

## BIOGEOGRAPHIC RELATIONSHIPS BETWEEN THE MEDITERRANEAN AND NORTH AMERICAN FLORAS: INSIGHTS FROM MOLECULAR DATA

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### Abstract

Molecular phylogenetic analysis has proven to be a powerful tool for inferring the evolutionary history of organisms. These methods can also be used to examine the origins and relationships of plant taxa that are disjunctly distributed between the Mediterranean region and North America. Examples are given from studies of *Pinus* (*Pinaceae*), *Datisca* (*Datisceae*), *Astragalus* (*Fabaceae*), and *Senecio* (*Asteraceae*). In *Pinus* subgenus *Pinus* and *Datisca*, Old World / New World disjunctions can be explained by vicariance dating back to the Late Cretaceous and Eocene, respectively. The intercontinental disjunct species pair *Senecio flavus* subsp. *breviflorus* and *S. mohavensis* probably originated through Holocene long-distance dispersal. The previously unsuspected relationship between the Mediterranean species *Astragalus echinatus* and the New World aneuploid *Astragalus* clade is supported by two independent data sets; however, its biogeographic origin remains unresolved. These examples illustrate the range of biogeographic questions that molecular phylogenetic methods can address.

### Introduction

In plant systematics, two significant advances have occurred over the past two decades: the development and acceptance of explicit phylogenetic approaches, and the use of nucleic acids as a source of comparative data. The phylogenetic analysis of molecular data has provided significant new insights into a broad range of biological questions (SOLTIS & al., 1992; AVISE, 1994). The application of molecular phylogenetic analysis can also provide important data for biogeographic investigations by 1) allowing the explicit evaluation of hypotheses of common ancestry, and 2) permitting an estimate of the relative age of a disjunction.

Plant geographers have long been interested in related plants with intercontinental disjunct geographic ranges (THORNE, 1972). Morphological similarity between plant species has been traditionally used to identify these geographic disjunctions. For example, similarities in vegetative and ovulate cone characteristics of *Pinus pinaster* Aiton have been used to associate this species with the New World diploxylon pines of subsection *Australes* in the classifications of VAN DER BURGH (1973), and KLAUS (1989). In contrast, this morphological similarity can be interpreted as resulting from convergent evolution, and thus *P. pinaster* has also been classified in a separate subsection from the New World diploxylon pines (LITTLE & CRITCHFIELD, 1969). In the absence of an explicit phylogenetic analysis, it is difficult to distinguish between the conflicting hypotheses of relationships represented by these classifications of *Pinus*. This distinction between common ancestry and convergent evolution is critical to plant

biogeography. Following the classifications of Van der Burgh and Klaus, one might ask "what is the biogeographic explanation for the disjunction between *P. pinaster* and its western hemisphere relatives?" However, following Little & Critchfield, there is nothing to explain, for *P. pinaster* and the New World diploxylon pines are considered unrelated. For this reason, it is essential that biogeographic investigations are based on robust phylogenetic frameworks.

ZUCKERKANDL & PAULING (1965) were the first to hypothesize that molecular divergence values could be used to estimate the time since two lineages last shared a common ancestor. Their "molecular clock" hypothesis predicted a linear correlation between the accumulation of neutral mutations and time elapsed since gene divergence. Three decades later, the reliability (and even the existence) of molecular clocks is still under debate (HILLIS & al., 1996). It is clear that precise molecular metronomes generally do not exist, and that the variance of time estimates is often too large to be meaningful. Furthermore, rates of molecular evolution can vary both within and among lineages (BOUSQUET & al., 1992), and among different molecules. Despite these weaknesses, a broad correlation between amount of divergence and geological time does exist (HILLIS & al., 1996). Thus molecular data can discriminate between recent and ancient divergence. This discrimination can provide evidence for dispersalist (recent) vs. vicariant (ancient) hypotheses of plant disjunctions.

Many plant disjunctions have been attributed to both dispersalist and vicariance explanations, depending on the weight given to different sources of evidence. For example, the disjunct presence of *Psilotum nudum* (L.) Pal. (*Psilotaceae*) in southwestern Spain is generally considered a Tertiary relict. On the other hand, *Psilotum* is found on Oceanic islands, indicating that its spores can be dispersed to great distances. This suggests that the Spanish population could be the result of recent long-distance dispersal. While a molecular divergence value might not allow a precise dating of such a disjunction, it can easily differentiate between these two equally plausible hypotheses. Using such an approach, RANKER & al. (1994) have shown that disjunct populations of *Asplenium adiantum-nigrum* L. in Colorado, USA, probably originated by long-distance dispersal.

### Applications of molecular phylogenetic analysis to plant biogeography

*Pinus* (*Pinaceae*). *Pinus* subgenus *Pinus* (hard or diploxylon pines) includes those species with two vascular bundles per needle and (usually) persistent fascicle sheaths. The subgenus is widely distributed in Eurasia and North and Central America. Molecular phylogenetic data strongly support the monophyly of the six subsections of New World diploxylon pines (KRUPKIN & al., 1996; PRICE & al., in press). Two biogeographic hypotheses can be made for the sister-group of this western hemisphere clade: The first is a relationship to subsect. *Sylvestres*, through *P. resinosa* Aiton and *P. tropicalis* Morelet, the only New World taxa of this primarily Eurasian subsection. This view is reflected in the classification of LITTLE & CRITCHFIELD (1969), who placed subsect. *Sylvestres* and the New World diploxylon pines in sect. *Pinus*. A second view is presented by VAN DER BURGH (1973) and KLAUS (1989) who argue for relationships between a group of Mediterranean pines and North and Central American species.

Molecular phylogenetic analysis of the nuclear ribosomal DNA internal transcribed spacer region (ITS) lends support to some of the relationships suggested by Van der Burgh and Klaus (LISTON & al., 1996). In the ITS phylogeny, the Himalayan *P. roxburghii* Sargent (subsect. *Canarienses*), shares a direct common ancestor with the New World diploxylon pines. *Pinus roxburghii* is also the sister species to a paraphyletic grade of Mediterranean pines including *P. canariensis* C. Smith, *P. halepensis* Miller, *P. pinea* L. and *P. pinaster*. This topology is consistent with an early differentiation of subgenus *Pinus* along the Tethys seaway during the Late Cretaceous (KLAUS, 1989; MILLAR, 1993). On the other hand, the ITS phylogeny does not support direct relationships between extant Mediterranean and New World pines. Thus the morphological similarities between *P. pinaster* and the New World diploxylon pines of subsections *Australes* and *Ponderosae* are apparently due to convergent evolution. Likewise, the putative morphological similarities between the Mexican haploxylon pine, *P. rzedowskii* Madrigal & Caballero and the ancestors of subsect. *Canarienses* (KLAUS, 1989) must be due to convergent evolution.

*Datisca* (*Datisceae*). The genus *Datisca* consists of two species: *D. cannabina* L., distributed from the east Mediterranean to central Asia; and *D. glomerata* (Presl) Baill., native to California, USA and northern Baja California, Mexico. Both species are herbaceous perennials of riparian habitats in winter-rainfall regions (LISTON & al., 1989a). They share morphological and anatomical features, the same chromosome number, and identical flavonoid profiles (see LISTON & al., 1989a, 1992). The two species differ in their breeding system: populations of *D. cannabina* are dioecious, while *D. glomerata* populations are androdioecious, consisting of male and hermaphrodite individuals (LISTON & al., 1990). Molecular phylogenetic analysis of chloroplast DNA indicates that the two taxa are sister species (RIESEBERG & al., 1992; SWENSEN & al., 1994).

In contrast to their morphological similarity, the two species of *Datisca* exhibit high levels of genetic divergence as measured by isozymes. The mean genetic identity among populations of the two species for 21 isozyme loci is only  $I = 0.142$  (LISTON & al., 1989a). Divergence estimates from chloroplast DNA restriction sites ( $0.87\% \pm 0.17\%$ ) and *rbcL* nucleotide sequence ( $0.84\%$ ) are remarkably similar (LISTON & al., 1992; SWENSEN & al., 1994). Using a rate of  $2 \times 10^{-10}$  substitutions per site per year (ALBERT & al., 1994), divergence estimates of 36.5-50.5 million years and 42 million years respectively are obtained, placing the origin of this intercontinental disjunction in the Eocene. Genetic identities are higher between North American and east Mediterranean populations of *Datisca* than between North American and Central Asian populations (Liston & al., 1989a). Land connections between North America and Europe existed at least until the middle Eocene (MCKENNA, 1983). These facts suggest that the connection among the ancestral *Datisca* populations was probably across the North Atlantic rather than Beringia.

*Astragalus* (*Fabaceae*). With between 2500-3000 species, *Astragalus* is generally acknowledged to be the largest genus of vascular plants (SANDERSON & LISTON, 1995). The genus is widely distributed throughout the temperate regions of the Northern Hemisphere and in temperate South America. However the greatest species diversity is concentrated in two geographic areas: the Intermountain region of western North

America and the steppes, mountains, and semi-deserts of Central Asia. Significant numbers of species are also found in the Mediterranean region and in California.

Cytological and molecular data provide convincing evidence that the North and South American members of the genus *Astragalus* constitute a monophyletic clade. The species of this "New World clade" are characterized by gametic chromosome numbers varying from  $n = 11$  to  $n = 15$  (SPELLENBERG, 1976). In contrast, most (ca. 90%) Eurasian species have gametic chromosome numbers of  $n = 8, 16, 24$ , etc. (ASHRAF & GOHIL, 1988). Note that 13 species of the "Old World clade" are also native to North America (BARNEBY, 1964). These species primarily belong to circumboreal species complexes, and are primarily distributed at higher latitudes and/or alpine habitats in North America. Polyploidy is widespread in the Old World species, and very uncommon (three known cases) in the New World. A base number of 8 is apparently plesiomorphic in the temperate herbaceous clade of legumes, and thus the New World chromosome numbers are assumed to result from aneuploidy.

Phylogenetic analysis of chloroplast DNA (LISTON, 1992; LISTON & WHEELER, 1994) and the nuclear ribosomal DNA internal transcribed spacer region (WOJCIECHOWSKI & al., 1993) provide independent support for the monophyly of New World *Astragalus*. What is the biogeographic origin of this New World clade? Unfortunately two factors complicates the molecular phylogenetic analysis required to answer this question. The first is the inability to thoroughly sample the genus, due to its large size. It is likely that some critical groups have not yet been sampled. A second difficulty is the relatively low levels of molecular divergence that have been found among the New World species. Although this can be interpreted as evidence of a recent origin for the New World clade, it also results in relatively unresolved and poorly supported hypotheses of relationships among species of this group. Despite these limitations, an interesting finding is the association of at least one Old World aneuploid species (the west Mediterranean *A. echinatus* Murray) with the New World aneuploids (LISTON & WHEELER, 1994; SANDERSON & WOJCIECHOWSKI, 1995). This hypothesis is strengthened now that it has been observed with two independent data sets (cpDNA and ITS). Like the members of the New World clade, *Astragalus echinatus* has an aneuploid chromosome number. However, none of its morphological features link it to the New World clade. For example, its fruits have unusual flattened processes that are unknown elsewhere in the genus. Due to the lack of resolution with the molecular data sets, it is unresolved whether *A. echinatus* is the sister taxon to the New World aneuploid clade or derived from within the clade. The former situation would be consistent with a Mediterranean origin for the New World *Astragalus*, while the latter would suggest long-distance dispersal from the western hemisphere to the Mediterranean. Additional molecular and taxonomic sampling will be necessary to discriminate between these scenarios.

*Senecio* (Asteraceae). *Senecio mohavensis* A. Gray is an annual species native to the Mohave and Sonoran deserts of southwestern North America. *Senecio mohavensis* is morphologically similar to *S. flavus* (Decne.) Schultz-Bip., an annual species distributed in the Saharo-Arabian and Namibian deserts of North Africa, southwest Asia, and southern Africa. In southwest Asia and southwestern North America the two species grow in similar habitats: desert washes and steep, often north-facing slopes. *Senecio*

*flavus* is also found in southern Spain. Isozyme analysis (LISTON & al., 1989b) revealed a high genetic identity ( $I = 0.952$ ) between the southwest Asian *S. flavus* subsp. *breviflorus* Kadereit and *S. mohavensis*. Furthermore, no cpDNA differences were observed between one population of *S. mohavensis* and two populations of *S. flavus* subsp. *breviflorus* (LISTON & KADEREIT, 1995). The lack of significant molecular divergence suggests that *S. mohavensis* originated via recent long-distance dispersal. This may be correlated with the Holocene origin of the current vegetation of the northern Sonoran desert (THORNE, 1986). The achenes of both species possess a pappus and are mucilaginous when wet; these features could aid in their dispersal. Although anthropogenic transport cannot be ruled out, the habitat and ecology of these taxa are not typical of colonizing species.

## Conclusions

The above examples illustrate a range of applications of molecular phylogenetic methods to questions of plant biogeography. As increasing numbers of taxa are subjected to molecular phylogenetic analysis, additional insights will be gained into plant geographic distributions. Several studies have examined the well-known eastern Asian - eastern North America disjunctions (reviewed in QIU & al., 1995). Additional taxa with Mediterranean - North American disjunctions that have been the subject of molecular phylogenetic analysis include *Styrax* (*Styracaceae*) (FRITSCH, 1996), *Allium* (*Alliaceae*) (SAMOYLOV & al., 1995), *Arbutus* (*Ericaceae*) (HILEMAN & al., 1995), genera of the *Papaveroideae* (*Papaveraceae*) (JORK & KADEREIT, 1995), *Centaurea* (*Asteraceae*) (SUSANNA & al., 1995), *Brachypodium* (*Poaceae*) (CATALAN & al., 1995), *Lavatera* (*Malvaceae*) (RAY, 1994), and *Hordeum* (*Poaceae*) (DOEBLEY & al., 1992). A synthesis of results from these and other studies will provide a better understanding of the biogeographic relationships of the Mediterranean flora.

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