plant biology

Plant Biology ISSN 1435-8603

RESEARCH PAPER

Genomic scanning using AFLP to detect loci under selection in the moss *Funaria hygrometrica* along a climate gradient in the Sierra Nevada Mountains, Spain

M. Magdy[1,2], O. Werner[1], S. F. McDaniel[3], B. Goffinet[4] & R. M. Ros[1]

1 Departamento de Biología Vegetal, Facultad de Biología, Universidad de Murcia, Murcia, Spain
2 Genetics Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
3 Biology Department, University of Florida, Gainesville, FL, USA
4 Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT, USA

Keywords
Adaptive variation; bryophytes; mantel tests; moss distribution.

Correspondence
M. Magdy, Genetics Department, Faculty of Agriculture, Ain Shams University, 68 Hadayek Shubra, 11241 Cairo, Egypt.
E-mail: m.elmosallamy@agr.asu.edu.eg

Editor
J. Keurentjes

Received: 13 March 2015; Accepted: 11 August 2015

doi:10.1111/plb.12381

ABSTRACT

The common cord moss *Funaria hygrometrica* has a worldwide distribution and thrives in a wide variety of environments. Here, we studied the genetic diversity in *F. hygrometrica* along an abiotic gradient in the Mediterranean high mountain of Sierra Nevada (Spain) using a genome scan method. Eighty-four samples from 17 locations from 24 to 2700 m were fingerprinted based on their amplified fragment length polymorphism (AFLP) banding pattern. Using PCA and Bayesian inference we found that the genetic diversity was structured in three or four clusters, respectively. Using a genome scan method we identified 13 outlier loci, which showed a signature of positive selection. Partial Mantel tests were performed between the Euclidean distance matrices of geographic and climatic variables, *versus* the pair-wise genetic distance of the AFLP dataset and AFLP-positive outliers dataset. AFLP-positive outlier data were significantly correlated with the gradient of the climatic variables, suggesting adaptive variation among populations of *F. hygrometrica* along the Sierra Nevada Mountains. We highlight the additional analyses necessary to identify the nature of these loci, and their biological role in the adaptation process.

INTRODUCTION

Funaria hygrometrica Hedw. (Funariaceae) is a cosmopolitan, hermaphroditic moss, common in disturbed habitats (Parihar 1961; Crum 1972; Shaw 1991; Hallingbäck & Hodgetts 2000). *F. hygrometrica* produces abundant sporophytes per population and large quantities of small spores that are easily airborne, two traits that are characteristic of a fugitive life strategy (During 1979). The high dispersal ability and fugitive characteristics make *F. hygrometrica* a suitable model to study the genetic variability and its correlation to changes in environmental factors (*e.g.* climate), especially to test the hypothesis of Baas-Becking (1934), reviewed in Fontaneto (2011), '*Everything is everywhere, but the environment selects*', whereby local adaption occurs within a widely distributed species with high dispersal capacity. We sought to test this hypothesis using amplified fragment length polymorphism (AFLP) within and between populations of *F. hygrometrica* in the Sierra Nevada of Spain.

The AFLP technique (Vos *et al.* 1995) has been widely used to characterise genetic diversity within and among populations, particularly in non-model organisms for which no prior DNA sequence information is available. Such wide multi-locus screening (also known as a genome scan) also allows for the identification of so-called outlier loci for which alleles are highly differentiated among populations, and hence potentially linked to adaptive divergence (Black *et al.* 2001; Luikart *et al.* 2003; Storz 2005). Those outlier loci, if involved in adaptation to local environmental conditions, would indeed be expected to exhibit increased differentiation among populations and decreased diversity within populations. AFLP-based genome scans revealed outlier loci linked to adaptation to altitude (Bonin *et al.* 2006; Poncet *et al.* 2010), host plants (Conord *et al.* 2006; Egan *et al.* 2008; Nosil *et al.* 2008; Manel *et al.* 2010), floral divergence (Herrera & Bazaga 2008), insecticide resistance (Paris *et al.* 2010), selection during domestication (Rossi *et al.* 2009) or ecotype divergence (Wilding *et al.* 2001; Campbell & Bernatchez 2004; Savolainen *et al.* 2006; Meyer *et al.* 2009). Overall, about 5% of loci show potential signatures of selection (Nosil *et al.* 2009), suggesting that the distribution of their allelic diversity is not random.

We tested for local adaptation in populations of *F. hygrometrica* sampled along an altitudinal gradient in the Sierra Nevada Mountains of Spain, one of highest mountain ranges of the Mediterranean region. The altitudinal range, southern latitude in the context of the European continent and complex topography account for the large climate diversity in the Sierra Nevada and the surrounding areas. Near the Mediterranean coast, subtropical plants like avocado (*Persea americana* Mill.) and cherimoya (*Anone cherimola* Mill.) are cultivated, whereas the summits are in some years covered with snow until July. The Sierra Nevada is located in the southern part of the Iberian Peninsula in the Mediterranean zone. Rainfall is minimal between May and October, and above 2000 m a.s.l., precipitation occurs mostly in the form of snow (Blanca *et al.* 2009).

Parts of the mountains were declared a natural park in 1989 and national park in 1999. *F. hygrometrica* was reported to be very common along the mountain range between 410–2861 m a.s.l. (Rams *et al.* 2014) and is also found frequently in coastal areas outside the protected area of Sierra Nevada.

The objectives of this study were to (i) characterise the genetic variation within and among populations of *F. hygrometrica* along broad ecological gradients, and (ii) test whether allelic differentiation can be correlated to, and hence be explained by, selection by climatic factors using the genome scan method.

MATERIAL AND METHODS

Material

A total of 84 samples from 17 locations were collected and ordered by their altitude above sea level in meters in the Sierra Nevada Mountains of Spain. Based on both altitude and geographic coordinates, the 17 locations were separated into four altitudinal groups (Table 1). Group 1 (G1) comprises seven lowland locations distributed between 27–657 m a.s.l. and between 36°43′–36°55′ N and 03°11′–03°28′ W; group 2 (G2) is four mid-land locations between 755–1328 m a.s.l. and between 37°08′–37°09′ N and 03°29′–03°32′ W; group 3 (G3) is two high mid-land locations between 1650–1667 m a.s.l. at approximately 37°07′ N and 03°26′ W; and group 4 (G4) is four highland locations between 2180–2700 m a.s.l. and situated between 37°04′–37°06′ N and 03°23′–03°25′ W.

In vitro cultivation

Spores were isolated from mature capsules and cultured *in vitro* under axenic conditions. Resulting protonemata were used for DNA extraction. The capsules of samples from locations 14, 15, 16 and 17 did not have viable spores, and DNA was extracted from the cleaned upper part of sterilised gametophytes. Samples were cultivated on Murashige and Skoog (MS; Murashige & Skoog 1962) nutrient media or solid Knop medium (KM; Knop 1884). Capsules and green gametophytes were surface-sterilised for 5 min using 0.5% sodium dichloroisocyanuric acid ($C_3Cl_2N_3NaO_3$). All cultures were kept in an incubator (Binder KB 720, Tuttlingen, Germany) at 22 ± 3 °C, and 16/8 h light/dark, supplied by cool-white fluorescent tubes at a photon fluency rate of 33.5–55 μmol $m^2 \cdot s^{-1}$ (Sabovljević *et al.* 2003).

Extraction of DNA

The DNA extractions were carried out using GenElute™ Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St. Louis, MO, USA, Cat# G2N350) following the manufacturer's manual. DNA was quantified using an Eppendorf spectrophotometer.

Amplified fragment length polymorphism analysis

The protocol follows Vos *et al.* (1995), with the primers fluorescently rather than radioactively labelled. All primers and adaptors were synthesised by Eurofins, Hamburg, Germany (Table 2). Twelve different selective PCR combinations (3 Eco+NNN fluorescently labelled forward primers × 4 Mse+NNN reverse primers) selected from Kamisugi *et al.* (2008) were tested using the original PCR programme (Vos *et al.* 1995). Amplified products were visualised by Secugen, S.L. sequencing service (Madrid, Spain), with an ABI3730 DNA analyser (Applied Biosystems, Carlsbad, CA, USA). A size standard (RoxGeneScan500) was added to the samples in order to estimate the size of the amplified fragments.

Data analysis

Loci scoring

The treatment of AFLP data is very sensitive to factors like small bands, stutter bands, dimers and background noise. To minimise the influence of such factors an automated AFLP scoring was performed using Peakscanner (Applied Biosystems) for peak analysis, and Rawgeno version 2 for band scoring and quality tests. Analysis of the AFLP data was based on the band-binary criterion (*i.e.* coding the locus as 1 when a band is present and 0 when it is absent) and processed according to Bonin *et al.* (2007). Bands with a frequency of more than 95% or <5% are often uninformative or misleading when included in the analyses (Cavers *et al.* 2005; Bonin *et al.* 2007) and were therefore excluded from further analysis using FAMD software (Schlüter & Harris 2006).

Population structure

To infer the pattern of population structure of the genetic diversity, we used a non-spatial Bayesian clustering method (*i.e.* Structure version 2.2; Pritchard *et al.* 2000; Hubisz *et al.* 2009). To determine the best number of clusters, three independent simulations were performed per number of sub-populations K (30 runs of K = 1–10). The optimal K number is determined based on the highest average of the estimated ln probability score that shows the lowest variance for each run. A burn-in period of 10,000 out of 100,000 Markov chain Monte Carlo (MCMC) iterations was chosen, and an admixture ancestry model with correlated allele frequencies was invoked. The bar plot of the Structure output was plotted in coloured groups according to the K number of sub-populations with the highest likelihood log. Fingerprint analysis with missing data (FAMD) software was used to perform principle components analyses (PCA), applying the default settings. The output graph was edited using Microsoft Excel 2013, and adjacent samples were defined as a cluster.

Outlier loci detection

Loci exhibiting F_{ST} values outside the 0.995 confidence interval were identified as positive outliers using the software Mcheza (Antao & Beaumont 2011), by performing 16 pair-wise analyses between the four altitudinal groups. Under the default parameters, Mcheza was run five times with 500,000 simulations. For each locus, Mcheza plots the estimated F_{ST} value against its heterozygosity (He) value. Positive outlier loci (*i.e.* those over the 99.5% limit) extracted from each run were compared with each other. Loci that constantly appeared to be outliers were included in further analyses.

Genetic differentiation and gene flow

Arlequin version 3.5 (Excoffier & Lischer 2010) was used to test the population genetic differentiation by performing an analysis of molecular variance (AMOVA). Fixation index F_{ST}

Table 1. Locations sampled in the Sierra Nevada Mountains of Spain and surroundings.

Groups	Location no.	Altitude	Geographic coordinates	Description	Collected samples (Sample no.)
G1	Loc_1	24 m	36°43′ 57.4″ N; 03°31′ 00.9″ W	Road N-340 km 330, direction to Motril coming from Granada. The sample was found in a shady corner on the roadside, near an artificial small water canal	1 (1)
	Loc_2	58 m	36°43′ 47.4″ N; 03°30′ 30.8″ W	Road N-340 km 334, direction to Motril coming from Granada. Samples were found in a palm nursery with highly moist soil	3 (2–4)
	Loc_3	58 m	36°45′4.6″ N; 03°11′ 59.6″ W	Road N-340 km 370, direction to Motril coming from Granada. Samples were found on the roadsides of 30-m length	2 (5–6)
	Loc_4	287 m	36°51′ 56.5″ N; 03°28′ 59.6″ W	Road A-346 km 9, direction to Órgiva coming from Granada. Samples were collected on asphalt and soil slope on the edge of the road	3 (7–9)
	Loc_5	382 m	36°52′ 19.8″ N; 03°28′ 24.3″ W	Road A-346 km 7, direction to Órgiva coming from Granada. Samples were collected around the edge of the road	8 (10–17)
	Loc_6	630 m	36°55′1.60″ N; 03°28′47.9″ W	Lanjarón city. Samples were found on a soil slope on the edge of the road, at the entrance to the city, the slope was nitrified	4 (18–21)
	Loc_7	657 m	36°55′1.40″ N; 03°28′17.0″ W	Out of Lanjarón city, road A-348, direction to Órgiva coming from Lanjarón. Samples were found on a soil slope on the edge of the road out of the city, the slope was nitrified and moist	3 (22–24)
G2	Loc_8	755 m	37°09′17.4″ N; 03°32′46.2″ W	Cenés de la Vega surroundings. Samples were found along the road and cliff edge in front of a restaurant	9 (25–33)
	Loc_9	757 m	37°09′ 41.9″ N; 03°31′27.2″ W	Road GR-420 km 1, direction to Pinos Genil coming from Cenés de la Vega. Samples were collected from the edge of the road in the proximity of a restaurant and farms	9 (34–42)
	Loc_10	1295 m	37°08′ 34.1″ N; 03°29′14.5″ W	Road A-395 km 15, up to Sierra Nevada crests. Samples were collected from the edge of the road, on soil slopes in the vicinity of La Higuera restaurant	10 (43–52)
	Loc_11	1328 m	37°08′22.7″ N; 03°29′03.5″ W	Road A-395 km 16, up to Sierra Nevada crests. Samples were collected from the edge of the road, on soil slopes in the vicinity of Los Puentes restaurant	10 (53–62)
G3	Loc_12	1650 m	37°07′58.0″ N; 03°26′20.6″ W	Road A-395, barranco de Las Víboras, up to Sierra Nevada crests. Samples were collected from the edge of the road, on soil slopes in the vicinity of Los Jamones restaurant	8 (63–70)
	Loc_13	1667 m	37°07′57.9″ N; 03°26′06.2″ W	Road A-395, centre for nature interpretation El Dornajo. Samples were collected on soils in proximity to the centre	6 (71–76)
G4	Loc_14	2180 m	37°05′ 35.7″ N; 03°23′54.5″ W	Ski resort Pradollano. Samples were collected on soils at the base of a chairlift machine and surrounding sites	2 (77–78)
	Loc_15	2220 m	37°06′47.9″ N; 03°25′10.0″ W	Road A-395, military mountain hostel. Samples were collected from soil slopes around the hostel	2 (79–80)
	Loc_16	2622 m	37°04′22.3″ N; 03°23′28.2″ W	Artificial lake at the base of the ski resort Borreguiles. Soil on rocks around the lake.	3 (81–83)
	Loc_17	2700 m	37°04′16.4″ N; 03°23′14.6″ W	Ski resort Borreguiles. The sample was collected on soil surrounding a chairlift machine	1 (84)

Locations are ordered by altitude, along with their description, positioning in geographic coordinates and number of collected samples.

Table 2. Sequence (5′–3′) of primers and adaptors used in the current study.

Primer/Adaptor	5′ - sequence - 3′	Primer/Adaptor	5′ - sequence - 3′
MseI-A1	GACGATGAGTCCTGAG	EcoRI-A1	CTCGTAGACTGCGTACC
MseI-A2	TACTCAGGACTCAT	EcoRI-A2	AATTGGTACGCAGTC
Mse-C	GATGAGTCCTGAGTAA**C**	Eco-A	GACTGCGTACCAATTC**A**
Mse-CAA	GATGAGTCCTGAGTAA**CAA**	Eco-ACA	***FAM-***GACTGCGTACCAATTC**ACA**
Mse-CTC	GATGAGTCCTGAGTAA**CTC**	Eco-AGG	***HEX-***GACTGCGTACCAATTC**AGG**
Mse-CAT	GATGAGTCCTGAGTAA**CAT**	Eco-ATA	***CY3-***GACTGCGTACCAATTC**ATA**
Mse-CTA	GATGAGTCCTGAGTAA**CTA**		

Selective nucleotides and fluorescent labels are in bold.

was used to estimate the part of the observed variance that corresponds to the variability within populations, even though for two populations only one individual was available (locations 1 and 17, unique samples). The significance of F_{ST} was tested with 10,000 permutations for both the complete data set and the detected AFLP outlier loci. Gene flow (Nm) based on F_{ST} value was estimated for both using AFLP-Surv (Vekemans *et al.* 2002).

Environmental variables

Climate variables of the Sierra Nevada and surrounding areas were estimated based on data gathered from meteorological

stations and then processed statistically to be more applicable in environmental studies. Worldclim is one of the basic international sources for such variables on a global scale (Hijmans *et al.* 2005). However, Benito *et al.* (2011) performed a more precise study, and estimated minimum and maximum temperature and precipitation values of different resolution specifically for the Sierra Nevada Mountains and surrounding areas. The climate variables were obtained from Dr. B. Benito (Granada University) as generic grids at 10-m resolution and extracted using DIVA-GIS version 7.4.0.1 (Hijmans *et al.* 2001). Geographic distance in kilometres was estimated with a distance calculator built in DIVA-GIS. Each variable was standardised using principal coordinates analysis PCO3 (Anderson 2003) by subtracting each value from the sample mean and dividing the result by the sample SD, a procedure known as *z*-score transformation. PCO3 was finally used to generate the Euclidean distance between the 17 sampled locations based on each environmental variable.

Correlation tests

The population genetic diversity of *F. hygrometrica* in the Sierra Nevada Mountains was subjected to correlation tests with spatial patterning of climatic variation (*i.e.* isolation by adaptation, IBA). Partial Mantel tests (to measure the association between two matrices while avoiding any correlation to a third matrix) were performed using GenAlEx version 6 (Peakall & Smouse 2006). The genetic distance matrices calculated with PCO3 using the Jaccard dissimilarity method of the scored bands, for the AFLP-neutral and AFLP-positive outlier loci datasets separately, were tested against the Euclidean distance of the climate variables while controlling of the geographic distance matrix. A Mantel test was then performed using the default preset values and parameters (9999 permutation steps) according to the software manual.

RESULTS

Band scoring quality and DNA polymorphism

The PCR amplification was successful for 12 primer pairs. We were able to accurately score bands for each primer pair between 150–600 bp, with up to 195 peaks per primer pair. Peak analysis and automated band scoring were successful and quality tests showed adequate quality of band scoring (data not shown). In total, 3057 loci were scored from all primer pairs for all 84 samples. Among these, 3056 loci were polymorphic, only one was monomorphic; 1411 loci were rare, characterised by band frequencies <5%, and 61 loci had band frequencies >95% among all samples. Thus, only 1584 loci were used for further analyses. The mean fragment size was ~321 bp ± 121 bp (±SD). The mean number of bands present per location was ~607 ± 93 bands. All samples presented a different multi-locus genotype and no AFLP bands of the final dataset (after excluding the rare or very frequent bands) showed the same individual pattern.

Population and genetic structure

The PCA graph (Fig. 1) of all individuals showed a distribution of the samples into three main clusters. Cluster A contained the highest number of samples (65), whereas cluster B had 12 samples and cluster C had seven samples. Although individuals from one locality tend to cluster together, individual locations harbour individuals that are clearly genetically distinct. For example, samples from location 12 (numbers 63–70) are scattered among clusters A and B.

By applying the criteria of the highest average estimated ln probability score and lowest variance, the most probable subpopulation number was K = 4, indicating that the samples are grouped into four main clusters. Bar plots showed different extents of admixture among clusters (Fig. 2). Each cluster contained individuals from different regions. Some individuals showed a mixed portion of different clusters (inferred by colour), which indicates a weak attachment to their assigned cluster, and that they are assigned to such a cluster only when the information about the sampling locations was included *a priori* in the analysis. Samples that are genetically related are occasionally from different geographic locations, while samples from the same location do not always group together genetically, as observed in the PCA (Fig. 3). Basically, Structure and PCA results were the same except for cluster A, which is split into two clusters (red and blue). The most scattered samples within each cluster in the PCA show a clear signal of mixed portions of different clusters in the Structure analysis.

Loci under selection

Detection of positive selection signatures

The AFLP data of *F. hygrometrica* were screened for outlier loci detection using the Mcheza software on the four altitudinal groups. Across the 16 pair-wise analyses between the four groups, 13 out of 1584 loci (1.8%) were identified as outlier loci under directional selection at the 99.5% confidence level (Fig. 4). The 13 loci appeared constantly above the 99.5% line as outlier loci among the four altitudinal groups in each run.

Genetic differentiation and gene flow

An AMOVA was used to measure changes in the pair-wise differentiation of the F_{ST} for the AFLP neutral loci (abbreviated as AFLP) dataset in comparison with the F_{ST} of AFLP 13 outlier loci (abbreviated as AFLP-13) dataset. F_{ST} changed from 0.23 ($P = 0.000$) in AFLP dataset to 0.65 ($P = 0.000$) in AFLP-13 dataset. In the AFLP dataset, F_{ST} partitions were 6.56% between groups, 16.38% among locations between groups and 77.05% within locations. In the AFLP-13 dataset, F_{ST} partitions were 36.77% between groups, 28.99% among locations between groups and 34.25% within locations (Table 3). The mean gene flow (Nm) estimated from F_{ST} for AFLP and AFLP-13 datasets was 1.88 and 0.30, respectively.

Correlation tests

Estimation of the correlation coefficient (r) through application of a partial Mantel test differed, depending on the dataset used. The correlation coefficient for each dataset was determined in comparison with the genetic distance. In addition, environmental variation was spatially structured using a Mantel test, in which only the minimum temperature and precipitation values showed a significant correlation to their geographic location (data not shown). The AFLP neutral loci dataset was tested separately from the AFLP 13 outlier loci dataset (abbre-

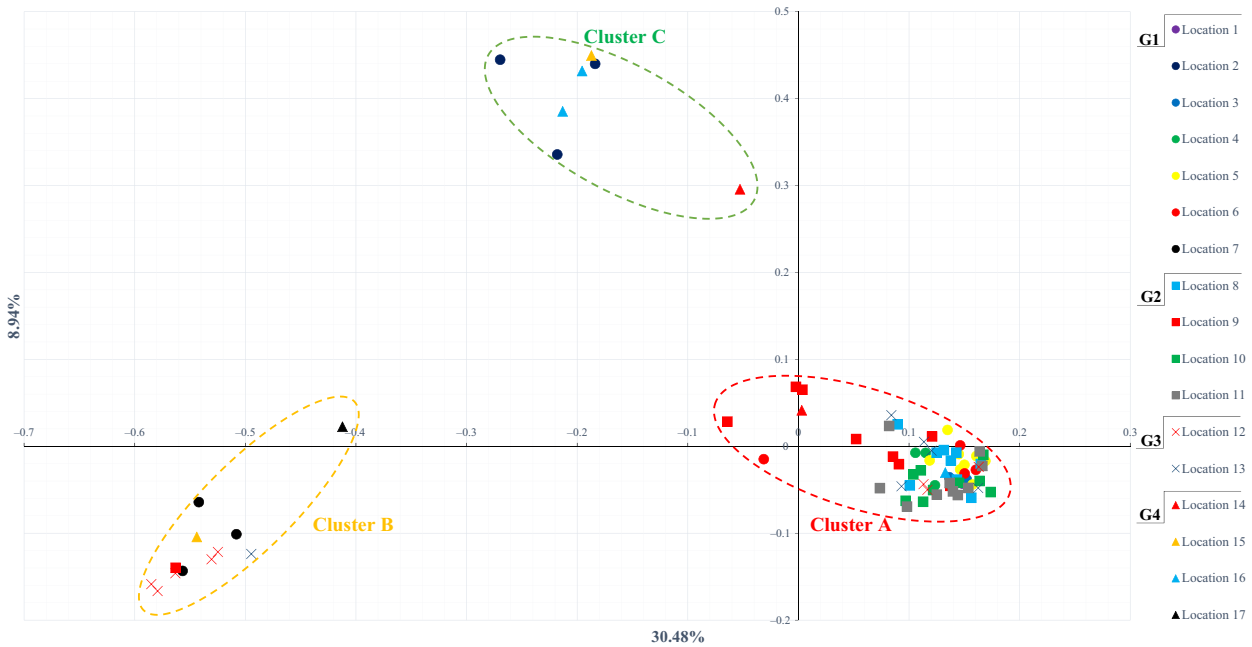

Fig. 1. PCA of AFLP-generated bands for 84 samples of *F. hygrometrica* from 17 locations. The variation revealed by two axes, x-axis of 30.48% and y-axis of 8.94%. Cluster A is defined by a red dotted circle in quadrat I, cluster B by an orange dotted circle in quadrat II and cluster C by a green dotted circle in quadrat II. All samples are coloured according to their altitudinal group (1, 2, 3 and 4).

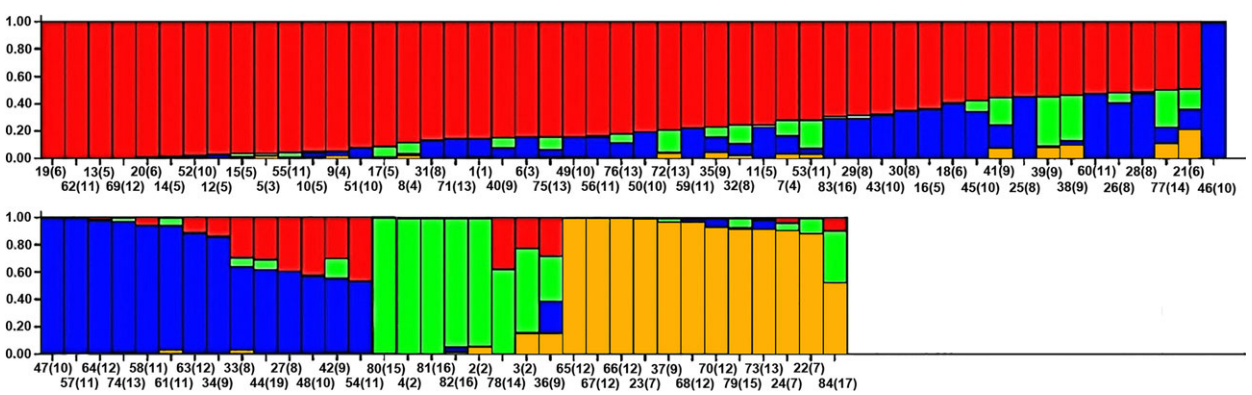

Fig. 2. AFLP marker-based Structure bar plot graph of K = 4, for 84 samples of *F. hygrometrica* from 17 locations. Samples are ordered by group assignment and locations are indicated in parentheses.

viated as AFLP-13). The AFLP-13 dataset had a positive significant correlation to the gradient of minimum temperature ($r = 0.41$, $P < 0.0001$), to the gradient of maximum temperature ($r = 0.37$, $P < 0.0001$) and to the gradient of precipitation values ($r = 0.42$, $P < 0.0001$). AFLP banding pattern did not show significant correlation to the tested climate variables (Table 4).

DISCUSSION

The AFLP technique generally provides a characterisation of genetic diversity based on a large number of markers scattered across the genome, enabling the detection of markers linked to a gene under selection (or potentially loci directly under selection). Here we characterise the genetic structure of populations of *F. hygrometrica* in the Sierra Nevada using ALFP and identify loci that show a signature for local adaptation.

Although it is possible to generate a large number of informative and reproducible markers using the AFLP technique, in comparison with other fingerprinting techniques (Jones *et al.* 1997), the success of this method may be compromised by several factors (Pompanon *et al.* 2005), such as quality control of the AFLP procedures, *e.g.* contamination (ensure the use of sterile material or *in vitro* axenic cultures; McDaniel *et al.* 2007), peak homoplasy, reliability of the method or genotyping errors (Bonin *et al.* 2004; Pompanon *et al.* 2005; Zartman *et al.* 2006; Paris *et al.* 2010). Such factors can be minimised using marker selection algorithms developed to optimise the challenging step of AFLP marker scoring by discarding biases due to subjective and unreliable personal procedures (Arrigo *et al.*

2009, 2012; Herrmann *et al.* 2010). Rawgeno version 2 (Arrigo *et al.* 2009) was built around an optimised and less time-consuming algorithm (Arrigo *et al.* 2012). Our procedure has taken into account these problems by using axenic cultures and an adequate protocol for AFLP quality control.

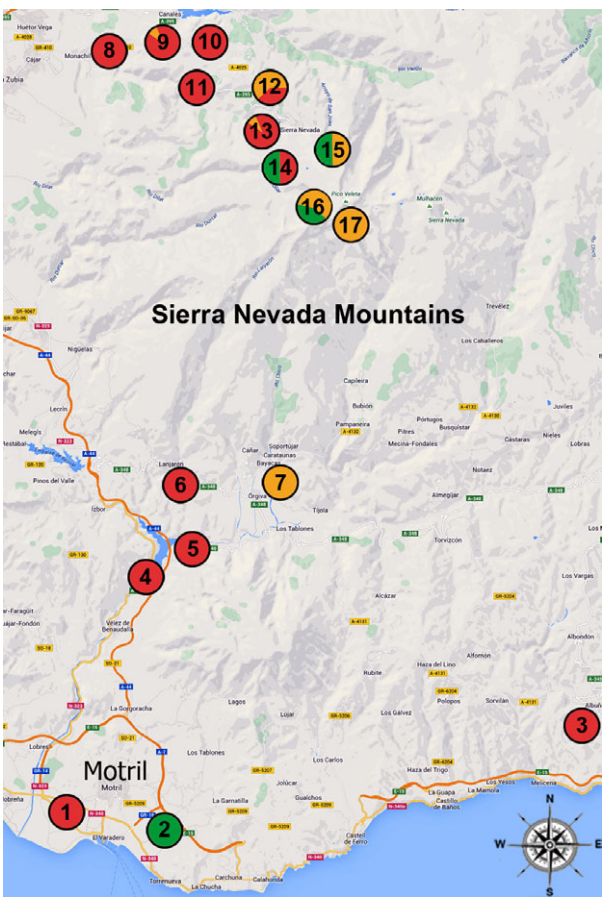

Fig. 3. Map of Sierra Nevada showing the 17 sampled locations of *F. hygrometrica* coloured by their assignment to one of the PCA clusters (red: cluster A, orange: cluster B, green: cluster C).

Table 3. Genetic differentiation inferred from AMOVA of F. hygrometrica based on the AFLP neutral loci dataset (a) and AFLP outlier loci dataset (b).

Source of variance	df	SS	Variance components	Percentage of variation (%)
(a)				
Between groups	3	2022.36	15.16	6.56%
Among locations within groups	13	4601.32	37.85	16.38%
Within locations	67	11927.78	178.02	77.05%
Total	83	18551.46	231.04	
(b)				
Between groups	3	42.47	0.58	36.77%
Among locations within groups	13	34.90	0.46	28.99%
Within locations	67	36.46	0.54	34.25%
Total	83	113.84	1.58	

The source of variance (between groups and, among and within locations), the degrees of freedom (df), sum of squares (SS), variance components and percentage of variation are shown.
F_{ST} = 0.23 (*P*-value < 0.000)
F_{ST} = 0.65 (*P*-value < 0.000)

In bryophytes, some reported AFLP marker data show low to moderate variable numbers of amplified bands using modifications to the original protocol designed for organisms with small genomes (*e.g.* mosses; Fernandez *et al.* 2006; Mikuláškova *et al.* 2012). In contrast, data obtained by Zartman *et al.* (2006) and McDaniel *et al.* (2007) in the bryophyte genera *Radula* and *Ceratodon* suggest that a high amount of AFLP bands can be obtained with three selective bases. Similar to the latter studies, in *F. hygrometrica* a large number of bands were amplified with three selective bases at the EcoRI and MseI primers, even though the genome of *F. hygrometrica* is small (0.4 pg; 1C; Voglmayr 2000; Beike *et al.* 2014), only twice the size of that of *Arabidopsis thaliana* (L.) Heynh. (Schmuths *et al.* 2004).

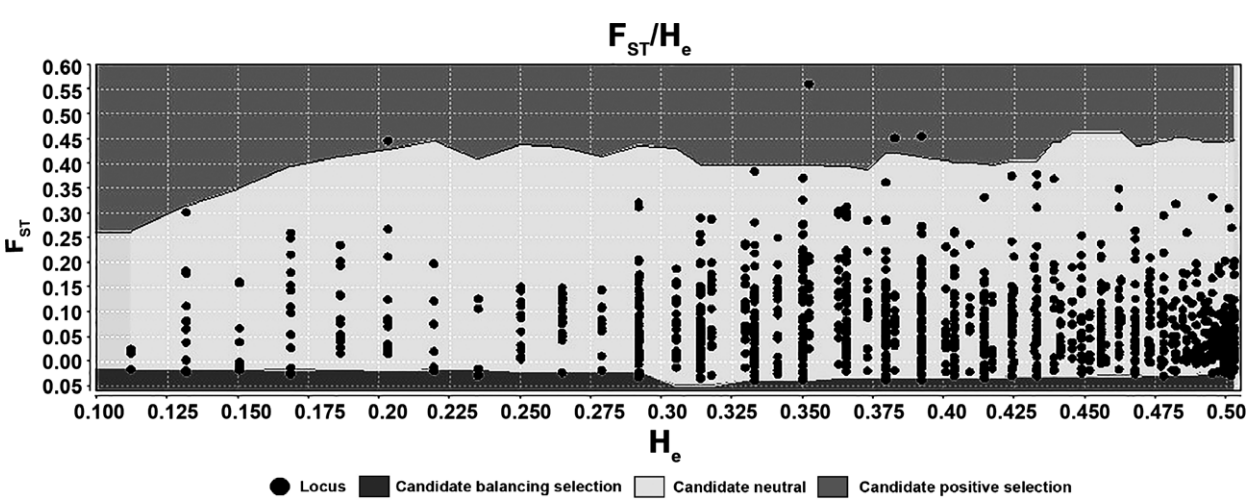

Fig. 4. Graphical plot produced by Mcheza of F_{ST} values against heterozygosity (H_e) for each of the 1584 AFLP loci scored for 84 samples of *F. hygrometrica*. Each dot indicates an AFLP locus; loci with the same value appear as one dot. The lower and higher zones represent the 0.5% and 99.5% confidence intervals, respectively. Loci in the dark grey zone above the 99.5% are regarded as outlier loci. Because some of the outlier loci overlap, fewer than 13 can be seen.

Table 4. Correlation coefficient (r) values and *P*-values of the partial Mantel test between Jaccard's distance-based matrices of different datasets (AFLP all loci, AFLP 13 positive outliers, AFLP of only neutral loci) and Euclidean matrices of the gradient of maximum temperature, minimum temperature and precipitation between 17 sampled location of *F. hygrometrica* in the Sierra Nevada.

Climate variable	Mantel test	*AFLP-all*	*AFLP-13*	*AFLP-neutral*
Maximum temperature	r	0.15	0.37	0.12
	P	0.07	<0.0001	0.14
Minimum temperature	r	−0.04	0.41	−0.04
	P	0.60	<0.0001	0.64
Precipitation	r	−0.06	0.42	−0.05
	P	0.48	<0.0001	0.52

We used a Bayesian-based method (Structure) and PCA to resolve patterns of genetic variation and assess genetic distances among groups of populations of *F. hygrometrica* in the Sierra Nevada. Mating between gametes produced from the same haploid individual results in complete homozygosity in a single generation (Hedrick 1987), resulting effectively in 'cloning' of the parent. Individual plants of *F. hygrometrica* are protandrous, they develop male and female sex organs in distinct clusters, with the former maturing first. Selfing reduces the genetic variation within populations through different mechanisms (Charlesworth 2003), yet inbreeding depression in sporophytes resulting from self-fertilisation has not been detected (Taylor *et al.* 2007). Although mechanisms to avoid selfing to some degree occur in bryophytes, selfing rates in hermaphroditic mosses are thought to be quite high (Eppley *et al.* 2007; McDaniel *et al.* 2010; Perroud *et al.* 2011). A model of colonisation where a location is reached by only one spore of *F. hygrometrica* and subsequently occupied only by descendants arising from selfing of this plant can clearly be excluded from our data, as most (or all) locations harbour plants that are genetically distinct, considering that within a range of 50–100 m the genetic diversity in *F. hygrometrica* is very high. Thus, multiple dispersal events and colonisation events account for the genetic diversity in locations in the Sierra Nevada Mountains. The data suggest that dispersal is high, at least at the scale investigated here, and hence potentially 'everything could be everywhere', if not subject to selection. In a system wherein dispersal is potentially high, the number of (dia)spores is very high and sexual reproduction is frequent (probably even outbreeding; see Perroud *et al.* 2011, on the Funariaceae *Physcomitrella patens*), the conditions are met for high genetic diversity to be subjected to selection driven by clearly contrasting environmental factors such as temperature and precipitation.

Local adaptation and directional selection should have locus-specific effects of reducing genetic diversity within populations and increasing differentiation between populations. Therefore, outlier loci characterised by unusually high levels of population differentiation are candidate loci for adaptation, and several studies have applied this approach (*e.g.* Bonin *et al.* 2006; Nosil *et al.* 2008; Manel *et al.* 2010). For example, a study of the genetic framework of adaptation to a gradient of altitude in the common frog (*Rana temporaria* L.) by Bonin *et al.* (2006) showed that approximately 2% of the AFLP loci exhibited altitudinal differentiation between highland, midland and lowland populations.

Whereas other studies detected evidence of selection using genetic data in mosses (*e.g.* Jules & Shaw 1994; McDaniel & Shaw 2005; McDaniel *et al.* 2008, 2013), the present study is the first to apply the F_{ST} outlier genome scan approach to identify loci under selection in this group of organisms. The 13 loci had higher genetic differentiation among locations than the putative neutral loci (28.99% for the outlier loci as compared to 16.38% for AFLP neutral loci) and decreased diversity within locations (from 77.05% for AFLP neutral loci to 34.25% AFLP 13 outlier loci).

The significant F_{ST} value of the neutral markers was 0.23, which matches the results of Shaw (1990) and Shaw & Bartow (1992). In contrast, Eppley *et al.* (2007) estimated a high F_{ST} value of 0.6 from allozyme data for *F. hygrometrica* samples from New Zealand, a result that may reflect the difference in the genetic marker used to estimate population differentiation, although in studies using allozyme and AFLP data simultaneously, neither of these methods results in systematically higher F_{ST} values than the other (Pérez-Collazos & Catalán 2006 *versus* Chung *et al.* 2004). The mean gene flow (Nm) estimated from F_{ST} for AFLP neutral loci and AFLP 13 outlier loci was 1.88 and 0.30, respectively, resulting in a homogenised pool of AFLP markers *versus* strong partitioning of alleles for the AFLP 13 outlier loci.

The Mantel test, which measures the association between two (simple) or three (partial) matrices, is known to overcome some of the problems inherent in explaining species–environment relationships (Mantel 1967; Legendre & Fortin 1989). The AFLP loci were significantly correlated to the gradient of minimum and maximum temperature in addition to precipitation ($P < 0.0001$). The correlation due to geographic distance was eliminated by the partial Mantel test (comparing the genetic distance against climatic variation while controlling the natural correlation between climatic variables and geographic distance), even if the gradient in maximum temperature showed no significant correlation to the geographic distance (data not shown).

In recent studies, apparently neutral DNA sequence markers (nrITS) showed differentiation along altitudinal gradients in the Spanish Sierra Nevada among populations of the moss *Bryum argenteum* Hedw. (Pisa *et al.* 2013). Similarly, microsatellite markers varied along altitudinal gradients in Hokkaido, Japan, in the mosses *Pleurozium schreberi* (Willd. ex Brid.) Mitt. and *Racomitrium lanuginosum* (Hedw.) Brid. (Korpelainen *et al.* 2012). These results might indicate that in moss species with potentially high dispersal ability, selection may favour different genotypes in adjacent locations, or frequency dependent selection may favour rare genotypes. The genetic markers used in the studies of *B. argenteum* (nrITS), *P. schreberi* and *R. lanuginosum* (microsatellites) are considered neutral and not affected by selection related to altitudinal gradients. However, in species that only rarely form sporophytes or that are monoecious and thus typically self-fertilising, the adaptive and non-adaptive genome regions are mostly linked, because genetic recombination is absent or less frequent than in species where sexual reproduction through outcrossing is typical. *F. hygrometrica* is monoecious and estimated to frequently undergo selfing (Eppley *et al.* 2007), so that although it forms sporophytes with a very high frequency, these may be mostly strictly homozygous, hence resulting in a spore mass characterised by a single haplotype. Our data suggest that in

F. hygrometrica sufficient outcrossing occurs to avoid a strict linkage between neutral and adaptive genome regions.

By comparing the Mantel test results of the AFLP neutral dataset with the AFLP 13 outlier loci, the correlation strength improved positively. This suggests that the detected outlier loci of *F. hygrometrica* are genetic regions that could be under selection and hence be relevant to adaptation to temperature and precipitation, the two environmental variables correlated to variation in allelic frequencies. Whether these are the actual loci under selection or merely linked and adjacent to the locus under selection is uncertain (Schlötterer 2003; Tollenaere *et al.* 2011). Although it is difficult to know the location and function of the loci involved in the adaptation to climatic factors, a genome scan of *F. hygrometrica* offers an opportunity to unravel the genetic basis of moss adaptation without prior knowledge about the genome sequences. In particular, approaches like RAD-Seq (Hohenlohe *et al.* 2012) can be used to generate random fragments that, unlike the AFLP procedure, contain DNA sequence tags that can be mapped back to the *Physcomitrella patens* genome (Rensing *et al.* 2008), thereby allowing the identification of genomic regions that are associated with local adaptation.

ACKNOWLEDGEMENTS

The authors thank the management group of the Sierra Nevada Natural Area for allowing us to collect samples in the national and natural parks, Dr. B. Benito (Granada University, Spain) for facilitating the climate variables and Dr. Jesús Muñoz (Madrid Botanical Garden, Spain) for valuable comments during preparation of the manuscript. This study was supported financially by the Spanish Ministries of Science and Innovation (Projects CGL2008-00275/BOS, CGL2011-22936/BOS) to R. M. Ros, O. Werner and S. F. McDaniel, and Foreign Affairs and Cooperation (Ph.D. grant to M. Magdy) and FEDER Funds of the EU. B. Goffinet was supported by US-NSF grant DEB–1146295.

REFERENCES

Anderson M.J. (2003) *PCO: a FORTRAN computer program for principal coordinate analysis*. Department of Statistics, University of Auckland, Auckland, New Zealand. Available from http://www.stat.auckland.ac.nz/~mja/Programs.htm.

Antao T., Beaumont M.A. (2011) Mcheza: a workbench to detect selection using dominant markers. *Bioinformatics*, **27**, 1717–1718.

Arrigo N., Tuszynski J.W., Ehrich D., Gerdes T., Alvarez N. (2009) Evaluating the impact of scoring parameters on the structure of intra-specific genetic variation using RawGeno, an R package for automating AFLP scoring. *BMC Bioinformatics*, **10**, 33.

Arrigo N., Holderegger R., Alvarez N. (2012) Automated scoring of AFLPs using RawGeno v 2.0, a Free R CRAN library. In: Pompanon F., Bonin A. (Eds), *Data production and analysis in population genomics*. Humana Press, New York, NY, USA, pp 155–175.

Baas-Becking L.G.M. (1934) *Geobiologie of inleiding tot de milieukunde*. W.P. Van Stockum & Zoon, Den Haag, the Netherlands, pp 263.

Beike A.K., von Stackelberg M., Schallenberg-Rüdinger M., Hanke S.T., Follo M., Quandt D., McDaniel S.F., Reski R., Tan B.C., Rensing S.A. (2014) Molecular evidence for convergent evolution and allopolyploid speciation within the *Physcomitrium–Physcomitrella* species complex. *BMC Evolutionary Biology*, **14**, 158.

Benito B., Lorite J., Peñas J. (2011) Simulating potential effects of climatic warming on altitudinal patterns of key species in Mediterranean-alpine ecosystems. *Climatic Change*, **108**, 471–483.

Black W.C., Baer C.F., Antolin M.F., DuTeau N.M. (2001) Population genomics: genome-wide sampling of insect populations. *Annual Review of Entomology*, **46**, 441–469.

Blanca G., Cabezudo B., Cueto M., Fernández López C., Morales Torres C. (2009) *Flora Vascular de Andalucía Oriental. Volumen 1: Selaginellaceae–Ceratophyllaceae*. Consejería de Medio Ambiente, Junta de Andalucía, Sevilla, Spain, pp 427.

Bonin A., Bellemain E., Bronken Eidesen P., Pompanon F., Brochmann C., Taberlet P. (2004) How to track and assess genotyping errors in population genetics studies. *Molecular Ecology*, **13**, 3261–3273.

Bonin A., Taberlet P., Miaud C., Pompanon F. (2006) Explorative genome scan to detect candidate loci for adaptation along a gradient of altitude in the common frog (*Rana temporaria*). *Molecular Biology and Evolution*, **23**, 773–783.

Bonin A., Ehrich D., Manel S. (2007) Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology*, **16**, 3737–3758.

Campbell D., Bernatchez L. (2004) Generic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. *Molecular Biology and Evolution*, **21**, 945–956.

Cavers S., Degen B., Caron H., Lemes M.R., Margis R., Salgueiro F., Lowe A.J. (2005) Optimal sampling strategy for estimation of spatial genetic structure in tree populations. *Heredity*, **95**, 281–289.

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.

Chung J.D., Lin T.P., Tan Y.C., Lin M.Y., Hwang S.Y. (2004) Genetic diversity and biogeography of *Cunninghamia konishii* (Cupressaceae), an island species in Taiwan: a comparison with *Cunninghamia lanceolata*, a mainland species in China. *Molecular Phylogenetics and Evolution*, **33**, 791–801.

Conord C., Lempérière G., Taberlet P., Després L. (2006) Genetic structure of the forest pest *Hylobius abietis* on conifer plantations at different spatial scales in Europe. *Heredity*, **97**, 46–55.

Crum H.A. (1972) The geographic origins of the mosses of North America's eastern deciduous forest. *Journal of the Hattori Botanical Laboratory*, **25**, 269–298.

During H.J. (1979) Life strategies of bryophytes: a preliminary review. *Lindbergia*, **5**, 2–18.

Egan S.P., Nosil P., Funk D.J. (2008) Selection and genomic differentiation during ecological speciation: isolating the contributions of host association via a comparative genome scan of *Neochlamisus bebbianae* leaf beetles. *Evolution*, **62**, 1162–1181.

Eppley S.M., Taylor P.J., Jesson L.K. (2007) Self-fertilization in mosses: a comparison of heterozygote deficiency between species with combined versus separate sexes. *Heredity*, **98**, 38–44.

Excoffier L., Lischer H.E.L. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, **10**, 564–567.

Fernandez C.C., Shevock J.R., Glazer A.N., Thompson J.N. (2006) Cryptic species within the cosmopolitan desiccation-tolerant moss *Grimmia laevigata*. *Proceedings of the National Academy of Sciences USA*, **103**, 637–642.

Fontaneto D. (2011) *Biogeography of microscopic organisms: is everything small everywhere?*. Cambridge University Press, New York, NY, USA, pp 365.

Hallingbäck T., Hodgetts N. (2000) *Mosses, liverworts, and hornworts: status survey and conservation action plan for bryophytes*. IUCN/SSC Bryophyte Specialist Group. IUCN, Gland, Switzerland, pp 106.

Hedrick P.W. (1987) Genetic load and the mating system in homosporous ferns. *Evolution*, **41**, 1282–1289.

Herrera C.M., Bazaga P. (2008) Population-genomic approach reveals adaptive floral divergence in discrete populations of a hawk moth-pollinated violet. *Molecular Ecology*, **17**, 5378–5390.

Herrmann D., Poncet B.N., Manel S., Rioux D., Gielly L., Taberlet P., Gugerli F. (2010) Selection criteria for scoring amplified fragment length polymorphisms (AFLPs) positively affect the reliability of population genetic parameter estimates. *Genome*, **53**, 302–310.

Hijmans R., Guarino L., Cruz M., Rojas E. (2001) Computer tools for spatial analysis of plant genetic resources data: 1. DIVA-GIS. *Plant Genetic Resources Newsletter*, **127**, 15–19.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G., Jarvis A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hohenlohe P.A., Catchen J., Cresko W.A. (2012) Population genomic analysis of model and nonmodel organisms using sequenced RAD tags. In: Pompanon F., Bonin A. (Eds), *Data production and analysis in population genomics*. Humana Press, New York, NY, USA, pp 235–260.

Hubisz M.J., Falush D., Stephens M., Pritchard J.K. (2009) Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources*, **9**, 1322–1332.

Jones C.J., Edwards K.J., Castaglione S., Winfield M.O., Sala F., van de Wiel C., Bredemeijer G., Vosman B., Matthes M., Daly A., Brettschneider R., Bettini P., Buiatti M., Maestri E., Malcevschi A., Marmiroli N., Aert R., Volckaert G., Rueda J., Linacero R., Vazquez A., Karp A. (1997) Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. *Molecular Breeding*, **3**, 381–390.

Jules E.S., Shaw A.J. (1994) Adaptation to metal-contaminated soils in populations of the moss, *Ceratodon purpureus*: vegetative growth and reproductive expression. *American Journal of Botany*, **81**, 791–797.

Kamisugi Y., von Stackelberg M., Lang D., Care M., Reski R., Rensing S.A., Cuming A.C. (2008) A sequence-anchored genetic linkage map for the moss, *Physcomitrella patens*. *The Plant Journal*, **56**, 855–866.

Knop W. (1884) Bereitung einer concentrierten Nährstofflösung für Pflanzen. *Die Landwirtschaftlichen Versuchs-Stationen*, **30**, 292–294.

Korpelainen H., Jägerbrand A., von Cräutlein M. (2012) Genetic structure of mosses *Pleurozium schreberi* (Willd. ex Brid.) Mitt. and *Racomitrium lanuginosum* (Hedw.) Brid. along altitude gradients in Hokkaido, Japan. *Journal of Bryology*, **34**, 309–312.

Legendre P., Fortin M. (1989) Spatial pattern and ecological analysis. *Vegetatio*, **80**, 107–138.

Luikart G., England P.R., Tallmon D., Jordan S., Taberlet P. (2003) The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics*, **4**, 981–994.

Manel S., Joost S., Epperson B.K., Holderegger R., Storfer A., Rosenberg M.S., Scribner K.T., Bonin A., Fortin M.-J. (2010) Perspectives on the use of landscape genetics to detect genetic adaptive variation in the field. *Molecular Ecology*, **19**, 3760–3772.

Mantel N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research*, **27**, 209–220.

McDaniel S.F., Shaw A.J. (2005) Selective sweeps and intercontinental migration in the cosmopolitan moss *Ceratodon purpureus* (Hedw.) Brid. *Molecular Ecology*, **14**, 1121–1132.

McDaniel S.F., Willis J.H., Shaw A.J. (2007) A linkage map reveals a complex basis for segregation distortion in an interpopulation cross in the moss *Ceratodon purpureus*. *Genetics*, **176**, 2489–2500.

McDaniel S.F., Willis J.H., Shaw A.J. (2008) The genetic basis of developmental abnormalities in interpopulation hybrids of the moss *Ceratodon purpureus*. *Genetics*, **179**, 1425–1435.

McDaniel S.F., von Stackelberg M., Richardt S., Quatrano R.S., Reski R., Rensing S.A. (2010) The speciation history of the *Physcomitrium–Physcomitrella* species complex. *Evolution*, **64**, 217–231.

McDaniel S.F., Neubig K.M., Payton A.C., Quatrano R.S., Cove D.J. (2013) Recent gene-capture on the UV sex chromosomes of the moss *Ceratodon purpureus*. *Evolution*, **67**, 2811–2822.

Meyer C.-L., Vitalis R., Saumitou-Laprade P., Castric V. (2009) Genomic pattern of adaptive divergence in *Arabidopsis halleri*, a model species for tolerance to heavy metal. *Molecular Ecology*, **18**, 2050–2062.

Mikuláśková E., Fér T., Kućabová V. (2012) The effect of different DNA isolation protocols and AFLP fingerprinting optimizations on error rate estimates in the bryophyte *Campylopus introflexus*. *Lindbergia*, **35**, 7–17.

Murashige T., Skoog F. (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, **15**, 473–497.

Nosil P., Egan S.P., Funk D.J. (2008) Heterogeneous genomic differentiation between walking-stick ecotypes: "isolation by adaptation" and multiple roles for divergent selection. *Evolution*, **62**, 316–336.

Nosil P., Funk D.J., Ortiz-Barrientos D. (2009) Divergent selection and heterogeneous genomic divergence. *Molecular Ecology*, **18**, 375–402.

Parihar N.S. (1961) *An introduction to embryophyta. Volume 1 bryophyta*. Central Book Depot, Allahabad, India, pp 331.

Paris M., Boyer S., Bonin A., Collado A., David J.-P., Despres L. (2010) Genome scan in the mosquito *Aedes rusticus*: population structure and detection of positive selection after insecticide treatment. *Molecular Ecology*, **19**, 325–337.

Peakall R.O.D., Smouse P.E. (2006) genalex 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Pérez-Collazos E., Catalán P. (2006) Palaeopolyploidy, spatial structure and conservation genetics of the narrow steppe plant *Vella pseudocytisus* subsp. *paui* (Vellinae, Cruciferae). *Annals of Botany*, **97**, 635–647.

Perroud P.-F., Cove D.J., Quatrano R.S., McDaniel S.F. (2011) An experimental method to facilitate the identification of hybrid sporophytes in the moss *Physcomitrella patens* using fluorescent tagged lines. *New Phytologist*, **191**, 301–306.

Pisa S., Werner O., Vanderpoorten A., Magdy M., Ros R.M. (2013) Elevational patterns of genetic variation in the cosmopolitan moss *Bryum argenteum* (Bryaceae). *American Journal of Botany*, **100**, 2000–2008.

Pompanon F., Bonin A., Bellemain E., Taberlet P. (2005) Genotyping errors: causes, consequences and solutions. *Nature Reviews Genetics*, **6**, 847–859.

Poncet B.N., Herrmann D., Gugerli F., Taberlet P., Holderegger R., Gielly L., Rioux D., Thuiller W., Aubert S., Manel S. (2010) Tracking genes of ecological relevance using a genome scan in two independent regional population samples of *Arabis alpina*. *Molecular Ecology*, **19**, 2896–2907.

Pritchard J.K., Stephens M., Rosenberg N.A., Donnelly P. (2000) Association mapping in structured populations. *American Journal of Human Genetics*, **67**, 170–181.

Rams S., Werner O., Ros R.M. (2014) Updated checklist of the bryophytes from the Sierra Nevada Mountains (S of Spain). *Cryptogamie, Bryologie*, **35**, 261–311.

Rensing S.A., Lang D., Zimmer A.D., Terry A., Salamov A., Shapiro H., Nishiyama T., Perroud P.-F., Lindquist E.A., Kamisugi Y., Tanahashi T., Sakakibara K., Fujita T., Oishi K., Shin-I T., Kuroki Y., Toyoda A., Suzuki Y., Hashimoto S.-I., Yamaguchi K., Sugano S., Kohara Y., Fujiyama A., Anterola A., Aoki S., Ashton N., Barbazuk W.B., Barker E., Bennetzen J.L., Blankenship R., Cho S.H., Dutcher S.K., Estelle M., Fawcett J.A., Gundlach H., Hanada K., Heyl A., Hicks K.A., Hughes J., Lohr M., Mayer K., Melkozernov A., Murata T., Nelson D.R., Pils B., Prigge M., Reiss B., Renner T., Rombauts S., Rushton P.J., Sanderfoot A., Schween G., Shiu S.-H., Stueber K., Theodoulou F.L., Tu H., Van de Peer Y., Verrier P.J., Waters E., Wood A., Yang L., Cove D., Cuming A.C., Hasebe M., Lucas S., Mishler B.D., Reski R., Grigoriev I.V., Quatrano R.S., Boore J.L. (2008) The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. *Science*, **319**, 64–69.

Rossi M., Bitocchi E., Bellucci E., Nanni L., Rau D., Attene G., Papa R. (2009) Linkage disequilibrium and population structure in wild and domesticated populations of *Phaseolus vulgaris* L. *Evolutionary Applications*, **2**, 504–522.

Sabovljević M., Bijelović A., Dragičević I. (2003) In vitro culture of mosses: *Aloina aloides* (K. F. Schultz) Kindb., *Brachythecium velutinum* (Hedw.) B. S. & G., *Ceratodon purpureus* (Hedw.) Brid., *Eurhynchium praelongum* (Hedw.) B. S. & G. and *Grimmia pulvinata* (Hedw.) Sm. *Turkish Journal of Botany*, **27**, 441–446.

Savolainen V., Anstett M.-C., Lexer C., Hutton I., Clarkson J.J., Norup M.V., Powell M.P., Springate D., Salamin N., Baker W.J. (2006) Sympatric speciation in palms on an oceanic island. *Nature*, **441**, 210–213.

Schlötterer C. (2003) Hitchhiking mapping – functional genomics from the population genetics perspective. *Trends in Genetics*, **19**, 32–38.

Schlüter P.M., Harris S.A. (2006) Analysis of multilocus fingerprinting data sets containing missing data. *Molecular Ecology Notes*, **6**, 569–572.

Schmuths H., Meister A., Horres R., Bachmann K. (2004) Genome size variation among accessions of *Arabidopsis thaliana*. *Annals of Botany*, **93**, 317–321.

Shaw A.J. (1990) Metal tolerances and co-tolerances in the moss *Funaria hygrometrica*. *Canadian Journal of Botany*, **68**, 2275–2282.

Shaw A.J. (1991) The genetic structure of sporophytic and gametophytic populations of the moss, *Funaria hygrometrica* Hedw. *Evolution*, **45**, 1260–1274.

Shaw A.J., Bartow S.M. (1992) Genetic structure and phenotypic plasticity in proximate populations of the moss, *Funaria hygrometrica*. *Systematic Botany*, **17**, 257–271.

Storz J.F. (2005) Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology*, **14**, 671–688.

Taylor P.J., Eppley S.M., Jesson L.K. (2007) Sporophytic inbreeding depression in mosses occurs in a species with separate sexes but not in a species with combined sexes. *American Journal of Botany*, **94**, 1853–1859.

Tollenaere C., Duplantier J.M., Rahalison L., Ranjalahy M., Brouat C. (2011) AFLP genome scan in the black rat (*Rattus rattus*) from Madagascar: detecting genetic markers undergoing plague-mediated selection. *Molecular Ecology*, **20**, 1026–1038.

Vekemans X., Beauwens T., Lemaire M., Roldán-Ruiz I. (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Molecular Ecology*, **11**, 139–151.

Voglmayr H. (2000) Nuclear DNA amounts in mosses (Musci). *Annals of Botany*, **85**, 531–546.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Friters A., Pot J., Paleman J., Kuiper M., Zabeau M. (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, **23**, 4407–4414.

Wilding C.S., Butlin R.K., Grahame J. (2001) Differential gene exchange between parapatric morphs of *Littorina saxatilis* detected using AFLP markers. *Journal of Evolutionary Biology*, **14**, 611–619.

Zartman C.E., McDaniel S.F., Shaw A.J. (2006) Experimental habitat fragmentation increases linkage disequilibrium but does not affect genetic diversity or population structure in the Amazonian liverwort *Radula flaccida*. *Molecular Ecology*, **15**, 2305–2315.