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.

Table of contents

Summary

The global decline of species and habitats makes e*x 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.

In meiner Dissertation untersuchte ich diese Aspekte bei Ackerwildkräutern, da diese in Mitteleuropa eine der meist bedrohten Artengruppen darstellen. Bedingt durch ihre vorwiegend annuelle Lebensform und die Fähigkeit zur Selbstbestäubung sollten Ackerwildkräuter besonders sensitiv auf zunehmende Habitatfragmentierung und damit einhergehende abnehmende Populationsgrößen reagieren. Bislang gibt es nur wenige Untersuchungen zur genetischen Struktur von Ackerwildkräutern. Deshalb habe ich die genetische Struktur und Diversität von 67 Freiland- und 20 Garten-Populationen von *Adonis aestivalis*, *Anagallis arvensis*, *Anagallis foemina*, *Bupleurum rotundifolium*, *Consolida regalis* und *Nigella arvensis* untersucht und die Diversitätswerte mit den Populationsgrößen und – für die *Ex situ* Populationen – mit der Kultivierungsdauer korreliert. Mit *Bupleurum rotundifolium* wurden Bestäubungsexperimente durchgeführt, um zu untersuchen, ob künstliche Kreuzbestäubung innerhalb von Populationen im Gegensatz zur künstlichen Selbstbestäubung eine höhere genetische Diversität bewirkt.

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 Übersichtsarbeit 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 Kultivierungsdauer 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.

E*x situ*- und *In situ*-Kultivierung sollten als sich gegenseitig ergänzende Ansätze betrachtet werden. So kann die Errichtung von Ackerrandstreifen und Feldflorareservaten Fragmentierungseffekte abschwächen und die Stabilisierung der Ackerwildkräuter *in situ* unterstützen. Bei Rekultivierungsprogrammen sollten mögliche unterschiedliche Herkünfte der Pflanzen beachtet werden. Für die *Ex situ*-Kultivierung sollten Samen von vielen Individuen innerhalb eines Herkunftsgebietes gesammelt und möglichst im Abstand von einigen Jahren mit neuem *In-situ*-Material aufgefrischt werden.

Ich fand keine eindeutigen Zusammenhänge zwischen Populationsgröße und Kultivierungsdauer 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.

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 senstive 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 (Bretschneider 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 *in situ* and *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 *in situ* after decades of agricultural intensification, and how these changed due to cultivation *ex situ*, I analyzed genetic structure and diversity of 67 *in situ* and 20 *ex situ* populations of six arable plant species and correlated the genetic data with population sizes (*in situ* and *ex situ*) and the duration of cultivation (*ex situ*). In a final step, a pollination experiment was performed to assess options for increasing genetic diversity *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 diaspore 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*   Figure 2: *Anagallis arvensis*

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)   Figure 4: *Bupleurum rotundifolium*

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*   Figure 6: *Nigella arvensis*

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.
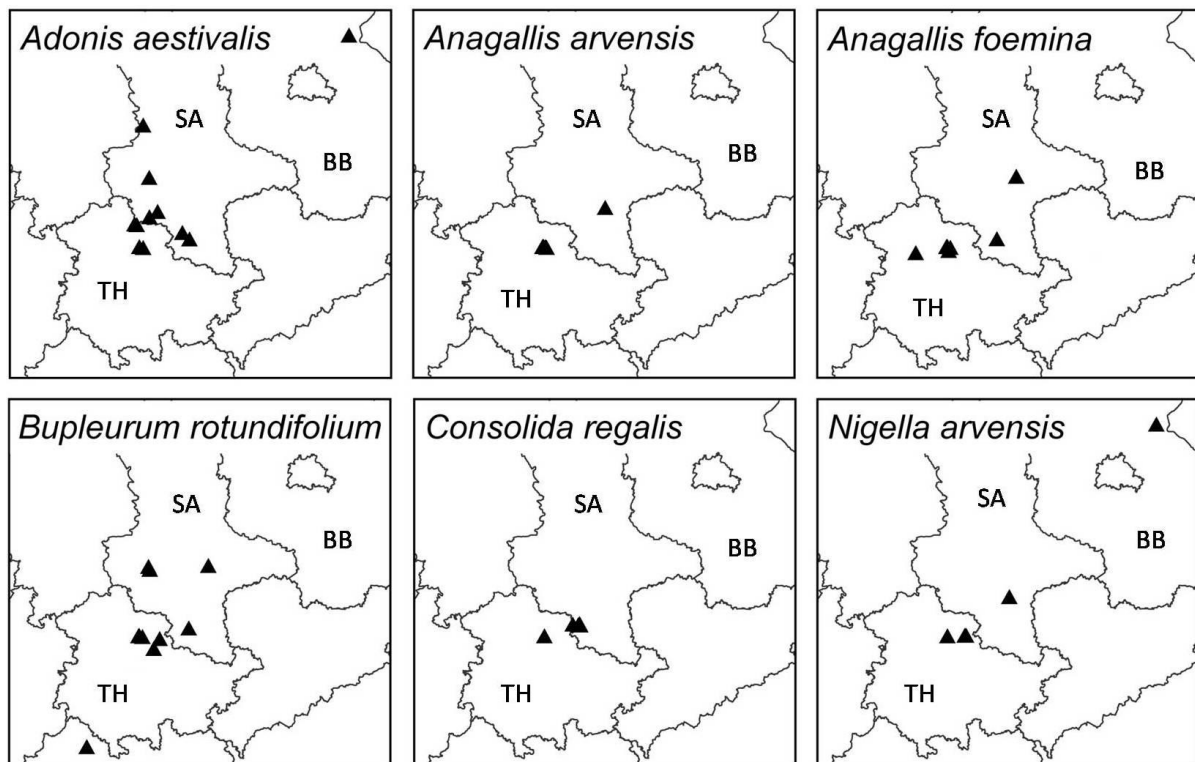
1.3 Study area

Figure 7: Location of sampled populations for the study species *Adonis aestivalis*, *Anagallis arvensis*, *Anagallis foemina*, *Bupleurum rotundifolium*, *Consolida regalis* and *Nigella arvensis* in the study area (SA=Saxony-Anhalt, TH=Thuringia, BB=Brandenburg).

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).
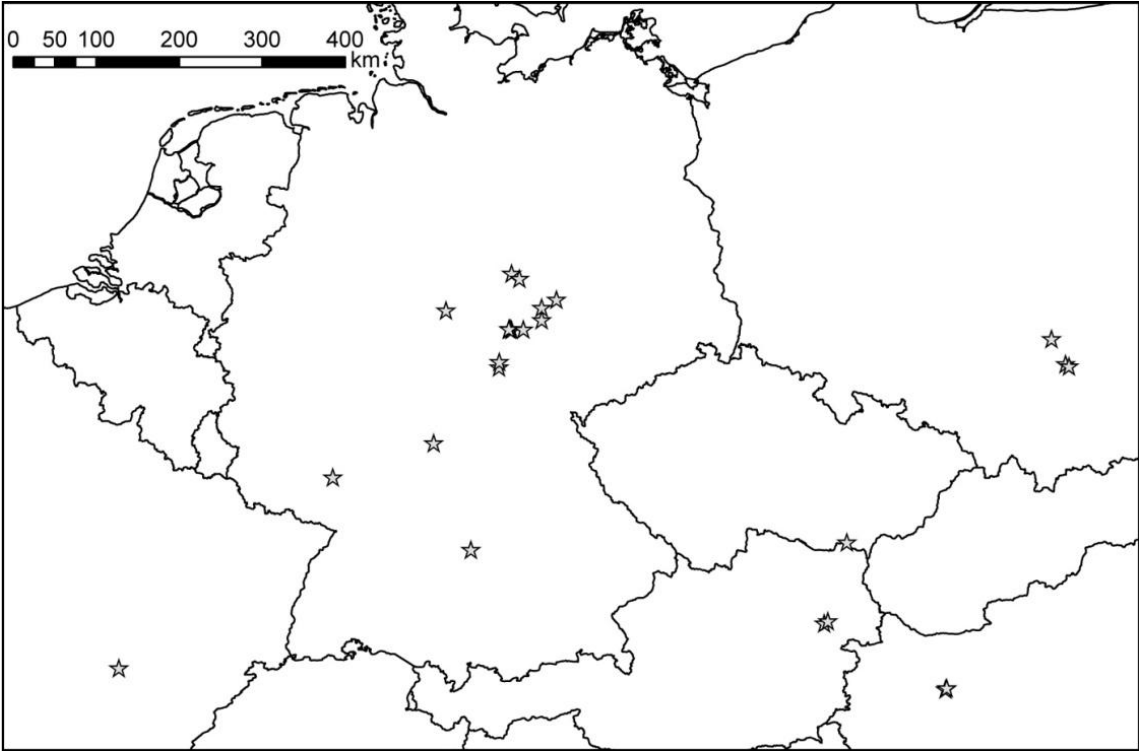
Figure 8: Location of the sampled *Bupleurum rotundifolium* populations (n=27).

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. Φ_{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.

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

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_{ST} -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%).

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 Φ_{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.

References

Aarssen, L.W. 2000. „Why are most selfers annuals? A new hypothesis for the fitness benefit of selfing". *Oikos* 89 (3): 606–612.

Aguilar, R., & L. Galetto. 2004. „Effects of forest fragmentation on male and female reproductive success in *Cestrum parqui* (Solanaceae)". *Oecologia* 138 (4): 513–520.

Akerreta, S., R. Y Cavero, V. López, & M. I Calvo. 2007. „Analyzing factors that influence the folk use and phytonomy of 18 medicinal plants in Navarra". *Journal of Ethnobiology and Ethnomedicine* 3 (16): 1–18.

Albrecht, H. 2005. „Development of arable weed seedbanks during the 6 years after the change from conventional to organic farming". *Weed Research* 45 (5): 339–350.

Albrecht, H., & E.M. Forster. 1996. „The weed seed bank of soils in a landscape segment in southern Bavaria—I. Seed content, species composition and spatial variability". *Plant Ecology* 125 (1): 1–10.

van Andel, J. 1998. „Intraspecific variability in the context of ecological restoration projects". *Perspectives in Plant Ecology, Evolution and Systematics* 1 (2): 221–237.

van Andel, J., & J. Aronson. 2012. *Restoration Ecology: The New Frontier*. Hoboken: Wiley & Blackwell.

Angeloni, F., N.J. Ouborg, & R. Leimu. 2011. „Meta-analysis on the association of population size and life history with inbreeding depression in plants". *Biological Conservation* 144 (1): 35–43.

Aranzana, M.J., T. Barreneche, & P. Arús. 2012. „Diversity analysis". In: *Genetics, Genomics and Breeding of Stone Fruits*: 55–75.

Ashton, P.S. 1988. „Conservation of biological diversity in botanical gardens". In *Biodiversity (EO Wilson & FM Peter, ed.)*. 269–278. Washington, DC, USA: National Academy Press.

Bachmann, U. 2007. „Die Gefährdung der Knäuel-Glockenblume *Campanula glomerata* L. in Deutschland". Halle, Saale: Martin-Luther Universität.

Bachmann, U., & I. Hensen. 2007. „Is declining *Campanula glomerata* threatened by genetic factors?" *Plant Species Biology* 22 (1): 1–10.

Baessler, C., S. Klotz, & W. Durka. 2010. „Temporal changes and spatial determinants of plant species diversity and genetic variation". *Long-Term Ecological Research*: 279–297.

Ballantyne, R., J. Packer, & K. Hughes. 2008. „Environmental awareness, interests and motives of botanic gardens visitors: Implications for interpretive practice". *Tourism Management* 29 (3): 439–444.

Balmford, A., L. Bennun, B. ten Brink, D. Cooper, I.M. Côté, P. Crane, A. Dobson, et al. 2005. „The convention on biological diversity's 2010 target". *Science* 307 (5707): 212–213.

Barroso, J., L. Navarrete, M.J. Sánchez del Arco, C. Fernandez-Quintanilla, P.J.W. Lutman, N.H. Perry, & R.I. Hull. 2006. „Dispersal of *Avena fatua* and *Avena sterilis* patches by natural dissemination, soil tillage and combine harvesters". *Weed Research* 46 (2): 118–128.

Becker, U., G. Colling, P. Dostal, A. Jakobsson, & D. Matthies. 2006a. „Local adaptation in the monocarpic perennial *Carlina vulgaris* at different spatial scales across Europe". *Oecologia* 150 (3): 506–518.

Becker, U., T. Reinhold, & D. Matthies. 2006b. „Effects of pollination distance on reproduction and offspring performance in *Hypochoeris radicata*: Experiments with plants from three European regions". *Biological Conservation* 132 (1): 109–118.

Bellard, C., C. Bertelsmeier, P. Leadley, W. Thuiller, & F. Courchamp. 2012. „Impacts of climate change on the future of biodiversity". *Ecology Letters* 15 (4): 365–377.

BfN. 2011. „floraweb.de".

BGCI. 2012. http://http://www.bgci.org/resources/1528/.

Biesmeijer, J.C., S.P.M. Roberts, M. Reemer, R. Ohlemüller, M. Edwards, T. Peeters, A.P. Schaffers, S.G. Potts, R. Kleukers, & C. D. Thomas. 2006. „Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands". *Science* 313 (5785): 351–354.

Bischoff, A. 2005. „Analysis of weed dispersal to predict chances of re-colonisation". *Agriculture, Ecosystems & Environment* 106 (4): 377–387.

Bischoff, A., T. Steinger, & H. Müller-Schärer. 2010. „The importance of plant provenance and genotypic diversity of seed material used for ecological restoration". *Restoration Ecology* 18 (3): 338–348.

Bittkau, C., & H.P. Comes. 2005. „Evolutionary processes in a continental island system: molecular phylogeography of the Aegean *Nigella arvensis* alliance (Ranunculaceae) Inferred from chloroplast DNA". *Molecular Ecology* 14 (13): 4065–4083.

Bogenrieder, A., & M. Bühler. 1991. „Zwischen Beharren und Wandel – Pflanzengesellschaften unter dem Einfluss des wirtschaftenden Menschen." *Das Marktgräferland* 81:25–64. Hoppe, A. (Publisher). Freiburg i. Br: Berichte der Naturforschenden Gesellschaft.

Bowman, G., C. Perret, S. Hoehn, D.J. Galeuchet, & M. Fischer. 2008. „Habitat fragmentation and adaptation: a reciprocal replant–transplant experiment among 15 populations of *Lychnis flos-cuculi*". *Journal of Ecology* 96 (5): 1056–1064.

Bretschneider, E. 1895. „Botanicon Sinicum: notes on Chinese botany from native and western sources". *Journal of the North China Branch of the Royal Asiatic Society* 29: 11–12.

Brooks, T.M., R.A. Mittermeier, C.G. Mittermeier, G.A.B. Da Fonseca, A.B. Rylands, W.R. Konstant, P. Flick, J. Pilgrim, S. Oldfield, & G. Magin. 2002. „Habitat loss and extinction in the hotspots of biodiversity". *Conservation Biology* 16 (4): 909–923.

Brown, A.H.D., & D.R. Marshall. 1995. „A basic sampling strategy: theory and practice". In *Collecting plant genetic diversity: techical guidelines*, 75–91. Wallingford, UK: CAB International.

Brush, S.B. 1994. „*In situ* conservation of landraces in centers of crop diversity". *Crop Science* 35 (2): 346–354.

Butchart, S.H.M., M. Walpole, B. Collen, A. van Strien, J.P.W. Scharlemann, R.E.A. Almond, J.E.M. Baillie, et al. 2010. „Global biodiversity: indicators of recent declines". *Science* 328 (5982): 1164–168.

Casas, A., J. Cruse-Sanders, E. Morales, A. Otero-Arnaiz, & A. Valiente-Banuet. 2006. „Maintenance of phenotypic and genotypic diversity in managed populations of *Stenocereus stellatus* (Cactaceae) by indigenous peoples in Central Mexico". *Biodiversity and Conservation* 15 (3): 879–898.

de Cauwer, B., D. Reheul, I. Nijs, & A. Milbau. 2008. „Management of newly established field margins on nutrient-rich soil to reduce weed spread and seed rain into adjacent crops". *Weed Research* 48 (2): 102–112.

CBD. 2012. „Convention on biological diversity". http://www.cbd.int/convention/text/.

Charlesworth, B. 2009. „Effective population size and patterns of molecular evolution and variation". *Nature Reviews Genetics* 10 (3): 195–205.

Charlesworth, D. 2003. „Effects of inbreeding on the genetic diversity of populations". *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 358 (1434): 1051–1070.

de Chazal, J., & M.D.A. Rounsevell. 2009. „Land-use and climate change within assessments of biodiversity change: A review". *Global Environmental Change* 19 (2): 306–315.

Cole, C.T. 2003. „Genetic variation in rare and common plants". *Annual Review of Ecology, Evolution, and Systematics* 34: 213–237.

CPC, Center for Plant Conservation. 2004. „Revised genetic sampling guidelines for conservation collections of rare and endangered plants". In Ex situ *plant conservation: Supporting species survival in the wild.* 419–441. Washington, DC: Island Press.

Crémieux, L., A. Bischoff, H. Müller-Schärer, & T. Steinger. 2010. „Gene flow from foreign provenances into local plant populations: Fitness consequences and implications for biodiversity restoration". *American Journal of Botany* 97: 94–100.

Crow, J.F. 1948. „Alternative hypotheses of hybrid vigor". *Genetics* 33: 477.

Dallimer, M., D. Tinch, S. Acs, N. Hanley, H.R. Southall, K.J. Gaston, & P.R. Armsworth. 2009. „100 years of change: examining agricultural trends, habitat change and stakeholder perceptions through the 20th century". *Journal of Applied Ecology* 46: 334–343.

Darwin, C. 1876. *The effects of cross and self fertilization in the vegetable kingdom*. London: John Murray.

Délye, C., J.A.J. Clément, F. Pernin, B. Chauvel, & V. Le Corre. 2010. „High gene flow promotes the genetic homogeneity of arable weed populations at the landscape level". *Basic and Applied Ecology* 11 (6): 504–512.

Donath, T.W., & R.L. Eckstein. 2008. „Bedeutung genetischer Faktoren für die Wiederansiedlung seltener Pflanzengemeinschaften". *Naturschutz und Landschaftsplanung* 40: 21–25.

Dulloo, M.E., D. Hunter, & T. Borelli. 2010. „*Ex situ* and *in situ* conservation of agricultural biodiversity: major advances and research needs". *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 38 (2): 123–135.

Duminil, J., O.J. Hardy, & R.J. Petit. 2009. „Plant traits correlated with generation time directly affect inbreeding depression and mating system and indirectly genetic structure". *BMC Evolutionary Biology* 9: 177–190.

Edmands, S. 2007. „Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management." *Molecular Ecology* 16 (3): 463–475.

Ehlers, B.K. 1999. „Variation in fruit set within and among natural populations of the self-incompatible herb *Centaurea scabiosa* (Asteraceae)". *Nordic Journal of Botany* 19 (6): 653–663.

Eichstaedt, U., & E.G. Mahn. 1993. „Comparative phytosociological investigations of windbreaks and other hedgerows of the Querfurter Platte (central German dry region)". *Phytocoenologia* 23: 519–537.

Eisto, A.-K., M. Kuitunen, A. Lammi, V. Saari, J. Suhonen, S. Syrjäsuo, & P.M. Tikka. 2000. „Population persistence and offspring fitness in the rare bellflower *Campanula cervicaria* in relation to population size and habitat quality". *Conservation Biology* 14 (5): 1413–1421.

Ellstrand, N.C., & D.R. Elam. 1993. „Population genetic consequences of small population size: implications for plant conservation". *Annual Review of Ecology and Systematics* 24: 217-242.

Enßlin, A., T.M. Sandner, & D. Matthies. 2011. „Consequences of *ex situ* cultivation of plants: Genetic diversity, fitness and adaptation of the monocarpic *Cynoglossum officinale* L. in botanic gardens". *Biological Conservation* 144 (1): 272–278.

Eriksson, A., B. Mehlig, M. Rafajlovic, & S. Sagitov. 2010. „The total branch length of sample genealogies in populations of variable size". *Genetics* 186 (2): 601–611.

Fahrig, L. 2007. „Non-optimal animal movement in human-altered landscapes". *Functional Ecology* 21 (6): 1003–1015.

Falk, D.A. 1990. „Integrated strategies for conserving plant genetic diversity". *Annals of the Missouri Botanical Garden* 77: 38–47.

Falk, D.A., & K.E. Holsinger. 1991. *Genetics and conservation of rare plants.* Oxford: Oxford University Press.

Fernández, J., & S.C. González-Martínez. 2009. „Allocating individuals to avoid inbreeding in *ex situ* conservation plantations: so far, so good". *Conservation Genetics* 10 (1): 45–57.

Fernández, J., & S.C. González-Martínez. 2010. „SOFSOG: a suite of programs to avoid inbreeding in plantation designs". *Molecular Ecology Resources* 10 (2): 393–396.

Fischer, M., & D. Matthies. 1998. „RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae)". *American Journal of Botany* 85 (6): 811–819.

Frank, D., H. Herdam, H. Jage, S. Klotz, F. Ratey, U. Wegener, E. Weinert, & W. Westhus. 1992. „Rote Liste der Farn- und Blütenpflanzen des Landes Sachsen-Anhalt". *Ber. Landesamt Umweltschutz Sachsen-Anhalt* 1: 44–63.

Frankel, O.H., A.H.D. Brown, & J.J. Burdon. 1995. *The conservation of plant biodiversity*. Cambridge: Cambridge University Press.

Frankel, O.H., & M.E. Soulé. 1981. *Conservation and evolution*. Cambridge: Cambridge University Press.

Frankham, R. 2005. „Genetics and extinction". *Biological Conservation* 126: 131–140.

Frankham, R., J.D. Ballou, & D.A. Briscoe. 2002. *Introduction to conservation genetics*. Cambridge: Cambridge University Press.

Frankham, R., J.D. Ballou, M.D.B. Eldridge, R.C. Lacy, K. Ralls, M.R. Dudash, & C.B. Fenster. 2011. „Predicting the probability of outbreeding depression". *Conservation Biology* 25: 465-475

Galmés, J., A. Abadía, H. Medrano, & J. Flexas. 2007. „Photosynthesis and photoprotection responses to water stress in the wild-extinct plant *Lysimachia minoricensis*". *Environmental and Experimental Botany* 60 (3): 308–317.

Gaut, B. 2012. „*Arabidopsis thaliana* as a model for the genetics of local adaptation". *Nature Genetics* 44 (2): 115–116.

Geiger, F., J. Bengtsson, F. Berendse, W.W. Weisser, M. Emmerson, M.B. Morales, P. Ceryngier, et al. 2010. „Persistent negative effects of pesticides on biodiversity and biological control potential on European farmland". *Basic and Applied Ecology* 11 (2): 97–105.

Giam, X., C.J.A. Bradshaw, H.T.W. Tan, & N.S. Sodhi. 2010. „Future habitat loss and the conservation of plant biodiversity". *Biological Conservation* 143 (7): 1594–1602.

Gibbs, P.E., & S. Talavera. 2001. „Breeding system studies with three species of *Anagallis* (Primulaceae): self-incompatibility and reduced female fertility in *A. monelli* L." *Annals of Botany* 88 (1): 139–144.

Gitzendanner, M.A., & P.S. Soltis. 2000. „Patterns of genetic variation in rare and widespread plant congeners". *American Journal of Botany* 87 (6): 783–792.

Glowka, L., F. Burhenne-Guilmin, & H. Synge. 1994. *A guide to the convention on biological diversity*. 30. World Conservation Union.

Godefroid, S., C. Piazza, G. Rossi, S. Buord, A.-D. Stevens, R. Aguraiuja, C. Cowell, et al. 2011a. „How successful are plant species reintroductions?" *Biological Conservation* 144 (2): 672–682.

Godefroid, S., S. Rivière, S. Waldren, N. Boretos, R. Eastwood, & T. Vanderborght. 2011b. „To what extent are threatened European plant species conserved in seed banks?" *Biological Conservation* 144 (5): 1494–1498.

Golding, J., S. Güsewell, H. Kreft, V.Y. Kuzevanov, S. Lehvävirta, I. Parmentier, & M. Pautasso. 2010. „Species-richness patterns of the living collections of the world's botanic gardens: a matter of socio-economics?" *Annals of Botany* 105 (5): 689–696.

Gómez, O.J., M.W. Blair, B.E. Frankow-Lindberg, & U. Gullberg. 2005. „Comparative study of common bean (*Phaseolus vulgaris* L.) landraces conserved *ex situ* in genebanks and *in situ* by farmers". *Genetic Resources and Crop Evolution* 52: 371–380.

Gómez-Campo, C. 2007. *A guide to efficient long term seed preservation*. Monographs ETSIA. Univ. Politécnica de Madrid 170: 1–17.

Goto, S., H. Iijima, H. Ogawa, & K. Ohya. 2011. „Outbreeding depression caused by intraspecific hybridization between local and nonlocal genotypes in *Abies sachalinensis*". *Restoration Ecology* 19 (2): 243–250.

Guerrant, E.O., K. Havens, & M. Maunder. 2004. Ex situ *plant conservation: supporting species survival in the wild*. Washington: Island Press.

Guo, J., Y. Wang, C. Song, J. Zhou, L. Qiu, H. Huang, & Y. Wang. 2010. „A single origin and moderate bottleneck during domestication of soybean (*Glycine max*): implications from microsatellites and nucleotide sequences". *Annals of Botany* 106 (3): 505–514.

Hamilton, M.B. 1994. „*Ex situ* conservation of wild plant species: time to reassess the genetic assumptions and implications of seed banks". *Conservation Biology* 8 (1): 39–49.

Hamrick, J. L., Y. B. Linhart, & J. B. Mitton. 1979. „Relationships between life history characteristics and electrophoretically detectable genetic variation in plants". *Annual Review of Ecology and Systematics* 10: 173–200.

Hamrick, J.L., & M.J.W. Godt. 1996. „Effects of life history traits on genetic diversity in plant species". *Philosophical Transactions: Biological Sciences* 351 (1345): 1291–1298.

Hannah, L., G.F. Midgley, T. Lovejoy, W.J. Bond, M. Bush, J.C. Lovett, D. Scott, & F.I. Woodward. 2002. „Conservation of biodiversity in a changing climate". *Conservation Biology* 16 (1): 264–268.

Hans, J. 2010. „Genetic structure of arable weed species, *Adonis aestivalis* and *Consolida regalis*, in relation to landscape structure". Dissertation. Carl von Ossietzky University of Oldenburg.

Hapca, A. 2011. „Invasive plant species, the enemy of biodiversity". *Bulletin UASVM Horticulture* 68 (2): 317–320.

Harel, D., C. Holzapfel, & M. Sternberg. 2011. „Seed mass and dormancy of annual plant populations and communities decreases with aridity and rainfall predictability". *Basic and Applied Ecology* 12 (8): 674–684.

Hartl, D.L., & A.G. Clark. 1997. *Principles of population genetics.* 3. Aufl. Sunderland, Massachusetts: Sinauer Associates.

Hawes, C., G.R. Squire, P.D. Hallett, C.A. Watson, & M. Young. 2010. „Arable plant communities as indicators of farming practice". *Agriculture, Ecosystems & Environment* 138 (1-2): 17–26.

Hensen, I., C. Kilian, V. Wagner, W. Durka, J. Pusch, & K. Wesche. 2010. „Low genetic variability and strong differentiation among isolated populations of the rare steppe grass *Stipa capillata* L. in Central Europe". *Plant Biology* 12 (3): 526–536.

Hensen, I., & C. Oberprieler. 2005. „Effects of population size on genetic diversity and seed production in the rare *Dictamnus albus* (Rutaceae) in central Germany". *Conservation Genetics* 6 (1): 63–73.

Hereford, J. 2010. „Does selfing or outcrossing promote local adaptation?" *American Journal of Botany* 97: 298–302.

Heywood, V.H. 2011. „The role of botanic gardens as resource and introduction centres in the face of global change". *Biodiversity and Conservation* 20 (2): 221–239.

Heywood, V.H., & J.M. Iriondo. 2003. „Plant conservation: old problems, new perspectives". *Biological Conservation* 113 (3): 321–335.

Hilbig, W. 2007. „Die Haftdolden-Gesellschaft der Kalkäcker in Thüringen - Erinnerungen an einstige Blütenpracht". *Landschaftspflege und Naturschutz in Thüringen* 44 (2): 76–83.

Hill, A.W. 1915. „The history and functions of botanic gardens". *Annals of the Missouri Botanical Garden* 2 (1/2): 185–240.

Hill, J.P., E.M. Lord, & R.G. Shaw. 1992. „Morphological and growth rate differences among outcrossing and self-pollinating races of *Arenaria uniflora* (Caryophyllaceae)". *Journal of Evolutionary Biology* 5 (4): 559–573.

Holmes, E.M. 1906. „Horticulture in relation to medicine". *Royal Horticultural Society* 31: 42–61.

Honnay, O., & H. Jacquemyn. 2007. „Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation". *Conservation Biology* 21 (3): 823–831.

Hufford, K.M., & S.J. Mazer. 2012. „Local adaptation and the effects of grazing on the performance of *Nassella pulchra*: implications for seed sourcing in restoration". *Restoration Ecology* 20 (6): 688–695.

Hurka, H., N. Friesen, P. Borgmann, & B. Neuffer. 2008. „Schutz und Erhalt pflanzengenetischer Vielfalt: *In situ*-und *Ex situ*-Maßnahmen". *Osnabrücker Naturwissenschaftliche Mitteilungen* 33 (34): 177–195.

Hurka, H., B. Neuffer, & N. Friesen. 2004. „Plant genetic resources in botanical gardens". *Acta Horticulturae* 651: 35–44.

Illig, H., & H.C. Kläge. 1994. „Zehn Jahre Feldflorareservat bei Luckau-Freesdorf". *Naturschutz und Landschaftspflege in Brandenburg* 3: 32–35.

Jacquemont, F., & A. Caparrós. 2002. „The convention on biological diversity and the climate change convention 10 years after Rio: towards a synergy of the two regimes?" *Review of European Community & International Environmental Law* 11 (2): 169–180.

Jacquemyn, H., R. Brys, D. Adriaens, O. Honnay, & I. Roldán-Ruiz. 2009. „Effects of population size and forest management on genetic diversity and structure of the tuberous orchid *Orchis mascula*". *Conservation Genetics* 10 (1): 161–168.

Jakobsson, A., & P. Dinnetz. 2005. „Local adaptation and the effects of isolation and population size–the semelparous perennial *Carlina vulgaris* as a study case". *Evolutionary Ecology* 19 (5): 449–466.

Jones, A.T., & M.J. Hayes. 1999. „Increasing floristic diversity in grassland: the effects of management regime and provenance on species introduction". *Biological Conservation* 87 (3): 381–390.

Katzir, N., V. Portnoy, G. Tzuri, D.M. Joel, & M. Castejón-Muñoz. 1996. „Use of random amplified polymorphic DNA (RAPD) markers in the study of the parasitic weed *Orobanche*". *Theoretical and Applied Genetics* 93 (3): 367–372.

Kawecki, T.J., & D. Ebert. 2004. „Conceptual issues in local adaptation". *Ecology Letters* 7 (12): 1225–1241.

Keller, L.F., & D.M. Waller. 2002. „Inbreeding effects in wild populations". *Trends in Ecology & Evolution* 17 (5): 230–241.

Keller, M., J. Kollmann, & P.J. Edwards. 2000. „Genetic introgression from distant provenances reduces fitness in local weed populations". *Journal of Applied Ecology* 37: 647–659.

Kew Royal Botanic Gardens. 2012. „Kew Royal Botanic Gardens: Kew's Millennium Seed Bank - seed count". http://http://www.kew.org/science-conservation/save-seed-prosper/millennium-seed-bank/about-the-msb/msb-seed-count/index.htm.

King, H., T.C.G. Rich, & L. Cordrey. 2007. „Comparing hand pollination success with natural pollination of wild asparagus *Asparagus prostratus*, at colonies on The Gower, Pembrokeshire, west Wales, and in Cornwall, south-west England". *Conservation Evidence* 4: 69–72.

van Kleunen, M., M. Fischer, & S.D. Johnson. 2007. „Reproductive assurance through self-fertilization does not vary with population size in the alien invasive plant *Datura stramonium*". *Oikos* 116 (8): 1400–1412.

Kohler, F., C. Vandenberghe, R. Imstepf, & F. Gillet. 2011. „Restoration of threatened arable weed communities in abandoned mountainous crop fields". *Restoration Ecology* 19 (101): 62–69.

Kolehmainen, J., H. Korpelainen, & P. Mutikainen. 2010. „Inbreeding and inbreeding depression in a threatened endemic plant, the African violet (*Saintpaulia ionantha* ssp. *grotei*), of the East Usambara Mountains, Tanzania". *African Journal of Ecology* 48 (3): 576–587.

Korneck, D., M. Schnittler, & I. Vollmer. 1996. „Rote Liste der Farn- und Blütenpflanzen (Pteridophyta et Spermatophyta) Deutschlands". *Schriftenreihe für Vegetationskunde* 28: 21–187.

Krauss, J., R. Bommarco, M. Guardiola, R.K. Heikkinen, A. Helm, M. Kuussaari, R. Lindborg, et al. 2010. „Habitat fragmentation causes immediate and time-delayed biodiversity loss at different trophic levels". *Ecology Letters* 13 (5): 597–605.

Kreyling, J., D. Thiel, K. Simmnacher, E. Willner, A. Jentsch, & C. Beierkuhnlein. 2012. „Geographic origin and past climatic experience influence the response to late spring frost in four common grass species in Central Europe". *Ecography* 35 (3): 268–275.

Lacy, R.C. 1987. „Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection and population subdivision". *Conservation Biology* 1: 143–158.

Lande, R. 1993. „Risks of population extinction from demographic and environmental stochasticity and random catastrophes". *American Naturalist*: 911–927.

Lande, R., & D.W. Schemske 1985. „The evolution of self-fertilization and inbreeding depression in plants. I. Genetic model". *Evolution*: 24–40.

Lauterbach, D., M. Burkart, & B. Gemeinholzer. 2012a. „Rapid genetic differentiation between *ex situ* and their *in situ* source populations: an example of the endangered *Silene otites* (Caryophyllaceae)". *Botanical Journal of the Linnean Society* 168 (1): 64–75.

Lauterbach, D., M. Ristow, & B. Gemeinholzer. 2011. „Genetic population structure, fitness variation and the importance of population history in remnant populations of the endangered plant *Silene chlorantha* (Willd.) Ehrh.(Caryophyllaceae)". *Plant Biology* 13 (4): 667–777.

Lauterbach, D., M. Ristow, & B. Gemeinholzer. 2012b. „Population genetics and fitness in fragmented populations of the dioecious and endangered *Silene otites* (Caryophyllaceae)". *Plant Systematics and Evolution* 298 (1): 155–164.

Leimu, R., & M. Fischer. 2008. „A meta-analysis of local adaptation in plants". *PLoS ONE* 3 (12): 1–8.

Leimu, R., & P. Mutikainen. 2005. „Population history, mating system, and fitness variation in a perennial herb with a fragmented distribution". *Conservation Biology* 19 (2): 349–356.

Leimu, R., P.I.A. Mutikainen, J. Koricheva, & M. Fischer. 2006. „How general are positive relationships between plant population size, fitness and genetic variation?" *Journal of Ecology* 94 (5): 942–952.

Leimu, R., P. Vergeer, F. Angeloni, & N.J. Ouborg. 2010. „Habitat fragmentation, climate change, and inbreeding in plants". *Annals of the New York Academy of Sciences* 1195: 84–98.

Leinonen, P.H., D.L. Remington, & O. Savolainen. 2011. „Local adaptation, phenotypic differentiation, and hybrid fitness in diverged natural populations of *Arabidopsis lyrata*". *Evolution* 65 (1): 90–107.

Leinonen, P.H., S. Sandring, B. Quilot, M.J. Clauss, T. Mitchell-Olds, J. Ågren, & O. Savolainen. 2009. „Local adaptation in European populations of *Arabidopsis lyrata* (Brassicaceae)". *American Journal of Botany* 96 (6): 1129–1137.

Lipowsky, A., B. Schmid, & C. Roscher. 2011. „Selection for monoculture and mixture genotypes in a biodiversity experiment". *Basic and Applied Ecology* 12 (4): 360–371.

Litterski, B., & U. Hampicke. 2008. „Naturschutz auf Ackerflächen". *Berichte des Institutes für Landschafts- und Pflanzenökologie der Universität Hohenheim* 17: 91–108.

Loveless, M.D., & J.L. Hamrick. 1984. „Ecological determinants of genetic structure in plant populations". *Annual Review of Ecology and Systematics* 15: 65–95.

Luijten, S.H., M. Kéry, J.G.B. Oostermeijer, & H. den Nijs. 2002 . „Demographic consequences of inbreeding and outbreeding in *Arnica montana*: a field experiment". *Journal of Ecology* 90(4): 593–603.

Mable, B.K., & A. Adam. 2007. „Patterns of genetic diversity in outcrossing and selfing populations of *Arabidopsis lyrata*". *Molecular Ecology* 16 (17): 3565–3580.

MacDaniels, L.H. 1930. „The possibilities of hand pollination in the orchard on a commercial scale". *Proceedings. American Society for Horticultural Science* 27:370–373.

Magnaghi, E., M. Frey, & K. Ariyoshi. 2007. „Hand-pollination of *Cardamine californica* improves seed set". *Native Plants Journal* 7 (3): 248–252.

Marsden-Jones, E.M., & P.E. Weiss. 1938. „The essential differences between *Anagallis arvensis* Linn. and *Anagallis foemina* Mill." *Proceedings of the Linnean Society of London* 150 (3): 146–155.

Marshall, E.J.P., & P. Brain. 1999. „The horizontal movement of seeds in arable soil by different soil cultivation methods". *Journal of Applied Ecology* 36 (3): 443–454.

Maunder, M. 1992. „Plant reintroduction: an overview". *Biodiversity and Conservation* 1 (1): 51–61.

Maunder, M., R.S. Cowan, P. Stranc, & M.F. Fay. 2001a. „The genetic status and conservation management of two cultivated bulb species extinct in the wild: *Tecophilaea cyanocrocus* (Chile) and *Tulipa sprengeri* (Turkey)". *Conservation Genetics* 2 (3): 193–201.

Maunder, M., A. Culham, B. Alden, G. Zizka, C. Orliac, W. Lobin, A. Bordeu, J.M. Ramirez, & S. Glissmann-Gough. 2000. „Conservation of the Toromiro tree: case study in the management of a plant extinct in the wild". *Conservation Biology* 14 (5): 1341–1350.

Maunder, M., S. Higgens, & A. Culham. 2001b. „The effectiveness of botanic garden collections in supporting plant conservation: a European case study". *Biodiversity and Conservation* 10 (3): 383–401.

Maxted, N., B.V. Ford-Lloyd, & J.G. Hawkes. 1997. „Complementary conservation strategies". In *Plant genetic conservation: The* in situ *approach*, 15–40. London: Chapman and Hall.

Mendel, J.G. 1866. „Versuche über Pflanzen-Hybriden". *Verhandlungen des naturforschenden Vereines in Brünn*.

Meyer, S., C. Leuschner, & T. van Elsen. 2008. „Sanctuaries for the segetal vegetation in Germany – inventory and new impulses by the project ,Biodiversity in agricultural landscapes'". *Journal of Plant Diseases and Protection* 21: 363–368.

Meyer, S., K. Wesche, B. Krause, & C. Leuschner. 2013. „Dramatic losses of specialist arable plants in Central Germany since the 1950s/60s – a cross-regional analysis". *Diversity and Distribution* 19: 1175–1187.

Meyer, S., K. Wesche, C. Leuschner, T. van Elsen, & J. Metzner. 2010. „A new conservation strategy for arable plant vegetation in Germany - the project ,100 fields for biodiversity'". *Plant Breeding and Seed Science* 61: 25–34.

Miller, B., W. Conway, R.P. Reading, C. Wemmer, D. Wildt, D. Kleiman, S. Monfort, A. Rabinowitz, B. Armstrong, & M. Hutchins. 2004. „Evaluating the conservation mission of zoos, aqua-

riums, botanical gardens, and natural history museums". *Conservation Biology* 18 (1): 86–93.

Mueller, F.G. 2000. „Does the convention on biodiversity safeguard biological diversity?" *Environmental Values* 9 (1): 55–80.

Negri, V., & B. Tiranti. 2010. „Effectiveness of *in situ* and *ex situ* conservation of crop diversity. What a *Phaseolus vulgaris* L. landrace case study can tell us". *Genetica* 138 (9-10): 985–998.

Nei, M., T. Maruyama, & R. Chakraborty. 1975. „The bottleneck effect and genetic variability in populations". *Evolution* 29 (1): 1–10.

Nunney, L. 2002. „The effective size of annual plant populations: the interaction of a seed bank with fluctuating population size in maintaining genetic variation". *The American Naturalist* 160 (2): 195–204.

Nybom, H. 2004. „Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants". *Molecular Ecology* 13 (5): 1143–1155.

Nybom, H., & I. Bartish. 2000. „Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants". *Perspectives in Plant Ecology, Evolution and Systematics* 3 (2): 93–114.

Oldfield, S.F. 2009. „Botanic gardens and the conservation of tree species". *Trends in Plant Science* 14 (11): 581–583.

Opgenoorth, L. 2009. „Identification and characterization of microsatellite marker in the tetra-ploid *Juniperus tibetica* Kom. using next generation sequencing". *Conservation Genetics Resources* 1 (1): 253–255.

Parzies, H.K., W. Spoor, & R.A. Ennos. 2000. „Genetic diversity of barley landrace accessions (*Hordeum vulgare* ssp. *vulgare*) conserved for different lengths of time in *ex situ* gene banks". *Heredity* 84: 476–486.

Paton, A., & E. Nic Lughadha. 2011. „The irresistible target meets the unachievable objective: what have 8 years of GSPC implementation taught us about target setting and achievable objectives?" *Botanical Journal of the Linnean Society* 166 (3): 250–260.

Patterson, K.J. 1988. „Effects of hand pollination on fruit set and fruit quality of the feijoa (*Acca sellowiana*)". *Australian Temperate Fruits Review Conference* 240: 197–200.

Pautasso, M., & I. Parmentier. 2007. „Are the living collections of the world's botanical gardens following species-richness patterns observed in natural ecosystems?" *Botanica Helvetica* 117 (1): 15–28.

Pauw, A. 2007. „Collapse of a pollination web in small conservation areas". *Ecology* 88 (7): 1759–1769.

Pennisi, E. 2010. „Tending the global garden". *Science* 329 (5997): 1274–1277.

Pérez-García, F., M.E. González-Benito, & C. Gómez-Campo. 2007. „High viability recorded in ultra-dry seeds of 37 species of Brassicaceae after almost 40 years of storage". *Seed Science and Technology* 35 (1): 143–153.

Pertoldi, C., R. Bijlsma, & V. Loeschcke. 2007. „Conservation genetics in a globally changing environment: present problems, paradoxes and future challenges". *Biodiversity and Conservation* 16 (14): 4147–4163.

Peterson, A., I.V. Bartish, & J. Peterson. 2008. „Effects of population size on genetic diversity, fitness and pollinator community composition in fragmented populations of *Anthericum liliago* L." *Plant Ecology* 198 (1): 101–110.

Picó, F.X., C. Mix, N.J. Ouborg, & J. van Groenendael. 2007. „ Multigenerational inbreeding in *Succisa pratensis*: effects on fitness components". *Biologia plantarum* 51 (1): 185–188.

Potts, S.G., J.C. Biesmeijer, C. Kremen, P. Neumann, O. Schweiger, & W.E. Kunin. 2010. „Global pollinator declines: trends, impacts and drivers". *Trends in Ecology & Evolution* 25 (6): 345–353.

Primack, R.B., & A.J. Miller-Rushing. 2009. „The role of botanical gardens in climate change research". *New Phytologist* 182: 303–313.

Prinz, K., K. Weising, & I. Hensen. 2010. „Genetic structure of coastal and inland populations of *Spergularia media* (L.) C. Presl (Caryophyllaceae) in Central Europe". *Conservation Genetics* 11 (6): 2169–2177.

Puchalski, J. 2004. „International programmes for seed preservation of European native plants". *Biuletyn Ogrodów Botanicznych Muzeów i Zbiorów* 13: 11–18.

Quilichini, A., M. Debussche, & J.D. Thompson. 2001. „ Evidence for local outbreeding depression in the Mediterranean island endemic *Anchusa crispa* Viv. (Boraginaceae)". *Heredity* 87(2): 190–197.

Raabová, J., Z. Münzbergová, & M. Fischer. 2007. „Ecological rather than geographic or genetic distance affects local adaptation of the rare perennial herb, *Aster amellus*". *Biological Conservation* 139 (3-4): 348–357.

Rae, D. 2011. „Fit for purpose: the importance of quality standards in the cultivation and use of live plant collections for conservation". *Biodiversity and Conservation* 20 (2): 241–258.

Rands, M.R.W., W.M. Adams, L. Bennun, S.H.M. Butchart, A. Clements, D. Coomes, A. Entwistle, et al. 2010. „Biodiversity conservation: challenges beyond 2010". *Science* 329 (5997): 1298–1303.

Reed, D.H., & R. Frankham. 2003. „Correlation between fitness and genetic diversity". *Conservation Biology* 17 (1): 230–237.

Rice, B.E., M.E. Smith, S.E. Mitchell, & S. Kresovitch. 2006. „Conservation and change: a comparison of *in situ* and *ex situ* conservation of Jala maize germplasm". *Crop Science* 46: 428–436.

Rogers, D.L. 2004. „Genetic erosion: no longer just an agricultural issue". *Native Plants Journal* 5 (2): 112–122.

Routley, M.B., K. Mavraganis, & C.G. Eckert. 1999. „Effect of population size on the mating system in a self-compatible, autogamous plant, *Aquilegia canadensis* (Ranunculaceae)". *Heredity* 82 (5): 518–528.

Rucińska, A., & J. Puchalski. 2011. „Comparative molecular studies on the genetic diversity of an *ex situ* garden collection and its source population of the critically endangered polish endemic plant *Cochlearia polonica* E. Fröhlich". *Biodiversity and Conservation* 20 (2): 401–413.

Salisbury, E.J. 1961. *Weeds and aliens*. London: Collins Publishers.

Savolainen, O., T. Pyhäjärvi, & T. Knürr. 2007. „Gene flow and local adaptation in trees". *Annual Review of Ecology, Evolution, and Systematics* 38 (1): 595–619.

Scarascia-Mugnozza, G.T., & P. Perrino. 2002. *The history of* ex situ *conservation and use of plant genetic resources*. Oxford: Cabi Publishing.

Schiebold, S., I. Hensen, K. Wesche, & M. Röser. 2009. „Extensive clonality of the endemic *Calamagrostis pseudopurpurea* Gerstl. ex OR Heine in central Germany revealed by RAPD markers". *Plant Biology* 11 (3): 473–482.

Schmidt, K., & K. Jensen. 2000. „Genetic structure and AFLP variation of remnant populations in the rare plant *Pedicularis palustris* (Scrophulariaceae) and its relation to population size and reproductive components". *American Journal of Botany* 87 (5): 678–689.

Schmidt, T., P. Arens, M.J.M. Smulders, R. Billeter, J. Liira, I. Augenstein, & W. Durka. 2009. „Effects of landscape structure on genetic diversity of *Geum urbanum* L. populations in agricultural landscapes". *Flora - Morphology, Distribution, Functional Ecology of Plants* 204 (7): 549–559.

Schoen, D.J., & A.H. Brown. 1991. „Intraspecific variation in population gene diversity and effective population size correlates with the mating system in plants". *Proceedings of the National Academy of Sciences of the United States of America* 88 (10): 4494–4497.

Schroeder, C.A. 1947. „Hand pollination of cherimoya improves fruit set". *California Avocado Society Yearbook* 32: 67–70.

Seaton, P.T., H. Hu, H. Perner, & H.W. Pritchard. 2010. „*Ex situ* conservation of orchids in a warming world". *The Botanical Review* 76 (2): 193–203.

Sharrock, S., & M. Jones. 2010. „Saving Europe's threatened flora: progress towards GSPC target 8 in Europe". *Biodiversity and Conservation* 20 (2): 325–333.

Sheridan, P.M., & D.N. Karowe, 2000. "Inbreeding, outbreeding, and heterosis in the yellow pitcher plant, *Sarracenia flava* (Sarraceniaceae), in Virginia". *American Journal of Botany* 87(11): 1628–1633.

Slotte, T., J.P. Foxe, K.M. Hazzouri, & S.I. Wright. 2010. „Genome-wide evidence for efficient positive and purifying selection in *Capsella grandiflora*, a plant species with a large effective population size". *Molecular Biology and Evolution* 27 (8): 1813–1821.

Spagnoletti-Zeuli, L., L. Sergio, & P. Perrino. 1995. „Changes in the genetic structure of wheat germplasm accessions during seed rejuvenation". *Plant Breeding* 114: 193–198.

Spielman, D., B.W. Brook, & R. Frankham. 2004. „Most species are not driven to extinction before genetic factors impact them". *Proceedings of the National Academy of Sciences of the USA* 101 (42): 15261–15264.

Squirrell, J., P.M. Hollingsworth, J. Sears, B. Banks, B. Ferry, & D.T. de Graaf. 2006. „Assessment of genetic diversity in populations of *Crepis foetida* L. (Asteraceae)". *Watsonia* 26: 121–126.

Stearn, W.T. 1971. „Sources of information about botanic gardens and herbaria". *Biological Journal of the Linnean Society* 3 (3): 225–233.

Storkey, J., S. Meyer, K.S. Still, & C. Leuschner. 2012. „The impact of agricultural intensification and land-use change on the European arable flora". *Proceedings of the Royal Society B* 279: 1421–1429.

Sutherland, W.J., W.M. Adams, R.B. Aronson, R. Aveling, T.M. Blackburn, S. Broad, G. Ceballos, et al. 2009. „One hundred questions of importance to the conservation of global biological diversity". *Conservation Biology* 23 (3): 557–567.

Svensson, R., & M. Wigren. 1986. „History and biology of *Consolida regalis* in Sweden". *Svensk Botanisk Tidskrift* 80 (1): 31–53.

Swanson, T. 1997. *Global action for biodiversity: an international framework for implementing the convention on biological diversity*. London: Earthscan. http://www.earthscan.co.uk/?tabid=1010.

Swarts, N.D., & K.W. Dixon. 2009. „Perspectives on orchid conservation in botanic gardens". *Trends in Plant Science* 14 (11): 590–598.

Thompson, K., J.P. Bakker, & R.M. Bekker. 1997. *The soil seed banks of North West Europe: methodology, density and longevity*. Cambridge: Cambridge University Press.

Thompson, P.A. 1972. „The role of the botanic garden". *Taxon* 21 (1): 115–119.

Touchell, D.H., M. Richardson, & K.W. Dixon. 1997. *Germplasm conservation guidelines for Australia*. Canberra: Australian Network for Plant Conservation.

van Treuren, R., R. Bijlsma, N.J. Ouborg, & W. van Delden. 1993. „The significance of genetic erosion in the process of extinction. IV. Inbreeding depression and heterosis effects caused by selfing and outcrossing in *Scabiosa columbaria*". *Evolution* 47 (6): 1669–1680.

de Vere, N., E. Jongejans, A. Plowman, & E. Williams. 2009. „Population size and habitat quality affect genetic diversity and fitness in the clonal herb *Cirsium dissectum*". *Oecologia* 159 (1): 59–68.

Vernesi, C., M.W. Bruford, G. Bertorelle, E. Pecchioli, A. Rizzoli, & H.C. Hauffe. 2008. „Where's the conservation in conservation genetics?" *Conservation Biology* 22 (3): 802–804.

Volis, S., & M. Blecher. 2010. „Quasi *in situ*: a bridge between *ex situ* and *in situ* conservation of plants". *Biodiversity and Conservation* 19 (9): 2441–2454.

Wagner, V., W. Durka, & I. Hensen. 2011. „Increased genetic differentiation but no reduced genetic diversity in peripheral vs. central populations of a steppe grass". *American Journal of Botany* 98 (7): 1173–1179.

Wäldchen, J. 2004. „Untersuchungen zur Lebensdauer der Diasporen ausgewählter Segetalpflanzen der Flora Thüringens." Diploma thesis. University of applied sience Eberswalde.

Wäldchen, J., J. Pusch, & V. Luthardt. 2005. „Zur Diasporen-Keimfähigkeit von Segetalpflanzen – Untersuchungen in Nord-Thüringen". *Beiträge zur Forstwirtschaft und Landschaftsökologie* 38: 145–156.

Walker, K.J., C.D. Preston, & C.R. Boon. 2009. „Fifty years of change in an area of intensive agriculture: plant trait responses to habitat modification and conservation, Bedfordshire, England". *Biodiversity and Conservation* 18 (13): 3597–3613.

Walz, U. 2008. „Monitoring of landscape change and functions in Saxony (Eastern Germany) - Methods and indicators". *Ecological Indicators* 8: 807–817.

Wania, A., I. Kuhn, 6 S. Klotz. 2006. „Plant richness patterns in agricultural and urban landscapes in Central Germany–spatial gradients of species richness". *Landscape and Urban Planning* 75 (1-2): 97–110.

Ward, C.D., C.M. Parker, & C.M. Shackleton. 2010. „The use and appreciation of botanical gardens as urban green spaces in South Africa". *Urban Forestry & Urban Greening* 9 (1): 49–55.

Watt, A.D., R.H.W. Bradshaw, J. Young, D. Alard, T. Bolger, D. Chamberlain, F. Fernández-González, R. Fuller, P. Gurrea, & K. Henle. 2007. „Trends in biodiversity in Europe and the impact of land-use change". *Biodiversity under Threat*. Issues in Environmental Science and Technology 25: 135–160.

Weeks, A.R., C.M. Sgro, A.G. Young, R. Frankham, N.J. Mitchell, K.A. Miller, M. Byrne, et al. 2011. „Assessing the benefits and risks of translocations in changing environments: a genetic perspective". *Evolutionary Applications* 4 (6): 709–725.

Welk, E. 2002. „Arealkundliche Analyse und Bewertung der Schutzrelevanz seltener Gefäßpflanzen Deutschlands". *Schriftenreihe für Vegetationskunde* 37: 1–337.

Westhus, W., & H.-J. Zündorf. 1993. „Rote Liste der Farn- und Blütenpflanzen (Pteridophyta et Spermatophyta) Thüringens". *Naturschutzreport* 5: 134–152.

Wicke, G. 1998. „Stand der Ackerstreifenprogramme in Deutschland". *Schriftenreihe der Landesanstalt für Pflanzenbau und Pflanzenschutz* 6: 55–84.

Willi, Y., J. van Buskirk, & M. Fischer. 2005. „A threefold genetic allee effect: population size affects cross-compatibility, inbreeding depression and drift load in the self-incompatible *Ranunculus reptans*". *Genetics* 169: 2255–2265.

Wright, S. 1931. „Evolution in Mendelian populations". *Genetics* 16 (2): 97–159.

Wright, S. 1938. „Size of population and breeding structure in relation to evolution". *Science* 87: 430–431.

Wyse Jackson, P.S. 2001. „An international review of the *ex situ* plant collections of the botanic gardens of the world". *Botanic Gardens Conservation* 3 (6): 22–33.

Wyse Jackson, P.S. 2009. „Growing an international movement for plant conservation and plant resource management: the development of the international botanic garden community". *Buletin Kebun Raya Indonesia* 12: 41–48.

Yashina, S., S. Gubin, S. Maksimovich, A. Yashina, E. Gakhova, & D. Gilichinsky. 2012. „Regeneration of whole fertile plants from 30,000-y-old fruit tissue buried in Siberian permafrost". *Proceedings of the National Academy of Sciences* 109 (10): 4008–4013.

Ye, Y.H., & N. Aoki. 2003. „Studies on the forcing in blueberry influences of the onset of forcing, cultivar, gibberellin treatment and hand pollination on harvesting time, yield and berry quality of blueberry (1)". *Journal of the Japanese Society of agricultural Technology Management* 10 (2): 81–87.

Young, A., T. Boyle, & T. Brown. 1996. „The population genetic consequences of habitat fragmentation for plants". *Trends in Ecology & Evolution* 11 (10): 413–418.

Zhang, J.-J., Q.-G. Ye, X.-H. Yao, & H.-W. Huang. 2010. „Spontaneous interspecific hybridization and patterns of pollen dispersal in *ex situ* populations of a tree species (*Sinojackia xylocarpa*) that is extinct in the wild". *Conservation Biology* 24 (1): 246–255.

Zhu, Q., X. Zheng, J. Luo, B.S. Gaut, & S. Ge. 2007. „Multilocus analysis of nucleotide variation of *Oryza sativa* and its wild relatives: severe bottleneck during domestication of rice". *Molecular Biology and Evolution* 24 (3): 875–888.

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:

Curriculum vitae

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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*?

C Brütting, I Hensen & K Wesche (2013): *Ex situ* cultivation affects genetic structure and diversity in arable plants. Plant Biology 15: 505-513

C Brütting, S Meyer, P Kühne, I Hensen & K Wesche (2012): Spatial genetic structure and low diversity of the rare arable plant *Bupleurum rotundifolium* L. indicate fragmentation in Central Europe. Agriculture, Ecosystem & Environment 161: 70-77

C Brütting, K Wesche, S Meyer & I Hensen (2012): Genetic diversity of six arable plants in relation to their Red List status. Biodiversity and Conservation 21 (3):745-761

C Bläß, K Ronnenberg, O Tackenberg, I Hensen & K Wesche (2010): The relative importance of different seed dispersal modes in dry Mongolian rangelands. Journal of Arid Environments 74: 991–997

C Bläß, K Ronnenberg, I Hensen & K Wesche (2008): Grazing impact on plant seed production in Southern Mongolia. Mongolian Journal of Biological Sciences 6 (1-2): 3-9

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:

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