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Gene flow and genetic structure of the aquatic macrophyte *Sparganium emersum* in a linear unidirectional river

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SUMMARY

1. River systems offer special environments for the dispersal of aquatic plants because of the unidirectional (downstream) flow and linear arrangement of suitable habitats.
2. To examine the effect of this flow on microevolutionary processes in the unbranched bur-reed (*Sparganium emersum*) we studied the genetic variation within and among nine (sub)populations along a 103 km stretch of the Niers River (Germany–The Netherlands), using amplified fragment length polymorphisms.
3. Genetic diversity in *S. emersum* populations increased significantly downstream, suggesting an effect of flow on the pattern of intrapopulation genetic diversity.
4. Gene flow in the Niers River is asymmetrically bidirectional, with gene flow being approximately 3.5 times higher in a downstream direction. The observed asymmetry is probably caused by frequent hydrochoric dispersal towards downstream locations on the one hand, and sporadic zoochoric dispersal in an upstream direction on the other. The spread of vegetative propagules (leaf and stem fragments) is probably not an important mode of dispersal for *S. emersum*, suggesting that gene flow is mainly via seed dispersal. Realized dispersal distances exceeded 60 km, revealing a potential for long-distance dispersal in *S. emersum*.
5. There was no correlation between geographical and genetic distances among the nine *S. emersum* populations (i.e. no isolation by distance), which may be due to the occurrence of long-distance dispersal and/or colonization and extinction dynamics in the Niers River.
6. Overall, the genetic population structure and regional dispersal patterns of *S. emersum* in the Niers River are best explained by a linear metapopulation model. Our study shows that flow can exert a strong influence on population genetic processes of plants inhabiting stream systems.

Keywords: assignment tests, asymmetric bidirectional dispersal, hydrochory, one-dimensional ecosystems, zoochory

Introduction

River systems offer special environments for the dispersal of aquatic plants because of the unidirectional nature of the water flow and linear arrangement of suitable habitats. Water-mediated dispersal (hydrochory) is considered to be the most important mechanism of plant dispersal in rivers (Sculthorpe,

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1967; Haslam, 1978). The seeds of many aquatic and riparian plants float, buoyancy being due to the structure of the seed coat. For instance, spongy or cork-like tissues can contain trapped air, external structures (hairs or wings) may increase the surface area-to-volume ratio and a waxy, cuticularized epidermis will render the seed coat water-impermeable (Sculthorpe, 1967). In addition, vegetative plant parts (e.g. leaf and stem fragments, stolons, rhizomes, tubers, turions) often float for long periods of time while remaining viable, thus potentially contributing to the (long-distance) dispersal among populations (Haslam, 1978; Boedeltje *et al.*, 2003, 2004; Riis & Sand-Jensen, 2006). Although the importance of hydrochoric dispersal was recognized a long time ago (Ridley, 1930), surprisingly little is known about the relative contribution of generative and vegetative propagules to the gene flow and genetic regional structure of plant populations on a whole catchment scale. Molecular techniques are particularly useful for addressing this question, because they enable a distinction between gene flow by seeds and plant fragments (Halkett, Simon & Balloux, 2005).

The flow and the presumed preponderance of hydrochoric dispersal, generates specific hypotheses concerning the expected pattern of gene flow and regional population structure of aquatic plants in streams and rivers. The hydrochoric spread of seeds and vegetative propagules from upstream to downstream may lead to an erosion of genetic diversity in upstream populations (due to a continuous loss of alleles to drift) and accumulation of genetic diversity in downstream populations (due to a continuous influx of alleles) (Ritland, 1989). While a few studies have indeed shown such a relationship between the position of plant populations along the longitudinal course of a river on the one hand and measures of genetic diversity within populations on the other (Gornall, Hollingsworth & Preston, 1998; Lundqvist & Andersson, 2001; Liu, Wang & Huang, 2006), most studies have failed to reveal such associations for reasons which remain, as yet, largely unknown (Ritland, 1989; Russel *et al.*, 1999; Tero *et al.*, 2003; Markwith & Scanlon, 2007).

In addition, different hypothetical models have been proposed about the patterns of dispersal and connectivity among plant populations in linear systems connected by one-way flow (summarized in Fig. 1): the first model represents a 'regional ensemble' (sensu Freckleton & Watkinson, 2002) of highly isolated, persistent populations without any present-day migration between them (Fig. 1a; Tero *et al.*, 2003). Models two and three assume that gene flow occurs only between (temporally persistent) neighbouring populations (Tero *et al.*, 2003; Markwith & Scanlon, 2007), with dispersal being unidirectional in model two (Fig. 1b) and bidirectional in model three (i.e. the 'classical stepping-stone' model; Fig. 1c). The next two models are metapopulation models in that they consist of a series of ephemeral, local populations connected by gene flow that is not restricted to neighbouring populations (Freckleton & Watkinson, 2002; Tero *et al.*, 2003), with dispersal being unidirectional in model four (Fig. 1d) and bidirectional in model five (i.e. the 'classical metapopulation' model; Fig. 1e) (Tero *et al.*, 2003; Liu *et al.*, 2006; Markwith & Scanlon, 2007). Finally, in the last model the patches exist as a 'spatially extended population' (sensu Freckleton & Watkinson, 2002), forming a single genetically uniform panmictic unit with high rates of gene flow among the patches (Fig. 1f; Tero *et al.*, 2003). The assessment of the most suitable linear population model requires a firm understanding of the migration patterns within the study area. However, little is known about the relative frequency and distance of up- and downstream

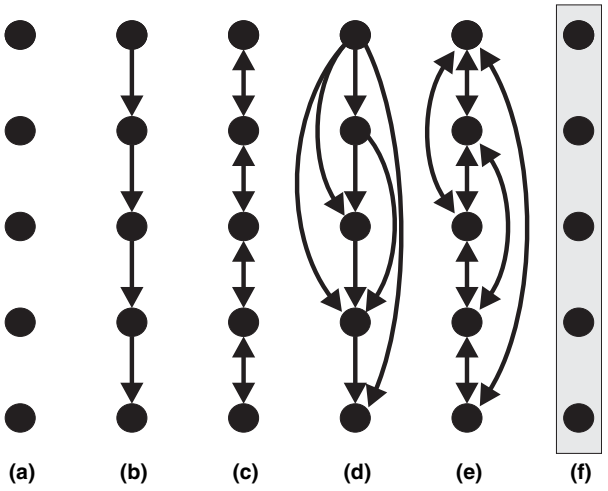

Fig. 1 Schematic representation of the six types of linear population models: (a) regional ensemble, (b) unidirectional gene flow, between neighbouring populations only, (c) classic stepping-stone population, (d) unidirectional gene flow, not restricted to neighbouring populations, (e) classic metapopulation and (f) spatially extended population (Freckleton & Watkinson, 2002; Tero *et al.*, 2003; Markwith & Scanlon, 2007).

dispersal events in linear systems such as rivers and streams (Tero *et al.*, 2003) or the mechanisms responsible for upstream plant dispersal in rivers (Pollux, Santamaría & Ouborg, 2005; Pollux *et al.*, 2006). These questions need to be addressed because these will largely determine the extent of connectivity among populations and hence the scale on which populations will act as independent evolutionary units (Barrett, Eckert & Husband, 1993).

Traditionally, Wright's *F*-statistics have been used to estimate the number of migrants exchanged among populations per generation from molecular data, as $F_{ST} = 1/(4N_em + 1)$, or equivalently $N_em = 1/4(1/F_{ST} - 1)$, with N_e being the effective population size of each population, m the migration rate between populations and, hence, N_em the effective number of migrants exchanged per population (Wright, 1951). The utility of these estimates, however, has been questioned because of the many underlying, biologically unrealistic, assumptions (Whitlock & McCauley, 1999; Neigel, 2002), among which is the assumed symmetry in the rate of gene flow among populations. The recent development of population assignment tests, however, provides a new approach for the assessment of asymmetric dispersal rates among populations based on molecular data (e.g. AFLPs and microsatellites) (Berry, Tocher & Sarre, 2004; Paetkau *et al.*, 2004; Manel, Gaggiotti & Waples, 2005).

To date few studies have examined the effect of unidirectional flow on microevolutionary processes in plants inhabiting stream systems on a whole catchment scale, using assignment analyses. In this study, we employ amplified fragment length polymorphisms (AFLP) markers and population assignment analyses to examine explicit hypotheses concerning the dispersal of aquatic plants along rivers. We choose the unbranched bur-reed *Sparganium emersum* Rehmann 1871 (=*S. simplex* Hudson 1778) (Sparganiaceae) because of the extensive knowledge available regarding its dispersal ecology. Field studies have shown that seeds and vegetative propagules of *S. emersum* are readily captured in traps, suggesting that both can potentially contribute to its dispersal (Boedeltje *et al.*, 2004). Both types of propagules can float for extended periods of time (up to 6 months and 10 weeks, respectively) while remaining viable, indicating that hydrochoric dispersal could take place over considerable distances (Barrat-Segretain, Bornette & Hering-Vilas-Bôas, 1998; Pollux, 2007; Pollux *et al.*, 2008). Furthermore, the seeds of *S. emersum* are internally dispersed by fish and waterfowl, hence potentially being dispersed upstream (Pollux *et al.*, 2005, 2006). Moreover, the transit times of these seeds when passing through the intestinal tract of animals suggest that such zoochory can result in considerable dispersal distances (13.5 and 3600 km for fish and waterfowl, respectively; Pollux *et al.*, 2007a; Pollux, 2007).

The purpose of the present study was to examine the extent and patterns of genetic variability within and among discrete (sub)populations of *S. emersum* along the Niers River (The Netherlands–Germany). Specifically, we asked: (i) What is the relative contribution of seeds and vegetative dispersal to gene flow among populations? (ii) What is the predominant direction of dispersal (e.g. unidirectional, symmetrically bidirectional, asymmetrically bidirectional)? (iii) What are the realized dispersal distances? (iv) Is there a positive correlation between the position of plant populations along the river course and within-population genetic diversity? (v) Which of the proposed linear population models (Fig. 1) is most consistent with the pattern of gene flow and genetic population structure of *S. emersum* in the Niers River?

Methods

Sampling procedure, DNA isolation and AFLP protocol

The Niers River (catchment area 1348 km^2) originates near Kuckum (Erkelenz, close to Mönchengladbach, Germany), flows through Germany (106 km) and The Netherlands (8 km) where it discharges into the Meuse River (near Gennep, The Netherlands) (Fig. 2). In autumn 2004, a total of 283 ramets of *S. emersum* were collected at nine locations along the Niers River (Table 1). In each location, plants were collected at 1–2 m intervals along linear transects running parallel to the shore. Plant samples were immediately transferred to 1.5 mL Eppendorf tubes and stored at −80 °C until DNA extraction. Genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen Benelux B.V., Venlo, The Netherlands). AFLP analyses were performed according to Vos *et al.* (1995) with minor modifications: selective amplification reactions were performed with two primer combinations: primer set 1 (*Eco*RI-ACC/*Mse*I-GCG) and

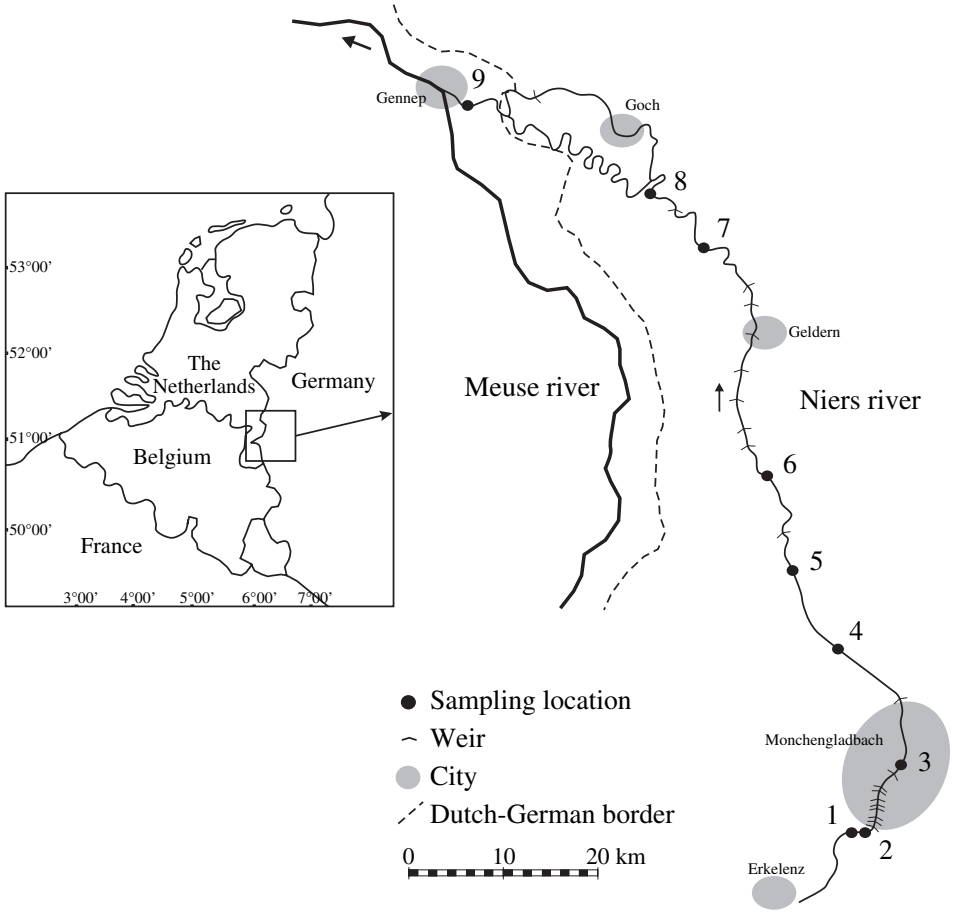

Fig. 2 Map of sampling locations of the nine *Sparganium emersum* populations in the Niers River (The Netherlands–Germany; see Table 1 for more details).

Table 1 Genetic and genotypic diversity statistics for nine *Sparganium emersum* populations along the Niers river

			Genotypic diversity			Genetic diversity*					
						Primer 1 (68)			Primer 2 (88)		
Populations	Location	Geographic Coordinates	N_r	G	P_G	N_{PL}	P_{PL} (%)	I (±SD)	N_{PL}	P_{PL} (%)	I (±SD)
1	Gödderath tunnel	51°7′51.19″N, 6°26′10.02″E	19	17	89.5	41	60.29	0.2323 (0.2479)	9	10.23	0.0314 (0.1079)
2	Gödderath sluis	51°7′51.36″N, 6°26′14.00″E	12	11	91.7	14	20.59	0.0700 (0.1624)	19	21.59	0.0654 (0.1432)
3	Kamphausen	51°8′16.97″N, 6°27′07.10″E	38	38	100	55	80.88	0.4345 (0.2592)	29	32.95	0.1687 (0.2673)
4	Hülsdonk	51°16′3324″N, 6°26′14.63″E	38	37	97.4	36	52.94	0.2072 (0.2537)	28	31.82	0.1230 (0.2164)
5	Oedt	51°19′2218N, 6°22′19.17″E	39	32	82.1	37	54.41	0.1825 (0.2331)	15	17.05	0.0576 (0.1556)
6	Wachtendonk	51°24′4218″N, 6°19′52.47″E	39	39	100	44	64.71	0.2247 (0.2333)	32	36.36	0.1349 (0.2246)
7	Kevelaer	51°35′1790″N, 6°15′12.09″E	36	36	100	57	83.82	0.3744 (0.2520)	44	50.00	0.1843 (0.2306)
8	Schloß Wissen	51°37′4941″N, 6°13′31.14″E	36	36	100	47	69.12	0.3091 (0.2699)	37	42.05	0.1651 (0.2476)
9	Gennep	51°42′21.61″N, 5°57′58.25″E	26	26	100	45	66.18	0.3154 (0.2766)	40	45.45	0.1894 (0.2490)
All populations			283	272	96.1	66	97.06	0.4468 (0.2037)	64	72.73	0.2465 (0.2057)

N_r, number of ramets sampled in each population; G, number of genotypes identified; P, proportion of distinguishable genotypes; N_{PL}, number of polymorphic loci; P_{PL} (%), percentage of polymorphic loci; I, Shannon's diversity index.
*Recurrent genotypes excluded.

primer set 2 (*Eco*RI-AC/*Mse*I-GCA). All PCR reactions were performed on a T3 thermocycler (Biometra®; Göttingen, Germany), using a ramping speed of 1 °C s^{-1}. Fragment separation took place on a model 4200IR^2 DNA Analyser (LI-COR, Lincoln, NE, U.S.A.), using 25 cm denaturing gels with 6.5% polyacrylamide. IRDye size standards (50–700 bp) were included for sizing of the fragments. AFLP band patterns were scored (1 as present, 0 as absent) using the SAGA™ Automated AFLP® Analysis Software (LI-COR). Thirty randomly selected samples were each analysed twice to test the reproducibility of the AFLP protocol. A total of 156 fragments were found that yielded clear and reproducible bands and these were retained for further statistical analyses.

Genetic analyses

Intrapopulation diversity. Within each population, clones were identified by searching for pairs of ramets with identical AFLP genotypes, using the program GENOTYPE (Meirmans & van Tienderen, 2004). Genotypic diversity within populations was calculated as the proportion of distinguishable genotypes $P_G = G/N_r$, with G being the number of identified genets and N_r the number sampled ramets (Ellstrand & Roose, 1987). Within populations, recurrent genotypes were removed from all further analyses. Genetic variation within populations was assessed by calculating Shannon's index of diversity (I; Shannon & Weaver 1949), the number of polymorphic loci (N_{PL}) and the percentage of polymorphic loci (P_{PL}), for each primer pair separately, using the software program POPGENE version 1.31 (Yeh *et al*., 1997). To assess whether the location of populations along the river course affected measures of genetic variation within populations, we performed linear regression analyses of P_{PL} on the geographic distance along the river course, for each primer pair separately, using SPSS 13.0 (SPSS Inc., Chicago, IL, U.S.A.).

Population structure. Several different approaches were used to assess the regional population structure. First, to test the null hypothesis that the nine populations constitute a single panmictic unit, an analysis of molecular variance (AMOVA) was performed to assess the degree of molecular variation within and among populations, using the program ARLEQUIN version 2.000 (Schneider, Roessli & Excoffier, 2000). Secondly, the level of genetic population subdivision was estimated by calculating pairwise genetic distances among populations (Excoffier, Smouse & Quattro, 1992), using ARLEQUIN version 2.000. Exact tests of population differentiation were calculated with the program Tools For Population Genetic Analysis (TFPGA) version 1.3 (Miller, 1999). Analyses were performed with pairwise combinations of populations (using 20 batches and 2000 permutations), based on observed marker frequencies and assuming linkage equilibrium among loci (Miller, 1999). The relationship between pairwise genetic distance (Φ_{ST}) and geographic distance was assessed with a Mantel test implemented in FSTAT version 2.9.3.2 (Goudet, 1995). Thirdly, the regional population structure was examined with STRUCTURE version 2.1 (Pritchard, Stephens & Donnelly, 2000), which employs a Markov chain Monte Carlo (MCMC) approach to cluster individuals into K panmictic groups without *a priori* assignment of individuals to geographical locations, by minimizing deviations from Hardy–Weinberg equilibrium and linkage equilibrium. In order to quantify the amount of variation of the likelihood for each K we performed a series of five 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 a no-admixture model (Pritchard & Wen, 2004) with correlated allele frequencies (Falush, Stephens & Pritchard, 2003), using a length of the burn-in and MCMC iterations of 10 000 each. Evanno, Regnaut & Goudet's (2005) *ad hoc* statistic, ΔK, was used to calculate the uppermost hierarchical level of structure (K).

Dispersal. To identify migration events between the study populations, as well as potential immigrants from outside our study populations, we used a frequency-based assignment procedure implemented in AFLPOP version 1.1 (Duchesne & Bernatchez, 2002). To assess the accuracy of the assignments, the allocation of individuals was performed in three different assignment analyses, each time using a different minimal log-likelihood difference (MLDs of 0, 1 and 2, respectively). An MLD of 0 means that a genotype is allocated to the population in which it has the highest likelihood, whereas an MLD of 2 means that a genotype has to be 10^2 times more likely to be found in population X than in any other population in order to be allocated to population X (Duchesne &

Bernatchez, 2002). The assignment outcome of an individual can be divided into either of four groups: First, correctly assigned individuals (CA), i.e. individuals unambiguously assigned to their population of origin (the likelihood is at least 10^{MLD} times higher in their own population as in the next most likely candidate population, and the *P*-value is larger than the threshold value of 0.001). Secondly, mismatched assigned individuals (MA), i.e. individuals unambiguously assigned to a study population other then their population of origin (likelihood more than 10^{MLD} times higher in one of the other study populations, and $P > 0.001$). Thirdly, ambiguously assigned individuals (AA), i.e. assigned to more than one study population (the difference in likelihoods of assignment between, at least, two study populations, is smaller than 10^{MLD}). Finally, non-assignable individuals (NA), i.e. individuals whose likelihoods are so low that associated *P*-values fall below the threshold value of 0.001; therefore, these individuals are likely to originate from populations other than the study populations (immigrants; Duchesne & Bernatchez, 2002; Berry *et al.*, 2004).

Results

Genotypic diversity within populations was high, with proportions of distinguishable genotypes (P_G) ranging from 82.1 to 100 (Table 1). Clones were found only within populations, not between them, suggesting that the interpopulation dispersal of vegetative propagules is uncommon. Genetic diversity within populations was also high, with a total percentage of polymorphic loci (P_{PL}) over all populations of 83.33% (mean = 44.80%; range = 21.15–64.74) and a Shannon's diversity index (*I*) of 0.3338 (mean = 0.1840; range = 0.0674–0.2846). Genetic diversity within populations (measured as P_{PL}, Table 1) tended to increase from upstream to downstream along the course of the Niers River (primer-pair 1: $R^2 = 0.211$, $P = 0.214$; primer-pair 2: $R^2 = 0.676$, $P = 0.007$; Fig. 3a).

The AMOVA analysis showed that the overall population differentiation was high ($\Phi_{ST} = 0.4032$, $P < 0.0001$), indicating that the populations did not form a single panmictic unit. Of the total genetic variation partitioned in the nine *S. emersum* populations, 40.32% was attributed to the differences among populations (d.f. = 8, $P < 0.00001$), whereas 59.68% was attributed to the differences among individuals within populations (d.f. = 263, $P = <0.00001$). The pairwise genetic distances (Φ_{ST}) between populations varied widely, ranging from 0.08964 (populations 5–6) to 0.58567 (populations 1–6; Table 2). Exact tests of pairwise population differentiation suggested that nearly all of the population pairs were significantly differentiated (at the $P < 0.001$ level), except for populations 8 and 9 ($P < 0.05$) and populations 5 and 6 ($P > 0.05$; Table 2). There was no significant association between pairwise genetic distances (Φ_{ST})

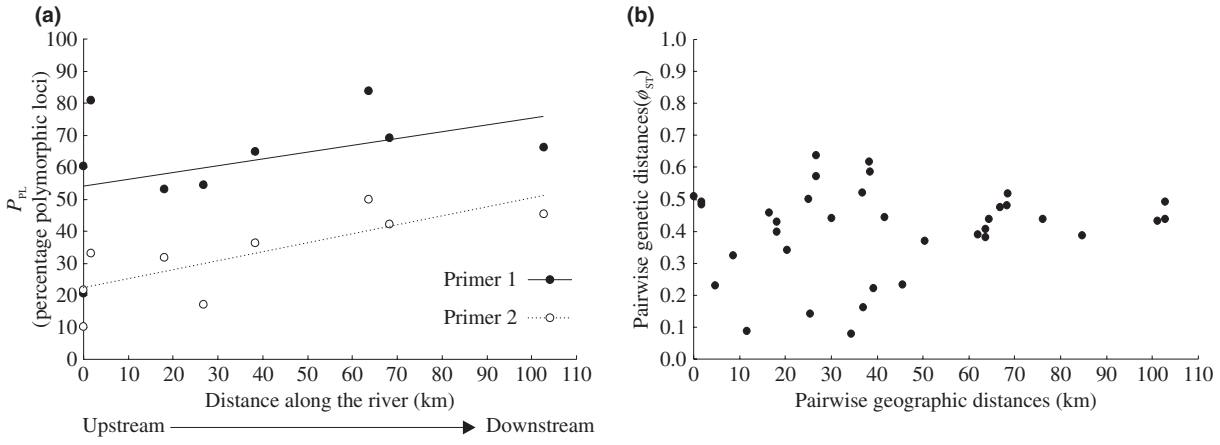

Fig. 3 (a) Percentage of polymorphic loci (P_{PL}) of primer-pair 1 (black circles) and primer-pair 2 (white circles) in nine *Sparganium emersum* populations along the Niers river. Lines represent the linear regressions for primer 1 (solid line) and primer 2 (dashed line) (see text for significancies). (b) Correlation between pairwise genetic (Φ_{ST}) and geographic distances among nine *S. emersum* populations along the Niers river showing an absence of isolation by distance (IBD).

Table 2 Pairwise genetic (Φ_{ST}; below the diagonal) and geographical distances (km; above the diagonal) for nine *Sparganium emersum* populations along the Niers river

Population	1	2	3	4	5	6	7	8	9
1		0.070	1.690	18.090	26.715	38.365	63.665	68.365	102.740
2	0.50926***		1.620	18.020	26.645	38.295	63.595	68.295	102.670
3	0.48407***	0.49379***		16.400	25.025	36.675	61.975	66.675	101.050
4	0.42987***	0.39784***	0.45886***		8.625	20.275	45.575	50.275	84.650
5	0.57406***	0.63893***	0.50278***	0.32618***		11.650	36.950	41.650	76.025
6	0.58567***	0.61751***	0.52105***	0.34119***	0.08964		25.300	30.000	64.375
7	0.40880***	0.38295***	0.39084***	0.23436***	0.16292**	0.14325*		4.700	39.075
8	0.51913***	0.48027***	0.47592***	0.36922***	0.44559***	0.44229***	0.22988***		34.375
9	0.49155***	0.43747***	0.43207***	0.38614***	0.43920***	0.43871***	0.22145***	0.08115	

*$P < 0.05$; ***$P < 0.001$.

and geographic distances (Mantel test: $R^2 = 0.0205$; $P = 0.4240$): At river distances <50 km the pairwise genetic distances (Φ_{ST}) were highly variable, while at distances >50 km the pairwise Φ_{ST} values were invariably high (Fig. 3b). The STRUCTURE version 2.1 analyses revealed a clear peak of Evanno *et al.*'s (2005) *ad hoc* statistic ΔK at $K = 5$, corresponding to a mean (±SD over five runs) $\Pr(X \mid K)$ of −9554.2 (±57.9), suggesting that the nine populations in the Niers River comprise of five clusters of populations: C1 (populations 1 and 2), C2 (population 3), C3 (population 4), C4 (populations 5–7) and C5 (populations 8 and 9) (Table 3; Fig. 4). The frequency-based assignment tests in AFLPOP version 1.1 show that 65.4–80.5% of the individuals were assigned to their population of origin (using an MLD of 0 and 2, respectively), 8.1–2.6% of the individuals were assigned to an upstream located population, 5.5–0.7% of the individuals to a downstream located population, and 6.25% of the individuals could not be assigned to any of the study populations (Table 4; Fig. 4).

Table 3 The proportion of individuals assigned to the five clusters (*K*)

	Inferred population clusters				
Population	C1	C2	C3	C4	C5
1	**1.000**	0.000	0.000	0.000	0.000
2	**1.000**	0.000	0.000	0.000	0.000
3	0.000	**0.987**	0.012	0.000	0.000
4	0.000	0.000	**0.757**	0.243	0.000
5	0.000	0.000	0.000	**0.952**	0.048
6	0.000	0.000	0.000	**0.956**	0.044
7	0.000	0.000	0.000	**0.641**	0.359
8	0.000	0.000	0.000	0.000	**1.000**
9	0.000	0.000	0.000	0.000	**1.000**

Based on Bayesian clustering analyses: proportions > 0.5 are given in bold.

Discussion

Within-population genetic diversity

Measures of within-population genetic diversity found for *S. emersum* (P_{PL}: 21.15–64.74%; *I*: 0.0674–0.2846) are comparable to those found for

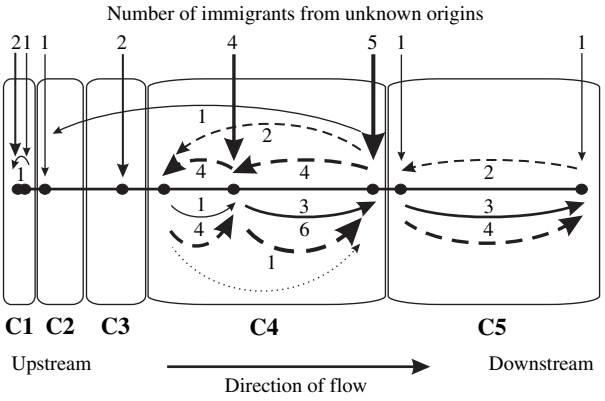

Fig. 4 Summary of the genetic structure of, and connectivity among, nine *Sparganium emersum* (sub)populations along a 103 km stretch of the Niers river. The thick black line represents the Niers river, the black dots indicate the location of the nine (sub)populations, the solid-lined boxes represent the five clusters (C1–C5) inferred from the Bayesian analyses, and the arrows indicate dispersal events between (sub)populations inferred from the population assignment analyses. Arrows to the left indicate dispersal in an upstream direction, arrows to the right dispersal in a downstream direction, solid arrows represent unambiguous dispersal events (i.e. MA individuals, using an MLD of 2), dashed arrows represent ambiguous dispersal events (i.e. MA individuals using an MLD of 0, but AA individuals using and MLD of 2), and vertical arrows represent dispersal events originating from unknown (non-studied) populations.

Table 4 Assignment of *S. emersum* individuals (n = 272) from nine locations in the Niers River

	To												
From	**1**	2	3	4	5	6	7	8	9	Total no individuals	CA (n)	MA (n)	AA (n)	NA (n)
1	**14, 14, 14**	1, 1, 1	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	17	14, 14, 14	1, 1, 1	0, 0, 0	2
2	0, 0, 0	**10, 10, 10**	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	11	10, 10, 10	0, 0, 0	0, 0, 0	1
3	0, 0, 0	0, 0, 0	**36, 36, 36**	0, 0, 0	0, 0, 0	0, 0, 0	1, 1, 1	0, 0, 0	0, 0, 0	38	36, 36, 36	1, 1, 1	0, 0, 0	1
4	0, 0, 0	0, 0, 0	0, 0, 0	**35, 35, 35**	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	37	35, 35, 35	0, 0, 0	0, 0, 0	2
5	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	**26, 22, 16**	4, 0, 0	2, 1, 0	0, 0, 0	0, 0, 0	32	26, 22, 16	6, 1, 0	0, 9, 16	0
6	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	5, 3, 1	**26, 23, 6**	4, 1, 0	0, 0, 0	0, 0, 0	39	26, 23, 6	9, 4, 1	0, 8, 28	4
7	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	1, 1, 0	9, 7, 3	**21, 19, 19**	0, 0, 0	0, 0, 0	36	21, 19, 19	10, 8, 3	0, 4, 9	5
8	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	**33, 31, 28**	2, 1, 0	36	33, 31, 28	2, 1, 0	0, 3, 7	1
9	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	7, 6, 3	**18, 15, 14**	26	18, 15, 14	7, 6, 3	0, 4, 8	1

Three different minimum log-likelihood differences (MLD) were used for the allocation of individuals (0, 1 and 2, respectively; see text for detailed explanation). The bold numbers show the number of correctly assigned individuals found in each population (recurrent genotypes excluded). CA, number of correctly assigned ($P > 0.001$, likelihood of assignment is highest to the population of origin); MA, mismatched assigned ($P > 0.001$, likelihood of assignment is highest in a population other than the population of origin); AA, ambiguously assigned ($P > 0.001$, but likelihood of assignment to the most likely and the second most likely population is smaller than $10^{(MLD)}$); NA, non-assigned ($P < 0.001$) individuals.

most riparian plants in other studies using AFLP-markers: e.g. *Silene tatarica* L. (P_{PL}: 25.9–54.9%; Tero *et al.*, 2003), *Helmholtzia glaberrima* Hook (P_{PL}: 46.8–68.0%; *I*: 0.239–0.352; Prentis *et al.*, 2004) and *Myricaria laxiflora* Franch. (P_{PL}: 12.1–39.7%; *I*: 0.138–0.407; Liu *et al.*, 2006), though much higher levels were reported for *Sisymbrium austriacum* Jacq. (P_{PL}: 74.4–87.8%; Jacquemyn *et al.*, 2006). Regression analyses of genetic diversity within *S. emersum* populations against their position along the Niers River revealed a significant increase of genetic diversity towards populations located downstream (Fig. 3a), consistent with Ritland's (1989) 'unidirectional diversity hypothesis'. Interestingly, although similar associations have been found in a few studies, e.g. in *Potamogeton coloratus* Hornem. (Gordano Valley, U.K.), *Angelica archangelica* L. (Vindel River, Sweden) and *M. laxiflora* (Yangtze River, China) (Gornall *et al.*, 1998; Lundqvist & Andersson, 2001; Liu *et al.*, 2006), the majority of studies failed to show any effect of unidirectional gene flow on the pattern of genetic variation along rivers, e.g. in *Mimulus caespitosus* Greene (mountain streams, WA, U.S.A.), *Calycophyllum spruceanum* Benth. (Amazon basin, Peru), *Bistorta vivipara* L. and *Viscaria alpina* L. (Vindel River, Sweden), *Populus nigra* L. (Drôme River, France), *S. tatarica* (Oulankajoki River, Finland), *H. glaberrima* (Toolona creek, Australia), *Boltonia decurrens* Torr. & Gray (Illinois and Mississippi Rivers, U.S.A.), *S. emersum* (Swalm and Rur Rivers, The Netherlands) and *Hymenocallis coronaria* Le Conte (Savannah, Flint and Cahaba Rivers, U.S.A.) (Ritland, 1989; Russel *et al.*, 1999; Lundqvist & Andersson, 2001; Imbert & Lefèvre, 2003; Tero *et al.*, 2003; DeWoody, Nason & Smith, 2004; Prentis *et al.*, 2004; Markwith & Scanlon, 2007; Pollux *et al.*, 2007b).

The reasons for these inconsistent findings among different studies remain obscure. There seem to be three possible explanations: (i) the occurrence of upstream dispersal, leading to the reintroduction of lost alleles in upstream located populations, may impede the erosion of genetic diversity in upstream river stretches (DeWoody *et al.*, 2004; Markwith & Scanlon, 2007). Nonetheless, despite the occurrence of upstream dispersal, a unidirectional diversity pattern was observed for *S. emersum* in our study (as well as for *M. laxiflora*; Liu *et al.*, 2006), indicating that other factors also play a role; (ii) the influence of unidirectional flow on linear diversity patterns may be more pronounced in species with a long-floating seeds

compared to species with short-floating seeds. A study by Lundqvist & Andersson (2001) in the Vindel River revealed the presence of a unidirectional diversity pattern for *A. archangelica* (which has seeds that can float for over 1 year) yet not for *B. vivipara* and *V. alpine* (which have seeds that float for <2 days). *Sparganium emersum*, like *A. archangelica*, has long-floating seeds (>6 months; Pollux *et al.*, 2008), which might explain the observed unidirectional diversity pattern for *S. emersum* in the Niers River. Notably, however, such unidirectional diversity patterns for *S. emersum* have not been observed in the nearby Swalm and Rur Rivers (Pollux *et al.*, 2007b), implying that seed buoyancy alone cannot explain these patterns; (iii) finally, differences in physical and morphological properties between rivers (e.g. flow regime, channel morphology, presence of dams) may influence plant dispersal patterns (Jansson, Nilsson & Renöfält, 2000; Merritt & Wohl, 2002) in turn leading to different diversity patterns along different rivers. Thus, we argue that upstream spread, long-distance dispersal and/or morphological differences between rivers may all contribute to the observed discrepancies among all these studies (see references above), leading to differences in linear diversity patterns both within and among riparian plant species as well as within and among river systems.

Genetic structure and isolation by distance

The Bayesian clustering approach grouped the 272 *S. emersum* individuals into five population clusters. This grouping was supported by the genetic distances (Φ_{ST}) between populations, which were considerably lower among populations within clusters compared to the high genetic distances among populations from different clusters. We found no significant relationship between pairwise genetic differentiation and geographical distances among populations (no isolation by distance pattern, IBD; Fig. 3b). The genetic differentiation among populations may be affected by a large number of different processes. Among these, three processes are particularly likely to disrupt or obscure potential positive relationships between genetic and geographic distances in rivers:

First, the presence of barriers to migration among populations may cause an absence of isolation by distance within a catchment (Koizumi, Yamamoto & Maekawa, 2006). IBD scatter–plot patterns may be significantly altered by the inclusion of just one or a few highly diverged populations that are isolated due to the presences of physical barriers to dispersal (e.g. dams, weirs, retention areas, isolated backwaters; Koizumi *et al.*, 2006). Although there are a number of small weirs (for the purposes of water level regulation) in the Niers River (Fig. 2), these can be freely passed by seeds and plant fragments (see Fig. 3 in Boedeltje *et al.*, 2003 for a picture of a weir similar to those found in the Niers River). Moreover, the regional genetic structure of *S. emersum* does not appear to be correlated with the locations of these weirs. Therefore, it seems highly unlikely that these weirs restrict gene flow of *S. emersum*.

Secondly, the occurrence of long distance dispersal may also cause a lack of isolation by distance within a catchment (Jacquemyn *et al.*, 2006; Markwith & Scanlon, 2007). Experimental studies indicate a propensity for long distance dispersal of *S. emersum*, both via hydrochory (Pollux *et al.*, 2008) and zoochory (Pollux *et al.*, 2005, 2007a). The results from the present study confirm that seed dispersal of *S. emersum* can take place over long distances (> 60 km). Hence, it is possible that long-distance dispersal contributed to the absence of IBD for *S. emersum* in the Niers River.

Finally, extinction and colonization dynamics may lead to the formation of an age structure, characterized by highly diverged young populations and less diverged older populations (Giles & Goudet, 1997; Pannell & Dorken, 2006). This may result in a wider (than expected under the absence of population turnover dynamics) scattering of pairwise data points around the mean trend line, potentially obscuring isolation by distance patterns (Jacquemyn *et al.*, 2006). The monitoring of 26 *S. emersum* populations, during May 2001–September 2006, did reveal the extinction of one population, indicating the occurrence of population turnover in the Niers River (B. Pollux, unpubl. data). Thus, it is possible that either, or both, the occurrence of long distance dispersal and population turnover dynamics may have contributed to the absence of an IBD pattern for *S. emersum* in the Niers River.

Bidirectional gene flow

In linear ecosystems, such as streams and rivers, generative and vegetative propagules can essentially be dispersed in either of three directions:

downstream, upstream or away from the river corridor (e.g. overland to nearby waterbodies). In this study, assignment tests show that 65.4% of all individuals were assigned to their population of origin, indicating that most seeds sink within their own population. This is consistent with the results from seed floating-experiments, which show that *S. emersum* produces two distinct types of seeds: i.e. short-floating seeds which sink within the population ensuring local recruitment (*c.* 70% of all seeds), and long-floating seeds which float for more than 6 months allowing for long-distance dispersal (*c.* 30%; Pollux *et al.*, 2008). Secondly, about 3.3% of the individuals were assigned to a population other than its population of origin, indicating dispersal among populations. Of these, 2.6% was due to dispersal in a downstream direction, while 0.7% was attributed to dispersal in an upstream direction. Hence, our results indicate that dispersal of *S. emersum* is asymmetric, with gene flow being proportionally higher in a downstream direction. This asymmetric bidirectional migration pattern inferred from population assignment analyses is consistent with those found for *S. tatarica* (Tero *et al.*, 2003) and *M. laxiflora* (Liu *et al.*, 2006). Interestingly, these findings are in stark contrast to the symmetric upstream–downstream migration patterns found for the riparian tree *P. nigra* along the Drôme River (Imbert & Lefèvre, 2003). Imbert & Lefèvre (2003) argued that dispersal among *P. nigra* populations essentially occurred through wind-mediated pollen flow (anemophily) leading to symmetric dispersal rates in upstream and downstream directions. This prompts the hypothesis that the occurrence of asymmetric bidirectional gene flow as found for *S. tatarica*, *M. laxiflora* and *S. emersum* (Tero *et al.*, 2003; Liu *et al.*, 2006; this study) is mainly related to the dispersal of generative and vegetative propagules, rather than pollen flow, with hydrochory being responsible for downstream dispersal and zoochory (e.g. fish- and waterfowl-mediated dispersal) for dispersal in an upstream direction (Pollux *et al.*, 2005, 2006, 2007b). Thirdly, approximately 25% of the individuals were assigned to more than one population. Notably, however, this occurred only between neighbouring (sub)populations within the population clusters, hence being consistent with the genetic structuring inferred from the Bayesian analyses and pairwise differentiation among (sub)populations (Fig. 4). Finally, a small proportion of the individuals (6.25%) was not assigned to any of the study populations (NA), and probably originated from non-sampled populations, either from the Niers River or from nearby lakes and rivers (i.e. inter-catchment dispersal; Pollux *et al.*, 2007b; Fér & Hroudová, 2008).

Of the 272 genotypes found in the Niers River, not a single one was found in more than one population. A study in the nearby Rur River showed that of the 248 genotypes found there, only 10 occurred in more than one population (Pollux *et al.*, 2007b). Surprisingly, both studies suggest that the dispersal of vegetative propagules is a relatively rare occurrence for *S. emersum*, despite the ability of its vegetative plant fragments to float and remain viable for up to 10 weeks (Barrat-Segretain *et al.*, 1998). Boedeltje *et al.* (2004) recently showed that seeds of *S. emersum* were far more frequently encountered in surface traps than its vegetative plant fragments, supporting our conclusion that the spread of vegetative propagules is a relatively unimportant mode of dispersal for *S. emersum*.

Linear population model

In this study, the inference of dispersal events between the (sub)populations, as well as the identification of immigrants originating from populations that were not sampled, argue against the existence of a regional ensemble for *S. emersum* in the Niers River (Fig. 1a). The inference of bidirectional long-distance dispersal, as well as the absence of a isolation by distance pattern, further argue against either a unidirectional or a classical stepping-stone model in which gene flow is restricted to between neighbouring populations (Fig. 1b,c; Tero *et al.*, 2003; Markwith & Scanlon, 2007). Moreover, the Bayesian inference of $K = 5$ population clusters, together with the pronounced genetic differentiation among sampling locations strongly argue against the existence of a single, panmictic, spatially extended population (Fig. 1f). Hence, the regional structure and migration patterns are most in agreement with a classical metapopulation model of *S. emersum* in the Niers River (Fig. 1e).

Linear population models (Fig. 1) will vary with the scale of study, the dispersal traits of the plant species and the physical and morphological features of the catchment. It is therefore difficult to extract general conclusions on plant dispersal from different studies. Most studies reject a classical stepping stone model for riparian plants (Fig. 1b,c), which assumes that

gene flow is restricted to neighbouring populations, because hydrochory can potentially lead to long-distance dispersal of generative and vegetative propagules (Kudoh & Whigham, 1997; Markwith & Scanlon, 2007). A linear metapopulation model with asymmetric bidirectional gene flow that is not restricted to neighbouring populations is the most commonly accepted migration model for riparian plants (Kudoh & Whigham, 2001; Tero *et al*., 2003; DeWoody *et al*., 2004; Prentis *et al*., 2004; Liu *et al*., 2006). Here, the skewness of dispersal is most likely to originate from frequent hydrochoric dispersal of seeds and vegetative propagules downstream and sporadic zoochoric dispersal upstream. Nonetheless, in some riparian trees (e.g. *P. nigra*) a metapopulation with symmetric dispersal may be more appropriate. These typically pertain to species in which wind-mediated pollen dispersal is the most important mode of interpopulation gene flow and the dispersal of seeds and vegetative propagules plays no significant role (Imbert & Lefèvre, 2003). It is important to note, however, that a single species may display markedly different linear population structures when inhabiting different river systems due to the locally reigning environmental conditions (e.g. channel morphology, presence of dispersal barriers, current velocity, light availability) that may affect both its reproductive strategy as well as its regional dispersal patterns (Pollux *et al*., 2007b; this study).

Finally, although, to date, most studies have focussed on linear models of connectivity in river systems (though see Kudoh & Whigham, 1997), streams and rivers are in fact not linear in nature, but instead consist of a hierarchical network of corridors and tributaries. The dendritic nature of riparian landscapes is likely to affect the patterns of connectivity as well as the dynamics and persistence of plant (meta)populations (Honnay, Verhaeghe & Hermy, 2001; Fagan, 2002; Muneepeerakul *et al*., 2007). Future studies, examining the gene flow and genetic structure of plant populations in these landscapes, are therefore likely to provide valuable new insights into the microevolutionary processes of plants inhabiting dendritic ecosystems.

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