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Phenotypic plasticity of the bud set process in a *Populus nigra* L. mapping pedigree and characterization of phenology in a *P. nigra* European association population

Settore Scientifico-Disciplinare AGR/05
Assestamento Forestale e Selvicoltura

Coordinatore:
Prof. Paolo De Angelis

Tutore:
Dr. Maurizio Sabatti

Dottorando:
Francesco Fabbrini

Co-Tutore:
Dr. Catherine Bastien
Dr. Muriel Gaudet

A Loretta,

… che è bella
come il primo fiocco di neve
che vede un bambino …

Abstract
Among forest trees, poplars are important components of riparian ecosystems and are now accepted by the scientific community as ideal model to study perennial plants. They offer several advantages as a model system, including rapid growth, ease of cloning, prolific sexual reproduction, small genomic size and facile transgenesis. This study has the objective to contribute to the knowledge of the genetic control of bud set (apical bud formation in the fall) in black poplar (*Populus nigra*). For this purpose the genetic variability and the broad-sense hereditability at individual and genotypic level, have been examined in a full-sib family (POP5) and in different european natural metapopulations of black poplar grown in a common garden experiment. In addition QTLs (Quantitative Trait Loci) analysis for the most discriminative traits of phenotypic variation in POP5 has been done using a genetic linkage map produced using 154 genotypes. The full-sib family was obtained crossing two parents selected in the germplasm collection (DISAFRI – University of Tuscia) from Italian natural populations and divergent for phenology and other adaptive traits. The full-sib family was planted in two sites in central (Viterbo 42°25'N, 12°05'E) and northern Italy (Cavallermaggiore 44°42'N, 07°40'E). The european natural populations were sampled in 4 different countries and 814 genotypes, from 14 metapopulations were planted in a common garden study in Northern Italy (Savigliano 44°36'N, 07°37'E). A randomized block design was defined for the establishment of the experimental plantations in each site. Six complete blocks were used with one replicated genotype randomly assigned to each block. The phenological study has been realized on the basis of a protocol designed to monitor six crucial phenological phases of bud set in black poplar. Data analysis have allowed to decompose the contribution of the different phases to the dynamics of bud set. Results showed that the onset of growth cessation is a quantitative trait under strong genetic control. Night length is the most important signal triggering the physiological process, but the role of other environmental factors such as temperature increase during the process. Taking advantage of two contrasting experimental sites a considerable role of GxS interactions has been found in all the different phenological phases and the low temperature seems to influence the sensitivity of some more plastic genotypes. The large number of QTLs mapped in the POP5 genetic map, each one characterized by small or modest effect, highlighted the complex nature of traits involved in the apical bud formation-maturation process.

Key-words: *Populus nigra* L., GxS interaction, photoperiod, bud set, heritability.

Riassunto
Tra le specie forestali i pioppi risultano essere un'importante componente degli ecosistemi ripariali e sono ad oggi accettati dalla comunità scientifica come modello ideale per lo studio delle piante perenni. Per tale scopo questo genere offre molti vantaggi come il rapido accrescimento, la facilità di clonazione, la semplice riproduzione gamica, un genoma di dimensioni ridotte e un'agevole produzione di organismi transgenici. L'obiettivo di questo lavoro è contribuire alla conoscenza del controllo genetico del bud set (formazione della gemma apicale autunnale) in pioppo nero (*Populus nigra* L.). A tale scopo sono state esaminate la variabilità genetica e l'ereditabilità in senso lato, a livello individuale e genotipico, in una famiglia full-sib di pioppo nero (POP5) e in differenti metapopolazioni europee della medesima specie cresciute nello stesso impianto sperimentale (common garden). In POP5 sono stati inoltre mappati i QTLs (Quantitative Trait Loci) limitatamente ai caratteri maggiormente discriminanti della variabilità fenotipica sulla base di una mappa genetica prodotta utilizzando 154 genotipi. La famiglia full-sib è stata ottenuta dall'incrocio di due parentali divergenti per fenologia ed altri caratteri adattativi e selezionati in una collezione di germoplasma (DISAFRI – Università della Tuscia) composta da popolazioni naturali raccolte in Italia. La famiglia full-sib è stata piantata in due siti, rispettivamente nell'Italia centrale (Viterbo 42°25'N, 12°05'E) e settentrionale (Cavallermaggiore 44°42'N, 07°40'E). Le popolazioni europee sono state raccolte in 4 differenti Paesi e 814 genotipi, provenienti da 14 metapopolazioni, sono state piantate nello stesso sito sperimentale dell'Italia settentrionale (Savigliano 44°36'N, 07°37'E). In ciascun sito è stato definito un disegno sperimentale a blocchi randomizzati. Sono stati utilizzati sei blocchi ed una replica di ciascun genotipo è stata assegnata casualmente a ciascun blocco. Lo studio fenologico è stato condotto sulla base di un protocollo realizzato allo scopo di monitorare sei specifiche fasi fenologiche caratterizzanti il bud set in pioppo nero. L'analisi dei dati ha permesso di scomporre il contributo delle differenti fasi nella dinamica del bud set. I risultati hanno mostrato che l'innesco della cessazione dell'accrescimento è un carattere quantitativo caratterizzato da un forte controllo genetico. La lunghezza della notte risulta essere il segnale più importante nell'innesco del processo fisiologico, ma il ruolo di altri fattori ambientali come la temperatura aumenta durante il processo. Grazie alla possibilità di studiare due siti sperimentali contrastanti si è potuto constatare un ruolo considerevole dell'interazione GxS in tutte le diverse fasi fenologiche e la bassa temperatura sembra influenzare la sensibilità di alcuni genotipi più plastici. L'alto numero di QTLs mappati nella mappa genetica di POP5, ciascuno caratterizzato da un basso o modesto effetto, sottolineano la complessa natura dei caratteri coinvolti nel processo di formazione-maturazione della gemma apicale.

Parole chiave: *Populus nigra* L., interazione GxS, fotoperiodo, bud set, ereditabilità.

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INTRODUCTION

Poplars are considered the fastest growing trees under temperate zones and are broadly used to produce a variety of wood-based products including paper, plywood, matches, light packaging materials or wood for bioenergy (Hansen, 1991; Heilman *et al.*, 1994; Zsuffa *et al.*, 1996). Among forest trees, they are important components of riparian ecosystems and are now accepted by the scientific community as a woody model tree which is necessary needed to study the unique processes that occur in perennial plants, such as secondary wood formation, bud set, bud flush and dormancy (Taylor, 2002). Poplars offer several advantages as a model system, including rapid juvenile growth (Bradshaw *et al.*, 2000 ; Dickmann, 2001b), high levels of natural variations in wild populations (Jansson and Douglas, 2007), easy of sexual and asexual propagation (Stettler and Bradshaw, 1996), small genomic size (about 550 million base pairs, 4× larger than *Arabidopsis* and one fortieth the size of the *Pinus* genome) (Taylor, 2002 ; Boerjan, 2005 ; Tuskan *et al.*, 2006), facile transgenesis, plant transformation and regeneration capabilities (Wullschleger *et al.*, 2002).

Populus nigra L. is a tree of ecological, social, and economic interest. It is a species of riparian woodlands that has been planted for centuries in urban areas as ornamental and landscaping and as windbreak and shelterbelt in rural areas. It is well suited also for riparian buffers for interception of contaminants before they reach the stream and for the control of erosion of banks streams and rivers maintaining a more natural landscape and saving money due to the low cost natural plant materials (Isebrands and Karnosky, 2001). *P. nigra* is one of the most important parents to produce euramerican hybrids in breeding programs to increase wood production and to be used in short rotation intensive culture.

Despite the enormous environmental advantages, the *P. nigra* riparian forests have been largely lost due to the spread of agriculture and other human activities. The genetic diversity, that is considered to be important for long-term survival of natural populations and for the ability to adapt to changing environmental conditions (Booy *et al.*, 2000), is seriously threatened (Cagelli and Lefèvre, 1995 ; Lefèvre *et al.*, 1998). Therefore, studies are necessary to get a better knowledge of the remaining natural populations of *P. nigra* to conserve and restore natural riparian ecosystems.

In the case of *Populus* several traits are likely to be important for local adaptation and phenology permits the trees to find the right compromise solution between the necessity to avoid the risk of frost damage, if the trees set buds too late or flush too early (Luquez *et al.*,

2008), and assure the maximization of the growth period (Kramer, 1996), especially for the short growing season of the northern latitudes. As a trait directly related to cold adaptation, the characterization of growth cessation is an important tool for the identification of ecotypes for conservation strategies, delineation of breeding zones and selection in breeding programs (Howe *et al.*, 2003 ; Rohde *et al.*, 2011). Daylength is the primarily used environmental signal for the growth cessation (Rohde and Bhalerao, 2007) even if the timing of bud formation are also influenced by other factors such as temperature (Kalcsits *et al.*, 2009), temperature x photoperiod (x population) interaction (Håbørg, 1972 ; Tanino *et al.*, 2010), nutrition and drought (Rohde *et al.*, 2000 ; Howe *et al.*, 2003). Given the influence of temperature on the phenology of trees, the cessation of growth and the bud formation may be affected by globally climatic warming and increase the risk of frost damage, the survival and eventually the distribution of forest trees, caused by a less closely adaptations to the local altered environment (Kramer, 1996 ; Kramer *et al.*, 2000).

In the light of these considerations, research efforts in order to understand the genetic basis of growth cessation and bud formation are of paramount importance. Besides, in this context efforts are required also to investigate the role of phenotypic plasticity, a source of variability that can contribute to short-term adaptation to a changing climate regime (Rohde *et al.*, 2011), and to evaluate the possibility of temperature-mediated plasticity in some genotypes that are more adapted to specific conditions.

The main objective of this work is to contribute to the knowledge of the genetic control of bud set applying a new detailed scoring system, analysing phenotypic plasticity of the bud set process in a full-sib family grown in two contrasting site and detecting quantitative trait loci (QTLs) for the most discriminative traits in term of phenotypic variation.

For these purposes the genetic variability (CV_g) and the broad-sense hereditability (H^2) at individual and genotypic level have been examined in a full-sib family of black poplar (POP5) and in different european natural metapopulations of the same species grown in a common garden experiment. The full-sib family was obtained from parents selected in a germplasm collection (DISAFRI – University of Tuscia) from Italian natural populations and divergent for phenology and other adaptive traits. The full-sib family was planted at two sites in central (Viterbo; 42°25’N, 12°05’E) and in northern (Cavallermaggiore; 44°42’N, 07°40’E) Italy. The european natural populations were sampled in 4 different countries and 814 genotypes, from 14 metapopulations, were planted in northern Italy (Savigliano; 44°36’N, 07°37’E). A randomized complete block design was defined for the establishment

of the experimental plantations in each site. Six complete blocks were used with one replicated genotype randomly assigned to each block. QTL analysis was based on 154 individuals. A genetic linkage map for the full-sib family POP5 has been produced by Gaudet and co-workers (2008) using MapMaker software version 3.0 (Lander *et al.*, 1987) and improved after the addition of new genotypes (154 vs. 92) (Gaudet, personal communication). The phenological study has been realized on the basis of a protocol (Rohde *et al.*, 2011) developed inside the POPYOMICS project (contract No. QLK5-CT-2001-00953, an EC funded research program: http://www.soton.ac.uk/~popyomic/) in order to improve the phenotypic resolution of the distinctive phases observed during the formation of the apical bud in autumn and to evaluate the duration of bud development as separate character.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/ 2007-2013) under the Project NOVELTREE (grant agreement n° 211868, May 2008 – April 2012). The project has been undertaken by fifteen european partners, from seven countries, within six work-packages. The main aim of this project is i) develop genetic tools for forest tree breeding and optimized management of genetic resources, especially high throughput phenotyping and genotyping for adaptive and productivity-related traits of interest, and ii) demonstrate novel/improved methods to breed trees with improved quality and productivity (other information on the web site: www.noveltree.eu).

1. STATE OF THE ART

1.1. Populus nigra L.

1.1.1. Taxonomy

Populus nigra L., commonly known as black poplar, is a pioneer angiosperm of the genus *Populus* L. (aspen, cottonwood, and poplars) which belongs, as well as the genus *Salix* L., to the family *Salicaceae*. The taxonomy hierarchy is:

Division	MAGNOLIOPHYTA
Class	MAGNOLIOPSIDA
Subclass	DILLENIIDAE
Order	SALICALES
Family	SALICACEAE
Genus	POPULUS
Species	POPULUS NIGRA

The genus includes nearly thirty species, according to a recent classification, but in the literature the number varies from 22 to about 85 due to the difficulties in delineating species boundaries and the misinterpretation of some hybrid (Eckenwalder, 1996 ; Dickmann, 2001a). The genus is naturally distributed in the temperate and cold regions of the northern hemisphere, from 30° to 70° of latitude, including Europe, Asia, north-Africa and America. Only a few numbers of species grow in subtropical areas and only *Populus ilicifolia* Engl., which is endemic to Kenya, grows in the equatorial region of East Africa (Browicz, 1966 ; Oballa, 1996).

Populus L. is historically divided into 5 morphologically and ecologically distinct sections which subsequently, with the addition of *Abaso*, have been brought into 6 (Table 1). The major barriers to hybridization in the genus lie between sections whereas poplar hybrids occur naturally between species within the same sections and between species in some combinations of sections (Figure 1) (Zsuffa, 1975 ; Eckenwalder, 1996 ; Dickmann, 2001a ; Rajora and Zsuffa, 1984).

Table 1. Proposed taxonomic classification of the genus *Populus* (Dickmann and Kuzovkina, 2008).

[b] Common names vary considerably depending upon language and locality.

[c] Formerly section *Leuce*.

Section	Taxon	English common name[b]	Notes and synonyms
Abaso	*P. mexicana* Wesmael	Yaqui cottonwood	Monotypic section
Turanga (Afro-Asian poplars)	*P. euphratica* Olivier	Euphrates poplar	Includes *P. diversifolia*
	P. ilicifolia (Engler) Rouleau	Kenyan poplar	Formerly synonymous with *P. euphratica*
	P. pruinosa Schrenk	Desert poplar	Formerly synonymous with *P. euphratica*
Leucoides (Swamp poplars)	*P. glauca* Haines	Asian swamp cottonwood	Formerly *P. wilsonii*
	P. heterophylla Linnaeus	Swamp cottonwood	
	P. lasiocarpa Oliver	Heart-leaf poplar	
Aigeiros (Cottonwoods, black poplar)	*P. deltoides* Marshall	Eastern cottonwood	Includes *P. sargentii*, *P. palmeri*, and *P. wislizenii*
	P. fremontii S. Watson	Fremont cottonwood	Includes *P. arizonica*
	P. nigra Linnaeus	Black poplar	
Tacamahaca (Balsam poplars)	*P. angustifolia* James	Narrowleaf cottonwood	
	P. balsamifera Linnaeus	Balsam poplar	Formerly *P. tacamahaca*
	P. cathayana Rehder	Cathay poplar	May be synonymous with *P. suaveolens*; includes *P. purdomii*
	P. ciliata Royle	Himalayan poplar	Heretofore in section *Leucoides*; the former *P. tristis* may be a hybrid with this species
	P. koreana Rehder	Korean poplar	Likely synonymous with *P. suaveolens* or *P. maximowiczii*
	P. laurifolia Ledebour	Laurel poplar	
	P. maximowiczii Henry	Japanese poplar	May be synonymous with *P. suaveolens*; includes *P. ussuriensis*
	P. simonii Carrière	Simon poplar	Includes *P. przewalskii* and *P. kangdingensis*
	P. suaveolens Fischer	Siberian poplar	
	P. szechuanica Schneider	Szechuan poplar	
	P. trichocarpa Torrey & Gray	Black cottonwood	May be synonymous with *P. balsamifera*
	P. yunnanensis Dode	Yunnan poplar	
Populus[c] (White poplars and aspens	*P. alba* Linnaeus	White poplar	
	P. guzmanantlensis Vazquez & Cuevas	Manantlán poplar	May be synonymous with *P. simaroa*
	P. monticola Brandegee	Baja poplar	A.k.a. *P. brandegeei*; may be naturalized *P. alba* var. *subintegerrima*
	P. simaroa Rzedowski	Balsas poplar	
	P. adenopoda Maximowicz	Chinese aspen	
	P. gamblei Haines	Himalayan aspen	
	P. grandidentata Michaux	Bigtooth aspen	
	P. sieboldii Miquel	Japanese aspen	Includes *P. jesoensis*
	P. tremula Linnaeus	Common aspen	Includes *P. davidiana* and *P. rotundifolia*
	P. tremuloides Michaux	Quaking aspen	

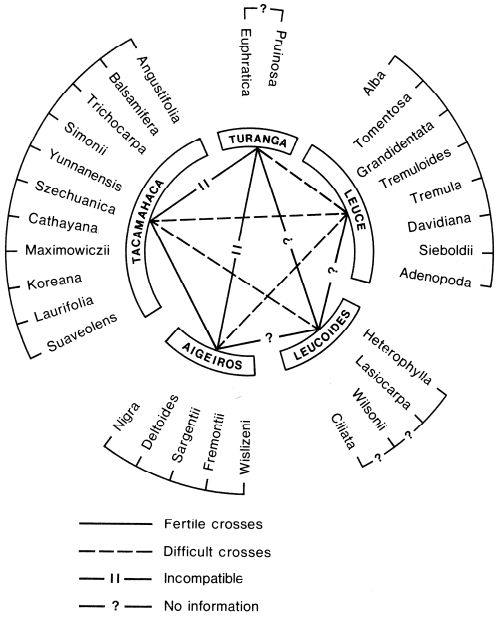


Figure 1. Interspecific breeding in the genus *Populus* (Zsuffa, 1975).
URL: http://na.fs.fed.us/spfo/pubs/silvics_manual/volume_2/populus/populus.htm

The sections *Aigeiros* and *Tacamahaca*, between which natural hybridization occurs (Zsuffa, 1975 ; Eckenwalder, 1984 ; Floate, 2004), comprise most of the species of economic importance. *Populus nigra* L. belong to the first section that includes also *Populus deltoides* Marshall, an important commercial timber species particularly in the southern U.S.. It has a substantial predisposition to make hybrid with *Populus trichocarpa* Torr. & Gray (producing *Populus x generosa* Henry) and *Populus nigra* L. (producing *Populus x canadensis* Moench), largely utilized in poplar cultures. However, the placement of *Populus nigra* L. into section *Aigeiros* remains controversial because its chloroplast DNA has ties to *Populus alba* L. of section *Populus* (Smith and Sytsma, 1990), it has a genetic affinity to the other species of section *Tacamahaca* and it is not clearly more similar to the northern American cottonwoods placed into the same section than some species of the balsam poplars in the section *Tacamahaca*. *Aigeiros* and *Tacamahaca* are two sections vegetatively and ecologically readily distinguishable but there are no clear differences in flowers and inflorescences between them (Eckenwalder, 1996). The taxonomy of the species is particularly complex because the wide range of distribution and the human responsibility in the diffusion of the species facilitate intermediate forms of spontaneous hybrid among varieties. In addition, different synonymous for the same varieties exist (Cagelli and Lefèvre, 1995).

The varieties known are: *P. nigra* var. *nigra* Schneid, *P. nigra* var. *italica* Duroi, *P. nigra* var. *betulifolia* (Pursh) Torr., *P. nigra* var. *pubescens* Parl. (= *P. nigra* var. *caudina* Ten.), *P. nigra* var. *thevestina* Dode, *P. nigra* var. *neapolitana* Ten., *P. nigra* var. *sinensis* Carr., *P. nigra* var. *sosnowskyi* Grossheim., *P. nigra* var. *afghanica* Aiton and Hemsl (Vietto, 2009).
More researches are needed to improve the resolution of poplar classification at section and species levels.

1.1.2. Distribution

Black poplar has a wide natural distribution area that extends from Ireland and the British Isles in the west to Kazakhstan and China in the east. It naturally grows all over Europe, from the Mediterranean in the south to approximately 64° latitude in the north, western Asia, form 30° latitude (Pakistan) in the south and 64° latitude (Russia, Severnaya river) in the north, and north Africa (Lefèvre *et al.*, 2001 ; Vietto, 2009 ; Vanden Broeck, 2003) (Figure 2).

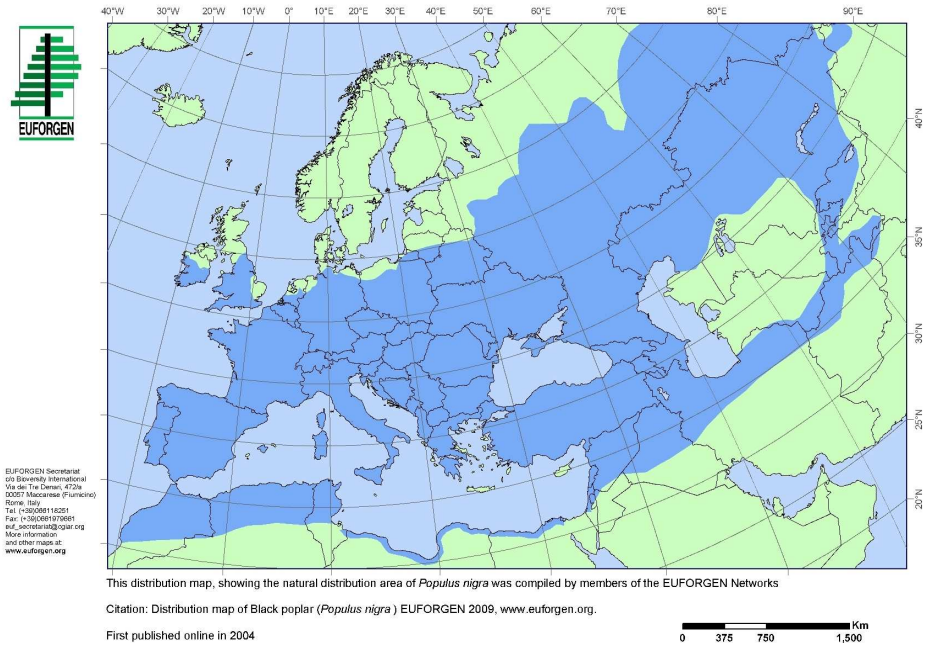


Figure 2. Distribution map of black poplar (*Populus nigra* L.), EUFORGEN 2009.
URL: http://www.euforgen.org/distribution_maps.html

In Italy the black poplar grows in all the national territory in cold and medium *Lauretum* and warm and cold *Castanetum* phytoclimatic zones.

Due to the fact that is an azonal species not linked to particular climatic area but only to the soil moisture, this native species grows along riparian ecosystem between the sea level up to 1.200 m a.s.l. (mountain range system of Alpi), 1.660 m a.s.l. (mountain range system of Appenini) (Vietto, 2009).

1.1.3. Biology and ecology

European black poplar is a typical heliophilous pioneer tree species that colonize open areas on european alluvial soils forming floodplain ecosystems. In softwood floodplain forests *P. nigra*, together with *Salix* species, plays an important role in the initial phase of development and it is a keystone species because it is highly adapted to water dynamics and sediment movement.

P. nigra is a rapid growth tree that can reach heights of 40 m and diameters over 2 m at maturity; individual trees can live 300 years (Weisgerber, 1999). It has a wide, rounded crown and a yellow-white juvenile bark that become dark grey and deeply fissured with age with many swellings on the trunk. The leaves of *Populus nigra* L. are dimorphic; pre-formed leaves are small (15-95 cm^2), dark green in colour, rhombic in shape, with a slightly lighter under-surface whereas neo-formed leaves are larger, broader than long, and oval in shape (Dickmann and Kuzovkina, 2008). Leaves has the lower angles rounded and the upper angle pointed, smooth on both sides, bluntly toothed margin and long and slender foot-stalks, so that they wave and flutter with the slightest draught of wind.

P. nigra is a dioecious and obligatory out-crossing species with female and male flowers on separate uni-sexual individuals. They reach reproductive age at 10-15 years old, even if a remarkable quantities of seed are generally produced after 20 years old (Stanton and Villar, 1996), but may be delayed by unfavourable environmental conditions. Males commonly initiate flowering before females, ensuring that pollen is in the air when the first females are receptive (Farmer and Pitcher, 1981). Both sexes flower in the early spring (March - April), approximately 1-2 weeks prior to leaf emergence (except in some subtropical species), during the flood peak period. Black poplar produces flowers from specialized buds containing preformed inflorescences, which are clustered in <10 cm pendulous green racemes (catkins, aments) on females and smaller reddish-purple on males (Figure 3), primarily located in the upper tree crown (Barsoum, 2001).

Significant variation exist within and between species concerning the time and duration of flowering that are related to both photoperiod and ambient temperatures (Pauley, 1950): at higher latitude and altitude flowering is delayed while under longer growing season and wormer conditions it occurs earlier.

Figure 3. *P. nigra* leaves, flowers, catkins and seeds: 1. Branch with male catkins (a - f) and a closed terminal vegetative bud (k). - 2. Branch with female catkins (g - i) and closed terminal vegetative bud (k). - 3. Branch with ripe fruit-catkins (a, b). - 4. Seedling with cotyledons and first ordinary leaves. - 5. Winter-branch. Photos and explanations from Borzan (2001).
URL: http://www.forestryimages.org/browse/detail.cfm?imgnum=1379035

Wind-dispersed pollen fertilizes ovule within 24 hours of arrival upon the receptive stigma and subsequent ovule ripening, seed maturation and dispersal occurring within 3-6 weeks following fertilization**.** Mature catkins are made up of many capsules, approximately 20-50, each developed from an individual flower, with about 4–5 small seeds (2 mm) per capsule with little or no endosperm (Braatne *et al.*, 1996).

Seed dispersal typically occurring in late spring and it is timed to coincide with the presence of ideal conditions for seed germination and seedling establishment after the declining of river flows and the exposure of freshly barren alluvial deposits that are ideal microsites for colonization by *P. nigra* via wind- and water-disseminated seed. The opening of fruit capsule and release of seeds can be either gradual or very rapid. The short seed life, generally less than 2 weeks following seed dispersal under natural conditions, is an important limiting factor in the black and once a seed became wet, viability will be lost in 2-3 days (Braatne *et al.*, 1996).

P. nigra is affected at all stages of its life cycle by hydrological conditions and the water availability of soil moisture is fundamental for successful regeneration, but prevailing anoxic conditions will be fatal. Poplar species are also capable of asexual (vegetative) reproduction that occur after damage to parent promoted by flood disturbances or extended periods of submergence that stimulate dormant primordia in roots and shoots to produce new shoots and roots (Barsoum, 2001).

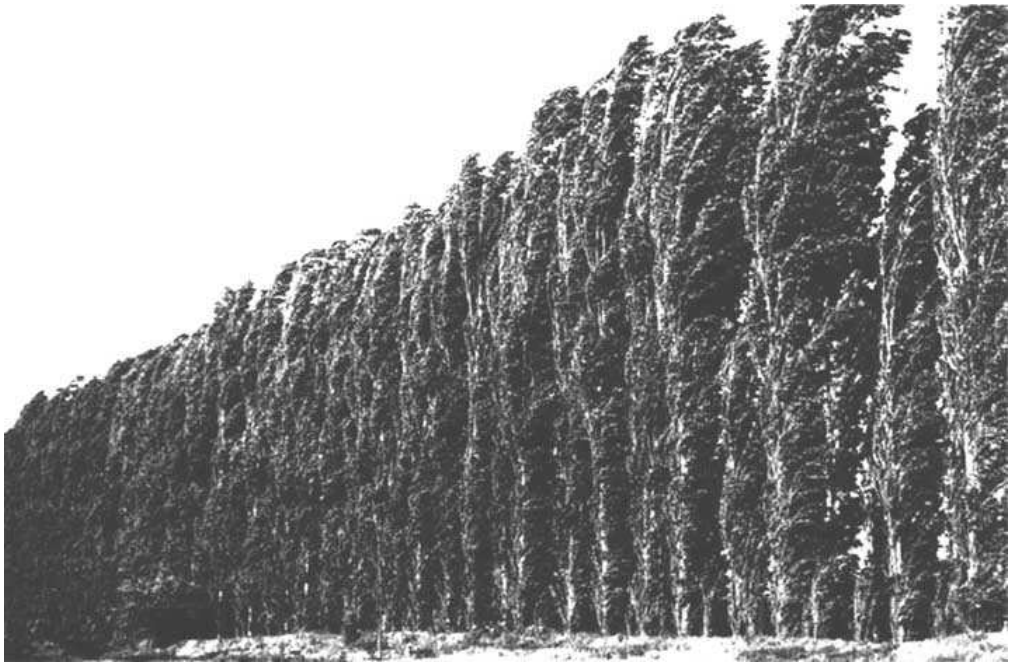
1.1.4. Importance and uses

1.1.4.1. Ecological importance of *Populus nigra* L.

Populus nigra L. has been planted for environmental purposes for centuries. Trees were commonly used for amenity plantings in urban areas as ornamentals and landscaping and as windbreaks and shelterbelts in rural areas.

Urban amenity planting provide environmental benefits like moderation of winter and summer climate, protection from heavy or cold wind, decrement of noise pollution, creation of wildlife habitat, improvement of the air quality and good psychological effect on people that feel more peaceful and serene. Farmstead windbreaks protect farm homes, buildings and orchards from winter winds (Picture 1), decrease noise pollution and dust, provide beautify landscape and wildlife habitat. Field shelterbelts protect crops by decreasing moisture loss and

soil erosion, increase crop yields by up to 20% by decreasing stress on the plants, increase biodiversity and populations of beneficial insects and produce biomass for wood and energy.
For these purposes it is essential the choice of the appropriate clone for the intended site and use, especially in order to ensure high levels of survival and growth. In the rural contest poplars are well suited also for riparian buffers for interception of contaminants before they reach the stream and, at the same time, provide improved wildlife habitat, increased biodiversity and decreased flood damage.
In both rural and urban contest poplars can be used to control erosion of banks streams and rivers maintaining a more natural landscape and saving money due to the low cost natural plant materials (Isebrands and Karnosky, 2001).



Picture 1. Row of *Populus* acting as windbreak near Oriashoro, Bulgaria.
URL: http://www.fao.org/docrep/62737e/62737e19.jpg

Poplars are also being used to recover polluted sites with heavy metals, pesticides, chlorinated solvents, hydrocarbons, excess nutrients and others pollutants because they can remove, degrade or contain chemical contaminants located in the soil by phytoremediation (Picture 2). 1.4 million sites in the EU have been identified as contaminate with organic and inorganic pollutants (Reichenauer, 2001) and this relatively new emerging technique is seen with interest by the world of research because is a promising cleanup solution for a wide variety of

pollutants and sites. Moreover, it is cheaper than conventional techniques (Chappell, 1997), like removal of the contaminated soil followed by the technical extraction of the pollutant or by deposition of the contaminated soil in a landfill, especially for a broad polluted area.

At last, but not less important, all these forms of poplar's utilization contribute also to carbon sequestration and provide a natural mitigation strategy in the storage of carbon dioxide (CO_2) in biomass and soil (Isebrands and Karnosky, 2001). For example, concerning the rural shelterbelt Kort and Turnock (1996) reported that a poplar 2.5 m spacing between trees shelterbelt would have an average above ground biomass of 174.8 tonnes per kilometre and would contain 84.2 tonnes of carbon.

Picture 2. Phytoremediation study using *Populus*.
URL: http://ideonexus.com/2008/05/02/let-the-phytoremediation-begin/

Despite the enormous environmental advantages the black poplar riparian forests, that naturally evolve towards hardwood formations (Vanden Broeck, 2003) and that are among the most biologically diverse in the area of the species (Dickmann and Kuzovkina, 2008), have been largely lost due to the spread of modern civilization. Various anthropic perturbations, like the management of riverbanks, channelization, canal and dyke construction, intensive grazing, wood cutting, expanding agricultural activities and the large utilization of the euramerican hybrids, black poplar natural habitat have been lost and genetic diversity, that is considered to be important for long-term survival of natural populations and for the ability to

adapt to changing environmental conditions (Booy *et al.*, 2000), is seriously threatened (Cagelli and Lefèvre, 1995 ; Lefèvre *et al.*, 1998).

Therefore, studies are necessary to get a better knowledge of the remaining natural populations of *P. nigra* and efforts to improve of poplar image in the public perception (Cagelli and Lefèvre, 1995) in order to conserve and restore natural riparian ecosystems.

Restoration programs will involve different approaches related to flow regulation, land-use policies, stream channel restoration and revegetation using a range of genotypes of native species in order to promote diversity (Braatne *et al.*, 1996). In this sense two main projects, "EUFORGEN" *P. nigra* network" and "EUROPOP", were started in order to work on the evaluation of the existing biodiversity of natural populations with the objectives of conservation and the restoration of the remaining natural riparian ecosystems.

1.1.4.2. Economic interest of *Populus nigra* L.

Concerning the economic point of view, *P. nigra* is one of the most important parent to produce euramerican hybrids in breeding programs to increase wood production, providing adaptive properties for various soil and climate conditions, excellent rooting ability of stem cuttings, high resistance to bacterial canker (*Xanthomonas populi* Ridé), fair resistance to Marssonina leaf spot (*Marssonina brunnea* (Ell. *et* Ev.) P. Magn.) and to poplar mosaic virus (Cagelli and Lefèvre, 1995). In the early 1980s, the ISP (*Istituto di Sperimentazione per la Pioppicoltura*) started long-term breeding programmes using a large number of native *P. nigra* and the best *P. deltoides* females in controlled crossing or open pollination (Vietto and Bianco, 2004). Recently intersectional hybrids between *P. nigra* and taxa in section *Tacamahaca*, including some Asian species like *P. maximowiczii* and the north American *P. trichocarpa*, have been successful and some clones show promise for plantation wood production (Dickmann and Kuzovkina, 2008).

Poplars are widely used in overseas countries as a source of timber for furniture manufacture, match-making and pulp. The most rapidly increasing use of the species today is in intensive culture for wood fibre and biomass for renewable, low-polluting energy (Picture 3). Thanks to the rapid juvenile growth and response to cultural treatments, the easy production of hybrids and the easy vegetative propagation, poplar's hybrids are ideal for short-rotation intensive-culture (Wyckoff and Zasada, 2007).

Short-rotation coppice cultures (SRCs) can be expected to play a major role in the production of biomass as a renewable energy source to substitute fossil fuels according to the "White Paper of the European Commission on renewable sources of energy". It set the target to increase energy by renewable sources up to 12% of the european energy consumption by 2010 in answer to the Kyoto's protocol disposition that scheduled (Article 3.1) a 8% reduction in annual greenhouse gas emissions compared to the 1990s emissions by 2008–2012 (Schulze *et al.*, 2002).

Picture 3. Harvest of poplar's short-rotation coppice cultures (SRCs).
URL:http://www.avanzi.unipi.it/ricerca/quadro_gen_ric/biomass_bioenergy/image biomass/Image19.jpg

1.1.4.3. Scientific importance of Genus

Regarding scientific importance of the genus, *Populus* was accepted by the scientific community as a woody model tree which is necessary needed to study the unique processes that occur in perennial plants, such as secondary wood formation, bud set, bud flush and dormancy (Taylor, 2002).

This genus has several advantages for biologic and genetics studies and it is considered primary model system because of the small and completely sequenced genome (about 550

million base pairs, 4× larger than Arabidopsis and one fortieth the size of the *Pinus* genome) (Taylor, 2002 ; Boerjan, 2005 ; Tuskan *et al.*, 2006), publicly accessible molecular markers, gene sequences, bacterial artificial chromosome (BAC) libraries, high-throughput plant transformation and regeneration capabilities (Wullschleger *et al.*, 2002). Other important attributes include the great diversity of species that cover vast geographic and elevation ranges (Brunner *et al.*, 2004) as adaptations to the diverse conditions, the high levels of natural variations in wild populations (Jansson and Douglas, 2007), inter-specific crossability and the ease of sexual and asexual propagation (Stettler and Bradshaw, 1996). Moreover the genus has phylogenomic proximity to well-studied angiosperms (Brunner *et al.*, 2004) and the rapid juvenile growth allow researchers to quickly measure short-term responses to abiotic and biotic factors (Bradshaw *et al.*, 2000 ; Dickmann, 2001b).

To give an indication of the reservoir of knowledge of poplar that have been accumulated up to 2001, Dickmann (2001b) searched the TREECD database (2001 CAB International, New York, NY) and founded that from 1939 over 20500 publications on some aspect of poplars appeared were done, and approximately 65% of these were printed in the last quarter of the past century.

1.2. Phenology and quantitative genetics

1.2.1. Tree Phenology

Trees of temperate and boreal zones must adapt themselves to persist periods that are unfavourable for growth, as well as resist to the strict winter climatic conditions, synchronizing the annual growth cycle with the seasonality (Ruttink *et al.*, 2007) in the context of a sessile existence. For this reason they have evolved a mechanism whereby they can switch between active growth during summer and dormancy in winter in response to the environmental signals (Figure 4).

Phenology is the study of recurring events that annually occur during the life cycle of plants and animals (Kramer, 1996), as a result of natural selection, and how they respond to seasonal change in their environment. The timing of bud set in the fall and bud flush during the spring represents a critical ecological and evolutionary trade-off between plant growth and survival (Horvath *et al.*, 2003 ; Howe *et al.*, 2003). Ruttink and co-worker (2007) describe the bud development during the autumn as compose of three processes: the bud formation, the acclimation to dehydration and cold, and finally the dormancy. In trees the activity of the bud

determines not only the extent of seasonal growth, which largely determines tree productivity and wood quality in temperate and boreal zones, but also growth habit and tree form (Rhode *et al.*, 2000).

Populus spp. are trees that show a continuous development during the growing season (indeterminate growth) and that respond to short daylength, the only environmental signal that is constant and predictable year after year, with growth cessation, the formation of terminal bud (autumn bud set) and dormancy induction (Howe *et al.*, 1996 ; Rohde *et al.*, 2002). Plants which have an indeterminate growth are most responsive to photoperiod (Rohde *et al.*, 2000) and the critical photoperiod, the largest one that induce the bud set response (Howe *et al.*, 1995), is not inherited in a simple Mendelian mode but as a quantitative trait with a large number of genes involved in the reaction (Pauley and Perry, 1954 ; Rohde *et al.*, 2000).

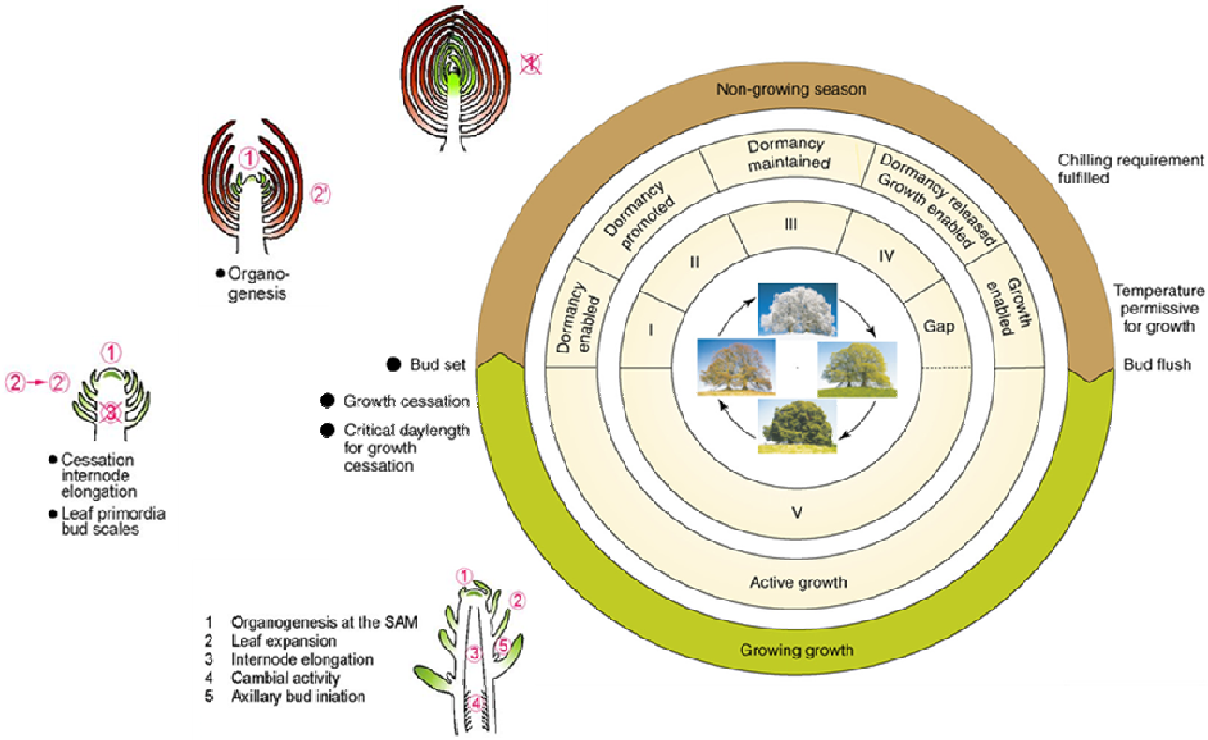


Figure 4. Modified picture of the synchronization of the annual growth cycle with the seasonality in *Populus* spp. The bud flush and bud set delimit the growing season. Non-growing season is characterised by different meristem stages: I, cessation of cell division; II, establishment of dormancy; III, maintenance of dormancy; IV, release from dormancy state; and V, resumption of cell division. 'Gap' between stage IV and V denotes the phase where growth does not occur because of purely environmental restraints (Rohde *et al.*, 2007). On the left are showed different significant moment during the bud formation process (Rohde *et al.*, 2000).

In the case of *Populus* several traits are likely to be important for local adaptation and phenology of bud permits the trees to find the right compromise solution between the necessity to avoid the risk of frost damage, if the trees set buds too late or flush too early (Luquez *et al.*, 2008), and assure the maximization of the growth season (Kramer, 1996), especially for the short growing season of the northern latitudes.

Common garden studies demonstrate that bud set in the fall and bud flush in the spring are correlated with the latitudinal and altitudinal origin of the trees (Frewen *et al.*, 2000 ; Howe *et al.*, 2003 ; Ingvarsson *et al.*, 2006) and trees from high latitudes and elevations are earlier relative to trees from lower latitudes and elevations (Pauley and Perry, 1954). Howe and co-workers (1995) found the northern ecotype show a longer critical photoperiod and a greater photoperiodic sensitivity, defined as the change response per unit change in photoperiod, than the southern ones. Some studies show that the latitude of origin is a factor which has strong effect on phenology. The days to bud set showed a significant clinal pattern with the variation in the latitude which explains most of the variation among populations in the timing of bud set (Ingvarsson *et al.*, 2006 ; Hall *et al.*, 2007) as adaptive answer to different length of the growing season.

For the growth cessation daylength is the primarily used environmental signal (Rohde *et al.*, 2007) even if the timing of bud formation are also influenced by other factors such as temperature (Kalcsits *et al.*, 2009), temperature x photoperiod (x population) interaction (Håbørg, 1972 ; Tanino *et al.*, 2010), nutrition and drought (Rohde *et al.*, 2000 ; Howe *et al.*, 2003). Given the influence of temperature on the phenology of trees, the cessation of growth and the bud formation may be affected by globally climatic warming and increase the risk of frost damage, the survival and eventually the distribution of forest trees, caused by a less closely adaptations to the local altered environment (Kramer, 1996 ; Kramer *et al.*, 2000); if some species will benefit from increasing temperatures and longer favourable season, others will disappear (Linderholm, 2006).

In this contest phenological observation are very important in a global-climate warming scenarios for a better understanding how different plant species respond to regional climate conditions and to climatic changes by shifting their phenological events (Chmielewski and Rötzer, 2001 ; Menzel, 2000). Johnesn and co-workers (2005a ; 2005b) had demonstrated that phenological process of bud formation in *Picea abies* (L.) Karst. is strongly affected for many year by temperature prevailing during zygotic and somatic embryogenesis and this memory has probably an epigenetic nature (Kvaalen and Johnsen, 2008), introducing a new font of

phenotypic variation and adaptive plasticity between generations for one of the most differentiated genetic trait in many temperate trees (Rohde and Junttila, 2008).

In Poplars the bud set in the fall and the cessation of growth are regulated by phytocrome (Howe *et al.*, 1996 ; Olsen *et al.*, 1997) *phy*A, *phy*B1, *phy*B2 (Howe *et al.*, 1998), with the mediation of poplar CO/FT module (Costant/Flowering Locust) (Böhlenius *et al.*, 2006). Given that the phenological process of bud set in the fall is a quantitative trait under moderate to strong genetic control (Li *et al.*, 1998 ; Howe *et al.*, 2000 ; Frewen *et al.*, 2000, Luquez *et al.*, 2008), in the last years a lot of researches are developing about the identification of genes involved in the genetic variation of bud set through quantitative traits loci (QTLs) analyses.

1.2.2. Quantitative genetics

Quantitative genetics is a science that study quantitative characters and seek to explain how variability in these is influenced by heritable and non-heritable factors. In fact quantitative traits, also called metric characters because their study depends on measurement instead of on counting, exhibit a continuous variation. The resultant normal distribution, which is described by its mean (mid-point) and by its characteristic variance (width), is due to the combination effect of many genes, each contributing a small amount (Figure 5), plus non-genetic causes, like environmental factors, that are non-heritable and reduces the efficiency of selection (Falconer, 1981). The study of quantitative traits is very important because they are adaptive and economic characters such as reproductive ability, diameter, height, growth, survival, bud set, bud flush, cold hardiness, drought hardiness, wood quality, disease resistance etc. In order to dissect quantitative traits and analyse the genetic proprieties of a population is necessary to distinguish two terms often used mistakenly: genotype and phenotype. The genotype is the genetic potential of a generic individual. It is determined by the genes that reside in chromosomes and it cannot be seen directly. The phenotype is the individual we directly see on the field, we measure and we work with. It is influenced by the genetic potential of the tree and by the environment in which the tree is growing (Zobel and Talbert, 1984). The phenotype is often indicated by the formula:

Formula 1: $$P = G + E$$

where P is the phenotype, G the genotype and E the environment.

The relative contribution of the genetic and environmental components to the phenotypic variability of a generic quantitative trait is described by heritability. This parameter measures the percentage of variation among trees that is genetic and range from 0 to 100% (0.00 to 1.00). It is of primarily importance in order to predict result in genetic improvement programs: low heritabilities make genetic improvement difficult.

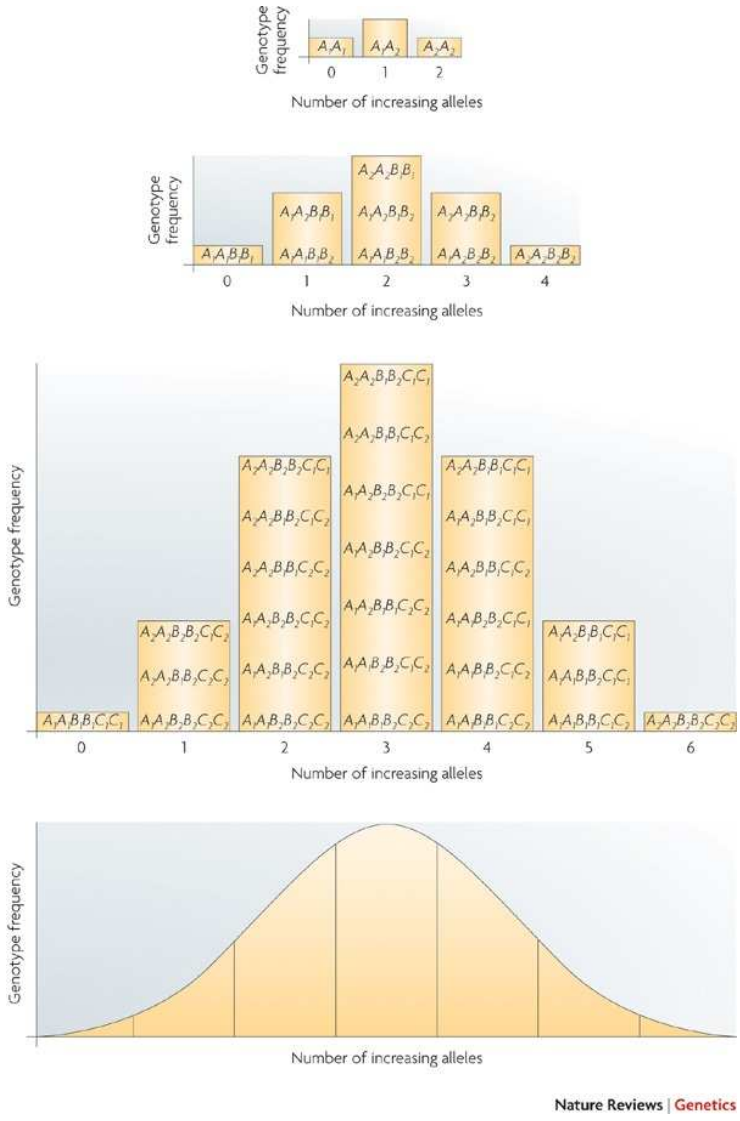


Figure 5. Effect of increasing number of alleles on genotype frequency (Plomin *et al.*, 2009).

In order to calculate heritability of a generic quantitative trait is necessary to measure the phenotypic variability by the Analysis of Variance, an analytical method developed by R.A. Fisher in the 1920s, and divide it into its genetic and environmental components by equating

genetic expectations (expected mean square) to observed mean square in a completely balanced experiment (Riemenschneider *et al.*, 1996).

Another source of variability is due to the genotype x environment interaction (Barcaccia and Falcinelli, 2005), term used to describe the situation where a number of genotypes respond differently to various environments. This may involve a change in the rank of the different individuals of a given population, which is the most important type of interaction to the tree breeder, or may simple involve a change in the variation from one site to other with no change in rank, situation that cause no problem for breeding because a well-performing genotype selected in one environment will remain well-performing when grown in a different environment. It can be of great benefit when maximum gains are desired in specific environment, but the GxE interaction can became a formidable barrier when an environmental stable genotype for a given trait is sought (Zobel and Talbert, 1984). A good method to reduce the genotype x environment interaction in breeding program is to select for genotypes that perform well and that show little interaction over different environmental condition; for this aim several statistics estimating stability (Wricke, 1962 ; Brancourt-Hulmel *et al.*, 1997 ; Mandel, 1969 ; Gauch, 1992) have been developed to understand which genotypes mainly contribute to the total GxE interaction in a given population studied. The genotype x environment source of phenotypic variability is considered very important and an understanding of the genetic control of this interaction is of great importance for quantitative genetics and its applications in breeding, conservation and theory of evolution (Rae *et al.*, 2008).

Following what we have previously mentioned, the total phenotypic variance includes the components due to the genotypic variability, environmental variability and the interaction between genotype and environment, as shown by the following formula:

Formula 2: $$\sigma^2_P = \sigma^2_G + \sigma^2_E + \sigma^2_{GE}$$

where σ^2_P is the phenotypic variability, σ^2_G and σ^2_E the genotypic and environmental variability respectively and σ^2_{GE} the genotype-environment interaction.

The genotypic variance can be further divided into the additive and the non-additive components. The additive variance**,** fixable by selection, is the variance in a trait that is due to the effects of each individual allele being added together, without any interaction with other alleles or genes. The non-additive genetic variance can be divided into two types: dominance

variance, that is due to the interaction between alleles at a gene locus and is not directly inherited from parent to offspring, and epistasis variance, which is due to the interaction between different gene loci (Zobel and Talbert, 1984). Following the previous distinction, the genetic variance component is described as:

Formula 3: $$\sigma^2_G = \sigma^2_D + \sigma^2_H + \sigma^2_I$$

where σ^2_D is the additive variance**,** σ^2_H is the dominance variance and σ^2_I the interaction component. In this way, the phenotypic variance could be expressed by the complete formula:

Formula 4: $$\sigma^2_P = \sigma^2_D + \sigma^2_H + \sigma^2_I + \sigma^2_E + \sigma^2_{GE}$$

Because the expression of a generic quantitative trait is a result both of the environment in which the trait is expressed and genes involved in this expression, it doesn't make sense to say of a particular phenotype that characterize an individual that genes are more important than environment or vice versa in determining it because it's not possible have this phenotype without both sources of variability. What is possible to measure is how much of the difference among individuals is attributable to differences in their genes by the heritability value. The concept of heritability, which express the proportion of variation in the population that is attributable to genetic differences among individuals and, therefore, a ratio of the degree to which parents pass their characteristics to their offspring, is one of the most used in quantitative genetics and it is of key importance in estimating gains that can be obtained from selection programs (Zobel and Talbert, 1984).

Two types of individual-tree heritability exist: *narrow-sense heritability* (h^2) and b*road-sense heritability* (H^2). The first one represents the ratio of additive genetic variance to total variance and it can be written as:

Formula 5: $$h^2 = \sigma^2_D / \sigma^2_P$$

or

Formula 6: $$h^2 = \sigma^2_D / \sigma^2_D + \sigma^2_H + \sigma^2_I + \sigma^2_E + \sigma^2_{GE}$$

where σ^2_D is the additive variance and σ^2_P the total phenotypic variance.

Narrow sense heritability can also be calculated directly from breeding experiments through the direct observation of results and, in this case, it is called “realized heritability”. The range of narrow-sense heritability varies from 0, when we have no additive variance, to 1, when we have only the additive component. Broad-sense heritability is defined as the ratio of total genetic variation in a population to the phenotypic variation and it can be written as:

Formula 7: $$H^2 = \sigma^2_G / \sigma^2_P$$

or

Formula 8: $$H^2 = \sigma^2_D + \sigma^2_H + \sigma^2_I / \sigma^2_D + \sigma^2_H + \sigma^2_I + \sigma^2_E + \sigma^2_{GE}$$

where σ^2_G is the genetic variance and σ^2_P the total phenotypic variance.

The biggest problem with broad sense heritability comes from that all genetic phenomena are included into a single σ^2_G factor. The broad-sense heritability limits are 0, when none of variation in a population is due to genetics, and 1, when we have not environmental effects on the variation. This kind of parameter is never lower than the narrow-sense heritability; if all the genetic variance is of the additive type, the two types of heritabilities are equal.

An important but often underestimate aspect of heritability is that they applies only to a particular population growing in a particular site at a particular moment because a generic quantitative trait may not be influenced by exactly the same genes when grow in different environmental or experimental conditions. The heritability value of a given trait also change with age when the environment change and when the genetic control of the character change as the tree mature (Zobel and Talbert, 1984).

Like heritability, tree improvers are really interest to understand the genetic correlations among traits because they indicate the proportion of variance that two traits share due to genetic causes and how the improvement of one character will cause simultaneous changes in other ones.

An observed phenotypic correlation between two quantitative traits may be due to genetic or environmental causes, and just as with phenotypic variances, there is a need to understand these underlying components that give rise to a phenotypic correlation. Genetic correlations, correlation of breeding values, and environmental correlations, correlation of environmental deviations together with non-additive genetic deviations, combine together to give phenotypic correlations. These two genetic parameters allow us to define independently the genetic and environmental causes of a correlation (Falconer, 1981).

Genetic correlations may be estimated as:

Formula 9: $r_g = \sigma_{g(xy)} / [\sigma^2_{g(x)} \times \sigma^2_{g(y)}]^{½}$

where $\sigma^2_{g(x)}$ is the genotypic variance component for the trait x, $\sigma^2_{g(y)}$ is the genotypic variance component for the trait y and $\sigma_{g(xy)}$ is the genetic covariance between the same traits.
By the same way we can estimate residual or environmental correlations as:

Formula 10: $r_w = \sigma_{w(xy)} / [\sigma^2_{w(x)} \times \sigma^2_{w(y)}]^{½}$

where $\sigma^2_{w(x)}$ is the residual variance component for the trait x, $\sigma^2_{w(y)}$ is the residual variance component for the trait y and $\sigma_{w(xy)}$ is the residual covariance between the same traits.
In some cases genetic and environmental correlations are similar but they could be very different in magnitude or opposite in sign and these show that genetic and environmental sources of variation affect the traits through different physiological mechanisms.

1.2.3. Quantitative traits loci (QTLs)

Quantitative trait loci (QTLs) are genomic regions associated with a phenotypic trait exhibiting continuous variation. The QTL mapping techniques were developed in the late 1980s with the advent of molecular markers and it allows us to dissect quantitative variation and understand the genetic control of such complex traits. This important tool has been used to look at a number of traits in *Populus* such as biomass (Rae *et al.*, 2008 ; Marron *et al.*, 2010), rust resistance (Newcombe *et al.*, 1996 ; Jorge *et al.*, 2005), canopy structure (Wu and Stettler, 1998), leaf traits (Wu *et al.*, 1997 ; Ferris *et al.*, 2002 ; Rae *et al.*, 2006), stem growth (Bradshaw and Stettler, 1995 ; Wullschleger *et al.*, 2005), growth rates (Wu *et al.*, 2003), bud dormancy (Frewen *et al.*, 2000), bud set (Marron *et al.*, 2010 ; Rohde *et al.*, 2011).
The mapping of quantitative trait loci is the statistical study of the alleles that occur in a locus and the phenotypes that they produce. They are shown as intervals across a chromosome, where the probability of association is plotted for each marker used in the mapping experiment (Bradshaw, 1996).
Understanding the genetic architecture of quantitative traits is important in a number of disciplines, including animal and plant breeding, medicine, plant evolution.

Mapping QTLs make possible the manipulation of genes in breeding programs. For every quantitative trait at the molecular level we wish to know the chromosomal location of each quantitative trait locus (QTL) affecting the trait, the magnitude of effect of each QTL on observed phenotype (many genes of small effect or few genes of large effect), the mode of gene action at each QTL (additive, dominant/recessive, over-dominant), the effect of interaction among different QTLs (epistasis) and the parent source of beneficial QTL alleles (Bradshaw, 1996). Moreover an important goal of genetic studies is try to quantify the number of QTLs that influence a quantitative trait, the number of alleles that each QTL has, the frequencies of the alleles in the population and the influence of each QTL and its alleles on the quantitative trait (Moore, 2003), in order to characterize the genetic architecture of the traits. The locations of QTLs in the genome can be determined by genetic mapping with respect to genetic markers (Figure 6).

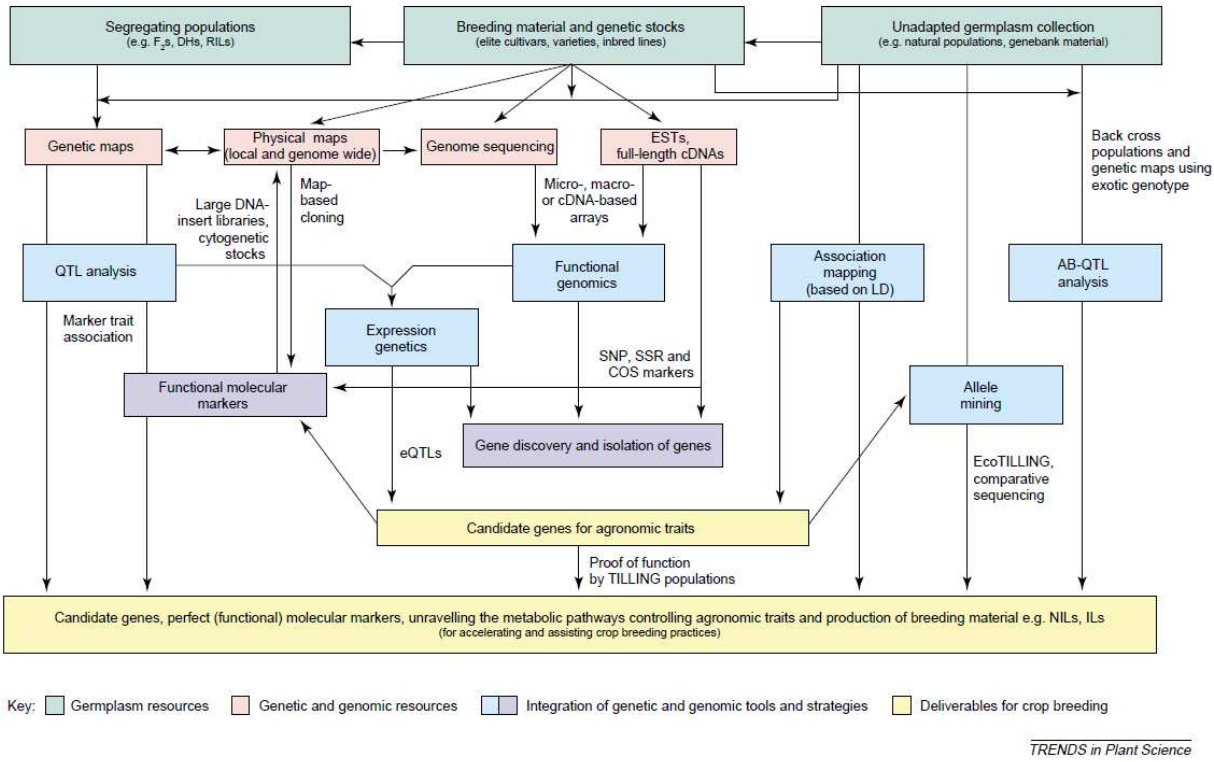


Figure 6. Different genetic and genomic strategies for crop improvement (Varshney *et al.*, 2005). Abbreviations: AB-QTLs, advanced backcross QTL; COS, conserved orthologous set; eQTLs, expression QTLs; ESTs, expressed sequence tags; LD, linkage disequilibrium; QTL, quantitative trait loci; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; TILLING, target induced local lesions in genome.

Genetic maps allow scientists to search for a specific gene somewhere within a vast genome of plants or animals through the help of genetic markers, genes or DNA sequence with a known location on a chromosome, that allow to access to the genes. In recent years, numerous genetic markers have been identified and mapped with molecular techniques making QTL mapping feasible for many organisms. The underlying idea is simple: if the inheritance of a genetic marker is associated consistently with the inheritance of a particular characteristic (such as increased height), then that marker must be linked to a QTL that affects height. The key is to have enough genetic markers so that QTLs can be detected throughout the genome (Pierce, 2002). When a QTL is found, it is often not the actual gene underlying the phenotypic trait, but rather a region of DNA that is closely linked with the gene.

Another use of QTLs is to identify candidate genes underlying a trait because once a region of DNA is identified as contributing to a phenotype, it can be sequenced and compared to a database of DNA for genes whose function is already known. For organisms whose genomes are known, one might try to exclude genes in the identified region whose function is known with some certainty not to be connected with the trait in question.

2. OBJECTIVE

Phenology is crucial to tree distribution and conservation and research efforts to understand its genetic basis are of paramount importance, especially in the light of climate change. In this direction our objectives are:

- to contribute to the knowledge of the genetic control of bud set applying a new detailed scoring system to assess the phenotypic and genetic variation of the different components of the process (Rohde *et al.*, 2011);
- to assess the bud set process in an intra-specific poplar pedigree grown in two contrasting sites and in different european metapopulations of black poplar;
- to analyse the response to the photoperiod and evaluate the role played by the temperature and the "environment" in the process;
- to analyse the phenotypic plasticity of the bud set process in two contrasting site, another short-term option for adaptation (global change) that remained largely uncharacterized for phenology traits;
- to detect quantitative trait loci (QTL) in the intra-specific pedigree for the most descriptive traits, using the available molecular genetic map (Gaudet *et al.*, 2008), and test the robustness of QTL in the mapping pedigree.

In perspective this work is aimed to contribute to the identification of one or more traits relevant as selection criteria and to provide molecular markers for marker-assisted selection (MAS) to be used in breeding programs.

3. MATERIAL AND METHODS – *P. nigra* full-sib family

3.1. Genetic material

An intra-specific *Populus* full-sib family was examined in this experiment. The family consisted of 162 black poplar (*Populus nigra* L.) genotypes resulting from an intra-specific cross (*P. nigra* x *P. nigra* family, POP5) between the *Populus nigra* L. "Poli" (father parent) from the southern Italy Policoro (40°09'N, 16°41'E) and the *Populus nigra* L. "58-861" (mother parent) from the northern Italy Val Cenischia (45°09'N, 07°01'E).
In order to cover a significant range of variability, parental genotypes were chosen according to their strong differences, tested in a common garden experiment, in morphological (Picture 4) and phenological traits.

Picture 4. Morphological differences between female (58-861, on the left) and male (Poli, on the right) parents of the *P. nigra* intra-specific cross.

The female parent has less branches and biggest leaves than the male parent as adaptive response to colder ad wet environment conditions of the native site of origin. The cross was realized in Italy by the Department of Forest Resources and Environment of the University of

Tuscia (DISAFRI, Viterbo) in 2001 (Sabatti, personal communication) and the collection of progeny is preserved in the experimental farm of the University.

3.2. Experimental plantations

The study was performed in two common garden experimental plantations established during April 2003 (Cavallermaggiore, CV) and April 2008 (Viterbo, VT) from 25 cm homogeneous hardwood cuttings planted at a spacing of 0.75 m in rows and 2 m between rows (Picture 5), accommodating an overall plant density of 6667 plants per ha.

Picture 5. Preparation of the two common garden experimental plantations established during April 2003 (Cavallermaggiore, on the left) and April 2008 (Viterbo, on the right).

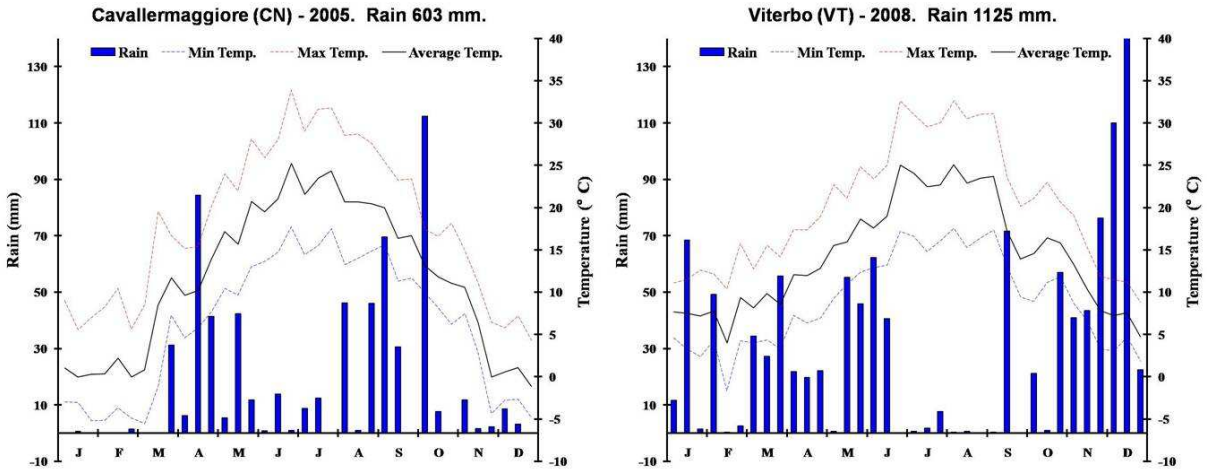
The field trials were established using a randomized complete blocks design with six blocks for CV and five blocks for VT. One replicate of each F_1 genotype and of the parents was randomly assigned to each block and a double border row, using clonal material of the same species, was planted around the plantation in each location to minimize border effect (Zavitkovski, 1981 ; van Heacke *et al.*, 1995).

The two plantations were irrigated, weeded and treated with pesticides and fungicides as needed during the two growing seasons in CV and the first one in VT. Systematic pruning was made during February 2005 (at the end of second growing season of the first coppice rotation) only for the northern site, and during the entire plantations duration new shoots were removed in both sites in order to keep only one stem on each stool to avoid light competition among shoots of a stool.

3.3. Sites description

The field trials were located in central (VT, Tevere valley; 42°25'N, 12°05'E) and northern Italy (CV, Po valley; 44°42'N, 07°41'E) with an elevation of 285 m above sea level for the second site and 310 m above sea level for the first one.

At the northern site in 2005 the mean meteorological conditions during the year was colder and the rainfall amounts were lower as compared to the central Italy location in 2008 (Figure 7), with more seasonality for the first location especially during spring and fall. The soil at the northern site was an alluvial soil with sandy-load texture while it was sandy silt in VT (Sabatti, personal communication).



Site	Mean daily temperature (°C)				Cumulative rainfall (mm)			
	Spring	Summer	Fall	Annual mean	Spring	Summer	Fall	Annual sum
Cavallermaggiore	15.50	21.37	7.67	12.20	237.40	215.60	147.40	602.60
Viterbo	14.65	22.87	11.81	14.50	324.00	83.00	500.40	1124.80

Figure 7. Meteorological characteristics of the two experimental sites in Italy where the POP5 Full-sib family was studied. Data were obtained in 2005 (CV) from a nearby meteorological station and in 2008 (VT) from the Department of Crop Production (DIPROV) of University of Tuscia.

3.4. Protocol of bud set measures

The phenological measurements of bud set were done according to the protocol developed inside the POPYOMICS project (contract No. QLK5-CT-2001-00953, an EC funded research

program: http://www.soton.ac.uk/~popyomic/) in order to improve the phenotypic resolution of the distinctive phases observed during the formation of the apical bud in autumn and to evaluate the duration of bud development as separate character.

For this protocol (Figure 8) six representative phases (excluding stage 3 which represent the shoot during vegetation) characterizing the development and maturation of apical bud (Rohde *et al.*, 2011) were defined. The protocol covers the bud set process form the stage 2.5, which represent the perception of critical day length and the onset of the phenological process, to the phase 0, the end of bud maturation.

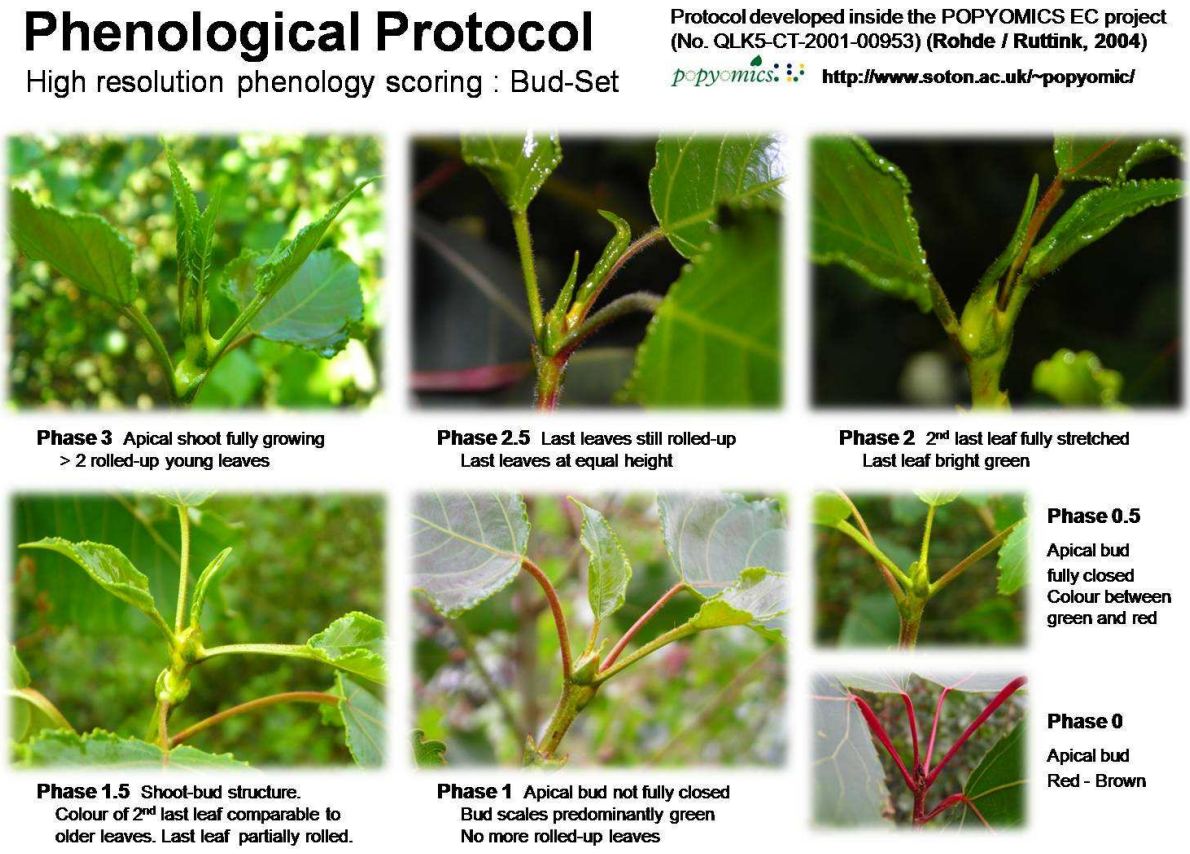


Figure 8. The high resolution bud set scoring protocol. The first stage (phase 3) represent the vegetative apex in fully growing stage. All pictures were made in individuals of *Populus nigra* L. during the phenological sampling.

Phases 1, 0.5, and sometime the stage 0, correspond to the most utilized model of bud set in some traditional studies of apical bud formation (Li *et al.*, 1998 ; Frewen *et al.*, 2000 ; Howe *et al.*, 2000 ; Rae *et al.*, 2004), whereas the phase 1.5 represent the critical transition from shoot to bud.

Since the last phase described in the protocol is only a change of colour, for our aim we decided to examine only the first five phases (from stage 2.5 to 0.5) and estimate indirect phenological traits such as the duration of the different phases described (*duration of phase 2.5*, *duration of phase 2*, *duration of phase 1.5* and *duration of phase 1*), *duration of subprocess 1* (cumulative days for each tree in phases 2.5, 2 and 1.5, i.e. the total amount of the duration of the phase 2.5 and phase 2), that essentially describe change in the apical shoot, and *duration of subprocess 2* (cumulative days for each tree in phases 1.5, 1 and 0.5, i.e. the total amount of the duration of the phase 1.5 and the phase 1) (Figure 9), refers to visible changes in the apical bud after the onset of its formation. These indirect traits were defined in order to widen the information that could be studied.

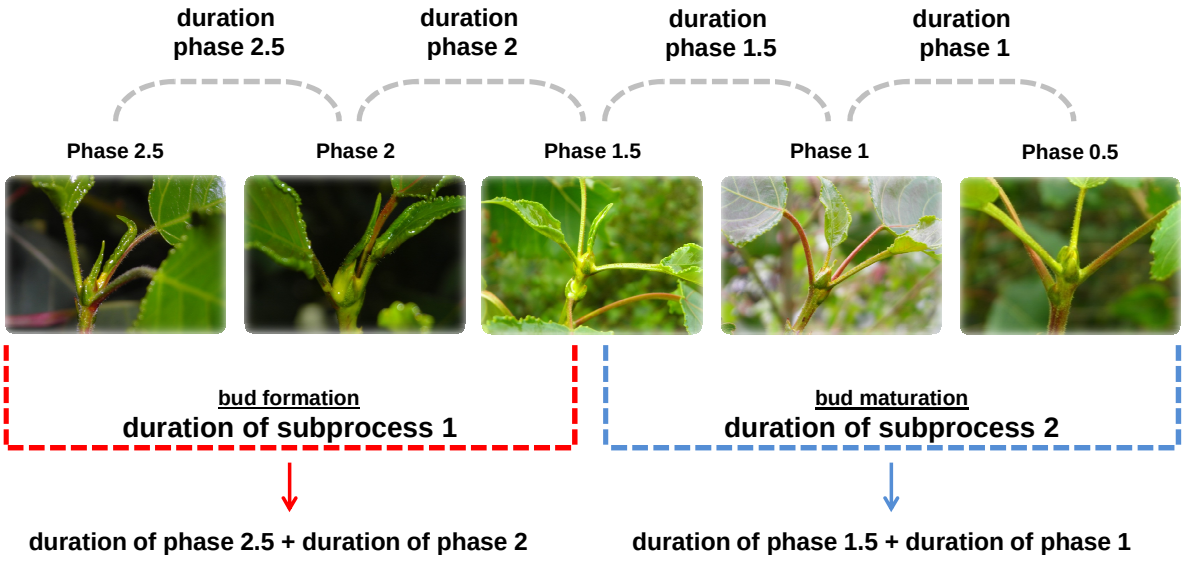


Figure 9. The different traits analysed in this work.

Moreover, only concerning the full-sib family POP5 experiment for which we had in both experimental trials an enough number of sampling days, the date of the year in which 50% of individuals had reached the onset of the phenological process and the day of transition to bud structure (score50%_2.5 and score50%_1.5) was calculated and the distribution of score analysed. These are two important breading values to compare different family and tools applicable in practical tree breeding strategies.

Phenological measurements for the northern location (CV) were started in autumn 2005, at the end of the first growing season of the second coppice rotation (i.e. Root 3 - Shoot 1, third growth year), and data were collected every two days from the beginning of September

(September 9, DOY = 252) up to the middle of October (October 13, DOY = 286). For the central Italian site (VT) bud set was scored in autumn 2008 (leap year) at the end of the first growing season after planting (Root 1 - Shoot 1) and data were collected every three days from September 15 (DOY = 259) up to October 20 (DOY = 294). On each observation day the appearance of apical buds was recorded by giving each tree a score according to the phenological protocol. All the trees that showed some stressful situation, likely to influence the natural phenological process or with damage to the apical meristem, were excluded from all measure.

3.5. Other traits measured

In Cavallermaggiore maximum stem height and circumference (Picture 6) measures were performed at the end of the first growing season of the second coppice rotation (January 2006, i.e. Root 3 – Shoot 1).

Picture 6. Maximum stem height (on the left) and circumference (on the right) measures in northern Italy.

For each tree in the six blocks of POP5 full-sib family in order to use these traits as covariate of phenological data and investigate the possible influence on the phenological process. Maximum stem height was measured with an accuracy of 1 centimetre using an extendable

aluminium pole and the stem circumference was measured at 1 m above ground level to the nearest millimetre using a tape measure. In Viterbo the maximum stem height and circumference were measured at the end of the first growing season after plantation (December 2008, i.e. Root 1 – Shoot 1) using the same protocol of measurement.

3.6. Organization of the database and statistical analyses

3.6.1. Data management and fitting

One database was developed for the same full-sib family at each location and all genotypes measured for which there were less than three observations for a trait were removed in a preliminary analysis.

For the evaluation of photoperiod on bud set process the night length, calculated as the difference between the times of sunset and sunrise (geographical data available in URL: http://aa.usno.navy.mil/), was accumulated from 1 July until the date of the end of the whole phenological process (CNL - cumulative night length) and, by the same way, to analyse the role of temperature on the dynamism of bud set, the daily minimum temperature below 10°C was cumulated from 1 July until the end of measurement (CMT_{10} - cumulative minimum temperature below 10°C). Original data, expressed as the number of days elapsed since December 31 of the previous year (DOY - day of the year), were converted using these two climatic parameters that allows us also to directly compare different field sites.

The raw data were fitting using a local polynomial regression (LOESS) (Cleveland *et al.*, 1992) that was identified, in our case, as the best fitting procedure for bud set dynamics (Bastien, personal communication) (Figure 10) in order to evaluate the bud scores that have not be observed in the field due to the speed of the phenological process.

3.6.2. Statistical analyses

Phenotypic fitted data were analysed using R software (version 2.6.0, A Language and environment Copyright, 2007) for windows (http://www.r-project.org). Prior to analysis of variance and to calculate genotype means for each site studied, when necessary original values were transformed using the Box-Cox procedure (Box and Cox, 1964), a procedure developed for estimating the best power transformation when one has no a-prior reason for

selecting a specific transformation (Figure 11), to ensure homoscedasticity and normality of the residuals.

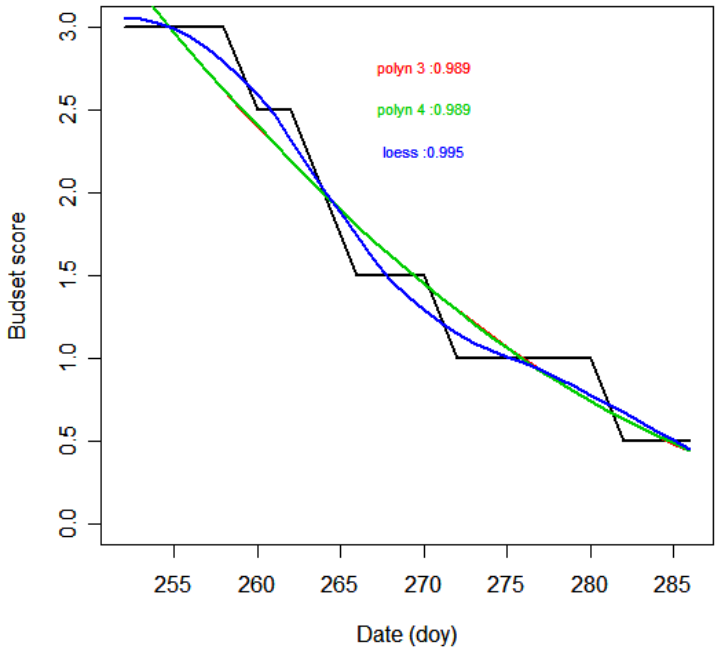


Figure 10. The result of fitting procedure concerning a generic individual. Figure shows the raw data trend (black broken line), 3th order polynomial regression (red line), 4th order polynomial regression (green line) and LOESS, locally weighted scatterplot smoothing (blue line).

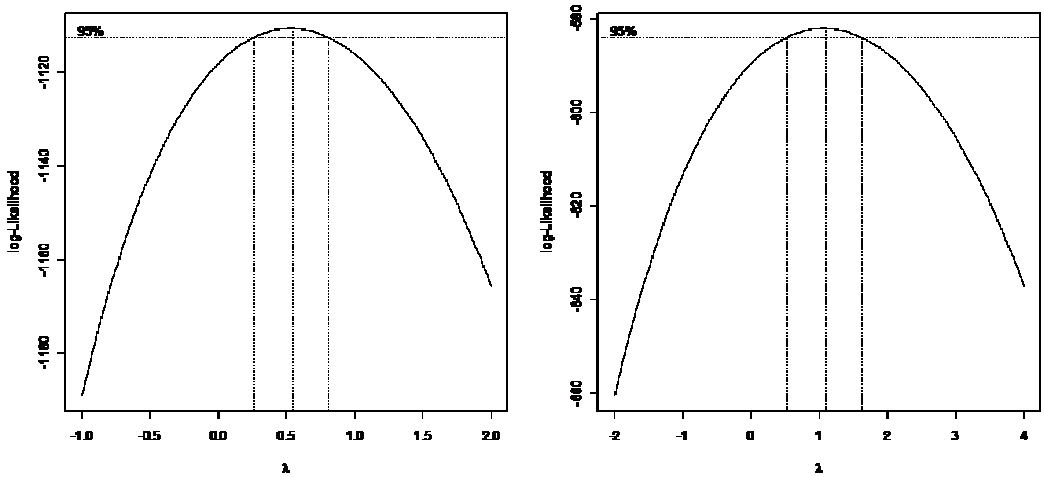


Figure 11. The result of estimating the best power transformation (x^{λ}) to ensure homoscedasticity and normality of the residuals for a generic trait. In this case the Box-Cox procedure suggests a power transformation of 0.5 (square root) to obtain the normality of residuals (on the right, $\lambda=1$).

For investigation of genotype and block effects in a within site analysis, data were evaluated by the following model of analysis of variance (ANOVA):

Formula 11: $$Y_{ij} = \mu + B_i + G_j + \varepsilon_{ij},$$

where Y_{ij} is the observation for the j^{th} genotype in the i^{th} block, μ is the general mean, B_i is the effect of the i^{th} block (fixed), G_j is the effect of the j^{th} genotype (random) and ε_{ij} is the error. When the within site block effect were significant, all individual values were adjusted as:

Formula 12: $$Y_{ij}' = Y_{ij} - B_i$$

where Y_{ij}' are individual values adjusted and B_i is the estimated effect of block i. The block effect and the differences between genotypes were considered significant when the *P*-value of the ANOVA *F*-test < 0.05 for all data and all different sites.

3.6.3. Genetic parameters

VARCOMP procedure (SAS VARCOMP; SAS Institute, 1999) of SAS® version 9.0 for Windows (SAS Institute Inc., Cary, North Carolina) was used to estimate variance components of random effect by the REstricted Maximum Likelihood (REML) method. Broad-sense heritability was calculated for each trait at individual (H^2_i) and genotypic (H^2_g) level using (Nyquist, 1991 ; Holland *et al.*, 2003):

Formula 13: $$H^2_i = \sigma^2_g / (\sigma^2_g + \sigma^2_e)$$

Formula 14: $$H^2_g = \sigma^2_g / (\sigma^2_g + (\sigma^2_e / r))$$

where σ^2_g is the genetic variance, σ^2_e is the residual variance and r is the mean number of individuals per genotype.

The heritability on a genotype basis is used to estimate the efficiency of clonal selection and give information about the precision of estimated genetic value when clone means are used as phenotypic predictors, and the heritability on an individual basis can be considered a reference value more easily comparable to literature values (Marron *et al.*, 2006). Standard errors of

broad-sense heritability were calculated using the method suggested by Singh and co-workers (1993). The coefficient of variation was used to compare the genetic variation of traits with different means and it was calculated at the genetic and residuals level using:

Formula 15: $CV_g = (\sigma_g / \mu) * 100$

Formula 16: $CV_r = (\sigma_r / \mu) * 100$

where μ is the phenotypic family mean, σ_g and σ_r the genetic and residual standard deviation of a trait respectively.

3.6.4. Principal component analysis

In order to simplify and improve the significance of genetic correlations, multivariate analyses, one for each experimental location, using principal component analysis (PCA) were performed on individual tree basis. PCA uses a set of linear transformations of the variables studied to create new uncorrelated variables called the principal components.
Our aim was to select the traits which account for most of the variance in the observed phenological process of bud set and describe genetic relationship between these. We used VARCOMP procedure (SAS PRINCOMP; SAS Institute, 1999) and PCA axes were performed based on the correlation matrix of the specified variables which provides a standardized measure across all traits.

3.6.5. Linear and genetic relationship between traits

Strength phenotypic dependence between two traits was estimated by Pearson's linear correlation coefficient:

Formula 17: $r_{(x,y)} = cov(x,y) / (\sigma_x \times \sigma_y)$

where cov(x,y) is the covariance of the two variables x and y and σ the standard deviation. Results were obtained on a genotypic-mean basis using the CORR procedure on SAS® (Shen and Lu, 2006).

The Pearson's correlation coefficient is +1 in the case of a perfect increasing linear relationship, −1 in the case of a perfect decreasing relationship and 0 when the variables are completely independent. All values between the minimum and maximum indicating the degree of linear dependence between the two variables tested.
Genetic correlations between traits within family were calculated using:

Formula 18: $$r_g = \sigma_{g(xy)} / [\sigma^2_{g(x)} \text{ x } \sigma^2_{g(y)}]^{½}$$

where $\sigma^2_{g(x)}$ is the genotypic variance components for the trait x, $\sigma^2_{g(y)}$ is the genotypic variance components for the trait y and $\sigma_{g(xy)}$ is the genetic covariance between the same traits (Pliura *et al.*, 2007), calculated according to the formula (Howe *et al.*, 2000):

Formula 19: $$\sigma_{g(xy)} = [(\sigma^2_{g(x+y)} - \sigma^2_{g(x)} - \sigma^2_{g(y)})/2]$$

Variance (Visscher, 1998) and standard deviation of genetic correlations was calculated using:

Formula 20: $$\sigma^2(r_g) = (1- r_g^2)^2/n_c$$

Formula 21: $$S.D. = [\sigma^2(r_g)]^{1/2}$$

where n_c r is the number of clones used to calculate the covariance components and r_g the genetic correlation coefficient.
The genetic correlation between performances of a phenological trait in the two sites was calculated from (Burdon, 1977):

Formula 22: $$r_{gab} = \rho / (H_{ga} \text{ x } H_{gb})$$

where ρ is the Spearman's correlation coefficient between the two sites (Formula 24), H_{ga} and H_{gb} the square root of genotypic heritabilities at sites a and b, respectively.

3.6.6. GxS interaction and plasticity of bud set

Phenotypic plasticity refers to the degree to which the phenotypic expression of a genotype varies under different environmental conditions (Bradshaw, 1965 ; Via, 1984 ; Sultan 1987 ; Callaway *et al.*, 2003). Plasticity has been studied most intensively in plants, which typically show dramatic effects of environment on growth and development (Sultan, 2000).

The larger the GxS interaction of a given genotype, the less stable and predictable is its performance in different environments. In order to evaluate and quantify genotype by site interaction, the following model of analysis of variance for among site comparisons was used:

Formula 23: $$Y'_{jk} = \mu + G_j + S_k + G_jxS_k + \varepsilon_{jk}$$

where Y'_{jk} are individual adjusted values for within site block effects (Formula 12), μ is the general mean, G is the genotype effect (random), S is the site effect (random) and GxS is the genotype by site interaction effect (random).

When the analysis of variance showed significant GxS interaction effects, the change in ranking of the different genotypes was evaluated by the Spearman rank that is the Pearson's correlation coefficient at the rank level (Soliani, 2005):

Formula 24: $$\rho = 1 - (6 \times \sum d^2_{Ri}) / (N^3 - N)$$

where d^2_{Ri} s he difference between each rank of corresponding values of x and y and N is the number of observation pairs.

In order to dissect the GxS interaction and understand how many genotypes and how much they contribute to this source of phenotypic variability, relative genotype ecovalence was performed.

This is a uni-parametric approach and a common measure for genotype stability (Wricke, 1962, Brancourt-Hulmel *et al.*, 1997) defined as:

Formula 25: $$W_j^r = \Sigma_k (GxS)_{jk}^2 / \Sigma_{jk} (GxS)_{jk}^2$$

where W_j^r is the relative ecovalence for a given genotype j evaluated in k sites.

It's moreover possible to evaluate the relative contribution of each site analysed in the interaction by the relative ecovalence of the site as:

Formula 26: $$W_k^r = \Sigma_j (GxS)_{jk}^2 / \Sigma_{jk} (GxS)_{jk}^2$$

where W_k^r is the relative ecovalence for a given site k.

3.6.7. QTLs analysis

QTL analysis was based on 154 individuals. A genetic linkage map for the full-sib family POP5 has been produced by Gaudet and co-workers (2008) using MapMaker software version 3.0 (Lander *et al.*, 1987) and improved after the addition of new genotypes (154 vs. 92) (Gaudet, personal communication). Since a QTL for a transformed trait is difficult to interpret and the loss of power is minor compared to nonparametric methods (Rebai, 1997), we used the block-adjusted genotype means of some selected more informative phenological phases for each site to map QTLs. Only genotypes represented by at least three surviving replicates were included in the QTL analysis.

Analyses were performed with MultiQTL software (http://www.multiqtl.com/; MultiQTL Ltd., Institute of Evolution, Haifa University, 31905 Haifa, Israel) for the parental maps separately using a pseudo-test-cross analysis (Grattapaglia and Sederoff, 1994). Permutation testing was used to establish the QTL significance thresholds in order to declaring a QTL present (1000 permutation, Churchill *et al.*, 1994). Single trait analysis was performed using the interval mapping approach followed by multiple interval mapping (MIM). In a first step the entire genome was scanned for QTL assuming a single model, whit a single QTL per linkage group (LG) as hypotheses H1. In a second step, the genome was scanned for QTL assuming a two-linked QTL model (hypothesis H2). Permutation tests were carried out to compare the hypothesis H1 vs. H0 (no QTL on the LG) and H2 vs. H0. When $p_{(H2vsH1)}<0.05$, further permutation tests were run to compare the hypotheses H2 vs. H1 to ensure that the two-linked QTL model fitted the data better than a single QTL model (Rae *et al*, 2008).

In a last step, multiple-interval mapping was carried out to ensure that all single and two-linked QTL identified in the single interval analysis were significant when multiple intervals were analyzed. Bootstrap analysis was done to estimate the 95% confidence interval.

4. RESULTS - *P. nigra* full-sib family

4.1. Survival

Survival rate of the plantation in VT three months after the establishment was 99% while it was lower in the northern site, close to 76%, after the coppicing of the plantation at the end of the first growing season in the second rotation (i.e. Root 3 - Shoot 1, third growing year), due to the different sprouting ability and competition among genotypes (Figure 12). Male southern parent "Poli" died after coppicing in all the six blocks in CV for a low growing rate, and it wasn't available for the autumnal phenological measurement.

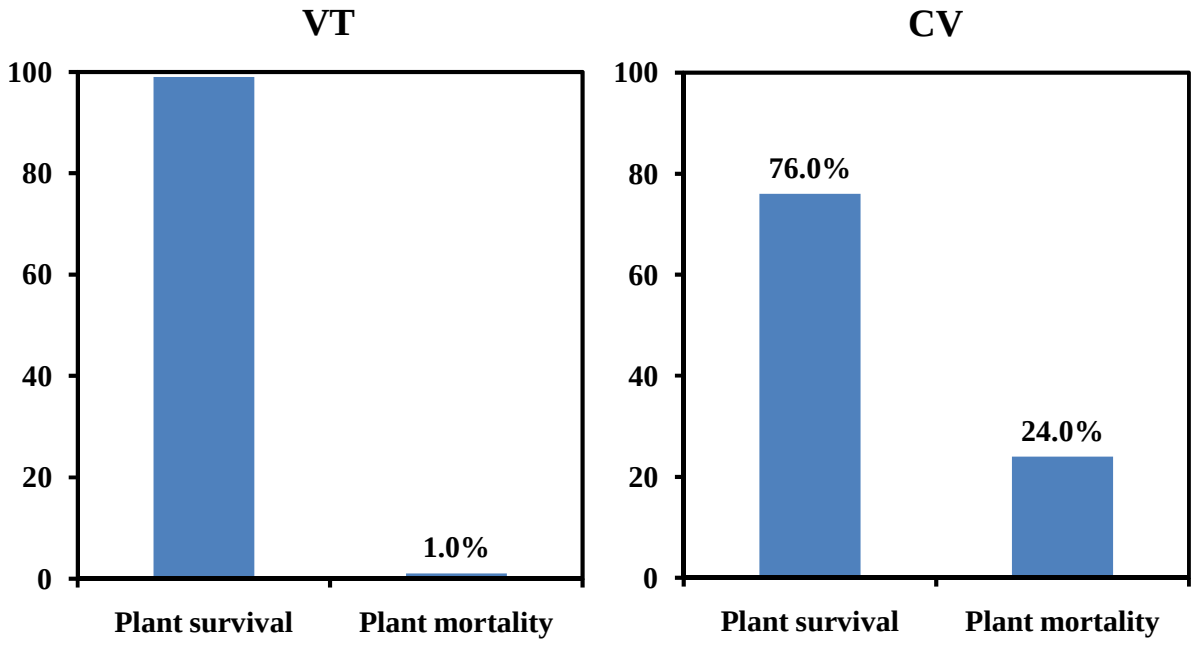


Figure 12. Plant survival observed in POP5 experimental plantations in VT at the end of the first growing season (Root 1 - Shoot 1, 2008), and in CV at the end of the first growing season of the second rotation (Root 3 - Shoot 1, 2005).

The mean number of available replicates for each genotype used for the phenological measurements in both site was between 4 and 5.

4.2. Temperature and photoperiod during the bud set period

The two experimental sites, which differ for 2° of latitude, didn't show clear distinction in photoperiod regime (Figure 13) around the onset of the autumnal phenological process of bud set. Considering the minimum daily temperature, the northern site during the measuring period in 2005 was colder than VT in 2008 and already reached a temperature below to 10°C only once, in the middle of August (Figure 13, B). Cumulative Minimum Temperature under 10°C (CMT_{10}) is a useful parameter to describe the trend of the temperature during the period and from figure 13 (B) is clear a faster accumulation of "cold" in CV (northern site) compared to VT, with the exception of a small period of three days (October 28, 29 and 30) for which a higher cumulative cold temperature was registered in VT.

The duration from growth cessation (phase 2.5) to visible bud-scales (phase 1.5), corresponding to subprocess 1, seems to start for each site (Figure 13, A) some days before the first peak of 10°C in September and, in particular, 3 days for CV and 2 days for VT. This trait was completed by all genotypes in VT after a minimum peak of 2.3°C registered in October 5, and it appeared shorter compared to the duration of subprocess 1 in the northern site.

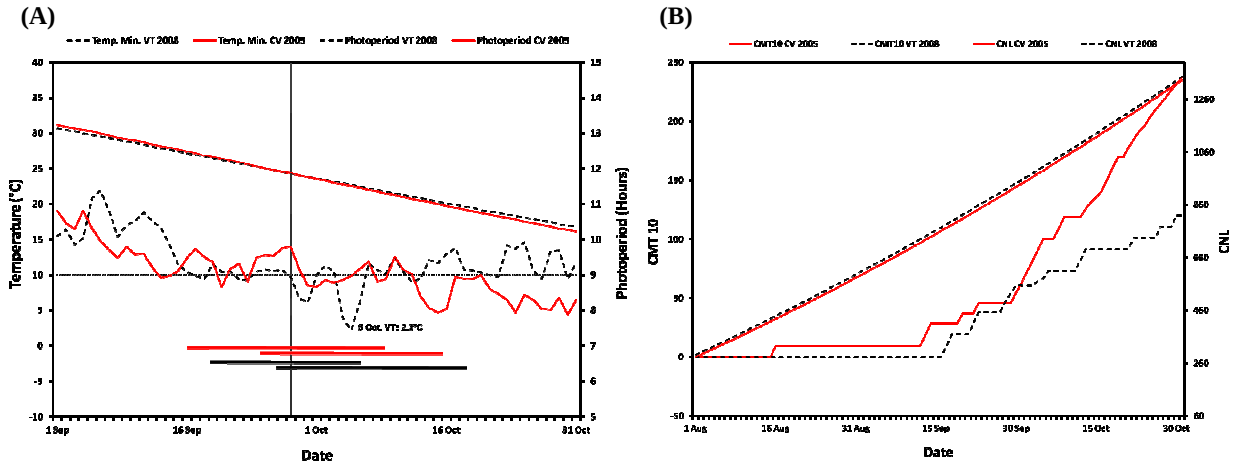


Figure 13. Climatic conditions in CV and VT during the bud set period. (A) Minimum temperature and day length for the two sites located at 44°42'N (CV) and 42°25'N (VT); subprocess 1 (upper bar) and subprocess 2 (lower bar) for the northern (red lines) and central (black lines) sites are shown. (B) CMT_{10} and CNL were calculated from July 1st for the two field sites.

4.3. Phenotypic dynamics and variation in bud set traits

Genotypic mean of each genotype in each sampling data were plotted in order to observe the trend of bud development in all the genotypes analysed at both sites (Figure 14, A and B). In the same figure horizontal dashed lines show the two critical points of bud set process, corresponding to the critical day length perception (stage 2.5) and the transition from shoot to bud (stage 1.5).

We observed smaller variations between genotypes on the phenological dynamics in VT than in CV and the male parent "Poli" was firmly the latest genotype for all the phases analysed. On the contrary, the northern parent "58-861" in VT was the earliest genotype to reach phase 2.5 but transgressive segregation was observed in the phases 1.5, 1 and 0.5.

Transgressive segregation of some early genotypes was observed in CV in the perception of critical day length at the onset of the bud set process (phase 2.5).

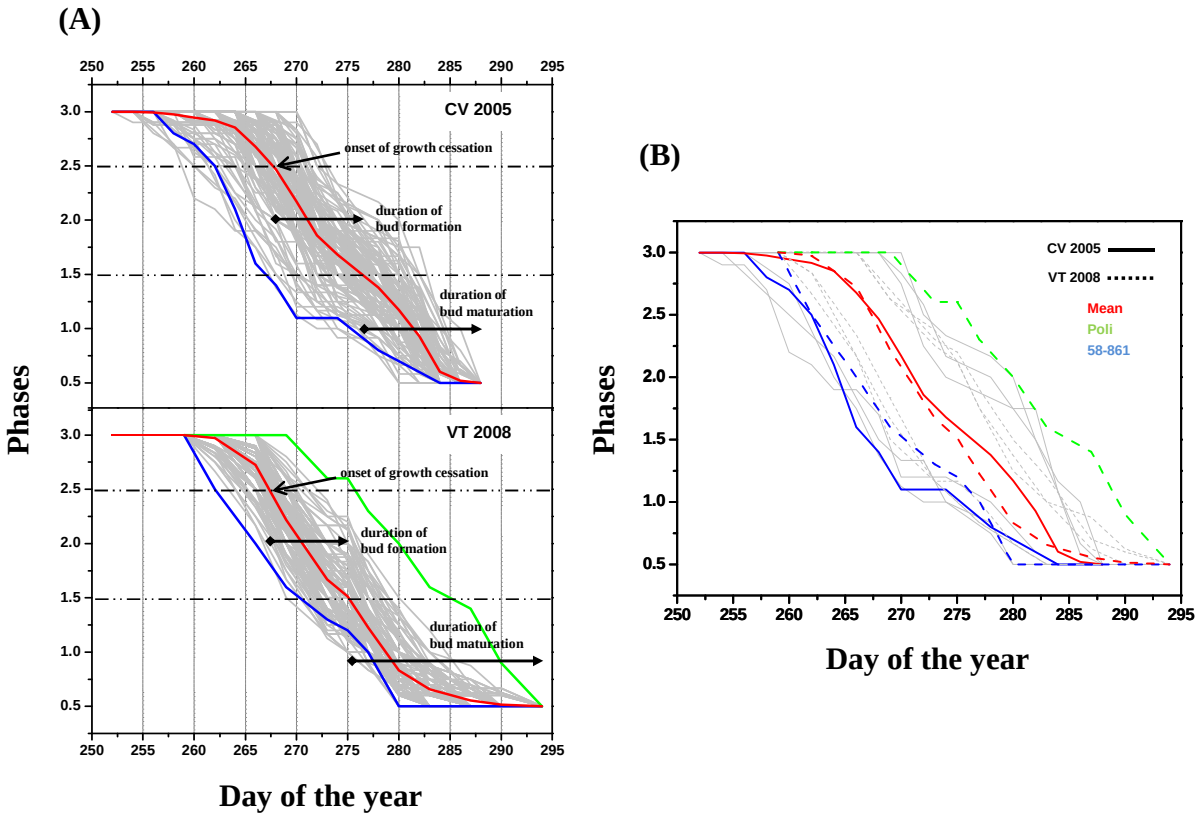


Figure 14. Trend of bud development at the two sites CV and VT. (A) Genotype means are showed for the progenies (grey lines), "58-861" (blue line) and "Poli" (green line). The full-sib family mean is indicated with a red line. (B) Five early and five late genotypes, population means and the parent values are shown.

Comparing the two sites, data showed a later onset of the bud set dynamics of genotypes in VT respect to the northern Italy site: the earliest genotype in CV reached phase 2.5 in DOY 262.9 (genotype 76) and in DOY 259 (Table 2, B) in the central Italy experimental field (genotype 36). This result is quite unexpectedly because in terms of CNL we had an onset of the bud set process after 762.2 and 809.4 hours in CV and in VT, respectively. Minimum temperature was the most contrasting environmental variable between the two planting sites. The plantation in VT experienced a minor CMT_{10} respect to CV (19.3 vs. 28.7) that is, effectively, a more cold and continental station.

Table 2. Duration of bud development. (A) Values expressed as DOY, CNL and CMT_{10} of phase 2.5, phase 0.5 and the total process length at family level. (B) Date in which the earliest genotype reached phase 2.5 (expressed using all the parameters considered) and the latest genotype phase 0.5; the duration of total process was calculated as the difference between the two extreme clones.

(A) **POPULATION MEAN**

Site	Phase 2.5			Phase 0.5			Total Process		
	DOY	CNL	CMT10	DOY	CNL	CMT10	Duration (Day)	Duration (CNL)	Duration (CMT)
CV 2005	267.65	864.23	45.11	286.45	1098.25	130.83	18.80	234.02	85.72
VT 2008	267.32	861.95	34.47	286.17	1093.54	84.07	18.85	231.59	49.60

(B)

Site	Early genotype			Late genotype					
	Phase 2.5			Phase 0.5			Total Process		
	DOY	CNL	CMT10	DOY	CNL	CMT10	Duration (Day)	Duration (CNL)	Duration (CMT)
CV 2005	259.03	762.21	28.70	288.54	1125.16	138.61	29.51	362.95	109.91
VT 2008	262.87	809.41	19.30	292.29	1174.63	91.50	29.42	365.22	72.20

However, these results were observed only at genotypic level as in the two sites was found, substantially, the same population mean, i.e. DOY 267, for the onset of the bud set process. This value is equivalent to the critical night length expressed in CNL amounting to about 864 and 862 hours in CV and VT, respectively (Table 2, A). The obtained results are in accord with the concept of critical night length for which we would expect approximately the same night length for the initiation of bud development in the same analysed population.

The latest genotypes that reached phase 0.5 in CV fully closed the apical bud on DOY 288.5 (genotype 25), earlier than the last one in VT, that reached the same phenological stage on DOY 292.3 (genotype 161), corresponding to 1125.2 and 1174.6 hours of CNL in CV and VT, respectively. Concerning the low temperatures experienced from July 1st up to the end of the bud set process, we had 138.6°C of CAT_{10} in the northern site versus 91.5°C in central Italy, with differences that could explain the minor night length cumulated up to the end of the total phenological process of bud formation in CV.

Even if differences on the onset of phase 0.5 between the two sites were found at genotypic level, the same population mean was observed in the two sites for the study parameters characterizing this phase (DOY 286.4 in CV and DOY 286.2 in VT), corresponding to a quite small difference in terms of effective night length (i.e. about 8 minutes) (Table 3).

Table 3. Night length of the days in which the different phases were reached at the two sites.

	Cavallermaggiore		Viterbo	
	DOY	Night Length	DOY	Night Length
Phase 2.5	267.6	11.57	267.3	11.54
Phase 2	271.8	12.09	271.1	12.05
Phase 1.5	275.9	12.24	274.6	12.15
Phase 1	280.3	12.37	278.7	12.26
Phase 0.5	286.5	12.55	286.2	12.47

CNL data were almost identical (1098.2 and 1093.5 for the northern and the central site, respectively) even if we registered a difference of 46.8°C of CAT_{10} (130.8 and 84.1 in CV and VT, respectively) between the two sites.

Very small differences in terms of CNL were also observed for the other phases during the bud set process. As showed in Table 3 each stage was reached at the two sites under a quite similar effective cumulative night length.

Very interesting are the results concerning the duration of the total bud set process for the full-sib family at the two sites.

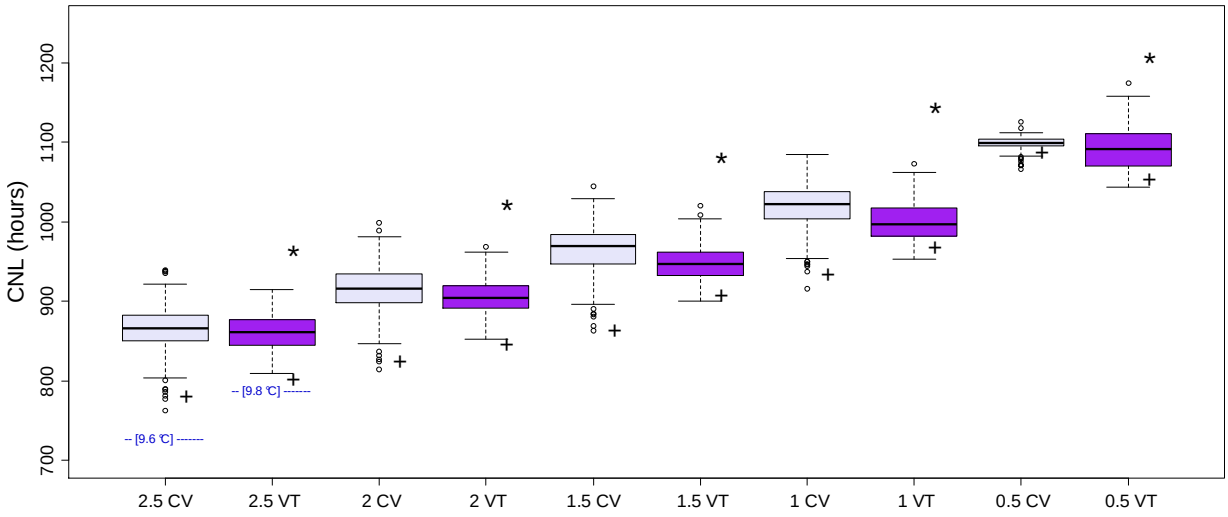


Figure 15. Genotypic variation from the onset of growth cessation (phase 2.5) to the end of the bud formation (phase 0.5) observed in CV and VT. Block-adjusted genotypic means expressed as CNL were used. The northern parent "58-861" is showed as "+", "Poli" as "*".

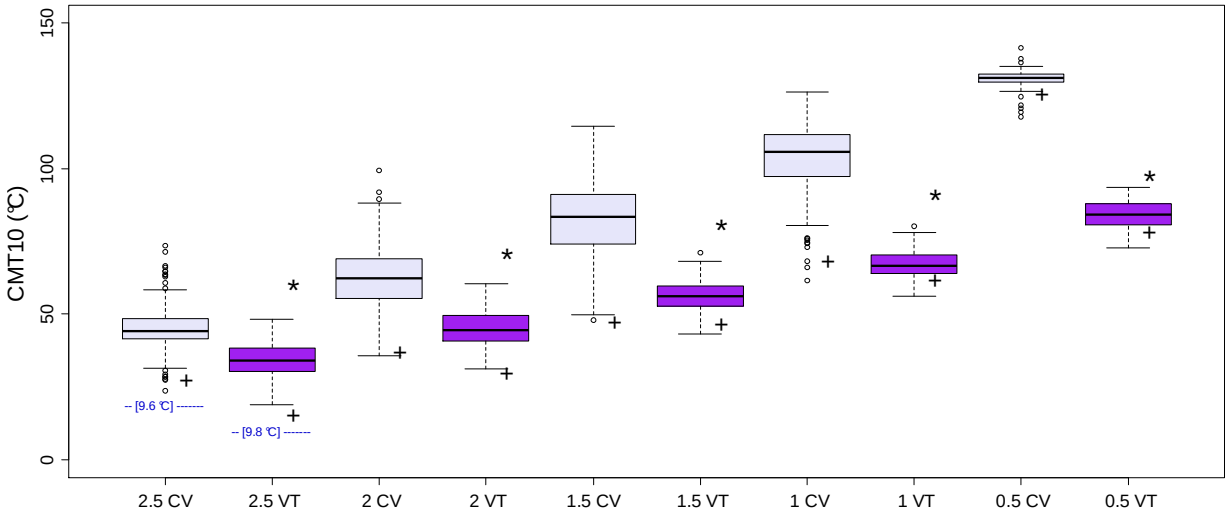


Figure 16. Genotypic variation from the onset of growth cessation (phase 2.5) to the end of the bud formation (phase 0.5) observed in CV and VT. Block-adjusted genotypic means expressed as CMT_{10} were used. The northern parent "58-861" is showed as "+", "Poli" as "*".

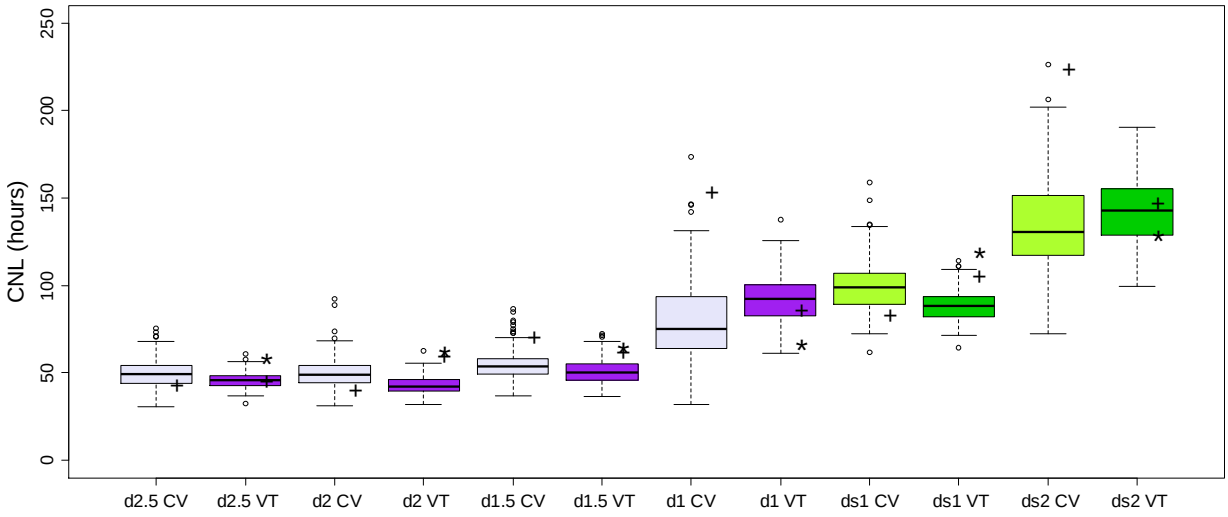


Figure 17. Genotypic variation of the duration of the different phases and of the two subprocesses observed CV and VT. Block-adjusted genotypic means expressed as CNL were used. The northern parent "58-861" is showed as "+", "Poli" as "*".

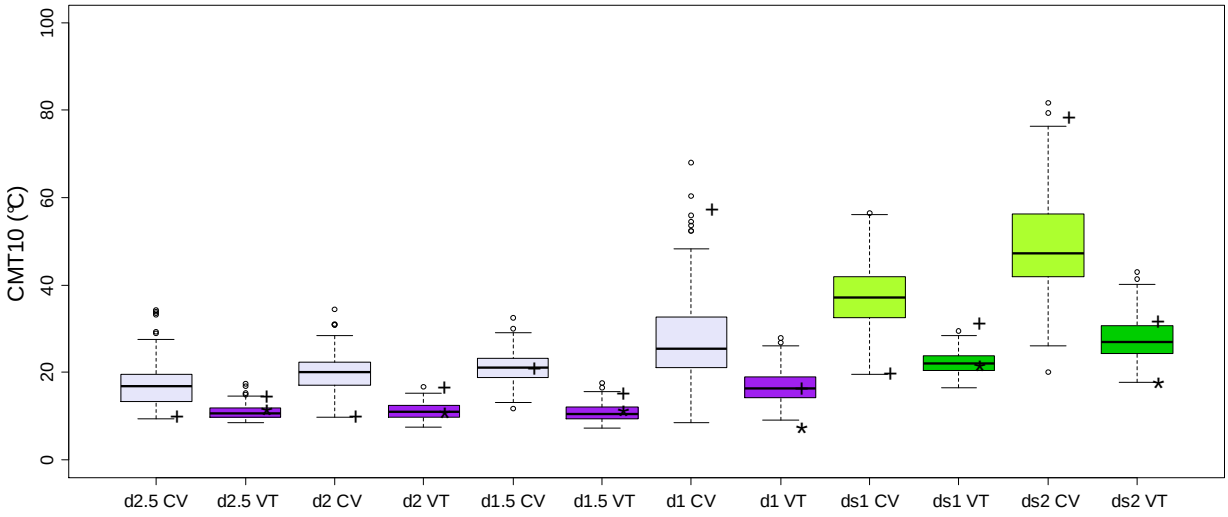


Figure 18. Genotypic variation of the duration of the different phases and of the two subprocesses observed CV and VT. Block-adjusted genotypic means expressed as CMT_{10} were used. The northern parent "58-861" is showed as "+", "Poli" as "*".

Data show the same duration amounting to 18.80 and 18.85 days for the northern and the central sites, respectively, corresponding to 234 and 231.6 hours of CNL, even if CV experienced a major "cold" sum (85.7 vs. 49.6 CMT_{10}).

The same behaviour was found also at genotypic level where the same duration in days, i.e. 29.5, of the total bud set process (from the onset of growth cessation of the earliest genotype to the last stage of the latest one) was found in both sites (Table 2, B). Small differences for CNL, amounting to about two hours (362.9 vs. 365.2), were observed between the sites and the minor value in CV is due to the longer night in the northern site after autumnal equinox. For the same trait a major divergence between the sites was found for CMT_{10} with a difference of 37.7°C (109.9 in CV and 72.2 in VT).

Genotypic variation of all the studied phenological phases during the bud set process was higher in CV except for the phase 0.5 that was characterized by a quite small variability (Figure 15 and 16). Another interesting difference between the sites was the presence of "outlier" precocious genotypes for all the phases only in CV. Data showed that when the growth cessation start, the different phenological stages follow each other constantly as a cascade process. Analysing the duration of the different phases, the same length for CNL was found within and between sites, except for the duration of phase 1 that was longer and more variable than the other duration traits in both sites (Figure 17 and 18). Pedigree POP5 spent, on average, 4.71 and 4.72 days (corresponding to 57.9 and 58.7 hours of CNL) in each phase in VT and CV, respectively. Duration of phase 1 showed a different behaviour and was characterized by a longer mean duration of 6.2 and 7.4 days (79.9 and 92.1 hours of CNL) in CV and VT, respectively.

Considering the duration of the two subprocesses described in the section Material and Methods, POP5 spent more time, 10.6 days in CV and 11.6 days d in VT, and then cumulated a major sum of night length and "cold" for the bud maturation (duration of subprocess 2) rather than for the bud formation (duration of subprocess 1) where 8.2 days and 7.3 days in CV and VT, respectively, were necessary to complete the subprocess. A major variability in the duration of bud maturation was observed, in both the experiments of this work.

4.4. Comparison of POP5 parents and family mean values

The parents of the POP5 pedigree originated in two contrasting environments spanning about 5° of latitude and their genotype was subjected to a different evolutionary history.

Unfortunately, both parents were available only in VT as "Poli" didn't sprout well after coppice in CV.

Table 4. Parents' values (i.e. means ± standard errors and significance differences between the two parents "58-861" and "Poli") and family genotypic variation (i.e. number of genotype analysed, average number of individuals per genotype, population means and range of genotypic variation) for POP5 grown in VT. The significance level of the F-test between the two parents for each trait is indicated as: ns = non significant, * = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001.

VT 2008		Parental values							Family phenotypic variation							
Trait	Climatic parameter	Poli	±	SE	58-861	±	SE	*F* test	N° genotype analysed	Mean no. replicates	General mean	±	SE	P	Min. genotype mean	Max. genotype mean
Phase 2.5	CNL	960.04	±	12.18	801.54	±	1.74	***	154	4.13	861.95	±	1.22	***	809.51	914.97
Phase 2	CNL	1016.16	±	12.17	846.86	±	4.68	***	154	4.13	907.51	±	1.22	***	852.37	968.87
Phase 1.5	CNL	1076.22	±	9.47	906.56	±	8.68	***	154	4.13	950.24	±	1.28	***	899.84	1020.49
Phase 1	CNL	1138.32	±	8.85	968.12	±	8.06	***	154	4.13	1001.44	±	1.45	***	952.48	1073.29
Phase 0.5	CNL	1202.04	±	7.63	1053.68	±	3.24	***	154	4.13	1093.54	±	2.22	***	1043.83	1175.05
Duration 2.5	CNL	56.12	±	3.03	45.32	±	3.12	*	154	4.13	45.56	±	0.32	**	32.52	60.93
Duration 2	CNL	59.76	±	4.16	59.70	±	6.33	NS	154	4.13	42.73	±	0.38	*	31.76	62.56
Duration 1.5	CNL	62.40	±	8.83	61.56	±	3.04	NS	154	4.13	51.20	±	0.58	NS	36.43	72.46
Duration 1	CNL	63.72	±	2.24	85.56	±	11.11	NS	154	4.13	92.10	±	2.11	NS	61.11	137.49
Dur.Sub.1	CNL	116.18	±	4.66	105.02	±	8.14	NS	154	4.13	88.29	±	0.66	**	64.28	114.03
Dur.Sub.2	CNL	126.12	±	9.79	147.12	±	11.91	NS	154	4.13	143.31	±	2.17	NS	99.25	190.42
Phase 2.5	CMT10	58.80	±	2.40	15.18	±	0.62	***	154	4.13	34.47	±	0.32	***	18.91	48.28
Phase 2	CMT10	69.42	±	2.14	29.78	±	1.43	***	154	4.13	45.43	±	0.34	***	31.05	60.56
Phase 1.5	CMT10	79.42	±	1.91	46.38	±	3.18	***	154	4.13	56.58	±	0.30	***	43.04	71.17
Phase 1	CMT10	89.82	±	0.88	61.72	±	1.50	***	154	4.13	67.34	±	0.25	***	56.19	80.15
Phase 0.5	CMT10	96.24	±	0.54	78.00	±	2.71	**	154	4.13	84.07	±	0.33	***	72.77	93.59
Duration 2.5	CMT10	10.62	±	0.61	14.60	±	0.88	**	154	4.13	10.96	±	0.11	*	8.52	17.44
Duration 2	CMT10	10.00	±	0.73	16.60	±	2.94	NS	154	4.13	11.15	±	0.14	**	7.55	16.67
Duration 1.5	CMT10	10.40	±	1.72	15.34	±	1.83	NS	154	4.13	10.76	±	0.13	***	7.30	17.53
Duration 1	CMT10	6.42	±	0.41	16.28	±	4.10	NS	154	4.13	16.72	±	0.25	*	9.05	27.79
Dur.Sub.1	CMT10	20.62	±	1.12	31.20	±	3.23	*	154	4.13	22.11	±	0.19	NS	16.52	29.46
Dur.Sub.2	CMT10	16.82	±	1.91	31.62	±	5.87	NS	154	4.13	27.49	±	0.31	**	17.75	42.94
score50%_2.5 (BS266)	DOY	3.00	±	0.00	2.00	±	0.00	***	154	4.13	2.73	±	0.01	***	2.13	3.03
score50%_1.5 (BS273)	DOY	2.60	±	0.10	1.30	±	0.12	***	154	4.13	1.67	±	0.02	***	1.11	2.40

Data showed strong differences between the two parental genotypes concerning the different date-of-onset traits analysed using climatic parameters (CNL and CMT_{10}) and for the date in which 50% of individuals reach phases 2.5 and 1.5 (score50%_2.5 and score50%_1.5). Not significant differences were observed for the duration traits, except for duration of phase 2.5 ($P \leq 0.05$ using CNL and $P \leq 0.01$ using CMT_{10}). The two subprocesses also showed not significant differences, at exception of the duration of subprocess 1 for CMT_{10} that showed only a few significant differences ($P \leq 0.05$) (Figure 15 to 18, Table 4).

The parents showed values at the two extremes of the distribution of the segregating family for all the date-of-onset traits and both climatic parameters in VT.

Table 5. Parent values (i.e. means ± standard errors and significance differences between the two parents "58-861" and "Poli") and family genotypic variation (i.e. number of genotypes analysed, average number of individuals per genotype, population means and range of genotypic variation) of POP5 grown in CV. It was not possible to perform the F-test between parents due to the absence of "Poli" (see Materials and Methods).

CV 2005		Parental values							Family phenotypic variation							
Trait	Climatic parameter	Poli	±	SE	58-861	±	SE	*F* test	N° genotype analysed	Mean no. replicates	General mean	±	SE	P	Min. genotype mean	Max. genotype mean
Phase 2.5	CNL	NA	±	NA	781.17	±	10.16	-	148	4.49	864.23	±	1.62	***	762.21	939.60
Phase 2	CNL	NA	±	NA	824.17	±	9.47	-	148	4.49	913.87	±	1.68	***	814.31	998.74
Phase 1.5	CNL	NA	±	NA	863.93	±	9.51	-	148	4.49	964.08	±	1.76	***	862.75	1044.86
Phase 1	CNL	NA	±	NA	934.33	±	20.75	-	148	4.49	1019.04	±	1.64	***	915.57	1084.96
Phase 0.5	CNL	NA	±	NA	1087.67	±	9.46	-	148	4.42	1098.25	±	0.63	***	1065.99	1125.65
Duration 2.5	CNL	NA	±	NA	43.00	±	2.49	-	148	4.49	49.64	±	0.58	***	30.80	75.58
Duration 2	CNL	NA	±	NA	39.76	±	3.56	-	148	4.49	50.21	±	0.60	***	31.04	92.42
Duration 1.5	CNL	NA	±	NA	70.40	±	14.61	-	148	4.49	54.95	±	0.58	***	37.01	86.59
Duration 1	CNL	NA	±	NA	153.34	±	17.27	-	148	4.42	79.95	±	1.43	***	32.17	173.40
Dur.Sub.1	CNL	NA	±	NA	82.76	±	5.43	-	148	4.49	99.85	±	1.02	***	61.84	159.11
Dur.Sub.2	CNL	NA	±	NA	223.74	±	2.95	-	148	4.42	134.91	±	1.59	***	72.27	226.15
Phase 2.5	CMT10	NA	±	NA	27.16	±	1.81	-	148	4.49	45.11	±	0.42	***	23.73	73.51
Phase 2	CMT10	NA	±	NA	37.10	±	1.93	-	148	4.49	62.34	±	0.62	***	35.68	99.29
Phase 1.5	CMT10	NA	±	NA	47.12	±	2.24	-	148	4.49	82.26	±	0.73	***	47.83	114.52
Phase 1	CMT10	NA	±	NA	68.08	±	8.47	-	148	4.49	103.36	±	0.66	***	61.44	126.16
Phase 0.5	CMT10	NA	±	NA	125.48	±	4.11	-	148	4.43	130.83	±	0.20	***	117.68	141.31
Duration 2.5	CMT10	NA	±	NA	9.94	±	0.34	-	148	4.49	17.23	±	0.32	***	9.46	34.33
Duration 2	CMT10	NA	±	NA	10.02	±	0.68	-	148	4.49	19.92	±	0.31	***	9.75	34.46
Duration 1.5	CMT10	NA	±	NA	20.96	±	6.60	-	148	4.49	21.10	±	0.25	***	11.64	32.54
Duration 1	CMT10	NA	±	NA	57.40	±	6.78	-	148	4.43	27.73	±	0.60	***	8.53	68.04
Dur.Sub.1	CMT10	NA	±	NA	19.96	±	0.95	-	148	4.49	37.15	±	0.49	***	19.45	56.38
Dur.Sub.2	CMT10	NA	±	NA	78.36	±	2.32	-	148	4.43	48.86	±	0.68	***	20.15	81.64
score50%_2.5 (BS268)	DOY	NA	±	NA	1.40	±	0.09	-	148	4.49	2.47	±	0.02	***	1.41	3.04
score50%_1.5 (BS274)	DOY	NA	±	NA	1.10	±	0.09	-	148	4.49	1.69	±	0.02	***	0.92	2.35

The southern genotype (i.e. "Poli") reached always later than "58-861" the onset of the different phases, and this northern parent maintained the performance as one of the early genotypes in CV. Regardless the climatic parameters used, "58-861" in the northern site showed transgressive segregation for all the different date-of-onset traits from the beginning to the end of the process (except for phase 1.5 CMT_{10}). This behaviour was observed in VT only during bud maturation (phases 1.5, 1 and 0.5) (Figure 15 and 16). On the contrary, "Poli" was firmly the latest genotype and it never showed transgressive segregation.

Concerning the two subprocesses, “58-861” was one of the most quick genotype to complete the subprocess 1 in the northern plantation but, on the other hand, was very slow to complete the subprocess 2 (Figure 17 and 18).
We didn’t observe the same behaviour in VT, where the parent “58-861” and “Poli” didn’t place the extreme values on the family distribution and showed similar durations of the two subprocesses.
The onset of the bud set process for the northern parent was 1.5 days earlier in CV than in VT (781.2 hours versus 801.5 hours of CNL, corresponding to a difference of 20.37 hours) and in this site comparison “58-861” remained, on average, 2.5 days earlier for the other phases. This situation changed for phase 0.5 that was about 2.5 days later in CV than in VT (data not shown). Besides, to reinforce the previous data, the “cold” experienced by the female parent at the onset of the process in the northern location was higher (27.2°C vs. 15.2°C) and it remained higher for each following phase (Table 4 and 5).
Concerning the length of the bud set process of the genotype “58-861”, the comparison of the duration of subprocess 1 between CV and VT was shorter of about two days, corresponding to 22.3 hours of CNL, in the northern site and there were about six days more for the duration of subprocess 2 (about 76.6 hours of CNL) between the same sites.
Genotype “58-861” presented a different length of the two subprocesses in both sites, with a longer duration of subprocess 2 in respect to subprocess 1. On the contrary, “Poli”, that was present only in VT, spent the same time for each of the two processes (about 9.5 days).

4.5. Within-site family variability and heritability

Presence of a substantial within-site genetic variation for the full-sib family was observed in this study (Table 6 and 7), due to the choice of parents originated from widely different sites.
Data showed higher values of genetic variability in the northern site than in the central one for all the traits and all the different climatic parameters, except for phase 0.5. The coefficient of genetic variation (CV_G) ranged from 1.7% (phase 0.5 CNL) to 2.2% (phase 2.5 CNL) in VT and from 0.5% (i.e. phase 0.5 CNL) to 3.5% (phase 2.5 CNL) in CV. The coefficient of residuals variation (CV_R) ranged from 2.6% (phase 2 CNL) to 4% (phase 0.5 CNL) in VT and from 1.4% (phase 0.5 CNL) to 3.6% (phase 1.5 CNL) in CV.
In general, the higher values of residual variation and the lower values of CV_G/CV_R ratio, defined to understand how CV_G changes in respect to CV_R, were found for duration traits

(Table 6 and 7), underlining the higher influence of environmental conditions on these characters.

Table 6. Family genetic variation: coefficients of genetic (CV_G) and residual (CV_R) variation, individual (H^2_{ind}) and genotypic (H^2_{gen}) broad-sense heritability for the *P. nigra* x *P. nigra* full-sib family grown in VT.

VT 2008		Family genetic variation				
Trait	Climatic parameter	CV_G (%)	CV_R (%)	CV_G/CV_R	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	CNL	2.22	2.76	0.802	0.39 ± 0.05	0.73 ± 0.03
Phase 2	CNL	2.17	2.59	0.837	0.40 ± 0.05	0.74 ± 0.03
Phase 1.5	CNL	2.16	2.62	0.822	0.39 ± 0.05	0.73 ± 0.03
Phase 1	CNL	2.02	3.04	0.665	0.30 ± 0.04	0.64 ± 0.04
Phase 0.5	CNL	1.71	3.96	0.432	0.16 ± 0.04	0.45 ± 0.04
Duration 2.5	CNL	6.16	19.90	0.310	0.09 ± 0.04	0.28 ± 0.04
Duration 2	CNL	5.58	21.46	0.260	0.07 ± 0.03	0.24 ± 0.04
Duration 1.5	CNL	4.35	28.80	0.151	0.04 ± 0.03	0.15 ± 0.04
Duration 1	CNL	3.69	28.59	0.129	0.03 ± 0.03	0.10 ± 0.04
Dur.Sub.1	CNL	5.03	17.43	0.289	0.08 ± 0.04	0.27 ± 0.04
Dur.Sub.2	CNL	4.19	25.37	0.165	0.04 ± 0.03	0.15 ± 0.04
Phase 2.5	CMT10	14.05	18.28	0.769	0.37 ± 0.05	0.71 ± 0.03
Phase 2	CMT10	11.89	14.35	0.828	0.40 ± 0.05	0.74 ± 0.03
Phase 1.5	CMT10	8.02	10.37	0.774	0.38 ± 0.05	0.72 ± 0.03
Phase 1	CMT10	5.45	7.86	0.693	0.32 ± 0.04	0.66 ± 0.04
Phase 0.5	CMT10	3.39	8.97	0.378	0.13 ± 0.04	0.38 ± 0.05
Duration 2.5	CMT10	7.17	24.14	0.297	0.05 ± 0.03	0.18 ± 0.04
Duration 2	CMT10	8.75	28.75	0.305	0.10 ± 0.04	0.31 ± 0.04
Duration 1.5	CMT10	10.67	28.94	0.369	0.12 ± 0.04	0.37 ± 0.05
Duration 1	CMT10	8.90	36.65	0.243	0.05 ± 0.03	0.17 ± 0.04
Dur.Sub.1	CMT10	3.41	20.57	0.166	0.03 ± 0.03	0.11 ± 0.04
Dur.Sub.2	CMT10	8.92	27.42	0.325	0.08 ± 0.04	0.27 ± 0.04
score50%_2.5 (BS266)	DOY	6.09	8.73	0.698	0.33 ± 0.04	0.67 ± 0.03
score50%_1.5 (BS273)	DOY	12.96	18.89	0.686	0.32 ± 0.04	0.66 ± 0.04

Data analysed using CNL showed a decreasing importance of genetic component of variance from the perception of critical night length (phase 2.5) to the end of bud maturation (phase 0.5) and between the score50%_2.5 and score50%_1.5.

Duration traits showed a different trend between the two sites. CV_G/CV_R ratio increased from the duration of phase 2.5 to phase 0.5 and between the first subprocess and the second one in CV. The tendency shows an opposite behaviour for the same traits in VT. CMT_{10} data presented higher values of CV_G and CV_R for the different traits in both sites (Table 6 and 7).

Table 7. Family genetic variation: coefficients of genetic (CV_G) and residual (CV_R) variation, individual (H^2_{ind}) and genotypic (H^2_{gen}) broad-sense heritability for the *P. nigra* x *P. nigra* full-sib family grown in CV.

CV 2005		Family genetic variation				
Trait	Climatic parameter	CV_G (%)	CV_R (%)	CV_G/CV_R	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	CNL	3.46	3.24	1.067	0.53 ± 0.04	0.84 ± 0.02
Phase 2	CNL	3.25	3.35	0.969	0.48 ± 0.05	0.81 ± 0.02
Phase 1.5	CNL	2.96	3.56	0.831	0.40 ± 0.05	0.75 ± 0.03
Phase 1	CNL	2.45	3.29	0.742	0.35 ± 0.05	0.70 ± 0.03
Phase 0.5	CNL	0.53	1.37	0.385	0.14 ± 0.04	0.43 ± 0.05
Duration 2.5	CNL	7.78	26.76	0.291	0.11 ± 0.04	0.36 ± 0.05
Duration 2	CNL	12.99	28.09	0.463	0.18 ± 0.04	0.49 ± 0.04
Duration 1.5	CNL	11.59	24.58	0.472	0.23 ± 0.04	0.57 ± 0.04
Duration 1	CNL	23.32	38.61	0.604	0.27 ± 0.04	0.62 ± 0.04
Dur.Sub.1	CNL	10.01	23.59	0.424	0.15 ± 0.04	0.44 ± 0.05
Dur.Sub.2	CNL	16.70	24.59	0.679	0.32 ± 0.05	0.67 ± 0.04
Phase 2.5	CMT10	16.72	17.06	0.980	0.51 ± 0.04	0.82 ± 0.02
Phase 2	CMT10	16.45	19.05	0.864	0.45 ± 0.05	0.79 ± 0.03
Phase 1.5	CMT10	13.69	17.89	0.765	0.37 ± 0.05	0.72 ± 0.03
Phase 1	CMT10	9.85	12.99	0.758	0.34 ± 0.05	0.70 ± 0.03
Phase 0.5	CMT10	1.30	3.51	0.369	0.17 ± 0.04	0.48 ± 0.05
Duration 2.5	CMT10	21.45	42.07	0.510	0.25 ± 0.04	0.60 ± 0.04
Duration 2	CMT10	13.61	36.74	0.370	0.15 ± 0.04	0.45 ± 0.05
Duration 1.5	CMT10	10.38	28.49	0.364	0.15 ± 0.04	0.44 ± 0.05
Duration 1	CMT10	30.79	45.27	0.680	0.29 ± 0.04	0.64 ± 0.04
Dur.Sub.1	CMT10	14.79	30.19	0.490	0.21 ± 0.04	0.54 ± 0.04
Dur.Sub.2	CMT10	19.68	29.11	0.676	0.31 ± 0.05	0.66 ± 0.04
score50%_2.5 (BS268)	DOY	12.83	13.57	0.946	0.47 ± 0.05	0.80 ± 0.03
score50%_1.5 (BS274)	DOY	14.67	21.19	0.693	0.33 ± 0.05	0.69 ± 0.03

Medium values of broad-sense heritability (H^2_{ind}) were observed for the onset of the phenological process (phase 2.5), the phase 2 and score50%_2.5 in CV ranging from 0.45 to 0.53 while lower values were observed in VT for heritabilities related to the same phases of each climatic parameter (0.33 – 0.40). Comparing the two sites, we observed the highest values of heritability in the northern site where the experimental plantation was at the second year of growth after coppice.

The transition from the shoot to the bud structure (i.e. phase 1.5 CNL and CMT_{10}) showed quite similar values between the two sites ($0.37<H^2_{ind}<0.40$; $0.72<H^2_{gen}<0.75$).

High values of broad-sense heritability at genotype level were observed for the trait score50%_2.5, especially for the northern site ($H^2_{gen}=0.80$), and similar values between sites for the score50%_1.5.

As expected, because of the low values of CV_G compared to CV_R, data show small levels of heritability in VT concerning duration traits and subprocesses analysed using CNL parameter ($0.03<H^2_{ind}<0.09$; $0.10<H^2_{gen}<0.28$). There was a decreasing importance of the genetic role for these traits during the bud set process in VT while low to moderate values of the full-sib family grown in the northern site ($0.11<H^2_{ind}<0.32$; $0.36<H^2_{gen}<0.67$) and an opposite trend of genetic importance during the process were found.

4.6. Genotype x Site interaction and genotype stability

All the sources of variability concerning analysed data expressed in CNL, were significant except the site effect for the induction of the apical bud formation process (i.e. phase 2.5) where any part of the phenotypic variation was due to the site effect, and the genotype by site interaction (GxS) for the duration of phase 2.5. Genotypic relative importance in the phenotypic variation, as well as the interaction GxS component, are considerable in all the different onset-of stage traits and for the two dates score50%_2.5 and score50%_1.5, with a firmly decreasing trend from the perception of critical night length to bud maturation. The phenotypic variation explained by genotype ranges from 29.6% (phase 2.5) to 5.8% (phase 0.5) and values from 19.0% (phase 2.5) to 9.2% (phase 0.5) are explained by GxS interaction. The Spearman rank coefficients between genotypic means at the two sites were positive, moderate and high significant, but they decreased during the process. Hence, there were changes in the ranking of the clones between the two sites and the general classification, mainly maintained at the onset of the bud set process, changed during it (Table 8).

Concerning duration traits, including the two overall subprocesses, we observed low levels of genotypic component ($0.8\%<\sigma^2_G/\sigma^2_P<8.9\%$) and high levels of residuals (up to 87.9%) in the phenotypic variation, with an increasing importance of GxS interaction during bud development (from 0.0% to 16.3%) that reached a value of 20.0% for subprocess 2. The low and often not significant values of Spearman's coefficient were a clear sign of strong random changes in genotype ranking. The relative importance of the site was high for all the phases and subprocesses analysed using the minimum temperature parameter (CMT_{10}) and, in particular, it increased from the onset of the process to the end of but formation (up to 97.2% of phenotypic variance explained). Using this parameter, the genetic relative contribution and the interaction source of variability found were generally low and, also in this case, the higher

value was shown by the first two onset-of stage traits, i.e. phase 2.5 (σ^2_G/σ^2_P = 16.6% and $\sigma^2_{GxS}/\sigma^2_P$ = 11.1%) and phase 2 (σ^2_G/σ^2_P = 12.8% and $\sigma^2_{GxS}/\sigma^2_P$ = 10.5%).

Table 8. Relative importance of site (σ^2_S), genetic (σ^2_G), genotype by site (σ^2_{GxS}) and residual (σ^2_ε) effects in the phenotypic variation (σ^2_P). Spearman rank coefficients were calculated from genotype means in CV and VT. All correlations are significant at P≤0.05.

		Variance components (%)							Spearman Rank	
		$\sigma2_S/\sigma2_P$		$\sigma2_G/\sigma2_P$		$\sigma2_{GxS}/\sigma2_P$		$\sigma2_\varepsilon/\sigma2_P$	Coeff.	P.Val.
Phase 2.5	CNL	0.0	NS	29.6	***	19.0	***	51.4	0.51	***
Phase 2.0		1.1	***	27.1	***	18.8	***	53.0	0.51	***
Phase 1.5		5.6	***	21.8	***	16.8	***	55.8	0.46	***
Phase 1.0		9.7	***	17.2	***	13.1	***	60.0	0.39	***
Phase 0.5		2.9	***	5.8	***	9.2	***	82.1	0.22	**
Duration 2.5		3.2	***	8.9	***	0.0	NS	87.9	0.27	**
Duration 2.0		14.5	***	2.9	***	7.7	**	74.8	0.09	NS
Duration 1.5		6.3	***	0.9	***	11.5	***	81.3	0.06	NS
Duration 1.0		12.4	***	1.9	***	16.3	***	69.4	0.08	NS
Dur. Subproc. 1		11.3	***	5.3	***	4.9	*	78.4	0.18	*
Dur. Subproc. 2		3.1	***	0.8	***	20.0	***	76.1	0.01	NS
Phase 2.5	CMT10	38.2	***	16.6	***	11.1	***	34.0	0.49	***
Phase 2.0		46.8	***	12.8	***	10.5	***	29.9	0.43	***
Phase 1.5		60.5	***	7.5	***	7.8	***	24.3	0.44	***
Phase 1.0		79.2	***	3.2	***	4.4	***	13.2	0.41	***
Phase 0.5		97.2	***	0.2	***	0.2	**	2.4	0.25	**
Duration 2.5		43.9	***	6.8	***	3.5	*	45.8	0.35	***
Duration 2.0		57.4	***	0.0	***	5.9	***	36.6	0.06	NS
Duration 1.5		71.3	***	0.6	***	3.4	***	24.8	0.10	NS
Duration 1.0		29.0	***	1.4	***	13.2	***	56.3	0.12	NS
Dur. Subproc. 1		58.0	***	1.4	***	5.4	***	35.1	0.16	NS
Dur. Subproc. 2		56.4	***	1.6	***	8.1	***	33.9	0.16	NS
score50%_2.5		17.5	***	21.5	***	13.0	***	48.0	0.47	***
score50%_1.5		0.0	NS	20.8	***	11.8	***	67.4	0.47	***

Spearman rank coefficients were positive and moderate for the different phases described in the phenological protocol but resulted very low and not significant for all the duration traits,

except for duration of phase 2. The relative ecovalence analysis showed that only a few genotypes contributed to the overall GxS interaction (Figure 19).

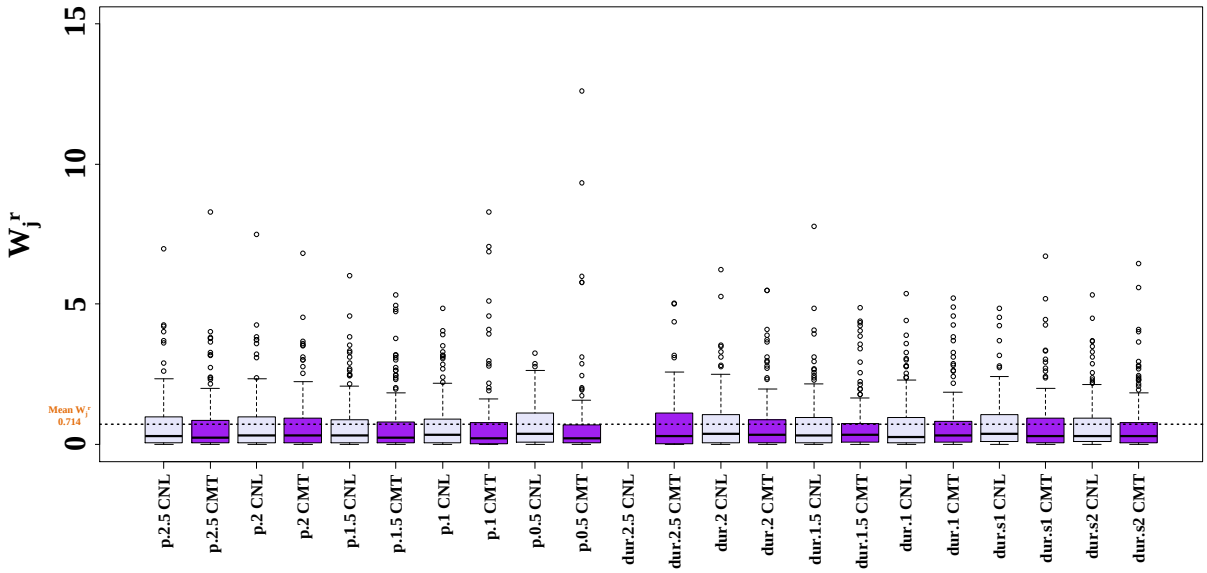


Figure 19. Genotype distribution of the relative genotype ecovalence (W_j^r) for the different phenological traits. Not significant GxS interaction for phase 2.5 analysed using CNL was found. p=phase X, dur=duration of phase X, dur.s = duration of subprocess X.

We found, on average, that selecting only five of the more plastic genotypes for the different traits analyzed, a contribution of 25% of the overall GxS interaction was explained.

4.7. Definition of the most descriptive traits based on phenotypic variation

In the two experimental sites all the different onset-of-stage traits resulted positively highly correlated among each other and especially between contiguous phases whereas highly negative or not correlations between these phases and the two subprocesses were found (Figure 20 and 21). The onset of growth cessation trait highly correlated negatively with the duration of subprocess 2 in CV (r=-0.84), while a low correlation was shown in VT (r=-0.24). We observed also good correlations between score50%_2.5, score50%_1.5 and all the onset-of-stage. Principal component analysis using the five onset-of-stage traits and the two subprocesses was used in order to avoid needless information such as high correlated or redundant traits in genetic analyses.

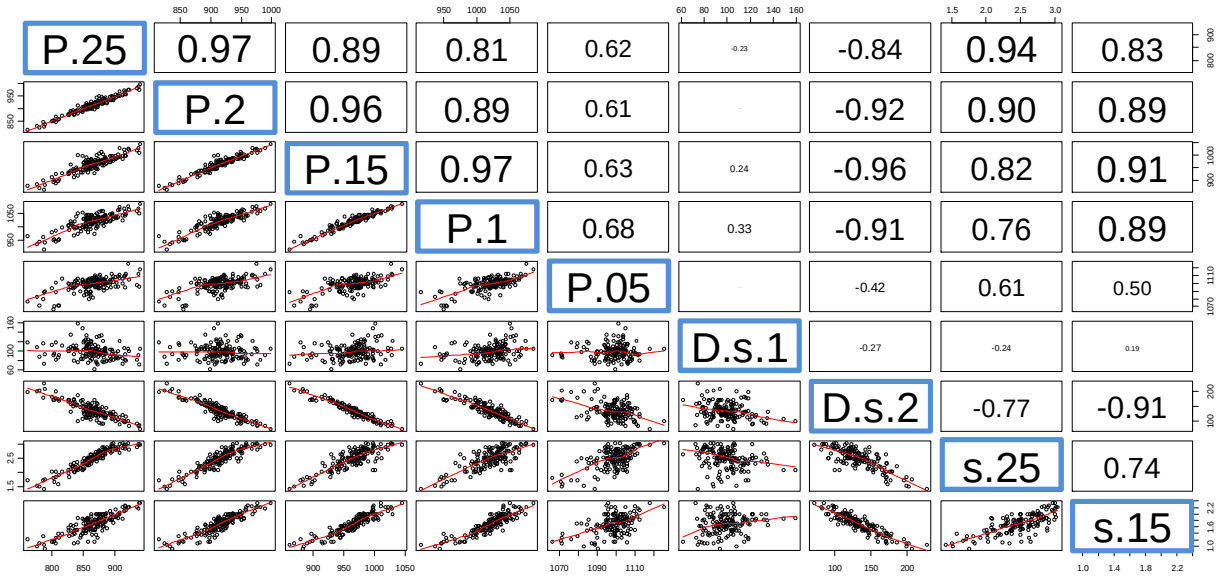


Figure 20. Pearson's phenotypic correlations between traits (CNL) of bud set based on genotypic means in CV. P=phase X, D.s. = duration of subprocess X, s.=date when approximately 50% of the individuals in the experiment have reached at least stage X.

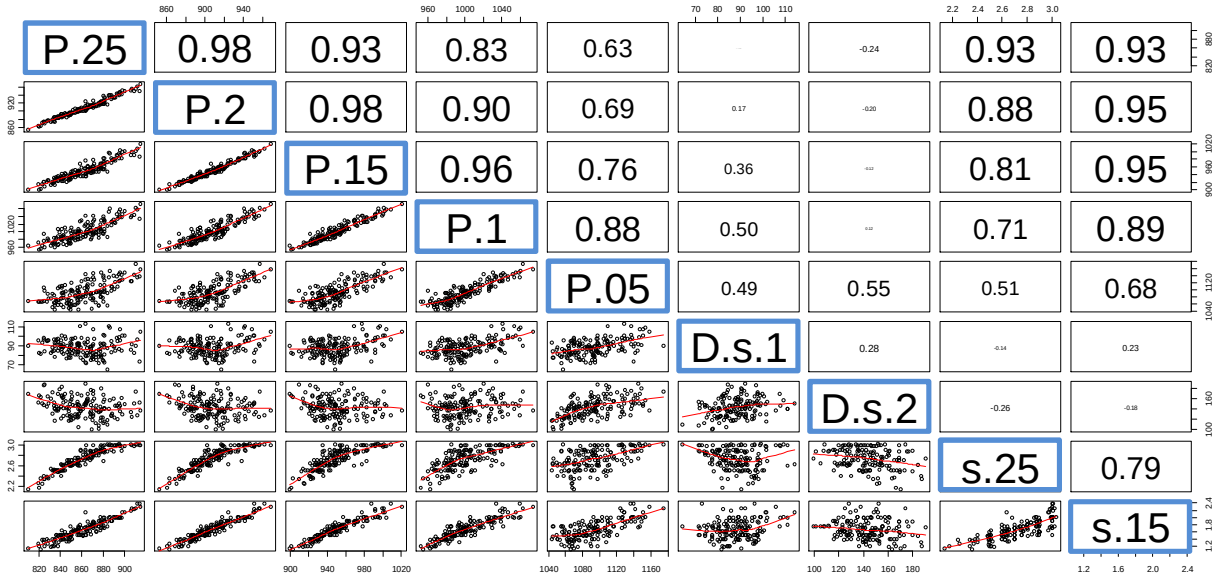


Figure 21. Pearson's phenotypic correlations between traits (CNL) of bud set based on genotypic means in VT. P=phase X, D.s. = duration of subprocess X, s.=date when approximately 50% of the individuals in the experiment have reached at least stage X.

This analysis allowed to identifying the most discriminative traits in term of phenotypic variation in the full-sib family. The different duration traits were not included because wholly represented by the duration of the two main subprocesses (i.e. duration of subprocess 1 and duration of subprocess 2). The phenotypic variation in CV and VT was divided into two major groups: Prin1, representing about 0.67% (CV) and 0.60% (VT) of the phenotypic variation and Prin2, accounting for about 0.18% (CV) and 0.26% (VT) of the same variation (Figure 22). Both sites showed that the onset-of-stage traits, really important for a deep physiological dissection of bud development, had a main contribute along the axis of Prin1.

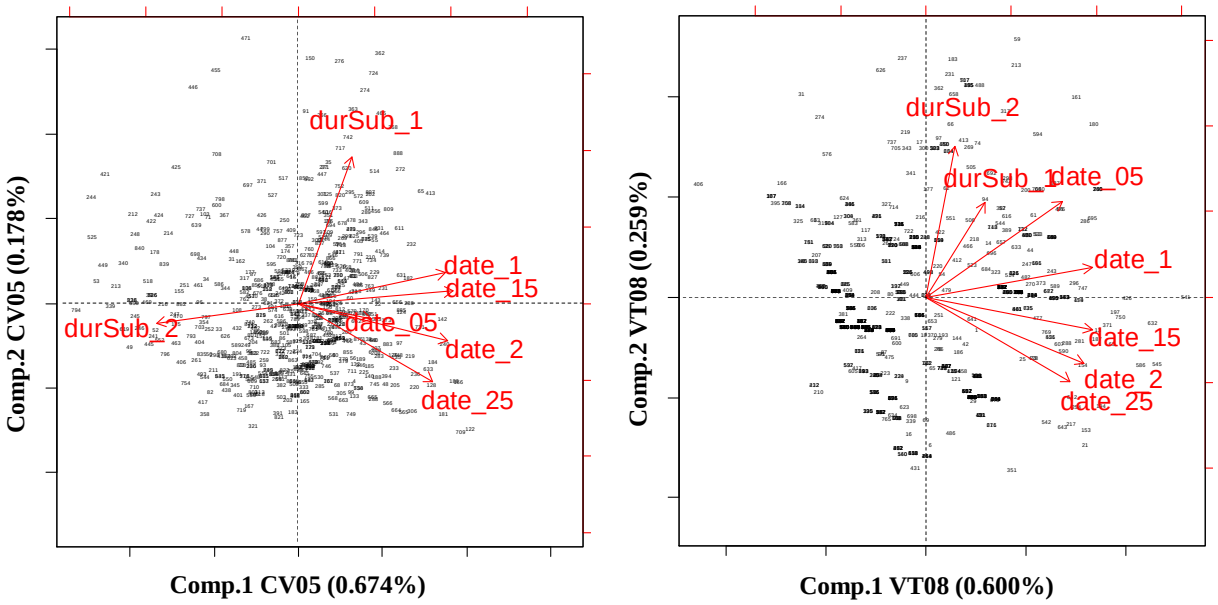


Figure 22. Principal component analysis (PCA) performed on different bud set traits for CV 2005 (on the left) and VT 2008 (on the right). Individual block-adjusted phenotypic values were used.

In CV, phases 2, 1.5 and 1 (CNL) had the major effect (0.440, 0.458 and 0.435 respectively, numeric data not shown) while duration of subprocess 2 for CNL a minor opposing effect on the Prin1 (-0.419). Duration of subprocess 1 contributed most to Prin2 with a minor opposing effect for phase 2.5 CNL (0.836 and -0.446 respectively).

In VT, phases 1.5 and 1 (0.473 and 0.471) had the major effect on the Prin1, with the duration of subprocesses 2 and 1 for CNL that showed the major effect for Prin2 (0.404 and 0.648), and a minor opposing effect for phase 2.5 CNL (-0.361). This analysis allowed to select common characters in the two sites, consisting in the onset of bud initiation (i.e. date 1.5) and the duration of the two subprocesses, to evaluate overlapping genomic region when genetic

traits architecture for the bud set process were studied. Additionally, the onset of growth cessation (i.e. phase 2.5) was included for its specific importance as date of perception of the critical night length representing the onset of the autumnal bud formation process.

4.8. Genetic correlations between bud set traits

As previously shown by linear correlations, all the different onset-of-stage traits resulted highly positively correlated among each other in the two experimental trials (Figure 23).

	phase 2.5	phase 2	phase 1.5	phase 1	phase 0.5	dur. subproc. 1	dur. subproc. 2	score50%_2.5	score50%_1.5	r_g between sites
phase 2.5		0.99 *0.00*	0.93 *0.01*	0.87 *0.02*	0.78 *0.03*	0.31 *0.07*	-0.92 *0.01*	0.97 *0.00*	0.93 *0.01*	0.66
phase 2	0.99 *0.00*		0.97 *0.00*	0.93 *0.01*	0.72 *0.04*	0.16 *0.08*	-0.98 *0.00*	0.95 *0.01*	0.96 *0.01*	0.66
phase 1.5	0.98 *0.00*	1.00 *0.00*		0.98 *0.00*	0.58 *0.05*	-0.08 *0.08*	-1.00 *0.01*	0.88 *0.02*	0.95 *0.01*	0.62
phase 1	0.96 *0.01*	0.99 *0.00*	1.00 *0.00*		0.84 *0.02*	-0.17 *0.08*	-0.94 *0.01*	0.82 *0.03*	0.92 *0.01*	0.58
phase 0.5	0.88 *0.02*	0.93 *0.01*	0.96 *0.01*	0.98 *0.00*		0.11 *0.08*	-0.87 *0.02*	1.00 *0.01*	0.35 *0.07*	0.51
dur. subproc. 1	-0.08 *0.08*	-0.21 *0.08*	-0.31 *0.07*	-0.42 *0.07*	-0.59 *0.05*		0.08 *0.08*	0.36 *0.07*	0.05 *0.08*	0.53
dur. subproc. 2	-0.56 *0.06*	-0.47 *0.06*	-0.37 *0.07*	-0.26 *0.08*	-0.08 *0.08*	0.74 *0.04*		-0.81 *0.03*	-1.00 *0.00*	0.04
score50%_2.5	0.98 *0.00*	0.96 *0.01*	0.93 *0.01*	0.89 *0.02*	0.77 *0.03*	0.05 *0.08*	-0.72 *0.04*		0.86 *0.02*	0.64
score50%_1.5	0.99 *0.00*	0.99 *0.00*	0.99 *0.00*	1.00 *0.00*	0.98 *0.00*	-0.23 *0.08*	-0.30 *0.07*	0.93 *0.01*		0.70

Figure 23. Genetic correlations between traits within sites (on the left) for CV (above the diagonal) and VT (below the diagonal) and genetic correlations between sites (on the right).

The duration of bud maturation (duration of subprocess 2) showed high negative correlations with the different onset-of-stage traits, especially in the northern site. On the contrary, subprocess 1 in the northern site, didn't show correlation with the duration of bud maturation (subprocess 2) (0.08±0.08), and had low and less stable correlations with the different phases ranging values from 0.31±0.07 (subprocess 1 vs. phase 2.5) to -0.17±0.08 (subprocess 1 vs. phase 1). No correlation was found between the onset of growth cessation and the duration of bud formation in VT (-0.08±0.08), with a trend that increased up to phase 0.5. In this experimental plantation the two subprocesses showed high positive correlations between each

other (0.74±0.04). Strong correlations between the dates score50%_2.5, score50%_1.5 and all the onset-of-stage traits were found, as a consequence of the high correlations among the observed traits. Medium values of correlation between the two sites were also observed concerning the different onset-of-stage (from 0.51 to 0.66), with a decreasing trend from the first to the last phase analyzed. This trend was a consequence of the similar tendency showed by the Spearman rank coefficient and the heritability at genotypic level. Medium values of correlation between the two sites were also observed in the duration of bud formation (0.53), whereas no correlation was showed between CV and VT concerning the duration of subprocess 2.

4.9. Number of QTLs and percentage of variance explained

Across the two sites, twenty-four QTLs were detected for the two onset-of-stage (phase 2.5 and 1.5) and the two duration traits (subprocess 1 and 2) previously selected. A single QTL explains on average 6.4% and 4.7% of the phenotypic variation (PEV) in CV and VT, respectively. The average sum of PEV per trait in the pedigree was 19.1% (CV) and 14.0% (VT) (Table 9).

For the onset of growth cessation in POP5 full-sib family, three QTLs were identified on the maternal "58-861" map and five on the paternal "Poli" map. The maximum PEV by individual QTL was found in the male parent in CV on LG Ia with 11.9% and in the female parent in VT on LG X, with 10.2%. The sum of PEV of the onset of growth cessation covered up to 55.2% in "Poli" and 37.2% in "58-861" (Table 10).

Seven QTLs for the onset of bud initiation were detected in the two sites. Five of these were inherited from the "Poli" male parent, and two from the female one. The maximum values of PVE was in "Poli" in the northern site (CV), where a QTL in LG VIb explained up to 12.6% of the phenotypic variance. For the "58-861" parent, a minor value of PEV, 11.3%, was found in the QTL of LG X in VT. The total PEV of this trait, representing the important transition from shoot to bud structure, covered up to 60.6% in "Poli" and 24.7% in "58-861".

For the duration of subprocess 1 (Table 9) four QTLs were mapped on "Poli" and two on "58-861". The maximum PVE% covered by an individual QTL was in the female parent in CV on LG XIII, with 10.7% of the phenotypic variance explained, whereas a minor value of 9.9% on LG Ic was found in "Poli" in the same site.

Table 9. Identified QTLs for selected traits related to phenology (two onset-of-stage and the two duration traits). For each QTL, the parent, the linkage groups (LG), the position of peak (in cM), the LOD (logarithm of the odds), the percentage of phenotypic variance explained (PEV) at each site (CV and VT), and the 95% confidence interval (CI) in cM are shown. LG refers to the map in Figure 24.

Parent	Trait	LG	LOD peak (cM)	Lod	PEV CV	PEV VT	95 CI (cM) start	95 CI (cM) end	Interval length (cM)
Poli	date2.5_cnl	1a	16.80	3.46	11.90	0.40	0.30	36.20	35.90
Poli	date2.5_cnl	4b	66.40	4.15	4.60	6.40	13.80	105.90	92.10
Poli	date2.5_cnl	11	41.00	2.94	4.90	4.30	8.30	122.10	113.80
Poli	date2.5_cnl	13	84.90	4.81	6.50	5.10	72.80	92.90	20.10
Poli	date2.5_cnl	17	63.80	3.30	2.60	8.50	25.10	105.90	80.80
Poli	date1.5_cnl	1a	10.70	3.31	9.90	0.40	0.00	31.50	31.50
Poli	date1.5_cnl	1b	84.40	3.14	4.10	3.60	46.40	109.00	62.60
Poli	date1.5_cnl	4b	6.60	4.64	9.10	5.90	0.00	78.00	78.00
Poli	date1.5_cnl	6b	6.90	4.50	12.60	0.10	0.00	18.35	18.35
Poli	date1.5_cnl	17	69.30	4.11	4.40	10.50	44.60	97.40	52.80
Poli	subproc1_cnl	1c	81.80	3.58	9.90	0.50	30.40	101.10	70.70
Poli	subproc1_cnl	6a	96.80	2.50	0.60	7.20	51.40	131.60	80.20
Poli	subproc1_cnl	6b	0.00	2.91	7.50	0.70	0.00	9.69	9.69
Poli	subproc1_cnl	19a	48.40	2.32	2.60	6.10	15.80	69.40	53.60
Poli	subproc2_cnl	1a	4.40	3.24	12.60	0.10	0.00	22.42	22.42
Poli	subproc2_cnl	6b	13.10	2.59	10.40	0.10	0.00	21.80	21.80
58-861	date2.5_cnl	10	126.60	5.80	6.50	10.20	111.00	136.20	25.20
58-861	date2.5_cnl	16	51.00	3.85	8.10	3.60	28.10	82.50	54.40
58-861	date2.5_cnl	3	31.50	3.40	0.30	8.50	0.00	97.10	97.10
58-861	date1.5_cnl	10	126.60	4.95	3.80	11.30	88.60	136.20	47.60
58-861	date1.5_cnl	16	52.00	2.84	6.60	3.00	17.80	86.00	68.20
58-861	subproc1_cnl	13	169.90	2.49	10.70	0.20	59.90	180.80	120.90
58-861	subproc1_cnl	7	48.90	2.77	0.00	9.30	27.30	57.70	30.40
58-861	subproc2_cnl	18	24.10	2.20	2.80	5.70	0.00	61.30	61.30

The total percentage of variance for this trait covered up to 35.1% in “Poli” and 20.2% in “58-861” (Table 10). A total of three QTLs for the duration of bud maturation were found between CV and VT. Two of them were inherited from “Poli” and one from “58-861”.

The maximum values of PVE% for an individual QTL was in “Poli” in CV where a QTL in LG Ia explained up to 12.6% of the phenotypic variance whereas, in “58-861” a percentage of 5.7 was found in VT in the only one detected QTL on LG XVIII.

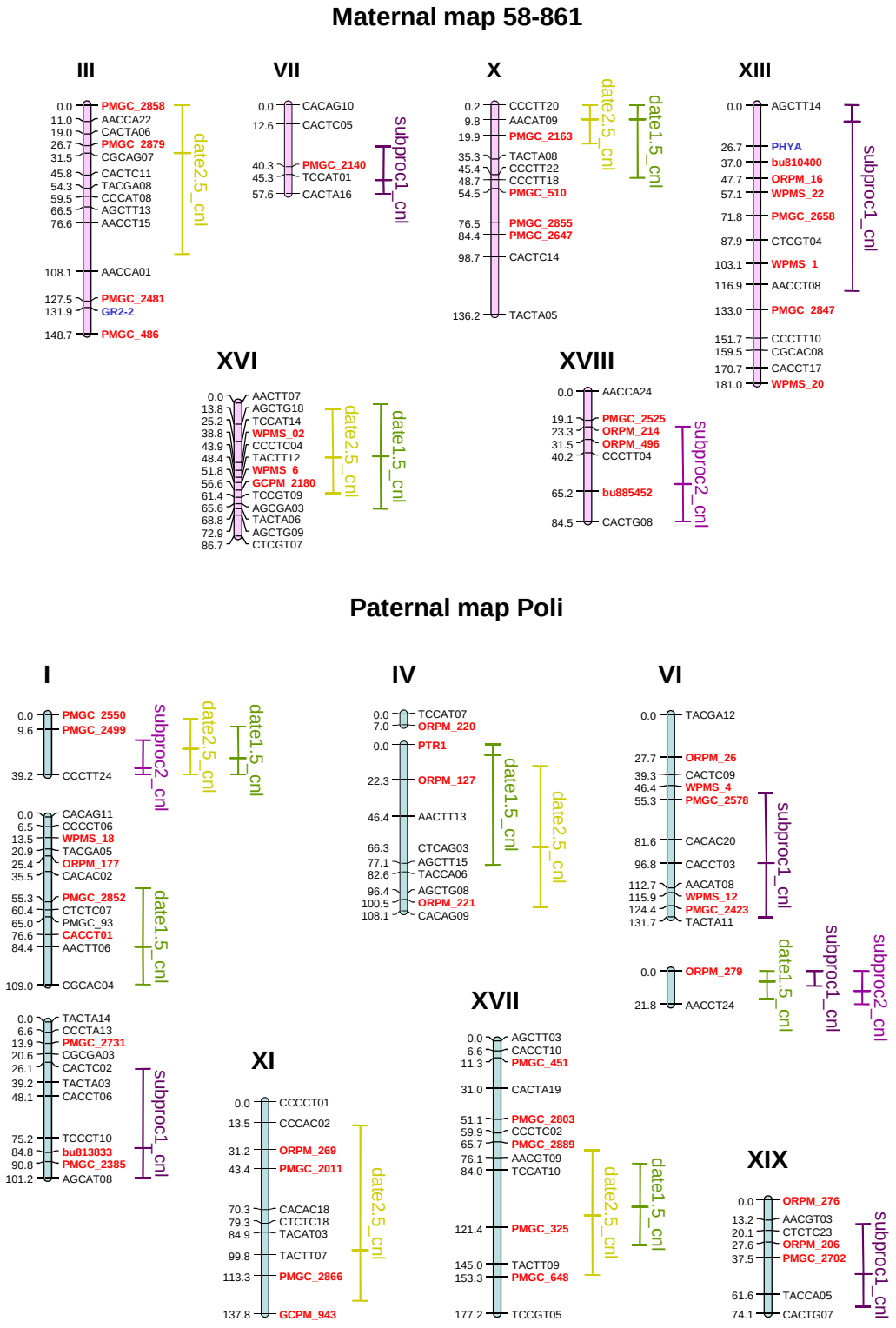


Figure 24. QTLs for the phenological traits selected. The length of the LG bars and QTL is proportional to the distance in cM (0.4 mm per cm). The middle segment on the line indicates the peak of the QTL, and the total length of the line represents the 95% confidence interval. AFLP markers are in black, microsatellite markers in red and SNP in blue. Names of markers and map distances (cM) are indicated on the right and left, respectively, of the linkage groups.

Data showed that a total of 23.2% in “Poli” and 8.5% in “58-861” of the phenotypic variance were covered for the duration of subprocess 2 (Table 10).

Table 10. Total percentage of phenotypic variance (%) explained by the QTLs per trait and per site for the POP5 full-sib family.

Trait	**Poli**			**58-861**		
	CV	VT	Σ sites	CV	VT	Σ sites
date2.5_cnl	30.5	24.7	55.2	14.9	22.3	37.2
date1.5_cnl	40.1	20.5	60.6	10.4	14.3	24.7
subproc1_cnl	20.6	14.5	35.1	10.7	9.5	20.2
subproc2_cnl	23.0	0.2	23.2	2.8	5.7	8.5

Various QTLs for the two onset-of-stage traits were often observed to tend to overlap, especially in the northern parent “58-861” (Figure 24).
Three QTLs were observed in “Poli” in the same small regions LG Ia and LG VIb.

4.10. Phenological vs. biometric traits

In order to investigate the possible influence of the tree dimensions on the phenological process, circumference was used as a covariate of some phenological traits studied.
To avoid redundant information, the selection of informative traits was considered such as the two onset-of-stage traits (phase 2.5 and 1.5) and the durations of the two subprocesses, not considering the height for its high correlation with circumference (Figure 25 and 26). Biometric traits did not influence the phenology during the bud set process in the fall, neither concerning the perception of the critical night length and the transition from the shoot to the bud structure, nor the duration of the bud formation and the following maturation.

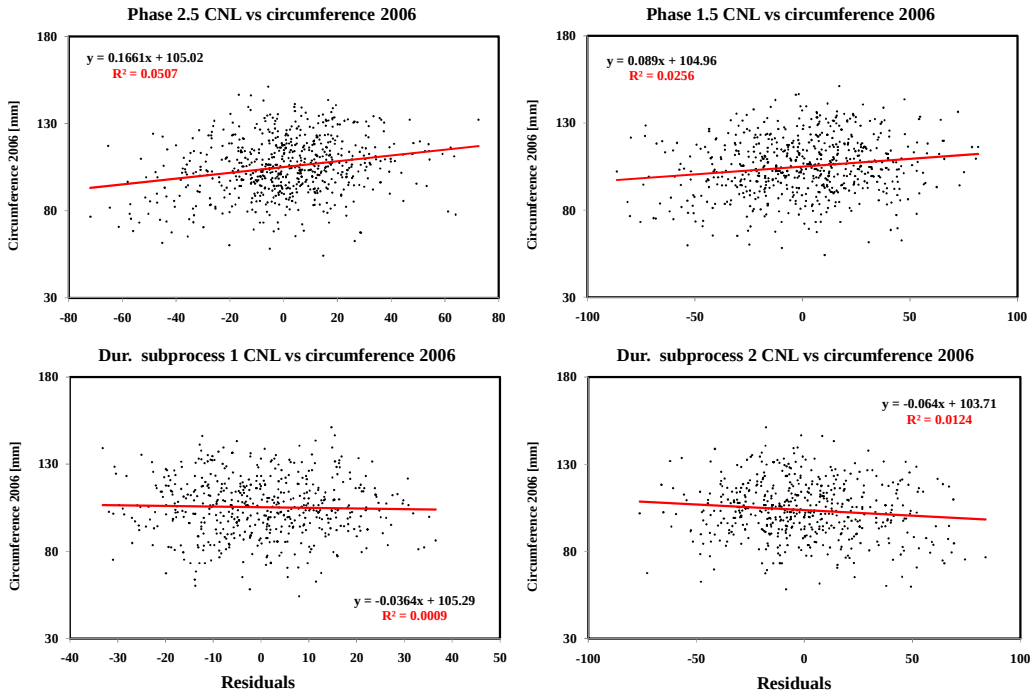


Figure 25. Linear regressions between circumference and informative phenological traits in CV.

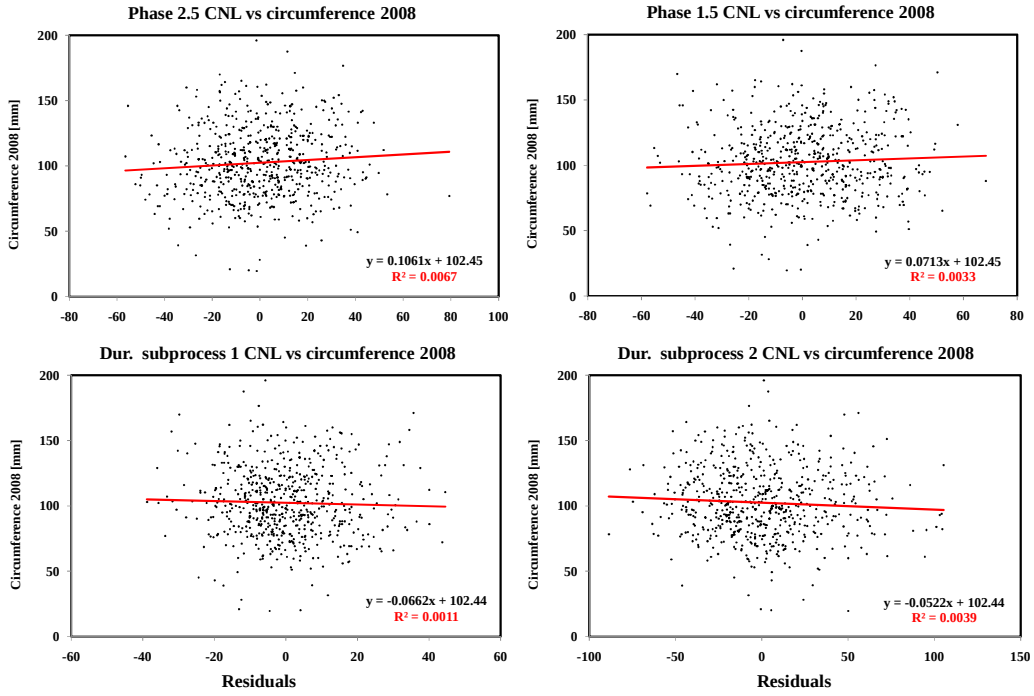


Figure 26. Linear regressions between circumference and informative phenological traits in VT.

5. DISCUSSIONS - *P. nigra* full-sib family

5.1. Dissecting the bud set process in POP5

This work investigated the genetic control and the phenotypic plasticity of bud set process in black poplar (*Populus nigra* L.) that is, among forest trees, an important component of riparian ecosystems and an ideal model tree for biological and genetic studies.

For this purpose an innovative protocol defined to dissect the process into different crucial stages involved in the process (Rohde *et al.*, 2011) and to evaluate the duration of bud development as separate characters was used to understand the relative contribution of each developing step through an in-depth analysis. This protocol represents "an excellent tool to clarify a previously opaque series of distinct developmental stages" (Bielenberg, 2011), seeing that in previous studies only one phase, in general the day in which there were visible stipules (Howe *et al.*, 1996 ; Howe *et al.*, 2000 ; Dillen *et al.*, 2009 ; Marron *et al.*, 2010) or evident terminal buds (Li *et al.*, 1998 ; Frewen *et al.*, 2000 ; Ingvarsson *et al.*, 2006 ; Luquez *et al.*, 2008), was taken into account.

Decreasing of photoperiod up to the reach of the critical night length, is considered to be the major factor influencing timing of bud set, although low temperatures and a multiplicity of other factors are also involved (Junttila, 1989, Howe *et al.*, 1995 ; Howe *et al.*, 1996 ; Olsen *et al.*, 1997 ; Howe *et al.*, 1998).

In this study, the onset of growth cessation, that in our experiments as well as the others onset-of-stage traits and the duration of bud development was not influenced by biometric traits, was recorded in the two contrasting sites and in two different years in the same day (DOY 267), corresponding to quite similar critical night lengths (11.57 and 11.54 hours in CV and VT, respectively). This result demonstrated that this environmental signal is the key factor in the initiation of bud development. Besides, also the onset of the different following phases in the bud formation process was reached in a similar effective night length. Only a maximum difference of about 10 minutes of CNL for the last phases (phase 1.5 and 1) was observed.

Temperature was the most contrasting environmental variable between the two sites, diverging of 2° of latitude and only 7 minutes of photoperiod per month, before and after autumnal equinox. For this reason, the differences found at genotypic levels, showed by the presence of outlier individuals in the cold northern site, were imputable to the lower

temperature experienced by the *P. nigra* full-sib family in CV than in VT. Temperature probably influenced the sensitivity of some genotypes in the onset of the different phases, and not the generic family behaviour. This is also the case of the parent "58-861" that showed an early onset of growth cessation in the northern site compared to VT. Taking into account some replicates available in Savigliano (association population experiment done in 2009 and presented in this thesis), the northern parent was found to reach the onset of growth cessation earlier in this site than the other two previously presented, at 11.24 hours, against 11.35 and 11.40 hours, respectively for CV and VT (corresponding to 35.20°, 27.16° and 15.18° of CMT_{10}, respectively).

The two parents, which resulted highly significantly different for all the onset-of-phase traits but not for the different durations, showed extreme performances. The segregating progenies showed a skewed distribution towards the northern early parent while "Poli", that is always late compared to "58-861", was firmly the latest genotype to set the terminal bud. On the contrary "58-861" often showed transgressive segregation for all the date-of-onset traits.

The differences in genetic variability observed between the two sites might be partially attributed to the age of the studied material, R1 – S1 (root 1 – shoot 1) in VT compared to the age R3 – S1 (root 3 – shoot 1) in CV, influenced by the propagation effects during the year of planting but less important in the following years.

The total duration of bud development was conserved among the two experimental trials and years (i.e. 18.8 days) at a population mean level, with more time spent in the more variable duration of phase 1. Taking into account genetic and phenotypic correlations, all the different phases were strong correlated each other indicating that once the process starts, all the different following phases, describing together a developmental sequence, proceed in a "cascade" fashion. This behaviour was probably due to the control of the different traits by the same genes.

The absence of differences between the two parents concerning duration traits indicate that the variability observed in the progeny for these traits was mainly due to environmental factors.

Even if the total duration of the process was conserved, the time spent in each subprocess depended on the site considered. On the whole, POP5 needed more time for the bud maturation process than for the bud formation in both sites. The obtained correlations showed a highly negative linkage between the onset of growth cessation and the duration of bud maturation at genotypic level in CV and a low negative correlation in VT. This result

indicates that the later the growth cessation occurred, the shorter the duration of bud maturation was, especially in the northern site.

The role of temperature also in this case appears to be very important at genotypic level, even if low negative (CV) or no correlation (VT) were observed between phase 2.5 and the duration of subprocess 1. In fact, individuals that resulted early and outliers to enter in phase 2.5 in the northern site, spent a few time on the bud formation and relatively more time for the bud maturation. An example is the plastic northern parent ("58-861") that was one of the early genotypes in both sites. It reached the onset of the growth cessation a little early in the colder site of northern Italy and spent less time for the subprocess 1 than for the second one. The behaviour of this genotype was a consequence of the sensibility to the low cumulated temperatures that characterized the northern site.

Subprocess 1 was mainly controlled by environmental factor, as showed by the low heritability values (0.44 and 0.27 at genotypic level in CV and VT, respectively) and the high relative contribution to phenotypic variation of the site (σ^2_S/σ^2_P = 11.3%). For this reason, it might be particularly important in the adaptation to short-term fluctuations of temperature and environments (Rohde *et al.*, 2011).

The onset-of-stage traits and the duration traits had not the same extended genetic control. All the phases showed high levels of genetic component of the phenotypic variance, especially concerning phase 2.5 (and score50%_2.5) for which was found up to 84% of the phenotypic variance due to the genetic variance in CV. The importance of the environmental factors increases from the onset of the process to the end of the bud maturation (form phase 2.5 to the phase 0.5).

Taking into account other studies, the presented results are partially comparable with values obtained in other three full-sib (two *P. deltoides* Bartr. x *P. trichocarpa* Torr. & Gray and one *P. deltoides* Bartr. x *P. nigra* L.) families studied in France (47°46′N, 1°52′E) (Rohde *et al.*, 2011), characterized by a completely inverse trend (higher values of H^2 for the last phases and minor for the onset of growth cessation). Rohde and colleagues (2011) showed also results obtained in european natural populations of *Populus nigra* evaluated in Belgium (50°46′N, 3°52′E) that showed higher values of H^2 than those obtained in VT and CV but characterized by the same trend (decreasing H^2 during the process).

Howe and co-workers (2000) found similar results (H^2_{ind}=0.48 and H^2_{gen}=0.75) in a F_2 family derived from a cross between two F_1 hybrids (*P. trichocarpa* Torr. & Gray x *P. deltoides* Bartr.), studied in Minnesota (44°59′N, 93°10′W) at the same latitude of CV (only phase 1

was comparable). POP5 was characterized by a lower genetic contribution of the phenotypic variation of phase 1 (H^2_{ind}=0.72 and H^2_{gen}=0.91) than the same F_2 family studied by Howe *et al*. (2000) in Oregon (44°34′N, 123°16′W), and previously described.

In another experiment, based on twelve accessions of *Populus tremula* (SwAsp Collection) evaluated in two common garden experiments in Ekebo (55.9°N, Sweden) and Sävar (63.4°N, Sweden), Luquez *et al.* (2008) found higher levels of broad-sense heritability (0.60 and 0.70, respectively) than those obtained in this study (only phase 0.5 was comparable).

Higher levels of broad-sense heritability were also documented by Li and co-workers (1998) in interspecific hybrids of *Populus tremuloides* Michx. and *Populus tremula* L. grown in Grand Rapids, Minnesota (47°14′N, 93°31′W), and near Rhinelander, Wisconsin (45°40′N, 89°25′W), in two following years (H^2_{year1}=0.78 and H^2_{year2}=0.79 for phase 0.5).

Unlike the phases, the duration traits showed a major phenotypic variance and minor contribution for the genetic component of the observed variance, as result of a high influence of environmental conditions on these traits. Despite this result, the duration of bud maturation, together with the onset of bud initiation (phase 1.5), was identified through the PCA analysis as the most discriminative trait in term of phenotypic variation in the POP5 family. The high environmental influence on duration traits compared to the onset-of-stage traits was also observed by Rohde and co-workers (2011) in other full-sib families studied in France, in the unique work published using different phases to dissect the bud set process.

Differences in the genetic correlations observed between the onset-of-stage traits and the two subprocesses comparing the two sites were expected considering that quantitative traits may interact in a different way with multiple genes codifying a character, when grown in different sites. For this reason, the heritability values are closely valid only for a particular population studied and grown in a particular site at a particular moment (Zobel and Talbert, 1984). Moreover, genetic correlations can alter the magnitude, and sometimes the sign, because they are measured in a given environment and can themselves be plastic if the environment is changed (Stearns *et al.*, 1991 ; Pigliucci, 2005).

5.2. The contribution of phenotypic plasticity to variation in bud set

Phenotypic plasticity is defined as the ability of a given genotype to produce different phenotypes in response to different environmental conditions (Bradshaw, 1965 ; Via, 1984 ; Sultan 1987 ; Callaway *et al.*, 2003). This term is generally used to indicate the property of

individual reaction norms, unlike “GxE interaction” that is usually referred to a statistical attribute of an entire population. This interaction, in fact, describes the situation where a number of genotypes respond differently to various environments (Pigliucci, 2005), giving impossible to predict the performance of the genotypes to different sites. Phenotypic plasticity is mostly uncharacterized for phenology traits even if it is an important short-term option for adaptation and of primary importance for a rational selection of ecotypes in breeding programs (Rohde *et al.*, 2011).

Data showed always high significant contribution of the GxS interaction in the phenotypic variation of all the different phases analysed with up to 19% of variability explained in the onset of growth cessation and a firmly decreasing trend observed during the process ($\sigma^2_{GXS}/\sigma^2_P$ = 9.2% for phase 0.5).

The relative contribution of the interaction component on the phenotypic variation observed on the phase 1, resulted perfectly comparable with the results documented by Luquez and co-workers (2008) (12.1% PVE_{prop}, p=<0.001) in a *Populus tremula* trial established using the Swedish Aspen Collection (SwAsp) tested in two different sites across Sweden.

The low level of the genotypic component and the high level of residuals in the phenotypic variation underline the importance of environmental factors on duration traits. For these traits a lower contribution of GxS interaction in the phenotypic variation than those observed in all the phases was found. The GxS interaction component partially increased from the bud formation subprocess ($\sigma^2_{GXS}/\sigma^2_P$ = 4.9%) to the subprocess 2. This second subprocess is a developmental period for which the genotypes of the analysed full-sib family tend to respond highly different to the various environments because up to 20% of the measured variability was due to the genotype x site interaction.

The Spearman’s rank coefficient allowed to identifying changes in the ranking of genotypes between the two sites. The general classification was mainly maintained at the onset of growth cessation underlining the predominant role of the “scale change” effect in the GxS interaction. Therefore, some genotypes more divergent in some environments take advantage (disadvantage) of an increasing (decreasing) environmental “signal” as showed for the female parent “58-861”, one of the most early clone in both sites but sensible to cold temperatures. The Spearman coefficient values for the different analysed phases, decreased during the process as a consequence of the increasing “rank change” effect important in the GxS interaction.

As the family means for onset of growth cessation among the two sites were mainly conserved showing the same values, the photoperiod can be considered the more crucial environmental signal in the control of the bud set in the full-sib materials analysed. Anyway the temperature, the most different parameter between the two sites, appeared the main factor triggering the change of rank and performance of the most plastic genotypes between sites, probably changing their sensibility to photoperiod. Low and not significant values of Spearman's coefficient were a clear sign of strong random changes in genotype ranking concerning the duration traits for which there was a major influence of the environmental conditions.

Because the high contribution of GxS interaction component in the observed variability, the often high broad-sense heritabilities estimated for the different onset-of-stage traits are certainly inflated to inclusion of GxS variance in the numerator of the equation (White *et al.*, 2007).

Analysing the phenotypic plasticity through the relative ecovalence analysis only a few genotypes were found to strongly contribute to the overall GxS interaction. Results showed that only 5 more plastic genotypes explained, on average, up to 25% of the GxS interaction component observed for a generic trait. The stability of each genotype changed depending from the phenological phase considered and the relative ecovalence analysis allowed to identify which genotype preserved the stability through the environments for the different phenological phases. This result represents a practical tool applicable in tree breeding strategies and in the genetic improvement providing the possibility to select genotypes to be utilized in different environments and avoiding the risk of unexpected performances like frost injury.

5.3. QTL regions detected

Variation of quantitative complex traits can be dissected by mapping quantitative trait loci (QTLs). The fact that many important economic and adaptive traits in forest trees appear to be under the control of many genes makes conventional breeding very difficult. This tool may provide opportunities for genetic improvement in Poplar that is widely used in the timber industry and as a model for the study of woody plants (Lin *et al.*, 2006). For this reason in the last decades dozens of QTL studies have been done to look at a number of traits in *Populus*, such as biomass (Rae *et al.*, 2008 ; Marron *et al.*, 2010), rust resistance (Newcombe *et al.*,

1996 ; Jorge *et al.*, 2005), canopy structure (Wu and Stettler, 1998), leaf traits (Wu *et al.*, 1997 ; Ferris *et al.*, 2002 ; Rae *et al.*, 2006), stem growth (Bradshaw and Stettler, 1995 ; Wullschleger *et al.*, 2005), growth rates (Wu *et al.*, 2003), bud dormancy (Frewen *et al.*, 2000), and bud set (Marron *et al.*, 2010 ; Rohde *et al.*, 2011).

Is very important to characterize the bud set process at molecular level especially in a breeding program context in order to find the right compromise between the necessity to avoid the risk of frost damage, if the trees set buds too late (or flush too early) assuring the maximization of the growing season length.

In this work the number of QTLs mapped varied among LGs. Those with more than 4 QTLs were LG I (5 QTLs) and LG VI (4 QTLs) (Figure 27), while in the LG XIII one QTL was found in both maps taken into account. Two LGs characterized by a major number of QTLs were identified in this work as the more promising region to check for candidate genes in the phenological process of bud set. As identified in a previous study (Ruttink *et al.*, 2007), these two LGs contain 77 (LG I) and 60 (LG VI) expressional candidate genes. LG III, VI and XV were also underlined by Rohde and colleagues (2011) taking into account several F_1 full-sib (*P. deltoides* Bartr. x *P. trichocarpa* Torr. & Gray, *P. nigra* L. x *P. nigra* L. – POP5 and *P. deltoides* Bartr. x *P. nigra* L.) families studied in France (47°46′N, 1°52′E) and in Italy (CV). In the cited work, a total of 53 QTLs were found, and 21 of them were mapped on six recurrent LGs, identifying important QTL regions for bud set.

LG III, IV, VII and XVIII observed in this study were also documented by Marron and co-workers (2010) that detected 10 QTLs controlling bud set studying two F_1 full-sib families of *P. deltoides* x *P. nigra* (2 QTLs) and of *P. deltoides* x *P. trichocarpa* (8 QTLs) grown in one of the two sites taken into account (CV). Moreover the LG III, which contains 44 expressional candidate genes (Ruttink *et al.*, 2007), resulted very interesting because QTLs for phenology were mapped on this LG in the current study and in the two others cited.

The large number of QTLs detected (especially concerning the onset-of-stage traits), each one characterized by small or modest effect, highlights the multigenic nature of these traits. QTLs for the two phases tended to overlap and this results were expected because of the correlated nature of the traits analyzed, which were part of a developmental process (Rohde *et al.*, 2011). A limited number of QTLs occurred on the female map for the duration of subprocess 2.

The total PVE explained by QTLs for each phenological trait in each site ranged from 0.2% to 40.1% suggesting that likely many QTLs, of smaller effect or localized in the genomic regions were not covered by the genetic maps, remaining undetected.

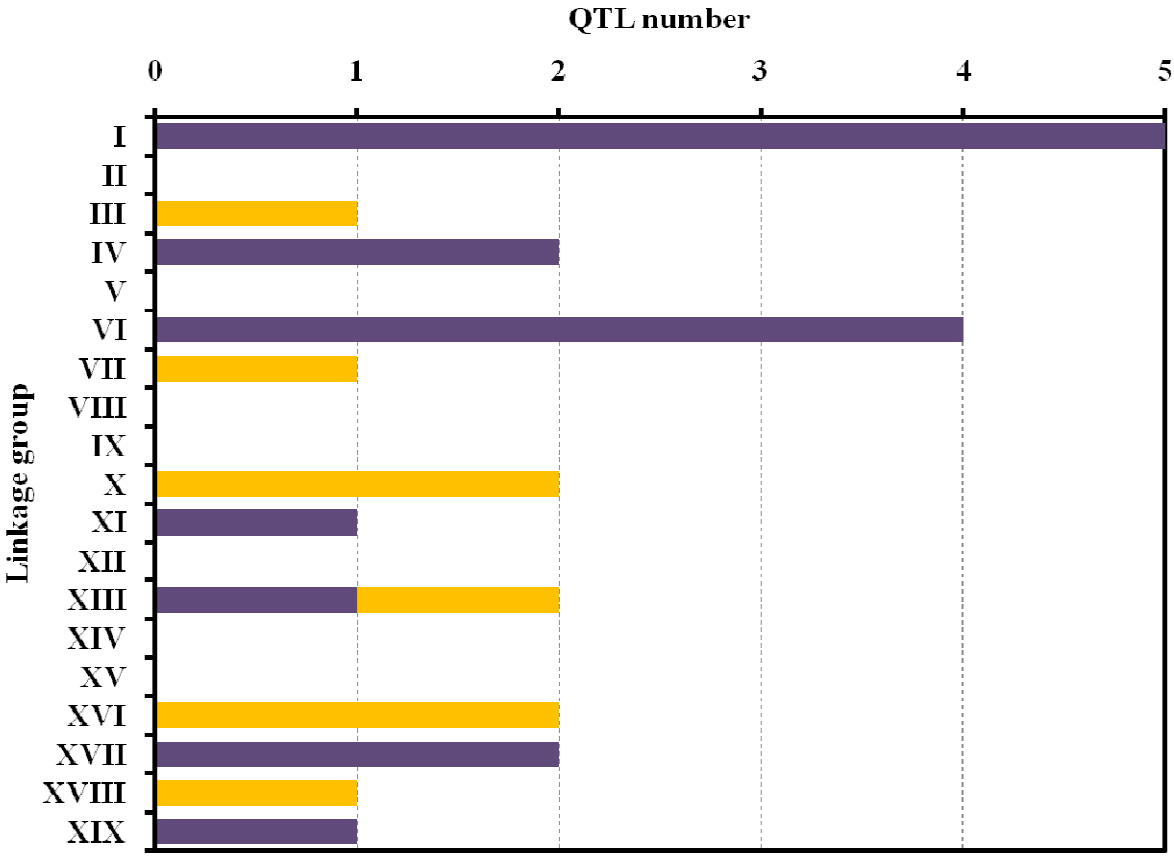


Figure 27. Number of QTLs by linkage groups detected for the bud formation process in the male (violet bars) and female (yellow bars) map.

Our study contribute to improve the knowledge of the genomic regions involved in the bud set in poplar and extend results by dissecting the process into some interesting components. Moreover, this study benefits of the availability of a new improved genetic linkage map produced by Gaudet and co-workers (Gaudet, personal communication) and of results obtained by the simultaneous treatment of data from two different sites. This approach provides an increased statistical power of QTL detection and a major accuracy of the estimates of QTL position and effect (Jansen *et al.*, 1995 ; Rae *et al.*, 2008).

6. CONCLUSIONS - *P. nigra* full-sib family

Although our results might be specific to the POP5 full-sib family in its specific experimental sites, some interesting general conclusions can be drawn:

- A new approach in the study of bud set offers the possibility of dissecting bud set into stages and durations (Rohde *et al.*, 2011) and to estimate their relative contribution. This is the major improvement compared to the previous studies.
- Night length, the most stable predictor of coming winter, is the crucial factor in the onset of growth cessation in poplar as shown by the stable pedigree mean behavior between environments.
- Temperature seems to influence the sensitivity of some segregant genotypes of POP5 in the onset of the different phases (see plasticity).
- The onset-of-stage traits, especially the onset of growth cessation, provide the higher contribution to the genetic variance in the total phenotypic variance and it decreases from the first to the last stage. Duration of traits appears to be highly influenced by the environmental factors, especially the duration of bud formation (duration of subprocess 1) that might be particularly important in the adaptation to short term fluctuations of temperature and environments (Rohde *et al.*, 2011).
- The timing of bud initiation (phase 1.5) and the duration of the two subprocesses were identified as the most discriminative traits in terms of phenotypic variation.
- *P. nigra* "58-861" (mother parent) from Val Cenischia (45°09'N, 07°01'E) in northern Italy, is better adapted to CV (Po valley; 44°42'N, 07°41'E) while *P. nigra* "Poli" (father parent) from Policoro (40°09'N, 16°41'E) in southern Italy, survived and grew well in VT (Tevere valley; 42°25'N, 12°05'E). The selected material has to be chosen with regard to the local conditions in which the plants will use.
- In the POP5 full-sib family the southern parent "Poli" is later than the northern one when tested in the same site. An expected phenotypic segregation and a transgressive segregation for all the onset-of-stage traits were observed in both sites.
- Bud set is a constant process starting from the perception of critical night length to the end of bud formation and the time spent for the bud maturation (duration of subprocess 2) is always longer than the time needed for bud formation (duration of subprocess 1).

- GxS interactions are considerable in all the different onset-of-stage traits, with a firmly decreasing trend from the perception of critical night length to the fully closed bud. An higher importance in the duration of bud maturation (duration of subprocess 2) than bud formation (duration of subprocess 1) was observed. The general classification of clones, that was mainly maintained at the onset of the process due to the more important “scale change” effect in the GxS, changes more during the bud development with an increasing role of the “rank change” effect in the interaction. Strong random changes were observed for the duration traits. The higher maintenance of ranking in the onset of growth cessation among sites, in spite of significant GxS interactions, suggests that this trait are mainly controlled by the same genes in the two sites.
- Phenotypic plasticity can contribute to short-term adaptation. The relative ecovalence analysis allow to identify which genotypes preserve the stability through environments as a practical tool applicable in tree breeding strategies and genetic improvement. On average, selecting only 5 more plastic genotypes concerning the different traits analyzed, a contribution of 25% of the overall GxS interaction was explained.
- The large number of QTLs mapped, each one characterized by small or modest effect, highlights the complex nature of these traits. QTLs for the two phases 2.5 and 1.5 tended to overlap due to the correlated nature of the analysed traits as part of the same developmental process. The low to moderate total percentage of phenotypic variance explained by QTLs for each trait and site, suggest that there are many QTLs of smaller effect contributing to the phenotypic variation still undetected.

7. MATERIAL AND METHODS - *P. nigra* european native populations

7.1. Genetic material

Genotypes utilized in this phenological experiment were sampled along riparian ecosystems in four different european countries. A total of 814 genotypes were randomly collected in 14 natural metapopulations (Table 11) consisting of a collection of sub-populations (Begon *et al.*, 2006) whose densities fluctuate independently of one another but that are linked by dispersal (Elkinton, 2000). A minimum of 30 genotypes, except for Durance and Stura which have respectively 10 and 29 ramets, were utilize for our aims.

Following the results of ANOVA analysis, we decided to consider for our aims Ticino North and Ticino South as the same metapopulation. These two provenances, as well as Drôme1, Drôme6 and Kühkopf, had been previously sampled and characterized with neutral markers (van Dam and Bordács, 2001 ; Imbert and Lefèvre, 2003 ; Smulders *et al.*, 2008). Others accessions, i.e. Durance, Loire East, Loire West and NL, are individual trees from narrow regions or singular genotypes (Storme *et al.*, 2004 ; Rohde *et al.*, 2011).

The populations taken into account ranged from 40°33’ (Basento) to 52°00’ (Netherlands) of latitude north and the sites of sampling were at one average altitude between 6 (Netherlands) and 1095 (Stura) meters above sea level (Figure 28).

The Italian metapopulations of Paglia and Basento were sampled and collected by the Department of Forest Resources and Environment (University of Tuscia, Viterbo) in 2007, along the homonymous rivers, in the regions of Lazio and Basilicata. A systematic sampling strategy was designed and one individual every 200 m was randomly sampled inside the populations or the mature cohort. To avoid hybrid cultivars of *Populus* spp. and the *Populus nigra* var. *italica*, only the trees which look morphologically like the black poplar, following morphological characters commonly used to discriminate hybrids and black poplar specimens, were taken into account.

7.2. Experimental plantation

A randomized complete block design was used for the establishment of the experimental plantation in the middle of April 2008 from 25 cm homogeneous hardwood cuttings, selected and labelled at the end of February 2008 and preserved at –2 °C until utilization.

Table 11. The different metapopulations analysed in this study. In the table is specified the country, sites and geographical coordinates in which the metapopulations were sampled, the name of the main metapopulations (Metapop.), the number of genotypes available for each site and the main population code utilized for the experiment management. For our aim, we considered Ticino North and Ticino South as the same metapopulation.

Code	Country	Metapop.	Genotype	Site	Latitude	Longitude	ALT
AST-xx	F	Adour	52	Assat	04314N	00018W	210
BLR-xx		Adour		Baliros	04313N	00018W	216
BDX-xx		Adour		Baudreix	04312N	00015W	238
CTR-xx		Adour		Cauterets	04253N	00006W	902
SSN-xx		Adour		Gave Mauleon	04309N	00054W	135
LRD-xx		Adour		Lourdes	04305N	00002W	375
OLO-xx		Adour		Sauveterre	04323N	00056W	52
TRB-xx		Adour		Tarbes	04313N	00004E	310
DRA-xx		Dranse	42	Dranse	04623N	00630E	374
DUR-xx		Durance	10	Durance	04347N	00530E	209
BSL-xx		Loire	200	Bonny	04734N	00249E	135
GLY-xx		Loire		Guilly	04748N	00217E	100
LOE-xx		Loire		Loire East	04719N	00255E	147
LOW-xx		Loire		Loire West	04716N	00046W	29
SPM-xx		Loire		St. Prive	04751N	00148E	90
VDL-xx		Loire		Val de Loire	04713N	00259E	154
NOH-xx		Nohede	41	Nohede	04237N	00217E	820
1-Axx		Ramieres	100	Drome1	04441N	00524E	470
1-Jxx		Ramieres		Drome1	04441N	00524E	470
6-Axx		Ramieres		Drome6	04445N	00455E	145
6-Jxx		Ramieres		Drome6	04445N	00455E	145
ERS-xx		Rhin	50	Erstein	04825N	00743E	150
RHN-xx		Rhin		Rhinau	04816N	00741E	160
STR-xx		Rhin		Strasbourg	04837N	00749E	135
TBG-xx		Rhin		Taubergiessen	04818N	00743E	155
ALL-xx		Val Allier	40	Val Allier	04624N	00319E	220
KUH-xx	G	Kuhkopf	46	Kuhkopf	04949N	00829E	90
NL-xxxx	N	NL	42	NL	05200N	00534E	6
BS-xx	I	Basento	34	Basento	04033N	01623E	118
PG-xx		Paglia	50	Paglia	04247N	01149E	278
ST-xx		Stura	29	Stura	04419N	00705E	1095
N-xx		Ticino North	42	Ticino North	04516N	00859E	70
SN-xx		Ticino South	36	Ticino South	04512N	00904E	60

Countries: F = France, G = Germany, N= Netherlands, I = Italy.

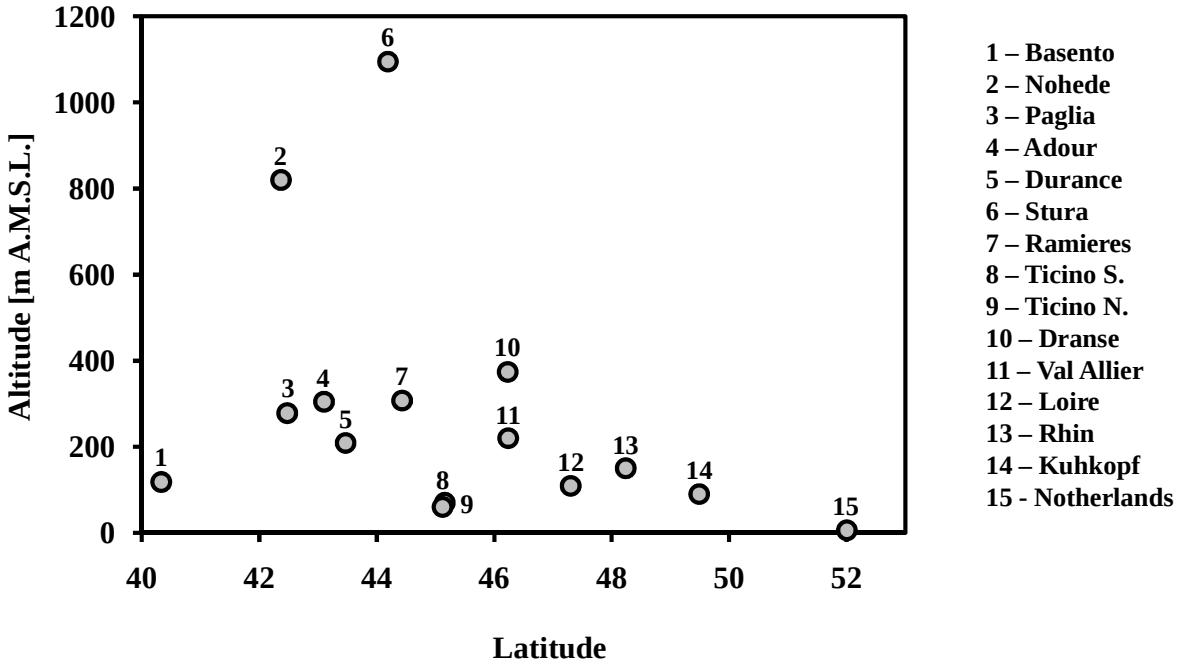


Figure 28. Latitude versus altitude of origin of the different metapopulations analysed.

Picture 7. The experimental plantation of european natural metapopulations in northern Italy. The cuttings were labelled and randomly planted in a randomized complete block layout.

The cuttings were planted at a spacing of 0.80 m in rows and 2.20 m between rows, accommodating an overall plant density of 5682 plants per ha (Picture 7). One individual of each genotype was randomly assigned to each of the six blocks available in the experimental field and a double border row, using *Populus nigra* L. “Jean Pourtet” and *Populus x*

canadensis Moench "Robusta", was planted respectively at the internal and external row of the plantation to minimize border effect.

The experimental site were irrigated every two weeks during the dry period, weeded and treated with pesticides and fungicides as needed especially to control damages from *Hyphantria cunea* Drury and *Chrysomela* (=*Melasoma*) *populi* L. The plantation was cut down in February 2009 (at the end of the first growing season) and new shoots were removed during the growing season in order to preserve only one stem on each stool for the phenological sampling.

7.3. Sites description

The experimental field was located in northern Italy (Savigliano, Po valley; 44°36'N, 07°37'E) with an elevation of 321 m above sea level.

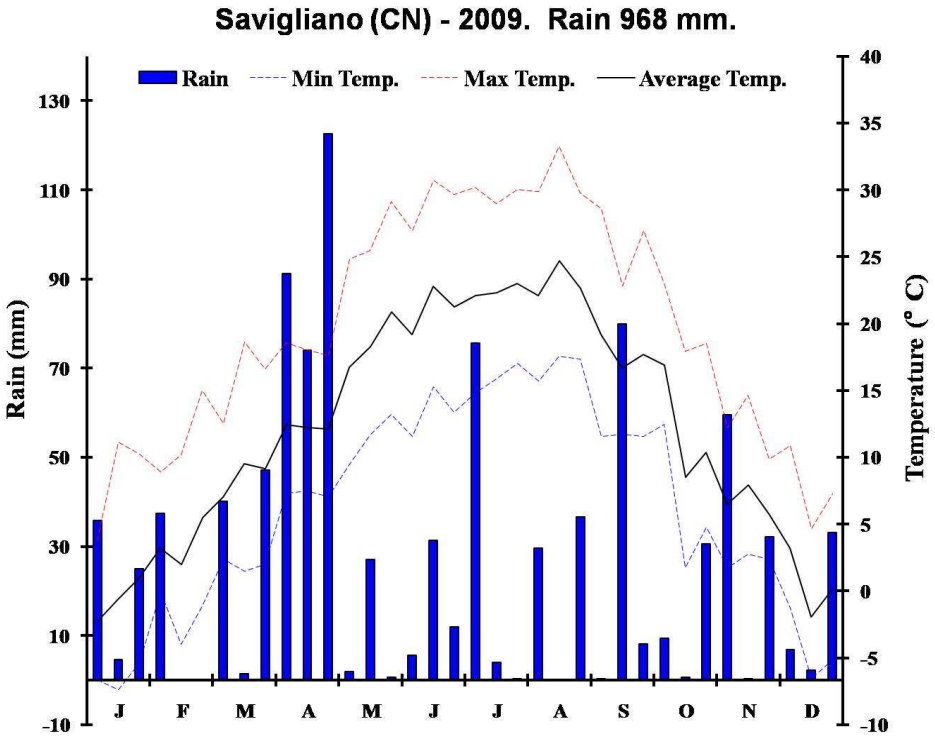


Figure 29. Meteorological characteristics of the experimental sites in Savigliano where the european natural populations where studied in a common garden experiment. Data were obtained in 2009 from a nearby meteorological station (Lagnasco, CN).

In Savigliano during 2009 the average temperature was 12.2 °C, whit the highest value in December 20 (-18 °C) and the maximum in August 19 (35.2 °C). The average rain was 968 mm; in this site there was not a strong dry period and the rainiest season was in spring (Figure 29). The soil at the experimental field was an alluvial soil with sandy-load texture.

7.4. Protocol of bud set measures

The phenological measurements of bud set were done according to the protocol described in material and methods of *P. nigra* full-sib family experiment (paragraph 3.4).
Bud set was scored in autumn 2009, at the end of the first growing season after the coppice (i.e. Root 2 - Shoot 1, second growth year). Due to the extent of the plantation and the quantity of the material, data were collected every 10-15 days by two sampling team, one every three blocks, from the middle of August (August 18, DOY = 230) up to the beginning of October (October 7, DOY = 280). In order to define the best sampling protocol, some genotypes are selected (Table 12) (Bastien, personal communication) and monitored in the field.

Table 12. Expected early, medium and late genotypes previously tested in a common garden experiment in Belgium. The control genotypes were used to control the onset of the phenological process in the collection and to decide the strategy of sampling. “Poli” (male parent of POP5 full-sib family), Bridge-19, Bridge-31, Bridge-41 (offspring of POP5 full-sib family) are planted in the experimental plantation as control genotypes.

Early Genotype	Medium Genotype	Late Genotype
LOW-05	DUR-03	POLI
LOW-06	DUR-06	Bridge-19
		Bridge-31
LOE-10	LOW-17	Bridge-41
LOE-17		
LOE-22	N-11	N-26
	N-15	N-35
KUH-44	N-45	N-57
KUH-47		
		6-A22
NL-1045		6-J05
NL-1682		6-J10
NL-1250		
NL-1171		

The fist sampling (DOY = 230) started three days after 50% of individuals of the 11 early control genotypes had reached phase 2.5. The third (DOY = 258) and the fourth (DOY = 272) sampling were done after the onset of the process in 50% of individuals of the medium and later genotypes. The second and the last sampling were performed in order to cover the great variability between metapopulations and to have, at the end of experiment, at least 2 phases scored for each individuals sampled. In total bud set was scored five times for all metapopulations and, in particular, in August 18 (DOY = 230), August 28 (DOY = 240), September 15 (DOY = 258), September 29 (DOY = 272) and October 07 (DOY = 280).

7.5. Other traits measured

In Savigliano height and circumference measures were done at the end of the first growing season of the second coppice rotation (December 2009, i.e. Root 2 – Shoot 1) for each individuals of the six blocks of the association populations experiment.
An extendable aluminium pole was used to measure stem height with an accuracy of 1 centimetre and the stem circumference was measured at 1 m above ground level to the nearest millimetre using a tape measure. This biometric traits utilized as covariate of the bud set allow us to understand the possible relationship between phenology and biometrics.

7.6. Organization of the database and statistical analyses

7.6.1. Data management and fitting

Data sampled were organized into a database and all genotypes measured for which there were less than three observations for a trait were preliminary removed.
In order to compare the different metapopulations tested as cumulative night length needed to onset bud growth cessation and bud formation, data were transformed using sunset and sunrise data and smoothed as reported in material and methods of *P. nigra* full-sib family study (paragraph 3.6.1).

7.6.2. Statistical analyses

Phenotypic fitted data were analysed using R software (version 2.6.0, A Language and environment Copyright, 2007) for windows (http://www.r-project.org).

Prior to analysis of data and because the large size of the single block of the replicated experiment, micro-environmental effects were tested and adjusted using Papadakis spatial correction (Papadakis, 1937 ; Papadakis, 1984). This method was applied to individual values and makes it possible to eliminate the micro-environmental effect for a given trait from the residual value of each neighbouring individuals included in an appropriate dimension grid.

In case of non-respect of the assumptions for the analysis of variance, original data were transformed using the best power transformation estimated by the Box-Cox procedure (Box and Cox, 1964).

Variation among genotypes of the same metapopulation for the different phenological traits considered in this study was analysed using a random-effects model in a metapopulation x metapopulation analysis:

Formula 27: $$Y'_{ijk} = \mu + G_{j(i)} + \varepsilon_{ijk},$$

where Y'_{ijk} is the individual values adjusted for micro-environmental effects using Papadakis spatial correction, μ is the general mean, G_j is the effect of the j^{th} genotype (random) of the i^{th} metapopulation and ε_{ijk} is the residual error term.

Differences among metapopulations were tested using a mixed-effects model performed for a between metapopulation analysis:

Formula 28: $$Y'_{ijk} = \mu + M_i + G_{j(i)} + \varepsilon_{ijk},$$

where Y'_{ijk} is the individual values adjusted for micro-environmental effects using Papadakis spatial correction, μ is the general mean, M_i is the effect of the i^{th} metapopulation (fixed), G_j is the effect of the j^{th} genotype (random) of the i^{th} metapopulation and ε_{ijk} is the residual error term. The differences between metapopulations and genotypes were considered significant when the *P*-value of the ANOVA *F*-test < 0.05 for all data analysed.

7.6.3. Genetic parameters

Genotype and residual variance components were estimated from the REstricted Maximum Likelihood (REML) (SAS VARCOMP; SAS Institute, 1999) method using the phenotypic values adjusted for micro-environmental effects, considering genotype as random factor (random-effect model in a metapopulation x metapopulation analysis) and metapopulation as fixed factor (mixed-effect model in a between metapopulations analysis).
Variance components were utilized to calculate broad-sense heritability at individual (H^2_i) and genotypic (H^2_g) level and the coefficient of genetic (CV_g) and residual (CV_r) variation as described in material and methods of *P. nigra* full-sib family (paragraph 3.6.3).

7.6.4. Principal component analysis

Multivariate analyses, one for each metapopulation and one including all the different metapopulations in the same analysis, using principal component analysis (PCA) were performed on individual tree basis in order to select the traits which account for the most of the variance in the observed data.
Results were evaluated by taking into account also the PCA obtained in the *P. nigra* full-sib family analysis (paragraph 3.6.4).

7.6.5. Linear and genetic relationship between traits

Linear and genetic correlations, using Pearson and REstricted Maximum Likelihood (REML) methods respectively, are calculated as described in the material and method paragraph concerning *P. nigra* full-sib family study (paragraph 3.6.5).

8. RESULTS - *P. nigra* european native populations

8.1. Survival

The survival of the *P. nigra* collection after the establishment of the plantation in April 2008 in Savigliano was 92.2% and it decreased of about 1% after the coppicing in 2009. An increasing latitudinal trend of survival was observed between the different metapopulations (Figure 30, A) and Basento, the more southern one, showed the low rooting ability, with 36.5% (Figure 30, B) of individuals died before the phenological sampling.

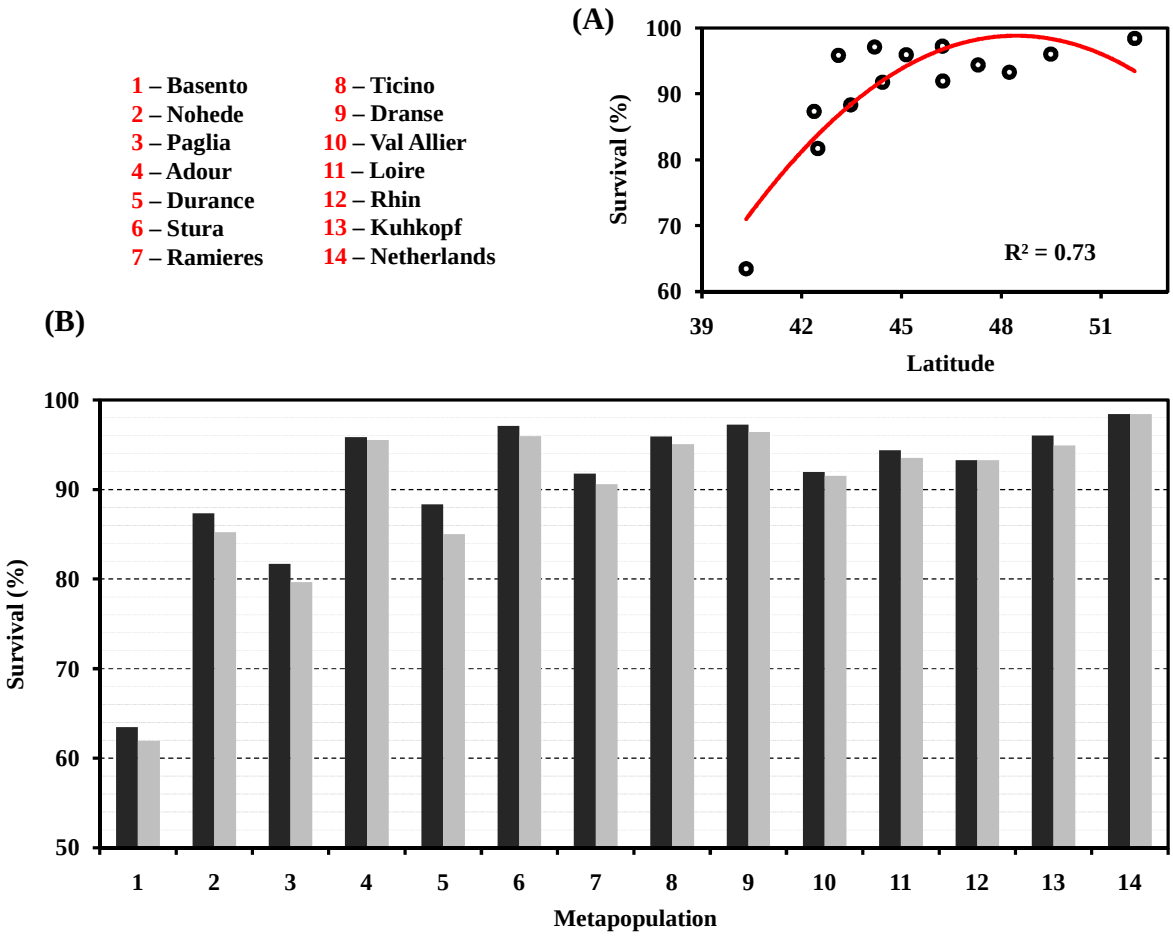


Figure 30. Individual survival observed in the different metapopulations taken into account. (A) Latitudinal trend of survival observed in the common garden experiment. (B) Individual survival after the establishment of the plantation in April 2008 (dark gray bars) and after coppicing in February 2009 (light gray bars).

Durance showed a minor ability to resprout after coppicing and there was a further mortality of 3.3% individuals. The mean number of available replicates for each genotype in the *P. nigra* european collection to be used for the phenological measurements was about 5.3.

8.2. Temperature and photoperiod during the bud set period

Minimum daily temperatures during the bud set sampling period in Savigliano reached a minimum of 10°C, used in previous study (Rohde *et al.*, 2011 ; bud set process of POP5 full-sib family in the first part of this thesis), only once on September, 5 (DOY 248) (Figure 31), and remained relatively high up to October, 11, before the end of the work.

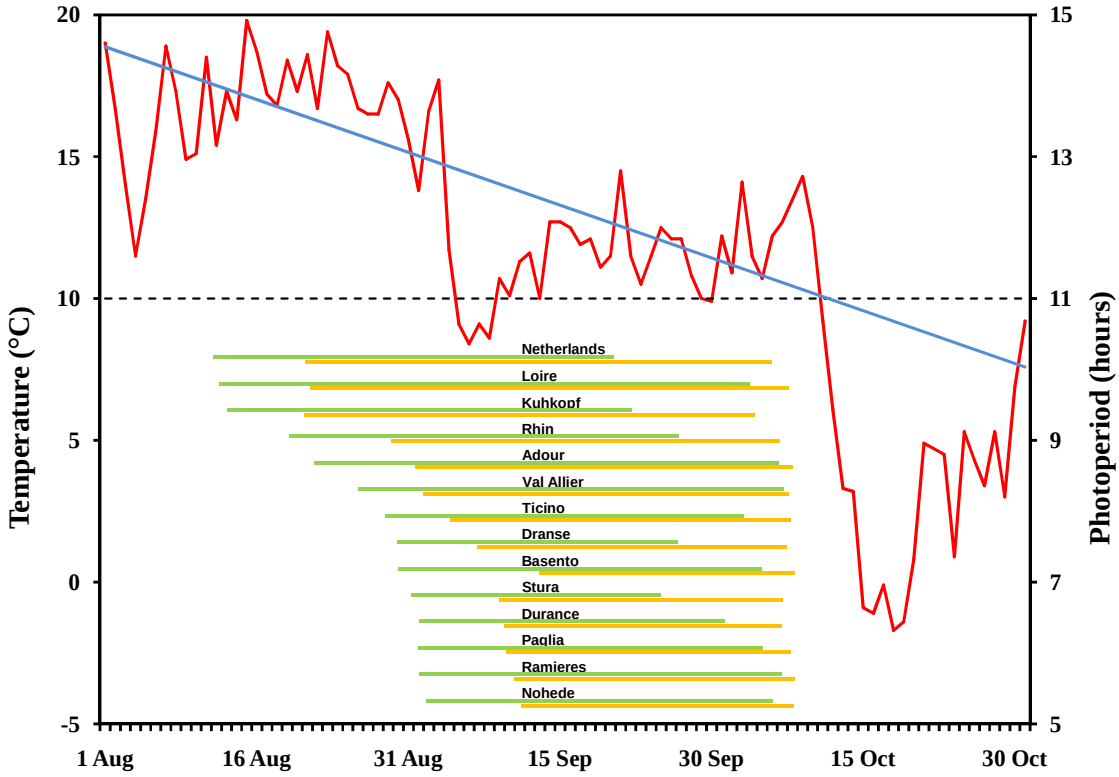


Figure 31. Minimum temperatures and photoperiod in Savigliano during the bud set period. Maximum genotypic range of duration of subprocess 1 (green line) and duration of subprocess 2 (orange line) of the studied populations are shown.

The phenological process started at genotypic level on August, 11, and the bud development process was monitored until the last genotype among all the different populations reached the fully closed bud stage (phase 0.5).

The genotypic variability was high in the northern and more precocious metapopulations and the onset of the second subprocess, taking into account the early genotype that reached phase 1.5, was closely related to the onset of growth cessation. The duration of subprocess 1, considered as the maximum genotypic range overlapping the extreme genotypes to reach the phases 2.5 and 1.5, resulted more variable between the different populations**.**

8.3. Progression of bud development and phenotypic variation

The observed difference between the earliest genotype (phase 2.5) of the collection, sampled in Netherlands (DOY 223.8, i.e. 385.7 CNL), and the latest one (phase 0.5) for the fully closed bud, belonging to the population of Basento (DOY 281.5, i.e. 1031.6 CNL), was about 57.7 days corresponding to a divergence of 645.9 hours of CNL (Table 13).

Table 13. Phenotypic variation in the *P. nigra* association population: number of genotypes analysed, average number of individuals per genotype, association population general means and range of genotypic variation are shown. Significance of differences between the different metapopulations for each trait is indicated as: ns = non significant, * = P ≤ 0.05, ** = P ≤ 0.01, *** = P ≤ 0.001. Data for each metapopulation in appendix 1.

CNL	Phenotypic variation among metapopulations							
Trait	N° genotype analysed	Mean no. replicates	General mean	±	SE	P	Min. genotype mean	Max. genotype mean
Phase 2.5	796	5.31	675.12	±	1.45	***	385.66	930.82
Phase 2	796	5.31	733.65	±	1.52	***	434.12	995.51
Phase 1.5	796	5.29	786.29	±	1.55	***	476.17	1016.03
Phase 1	785	5.29	852.28	±	1.56	***	530.14	1022.62
Phase 0.5	750	5.15	953.42	±	1.08	***	673.49	1031.59
Duration 2.5	796	5.31	58.66	±	0.35	***	27.00	125.19
Duration 2	796	5.29	53.71	±	0.29	***	29.04	96.17
Duration 1.5	785	5.29	68.26	±	0.58	***	32.16	185.71
Duration 1	750	5.15	112.10	±	0.87	***	34.24	344.57
Dur.Sub.1	796	5.29	112.25	±	0.57	***	56.35	200.41
Dur.Sub.2	750	5.15	180.53	±	0.99	***	69.66	404.17

If ordered according to their latitude of origin, all the metapopulations showed a strong clinal response to the perception of the critical night length (Pauley & Perry, 1954 ; Ingvarsson *et al.*, 2006 ; Hall *et al.*, 2007; Luquez *et al.*, 2008 ; Rohde *et al.*, 2011) (Figure 32, C).

High phenotypic variability was observed within the different metapopulations for this trait (Figure 32, B) because of a difference between the earliest and the latest genotype amounting up to 452.5 (corresponding to 41.7 days) and 405.8 (i.e. 36.4 days) CNL for Loire and Adour populations, respectively, taking into account also outliers genotypes. For the same trait, the less variable metapopulation was Stura for which were observed 168 hours CNL (15.2 days) of difference between the two extreme genotypes in the population distribution.

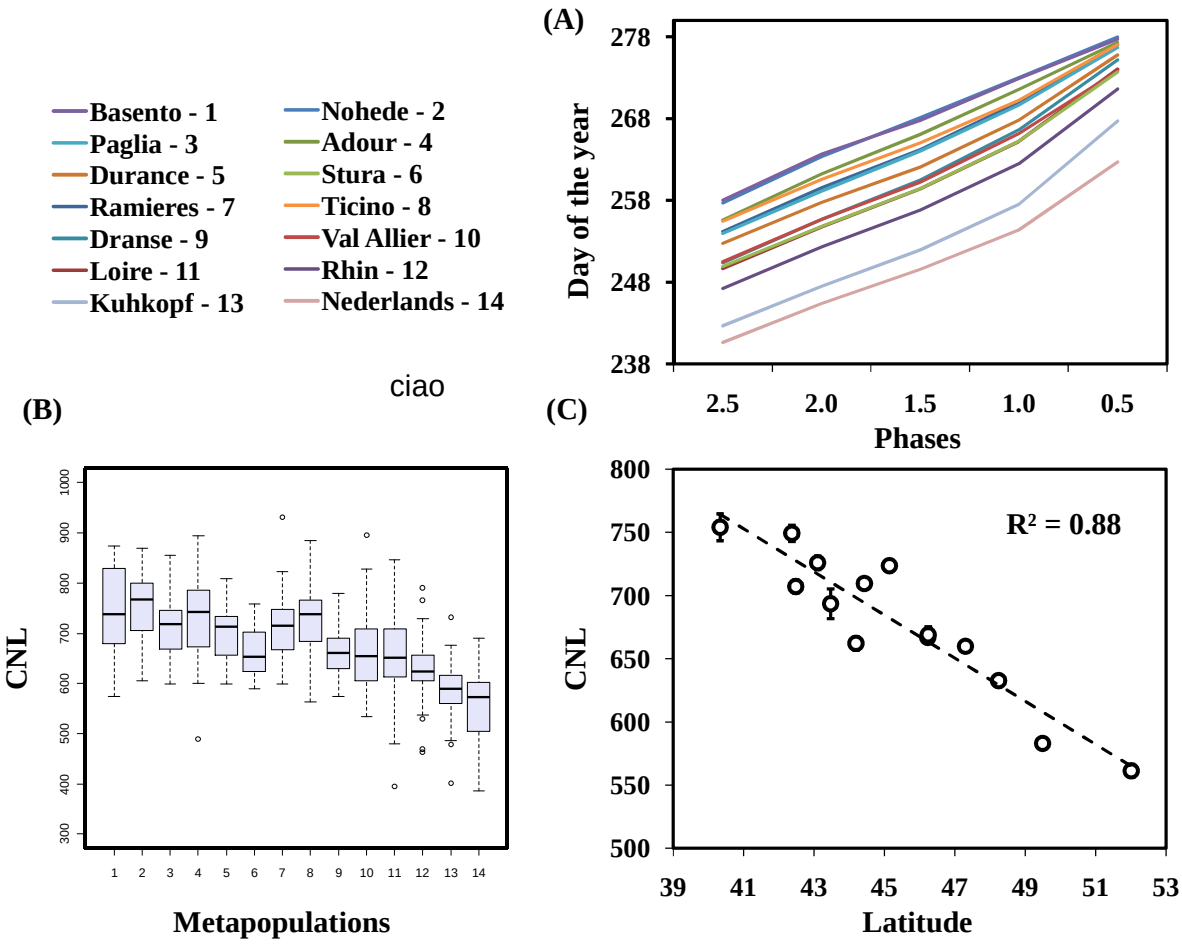


Figure 32. Bud development and onset of growth cessation in the different metapopulations analysed based on genotypic means. (A) Progression of the bud process from the perception of critical night length (phase 2.5) to the fully closed bud (phase 0.5). (B) Phenotypic variation in the onset of growth cessation (CNL - phase 2.5). Metapopulations are numbered and ordered according to their latitude of origin. (C) Regression between populations means for CNL - phase 2.5 and latitude.

The earliest metapopulation, Netherlands, onset the bud formation process on day 240.6, corresponding to 561.3 hours of CNL, whereas Basento, the last to start growth cessation, reached phase 2.5 on day 258, corresponding to 754.1 hours of CNL. A difference of 18 days, about 193 hours of cumulated night lengths were observed among Netherlands and Basento for the trait growth cessation, corresponding to about 1.5 days for each degree of latitude.

The average duration of the phenological process of bud set was 22.8 days in the studied *P. nigra* association population. It ranged between 19.7 days for Basento (southern Italy) and 25 days for Kuhkopf (Germany). Analysing data at genotypic level, a major range from the earliest genotype reaching phase 2.5 and the latest to the phase 0.5 was observed, spanning between 35.8 days for Durance (corresponding to 420.5 CNL) and 56.5 days concerning Loire (i.e. 635.2 CNL). A constant trend in the process was observed for the different populations and the more northern ones tended to change behaviour and to reduce the speed after the onset of transition from the shoot to the bud structure (phase 1.5) (Figure 32, A).

Concerning the two subprocesses, a latitudinal trend in the duration of both subprocesses was monitored (Figure 33, A). The time needed for the bud formation (subprocess 1) decreases moving from southern to northern (R^2=0.66) locations whereas the bud maturation period has an inverse trend, with more time required for the metapopulations sampled in the northern sites than southern ones to complete the bud maturation (R^2=0.52). Minor differences in the duration of bud formation were observed among metapopulations (Figure 33, B). The mean duration of the first subprocess was 9.8 days, corresponding to an amount of 112.2 hours of CNL. Netherlands and Nohede were the extreme metapopulations to complete this trait, with 8.9 and 10.8 days (i.e. 97.5 and 126.1 hours of CNL) respectively. Concerning the second subprocess (Figure 33, C), the average duration was 14.3 days, corresponding to an amount of 180.5 hours of CNL. Kuhkopf, that experienced 15.8 days (corresponding to 217.4 hours of CNL experienced) to complete the bud maturation, and Ticino, 12.5 days (i.e. 153.4 of CNL), represented the extreme points of the range distribution for this trait.

Regarding the within population variability, the smaller differences were found for Durance that showed a range of 3.8 days, corresponding to 49.6 hours of CNL, to complete the bud formation and 5.3 days, about 71.5 hours of CNL, to finish the maturation process. The more variable metapopulation observed in the association population was Basento with 10.7 days necessary to complete the subprocess 1 between the fastest and the slowest genotype (corresponding to 116.7 hours of CNL).

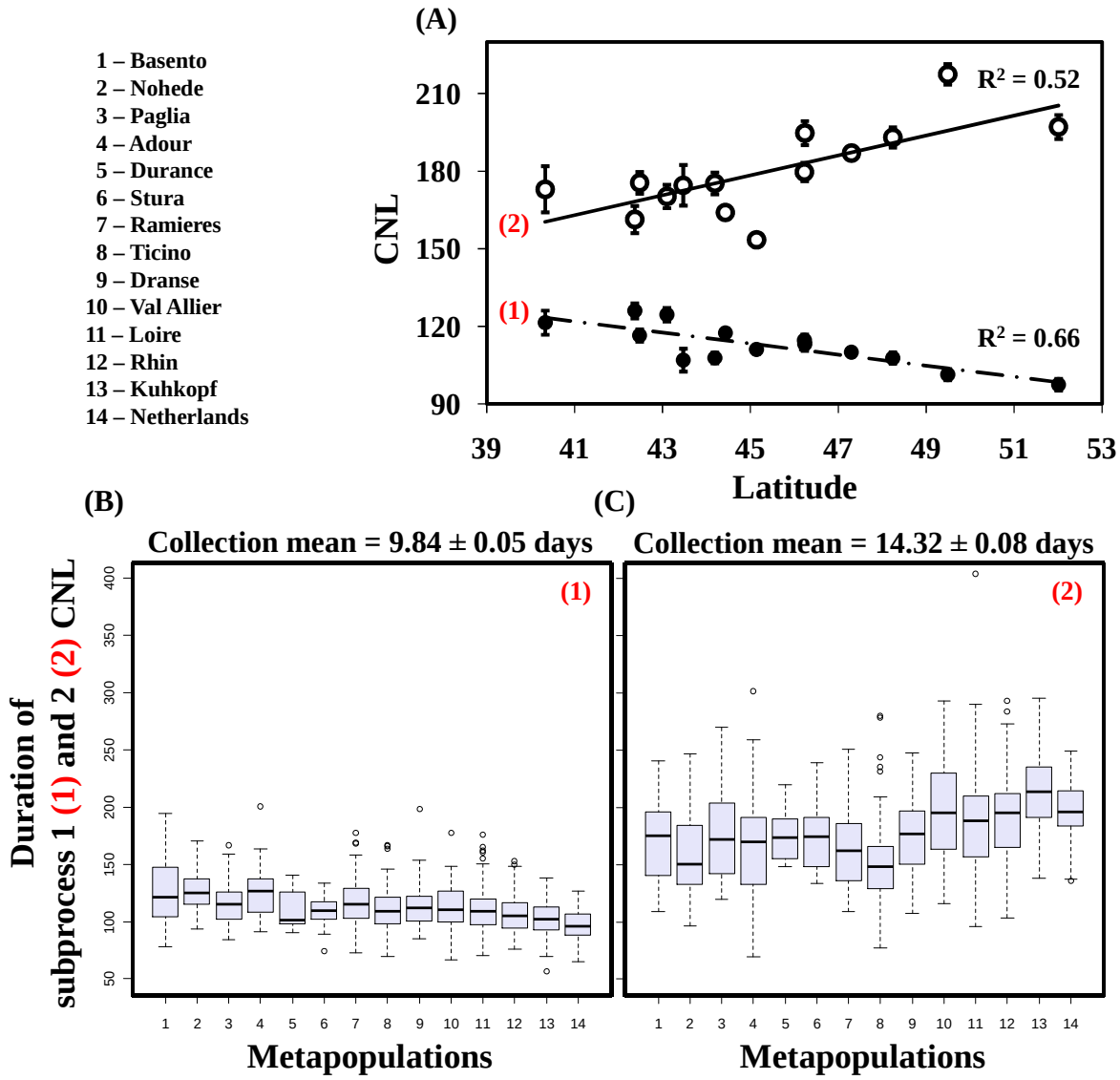


Figure 33. Duration of bud formation (subprocess 1) and bud maturation (subprocess 2) for the different metapopulations analysed based on genotypic means expressed as CNL. (A) Latitudinal trend of the two subprocesses. (B) Phenotypic variation in the duration of bud formation (1). Metapopulations are numbered and ordered according to their latitude of origin. (C) Phenotypic variation in the duration of bud maturation (2).

Loire resulted the more variable population in the bud maturation (subprocess 2), with 22.7 days of difference between the two extreme genotypic performances (corresponding to 308.3 hours of CNL).

In all the different cases and for all the different metapopulations considered, the duration necessary to complete the bud formation (subprocess 1) was lower than the duration spent in the bud maturation (subprocess 2).

8.4. Genetic variability and heritability

The data showed high broad-sense heritabilities at individual at genotypic levels for all the onset-of-stage traits and lower values concerning the different duration traits.

The presence of a substantial genetic variation in the analysed material was observed. CV_G varied between 3.6% (phase 0.5) to 8.9% (phase 2.5) concerning the onset-of-stage traits and from 10.2% (duration phase 2) to 22.4% (duration phase 1) for the different duration traits. The coefficient of residual variation (CV_R) was higher than CV_G only for the last two phases (phase 1 and phase 0.5) and for all the duration traits. It ranged from 4.9% (phase 0.5) to 8.1% (phase 2.5) concerning phases and from 28.8% (duration of subprocess 2) and 51.3% (duration 1.5) (Table 14) for duration traits.

Table 14. Coefficients of genetic (CV_G) and residual (CV_R) variation, individual (H^2_{ind}) and genotypic (H^2_{gen}) broad-sense heritability among the metapopulations analysed using CNL. Data for each metapopulation in appendix 1.

CNL	Genetic variation among metapopulations				
Trait	CV_G (%)	CV_R (%)	CV_G/CV_R	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	8.86	8.11	1.09	0.55 ± 0.02	0.86 ± 0.01
Phase 2	8.52	7.68	1.11	0.55 ± 0.02	0.87 ± 0.01
Phase 1.5	7.95	7.34	1.08	0.54 ± 0.02	0.86 ± 0.01
Phase 1	6.92	7.07	0.98	0.48 ± 0.02	0.83 ± 0.01
Phase 0.5	3.62	4.90	0.74	0.35 ± 0.02	0.73 ± 0.01
Duration 2.5	11.88	36.36	0.33	0.13 ± 0.01	0.44 ± 0.02
Duration 2	10.23	32.76	0.31	0.11 ± 0.01	0.41 ± 0.02
Duration 1.5	18.58	51.35	0.36	0.10 ± 0.01	0.37 ± 0.02
Duration 1	22.39	39.95	0.56	0.21 ± 0.02	0.58 ± 0.02
Dur.Sub.1	10.49	30.42	0.34	0.13 ± 0.01	0.44 ± 0.02
Dur.Sub.2	16.20	28.76	0.56	0.23 ± 0.02	0.61 ± 0.02

In general, data showed very high levels of phenotypic variation and lower contribution of genetic variation for traits describing the duration of phenological events comparing to date-of-onset traits (Figure 34, A). Higher CV_G/CV_R ratio was observed for the duration of bud

maturation than for the bud formation. For the phenotypic variance was partitioned to genetic variance in a range between 73% and 87% for the various onset-of-stage traits whereas this interval was minor for traits related to duration and explaining between 37% to 61% of the total variance. Individual values of broad-sense heritability for all the analysed traits were, as expected, from low to intermediate, ranging from 0.1 and 0.55 (Table 14). Heritability (H^2_{ind}) values remained medium only for the onset-of-stage traits and, in particular, in the first part of the process.

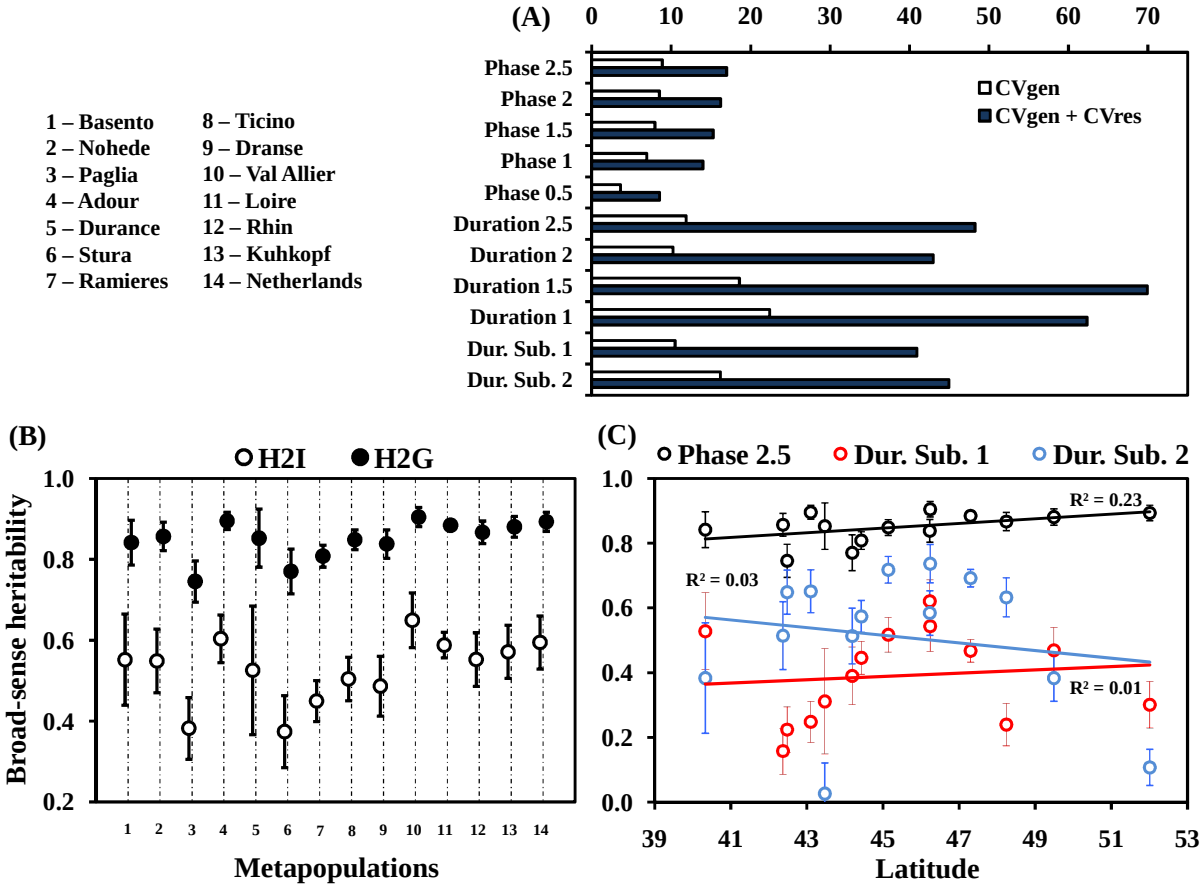


Figure 34. Genetic variation and broad-sense heritability of the bud set process. (A) The bars represent the amount of phenotypic (blue) and genetic (white) variation of the *P. nigra* association population. (B) Broad sense heritability at individual (white dots) and genotypic (black dots) basis for the onset of growth cessation (phase 2.5). (C) Latitudinal trend of (H^2_{gen}) for the growth cessation (black dots and line), the duration of bud formation (red dots and line) and the duration of bud maturation (blue dots and line).

Regarding the different metapopulations analysed the onset of growth cessation (phase 2.5) was found to account up to 90% of the phenotypic variance due to the genetic component in

Val Allier (H^2_{gen}=0.91), Adour (H^2_{gen}=0.90) and Netherlands (H^2_{gen}=0.89) (Figure 34, B), whereas the minor contribution of genetic effects was showed by the population sampled along the Italian Paglia river (H^2_{gen}=0.75).

Genotypic broad-sense heritability for the two subprocesses varied considerably with the metapopulations analysed and it ranged from 0.16 (Nohede) to 0.62 (Dranse), concerning duration of bud formation, and from 0.11 (Netherlands) to 0.74 (Val Allier) for the duration of bud maturation. H^2_{gen} close to 0.03 was found for Durance, but only 8 genotypes were available for the evaluation of the duration of subprocess 2.

No strong clinal variation for the broad-sense heritability was shown, especially concerning the duration of the two subprocess, whereas a slight trend for the onset of growth cessation (R^2=0.23) was observed, with a southern-northern increasing trend of the genetic contribution to the phenotypic variation (Figure 34, C).

8.5. Selection of descriptive traits based on phenotypic variation

Analysing all trees available in the association population experimental plantation, data showed high positively correlations among all the different onset-of-stage traits (0.84<r<0.99), especially between nearby phases (Figure 35).

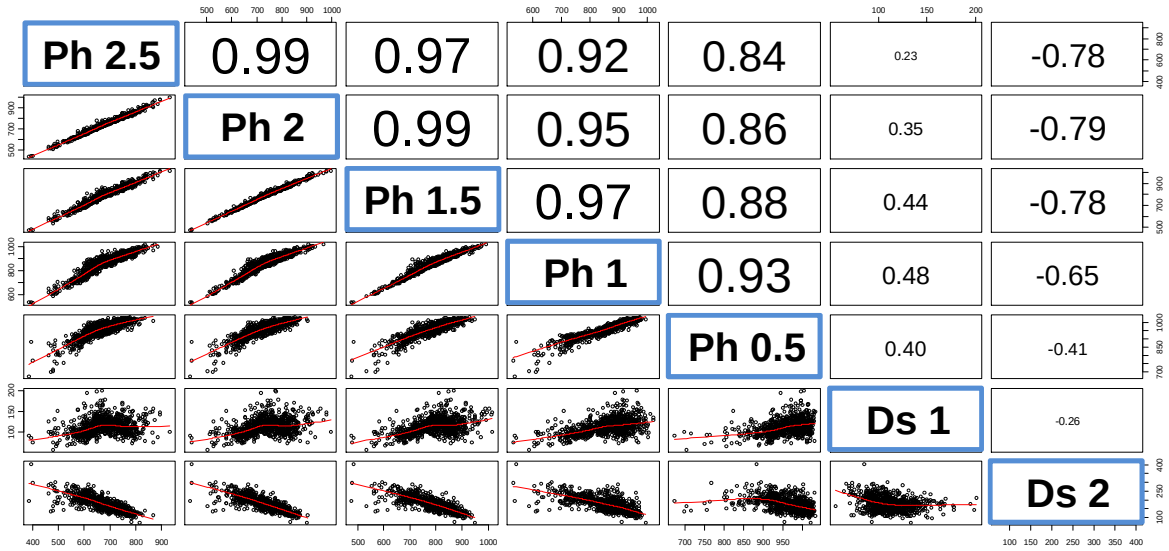


Figure 35. Pearson's phenotypic correlations among traits of bud set based on genotypic means. P=phase X and D.s. = duration of subprocess X. Data for each metapopulation in appendix 2.

Concerning the two subprocesses, data showed high negative correlations between the duration of subprocess 2 and the different phases (-0.41<r<-0.79), especially among those traits describing the early stages of the bud set process (-0.78<r<-0.79). Low positive correlations among the phases and the duration of bud formation (0.23<r<0.48) were also observed. Correlations among phases and subprocesses, which resulted negatively related to each other (r=-0.26), showed an inverse trend decreasing from the correlation between phase 2.5 vs. the duration of subprocess 2 (r=-0.78) and phase 0.5 vs. duration of subprocess 2 (r=-0.41).

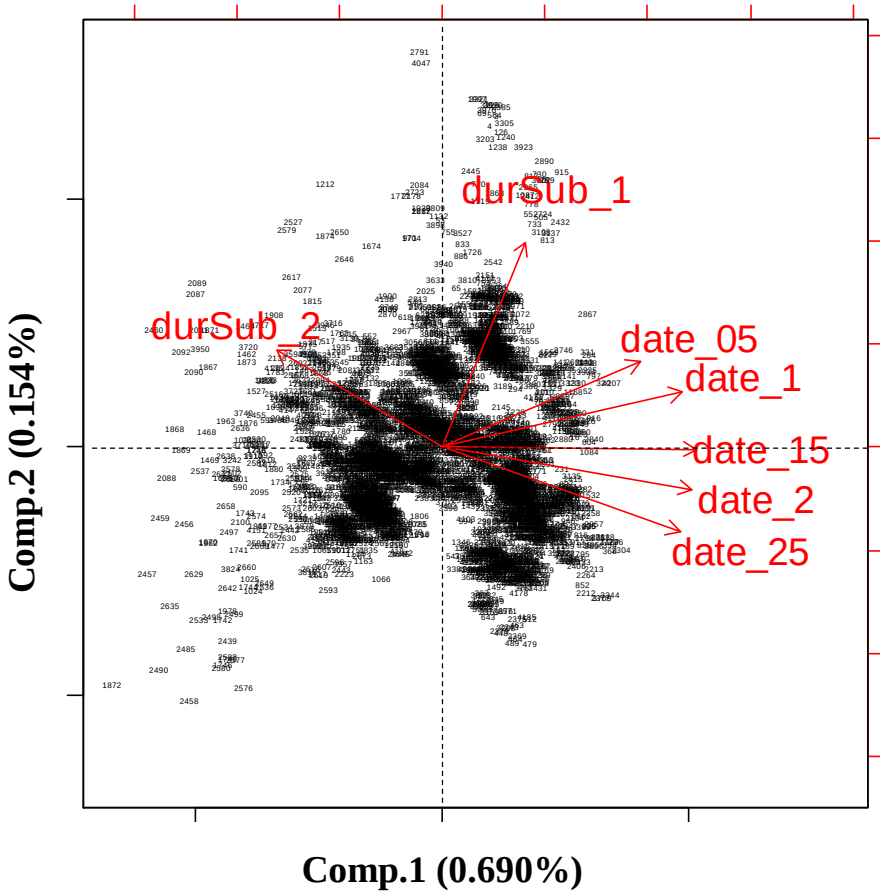


Figure 36. Principal component analysis (PCA) performed on different bud set traits for the *P. nigra* association population studied in Savigliano. Individual adjusted phenotypic values for micro-environmental effects were used.

Successively, an increase of the correlation between phase 2.5 vs. duration of subprocess 1 (r=0.23) and between phase 1 vs. duration of subprocess 1 (r=0.48) was observed, with an exception in the lower correlation between phase 0.5 vs. duration of subprocess 1 (r=0.40). As

all the considered traits described a developmental process and for this reason tended to be related, a principal component analysis using the five onset-of-stage and the two subprocesses transformed in CNL was performed to identify the most discriminative traits in terms of phenotypic variation and to avoid needless or redundant information. The different duration traits were not included because they are wholly represented by the duration of the bud formation and the duration of bud maturation (i.e. duration of subprocess 1 and duration of subprocess 2, respectively).

The phenotypic variation measured in Savigliano was divided into two major groups: Prin1, which explained 0.69% of the phenotypic variation and Prin2 about the 0.15% (Figure 36). The onset-of-stage traits, that are really important for a deep physiological dissection of bud development, provided a main contribution to Prin1. Results showed that phases 2 and 1.5 had the major effect with values of 0.446 and 0.453, respectively) (numeric data not shown) while duration of subprocess 2 (CNL) presented a minor opposing effect on the axis of Prin1 (-0.295). Duration of subprocess 1 contributed mainly to Prin2 (0.772) with a minor opposing effect for phase 2.5 CNL (-0.318).

Based on this results the onset of bud initiation (date 1.5) and the two subprocesses were selected. In addition, it was decided to include the onset of growth cessation (i.e. phase 2.5) because of its specific importance as the date of perception of critical night length.

8.6. Genetic correlations between bud set traits

As previously shown by Pearson's linear correlations, all the different onset-of-stage traits were highly positively correlated among each other ($0.76\pm0.02<r_g<1.00\pm0.00$) (Figure 37), especially between nearby phases.

The duration of the two subprocesses was genetically positively related ($r_g=0.30\pm0.03$) and both these traits showed negative correlations with all the different onset-of-stage traits. The duration of subprocess 2 (the bud maturation period) showed high negative correlations with the different onset-of-stage traits ($-0.67\pm0.02< r_g<-0.83\pm0.01$), whit the maximum value observed for the correlation between phase 2 vs. duration of subprocess 2.

The bud formation subprocess (i.e. the first subprocess) had lower correlation with the different phases, and values ranged from -0.53±0.03 (subprocess 1 vs. phase 2.5) to -0.73±0.02 (subprocess 1 vs. phase 2). The duration of the bud maturation (subprocess 2) was always highly negatively correlated with the onset of growth cessation ($-1.00\pm0.00<r_g<-$

0.53±0.11) and bud initiation (-1.00±0.00< r_g<-0.67±0.10) in the different studied metapopulations (Figure 38). An exception was the population sampled in the Netherlands that didn't show any correlation of subprocess 2 with phase 2.5 (r_g=0.13±0.15), nor with phase 1.5 (r_g=0.07±0.15).

	phase 2.5	phase 2	phase 1.5	phase 1	phase 0.5	Dur. Subproc. 1	Dur. Subproc. 2
phase 2.5		1.00 *0.00*	0.98 *0.00*	0.92 *0.01*	0.82 *0.01*	-0.53 *0.03*	-0.79 *0.01*
phase 2	0.93 *0.01*		0.97 *0.00*	0.90 *0.01*	0.76 *0.02*	-0.73 *0.02*	-0.83 *0.01*
phase 1.5	0.81 *0.01*	0.94 *0.00*		0.92 *0.01*	0.78 *0.01*	-0.61 *0.02*	-0.82 *0.01*
phase 1	0.58 *0.02*	0.70 *0.02*	0.81 *0.01*		0.82 *0.01*	-0.63 *0.02*	-0.79 *0.01*
phase 0.5	0.41 *0.03*	0.43 *0.03*	0.49 *0.03*	0.69 *0.02*		-0.57 *0.02*	-0.67 *0.02*
Dur. Subproc. 1	0.18 *0.03*	-0.15 *0.03*	-0.38 *0.03*	-0.47 *0.03*	-0.19 *0.04*		0.30 *0.03*
Dur. Subproc. 2	-0.50 *0.03*	-0.61 *0.02*	-0.61 *0.02*	-0.28 *0.03*	0.29 *0.03*	0.22 *0.03*	

Figure 37. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits of the *P. nigra* collection studied. Data for each metapopulation in appendix 3.

Data didn't show a clinal variation of the genetic correlations between the onset of growth cessation, the onset of bud initiation and the duration of bud maturation: only 23% of the variability showed in the plot between phase 2.5 and duration of subprocess 2 is accounted by the linear model (Figure 38, A), and the coefficient of determination was 0.35, concerning linear regression of onset of bud initiation vs. duration of bud maturation (Figure 38, B). On the contrary, a latitudinal trend in the genetic correlation between the duration of bud formation and the perception of the critical night length (R^2=0.60) was observed, with values of r_g found more variable than those showed in the relationship between phase 2.5 and the duration of subprocess 2.

The decreasing trend showed in Figure 38 (A) is characterized by positive moderate genetic correlations ($0.19\pm0.13<r_g<0.51\pm0.14$) of the southern metapopulations (Basento, Paglia, Adour, Stura, Ramieres and Ticino), with the exception of Nohede, and become negative and high for the northern ones (up to $r_g=-0.89\pm0.03$ for Netherlands).

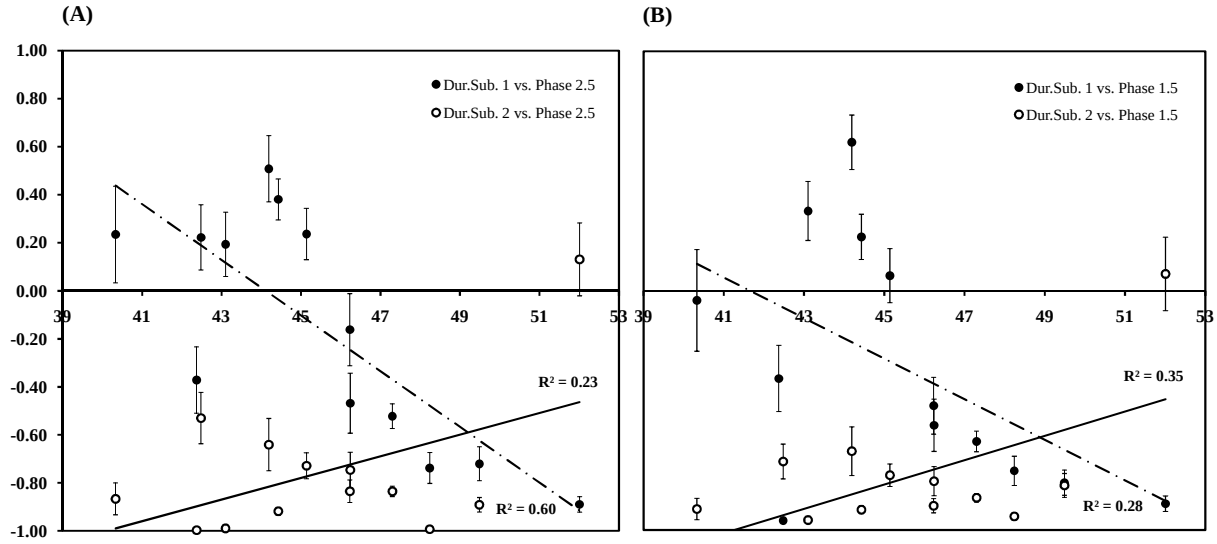


Figure 38. Latitudinal trend of genetic correlations. (A) Correlations between duration of subprocess 1, duration of subprocess 2, and the onset of growth cessation (phase 2.5). (B) Correlations of the two subprocesses and the onset of bud initiation (phase 1.5).

No clinal variation was found in the genetic correlation between the duration of bud formation and the onset of bud initiation ($R^2=0.28$).

8.7. Phenological vs. biometric traits

In order to test the possible relationship between the tree architecture and the phenological process, circumference was used as covariate of the more descriptive phenological traits analyzed.

To avoid redundant information, it was decided not to consider height which is highly correlated with circumference.

As shown in the plot (Figure 39), circumference did not influence phenology neither in the onset of growth cessation, nor for the onset of bud initiation and in the duration of bud formation and maturation.

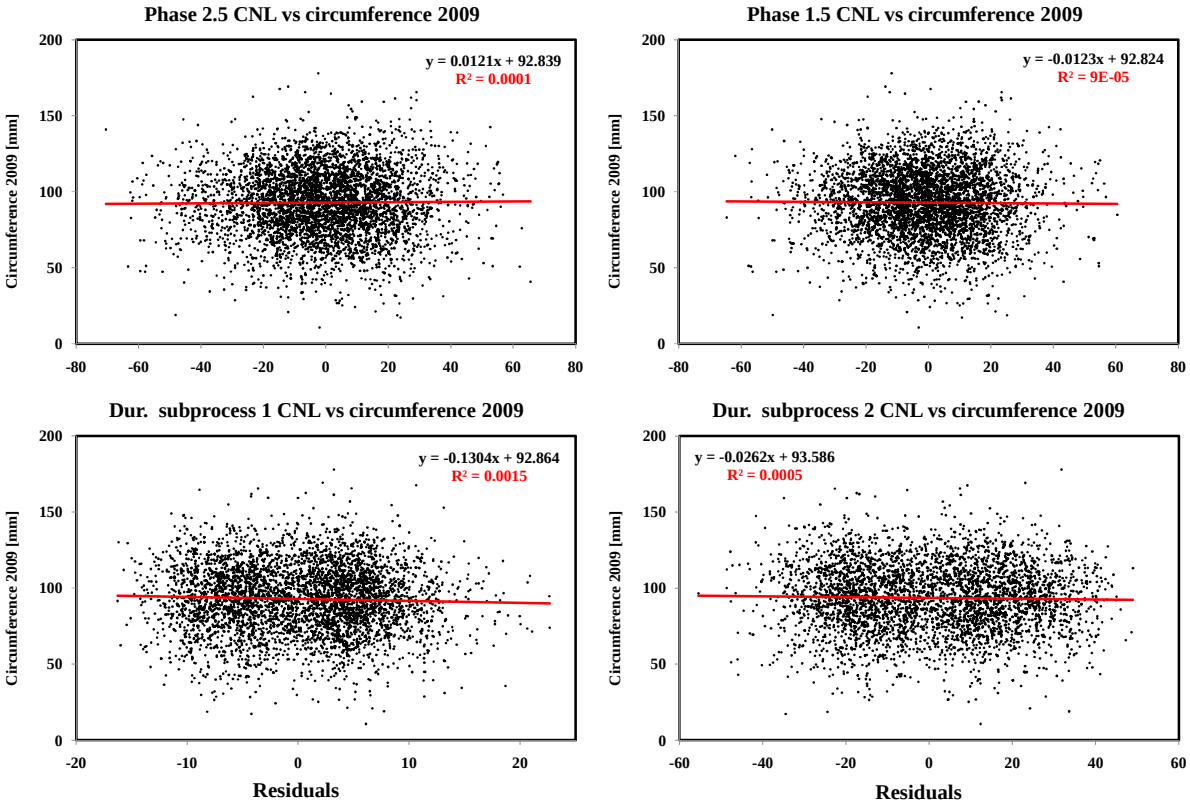


Figure 39. Linear regressions between circumference and some more descriptive phenological traits for *P. nigra* collection.

9. DISCUSSIONS - *P. nigra* european native populations

9.1. Latitudinal clines in growth cessation and bud set dynamic

The target of this work was to assess the bud set process in different metapopulations of european black poplar (*Populus nigra* L.) and investigate where the genetic variation reside. This important phenological trait is a critical ecological and evolutionary trade-off between survival and growth in trees of temperate zones (Horvath *et al.*, 2003 ; Howe *et al.*, 2003) and a local adaptive response to latitudinal changes in the duration of the favourable growing season (Hall *et al.*, 2007).

A detailed scoring system dissecting the different aspects of the bud development, from the onset of growth cessation to the fully closed bud, was adopted to analyse separately the growth cessation (phase 2.5) from the bud formation process (Rohde *et al.*, 2011).

All the studied metapopulations were not different in a random way. In fact, if ordered according to their latitude of origin, they showed a strong clinal response to the critical night length (Pauley & Perry, 1954 ; Ingvarsson *et al.*, 2006 ; Hall *et al.*, 2007; Luquez *et al.*, 2008 ; Rohde *et al.*, 2011) characterizing their native location. About 90% of the observed variation in the onset of growth cessation among the natural accessions was explained by the latitude of origin. This trend provided indirect evidence that the perception of critical night length and the onset of growth cessation is under strong natural selection (Howe *et al.*, 2003). Local adaptation complicated the establishment of the common garden study because genotypes sampled in southern location (i.e. Basento and Nohede) grew slowly in the continental location of Savigliano. The planting site might also affect the natural performances of more oceanic accessions.

The onset of growth cessation as well as other onset-of-stage traits and the duration of bud development, were not influenced by biometric traits. Phase 2.5 exhibited a great genetic variation because the existence of 18 days of differences between the earliest and the latest accession. High differences were documented by Rohde and colleagues (2011) that observed 27 days between the two extreme populations in a common garden experiment established in Belgium and by Ingvarsson and collaborators (2006) that in a growth chamber experiment found 48 days of difference among 12 accessions of *Populus tremula* (SwAsp Collection) sampled across 10° latitude in Sweden.

In this study, based on the *P. nigra* association population, a clinal variation was observed underling a trend of 1.5 days per degree of latitude. Genotypic continuous distribution in the time of growth cessation in the different metapopulations, emphasize the genetic control by a large number of genes, each one with a small effect on the phenotype. All the accessions showed a constant trend in the bud set process.

The different analysed phases described a developmental "cascade" sequence and were strongly correlated each other at genetic (r_g>0.76) and phenotypic (r>0.84) level. For this reason, it should be hypothesised that the different stages were controlled by the same genes.

The total duration of the bud set process was characterized by small differences between population means (5 days between the quickest and the slowest metapopulation) whereas a large within metapopulation genotypic variability (up to 57 days for Loire) was observed. The clinal variation found in the duration of bud formation, for which in this study 66% of the variation was explained by the latitude of origin, was also documented by Rohde and co-workers (2011) in *Populus nigra* and by Junttila *et al.* (2003) in *Betula alba*, under controlled growth conditions. In general, the southern accessions complete bud formation process (subprocess 1) slower than the northern ones.

An inverse latitudinal cline trend was showed for the bud maturation process. It was longer for this trait and more variable than the duration of subprocess 1, in all the different studied *P. nigra* metapopulations.

Phenotypic correlations showed for all the different accessions (except for the Netherlands) an inverse relationship: the later the onset of growth cessation, the shorter the duration of bud maturation.

9.2. Genetic characterization of the bud set process

Results showed low levels of phenotypic variation and a relative high contribution of genetic variation to all the phases studied.

Across the collection, up to 87% of the phenotypic variance measured in the onset of growth cessation was partitioned to genetic variance. Broad-sense heritability reached maximum values of about 0.90 in some accessions and a slight increasing latitudinal trend for this parameter (R^2=0.23), from south to north, was observed. A decreasing trend from the phase 2.5 to the phase 0.5 concerning the genetic importance of the phenotypic variability across the

collection was found, underling an increasing role of the environment during the bud set process.

Taking into account the between population analyses, results showed lower values of broad sense heritability in this study compared to those obtained by Rohde and colleagues (2011) (values always >0.90 in all onset-of-stage traits) in natural populations of *Populus nigra* evaluated in Belgium (50°46′N, 3°52′E). However, a common decreasing trend of H^2 during the process was shown. Partially comparable results, characterized by an inverse trend (higher values of H^2 for the last phases), were obtained in the F_1 full-sib families (two *P. deltoides* Bartr. x *P. trichocarpa* Torr. & Gray and one *P. deltoides* Bartr. x *P. nigra* L.) studied in France (47°46′N, 1°52′E) (Rohde *et al.*, 2011).

Luquez and co-workers (2008) showed similar levels of broad-sense heritability to those obtained in this study (only phase 0.5 was comparable), analysing twelve populations of *Populus tremula* (SwAsp Collection) in two common garden experiments in Sweden (Ekebo, 55.9°N; Sävar 63.4°N), amounting to 0.60 and 0.70, respectively for the two sites.

In this *P. nigra* experiment a lower genetic contribution in the phenotypic variation of phase 1 was found comparing the results (H^2_{ind}=0.72 and H^2_{gen}=0.91) showed by Howe *et al.* (2000) in a F_2 family, derived from a cross between two F_1 hybrids (*P. trichocarpa* Torr. & Gray x *P. deltoides* Bartr.), studied in Oregon (44°34′N, 123°16′W). In a similar study on the same F_2 family grown in Minnesota (44°59′N, 93°10′W), Howe and colleagues (2000) observed similar results (H^2_{ind}=0.48 and H^2_{gen}=0.75) (only phase 1 was comparable) to those obtained in this study.

Comparable levels of broad-sense heritability for phase 0.5, with values close to 0.80, were also documented by Li and co-workers (1998) in interspecific hybrids of *Populus tremuloides* Michx. x *Populus tremula* L. grown near Rhinelander, Wisconsin (45°40′N, 89°25′W), and Grand Rapids, Minnesota (47°14′N, 93°31′W).

Higher phenotypic variances and lower values of genetic contribution to the measured variance were observed for the duration characters than in all the phases, as a result of the high influence of the environmental conditions on the duration traits. This result was documented also by Rohde and co-workers (2011) in the natural populations of the same species studied in Belgium, in the only published work using phases and duration as distinct traits. Despite the influence of the environment, the duration of subprocess 2, with the onset of bud initiation (phase 1.5), were identified as the most discriminative trait in terms of phenotypic variation using PCA approach.

Broad-sense heritability at genotypic level for the two subprocesses varied considerably with the analysed accession and no strong clinal variation for H^2 was shown.

A latitudinal trend of the genetic correlations between the duration of bud formation and the perception of the critical night length (R^2=0.60) were observed, with a decreasing tendency from positive moderate genetic correlations, found in the southern metapopulations, to the high negative correlations, showed in the more northern accessions. High negative correlations between the growth cessation and the duration of bud maturation (excluding Netherland) were always observed.

As a trait directly related to the adaptation, the genetic characterization of the growth cessation and the behavior of the bud formation-maturation process is of key importance in the identification of plant material for conservation strategies and an important tool to selecting trees in breeding programs. Genetic parameters like heritability and genetic correlation are of paramount importance to investigate where the genetic variation for a specific quantitative trait resides and to predict correlated responses between traits or provide indirect selection.

10. CONCLUSIONS - *P. nigra* european native populations

Although our results might be specific to the different metapopulations studied in the experimental site of Savigliano, some interesting conclusions can be drawn:

- The innovative protocol used in this study offers the possibility of dissecting the bud setting process in the fall into phases and durations (Rohde *et al.*, 2011) and characterize their relative contribution. This is the major improvement compared to the previous study.
- Night length is the crucial factor in the onset of growth cessation in poplar as shown by the strong latitudinal cline (1.5 days/lat of difference between accessions).
- The onset-of-stage traits (especially phase 2.5) are characterized by the higher genetic contribution in the total phenotypic variance and it decreases during the process (from the first to the last phase). Duration of traits appears to be highly influenced by the environmental factors, especially the duration of bud formation (duration of subprocess 1) that might be particularly important in the adaptation to short term fluctuations of temperature and environments (Rohde *et al.*, 2011).
- The timing of bud initiation (phase 1.5) and the duration of bud formation and maturation (subprocesses 1 and 2) were identified as the most discriminative traits in terms of phenotypic variation.
- Bud set is a constant process from the onset of growth cessation to the end of bud maturation and the time spent for the duration of subprocess 2 (bud maturation) is always longer than the time needed for the duration of subprocess 1 (bud formation). This last trait differs only a few days among the different metapopulations and the northern accessions complete this duration stage slightly faster than the southern ones (Rohde *et al.*, 2011) (*Betula alba*, Junttila *et al.*, 2003).
- As a trait directly related to the adaptation, the characterization of growth cessation in different european metapopulations is an important tool for the identification of the accessions for conservation strategies and the delineation of breeding zones (Howe *et al.*, 2003). The study and characterization of material at metapopulation level is a key factor for *in situ* conservation activities, because black poplar naturally forms network of inter-linked local populations rather than small isolated stands (Lefèvre *et al.*, 2001).

11. GENERAL CONCLUSIONS AND PERSPECTIVES

Phenological process of bud set is of paramount importance for local adaptation and permits the trees of the temperate zones to survive cold winters.

The high number of phenotypic observations showed in this study were drawn thanks to the new approach in the study of bud set offered by the protocol defined by Rohde and colleagues (2011), as the major improvement compared to the previous works.

We showed that the onset of growth cessation is a quantitative trait under strong genetic control. The large number of QTLs mapped in the POP5 genetic map, each one characterized by small or modest effect, highlighted the complex nature of traits involved in the bud formation-maturation process. Night length is the crucial signal triggering the physiological process, but the role of other environmental factors increase during the process. Taking advantage of two contrasting environments we found that temperature, one of the more contrasting environmental variables between sites, seems to influence the sensitivity of some genotypes. The between sites analysis of POP5 highlighted the considerable role of GxS interactions in all the different onset-of-stage traits. The interaction between sites allow to decompose the more important “scale change” effect in the onset of growth cessation and an increasing role of “rank change” effect during the process. We noted that a number of genotypes are more responsible of the GxS interaction observed. The study of the contribution of phenotypic plasticity to variation in bud set is of key importance because it can contribute to short-term adaptation.

Quite similar results concerning the higher values of heritability in the onset-of-stage traits rather than in the durations, and the decreasing trend of the importance of genetic component of variance from the onset of growth cessation to the fully closed bud, were observed both in the pedigree and in the association population study.

The approach based on heterogeneous genetic material is essential in order to better characterize a physiological process like the bud set. We took advantage of the availability of a genetic linkage map based on 154 full-sib individuals which can be considered as an artificial simplified metapopulation. The study of different natural metapopulations allows to take advantage of the genetic and phenotypic diversity found in natural stands. In perspective, the simultaneous study of the molecular regulators of bud set process in natural and model populations will permit to find shared and more robust QTLs regions essential to check for candidate genes. QTLs mapping in a genome-sequenced species give the opportunity to

integrate the large data sets of global transcriptome profiling into the experimental trials (Bielenberg, 2011).

The project NOVELTREE is the first project in Europe structured to create new and improved breeding strategies integrating molecular biology with the breeding programs. For this purposes the southern POP5 parent ("Poli"), two different clones ("BEN3" and "71077-380") and 50 additional individuals belonging to the european metapopulations and spanning the latitudinal range of twelve degrees of latitude of the *P. nigra* association population were resequenced at 50×, 20× and 2× coverage, respectively. Within the project SNP markers for candidate genes associated to phenology were selected. A strategy of genotyping the whole *P. nigra* association population and the POP5 pedigree is actually under evaluation using the technology SNP-array ILLUMINA. This activity will allow to find a strong association of candidate genes for phenology using the *P. nigra* association genetics study and to validate it with the pedigree approach.

A further help will be provided by the availability of the draft sequence (50×) of "Poli" that in a short time will be the reference sequence for the *P. nigra* genome.

The proposed strategy is being developed within a consortium formed among the NOVELTREE, ENERGYPOPLAR and EVOLTREE projects with the aim to elucidate genotype-phenotype association studies and the objective to develop a Marker-Assisted-Selection (MAS) breeding program in poplar.

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13. APPENDIX 1

Basento		Metapopulation phenotypic variation							Metapopulation genetic variation				
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	± SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	DOY	22	4.32	258.00	± 0.94	***	241.98	268.51	2.72	2.44	0.55 ± 0.11	0.84 ± 0.05
Phase 2	DOY	22	4.32	263.67	± 0.93	***	251.33	276.14	2.49	2.47	0.50 ± 0.12	0.81 ± 0.06
Phase 1.5	DOY	22	4.14	267.78	± 0.86	***	255.92	278.22	2.25	2.20	0.52 ± 0.12	0.81 ± 0.06
Phase 1	DOY	21	4.05	272.88	± 0.74	***	261.63	280.45	1.50	2.04	0.36 ± 0.13	0.70 ± 0.10
Phase 0.5	DOY	14	3.57	277.68	± 0.58	*	272.28	281.46	0.76	1.29	0.26 ± 0.16	0.56 ± 0.16
Duration 2.5	DOY	22	4.32	5.67	± 0.28	**	3.27	9.26	16.86	44.95	0.23 ± 0.11	0.56 ± 0.11
Duration 2	DOY	22	4.14	4.83	± 0.21	*	3.17	7.99	15.50	38.78	0.19 ± 0.11	0.49 ± 0.12
Duration 1.5	DOY	21	4.05	5.80	± 0.37	*	2.59	10.53	21.46	54.12	0.15 ± 0.11	0.42 ± 0.13
Duration 1	DOY	14	3.57	7.97	± 0.50	NS	4.81	10.84	7.45	44.18	0.03 ± 0.11	0.09 ± 0.13
Dur.Sub.1	DOY	22	4.14	10.39	± 0.41	**	6.49	17.23	17.63	32.96	0.23 ± 0.11	0.55 ± 0.12
Dur.Sub.2	DOY	14	3.57	14.35	± 0.75	NS	9.10	20.81	13.99	34.50	0.14 ± 0.14	0.37 ± 0.17
Phase 2.5	CNL	22	4.32	754.08	± 10.70	***	574.91	874.02	10.57	9.53	0.55 ± 0.11	0.84 ± 0.06
Phase 2	CNL	22	4.32	820.00	± 10.83	***	677.82	966.80	9.33	9.28	0.50 ± 0.12	0.81 ± 0.06
Phase 1.5	CNL	22	4.14	868.29	± 10.15	***	729.93	992.90	8.24	8.00	0.52 ± 0.12	0.82 ± 0.06
Phase 1	CNL	21	4.05	929.06	± 8.91	***	794.46	1020.71	5.33	7.21	0.37 ± 0.13	0.71 ± 0.09
Phase 0.5	CNL	14	3.57	988.11	± 7.17	*	925.11	1031.59	2.50	4.54	0.25 ± 0.16	0.54 ± 0.16
Duration 2.5	CNL	22	4.32	66.01	± 3.18	**	39.58	101.86	15.49	44.51	0.21 ± 0.11	0.54 ± 0.11
Duration 2	CNL	22	4.14	56.97	± 2.47	*	38.68	92.81	15.38	38.49	0.18 ± 0.11	0.48 ± 0.12
Duration 1.5	CNL	21	4.05	69.33	± 4.34	.	32.16	121.73	20.26	54.15	0.14 ± 0.10	0.39 ± 0.13
Duration 1	CNL	14	3.57	97.51	± 5.94	NS	58.50	133.19	6.64	43.09	0.02 ± 0.09	0.08 ± 0.09
Dur.Sub.1	CNL	22	4.14	121.44	± 4.66	*	78.04	194.70	16.58	32.83	0.21 ± 0.11	0.53 ± 0.12
Dur.Sub.2	CNL	14	3.57	172.98	± 8.92	NS	109.27	240.80	14.11	33.84	0.15 ± 0.14	0.38 ± 0.17

Figure 40. Phenotypic and genetic variation for Basento (40.33°N). Data are expressed as cumulative night length.

Nohede		Metapopulation phenotypic variation							Metapopulation genetic variation				
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	± SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	DOY	39	4.92	257.65	± 0.57	***	244.64	267.96	2.29	2.05	0.55 ± 0.08	0.86 ± 0.03
Phase 2	DOY	39	4.92	263.34	± 0.57	***	249.14	274.83	2.31	1.91	0.60 ± 0.07	0.88 ± 0.03
Phase 1.5	DOY	39	4.79	268.08	± 0.52	***	254.28	279.02	2.00	1.79	0.56 ± 0.08	0.86 ± 0.03
Phase 1	DOY	35	4.83	272.99	± 0.46	***	261.42	280.53	1.41	1.67	0.42 ± 0.09	0.78 ± 0.05
Phase 0.5	DOY	26	4.46	277.96	± 0.37	*	272.30	281.16	0.59	1.31	0.17 ± 0.09	0.48 ± 0.11
Duration 2.5	DOY	39	4.92	5.69	± 0.15	NS	4.16	8.66	5.42	35.53	0.06 ± 0.06	0.25 ± 0.08
Duration 2	DOY	39	4.79	5.12	± 0.15	.	3.53	8.10	8.59	39.23	0.07 ± 0.06	0.26 ± 0.08
Duration 1.5	DOY	35	4.83	5.83	± 0.21	NS	3.72	8.83	12.82	44.62	0.06 ± 0.06	0.25 ± 0.08
Duration 1	DOY	26	4.46	7.38	± 0.27	*	4.47	10.91	13.65	36.77	0.13 ± 0.09	0.39 ± 0.11
Dur.Sub.1	DOY	39	4.79	10.76	± 0.25	NS	7.78	14.68	2.16	31.99	0.04 ± 0.05	0.16 ± 0.07
Dur.Sub.2	DOY	26	4.46	13.28	± 0.45	*	7.97	19.01	13.51	33.98	0.14 ± 0.09	0.42 ± 0.11
Phase 2.5	CNL	39	4.92	749.35	± 6.47	***	604.55	868.92	8.94	8.00	0.55 ± 0.08	0.86 ± 0.04
Phase 2	CNL	39	4.92	815.53	± 6.60	***	653.78	952.49	8.69	7.14	0.60 ± 0.07	0.88 ± 0.03
Phase 1.5	CNL	39	4.79	871.37	± 6.19	***	710.44	1003.92	7.35	6.50	0.57 ± 0.08	0.86 ± 0.03
Phase 1	CNL	35	4.83	931.51	± 5.45	***	805.34	1022.62	4.81	5.93	0.40 ± 0.09	0.76 ± 0.06
Phase 0.5	CNL	26	4.46	992.32	± 4.10	*	935.45	1027.87	1.80	4.08	0.16 ± 0.09	0.46 ± 0.11
Duration 2.5	CNL	39	4.92	66.28	± 1.72	.	48.52	100.35	7.31	35.27	0.08 ± 0.06	0.29 ± 0.08
Duration 2	CNL	39	4.79	60.44	± 1.74	NS	42.36	92.17	6.24	38.91	0.06 ± 0.06	0.22 ± 0.08
Duration 1.5	CNL	35	4.83	71.12	± 2.77	NS	44.69	105.18	11.96	49.26	0.05 ± 0.06	0.20 ± 0.08
Duration 1	CNL	26	4.46	89.75	± 3.17	*	53.00	143.66	15.72	34.65	0.16 ± 0.09	0.47 ± 0.11
Dur.Sub.1	CNL	39	4.79	126.05	± 2.91	NS	93.63	170.53	6.23	31.55	0.04 ± 0.05	0.16 ± 0.07
Dur.Sub.2	CNL	26	4.46	161.32	± 5.25	**	96.57	247.06	15.31	31.55	0.19 ± 0.10	0.51 ± 0.11

Figure 41. Phenotypic and genetic variation for Nohede (42.37°N). Data are expressed as cumulative night length.

Paglia		Metapopulation phenotypic variation							Metapopulation genetic variation				
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	± SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	DOY	49	4.73	253.95	± 0.46	***	244.21	266.85	1.71	2.17	0.38 ± 0.08	0.75 ± 0.05
Phase 2	DOY	49	4.73	259.14	± 0.47	***	248.58	272.19	1.79	2.12	0.41 ± 0.08	0.77 ± 0.05
Phase 1.5	DOY	49	4.73	264.03	± 0.48	***	252.62	278.26	1.85	2.06	0.45 ± 0.08	0.79 ± 0.04
Phase 1	DOY	47	4.74	269.63	± 0.43	***	257.40	277.56	1.42	1.88	0.36 ± 0.08	0.72 ± 0.06
Phase 0.5	DOY	45	4.49	276.66	± 0.37	***	265.01	280.64	1.03	1.58	0.28 ± 0.08	0.64 ± 0.07
Duration 2.5	DOY	49	4.73	5.22	± 0.12	NS	4.06	8.84	7.86	32.85	0.02 ± 0.05	0.10 ± 0.06
Duration 2	DOY	49	4.73	4.86	± 0.12	NS	3.46	8.07	7.27	37.60	0.05 ± 0.05	0.19 ± 0.07
Duration 1.5	DOY	47	4.74	6.09	± 0.21	***	3.28	11.53	24.91	45.74	0.22 ± 0.07	0.58 ± 0.07
Duration 1	DOY	45	4.49	7.99	± 0.23	NS	5.04	14.27	11.25	39.61	0.06 ± 0.06	0.22 ± 0.08
Dur.Sub.1	DOY	49	4.73	10.08	± 0.21	NS	7.55	14.23	5.47	31.06	0.03 ± 0.05	0.13 ± 0.06
Dur.Sub.2	DOY	45	4.49	14.10	± 0.35	***	9.82	23.20	18.64	30.53	0.24 ± 0.08	0.59 ± 0.07
Phase 2.5	CNL	49	4.73	707.14	± 5.14	***	599.27	855.01	6.88	8.75	0.38 ± 0.08	0.75 ± 0.05
Phase 2	CNL	49	4.73	766.77	± 5.38	***	647.12	919.53	6.92	8.25	0.41 ± 0.08	0.77 ± 0.05
Phase 1.5	CNL	49	4.73	823.61	± 5.58	***	692.26	994.84	6.95	7.74	0.45 ± 0.08	0.79 ± 0.04
Phase 1	CNL	47	4.74	893.76	± 4.97	***	744.52	985.09	4.99	6.53	0.34 ± 0.08	0.71 ± 0.06
Phase 0.5	CNL	45	4.49	982.00	± 3.16	***	911.71	1026.23	2.09	4.05	0.19 ± 0.07	0.52 ± 0.08
Duration 2.5	CNL	49	4.73	59.93	± 1.34	NS	44.90	105.15	9.69	32.80	0.06 ± 0.05	0.22 ± 0.07
Duration 2	CNL	49	4.73	56.58	± 1.45	NS	39.73	96.17	8.05	38.17	0.06 ± 0.05	0.24 ± 0.07
Duration 1.5	CNL	47	4.74	76.24	± 3.13	***	38.55	163.46	29.21	54.03	0.24 ± 0.07	0.59 ± 0.07
Duration 1	CNL	45	4.49	99.57	± 2.83	***	62.04	171.69	21.17	33.98	0.21 ± 0.07	0.55 ± 0.08
Dur.Sub.1	CNL	49	4.73	116.50	± 2.44	NS	84.13	166.73	7.00	31.15	0.06 ± 0.05	0.22 ± 0.07
Dur.Sub.2	CNL	45	4.49	175.52	± 4.18	***	119.77	269.65	18.72	28.28	0.29 ± 0.08	0.65 ± 0.07

Figure 42. Phenotypic and genetic variation for Paglia (42.48°N). Data are expressed as cumulative night length.

Adour		Metapopulation phenotypic variation							Metapopulation genetic variation				
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	± SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	DOY	52	5.62	255.57	± 0.49	***	233.76	270.14	2.53	2.04	0.61 ± 0.06	0.90 ± 0.02
Phase 2	DOY	52	5.62	261.21	± 0.49	***	238.27	276.87	2.53	1.99	0.62 ± 0.06	0.90 ± 0.02
Phase 1.5	DOY	52	5.54	266.06	± 0.47	***	243.76	279.68	2.34	1.94	0.58 ± 0.06	0.89 ± 0.02
Phase 1	DOY	50	5.50	271.58	± 0.42	***	250.07	279.61	1.92	1.72	0.53 ± 0.07	0.86 ± 0.03
Phase 0.5	DOY	44	4.80	277.29	± 0.34	***	266.39	281.24	1.00	1.43	0.34 ± 0.08	0.72 ± 0.06
Duration 2.5	DOY	52	5.62	5.64	± 0.14	NS	4.08	9.35	10.41	40.11	0.05 ± 0.04	0.22 ± 0.06
Duration 2	DOY	52	5.54	5.10	± 0.13	*	3.44	8.22	10.48	40.86	0.09 ± 0.05	0.34 ± 0.07
Duration 1.5	DOY	50	5.50	6.03	± 0.19	**	3.26	10.17	12.76	50.34	0.13 ± 0.05	0.44 ± 0.07
Duration 1	DOY	44	4.80	7.67	± 0.26	***	2.77	16.56	22.33	43.12	0.19 ± 0.07	0.53 ± 0.08
Dur.Sub.1	DOY	52	5.54	10.72	± 0.22	.	7.62	17.36	9.19	34.25	0.06 ± 0.05	0.25 ± 0.06
Dur.Sub.2	DOY	44	4.80	13.84	± 0.37	***	5.66	22.79	17.09	35.38	0.21 ± 0.07	0.56 ± 0.07
Phase 2.5	CNL	52	5.62	725.96	± 5.46	***	488.71	894.50	9.99	8.10	0.60 ± 0.06	0.90 ± 0.02
Phase 2	CNL	52	5.62	791.08	± 5.66	***	535.82	977.44	9.61	7.56	0.61 ± 0.06	0.90 ± 0.02
Phase 1.5	CNL	52	5.54	847.80	± 5.56	***	594.16	1012.43	8.61	7.18	0.58 ± 0.06	0.89 ± 0.02
Phase 1	CNL	50	5.50	915.82	± 4.93	***	664.38	1010.99	6.59	6.03	0.50 ± 0.07	0.84 ± 0.03
Phase 0.5	CNL	44	4.80	987.01	± 3.17	***	894.18	1028.75	2.81	3.69	0.35 ± 0.08	0.72 ± 0.06
Duration 2.5	CNL	52	5.62	65.17	± 1.59	.	46.78	106.03	11.26	40.07	0.06 ± 0.05	0.27 ± 0.06
Duration 2	CNL	52	5.54	59.75	± 1.49	.	40.79	95.62	10.04	41.17	0.07 ± 0.05	0.29 ± 0.07
Duration 1.5	CNL	50	5.50	74.07	± 2.55	**	40.07	131.36	16.40	54.67	0.12 ± 0.05	0.42 ± 0.07
Duration 1	CNL	44	4.80	94.43	± 3.11	***	34.24	231.39	27.26	38.73	0.29 ± 0.08	0.66 ± 0.06
Dur.Sub.1	CNL	52	5.54	124.50	± 2.60	NS	90.81	200.41	9.26	34.23	0.06 ± 0.05	0.25 ± 0.06
Dur.Sub.2	CNL	44	4.80	170.22	± 4.50	***	69.66	301.84	20.26	32.63	0.28 ± 0.08	0.65 ± 0.07

Figure 43. Phenotypic and genetic variation for Adour (43.10°N). Data are expressed as cumulative night length.

Durance		Metapopulation phenotypic variation							Metapopulation genetic variation				
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	± SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	DOY	9	5.22	252.73	± 1.05	***	244.17	262.91	2.16	2.07	0.52 ± 0.16	0.85 ± 0.07
Phase 2	DOY	9	5.22	257.74	± 1.04	***	248.51	270.79	2.32	1.86	0.61 ± 0.15	0.89 ± 0.06
Phase 1.5	DOY	9	5.22	262.06	± 1.00	***	252.56	274.42	2.17	1.75	0.64 ± 0.14	0.90 ± 0.05
Phase 1	DOY	9	5.22	267.81	± 0.90	***	257.86	277.96	1.90	1.49	0.64 ± 0.14	0.90 ± 0.05
Phase 0.5	DOY	8	5.50	275.77	± 0.69	***	269.72	279.93	1.09	1.28	0.43 ± 0.17	0.81 ± 0.09
Duration 2.5	DOY	9	5.22	4.97	± 0.27	.	3.98	7.99	16.57	34.01	0.15 ± 0.14	0.47 ± 0.16
Duration 2	DOY	9	5.22	4.33	± 0.18	NS	3.54	5.50	3.13	28.29	0.01 ± 0.10	0.06 ± 0.11
Duration 1.5	DOY	9	5.22	5.67	± 0.41	.	3.23	9.57	10.64	48.44	0.11 ± 0.13	0.39 ± 0.17
Duration 1	DOY	8	5.50	8.70	± 0.44	NS	7.45	11.69	8.22	32.33	0.03 ± 0.10	0.13 ± 0.14
Dur.Sub.1	DOY	9	5.22	9.32	± 0.39	NS	7.78	11.60	8.27	27.27	0.07 ± 0.12	0.28 ± 0.16
Dur.Sub.2	DOY	8	5.50	14.51	± 0.65	NS	12.66	18.01	2.64	29.63	0.01 ± 0.10	0.04 ± 0.11
Phase 2.5	CNL	9	5.22	693.50	± 11.79	***	598.77	809.34	8.85	8.41	0.53 ± 0.16	0.85 ± 0.07
Phase 2	CNL	9	5.22	750.68	± 11.93	***	645.90	903.35	9.23	7.25	0.62 ± 0.14	0.89 ± 0.05
Phase 1.5	CNL	9	5.22	800.48	± 11.62	***	690.91	948.15	8.39	6.62	0.70 ± 0.13	0.92 ± 0.04
Phase 1	CNL	9	5.22	870.67	± 10.61	***	749.71	992.77	7.05	5.27	0.70 ± 0.13	0.92 ± 0.04
Phase 0.5	CNL	8	5.50	965.07	± 8.51	***	891.44	1019.23	3.87	4.48	0.44 ± 0.17	0.81 ± 0.09
Duration 2.5	CNL	9	5.22	56.77	± 3.13	.	45.91	95.55	18.50	34.13	0.16 ± 0.14	0.50 ± 0.16
Duration 2	CNL	9	5.22	49.99	± 2.01	NS	43.98	61.89	2.30	27.44	0.02 ± 0.09	0.08 ± 0.09
Duration 1.5	CNL	9	5.22	69.52	± 6.20	NS	40.78	111.84	4.83	60.98	0.08 ± 0.09	0.31 ± 0.09
Duration 1	CNL	8	5.50	102.06	± 4.82	NS	89.06	137.91	10.22	29.78	0.06 ± 0.12	0.26 ± 0.16
Dur.Sub.1	CNL	9	5.22	106.95	± 4.42	NS	90.75	140.35	9.23	27.00	0.08 ± 0.12	0.31 ± 0.16
Dur.Sub.2	CNL	8	5.50	174.51	± 7.88	NS	148.54	220.09	3.56	29.77	0.00 ± 0.09	0.03 ± 0.09

Figure 44. Phenotypic and genetic variation for Durance (43.47°N). Data are expressed as cumulative night length.

Stura		Metapopulation phenotypic variation							Metapopulation genetic variation				
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	± SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	DOY	29	5.62	249.93	± 0.49	***	243.35	258.57	1.54	2.00	0.37 ± 0.09	0.77 ± 0.06
Phase 2	DOY	29	5.62	254.82	± 0.51	***	248.14	263.66	1.59	2.01	0.38 ± 0.09	0.78 ± 0.05
Phase 1.5	DOY	29	5.62	259.42	± 0.52	***	251.94	267.99	1.60	2.00	0.41 ± 0.09	0.79 ± 0.05
Phase 1	DOY	29	5.62	265.22	± 0.55	***	256.24	272.74	1.57	2.15	0.37 ± 0.09	0.77 ± 0.06
Phase 0.5	DOY	29	5.62	273.69	± 0.46	***	266.35	279.94	1.29	1.75	0.38 ± 0.09	0.77 ± 0.05
Duration 2.5	DOY	29	5.62	4.93	± 0.10	.	3.53	6.08	6.88	25.19	0.08 ± 0.06	0.33 ± 0.09
Duration 2	DOY	29	5.62	4.59	± 0.09	*	3.23	5.82	7.69	25.02	0.09 ± 0.07	0.37 ± 0.09
Duration 1.5	DOY	29	5.62	5.72	± 0.22	NS	4.08	9.78	12.71	47.07	0.06 ± 0.06	0.26 ± 0.08
Duration 1	DOY	29	5.62	8.50	± 0.28	NS	6.25	11.70	6.08	40.90	0.02 ± 0.05	0.11 ± 0.07
Dur.Sub.1	DOY	29	5.62	9.52	± 0.18	.	6.75	11.60	6.84	23.31	0.09 ± 0.07	0.35 ± 0.09
Dur.Sub.2	DOY	29	5.62	14.24	± 0.35	NS	11.69	19.61	7.03	30.22	0.03 ± 0.06	0.16 ± 0.08
Phase 2.5	CNL	29	5.62	662.15	± 5.43	***	590.34	758.38	6.43	8.33	0.37 ± 0.09	0.77 ± 0.06
Phase 2	CNL	29	5.62	717.16	± 5.74	***	642.54	818.11	6.38	8.04	0.39 ± 0.09	0.78 ± 0.05
Phase 1.5	CNL	29	5.62	769.54	± 5.93	***	684.80	869.49	6.23	7.68	0.41 ± 0.09	0.80 ± 0.05
Phase 1	CNL	29	5.62	838.14	± 6.45	***	732.21	927.14	5.84	7.94	0.40 ± 0.09	0.79 ± 0.05
Phase 0.5	CNL	29	5.62	945.23	± 4.69	***	876.65	1016.92	3.92	4.99	0.42 ± 0.09	0.80 ± 0.05
Duration 2.5	CNL	29	5.62	55.46	± 1.17	*	38.51	68.55	8.02	25.69	0.10 ± 0.07	0.38 ± 0.09
Duration 2	CNL	29	5.62	52.33	± 1.07	*	36.00	65.29	7.86	24.90	0.10 ± 0.07	0.39 ± 0.09
Duration 1.5	CNL	29	5.62	67.58	± 2.80	.	44.28	114.63	14.74	50.88	0.08 ± 0.06	0.32 ± 0.09
Duration 1	CNL	29	5.62	107.76	± 3.73	**	76.03	182.10	16.39	41.16	0.13 ± 0.07	0.46 ± 0.09
Dur.Sub.1	CNL	29	5.62	107.80	± 2.09	*	74.46	133.27	7.56	23.63	0.10 ± 0.07	0.39 ± 0.09
Dur.Sub.2	CNL	29	5.62	175.30	± 4.25	**	133.57	238.82	12.55	28.37	0.16 ± 0.07	0.51 ± 0.09

Figure 45. Phenotypic and genetic variation for Stura (44.19°N). Data are expressed as cumulative night length.

Ramieres		Metapopulation phenotypic variation							Metapopulation genetic variation				
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	± SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	DOY	100	5.16	254.18	± 0.30	***	244.24	273.12	1.80	2.02	0.45 ± 0.05	0.81 ± 0.03
Phase 2	DOY	100	5.16	259.53	± 0.30	***	249.41	278.35	1.68	2.02	0.41 ± 0.05	0.78 ± 0.03
Phase 1.5	DOY	100	5.14	264.19	± 0.29	***	253.67	279.97	1.47	1.98	0.36 ± 0.05	0.74 ± 0.03
Phase 1	DOY	99	5.16	269.98	± 0.26	***	258.53	277.01	1.20	1.84	0.30 ± 0.05	0.68 ± 0.04
Phase 0.5	DOY	97	4.84	277.02	± 0.18	***	268.82	281.24	0.61	1.23	0.20 ± 0.05	0.54 ± 0.05
Duration 2.5	DOY	100	5.16	5.41	± 0.10	***	3.18	9.52	12.43	38.56	0.13 ± 0.04	0.44 ± 0.05
Duration 2	DOY	100	5.14	4.76	± 0.07	***	2.92	8.20	10.34	31.54	0.15 ± 0.04	0.48 ± 0.05
Duration 1.5	DOY	99	5.16	5.82	± 0.13	***	3.26	11.73	15.07	47.49	0.13 ± 0.04	0.43 ± 0.05
Duration 1	DOY	97	4.84	7.81	± 0.14	***	4.60	13.10	17.50	34.61	0.19 ± 0.05	0.54 ± 0.05
Dur.Sub.1	DOY	100	5.14	10.17	± 0.14	***	6.06	15.52	11.19	30.07	0.16 ± 0.04	0.49 ± 0.05
Dur.Sub.2	DOY	97	4.84	13.65	± 0.21	***	8.71	20.95	16.24	28.50	0.24 ± 0.05	0.60 ± 0.05
Phase 2.5	CNL	100	5.16	709.60	± 3.39	***	599.51	930.82	7.29	8.07	0.45 ± 0.05	0.81 ± 0.03
Phase 2	CNL	100	5.16	771.04	± 3.45	***	656.50	995.51	6.55	7.80	0.43 ± 0.05	0.79 ± 0.03
Phase 1.5	CNL	100	5.14	825.26	± 3.35	***	703.77	1016.03	5.53	7.42	0.36 ± 0.05	0.74 ± 0.03
Phase 1	CNL	99	5.16	893.62	± 3.14	***	759.53	979.41	4.33	6.67	0.29 ± 0.05	0.68 ± 0.04
Phase 0.5	CNL	97	4.84	979.47	± 2.10	***	917.69	1028.36	1.99	4.20	0.18 ± 0.05	0.51 ± 0.05
Duration 2.5	CNL	100	5.16	62.12	± 1.10	***	38.15	104.80	11.77	38.60	0.11 ± 0.04	0.40 ± 0.05
Duration 2	CNL	100	5.14	55.36	± 0.81	***	34.78	95.38	9.51	31.82	0.13 ± 0.04	0.44 ± 0.05
Duration 1.5	CNL	99	5.16	68.77	± 1.52	***	37.96	138.10	14.08	47.91	0.10 ± 0.04	0.37 ± 0.05
Duration 1	CNL	97	4.84	95.34	± 1.68	***	56.80	158.02	17.25	34.01	0.19 ± 0.05	0.53 ± 0.05
Dur.Sub.1	CNL	100	5.14	117.48	± 1.66	***	72.52	177.35	10.43	30.23	0.14 ± 0.04	0.45 ± 0.05
Dur.Sub.2	CNL	97	4.84	164.02	± 2.42	***	108.54	250.48	15.24	28.06	0.22 ± 0.05	0.57 ± 0.05

Figure 46. Phenotypic and genetic variation for Ramieres (44.43°N). Data are expressed as cumulative night length.

Ticino		Metapopulation phenotypic variation							Metapopulation genetic variation								
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	±	SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind}	±	SE	H^2_{gen}	±	SE
Phase 2.5	DOY	78	5.51	255.44	±	0.32	***	240.75	269.34	1.88	1.87	0.50	±	0.05	0.85	±	0.02
Phase 2	DOY	78	5.51	260.54	±	0.31	***	244.07	273.30	1.75	1.75	0.49	±	0.05	0.84	±	0.03
Phase 1.5	DOY	78	5.51	265.03	±	0.30	***	247.27	276.48	1.67	1.69	0.48	±	0.05	0.84	±	0.03
Phase 1	DOY	78	5.51	270.21	±	0.28	***	253.84	278.77	1.43	1.57	0.45	±	0.05	0.82	±	0.03
Phase 0.5	DOY	75	5.43	277.04	±	0.22	***	262.91	280.72	1.07	1.23	0.42	±	0.06	0.80	±	0.03
Duration 2.5	DOY	78	5.51	5.11	±	0.10	***	3.24	8.40	13.04	37.14	0.16	±	0.05	0.52	±	0.05
Duration 2	DOY	78	5.51	4.46	±	0.07	***	2.90	7.02	12.54	30.78	0.18	±	0.05	0.55	±	0.05
Duration 1.5	DOY	78	5.51	5.19	±	0.12	***	2.72	9.56	16.29	43.89	0.16	±	0.05	0.50	±	0.05
Duration 1	DOY	75	5.43	7.36	±	0.14	***	3.71	13.87	18.47	33.61	0.21	±	0.05	0.59	±	0.05
Dur.Sub.1	DOY	78	5.51	9.57	±	0.16	***	6.11	14.57	12.64	31.15	0.17	±	0.05	0.52	±	0.05
Dur.Sub.2	DOY	75	5.43	12.51	±	0.21	***	6.49	23.75	17.87	29.05	0.25	±	0.05	0.64	±	0.05
Phase 2.5	CNL	78	5.51	723.71	±	3.65	***	562.99	885.40	7.48	7.42	0.50	±	0.05	0.85	±	0.02
Phase 2	CNL	78	5.51	782.56	±	3.55	***	599.00	933.99	6.68	6.69	0.49	±	0.05	0.84	±	0.03
Phase 1.5	CNL	78	5.51	835.01	±	3.52	***	633.92	972.79	6.16	6.25	0.48	±	0.05	0.83	±	0.03
Phase 1	CNL	78	5.51	897.38	±	3.25	***	707.56	1002.44	4.98	5.67	0.43	±	0.06	0.81	±	0.03
Phase 0.5	CNL	75	5.43	982.55	±	2.33	***	897.41	1023.91	2.88	3.82	0.35	±	0.06	0.75	±	0.04
Duration 2.5	CNL	78	5.51	58.97	±	1.09	***	35.34	94.51	12.24	36.30	0.16	±	0.05	0.51	±	0.05
Duration 2	CNL	78	5.51	52.22	±	0.84	***	34.18	82.67	12.31	30.90	0.18	±	0.05	0.55	±	0.05
Duration 1.5	CNL	78	5.51	62.44	±	1.56	***	33.91	128.87	17.65	48.85	0.13	±	0.04	0.45	±	0.05
Duration 1	CNL	75	5.43	91.63	±	1.90	***	45.05	208.16	23.19	34.85	0.27	±	0.05	0.67	±	0.05
Dur.Sub.1	CNL	78	5.51	111.12	±	1.77	***	69.83	167.09	12.04	30.82	0.16	±	0.05	0.52	±	0.05
Dur.Sub.2	CNL	75	5.43	153.44	±	2.61	***	77.39	280.07	20.55	27.55	0.32	±	0.06	0.72	±	0.04

Figure 47. Phenotypic and genetic variation for Ticino (45.14°N). Data are expressed as cumulative night length.

Dranse		Metapopulation phenotypic variation							Metapopulation genetic variation							
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	±	SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind}	±	SE	H^2_{gen}	±	SE
Phase 2.5	DOY	42	5.48	250.36	±	0.40	***	241.89	260.36	1.67	1.73	0.48	±	0.07	0.84	±	0.04
Phase 2	DOY	42	5.48	255.66	±	0.41	***	245.79	265.57	1.71	1.77	0.48	±	0.07	0.84	±	0.04
Phase 1.5	DOY	42	5.48	260.47	±	0.43	***	249.90	269.59	1.71	1.81	0.47	±	0.07	0.83	±	0.04
Phase 1	DOY	42	5.48	266.62	±	0.44	***	254.80	274.73	1.72	1.85	0.47	±	0.07	0.83	±	0.04
Phase 0.5	DOY	42	5.43	275.18	±	0.33	***	263.37	280.46	1.14	1.38	0.40	±	0.08	0.78	±	0.05
Duration 2.5	DOY	42	5.48	5.28	±	0.12	***	3.95	11.13	18.92	29.50	0.20	±	0.07	0.57	±	0.07
Duration 2	DOY	42	5.48	4.79	±	0.09	***	3.60	6.61	11.73	25.08	0.18	±	0.07	0.54	±	0.07
Duration 1.5	DOY	42	5.48	6.15	±	0.20	**	4.07	9.89	18.49	44.80	0.12	±	0.06	0.43	±	0.08
Duration 1	DOY	42	5.43	8.67	±	0.23	***	5.12	13.15	18.80	34.59	0.22	±	0.07	0.61	±	0.07
Dur.Sub.1	DOY	42	5.48	10.08	±	0.19	***	7.62	17.36	14.78	25.17	0.22	±	0.07	0.61	±	0.07
Dur.Sub.2	DOY	42	5.43	14.84	±	0.30	***	9.09	20.59	12.28	28.38	0.16	±	0.06	0.50	±	0.07
Phase 2.5	CNL	42	5.48	666.82	±	4.38	***	574.12	779.45	6.95	7.14	0.49	±	0.07	0.84	±	0.04
Phase 2	CNL	42	5.48	726.57	±	4.70	***	616.38	840.90	6.83	7.04	0.48	±	0.07	0.84	±	0.04
Phase 1.5	CNL	42	5.48	781.67	±	4.93	***	661.14	888.87	6.59	6.96	0.47	±	0.07	0.83	±	0.04
Phase 1	CNL	42	5.48	855.38	±	5.25	***	716.37	950.95	6.21	6.94	0.45	±	0.08	0.82	±	0.04
Phase 0.5	CNL	42	5.43	959.76	±	3.54	***	852.37	1023.80	3.43	4.40	0.38	±	0.08	0.77	±	0.05
Duration 2.5	CNL	42	5.48	59.49	±	1.37	***	42.82	125.19	19.21	29.57	0.21	±	0.07	0.59	±	0.07
Duration 2	CNL	42	5.48	54.99	±	1.02	***	40.52	74.32	11.91	25.52	0.18	±	0.07	0.55	±	0.07
Duration 1.5	CNL	42	5.48	73.60	±	2.62	**	49.48	136.92	19.40	50.45	0.12	±	0.06	0.43	±	0.08
Duration 1	CNL	42	5.43	105.57	±	2.83	***	60.59	189.67	20.94	34.77	0.24	±	0.07	0.64	±	0.06
Dur.Sub.1	CNL	42	5.48	114.64	±	2.24	***	85.25	198.62	15.09	25.53	0.23	±	0.07	0.62	±	0.07
Dur.Sub.2	CNL	42	5.43	179.66	±	3.60	***	107.26	247.69	13.79	27.06	0.21	±	0.07	0.58	±	0.07

Figure 48. Phenotypic and genetic variation for Dranse (46.23°N). Data are expressed as cumulative night length.

Val Allier		Metapopulation phenotypic variation							Metapopulation genetic variation				
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	± SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	DOY	39	5.13	250.50	± 0.58	***	238.09	270.24	2.66	1.98	0.64 ± 0.07	0.90 ± 0.02
Phase 2	DOY	39	5.13	255.68	± 0.60	***	241.25	276.98	2.70	2.01	0.64 ± 0.07	0.90 ± 0.02
Phase 1.5	DOY	39	5.08	260.23	± 0.59	***	244.57	279.98	2.56	2.01	0.62 ± 0.07	0.89 ± 0.03
Phase 1	DOY	38	5.13	266.15	± 0.58	***	249.89	277.94	2.18	2.12	0.51 ± 0.08	0.84 ± 0.04
Phase 0.5	DOY	36	5.00	273.80	± 0.49	***	262.39	280.42	1.52	1.83	0.41 ± 0.09	0.78 ± 0.05
Duration 2.5	DOY	39	5.13	5.19	± 0.14	***	3.02	9.22	16.86	34.51	0.24 ± 0.08	0.62 ± 0.07
Duration 2	DOY	39	5.08	4.80	± 0.11	.	3.30	6.43	6.60	32.18	0.07 ± 0.06	0.28 ± 0.08
Duration 1.5	DOY	38	5.13	6.16	± 0.23	*	3.65	12.29	18.02	48.27	0.11 ± 0.06	0.38 ± 0.08
Duration 1	DOY	36	5.00	8.68	± 0.28	**	5.00	13.18	16.85	39.45	0.17 ± 0.07	0.50 ± 0.08
Dur.Sub.1	DOY	39	5.08	9.95	± 0.23	**	6.32	15.36	12.21	29.77	0.16 ± 0.07	0.50 ± 0.08
Dur.Sub.2	DOY	36	5.00	14.94	± 0.38	***	9.75	22.01	15.16	30.66	0.19 ± 0.08	0.54 ± 0.08
Phase 2.5	CNL	39	5.13	669.01	± 6.45	***	534.39	895.01	11.15	8.19	0.65 ± 0.07	0.90 ± 0.02
Phase 2	CNL	39	5.13	727.58	± 6.87	***	568.13	977.69	10.88	8.01	0.65 ± 0.07	0.90 ± 0.02
Phase 1.5	CNL	39	5.08	779.54	± 6.79	***	603.62	1015.85	9.94	7.74	0.62 ± 0.07	0.89 ± 0.03
Phase 1	CNL	38	5.13	849.50	± 6.80	***	661.43	990.67	8.08	7.79	0.52 ± 0.08	0.85 ± 0.04
Phase 0.5	CNL	36	5.00	958.36	± 3.61	***	887.60	1021.05	2.83	4.18	0.31 ± 0.08	0.70 ± 0.07
Duration 2.5	CNL	39	5.13	58.59	± 1.63	***	31.86	105.03	18.05	34.90	0.27 ± 0.08	0.66 ± 0.07
Duration 2	CNL	39	5.08	55.02	± 1.31	*	35.00	79.72	8.22	32.50	0.10 ± 0.06	0.35 ± 0.08
Duration 1.5	CNL	38	5.13	72.84	± 2.83	*	41.36	156.93	21.22	49.85	0.11 ± 0.06	0.39 ± 0.08
Duration 1	CNL	36	5.00	121.17	± 4.25	***	60.00	226.33	29.45	36.74	0.38 ± 0.09	0.75 ± 0.06
Dur.Sub.1	CNL	39	5.08	113.14	± 2.65	***	66.89	177.58	13.30	30.10	0.19 ± 0.07	0.54 ± 0.08
Dur.Sub.2	CNL	36	5.00	194.71	± 4.64	***	115.62	293.22	19.14	25.59	0.36 ± 0.09	0.74 ± 0.06

Figure 49. Phenotypic and genetic variation for Val Allier (46.24°N). Data are expressed as cumulative night length.

Loire		Metapopulation phenotypic variation							Metapopulation genetic variation							
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	±	SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind}	±	SE	H^2_{gen}	±	SE
Phase 2.5	DOY	199	5.37	249.69	±	0.23	***	224.49	266.16	2.31	1.94	0.59	±	0.03	0.88	±	0.01
Phase 2	DOY	199	5.37	254.74	±	0.24	***	228.96	272.29	2.37	1.92	0.60	±	0.03	0.89	±	0.01
Phase 1.5	DOY	199	5.37	259.39	±	0.24	***	233.22	275.81	2.35	1.94	0.60	±	0.03	0.89	±	0.01
Phase 1	DOY	199	5.36	265.20	±	0.24	***	238.81	278.99	2.24	1.96	0.56	±	0.03	0.87	±	0.01
Phase 0.5	DOY	196	5.27	274.02	±	0.19	***	258.08	280.98	1.47	1.64	0.45	±	0.04	0.81	±	0.02
Duration 2.5	DOY	199	5.37	5.05	±	0.05	***	3.26	8.28	10.32	31.75	0.11	±	0.03	0.41	±	0.04
Duration 2	DOY	199	5.37	4.65	±	0.05	***	3.19	7.90	9.86	30.30	0.10	±	0.03	0.38	±	0.04
Duration 1.5	DOY	199	5.36	5.82	±	0.08	***	3.04	11.85	15.62	43.78	0.13	±	0.03	0.45	±	0.03
Duration 1	DOY	196	5.27	9.22	±	0.12	***	4.46	25.16	21.92	37.48	0.22	±	0.03	0.59	±	0.03
Dur.Sub.1	DOY	199	5.37	9.70	±	0.09	***	6.59	15.31	9.80	28.00	0.11	±	0.03	0.40	±	0.04
Dur.Sub.2	DOY	196	5.27	15.14	±	0.15	***	8.25	30.97	16.32	28.36	0.24	±	0.03	0.62	±	0.03
Phase 2.5	CNL	199	5.37	659.87	±	2.55	***	394.25	846.74	9.65	8.13	0.59	±	0.03	0.88	±	0.01
Phase 2	CNL	199	5.37	716.70	±	2.69	***	439.02	920.70	9.50	7.71	0.60	±	0.03	0.89	±	0.01
Phase 1.5	CNL	199	5.37	769.82	±	2.78	***	482.65	964.47	9.10	7.49	0.60	±	0.03	0.89	±	0.01
Phase 1	CNL	199	5.36	839.16	±	2.85	***	540.32	1004.15	8.34	7.36	0.56	±	0.03	0.87	±	0.01
Phase 0.5	CNL	196	5.27	950.97	±	1.84	***	820.70	1029.43	4.01	4.76	0.42	±	0.04	0.79	±	0.02
Duration 2.5	CNL	199	5.37	56.86	±	0.59	***	34.65	95.13	11.71	32.00	0.15	±	0.03	0.48	±	0.03
Duration 2	CNL	199	5.37	53.16	±	0.53	***	33.91	93.29	10.72	30.55	0.12	±	0.03	0.43	±	0.04
Duration 1.5	CNL	199	5.36	69.57	±	1.17	***	36.63	185.71	20.29	51.23	0.13	±	0.03	0.44	±	0.04
Duration 1	CNL	196	5.27	116.56	±	1.66	***	53.30	344.57	27.32	36.70	0.30	±	0.03	0.69	±	0.03
Dur.Sub.1	CNL	199	5.37	110.03	±	1.02	***	70.40	176.29	10.90	28.29	0.14	±	0.03	0.47	±	0.03
Dur.Sub.2	CNL	196	5.27	186.98	±	1.88	***	95.90	404.17	17.95	26.71	0.30	±	0.03	0.69	±	0.03

Figure 50. Phenotypic and genetic variation for Loire (47.30°N). Data are expressed as cumulative night length.

Rhin		Metapopulation phenotypic variation							Metapopulation genetic variation				
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	± SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	DOY	50	5.28	247.24	± 0.42	***	231.28	261.42	2.13	1.90	0.56 ± 0.07	0.87 ± 0.03
Phase 2	DOY	50	5.28	252.31	± 0.44	***	236.22	265.65	2.15	2.00	0.54 ± 0.07	0.86 ± 0.03
Phase 1.5	DOY	50	5.28	256.80	± 0.46	***	241.30	269.68	2.13	2.09	0.51 ± 0.07	0.84 ± 0.03
Phase 1	DOY	50	5.28	262.45	± 0.48	***	246.97	274.28	2.06	2.25	0.45 ± 0.07	0.81 ± 0.04
Phase 0.5	DOY	50	5.26	271.61	± 0.42	***	258.16	279.81	1.44	2.07	0.33 ± 0.07	0.72 ± 0.05
Duration 2.5	DOY	50	5.28	5.09	± 0.13	NS	3.58	7.61	4.09	41.78	0.04 ± 0.04	0.17 ± 0.06
Duration 2	DOY	50	5.28	4.52	± 0.08	.	3.32	6.29	7.31	27.90	0.06 ± 0.05	0.25 ± 0.07
Duration 1.5	DOY	50	5.28	5.62	± 0.16	*	3.25	8.90	9.10	46.26	0.07 ± 0.05	0.29 ± 0.07
Duration 1	DOY	50	5.26	9.30	± 0.27	***	5.08	18.62	21.67	42.71	0.17 ± 0.06	0.51 ± 0.07
Dur.Sub.1	DOY	50	5.28	9.60	± 0.19	NS	6.92	13.33	6.20	31.85	0.04 ± 0.04	0.16 ± 0.06
Dur.Sub.2	DOY	50	5.26	14.86	± 0.32	***	8.36	24.23	14.48	32.20	0.16 ± 0.06	0.50 ± 0.07
Phase 2.5	CNL	50	5.28	632.62	± 4.59	***	463.08	790.42	9.05	8.15	0.55 ± 0.07	0.87 ± 0.03
Phase 2	CNL	50	5.28	689.12	± 4.96	***	512.29	841.41	8.79	8.21	0.53 ± 0.07	0.86 ± 0.03
Phase 1.5	CNL	50	5.28	739.98	± 5.24	***	565.85	889.84	8.38	8.28	0.51 ± 0.07	0.84 ± 0.03
Phase 1	CNL	50	5.28	805.92	± 5.64	***	629.05	943.18	7.79	8.59	0.45 ± 0.07	0.81 ± 0.04
Phase 0.5	CNL	50	5.26	932.44	± 3.39	***	852.83	1016.29	3.09	5.07	0.30 ± 0.07	0.69 ± 0.05
Duration 2.5	CNL	50	5.28	56.62	± 1.48	.	38.85	84.15	6.14	42.16	0.07 ± 0.05	0.27 ± 0.07
Duration 2	CNL	50	5.28	51.18	± 0.92	*	36.86	74.13	7.95	28.23	0.07 ± 0.05	0.27 ± 0.07
Duration 1.5	CNL	50	5.28	65.57	± 2.18	*	38.18	131.99	14.92	52.02	0.08 ± 0.05	0.31 ± 0.07
Duration 1	CNL	50	5.26	128.33	± 3.89	***	63.26	230.82	23.83	43.27	0.22 ± 0.06	0.59 ± 0.06
Dur.Sub.1	CNL	50	5.28	107.76	± 2.20	.	75.98	153.10	7.28	32.38	0.06 ± 0.05	0.24 ± 0.07
Dur.Sub.2	CNL	50	5.26	193.04	± 3.96	***	103.56	292.89	16.73	29.02	0.25 ± 0.07	0.63 ± 0.06

Figure 51. Phenotypic and genetic variation for Rhin (48.24°N). Data are expressed as cumulative night length.

Kuhkopf		Metapopulation phenotypic variation							Metapopulation genetic variation				
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	± SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	DOY	46	5.54	242.66	± 0.40	***	225.23	256.26	2.03	1.73	0.58 ± 0.07	0.88 ± 0.03
Phase 2	DOY	46	5.54	247.49	± 0.42	***	229.08	260.91	2.10	1.72	0.60 ± 0.06	0.89 ± 0.02
Phase 1.5	DOY	46	5.54	251.94	± 0.43	***	232.73	265.02	2.15	1.73	0.62 ± 0.06	0.90 ± 0.02
Phase 1	DOY	46	5.54	257.51	± 0.48	***	237.81	269.78	2.26	2.04	0.56 ± 0.07	0.88 ± 0.03
Phase 0.5	DOY	46	5.54	267.70	± 0.51	***	252.07	277.12	2.00	2.32	0.42 ± 0.07	0.80 ± 0.04
Duration 2.5	DOY	46	5.54	4.80	± 0.12	*	2.60	7.63	9.98	39.45	0.11 ± 0.05	0.40 ± 0.07
Duration 2	DOY	46	5.54	4.42	± 0.10	*	2.81	7.51	11.25	34.88	0.10 ± 0.05	0.39 ± 0.07
Duration 1.5	DOY	46	5.54	5.52	± 0.16	NS	4.07	9.05	7.54	45.90	0.00 ± 0.04	0.01 ± 0.04
Duration 1	DOY	46	5.54	10.25	± 0.30	*	5.92	16.40	14.24	44.59	0.07 ± 0.05	0.29 ± 0.07
Dur.Sub.1	DOY	46	5.54	9.21	± 0.19	**	5.44	12.39	9.79	32.05	0.11 ± 0.06	0.41 ± 0.07
Dur.Sub.2	DOY	46	5.54	15.78	± 0.34	.	10.88	23.34	8.52	33.78	0.06 ± 0.05	0.26 ± 0.07
Phase 2.5	CNL	46	5.54	582.95	± 4.32	***	400.82	731.60	9.03	7.82	0.57 ± 0.07	0.88 ± 0.03
Phase 2	CNL	46	5.54	635.56	± 4.56	***	439.49	786.49	8.91	7.41	0.60 ± 0.06	0.89 ± 0.02
Phase 1.5	CNL	46	5.54	685.04	± 4.82	***	476.88	834.41	8.79	7.18	0.63 ± 0.06	0.90 ± 0.02
Phase 1	CNL	46	5.54	749.95	± 5.72	***	530.14	890.46	8.99	8.41	0.56 ± 0.07	0.88 ± 0.03
Phase 0.5	CNL	46	5.54	902.32	± 4.56	***	704.87	995.04	4.88	6.45	0.34 ± 0.07	0.74 ± 0.05
Duration 2.5	CNL	46	5.54	52.36	± 1.35	**	27.00	84.05	10.75	39.86	0.13 ± 0.06	0.45 ± 0.07
Duration 2	CNL	46	5.54	49.02	± 1.13	**	29.04	82.30	12.00	34.65	0.14 ± 0.06	0.47 ± 0.07
Duration 1.5	CNL	46	5.54	64.41	± 2.38	NS	44.41	129.06	16.10	56.89	0.03 ± 0.04	0.13 ± 0.06
Duration 1	CNL	46	5.54	153.18	± 4.23	**	74.74	239.84	17.05	40.80	0.13 ± 0.06	0.45 ± 0.07
Dur.Sub.1	CNL	46	5.54	101.36	± 2.16	**	56.35	138.36	10.64	32.34	0.14 ± 0.06	0.47 ± 0.07
Dur.Sub.2	CNL	46	5.54	217.41	± 3.98	*	138.52	295.21	9.28	27.73	0.10 ± 0.05	0.38 ± 0.07

Figure 52. Phenotypic and genetic variation for Kuhkopf (49.49°N). Data are expressed as cumulative night length.

Nederlands		Metapopulation phenotypic variation							Metapopulation genetic variation				
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	± SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind} ± SE	H^2_{gen} ± SE
Phase 2.5	DOY	42	5.71	240.62	± 0.47	***	223.77	252.49	2.36	1.93	0.60 ± 0.07	0.89 ± 0.02
Phase 2	DOY	42	5.71	245.36	± 0.48	***	228.61	258.05	2.38	1.85	0.62 ± 0.06	0.90 ± 0.02
Phase 1.5	DOY	42	5.71	249.55	± 0.49	***	232.73	263.34	2.43	1.87	0.63 ± 0.06	0.91 ± 0.02
Phase 1	DOY	42	5.71	254.38	± 0.54	***	238.35	270.57	2.58	2.04	0.62 ± 0.06	0.90 ± 0.02
Phase 0.5	DOY	42	5.71	262.70	± 0.57	***	249.02	279.03	2.46	2.30	0.53 ± 0.07	0.87 ± 0.03
Duration 2.5	DOY	42	5.71	4.75	± 0.14	NS	3.13	6.84	10.07	45.48	0.05 ± 0.05	0.22 ± 0.07
Duration 2	DOY	42	5.71	4.18	± 0.08	NS	3.09	5.62	6.91	30.06	0.05 ± 0.05	0.23 ± 0.07
Duration 1.5	DOY	42	5.71	4.84	± 0.13	NS	3.40	7.48	9.26	41.02	0.01 ± 0.04	0.07 ± 0.05
Duration 1	DOY	42	5.71	8.36	± 0.27	***	4.46	15.63	22.42	45.16	0.17 ± 0.06	0.54 ± 0.07
Dur.Sub.1	DOY	42	5.71	8.94	± 0.20	NS	6.16	11.55	2.79	35.28	0.04 ± 0.05	0.19 ± 0.06
Dur.Sub.2	DOY	42	5.71	13.20	± 0.31	***	7.65	20.09	15.72	32.73	0.18 ± 0.06	0.55 ± 0.07
Phase 2.5	CNL	42	5.71	561.28	± 5.05	***	385.66	690.02	10.75	8.87	0.59 ± 0.07	0.89 ± 0.02
Phase 2	CNL	42	5.71	612.54	± 5.20	***	434.12	753.21	10.36	8.08	0.62 ± 0.06	0.90 ± 0.02
Phase 1.5	CNL	42	5.71	658.68	± 5.47	***	476.17	814.67	10.19	7.86	0.63 ± 0.06	0.91 ± 0.02
Phase 1	CNL	42	5.71	712.31	± 6.12	***	536.56	899.05	10.43	8.29	0.61 ± 0.06	0.90 ± 0.02
Phase 0.5	CNL	42	5.71	855.07	± 6.56	***	673.49	1006.51	7.62	9.11	0.44 ± 0.07	0.82 ± 0.04
Duration 2.5	CNL	42	5.71	51.31	± 1.50	*	32.89	74.87	12.75	45.38	0.07 ± 0.05	0.31 ± 0.07
Duration 2	CNL	42	5.71	46.01	± 0.94	*	32.60	61.21	9.11	30.31	0.08 ± 0.05	0.34 ± 0.07
Duration 1.5	CNL	42	5.71	53.83	± 1.59	NS	36.95	86.77	12.15	44.02	0.03 ± 0.05	0.14 ± 0.06
Duration 1	CNL	42	5.71	143.10	± 4.93	NS	75.94	203.84	7.67	52.88	0.04 ± 0.05	0.20 ± 0.07
Dur.Sub.1	CNL	42	5.71	97.45	± 2.25	.	64.81	126.38	6.12	35.32	0.07 ± 0.05	0.30 ± 0.07
Dur.Sub.2	CNL	42	5.71	197.07	± 4.61	NS	136.16	249.05	3.09	36.14	0.02 ± 0.04	0.11 ± 0.06

Figure 53. Phenotypic and genetic variation for Netherlands (52.01°N). Data are expressed as cumulative night length.

Between		Metapopulation phenotypic variation							Metapopulation genetic variation							
Trait	Climatic parameter	N° genotype analysed	Mean no. replicates	General mean	±	SE	P	Min. genotype mean	Max. genotype mean	CV_G (%)	CV_R (%)	H^2_{ind}	±	SE	H^2_{gen}	± SE
Phase 2.5	DOY	796	5.31	251.04	±	0.13	***	223.77	273.12	2.15	1.96	0.54	±	0.02	0.86	± 0.01
Phase 2	DOY	796	5.31	256.21	±	0.13	***	228.61	278.35	2.15	1.94	0.55	±	0.02	0.87	± 0.01
Phase 1.5	DOY	796	5.29	260.79	±	0.13	***	232.73	279.98	2.08	1.92	0.54	±	0.02	0.86	± 0.01
Phase 1	DOY	785	5.29	266.32	±	0.13	***	237.81	280.53	1.89	1.91	0.48	±	0.02	0.83	± 0.01
Phase 0.5	DOY	750	5.15	273.96	±	0.11	***	249.02	281.46	1.36	1.66	0.40	±	0.02	0.77	± 0.01
Duration 2.5	DOY	796	5.31	5.18	±	0.03	***	2.60	11.13	11.23	36.33	0.11	±	0.01	0.41	± 0.02
Duration 2	DOY	796	5.29	4.67	±	0.02	***	2.81	8.22	9.98	32.48	0.11	±	0.01	0.39	± 0.02
Duration 1.5	DOY	785	5.29	5.72	±	0.04	***	2.59	12.29	15.62	45.93	0.11	±	0.01	0.40	± 0.02
Duration 1	DOY	750	5.15	8.56	±	0.06	***	2.77	25.16	19.05	39.19	0.16	±	0.02	0.50	± 0.02
Dur.Sub.1	DOY	796	5.29	9.84	±	0.05	***	5.44	17.36	10.09	30.28	0.11	±	0.01	0.40	± 0.02
Dur.Sub.2	DOY	750	5.15	14.32	±	0.08	***	5.66	30.97	15.29	30.42	0.19	±	0.02	0.55	± 0.02
Phase 2.5	CNL	796	5.31	675.12	±	1.45	***	385.66	930.82	8.86	8.11	0.55	±	0.02	0.86	± 0.01
Phase 2	CNL	796	5.31	733.65	±	1.52	***	434.12	995.51	8.52	7.68	0.55	±	0.02	0.87	± 0.01
Phase 1.5	CNL	796	5.29	786.29	±	1.55	***	476.17	1016.03	7.95	7.34	0.54	±	0.02	0.86	± 0.01
Phase 1	CNL	785	5.29	852.28	±	1.56	***	530.14	1022.62	6.92	7.07	0.48	±	0.02	0.83	± 0.01
Phase 0.5	CNL	750	5.15	953.42	±	1.08	***	673.49	1031.59	3.62	4.90	0.35	±	0.02	0.73	± 0.01
Duration 2.5	CNL	796	5.31	58.66	±	0.35	***	27.00	125.19	11.88	36.36	0.13	±	0.01	0.44	± 0.02
Duration 2	CNL	796	5.29	53.71	±	0.29	***	29.04	96.17	10.23	32.76	0.11	±	0.01	0.41	± 0.02
Duration 1.5	CNL	785	5.29	68.26	±	0.58	***	32.16	185.71	18.58	51.35	0.10	±	0.01	0.37	± 0.02
Duration 1	CNL	750	5.15	112.10	±	0.87	***	34.24	344.57	22.39	39.95	0.21	±	0.02	0.58	± 0.02
Dur.Sub.1	CNL	796	5.29	112.25	±	0.57	***	56.35	200.41	10.49	30.42	0.13	±	0.01	0.44	± 0.02
Dur.Sub.2	CNL	750	5.15	180.53	±	0.99	***	69.66	404.17	16.20	28.76	0.23	±	0.02	0.61	± 0.02

Figure 54. Phenotypic and genetic variation for the *P. nigra* collection. Data are expressed as cumulative night length.

14. APPENDIX 2

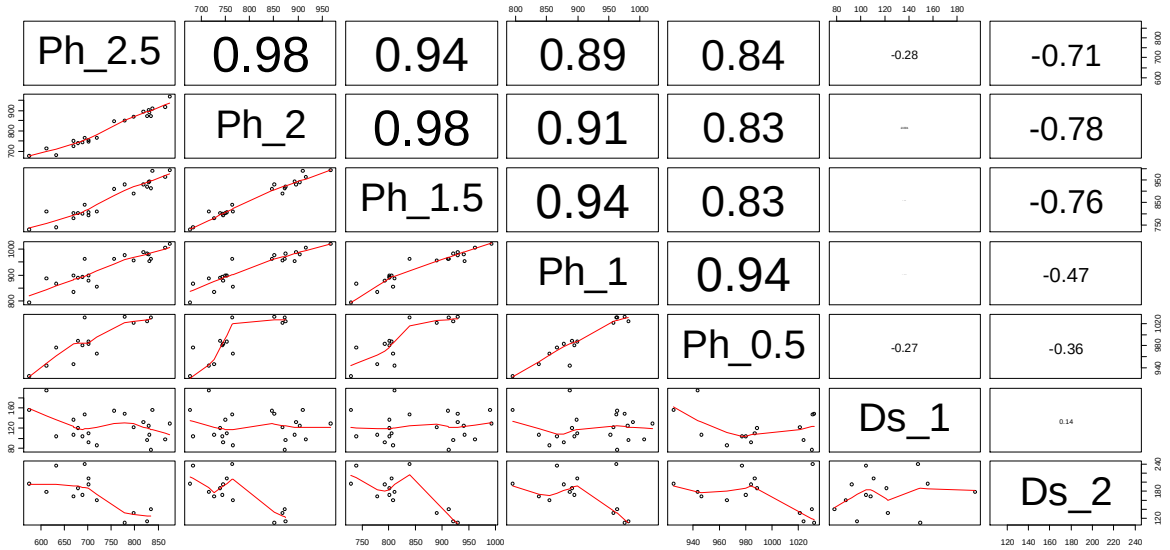


Figure 55. Pearson's phenotypic correlations between traits of bud set process for Basento based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

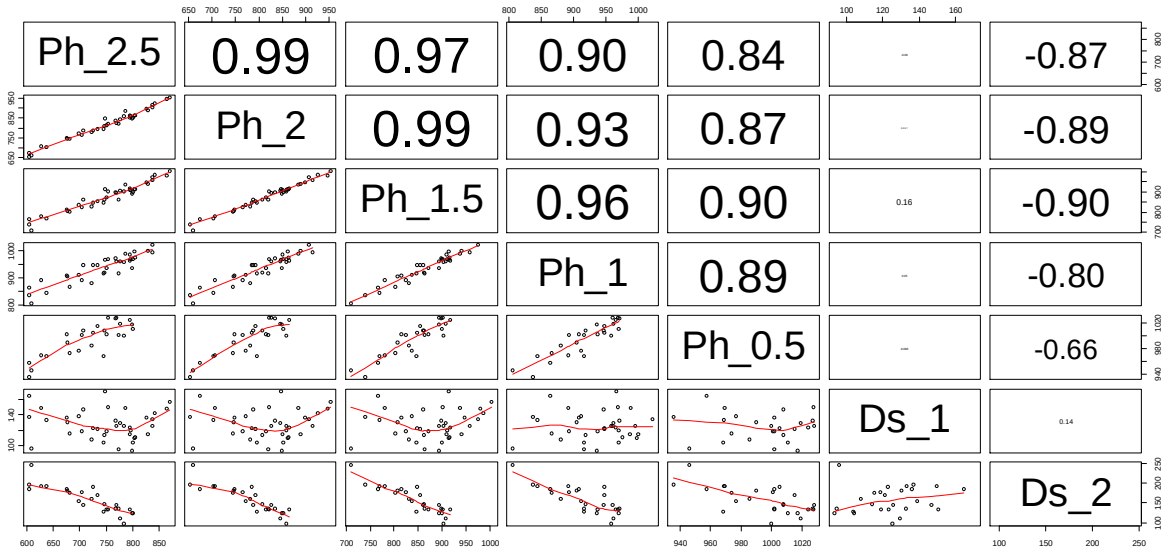


Figure 56. Pearson's phenotypic correlations between traits of bud set process for Nohede based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

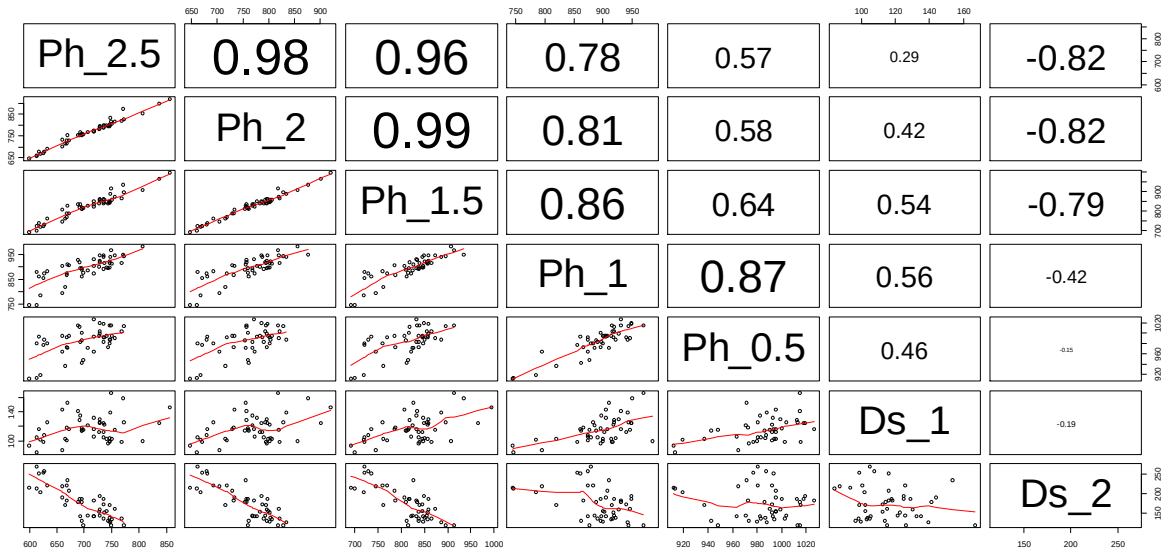


Figure 57. Pearson's phenotypic correlations between traits of bud set process for Paglia based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

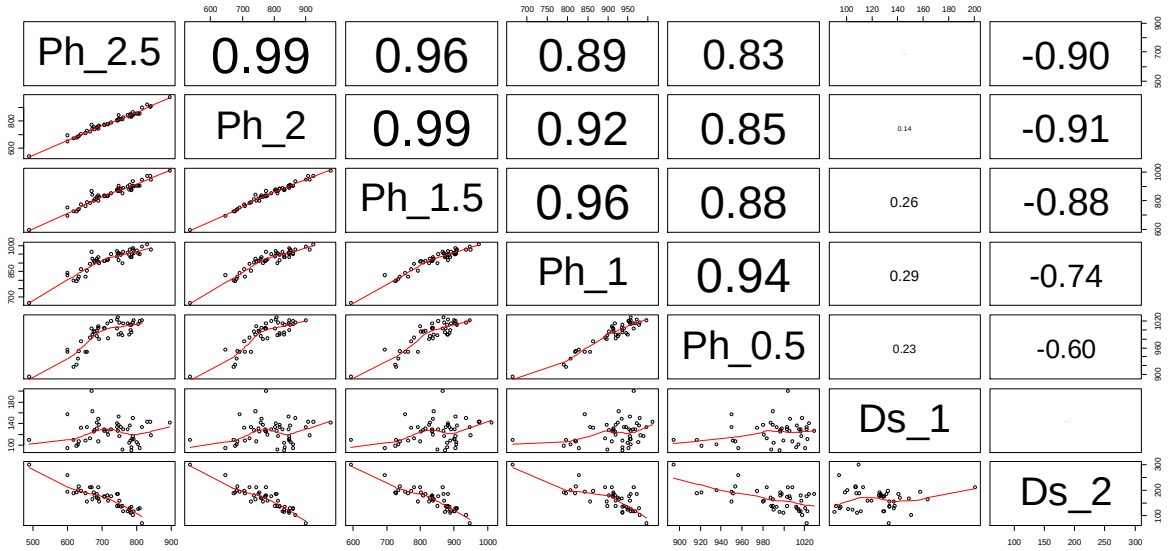


Figure 58. Pearson's phenotypic correlations between traits of bud set process for Adour based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

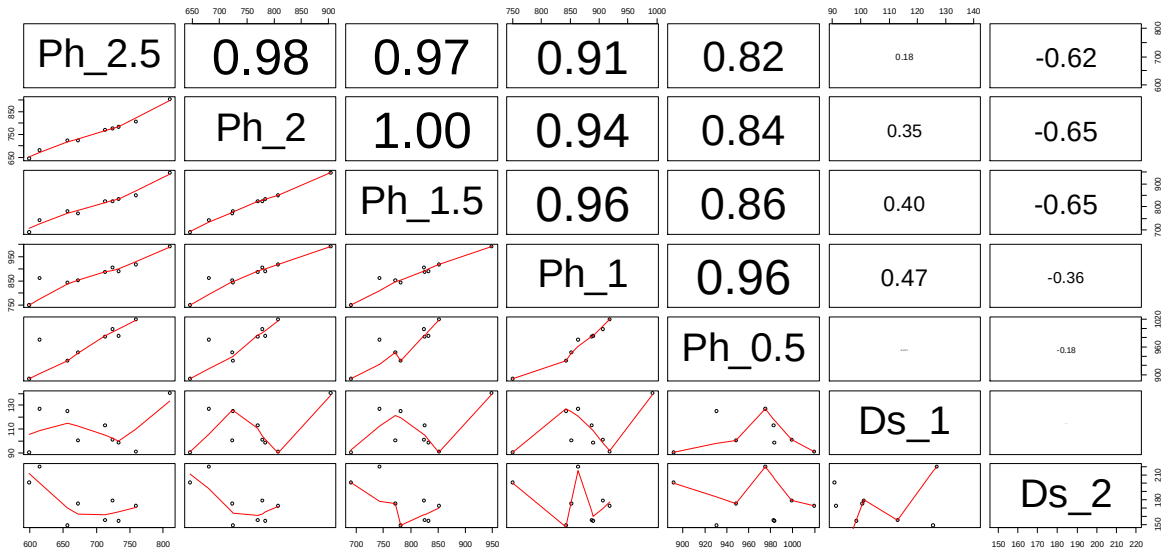


Figure 59. Pearson's phenotypic correlations between traits of bud set process for Durance based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

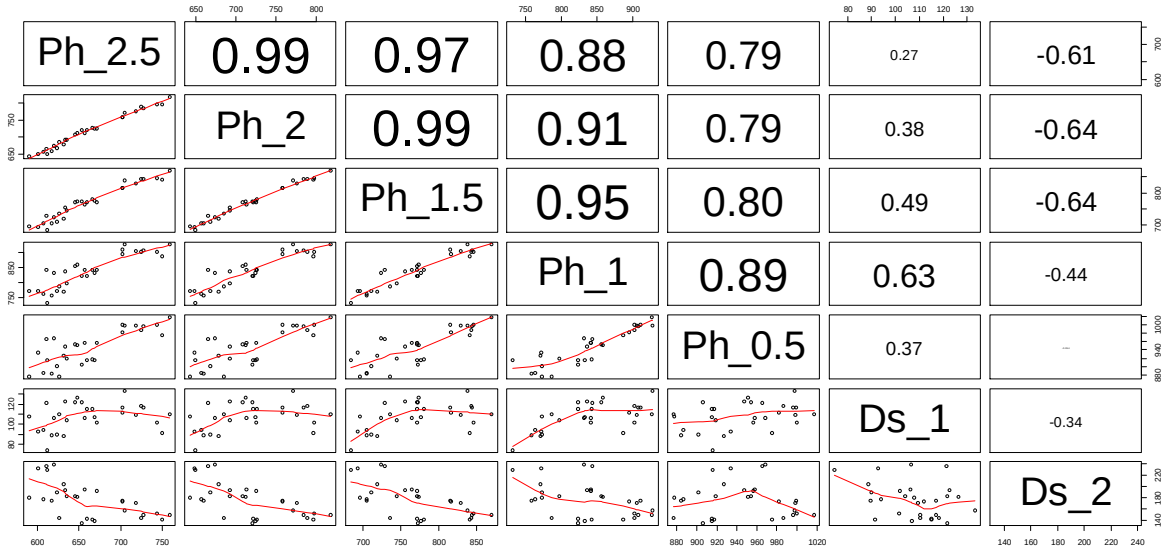


Figure 60. Pearson's phenotypic correlations between traits of bud set process for Stura based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

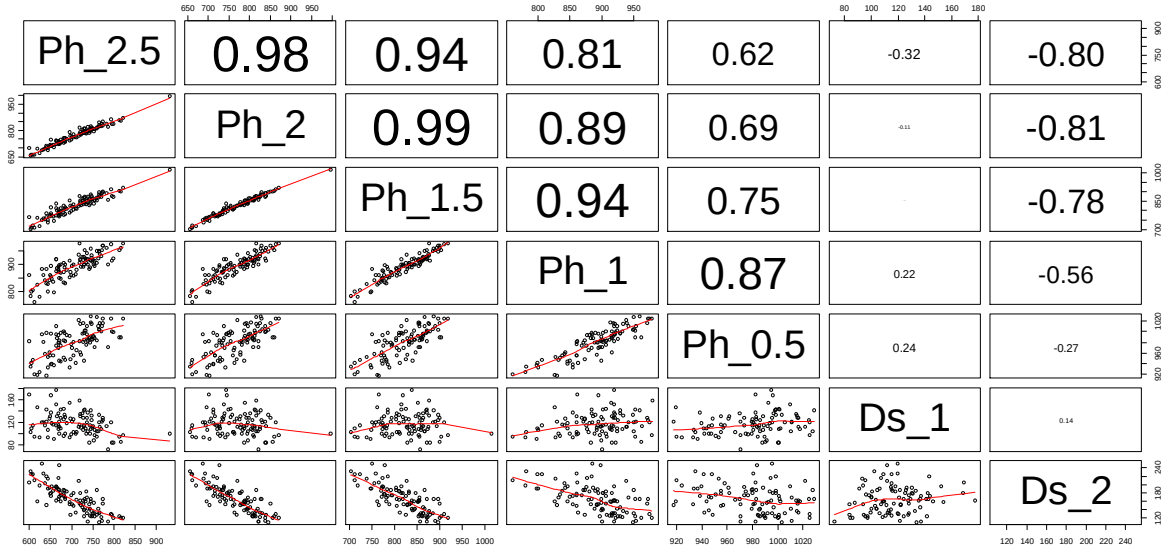


Figure 61. Pearson's phenotypic correlations between traits of bud set process for Ramieres based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

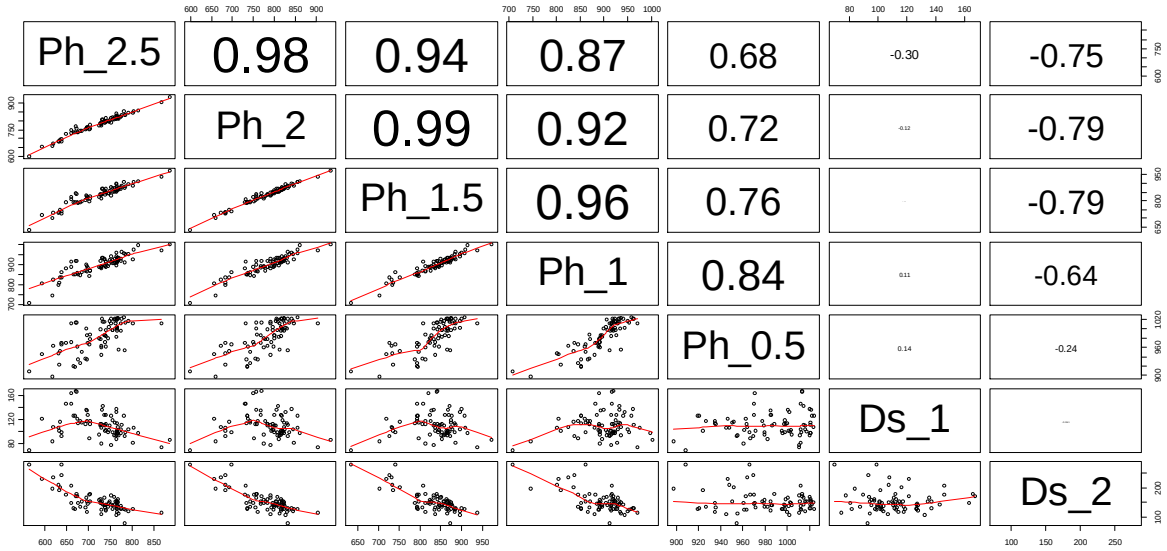


Figure 62. Pearson's phenotypic correlations between traits of bud set process for Ticino based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

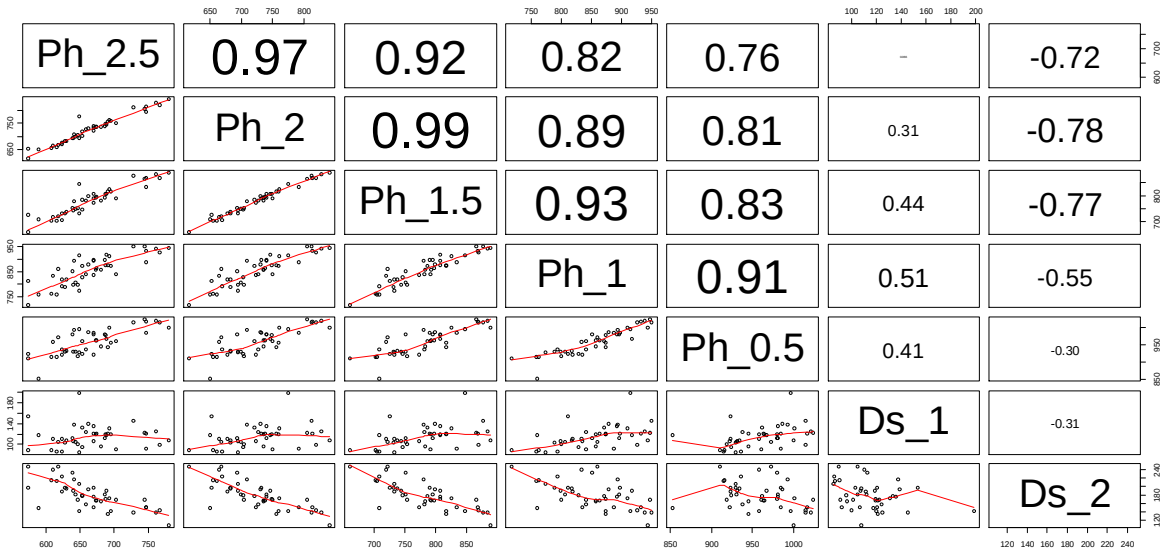


Figure 63. Pearson's phenotypic correlations between traits of bud set process for Drance based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

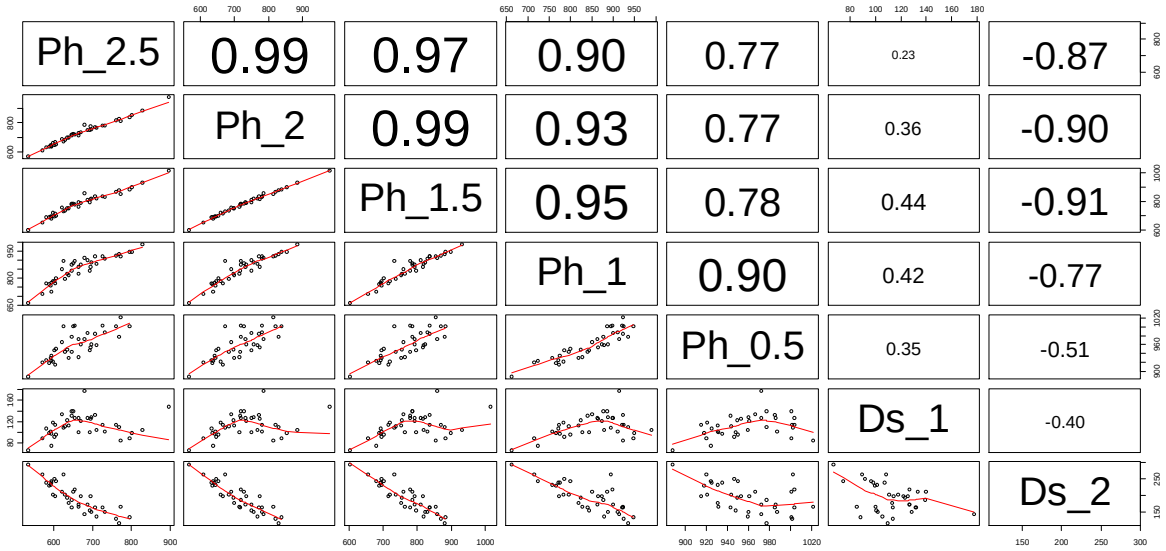


Figure 64. Pearson's phenotypic correlations between traits of bud set process for Val Allier based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

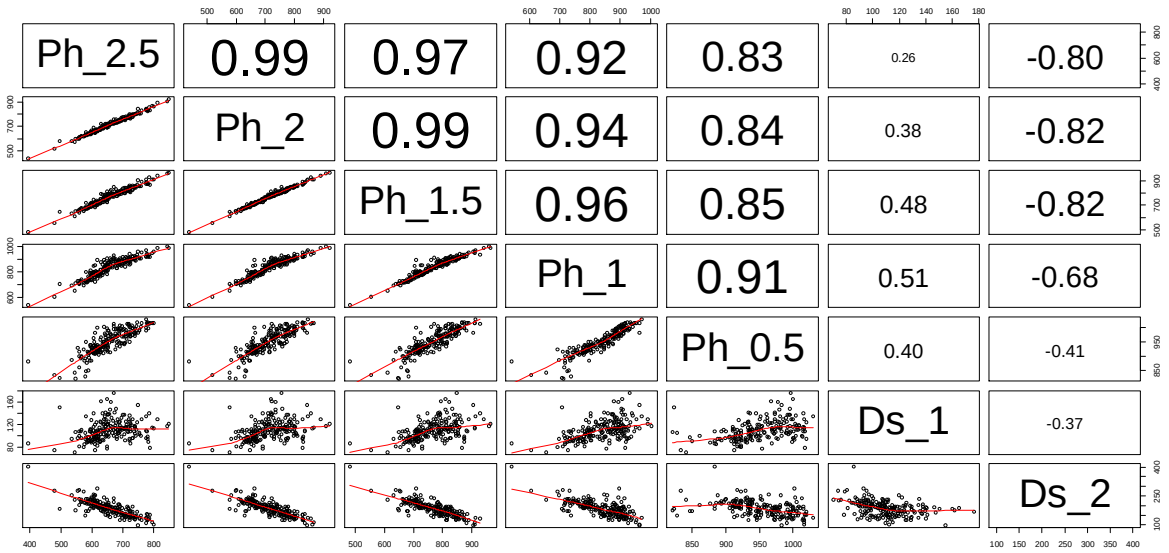


Figure 65. Pearson's phenotypic correlations between traits of bud set process for Loire based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

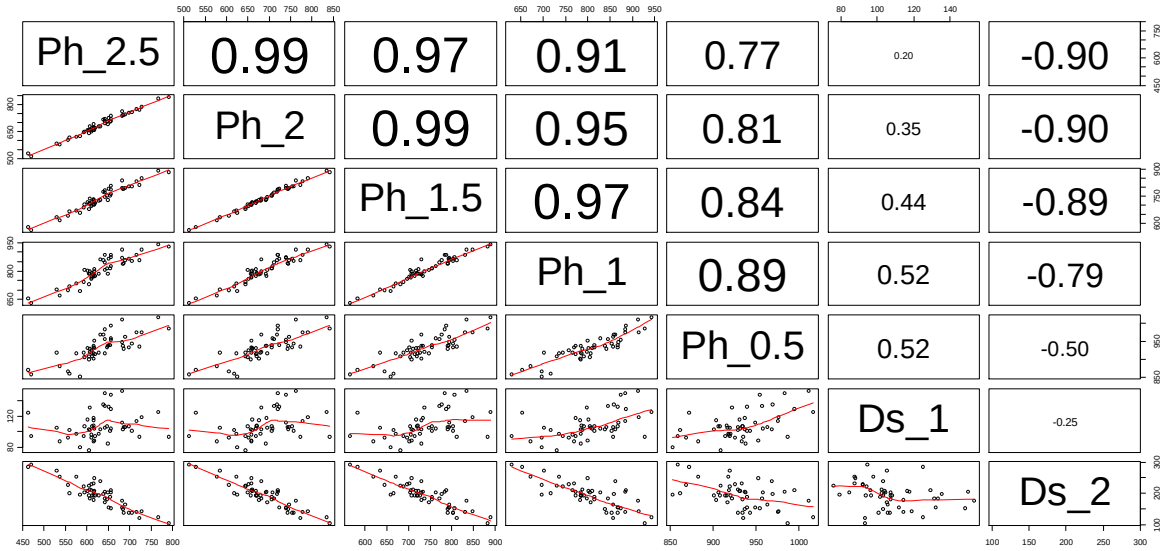


Figure 66. Pearson's phenotypic correlations between traits of bud set process for Rhin based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

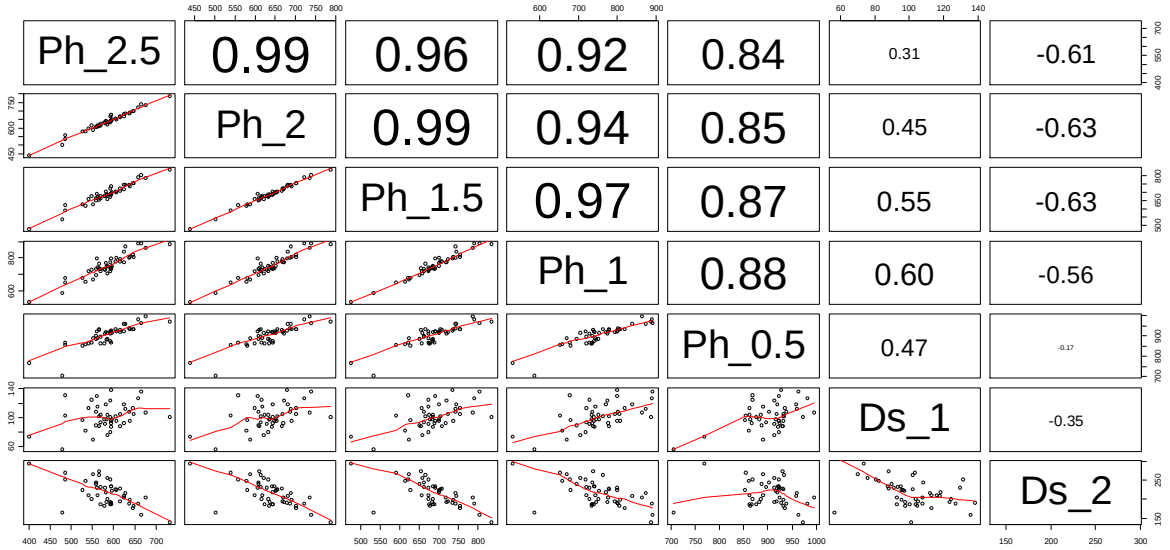


Figure 67. Pearson's phenotypic correlations between traits of bud set process for Kuhkopf based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

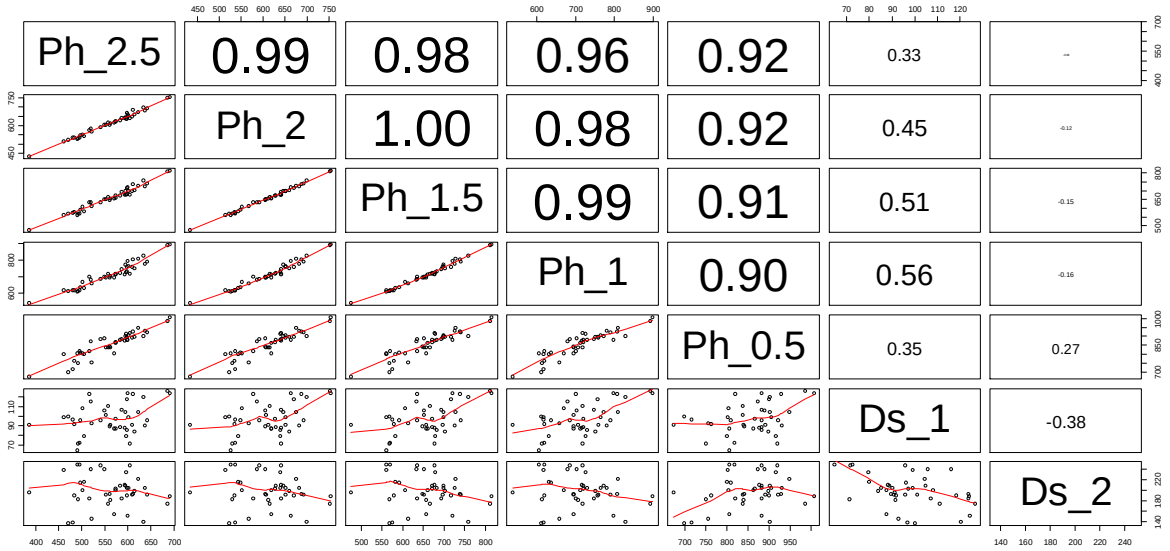


Figure 68. Pearson's phenotypic correlations between traits of bud set process for Netherlands based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

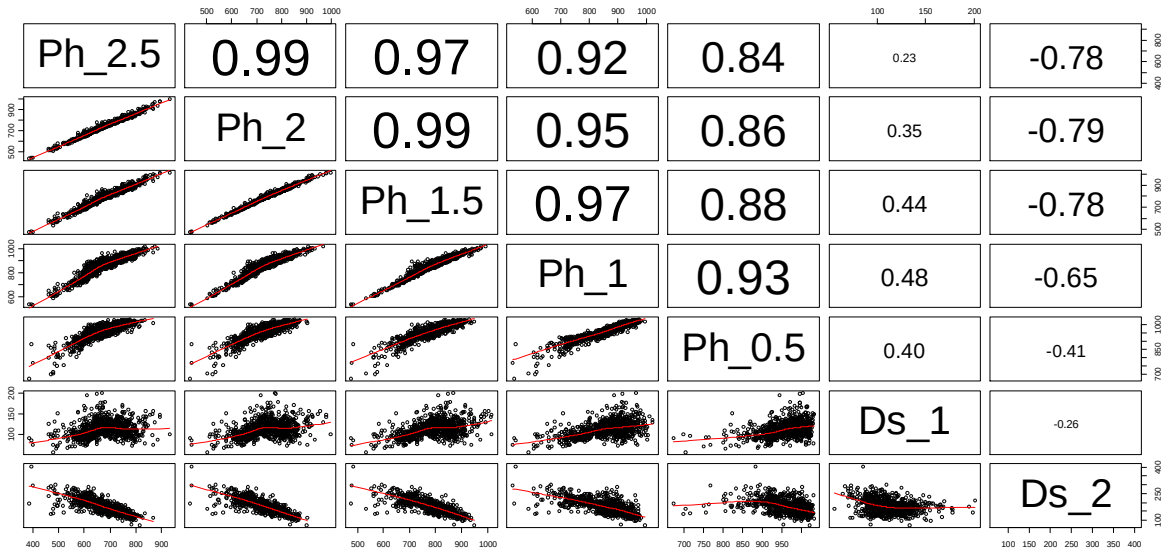


Figure 69. Pearson's phenotypic correlations between traits of bud set process for the *P. nigra* collection based on genotypic means (CNL data). Ph_x = phase X and Ds_x = duration of subprocess X.

15. APPENDIX 3

	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		0.99 *0.00*	0.90 *0.04*	0.84 *0.06*	-0.17 *0.26*	0.23 *0.20*	-0.87 *0.07*
Phase 2	0.93 *0.03*		0.90 *0.04*	0.83 *0.07*	-0.82 *0.09*	0.09 *0.21*	-0.79 *0.10*
Phase 1.5	0.78 *0.08*	0.86 *0.06*		0.82 *0.07*	-1.46 *0.30*	-0.04 *0.21*	-0.91 *0.04*
Phase 1	0.57 *0.15*	0.64 *0.13*	0.83 *0.07*		-1.43 *0.28*	0.12 *0.21*	-0.94 *0.03*
Phase 0.5	0.02 *0.27*	-0.10 *0.26*	-0.31 *0.24*	-0.31 *0.24*		-0.49 *0.20*	-1.28 *0.17*
dur.subproc. 1	0.28 *0.20*	-0.03 *0.21*	-0.22 *0.20*	-0.41 *0.18*	0.14 *0.26*		-0.53 *0.19*
dur.subproc. 2	-0.52 *0.20*	-0.69 *0.14*	-0.66 *0.15*	-0.22 *0.25*	0.29 *0.24*	0.19 *0.26*	

Figure 70. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Basento.

	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		1.00 *0.00*	0.90 *0.03*	0.78 *0.07*	NA *NA*	-0.37 *0.14*	-1.00 *0.00*
Phase 2	0.92 *0.02*		0.86 *0.04*	0.72 *0.08*	NA *NA*	-0.46 *0.13*	-1.01 *0.00*
Phase 1.5	0.75 *0.07*	0.87 *0.04*		0.73 *0.08*	NA *NA*	-0.37 *0.14*	-1.01 *0.00*
Phase 1	0.51 *0.12*	0.61 *0.11*	0.78 *0.07*		NA *NA*	-0.06 *0.17*	-1.06 *0.02*
Phase 0.5	-0.08 *0.19*	0.13 *0.19*	0.49 *0.15*	-0.52 *0.14*		NA *NA*	NA *NA*
dur.subproc. 1	0.38 *0.14*	0.06 *0.16*	-0.24 *0.15*	-0.29 *0.16*	0.62 *0.12*		-0.49 *0.15*
dur.subproc. 2	-0.58 *0.13*	-0.73 *0.09*	-0.73 *0.09*	-0.41 *0.16*	0.18 *0.19*	0.23 *0.19*	

Figure 71. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Nohede.

	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		1.00 *0.00*	1.00 *0.00*	0.70 *0.07*	-0.39 *0.13*	-0.92 *0.02*	-0.86 *0.04*
Phase 2	0.95 *0.01*		1.00 *0.00*	0.69 *0.08*	-0.47 *0.12*	-0.94 *0.02*	-0.83 *0.05*
Phase 1.5	0.83 *0.04*	0.94 *0.02*		0.71 *0.07*	-0.53 *0.11*	-0.96 *0.01*	-0.71 *0.07*
Phase 1	0.57 *0.10*	0.66 *0.08*	0.72 *0.07*		-1.20 *0.07*	-0.97 *0.01*	-0.58 *0.10*
Phase 0.5	0.33 *0.13*	0.24 *0.14*	0.22 *0.14*	-0.13 *0.15*		-1.73 *0.30*	-0.44 *0.12*
dur.subproc. 1	0.22 *0.14*	-0.04 *0.14*	-0.31 *0.13*	-0.33 *0.13*	-0.23 *0.14*		-0.02 *0.15*
dur.subproc. 2	-0.53 *0.11*	-0.61 *0.09*	-0.63 *0.09*	-0.25 *0.14*	0.21 *0.14*	0.23 *0.14*	

Figure 72. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Paglia.

	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		0.99 *0.00*	0.95 *0.01*	0.82 *0.05*	0.23 *0.14*	0.19 *0.13*	-0.99 *0.00*
Phase 2	0.90 *0.03*		0.90 *0.03*	0.77 *0.06*	-0.67 *0.08*	0.26 *0.13*	-0.98 *0.01*
Phase 1.5	0.74 *0.06*	0.89 *0.03*		0.83 *0.04*	-0.77 *0.06*	0.33 *0.12*	-0.96 *0.01*
Phase 1	0.51 *0.10*	0.63 *0.08*	0.75 *0.06*		-0.98 *0.01*	0.75 *0.06*	-0.95 *0.01*
Phase 0.5	0.17 *0.15*	-0.12 *0.15*	0.00 *0.15*	-0.63 *0.09*		-0.15 *0.15*	-0.99 *0.00*
dur.subproc. 1	-0.26 *0.13*	0.09 *0.14*	0.39 *0.12*	0.25 *0.13*	-0.44 *0.12*		-0.65 *0.09*
dur.subproc. 2	-0.54 *0.11*	-0.70 *0.08*	-0.76 *0.06*	-0.36 *0.13*	0.24 *0.14*	0.32 *0.14*	

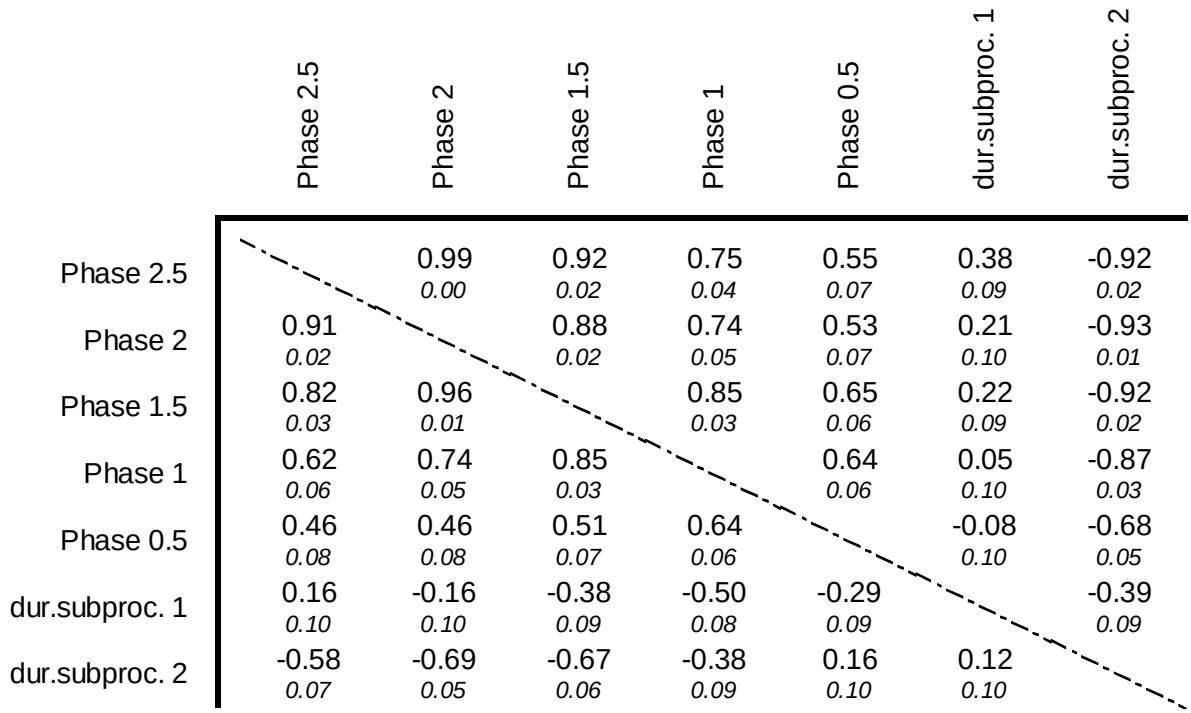
Figure 73. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Adour.

	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		0.99 *0.01*	0.96 *0.03*	0.98 *0.01*	0.72 *0.17*	-0.20 *0.32*	NA *NA*
Phase 2	0.94 *0.04*		0.99 *0.01*	1.02 *0.01*	0.56 *0.24*	-0.40 *0.28*	NA *NA*
Phase 1.5	0.90 *0.06*	0.98 *0.02*		1.01 *0.01*	0.20 *0.34*	-0.66 *0.19*	NA *NA*
Phase 1	0.52 *0.24*	0.54 *0.24*	0.61 *0.21*		0.35 *0.31*	-0.74 *0.15*	NA *NA*
Phase 0.5	0.37 *0.30*	0.39 *0.30*	0.46 *0.28*	0.77 *0.14*		0.21 *0.34*	NA *NA*
dur.subproc. 1	0.37 *0.29*	0.10 *0.33*	-0.01 *0.33*	-0.10 *0.33*	0.02 *0.35*		NA *NA*
dur.subproc. 2	-0.63 *0.22*	-0.60 *0.22*	-0.55 *0.25*	-0.07 *0.35*	0.39 *0.30*	-0.06 *0.35*	

Figure 74. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Durance.

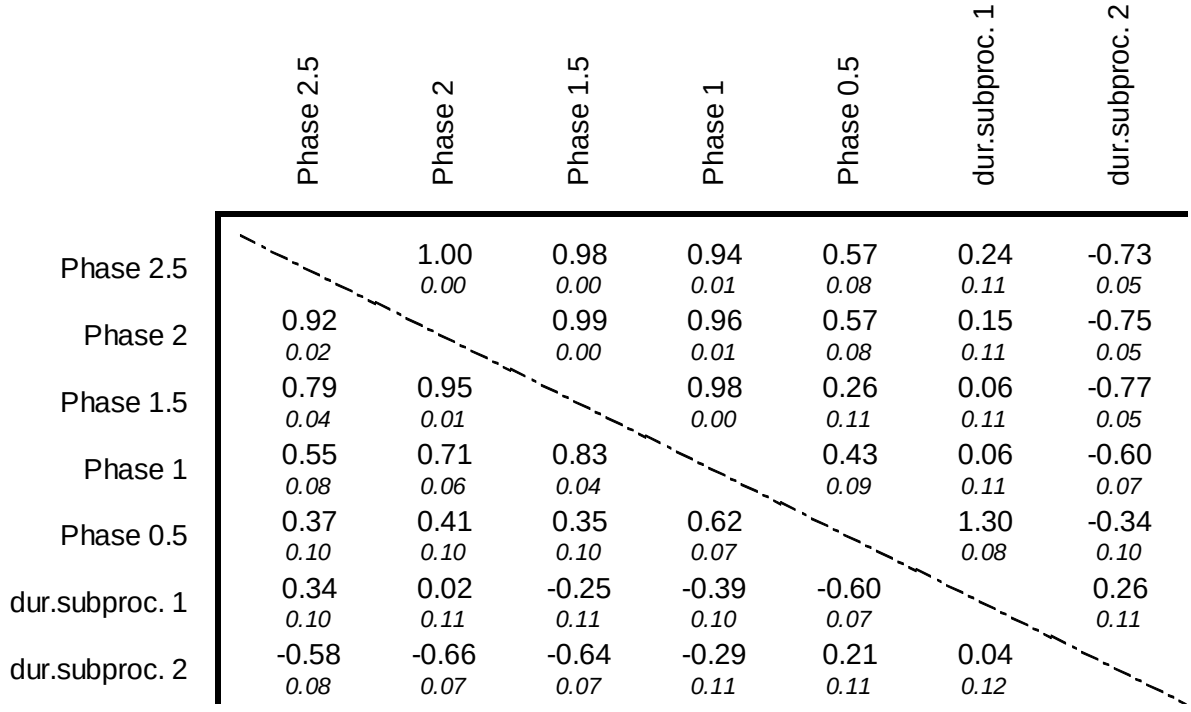
	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		1.00 *0.00*	0.99 *0.00*	0.93 *0.03*	0.88 *0.04*	0.51 *0.14*	-0.64 *0.11*
Phase 2	0.97 *0.01*		1.00 *0.00*	0.94 *0.02*	0.87 *0.05*	0.56 *0.13*	-0.67 *0.10*
Phase 1.5	0.91 *0.03*	0.98 *0.01*		0.97 *0.01*	0.88 *0.04*	0.62 *0.11*	-0.67 *0.10*
Phase 1	0.68 *0.10*	0.76 *0.08*	0.83 *0.06*		0.95 *0.02*	0.75 *0.08*	-0.49 *0.14*
Phase 0.5	0.51 *0.14*	0.54 *0.13*	0.59 *0.12*	0.81 *0.06*		0.49 *0.14*	-0.22 *0.18*
dur.subproc. 1	-0.07 *0.18*	0.16 *0.18*	0.33 *0.16*	0.49 *0.14*	0.28 *0.17*		-0.53 *0.13*
dur.subproc. 2	-0.59 *0.12*	-0.64 *0.11*	-0.62 *0.11*	-0.28 *0.17*	0.22 *0.18*	-0.17 *0.18*	

Figure 75. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Stura.



	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		0.99 *0.00*	0.92 *0.02*	0.75 *0.04*	0.55 *0.07*	0.38 *0.09*	-0.92 *0.02*
Phase 2	0.91 *0.02*		0.88 *0.02*	0.74 *0.05*	0.53 *0.07*	0.21 *0.10*	-0.93 *0.01*
Phase 1.5	0.82 *0.03*	0.96 *0.01*		0.85 *0.03*	0.65 *0.06*	0.22 *0.09*	-0.92 *0.02*
Phase 1	0.62 *0.06*	0.74 *0.05*	0.85 *0.03*		0.64 *0.06*	0.05 *0.10*	-0.87 *0.03*
Phase 0.5	0.46 *0.08*	0.46 *0.08*	0.51 *0.07*	0.64 *0.06*		-0.08 *0.10*	-0.68 *0.05*
dur.subproc. 1	0.16 *0.10*	-0.16 *0.10*	-0.38 *0.09*	-0.50 *0.08*	-0.29 *0.09*		-0.39 *0.09*
dur.subproc. 2	-0.58 *0.07*	-0.69 *0.05*	-0.67 *0.06*	-0.38 *0.09*	0.16 *0.10*	0.12 *0.10*	

Figure 76. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Ramieres.



	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		1.00 *0.00*	0.98 *0.00*	0.94 *0.01*	0.57 *0.08*	0.24 *0.11*	-0.73 *0.05*
Phase 2	0.92 *0.02*		0.99 *0.00*	0.96 *0.01*	0.57 *0.08*	0.15 *0.11*	-0.75 *0.05*
Phase 1.5	0.79 *0.04*	0.95 *0.01*		0.98 *0.00*	0.26 *0.11*	0.06 *0.11*	-0.77 *0.05*
Phase 1	0.55 *0.08*	0.71 *0.06*	0.83 *0.04*		0.43 *0.09*	0.06 *0.11*	-0.60 *0.07*
Phase 0.5	0.37 *0.10*	0.41 *0.10*	0.35 *0.10*	0.62 *0.07*		1.30 *0.08*	-0.34 *0.10*
dur.subproc. 1	0.34 *0.10*	0.02 *0.11*	-0.25 *0.11*	-0.39 *0.10*	-0.60 *0.07*		0.26 *0.11*
dur.subproc. 2	-0.58 *0.08*	-0.66 *0.07*	-0.64 *0.07*	-0.29 *0.11*	0.21 *0.11*	0.04 *0.12*	

Figure 77. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Ticino.

	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		0.97 *0.01*	0.94 *0.02*	0.87 *0.04*	0.87 *0.04*	-0.16 *0.15*	-0.84 *0.05*
Phase 2	0.94 *0.02*		0.99 *0.00*	0.94 *0.02*	0.91 *0.03*	-0.37 *0.13*	-0.89 *0.03*
Phase 1.5	0.84 *0.05*	0.96 *0.01*		0.96 *0.01*	0.92 *0.02*	-0.48 *0.12*	-0.90 *0.03*
Phase 1	0.57 *0.10*	0.68 *0.08*	0.78 *0.06*		0.93 *0.02*	-0.59 *0.10*	-0.79 *0.06*
Phase 0.5	0.32 *0.14*	0.40 *0.13*	0.49 *0.12*	0.78 *0.06*		-0.48 *0.12*	-0.69 *0.08*
dur.subproc. 1	0.00 *0.15*	-0.30 *0.14*	-0.52 *0.11*	-0.57 *0.10*	-0.39 *0.13*		0.41 *0.13*
dur.subproc. 2	-0.64 *0.09*	-0.70 *0.08*	-0.64 *0.09*	-0.20 *0.15*	0.30 *0.14*	0.20 *0.15*	

Figure 78. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Dranse.

	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		0.99 *0.00*	0.92 *0.02*	0.81 *0.05*	0.64 *0.10*	-0.47 *0.13*	-0.75 *0.07*
Phase 2	0.93 *0.02*		0.92 *0.02*	0.82 *0.05*	0.66 *0.09*	-0.57 *0.11*	-0.77 *0.07*
Phase 1.5	0.83 *0.05*	0.96 *0.01*		0.85 *0.05*	0.67 *0.09*	-0.56 *0.11*	-0.80 *0.06*
Phase 1	0.60 *0.10*	0.73 *0.08*	0.85 *0.05*		0.84 *0.05*	-0.47 *0.13*	-0.77 *0.07*
Phase 0.5	0.39 *0.14*	0.44 *0.14*	0.51 *0.12*	0.67 *0.09*		-0.42 *0.14*	-0.73 *0.08*
dur.subproc. 1	0.17 *0.16*	-0.15 *0.16*	-0.40 *0.13*	-0.54 *0.12*	-0.28 *0.15*		0.49 *0.13*
dur.subproc. 2	-0.62 *0.10*	-0.69 *0.09*	-0.67 *0.09*	-0.35 *0.15*	0.17 *0.16*	0.17 *0.16*	

Figure 79. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Val Allier.

	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		1.00 *0.00*	0.99 *0.00*	0.96 *0.01*	0.80 *0.03*	-0.52 *0.05*	-0.84 *0.02*
Phase 2	0.94 *0.01*		1.00 *0.00*	0.96 *0.00*	0.45 *0.06*	-0.57 *0.05*	-0.87 *0.02*
Phase 1.5	0.85 *0.02*	0.96 *0.01*		0.97 *0.00*	0.44 *0.06*	-0.63 *0.04*	-0.87 *0.02*
Phase 1	0.59 *0.05*	0.71 *0.04*	0.80 *0.03*		0.30 *0.07*	-0.64 *0.04*	-0.81 *0.02*
Phase 0.5	0.40 *0.06*	0.44 *0.06*	0.47 *0.06*	0.62 *0.04*		-0.74 *0.03*	-0.64 *0.04*
dur.subproc. 1	0.12 *0.07*	-0.16 *0.07*	-0.39 *0.06*	-0.47 *0.05*	-0.36 *0.06*		0.51 *0.05*
dur.subproc. 2	-0.59 *0.05*	-0.66 *0.04*	-0.64 *0.04*	-0.27 *0.07*	0.27 *0.07*	0.17 *0.07*	

Figure 80. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Loire.

	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		1.00 *0.00*	1.00 *0.00*	0.98 *0.01*	0.86 *0.04*	-0.74 *0.06*	-0.99 *0.00*
Phase 2	0.90 *0.03*		1.00 *0.00*	0.99 *0.00*	0.91 *0.02*	-0.73 *0.07*	-0.97 *0.01*
Phase 1.5	0.80 *0.05*	0.97 *0.01*		0.99 *0.00*	0.95 *0.01*	-0.75 *0.06*	-0.94 *0.02*
Phase 1	0.63 *0.09*	0.78 *0.06*	0.86 *0.04*		0.97 *0.01*	-0.83 *0.04*	-0.88 *0.03*
Phase 0.5	0.39 *0.12*	0.45 *0.11*	0.49 *0.11*	0.64 *0.08*		-1.25 *0.08*	-0.79 *0.05*
dur.subproc. 1	-0.01 *0.14*	-0.36 *0.12*	-0.55 *0.10*	-0.63 *0.08*	-0.25 *0.13*		0.39 *0.12*
dur.subproc. 2	-0.53 *0.10*	-0.67 *0.08*	-0.67 *0.08*	-0.42 *0.12*	0.28 *0.13*	0.40 *0.12*	

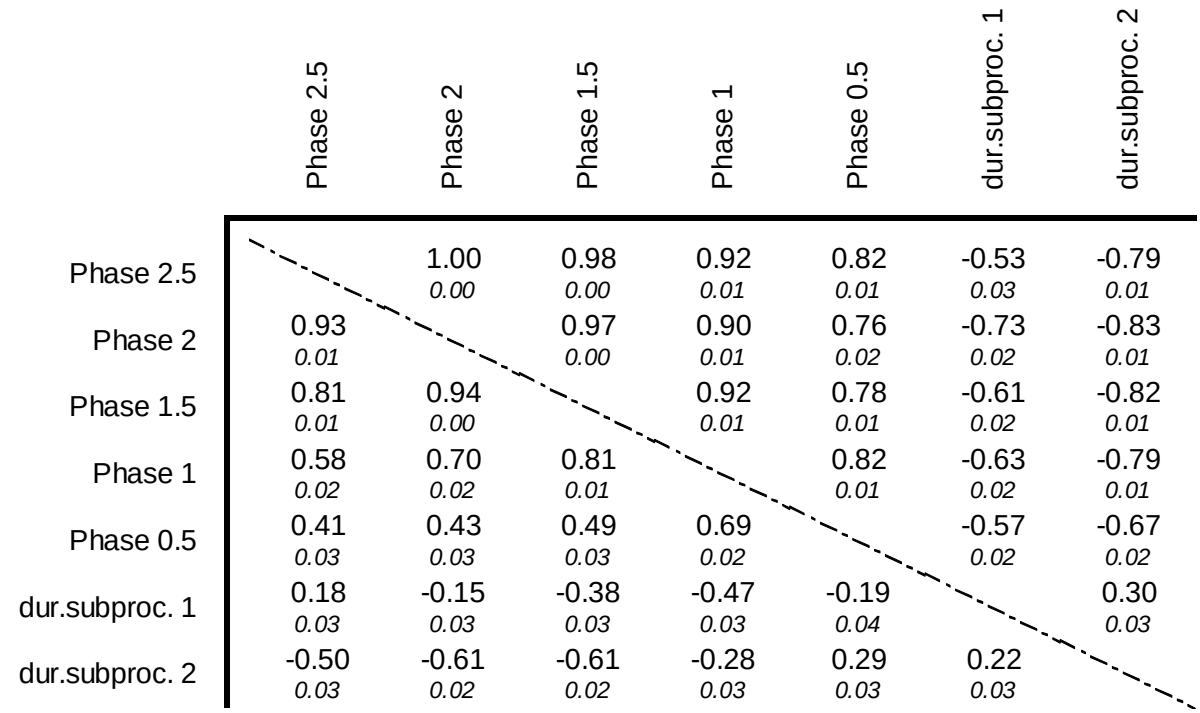
Figure 81. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Rhin.

	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		1.00 *0.00*	0.99 *0.00*	0.98 *0.01*	0.96 *0.01*	-0.72 *0.07*	-0.89 *0.03*
Phase 2	0.90 *0.03*		1.00 *0.00*	0.99 *0.00*	0.97 *0.01*	-0.75 *0.07*	-0.85 *0.04*
Phase 1.5	0.76 *0.06*	0.94 *0.02*		1.00 *0.00*	0.99 *0.00*	-0.80 *0.05*	-0.81 *0.05*
Phase 1	0.50 *0.11*	0.66 *0.08*	0.80 *0.05*		1.00 *0.00*	-0.83 *0.05*	-0.82 *0.05*
Phase 0.5	0.41 *0.12*	0.41 *0.12*	0.40 *0.12*	0.54 *0.11*		-0.90 *0.03*	-0.75 *0.06*
dur.subproc. 1	0.22 *0.14*	-0.16 *0.14*	-0.43 *0.12*	-0.54 *0.10*	-0.02 *0.15*		0.35 *0.13*
dur.subproc. 2	-0.24 *0.14*	-0.38 *0.13*	-0.43 *0.12*	-0.20 *0.14*	0.58 *0.10*	0.33 *0.13*	

Figure 82. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Kuhkopf.

	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		1.00 *0.00*	1.00 *0.00*	1.00 *0.00*	0.96 *0.01*	-0.89 *0.03*	0.13 *0.15*
Phase 2	0.89 *0.03*		1.00 *0.00*	1.00 *0.00*	0.97 *0.01*	-0.88 *0.03*	0.13 *0.15*
Phase 1.5	0.77 *0.06*	0.96 *0.01*		1.00 *0.00*	0.97 *0.01*	-0.89 *0.03*	0.07 *0.15*
Phase 1	0.57 *0.11*	0.78 *0.06*	0.90 *0.03*		0.99 *0.00*	-0.89 *0.03*	0.05 *0.15*
Phase 0.5	0.51 *0.11*	0.46 *0.12*	0.45 *0.12*	0.41 *0.13*		-0.93 *0.02*	0.11 *0.15*
dur.subproc. 1	0.25 *0.14*	-0.16 *0.15*	-0.39 *0.13*	-0.58 *0.10*	0.10 *0.15*		0.28 *0.14*
dur.subproc. 2	0.02 *0.15*	-0.15 *0.15*	-0.19 *0.15*	-0.18 *0.15*	0.72 *0.07*	0.36 *0.13*	

Figure 83. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for Netherlands.



	Phase 2.5	Phase 2	Phase 1.5	Phase 1	Phase 0.5	dur.subproc. 1	dur.subproc. 2
Phase 2.5		1.00 *0.00*	0.98 *0.00*	0.92 *0.01*	0.82 *0.01*	-0.53 *0.03*	-0.79 *0.01*
Phase 2	0.93 *0.01*		0.97 *0.00*	0.90 *0.01*	0.76 *0.02*	-0.73 *0.02*	-0.83 *0.01*
Phase 1.5	0.81 *0.01*	0.94 *0.00*		0.92 *0.01*	0.78 *0.01*	-0.61 *0.02*	-0.82 *0.01*
Phase 1	0.58 *0.02*	0.70 *0.02*	0.81 *0.01*		0.82 *0.01*	-0.63 *0.02*	-0.79 *0.01*
Phase 0.5	0.41 *0.03*	0.43 *0.03*	0.49 *0.03*	0.69 *0.02*		-0.57 *0.02*	-0.67 *0.02*
dur.subproc. 1	0.18 *0.03*	-0.15 *0.03*	-0.38 *0.03*	-0.47 *0.03*	-0.19 *0.04*		0.30 *0.03*
dur.subproc. 2	-0.50 *0.03*	-0.61 *0.02*	-0.61 *0.02*	-0.28 *0.03*	0.29 *0.03*	0.22 *0.03*	

Figure 84. Genetic correlations (above the diagonal) and residual correlations (below the diagonal) between traits for the collection of *P. nigra* studied.

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