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Contributions of candidate-gene research to understanding the role of genetic diversity in the adaptive response of Mediterranean conifers to drought

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One major feature of Mediterranean climates is the existence of seasonal drought. For those trees which seeds germinate in spring or early summer, severe droughts result in high mortality at early stages of development and may prevent natural regeneration of the forest; also adult trees may die, in particular when droughts are recurrent and acute, which in turn attracts pests and diseases, resulting in forest decline. Moreover, an indirect effect of drought is the increase in size, intensity and recurrence of forest fires, constituting one of the major threats to survival of Mediterranean forests. Common garden experiments (provenance tests and combined provenance-progeny tests) have shown notable genetic variation on drought response for forest trees at the species, population and individual levels, as well as differences in drought-response mechanisms (e.g. biomass allocation vs. water-use efficiency). For instance, studies reporting estimates of additive variance for water use efficiency (WUE) at the population and family level in Mediterranean pines have shown phenotypic clines from south to north in Aleppo pine, a xeric species with wide distribution in the Mediterranean (VOLTAS *et al.* 2008). New approaches based on DNA sequencing of candidate genes (i.e. genes potentially involved in the expression of a trait of interest) hold promise to evaluate the levels of

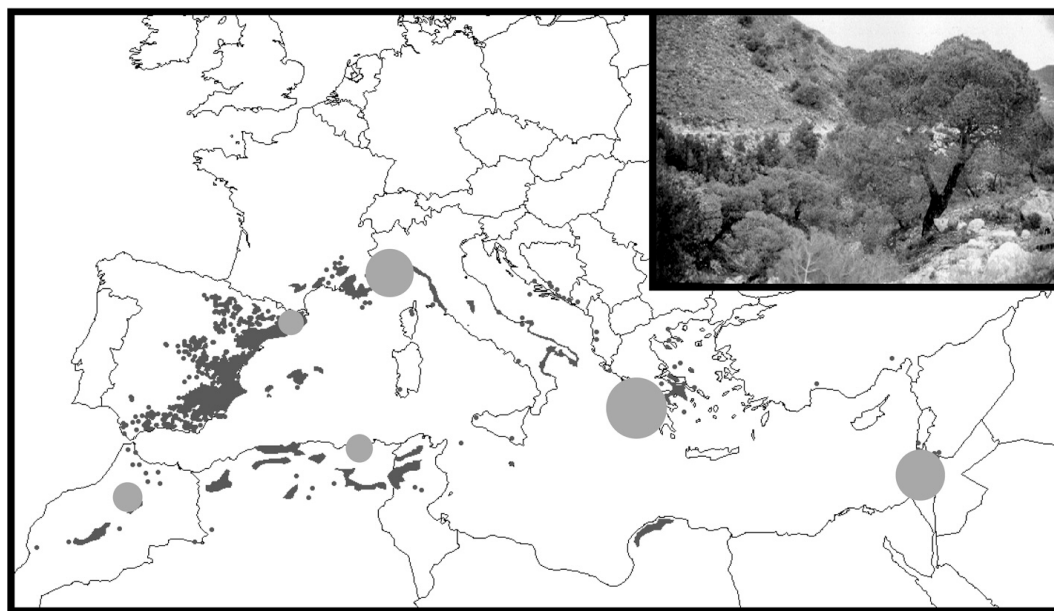
adaptive genetic variation present in Mediterranean forest trees. The existence of genetic diversity for drought-related traits (and the underlying molecular variation) is important as adaptation mostly relies on standing genetic variation (and less in new mutations). Moreover, given the rapid climate change expected in the near future — especially in the Mediterranean basin where a substantial decrease in precipitation and a pronounced warming is expected (GIORGI and LIONELLO 2008) — the ability of forest trees for short-term genetic change is of paramount importance. This fact is even more true if we consider that recent estimates of forest tree migration rates and climate change velocity suggest that most forest trees will not be able to migrate at the necessary speed to track ecological optima, the only other alternative left being either to adapt in situ or to go extinct (PETIT *et al.* 2008; AITKEN *et al.* 2008).

In this paper, we present a brief summary of our on-going population genetics studies on drought-response candidate genes for two widespread Mediterranean pines, the maritime (*Pinus pinaster* Aiton) and the Aleppo (*Pinus halepensis* Mill.) pines. Maritime (or cluster) pine is restricted to the Western Mediterranean, its southern natural populations reaching the High Atlas in Morocco. Three main gene pools have been identified in this species based on neutral molecular markers (BURBAN & PETIT 2003; BUCCI *et al.* 2008): a western group (most of the Iberian

Peninsula and western France), an eastern group (Corsica, Mediterranean France, Italy and Tunisia) and, curiously, an additional group composed by highly-differentiated populations from Morocco. Fossil evidence and phylogenetic inference have shown that this species has long-term presence in the western Mediterranean, in particular in the Iberian Peninsula (JARAMILLO-CORREA *et al.* 2010). Aleppo pine, on the contrary, seems to be a recent colonizer of the western Mediterranean, which may have important consequences for its adaptability within this range as colonization was accompanied by a substantial reduction of its genetic variation (see below). Still, this species harbors large amounts of genetic diversity within its eastern native distribution (mainly Greece and, more marginally, Turkey).

The increasing availability of genomic resources in conifer species makes approaches based on candidate gene sequencing appealing, despite the large genome size of these species (ca. seven times the human genome). Currently, different American and European initiatives have been launched to obtain the first complete sequence of a conifer genome. Our approach is based on the selection of candidate genes for relevant adaptive traits, in this case response or tolerance to drought. In the pine studies reported here (see details in GRIVET *et al.* 2009, 2010 and JARAMILLO-CORREA *et al.* 2010), we have focused on gene families that are known to be over-expressed during drought

Fig. 1: Distribution map of *P. halepensis* (kindly provided by EUFORGEN, the European program for Conservation of Forest Genetic Resources) along with the average nucleotide diversity (estimated by π , the within-population average pairwise difference across sequences) of ten drought-response candidate genes (the size of the circles is proportional to the level of nucleotide diversity).



stress and the subsequent recovery phase (such as different dehydrins) as well as some genes with major roles in more general pathways for stress response (such as 4-coumarate:CoA ligase, 4CL, gene). These genes have also some functional links with general drought responses that have been reported in model species, in particular *Arabidopsis* and different crops. For example, the dehydrins act as structural stabilizers with chaperone-like properties in several plant species (CLOSE 1997). Once a set of relevant genes is selected, we then proceeded to obtain a sample of sequences at the population level (typically, ten megagametophytes, the haploid tissue in pine seeds, are sequenced for each population) covering the full range of the species and targeting contrasted populations. For instance, 77 to 122 individuals (depending on the gene) were sequenced in maritime pine including populations from the humid coastal Atlantic range (1235 mm of annual rainfall) and the arid one in southern Spain (357 mm of annual rainfall). Finally, a series of new generation neutrality tests (such as Zeng's compound tests, ZENG *et al.* 2007) were performed to detect signatures of selection acting at different temporal scales; we also searched for correlations between allele/haplotype frequencies in candidate genes and climate variables under the assumption that such correlations may unveil genes underlying adaptation to environmental heterogeneity.

Higher levels of nucleotide diversity in candidate genes for drought response were present in *P. pinaster* than in *P. halepensis*, despite its narrower range in the Mediterranean. Differences across species were also reflected in the haplotype distribution for each tree species, with *P. pinaster* showing many different haplotypes at similar frequencies and *P. halepensis* showing fewer haplotypes with only one that is common or even fixed. The low levels of nucleotide diversity in Aleppo pine are more noticeable in its western distribution (see Figure 1 and GRIVET *et al.* 2009) where most genes were fixed or almost fixed for particular haplotypes (Figure 2), a probable consequence of long-range colonization of the Western Mediterranean from ancient Aleppo pine populations in Greece and Turkey and a more acute impact of the Ice Ages in this range of the species. Molecular analyses also revealed intense and relatively recent bottlenecks in Aleppo pine as well as a time of split between North-African and Iberian populations of the species well predating the Last Glacial Maximum albeit not as old as the one estimated for maritime pine (JARAMILLO-CORREA *et al.* 2010). In contrast, maritime pine seems to harbor large amounts of diversity for these genes due to a more stable demography; in addition, because of its more mesic distribution, higher environmental heterogeneity (from xeric to humid) would have resulted in



Fig. 2: Haplotype distribution of the candidate gene *dhnl1* superimposed on the distribution map of *P. halepensis*. Notice the low diversity for this gene in the western area.

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contrasted selective pressures that may have increased general levels of diversity at candidate genes.

Drought response is a very complex trait, as forest trees use very different strategies to confront drought. In addition, response to drought involves several cross-talking and incompletely known metabolic pathways. This obvious “polygenic” nature of drought response predicts the existence of only few genes with major effects whereas most of the drought-related processes would be controlled by large amounts of genes with minor effects and their interactions. Extensive candidate gene dissection of drought traits is still lacking for most forest trees, but judging from other better-known traits, polymorphisms in candidate genes with phenotypic effects higher than 5% of the trait variance would be rare (see, for instance, GONZÁLEZ-MARTÍNEZ *et al.* 2007 for wood property traits or HOLLIDAY *et al.* 2010 for bud set and cold tolerance). Nevertheless, using a wide range of neutrality tests, we found some of the candidate genes studied to evolve in non-neutral patterns. Interestingly, two of them showed statistical correlation with temperature variables, in particular with extremely high or low temperatures, and may constitute valuable tools for monitoring adaptive genetic diversity in these two Mediterranean pines.

Conclusions

Although, so far, discovery of adaptive variation at the molecular level (i.e. functional markers) is progressing slowly in non-model species, early works as the one presented here show the great potential of these tools in detecting valuable populations for genetic conservation as well as the environmental drivers promoting adaptation in forest trees. The development of new high-throughput sequencing and genotyping techniques will facilitate in the near future the extension of candidate gene approaches to full-genome screens. Research based on functional markers also holds promise for phenotype prediction in breeding programs and operative plantations. In the future, it is expected that molecular techniques based on candidate gene polymorphisms will be used to monitor changes in genetic composition of populations through time and space, and for

the evaluation of the genetic response of forest trees to impending climate and environmental change.

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