Historical biogeography and population genetics of the rare fern,

Asplenium adiantum-nigrum L.

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Abstract

Asplenium adiantum-nigrum is one of the rarest ferns in North America, occurring only in Boulder County, CO, Eldon Mountain, AZ, Zion National Park, UT, and the states of Chihuahua and Tamaulipas, Mexico. The species also occurs in the Hawaiian Archipelago, Eurasia, and Africa where it is more common. The present study was undertaken to determine 1) the mode of origin of the North American populations (i.e., remnants of a once more widely dispersed population or established via long-distance dispersal) and, 2) the phylogenetic distinctness of the Boulder population which has occasionally been treated as a separate species (_A_. _andrewsii_). Our results are more consistent with the hypothesis that North American populations arose from at least two independent long-distance dispersal events with subsequent intracontinental dispersal establishing new populations. We suggest that the Boulder population is not sufficiently genetically distinct from other populations to merit separate specific status.

Asplenium adiantum-nigrum ("Black Spleenwort") is one of the rarest ferns in North America where populations are known only from Boulder County, Colorado, Zion National Park, Utah, Eldon Mountain (near Flagstaff), Arizona, and several locations in the Mexican states of Chihuahua and Tamaulipas. Outside of North America A. *adiantum-nigrum* is known from the Hawaiian Islands, Europe, Africa, and Asia. Manton (1950) demonstrated that A. *adiantum-nigrum* is a tetraploid with n = 72 pairs of chromosomes at meiosis. Shivas (1969) provided cytological evidence from experimental hybridizations suggesting that this tetraploid is of hybrid origin, resulting from the hybridization of two European diploids, A. *onopteris* L. and A. *cuneifolium* Viv. The original diploid hybrid is presumed to have been sterile but through chromosomal duplication (i.e., such as by nondisjunction at meiosis) could have ultimately produced a fertile allotetraploid (see Manton, 1950, for other examples). Nothing is known of the historical biogeography of this species and, in particular, of the origin of the North American populations. These populations could have arisen via long-distance spore dispersal from Old World or Pacific sources or may be relictual populations from a once more widespread species.

The present study of A. *adiantum-nigrum* was undertaken to assess 1) two competing hypotheses on the mode of origin of the North American populations (i.e., remnants of a once more widely dispersed population or established via long-distance dispersal) and 2) the phylogenetic distinctness of the Boulder population which has occasionally been treated as a separate species (A. *andrewsii* Nelson; Nelson, 1904).

Materials and Methods

Leaves were collected from 21 individuals from each of the populations at Zion National Park, Utah (vicinity of Canyon Overlook Trail and Emerald Pools) and Eldon Mountain, Arizona (bordering the city of Flagstaff). Twenty individuals were sampled from the population in Boulder County, Colorado (White Rocks area). Leaves were kept on ice in plastic bags until processing for electrophoresis. Voucher specimens were not collected because they already exist

for this rare species and are housed in the herbaria of the University of Colorado (COLO), University of Wyoming (RM), U. S. National Herbarium (US), Colorado State University (CS), University of Arizona (ARIZ), Northern Arizona University (ASC), New York Botanical Garden (NY), and University of Utah (UT). Fragments of fertile leaves obtained from a population in the State of Chihuahua, Mexico (Mpio. Bocoyna, SE of Creel; Bye #18331) were kindly provided by Dr. Robert A. Bye, Jr. Spores were sown in petri dishes on an agar growth medium following Windham, Wolf, and Ranker (1986) to obtain gametophytes for isozymic analyses. Viable spores were also obtained from herbarium specimens collected in Mexico in 1977 (Bye 7384 from Mpio. Guachochic; COLO accession no. 352634)and in the Caucasus Mountains in 1981 (Vasák s.n.; COLO accession no. 441033) and sown on agar growth medium. Although the small samples available from Mexico and the Caucasus do not allow estimation of intrapopulational variability, they are sufficient to provide adequate preliminary estimates of genetic similarity among populations (see Nei, 1978).

Samples were also collected from nine localities on the island of Hawaii, State of Hawaii. Detailed results from isozymic analyses of these populations are part of a separate study and will be presented elsewhere (Ranker, Floyd, and Trapp, unpublished data) and only general comparisons will be made in the present context with Hawaiian populations.

Horizontal starch-gel electrophoresis was conducted extracts of collected leaf material and cultured gametophytes following Ranker, Haufler, Soltis, and Soltis (1989). Results were obtained from 11 enzyme systems including aldolase (ALD), fructose-bisphosphatase (FBP), glutamate oxaloacetate transaminase (GOT), hexokinase (HK), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (6-PGDH), phosphoglucose isomerase (PGI), shikimate dehydrogenase (SkDH), and triosephosphate isomerase (TPI).

Nei's unbiased genetic identity coefficient (I; Nei, 1978) and Rogers' genetic similarity coefficient (S; Rogers, 1972) were calculated for all pairs of populations.

Results

Among the 11 enzyme systems surveyed, 17 putative loci were scored. Populational allele frequencies are presented in Table 1. The samples from Zion and Eldon exhibited no within- or between-population variability and were fixed for a single allele at each of eight loci (Fbp-1, Hk, Idh, Mdh-2, 6-Pgdh-1, 6-Pgdh-2, Pgi-1, and Skdh). At the remaining nine loci, all samples from Zion and Eldon expressed multiple-banded patterns that were interpreted as fixed heterozygosity (i.e., two alleles per locus per individual). Presumably these latter loci represent cases where the parental diploid taxa of A. adiantum-nigrum possessed different alleles that were combined in the allotetraploids. Values of Nei's I and Rogers' S were both 1.000 between the Zion and Eldon populations (Table 2).

The Boulder sample was nearly identical to those from Zion and Eldon, expressing a difference at only a single locus. Whereas all samples from Zion and Eldon and 18 of the individuals from Boulder expressed a heterozygotic genotype for Lap (combining alleles 1 and 4), two individuals from the latter population were homozygous for allele 1 at this locus. No alleles were unique to mainland U. S. populations. Rogers' S between Boulder and each of Zion and Eldon was 0.997 (Table 2) which seems to have been a more sensitive indicator of the difference at Lap than Nei's I (1.000 for both comparisons).

The samples from Hawaii exhibited more allozymic variability than the mainland U. S. populations but generally showed high degrees of genetic similarity to them. Samples from Hawaii were invariant at eight loci (Table 1), four of which showed fixed homozygosity and four of which showed fixed heterozygosity. The remaining loci exhibited varying degrees of variability within and among populations. Two of the heterozygous loci expressed three alleles and one expressed four alleles across individuals and populations, whereas only two alleles per locus were expressed at heterozygous loci in the mainland U. S. populations. Eight alleles unique to Hawaiian populations (so-called "private alleles") were observed at six loci (Table 1). Three of those private alleles (at Hk, Skdh, and Tpi-2) were found at only one locality of the

nine populations sampled on the island of Hawaii and were always at frequencies of less than 0.05. The remaining five private alleles were discovered at two or more local populations and ranged in frequency from 0.028 to 0.409. The two private alleles observed at _Idh_ (alleles 1 and 3) were found in all Hawaiian populations sampled. The mean values of I and S (Table 2) between Hawaii and the mainland U. S. were 0.988 and 0.956, respectively. At two loci (_Hk_ and _Pqi-2_) alleles were shared uniquely between Hawaii and mainland U. S. populations. Allele 3 at _Hk_ was fixed in all mainland U. S. samples and was nearly fixed in all Hawaii populations. At _Pqi-2_ all samples from the mainland U. S. and most of those from Hawaii were fixed heterozygotes for alleles 2 and 4 which were uniquely shared between these two regions. A subset of individuals from three Hawaiian populations were homozygotes for allele 2.

The samples from Mexico and the Caucasus showed the greatest amount of genetic divergence compared to each other and relative to all other populations sampled. Five private alleles were found in the Mexican samples at four loci and six such alleles were discovered in the Caucasus sample at three loci (Table 1). Mean values of I and S between the Mexican samples and mainland U. S. were 0.684 and 0.679, respectively (Table 2), and those between the Caucasus and mainland U. S. were 0.651 and 0.678. The mean values of I and S between Hawaii and Mexico were 0.651 and 0.641, respectively, and those between Hawaii and the Caucasus were 0.624 and 0.650, respectively. The values of I and S between Mexico and the Caucasus were 0.626 and 0.609, respectively.

Discussion

The complete (or nearly complete) genetic identity among the three mainland U. S. populations of _A._ _adiantum-nigrum_ (Boulder, Eldon, and Zion) suggests that they share a common and recent evolutionary history. All of the individuals sampled from Zion and Eldon and most of those from Boulder may be descendants of a single hybrid individual that combined the particular parental genotypes expressed in those populations. The production of such an individual in one locality with subsequent dispersal to the present population sites would account for the observed distribution of this genotype in North America. Alternatively,

the existing populations could be remnants of a once more widespread, southern Rocky Mountain population most of which has gone extinct. Even under the latter scenario, however, one must invoke dispersal from a single original point of hybridization with subsequent reduction in population size. The variant _Lap_ genotype observed in two of the samples from the Boulder population may have arisen via a second hybridization event or could have been produced by the genetic silencing of allele 4 at this locus in an individual plant. The data are insufficient to distinguish these two alternatives.

The fact that the most common genotypes in individuals from Hawaii were shared with those sampled from the mainland U. S. suggests a common evolutionary origin for the populations in these two regions. This hypothesis is strongly supported by the presence of high frequency alleles that are uniquely shared between these two regions (i.e., at _Hk_ and _Pgi-2_). Since the Hawaiian Islands arose through suboceanic volcanism in the mid-Pacific, we know that _A_. adiantum-nigrum is capable of successful long-distance migration and colonization of previously uninhabited sites. Whether the mainland U. S. populations served as a source of migrants for the colonization of Hawaii (or vice-versa) or both regions received migrants from a third common source can not be completely resolved from the data. Given that mainland U. S. populations contain only a subset of the genetic variability present in Hawaiian populations, however, it is more likely that migrants from Hawaii were the source of colonists for the mainland rather than the other way around.

The extreme genetic difference among populations from the mainland U. S. and Hawaii versus those from Mexico and Asia suggests that each population (or set of populations) originated independently from the others. The presence of unique genotypes at single loci and across loci in each region (combining Hawaii and mainland U. S.) provides strong evidence that each set of populations arose from separate hybridization events between individuals of the parental species of different genetic composition. The present European distribution of the presumed parental species (_A_. onopteris and _A_. cuneifolium) suggests that hybrid individuals originally arose in Europe with subsequent dispersal events to Asia,

Hawaii, and North America. Alternatively, the parental diploids could have occurred in the continental regions where the tetraploids presently occur, with hybrids being produced _in situ_ and subsequent extinction of the diploids from all regions but Europe. The nearly complete lack of genetic variability in U. S. mainland populations combined with the presence of variability among populations from different geographic regions (thus, indicating the polymorphic nature of the ancestral diploids) argues against the alternative hypothesis of multiple _in situ_ hybridizations at the present North American localities. Although the alternative hypothesis can not be completely excluded, under that hypothesis one might expect more genetic variability within populations of A. adiantum-nigrum than has been observed in the present study.

The recognition of the Boulder population as a species distinct from other populations of A. adiantum-nigrum (as A. andrewsii; Nelson, 1904) appears to be unwarranted in light of the present genetic data. It is worth noting, however, that the only genetic variability discovered among mainland U. S. populations was found in the Boulder population. The unique genetic attributes of this population should be noted by those concerned with the conservation management of this rare species.

Acknowledgments

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Table 1. Allele frequencies in populations surveyed. Those for Hawaii represent means of nine populations. N = sample size per population per locus. Alleles unique to a region are marked with asterisks.

Population

Locus	Boulder	Zion	Eldon	Mexico	Caucasus	Hawaii
Ald
(N)	20	21	21	2	1	236
1	0.500	0.500	0.500	0.500	0.500	0.498
2	0.500	0.500	0.500	0.500	0.500	0.502
Fbp-1
(N)	20	21	21	2	1	232
1	0.000	0.000	0.000	0.000	0.000	0.043*
2	1.000	1.000	1.000	1.000	1.000	0.957
Got-1
(N)	20	21	21	2	1	236
1	0.000	0.000	0.000	0.000	0.500*	0.000
2	0.500	0.500	0.500	0.500	0.000	0.648
3	0.000	0.000	0.000	0.000	0.500*	0.000
4	0.500	0.500	0.500	0.500	0.000	0.352
Hk
(N)	20	21	21	2	1	236
1	0.000	0.000	0.000	0.500*	0.000	0.000
2	0.000	0.000	0.000	0.500	1.000	0.000
3	1.000	1.000	1.000	0.000	0.000	0.998
4	0.000	0.000	0.000	0.000	0.000	0.002*

Table 1. continued

			Population			
Locus	Boulder	Zion	Eldon	Mexico	Caucasus	Hawaii
Idh						
(N)	20	21	21	2	1	219
1	0.000	0.000	0.000	0.000	0.000	0.247*
2	1.000	1.000	1.000	1.000	1.000	0.507
3	0.000	0.000	0.000	0.000	0.000	0.247*
Lap						
(N)	20	21	21	2	1	236
1	0.550	0.500	0.500	0.000	0.500	0.500
2	0.000	0.000	0.000	0.500	0.500	0.000
3	0.000	0.000	0.000	0.000	0.000	0.047*
4	0.450	0.500	0.500	0.500	0.000	0.453
Mdh-1						
(N)	20	21	21	2	1	236
1	0.500	0.500	0.500	0.000	0.500	0.500
2	0.000	0.000	0.000	1.000*	0.000	0.000
3	0.500	0.500	0.500	0.000	0.500	0.500
Mdh-2						
(N)	20	21	21	2	1	236
1	1.000	1.000	1.000	0.000	1.000	1.000
2	0.000	0.000	0.000	1.000*	0.000	0.000
Mdh-3						
(N)	20	21	21	2	1	236
1	0.500	0.500	0.500	0.500	0.500	0.500
2	0.500	0.500	0.500	0.500	0.500	0.500

Table 1. continued

Population

Locus	Boulder	Zion	Eldon	Mexico	Caucasus	Hawaii
6-Pgdh-1						
(N)	20	21	21	2	1	236
1	1.000	1.000	1.000	1.000	1.000	1.000
6-Pgdh-2						
(N)	20	21	21	2	1	236
1	1.000	1.000	1.000	1.000	1.000	1.000
6-Pgdh-3						
(N)	20	21	21	2	1	236
1	0.500	0.500	0.500	0.500	0.500	0.500
2	0.500	0.500	0.500	0.500	0.500	0.500
Pgi-1						
(N)	20	21	21	2	1	236
1	0.000	0.000	0.000	1.000*	0.000	0.000
2	0.000	0.000	0.000	0.000	1.000*	0.000
3	1.000	1.000	1.000	0.000	0.000	1.000
Pgi-2						
(N)	20	21	21	2	1	236
1	0.000	0.000	0.000	0.500	0.500	0.000
2	0.500	0.500	0.500	0.000	0.000	0.521
3	0.000	0.000	0.000	0.500	0.500	0.000
4	0.500	0.500	0.500	0.000	0.000	0.479
Skdh						
(N)	20	21	21	2	1	236
1	1.000	1.000	1.000	0.500	1.000	0.987
2	0.000	0.000	0.000	0.000	0.000	0.013*
3	0.000	0.000	0.000	0.500*	0.000	0.000

Table 1. continued

Population

Locus	Boulder	Zion	Eldon	Mexico	Caucasus	Hawaii
Tpi-1						
(N)	20	21	21	2	1	236
1	0.000	0.000	0.000	0.000	0.500*	0.000
2	0.500	0.500	0.500	0.500	0.000	0.500
3	0.000	0.000	0.000	0.000	0.500*	0.000
4	0.500	0.500	0.500	0.500	0.000	0.500
Tpi-2						
(N)	20	21	21	2	1	236
1	0.000	0.000	0.000	0.000	0.000	0.002*
2	0.500	0.500	0.500	0.500	0.000	0.498
3	0.000	0.000	0.000	0.000	1.000*	0.000
4	0.000	0.000	0.000	0.000	0.000	0.047*
5	0.500	0.500	0.500	0.500	0.000	0.453

Table 2. Matrix of Nei's genetic identity (above diagonal) and Roger's genetic similarity (below diagonal) coefficients between pairs of populations.

Population	Boulder	Zion	Eldon	Mexico	Caucasus	Hawaii
Boulder	•••••	1.000	1.000	0.683	0.652	0.988
Zion	0.997	•••••	1.000	0.685	0.650	0.988
Eldon	0.997	1.000	•••••	0.685	0.650	0.988
Mexico	0.678	0.680	0.680	•••••	0.626	0.651
Caucasus	0.679	0.677	0.677	0.609	•••••	0.624
Hawaii	0.956	0.956	0.956	0.641	0.650	•••••