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THE ROLE OF THE SOIL DIASPORE BANK AS AN INTEGRAL PART OF THE LIFE HISTORY OF THE ECOLOGICALLY EXTREMELY SPECIALISED EPHEMERAL, SEMI-AQUATIC PLANT SPECIES *CYPERUS FUSCUS*

Dissertation
for obtaining his doctorate degree
at the University of Natural Resources and Life Sciences, Vienna (Institute of Botany)

Submitted by
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“Science is organized knowledge. Wisdom is organized life.”

Imanuel Kant

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Preface

In front of you lies the dissertation thesis “The role of the soil diaspore bank as an integral part of the life history of the ecologically extremely specialised ephemeral, semi-aquatic plant species *Cyperus fuscus*”, submitted for the degree of “Dr. nat. techn” at the University of Natural Resources and Life Sciences (BOKU) in Vienna.

The research described herein was conducted under the supervision of Prof. Dr. Karl-Georg Bernhardt at the Department of Integrative Biology and Biodiversity Research at the Institute of Botany between July 2012 and July 2017. The project was funded by the Austrian Science Fund (FWF) under the project number P 24558-B16.

The research wasn’t easy, but we could complete the project and answer the posed questions.

This work is to the best of my knowledge original, except where acknowledgements and references are made to previous work. Neither this, nor any substantially similar dissertation has been or is being submitted for any other degree or other qualification at any other university.

Parts of the work have been/will be presented in the following publications:

Böckelmann, J; Wieser, D; Tremetsberger, K; Šumberová, K; Bernhardt, KG

Isolation of nuclear microsatellite markers for *Cyperus fuscus* (Cyperaceae).

Appl. Pant Sci. 2015; 3(11): 1500071

Böckelmann, J; Tremetsberger, K; Grausgruber, H.; Šumberová, K; Bernhardt, KG

Fitness and growth of the ephemeral mudflat species *Cyperus fuscus* in river and anthropogenic habitats in response to fluctuating water levels.

Flora – http://dx.doi.org/doi:10.1016/j.flora.2017.07.012

Böckelmann, J; Tremetsberger, K; Kohl, G.; Šumberová, K; Bernhardt, KG

The distribution of neutral genetic variation in an ephemeral mudflat species: the role the soil seed bank and dispersal in river and secondary anthropogenic habitats.

Molecular Ecology – in preparation

Additionally, it was presented on several international conferences.

I hope you enjoy reading.

Diplom Biologe Jörg Böckelmann

Vienna, August, 2017

Zusammenfassung

Das Braune Zypergras *Cyperus fuscus* L. (Cyperaceae) ist ein im Mittelmeergebiet und gemäßigten Eurasien heimischer Vertreter der Sauergräser. Die kleine sommeranuelle Pflanze ist ein typischer Vertreter der Pioniervegetation der Schlammlingsfluren entlang der Uferbereiche naturnaher Flüsse und schließt seinen Lebenszyklus während des periodischen Trockenfallens dieser Uferbereiche ab. Die Art selbst ist nicht selten, aber das primäre Habitat mit zahlreichen seltenen Arten ist global bedroht und gehört zu den prioritär zu schützenden Lebensraumtypen der FFH Richtlinie. *Cyperus fuscus* wächst außerdem in sekundären anthropogenen Habitaten wie den traditionell bewirtschafteten Fischteichen und den zur Fischernte verwendeten künstlichen Fischhältern mit unterschiedlichen hydrologischen Bedingungen und einem historischen Verbreitungsschwerpunkt in der Tschechischen Republik.

Die kurzlebigen Arten sind an die regelmäßig auftretenden Überstauungen angepasst, nicht geeignete Zeiträume werden jedoch durch die Erhaltung einer enormen Diasporenbank im Boden überdauert. Wenn Arten neue Habitate mit veränderten Wachstumsbedingungen besiedeln, kann es sein, dass die neue Umwelt andere als die etablierten Genotypen selektiert.

Das Wachstum von *C. fuscus* wurde in über 30 zu den drei Habitattypen (naturnahe Flüsse, Fischteiche und Fischhälter) gehörigen Populationen in Zentraleuropa direkt im Gelände untersucht. Pflanzen, die aus den Bodenproben und den reifen Samen der Oberflächenpopulation angezogen wurden, wurden eine Generation geselbstet und danach wurde die Variation und Plastizität in Wachstums- und Fitnessmerkmalen unter standardisierten Bedingungen in einem Keim- und Überstauungsexperiment mit drei verschiedenen hydrologischen Regimen verglichen. Seit dem jüngsten Quantensprung in der Sequenziertechnologie ist es heute möglich, etablierte Methoden der Populationsgenetik auch an *non-model* Organismen anzuwenden. Ich untersuchte die genetische Diversität dieser Populationen, inklusive der Diasporenbank und zusätzlichen 49 Populationen im größeren europäischen Maßstab mit 21 neu entwickelten Mikrosatellitenmarkern.

Samen von Pflanzen die aus der Diasporenbank herangezogen wurden, keimten schneller als die Samen der an der Oberfläche beprobten Individuen, was nahelegt, dass die kurzzeitige Selektion von Genotypen durch die besonderen Bedingungen am Standort während der Keimung beeinflusst wird. Ich konnte zeigen, dass auch *C. fuscus* der typischen Reaktion überstauungstoleranter Pflanzen, der *low-oxygen escape* Strategie folgt, um die negativen Effekte einer temporären Überstauung, wie sie an Flüssen im Sommer typisch ist, zu vermeiden. Die überstauten Pflanzen bildeten weniger und dafür längere Blätter aus. Meine Ergebnisse zeigten, dass die Unterschiede im Wachstum zwischen den natürlichen und anthropogenen Habitaten auf den Standortfaktoren am Wuchsort (z.B. hohe Nährstoffverfügbarkeit an den Fischteichen) beruhen als auch genetisch fixiert sein müssen. Unter standardisierten Bedingungen unterschieden sich die Pflanzen aus den verschiedenen Habitaten nicht nur in Bezug auf die Mittel der Merkmale, als auch in der Plastizität. Das kann durch eine divergente Selektion auf die durch Überstauung ausgelöste Plastizität in den Habitaten erklärt werden. Pflanzen der naturnahen Flusspopulationen zeigten generell die beste Performance im Experiment und konnten die fluktuierenden Wasserlevel besser ertragen als die Pflanzen aus den anthropogenen Habitaten, während die Pflanzen an den Fischteichen im Gelände die größte Fitness zeigten.

Die Flusspopulationen sind außerdem auch genetisch diverser als die Populationen der anthropogenen Habitate. Meine Ergebnisse zeigen, dass sich die Populationen in den sekundären Standorten an die Bedingungen, die die Fischzucht seit Jahrhunderten bietet, angepasst haben. Ich konnte keine weitere Differenzierung zwischen der Diasporenbank und der Oberflächenpopulation nachweisen. Auch in Hinsicht der genetischen Diversität konnten keine Unterschiede festgestellt werden. Das legt nahe, dass die Diasporenbank genetische Variation früherer Generationen speichert und zusätzlich durch jährlich Ausbreitung von Diasporen durch Hydrochorie, Wasservögel oder auch Fische bereichert wird.

Die Art zeigt kaum genetische geographische Struktur in ganz Europa, was auf eine hohe Ausbreitungsfähigkeit hinweist. Ein Nord-Süd-Gradient in der genetischen Variation deutet auf eine nacheiszeitliche Besiedlung von Mittel- und Nordeuropa aus südlichen Refugien hin. Die Ergebnisse unterstreichen die Bedeutung der Erhaltung von unberührten Flusslebensräumen zusätzlich zu sekundären Lebensräumen für die Erhaltung der gesamten Variation und damit des adaptiven Potentials der Pioniervegetation von Uferbereichen.

Schlüsselworte: Lokale Adaptionen; Phänotypische Plastizität bei Überflutung; Samenbank; Schlammlingsflure; Sekundäre Habitate;

Abstract

The brown galingale Cyperus fuscus L. (Cyperaceae) is a graminoid native to the Mediterranean and temperate Eurasian region. This small summer-annual plant is a typical pioneer of land interface zones of rivers, where it grows during periodic drying of these zones. The species itself is not endangered, but its primary habitats are declining on a global scale and belong to the priority habitats of the European Habitats Directive and include many rare species. *C. fuscus* is also found in secondary anthropogenic habitats such as traditionally used fishponds and fish storage ponds with different hydrological regimes and with a historical distribution hotspot in the Czech Republic.

Short-lived species are adapted to regularly occurring flooding stress, but unsuitable conditions are survived through maintenance of an enormous soil seed bank. When species colonise different habitats with altered growing conditions, the new environment might select for genotypes that might differ from those suitable for the original habitat.

I surveyed growth of C. fuscus in over 30 localities belonging to the three habitat types (natural-near rivers, fishponds and fish storage ponds) in Central Europe. Variation and plasticity in traits related to fitness and growth of plants germinated from the soil seed bank and established plants from river and secondary anthropogenic habitats were compared in a germination and environmental manipulation experiment involving three different water regimes after one generation of selfing. Since the recent quantum leap in sequencing technology, it is feasible to apply established methods of population genetics to non-model organisms. An analysis of genetic diversity was performed with 21 newly developed microsatellite markers on the same localities, including the soil seed bank, and additionally on 49 populations on a European scale.

Seeds of plants derived from the persistent soil seed bank germinated faster than seeds of plants derived from established plants suggesting that short-term selection of genotypes is mediated by the particular conditions on the site during germination. I showed that C. fuscus adopts a low-oxygen escape strategy characteristic of flood-tolerant species to avoid the negative effects of temporary partial submergence that might occur during summer floods by growing higher and developing longer and narrower leaves. My results suggest that differences in growth between primary and secondary habitats (rivers, fishponds, and fish storage ponds) are both genetically fixed and caused by the growing conditions at the localities (e.g. high nutrient availability in fishponds). In a controlled environment, plants originating from different habitats differed in both trait means and plasticities, indicating that divergent selection on flooding induced plasticity had taken place. Plants from river habitats performed better in general and responded better to high and fluctuating water levels than plants from secondary habitats while plants from fishponds showed the best performance in the field. River populations are also more genetically variable than populations in anthropogenic habitats. My results suggest that populations have adapted to conditions at secondary habitats provided by the fish farming during the last centuries. There is no other differentiation between the soil seed bank and the yearly surface population. There is also no difference in the amount of genetic variation present in the soil seed bank or surface population. This suggests that the soil seed bank is a reservoir of genetic variation storing genotypes from earlier generations, which is enriched every year by immigration of seeds dispersed by running water, waterfowl, or fish transport.

Little genetic geographic structure exists in the species throughout Europe, suggesting high ability for dispersal, most probably by waterfowl. A north-south gradient in genetic variation suggests postglacial colonisation of central and northern Europe from southern refugia. To my knowledge, this is the first ephemeral mudbank species, whose populations are isolated in time and space, which has been analysed for phenotypic and genetic variation across a larger geographic area. The results highlight the importance of preserving pristine river habitats, in addition to secondary habitats, for conservation of the entire variation and hence adaptive potential of pioneer vegetation of land interface zones of wetland habitats.

Keywords: Local adaptation; Mudflat species; Phenotypic plasticity to flooding; Secondary habitats; Soil seed bank

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1 Introduction

Habitat loss in wetlands

Habitat loss and degradation are the primary environmental causes of biodiversity decline at local, regional and global scales (SALA ET AL. 2000; VAN VUUREN ET AL. 2006). Freshwater ecosystems may well be the most endangered ecosystems and wetland habitats generally face a dramatic decline (DUDGEON ET AL. 2006). Regulations of the majority of the world's rivers has led to a dramatic decline of floodplain habitats (GRASS ET AL. 2014; HEIN ET AL. 2016). They are among the most threatened habitats. Up to 90% of floodplains are functionally extinct in Europe and North America, so that a large proportion of the aquatic and temporary wetland biodiversity is nowadays concentrated in small water bodies distributed in the landscape (TOCKNER AND STANFORD 2002; DAVIES ET AL. 2004; RICHERT ET AL. 2016).

Riparian strips

Riparian strips are found at the borderline between terrestrial and aquatic habitats. These semi-aquatic habitats are characterised by an intensive disturbance regime of changing water levels with periodic flooding and drying up. Communities of dwarf rushes (class Isoëto-Nanojuncetea) develop exclusively during periods of drying up and are usually adapted to the temporal isolation effect through maintenance of an enormous soil seed bank (BERNHARDT AND POSCHLOD 1993; THOMPSON ET AL. 1997; DEIL 2005). The highly specialized vegetation type is dominated by a considerable amount of mainly wetland annuals with short life cycles, which are therefore different from the characteristic shores of standing freshwaters, which are typically species poor and dominated by single species. The summer-annual and self-compatible brown galingale *Cyperus fuscus* (*Cyperaceae*) L. is a typical representative of ephemeral mudflat communities (EAST 1940). The highly specialized species itself is not threatened, but its primary habitat along rivers is a priority habitat of the European Habitat Directive (code: 3130) and includes many rare species (DEIL 2005; ALTENFELDER ET AL. 2014; RICHERT ET AL. 2016).

Secondary "rescue" habitats (general)

For both species and ecosystems, many of the changes projected for the 21st century can best be described as shifts in potential distribution, with favourable conditions vanishing in some places, which may cause local extinctions, and appearing in new places, which may result in colonizations (PEREIRA ET AL. 2010). Possible secondary "rescue" (substitute) habitats of endangered species are therefore crucial for the conservation of biodiversity, whereby the colonization of substitute habitats can be spontaneous (RICHERT ET AL. 2016) or targeted by man (HALBUR ET AL. 2014). However, when species from an endangered habitat find refuge in a different habitat type, conditions in the new environment might select for genotypes, which differ from those suitable for the original habitat. Evolutionary forces that shape the genetic structure of species may be altered between near-natural and anthropogenic habitats and may provide opportunities for adaptive niche shifts (KAMDEM ET AL. 2012). Consequently, species persist, but with a modified genetic architecture.

Fishponds / fish storage ponds

Artificial fishpond and fish storage pond systems with a historical Central European distribution hotspot in the Czech Republic provide a rich mosaic of different wetland habitats with relatively natural features suitable as substitute habitats for threatened mudflat species (KVĚT AND JENIK 2002; ŠUMBEROVÁ ET AL. 2012; WEZEL ET AL. 2014; RICHERT ET AL. 2016). Along rivers, the level of streaming water can change fast and dynamically and is therefore unpredictable (HEJNÝ 1960). In contrast, the secondary ponds have to face regular and managed changes of water level by man and climate (KVĚT ET AL. 2002). The hydrological regime also differs between the two secondary pond types (ŠUMBEROVÁ ET AL. 2006, 2012).

Flooding

Flooding stress occurring regularly in wetland ecosystems varies in seasonal timing, duration, water depth, and frequency. For many plant species, flooding has a devastating effect on performance, but wetland species are adapted to survive temporary submergence. Evolution of suites of traits is evident in wild wetland species as well as in cultivated species like rice, adapted to particular flooding regimes (COLMER AND VOESENEK 2009; VOESENEK ET AL. 2014). The main reason why flooding hampers plant performance is a negative effect on photosynthesis due to slower gas exchange and reduced light availability. Wetland species are thus usually characterised by an adaptive plastic response to flooding involving rapid shoot elongation to restore air contact and the formation of large portions of aerenchyma to facilitate internal gas diffusion (low-oxygen escape strategy; KENDE ET AL. 1998; VRETARE 2001; BAILEY-SERRES AND VOESENEK 2008). This response seems to be favourable in environments with shallow and prolonged floods (VOESENEK ET AL. 2004). Alternatively, wetland plants may adopt a quiescence strategy by dampening energetically expensive processes such as growth, allowing endurance of deep floods or floods short in duration (e.g., VOESENEK ET AL. 2004; AKMAN ET AL. 2012; VAN VEEN ET AL. 2013).

Soil seed bank

Mudflat species with short life cycles survive unsuitable flooded periods in these habitats through maintenance of an enormous soil seed bank (= soil diaspore bank; BERNHARDT 1993; BISSELS ET AL. 2005; DEIL 2005). Soil seed banks contribute to the overall genetic variation of a given species to a differing extent depending on the amount of diaspores in the soil and the type of soil seed bank (transient or persistent; THOMPSON ET AL. 1997). The soil seed bank of *C. fuscus* has been classified as transient (THOMPSON ET AL.1997; based on a single record) or short-term persistent (WEYEMBERGH ET AL. 2004). Irrespective of the type of soil seed bank of *C. fuscus*, most of the typical Isoëto-Nanojuncetea plant species build up long-term persistent soil seed banks, where the seeds stay viable for decades or even more than hundred years (SALISBURY 1970; THOMPSON ET AL. 1997; POSCHLOD 1993; DEIL 2005; WEYEMBERGH ET AL. 2004). The presence of long-lasting viable seeds results in a complex age structure of the soil seed bank. Aging of the seeds, which is accompanied by chromosome breakage and genic mutation through progressive cleavage of DNA, may be a source of genetic novelty (LECK 1989; LEVIN 1990). Either way, the soil seed bank has to be considered as one of the key life history traits of this vegetation type (VON LAMPE 1996; BASKIN AND BASKIN 2014). The evolutionary and ecological consequences of the soil seed bank are manifold. It may function as a genetic memory by storing genetic variability and hence local adaptation to habitat in viable seeds (LECK ET AL. 1989; MORRIS ET AL. 2002; HONNAY ET AL. 2008; MANDÁK ET AL. 2012). The soil seed bank of annual plants may eliminate the selective impact of environmental conditions that fluctuate randomly from year to year and may retard the response to constant selection (TEMPLETON AND LEVIN 1979; LEVIN 1990). By increasing the effective population size, the soil seed bank may also protect populations from genetic drift (NUNNEY 2002; LUNDEMO ET AL. 2009; FALAHATI-ANBARAN ET AL. 2011). The rapidly germinating seeds of most emergent wetland plants, which lack inborn dormancy, but possess a non-deep physiological dormancy, is forced by external factors, such as darkness, lack of oxygen, and lack of temperature oscillations during flooding. Physiological dormancy is broken when the water recedes, so that seeds germinate on a huge scale (LECK 1989; BERNHARDT AND POSCHLOD 1993; BASKIN AND BASKIN 2014). Soil seed banks of freshwater wetlands along rivers and lakeshores show the highest seed densities compared to other habitats. They can contain up to 59 different species and 377,041 seeds per m^2. Composition and density show very high variation within and between habitats, with declining values with increasing salinity. Viable seeds can be found even in 1.25 m depth and reach ages of up to 400 years. In lakeshores, howere, usally 80% of the viable seeds are found within the upper 5 cm of the soil (LECK 1989; BERNHARD ET AL. 2005).

Dispersal / metapopulation

Hydrochory and epi- and endozoochorous ornithochory are likely dispersal strategies of wetland plants and even dispersal between continents is increasingly discussed (HOHENSEE AND FREY 2001; FIGUEROLA AND GREEN 2002; SOONS ET AL. 2008; VIANA ET AL. 2016). The dispersal ability of a species' seeds and so the accessibility of new habitats plays the most important role in postglacial migration after climate itself (NORMAND ET AL. 2011). Gene flow between suitable habitat patches in the form of pollen and seeds connects populations with each other. The spatial separation of populations influencing rates of gene flow among populations was already a key element in early concepts and models of population ecology and genetics (WRIGHT 1931, 1943). The metapopulation model, which has been successfully applied to many short-lived mobile animals (mostly invertebrates), describes how discrete local populations, that are present temporally in a highly fragmented habitat patches are connected through migration. According to the metapopulation model, local extinctions and (re-)colonisations are recurrent rather than unique events (HANSKI AND SIMBERLOFF 1997), especially in habitats with high levels of physical disturbance. Geographic isolation of traditionally used ponds results in an island-like status of the ponds (OOSTERMEIJER ET AL. 1996; DURKA 2000; POSCHLOD 2000) and recent studies have shown a progressive fragmentation and isolation of populations in Central Europe (TRAXLER 1991; POSCHLOD 1993; BERNHARDT 1999; BERNHARDT ET AL. 2004, 2005; KOCH ET AL. 2005). A prominent example of a plant metapopulation is provided by the annual emergent aquatic plant *Eichhornia paniculata*, which occurs in transient pools in NE Brazil (BARRETT AND HUSBAND 1997; HUSBAND AND BARRETT 1998). The species does not maintain a permanent soil seed bank. It is thought that seeds are dispersed by birds and cattle as well as through flash floods in the rainy season. So far, however, accounts of plant metapopulations remain scarce (FRECKLETON AND WATKINSON 2002; HONNAY ET AL. 2009). The presence of a soil seed bank complicates the metapopulation model in plants, since recruitment from the soil seed bank can be thought of as dispersal through time (FRECKLETON AND WATKINSON 2002; MCCAULEY 2014).

Local adaptation / phenotypic plasticity / transgenerational effects

Reciprocal transplant and common garden experiments have repeatedly shown that plant populations are adapted to local environmental conditions (e.g., CLAUSEN ET AL. 1941; LEIMU AND FISCHER 2008; RICE AND KNAPP 2008; ÅGREN AND SCHEMSKE 2012). Evolutionary ecologists working across diverse systems for decades have provided mounting evidence that even microgeographic adaptation is more widespread than it is commonly appreciated (LEKBERG ET AL. 2012; RICHARDSON ET AL. 2014). Evolution can lead to specialization, generalization or adaptive phenotypic plasticity (ANDERSON ET AL. 2014). In strongly divergent environments, particularly when conditions change slowly relative to the lifespan of an organism, specialization through local adaptation of ecotypes to a narrow range of conditions is in favour. Generalization and adaptive phenotypic plasticity may evolve, when the individuals must face multiple environmental conditions during their or their parents' lifetime (ANDERSON ET AL. 2014). With generalization, an intermediate phenotype persists in all environments. With adaptive phenotypic plasticity, individual genotypes adjust their phenotypes to suit specific conditions. Variation and plasticity in phenotypic and life history traits are important factors of a plant's ability to survive in a heterogeneous environment (BRADSHAW 1965; JAIN 1979; LLOYD 1984; PIGLIUCCI 2001). Variation in phenotypic plasticity might also play a major role in adaptation processes of these ephemeral, semi-aquatic species, which need to deal with extreme ecological conditions (BERNHARDT 1993; BERNHARDT AND POSCHLOD 1993; VON LAMPE 1996). In order to cope with situations of unpredictable flooding and drying-up along the river shorelines, plants of dwarf rush communities are known for their high phenotypic plasticity in growth (VON LAMPE 1996) and germination (CARTA ET AL. 2013). The phenotype of an individual can, however, also be affected by the environment of its parents and grandparents, a phenomenon commonly referred to as transgenerational effects (TE). These effects, if not properly acknowledged respectively controlled, may impede the interpretation of ecological studies (LATZEL 2015).

Both paternal and maternal environments may contribute to transgenerational plasticity, but maternal effects are typically greater than paternal effects, because the offspring's early growth takes place on the maternal plant (GALLOWAY 2005). The modification of the seed and its storage reserves on the one hand as well as altered DNA methylation and smRNA silencing pathways on the other hand have been invoked as underlying mechanisms (ROACH AND WULFF 1987; BOYKO ET AL. 2010). TE mediated by the modification of the seed should play a particularly significant role during the early stages of plant development (BISCHOFF AND MÜLLER-SCHÄRER 2010; LATZEL 2015). In contrast, local adaptation is expressed at all stages of life history, and may become manifest most strongly in traits associated with reproduction (e.g., RICE AND KNAPP 2008; RICE ET AL. 2013).

Genetic / epigenetic diversity / phylogeography

Genetic diversity potentially affects a wide range of population, community and ecosystem processes in direct and indirect ways (HUGHES ET AL. 2008). Low genetic diversity is likely to reduce fitness, productivity, and to restrict a species' ability to respond to changing environmental conditions through adaptation and selection (HUGHES ET AL. 2008; HENSEN ET AL. 2010). Absence of gene flow between plant populations leads to high differentiation between populations, and low within-population diversity can be a result of bottlenecks through founder effects in the past (HENSEN ET AL. 2010). In contrast, a high dispersal rate may counteract these effects even in obligately selfing species like *Arabidopsis thaliana* (FALAHATI-ANBARAN ET AL. 2014). Self-compatibility is also expected to influence a number of important features of population genetic structure and diversity. Homozygosity increases as a function of the selfing rate and thus reduces effective population size, up to twofold with complete selfing. Further, linkage disequilibrium increases (NESS ET AL. 2010). In outcrossing species, genetic variation is usually high within populations, and gene flow counteracts genetic differentiation among populations, whereas inbreeding species exhibit higher differentiation among populations (NYBOM 2004; GALEUCHET ET AL. 2005; HENSEN ET AL. 2010). The storage of seeds in the soil seed bank after (long-distance) dispersal events might also enrich the gene pool and might be responsible for genetic novelties through new genotypes (OUBORG ET AL. 1999).

The well-documented model plant *A. thaliana* is annual and self-compatible, showing most of the variation between populations. However, variation and heterozygosity are generally low. The closely related *A. lyatra*, on the other hand, is perennial and not able to self-pollinate, which is expressed in higher heterozygosity and high within-population diversity. Even though Scandinavian populations of *A. lyatra* are genetically less diverse and highly differentiated from cenral Europe, they do not show the typical signature of a leading edge postglacial re-colonisation strategy, which implies that this species survived the Ice Age in ice-free habitats north of the Alps, whereas *A. thaliana* recolonised Europe from at least two southern refugia (CLAUSS AND MITCHELL-OLDS 2006).

In the last decade, epigenetic variation underlying differentiation and adaptation of species is increasingly discussed. There is evidence that ecologically relevant traits can be acquired even in the absence or independently of genetic variation (BOSSDORF ET AL. 2008). Environmental, developmental, physiological, and other factors may not only cause genetic changes, but they may also directly cause epigenetic changes (LANG ET AL. 2016). Methylation of DNA, one of three elucidated epigenetic mechanisms, has been studied extensively over the last two decades. However, it remains poorly understood how these patterns may contribute to phenotypic diversity in natural populations.

Phylogeography describes the historical processes that may be responsible for the geographic distributions of ecspecially closly related individuals (ARVISE 2000). Enviromental changes lead to expansion or contraction of species range and affect the species distribution (COLIN AND EGUIARTE 2016). Especially the climatic changes of the Quarternary have long been an issue and had significant influence on plant distribution and evolution (COMES AND KEDEREIT 1998). As genetic data have become easier and less expensive to gather, the field of phylogeography has experienced an explosion in the number and variety of

methodological approaches to species delimitation (CARSTENS ET AL. 2013, MCCORMACK ET AL. 2013)

Microsatellites

Different types of markers provide different types of information. Microsatellites, or simple sequence repeats (SSRs), have long played a major role in genetic studies due to their typically high polymorphism (KALIA ET AL. 2011; HODEL ET AL. 2016). Since the quantum leap of sequencing technology (MARGULIES ET AL. 2005), the first genetic studies of ecologically well-characterised species without previous genome information came out in 2007 (EKBLOM AND GALINDO 2011). Even though newer methods with wide applicability like genotyping-by-sequencing (GBS) are increasing in popularity, microsatellites will continue to play an important role as effective and cost-efficient neutral markers that are transferable across closely related species (KALIA ET AL. 2011; HODEL ET AL. 2016). The usefulness of microsatellites is, however, compromised in polyploid organisms, because it is difficult or impossible to determine the allele copy number in partially heterozygous genotypes and because inheritance patterns are complex (BRUVO ET AL. 2004; DE SILVA ET AL. 2005). Diploid as well as tetraploid plants of *Cyperus fuscus* have been reported (2*n* = 36 and 72 chromosomes; KRAHULCOVÁ 2003), but the possible occurrence of diploids and tetraploids in the study region was unknown at the beginning of the thesis. Differences in ploidy level of a species are influencing the practicability of studies of genetic diversity, so that the knowledge of the ploidy level is a necessary pre-requisite for microsatellite genotyping studies. Especially in invasion biology a polypoidisation as an important factor of invasion success is discussed (TE BEEST ET AL. 2012).

2 Objectives and definition of the topic

*Cyperus fuscus w*as used as a model and typical representative of the ephemeral dwarf rush communities, original growing in the rare and threatened habitat type along the shores of natural-near rivers. I addressed several questions, which are grouped under four objectives. To my knowledge, this is the first study of genetic diversity of a species of this community of highly specialised semi-aquatic plant species, especially under consideration of the secondary anthropogenic habitats and the role of the soil seed bank.

Objective 1 was to develop new microsatellite (MS) markers for this non-model species in order to study its genetic diversity.

Question 1.1. Is the design of at least 12 polymorphic MS markers with the help of next-generation sequencing possible?

Question 1.2. The price for genotyping an individual drops when multiplexing is used. Are there practicable combinations of primers to analyse groups of markers in a single PCR reaction?

Question 1.3. Differences in ploidy level of a species are influencing the practicability of studies of genetic diversity. Are there differences in ploidy level in *C. fuscus*? What is the exact genome size of this species?

Objective 2 is fundamental to this thesis. It aimed at shedding light on the soil seed bank as an integral part of the life cycle of this ephemeral plant species.

Question 2.1. Is the soil seed bank of *C. fuscus* transient or persistent?

Question 2.2. Are there any differences in germination and growth under standardised environmental conditions between seeds originating from the soil seed bank and those harvested from the above-ground populations?

Question 2.3. Does the genetic composition of the above-ground populations differ from their soil seed bank? If the soil seed bank proves to be persistent, is it functioning as a “genetic memory”?

Objective 3 was to examine the growth and genetic diversity of *Cyperus fuscus* and its soil seed bank within its primary river habitats and secondary artificial fishpond and fish storage pond systems with different hydrological regime. Water levels in rivers change fast and unpredictably, whereas the regime in fishponds is more predictable with regular dried-out periods in summer. Fish storage ponds are without water most of the time. In addition to the relatively dry conditions in the artificial concrete basins, the vegetation is controlled by mowing, grazing and increasingly with the help of herbicides. Preliminary observations point to a higher degree of **phenotypic variation/plasticity** in populations of ephemeral freshwaters than of permanently watered ponds.

Question 3.1. Do rivers as primary habitats show higher biodiversity than secondary ponds?

Question 3.2. Are there any differences in growth traits and the zonation of the soil seed bank between the three habitat types detectable in the field?

Question 3.3. What is the response of *C. fuscus* to temporary flooding? Do plants from the three habitat types persistently vary in their response to flooding?

Question 3.4. Do any differences in growth under field conditions persist under standardised conditions in the flooding experiment? Does the species show local adaptation to habitat conditions?

Question 3.5. Do populations from river and anthropogenic habitats differ in their level of variation within and among populations due to altered levels of gene flow? Are they genetically differentiated due to differential selection pressures?

Objective 4. The objective was to study the population structure and history of C. fuscus across a larger European scale.
The questions above have been studied in Central Europe, but *C. fuscus*, as many other mudflat species, occurs sporadically all over Europe—for short periods of time and at different points in time—meaning that populations are isolated spatially and temporally from each other.

Question 4.1. Are populations across a larger geographic area genetically differentiated due to the action of drift and/or selection and lack of gene flow among sites or are populations of distant sites genetically similar due to high levels of gene flow?

Question 4.2. Hydrochory with flowing water is the most forward explanation for dispersal of this species, but is theoretically unidirectional and just within river systems. Are there also indications for different dispersal vectors?

Question 4.3. Are there indications whether *C. fuscus* survived the Ice Age north of the Alps or in Mediterranean ice free refugia?

Question 4.4. Is the metapopulation model applicable for populations of *C. fuscus*, which disperses not just in space, but also in time?

3 Data basis, material and methods

3.1 Study species

The brown galingale *Cyperus fuscus* (*Cyperaceae*) is a summer-annual, self-compatible graminoid native to the Mediterranean Region and temperate Eurasia (EAST 1940). The small achenes have no particular dispersal features and are supposedly dispersed by wind, water, and in mud carried away by animals (e.g., waterfowl) or humans (e.g., on footwear or vehicles; VON LAMPE 1996; ŠUMBEROVÁ ET AL. 2012). The species was apparently introduced into North America in the late 1800s, where it steadily moved southward and westward. BRYSON AND CARTER (2010) indicate the species as a potential threat to natural plant communities and rice agriculture in six US states. *Cyperus fuscus* grows on muddy, sandy or gravelly substrats in habitats subjected to changing water levels and characterized by low competition of perennial plants. As a small ephemerophyte, it is a typical component of amphibious short annual vegetation, pioneer of land interface zones of rivers, lakes, pools and ponds, where it grows during periodic drying of these waters (class Isoëto-Nanojuncetea Br.-Bl. et Tx. 1943). The species can also be found in secondary habitats like gravel pits, wet fields as well as traditionally used fishponds and fish storage ponds, which are mainly used for breeding of common carp (*Cyprinus carpio* L.) in the Czech Republic and some other regions of Europe. In the Old World, the species itself is not threatened, but its habitat is a priority habitat of the European Habitats Directive (code: 3130) and includes many rare species (DEIL 2005; ALTENFELDER ET AL. 2014). As other mudflat species, *C. fuscus* is maintaining an enormous soil seed bank, out of which seeds germinate under favourable conditions (BASKIN AND BASKIN 2014).

Figure 3-1 Historical illustration of *Cyperus fuscus* (left) and *C. glomeratus* (right) Original book source: Prof. Dr. Otto Wilhelm Thomé Flora von Deutschland, Österreich und der Schweiz 1885, Gera, Germany Permission granted to use under GFDL by Kurt Stueber Source: www.biolib.de.

Figure 3-2 Photo of *C. fuscus* in its primary habitat on a stony riverbank.

3.2 Characterization of the studied habitats

Every sampled locality is unique, but still a typical variant of one of the three habitat types—amphibious shorelines of rivers, fishponds, and fish storage ponds—that face different hydrologic regimes. Suitable river habitats have become rare due to regulation of the water flow in the last centuries (e.g., HEIN ET AL. 2016). The level of the streaming water is changing fast and dynamically and is therefore unpredictable, but the mudbanks are usually exposed in later summer (HEJNÝ 1960). Because of the scarcity and ephemerality of mudbanks along rivers, it was hard to find suitable populations within one growing season in the study region. Moreover, river habitats in the South Bohemian Region, where the density of anthropogenic habitats is highest, are not suitable for *C. fuscus* because of the acidic granite substrate and the small size of the rivers. The two secondary habitats differ from rivers and from each other. Historical, semi-intensively managed fishponds face regular and managed changes of water level by man and climate (KVĚT ET AL. 2002). The sapropelic mud is usually thick, nutrient rich and exposed for a few months in summer. Fishponds can still be referred to as relatively natural habitats for mudflat species. After fish harvest in autumn, the fish is stored alive in the fish storage ponds before it is sold. Flooding and management is based on ad hoc decisions of the fish farmers. The eventual utilization as summer storage ponds makes the flooding regime even less predictable. Fish storage ponds can be flooded within hours and emptied after days or weeks. Most of the year, however, these concrete tanks with stony ground are without water. Management of fish storage ponds is aimed at maintenance of low-nutrient conditions and elimination of muddy sediments with high water capacity. High ground temperatures during summer may create drought stress. To maintain the basins, the ground vegetation is removed by grazing, mowing or, more recently, herbicides (ŠUMBEROVÁ ET AL. 2006, 2012).

3.3 Field studies and sampling for experiments

From July to October 2012, my collaborators and I visited 33 localities of the three different habitat types, rivers (11 localities), fishponds (10;), and fish storage ponds (12), in Austria, the Czech Republic, Poland and Slovakia (Table 3-1, Map Fig. 3-3; exemplary photos of habitat types Fig. 3-4 – 3-11). At each locality, we established three 1 m2 plots within the C. fuscus population. On each of these 1 m2 plots, vegetation relevés according to the method of Braun-Blanquet (1951) were performed. After that, preferably twelve ripe plants were collected. The roots of these plants were rinsed out in the field and the shoot height was measured. Thereafter, the plants (1162 in total) were dried at ambient temperature. Their shoot and root biomass as well as the number of culms with inflorescences were determined. After that, plants were stored at 6°C in the dark for six months until seeds were taken for further experiments. The relevés digitalized and imported into Czech National Phytosociological Database (CHYTRÝ AND RAFAJOVÁ 2003). The species numbers have been evaluated and compared with respect to the three habitat types.

Further, five soil samples, each with two fractions, 0-5 cm deep soil (shallow seed bank) and 5-15 cm deep soil (deep seed bank), were taken in every 1 m^2 plot, four samples in the corners and the fifth in the middle (Fig. 3-12). The first 5 mm of topsoil were removed and the shallow seed bank was sampled with a metal square (10 cm × 10 cm). The deep seed bank was abstracted with a shovel. After removal of plant fragments and stones, the soil samples were cooled for the rest of the field trip and then stored at 6°C in the dark for approximately six months.

Weight of the dried plants and number of inflorescences have been evaluated by Hadi Hassanbeikborojerdi and used for his master thesis “Fitnessfaktoren des Sumpfgrases Cyperus fuscus in drei verschiedenen Landnutzungstypen.” finished in 2015 at the Botanical Institute of Boku University.

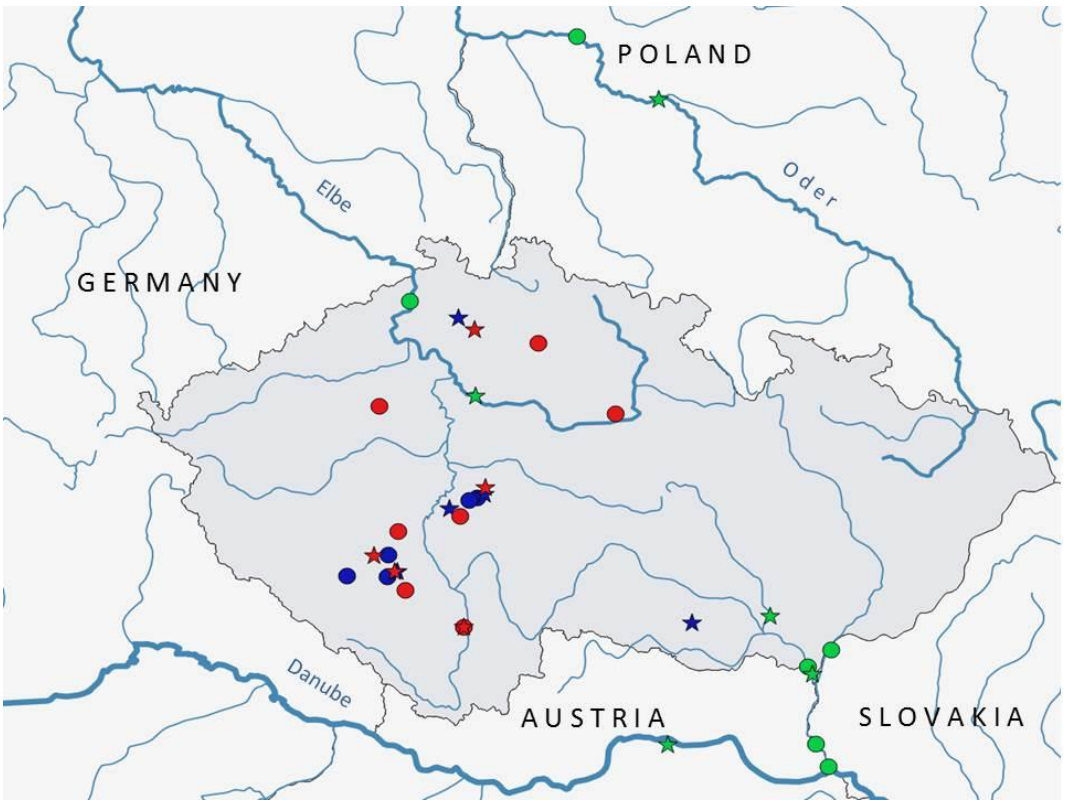

Figure 3-3 Map of study sites of *Cyperus fuscus* populations in Central Europe. Circles represent sites investigated in the field only. Stars represent sites further analysed in the germination and environmental manipulation (flooding) experiment. Green symbols represent near-natural sites along rivers (n = 11), blue symbols fishponds (n = 10), and red symbols fish storage ponds (n = 12). Details of the corresponding localities are shown in Table 3-1.

Figure 3-4 Dense vegetation on the exposed bottom of the fishpond Novazámecký that had been flooded for over twenty years (photo by K. Tremetsberger).

Figure 3-5 Stony mudbank along the near-natural river March in Austria (NN08).

Figure 3-6 Eutrophic oxbow of the river March in the Czech Republic (NN04).

Figure 3-7 Fortified riverbank at the river Vlatva near Bukol (Czech Republic; photo by K. Tremetsberger).

Figure 3-8 Exposed bottom of a fishpond near Libohošť, Czech Republic (P03).

Figure 3-9 Exposed bottom of the fishpond Velkorojický near Rojice, Czech Republic (P05).

Figure 3-10 Mown fish storage pond in Rojice, Czech Republic (SP09).

Figure 3-11 Fish storage pond in Semovice, Czech Republic (SP06).

Figure 3-12 Method for sampling of soil seed bank.

3.4 Next generation sequencing and isolation of nuclear microsatellite markers

Plants were grown in the greenhouse from ripe seeds collected in the field (Appendix 10.1). Genomic DNA of fresh leaves from one plant was extracted with the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions and sent to LGC Genomics (Berlin, Germany) for next-generation sequencing (NGS) on a Genome Sequencer FLX Titanium Instrument (454 Life Sciences, a Roche Company, Branford, Connecticut, USA). MSATCOMMANDER version 0.8.2 (FAIRCLOTH 2008) was used to detect sequences with simple sequence repeat (SSR) motifs (options: dinucleotide repeats ≥10 repeat units, tri- and tetranucleotide repeats ≥6 repeat units, combine multiple arrays within a sequence if within 50 bp distance). Primers for microsatellite-containing sequences were also designed in MSATCOMMANDER using Primer3 (ROZEN AND SKALETSKY 1999), with a GTTT PIG-tail (BROWNSTEIN ET AL. 1996) added to the 5′-end of one primer and a CAG or M13R tail (CAG: 5′-CAGTCGGGCGTCATCA-3′; M13R: 5′-GGAAACAGCTATGACCAT-3′) added to the 5′-end of the other primer (SCHUELKE 2000). PCR amplifications were performed in a 25 µL final volume of REDTaq ReadyMix PCR Reaction Mix (Sigma-Aldrich, St. Louis, Missouri, USA) with 0.40 µM 5′-FAM-labeled universal CAG or M13R primer, 0.40 µM GTTT-tailed primer, 0.04 µM CAG- or M13R-tailed primer, and 1 µL diluted DNA extract (2–20 ng DNA). Reactions were performed using a touchdown PCR protocol in an Eppendorf Mastercycler gradient (Eppendorf, Hamburg, Germany), with an initial 5 min of denaturation at 95°C; 24 cycles with denaturation at 95°C for 45 s, annealing at 63–48.6°C (0.6°C decrease per cycle) for 90 s, and extension at 72°C for 60 s; 19 cycles with denaturation at 95°C for 45 s, annealing at 50°C for 90 s, and extension at 72°C for 60 s; and a final extension at 72°C for 5 min and 60°C for 30 min. Amplified fragments were analysed on a 3500 Genetic Analyzer (Applied Biosystems, Foster City, California, USA) and sized using GeneMarker 2.4 (SoftGenetics, State College, Pennsylvania, USA). The markers were tested on seven individuals from different localities (Appendix 10.1). Seven loci could be unambiguously

scored in all seven test individuals. A second NGS run of an SSR-enriched library was performed at ecogenics (Balgach, Switzerland), starting from a mix of genomic DNA of two individuals (Appendix 10.1). Size-selected fragments from genomic DNA were enriched for SSR content by using magnetic streptavidin beads and biotin-labeled CT, GT, AAG, and ATGT repeat oligonucleotides. The SSR-enriched library was analysed on a Roche 454 platform using the GS FLX Titanium reagents (454 Life Sciences, a Roche Company). Ecogenics sent 80 primer pairs also designed with Primer3, containing an M13 tail at the 5′-end of the forward primer (5′-TGTAAAACGACGGCCAGT-3′; SCHUELKE 2000) and no PIG-tail. For primer testing, the concentrations and volumes for PCR were the same as above, but I used JumpStart REDTaq ReadyMix Reaction Mix (Sigma-Aldrich) and a regular PCR protocol, with an initial 5 min of denaturation at 95°C; 38 cycles of denaturation at 95°C for 45 s, annealing at 56°C for 60 s, and extension at 72°C for 1 min; and a final extension at 72°C for 5 min and 60°C for 30 min. Polymorphic markers were selected for further analysis and combined into four multiplex PCRs with Multiplex Manager version 1.0 (HOLLELEY AND GEERTS 2009; PCR multiplex sets 1–4 in Table 2). The remaining loci are shown in Appendix 2. For application of PCR multiplex sets 1–4 to a larger number of individuals, a GTTT PIG-tail was added to the reverse primers (as for primers with the prefix Cf). For multiplex PCR reactions, the forward primers were directly labeled with a fluorescent dye at the 5′-end (Table 3-2).
David Wieser helped with the detection and testing of the microsatellite motivs and finished his master thesis “Mikrosatelliten-Entwicklung für die einjährige Schlammlingsart Cyperus fuscus.” in 2015.

3.5 **Genome size and chromosome counts**

The following measurements have been performed within the project CZ 13/2012 “Polyploidy, ecological niche and demographic development of the wetland annual plant species *Cyperus fuscus*” funded by the Austrian Agency for International Cooperation in Education and Research (OeAD GmbH). Genome size measurements were mainly carried out by Soňa Píšová and chromosome counts by Karin Tremetsberger. Genome size measurements using propodium iodide staining and chromosome counts were performed at the University of Vienna (laboratory of H. Weiss-Schneeweiss).

Relative and absolute DNA content has been measured from fresh leaves of individual plants by using two different flow cytometric methods: (1) 4',6-diamidino-2-phenylindole (DAPI) staining and (2) propidium iodide (PI) staining. DAPI staining was used for rough overview of the relative DNA content of a large number of individuals, whereas PI staining was used for exact determination of the absolute genome size of a limited number of individuals. Fresh leaves of *Solanum pseudocapsicum* (1.295 pg/1C; TEMSCH ET AL. 2010) were used as an internal size standard for both methods.

For DAPI measurements, fresh leaves were prepared by using the CyStain® UV Precise P kit (Partec), following the manufacturer’s instructions. Fresh leaves of the sample and the internal size standard were chopped together with a razor blade in 400 µl extraction buffer. After c. 1 min incubation, the suspension containing cell walls and cell content incl. nuclei was filtered through a CellTrics filter (Partec; mesh size 50 µm). The filtered suspension was incubated with 1.6 ml staining buffer for c. 1 min or longer. Measurements were done in a Partec Ploidy Analyser equipped with a HBO 100 long life mercury lamp (Partec).

For PI measurements, Otto et al.’s (1981) buffer was used for extraction of nuclei following the chopping method of Galbraith et al. (1983). After chopping, the suspension was filtered through a 30 µm nylon mesh (Sefar AG, Rüschlikon, Switzerland). Thereafter, 50 µl RNase A was added. Digestion of RNA took place at 37°C for 30 min. in a water bath. After digestion, the suspension was supplied with 4 ml propidium iodide solution (pH ≈ 9.5) and incubated in the dark at room temperature for at least 20 minutes or at 4°C overnight. Measurements were carried out in a CyFlow® ML flow cytometer (Partec) equipped with a Cobolt Samba

green laser (Cobolt). For both, DAPI and PI methods, values for mean and CV of peaks were obtained with the software FloMax ver. 2.81 (© 2010 by QA GmbH, Münster, Germany).

For chromosome counts, seeds were germinated on filter paper in petri dishes in a Sanyo MLR-352 environmental test chamber ("day": 35°C/12 hours/light [15 fluorescent lamps FL40SS-W/37 on]; "night": 15°C/12 hours/dark) and watered with distilled water. After 7-8 days (c. 1-3 hours after start of the "day" conditions), radicles were cut off from the seedlings and pre-treated with 0.002 M 8-hydroxychinoline for 4 hours at 12°C (or for 4 hours on ice and at 4°C overnight thereafter). Thereafter, the radicles were fixed in freshly made absolute ethanol: glacial acetic acid (3:1) and kept at −20°C until preparation.

Chromosome counts have been attempted by using two methods: (1) Feulgen staining and (2) enzymatic digestion and squashing as described by Schwarzacher and Heslop-Harrison (2000). The Feulgen staining method proved to be not suitable for such small chromosomes as encountered in *Cyperus fuscus*. Therefore, chromosomes had to be prepared by the enzymatic digestion and squashing method following the protocol of H. Weiss-Schneeweiss (e.g. SCHÖNSWETTER ET AL. 2007). To this aim, fixed root tips were kept in citric acid-trisodium citrate buffer (pH 4.8) for 20 min. Thereafter, the citrate buffer was replaced with enzyme mixture (1% [w/v] cellulase Onozuka [Serva, Heidelberg, Germany], 0.4% [w/v] pectolyase [Sigma-Aldrich], 0.4% [w/v] cytohelicase [Sigma-Aldrich], in citrate buffer, pH 4.8, pre-warmed at 37°C) and the root tips were incubated for 30 min at 37°C in an incubator, until the root material was loose. After removal of the enzyme mixture, the root tips were kept in citrate buffer for a minimum of 30 min and up to several hours to stop the enzymatic reaction, until squashing was performed. For squashing, root tips were transferred to a drop of 60% acetic acid on a slide (alternatively, 45% acetic acid could also be used), dissected using entomological needles under a stereo microscope and squashed after having been covered with a cover slip. Preparations were frozen on a cooling plate, air dried after cover slip removal, and stored at −20°C until observation. After application of 9 µl of Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA) with 2 µg/ml DAPI to the dry preparations, these were screened for well-spread mitotic metaphases under a Zeiss (Oberkochen, Germany) Axio Imager.M2 epifluorescence microscope equipped with an AxioCam HRm camera. Images were acquired with the Zeiss AxioVision SE64 software.

Table 3-1 Locations of the 33 populations of *Cyperus fuscus* in Central Europe according to three habitat types.

Locality, collectors, voucher number (WHB[1]) and collection date	Coordinates	Sea level	Germination and flooding experiment
Rivers			
Czech Republic, Central Bohemian Region, Záryby: shoreline of river Labe (JB, KŠ, KT, PK, SP, ZH[2]; WHB 62957; 21.08.2012)	N 50° 13.424' E 14° 37.717'	168 m	Included
Czech Republic, South Moravian Region, Lanžhot: shoreline of river Dyje (JB, KŠ, KT; WHB 62982; 10.09.2012)	N 48° 42.710' E 16° 54.169'	152 m	Not included
Czech Republic, South Moravian Region, Lanžhot: river arm of river Dyje (JB, KŠ, KT[2]; WHB 62981; 10.09.2012)	N 48° 40.354' E 16° 55.442'	150 m	Included
Czech Republic, South Moravian Region, Moravská Nová Ves: oxbow of river Morava (JB, KŠ, KT[2]; WHB 62980; 11.09.2012)	N 48° 47.079' E 17° 4.793'	162 m	Not included
Czech Republic, South Moravian Region, Velké Němčice: alluvial sediments of river Svratka (JB, KŠ, KT[2]; WHB 62979; 11.09.2012)	N 48° 59.056' E 16° 39.894'	183 m	Included
Czech Republic, Ústí nad Labem Region, Nebočady: artificial pool of river Labe (JB, KŠ, PK, SP[2]; WHB 62978; 13.09.2012)	N 50° 43.767' E 14° 11.222'	130 m	Not included
Austria, Lower Austria, Zwentendorf: shoreline of river Traisen[4] (JB[2]; WHB 62956; 18.09.2012)	N 48° 22.245' E 15° 50.283'	183 m	Included
Austria, Lower Austria, Markthof: sidearm of river March (JB, KŠ, PK[2]; WHB 62974; 05.10.2012)	N 48° 11.485' E 16° 58.302'	126 m	Not included
Poland, Lower Silesia, Borków: artificial pool of river Odra (JB, KŠ, PK[2]; WHB 62973; 02.10.2012)	N 51° 40.477' E 16° 12.239'	76 m	Included
Poland, Lower Silesia, Cigacice: artificial pool of river Odra (JB, KŠ, PK[2]; WHB 62955; 03.10.2012)	N 52° 1.883' E 15° 36.659'	55 m	Included (only germination experiment)
Slovakia, Bratislava Region, Vysoká pri Morave: river arm of river Morava (JB, KŠ, PK[2]; WHB 62972; 05.10.2012)	N 48° 18.739' E 16° 54.224'	155 m	Not included
Fishponds			
Czech Republic, Liberec Region, Zahrádky: fishpond Novozámecký, used for marketable fish, dried after many years due to dam reconstruction (JB, KŠ, KT[2]; WHB 62967; 10.07.2012)	N 50° 37.687' E 14° 32.595'	252 m	Included
Czech Republic, Central Bohemian Region, Petrovice: fishpond Horní Petrovický, used for fish fry, regularly dried in summer (JB, KŠ, KT[2]; WHB 62965; 11.07.2012)	N 49° 43.099' E 14° 39.030'	400 m	Included

Czech Republic, Central Bohemian Region, Libohošť: fishpond Libohošťský, used for fish fry, regularly dried in summer (JB, KŠ, KT^2; WHB 62964; 11.07.2012)	N 49° 42.057' E 14° 35.398'	374 m	Not included
Czech Republic, South Bohemian Region, Chrášťovice: fishpond Chválovec, used for fish fry, regularly dried in summer (JB, KŠ, KT^2; WHB 62962; 13.07.2012)	N 49° 19.262' E 13° 53.836'	466 m	Not included
Czech Republic, South Bohemian Region, Rojice: fishpond Velkorojický, used for marketable fish, low water level after about 5 years (JB, KŠ, KT^2; WHB 62961; 13.07.2012)	N 49° 20.998' E 13° 56.540'	457 m	Included
Czech Republic, Plzeň Region, Smrkovec: fishpond Velký Smrkovec, used for marketable fish, regularly with low water level due to small inflow (JB, KŠ^2; WHB 62997; 24.07.2012)	N 49° 20.211' E 13° 35.915'	473 m	Not included
Czech Republic, South Bohemian Region, Skaličany: fishpond Pýcha, used for fish fry, regularly dried in summer (JB, KŠ^2; WHB 62996; 24.07.2012)	N 49° 26.078' E 13° 54.699'	489 m	Not included
Czech Republic, Central Bohemian Region, Sedlečko: fishpond Velký Sedlečský, used for marketable fish, in biennial intervals with low water level (JB, KB^2; WHB 62994; 27.07.2012)	N 49° 41.614' E 14° 32.091'	447 m	Not included
Czech Republic, Central Bohemian Region, Solopysky: fishpond Dolní Solopyský, management unknown, very often low water level (JB, KB^2; WHB 62992; 02.08.2012)	N 49° 39.217' E 14° 23.095'	379 m	Included
Czech Republic, South Moravian Region, Křepice: unnamed small village fishpond, drained after many years due to dam reconstruction (KB^2; WHB 62983; 05.09.2012)	N 48° 59.193' E 16° 5.641'	334 m	Included
Fish storage ponds			
Czech Republic, Pardubice Region, Lázně Bohdaneč: fish storage ponds with long summer drainage, mowing and herbicide spraying (started 2012) (JB, KŠ, KT^2; WHB 62970; 09.07./20.08.2012)	N 50° 4.994' E 15° 39.887'	220 m	Not included
Czech Republic, Liberec Region, Doksy: fish storage ponds with long summer drainage and grazing (JB, KŠ, KT^2; WHB 62969; 10.07.2012)	N 50° 33.830' E 14° 39.532'	266 m	Included
Czech Republic, Central Bohemian Region, Mšec: fish storage ponds with long summer drainage and herbicide spraying (JB, KŠ, KT^2; WHB 62963; 12.07.2012)	N 50° 11.815' E 13° 54.651'	410 m	Not included
Czech Republic, South Bohemian Region, Hluboká nad Vltavou: fish storage pond with short summer drainage and herbicide spraying	N 49° 2.624' E 14° 25.952'	376 m	Included

(JB, KŠ, KT[2]; WHB 62960; 14.07.2012)			
Czech Republic, Central Bohemian Region, Nedrahovice: fish storage ponds with short to long summer drainage and herbicide spraying (JB, KB[2]; WHB 62993; 01.08.2012)	N 49° 36.856' E 14° 27.600'	360 m	Not included
Czech Republic, Central Bohemian Region, Semovice: fish storage pond with short summer drainage and occasional herbicide spraying (JB, KB[2]; WHB 62959; 06.08.2012)	N 49° 45.067' E 14° 39.655'	357 m	Included
Czech Republic, South Bohemian Region, Hluboká nad Vltavou: fish storage pond with long summer drainage and herbicide spraying (JB, KB, KŠ[2]; WHB 62991; 07.08.2012)	N 49° 2.686' E 14° 25.991'	372 m	Not included
Czech Republic, South Bohemian Region, Čejetice: fish storage pond with long summer drainage and mowing (JB, KŠ[2]; WHB 62958; 08.08.2012)	N 49° 14.973' E 14° 1.331'	387 m	Not included
Czech Republic, South Bohemian Region, Rojice: fish storage pond with long summer drainage, mowing and low-intensity poultry grazing (JB, KŠ[2]; WHB 62989; 08.08.2012)	N 49° 20.842' E 13° 56.903'	450 m	Included
Czech Republic, Central Bohemian Region, Dobrá Voda: fish storage pond with long summer drainage and sheep grazing (JB[2]; WHB 62988; 09.08.2012)	N 49° 33.247' E 13° 59.790'	449 m	Not included
Czech Republic, South Bohemian Region, Tchořovice: fish storage pond with long summer drainage and mowing (JB[2]; WHB 62987; 09.08.2012)	N 49° 26.115' E 13° 48.442'	447 m	Included
Czech Republic, Central Bohemian Region, Střehom: fish storage ponds with short summer drainage, mowing and herbicide spraying (JB, KŠ, KT, SP[2]; WHB 62985; 23.08.2012)	N 50° 28.341' E 15° 7.952'	252 m	Not included

[1]WHB = Herbarium of the University of Natural Resources and Life Sciences, Vienna.

[2]Collectors: JB = Jörg Böckelmann, KB = Kateřina Bubíková, KŠ = Kateřina Šumberová, KT = Karin Tremetsberger, PK = Pavel Kúr, SP = Soňa Píšová, ZH = Zdenka Hroudová, ZK = Zygmunt Kącki.

[3]Traits of plants collected in the field not measured.

3.6 Sampling and DNA extraction for analysis of genetic diversity

I analysed 31 populations of the three habitat types (rivers, fishponds and fish storage ponds) and, in addition, 49 populations on a European scale for their genetic diversity (Table 3-1 and 3-5; Fig. 3-13). For the analysis of habitat types, the soil samples from the two depths and the ripe seeds were collected in summer 2012 and stored in the dark at 6 °C for approximately six months. In April 2013, the soil samples of the same plot and fraction were pooled and carefully homogenized before being spread on sterile sand. Ideally, 15 emerging *C. fuscus* seedlings have been pricked in a single pot. Later-on, a leaf sample was taken and dried on silica gel. All other emerging seedlings than *C. fuscus* have been determined, counted and discarded. For the ripe seeds collected in the field, the same procedure was applied. The DNA of the silica-dried leaf samples has been extracted with the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions. Every extract of genomic DNA was checked on 1% agarose gel and concentration was measured on NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

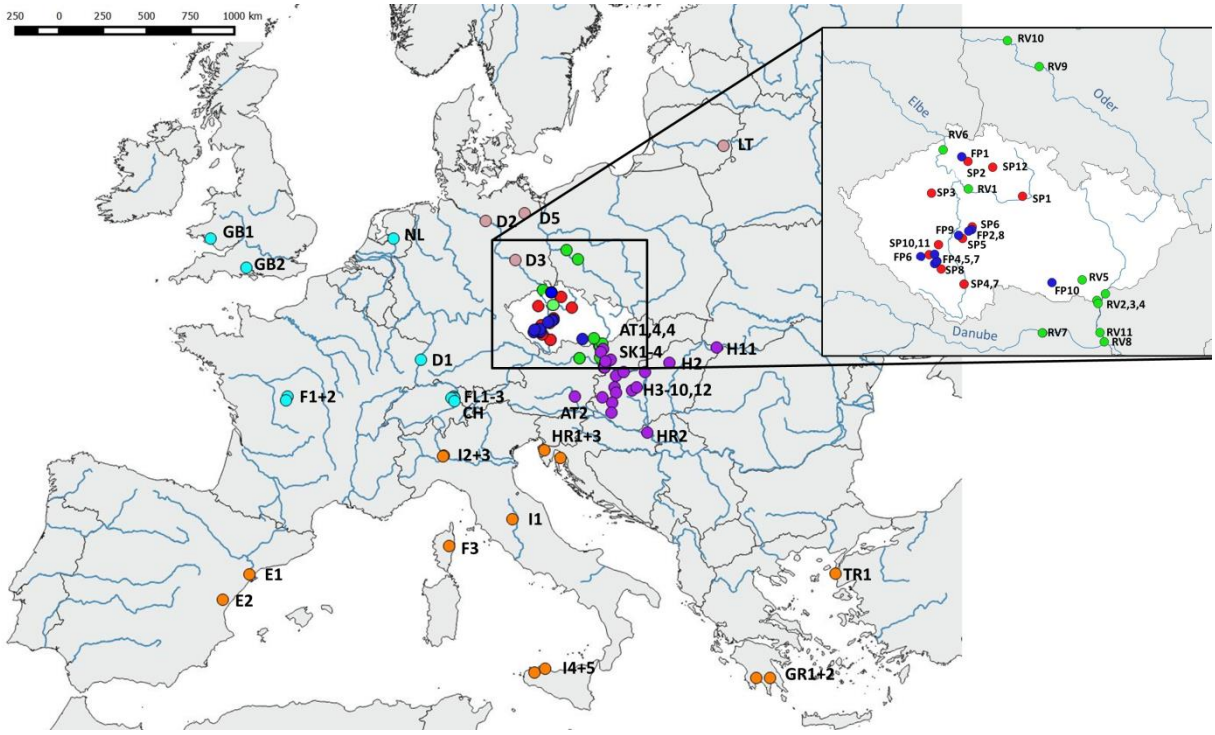

Figure 3-13 Map of study sites of *Cyperus fuscus* populations across Europe. Colour coding refers to the coarse geographical European regions. Blue, green and red dots refer to habitat types (mainly assigned to the Bohemian Massif region; see Fig 3-10 and Table 3-1). Orange dots refer to Mediterranean Europe, blue dots to Western Europe, pink dots to Northern Europe and lilac dots to South-Eastern Europe. Details of the corresponding localities are shown in Tables 3-1 and 4-5.

3.7 Population genetic analysis

For the population level analysis, only localities with at least five amplified individuals have been considered. These localities were assigned to five coarse geographical regions (Fig. 3-13). Individuals from the two different depths of the soil seed bank were combined. An assumed null allele in one marker (Cypfus_2663) was treated as missing data. After this cleaning of the dataset, a final dataset of 1429 plants belonging to 103 populations (with the soil seed bank treated as separate population) belonging to five coarse geographical regions remained for further analysis.

For investigation of the genetic differentiation within and between the three habitat types as well as within and between the above-ground population and the soil seed bank, two additional datasets were created. The habitat dataset contained 988 individuals. After exclusion of one fishpond, in which just one individual from the soil seed bank could be genotyped successfully, the seed bank dataset contained 959 individuals. For all populations, observed and expected heterozygosities (H_o, H_e) and the fixation index (F_{IS}) were calculated using GenAlEx 6.5 (PEAKALL AND SMOUSE 2006, 2012). Because of the different sample sizes, allelic richness (AR) and private allelic richness (priv. AR) were calculated using the rarefaction method in the program HP-Rare (KALINOWSKI 2005) based on 5 individuals (10 genes). For the evaluation of the differences in AR and priv. AR between coarse geographic regions, the maximum number of individuals for rarefaction was set to 66 (132 genes). For the evaluation of the differences in AR and priv. AR between the above-ground population and the soil seed bank, the values were calculated separately for each locality with the same program.

Analyses of Molecular Variance (AMOVA) and a test on deviation from Hardy-Weinberg-equilibrium (HWE) were conducted in Arlequin software Vers 3.5 (EXCOFFIER AND LISCHER 2010). For HWE, a Markov chain with 1,000,000 steps following 1,000,000 dememorisation steps, was calculated. For AMOVA, the F_{ST} distance matrix and 10,000 permutation steps have been used as measure of differentiation, to assess the distribution of genetic variation within and among several groupings. Like for the genetic diversity, different datasets were used for analysis and each dataset has been adapted trough the elimination of some individuals to meet the requirements of the G statistics before calculation, while the permutation steps for different AMOVAs were identical (10,000 steps). The calculation was performed on a locus by locus basis. Individuals with up to 20% missing data were used. Differences between habitats (A; incl. the soil seed bank), river systems (B1 across Europe excl. the soil seed bank; B2 just river localities from habitat dataset incl. the soil seed bank), the above-ground population and the soil seed bank (C), and coarse geographical regions excl. the soil seed bank (D) were calculated. After the calculations of the AMOVAs as described above, a pairwise comparison for the habitat and soil seed bank datasets was performed for every locality and afterwards means over habitat types have been calculated. Settings were the same and the mean variation was calculated among populations within each habitat type and between the above-ground population and the soil seed bank. The genetic distances (F_{ST}) between all pairs of populations were calculated for two of the datasets used for AMOVA (A and D) using GenAlEx 6.5 (PEAKALL AND SMOUSE 2006, 2012). These two distance matrices were used to produce two neighbour-joining trees with SPLITSTREE Vers 4.14.4 (HUSON AND BRYANT 2006).

To check if the set of SSRs obtained were informative enough to discriminate geographical patterns, I tested whether genetic structure reflects the geographical distribution of the samples by using STRUCTURE Vers. 2.3.4 (HUBISZ ET AL. 2009) without prior identification of populations or locations. A dataset of 1119 individuals containing all individuals analysed, but excluding the plants from the soil seed bank (they were not sampled across Europe), was used. The number of assumed clusters (K), was set from 2-60 and every run was repeated three times. For each K, I ran the program for 1,000,000 MCMC generations after an initial burn-in of 100,000 iterations. K was chosen based on the second order rate of change of the log likelihood function with respect to K (EVANNO ET AL. 2005) using STRUCTURE HARVESTER (EARL AND VON HOLDT 2012).

For evalution of the role of the soil seed bank, a pairwise comparison was performed for every locality corresponding to one of the three habitat types (31 datasets corresponding to 31 localities). The assumed number of clusters was set from K = 1-2 to examine the differentiation between the above-ground population and the soil seed bank.

Because the data were normally distributed, differences in genetic diversity estimates between groups were evaluated by means of linear mixed models using the procedure MIXED of SAS 9.4 (SAS Institute, Cary, USA). For analysis of differences within and between habitat types as well as between the soil seed bank and the above-ground populations, habitat, fraction (soil seed bank or above-ground population) and their

interaction were used as fixed factors. For analysis of differences between geographical regions, only this term was used as a fixed factor.

Bayesian Interference of recent migration was performed on the same data sets. I used the program BayesAss Edition 3 (WILSON AND RANNALA 2003) to detect first and second generation migrants from the genotyped dataset.

3.8 Preparation of seed material for flooding and germination experiments

In April 2013, the five soil samples of each plot and fraction were pooled and carefully homogenized. Six hundred grams of the resulting mixed soil sample were spread out as a 5 mm thin layer on a 3 cm layer of sterile sand in the glasshouse of the University of Natural Resources and Life Sciences, Vienna, Austria, in a single 54 cm × 31 cm plastic tray (seedling emergence method; THOMPSON ET AL. 1997; BERNHARDT ET AL. 2008; Fig. 3-14). The temperature was set to 28°C to stimulate the germination of seeds of *C. fuscus* (VON LAMPE 1996; PIETSCH 1999) and the trays were watered daily until August 2013. Seedlings of all species emerging from the soil samples were counted and discarded, except those of *C. fuscus*. The number of emerging seedlings per g of sampled soil was analysed and evaluated according to soil fractions and habitats. When possible, 15 individuals of *C. fuscus* from every pooled soil sample were pricked and individually grown in plastic pots with a diameter of 9 cm filled with standardized soil mixture (two parts fertilized peat soil and one part sand). Plastic cups with a volume of 500 ml and self-made vents closed with tea filters were used to self the single plants to obtain homogenous seed material for the experiments. The cup fitted perfectly on the pots, so that the transfer of pollen between plants was very unlikely. At the same time, seeds from the plants collected in the field were germinated under the same conditions, pricked and subjected to the same procedure as the plants that emerged from the soil samples. The ripe seeds were harvested from the selfed mother plants in October/November 2013 and stored at 6°C in the dark until May 2014. For a better overview of the complex experimental steps, a flow chart is shown (Fig. 3-15).

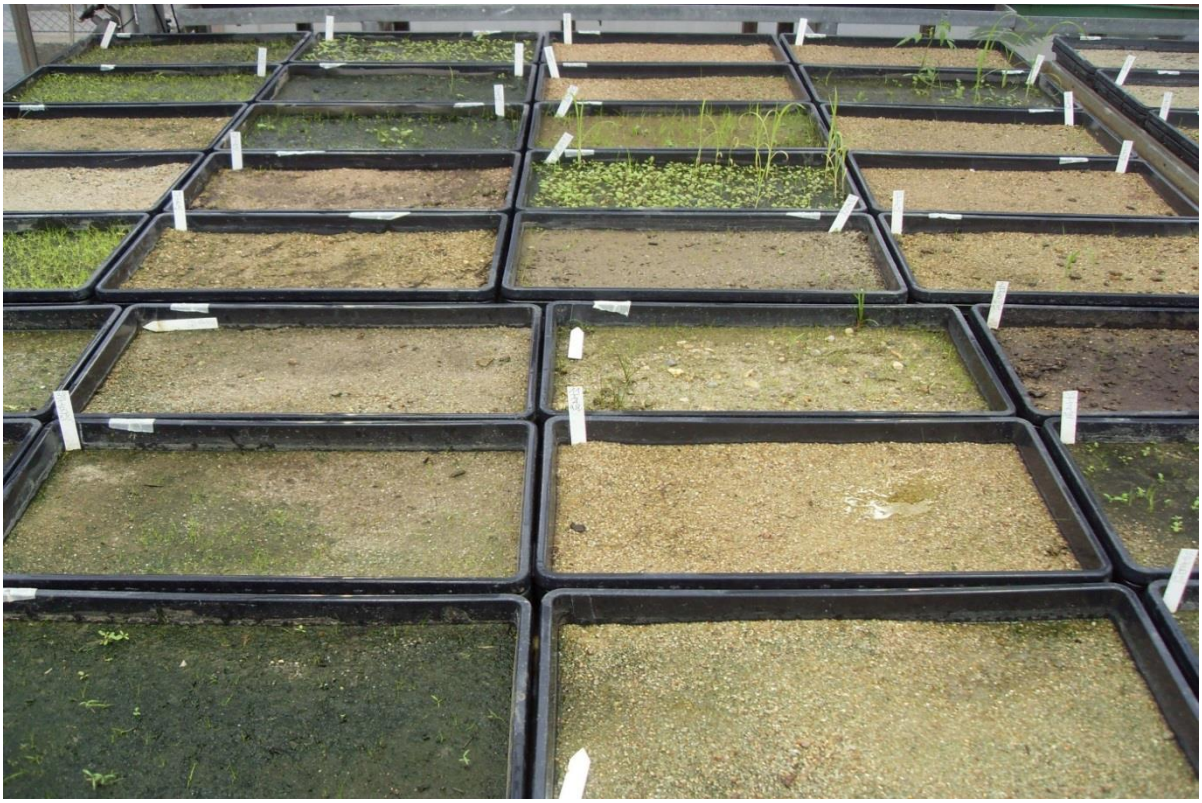

Figure 3-14 Soil seed bank analysis on sand in the BOKU glasshouse.

3.9 Germination and environmental manipulation (flooding) experiments

In May 2014, I chose 16 localities (five resp. six of each habitat type; Table 3-1) with at least five mother plants with ripe seeds from the three fractions (established plants, shallow seed bank, and deep seed bank). A germination experiment was performed on possibly 50 seeds of each of 238 mother plants in plastic Petri dishes with a diameter of 9 cm on filter paper watered with Milli-Q® (Merck Millipore, Darmstadt, Germany) water. The experiment was performed in a Versatile Environmental Test Chamber MLR-352-PE (Panasonic, Osaka, Japan) with a day/night rhythm of 14/10 h and a high difference of 35°C day and 10°C night temperature to trigger germination (VON LAMPE 1996). Germination was monitored for four weeks, daily in the first week and every two days later-on.

The first 18 germinated seeds of each mother plant were planted in three 9 cm plastic pots with the same substrate as above and grown in the glasshouse under similar conditions. In June, six daughter plants of each of 225 (out of the 238) mother plants from 15 localities (five of each habitat type; Table 3-1) were planted into individual 9 cm pots, again with similar substrate. To minimize maternal effects, seedlings of the same size were selected. This set of 1350 plants corresponding to 225 families (15 families, each consisting of six daughter plants of one mother plant, of each of the 15 chosen localities) was used for an environmental manipulation experiment (Fig. 3-15 - 3-17). For each locality, 15 plants (one daughter plant from each family of the respective locality) were placed in a plastic tray (six such trays per locality). Two of the six trays per locality were subjected to one of three different watering treatments (no flooding, moderate flooding, and severe flooding).

The watering treatments were chosen to simulate field conditions in the different habitats. In the treatment without flooding, the plastic trays had draining holes 1 cm above the ground. The plants were watered daily without being flooded. On very hot summer days, it could happen that the soil dried up superficially, as it may occur in fish storage ponds. The two flooding treatments were chosen to simulate conditions at fishpond and river habitats. The severe flooding treatment with two consecutive phases of submergence may occasionally occur at river habitats, where the water level can vary greatly and rapidly. To simulate flooding, three plastic trays with vents at the bottom were placed on a free hanging metal rack in a 400 l plastic tank (twenty such tanks in total). The height of the rack was adjustable with an accuracy of 1 cm with a galvanized steel chain on the four corners. The tanks were filled with water and the water level was kept stable by a draining hole on a fixed height. The water in the tanks was oxygenized with an air compressor for 8 h per day. Further, I replenished the tanks with fresh water for twenty minutes every two weeks. Replicates of the same locality were always placed in different tanks. In the moderate flooding treatment, plants were flooded for 6 cm (measured from the top of the pot) for four weeks after slowly submerging the trays (1 cm per day). In the severe flooding treatment, the same procedure was adopted, but the plants were lowered to a depth of 12 cm. After four weeks of submergence, the plants were brought to the surface at the same speed (1 cm per day). After two weeks at the surface, the plants of the severe flooding treatment were again flooded, now with to a depth of 6 cm for four weeks, whereas the plants of the moderate flooding treatment stayed at the surface. Every two weeks, a liquid organic-mineral fertilizer (Blumendünger mit Guano, Compo, Münster, Germany) containing a high portion of guano, 4% total N (nitrate and ammonium), 5% P_2O_5, 6% K_2O, 0.01% B, 0.002% Cu, 0.02% Fe, 0.01% Mn, 0.001% Mo, and 0.002% Zn was applied. In the control treatment, 30 ml of fertilizer was given to each tray. The 400 l tanks got the quadruple amount.

In the three months of the experiment, there were three monitoring dates, the first before any treatment was carried out (12–18 June 2014), the second, when the plants were brought to the surface after four weeks (21–31 July 2014), and the third at the end of the experiment (26 August–10 September 2014). At every monitoring date, I measured various plant traits to assess fitness and growth under different watering treatments, namely the height of the erected plant (i.e. the height of the highest culm), the number of culms, the number of leaves, the number of culms with inflorescences, the length of the longest leaf, and the width of the two widest leaves at the basis. A vitality index based on the overall impression (colour, health

status) ranging from 1 (nearly dead) to 9 (vigorous) was determined for each plant at every monitoring date. At the last monitoring date, the shoots were harvested and dried at 70°C, and the shoot biomass was determined.

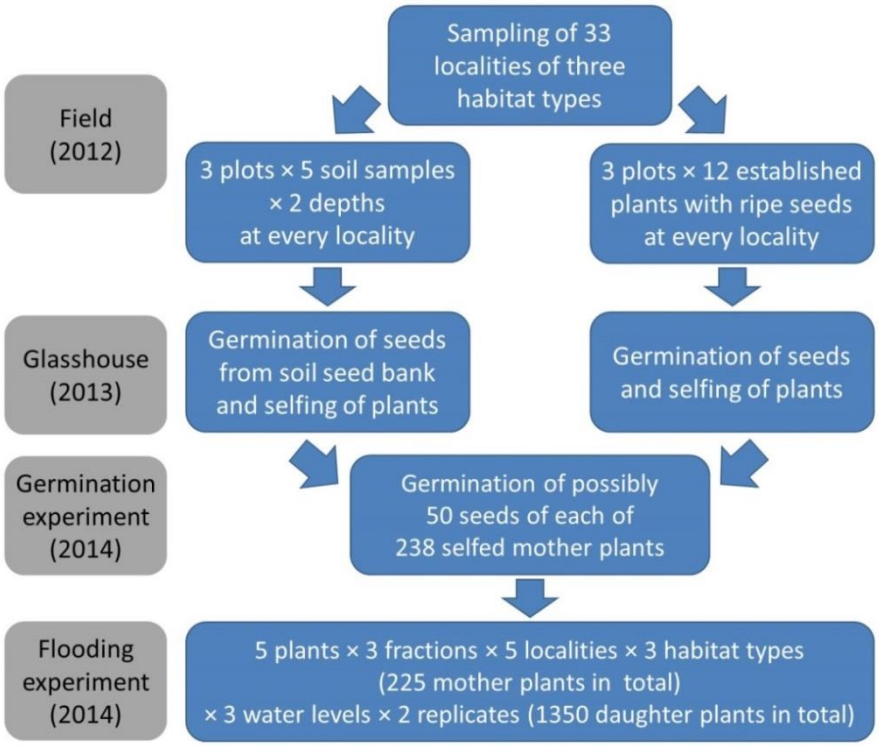

Figure 3-15 Flowchart of the experimental steps for the germination and flooding experiments.

Figure 3-16 Overview over flooding experiment.

Figure 3-17 Detail of one tank at the beginning of the experiment. Chains were used to regulate the depth of flooding, whereas the tube regulated the water level in the tank. The black hose aereted the water.

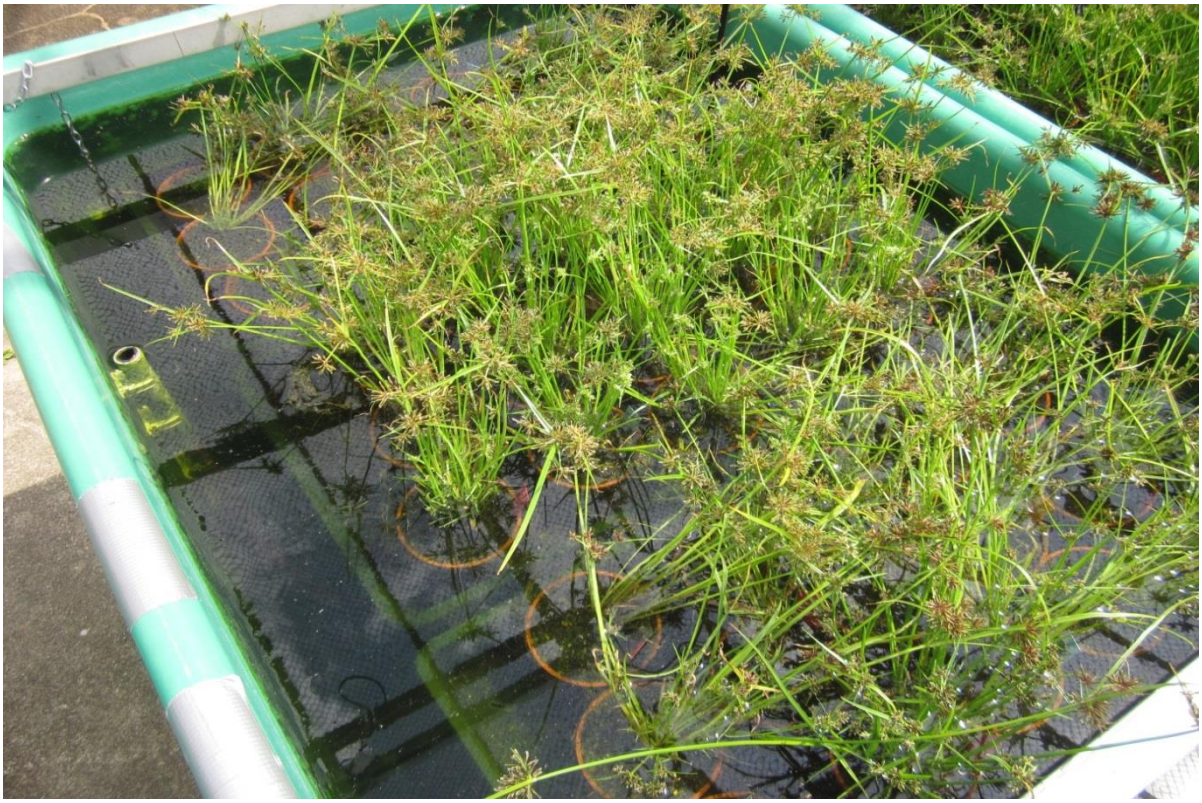

Figure 3-18 Detail of flooded plants.

3.10 Statistical analysis of the flooding and germination experiments

Differences in traits and their plasticities were evaluated by means of linear mixed models and generalized linear mixed models. For analysis of field data, habitat was treated as fixed factor and site within habitat as random factor. For analysis of germination data, habitat, fraction and their interaction were treated as fixed factors, and site within habitat as random factor. Both data sets were not normally distributed, even after the usual transformation, and the variances were heterogeneous. Therefore, generalized linear mixed models were calculated using the procedure GLIMMIX of SAS version 9.4 (SAS Institute, Cary, North Carolina, USA). A gamma distribution and a log link function were assumed and a Wald-*Z* test for random factors was performed. Differences between least square means were tested for significance by Tukey-Kramer post-hoc tests.

Residuals of the data of the flooding experiment were normally distributed, so that this data set was analysed by linear mixed models (procedure MIXED of SAS 9.4) using treatment as third fixed factor in addition to habitat and fraction. The two-way interactions habitat × treatment, fraction × treatment and habitat × fraction were also included. Site within habitat and its interactions with treatment and fraction were treated as random factors. Since the date when the individual plant was measured and so the number of days since germination had a significant influence, it was used as a covariate. The use of means of the two biological replicates in different tanks did not change the results of the statistical analysis, so that the original data set of 1350 plants was used for analysis. For every plant trait measured in the experiment, the individual statistical model with the smallest Akaike Information Criterion (AIC) was chosen. To examine the significance of the random factors, a likelihood ratio test was performed (GALWEY 2006).

Johanna Höggerl helped was a big help in handling that huge amount of plants in the experiment and finished her master thesis “Untersuchung des Wachstums des Braunen Zypergrases (Cyperus fuscus) aus verschiedenen Landnutzungen in drei verschiedenen Wasserlevels.” in 2015 at the Botanical Institute.

4 Results

4.1 NGS, primer design and multiplexing

In this first run, 143,027 sequence reads with an average length of 238 bp were obtained (Table 4-1). NGS data are deposited in the GenBank Sequence Read Archive (BioProject no. PRJNA275048). Due to the shortness of the sequences (range = 7–762 bp, mean = 238 bp), only 101 out of the 520 SSR-containing sequences were suitable for primer design. Four of these were applied to a larger number of individuals (primers with the prefix Cf in Table 4-2; remaining loci are shown in Appendix 2).

The second SSR enriched sequencing event obtained 4877 reads with a mean length of 415 bp in total and have also been deposited in the GenBank Sequence Read Archive (BioProject no. PRJNA275048), of which 967 contained SSR motifs (MSATCOMMANDER search and primer design settings same as above; Table 4-1). Four hundred ninety-four reads were suitable for primer design. Of the 80 pre-designed primers sent by ecogenics 22 showed no PCR product or had a weak signal, failures, or were unspecific. The remaining 58 markers showed clear peaks. Ten of these were monomorphic and 48 polymorphic. Seventeen polymorphic markers were selected for further analysis and combined into four multiplex PCRs with Multiplex Manager version 1.0 (Holleley and Geerts 2009; PCR multiplex sets 1–4 in Table 4-2). These markers are published in Applications in Plant Sciences 3(11) (BÖCKELMANN ET AL. 2015). The remaining loci are shown in Appendix 2.

Table 4-1 Characteristics of the two 454 GS FLX Titanium sequencing runs.

	Total no. of reads	Range of read lengths (bp)	Average read length (± SD; bp)	GC content (%)	SSR-containing sequences (total no. of SSRs encountered)	No. of reads useful for primer design
First run	143,027	7-762	238 (± 130)	40.2	520 (539)	101
Second run	4,877	34-801	415 (± 165)	40.7	967 (990)	494

Note: In the first run, a crude extract of genomic DNA of a single *Cyperus fuscus* individual was used.

In the second run, an enriched library, generated from genomic extracts of two *C. fuscus* individuals,

was used. See Appendix 2 for origin of sequenced individuals

Table 4-2 Characteristics of 21 SSR markers developed for *Cyperus fuscus*. GTTT-PIGtails (BROWNSTEIN ET AL. 1996), M13R-tails (5′-GGAAACAGCTATGACCAT-3′; primer set 1) and M13-tails (5′-TGTAAAACGACGGCCAGT-3′; primer set 3) added to the 5′ ends of primers are underlined. Fluorescent dyes at the 5′ ends of M13R- and M13-primers (primer sets 1 and 3) and forward primers (primer sets 2-5) are in italics. The allele range is based on seven test individuals (Appendix 1).

Locus	Primer sequence (5′ → 3′)	Repeat motif	*A*	Allele range	EMBL accession no.
Primer set 1 (no multiplex in PCR, first NGS run)					
Cf_008	F: GGAAACAGCTATGACCATAGATAATTAACGGATCAGGGACG	$(AG)_{11}$	4	312-344	LN848930
	R: GTTTGAGACAGATTACTCACCTCTCAAG				
	M13R: *ATTO 565*-GGAAACAGCTATGACCAT				
Cf_017	F: GGAAACAGCTATGACCATGAGGCAATAGAAATTGTTGGAG	$(CTTT)_{13}$	3	218-242	LN848931
	R: GTTTACGAAATGAGGAGCCATAACTG				
	M13R: *ATTO 550*-GGAAACAGCTATGACCAT				
Cf_019	F: GTTTAATTGTCAGGCCACATGCC	$(CTT)_7 + (CTT)_6$	2	184-205	LN848932
	R: GGAAACAGCTATGACCATACAGGGAGCAACCTGAGC				

	M13R: *FAM*-GGAAACAGCTATGACCAT				
Cf_104	F: GGAAACAGCTATGACCATGACAGAAGATGAATTAAGGCCAC	$(GT)_{14}$	2	180-184	LN848934
	R: GTTTCGATGACAGTTTAAAGGTCCAG				
	M13R: *Yakima Yellow*-GGAAACAGCTATGACCAT				
Primer set 2 (PCR multiplex 1, second NGS run)					
Cypfus_0173	F: *ATTO 532*-CGCCAAAGGAGAATGAGGTG	$(GAA)_9$	3	189-201[1]	LN848937
	R: GTTTATCGAACAATCCGATCTCGC				
Cypfus_0551	F: *ATTO 565*-TTGCCACATTGACGCACAC	$(TGTA)_9$	2	205-229[1]	LN848938
	R: GTTTAGCGTGCTATTTACAACCTTGG				
Cypfus_1207	F: *FAM*-ATCTCTTCACTCCCGCCATC	$(CAG)_7$	3	138-150[1]	LN848946
	R: GTTTGGAGTAAACCACGGACTCG				
Cypfus_2506	F: *ATTO 550*-ACCCTAACGACTGCATCACC	$(TTC)_{12}$	4	218-245[1]	LN848954
	R: GTTTAAATCTTGCCGTCTTCACCG				
Primer set 3 (PCR multiplex 2, second NGS run)					
Cypfus_3114	F: *ATTO 565*-TCCCGACTTCCTCCCAATTC	$(CT)_{15}$	4	160-180[1]	LN848967
	R: GTTTAGCTCGCAGCATACCTAGAC				
Cypfus_3300	F: *ATTO 550*-TTTTGTTCTGGTTCCACGGG	$(GTAT)_{13}$	3	232-248[1]	LN848971

	R: GTTTAGGTCCTCATTCTCTTCACCG				
Cypfus_4093	F: TGTAAAACGACGGCCAGTGTCTCTCCAAACAGGAGGGC	$(GA)_{13}$	2	94-98[1]	LN848986
	R: GTTTGTACAGGTAAGCGCAAGAGC				
	M13: *FAM*-TGTAAAACGACGGCCAGT				
Cypfus_4216	F: *FAM*-GTTGTGAAAACCCTAGGCGG	$(TTC)_{20}$	5	183-213[1]	LN848989
	R: GTTTATTGAGGCCAGCACAACAAC				
Cypfus_4666	F: *Yakima Yellow*-GGGTGTTTGCATGACTGTAGC	$(TATG)_7$	3	189-221[1]	LN848995
	R: GTTTCGTAAGGGTACATAAGTCGATCC				
Primer set 4 (PCR multiplex 3, second NGS run)					
Cypfus_2663	F: *Yakima Yellow*-TGCAATTAAAGCCGTCCCAG	$(CATA)_7$	3	230-242[1]	LN848957
	R: GTTTACCTCCCTATGAGGTTCTTTAGC				
Cypfus_2987	F: *ATTO 550*-ACGGATTCCTTCTCACACCC	$(CTT)_9$	4	249-264[1]	LN848964
	R: GTTTGCACGATGCTGCCTATACTTG				
Cypfus_3212	F: *ATTO 565*-ACACCTAAAAGCGAAAGCGG	$(AAG)_8$	3	209-227[1]	LN848969
	R: GTTTGACCGAAAGACGCTTGGAAC				
Cypfus_3921	F: *FAM*-ATGGATGACGAGGAGGTTGG	$(CGC)_8$	3	261-270[1]	LN848982
	R: GTTTGTAGAGGGAGGTTGGTAGCG				

Primer set 5 (PCR multiplex 4, second NGS run)					
Cypfus_2257	F: *FAM*-AACCAGAGAAGTCCAGGTGC	$(CT)_{13}$	3	230-236[1]	LN848952
	R: GTTTGGGTCCCAGTCTCTGACATC				
Cypfus_2993	F: *ATTO 550*-ATCGACTGCAAAGCATAGGG	$(GAA)_8$	3	141-162[1]	LN848965
	R: GTTTGGCCTCGGTCAGTTCTAC				
Cypfus_3218	F: *ATTO 565*-TGTCCTCCTCTCCAACAAGC	$(CTT)_9$	3	163-193[1]	LN848970
	R: GTTTGAAATTCAACGGAGAGCGGG				
Cypfus_4236	F: *Yakima Yellow*-GCTGTACGTGGAGAGAGGAG	$(AG)_{12}$	3	176-184[1]	LN848990
	R: GTTTAAATCCACCGTCGCAAATCC				

Note: *A* = number of alleles sampled.

[1]Length of PCR products is without PIGtail, but with M13-tail (as for other loci resulting from the second NGS run in Appendix 2)

4.2 **Genome size and chromosome counts**

Unequivocal chromosome counts have been obtained from seven populations of *Cyperus fuscus* within the framework of the project CZ 13/2012 “Polyploidy, ecological niche and demographic development of the wetland annual plant species *Cyperus fuscus*” funded by the Austrian Agency for International Cooperation in Education and Research (one from Austria, three from the Czech Republic, one from Hungary, and two from Slovakia; see Appendix 2). They all have 2*n* = 36 chromosomes. Some additional counts were obtained, which all had 2*n* = 36 ± 2 chromosomes, but these were not included in Annex 2, because the metaphases were not good enough to allow exact determination of the chromosome number. Figures 4-2 – 4-3 show mitotic metaphases in *C. fuscus*.

Annex 2 shows the populations assessed along with the genome size and chromosome number. In contrast to the expectation derived from previous publication records (2*n* = 36 for the Czech Republic, 2*n* = 72 for Slovakia; KRAHULCOVÁ 2003, MÁJOVSKÝ ET AL. 1987), no substantial variation in genome size has been found. The exact genome size of *Cyperus fuscus* with 2*n* = 36 chromosomes is 0.24 pg/1C. The variation encountered in the total species does not exceed the variation encountered among individuals within populations, indicating that there is no variation in chromosome number among the populations revised.

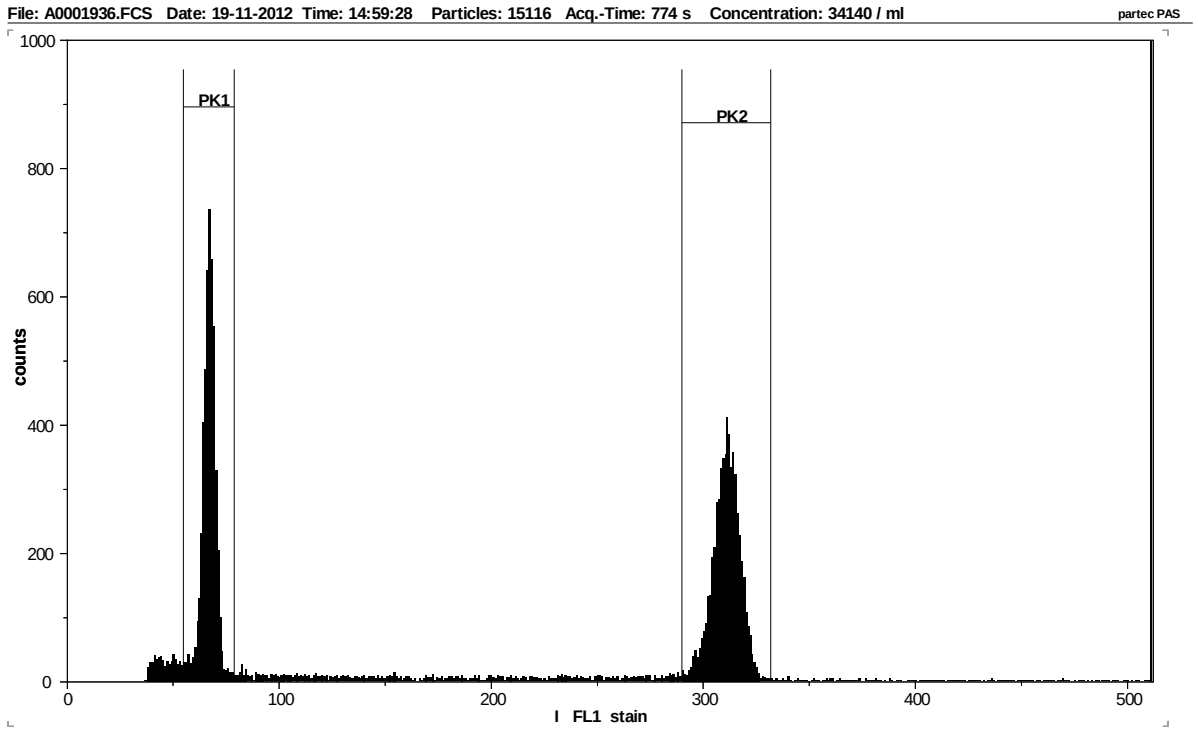

Figure 4-1 Flow cytometric analysis (DAPI staining) of *Cyperus fuscus* from an abandoned quarry (Hořátev, Nymburk, Czech Republic, J. Böckelmann, Z. Hroudová, S. Píšová, K. Tremetsberger, 24.08.2012; peak 1) and *Solanum pseudocapsicum* (internal size standard; peak 2). Measurement performed by S. Píšová.

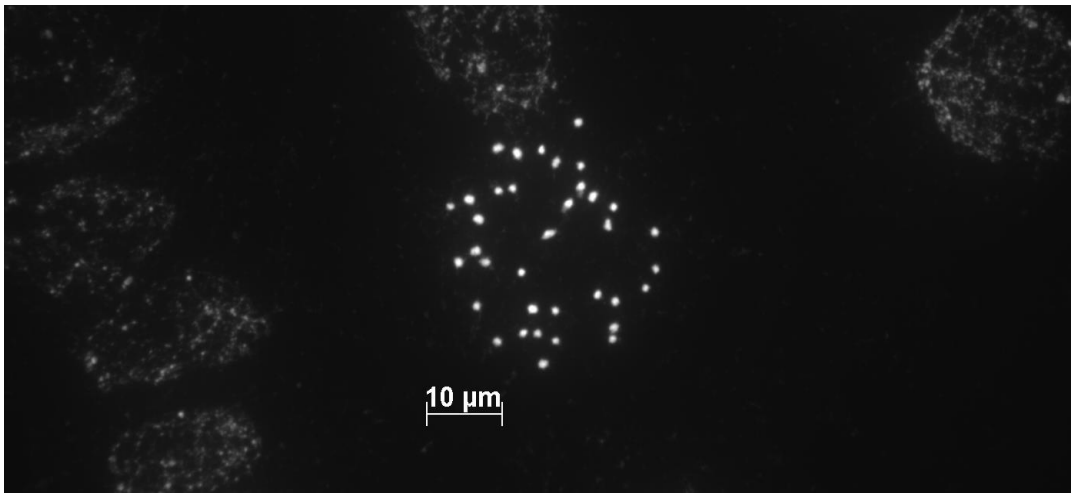

Figure 4-2 Mitotic metaphase of *Cyperus fuscus* from the pond Vodná Nádrž Horná Studená Voda, NW of Tomky, Slovakia (P. Kúr, K. Tremetsberger, 20.09.2013; 2*n* = 36). Magnification 100×. Preparation and photograph by K. Tremetsberger.

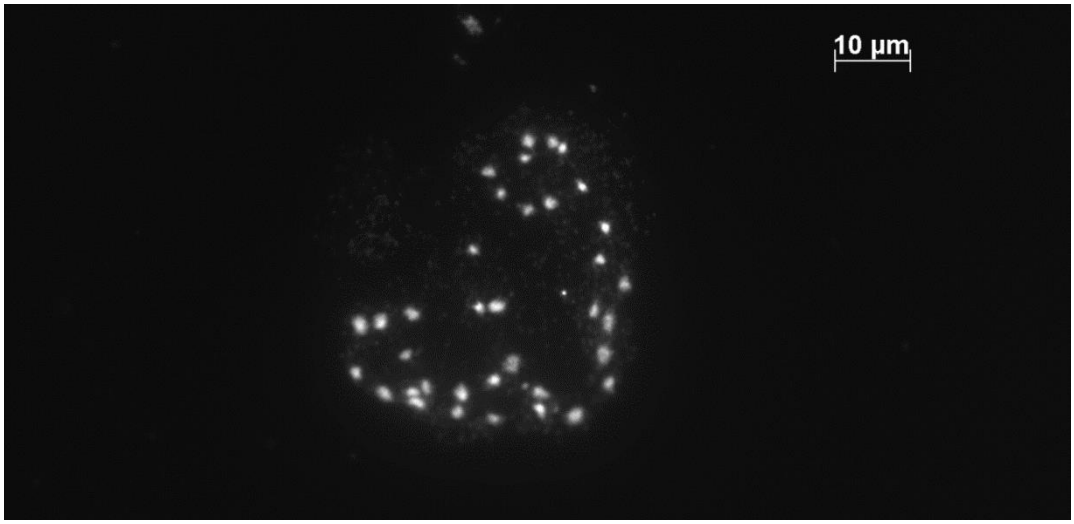

Figure 4-3 Mitotic metaphase of *Cyperus fuscus* from the pond Kuchajda, Bratislava, Slovakia (P. Kúr, K. Tremetsberger, 19.09.2013; 2*n* = 36). Magnification 100×. Preparation and photograph by K. Tremetsberger.

4.3 **Growth traits of plants sampled in the field**

The generalized linear mixed models revealed a significant effect of habitat on all traits of plants sampled in the field (Table 4-3, Fig. 4-4). The sampling site also had a highly significant influence in all cases. The height of plants from fishponds was significantly greater than that of plants from fish storage ponds ($\chi^2_{2,\ 29.01}$ = 6.60, P = 0.0368). Plants from rivers had an intermediate height. The number of culms with inflorescences ($\chi^2_{2,\ 28.98}$ = 9.23, P = 0.0099) was significantly greater in plants from fishponds and rivers than in plants from fish storage ponds. Shoot biomass ($\chi^2_{2,\ 28.99}$ = 8.60, P = 0.0135) and root biomass ($\chi^2_{2,\ 28.98}$ = 6.74, P = 0.0345) both showed the same pattern as plant height, with plants from fishponds having a significantly greater shoot and root biomass than plants from fish storage ponds, and plants from rivers having an intermediate biomass. The shoot/root ratio ($\chi^2_{2,\ 29.02}$ = 8.76, P = 0.0125) was significantly greater in plants from rivers than in plants from fish storage ponds, with plants harvested in fishponds having an intermediate ratio.

Table 4-3 Results of the generalized linear mixed models used to investigate the effect of habitat on plant traits in the field. For the fixed factor habitat, I report the χ^2-values and their associated *P*-values (in brackets). *Z*-values and their associated *P*-values are reported for random factors.

Source of variation	Plant height	No. of culms with infloresc.	Shoot biomass	Root biomass	Shoot/root ratio
Habitat	**6.60**	**9.23**	**8.60**	**6.74**	**8.76**
(df = 2)	**(0.0368)**	**(0.0099)**	**(0.0135)**	**(0.0345)**	**(0.0125)**
Site (within habitat)	**3.74**	**3.71**	**3.73**	**3.69**	**3.40**
(df = 1)	**(<.0001)**	**(0.0001)**	**(<.0001)**	**(0.0001)**	**(0.0003)**

df = degrees of freedom

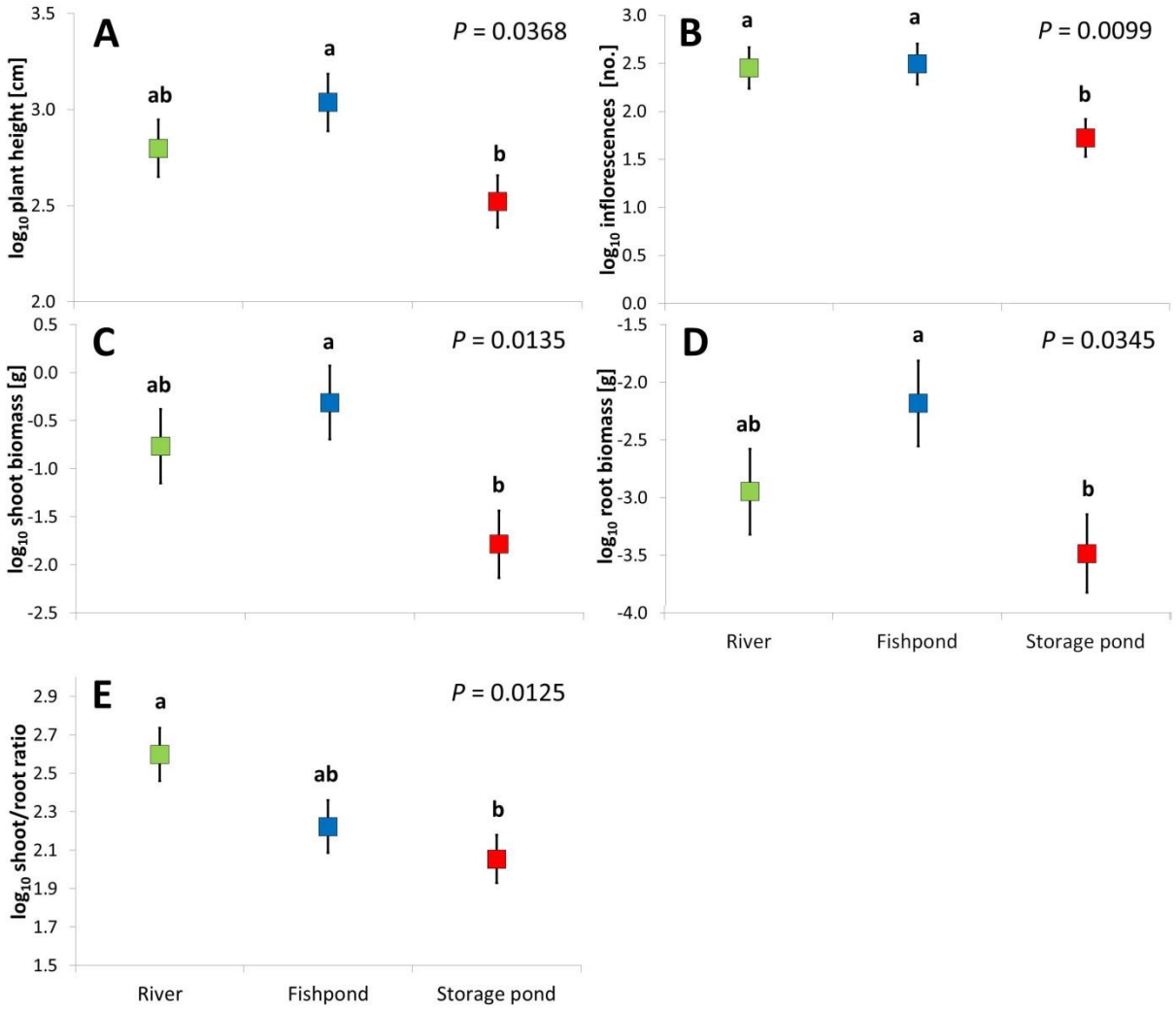

Figure 4-4 Significant differences among plants of the three habitat types harvested in the field. Least square means and standard errors of the generalized linear mixed models are shown: (A) plant height, (B) number of culms with inflorescences, (C) shoot biomass, (D) root biomass, and (E) shoot/root ratio. Letters denote significant differences in least square means (Tukey-Kramer adjustment for multiple comparisons: $P < 0.05$).

4.4 Species number and amount of seedlings emerging from the soil seed bank in the different habitat types

The average number of species within the three vegetation relevés in each locality differed marginally, but not significantly (Fig. 4-5). Within the vegetation at rivers, 18 species were detectable on average, whereas 13 different species emerged on average from the mud samples under glasshouse conditions. At the dried shores of fishponds, the highest average number of species was found in the vegetation (20). Less than 50% of these were detectable in the soil seed bank analysis (9 species on average). With an average number of 20 species in the relevés, fish storage ponds were also relatively species rich. Twelve species emerged from the soil samples on average in this habitat type.

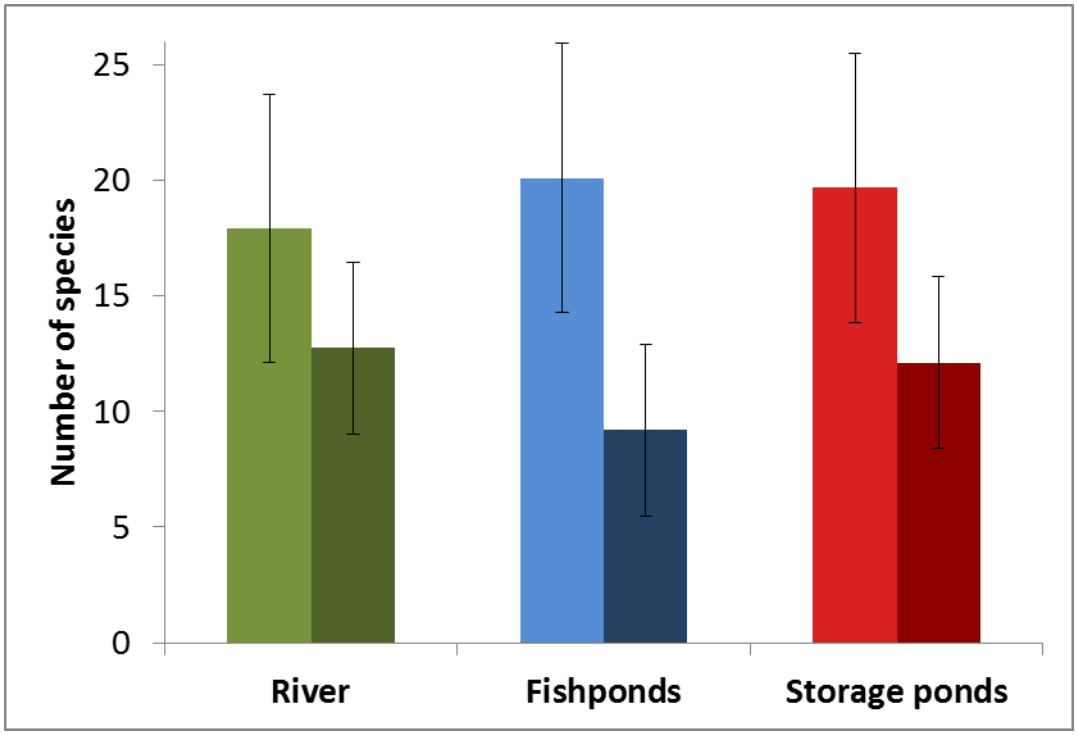

Figure 4-5 Average number of species within the above-ground vegetation vs. emerged from the soil seed bank displayed by habitat type.

The amount of emerged seeds from the soil seed bank samples from the three habitat types differed significantly (Fig. 4-6; P = 0.0423). The primary river habitats showed the significantly highest amount (median of 617 emerging plants per liter soil), but they also exhibited extremely high variance between the localities. The amount of emerging plants from the soil samples of the secondary habitats did not differ significantly from each other. The median of emerging individuals out of fishpond soil was 166. The median of emerging individuals out of fish storage pond soil was slightly higher (188), but the variance in this unnatural habitat was also higher than in fishponds.

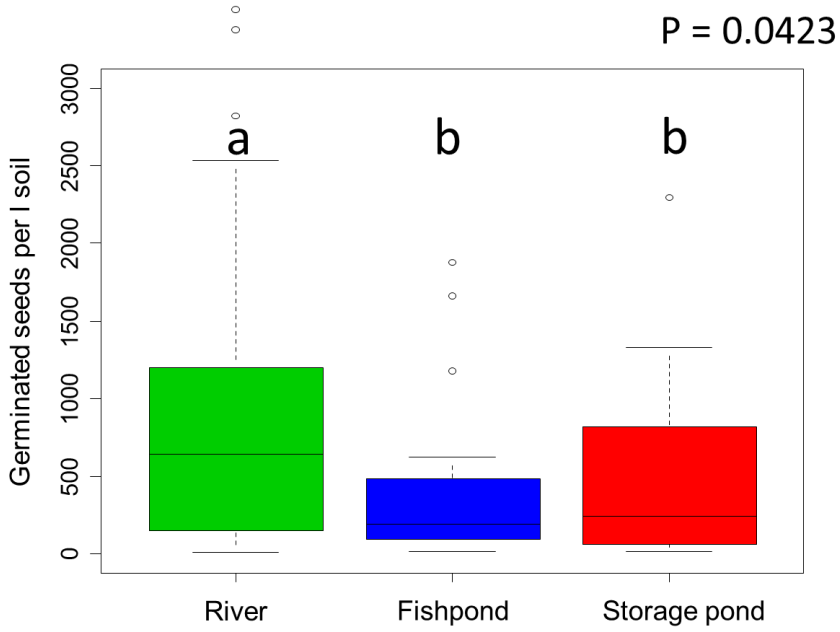

Figure 4-6 Differences of ANOVA between habitat types for the amount of emerging seedlings per l substrate in the soil seed bank analysis. Letters denote significant differences in least square means (Tukey-Kramer adjustment for multiple comparisons: $P < 0.05$).

With a median of 515 emerging seeds out of one liter soil sample of the shallow seed bank, the amount and also the variance were significantly higher than in the deep soil seed bank, which showed a median of 119 emerging seedlings per liter (Fig. 4-7; $P = 0.00728$). We found 6.6 ± 8.7 seedlings (mean ± standard deviation) in 10 ml of shallow soil compared to 4.2 ± 6.9 seedlings in the same volume of deeper soil.

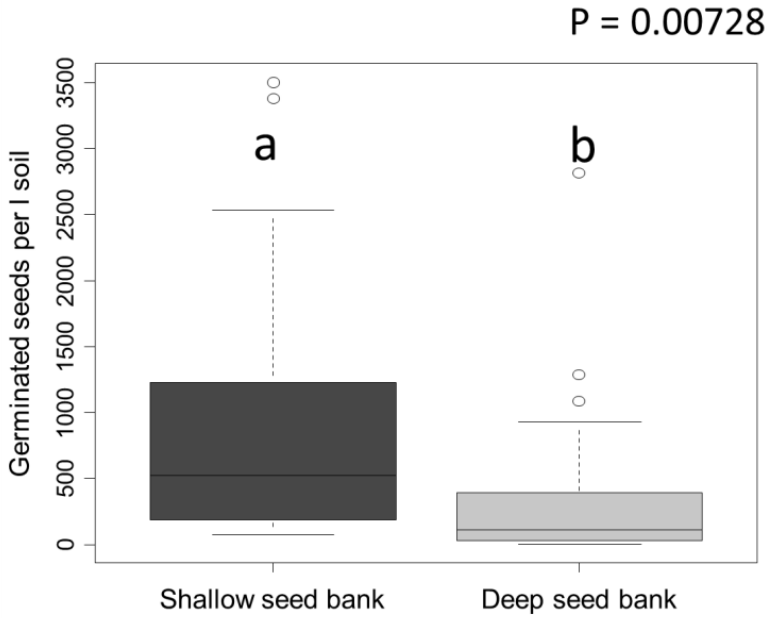

Figure 4-7 Differences of ANOVA between shallow and deep seed bank for the amount of emerging seedlings per litre substrate in the soil seed bank analysis. Letters denote significant differences in least square means (Tukey-Kramer adjustment for multiple comparisons: $P < 0.05$).

4.5 **Population genetic structure**

All of the 21 polymorphic microsatellite markers included in the analysis were polymorphic (63.3% polymorphic markers per population on average) and showed high amplification success, so that all have been used for further analysis. The number of alleles per marker ranged from 5 to 38 (mean = 11.9). 251 different alleles were recorded at all. Around 50% of the populations (soil seed bank as separate populations) showed significant deviation from HWE ($P < 0.05$) and only one marker showed a suspected null allele at low frequency. All scored plants were diploid and did not show more than two distinct peaks.

Genetic diversity

The genetic diversity of the habitat localities is shown in Table 4-4. Values for the European populations are given in Table 4-5. Across the populations of the three habitat types and two fractions (soil seed bank and above-ground population), the allelic richness ranged from just one to 3.16 alleles. Private allelic richness ranged from 0 – 1.59. The observed heterozygosity with a range of 0 – 0.35 is considerably lower than the expected heterozygosity with values up to 0.58, explaining the high fixation index with a mean of 0.67. River populations showed significantly highest values in all analysed parameters, whereas the two anthropogenic habitats did not differ significantly on their lower levels of diversity (Fig. 4-8 A, B). No significant differences in genetic diversity measures have been detected between the sampled above-ground populations and their corresponding soil seed bank populations in all three habitat types. Also, the overall means showed nearly the same values. The same analysis was performed with a reduced dataset of just 16 out of 31 locations, which contained at least ten amplified individuals per population and fraction. The values of genetic diversity measures have been similar, but the difference between rivers and the two pond types was only marginally significant due to weaker statistical power.

The mainly anthropogenic populations sampled over Central Europe also showed low levels of genetic diversity, but higher private allelic richness than the habitat localities (AR = 1 – 2.91; priv. AR = 0 – 0.66; H_o = 0 – 0.14; H_e = 0 – 0.51; F_{IS} = -0.11 – 1). Populations in southeastern Europe showed significantly higher values (AR = 2.21; H_e = 0.352) than populations in northern and western Europe. The proportion of private alleles was highest (0.19) in the Mediterranean populations (Table 4-5, Fig. 4-8 C, D). The results are based on a HP-Rare analysis with at least 5 individuals per population. When just the above-ground populations of the three habitat types were taken into account, a rarefaction analysis based on 68 individuals per region showed even clearer results. Allelic richness and private allelic richness were highest in the Mediterranean region (7.8 and 2.48), intermediate in southeastern Europe (6.7 and 1.02), and on the same low level in the other three regions (western Europe: 4.11 and 0.14; Bohemian Massif: 3.9 and 0.22; northern Europe 4.13 and 0.16; Fig. 4-9).

We performed five independent AMOVAs using different groups (according to Table 4-4 and 4-5). In the comparison of genetic differentiation between the groupings, most variation was detectable within the populations (39.3% - 75.8%) and among populations within the same groups (19 – 46.8 %). In the data set with the highest amount of individuals and largest geographical scale, the maximum value of 13.8% variation was found between regions. The three different habitat types including the soil seed bank varied with 8.1%, whereas the difference between the above-ground populations and their soil seed bank was even lower (just 5.9%). Remarkably, 12% variation was found between the European river systems (Table 4-6). This value shrank to 5.2%, when just the rivers of the habitat dataset were taken into account.

The two neighbour-joining networks (equal angles, F_{ST} distance matrix imported directly) show differentiation of the populations (Fig. 4-10 and 4-11). At the European scale, 1019 individuals were used and the base of the tree has a “bush-like” structure with low bootstrap support, but relatively long branches leading to the individual populations (Fig. 4-11). A coarse geographical pattern is visible: The western European populations group with individuals from the Mediterranean area despite of the spatial distance between sampled populations. Most of the populations of the southeastern region group together. Most, but not

all of the above-ground populations of the three habitat types are close to each other. One cluster of closely related populations appears most prominently on one single branch (fishponds 4, 5, 6, 7 and storage ponds 8, 9 and 11). The same branching pattern is visible in Fig. 4-11. Most of the river populations cluster together and in most of the cases, above-ground populations and the soil seed bank from the same locality are located in close proximity on the network.

Bayesian methods implemented in STRUCTURE (Hubisz et al. 2009) revealed K = 41 as the most likely number of clusters (Delta K = 71.4). In Fig. 4-14, the structure of the above-ground populations across Europe is shown for K = 17, 28, 36, and 41 (which all have comparatively high Delta K values). The populations are ordered by their belonging to geographical regions.

The results of the pairwise STRUCTURE analyses of the soil lseed bank vs. above-ground populations within the three habitat types for an assumed value of K = 2 are shown in Fig. 4-12. With a few exceptions (e.g. FP10), there is no differentiation between the soil seed bank and the above-ground population detectable.

The sampling scheme over Europe was less dense, so that the results of the migrant analysis are just shown for the three habitat types and their soil seed bank (Fig. 4-13). On the first sight, it is visible that most seeds originated from their proper locality resp. soil seed bank (diagonal line). It is also obvious that most seeds are dispersed in spatial proximity. Less dispersal is detectable between primary and secondary habitats than within the rivers, which is also underpinned by the structure analysis (Fig. 4-15). The same clusters of very closely related ponds detectable in the NJ-networks (Fig. 4-10 and 4-11) are also visible in the migrant analysis. On a closer look, there are also some migrants detectable that stem from populations, which are far away from the receiving population. Source and receiving populations may even be located in different river systems. Examples for that are found between river populations at the rivers Oder (RV 9 and 10), Elbe (RV 1 and 6), and Danube (all other RV). Most of these migrants represent less than 1% of the individuals analysed.

Table 4-4 Geographical position and measures of genetic diversity based on 21 microsatellite loci of sampled populations according to three habitat types: N, number of samples analysed; AR, Allelic richness; priv AR, Private allelic richness; H_o, observed heterozygosity; H_e, expected heterozygosity and F_{IS}, inbreeding coefficient ($P < 0.001$, * $P < 0.05$).**

Population	Coordinates		Country/Altidude	Geogr. region/ River system	Sample size	pairwise AR (based on 10 genes)	pairwise priv. PAR (based on 10 genes)	H_o	H_e	F_{IS}
	X	Y								
				River						
RV01_AG	50.223828	14.628617	CZ	Bohemian Massif	15	2.20 ± 0.65	0.61 ± 0.47	0.04 ± 0.01	0.35 ± 0.04	0.88 ± 0.03*
RV01_S			168m	Elbe	8	1.90 ± 0.80	0.31 ± 0.47	0.01 ± 0.01	0.25 ± 0.05	0.96 ± 0.02*
RV02_AG	48.711833	16.902817	CZ	Southeastern Europe	16	2.93 ± 0.72	0.93 ± 0.92	0.08 ± 0.01	0.54 ± 0.03	0.86 ± 0.02***
RV02_S			152m	Danube	5	2.57 ± 0.75	0.57 ± 0.72	0.14 ± 0.03	0.45 ± 0.04	0.63 ± 0.09
RV03_AG	48.672567	16.924033	CZ	Southeastern Europe	15	2.99 ± 0.97	0.58 ± 0.21	0.13 ± 0.02	0.58 ± 0.03	0.77 ± 0.03**
RV03_S			150m	Danube	20	2.85 ± 0.91	0.44 ± 0.34	0.15 ± 0.02	0.56 ± 0.03	0.74 ± 0.03**
RV04_AG	48.784650	17.079883	CZ	Southeastern Europe	24	3.1 0 ± 0.83	0.72 ± 0.17	0.29 ± 0.02	0.58 ± 0.03	0.49 ± 0.03*
RV04_S			162m	Danube	14	2.95 ± 0.91	0.57 ± 0.35	0.21 ± 0.03	0.54 ± 0.00	0.6 ± 0.04
RV05_AG	48.984267	16.664900	CZ	Southeastern Europe	15	2.79 ± 0.18	0.77 ± 0.03	0.05 ± 0.01	0.47 ± 0.04	0.89 ± 0.03**
RV05_S			183m	Danube	15	2.49 ± 0.73	0.47 ± 0.07	0.09 ± 0.01	0.47 ± 0.04	0.77 ± 0.05
RV06_AG	50.729450	14.187033	CZ	Bohemian Massif	15	2.69 ± 0.14	0.64 ± 0.14	0.18 ± 0.02	0.48 ± 0.04	0.62 ± 0.05
RV06_S			130m	Elbe	5	2.19 ± 0.68	0.14 ± 0.19	0.01 ± 0.01	0.44 ± 0.04	0.99 ± 0.01*
RV07_AG	48.370750	15.838056	AT	Southeastern Europe	15	1.83 ± 0.00	0.11 ± 0.00	0.07 ± 0.02	0.26 ± 0.03	0.74 ± 0.05*
RV07_S			183m	Danube	17	2.04 ± 0.57	0.32 ± 0.20	0.05 ± 0.01	0.42 ± 0.04	0.90 ± 0.02***

RV08_AG	48.372300	15.831817	AT	Southeastern Europe	14	3.16 ± 0.77	0.78 ± 0.54	0.07 ± 0.02	0.57 ± 0.03	0.89 ± 0.03***
RV08_S			187m	Danube	8	2.92 ± 1.11	0.55 ± 0.67	0.21 ± 0.03	0.55 ± 0.04	0.59 ± 0.05
RV09_AG	51.674617	16.203983	POL	Northern Europe	17	2.54 ± 0.68	0.41 ± 0.04	0.35 ± 0.03	0.47 ± 0.04	0.25 ± 0.04
RV09_S			76m	Oder	15	2.53 ± 0.59	0.40 ± 0.13	0.20 ± 0.02	0.47 ± 0.04	0.56 ± 0.05
RV10_AG	52.031383	15.610983	POL	Northern Europe	15	2.46 ± 0.84	0.86 ± 0.81	0.20 ± 0.02	0.42 ± 0.05	0.53 ± 0.04
RV10_S			55m	Oder	6	1.69 ± 0.63	0.08 ± 0.19	0.12 ± 0.03	0.25 ± 0.05	0.39 ± 0.09
RV11_AG	48.312317	16.903733	SK	Southeastern Europe	13	2.58 ± 0.62	0.54 ± 0.57	0.14 ± 0.02	0.45 ± 0.03	0.65 ± 0.06
RV11_S			155m	Danube	8	2.57 ± 0.79	0.52 ± 0.61	0.20 ± 0.03	0.48 ± 0.04	0.55 ± 0.05
FP01_AG	50.628117	14.54325	CZ	Bohemian Massif	22	2.08 ± 0.59	0.34 ± 0.05	0.14 ± 0.02	0.39 ± 0.04	0.63 ± 0.04*
FP01_S			252m	Elbe	26	1.94 ± 0.36	0.19 ± 0.02	0.09 ± 0.02	0.34 ± 0.04	0.74 ± 0.05*
FP02_AG	49.718317	14.6505	CZ	Bohemian Massif	22	1.22 ± 0.32	0.09 ± 0.00	0.01 ± 0.01	0.06 ± 0.03	0.91 ± 0.03*
FP02_S			400m	Elbe	34	1.34 ± 0.34	0.21 ± 0.00	0.00 ± 0.00	0.09 ± 0.02	0.98 ± 0.01**
FP03_AG	49.70095	14.589967	CZ	Bohemian Massif	20	2.08 ± 0.52	0.14 ± 0.00	0.05 ± 0.01	0.41 ± 0.04	0.86 ± 0.04**
FP03_S			374m	Elbe	21	2.28 ± 0.5	0.33 ± 0.01	0.17 ± 0.02	0.45 ± 0.04	0.60 ± 0.05
FP04_AG	49.321033	13.897267	CZ	Bohemian Massif	17	n.a.	n.a.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
FP04_S			466m	Elbe	1	n.a.	n.a.	n.a.	n.a.	n.a.
FP05_AG	49.349967	13.942333	CZ	Bohemian Massif	16	1.04 ± 0.18	0.04 ± 0.03	0.00 ± 0.00	0.01 ± 0.01	0.64 ± 0.01*
FP05_S			457m	Elbe	25	1.17 ± 0.19	0.17 ± 0.00	0.00 ± 0.00	0.04 ± 0.01	0.86 ± 0.07
FP06_AG	49.33685	13.598583	CZ	Bohemian Massif	15	1.04 ± 0.14	0.04 ± 0.14	0.00 ± 0.00	0.01 ± 0.01	0.48 ± 0.16
FP06_S			473m	Elbe	6	1.00 ±	0.00 ±	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

						0.00	0.00			
FP07_AG	49.434633	13.91165	CZ	Bohemian Massif	23	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
FP07_S			489m	Elbe	12	1.54 ± 0.51	0.54 ± 0.2	0.03 ± 0.02	0.18 ± 0.04	0.75 ± 0.09
FP08_AG	49.693567	14.53485	CZ	Bohemian Massif	16	1.13 ± 0.24	0.13 ± 0.24	0.00 ± 0.00	0.03 ± 0.01	1.00 ± 0.00*
FP08_S			447m	Elbe	9	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
FP09_AG	49.653617	14.384917	CZ	Bohemian Massif	13	1.96 ± 0.59	0.14 ± 0.27	0.02 ± 0.01	0.37 ± 0.05	0.95 ± 0.02**
FP09_S			379m	Elbe	10	1.91 ± 0.53	0.08 ± 0.19	0.00 ± 0.00	0.35 ± 0.04	1.00 ± 0.00**
FP10_AG	48.986553	16.094016	CZ	Southeastern Europe	14	1.03 ± 0.11	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	-0.04 ± 0.00
FP10_S			334m	Danube	5	2.62 ± 0.80	1.59 ± 0.79	0.12 ± 0.03	0.50 ± 0.04	0.77 ± 0.06
				Storage Ponds						
SP02_AG	50.563833	14.658867	CZ	Bohemian Massif	22	1.97 ± 0.82	0.28 ± 0.21	0.05 ± 0.01	0.33 ± 0.05	0.81 ± 0.06
SP02_S			266m	Elbe	27	2.11 ± 0.91	0.43 ± 0.26	0.04 ± 0.01	0.35 ± 0.06	0.85 ± 0.03**
SP03_AG	50.196917	13.91085	CZ	Bohemian Massif	28	1.56 ± 0.58	0.06 ± 0.12	0.02 ± 0.01	0.2 ± 0.05	0.88 ± 0.03**
SP03_S			418m	Elbe	9	1.58 ± 0.64	0.09 ± 0.15	0.03 ± 0.01	0.21 ± 0.05	0.89 ± 0.03*
SP04_AG	49.043733	14.432533	CZ	Bohemian Massif	20	1.95 ± 0.50	0.69 ± 0.08	0.03 ± 0.01	0.3 ± 0.03	0.90 ± 0.03**
SP04_S			376m	Elbe	27	1.33 ± 0.36	0.07 ± 0.00	0.02 ± 0.01	0.09 ± 0.02	0.76 ± 0.03*
SP06_AG	49.751117	14.660917	CZ	Bohemian Massif	5	2.69 ± 0.75	0.64 ± 0.73	0.04 ± 0.02	0.41 ± 0.03	0.92 ± 0.04
SP06_S			357m	Elbe	5	2.19 ± 0.68	0.14 ± 0.19	0.10 ± 0.02	0.31 ± 0.04	0.67 ± 0.09
SP07_AG	49.044767	14.433183	CZ	Bohemian Massif	14	1.20 ±	0.05 ±	0.00 ± 0.00	0.05 ± 0.02	1.00 ± 0.00*

						0.33	0.14			
SP07_S			372m	Elbe	14	1.20 ± 0.41	0.05 ± 0.23	0.01 ± 0.01	0.08 ± 0.03	0.87 ± 0.05*
SP08_AG	49.24955	14.022183	CZ	Bohemian Massif	27	1.38 ± 0.31	0.37 ± 0.11	0.00 ± 0.00	0.08 ± 0.02	0.98 ± 0.02**
SP08_S			387m	Elbe	20	1.02 ± 0.10	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	-0.05 ± 0.01
SP09_AG	49.347367	13.948383	CZ	Bohemian Massif	30	1.16 ± 0.21	0.13 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.86 ± 0.07
SP09_S			450m	Elbe	13	1.29 ± 0.40	0.26 ± 0.14	0.00 ± 0.00	0.06 ± 0.02	0.95 ± 0.03*
SP10_AG	49.554117	13.9965	CZ	Bohemian Massif	34	1.19 ± 0.37	0.10 ± 0.00	0.01 ± 0.00	0.07 ± 0.03	0.51 ± 0.11
SP10_S			449m	Elbe	16	1.11 ± 0.30	0.02 ± 0.00	0.00 ± 0.00	0.05 ± 0.03	0.66 ± 0.13
SP11_AG	49.43525	13.807367	CZ	Bohemian Massif	15	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SP11_S			447m	Elbe	16	1.05 ± 0.17	0.05 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.3 ± 0.10
SP12_AG	50.47235	15.132533	CZ	Bohemian Massif	13	1.78 ± 0.63	0.32 ± 0.41	0.02 ± 0.01	0.23 ± 0.04	0.88 ± 0.06
SP12_S			252m	Elbe	8	1.76 ± 0.73	0.29 ± 0.41	0.02 ± 0.01	0.23 ± 0.05	0.83 ± 0.08

MEAN RV AG						2.66 ± 0.58	0.63 ± 0.35	0.15 ± 0.02	0.47 ± 0.04	0.69 ± 0.04
MEAN RV S						2.43 ± 0.77	0.4 ± 0.36	0.13 ± 0.02	0.44 ± 0.04	0.70 ± 0.05
MEAN RV						2.54 ± 0.68	0.51 ± 0.36	0.14 ± 0.02	0.46 ± 0.04	0.69 ± 0.04
Mean FP AG						1.4 ± 0.3	0.1 ± 0.08	0.03 ± 0.01	0.14 ± 0.02	0.60 ± 0.03
Mean FP S						1.64 ± 0.36	0.35 ± 0.13	0.05 ± 0.01	0.22 ± 0.03	0.63 ± 0.04
Mean FP						1.49 ± 0.31	0.22 ± 0.11	0.03 ± 0.01	0.16 ± 0.02	0.58 ± 0.03

Mean SP AG	1.59 ± 0.45	0.26 ± 0.18	0.02 ± 0.01	0.17 ± 0.03	0.77 ± 0.04
Mean SP S	1.46 ± 0.47	0.14 ± 0.14	0.02 ± 0.01	0.14 ± 0.03	0.67 ± 0.06
Mean SP	1.53 ± 0.46	0.2 ± 0.16	0.02 ± 0.01	0.16 ± 0.03	0.72 ± 0.05
MEAN AG	1.92 ± 0.45	0.35 ± 0.21	0.06 ± 0.01	0.26 ± 0.03	0.67 ± 0.04
MEAN S	1.87 ± 0.55	0.3 ± 0.22	0.07 ± 0.01	0.27 ± 0.03	0.67 ± 0.05

Table 4-5 Geographical location and measures of genetic diversity based on 21 microsatellite loci of sampled populations across Europe. AR, Allelic richness; Private allelic richness; H_O, observed heterozygosity; H_e, expected heterozygosity and F_{IS}, inbreeding coefficient ($P < 0.00001$, * $P < 0.05$). Calculations just for populations with at least five positively genotyped individuals.**

Population	Coordinates		Country	Altidude	River system	Sample size	Geogr. region	Allelic richness	Private allelic richness	H_o	H_e	F_{IS}
	X	Y										
AT1	47.989194	17.039056	Austria	133	Donau	12	Southeastern Europe	2.74 ± 1.03	0.09 ± 0.29	0.00 ± 0.00	0.46 ± 0.05	1.00 ± 0.00**
AT2	47.082022	15.4564	Austria	381	n.a.	9	Southeastern Europe	1.14 ± 0.31	0.04 ± 0.15	0.04 ± 0.02	0.03 ± 0.02	-0.11 ± 0.01
AT3	47.963611	17.069139	Austria	132	Donau	10	Southeastern Europe	2.29 ± 0.80	0.05 ± 0.17	0.00 ± 0.00	0.4 ± 0.04	1.00 ± 0.00**
AT4	47.973194	17.052944	Austria	133	Donau	17	Southeastern Europe	2.74 ± 0.80	0.04 ± 0.12	0.09 ± 0.01	0.51 ± 0.04	0.79 ± 0.05*
AT5	47.9725	17.053083	Austria	133	Donau	20	Southeastern Europe	2.69 ± 1.05	0.01 ± 0.05	0.05 ± 0.01	0.44 ± 0.05	0.89 ± 0.02***
CH	47.222433	9.477733	Switzerland	439	Rhine	12	Western Europe	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CORSE	42.2114823	9.31803703	Corsica	162	n.a.	11	Mediterranean Sea	1.47 ± 0.65	0.1 ± 0.26	0.01 ± 0.01	0.15 ± 0.04	0.92 ± 0.03**
D1	48.430819	7.763239	Germany	144	Rhine	9	Western Europe	2.06 ± 0.89	0.02 ± 0.1	0.01 ± 0.01	0.31 ± 0.06	0.98 ± 0.01**
D2	53.145047	11.207472	Germany	19	Elbe	11	Northern Europe	2.43 ± 0.68	0.00 ± 0.00	0.01 ± 0.01	0.43 ± 0.04	0.99 ± 0.01**
D3	51.785786	12.791175	Germany	73	Elbe	7	Northern Europe	1.65 ± 0.83	0.07 ± 0.22	0.01 ± 0.01	0.22 ± 0.06	0.97 ± 0.02*
D5	53.352514	13.407528	Germany	110	Elbe	10	Northern Europe	1.68 ± 0.44	0.00 ± 0.00	0.03 ± 0.01	0.25 ± 0.04	0.90 ± 0.04*
ESP1	40.816533	0.521517	Spain	7	n.a.	12	Mediterranean Sea	1.09 ± 0.29	0.05 ± 0.22	0.00 ± 0.00	0.04 ± 0.03	1.00 ± 0.00**
ESP2	39.854333	-0.475217	Spain	305	n.a.	11	Mediterranean Sea	2.74 ± 0.67	0.66 ± 0.62	0.03 ± 0.02	0.51 ± 0.04	0.94 ± 0.03***
F1	46.863008	1.255556	France	117	n.a.	10	Western Europe	1.56 ± 0.54	0.00 ± 0.01	0.04 ± 0.01	0.18 ± 0.04	0.67 ± 0.08

F2	46.731911	1.205794	France	98	n.a.	9	Western Europe	1.20 ± 0.37	0.00 ± 0.00	0.01 ± 0.01	0.05 ± 0.02	0.92 ± 0.04
FL1	47.187217	9.49225	Liechtenstein	445	Rhine	12	Western Europe	1.94 ± 0.48	0.01 ± 0.04	0.00 ± 0.00	0.25 ± 0.03	1.00 ± 0.00*
FL2	47.1844	9.35935	Liechtenstein	1309	n.a.	12	Western Europe	1.55 ± 0.55	0.00 ± 0.00	0.00 ± 0.00	0.21 ± 0.05	1.00 ± 0.00**
FL3	47.06335	9.501667	Liechtenstein	474	Rhine	12	Western Europe	1.00 ± 0.00	0.01 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
GB01	51.71605	-3.969489	Great Britain	205	n.a.	**1**	Western Europe	n.a.	n.a.	n.a.	n.a.	n.a.
GB02	50.959314	-1.769404	Great Britain	33	n.a.	10	Western Europe	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
GR01	37.048611	22.030556	Greece	2	n.a.	**1**	Mediterranean Sea	n.a.	n.a.	n.a.	n.a.	n.a.
GR02	36.973611	22.580556	Greece	93	n.a.	**3**	Mediterranean Sea	n.a.	n.a.	n.a.	n.a.	n.a.
HR01	45.074653	14.567875	Croatia	112	n.a.	18	Mediterranean Sea	1.14 ± 0.36	0.15 ± 0.36	0.04 ± 0.03	0.07 ± 0.04	0.35 ± 0.13
HR02	45.345611	13.829583	Croatia	9	n.a.	9	Southeastern Europe	2.78 ± 0.76	0.12 ± 0.26	0.00 ± 0.00	0.28 ± 0.05	1.00 ± 0.00
HR03	45.625875	18.813597	Croatia	78	Donau	12	Mediterranean Sea	1.88 ± 0.8	0.09 ± 0.28	0.14 ± 0.02	0.50 ± 0.04	0.69 ± 0.06**
HUN01	47.1049	18.3223	Hungary	177	Donau	11	Southeastern Europe	2.37 ± 0.68	0.08 ± 0.22	0.00 ± 0.00	0.40 ± 0.04	1.00 ± 0.00**
HUN02	47.180458	18.539744	Hungary	102	n.a.	11	Southeastern Europe	2.54 ± 0.66	0.00 ± 0.00	0.10 ± 0.02	0.48 ± 0.04	0.81 ± 0.03*
HUN03	47.665025	19.079508	Hungary	105	Donau	**4**	Southeastern Europe	n.a.	n.a.	n.a.	n.a.	n.a.
HUN04	47.643744	17.607969	Hungary	111	Donau	12	Southeastern Europe	2.19 ± 0.91	0.05 ± 0.20	0.00 ± 0.00	0.33 ± 0.06	1.00 ± 0.00**
HUN05	47.277894	17.462881	Hungary	156	Donau	8	Southeastern Europe	1.99 ± 0.93	0.09 ± 0.26	0.02 ± 0.01	0.28 ± 0.05	0.87 ± 0.07
HUN06	47.097072	17.524164	Hungary	215	n.a.	9	Southeastern Europe	2.01 ± 0.86	0 ± 0	0.01 ± 0.01	0.33 ± 0.06	0.99 ± 0.01**
HUN07	46.764367	17.270086	Hungary	106	Donau	**2**	Southeastern Europe	n.a.	n.a.	n.a.	n.a.	n.a.
HUN08	46.979669	16.831636	Hungary	154	Donau	6	Southeastern Europe	1.90 ± 0.62	0.05 ± 0.22	0 ± 0	0.33 ± 0.05	1 ± 0*

HUN09	46.446861	17.194894	Hungary	123	Donau	7	Southeastern Europe	2.47 ± 1.04	0.04 ± 0.2	0.05 ± 0.02	0.42 ± 0.06	0.84 ± 0.06
HUN10	47.740969	18.002042	Hungary	119	Donau	7	Southeastern Europe	2.49 ± 0.64	0.00 ± 0.01	0.02 ± 0.02	0.49 ± 0.03	0.96 ± 0.03*
HUN11	48.107056	22.831131	Hungary	118	Donau	7	Southeastern Europe	1.73 ± 0.68	0.00 ± 0.00	0.02 ± 0.01	0.23 ± 0.05	0.88 ± 0.07
HUN12	47.853889	20.329881	Hungary	149	Donau	8	Southeastern Europe	1.93 ± 0.68	0.05 ± 0.22	0.01 ± 0.01	0.3 ± 0.05	0.99 ± 0.01*
ITA01	43.105897	12.1865	Italy	262	n.a.	12	Mediterranean Sea	2.42 ± 0.8	0.18 ± 0.36	0.01 ± 0.01	0.38 ± 0.05	0.97 ± 0.02*
ITA02	45.236	9.006417	Italy	70	n.a.	9	Mediterranean Sea	2.91 ± 0.97	0.22 ± 0.57	0.09 ± 0.02	0.5 ± 0.04	0.81 ± 0.05*
ITA03	45.213667	8.982833	Italy	71	n.a.	**2**	Mediterranean Sea	n.a.	n.a.	n.a.	n.a.	n.a.
ITA04	37.986583	12.900389	Italy	106	n.a.	10	Mediterranean Sea	1.00 ± 0.00	0.01 ± 0.03	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
ITA05	38.109961	13.379775	Italy	5	n.a.	11	Mediterranean Sea	1.66 ± 0.67	0.27 ± 0.45	0.11 ± 0.03	0.22 ± 0.05	0.45 ± 0.10
LT01	54.73125	25.300639	Lithua	99	n.a.	15	Northern Europe	1.02 ± 0.07	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-0.03 ± 0.00
NL01	52.4969	6.0599	Netherlands	6	Rhine	10	Western Europe	1.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SK01	48.215231	17.415019	Slovakia	125	Donau	10	Southeastern Europe	2.31 ± 0.82	0.00 ± 0.00	0.05 ± 0.01	0.43 ± 0.05	0.85 ± 0.05
SK02	48.5841	17.07905	Slovakia	175	Donau	10	Southeastern Europe	2.50 ± 0.76	0.00 ± 0.00	0.02 ± 0.01	0.44 ± 0.05	0.97 ± 0.02**
SK03	48.493911	16.960581	Slovakia	149	Donau	8	Southeastern Europe	1.46 ± 0.65	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.06	1.00 ± 0.00**
SK04	48.170983	17.1431	Slovakia	134	Donau	9	Southeastern Europe	1.87 ± 0.41	0.00 ± 0.00	0.02 ± 0.01	0.25 ± 0.03	0.92 ± 0.03
TR1	39.920969	26.156913	Turkey	20	n.a.	**1**	Mediterranean Sea	n.a.	n.a.	n.a.	n.a.	n.a.

Table 4-6 Summary of analyses of molecular variance (AMOVA) of *C. fuscus* (all F_{ST} values are significant with $P < 0.0001$). Groupings according to Tables 4-4 and 4-5.

	No. of pop.	No. of ind.	Overall F_{ST}	Mean Percentage Variation		
				Among groups	Among Pop.	Within pop.
A Habitats incl. soil seed bank	31	984	0.508	8.1	42.7	49.2
B1 River systems excl. soil seed bank	56	810	0.550	12.2	42.9	44.9
B2 River systems (RV) incl. soil seed bank	31	295	0.241	5.2	19.0	75.8
C Soil seed bank vs. above-ground population	61	935	0.508	5.9	44.9	49.2
D Geographic regions excl. soil seed bank (based on 17 marker)[1]	80	1019	0.607	13.8	46.8	39.3

[1] Four markes have been discarded to meet the requierments for the AMOVA statistics. One is a assumed NULL allel and the other three were missing data in populations with small amount of genotyped individuals.

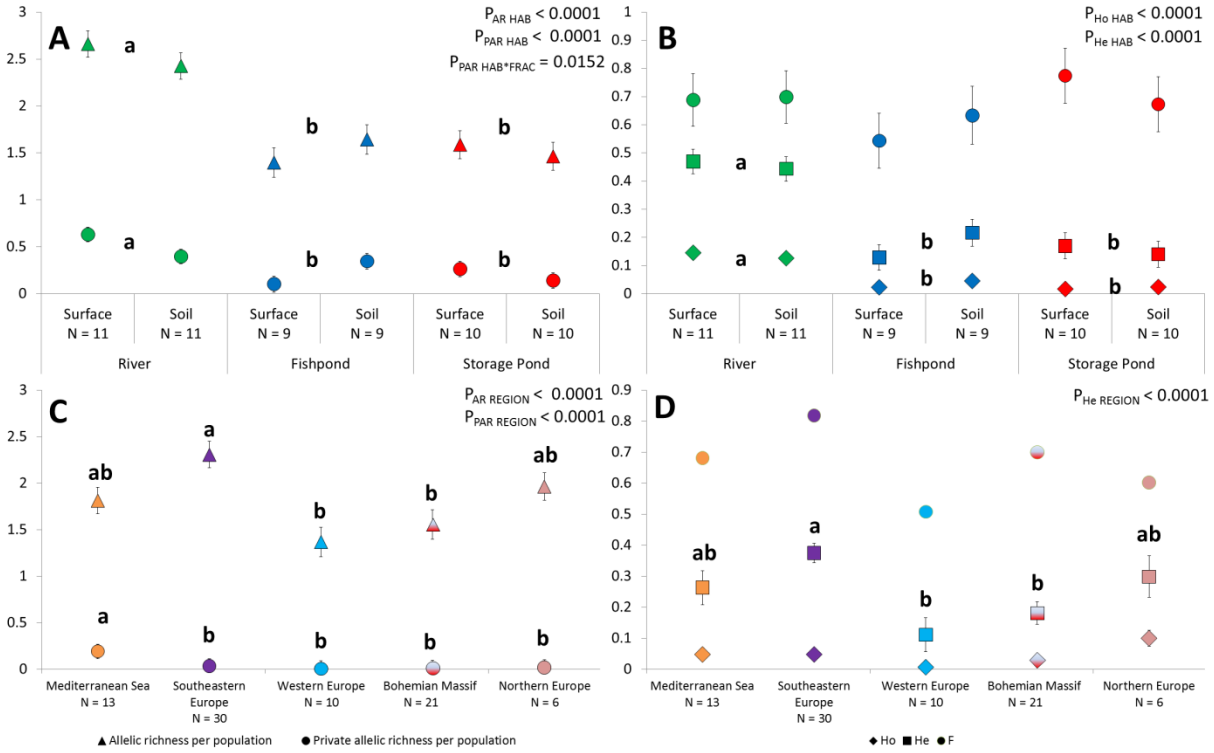

Figure 4-8 Mean genetic diversity calculated with HP-Rare based on five individuals per population for habitats (green – river, blue – fishponds, red – storage ponds; A and B; above-ground population vs. soil seed bank) and regions (C and D; just above-ground populations of habitat dataset included in analysis).

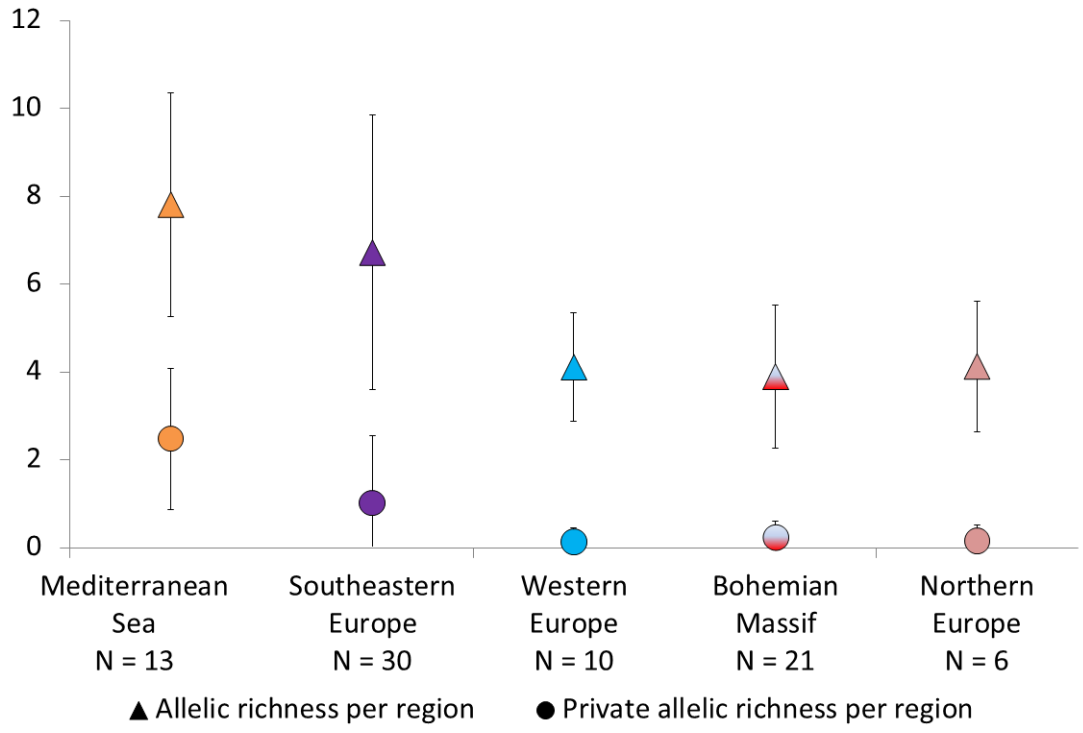

Figure 4-9 Allelic and private allelic richness of European regions calculated with HP-Rare based on 136 genes per region. N = number of included populations according to coarse regions.

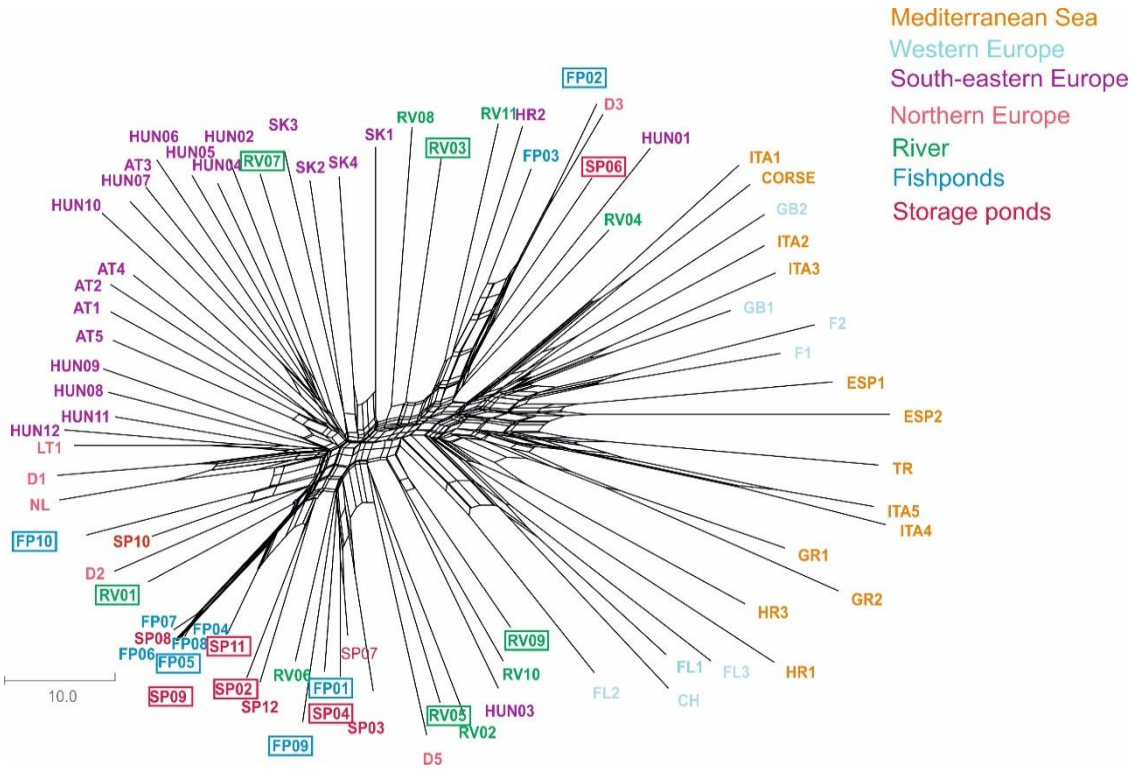

Figure 4-10 Neighbour-joining network based on genetic distances of all sampled populations. Localities with boxes have been included in the environmental manipulation (flooding) experiment. For further information on geographic provenances of localities and their genetic diversities, see Tables 3-1 and Tables 4-3/4-4.

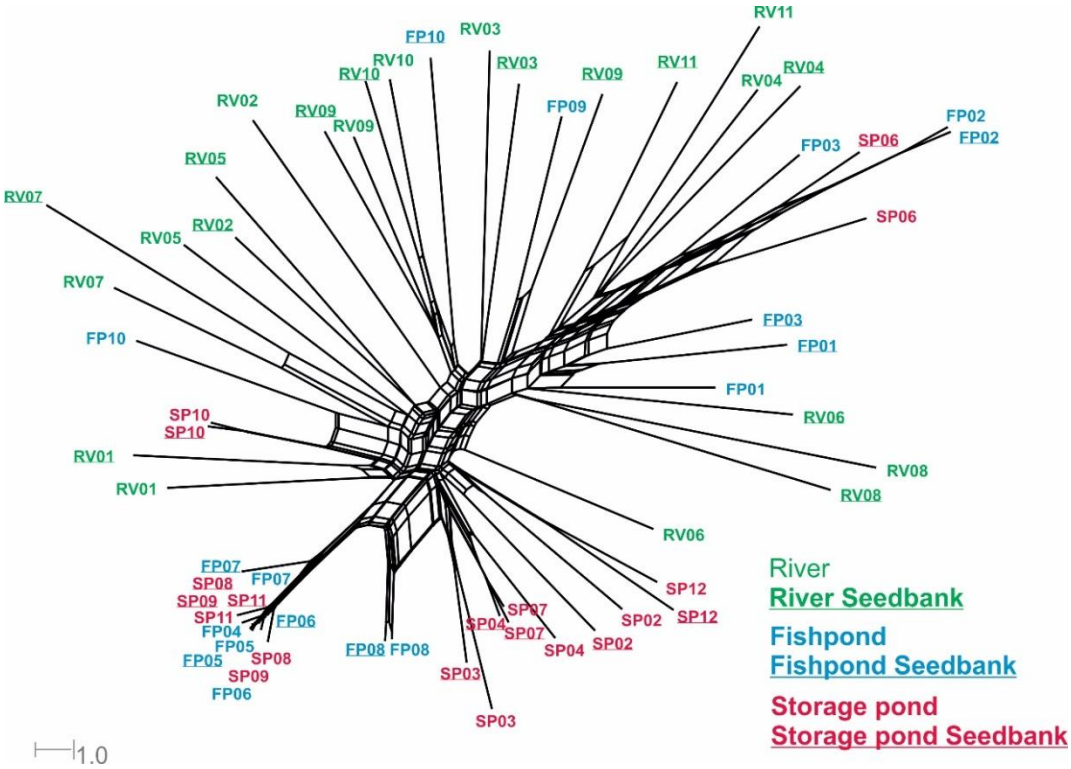

Figure 4-11 Neighbour-joining network based on genetic distances of populations according to three habitat types. Each locality is divided in the above-ground population and the sampled individuals from the soil seed bank (underlined). For further information on the geographic provenances of the localities and their genetic diversity, see Tables 3-1 and 4-3.

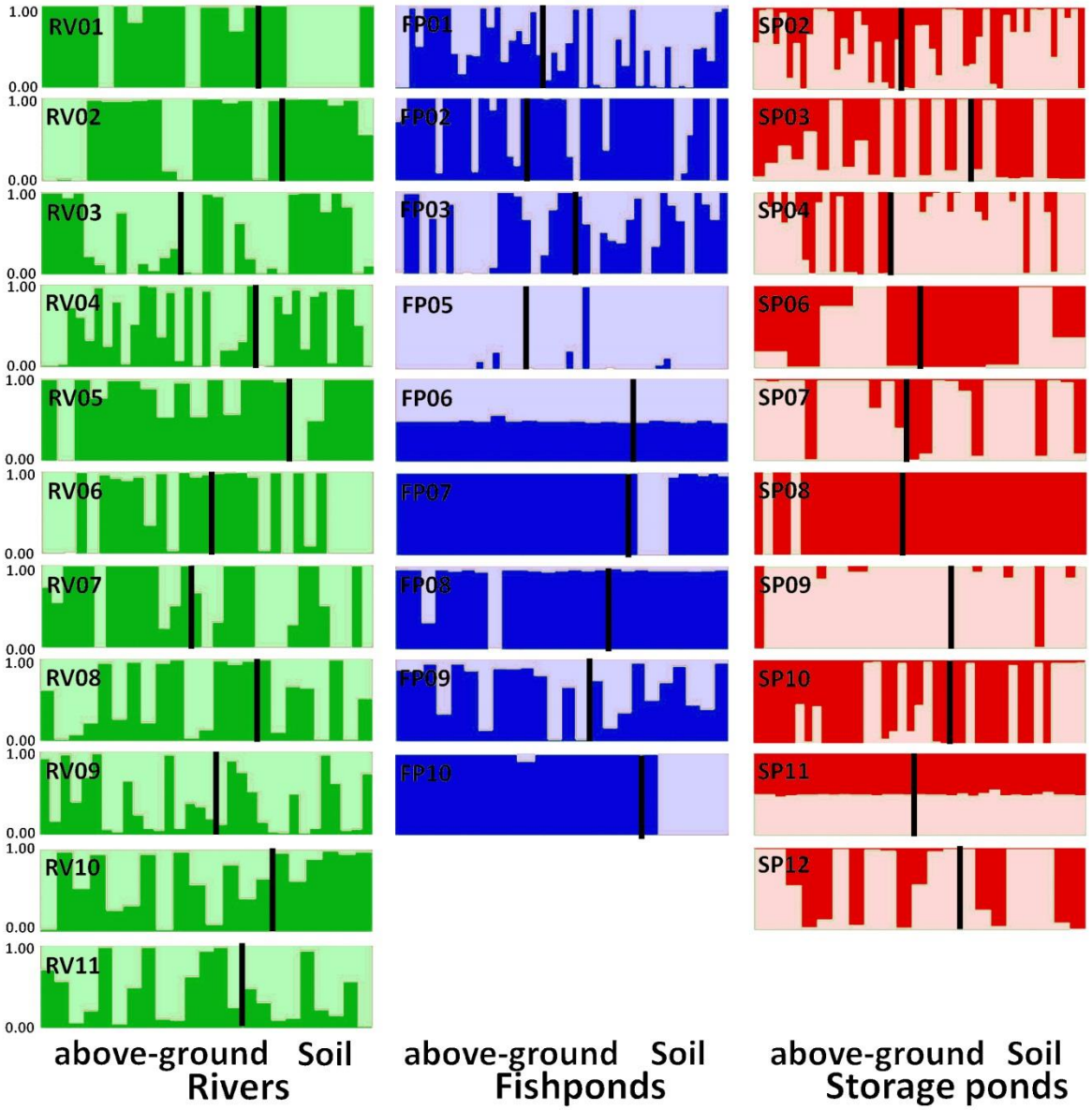

Figure 4-12 Pairwise STRUCTURE analysis (*K* = 2) of each locality according to three habitat types with at least five analysed individuals in the above-ground population and the soil seed bank.

	FP1	FP2	FP3	FP4	FP5	FP6	FP7	FP8	FP9	FP10	RV1	RV2	RV3	RV4	RV5	RV6	RV7	RV8	RV9	RV10	RV11	SP2	SP3	SP4	SP6	SP7	SP8	SP9	SP10	SP11	SP12
F1A	69.1	0.0	3.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.2	0.0	0.0	3.5	2.7	20.5	0.0	0.1	0.0	0.0	0.0	0.0	0.0
F1S	85.9	0.0	1.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0	6.1	0.5	2.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0
F2A	0.0	99.7	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F2S	0.0	97.1	2.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F3A	0.0	8.8	75.4	0.0	4.9	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	9.5	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F3S	0.0	8.1	67.3	0.0	0.0	0.0	0.0	14.7	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.2	6.8	0.0	1.1	0.0	0.0	1.5	0.0	0.0
F4A	0.0	0.0	0.0	4.3	80.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.5	3.0	0.0	0.5	0.0
F5A	0.0	0.0	0.0	0.0	99.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.1	0.0	0.1	0.0
F5S	0.0	0.0	0.0	0.0	95.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4	0.0	0.0	0.0	0.0	1.0	0.2	0.0	0.0	0.0
F6A	0.0	0.0	0.0	0.4	87.9	1.8	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.8	1.0	0.0	0.8	0.0
F6S	0.0	0.0	0.0	0.2	85.0	3.3	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.6	0.6	0.0	4.3	0.0
F7A	0.0	0.0	0.0	0.0	46.8	0.0	28.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	21.5	2.8	0.0	0.0	0.0
F7S	0.0	0.0	0.0	0.0	24.9	0.0	25.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	29.6	0.0	2.9	15.9	0.7	0.0	0.0	0.0
F8A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	99.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
F8S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F9A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.7	92.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F9S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F10A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F10S	15.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.4	0.6	0.0	0.3	21.2	2.1	0.0	0.0	0.0	36.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R1A	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	86.1	0.0	0.0	0.0	3.5	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R1S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	87.5	0.0	0.0	0.0	0.0	0.0	12.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R2A	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.9	72.5	0.2	3.0	5.8	1.3	0.0	0.2	4.4	0.0	0.1	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R2S	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	1.3	0.0	13.4	32.8	15.9	17.8	7.4	0.0	0.9	0.0	0.0	0.7	0.0	0.0	0.0	1.5	0.1	0.0	0.0	3.6	2.3	1.1	0.0
R3A	8.6	0.0	3.1	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	82.7	0.0	5.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R3S	4.0	0.0	9.7	0.0	0.0	0.0	0.0	0.2	1.1	0.0	4.6	0.3	64.6	3.6	8.3	0.3	1.6	0.4	0.3	0.0	0.1	0.6	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.1
R4A	0.0	5.5	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	1.2	7.7	62.5	9.5	0.4	0.0	0.2	3.6	0.4	4.9	0.0	0.6	0.1	0.0	0.0	0.2	0.0	0.3	0.3	0.0
R4S	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	7.7	83.2	2.0	0.0	0.0	0.0	0.7	0.0	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R5A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.7	5.0	85.5	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	6.9	0.0	0.0	0.0	0.0	0.3	0.0	0.0
R5S	0.0	0.0	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	3.8	0.3	84.0	0.4	0.0	0.0	4.6	0.0	0.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R6A	11.4	0.0	5.6	0.0	5.3	0.0	0.0	0.1	5.4	0.0	0.2	0.0	0.7	3.0	0.0	21.2	0.0	0.0	2.7	0.1	0.0	6.5	21.2	3.5	0.0	0.0	1.5	0.0	8.9	0.1	0.1
R6S	0.1	0.0	19.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	59.8	0.0	0.0	0.0	0.0	0.0	4.7	13.5	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R7A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	0.1	0.0	0.0	0.1	0.0	93.3	0.0	0.0	0.0	0.0	0.8	0.1	0.0	0.0	0.1	0.0	0.0	1.2	0.0	0.5
R7S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.1	0.0	0.0	0.2	0.0	97.5	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R8A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	1.6	0.0	0.2	1.0	4.1	0.2	0.0	87.6	0.0	0.0	0.0	0.0	0.4	3.4	0.0	0.4	0.0	0.0	0.3	0.0	0.0
R8S	5.6	0.0	0.3	0.0	0.0	0.0	0.0	0.0	1.1	0.0	5.3	1.6	1.1	3.9	11.3	0.0	0.0	66.1	3.5	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R9A	6.6	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.2	0.3	0.0	0.0	0.0	0.0	88.2	0.5	0.3	0.1	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R9S	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	91.7	1.2	0.0	0.0	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R10A	7.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.6	82.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R10S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R11A	0.0	4.6	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	6.6	0.0	0.0	0.0	0.5	0.0	0.0	86.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R11S	0.8	0.0	3.6	0.0	0.0	0.0	0.0	0.0	0.4	12.5	0.3	0.0	0.8	0.0	0.2	1.3	0.0	9.0	0.1	0.0	64.5	0.6	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S2A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	93.7	1.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.5
S2S	0.0	0.0	0.0	0.0	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	86.3	0.0	3.3	0.0	0.2	0.5	0.0	6.4	0.0	0.1
S3A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.8	93.1	2.5	0.0	0.0	0.0	0.0	0.4	0.0	0.0
S3S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.2	80.8	0.8	0.0	0.0	0.0	0.0	6.2	0.0	0.0
S4A	25.9	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	73.2	0.0	0.0	0.0	0.0	0.3	0.0	0.0
S4S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	99.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0
S6A	0.0	40.0	39.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.9	0.8	0.1	0.0	0.0	0.0	0.0	0.0
S6S	3.5	57.4	20.0	0.0	0.0	0.0	0.0	0.0	16.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
S7A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	70.0	0.0	29.3	0.0	0.0	0.0	0.0	0.0
S7S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	65.5	0.0	34.0	0.0	0.0	0.0	0.0	0.0
S8A	0.0	0.0	0.0	0.0	13.4	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	79.2	0.0	0.0	0.0	0.0
S8S	0.0	0.0	0.0	0.0	19.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	80.5	0.3	0.0	0.0	0.0
S9A	0.0	0.0	0.0	0.0	21.8	0.0	0.1	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	7.6	67.2	0.0	0.0	0.0
S9S	0.0	0.0	0.0	0.0	20.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.6	0.0	0.0	7.5	64.6	0.0	0.0	0.0
S10A	0.0	0.0	0.0	0.0	1.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.3	97.1	0.0	0.0
S10S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0
S11A	0.0	0.0	0.0	0.0	67.6	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.2	1.9	0.0	19.0	0.0
S11S	0.0	0.0	0.0	0.1	64.7	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	16.8	1.7	0.0	16.6	0.0
S12A	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	85.6
S12S	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.5	0.0	0.0	0.0	0.0	10.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	77.2

Figure 4-13 Results of the migrant analysis between localities according to the three habitat types. Numbers correspond to percentage of origin. Localities and their genetic diversities are given in Tables 3-1 and 4-4. A – above-ground populations; F – Fishpond; R – River; S – Fish storage pond; S – Soil seed bank.

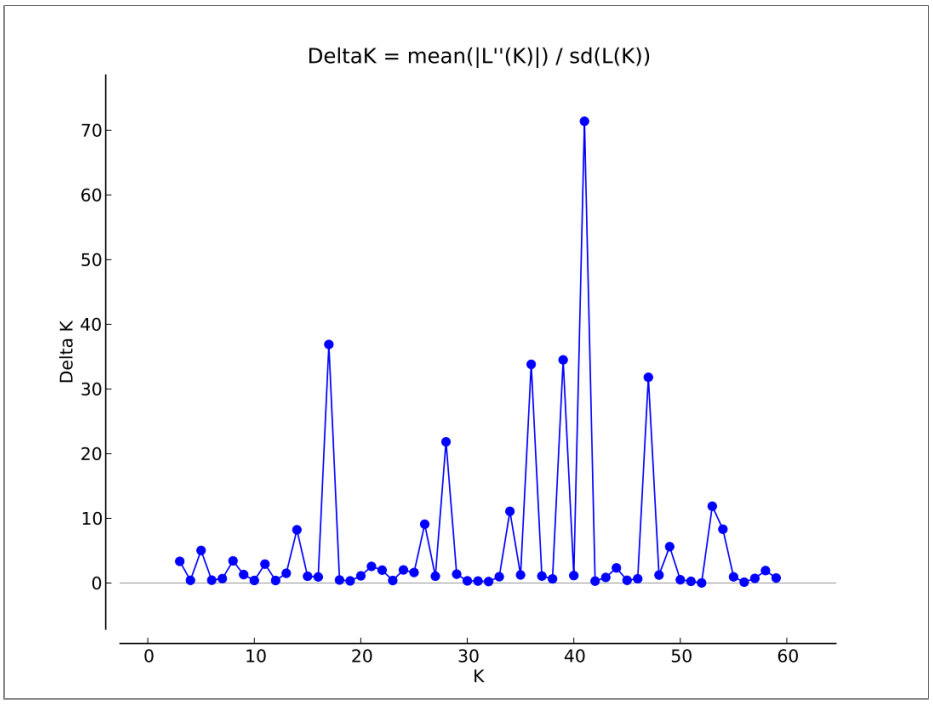

Figure 4-14 Delta *K* diagram of the STRUCTURE run of all sampled individuals exclusive the soil seed bank (Evanno et al. 2005). Barplots for *K* = 17, 28, 36, and 41 are shown in Figure 4-15.

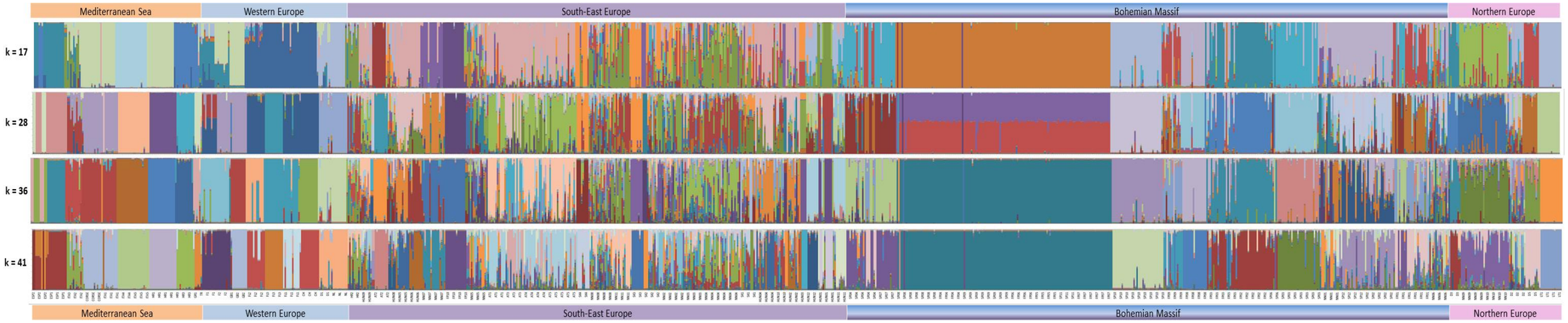

Figure 4-15 STRUCTURE analysis of *K* = 17, 28, 36, and 41

4.6 **Germination experiment**

The generalized linear mixed models did not reveal a significant effect of habitat on germination time or rate. Fraction, on the other hand, had a significant effect on germination time ($\chi^2_{2, 216.1} = 13.93$, $P = 0.0009$) and a marginally significant effect on germination rate ($\chi^2_{2, 216.1} = 5.09$, $P = 0.0784$; Table 4-7). The sampling site also had a significant effect. Seeds of plants derived from the deep and shallow soil seed bank germinated significantly faster than seeds of plants derived from established plants (Fig. 4-16). Similarly, seeds of plants derived from the soil seed bank germinated at a higher rate than seeds of plants derived from established plants.

Table 4-7 Results of the generalized linear mixed models used to investigate the effect of habitat, fraction and their interaction on germination traits. For each fixed factor, I report the χ^2-values and their associated *P*-values (in brackets). *Z*-values and their associated *P*-values are reported for random factors.

Source of variation	Germination time	Germination rate
Habitat	4.34	4.28
(df = 2)	(0.1141)	(0.1178)
Fraction	**13.93**	5.09
(df = 2)	**(0.0009)**	(0.0784)
Habitat × fraction	7.80	7.57
(df = 4)	(0.0993)	(0.1088)
Site (within habitat)	**2.34**	**2.31**
(df = 1)	**(0.0096)**	**(0.0103)**

df = degrees of freedom

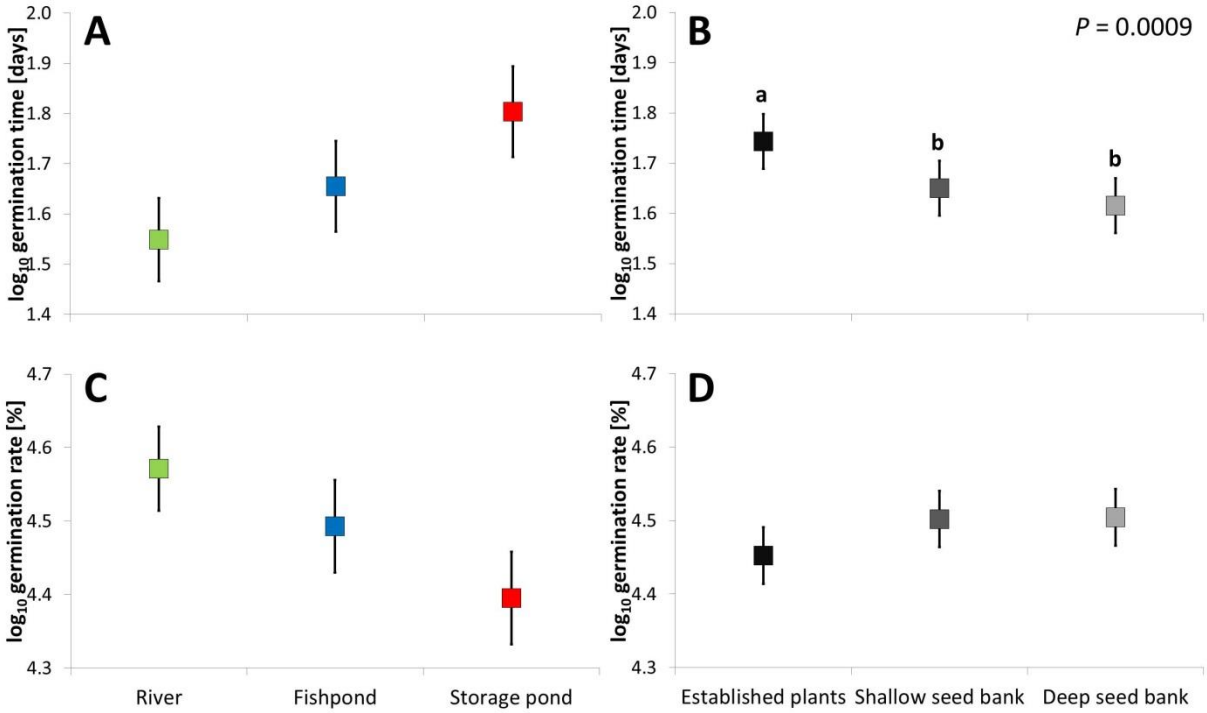

Figure 4-16 Germination time and rate of seeds in relation to their habitat (river, fishpond and fish storage pond; A, C) and seed origin (established plants from the above-ground population, shallow seed bank, deep seed bank; B, D). Least square means and standard errors of the generalized linear mixed models are shown. Letters denote significant differences in least square means of the generalized linear mixed models (Tukey-Kramer adjustment for multiple comparisons: $P < 0.05$).

4.7 **Flooding experiment**

Just three of the 900 flooded plants (out of the 1350 total plants) died in the experiment and all other plants completed their life cycle. There were no significant differences between habitats at the first monitoring, i.e. before the plants were submerged (Appendix 4). The covariate “days since germination” was highly significant in most models. The random factor site as well as its interaction with treatment and fraction were also significant in most models (Table 4-8).

Effect of water level

At both the second and third monitoring, treatment had a highly significant effect on all examined variables. Plant height was highest under severe flooding and lowest without flooding, at both the second and third monitoring (Fig. 4-17 A, B). At both monitoring dates, moderately flooded plants had the longest leaves, and non-flooded plants the shortest (Fig. 4-17 C, D). The latter plants had the widest leaves and were most vital, whereas severely flooded plants had the narrowest leaves and were least vital (Fig. 4-17 E-H). At the second monitoring, non-flooded and moderately flooded plants had the highest number of culms (Fig. 4-18 A), but at the third monitoring, the moderately flooded plants had the highest number of culms compared to the other two treatments (Fig. 4-18 B). Non-flooded plants had most culms with inflorescences and most leaves at the second monitoring (Fig. 4-18 C, E). At the third monitoring, however, the moderately flooded plants caught up with the non-flooded plants in this respect (Fig. 4-18 D, F). Regarding shoot biomass, moderately flooded plants performed best and non-flooded plants worst (Fig. 4-18 G).

Effect of fraction

The fractions did not show any significant differences in the flooding experiment (Appendix 5). Merely the interaction of fraction with habitat showed some effects, but no significant differences were detectable after Tukey-Kramer adjustment for multiple comparisons between least square means.

Effect of habitat

At the first monitoring, no significant effects of habitat were found. Interestingly, however, the share of already flowering plants was greatest for plants from fish storage ponds (least square mean ± standard error = 63.5 ± 11.8%), followed by plants from fishponds (47.5 ± 11.8%) and, lastly, rivers (31.3 ± 11.8%; Appendix 4). With a *P*-value of 0.1575, these differences were, however, not significant.

At the second monitoring, a significant effect of habitat on the number of culms was found. Plants from rivers had a significantly higher number of culms than plants from fish storage ponds (Fig. 4-18 A). Both groups did not significantly differ from fishpond plants, which had an intermediate number of culms. At the harvest of the plants (third monitoring), a significant effect of habitat was found on the number of culms (as at the second monitoring) as well as on the number of culms with inflorescences (Fig. 4-18 B, D). The pattern found for both traits was the same as at the second monitoring.

Significant interactions of habitat and treatment were found for the number of leaves and vitality at the third monitoring. Both traits showed a similar pattern (Fig. 4-17 H, 4-18 F). Remarkably, plants from fishponds performed best in the treatment without any flooding, but plants from rivers performed best in both the moderate and severe flooding treatments. Several other traits related to fitness, namely the number of culms, the number of culms with inflorescences, and shoot biomass, showed the same pattern, but the differences were not statistically significant (Fig. 4-18 B, D, G).

Table 4-8 Results of the linear mixed models used to investigate the effects of habitat, fraction and watering treatment on plant traits in the environmental manipulation (flooding) experiment. For random, fixed factors and their interactions χ^2-values and associated *P*-values (in brackets) are reported. Days since germination was used as a covariate.

Source of variation	Plant height	Leaf length	Leaf width	Vitality	No. of culms	Prop. of flow. plants	No. of culms with infloresc.	No. of leaves	Shoot biomass
First monitoring (*n* = 1350)									
Habitat	1.64	1.42	0.10	0.44	0.53	3.70	0.55	0.23	n. a.
(df = 2)	(0.4401)	(0.4909)	(0.9494)	(0.8025)	(0.7659)	(0.1575)	(0.7584)	(0.8912)	
Fraction	0.40	0.82	1.28	0.64	0.19	0.15	1.39	0.29	n. a.
(df = 2)	(0.8171)	(0.6649)	(0.5262)	(0.7258)	(0.9075)	(0.9258)	(0.4987)	(0.8656)	
Habitat × fraction	**17.54**	**14.23**	**13.55**	7.40	3.26	2.58	5.72	1.23	n. a.
(df = 4)	**(0.0015)**	**(0.0066)**	**(0.0089)**	(0.1161)	(0.5159)	(0.6311)	(0.2212)	(0.8730)	
Site (within habitat)	**17.0**	**22.5**	**34.3**	**13.2**	**6.3**	**11.8**	**29.7**	**12.0**	n. a.
(df = 1)	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(0.0003)**	**(0.0121)**	**(0.0006)**	**(<.0001)**	**(0.0005)**	
Site × fraction	**54.3**	**53.1**	**6.3**	**17.4**	**42.4**	**104.1**	**72.5**	**39.2**	n. a.
(df = 2)	**(<.0001)**	**(<.0001)**	**(0.0121)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	
Days since germ.	**364.81**	**187.59**	**180.83**	**648.38**	**591.38**	**34.70**	**75.02**	**722.60**	n. a.
(df = 1)	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	
Second monitoring (*n* = 1349)									
Habitat	2.77	1.71	1.09	2.63	**13.93**	n. a.	2.14	4.55	n. a.
(df = 2)	(0.2498)	(0.4256)	(0.5798)	(0.2686)	**(0.0009)**		(0.3428)	(0.1030)	
Fraction	0.69	0.69	2.20	0.75	1.01	n. a.	3.18	0.98	n. a.
(df = 2)	(0.7071)	(0.7094)	(0.3335)	(0.6875)	(0.6039)		(0.2035)	(0.6130)	
Treatment	**769.03**	**149.73**	**51.64**	**154.96**	**16.86**	n. a.	**57.95**	**78.24**	n. a.
(df = 2)	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(0.0002)**		**(<.0001)**	**(<.0001)**	
Habitat × treatment	6.42	0.78	2.25	5.26	1.32	n. a.	1.51	4.80	n. a.
(df = 4)	(0.1699)	(0.9416)	(0.6907)	(0.2616)	(0.8584)		(0.8256)	(0.3086)	
Fraction × treatment	0.58	4.12	2.25	0.41	4.73	n. a.	3.30	1.94	n. a.
(df = 4)	(0.9651)	(0.3905)	(0.6893)	(0.9816)	(0.3157)		(0.5096)	(0.7474)	
Habitat × fraction	2.82	9.16	**13.65**	6.30	1.23	n. a.	6.04	5.44	n. a.
(df = 4)	(0.5878)	(0.0573)	**(0.0085)**	(0.1782)	(0.8738)		(0.1960)	(0.2455)	

Site (within habitat)	**28.6**	**28.6**	**6.6**	**15.9**	.	n. a.	2.6	**10.4**	n. a.
(df = 1)	**(<.0001)**	**(<.0001)**	**(0.0102)**	**(0.0001)**	.		(0.1069)	**(0.0013)**	
Site × treatment	**12.3**	**29.3**	**44.3**	**40.3**	**84.5**	n. a.	**117.3**	**78.0**	n. a.
(df = 2)	**(0.0005)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**		**(<.0001)**	**(<.0001)**	
Site × fraction	**42.7**	**50.1**	**19.7**	**22.0**	**51.0**	n. a.	**33.8**	**43.7**	n. a.
(df = 2)	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**		**(<.0001)**	**(<.0001)**	
Days since germ.	**11.37**	**32.92**	**16.96**	**4.84**	**4.78**	n. a.	**17.11**	**7.86**	n. a.
(df = 1)	**(0.0007)**	**(<.0001)**	**(<.0001)**	**(0.0279)**	**(0.0287)**		**(<.0001)**	**(0.0051)**	
Third monitoring (*n* = 1347)									
Habitat	2.12	2.53	0.24	4.71	**6.97**	n. a.	**8.15**	5.34	3.32
(df = 2)	(0.3464)	(0.2819)	(0.8886)	(0.0947)	**(0.0307)**		**(0.0170)**	(0.0692)	(0.1901)
Fraction	1.03	1.05	1.72	0.80	1.77	n. a.	1.64	1.45	0.79
(df = 2)	(0.5984)	(0.5902)	(0.4233)	(0.6688)	(0.4119)		(0.4403)	(0.4834)	(0.6724)
Treatment	**319.64**	**108.23**	**104.26**	**18.37**	**12.19**	n. a.	**13.00**	**23.81**	**33.59**
(df = 2)	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(0.0001)**	**(0.0023)**		**(0.0015)**	**(<.0001)**	**(<.0001)**
Habitat × treatment	2.95	1.72	5.06	**12.42**	5.75	n. a.	5.04	**10.34**	4.84
(df = 4)	(0.5661)	(0.7877)	(0.2816)	**(0.0145)**	(0.2190)		(0.2830)	**(0.0350)**	(0.3040)
Fraction × treatment	0.32	2.42	1.53	0.68	3.48	n. a.	4.07	2.15	1.77
(df = 4)	(0.9886)	(0.6583)	(0.8211)	(0.9535)	(0.4811)		(0.3967)	(0.7073)	(0.7781)
Habitat × fraction	2.23	6.31	**10.22**	2.21	1.20	n. a.	1.02	1.76	2.67
(df = 4)	(0.6932)	(0.1773)	**(0.0369)**	(0.6964)	(0.8773)		(0.9064)	(0.7795)	(0.6153)
Site (within habitat)	**27.2**	**27.6**	**22.6**	**7.8**	.	n. a.	.	3.0	**7.3**
(df = 1)	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(0.0052)**	.		.	(0.0833)	**(0.0069)**
Site × treatment	**40.8**	**29.7**	**47.9**	**62.3**	**39.6**	n. a.	**37.7**	**25.6**	**29.8**
(df = 2)	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**		**(<.0001)**	**(<.0001)**	**(<.0001)**
Site × fraction	**73.6**	**22.2**	**20.8**	**60.7**	**64.7**	n. a.	**56.1**	**64.5**	**150.6**
(df = 2)	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**	**(<.0001)**		**(<.0001)**	**(<.0001)**	**(<.0001)**
Days since germ.	**26.39**	**135.58**	**0.17**	**35.25**	**14.57**	n. a.	**10.51**	**22.96**	**12.44**
(df = 1)	**(<.0001)**	**(<.0001)**	**(0.6801)**	**(<.0001)**	**(0.0001)**		**(0.0012)**	**(<.0001)**	**(0.0004)**

df = degrees of freedom

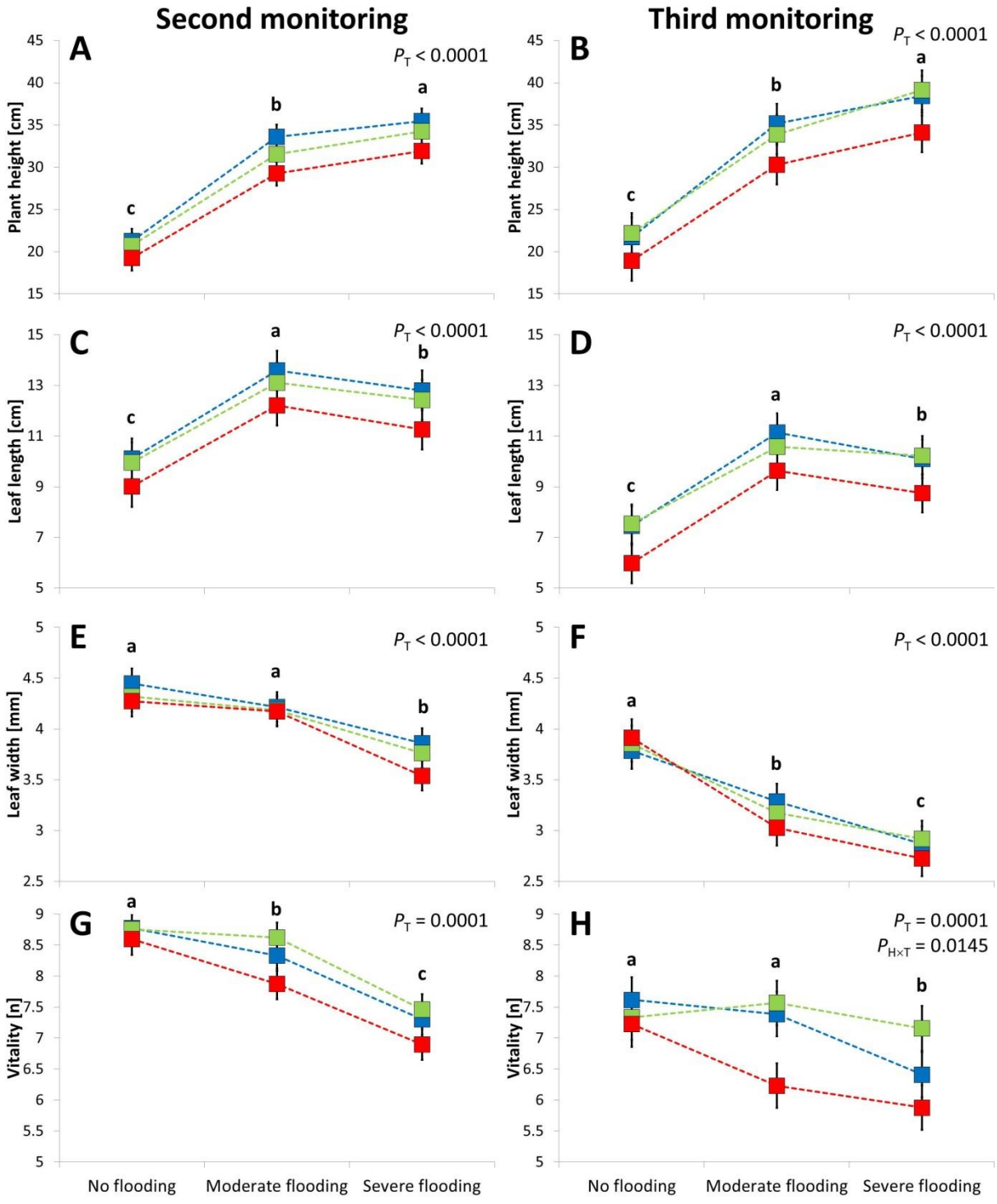

Figure 4-17 Interaction of habitat with water treatment in the flooding experiment at the second and third monitoring (harvest; green, rivers; blue, fishponds; red, fish storage ponds; see Fig. 4-18 for legend). Least square means and standard errors of the linear mixed models are shown for traits reflecting response to flooding—plant height (A, B), leaf length (C, D), and leaf width (E, F)—and vitality (G, H). Letters denote significant differences in least square means (Tukey-Kramer adjustment for multiple comparisons: $P < 0.05$; P_T, P-value of treatment; $P_{H\times T}$, P-value of the interaction of habitat and treatment).

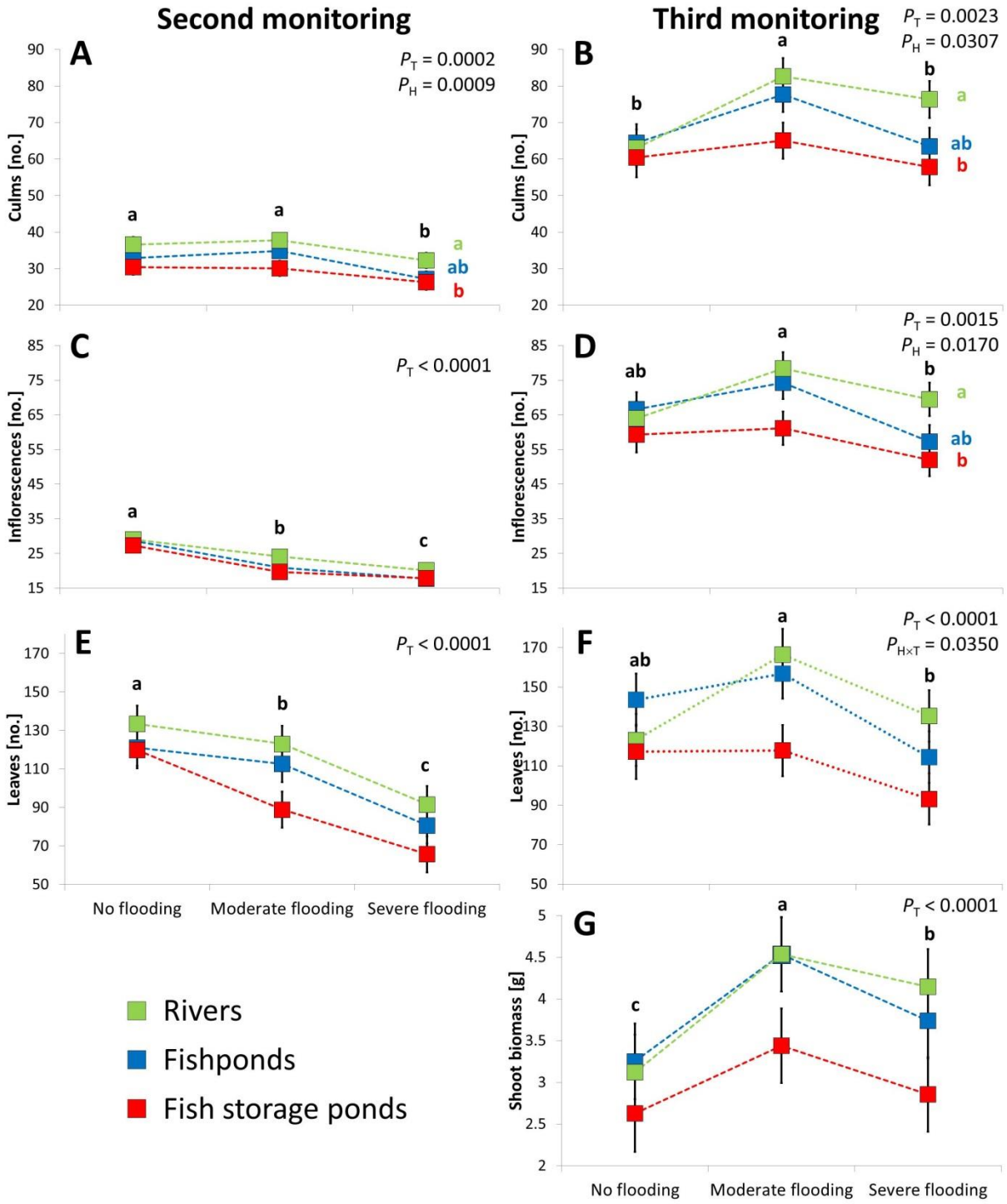

Figure 4-18 Interaction of habitat with water treatment in the flooding experiment at the second and third monitoring (harvest). Least square means and standard errors of the linear mixed models are shown for traits related to fitness: number of culms (A, B), number of culms with inflorescences (C, D), number of leaves (E, F), and shoot biomass (G). Letters denote significant differences in least square means (Tukey-Kramer adjustment for multiple comparisons: $P < 0.05$; P_T, P-value of treatment; P_H, P-value of habitat; $P_{H\times T}$, P-value of the interaction of habitat and treatment).

5 Discussion

In the following section, the results will be discussed according to the questions stated at the beginning. The discussed aspects are overlapping, but I decided to divide this section into blocks according to the concepts of the questions. Subheadings separate these concepts and at the beginning of most subsections, the questions and the major associated results will be repeated.

5.1 Development of microsatellite markers

(1.1) Is the design of at least 12 polymorphic MS markers with the help of next-generation sequencing possible?

After two independent next-generation sequencing events from two different commercial suppliers on the Roche 454 platform, we published 21 suitable markers for *C. fuscus* and 39 further polymorphic primer pairs were found (BÖCKELMANN ET AL. 2015; Table 4-2; Appendix 2). The 21 markers were tested on two populations and seven geographically seperated test individuals, whereas the 39 additional primer pairs have just been tested on the seven test indivduals.

High-throughput sequencing has a significant impact on genomic research and is giving new opportunities for locating microsatellites in non-model and not-previously-sequenced organisms (e.g. SELKOE AND TOONEN 2006; TAKAYAMA ET AL. 2011; HODEL ET AL. 2016). Before the next-generation sequencing thechnique came up, the identification of SSR motifs has long been a bottle-neck for studying non-model organisms (GUICHOUX ET AL. 2011). The choice of techniques is depending on the objective of the study, pre-known genomic information and the funding situation. For small projects with short funding, the investment in a genomic dataset is not cost-efficient, especially when researchers still have to acquire the data-managing skills. When a project requires a high number of individuals, but not loci, microsatellites are still in favour, as the RAD-sequencing of just 96 individuals has a similar price as the genotyping of the same sample with 12-15 microsatellites (HODEL ET AL. 2016).

A big advantage of this classical marker system, which has been used for a long time, is the availability of extensive literature (e.g. GOLDSTEIN AND SCHLÖTTERER 1999) with current updates (e.g. GUICHOUX ET AL. 2011). Even guides for ecologists (e.g. SELKOE AND TOONEN 2006; GARDENER ET AL. 2011) and non-model organisms (e.g. TAKAYAMA ET AL. 2011) are accessible and describing the caveats. The relatively new technology of RAD-sequencing, which is based on restriction sites of enzymes like AFLPs, on the other hand, showed several problems, of which not all could be solved satisfactorily so far (e.g. ARNOLD et al. 2013; ANDREWS 2016).

To rely on the results of a commercial company is generally problematic due to possible mistakes that are out of the control of the researchers. For this project, the first sequencing run was way less successful than the second run. Whether the problem was the quality of the sequencing itself, the DNA extraction or the data mining with MSATCOMMANDER (FAIRCLOTH 2008) is not clear at this point of the project. The second sequencing was performed after enrichment for four widespread repeats in plant genomes on just a 1/16 of a titer plate. while the data-handling and primer design of 80 suitable was also performed by the company. This service led to a 70% higher price, but at least for our situation it saved a lot of time, was comparably convenient and showed very satisfying results.
Few genetic markers, if any, played such a big role in population genetics as co-dominant and highly variable markers like microsatellite markers (ELLEGREN 2004). With time, a better understanding of the new methods and their limitations will be gathered and they will eventually replace the classic markers and technologies like genotyping by sequencing. Mean wile, some efforts have been made to genotype markers such as microsatellites with NGS platforms (VARTIA ET AL. 2016).

The 21 polymorphic loci developed in this study will be useful for studying genetic diversity of C. fuscus and the role of the soil seed bank in the life cycle of this ephemeral plant in natural and anthropogenic habitats.

(1.2) The price for genotyping an individual drops when multiplexing is used. Are there practicable combinations of primer to analyse groups of markers in a single PCR reaction?

Microsatellites are comparatively easy to automate and with multiplex amplification up to five loci can be amplified in one single PCR reaction (GOLDSTEIN AND SCHLÖTTERER 1999). In the last years, methods for multiplexing PCR improved considerably and thereby genotyping costs decreased, while throughput increased. Due to the use of sensitive capillary electrophoresis machines and increasing numbers of fluorochromes, under ideal circumstances even a combination of up to 40 multiplexed markers in one single reaction is possible. Nevertheless, between 2009 and 2010 real multiplexing was used in just 42% of surveyed SSR studies published in *Molecular Ecology*, which illustrates the still limited prevalence of this technique. This is surprising, because the final cost of genotyping one sample can be reduced to a small fraction of the price, when multiplexing is used. For high-quality multiplex SSRs, a stringent selection of markers is necessary. Primer pairs amplifying fragments of contrasted non-overlapping sizes can be used with a single dye. Also, the multiplexed primer pairs should have a similar annealing temperature. A variety of programs exists to choose such pairs (GUICHOUX ET AL. 2011). The program MULIPLEX MANAGER (HOLLEY AND GEERTS 2009) has the advantage that it eliminates combinations of primers with potential primer-dimer interactions. After sequences of the primer pairs had been entered into the program, an easy and user-friendly output showed the best combination of the primers. Every single marker derived from the first sequencing (Cf at the beginning of the name) was amplified in a separate PCR reaction and so, the PCR products had to be transferred onto one sequencing plate one by one. Multiplexing not just reduced the costs, but also the time investment massively and so, the use of this technology is strongly recommended. An overview of the used multiplex sets is shown in Table 4-2.

(1.3) Are there differences in ploidy level in *C. fuscus*? What is the exact genome size of this species?

Polyploidy is recognized as one major evolutionary force not only in plants, but also in all eukaryotes (SOLTIS ET AL. 2014). In plants, it is a common mode of speciation and can have far-reaching consequences for plant ecology (SEGRAVES AND ANNEBERG 2016). The importance of ancient polyploidy became apparent, when the complete genome of *Arabidopsis thaliana* was sequenced in 2003 by BENNETT ET AL. and numerous duplicated genes in this “undoubtedly” diploid organism have been found. Recent investigations even indicate that whole genome duplications are ubiquitous in angiosperms (SOLTIS ET AL. 2014). Beside the evolutionary role of polyploidization, it aggravates the determination of genotypes in microsatellite analysis because of the complex inheritance patterns in partially heterozygous individuals (BRUVO ET AL. 2004; DE SILVA ET AL. 2005). Because of the ambiguous remarks in the literature about the ploidy level of *C. fuscus*, the project “Polyploidy, ecological niche and demographic development of the wetland annual plant species *Cyperus fuscus*” (CZ 13/2012, granted to K. Tremetsberger) was additionally performed to clarify these uncertainties.

The sampling of populations for flow cytometry was rather dense in the Czech Republic, eastern Austria, western Slovakia and western Hungary and the genome size obtained relates to 2*n* = 36 chromosomes, so that the count of 2*n* = 72 chromosomes from Lakšárska

Nová Ves, Slovakia (MÁJOVSKÝ ET AL. 1987) remains an erratic count that could not be found again. P. Kúr and K. Tremetsberger searched for *Cyperus fuscus* in this area, but could not find it again there. The closest sampled population with similar ecological conditions (acidic sand) is from the pond Vodná Nádrž Horná Studená Voda, NW of Tomky, Slovakia (P. Kúr, K. Tremetsberger, 20.09.2013) and has 2*n* = 36 chromosomes (Figure 4-2). In the genotyping of over 1000 individuals, also no polyploid individuals have been detected.

Results of genome size measurements along with chromosome numbers are given in Appendix 3. In contrast to the initial expectation, no substantial variation in genome size has been found. The exact genome size of *Cyperus fuscus* with 2*n* = 36 chromosomes is 0.24 pg/1C. The variation encountered in the total species does not exceed the variation encountered among individuals within populations, indicating that there is no variation in chromosome number among the populations revised.

The term genome can refer to either the entire nuclear DNA or just some parts of it, and a completely sequenced genome is relative, because of copy numbers of genes, centromere regions and non-nuclear DNA (BENNETT ET AL. 2003). *Arabidopsis thaliana* was chosen as a model organism because of reasons like the fast generation cycle and the easy manipulation of the flowers. Another important motivation was the comparable small genome of this species. Already in 1984, the first estimations of genome size (0.082 pg/1C) were published. From the recent perspective, it was quite an underestimation. The newest estimations of different accessions, also gained through flow cytometry and propidium iodide staining, are between 0.153 and 0.167 pg/1C (BENNETT ET AL. 2003). The genome of *Cyperus fuscus* is rather small, but still larger than the *A. thaliana* genome. Genome size generally shows a very large diversity and especially in plants, nuclear DNA amounts show a 2400-fold variation with C-values ranging from 0.065 to 152.2 pg (PELLICER ET AL. 2010). Wetland and aquatic plants seem to have relatively small genomes ranging from 0.22 to 21.53 pg/1C and according to the genome size categories in plants by LEITCH ET AL. (2005) 33 out of 55 (60%) of surveyed species showed very small genome sizes. Three quarters of the genome size values of wetland and aquatic plants belong to the lowest values for angiosperms (HIDALGO ET AL. 2015). Annual plants often exhibit lower values than perennials. In the study of HIDALGO ET AL. (2015), however, this difference was not significant, but plants that are able to colonise both, aquatic and wetland habitats, showed significantly higher values than plants the colonise just one of these habitats. The large genome constraint hypothesis suggests that large genomes are underrepresented in extreme habitats, to which the aquatic habitats can be referred (KNIGHT ET AL. 2005). But wetland plants even seem to be more constrained towards small genome sizes than aquatic plants, probably due to the fact that these species face extreme aquatic and very dry conditions (HIDLAGO ET AL. 2015).

5.2 Soil seed bank

A fundamental objective of this thesis was to shed light on the soil seed bank as an integral part of the life cycle of the ephemeral plant species *Cyperus fuscus*.

(2.1) Is the soil seed bank of *C. fuscus* transient or persistent?

The scarce literature about *Cyperus fuscus* showed two contradictory statements about the type of the soil seed bank, but mudflat species are known for building up a long-term persistent soil seed banks (SALISBURY 1970; THOMPSON ET AL. 1997; POSCHLOD 1993; WEYEMBERGH ET AL. 2004; DEIL 2005; ŠUMBEROVÁ ET AL. 2012). During this study, I found evidence to state that the actual type of *C. fuscus* is persistent. The occurrence of seeds below dense perennial vegetation or on the bottoms of ponds not drained for several years (fishpond Novozámecký), i.e. in conditions where the recent reproduction of the species has not been feasible (ŠUMBEROVÁ ET AL. 2012), and the relative abundance of viable seeds in the deep soil layers suggest the presence of a long-term persistent soil seed bank also for *C. fuscus*. For persistence, seed longevity is of major interest and as most annual plants, *C.*

fuscus produces large amounts of small seeds (BRYSON AND CARTER 2010). The wet-dry cycling typical for the habitat of *C. fuscus* (LONG ET AL. 2015) and the deep burial (ZAGHLOUL 2013) can extend seed longevity, but deeply buried seeds can also become unavailable for rapid germination (HONNAY ET AL. 2008).

The very high germination rates in the experiment after dark and cold storage and the huge amounts of seeds emerging from the soil additionally suggest that this species also does not show a deep physiological dormancy. In my experiment, a small amount of seeds germinated in the spike directly after seed ripening, which makes an obligatory physiological dormancy for *C. fuscus* very unlikely. VON LAMPE (1996) made the same observation.

Out of ten emergent *Cyperus* species, seven are listed as physiologically dormant and the remaining three as non-dormant (BASKIN AND BASKIN 2014). For most mudflat species examined in SALISBURY (1970), freshly ripened seeds are able to germinate immediately after harvest, but there is a huge variation between species and even between individuals of the same species. *Bidens frondosa* is even forming different seed morphotypes with different states of dormancy (BRÄNDEL 2004).

(2.2) Are there any differences in germination and growth under standardised environmental conditions between seeds originating from the soil seed bank and those harvested from the above-ground populations?

Germination rates reported for *C. fuscus* range from 39 to 77% (SALISBURY 1970), which is supported by the findings in this experiment, in which *C. fuscus* showed germination rates between 21 and 100%. The mean germination rate of 90% after dry, cold and dark storage on the other is slightly higher in the experiment than in the literature SALISBURY (1970). Light, temperature and water are the main factors regulating dormancy and germination of seeds of mudflat species (HEJNÝ 1960; SALISBURY 1970; VON LAMPE 1996; BASKIN AND BASKIN 2014). When the mud is exposed, the temperature rises and a large difference between day and night temperature triggers germination of mudflat species (VON LAMPE 1996; PIETSCH 1999; WANG ET AL. 2014).

The only significant difference between fractions (soil vs. above-ground population) was found in germination time. Seeds originating from ripe plants collected in the field germinated slower than seeds originating from the soil fractions. Because the seeds used in the experiment had the same age and experienced the same storage conditions, I can exclude differences in their state of dormancy as underlying reason. The maternal environment is known to influence seed fitness and the early stages of plant development (GALLOWAY 2005; BISCHOFF AND MÜLLER-SCHÄRER 2010; BASKIN AND BASKIN 2014). Here, however, I can exclude effects of the parental (but not of the grandparental) generation on germination, because it grew under similar conditions in the glasshouse. I therefore suggest that the observed differences in germination are mainly under genetic control.

(2.3) Does the genetic composition of the above-ground populations differ from their soil seed bank? Does the soil seed bank function as a "genetic memory"?

Despite the differences in germination speed, I found no significant genetic differentiation between the standing above-ground population and the sampled soil seed bank in any of the examined habitat types. Just 5.9% of the total genetic variation was attributable to the difference between the soil seed bank and the standing above-ground population. With an F_{ST}-value of 0.51 and an associated *P*-value < 0.0001, the null hypothesis of random grouping has to be accepted. HONNAY ET AL. (2009) questioned the reliability of snapshot F_{ST}-values to infer population dynamics in a three year-study on stony riverbanks. The study design demanded the sampling of the soil seed bank (exclusively the upper five mm were

discarded) and of ripe seeds of the above-ground population at the same time, so that this study is just a snapshot of just one year.

There is evidence that soil seed banks of annual species increase effective population size (HONNAY ET AL. 2008) and an increased population size is generally positively correlated with higher fitness and genetic diversity, but this is not the case for self-compatible species (LEIMU ET AL. 2006). TEMPLETON AND LEVIN (1979) postulated an accumulation of genotypes in the soil, but I could not find any evidence for that. HONNAY ET AL. (2008) also found no accumulation, even though 13 of 42 studies on fragmented habitats showed differences in genetic diversity between the soil seed bank and the above-ground populations. For the rare and endemic *Astragalus bibullatus*, on the other hand, seeds from the deeper seed bank layers, so supposedly of older age, have been formed under conditions of higher gene flow, whereas inbreeding seems to play a growing role for newer generations. The seed bank also showed a higher diversity, which can be interpreted as evidence for a historical genetic memory or as the result of differential selection (MORRIS ET AL. 2002).

Especially in fast changing environments, selection on germination and growth is an important factor and acts directly or indirectly as a filter on alleles present in the above-ground population. For the same reason, continuing comparisons of genetic diversity between soil seed bank and above-ground population should be encouraged under different selection regimes (HONNAY ET AL. 2008). If the above-ground population would be non-random sample of the soil seed bank, self-thinning after mass germination, which is an important feature of ephemeral wetland species that rapidly colonise suitable habitats, may be the major selective force (MANDÁK ET AL. 2006). Many studies on annual wetland plants suggest that even after a mass germination event, a much higher proportion of seeds remains in the soil than germinates (LECK AND BROCK 2000; DEIL 2005; BERNHARDT ET AL. 2008). In my experiment, the amount of viable, but not germinated seeds in the soil remained unclear. Moreover, the use of the seedling emergence method to obtain *C. fuscus* plants from the soil may have led to a bias towards genotypes that are best adapted to germinate under the specific glasshouse conditions (e.g., temperature, air moisture). Similarly, the specific conditions on the site in the given period including the weather situation (e.g., FERNÁNDEZ-PASCUAL ET AL. 2013) could support the germination of particular genotypes of *C. fuscus* and suppress the germination of other genotypes, and thus function as an ecological filter. This supports the view that the soil seed bank stores genetic variability, as a result of the accumulation of seeds of the yearly cohorts (established plants), to ensure germination and establishment of above-ground populations under various environmental conditions and thus plays an important role not only as a way to survive unsuitable conditions (LECK 1989; MANDÁK ET AL. 2012). Selection of the plants established every year is thus mediated by on site conditions during germination. Variation in possible short-term filtering of genotypes by fluctuating environmental factors has not yet been studied in mudflat species and desires further attention.

The significant differences between soil seed bank and above-ground population in germination speed, which is supposedly under genetic control, could not be shown with microsatellite markers. Evolution of traits influenced by single or multiple changes in regulatory genes are hard to detect with microsatellites because of the neutral nature of these markers. The role of the soil seed bank should not be neglected, not just as a fundamental part of life cycle, but also from the adaptive potential, which has become at least punctually visible in this study.

5.3 **Primary river and secondary pond habitats**

Another goal of the thesis was to examine the growth and the genetic diversity of *Cyperus fuscus* and its soil seed bank within its **primary river habitats and secondary artificial pond and storage pond systems** with different hydrological regime. Water levels in rivers change fast and unpredictably, whereas regime in fishponds is more predictable with regular dried-out periods in summer. Fish storage ponds are without water most of the time and in

addition to the relatively dry conditions in the artificial concrete basins, vegetation is controlled by mowing, grazing and increasingly with help of herbicides. Preliminary observations point to a higher degree of **phenotypic variation/plasticity** in populations of ephemeral freshwaters than of permanently watered ponds.

(3.1) Do rivers as primary habitats show higher biodiversity than secondary ponds?

Secondary habitats harboured slightly more species than the primary river habitats. Along rivers, I found on average 17 species per 1 m^2 relevés, whereas 20 species could be found on average in the pond types.

In a study by ŠUMBEROVÁ ET AL. focusing on the differences between the vegetation of the two pond types, comparable species numbers have been found. In fish storage ponds, 21 species where found, whereas in fishponds in the same area, just 17 species were detected. The differences have been explained with a higher number of ruderals and non-native species in fish storage ponds, because they are mainly located within or near settlements with gardens, which was also detectable by a higher Sørensen dissimilarity index. The Ellenberg values displayed drier and more continental conditions in fish storage ponds, whereas at fishponds, nutrient values were higher (ŠUMBEROVÁ ET AL. 2006). In another study of 180 French fishponds along a fish-farming intensification gradient, no connection between intensity and macrophyte biodiversity was found. Even intensively used ponds showed high richness of protected plant species if shallow littoral areas were present and ponds were periodically drained (BROYER AND CURTET 2012). In the same region, another study found comparable results despite the assumption that nutrient-rich water bodies are associated with low species richness. And even if some fishponds are macrophyte poor, the regional diversity in the landscape is high because of a high number of scattered ponds collectively contributing to it (WEZEL ET AL. 2014). Biodiversity of aquatic plants in fishponds on the other hand is, however, negatively affected by nutrient load in the water. Weather conditions in spring seem to regulate the competition between aquatic plants and phytoplankton (VANACKER ET AL. 2016).

Suitable primary river populations were generally hard to find and are mostly located in larger distance to settlements, but often within agricultural landscapes. Despite many adjustments in agricultural policy in Europe, intensification on the one hand and abandonment of land use on the other hand are reducing the biological diversity. This impact also extends to aquatic systems (STOATE ET AL. 2009). The impact of agriculture through nitrogen deposition is one of the main drivers in species decline of grasslands (KLEJIN ET AL. 2009), but also for aquatic ecosystems (RIIS AND SAND JENSEN 2001). European lowland rivers faced a dramatic decline in plant biodiversity due to eutrophication and physical disturbance. In Danish rivers, particularly the large group of *Potamogeton* species became extremely less diverse in the last century and the majority of the stands are now dominated by few species adapted to frequent disturbance and eutrophic conditions. The decline of biodiversity in river habitats is partly explained by the decline in species richness of now eutrophic upstream lakes. The loss of suitable habitats and the strong anthropogenic impacts have driven several European aquatic species close to extinction (RIIS AND SAND-JENSEN 2001). But even though vegetation richness is comparably poor in small rivers within intensive agricultural landscapes, they are still a considerable contribution to biodiversity in these areas (BOUTIN ET AL. 2002).

A direct comparison of the mudflat species richness at fishponds and along rivers is to my knowledge not available, but secondary habitats seem to be particularly important for the species of this rare and declining vegetation type. In addition to these artificial fishponds (e.g. ŠUMBEROVÁ ET AL. 2006, 2012), also the historic water system of a mining region (JOHN ET AL. 2010), water reservoirs (RICHERT ET AL. 2016), or temporary ponds in wet fields (ALTENFELDER ET AL. 2014) can be suitable for the growth of the Isoëto-Nanojuncetea species. Muddy river deposits are also the primary habitats of the rare *Coleanthus subtilis.* But almost all are lost due regulations and natural populations can be found just in Siberian streams (RICHERT ET AL. 2016).

(3.2) Are there any differences in growth traits and the zonation of the soil seed bank between the three habitat types detectable in the field?

Plants of the three habitat types differed significantly in all investigated traits except for plant height, which was just marginal significant. Plants from fishponds showed the best performance in growth traits, but did not differ from river plants significantly. Plants growing in artificial fish storage ponds showed significantly worse growth traits than plants from both other habitat types (Fig. 4-4).

The low shoot-root ratio of plants from fish storage ponds is interpreted as an adaption to the growing conditions in the basins, which are without water most time of the year and usually rather dry in comparison to fishponds or near-natural sites (ŠUMBEROVÁ ET AL. 2006, 2012). In addition, some of them are treated with herbicides, mown or grazed by animals. A shift of biomass to the roots is one of the most important adaptations of plants to water stress (POORTER AND NAGEL 2000). The small size and low weight of plants from fish storage ponds must also be an adaptation to the growing conditions. VON LAMPE (1996) demonstrated that plants that face unfavourable growing conditions at the beginning of their life follow a pessimistic strategy resulting in a dwarf phenotype, in which plants reach fertility in a state that corresponds to the juvenile state. HEJNÝ (1960) also described a dwarf growth form of *C. fuscus* mainly explained by dry and compressed soil. Typical mudflat species like *Chenopodium rubrum* and *Bidens cernua* also exhibit massive differences in height and biomass, depending on whether they grow under dry or wet conditions (SALISBURY 1970). Similarly, *Coleanthus subtilis* produces less, but fast ripening seeds when growing under suboptimal conditions (BERNHARDT 2005). Flooding and management in fish storage ponds are based on ad hoc decisions of fish farmers and can be very unpredictable.

The plants from fishponds and near-natural habitats not facing these unfavourable conditions showed a better overall performance. Plants from fishponds showed the highest shoot biomass in the field. This could not be reproduced in the experiment (see question 3.4), which suggests that fishponds offer better growing conditions for *C. fuscus*. The high shoot-root ratio of plants from near-natural habitats suggests that water availability is not the limiting factor in river habitats. Rather, the better nutrient supply (DYKYJOVÁ AND KVĚT 1987; MCCONNAUGHAY AND COLEMAN 1999) and the regular and managed change of water level seem to lead to vigour of plants in fishponds (DYKYJOVÁ AND KVĚT 1987; ŠUMBEROVÁ ET AL. 2012). The nutrient load in exposed mud and especially fishpond water is extremely high (SALISBURY 1970; POTUŽÁK ET AL. 2016). Even if nutrient concentrations in fishponds were declining at the end of the last century, they are still very high compared to rivers (HROUDOVÁ AND ZÁKRAVSKÝ 1999; ŠUMBEROVÁ ET AL. 2006). On the contrary, management of fish storage ponds is aimed at maintenance of low-nutrient conditions and elimination of muddy sediments with high water capacity (ŠUMBEROVÁ ET AL. 2006). This is likely to reduce the performance of *C. fuscus*. A higher amount of nutrients also leads to higher above-ground biomass of *Typha angustifolia* (STEINBACHOVÁ-VOJTÍŠKOVÁ 2006) and 14 other wetland species (GÜSEWELL ET AL. 2003).

Over 80% of the seeds of soil seed banks of lakeshores, temporary ponds, and freshwater tidal wetlands can usually be found within the upper 5 cm of soil (NICHOLSON AND KEDDY 1983; LECK 1989). In *C. fuscus*, most of the seeds (67.5% on average) were found in the upper 5 cm of soil; only 32.5% were buried deeper. Seeds can fall into cracks in the soil, rain water can wash them into microsites or they can be buried by flooding or faunal activity (BASKIN AND BASKIN 2014). Especially in fish-farming, the activity of stocked carp as heavy bioturbators, however, can also be used to explain this finding in the fishponds and fish storage ponds (RITVO ET AL. 2004). In the fish storage ponds, fish stock is very high and the fish is usually not fed until it is sold, which leads to even higher perturbation. Indeed, the proportion of *C. fuscus* seedlings emerging from the deeper soil fraction is higher in fish storage ponds (37.9%) than in fishponds (20.2%). The highest proportion, however, is in near-natural sites (39.4%), where beetles (BERNHARDT 1995), waterfowl (SALISBURY 1970;

VAN LEEUWEN ET AL. 2012), and wild pigs (NEUGEBAUER 2003) can also act as bioturbators beside fish and current of flowing water (BOEDELTJE ET AL. 2015).

(3.3) What is the response of *C. fuscus* to temporary flooding? Do plants from the three habitat types persistently vary in their response to flooding?

Response to flooding

Cyperus fuscus follows a low-oxygen escape strategy to avoid the negative effects of partial submergence by growing taller when submerged (VOESENEK ET AL. 2004; BAILEY-SERRES AND VOESENEK 2008). The severer the flooding, the taller the plants grew. Leaves responded in a similar way, but leaf length did not further increase under severe flooding in comparison to moderate flooding, as it was the case for the culms, possibly due to reduced carbohydrate reserves to invest in longer leaves.

The energy investment for growing taller was potentially compensated by the production of fewer culms (evident when comparing the moderate and severe flooding treatments), as also described for the perennial wetland plant *Carex secta* (SORRELL ET AL. 2012). The production of leaves and leaf width were reduced under flooding as well. Phenotypic plasticity can be costly if it requires investment into the organ responding plastically to its environment (HUBER ET AL. 2012). The results suggest that the submerged plants allocated resources to increase the height of their culms, at the expense of producing more culms and, most notably, leaves. This reallocation makes sense in the light of energy management and reproductive success. I hypothesize that the green culms are similarly well capable of photoautotrophic nutrition as leaves, facilitating the reallocation of resources from leaves to culms. Further, the terminal flowers producing seeds project beyond the water surface with this strategy.

The non-flooded, but daily watered plants performed better than the flooded plants in most traits related to fitness at the second monitoring, supposedly because the flooded plants needed resources to respond to submergence. At the harvest, however, this was no longer the case. The non-flooded plants even had the lowest biomass. I suspect that the hot summer days in the last phase of the experiment have led to superficial drying out of the soil in the treatment without flooding in the afternoon. This indicates that even a minor drought stress is more critical for the growth of *C. fuscus* than moderate flooding, which can be expected for wetland plants (e.g., KIRKMAN AND SHARITZ 1993).

In the genus *Cyperus*, only the perennial *C. rotundus*, a major weed of crops and vegetables, has been investigated for its response to flooding so far (FUENTES ET AL. 2010). Along with larger air spaces in the culms and larger tubers with higher carbohydrate content, a steady activity of alcohol dehydrogenase in the roots as a measure of sustained anaerobic respiration under hypoxia characterizes the flood-tolerant ecotype of *C. rotundus*. Such additional possible responses described for various perennial wetland plants (e.g., rice; VOESENEK AND BAILEY-SERRES 2015) were not in the focus of this study. Nevertheless, my results confirm that *C. fuscus* as an annual wetland plant is able to modify its morphology to cope with flooding like perennials, at least in nutrient-rich environments such as in this experiment (see also VOESENEK ET AL. 2004; SONG ET AL. 2015).

The experimental data did not confirm the initial hypothesis that plasticity to flooding should be disfavored in fish storage pond populations. Plants from fish storage ponds did follow the low-oxygen escape strategy typical for ephemeral mudflat species in their natural habitat (VOESENEK ET AL. 2004). Similarly, flooding-induced plasticity did not vary among habitat types in *Rumex palustris* (CHEN ET AL. 2009) and *Solanum dulcamara* (ZHANG ET AL. 2016). The management (including the flooding) even within a single fish storage pond complex is very variable and, at times, less predictable than in river and fishpond habitats. Some of the fish storage ponds may be flooded suddenly for several days and then again exposed. It remains to be tested whether the response to flooding is associated with higher

costs in resource consumption in fish storage ponds than in the natural habitat (VAN KLEUNEN ET AL. 2000; PIGLIUCCI 2001).

(3.4) Do the differences in growth under field conditions persist under standardised conditions in the flooding experiment? Does the species show local adaptation to habitat conditions?

Persistence of traits in experiment

Significant differences in growth between the habitat types were found not just in the field, but also—at least for culm-related traits—under standardised growing conditions. This suggests that some differences are caused by the growing conditions at the localities (e.g., height) and some are genetically fixed, i.e. local adaptations (e.g., number of culms with inflorescences; ERFMEIER ET AL. 2011; RICE ET AL. 2013; ANDERSON ET AL. 2014).

The different number of sites included might explain why the differences between the habitat types are not as clear-cut in the experiment (with only five sites per habitat type) as in the field (10-12 sites per habitat type). Especially in multivariate designs a high number of replicas are necessary to filter between noise and differentiating factors (LEYER AND WESCHE 2007). Transgenerational effects (TEs), where the phenotype of an individual is affected by the environment of its parents and grandparents could underlie some of the results, but I controlled for parental effects via one generation of selfing (LATZEL 2015). TEs should therefore be negligible compared to local adaptation to growing conditions in the different habitat types as underlying mechanism of the observed differences between them in the experiment.

The significant habitat × treatment effect found for some traits related to fitness in the experiment (vitality, number of leaves) could be the result of a reduced growth rate under stressful environmental conditions, which is a pre-requisite for local adaptation (PIGLIUCCI 2001; KAWECKI AND EBERT 2004). Plants from rivers were superior in both flooding treatments, whereas plants from fishponds were superior in the treatment without flooding at the third monitoring (i.e. a treatment that might have implied a minor drought stress). This supports the hypothesis that the populations have adapted to their local conditions, i.e. water-logged or flooded soil in river habitats and superficially dry soil in fishpond habitats. Plants from fish storage ponds also performed best in the treatment without flooding (e.g., with respect to vitality), though on a lower overall level, suggesting that they can cope well with a minor drought stress too. This may imply a higher cost of flooding-induced shoot elongation for plants from anthropogenic habitats than from river habitats or—vice versa—a higher cost of drought-induced plastic responses (e.g., intensified root growth) for plants from river habitats than from anthropogenic habitats. Alternatively—if one interprets the traits showing a habitat × treatment effect not just as indicators of fitness, but also as potentially adaptive traits—the habitat × treatment effect may also suggest that divergent selection on flooding induced plasticity has taken place in the different habitats. To definitively assess local adaptation, however, reciprocal transplant experiments as a complement to my experiment are essential (PIGLIUCCI 2001; KAWECKI AND EBERT 2004; LEIMU AND FISCHER 2008).

Adaptions to habitats

Rivers

The experimental data confirmed the expectation that plants from rivers should be fitter than plants from anthropogenic habitats in the moderate and severe flooding treatments simulating conditions at rivers, where water levels can change fast and unpredictably (COLMER AND VOESENEK 2009).

Fishponds

The experiment further confirmed the assumption above that the main factor explaining the very good performance of *C. fuscus* at fishponds in the field seems to be the high nutrient availability in this habitat type.

Fish storage ponds

The overall bad performance of plants from fish storage ponds could rest on the fact that I might have neglected some key environmental factors (such as management measures) in fish storage ponds in the experiment, which was primarily designed to test for the adaptation to flooding of plants at rivers (KAWECKI AND EBERT 2004). VON LAMPE (1996) demonstrated that plants facing unfavorable growing conditions at the beginning of their life adopt a "pessimistic strategy", i.e. a plastic response resulting in an early flowering dwarf phenotype. The results show that plants from fish storage ponds display a "dwarf" phenotype not just in the field, but also under experimental conditions, suggesting that dwarfism has become an adaptive strategy. Plants from fish storage ponds also started to flower earlier than the other plants in the experiment. By staying small and accelerating their life cycle, plants in fish storage ponds may be able to reproduce before drying out or being killed by management measures such as grazing, mowing, or application of herbicides (ŠUMBEROVÁ ET AL. 2006, 2012). They thus seem to be optimally adapted to poor-quality habitats. The shift of biomass to the roots observed in plants from fish storage ponds also indicates that water and/or nutrients are limiting at the site (POORTER AND NAGEL 2000). Along rivers, where the low water level usually continues until late autumn, plants may have more time to reproduce, but in fish storage ponds, an even faster seed production seems to be in favour. A similar strategy has been observed in an annual sunflower species (MOYERS AND RIESEBERG 2016), where divergent selection in coastal barrier islands is thought to have led to a distinct short and early flowering life history syndrome.

The findings suggest that fish storage ponds provide consistently different selection pressures when compared to rivers. Fish farming can thus serve as an excellent model system to study how specific selection pressures have led to phenotypic differentiation. Adaptation to anthropogenic environments has also been suggested for *C. rotundus*, where the recently evolved lowland ecotype is thought to have acquired numerous adaptive traits under the selection pressure of repeated management practices in flooded rice fields in the Philippines (FUENTES ET AL. 2010).

(3.5) Do populations from river and anthropogenic habitats differ in their level of variation within and among populations due to altered levels of gene flow? Are they genetically differentiated due to differential selection pressures?

Near-natural river habitats showed—with the exception of the F_{ST}-value—significantly higher values for genetic diversity than the two anthropogenic pond types which did not differ from each other. The variation within and among the individual river sites is also larger (Fig. 4-8; A, B). In the neighbour-joining network, some ponds cluster together, but no clear differentiation between habitat types is detectable (Fig. 4-11).

For declining species in fragmented landscapes, numerous studies on genetic diversity and gene flow are available (e.g. GALEUCHET ET AL. 2005). That is an expedient approach, due to the fact that habitat loss is the main driver for endangered species decline (VAN VUUREN ET AL. 2006). Freshwater wetland habitats generally (DUDGEON ET AL. 2006) and particularly riverine floodplains (TOCKNER & STANFORD 2002) face a dramatic decline. In widely anthropogenically influenced and fragmented Central Europe, the role of man-made wetland habitats possibly harbouring rare and endangered species should not be neglected (KVĚT ET AL. 2002; DEIL 2005; VEGVARI ET AL. 2015; RICHERT ET AL. 2016). In Europe, almost all current habitats of the highly endangered *Coleanthus subtilis* are anthropogenic, whereas trough regulation nearly all primary habitats in rivers are lost and natural populations can now be found along Siberian streams (RICHERT ET AL. 2016). Especially old artificial pond systems in

the Czech Republic enable the existence of biotopes and communities of more or less natural character and whole regions are internationally regarded by the UNESCO project *Man and Biosphere* (KVĚT ET AL. 2002). Growth of *Cyperus fuscus* at fishponds in the field was not different from river habitats, species richness is even higher and fish storage ponds showed the worst performance in growth in the field and under standardised environment. Most importantly, I found evidence for phenotypic differentiation regarding tolerance of flooding events that occur regularly in primary river habitats suggesting that adaptive evolution is taking place. Understanding the threats on population viability requires considering not only population sizes, but also the genetic diversity of populations (OLIVIERI ET AL 2016).

From the genetic diversity point of view, secondary habitats must now be regarded as relatively impoverished. Studies on differences in genetic diversity between primary and anthropogenic secondary habitats seem to be rare anyway and should be encouraged. A significant loss of diversity in secondary habitats without a clear differentiation is to my knowledge not published yet. REISCH (2007) found slightly, but not significantly higher diversity in natural populations, but a clear differentiation on a small scale in the selfing annual *Saxifraga tridactylites* that expends its range along railroads and interprets the on-going differentiation as an evolutionary process. In a study of *Orchis militaris* with special regard to the age differences of populations in near-natural and secondary habitats, no genetic divergence could be found, which suggests that genetic variation was not lost during the colonisation event (ILVES ET AL. 2015). In a study of *Gentiana pannonica*, on the other hand, authors could show a low within and high among population variation in secondary habitats as a result of bottlenecks through historical processes (HOFHANZLOVÁ & FÉR 2009).

In African rainforest, an on-going cryptic speciation through ecological divergence of two molecular forms of the most important afro-tropical malaria mosquito between rural and anthropogenic habitats could be proved (KAMDEM ET AL. 2012). Either way, genetic differentiation is mainly a result of reduced gene flow of pollen or seeds (GALEUCHET ET AL. 2005). The amount and the spatial distribution of genetic variation belong to the key elements to determinate viability and survival of endangered plant species (ILVES ET AL. 2015). Rivers and secondary habitats show fundamental differences in regional dynamics and dispersal. In rivers, populations are connected through running water, seeds and even plants can be transported and new habitats can be generated (HEJNÝ 1960; HONNAY ET AL. 2009; NILSSON ET AL. 2010).

Dispersal of seeds with flowing water is the most straight-forward explanation for these results (BURKART 2001; NILSSON ET AL. 2010) and provides a basis for explaining species distributions (JOHANSSON ET AL. 2004). Spatial aggregation of genetically related individuals may be reduced by hydrochory and genetic diversity should be increased in populations receiving many diaspores (NILSSON ET AL. 2010). In contrast to other dispersal vectors, transport by water leads to suitable wet sites by higher chance, but on the other hand, dispersal is limited to areas connected to seed source by surface water and often unidirectional. Zoochory with birds shows intermediate dispersal success between completely random transport with wind and limited dispersal with flowing water (SOONS ET AL. 2008).

Pond systems are also connected to each other through a system of channels (KVĚT ET AL. 2002; ŠUMBEROVÁ ET AL. 2006, 2012), but water is mainly standing and water flow is regulated by fish farmers. Dispersal of seeds between pond systems with mud on cars, boots and tools of the fish farmers could also be proven (ŠUMBEROVÁ ET AL. 2012). Dispersal between secondary and river habitats should therefore be less frequent and vanished genetic diversity could be caused by bottlenecks followed (e.g. HENSEN ET AL. 2010).

Cyperus fuscus prefers mineral-rich calcareous soils especially in northern parts of its distribution range (HEJNÝ 1960). Areas in Southern Bohemia, where most of the studied fishpond systems in the Czech Republic are located, are formed by acidic, mineral-poor bedrock and the species was reported as rare until the 1950s, occurring mainly in eutrophic water bodies in settlements (HEJNÝ 1960; ŠUMBEROVÁ AND HRIVNÁK 2013). As a

consequence of overall eutrophication and soil chemistry changes associated with fish farming intensification, the number of records considerably increased since then (ŠUMBEROVÁ 2003; ŠUMBEROVÁ AND HRIVNÁK 2013). While some populations in river alluvia got lost, *C. fuscus* is classified as vulnerable (GRULICH 2012), but survived in secondary habitats with a changed genetic architecture. A revision of 20 herbaria in Austria, Slovakia and the Czech Republic has been performed in a side project (CZ 13/2012 funded by the Austrian Agency for International Cooperation in Education and Research and performed in collaboration with various Czech colleagues). The main goal was to to get a good information base to assess the historical demographic development of *Cyperus fuscus*. The revision has also delivered information on the decrease of suitable natural habitats for *Cyperus fuscus* (river banks) and supports my findings. It also provides information on the importance of secondary habitats (e.g. fishponds) for the actual distribution of *Cyperus fuscus*. The theory of man-made expansion is additionally supported by a noteworthy clustering of also spatially close ponds in the Bohemian Massif cultivated by the same company (Fig. 4-10 and 4-11), which can also be the result of enhanced gene flow of seeds by fish farmers within their managed pond system (ŠUMBEROVÁ ET AL. 2012). Fishponds are the most probable source of diaspores for fish storage ponds before the establishment of soil seed banks at the bottoms of the fish storage ponds. In this context, epi- and endoichthyochory of diaspores with fish into fish storage ponds is discussed (ŠUMBEROVÁ ET AL. 2006).

Intraspecific phenotypic variation is controlled by natural genetic and epigenetic variation. Especially DNA methylations, as one of three elucidated epigenetic mechanisms (BOSSDORF ET AL. 2008), have been extensively studied in the last two decades. These patterns change in response to environmental stimuli (LANG 2016) and are heritable so that we might need to expand our concept of variation and evolution in natural populations (BOSSDORF ET AL. 2008).

5.4 **European approach**

The questions above have been studied in Central Europe, but *C. fuscus*, as many other mudflat species, occurs sporadically all over Europe—for short periods of time and at different points in time—meaning that populations are isolated spatially and temporally from each other.

(4.1) The question therefore was whether populations across a larger geographic area are genetically differentiated due to the action of drift and/or selection and lack of gene flow among sites or whether populations of distant sites are genetically similar due to high levels of gene flow?

To the best of my knowledge, this is the first study of genetic variation of a typical mudflat species. I found comparable values of genetic variation within populations of *Cyperus fuscus* (mean expected heterozygosity in above-ground populations across Europe = 0.27 [range = 0.00–0.58]; Table 4-5) as reported for other annual (to perennial), self-compatible plants such as *Arabidopsis thaliana* (average genetic diverstiy index equivalent to expected heterozygosity for diploid species = 0.43 [range = 0.00–0.80]; LORIDON ET AL. 1998) and *Medicago lupulina* (mean expected heterozygosity = 0.25 [range = 0.06–0.40]; Yan *et al.* 2009). The fixation index F_{IS} of *C. fuscus* (mean = 0.70 [range = -0.11–1.00]; Table 4-5) is slightly lower than the one reported for *M. lupulina* (mean = 0.92 [range = 0.74–1.00]; YAN ET AL. 2009), but still suggests a high selfing rate. The usually large sizes of the sampled populations make the mating of close relatives less likely than a high selfing rate with occasional outcrossing in *C. fuscus*.

Inbreeding species generally exhibit higher differentiation among populations, whereas outcrossing species usally show high within-population variation counteracted by gene flow (GALEUCHET ET AL. 2005; CLAUSS AND MITCHELL-OLDS 2006; HENSEN ET AL. 2010). *Arabidopsis thaliana* shows the same pattern of a bush-like network with populations on relatively long branches and the lack of phylogeographic structure is explained by a rapid

expansion of the species with strong involvement of human-induced migrations (KOORNNEEF ET AL. 2004). Patterns of genetic homogeneity seem to be the exception, but new technologies may reveal the structure of more non-model organisms. Comparable results are known e.g. for the North American *Achillea millefolium* complex (RAMSEY ET AL. 2008), the annual and range expanding *Ceratocapnos claviculata* (VOSS ET AL. 2012), and the annual, predominantly selfing *Brachypodium stacei* (SHIPOSHA ET AL. 2016). *Jatropha curcas*, an important oil plant, even shows no differences in genetic diversity despite different phenotypes between continents, which may be based on epigenetic variation (YI ET AL. 2010).

(4.2) Hydrochory with flowing water is the most forward explanation for dispersal of this species, but is theoretically unidirectional and just within river systems. Are there also indications for different dispersal vectors?

Dispersal of diaspores is crucial for gene flow and colonisation (SOONS ET AL. 2008) and dispersal capacity in ephemeral wetland vegetation is high (DEIL 2005). For a selfing species, a high degree of differentiation would be expected, but efficient seed dispersal can counteract population turnover (CHAUVET 2004; FALAHATI-ANBARAN ET AL. 2014). Beside hydrochory as a dispersal vector (NILSSON ET AL. 2010), waterfowl species are regular and efficient seed dispersers especially for plant species of dynamic and ephemeral wetland habitats (SOONS ET AL. 2008). Long distance dispersal with migratory birds is recently also increasingly discussed (VIANA ET AL. 2016). In a ten year-study of South Bohemian fishponds, 70 different water and wetland birds have been documented (MUSIL AND FUCHS 1994) and fishponds are even more attractive for breeding than the also important sand pits (KAMENÍKOVÁ AND RAJCHARD 2013). For mallards (*Anas platyrhynchos*), a common and highly omnivorous duck species that is also frequent at rivers and fishponds (BÍLÝ ET AL. 2008), research on dispersal of wetland plants is available. Ducks transport seeds of *Carex bohemica* exozoochorously on feathers and crusts of mud (HOHENSEE AND FREY 2001) and viable seeds of 19 of 23 tested common wetland species endozoochorously, potentially in reasonable amounts over large scales. The smallest seeds showed the highest potential to be dispersed, whereas the coat thickness played a minor role (SOONS ET AL. 2008). *Cyperus fuscus* produces an enormous amount of small nutlets (BRYSON AND CARTER 2010) so that ducks should be the ideal dispersers for this species. Fish farmers are documented dispersers because seeds are transported between ponds in mud with boots, tools and cars (ŠUMBEROVÁ ET AL. 2012). Transport of seeds by fish are also documented even over long distances (ŠUMBEROVÁ ET AL. 2006). Long distance seed dispersal is of critical importance for plant distribution and better data has to be collected from the tails of the seed dispersal curves. Genetic methods provide a broadly applicable way to monitor these comparably rare events (CAIN ET AL. 2000). Migrant analysis (Fig. 4-13) shows that most of the seeds are dispersed in close vicinity or derived from the soil seed bank, but some of the receiving populations are in great distance and not connected through water ways. As hydrochory with floating water between the rivers Odra (RV 9 and 10), Elbe (RV 1 and 6) and Danube is physically impossible, dispersal must have been with other vectors. Even though these migrants constitute less than 1% of the dataset, it shows the high importance of long-distant dispersal.

(4.3) Are there indications whether *C. fuscus* survived the Ice Age north of the Alps or in Mediterranean ice free refugia?

The star-like neighbour-joining network of *Cyperus fuscus* over a large scale shows no clear association between geographical origin and genetic distances (Fig. 4-10), but I found some indication for higher genetic diversity in populations from the Mediterranean region and from southeastern Europe (Fig.4-9). For *A. thaliana*, which shows the same bush-like network pattern (KOORNNEEF ET AL. 2004), re-colonisation of Central Europe from Asia and

Pleistocene refugia south of Alps are discussed (SHARBEL ET AL. 2000). In a review of studies comparing genetic diversity in central and peripheral populations, 64% detected a decline of diversity at range limits (ECKERT ET AL. 2008). Further, epigenetic variation is influencing the phenotypic diversity of *A. thaliana* (LANG ET AL. 2016). European southern glacial refugia could be postulated in the Iberian Peninsula, Italy and the Balkans (COMES AND KADEREIT 1998) for a range of plant species (NORMAND ET AL. 2011; SANTISO ET AL. 2016). For the annual *C. fuscus*, a survival of the Ice Age outside of these supposed refugia seems very unlikely depending on regular flooding of the habitat. This study focused on the three different habitat types with dense and intensively sampled localities. To verify the hypothesis that *C. fuscus* survived the Ice Age south of the Alps in Mediterranean refugia, considerably more populations and individuals need to be sampled, especially in the assumed refugia and at the edges of the species distribution.

(4.4) Is the metapopulation model applicable for *C. fuscus* populations, which are dispersed not just in space, but also in time?

While especially in animal ecology, the connection of populations to metapopulations through the movement of individuals is discussed intensively (e.g. LEVINS 1969; OKUBO AND LEVIN; 1980, JOHNSON ET AL. 1992; HANSKI AND SIMBERLOFF 1997), there are to my knowledge not many true accepted plant metapopulations. The presence of viable seeds in the soil seed bank or as dispersal propagules, the immobility of individuals, and the spatial distribution of plant populations make them more complicated to apply the metapopulation model. But if special features of plants like dormancy, restricted dispersal and local adaptation are incorporated, new insights on plant metapopulations can be gained (HUSBAND AND BARRETT 1996). For *Eichhornia paniculate*, a wetland annual, a plant metapopulation was documented for the first time in a 7 year-period (BARRETT AND HUSBAND 1998). For the also annual *Erysimum cheiranthoides* growing on stony river banks, the metapopulation dynamics was examined (HONNAY ET AL. 2009). In both studies, the authors could neglect the role of the seed bank. Even though the geographical structure of populations varies along a continuum of patchiness in space and time in *Cyperus fuscus* (BARRETT AND HUSBAND 1998), it cannot be seen as a “true” metapopulation because of the fundamental role of the soil seed bank. Further, this study just contains a snap-shot of one year, so recurrent extinctions and re-colonisations were not examined (HONNAY ET AL. 2009). Most likely, populations growing along near-natural rivers show metapopulation dynamics, because the activity of water at near-natural rivers leads to regular destruction and creation of new habitat patches and dispersal is frequent (FRECKLETON AND WATKINSON 2002; HONNAY ET AL. 2009). In a review on existing studies on plant metapopulations, of which only 5 of 28 claim for this status, the actually measured important features are colonisation, extinction, re-colonisation, and patch density. Further, the authors criticise that the regional distribution and dynamics of species, which is hard to measure, is not taken into account adequately. According to the metapopulation model, conservation efforts for threatened species need to be directed towards regionally available suitable habitats. In contrast, when metapopulation processes are weak, conservation should be directed towards the population level (FRECKELTON AND WATKINSON 2002). These considerations are relatively new, since conservation biology largely ignored evolutionary reflections. Moreover, genetic studies in the 90ies have been rare and included at best inbreeding depression with neutral markers. Metapopulations have been first acknowledged from a demographic point of view, but soon mainly theoretical models, but also experimental studies focussing on dispersal and other life history traits followed. In this context, evaluation of vulnerability of rare plant species has to be assessed differently (KUUSSAARI ET AL. 2009; OLIVIERI ET AL. 2015). Recently, molecular methods allowed to infer the long- and short-term history of gene flow among populations and colonisation-extinction dynamics of subdivided plant populations on different scales, fuelling new insights in highly dynamic plant metapopulations (LOWE AND ALLENDORF 2010; PANNELL AND FIELDS 2013).

6 Conclusion

In such dense populated areas as Central Europe, unaffected primary habitats are nearly extinct. Especially near-natural rivers are declining and the majority of them are regulated, which led to a dramatic loss in natural semiaquatic habitats. The role of secondary anthropogenic habitats is therefore of the utmost importance for environmental conservation and the Czech historical fishpond system provides a landscape with a rich mosaic of functional wetland habitats with relatively natural features for a wide range of species. The species rich habitats along the dried shores of fishponds seemed to provide excellent habitats for Cyperus fuscus as a typical representative of this highly specialised vegetation type and plants even performed best in the field. Especially in times of declining biodiversity, the fishponds are certainly worthy of protection, but my results also suggest that the plants growing in these substitute habitats may have lost some adaptations to their original habitat and gained divergent adaptations in return. These pond systems provide an excellent model to examine this on-going evolution and also play an important role in socio-economic aspects for the region.

Additionally, secondary habitats have to be seen as impoverished from the genetic point of view. Even highly degraded rivers are genetically more diverse than anthropogenic habitats. This is an excellent example for the complexity of such processes and the difficulty to assess consequences for the conservation of biodiversity. The conservation of rare habitats seems to be more important than the conservation of populations in this context. On the other hand, conservation biology shouldn't consist on conserving habitats and species alone, but should also promote the evolutionary potential at all levels of biodiversity.

The relatively low genetic differentiation on a large spatial scale can be explained by the high dispersal capacity of the species, but it was not easy to find comparable examples in the literature. Even though molecular research is not a young discipline anymore, it already outgrew its "children shoes". But the next-generation-sequencing finally enabled me to intensively research an ecologically relevant species. I expect a huge amount of studies in the next years and probably we have to re-think our understanding of genetic diversity, adaptation and functioning of the genome (again). To my best knowledge, this is the first study on genetic variation of a typical mudflat species.

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10 Appendix

Appendix 1. Voucher information for *Cyperus fuscus* populations used in this study. All vouchers are deposited at the Institute of Botany, University of Natural Resources and Life Sciences, Vienna (WHB). Individuals were grown from seeds in the greenhouse.

Voucher no. (WHB)	Collection locality	Geographic coordinates	No. of individuals
Used for first NGS run at LGC Genomics (Berlin, Germany)			
62957	Czech Republic, Záryby	50°13.424' N, 14°37.717' E	1
Used for second NGS run at ecogenics (Balgach, Switzerland)			
62959	Czech Republic, Semovice	49°45.067' N, 14°39.655' E	1
62987	Czech Republic, Tchořovice	49°26.115' N, 13°48.442' E	1
Test individuals for screening of primer pairs			
62963	Czech Republic, Mšec	50°11.815' N, 13°54.651' E	1
62960	Czech Republic, Hluboká nad Vltavou	49°02.624' N, 14°25.952' E	1
62996	Czech Republic, Smrkovec	49°26.078' N, 13°54.699' E	1
62982	Czech Republic, Břeclav	48°42.710' N, 16°54.169' E	1
62979	Czech Republic, Velké Němčice	48°59.056' N, 16°39.894' E	1
62973	Poland, Borków	51°40.477' N, 16°12.239' E	1
62955	Poland, Cigacice	48°18.739' N, 16°54.224' E	1

Appendix 2. Characteristics of 39 additional SSR loci with flanking regions useful for primer design in *Cyperus fuscus*. GTTT-PIGtails (Brownstein et al., 1996), CAG- and M13R-tails (CAG: 5′-CAGTCGGGCGTCATCA-3′; M13R: 5′-GGAAACAGCTATGACCAT-3′; only in Cf_007, Cf_020 and Cf_112) and M13-tails (5′-TGTAAAACGACGGCCAGT-3′) added to the 5′ ends of primers are underlined. The allele range is based on seven test individuals (Appendix 1).

Locus	Primer sequence (5′ → 3′)	Repeat motif	*A*	Allele range	EMBL accession no.
First NGS run					
Cf_007	F: CAGTCGGGCGTCATCAGAAGTGTATATTGAGATTAGGAGCC	$(AT)_{11}$	3	274-286	LN848929
	R: GTTTGGCTAGATCCAAATGGCGG				
Cf_020	F: GGAAACAGCTATGACCATCTGCTGCCACCATTTCGAG	$(GGT)_5$ + $(GGT)_5$	1	273	LN848933
	R: GTTTAGGCTCAACCCTATGCACC				
Cf_112	F: GTTTGTGGTGTGGCAGGAAGGG	$(AATG)_7$	1	203	LN848935
	R: CAGTCGGGCGTCATCAGTCAGCTGTCAATCTGCACC				
Second NGS run					
Cypfus_0023	F: TGTAAAACGACGGCCAGTCTGCCTTCGATGAACTCCTG	$(AGA)_7$	2	180-183	LN848936
	R: TCTTGTTCGGCGTCTAACCC				
Cypfus_0563	F: TGTAAAACGACGGCCAGTGAGAAGCGGGCATTCATCAG	$(TC)_{12}$	3	139-143	LN848939
	R: TATCCTCAGCTCCGTGTGTG				

Cypfus_0568	F: TGTAAAACGACGGCCAGTCTGAGTCCCATGTCTCCTCC	$(CT)_{13}$	3	153-175	LN848940
	R: TGGTAATGCTCCATGCAAAGAC				
Cypfus_0604	F: TGTAAAACGACGGCCAGTCACAGCTAGTGCAGTCAACG	$(GA)_{18}$	4	160-170	LN848941
	R: TGAGAAGTCGAGAGGAACGG				
Cypfus_0785	F: TGTAAAACGACGGCCAGTAGGCGAGCTAGAGAAATGGG	$(AGA)_{8}$	2	152-155	LN848943
	R: GAGGCGCCATCGATTCTTTC				
Cypfus_1174	F: TGTAAAACGACGGCCAGTCCCAACTGGAGCAAAGAAGC	$(TC)_{12}$	2	226-228	LN848945
	R: GCGGAAGTAGTTCAGGCAAC				
Cypfus_1302	F: TGTAAAACGACGGCCAGTTTAACCAGGTCTCGTGGTCG	$(TACA)_{12}$	2	162-166	LN848947
	R: ACAAAAGAGGCCGGATAGGC				
Cypfus_1319	F: TGTAAAACGACGGCCAGTAGAGGTTATTTGGCCCCAGC	$(TATG)_{8}$	1	154	LN848948
	R: AGTGTTTGGCATGGGCTTTC				
Cypfus_1818	F: TGTAAAACGACGGCCAGTTCGCAGTTACGATAGGTACTC	$(CA)_{12}$	2	109-121	LN848949
	R: CATGGACGTGTCAAACAAAGC				
Cypfus_1819	F: TGTAAAACGACGGCCAGTAGTGGACAAGGTCAAGAGGG	$(GAA)_{8}$	2	207-210	LN848950
	R: CCATTGGGAGTCAAAGCCAC				
	R: GATGCGAGGTTTAAGCAGGG				

Cypfus_1966	F: TGTAAAACGACGGCCAGTATGGCATCGCAATCAACCAG	$(GAA)_8$	3	216-222	LN848951
	R: GATGCGAGGTTTAAGCAGGG				
Cypfus_2381	F: TGTAAAACGACGGCCAGTGCACGTAACTTCCTTCTAGTGG	$(TATG)_{18}$	3	191-263	LN848953
	R: TGGAAATAACTAGCTCACCACAC				
Cypfus_2517	F: TGTAAAACGACGGCCAGTTGAGCTGCAACCAATCAAGC	$(GAA)_7$	2	213-216	LN848955
	R: TGTGCTGCCAGTTTTCCAAG				
Cypfus_2640	F: TGTAAAACGACGGCCAGTATCAAAACCCATCGCACTCC	$(AGA)_7$	1	122	LN848956
	R: CGCTTATGCGCAAACAAACC				
Cypfus_2806	F: TGTAAAACGACGGCCAGTGCCTGATAAAGCATGTGACCG	$(AG)_{12}$	3	187-193	LN848958
	R: TCGAATTGACACCATGCCTC				
Cypfus_2832	F: TGTAAAACGACGGCCAGTAGCACAAGTTGGGTCTCCTC	$(GAA)_7$	1	173	LN848959
	R: TTGATCACCCCCACTAAGGC				
Cypfus_2855	F: TGTAAAACGACGGCCAGTCAGCGGAAGGGAAGATTTCG	$(CTT)_7$	3	202-215	LN848960
	R: CTCAGCCATCTCAATCACCG				
Cypfus_2888	F: TGTAAAACGACGGCCAGTTGCTCCGCTTCTATTTTGCTC	$(CTT)_{12}$	2	151-154	LN848961
	R: GACCGAAGCTGCTGATTTCC				

Cypfus_2891	F: TGTAAAACGACGGCCAGTGGACTGGTTTAGAAATGTGTGC	$(CT)_{12}$	1	255	LN848962
	R: TTTTGGCAACGTGAAAGTGC				
Cypfus_2898	F: TGTAAAACGACGGCCAGTAAAAACAGCTGAATCGGGGC	$(TTC)_9$	3	226-247	LN848963
	R: CTGCAGACCCATCTCTCTCC				
Cypfus_3033	F: TGTAAAACGACGGCCAGTTATGGCGCTGGAGGAGAAAG	$(CTT)_{10}$	2	220-226	LN848966
	R: CGTGTCGTAAGGCAGAAAATAAAATC				
Cypfus_3195	F: TGTAAAACGACGGCCAGTGGGGAGGAGAGTTCCTTGAC	$(AG)_{12}$	1	197	LN848968
	R: CTTCAGTGATCCCATGTGGC				
Cypfus_3323	F: TGTAAAACGACGGCCAGTCCTAGCACTTGCAAAGGGTG	$(AAG)_7$	1	217	LN848972
	R: CGCCCCTTTTCGTATTGTCC				
Cypfus_3372	F: TGTAAAACGACGGCCAGTCAGCTCCACGATACTCGATTG	$(GAA)_8$	2	255-258	LN848974
	R: AAGGGACTCAATATCGCCCC				
Cypfus_3416	F: TGTAAAACGACGGCCAGTTCTTCAAAACTTGCCTATGGGTC	$(GAGT)_7$	3	238-244	LN848975
	R: TGTGCAGACATTTGAGGAAGC				
Cypfus_3423	F: TGTAAAACGACGGCCAGTTCTGTCCTCCTCGCTCAATC	$(GAA)_7$	2	193-202	LN848976
	R: TCAAACCAAGTAATTTTCCAAAGAG				

Cypfus_3542	F: TGTAAAACGACGGCCAGTGCGGGACTTCCATTCCATTC	$(TCT)_{15}$	3	241-253	LN848977
	R: GGTAGACGGCGCTTTTTGAG				
Cypfus_3597	F: TGTAAAACGACGGCCAGTTGCATGTTCACTTCTGGTGC	$(GAA)_{7}$	1	181	LN848978
	R: CACCTTCTGCTGCTCAATCG				
Cypfus_3776	F: TGTAAAACGACGGCCAGTTCGGTAATATACTTTGGGTCAGC	$(CT)_{12}$	2	245-249	LN848979
	R: GAACGGGAAACAAGACGCTC				
Cypfus_3864	F: TGTAAAACGACGGCCAGTCGAAGAATTTTCCCACCCCG	$(CT)_{14}$	3	210-218	LN848980
	R: CCGTTAAACAGGTCCGAAGC				
Cypfus_3873	F: TGTAAAACGACGGCCAGTGAAAAGACAGATGCCTCCGC	$(GAA)_{8}$	3	172-211	LN848981
	R: CCGCCTCTACCAGATACTGC				
Cypfus_4041	F: TGTAAAACGACGGCCAGTAGGTGGAAGTAGGAAGCCAG	$(GAA)_{9}$	1	176	LN848983
	R: CATTTGCAGCCCCATCCTTC				
Cypfus_4074	F: TGTAAAACGACGGCCAGTTTGCAAATGGGCACAGGAAG	$(TC)_{13}$	4	184-198	LN848985
	R: CCTAATAAAGGTAGGACAGAGCG				
Cypfus_4102	F: TGTAAAACGACGGCCAGTTGGGCGTTCTCAAATCAAAGAG	$(GA)_{13}$	1	260	LN848987
	R: GGGGCCCACTGAAGAAAAAG				

Cypfus_4240	F: TGTAAAACGACGGCCAGTCTTCTTCATTTCCCGCACCC	$(TACA)_7$	2	251-255	LN848991
	R: GCCACCTGCATTCATCATCC				
Cypfus_4347	F: TGTAAAACGACGGCCAGTTCATTTCAACTCGGAATCCTCTAC	$(TGTA)_7$	2	252-256	LN848992
	R: CAACTACAACCGGCACCTTC				
Cypfus_4468	F: TGTAAAACGACGGCCAGTCGAATCTGAGAAGCGCTGTG	$(CT)_{12}$	3	259-275	LN848993
	R: ACTCATCGCTTGAGAGGCAG				
Cypfus_4479	F: TGTAAAACGACGGCCAGTTGGGTGCCAAACAAAAATTGG	$(AAG)_8$	2	158-233	LN848994
	R: AGATATCAAAAGCAACCGACCC				
Cypfus_4799	F: TGTAAAACGACGGCCAGTTATGGGCTTCCCGTCTTCTG	$(AAG)_7$	2	248-251	LN848996
	R: CTGTCATGCTCGACACCAAG				
Cypfus_4849	F: TGTAAAACGACGGCCAGTAATGAAGAGCGCACCAATCG	$(GA)_{12}$	1	158	LN848997
	R: ACAATACATTCCTCGGTTAGACAG				

Note: *A* = number of alleles sampled.

Appendix 3 Populations used for determination of genome size and counting of chromosome number.

Country	Region	District	Locality	Collection date	Collector	Latitude, Longitude	Altitude	Sample/ standard (DAPI; mean)	Sample/ standard (DAPI; SD)	No. of ind. analysed (DAPI)	Sample/ standard (PI; mean)	Sample/ standard (PI; SD)	No. of ind. analysed (PI)	1C (pg)	No. of chr. (2n)
Austria	Lower Austria	Melk	Muddy bank of river Donau/Danube, Luberegg	11.08.2003 15.08.2003	K.-G. Bernhardt	N 48°13'59" E 15°18'56"	218 m	0,198	0,007	10	--	--	--	--	--
Austria	Lower Austria	Tulln	Oxbow between rivers Donau/Danube and Traisen, NW of Zwentendorf an der Donau	08.10.2013	S. Hameister	N 48°22'40" E 15°48'18"	185 m	--	--	--	0,180	0,001	2	0,230	--
Austria	Lower Austria	Tulln	Oxbow between rivers Donau/Danube and Traisen, NW of Zwentendorf an der Donau	18.09.2012	J. Böckelmann	N 48°22'20" E 15°49'55"	187 m	--	--	--	--	--	--	--	36
Austria	Lower Austria	Tulln	Traisen canal, NW of Zwentendorf an der Donau	18.09.2012	J. Böckelmann	N 48°22'14" E 15°50'17"	182 m	0,210	0,006	12	--	--	--	--	--
Austria	Lower Austria	Tulln	Muddy lane close to Traisen canal, NW of Zwentendorf an der Donau	15.08.2013	K.-G. Bernhardt, E. Naumer-Bernhardt	N 48°22'14" E 15°48'54"	183 m	--	--	--	0,180	0,003	3	0,231	--
Austria	Lower Austria	Tulln	Farmland, NW of Zwentendorf an der Donau	15.08.2013	K.-G. Bernhardt, E. Naumer-Bernhardt	N 48°21'06" E 15°53'29"	182 m	--	--	--	0,179	0,002	3	0,230	--
Austria	Lower Austria	Tulln	Rain retention basin, SE of Moosbierbaum	01.10.2011	K.-G. Bernhardt	N 48°18'14" E 15°54'28"	178 m	0,212	0,012	8	--	--	--	--	--
Austria	Lower Austria	Gänserndorf	Branch of river March/Morava, E of Markthof	14.07.2011	K.-G. Bernhardt, E. Döltl, K. Tremetsberger, M. Wernisch	N 48°11'34" E 16°58'12"	138 m	0,216	--	1	--	--	--	--	--
Austria	Lower Austria	Gänserndorf	Branch of river March/Morava, E of Markthof	19.09.2012 05.10.2012	J. Böckelmann, P. Kúr, K. Šumberová	N 48°11'29" E 16°58'18"	137 m	0,211	0,003	6	--	--	--	--	--
Austria	Vienna	21st district (Floridsdorf)	Pool close to the Marchfeldkanal	25.10.2012	K. Tremetsberger	N 48°17'31" E 16°25'36"	159 m	0,202	0,006	8	--	--	--	--	--
Austria	Burgenland	Neusiedl am See	Oxbow of river Leitha, E of Zurndorf	25.09.2013	K.-G. Bernhardt	N 47°58'24" E 17°03'11"	126 m	--	--	--	0,183	0,001	3	0,234	--
Austria	Burgenland	Neusiedl am See	Farmland, E of Zurndorf	07.08.2013	K.-G. Bernhardt K. Tremetsberger	N 47°59'21" E 17°02'21"	132 m	0,183	0,006	6	--	--	--	--	--

Country	Region	District	Locality	Collection date	Collector	Latitude, Longitude	Altitude	Sample/ standard (DAPI; mean)	Sample/ standard (DAPI; SD)	No. of ind. analysed (DAPI)	Sample/ standard (PI; mean)	Sample/ standard (PI; SD)	No. of ind. analysed (PI)	1C (pg)	No. of chr. (2*n*)
Austria	Burgenland	Neusiedl am See	Farmland close to river Leitha, N of Nickelsdorf	07.08.2013	K.-G. Bernhardt, T. Klute, K. Lapin, L. Schober, N. Stöckl, K. Tremetsberger	N 47°57'49" E 17°04'09"	130 m	0,194	0,005	7	--	--	--	--	--
Croatia	Osijek-Baranja County	Bilje	Canal, S of Podunavlje	15.10.2011	M. & K. Wernisch	N 45°37'33" E 18°48'49"	78 m	0,202	0,009	8	--	--	--	--	--
Croatia	Istria County	Motovun	Mirna canal, N of Motovun	29.08.2013	K.-G. Bernhardt, E. Naumer-Bernhardt	N 45°20'44" E 13°49'47"	16 m	--	--	--	0,182	0,004	3	0,233	--
Czech Republic	South Bohemian Region	České Budějovice	Storage pond (no. 20), Hluboká nad Vltavou	01.10.2008	K. Šumberová	N 49°02'41" E 14°25'58"	374 m	0,204	0,005	10	--	--	--	--	--
Czech Republic	South Bohemian Region	České Budějovice	Storage pond (no. 22), Hluboká nad Vltavou	14.07.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 49°02'37" E 14°25'57"	379 m	0,219	0,004	5	0,187	0,001	3	0,239	--
Czech Republic	South Bohemian Region	České Budějovice	Storage pond (no. 30), Hluboká nad Vltavou	22.08.2009	K. Šumberová	N 49°02'40" E 14°26'00"	376 m	0,207	0,005	9	--	--	--	--	--
Czech Republic	South Bohemian Region	České Budějovice	Storage pond (no. 32), Hluboká nad Vltavou	07.08.2012	J. Böckelmann, K. Bubíková, K. Šumberová	N 49°02'41" E 14°25'59"	374 m	0,216	0,003	5	--	--	--	--	--
Czech Republic	South Bohemian Region	České Budějovice	Storage pond (no. 33), Hluboká nad Vltavou	09.09.2008	K. Šumberová	N 49°02'43" E 14°26'00"	373 m	0,208	0,006	10	--	--	--	--	--
Czech Republic	South Bohemian Region	České Budějovice	Pond Vlhlavský, Vlhlavy	14.07.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 49°03'14" E 14°17'15"	403 m	0,213	0,007	2	--	--	--	--	--
Czech Republic	South Bohemian Region	Písek	Storage pond (no. 7A), Kestřany	12.08.2008	K. Šumberová	N 49°16'19" E 14°04'22"	377 m	0,197	0,005	6	--	--	--	--	--
Czech Republic	South Bohemian Region	Písek	Pond Velký Hánovec, S of Písek city	25.08.2011	K. Šumberová	N 49°17'33" E 14°08'54"	372 m	0,193	0,006	2	--	--	--	--	--

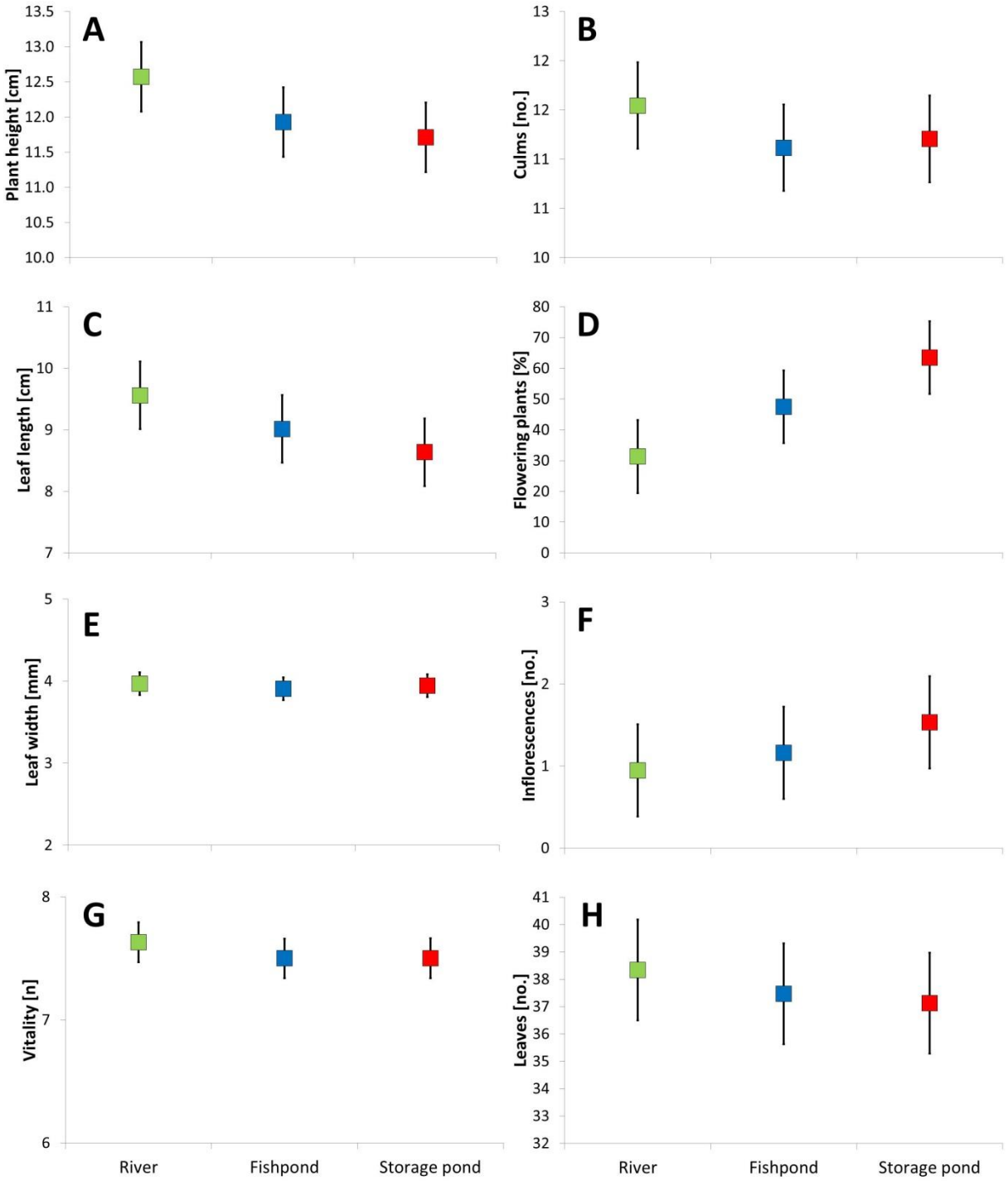
Country	Region	District	Locality	Collection date	Collector	Latitude, Longitude	Altitude	Sample/ standard (DAPI; mean)	Sample/ standard (DAPI; SD)	No. of ind. analysed (DAPI)	Sample/ standard (PI; mean)	Sample/ standard (PI; SD)	No. of ind. analysed (PI)	1C (pg)	No. of chr. (2n)
Czech Republic	South Bohemian Region	Strakonice	Pond Velkorojický, Rojice	13.07.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 49°21'00" E 13°56'32"	457 m	0,213	0,001	6	0,188	0,003	3	0,240	--
Czech Republic	South Bohemian Region	Strakonice	Storage pond, Rojice	08.08.2012	J. Böckelmann, K. Bubíková, K. Šumberová	N 49°20'51" E 13°56'54"	453 m	0,217	--	1	--	--	--	--	--
Czech Republic	South Bohemian Region	Strakonice	Pond Pýcha, S of Skaličany	24.07.2012	J. Böckelmann, K. Bubíková	N 49°26'05" E 13°54'42"	443 m	0,219	--	1	--	--	--	--	--
Czech Republic	South Bohemian Region	Strakonice	Storage pond, Tchořovice	09.08.2012	J. Böckelmann, K. Bubíková	N 49°26'07" E 13°48'27"	454 m	0,220	--	1	--	--	--	--	--
Czech Republic	Central Bohemian Region	Rakovník	Storage pond, Mšec	26.07.2012	J. Böckelmann, K. Bubíková	N 50°11'49" E 13°54'39"	408 m	0,214	0,005	4	--	--	--	--	36
Czech Republic	Central Bohemian Region	Rakovník	Pond Krtský	12.07.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 50°04'47" E 13°26'45"	500 m	--	--	--	0,188	0,001	3	0,240	--
Czech Republic	Central Bohemian Region	Benešov	Pond Horní petrovický, Petrovice	11.07.2012	J. Böckelmann, Z. Hroudová, K. Šumberová, K. Tremetsberger	N 49°43'06" E 14°39'02"	402 m	0,214	0,003	6	--	--	--	--	--
Czech Republic	Central Bohemian Region	Benešov	Pond Velký sedlečský, Sedlečko	27.07.2012	J. Böckelmann, K. Bubíková	N 49°41'37" E 14°32'05"	445 m	0,209	0,002	2	--	--	--	--	--
Czech Republic	Central Bohemian Region	Příbram	Storage pond, Nedrahovice	01.08.2012	J. Böckelmann, K. Bubíková	N 49°36'51" E 14°27'36"	357 m	0,213	--	1	--	--	--	--	--
Czech Republic	Central Bohemian Region	Příbram	Pond Dolní solopyský, Solopysky	02.08.2012	J. Böckelmann, K. Bubíková	N 49°39'13" E 14°23'06"	382 m	0,209	--	1	--	--	--	--	--
Czech Republic	Central Bohemian Region	Příbram	Storage pond, Dobrá Voda	09.08.2012	J. Böckelmann, K. Bubíková	N 49° 33'15" E 13°59'47"	454 m	0,212	--	1	--	--	--	--	--

Country	Region	District	Locality	Collection date	Collector	Latitude, Longitude	Altitude	Sample/ standard (DAPI; mean)	Sample/ standard (DAPI; SD)	No. of ind. analysed (DAPI)	Sample/ standard (PI; mean)	Sample/ standard (PI; SD)	No. of ind. analysed (PI)	1C (pg)	No. of chr. (2*n*)
Czech Republic	Central Bohemian Region	Mělník	Oxbow of river Labe/Elbe, NW of Liběchov	22.08.2012	J. Böckelmann, Z. Hroudová, S. Píšová, K. Šumberová, K. Tremetsberger	N 50°24'53" E 14°25'48"	155 m	0,196	--	1	--	--	--	--	--
Czech Republic	Central Bohemian Region	Mladá Boleslav	Storage pond, Střehom	23.08.2012	J. Böckelmann, Z. Hroudová, S. Píšová, K. Šumberová, K. Tremetsberger	N 50°28'20" E 15°07'57"	252 m	0,219	--	1	--	--	--	--	--
Czech Republic	Central Bohemian Region	Mladá Boleslav	Pond Rejšický, Rejšice	10.07.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 50°19'08" E 14°58'40"	214 m	0,212	0,003	4	--	--	--	--	--
Czech Republic	Central Bohemian Region	Nymburk	Abandoned quarry, Hořátev	24.08.2012	J. Böckelmann, Z. Hroudová, S. Píšová, K. Tremetsberger	N 50°08'55" E 15°03'05"	186 m	0,215	--	1	--	--	--	--	--
Czech Republic	Hradec Králové Region	Jičín	Unnamed pond near Kopidlno	09.07.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 50°20'18" E 15°14'52"	237 m	--	--	--	0,190	0,001	2	0,243	--
Czech Republic	Plzeň Region	Klatovy	Pond Benátka near Velký Bor	13.07.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 49°22'13" E 13°41'00"	465 m	0,213	0,005	4	0,188	0,002	3	0,240	--
Czech Republic	Plzeň Region	Klatovy	Pond Velký Smrkovec, Smrkovec	24.07.2012	J. Böckelmann, K. Bubíková	N 49°20'13" E 13°35'55"	470 m	0,210	--	1	--	--	--	--	--
Czech Republic	Pardubice Region	Pardubice	Storage pond, Lázně Bohdaneč	09.07.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 50°04'60" E 15°39'53"	220 m	0,210	--	1	--	--	--	--	--
Czech Republic	Liberec Region	Česká Lípa	Pond Novozámecký, Zahrádky	10.07.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 50°37'41" E 14°32'36"	252 m	0,217	--	1	--	--	--	--	--
Czech Republic	South Moravian Region	Znojmo	Unnamed pond, Křepice	05.09.2012	K. Bubíková	N 48°59'12" E 16°05'38"	336 m	0,211	0,005	6	--	--	--	--	--
Czech Republic	South Moravian Region	Břeclav	River Dyje/Thaya, W of Lanžhot	10.09.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 48°42'43" E 16°54'10"	155 m	0,216	--	1	--	--	--	--	--

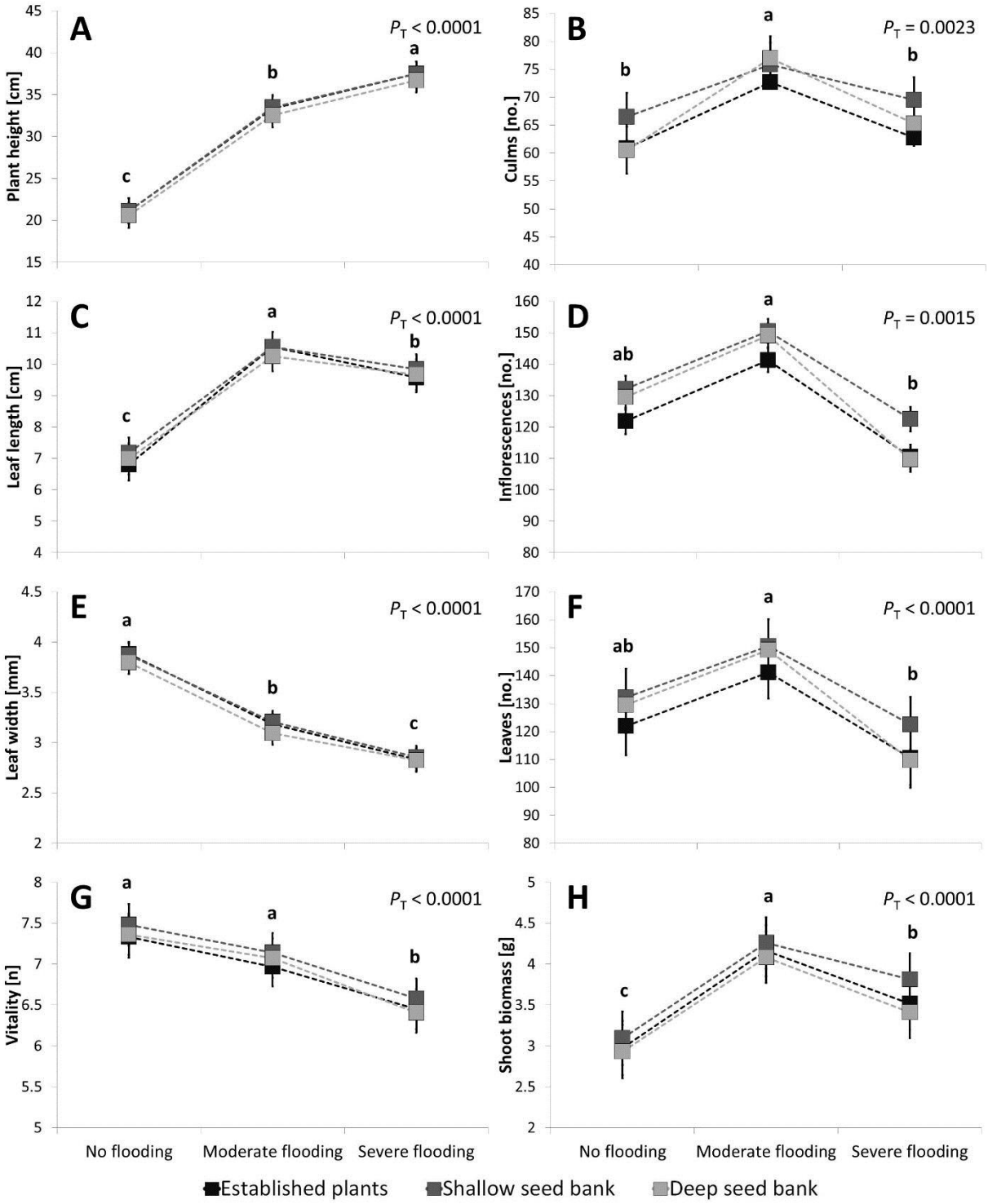
Country	Region	District	Locality	Collection date	Collector	Latitude, Longitude	Altitude	Sample/ standard (DAPI; mean)	Sample/ standard (DAPI; SD)	No. of ind. analysed (DAPI)	Sample/ standard (PI; mean)	Sample/ standard (PI; SD)	No. of ind. analysed (PI)	1C (pg)	No. of chr. (2*n*)
Czech Republic	South Moravian Region	Břeclav	Oxbow of river Dyje/Thaya, SW of Lanžhot	10.09.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 48°40'21" E 16°55'27"	153 m	0,215	--	1	--	--	--	--	--
Czech Republic	South Moravian Region	Břeclav	River Svratka, Velké Němčice	11.09.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 48°59'03" E 16°39'54"	178 m	0,215	0,004	8	--	--	--	--	--
Czech Republic	South Moravian Region	Brno-venkov	River Jihlava, Medlov	11.09.2012	J. Böckelmann, K. Šumberová, K. Tremetsberger	N 49°02'07" E 16°30'57"	185 m	0,217	0,003	6	--	--	--	--	--
Czech Republic	Ústí nad Labem Region	Děčín	Artificial basin of river Labe/Elbe, Nebočady	13.09.2012	J. Böckelmann, Z. Hroudová, S. Píšová, K. Šumberová	N 50°43'46" E 14°11'13"	378 m	0,216	0,002	5	--	--	--	--	36
Czech Republic	Ústí nad Labem Region	Ústí nad Labem	River Labe/Elbe, Ústí nad Labem	01.10.2012	J. Böckelmann, P. Kúr, K. Šumberová	N 50°39'28" E 14°02'46"	134 m	0,214	0,004	6	--	--	--	--	--
Czech Republic	Prague	Vinoř	Vernal pool in fallow land, Ctěnice	21.08.2012	J. Böckelmann, Z. Hroudová, S. Píšová, K. Šumberová, K. Tremetsberger	N 50°08'57" E 14°33'38"	238 m	0,215	0,001	6	--	--	--	--	--
Hungary	Western Transdanubia	Győr-Moson-Sopron	Wet, disturbed sand, N of Ménfőcsanak	17.10.2012	A. Mesterházy	N 47°38'37" E 17°36'29"	112 m	--	--	--	0,185	0,003	3	0,237	--
Hungary	Western Transdanubia	Vas	Unnamed pond, SW of Győrvár	14.10.2012	A. Mesterházy	N 46°58'47" E 16°49'54"	154 m	--	--	--	0,179	0,004	3	0,229	--
Hungary	Western Transdanubia	Zala	Canal near Lake Balaton, Zámor	08.09.2012	A. Mesterházy	N 46°45'52" E 17°16'12"	105 m	--	--	--	0,177	0,005	3	0,227	--
Hungary	Central Transdanubia	Komárom-Esztergom	Rivulet Concó, N of Ács	16.10.2012	A. Mesterházy	N 47°44'27" E 18°00'07"	110 m	0,192	--	1	0,181	0,001	3	0,232	36
Hungary	Central Transdanubia	Veszprém	Unnamed pond, Nóráp	17.10.2012	A. Mesterházy	N 47°16'40" E 17°27'46"	146 m	--	--	--	0,186	0,002	3	0,238	--
Hungary	Central Transdanubia	Veszprém	Unnamed pond, Tósokberénd	14.10.2012	A. Mesterházy	N 47°05'49" E 17°31'27"	211 m	--	--	--	0,181	0,005	3	0,231	--
Hungary	Central Transdanubia	Fejér	Unnamed pond, NW of Dinnyés	12.09.2012	A. Mesterházy	N 47°10'50" E 18°32'23"	103 m	--	--	--	0,180	0,003	3	0,230	--

Country	Region	District	Locality	Collection date	Collector	Latitude, Longitude	Altitude	Sample/ standard (DAPI; mean)	Sample/ standard (DAPI; SD)	No. of ind. analysed (DAPI)	Sample/ standard (PI; mean)	Sample/ standard (PI; SD)	No. of ind. analysed (PI)	1C (pg)	No. of chr. (2*n*)
Hungary	Southern Transdanubia	Somogy	Storage pond, NW of Varászló	11.10.2012	A. Mesterházy	N 46°26'49" E 17°11'42"	125 m	--	--	--	0,178	0,001	3	0,228	--
Hungary	Central Hungary	Pest	River Duna/Danube, Szentendre	11.09.2012	A. Mesterházy	N 47°39'54" E 19°04'46"	102 m	--	--	--	0,179	0,002	3	0,229	--
Hungary	Northern Hungary	Heves	Ditch, Egerszalók	06.10.2012	A. Mesterházy	N 47°51'14" E 20°19'48"	150 m	--	--	--	0,182	0,001	3	0,233	--
Hungary	Northern Great Plain	Szabolcs-Szatmár-Bereg	River Tisza, Tiszabecs	10.10.2012	I. Varga	N 48°06'25" E 22°49'52"	113 m	--	--	--	0,181	0,003	3	0,231	--
Italy	Lombardy	Pavia	Bank of river Ticino	31.08.2013	K.-G. Bernhardt, E. Naumer-Bernhardt	N 45°14'10" E 09°00'23"	65 m	--	--	--	0,183	0,000	2	0,234	--
Italy	Umbria	Perugia	Lago Trasimeno, San Savino	19.09.2012	F. Landucci	N 43°06'21" E 12°11'11"	256 m	0,214	0,005	6	0,182	0,002	3	0,233	--
Italy	Sicily	Palermo	River Oreto, Palermo	13.09.2013	K.-G. Bernhardt	N 38°06'36" E 13°22'47"	9 m	--	--	--	0,184	0,002	3	0,235	--
Poland	Lower Silesian Voivodeship	Głogów	Artificial basin of river Odra/Oder near Borków	02.10.2012	J. Böckelmann, Z. Kącki, P. Kúr, K. Šumberová	N 51°40'29" E 16°12'14"	75 m	0,216	0,002	6	--	--	--	--	--
Poland	Lubusz Voivodeship	Zielona Góra	Basin of river Odra/Oder near Cigacice	03.10.2012	J. Böckelmann, Z. Kącki, P. Kúr, K. Šumberová	N 52°01'53" E 15°36'40"	52 m	0,213	0,003	5	--	--	--	--	--
Slovakia	Bratislava Region	Malacky	Pond at Camping Rudava, SW of Malé Leváre	19.09.2013	P. Kúr, K. Tremetsberger	N 48°29'38" E 16°57'38"	147 m	--	--	--	0,182	0,001	3	0,233	--
Slovakia	Bratislava Region	Malacky	River Rudava near Veľké Leváre	04.10.2012	J. Böckelmann, P. Kúr, K. Šumberová	N 48°29'19" E 17°00'34"	152 m	0,214	0,003	5	--	--	--	--	--
Slovakia	Bratislava Region	Malacky	Oxbow of river Morava/March near Vysoká pri Morave	05.10.2012	J. Böckelmann, P. Kúr, K. Šumberová	N 48°18' 44" E 16°54'13"	143 m	0,215	0,002	7	--	--	--	--	--
Slovakia	Bratislava Region	Bratislava	Pond Kuchajda	19.09.2013	P. Kúr, K. Tremetsberger	N 48°10'16" E 17°08'35"	133 m	--	--	--	--	--	--	--	36
Slovakia	Bratislava Region	Senec	Pond Slnečné jazerá, Senec	20.09.2013	P. Kúr, K. Tremetsberger	N 48°12'55" E 17°24'54"	122 m	--	--	--	0,185	0,002	2	0,237	--

Country	Region	District	Locality	Collection date	Collector	Latitude, Longitude	Altitude	Sample/ standard (DAPI; mean)	Sample/ standard (DAPI; SD)	No. of ind. analysed (DAPI)	Sample/ standard (PI; mean)	Sample/ standard (PI; SD)	No. of ind. analysed (PI)	1C (pg)	No. of chr. (2*n*)
Slovakia	Trnava Region	Senica	Pond Vodná Nádrž Horná Studená Voda, NW of Tomky	20.09.2013	P. Kúr, K. Tremetsberger	N 48°35'03" E 17°04'45"	169 m	--	--	--	0,180	0,001	3	0,231	36
Slovakia	Trenčín Region	Ilava	Gravel pit, NW of Dubnica nad Váhom	10.10.2013	P. Kúr, S. Píšová	N 48°58'06" E 18°08'42"	224 m	--	--	--	0,178	0,001	3	0,228	--
Slovakia	Trenčín Region	Ilava	Muddy path, E of Horná Poruba	10.10.2013	P. Kúr, S. Píšová	N 48°56'36" E 18°18'47"	489 m	--	--	--	0,180	0,003	3	0,230	--
Slovakia	Trenčín Region	Púchov	Gravel pit, SE of Lednické Rovné	10.10.2013	P. Kúr, S. Píšová	N 49°03'45" E 18°17'48"	249 m	--	--	--	0,182	0,007	3	0,233	--
Slovakia	Nitra Region	Nové Zámky	River Dunaj/Danube, S of Chľaba	11.10.2013	P. Kúr, S. Píšová	N 47°49'12" E 18°49'44"	103 m	--	--	--	0,180	0,003	3	0,230	--



Appendix 4. Differences among habitat types in the flooding experiment at the first monitoring (before any treatment had taken place). Least square means and standard errors of the linear mixed models are shown: (A) plant height, (B) number of culms, (C) leaf length, (D) proportion of flowering plants, (E) leaf width, (F) number of culms with inflorescences, (G) vitality, and (H) number of leaves.



Appendix 5. Interaction of fraction and water treatment in the flooding experiment at harvest (third monitoring). Least square means and standard errors of the linear mixed models are shown: (A) plant height, (B) number of culms, (C) leaf length, (D) number of culms with inflorescences, (E) leaf width, (F) number of leaves, (G) vitality, and (H) shoot biomass. Letters denote significant differences in least square means (Tukey-Kramer adjustment for multiple comparisons: $P < 0.05$; P_T, P-value of treatment).

11 Lebenslauf/CV (einseitig)

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Name	Jörg Böckelmann
Geburtstag	29. Juni 1983
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