ORIGINAL ARTICLE

Post-glacial dispersal, rather than *in situ* glacial survival, best explains the disjunct distribution of the Lusitanian plant species *Daboecia cantabrica* (Ericaceae)

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ABSTRACT

Aim The distribution of the Lusitanian flora and fauna, species which are found only in southern and western Ireland and in northern Spain and Portugal but which are absent from intervening countries, represents one of the classic conundrums of biogeography. The aim of the present study was to determine whether the distribution of the Lusitanian plant species *Daboecia cantabrica* was due to persistence in separate Irish and Iberian refugia, or has resulted from post-glacial recolonization followed by subsequent extinction of intervening populations.

Location Northern Spain and Co. Galway, western Ireland.

Methods Palaeodistribution modelling using MAXENT was employed to identify putative refugial areas for *D. cantabrica* at the Last Glacial Maximum (LGM). Phylogeographical analysis of samples from 64 locations in Ireland and Spain were carried out using a chloroplast marker (*atp*B–*rbc*L), the nuclear ITS region, and an anonymous nuclear single-copy locus.

Results The palaeodistribution model indicated areas with a high probability of survival for *D. cantabrica* at the LGM off the western coast of Galicia in Spain, and in the Bay of Biscay. Spanish populations exhibited substantially higher genetic diversity than Irish populations at all three loci, as well as geographical structuring of haplotypes within Spain consistent with divergence in separate refugia. Spanish populations also exhibited far more endemic haplotypes. Divergence time between Irish and Spanish populations associated with the putative Biscay refugium was estimated as 3.333–32 ka.

Main conclusions Our data indicate persistence by *D. cantabrica* throughout the LGM in two separate southern refugia: one in western Galicia and one in the area off the coast of western France which now lies in the Bay of Biscay. Spain was recolonized from both refugia, whilst Ireland was most likely recolonized from the Biscay refugium. On the balance of evidence across the three marker types and the palaeodistribution modelling, our findings do not support the idea of *in situ* survival of *D. cantabrica* in Ireland, contrary to earlier suggestions. The fact that we cannot conclusively rule out the existence of a small, more northerly refugium, however, highlights the need for further analysis of Lusitanian plant species.

Keywords

Daboecia cantabrica, Devensian glaciations, Ireland, Last Glacial Maximum, Lusitanian flora, palaeodistribution modelling, phylogeography, Spain, St Daboec's heath.

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INTRODUCTION

Species with disjunct distributions have long been of great interest to biogeographers. One such group, the Lusitanian flora and fauna, represents one of the classic puzzles of biogeography. These organisms are found in southern and western Ireland and in northern Spain and Portugal (and occasionally Brittany) but are largely or completely absent from intervening countries, including England. This enigmatic element of the European biota includes several plant species such as the strawberry tree, *Arbutus unedo*, several heaths (*Daboecia cantabrica*, *Erica erigena* and *E. mackayana*) and the carnivorous large-flowered butterwort, *Pinguicula grandiflora*, as well as a few invertebrates such as the Kerry slug, *Geomalacus maculosus*, and the Pyrenean glass snail, *Semilimax pyrenaicus* (Praeger, 1933, 1939; Webb, 1983).

Debate concerning the origin and distribution of the Lusitanian element has been going on since the mid-19th century. It is now accepted that the present-day distributions of many species have resulted from demographic changes during the ice ages of the Quaternary period (i.e. the past 2.6 Myr; Webb & Bartlein, 1992; Hewitt, 2003). At the height of the glaciations, most species' ranges contracted, with populations persisting in climatically suitable refugia, usually located south of the ice sheets and permafrost (Taberlet *et al.*, 1998; Hewitt, 1999; Provan & Bennett, 2008; Stewart *et al.*, 2010). In 1846, however, the naturalist Edward Forbes suggested that whilst most of the flora and fauna of the British Isles recolonized after the Last Glacial Maximum (LGM, *c.* 18–21 ka), *in situ* survival of the Irish species belonging to the Lusitanian element was the only explanation for their observed disjunct distribution (Forbes, 1846).

The subsequent controversy surrounding Forbes' suggestion centred on the biological and geological feasibility of such persistence at the height of the last ice age. Botanists such as Clement Reid insisted that only the hardiest of Arctic plants could have survived in ice-free areas of Ireland, and that long-distance dispersal from Iberian refugia was a more likely source of the Irish element of the Lusitanian flora (Reid, 1913), although others doubted that the seeds of these species possessed the necessary capacity for such dispersal (e.g. Praeger, 1933). Furthermore, emerging geological evidence around the same time suggested that the ice coverage of Ireland was so complete that such ice-free areas were unlikely to have existed, even on the extended areas of dry land to the south and west of the island resulting from the drop in sea levels at the LGM (Charlesworth, 1931). Even today, the extent and dynamics of the British–Irish Ice Sheet during the last glaciations remains a matter of ongoing debate (Bowen *et al.*, 2002; Knight *et al.*, 2004; Sejrup *et al.*, 2005; Ó Cofaigh & Evans, 2007; Scourse *et al.*, 2009; Clark *et al.*, 2012; Ó Cofaigh *et al.*, 2012).

Over the last 25 years, phylogeographical analyses have shed much light on the glacial and post-glacial histories of many species. The early phylogeographical studies on the biota of Europe confirmed the previously proposed existence of southern refugia for many species, based on palynological and macrofossil analyses, and indicated that the peninsulas of Iberia, Italy and the Balkans had acted as major refugial areas for a wide variety of plants and animals at the LGM (Taberlet *et al.*, 1998; Hewitt, 1999). Recent studies, however, have suggested that many species may have persisted through glacial periods, and in particular the LGM, in cryptic refugia at higher latitudes (reviewed in Stewart & Lister, 2001; Bennett & Provan, 2008; Provan & Bennett, 2008). In addition, phylogeography has also been used to gain important insights into the roles of the glaciations in shaping disjunct species' distributions (Beatty & Provan, 2010, 2011).

In the present study we have used evidence from palaeo-distribution modelling and phylogeographical analyses to explain the current disjunct distribution of *Daboecia cantabrica* (Huds.) K. Koch, a plant species found almost exclusively in northern Spain and Co. Galway, western Ireland (with the exception of a very few sporadic patches in western France; Fig. 1a). We tested the three main hypotheses that have been proposed previously to explain the distribution of Lusitanian plants (Forbes, 1846; Reid, 1913; Woodell, 1958; Coxon & Waldren, 1995; Rowe *et al.*, 2006): (1) recolonization of Ireland from an Iberian refugium followed by extinction of intervening populations, which will have resulted in Irish populations exhibiting a subset of the genotypes found in Iberian populations, or closely related genotypes, that can be dated to several thousand years ago, i.e. coincident with the retreat of the ice sheets after the LGM; (2) anthropogenic introduction from Iberia, which will have resulted in Irish populations exhibiting an extremely reduced subset of the genotypes found in Iberia that can be dated to several hundred years ago; and (3) *in situ* survival in both Ireland and Iberia, which will be reflected in distinct genetic lineages in Ireland and Iberia, with the separation of these lineages predating the LGM, and most likely coincident with the Eemian interglacial (115–130 ka). The expected patterns of genetic variation and divergence (after Provan & Bennett, 2008) under each of the three scenarios are summarized in Table 1.

MATERIALS AND METHODS

Study species

Daboecia cantabrica is an evergreen woody shrub which grows on acid heath in areas of high (> 1500 mm/year) rainfall and has a restricted disjunct distribution. The genus *Daboecia* is considered monospecific, with '*D. azorica*', which is found in the Azores, classified as a subspecies of *D. cantabrica* (McClintock, 1989). The species has hermaphroditic flowers which are generally bee-pollinated, although it is not known if self-pollination occurs (Woodell, 1958). The seeds are very light, and are easily dispersed by wind.

Sampling and DNA extraction

Samples of *Daboecia cantabrica* were collected from 11 locations across the species' Irish range in Co. Galway. Leaf

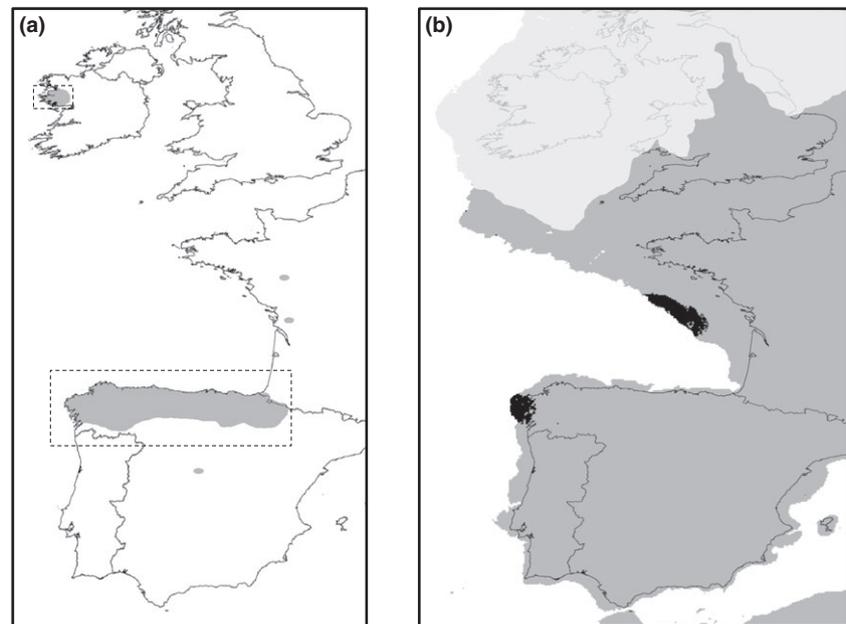**Figure 1** (a) Present-day distribution of *Daboecia cantabrica* [shaded; based on Webb, 1983, and the Global Biodiversity Information Facility (http://data.gbif.org/)] in western Europe. Dashed areas show the regions highlighted in Fig. 3a–c. (b) Palaeodistribution model (black) for *D. cantabrica* at the Last Glacial Maximum (LGM, *c.* 21 ka). The limits of the British–Irish Ice Sheet at the LGM (after Sejrup *et al.*, 2005) are also indicated in light grey, with areas of dry land at the LGM indicated by dark grey.

Table 1 Summary of expected and observed patterns of genetic diversity and divergence under the three scenarios explaining the origin of the Irish populations of *Daboecia cantabrica* (see Introduction for details), and values calculated for the three markers studied.

		Feature				
			Genetic diversity		Unique haplotypes	
Scenario	Divergence time		East Spain	Ireland	East Spain	Ireland
Post-LGM recolonization	$\leq$ *c.* 21 ka		High	Low	Many	Few
Anthropogenic introduction	$\leq$ *c.* 2 ka		High	Low	Many	Few
In situ persistence	> *c.* 115 ka		High	High	Many	Many
Marker						
atpB–rbcL	3.333–10 ka		4/0.376[a]	2/0.092	3	1
ITS	20.512–32 ka		13/0.449	3/0.589	11	1
DC1A02	N/A		26/0.689	6/0.102	23	3

ITS, internal transcribed spacer region; DC1A02, an anonymous single-copy nuclear DNA locus.

N/A, not applicable.

[a]Number of haplotypes/gene diversity.

samples were also obtained from herbarium specimens from a further seven locations in Ireland and from 46 locations spanning the species' entire Spanish distribution (see Appendix S1 in Supporting Information for details of locations). DNA was extracted from field-collected material using a modified CTAB (cetyl trimethyl ammonium bromide) protocol (Doyle & Doyle, 1987) and from herbarium samples using Qiagen DNeasy kits.

Palaeodistribution modelling

Palaeodistribution modelling was carried out to determine suitable climate envelopes for *D. cantabrica* at the LGM (*c.* 21 ka) using the maximum entropy approach implemented in the software package MAXENT 3.2.1 (Phillips *et al.*, 2006). Species occurrence data between 1950 and 2000 (312 occurrences) were downloaded from the Global Biodiversity Information Facility data portal (http://www.gbif.org/).

A distribution model based on the 19 BIOCLIM variables in the WorldClim data set (1950–2000; Hijmans *et al.*, 2005) was generated at 2.5-arc-minute resolution using MAXENT with the default parameters for convergence threshold (10^{-5}) and number of iterations (500), and projected onto reconstructed LGM data [Community Climate System Model (CCSM); Palaeoclimate Modelling Intercomparison Project Phase II: http://pmip2.lsce.ipsl.fr/] to identify potential refugial areas. Duplicate records from the same locality were removed to reduce the effects of spatial autocorrelation. A presence/absence threshold based on the maximum test sensitivity and specificity was used to indicate areas of suitable climate at the LGM (Cantor *et al.*, 1999; Liu *et al.*, 2005).

Chloroplast *atp*B–*rbc*L sequencing

In total, 222 samples were sequenced for the chloroplast *atp*B–*rbc*L intergenic spacer. For the majority of the samples, a

c. 710-bp product was amplified using the following primers: DC-atpB 5′–AAAAAGTCAATATTAGGGCGAAA–3′ and DC-rbcL 5′–AGCAGGGTCTACTCGACACG–3′ (designed from an existing *D. cantabrica* sequence, GenBank accession number AY520758; McGuire & Kron, 2005). For herbarium samples from which the complete product could not be amplified in a single polymerase chain reaction (PCR), the region was amplified as two overlapping fragments using the above primers and two internal primers (DC-atpB-IN-R 5′–GAAAAATAGGTGGAATTCGTCTATG–3′ and DC-rbcL-IN-F 5′–CATAGACGAATTCCACCTATTTTC–3′). PCR was carried out on a MWG Primus thermal cycler (Ebersberg, Germany) using the following parameters: initial denaturation at 94 °C for 3 min followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. PCR was carried out in a total volume of 20 μL containing 200 ng genomic DNA, 10 pmol of each primer, 1× PCR reaction buffer, 200 μM each dNTP, 2.5 mM $MgCl_2$ and 0.5 U GoTaq Flexi DNA polymerase (Promega, Sunnyvale, CA). 5 μL volumes of PCR products were resolved on 1.5% agarose gels and visualized by ethidium bromide staining, and the remaining 15 μL were ExoSAP-purified and sequenced in both directions using the BigDye sequencing kit v3.1 (Applied Biosystems, Foster City, CA) and run on an AB 3730XL DNA analyser (Life Technologies, Carlsbad, CA).

Nuclear ITS sequencing

In total, 215 samples were sequenced for the nuclear internal transcribed spacer (ITS) region. For the majority of the samples, a *c.* 640-bp product was amplified using the following primers: DC-ITS-F 5′–TCGATAACCTGACGATCAGAAAA–3′ and DC-ITS-R 5′–ACTTGAGGTACCACAATTGAGG–3′ (designed from an existing *D. cantabrica* sequence, GenBank accession number AY520786; McGuire & Kron, 2005). For herbarium samples from which the complete product could not be amplified in a single PCR, the region was amplified as two overlapping fragments using the above primers and two internal primers (DC-ITS-IN-R 5′–GCAATTCACACCAAGTATCGC–3′ and DC-ITS-IN-F 5′–GCGATACTTGGTGTGAATTGC–3′). PCR and sequencing were carried out as described above.

Single-copy nuclear DNA (scnDNA) sequencing

Primers to amplify an anonymous single-copy nuclear DNA locus (DC1A02) were developed using the ISSR cloning method described in Beatty *et al.* (2010). The 353-bp region was sequenced in 231 individuals. The primers used were DC1A02-F (5′–CTTCACGTATGTCGCAGGAA–3′) and DC1A02-R (5′–TGGCATCGTATAACCAAATCC–3′). For herbarium samples from which complete products could not be amplified in a single PCR, regions were amplified as two overlapping fragments using the above primers and internal reverse and forward primers (DC1A02-IN-R 5′–GTATG-

GAAGTAACCCATTGAAAC–3′, DC1A02-IN-F 5′–GTTTCAATGGGTTACTTCCATAC–3′). PCR and sequencing were carried out as described above.

Phylogeographic analysis

For the two nuclear loci, potential recombination was assessed using the Hudson & Kaplan (1985) test in the software package DnaSP 5.10 (Librado & Rozas, 2009). In the absence of recombination in either region, haplotypes were resolved for individuals exhibiting two or more heterozygous positions using Phase 2.1 (Stephens & Donnelly, 2003) implemented in DnaSP. DNA sequences were aligned in BioEdit 7.0.9.0 (Hall, 1999). Median-joining networks for all three loci were constructed using Network 4.5.1.6 (http://www.fluxus-engineering.com/). Any reticulations in the networks were broken following the rules described in Pfenninger & Posada (2002).

Because the data suggested separate glacial histories for eastern and western Spanish populations, the Spanish samples were split into two groups corresponding to the two putative refugia associated with Galicia and the Bay of Biscay (see Results and Discussion). We performed a spatial analysis of molecular variance (SAMOVA) using the software package samova 1.0 (Doupanloup *et al.*, 2002) for each of the three data sets. This program uses a simulated annealing approach based on genetic and geographical data to identify groups of related populations. The program was run for 10,000 iterations for *K* = 2 to 10 groups, from 200 initial conditions, and the most likely structure was identified using the maximum value of Φ_{CT} (the proportion of genetic variation between groups of populations) that did not include any groups of a single population.

Levels of haplotype diversity (*H*) and nucleotide diversity (π) were calculated for all three groups (Ireland, West Spain and East Spain) using DnaSP. The divergence time (*T*) between Irish and East Spain populations was estimated by calculating Nei's genetic distance (D_A) using DnaSP, and by using the formula $T = D_A/2 \mu$ (Nei & Kumar, 2000), where μ is the mutation rate per site per year. Mutation rates of $1.0–3.0 \times 10^{-9}$ (Wolfe *et al.*, 1987) and $5.0–7.8 \times 10^{-9}$ (Kay *et al.*, 2006) were used for the chloroplast *atpB–rbc*L and nuclear ITS regions, respectively. As we had no information for the mutation rate of the anonymous DC1A02 single copy nuclear locus, divergence times were not calculated based on this region. Finally, levels of differentiation (Φ_{ST}) between the three groups were estimated by an analysis of molecular variance (AMOVA) using the software package Arlequin 3.01 (Excoffier *et al.*, 2005).

RESULTS

Palaeodistribution modelling

For the palaeodistribution model generated by Maxent using the 19 BIOCLIM variables, the area under the receiver

operating characteristic curve (AUC) value of 0.999 indicated a better than random prediction. The model indicated two substantial areas with a high probability of survival for *D. cantabrica* at the LGM based on the maximum test sensitivity and specificity threshold of 0.42 (Fig. 1b). One of these was located off the western coast of Galicia in Spain, whilst the other was located in the Bay of Biscay off the western coast of France.

Phylogeography of *D. cantabrica*

Nine, 18 and 33 haplotypes were identified for the chloroplast *atp*B–*rbc*L intergenic spacer, the nuclear ITS region and the anonymous DC1A02 single copy nuclear locus, respectively (GenBank accession numbers JX262430–JX262489). The haplotype networks for all three markers indicated a split, to a greater or lesser degree (one mutation for ITS, two mutations for *atp*B–*rbc*L and six mutations for DC1A02), between the haplotype depicted in dark blue in each network and its associated haplotypes, and the remaining haplotypes (Fig. 2). This was generally reflected in the geographical distribution of the haplotypes, with the dark blue haplotype and its associated haplotypes being primarily restricted to Galicia, and being absent from eastern Spain and Ireland (Fig. 3a–c).

Based on the results of the SAMOVA analyses from all three loci (Table 2), and given the fact that chloroplast markers retain the signals of post-glacial recolonization from refugia more effectively than their nuclear counterparts (Ennos *et al.*, 1999), populations belonging to two of the three groups identified by the chloroplast SAMOVA and which were composed solely or almost exclusively of 'western' chloroplast haplotypes, indicated by the three shades of blue in Fig. 3a, were designated as belonging to the West Spain group associated with the putative Galician refugium (Carnota, Bainas, Barbazàn, Enxo, Devesa, Santiago, As Pontes, Friol, Puerto del Barquero, Sierra de San Mamed, Villasinde, Villanueva, Argayo del Sil and Cangas de Narcia). Four (Devesa, Enxo, Villanueva and Villasinde) and two (Enxo and Bainas) of these populations also formed the distinct groups in the SAMOVA analyses based on the ITS and DC1A02 loci, respectively.

Higher numbers of haplotypes, both total and private, were found in both the West Spain and East Spain populations compared to Irish populations at all three loci (Table 3). Levels of haplotype diversity (H) and nucleotide diversity (π) were higher in West Spain and East Spain than in Ireland for the chloroplast *atp*B–*rbc*L locus (H: 0.301 and 0.376 vs. 0.092; π: 0.0021 and 0.0002 vs. 0.0001) and the nuclear DC1A02 locus (H: 0.696 and 0.689 vs. 0.102; π: 0.0105 and 0.0051 vs. 0.0006). For the nuclear ITS locus, diversity levels for Ireland ($H = 0.589$; $\pi = 0.0011$) were intermediate between those found in West and East Spain (H: 0.659, 0.449; π: 0.0021, 0.0009). The estimate of the time since the initial divergence of the Ireland and East Spain groups of populations, which both appear to have originated from the same refugium in the Bay of Biscay, was calculated
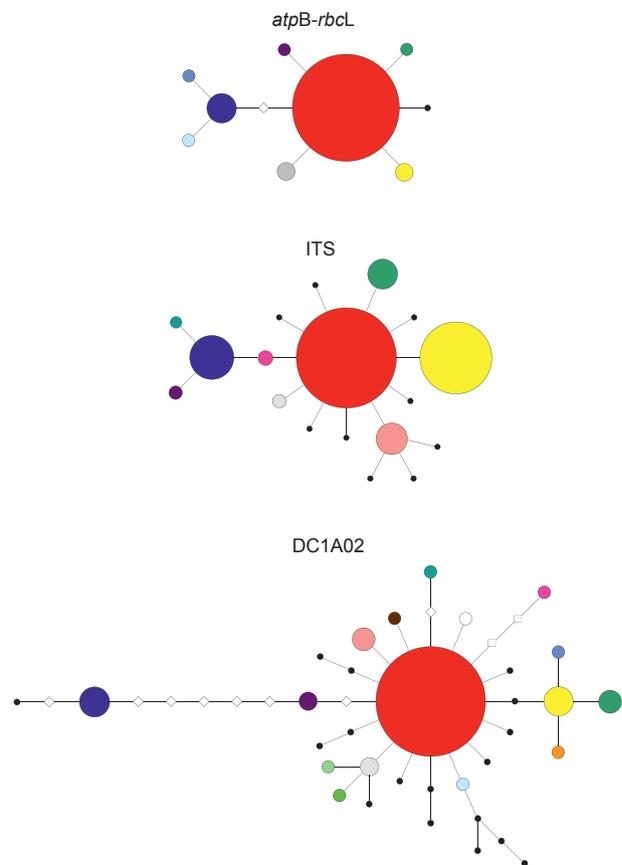

Figure 2 Haplotype networks for chloroplast *atp*B–*rbc*L region (top), nuclear ITS region (middle) and nuclear DC1A02 region (bottom) for *Daboecia cantabrica* in western Europe. Circle sizes are approximately proportional to haplotype frequency. Open diamonds represent missing haplotypes and small black circles represent unique haplotypes, i.e. those found in a single individual.

as 3.333–10 ka for *atp*B–*rbc*L and as 20.512–32 ka for ITS. Finally, the results of the AMOVA for all three loci further highlighted the closer relationships of East Spain populations to Ireland populations than to West Spain populations (Table 4).

DISCUSSION

The phylogeographical data presented here support the palaeodistribution model of persistence by *Daboecia cantabrica* throughout the LGM in two separate southern refugia, one in western Galicia and one in an area off the coast of western France which now lies in the Bay of Biscay. A previous palaeodistribution modelling study on 19 Iberian tree species also indicated that the most suitable climatic conditions for survival were found in the north-west and north-east of Spain (Benito Garzón *et al.*, 2007). In the present study, all three markers indicated a divergent haplotype or group of haplotypes that were restricted to Galicia and neighbouring provinces. This is particularly evident for the chloroplast locus *atp*B–*rbc*L, where the three haplotypes depicted in
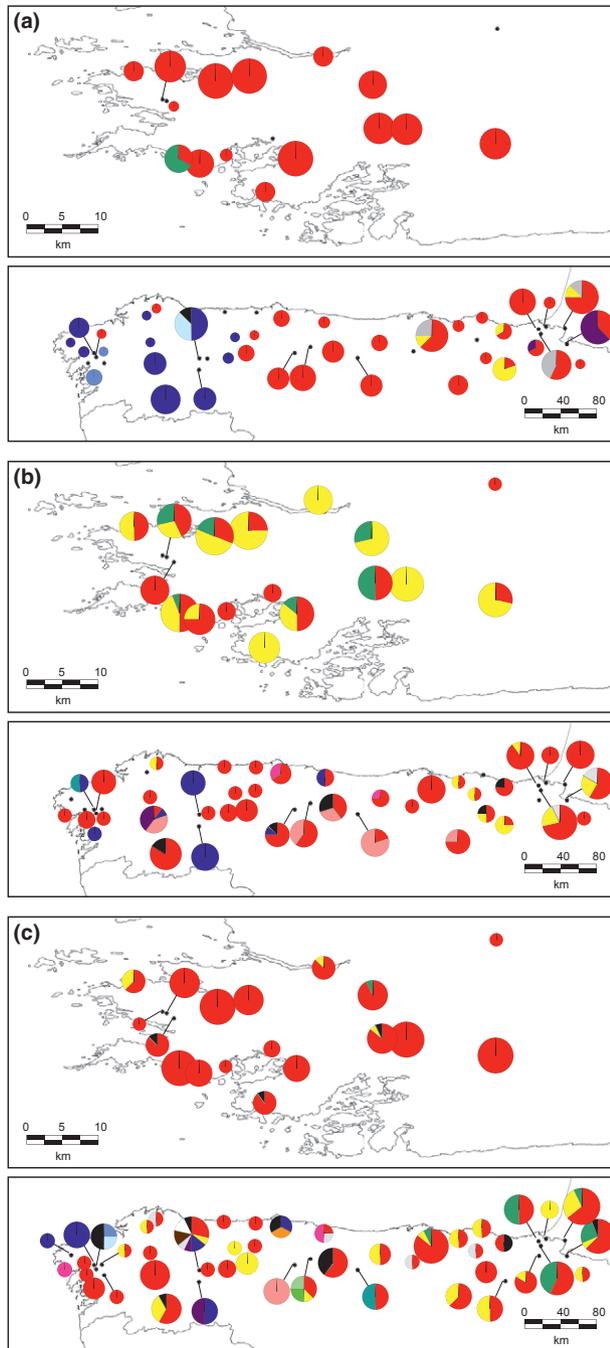
Figure 3 Haplotype distributions for (a) the chloroplast *atp*B–*rbc*L region, (b) the nuclear ITS region, and (c) the nuclear DC1A02 region for *Daboecia cantabrica* in western Europe. Pie chart sizes are approximately proportional to sample size, with the smallest circles representing *n* = 1 (chloroplast locus) or *n* = 2 (nuclear loci) and the largest representing *n* = 8 (chloroplast locus) or *n* = 16 (nuclear loci).

various shades of blue were separated from the remaining haplotypes by a missing intermediate. With the exception of three individual plants, which exhibited either the predominant red haplotype or a unique haplotype (black), all samples from Galicia and western León exhibited one of these

blue haplotypes, which were not found further east. This high level of genetic structuring is frequently observed in chloroplast markers, which in hermaphroditic species such as *D. cantabrica*, have half the effective population size of biparentally inherited nuclear markers and are thus more prone to the effects of genetic drift, as well as being dispersed only via seeds in angiosperms. Consequently, they are expected to retain the genetic signatures of refugial areas and recolonization routes for longer than nuclear markers (Ennos *et al.*, 1999). Nevertheless, the two nuclear loci used in the present study, which are transmitted via pollen as well as seed, also exhibited separate groups of 'eastern' and 'western' haplotypes, consistent with recolonization from two separate refugia. Thus, *D. cantabrica* represents a further addition to the list of species that have persisted through the glacial periods in 'refugia within refugia' (Gómez & Lunt, 2007), particularly within Iberia, although technically the palaeodistribution model suggests that the eastern refugium was located to the north of Spain in the Bay of Biscay. Again, this is particularly evident for the chloroplast marker, which exhibits a general decrease in levels of within-population diversity from east to west, characteristic of post-glacial spread from the eastern (Biscay) refugium. Recolonization from the western (Galician) refugium would appear to have been more limited, and is probably consistent with this refugium being much smaller than its eastern counterpart, as is evident from the lower numbers of haplotypes exhibited at all three loci. It is likewise possible that the high nucleotide diversity (π) values for the two nuclear markers in the West Spain populations might reflect admixture of haplotypes from both refugia (Petit *et al.*, 2003) in some of the east Galicia populations, given that nuclear markers are transmitted via both pollen and seed.

Of the scenarios proposed to explain the history of Irish populations of *D. cantabrica* (see Introduction), our data strongly support post-glacial recolonization from a southern refugium, rather than *in situ* persistence throughout the LGM. Populations in Ireland exhibit greatly reduced genetic diversity compared to their Spanish counterparts, characteristic of the founder effects associated with leading-edge colonization patterns (Hewitt, 1999; Provan & Bennett, 2008). The differences in haplotype frequency at the ITS locus, where Irish populations exhibit a high proportion of the yellow haplotype, as well as a haplotype (depicted in green) not found in Spain, is probably a stochastic consequence of different founder effects during separate northward and southwestward recolonization. The high frequency of the green haplotype is responsible for the high gene diversity value for the ITS region in Ireland, as well as the difference between the divergence time of Irish and East Spain populations compared with that calculated for the chloroplast *atp*B–*rbc*L region. Alternatively, the unique haplotypes found in Ireland at all three loci could represent recent, geographically localized mutations (Provan *et al.*, 2005). Although the location of the refugium identified by the palaeodistribution model now lies under water, further light could be shed on the

Table 2 Results of the SAMOVA analyses for Spanish populations of *Daboecia cantabrica*.

Marker	No. of groups	Φ_{CT}	Groups
*atp*B–*rbc*L	3	0.866	Devesa, Santiago
			Carnota, Baiñas, Barbazàn, Enxo, As Pontes, Friol, Puerto del Barquero, Sierra de San Mamed, Villasinde, Villanueva, Argayo del Sil, Cangas de Narcia
			The rest
ITS	2	0.743	Enxo, Devesa, Villasinde, Villanueva
			The rest
DC1A02	2	0.784	Enxo, Baiñas
			The rest

genetic composition of the putative source population by examining samples from France, which unfortunately were not available for the present study. It is clear, however, that the western refugium in the Galicia area was highly unlikely to be the source of extant Irish *D. cantabrica*.

Seeds of *D. cantabrica* have been recorded in interglacial deposits from Ireland (Woodell, 1958; Coxon, 1996), but these correspond to the Gortian interglacial (*c.* 428–302 ka). Although no such deposits have been discovered in Ireland for more recent interglacial periods, it seems likely that the species was extirpated from Ireland during one or more subsequent glaciations and has recolonized since the LGM. Early models of the British–Irish Ice Sheet indicated potential ice-free areas in southern Ireland and in the Celtic Sea (Bowen *et al.*, 2002), but recent reconstructions suggest more extensive coverage by ice sheets (e.g. Sejrup *et al.*, 2005; Ó Cofaigh & Evans, 2007; Clark *et al.*, 2012), contrary to suggestions of

the existence of a local refugium near the coast of Ireland (Coxon & Waldren, 1995; Rowe *et al.*, 2006). Thus, a 'tabula rasa' scenario for *D. cantabrica* indicated by our phylogeographical data, at least as far as Ireland and its adjoining continental shelf is concerned, is consistent with current geological scenarios for the Devensian glaciations.

Expansion from a refugium further north of the Biscay refugium in the exposed land area between France and Ireland was proposed in the only previous study on a species with a Lusitanian distribution, the natterjack toad, *Bufo calamita* (Rowe *et al.*, 2006), although this species is also found in intervening regions, including France and England. A northern refugium was also suggested for the herb *Meconopsis cambria*, which has a similar disjunct distribution in northern Spain, France, western England and Wales, and Ireland (Valtueña *et al.*, 2012). Coxon & Waldren (1995) suggested that *D. cantabrica* could have persisted to the south or south-east of Ireland in a refugium, but the extreme founder effect revealed by the phylogeographical analysis, coupled with the palaeodistribution model, suggest that this putative refugium was located much further south, and cannot be classed as a 'local' refugium per se. Nevertheless, the existence of unique haplotypes in Irish populations at all three loci studied means that we cannot completely discount recolonization from a smaller refugium located closer to Ireland. Although such a refugium was not indicated by the palaeodistribution model, it is conceivable that the few unique haplotypes found in Ireland originated from an 'unstable' northern refugium on the continental shelf, perhaps where the probability of occurrence in the model lies just below the presence threshold. Given that such a refugium would probably have been rather small, haplotypes not present in the Biscay refugium could conceivably have been lost via genetic drift, which could also explain the greatly reduced diversity in Ireland. The occurrence of another plant species that exhibits

Table 3 Diversity statistics for *Daboecia cantabrica* by region.

Locus	West Spain (Galician refugium)				East Spain (Biscay refugium)				Ireland			
	n	h[a]	H	π	n	h[a]	H	π	n	h[a]	H	π
*atp*B–*rbc*L	42	4 (4)	0.301	0.0002	96	4 (3)	0.376	0.0006	84	2 (1)	0.092	0.0001
ITS	64[b]	7 (3)	0.659	0.0021	178[b]	13 (11)	0.449	0.0009	190[b]	3 (1)	0.589	0.0011
DC1A02	74[b]	10 (2)	0.696	0.0105	198[b]	26 (23)	0.689	0.0051	190[b]	6 (3)	0.102	0.0006

n, number of individuals; h, number of haplotypes observed; H, haplotype diversity; π, nucleotide diversity.
[a]Numbers in parentheses indicate private haplotypes.
[b]Two gene copies sequenced per diploid individual.

Table 4 Pairwise Φ_{ST} values based on an analysis of molecular variance (AMOVA) for *Daboecia cantabrica* by region.

	*atp*B–*rbc*L				ITS				DC1A02		
	W Spain	E Spain	Ireland		W Spain	E Spain	Ireland		W Spain	E Spain	Ireland
W Spain	–			W Spain	–			W Spain	–		
E Spain	0.775	–		E Spain	0.436	–		E Spain	0.164	–	
Ireland	0.835	0.053	–	Ireland	0.522	0.241	–	Ireland	0.390	0.109	–

a Lusitanian distribution, *Euphorbia hyberna* (Irish spurge), in the extreme south-west of England, could be due to recolonization from such a refugium, and further phylogeographical work on this particular species would be of interest.

Of the two possible 'recolonization' scenarios previously proposed for Ireland (i.e. natural versus anthropogenic), the divergence time between Spanish and Irish populations would appear to preclude anthropogenic introduction because the divergence of the Irish and Spanish populations pre-dates *c.* 3 ka. It had previously been suggested that trade between Ireland and Iberia could have introduced Lusitanian species (Corbet, 1961, 1962) including another heath, *Erica erigena* (Foss *et al.*, 1987), and there are records of plants being deliberately introduced to Ireland by monks in medieval times (Smith & Waldren, 2010), but the time-scales estimated in the present study are inconsistent with these scenarios. Natural dispersal could have involved gradual northward spread following the LGM, or one or more episodes of long-distance dispersal, although it is difficult to discriminate between the two. Seeds of *D. cantabrica* are small enough to be dispersed by wind, but Woodell (1958) has noted that although *D. cantabrica* is abundant in western Galway, the species is not found on islands a few miles off the coast. Water-mediated dispersal has been deemed extremely unlikely (Praeger, 1933), leaving transport by birds as the only other possible method for long-distance dispersal (Reid, 1913; Praeger, 1933).

CONCLUSIONS

The *in situ* survival of the Lusitanian flora in Ireland during the last glaciations, originally proposed by Forbes over 150 years ago and more recently advocated for *Daboecia cantabrica* in particular by Woodell (1958) and Webb (1983), is not supported by the findings of the present study. Woodell (1958, p. 215) stated that '[c]onceivably, *Daboecia* could have disappeared from the Irish flora during a later glaciation, and migrated back in post-glacial times, but this hardly seems likely', whilst Webb (1983, p. 15) believed that periglacial survival is '… well worth considering as the least improbable hypothesis for [*Daboecia*]'. These views would appear to be misguided, as our combination of palaeodistribution modelling and phylogeographical analysis indicates that post-glacial recolonization of Ireland from a southern refugium is the most likely scenario. Nevertheless, as highlighted by Coxon & Waldren (1995), different species of the Lusitanian flora (including, possibly, *Euphorbia hyberna*) might have different glacial histories. Future phylogeographical studies on these species will undoubtedly indicate whether that of *D. cantabrica* is typical.

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SUPPORTING INFORMATION

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

Appendix S1 Populations of *Daboecia cantabrica* analysed in this study.

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