New Phytologist

Macroecological patterns of genetic structure and diversity in the aquatic moss *Platyhypnidium riparioides*

V. Hutsemekers[1], O. J. Hardy[2], P. Mardulyn[2], A. J. Shaw[3] and A. Vanderpoorten[1]

[1]Université de Liège, Institut de Botanique, B22 Sart Tilman, B-4000 Liège1, Belgium; [2]Université Libre de Bruxelles, Ecoéthologie Evolutive, CP 160/12, 50 Av F Roosevelt, B-1050 Brussels, Belgium; [3]Department of Biology, Duke University, Durham, NC 27708, USA

Author for correspondence:
Virginie Hutsemékers
Tel: 00 32 4 366 38 42
Email: vhutsemekers@ulg.ac.be

Received: *22 July 2009*
Accepted: *4 October 2009*

New Phytologist (2010) **185**: 852–864
doi: 10.1111/j.1469-8137.2009.03094.x

Key words: bryophytes, dispersal, ecotype, genetic structure, isolation by distance, Mantel test, *Platyhypnidium,* spatial analysis of molecular variance.

Summary

• Genetic diversity and structure are described in the aquatic moss *Platyhypnidium riparioides* to assess its dispersal ability at a regional scale and to determine whether patterns of genetic differentiation correlate with environmental variation.

• Variation at six nuclear microsatellite loci from 50 populations in southern Belgium was investigated through Mantel tests, partial Mantel tests and spatial analysis of molecular variance.

• Overall patterns of genotypic variation showed strong differentiation among populations at a regional scale (F_{ST} = 0.57). The high values of F_{IS} observed within populations at both the ramet and genet levels, and the higher proportion of ramets with the same genotype than expected by chance, all point to a strongly clonal or selfing mating system. A genetic discontinuity was identified between northern and southern groups of populations. Within each group, F_{ST} and geographical distances were significantly correlated. Partial Mantel tests suggest that genetic and ecological distances are significantly correlated in the southern group.

• The results point to strong dispersal limitation at the landscape scale and suggest that the southern and northern groups experienced different histories. Within the former, the correlation between genetic and ecological variation is suggestive of reproductive isolation among ecotypes.

Introduction

The regulation of large rivers for flood control, the production of hydroelectricity and navigation has caused irreversible damage to freshwater ecosystems at a global scale (Naiman, 1992). In particular, the dramatic industrial development of alluvial floodplains has substantially contributed to a spectacular decrease in water quality that culminated in the 1970s–1980s (Malle, 1994). Subsequent ecological disasters have led to a sudden realization of the vulnerability of these ecosystems, and the launch of several international agreements aimed at the improvement of water quality and protection of aquatic ecosystems (Malle, 1996). Although water quality has since substantially improved in large river ecosystems, long-term monitoring studies indicate that pollution levels remain much higher than before the industrial era (Cun *et al.*, 1997). During the past decade, the attention of European countries has therefore focused on eutrophication problems, largely using biological indicators of water quality (Kelly & Whitton, 1998), following the recommendations of the United Nations' Educational, Scientific and Cultural Organization's Man and Biosphere program (http://www.unesco.org/mab/index.htm).

Among the organisms that have been most widely used for biomonitoring, aquatic mosses offer a number of advantages. Because their leaves always lack stomata and are most often unistratose, they offer a large surface area, whilst lacking the complex regulation mechanisms of angiosperms, and are thereby excellent bioaccumulators of pollutants (for a review, see Markert *et al.*, 2003). Their communities also segregate along an upstream–downstream gradient of habitat conditions and water quality, which has promoted their use as direct bioindicators (Vanderpoorten, 2003). Biomonitoring using aquatic bryophytes suffers, however, from several shortcomings associated with their large phenotypic variability, rendering species' identification difficult at times. For example, the moss *Platyhypnidium riparioides*, which is among the most common aquatic species in the circumboreal region, is extremely variable morphologically,

to the extent that it has been suggested that the taxon should be divided into infraspecific taxa or even multiple species (Wehr & Whitton, 1986).

A second shortcoming associated with the use of aquatic bryophytes for bioindication is that the same species can exhibit different ecological responses in different geographical regions (Vanderpoorten & Durwael, 1999). Similar modulations of the response to environmental variation were observed along temperature (Kallio & Saarnio, 1986) and light (Ueno *et al.*, 2006) gradients, suggesting that mosses are either able to adapt locally or are physiologically plastic. In the liverwort *Marchantia polymorpha* and the moss *Funaria hygrometrica*, for instance, populations vary in their degree of tolerance to heavy metals, and these differences are directly related to the levels of soil contamination (for a review, see Shaw, 1988). Subtle morphological variation in peat mosses along a gradient of water availability has similarly been shown to be adaptive (Såstad & Flatberg, 1994; Såstad *et al.*, 1999). In contrast with flowering plants, wherein high levels of tolerance to metals are almost always restricted to plants growing in contaminated environments (Antonovics *et al.*, 1971; Baker, 1987), evidence for local adaptation in bryophytes is, however, uncommon (Shaw, 1988; Shaw & Albright, 1990). It seems that, in some species, inherent, relatively high levels of tolerance have impeded the evolution of specialized races (Shaw *et al.*, 1989; Shaw & Albright, 1990). Although bryophytes are not genetically depauperate and, indeed, display levels of genetic diversity comparable with angiosperms (see Shaw, 2009 for review), it is not clear whether much of this variation is adaptive to specific environments (Shaw, 1992).

One reason why local differentiation and adaptation may be infrequent in bryophytes is that their high dispersal potential is a strong homogenizing force that prevents genetic divergence. With their range of asexual propagules, whose diversity is unparalleled among land plants (Duckett & Ligrone, 1992), and tiny spores, bryophytes indeed seem to be well adapted for dispersal. Nevertheless, studies on bryophyte dispersal are conflicting. Observations of the colonization of artificial substrates clearly point to a high dispersal ability over tens to hundreds of kilometers within a few decades (Miller & McDaniel, 2004; Hutsemekers *et al.*, 2008a). Efficient dispersal at the landscape scale and the ability to navigate between spatially separated habitats have been further invoked by Pharo *et al.* (2004, 2005) to explain the lack of relationship between habitat fragmentation and the composition and abundance of species' communities. This interpretation is shared by Hazell *et al.* (1998), who observed that the colonization of aspens by epiphytes was not more effective within clusters of aspen than among solitary trees, suggesting that long-distance dispersal was sufficiently effective to obliterate the effects of fragmentation.

An increasing body of literature, by contrast, points to a positive effect of connectivity, and emphasizes the effects of dispersal limitation and metapopulation dynamics on community species' richness (Zartman & Shaw, 2006; Pharo & Zartman, 2007; Virtanen & Oksanen, 2007). This hypothesis is supported by the fact that bryophytes often exhibit aggregated distribution patterns (Snäll *et al.*, 2003, 2004b, 2005; Löbel *et al.*, 2006). Indeed, pairs of individuals of the epiphytic mosses *Orthotrichum speciosum* and *O. obtusifolium*, separated by a distance of up to 350 m, tend to exhibit more genetic similarity than individuals separated by a greater distance, suggesting that isolation by distance operates at this scale (Snäll *et al.*, 2004a). Shaw *et al.* (2009) similarly found a weak, but significant, correlation between genetic and geographical differences between plants of *Sphagnum torreyanum*.

In this article, we describe genetic structure and variation in the aquatic moss *P. riparioides* at a regional scale (south Belgium) using microsatellite markers to contrast different interpretations regarding the dispersal ability and potential for local adaptation. If, as a result of divergent selection between environments, successful gene flow indeed only or mostly occurs among populations from similar habitat conditions, genetic divergence accumulates first in selected and neutral loci through direct linkage or genetic hitchhiking (Hamrick, 1989; Slatkin, 1995b; Beaumont & Nichols, 1996; Vitalis & Couvet, 2001) and, subsequently, throughout the genome by drift. In such a scenario of ancient divergence, we expect to observe a significant correlation between environmental and neutral genetic variation (Charlesworth *et al.*, 1997), as evidenced by an important body of literature on ecotypic differentiation (e.g. Van Rossum *et al.*, 1997; Vekemans & Lefebvre, 1997; Badri *et al.*, 2008; Barker *et al.*, 2009; Nielsen *et al.*, 2009). By contrast, the absence of any correlation between ecological and neutral genetic distances can reflect an absence of local adaptation, recent divergence or recurrent gene flow among populations.

Gene flow and, hence, mating system are thus key factors for the detection of the signature of local adaptation in genetically neutral variation, and we therefore attempted to answer the following questions. To what extent is gene flow limited by geographical discontinuities and/or distance in *P. riparioides* at the regional scale? Is the mating system of this species mostly clonal or autogamous, or does, by contrast, extensive allogamy favour the exchange of genetic material among populations? Does any signal of ecotypic differentiation emerge from the correlation between genetic and ecological variation?

Materials and Methods

Study species and sampling

The aquatic moss *Platyhypnidium riparioides* (Hedw.) Dixon (= *Rhynchostegium riparioides*) was selected as a

model for this study. It occurs widely throughout circumboreal areas and in a wide range of water physicochemistries and pollution levels (Dierßen, 2001), making it an appropriate model to investigate the genetic structure at different spatial scales and the microevolutionary responses to environmental variation.

The study was conducted in southern Belgium (Wallonia) in an area of 16 844 km^2 (Fig. 1). The area, which encompasses two hydrographic networks drained by the Meuse and Escaut rivers, offers several advantages for the investigation of the impact of water chemistry and pollution level on the evolution of aquatic bryophytes. The two rivers exhibit contrasting physicochemistries, and detailed information on their past and present water quality is available. River regulation for flood control, hydroelectricity production, navigation and, in particular, the industrialization of the alluvial plains led the Escaut basin, the Sambre River and the areas located northwards to experience dramatic pollution peaks during the 1970s, and to the complete disappearance of bryophytes locally. By contrast, the areas located south of the line formed by the Sambre and Meuse rivers was much less affected (Empain, 1977; Vanderpoorten, 1999).

Fifty sites were selected in order to cover a wide range of water physicochemistry and pollution levels, as measured annually on a monthly basis by the ministry of the Walloon region. Each site was characterized by the average concentration of each of 21 physicochemical parameters over 4–12 months in 2006 (Table 1). All of these environmental parameters are spatially autocorrelated and difficult to dissociate. The ecological information was summarized in a macroecological gradient by performing a principal component analysis (PCA) on the covariance matrix (hereafter, environmental PCA). The first two axes, which explained 67.17% and 11.41% of the total physicochemical variance, respectively, were used as a compound indicator of water quality. The first axis was most notably correlated with calcium (r = 0.90) and sulfates (r = 0.92), and the second with chlorides (r = 0.70) and pheopigments (r = 0.59) (Table 1). Each site was also characterized by the distance separating it from neighbouring sites. In order to determine whether the species preferentially dispersed by water along an upstream–downstream gradient, or by air, two types of distance matrix among sites were computed: one recording the nearest aerial distance between sites, and the other recording pairwise distances following the river course.

At each sampling site, at least 16 gametophyte individuals of *P. riparioides* were collected. Individuals were sampled along a transect of 1–20 m depending on the population size. Because *P. riparioides* is a creeping, pleurocarpous moss, the identification of separate genetic individuals (genets) can be difficult, but it was ensured that the collections had no physical connections between them. Each specimen was placed directly in an Eppendorf tube and kept fresh pending DNA extraction.

DNA extraction and genotyping

Total DNA was extracted from young, fresh shoot apices using Doyle & Doyle's (1990) standard cetyltrimethylammonium bromide protocol, and resuspended in 100 μl of sterile water. All individuals were genotyped for six polymorphic microsatellite nuclear loci: R3, R9, R11, R13, R14 and R17 (Hutsemekers *et al.*, 2008b). PCR amplifications were performed with 2 μl of DNA, 0.5 μl of each 10 μM primer [including a fluorescent dye HEX (R3 and R11) or FAM (R9, R13, R14 and R17) labelled on the forward primers], 1.25 μl of each 2.5 mM deoxynucleoside triphos-

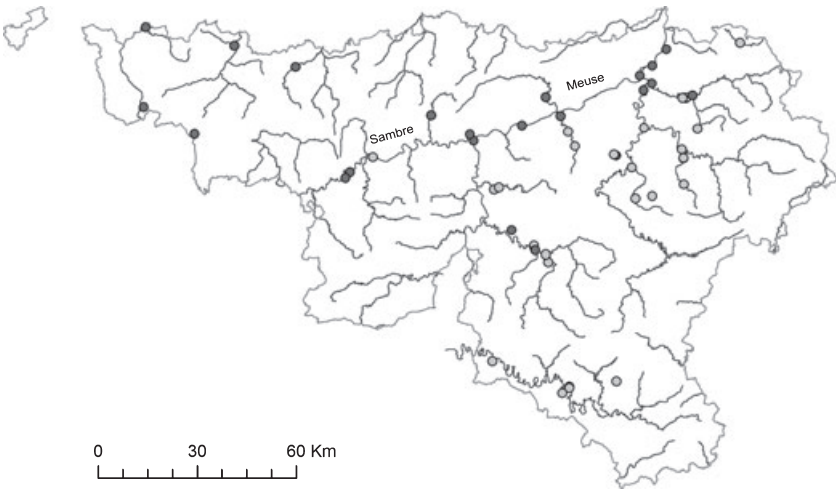

Fig. 1 Spatial analysis of molecular variance displayed by six nuclear microsatellite loci in 50 populations of the aquatic moss *Platyhypnidium riparioides* in southern Belgium. The distribution of the populations to one of two groups (black and light grey dots) was identified by the spatial analysis of molecular variance (SAMOVA).

Table 1 Average (± SD) of the 21 physicochemical parameters measured over 4–12 months in 2006 at the 50 sampling sites (data from the Ministry of the Walloon Region)

Physicochemical parameter	Unit	Average	Average group, N	Average group, S	*P* value (ANOVA)	Correlation with PCA1	Correlation with PCA2
Nitrogen in ammonia	mg N l^{-1}	0.41 ± 0.8	0.79 ± 1.1	0.09 ± 0.1	*	0.81**	NS
Calcium	mg l^{-1}	63.57 ± 37.2	77.24 ± 34.7	46.63 ± 34.6	*	0.90**	NS
Organic carbon	mg C l^{-1}	3.39 ± 1.5	4.27 ± 1.7	2.72 ± 0.9	**	0.76**	NS
Chlorophyll *a*	μg l^{-1}	4.85 ± 5.1	6.34 ± 5.8	2.86 ± 3.4	*	0.59**	NS
Chlorides	mg l^{-1}	32.59 ± 27.2	45.21 ± 35.2	22.60 ± 11.5	*	0.71**	0.70**
Soluble copper	μg l^{-1}	0.99 ± 0.6	1.24 ± 0.6	0.74 ± 0.5	*	0.57**	0.33*
DCO[1]	mg l^{-1}	13.92 ± 7.8	17.69 ± 9.3	11.17 ± 4.6	**	0.75**	NS
DBO5[2]	mg l^{-1}	2.14 ± 2.0	2.86 ± 2.5	1.23 ± 0.6	**	0.83**	NS
Organic pollution index[3]	–	3.78 ± 0.8	3.41 ± 0.8	4.24 ± 0.4	**	−0.90**	NS
Quality index[4]	–	4.14 ± 1.8	4.88 ± 2.2	3.36 ± 0.5	*	0.80**	NS
Magnesium	mg l^{-1}	9.63 ± 5.2	10.52 ± 5.0	8.40 ± 5.1	NS	0.83**	NS
Matter in suspension	mg l^{-1}	19.13 ± 20.7	26.64 ± 26.4	10.57 ± 4.6	*	0.67**	NS
Nitrates	mg N l^{-1}	4.03 ± 1.4	3.86 ± 1.6	3.92 ± 1.5	NS	0.43*	NS
Nitrites	mg N l^{-1}	0.05 ± 0.1	0.08 ± 0.1	0.02 ± 0.03	**	0.88**	NS
Orthophosphates	mg P l^{-1}	0.15 ± 0.2	0.19 ± 0.2	0.07 ± 0.06	**	0.90**	NS
Oxygen dissolved	mg l^{-1}	10.64 ± 1.8	10.29 ± 2.6	11.01 ± 0.6	NS	−0.46*	NS
pH	–	7.85 ± 0.2	7.87 ± 0.2	7.83 ± 0.3	NS	NS	NS
Pheopigments	μg l^{-1}	2.11 ± 1.8	2.38 ± 2.1	1.84 ± 1.3	NS	0.53**	0.59**
Phosphorus total	mg P l^{-1}	0.18 ± 0.2	0.28 ± 0.3	0.10 ± 0.07	**	0.89**	NS
Saturation in oxygen	%	94.76 ± 15.9	92.18 ± 22.9	97.06 ± 3.9	NS	−0.42*	NS
Sulfates	mg l^{-1}	38.75 ± 33.8	54.76 ± 41.6	24.55 ± 18.4	**	0.92**	NS

Physicochemical characteristics for the two ecoregions (southern and northern groups) identified by the spatial analysis of molecular variance of allelic frequencies at six nuclear microsatellite loci in *Platyhypnidium riparioides* (see Fig. 1) are also provided, and the significance of the average difference in concentrations between the two regions was tested by a one-way analysis of variance, whose *P* value is given in the sixth column. Correlations of the physicochemical parameters with the first and second axes of the environmental principal components analysis (PCA) are given in the two extreme right-hand columns.

NS, not significant.

[1], Chemical demand for oxygen (quantity of oxygen necessary for total chemical oxidation of the organic matter of a sample of water).

[2], Biological demand for oxygen in 5 d (quantity of oxygen necessary for microorganisms to oxidize the organic matter of a sample of water in 5 d in the dark at 20°C).

[3], Organic pollution index: average value of DBO5, ammonium, nitrites and *o*-phosphates (Leclercq & Vandevenne, 1987).

[4], Quality index: index related to the number of organisms collected and their degree of sensitivity to pollution.

*, **, Significance at the 0.05 and 0.01 levels, respectively.

phate, 3 μl of 25 mM $MgCl_2$, 1.25 μl of buffer 10 ×, 0.1 μl of 5 U μl^{-1} *Taq* polymerase (Roche) and 3.9 μl of water. Thermocycling consisted of a denaturation step of 5 min at 94°C; 35 cycles of 45 s at 94°C, 45 s at 50°C and 90 s at 72°C; and finally an elongation step of 7 min at 72°C. PCR products were genotyped using 2 μl of amplified DNA, 13.5 μl of formamide and 0.5 μl of ROX 350 size standard on an ABI 310 sequencer (Applied Biosystems Inc, Foster City, California, USA). The data were exported into GENEMAPPER version 3.7 (Applied Biosystems Inc, Foster City, California, USA) for allele sizing.

Data analysis

Because a wide range of chromosome numbers and ploidal levels have been documented for *P. riparoides* on the basis of chromosome counts (Fritsch, 1991), the relative DNA content per cell of three to four specimens from *c.* 30 populations at random was determined by flow cytometry with a Partec PA flow cytometer (Partec, Münster, Germany).

Missing data and null alleles

The dataset included a proportion of missing data ranging between 7 and 24% per locus, raising the possibility of the presence of null alleles. Because *P. riparioides* is a monoicous species, whose mating system is characterized by substantial levels of inbreeding, none of the investigated populations was at Hardy–Weinberg equilibrium at any locus, making it impossible to calculate the expected frequency of null alleles based on the observed deficiency in heterozygotes (Brookfield, 1996). Two analyses were therefore employed to estimate the potential influence of null alleles in the observed deviations from Hardy–Weinberg equilibrium. First, we calculated the value of the selfing rate under the assumption of inbreeding only, with the software RMES (David *et al.*, 2007), and compared the value

obtained with the selfing rate s derived from F_{IS} [$F_{IS} = s(2 - s)$]. Second, in order to determine whether missing data (and potentially null alleles) may influence our estimate of inbreeding, we compared two groups of populations characterized by contrasted levels of missing data. In the present data, the threshold of 9% of missing data was used to obtain two groups of populations of similar size, including 26 and 24 populations.

Genetic diversity

Population polymorphism was described by the mean number of alleles observed (A_e), and the expected and observed heterozygosity per population (H_e and H_o), using the software GENETIX 4.05 (Belkhir *et al.*, 1996–2004). Departures from Hardy–Weinberg equilibrium among loci and populations were assessed using GENEPOP version 4.0.7 (Raymond & Rousset, 1995) with 10 000 dememorization steps, 20 batches and 5000 iterations per batch.

Rare alleles were calculated on the basis of a frequency of < 0.01 in each population.

Population differentiation

F statistics (*sensu* Weir & Cockerham, 1984), including F_{IS}, F_{IT} and F_{ST}, were computed for each locus and over all loci using the software SPAGeDi 1.2 (Hardy & Vekemans, 2002). The significance of *F* statistics was determined by means of 1000 permutations of individuals (F_{ST}) or of gene copies (F_{IS}, F_{IT}). Pairwise F_{ST} values between all pairs of populations were calculated using GENETIX 4.05 (Belkhir *et al.*, 1996–2004).

All individuals exhibiting the same multilocus genotype were interpreted as clones (Luttikhuizen *et al.*, 2007). To ensure that two individuals did not display the same genotype just by chance, we modelled, for each population, the probability of twice sampling identical genotypes from a theoretical nonclonal population (P_e), and compared it with the observed probability (P_o):

$$P_e = (\Sigma fa_i^2)(\Sigma fb_i^2)(\Sigma fc_i^2)\ldots,$$

where fa_i, fb_i, fc_i, … are the frequencies of the single-locus genotype i at loci a, b, c, …;

$$P_o = \Sigma fg_i^2,$$

where fg_i is the frequency of the multilocus genotype i.

All individuals with missing data were removed from the analysis. In order to evaluate the extent to which the observed populations exhibited a clonal structure, the proportion N_g/N, where N_g is the number of distinct multilocus genotypes and N is the number of individuals, was calculated for each population. A predominantly asexual structure is characterized by an N_g/N ratio approaching zero, whereas a purely sexual structure approaches unity (Ellstrand & Roose, 1987).

In order to determine the extent to which clonality influences the *F* statistics, the latter were recalculated, keeping only a single individual per multilocus genotype in each population, and populations with at least four distinct genotypes. This approach represents a compromise between the necessity to include populations with multiple genotypes and having sufficient populations in the analysis (27).

Geographical patterns

The mating system of *P. riparioides* is characterized by strong selfing (see Results section), making it impossible to use techniques such as Hardy–Weinberg equilibrium as the criterion to define groupings (e.g. Pritchard *et al.*, 2000). Therefore, the global geographical structure in the data was explored by means of a spatial analysis of molecular variance (SAMOVA), as implemented by the program SAMOVA 1.0 (Dupanloup *et al.*, 2002). This analysis defines groups of populations that are geographically homogeneous and maximally genetically differentiated from each other. The method is based on a simulated annealing procedure that aims to maximize the proportion of total genetic variance as a result of differences between geographical groups of populations. All individuals with missing data for more than two loci were removed from the dataset prior to analysis. Each group identified by SAMOVA was characterized by its genetic diversity: A_e, A_n (number of alleles per locus), H_e, *F* statistics. Differences in mean genetic diversity among regions were tested using a one-way analysis of variance. To compare with the structure identified by SAMOVA, we also performed a PCA of the populations based on their allelic frequencies (hereafter, genetic PCA). This ordination technique, which, as opposed to SAMOVA, does not take geographical information into account, was conducted with the software PCAGEN 1.2.1 (Goudet, 1999).

Mantel tests were used to measure the correlation between genetic and geographical distances among populations within each region identified by SAMOVA and for the global dataset. F_{ST} was used as a measure of genetic distances among pairs of populations. The linearity of the relationship between F_{st} and geographical distances was inspected graphically, and the transformation $F_{ST}/(1 - F_{ST})$ was applied when necessary. Geographical distances included pairwise shortest distances among populations and two kinds of distances along the river course. In the first (R1), all pairwise distances were computed, whereas, in the second (R2), only distances from upstream populations to downstream populations were included, with other pairwise distances coded as missing. Mantel tests were performed with SPAGeDI 1.2 and GENETIX 4.5 –

depending on the kind of genetic distance matrix – and the significance of the correlation values was tested by 10 000 random permutations.

Evaluation and comparison of mutation and dispersal rates were obtained by calculating global R_{ST} and F_{ST} values (Slatkin, 1995a; Michalakis & Excoffier, 1996). R_{ST} is a measure of genetic differentiation among populations, analogous to F_{ST}, but taking into account phylogenetic relationships between alleles, assuming the stepwise mutation model of microsatellites. An interesting property is that $R_{ST} > F_{ST}$ when a phylogeographic structure exists; that is, when the mutation rate is higher than the dispersal rate (Hardy *et al.*, 2003). The hypothesis that $R_{ST} > F_{ST}$ was tested by computing the distribution of R_{ST} for 1000 permutations of allele sizes among alleles in each locus, as implemented by SPAGeDi 1.2. The hypothesis was accepted if the observed R_{ST} value was higher than at least 95% of the values obtained after permutation.

Effects of environmental variation

In order to investigate whether patterns of genetic variation correlate with patterns of environmental variation, we calculated the correlation coefficients between the scores of the populations on the first axes of the environmental PCA with those of the genetic PCA.

Mantel tests were also performed between pairwise F_{ST} and pairwise differences in population scores along the first two environmental PCA axes. In order to remove the potentially confounded signal of geographical and ecological information, partial Mantel tests were used. The latter tests the correlation between two matrices (for example, genetic and ecological matrices), whilst controlling for the information present in the third matrix (for example, a geographical matrix). Partial Mantel tests were performed with the software ZT (Bonnet & Van de Peer, 2002), and the significance of the correlations was tested by means of 10 000 randomization runs.

Results

Ploidal level

The analysis revealed that all specimens had the same ploidal level. As many individual gametophytes exhibited two alleles per locus, the data matrix was coded as diploid, which means that individuals, for which only one allele was detected at a given locus, were considered to be homozygous at that locus.

Genetic diversity and variation at the population level

The patterns of genetic diversity revealed by the six microsatellite loci are summarized in Table 2. The microsatellite loci varied considerably in their level of diversity. The number of alleles per locus ranged from 16 (R17) to four (R9) (Table 3) for a total of 50 observed alleles across the six sampled loci. Mean within-population gene diversity per locus (H_e) ranged from 0.355 (R14) to 0.867 (R17), and the mean number of alleles per locus from 1 to 3.17 (Table 2).

None of the populations exhibited fixed heterozygosity at any locus. Significant departures from Hardy–Weinberg equilibrium were observed for most of the populations owing to a general deficit in heterozygotes: 32 populations of 36 for locus R3, all populations for R9, 21 of 36 for R11, 24 of 29 for R13, 24 of 27 for R14 and 34 of 39 for R17 ($P < 0.05$). No within-population significant heterozygote excesses were observed. The global F_{IS} across all loci and populations was 0.80. Within-population F_{IS} values ranged between 0.21 and 1.00 ($P < 0.001$). Although F statistic values may be overestimated because of the presence of null alleles (Chapuis & Estoup, 2007), the results of RMES analysis (estimation of selfing rate $s = 0.7$; whereas $s = 0.88$ when derived from F_{IS}) and the F_{IS} values of the two groups with contrasting levels of missing data ($F_{IS} = 0.80$ for both groups) clearly indicated that their influence on the level of inbreeding and on F statistics was negligible.

Over the 50 populations from southern Belgium, the average of N_g/N was 0.42 ± 0.19. The observed probability of two individuals with the same genotype per population was much higher than the expected probability in 37 of the 40 populations with at least two polymorphic loci. F_{IS} values remained highly significant ($P < 0.005$) at the genet level. Global F_{IS} over the 27 populations studied was 0.78, against 0.81 at the ramet level for the same populations.

Patterns of genetic differentiation

Populations exhibited a high degree of genetic differentiation, with global multilocus F_{ST} values of 0.57 ($P < 0.001$) and 0.36 ($P < 0.001$) at the ramet and genet levels, respectively (Table 3). Two spatial groups of populations, north and south of the Meuse and Sambre rivers (hereafter, the Sambre and Meuse ridge), were identified by SAMOVA (Fig. 1). Genetic differentiation between the northern and southern groups was significant ($F_{ST} = 0.16$; $P < 0.001$). PCA of the allelic frequencies was consistent with the identification of northern and southern groups (Fig. 2). Along PCA1, which accounted for 24.85% of the total genetic variance, populations of the southern and northern groups tended to have negative vs. positive values, respectively. The correlation between the score of a population along the axis and its assignment to the southern and northern groups according to SAMOVA was 0.66 ($P < 0.001$).

Genetic diversity was higher in the northern group, with a total of 46 alleles, compared with 38 in the southern

Table 2 Name, abbreviation, hydrographical basin, geographical coordinates, sample size (N), expected (H_e) and observed (H_o) heterozygosity, mean number of alleles per locus (A_e) and inbreeding coefficient (F_{IS}) and estimation of clonality (N_g/N) of *Platyhypnidium riparioides* populations collected at 50 sampling sites in southern Belgium

				Coordinates							
Abbr.	River	Basin	Group	Longitude	Latitude	N	H_e	H_o	A_e	F_{IS}	Ng/N
3BOI	3 Bois	Meuse	S	5°46′22.8″	50°33′41.2″	16	0.12	0.01	1.83	0.90***	0.29
ABS	Affl. Bock	Meuse	S	4°56′51.2″	50°19′04.4″	16	0.21	0.00	1.67	1.00***	0.19
ACO	Amblève	Meuse	S	5°35′27.2″	50°28′54.1″	16	0.40	0.00	2.17	1.00***	0.31
AER	Aisne	Meuse	S	5°32′54.2″	50°17′20.6″	16	0.17	0.05	1.83	0.75***	0.46
AFFNEB	Affl. Neblon	Meuse	N	5°28′10.2″	50°24′23.9″	20	0.15	0.06	2.17	0.62***	0.35
AIB	Aisne	Meuse	S	5°32′07.3″	50°22′24.0″	16	0.22	0.02	2.17	0.90***	0.31
ALI	Affl. Lienne	Meuse	S	5°45′27.1″	50°23′46.7″	16	0.20	0.03	2.17	0.85***	0.44
AND	Meuse	Meuse	N	5°04′11.1″	50°29′33.0″	16	0.41	0.02	1.83	0.95***	0.23
ASS	Affl. Vierre	Meuse	S	5°27′22.0″	49°47′24.2″	16	0.33	0.25	2.50	0.30 NS	0.69
ATG1	Antrogne	Meuse	S	5°15′27.4″	49°46′39.1″	20	0.00	0.00	1.00	–	0.08
ATG2	Affl. Antrogne	Meuse	–	5°15′24.7″	49°46′25.9″	20	0.12	0.01	1.33	0.93***	0.38
AUL	Sambre	Meuse	N	4°19′58.7″	50°22′00.6″	16	0.36	0.06	2.33	0.84***	0.58
AVE	Ave	Meuse	S	5°10′27.8″	50°07′06.8″	8	0.11	0.10	1.67	0.08 NS	0.50
BAI	Amblève	Meuse	N	5°46′25.7″	50°33′45.9″	16	0.31	0.09	2.83	0.73***	0.53
BERN	Canal Bernissart	Meuse	N	3°40′05.5″	50°28′09.4″	23	0.06	0.05	1.67	0.22 NS	0.29
BRA	Lienne	Meuse	S	5°45′26.3″	50°19′28.4″	16	0.17	0.01	1.67	0.94***	0.19
BS	Bock	Meuse	S	4°58′02.7″	50°19′26.1″	16	0.36	0.03	2.33	0.91***	0.62
CHA	Sambre	Meuse	S	4°25′49.1″	50°24′28.5″	16	0.00	0.00	1.00	–	0.10
DENDRE	Dendre	Escaut	N	3°50′06.5″	50°42′42.4″	25	0.11	0.07	1.83	0.39*	0.29
ESC	Escaut	Escaut	N	3°27′15.3″	50°45′37.7″	16	0.16	0.05	2.33	0.68***	0.38
GCO	Sambre	Meuse	–	4°18′50.4″	50°21′05.6″	16	0.22	0.00	1.67	1.00***	–
GUEUL	Gueule	Meuse	S	6°00′35.1″	50°42′34.8″	16	0.07	0.04	1.50	0.54 NS	0.20
HBM	Semois	Meuse	S	5°13′42.1″	49°45′34.7″	20	0.36	0.16	2.17	0.64***	–
HOY	Hoyoux	Meuse	S	5°15′56.6″	50°28′24.8″	16	0.39	0.00	2.67	1.00***	0.62
HUCC	Mehaigne	Meuse	N	5°10′16.9″	50°34′10.7″	20	0.30	0.01	2.50	0.97***	0.45
HUY	Meuse	Meuse	N	5°14′01.0″	50°31′01.0″	20	0.22	0.00	2.00	1.00***	0.32
HWI	Hoegne	Meuse	S	5°49′11.4″	50°28′34.8″	16	0.15	0.02	2.17	0.86***	0.29
IWEN	Iwenne	Meuse	N	5°01′13.2″	50°12′22.5″	16	0.35	0.06	2.83	0.83***	0.81
JUL	Julienne	Meuse	N	5°41′36.0″	50°41′45.0″	15	0.22	0.02	1.83	0.92***	0.56
LESVIL	Lesse	Meuse	S	5°06′53.9″	50°09′32.2″	16	0.05	0.00	1.17	1.00***	0.13
LHO	Lhomme	Meuse	S	5°09′50.8″	50°08′21.2″	16	0.04	0.03	1.50	0.21 NS	0.25
LIRA	Lienne	Meuse	N	5°45′27.0″	50°23′46.8″	16	0.13	0.04	2.00	0.70***	0.36
LOU	Trou du loup	Meuse	S	5°37′20.4″	50°17′41.7″	16	0.22	0.12	1.83	0.50***	0.50
MEFRAG	Meuse	Meuse	N	5°34′40.8″	50°37′28.6″	16	0.38	0.10	2.67	0.77***	0.64
MEMONS	Meuse	Meuse	N	5°37′50.4″	50°39′05.8″	16	0.21	0.17	1.83	0.27 NS	–
MENAM	Meuse	Meuse	N	4°51′40.6″	50°27′04.4″	16	0.39	0.17	2.33	0.59***	0.64
MOD	Hoyoux	Meuse	S	5°17′42.2″	50°26′01.6″	20	0.18	0.00	1.67	1.00***	0.20
NAM	Sambre	Meuse	N	4°50′48.3″	50°28′11.8″	20	0.08	0.04	1.83	0.58*	0.19
NAZ	Amblève	Meuse	S	5°45′03.7″	50°25′16.2″	16	0.39	0.01	2.17	0.97***	0.54
NEBLON	Neblon	Meuse	S	5°27′37.6″	50°24′36.5″	20	0.33	0.02	2.67	0.94***	0.69
ORN	Orneau	Meuse	N	4°40′50.3″	50°31′21.7″	16	0.36	0.05	3.17	0.86***	0.62
OURTILF	Ourthe	Meuse	N	5°35′33.4″	50°35′04.3″	16	0.38	0.18	2.17	0.63**	–
PEP	Hoegne	Meuse	N	5°48′04.9″	50°34′05.9″	16	0.39	0.02	2.00	0.95***	0.50
PER	Escaut	Escaut	N	3°26′57.6″	50°32′28.5″	16	0.31	0.13	2.33	0.60***	0.70
REB	Rebais	Meuse	S	4°56′03.0″	49°50′59.2″	16	0.22	0.00	1.60	1.00***	–
SENNE	Senne	Escaut	N	4°05′57.2″	50°39′14.5″	23	0.22	0.03	2.17	0.88***	0.36
SON	Trou du Renard	Meuse	S	5°45′27.6″	50°33′40.4″	16	0.22	0.07	1.83	0.69***	0.53
VACHAU	Vachau	Meuse	N	5°06′55.5″	50°09′46.4″	16	0.25	0.00	2.50	1.00***	0.43
VESDRE	Vesdre	Meuse	N	5°37′41.1″	50°36′10.7″	15	0.38	0.02	2.33	0.94***	0.36
WIMB	Wimbe	Meuse	N	5°07′12.3″	50°09′05.2″	16	0.43	0.10	2.33	0.79***	0.86

NS, not significant.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

group. H_e values were 0.55 (mean H_e per population, 0.27) and 0.51 (mean H_e per population, 0.20) in the north and south, respectively ($P = 0.05$). However, the northern group showed slightly less differentiation ($F_{ST} = 0.50$, $P < 0.001$) than the southern group ($F_{ST} = 0.58$, $P < 0.001$) among populations. The mean number of alleles per

Table 3 *F* statistics, number of alleles per locus (A_n) and expected heterozygosity (H_e) for all loci and at each of six microsatellite loci for a sample of 50 populations of *Platyhypnidium riparioides* in southern Belgium

Locus	F_{IS}	F_{ST}	A_n	H_e
All loci	0.80	0.57	50	0.57
R3	0.90	0.61	7	0.67
R9	0.97	0.63	4	0.60
R11	0.79	0.55	12	0.39
R13	0.46	0.50	6	0.59
R14	0.80	0.44	5	0.36
R17	0.89	0.62	16	0.87

population was higher in the southern (2.24) than the northern (1.87) group ($P < 0.01$). The genetic differentiation was paralleled by a significant difference in water quality north and south of the Sambre and Meuse ridge. In fact, 15 of the 21 investigated physicochemical factors reached significantly higher concentrations in the north than in the south ($P < 0.05$) (Table 1).

Isolation by distance

The regression between pairwise F_{ST} and shortest geographical distances among all populations showed a typical isolation by distance pattern (Fig 3). Both the pairwise F_{ST} and $F_{ST}/(1 - F_{ST})$ matrices were significantly correlated with the matrix of shortest geographical distances among populations [$r = 0.151$; $P = 0.023$ for F_{ST} and $r = 0.162$; $P = 0.013$ for $F_{ST}/(1 - F_{ST})$]. The correlation between the pairwise F_{ST} matrix and the matrix of pairwise shortest geographical distances remained significant within each of the two groups defined by SAMOVA ($P < 0.05$; Fig 3). By contrast, no significant correlation was found between the matrices of genetic distances and both matrices of distances following the river network ($r = 0.085$; $P = 0.09$ between F_{ST} and R1; $r = 0.149$; $P = 0.13$ between F_{ST} and R2). No phylogeographical signal was detected in the data, as R_{ST} values were never significantly different from F_{ST} values.

Ecological patterns

The score for each site on the first axis of the PCA of environmental variables was significantly correlated with the first axis of the PCA based on allele frequencies ($r = 0.60$; $P < 0.001$), but not with the second axis. Similarly, the Mantel test between the pairwise F_{ST} and the pairwise score differences along the first axis of the ecological PCA was significant ($r = 0.243$; $P = 0.005$; second axis $P > 0.05$). Although the matrices of pairwise geographical and ecological distances were significantly correlated ($r = 0.5$; $P = 0.001$), partial Mantel tests suggested that the correlation between the genetic and ecological distance matrices remained significant after removal of the geographical component of the genetic distance matrix ($r = 0.16$, $P = 0.03$). In contrast, when the ecological component of the genetic distance matrix was removed, the residual signal was not significantly correlated with the pairwise geographical distance matrix ($P = 0.16$).

When the same analyses were performed within each of the southern and northern groups, the Mantel tests revealed that genetic variation was significantly correlated with the ecological gradient in the southern group ($r = 0.303$;

Fig. 2 Principal components analysis of allele frequencies at six nuclear microsatellite loci in 50 populations of the aquatic moss *Platyhypnidium riparioides* in southern Belgium. Groups S and N represent the groups of populations identified by a spatial analysis of molecular variance (SAMOVA; see Fig. 1). See Table 2 for population identification.

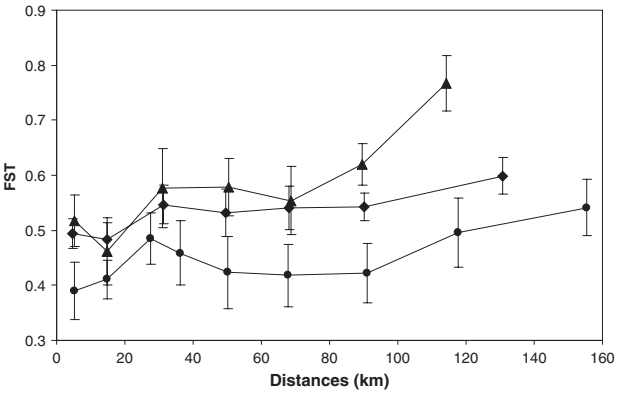

Fig. 3 Relationship between pairwise F_{ST} values calculated from the allele frequencies at six nuclear microsatellite loci and pairwise nearest distances among 50 populations of *Platyhypnidium riparioides* from southern Belgium. Mean pairwise F_{ST} values were calculated for different classes of distances ranging from 10 to 150 km, for the global dataset (diamonds) and within each of the southern (triangles) and northern (circles) groups identified by the spatial analysis of molecular variance (SAMOVA; see Fig. 1).

$P = 0.009$), but not in the northern group ($P = 0.13$). These correlations between the genetic and ecological matrices remained identical when the geographical signal was first removed from the genetic distance matrix. In contrast, when the ecological component of the genetic distance matrix was removed, the partial Mantel tests between the geographical and residuals of the genetic distance matrices remained significant in both the northern and southern groups ($r = 0.276$; $P = 0.015$ and $r = 0.326$; $P = 0.004$ for the northern and southern groups, respectively).

Discussion

Origin of polyploidy in *P. riparioides*

Flow cytometry revealed that all individuals exhibited the same ploidal level and, owing to the presence of heterozygous profiles in the data, individual gametophytes were considered to be diploid. Heterozygosity was, however, not fixed at any locus for any population. *Platyhypnidium riparioides* is therefore interpreted as an autopolyploid (see Soltis & Rieseberg, 1986; Arft & Ranker, 1998; Såstad *et al.*, 2001; Berglund *et al.*, 2006). In allopolyploids, by contrast, parental chromosomes may be so divergent that bivalent formation at meiosis only occurs between chromosomes that originate from each parental genome, and therefore they segregate in a disomic pattern, keeping fixed the different alleles of each parent. In the present data, the observed range of homo- and heterozygous genotypes is rather suggestive of tetrasomic inheritance, a condition that is thought to characterize *c.* 80% of moss species (Wyatt *et al.*, 1988; but see Shaw, 2009). Although an autotetraploid origin may obscure predictions regarding demographic aspects because, owing to the 'double reduction' specific to autopolyploids, the assortment of homologous chromosomes into gametes is not necessarily random (Ronfort *et al.*, 1998), it does not affect the interpretation of diversity patterns and population differentiation.

Patterns of genetic diversity and structure in *P. riparioides*

The overall pattern of genotypic variation in *P. riparioides* suggests strong differentiation among populations at a regional scale ($F_{ST} = 0.57$, $P < 0.001$). Such strong differentiation can be attributed to three main factors, including mating system, dispersal ability and diversifying selection (Hamrick & Godt, 1996; Duminil *et al.*, 2007).

The high values of F_{IS} observed within populations at both the ramet and genet levels, together with the deficits in heterozygotes observed, point to clonal reproduction and/or high levels of intragametophytic self-fertilization. A mating system with predominant selfing would not be surprising in *P. riparoides*, which has bisexual gametophytes with male and female gametangia borne in separate buds on a single plant (autoicous). Because egg and sperm are produced mitotically by moss gametophytes, intragametophytic selfing yields completely homozygous sporophytes. In addition, the higher proportion of ramets with the same genotype than expected by chance supports the view that the fairly low N_g/N ratio (0.42) observed is indicative of strong clonality. Although *P. riparioides* does not produce specialized vegetative dispersal structures, it is likely that gametophyte fragments detached from the main stem are able to develop into new clonal individuals. Both clonality and inbreeding can strongly influence population differentiation, because the effective size of each population is small. As a result, within-population variation is reduced and differentiation among populations increases, resulting in high F_{ST} values (Jain, 1983; Hamrick & Godt, 1989; Charlesworth, 2003). The observed global F_{ST} value is, in fact, comparable with that found in autogamous angiosperms (Hamrick & Godt, 1989).

Landscape-scale genetic variation in *P. riparioides* is, however, not random. A genetic discontinuity was identified between northern and southern populations within the study area (F_{ST} between the northern and southern regions, 0.57). This differentiation has been identified in other organisms as an important ecological discontinuity (Tournay, 1968; Van Rompaey & Delvosalle, 1979; Lambinon *et al.*, 2004), and might be explained by the different histories of the populations in the two areas. Some rivers in the northern area experienced dramatic pollution peaks in the 1960s–1970s, during which bryophytes were decimated in many places (Empain, 1977). Populations of the northern area could thus be characterized by a recent history of recolonization. However, a severe population bottleneck in the

northern region is inconsistent with the numbers of rare alleles detected in this study. In fact, 22 northern populations had rare alleles (with a frequency of < 0.01), when compared with only 10 in the southern region. One explanation is that the high allelic richness found in the northern area is of allochtonous origin. Habitat disturbance owing to high pollution peaks would have subsequently opened up opportunities for long-distance dispersers that would have been prevented from colonization in the biologically saturated southern area. This remains speculative, and detailed demographic analyses of populations that experienced contrasting pollution levels are currently ongoing.

Within the overall southern Belgium region, Mantel tests between geographical and genetic distance matrices show evidence of an increase in genetic differentiation with distances ranging from 20 to 140 km. These results are suggestive of a global dispersal limitation at the regional scale. Many other aquatic organisms exhibit the same pattern. Hughes (2007) reviewed genetic data for a range of freshwater species, including insects, fish, molluscs and crustacean species, and found that, except for aquatic insects with a flying adult stage, all were characterized by high levels of genetic differentiation among populations, revealing negligible dispersal across catchment boundaries. Even for crustacean species, which are believed to be capable of terrestrial dispersal, dispersal among streams within catchments was more limited than previously thought (for a review, see Clarke *et al.*, 2008).

The low level of gene flow implied by the strong differentiation among populations of *P. riparioides* within tens of kilometers of one another was, however, unexpected (average F_{ST} for populations < 10 km distant: 0.46 for linear distances and 0.49 for nearest geographical distances). Like other moss species, *P. riparioides* probably disperses vegetatively by gametophyte fragmentation. In addition, although its gametophyte is clearly adapted for growth in running water, the sporophyte of *P. riparioides* is produced during periods of low water discharge and is most often emergent. The capsule, and especially the peristome, composed of two concentric rings of hygroscopic teeth to effect spore liberation, are typical for terrestrial species of the same family (Vitt & Glime, 1984). The results of the Mantel tests employing shortest vs. linear distances along the water course show that only the former are significantly correlated with the genetic distances. This suggests that wind- rather than water-disseminated diaspores primarily contribute to the dispersal of the species.

Spore-reproducing organisms, and bryophytes in particular, are usually considered to be good dispersers, as suggested by the low levels of geographical differentiation revealed by many phylogeographical studies (van der Velde & Bijlsma, 2003; Werner & Guerra, 2004) and indirect measurements of intercontinental gene flow (McDaniel & Shaw, 2005; Szövényi *et al.*, 2008; Vanderpoorten *et al.*, 2008). In fact, the long-distance dispersal ability of bryophytes, an alternative explanation to ancient vicariance for broad intercontinental distributions of many species (for a review, see Shaw, 2001), has been increasingly acknowledged (Muñoz *et al.*, 2004; Aryanti & Gradstein, 2007; Peat *et al.*, 2007). Dispersal has been identified as one of the reasons why endemism rates in the bryophyte flora are consistently lower than in angiosperms (Vanderpoorten & Long, 2006; Vanderpoorten *et al.*, 2007, 2008). At the landscape scale, observations of colonization rates for alien substrates clearly suggest that many species are able to cross an apparently hostile landscape over tens to hundreds of kilometers within a 10–60 yr time frame (Miller & McDaniel, 2004; Hutsemekers *et al.*, 2008a). It has, on this basis, been suggested that many bryophytes have the mobility to overcome dispersal problems posed by fragmented landscapes (Hazell *et al.*, 1998; Moen & Jonsson, 2003; Pharo *et al.*, 2004, 2005).

Our results show that, although discrete long-distance dispersal is likely to account for most of the observed disjunct distributions of bryophytes (see Devos & Vanderpoorten, 2009 for review), the hypothesis that dispersal erases any biogeographical pattern (van Zanten & Pócs, 1981; Wolf *et al.*, 2001) can definitely be ruled out in the case of *P. riparioides*. The strongly structured pattern observed here is consistent with recent observations on the aggregated spatial structure of epiphytes in the landscape (Snäll *et al.*, 2003, 2004a, 2005; Löbel *et al.*, 2006). Using amplified fragment length polymorphism markers, Snäll *et al.* (2004a) confirmed that genetic variation in the moss *Neckera pennata* is not randomly distributed across the landscape, as would be expected if the species had a high rate of migration. Rather, offspring from the same parental source tend to occur most frequently in close proximity to the latter. This genetic structure was observed on a scale up to 350 m, and supports the hypothesis that some species have a restricted routine dispersal range. Hence, in mosses, distance-limited dispersal of diasporas, causing substantial geographical structure at a regional scale, is not incompatible with the possibility that occasional, very long dispersal events homogenize the genetic structure at an intercontinental scale.

Perhaps one reason for the failure of *P. riparioides* to disperse more freely at the landscape scale is that immigrants from some habitats are selected against in other habitats, reducing the effective migration rates. Indeed, although these results must be interpreted with caution, because partial Mantel tests are not exact and may be liberal (Raufaste & Rousset, 2001), our analyses suggest that genetic variation is significantly correlated with the global index of water quality derived from the environmental PCA in the southern ecoregion, but not in the northern one. This observation is consistent with the interpretation of different origins of the southern and northern groups described above. Populations in the south, which were less affected by the

pollution peaks of the 1960s–1970s than were those in the north, are likely to have had a much more ancient origin. This could allow for differentiation among ecologically specialized populations even in neutral markers.

The correlation between ecological and genetic variation observed here was unexpected for two reasons. First, even if certain traits are under divergent selection, dispersal would result in low differentiation at neutral loci. In the case of *P. riparioides*, however, genetic divergence is probably maintained by a limited dispersal ability and a mostly autogamous and/or clonal mating system. Second, although evidence for adaptation to high metal concentrations was found in the moss *Funaria hygrometrica* (Shaw, 1988), the general idea that emerges from the data currently available on bryophyte adaptation is that many bryophytes exhibit 'general-purpose genotypes' (Shaw, 1993). This means that, as opposed to angiosperms, they do not tend to develop adapted races, but rather display an inherent broad ability to tolerate high pollution levels: that is, they are capable of modifying their physiology in response to environmental conditions. For example, the aquatic moss *Fontinalis antipyretica* displays a fairly narrow temperature range, but is capable of acclimatization to moderately high temperatures. Although this moss normally exhibits an optimum temperature of *c.* 15°C, a period at 20–25°C eventually results in a shift in the compensation point, that is in a reduction of the respiration rate at the new temperature, apparent after *c.* 10 d (Carballeira *et al.*, 1998). The idea that a widespread aquatic moss such as *P. riparioides* is able to locally adapt to its environment suggests that bryophytes are, like angiosperms, able to develop local 'races' in response to environmental variation. If this hypothesis is confirmed by additional experimental work, it may have important consequences for the understanding of how environmental variation affects speciation in bryophytes, and may, in particular, help us to understand the origin of the increasing numbers of 'cryptic' bryophyte species that have been reported recently (Hedenäs & Eldenäs, 2007; Wachowiak *et al.*, 2007; Hedenäs, 2008).

Acknowledgements

Many thanks are due to I. Olivieri, P. Szovényi, J. Tigel Pourtois and H. Hürner, as well as three anonymous reviewers, for their constructive comments on a first draft of this article. Financial support from the Aquapôle, the Wallonie-Bruxelles International (WBI), and the Belgian Fund for Scientific Research (F.R.S.-FNRS) is gratefully acknowledged.

References

Antonovics J, Bradshaw AD, Turner RG. 1971. Heavy metal tolerance in plants. *Advances in Ecological Research* **7**: 1–85.

Arft AM, Ranker TA. 1998. Allopolyploid origin and population genetics of the rare orchid *Spiranthes diluvialis*. *American Journal of Botany* **85**: 110–122.

Aryanti NS, Gradstein SR. 2007. Wallace's line and the distribution of the liverworts of Sulawesi. *Cryptogamie Bryologie* **28**: 3–14.

Badri M, Zitoun A, Ilahi H, Huguet T, Aouani ME. 2008. Morphological and microsatellite diversity associated with ecological factors in natural populations of *Medicago laciniata* Mill. (Fabaceae). *Journal of Genetics* **87**: 241–255.

Baker AJM. 1987. Metal tolerance. *New Phytologist* **106**: 93–111.

Barker JSF, Frydenberg J, Gonzalez J, Davies HI, Ruiz A, Sorensen JG, Loeschcke V. 2009. Bottlenecks, population differentiation and apparent selection at microsatellite loci in Australian *Drosophila buzzatii*. *Heredity* **102**: 389–401.

Beaumont MA, Nichols RA. 1996. Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London Series B-Biological Sciences* **263**: 1619–1626.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F. 1996–2004. *GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations*. Montpellier (France): Laboratoire Génome, Populations, Interactions, CNRS UMR 5171, Université de Montpellier II.

Berglund ABN, Saura A, Westerbergh A. 2006. Electrophoretic evidence for disomic inheritance and allopolyploid origin of the octoploid Cerastium alpinum (Caryophyllaceae). *Journal of Heredity* **97**: 296–302.

Bonnet E, Van de Peer Y. 2002. ZT: a software tool for simple and partial Mantel tests. *Journal of Statistical Software* **7**: 1–12.

Brookfield JFY. 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology* **5**: 453–455.

Carballeira A, Diaz S, Vazquez MD, Lopez J. 1998. Inertia and resilience in the responses of the aquatic bryophyte *Fontinalis antipyretica* Hedw. to thermal stress. *Archives of Environmental Contamination and Toxicology* **34**: 343–349.

Chapuis MP, Estoup A. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* **24**: 2465–2473.

Charlesworth D. 2003. Effects of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **358**: 1051–1070.

Charlesworth B, Nordborg M, Charlesworth D. 1997. The effects of local selection, balanced polymorphism and background selection on equilibrium patterns of genetic diversity in subdivided populations. *Genetical Research* **70**: 155–174.

Clarke A, MacNally R, Bond N, Lake PS. 2008. Macroinvertebrate diversity in headwater streams: a review. *Freshwater biology* **53**: 1707–1721.

Cun C, Bousquet G, Vilagines R. 1997. A 90-year record of water quality data of Paris Seine and Marne rivers. *Journal of Water Supply: Research and Technology – Aqua* **46**: 150–164.

David P, Pujol B, Viard F, Castella V, Goudet V. 2007. Reliable selfing rate estimates from imperfect population genetic data. *Molecular Ecology* **16**: 2474–2487.

Devos N, Vanderpoorten A. 2009. Range disjunctions, speciation, and morphological transformation rates in the liverwort genus *Leptoscyphus*. *Evolution* **63**: 779–792.

Dierßen K. 2001. Distribution, ecological amplitude and phytosociological characterization of European bryophytes. *Bryophytorum Bibliotheca* **56**: 1–289.

Doyle JJ, Doyle JL. 1990. Isolation of plant DNA from fresh tissue. *Focus* **12**: 13–15.

Duckett JG, Ligrone R. 1992. A survey of diaspore liberation mechanisms and germination patterns in mosses. *Journal of Bryology* **17**: 335–354.

Duminil J, Fineschi S, Hampe A, Jordano P, Salvini D, Vendramin GG, Petit RJ. 2007. Can population genetic structure be predicted from life-history traits? *American Naturalist* **169**: 662–672.

Dupanloup I, Schneider S, Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* **11**: 2571–2581.

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

Empain A. 1977. *Ecologie des populations de bryophytes aquatiques de la Meuse, de la Sambre et de la Somme. Relations avec la qualité des eaux, écophysiologie comparée, et étude de la contamination par métaux lourds*. PhD thesis, University of Liège, Belgium.

Fritsch R. 1991. Index to bryophyte chromosome counts. *Bryophytorum Bibliotheca* **40**: 1–352.

Goudet J. 1999. *PCAGEN, A computer package which performs principal component analysis (PCA) on gene frequency data*. Available from URL: http://www2.unil.ch/popgen/softwares/pcagen.htm.

Hamrick JL. 1989. Isozymes and analyses of genetic structure of plant populations. In: Soltis D, Soltis P, eds. *Isozymes in plant biology*. Portland, OR, USA: Discorides Press, 87–105.

Hamrick JL, Godt MJ. 1989. Allozyme diversity in plant species. In: Brown AHD, Clegg MT, Kahler AL, Weir BS, eds. *Plant population genetics, breeding and germplasm resources*. Sunderland, MA, USA: Sinauer Associates, 43–63.

Hamrick JL, Godt MJW. 1996. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **351**: 1291–1298.

Hardy OJ, Vekemans X. 2002. SPAGeDI: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**: 618–620.

Hardy OJ, Charbonnel N, Freville H, Heuertz M. 2003. Microsatellite allele size: a simple test to assess their significance on genetic differentiation. *Genetics* **163**: 1467–1482.

Hazell P, Kellner O, Rydin H, Gustafsson L. 1998. Presence and abundance of four epiphytic bryophytes in relation to density of aspen (*Populus tremula*) and other stand characteristics. *Forest Ecology and Management* **107**: 147–158.

Hedenäs L. 2008. Molecular variation and speciation in *Antitrichia curtipendula* s.l. (Leucodontaceae, Bryophyta). *Botanical Journal of the Linnean Society* **156**: 341–354.

Hedenäs L, Eldenäs P. 2007. Cryptic speciation, habitat differentiation, and geography in *Hamatocaulis vernicosus* (Calliergonaceae, Bryophyta). *Plant Systematics and Evolution* **268**: 131–145.

Hughes JM. 2007. Constraints on recovery: using molecular methods to study connectivity of aquatic biota in rivers and streams. *Freshwater Biology* **52**: 616–631.

Hutsemekers V, Dopagne C, Vanderpoorten A. 2008a. How far and how fast do bryophytes travel at the landscape scale? *Diversity and Distributions* **14**: 483–492.

Hutsemekers V, Risterucci AM, Ricca M, Boles S, Hardy OJ, Shaw AJ, Vanderpoorten A. 2008b. Identification and characterization of nuclear microsatellite loci in the aquatic moss *Platyhypnidium riparioides* (Brachytheciaceae). *Molecular Ecology Resources* **8**: 1130–1132.

Jain SK. 1983. Breeding systems and the dynamics of plant populations. In: Chopra VL, Joshi BC, Sharma RP, Bansal HC eds. *Genetics: new frontiers*. New Delhi, India: Oxford and IBH Publishing Co, 291–316.

Kallio P, Saarnio E. 1986. The effect on mosses of transplantation to different latitudes. *Journal of Bryology* **14**: 159–178.

Kelly MG, Whitton BA. 1998. Biological monitoring of eutrophication in rivers. *Hydrobiologia* **384**: 55–67.

Lambinon J, Delvosalle L, Duvigneaud J. 2004. *Nouvelle flore de la Belgique, du Grand-Duché de Luxembourg, du Nord de la France et des régions voisines*. 5th edn. Meise, Belgium: Jardin Botanique National de Belgique.

Leclercq L, Vandevenne L. 1987. Impact d'un rejet d'eau chargée en sel e d'une pollution organique sur les peuplements de diatomées de la Gander (Grand-Duché de Luxembourg). *Cahiers de Biologie Marine* **28**: 311–318.

Löbel S, Snäll T, Rydin H. 2006. Metapopulation processes in epiphytes inferred from patterns of regional distribution and local abundance in fragmented forest landscapes. *Journal of Ecology* **94**: 856–868.

Luttikhuizen PC, Stift M, Kuperus P, van Tienderen PH. 2007. Genetic diversity in diploid vs. tetraploid *Rorippa amphibian* (Brassicaceae). *Molecular Ecology* **16**: 3544–3553.

Malle KG. 1994. Accidental spills – frequency, importance, control, counter measures. *Water Science and Technology* **29**: 149–164.

Malle KG. 1996. Cleaning-up the river Rhine. *Scientific American* **274**: 55–59.

Markert BA, Breure AM, Zechmeister HG. 2003. *Bioindicators and biomonitors. Principles, concepts and applications*. Oxford, UK: Elsevier Press.

McDaniel SF, Shaw AJ. 2005. Selective sweeps and intercontinental migration in the cosmopolitan moss *Ceratodon purpureus* (Hedw.) Brid. *Molecular Ecology* **14**: 1121–1132.

Michalakis Y, Excoffier L. 1996. A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics* **142**: 1061–1064.

Miller NG, McDaniel SF. 2004. Bryophyte dispersal inferred from colonization of an introduced substratum on Whiteface Mountain, New York. *American Journal of Botany* **91**: 1173–1182.

Moen J, Jonsson BG. 2003. Edge effects on liverworts and lichens in forest patches in a mosaic of boreal forest and wetland. *Conservation Biology* **17**: 380–388.

Muñoz J, Felicisimo AM, Cabezas F, Burgaz AR, Martinez I. 2004. Wind as a long-distance dispersal vehicle in the Southern Hemisphere. *Science* **304**: 1144–1147.

Naiman RJ. 1992. *Watershed management: balancing sustainability with environmental changes*. New York, NY, USA: Springer Verlag.

Nielsen EE, Wright PJ, Hemmer-Hansen J, Poulsen NA, Gibb LM, Meldrup D. 2009. Micro geographical population structure of cod *Gadus morhua* in the North Sea and west of Scotland: the role of sampling loci and individuals. *Marine Ecology Progress Series* **376**: 213–225.

Peat HJ, Clarke A, Convey P. 2007. Diversity and biogeography of the Antarctic flora. *Journal of Biogeography* **34**: 132–146.

Pharo EJ, Zartman CE. 2007. Bryophytes in a changing landscape: the hierarchical effect of habitat fragmentation on ecological and evolutionary processes. *Biological Conservation* **135**: 315–325.

Pharo EJ, Lindenmayer DB, Taws N. 2004. The effects of large-scale fragmentation on bryophytes in temperate forests. *Journal of Applied Ecology* **41**: 910–921.

Pharo EJ, Kirkpatrick JB, Gilfedder L, Mendel L, Turner PAM. 2005. Predicting bryophyte diversity in grassland and eucalypt-dominated remnants in subhumid Tasmania. *Journal of Biogeography* **32**: 2015–2024.

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

Raufaste N, Rousset F. 2001. Are partial Mantel tests adequate? *Evolution* **55**: 1703–1705.

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

Ronfort JL, Jenczewski E, Bataillon T, Rousset F. 1998. Analysis of population structure in autotetraploid species. *Genetics* **150**: 921–930.

Såstad SM, Flatberg KI. 1994. Leaf size and shape in the *Sphagnum recurvum* complex – taxonomic significance and habitat variation. *Journal of Bryology* **18**: 261–275.

Såstad S, Pederson B, Digre K. 1999. Habitat-specific genetic effects on growth rate and morphology across pH and water-level gradients within a population of the moss *Sphagnum angustifolium* (Sphagnaceae). *American Journal of Botany* **86**: 1687–1698.

Såstad SM, Stenoien HK, Flatberg KI, Bakken S. 2001. The narrow endemic *Sphagnum troendelagicum* is an allopolyploid derivative of the widespread *S. balticum* and *S. tenellum*. *Systematic Botany* **26**: 66–74.

Shaw AJ. 1988. Genetic variation for tolerance to copper and zinc within and among populations of the moss, *Funaria hygrometrica* Hedw. *New Phytologist* **109**: 211–222.

Shaw AJ. 1992. The evolutionary capacity of bryophytes and lichens. In: Bates JW, Farmer AM, eds. *Bryophytes and lichens in a changing environment*. Oxford, UK: Clarendon Press, 362–380.

Shaw AJ. 1993. Morphological uniformity among widely disjunct populations of the rare copper moss, *Scopelophila cataractae* (Pottiaceae). *Systematic Botany* **18**: 525–537.

Shaw AJ. 2001. Biogeographic patterns and cryptic speciation in bryophytes. *Journal of Biogeography* **28**: 253–261.

Shaw AJ. 2009. Bryophyte species and speciation. In: Goffinet B, Shaw AJ eds. *Bryophyte biology*, 2nd edn. Cambridge: Cambridge University Press, 445–485.

Shaw AJ, Albright DL. 1990. Potential for the evolution of heavy-metal tolerance in *Bryum argenteum*, a moss. 2. Generalized tolerances among diverse populations. *The Bryologist* **93**: 187–192.

Shaw AJ, Beer SC, Lutz J. 1989. Potential for the evolution of heavy-metal tolerance in *Bryum argenteum*, a moss. I. Variation within and among populations. *The Bryologist* **92**: 73–80.

Shaw B, Terracciano S, Shaw AJ. 2009. A genetic analysis of two recently described peat moss species, *Sphagnum atlanticum* and *S. bergianum* (Sphagnaceae). *Systematic Botany* **34**: 6–12.

Slatkin M. 1995a. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**: 457–462.

Slatkin M. 1995b. Hitchhiking and associative overdominance at a microsatellite locus. *Molecular Biology and Evolution* **12**: 473–480.

Snäll T, Ribeiro PJ, Rydin H. 2003. Spatial occurrence and colonizations in patch-tracking metapopulations of epiphytic bryophytes: local conditions vs dispersal. *Oikos* **103**: 566–578.

Snäll T, Fogelqvist J, Bibeiro PJ, Lascoux L. 2004a. Spatial genetic structure in two congeneric epiphytes with different dispersal strategies analysed by three different methods. *Molecular Ecology* **13**: 2109–2119.

Snäll T, Hagstrom A, Rudolphi J, Rydin H. 2004b. Distribution pattern of the epiphyte *Neckera pennata* on three spatial scales – importance of past landscape structure, connectivity and local conditions. *Ecography* **27**: 757–766.

Snäll T, Ehrlen J, Rydin H. 2005. Colonization–extinction dynamics of an epiphyte metapopulation in a dynamic landscape. *Ecology* **86**: 106–115.

Soltis DE, Rieseberg LH. 1986. Autopolyploidy in *Tolmiea menziesii* (Saxifragaceae): genetic insights from enzyme electrophoresis. *American Journal of Botany* **73**: 310–318.

Szövényi P, Terracciano S, Ricca M, Shaw AJ. 2008. Recent divergence, intercontinental dispersal and shared polymorphisms are shaping the genetic structure of amphi-Atlantic peatmoss populations. *Molecular Ecology* **17**: 5364–5377.

Tournay R. 1968. Le Brome des Ardennes "*Bromus ardennensis*" et ses proches *B. secalinus* et *B. grossus*. *Bulletin du Jardin Botanique de l'Etat à Bruxelles* **38**: 295–380.

Ueno T, Bekku Y, Uchida M, Kanda H. 2006. Photosynthetic light responses of a widespread moss, *Sanionia uncinata*, from contrasting water regimes in the high Arctic tundra, Svalbard, Norway. *Journal of Bryology* **28**: 345–349.

Van Rompaey E, Delvosalle L 1979. *Atlas de la Flore belge et luxembourgeoise, Ptérido-phytes et Spermatophytes*. 2nd edn. Meise, Belgium: Jardin botanique national de Belgique.

Van Rossum F, Vekemans X, Meerts P, Gratia E, Lefebvre C. 1997. Allozyme variation in relation to ecotypic differentiation and population size in marginal populations of *Silene nutans*. *Heredity* **78**: 552–560.

Vanderpoorten A. 1999. Aquatic bryophytes for a spatio-temporal monitoring of the water pollution of the rivers Meuse and Sambre (Belgium). *Environmental Pollution* **104**: 401–410.

Vanderpoorten A. 2003. Hydrochemical determinism, ecological polymorphism and indicator values of aquatic bryophytes for water quality. In: Ambasht RS, Ambasht NK eds. *Applied aquatic ecology*. Dordrecht, the Netherlands: Kluwer Academic Publishers, 65–96.

Vanderpoorten A, Durwael L. 1999. Trophic response curves of aquatic bryophytes in lowland calcareous streams. *The Bryologist* **102**: 720–728.

Vanderpoorten A, Long DG. 2006. Budding speciation and neotropical origin of the Azorean endemic liverwort, *Leptoscyphus azoricus*. *Molecular Phylogenetics and Evolution* **40**: 73–83.

Vanderpoorten A, Rumsey FJ, Carine MA. 2007. Does Macaronesia exist? Conflicting signal in the bryophyte and pteridophyte floras. *American Journal of Botany* **94**: 625–639.

Vanderpoorten A, Devos N, Goffinet B, Hardy OJ, Shaw AJ. 2008. The barriers to oceanic island radiation in bryophytes: insights from the phylogeography of the moss *Grimmia montana*. *Journal of Biogeography* **35**: 654–663.

Vekemans X, Lefebvre C. 1997. On the evolution of heavy-metal tolerant populations in *Armeria maritima*: evidence from allozyme variation and reproductive barriers. *Journal of Evolutionary Biology* **10**: 175–191.

van der Velde M, Bijlsma R. 2003. Phylogeography of five *Polytrichum* species within Europe. *Biological Journal of the Linnean Society* **78**: 203–213.

Virtanen R, Oksanen J. 2007. The effects of habitat connectivity on cryptogam richness in boulder metacommunity. *Biological Conservation* **135**: 415–422.

Vitalis R, Couvet D. 2001. Two-locus identity probabilities and identity disequilibrium in a partially selfing subdivided populations. *Genetical Research* **77**: 67–81.

Vitt DH, Glime JM. 1984. The structural adaptations of aquatic *Musci*. *Lindbergia* **10**: 95–110.

Wachowiak W, Baczkiewicz A, Chudzinska E, Buczkowska K. 2007. Cryptic speciation in liverworts – a case study in the *Aneura pinguis* complex. *Botanical Journal of the Linnean Society* **155**: 273–282.

Wehr JD, Whitton BA. 1986. Ecological factors relating to morphological variation in the aquatic moss *Rhynchostegium riparioides*. *Journal of Bryology* **14**: 269–280.

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

Werner O, Guerra J. 2004. Molecular phylogeography of the moss *Tortula muralis* Hedw. (Pottiaceae) based on chloroplast rps4 gene sequence data. *Plant Biology* **6**: 147–157.

Wolf PG, Schneider H, Ranker TA. 2001. Geographic distributions of homosporous ferns: does dispersal obscure evidence of vicariance? *Journal of Biogeography* **28**: 263–270.

Wyatt R, Rdrzykoski IJ, Stoneburner A, Bass HW, Galau GA. 1988. Allopolyploidy in bryophytes: multiple origins of *Plagiomnium medium*. *Proceedings of the National Academy of Sciences, USA* **85**: 5601–5604.

van Zanten BO, Pócs T. 1981. Distribution and dispersal of bryophytes. *Advances in Bryology* **1**: 479–562.

Zartman CE, Shaw AJ. 2006. Metapopulation extinction thresholds in rain forest remnants. *The American Naturalist* **167**: 177–189.