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Genetic variation in the allotetraploid *Dryopteris corleyi* (Dryopteridaceae) and its diploid parental species in the Iberian Peninsula[1]

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Studies on genetic diversity help us to unveil the evolutionary processes of species and populations and can explain several traits of diploid–polyploid complexes such as their distributions, their breeding systems, and the origin of polyploids. We examined the allozyme variation of *Dryopteris aemula* and *D. oreades*, diploid ferns with highly fragmented habitats, and the allotetraploid *D. corleyi* to (1) analyze the putative relationship between both diploids and the tetraploid, (2) compare the levels of genetic variation among species and determine their causes, and (3) assess the breeding system of these taxa. The allozymic pattern of *D. corleyi* confirms that it derived from *D. aemula* and *D. oreades*. The lack of genetic diversity in *D. aemula*, a species of lowland habitats, may be due to genetic drift associated with the contraction of populations in the last glaciation. By contrast, the alpine *D. oreades* had moderate intrapopulation genetic variation, which may derive from the expansion of populations during the last glaciation. In the latter species, low interpopulational variation suggested effective gene flow (spore exchange), and genotype frequencies in Hardy–Weinberg equilibrium indicated cross-fertilization of gametophytes. Evolutionary history appears to be an essential element in the interpretation of genetic variation of highly fragmented populations.

Key words: allozymes; breeding system; Dryopteridaceae; *Dryopteris*; ferns; fragmentation; genetic diversity; genetic drift; glacial refugia; Iberian Peninsula; polyploidy.

Genetic diversity maintains the evolutionary potential of taxa to adapt to changing environmental conditions (Ellstrand and Elam, 1993; Lande and Shannon, 1996), and the knowledge of how much genetic diversity there is and how it is distributed provides important insights into the evolutionary processes that species and populations undergo. Genetic diversity arises through mutation in local populations, is maintained and spread via gene flow among individuals, and decreases through inbreeding, genetic drift, and genetic bottlenecks (Usher, 1997). Natural or anthropogenic habitat fragmentation during the evolutionary history of species is currently regarded as one of the main threats to genetic diversity (e.g., Peterson et al., 2008). Fragmentation leads to smaller patches of suitable habitat, thereby reducing population sizes and increasing distances among remaining populations, which restricts interpopulational gene flow (Young et al., 1996; Usher, 1997). Under these conditions, characterized by the low probability of "genetic rescue" via gene flow, random genetic drift can lead to the fixation of different alleles at each population (Barrett and Kohn, 1991; Robichaux et al., 1997). Consequently, drift not only causes genetic differentiation among populations, but also leads to a loss of genetic diversity both at the population and species levels.

Ferns have been the target of numerous studies on genetic diversity because of their unusual life histories, potential for long-distance dispersal, and frequently fragmented populations (e.g., Pajarón et al., 1999; Landergott et al., 2001; Schneller and Liebst, 2007). The life history of these organisms typically consists of a haploid stage, the gametophyte, and a diploid stage, the sporophyte, which are independent of each other. Homosporous fern gametophytes are potentially hermaphroditic and can self-fertilize, yielding completely homozygous sporophytes, thus having the possibility to found a population from a single spore. However, rather than selfing, many species tend to cross-fertilize even if their gametophytes can express both sexes (Ranker and Geiger, 2008). Cross-fertilizations are facilitated by antheridiogens, gibberellin-related pheromones that are released by archegoniate gametophytes and influence presexual gametophytes by hindering their growth and inducing male sex expression (Yamane, 1998; Tanurdzic and Banks, 2004).

Hybridization and polyploidy have played a pre-eminent role in fern species diversification (Soltis and Soltis, 1989). Allopolyploids, i.e., interspecific hybrids which have undergone a genome duplication, can normally produce balanced, viable gametes (Manton, 1950; Comai, 2000). As a result of nonsegregation of homeologous chromosomes during meiosis, they are often reported to have "fixed heterozygosity" (e.g., Glover and Abbott, 1995; Nelson and Elisens, 1999). Genetic diversity of allopolyploid species can increase when multiple origins from different diploid parents take place, with each subsequent origin potentially adding new alleles to the previous pool of the allopolyploid (Werth et al., 1985).

With roughly 225 species described throughout the world (Hoshizaki and Wilson, 1999), woodferns (genus *Dryopteris*) comprise one of the most diversified groups among homosporous ferns. Hybridization and allopolyploidy have been major factors in shaping evolutionary affinities within *Dryopteris*, with numerous diploid–polyploid complexes identified (e.g., Manton, 1950; Darnaedi et al., 1990; Werth, 1991). *Dryopteris corleyi* Fraser-Jenkins is an allotetraploid endemic to the northern Iberian Peninsula. Morphological and phytochemical characters (Fraser-Jenkins, 1982; Fraser-Jenkins and Widén, 1983) indicate that it derived from *D. aemula* (Aiton) Kuntze and

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D. oreades Fomin, two diploids with a highly fragmented distribution in the Iberian Peninsula. A partial phylogeny of the genus including *D. corleyi* and *D. aemula* supports the close relationship between these species (Geiger and Ranker, 2005). Using allozyme electrophoresis, we addressed the following questions: (1) Did *Dryopteris corleyi* derive from *D. aemula* and *D. oreades*? (2) How much genetic variation is distributed within and among the populations, and what are their principal determinants? (3) What are the mating systems of these species?

MATERIALS AND METHODS

Studied species—Three species were sampled for this study: the tetraploid *Dryopteris corleyi* ($2N = 4x = 164$; Fraser-Jenkins and Gibby, 1986) and the sexual diploids *D. aemula* ($2N = 2x = 82$; Manton, 1950) and *D. oreades* ($2N = 2x = 82$; Manton, 1950). *Dryopteris corleyi*, endemic to a narrow coastal strip of the northern Iberian Peninsula, inhabits heathland banks and anthropogenic habitats such as eucalyptus and pine plantations from 50 to 650 m a.s.l. (Salvo and Arrabal, 1986). *Dryopteris aemula* is present in western Europe, from Scotland to the Azores, Canary and Madeira archipelagos, and appears also in warm, oceanic enclaves in Turkey and Transcaucasia; in the Iberian Peninsula, it inhabits temperate deciduous forests within narrow, north-oriented valleys from sea level to 900 m a.s.l. (Salvo and Arrabal, 1986). These lowland forests have been heavily fragmented and transformed into cattle pastures in historical times (Izco, 1994). *Dryopteris oreades* is distributed in the mountains of western, southern, and central Europe, and appears also in Turkey and the Caucasus mountains; in the Iberian Peninsula, it occurs in open noncalcareous rocky areas and screes that are snow-covered in winter, from 600 to 2400 m a.s.l. (Salvo and Arrabal, 1986).

Plant material—Leaf fragments were sampled from 41 individuals from each of three wild populations of each species as listed in Table 1. For each individual, 1–2 pinnules were collected and stored in plastic bags for 4–6 d at 5°C until enzyme extraction. Pinnules were ground in polyvinylpyrrolidone-phosphate buffer (Soltis et al., 1983), and the resulting extracts were absorbed onto 4 cm × 6 cm rectangles of Whatman no. 3 chromatography paper and frozen at –80°C until electrophoresis.

Allozyme electrophoresis—Electrophoreses were conducted in 12.5% starch gels as per Soltis et al. (1983) and Haufler (1985). Ten enzymatic systems were studied, with nomenclature following Acquaah (1992): aspartate aminotransferase (AAT), hexokinase (HEX), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM) and shikimate dehydrogenase (SKDH). Genotypes were deduced from the electrophoretic phenotypes on the basis of the quaternary structure and subcellular locations reported for other plant species (Gottlieb, 1982; Weeden and Wendel, 1989). Presumed loci were numbered consecutively from anode to cathode; alleles were named alphabetically from anode to cathode.

TABLE 1. Populations sampled to study allozyme variation of the three *Dryopteris* species in the Iberian Peninsula.

Population	Locality	Latitude, Longitude	Altitude (m a.s.l.)
D. aemula			
A1	A Coruña, Fragas do Eume	43°14′24″N, 8°30′19″W	50
A2	Asturias, Santianes del Agua	43°25′42″N, 5°20′40″W	50
A3	Gipuzkoa, Mutriku	43°18′40″N, 2°24′51″W	90
D. oreades			
O1	Madrid, Puerto de Navafría	40°59′60″N, 3°49′36″W	2060
O2	León, Puerto de Ancares	42°50′59″N, 6°49′40″W	1790
O3	Burgos, Lagunas altas de Neila	42°30′40″N, 3°30′59″W	1930
D. corleyi			
AO1	Asturias, between Purón and La Borbolla	43°23′22″N, 4°41′90″W	50
AO2	Asturias, Pendueles	43°23′57″N, 4°38′85″W	75
AO3	Asturias, between Buelna and Santiuste	43°23′27″N, 4°35′49″W	65

Statistical analysis—The mean number of alleles per locus (A) and the percentage of polymorphic loci following the 0.99 criterion ($P_{0.99}$) were calculated for each population and averaged for each of the three species. For polymorphic loci, we determined the observed heterozygosity (H_o), the expected heterozygosity under Hardy–Weinberg equilibrium (H_e), and Wright's (1943) fixation index $F = 1 - H_o/H_e$. The statistical significance of F was tested by χ^2 tests. We used the statistics H_I, H_S, H_T, F_{IS}, F_{IT} and F_{ST} to portray genetic diversity and population structure. Values of F_{ST} were averaged over loci to calculate G_{ST} as per Hamrick and Godt (1989). Additionally, as suggested by Culley et al. (2002), G_{ST} was also calculated following Nei's (1973) method. Nei's (1978) genetic distance D was calculated for all population pairs within species.

RESULTS

Band interpretation—The 10 enzymatic systems provided a total of 14 interpretable loci: *Aat*, *Hex*, *Idh*, *Lap*, *Mdh-1*, *Mdh-2*, *Mdh-3*, *Me*, *6-Pgd-1*, *6-Pgd-2*, *Pgi*, *Pgm-1*, *Pgm-2*, and *Skdh* (Fig. 1). Some of these loci were not interpretable for some species or populations. Locus *Aat* could not be interpreted in populations O2 and O3 due to extremely faint bands in all individuals. *Mdh-2* could only be interpreted in *D. oreades* as presenting one expressed allele and one null allele; therefore, it did not permit a distinction between *aa* homozygotes and *an* (*n* = null allele) heterozygotes. Because no bands appeared in *Mdh-2* for *D. aemula* and *D. corleyi*, *Mdh-2* was considered to be uninterpretable for these species. *Me* could not be interpreted in *D. corleyi*; most individuals had unreliable, blurry bands. These problematic loci were included in calculations only if they provided valid data.

For loci *Hex*, *Idh*, *Lap*, *Mdh-1*, *6-Pgd-1*, *Pgm-1*, and *Sdh*, *D. corleyi* showed a fixed (i.e., nonsegregative among individuals) heterozygosity allozymic pattern, which could be readily interpretable as the sum of patterns of *D. aemula* and *D. oreades* (Fig. 1). For the remaining loci, *D. corleyi* was monomorphic for the same bands as *D. aemula*, and those bands were also present in some *D. oreades* individuals. All alleles present in the tetraploid were present in at least one of the two putative parents, or in other words, no orphan alleles were detected.

Genetic variation—In *D. aemula*, the 13 resolved loci were monomorphic, fixed for the same alleles in all individuals from all three populations (Fig. 1). Thus, the values of A and $P_{0.99}$ averaged over populations were 1.00 and 0.0, respectively (Table 2).

In *D. oreades*, seven of the 14 resolved loci showed polymorphism (Fig. 1). A and $P_{0.99}$ averaged over populations were moderate values (Table 2). The values of G_{ST} calculated as per Nei (1973) and as per Hamrick and Godt (1989) (Table 3) were, respectively, 0.052 and 0.057. The genetic distances between population pairs were very low: $D_{O1\text{-}O2} = 0.005$, $D_{O1\text{-}O3} = 0.016$, and $D_{O2\text{-}O3} = 0.011$.

In *D. corleyi*, only one of the 12 loci resolved, *6-Pgd-1*, was polymorphic (Fig. 1). Loci showing fixed heterozygosity were regarded as monomorphic. The value of A averaged over populations was similar to that of *D. oreades*, but the value of $P_{0.99}$ was quite low (Table 2). Some individuals were heterozygous

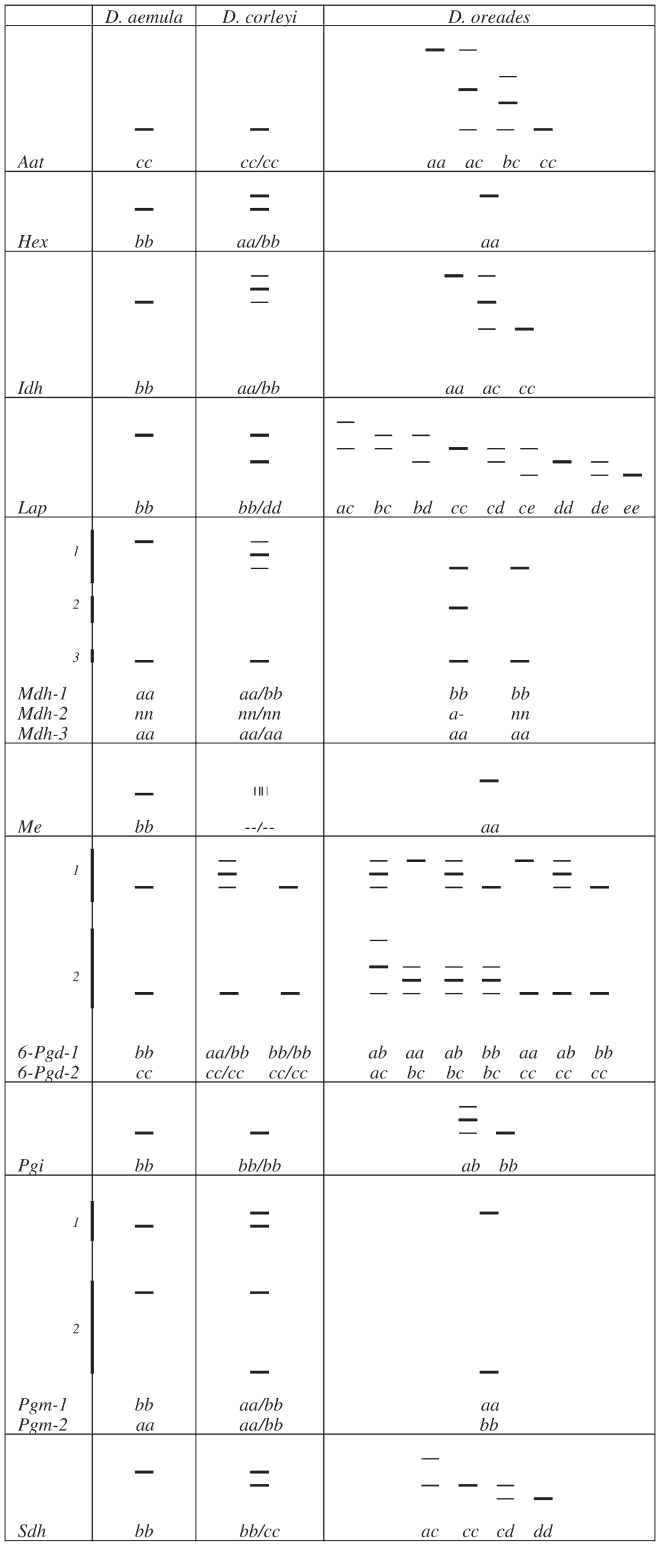

Fig. 1. Zymograms of the 10 enzymatic systems studied and genotype interpretation in the three *Dryopteris* species. Thin horizontal lines represent single allele dosage; thick horizontal lines represent double allele dosage; vertical lines represent unresolved loci; -, unresolved allele; ***n***, null allele. Anode toward top of the figure.

TABLE 2. Genetic variation statistics for the three *Dryopteris* species in the Iberian Peninsula. For each population, 41 individuals were sampled. *A*, mean number of alleles per locus; $P_{0.99}$, percentage of polymorphic loci following the 0.99 criterion; SD, standard deviation.

Species	Population	*A*	$P_{0.99}$
D. aemula	A1	1.00	0.0
	A2	1.00	0.0
	A3	1.00	0.0
Mean (SD)		1.00 (0.00)	0.0 (0.0)
D. oreades	O1	1.69	50.0
	O2	1.33	38.5
	O3	1.83	46.2
Mean (SD)		1.62 (0.26)	44.9 (5.9)
D. corleyi	AO1	1.58	0.0
	AO2	1.67	8.3
	AO3	1.67	8.3
Mean (SD)		1.64 (0.05)	5.6 (4.8)

for locus *6-Pgd-1*, with alleles present both in *D. aemula* and *D. oreades*, and the remaining individuals were homozygous for the allele fixed in *D. aemula* (Fig. 1). The observed frequencies of these heterozygotes in populations AO1, AO2 and AO3 were 0.00, 0.22 and 0.90, respectively. Given that *a–/b–* heterozygotes for *6-Pgd-1* might theoretically be *aa*/*ab*, *aa*/*bb* or *ab*/*bb*, and that these genotypes could not be reliably distinguished in gels, genotypic and allelic frequencies and statistics based on them could not be calculated.

Hardy–Weinberg equilibrium—In *D. oreades*, most polymorphic loci were in Hardy–Weinberg equilibrium in the three populations (Table 4). The only exceptions were *6-Pgd-2*, which had a significant excess of heterozygotes in population O1, and *6-Pgd-1* and *Idh*, which had a significant deficit of heterozygotes in populations O2 and O3, respectively. Hardy–Weinberg equilibrium could not be tested in *D. aemula* or *D. corleyi*, as a result of the absence of genetic variation in *D. aemula* and the impossibility of assigning genotypes for the *6-Pgd-1* locus of *D. corleyi*, as described earlier.

DISCUSSION

***Origin of* D. corleyi**—Our data strongly support the hypothesis that *D. aemula* and *D. oreades* gave rise to *D. corleyi* via allopolyploidization. First, the allozyme banding pattern of *D. corleyi* showed fixed heterozygosity. Second, this pattern was readily interpretable as the additive pattern of *D. aemula* and *D. oreades.* And third, all alleles present in *D. corleyi* were present in at least one of the two putative parents. This absence of orphan alleles in *D. corleyi* can be due to the lack of evolutionary time for genetic divergence between the allotetraploid and its diploid parents to occur, thus also hinting at a recent origin of this species. This conclusion agrees with several traits of *D. corleyi*, including narrow distribution range, occupation of recent habitats (Mayor and Fernández, 1988), low genetic divergence from *D. aemula* at the chloroplast DNA level (Geiger and Ranker, 2005), and highly variable percentage of spore abortion (Quintanilla and Escudero, 2006). We must remark, however, that the sampling range of our study, restricted to Iberian populations only, may be insufficient to provide evidence of local formation of *D. corleyi* in its current distribution area. If this species had a recent origin, a wider sampling including several non-Iberian populations of *D. aemula* and *D. oreades* might

TABLE 3. *H* and *F* statistics for three *Dryopteris oreades* populations from the Iberian Peninsula. H_I, H_S, and F_{IS} are averaged over populations. The mean value of F_{ST} equals G_{ST} calculated as per Hamrick and Godt (1989). Loci *Hex*, *Mdh-1*, *Mdh-3, Me, Pgm-1* and *Pgm-2* were monomorphic in all populations and thus $H_I = H_S = H_T = 0$. Loci *Aat* and *Mdh-2* were excluded from calculations (see Results).

Locus	H_I	H_S	H_T	F_{IS}	F_{ST}	F_{IT}
Idh	0.130	0.185	0.226	0.298	0.181	0.425
Lap	0.423	0.469	0.484	0.099	0.030	0.126
6-Pgd-1	0.390	0.438	0.439	0.108	0.003	0.111
6-Pgd-2	0.260	0.211	0.227	–0.234	0.072	–0.144
Pgi	0.016	0.016	0.016	–0.025	0.016	–0.008
Skdh	0.049	0.061	0.063	0.201	0.037	0.231
Mean	0.106	0.115	0.122	0.075	0.057	0.126

reveal additional alleles that could confirm an Iberian origin of *D. corleyi*.

We found two allozymic phenotypes in locus *6-Pgd-1* of *D. corleyi*: *bb*/*bb* and *a*–/*b*– (Fig. 1). Given that in all *D. aemula* individuals sampled, the allele *b* was fixed, the most likely interpretation is that *a*–/*b*– individuals of *D. corleyi* actually represent genotypes *aa*/*bb* and *ab*/*bb*. Under this assumption, *D. oreades* would have contributed alleles *a* and *b* in at least two separate origins of *D. corleyi.* However, the supposition of multiple origins for *D. corleyi* based only on the polymorphism in the *6-Pgd-1* locus seems unwarranted because other explanations, such as postpolyploidization mutations resulting in different allozymic phenotypes or repeated origin in a hybrid swarm from the same diploid parents, are possible (Vogel et al., 1999). Additionally, two routes of polyploid formation involving unreduced gametes, rather than diploid hybridization and subsequent polyploidization, could explain our results under the supposition of a single hybridization event (e.g., Gastony, 1986; Ramsey and Schemske, 1998). The first route would consist of the direct union of two abnormally produced diploid gametes, one *bb* (from *D. aemula*) and one *ab* (from *D. oreades*). The second one would involve a triploid bridge, where an unreduced *ab* gamete from a heterozygous diploid would combine with a normal *b* gamete; the resulting *ab/b* triploid would be mostly sterile but could eventually produce a functional unreduced triploid spore, whose gametes could combine with a new *b* gamete. In both cases, the resulting initial *ab/bb* sporophyte would produce *a/b* and *b/b* gametophytes and gametes, which could then generate *aa/bb*, *ab/bb*, and/or *bb/bb* sporophytes.

***Genetic diversity*—**We found very distinct genetic diversities in the diploid species studied, with extremely low levels in *D. aemula* and moderate levels in *D. oreades.* The disparate evolutionary histories of these species can explain these results. Concretely, their different environmental requirements could have determined their response to glacial–interglacial cycles. During the last glacial stage (the Würm glaciation), lowland species, such as *D. aemula*, would have withdrawn to warmer southern refugia in the Iberian Peninsula or Macaronesia, whereas the populations of alpine species, such as *D. oreades*, would have expanded across the Iberian Peninsula. At the end of the glacial stage, the generalized rise of temperatures in the Iberian Peninsula would have facilitated the spread of lowland taxa from warm refugia and the retreat of alpine taxa to cooler altitudes and latitudes, as explained by the contraction–expansion model suggested by Hewitt (1996).

The outcome of these events for lowland taxa would be a general genetic impoverishment due to population fragmentation and extinction during the ice spread, genetic drift in isolated refugial populations, and founder effects during the subsequent Holocene expansion (Hewitt, 1996). Our results for *D. aemula* agree with those found in other Macaronesian relict ferns such as *Trichomanes speciosum*, *Culcita macrocarpa*, and *Woodwardia radicans* (Rumsey et al., 1999; Quintanilla et al., 2007). These species have similar ecological requirements and are often found sharing habitats with *D. aemula* in the northern Iberian Peninsula, and also have a very low genetic diversity at the allozyme level in fragmented populations close to their distribution limits. Some northern Iberian coastal spots could have acted as warm refugia for temperate Atlantic taxa during glacial maxima, as suggested by the presence of pollen of trees such as *Quercus*, *Alnus*, and *Corylus* usually found sharing habitats with *D. aemula*, *C. macrocarpa*, and *W. radicans* in Würmian sediments in the northern Iberian coast (Mary et al., 1975, 1977; Ramil-Rego et al., 1998). Preliminary results obtained with microsatellite markers confirm that genetic diversity in Iberian populations of *D. aemula* is very low (A. Jiménez, L. G. Quintanilla, and D. Csencsics and R. Holderegger [WSL Swiss Federal Research Institute], unpublished manuscript).

On the other hand, alpine species would have sustained high levels of genetic diversity during the last glaciation due to population expansion, arrival of new alleles from northern latitudes and abundant gene flow among populations. After the retreat of ice, remaining, shrunken populations would then begin to experience a loss of genetic diversity and an increase of genetic divergence among them because of fragmentation-driven genetic drift (Hewitt, 1996; Knowles and Richards, 2005). The presently high fragmentation in the southern distribution limit of *D. oreades* in the Iberian Peninsula could be expected to determine low within-population genetic diversity and high genetic distances among populations, as observed in other plants with fragmented distributions (e.g., Landergott et al., 2001; Lönn and Prentice, 2002; Jump and Peñuelas, 2006). However, according to our results, this is not the case. Genetic diversity values averaged for the studied populations ($A = 1.62$, $P_{0.99} = 44.9$; Table 2) do not depart greatly from those reported for other fern species ($A = 1.6$, $P_{0.99} = 38.4$, Ranker and Geiger, 2008) and are only slightly lower than those of seed plants ($A = 1.95$, $P_{0.99} = 50.5$, Hamrick and Godt, 1989). G_{ST} and D values were low, suggesting abundant interpopulation gene flow and low genetic differentiation among populations, in accordance with the expectations for long-lived plant species with potential for long-range gene movement (Hamrick and Godt, 1989). It appears, therefore, that long-distance spore dispersal can be instrumental in delaying the drift-mediated erosion of genetic diversity in *D. oreades*. Wind-driven homosporous fern spores can travel distances of up to thousands of kilometers (Muñoz et al., 2004) and have been previously reported to play an important role in shaping the genetic diversity of several species (e.g.,

TABLE 4. Observed (H_o) and expected (H_e) heterozygosities and fixation indexes (F) for three *Dryopteris oreades* populations from the Iberian Peninsula. *, significant deviation from Hardy–Weinberg equilibrium; n.r., not resolved; —, monomorphic population.

Locus	Population O1			Population O2			Population O3		
	H_o	H_e	F	H_e	H_o	F	H_e	H_o	F
Aat	0.390	0.405	0.035	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
Idh	0.000	0.000	—	0.122	0.115	–0.065	0.268	0.442	0.393*
Lap	0.415	0.487	0.148	0.366	0.356	–0.028	0.488	0.565	0.137
6-Pgd-1	0.439	0.414	–0.060	0.317	0.457	0.306*	0.415	0.442	0.061
6-Pgd-2	0.512	0.381	–0.344*	0.112	0.115	–0.065	0.146	0.137	–0.067
Pgi	0.049	0.048	–0.025	0.000	0.000	—	0.000	0.000	—
Sdh	0.024	0.024	–0.012	0.000	0.000	—	0.122	0.159	0.234

Wolf et al., 1991; Sciarretta et al., 2005; Schneller and Liebst, 2007). Even though the great majority of released spores fall near the source plant (Raynor et al., 1976), very low levels of gene flow via long-distance dispersal can offset the loss of variation due to genetic drift and reduce genetic distances among populations (Ellstrand and Elam, 1993). Consequently, occasional arrival of wind-dispersed spores from other populations can maintain the levels of genetic variation observed in *D. oreades*. This result is in accordance with those for other alpine ferns that may have undergone similar evolutionary events, such as *Cryptogramma crispa* in the Iberian Peninsula (Pajarón et al., 1999) and *Athyrium distentifolium* in North America (Woodhead et al., 2005).

The allotetraploid *D. corleyi* presented little genetic variation, with only one polymorphic locus, *6-Pgd-1*. Although the average number of alleles per locus is similar to the average value reported for seed plants and other ferns (Hamrick and Godt, 1989; Ranker and Geiger, 2008), this comparison is debatable as *A* is intrinsically inflated for allopolyploids due to fixed heterozygosity. Given the efficient wind spore dispersal in ferns and the short distances separating the three *D. corleyi* populations studied (AO1-AO2: 5 km; AO1-AO3: 7 km; AO2-AO3: 4 km), one might expect comparable proportions of *a–/bb* and *bb/bb* genotypes at the *6-Pgd-1* locus in the three populations. However, the *a–/bb* genotype is absent in population AO1 and represents only 22% of genotypes in population AO2, whereas in AO3 it is present in 90% of the sampled individuals. This uneven distribution may obey a recent appearance of the *a–/bb* genotype or to populations having been founded by individuals with different genotypes.

Breeding systems—For *D. oreades*, the fact that most polymorphic loci were in Hardy–Weinberg equilibrium in all three populations, with *F* values close to zero (Table 4), strongly supports the hypothesis that this woodfern is mostly an outbreeder, as also suggested by the presence of an antheridiogen system operating in this species (Jiménez et al., 2008). This result is in accordance with the observations for most diploid fern species (Masuyama and Watano, 1990). The lack of genetic variation in *D. aemula* and the impossibility to score *6-Pgd-1* genotypes in *D. corleyi* prevented deducing the breeding system of these species. The presence of an antheridiogen system in these species should favor outcrossing in the studied populations (Jiménez et al., 2008).

Conclusions—*Dryopteris corleyi* originated by allopolyploidization from the diploids *D. aemula* and *D. oreades.* The absence of genetic divergence from its parental species indicates a recent origin of the polyploid, although a local Iberian origin cannot be confirmed. The lack of genetic variation in *D. aemula* seems to correspond to the influence of Pleistocene glaciations. On the other hand, *D. oreades* harbors a moderate genetic diversity within its populations, probably as a consequence of windborne spore dispersal delaying the genetic drift that should accompany the natural fragmentation of its habitat. The genotypic frequencies of *D. oreades* are in Hardy–Weinberg equilibrium, thus confirming that this species frequently outcrosses in nature. As shown by this work, the occurrence of population contraction–expansion cycles can explain the amount and distribution of genetic diversity in current fern populations. Further studies on seed plants comparing lowland and alpine species with fragmented distributions may also arrive at the conclusion that particular evolutionary histories can be,

rather than the fragmentation-mediated processes, the main factor shaping intra- and interpopulational genetic diversity.

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