The potential of polyploidy to counteract the negative consequences of demographic disequilibria in colonizing populations - empirical insights from diploid and tetraploid *Centaurea stoebe*

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Photo: Green house experiment with diploid and tetraploid *Centaurea stoebe*. Botanical Garden in Halle, 2013.

"I do not pretend to indicate the exact lines and means of migration, or the reason why certain species have been modified and have given rise to new groups of forms, and others have remained unaltered. We cannot hope to explain such facts, until we can say why one species and not another becomes naturalized by man's agency in a foreign land'

Charles Robert Darwin (1859): ***On the Origin of Species.***

Contents

Chapter 1

GENERAL INTRODUCTION

1.1 Preface: The motivation of my PhD-thesis

The invasion of non-native species dramatically contributes to ecosystem alterations (Simberloff *et al.* 2013; Galiana *et al.* 2014) and as such represents a global threat to the environment, public health and economy (Pimentel *et al.* 2005; Pimentel 2011; Richardson and Ricciardi 2013). Understanding the success of invasive species has consequently become a major goal in ecological research, which manifests in a rapidly accumulating number of studies on invasive species during the past decades (Gurevitch *et al.* 2011). Nonetheless, we are still very limited in predicting the invasiveness of species and populations (Richardson and Pyšek 2012; Kueffer *et al.* 2013).

In this PhD-thesis, I address the phenomenon that **polyploid plants are more likely to become invasive than diploid ones** (Pandit *et al.* 2011). Although first attempts to explain this pattern generated remarkable progress (te Beest *et al.* 2011), an in-depth understanding is still lacking (Bock *et al.* 2015). Generally, to evaluate why some species groups infest new ranges, whereas others are strictly bounded to their native distribution, one has to consider processes that impede biological invasions. In fact, the vast majority of unintentionally introduced species fail to establish in a novel range (Sax and Brown 2000) owing to specific invasion filters that restrict invasion success (Theoharides and Dukes 2007). More specifically, for non-native species it is particularly to successfully pass the repeated colonization events during both the early phases of invasion and at leading edges of invasive spread (Schrieber and Lachmuth 2016). In this context, population genetic determinants of colonization success are of extraordinary interest, because genetic founder effects may cause negative feedback on the demography of colonizing populations (Excoffier *et al.* 2009; Crawford and Whitney 2010; Firestone and Jasieniuk 2012a).

In this thesis, I investigate the founder potential of the diploid and the tetraploid cytotype of *Centaurea stoebe*. This polyploid complex shows a striking cytotype shift between the native and the invasive range, and has thus been highlighted as an ***"excellent model system for evaluating the role of polyploidy in plant invasions"*** (te Beest *et al.* 2011): diploids prevail over tetraploids in the native range, but only tetraploids were able to colonize the novel range. The results of my thesis specifically **contribute to explaining this cytotype shift**, an issue that has been under intense investigations throughout the past decade. More generally, my thesis demonstrates that unraveling the interplay of polyploidy with early-acting invasion filters can substantially enhance our **mechanistic understanding of the overrepresentation of polyploids among invasive species**.

In the following, I introduce the major ecological and population genetic theory underlying my thesis. Subsequently, a general concept synthesizes the distinct aims of my studies. Within the introduction, keywords are highlighted in bold at first mentioning and defined in a footnote glossary that aims to support an unambiguous handling of these central terms throughout my thesis.

1.2 Population dynamics across invasion stages

The massive quantity of invasion studies resulted in ever-emerging new hypotheses that were confirmed and rejected by highly contradictory findings across case studies and series of meta-analyses (Blackburn *et al.* 2011). Such contrasting outcomes result to a large extend from indiscriminate investigations, which ignore the distinct invasion stage of the study system (Broennimann *et al.* 2014). According to Richardson *et al.* (2000), biological invasions can be roughly outlined into three main stages: introduction (Fig. 1.1: A1 + A2), naturalization (A3) and invasive spread (A4). Additionally, Dietz and Edwards (2006) proposed to distinguish primary invasion (A1 - A4) predominantly taking place in ruderal habitats from secondary invasion (B1 - B2) occurring in more natural and competitive communities. Since I particularly address factors that hamper the establishment of exotics (e.g. in diploid as compared to tetraploid *Centaurea stoebe*), my thesis focuses on primary invasion.

Concerning the introduction stage, I follow Theoharides and Dukes (2007) and distinguish between the transport of propagules that arrive the introduced range (i.e. initial introduction; A1) and the primary colonization stage (A2), where the offspring of the introduced propagules build up founder populations with highly fluctuating population sizes. For primary naturalization, I consider the establishment of self-perpetuating populations that show rather constant population sizes (Prentis *et al.* 2008), which is frequently associated with a **lag phase**[1]. The subsequent primary invasive spread begins mostly unnoticed until the often rapid expansion leads to the local displacement of indigenous species (Rundel *et al.* 2014). In each stage, certain key processes may act as **invasion filters**[2] (Fig. 1.2), and it is exactly the understanding of these filters, which may enhance our mechanistic knowledge and predictive abilities of invasion success (Theoharides and Dukes 2007).

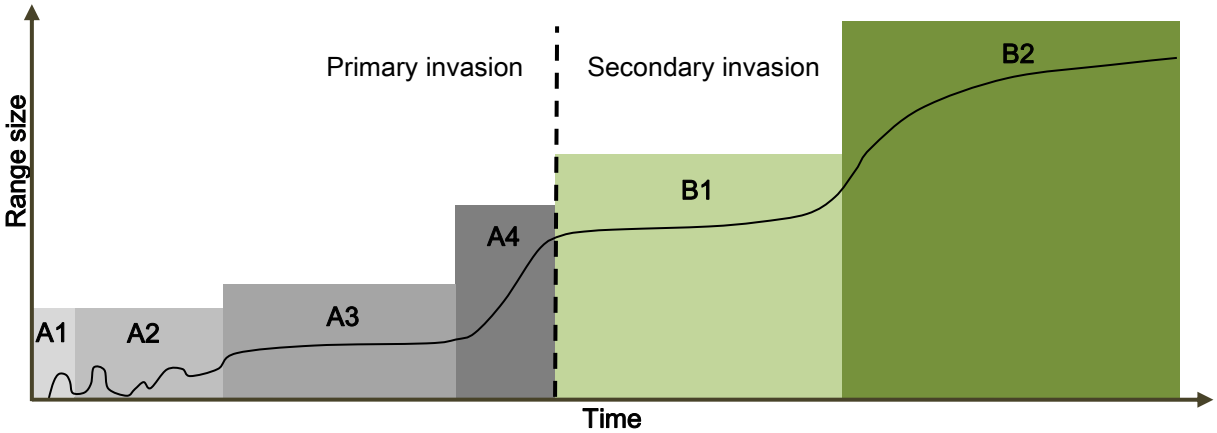


Fig. 1.1 Range dynamics of a non-native species across typical invasion stages, synthesized from four widely accepted invasion stage concepts (Richardson *et al.* 2000; Dietz and Edwards 2006; Theoharides and Dukes 2007; Prentis *et al.* 2008). The stages are assigned to primary invasion (A1 - A4) and secondary invasion (B1 - B2). A1, initial introduction; A2, primary colonization; A3, primary naturalization; A4, primary invasive spread; B1, secondary naturalization; B2, secondary invasive spread.

1 phase during the invasion history, where steady occurrences are restricted to just a small number of locations and / or habitat types and show only negligible range expansion over several generations (Hyndman *et al.* 2015)

2 abiotic or biotic factor that prohibit or limit invasion success (Theoharides and Dukes 2007)

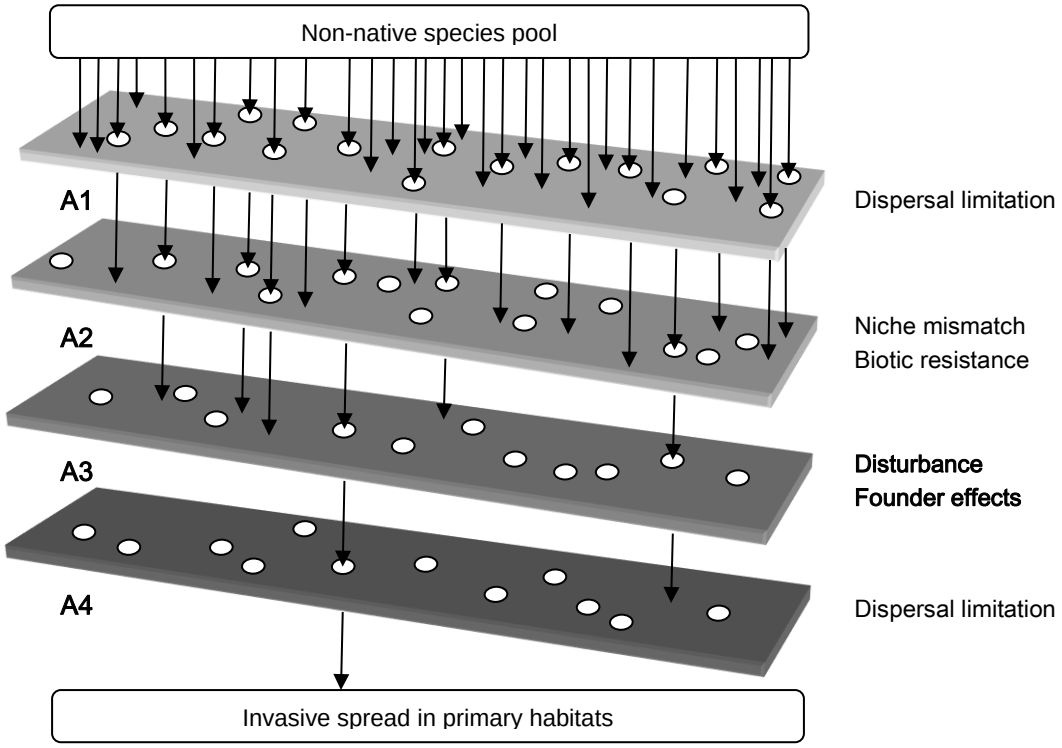


Fig. 1.2 Sequential invasion filters across the typical stages of primary invasion. The non-exhaustive selection of filters is based on the concept proposed in Rius and Darling (2014). The figure illustrates the complexity of the process of becoming invasive, whereby the underlying mechanisms correspond to the community assembly theory (see Tilman 2004 for details). The filters highlighted in bold (**disturbance** and **founder effects**) display the main aspects of my thesis. Note that the different filters are not mutually exclusive and may act simultaneously across various stages. The outlined stages correspond to Fig. 1.1: A1, initial introduction; A2, primary colonization; A3, primary naturalization; A4, primary invasive spread.

The transfer of propagules into the non-native range is a fundamental prerequisite for biological invasions (Lockwood *et al.* 2005), which is usually hindered by considerable geographic barriers (i.e. dispersal limitation, Fig. 1.2). However, sufficient **propagule pressure**[3] is mandatory to ensure that enough propagules arrive at suitable micro-habitats allowing for germination (Colautti *et al.* 2006). For successful establishment and vital plant growth, a certain extent of **pre-adaptation**[4] of the introduced individuals to the conditions in the novel range is required (i.e. matching of the environmental niche; Petitpierre *et al.* 2012; Strubbe *et al.* 2013).

In general, colonization is a central element of invasion dynamics, not only following the initial introduction, but also following dispersal within the novel range (Warren *et al.* 2013). Indeed, it is of fundamental importance to understand that the population dynamics of introduced species are inconsistent across space and time (Essl *et al.* 2009): while the invasions´ core areas have already been occupied by large and naturalized populations, the leading edges of the invasion front have more recently been colonized by small founder populations, which may thus still face early-acting invasion filters. Moreover, the realized niche of invaders may change in the course of their invasion history (Tingley *et al.* 2014; Essl *et al.* 2015). For example, during the transition from primary to secondary invasions (see Fig. 1.1 A4 - B1), occupied habitat usually change from

3 the total number of individuals introduced into a non-native region, which includes both the number of separate introductions and the number of individuals in each of these introduction events (Lockwood *et al.* 2009)

4 a situation, where introduced (naïve) genotypes match by chance the environmental niche of the habitat, to which they were introduced (Dlugosch and Parker 2007)

ruderal to more natural sites (Box 1.1), and such habitat switches may somewhat reflect a separate invasion within the exotic range, which again may involve severe colonization events.

Ruderal habitats are much more likely to be colonized during primary invasions than natural habitats, because the latter are usually inhabited by rather intact resident plant communities that show a high biotic resistance against introduced species owing to competitive effects (Levine *et al.* 2004; Mitchell *et al.* 2006; Byun *et al.* 2013). In ruderal habitats, human-mediated disturbance can reduce such competition leading to temporary alterations of resource availability (D´Antonio *et al.* 1999; Parepa *et al.* 2013). The invasiveness of species is known to be highly correlated with their ability to quickly occupy such temporary vacant sites (Simberloff 2009). Thus, most invaders are excellent colonizers, often referred to as "ideal weeds" (*sensu* Baker 1965) with great dispersal capacities, fast germination and high relative grow rates (Sutherland 2004; Van Kleunen *et al.* 2010; 2015). Besides the positive aspects of stress releases (see above), frequent anthropogenic disturbance may also cause stress in terms of physical damage on plant individuals, which can reduce plant fitness (Kallimanis *et al.* 2005). Moreover, ruderal sites are rather erratic environments, where strong disturbance events, such as mowing, ploughing, bituminization or fertilization, can entirely transform habitats, which can result in considerable demographic oscillations. The ability of species or populations to deal with the specific positive (e.g. competition release) and negative conditions (e.g. physical damage) in ruderal habitats, can crucially determine their pre-adaptation to become invasive (see anthropogenically induced adaptation to invade (AIAI) theory; Hufbauer *et al.* 2012).

Box 1.1 Ecological characteristics of the ruderal habitats of primary invasion.

In conclusion, the invasion histories of species are often characterized by repeated colonization events. Colonization, in turn, is considered to be a highly critical stage, because it is frequently associated with substantial demographic disequilibria (Schrieber and Lachmuth 2016). Thus, the ability to cope with the resultant **founder effects**[5], which - as a potent invasion filter - may reveal high explanatory power to understand biogeographical distribution patterns (Theoharides and Dukes 2007). Founder effects are therefore the central objective of my studies. The mechanisms potentially underlying this particular filter are briefly introduced in the next section and discussed in more detail in the specific studies of my thesis (chapters 2-4).

1.3 Founder populations and the genetic paradox of invasions

Founder populations often exhibit a small population size and their initial genetic diversity mostly represents only a subset of the source populations (Price and Sol 2008; Dlugosch and Parker 2008; Wilson *et al.* 2009; Firestone and Jasieniuk 2012b). Such small populations can be prone to progressive reduction of population size due to ecological Allee-effects (Taylor and Hastings 2005; Dennis *et al.* 2015). Moreover, they are likely to face dramatic fluctuations in population size as a result of environmental stochasticity (Engen *et al.* 2005), particularly in ruderal

5 a demographic bottleneck resulting from the colonization of a founder population that shows a considerably smaller population size than its source population (Dlugosch and Parker 2008)

populations (see Box 1.1). Additionally, founder populations are often spatially isolated, e.g. after propagule transfer across geographical barriers or occasional long-distance dispersal within ranges (Excoffier *et al.* 2009). In such small and isolated populations, **genetic bottlenecks**[6] reduce genetic diversity and simultaneously increase relatedness among individuals (Young *et al.* 1996). Both of these factors can reduce population growth for three main reasons (Fig. 1.3).

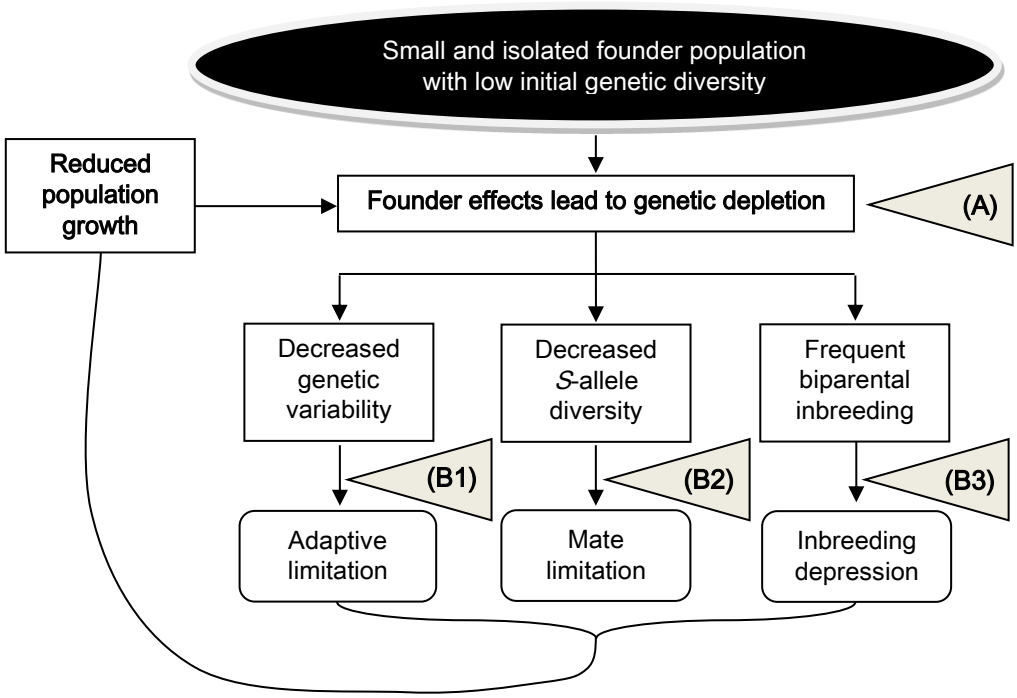


Fig. 1.3 Negative consequences of founder effects on population growth in self-incompatible plants. The gray-stained triangles reflect mechanisms that antagonize founder effects at different scales: (A) counteracting genetic depletion, (B1 - B3) counteracting the specific consequences of genetic depletion (both aspects are explained in Box 1.2 on the next page).

(1) Low genetic variation may reduce the adaptive abilities to respond to both various and changing environmental conditions (Prentis *et al.* 2008). Therefore, genetically depauperate populations may be restricted to occupy only micro-habitats and locations that match the niches of the available genotypes (Barrett and Schluter 2008).

(2) In founder populations of self-incompatible species, increasing relatedness among mating partners and / or a stochastic loss of ***S*-alleles**[7] in the populations´ gene pool can lead to mate limitation (Thrall *et al.* 2014). Asteraceae species, such as diploid and tetraploid *C. stoebe*, show **sporophytic self-incompatibility**[8], where fertilization is prevented when the mating partners share at least one dominantly expressed *S*-allele (Gonthier *et al.* 2013).

(3) Increasing relatedness among individuals inevitably leads to increasing biparental **inbreeding**[9] (Loeschcke *et al.* 2013). A recent meta-analysis of Angeloni *et al.* (2011) proved that

6 a sudden and significant reduction in population size, which results in considerable genetic depletion (Wilson *et al.* 2009)
7 non-recombining haplotype of a pollen- and a pistil-expressed *S*-gene (Brennan *et al.* 2013)
8 system, where the gametophytic pollen phenotype is determined by the sporophyte, which potentially leads to the exposition of all sporophytic *S*-alleles unless dominance hierarchies suppress the expression of recessive *S*-alleles (Busch *et al.* 2014)
9 any mating of individuals that increases homozygosity as compared to non-assortative mating across the entire population (Keller and Waller 2002)

for the great majority of plants, inbreeding causes severe **inbreeding depression**[10], which mainly arises from the homozygosity of recessive deleterious alleles or allele combinations (i.e. genetic load; Charlesworth and Willis 2009). The detrimental effects of inbreeding may be accelerated under stress (e.g. physical damage in ruderal habitats; Box 1.1) due to **negative inbreeding-environment interactions**[11] (IxE interactions; Fox and Reed 2011).

These three mechanisms may consequently imply further reduction of the population growth rates, which can ultimately lead to further reductions of population size and therefore, to the initiating of extinction vortex dynamics (Coron 2014). However, invasive species are rapidly expanding worldwide, despite the fact that series of founder events are likely to occur throughout the invasion history (see previous section) – a conundrum which is coined in the term the genetic paradox of invasions (Allendorf and Lundquist 2003). Several mechanisms are proposed to counteract genetic depletion and / or its negative consequences on population growth (Box 1.2).

(A) Avoiding genetic depletion	**(B1)** Counteracting adaptive limitation
Mass introduction of propagules may lead to only negligible reductions of effective population sizes as compared to the native source populations (Roman and Darling 2007), while multiple introductions with subsequent **population admixture**[12] may restore genetic diversity (Verhoeven *et al.* 2011). In predominantly selfing (e.g. Okada *et al.* 2013) or asexually reproducing plant invaders (e.g. Clark *et al.* 2012), immediate effects of population size on heterozygosity are avoided (Silvertown and Charlesworth 2009). Increasing longevity is assumed to decelerate genetic depletion (Austerlitz *et al.* 2000).	High phenotypic plasticity (Luquet *et al.* 2011) and epigenetically-acting adaptation (Rollins *et al.* 2015) can maintain variability in spite of low genetic diversity. Phenotypic plasticity (i.e. general-purpose genotype) may reduce the necessity of variance (Davidson *et al.* 2011). **(B2)** Counteracting mate limitation **Negative frequency-dependent selection**[13] can maintain *S*-allele diversity during bottlenecks (Stoeckel *et al.* 2012), and extensive **dominance interactions among *S*-alleles**[14] may increase mate availability in founder populations that exhibit low *S*-allele diversity (Brennan *et al.* 2006). **(B3)** Counteracting inbreeding depression **Purging of genetic load**[15] (Fountain *et al.* 2014) and **positive IxE interactions**[16] (Schrieber *et al.* submitted) were shown to alleviate inbreeding depression during colonization.

Box 1.2 Mechanisms that counteract genetic depletion (A) and its negative consequences for population growth (B1 - B3). Note that all mechanisms that counteract genetic depletion are anticipated to consequently alleviate its consequences.

10 the fitness decrements suffered in inbred offspring, when compared to offspring from random mating within the same population (Pekkala *et al.* 2014)

11 increased inbreeding depression in individuals that face external stress compared to benign conditions (Reed *et al.* 2012)

12 the mixture of genetically distinct lineages due to a secondary contact after numerous generations of isolation (Havrdová *et al.* 2015)

13 selection advantage of rare *S*-alleles or new *S*-alleles that arise via mutation or immigration (Herman *et al.* 2012)

14 a mechanism that suppresses the expression of recessive *S*-alleles, which reduces the effective number of *S*-alleles per individual and therefore reduces the probability of matching *S*-alleles between mating partners (de Nettancourt 2013)

15 removal of deleterious recessive alleles in inbred populations, as homozygosity exposed their detrimental effects to purifying selection (Larsen *et al.* 2011)

16 a mechanism that reduces inbreeding depression, where individuals face a stress-release (benign environmental conditions) that enhance their ability to circumvent internal stress caused by inbreeding (Schrieber and Lachmuth 2016)

Since such mechanisms as described in Box 1.2 were frequently reported in successful invaders (reviewed in Estoup *et al.* 2016 and in Schrieber and Lachmuth 2016), it has been suggested that the ecological significance of the genetic paradox of invasions might have been overestimated (Frankham 2004; Roman and Darling 2007; Hufbauer 2008). However, this conclusion seems at least questionable. In fact, the frequent occurrence of these mechanisms reveals the opposite: genetic depletion matters, because it appears to select species that are able to handle demographic disequilibria. In other words, founder effects may operate as an invasion filter against species, which do not possess efficient mechanisms to endure critical phases of small population sizes.

Accordingly, it is notable that the majority of plant invaders shows similar or even increased genetic diversity in the introduced as compared to the native range (reviewed in Uller and Leimu 2011), whereas mathematical principles suggest that a loss of genetic diversity is most likely in the course of colonization (Hartl and Clark 1989). However, descriptive comparisons of genetic diversity between invasive vs. native populations are mostly conducted for species that are already recognized as notorious weeds. While current genetic diversity is often not reduced in such far-advanced invasion stages, initial colonization and range expansion may have involved severe bottlenecks, because contrasting demographic events as bottlenecks and population admixture can occur consecutively (Chun *et al.* 2010; Keller *et al.* 2012 a).

As such, if founder populations can outlast detrimental founder effects, external gene flow may restore genetic diversity. Indeed, multiple introductions are the rule and not the exception in biological invasions (Bossdorf *et al.* 2005), and population admixture is nowadays widely accepted to promote invasiveness (e.g. Okada *et al.* 2007; Culley and Hardiman 2008; Schierenbeck and Ellstrand 2008; Hovick and Whitney 2014). Importantly, population admixture is assumed to be more favored in the introduced than in the native range, because negative consequences of admixture, such as dilution of the locally adapted gene pool (reviewed in Rius and Darling 2014) are less important in exotic populations that usually did not evolve strong local adaptation (Verhoeven *et al.* 2011). Instead, the benefits of restoring genetic diversity prevail in genetically depauperate founder populations. Particularly within the first generations after admixture, intraspecific hybrids of genetically differentiated populations may show heterosis due to massive increases in heterozygosity (Shapira *et al.* 2014). Moreover, such recombination generates novel genetic variation that may stimulate **rapid adaptive changes**[17] (Handley *et al.* 2011). Both mechanisms are frequently attributed to boost growth rates of previously inconspicuous populations (i.e. “catapult effect”), which may help to understand the transition from lag-phases to invasive spread (Drake 2006). Considering the obviously relevant ability to outlast critical phases of demographic disequilibria (Fig. 1.4, A2), I elaborate in the following section, how **polyploidy**[18] may affect the population genetic consequences of colonization.

17 genetically based trait shifts, which establish within few generations in the gene pool of populations that find themselves subjected to suddenly changing selective pressures (Sax *et al.* 2007)

18 the occurrence of more than two chromosome sets in one nucleus (Lavania 2015)

1.4 Polyploidy and invasions

Polyploidy has often been emphasized to promote plant invasions, but the underlying mechanisms remain under controversial discussion (Aboucaya *et al.* 2002; Pandit *et al.* 2006; 2011; 2014; Küster *et al.* 2008; te Beest *et al.* 2011; Bock *et al.* 2015; Suda *et al.* 2015). Polyploidization is characterized by sudden genome-wide changes and is thus considered as one of the most important drivers of plant evolution (Jiao *et al.* 2011; Soltis *et al.* 2014; but see Mayrose *et al.* 2015). Specifically, polyploidization fundamentally alters gene expression (Parisod 2012; Soltis *et al.* 2015), which affects the physiology (Dudits *et al.* 2016), morphology (Huang *et al.* 2015), life history (Larkin *et al.* 2016) and ultimately the ecological niche of an organism (Sonnleitner *et al.* 2016). Within **polyploid complexes**[19], polyploids often show broader ecological amplitudes than their diploid conspecies (Soltis *et al.* 2004) and tend to occur at more extreme and marginal habitats, where they drive range expansion (te Beest *et al.* 2011).

Many factors which determine the general success of polyploids, have also been shown to increase invasiveness. Particularly, the invasiveness of polyploid species is highlighted to benefit from their large genome sizes that inherently result from the multiplication of the chromosome set (Pandit *et al.* 2014; Suda *et al.* 2015). Increasing genome size goes along with increasing cell size (Gregory 2001), which may lead to increasing plant body size (Otto 2007) and increasing competitive ability (te Beest *et al.* 2011). Meanwhile, polyploidy was frequently recognized as beneficial for colonization abilities (e.g. Brochmann *et al.* 2004; Prentis *et al.* 2008), which could be related to the fact that polyploids are commonly expected to maintain higher genetic diversity than diploids (Soltis and Soltis 2000). In addition to higher initial genetic diversity, Box 1.3 shows that polyploidy can have important effects on genetic depletion (e.g. strength of **genetic drift**[20]) and its consequences (e.g. inbreeding depression).

(A) Avoiding genetic depletion	(B1) Counteracting adaptive limitation
The negative effects of genetic drift on genetic diversity, that are, the loss of alleles and increasing homozygosity across the gene pool of populations, are reduced due to the segregation of multiple chromosome sets (Hartl and Clark 1989).	The novel recombination of genome parts can stimulate adaptive capabilities (Prentis *et al.* 2008). Accelerating mate limitation (B2) The higher number of alleles per locus may result in more *S*-alleles per individual, which potentially increases the probability that mating partners show identical *S*-alleles (Pickup and Young 2007). (B3) Counteracting inbreeding depression The multiplied number of allele copies per locus may increase the masking of genetic load (Eliášová *et al.* 2013).

Box 1.3 The influence of polyploidy on genetic depletion (A) and its negative consequences for population growth (B1 - B3). Note that all mechanisms that counteract genetic depletion are anticipated to consequently alleviate its consequences.

19 a taxa, which comprises closely related conspecies that differ in their ploidy level (Kolář *et al.* 2015)

20 random fluctuations of allele frequencies in a gene pool, which can reduce genetic diversity due to the extinction or fixation of alleles, particularly in small populations (Ewens 2012)

However, the in Box 1.3 described effects of polyploidy can considerably vary among polyploid species. The genetic diversity of polyploids may be crucially affected by the mode of polyploidization, which can be either **autopolyploid**[21] or **allopolyploid**[22]. In allopolyploids, **disomic inheritance**[23] can result in fixed heterozygosity, which can ultimately conserve heterosis for several generations (García-Verdugo *et al.* 2013). Thereby, at loci that show disomic inheritance, the effects of genetic drift on homozygosity (Box 1.3, (A1)) are rather similar to diploids, as such loci virtually show the segregation of two independent diploid genomes (i.e. "functional diploid"; Le Comber *et al.* 2010). Thus, the deceleration of genetic drift is most pronounced under **polysomic inheritance**[24], which is most likely to occur in autopolyploid genomes (Ronfort *et al.* 1998). However, polysomic inheritance may evolve from disomic inheritance, and *vice versa* (Roux and Pannell 2015), which leads to mixed inheritance patterns across different loci in autopolyploids and in allopolyploids. Importantly, with increasing time elapsed since polyploidization, genomic downsizing causes the successive loss of duplicated regions throughout a polyploid genome, which makes polyploids increasingly similar to their diploid ancestors (i.e. diploidization; Ohno 2013; Douglas *et al.* 2015).

Moreover, the propagated enhancements in mate limitation (Box 1.3, (B2)) can be diminished by higher *S*-allele diversity in polyploids than diploids (Pickup and Young 2007). The counteracting effect of polyploidy on inbreeding depression (Box 1.3, (B3)) may decrease with increasing evolutionary age of the polyploid (Galloway and Etterson 2007). In particular, polyploids may accumulate genetic load more rapidly than diploids (Otto and Whitton 2000; Otto 2007), because the more effective masking of mutations may allow mutations to spread until the mutation-selection equilibrium is approximated (Ozimec and Husband 2011).

Interestingly, IxE interactions have not yet been compared between cytotypes. Negative IxE interactions may be less pronounced in polyploids than diploids, if polyploids are generally less vulnerable to the detrimental effects of inbreeding. Furthermore, polyploids often show increased longevity (te Beest *et al.* 2011), and increased longevity itself may alleviate genetic depletion and therefore its negative consequences (see Box 1.2). This emphasizes that multiple antagonists of genetic depletion are mutually non-exclusive, which should be considered in studies on colonization genetics.

21 an organism that shows multiplied number of chromosomes sets, which results from whole genome duplication without hybridization with another species (Rensing *et al.* 2013)

22 an organism with a genome, which contains two or more genetically distinct chromosome sets due to the hybridization between different parental species (Combes *et al.* 2013)

23 phenomenon during meiosis in most allopolyploids, where the two homologous chromosomes of each contributing parental species (instead of the homeologous chromosomes) preferentially pair to each other (Roux and Pannell 2015)

24 phenomenon during meiosis in most autopolyploids, where the chromosome segregation is characterized by no preferentially pairing of homologous chromosomes (Stift *et al.* 2008); in tetraploids specifically called tetrasomic inheritance

1.5 The model *Centaurea stoebe s.l.*

Centaurea stoebe s.l. L. (syn. *C. maculosa* Lam.; spotted knapweed; Asteraceae) is taxonomically subdivided in diploid *C. stoebe* L. subsp. *stoebe* (2n = 2x = 18) and tetraploid *C. stoebe* L. subsp. *micranthos* (Gugler) Hayek (2n = 4x = 36) (Ochsmann 2000). Tetraploids originated from allopolyploidization, yet, the second parental species remains unknown (Mráz *et al.* 2012a). Gene flow between cytotypes is assumed to be almost absent due to the infertility of triploid hybrids (Mráz *et al.* 2012b). Since the taxonomic status of this polyploid complex is unresolved, I regard *C. stoebe* s.l. as the taxonomic entity for my PhD thesis.

In the native range, diploids are more common in Western, Northern and Central Europe, whereas tetraploids prevail in South Eastern Europe (Španiel *et al.* 2008), however, interestingly, tetraploids presently expand towards Central Europe, which increases the overlap of the cytotypes´ ranges and generates mixed-ploidy populations (Korneck 2006; Welss *et al.* 2008; Otisková *et al.* 2014). Overall, diploids represent the majority cytotype in the native range (Broennimann *et al.* 2014). It is assumed that both cytotypes were initially introduced to North America (Treier *et al.* 2009), but to date, only the occurrence of tetraploids has been recorded (Mráz *et al.* 2011). This cytotype shift results in three **geo-cytotypes**[25] (GCTs): native diploids (EU2x), native tetraploids (EU4x) and invasive tetraploids (NA4x), which motivated a series of studies to investigate pre-adaptive differences between the cytotypes (Box 1.4).

The most important differences between the cytotypes is that diploids are predominantly monocarpic, whereas tetraploids show a polycarpic life cycle (Henery *et al.* 2010). Both cytotypes occupy relatively similar habitats (Ochsmann 2000), but diploids prevail in natural and tetraploids in ruderal habitats (Otisková *et al.* 2014). The polycarpy of tetraploids has been postulated to promote disturbance tolerance and therefore their invasiveness (Mráz *et al.* 2012b). However, in a clipping experiment, the response to physical damage was similarly pronounced in both cytotypes (Thébault and Buttler 2009). Moreover, EU4x was found to show greater plasticity (Hahn *et al.* 2012b), higher seed survival in the soil bank (Hahn *et al.* 2013) and a higher life span seed production (Broz *et al.* 2009) than EU2x. According to the European distribution of both cytotypes, tetraploids were considered to be better pre-adapted to the dry and continental climate in large parts of North America (Treier *et al.* 2009). This was related to differences in several leaf traits that were assumed to enhance drought tolerance (Henery *et al.* 2010). However, latest experimental results suggest that drought tolerance does not differ between cytotypes, but is instead correlated with latitudinal clines within cytotypes (Mráz *et al.* 2014). In addition, a current data set of herbarium specimen shows that the most continental areas in the entire native distribution (e.g. Central Russia) seem to be predominantly occupied by diploids (Rosche, C. and Mráz, P., unpublished data). Differences in the genetic consequences of founder events may offer additional explanations for the invasion success of tetraploids that are not mutually exclusive with previous suggestions.

Box 1.4 Pre-adaptive differences between native tetraploids and diploids that may contribute to explain the cytotype shift between the ranges of *Centaurea stoebe*.

25 distribution pattern describing the occurrence of a cytotype in a given continent or range (Hahn and Müller-Schärer 2013)

Both diploids and tetraploids are strictly self-incompatible, show similar pollinator spectra (dominated by Hymenoptera; Mráz *et al.* 2012b), and the same seed dispersal syndrome (barochory; Hahn *et al.* 2013). This lack of achene dispersal vectors can result in the spatial accumulation of siblings, which may increase biparental inbreeding (Richards and Ritland 2000). However, opportunistic myrmecochory is reported (Jensen and Six 2006), which potentially drives within-population gene flow, but estimations of the small-scale genetic structure of natural *C. stoebe* populations are lacking.

Previous population genetic analyses found significantly higher expected heterozygosity in NA4x than EU4x for microsatellites (Marrs *et al.* 2008), but cpDNA results (Hufbauer and Sforza 2008) and AFLP analyses (Mráz, P. and Müller-Schärer, H., unpublished results) suggested opposite trends. In addition, previously published studies included only two diploid populations, which averted any across-cytotype comparison. Additional population genetic studies with larger sample sizes and a more balanced sampling design may thus yield new insights in the genetic structure in both ranges. Nevertheless, the studies of Hufbauer and Sforza (2008) and Marrs *et al.* (2008) revealed important information on the invasion history of NA4x as they revealed multiple introductions from different sources of the native range and subsequent admixture.

The first records of *C. stoebe* in North America date to the late 19th century (Roche and Roche 1991). Afterwards, NA4x faced a lag-phase of 50 years until it expanded rapidly throughout large parts of North America (Broennimann *et al.* 2014). The rapid invasive spread took place along two separate main invasion routes: one expanding from the East coast and one from the West coast (Hordijk and Broennimann 2012). This invasive expansion required a strong climatic niche shift towards a more continental and dry climate in Central North America (Broennimann *et al.* 2007; 2012), which may be explained by rapid adaptive changes that may have occurred during the lag-phase (Broennimann *et al.* 2014). Further evidence for rapid adaptation was found in NA4x, as this GCT shows higher population growth rates (Hahn *et al.* 2012a), increased competitive abilities (Ridenour *et al.* 2008), higher seedling emergence (Hahn *et al.* 2013) and a more pronounced polycarpic life cycle (Henery *et al.* 2010) than EU4x. For my PhD study, I particularly investigate NA4x populations from North West America. In this region, *C. stoebe* is among the most destructive weeds (Reinhart and Rinella 2010; Ortega and Pearson 2011; Maron *et al.* 2013) as it can significantly alter community composition and productivity (Maron and Marler 2008), and often builds up virtual monocultures with up to 100 plants per m^2 (Müller-Schärer 1991). Due to its enormous ecological and economical impact, *C. stoebe* became one of the most prominent model systems in invasion biology that has been extensively studied since decades. Although tetraploids have been observed occasionally in Australia (Hufbauer and Sforza 2008), I am not aware of any non-native occurrence of *C. stoebe* that currently exists outside of North America. The most important subjects that were investigated in the recent literature, but are not under specific consideration in my studies, are summarized in the Appendix (Box A1.1).

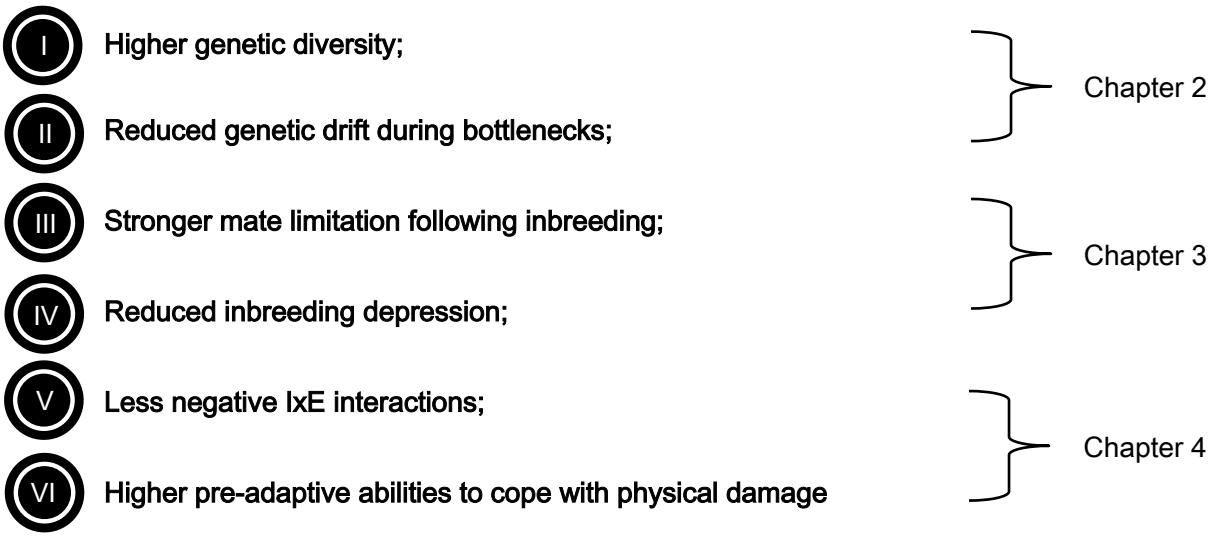
1.6 Principle objectives and concept of the thesis

The general introduction is followed by three separate studies that address the specific goals of my thesis. In particular, I elaborate how the GCTs differ in their response to distinct ecological and population genetic processes that may typically occur in the course of a primary colonization. In **chapter 2**, I investigate the population genetic structure and genetic diversity of 56 *C. stoebe* populations (at least 18 per GCT). The roles of population size and habitat type (ruderal vs. natural populations) in determining genetic diversity are under special consideration. Moreover, I highlight how the small-scale genetic structure within populations may be affected by longevity, range and ploidy level.

In **chapter 3**, a crossing experiment is presented that involved offspring from 42 of the 56 populations from the previous chapter. Pollination success of inbreeding and outcrossing is evaluated and the fitness of the resultant F1-offspring is estimated. Particularly, I test for differences in the response to breeding treatment between GCTs and for evidence of purging in populations that had undergone frequent inbreeding in their population history.

In **chapter 4**, the F1-offspring from the previous chapter is used in a clipping experiment (37 populations). I investigate whether the response to clipping differs between habitat types and / or between GCTs. Moreover, I examine IxE interactions (i.e. the response of inbred vs. outbred offspring to clipping), and assess whether these IxE interactions differ between GCTs.

Across all three studies, the comparison between the GCTs enables me to gain knowledge on 1) post-introduction processes in tetraploids (e.g. rapid adaptive and / or non-adaptive changes in NA4x as compared to EU4x), and especially 2) pre-adaptive differences between the cytotypes in their colonization capabilities (i.e. EU4x vs. EU2x). The later aspect represents the main interest of my studies as it promises to reveal mechanisms, which may contribute to the cytotype shift in *C. stoebe*. Therefore, my thesis stresses the following working hypotheses (see also Fig. 1.5). **Compared to diploids, polyploids show:**



The results of my studies are synthesized in a general discussion, which demonstrates how my thesis adds to previous studies that examined the biology of the cytotype shift in *C. stoebe*.

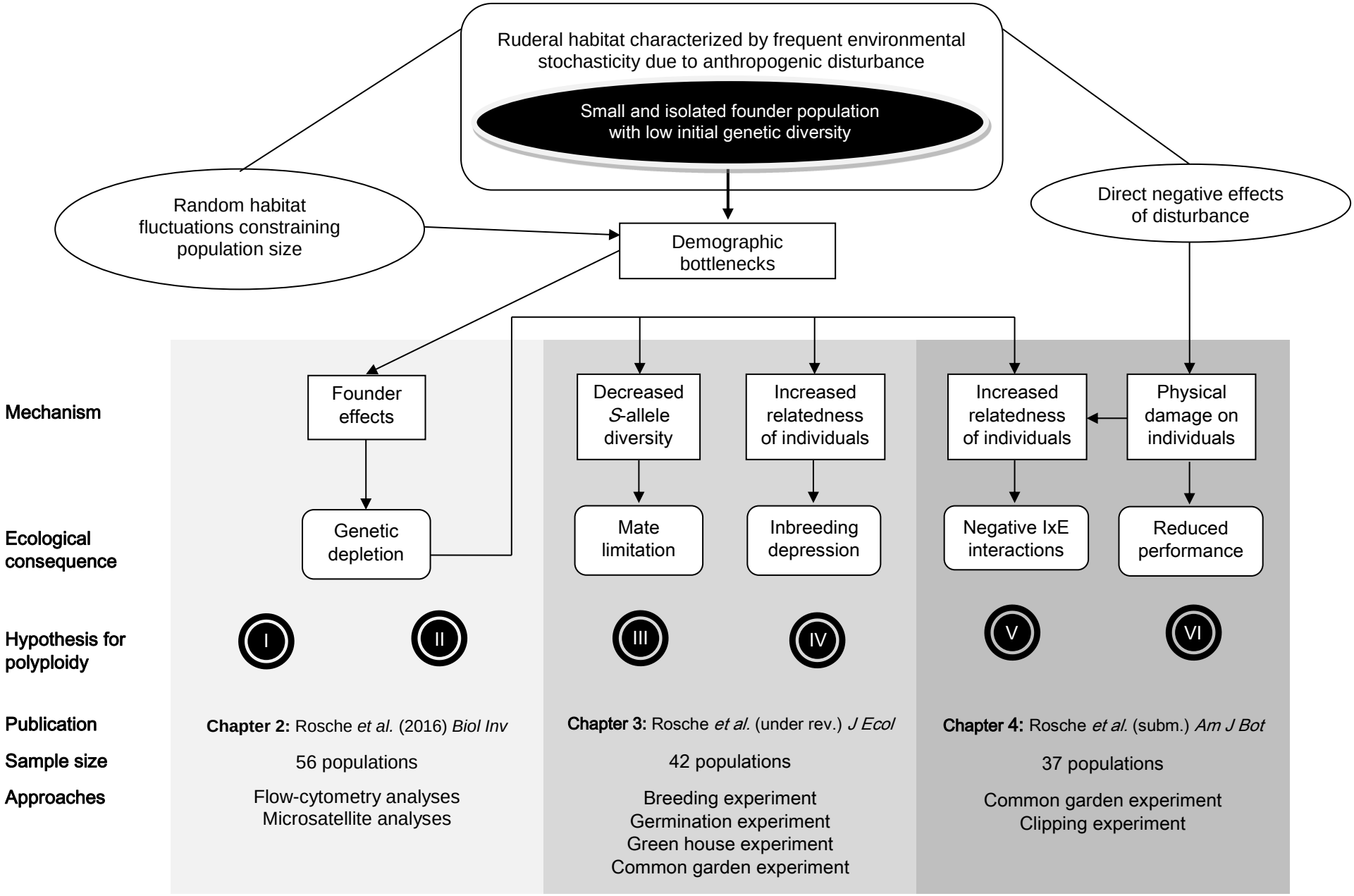


Fig. 1.5 General concept of the three separate studies of this cumulative dissertation. Note that the studies are built upon each other (e.g. sampled populations).

Chapter 2

COLONIZATION GENETICS OF THE THREE GEO-CYTOTYPES

This chapter has been published in *Biological Invasions* as:
Rosche C, Durka W, Hensen I, Mráz P, Hartmann M, Müller-Schärer H, Lachmuth S (2016): The population genetics of the fundamental cytotype-shift in invasive *Centaurea stoebe* s.l.: genetic diversity, genetic differentiation and small-scale genetic structure differ between cytotypes but not between ranges. DOI: 10.1007/s10530-016-1133-2

2.1 Abstract

Polyploids are overrepresented in invasive species. Yet, the role of genetic diversity and drift in colonization success of polyploids remains unclear. Here, we investigate genetic diversity, genetic differentiation and small-scale genetic structure in our model system, the three geo-cytotypes of *Centaurea stoebe*: monocarpic diploids and polycarpic (allo)tetraploids coexist in the native range (Eurasia), but only tetraploids are reported from the invasive range (North America). For each geo-cytotype, we investigated 18 to 20 populations varying in size and habitat type (natural vs. ruderal). Population genetic analyses were conducted at eight microsatellite loci.

Compared to diploids, tetraploids revealed higher genetic diversity and lower genetic differentiation, whereas both were comparable in tetraploids between both ranges. Within spatial distances of a few meters, diploid individuals were more strongly related to one another than tetraploids. In addition, expected heterozygosity in diploids increased with population size and was higher in natural than in ruderal habitats. However, neither relationship was found for tetraploids.

The higher genetic diversity of tetraploid *C. stoebe* may have enhanced its colonization abilities, if genetic diversity is correlated with fitness and adaptive capabilities. Furthermore, the inheritance of a duplicated chromosome set as well as longevity and frequent gene flow reduces drift in tetraploids. This counteracts genetic depletion during initial introductions and in subsequent phases of small or fluctuating population sizes in ruderal habitats. Our findings advocate the importance of studying colonization genetic processes to gain a more mechanistic understanding of the role of polyploidy in invasion dynamics.

Keywords *Centaurea stoebe,* colonization, genetic diversity, geo-cytotype, biological invasion, polyploidy

2.2 Introduction

Biological invasions are ecological enigmas: while some non-native species attain astoundingly high abundances in the introduced range (e.g. Shah *et al.* 2014); the vast majority of exotics fail to establish (Sax and Brown 2000). Understanding the mechanisms that determine the success of invaders has consequently attracted major interest in ecological research (Simberloff *et al.* 2013). More recently, polyploidy has received increasing attention as it may promote founder and invasion success (Pandit *et al.* 2014; Bock *et al.* 2015). Polyploids frequently possess broader ecological amplitudes and higher plasticity than diploids (Soltis *et al.* 2014). Both may be related to the high genetic diversity in polyploids, which often considerably exceeds that of their diploid ancestors (reviewed in Soltis and Soltis 2000).

Genetic diversity is considered to be a key determinant of invasion dynamics, largely because it inherently affects population growth rates and the adaptive potential of exotics (Forsman 2014). However, dispersal limitation may result in a random loss of overall allele diversity in the introduced range, and colonization usually involves founder effects, including genetic drift and inbreeding (Dlugosch and Parker 2008; Hufbauer *et al.* 2013; Szűcs *et al.* 2014). More specifically, colonization and initial range expansion mostly take place at ruderal sites, where higher disturbance frequencies ensure great resource supply (Dietz and Edwards 2006). However, such environmental stochasticity may cause more frequent fluctuations in population size and consequent genetic bottlenecks than in natural habitats. Nevertheless, invasion dynamics are, at least in the long term, not inevitably restricted by massive reductions in genetic diversity (reviewed in Uller and Leimu 2011). Since multiple introductions are rather the rule than the exception (Dlugosch and Parker 2008), invasions may involve both, the fission and fusion of native source gene pools (Keller and Taylor 2010). The resultant admixture of previously isolated gene pools may boost spread by counteracting genetic depletion (Verhoeven *et al.* 2011). Thus, traits that decelerate the loss of genetic diversity may ultimately facilitate invasion success as they may help founder populations persist though colonization bottlenecks until the gene pool can be restored (Theoharides and Dukes 2007).

Polyploidy antagonizes genetic depletion (Soltis *et al.* 2014), because genetic drift is known to affect polyploid genomes less strongly than diploid ones due to inheritance of a duplicated set of chromosomes per gamete (Ronfort *et al.* 1998). In addition, polyploidy often involves a switch from annual to perennial life history (te Beest *et al.* 2011), which can influence the small-scale genetic structure. Under fluctuating population sizes, perennials may exhibit less frequent (biparental) inbreeding and reduced drift due to their longevity and overlapping generations (Nybom 2004). However, population genetic consequences of ploidy level are poorly understood and can best be investigated within polyploid complexes that comprise diploid and polyploid subspecies (Hardy and Vekemans 2001).

In this study, we examined the genetic structure of spotted knapweed, *Centaurea stoebe* s.l. L. (Asteraceae, syn. *C. maculosa* Lam.), which constitutes a polyploid complex including a diploid (predominantly) monocarpic cytotype and a polycarpic tetraploid cytotype (Mráz *et al.* 2011). The complex is native to Europe and Asia Minor with diploids representing the majority of native populations (Broennimann *et al.* 2014). Both cytotypes occupy relatively similar habitats (i.e. dry natural and ruderal sites), but tetraploids are more frequent at ruderal sites (Treier *et al.* 2009; Otisková *et al.* 2014). Remarkably, so far, only tetraploids have been reported from the non-native range (Mráz *et al.* 2011). As such, we distinguish three geo-cytotypes (GCTs, defined by ploidy level and range) as follows: native diploid (EU2x), native tetraploid (EU4x), and invasive tetraploid (NA4x). Te Beest *et al.* (2011) highlighted this cytotype shift as "*an excellent model system for evaluating the role of polyploidy in plant invasions*".

A previous microsatellite study from Marrs *et al.* (2008) particularly focused on the introduction history of tetraploid *C. stoebe.* Although it included two diploid populations, it did not allow for and did not aim at drawing conclusions about population genetic differences between the two cytotypes. In contrast, we examine the interplay of polyploidy, longevity and demographic history for overcoming founder effects and use the *C. stoebe* complex as a model system to highlight the relevance of polyploidy for the population genetics of colonizing species. Despite increasing awareness of the significance of polyploidy in invasions (e.g. Pandit *et al.* 2011; 2014), population genetic studies on GCTs are surprisingly scarce (but see Schlaepfer *et al.* 2008; Ferrero *et al.* 2015). Our investigations were directed by the following hypotheses:

1) Tetraploids of *C. stoebe* reveal higher genetic diversity than diploids. Current genetic diversity is not reduced in NA4x compared to EU4x.

2) Among population differentiation is stronger in diploids than in tetraploids. Tetraploids are more strongly differentiated in the native range.

3) Within populations, diploid individuals are more closely related on a small spatial scale than tetraploid individuals.

4) Within GCTs, genetic diversity increases with population size. Natural populations reveal higher genetic diversity than ruderal populations.

Our analyses will contribute to better understanding of the cytotype shift in *C. stoebe* and may provide important implications for polyploid vs. diploid range dynamics in general.

2.3 Materials and Methods

The model system *Centaurea stoebe* s.l.

Diploid *C. stoebe* L. subsp. *stoebe* and tetraploid *C. stoebe* L. subsp. *micranthos* (Gugler) Hayek exhibit a strong reproductive barrier (Mráz *et al.* 2012b). Based on cloned internal transcribed spacers, Mráz *et al.* (2012a) showed that the tetraploid cytotype originated from allopolyploidization events, which occurred within the last 2 mya, but, the second closely related

parental taxon has not yet been identified. Despite the allopolyploid origin of tetraploids, Mráz *et al.* (unpublished data) found clear evidence for tetrasomic inheritance when they screened the inheritance of four microsatellite loci in controlled crosses of tetraploid plants. Due to its complexity, the nomenclature of both taxa remains unresolved, and the cytotypes are mainly treated at the subspecies level (Mráz *et al.* 2011). Both cytotypes are strictly self-incompatible; and they have similar gene dispersal capabilities: small Hymenoptera are considered as main pollinators (Mráz *et al.* 2012b), and achenes are dispersed by barochory with no differences in falling velocity between GCTs (Hahn *et al.* 2013). Although tetraploids are polycarpic, neither cytotype shows vegetative propagation.

In the native range, diploids are more common in central Europe, while tetraploids prevail in south-eastern Europe (Broennimann *et al.* 2014). However, their native distributions overlap widely and include several mixed-ploidy populations (Mráz *et al.* 2012b). Moreover, EU4x recently expanded towards central Europe (Ochsmann 2000). In the invasive range, the first recorded introductions of NA4x were in the late 19th century, followed by a lag-phase of 50 years. Subsequently, the species spread rapidly along ruderal transport corridors of two separate invasion routes: one expanding from the east coast and one from the west coast (Broennimann *et al.* 2014). Nowadays, *C. stoebe* is a widespread, notorious weed that causes tremendous economic damage in the North American grasslands (Corn *et al.* 2006).

Sampling

Extensive field sampling was undertaken across large parts of the native distribution and of the western invasion route in North America, where *C. stoebe* is regarded as one of the most noxious invaders (Maron *et al.* 2013). Between 2012 and 2014, we sampled at least 18 populations of each GCT. We estimated population size as the number of flowering individuals. Therefore, we counted every flowering individual in populations with up to 500 individuals and rounded the counts. In larger populations, we counted 500 individuals in an area of representative density and extrapolated the population size to the entire population area. All sites were classified according to the European classification system of habitats (EUNIS 2008). Following the protocol of Broennimann *et al.* (2014), natural and semi-natural grasslands (EUNIS-category E), natural rocky outcrops (H), and diluvial sediments (C) were considered as (semi-)natural (for reason of simplicity referred to as “natural” throughout this manuscript). Agricultural (I), artificial and industrial habitats (J) were considered as ruderal. We collected leaf samples for genetic analysis and, if available, seeds for flow cytometry, from 19-31 haphazardly selected adult individuals per population equally distributed across the population. If populations consisted of fewer than 20 adults, we added samples from rosettes.

Flow cytometry and microsatellite amplification

The ploidy level of all populations was assessed by applying the identical protocol as in Mráz *et al.* (2011). For population genetic analyses of mixed-ploidy populations, we only made use of leaf samples from individuals for which we determined the majority cytotype. Thus, the microsatellite analyses concerned only one cytotype per population, as sample size of the minority cytotype was commonly too low (Table 2.1), and gene flow between the cytotypes was shown to be almost absent (Mráz *et al.* 2012b).

Microsatellite amplification and genotyping

We extracted DNA from 10–15 mg of lyophilized leaf tissue with the DNeasy 96 Plant Kit (Qiagen) following the manufacturer's protocol. We tested ten already established microsatellites, eight of which appeared to be highly polymorphic, and showed clear single bands for each allele: CM-730, CM-8337, CM-1922, CM-10060 described in Mráz *et al.* (2012b), and CM17, 42 CM27, CM26, CD9 from Marrs *et al.* (2008). Amplification was accomplished with M13R- or CAG-tailed primers in three multiplex PCR reactions. The final volume was 5 µL containing 3 µL QIAGEN Multiplex PCR kit, ~20 ng genomic DNA and 1 µL Mastermix. Mastermix contained 0.25 µM of forward primer (either CAG- or M13R-tailed), 0.25 µM reverse primer and 0.25 µM of the fluorescent-labeled CAG or M13R primer. We applied a touchdown PCR with following conditions: 95 °C for 15 min; 20 cycles of 94 °C for 30 s, 60 °C for 60 s (with an increment of −0.5 °C per cycle), 72 °C for 90 s; 20 cycles of 94 °C for 30 s, 50 °C for 60 s, 72 °C for 90 s; and finally, an elongation step of 10 min at 72 °C. Electropherograms were obtained by migration of amplification products on an ABI 3130 genetic analyzer (Applied Biosystems) with LIZ-500 (internal size standard). To bin the allele sizes, we used GeneMapper 5.0 (Applied Biosystems). 67 samples were deleted from the final data set, because more than one locus failed to amplify. The remaining proportion of missing loci was 2.5 %. In total, we genotyped 1,321 individuals. We confirmed reliable and stable patterns at each SSR locus by repeating the amplification of 48 identical samples. Without segregation analysis, peak intensities are not reliable to estimate the quantum of null alleles or allelic doses (Dufresne *et al.* 2014; Blanchet *et al.* 2014). Exact genotypes of polyploids can be assigned only when marker phenotypes show a single allele or the number of alleles equals the ploidy level. We therefore choose programs that are robust for dealing with genotype uncertainty and occurrence of null alleles.

Note that allopolyploidy may result in disomic inheritance, which leads to biased population genetic parameter estimates when calculated under the assumption of tetrasomy (for details see Meirmans and van Tienderen 2013). However, in accordance with the above mentioned inheritance screening of microsatellites by Mráz *et al.* (unpublished data), we also found strong evidence for tetrasomic inheritance, as we did not find fixed heterozygosity at any of our eight loci. Thus, disomic inheritance seems rather unlikely, at least for large parts of the genome of tetraploid *C. stoebe*.

Genetic diversity within populations

Allelic richness (A_R, i.e. number of alleles rarefied to the minimum sample size of 19 individuals per population), was calculated with SPAGeDi 1.4 (Hardy and Vekemans 2002). Expected heterozygosity (H_e) was estimated using the unbiased estimator of Nei (1978) correcting for sample size in SPAGeDi for diploids. For tetraploids, we estimated H_e in ATetra 1.3 (van Puyvelde *et al.* 2010) by 10,000 Markov Chain Monte Carlo (MCMC) iterations (accounting for different probabilities of allele copy number combinations in partial heterozygotes). We further determined the numbers of private alleles per population, range and cytotype in R 3.12 (R development core team 2015). To test whether rare alleles were more frequent in EU4x than in NA4x, we calculated frequency down-weighted marker values (*DW*) per population according to Schönswetter and Tribsch (2005), and compared $\log_e$ (*DW*) between both tetraploid ranges in a linear model.

Geographic distribution of genetic clusters

We studied the among-population genetic structure with Structure 2.3.4 (Pritchard *et al.* 2000) employing a Bayesian assignment analysis. We performed two admixture models with correlated allele frequencies, one for each cytotype. Data were coded as co-dominant allele matrix. To handle genotypes with ambiguous allele copy numbers, we analyzed the tetraploid subset with the recessive allele option.

We ran both models with 20 replicate chains of 100,000 MCMC iterations after discarding 100,000 burn-in iterations for each K (i.e. number of genetically distinct partitions). The most likely partitioning was determined according to Evanno *et al.* (2005) using Structure Harvester (Earl and vonHoldt 2012). Tested K values ranged from $K = 1$ to $K = 18$ for the tetraploid subset, and to $K = 20$ for the diploid subset. Individual as well as population mean posterior assignment probabilities were inferred with CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007). Barplots of individual assignments were illustrated in Distruct 1.1 (Rosenberg 2004). We visualized mean population cluster memberships as barplots and plotted them on a geographic map in ArcMap 10.1 (ESRI). We attributed a population or an individual to a distinct cluster when its membership probability (*qK*) was higher than an arbitrary threshold of 80% (Zorić *et al.* 2012). Mean assignment probabilities of populations were regressed against longitude and latitude in linear models. We further estimated an admixture index (H_A) according to Keller and Taylor (2010), and tested for differences in degree of admixture within individuals in NA4x and EU4x in a linear mixed effect model with population set as a random effect (package lme4 in R; Bates *et al.* 2014).

Since Structure may be less reliable when dealing with different ploidy levels (Dufresne *et al.* 2014), we assessed genetic similarity between the GCTs with a principal component analysis (PCA) to illustrate gene flow between cytotypes. We used Bruvo distances (Bruvo *et al.* 2004)

calculated with the R-package polysat (Clark and Jaseniuk 2011), which is particularly recommended for analyzing mixed-ploidy data (Dufresne *et al.* 2014).

Differentiation among populations

To quantify genetic differentiation of populations, we calculated the most frequently used estimator of F_{ST} (Nei 1978), and ρ_{ST} (Ronfort *et al.* 1998) in SPAGeDi. ρ_{ST}-statistics exhibit identical expectations for population differentiation in different ploidy levels under identical gene flow conditions (Hardy and Vekemans 2001). Moreover, while F_{ST} may be underestimated under disomic inheritance (Dufresne *et al.* 2014), ρ_{ST} was shown to be least sensitive to ploidy level and double reduction rate and, consequently, mode of inheritance (Meirmans and van Tienderen 2013).

Small-scale genetic structure within populations

In 30 populations (ten populations of each GCT, see Table 2.1), we geo-referenced all sampled individuals. To compare small-scale genetic structure of diploids and tetraploids, we used the coefficient of relationship (ρ), as it is not affected by reduced drift in polyploids (Hardy and Vekemans 2001). We computed pair-wise ρ_{ij} by applying Moran´s *I* statistics in SPAGeDi. Spatial distance was divided into distinct distance intervals that ensured a high spatial resolution and a sufficient number of individual pairs per distance class (i.e. 5, 10, 20, 40, 80, 160, and 320 meters). To illustrate whether the within population structure differed between GCTs, an averaged ρ was computed for each given distance interval over all pair-wise comparisons within GCTs and plotted against spatial distance in correlograms. Within each GCT, significance of each mean ρ per class was tested with 1,000 permutations of multilocus genotypes. We correlated matrices of pair-wise ρ_{ij} and $\log_e$ spatial distances for each population and for each GCT, and tested b_{log} (i.e. slope of the regression) with Mantel tests (1,000 randomizations).

Influence of population size and habitat on genetic diversity

To analyze differences in genetic diversity (H_e, A_R, both untransformed) between GCTs and habitat type, we used ANOVAs including GCT and habitat, as well as their interaction, as fixed effects in R. Moreover, we performed ANCOVAs to analyze the effects of centered $\log_e$ population size, GCT and their interaction on genetic diversity. Transformation decisions were based on graphical assessment of normality of errors and homogeneity of variance (i.e. model checking plots; Crawley 2014). Significance of all terms was tested with *F*-tests (type III sums of squares; R-package car; Fox and Weisberg 2010). For significant terms of the ANOVA models, pair-wise comparisons among factor levels were performed with Tukey post-hoc tests (R-package multcomp; Hothorn *et al.* 2008). When the interaction of GCT and $\log_e$ population size was significant in the ANCOVAs, we fitted single linear models to assess the significance of $\log_e$ population size on genetic diversity for the single GCTs. To assess potentially confounding

relationships between population size and other explanatory variables, we tested whether $\log_e$ population size depended on GCT, habitat or their interaction. While population size differed between habitats within EU4x (ruderal < natural; $F_{1,18}$ = 5.73, P < 0.05), all other tested potentially confounding relationships were non-significant.

2.4 Results

Cytotype distribution

All North American samples were tetraploid (Table 2.1). In Europe, we confirmed 18 populations to consist exclusively of diploids and 15 populations to be tetraploid. Five populations consisted of both cytotypes, from which two were dominated by diploids and three by tetraploids. We did not observe any triploid individual.

Genetic diversity within populations

We recorded a total of 115 alleles, 16 of which were found exclusively in tetraploids, and two in diploids only. Within diploids, we determined 17 private alleles (i.e. found only within a single sample location) in ten populations. For tetraploids, 14 private alleles were found in ten populations. At the continent scale, more alleles were unique to EU4x (16) than to NA4x (4), and *DW* was significantly higher ($F_{1,34}$ = 6.03; P < 0.05) in EU4x (mean = 4.55; range = 1.96 to 11.03; Table 2.1) than in NA4x (mean = 3.16; range = 1.68 to 5.57). A_R ranged from 2.58 to 6.04 in EU2x (mean = 4.39), from 4.74 to 6.39 in EU4x (mean = 5.64), and from 3.95 to 6.84 in NA4x (mean = 5.69). We estimated an average H_e of 0.6 in EU2x (0.32-0.75), 0.74 in EU4x (0.62-0.79) and 0.75 in NA4x (0.62-0.82). Both A_R and H_e differed highly significantly between GCTs (P < 0.001). Posthoc-tests for the main effect of GCT consistently revealed significantly lower values in EU2x compared to EU4x and NA4x, whereas EU4x did not differ from NA4x in both cases.

Geographic distribution of genetic clusters

The Bayesian inferences of genetic structure revealed an optimal number of two clusters for the diploid data set, and four clusters for the tetraploid subset (Appendix, Fig. A2.1). Within the diploid data set, the first cluster (dip1, Fig. 2.1) was more abundant in southern populations (decreasing with latitude, $F_{1,18}$ = 27.04, P < 0.001), while dip2 mainly occurred in northern populations. 12 out of 20 EU2x populations were concerned as distinct, because they showed an assignment to one of both clusters that was higher than the arbitrary threshold of 80 % (Zorić *et al.* 2012). At the individual level, 79.2 % of all diploid individuals were assigned to a distinct cluster (Fig. 2.2). For tetraploids, the genetic structure was considerably more ambiguous, as the majority of populations (30 out of 36) comprised mixtures of different clusters (Fig. 2.1). We found significant relationships of cluster membership coefficients with longitude in EU4x (tet 3:

$F_{1,16}$ = 8.33, P < 0.05; tet 4: $F_{1,16}$ = 7.53, P < 0.05) and with latitude in NA4x (tet 1: $F_{1,16}$ = 5.9, P < 0.05; tet 2: $F_{1,16}$ = 7.34, P < 0.05; tet 4: $F_{1,16}$ = 12.79, P < 0.01). The proportion of individuals belonging to a distinct cluster was 28.4 % for EU4x and 21.2 % for NA4x (Fig. 2.2). There was no significant difference ($\chi^2_{(1)}$ = 2.91, P = 0.09) in degree of admixture between diploids (mean H_A = 0.51, Table 2.1) and tetraploids (mean H_A = 0.64). The level of admixture did not differ ($\chi^2_{(1)}$ = 0.35, P = 0.55) between EU4x (mean H_A = 0.61) and NA4x (mean H_A = 0.66).
The PCA revealed a strict separation of both cytotypes. We found two clusters, one including EU2x and one including both tetraploid GCTs (Appendix Fig. A2.2). NA4x and EU4x did not show clear separation from one another.

Differentiation among populations

The overall population structure was significant in all GCTs (P < 0.001, Table 2.2). Global differentiation of diploids (F_{ST} = 0.17; ρ_{ST} = 0.24) was substantially higher than that of tetraploids (F_{ST} = 0.07; ρ_{ST} = 0.13). Differentiation was almost identical among EU4x populations (F_{ST} = 0.07; ρ_{ST} = 0.13) compared to NA4x (F_{ST} = 0.07; ρ_{ST} = 0.12).

Small-scale genetic structure within populations

Diploid individuals showed higher spatial autocorrelation within the first two distance classes than tetraploids (Fig. 2.3). Overall, we observed similar patterns of small-scale genetic structure across all GCTs with the highest relationship coefficients occurring in the first distance intervals, and decreasing continuously thereafter with increasing spatial distance. All observed mean coefficients of relationship per distance class were highly significant in all GCTs (P < 0.001). The correlation of pair-wise ρ_{ij} and $\log_e$ spatial distances was highly significant in all GCTs (EU2x: b_{log} = -0.042, P < 0.001; EU4x: b_{log} = -0.028, P < 0.001; NA4x: b_{log} = -0.32, P < 0.001) and significant in the majority of populations (Table 2.1).

Influence of population size and habitat on genetic diversity

We observed a significant interaction in the effects of population size and GCT on H_e ($F_{2,50}$ = 9.04, P < 0.001; Fig. 2.4a) and A_R ($F_{2,50}$ = 4.63, P < 0.05). Specifically, we found a positive effect of population size on H_e in EU2x ($F_{1,18}$ = 18.78, P < 0.001), while there was no significant relationship in any of the two tetraploid GCTs. Allelic richness revealed similar patterns and was positively related to population size in EU2x ($F_{1,18}$ = 20.94, P < 0.01) and NA4x ($F_{1,16}$ = 6.99, P < 0.05), but not in EU4x. In addition, we found a significant interaction between habitat and GCT for H_e ($F_{2,50}$ = 4.31, P < 0.05; Fig. 2.4b) and A_R ($F_{2,50}$ = 3.26, P < 0.05). In particular, H_e was significantly smaller in ruderal compared to natural habitats within EU2x (t = 3.24, P < 0.05), but it did not differ significantly within both of the tetraploid GCTs. Allelic richness showed a similar pattern although the difference among habitats within EU2x was non-significant (t = 2.16, P = 0.28).

Table 2.1 Investigated populations: characteristics, ploidy levels, indices for genetic diversity and small-scale genetic structure

ID	Country, Locality, GPS (°N,°E)	Habitat Type	Population size	4x	2x	Sample size	H_e	A_R	*DW*	H_A	b_{log}
Native range, diploid (EU2x)											
1	DE, Federow, 53.48°, 12.76°	ruderal	40	0	22	20	0.32	2.58	n.e.	0.05	-0.004
2	DE, Feldberg, 53.32°, 13.43°	ruderal	250	0	27	27	0.55	3.57	n.e.	0.14	n.e.
3	DE, Hillersleben, 52.29°, 11.48°	ruderal	10	0	15	24	0.56	3.34	n.e.	0.15	n.e.
4	DE, Steinthaleben, 51.39°, 11.04°	natural	500	0	22	27	0.64	5.09	n.e.	0.86	n.e.
5	DE, Lieskau, 51.5°, 11.86°	natural	200	0	24	29	0.61	4.43	n.e.	0.93	n.e.
6	DE, Amselgrund, 51.5°, 11.94°	natural	500	0	25	23	0.63	4.51	n.e.	0.54	-0.041*
7	DE, Neue Göhle, 51.23°, 11.78°	ruderal	250	0	20	21	0.47	3.25	n.e.	0.07	n.e.
8	DE, Bautzen, 51.18°, 14.42°	ruderal	9	0	9	26	0.41	3.23	n.e.	0.18	n.e.
9	DE, Isteiner Klotz, 47.66°, 7.53°	natural	200	0	20	20	0.58	3.72	n.e.	0.82	n.e.
10	CH, Ramosch, 46.83°, 10.4°	natural	250	0	33	23	0.69	5.53	n.e.	0.31	-0.028
11	IT, Castelle Penede, 45.88°, 10.89°	natural	140	0	32	23	0.67	4.39	n.e.	0.28	-0.037*
12	IT, Rafenstein, 46.53°, 11.36°	natural	250	0	30	29	0.73	6.04	n.e.	0.54	-0.006
13	CZ, Rájov, 48.84°, 14.37°	natural	10	0	12	31	0.49	3.11	n.e.	0.06	n.e.
14	AT, Völkermarkt, 46.65°, 14.91°	ruderal	150	0	30	24	0.67	5.24	n.e.	0.69	-0.071***
15	SI, Murska Sobota, 46.63°, 16.21°	ruderal	50	0	9	20	0.54	3.66	n.e.	0.95	n.e.
16	SK, Sandberg, 48.2°, 16.97°	natural	1,000	2	31	20	0.71	5.29	n.e.	0.93	-0.002
17	HU, Balatongyörök, 46.76°, 17.34°	ruderal	600	0	31	25	0.67	5.4	n.e.	0.83	-0.029*
18	HU, Csepel Island, 47.33°, 18.95°	ruderal	400	6	27	27	0.70	5.63	n.e.	0.85	-0.019*
19	SK, Gelnica, 48.85°, 20.93°	ruderal	50	0	15	24	0.54	4.16	n.e.	0.58	-0.085**
20	RO, Valea lui David, 47.2°, 27.47°	natural	2,000	0	24	21	0.75	5.86	n.e.	0.35	n.e.

Table 2.1 continued.

ID	Country, Locality, GPS (°N,°E)	Habitat Type	Population size	4x	2x	Sample size	H_e	A_R	*DW*	H_A	b_{log}
Native range, tetraploid (EU4x)											
21	DE, Lichterfelde Süd, 52.4°, 13.31°	ruderal	750	24	0	30	0.77	5.96	5.12	0.78	-0.027*
22	DE, Dieskau, 51.44°, 12.04°	ruderal	300	30	0	23	0.62	4.75	2.73	0.33	-0.007
23	DE, Pratzschwitz, 50.97°, 13.9°	ruderal	70	25	5	21	0.74	5.5	4.84	0.57	n.e.
24	DE, Nürnberg, 49.39°, 11.08°	ruderal	200	33	0	25	0.74	6.05	3.01	0.68	-0.029*
25	AT, Starkenbach, 47.19°, 10.64°	ruderal	1500	32	0	28	0.73	5.41	3.22	0.62	-0.03*
26	AT, Krems, 48.43°, 15.65°	ruderal	400	33	0	26	0.78	6.26	7.2	0.41	-0.005
27	SK, Závod, 48.52°, 17.02°	ruderal	200	33	0	23	0.77	5.6	2.96	0.73	-0.052***
28	SK, Trnava, 48.38°, 17.6°	ruderal	200	23	0	25	0.75	5.94	8.11	0.61	-0.044*
29	SK, Nové Mesto, 48.76°, 17.84°	ruderal	50	25	0	21	0.66	4.74	3.56	0.62	-0.022
30	HU, Tapolca, 46.88°, 17.43°	ruderal	25	22	10	19	0.78	6.01	2.29	0.85	n.e.
31	HU, Gellérthegy, 47.49°, 19.05°	natural	1,000	30	0	26	0.79	6.2	11.03	0.82	n.e.
32	RO, Urziceni Pădure, 47.7°, 22.44°	natural	2,000	28	0	20	0.77	6.39	4.24	0.79	-0.007
33	RO, Cheile Turzii, 46.56°, 23.7°	natural	500	31	2	23	0.77	6.08	3.89	0.82	n.e.
34	RO, Poșaga de Jos, 46.43°, 23.45°	natural	400	28	0	25	0.77	5.68	4.92	0.86	-0.02*
35	RO, Oprișeni, 47.48°, 26.27°	natural	750	26	0	24	0.70	5.24	3.69	0.47	n.e.
36	RO, Lepșa, 45.94°, 26.59°	natural	800	15	0	20	0.71	4.88	1.96	0.12	n.e.
37	RO, Paraul Cacaina, 47.19°, 27.59°	natural	750	25	0	20	0.68	5.23	3.92	0.3	n.e.
38	MD, Tiraspol, 46.87°, 29.58°	natural	600	24	0	19	0.76	5.63	5.16	0.6	n.e.

Table 2.1 continued.

ID	Country, Locality, GPS (°N,°E)	Habitat Type	Population size	4x	2x	Sample size	H_e	A_R	*DW*	H_A	b_{log}
Invasive range, tetraploid (NA4x)											
39	CA, Tsawwassen, 49.02°, -123.11°	ruderal	1500	22	0	28	0.78	6.44	4.66	0.71	-0.033**
40	US, Seattle, 47.48°, -122.24°	ruderal	155	18	0	22	0.72	5.26	2.11	0.59	n.e.
41	US, Stevens Pass, 47.79°, -120.89°	ruderal	300	28	0	26	0.76	6.28	3.23	0.81	-0.018
42	CA, Kamloops, 50.71°, -120.37°	ruderal	2500	20	0	26	0.77	6.19	3.43	0.87	-0.041***
43	CA, Revelstoke, 51.02°, -118.21°	natural	80	27	0	25	0.77	6.21	3.74	0.77	-0.052**
44	US, Coeur d´Alene, 47.65°, -116.72°	natural	20,000	22	0	26	0.77	6.24	4.49	0.87	0.003
45	US, Emerald Creek, 47.07°, -116.33°	ruderal	5,000	25	0	31	0.78	6.47	4.49	0.82	-0.034**
46	CA, Okotoks, 50.72°, -113.95°	ruderal	6	11	0	22	0.70	4.61	1.68	0.39	n.e.
47	US, Logan Pass, 48.7°, -113.69°	natural	1500	25	0	26	0.66	4.29	2.46	0.57	n.e.
48	US, Missoula, 46.87°, -113.99°	ruderal	12,000	21	0	22	0.77	6.28	5.57	0.83	-0.024*
49	US, Salmon Lake, 47.12°, -113.43°	natural	25,000	22	0	21	0.82	6.84	3.64	0.82	-0.002
50	US, Butte, 46°, -112.61°	ruderal	120	21	0	20	0.79	6.6	2.91	0.74	n.e.
51	CA, Manyberries, 49.43°, -110.72°	ruderal	22	22	0	23	0.79	6.15	3.09	0.66	n.e.
52	US, Big Sandy, 48.19°, -110.11°	natural	14	19	0	20	0.76	5.38	2.29	0.69	n.e.
53	US, West Yellowstone, 44.7°, -111.1°	natural	10	10	0	19	0.62	3.95	2.36	0.23	n.e.
54	US, Kendalls´, 45.53°, -111.18°	natural	300	22	0	23	0.72	5.05	2.84	0.2	-0.005
55	US, Burke Park, 45.67°, -111.03°	ruderal	160	20	0	19	0.72	5.21	1.94	0.49	n.e.
56	US, Mammoth, 44.97°, -110.69°	natural	250	13	0	20	0.74	5.08	1.89	0.65	-0.073**

ID: population ID, 4x: tetraploid individuals identified by flow cytometry, 2x: diploid individuals

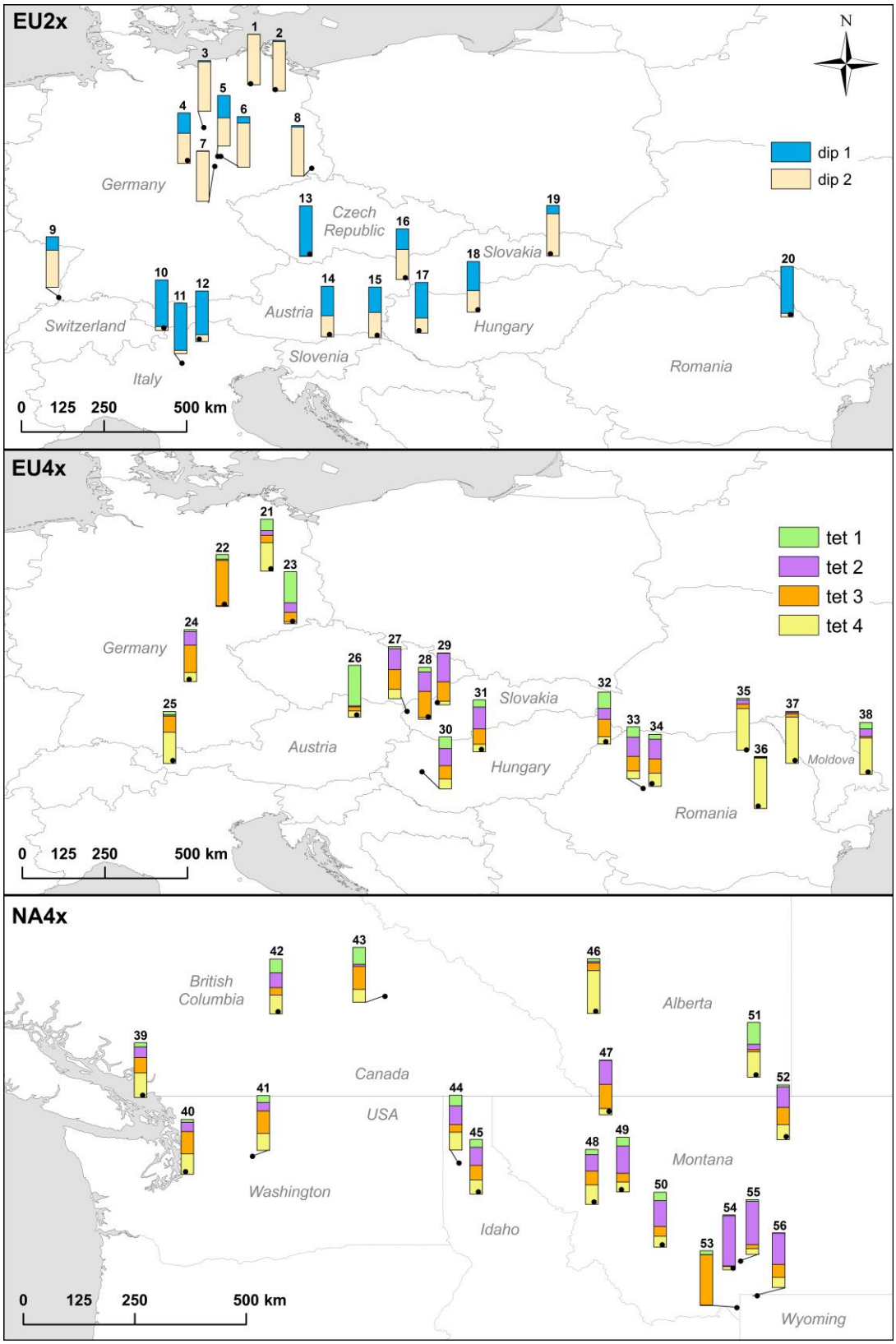


Fig. 2.1 Maps of the sampled *Centaurea stoebe* populations including barplots of the Structure results, subdivided by the geo-cytotypes (EU2x = native range, diploid; EU4x = native range, tetraploid; NA4x = invasive range, tetraploid). Two separate Structure analyses revealed two clusters for the diploid and four clusters for the tetraploid data set. The stacked barplots show proportions of populations' posterior assignment probabilities to the different genetic clusters. Population IDs are given in Table 2.1. Note that we only analyzed samples from the majority cytotype in mixed-ploidy populations (i.e. 16, 18, 23, 30 and 33).

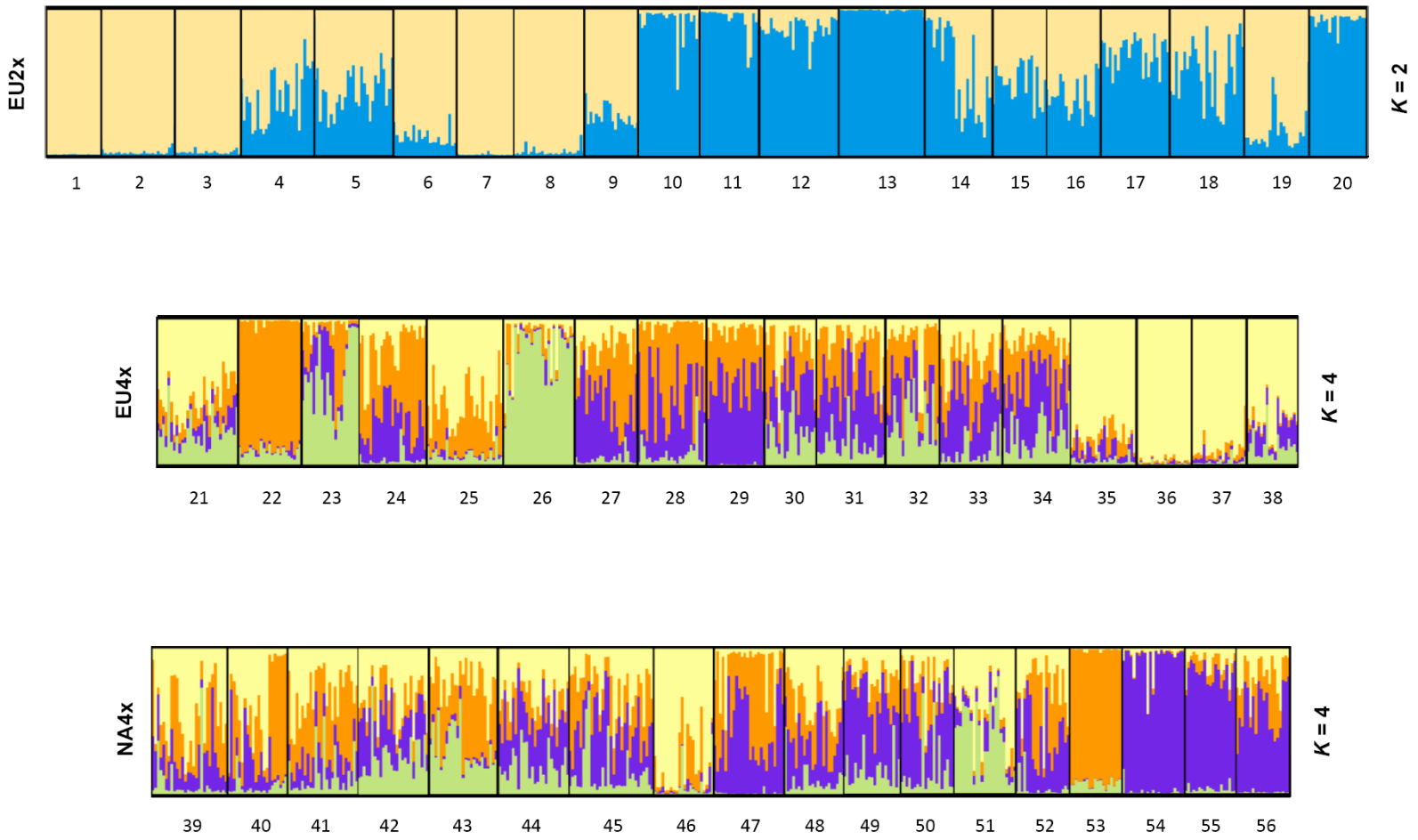


Fig. 2.2 Stacked barplots of individual posterior assignment probabilities to the clusters identified in the Structure analyses, subdivided by the geo-cytotypes (EU2x = native range, diploid; EU4x = native range, tetraploid; NA4x = invasive range, tetraploid). Two separate Structure analyses revealed two clusters for the diploid and four clusters for the tetraploid data set, respectively. Population IDs are given in Table 2.1. Note that we only analyzed samples from the majority cytotype in mixed-ploidy populations (i.e. 16, 18, 23, 30 and 33)

Table 2.2 Genetic differentiation of the populations: F_{ST}-statistics and ρ_{ST}-statistics

Group	F_{ST}	ρ_{ST}
total	0.114	0.204
4x total	0.072	0.127
EU4x	0.073	0.131
NA4x	0.069	0.122
EU2x	0.168	0.238

total: all samples, 4x total: all tetraploid samples, EU4x: native tetraploid, NA4x: invasive tetraploid, EU2x: native diploid

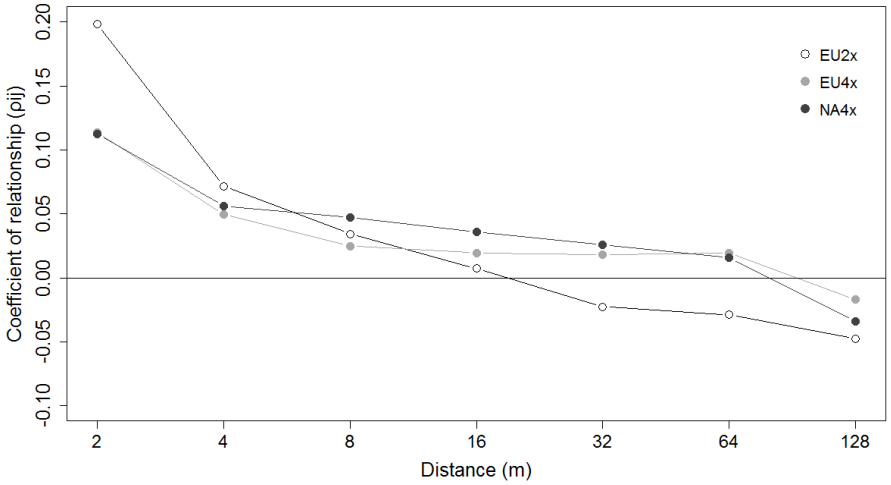


Fig. 2.3 Correlogram of averaged coefficients of relationship between individuals per distance class (log scale). Coefficients were computed for pairs of individuals within 30 populations in SPAGeDi [10 per geo-cytotype (GCT)]. Colors of dots correspond to the GCTs [white = EU2x (native range, diploid); light grey = EU4x (native range, tetraploid); dark grey = NA4x (invasive range, tetraploid); see legend]. The solid horizontal line (y = 0) represents the average relationship between individuals of the overall gene pool within GCTs under Hardy-Weinberg equilibrium. All observed coefficients of relationship per distance class were highly significant higher than y = 0 ($P < 0.001$, 1,000 permutations). Sample size for each distance within each GCT was $N > 80$. Mantel Tests revealed highly significant slopes (b_{log}) for the regression between relationship coefficients and spatial distance in all GCTs (see legend)

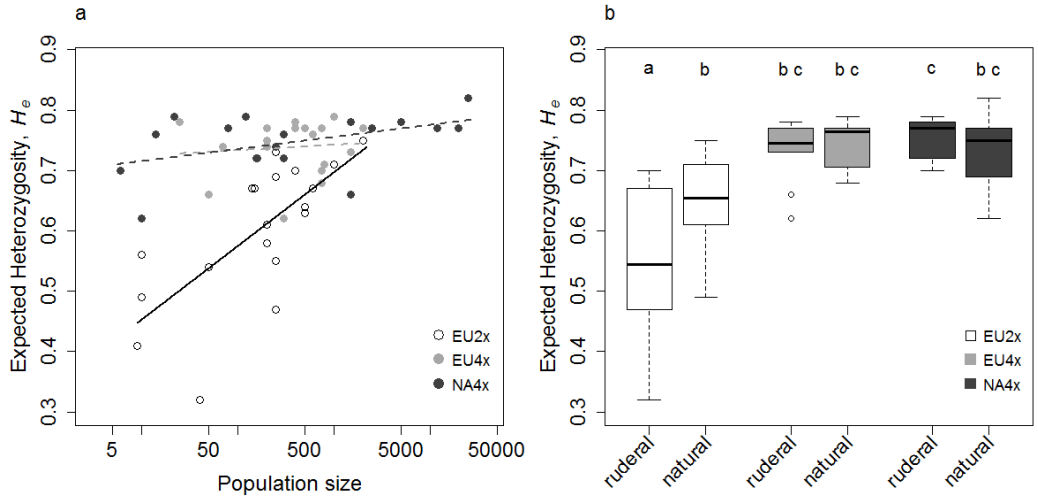


Fig. 2.4 Genetic diversity in relation to population size and habitat type. **a** Expected heterozygosity (H_e) in relation to the interaction of geo-cytotype (GCT) and population size. Lines represent predictions of the respective models as follows: solid = EU2x (significant; $F_{1,18} = 18.78$, $P < 0.001$), light grey dashed = EU4x (non-significant), dark grey dashed = NA4x (non-significant). **b** H_e in relation to the interaction of GCT and habitat. Boxplot symbols represent following statistics: bold line = median; box = interquartile range; whiskers = 1.5 times the inter-quartile range or the range of data, whichever is smaller; points = outliers. Groups a, b, and c are based on pair-wise comparisons with Tukey post-hoc tests (significance level $P < 0.05$). Colors in **a** and **b** correspond to the GCTs [white = EU2x (native range, diploid); light grey = EU4x (native range, tetraploid); dark grey = NA4x (invasive range, tetraploid); see legend]

2.5 Discussion

Consequences of polyploid formation for genetic diversity

Our results confirmed the hypothesis that, in *C. stoebe*, tetraploids sustain higher genetic diversity than diploids. Since 16 of 115 alleles were unique to the tetraploid cytotype, the high genetic diversity may have resulted from hybridization with a second divergent parental species as suggested by Mráz *et al.* (2012a). The few alleles unique to diploids (i.e. 2 of 115) suggest that polyploidization occurred on multiple occasions, which aligns with the currently prevalent opinion that the majority of polyploids originated from multiple polyploidization events (Soltis *et al.* 2014). The PCA that investigated genetic structure across the GCTs showed a strict separation of both cytotypes into two clusters, which supports previous findings of a strong reproductive isolation between diploid and tetraploid *C. stoebe* (Mráz *et al.* 2012b).

Most studies on polyploid complexes have revealed higher genetic diversity in polyploids than in diploids (e.g. Eliášová *et al.* 2013 and references therein; but see Ferriol *et al.* 2014). However, we explicitly investigated this difference with a focus on GCTs. This is particularly important regarding the correlation between genetic diversity and invasion success (Forsman 2014).

In *C. stoebe,* higher genetic diversity in tetraploids may account for broader adaptive capabilities, which may have enabled tetraploids to adapt to novel conditions in the non-native range with a remarkable climatic niche shift towards a drier and more continental climate in NA4x as

compared to EU4x (see Treier *et al.* 2009). In addition, heterozygosity masks recessive deleterious mutations, which may result in lower inbreeding depression compared to diploids under the same level of inbreeding (Eliášová *et al.* 2013). Such genetic processes may, in concert, have led to higher population growth rates of tetraploids, which Hahn *et al.* (2012a) recently recorded in a common garden study with artificial populations of the three GCTs.

The role of multiple introductions and admixture in genetic diversity of NA4x

In accordance with our hypothesis, genetic diversity between both tetraploid ranges was comparable. Previous population genetic studies on tetraploid *C. stoebe* revealed different outcomes. While Marrs *et al.* (2008) reported significantly higher expected heterozygosity for microsatellite loci in NA4x than EU4x, chloroplast haplotype diversity was found to be substantially lower (Hufbauer and Sforza 2008). In contrast to Marrs *et al.* (2008), genetic diversity of NA4x did not exceed that of EU4x, which may be related to different sampling designs. Marrs *et al.* (2008) analyzed considerably fewer samples per population in EU4x than in NA4x, and they included one mixed-ploidy population and two EU2x populations in their estimation of native genetic diversity without explicitly distinguishing between diploids and tetraploids.

Our Structure analysis supports that high genetic diversity of NA4x is a result of multiple introductions, most likely from different parts of the native range (see also Hufbauer and Sforza 2008; Marrs *et al.* 2008). As multiple introductions are common, high genetic diversity in NA4x corresponds to numerous studies on invasive plants (e.g. Kelager *et al.* 2012; Bousset *et al.* 2013). However, even if current genetic diversity is not reduced in later invasion phases, initial colonization and range expansion may still have involved bottlenecks, as contrasting demographic events (e.g. bottlenecks and admixture) may act simultaneously at different points in space and time (Keller *et al.* 2012). The considerably higher number of alleles unique to EU4x than to NA4x, along with the significantly higher *DW* of EU4x, at least suggests that rare alleles were not exhaustively carried to the exotic range. The loss of rare alleles shows evidence of bottlenecks in the past (Comps *et al.* 2001). When populations face bottlenecks, they most likely undergo strong selection towards restoring heterozygosity (Theoharides and Dukes 2007). Moreover, benefits of admixture may rule out disadvantages from introgression of maladapted genotypes in the new range, where local adaptation is rather weak compared to the native range (Verhoeven *et al.* 2011). We indeed found a substantially higher frequency of admixed individuals in NA4x (27%) than in EU4x (22%), but the difference in H_A remained non-significant due to large among-population variation. Nevertheless, comparable genetic diversity of NA4x and EU4x indicates that the ability of polyploids to maintain or restore high genetic diversity may be crucial for colonization of new ranges in *C. stoebe*.

Influences of ploidy level, life history and range dynamics on differentiation

In line with our third hypothesis, differentiation was stronger among diploid than among tetraploid populations. Due to reduced genetic drift in polyploids (Ronfort *et al.* 1998), higher F_{ST}-values of diploids are common in polyploid complexes (e.g. Eliášová *et al.* 2013 and references therein). However, in contrast to previous investigations (e.g. Hardy and Vekemans 2001; Eliášová *et al.* 2013), in our study, ρ_{ST}-statistics also revealed remarkably stronger differentiation among diploids than among tetraploids, which cannot be accounted for by mathematical principles of differences in drift (Meirmans and van Tienderen 2013). In addition, other mechanisms that influence demographic history have to be considered.

Firstly, in *C. stoebe*, tetraploids are, unlike the monocarpic diploids, characterized by increased longevity (Mráz *et al.* 2011) and a meta-analysis confirmed weaker differentiation among polycarpic populations due to trans-generational gene flow and less frequent inbreeding (Nybom 2004). Particularly, during events that lead to a short-term reduction of flowering mating partners (e.g. mowing), drift will be reduced in species that do not necessarily have to reproduce sexually every year. Instead polycarpic species may outlast such events as rosettes and can reproduce sexually after re-sprouting in the following vegetation period. Quantitative information about specific disturbance regimes (e.g. mowing frequencies) were not available for our populations, but should be included in more mechanistic investigations of this aspect in future studies.

Secondly, while tetraploids in both ranges recently expanded their range with potentially high gene flow among populations, EU2x show a more stable and scattered distribution, which may hamper gene flow (Mráz *et al.* 2014). Accordingly, our Structure analyses showed that the vast majority of tetraploid populations were assigned to mixed clusters, while most diploid populations were assigned to a distinct cluster. The distribution of clusters was differentiated along a north-south gradient in EU2x and in NA4x, which corresponds to findings of Mráz *et al.* (2014) in phenotypic trait variation that was largely explained by latitudinal clines.

Signatures of range dynamics in the differentiation within tetraploid ranges

We found a rather weak differentiation in both ranges, which, in contrast to our expectations, did not differ between NA4x and EU4x (see also Marrs *et al.* 2008). In North America, low differentiation can be explained by multiple introductions with repeated admixture events and by huge metapopulation sizes, as *C. stoebe* is highly abundant across our invasive study area (Maron *et al.* 2013). The weak differentiation in EU4x may result from a recent range expansion from its presumed ancestral region in south-eastern Europe towards central Europe (Mráz *et al.* 2014), mainly into ruderal habitats (Otisková *et al.* 2014). We indeed recorded a switch from natural sites inhabited by all our investigated populations east of the 19th longitudinal degree to ruderal habitats west of it. This corresponds to the longitudinal gradient in the cluster distribution of EU4x. Within tetraploids, we found a large among-population variation in H_A, whereby populations at the margins of the sampled distribution range tended to be less admixed. In

particular, the most obvious geographical signal of our tetraploid Structure analysis was that four south-eastern EU4x populations form a common gene pool. On the opposite, the ruderal populations in central Europe were rather admixed (to a similar extent as in NA4x). Thus, recent range expansions in both ranges may have led to similar conditions of ongoing admixture. Human-mediated long distance dispersal may facilitate such high gene flow, with railways and roads serving as the most probable dispersal corridors (Broennimann *et al.* 2014).

Small-scale genetic structure suggests biparental inbreeding and spatial cytotype segregation.
As hypothesized, tetraploids exhibited smaller relationship coefficients at short distances than diploids. Since both cytotypes have comparable pollen and achene dispersal agents (Mráz *et al.* 2012b; Hahn *et al.* 2013), and ρ_{ij}-statistics account for differences in drift between ploidy levels, this outcome may have resulted from less frequent inbreeding in tetraploids due to their polycarpic life cycle. In addition, other, mutually non-exclusive mechanisms, e.g. seed or pollen number per lifetime, community diversity or plant density, may influence gene dispersal distances (Zeng *et al.* 2011).
Nonetheless, the relatedness of individuals significantly decreased with spatial distance displaying a certain level of biparental inbreeding in all GCTs. In the polyploid complex of *Centaurea jacaea*, Hardy and Vekemans (2001) found similar results that coincide with barochory and with pollinators that normally travel short distances. They argued that these modes of gene dispersal seem to maintain mixed-ploidy populations via spatial segregation of cytotypes. Indeed, *C. stoebe* exhibits equal pollen and seed dispersal capabilities, and spatial segregation prevails in mixed-cytotype populations (Mráz *et al.* 2012b).

Contrasting influences of population size and habitat on genetic diversity between the cytotypes
In line with our hypotheses, H_e increased with population size, and natural populations revealed higher genetic diversity than ruderal populations in diploids. However, neither population size nor habitat influenced H_e in tetraploids. In the native range, this ability to buffer fluctuating population sizes may contribute to the ecological prevalence of tetraploids in ruderal habitats with high environmental stochasticity as observed by Broennimann *et al.* (2014) and Otisková *et al.* (2014). Such ruderal EU4x populations contemporarily face conditions of human-altered habitats, which may result in increased pre-adaptation to highly disturbed habitats of primary invasion (i.e. anthropogenically induced adaptation to invade theory; Hufbauer *et al.* 2012).
The considerably weaker influence of population size and disturbance regime on genetic diversity in tetraploids than in diploids may particularly result from: a) reduced drift in polyploids due to mathematical principles of inheritance, b) recent range expansion in NA4x and EU4x with high connectivity among populations, and c) increased longevity in polyploids. These processes, in concert, can decelerate genetic depletion through bottlenecks, and thus enhance the probability of tetraploid founder populations surviving and persisting until admixture restores the

gene pool. Such a scenario corresponds to the observed lag phase in *C. stoebe* (Broennimann *et al.* 2014). At the same time, diploids are prone to a higher susceptibility to genetic drift. Under small or fluctuating population sizes, this may ultimately lead to extinction of populations during colonization (Szűcs *et al.* 2014).

Besides polyploidy, asexual propagation can help to avoid loss of genetic diversity during demographic bottlenecks (Cosendai *et al.* 2013; Stein *et al.* 2014). Moreover, there are species that became invasive despite exhibiting strong reductions of genetic diversity in the non-native range (reviewed in Uller and Leimu 2011). However, for the majority of species, mechanisms that maintain high levels of genetic diversity enhance their invasiveness (Forsman 2014), and this should be particularly the case for obligate outcrossers that show no vegetative spread.

Conclusions and perspectives

It is increasingly clear that genetic bottlenecks occur far more frequently during biological invasions than suggested by rather simplistic native vs. introduced comparisons of mean genetic diversity at an advanced temporal stage of invasion (Keller *et al.* 2012). More emphasis should therefore be put on identifying mechanisms, such as polyploidy and longevity, that help founder populations to persist until gene flow and genetic admixture occur. Although several examples of cytotype shifts between native and invasive ranges have been reported (reviewed in te Beest *et al.* 2011), we are the first to explicitly show how differences in genetic diversity and drift between GCTs may relate to colonization success.

Our results highlight a) the higher initial genetic diversity of tetraploids than diploids in *C. stoebe*, and b) the ability of tetraploids to counteract genetic depletion in phases of small or fluctuating population sizes in ruderal habitats. While polycarpic tetraploid founder populations have an enhanced probability to outlast several generations of demographic disequilibrium, monocarpic diploids may be excluded from non-native ranges by lower initial genetic diversity, more frequent inbreeding and stronger drift.

Our analyses help explaining the outstanding invasion success of tetraploid *C. stoebe* on the one hand and the apparent lack of diploids in North America on the other hand. In addition, they provide important insights towards a more mechanistic understanding of the general colonization advantage of polyploids. We are, however, well aware that purely observational studies cannot unequivocally identify drivers of invasions. More colonization genetic studies on polyploid complexes are required to test any generality and limitations in our results. Moreover, identifying the second parental species involved in the origin of allotetraploid *C. stoebe* s.l. may help to understand the relative importance of hybridization in generating ecological and evolutionary change.

Acknowledgements

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Chapter 3

CONSEQUENCES OF INBREEDING IN *CENTAUREA STOEBE*

This chapter is under revision in *Journal of Ecology* as:
Rosche C, Hensen I, Mráz P, Durka W, Hartmann M & Lachmuth S (under revision): Invasion success in polyploids: the role of inbreeding in the contrasting colonization abilities of diploid vs. tetraploid populations of *Centaurea stoebe* s.l.

3.1 Abstract

As a consequence of founder effects, there are two reasons why inbreeding can hamper colonization success. First, in self-incompatible species, inbreeding may result in decreased cross-compatibility, mainly due to the sharing of identical *S*-alleles between closely related mating partners. Second, inbreeding can reduce fitness of inbred relative to outbred offspring (i.e. inbreeding depression). Polyploids often show reduced inbreeding depression compared to diploids, which may contribute to the overrepresentation of polyploids among invasive species. This is the first study that tests how the effects of inbreeding differ between geo-cytotypes (i.e. ploidy levels within a given range).

Our model organism, *Centaurea stoebe*, is strictly self-incompatible and comprises three geo-cytotypes: diploids are more frequent than tetraploids in the native range, while only tetraploids occur in the invasive range. We conducted a breeding experiment (sib-mating vs. outcrossing) with 14 native diploid, 13 native tetraploid and 15 invasive tetraploid populations. We recorded cross-compatibility and estimated a cumulative index for offspring fitness. Since frequent inbreeding can result in purging of genetic load responsible for inbreeding depression, our analyses included a metric for within-population relatedness, based on eight microsatellite markers, to assess the effect of purging.

Inbreeding was found to reduce cross-compatibility, which was similarly pronounced in diploids and tetraploids. It also caused inbreeding depression in cumulative fitness, which was significant in diploids but not in tetraploids. No evidence of purging was observed as inbred fitness was not affected by within-population relatedness.

Our results provide new insights into the contrasting invasion success of the cytotypes of *C. stoebe*. As the effects of cross-compatibility and purging were comparable between cytotypes, both processes can be ruled out to affect the colonization success of diploids vs. tetraploids. Our findings are consistent with the hypothesis that polyploidy increases the masking of recessive mutations, which maintains high fitness in inbred tetraploids and may thus facilitate colonization of new ranges. We highlight that reduced inbreeding depression may add to previously acknowledged advantages of polyploids in range expansions, a mechanism that may hitherto have been underestimated due to a lack of data on variation in inbreeding depression across geo-cytotypes.

Key words: coefficient of relationship, founder effects, genetic bottleneck, geo-cytotype, inbreeding depression, invasion ecology, purging, sporophytic self-incompatibility, *S*-alleles, spotted knapweed

3.2 Introduction

Polyploids are understood to be more likely to become invasive than diploids (Pandit *et al.* 2011), and growing emphasis is being placed on identifying the mechanisms responsible for such (te Beest *et al.* 2011; Pandit *et al.* 2014). Compared to diploids, polyploids often exhibit longer life spans, greater competitive ability and wider ecological niches (te Beest *et al.* 2011). However, empirical investigations focusing on the significance of polyploidy in buffering the negative effects of founder events are lacking, despite the accumulating evidence that negative consequences of founder effects may play a major role in invasion dynamics by considerably reducing establishment success, population growth and, ultimately, spread rates (e.g. Murren & Dudash 2012; Mullarkey *et al.* 2013; Szűcs *et al.* 2014). During initial colonization and subsequent range expansion at the leading edges (Slatkin & Excoffier 2012; Hufbauer *et al.* 2013), small and isolated founder populations may undergo frequent mating among relatives, which increases homozygosity (i.e. inbreeding). In strictly self-incompatible species, such biparental inbreeding may reduce population growth for two reasons: 1) Cross-compatibility may decrease, mainly as a consequence of the sharing of identical *S*-alleles between closely related mating partners; and 2) Offspring fitness can be reduced (i.e. inbreeding depression).

Self-incompatibility is a genetically controlled pollen-pistil cell-cell recognition system to avoid self-fertilization that is realised by different mechanisms across plant taxa (Franklin-Tong 2008). For instance, in Asteraceae, sporophytic self-incompatibility is controlled by an *S*-locus consisting of a pistil-expressed and a pollen-expressed *S*-gene, which together form a non-recombining *S*-haplotype (i.e. the *S*-allele; Brennan *et al.* 2013). In addition to inhibiting of self-pollination, crosses between mating partners are prevented when partners share identical *S*-alleles. Mating among close relatives increases the risk of *S*-alleles being shared, as such, inbreeding and / or a stochastic loss of *S*-alleles in bottlenecked populations may reduce cross-compatibility among mating partners (Brennan *et al.* 2006; Wagenius *et al.* 2007). However, strong negative frequency-dependent selection favours high *S*-allele diversity, and dominance interactions can conceal recessive *S*-alleles in systems with sporophytic self-incompatibility (Busch *et al.* 2014). Both phenomena increase cross-compatibility among mating partners (Brennan *et al.* 2013), which facilitates reproduction in small populations but also allows biparental inbreeding, which can lead to inbreeding depression.

Inbreeding depression, i.e. the reduction of fitness in inbreds compared to outbreds, is predominantly based upon the homozygous expression of recessive deleterious alleles (reviewed in Charlesworth & Willis 2009). The genome-wide portion of deleterious recessive alleles is defined as the genetic load. Dominant alleles are usually non-deleterious and mask the genetic load (Hedrick *et al.* 2016). Due to their multiplied chromosome sets and consequently higher number of alleles per locus, polyploids may mask recessive deleterious mutations more efficiently than diploids resulting in lower inbreeding depression (reviewed in Soltis & Soltis

2000). However, when approximating the mutation-selection equilibrium over a longer time period, a more efficient masking of recessive deleterious mutations in polyploids can lead to a greater accumulation of deleterious alleles in polyploid than in diploid genomes (Ronfort *et al.* 1998). In accordance, among the few studies addressing inbreeding in polyploid complexes, some have confirmed lower levels of inbreeding depression in polyploids (e.g. Eliášová *et al.* 2013), while others found comparable inbreeding depression between diploids and polyploids (e.g. Galloway & Etterson 2007).

In addition to ploidy level, the demographic history of populations can affect the present-day degree of inbreeding depression. Population bottlenecks induce genetic drift and inbreeding, both of which can augment within-population relatedness and, at the same time, can reduce inbreeding depression. In particular, successive generations of inbreeding can expose deleterious mutations to selection in recessive homozygotes, which can lead to their selective removal (i.e. purging; Crnokrak and Barrett 2002). Purging in turn reduces genetic load in the gene pools of populations and increases fitness of inbreds. More specifically, during colonization processes that involve series of demographic bottlenecks, purging may successively diminish inbreeding depression (Pujol *et al.* 2009). As such, demographic disequilibria in the course of biological invasions may result in lower inbreeding depression in invasive than native populations (Facon *et al.* 2011).

For the present study, we investigated the consequences of inbreeding on cross-compatibility and fitness in spotted knapweed (*Centaurea stoebe* s.l. L.; Asteraceae; syn. *C. maculosa* Lam.), a strictly self-incompatible polyploid complex consisting of the diploid *C. stoebe* ssp. *stoebe* and the tetraploid *C. stoebe* ssp. *micranthos* (Mráz *et al.* 2011). A fundamental cytotype shift between its invasive and native range makes *C. stoebe* an excellent model for studying the relevance of polyploidy in colonization success (te Beest *et al.* 2011): whereas diploids represent the majority cytotype in the native Eurasian range (Broennimann *et al.* 2014), exclusively tetraploids became established in North America (Mráz *et al.* 2011). Thus, three geo-cytotypes (GCTs) can be defined by ploidy level and range: native diploids (EU2x), native tetraploids (EU4x) and invasive tetraploids (NA4x). We performed inbred and outbred crosses in 42 *C. stoebe* populations varying in range, cytotype and degree of natural inbreeding (i.e. within-population relatedness). We then assessed the cross-compatibility of inbred vs. outbred crosses and recorded inbreeding depression in offspring fitness.

The consequences of inbreeding on cross-compatibility or offspring fitness have, to our knowledge, never been investigated in a model system comprising distinct GCTs. This first study in that context was driven by the following hypotheses:

(1) Cross-compatibility is reduced in inbred crosses due to the sharing of *S*-alleles between mating partners; (2) Outcrossed progenies outperform inbred progenies, which represents inbreeding depression; (2a) Inbreeding depression decreases with increasing degree of natural inbreeding (i.e. within-population relatedness) due to purging; (2b) Inbreeding depression is

weaker in NA4x than in EU4x as a result of purging during the colonization of the invaded range; and (2c) Inbreeding depression is lower in tetraploids than in diploids due to the higher probability of deleterious recessive mutations being masked in tetraploids.
It is anticipated that results will shed light on the potential significance of inbreeding in the cytotype shift in *C. stoebe* and provide new empirical insights into the role of polyploidy in colonization.

3.3 Materials and Methods

The study system *Centaurea stoebe* s.l.
The two cytotypes of *C. stoebe* are reproductively isolated due to the strongly reduced viability of progeny of interploidy crosses because of unbalanced ratio between male and female genomes in the endosperm (i.e. triploid block; Marks 1966), and the infertility of extremely rare triploid hybrids (Mráz *et al.* 2012a). The tetraploid cytotype is considered to be a very young neo-polyploid that originated from the hybridization between the diploid cytotype and an as yet unknown but closely related parental taxon (Mráz *et al.* 2012b). Although allopolyploidy may lead to the independent segregations of the two parental genomes (i.e. disomic inheritance; Barcaccia *et al.* 2014), tetraploid *C. stoebe* shows tetrasomic inheritance of microsatellite alleles (Rosche *et al.* 2016).
Diploids and tetraploids are similar in their morphology (Mráz *et al.* 2011) and have comparable ecological amplitudes: both occupy dry, (semi)-natural (e.g. rocks, steppe slopes, dry grasslands) and ruderal habitats (Ochsmann 2000). The most important difference is that tetraploids are polycarpic, whereas diploids are predominantly monocarpic (Mráz *et al.* 2011). Both cytotypes are strictly self-incompatible; they are mainly pollinated by Hymenoptera (Mráz *et al.* 2012a) and disperse their achenes via barochory (Hahn *et al.* 2013). These gene dispersal agents generate moderate levels of natural inbreeding within populations in all three GCTs (Rosche *et al.* 2016).
The protandric florets of *C. stoebe* open successively towards the capitulum centre. Pollen is available for 1-4 days in each capitulum. Each floret exposes pollen for about 24 h, afterwards, the stigma becomes receptive for about 12-36 h (personal observations).
Tetraploids were introduced to North America in the late 19th century. Following a lag-phase of 50 years (Broennimann *et al.* 2014), tetraploid *C. stoebe* has become one of the most noxious weeds in North America (Maron *et al.* 2013) and causes enormous economic damage (Corn *et al.* 2006).

Sampling

Covering large parts of the ranges of all GCTs, we sampled 14 populations of EU2x, 13 of EU4x and 15 of NA4x (Fig. 3.1; Table A3.1 in Appendix).

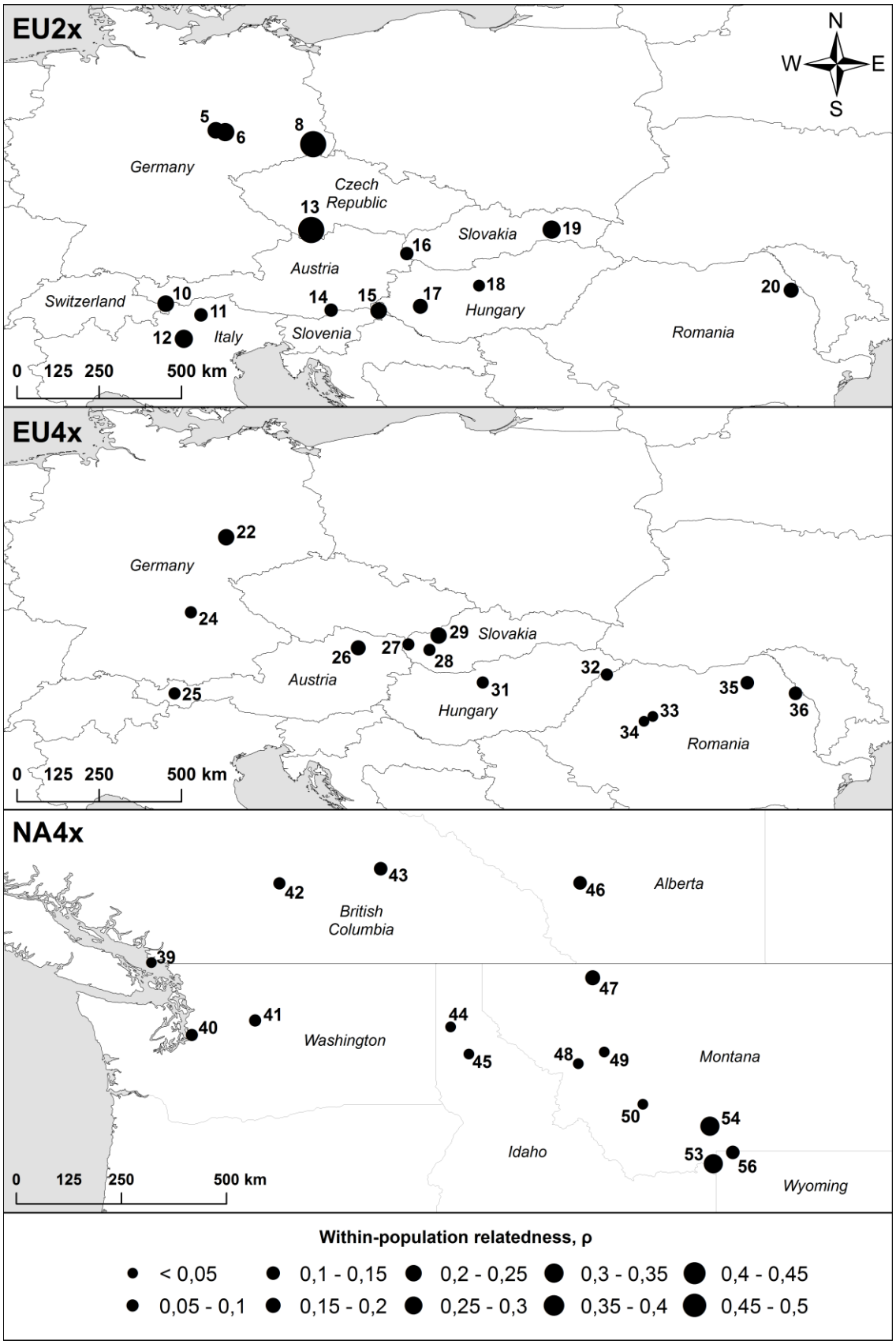

Fig. 3.1 Distribution of the *Centaurea stoebe* populations involved in the crossing experiments. The figure is divided into three maps, each of which corresponds to a geo-cytotype (EU2x, native diploid; EU4x, native tetraploid; NA4x, invasive tetraploid). The size of the dots refers to the within-population relatedness inferred from eight microsatellite markers. Further population characteristics can be found in Table A3.1.

We collected matured capitula from four plants per population (i.e. four seed families). To reduce the probability of sampling close relatives, seed families were chosen in such a way so as to maximise the spatial distance between them. Sufficient replication at the population level with broad environmental gradients among populations is required to representatively assess inbreeding depression at the GCT level, because inbreeding depression may vary substantially across populations depending on the history of natural inbreeding and prevailing environmental conditions (Leimu *et al.* 2008). Thus, we aimed at increasing the number of populations rather than seed families per population. Data on the ploidy level of the seed families (based on flow-cytometry) and population genetic data (based on eight microsatellites) were available from Rosche *et al.* (2016). Within each GCT, we calculated pair-wise coefficients of relationship between all individuals (ρ_{ij}) using SPAGeDi 1.4 (Hardy and Vekemans 2002). We then averaged ρ within populations to establish an estimate of within-population relatedness with a view to describing the history of genetic drift and natural inbreeding within each population (see Table A3.1 for ρ of each study population). The ρ-statistics approach was used as it provides estimates that are the most comparable across different ploidy levels (Dufresne *et al.* 2014).

Breeding experiment

In October 2012, ten achenes per seed family were germinated on water-filled Petri dishes. Emerged seedlings were placed on planting trays and grown in the greenhouse (25/15 °C day/night with a 16 h photoperiod). After six months, plants were transferred to the Botanical Garden in Halle (51.49°N; 11.96°E) where they received ample water supply and phosphorous fertilization in order to promote flowering (Kamasol Brilliant Rot, Compo Expert, applied according to manufacturer's instructions).

To avoid adverse environmental conditions, crosses were conducted in the greenhouse. Three individuals per seed family were used in hand-pollination breeding treatments with two levels: bi-parental inbreeding via sib-mating (at least half-sib) vs. within-population outbreeding. Each plant of the parental generation was crossed with two plants from the same seed family and two from another seed family. Thus, four crosses per individual were conducted resulting in 12 inbred and 12 outbred lineages per population (Fig. A3.1). Where necessary, the breeding design was adjusted according to the availability of flowering plants per seed family (see Table A3.2). To prevent cross-pollination by insects, capitula were covered with mesh bags before anthesis and remained bagged until achenes had ripened. Crosses were conducted reciprocally by rubbing the respective capitula together with each plant serving as a pollen donor (paternal) and as a pollen acceptor (maternal). Each cross was initiated when pollen developed on both mating capitula and was carried out daily until stigmas were no longer receptive. Where necessary, other available pollen donor capitula from the same donor plant were used to ensure exhaustive pollination. A total of 1,780 crosses were achieved. Thirty capitula from 30 separate individuals

kept in closed mesh bags were checked for autogamous selfing. None of them set any fruit, indicating strict self-incompatibility in *C. stoebe*.

Survey of cross-compatibility

In October 2013, we recorded cross-compatibility among individuals. Partners were considered cross-compatible when at least one floret produced a fertile achene (i.e. darkish and full-sized). It is noted that in addition to sharing *S*-alleles, cross-compatibility may also be influenced by inbreeding depression, which may reduce the number of fertile achenes during early seed development (Harder *et al.* 2012). A total absence of fertile achenes is, nonetheless, rather unlikely to be attributed to inbreeding depression, because each achene exhibits a different recombination of the involved parental genomes and not all combinations should result in the abortion of a fertilized ovule due to inbreeding depression. Therefore, our results for cross-compatibility may be attributed to the effects of self-incompatibility.

Survey of fitness components

For the survey of fitness components, where available, we chose six inbred and six outbred lineages per population (482 lineages; 76 EU2x inbred, 80 EU4x inbred, 85 NA4x inbred, 81 EU2x outbred, 80 EU4x outbred, 87 NA4x outbred; Table A3.2). For each population, we chose lineages that represented each of the seed families as equally as possible. The seeds from the lineages were then used for a germination experiment. Where available, ten achenes per lineage were germinated in water-filled Petri dishes placed in germination chambers (20°C/10°C with a 12 h photoperiod). For logistical reasons, we split the germination experiment into three separate runs: germination of the first cohort was initiated on October 20th, the second on October 27th and the third on November 11th. GCTs and breeding treatments were distributed equally across cohorts (Table A3.2). We recorded the total number of seeds that germinated within 12 days (i.e. germination success).

The seedlings of 430 lineages belonging to the first two germination cohorts were pricked out on planting trays immediately upon germination and moved to the greenhouse (25°C/15°C with a 12 h photoperiod). Five planted seedlings from each lineage were then randomly chosen for the subsequent assessment of fitness components, with the remaining seedlings serving as backups and grown under the same conditions. Within the first five days, all dead individuals were replaced with randomly chosen backup seedlings of the same lineage (where available). Juveniles were then re-potted into 0.8 L pots after three weeks and into 2.2 L pots after nine weeks.

In April 2014, the pots were transferred to the experimental site in the Botanical Garden in Halle. To simulate ruderal habitats typical of initial colonization stages (i.e. less competition, high radiation, drought exposure, low soil depth; Davis *et al.* 2000), we excluded competitors every three weeks, exposed the plants to full sunlight, watered them only in times of extreme drought

and used thick plastic planes to avoid attachment of roots with ground soil. In August 2014, we recorded whether plants had survived and whether they had flowered, in which case we counted the number of capitula per plant (including flowering capitula, matured capitula and buds >3 mm according to Mráz *et al.* 2011). Throughout the experiment, the Petri dish and pot positions were frequently randomised. In accordance with Oakley & Winn (2012), we calculated a composite index of fitness over one vegetation period, i.e. cumulative fitness as a product of germination success, survival, flowering probability and number of capitula.

Statistical analyses

Statistical analyses were performed using the *lme4*-package (Bates *et al.* 2014) implemented in R 3.2.3 (R development core team 2015). For responses with Gaussian error distribution, we used linear mixed-effects models. Decisions on the transformation of variables followed graphical assessment of variance homogeneity and normality of errors (Crawley 2015). For responses with binomial or Poisson error distribution, we used generalised linear mixed-effects models. All models were fitted with a maximum likelihood approach. In order to identify the minimal adequate models, we removed non-significant fixed effects in a stepwise backward manner based on χ^2-tests.

We analysed the following response variables (see Table 3.1): cross-compatibility (binomial) and the fitness components of the offspring (germination success (binomial), survival (binomial), flowering probability (binomial), $\log_e$ number of capitula (Gaussian), square-root cumulative fitness (Gaussian)). The models for all responses included an interaction of breeding treatment and GCT, an interaction of breeding treatment and $\log_e$ ρ (centred and scaled) and the respective main effects. In addition, latitude (centred and scaled) was included to account for the fact that *C. stoebe* shows adaptive differentiation along latitudinal environmental clines (Mráz *et al.* 2014). Germination cohort and the nested factors of pollen donor individual within donor seed family within donor population as well as of pollen acceptor individual within acceptor seed family within acceptor population were set as random effects.

3.4 Results

Inbreeding and cross-compatibility

Cross-compatibility, expressed as a probability to produce at least one well-developed achene per capitulum after crosses, was significantly influenced by the interaction of within-population relatedness (ρ) and breeding treatment ($\chi^2_{(1)} = 4.77$, $P < 0.05$, Table 3.1): overall, cross-compatibility was predominantly higher in outbred than inbred crosses, and it decreased with increasing ρ, with the decline being more pronounced in outbred than in inbred crosses (Fig. 3.2). Moreover, cross-compatibility differed significantly between GCTs ($\chi^2_{(1)} = 6.46$, $P < 0.05$). The cross-compatibility of EU2x significantly exceeded that of NA4x, while the other GCT

combinations did not show any differences. The means of cross-compatibility over breeding treatment x GCT are given in Table A3.3.

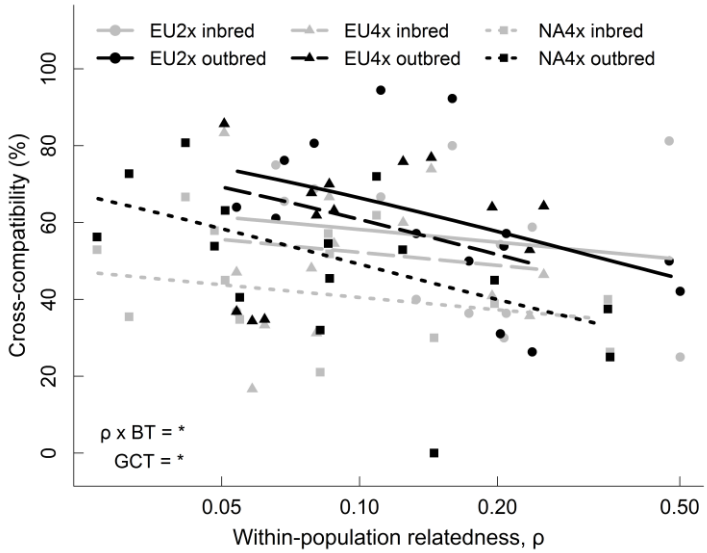

Fig. 3.2 Cross-compatibility as a function of geo-cytotype and of breeding treatment in interaction with within-population relatedness (ρ). Observation points represent means over populations and breeding treatments, and lines represent predictions of the minimal adequate model. EU2x, native diploid; EU4x, native tetraploid; NA4x, invasive tetraploid; ρ x BT, interaction of within-population relatedness and breeding treatment; GCT, geo-cytotype; significance level: *, P < 0.05.

Inbreeding and fitness components

All fitness components along with the cumulative fitness were lower in inbred than in outbred progeny (Fig. A3.2). The strongest inbreeding depression among the fitness components was found for the number of capitula. The factors that influenced the degree of inbreeding depression differed among the fitness components considered (Table 3.1).

Flowering probability was the only fitness component that was exclusively affected by main effects and not by any of the interactions. Flowering probability was higher in outbreds than in inbreds ($\chi^2_{(1)}$ = 4.5, P < 0.05) and it differed among GCTs ($\chi^2_{(1)}$ = 31.18, P < 0.001): both tetraploid GCTs flowered more frequently than diploids, while there were no differences between tetraploid GCTs.

The interaction of breeding treatment with ρ significantly influenced the number of capitula ($\chi^2_{(1)}$ = 5.25, P < 0.05) and the cumulative fitness ($\chi^2_{(1)}$ = 4.82, P < 0.05). The number of capitula was slightly higher in outbreds than in inbreds (Fig. 3.3a). While for outbreds the number of capitula decreased significantly with increasing ρ, inbreds were almost unaffected by ρ. Cumulative fitness was predominantly lower in inbred than outbred progeny, which corresponds to inbreeding depression (Fig. 3.3b). The magnitude of inbreeding depression became smaller with increasing ρ, but such decreasing difference between inbred and outbred fitness could not be ascribed to purging, because the fitness of inbred progeny did not increase with increasing ρ.

Table 3.1. Analyses testing for the interactive effects of breeding treatment and geo-cytotype and the interactive effects of breeding treatment and within-population relatedness on cross-compatibility and fitness components of *Centaurea stoebe*. The table gives parameter estimates from the respective minimal adequate mixed-effects models for each fixed effect (with the second level of factors subscripted). Parameter estimates of significant fixed effect terms are bold. Parameter estimates of main effects involved in significant interaction are provided, but their significance has not been tested (Crawley 2014). Where geo-cytotype or its interactions were significant, the significance level does not correspond to the particular pair-wise comparison, but to the overall fixed effect. Note that $\log_e$ ρ and latitude were scaled and centred in the models. Variance estimates are given for random effects, and the number of groups at each random effect level is given in parentheses.

Fixed effects	cross-compatibility	germination success	survival	flowering probability	$\log_e$ no. capitula	sqrt cumulative fitness
(Intercept)	0.331	2.134	-0.432	0.48	2.1	1.102
Latitude	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
$\log_e$ ρ	-0.133 [a]	n.s.	n.s.	n.s.	-0.003 [a]	0.006 [a]
BT $_{Outbred}$	0.35 [a]	2.1 [a]	0.481 [a]	**0.356 ***	0.079 [a]	0.868 [a]
GCT $_{EU4x}$	**-0.243** *	0.207 [a]	0.488 [a]	**1.457 *****	n.s.	0.647 [a]
GCT $_{NA4x}$	**-0.716** *	0.534 [a]	0.52 [a]	**1.465 *****	n.s.	0.675 [a]
BT $_{Outbred}$: $\log_e$ ρ	**-0.233** *	n.s.	n.s.	n.s.	**-0.12 ***	**-0.209 ***
GCT $_{NA4x}$: BT $_{Outbred}$	n.s.	**-1.389 *****	**-0.516 ***	n.s.	n.s.	**-0.739 ****
GCT $_{EU4x}$: BT $_{Outbred}$	n.s.	**-0.631*****	**-0.481 ***	n.s.	n.s.	**-0.594 ****
Random effect variances						
Acceptor: Population	0.15 (42)	0.00 (42)	0.00 (37)	0.03 (37)	0.00 (37)	0.00 (37)
Acceptor: Population/SF	0.22 (146)	0.58 (136)	0.03 (120)	0.00 (120)	0.02 (120)	0.00 (120)
Acceptor: Population/SF/Ind	0.19 (476)	0.00 (312)	0.00 (278)	0.00 (267)	0.00 (254)	0.00 (279)
Donor: Population	0.11 (42)	0.00 (42)	0.00 (37)	0.09 (37)	0.00 (37)	0.00 (37)
Donor: Population/SF	0.05 (146)	0.14 (137)	0.00 (120)	0.05 (119)	0.00 (118)	0.02 (120)
Donor: Population/SF/Ind	0.11 (472)	0.74 (316)	0.00 (281)	0.00 (270)	0.00 (257)	0.00 (281)
Germination cohort	n.t.	0.00 (3)	0.00 (2)	0.00 (2)	0.00 (2)	0.00 (2)
Residuals	n.e. (1787)	n.e. (481)	n.e. (1927)	n.e. (971)	0.5 (793)	0.83 (432)

GCT, geo-cytotype; NA4x, invasive tetraploid; EU4x, native tetraploid; BT, breeding treatment; SF, seed family; Ind, individual; sqrt, square root; ρ, within-population relatedness; n.s., not significant; n.e., not estimated; n.t., not tested; a, main effect in significant interaction; significance levels: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

The interaction of GCT and breeding treatment significantly influenced germination success ($\chi^2_{(1)} = 11.41$, $P < 0.01$), survival ($\chi^2_{(1)} = 6.04$, $P < 0.05$) and cumulative fitness ($\chi^2_{(1)} = 11.54$, $P < 0.01$). The germination success of outbreds did not markedly differ among GCTs. In EU2x and in EU4x, the germination success of outbreds was significantly higher than that of inbreds, but there was no such difference in NA4x (Fig. 3.4a). Survival was similar across GCTs. While outbreds showed significantly higher survival than inbreds in diploids, both tetraploid GCTs did not reveal this pattern (Fig. 3.4b). Outbred cumulative fitness did not differ between GCTs. In contrast, inbred fitness differed among GCTs, with both tetraploid GCTs outperforming diploids. In particular, for diploids, inbred fitness was significantly lower than outbred fitness, which was not the case for both tetraploid GCTs (Fig. 3.4c). Mean values for the cumulative fitness and each fitness component divided by breeding treatment x GCT are given in Table A3.3.

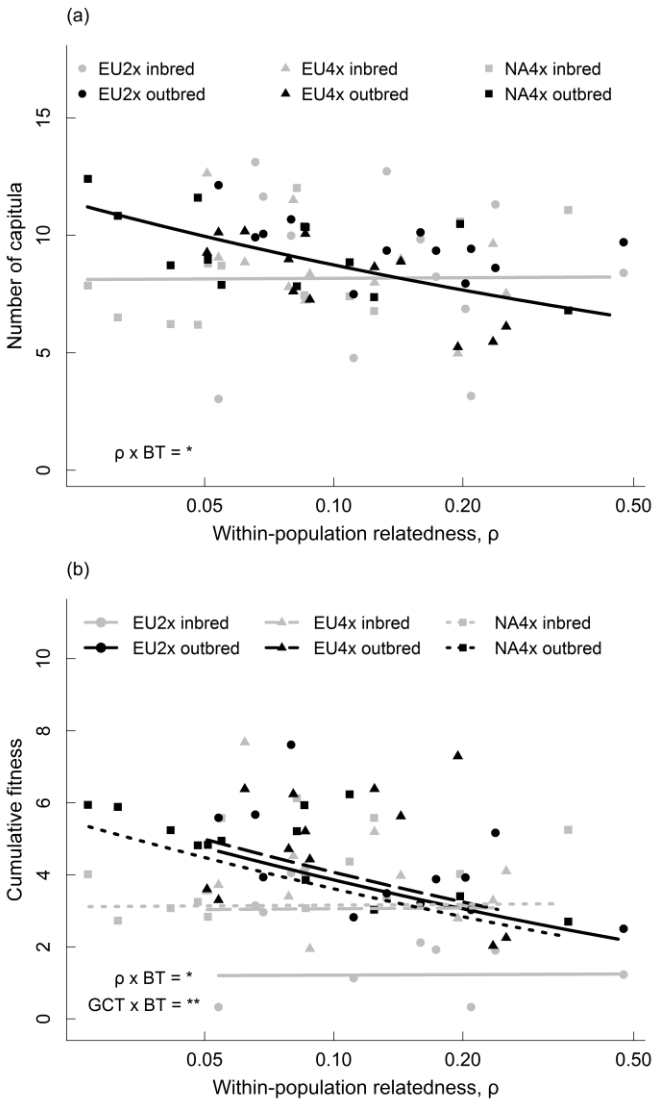

Fig. 3.3 Interactive effects of within-population relatedness and breeding treatment on (a) number of capitula and (b) cumulative fitness. Observation points represent means over populations and breeding treatments and lines represent predictions of the minimal adequate model. Note that in (a), lines reflect the predictions of the minimal adequate model for inbreds (grey) and outbreds (black), while in (b), lines reflect the predictions of the minimal adequate model for inbreds and outbreds within each geo-cytotype (see legend). EU2x, native diploid; EU4x, native tetraploid; NA4x, invasive tetraploid; ρ x BT, interaction of within-population relatedness and breeding treatment; GCT x BT, interaction of geo-cytotype and breeding treatment; significance levels: *, $P < 0.05$; **, $P < 0.01$.

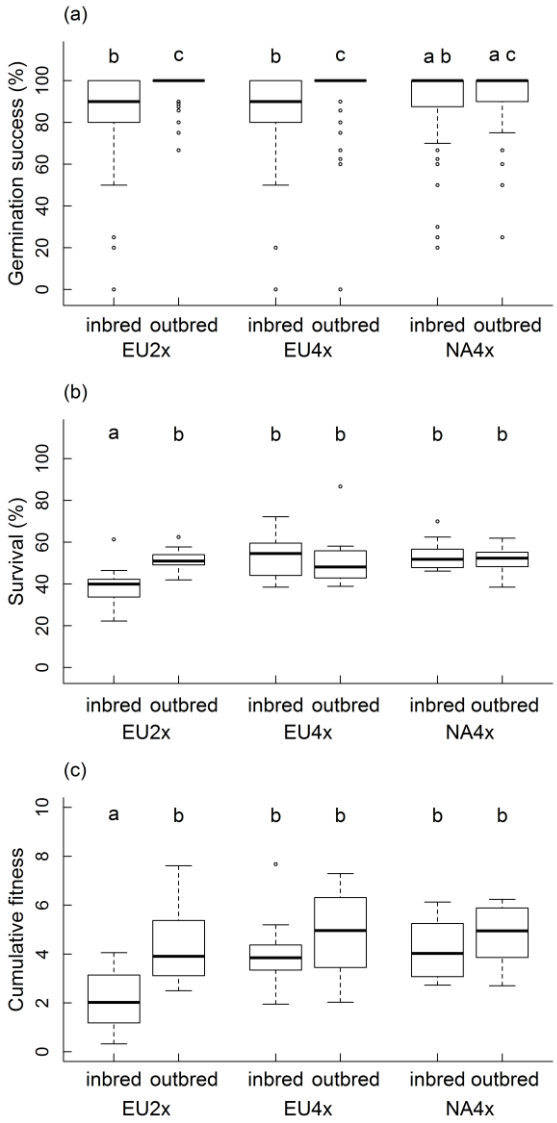

Fig. 3.4 Interactive effects of geo-cytotype and breeding treatment on (a) germination success, (b) survival and (c) cumulative fitness. Groupings (lower case letters) are based on Tukey post-hoc tests (pair-wise comparisons with a significance level of $P < 0.05$). EU2x, native diploid; EU4x, native tetraploid; NA4x, invasive tetraploid.

3.5 Discussion

In accordance with our main hypotheses, we found that inbreeding reduces (1) cross-compatibility and (2) offspring fitness (i.e. inbreeding depression) in *C. stoebe*. In contrast to that set-out in our hypotheses (2a) and (2b), the strength of inbreeding depression was neither influenced by purging nor range, respectively. In accordance with our last hypothesis (2c), inbreeding depression was more pronounced in diploids than in tetraploids.

Impact of inbreeding on cross-compatibility

Experimental inbreeding (breeding treatment) as well as increasing natural inbreeding (within-population relatedness) reduced cross-compatibility. The latter effect was significantly more pronounced in outbreds than in inbreds, because with increasing relatedness among mating partners, outcrossing becomes more similar to sib-mating (Angeloni *et al.* 2011). In particular, for the *C. stoebe* populations that exhibited the highest within-population relatedness, we found nearly identical cross-compatibility in inbred as compared to outbred crosses. Previous studies confirmed that small populations with closely related individuals show reduced cross-compatibility (Willi *et al.* 2005; Young and Pickup 2010), and that reduced *S*-allele pools can considerably decrease colonization success (Wagenius *et al.* 2007).

Cross-compatibility did not differ between EU2x and EU4x and the response of cross-compatibility to breeding treatment did not differ between GCTs. So far, exclusively Pickup & Young (2007) investigated self-incompatibility within a polyploid complex in Asteraceae (*Rutidosis leptorrhynchoides*) and they also found comparable cross-compatibility in different cytotypes. Our result appears to contradict the fact that polyploids have multiplied numbers of *S*-alleles per individual compared to diploids, which might lead to a higher probability of matching *S*-alleles between mating partners. However, this assumption will only be valid if *S*-alleles act co-dominantly, whereas dominance interactions can dramatically change the consequences of *S*-alleles on cross-compatibility (Busch *et al.* 2014), which restricts any conclusions being drawn on potential differences in cross-compatibility between the cytotypes. Extensive dominance interactions are frequent in Asteraceae (Brennan *et al.* 2013), which may, in concert with the higher *S*-allele diversity in polyploids than diploids, counteract the disadvantage of more *S*-alleles in polyploids (Pickup & Young 2007). Both high *S*-allele diversity and dominance may further foster the overall moderate cross-compatibility, even in populations with high within-population relatedness and following inbred crosses (Busch *et al.* 2014). During founder events, this has positive demographic effects due to increased reproduction, but it may also have negative consequences with respect to the occurrence of inbreeding depression.

Impact of inbreeding on offspring fitness

Inbreeding reduced fitness, with inbreeding depression being largely consistent across fitness components. This aligns with theoretical predictions (Charlesworth & Willis 2009) and the vast majority of studies on inbreeding effects on fitness (reviewed in Angeloni *et al.* 2011). However, the effects of cytotype, range and within-population relatedness on inbreeding depression differed considerably among traits. Since composite fitness indices are more substantial than single stage fitness proxies (Oakley & Winn 2012), the following focuses on inbreeding depression in cumulative fitness.

Differences in cumulative fitness between inbreds and outbreds decreased significantly with increasing within-population relatedness. However, as opposed to being positively related to within-population relatedness, inbred fitness remained constant across the range of within-population relatedness. This finding refutes the hypothesis that purging represents the mechanism that accounts for the decreasing inbreeding depression. Several studies suggested that substantial genome-wide effects of purging occur only under specific conditions, such as intermediate and constant bottlenecks over several generations (e.g. Keller *et al.* 2012b; Kennedy *et al.* 2014). As such, the observed reduction in inbreeding depression in our study may have resulted from random genetic drift, which may exclude rare, strongly deleterious alleles and may fix mildly deleterious alleles (i.e. drift load, which reduces the fitness of outbreds; Leimu *et al.* 2006; Willi *et al.* 2013). Thus, in both types of alleles, inbreeding results in the same phenotypic effect as compared to random mating (Oakley & Winn 2012). In addition, drift increases relatedness among mating partners. The higher the within-population relatedness, the more similar random mating (i.e. outcrossing) to mating among relatives (i.e. inbreeding) becomes. Therefore, outcrossing can result in similar homozygosity as sib-mating (Angeloni *et al.* 2011), which aligns with the comparable cumulative fitness observed between outbreds and inbreds in aforementioned populations of high within-population relatedness. The number of capitula was the only fitness component that contributed to the relationship between inbreeding depression and within-population relatedness. Since selection against mutations is weakest in late life-stages (Angeloni *et al.* 2011), drift load may have especially accumulated in genes that are related to the number of capitula.

Furthermore, inbreeding depression in cumulative fitness was not affected by range. In fact, we expected lower inbreeding depression due to frequent purging in NA4x, if invasive populations had experienced severe colonization bottlenecks in their invasion history, as shown e.g. for *Harmonia axyridis* (Facon *et al.* 2011). However, we found purging to be rather inefficient in *C. stoebe* (see above). Moreover, recent microsatellite data suggests that colonization bottlenecks in NA4x were counteracted by multiple introductions (Rosche *et al.* 2016). Thus, frequent population admixture apparently restored genetic diversity, obviously including genetic load. In addition, Broennimann *et al.* (2014) demonstrated that parts of the “native” range (i.e. Central Europe) were colonized mainly within the last century along ruderal sites, a situation that

corresponds to the colonization history of NA4x, which may have led to partly comparable population histories in both ranges. Nevertheless, the reduction in germination success was significantly smaller in NA4x as compared to EU4x. This may result from range-specific purging of genetic load, which may be amplified to reflect a strong r-selection in favour of fast and successful recruitment (Lachmuth *et al.* 2011).

In accordance with our last hypothesis, inbreeding depression in cumulative fitness was significant in diploids but not in tetraploids, with survival having contributed most to this effect. Increased homozygosity of deleterious recessive alleles may have resulted in increasing mortality in the inbred diploids, whereas the whole genome duplication obviously increased the masking of genetic load in the polyploids. Most, but not all, of the previous studies that have tested variation in inbreeding depression between cytotypes are concordant with our results (reviewed in Soltis & Soltis 2000). According to Galloway & Etterson (2007), two processes in polyploid speciation may lead to inconsistent results among inbreeding studies in polyploid complexes. First, under perfect tetrasomic inheritance, the probability of becoming homozygote is reduced by 50% in tetraploids compared to diploids (Bever & Felber 1992), whereas disomic inheritance of two rather independent genome parts would not lead to such effective masking of recessive deleterious alleles (Barringer and Geber 2008). Second, with increasing evolutionary age, polyploids accumulate detrimental alleles (Ronfort *et al.* 1998). The large reductions of inbreeding depression in tetraploid *C. stoebe* may have therefore resulted from both its tetrasomic inheritance (Rosche *et al.* 2016) and its neopolyploid origin (Mráz *et al.* 2011).

Potential consequences of polyploidy in colonization capacity

Despite growing awareness of the significance of both polyploidy and inbreeding in biological invasions, this is the first study to address the consequences of mating among relatives in a GCT system. Our results clearly demonstrate that inbreeding reduces cross-compatibility and fitness in *C. stoebe*, which may partly explain the 50 years of lag-phase during the species´ invasion of North America (Broennimann *et al.* 2014). Subsequently, the reported population admixture in the invasion history of NA4x (Rosche *et al.* 2016) may have led to genetic rescue of genetically depleted populations (Frankham 2016).

The effects of inbreeding on cross-compatibility did not differ between cytotypes. However, the polycarpic life-cycle may enable tetraploids to outlast flowering seasons in the absence of compatible mating partners. In subsequent flowering seasons, polycarpic tetraploids may, in contrast to predominantly monocarpic diploids (Mráz *et al.* 2011), reproduce successfully when immigrating diaspores enlarge the *S*-allele pool. This may, in concert with the higher first year flowering probability (our results; Mráz *et al.* 2011) and higher life-time seed output of tetraploids vs. diploids (Broz *et al.* 2009), have enhanced the colonization success of tetraploid *C. stoebe* populations.

Most importantly, our results show that polyploidy significantly counteracts inbreeding depression, which in turn provides a strong indication of a reduction of the negative demographic consequences of inbreeding in tetraploid founder populations. Thus, tetraploid founder populations may have been more likely to outlast critical early invasion phases until the influx of new genetic material allowed the sudden spread of the species across North America. Diploids, in contrast, face substantial inbreeding depression, and they cannot easily endure seasons with *S*-allele-mediated restrictions of mate availability. During phases of demographic disequilibrium, such disadvantages may ultimately exclude small diploid founder populations from a novel range, even before their gene pools can be restored via multiple introductions. Thus, reduced inbreeding depression in tetraploids helps explain the drastic cytotype shift between the native and invasive ranges of *C. stoebe*, and in addition, the recent spread of tetraploids in the native range (Mráz *et al.* 2014).

Perspectives

More generally, reduced inbreeding depression in polyploids may add an important keystone to the explanation for the overrepresentation of polyploids among invasive species. More studies on polyploid complexes with polyploids of varying age since speciation and differing mode of inheritance should therefore test the representativeness of our results. Furthermore, future studies should aim at disentangling the relative contribution of self-incompatibility and inbreeding depression on reproductive output. Such studies should apply *S*-locus genotyping combined with parent diallel-estimations across a large number of within-population crosses in order to estimate the number, segregation and dominance interactions of *S*-alleles (see Brennan *et al.* 2013). In addition, studies on inbreeding-environment-interactions should address the extent of inbreeding depression under varying environmental scenarios (Prill *et al.* 2014). Modelling approaches may help to reveal how inbreeding interacts with contrasting life histories to shape demographic rates and population growth in the three GCTs (Smallegange & Coulson 2013).

Acknowledgements

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Chapter 4

INBREEDING-ENVIRONMENT-INTERACTIONS AND PRE-ADAPTATION

This Chapter is has been submitted to *American Journal of Botany* as:
Rosche C, Hensen I, Lachmuth S (submitted): The potential role of pre-adaptation and inbreeding-environment interactions for colonization of disturbed habitats: an experimental study with diploid and tetraploid *Centaurea stoebe*

4.1 Abstract

Primary colonization in invasive ranges most commonly occurs in ruderal habitats, where disturbance may cause physical damage in plants. The tolerance to such disturbance may differ between cytotypes and among populations as a result of differing evolutionary histories. Moreover, founder populations often experience inbreeding depression, the effects of which may increase through physical damage as a result of inbreeding-environment interactions. We aimed at understanding how such colonization processes differ between diploid and tetraploid *Centaurea stoebe* populations, with a view to understanding why only tetraploids are invasive.

We conducted a clipping experiment (freq.: 0, 1 or 2 times in the growing season) on inbred vs. outbred offspring originating from 37 *Centaurea stoebe* populations of varying cytotype, range and habitat type (natural vs. ruderal). Aboveground biomass was harvested at the end of the vegetation period while re-sprouting success was recorded the following spring.

Clipping reduced re-sprouting success and biomass, which was significantly more pronounced in natural than in ruderal populations. Inbreeding depression was not detected under benign conditions, but became increasingly apparent in biomass when plants were clipped. The effects of clipping and inbreeding did not differ among cytotypes.

Adaptive differentiation in disturbance tolerance was greater among populations than between cytotypes, which highlights the potential of pre-adaptation in ruderal populations during early colonization on anthropogenically disturbed sites. While the consequences of inbreeding increased through clipping-mediated stress, they were comparable between cytotypes, and do consequently not contribute to understanding the cytotype shift in the invasive range.

Keywords: AIAI hypothesis; Asteraceae; biological invasions; clipping; geo-cytotype; habitat type; inbreeding depression; polyploidy; rapid evolution; spotted knapweed.

4.2 Introduction

Understanding the mechanisms that promote or suppress biological invasions is a major challenge in ecology (Gurevitch *et al.*, 2011). It is widely documented that polyploidy increases colonization success, but surprisingly little is known about the processes underlying this phenomenon (Prentis *et al.*, 2008; te Beest *et al.*, 2011; Bock *et al.*, 2015). However, filters that act in the early stages of primary colonization can determine whether non-native species fail or succeed to establish in an exotic range, and thus, they should help explaining the outcome of invasions (Theoharides and Dukes, 2007). In the present study, we investigate how the success of polyploids may be mediated by two distinct processes that are of great importance to colonization success: 1) pre-adaptation to environmental conditions in the non-native habitat (Hufbauer *et al.*, 2012; Fridley, 2013); and 2) the avoidance of inbreeding depression in founder populations (Fauvergue *et al.*, 2012; Hufbauer *et al.*, 2013).

Primary colonization following initial introduction to the non-native range as well as during subsequent invasive range expansion at the spread front usually takes place in ruderal habitats (Dietz and Edwards, 2006). This habitat preference results from the facilitative effect of anthropogenic disturbance on invasions (e.g., Milbau *et al.*, 2013; Shah *et al.*, 2014; Jauni *et al.*, 2015). In particular, disturbance can generate fluctuations in resource availability (Davis *et al.*, 2000) and may result in a release from competitors (Catford *et al.*, 2012; Maron *et al.*, 2014). On the other hand, human-mediated disturbance regimes, such as frequent mowing, trampling or traffic, can cause physical damage to plants that reduces individual performance, which can in turn lead to the extinction of founder populations (Kallimanis *et al.*, 2005). Thus, ruderal species that are pre-adapted to sporadic physical damage (i.e. disturbance tolerance) are regarded as being more successful at colonizing non-native ranges (Baker, 1974; Di Castri, 1989). As such, within polyploid complexes comprising conspecies with differing ploidy levels, the cytotype more pre-adapted to ruderal conditions may prevail over the less pre-adapted cytotype in the invasive range (Mráz *et al.*, 2012a). Similarly, within each cytotype, populations may exhibit local adaptation to their specific habitat conditions (Mráz *et al.*, 2014). As a consequence, offspring from native ruderal populations may be more pre-adapted to colonize human-altered sites in the novel range than offspring from natural or semi-natural populations, because ruderal populations may have experienced more frequent anthropogenic disturbance in their recent population history (see anthropogenically induced adaptation to invade (AIAI)-theory; Hufbauer *et al.*, 2012). Furthermore, where the occupied habitats in the invasive range are generally characterized by more prevalent disturbance than those in the native range, rapid adaptive evolution (Sax *et al.*, 2007; Prentis *et al.*, 2008) may increase disturbance tolerance in invasive as compared to native populations.

Moreover, introduced or spreading populations that surpass environmental filters are often subjected to repeated demographic bottlenecks, which involve an increasing frequency of

inbreeding among close relatives (Marchini *et al.*, 2015; Schrieber and Lachmuth, 2016). Inbreeding often reduces offspring fitness (i.e. inbreeding depression), which mainly arises from the homozygosity of recessive deleterious alleles (Charlesworth and Willis, 2009). The extent of inbreeding depression can be substantially affected by environmental conditions through so-called inbreeding-environment interactions (IxE interactions; Liao and Reed, 2009). Particularly, inbreeding depression is known to be amplified in harsh as compared to benign environments (reviewed in Fox and Reed, 2011). Stress fundamentally alters gene expression by inducing a broad variety of stress responses (Atkinson, 2012). If recessive deleterious alleles occur in such stress responses and are homozygote due to inbreeding, their detrimental effects become apparent when environment-specific responses are urgently needed (i.e. conditionally deleterious alleles; Vermeulen *et al.*, 2014). In addition, as inbreeding represents a stressor in itself and pushes organisms to their physiological limits, it may crucially limit their capacity to respond to additional external stressors or vice versa (Prill *et al.*, 2014; Menzel *et al.*, 2015). Under more benign environmental conditions, inbreeding depression can be much lower (Murren and Dudash, 2012; Vermeulen *et al.*, 2013), which may enable founder populations to spread, in spite of inbreeding. Alternatively, negative IxE interactions can strengthen inbreeding depression and prevent the establishment of inbred founder populations when the non-native environment is stressful (Hufbauer *et al.*, 2013, Szűcs *et al.*, 2014), which, in ruderal habitats of primary colonization, may be specifically pronounced under physical damage.

Polyploids often show reduced inbreeding depression compared to diploids (reviewed in Soltis and Soltis, 2000), because the multiplication of chromosome copies can reduce homozygosity, which potentially enhances the masking of recessive deleterious alleles (Eliášová *et al.*, 2013). However, variation in IxE interactions between cytotypes has never been addressed, nor has it been investigated in relation to anthropogenic disturbance. At the same time, cytotypes may also differ in their adaptation to disturbance in ruderal founder populations, which may, in turn, depend on range and habitat type of the source populations. Here, we present the first empirical research into such complex interactions between genetic and ecological determinants of colonization success. Our model system, *Centaurea stoebe* s.l. L (Asteraceae; spotted knapweed; syn. *C. maculosa* Lam.), is a polyploid complex consisting of a diploid and a tetraploid conspecies (Mráz *et al.*, 2011). While diploids represent the majority cytotype in the native Eurasian range, only tetraploids have been recorded in the invasive North American range (Mráz *et al.*, 2011). This apparent shift in cytotype distribution has resulted in the establishment of three so-called geo-cytotypes (GCTs): native diploids (EU2x), native tetraploids (EU4x), and invasive tetraploids (NA4x). A previous study revealed considerable inbreeding depression in *C. stoebe*, which was significantly more pronounced in diploids than in tetraploids (Rosche *et al.*, submitted). Ecological niches are rather similar between cytotypes (Ochsmann, 2000), with diploids being more frequent in drier, (semi)-natural sites and tetraploids prevailing in ruderal habitats (Otisková *et al.*, 2014). Despite such apparent habitat affiliation between cytotypes,

Thébault and Buttler (2009) found that both cytotypes share similar responses to artificial disturbance. However, this result may be biased in that they did not account for habitat differences within cytotypes, as Mráz *et al.* (2014) later found that adaptive differentiation within cytotypes can override the effects of stress tolerance between diploid and tetraploid *C. stoebe*.

To gain a more complete picture of processes determining the colonization success of diploid vs. tetraploid *C. stoebe*, we applied a clipping experiment with inbred vs. outbred lineages from at least 12 populations per GCT. Each GCT comprised ruderal and natural populations (*sensu* Broennimann *et al.*, 2014, see methods for details). Our experiment addressed the following hypotheses:

H1: Disturbance regimes experienced in a population's history affect its adaptive response to experimental clipping. More precisely, disturbance tolerance is:

H1a: higher in tetraploids than diploids, which gives tetraploids a pre-adaptive advantage with respect to colonizing of frequently disturbed habitats;

H1b: higher in NA4x than EU4x due to rapid adaptation to ruderal conditions during the colonization of the invasive range; and

H1c: higher in offspring from ruderal populations than from natural populations.

H2: Inbreeding reduces plant performance. More specifically:

H2a: clipping amplifies the negative effects of inbreeding through IxE interactions; and

H2b: such negative IxE interactions are stronger in diploids than in tetraploids.

The results of our study should shed light on how adaptive disturbance tolerance and IxE interactions vary among and within GCTs and how this potentially affects the contrasting capabilities of diploid vs. tetraploid *C. stoebe* in its colonization of strongly disturbed habitats.

4.3 Materials and methods

Model system

Diploid *C. stoebe ssp. stoebe* and tetraploid *C. stoebe ssp. micranthos* are reproductively isolated from one another (Mráz *et al.*, 2012a). Tetraploid *C. stoebe* originated from hybridization between diploids and a closely related, yet unknown taxon (Mráz *et al.*, 2012b). The cytotypes differ in their life histories, with diploids showing a predominantly monocarpic lifecycle while tetraploids are short-lived perennials (Mráz *et al.*, 2011). Both cytotypes show barochory, they are strictly self-incompatible and mainly pollinated by hymenoptera (Mráz *et al.*, 2012a), which, in concert, results in moderate levels of biparental within-population inbreeding across all three GCTs (Rosche *et al.*, 2016).

In North America, the invasion of tetraploids involved a lag-phase of 50 years (Broennimann *et al.*, 2014), which was likely encompassed by adaptive shifts towards a more continental climatic niche (Broennimann *et al.*, 2007) and by founder effects, leading to a temporary loss of genetic diversity (Rosche *et al.*, 2016) and pronounced inbreeding depression (Rosche *et al.*, submitted).

Nowadays, as one of the most noxious North American invaders, tetraploid *C. stoebe* has a tremendous economic impact on agriculture and livestock farming (Corn *et al.*, 2006). The overwhelming success of NA4x has frequently been related to a combination of both the better pre-adaptation to the novel range in tetraploids compared to diploids, and the rapid adaptive divergence of NA4x from the ancestral EU4x (e.g., Treier *et al.*, 2009; Henery *et al.*, 2010; Hahn and Müller-Schärer, 2013). Furthermore, disturbance is assumed to support the establishment of *C. stoebe* within natural communities in North America (Emery and Rudgers, 2012). Particularly, removal of above-ground was found to increase recruitment success of *C. stoebe* seedlings (Maron *et al.*, 2013).

Sampling

The 37 populations assessed in this study were sampled in summer 2012 and comprised 12 EU2x (5 ruderal, 7 natural), 12 EU4x (7 ruderal, 5 natural) and 13 NA4x (7 ruderal, 6 natural) populations (Fig. 4.1). For each population, we collected achenes from four separated individuals (i.e. seed families). To avoid sampling closely related seed families, we maximized the spatial distance between the sampled maternal plants (see Rosche *et al.*, submitted). The cytotype of each seed family was determined using flow-cytometry, in accordance with the protocol set out by Mráz *et al.* (2011). Habitat classification of the sampled populations was conducted and documented in Rosche *et al.* (2016) and followed the European classification system of habitats (EUNIS, 2008). According to the protocol of Broennimann *et al.* (2014), artificial and industrial habitats (EUNIS-category J) as well as agricultural sites (I) were regarded as ruderal, whereas populations from natural and diluvial sediments (C), natural and semi-natural grasslands (E) or rocky outcrops (H) were considered as (semi-) natural (hereafter referred to as “natural”). The respective habitat type, GPS coordinates and locality for each population are listed in Table A4.1 (see Appendix).

Origin of inbred and outbred lineages

Achenes of the seed families were germinated in autumn 2012. From the emerged seedlings, we cultivated up to five individuals from each seed family (i.e. P-generation) then, in summer 2013, we conducted a crossing experiment with this P-generation. We applied a two-level breeding treatment: inbreeding (i.e. sib-mating within seed families) vs. outbreeding (i.e. crosses between seed families within a population; for details, see Rosche *et al.*, submitted). In total, we conducted 1780 hand-pollinated crosses. After the capitula had matured, we found that 53.5 % of the crosses yielded achenes, from which we chose up to six inbred and six outbred F1-lineages from each population (i.e. achenes that resulted from a distinct cross). Within populations, we selected lineages that included all seed families as equally as was applicable (see Table A3.2). Up to ten achenes per lineage were germinated in October 2013, from which up to five F1-seedlings per lineage were planted on planting trays and raised in 2.2 L pots in the greenhouse

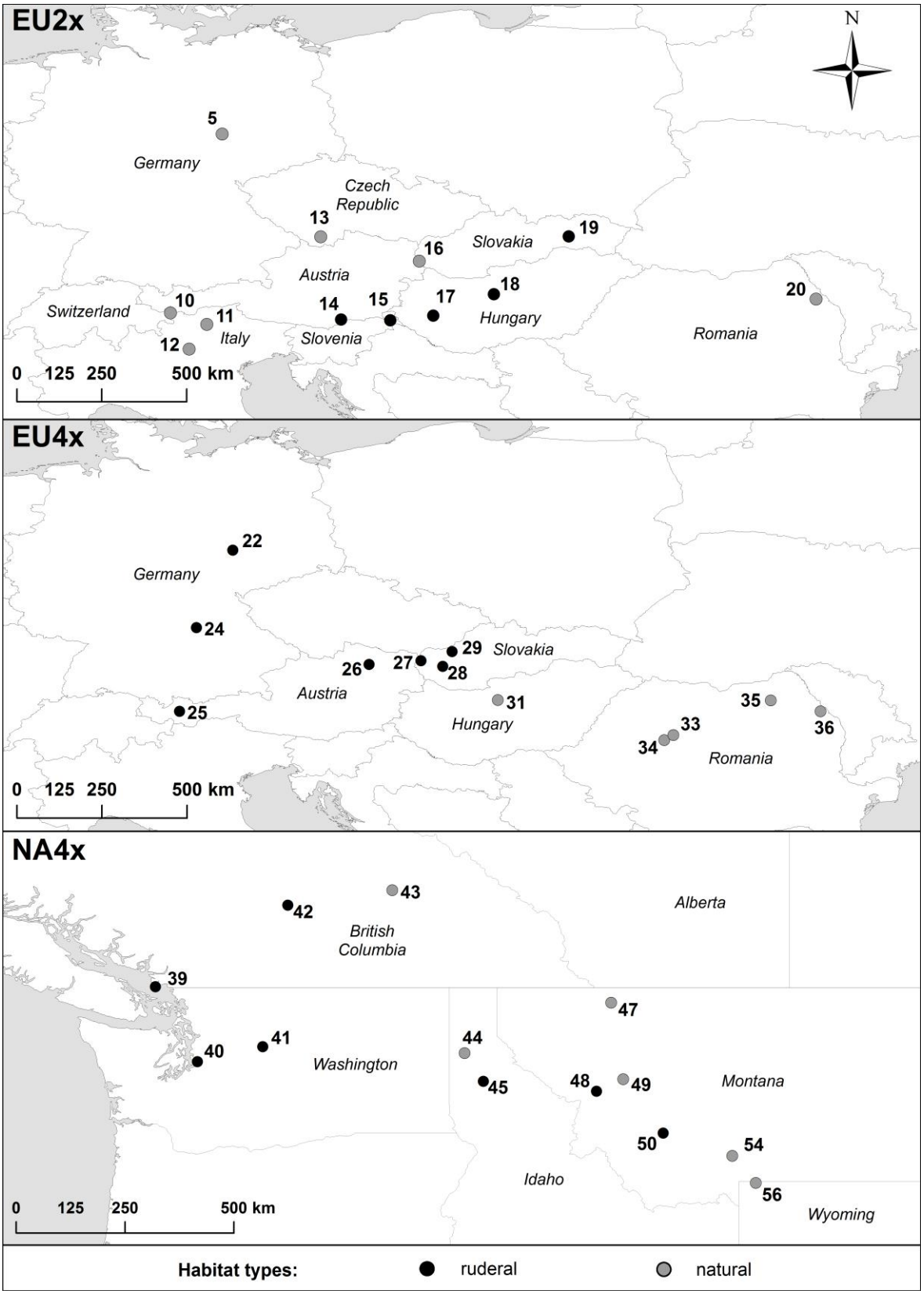

Fig. 4.1 Geographical distribution of the 37 *Centaurea stoebe* populations that were involved in the clipping experiment. Each geo-cytotype distribution is shown in a separate map. Further information on the populations is available in Appendix S1. Geo-cytotypes: EU2x: native diploid, EU4x: native tetraploid, NA4x: invasive tetraploid.

(in total: 1927 individuals from 430 lineages). In April 2014, pots were moved to the Botanical Garden in Halle (51.49°N; 11.96°E). We excluded competitors across a three week cycle to expose the plants to low levels of competition and full sunlight, which resembles the conditions they experience in ruderal habitats. Impermeable plastic planes were placed below pots to avoid any attachment of roots to ground soil, and plants were watered only when they started to wilt. More detailed information on the conditions in the germination chamber, green house and common garden during the cultivation of the P-generation as well as the F1-generation can be found in Rosche *et al.* (submitted). In August 2014, we recorded 959 individuals that had survived, determined their flowering status (i.e. flowering or not flowering) and estimated the cumulative fitness of each lineage as a product of germination success, survival probability, flowering probability and number of capitula. This estimate of fitness was obtained to quantify inbreeding depression in Rosche *et al.* (submitted) and was not used for our present study. Instead, here we particularly focused on the effects of the clipping treatment, which we applied immediately after obtaining cumulative fitness.

Clipping experiment

In August 2014, we initiated our clipping experiment with 396 lineages that contained the remaining 959 individuals (124 EU2x inbred, 170 EU2x outbred, 160 EU4x inbred, 178 EU4x outbred, 156 NA4x inbred, 171 NA4x outbred). The clipping treatment simulated physical damage (e.g., mowing, trampling, traffic), which represents typical disturbance-mediated stress in ruderal habitats of *C. stoebe* (Thébault and Buttler, 2009). Within populations, individuals from the different lineages were assigned randomly and in equal numbers to one of the following treatments: CT0: control group without clipping, CT1: single clipping on August 25th and CT2: clipping on August 25th and September 22th (Table A3.2). CT0 simulated benign conditions, CT1 simulated a single and harsh disturbance event, and CT2 simulated repeated physical damage. Biomass from the clipping treatments was discarded and not documented. All clippings were applied at 2 cm above the root collar. For rosettes, we grasped all leaves and stretched them vertically upwards to cut them at 2 cm above the root collar. On October 27th, the above-ground biomass of all plants was harvested by cutting all individuals at ground level. The harvested biomass was dried at 80°C for 24 h and then weighted. In April 2015, we surveyed whether plants re-sprouted (i.e. re-spouting success). Throughout the entire experiment (germination chamber, green house and common garden), the positions of all F1-individuals were haphazardly distributed and frequently randomized. The variables of biomass and re-sprouting success were assumed to represent estimates of disturbance tolerance. We did not consider information on flowering or survival to describe the response to clipping (i.e. disturbance tolerance), because within the timeframe of the beginning of the clipping treatments and the harvest of the aboveground biomass, only seven individuals started to flower and only 12 plants died. However,

information on the flowering status (before initiating the clipping treatment) was used to account for life cycle status of the individuals in the statistical analyses (see below).

Statistical analyses

All statistical analyses were performed in R 3.2.3 (R development core team, 2015) using the R package lme4 (Bates *et al.*, 2014). In accordance with Crawley (2015), transformation decisions were based on model-checking plots of variance, homogeneity and normality of errors. To test our hypotheses concerning adaptive processes (i.e. adaptation models), we used a dataset consisting exclusively of data from outbred individuals. To test our hypotheses concerning the consequences of inbreeding (i.e. inbreeding models), we used the full dataset including data from inbred and outbred individuals. The adaptation models included the three-way interaction of clipping treatment with GCT and habitat type, whereas the inbreeding models included the three-way interaction of clipping treatment with GCT and breeding treatment. In addition, all models included all possible two-way interactions and main effects of the explanatory variables as well as two potentially confounding variables: 1) Latitude (centered and scaled), as *C. stoebe* shows adaptive differentiation along latitudinal clines (Mráz *et al.*, 2014); and 2) Flowering status, as individuals that invested resources in flowering may exhibit a reduced ability to cope with clipping stress. Both the adaptation and the inbreeding models were run for the two response variables representing disturbance tolerance: 1) Biomass (Gaussian error distribution, square root-transformed), using linear mixed-effects models, and 2) Re-sprouting success (binomial distribution), using generalized linear mixed-effects models. Minimal adequate models were obtained by employing step-wise backward removal of non-significant fixed effects based on χ^2-tests. Pollen donor individual (nested in donor seed family, nested in donor population), and pollen acceptor individual (nested in acceptor seed family, nested in acceptor population) were set as random effects.

4.4 Results

Overall, increasing clipping intensity considerably reduced biomass and re-sprouting success. Our focus was to investigate how this decreasing performance varied between GCTs, breeding treatments and habitat types and / or their interactions. The parameter estimates of significant fixed effects are presented in Table 4.1 for the adaptation models (using the outbred dataset) and Table 4.2 for the inbreeding models (using the full dataset).

Table 4.1 Statistical analyses investigating adaptation to disturbance (i.e. adaptation models) in *Centaurea stoebe* using the outbred dataset (i.e. exclusively data from inbreds). The table shows the model structure of the maximal models for analyzing the interactive effects of clipping treatment with geo-cytotype and habitat type on the response variables of biomass and re-sprouting success.

Fixed effects	Sqrt biomass (g)	Re-sprouting success
(Intercept)	1.463	— 1.069
Flowering status	**0.338 *****	**— 0.803 ****
Latitude	n.s.	n.s.
GCT_{NA4x}	n.s.	**1.373 *****
GCT_{EU4x}	n.s.	**1.606 *****
$HT_{Natural}$	— 0.005 [a]	— 0.38 [a]
CT_{CT1}	— 0.033 [a]	— 0.507 [a]
CT_{CT2}	— 0.191 [a]	— 0.468 [a]
GCT_{EU4x} : CT_{CT1}	n.s.	n.s.
GCT_{EU4x} : CT_{CT2}	n.s.	n.s.
GCT_{NA4x} : CT_{CT1}	n.s.	n.s.
GCT_{NA4x} : CT_{CT2}	n.s.	n.s.
GCT_{EU4x} : $HT_{Natural}$	n.s.	n.s.
GCT_{NA4x} : $HT_{Natural}$	n.s.	n.s.
CT_{CT1} : $HT_{Natural}$	**— 0.179 ****	**0.073 ***
CT_{CT2} : $HT_{Natural}$	**— 0.215 ****	**— 1.536 ***
GCT_{EU4x} : CT_{CT1} : $HT_{Natural}$	n.s.	n.s.
GCT_{EU4x} : CT_{CT2} : $HT_{Natural}$	n.s.	n.s.
GCT_{NA4x} : CT_{CT1} : $HT_{Natural}$	n.s.	n.s.
GCT_{NA4x} : CT_{CT2} : $HT_{Natural}$	n.s.	n.s.

Random effect variances	Sqrt biomass (g)	Re-sprouting success
$Population_{Acceptor}$	0.000 (37)	0.000 (37)
$Population_{Acceptor}$ / $SF_{Acceptor}$	0.000 (115)	0.000 (115)
$Population_{Acceptor}$ / $SF_{Acceptor}$ / $Individual_{Acceptor}$	0.004 (186)	0.000 (186)
$Population_{Donor}$	0.013 (37)	0.000 (37)
$Population_{Donor}$ / SF_{Donor}	0.000 (113)	0.000 (113)
$Population_{Donor}$ / SF_{Donor} / $Individual_{Donor}$	0.000 (176)	0.000 (176)
Residuals	0.109 (519)	n.e.

Notes: CT: clipping treatment, GCT: geo-cytotype, HT: habitat type, SF: seed family, CT1: single clipping, CT2: double clipping, NA4x: invasive tetraploids, EU4x: native tetraploids, n.s.: not significant, n.e.: not estimated, sqrt: square root, a: fixed main effect in significant interaction, significance levels: *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$. Parameter estimates for significant fixed effect terms are given in bold. Parameter estimates for main effects that form part of a significant interaction are not bold, while the significance of those main effects was not tested (see Crawley, 2015). The second level of factors is given in subscript. Where a factor had more than two levels, the significance level refers to the overall fixed effect and accordingly does not correspond to the pair-wise comparison between two distinct levels. Latitude was scaled and centered in both models. Random effect variances including their number of observations are presented in parentheses.

Table 4.2 Statistical analyses investigating the effects of the clipping x inbreeding interaction (i.e. inbreeding models) in *Centaurea stoebe* using the full dataset (i.e. data from inbreds and outbreds). The table shows the model structure of the maximal models for analyzing the interactive effects of clipping treatment with geo-cytotype and breeding treatment on the response variables of biomass and re-sprouting success.

Fixed effects	Sqrt biomass (g)	Re-sprouting success
(Intercept)	1.458	— 1.608
Flowering $_{yes}$	**0.283 *****	**— 0.446 ***
Latitude	n.s.	n.s.
GCT $_{NA4x}$	n.s.	**1.469 *****
GCT $_{EU4x}$	n.s.	**1.438 *****
CT $_{CT1}$	— 0.317 [a]	**— 0.266 *****
CT $_{CT2}$	— 0.405 [a]	**— 0.845 *****
BT $_{Outbred}$	0.048 [a]	n.s.
GCT $_{NA4x}$:BT $_{Outbred}$	n.s.	n.s.
GCT $_{EU4x}$:BT $_{Outbred}$	n.s.	n.s.
GCT $_{EU4x}$:CT $_{CT1}$	n.s.	n.s.
GCT $_{EU4x}$:CT $_{CT2}$	n.s.	n.s.
GCT $_{NA4x}$:CT $_{CT1}$	n.s.	n.s.
GCT $_{NA4x}$:CT $_{CT2}$	n.s.	n.s.
BT $_{Outbred}$:CT $_{CT1}$	**0.203 *****	n.s.
BT $_{Outbred}$:CT $_{CT2}$	**0.112 *****	n.s.
GCT $_{NA4x}$:BT $_{Outbred}$:CT $_{CT1}$	n.s.	n.s.
GCT $_{EU4x}$:BT $_{Outbred}$:CT $_{CT1}$	n.s.	n.s.
GCT $_{NA4x}$:BT $_{Outbred}$:CT $_{CT2}$	n.s.	n.s.
GCT $_{EU4x}$:BT $_{Outbred}$:CT $_{CT2}$	n.s.	n.s.

Random effect variances	Sqrt biomass (g)	Re-sprouting success
Population $_{Acceptor}$	0.002 (37)	0.008 (37)
Population $_{Acceptor}$ / SF $_{Acceptor}$	0.000 (120)	0.000 (120)
Population $_{Acceptor}$ / SF $_{Acceptor}$ / Individual $_{Acceptor}$	0.001 (266)	0.008 (266)
Population $_{Donor}$	0.011 (37)	0.005 (37)
Population $_{Donor}$ / SF $_{Donor}$	0.000 (119)	0.000 (119)
Population $_{Donor}$ / SF $_{Donor}$ / Individual $_{Donor}$	0.000 (269)	0.000 (269)
Residuals	0.106 (959)	n.e.

Notes: CT: clipping treatment, GCT: geo-cytotype, BT: breeding treatment, SF: seed family, CT1: one time clipping, CT2: two times clipping, NA4x: invasive tetraploids, EU4x: native tetraploids, n.s.: not significant, n.e.: not estimated, sqrt: square root, a: fixed main effect in significant interaction, significance levels: *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$. Parameter estimates for significant fixed effect terms are given in bold. Parameter estimates for main effects that form part of a significant interaction are not bold, while the significance of those main effects was not tested (see Crawley, 2015). The second level of factors is given in subscript. Where a factor had more than two levels, the significance level refers to the overall fixed effect and accordingly does not correspond to the pair-wise comparison between two distinct levels. Latitude was scaled and centered in both models. Random effect variances including their number of observations are presented in parentheses.

The effects of geo-cytotype on disturbance tolerance

GCT had no effect on biomass, but it significantly influenced re-sprouting success (outbred dataset: $\chi^2 = 27.79$, df = 1, $P < 0.001$, Fig. 4.2a; full dataset: $\chi^2 = 34.51$, df = 1, $P < 0.001$, Fig. 4.2b). For both datasets, we consistently found that both NA4x and Eu4x showed significantly higher re-sprouting success than EU2x, whereas the two tetraploid GCTs did not differ from one another. With respect to both biomass and re-sprouting success, we found no significant two-way interactions between GCT with habitat type (outbred dataset), GCT with breeding treatment (full dataset) or GCT with clipping treatment (for both datasets). Likewise, we found no significant three-way interaction between GCT, clipping treatment and habitat type for both the outbred dataset and the full dataset.

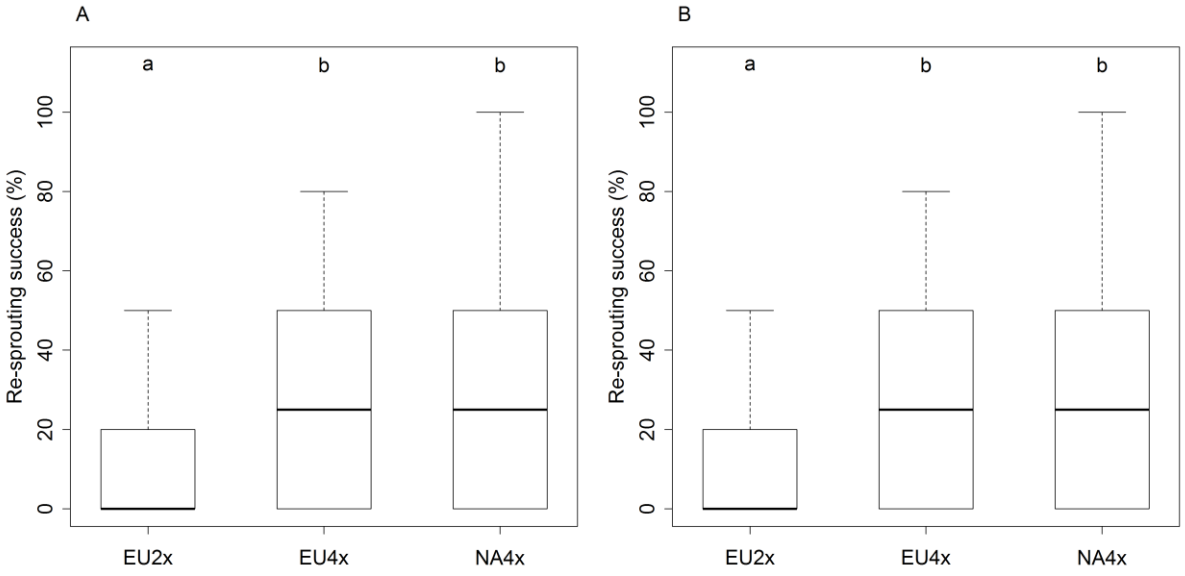

Fig. 4.2 Effects of geo-cytotype on re-sprouting success of *Centaurea stoebe*. A: Outbred dataset of the adaptation model. B: Full dataset of the inbreeding model. Groupings (lower case letters) are based on Tukey post-hoc tests (pair-wise comparisons with a significance level of $P < 0.05$). Geo-cytotypes: EU2x: native diploid, EU4x: native tetraploid, NA4x: invasive tetraploid.

Interaction between clipping treatment and habitat type

The interaction of habitat type and clipping treatment significantly affected biomass ($\chi^2 = 10.04$, df = 1, $P < 0.01$) and re-sprouting success ($\chi^2 = 7.43$, df = 1, $P < 0.05$). Overall, biomass was higher in ruderal than in natural populations, and it decreased with increasing clipping intensity (Fig. 4.3a). Biomass of individuals from natural habitats showed a stronger response to the clipping treatments than those from ruderal habitats. Without clipping (i.e. under CT0), there was no difference in biomass between populations from ruderal and natural habitats. In contrast, under both CT1 and CT2, biomass from ruderal populations was significantly higher than that from natural populations. Similarly, re-spouting success was higher overall in ruderal than in natural populations, and it decreased with increasing clipping intensity (Fig. 4.3b). Individuals from natural habitats showed a stronger response to clipping treatment than those from ruderal

habitats. In both habitat types, re-sprouting success was comparable under CT0 and CT1. In contrast, in natural populations under CT2, re-sprouting success was significantly reduced compared that in CT0, but this relationship was not found for ruderal populations, where re-sprouting success was comparable across all clipping treatments.

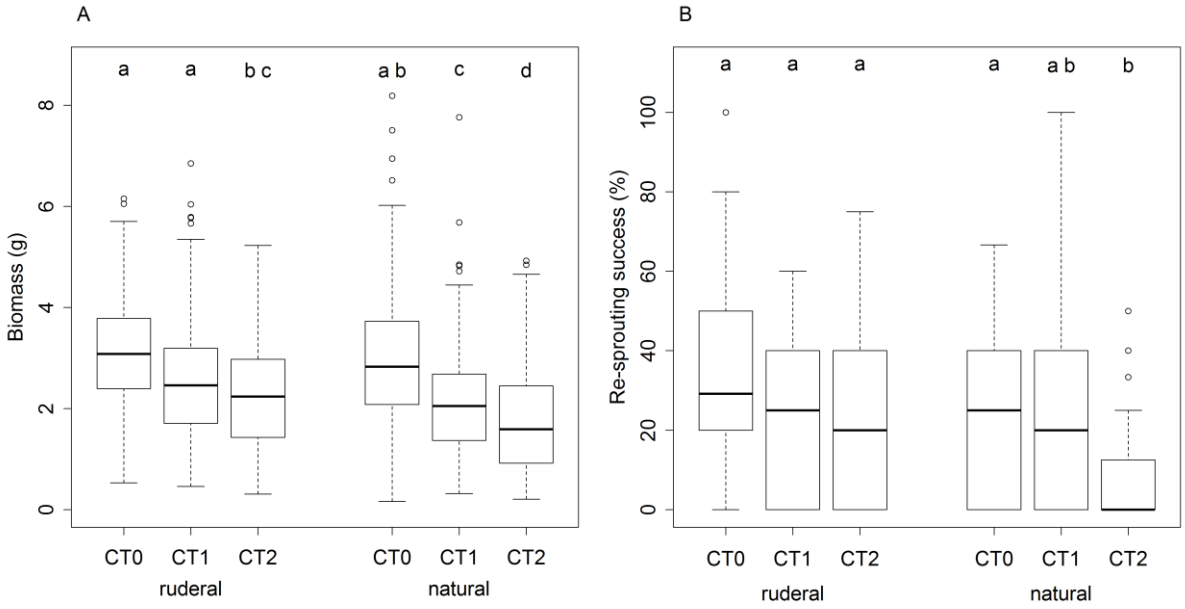

Fig. 4.3 Interactive effects of clipping treatment and habitat type (natural vs. ruderal) on performance of *Centaurea stoebe*. A: Biomass. B: Re-sprouting success. Groupings (lower case letters) are based on Tukey post-hoc tests (pair-wise comparisons with a significance level of $P < 0.05$). CT0: no clipping, CT1: single clipping, CT2: double clipping.

Interaction between clipping treatment and breeding treatment

Breeding treatment in interaction with clipping treatment significantly influenced biomass (χ^2 = 15.26, df = 1, $P < 0.001$). Overall, biomass was slightly higher in outbreds than in inbreds and it decreased with increasing clipping intensity (Fig. 4.4). Inbreds showed a stronger response to clipping treatment than outbreds. Without clipping, there was no difference between inbred and outbred biomass, whereas under both CT1 and CT2, the biomass of outbreds was significantly higher that of inbreds. As such, inbreeding depression in biomass was only apparent under stressful conditions of physical damage, but not in the relatively benign environment without clipping.

Potentially confounding variables

Latitude had no effect in any tested model. In contrast, flowering status (before initiating the clipping treatments) significantly affected both biomass and re-sprouting success. Flowering individuals had a higher biomass than those that did not flower, which was found for both the full dataset (χ^2 = 74.34, df = 1, $P < 0.001$) and the outbred dataset (χ^2 = 98.77, df = 1, $P < 0.001$). However, non-flowering individuals showed a significantly higher re-sprouting success than

individuals that flowered (full dataset: $\chi^2 = 4.42$, df = 1, $P < 0.05$; outbred dataset: $\chi^2 = 7.91$, df = 1, $P < 0.01$).

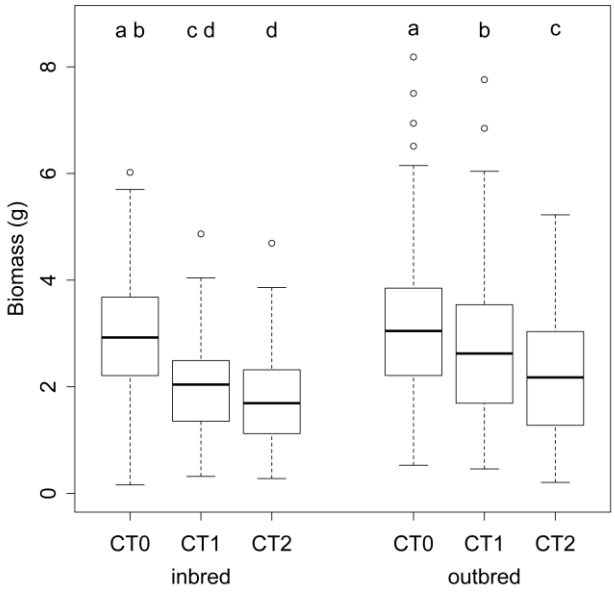

Fig. 4.4 Interactive effects of clipping treatment and breeding treatment (outbred vs. inbred) on biomass of *Centaurea stoebe*. Groupings (lower case letters) are based on Tukey post-hoc tests (pair-wise comparisons with a significance level of P < 0.05). CT0: no clipping, CT1: single clipping, CT2: double clipping.

4.5 Discussion

Experimental clipping, as a simulation of physical damage resulting from anthropogenic disturbance (see Thébault and Buttler, 2009), substantially reduced biomass and next spring re-sprout success in *C. stoebe.* In contrast to our hypotheses H1a and H1b, we found comparable disturbance tolerances between cytotypes and ranges, respectively. As expected (H1c), ruderal populations showed higher disturbance tolerance than natural populations. The effects of inbreeding were, as hypothesized (H2a), more pronounced under clipping application through IxE interactions. These IxE interactions were, against our expectation (H2b), comparable between cytotypes.

Disturbance tolerance across cytotypes

The responses of biomass and re-sprouting success to clipping were comparable between cytotypes, which aligns with previous findings of Thébault and Buttler (2009), who reported the same observations for survival and flowering in their clipping experiment. Considering the prevalence of tetraploids over diploids in ruderal sites (Broennimann *et al.*, 2014; Otisková *et al.*, 2014), these results contradict an anticipated pre-adaptive advantage of tetraploids to colonize highly disturbed habitats and, moreover, they do not correspond with several previous studies that found evidence that pre-adaptation enhances the invasivity of EU4x compared to EU2x (e.g.,

for competitive ability, Thébault *et al.*, 2010; for phenotypic plasticity, Hahn *et al.*, 2012b). Furthermore, GCTs did not differ in biomass, which may reflect the broad similarities in morphology and ecology between GCTs (Ochsmann, 2000; Španiel *et al.*, 2008). However, although the response of re-sprouting to clipping was similarly pronounced between GCTs, re-sprouting was generally much higher in tetraploids, which is most likely explained by their polycarpic life cycle (Mráz *et al.*, 2011). Following strong disturbance events (e.g. mowing), re-sprouting can maintain constant population sizes, which may contribute to the success of tetraploids in more erratic ruderal habitats. Such properties that favor the ruderal strategy are assumed to have an eminent impact on the cytotype shift in *C. stoebe* between the native and introduced range (see Mráz *et al.*, 2012a).

Adaptive differentiation between habitat types

Biomass and re-sprouting success were less affected by clipping in ruderal than in natural populations, which may reflect adaptation to frequent disturbance in the population history. Since anthropogenic physical damage can restrict colonization success of founder populations (Kallimanis *et al.*, 2005), such (pre-)adaptation to disturbance may facilitate a population through the critical stages of primary colonization during the initial introduction as well as at the expanding edges of the invaded range (Bossdorf *et al.*, 2008; Lee and Gelembiuk, 2008). Adaptive shifts that increase the invasiveness of populations may occur in native populations prior to colonization of the exotic range and / or during the course of invasion, due to rapid adaptive changes (Rey *et al.*, 2012). Evidence for rapid adaptation was recently detected in NA4x (e.g., for competitive ability, Ridenour *et al.*, 2008; for seed mass, Hahn *et al.*, 2013), however, a post-introduction scenario for adaptation to disturbance is unlikely, because both ranges show similar distributions of populations in ruderal and natural habitats (Broennimann *et al.*, 2014). Indeed, disturbance tolerance did not differ between EU4x and NA4x, and thus, the increased disturbance tolerance in ruderal populations most likely resulted from adaptation in the native range. According to the AIAI-theory (Hufbauer *et al.*, 2012), this may provide an a priori advantage in the colonization of disturbed habitats during the primary colonization of a non-native range. Meanwhile, our results coincide with a recent study of Mráz *et al.* (2014), who showed that drought tolerance in *C. stoebe* did not differ between GCTs but rather along latitudinal clines within GCTs due to local adaptation to precipitation. Similarly, in our experiment, disturbance tolerance was affected by the local conditions of the respective stress factor (i.e. physical damage in ruderal habitats). Thus, in both types of stress tolerance, local adaptation within GCTs appears to have been more important than any adaptive divergence between GCTs.

Effects of inbreeding-environment interactions across cytotypes

While inbreeding had no effects under benign conditions, inbreeding depression in biomass became apparent under clipping due to IxE interactions. This result aligns with the expectation

that stress augments the magnitude of inbreeding depression (Reed *et al.*, 2012) and clearly demonstrates the importance of considering varying environmental conditions when studying the consequences of inbreeding in distinct habitats or scenarios. Accordingly, the studies of Hufbauer *et al.* (2013) and Szűcs *et al.* (2014) illustrate how novel environments may interact with inbreeding to restrict colonization success. At the same time, our results indicate that inbreeding can lead to reduced disturbance tolerance. The homozygote expression of conditionally deleterious alleles may hinder stress responses in inbreds, as previously demonstrated e.g. for heat-sensitivity (Pedersen *et al.*, 2009) or herbivory tolerance (Karyat *et al.*, 2012, 2013). In addition, the genome-wide expression of genetic load may cause considerable physiological dysfunctions in inbreds (reviewed in Kristensen *et al.*, 2010), which may lead to a reduced ability to manage additional environmental stress (e.g., Prill *et al.*, 2014; but see Franke and Fischer, 2013).

Re-sprouting success was however not affected by inbreeding, neither under benign conditions nor under clipping. Moreover, contrary to our findings in a previous study (Rosche *et al.*, submitted), inbreeding generally had rather weak effects on plant performance, and for both response variables, the across-environment effects of inbreeding were similarly pronounced between GCTs. Inbreeding depression acts trait-specifically (Mikkelsen *et al.*, 2009; Angeloni *et al.*, 2011) and as such, our contrasting results may reflect the effect of the distinct variables we investigated. In fact, composite estimates of fitness including several life stage traits may provide more informative estimates of inbreeding depression than single trait observations (Oakley and Winn, 2012), but such data were not available for the present study (see methods). More importantly, since the clipping treatment was initiated when plants were older than ten months, previously-acting selective death may have excluded inbred individuals with very low fitness from our dataset. Thus, while our results could not confirm that IxE interactions differentially affect colonization success of the cytotypes of *C. stoebe* in anthropogenically disturbed habitats, further investigation is needed to clarify whether IxE interactions in composite fitness across the whole life cycle differ between GCTs of *C. stoebe*.

Conclusions and perspectives

Colonization success of founder populations can be crucially affected by pre-adaptation to ruderal conditions (Hufbauer *et al.*, 2012) and IxE interactions (Szűcs *et al.*, 2014). Our results did not reveal significant differences between diploid and tetraploid *C. stoebe* neither in their responses to clipping nor in their response to inbreeding. However, disregarding clipping and breeding treatment, our data showed generally higher re-spouting success in tetraploids, and we therefore regard longevity to be one of the potential drivers of the cytotype shift in *C. stoebe.* Its distinct influence in ruderal systems may be further addressed in long-term studies on population growth under continuous disturbance regimes and with demographic modeling approaches (Smallegange and Coulson, 2013).

In addition, we can conclude that the increased disturbance tolerance in offspring from ruderal populations over their counterparts from natural habitats may increase their capability to colonize ruderal sites. We therefore highlight that adaptive differentiation can be more pronounced among populations than between cytotypes. With a view to avoiding any potentially misleading conclusions from rather simplistic diploid vs. tetraploid comparisons, future experimental studies investigating potential differences between cytotypes may require the consideration of the broad variance in population characteristics to account for varying evolutionary histories between habitat types. Moreover, our results add to the growing body of studies suggesting that inbreeding depression varies among environments. Considering the definite negative effects of inbreeding in our previous study (Rosche *et al.*, submitted), future studies testing the effects of inbreeding on composite fitness indices under various environments will clearly deepen our mechanistic understanding of the role of inbreeding depression with respect to colonization success in diploid and tetraploid *C. stoebe*.

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Chapter 5

GENERAL DISCUSSION

More than 50 years ago, *The genetics of colonizing species* edited by Baker and Stebbins (1965) launched a new era in invasion biology, that is, the scientific effort to identify drivers of colonization success. Over the years, it became increasingly clear that primary colonization is a central and repetitive element of biological invasions, and that this demographic process often involves founder effects that act as an invasion filter (e.g. Firestone and Jasieniuk 2012b; Mullarkey *et al.* 2013; Dlugosch *et al.* 2015; Schrieber and Lachmuth 2016). Mechanisms that help overcoming this filter may, therefore, enhance the probability of species to establish in a non-native range (Theoharides and Dukes 2007). Throughout the three preceding chapters, I applied a broad range of methods in a concerted manner to study how polyploidy can affect colonization success. My results portray a differentiated picture of **population genetic determinants of colonization capabilities in diploid and tetraploid *Centaurea stoebe*** (Table 5.1).

No.	Hypothesis concerning	Hypothesized outcome	Result	Effect for colonization abilities of polyploids
I	Genetic diversity	*4x > 2x*	**4x > 2x**	+
II	Genetic drift during bottlenecks	*4x < 2x*	**4x < 2x**	+
III	Mate limitation following inbreeding	*4x > 2x*	**4x = 2x**	0
IV	Inbreeding depression	*4x < 2x*	**4x < 2x**	+
V	Negative IxE interactions	*4x < 2x*	**4x = 2x**	0
VI	Pre-adaptive abilities to physical damage	*4x > 2x*	**4x = 2x**	0

Table 5.1 Key results of my PhD studies concerning the colonization capabilities of diploid vs. tetraploid *Centaurea stoebe*. Roman numerals refer to the specific working hypothesis as formulated in section 1.6. The previously hypothesized outcomes (italicized) are compared with the results found in the three preceding studies (given in bold). Effects on colonization abilities in tetraploids as compared to diploids (gray stained circles): +, positive effect; 0, no effect. No, number of the working hypothesis; 4x, tetraploid; 2x, diploid.

5.1 Synthesis: Novel insights into the biology of the cytotype shift in *Centaurea stoebe s.l.*

In chapter 2, I found tetraploids to maintain significantly **higher genetic diversity** than diploids (Table 2.1). This is likely to promote the cytotype shift between the ranges of *C. stoebe* because high genetic diversity is generally assumed to be beneficial for biological invasions (Firestone and Jasieniuk 2012c; Forsman 2014), and, more specifically, for colonization success (Blackburn *et al.* 2015). In particular, high initial genetic diversity ensures the raw genetic material for adaptation and decreases the risk to suffer from genetic depletion in later invasion stages (see Fig. 1.3). Furthermore, genetic diversity was strongly correlated with population size in EU2x, but

such relation was not apparent in both tetraploid GCTs (Fig. 2.4a). Thus, in addition to the higher initial genetic diversity in tetraploid founder populations, **polyploid genomes face decelerated genetic drift** as compared to diploid genomes during successive bottlenecks (Ronfort *et al.* 1998). This can be particularly important for founder populations as they frequently show small and / or fluctuating population sizes (Dlugosch and Parker 2008). In addition, anthropogenic disturbance may intensify such population size oscillations in the ruderal habitats of primary colonization (Davies *et al.* 2016). Accordingly, genetic diversity was reduced in ruderal as compared to natural populations in EU2x (Fig. 2.4b). However, such correlation was not detected in both tetraploid GCTs, which again underscores the ability of tetraploids to maintain high genetic diversity in times of demographic disequilibria. Moreover, the analyses of the small-scale-genetic structure revealed that, in all GCTs, considerable biparental inbreeding was occurs within natural populations (Fig 2.3). Furthermore, I obtained no reductions in genetic diversity in NA4x as compared to EU4x, but found more pronounced population admixture in NA4x than EU4x populations (Figs. 2.1 and 2.2). The **extensive admixture of NA4x** populations supports previous studies (Hufbauer and Sforza 2008; Marrs *et al.* 2008) that suggested **multiple introductions** in the invasion history of NA4x. I postulated that frequent population admixture ensured high genetic diversity in NA4x, which may have been an important pre-requisite for its rapid expansion. This assumption marked the starting point for unraveling specific mechanisms that may decrease population growth in periods of genetic depletion.

As such, I found in chapter 3 that **pollination success was diminished by inbreeding** and that for outcrossings, pollination success decreased with increasing within-population relatedness (Fig. 3.2). These results confirm that with increasing degree of relatedness, mating partners show an increasing likelihood of sharing identical *S*-alleles (Busch and Schoen 2008; Williams *et al.* 2013). The resultant mate limitation is known to dramatically affect population growth in bottlenecked populations (Levin *et al.* 2009), which can markedly restrict colonization success (Wagenius *et al.* 2007). Importantly, mate limitation was **similarly pronounced in both cytotypes**, which was also detected for diploid vs. tetraploid *Rutidosis leptorrhynchoides* in the only previous study that similarly compared cross-compatibilities in different cytotypes (Pickup and Young 2007). Thus, in both studies the anticipated disadvantage of polyploids (i.e. having an increasing risk to share common *S*-alleles due to a higher number of *S*-alleles per individual) was rejected. This may be attributed to high *S*-allele diversity in tetraploids and the decelerated effects of drift on *S*-allele diversity in polyploid genomes during bottlenecks (Pickup and Young 2007). Furthermore, chapter 3 revealed that **inbreeding reduced offspring fitness**, and this inbreeding depression was significantly **more pronounced in diploids** than in tetraploids (Fig. 3.4). Obviously, the higher number of alleles per locus in polyploids increased the masking of genetic load (see Eliášová *et al.* 2013), which can have essential implications for the success of founder populations as severe inbreeding depression is regarded as a major colonization filter (Hufbauer *et al.* 2013; Szűcs *et al.* 2014).

In chapter 4, I found that clipping (which simulated physical damage as negative consequence of disturbance) reduces performance, but there were **no differences in disturbance tolerance between cytotypes** (Table 4.1). This outcome runs against the assumption of a pre-adaptive advantage in tetraploids. Particularly, I previously expected that tetraploid populations should be more frequently confronted with anthropogenic disturbance in their evolutionary histories than diploids, since they prevail at ruderal sites (Otisková *et al.* 2014). However, large within-GCT variation resulting from differentiation between habitat types accounted for differences in disturbance tolerance among populations (Fig. 4.3). Additionally, chapter 4 showed that the negative impacts of inbreeding on offspring fitness are fostered under stressful conditions (i.e. clipping; Fig. 4.4), which adds to the growing body of studies that confirm the consequences of inbreeding to be context-specific (reviewed in Fox and Reed 2011). The **negative IxE interactions were comparably pronounced in both cytotypes** and can consequently not contribute to explaining the cytotype shift in *C. stoebe*.

In conclusion, my studies elucidated **multiple benefits of tetraploid over diploid *C. stoebe* in counteracting the negative effects of genetic depletion on population growth** (Table 5.1). Understanding the **additive nature** of these effects is crucial, because they act in **a hierarchical manner of three levels** (Fig. 5.1): 1) If initial genetic diversity is high, the strength of successive genetic depletion in subsequent founder events is reduced. 2) If mechanisms decelerate genetic depletion, its consequences are reduced. 3) If mechanisms alleviate the consequences of genetic depletion, population growth rates remain largely unaffected. I elaborated that polyploidy can simultaneously counteract negative founder effects at all three levels.

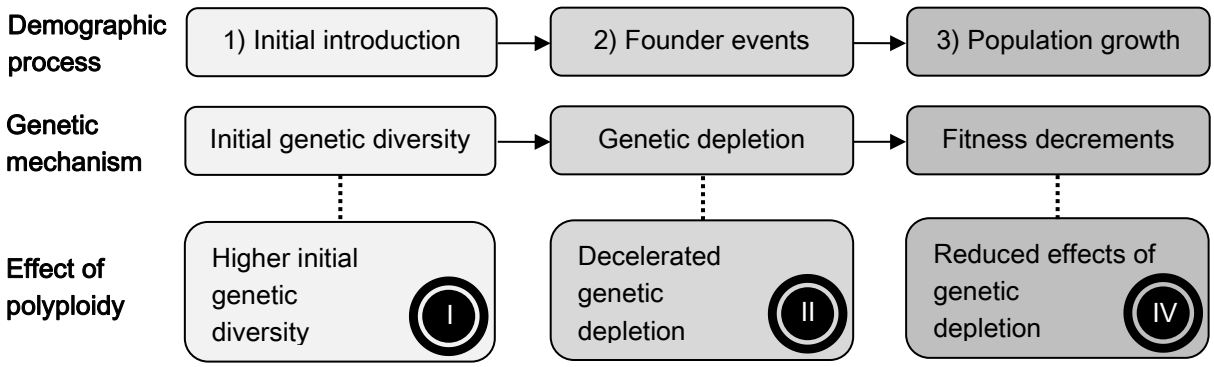

Fig. 5.1 Hierarchical components of the successive population genetic mechanisms that enhance the colonization capabilities in tetraploid as compared to diploid *Centaurea stoebe*. The mechanisms act at three levels of demographic processes. Black circles refer to the number of the working hypotheses (see Table 5.1).

Importantly, polycarpy can add to the decelerating effects of genetic drift in polyploids (see ρ-statistics; Table 2.2, Fig. 2.3). The higher re-sprouting of polycarpic tetraploids than monocarpic diploids (Fig. 4.2) reduces random fluctuations in population sizes, especially in erratic environments (e.g. re-sprouting can restore the number of flowering individuals following strong mowing events). More specifically, under fluctuations of mate availbility (e.g. temporary reductions of the *S*-allele pool), polycarpic individuals can persist without sexual reproduction

and may find suitable mating partners in the following season (Wagenius *et al.* 2007). Thus, **polycarpy is illustrated to facilitate the beneficial effects of polyploidy** as it helps to maintain genetic diversity during demographic oscillitations (see also Nybom 2004).

Nevertheless, it is pertinent to recognize that tetraploids can be prone to genetic depletion in early invasion stages (chapter 2) and that they suffer from its consequences (chapters 3 and 4). In fact, tetraploid *C. stoebe* occupies a tremendous ecological and climatic niche space across the North American continent (Broennimann *et al.* 2007; 2012), and it is one of the most devastating weeds in large parts of its invasive range (see Box A1.1). Thus, it seems astounding that its invasion is currently not a global, but exclusively a North American phenomenon. For example, tetraploid *C. stoebe* was introduced in Canberra and its surroundings (see Hufbauer and Sforza 2008), but the species never established in Australia (NSW Invasive Plants & Animals Enquiry 2016). Consequently, stochastic processes such as founder effects are likely to contribute, at least partially, to the rather restricted distribution at the global scale. However, if founder populations of tetraploid *C. stoebe* outlast early-acting invasion filters, **selection towards restoring genetic diversity** can strongly favor population admixture (see Verhoeven *et al.* 2011), which can ultimately initiate a "catapult effect" in population growth (see Fig. 5.2a).

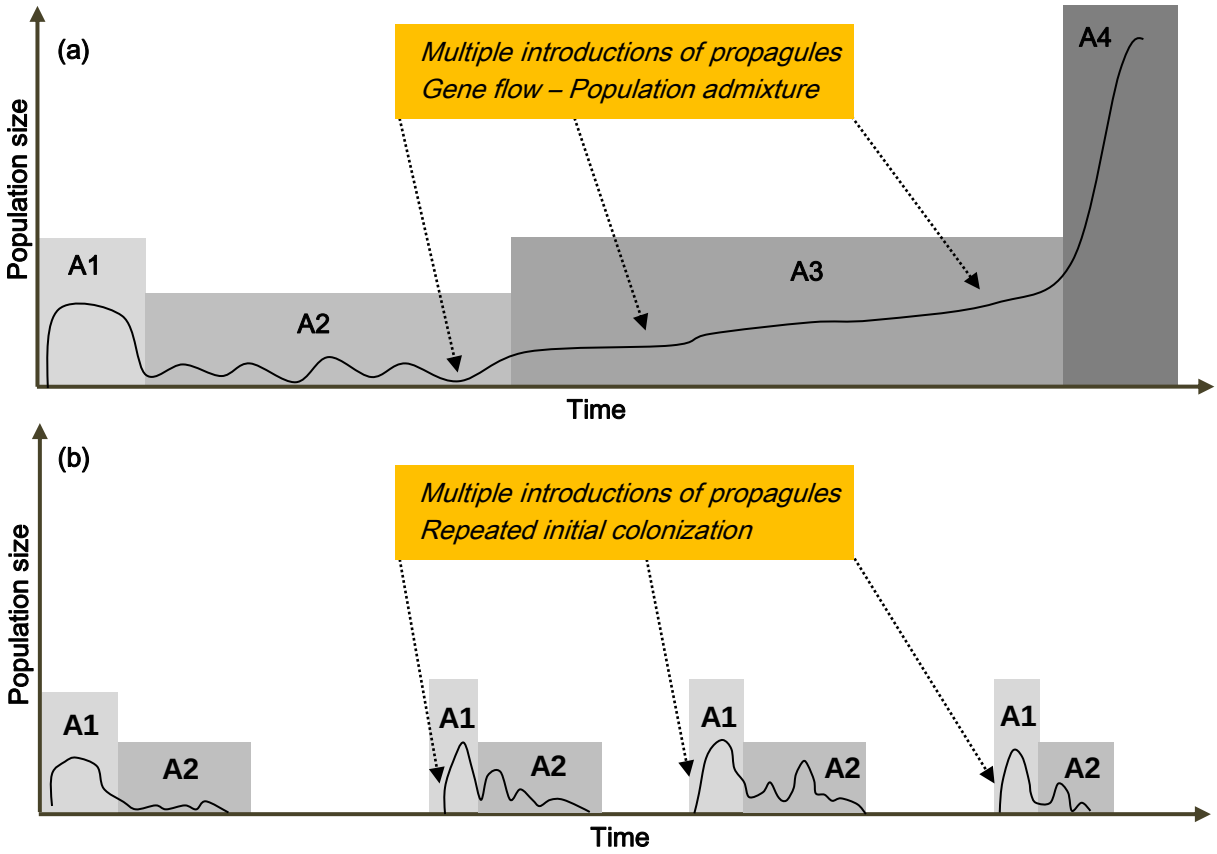

Fig. 5.2 Putative population size dynamics of (a) tetraploid and (b) diploid founder populations of *Centaurea stoebe* across the typical stages of an invasion. The tetraploid scenario (a) shows initial fluctuations in population growth rate due to genetic depletion, which is counteracted by population admixture following multiple introductions. In contrast, the diploid scenario (b) shows that the founder population does not endure the critical colonization stage and thus, multiple introductions result in new (separate) initial introductions, which again fail to establish self-perpetuating populations. Note that the stages are concordant with the invasion stages of Fig. 1.1: A1, initial introduction; A2, primary colonization; A3, primary naturalization; A4, primary invasive spread.

Successful establishment in a non-native range is not simply predefined by species identity, but founder populations may fail or succeed according to multi-dimensional acting key filters (see Fig. 1.2) – a process that follows distinct probabilistic rules under certain scenarios. I argue that the overcoming of early invasion stages seems critical for *C. stoebe*. By that, the lag-phase observed in NA4x (Broennimann *et al.* 2014) may coincide with genetic depletion in the beginning of the invasion, which was followed by multiple introductions that restored genetic diversity (Prentis *et al.* 2008). Note that such a postulated development of founder populations largely correlates with the previously outlined range size dynamics across the invasion stages (compare Figs. 1.1 and 5.2a). More theoretically, the presented scenarios may also imply that overcoming founder effects can initiate further invasions of *C. stoebe* at the global scale (for areas, where the environmental niche matches). Management measures should therefore consider to eradicate initial founder populations of (both cytotypes of) *C. stoebe,* particularly where the species is not established yet. In regions where *C. stoebe* is already more widespread, purposeful reductions of population sizes combined with inhibition of gene flow between populations may facilitate other commonly applied measures to repress its abundance.

The most important finding of my PhD thesis is that the tetraploid subspecies is much more likely to endure phases of demographic disequilibria than diploids. While tetraploids have a great potential to buffer founder effects (Fig. 5.1), diploid founder populations may face more urgent issues when experiencing founder effects, which increases the probability of local extinction, even before new genetic material may restore genetic diversity (compare Figs. 5.2a and 5.2b). My findings add to previous studies that emphasized various pre-adaptive traits of tetraploids to potentially increase their invasiveness as compared to diploids (see Box 1.4). All of these mechanisms are mutually non-exclusive from another, and can interact with the presented colonization genetic processes (e.g. through IxE interactions). My thesis consequently enhances the knowledge on the ecology of one of the worldwide most aggressive weeds. Further results on the biology of *C. stoebe*, which are apart my main focus, are summarized in Box 5.1.

The investigated EU4x populations show a striking switch from occupying natural habitats east of the 19th longitudinal degree to ruderal habitats west of it (chapter 2). This switch underscores that the recent expansion of EU4x towards Central Europe takes place predominately in ruderal habitats (see also Otisková *et al.* 2014). Moreover, I did not find evidence for purging of genetic load under natural conditions in *C. stoebe* populations (chapter 3). This finding aligns with current concepts, which suggest that efficient purging is not an omnipresent phenomenon as soon as inbreeding occurs, but depends on specific conditions in the population history (see Keller *et al.* 2012b). Pre-adaptive divergence in disturbance tolerance was found between ruderal and natural populations (chapter 4). According to the AIAI-theory Hufbauer *et al.* (2012), such pre-adaptation to human-modified environments can crucially increase invasiveness in introduced offspring descending from native ruderal populations as compared to offspring from natural populations.

Box 5.1 Findings of the preceding chapters, which were not in the main scope of my thesis, but revealed novel information on the biology of *Centaurea stoebe*.

5. 2 Conclusions and future challenges: Implications for invasion biology and more general eco-evolutionary processes

My thesis facilitates a deeper understanding of the overrepresentation of polyploids among invasive species, because polyploid complexes are the most appropriate model systems to detect mechanisms that generate this biogeographical pattern (te Beest *et al.* 2011). Particularly, I highlight three levels (Fig. 5.1), at which polyploidy can support the outlasting of founder effects. My findings are not purely case-specific, because theoretical assumptions underline that, at all three propagated levels, polyploids are likely to show on average 1) higher initial genetic diversity (Soltis and Soltis 2000), 2) reduced effects of genetic drift (Ronfort *et al.* 1998) and 3) reduced inbreeding depression (Soltis and Soltis 2000) as compared to diploids. The representativeness and limitations of my results urgently need to be validated with additional case studies, meta-analyses and modeling approaches. Suitable model species include several other prominent examples of polyploid complexes, in which the ratio of polyploid to diploid populations is highly increased in the invasive range as compared to the native range: e.g. *Senecio inaequidens* (Lafuma *et al.* 2003), *Lythrum salicaria* (Kubátová *et al.* 2008)*, Brachypodium distachyon* (Bakker *et al.* 2009), *Vicia cracca* (Trávníček *et al.* 2010) and *Oxalis pes-caprae* (Castro *et al.* 2009).

If reduced negative effects of genetic depletion may indeed significantly contribute to the overrepresentation of polyploids, such relationship may, in turn, support another, **more general implication on the invasiveness of species**, that is: mechanisms that help to endure founder effects might enhance the likelihood to become invasive (Allendorf and Lundquist 2003). In this context, some previous opinions doubted the ecological importance of the genetic paradox of invasions (Roman and Darling 2007; Hufbauer 2008). However, together with several experimental studies of the last years (e.g. Firestone and Jasieniuk 2012a; 2012b; 2012c; Mullarkey *et al.* 2013; Hufbauer *et al.* 2013; Szűcs *et al.* 2014; Schrieber *et al.* submitted), the results of my work demonstrate that genetic depletion can obviously play a role for shaping colonization success. These findings add to latest research concepts (Estoup *et al.* 2016; Schrieber and Lachmuth 2016), which should stimulate further lively debates on the significance of the genetic paradox of invasions for species distribution. Thereby, it is necessary to understand that comparable levels of current genetic diversity between introduced and invasive ranges do not inevitably proof the absence of bottlenecks during initial stages of invasion (Keller *et al.* 2012a). More effort should therefore be invested to understand how antagonists of genetic depletion can help founder populations to persist in periods of bottlenecks until gene flow and genetic admixture may occur. In addition to polyploidy, such antagonists include mass introduction, purging of genetic load, positive IxE interactions, polycarpy, etc. (see Box 1.3 for details). Interestingly, various successful invaders avoid potential reductions in genetic diversity in introduced as compared to their ancestral native populations due to predominately asexual reproduction, e.g. clonal propagation (Ahmad *et al.* 2008; Okada *et al.* 2009a), apomixis (Okada

et al. 2009b; Clark and Jasieniuk 2012) and selfing (Okada *et al.* 2013; 2015). All of such reproductive traits that counteract genetic depletion potentially favor successful colonization. Mechanistic frameworks that include information on species´ abilities to deal with founder effects can considerably improve our knowledge on species probabilities to become invasive.

Meanwhile, neutral genetic diversity estimated with relatively few markers does not necessarily correspond to quantitative trait variation (Estoup *et al.* 2016). Next generation sequencing (NGS) approaches may help estimating gene expression variation in native and introduced populations and can identify candidate genes that encounter rapid evolutionary changes (Egan *et al.* 2012; Peng *et al.* 2014; Rius *et al.* 2015). In addition, NGS data may decode the distribution of genetic load, which can unravel the molecular bases of 1) inbreeding depression in distinct populations (Hedrick *et al.* 2016) and of 2) the consequences of inbreeding across varying environmental scenarios (e.g. identifying conditionally deleterious genetic load; see Kariyat *et al.* 2012).

Additional experiments on IxE interactions should be conducted with manipulations of further biotic interactions that specifically characterize biological invasions (e.g. different herbivory regimes in native and introduced ranges). Such approaches can gain important knowledge on the eco-evolutionary interplay of bottlenecks and invasions (Schrieber *et al.* submitted). Experiments with artificial founder populations can deeply improve our understanding of colonization success (Hahn *et al.* 2012a; Firestone and Jasieniuk 2012a; Hufbauer *et al.* 2013). Such experiments should be conducted with inbred and outbred offspring for numerous generations (Estoup *et al.* 2016).

Moreover, the large among-population variation in NA4x, as found in genetic diversity (chapter 2), the extent of inbreeding depression (chapter 3) or disturbance tolerance (chapter 4) confirms that it remains indispensible to realize that invasive populations are not equal, and not static (Lachmuth 2012). Instead, invasions need to be regarded as spatio-temporally inconsistent with different invasive populations facing different key processes in their respective invasion stages (see Fig. 1.2). In addition, similar between-population variance may be found in the native range (Broennimann and Guisan 2008). However, numerous studies still test complex invasion hypotheses, while they ignore within-range variances in demographic and evolutionary history of populations. Where populations are sampled without considering discrete invasion stages, native vs. introduced approaches can fail to yield meaningful results (Broennimann *et al.* 2014).

My study adds to the current research in invasion biology. At the same time, biological invasions are big **unplanned experiments**, which enable scientists **to study complex principles in ecology and evolution** (Sax *et al.* 2007; Santos *et al.* 2012). In fact, biological invasions are just one piece of a wide spectrum of species movements (Hoffmann and Courchamp 2016). Thus, colonization processes, including the action of colonization filters, occur continuously throughout the entire distribution area of species, particularly in times of global change (Leimu *et al.* 2010; Brandvain and Wright 2016). Additionally, demographic fluctuations are not unique to colonization events, but permanently take place in the course of habitat fragmentation and ecosystem alterations

(Fischer and Lindenmayer 2007). As such, all of the above drawn conclusions can directly be applied in any research that considers demographic fluctuations, including conservation biology. In that context, it is notable that a meta-analysis of Pandit *et al.* (2011) found that diploids are more likely to be endangered than polyploids. Thus, my PhD thesis may also provide important insights in more general mechanisms in ecology and evolution. This aligns with the quotation form Charles Darwin (see page 3), who literally stated that our understanding of evolutionary processes seems rather limited *"until we can say why one species and not another becomes naturalized by man's agency in a foreign land".*

Chapter 6

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Chapter 7

SUMMARY / ZUSAMMENFASSUNG

SUMMARY

Premise of the studies: Polyploid plants show a higher probability to become invasive than diploids, however, the underlying mechanisms are not fully understood. Early-acting invasion filters may offer high explanatory power in understanding such biogeographical patterns, because they can inherently determine whether founder populations fail or succeed in colonizing a novel range. In addition, colonization events and their associated filters occur repeatedly in the course of range expansions (e.g. towards the expansion front). Surprisingly, the interplay of ecological and population genetic determinants of colonization success have not yet been addressed empirically in the context of the overrepresentation of polyploids among invasive species. The typical habitats of primary colonization are ruderal sites, which are characterized by high levels of anthropogenic disturbance that can cause physical damage reducing plant performance. Moreover, colonization often involves founder effects, which may result in severe genetic depletion. Such loss of genetic diversity can cause reduced adaptive capabilities, mate limitation (in self-incompatible plants), and inbreeding depression, which may be amplified under stress (e.g. physical damage) due to negative inbreeding-environment interactions. With this thesis, I aim to contribute to a more complete understanding of the processes that shape the colonization success of polyploids.

Materials and methods: My model organism, the polyploid complex *Centaurea stoebe* s.l., comprises three so-called geo-cytotypes: monocarpic diploids are more frequent than polycarpic (allo)tetraploids in the native range (Eurasia), whereas only tetraploids are reported from the invasive range (North America). To gain novel insights in this cytotype shift, I applied a broad variety of methods in a concerted manner, including flow-cytometry and microsatellite analyses (chapter 2) as well as a breeding experiment (chapter 3). Pollination success of outcrossings and sib-matings was recorded and fitness of the resultant inbred and outbred offspring was surveyed over one vegetation period (first months in the green house, then in the common garden). Subsequently, a clipping experiment was applied with this offspring (chapter 4). After harvesting final biomass, re-sprouting success was recorded in the following spring.

Key results: Compared to diploids, tetraploids maintain higher genetic diversity and face reduced genetic drift during genetic bottlenecks. Analyses of the small-scale genetic structure revealed that biparental inbreeding is common in natural populations of all three geo-cytotypes. Experimental inbreeding reduced pollination success to a comparable extent in both cytotypes, and caused inbreeding depression in offspring fitness, which was significantly more pronounced in diploids than in tetraploids. Clipping reduced performance and accelerated inbreeding depression in biomass, whereby both of these effects were similarly pronounced in both cytotypes. Re-sprouting in the following vegetation period was generally higher in tetraploids than

diploids (unaffected from both breeding and clipping treatment). Between native and invasive tetraploids, I did not observe differences in any of the above mentioned results.

Synthesis: My results suggest that founder effects may critically decrease population growth in both cytotypes of *C. stoebe*. In tetraploids, this may have contributed to the lag-phase of 50 years following the initial colonization of North America. Subsequently, multiple introductions with frequent population admixture may have facilitated the rapid spread of tetraploids across the non-native range. Most importantly, I highlight that the tetraploid subspecies is significantly more likely to overcome phases of demographic disequilibria than diploids. In particular, tetraploid founder populations may show high initial genetic diversity and they have a great potential to counteract genetic impoverishment and its harmful consequences. In addition, the polycarpy of tetraploids can support the outlasting of founder effects. Specifically, following strong disturbance events, re-sprouting in the next vegetation period can ensure constant population sizes in erratic environments. All of these aspects may enhance the colonization capabilities of tetraploids. In contrast, diploid populations may exhibit small initial genetic diversity and are prone to a high susceptibility to genetic depletion and its consequences. Under small or fluctuating population sizes, this may ultimately lead to the extinction of diploid founder populations during colonization. Therefore, my results substantially contribute to explaining the contrasting invasion success of the cytotypes of *C. stoebe*.

Major conclusions: My findings highlight the importance of studying colonization genetic processes with a view to gaining a more mechanistic knowledge on the role of polyploidy in plant invasions. Meanwhile, theoretical assumptions underline that my findings are not simply case-specific. Thus, my results may indeed contribute to understanding the overrepresentation of polyploids among invasive species. The relevance of the illustrated genetic processes in shaping the colonization success of polyploids may hitherto have been underestimated due to a lack of data on genetic depletion and its consequences in model systems that show distinct geo-cytotypes. Further studies on such model systems are necessary to test for generality and limitations in my results. Moreover, future studies should be complemented by long-term studies on population growth under various environmental conditions (e.g. testing further ecological meaningful inbreeding-environment interactions). In addition, next generation sequencing and modeling approaches seem promising for completing the picture on demographic dynamics in polyploid vs. diploid founder populations.

Keywords: biological invasions, clipping, colonization, founder effects, genetic drift, geo-cytotype, inbreeding depression, inbreeding-environment interactions, natural habitat, pre-adaptation, rapid evolution, ruderal habitat, sporophytic self-incompatibility, spotted knapweed

ZUSAMMENFASSUNG

Theoretischer Hintergrund: Polyploide Pflanzen neigen stärker als diploide Pflanzen dazu, invasiv zu werden, jedoch sind die zugrunde liegenden Mechanismen bisher unzureichend erforscht. Invasionsfilter, die in frühen Invasionsstadien agieren, beeinflussen in hohem Maße, ob Gründerpopulationen ein neues Areal erfolgreich kolonisieren. Daher trägt das Wissen über die Funktionsweise dieser Filter entscheidend dazu bei, biogeographische Verbreitungsmuster zu verstehen. Erstaunlicherweise gibt es bisher keine empirischen Untersuchungen, welche das Zusammenspiel von ökologischen und populationsgenetischen Kolonisationsprozessen mit dem Invasionserfolg polyploider Pflanzen in Verbindung gebracht haben. Während der Primärkolonisation werden bevorzugt ruderale Standorte besiedelt, in denen starke anthropogene Störung zu physischen Verletzungen von Pflanzen führen kann. Des Weiteren beinhalten Kolonisationsereignisse häufig Gründereffekte, welche die genetische Diversität verringern können. Dadurch kann es zu reduzierter Adaptationsfähigkeit und den Verlust geeigneter Fortpflanzungspartner in Gründerpopulationen selbstinkompatibler Pflanzen kommen. Zudem kann Inzuchtdepression auftreten, deren Auswirkung als Folge von Inzucht-Umwelt-Interaktionen verstärkt werden kann (z.B. durch physische Verletzungen). Diese Doktorarbeit soll dazu beitragen, ein besseres Verständnis über die Prozesse zu erlangen, die den Kolonisationserfolg polyploider Arten unterstützen.

Material und Methoden: Mein Modellsystem, der polyploide Artkomplex *Centaurea stoebe* s.l., beinhaltet drei Geo-cytotypen: Die diploide Unterart kommt häufiger im nativen Areal (Eurasien) vor als die allo-tetraploide Unterart, wohingegen im invasiven Areal (Nord-Amerika) bisher ausschließlich Vorkommen der tetraploiden Subspezies dokumentiert wurden. Um einen tieferen Einblick in dieses Verbreitungsmuster zu erlangen, wurden in meinen Studien vielfältige Methoden angewendet, unter anderem Flowcytometrie- und Mikrosatelliten-Analysen (Kapitel 2) sowie ein Kreuzungsexperiment (Kapitel 3). Der Bestäubungserfolg von Auskreuzungen und Inzuchtbehandlungen wurde dokumentiert und die Fitness der resultierenden F1-Nachkommen bestimmt. Mit den F1-Nachkommen wurde danach ein Clipping-Experiment durchgeführt (Kapitel 4). Daran anschließend wurde die Biomasse geerntet und im darauffolgenden Frühling der Wiederaustrieb der Individuen beobachtet.

Schlüsselergebnisse: Tetraploide Populationen wiesen im Vergleich zu diploiden Populationen eine höhere genetische Diversität und verringerte Auswirkungen genetischer Drift auf. Die Analyse der spatialen genetischen Struktur innerhalb natürlicher *C. stoebe*-Populationen zeigte, dass in allen drei Geo-cytotypen häufig biparentale Inzucht auftritt. Experimentelle Inzucht resultierte in verringertem Bestäubungserfolg als bei Auskreuzungen, wobei dieser Effekt in beiden Cytotypen gleich stark ausgeprägt war. Außerdem wurde Inzuchtdepression in den F1-Nachkommen festgestellt, welche signifikant stärker in Diploiden als in Tetraploiden war. Clipping verringerte die Fitness und verstärkte Inzuchtdepression. Beide Effekte unterschieden sich jedoch nicht zwischen den beiden Cytotypen. Der Wiederaustrieb im Frühjahr war höher in

Tetraploiden als in Diploiden (unabhängig der Kreuzungs- und Clippingbehandlung). Zwischen nativen and invasiven Tetraploiden wurden keine signifikanten Unterschiede in einem der obengenannten Ergebnisse festgestellt.

Synthese: Gründereffekte können in beiden Cytotypen von *C. stoebe* zu drastischen Verringerungen der Populationswachstumsraten führen. Dies könnte zu der 50 Jahre langen Lag-Phase während der invasiven Besiedlung der tetraploiden Subspezies in Nord Amerika beigetragen haben. Anschließend haben möglicherweise wiederholte Einführungen und die Vermischung unterschiedlicher Genpoole die rasante Ausbreitung der Art über den nordamerikanischen Kontinent begünstigt. Vor allem zeigte sich, dass die tetraploide Unterart signifikant bessere Fähigkeiten hat Gründereffekte zu überstehen als die diploide Subspezies. Neben der höheren genetischen Diversität waren die negativen genetischen Konsequenzen genetischer Verarmung in Tetraploiden vergleichsweise schwach ausgeprägt. Zudem hilft der ausdauernde Lebenszyklus demographische Oszillationen in tetraploiden Populationen zu dämpfen, indem beispielsweise nach starken Störungsereignissen durch den Wiederaustrieb im darauffolgenden Jahr die Populationsgrößen relativ konstant gehalten werden. Im Gegensatz dazu weisen diploide Populationen geringere genetische Diversität auf und sind zudem anfälliger für die negativen Konsequenzen genetischer Verarmung. Im Zuge kleiner und/oder schwankender Populationsgrößen kann dies zum Erlöschen diploider Gründerpopulationen führen. Die Ergebnisse meiner Arbeit tragen folglich substantiell dazu bei, das Verbreitungsmuster der beiden Cytotypen von *C. stoebe* zu erklären.

Hauptschlussfolgerungen: Kolonisationsgenetische Prozesse können einen bedeutenden Beitrag für das Verständnis liefern, weshalb polyploide Pflanzen eine erhöhte Wahrscheinlichkeit haben invasiv zu werden. Theoretische Annahmen unterstützen meine Thesen und verdeutlichen, dass meine erlangten Erkenntnisse nicht rein fall-spezifisch sind. Die ökologische Relevanz der Mechanismen, die in meiner Arbeit hervorgehoben wurde, wurde bisher gänzlich unterschätzt, da es keine ähnlichen Studien mit Modellsystemen gab, die ein solches Geo-cytotyp-Verbreitungsmuster aufweisen. Nachfolgende Studien sollten die Generalisierbarkeit und Einschränkungen meiner Ergebnisse überprüfen. Solche Studien sollten Langzeituntersuchungen von Populationswachstumsraten unter variierenden Umweltbedingungen einbeziehen (z.B. um andere kolonisations-relevante Umwelt-Inzucht-Interaktionen zu quantifizieren). Next-Generation-Sequenzierungsmethoden und Modellierungsansätze können das Verständnis der Gründerpopulationsdynamiken in polyploiden und diploiden Arten bedeutend voran bringen.

Schlüsselwörter: Biologische Invasionen, gefleckte Flockenblume, genetische Drift, Geo-cytotyp, Gründereffekte, Inzuchtdepression, Inzucht-Umwelt-Interaktionen, Kolonisation, Mikroevolution, natürliches Habitat, Pre-adaptation, ruderales Habitat, sporophytische Selbstinkompatibilität

Chapter 8

APPENDIX

A1: Supplemental Information Chapter 1

Although enormous efforts were undertaken to control tetraploid *C. stoebe* (Maddox 1982; Sheley *et al.* 1998; Story *et al.* 2000; Story *et al.* 2006), it represents a notorious invader that is estimated to cause economical damage of $150*10^6$ US $ per year (Van Driesche *et al.* 2002). The low nutritious value and high catechin content lead to the avoidance by grazing life stock (Campobasso *et al.* 1994). Tetraploid *C. stoebe* can have a devastating impact on local plant diversity, as it is known to outcompete natives, especially in North West America (Ridenour and Callaway 2001; Mangold and Sheley 2008; Callaway *et al.* 2011). Studies on the competitive consequences of *C. stoebe* investigated differences in net competitive interactions of native vs. non-native communities (Callaway and Aschehoug 2000; He *et al.* 2009; Thorpe *et al.* 2009; Knochel and Seastedt 2010; Callaway *et al.* 2011; Aschehoug *et al.* 2012; Sun *et al.* 2015a; 2015b), and, on the other hand, identified several native competitors or plant communities that can hamper the invasive success of NA4x (Pokorny *et al.* 2005; Maron and Marler 2008; Reinhart and Rinella 2010; Emery and Rudgers 2012; Metlen *et al.* 2013; Metlen and Callaway 2014). The overwhelming success of NA4x in suppressing native species is frequently attributed to allelochemical root exudates affecting competitors (Callaway and Aschehoug 2000; Bais *et al.* 2003; Thorpe *et al.* 2009), which yielded in the postulation of the well-known novel weapons hypothesis (Callaway and Ridenour 2004). However, whether the amounts of (±)-catechin produced by *C. stoebe in vivo* are allelopathic enough to represent a key mechanism that explains the species´ invasion success is under controversial debate (Blair *et al.* 2006; Blair *et al.* 2008; Bais and Kaushik 2010). Meanwhile, disturbance has been shown to support the establishment of *C. stoebe* into intact communities in the invasive range (Emery and Rudgers 2012; Maron *et al.* 2013). Further previous studies on *C. stoebe* investigated the effects of herbicides (Sheley *et al.* 2000; 2004; Ortega and Pearson 2010; 2011; MacDonald *et al.* 2013), fungal endophytes (Shipunov *et al.* 2008; Newcombe *et al.* 2009; Xiao *et al.* 2012; Aschehoug *et al.* 2012), root-associated herbivores (Müller 1989a; 1989b; Steinger and Müller-Schärer 1992; Collins and Müller-Schärer 2012; Hahn *et al.* 2012; Mosley *et al.* 2015), capitula feeders (Corn *et al.* 2006; 2007; Story *et al.* 2008; Knochel and Seastedt 2010; Knochel *et al.* 2010; Ortega *et al.* 2012), grazing (Sheley *et al.* 2004; Thrift *et al.* 2008; Mosley *et al.* 2015), precipitation (Corn *et al.* 2007; Maines *et al.* 2013a; 2013b), mycorrhizal fungi (Zabinski *et al.* 2002; Callaway *et al.* 2004a; Harner *et al.* 2009; Emery and Rudgers 2012; Maron *et al.* 2013), fire regimes (Emery and Gross 2005; MacDonald *et al.* 2007; Vermeire and Rinella 2009), and nutrient availability (Jacobs *et al.* 2000; Suding *et al.* 2004; Maron and Marler 2008; He *et al.* 2012) on the performance of *C. stoebe* populations. Moreover, tetraploid *C. stoebe* was shown to alter the microbial soil community (Ridenour and Callaway 2003; Callaway *et al.* 2004b; Mummey and Rillig 2006), and as a consequence, the nutrient availability (Thorpe *et al.* 2006).

Box A1.1 Literature overview of topics that have been examined in Centaurea stoebe, but were not considered in this PhD-thesis, mainly because they targeted specific biotic interactions in far-advanced invasion stages. Due to the huge number of publications on *Centaurea stoebe*, this box does not cover all topics, and within each objective, the given citations represent only a subset of the studies that were conducted.

A2: Supplemental Information Chapter 2

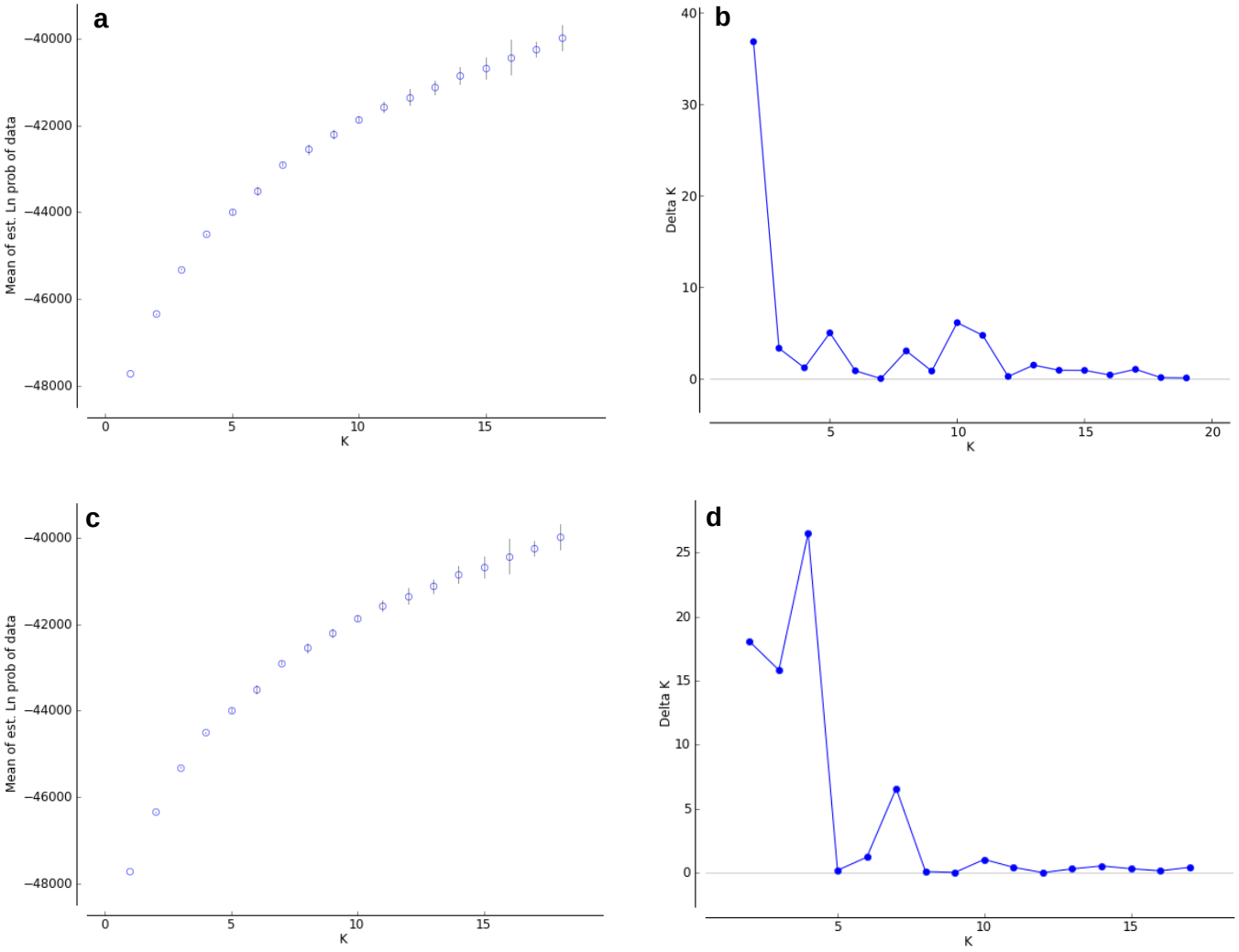

Fig. A2.1 Bayesian inference to estimate the most likely partitioning (*K*) in the Structure analyses. **a** Log-likelihood for given *K* clusters obtained through 20 runs with the diploid data set. **b** Delta *K* statistics of Evanno *et al.* (2005) to identify the most probable *K* in the diploid data set. **c** Log-likelihood for given *K* clusters obtained through 20 runs with the tetraploid data set. **d** Delta *K* statistics of Evanno *et al.* (2005) to identify the most probable *K* in the tetraploid data set. All figures were illustrated using Structure Harvester (Earl and vonHoldt 2012).

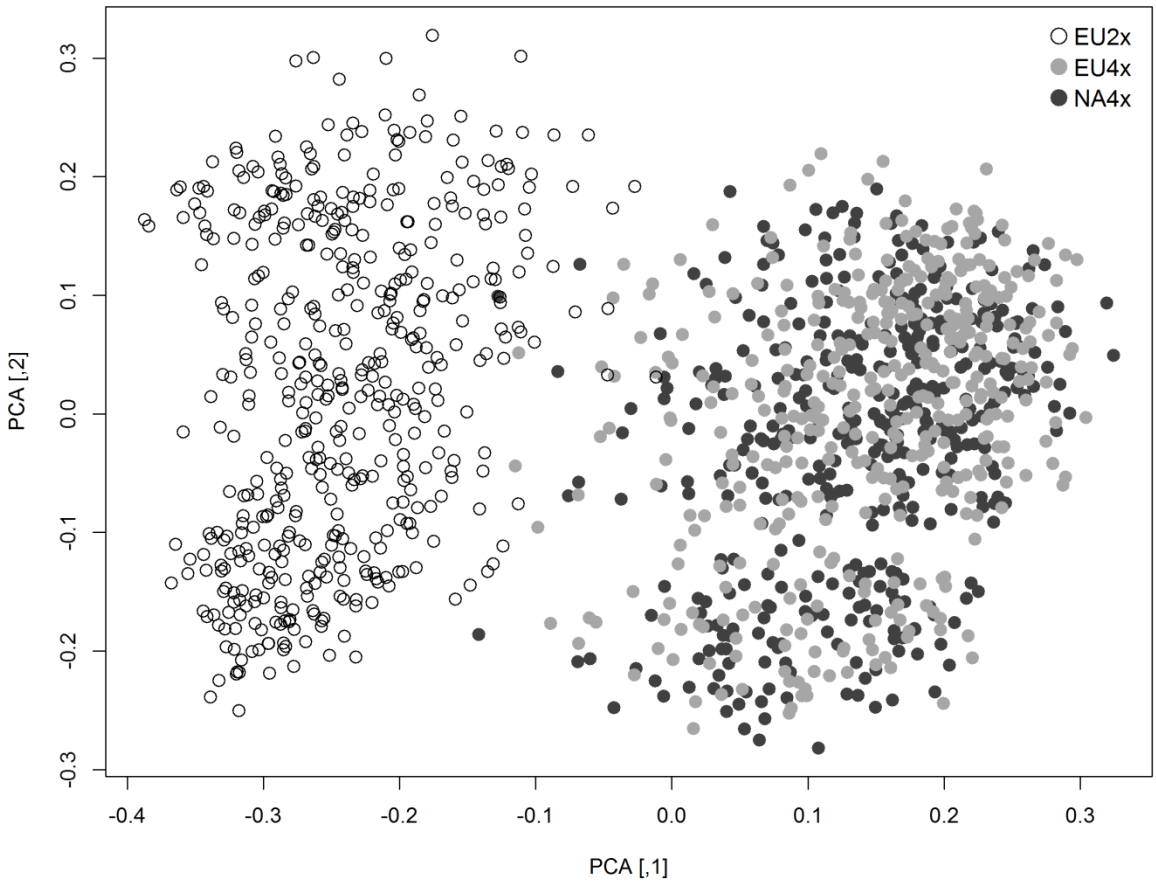

Fig. A2.2 Principal Component Analysis (PCA) of the full data set including all three geo-cytotypes. Colors correspond to the geo-cytotypes [white = EU2x (native range, diploid); light grey = EU4x (native range, tetraploid); dark grey = NA4x (invasive range, tetraploid); see legend]. Note that we only analyzed samples from the majority cytotype in mixed-ploidy populations (i.e. 16, 18, 23, 30 and 33).

A3: Supplemental Information Chapter 3

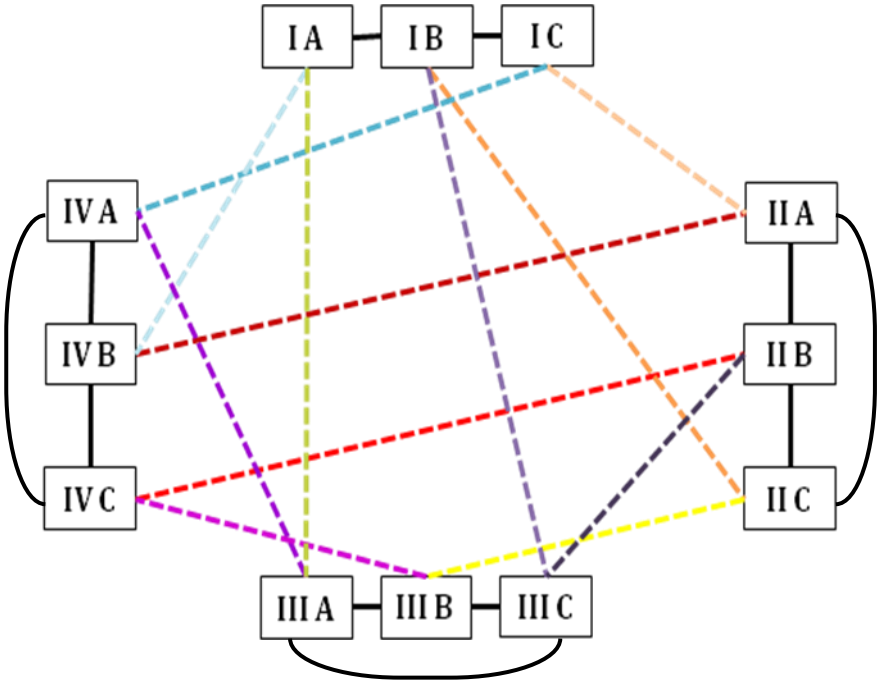

Fig. A3.1 Scheme of the experimental crosses performed within each population. Roman numerals refer to seed families within a population, whereas letters refer to individuals within the seed family. Black solid lines refer to inbred crosses and colored dashed lines refer to outcrosses. For biparental inbreeding, we crossed each individual with two siblings from the same seed family. For outbreeding, each individual was crossed with two individuals from other seed families from the population. The breeding design varied among populations according to the availability of flowering plants per seed family (see Table A3.2).

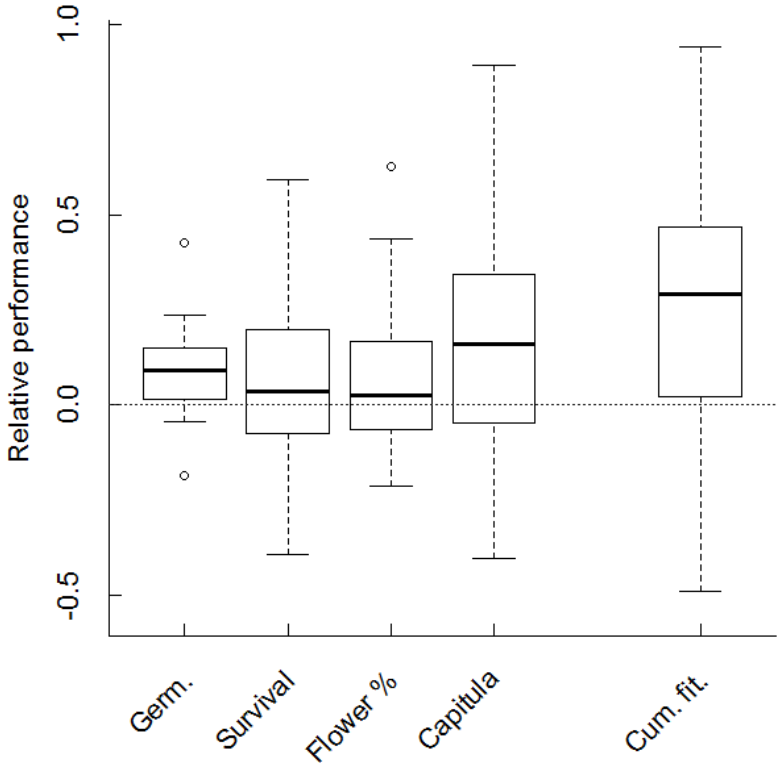

Fig. A3.2. Relative performances of inbred vs. outbred progeny in four performance traits and in cumulative fitness. Relative performance was calculated according to Angeloni, Ouborg & Leimu (2011) as relative performance = (W_o–W_i)-1 if $W_i > W_o$ or relative performance = 1−(W_i−W_o), if $W_i < W_o$. W_i is the performance of inbred progeny and W_o the performance of outbred progeny. This index does not require the specification of a particular inbreeding level. Instead, relative performance provides a relative estimate of inbred vs. outbred performance, which can be compared between traits. Relative performance values > 0 (i.e. above the gray dotted line of y = 0) indicate inbreeding depression, whereas relative performance values < 0 indicate outbreeding depression (Angeloni, Ouborg & Leimu 2011). All traits showed positive relative performance and thus evidence for inbreeding depression. A linear model revealed that relative performance did not differ among fitness components ($F_{3,149}$ = 1.23, P > 0.05). Cumulative fitness was not included in the statistical analysis because it represents the product of the single fitness components (germination success, survival, flowering probability and number of capitula). Germ., germination success; Flower %, flowering probability; Capitula, number of capitula; Cum. fit., cumulative fitness.

Table A3.1 Populations involved in the breeding experiment. Population numbers correspond to those in Rosche *et al.* (re-submitted). Asterisks highlight populations for which we only recorded cross-compatibility and germination success but no other fitness components. Relative performance was calculated according to Angeloni, Ouborg & Leimu (2011) as relative performance = (W_o–W_i)-1 if W_i > W_o or relative performance = 1−(W_i−W_o), if W_i < W_o. W_i is the performance of inbred progeny and W_o the performance of outbred progeny. The given relative performance was estimated for the cumulative fitness, which is the product of the single fitness components (germination success, survival, flowering probability and number of capitula).

Population	Country	Locality	Latitude	Longitude	ρ	RP
Native range, diploid (EU2x)						
5	DE	Sachsen-Anhalt, Lieskau	51.50	11.86	0.16	0.34
6 *	DE	Sachsen-Anhalt, Amselgrund	51.50	11.94	0.21	n.e.
8 *	DE	Sachsen, Bautzen	51.18	14.42	0.50	n.e.
10	CH	Graubünden, Ramosch	46.83	10.40	0.17	0.51
11	IT	Trentino-Alto Adige, Castelle Penede	45.88	10.89	0.24	0.63
12	IT	Trentino-Alto Adige, Rafenstein	46.53	11.36	0.07	0.45
13	CZ	Jihočeský, Rájov	48.84	14.37	0.47	0.51
14	AT	Kärnten, Völkermarkt	46.65	14.91	0.07	0.25
15	SI	Murska Sobota, Murska Sobota	46.63	16.21	0.20	0.20
16	SK	Bratislavský, Sandberg	48.20	16.97	0.08	0.47
17	HU	Zala, Balatongyörök	46.76	17.34	0.11	0.60
18	HU	Pest, Csepel Island	47.33	18.95	0.05	0.94
19	SK	Košický, Gelnica	48.85	20.93	0.21	0.89
20	RO	Iaşi, Valea lui David	47.20	27.47	0.13	0.04
Native range, tetraploid (EU4x)						
22	DE	Sachen-Anhalt, Dieskau	51.44	12.04	0.25	-0.45
24	DE	Bayern, Nürnberg	49.39	11.08	0.08	0.28
25	AT	Tirol, Starkenbach	47.19	10.64	0.08	0.27
26	AT	Niederösterreich, Krems	48.43	15.65	0.19	0.62
27	SK	Bratislavský, Závod	48.52	17.02	0.06	-0.17
28	SK	Trnavský, Trnava	48.38	17.60	0.09	0.19
29	SK	Trenčiansky, Nové Mesto nad Váhom	48.76	17.84	0.24	-0.38
31	HU	Budapest, Gellérthegy	47.49	19.05	0.09	0.56
32 *	RO	Satu Mare, Urziceni Pădure	47.70	22.44	0.06	n.e.
33	RO	Cluj, Cheile Turzii	46.56	23.70	0.05	-0.11
34	RO	Alba, Poşaga de Jos	46.43	23.45	0.05	0.02
35	RO	Suceava , Oprişeni	47.48	26.27	0.12	0.19
37	RO	Iaşi, Parâul Cacaina	47.19	27.59	0.14	0.29
Invasive range, tetraploid (NA4x)						
39	CA	British Columbia, Tsawwassen	49.02	-123.11	0.05	-0.11
40	US	Washington, Seattle	47.48	-122.24	0.08	-0.15
41	US	Washington, Stevens Pass	47.79	-120.89	0.09	0.31
42	CA	British Columbia, Kamloops	50.71	-120.37	0.09	0.20
43	CA	British Columbia, Revelstoke	51.02	-118.21	0.12	-0.46
44	US	Idaho, Coeur d´Alene	47.65	-116.72	0.04	0.41
45	US	Idaho, Emerald Creek	47.07	-116.33	0.03	0.32
46 *	CA	Alberta, Okotoks	50.72	-113.95	0.15	n.e.
47	US	Montana, Glacier National Park	48.70	-113.69	0.20	-0.15
48	US	Montana, Clark Fork River	46.87	-113.99	0.03	0.54
49	US	Montana, Salmon Lake	47.12	-113.43	0.05	0.33
50	US	Montana, Butte	46.00	-112.61	0.05	0.41
53 *	US	Montana, West Yellowstone	44.73	-111.11	0.35	n.e.
54	US	Montana, Kendalls´ Ranch	45.53	-111.18	0.35	-0.49
56	US	Wyoming, Mammoth	44.97	-110.69	0.11	0.30

ρ, within-population relatedness; RP, relative performance of the cumulative fitness; AT, Austria; CA, Canada; CH, Switzerland; CZ, Czech Republic; DE, Germany; HU, Hungary; IT, Italy; RO, Romania; SI, Slovenia; SK, Slovakia; US, USA; n.e., not estimated.

Table A3.2 Inbred and outbred lineages from which cross-compatibility and fitness components were obtained. The table shows the paternal (donor) and maternal (acceptor) mating partners of the lineages. Asterisks highlight populations for which we only recorded cross-compatibility and germination success but no other fitness components. Mating partners are encoded as follows: underscores separate information on population, seed family and individual within seed family; Arabic numerals refer to populations, Roman numerals refer to seed families and letters refer to individuals within each seed family. Numbers of populations correspond to those in Table A3.2. Cohort 1, 2, 3 refers to the start of the germination experiment, which took place on October 20th, October 27th and November 11th, respectively.

Population	Donor	Acceptor	Cohort	Lineage ID
Native range, diploid (EU2x), inbred				
5	5_I_C	5_I_R	1	i5_1
5	5_I_B	5_I_C	1	i5_2
5	5_II_F	5_II_E	1	i5_3
5	5_II_E	5_II_G	1	i5_4
5	5_III_A	5_III_F	1	i5_5
6 *	6_I_L	6_I_K	3	i6_1
6 *	6_IV_A	6_IV_E	3	i6_2
6 *	6_I_L	6_I_H	3	i6_3
8 *	8_I_B	8_I_A	3	i8_1
8 *	8_II_G	8_II_R	3	i8_2
8 *	8_II_J	8_II_R	3	i8_3
8 *	8_III_A	8_III_B	3	i8_4
10	10_I_G	10_I_N	1	i10_1
10	10_I_A	10_I_N	1	i10_2
10	10_II_N	10_II_C	1	i10_3
10	10_II_A	10_II_C	1	i10_4
10	10_IV_A	10_IV_N	2	i10_5
11	11_I_I	11_I_G	1	i11_1
11	11_I_G	11_I_I	1	i11_2
11	11_II_I	11_II_A	1	i11_3
11	11_III_C	11_III_H	1	i11_4
11	11_III_H	11_III_C	1	i11_5
11	11_III_J	11_III_H	1	i11_6
11	11_II_H	11_II_N	2	i11_7
12	12_II_A	12_II_E	1	i12_1
12	12_II_E	12_II_F	1	i12_2
12	12_11J	12_11D	1	i12_3
12	12_11P	12_11B	1	i12_4
12	12_III_H	12_III_D	1	i12_5
12	12_III_B	12_III_D	1	i12_6
13	13_I_A	13_I_G	2	i13_1
13	13_I_D	13_I_A	2	i13_2
13	13_III_A	13_III_H	2	i13_3
13	13_III_F	13_III_A	2	i13_4
13	13_IV_G	13_IV_E	2	i13_5
13	13_IV_H	13_IV_G	2	i13_6
14	14_I_G	14_I_J	1	i14_1
14	14_I_J	14_I_F	1	i14_2
14	14_III_J	14_III_B	1	i14_3
14	14_III_A	14_III_J	1	i14_4
14	14_IV_B	14_IV_J	1	i14_5
14	14_IV_E	14_IV_J	1	i14_6
15	15_I_M	15_I_P	1	i15_1
15	15_I_M	15_I_J	1	i15_2
15	15_II_P	15_II_K	1	i15_3
15	15_II_P	15_II_J	1	i15_4
15	15_IV_P	15_IV_N	1	i15_5
15	15_IV_D	15_IV_F	1	i15_6

16	16_I_E	16_I_i	1	i16_1
16	16_I_D	16_I_E	1	i16_2
16	16_II_F	16_II_B	1	i16_3
16	16_II_N	16_II_G	1	i16_4
16	16_IV_J	16_IV_B	1	i16_5
16	16_IV_N	16_IV_H	1	i16_6
17	17_II_F	17_II_A	1	i17_1
17	17_II_C	17_II_E	1	i17_2
17	17_III_A	17_III_D	1	i17_3
17	17_III_E	17_III_D	1	i17_4
17	17_IV_D	17_IV_K	1	i17_5
17	17_IV_A	17_IV_J	1	i17_6
18	18_I_J	18_I_A	1	i18_1
18	18_II_B	18_II_F	1	i18_2
18	18_II_A	18_II_F	1	i18_3
18	18_IV_E	18_IV_B	1	i18_4
18	18_III_N	18_III_A	1	i18_5
18	18_II_B	18_II_E	1	i18_6
18	18_III_N	18_III_F	2	i18_7
19	19_I_a	19_I_i	1	i19_1
19	19_II_r	19_II_e	2	i19_2
19	19_III_D	19_III_A	2	i19_3
19	19_IV_B	19_IV_A	2	i19_4
19	19_IV_R	19_IV_B	2	i19_5
20	20_I_C	20_I_A	2	i20_1
20	20_I_N	20_I_A	2	i20_2
20	20_II_B	20_II_A	2	i20_3
20	20_IV_N	20_IV_J	2	i20_4

Native range, tetraploid (EU4x), inbred

22	22_I_A	22_I_J	1	i22_1
22	22_I_B	22_I_A	1	i22_2
22	22_I_J	22_I_B	1	i22_3
22	22_II_A	22_II_C	1	i22_4
22	22_IV_C	22_IV_F	1	i22_5
22	22_IV_F	22_IV_C	1	i22_6
24	24_II_A	24_II_N	1	i24_1
24	24_II_D	24_II_F	1	i24_2
24	24_III_B	24_III_R	1	i24_3
24	24_III_C	24_III_B	1	i24_4
24	24_I_E	24_I_C	1	i24_5
24	24_II_N	24_II_F	1	i24_6
25	25_I_B	25_I_E	1	i25_1
25	25_III_B	25_III_F	1	i25_2
25	25_III_E	25_III_B	1	i25_3
25	25_IV_J	25_IV_C	1	i25_4
25	25_IV_C	25_IV_N	1	i25_5
26	26_I_F	26_I_A	1	i26_1
26	26_II_E	26_II_D	1	i26_2
26	26_IV_H	26_IV_F	1	i26_3
26	26_II_C	26_II_D	1	i26_4
26	26_IV_F	26_IV_B	1	i26_5
26	26_III_R	26_III_B	1	i26_6
27	27_II_B	27_II_F	1	i27_1
27	27_II_B	27_II_C	1	i27_2
27	27_III_B	27_III_C	1	i27_3
27	27_IV_D	27_IV_E	1	i27_4
27	27_IV_E	27_IV_D	1	i27_5
27	27_IV_B	27_IV_D	1	i27_6
28	28_I_N	28_I_G	1	i28_1
28	28_II_E	28_II_D	1	i28_2

28	28_III_C	28_III_D	2	i28_3
28	28_I_N	28_I_C	2	i28_4
28	28_III_D	28_III_N	2	i28_5
28	28_III_N	28_III_C	2	i28_6
29	29_I_A	29_I_N	2	i29_1
29	29_III_E	29_III_N	2	i29_2
29	29_I_N	29_I_A	2	i29_3
29	29_III_C	29_III_E	2	i29_4
29	29_IV_A	29_IV_B	2	i29_5
31	31_I_F	31_I_A	1	i31_1
31	31_I_A	31_I_F	1	i31_2
31	31_I_F	31_I_D	1	i31_3
31	31_II_D	31_II_F	1	i31_4
31	31_III_B	31_III_A	2	i31_5
31	31_IV_C	31_IV_A	2	i31_6
32 *	32_II_C	32_II_D	2	i32_1
32 *	32_II_F	32_II_B	3	i32_2
32 *	32_III_J	32_III_M	3	i32_3
32 *	32_IV_R	32_IV_K	3	i32_4
33	33_I_N	33_I_P	1	i33_1
33	33_II_P	33_II_J	1	i33_2
33	33_II_P	33_II_M	1	i33_3
33	33_III_N	33_III_D	1	i33_4
33	33_IV_R	33_IV_N	2	i33_5
34	34_II_F	34_II_C	1	i34_1
34	34_II_J	34_II_F	1	i34_2
34	34_IV_J	34_IV_N	1	i34_3
34	34_IV_J	34_IV_A	1	i34_4
34	34_III_A	34_III_F	1	i34_5
34	34_III_F	34_III_D	1	i34_6
35	35_II_N	35_II_E	1	i35_1
35	35_II_J	35_II_N	1	i35_2
35	35_III_A	35_III_B	1	i35_3
35	35_III_D	35_III_A	1	i35_4
35	35_IV_N	35_IV_E	1	i35_5
35	35_IV_E	35_IV_F	1	i35_6
37	37_I_E	37_I_A	1	i37_1
37	37_I_F	37_I_E	1	i37_2
37	37_II_B	37_II_E	1	i37_3
37	37_II_C	37_II_B	1	i37_4
37	37_III_N	37_III_A	1	i37_5
37	37_III_C	37_III_N	1	i37_6

Invasive range, tetraploid (NA4x), inbred

39	39_II_D	39_II_F	1	i39_1
39	39_II_D	39_II_A	1	i39_2
39	39_III_A	39_III_D	1	i39_3
39	39_III_D	39_III_B	1	i39_4
39	39_IV_C	39_IV_D	1	i39_5
39	39_IV_D	39_IV_E	1	i39_6
40	40_I_R	40_I_D	1	i40_1
40	40_II_F	40_II_G	1	i40_2
40	40_III_B	40_III_C	1	i40_3
40	40_II_F	40_II_C	1	i40_4
40	40_I_D	40_I_A	2	i40_5
40	40_III_A	40_III_R	2	i40_6
41	41_I_R	41_I_C	1	i41_1
41	41_I_D	41_I_A	1	i41_2
41	41_III_B	41_III_N	1	i41_3
41	41_III_B	41_III_F	1	i41_4
41	41_IV_E	41_IV_C	1	i41_5

41	41_IV_E	41_IV_B	1	i41_6
42	42_II_C	42_II_A	1	i42_1
42	42_II_D	42_II_G	1	i42_2
42	42_III_A	42_III_D	1	i42_3
42	42_15N	42_15G	1	i42_4
42	42_15F	42_15G	1	i42_5
42	42_III_F	42_III_G	1	i42_6
43	43_I_C	43_I_N	1	i43_1
43	43_I_B	43_I_C	1	i43_2
43	43_II_A	43_II_C	1	i43_3
43	43_II_B	43_II_A	1	i43_4
43	43_III_M	43_III_J	1	i43_5
43	43_III_M	43_III_P	1	i43_6
44	44_I_E	44_I_D	1	i44_1
44	44_I_F	44_I_E	1	i44_2
44	44_II_C	44_II_D	1	i44_3
44	44_II_D	44_II_E	1	i44_4
44	44_IV_C	44_IV_B	1	i44_5
44	44_IV_B	44_IV_D	1	i44_6
45	45_I_D	45_I_C	1	i45_1
45	45_I_D	45_I_R	1	i45_2
45	45_II_C	45_II_D	1	i45_3
45	45_IV_C	45_IV_J	1	i45_4
45	45_IV_C	45_IV_E	1	i45_5
45	45_II_R	45_II_C	1	i45_6
46 *	46_II_D	46_II_C	3	i46_1
46 *	46_III_D	46_III_B	3	i46_2
46 *	46_II_C	46_II_B	3	i46_3
46 *	46_IV_A	46_IV_B	3	i46_4
47	47_I_F	47_I_C	1	i47_1
47	47_II_A	47_II_C	1	i47_2
47	47_II_D	47_II_A	1	i47_3
47	47_III_C	47_III_A	1	i47_4
47	47_III_A	47_III_B	1	i47_5
47	47_I_F	47_I_A	1	i47_6
48	48_I_G	48_I_C	1	i48_1
48	48_I_C	48_I_H	1	i48_2
48	48_III_B	48_III_A	1	i48_3
48	48_IV_B	48_IV_C	1	i48_4
48	48_IV_E	48_IV_B	1	i48_5
48	48_III_C	48_III_B	1	i48_6
49	49_II_B	49_II_N	1	i49_1
49	49_II_N	49_II_A	1	i49_2
49	49_III_A	49_III_B	1	i49_3
49	49_III_D	49_III_E	1	i49_4
49	49_IV_N	49_IV_A	1	i49_5
49	49_IV_A	49_IV_N	1	i49_6
50	50_I_C	50_I_E	1	i50_1
50	50_II_R	50_II_C	1	i50_2
50	50_I_E	50_I_C	1	i50_3
50	50_III_A	50_III_R	1	i50_4
50	50_III_R	50_III_A	1	i50_5
50	50_III_E	50_III_A	1	i50_6
53 *	53_I_D	53_I_C	2	i53_1
53 *	53_I_E	53_I_C	3	i53_2
53 *	53_I_C	53_I_D	3	i53_3
53 *	53_IV_K	53_IV_A	3	i53_4
54	54_I_D	54_I_B	2	i54_1
54	54_II_D	54_II_B	2	i54_2
54	54_I_A	54_I_D	2	i54_3
54	54_IV_A	54_IV_B	2	i54_4

54	54_IV_N	54_IV_B	2	i54_5
56	56_I_F	56_I_B	1	i56_1
56	56_III_B	56_III_A	1	i56_2
56	56_III_A	56_III_D	1	i56_3
56	56_IV_D	56_IV_E	1	i56_4
56	56_IV_B	56_IV_D	1	i56_5
56	56_IV_F	56_IV_D	1	i56_6

Native range, diploid (EU2x), outbred

5	5_I_C	5_III_F	1	o5_1
5	5_I_R	5_II_G	1	o5_2
5	5_II_F	5_I_C	1	o5_3
5	5_II_G	5_III_J	1	o5_4
5	5_III_F	5_I_A	1	o5_5
5	5_III_J	5_II_A	1	o5_6
6 *	6_IV_A	6_I_K	3	o6_1
6 *	6_III_B	6_IV_A	3	o6_2
6 *	6_I_K	6_IV_A	3	o6_3
6 *	6_IV_A	6_III_B	3	o6_4
8 *	8_III_C	8_II_J	3	o8_1
8 *	8_I_A	8_IV_B	3	o8_2
8 *	8_II_G	8_IV_A	3	o8_3
8 *	8_I_A	8_II_J	3	o8_4
8 *	8_I_B	8_III_B	3	o8_5
8 *	8_III_B	8_I_C	3	o8_6
10	10_I_F	10_IV_N	1	o10_1
10	10_I_G	10_II_B	1	o10_2
10	10_II_N	10_I_F	1	o10_3
10	10_II_N	10_IV_A	1	o10_4
10	10_IV_A	10_II_G	1	o10_5
11	11_I_G	11_II_N	1	o11_1
11	11_III_C	11_I_A	1	o11_2
11	11_III_J	11_II_N	1	o11_3
11	11_I_G	11_III_J	1	o11_4
11	11_II_N	11_III_J	1	o11_5
11	11_07N	11_III_H	2	o11_6
12	12_II_F	12_III_E	1	o12_1
12	12_II_E	12_11J	1	o12_2
12	12_11D	12_II_C	1	o12_3
12	12_11P	12_III_B	1	o12_4
12	12_III_B	12_II_F	1	o12_5
12	12_III_D	12_11P	1	o12_6
13	13_I_G	13_IV_E	2	o13_1
13	13_III_A	13_I_G	2	o13_2
13	13_III_F	13_I_A	2	o13_3
13	13_III_F	13_IV_G	2	o13_4
13	13_IV_H	13_III_H	2	o13_5
14	14_I_H	14_III_A	1	o14_1
14	14_I_F	14_IV_N	1	o14_2
14	14_III_J	14_I_G	1	o14_3
14	14_III_G	14_IV_E	1	o14_4
14	14_IV_B	14_I_G	1	o14_5
14	14_IV_E	14_III_J	1	o14_6
15	15_I_M	15_IV_E	1	o15_1
15	15_II_J	15_IV_D	1	o15_2
15	15_II_P	15_05N	1	o15_3
15	15_II_J	15_IV_F	1	o15_4
15	15_IV_G	15_05N	1	o15_5
15	15_IV_N	15_II_P	1	o15_6
16	16_I_D	16_IV_N	1	o16_1
16	16_I_D	16_II_F	1	o16_2

16	16_05A	16_I_i	1	o16_3
16	16_05A	16_I_E	1	o16_4
16	16_II_G	16_IV_N	1	o16_5
16	16_II_F	16_IV_H	1	o16_6
16	16_IV_H	16_I_E	1	o16_7
16	16_IV_H	16_II_F	1	o16_8
17	17_II_E	17_IV_J	1	o17_1
17	17_II_F	17_III_D	1	o17_2
17	17_III_A	17_II_C	1	o17_3
17	17_III_A	17_IV_J	1	o17_4
17	17_IV_A	17_III_E	1	o17_5
17	17_IV_D	17_II_F	1	o17_6
18	18_III_E	18_I_N	1	o18_1
18	18_III_N	18_II_F	1	o18_2
18	18_II_B	18_III_F	1	o18_3
18	18_IV_J	18_I_N	1	o18_4
18	18_II_B	18_I_N	1	o18_5
18	18_I_J	18_IV_J	1	o18_6
19	19_I_a	19_II_r	1	o19_1
19	19_I_a	19_IV_a	1	o19_2
19	19_III_r	19_IV_a	1	o19_3
19	19_II_r	19_III_a	1	o19_4
19	19_IV_b	19_I_a	1	o19_5
19	19_IV_a	19_I_i	1	o19_6
20	20_I_N	20_IV_J	2	o20_1
20	20_I_C	20_IV_J	2	o20_2
20	20_IV_J	20_II_F	2	o20_3
20	20_IV_N	20_I_A	2	o20_4
20	20_II_F	20_I_A	2	o20_5

Native range, tetraploid (EU4x), outbred

22	22_I_J	22_IV_F	1	o22_1
22	22_I_B	22_II_B	1	o22_2
22	22_II_E	22_I_A	1	o22_3
22	22_IV_F	22_II_B	1	o22_4
22	22_IV_C	22_I_B	1	o22_5
22	22_II_B	22_IV_N	1	o22_6
24	24_II_A	24_III_B	1	o24_1
24	24_II_N	24_I_C	1	o24_2
24	24_III_C	24_II_N	1	o24_3
24	24_III_R	24_I_C	1	o24_4
24	24_I_E	24_II_A	1	o24_5
24	24_I_C	24_III_R	1	o24_6
25	25_I_B	25_III_B	1	o25_1
25	25_I_B	25_IV_J	1	o25_2
25	25_III_F	25_I_E	1	o25_3
25	25_III_A	25_IV_J	1	o25_4
25	25_IV_C	25_I_B	1	o25_5
25	25_IV_J	25_III_F	1	o25_6
26	26_IV_H	26_III_J	1	o26_1
26	26_IV_C	26_I_F	1	o26_2
26	26_IV_H	26_II_E	1	o26_3
26	26_III_R	26_IV_F	1	o26_4
26	26_II_E	26_III_B	1	o26_5
26	26_II_E	26_I_F	1	o26_6
27	27_III_B	27_II_B	1	o27_1
27	27_III_C	27_IV_E	1	o27_2
27	27_II_F	27_III_B	1	o27_3
27	27_II_B	27_IV_E	1	o27_4
27	27_IV_D	27_III_C	1	o27_5
27	27_IV_B	27_II_B	1	o27_6

27	27_III_E	27_II_F	2	o27_7
28	28_I_C	28_III_C	1	o28_1
28	28_II_E	28_III_D	1	o28_2
28	28_I_N	28_II_E	1	o28_3
28	28_III_C	28_I_C	2	o28_4
28	28_III_C	28_II_D	2	o28_5
28	28_III_D	28_I_G	2	o28_6
28	28_II_D	28_I_N	2	o28_7
29	29_III_N	29_I_A	2	o29_1
29	29_I_N	29_III_N	2	o29_2
29	29_III_E	29_I_A	2	o29_3
29	29_III_N	29_IV_A	2	o29_4
29	29_IV_B	29_III_E	2	o29_5
29	29_IV_A	29_I_N	2	o29_6
31	31_III_E	31_I_D	1	o31_1
31	31_IV_A	31_II_F	1	o31_2
31	31_I_D	31_III_E	1	o31_3
31	31_III_E	31_II_F	1	o31_4
31	31_I_F	31_III_E	1	o31_5
31	31_I_D	31_II_F	1	o31_6
31	31_II_A	31_I_F	2	o31_7
32 *	32_04N	32_III_J	2	o32_1
32 *	32_II_D	32_III_M	3	o32_3
32 *	32_04N	32_III_P	3	o32_4
32 *	32_04N	32_III_M	3	o32_5
32 *	32_04N	32_II_F	3	o32_6
33	33_I_P	33_III_D	1	o33__1
33	33_I_N	33_III_E	1	o33__2
33	33_I_J	33_II_P	1	o33__3
33	33_III_N	33_II_P	2	o33__4
34	34_I_J	34_III_F	1	o34_1
34	34_I_F	34_IV_A	1	o34_2
34	34_IV_J	34_III_D	1	o34_3
34	34_IV_N	34_I_C	1	o34_4
34	34_III_A	34_IV_N	1	o34_5
34	34_III_D	34_I_C	1	o34_6
35	35_II_A	35_III_D	1	o35_1
35	35_II_E	35_IV_E	1	o35_2
35	35_III_B	35_IV_E	1	o35_3
35	35_III_A	35_II_N	1	o35_4
35	35_IV_E	35_II_E	1	o35_5
35	35_IV_N	35_III_B	1	o35_6
35	35_II_E	35_I_R	1	o35_7
35	35_III_A	35_IV_F	1	o35_8
37	37_I_A	37_III_N	1	o37_1
37	37_I_E	37_II_C	1	o37_2
37	37_II_B	37_I_E	1	o37_3
37	37_II_B	37_III_A	1	o37_4
37	37_III_C	37_I_E	1	o37_5
37	37_III_N	37_II_C	1	o37_6

Invasive range, tetraploid (EU4x), outbred

39	39_II_D	39_IV_D	1	o39_1
39	39_02E	39_IV_D	1	o39_2
39	39_III_D	39_II_A	1	o39_3
39	39_III_A	39_02P	1	o39_4
39	39_IV_D	39_III_A	1	o39_5
39	39_IV_C	39_III_D	1	o39_6
40	40_I_A	40_II_C	1	o40_1
40	40_II_F	40_I_R	1	o40_2
40	40_III_B	40_II_F	1	o40_4

40	40_II_G	40_III_C	1	o40_5
40	40_I_R	40_II_C	1	o40_6
40	40_I_D	40_III_C	1	o40_7
41	41_I_J	41_III_N	1	o41_1
41	41_I_C	41_IV_B	1	o41_2
41	41_III_B	41_I_R	1	o41_3
41	41_III_B	41_I_C	1	o41_4
41	41_IV_E	41_III_F	1	o41_5
41	41_IV_E	41_I_R	1	o41_6
42	42_II_D	42_III_D	1	o42_1
42	42_II_A	42_15F	1	o42_2
42	42_15N	42_II_D	1	o42_3
42	42_15N	42_III_G	1	o42_4
42	42_III_D	42_II_D	1	o42_5
42	42_III_F	42_15C	1	o42_6
42	42_III_A	42_II_A	1	o42_7
43	43_I_B	43_III_M	1	o43_1
43	43_I_C	43_III_P	1	o43_2
43	43_I_N	43_II_A	1	o43_3
43	43_III_M	43_I_B	1	o43_4
43	43_III_P	43_II_C	1	o43_5
43	43_II_A	43_I_N	1	o43_6
44	44_I_D	44_IV_B	1	o44_1
44	44_I_F	44_II_E	1	o44_2
44	44_II_C	44_IV_R	1	o44_3
44	44_II_D	44_I_E	1	o44_4
44	44_IV_D	44_I_E	1	o44_5
44	44_IV_C	44_II_F	1	o44_6
45	45_I_C	45_II_A	1	o45_1
45	45_I_D	45_II_B	1	o45_2
45	45_IV_J	45_I_C	1	o45_3
45	45_IV_C	45_II_B	1	o45_4
45	45_I_D	45_IV_C	1	o45_5
45	45_II_B	45_I_D	1	o45_6
45	45_II_A	45_I_C	1	o45_7
46 *	46_IV_A	46_06F	3	o46_1
46 *	46_06B	46_II_R	3	o46_2
46 *	46_II_A	46_06B	3	o46_3
46 *	46_II_R	46_IV_B	3	o46_4
47	47_I_C	47_III_A	1	o47_1
47	47_I_F	47_II_A	1	o47_2
47	47_II_B	47_I_C	1	o47_3
47	47_III_C	47_II_B	1	o47_4
47	47_III_A	47_II_A	1	o47_5
47	47_III_B	47_I_B	1	o47_6
48	48_I_G	48_IV_A	1	o48_1
48	48_I_B	48_III_A	1	o48_2
48	48_III_D	48_IV_B	1	o48_3
48	48_III_E	48_21B	1	o48_4
48	48_IV_C	48_III_E	1	o48_5
48	48_21B	48_I_H	1	o48_6
49	49_II_N	49_IV_F	1	o49_1
49	49_II_A	49_IV_B	1	o49_2
49	49_IV_A	49_III_D	1	o49_3
49	49_III_E	49_IV_A	1	o49_4
49	49_III_D	49_IV_N	1	o49_5
49	49_III_E	49_II_B	1	o49_6
49	49_IV_N	49_III_D	1	o49_7
50	50_II_F	50_I_R	1	o50_1
50	50_I_E	50_III_R	1	o50_2
50	50_I_E	50_II_R	1	o50_3

50	50_I_C	50_II_R	1	o50_4
50	50_III_E	50_II_E	1	o50_5
50	50_II_F	50_III_A	1	o50_6
53 *	53_IV_A	53_I_D	2	o53_1
53 *	53_I_D	53_IV_K	3	o53_2
53 *	53_IV_A	53_01B	3	o53_3
54	54_IV_B	54_I_F	2	o54_1
54	54_IV_B	54_II_B	2	o54_2
54	54_I_B	54_IV_D	2	o54_3
54	54_IV_A	54_I_B	2	o54_4
54	54_II_F	54_I_D	2	o54_5
56	56_I_F	56_IV_E	1	o56_1
56	56_III_A	56_I_F	1	o56_2
56	56_III_D	56_I_B	1	o56_3
56	56_III_D	56_IV_E	1	o56_4
56	56_IV_E	56_III_B	1	o56_5
56	56_IV_F	56_I_B	1	o56_6

Table A3.3 Means and standard deviations of the cross-compatibility, the fitness components and the cumulative fitness over geo-cytotype x breeding treatment combinations.

Fixed effects	cross-compatibility	germination success	survival	flowering probability	number of capitula	cumulative fitness
mean ± standard deviation						
overall	0.53 ± 0.5	0.91 ± 0.16	0.5 ± 0.5	0.82 ± 0.39	10.56 ± 6.27	4.08 ± 3.48
EU2x inbred	0.56 ± 0.5	0.85 ± 0.19	0.4 ± 0.49	0.6 ± 0.49	10.71 ± 6.15	2.12 ± 2.38
EU2x outbred	0.6 ± 0.49	0.97 ± 0.07	0.52 ± 0.5	0.71 ± 0.46	12.08 ± 7.22	4.4 ± 3.72
Eu4x inbred	0.49 ± 0.5	0.87 ± 0.18	0.52 ± 0.5	0.86 ± 0.35	10.01 ± 5.38	3.99 ± 3.21
EU4x outbred	0.61 ± 0.49	0.95 ± 0.14	0.54 ± 0.5	0.91 ± 0.29	10.12 ± 7.31	4.87 ± 4.31
NA4x inbred	0.45 ± 0.5	0.88 ± 0.2	0.53 ± 0.5	0.88 ± 0.29	9.35 ± 5	4.05 ± 3.19
NA4x outbred	0.5 ± 0.5	0.94 ± 0.13	0.52 ± 0.5	0.89 ± 0.32	11.3 ± 5.9	4.83 ± 3.07

EU2x, native diploid; EU4x, native tetraploid; NA4x, invasive tetraploid.

A4: Supplemental Information Chapter 4

Table A4.1 Populations that were involved in the clipping experiment.

Population	Country	Locality	Habitat	Latitude	Longitude
Native range, diploid (EU2x)					
5	DE	Sachen-Anhalt, Lieskau	n	51.50	11.86
10	CH	Graubünden, Ramosch	n	46.83	10.40
11	IT	Trentino-Alto Adige, Castelle Penede	n	45.88	10.89
12	IT	Trentino-Alto Adige, Rafenstein	n	46.53	11.36
13	CZ	Jihočeský, Rájov	n	48.84	14.37
14	AT	Kärnten, Völkermarkt	r	46.65	14.91
15	SI	Murska Sobota, Murska Sobota	r	46.63	16.21
16	SK	Bratislavský, Sandberg	n	48.20	16.97
17	HU	Zala, Balatongyörök	r	46.76	17.34
18	HU	Pest, Csepel Island	r	47.33	18.95
19	SK	Košický, Gelnica	r	48.85	20.93
20	RO	Iași, Valea lui David	n	47.20	27.47
Native range, tetraploid (EU4x)					
22	DE	Sachen-Anhalt, Dieskau	r	51.44	12.04
24	DE	Bayern, Nürnberg	r	49.39	11.08
25	AT	Tirol, Starkenbach	r	47.19	10.64
26	AT	Niederösterreich, Krems	r	48.43	15.65
27	SK	Bratislavský, Závod	r	48.52	17.02
28	SK	Trnavský, Trnava	r	48.38	17.60
29	SK	Nové Mesto nad Váhom	r	48.76	17.84
31	HU	Budapest, Gellérthegy	n	47.49	19.05
33	RO	Cluj, Cheile Turzii	n	46.56	23.70
34	RO	Alba, Poșaga de Jos	n	46.43	23.45
35	RO	Suczawa, Oprișeni	n	47.48	26.27
37	RO	Iași, Paraul Cacaina	n	47.19	27.59
Invasive range, tetraploid (NA4x)					
39	CA	British Columbia, Tsawwassen	r	49.02	-123.11
40	US	Washington, Seattle	r	47.48	-122.24
41	US	Washington, Stevens Pass	r	47.79	-120.89
42	CA	British Columbia, Kamloops	r	50.71	-120.37
43	CA	British Columbia, Revelstoke	n	51.02	-118.21
44	US	Idaho, Coeur d´Alene	n	47.65	-116.72
45	US	Idaho, Emerald Creek	r	47.07	-116.33
47	US	Montana, Glacier National Park	n	48.70	-113.69
48	US	Montana, Clark Fork River	r	46.87	-113.99
49	US	Montana, Salmon Lake	n	47.12	-113.43
50	US	Montana, Butte	r	46.00	-112.61
54	US	Montana, Kendalls´ Ranch	n	45.53	-111.18
56	US	Wyoming, Mammoth	n	44.97	-110.69

Notes: Population numbers correspond to those in Rosche *et al.* (2016). Habitat: habitat type, n: natural, r: ruderal, AT: Austria, CA: Canada, CH: Switzerland, CZ: Czech Republic, DE: Germany, HU: Hungary, IT: Italy, RO: Romania, SI: Slovenia, SK: Slovakia, US: USA.

Appendix A4.2. Inbred and outbred lineages that were involved in the clipping experiment.

Population	Donor	Acceptor	Charge	Lineage ID	CT 0	CT 1	CT 2
Native range, diploid (EU2x), inbred							
5	5_I_B	5_I_C	1	i5_2	1	0	1
5	5_II_F	5_II_E	1	i5_3	1	0	1
5	5_II_E	5_II_G	1	i5_4	0	2	0
5	5_III_A	5_III_F	1	i5_5	1	0	1
10	10_I_G	10_I_N	1	i10_1	1	0	0
10	10_I_A	10_I_N	1	i10_2	0	1	0
10	10_II_N	10_II_C	1	i10_3	3	1	0
10	10_II_A	10_II_C	1	i10_4	1	0	0
11	11_I_I	11_I_G	1	i11_1	1	0	0
11	11_I_G	11_I_I	1	i11_2	0	2	1
11	11_II_I	11_II_A	1	i11_3	1	1	1
11	11_III_C	11_III_H	1	i11_4	1	1	1
11	11_III_H	11_III_C	1	i11_5	2	1	0
11	11_III_J	11_III_H	1	i11_6	0	0	2
12	12_II_A	12_II_E	1	i12_1	1	0	1
12	12_II_E	12_II_F	1	i12_2	1	2	1
12	12_11J	12_11D	1	i12_3	0	1	0
12	12_11P	12_11B	1	i12_4	0	0	1
12	12_III_H	12_III_D	1	i12_5	0	2	0
12	12_III_B	12_III_D	1	i12_6	2	0	1
13	13_I_D	13_I_A	2	i13_2	1	0	0
13	13_III_A	13_III_H	2	i13_3	1	1	1
13	13_III_F	13_III_A	2	i13_4	0	1	0
13	13_IV_G	13_IV_E	2	i13_5	1	0	2
13	13_IV_H	13_IV_G	2	i13_6	0	2	0
14	14_I_J	14_I_F	1	i14_2	1	1	0
14	14_IV_B	14_IV_J	1	i14_5	1	1	0
14	14_IV_E	14_IV_J	1	i14_6	1	1	3
15	15_I_M	15_I_P	1	i15_1	0	1	0
15	15_I_M	15_I_J	1	i15_2	2	0	1
15	15_II_P	15_II_J	1	i15_4	1	1	1
15	15_IV_P	15_IV_N	1	i15_5	1	1	1
15	15_IV_D	15_IV_F	1	i15_6	0	1	1
16	16_I_E	16_I_i	1	i16_1	2	1	1
16	16_I_D	16_I_E	1	i16_2	0	0	1
16	16_II_F	16_II_B	1	i16_3	2	1	1
16	16_II_N	16_II_G	1	i16_4	0	1	1
16	16_IV_J	16_IV_B	1	i16_5	1	1	2
16	16_IV_N	16_IV_H	1	i16_6	1	1	1
17	17_II_F	17_II_A	1	i17_1	1	0	0
17	17_II_C	17_II_E	1	i17_2	0	1	0
17	17_III_A	17_III_D	1	i17_3	0	0	2
17	17_III_E	17_III_D	1	i17_4	1	1	1
17	17_IV_A	17_IV_J	1	i17_6	2	1	1
18	18_II_B	18_II_F	1	i18_2	0	1	1
18	18_II_A	18_II_F	1	i18_3	1	1	1
18	18_IV_E	18_IV_B	1	i18_4	0	1	0
18	18_III_N	18_III_A	1	i18_5	1	0	0
18	18_II_B	18_II_E	1	i18_6	1	1	0
18	18_III_N	18_III_F	2	i18_7	0	0	1
19	19_I_a	19_I_i	1	i19_1	0	1	0
19	19_II_r	19_II_e	2	i19_2	1	1	0
19	19_IV_B	19_IV_A	2	i19_4	0	0	1

20	20_I_C	20_I_A	2	i20_1	1	0	1
20	20_I_N	20_I_A	2	i20_2	1	2	0
20	20_II_B	20_II_A	2	i20_3	0	0	1
20	20_IV_N	20_IV_J	2	i20_4	0	1	0
Native range, tetraploid (EU4x), inbred							
22	22_I_A	22_I_J	1	i22_1	1	1	1
22	22_I_B	22_I_A	1	i22_2	0	0	1
22	22_I_J	22_I_B	1	i22_3	0	1	1
22	22_IV_C	22_IV_F	1	i22_5	2	2	1
22	22_IV_F	22_IV_C	1	i22_6	2	1	1
24	24_II_A	24_II_N	1	i24_1	1	1	1
24	24_II_D	24_II_F	1	i24_2	0	1	0
24	24_III_B	24_III_R	1	i24_3	2	1	1
24	24_I_E	24_I_C	1	i24_5	1	1	1
24	24_II_N	24_II_F	1	i24_6	0	1	1
25	25_I_B	25_I_E	1	i25_1	1	1	1
25	25_III_B	25_III_F	1	i25_2	1	1	1
25	25_III_E	25_III_B	1	i25_3	1	1	1
25	25_IV_J	25_IV_C	1	i25_4	1	1	1
26	26_I_F	26_I_A	1	i26_1	0	0	1
26	26_II_E	26_II_D	1	i26_2	0	1	0
26	26_IV_H	26_IV_F	1	i26_3	2	1	0
26	26_II_C	26_II_D	1	i26_4	1	1	1
26	26_IV_F	26_IV_B	1	i26_5	2	2	1
26	26_III_R	26_III_B	1	i26_6	0	1	2
27	27_II_B	27_II_F	1	i27_1	1	0	0
27	27_II_B	27_II_C	1	i27_2	2	1	1
27	27_III_B	27_III_C	1	i27_3	0	1	1
27	27_IV_E	27_IV_D	1	i27_5	1	2	2
27	27_IV_B	27_IV_D	1	i27_6	0	1	0
28	28_II_E	28_II_D	1	i28_2	2	0	1
28	28_III_C	28_III_D	2	i28_3	0	2	0
28	28_I_N	28_I_C	2	i28_4	1	1	1
28	28_III_D	28_III_N	2	i28_5	1	1	1
28	28_III_N	28_III_C	2	i28_6	1	1	2
29	29_I_A	29_I_N	2	i29_1	1	1	0
29	29_III_E	29_III_N	2	i29_2	0	0	1
29	29_I_N	29_I_A	2	i29_3	1	1	1
29	29_III_C	29_III_E	2	i29_4	1	1	1
29	29_IV_A	29_IV_B	2	i29_5	0	1	0
31	31_I_F	31_I_A	1	i31_1	2	2	1
31	31_I_A	31_I_F	1	i31_2	1	0	1
31	31_I_F	31_I_D	1	i31_3	0	2	1
31	31_III_B	31_III_A	2	i31_5	1	1	0
31	31_IV_C	31_IV_A	2	i31_6	0	0	1
33	33_I_N	33_I_P	1	i33_1	1	0	0
33	33_II_P	33_II_J	1	i33_2	1	2	0
33	33_II_P	33_II_M	1	i33_3	1	1	1
33	33_III_N	33_III_D	1	i33_4	1	1	2
33	33_IV_R	33_IV_N	2	i33_5	0	0	1
34	34_II_F	34_II_C	1	i34_1	1	1	1
34	34_II_J	34_II_F	1	i34_2	2	0	0
34	34_IV_J	34_IV_N	1	i34_3	0	0	1
34	34_IV_J	34_IV_A	1	i34_4	2	1	0
34	34_III_A	34_III_F	1	i34_5	1	0	0
34	34_III_F	34_III_D	1	i34_6	1	1	1
35	35_II_N	35_II_E	1	i35_1	1	0	0
35	35_II_J	35_II_N	1	i35_2	0	1	1

35	35_III_A	35_III_B	1	i35_3	1	1	1
35	35_III_D	35_III_A	1	i35_4	1	2	1
35	35_IV_N	35_IV_E	1	i35_5	1	1	0
35	35_IV_E	35_IV_F	1	i35_6	1	0	2
37	37_I_E	37_I_A	1	i37_1	0	0	1
37	37_I_F	37_I_E	1	i37_2	1	2	1
37	37_II_B	37_II_E	1	i37_3	1	2	0
37	37_II_C	37_II_B	1	i37_4	1	0	1
37	37_III_C	37_III_N	1	i37_6	1	1	1
Invasive range, tetraploid (NA4x), inbred							
39	39_II_D	39_II_F	1	i39_1	0	0	1
39	39_II_D	39_II_A	1	i39_2	2	0	0
39	39_III_A	39_III_D	1	i39_3	1	1	1
39	39_III_D	39_III_B	1	i39_4	0	1	1
39	39_IV_C	39_IV_D	1	i39_5	0	1	1
40	40_I_R	40_I_D	1	i40_1	1	1	1
40	40_II_F	40_II_G	1	i40_2	1	1	1
40	40_III_B	40_III_C	1	i40_3	2	1	1
40	40_II_F	40_II_C	1	i40_4	0	0	1
41	41_I_R	41_I_C	1	i41_1	0	1	0
41	41_I_D	41_I_A	1	i41_2	1	1	1
41	41_III_B	41_III_F	1	i41_4	1	2	2
41	41_IV_E	41_IV_B	1	i41_6	2	1	1
42	42_II_C	42_II_A	1	i42_1	0	1	1
42	42_II_D	42_II_G	1	i42_2	2	0	0
42	42_III_A	42_III_D	1	i42_3	1	2	0
42	42_15N	42_15G	1	i42_4	0	0	2
42	42_15F	42_15G	1	i42_5	1	0	0
42	42_III_F	42_III_G	1	i42_6	0	0	1
43	43_I_C	43_I_N	1	i43_1	1	1	1
43	43_I_B	43_I_C	1	i43_2	0	0	1
43	43_II_A	43_II_C	1	i43_3	1	1	1
43	43_II_B	43_II_A	1	i43_4	1	0	0
43	43_III_M	43_III_J	1	i43_5	1	1	2
43	43_III_M	43_III_P	1	i43_6	1	1	0
44	44_I_F	44_I_E	1	i44_2	1	2	1
44	44_II_C	44_II_D	1	i44_3	1	0	1
44	44_II_D	44_II_E	1	i44_4	1	1	0
44	44_IV_C	44_IV_B	1	i44_5	0	0	1
44	44_IV_B	44_IV_D	1	i44_6	1	1	1
45	45_I_D	45_I_C	1	i45_1	0	0	1
45	45_I_D	45_I_R	1	i45_2	1	2	0
45	45_II_C	45_II_D	1	i45_3	2	1	2
45	45_IV_C	45_IV_J	1	i45_4	1	1	1
45	45_IV_C	45_IV_E	1	i45_5	1	1	0
45	45_II_R	45_II_C	1	i45_6	0	1	1
47	47_II_A	47_II_C	1	i47_2	1	0	1
47	47_II_D	47_II_A	1	i47_3	1	2	1
47	47_III_A	47_III_B	1	i47_5	1	0	1
47	47_I_F	47_I_A	1	i47_6	1	1	1
48	48_I_G	48_I_C	1	i48_1	1	1	0
48	48_I_C	48_I_H	1	i48_2	0	0	2
48	48_III_B	48_III_A	1	i48_3	1	1	0
48	48_IV_B	48_IV_C	1	i48_4	0	1	0
48	48_IV_E	48_IV_B	1	i48_5	1	1	1
48	48_III_C	48_III_B	1	i48_6	1	1	1
49	49_II_B	49_II_N	1	i49_1	1	0	1
49	49_II_N	49_II_A	1	i49_2	1	1	1

49	49_III_A	49_III_B	1	i49_3	0	1	1
49	49_III_D	49_III_E	1	i49_4	1	0	0
49	49_IV_N	49_IV_A	1	i49_5	1	1	1
49	49_IV_A	49_IV_N	1	i49_6	1	1	1
50	50_I_C	50_I_E	1	i50_1	0	0	1
50	50_II_R	50_II_C	1	i50_2	2	1	1
50	50_I_E	50_I_C	1	i50_3	1	2	0
50	50_III_A	50_III_R	1	i50_4	0	0	1
54	54_I_D	54_I_B	2	i54_1	0	0	1
54	54_II_D	54_II_B	2	i54_2	1	1	0
54	54_I_A	54_I_D	2	i54_3	1	0	0
54	54_IV_A	54_IV_B	2	i54_4	0	0	1
56	56_I_F	56_I_B	1	i56_1	1	0	1
56	56_III_B	56_III_A	1	i56_2	2	1	0
56	56_III_A	56_III_D	1	i56_3	2	1	1
56	56_IV_D	56_IV_E	1	i56_4	0	1	1
56	56_IV_B	56_IV_D	1	i56_5	0	1	1
56	56_IV_F	56_IV_D	1	i56_6	1	1	2
Native range, diploid (EU2x), outbred							
5	5_I_C	5_III_F	1	o5_1	0	1	1
5	5_I_R	5_II_G	1	o5_2	1	1	1
5	5_II_F	5_I_C	1	o5_3	0	1	0
5	5_II_G	5_III_J	1	o5_4	1	1	0
5	5_III_F	5_I_A	1	o5_5	1	1	0
5	5_III_J	5_II_A	1	o5_6	1	0	2
10	10_I_F	10_IV_N	1	o10_1	1	0	0
10	10_I_G	10_II_B	1	o10_2	0	1	1
10	10_II_N	10_I_F	1	o10_3	1	1	1
10	10_II_N	10_IV_A	1	o10_4	2	1	0
10	10_IV_A	10_II_G	1	o10_5	0	2	2
11	11_I_G	11_II_N	1	o11_1	1	1	1
11	11_III_C	11_I_A	1	o11_2	0	1	1
11	11_III_J	11_II_N	1	o11_3	0	1	0
11	11_I_G	11_III_J	1	o11_4	2	0	1
11	11_II_N	11_III_J	1	o11_5	1	1	0
11	11_07N	11_III_H	2	o11_6	1	1	2
12	12_II_F	12_III_E	1	o12_1	1	0	1
12	12_II_E	12_11J	1	o12_2	1	1	0
12	12_11D	12_II_C	1	o12_3	1	1	1
12	12_11P	12_III_B	1	o12_4	1	1	1
12	12_III_B	12_II_F	1	o12_5	1	0	1
12	12_III_D	12_11P	1	o12_6	0	1	0
13	13_I_G	13_IV_E	2	o13_1	0	1	1
13	13_III_A	13_I_G	2	o13_2	1	1	1
13	13_III_F	13_I_A	2	o13_3	1	0	0
13	13_III_F	13_IV_G	2	o13_4	1	0	1
14	14_I_H	14_III_A	1	o14_1	1	0	1
14	14_I_F	14_IV_N	1	o14_2	0	1	1
14	14_III_J	14_I_G	1	o14_3	1	1	0
14	14_III_G	14_IV_E	1	o14_4	1	1	1
14	14_IV_B	14_I_G	1	o14_5	1	1	1
14	14_IV_E	14_III_J	1	o14_6	1	1	1
15	15_I_M	15_IV_E	1	o15_1	1	0	1
15	15_II_J	15_IV_D	1	o15_2	1	0	1
15	15_II_P	15_05N	1	o15_3	0	1	0
15	15_II_J	15_IV_F	1	o15_4	2	1	1
15	15_IV_G	15_05N	1	o15_5	1	1	1
15	15_IV_N	15_II_P	1	o15_6	0	1	1

16	16_I_D	16_IV_N	1	o16_1	1	1	2
16	16_I_D	16_II_F	1	o16_2	0	1	0
16	16_05A	16_I_i	1	o16_3	2	1	1
16	16_05A	16_I_E	1	o16_4	1	2	1
16	16_II_G	16_IV_N	1	o16_5	1	2	1
16	16_II_F	16_IV_H	1	o16_6	1	1	2
16	16_IV_H	16_I_E	1	o16_7	2	0	0
16	16_IV_H	16_II_F	1	o16_8	0	1	1
17	17_II_E	17_IV_J	1	o17_1	1	1	0
17	17_II_F	17_III_D	1	o17_2	0	1	1
17	17_III_A	17_II_C	1	o17_3	0	1	0
17	17_III_A	17_IV_J	1	o17_4	1	2	2
17	17_IV_A	17_III_E	1	o17_5	1	0	1
17	17_IV_D	17_II_F	1	o17_6	1	1	2
18	18_III_E	18_I_N	1	o18_1	1	1	0
18	18_III_N	18_II_F	1	o18_2	0	1	1
18	18_II_B	18_III_F	1	o18_3	2	0	2
18	18_IV_J	18_I_N	1	o18_4	1	0	0
18	18_II_B	18_I_N	1	o18_5	1	1	1
18	18_I_J	18_IV_J	1	o18_6	0	2	1
19	19_I_a	19_II_r	1	o19_1	1	1	1
19	19_I_a	19_IV_a	1	o19_2	0	1	0
19	19_III_r	19_IV_a	1	o19_3	0	0	1
19	19_II_r	19_III_a	1	o19_4	2	1	1
19	19_IV_b	19_I_a	1	o19_5	1	0	1
19	19_IV_a	19_I_i	1	o19_6	0	1	0
20	20_I_N	20_IV_J	2	o20_1	1	0	1
20	20_I_C	20_IV_J	2	o20_2	0	1	1
20	20_IV_J	20_II_F	2	o20_3	1	1	0
20	20_IV_N	20_I_A	2	o20_4	0	0	2
20	20_II_F	20_I_A	2	o20_5	2	1	0
Native range, tetraploid (EU4x), outbred							
22	22_I_B	22_II_B	1	o22_2	2	1	1
22	22_II_E	22_I_A	1	o22_3	1	1	1
22	22_IV_F	22_II_B	1	o22_4	0	0	1
22	22_IV_C	22_I_B	1	o22_5	0	2	1
22	22_II_B	22_IV_N	1	o22_6	1	1	0
24	24_II_A	24_III_B	1	o24_1	2	1	1
24	24_II_N	24_I_C	1	o24_2	1	1	1
24	24_III_C	24_II_N	1	o24_3	0	1	2
24	24_III_R	24_I_C	1	o24_4	2	0	0
24	24_I_E	24_II_A	1	o24_5	0	1	1
24	24_I_C	24_III_R	1	o24_6	0	1	0
25	25_I_B	25_III_B	1	o25_1	0	1	0
25	25_I_B	25_IV_J	1	o25_2	0	0	2
25	25_III_F	25_I_E	1	o25_3	1	2	0
25	25_III_A	25_IV_J	1	o25_4	1	0	0
25	25_IV_C	25_I_B	1	o25_5	1	0	1
26	26_IV_H	26_III_J	1	o26_1	2	1	2
26	26_IV_C	26_I_F	1	o26_2	1	3	1
26	26_IV_H	26_II_E	1	o26_3	2	1	1
26	26_III_R	26_IV_F	1	o26_4	1	1	2
26	26_II_E	26_III_B	1	o26_5	2	0	1
26	26_II_E	26_I_F	1	o26_6	1	2	2
27	27_III_B	27_II_B	1	o27_1	1	0	0
27	27_III_C	27_IV_E	1	o27_2	0	2	0
27	27_II_F	27_III_B	1	o27_3	0	0	1
27	27_IV_B	27_II_B	1	o27_6	1	1	0

27	27_III_E	27_II_F	2	o27_7	0	0	1
28	28_I_C	28_III_C	1	o28_1	0	1	1
28	28_II_E	28_III_D	1	o28_2	2	2	1
28	28_I_N	28_II_E	1	o28_3	1	0	0
28	28_III_C	28_I_C	2	o28_4	1	2	2
28	28_III_C	28_II_D	2	o28_5	0	1	1
28	28_III_D	28_I_G	2	o28_6	2	1	0
28	28_II_D	28_I_N	2	o28_7	0	0	1
29	29_III_N	29_I_A	2	o29_1	0	1	1
29	29_I_N	29_III_N	2	o29_2	1	1	0
29	29_III_E	29_I_A	2	o29_3	1	1	1
29	29_III_N	29_IV_A	2	o29_4	1	1	0
29	29_IV_B	29_III_E	2	o29_5	1	0	1
29	29_IV_A	29_I_N	2	o29_6	0	0	1
31	31_III_E	31_I_D	1	o31_1	2	1	1
31	31_IV_A	31_II_F	1	o31_2	0	0	2
31	31_I_D	31_III_E	1	o31_3	0	1	0
31	31_III_E	31_II_F	1	o31_4	1	0	0
31	31_I_F	31_III_E	1	o31_5	2	1	0
31	31_I_D	31_II_F	1	o31_6	1	2	1
31	31_II_A	31_I_F	2	o31_7	0	0	1
33	33_I_P	33_III_D	1	o33__1	0	1	1
33	33_I_N	33_III_E	1	o33__2	1	0	0
33	33_III_N	33_II_P	2	o33__4	1	2	1
34	34_I_J	34_III_F	1	o34_1	0	1	1
34	34_I_F	34_IV_A	1	o34_2	0	1	1
34	34_IV_J	34_III_D	1	o34_3	2	1	0
34	34_IV_N	34_I_C	1	o34_4	1	1	1
34	34_III_A	34_IV_N	1	o34_5	1	0	0
34	34_III_D	34_I_C	1	o34_6	0	0	1
35	35_II_A	35_III_D	1	o35_1	0	1	1
35	35_II_E	35_IV_E	1	o35_2	1	0	1
35	35_III_B	35_IV_E	1	o35_3	0	3	0
35	35_III_A	35_II_N	1	o35_4	2	1	1
35	35_IV_E	35_II_E	1	o35_5	1	0	2
35	35_IV_N	35_III_B	1	o35_6	2	0	1
35	35_II_E	35_I_R	1	o35_7	1	1	2
35	35_III_A	35_IV_F	1	o35_8	1	1	0
37	37_I_A	37_III_N	1	o37_1	1	0	1
37	37_I_E	37_II_C	1	o37_2	0	0	1
37	37_II_B	37_I_E	1	o37_3	1	1	1
37	37_II_B	37_III_A	1	o37_4	1	0	1
37	37_III_C	37_I_E	1	o37_5	2	2	1
37	37_III_N	37_II_C	1	o37_6	1	3	1
Invasive range, tetraploid (EU4x), outbred							
39	39_II_D	39_IV_D	1	o39_1	1	0	1
39	39_02E	39_IV_D	1	o39_2	1	1	0
39	39_III_D	39_II_A	1	o39_3	1	1	2
39	39_IV_D	39_III_A	1	o39_5	1	1	2
39	39_IV_C	39_III_D	1	o39_6	1	1	0
40	40_I_A	40_II_C	1	o40_1	2	2	0
40	40_II_F	40_I_R	1	o40_2	0	2	0
40	40_III_B	40_II_F	1	o40_4	1	0	1
40	40_II_G	40_III_C	1	o40_5	0	0	1
40	40_I_R	40_II_C	1	o40_6	1	0	1
40	40_I_D	40_III_C	1	o40_7	0	0	1
41	41_I_J	41_III_N	1	o41_1	3	0	2
41	41_III_B	41_I_R	1	o41_3	1	1	0

41	41_III_B	41_I_C	1	o41_4	0	1	1
41	41_IV_E	41_III_F	1	o41_5	0	0	1
41	41_IV_E	41_I_R	1	o41_6	1	3	1
42	42_II_D	42_III_D	1	o42_1	1	1	0
42	42_II_A	42_15F	1	o42_2	0	1	1
42	42_15N	42_III_G	1	o42_4	1	0	0
42	42_III_D	42_II_D	1	o42_5	1	1	1
42	42_III_F	42_15C	1	o42_6	0	1	1
43	43_I_B	43_III_M	1	o43_1	1	0	1
43	43_I_C	43_III_P	1	o43_2	0	1	1
43	43_I_N	43_II_A	1	o43_3	1	0	0
43	43_III_M	43_I_B	1	o43_4	0	1	1
43	43_III_P	43_II_C	1	o43_5	1	0	0
43	43_II_A	43_I_N	1	o43_6	0	2	0
44	44_I_D	44_IV_B	1	o44_1	1	0	1
44	44_I_F	44_II_E	1	o44_2	1	2	1
44	44_II_C	44_IV_R	1	o44_3	1	1	1
44	44_II_D	44_I_E	1	o44_4	0	1	0
44	44_IV_D	44_I_E	1	o44_5	2	1	1
44	44_IV_C	44_II_F	1	o44_6	0	0	1
45	45_I_C	45_II_A	1	o45_1	1	3	0
45	45_I_D	45_II_B	1	o45_2	1	0	2
45	45_IV_J	45_I_C	1	o45_3	0	1	1
45	45_IV_C	45_II_B	1	o45_4	1	0	0
45	45_I_D	45_IV_C	1	o45_5	0	1	1
45	45_II_B	45_I_D	1	o45_6	1	0	1
45	45_II_A	45_I_C	1	o45_7	1	1	0
47	47_I_C	47_III_A	1	o47_1	1	2	0
47	47_I_F	47_II_A	1	o47_2	2	1	2
47	47_II_B	47_I_C	1	o47_3	0	1	1
47	47_III_C	47_II_B	1	o47_4	1	0	1
47	47_III_A	47_II_A	1	o47_5	1	0	0
47	47_III_B	47_I_B	1	o47_6	0	0	1
48	48_I_G	48_IV_A	1	o48_1	0	0	2
48	48_I_B	48_III_A	1	o48_2	1	1	0
48	48_III_D	48_IV_B	1	o48_3	2	0	0
48	48_III_E	48_21B	1	o48_4	1	2	1
48	48_IV_C	48_III_E	1	o48_5	0	1	1
48	48_21B	48_I_H	1	o48_6	1	0	1
49	49_II_N	49_IV_F	1	o49_1	3	0	0
49	49_II_A	49_IV_B	1	o49_2	2	0	0
49	49_IV_A	49_III_D	1	o49_3	1	1	0
49	49_III_E	49_IV_A	1	o49_4	1	0	0
49	49_III_D	49_IV_N	1	o49_5	2	0	1
49	49_III_E	49_II_B	1	o49_6	0	1	0
49	49_IV_N	49_III_D	1	o49_7	1	0	0
50	50_II_F	50_I_R	1	o50_1	0	1	1
50	50_I_E	50_III_R	1	o50_2	1	1	0
50	50_I_E	50_II_R	1	o50_3	1	1	2
50	50_I_C	50_II_R	1	o50_4	1	0	0
50	50_III_E	50_II_E	1	o50_5	0	1	1
50	50_II_F	50_III_A	1	o50_6	1	1	0
54	54_IV_B	54_I_F	2	o54_1	0	2	0
54	54_IV_B	54_II_B	2	o54_2	1	0	1
54	54_I_B	54_IV_D	2	o54_3	1	1	1
56	56_I_F	56_IV_E	1	o56_1	1	2	1
56	56_III_A	56_I_F	1	o56_2	0	1	1
56	56_III_D	56_I_B	1	o56_3	2	2	1
56	56_III_D	56_IV_E	1	o56_4	2	1	1

56	56_IV_E	56_III_B	1	o56_5	1	0	1
56	56_IV_F	56_I_B	1	o56_6	0	0	1

Notes: The table shows lineages, which originated from the experiments in Rosche *et al.* (submitted). Lineages resulted from crosses between the maternal (acceptor) and the paternal (donor) mating partners. Code of each mating partner (individual ID for donor and acceptor) is as follows: information on population, seed family and individual (within seed family) are separated by underlines; arabic numerals correspond to populations that are given in Appendix A4.1, roman numerals correspond to seed families and letters refer to distinct individuals. Germination was conducted in two separate runs: a first germination cohort was initiated on October 20th and a second on October 27th. Breeding treatments, habitat types and geo-cytotypes were equally distributed across germination cohorts. We did not include information about the germination cohort, because it did not explain any variation (data not shown), and because flowering status reflects a more substantial predictor of life stage. Lineage ID was encoded as follows: i for inbred or o for outbred followed by population number, underline and lineage number within the population. The table gives the number of individuals per lineage that were assigned to each of the three clipping treatments. CT0: no clipping, CT1: single clipping, CT2: double clipping.

A5: Personal contributions to the manuscripts

Chapter 2 Rosche C, Durka W, Hensen I, Mráz P, Hartmann M, Müller-Schärer H, Lachmuth S (2016) The population genetics of the fundamental cytotype-shift in invasive *Centaurea stoebe* s.l.: genetic diversity, genetic differentiation and small-scale genetic structure differ between cytotypes but not between ranges. DOI: 10.1007/s10530-016-1133-2

Field work	Christoph Rosche (75%), Matthias Hartmann & Patrik Mráz (together 25%)
Laboratory work	Christoph Rosche (65%), Melanie Laudien, Matthias Hartmann, & Patrik Mráz (together 35%)
Analysis	Christoph Rosche (70%), Susanne Lachmuth, Walter Durka & Patrik Mráz (together 30%)
Writing	Christoph Rosche (80%), corrections by: Susanne Lachmuth, Walter Durka, Isabell Hensen, Patrik Mráz, Matthias Hartmann & Heinz Müller-Schärer (together 20%)

Chapter 3 Rosche C, Hensen I, Mráz P, Durka W, Hartmann M, Lachmuth S (under revision in *Journal of Ecology*) Invasion success in polyploids: the role of inbreeding in the contrasting colonization abilities of diploid versus tetraploid populations of *Centaurea stoebe* s.l.

Laboratory work	Christoph Rosche (60%), Matthias Hartmann, Janin Hochheimer, Elisa Schütz & Adrian Schaar (together 40%)
Analysis	Christoph Rosche (65%), Susanne Lachmuth (35%)
Writing	Christoph Rosche (80%), corrections by: Susanne Lachmuth, Isabell Hensen, Patrik Mráz, Walter Durka & Matthias Hartmann (together 20%)

Chapter 4 Rosche C, Hensen I, Lachmuth S (submitted to *American Journal of Botany*) The potential role of pre-adaptation and inbreeding-environment interactions for colonization of disturbed habitats: an experiment with diploid and tetraploid *Centaurea stoebe*

Laboratory work	Christoph Rosche (90%), Stephanie Schmidt (10%)
Analysis	Christoph Rosche (90%), Susanne Lachmuth (10%)
Writing	Christoph Rosche (80%), corrections by: Susanne Lachmuth & Isabell Hensen (together 20%)

A6: Curriculum vitae

* 26.01.1985

Education

Since 04/2012	MLU Halle: Ph.D. studies
10/2005 – 09/2011	MLU Halle: Studies in Biology Graduation: Diploma-Biologist (1.1) Diploma thesis: Genetic diversity and structure of small and fragmented *Antennaria dioica* populations.

Employments

Since 01/2016	Senckenberg Museum Natural History Görlitz (Section Phanerogams I): Research associate BMBF / FONA project KULUNDA
Since 04/2015	MLU Halle (Plant Ecology Group): Research associate
09/2014 – 03/2015	Charles University Prague (Herbarium of the Charles University): Project position
05/2007 – 09/2014	FED Leipzig, HBW Halle, Lebens(t)raum Halle: Volunteering
09/2008 – 05/2012	UFZ Halle (Community Ecology Group), MLU Halle (Geobotany Group), MLU Halle (Developmental Genetics), MLU Halle (Plant Ecology Group), IPB Halle (Secondary Metabolism Group): Scientific assistance in various labs

Acquisition of research grants

Project grant of the German Academic Exchange Service (DAAD): 10/2014 – 03/2015: “Native range dynamics of two cytotypes of *Centaurea stoebe*.” Project coordinator together with Dr. Mráz, Charles University of Prague (Czech Republic). 7,500 €.

Indo-German Joint Research Collaboration between Department of Science & Technology, Government of India (DST) and DAAD (04/2014 – 08/2016): “Towards a better mechanistic understanding of colonization: Biogeographic analysis and population genetics of highly invasive Asteraceae.” Project coordinator together with Dr. Shah, University of Kashmir (India). 20,000 €.

DAAD travel grant for research in USA and Canada 09/2012 – 10/2012: Funding of travel expenses of my Ph.D. studies. 1,500 €.

Scholarship of the federal state Saxony-Anhalt (04/2012 – 09/2014): Funding of my Ph.D. studies. 22,500 €.

A7: Publications and scientific talks

List of publications

Rosche C, Durka W, Hensen I, Mráz P, Hartmann M, Müller-Schärer H, Lachmuth S (2016): The population genetics of the fundamental cytotype-shift in invasive *Centaurea stoebe* s.l.: genetic diversity, genetic differentiation and small-scale genetic structure differ between cytotypes but not between ranges. DOI: 10.1007/s10530-016-1133-2

Rosche C, Schrieber K, Hirsch H, Blachnik T, Träger S, Richter F, Seidler G, Hensen I (2015): Verringerte sexuelle Reproduktionsfähigkeit oligoklonaler Populationen von *Antennaria dioica* (L.) GAERTNER. *Hercynia* 47: 59-86.

Shah MA, Callaway RM, Shah T, Houseman GR, Pal RW, Xiao S, Luo W, Rosche C, Reshi ZA, Khasa DP, Chen S (2014): *Conyza canadensis* suppresses plant diversity in its nonnative ranges but not at home: a transcontinental comparison. *New Phytologist* 202: 1286–1296.

Stein K, Rosche C, Hirsch H, Kindermann A, Köhler J, Hensen I (2014): The influence of forest fragmentation on clonal diversity and genetic structure in *Heliconia angusta*, an endemic understorey herb of the Brazilian Atlantic rain forest. *Journal of Tropical Ecology* 30: 199–208.

Cosendai A-C, Wagner J, Ladinig U, Rosche C, Hörandl E (2013): Geographical parthenogenesis and population genetic structure in the alpine species *Ranunculus kuepferi* (Ranunculaceae). *Heredity* 110: 560–56.

Submitted manuscripts

Rosche C, Hensen I, Mráz P, Durka W, Hartmann M, Lachmuth S (submitted 02/02/2016) Invasion success in polyploids: the role of inbreeding in the contrasting colonization abilities of diploid versus tetraploid populations of *Centaurea stoebe* s.l. *Journal of Ecology.*

Rosche C, Hensen I, Lachmuth S (submitted 14/04/2016): The potential role of pre-adaptation and inbreeding-environment interactions for colonization of disturbed habitats: an experimental study with diploid and tetraploid *Centaurea stoebe*. *American Journal of Botany.*

Heinicke S, Hensen I, Rosche C, Lachmuth S, Hanselmann D, Shavrova P, Silantyeva MM, Wesche K (minor revision): Effects of habitat fragmentation and environmental conditions on genetic diversity and germination of Siberian *Stipa pennata* populations. *Flora*.

Nagy DU, Stranczinger S, Godi A, Weisz A, Rosche C, Suda, J, Mariano M, Pal RW (submitted 26/02/2016): How ploidy level influences performance: a case study with two geo-cytotypes of *Solidago gigantea* Aiton (Asteraceae). *Preslia*.

Al-Gharaibeh MM, Hamasha HR, Rosche C, Alrababah MA, Hensen I (submitted 23/03/2016): Environmental gradients shape the genetic structure of two medicinal *Salvia* species in Jordan. *Plant Biology*.

Schrieber K, Rosche C, Schleuning, M, Hensen I, Lachmuth S (submitted 10/09/2015): Sex differentiated responses to inter-specific competition in an endangered dioecious dry grassland species. *Flora*.

Conference contributions

Guggenberger G, Bischoff N, Rosche C, *et al.* (2016): Impact of land use management on soil quality along a climatic gradient in the Kulunda steppe, western Siberia – Challenges and possible solutions. *Final Conference on Sustainable Land Management BMBF Research Programme Sustainable Land Management - Challenges and Opportunities*. Berlin (Germany).

Rosche C, Lachmuth S, Hensen I (2015): Population genetic determinants of the cytotype shift in *Centaurea stoebe*. Workshop on biological invasions at a biogeographic scale. Pécs (Hungary).

Rosche C, Hensen I, Lachmuth S (2015): Invasion success of polyploids: The role of inbreeding depression for the contrasting colonization ability of diploid and tetraploid *Centaurea stoebe*. *iDiv Annual Conference*. Leipzig (Germany).

Hartmann M, Rosche C, Hensen I, Mráz P, Schaar A, Hochheimer J, Lachmuth S (2014): Variation in inbreeding depression between geo-cytotypes of highly invasive *Centaurea stoebe* s.l. *International Symposium of the German Botanical Society (DBG)*. Dresden (Germany)

Rosche C, Schrieber K, Lachmuth S, Hensen I (2011): Genetic diversity and fitness in endangered Antennaria dioica populations. *Annual conference of the Ecological Society of Germany, Austria and Switzerland (GFÖ)*. Oldenburg (Germany).

Schrieber K, Rosche C, Lachmuth S, Schleuning M, Hensen I (2011): The influence of environmental conditions on the sex ratio of *Antennaria dioica*. *Annual conference of the Ecological Society of Germany, Austria and Switzerland (GFÖ)*. Oldenburg (Germany).

Rosche C, Schrieber K, Lachmuth S, Hensen I (2011): Zur Populationsökologie von *Antennaria dioica*. *Annual Meeting of the Ziel 3 / Cíl 3 program*. Ústí nad Labem (Czech Republic).

Invited talks

Charles University Prague (2015): Colonization success of polyploids: insights from the cytotype shift in *Centaurea stoebe* s.l. *Colloquium contribution*. Prague (Czech Republic).

University of Kashmir (2015): Reduced founder effects in tetraploid vs. diploid Centaurea stoebe s.l. *Colloquium contribution*. Srinagar (India).

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A9: Eigenständigkeitserklärung

Hiermit erkläre ich, dass ich die vorliegende Doktorarbeit mit dem Titel „The potential of polyploidy to counteract the negative consequences of demographic disequilibria in colonizing populations - empirical insights from diploid and tetraploid *Centaurea stoebe*“ eigenständig und ohne fremde Hilfe verfasst sowie keine anderen als die 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. Ich erkläre weiterhin, dass ich mich bisher noch nie um einen Doktorgrad beworben habe. Die vorliegende Doktorarbeit wurde bis zu diesem Zeitpunkt weder bei der Naturwissenschaftlichen Fakultät I Biowissenschaften der Martin-Luther-Universität Halle-Wittenberg noch einer anderen wissenschaftlichen Einrichtung zum Zweck der Promotion vorgelegt.

Christoph Rosche; Halle (Saale), 14.04.2016