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GENETICALLY DEPAUPERATE BUT WIDESPREAD: THE CASE OF AN EMBLEMATIC MEDITERRANEAN PINE

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Genetic variation is generally considered a prerequisite for adaptation to new environmental conditions. Thus the discovery of genetically depauperate but geographically widespread species is unexpected. We used 12 paternally inherited chloroplast microsatellites to estimate population genetic variation across the full range of an emblematic circum-Mediterranean conifer, stone pine (*Pinus pinea* L.). The same chloroplast DNA haplotype is fixed in nearly all of the 34 investigated populations. Such a low level of variation is consistent with a previous report of very low levels of diversity at nuclear loci in this species. Stone pine appears to have passed through a severe and prolonged demographic bottleneck, followed by subsequent natural- and human-mediated dispersal across the Mediterranean Basin. No other abundant and widespread plant species has as little genetic diversity as *P. pinea* at both chloroplast and nuclear markers. However, the species harbors a nonnegligible amount of variation at adaptive traits. Thus a causal relationship between genetic diversity, as measured by marker loci, and the evolutionary precariousness of a species, cannot be taken for granted.

KEY WORDS: **Chloroplast microsatellites, conservation genetics, diversity depletion, human impact, *Pinus pinea*.**

The existence of genetically depauperate species has long puzzled and challenged geneticists. A lack of variation is sometimes viewed as a negative result, with the risk that results remain unreported (Amos and Balmford 2001). Lack of variation also makes it difficult to apply the usual arsenal of analytical methods for population genetic inferences (Soltis et al. 1992). But most important, the finding of species that are largely devoid of genetic variation questions the "central dogma of conservation genetics": the belief that genetic diversity is essential and worthy of conservation (Lehman 1998). One of the most famous cases of a genetically depauperate species is that of the African cheetah (O'Brien et al. 1985). This species has experienced a prolonged bottleneck

resulting in extreme inbreeding, probably during the Pleistocene, when large mammals such as the giant ground sloth or cave bears became extinct (O'Brien 1994). Although the cheetah has regained a large distribution range, it has poor sperm quality and poor reproductive success in captivity, stimulating the interest in the role of genetics in conservation (Lehman 1998; Amos and Balmford 2001). However, other species that are also nearly completely devoid of genetic variation, such as the northern elephant seal, do not seem to suffer from fertility problems (Bonnell and Selander 1974). This has led some authors to conclude that continued demographic recovery of a species may owe more to immediate nongenetic factors, such as local availability of food resources, than to the level of genetic variability (Weber et al. 2004).

Trees provide an excellent opportunity to test such hypotheses. Given their larger effective population sizes, higher reproductive capacity and greater longevity, trees should be less prone than large animal species to prolonged bottlenecks (Petit and Hampe 2006), as testified by their typically high levels of genetic diversity (Hamrick et al. 1992). If they do experience bottlenecks and massive diversity loss, can they recover and thrive, as observed in some large mammals? Such cases should be particularly useful for testing the conservationists' dogma.

Here we focus on an emblematic Mediterranean tree, the stone pine or umbrella pine (*Pinus pinea* L.). Humans have long relied on its nutritious nuts. The oldest stone pine fossil remains are associated with Neanderthal groups in the south of the Iberian Peninsula: cone and wood charcoal fragments dated to 49,200 Before Present (BP) have been found in Gibraltar (Prada et al. 1997; Finlayson et al. 2006). In a nearby site, the Nerja cave (Málaga, southern Spain), large amounts of *P. pinea* charcoal and cone fragments have been discovered that date back from the Upper Paleolithic (older than 18,940 BP) to the Neolithic (Badal 1998), suggesting that the nuts were a major source of food for humans at that time. The earliest reported date of *P. pinea* cultivation is 3000 BP (Prada et al. 1997; Martínez and Montero 2004). The nuts became the object of an important trade and were exported by the Phoenicians and the Romans as far as Egypt or England. Currently, stone pine has a scattered distribution throughout the Mediterranean Basin (Fig. 1), covering ~650,000 ha (representing tens of millions of trees) from sea level up to 1000 meters (Quézel and Médail 2003).

We use paternally inherited chloroplast microsatellites to study the genetic variation and possible origin of *P. pinea* forests situated across the Mediterranean, as these markers have proven useful to study the geographic distribution of plant genetic diversity (Powell et al. 1995; Vendramin et al. 1999, 2000). We present evidence for an almost complete lack of chloroplast-microsatellite variation across the full species range, a feature never encountered

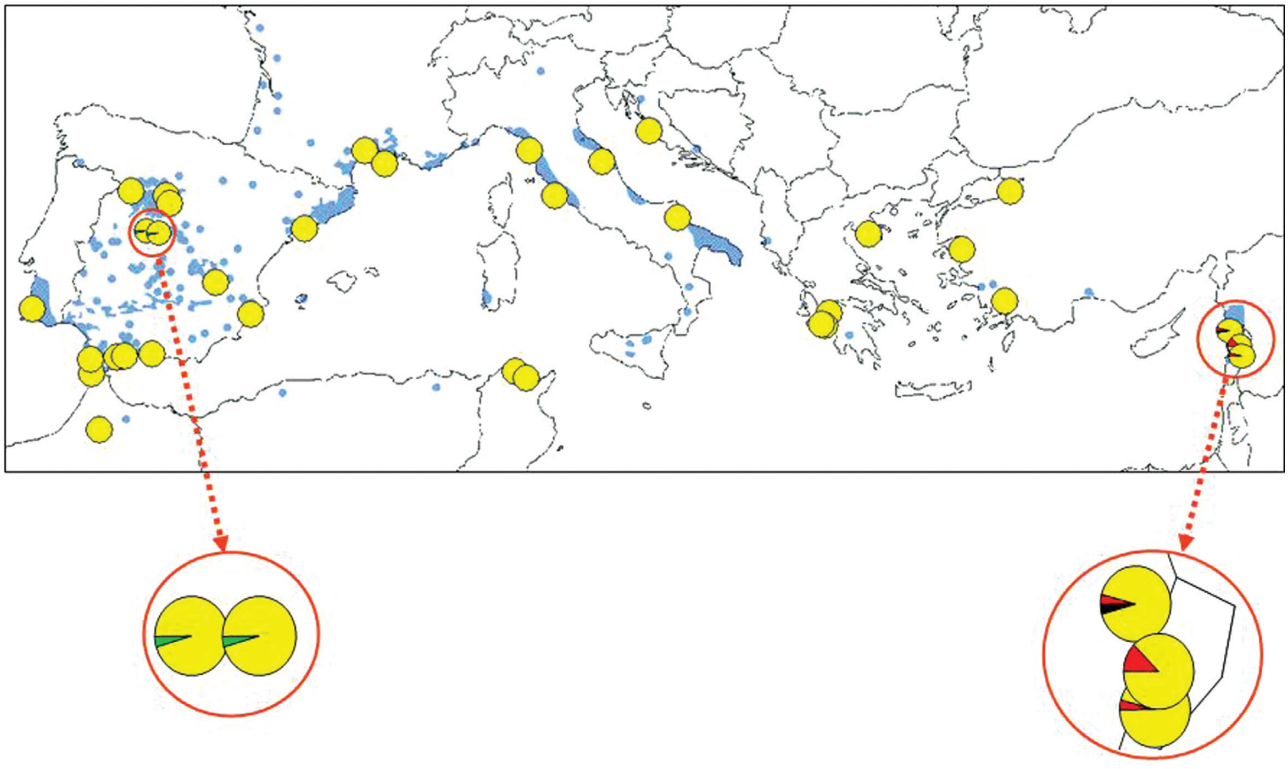

Figure 1. Chloroplast DNA variation in *Pinus pinea* populations across its range. Only four haplotypes were identified when surveying 13 microsatellites: H1: yellow, H2: red, H3: green, H4: black.

so far in any other widespread plant species. These results are consistent with an earlier report based on allozyme markers that detected only one polymorphic locus among 32 scored loci (Fallour et al. 1997). To provide a baseline for comparison with *P. pinea*, we searched the literature to identify other cases of genetically depauperate plants. This search indicates that *P. pinea* is truly exceptional among widespread, sexually reproducing plant species for its low level of genetic diversity. We discuss factors that might account for the remarkable "reversal of fortune" of this species, that is the recovery of a large circum-Mediterranean range after the demographic bottleneck that resulted in a nearly complete loss of genetic diversity.

Materials and Methods

STUDY SPECIES

Stone pine is one of the pine species most readily identified, given its distinctive umbrella-shaped crown. Trees of that species can withstand strong and salty winds. They can grow on sandy soils and sandstones, and their thick bark and absence of low branches make them resistant to ground fires (Rigolot 2004; Tapias et al. 2004). Today, *P. pinea* is distributed across the Mediterranean region and has recently been naturalized in other regions with Mediterranean climates, such as South Africa (Cape Province) where it is considered an invasive (Richardson et al. 1990). Stone pine is also the object of several genetic improvement programs focusing on nut and wood production (Court-Picon et al. 2004; Mutke et al. 2005).

CHLOROPLAST DNA STUDY

We collected seeds or needles from 34 natural populations distributed across the full range of *P. pinea* (Table 1). Sampled trees (24 individuals per population) were at least 50 m from one another. We isolated DNA from embryos or needles following the protocols of Doyle and Doyle (1990), Dellaporta et al. (1983), or with the Plant DNeasy kit (QIAGEN). We screened most samples with 12 chloroplast microsatellite primers designed on the basis of the complete chloroplast DNA (cpDNA) sequence of *P. thunbergii*: Pt1254, Pt9383, Pt15169, Pt26081, Pt30204, Pt36480, Pt41093, Pt63718, Pt71936, Pt79951, Pt87268, and Pt109567 (Vendramin et al. 1996). However, five Spanish populations (SP3-7; see Table 1) were screened with only five markers (Pt1254, Pt30204, Pt36480, Pt71936, and Pt87268), but these markers included all chloroplast microsatellites regions found to be polymorphic. PCR amplification and sizing conditions were as described in Vendramin et al. (1996). We repeated sizing at least twice. We cloned the fragments of the different haplotypes into plasmid vectors and then sequenced them with an ALF Express *Amersham Biosciences* sequencer to confirm both the presence of the microsatellites in the amplified fragments and the fact that variation in length was due to different numbers of repeats within the microsatellite regions. We obtained haplotype diversity estimates with the GenAlEx software (Peakall and Smouse 2006).

We used a double standardization procedure to check for the possible effects of differences in sampling and microsatellite length when comparing diversity of *P. pinea* with that of other conifer species. First, we estimated the expected number of different alleles in equal-sized samples of 100 ($\hat{A}_{[100]}$) with the program RAREFAC (www.pierroton.inra.fr/genetics/labo/Software/). Second, we standardized this measure of allelic richness to a mean number of microsatellite repeats of 10 ($\hat{A}_{[100,10]}$) (Petit et al. 2005, eq. 1).

Table 1. Variation at chloroplast microsatellites in *Pinus pinea* compared to that in other conifers

	Pinus pinea	Other conifers[a]
Number of loci per species (>6 repeats)	13	4.9
Proportion of polymorphic loci[b]	23%	71%
H (haplotypic diversity)	0.019	0.37
$\hat{A}$ (mean number of alleles/locus)	1.23	4.62
$\hat{A}_{[100]}$[c]	1.06	3.90
$\hat{A}_{[100;10]}$[d]	1.08	2.58
Mean number of repeats per locus	10.0	13.0
Maximum number of repeats	14.0	15.1

[a] Data for eight species combined: *Abies alba*, *Picea abies*, *Pinus brutia*, *P. cembra*, *P. halepensis*, *P. lambertiana*, *P. mugo*, and *P. pinaster*; see Petit et al. (2005).
[b] No threshold used.
[c] Mean number of alleles per locus in equal-sized samples of 100 individuals.
[d] Mean number of alleles per locus in equal-sized samples of 100 individuals after standardization to a mean number of repeats per locus of 10.

COMPARISON WITH OTHER GENETICALLY DEPAUPERATE PLANTS

Genetically depauperate plant species were defined as those with overall species nuclear diversity *H* lower than 0.05; this corresponds to the heterozygosity of a locus whose most frequent allele exceeds 0.97. Other requirements were that estimates should be based on a minimum of 10 loci and on samples originating from multiple populations, except for species growing in a single locale. We also noted a number of potential explanatory attributes such as plant size, woody/herbaceous habit, geographic range size, native or introduced status, existence of asexual reproduction (through apomixis or vegetative growth), and mating system.

Results and Discussion

1. THE EXTREMELY LOW GENETIC DIVERSITY OF *P. PINEA*

In their investigation of range-wide allozyme diversity in *P. pinea*, Fallour et al. (1997) found only one polymorphic locus (with just

two alleles) out of 32 loci tested (overall diversity $H = 0.015$; recomputed from their data). This diversity value is an order of magnitude lower than the mean value observed in other species of the genus *Pinus* ($H = 0.157$, $N = 93$; Hamrick et al. 1992). Our cpDNA survey extends this finding to the chloroplast genome. The sequencing of 12 cpDNA regions in 40 individuals revealed a total of 13 mononucleotide stretches with >6 repeats (one region did not have any microsatellite motif and two had two mononucleotide stretches each). Only three of the 13 mononucleotide stretches were polymorphic in the overall sample, each with two size variants differing from each other by one repeat, which combined into four different haplotypes (online Supplementary Table S1). All investigated populations were fixed for the same haplotype except the three populations from Lebanon where two additional low-frequency haplotypes were detected, and two populations from central Spain where one additional haplotype was found, also at very low frequency (Fig. 1). Hence, cpDNA diversity is extremely low in this species: total haplotype diversity is only 0.019 (compared to an average of 0.37 in other conifers, see Table 1) and standardized allelic richness per microsatellite locus was 1.08 (compared to 2.58 in other conifers). Sequencing of the cpDNA fragments indicated that the lack of polymorphism is not due to loss or interruption of repeat motifs. Further analyses and standardization showed that the lower diversity of stone pine is neither due to ascertainment bias caused by shorter alleles (i.e., a lower number of repeats in stone pine microsatellite stretches) nor due to different sample sizes compared to other conifers (Table 1).

In line with the reduced genetic diversity of *P. pinea* detected with allozymes (Fallour et al. 1997) and chloroplast microsatellites (this study), previous investigations found no significant variation in seed fatty acids composition (Nasri et al. 2005) and oil unsaponifiable matter (Nasri et al. 2007), even though these compounds are generally variable in plants (e.g., Linder 2000). Similarly, no variation exists in the response of *P. pinea* to the rust *Cronartium flaccidum*, as all plants tested are highly susceptible to this fungus; this contrasts with the situation in *Pinus pinaster*, a closely related Mediterranean conifer (Raddi et al. 1979). However, provenance tests involving material from across the range have revealed some differences in other adaptive traits (e.g., Bellefontaine et al. 1980; Court-Picon et al. 2004). Moreover, estimates of broad-sense heritability (H^2: the proportion of phenotypic variation in a population attributable to genetic variation among individuals) obtained in a grafted clone bank ($H^2 = 0.17$ for cone weight and 0.20 for seed output, Mutke et al. 2005) are significant, although lower than the mean heritability found in the literature (mean $H^2 = 0.32$ based on 504 estimates from 49 studies of plants, Geber and Griffen 2003). This indicates that, at least at some traits, there is a nonnegligible store of variation available for selection to act upon in this species.

2. COMPARISON WITH OTHER GENETICALLY DEPAUPERATE PLANT SPECIES

The finding of reduced levels of marker diversity in *P. pinea* can best be appreciated in a comparative framework. We therefore conducted a literature survey of studies reporting genetically depauperate wild plant species based on allozyme data. Allozyme markers have been used extensively, providing comparable genetic data from a wide variety of plants. Species lacking allozyme diversity were estimated to represent about 4% of the cases, a small but significant number (Godt and Hamrick 1997). In agreement with this finding, our literature survey identified as many as 96 species with H (species level diversity) below 0.05 (online Supplementary Table S2). However, further analysis indicated that genetically depauperate plants with attributes similar to those of *P. pinea* are truly exceptional. First, 26 species (27% of the total) identified can reproduce clonally. In these species, the lack of diversity can stem from a single clone spreading across a large area. For instance, the English elm (*Ulmus procera*), widespread across Europe, was recently shown to be a 2000-year-old Roman clone (Gil et al. 2004). Second, 34 species (35%) are predominantly self-fertilizing. Selfing plants are not less diverse than allogamous ones at the species level (Hamrick et al. 1992). However, this mode of reproduction allows a single "pure line" to persist or expand, potentially accounting for the finding of genetically depauperate selfing plants. Third, the list includes 59 endemics (61%), some growing in a single population, often in isolated places such as in islands. Finally, 15 cases involve introduced and/or weedy species sampled only in their introduced range.

Excluding all these categories leaves only nine plant species (Table 2). Three of these have an overall genetic diversity lower than that of *P. pinea*, although the only truly abundant and widespread species that may potentially rival with *P. pinea* in terms of genetic uniformity is *Pinus resinosa*, a widespread North American pine. Several studies have confirmed the nearly complete lack of diversity of *P. resinosa* at nuclear loci (Allendorf et al. 1982; Simon et al. 1986; Mosseler et al. 1992; De Verno and Mosseler 1997). As with *P. pinea*, this discovery "seems to conflict with the fact that this species consists of million of individuals occupying a vast range" (Walter and Epperson 2001).

Chloroplast microsatellites have higher mutation rates than allozymes (Provan et al. 1999) and are typically highly variable in conifers (e.g., Powell et al. 1995; Vendramin et al. 1996; Petit et al. 2005). For instance, 25 different chloroplast-microsatellite haplotypes were found in the circum-Mediterranean pine *P. halepensis*, a xerothermic species closely related to *P. pinea* that has low allozyme diversity (Bucci et al. 1998). Even in *P. resinosa*, which completely lacks allozyme diversity, nine of 11 chloroplast microsatellite loci investigated were polymorphic and up to 23 haplotypes were identified (Echt et al. 1998; Walter and Epperson 2001). Hence, the reduced chloroplast diversity found in *P. pinea*

Table 2. List of genetically depauperate but widespread plant species, excluding clonal and self-fertilizing plants, ranked by order of increasing diversity.

Species[a]	Family	Stature[b]	Habit[c]	Pop[d]	Loci[e]	H_{es}^{f}	Reference
Pinus resinosa	Pinaceae	32	W	2	27	0.001	Allendorf et al. 1982
Berchemia berchemiaefolia	Rhamnaceae	17	W	4	14	0.001	Lee et al. 2003
Schwalbea americana	Scrophulariaceae	0.6	H	13	15	0.006	Godt and Hamrick 1998
Pinus pinea	Pinaceae	30	W	17	32	0.015	Fallour et al. 1997
Lespedeza capitata	Fabaceae	1.6	H	12	34	0.020	Cole and Biesboer 1992
Juglans cinerea	Juglandaceae	30	W	9	12	0.029	Morin et al. 2000
Heuchera americana	Saxifragaceae	0.6	H	12	14	0.039	Soltis 1985
Desmodium nudiflorum	Fabaceae	0.29	H	5	13	0.043	Schaal and Smith 1980
Tsuga canadensis	Pinaceae	35	W	17	10	0.043	Zabinski 1992

[a]Trees are underlined.
[b]Stature in meters.
[c]Habit (W: woody, H: herbaceous).
[d]Pop: number of populations sampled.
[e]Loci: number of loci scored.
[f]H_{es}: expected heterozygosity at the species level.

(only four haplotypes, including three that are very rare) is particularly remarkable. To our knowledge, the only conifer in which a lower level of chloroplast microsatellites variation than *P. pinea* has been detected is *P. torreyana*, a Californian narrow endemic (Provan et al. 1999).

3. ACCOUNTING FOR THE LOW GENETIC DIVERSITY BUT BROAD GEOGRAPHIC DISTRIBUTION OF *P. PINEA*

To understand the causes of the uniquely low diversity at both chloroplast and nuclear markers in *P. pinea*, we first outline those factors that might have contributed to the initial loss of diversity. We then focus on those that can explain its subsequent range expansion despite a near absence of genetic diversity.

The loss of diversity

Of great significance is the fact that only limited genetic differentiation exists among *P. pinea* populations (Fallour et al. 1997; Nasri et al. 2005, 2007). If diversity had been lost independently in different parts of the range, different alleles would have been fixed in different areas, as observed in *P. torreyana* in California (Ledig and Conkle 1983). The three rare cpDNA haplotypes from Lebanon and central Spain, all differing by a single mutation from the ubiquitous haplotype, do not point to independent survival of haplotypes that existed prior to the bottleneck but rather to post-bottleneck mutations in areas of expansion (Slatkin and Hudson 1991). In contrast, the single allozyme polymorphism identified, malic enzyme, with two widely distributed alleles at frequency of 0.4 and 0.6 (Fallour et al. 1997), would predate the bottleneck. Altogether, this suggests that a range-wide decline resulting in the survival of a single (i.e., geographically circumscribed) population took place at some point(s) during the species history.

The rate of diversity loss following a bottleneck can be estimated as follows:

$$H_t/H_0 = (1 - 1/[2N_e])t \quad (1)$$

where H_t is the heterozygosity at generation t, H_0 the initial heterozygosity, and N_e the genetically effective population size (Frankham 2005). As discussed previously, there is a 10-fold difference in allozyme diversity between *P. pinea* and other pines. By assuming that *P. pinea* had a prebottleneck diversity level typical of that of other pines, equation (1) indicates that 22 generations would be necessary to reduce diversity to 10% of its initial value with $N_e = 5$, and as many as 45 generations with $N_e = 10$. In view of the long generation times of trees (see Hailer et al. 2006 for an analysis of the consequences of bottlenecks on long-lived organisms), these estimates suggest that one or more drastic and prolonged declines took place that affected the entire species.

However, the loss of diversity might have been accelerated by the unusual mating system of the species. Trees normally experience strong inbreeding depression, resulting in the elimination of all inbred progeny prior to maturity (Petit and Hampe 2006; Scofield and Schultz 2006). Interestingly, along with *P. resinosa* (Fowler 1964; Mosseler et al. 1991, 1992; Echt et al. 1998), *P. pinea* is one of the very few tree species that are largely devoid of inbreeding depression: even two successive generations of self-pollination do not appreciably reduce height growth in this species (Ammannati 1988). During a bottleneck, the effects of inbreeding depression begin very early, within a few generations, whereas genetic diversity is affected much more slowly (Amos and Balmford 2001). If a tree species is purged from its inbreeding depression in the course of the bottleneck, by exposing deleterious alleles

to selection, tolerance to selfing should appear, as observed in *P. pinea*. This in turn should accelerate genetic drift and diversity loss, compared to estimates obtained with equation (1) (effective population size is divided by two in a completely selfing species, compared to an outcrossing species with the same census size).

The survival of the species during the last glacial period not only in southern Spain but also in other regions such as southeastern France (where fossil wood remains of *P. pinea* dated at 20,300 BP have been found; Bazile-Robert 1981) implies that the bottleneck predates the Last Glacial Maximum, before humans could have had a major impact on forests (although they might have acted indirectly, for example by increasing fire frequencies in the region).

Natural climatic fluctuations during the Quaternary could have resulted in the prolonged bottleneck experienced by *P. pinea*. Xerothermic conifer species, such as *P. pinea*, tend to have less gene diversity than their mesothermic counterparts (Fady 2005), probably as a consequence of the greater magnitude of population reductions and expansions of these species in relation to glacial–interglacial episodes. In *P. torreyana*, a genetically depauperate coastal pine with ecological requirements similar to those of *P. pinea*, ice ages have also been suggested to play a role in the species decline (Provan et al. 1999; Ledig and Conkle 1983).

Finally, in contrast to the majority of pines, whose seeds are adapted to dispersal by wind, the ability of *P. pinea* and *P. torreyana* to disperse seeds depends on the presence of mutualistic animals, such as birds. The scarcity of seed dispersers during critical periods of the species history may have compromised their ability to colonize new territories, making them more susceptible to range contractions and ultimately affecting their genetic diversity (Ledig and Conkle 1983, Ledig et al. 1999).

Demographic expansion of P. pinea

The most unusual finding of this study is not the bottleneck and subsequent loss of diversity per se but the fact that *P. pinea* has retained a low level of diversity while spreading across a diverse and fragmented region. The maintenance of such a low level of genetic diversity implies that the expansion of *P. pinea* was recent or that mutation rates are low enough. The expansion of *P. pinea* across the Mediterranean must have started at least 3000 years ago, when humans began to cultivate the species (Badal 1998). However, it could have started much earlier if the observations of the species' presence in at least two distant locations (southern Spain and southeastern France) during the last ice age (Bazile-Robert 1981; Finlayson et al. 2006) are confirmed or if claims that the species is native not only to the western Mediterranean but also to the eastern Mediterranean Basin hold true (Barbéro et al. 1998). Thus, *P. pinea* has been unable to regain much genetic diversity several hundreds or more probably thousands of generations after its decline. In North America, *P. resinosa* has also maintained very low levels of genetic variation at nuclear markers for a long period of time, because the species survived in multiple glacial refugia during the last ice age (Boys et al. 2005; Walter and Epperson 2005). Hence, low evolutionary rates seem necessary to account for the existence of such widespread yet genetically depauperate species. Substitution rates are known to be particularly low in trees (Petit and Hampe 2006), including pines (Willyard et al. 2006), a likely consequence of their long generation time (Kay et al. 2006; Petit and Hampe 2006), which can potentially account for the relatively high proportion of trees among widespread yet genetically depauperate plant species.

Although a reduced evolutionary rate should help preserve a low level of diversity after the expansion, it does not account for the success of the expansion itself. For this, a number of not mutually exclusive explanations can be suggested. First, the expansion may owe more to the idiosyncratic presence of a suitable disperser than to the existence of a large store of genetic variation. In this respect, humans have played a significant role by cultivating the species for at least three millennia. Second, the striking rebound of *P. pinea* may be in part attributed to the loss of specific parasites and diseases during the bottleneck phase (Amos and Balmford 2001). This "release from enemies" hypothesis is similar to that proposed for the success of invasive species in their introduced range (Keane and Crawley 2002). Today *P. pinea* is known to have comparatively few parasites and diseases (Fady et al. 2004). Third, successful adaptation to new environmental conditions encountered during the expansion depends on the presence of variation at phenotypic traits, not on marker diversity. The small but nonnegligible amount of heritable variation found at adaptive traits (Mutke et al. 2005) could have played a role in the successful expansion of the species. The situation is similar in the genetically depauperate *P. resinosa*, for which heritable variation "compares favorably with that of other pines" for critical characters such as growth rate or phenology (David et al. 2003), although for other traits the species has been considered relatively homogenous (Fowler and Lester 1970). These findings are not that surprising: heritability of traits and genetic diversity evaluated by molecular markers are poorly, if at all, correlated (Reed and Frankham 2001; see also González-Martínez et al. [2004] for a case study in a conifer). Balancing- or frequency-dependent selection should better preserve quantitative genetic variation than molecular diversity during bottlenecks (Lynch 1996; Reed and Frankham 2001), possibly accounting for the poor correlation. In addition, the recovery of genetic variance at quantitative traits is typically faster than the increase in mean heterozygosity (Willis and Orr 1993). Epigenetic variation can also accumulate quickly after a bottleneck (Rapp and Wendel 2005); it might therefore be responsible for some of the differences among trees identified in clonal tests. Finally, *P. pinea* has a high level of phenotypic plasticity (Mutke et al. 2005), which could

have helped it colonize new environments despite reduced genetic variation.

Conclusion

Although there is little doubt that genetic variation is the raw material for adaptation, the connections between neutral diversity, quantitative trait variation, and adaptability are not straightforward. As a consequence, a causal relationship between genetic variability, as measured by marker loci, and the evolutionary precariousness of a species, cannot be taken for granted (Lehman 1998). This study illustrates the great potential of genetically depauperate species for critically evaluating some of the classical assumptions of conservation genetics. However, we should keep in mind that even if some species can survive and thrive following a severe population bottleneck (such as stone pine, red pine, or the northern elephant seal), these represent the few lucky ones rather than the norm (Godt and Hamrick 1997). Moreover, the time needed to reestablish variation is considerable, especially in long-lived species, and it is unclear how such species will react to novel environmental conditions, not experienced during the bottleneck or recovery phase.

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Supplementary Material

The following supplementary material is available for this article:

Table S1. *Pinus pinea* populations analyzed (sample size = 24 individuals in all populations), their location and haplotype counts.

Table S2. Characteristics of genetically depauperate plant species.

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