Molecular Ecology (2007) **16**, 313–325 doi: 10.1111/j.1365-294X.2006.03146.x

Reproductive strategy, clonal structure and genetic diversity in populations of the aquatic macrophyte *Sparganium emersum* in river systems

B. J. A. POLLUX,*† M. D. E. JONG,* A. STEEGH,* E. VERBRUGGEN,* J. M. VAN GROENENDAEL* and N. J. OUBORG*

**Section of Molecular Ecology, Department of Aquatic Ecology & Environmental Biology, Institute for Wetland and Water Research, Radboud University Nijmegen, Toernooiveld 1, NL-6525 ED Nijmegen, The Netherlands, †Department of Plant–Animal Interaction, Centre for Limnology, Netherlands Institute of Ecology (NIOO-KNAW), Maarssen, The Netherlands*

Abstract

Many aquatic and riparian plant species are characterized by the ability to reproduce both sexually and asexually. Yet, little is known about how spatial variation in sexual and asexual reproduction affects the genotypic diversity within populations of aquatic and riparian plants. We used six polymorphic microsatellites to examine the genetic diversity within and differentiation among 17 populations (606 individuals) of *Sparganium emersum*, in two Dutch-German rivers. Our study revealed a striking difference between rivers in the mode of reproduction (sexual vs. asexual) within *S. emersum* populations. The mode of reproduction was strongly related to locally reigning hydrodynamic conditions. Sexually reproducing populations exhibited a greater number of multilocus genotypes compared to asexual populations. The regional population structure suggested higher levels of gene flow among sexually reproducing populations compared to clonal populations. Gene flow was mainly mediated via hydrochoric dispersal of generative propagules (seeds), impeding genetic differentiation among populations even over river distances up to 50 km. Although evidence for hydrochoric dispersal of vegetative propagules (clonal plant fragments) was found, this mechanism appeared to be relatively less important. Bayesian-based assignment procedures revealed a number of immigrants, originating from outside our study area, suggesting intercatchment plant dispersal, possibly the result of waterfowl-mediated seed dispersal. This study demonstrates how variation in local environmental conditions in river systems, resulting in shifting balances of sexual vs. asexual reproduction within populations, will affect the genotypic diversity within populations. This study furthermore cautions against generalizations about dispersal of riparian plant species in river systems.

Keywords: dispersal, hydrochory, sexual reproduction, vegetative reproduction, waterfowl, zoochory

Received 20 June 2006; revision accepted 1 September 2006

Introduction

Rivers offer special environments to aquatic and riparian plants, due to the one-dimensional linear arrangement of suitable habitats, the continuous subjection to the hydraulic forces of water currents and the unidirectional nature of the water flow. Knowledge about the processes that determine the genetic structure of populations (e.g. life form, reproductive biology, clonal propagation, dispersal mechanisms) is essential for understanding the scale over which dispersal, genetic drift and selection operate (Slatkin 1985; Heywood 1991; Ouborg *et al*. 1999).

Most aquatic and riparian plant species are characterized by the ability to reproduce sexually via seeds, and asexually via stolons, runners, tubers, etc. (Barrett *et al*. 1993). Some studies suggest that the age of plant populations affects the mode of reproduction, although opposing views exist on the underlying mechanisms that might determine the mode of reproduction (Piquot *et al*. 1998; Sun *et al*. 2001). Other studies have shown that the relative proportions of sexual vs. asexual reproduction varies widely within a

Correspondence: B.J.A. Pollux, Fax: +31 (0)24 3653047; E-mail: b.pollux@science.ru.nl

plant species, due to variations in environmental parameters (Honnay & Bossuyt 2005). Within the geographical range of a species, for example, plants may increasingly suffer from physiological stress near the boundaries of their geographical range, leading to reduced sexual reproduction (decreased flower, fruit and seed production) or seedling recruitment (Cox & Moore 1980; Dorken & Eckert 2001; Eckert 2002; Lui *et al*. 2005). In temperate deciduous forests, moreover, the relative investment in sexual vs. clonal reproduction has been shown to vary in response to spatial heterogeneity of light conditions and soil moisture content: Kudoh *et al*. (1999) found that sexual reproduction of *Uvularia perfoliata* was restricted to high-light conditions (in gap sites), whereas under low-light conditions (in closed-canopy sites) plants reproduced clonally; and Jacquemyn *et al*. (2006) showed that sexual reproduction of *Paris quadrifolia* was primarily found in moist and relatively productive sites, while under stressful conditions (i.e. in dry and relatively unproductive sites) sexual reproduction and seedling recruitment was suppressed. In aquatic systems, spatial variation in water depth and current velocity have also been known to affect the mode of reproduction within populations of several different plant species, by limiting the plants' ability to produce emergent flower-bearing stems in deep habitats or fast-running streams (Haslam 1978; Van Wijk 1988; Boeger & Poulson 2003).

The mode of reproduction (sexual vs. asexual) is likely to have important effects on the spatial distribution of genetic variation within and among plant populations in rivers (Ellstrand & Roose 1987; Widén *et al*. 1994; Honnay & Bossuyt 2005). First, sexual reproduction is likely to enhance the level of gene flow among populations via seed dispersal. The level of connectivity among riverine plant populations will, to a large extent, determine their genetic structure (Tero *et al*. 2003). In plant species with hydrochory as their main dispersal strategy, unidirectional gene flow may be expected to lead to erosion of genetic diversity in upstream river stretches and accumulation of genetic diversity in downstream stretches (Barrett *et al*. 1993). Such associations have, however, rarely been found (Gornall *et al*. 1998; Lundqvist & Andersson 2001; Liu *et al*. 2006). Second, the occurrence of modular clonal units (ramets) originating from the same sexually produced offspring (genets) will directly affect the genotypic diversity within populations (Ellstrand & Roose 1987; Widén *et al*. 1994; Honnay & Bossuyt 2005). Thus, insight into how spatial variation in sexual and asexual reproduction varies across environmental parameters will help understanding the genetic structure of (facultatively clonal) plant populations in river systems.

In this study, we employed microsatellite analysis to examine the genotypic diversity within and genetic differentiation among 17 populations of *Sparganium emersum* in two different rivers, the Swalm and Rur rivers (Germany–the Netherlands). These two rivers differ widely in their hydrodynamic regime. Several studies have shown that aquatic and riparian plants respond to increased water velocities through plastic morphological changes in order to reduce mechanical damage (Chambers *et al*. 1991; Schutten & Davy 2000), affecting their ability for sexual reproduction (Haslam 1978; Boeger & Poulson 2003). We hypothesized that spatial variation in current velocity within and between river systems would affect the mode of reproduction within populations, in turn affecting the intrapopulation genotypic diversity. The objectives of this study were therefore to determine: (i) how hydrodynamic conditions experienced by the plant populations affect their morphology, and consequently their ability for sexual vs. asexual reproduction; (ii) the extent and patterns of microsatellite variability within and among *S. emersum* populations; and (iii) whether the genetic and genotypic diversity within populations reflects a local balance between sexual and asexual reproduction.

Materials and methods

Study species

Unbranched burreed, *Sparganium emersum* Rehmann 1871 (*Sparganium simplex* Hudson 1778) (Sparganiaceae), is an aquatic vascular macrophyte, that is widely distributed throughout Eurasia and North America (Cook & Nicholls 1986). It typically grows in a narrow band at the margins of rivers, streams and canals that are characterized by shallow, slow flowing, nutrient-rich waters. *Sparganium emersum* is a monoecious and protandrous species (Sargent & Otto 2004). *Sparganium emersum* flowers from June to August, and its flowers are mainly wind-pollinated (Sargent & Otto 2004). The seeds are released in autumn and are mainly dispersed by water currents and waterfowl species (Boedeltje *et al*. 2004; Pollux *et al*. 2005). Vegetative plant fragments are also dispersed by water currents, remaining viable and capable of establishment even after floating for up to 10 weeks (Barrat-Segretain & Amoros 1996; Barrat-Segretain *et al*. 1998, 1999). *Sparganium emersum* is also capable of asexual (clonal) reproduction through the production of stolons, from which new ramets emerge (Cook & Nicholls 1986).

Study sites

The river Rur (catchment surface area of 2340 km^2; Fig. 1) originates in the Ardennes Mountains near the Belgian border [at 650 m above sea level (a.s.l.)], floats through Germany (143.5 km) and the Netherlands (21.5 km), where it discharges in the river Meuse (at 16.8 m a.s.l.). The channel width varies between 20 and 40 m. The seasonal hydrology is highly dynamic, with discharge ranging from 9.5 m^3/s to 123 m^3/s. The profile of the channel bed is

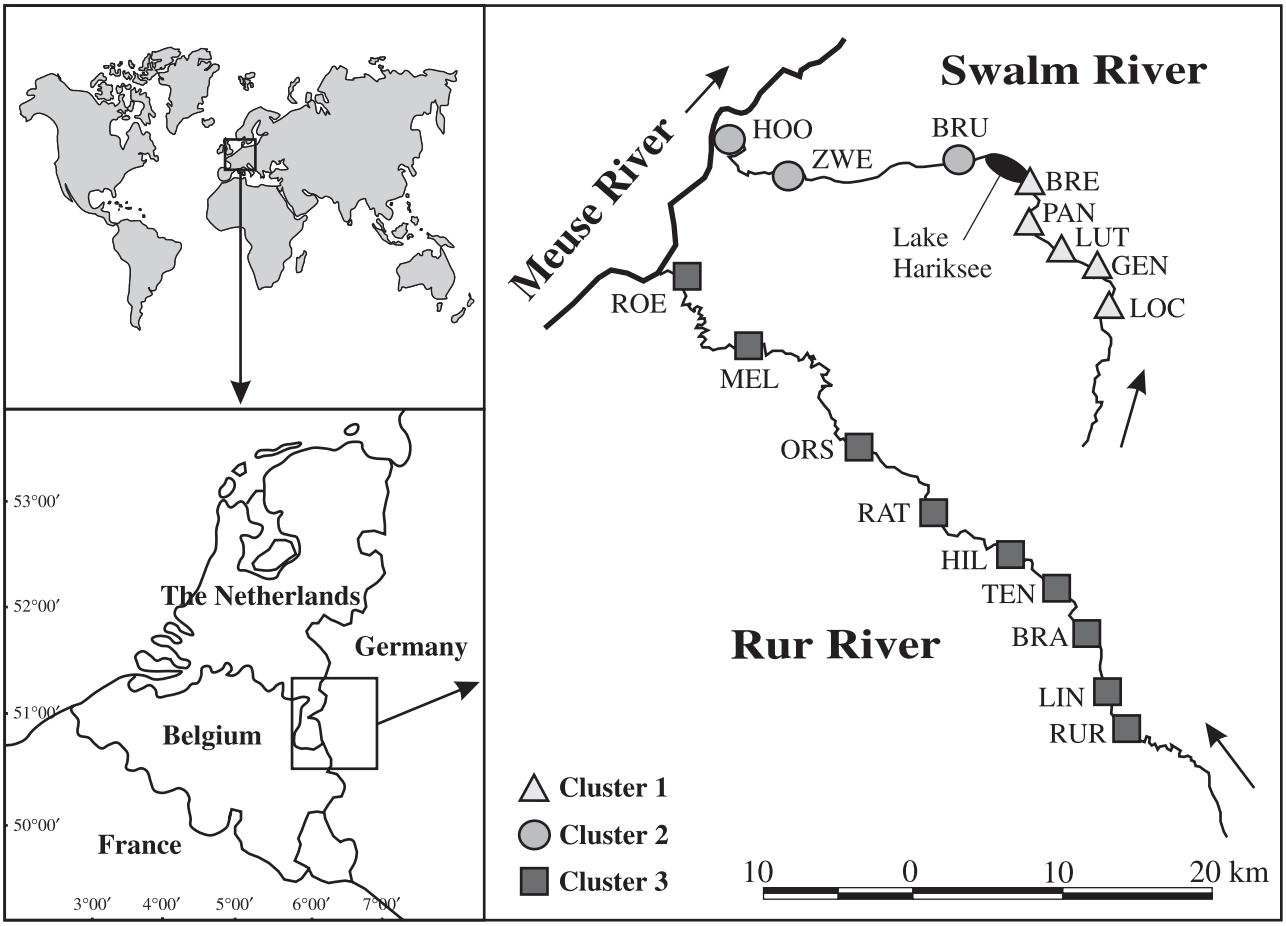

Fig. 1 Map showing the 17 sampling locations of the unbranched burreed (*Sparganium emersum*) in the Swalm and Rur rivers (the Netherlands-Germany). The three different clusters, inferred from the Bayesian clustering analysis, have been indicated by different symbols.

characterized by gradually sloping riverbanks running down to a depth of 2 m. The river Swalm (catchment surface area of 277 km^2; Fig. 1) originates near the city of Wegberg (Germany) (at 85 m a.s.l.), flows through Germany (31 km) and the Netherlands (12.2 km), where it discharges into the river Meuse (at 14 m a.s.l.). The channel width varies between 3 and 10 m and discharge ranges from 0.5 m^3/s to 15 m^3/s. The profile of the channel bed is characterized by steep slopes and a uniform channel depth of approximately 0.5 m. In the middle of its course lies Lake Hariksee, a large shallow lake formed after peat excavations in the 19th century.

Field survey

During 12–23 September 2005, plant density and proportion of flowering plants (%) were determined for each of the 17 study populations by counting the number of plants, and the number of flowering plants, within five randomly selected 0.4 × 0.4 m^2 areas in each population. Plant biomass was assessed by measuring the dry weight, on a microbalance (Sartorius LP620P), of 10 randomly collected plants after oven drying for 24 h at 55 °C. The number of seeds and seed heads per plant were inferred from up to 10 flowering plants randomly collected from each population. In addition, water velocities among plants were determined by measuring water velocity at five randomly selected locations within each plant population, at 5 cm below the water surface, using a SENSA-RC2 Water Velocity Meter (Aqua Data Services Ltd, Aquatec House). Differences in plant density and plant biomass between rivers were tested for significance by means of a One-Way ANOVA. Prior to the analyses, all data were log10-transformed to assure homoscedasticity and normality of residuals. All analyses were performed with STATISTICA 6.0 (StatSoft Inc).

Sample collection, DNA extraction, polymerase chain reaction (PCR) amplification and microsatellite analysis

In July 2003, a total of 606 *S. emersum* plants were collected from eight discrete locations (covering all populations) in the Swalm River and nine discrete locations (comprising

Table 1 Population characteristics (mean ± SD) and genotypic diversity statistics for the 17 *Sparganium emersum* populations in the Swalm and Rur rivers (N_r = number of ramets sampled in each population, G = number of unique genotypes identified, G_L = the number of local, i.e. unique, genotypes, P = the proportion of distinguishable genotypes and D = Simpson's diversity index)

			Population characteristics						Genotypic diversity				
River	Population		Water velocity (m/s)	Plant density (m^{-1})	Plant biomass (g)	Proportion flowering plants (%)	Number of seed heads	Number of seeds	N_r	G	G_L	P	D
Swalm	1	LOC	0.384 (0.09)	243.8 (91)	0.54 (0.4)	0	0	0	30	1	0	0.033	0
	2	GEN	0.480 (0.03)	281.3 (72)	0.31 (0.3)	0	0	0	33	2	1	0.061	0.1174
	3	LUT	0.547 (0.09)	327.1 (103)	0.23 (0.1)	0	0	0	33	1	0	0.030	0
	4	PAN	0.447 (0.06)	343.8 (27)	0.38 (0.2)	0	0	0	35	1	0	0.029	0
	5	BRE	0.373 (0.08)	387.5 (147)	0.41 (0.2)	0	0	0	35	11	11	0.314	0.5731
	6	BRU	0.333 (0.10)	183.3 (22)	0.45 (0.2)	0	0	0	33	13	13	0.394	0.8845
	7	ZWE	0.507 (0.13)	214.6 (19)	0.51 (0.2)	0	0	0	35	1	0	0.029	0
	8	HOO	0.260 (0.12)	243.8 (50)	0.44 (0.2)	0	0	0	35	1	0	0.029	0
Rur	1	RUR	0.060 (0.01)	95.2 (21)	1.97 (1.3)	0.44 (0.2)	2.67 (0.8)	188.43 (75.4)	20	1	1	0.050	0
	2	LIN	0.058 (0.02)	97.9 (53)	1.77 (0.5)	0.39 (0.1)	3.00 (0.7)	139.10 (111.2)	40	37	35	0.925	0.995
	3	BRA	0.042 (0.01)	133.3 (38)	1.73 (0.5)	0.23 (0.1)	3.22 (0.7)	233.67 (60.5)	40	36	30	0.900	0.991
	4	TEN	0.029 (0.00)	120.8 (51)	2.24 (1.0)	0.66 (0.0)	2.80 (0.6)	170.00 (64.7)	40	38	30	0.950	0.997
	5	HIL	0.052 (0.01)	100.7 (34)	2.60 (0.7)	0.30 (0.2)	4.00 (1.0)	412.12 (137.0)	39	21	18	0.538	0.896
	6	RAT	0.113 (0.03)	120.8 (42)	0.69 (0.2)	0	0	0	40	32	27	0.800	0.988
	7	ORS	0.115 (0.04)	72.9 (22)	1.60 (0.3)	0.21 (0.1)	2.83 (0.7)	114.67 (43.2)	39	30	23	0.769	0.974
	8	MEL	0.047 (0.01)	104.2 (38)	2.84 (0.9)	0.32 (0.1)	3.29 (0.5)	197.14 (79.5)	39	32	29	0.821	0.989
	9	ROE	0.034 (0.01)	56.7 (12)	2.96 (1.0)	0.83 (0.2)	3.72 (0.7)	340.16 (108.8)	40	21	19	0.525	0.959

the nine biggest populations) in the Rur River (Fig. 1, Table 1). In each site, plants were collected at 1–2-m intervals along a linear transect running parallel to the shore. Plant samples were immediately transferred to a 1.5 mL Eppendorf tube and stored at –80 °C until the DNA extraction. Genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN). Each individual was screened using six microsatellite primer pairs (SEM01, SEM05, SEM08, SEM12, SEM14 and SEM15; Pollux & Ouborg 2006). Fragments were analysed on a model 4200 IR2 DNA Analyser (LI-COR) using the SAGA Automated Microsatellite Analysis Software Version 2.1 (Li-cor).

Genetic and genotypic diversity

We used a number of standard measures to describe the clonal structure of each population. The proportion of distinguishable genets was calculated as: $P = G/N_r$, where G is the number of distinguishable genotypes and N_r the total number of sampled ramets (Ellstrand & Roose 1987). Second, for each population we determined the number of local (i.e. unique) genotypes (G_L) (Ellstrand & Roose 1987). Third, we calculated Simpson's diversity index (D; Simpson 1949) corrected for finite sample sizes as: $D = 1 - \Sigma\{[n_i(n_i - 1)]/[N_r(N_r - 1)]\}$, where n_i is the number of individuals with the same genotype and N_r the number of ramets sampled (Widén *et al*. 1994). A Mann–Whitney U-test was used to assess whether genotypic variation within populations (G, G_L, P and D) differed between rivers. The number of unique genotypes possible was calculated as: $N_g = \Pi[a_i(a_i + 1)]/2$, where a_i is the number of alleles detected at the ith locus (Widén *et al*. 1994). In addition, we calculated the probability that two individual ramets with the same multilocus genotype originated from the same genet as: $P_{gen} = (\Pi\, p_i q_i) 2^h$, where p_i and q_i is the frequency of the two alleles at the ith locus and h is the number of heterozygous loci represented in the genotype (Parks & Werth 1993; but see Gregorius 2005). If $P_{gen} < 0.001$ for a given genotype, then ramets carrying this genotype were assigned to the same genet. Recurring genotypes within populations were excluded from all further analyses.

The number of alleles (A) and expected and observed heterozygosity (H_E and H_O) were obtained using the POPGENE version 1.31 computer program (Yeh *et al*. 1997). GENEPOP version 3.4 (Raymond & Rousset 1995) was used to calculate the inbreeding coefficient (F_{IS}) for each locus in each population, and to test for linkage disequilibrium for all pairs of loci. Conformance to Hardy–Weinberg equilibrium was determined by assessing the significance of the F_{IS} values by means of Fisher's exact tests implemented in the GENEPOP version 3.4 program, with specified Markov chain parameters of 5000 dememorization steps, followed by 1000 batches of 5000 iterations per batch. The sequential Bonferroni correction was applied to obtain critical confidence limits for multiple comparisons, with an initial $\acute{a}$ of 0.05 (Holms 1979).

To examine whether there was any accumulation of genetic diversity in downstream populations we tested for associations between genotypic (G, G_L, P and D) and genetic (A, H_E and H_O) parameters and the position of populations along the course of the river (expressed in metres from the most upstream to the most downstream population), by means of separate regression analyses.

Bayesian-based inference of population structure

We employed several methods to assess population structure. First, the genetic structure of the populations was examined with two fully Bayesian clustering methods: BAPS (Bayesian Analysis of Population Structure) version 3.1 (Corander *et al*. 2003, 2004) and STRUCTURE version 2.1 (Pritchard *et al*. 2000). BAPS version 3.1 estimates hidden population substructure by clustering populations (i.e. geographical sampling locations) into panmictic groups [having a range of reasonable values of (1, N_P), with N_P representing the total number of geographical sampling locations], based on expected Hardy–Weinberg equilibrium and linkage equilibrium between loci within each of the observed populations. BAPS version 3.1 uses stochastic optimization, as opposed to the Markov chain Monte Carlo (MCMC) algorithm used in BAPS 2.0, to infer the posterior mode of the genetic structure (Corander *et al*. 2006). In addition, we used STRUCTURE version 2.1 to obtain a separate insight into how the genetic variation is organized based on the clustering of individuals (rather than populations) without prior information on the population of origin. STRUCTURE version 2.1 uses a Bayesian MCMC approach to cluster individuals into K panmictic groups, by minimizing deviations from Hardy–Weinberg equilibrium and linkage equilibrium. The program calculates an estimate of the posterior probability of the data for a given K, $\Pr(X|K)$ (Pritchard *et al*. 2000). In order to quantify the amount of variation of the likelihood for each K we performed a series of 10 independent runs for each value of K, with K ranging from 1 to the number of geographical sampling locations (N_P) plus one. We assumed an admixture model with correlated allele frequencies, using a length of the burn-in and MCMC iterations of 10 000 each. Longer burn-in and MCMC iterations did not significantly change the results. It has been shown that in many cases $\Pr(X|K)$ may still increase slightly, even after the real K is reached (Pritchard & Wen 2004; Evanno *et al*. 2005), making inferences of K solely based on the highest values of $\Pr(X|K)$ difficult. We therefore used Evanno *et al*.'s (2005) *ad hoc* statistic, ΔK, which is based on the second order rate of change of $\Pr(X|K)$ with respect to K $\{\Delta K = m[|L(K+1) - 2L(K) + L(K-1)|]/s[L(K)]\}$. This *ad hoc* statistic ΔK should show a clear peak at the uppermost hierarchical level of structure at the true value of K (see Evanno *et al*. 2005; for a detailed description).

BAPS and STRUCTURE are fully Bayesian approaches, implicitly assuming that all true populations of origin have been sampled (Manel *et al*. 2002, 2005). As a result, they do not take into account that some individuals may originate (as a result of recent migration) from source locations outside the studied sampling area. To identify potential immigrants from outside our river systems we used Rannala & Mountain's (1997) partial exclusion Bayesian-based assignment method, implemented in GENECLASS version 2.0c (Piry *et al*. 2004), to compute the likelihood of each individual's genotype into each of the inferred clusters. To avoid possible bias as a result of 'self assignment' the 'leave-one-out' procedure was followed, which excludes the tested individual when calculating the allele frequency distribution of their own population. We used the Monte Carlo resampling method by Paetkau *et al*. (2004) implemented in the GENECLASS software, to generate a statistical threshold (using a number of simulated individuals of 10 000) beyond which individuals, whose multilocus genotypes lie outside the 95% likelihood of a population, are likely to be excluded from that population, i.e. they were considered to be immigrants (Berry *et al*. 2004).

Isolation by distance

We followed the method proposed by Rousset (1997) to test the null hypothesis of a single migrant pool over a whole river system against isolation by distance (IBD). Two different distance measures were used to estimate genetic distances among populations: First, traditional F-statistics were used to estimate $F_{ST}/(1 - F_{ST})$ among populations according to Weir & Cockerham (1984), using FSTAT version 2.9.3.2 (Goudet 1995); second, Bayesian-based assignment procedures (Rannala & Mountain 1997) were used to calculate D_{LR}–distances among populations, using the program SPASSIGN (Pálsson 2004). D_{LR}-values (i.e. genotype likelihood ratio distances) represent the average orders of magnitude of the likelihood that the genotypes of individuals, of two populations being compared, are to occur in the individuals' own population, rather than in the other population (Paetkau *et al*. 1997). D_{LR}-values therewith represent an assignment-based measure of distance among populations (Pálsson 2004). A Mantel test was used to test for the presence of isolation by distance, using FSTAT version 2.9.3.2 (Goudet 1995).

Results

Field survey

We found significant differences in plant density, plant morphology and plant biomass within *Sparganium emersum* populations between the Swalm and Rur rivers (Table 1). In the river Swalm plant populations had a significantly

higher mean (SD) plant density compared to plant populations in the river Rur (278.15 ± 68.8 and 100.28 ± 24.1 plants m^{-2}, respectively; d.f. = 1, $F = 64.71$, $P < 0.001$). We also observed differences in plant morphology between rivers: in the river Swalm, only submerged plants were found (i.e. with very fragile, thin and flexible ribbon-formed leaves), whereas in the river Rur, both submerged and emergent plants were observed (i.e. the latter having sturdy, erect, emergent leaves and often a thick flowering stem). This difference in plant morphology was expressed in observed differences in plant biomass between rivers, with significantly lower plant biomass (dry weight) found in populations of the river Swalm, compared to the river Rur (0.41 ± 0.1 and 2.04 ± 0.7 g/plant, respectively; d.f. = 1, $F = 74.49$, $P < 0.001$). These differences in plant density, biomass and morphology coincided with an approximately 10-fold higher stream velocity within plant populations in the river Swalm compared to the river Rur (Table 1).

The differences in plant morphology also reached expression in observed differences in sexual reproduction between rivers, as assessed by the proportion of flowering plants and the seed production per plant. Notably, sexual reproduction was not observed in any of the populations in the river Swalm, whereas it was observed in all, but one (population RAT), of the populations in the river Rur (Table 1).

Genetic and genotypic diversity

The total number of alleles observed per locus in the overall sample of 606 individuals ranged from 8 (SEM14) to 17 (SEM05), with an overall total of 77 alleles scored over six loci (Appendix). Significant departures from Hardy–Weinberg equilibrium were observed in 21 of the 60 single-locus exact tests after sequential Bonferroni correction (populations with $N < 2$ genets not considered; Appendix). There was no evidence for linkage between any of the pairs of loci. Negative overall F_{IS} values were observed in all populations, however, a Hardy–Weinberg global test for heterozygote excess on F_{IS} values across loci revealed a significant heterozygote excess for only two populations in the river Rur (RAT and ORS; $P < 0.05$).

The theoretical number of possible genotypes (N_g), with the six loci used, was 3.26×10^{11}. The P_{gen} values for each multilocus genotype ranged from 1.33×10^{-17}–4.72×10^{-4}. Since, the P_{gen} values did not exceed the threshold of 0.001 for any given genotype, the microsatellite loci used in this study allowed the unequivocal assignment of ramets to clones. There was a large difference in genotypic diversity between the two rivers (Table 1). Compared to plant populations in the Rur River, populations in the Swalm River displayed a significantly lower mean (± SD) number of genotypes G (27.5 ± 12 and 3.9 ± 5, respectively; Mann–Whitney U-test, $U = 5.500$, $P = 0.003$), number of local genotypes G_L (23.6 ± 10 and 3.1 ± 6; $U = 2.500$, $P = 0.001$), proportion of distinguishable genotypes P (0.70 ± 0.3 and 0.11 ± 0.1; $U = 3.000$, $P = 0.001$) and Simpsons' diversity index D (0.87 ± 0.3 and 0.20 ± 0.3; $U = 5.500$, $P = 0.003$). Almost all populations in the Rur River consisted of a large number of genotypes, most of which were unique for that population (Table 1). Of the 248 multilocus genotypes that were found in the Rur River, only 10 occurred in more than one population. These ramet-pairs with identical multilocus genotypes were not restricted to neighbouring populations, but were randomly found between population pairs (regardless of their proximity to each other). In contrast, the populations in the river Swalm were either monoclonal or consisted of a few genotypes only. Moreover, a clear spatial separation of genotypes was observed in the Swalm River: the five populations in the river Swalm situated upstream of Lake Hariksee were dominated by a single genotype, while the three populations situated downstream of Lake Hariksee were also dominated by a single, although different, genotype. Only in the two populations lying at the upstream and downstream edge of Lake Hariksee (BRE and BRU, respectively), a few other genotypes were found (Table 1).

Regression analyses did not reveal any significant associations between genetic (A, H_E and H_O) or genotypic (G, G_L, P and D) parameters and the position of populations along the course of either the Rur or Swalm rivers ($P > 0.05$ for all regressions), indicating that there was no accumulation of genetic diversity in downstream populations.

Bayesian inference of population structure

The BAPS (Corander *et al.* 2004), which used the geographical information given by the sampling location, revealed a strong optimal partitioning of the 17 populations into three clusters (Table 2): cluster 1, consisting of all nine populations of the Rur River; cluster 2, consisting of populations 1–5 of the Swalm River; and cluster 3, consisting of populations 6–8 of the Swalm River. The absolute values of changes in the logarithm of the marginal likelihoods (logml) ranged from 25 to 365 (much larger then the threshold value of 2.3 given by Corander & Marttinen 2005), indicating that the optimal partitioning into these three groups was very stable (Table 2). When analysing the data of the two rivers pooled together, the STRUCTURE (Pritchard *et al.* 2000) analysis could not infer an optimal structuring into K populations: Ln(K) kept increasing with increasing K, even at $K > N_P$, and no clear peak was found after applying Evanno *et al.*'s (2005) posterior ΔK statistic. However, when the number of K populations was estimated for each river separately, the results were very consistent with the outcome of BAPS. For the nine populations of the river Rur an optimal partitioning of $K = 2$ clusters was found (Fig. 2a). The results in Table 3 show that for $K = 2$, the populations are roughly symmetrically assigned to the

Table 2 Population structure of the 17 *Sparganium emersum* populations (Rur and Swalm rivers), inferred from the BAPS analyses. Given are the goodness-of-fit levels, in terms of changes in the natural logarithm of the marginal likelihood of the data (logml-values) if group *i* is moved to cluster *j*, for the optimal clustering solution of BAPS (Corander & Marttinen 2005)

		Inferred population clusters		
River	Population	1	2	3
Rur	RUR	**0**	−25.2	−35.5
	LIN	**0**	−184.5	−319.2
	BRA	**0**	−222.8	−365.0
	TEN	**0**	−228.2	−338.9
	HIL	**0**	−126.8	−242.6
	RAT	**0**	−175.4	−314.2
	ORS	**0**	−198.2	−318.2
	MEL	**0**	−191.9	−353.5
	ROE	**0**	−160.5	−255.8
Swalm	LOC	−46.1	**0**	−35.6
	GEN	−77.0	**0**	−46.8
	LUT	−46.1	**0**	−35.6
	PAN	−46.1	**0**	−35.6
	BRE	−209.5	**0**	−145.2
	BRU	−485.2	−164.6	**0**
	ZWE	−63.2	−37.6	**0**
	HOO	−63.2	−37.6	**0**

two clusters. The results are therefore more in favour of considering the populations of the river Rur as one single population, i.e. $K = 1$, rather than two separate clusters (Pritchard & Wen 2004). For the eight populations of the river Swalm an optimal partitioning of $K = 3$ cluster was found (Fig. 2b). However, when viewing the proportions of individuals greater than 0.5 assigned to each of the three clusters (Table 3), the analysis seems more in favour of two distinct clusters: populations 1–5 and populations 6–8 (Table 3).

The results of both the BAPS and STRUCTURE analyses therefore support a partitioning of the 17 populations into three distinct clusters: cluster 1 (population 1–9 of the Rur), cluster 2 (population 1–5 of the Swalm) and cluster 3 (population 6–8 of the Swalm). The GENECLASS analysis identified seven individuals that could not be assigned to any of the three clusters (three individuals from cluster 1, two from cluster 2, and two from cluster 3), indicating recent immigration events from sources outside our study area (Table 4).

Table 4 The proportion of *Sparganium emersum* individuals assigned to each of the three clusters using GENECLASS 2.0 (Piry *et al*. 2004). NA represents the proportion of individuals that was not assignable to any of the three clusters ($P < 0.05$)

	C1	C2	C3	NA
C1	0.984	0	0	0.016
C2	0	0.875	0	0.125
C3	0	0	0.933	0.067

Mantel tests did not reveal a significant relationship between geographical and genetic distances among populations of the river Rur, for either the Bayesian-based D_{LR} distances ($r = 0.157$, $P = 0.4365$) or the $F_{ST}/(1 - F_{ST})$ distances ($r = 0.242$, $P = 0.225$). The low F_{ST}-values among populations in the Rur (ranging from −0.0009 to 0.0577) and the absence of isolation by distance, concur with the conclusion inferred from the BAPS and STRUCTURE analyses, that the nine populations of the Rur should be viewed as a single population.

Discussion

Genotypic diversity within populations in relation to mode of reproduction

In riverine habitats, hydraulic forces from water currents may have a large impact on plant morphology. Riparian plants respond to increasing water velocity through plastic morphological changes in order to reduce mechanical

Table 3 The proportion of individuals from each sample location assigned to each of the clusters (*K*) inferred from the STRUCTURE analysis, for each river separately. Proportions greater than 0.5 are shown in bold. (N_{gen} = the number of genets in each population; see Table 1)

Inferred population clusters Rur				Inferred population clusters Swalm				
Population	N_{gen}	1	2	Population	N_{gen}	1	2	3
RUR	1	0.013	**0.987**	LOC	1	**0.992**	0.004	0.003
LIN	37	0.477	**0.523**	GEN	2	**0.981**	0.004	0.015
BRA	36	**0.543**	0.457	LUT	1	**0.993**	0.004	0.004
TEN	38	0.500	0.500	PAN	1	**0.994**	0.003	0.003
HIL	21	0.355	**0.645**	BRE	11	**0.560**	0.430	0.009
RAT	32	**0.657**	0.343	BRU	13	0.012	0.005	**0.983**
ORS	30	0.470	**0.530**	ZWE	1	0.006	0.005	**0.989**
MEL	32	**0.607**	0.393	HOO	1	0.008	0.004	**0.988**
ROE	21	0.418	**0.582**					

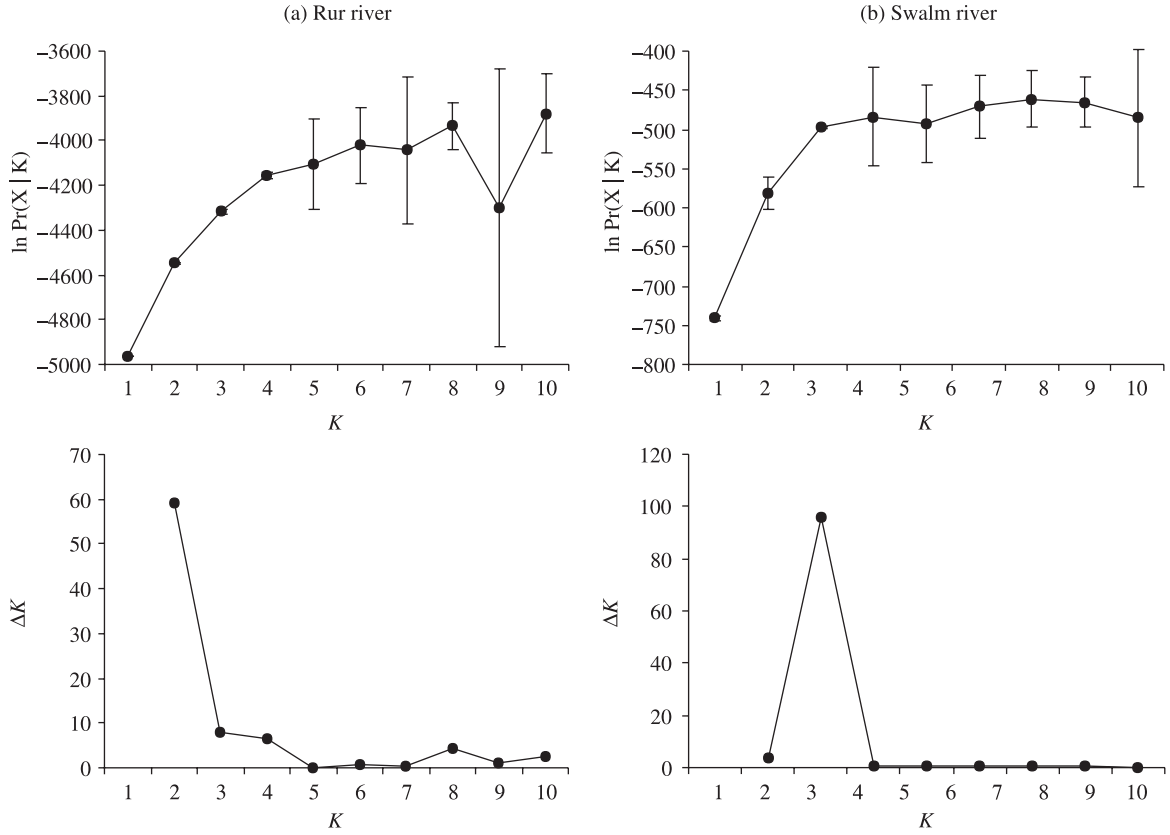

Fig. 2 Results of Bayesian clustering (STRUCTURE, Pritchard *et al*. 2000) of *Sparganium emersum* individuals from (a) the Rur and (b) the Swalm rivers. The upper graphs give the mean ln Pr(*X*|*K*) (± SD) over 10 runs for each value of *K*. The lower graphs give Evanno *et al*.'s (2005) *ad hoc* statistic Δ*K*, showing a peak at the uppermost level of structure at the true value of *K*.

damage (e.g. reduction of plant size and biomass, decreased spacer length leading to higher plant density, increased stem and leaf flexibility reducing rigidity and frontal area) (Chambers *et al*. 1991; Schutten & Davy 2000; Boeger & Poulson 2003; Puijalon & Bornette 2004; Puijalon *et al*. 2005).

Likewise, *Sparganium emersum* will, when subjected to different hydrodynamic conditions, form plants with different morphologies: totally submerged plants in high velocity areas and emergent plants in slow flowing areas (Haslam 1978; Ságová-Marečková & Květ 2002). In the Swalm River, characterized by an approximately 10-fold higher flow velocity compared to the Rur River, only submerged plants were observed, displaying typical morphological adaptations to withstand the associated pulling forces of the water, i.e. reduced plant size and above ground biomass and increased plant density (resulting in a more compact growth form reducing forces on individual ramets) and short, thin and flexible leaves (reducing drag stress; Sand-Jensen 1998). These morphological differences have consequences for the plants' ability for sexual reproduction (Haslam 1978; Boeger & Poulson 2003); since *S. emersum* relies on wind-mediated pollen dispersal, submerged plants are not capable of sexual reproduction.

This difference in the mode of reproduction between *S. emersum* populations of the Swalm and Rur rivers corresponds to a remarkable difference in genotypic diversity. In the Swalm River, the high water velocities induce morphological adaptations that prevent plants from emerging from the water, limiting their ability for sexual reproduction, and ultimately leading to low genotypic diversity within *S. emersum* populations. Whereas, in the Rur River the occurrence of low-velocity patches allows plants to emerge from the water and reproduce sexually, effectively leading to high genotypic diversity within *S. emersum* populations.

Regional population structure in the Rur River

The local mode of reproduction also has an impact on the regional dispersal processes. In the Rur River, where populations were reproducing sexually, the Bayesian-based inference of population structure as well as the low pairwise

genetic distances (F_{ST} values), indicate little genetic differentiation among the nine *S. emersum* populations. The results strongly suggest that the nine populations of the Rur River should be viewed as a single population, with high levels of gene flow occurring between them, in spite of large distances (up to 50 km). The high levels of gene flow most likely arise from hydrochoric dispersal of generative propagules (seeds) between the *S. emersum* populations: (i) sexual reproduction was observed in all of the studied populations (this study); (ii) seed buoyancy experiments have shown that *S. emersum* plants produce long-floating seeds (floating durations ranging from a few days up to several months; Pollux, unpublished); and (iii) germination experiments have shown that seeds remain viable regardless of the duration of their buoyancy (Pollux, unpublished).

Of the 248 genotypes found in the Rur River, only 10 were found in more than one population. This spatial separation of ramets indicates dispersal between populations by means of vegetative propagules (Nilsson *et al*. 1991; Boedeltje *et al*. 2004). The detection of a small number of identical genotypes, however, suggests that dispersal of vegetative propagules is a relatively rare event (Kitamoto *et al*. 2005).

Regression analyses of genetic and genotypic diversity parameters within populations against the position of *S. emersum* populations along the Rur River did not reveal any significant relationships, indicating that there was no accumulation of diversity towards downstream located *S. emersum* populations. Although such associations have been found in a few studies, e.g. in *Potamogeton coloratus* (Gordano Valley, UK), *Angelica archangelica* (Vindel River, Sweden) and *Myricaria laxiflora* (Yangtze River, China) (Gornall *et al*. 1998; Lundqvist & Andersson 2001; Liu *et al*. 2006), most studies failed to reveal any effect of unidirectional gene flow on the pattern of genetic variation along rivers, e.g. in *Mimulus caespitosus* (mountain streams, Washington, USA), *Calycophyllum spruceanum* (Amazon basin, Peru), *Bistorta vivipara* and *Viscaria alpina* (Vindel River, Sweden), *Populus nigra* (Drôme River, France), *Silene tatarica* (Oulankajoki River, Finland) or *Helmholtzia glaberrima* (Toolona creek, Australia) (Ritland 1989; Russel *et al*. 1999; Lundqvist & Andersson 2001; Imbert & Lefèvre 2003; Tero *et al*. 2003; Prentis *et al*. 2004). This lack of genetic erosion in upstream areas may be related to dispersal in an upstream direction, either by means of wind-mediated pollen dispersal or animal-mediated seed dispersal, resulting in the introduction of alleles from downstream to upstream areas (Pollux *et al*. 2005). Several genetic studies have provided evidence for waterfowl-mediated seed dispersal in aquatic plant species (Mader *et al*. 1998; King *et al*. 2002), and a few studies have provided evidence for the occurrence of upstream dispersal events in river systems (Imbert & Lefèvre 2003; Tero *et al*. 2003).

Regional population structure in the Swalm River

The spatial distribution of genotypes, as well as the Bayesian-based inference of population structure, suggests that the eight populations of the Swalm River were (i) monoclonal or dominated by a few genotypes only; and (ii) divided in two independent genetic neighbourhoods, separated by Lake Hariksee.

Two contrasting hypotheses that might explain the emergence of such a population structure are conceivable. First, the six monoclonal populations (together comprising only three genotypes) in the Swalm River may have originated from introductions of a very few individuals to the upper and lower reaches of the Swalm River, which were then followed by local clonal growth. Moreover, plant fragments of *S. emersum* are positively buoyant and have highly regenerative abilities (Barrat-Segretain *et al*. 1998, 1999) and although hydrochoric dispersal of clonal plant fragments may be a relatively infrequent mechanism of dispersal for *S. emersum* (see above; Boedeltje *et al*. 2004), it may, in the absence of seed dispersal, still lead to succesfull colonization of suitable habitat patches (Barrett *et al*. 1993; Kitamoto *et al*. 2005). The hydrochoric dispersal of clonal plant fragments therefore offers a likely explanation why several of the discrete monoclonal populations in the Swalm River, situated (tens of) kilometres apart, consisted of the same genotype (i.e. populations 1–5 and 6–8, respectively). Second, the populations in the Swalm River may originally have consisted of genotypically diverse populations. In a prolonged absence of sexual reproduction (due to a suppression by environmental conditions, see above), genetic processes, such as genetic drift and selection, may subsequently have resulted in the broad dominance of best-fitted 'single-purpose genotypes' (*sensu* Barrett *et al*. 1993; Honnay & Bossuyt 2005). Less adapted clones may have become outcompeted by ramets of more adapted genotypes, ultimately leading to monoclonal populations (Honnay & Bossuyt 2005). However, we found no evidence that, in the past, the hydrological regime in the Swalm River would have allowed sexual reproduction of *S. emersum*, potentially arguing against hypothesis 2. Unfortunately, as historical information about the genetic structure of populations in the Swalm River is not available, we are unable to reliably state which of the two proposed hypotheses is most likely true.

Dispersal between river systems

In both the Rur and Swalm rivers, the GENECLASS analysis revealed a total of seven possible immigrants originating from outside our study area. These immigrants most likely originated from nearby lowland rivers and streams, where *S. emersum* is a common species. Although the vector of dispersal remains unknown, it is known that (i) in these

lowland rivers and streams many waterfowl species are, during fall and winter, feeding on seeds of aquatic plants (e.g. *Sparganium* spp.); and that (ii) ingested *S. emersum* seeds can be internally transported by waterfowl, while remaining viable after gut passage (Pollux *et al*. 2005 and references therein). We therefore suggest that waterfowl-mediated seed dispersal is the most likely vector for plant dispersal between different river systems.

General conclusions

This study shows that spatial heterogeneity in the hydrodynamic regime may induce local differences in the mode of reproduction (sexual vs. asexual) in riparian plant species (e.g. *S. emersum*, *Sagittaria sagittifolia*, *Berula erecta*, *Veronica anagallis-aquatica*; Haslam 1978; Ságová-Marečková & Květ 2002; Boeger & Poulson 2003; Puijalon, unpublished), affecting both the clonal structure and genetic diversity within populations, as well as the regional population structure. The outcome of this study, furthermore, shows that the clonal structure and dispersal processes of riparian plants may differ greatly between river systems, depending on differences in environmental conditions between rivers (see also Kitamoto *et al*. 2005).

Acknowledgements

We thank J. Corander for his comments on the BAPS analysis, and M. Klaassen and two anonymous reviewers for their valuable comments on an earlier version of the manuscript. The plant collections and the fieldwork were conducted under permit T-2003.049 of the Water Board Roer & Overmaas. This is publication 3911 of the Netherlands Institute of Ecology (NIOO-KNAW).

References

Barrat-Segretain MH, Amoros C (1996) Recolonization of cleared riverine macrophyte patches: importance of the border effect. *Journal of Vegetation Science*, **7**, 769–776.

Barrat-Segretain MH, Bornette G, Hering-Vilas-Bôas A (1998) Comparative abilities of vegetative regeneration among aquatic plants growing in disturbed habitats. *Aquatic Botany*, **60**, 201–211.

Barrat-Segretain MH, Henry CP, Bornette G (1999) Regeneration and colonization of aquatic plant fragments in relation to the disturbance frequency of their habitats. *Archiv Fur Hydrobiologie*, **145**, 111–127.

Barrett SCH, Eckert CG, Husband BC (1993) Evolutionary processes in aquatic plant populations. *Aquatic Botany*, **44**, 105–145.

Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? *Molecular Ecology*, **13**, 551–561.

Boedeltje G, Bakker JP, Ten Brinke A, Van Groenendael JM, Soesbergen M (2004) Dispersal phenology of hydrochorous plants in relation to discharge, seed release time and buoyancy of seeds: the flood pulse concept supported. *Journal of Ecology*, **92**, 786–796.

Boeger MRT, Poulson ME (2003) Morphological adaptations and photosynthetic rates of amphibious *Veronica anagallis-aquatica* L. (Scrophulariaceae) under different flow regimes. *Aquatic Botany*, **75**, 123–135.

Chambers PA, Prepas EE, Hamilton HR, Bothwell ML (1991) Current velocity and its effect on aquatic macrophytes in flowing waters. *Ecological Applications*, **1**, 249–257.

Cook CDK, Nicholls MS (1986) A monographic study of the genus *Sparganium* (Sparganiaceae). Part 1. Subgenus *Xanthosparganium* Holmberg. *Botanica Helvetica*, **96**, 213–267.

Corander J, Marttinen P (2005) *BAPS: Bayesian Analysis of Population Structure Manual Version 3.2*. Department of Mathematics, University of Helsinki, Helsinki, Finland.

Corander J, Waldmann P, Sillanpää MJ (2003) Bayesian analysis of genetic differentiation between populations. *Genetics*, **163**, 367–374.

Corander J, Waldmann P, Marttinen P, Sillanpää MJ (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics*, **20**, 2363–2369.

Corander J, Marttinen P, Mäntyniemi S (2006) Bayesian identification of stock mixtures from molecular marker data. *Fishery Bulletin*, in press.

Cox CB, Moore PD (1980) *Biogeography: An Ecological and Evolutionary Approach*. Blackwell Scientific Publications, Oxford.

Dorken ME, Eckert CG (2001) Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *Journal of Ecology*, **89**, 339–350.

Eckert CG (2002) The loss of sex in clonal plants. *Evolutionary Ecology*, **15**, 501–520.

Ellstrand NC, Roose ML (1987) Patterns of genotypic diversity in clonal plant species. *American Journal of Botany*, **74**, 123–131.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Gornall RJ, Hollingsworth PM, Preston CD (1998) Evidence for spatial structure and directional gene flow in a population of an aquatic plant, *Potamogeton coloratus*. *Heredity*, **80**, 414–421.

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate *F*-statistics. *Journal of Heredity*, **86**, 485–486.

Gregorius HR (2005) Testing for clonal propagation. *Heredity*, **94**, 173–179.

Haslam SM (1978) *River Plants*. Cambridge University Press, Cambridge.

Heywood JS (1991) Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics*, **22**, 335–355.

Holms S (1979) A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, **6**, 65–70.

Honnay O, Bossuyt B (2005) Prolonged clonal growth: escape route of route to extinction? *Oikos*, **108**, 427–432.

Imbert E, Lefèvre F (2003) Dispersal and gene flow of *Populus nigra* along a dynamic river system. *Journal of Ecology*, **91**, 447–456.

Jacquemyn H, Brys R, Honnay O, Hermy M, Roldán-Ruiz I (2006) Sexual reproduction, clonal diversity and genetic differentiation in patchily distributed populations of the temperate forest herb *Paris quadrifolia* (Trilliaceae). *Oecologia*, **147**, 434–444.

King RA, Gornall RJ, Preston CD, Croft JM (2002) Population differentiation of *Potamogeton pectinatus* in the Baltic Sea with reference to waterfowl dispersal. *Molecular Ecology*, **11**, 1947–1956.

Kitamoto N, Honjo M, Ueno S *et al*. (2005) Spatial genetic structure among and within populations of *Primula sieboldii* growing beside separate streams. *Molecular Ecology*, **14**, 149–157.

Kudoh H, Shibaike H, Takasu H, Whigham DF, Kawano S (1999) Genet structure and determinants of clonal structure in a temperate deciduous woodland herb, *Uvularia perfoliata*. *Journal of Ecology*, **87**, 244–257.

Liu Y, Wang Y, Huang H (2006) High interpopulation genetic differentiation and unidirectional linear migration patterns in *Myricaria laxiflora* (Tamaricaceae), an endemic riparian plant in the Three Gorges Valley of the Yangtze river. *American Journal of Botany*, **93**, 206–215.

Lui K, Thompson FL, Eckert CG (2005) Causes and consequences of extreme variation in reproductive strategy and vegetative growth among invasive populations of a clonal aquatic plant, *Botumus umbellatus* L. (Botumaceae). *Biological Invasions*, **7**, 427–444.

Lundqvist E, Andersson E (2001) Genetic diversity in populations of plants with different breeding and dispersal strategies in a free-flowing boreal river system. *Hereditas*, **135**, 75–83.

Mader E, van Vierssen W, Schwenk K (1998) Clonal diversity in the submerged macrophyte *Potamogeton pectinatus* L. inferred from nuclear and cytoplasmic variation. *Aquatic Botany*, **62**, 147–160.

Manel S, Berthier P, Luikart G (2002) Detecting wildlife poaching: identifying the origin of individuals with Bayesian assignment tests and multilocus genotypes. *Conservation Biology*, **16**, 650–659.

Manel S, Gaggiotti OE, Waples RS (2005) Assignment methods: matching biological questions with appropriate techniques. *Trends in Ecology & Evolution*, **20**, 136–142.

Nilsson C, Gardfjell M, Grelsson G (1991) Importance of hydrochory in structuring plant-communities along rivers. *Canadian Journal of Botany*, **69**, 2631–2633.

Ouborg NJ, Piquot Y, van Groenendael JM (1999) Population genetics, molecular markers and the study of dispersal in plants. *Journal of Ecology*, **87**, 551–568.

Paetkau D, Waits LP, Clarkson PL, Craighead L, Strobeck C (1997) An empirical evaluation of genetic distance statistics using microsatellite data from bear (Ursidae) populations. *Genetics*, **147**, 1943–1957.

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology*, **13**, 55–65.

Pálsson S (2004) Isolation by distance, based on microsatellite data, tested with spatial autocorrelation (SPAIDA) and assignment test (SPASSIGN). *Molecular Ecology Notes*, **4**, 143–145.

Parks JC, Werth CR (1993) A study of spatial features of clones in a population of Bracken Fern, *Pteridium aquilinum* (Dennstaedtiaceae). *American Journal of Botany*, **80**, 537–544.

Piquot Y, Petit D, Valero M, Cuguen J, de Laguerie P, Vernet P (1998) Variation in sexual and asexual reproduction among young and old populations of the perennial macrophyte *Sparganium erectum*. *Oikos*, **82**, 139–148.

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity*, **95**, 536–539.

Pollux BJA, Ouborg NJ (2006) Isolation and characterization of microsatellites in *Sparganium emersum* and cross-species amplification in the related species *S. erectum*. *Molecular Ecology Notes*, **5**, 530–532.

Pollux BJA, Santamaría L, Ouborg NJ (2005) Differences in endozoochorous dispersal between aquatic plant species, with reference to plant population persistence in rivers. *Freshwater Biology*, **50**, 232–242.

Prentis PJ, Vesey A, Meyers NM, Mather PB (2004) Genetic structuring of the stream lily *Helmholtzia glaberrima* (Philydraceae) within Toolona Creek, south-eastern Queensland. *Australian Journal of Botany*, **52**, 201–207.

Pritchard JK, Wen W (2004) *Documentation for* STRUCTURE *Software: Version 2*. University of Chicago, Chicago, Illinois.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Puijalon S, Bornette G (2004) Morphological variation of two taxonomically distant plant species along a natural flow velocity gradient. *New Phytologist*, **163**, 651–660.

Puijalon S, Bornette G, Sagnes P (2005) Adaptations to increasing hydraulic stress: morphology, hydrodynamics and fitness of two higher aquatic plant species. *Journal of Experimental Botany*, **56**, 77–786.

Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences, USA*, **94**, 9197–9201.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): a population genetics software for exact test and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Ritland K (1989) Genetic differentiation, diversity, and inbreeding in the mountain monkeyflower (*Mimulus caespitosus*) of the Washington Cascades. *Canadian Journal of Botany*, **67**, 2017–2024.

Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics*, **145**, 1219–1228.

Russel JR, Weber JC, Booth A, Powell W, Sotelo-Montes C, Dawson IK (1999) Genetic variation of *Calycophyllum spruceanum* in the Peruvian Amazon Basin, revealed by amplified fragment length polymorphism (AFLP) analysis. *Molecular Ecology*, **8**, 199–204.

Ságová-Marećková M, Květ J (2002) Performance of *Sparganium emersum* Rehm. Shoots in response to sediment quality. *Hydrobiologia*, **479**, 131–141.

Sand-Jensen K (1998) Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. *Freshwater Biology*, **39**, 663–679.

Sargent RD, Otto SP (2004) A phylogenetic analysis of pollination mode and the evolution of dichogamy in angiosperms. *Evolutionary Ecology Research*, **6**, 1183–1199.

Schutten J, Davy AJ (2000) Predicting the hydraulic forces on submerged macrophytes from current velocity, biomass and morphology. *Oecologia*, **123**, 445–452.

Simpson EH (1949) Measurement of diversity. *Nature*, **163**, 668.

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393–430.

Sun S, Gao X, Cai Y (2001) Variations in sexual and asexual reproduction of *Scirpus mariqueter* along an elevational gradient. *Ecological Research*, **16**, 263–274.

Tero N, Aspi J, Siikamäki P, Jäkäläniemi A, Tuomi J (2003) Genetic structure and gene flow in a metapopulation of an endangered plant species, *Silene tatarica*. *Molecular Ecology*, **12**, 2073–2085.

Van Wijk RJ (1988) Ecological studies on *Potamogeton pectinatus* L. I. General characteristics, biomass production and life cycles under field conditions. *Aquatic Botany*, **31**, 211–258.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Widén B, Cronberg N, Widén M (1994) Genotypic diversity, molecular markers and spatial distribution of genets in clonal plants. A literature survey. *Folia Geobotanica et Phytotaxonomica*, **29**, 245–263.

Yeh FC, Yang R, Boyle TBJYeZ, Mao JX (1997) *POPGENE: The User-Friendly Shareware for Population Genetic Analysis*. Molecular Biology and Biotechnology Centre, University of Alberta, Alberta, Canada.

This study is part of Bart Pollux's PhD thesis on the population genetics of aquatic plants in river systems. Maaike de Jong, Anneke Steegh and Erik Verbruggen conducted part of the laboratory work as part of their undergraduate studies in biology. Jan van Groenendael is head of the Department of Ecology and interested in the population biology and dynamics of plant species and the conservation of biodiversity in fragmented landscapes. Joop Ouborg is an Associate Professor who is mainly interested in the population genetics and eco-genomics of plants.

Appendix

Number of alleles (A), expected (H_E) and observed (H_O) heterozygosities, and deviations from HWE (F_{IS}) according to Weir & Cockerham (1984). Values in bold indicate samples which deviate significantly from HWE ($P < 0.05$) after sequential Bonferroni corrections. All calculations are based on genet-level analyses (N_{gen} = the number of distinguishable genets in each population; note that several populations consist of only one genet; see Table 1)

		Swalm River								Rur River									
		LOC	GEN	LUT	PAN	BRE	BRU	ZWE	HOO	RUR	LIN	BRA	TEN	HIL	RAT	ORS	MEL	ROE	
Locus	N_{gen}	1	2	1	1	11	13	1	1	1	37	36	38	21	32	30	32	21	A_{Tot}
SEM01	A	2	2	2	2	3	6	2	2	2	7	5	5	3	4	6	4	4	11
	H_E	1.0000	0.6667	1.0000	1.0000	0.6474	0.8308	1.0000	1.0000	1.0000	0.6467	0.6786	0.5407	0.5528	0.5352	0.5717	0.4981	0.7094	
	H_O	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.6053	0.7568	0.6316	0.4762	0.7500	0.7097	0.6857	0.8800	
	F_{IS}	—	—	—	—	−0.593	−0.214	—	—	—	0.054	−0.109	−0.171	0.142	−0.390	−0.246	−0.391	**−0.229**	
SEM05	A	2	2	2	2	4	3	2	2	2	6	9	12	7	8	8	9	8	17
	H_E	1.0000	0.6667	1.0000	1.0000	0.7835	0.6431	1.0000	1.0000	1.0000	0.6774	0.7653	0.7969	0.7026	0.7184	0.7097	0.8334	0.7967	
	H_O	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8286	0.9444	0.9355	1.0000	1.0000	1.0000	1.0000	0.9524	
	F_{IS}	—	—	—	—	**−0.294**	−0.592	—	—	—	−0.213	**−0.247**	−0.177	−0.425	**−0.396**	**−0.421**	−0.199	−0.174	
SEM08	A	2	2	2	2	5	3	2	2	2	8	10	11	8	7	8	9	8	16
	H_E	1.0000	0.6667	1.0000	1.0000	0.8235	0.6769	1.0000	1.0000	1.0000	0.8477	0.8736	0.8641	0.7944	0.8131	0.8519	0.8396	0.8670	
	H_O	1.0000	1.0000	1.0000	1.0000	0.8889	0.7692	1.0000	1.0000	1.0000	0.9211	0.9412	1.0000	1.0000	0.9091	0.9643	0.8788	0.9583	
	F_{IS}	—	—	—	—	**−0.085**	**−0.143**	—	—	—	**−0.085**	**−0.075**	**−0.160**	**−0.239**	**−0.133**	−0.135	**−0.078**	**−0.086**	
SEM12	A	2	3	2	2	3	3	1	1	2	5	4	5	3	4	4	5	4	10
	H_E	1.0000	0.8333	1.0000	1.0000	0.5750	0.6615	0.0000	0.0000	1.0000	0.3347	0.4532	0.3446	0. 1991	0.4344	0.3435	0.2691	0.6082	
	H_O	1.0000	0.5000	1.0000	1.0000	0.7500	0.0769	0.0000	0.0000	1.0000	0.3784	0.5405	0.3421	0.1053	0.5357	0.3929	0.2941	0.7917	
	F_{IS}	—	—	—	—	−0.333	**0.888**	—	—	—	−0.137	−0.162	0.007	0.477	−0.239	−0.147	−0.085	−0.265	
SEM14	A	2	2	2	2	3	7	2	2	2	5	5	6	3	3	4	5	4	8
	H_E	1.0000	0.6667	1.0000	1.0000	0.6474	0.8431	1.0000	1.0000	1.0000	0.6774	0.6513	0.6414	0.5220	0.5499	0.5717	0.5834	0.6910	
	H_O	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.8286	0.9630	0.8000	0.8421	0.9310	0.7931	0.8824	0.8500	
	F_{IS}	—	—	—	—	−0.593	−0.195	—	—	—	0.110	−0.488	−0.252	−0.624	**−0.714**	−0.397	−0.539	−0.220	
SEM15	A	2	2	2	2	4	4	1	1	1	5	8	7	5	5	6	5	4	15
	H_E	1.0000	0.6667	1.0000	1.0000	0.7273	0.4831	0.0000	0.0000	0.0000	0.7031	0.7559	0.7419	0.6307	0.6769	0.6732	0.7669	0.6738	
	H_O	1.0000	1.0000	1.0000	1.0000	0.7273	0.5385	0.0000	0.0000	0.0000	0.9167	0.7778	0.9189	1.0000	1.0000	0.9677	0.8000	0.5833	
	F_{IS}	—	—	—	—	0.0000	−0.120	—	—	—	−0.308	**−0.093**	−0.243	**−0.573**	**−0.497**	**−0.448**	**−0.076**	**0.022**	
Overall	H_E	1.0000	0.6905	1.0000	1.0000	0.7274	0.5912	0.5714	0.5714	0.7143	0.6597	0.7017	0.6649	0.6006	0.6323	0.6291	0.6503	0.7087	
	H_O	1.0000	0.9286	1.0000	1.0000	0.8705	0.6264	0.5714	0.5714	0.7143	0.6946	0.7729	0.7405	0.7272	0.8483	0.8087	0.7411	0.7908	
	F_{IS}	—	—	—	—	−0.196	−0.060	—	—	—	−0.042	−0.116	−0.116	−0.200	**−0.353**	**−0.292**	−0.158	−0.119	