen Gärten waren hinsichtlich ihrer Herkunft schlecht dokumentiert oder stammten aus
anderen Botanischen Gärten. Künstlich kreuzbestäubte Individuen von *B. rotundifolium* zeigten keine höhere genetische Diversität als künstlich selbstbestäubte.


Ich fand keine eindeutigen Zusammenhänge zwischen Populationsgröße und Kultivierungsduer mit der genetischen Diversität. Möglicherweise reagieren Ackerwildkräuter weniger empfindlich auf eine abnehmende Populationsgröße, was auf eine Anpassung an die für gewöhnlich sehr instabilen Habitate zurückzuführen sein könnte. Dennoch sollte über eine Ausdehnung der Generationszeit durch das Lagern bei kalten Temperaturen nachgedacht werden, da dies eine kostengünstige Alternative zur jährlichen Kultivierung darstellt. Auch ist sie für meine untersuchten Arten, die im Feld persistente Samenbanken anlegen, besonders geeignet. Für Wiederansiedelungsmaßnahmen sollten die betreffenden *Ex situ*-Populationen vorher auf genetische Diversität und Ähnlichkeit zu Wildpopulationen getestet werden. Künstliche Bestäubung kann bislang nicht als ein vielversprechendes Instrument in der *Ex situ*-Kultivierung empfohlen werden: Der Aufwand ist relativ hoch im Vergleich zur gängigen Praxis, sich auf die “natürliche” Bestäubung zu verlassen und der Nutzen ist in Anbetracht der begrenzt verfügbaren Daten fraglich.
Chapter 1

1.1 General introduction

The worldwide loss of biodiversity and the decline of species (Butchart et al. 2010; Rands et al. 2010) is one of the major currently pressing environmental problems. This seems to be most severe in tropic regions as these hotspots of biodiversity showed the highest number of losses (Brooks et al. 2002). Even in less diverse Central Europe, more and more species are threatened (Watt et al. 2007). Reasons are mainly habitat loss (Giam et al. 2010; Krauss et al. 2010), fragmentation or management intensification (de Chazal & Rounsevell 2009), as well as invasive species (Hapca 2011) and climate change (Hannah et al. 2002; Bellard et al. 2012).

In Central Europe arable plant communities belong to the most threatened vegetation types (Hilbig 2007; Meyer et al. 2008). In the last 50 years agricultural land, the largest land cover type in Central Europe (Young et al. 2005; Schmidt et al. 2009), has undergone enormous changes (Walz 2008; Dallimer et al. 2009; Walker et al. 2009; Baessler et al. 2010). The increase of highly productive areas, the levelling of habitats, declines in crop diversity, use of pesticides, increasing nitrogen input and the purification of seeds during the last decades has decimated and often eliminated populations of sensitive arable plants (Geiger et al. 2010; Hawes et al. 2010; Storkey et al. 2012; Meyer et al. 2013).

Among arable plant species threat levels differ widely, with several being still common and widespread, while a large number of others is now Red Listed on the regional level (Korneck et al. 1996). Besides, arable plants should be relatively sensitive to genetic problems: plants are short-lived and mainly selfing, populations have often become small and are increasingly isolated. Studies on the genetic structure of arable plants have been relatively sparse, however (Katzir et al. 1996; Schmidt et al. 2009; Délye et al. 2010; Hans 2010). In addition, assessments of different provenance groups are needed because provenance groups can differ in their genetic diversity and with respect to plant fitness in the field (van Andel 1998; Jones & Hayes 1999; Keller et al. 2000). Provenances groups also matter in restoration programmes were intraspecific hybridisation between local and nonlocal genotypes could lead to a negative impacts of outbreeding depression (Edmands 2007; Crémieux et al. 2010; Goto et al. 2011).

The observed decline of plant species leads to the question of how to conserve populations, including their adaptive potential (Heywood & Iriondo 2003). Since the
Convention on Biological Diversity was adopted at the conference of Rio de Janeiro in 1992 (Glowka et al. 1994; CBD 2012), discussions on the approaches to the preservation of genetic diversity, species diversity and ecosystem diversity have been intensified (e.g. Frankel et al. 1995; Swanson 1997; Mueller 2000; Jacquemont & Caparrós 2002; Balmford et al. 2005; Sutherland et al. 2009). Recent studies increasingly address the importance of genetic diversity for species conservation, because as levels of threat are often related to the genetic structure of a given species (Spielman et al. 2004), many threatened plants species are expected to have unfavourable genetic structures. This has so far hardly been tested, although it is known that genetic structure and fitness are correlated (e.g. Fischer & Matthies 1998; Ehlers 1999; Schmidt & Jensen 2000; Frankham 2005).

Currently, conservation techniques can be grouped into two basic strategies: in situ and ex situ (Maxted et al. 1997; Dulloo et al. 2010). Conservation of threatened species in situ is generally preferred (Guerrant et al. 2004) because evolutionary processes are more likely to remain dynamic (Brush 1994). Besides, habitats and ecosystems and not only single species are conserved (CBD 2012). With the actual problems of biodiversity and habitat loss, however, ex situ cultivation such as in botanical gardens is increasingly becoming important for protecting a species from extinction (Squirrell et al. 2006; Seaton et al. 2010; Zhang et al. 2010; Rucińska & Puchalski 2011), and for providing sources for potentially reintroduction schemes (Maunder 1992; Oldfield 2009; Godefroid et al. 2011a).

This poses new challenges for botanical gardens, which have repeatedly had to serve new purposes in their long history: The first botanical garden of which there is any representation, the Royal Garden of Thotmes III of about the year 1000 B.C. (Holmes 1906), probably was a pleasure garden without any economic importance (Hill 1915). The idea of cultivating plants for their economic or medicinal value potentially arose in China where monastic herb gardens originated (Bretscheider 1895). The first botanical gardens in the modern sense were founded between the middle and the end of the 16th century in Pisa, Padua, Florence, Bologna, Leyden, Leipzig, Paris, Montpellier and Heidelberg (Stearn 1971), and were often established in connection with the medical faculties of the resident universities. During this time, several gardens embarked on scientific research, mainly in taxonomic botany (Wyse Jackson 2009). Shortly after that, due to the worldwide travelling and discoveries, especially new and rare ornamental
plant species without medicinal value were cultivated in the gardens (Hill 1915). At that
time, displaying the greatest possible diversity of plant species in cultivation became the
main aim (Thompson 1972), thereby focusing on quantity instead of quality.

Nowadays, in addition to scientific research, education and recreation (Ballantyne et al.
2008; Wyse Jackson 2009; Ward et al. 2010; BGCI 2012), a major task of botanical
gardens is the conservation of species diversity (Ashton 1988; Miller et al. 2004; Pennisi
2010). Up to now approximately 2500 botanical gardens in 156 countries (Pautasso &
Parmentier 2007) cultivate about 80000 taxa worldwide which is approximately one
quarter of all vascular plants in the world (Wyse Jackson 2001). Many of these taxa are
threatened (Sharrock & Jones 2010) and some are even extinct in the wild (Maunder et
al. 2000; Maunder et al. 2001a; Galmés et al. 2007). Thus, botanical gardens host the
world’s largest ex situ collections of plant biodiversity (Golding et al. 2010), and play a
key role in the conservation of plant biodiversity (Primack & Miller-Rushing 2009;
Swarts & Dixon 2009). Given that one target of the Global Strategy for Plant
Conservation of 2020 is to cultivate 75% of world’s threatened plant species ex situ
(Paton & Lughadha 2011), ex situ collections can be expected to expand.

Most of the cultivated taxa in botanical gardens are, however, held in a small number of
individuals (Maunder et al. 2001b; Hurka et al. 2004; Fernández & González-Martínez
2010). For example, Enßlin et al. (2011) counted just 4-25 individuals in ex situ
collection of Cynoglossum officinale in 12 different botanical gardens, while Lauterbach
et al. (2012a) found 20-40 individuals for Silene otites in 3 different botanical gardens.
Small populations often suffer from demographic and environmental stochasticity
(Lande 1993) as well as from genetic problems (Ellstrand & Elam 1993; Leimu et al.
2006). The first two problems are less severe in botanical gardens as the plant species
there are planted in favorable and relatively stable conditions (Primack & Miller-
Rushing 2009). Genetic problems may still occur including exposure of populations to
inbreeding depression, accumulation of new mildly deleterious mutations or losses of
 genetic diversity by genetic drift (Frankham et al. 2002). In that sense, ex situ
populations may face the same problems as any small and isolated in situ population.

As the extent of genetic drift and inbreeding commonly increases with the number of
generation cycles (Spagnoletti-Zeuli et al. 1995; Hartl & Clark 1997), the duration of
cultivation cycles, should have further negative impacts on genetic diversity. In botanical
gardens ex situ cultivation often lasts over a long time essentially always propagating the
same accessions. For example Enßlin et al. (2011) showed that their study species had been cultivated in most botanical gardens for several decades. In the long run, such effects render the importance of botanical gardens for species conservation questionable.

Measures to counteract these genetic problems such as artificial cross-pollination are poorly tested with respect to both \textit{in situ} and \textit{ex situ} cultivation, although it is known that crossbred plants can show heterosis (e.g. van Treuren et al. 1993; Keller & Waller 2002). Studies on the effects of hand pollination have been performed since decades (Mendel 1866; MacDaniels 1930; Schroeder 1947) and have shown for example that cross pollination by hand can increase the number of seeds (King et al. 2007; Magnaghi et al. 2007) or the fruit size (Patterson 1988; Ye & Aoki 2003). In contrast, it is less well known if cross pollination by hand within or across populations can also increase genetic diversity.

To understand patterns of arable plant species' genetic diversity and structure \textit{in situ} after decades of agricultural intensification, and how these changed due to cultivation \textit{ex situ}, I analyzed genetic structure and diversity of 67 \textit{in situ} and 20 \textit{ex situ} populations of six arable plant species and correlated the genetic data with population sizes (\textit{in situ} and \textit{ex situ}) and the duration of cultivation (\textit{ex situ}). In a final step, a pollination experiment was performed to assess options for increasing genetic diversity \textit{ex situ}. Although artificial introduction of different genetic material is reported to cause positive heterosis effects (Darwin 1876; Sheridan & Karowe 2000; Luijten et al. 2002), discussion on threats of outbreeding depression have not stopped (e.g. Edmands 2007; Frankham et al. 2011)). This makes simply cross-pollination among different accessions a questionable approach. We thus tested the effects if individuals were crossed within a population with the aim to increase the effective population size.

All genetic analyses were conducted with RAPDs and AFLPs, which are both anonymous markers. They allow limited inferences on heterozygosity and (should) represent variation in the neutral genome only. RAPDs may also not be suitable for the detection of very small differences in genetic structure. My estimates of, for example, genetic drift are thus rather conservative. Nonetheless, reviews (e.g. Nybom & Bartish 2000; Nybom 2004) and a large number of successful applications (e.g. Schiebold et al. 2009; Hensen et al. 2010; Prinz et al. 2010; Wagner et al. 2011) lend much support and confidence to the RAPD- and AFLP-based approach adopted here.
Specifically, my PhD project addressed the following questions:

1. Does genetic diversity and structure of *in situ* populations depend on rarity and threat of a plant species?

2. Are there different provenance groups of populations and do they differ in their genetic diversity?

3. Does genetic diversity decrease with population size *in situ* or *ex situ*?

4. Do populations from botanical gardens have lower genetic diversity than *in situ* populations?

5. Does genetic diversity decrease with the duration of *ex situ* cultivation?

6. Does artificial cross-pollination increase genetic diversity compared to self-pollinated individuals?
1.2 Study species

The study species are all annual arable plants. Annuals are excellent model species because of their short generation cycle, and thus relatively rapid responses to environmental changes like increasing nitrogen input or *ex situ* cultivation. As most of the analyzed annuals are self-compatible inbreeding is a prospective risk. All of the study species can be found in adequate population numbers in the study area (see below). Besides, I also paid attention that the study species at least partly differ in their frequency of occurrence and Red list values.

*Adonis aestivalis* L. (Figure 1) is a moderately common Ranunculaceae that is declining in occurrence in Central Europe. Stems are 20-45 cm high; the dipetalous flowers are yellow or red with a black patch at their base. Flowering occurs between May and July while diasporate dispersal occurs between June and August. *Adonis aestivalis* is self- and insect-pollinated, and self-dispersed. It produces 50 to 100 seeds per plant, and thousand-seed weight is between 7.4 and 12 g. Germany’s responsibility for conservation is low (Welk 2002); Red list status for Germany and the main study area of Thuringia and Saxony-Anhalt is vulnerable (Frank et al. 1992; Westhus & Zündorf 1993; Korneck et al. 1996).

![Figure 1: Adonis aestivalis](image1.png)

![Figure 2: Anagallis arvensis](image2.png)

The Primulaceae *Anagallis arvensis* L. (Figure 2) is 5-30 cm high; flowers occur between May and October and are usually red or, less commonly, blue. Diaspore dispersal occurs
between June and October. The insect- and self-pollinated and wind-dispersed plant species produces 200 to 450 seeds per plant and thousand-seed weight is about 0.4 g. Red List status on both federal and state level is least concern indicating that there is no need for conservation strategies at the moment.

The Primulaceae *Anagallis foemina* Mill. (Figure 3) is much rarer in Central Europe. Its flowers are always dark blue, and its leaves are more slender. Flowering and diaspore dispersal are both between June and September. The insect- and self-pollinated and wind-dispersed plant species produces 200 to 300 seeds per plant and thousand-seed weight is between 0.6 and 0.7 g. Red List status is least concern for Germany and Thuringia and vulnerable for Saxony-Anhalt. Responsibility of Germany for both *Anagallis* species was not assessed by Welk (2002). Probably the abundance of *A. foemina* is overestimated (Frank pers comm.) as it is often mistaken for a bluish flowering colour variety of *A. arvensis* (Akerreta et al. 2007).

![Figure 3: Anagallis foemina (Image: Alex Urner)](image1)

![Figure 4: Bupleurum rotundifolium](image2)

*Bupleurum rotundifolium* L. (Apiaceae) is very rare within the study area and critically endangered in Germany and Saxony-Anhalt (Red list Thuringia: endangered). With respect to its overall distribution range, Germany has responsibility for its conservation (Welk 2002). Stems are 15-60 cm high and flowers are yellowish (Figure 4). Flowering and diaspore dispersal take place between June and August. The insect- and self-
pollinated, and wind- and water-dispersed plant species produces 50 to 100 seeds per plant and thousand-seed weight is between 2.3 and 2.9 g.

The Ranunculaceae *Consolida regalis* L. (Figure 5) is moderately common but has declined in occurrence in the last few decades. Its stems are 20-40 cm high and its flowers are blue. Flowering and diaspore dispersal of the insect-pollinated and self-dispersed plant species occurs between May and August. It produces 100 to 500 seeds per plant, and thousand-seed weight is between 1 and 1.8 g. Germany’s responsibility for conservation is low (Welk 2002), while Red list status for Germany is vulnerable. Red list status of Thuringia and Saxony-Anhalt is least concern (Frank et al. 1992; Westhus & Zündorf 1993; Korneck et al. 1996).

![Figure 5: Consolida regalis](image1)

![Figure 6: Nigella arvensis](image2)

In contrast, the 10-30 cm high bluish-white (Figure 6) *Nigella arvensis* L. (Ranunculaceae) is a very rare species in Central Germany (Red list Thuringia: critically endangered; Red list Germany and Saxony-Anhalt: endangered). The species is declining in occurrence and Germany is responsible for its conservation (Welk 2002). Flowering and seed dispersal of the insect- and self-pollinated and wind-dispersed plant species occurs between June and September. It produces 100 to 300 seeds per plant and thousand-seed weight is around 0.9 g.
1.3 Study area

The main study area is the Central German limestone and loam region in Saxony-Anhalt and Thuringia (Figure 7), where the collection of mature seeds at almost the same point of time could be guaranteed. The landscape is part of the so called "Mitteldeutsches Trockengebiet", where precipitation is less than 500mm/year, due to its location in the rain shadow of the Harz Mountains (Eichstaedt & Mahn 1993; Wania et al. 2006). Wherever possible, sampling was extended so that there are also some samples collected in Bavaria and Brandenburg.

For *Bupleurum rotundifolium* sampling was extended to entire Central Europe as this species was selected for further investigations on genetic structure and also for the pollination experiment. In addition to Central Germany, seeds were also collected in Austria, Czech Republic, France, Hungary, Poland and south-western Germany (Figure 8).
Figure 8: Location of the sampled *Bupleurum rotundifolium* populations (n=27).
1.4 Aim and structure of the study

The first aim of this study were to understand patterns of arable plant species’ genetic diversity and structure in situ after decades of agricultural intensification, and how these changed due to cultivation ex situ. Moreover, my PhD study provided an insight into genetic diversity and structure of rare and threatened *Bupleurum rotundifolium* in local populations all over Central Europe, thereby assessing the importance of provenance groups. Finally, I conducted experimental hand-pollination in order to increase genetic diversity ex situ. Overall, results of this research provide the first cross-taxon assessment of genetic structures in arable plants, and evaluate the potential role botanical gardens may have in preserving and increasing genetic diversity. This will ultimately help to understand and protect threatened arable plants both in situ and ex situ. The study is structured in 4 results chapters:

Following the introductory parts, the second chapter of this thesis presents a general survey of arable plant species’ genetic diversity and structure in situ. It is asked, if different levels of threat, which are conventionally reflected in a species’ Red List status, or population sizes are related to the genetic diversity and structure of the species.

The third chapter focuses on the rare and threatened *Bupleurum rotundifolium*. Genetic diversity and structure is analyzed with an extended data set assembled across Central Europe. Moreover, the chapter questions if there are different provenance groups and if they differ in their genetic diversity.

The fourth chapter compares in situ and ex situ populations of the study species to assess whether ex situ cultivation affects genetic diversity. In addition, it is asked if genetic diversity decrease with the population size or the duration of cultivation.

The fifth chapter describes the results of a pilot study on artificial cross-pollination among individuals within a given population. It is analyzed if hand pollination increases genetic diversity in ex situ populations of *Bupleurum rotundifolium*. Pollen was experimentally exchanged among individuals of a given population to avoid potential risks of outbreeding.
Genetic diversity of six arable plants in relation to their Red List status

with Karsten Wesche, Stefan Meyer and Isabell Hensen


Abstract:
In Central Germany and throughout Europe, arable plants count among some of the most endangered plant species. Over the last few decades, the number and size of populations have been in sharp decline due to modern land use techniques, including the application of fertilizers, herbicide use and seed cleaning procedures. As arable plant species are underrepresented in population genetic studies, it is unknown whether agricultural intensification has affected the extant populations, and whether genetic structure varies among species with differing vulnerability in respect of their Red List status. We sampled 53 populations from 6 arable plant species throughout Central Germany. Random amplified polymorphic DNA analyses (RAPD) were applied to calculate measures of genetic diversity at the population level and genetic differentiation. Genetic diversity was found to be lowest in Bupleurum rotundifolium and Anagallis foemina, and highest in Consolida regalis and Nigella arvensis. The highest levels of genetic differentiation were observed among populations of An. foemina and B. rotundifolium but within populations in all other species. \( \Phi_{ST} \) values differed strongly ranging between 0.116 for C. regalis and 0.679 for An. foemina. Patterns of genetic structure were related to the Red List status for all the species studied except An. foemina, for which it should consequently be raised. Our data confirm that even relatively recent threats are accompanied by detrimental genetic structure. As losses of populations and increased fragmentation have occurred in all common and uncommon species, the situation for arable plants could change for the worse in the following decades, highlighting the need for consistent monitoring.

Keywords: Adonis aestivalis, Anagallis arvensis, Anagallis foemina, arable plants, Bupleurum rotundifolium, Central Germany, conservation genetics, Consolida regalis, Nigella arvensis, RAPD
Spatial genetic structure and low diversity of the rare arable plant

*Bupleurum rotundifolium* L. in Central Europe

with Stefan Meyer, Pierre Kühne, Isabell Hensen and Karsten Wesche

Agriculture, Ecosystem and Environment 161: 70-77 (2012)

**Abstract:**

Many threatened plant species have genetic structures indicating effects of fragmentation, and in Central Europe arable plants are among the most threatened taxa. Although this threat has developed only recently in the course of agricultural intensification, their annual life form makes arable plants relatively sensitive to increasing fragmentation and decreasing population sizes. One of Central Europe's rarest arable plant species is *Bupleurum rotundifolium* (Apiaceae). To analyze the genetic structure and diversity of this species we sampled 27 populations of *B. rotundifolium* in Central Europe, and assessed genetic structure by amplified fragment length polymorphism (AFLP) fingerprinting.

Ordination, clustering and also Bayesian analysis suggested that most of the populations of the Eastern part of Germany formed one cluster, and most of the Western German populations as well as populations from outside Germany built another group. The two clusters accounted for 24% of differences in genetic structure of *B. rotundifolium* populations, while there was relatively strong differentiation among (41% variance) and within populations of a given group (35% variance). The overall Φ<sub>ST</sub>-value was very high (0.65) and there was evidence for isolation-by-distance. Values of genetic diversity were very low for *B. rotundifolium*. The proportion of polymorphic loci per population varied between 9.4% and 38.7%, with those from eastern Germany being significantly less diverse (mean 19.1% vs. 25.5%).

**Keywords:** AFLP, genetic diversity, isolation by distance, red list, arable weeds
Ex situ cultivation affects genetic structure and diversity of arable plants

with Isabell Hensen and Karsten Wesche


Abstract:
Worldwide, botanical gardens cultivate around 80,000 taxa, corresponding to approximately one quarter of all vascular plants. Most of the cultivated taxa are, however, held in a small number of collections, and mostly only in small populations. Lack of genetic exchange and stochastic processes in small populations make them susceptible to detrimental genetic effects, which is most severe in annual plant species as sowing cycles are often short.

In order to assess whether ex situ cultivation affects genetic diversity of annuals, five annual arable plant species with similar breeding systems were assessed with 42 in situ populations being compared to 20 ex situ populations using a Random Amplified Polymorphic DNA (RAPD) analysis approach.

Population sizes tended to be lower under ex situ cultivation and levels of genetic diversity tended to be lower in four of the five species with differences being significant in two of these. Ex situ populations also showed incomplete representation of alleles found in the wild. The duration of cultivation did not show a clear effect on genetic diversity.

Results imply that cultivation strategies resulted in altered genetic structures in the garden populations. The effects were not unequivocally pronounced suggesting that garden cultivation at least partly resembles conditions in the field. Altered genetic structures nonetheless imply that conservation strategies in the involved gardens may need improvement with cool storage of seeds being an option, that is surprisingly not followed in the studied ex situ collections which may reflect the additional aim of displaying living plant populations in the public gardens.

Keywords: annuals, botanical garden, conservation genetics, ex situ cultivation, genetic diversity, random amplified polymorphic DNA (RAPD) analysis, species conservation
Does artificial cross-pollination increase genetic diversity compared to self-pollinated individuals of *Bupleurum rotundifolium*?

with Pierre Kühne, Isabell Hensen and Karsten Wesche

Manuscript

**Abstract:**

The dramatic decline of species around the world makes *ex situ* cultivation in botanical gardens becoming increasingly important. In contrast to the huge number of different plant species kept in botanical gardens, the number of generative individuals per species is often limited. These small populations are especially vulnerable because of e.g. stochastic demographic events as well as genetic effects. So far, many studies detected a lower genetic diversity with *ex situ* cultivation, especially for annuals. Measures to counteract these genetic problems such as artificial cross-pollination were occasionally tested, and it is known that crossbred plants may show heterosis effects like increased seed numbers or fruit sizes. Outbreeding depression has, however, also been described and it is thus poorly known if cross-pollination by hand is a suitable tool to improve genetic diversity of a given species.

In order to minimise threat of outbreeding depression and genetic swamping, we conducted a pilot study experimentally cross-pollinating within populations only, with the aim to increase effective population sizes. We chose the annual and highly selfing plant species *Bupleurum rotundifolium* L. for this experiment. Differences in genetic diversity were measured between populations subjected to artificial cross-pollination or selfing.

Amplified fragment length polymorphisms DNA (AFLP) fingerprinting supported the notion of low genetic diversity in *ex situ* populations of this species, but offspring of cross-pollinated individuals did not show higher genetic diversity than those originating from self-pollination. These results are discussed with respect to potential shortcomings of the experimental approach.

**Keywords:** AFLP, *Bupleurum rotundifolium*, conservation genetics, cross-pollination, *ex situ* cultivation, genetic diversity, hand pollination, selfing
6.1 Key findings

The presented studies concentrated on genetic structures in six typical arable plant species of Central Europe. Studies on in situ and ex situ populations as well as the pollination experiment provided the following main results (see key questions chapter 1):

1. Genetic diversity and structure of in situ populations in Central Germany was found to be very low especially in rare and threatened arable plant species. The occurrence of Anagallis foemina is largely overestimated.

2. This was also evident in a larger scale survey of Bupleurum rotundifolium, which revealed different provenance groups of populations that also differed in their genetic diversity.

3. Genetic diversity was correlated to population size in only one species in situ and ex situ.

4. In several species, genetic diversity and structure was negatively affected by ex situ cultivation.

5. Genetic diversity was not affected by the duration of ex situ cultivation.

6. Our pilot study implied that cross-pollination within populations did not increase genetic diversity compared to self-pollinated individuals. We thus did not conduct a larger experiment on this issue.

6.2 Overall discussion

Low genetic diversity and structure of rare arable plants in situ

My PhD project supported worries about detrimental genetic structures in declining arable weed vegetation. Common arable plant species had more favourable genetic structures than rare ones as shown by lower $\Phi_{ST}$ values and, to a lesser extent, slightly
higher genetic diversity on the population level (Chapter 2). The data corresponds to meta-analyses between rare and widespread congeners, which showed rarer species having a lower diversity and structure at all measured levels (Gitzendanner & Soltis 2000; Cole 2003). In the present thesis, this pattern holds largely true even if species from different taxonomical groups are compared. This also supports the general idea that genetic factors are associated with extinction threat (Spielman et al. 2004). Ultimately, results imply that genetic factors should not be ignored in conservation, and that conservation genetics is in an issue in spite of voices claiming its irrelevance (Pertoldi et al. 2007; Vernesi et al. 2008).

The reason for lower genetic diversity and structure could be the breeding system (Loveless & Hamrick 1984; Schoen & Brown 1991; Charlesworth 2003; Mable & Adam 2007). Nybom and Bartish (2000) presented a literature review showing that selfing taxa have a lower genetic diversity than species with a mixed or outcrossing breeding system. For my study species, this would imply that *Anagallis foemina* and *Bupleurum rotundifolium* are largely selfing, while *Adonis aestivalis* and *Anagallis arvensis* tend to have a mixed breeding system, and *Consolida regalis* and *Nigella arvensis* are more outcrossing species. This assumption holds certainly true for *Consolida regalis* because it is not self-pollinating (Svensson & Wigren 1986). As inflorescences of outcrossing taxa are generally larger than inflorescences of selfing species (Hill et al. 1992; Aarssen 2000), this assumption may also hold true for *Nigella arvensis* which has relatively large flowers and a relatively high genetic diversity. Indeed, *Nigella arvensis* has been described as self-compatible but mainly outcrossing (Bittkau & Comes 2005). *Anagallis arvensis* and *Anagallis foemina* are mainly selfing (Marsden-Jones & Weiss 1938; Gibbs & Talavera 2001), but they differ hugely in genetic diversity. In *Anagallis arvensis* the at least moderately high values for genetic diversity may be a consequence of the species still occurring in a larger number of populations, while *Anagallis foemina* is rare and populations are thus fragmented (Chapter 2). Current Red List assessments seem to present an overly optimistic picture (Frank et al. 1992; Westhus & Zündorf 1993; Korneck et al. 1996). Most likely the bluish flowering and more common *An. arvensis* is often mistakenly reported as *An. foemina* in surveys (Akerreta et al. 2007).

As fragmentation could lead to limited gene flow via pollen or seeds (Aguilar & Galetto 2004; Leimu et al. 2010), lower genetic diversity and structure in rare species could also be caused by higher fragmentation (Honnay & Jacquemyn 2007). In fragmented
populations of insect-pollinated species, pollinators struggle to reach the small and more distant populations (Pauw 2007). In addition, pollinators decline globally in abundance (Potts et al. 2010). The generally low seed dispersal rates and distances in arable plant species (Bischoff 2005; de Cauwer et al. 2008) further decrease because of restricted animal movement (Fahrig 2007), and because of modern seed cleaning procedures. Seed dispersal via farming machinery remains another possible option, but most seeds are dispersed over only very short distances in this way (Marshall & Brain 1999; Barroso et al. 2006).

**Provenance groups are important in arable weed conservation.**

My data imply that there are two provenance groups for Bupleurum rotundifolium in Central Europe, and further show that there can also be differences in genetic diversity values within these provenance groups (Chapter 3).

Although the importance of genetic aspects in practical nature conservation was mentioned decades ago (Frankel & Soulé 1981), the importance of genetic factors for e.g. recolonisation projects of rare plant species has not been widely perceived until quite recently (Donath & Eckstein 2008; Weeks et al. 2011; van Andel & Aronson 2012). Transplantation experiments have shown that plant fitness in the field (e.g. seedling survivorship, seed mass and plant biomass) is in most cases significantly lower if the seed material is from distant provenances (van Andel 1998; Jones & Hayes 1999; Keller et al. 2000; Leimu & Fischer 2008).

The reason is the lack of local adaptation (Leinonen et al. 2011) which is defined as a situation where resident genotypes have a higher relative fitness in their local habitat compared to genotypes originating from other habitats (Kawecki & Ebert 2004). The effects are likely due to ecological differences that tend to increase with geographical distances between regions (Becker et al. 2006a; Raabová et al. 2007; Bowman et al. 2008). Local adaptation can often be found in fragmented populations (e.g. Jakobsson & Dinnetz 2005) as the extent of local adaptation is depending on gene flow and selection (Savolainen et al. 2007). Local adaptation can be found in annual (Slotte et al. 2010; Harel et al. 2011; Gaut 2012) as well as perennial species (Leinonen et al. 2009; Kreyling et al. 2012) and does not seem to be affected by breeding system (Hereford 2010). In contrast, local adaptation is more commonly found in large populations (>1000 flowering individuals) than in small ones (Leimu & Fischer 2008). Effects may be even
stronger than reflected in the literature, because local adaptations are sometimes undetectable in the first years after transplantation, when most studies are typically conducted (Lipowsky et al. 2011; Hufford & Mazer 2012).

Intraspecific hybridisation between local and nonlocal genotypes could therefore lead to a negative impact because of outbreeding depression (Crémieux et al. 2010; Goto et al. 2011). The newly combined alleles from populations adapted to different environments are then not well adapted to either environment (Frankham et al. 2002). Restoration schemes with plants from different provenances thus have to find a balance between positive heterosis effects and negative outbreeding effects (Edmands 2007; Frankham et al. 2011). Genetic diversity values in different provenance groups, like in Bupleurum rotundifolium (Chapter 3), should also be taken into account, as Bischoff et al. (2010) detected an even higher impact of low genetic diversity values than of local adaptations due to plant fitness.

**Effects of population sizes on genetic diversity**

In my PhD project I found only limited effects of population sizes on genetic diversity. Correlations between population size and genetic diversity within species *in situ* and between *in situ* and *ex situ* were low and not significant. The only exception was *Nigella arvensis*, where larger populations had higher within-population diversity (Chapter 2 and 3).

This is in great contrast to a huge number of studies detecting a correlation between population size and genetic diversity both *in situ* (e.g. Hensen & Oberprieler 2005; Leimu et al. 2006; Peterson et al. 2008; Jacquemyn et al. 2009; de Vere et al. 2009) and also *ex situ* (Parzies et al. 2000; Gómez et al. 2005; Lauterbach et al. 2012a). Genetic drift and inbreeding increase with reduced population size (Ellstrand & Elam 1993; Willi et al. 2005; Leimu et al. 2010; Angeloni et al. 2011), and can lead to lower genetic diversity (Lacy 1987; Frankham et al. 2002; Charlesworth 2003). Thus, small population sizes could explain the lower genetic diversity in the *ex situ* cultivations of *Nigella arvensis*.

In the literature there are, however, also studies that did not detect a correlation between population size and genetic diversity (Leimu & Mutikainen 2005; Bachmann & Hensen 2007; Hensen et al. 2010; Lauterbach et al. 2011) or inbreeding (Routley et al. 1999; van Kleunen et al. 2007). This is also the case in most of my study species. One
possible explanation is that declining population sizes may not trigger decreases in genetic diversity over an evolutionary relatively short period of time (Ellstrand & Elam 1993; Bachmann 2007; Lauterbach et al. 2012b). Fragmentation effects in arable plants are also a rather young phenomenon (Dallimer et al. 2009; Walker et al. 2009; Meyer et al. 2013). Besides, many studies are based on just those population sizes in one year of analysis, and may not reflect bottlenecks occurring during potentially fluctuating populations (Bachmann 2007).

Because not all members of a population are reproductively active due to e.g. environmental but also biological constraints (Charlesworth 2009), Wright (1931) introduced the concept of the effective population size $N_e$, which is defined as “the number of breeding individuals in an idealized population that would show the same amount of dispersion of allele frequencies under random genetic drift or the same amount of inbreeding as the population under consideration” (Wright 1938). On average, the long-term effective population size has been estimated to be one-tenth of the actual population size (Frankham et al. 2002). Population size, however, often varies over time (Eisto et al. 2000; Eriksson et al. 2010) and especially in annual plants, population sizes fluctuate strongly from year to year (Nunney 2002). When population sizes are not constant, the effective population size is equal to the harmonic mean of the population size (Wright 1938; Nei et al. 1975) and as a result very close to the size of the generation with the smallest effective population size (Frankham et al. 2002). Thus, the effective population sizes of arable plants in fluctuating in situ populations and more stable but smaller ex situ populations may be approximately equal and therefore hardly lead to differences in genetic diversity values.

Taken together, it seems that population sizes in arable plants have limited impact on genetic diversity. Effective population sizes in situ are at least periodically small because of fluctuating population sizes and thus probably differ not much from effective population sizes ex situ.

*Effects of ex situ cultivation on genetic diversity*

My data demonstrate that most of the analyzed species tended to have lower genetic diversity values with ex situ cultivation compared to wild populations. Even more pronounced was the fact that the ex situ populations had a reduced set of alleles compared to wild populations (Chapter 4).
There are several possible explanations for altered genetic structures and lower genetic diversity values of ex situ populations. Firstly, an inefficient sampling of genetic polymorphisms during the establishment of the ex situ collection could have caused a bottleneck situation (Hamilton 1994; Rogers 2004). Bottlenecks were indeed detected in a number of studies on ex situ conservation of plants (Zhu et al. 2007; Guo et al. 2010; Negri & Tiranti 2010), and may cause lower genetic diversity and insufficient representation of alleles in garden populations.

Secondly, the lower genetic variability could be caused by genetic drift. Ex situ populations in botanical gardens are often small (Enßlin et al. 2011; Lauterbach et al. 2012a), facing a higher risk for genetic drift. For my analyzed species population sizes in botanical gardens were mostly just between five and fifty individuals; and thus were predominantly smaller than in situ populations that may cover an entire field (Chapter 3). The missing correlation between population size and genetic diversity in most of my species (Chapter 2 and 3, see also above), however, renders population size as an explanation for low genetic diversity in ex situ populations fairly unlikely.

A third explanation for the presented results could be increased selfing and inbreeding (Fernández & González-Martínez 2009), which reduces the number of polymorphic loci and could also lead to inbreeding depression (Kolehmainen et al. 2010).

A wider comparison of my results with 32 other studies on genetic diversity with in situ and ex situ cultivation of plants indicated that life form and breeding system have a significant impact on genetic diversity (Chapter 4). The only species showing a higher genetic diversity with ex situ cultivation was Stenocereus stellatus, a perennial and self-incompatible plant (Casas et al. 2006). Species showing no difference between in situ and ex situ cultivation are mainly perennial and outcrossing. The majority of species showed lower genetic diversity values with ex situ cultivation, and was mainly short-lived and frequently self-pollinating.

This corresponds to well-established theory: a review including 229 studies on annuals also found lower genetic diversity in annuals than in perennials (Hamrick & Godt 1996). It is also widely accepted that selfing species show lower genetic diversity than outcrossing species (Loveless & Hamrick 1984; Schoen & Brown 1991; Nybom & Bartish 2000; Charlesworth 2003; Mable & Adam 2007). This suggests that diversity of annual
and self-compatible species like most arable plants are especially negatively affected by *ex situ* conservation.

Not all of my study species were similarly negatively affected by *ex situ* cultivation (Chapter 4). This could be due to the limited number of replicates in some species which limited statistical power. Three or four *ex situ* collections are not likely to adequately represent allelic diversity in the wild, which is supported by the AMOVA and the analysis of loci present in the entire *ex situ* population (Chapter 4). Apart from these statistical considerations, the low number of *ex situ* populations present in European botanical gardens also poses a fundamental problem for conservation (Maunder et al. 2001b). In addition, the majority of threatened taxa in botanical gardens has been founded from non-wild origin accessions and/or poorly documented sources (Maunder et al. 2001b). The striking lack of information on source populations as well as the conservation history in the botanical gardens casts doubt on the value of using such *ex situ* populations for potential reintroductions due to genetic erosion, adaptation, hybridisation or unconscious selection of gardeners. Most of the analyzed *ex situ* populations should be tested for fitness and similarity to wild populations before they were brought to the field.

*Effects of the duration of ex situ cultivation on genetic diversity.*

Duration of cultivation did not show any effect on genetic diversity (Chapter 3), which contrasts with studies that found declining genetic diversity with the duration of cultivation (Hamrick et al. 1979; Parzies et al. 2000; Rice et al. 2006; Enßlin et al. 2011). The extent of genetic drift and inbreeding commonly increases with the duration of cultivation (Hartl & Clark 1997) as the duration correlates with the number of generation cycles (Spagnoletti-Zeuli et al. 1995). This should be especially severe in short lived species (Duminil et al. 2009) if they are generated every year, which was the case in those populations, studied here. Duration of *ex situ* cultivation in the botanical gardens was usually more than ten and often more than 20 years (Chapter 3) corresponding to more than 10 or even 20 generations. Moreover, garden populations were sometimes established from other garden populations, so that total cultivation time may have been much longer. I nonetheless found no evidence for any effect of cultivation duration on genetic diversity, although my data have to be taken with care due to limited number of sampled populations.
Arable plant species usually occur under environmental conditions that are relatively easily mimicked in botanical gardens (intense cultivation, relatively high fertility and high levels of disturbance). Potentially unwanted selection effects may therefore be limited, compared to plants with more specialised habitat requirements. In addition, this suggests again that small population sizes are to some extent of minor importance for maintaining genetic diversity. The even so observed differences in genetic diversity and structure *in situ* and *ex situ* are therefore probably mainly caused by bottleneck effects rather than drift or inbreeding.

Nonetheless, the extension of generation time by dry storage of seeds at cold temperatures still offers a cheap and recommended alternative to cultivation (Guerrant et al. 2004), and would also be suitable for my species that form large seed banks even in the wild (Wäldchen et al. 2005; Kohler et al. 2011): seeds of *Adonis aestivalis* showed for example a longevity of more than 50 years (Wäldchen 2004), seeds of *Anagallis arvensis* and *Anagallis foemina* of more than 100 years (Salisbury 1961; Bogenrieder & Bühler 1991), seeds of *Bupleurum rotundifolium* of at least 12 years (Wäldchen et al. 2005), seeds of *Consolida regalis* of more than 11 years (Thompson et al. 1997) and seeds of *Nigella arvensis* of more than 50 years (Wäldchen 2004).

That cold storage of seeds can extend generation time even up to 30,000 years was shown for *Silene stenophylla* (Yashina et al. 2012). Cold storage is, however, apparently not frequently employed by those German gardens that were approached for this study. In contrast, many botanic gardens around the world are very focused on seed banking threatened species under conditions that will maintain long-term viability of the seeds (Falk 1990; Touchell et al. 1997; Puchalski 2004). Kew’s Millennium Seed Bank, for example, stores more than 1.8 billion seeds of more than 30,000 species of which twelve species are listed as globally extinct in the wild (Kew Royal Botanic Gardens 2012). National and regional genebanks for agricultural plants in Germany can also serve as a model for long-term conservation as they store over 6 million accessions *ex situ* (Scarascia-Mugnozza & Perrino 2002).

### Pollination experiment

We tested whether artificial cross-pollination within populations of *B. rotundifolium* could help to sustain genetic diversity without involving the risk of outbreeding depression involved with cross-pollination across different populations. In *ex situ*
populations, artificial cross-pollination within populations did not result in higher genetic diversity compared to self-pollinated individuals (Chapter 5). The study had pilot character and reasons for this detected pattern are thus not fully clear. The occurrence of purging, i.e. the preferential elimination of recessive deleterious alleles in inbred lines which leads to occasional superior performance of selfed individuals (Crow 1948; Lande & Schemske 1985), could explain the equally high values of genetic diversity after selfing. Due to the limited sample size results could, however, also be partly caused by random effects. Apart from that it is also possible that it takes several generations of enforced selfing and outcrossing to find some effects of the different treatments (e.g. Picó et al. 2007). On the other hand there are even more studies that found effects of pollination experiments after only one generation for both inbreeding (e.g. Becker et al. 2006b; Picó et al. 2007) and outbreeding (e.g. Quilichini et al. 2001). In these cases, outbreeding effects were usually tested across populations.

In any case, in view of our question we had to conclude that no evidence was found for superior genetic diversity after artificial cross populations. With respect to these first results it seemed unwise to spend more time and money in a larger experiment. A subsequent experiment would have been much larger because pollination networks should be recorded on an individual base, allowing to assess potential effects in more details. Perhaps even more crucial is the application of a different marker system. Codominant markers like microsatellites are now much easier available than they used to be 6 years ago at the start of the present thesis (e.g. Opgenoorth 2009; Aranzana et al. 2012), and would allow to get a better impression of the heterozygosity. With the availability of next-generation sequencing, even other approaches like sequencing larger DNA sets and using e.g. SNPs is also becoming feasible now (Aranzana et al. 2012).

Practical considerations

As ex situ and in situ conservation are complementary approaches and not mutually exclusive (Volis & Blecher 2010) it is absolutely necessary to rethink protection efforts in the botanical gardens as well as in the field. My data add a genetic base for this repeatedly made claim; and more or less generally poor genetic structures found for both ex situ and in situ populations suggest that current conservation schemes are far from sufficient.
In situ conservation suffered from several constraints. In the last decades the establishment of field margin strips (Wicke 1998) or field flora reserves (Illig & Kläge 1994) supported the stabilization of arable plants in situ. Because of highly bureaucratic procedures or declining funds most of these projects are now finished (Meyer et al. 2010). In addition, prices for agricultural land and products have risen, putting conservation management under increasing competition with intensive land use (Litterski & Hampicke 2008). A new conservation project for arable plants, the so called “100 fields for biodiversity”, aims at building up a network of at least 100 conservation fields under long-term protection (Meyer et al. 2010), which is a great step forward. In most cases these protected fields are, however, far away from each other so that genetic exchange between populations is rarely feasible.

My data give evidence to suggest that population sizes are of minor importance for some arable plant species. For those species it seems to be more crucial to preserve several stands, possibly including even small population ideally being close enough for genetic exchange. Another practical implication is that the occurrence of Anagallis foemina is largely overestimated. Current Red List status should consequently be raised.

As the soil seed bank of especially conventionally managed fields is often depleted (Albrecht & Forster 1996; Albrecht 2005), potential restoration programmes would often have to rely on seed material brought in from other fields. The different provenance groups of plant species should then be taken into account. Ideally, genetic studies should be conducted for any given case. Given that this is not realistic, results from chapter 3 should prompt practitioners to avoid transport of material over exceedingly large distances.

The overall critical genetic structures of most arable plant species will it nonetheless make necessary to extend ex situ protection for rare and threatened arable plants. For establishing and maintaining ex situ cultivations the basic recommendations are already known (Guerrant et al. 2004; Volis & Blecher 2010; Rae 2011) but up to now suggestions for the establishment of ex situ populations have rarely been adopted (Heywood 2011). My PhD project basically supports this finding. Bottleneck effects provide the most straightforward explanation for the observed patterns and thus point to shortcomings during establishment of ex situ collections. Clearly, seeds should be collected from many different individuals within a given population. Falk and Holsinger (1991) for example recommended collecting 1 to 20 seeds from 10 to 50 sampled
individuals from 5 separate populations. Brown and Marshall (1995) as well as the Center for Plant Conservation (2004) suggest collecting seeds from 50 individuals out of 50 populations per ecogeographic region. Refreshing *ex situ* collections every few years with new *in situ* material is another (though relatively costly) measure but should be considered if the cultivated populations are to reflect the natural diversity. Ideally, fresh material should come from the source population.

I found less clear evidence for effects of generation time and population size. Arable plants may be less sensitive to typical trends in small populations, possibly reflecting adaptations to their frequently instable habitats. Nonetheless, the extension of generation time by dry storage of seeds at cold temperatures still offers a cheap and recommended alternative to cultivation (Guerrant et al. 2004), and would also be suitable for my species that form large seed banks even in the wild (Wäldchen et al. 2005). Another successful long term seed preservation method is the ultradrying of seeds with e.g. silica gel down to a moisture content of 1-3% (Gómez-Campo 2007; Pérez-García, et al. 2007).

Only 27% of the taxa listed on the European threatened plant list are stored in European seed banks and, considering the number of accessions per species and the number of seeds per accession, at least two thirds of them suffer from unacceptably low genetic diversity (Godefroid et al. 2011b). This should raise concern of conservationists, as low genetic diversity leads to limited fitness (Keller & Waller 2002; Reed & Frankham 2003; Leimu et al. 2006). Improved collection efforts are crucial in this respect, while options to improve cultivation in the broad sense are probably limited. Facilitating pollination among the already available plants of a given population can so far not be recommended as a promising tool for *ex situ* conservation: The effort is relatively high compared to the usual practice of simply relying on “natural” pollination, and effects are unclear with respect to the limited available data. Artificial cross-pollination between populations is more likely to increase gene diversity on the population level. It is, however, also time consuming, and may additionally involve the risk of outbreeding depression.

In any case, *ex situ* conservation is resource-consuming and conservation efforts may require a clear strategy with setting priorities on for example locally threatened plants (Rae 2011). Such a strategy could be based on alternate seeding of target species on the available space, while keeping seeds of other species under cold storage. This would allow showing displays and still having relatively large populations in the generative
phase. In addition, the construction of a network of gene banks for wild plant species should be promoted (Hurka et al. 2008). The role of botanical gardens for conserving plant species and their genetic diversity as well as education and recreation could thus not only be maintained, but largely improved.
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Erklärung über den persönlichen Anteil an den Publikationen

Die folgende Auflistung gibt einen Überblick über meinen persönlichen Anteil an den hier zusammengestellten Publikationen mit Koautorenschaft:

“Genetic diversity of six arable plants in relation to their Red List status”
Datenerhebung: 90% (40% der Proben gesammelt von S Meyer)
Datenanalyse: 95% (eine Abbildung erstellt von K Wesche)
Schriftliche Umsetzung: 90% (Korrekturen durch I Hensen, S Meyer und K Wesche)

“Spatial genetic structure and low diversity of the rare arable plant Bupleurum rotundifolium L. indicate fragmentation in Central Europe”
Datenerhebung: 50% (50% der Proben gesammelt von S Meyer, 50% Laborarbeit von P Kühne)
Datenanalyse: 100%
Schriftliche Umsetzung: 95% (Korrekturen durch I Hensen, S Meyer und K Wesche)

“Ex situ cultivation affects genetic structure and diversity in arable plants”
Datenerhebung: 95% (20% der Proben gesammelt von S Meyer)
Datenanalyse: 100%
Schriftliche Umsetzung: 95% (Korrekturen durch I Hensen und K Wesche)

“Does artificial cross-pollination increase genetic diversity compared to self-pollinated individuals of Bupleurum rotundifolium?”
Datenerhebung: 50% (50% Laborarbeit von P Kühne)
Datenanalyse: 95% (Korrekturen durch K Wesche)
Schriftliche Umsetzung: 95% (Korrekturen durch I Hensen und K Wesche)

Halle (Saale), den 05.06.2013

Unterschrift:
Appendix

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Unterschrift:
Publikationen

C Brütting, P Kühne, I Hensen & K Wesche (Manuskript): Does artificial cross-pollination increase genetic diversity compared to self-pollinated individuals of *Bupleurum rotundifolium*?


Halle (Saale), den 05.06.2013

Unterschrift:
Eigenständigkeitserklärung

Hiermit erkläre ich, dass diese Arbeit nicht bereits zu einem früheren Zeitpunkt der Naturwissenschaftlichen Fakultät I – Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg oder einer anderen wissenschaftlichen Einrichtung zur Promotion vorgelegt wurde.

Darüber hinaus erkläre ich, dass ich die vorliegende Arbeit eigenständig und ohne fremde Hilfe verfasst sowie keine anderen als im Text angegebenen Quellen und Hilfsmittel verwendet habe. Textstellen, welche aus verwendeten Werken wörtlich oder inhaltlich übernommen wurden, wurden von mir als solche kenntlich gemacht.

Im Übrigen erkläre ich, dass ich mich noch nie um einen Doktorgrad beworben habe.

Halle (Saale), den 05.06.2013

Unterschrift:
Danksagung

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