Molecular Phylogenetics and Evolution 68 (2013) 555–566

Contents lists available at SciVerse ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

# Adaptive evolution of Mediterranean pines

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ARTICLE INFO

Article history:
Received 21 August 2012
Revised 10 December 2012
Accepted 31 March 2013
Available online 13 April 2013

Keywords:
Phylogeny
Adaptation
Candidate genes
Coevolution
Phenotypic traits
*Pinus*

## ABSTRACT

Mediterranean pines represent an extremely heterogeneous assembly. Although they have evolved under similar environmental conditions, they diversified long ago, *ca.* 10 Mya, and present distinct biogeographic and demographic histories. Therefore, it is of special interest to understand whether and to what extent they have developed specific strategies of adaptive evolution through time and space. To explore evolutionary patterns, the Mediterranean pines' phylogeny was first reconstructed analyzing a new set of 21 low-copy nuclear genes with multilocus Bayesian tree reconstruction methods. Secondly, a phylogenetic approach was used to search for footprints of natural selection and to examine the evolution of multiple phenotypic traits. We identified two genes (involved in pines' defense and stress responses) that have likely played a role in the adaptation of Mediterranean pines to their environment. Moreover, few life-history traits showed historical or evolutionary adaptive convergence in Mediterranean lineages, while patterns of character evolution revealed various evolutionary trade-offs linking growth-development, reproduction and fire-related traits. Assessing the evolutionary path of important life-history traits, as well as the genomic basis of adaptive variation is central to understanding the past evolutionary success of Mediterranean pines and their future response to environmental changes.

## 1. Introduction

Mediterranean pines (section *Pinus*, subsection *Pinaster*) represent an extremely heterogeneous and interesting assembly, which ranges from shore and island pines to mountain pines in the areas surrounding the Mediterranean (Klaus, 1989; Richardson, 1998; Fig. 1). These pines are adapted to extremely variable, and frequently extreme, environments. Very often, in Mediterranean forests, different pine species co-occur and thus, they may have adapted to similar environmental conditions. As these taxa are also phylogenetically close, we could expect them to share common adaptive strategies. However, since Mediterranean pines diversified *c.* 10 Mya (divergence time between the subsections *Pinaster* and *Pinus*; Gernandt et al., 2008), each taxon has distinct biogeographic and demographic histories (e.g. Grivet et al., 2011; Fady, 2012), and may thus have responded quite differently to selective pressures. Therefore, we could also expect each individual taxon to have evolved following its own trajectory. It is thus of special interest to understand whether and how Mediterranean pines have developed specific strategies of adaptive evolution through time and space, and which genes and phenotypic traits are involved in this process.

To explore evolution patterns in the Mediterranean pines, two (independent) sets of common characters were examined, one at the molecular level (candidate genes) and the other at the phenotypic level (life-history traits). Although some of these genes may be directly involved in a given phenotype under study (for example, *4-coumarate-CoA ligase* (*4cl*) with wood density; see Fig. 2 in González-Martínez et al., 2007) currently there is not enough evidence to establish a clear association between genes and phenotypes. Moreover, allelic effects on phenotypes would most probably be polygenic, and strong association between genes and phenotypes is not expected (Rochman, 2012). Genes involved in species adaptation are still largely unknown, but examining genes that are differentially expressed under biotic (e.g. pathogen attack) and abiotic stress (such as high/low temperatures, and deficit/surplus of water, minerals or micronutrients), may help determine those that play a role in adaptive evolution. Examining the same set of genes across the Mediterranean pine taxa should allow discovering not only nucleotide similarities or the genetic variation that underlies differences among pine species, but also identifying

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http://dx.doi.org/10.1016/j.ympev.2013.03.032

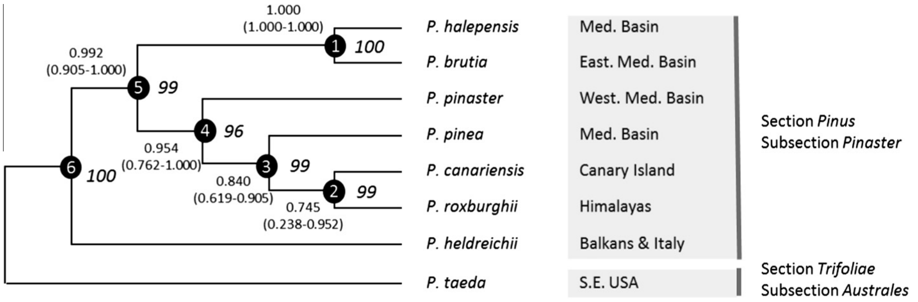

**Fig. 1.** Phylogenic trees inferred using 21 low-copy nuclear genes for the seven Mediterranean pine species and the American outgroup. Numbers in bold indicate the probability of the partition for each branch; decimals indicate the sample-wide mean concordance factor (with 95% credibility); numbers in parenthesis correspond to the standard deviation of sample-wide mean concordance factor across runs; circled digits indicate nodes' identity. The biogeographic region of origin (after Richardson, 1998) is also given.

selection events that have affected one particular branch of the gene tree (Yang, 1998), or/and particular sites under selection (Nielsen and Yang, 1998). The phylogenetic approach employed to detect footprints of selection complements previous population-genetic studies performed at the within species level for different Mediterranean pines (Eveno et al., 2008 and Pot et al., 2005 for *P. pinaster*; Grivet et al., 2009 and Grivet et al., 2011 for *P. pinaster* and *P. halepensis*). This approach examines adaptive evolution of candidate genes at a wider evolutionary scale across several taxa within the subsection *Pinaster*, and sheds some light on the features that make species similar to each other or unique.

Life-history traits have a direct bearing on fitness and thus direct consequences on species adaptive evolution (Stearns, 1977). Because pines of the Mediterranean Basin grow in rather extreme environments, they have developed specific strategies to face them, especially against drought and fire, the main disturbance factors in this region (Tapias et al., 2004, and references therein). Adaptation of Mediterranean conifers to their environment involves a suite of phenotypic traits that have been postulated as central for understanding their life-history strategies. These traits are related to vegetative growth (Grotkopp et al., 2002), seedling allometry and development (e.g. Strauss and Ledig, 1985), sexual reproduction (e.g. Tapias et al., 2001; Ne'eman et al., 2011), seed dispersal ability (e.g. Benkman, 1995) and, particularly, tolerance to fire (Keeley and Zedler, 1998; Schwilk and Ackerly, 2001; Pausas et al., 2004; He et al., 2012) and drought (e.g. Martínez-Vilalta et al., 2004). Wood physical properties have also received much attention recently, since a close link with contrasted life histories has been shown in different tree species (e.g. Poorter et al., 2010). Finally, genome size has been suggested to play a pivotal role in determining life-history traits in many organisms (e.g. Grotkopp et al., 2004). Our knowledge about the correlated evolution of these traits and their underlying molecular bases are still fairly limited. Furthermore, the lack of a consistent phylogeny for Mediterranean pines has prevented unveiling the evolutionary relevance of phenotypic traits.

Reconstructing underlying evolutionary processes requires robust inference of phylogenetic relationships. In the last decades, numerous studies have attempted the construction of pine phylogenies (including all or part of the Mediterranean pines), using chloroplast markers (e.g. Wang et al., 1999; Eckert and Hall, 2006; Parks et al., 2009), nuclear markers (e.g. Liston et al., 1999; Syring et al., 2005), a combination of molecular and non-molecular data (e.g. Gernandt et al., 2008), or combining phylogenetic trees

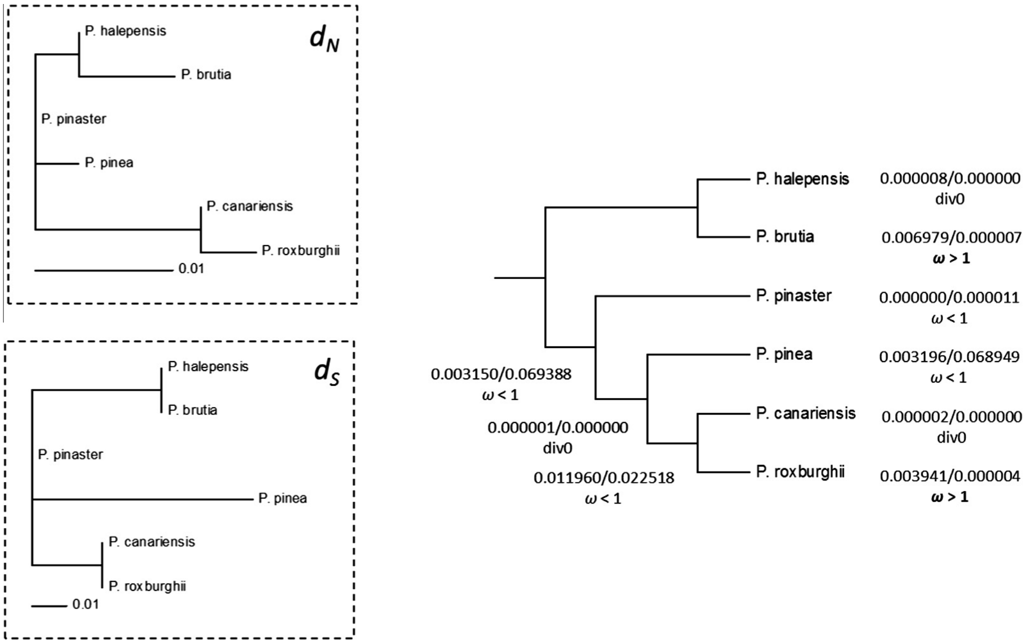

**Fig. 2.** Non-synonymous ($d_N$) and synonymous ($d_S$) values along with their ratio, $\omega = d_N/d_S$ for locus *lp31*, plotted for each branch of the consensus phylogenetic tree of the Mediterranean pines' clade. In bold: branches for which $\omega > 1$; div 0: the ratio could not be computed because the denominator equals zero.