Gene conservation in pine species

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Abstract

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Since a genetic resource is a collection of biological material containing either specific or particularly variable genetic variation, several cases can be found in European and Mediterranean pine species that meet these requirements and might be included in gene conservation programmes. On the basis of their geographic distribution, European forest trees may show different patterns of variation among and within populations, as is inferred from isozyme markers: they may present several examples of populations carrying specific genetic variation that can therefore be looked upon as genetic resources. Other genetic markers (morphological, physiological and biochemical) are also useful to indicate existing differentiation among populations and provenances. In this paper, some significant examples are given for the following species: *Pinus sylvestris* L., *Pinus leucodermis* Ant., *Pinus nigra* Arn., *Pinus cembra* L., *Pinus halepensis* Mill. For each of them the proposed form of preservation is mainly the dynamic *in situ* conservation, which makes it possible to preserve the genetic adaptability of populations in changing environments.

Introduction

The concept of 'gene conservation' requires a definition of the 'gene resources' to be preserved. A gene resource can be defined as a collection of biological material containing either specific or particularly variable genetic information (Ziehe & al. 1989). On the basis of this definition, we can find several examples of gene resources in European and Mediterranean pine species.

Depending on their geographic distribution, European forest trees may show different patterns of intra- and inter-population variation, as is inferred from isozyme markers (Müller-Starck & al. 1992); populations that are derived from different glacial refugia, or belong to different subspecies or races, to different areas of discontinuous ranges, to species with extremely small ranges, or are locally isolated: all of them can carry specific genetic information and can therefore be looked upon as gene resources. This variation can also be studied through other markers, either morphological, physiological, or biochemical (terpenes and antigens), that sometimes indicate better than isozymes existing differentiation among populations and provenances (Prus-Glowacki 1991).

The genus *Pinus* covers a very wide region in the Northern Hemisphere, mainly the north temperate zone (Fig. 1). It is one of the most important forest genera, and includes more than 100 species; of these, about 13 are found in Europe – but this number changes in accordance with different systematic classifications.

The following European species, will be discussed in connection with gene conservation problems: *Pinus sylvestris* L., *P. leucodermis* Ant., *P. nigra* Arn., *P. cembra* L., *P. halepensis* Miller.

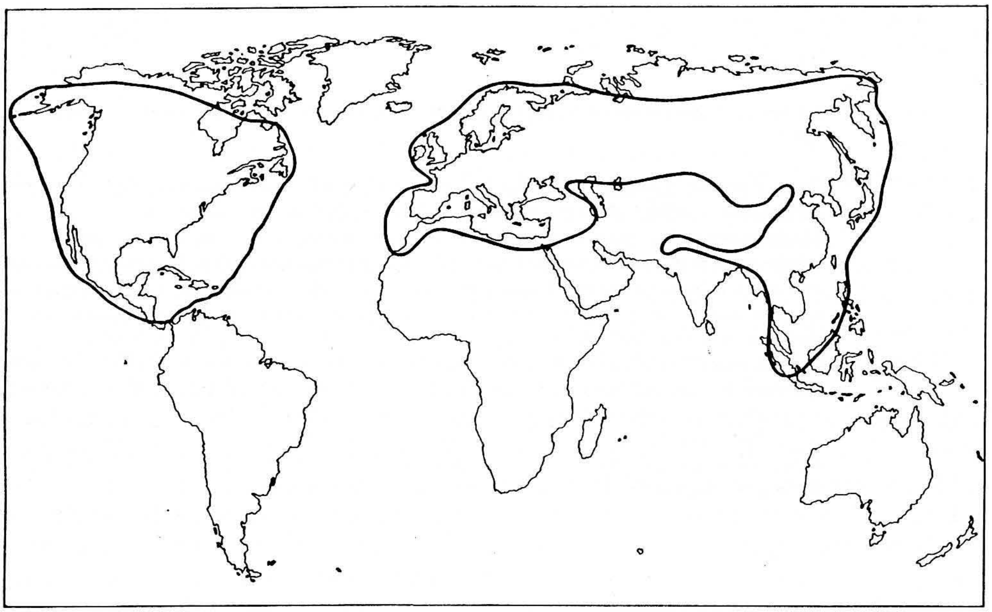

Fig. 1. The natural distribution area of the genus *Pinus* (after Mirov 1967).

Scots pine

Scots pine (*Pinus sylvestris* L.) occupies a larger area than any other pine species, and even any other species in the whole *Pinaceae* family (Mirov 1967, Boratynski 1991, Fig. 2).

Because of such a wide range, with very different environmental conditions, and because of the long history of this pine, a large amount of intraspecific variation is expected to occur. Several international provenance experiments on morphological (growth) traits have shown the existence, in the Baltic countries, of races endowed with great adaptability: these are important genetic resources, not only biologically but also economically. The above studies have also shown some marked differences among various provenances (Giertych 1991).

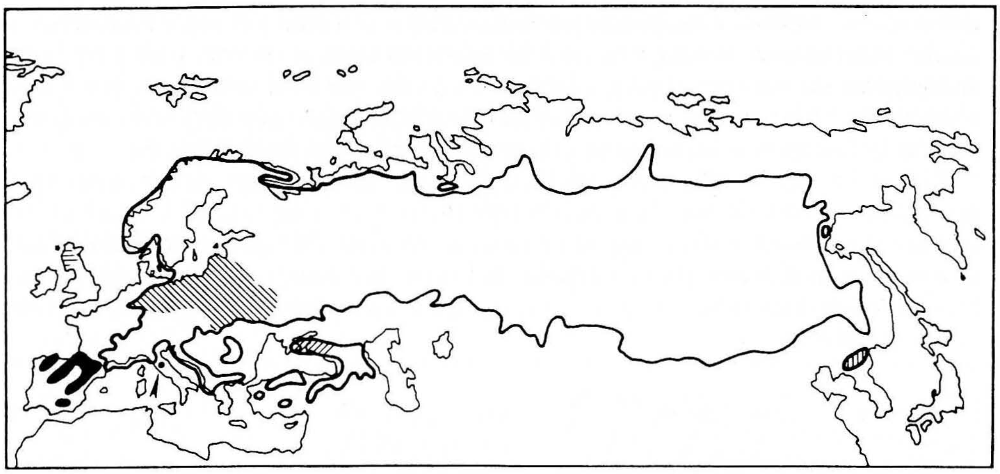

Fig. 2. The natural distribution area of *Pinus sylvestris* L. (after Boratynski 1991). The hatched and black areas and the small arrow (close to Italy) point to the locations of some subspecies, races and populations which are important for gene resource conservation (see text).

Geographical differentiation in the Scots pine's natural range has also been shown by means of biochemical markers, namely antigens (immunochemical techniques), terpenes and isozymes (Prus-Glowacki 1991), and physiological traits (Oleksyn & al. 1992).

Szmidt & Wang (1993) studied several populations of each of the following four taxonomic groups of *P. sylvestris* by using isozymes and RFLP of paternally inherited chloroplast DNA: var. *lapponica* (Fries) Hartman from Sweden, var. *armena* K. Koch from Turkey, var. *mongolica* Litvinov from China, var. *sylvestriformis* Takenouchi from China, together with some populations of the species *Pinus densiflora* Sieb. & Zucc. from China and Japan. In spite of the very long geographical distance between the populations of the varieties *lapponica, armena* and *mongolica*, these three taxa have not diverged considerably from one another, while the populations belonging to the variety *sylvestriformis* are highly differentiated from the rest, notwithstanding their geographical proximity to variety *mongolica*, and are genetically closer to the populations studied of *P. densiflora*. These results confirmed previous findings (Wang & al. 1991), suggesting that gene introgression between *P. densiflora* and *P. sylvestris* var. *mongolica* gave rise to the variety *sylvestriformis*, which thus becomes extremely valuable for genetic resource conservation (Fig. 2), both for its differentiation and for its higher levels of intrapopulational allozyme variability.

Goncharenko & al. (1995) compared several populations belonging to five subspecies of *P. sylvestris* and growing in the territory of the former Soviet Union, by means of isozyme analysis. They observed that only the geographically isolated var. *hamata* (Steven) Fomin, occurring in Caucasus and Crimea (Fig. 2), has differentiated enough to be considered as a distinct taxon, while the other subspecies exhibit a low differentiation between them, in spite of the great geographical distances separating them.

Prus-Glowacki & Stephan (1994) compared 7 Spanish and 16 North and East Europe populations by means of isozymes. They found that the Spanish populations are strongly differentiated, especially the population representing the southern border of the species' natural range (Sierra Nevada; Fig. 3). The results obtained agree well with their being attributed to the varieties *iberica* Svoboda, *pyrenaica* Svoboda and *nevadensis* Christ respectively. These are scattered and isolated populations and, according to these results, must be looked upon as important genetic resources within this species (Fig. 3).

Kinloch & al. (1986), using monoterpene and isozyme loci, found that some populations in northwestern Scotland (Wester Ross) were differentiated from all others and from each another. They suggest an endemic origin of the Caledonian race of Scots pine from more than one glacial refugium, and since the Scottish populations studied are relict and show high values of heterozygosity, they require protection, especially the more differentiated ones.

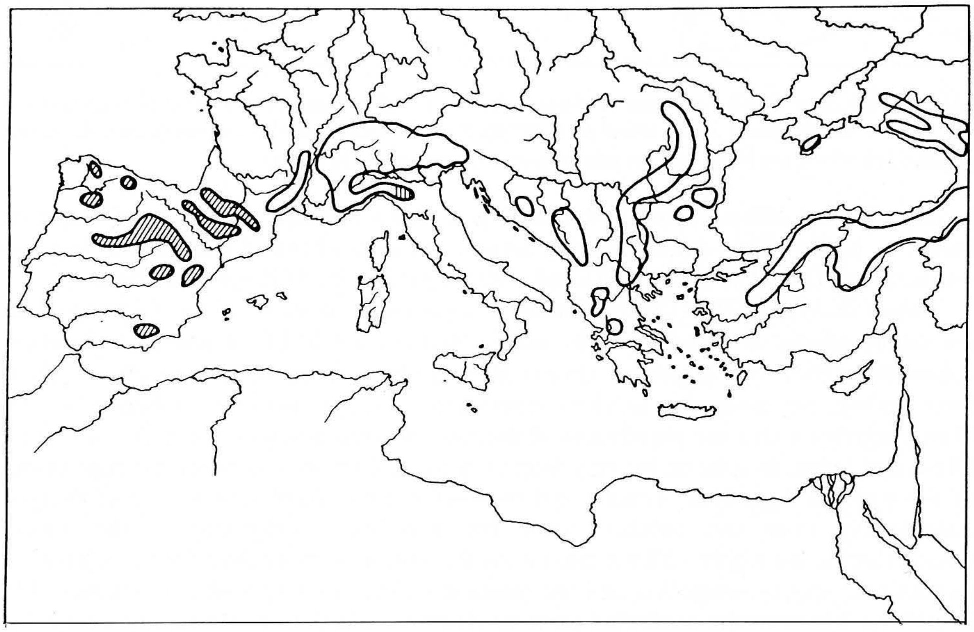

Fig. 3. The natural distribution area of *Pinus sylvestris* L. in the Mediterranean region (after Mirov 1967). The hatched areas mark the locations of some populations which are important for gene resource conservation (see text).

In Italy, Scots pine is present on the Alps and on the Northern Apennines (Fig. 3). The Apennine range consists of scattered and relict populations which bear witness to the climatic events of the inter- and postglacial periods (Agostini 1972). Preliminary results obtained by Puglisi & Cifarelli (unpublished data), in a study on enzyme polymorphism of seven Alpine populations and one Apennine population, show that the latter is clearly differentiated from the Alpine populations, which are all alike. This clear differentiation of the Appenine population indicates an independent evolutionary history of this population and the Alpine populations; and indicates that the postglacial recolonization of the Alps did not start from the refugia on the Northern Apennines and on the Tyrrhenian coasts near Viareggio (Giacomini 1958). The relict population investigated, whose intrapopulation genetic variation is as high as in the Alpine populations, is small and completely surrounded by cultivated stands. Since it is an official seed stand, it is protected by existing laws; however, due to its genetic value, more specific protection measures should be introduced, to include other Apennine relict populations as well.

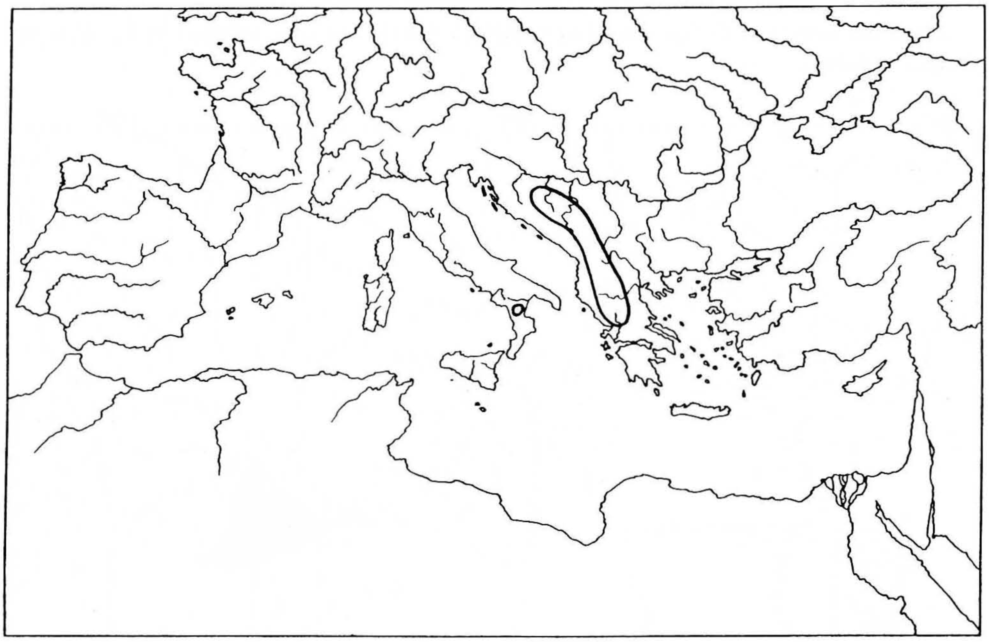

Fig. 4. The natural distribution area of *Pinus heldreichii* Christ. and *Pinus leucodermis* Ant. (after Mirov 1967).

Pinus leucodermis

In past times *Pinus leucodermis* Ant. used to be considered as a variety of *Pinus heldreichii* Christ., and later as a distinct species (Avolio 1984). Its natural range is discontinuous. It occurs mainly in the Balkan region, and as a volunteer species also in a small area in Southern Italy, at the border between Basilicata and Calabria (Fig. 4). Here, only some minor and isolated relict populations survive, though in the past the Italian range was probably much wider (Avolio 1984, Fig. 5). Boscherini & al. (1994) studied five Italian and two Greek populations (of which one is classified by some authors as *P. heldreichii*). They found that allozyme variation within and among populations is rather low, nevertheless the sampled Greek populations tend to differentiate from the Italian ones; RFLP analysis of chloroplast DNA, which is very useful in taxonomic studies on pine species, showed no differences between the populations sampled, so that they may belong to the same biological species, in accordance with Mirov's opinion (1967) who regarded *P. leucodermis* and *P. heldreichii* as conspecific.

Generally, species with very small ranges exhibit a relatively high differentiation among populations, though this does not apply to *P. leucodermis* (Müller-Starck & al. 1992). Possibly, its relatively low variation results from past genetic 'bottlenecks' of local populations (Boscherini & al. 1994).

Because of its small and discontinuous range, both in Italy and in the Balkans, *P. leucodermis* is an endangered species needing protective measures.

The most important Italian population (Mt. Pollino) is in fact included in the National Park of Calabria.

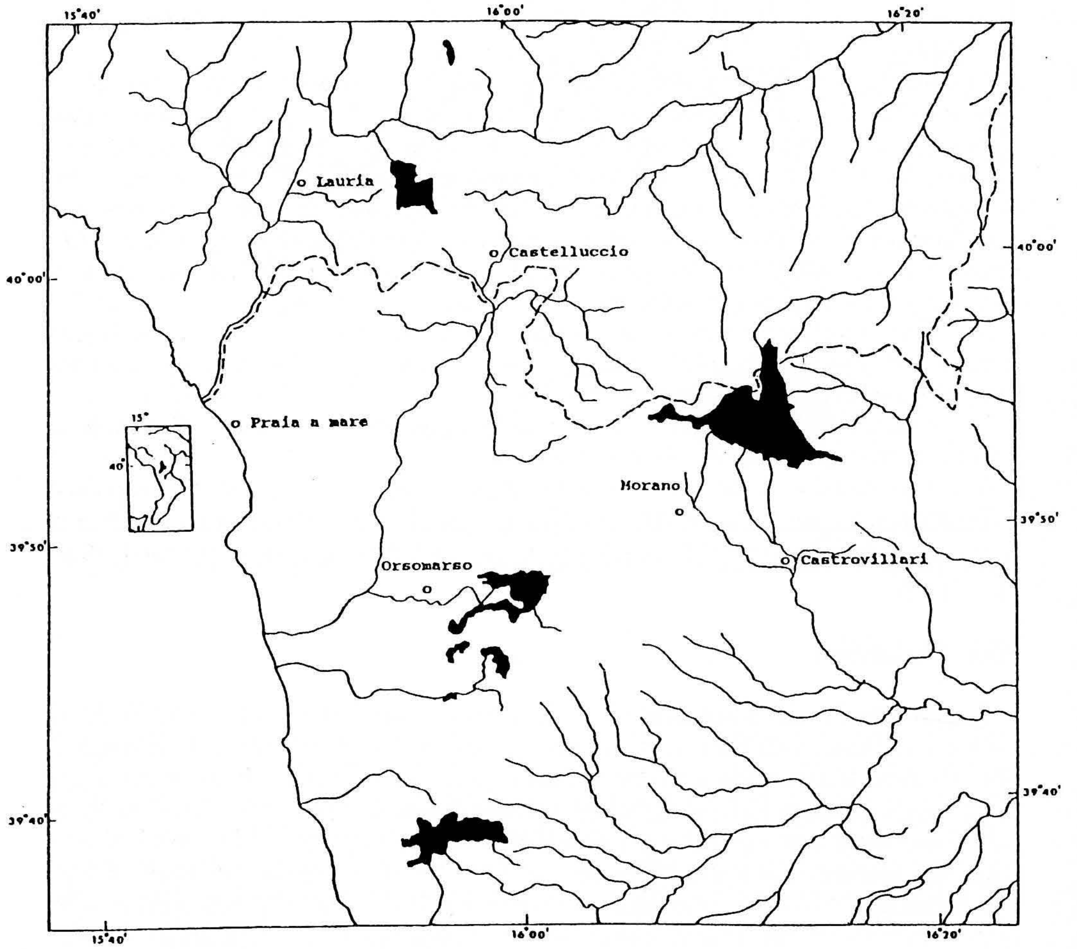

Fig. 5. The natural distribution area of *Pinus leucodermis* Ant. in Southern Italy (black areas; after Avolio 1984).

Black pine

Pinus nigra Arn. is a collective species with a wide and discontinuous natural distribution in south Europe and Turkey (Fig. 6).

The geographical variation among provenances, studied on the basis of morphological and anatomical traits (Gellini 1968, Arbez & Millier 1971, Wheeler & al. 1976), as well as physiological traits (De Philippis 1937), is relatively high. Significant differences among provenances were also found by means of terpene markers (Arbez & al. 1974, Fineschi & Grossoni 1981, Paci & al. 1989).

A relatively high differentiation among the 'small range species' – or subspecies –of this collective species, and among some populations within subspecies, was detected also by means of isozyme markers (Fineschi 1983, 1984, Nikolic & Tucic 1983, Scaltsoyiannes & al. 1994).

In Central Italy, there is a small population of black pine called "Villetta Barrea" black pine that Fukarek (1958) considers to be a subspecies of the Austrian pine (*Pinus nigricans* subsp. *italica* Hosschstett.). In several researches carried out using different markers, the present authors confirmed Fukarek's classification However, they also observed that this subspecies exhibits some intermediate features between the Austrian and the Calabrian pine, *Pinus nigra* subsp. *calabrica* (De Philippis 1937, Gellini 1968, Fineschi 1983, 1984, Paci & al. 1989). This important and isolated population is fortunately located inside the National Park of Abruzzo.

Fineschi (1984), by means of the allozyme locus SKDH-B, observed that a Tuscan isolated and small group of *P. laricio* could probably be attributed to the Corsican subspecies (subsp. *corsicana* Loud).

Recently, Scaltsoyiannes & al. (1994) have again found *P. laricio* (Corsican & Calabrian) to be clearly distinct from *P. nigricans*.

The discontinuity of the black pine geographic range and the sometimes marked differentiation among its taxonomic entities and populations make this species very rich in genetic resources that need appropriate protection and, therefore, a greater research effort for the future.

Swiss stone pine

Pinus cembra L. has a discontinuous range mainly in the Alpine region, separated into relatively small and isolated zones (Fig. 7). Szmidt (1982) studied 10 populations distributed inside this range and an Asiatic one – which is attributed by the author to the same species, but belongs to the closely related species *Pinus sibirica* Du Tour (Krutovskii & al. 1990) – and found a very wide differentiation among some of them, especially in a Romanian isolated population and in the Asiatic one, as shown by values of genetic distance by Nei (1972); genetic differentiation among populations from Tatra Mts. and the Alps is smaller but still remarkable compared to populations of other conifer species. Values of gene diversity within populations (expected heterozygosity) are similar to those found in most conifers.

Because of its discontinuous natural range and the wide differentiation among populations, *P. cembra* deserves a more detailed survey of its variation.

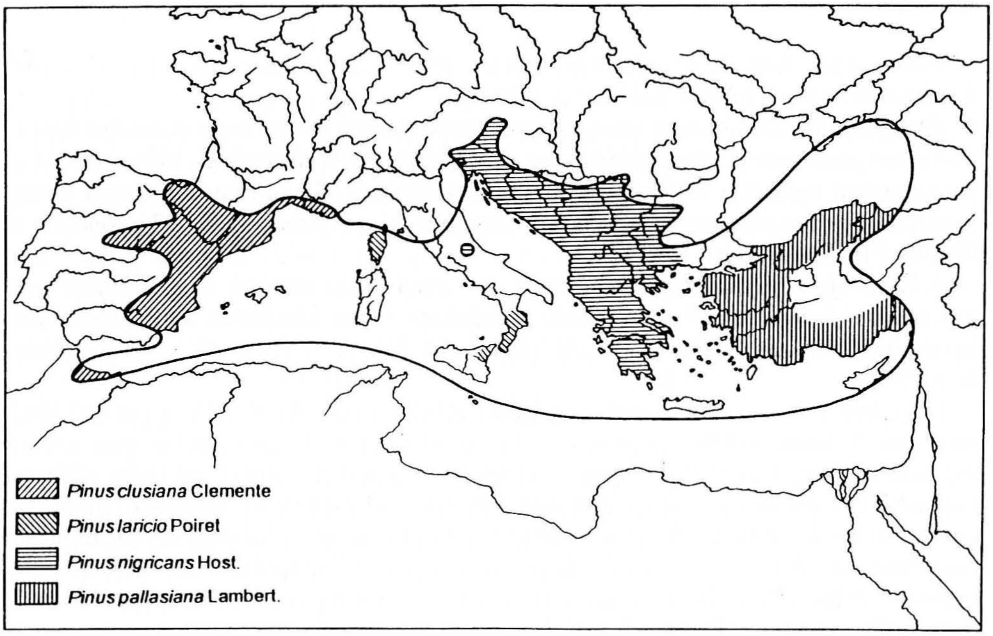

Fig. 6. The natural distribution area of *Pinus nigra* Arn. (after Mirov 1967). The hatched areas mark the natural ranges of different subspecies.

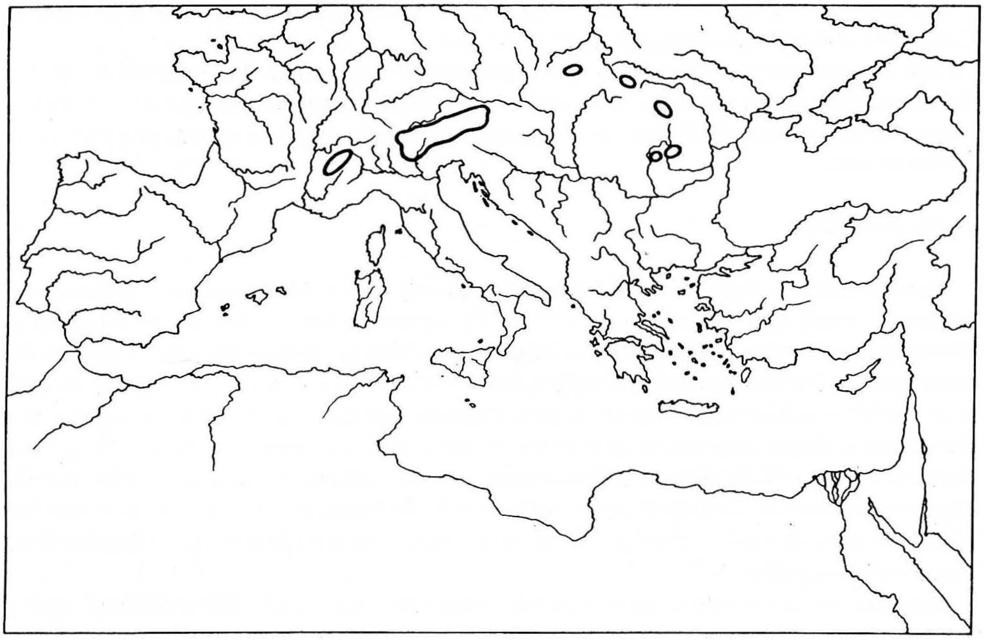

Fig. 7. The natural distribution area of *Pinus cembra* L. (after Mirov 1967).

Aleppo pine

Pinus halepensis Mill. is the most widely distributed pine of the Mediterranean region (Fig. 8). Using isozymes, Schiller & al. (1986) studied 19 populations representative of its natural range (most of them had been sampled for an international FAO research project on provenances of Aleppo pine and *Pinus brutia* Ten.).; They found low values of genetic diversity within populations, but were able to subdivide them into two groups: western and eastern Mediterranean. The former group was further subdivided into four races, one of which, from Eastern Europe, consists of populations introgressed with alleles from *P. brutia* therefore exhibiting higher values of genetic variation within populations. The other came from southern Italy (Gargano, Puglia), Albania, and Greece. These populations would be worthy of protection.

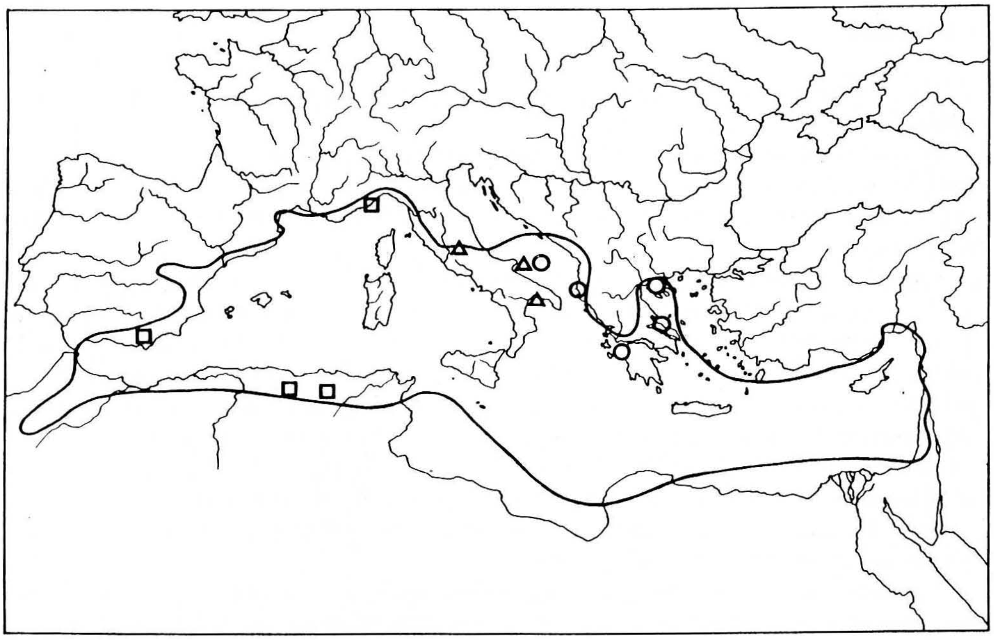

Fig. 8. The natural distribution area of *Pinus halepensis* Mill. (after Mirov 1967). Squares, triangles and circles point to the populations, belonging to three differentiated groups recognized by Calamassi & al. (1988). In particular, circles point to populations belonging to the "eastern European race", differentiated and introgressed with alleles from *Pinus brutia* Ten., found out by Schiller & al. (1986) using isozymes. The Albanian population was studied only by the latter. The population from Gargano (Southern Italy) is marked by two symbols because it was differently classified in the two quoted papers (see text).

Calamassi & al. (1988) studied some anatomical and morphological needle traits on samples picked from plants grown in a plantation close to Florence, which was established using seeds collected in *P. halepensis* the species natural range for the above-mentioned FAO project. Some populations sampled were the same as those studied by Schiller & al.

(1986). Results obtained by different multivariate analysis methods showed a sharp subdivision of the ten investigated populations into three well defined groups: populations from Greece, from Central and Southern Italy, and from the western sector of the range that includes Northern Italy, Spain and Algeria. It is of interest to note that some populations, common to both researches, were differently grouped as a result of using different markers (Fig. 8).

Significant differences among provenances were found for germination and resistance to water stress of seeds and seedlings (Calamassi & al. 1980, Falusi & al. 1983).

In conclusion, since this is an important species for Mediterranean countries, further studies may be useful to improve knowledge of the genetic structure of the more differentiated populations, and to find, elsewhere in the natural range, other populations endowed with specific genetic information, if any, in order to include them in genetic resource conservation programmes.

Conclusions

The above-mentioned examples are partial, but they help showing that gene conservation in pine species requires:

a) a greater effort in research on population genetics of these species;

b) an integration of results obtained by means of different markers. A synthesis may be important in order to indicate gene resources to be protected under gene conservation programs.

Specific features of forest tree species and the environmental condition of present-day world suggest, in particular, forms of dynamic *in situ* conservation, in order to preserve the genetic adaptability of tree populations under conditions of global environmental change. This kind of conservation allows populations to evolve and therefore to preserve their genetic variation, which originates from mutation, migration (gene flow) and sexual reproduction, by means of a continuous adaptation to spatial and temporal heterogeneity of environmental conditions. It involves the presence of a genetic load, that is less fit genetic information which provides the basis for adaptation to future environmental changes; it is reduced by selection, so it cannot become excessive lowering population adaptability (Gregorius 1989, 1991, Ziehe & al. 1989).

Measures of dynamic *in situ* conservation applied to minor and isolated relict populations — like Swiss stone pine in several parts of its range, *Pinus leucodermis* in Southern Italy and in some zones of its Balkanic range (whose gene resources need to be more studied), black pine in Central Italy and Scots pine in the Northern Apennines and in Scotland — should be matched with some appropriate forms of dynamic *ex situ* conservation, in order to ensure preservation of their gene resources in case adult trees should disappear altogether without any natural regeneration, as — for instance — following extended and recurrent fires, which are so common in Mediterranean regions. As a matter of fact, experimental plots and reafforestations of *Pinus leucodermis*, established in Southern Italy in the neighbourhood of some natural populations and using locally collected seeds, can be useful for gene conservation, just as are provenance trials for other species (Scots pine, black pine and Aleppo pine, for instance).

Forms of static conservation are hardly applicable to forest tree species because of their biological features — especially generation length and reproductive system — and could lead to the accumulation of an excessive genetic load or to gene loss due to drift effects, and therefore to lower adaptability. Nevertheless, in some particular cases and in situations of emergency, conservation of seeds or other reproductive material may be very important, but such solutions cannot be applied on a large scale and in any case such materials should be regenerated as soon as possible (Gregorius 1989, 1991, Ziehe & al. 1989).

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