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Deliverable D3.4

Trait proxy identification (relationship of phenotypes from WP2 and NIRS from WP3) & heritability estimates of trait proxy for the remaining species

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1 Summary

One of the objectives of WP3 is to forecast the adaptability of forest genetic resources by estimating selection gradient (trait-fitness relationships) and heritability (how much of the variation in a trait is due to variation in genetic factors) of selected traits of four species (*Populus nigra*, *Pinus pinaster*, *Fagus sylvatica*, *Pinus sylvestris*) collected in two natural populations growing in contrasted environments. To reach that objective, quantitative-genetic theory approaches are being used, which require three sets of data: phenotypic data, genetic data and environmental data. The originality of the project lies in i) estimating selection gradient and heritability in natural populations (as opposed to common gardens where the environmental conditions are controlled); ii) producing proxies of hard-to-measure traits (measured in WP2 in a subset of samples) in thousands of individuals from natural populations, via Near Infrared Spectra (NIRS). D3.4 is a follow up of D3.3 and at this stage of the project data production is heterogeneous across sites and species. We present results for the most advanced species, *Pinus pinaster* and *Populus nigra*.

2 Introduction

In situ estimates of selection gradient and heritability require the production, processing and integration of multiple datasets - namely genetic, phenotypic and environmental - in order to control for various factors that may bias the output. More specifically, there is a need to integrate relationship among individuals (via pedigree or genetic relationship matrix) as well as proxies to control environmental conditions. The production of these datasets requires the coordination of many partners and the use of various approaches:

- Phenotypic traits: hard traits (together with NIRS measurements) are produced by WP2 in a subset of 10 individuals across 15 populations distributed in the species distribution range. The relationship between hard traits and NIRS is then applied to the 500 individuals of each GCU in order to produce hard traits for the 500 individuals.
- Genetic data: genomic data are produced by WP4 for the 500 adults and 250 juveniles of each GCU. This data is used to estimate the genetic relationship across adults required to estimate the heritability. It is also used to produce individual fecundity estimates (i.e. fitness) comparing adults and juveniles genetic data.
- Environmental data: measured by Unmanned Aerial Vehicle - UAV (e.g. soil humidity, competition index, etc…), they are needed to control the effect of environmental heterogeneity in the field.

In natural populations, the spatial distributions of individuals could be not random and thus, environmental, spatial and genetic parameters could be correlated. Accurate information on the genetic relationship among individuals is essential to elucidate the genetic basis of complex traits and the capacity of the populations to genetically respond to environmental changes. Furthermore, considering the spatial distribution and the environmental resemblance among individuals improves the precision and accuracy of the estimated additive genetic variance.

Following the methodological framework employed by Alexandre et al., 2020 we considered the autocorrelation of the spatial distribution of the individuals and include the environmental variables collected by UAV flights in the populations as covariates in the models to account for the environmental effects over the resemblance of phenotypic values among individuals.

This procedure allow to better characterize the contribution of genes to the observed phenotypes.

3 Results

3.1 Trait proxy identification (relationship of phenotypes from WP2 and NIRS from WP3)

In agreement with WP2, 10 traits (measured on 10 trees from 15 GCUs = 150 trees) were contemplated within WP3, together with Near InfraRed Spectra (NIRS) (measured on 1000 trees from 2 GCUs = 2000 trees). The relationship between these traits and NIRS were explored in order to predict traits in 2000 trees. Besides these 10 hard traits, others soft traits are available: DBH, height, slenderness, crown size, seed count and wood density. Below we present the significant relationship for each species. Outcome for each species are presented below.

- *Pinus pinaster*

The available phenotypic traits for maritime pine are included in Table 1. Three of the traits are estimated by using UAV (HT, CA and Number of cones), 5 of them by direct measurement (HT, DBH, HSC, Slenderness and WD, where HT serve to check the values obtained by UAV) and four traits estimated successfully by NIRS (SLA, G_{min}, P12 and P50). Details for NIRS analyses (calibration and prediction) are available in D3.2.

Table 1. Description of the phenotypic traits used to estimate selection gradients in maritime pine.

Trait	Definition	Units	Origin
HT	Total height of the tree	m	Direct/UAV
DBH	Diameter at breast height (1.30 m)	m	Direct
Slen	Slenderness index (ratio of HT and DBH)		Direct
HSC	Height at the start of the crown	m	Direct
CA	Sun-exposed tree crown area	m^2	UAV
NC	Number of cones	n	UAV
WD	Wood density	kg/m3	Direct
SLA	Specific leaf area	m^2/kg	NIRS
G_{min}	Leaf minimum conductance	$mmol\ m^{-2}\ s^{-1}$	NIRS
P12	Xylem pressure inducing 12% loss of hydraulic conductivity due to embolism	MPa	NIRS

P50	Xylem pressure inducing 50% loss of hydraulic conductivity due to embolism	MPa	NIRS

- *Populus nigra*

The available phenotypic traits for *Populus nigra* are included in Table 2. One trait (HT) was estimated by using UAV, 2 of them by direct measurement (DBH, and Slenderness) and two traits estimated successfully by NIRS (SLA and P50). Details for NIRS analyses (calibration and prediction) are available in D3.2.

Table 2. Description of the phenotypic traits used to estimate selection gradients in maritime pine.

Trait	**Definition**	**Units**	**Origin**
HT	Total height of the tree	m	UAV
DBH	Diameter at breast height (1.30 m)	m	Direct
Slen	Slenderness index (ratio of HT and DBH)		Direct
SLA	Specific leaf area	m^2/kg	NIRS
P50	Xylem pressure inducing 50% loss of hydraulic conductivity due to embolism	MPa	NIRS

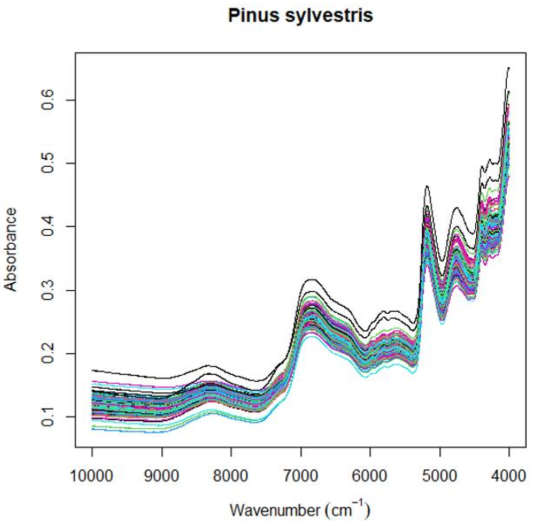
- *Pinus sylvestris*

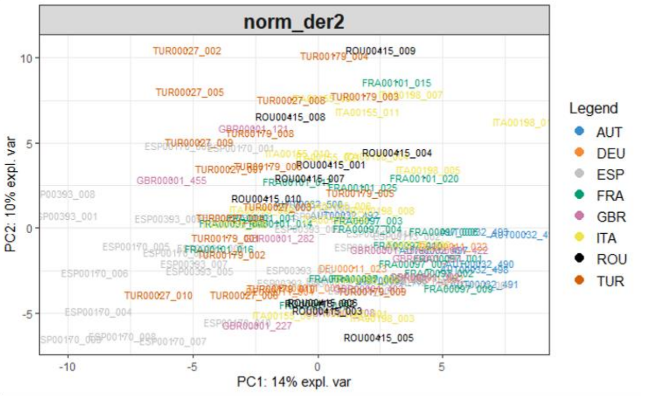
The available phenotypic traits for *Pinus sylvestris* are included in Table 3. One trait (HT) was estimated by using UAV, while two of them by direct measurement (DBH, and Slenderness) and two traits estimated successfully by NIRS (SLA and P50).

Table 3. Description of the phenotypic traits used to estimate selection gradients in Scots pine

Trait	Definition	Units	Origin
HT	Total height of the tree	m	Direct/UAV
DBH	Diameter at breast height (1.30 m)	m	Direct
Slen	Slenderness index (ratio of HT and DBH)		Direct
HSC	Height at the start of the crown	m	Direct
WD	Wood density	kg/m3	Direct

NIRS analyses (data acquisition, calibration and prediction) are ongoing on both short cores and branch segments using a benchtop spectrometer, the FT-NIR Perkin Elmer Frontier. Our exploration analysis revealed contrasted behaviour according to the country. We are currently finishing the acquisition of spectra on ground branches before developing the predictive models for traits of interest (10 traits measured in WP2).





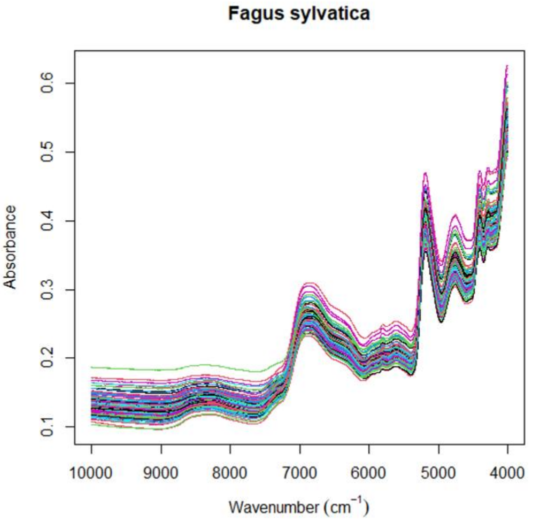
- *Fagus sylvatica*

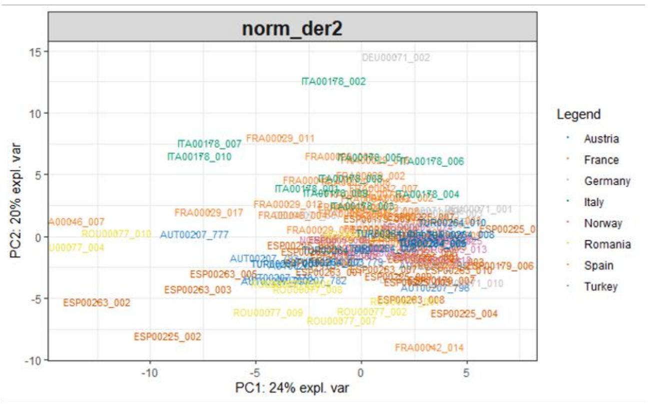
The available phenotypic traits for *Fagus sylvatica* are included in Table 4. One trait (HT) was estimated by using UAV, while two of them by direct measurement (DBH, and Slenderness).

Table 4. Description of the phenotypic traits used to estimate selection gradients in beech

Trait	Definition	Units	Origin
HT	Total height of the tree	m	Direct/UAV
DBH	Diameter at breast height (1.30 m)	m	Direct
Slen	Slenderness index (ratio of HT and DBH)		Direct
HSC	Height at the start of the crown	m	Direct
WD	Wood density	kg/m3	Direct

NIRS analyses (data acquisition, calibration and prediction) are ongoing on both short cores and branch segments using a benchtop spectrometer, the FT-NIR Perkin Elmer Frontier. Short core samples and branches were finely ground before collecting NIR spectra (64 scans per sample). Exploratory analysis has enabled us to highlight samples clustering by country and GCU. Identified outliers will be removed before the development of prediction models. We are currently finishing the acquisition of spectra on ground branches before developing the predictive models for traits of interest (10 traits measured in WP2).





3.2 In situ heritability estimates

Once phenotypic traits are available, and their relationship with fitness is demonstrated (through selection gradients, see 3.2), it is crucial to estimate how much phenotypic variance is genetically determined and heritable and able to respond to natural selection (i.e. heritability). Below we present the outcome for the two species for which we have complete datasets (genetic, phenotypic and environmental) at this stage. The methodology employed here will be easily applicable to the other GCUs included in WP3 once complete and curated datasets are available.

The methodology employed for this analysis can be found in Deliverable D3.3. Due to an improvement of precision on field coordinates and sex identification with molecular markers, results have changed from previous versions.

- *Pinus pinaster*

The estimates are shown in Table 5.

Table 5. Heritability estimates and confidence intervals [C.low- C.high] for the studied traits in the two *Pinus pinaster* GCUs. Null models included solely the genetic relationship matrix among individuals (GRM). Full models included the GRM plus splines correcting for the spatial position of the individuals and environmental variables related with temperature, precipitation and solar radiation. In bold, heritability estimates with C.low higher than 0.01.

	France		**Italy**	
Trait	Null model	Full model	Null model	Full model
Tree height				
	0.176	0.142	**0.468**	0.033
	[0.002-0.438]	[0.001-0.410]	[0.282-0.659]	[0.000-0.159]
Diameter at breast height				
	0.237	**0.287**	0.119	0.136
	[0.005-0.521]	[0.017-0.561]	[0.000-0.373]	[0.001-0.396]
Slenderness index				
	0.163	0.140	0.145	0.069
	[0.001-0.455]	[0.000-0.421]	[0.001-0.402]	[0.000-0.270]
Crown area				
	0.061	0.132	0.055	0.051
	[0.000-0.253]	[0.000-0.434]	[0.000-0.213]	[0.000-0.200]
Wood density				
	0.254	0.206	**0.246**	0.105
	[0.008-0.528]	[0.002-0.486]	[0.018-0.508]	[0.000-0.382]
Number of cones				
	0.167	0.148	**0.232**	0.199
	[0.002-0.438]	[0.001-0.431]	[0.018-0.483]	[0.005-0.456]
P50				
	0.290	**0.355**	**0.270**	**0.271**
	[0.017-0.571]	[0.040-0.638]	[0.083-0.483]	[0.070-0.497]
P12				
	0.120	0.158	**0.286**	**0.229**
	[0.000-0.390]	[0.000-0.488]	[0.092-0.504]	[0.027-0.468]
Specific leaf area				
	0.214	0.168	**0.446**	**0.362**
	[0.004-0.483]	[0.001-0.452]	[0.248-0.634]	[0.136-0.580]

- *Populus nigra*

For this species, we have used the traits defined in Table 2. We have found many potential factors that can have an impact on the estimates of heritability which we have decided to study in detail. In dioecious species such as *P. nigra*, the genetic architecture of traits may be different among sexes, thus, we performed the analysis not accounting for the sex of the individuals (Column *General* in Tables 6 and 7) and accounting for it (Columns *Female* and *Male* in Tables 6 and 7). Overall, we found that heritability estimates are higher in males than females in both populations.

Besides, the molecular analysis identified a notable number of clones within the sampled individuals. We performed separated analysis including and excluding clonal individuals in the genetic relationship matrix (GRM) to investigate how this affected the results. Overall, we found that including clones increased the heritability estimates and reduced the confidence intervals.

Table 6. Heritability estimates and confidence intervals [C.low- C.high] for the studied traits in the Austrian *Populus nigra* GCU. Separated analysis were performed including and excluding clonal individuals in the genetic relationship matrix (GRM). Null models included only the GRM. Full models included the GRM plus splines correcting for the spatial position of the individuals and environmental variables related with temperature, precipitation and solar radiation. In bold, heritability estimates with C.low higher than 0.01.

	Null model			**Full model**		
Trait	General	Female	Male	General	Female	Male
GRM with clones						
Tree height						
	0.622	**0.584**	**0.638**	**0.364**	0.173	**0.496**
	[0.481-0.735]	[0.336-0.773]	[0.456-0.776]	[0.169-0.538]	[0.002-0.454]	[0.269-0.674]
Diameter at breast height						
	0.335	0.114	**0.677**	**0.264**	0.105	**0.442**
	[0.136-0.517]	[0.001-0.333]	[0.448-0.832]	[0.043-0.465]	[0.001-0.325]	[0.207-0.630]
Slenderness index						
	0.382	**0.342**	**0.411**	**0.222**	0.200	**0.581**
	[0.248-0.513]	[0.117-0.540]	[0.239-0.579]	[0.036-0.399]	[0.005-0.437]	[0.280-0.782]
Crown area						
	0.553	**0.467**	**0.629**	**0.481**	**0.393**	0.205
	[0.379-0.694]	[0.190-0.689]	[0.382-0.794]	[0.277-0.648]	[0.097-0.640]	[0.008-0.432]
P50						
	0.556	**0.467**	**0.605**	**0.374**	0.186	**0.562**
	[0.414-0.675]	[0.195-0.672]	[0.435-0.741]	[0.179-0.542]	[0.000-0.523]	[0.279-0.758]
GRM without clones						
Tree height						
	0.428	**0.409**	**0.481**	0.215	0.24	0.332
	[0.176-0.655]	[0.062-0.797]	[0.104-0.847]	[0.002-0.526]	[0.002-0.676]	[0.007-0.790]
Diameter at breast height						
	0.202	0.319	0.151	0.120	0.186	0.165
	[0.004-0.440]	[0.006-0.731]	[0.001-0.511]	[0.000-0.395]	[0.001-0.657]	[0.000-0.573]
Slenderness index						
	0.484	**0.599**	**0.359**	0.342	**0.511**	0.263
	[0.223-0.717]	[0.201-0.921]	[0.031-0.776]	[0.007-0.702]	[0.014-0.999]	[0.001-0.808]
Crown area						
	0.422	**0.385**	**0.507**	0.133	0.127	0.391
	[0.162-0.683]	[0.025-0.820]	[0.084-0.899]	[0.000-0.465]	[0.000-0.536]	[0.002-0.938]
P50						
	0.170	0.067	**0.915**	0.112	0.048	0.782
	[0.000-0.515]	[0.000-0.324]	[0.441-1.000]	[0.000-0.404]	[0.000-0.265]	[0.165-0.998]

Table 7. Heritability estimates and confidence intervals [C.low- C.high] for the studied traits in the Spanish *Populus nigra* GCU. Separated analysis were performed including and excluding clonal individuals in the genetic relationship matrix (GRM). Null models included only the GRM. Full models included the GRM plus splines correcting for the spatial position of the individuals and environmental variables related with temperature, precipitation and solar radiation. In bold, heritability estimates with C.low higher than 0.01.

Trait	**Null model**			**Full model**		
	General	Female	Male	General	Female	Male
GRM with clones						
Tree height						
	0.234	0.071	**0.508**	0.039	0.074	0.105
	[0.015-0.482]	[0.000-0.335]	[0.165-0.775]	[0.000-0.192]	[0.000-0.424]	[0.000-0.384]
Diameter at breast height						
	0.047	0.139	0.142	0.047	0.151	0.128
	[0.000-0.253]	[0.000-0.725]	[0.000-0.546]	[0.000-0.252]	[0.000-0.653]	[0.000-0.493]
Slenderness index						
	0.289	0.195	**0.409**	0.114	0.095	0.234
	[0.021-0.543]	[0.001-0.549]	[0.053-0.709]	[0.000-0.389]	[0.000-0.417]	[0.002-0.581]
Crown area						
	0.034	0.03	0.263	0.046	0.064	0.115
	[0.000-0.167]	[0.000-0.161]	[0.001-0.675]	[0.000-0.175]	[0.000-0.256]	[0.000-0.480]
P50						
	0.348	0.198	**0.462**	0.137	0.116	0.262
	[0.102-0.566]	[0.004-0.546]	[0.082-0.723]	[0.001-0.388]	[0.000-0.509]	[0.001-0.617]
GRM without clones						
Tree height						
	0.182	0.085	**0.399**	0.035	0.081	0.075
	[0.002-0.438]	[0.000-0.386]	[0.024-0.761]	[0.000-0.172]	[0.000-0.396]	[0.000-0.327]
Diameter at breast height						
	0.050	0.203	0.101	0.052	0.220	0.095
	[0.000-0.258]	[0.000-0.790]	[0.000-0.434]	[0.000-0.267]	[0.000-0.827]	[0.000-0.417]
Slenderness index						
	0.179	0.145	0.355	0.092	0.103	0.241
	[0.001-0.484]	[0.001-0.514]	[0.010-0.798]	[0.000-0.375]	[0.000-0.476]	[0.001-0.704]
Crown area						
	0.022	0.029	0.146	0.047	0.065	0.125
	[0.000-0.116]	[0.000-0.157]	[0.000-0.578]	[0.000-0.181]	[0.000-0.249]	[0.000-0.523]
P50						
	0.342	0.271	0.400	0.140	0.158	0.269
	[0.075-0.593]	[0.007-0.712]	[0.006-0.820]	[0.001-0.402]	[0.000-0.644]	[0.000-0.791]

3.3 Delay and corrective measures

As presented in the Second Periodic Report (M36), datasets in WP3 have been produced at different paces depending on the species. We decided to develop the methodologies on two species in parallel (*Pinus pinaster* and *Populus nigra*) so that we could apply them readily on the two others species (*Pinus sylvestris* and *Fagus sylvatica*) upon completion of data production. At this stage of the project we have the following datasets:

- *P. pinaster*: since genetic, phenotypic and environmental data are available. We were able to estimate NIRS trait proxies as well as heritability estimates.
- *P. nigra*: since genetic, phenotypic and environmental data are available. We were able to estimate NIRS trait proxies as well as heritability estimates.
  P. sylvestris and F. sylvatica: environmental data from UAV will be available by the end of the Sumer 2024. Genetic and phenotypic data are available. However, because of the methodological limitations encountered with *P. pinaster* samples (see Report M36 on Trait Proxy Identification), NIR spectra acquisition on both species had to be interrupted in order to finalize NIRS prediction for *P. pinaster*, one of our model species. NIRS acquisition and calibration are ongoing and we expect to finalize NIRS prediction by autumn 2024. Once the datasets will be available, the methodologies will be readily applied to the remaining species.

4 Conclusions

We have shown for two of the four species included in the WP that we are able to combine different datasets and methods to successfully estimate phenotypic traits of importance in the adaption of tree populations together with their heritability. We observe significant heritability values for most of the traits depending on the model used. These results highlight important local and species effects.

These results should be confirmed with the other species as soon as the data will be available. Of particular interest are the effect of species and site effects in the estimation of the adaptive potential of the populations and their implications in conservation of the genetic resources.

5 Partners involved in the work

INRAE, INIA-CSIC, CETEMAS, CITA, CNR, UMR, BFW, UK-CEH, LUKE, GIS, CREAF