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Spatiotemporal structure of genetic variation of a spreading plant metapopulation on dynamic riverbanks along the Meuse River

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Long-distance seed dispersal is a crucial determinant of within-population genetic variability and among-population genetic differentiation in plant metapopulations undergoing recurrent local extinctions and (re-)colonization. We investigated the spatial and temporal structure of genetic variation in a metapopulation of *Sisymbrium austriacum* located along a dynamic river system using dominant AFLP markers. Data on riverbank dynamics and colonization history allowed separating populations based on their age (≤ 5 *vs* >5 years old). Bayesian analysis of population genetic structure indicated that populations were significantly differentiated from each other, but Mantel tests revealed that there was no relationship between pairwise geographic and genetic distances, suggesting that long-distance seed dispersal partly determines spatial genetic structure. Recent populations were less differentiated from each other than old populations. Analysis of molecular variance (AMOVA) indicated that both spatial factors and population age significantly determined genetic diversity, the effects of age being more important than spatial location. Clustering analysis revealed five large clusters, which were related primarily to population age and to a minor extent to geographical location. Our results indicate that the recurrent formation and destruction of riverbank habitats following peak flow events have a large impact on genetic diversity of riparian plant species.
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Introduction

Seed dispersal is a crucial component for the persistence and structure of plant metapopulations (Hanski, 2001; Higgins and Cain, 2002), because it affects the rate of population spread (Clark, 1998; Higgins and Richardson, 1999; Neubert and Caswell, 2000), recruitment patterns and colonization of empty habitat patches (Ehrlén and Eriksson, 2000; Jacquemyn *et al*, 2003) and species survival in patchy landscapes (Verheyen *et al*, 2004). Although new methods have been developed to determine levels of seed dispersal (Nathan *et al*, 2003; Tackenberg, 2003; Katul *et al*, 2005), estimating the long end of the dispersal tail remains challenging. This has become especially clear when it was shown that nonstandard mechanisms of seed dispersal may be far more important in determining long-distance seed dispersal and spatial spread of plant species than one would expect based on morphological dispersal syndromes alone (Higgins *et al*, 2003).

Molecular markers and the resulting measures of gene diversity and structure may offer some additional information on the relative importance of long- and short-distance dispersal in structuring plant metapopulations (Ouborg *et al*, 1999; Cain *et al*, 2000; Nathan *et al*, 2003). Indeed, next to its demographic consequences, long-distance seed dispersal also affects genetic diversity and structure of plant metapopulations (Ibrahim *et al*, 1996; Austerlitz and Garnier-Géré, 2003; Bohrer *et al*, 2005). In general, the genetic consequences of colonization and migration in metapopulations have been well studied (eg Slatkin, 1977, 1993; Wade and McCauley, 1988; Whitlock and McCauley, 1990; Pannell and Charlesworth, 2000), yet most of these models do not discriminate between short- and long-distance dispersal. However, incorporating the formal distinction between short- and long-distance dispersal in genetic metapopulation models largely affects the outcome of colonization–extinction dynamics on genetic diversity and differentiation.

Le Corre and Kremer (1998), for example, showed that the genetic consequences of a range of expansion of a metapopulation depended on the relative magnitudes of the number of colonists and migrants and on the age structure that is established during colonization. In linear-stepping stone models, in which short distance gene flow is prevalent, the cumulative effects of founder events were strongest, whereas in the island model in which long-distance gene flow is the predominant mode of gene dispersal, the effects were weaker. In addition, Bohrer *et al* (2005), using a spatially explicit simulation model to investigate the effects of long-distance dispersal

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on total and within-population genetic diversity and among-population genetic differentiation in a metapopulation undergoing different rates of local extinction, showed that the effects of long-distance dispersal were dependent on the extinction rate and initial population conditions. In initially 'well-mixed' populations, total and within-population diversity decreased when long-distance dispersal was the prevailing mode of dispersal, whereas in drifted populations (ie populations with initially skewed genotype distributions), long-distance seed dispersal maintained genetic variability. Genetic differentiation, on the other hand, increased under long-distance seed dispersal at intermediate to high extinction rates, but at low extinction rates long-distance seed dispersal prevented genetic differentiation.

In plant metapopulations, genetic diversity and differentiation may also be affected by pollen flow as gene flow may occur through the transfer of both seeds and pollen (McCauley, 1994). Whereas long-distance seed dispersal mostly results in decreased within-population genetic diversity and increased differentiation, pollen flow after population foundation may quickly erode any signatures left in patterns of genetic diversity arising from colonization by seeds. However, the way in which pollen flow affects genetic differentiation largely depends on colonization patterns by seeds. In diffusive colonization models, in which newly founded populations are adjacent to existing populations, pollen flow results in strong homogenization compared to models in which rare long-distance colonization events result in newly founded populations that remain separated by gaps for such a long time that significant differentiation occurs and any possible homogenization by pollen flow is prevented (Austerlitz and Garnier-Géré, 2003).

Dynamic river systems are excellent model systems to study the effects of metapopulation turnover on genetic diversity and differentiation (Kudoh and Whigham, 1997, 2001; Tero *et al*, 2003; DeWoody *et al*, 2004) and to evaluate the relative importance of short- and long-distance seed dispersal in determining long-term persistence of the metapopulation (Menges, 1990; Ouborg, 1993; Jäkäläniemi *et al*, 2005). Indeed, ephemeral habitat patches and recurrent local extinction and colonization are characteristic of these dynamic river systems (Lytle and Poff, 2004). In this study, we investigated the structure of genetic variation among and within populations of the short-lived plant species *Sisymbrium austriacum* Jacq. using dominant AFLP markers. This species, originally occurring in the Pyrenees and the Alps, has recently (from the early seventies onwards) colonized dynamic riverbanks along the Meuse River in Belgium and the Netherlands. Known data on riverbank dynamics and the history of population colonization of the species allowed us to distinguish old populations (>5 years old) displaying low extinction probabilities from recent populations (≤ 5 years old) with high extinction probabilities. More specifically, the following questions were addressed: (1) How is genetic diversity partitioned among young and old populations within this metapopulation? (2) Does the spatial location of populations affect genetic diversity and differentiation? (3) Do the colonization and spatial spread of *S. austriacum* reflect higher proportions of short- *versus* long-distance dispersal events?

Materials and methods

Study species

S. austriacum (jeweled rocket) (Brassicaceae) is a small, biennial or perennial, diploid ($2n = 14$) species originally occurring in the mountainous regions of South and Mid-Europe and the Pyrenees (Hegi, 1986). In its original distribution area, *S. austriacum* is a typical pioneer plant species that colonizes organic disposals on rocky soils, often in shady places or under inclining rocks. Plant height varies between 30 and 60 (80) cm. The plant is biennial, but may survive for longer time periods (more than 5 years) (K Van Looy, personal observation). In this case, old individuals can easily be recognized in the field by their large rooting system (more than 2 cm in diameter) and more than eight flower branches. Flowers are self-compatible and strictly pollinated by insects (bees and syrphid flies). The species flowers from June till September and seeds (mean seed weight: 0.3 mg) are dispersed by the wind (anemochory) or fall directly on the ground (barochory). In addition, seeds may be transported by water through the transfer of flowering branches.

Study system and study populations

The study area is situated in the Meuse River valley situated on the border between Belgium and the Netherlands. The Meuse River is for the largest part of its course a regulated river, but in the study area, an ongoing project aims at restoring the natural flow regime of the river. In Belgium, *S. austriacum* was first observed in 1824 by Lejeune on the shores of the Vesder River, one of the Ardennes' tributaries of the Meuse, where it was most likely introduced as a result of the transport of sheep wool (Van Landuyt *et al*, 2006). Probably, seeds germinated along the river and the species slowly expanded its range northwards. The first *S. austriacum* populations have been observed in the Meuse River some 30 years ago, although at that time populations were not persisting. However, during the last 10 years, several permanent populations have been established (see below). Along the Meuse River, the species mainly occurs in warm and sunny places on sand or gravel.

During the last 25 years, at least five (1982, 1993, 1995, 2000 and 2002) major water flushes (more than 2.200 m^3/s) occurred, resulting in overbank depositions of sand and gravel banks. Colonization of these sediment zones resulted in more or less permanent populations, in contrast to populations occurring on riverbanks in the immediate neighborhood of the river, where populations are rather short-lived and subject to yearly fluctuations of water levels. In 2004, 14 populations were found in this dynamic river system (Figure 1), five of which were located outside the regular influence of the river, were more than 5 years old and considered persisting populations and nine that were located in the immediate neighborhood of the river and were less than 5 years old. For each population, we determined population size by counting the number of flowering individuals. Most populations were small and consisted of less than 100 flowering individuals. The straight-line distance between populations was determined directly on the basis of site coordinates (mean distance: 7.23 km, range: 0.53–16.95 km) (Figure 1). Besides, distances between populations were also measured along the river using GIS

Figure 1 Study area and location of the 14 study populations of *S. austriacum* along the Meuse River. The arrow depicts the direction of the water flow.

(ArcView 3.2). In this case, distances between populations ranged from 0.56 to 25.60 km (mean: 9.77 km).

Sampling scheme for genetic analyses and AFLP protocol

In Spring 2004, a total of 242 individuals was sampled from the 14 populations. Individuals were sampled from the entire area occupied by the population in order to avoid the effects of population substructure. Young leaf material was collected and immediately frozen in liquid nitrogen. Before DNA extraction, leaf material was freeze-dried and homogenized with a mill (Retsch MM 200) to a fine powder. Total DNA was extracted from 20 mg of lyophilized leaf material using Dneasy Plant Mini Kit (Qiagen). After extraction, DNA concentrations were estimated on 1.0% (w/v) agarose gels.

AFLP analysis was carried out according to Vos *et al* (1995). The enzymes *Eco*RI and *Mse*I were used for DNA digestion. Each individual plant was fingerprinted with four combinations: *Eco*RI-AAG/*Mse*I-CAT, *Eco*RI-ATC/*Mse*I-CTA, *Eco*RI-AAG/*Mse*I-CAG and *Eco*RI-ATC/*Mse*I-CCA. Fragment separation and detection took place on a Nen IR^2 DNA analyzer (Licor) using 36 cm denaturing gels with 6.5% polyacrylamide. IRDye size standards (50–700 bp) were included for sizing of the fragments. AFLP patterns were scored using the SAGAmx.software from Licor. We scored the presence or absence of each marker in each individual plant. Twenty randomly selected samples where loaded three times to infer reproducibility of the AFLP protocol. Average similarity between replicated samples was very high (more than 95%).

Data analysis

Population genetic structure was analysed using the Bayesian methods proposed by Holsinger *et al* (2002) and Holsinger and Wallace (2004). These methods allow direct estimates of F_{ST} from dominant markers without previous knowledge of the degree of within-population inbreeding and they do not assume that genotypes are in Hardy–Weinberg equilibrium. Although model outcomes for f (an estimate of F_{IS}) may not always be very accurate, results for θ^B (an estimate of F_{ST}) are mostly very informative (Holsinger *et al*, 2002). We tested several models using Hickory version 1.0: (i) a full model with noninformative priors for f and θ^B, (ii) a model in which $f = 0$ and (iii) a model in which $\theta^B = 0$. The three models were compared using the deviance information criterion (DIC) (Holsinger and Wallace, 2004). The model with the smallest DIC value was chosen. We also calculated $I_E(x)$, an often used measure of the information provided about a parameter in Bayesian inference (Holsinger and Wallace, 2004), for f and θ^B. Analyses were performed for all populations, and for young, dynamic and old, persisting populations separately. Several runs were conducted with default sampling parameters (burn-in = 50 000, sample = 250 000, thin = 50) to ensure that the results were consistent.

To investigate the relative importance of spatial and temporal dynamics on genetic structure, we used AMOVA (Excoffier *et al*, 1992). Total genetic diversity was partitioned among groups of populations, among populations within groups and within populations by carrying out a hierarchical AMOVA on Euclidean pairwise distances among individuals using GenAlEx v. 6 (Peakall and Smouse, 2005). Three different models were tested: a model without any structure, a model in which an age structure was incorporated and a model in which spatial structure was included. In the second model, two subgroups were defined, old, persisting populations, and young dynamic populations. Spatial groups were defined based on their geographical position along the river: upper (Elsloo, Meers 1, Meers 2 and Meers 3), middle (Maasband, Mazenhoven, Meeswijk, Kerkeweerd 1, Kerkeweerd 2 and Kerkeweerd 3) and lower course populations (Elereert, Heppeneert, Roosteren 1 en Roosteren 2) (Figure 1). Significance of the three models was determined using a permutation test ($n = 9999$ permutations).

Pairwise genetic distances (Φ_{ST}) among the 14 *S. austriacum* populations and their levels of significance were also determined from the AMOVA using GenAlEx v. 6 (Peakall and Smouse, 2005). To illustrate the relationship among populations based on their pairwise genetic distances, an UPGMA dendrogram between all populations was constructed using Nei's unbiased genetic distance. Phylip 3.6.15 (Felsenstein, 1993) was used to construct the dendrogram. Pairwise genetic distances were plotted against geographic distances to test for regional equilibrium and to evaluate the relative influences of gene flow and drift on genetic structure (Hutchison and Templeton, 1999). Significance of the observed relationships was obtained by using a Mantel test (Mantel, 1967). A total of 5000 random permutations were performed.

Three measures of within-population genetic diversity were estimated: the number of polymorphic loci, Nei's gene diversity and the Bayesian estimate of gene diversity. Nei's gene diversity was calculated using AFLPsurv v.1.0 (Vekemans *et al*, 2002). Estimates of allelic frequencies at AFLP loci were calculated using the square root method. Although this method may lead to biased results of averaged estimates of heterozygosity and genetic differentiation when the number of polymorphic loci is low (Lynch and Milligan, 1994; Zhivotovsky, 1999), Krauss (2000) has shown that in highly polymorphic data sets, no statistical difference between methods was found. After estimation of allele frequencies, statistics of gene diversity and population genetic structure were computed according to Lynch and Milligan (1994). For each population, we calculated the number and proportion of polymorphic loci at the 5% level and Nei's gene diversity (H_j). Bayesian estimates of gene diversity (H_{eH}) were calculated using Hickory v.1.0. Differences among young and old populations in the percentage of polymorphic loci and both measures of gene diversity were investigated using *t*-tests. To investigate whether population size was related to the percentage polymorphic loci and both measures of gene diversity, Pearson's product moment correlations were used.

Results

Genetic structure and among-population differentiation

The four primer combinations resulted in 81 unambiguously scorable bands. No monomorphic bands were scored. Band frequencies ranged from 18.59 to 93.80 and each examined individual showed a unique banding pattern. The Bayesian analysis of population structure indicated significant differentiation among populations. Based on the DIC values of the three models tested, it was concluded that the full model performs far more better than the alternative models in which $f=0$ or $\theta^B=0$ (Table 1). In the full model, the posterior mean of θ^B over all populations was 0.0826 (SD: 0.0085) and significantly different from zero (95% confidence interval: 0.0662–0.0983). The point estimate of f was 0.4008 (SD: 0.1739) with a 95% confidence interval (0.0452–0.7001). The information provided by the data $I_E(x)$ was larger for θ^B than for f (3.356 and 0.356, respectively). The traditional estimate of F_{ST} calculated from allele frequencies assuming Hardy–Weinberg equilibrium was slightly higher than the Bayesian estimate ($F_{ST}=0.0968$). The AMOVA ($\Phi_{ST}=0.0905$) estimate gave a value somewhere between the Bayesian and the traditional estimate. Estimates of θ^B for young and old populations are also given in Table 1. In both cases, the full model was preferred above the alternative models. The posterior mean of θ^B over old populations was 0.0868 (SD: 0.0106, 95% confidence interval: 0.0682–0.1085), whereas in young populations, this was slightly lower (posterior mean: 0.0717; SD: 0.0087, 95% confidence interval: 0.0558–0.0894).

Results of the AMOVA analysis further indicated that both spatial and temporal variables significantly determined genetic variation in *S. austriacum* (Table 2). The

Table 1 Model comparison of posterior means of f (an estimate of F_{IS}) and θ^B (an estimate of F_{ST}) for all populations and young and old populations separately

Model	f	θ^B	*DIC*
All populations			
Full model	0.4008	0.0826	**4485.1198**
$f=0$		0.0655	4533.9477
$\theta^B=0$	0.4290		5516.8933
Young populations			
Full model	0.2998	0.0717	**2568.2319**
$f=0$		0.0597	2583.9290
$\theta^B=0$	0.4790		3073.7379
Old populations			
Full model	0.3011	0.0868	**1952.0403**
$f=0$		0.0725	1965.3213
$\theta^B=0$	0.4443		2431.8826

Model selection was based on the DIC. Best models are indicated in bold.

Table 2 Effects of spatial and temporal colonization dynamics on population genetic variation among 242 *S. austriacum* individuals located in 14 spatially separated populations as determined by AMOVA

Source	*d.f.*	*Sum of squares*	*Variance components*	*% of the total variance*	P-*value*
No regional or temporal structure					
Among populations	13	438.887	1.237	9.05	<0.001
Within populations	228	2833.137	12.426	90.95	<0.001
Total	241	3272.025			
Regional structure					
Among groups	2	76.615	0.070	0.52	0.009
Among populations within groups	11	362.273	1.188	8.68	<0.001
Within populations	228	2833.137	12.426	90.80	<0.001
Total	241	3272.025			
Temporal structure					
Among age groups	1	89.781	0.552	3.96	<0.001
Among populations within age groups	12	349.106	0.967	6.94	<0.001
Within populations	228	2833.137	12.426	89.11	<0.001
Total	241	3272.025			

Table 3 Pairwise genetic distances (Φ_{ST}) among 14 *S. austriacum* populations

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
1		***	***	***	***	***	***	NS	***	***	NS	***	***	***
2	0.060		***	***	***	***	***	***	**	***	*	***	***	***
3	0.156	0.113		**	***	***	***	***	***	***	***	***	***	***
4	0.164	0.115	0.029		***	***	***	***	***	***	***	***	***	***
5	0.185	0.111	0.130	0.118		NS	*	***	***	**	***	***	***	***
6	0.192	0.133	0.106	0.095	0.024		NS	***	***	***	***	***	***	***
7	0.152	0.072	0.100	0.121	0.034	0.007		***	**	**	***	**	***	***
8	0.007	0.055	0.137	0.166	0.176	0.186	0.129		***	***	*	***	***	***
9	0.075	0.034	0.070	0.073	0.085	0.087	0.052	0.058		NS	**	*	*	**
10	0.099	0.058	0.101	0.080	0.053	0.081	0.047	0.085	0.011		***	*	*	***
11	0.016	0.035	0.134	0.154	0.188	0.177	0.135	0.038	0.066	0.110		***	***	***
12	0.124	0.105	0.104	0.094	0.073	0.067	0.060	0.143	0.030	0.028	0.131		NS	NS
13	0.109	0.077	0.097	0.090	0.068	0.058	0.048	0.113	0.037	0.029	0.112	0.003		NS
14	0.111	0.078	0.090	0.086	0.100	0.089	0.060	0.123	0.046	0.055	0.101	0.020	0.004	

Values and levels of significance are given in the lower left and upper right triangle, respectively. Significances were based on random permutations ($N = 9999$) and indicate the probability that that random Φ_{ST} values are higher than the observed values.
NS: not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

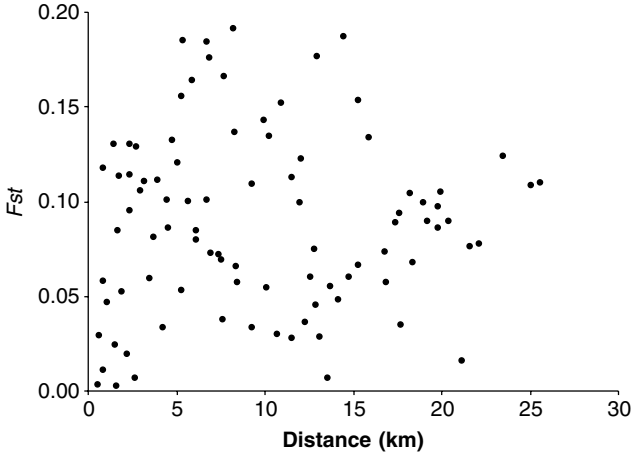

Figure 2 The relationship between pairwise genetic (F_{ST}) and geographic distances for 14 populations of *S. austriacum* along the Meuse River.

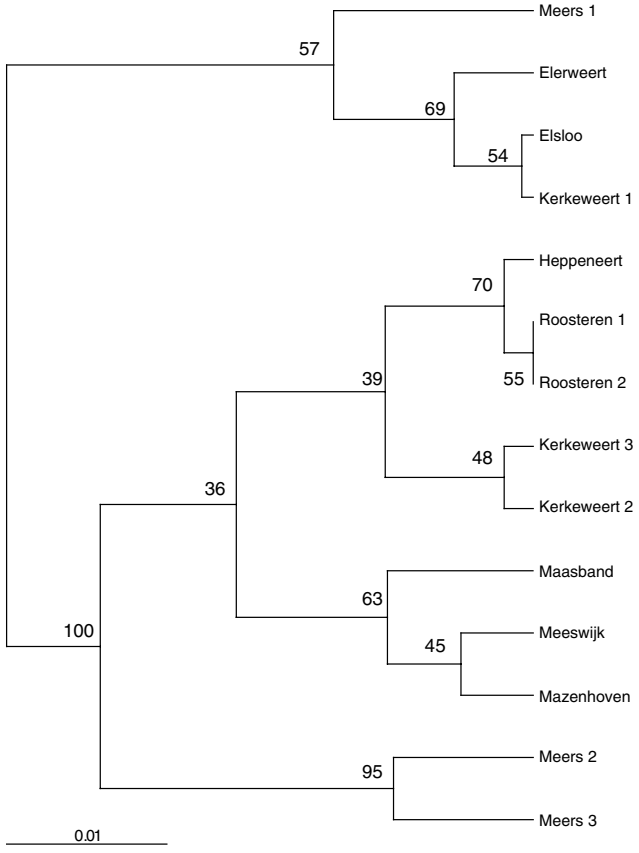

Figure 3 UPGMA dendrogram of the 14 populations of *S. austriacum* based on Nei's unbiased genetic distance. Bootstrap values (based on 1000 iterations) are indicated for each node.

regional component was, however, less pronounced than the temporal component. Pairwise Φ_{ST} values (14 populations, 91 pairs) ranged from 0.003 to 0.192; 82 (90%) *P*-values were significant at the 5% level (Table 3). Only in the case of nearby populations, pairwise Φ_{ST} were not significant. Pairwise geographic distances (both straight-line distances and distances measured along the river) and pairwise genetic distances were not significantly ($P > 0.05$) related ($r_M = 0.007$ and 0.016, respectively) (Figure 2).

The UPGMA dendrogram showed that old populations (Elsloo, Meers 1, Kerkeweert 1 and Elerweert) formed a clearly distinct group in the UPGMA dendrogram. Furthermore, nearby, young populations often formed clusters (eg Mazenhoven–Meeswijk–Maasband, Heppeneert–Roosteren1–Roosteren2, Kerkeweert 2–Kerkeweert3) (Figure 3).

Total and within-population genetic diversity

The proportion of polymorphic loci ranged from 74.4 to 87.8 (Table 4). Total gene diversity and average gene diversity measured over all loci and all populations was high ($H_t = 0.3791$, $H_w = 0.3424$). Within-population diversity ranged from 0.3286 to 0.3621 (Table 4). The Bayesian estimate resulted in similar values. There was no significant ($r_s = 0.13$, $P > 0.05$) relationship between population size and the proportion of polymorphic loci or gene diversity. Within-population diversity was also not significantly ($P > 0.05$) different between old

Table 4 Characteristics of the 14 *S. austriacum* populations

Nr	*Population*	*Population size*	*Age*	n	P	H_j	H_{eH}
1	Elsloo	96	2	17	81.7	0.3551	0.3493
2	Meers 1	49	2	18	79.3	0.3472	0.3420
3	Meers 2	37	1	25	82.9	0.3352	0.3377
4	Meers 3	51	2	19	79.3	0.3286	0.3388
5	Maasband	65	1	16	79.3	0.3321	0.3376
6	Mazenhoven	551	1	15	87.8	0.3621	0.3482
7	Meeswijk	28	1	15	78.0	0.3320	0.3424
8	Kerkeweerd 1	44	2	15	76.8	0.3394	0.3472
9	Kerkeweerd 2	65	1	15	75.6	0.3339	0.3416
10	Kerkeweerd 3	56	1	14	74.4	0.3340	0.3459
11	Elerweert	161	2	15	80.5	0.3503	0.3509
12	Heppeneert	49	1	19	79.3	0.3440	0.3399
13	Roosteren 1	66	1	20	85.4	0.3588	0.3513
14	Roosteren 2	75	1	20	82.9	0.3412	0.3399

Nr: population identifier; population size: total number of flowering individuals counted in Spring 2004; age: 1 (young populations: $\leq$5 years old) and 2 (old populations: >5 years old); *n*: sample size for AFLP analysis; *P*: proportion of polymorphic loci; H_j and H_{eH}: measures of within-population genetic diversity (see text for further details).

populations and recent populations (0.3441 and 0.3415, respectively).

Discussion

Genetic structure and among-population differentiation

In *S. austriacum*, most genotypic diversity was found within populations. Nevertheless, genetic differentiation among populations was highly significant and the observed θ^B value (a Bayesian analog of the traditional F_{ST}-statistic) = 0.083 indicates moderate genetic differentiation among populations. Most pairwise Φ_{ST} values were also significantly different from zero. The observed value of population genetic differentiation is similar to that of other plant species growing along riverbanks. Kudoh and Whigham (1997), for example, found an F_{ST} of 0.062 in the wetland macrophyte *Hibiscus moscheutos*. In *Boltonia decurrens*, a threatened floodplain species that requires regular flooding for suitable habitat and seed dispersal, an $F_{ST} = 0.098$ was observed (DeWoody *et al*, 2004). In *Populus nigra*, a riparian pioneer tree species that was studied along the Drôme River, the F_{ST} value was 0.047 (Imbert and Lefèvre, 2002). Only in the case of the riparian plant *Silene tatarica*, genetic differentiation appeared to be substantially higher ($\theta^B = 0.287$) (Tero *et al*, 2003).

Recent populations located in the immediate vicinity of the river, where extinction–colonization events are recurrent features of the species ecology, had lower levels of genetic differentiation than populations located in the winter bed of the river. Furthermore, the temporal aspect of colonization dynamics appeared to be more important than the spatial aspect. These results suggest that different colonization events may have led to distinct patterns of genetic variation between old and younger populations, and that gene exchange between young and old populations may be limited. Because water flushes larger than 2.000 m^3/s occur once every 3 or 4 years, older persisting populations may be less connected to the metapopulation than populations located on lower river banks that are submerged each year. In *Hibiscus moscheutos*, populations somewhat isolated from the tidal creek also showed significant genetic structuring, indicating reduced gene flow among these sites (Kudoh and Whigham, 1997).

Our results further suggest that founder effects during colonization do not cause increased differentiation among colonist populations. Similar results were obtained by Litrico *et al* (2005) for the pioneer tree species *Antirhea borbonica* in Réunion Island and by Jacquemyn *et al* (2004) for the forest herb *Primula elatior*. The moderate genetic differentiation and the fact that extinction–colonization dynamics do not result in increased differentiation among populations also suggest that individuals who founded these populations originated from multiple source populations rather than from a single source population. Mixing of seeds from different source populations through water transport may not be uncommon in natural river systems (Kudoh and Whigham, 2001). DeWoody *et al* (2004), for example, showed that colonization events in a metapopulation of *B. decurrens* involved seeds from at least three to five source populations. Similarly, colonization of islands by the weedy *Silene dioica* through water transport also involved a mixture from several locations (Giles and Goudet, 1997).

Extinction–colonization dynamics and within-population genetic diversity

Levels of genetic diversity were high ($H_w = 0.3424$) and were not related to population size. These results confirm that the studied *S. austriacum* populations are likely to be of recent common ancestry since it takes time for diversity to be lost. Within-population genetic diversity and total genetic diversity were also not significantly different between recently founded populations ($H_w = 0.3415$) and older and more stable populations ($H_w = 0.3441$), suggesting that recurrent extinction–colonization events did not result in decreased genetic diversity. These results contrast with metapopulation theory. In general, extinction–colonization dynamics have been shown to reduce the within and total–metapopulation genetic diversity (McCauley, 1991; Pannell and Charlesworth, 2000). However, these results confirm our previous findings that in *S. austriacum* minimal founder effects appear to accompany the

formation of new populations and that the vast majority of the total genetic variation is shared among all populations.

Importance of long-distance seed dispersal

The lack of isolation by distance and a weak effect of regional structure in the AMOVA analysis point to a weak spatial genetic structure, which suggests that long-distance seed dispersal occurs within the region. Hydrochory can result in effective long-distance seed dispersal (eg Kudoh and Whigham, 1997, 2001) and seeds may be transported over hundreds of meters to several kilometers (Waser *et al*, 1982; Lonsdale, 1993), especially if river systems display large variances in water discharge due to high volume rain events (Boedeltje *et al*, 2004). Extensive pollen flow, on the other hand, is an unlikely explanation for the lack of relationship between pairwise genetic and geographic distances, since *S. austriacum* is mainly pollinated by bees and syrphid flies and pollen flow is therefore most likely to be restricted to within-population pollen transfer, or to pollen transfer among nearby populations. Indeed, most studies investigating pollen flow found that pollinating insects rarely travel distances larger than 1 km (eg Steffan-Dewenter and Tscharntke, 1999; Richards *et al*, 1999). These results are also in agreement with other studies that have investigated spatial genetic structure of riparian plant species. Kudoh and Whigham (1997), for example, found high gene flow among populations that were adjacent to the tidal stream, indicating that these populations may function as a single genetic metapopulation by extensive and long-distance seed exchanges between them. Similarly, Tero *et al* (2003) also found no isolation by distance and no regional structure indicating frequent long-distance seed exchange.

On the other hand, nearby populations formed clear clusters in the UPGMA dendrogram, which suggests that short-distance seed dispersal or pollen flow among nearby populations may affect the structure of genetic variation in the studied metapopulation. Most of these populations were found on dynamic banks and were less than 5 years old. Another plausible explanation would be that these populations were formed at the same time by the same founding groups. The pairwise Φ_{ST} values between nearby populations indeed indicate very low differentiation among these populations, which confirms the minimal impact of founder effects in the studied system.

Conclusion

Previous research has demonstrated that incorporation of an age structure in plant population genetic research may reveal important information on the processes structuring genetic diversity in dynamic systems (eg McCauley *et al*, 1995; Giles and Goudet, 1997; Jacquemyn *et al*, 2004; Litrico *et al*, 2005). The structure of genetic variation observed in the metapopulation of *S. austriacum* indicates that the impact of founder effects is minimal. The spatial pattern of genetic variation in *S. austriacum* suggests that both short- and long-distance seed dispersal shape variation in genetic diversity. Short- and long-distance seed dispersal represent the extreme ends of the dispersal spectrum, and in natural metapopulations, both are likely to occur. The temporal pattern of genetic variation suggests that limited amounts of gene exchange between old and young populations have led to distinct temporal patterns of genetic variation.

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