Journal of Biogeography (J. Biogeogr.) (2011) 38, 631–639

ORIGINAL ARTICLE

Macaronesia: a source of hidden genetic diversity for post-glacial recolonization of western Europe in the leafy liverwort *Radula lindenbergiana*

Benjamin Laenen[1]*, Aurélie Désamoré[1], Nicolas Devos[1], A. Jonathan Shaw[2], Juana Maria González-Mancebo[3], Mark A. Carine[4] and Alain Vanderpoorten[1]

[1]*University of Liège, Institute of Botany, Liège, Belgium,* [2]*Department of Botany, Duke University, Durham, NC, USA,* [3]*Department of Botany, University of La Laguna, Tenerife, Canary Islands, Spain,* [4]*Department of Botany, The Natural History Museum, London, UK*

ABSTRACT

Aim Bryophytes exhibit apparently low rates of endemism in Macaronesia and differ from angiosperms in their diversity patterns by the widespread occurrence of endemics within and among archipelagos. This paper investigates the phylogeography of the leafy liverwort *Radula lindenbergiana* to determine: (1) whether or not morphologically cryptic diversification has occurred in Macaronesia, and (2) the relationships between Macaronesian and continental populations.

Location Macaronesia, Europe, Africa.

Methods Eighty-four samples were collected across the species' distribution range and sequenced at four chloroplast DNA (cpDNA) loci (*atp*B–*rbc*L, *trn*G, *trn*L and *rps*4). Phylogenetic reconstructions and Bayesian ancestral area reconstructions were used in combination with population genetics statistics (H, N_{ST}, F_{ST}) to describe the pattern of present genetic diversity in *R. lindenbergiana* and infer its biogeographic history.

Results Patterns of genetic diversity in *R. lindenbergiana* exhibit a striking westwards gradient, wherein haplotype (0.90) and nucleotide (0.0038 ± 0.0019) diversity peak in Macaronesia, with a substantial endemic component. We found 20.9% of the genetic variance between biogeographic regions, and most pairwise F_{ST} comparisons between regions are significantly different from zero. The global N_{ST} (0.78) is significantly higher than the global F_{ST} (0.20), providing evidence for the presence of phylogeographic signal in the data. Ancestral area reconstructions suggest that the haplotypes currently found in western Europe share a Macaronesian common ancestor.

Main conclusions The haplotype diversification exhibited by *R. lindenbergiana* in Macaronesia is comparable to that reported for many angiosperm groups at the species level. The apparent lack of radiation among Macaronesian bryophytes may thus reflect the reduced morphology of bryophytes in comparison with angiosperms. The high diversity found among Macaronesian haplotypes, especially in Madeira and the Canary Islands, and the significant N_{ST}/F_{ST} ratio between Macaronesia and all the other biogeographic regions (an indication that mutation rate exceeds dispersal rates) suggest that Macaronesian archipelagos could have served as a refugium during the Quaternary glaciations. Many haplotypes currently found in Europe share a Macaronesian common ancestor, and this further suggests that Macaronesia might have played a key role in the back-colonization of the continent.

Keywords

Back-colonization, bryophyte, island biogeography, liverwort, Macaronesia, Mediterranean, phylogeography, refugium.

*Correspondence: Benjamin Laenen, Institut de Botanique, B22, Sart Tilman, Université de Liège, B4000, Liège1, Belgium.
E-mail: benjamin.laenen@doct.ulg.ac.be

Journal of Biogeography

INTRODUCTION

The Macaronesian region comprises the mid-Atlantic volcanic, oceanic archipelagos of the Azores, Madeira, Selvagem Islands, Canaries and Cape Verde. The endemic angiosperm flora of Macaronesia has been intensively studied, not least because of its high rates of endemism (ranging between 31% and 50% in Madeira and the Canaries, respectively; Vanderpoorten *et al.*, in press), spectacular evolutionary radiations and the distinctive spectrum of growth forms in the endemic flora (e.g. Shmida & Werger, 1992).

Spore-dispersing plants have been much less intensively studied than angiosperms, but their regional diversity patterns and relationships between island and continental floras appear to be markedly different from those observed in angiosperms. Thus, the Macaronesian bryophyte floras exhibit very low levels of endemism. The Madeiran liverwort flora exhibits 6% endemism, the highest level of endemism among bryophyte groups (mosses, liverworts, hornworts) throughout the region. Patterns of endemism in the bryophyte flora differ from those exhibited in angiosperms in that endemic species tend to be widespread, both within and among archipelagos. Indeed, whilst single-island endemics comprise the bulk of the endemic element within angiosperms (e.g. 70% in the Canary Islands), they represent only 37.5% of the endemic liverworts in Macaronesia (Vanderpoorten *et al.*, in press).

Based on an analysis of floristic relationships of the moss floras of the Macaronesian archipelagos, Vanderpoorten *et al.* (2007) interpreted extant Macaronesian distributions in terms of dynamic interchange of taxa (i.e. dispersal) between the archipelagos and the near continents rather than relictualism. They further suggested that dispersal between islands, between archipelagos, and between the archipelagos and the Atlantic and Mediterranean seaboards of Europe and North Africa probably inhibits genetic isolation and speciation, resulting in the low levels of endemism observed and the widespread distribution of bryophyte endemics. In such a scenario, and in contrast to angiosperms, endemics would most probably evolve following rare and stochastic long-distance dispersal events (Vanderpoorten & Long, 2006). Cryptic speciation has, however, been increasingly documented in bryophytes (see Shaw, 2001; and Heinrichs *et al.*, 2009a,b, for review) and may offer an alternative explanation for the low rates of endemism observed in the Macaronesian bryophyte flora.

Molecular analyses of non-endemic elements of the Macaronesian cryptogamic floras provide strong evidence for the lack of geographic isolation and strong connectivity both among archipelagos and between archipelagos and continents. For instance, phylogeographic structure in the moss *Grimmia montana* Bruch & Schimp. (Vanderpoorten *et al.*, 2008) suggests that the Macaronesian archipelagos were colonized several times independently from different continents, and substantial gene flow was documented between the Canary Islands and south-western Europe in the liverwort *Porella canariensis* (F.Weber) Underw. (Freitas & Brehm, 2001).

The significance of dispersal in shaping extant bryophyte distributions in Macaronesia has further implications for our understanding of their biogeographic relationships with nearby continents. Bellemain & Ricklefs (2008); see also Heaney, 2007, and Caujape-Castells, in press) recently argued that islands are not necessarily 'the end of the colonization road' and that retro-colonization of continental areas from islands can and does occur. For Macaronesian angiosperm genera, molecular phylogenetic data suggest that the interchange of biota between continent and islands has been limited and that, for the most part, the interchange has been unidirectional, from continent to islands. Retro-colonization of continental areas from Macaronesia is consistent with the patterns observed in only 4% of groups studied to date (Carine *et al.*, 2010). Given the potentially greater dispersal ability of bryophytes (see Vanderpoorten *et al.*, 2010, for review) and the lower levels of competition within bryophyte communities (Rydin, 2009), the bidirectional interchange of propagules between islands and continents may be more likely in bryophytes than in angiosperms. Indeed, given that the climate of the Macaronesian islands would have been buffered by their oceanic location, it is possible that taxa may have persisted in the islands during glacial periods and that the archipelagos may have served as a source for the post-glacial colonization of continental areas.

In the present paper we investigate the phylogeography of the leafy liverwort *Radula lindenbergiana* Gottsche ex Hartm., which is widely distributed across Macaronesia and also occurs in North America, Eurasia and South Africa, to address the following questions.

1. Is there evidence for morphologically cryptic diversification in *R. lindenbergiana* in Macaronesia?

2. What is the significance of the Macaronesian populations in the present patterns of genetic diversity and variation at the scale of its total distribution range? Has Macaronesia been the 'end of the colonization road' for *R. lindenbergiana* or have the islands served as a source of genetic diversity for the back-colonization of the continent?

MATERIALS AND METHODS

Sampling strategy and molecular protocols

Eighty-four specimens of *R. lindenbergiana* were sampled from herbaria and field collections. *Radula complanata* (L.) Dumort., which is sister to *R. lindenbergiana* (see Devos *et al.*, in press; Stech *et al.*, 2010), was used as outgroup. The sampling was organized in order to cover the entire range of the species, including Europe, North Africa, South Africa, Caucasus, Russia and Macaronesia (Fig. 1 and Appendix S1 in Supporting Information). The species was also reported with doubts from a single North American locality (Schuster, 1980) and from eastern Asia, where it has mostly been confused with *Radula constricta* Steph. Accessions from those areas were therefore excluded.

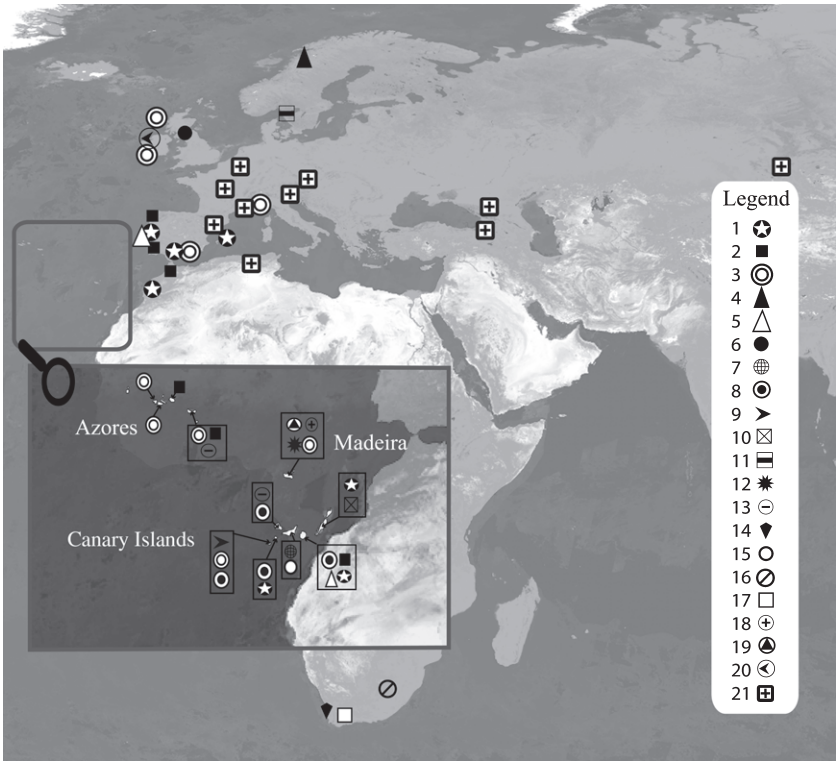

Figure 1 Sampling and haplotype distribution of 84 specimens of the liverwort *Radula lindenbergiana* across its distribution range sequenced at the chloroplast DNA (cpDNA) loci *trn*L, *trn*G, *rps*4 and the *atp*B–*rbc*L intergenic spacer. See Appendix S1 for voucher information and GenBank accession numbers.

Samples were frozen using liquid nitrogen and ground with a Genogrinder 2000 (BT&C/OPS Diagnostics, Lebanon, NJ, USA). DNA extraction was performed with the DNeasy Plant Minikit (Qiagen Benelux B.V., Venlo, The Netherlands) and eluted in 100 μL of water. Each specimen was genotyped at four chloroplast loci that show the appropriate levels of variation in *Radula* Dumort. (Devos *et al.*, in press): the *atp*B–*rbc*L intergenic spacer, the *trn*G region, the *trn*L region and the *rps*4 gene. As the chloroplast is maternally inherited in bryophytes (Natcheva & Cronberg, 2007), information retrieved from analyses of chloroplast DNA (cpDNA) sequences only retraces the history of the maternal lineages, but in the present case may provide a good proxy for the species' evolutionary history. Indeed, in bryophytes sperm dispersal ranges are extremely short owing to the need for a continuous film of water between antheridia and archegonia, making any sperm-mediated gene flow almost impossible over long distances.

The universal primers described by Shaw *et al.* (2003) were initially used but, owing to difficulties in amplifying *trn*L for a range of accessions, a specific primer pair (lind_trnL_F 5′-TCAGGGAAACCTAGGGTGAA-3′ and lind_trnL_R 5′-CC GGCAATTTTTGTTTCTGT-3′) was designed within conserved regions at the 5′ and 3′ ends of the locus from available sequences obtained using universal primers.

Amplification of the targeted loci was performed by polymerase chain reaction (PCR) using the following settings: 6.775 μL RNase-free H_2O, 1.5 μL buffer 10 × supplied with the Taq polymerase enzyme, 2.4 μL of a solution containing each nucleotide (1 mM each), 0.6 μL of 50 mM $MgCl_2$, 0.75 μL of each primer (10 μm), 1.125 μL of bovine serum albumin (BSA) and 0.3 μL of Taq polymerase. One microlitre of DNA was added for a total of 15 μL per sample. Higher concentrations of $MgCl_2$ were used for old herbarium specimens. The PCR included one cycle of denaturation at 95 °C for 2 min, 35 cycles of 30 s denaturation at 95 °C, 45 s of annealing at 50 °C, 2 min of extension at 72 °C followed by 7 min at 72 °C. Amplification fragments were purified with a solution of 0.2 μL exonuclease, 0.2 μL phosphatase and 2.4 μL H_2O for a total of 3 μL per sample prior to sequencing.

Sequences (see Appendix S1 for GenBank accession numbers) were aligned automatically using the contig option of Sequencher 3.1 (Schneider, 1998) and gaps were inserted where necessary to conserve homology among sequences. Individual electropherograms were rechecked at each variable site.

All individuals displaying identical sequences across the four loci were assigned to a single haplotype. The occurrence of each haplotype was scored for each of the following five regions, which corresponds to the classical biogeographic scheme of van der Wijk *et al.* (1969): Macaronesia (including the Azores, Canary Islands and Madeira), North Africa, Europe, South Africa and western Asia.

Data analysis

Haplotype diversity (*H*) (Nei, 1987) and nucleotide diversity (π) (Tajima, 1983) were computed for each of the five biogeographic regions defined above. Both statistics were computed with Arlequin 3.1. (Schneider *et al.*, 2000;

Excoffier *et al.*, 2005) Genetic variation was partitioned within and among biogeographic regions by an analysis of molecular variance (AMOVA) (Weir & Cockerham, 1984; Weir, 1996) as implemented by Arlequin 3.1. We calculated pairwise F_{ST} among regions and tested their significance by means of 999 random permutations of individuals among regions. The presence of phylogeographic signal in the data was explicitly tested by contrasting N_{ST} and F_{ST} values among geographic regions. N_{ST} is a measure of genetic differentiation among populations analogous to F_{ST} but taking into account the phylogenetic relationships between alleles (Pons & Petit, 1996). Here, N_{ST} values were computed from a Tamura and Nei distance matrix obtained from DnaSP 5.0 (Librado & Rozas, 2009). An interesting property is that $N_{ST} > F_{ST}$ when phylogeographic signal exists; that is, when distinct alleles sampled from within populations are phylogenetically closer, on average, than alleles sampled from different populations, indicating that the mutation rate exceeds the dispersal rate (Pons & Petit, 1996). The hypothesis that $N_{ST} > F_{ST}$ was tested by computing the distribution under the null hypothesis by conducting 1000 permutations of rows and columns of the distance matrix among haplotypes, as implemented by SPAGeDi 1.2 (Hardy & Vekemans, 2002).

Phylogeographic relationships among haplotypes were inferred using a model-based Bayesian inference. The GTR+I nucleotide substitution model was selected based upon the Akaike information criterion as implemented by Modeltest 3.7 (Posada & Buckley, 2004). This model was implemented by the Markov chain Monte Carlo (MCMC) of MrBayes 3.2 (Huelsenbeck & Ronquist, 2005). Trees and model parameters were sampled every 10,000 generations out of three independent chains of 10,000,000 iterations each. Convergence of the MCMCs was estimated in three ways. First, the standard deviation of split frequencies was <0.01 after 10,000,000 generations. Second, visual inspection of the plot of the log-likelihood score at each sampling point suggested that the four chains reached stationarity. Third, the posterior probability plots of all splits for paired MCMC runs showed high correlation, which indicates convergence among the four chains (Nylander *et al.*, 2008). The trees of the 'burn-in' for each run were excluded from the tree set, and the remaining trees from each run were combined to form the full sample of trees assumed to be representative of the posterior probability distribution. The monophyly of the Macaronesian haplotypes was tested by running new sets of analyses, wherein the MCMCs were forced to sample only trees that are compatible with a monophyletic Macaronesia. We used Bayes factors, which are estimated by twice the difference in the harmonic mean of the log-likelihood between the constrained and unconstrained analyses, to test whether the constraint induced a significant decrease in log-likelihood. Threshold values of the Bayes factors of 2, 5 and 10 are considered as evidence, strong evidence and very strong evidence, respectively, for one hypothesis over another (Pagel & Meade, 2004).

Finally, the consensus tree built in MrBayes was used to retrace ancestral distribution areas based upon a model-based Bayesian inference, as implemented by BayesTraits 1.0 (Pagel & Meade, 2006). This approach offers several advantages, including the incorporation of branch length information to determine the probabilities of change and the integration of both mapping and phylogenetic uncertainty in the estimates, over increasingly questioned parsimony-based approaches (Kodandaramaiah, 2009). As opposed to the latter, and like most recently developed programs for inferring historical distribution ranges (Ree & Smith, 2008; Sanmartín *et al.*, 2008), this approach is parametric and is most similar to Sanmartín *et al.*'s (2008) model in the absence of information on island carrying capacity and dispersal rates. As 'widespread taxa are uninformative by comparison to area relationships derived from endemic taxa' and 'introduce complications into biogeographic analysis because they obscure resolution and introduce redundancy by representing areas more than once on the terminals of any given cladogram' (Humphries & Parenti, 1999, p. 67), each haplotype was assigned to one of four categories depending on its endemic occurrence within Macaronesia (1), Europe (2), South Africa (3) (North Africa and western Asia lacking any endemic haplotypes), or in more than one region, which we refer to as combined distributions (4). A model implementing a forward and backward transition rate between each pair of regions was then employed to reconstruct ancestral range distributions onto the phylogeny. This model was implemented by a MCMC of 50,000,000 generations that was sampled every 10,000 generations. At each iteration, the chain proposes a new combination of rate parameters and randomly selects a new tree from the Bayesian sample. The likelihood of the new combination is calculated and this new state of the chain is accepted or rejected following evaluation based on the Metropolis–Hastings term. In the absence of information on rate parameters, the latter were sampled from flat, uniform distribution priors ranging between 0 and 100. The trees and rate parameters sampled from the posterior probability distribution were used to reconstruct, at each node of interest, the probability of occurrence within each of the four geographic areas. In order to circumvent the issues associated with the fact that not all of the trees necessarily contain the internal nodes of interest, reconstructions were performed using a 'most recent common ancestor' (MRCA) approach that identifies, for each tree, the MRCA of a group of species and reconstructs the state at the node, then combines this information across trees (Pagel & Meade, 2004).

RESULTS

The data matrix included 26 variable sites that included five singletons and five indels. This variation allowed the identification of 21 haplotypes. Four haplotypes exhibit a wide distribution range (Fig. 1). Haplotype 21 is present in France, Belgium and the Czech Republic and extends to western Asia. Haplotypes 1, 2 and 3 have a more south-westerly distribution. Haplotype 1 is distributed in Spain, Mallorca, Morocco and the Canaries. Haplotype 2 is present in western Spain, northern

Morocco and the Azores, whereas haplotype 3 is distributed in Spain, south-eastern France, the UK, the Azores, Madeira and the Canaries. Two endemic haplotypes (4 and 11) are found in Scandinavia, one in Scotland (6), one in Tenerife (7), two in Madeira (12 and 18), one in Ireland (20) and one in Gran Canaria (15). Haplotype 5 is present in Portugal and Gran Canaria, whereas haplotypes 8 and 10 are only present in the Canary Islands, where they occur on Gran Canaria, La Gomera, El Hierro (haplotype 8) and La Palma, Fuerteventura (haplotype 10), respectively. Finally, haplotype 13 is shared between Sao Miguel (Azores) and La Palma (Canaries).

Patterns of genetic diversity in *R. lindenbergiana* exhibit a striking westward gradient, wherein haplotype (0.90) and nucleotide (0.0038 ± 0.0019) diversity peak in Macaronesia (Table 1). The latter includes nine endemic haplotypes (7, 8, 9, 10, 12, 13, 15, 18 and 19), among which five are endemic to the Canaries (7, 8, 9, 10 and 15), three to Madeira (12, 18 and 19) and the last one (13) is shared between the Canaries and the Azores. The two regions with the lowest haplotype diversities are North Africa (0.50) and western Asia, with a haplotype and nucleotide diversity of zero because only one haplotype (21), which is shared with Europe, is found in that region.

AMOVA revealed that 20.9% of the genetic variance is found among biogeographic regions, and this global differentiation is significant at the 0.001 level. Most pairwise F_{ST} comparisons are significantly different from 0 (Table 2). The global N_{ST} is 0.78 ($P < 0.001$) and is significantly higher than the global F_{ST} (0.20; $P < 0.001$) at the 0.001 significance level, providing evidence for the presence of phylogeographic signal in the data. Pairwise comparisons of N_{ST} and F_{ST} values indicate that a significant phylogeographic signal is only present between Macaronesia and three other regions – Europe, western Asia and South Africa – and between South Africa and the two other regions – Europe and North Africa (Table 2).

Phylogeographic relationships among haplotypes are depicted in the 50% majority-rule consensus of the trees sampled from the posterior probability (PP) distribution (Fig. 2). The analysis resolved four major clades. Clade I, which has a PP of 1.00, is composed of the European and Asian haplotype 21 and the European endemic haplotype 4. Clade I is sister to a large polytomy with a PP of 0.84 including clades II, III and V. Clade II, whose comparatively long branch is supported by a PP of 1.00, is exclusively composed of South African haplotypes (14, 16 and 17). Clade V, which is supported with a PP of 0.98, almost exclusively includes Macaronesian endemic haplotypes except for haplotype 3, which is shared by Macaronesia and Europe. Clade III (PP = 0.89) includes a mix of endemic and more widespread haplotypes. There are four Macaronesian endemic haplotypes (7 from Gran Canaria, 15 from Tenerife, 18 and 19 from Madeira) within clade III, as well as the European endemic haplotypes 11 and 20. Within clade III, clade IV, supported with a PP of 0.74, is formed by haplotypes 2, 7, 11 and 20. The hypothesis that all Macaronesian haplotypes are monophyletic was rejected by the constrained analysis (Bayes factor = 4.86).

The reconstructions of ancestral distribution areas onto the trees sampled from the PP distribution (Fig. 2) identify Europe as the ancestral state at the root with a PP of 0.83. The MRCA

Table 1 Sample size, number of haplotypes per region, haplotype diversity (H) and nucleotide diversity (π) with their respective standard deviations (SD) per biogeographic region within a sample of 84 individuals of the liverwort *Radula lindenbergiana* collected throughout its distribution range and sequenced at *trn*L, *trn*G, *rps*4 and the *atp*B–*rbc*L intergenic spacer.

	Sample size	Number of haplotypes	Haplotype diversity (SD)	Nucleotide diversity (SD)
Macaronesia	39	13	0.90 (0.03)	0.0038 (0.0019)
South Africa	4	3	0.83 (0.22)	0.0013 (0.0010)
Europe	28	9	0.78 (0.06)	0.0025 (0.0014)
North Africa	4	2	0.50 (0.27)	0.0002 (0.0003)
Western Asia	9	1	0.00 (0.00)	0.0000 (0.0000)

Table 2 Pairwise F_{ST} (below) and N_{ST} (above) values in Macaronesia, North Africa, Europe, western Asia and South Africa in the liverwort *Radula lindenbergiana* based on the sequencing of 84 specimens at the chloroplast DNA (cpDNA) loci *trn*L, *trn*G, *rps*4 and the *atp*B–*rbc*L intergenic spacer. The *P*-values associated with the *F* statistics indicate the probability that $F_{ST} = 0$ after 999 permutations of specimens among biogeographic regions, whereas those associated with the *N* statistics indicate the probability that N_{ST} does not significantly differ from F_{ST} after 999 permutations of the matrix of genetic distances among haplotypes (see text for details).

	North Africa	Western Asia	South Africa	Europe	Macaronesia
North Africa	–	n.s.	0.90*	n.s.	n.s.
Western Asia	0.85***	–	n.s.	n.s.	0.84**
South Africa	N.S.	0.74**	–	0.77**	0.83***
Europe	0.21*	0.20**	0.20**	–	0.30**
Macaronesia	0.13*	0.41***	0.13*	0.10***	–

n.s., $P > 0.05$; *$P < 0.05$l; **$P < 0.01$; ***$P < 0.001$.

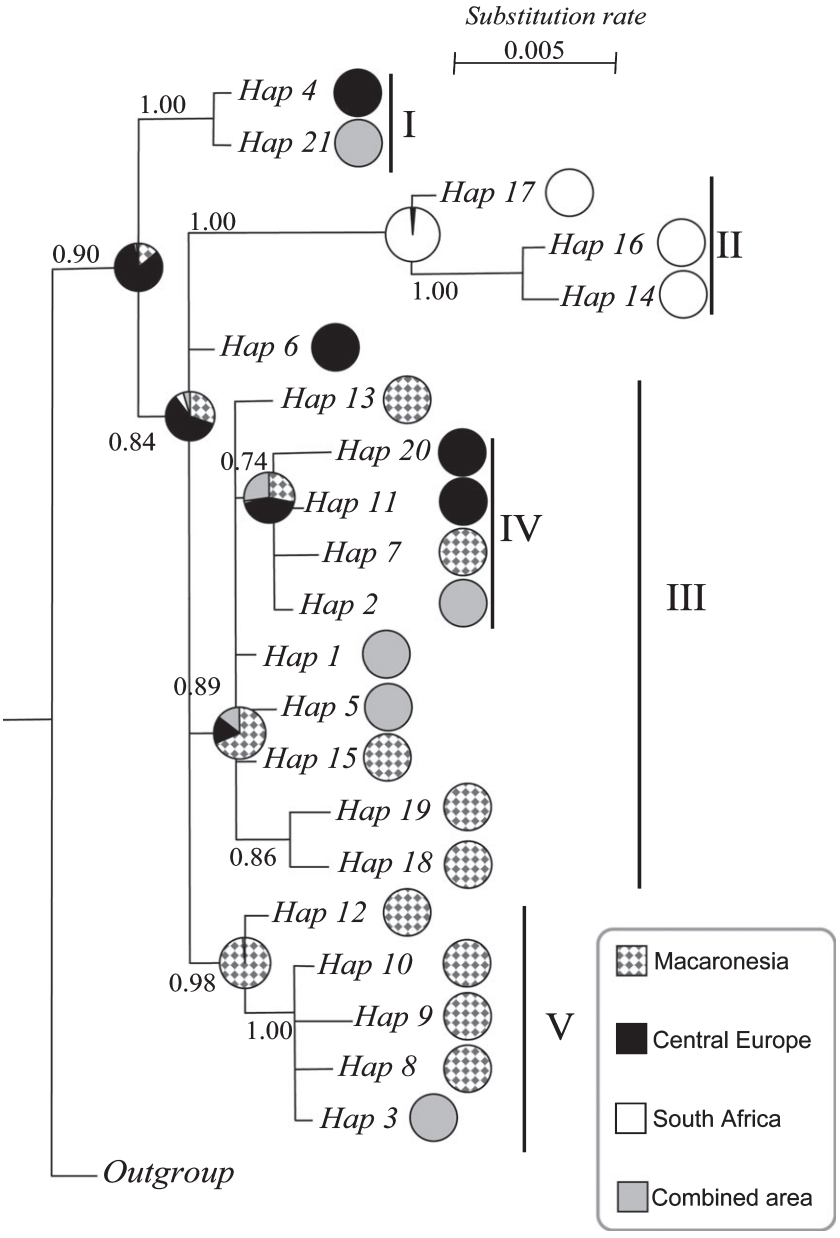

Figure 2 Fifty per cent majority-rule consensus with branch lengths averaged across the trees of the posterior probability distribution from a Bayesian analysis of 84 specimens of the liverwort *Radula lindenbergiana* sampled across its entire distribution range and sequenced at four chloroplast DNA (cpDNA) loci. Sequences of *Radula complanata* were used as outgroups. Labelled clades are described in the text. The number below the branches corresponds to their posterior probabilities. Pie diagrams at terminal nodes indicate actual haplotype distributions, whilst those at internal nodes are estimates of ancestral distribution areas within: (1) Macaronesia, (2) South Africa, (3) Eurasia, and (4) any combination of these three areas.

of clades II, III and V is also reconstructed as mainly European, but with much less support (PP = 0.59). The ancestral distribution areas of the MRCAs of clades II and V are unambiguously reconstructed as South African (PP = 0.97) and Macaronesian (PP = 0.98), respectively. Clade III is similarly reconstructed as Macaronesian in origin, but with a lower PP of 0.68. The reconstruction at clade IV is ambiguous and involves three areas with similar PPs.

DISCUSSION

Radula lindenbergiana displays a remarkable westward gradient of genetic diversity. In fact, only a single haplotype is dominant across eastern Eurasia, suggesting survival of a much reduced population within a refugium with subsequent fast recolonization. In this regard, the dual reproductive mode of *R. lindenbergiana*, which frequently produces sporophytes (despite its dioecious condition) and masses of asexual gemmae, might be significant in explaining its ability to disperse rapidly when oceanic barriers to long-distance dispersal are absent.

Westwards, the genetic diversity of *R. lindenbergiana* peaks in Macaronesia, wherein haplotype diversity reaches 0.90. The haplotype diversification exhibited by *R. lindenbergiana* in Macaronesia is comparable to that reported for many angiosperm groups at the species level (Carine *et al.*, 2010). The apparent lack of radiation among Macaronesian bryophytes (as evidenced by morphology) may thus reflect the reduced morphology of bryophytes in comparison with angiosperms. In bryophytes, the diversification of bryophyte lineages is in

fact not necessarily paralleled by morphological differentiation, and this phenomenon, known as cryptic speciation (Shaw, 2001), has increasingly been reported (Heinrichs *et al.*, 2009a,b).

In contrast to the situation in angiosperms, wherein the bulk of Canary Islands endemics are restricted to a single island (Carine & Schaefer, 2009), the Canarian endemic haplotypes of *R. lindenbergiana* occur across several islands. Dispersal limitations among islands thus do not seem to account for the observed patterns of island diversification in the Canaries. One interpretation of the origin of multiple Canarian endemic lineages within *R. lindenbergiana* is that the latter evolved through adaptive radiations favoured by the presence and dynamic nature of a high number of niches on those volcanic islands (Carine *et al.*, 2010), where volcanism and perturbations can create new opportunities for species to diversify (Roderick & Gillespie, 1998). The dynamic nature of oceanic island habitats might in fact be a crucial feature for the diversification of a pioneer species such as *R. lindenbergiana*. It is notable that this species is amongst the most common leafy liverwort in the Canaries, where it can be found across a very wide range of habitats, from dry, xeric exposed lowland rock outcrops within sub-desertic woody *Euphorbia* L. vegetation, epiphytic or even epiphyllous in the laurel forest, to higher vegetation belts such as pine forest. Such a wide ecological range might have promoted the evolution of several strains, as recently demonstrated in the aquatic moss *Platyhypnidium riparioides* (Hedw.) Dixon within a heterogeneous landscape (Hutsemékers *et al.*, 2010).

No endemic haplotypes of *R. lindenbergiana* were identified within the Azores, which is consistent with the observation that angiosperm radiations in that archipelago are limited (Carine & Schaefer, 2009). One interpretation for the lack of endemic radiation on the Azores is that colonization of the archipelago occurred more recently. In this regard, it is notable that we only observed *R. lindenbergiana* on secondary habitats in the Azores, e.g. on volcanic rock walls among pasture or even in botanical gardens, but never in laurel forests, where the species is amongst the most dominant leafy liverwort in the Canary Islands and Madeira. Furthermore, despite targeted fieldwork, we did not find *R. lindenbergiana* on the island of Flores, which is the westernmost island and arguably the one characterized by the lowest levels of human disturbance. These observations are consistent with the idea that *R. lindenbergiana* has colonized the Azores very recently, and has perhaps been accidentally introduced.

Pairwise N_{ST}/F_{ST} ratios were significant only between Macaronesia and all the other biogeographic regions apart from North Africa, indicating that mutations occur at a faster rate than migration events. Together with the high diversity found among Macaronesian haplotypes, especially in Madeira and the Canary Islands, this suggests that Macaronesian archipelagos are a reservoir of diversity for the species and could have served as a refugium during the Quaternary glaciations. Many haplotypes currently found in Europe share a Macaronesian common ancestor, and this further suggests that Macaronesia might have played a key role in the back-colonization of the continent. The results of the present analyses indeed suggest that Europe has been colonized from Macaronesia at least twice along different routes, once by a member of clade V and the other time by members of clade III, three of which (1, 2 and 5) are shared between Macaronesia, Europe and/or North Africa. This scenario is fully compatible with the existence of frequent depressions moving rapidly eastwards at relatively low altitude (3000 m) from the American coast and the existence of tropical cyclones of west Caribbean origin that can carry even large propagules (Schaefer, 2003). Altogether, these observations emphasize the major role of the Atlantic islands as sinks of biodiversity for the post-glacial recolonization of Europe.

Many bryophyte species exhibit a striking hyper-Atlantic distribution pattern (Hill & Preston, 1998; Rothero, 2005) that is often disjunct between Macaronesia and the westernmost fringe of Europe (e.g. the mosses *Dicranum scottianum* Turner ex Scott and *Myurium hochstetteri* (Schimp.) Kindb. and the liverworts *Saccogyna viticulosa* (L.) Dumort., *Leptoscyphus cuneifolius* (Hook.) Mitt., *Radula carringtonii* J.B. Jack and *Radula holtii* Spruce). Such distributions may reflect a similar history with Macaronesia serving as a refugium and a source area for the post-glacial recolonization of Europe. In *R. lindenbergiana*, and potentially other bryophyte species, Bellemain & Ricklefs (2008) are correct: the Macaronesian archipelagos are not the end of the colonization road.

ACKNOWLEDGEMENTS

B.L., A.D., N.D. and A.V. acknowledge financial support from the Belgian Funds for Scientific Research and the Fonds Léopold III. Many thanks are due to Jan Kucera, Beata Papp, Frank Müller and the curators of the National Botanic Garden of Belgium, the Royal Botanic Garden Edinburgh, Universidad Politécnica de Madrid and Universidad de Murcia for the loan of specimens. This manuscript was completed whilst A.V. was a Synthesys visitor at the Natural History Museum, London.

REFERENCES

Bellemain, E. & Ricklefs, R.E. (2008) Are islands the end of the colonization road? *Trends in Ecology and Evolution*, **23,** 536–537.

Carine, M.A. & Schaefer, H. (2009) The Azores diversity enigma: why are there so few Azorean endemic flowering plants and why are they so widespread? *Journal of Biogeography*, **37,** 77–89.

Carine, M.A., Santos Guerra, A., Guma, I.R. & Reyes-Betancort, J.A. (2010) Endemism and evolution of the Macaronesian flora. *Beyond cladistics: the branching of a paradigm* (ed. by D.M. Williams and S.K. Knapp), pp. 101–124. University of California Press, Berkeley, CA.

Caujape-Castells, J. (in press) Jesters, red queens, boomerangs and surfers: a molecular outlook on the diversity of the

Canarian endemic flora. *Plants and islands*, 2nd edn (ed. by D. Bramwell). Cambridge University Press, Cambridge.

Devos, N., Renner, M., Gradstein, S.R., Shaw, A.J., Laenen, B. & Vanderpoorten, A. (in press) Evolutionary significance and trade-offs in life-history traits associated to ecological specialization and gender evolution in the liverwort genus *Radula*. *Evolution*.

Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1,** 47–50.

Freitas, H. & Brehm, A. (2001) Genetic diversity of the Macaronesian leafy liverwort *Porella canariensis* inferred from RAPD markers. *Journal of Heredity*, **92,** 339–345.

Hardy, O.J. & 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.

Heaney, L.R. (2007) Is a new paradigm emerging for oceanic island biogeography? *Journal of Biogeography*, **34,** 753–757.

Heinrichs, J., Hentschel, J., Feldberg, K., Bombosch, A. & Schneider, H. (2009a) Phylogenetic biogeography and taxonomy of disjunctly distributed bryophytes. *Journal of Systematics and Evolution*, **47,** 497–508.

Heinrichs, J., Klugmann, F., Hentschel, J. & Schneider, H. (2009b) DNA taxonomy, cryptic speciation and diversification of the Neotropical-African liverwort, *Marchesinia brachiata* (Lejeuneaceae, Porellales). *Molecular Phylogenetics and Evolution*, **53,** 113–121.

Hill, M.O. & Preston, C.D. (1998) The geographical relationships of British and Irish bryophytes. *Journal of Bryology*, **20,** 127–226.

Huelsenbeck, J.P. & Ronquist, F. (2005) Bayesian analysis of molecular evolution using MrBayes. *Statistical methods in molecular evolution* (ed. by R. Nielsen), pp. 183–232. Springer, New York.

Humphries, C.J. & Parenti, L.R. (1999) *Cladistic biogeography*, 2nd edn. Oxford University Press, Oxford.

Hutsemékers, V., Hardy, O.J., Mardulyn, P., Shaw, A.J. & Vanderpoorten, A. (2010) Macroecological patterns of genetic structure and diversity in the aquatic moss *Platyhypnidium riparioides*. *New Phytologist*, **183,** 852–864.

Kodandaramaiah, U. (2009) Use of dispersal–vicariance analysis in biogeography – a critique. *Journal of Biogeography*, **37,** 3–11.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25,** 1451–1452.

Natcheva, R. & Cronberg, N. (2007) Maternal transmission of cytoplasmic DNA in interspecific hybrids of peat mosses, *Sphagnum* (Bryophyta). *Journal of Evolutionary Biology*, **20,** 1613–1616.

Nei, M. (1987) *Molecular evolutionary genetics*. Columbia University Press, New York.

Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics*, **24,** 581–583.

Pagel, M. & Meade, A. (2004) A phylogenetic mixture model for detecting pattern-heterogeneity in gene sequence or character-state data. *Systematic Biology*, **53,** 571–581.

Pagel, M. & Meade, A. (2006) Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *The American Naturalist*, **167,** 808–825.

Pons, O. & Petit, R.J. (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics*, **144,** 1237–1245.

Posada, D. & Buckley, T.R. (2004) Model selection and model averaging in phylogenetics: advantages of the AIC and Bayesian approaches over likelihood ratio tests. *Systematic Biology*, **53,** 793–808.

Ree, R.H. & Smith, S.A. (2008) Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, **57,** 4–14.

Roderick, G.K. & Gillespie, R.G. (1998) Speciation and phylogeography of Hawaiian terrestrial arthropods. *Molecular Ecology*, **7,** 519–531.

Rothero, G.P. (2005) Oceanic bryophytes in Atlantic woodlands. *Botanical Journal of Scotland*, **57,** 135–140.

Rydin, H. (2009) Population and community ecology of bryophytes. *Bryophyte biology*, 2nd edn (ed. by B. Goffinet and A.J. Shaw), pp. 393–445. Cambridge University Press, Cambridge.

Sanmartín, I., van der Mark, P. & Ronquist, F. (2008) Inferring dispersal: a Bayesian approach to phylogeny-based island biogeography, with special reference to the Canary Islands. *Journal of Biogeography*, **35,** 428–449.

Schaefer, H. (2003) The chorology and diversity of the Azorean flora. *Dissertationes Botanicae*, **374,** 1–130.

Schneider, I. (1998) Thermal cycler innovations: expanded applications and increased performance. *Genetic Engineering News*, **18,** 24.

Schneider, S., Roessli, D. & Excoffier, L. (2000) *Arlequin, version 2.0: a software for population genetic data analysis*. University of Geneva, Genetics and Biometry Laboratory, Geneva.

Schuster, R. (1980) *The Hepaticae and Anthocerotae of North America*, Vol. 4. Columbia University Press, New York.

Shaw, A.J. (2001) Biogeographic patterns and cryptic speciation in bryophytes. *Journal of Biogeography*, **28,** 253–261.

Shaw, A.J., Cox, C.J. & Boles, S.B. (2003) Polarity of peatmoss (*Sphagnum*) evolution: who says bryophytes have no roots? *American Journal of Botany*, **90,** 1777–1787.

Shmida, A. & Werger, M. (1992) Growth form diversity on the Canary Islands. *Vegetatio*, **102,** 183–199.

Stech, M., Sim-Sim, M., Esquível, M.G., Luís, L., Fontinha, S., Lobo, C., Garcia, C., Martins, S., Vieira, C., Barroso, J., Pedro, L.G. & Figueiredo, A.C.S. (2010) Molecular, phytochemical and morphological characterization of the liverwort genus *Radula* in Portugal (mainland, Madeira, Azores). *Systematics and Biodiversity*, **8,** 257–268.

Tajima, F. (1983) Evolutionary relationship of DNA sequences in finite populations. *Genetics*, **105,** 437–460.

Vanderpoorten, A. & Long, D.G. (2006) Budding speciation and Neotropical origin of the Azorean endemic liverwort, *Leptoscyphus azoricus*. *Molecular Phylogenetics and Evolution*, **40,** 73–83.

Vanderpoorten, A., Rumsey, F.J. & Carine, M.A. (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, O.J. & Shaw, A.J. (2008) The barriers to oceanic island radiation in bryophytes: insights from the phylogeography of the moss *Grimmia montana*. *Journal of Biogeography*, **35,** 654–663.

Vanderpoorten, A., Gradstein, S.R., Carine, M. & Devos, N. (2010) The ghosts of Gondwana and Laurasia in modern liverwort distributions. *Biological Reviews*, **85,** 471–487.

Vanderpoorten, A., Laenen, B., Rumsey, F., González-Mancebo, J.M., Gabriel, R. & Carine, M.A. (in press) Dispersal, diversity and evolution of the Macaronesian cryptogamic floras. *Plants and islands*, 2nd edn (ed. by D. Bramwell). Cambridge University Press, Cambridge.

Weir, B.S. (1996) *Genetic data analysis III: methods for discrete population genetic data*. Sinauer Associates, Sunderland, MA.

Weir, B.S. & Cockerham, C.C. (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38,** 1358–1370.

van der Wijk, R., Margadant, W.D. & Florschütz, P.A. (1969) *Index Muscorum*, Vol. 5 [T–Z, Appendix]. International Bureau for Plant Taxonomy and Nomenclature of the International Association for Plant Taxonomy, Utrecht.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Voucher information and GenBank accession numbers for the 84 individuals of *Radula lindenbergiana* sampled for this study.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be reorganized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

BIOSKETCHES

Benjamin Laenen and **Aurélie Désamoré** are PhD students at the University of Liège, Belgium. Benjamin Laenen currently focuses on the influence of mating systems on the evolutionary history of bryophytes. Aurélie Désamoré is interested in plant biogeography and population genetics, and is currently working on biogeographic patterns in European bryophytes.

Author contributions: A.V. conceived the project. J.M. G-M. and N.D. provided material and assisted with the manuscript. A.D., B.L., N.D. and A.V. collected and analysed the data. A.D., B.L., A.V. and M.C. wrote the manuscript. A.D. and B.L. contributed equally to this paper as senior authors.

Editor: José Maria Fernández-Palacios