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Low genetic and high environmental diversity at adaptive traits in *Pinus pinea* from provenance tests in France and Spain

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Abstract. Mediterranean Stone pine (*Pinus pinea* L.) is a native species of the Mediterranean region, widely used for reforestation. It is characterised by a low genetic variation both at phenotypic traits and molecular markers. In the early nineties, experimental plots were established in several countries bordering the Mediterranean Sea for studying the distribution of genetic variability among provenances in adaptative traits as survival, vegetative and reproductive phase change, phenology and growth. Here we report results of the first decade at seven trial sites in France and Spain. The survival, ontogeny and growth patterns were homogeneous among provenances but differed among sites. On the other hand, the phenotypic plasticity of the species and its sensitivity to microsite (soil) variation produced strong spatial autocorrelations of the response variables, both between and within sites, which masked greatly the variation between provenances. Nearest-neighbours adjustments that captured the spatial pattern at each site reduced the error term and increased the significance of the geographic genetic variation considerably. Comparison of the adjusted average heights of each provenance showed a common trend between sites, with a lightly more vigorous growth in plants from colder or inland provenances. These results confirm the conclusions obtained in earlier analyses of the Spanish test sites, where these provenances showed also an earlier spring shoot phenology and a stronger tendency to polycyclism.

Keywords. *Pinus pinea* – Common garden experiments – Phenotypic plasticity – Nearest neighbours adjustment – Iterative spatial analysis.

Faible variabilité génétique et forte variabilité environnementale des caractères adaptatifs de Pinus pinea *en plantation comparative en France et en Espagne*

Résumé. *Le pin pignon (*Pinus pinea *L.) est un arbre forestier endémique du bassin méditerranéen, largement utilisé en reboisement. Il se caractérise par une faible diversité génétique de ses caractères morphologiques et une faible variabilité génétique au niveau des marqueurs moléculaires. Au début des années 1990, plusieurs tests de provenance ont été installés conjointement dans plusieurs pays méditerranéens pour évaluer la structure de la diversité génétique de caractères importants pour l'adaptation : survie, changements ontologiques entre les phases végétatives et reproductives, phénologie et croissance. Dans cet article, nous présentons les résultats d'analyses de la variabilité de ces caractères à l'issue d'une décennie de croissance dans 7 des tests de provenance installés en France et en Espagne. Les différentes provenances ne montrent pas de différences significatives pour la survie, ni pour l'ontogénie de la croissance, alors que les différences sont fortes entre sites. Les génotypes montrent une forte plasticité phénotypique en réponse aux variations de sol entre différents sites et à l'intérieur des sites, qui masque la variabilité entre provenances. Les ajustements aux plus proches voisins qui permettent de prendre en compte les effets spatiaux à l'intérieur des sites ont permis de mettre en évidence une diversité génétique significative entre provenances. La comparaison des moyennes de provenance ajustées montre une croissance plus vigoureuse des provenances issues des sites les plus froids et les plus intérieurs. Ces résultats confirment des résultats plus anciens issus des seuls tests de provenance espagnols, dans lesquels ces provenances montraient un débourrement printanier plus précoce et une tendance plus marquée au polycyclisme.*

Mots-clés. Pinus pinea *– Test de provenance – Plasticité phénotypique – Ajustement au plus proche voisin – Analyse spatiale itérative.*

I – Introduction

Mediterranean Stone pine (*Pinus pinea* L.) is a native species widely used for protective or restoring afforestations in the Mediterranean region, and occasionally in other similar climate zones around the world, aiming soil protection, ecosystem restoration and sustainable production of both pine nut and timber. Its area around the Mediterranean Sea has more than doubled in the last two centuries as results of these reforestations, stone pine-dominated forests occupying currently about 0.75 million hectares, sparsely spread from the Atlantic coast in Portugal to the shores of the Black Sea and the Mount Lebanon, and also at the southern shore of the Mediterranean Sea, where it is supposed to have been introduced only in the XX century, as well as in other bioregions with similar climates, such as California, Chile, South Africa, or Australia. In other climate zones like northern Iran or even Ethiopia, and in temperate areas like Great Britain, New Zealand, or Argentina, stone pine is an exotic restricted mainly to ornamental uses in gardening, though in some regions its growth outperforms greatly the original Mediterranean populations on semiarid or dry Mediterranean sites, reaching dominant heights up to 35 m (Mutke *et al*., 2012).

Though since Antiquity, the stone pine has been a cultural species in the Mediterranean due to its ornamental value and its edible kernels, up to present most of its stands are natural or naturalised forests or rural groves, not horticultural plantations based on defined cultivars. However, no phylogenetic structure has been found within or among its widely separated, most ancient populations, because all of them are fixed to a same haplotype, at least for nearly all studied cpDNA and allozyme markers (Fallour *et al*., 1997; Vendramin *et al*., 2008). This near absence of genetic variation for molecular markers among populations, very rare for a widespread forest tree species, has also been observed in phenotypic traits and is attributed to its putative human spread by seed transfer from a narrow source population (Court-Picon *et al*., 2004; Gordo *et al*., 2007). It might be also related to its strong phenotypic plasticity that allows for a high adaptative potential in spite of a low genetic diversity, *e.g.* resulting in high survival rates even in severe environments, though with strong ontogenic delays as pay-off, such as retarded growth or phase changes (Mutke *et al*., 2010). In view of on-going and further expected climatic changes, the relevance of both plasticity and genetic diversity for the adaptive potential of forest species is a current issue of research, as well as the future risks of population decline or extinction at the rear edge of species' distribution under changing conditions (Matyas *et al*., 2009). In this context, Mediterranean stone pine might be an interesting model species for studying the role of phenotypic plasticity (and e.g. its epigenetic mechanisms) when genetic differentiation is nearly absent.

In the early nineties, as cooperation within the FAO framework *Silva Mediterranea* and coordinated by French INRA, *Pinus pinea* seed lots were exchanged and experimental plots of a reciprocal provenance test were established in several countries bordering the Mediterranean Sea. The objective was to study the genetic variability between provenances in adaptative traits such as survival, phenology or growth. Several preliminary results have been published already for the test sites in France (Court-Picon *et al*., 2004), Portugal (Carneiro *et al*., 2006), Spain (Mutke *et al*., 2010), Maroc (Sbay, 2000), and Tunisia (Khaldi *et al*., 2009), and current results for Portuguese and Turkish test sites are presented in this issue of *Options Mediterrenéennes* (Carrasquinho and Gonçalves, 2012; Acar *et al*., 2012). The present communication puts together comparative results of the first fifteen years at seven trial sites in France and Spain.

II – Materials and methods

The tested accessions correspond to 40 provenances from Lebanon, Turkey, Greece, Italy, France, Spain, Portugal, Maroc, and Tunisia (Fig. 1), though only eight provenances are represented at all seven analysed sites (three in France and four in Spain), whereas other seven accessions (hence summing up fifteen) are present at least in five test sites in both countries (Table 1).

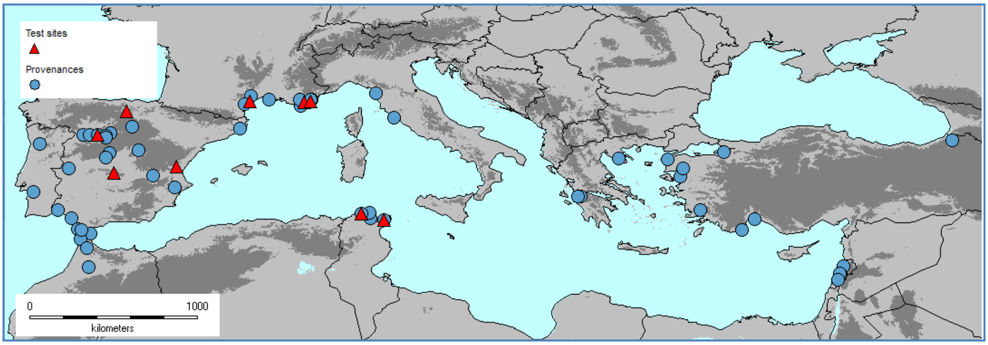

Fig. 1. Mediterranean stone pine provenances and analysed test sites of the provenance trial (also the discussed two Tunisian test sites are included in the map).

The repeated measurements at each site included the survival, occurrence of vegetative and reproductive phase changes, phenology, height and diameter. Given that no significant differences in survival or ontogeny were found between accessions, the present analysis centres on height growth.

Due to the presence of strong spatial autocorrelation (microsite pattern in mosaic) at the French and Spanish test sites, where the spatial individual tree position (coordinates) was included in the dataset, the analysis of variance adjusting a general linear model integrated an Iterative Spatial Analysis (ISA). For this, a Nearest Neighbourhood adjustment (NNA) used iteratively the mean residual values for the eight adjacent plant positions as covariate proxy for estimating a local microsite effect for each tree, following the methods used in the previous work on former measurements of the Spanish test sites (Mutke *et al.*, 2010) and on other genetic field tests (cf. Mutke *et al*., 2007).

Planting and measurement dates differed between test sites, so only the most recent height at each site were analysed for this study. In Spain, test sites Tordesillas and Trespaderne were measured last in 2007 at age 13 years, Quintos de Mora in 2008 (14 yrs.), whereas Cucalón has not been re-measured since 2003 (9 yrs.; Mutke *et al.*, 2010). In France, test site La Gaillarde was measured last in 2002 (8 yrs.), Sainte Baume in 2003 (10 yrs.) and Le Treps in 2010 (16 yrs.).

The adjusted provenance mean values at each site were compared in a Principal Component Analysis, including also the provenances mean values estimated at two Tunisian plots, Jbel Essmaa and Oued El Bir, at age 9 and 10 by Khaldi *et al.* (2009) adjusting a general linear model without spatial analysis.

Table 1. 34 accessions. mean annual temperature and rainfall and presence at the seven test sites and at two Tunisian sites

Provenance	Country	MAT	MAR	CUC	TOR	TRE	QUI	LT	StB	LG	TN
Chalkidiki Agios Nic	GR	15.0	439	1	1	1	1	1	1	1	1
Feniglia 67	I	15.8	680	1	1	-	-	1	1	1	1
Beit Mounzer (Mad. J.)	RL	13.3	1030	1	1	-	-	1	1	1	1
Kornaele	RL	13.2	1148	1	1	1	1	1	1	1	-
Qsaibe	RL	17.1	1098	1	1	-	-	1	1	1	1
Bkassine	RL	14.3	977	1	1	-	-	1	1	1	1
Antalya-Kumluca-Kalm.	TR	18.6	1030	1	1	1	1	1	1	1	1
Yalova-Dumanlidag	TR	11.7	754	1	1	1	1	1	-	1	-
Canakkale-Eceabat	TR	15.0	626	1	1	1	1	1	1	1	1
Mugla-Yatagan-Katran	TR	16.3	1202	1	1	1	1	1	1	1	1
St. Raphaël (Var)	F	14.0	848	1	1	1	1	1	-	1	1
Villeneuvette (Hér.)	F	13.5	775	1	1	1	1	1	1	1	1
Saintes-Maries	F	14.3	543	1	1	1	1	1	1	1	1
St. Aygulf (Var)	F	14.6	848	1	1	1	1	1	-	1	1
Brignoles- Le Val	F	13.1	813	1	1	1	1	1	1	1	1
Alcacer do Sal	P	16.3	589	1	1	1	1	-	-	-	-
Serra do Minho	P	14.0	1296	1	1	1	1	-	-	-	-
Cogeces de Iscar (VA)	E	12.2	455	1	1	1	1	-	-	-	-
Valorio, fondo (ZA)	E	12.3	359	1	1	1	1	-	-	-	-
Valorio, ladera (ZA)	E	12.3	359	1	1	1	1	-	-	-	-
Toro (ZA)	E	12.0	366	1	1	1	1	-	-	-	-
Vega Sicilia (VA)	E	11.3	461	1	1	1	1	-	-	-	-
Tordesillas (VA)	E	12.0	432	1	1	1	1	-	-	-	-
Portillo (VA)	E	11.6	470	1	1	1	1	-	-	-	-
Íscar (VA)	E	12.2	440	1	1	1	1	-	-	-	-
Cadalso (M)	E	13.6	687	1	1	1	1	-	-	-	-
Budia (GU)	E	12.9	530	1	1	-	-	-	-	-	-
Biar (A)	E	12.9	457	1	1	1	1	-	-	-	-
Tarazona de la Mancha	E	13.4	453	1	1	1	1	-	-	-	-
Parafrugell (GI)	E	15.3	665	1	1	1	1	-	-	-	-
Garrovillas (CC)	E	16.4	574	1	1	1	1	-	-	-	-
Doñana (CA)	E	17.5	575	1	1	1	1	-	-	-	-
Las Lomas (CA)	E	17.9	550	1	1	1	1	-	-	-	-
Cartaya (H)	E	18.1	500	1	1	1	1	-	-	-	-

MAT mean annual temperature; MAR mean annual rainfall; Presence at test sites: CUC Cucalón (Spain); TOR Tordesillas (Spain); TRE Trepaderne (Spain); QUI Quintos de Mora (Spain); LT Le Treps (France); StB Sainte Baume (France); LG La Gaillarde (France); TN Tunisia (Khaldi *et al.,* 2009).

III – Results and discussion

Height growth differed significantly between provenances, though this genetic variability (at each sites, 3-12% of the phenotypic variance) was quite minor than the common, stable reaction norm in dependence on site (among trials) and microsite, which produced strong spatial autocorrelations of tree height that masked those genetic differences.

Table 2. Adjusted mean height (cm) of 34 accessions at the test sites where they are present

Provenance	CUC	TOR	TRE	QUI	LT	StB	LG	Tun
Chalkidiki Agios Nic	173	468	107	200	390	142	142	196
Feniglia 67	228	482	-	-	420	156	142	213
Beit Mounzer (Mad. J)	198	468	-	-	400	159	153	214
Kornaele	190	441	100	196	405	145	135	-
Qsaibe	192	443	-	-	395	153	144	200
Bkassine	182	423	-	-	397	158	114	201
Antalya-Kumluca-Kalm	174	405	95	194	379	150	113	202
Yalova-Dumanlidag	185	455	78	196	392	-	132	-
Canakkale-Eceabat	190	456	101	199	393	142	124	204
Mugla-Yatagan-Katran	159	432	97	163	383	139	118	207
St. Raphaël (Var)	175	418	114	200	379	-	123	207
Villeneuvette (Hérau	174	456	101	196	396	149	128	210
Saintes-Maries (B.du	192	459	125	188	391	158	122	196
St. Aygulf (Var)	186	438	112	210	381	-	137	211
Brignoles- Le Val	204	470	121	200	415	163	134	217
Alcacer do Sal	183	434	107	179	-	-	-	-
Serra do Minho	186	480	122	193	-	-	-	-
Cogeces de Iscar (VA)	197	482	126	212	-	-	-	-
Valorio, fondo (ZA)	202	478	126	200	-	-	-	-
Valorio, ladera (ZA)	216	502	138	201	-	-	-	-
Toro (ZA)	211	481	150	207	-	-	-	-
Vega Sicilia (VA)	211	467	127	200	-	-	-	-
Tordesillas (VA)	192	473	128	200	-	-	-	-
Portillo (VA)	179	450	120	201	-	-	-	-
Íscar (VA)	200	461	127	214	-	-	-	-
Cadalso (M)	205	482	129	195	-	-	-	-
Budia (GU)	172	473	-	-	-	-	-	-
Biar (A)	193	468	107	224	-	-	-	-
Tarazona de la Mancha	193	484	112	202	-	-	-	-
Parafrugell (GI)	195	470	127	200	-	-	-	-
Garrovillas (CC)	170	435	112	185	-	-	-	-
Doñana (CA)	173	416	111	197	-	-	-	-
Las Lomas (CA)	190	442	97	209	-	-	-	-
Cartaya (H)	192	420	95	192	-	-	-	-
Adj. minimum mean height	159	405	78	163	379	139	113	196
Adj. minimum mean height	228	502	150	224	420	163	153	217
Observed minimum height	27	110	25	115	175	26	40	-
Observed máximum height	352	660	310	520	720	360	210	-
Site Mean	190	456	114	198	394	151	131	206

The residual analysis of a General Linear Model without spatial adjustment for each test site showed that the original experimental designs in blocks were not able to capture this variation at smaller scales. After applying instead of the pre-blocking nearest-neighbours adjustments (NNA), the spatial patterns accounted for 73-78% of the overall variance at each site, and the significance of the geographic genetic variation increased considerably (not so much due to changes in the adjusted provenance means than due to the huge reduction of the residual noise, thus narrowing confidence intervals), showing a common trend between sites.

At the Spanish test sites, the conclusions coincide with previous ones from 2003 (Mutke *et al.,* 2010): most provenances from inner Spain and one from France and the Italian one achieved the highest growths, whereas especially warmer, coastal provenances from southern Turkey, southern Spain and the French Côte d’Azur grew less (Table 2).

Applying a Principal Component Analysis of the adjusted mean tree heights for 29 provenances only from the four Spanish test sites, only the first component *CP1Sp* was retained. It accounted for 65% of the observed variance and correlated significantly with the mean annual temperature (MAT) in origin as climate proxy for each provenance (coefficient of correlation r = -0.68). The colder provenances did not only grow more, but showed also an earlier spring flush and higher tendency to form Lammas shoots (polycyclism) (Mutke *et al.*, 2010).

Analysing only the eight provenances present at all seven studied test sites in both countries, the first principal component accounted for 58% of the variance (55% when including the provenance means at Tunisian sites). This first principal component had a narrow correlation (0.98) with the values of *CP1Sp* from Spain and an negative correlation with mean annual temperature (MAT) in origin even stronger than the former variate (r=-0.82). Actually, the correlations of the adjusted provenance mean heights at each test with MAT ranged from -0.25 (La Gaillarde) to -0.65 (Tordesillas), -0.41 for the mean heights adjusted in Tunisia.

IV – Conclusions

In spite of the reduced molecular base of genetic variation in neutral molecular markers described for *Pinus pinea* (Vendramin *et al.*, 2008), the study revealed adaptative differences among populations, with the same trend observed already in a former analysis of the Spanish test sites, with lower growth in accessions from warmer origins (Mutke *et al.,* 2010). But the phenotypic plasticity of the species, especially sensitivity to soil variation, produced strong spatial autocorrelations of the response variables that masked greatly this variation, requiring nearest-neighbours adjustments for capturing the spatial pattern at each site.

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