Study of plant dispersal in river corridors using molecular markers

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Ph.D. Thesis

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Contents

I declare that this thesis or any part of it was never submitted to obtain any other academic degree.

Summary

The thesis presents how the use of molecular markers (AFLPs and microsatellites) can help when studying plant dispersal in river systems. Analysis and interpretation of the spatial pattern of genetic variation allowed to address and discuss following aspects of long-distance dispersal in these linearly structured systems: (1) the extent of long-distance dispersal, (2) the intensity of vegetative long-distance dispersal, (3) unidirectional transport along the streams, and (4) dispersal among rivers.

The first part of the PhD. thesis presents several general aspects of plant dispersal, methodological approaches used to detect dispersal, and possibilities of analysis and interpretation of the molecular data. It also gives a short introduction to the methodology used in the particular studies, summarizes the results of all studies, and discusses how differences detected by molecular markers correspond to dispersal possibility (*e.g.,* by water, by wind) of the selected species.

The second part contains a set of four papers, each focusing on a detailed survey of dispersal possibilities of one of four plant species within the river system of the Cidlina, the Mrlina and partly also the Labe Rivers (Czech Republic). Above-mentioned aspects of dispersal in river systems are further discussed in the light of the reproduction strategy and dispersal traits of the species under study.

Paper I presents the application of AFLPs to trace within and among river dispersal of *Sparganium erectum* L. in the river system of the Cidlina and the Mrlina Rivers. A spatial genetic structure has been found, and the dispersal seems to be restricted to within a distance of 30 km. Certain genotypes were detected in both rivers, indicating past inter-river dispersal.

In paper II, a very low intensity of vegetative long-distance dispersal of *Nuphar lutea* (L.) Smith. was detected using microsatellite markers, Although the seeds of this species are unable to float in water, repeated dispersal over tens of kilometers was detected.

Microsatellite markers revealed dispersal both within and among rivers in *Phragmites australis* (Cav.) Trin. ex Steud and *Typha latifolia* L. (papers III and IV), which is in agreement with their easy spread by seeds. Paper IV also addresses the detection of insufficient marker variability to distinguish all possible genotypes in *T. latifolia*.

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Introduction

Plant dispersal and its detection

Plants are typically sessile organisms without the possibility of active movement. The spread of plant species and the exchange of genetic information among populations happens via diaspores (*i.e.*, propagules, (specialized) particles used for dispersal around maternal individuals) and pollen grains. Propagules may be either vegetative (*e.g.*, bulbils or turions) or generative (seeds or fruits). Different types of dispersal have been recognized previously (*e.g.*, Ridley 1930). Propagules may be carried by wind (anemochory), water (hydrochory), animals (zoochory), or humans (anthropochory). Many morphological and anatomical adaptations have evolved in different plant species, which allow for simple dispersal of specialized propagules. In particular, long-distance dispersal is often related to some adaptation of diaspores, such as their low specific weight, floating ability or the presence of a spiny surface on seeds or fruits (Willson & Traveset 2000). The type, rate and frequency of dispersal strongly affect the distribution of the species and the patterns of genetic variation both within and among populations.

Seed dispersal has long been a topic of interest (Ridley 1930, Willson & Traveset 2000). Identification of the 'seed shadow' (Janzen 1971) or 'dispersal kernel' (*e.g.*, Levin & Kerster 1974), which is the spatial redistribution of dispersed seeds around the source, is crucial for understanding ecological processes at the landscape level. The number of dispersed seeds, as well as the distance and directionality of their transport, is significant to ecological questions. The majority of seeds disperse to relatively short distances; the seed number/distance relationship is defined as leptokurtic, having a higher peak and a longer tail than the normal distribution (Willson & Traveset 2000). Currently, several approaches for studying plant dispersal have been proposed (Nathan 2001, Nathan et al. 2003, Ouborg & Eriksson 2004, Bullock et al. 2006):

(1) Tracking propagules during dispersal. This is theoretically the best method because it provides exact individual dispersal distances and should allow long-distance dispersal events to be detected (Nathan et al. 2003). This method can be achieved only for large and easily visible propagules (Bullock et al. 2006); sometimes seed mimics have been used to test the performance of the dispersal vector (*e.g.*, rivers; Nilsson et al. 1991). The main disadvantage is that it is complicated or even impossible to document propagule redistribution over long distances (Soons et al. 2004).

(2) Trapping methods (mark-recapture techniques) are widely used to quantify dispersal in animal ecology. Marked individuals are released after capture and, from the pattern of re-captured individuals, dispersal is quantified. Similarly, diverse seed traps have been used in plant ecology (*e.g.*, Craddock & Huenneke 1997, Chabrerie & Alard 2005, Bullock et al. 2006) to identify the number of seeds that have traveled various distances from a seed source.

(3) Mathematical models describe the movement of propagules around their sources. Several dispersal models differing in complexity have been recently developed (Jongejans et al. 2008). Empirical models deal with obtaining the correct shape for the dispersal kernel (Nathan & Muller-Landau 2000). Mechanistic dispersal models are parameterized with independent data and predict dispersal distances in a landscape (Levin et al. 2003). Such models are developed, *e.g.*, for wind dispersal (Nathan et al. 2002, Tackenberg 2003), and they may well predict the actual dispersal over short distances. They are often highly sensitive to variation in the tail of the dispersal kernel (Caswell et al. 2003, Bullock et al. 2006).

(4) Genetic methods applied to dispersal ecology gave rise to testing hypotheses at different spatial and temporal scales (Ouborg et al. 1999). Different techniques (mainly molecular markers) have been used to get genetic information from individuals and populations. To solve questions about propagule dispersal, two main frameworks have been developed – direct and indirect. The direct approach to dispersal ecology allows the measurement of contemporary gene flow in the landscape (Sork et al. 1999). It requires complete sampling of all possible maternal sources for all analyzed propagules (parentage analyses), or it uses statistical detection of recently migrated individuals (assignment tests). Widely used indirect quantification of gene flow is based on the distribution of genetic variation of sampled individuals in a landscape by measuring the average dispersal in the past (historical gene flow). Only those techniques that use molecular information are truly effective for detecting long-distance dispersal events.

Molecular markers

Many methods have been developed to obtain information about the genetic similarity of individuals or populations. Many molecular markers (*e.g.*, alleles, presence of restriction fragments, DNA sequences, etc.) can be used for various purposes, including the study of dispersal. Most molecular markers are assumed to be neutral or at least nearly neutral, and the majority of evolutionary changes and variability within populations and species are caused by random genetic drift of selectively neutral alleles (Hartl & Clark 1997). Since the degree of genetic similarity among individuals corresponds to a kinship among individuals, the spatial pattern of

genetic variation can be explained in terms of the exchange of genetic information (*e.g.*, dispersal of propagules among populations).

Molecular markers differ in observed variability, heritability (dominant or codominant), recombination during sexual reproduction and mutation rate. Codominant markers (such as isozymes or microsatellites) can differentiate homozygotes and heterozygotes from each other, and they are particularly useful for population studies. Dominant markers (*e.g.*, RAPDs or AFLPs) explain only the presence or absence of variation, but they can be reasonably useful due to their variability. These markers have all been widely used for the study of plant dispersal (Ouborg et al. 1999). Two types of molecular markers (AFLPs and microsatellites) are described in the subsequent paragraphs. These markers are highly reliable and highly polymorphic, and they allow different genetic individuals to be recognized.

AFLPs

This method is an ingenious combination of RFLP and PCR methods (Vos et al. 1995) with several major advantages, which make it a powerful tool for genetic analyses (Mueller & Wolfenbarger 1999). The high multiplex ratio (the ability to amplify several DNA markers in a single PCR reaction), the lack of required previous knowledge of DNA sequences, robustness, reliability, and largely random distribution across the genome (Bonin et al. 2007), make this technique optimal for performing detailed population studies. The main disadvantage is the dominant nature of the marker (heterozygotes cannot be distinguished from homozygotes).

Microsatellites

Tandem repetitions of short DNA sequence (1-6 bp) are called microsatellites or simple sequence repeats (SSRs; Jarne & Lagoda 1996). The observed variability is the number of repetitions, which corresponds to the length of the PCR product(s). Microsatellites are typically highly polymorphic codominant markers. This allows the detection of both alleles at the locus (for diploid species) and the direct estimation of heterozygosity and related indices of genetic variation. Individual loci are usually widely distributed within the genome. A prior knowledge of sequences of the flanking regions used for primer design is necessary for microsatellite analysis. This may be obtained by intensive cloning and sequencing. Due to the high mutation rate of microsatellites, specific statistical techniques were developed to reflect mutation models (*e.g.*, stepwise mutation model, SMM). Differentiation statistics, estimated from microsatellite allele frequencies or repeat lengths, are expected to be one of the most valuable tools for studying moderately structured populations (Balloux & Lugon-Moulin 2002).

Several technical difficulties (*e.g.*, with the interpretation of electropherograms), such as stutter bands and/or so-called '+A bands' (Dewoody et al. 2006), can be handled by carefully comparing all the data. For diploid individuals, one or two alleles per locus can be distinguished. In such cases, exact genotypes can be defined. For cases of one detected allele, homozygosity is assumed. When analyzing polyploids, more than two alleles per locus are expected. Due to PCR-derived techniques, the intensity of individual alleles cannot be solely interpreted in terms of allele number in the genotype. Hence, the exact genotype definition is impossible for heterozygotes. Several methods are used to deal with this problem. (1) All alleles are coded as presence/absence (alleles and data are analyzed and interpreted as dominant markers (Becher et al. 2000). (2) Unknown alleles are marked as missing data (*e.g.*, when three alleles are observed in tetraploid individuals, the forth allele is one of the three but unknown; Saltonstall 2003).

Analysis of pattern of genetic variation

Patterns of genetic variation obtained using molecular markers can be used for indirect estimation of gene flow among populations, and several statistical approaches have been developed for this purpose (Neigel 1997, Ouborg et al. 1999). Traditionally, the level of genetic differentiation is quantified by a parameter, F_{ST} (Wright 1978), which is the standardized among-population variance in allele frequency. Various methods of estimating F_{ST} have been proposed (Weir & Cockerham 1984) and simulation studies (Slatkin & Barton 1989) have shown reasonable accuracy for indirect methods of gene flow estimation. All estimations are based on the assumption of equilibrium between genetic drift and gene flow. This assumption allows the interpretation of differences among populations (summarized in F_{ST}) in terms of the intensity of propagule exchange. Several population genetics models have been described and used, mainly the island model (Wright 1931) and the stepping-stone model (Kimura 1953). These models specify how much dispersal must have occurred to cause the observed pattern of genetic differentiation. In addition to this approach of defining the number of exchanged propagules, several other methods have been developed to evaluate population structure and to allow the interpretation of dispersal among populations:

(1) Analysis of molecular variance (AMOVA; Excoffier al. 1992) enables the comparison of the proportion of genetic variation among geographic regions, among populations within regions and within populations. An analogue of F_{ST} as a measure of overall genetic differentiation can be inferred as well. This technique handles differences among individuals based either on allele frequencies or allele lengths (in the case of microsatellites).

(2) Bayesian individual-based clustering (*e.g.*, Corander & Marttinen 2005) provides inference of the genetic structure using stochastic optimization (Corander et al. 2006). This method searches for the *a priori* unspecified number of clusters of individuals with the highest natural logarithm of the marginal likelihood of the data, giving a clustering of individuals and the posterior probability that is most likely. Projection of the distribution of the resulting clusters onto the real landscape provides a spatial pattern of possible connections among populations, including long-distance dispersal events.

(3) The relationship between pair-wise genetic and spatial distances can be tested using spatial autocorrelation methods (Heywood 1991, Wagner et al. 2005). Spatial genetic structure (nonrandom spatial distribution of genotypes; Vekemans & Hardy 2004) results from different processes, including limited gene flow or genetic drift. Under isolation-by-distance pattern (*i.e.*, limited gene dispersal), the probability of similarity between two individuals decreases with their geographic distance, and, at drift-dispersal-mutation equilibrium, this function depends on gene dispersal (Vekemans & Hardy 2004). Pair-wise genetic distance among individuals is summarized by kinship or relationship coefficients and may be expressed as a function of geographic distance. To visualize nonrandom spatial distribution of genotypes, spatial autocorrelograms are commonly used. Statistical tests of the structure are often performed using Mantel permutation tests (Legendre & Legendre 1998, Smouse & Peakall 1999). Although all methods have been proposed for continuous populations, results seem robust to spatial clustering of individuals (Barton et al. 2002).

Plant dispersal in a riverine landscape

The study of plant dispersal is particularly attractive in a heterogeneous landscape where suitable sites are connected with 'dispersal corridors.' If these corridors serve as dispersal routes for the majority of dispersed seeds, the distribution of genetic variability in a landscape is shaped by the structure of these corridors. Examples of such a system are riverine corridors with tributaries. Because of the flowing water, rivers serve as effective pathways for linear plant dispersal (Johansson et al. 1996, Andersson et al. 2000, Jansson et al. 2005). Hydrochory (dispersal by water; van der Pijl 1969) is presumably effective for dispersal because rivers connect all possible safe-sites for germination and suitable sites for the development of new populations.

Many species that are restricted to riparian biotopes or those that occur mainly in river corridors (Burkart 2001) have adaptations for water dispersal, *i.e.*, prolonged buoyancy (floating ability) of their propagules (Praeger 1913, Ridley

Table 1 Results of studies dealing with plant dispersal in river systems with respect to detection of long-distance dispersal, vegetative long-distance dispersal, unidirectional dispersal, and inter-river dispersal.

Species	Locality	Country	Seed buoyancy	Molecular method	Long-distance dispersal	Vegetative long-distance dispersal	Unidirectional dispersal	Inter-river dispersal	Reference
Phragmites australis	Charles River	USA	intermediate	RAPD	yes	no	-	limited	Keller 2000
Iris pseudacorus	Ijzer and Demer river	Belgium	high	AFLP	yes	no	-	limited	Lamote et al. 2002
Mimulus caespitosus	Washington cascades	USA		allozymes	yes	-	no	limited	Ritland 1989
Calycophyllum spruceanum	Amazon basin	Peru	high	AFLP	yes	-	not clear	yes	Russel et al. 1999
Hibiscus moscheutos	Rhode River	USA	high	allozymes	yes	-	-	-	Kudoh & Whigham 1997
Viscaria alpina	Vindel river	Sweden	low	allozymes	yes	-	-	yes	Lundquist & Andersson 2001
Angelica archangelica	Vindel river	Sweden	high	allozymes	yes	-	yes	yes	Lundquist & Andersson 2001
Typha minima	Rhine River	Switzerland, Austria	intermediate	allozymes	yes	yes	no	-	Galeuchet et al. 2002
Oryza glumaepatula	Rio Negro, Rio Solimões	Brazil		allozymes	yes	no	yes	limited	Akimoto et al. 1998
Vallisneria spinulosa	Yangtze river	China	high	allozymes	yes	yes, but rare	no	-	Chen et al. 2007
Arundo donax	Santa Ana River	USA		allozymes, RAPD	yes	yes	-	yes	Khudamrongsawat et al. 2004
Sparganium emersum	Rur River, Swalm River	Germany, Netherlands	high	SSRs	yes	yes, but rare	no	yes	Pollux et al. 2007
Corrigiola litoralis	Loire, Rhine, Weser, Elbe	France, Germany		allozymes	yes	-	not clear	limited	Durka 1999
Potamogeton maackianus	Yangtze river	China		RAPD	no	no	-	-	Li et al. 2004
Solanum carolinense	Takano River	Japan		AFLP	no	yes	no	-	Imaizumi et al. 2006
Silene tatarica	Oulankajoki River	Finland		AFLP	limited	no	no	-	Tero et al. 2003
Zostera japonica	Ohashi River	Japan		allozymes	limited	yes	-	-	Araki & Kunii 2006
Helmholtzia glaberrima	Albert River	Australia	high	AFLP	limited	-	not clear	limited	Prentis & Mather 2008
Populus nigra	Dutch rivers	Netherlands		AFLP	limited	no	-	-	Arens et al. 1998
Populus nigra	Drôme river	France		SSRs	limited	-	no	-	Imbert & Lefèvre 2003
Hymenocallis coronaria	Savannah River	USA	low	SSRs	limited	-	yes/no	-	Markwith & Scanlone 2007
Carapa guianensis	Amazon River basin	Brazil, Venezuela	high	PCR-RFLP cpDNA	limited	-	not clear	-	Cloutier et al. 2005
Myricaria laxiflora	Yangtze river	China	high	AFLP	limited	no	yes	-	Liu et al. 2006
Potamogeton coloratus	Gordano Valley	UK		allozymes	-	-	yes	-	Gornall et al. 1998
Bistorta vivipara	Vindel river	Sweden	low	allozymes	-	yes	-	yes	Lundquist & Andersson 2001
Acorus gramineus	Taiwan	Taiwan		RAPD	-	-	-	limited	Liao & Hsiao 1998

1930). To what extent the buoyancy of propagules is reflected by species distribution patterns in the landscape has been studied extensively in recent decades. However, no clear relationship between seed floating ability and the frequency of occurrence along rivers was found (Johansson et al. 1996, Danvind & Nillson 1997, Nilsson et al. 2002).

Studies that used molecular methods to detect plant dispersal in river corridors are summarized in Table 1. Applying diverse markers, the authors analyzed the genetic diversity of river plants and their results partially answered the following aspects of dispersal in rivers: long-distance dispersal, connectivity among different rivers, unidirectional dispersal and the possibility of vegetative long-distance dispersal. The majority of these studies confirmed the existence of long-distance dispersal along river corridors and also the occurrence of among-river propagule transport. However, among-river propagule transport seems to be limited in systems studied to date. Only five studies confirmed the effect of unidirectional river flow on the genetic diversity along with higher diversity in the lower reaches of the rivers (Akimoto et al. 1998, Gornall et al. 1998, Lundquist & Andersson 2001, Liu et al. 2006, Markwith & Scanlone 2007). Seven studies detected the possibility of vegetative dispersal among distant populations. Four of these used allozymes to define multilocus genotypes (Lundquist & Andersson 2001, Galeuchet et al. 2002, Araki & Kunii 2006, Chen et al. 2007), and there was a high probability that there was insufficient variability to detect all possible genotypes, so the amount of vegetative long-distance transport was overestimated.

Nevertheless, little attention has been paid to how much seed buoyancy affects the dispersal of aquatic plants within and among river corridors, and some comparative studies of plant species that differ in their dispersal abilities are needed. This thesis contributes such a study, as several species with contrasting seed dispersal abilities were compared.

Aims of the work

Even though there are several studies attempting to describe principles of plant dispersal in river corridors (summarized in Table 1), there are very few studies that compare dispersal for different species in the same river systems. Differences in spatial distribution of genetic variation among species can be clearly explained by different dispersal abilities. In this thesis, four species with different dispersal abilities (see below) are studied. The following aspects of plant dispersal in rivers are explained and discussed:

(1) *Long-distance dispersal*. Only molecular studies can give more detail about the distance and frequency of long distance dispersal. Spatial autocorrelation analysis of

genetic variation (Sokal & Oden 1978) reveals if and at which spatial scale there is a correlation between genetic and geographic distance, which would indicate restricted gene flow. The extent of spatial autocorrelation can be a rough approximation of the range of long-distance dispersal.

Question: How strong is the relationship between geographic and genetic distance? At what distance are individuals still positively autocorrelated?

(2) *Vegetative long-distance dispersal*. Many river plants can disperse vegetatively with specialized propagules, detached portions of rhizomes or even un-rooted whole plants (Arber 1920, Haslam 1972). The actual role of clonal long-distance dispersal in river plants is still poorly understood, but it can be detected when exactly the same genotypes in distant populations are found.

Question: Do identical genotypes occur in distant localities along the stream? What is the rate of vegetative and generative dispersal?

(3) *Unidirectional dispersal*. The majority of genotypes should be dispersed to lower reaches of the river if steady downstream dispersal represents the only dispersal method for water plants. This is not often the case ('drift paradox'; Hersey et al. 1993) and animal-mediated upstream dispersal is proposed to explain the paradox (Pollux et al. 2005). Predominant downstream dispersal would contribute to an increase in genetic diversity in lower parts of the river.

Question: Is genetic variation higher within lower parts of rivers, indicating that unidirectional (downstream) dispersal along the stream has occurred?

(4) *Dispersal among rivers and their tributaries*. It is almost impossible to get information about successful dispersal of plants among different river systems without using molecular methods. The spatial distribution of genetic variation reflects dispersal of propagules in a landscape. Animal or wind-mediated dispersal may represent a possible connection among different river systems (Figuerola & Green 2002, Mueller & Van der Valk 2002, Pollux et al. 2006). Little is known about the actual gene exchange among rivers that are not connected. Detailed analyses of spatial patterns of genetic variation allow the determination of whether rivers are the main dispersal corridors for water plants or whether between-river dispersal must be considered as well.

Question: Can we find genetically similar individuals in different streams, which would indicate dispersal among rivers? Are individuals from different rivers genetically isolated?

Four plant species were selected to study dispersal in three adjacent river catchments in the Czech Republic (Fig. 1). Selected species differed in their dispersal possibilities (Table 2). Two species (*Sparganium erectum* and *Nuphar lutea*) are

assumed to be predominantly dispersed by flowing water (hydrochory), while *Phragmites australis* and *Typha latifolia* are dispersed mainly by wind (anemochory). Moreover, propagules of *S*. *erectum* float well, while those of *N*. *lutea* do not.

These four species differing in many life history traits present a small sample to make general conclusions about the effects of traits on the dispersal throughout the landscape. Nonetheless, the final comparison of results is followed by a discussion about seed buoyancy and the possibility of wind dispersal affecting the spatial distribution of genetic variation in this riverine landscape.

Fig 1 Location of the three river systems within the Czech Republic: 1) the Cidlina River, 2) the Mrlina River, and 3) the Labe River.

Material and methods

Studied plants, area of interest

Bur reed, *Sparganium erectum* L., grows along slowly flowing rivers. It is a rhizomatous perennial that grows along riverbanks or forms large stands tens of meters in length. It is diploid, 2n=30 (Harada 1947), wind pollinated, and self-compatible (Cook & Nicholls 1987). Generative dispersal is facilitated by floating fruits. Vegetative spread is also possible through detached portions of rhizomes or by entire plants that have been uprooted, *e.g.*, during major floods (Hejný 1960).

Table 2 Plant species selected for the study of dispersal in river systems with respect to dispersal type and dispersal ability.

		Dispersal ability	
		good	weak
Dispersal type	by water	*Sparganium erectum*	*Nuphar lutea*
	by wind	*Phragmites australis* *Typha latifolia*	–

Yellow pond-lily, *Nuphar lutea* (L.) Smith, inhabits standing or slowly flowing water in calm bays or rivers with depths of 0.8 m to 2 m (Casper & Krausch 1981). It is diploid, 2n=34 (Heslop-Harrison 1955), insect pollinated and self-compatible. Vegetative reproduction of *N*. *lutea* takes place through a branching rhizome system. Rhizome fragments occasionally break away and may be found floating on the water (Arber 1920). Long-distance dispersal by seeds is limited because of their low buoyancy (Heslop-Harrison 1955, Meusel & Mühlberg 1965), but the whole fruits released from peduncles, loculi containing seeds, or the seeds surrounded by mucilaginous tissue are able to float for a short period after fruit wall disintegration (Smits et al. 1989, Hart & Cox 1995). Hence, seed dispersal by water is believed to be possible if seeds are transported by stream at the riverbed with other sediment particles (Lhotská & Kopecký 1966), but no clear evidence has been reported so far.

Common reed, *Phragmites australis* (Cav.) Trin. ex Steud, is a perennial wetland grass typically forming dense monodominant stands in the littoral belt of standing waters, in wet field depressions (wet meadows) and on banks of rivers and streams. Studied individuals were tetraploid, 2n=48 (Clevering and Lissner 1999). The species reproduces vegetatively and generatively. Vegetative reproduction within populations leads to the formation of clones, but spread by rhizome fragments through water drift has also been observed (Haslam 1972). Seed dispersal by wind is facilitated by the presence of pappus, and long-distance dispersal is expected. Dispersal through running water was also reported (Boedeltje et al. 2004); seeds can float for several days (Morton and Hogg 1989).

Broadleaved cattail, *Typha latifolia* L., is a wetland plant growing in shallow waters of ponds, channels and slowly flowing rivers. It forms dense stands through vegetative reproduction (rhizomes). The species is diploid, 2n=30 (Smits 1967), and wind pollinated. Generative reproduction is supported by the production of a large number of seeds that are easily dispersed by the wind. Seeds may also float in the water (Neff & Baldwin 2005).

The study area is situated in eastern and central Bohemia, Czech Republic (Fig. 1). It comprises catchments of two small rivers, the Cidlina and the Mrlina Rivers, both of which are tributaries of the Labe River. Several sites from the Labe River, the Jizera River and the Ohře River were included in the sampling for some

species. In this area, all four species are commonly distributed in slowly flowing parts of the rivers and larger tributaries (Rydlo 1990, 1991).

Sampling design

Due to the complicated definition of a single population, we collected individuals at so-called sampling sites. These were defined as one to several stands of the species growing along both sides of the river segment (several hundred meters long). At each sampling site, one to six individuals were collected depending on the abundance of the species at the site. Using this sampling design, collecting the same clonal individual twice was minimized. Since small and uneven sample sizes may have a strong effect on analyses made on the population (= sampling site) level, for some species, the sites were geographically grouped into 'river sections', and some analyses were made at this level only. To avoid pseudoreplication, all recurring genotypes within river sections were removed prior to subsequent analyses.

Molecular and statistical methods

Total DNA was extracted using the CTAB method (Doyle & Doyle 1987). The AFLP approach was used for *S*. *erectum*, and the remaining three species were analyzed using nuclear microsatellites. Since microsatellite analyses require species-specific primers, already-published primers were used for *N*. *lutea* (Ouborg et al. 2000), *P*. *australis* (Saltonstall 2003) and *T*. *latifolia* (Tsyusko-Omeltchenko et al. 2003). For details about these analyses see papers I-IV.

Molecular techniques allowed for the discrimination of genets and the recognition of common clonal origins of the samples. For AFLPs, a threshold value for genetic identity was used to define clones (Lamote et al. 2002; see paper I for details). Samples analyzed using SSR loci were considered clones only when they had identical multilocus genotypes. Detection of the same genotype in different populations was evidence for long-distance vegetative dispersal (Question 2).

Genetic diversity within populations was measured using the Shannon diversity index (Shannon & Weaver 1949) or the average gene diversity over loci (Nei 1987). An increase in genetic diversity downstream (as a result of prevailing unidirectional dispersal) was tested using correlations of the position of the population in the stream (*e.g.*, with the distance from the river source). This was used to answer Question 3.

To investigate population structure, Bayesian clustering of individuals (Corander & Marttinen 2005) was carried out. For each species, the distribution of Bayesian clusters was plotted onto the actual river landscape using ArcGIS 3.2 (ESRI). Interpretation of this pattern allowed us to answer Questions 1 and 4.

Table 3 Summary of the results obtained in papers included in this thesis. Results are presented according to the four aspects of plant dispersal formulated in the Introduction. See papers I-IV for detailed descriptions of all analyses.

Dispersal type		Species	Populations	Individuals	Molecular approach	Long-distance dispersal		Vegetative long-distance dispersal	Unidirectional dispersal - correlation of genetic diversity with the stream position			Among-river dispersal	
						Positive spatial autocorrelation	Mantel test	Minimum of detected events[+]	Cidlina	Mrlina	Labe	Minimum of detected events[++]	Differences among rivers
by water (hydrochory)	floating propagules	*Sparganium erectum*	66	258	AFLP	20-30 km	0.23 (0.20)[‡]	6	0.24	0.26	-	6	0.4%
	non-floating propagules	*Nuphar lutea*	44	156	SSRs	15-25 km	0.29 (0.36)[‡]	1	0.52	0.27[*]	0.38[*]	6	7.5%
by wind (anemochory)		*Phragmites australis*	78	189	SSRs	5-7 km	0.09	4	0.00[*]	0.04[*]	0.08[*]	15	6.0%
		Typha latifolia	24	56	SSRs	5 km	0.01	7[**]	-	-	-	3	1.0%

[+] Number of identical multilocus genotypes found in different populations
[++] Number of Bayesian clusters found in more river systems
[*] Not included in the papers (mainly due to small population sizes)
[**] Probably overestimated due to insufficient resolution of the marker system (see paper IV for details)
[‡] First value for the analysis with direct-line geographical distances, value in parenthesis for analysis with along-stream distances

To understand the level and partitioning of genetic variation into geographic groups, analysis of molecular variance (AMOVA; Excoffier al. 1992) was performed. The percentage of total variance due to the separation of individuals in different river catchments was interpreted as an indication of dispersal among rivers (Question 4).

The relationship between pairwise genetic and spatial distances was tested using Mantel tests (Legendre & Legendre 1998) and spatial autocorrelation methods (Heywood 1991, Wagner et al. 2005). In both approaches, we used up to three types of geographic distances among individuals: (1) direct-line distances, (2) along-river distances and (3) along-river distances while assuming only downstream dispersal. The third distance type produced a matrix with missing data because distances between localities with no flowing water connection were undefined. This matrix was analyzed only using spatial autocorrelation. The extent of autocorrelation was an indication of the range of long-distance dispersal (Question 1).

Results and discussion

Altogether, 258 samples from 66 sites of *Sparganium erectum*, 156 samples from 44 sites of *Nuphar lutea*, 189 samples from 78 sites of *Phragmites australis*, and 56 samples from 24 sites of *Typha latifolia,* were analyzed. The results are summarized in Table 3. The interpretation of the data answered questions about plant dispersal in river systems as described below.

Long-distance dispersal

Mantel correlations of matrices of genetic and geographic distances among individuals provided basic information about nonrandom spatial distribution of genotypes in the riverine landscape. The greater the correlation found, the higher the genetic similarity was among individuals from geographically close localities. Individuals from distant places were genetically less similar. A higher correlation indicates spatially restricted long-distance seed or pollen dispersal.

In the species adapted to wind dispersal, the correlation was low (0.09 in *P. australis*; 0.01 in *T. latifolia*) and not significant. The remaining two species with water-dispersed propagules displayed higher and significant correlations (0.23 in *S. erectum*; 0.36 in *N. lutea*). This finding is congruent with the hypothesis that

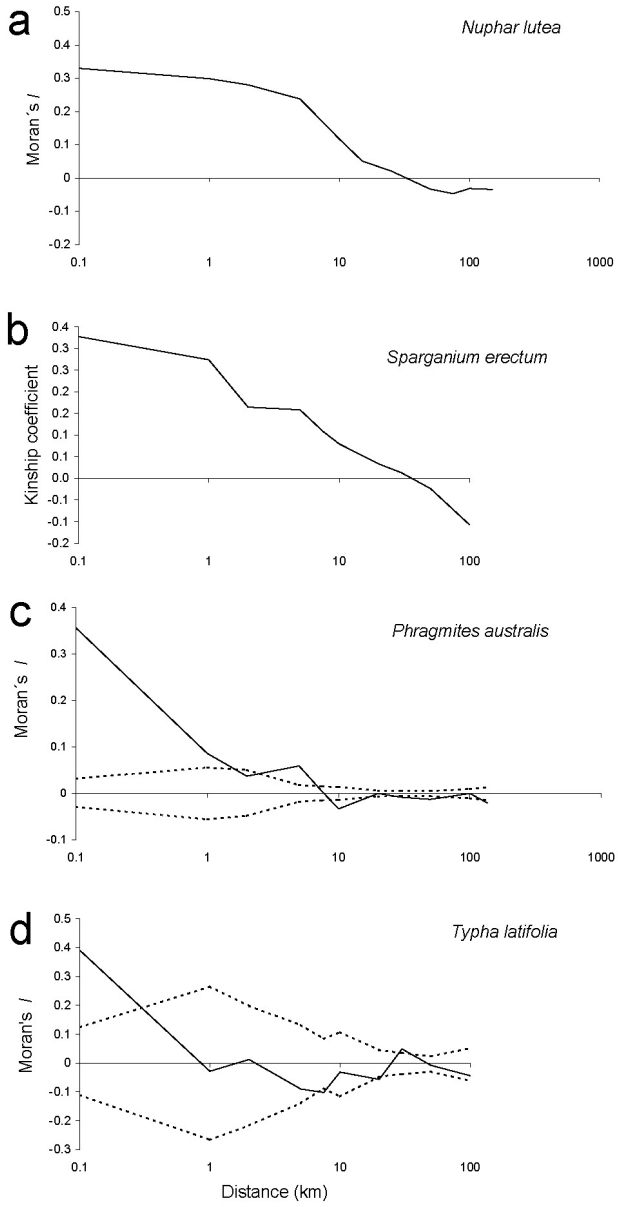

Fig 2 Results of spatial autocorrelation analyses for four species described in this thesis. The average values of Moran's *I* or kinship coefficients are given for distance classes up to 150 km. The first distance class corresponds to the autocorrelation within sampling sites. See papers I-IV for details.

buoyant *S*. *erectum* seeds are effectively dispersed over longer distances in the river than non-buoyant *N*. *lutea* seeds.

Differences in long-distance dispersal of propagules can also be demonstrated using spatial autocorrelograms. For *S*. *erectum* and *N*. *lutea,* genetic similarity decreased with increasing spatial pair-wise distances among individuals and did not

level off at greater distances (Fig. 2a, b). Significant positive autocorrelation among individuals existed up to 30 km (*S*. *erectum*) or 25 km (*N*. *lutea*). For *P*. *australis,* this positive correlation extended up to 5 km, and correlation coefficients for greater distant classes were not significant (Fig. 2c). This implies random distribution of individuals, and it can be interpreted as spatially unrestricted long-distance dispersal. In *T*. *latifolia,* the significant positive autocorrelation existed only within populations (Fig. 2d).

Long-distance dispersal exists both along and among rivers, as shown by the geographic distribution of genetically related multilocus genotypes (*i.e.*, groups defined by the Bayesian clustering method). For example, some genotypes of *N*. *lutea* were dispersed along the Mrlina River to the Labe River and further downstream (paper II, Fig. 2). Also in *S*. *erectum,* some genotypes showed along-stream spread in the Cidlina River (paper I, Fig. 2). The same is true for several groups of genotypes within the *P*. *australis* samples (paper III, Fig. 2). Among-river dispersal is further discussed below.

It can be concluded that dispersal of water-dispersed species (*S*. *erectum*, *N*. *lutea*) is spatially restricted, while wind-dispersed species (*P*. *australis*, *T*. *latifolia*) are probably regularly dispersed without regard to distance. Non-floating propagules of *N*. *lutea* may be dispersed over shorter distances than buoyant propagules of *S*. *erectum,* but the difference in propagule buoyancy between these two species probably plays a minor role in dispersal efficiency.

Vegetative long-distance dispersal

Unambiguous evidence of vegetative dispersal among populations was the presence of the same multilocus genotype in different populations. In *S*. *erectum,* six genotypes were found in more than one population, predominantly in lower parts of the Cidlina and the Mrlina Rivers (paper I, Fig. 4). Thus, long-distance vegetative dispersal takes place to some extent in this species. For *N*. *lutea,* only one genotype was found in two different populations. This suggests a surprisingly low proportion of this type of dispersal contributing to the successful spread of this species. Four identical multilocus genotypes distributed in two populations were detected in *P*. *australis*. For *T*. *latifolia,* frequent vegetative among-river transport may explain the distribution of identical multilocus genotypes. This is most likely overestimated since the resolution of markers was not sufficient to detect all possible genotypes (see paper IV).

In spite of the prolonged floating time of the vegetative parts of plants in the water (Morton & Hogg 1989), successful long-distance dispersal is relatively infrequent in all species studied. There are several possible explanations. First,

dispersing rhizomes are not distributed to suitable sites for further establishment. Since they float well, they cannot reach the river bottom where they could root, but they frequently settled on riverbanks where they subsequently dry out and die, or they may be damaged, *e.g.*, by rodents. Second, due to the limited number of analyzed individuals for each species, we covered the long-distance vegetative dispersal question only partially. If we had analyzed more data from this area, we may find stronger evidence for this dispersal type. Third, a strict criterion for genotype definition may underestimate the proportion of vegetative dispersal. The mutation rate of microsatellite loci cannot be excluded in long-lived clonal plants (Cloutier et al. 2003, Lian et al. 2004), so some individuals may differ genetically even though they are of clonal origin.

Diversity along streams and unidirectional dispersal

We found a tendency to greater within-population genetic diversity in downstream parts of both rivers for *S. erectum* (r^2=0.24, p=0.009 for the Cidlina River; r^2=0.26, p=0.107 for the Mrlina River; paper I, Fig. 3). For *N. lutea,* there was greater within-population diversity in the Cidlina River (r^2=0.52, p=0.106). Non-significance in the correlations may be due to the low sample size; only populations with at least three individuals were included. In these species, unidirectional dispersal downstream (probably mainly by water) predominates over occasional upstream dispersal (probably by animals). On the contrary, in wind-dispersed species (*P. australis*, *T. latifolia*), no correlations were found, and these species were dispersed in spite of the direction of river flow. There is a clear relationship between dispersal type (by water or by wind) and the presence of a linear gradient of within-population genetic diversity caused by the movement of the water.

Dispersal between rivers and their tributaries

Dispersal among river catchments can be described by the number of Bayesian clusters of genetically related individuals, with individuals originating from different catchments. Six of the eight clusters defined for *S. erectum* were found in both the Cidlina and the Mrlina River systems (paper I, Fig. 2). Therefore, at least six dispersal events between these catchments in the past may explain the present-day pattern of genetic variation. For the interpretation of the spatial pattern of genetic variation in *N. lutea,* six dispersal events would have been necessary (paper II, Fig. 2), and 15 would have been necessary in the case of wind-dispersed *P. australis* (paper III, Fig. 2b).

The percentage of the total variation due to the separation of individuals from different rivers (computed by AMOVA) is another parameter connected with the rate

of propagule exchange among rivers. Here, there was a difference among the studied species with regard to this variation. Analysis of *S. erectum* and *T. latifolia* revealed negligible differentiation among rivers, whereas the analysis of *N. lutea* and *P. australis* detected 7.5 and 6% differences among rivers, respectively. The question remains as to whether the present genetic structure is a result of a high rate of seed dispersal among river systems (in the past or ongoing) or a result of low genetic exchange of genotypes that subsequently spread widely in the other river basin. Negligible differentiation among rivers may also be a consequence of a common colonization history of the area.

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