

Polyploidy, phylogeography and Pleistocene refugia of the rockfern *Aleixaceae*: evidence from chloroplast DNA

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*C. *Aleixaceae*, D. *Aleixaceae*, B. *Aleixaceae*, H. *Aleixaceae*, L. *Aleixaceae*, K; †E. *Aleixaceae*, L. *Aleixaceae*, D. *Aleixaceae*, G. *Aleixaceae*, B. *Aleixaceae*, H. *Aleixaceae*; ‡D. *Aleixaceae*, G. *Aleixaceae*, C. *Aleixaceae*, C. *Aleixaceae*, K; ¶B. *Aleixaceae*, G. *Aleixaceae*, E. *Aleixaceae*, K

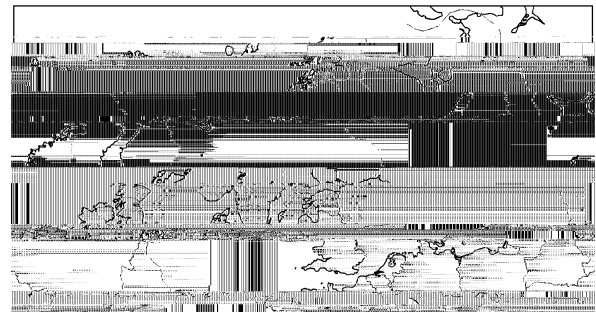
Abstract

Chloroplast DNA sequences were obtained from 331 *Aleixaceae* plants representing 143 populations from throughout the range of the complex in Europe, plus outlying sites in North Africa and the near East. We identified nine distinct haplotypes from a 900 bp fragment of the *L- F* gene. Tetraploid populations were encountered throughout Europe and



Fig. 1 Diploid *A. ...* growing among rocks in coastal Croatia.

on the underside with pale reddish-brown scales (hence the common name, rustyback) (Fig. 1). *A. ...* inhabits base-rich rocks and mortared walls in Europe and north Africa, and the near East, expanding eastwards in scattered populations as far as western China. The taxon is a complex comprising diploid, tetraploid and hexaploid forms that are generally treated as subspecies (Lovis 1977). The tetraploid *A. ...* ssp. *...* is the most common and widespread, occurring throughout the range from the Canary Islands in the west to China in the east (Fig. 2). The diploid, *A. ...* supsp. *...* (DE Meyer) Greuter & Burdet (1980), is known mainly from central eastern Europe (Croatia, Bulgaria, Hungary and Romania) with, as far as is known, peripheral populations in Italy, Greece and Turkey. Hexaploids have been reported from Sicily, Greece and Cyprus but there are few records (Vida 1963; Vianne *et al.* 1993; Pintér *et al.* 2002).



The distribution of diploid and tetraploid *A. ...* suggests that they have different colonizing abilities (Fig. 2). Much of the modern range of tetraploid *A. ...* is in regions that were glaciated or subject to periglacial conditions during the Pleistocene, implying range expansion in the Holocene. The modern occupation of walls is testimony to the colonizing ability of tetraploid *A. ...*, and contrasts with the localized distribution of diploid *A. ...*. This pattern fits well with the differing breeding systems of the two ploidy levels inferred from allozyme data: diploids are out-breeders and tetraploids are highly inbred (unpublished data).

Several Pleistocene glacial refugia have been proposed for the European biota (Taberlet *et al.* 1998; Hewitt 1999, 2000). The consensus from previous molecular studies is that taxa emerged from one or more of three southern areas (Iberia, Italy, Balkans) following the last glacial, and presumably also during interglacials. However, congruence

Fig. 2 European distribution of diploid (a) and tetraploid (b) *A. ...*. (Images provided by Paul Williams, Natural History Museum- Worldmap/Atlas Florae Europae, revised from Jalas & Suominen 1972).

in phylogeographical patterns is observed only on a broad

Biosystems). Sequence alignments used SEQED Version 1.03 or SEQUENCHER Version 3.0.

Ploidy was determined for the majority of material using the evidence of allozyme banding patterns (Vogel *et al.* 1999a; Suter *et al.* 2000; unpublished data) and confirmed for a subsample of specimens using cytology (Manton 1950) and spore measurement (Vida 1963; Nyárády & Vicol 1967; Pintér 1995a).

Results

S. ...

Primers Fern-1 and f amplified a fragment of ≈ 900 bp comprising the chloroplast *L*

We found that most populations of *A. ...* we examined from the Balkans were diploids but that diploid populations were less common in Italy and Greece, and absent from most of western Europe including Spain (Figs 3b and 5a). Of seven diploid cpDNA lineages, four occurred in the Balkans, one (red) being shared with Italy (mainland and Sicily) and Kefhalonia (Greece), and three being endemic (orange, turquoise, pink). Greece has two endemic diploid lineages (yellow in Peloponnisos, blue in Crete) and polyploid derivatives of these which are restricted to Greece or the southern Mediterranean, and Sicily has an endemic diploid haplotype (brown) (Figs 3b and 5a). Excluding the Mediterranean lineages, the overwhelming majority of diploids were red (89%) and we found no diploid with the green haplotype.

The majority of north Africa, western Europe including the Canary Islands, Morocco, Spain and France contained no diploid populations of *A. ...*, but tetraploids of three lineages (green, turquoise, red) were widespread (Figs 4 and 5b). Despite the absence of diploids to the west, haplotype diversity in Spain and mainland Italy are the

same, and higher than tetraploid haplotype diversity in the Balkans. In the west the number of tetraploid haplotypes diminishes northwards, three in Spain, two in France, one in the UK (Fig. 4). We calculated regional diversity as

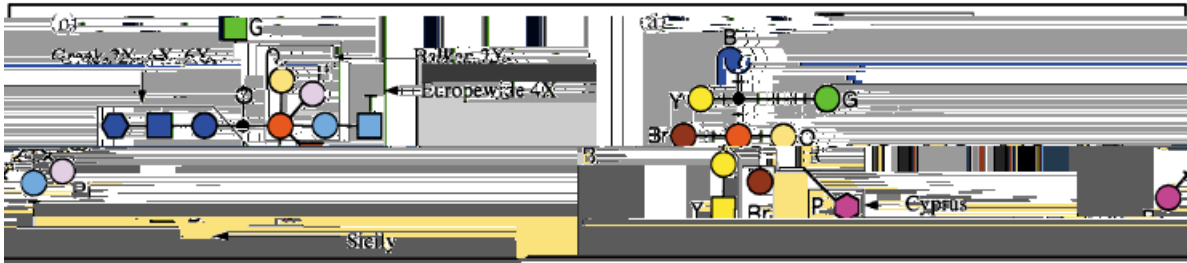


Fig. 3 Minimum spanning trees of nine *A. nemorosum* haplotypes (a), and observed haplotype/ploidy combinations (b). Geographic ranges of these are indicated.

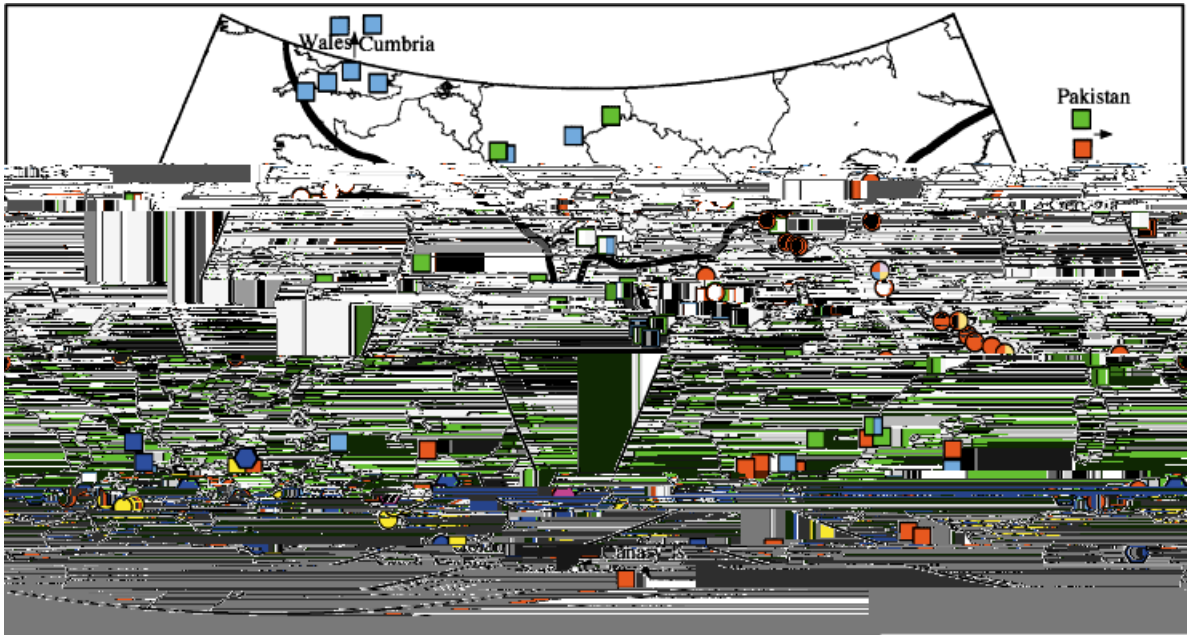


Fig. 4 Distribution of *A. nemorosum* L haplotypes in populations of *A. nemorosum* in and around Europe. Symbol colour indicates haplotype and symbol shape indicates ploidy (circle, 2 \times ; square, 4 \times ; hexagon, 6 \times). Polymorphic populations are indicated by combination of fill colour. The approximate extent of the Pleistocene periglacial (including montane glaciers in Italian Alps) is indicated by a black line (redrawn from Taberlet *et al.* 1998).

... 1999; Segraves *et al.* 1999; Sharbel & Mitchell-Olds 2001; Yamane & Ohnishi 2001), polyploidy can evolve many times. Current evidence indicates that gene flow between ploidal levels in *A. nemorosum* is predominantly unidirectional and occurs only during the generation of tetraploids from diploids; most spores produced by triploid hybrids are sterile (but see Pintér 1995b) or may result in hexaploid formation via unreduced gametes (see Reichstein 1981). Populations of mixed ploidy were not observed in *A. nemorosum*, and few triploid hybrids have been encountered, apparently arising from rare migration events (unpublished data). Introgression into an established diploid or tetraploid population by spores from the alternative ploidal level is unlikely. However, we have evidence of some diploid and tetraploid populations with more than one haplotype, and although tetraploids are primarily inbreeders, gene flow between haplotypes has been detected with allozymes (unpublished data).

Several attributes of polyploids have been cited as potentially giving them competitive advantages over diploid relatives (e.g. increased heterozygosity, allelic diversity and enzyme multiplicity) (Soltis & Soltis 2000). However, for ferns the potential for intragametophytic selfing that can arise in polyploids offers an obvious and profound advantage in colonization and establishment (Schneller & Holderegger 1996), especially in the context of large-scale shifts in habitat availability such as would have resulted from glacial cycling in the Pleistocene. Long-distance, single-spore colonization is very probably the principal reason for the geographical success of tetraploid *A. nemorosum*, which has been able to take advantage of virgin habitat revealed by the retreat of Pleistocene glaciers and periglacial conditions. This advantage operates where new habitat is available because minority cytotype exclusion is expected to prevent invasion of existing diploid populations (Levin 1975; Vogel

presence/absence of haplotype–ploidy combinations in *A. ...* by region, with diversity scores for each region adjusted for the frequency of their occurrence among regions)

	2×T	2×R	2×B	2×Y	2×Br	2×O	2×Pi	4×G	4×T	4×R	4×B	4×Y	6×B	6×Pu	Diversity score
region															
UK	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0.07
Cyprus	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0.21
France+	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0.21
Spain	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0.21
Italy	0	1	0	0	0	0	0	1	1	1	0	0	0	0	0.29
Sicily	0	1	0	0	1	0	0	0	1	1	0	0	1	0	0.36
Balkans	1	1	0	0	0	1	1	1	0	1	0	0	0	0	0.43
Greece	0	1	1	1	0	0	0	0	1	1	1	1	1	0	0.57
Rarity weighting	8	2	8	8	8	8	8	2	1.6	1.1	8	8	2.7	8	
															Rarity adjusted diversity
UK	0	0	0	0	0	0	0	0	1.6	0	0	0	0	0	0.11
Cyprus	0	0	0	0	0	0	0	0	0	1.1	0	0	2.7	8	0.84
France+	0	0	0	0	0	0	0	2	1.6	1.1	0	0	0	0	0.34
Spain	0	0	0	0	0	0	0	2	1.6	1.1	0	0	0	0	0.34
Italy	0	2	0	0	0	0	0	2	1.6	1.1	0	0	0	0	0.48
Sicily	0	2	0	0	8	0	0	0	1.6	1.1	0	0	2.7	0	1.10
Balkans	8	2	0	0	0	8	8	2	0	1.1	0	0	0	0	2.08
Greece	0	2	8	8	0	0	0	0	1.6	1.1	8	8	2.7	0	2.81

1999b; and references therein). The of having multiple genomic copies and recombination among polyploids is a sufficient fitness benefit to overcome restricted circumstances (Felber 1991; but 1996). In plants in general, polyploidy confer a consistent advantage, such as severe ecological or climatic conditions (Stebbins 1985). In *A. ceterach*, there is little evidence that tetraploids have been able to outcompete those that are already established (Fig. 5). Inbreds will also be free from the negative effect of inbreeding depression which outcrossing diploids on the edge of their range suffer (Peck *et al.* 1998).

DISCUSSION: *A. ceterach*

Our data reveal two distinct phylogeographic patterns within the *A. ceterach* polyploid complex: one for diploid and polyploid taxa. Within this complex, a clear distinction is evident in the geographical distribution of the diploid and polyploid taxa of central and southern Europe. As obligate outcrossers, diploid *A. ceterach* is expected to have limited ability of establishment at new sites, the existence of long-lived lineages comprising endemic and/or multiple lineages is strong evidence of long-term occupation of the area. These characteristics are seen in the distribution of the diploid in Europe (Pannonian-Balkans and Greece) and that these regions were refugia for diploid *A. ceterach*. Pannonian-Balkans, Kephallonia (Greece), Sicily and the Iberian Peninsula are linked by the co-occurrence of red diploids, which might be evidence of a now fragmented distribution, but in other respects these regions are quite distinct.

In the Pannonian-Balkans, tetraploid polyploids are comparatively rare and comprise lineages that are widely spread across Europe. Although two of these lineages are represented in Balkan diploids (turquoise, red and green), polyploids are not necessarily recently derived from diploids. In Hungary, allozyme analysis of all known polyploid *A. ceterach* indicates that red tetraploids share haplotypes that are not found in the present red diploids (our unpublished data). This implies that if, as it appears, the Pannonian-Balkans was the initial source of the widespread red tetraploids, they probably did not arise via hybridization (i.e. not following the last glacial). Alternatively, the red haplotype may have evolved independently in different regions. The common tetraploids (red and green) have reinvaded Hungary in just the same manner as the red diploids colonized the rest of Europe, perhaps from Western Europe, a refugia in Spain and/or Italy. The rarity (and possible absence) in one instance) of the turquoise and green haplotypes among diploids in the Pannonian-Balkans is consistent with the notion of an earlier origin of the 'red' haplotype.

- Soltis DE, Soltis PS (1987) Polyploidy and breeding systems in homosporous Pteridophyta: a reevaluation. *Annals of the Missouri Botanical Garden*, **130**, 219–232.
- Soltis DE, Soltis PS (1993) Molecular data and the dynamic nature of polyploidy.

