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Larch hybrid vigor and role of the phenotypic plasticity in the construction of heterosis

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1. Introduction

1.1. Context

1.1.1. Hybridization as a breeding strategy in forestry

Plant kingdom challenges the definition of biological species, due to the outstanding propensity of most plant 'species' to hybridize and to produce fertile offspring. This is particularly true for many forest trees, leading Whites *et al.* (2007) to the conclusion that 'natural hybridization in the genus *Quercus* (oak) is so ubiquitous that is is difficult to apply the biological species concept to this taxon. Despite the propensity of oaks species to naturally hybridize, strong selection forces maintain their identity through generations. Natural hybridization is a powerful evolutionary tool. On one hand, punctual hybridization events between sympatric species can allow the capture of valuable genes from one species by the other *via* the phenomenon of introgression. On the other hand, a hybrid population can diverge from the parental pools, to ultimately produce a new species: this is the reticulate evolution process. Both examples are illustrated for instance in the evolutionary histories of the genera *Pinus*, *Populus* and *Eucalyptus* (reviewed by White *et al.* 2007).

This predisposition to hybridization explains why so many interspecific combinations can be easily obtained as forestry genetic material by means of artificial hybridization. Nevertheless, not all combinations present an actual interest for forestry, and more than 95% of forest plantations are planted with pure species (White *et al.* 2007). Still, forest tree hybrids from several genus are already planted and raise interest. Planted forest tree hybrids concern, among others, the genera *Acacia*, *Araucaria*, *Eucalyptus*, *Larix*, *Picea*, *Pinus*, *Populus* (Nikles and Griffin 1991; Dungey 2001). Hybrids can be first (F_1) or second (F_2) generation, back-crosses (*i.e.* crosses between F_1 and one parental species), 3- or 4-way hybrids (*i.e.* crosses between F_1 and a third species or another F_1), though in practice, F_1 hybrids are by far the most frequent in forestry. The main motivation for growing hybrids are the phenomenon of heterosis, or hybrid vigor, and the phenomenon of complementarity. The former refers to a superiority of the hybrid over its parental species, especially in growth, whereas the latter indicates a transmission from one or both parental species of favorable traits, *e.g.* disease or pest resistance (Nikles and Griffin 1991; Sylvestre-Guinot *et al.* 1999; White *et al.* 2007). Especially, the hybrid may inherit an

environmental tolerance from one parental species or population (like the *Eucalyptus* and *Araucaria* hybrids (Nikles and Griffin 1991)), or be better adapted outside the natural range of the parental species (like the *Pinus* hybrids (Dungey 2001)).

Hybridization offers many opportunities and a wide range of potentially valuable genetic materials for forestry, but some critical obstacles to hybrid breeding should be kept in mind (reviewed by Dungey 2001). First, it is often more difficult to produce hybrid seeds, as intra-species combinations can be favored by phenological, physical or selective barriers. A simple example is that different larch species tend not to flower at the same time. In the most extreme cases, hybrid seeds and embryos may be less viable. Second, a strategy for hybrid breeding is necessarily more complex and more expensive than a pure species one. Along with breeding, the deployment of hybrid material to commercial production is also a key issue. For this latter reason, the availability and the ease of efficient vegetative propagation for *Eucalyptus* and *Populus* contribute to explain the success of the hybrids from these genera (White *et al.* 2007).

1.1.2. Larch species and hybrids

The genus *Larix*, from the Pinaceae family, is composed of 10 to 15 extant species: at least 7 species from Eurasia and 3 species from North America (LePage and Basinger 1995; Semerikov *et al.* 2003). Some species cover continent-scale distributions in Asia (*L. gmelinii*, *L. sibirica*) and in North America (the tamarack *L. laricina*), whereas the other species have narrower distributions. The phylogeny of the genus *Larix* and the exact number of species and sub-species underwent major revisions lately (LePage and Basinger 1995; Semerikov and Lascoux 1999; Semerikov *et al.* 2003). It is now assumed that the genus is divided into 2 major clads, the North American species on one side and the Eurasian species on the other.

Under boreal and temperate climates, winter represents a danger for trees as the soil water freezes and becomes unavailable for plants. If the weather is warm but the ground is frozen, trees can undergo a possibly fatal drought stress. Under this selective pressure, boreal and temperate trees have evolved two main strategies. Broad-leaves trees are often deciduous, meaning that they drop their leaves in winter. As a result, their transpiration during winter is null. On the other hand, the conifers are often evergreen, but their leaves are reduced to needles that limit the transpiration and therefore keep them

safe from drought. Larches present the uncommon feature to cumulate both strategies, so that their leaves are deciduous needles. The cumulation of both strategies contributes to explain the ability of larch to thrive in the most extreme environments, under icy climates and/or at very high altitude. The European larch *Larix decidua* is naturally distributed between 300 and 2400 meters high, in the Alps and in central Europe. The Japanese larch *L. kaempferi* grows on top of volcanoes on Hondo Island, between 1200 and 2600 meters high (Riou-Nivert 2001).

In France, European larch wood is famous for its quality. This wood, reddish in color, is very durable and thereby it has a long tradition as a construction material for chalets in the Alps. The interest for European larch wood properties motivated the first attempts to extend its cultivation beyond its natural range, especially in lowland and more oceanic conditions. Unfortunately, the Alpine variety of European larch did not thrive in these new environments. Beside the poor performances of the Alpine European larch, this variety also suffers from canker (*Lachnellula willkommii*) that appears as a major sanitary issue. Two solutions are now simultaneously considered for cultivation of larch in Western Europe (Pâques 2001): the prospection of other origins of European larch, especially from Central Poland, Sudetan Mountains and Lower Austria; and the hybridization with other species of larch.

The latter solution presents a special interest due to the complementation of traits and to the phenomenon of heterosis which appears with some larch interspecific combinations. In particular, all inter-specific hybrids between *L. decidua*, *L. kaempferi* and *L. laricina* show heterosis on their juvenile growth traits, at least up to 5 years old (Baltunis *et al.* 1998). After 8 years of growth, the hybrids involving tamarack (*L. laricina*) do not reach the performance of the hybrids between European and Japanese larches. Hybrids involving tamarack also display a poor stem form (Pâques 1992). Moreover, it has been recently demonstrated that heterosis from the hybrid between *L. decidua* and *L. kaempferi* was still present and strong in adult trees (Greenwood *et al.* 2015). As a conclusion, the hybrid between European and Japanese larches is highly promising for forestry in Western Europe and in North America. Beside heterosis, this hybrid inherits from *L. kaempferi* resistance to canker (Sylvestre-Guinot *et al.* 1999) and a better behavior towards meria (*Meria laricis*) (Philippe *et al.* 2016) thus presenting a solution to these sanitary issues under oceanic conditions.

1.1.3. Hybrid larch breeding

The breeding of forest trees can hardly be compared to that of other crops, and presents some inherent difficulties due to their massive stature and their long lifespans (Bastien 2015). Indeed, tree breeding demands both a lot of time and a lot of space: time, because reliable evaluations of trees' genetic merit must be inferred from old enough progeny trials. Moreover, trees have a late sexual maturity that must be reached prior to mating and recombination cycle; and space, because these cumbersome organisms must be tested in large-scale experiments and because a tree genetic collection is a wide set-up. In addition to these considerations, forest progeny trials have their own specific constraints. Competition between neighboring trees is likely the main issue, obliging the experimenter to thin the trials on a regular basis. This is especially true for larch which is a strict light-demanding species. Also, the consideration of micro- and macro- environmental influences on the growth is of first importance. The micro-environmental influences manifest in trials as a noise, masking the genetic contrasts between trees. The macro-environmental influences are even more worrying as the genetic performances assessed in one trial may not inform properly on the performances in other regions.

The breeding of hybrid crops also requires more space, money and imagination than the breeding of single breeds or species. Indeed, in the case of first generation hybrids (F_1) the breeder has to manage two parental genetic pools, and must evaluate them on their ability to produce a third genetic pool, namely the hybrid population (Gallais 2009).

Interspecific hybridization among larch species has for long attracted foresters since the first discovery of hybrids (European × Japanese larches) in Scotland at the beginning of the 20th century. Their exceptional growth compared to their parents encouraged breeders to create new hybrids and foresters to extend larch plantations well-beyond native European larch areas. Syrach (1956) in Denmark really boosted research and breeding activities around interspecific breeding in larch and some of the hybrid varieties created under his impulse are still nowadays of great commercial value. Interspecific breeding of larch started much later in France with first hybrids created and tested in the late 70s. French hybrid larch genetic program started in 1979. The main traits this program aims to control are (Bastien 2015; Pâques *et al.* 2016):

- Architecture, especially the stem straightness. Flexuosity is a real problem in larch, as some trees produce twisted stems that can hardly be processed by the sawmill.

- Wood productivity. As in most (if not all) agricultural breeding program, yield is a major trait of interest.
- Wood properties, such as mechanical properties and durability. In particular, it is important that a possible gain in productivity does not come with an unfordable loss in wood quality.
- Resistance to pathogens, especially canker but also meria. The sudden oak death (*Phytophthora ramorum*) just arrived in French Brittany and it is known to affect *L. kaempferi* in Great-Britain. It may be a major concern in a near future.
- Plasticity and adaptability. Phenotypic plasticity is a broad topic, that is especially under the scope of this dissertation and will be treated later on. Adaptability can be illustrated by phenology traits, such as bud flush and bud set. Indeed, due to climate change, the synchronism between phenology and the climate could be compromised, opening the door to climatic threats like frost. Resistance to cavitation is also a major question, in the context where climate change increases intensity and duration of drought stresses (Sánchez *et al.* 2013).

Hybrid larch superiority over its parents was already quite well documented when the INRA program started in 1979 and some evidence of its vigor were already observable in some scattered plots. This motivated the creation (period 1979-1985) by control crossing of several hundreds of hybrid full-sib combinations which have been progeny tested across France. The prime objective was to select some best families and to rapidly propose to foresters forest reproductive material (FRM), independently of external sources available abroad. This opportunistic strategy based on parental species flowering ability and a cross-test-select approach was judged far from optimal. The random implication of phenotypically selected parents led to many progenies which do not meet the necessary criteria for FRM selection. Nevertheless, this first phase was rich for a better understanding of the behavior and performances of hybrids as well as in the knowledge of its quantitative genetic parameters. This motivated a second phase (from 1986 up to now) including the systematic testing of the genetic value of parental clones from the European larch and Japanese larch breeding populations and the creation of dedicated mating plans to better document hybrid vigor and understand its genetic determinism in first and advanced generations.

Hybrid deployment requires to produce seeds and that is not a trivial task. Open-pollinated orchards gathering both European and Japanese larches naturally produce between 15% and 90% hybrid seeds. Pollen supplementation, *i.e.* collecting the pollen

from one parent (usually the Japanese larch) to pollinate the other (usually the European larch) yields 90% to 95% hybrid seeds (Pâques *et al.* 2016). This idea led to the creation of the first French hybrid larch orchard, in 1978: 'Lavercantière' (FH201-Lavercantière-PF). This variety is the copy of a combination used in a Danish orchard (FP201DK) but using pollen supplementation instead of open pollination. The combination consists in a European larch clone, called #35, that is pollinated by a Japanese larch full-sib family. The clone #35 presents a fair growth, but overall an outstanding stem straightness that is transmitted to its offspring. More recently, in 2000, a second French variety was approved: 'Rêve Vert'. The same European larch clone, #35, is still used as the female parent but this time the pollen comes from 12 Japanese larches of different geographical origin. In comparison to Lavercantière, the goal was to increase the genetic variability on the Japanese larch side. The clone #35 still contributes to the offspring stem straightness.

Vegetative propagation is an efficient option for rapid dissemination of gain, notably when elite genotypes are difficult to obtain. Unfortunately, old larch trees cannot be reproduced efficiently by cuttings, as cuttings poorly root and grow plagiotropic. For this reason, bulk multiplication was developed for hybrid larch (Verger and Pâques 1993) especially for the Rêve Vert variety. Bulk multiplication consists in a three-step approach: (i) a half- or full-sib family is produced by pollen supplementation, to produce hybrid seeds; (ii) these seeds are grown to produce mother plants, that are tested to ensure that they are indeed hybrids (*e.g.* Acheré *et al.* 2004; or Wagner *et al.* 2012); (iii) cuttings are collected from these young mother plants, thus producing non-plagiotropic saplings. The interests of bulk multiplications are, on one hand, the possibility to guarantee a 100% hybrid progeny; and on the other hand, the possibility to rapidly change the combination of parents used to generate the mother plants. The latter point is of special interest, as the set-up of a traditional seed orchard is both time and resources consuming. The possibility for rapid turn-overs allows faster deployment of the improved genetic material, and can also be used to vary the parents combination in order to manage more efficiently the genetic diversity. The major limitation of the bulk multiplication is the raising of costs, that increases the price of the plants by around 150% in comparison to pollen-supplemented seeds. Another option for vegetative multiplication is the somatic embryogenesis, that has also been investigated (Lelu-Walter *et al.* 2015) but leads to the same problem of costs raising. Moreover, there remain some uncertainties about the potential epigenetic modifications of the *ex vitro* plantlets: this is a current investigation for larch as for other forest trees.

1.1.4. The problem

In forestry, hybridization is understood as interspecific or inter-populations, thus among highly heterozygous individuals, and it is frequent to consider the 'hybrid superiority' as the whole package made of heterosis, complementation and transfer of favorable traits (such as the transfer of canker resistance from Japanese larch to the hybrid). Thereby, this hybrid superiority is often the result of a comparison between an elite hybrid and an elite representative of the pure species, without necessarily paying attention to the kinship between them; The result is that this information is of no help to dissect the genetic causal components of this superiority (Hinkelmann 1974). On the other hand, *sensu stricto* heterosis *i.e.* the superiority of the hybrid with respect to its parents, remains poorly studied in forestry due to the scarcity of experiments involving both forest tree hybrids and their parental control (Dungey 2001). This situation is responsible for an overall lack of information on heterosis in forestry, lack that has especially been emphasized for hybrid larch (Pâques 1989). Aside a proper mating design, it is also necessary to assess heterosis on old enough trees. Indeed, during the first years of growth after plantation, it is likely that the ranking of trees is unstable due to different dynamics. The first long-term analysis of a proper intra- and inter-specific mating design for larch was published a few years ago and confirmed the persistence of hybrid larch heterosis on mature trees (Greenwood *et al.* 2015). Another dimension that has to be explored to properly guide a breeding program as its setting is the stability and the plasticity of hybrid trees as function of the environment, and this is particularly the topic we are going to study throughout this dissertation. Hybridization and phenotypic plasticity are going to be studied from the perspective of the quantitative genetics theory, with the goal to draw practical implications for the hybrid larch breeding program.

1.2. Hybridization

Hybridization is one of the first and most intuitive tools available for the study of heredity. For instance, hybridization and recombination between pea lines is at the origin of the understanding of the mechanism of genes transmission (Mendel 1865). The increase in vigor of out-crossed plants in comparison to the inbred ones was also noticed early (Darwin 1876) and later named 'heterosis' (Shull 1914). Hybridization lays on a general principle: two distinct genetic pools are crossed to produce first generation (F_1) hybrids. The parental strains can be inbred lines (like Mendel's peas), heterozygous individuals, populations, or species (like Shull's *Capsella* ssp., and overall, like hybrid larch).

1.2.1. Heterosis

Hybridization can lead to a phenomenon of hybrid vigor, or heterosis, expressed in quantitative traits. Hybrid vigor is defined as the manifestation of an increased vigor in growth/production, or an increase in fitness in the hybrid in comparison to its parents. The notion of heterosis is almost synonymous but a bit broader, as it includes hybrid vigor but also physiological superiority of the hybrid that is not necessarily visible by the naked eye (Shull 1914, 1948). By proposing the word 'heterosis', Shull wanted to provide a 'free from every hypothesis' concept, unlike for instance the word 'heterozygosis' that explicitly refers to a genetic causality. An important consideration is that heterosis, or hybrid vigor, is always a superiority of the hybrid in comparison to the parents, therefore, there is no such thing as a 'negative heterosis' (we should use *e.g.* 'hybrid depression' instead). Hybridization is now a common breeding strategy in several crops. Historically, corn has been one of the first and most famous example (Shull 1910; Janick 1999). More formally, heterosis may be defined as the ability of the hybrid to outperform the average or the best of its parents. In the context of hybridization between pure lines, the opposite of heterosis is the inbreeding depression.

There is not a single, but a whole range of mechanisms behind heterosis. Basically, they fall into one of these two categories: the ones that involve dominance, and the ones that do not. First, let's define clearly the molecular dominance. Let A_1 and A_2 be two alleles at the same locus, with A_2 the favorable allele, that is, the allele that increases the trait of interest. The genotypic value of each homozygote are respectively $-a$ and $+a$, and that of

the heterozygous is *d*. In Fig. 1, we represent all the possible cases of dominance (Falconer and Mackay 1996). If there is no dominance, each allele A_2 increases additively the trait by the quantity *a*. Otherwise, dominance might be positive or negative, though in practice it is mostly positive. This can be understood at two levels: (i) at the molecular level, as the functional version of a gene (A_2, in this example) has the ability to restore a metabolic pathway even if the second allele (A_1) is defective; (ii) at the ecological level, indeed in the case of negative dominance the unfavorable allele (A_1) is particularly exposed to natural selection in both the unfavorable homozygote and the heterozygous (A_1A_1 and A_1A_2), and therefore it should be strongly selected against (Lerner 1954; Gallais 2009). The positive dominance can be partial ($d \in]0, +a[$), complete ($d = +a$), or can outperform the favorable homozygote ($d > +a$), the latter case being called overdominance.

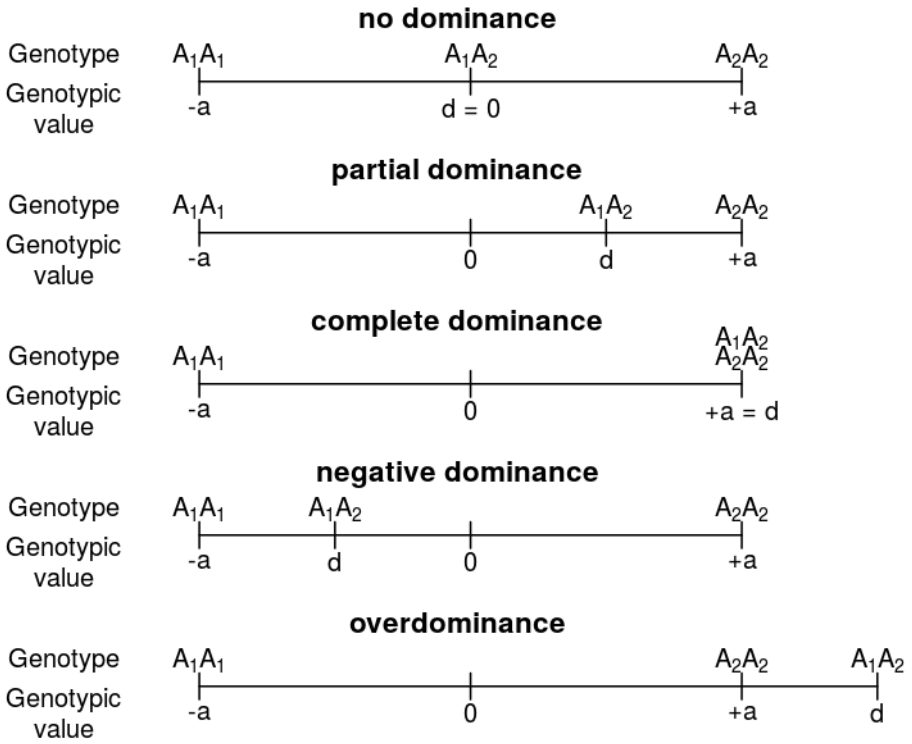

Figure 1: Illustration of the different types of dominance, from Falconer and Mackay (1996 Fig. 2.1 and 7.1)

Overdominance leads to heterosis in a trivial way. Indeed, in presence of overdominance at a locus, there is no way for any homozygous to outperform the heterozygous at this locus. Complete and partial dominance can also lead to heterosis, especially best-parent heterosis, but this involves several loci. In this case, the integration at several loci of positive dominance leads to a higher phenotypic value for the trait of interest in the hybrid than in the parents. In other words, the hybrid, which is more heterozygous than the parents, has the metabolic pathways that are defective in one parental line / population / species complemented by the functional alleles from the other parental line / population / species (Gallais 2009). Finally, two loci can be closely linked on the genome, with one favorable allele at locus 1 linked to an unfavorable one at locus 2 and reciprocally. In such a situation, the cluster made by these two loci acts as a single locus, that, due to dominance at locus 1 and 2, may show an overdominance pattern: this is the pseudo-overdominance hypothesis. The hypotheses based on dominance have the longest history and have traditionally been proposed as the main explanations for heterosis. The involvement of dominance and overdominance have been confirmed by QTL analysis in hybrid maize and rice, as reviewed by Lippman and Zamir (2007) and Chen (2013). The use of introgression lines, *i.e.* lines in which the genome is introgressed, segment by segment, into another line also unraveled the existence of dominant and overdominant loci, with preferentially overdominant loci for traits associated to reproductive fitness in tomato (reviewed by Lippman and Zamir 2007).

However, the dominance theory is not sufficient to fully explain the complex phenomenon of heterosis. On one hand, the selective breeding that has been performed on pure lines for decades should have started to purge their genetic load, therefore, if complementation was leading the phenomenon of heterosis, the level of heterosis would have been expected to decrease with time. However, it slightly increased instead (reviewed by Birchler *et al.* 2003; and Chen 2013). On the second hand, complementation alone cannot explain the phenomenon of progressive heterosis in polyploids, that is, the increase of vigor due to a progressive increase in heterozygosity in polyploids (*e.g.* for an autotetraploids, the increase in heterosis from AAAA to AABB to ABCD) (Birchler *et al.* 2003; Lippman and Zamir 2007; Chen 2013). Moreover, the decrease in vigor of polyploids due to inbreeding depression is faster than what could be expected from homozygosity of alleles alone.

Regulation of gene expression is more and more frequently suggested to play a major role in the manifestation of heterosis. Birchler *et al.* (2003) suggested regulatory

genes to be dosage-dependent, and favored by heterozygous states. In other words, an intermediate dose would be more effective than the plain dose provided by homozygosity at a locus. Ultimately, these regulatory genes control the expression of the quantitative traits that manifest hybrid vigor. The level of gene expression in the hybrids can be additive or non-additive, and is actually more often non-additive in inter-specific than in intra-specific hybrids (reviewed by Chen 2013). Many mechanisms are involved in the expression of genes. Chen (2013) reviewed the expressional change (both increases and decreases) of small interfering RNAs in arabidopsis, maize and rice hybrids with respect to the parental strains. He also found changes in the DNA methylation levels (especially increases) in arabidopsis and rice hybrids, and additive transmission (mid-parent value) to slightly non-additive levels of histones in arabidopsis and rice hybrids. Possibly, the down-regulation of genes in the hybrids with respect to the parental strains could cause an important reduction in the energy-consuming processes of their protein metabolism, and therefore, be a component of the overall heterosis (Baranwal *et al.* 2012). It has been suggested that some overdominant loci, involved in the regulation of a gene network, may have been naturally selected with the effect to favor heterozygosity (Birchler *et al.* 2003; Lippman and Zamir 2007). This evolutionary consideration is interesting, however it seems unlikely that it could contribute to explain heterosis observed in inter-specific hybrids that do not naturally occur, as such hybrids have never been directly exposed to natural selection.

These molecular considerations apart, heterosis can also arise from the simple complementarity between multiplicative traits. A practical example is the bunch yield of oil palm inter-population hybrids (*e.g.* Cros 2014; Marchal 2014; Marchal *et al.* 2016). One population of oil palm produces a few but heavy bunches, and the second population produces many bunches that are lighter. Bunch number and weight are additively inherited, and the hybrid between these two populations produces an intermediate number of bunches of intermediate weight thus maximizing the yield. In this case, the complementarity between traits leads to heterosis without the need for molecular dominance. However, as the parents performances are field tested in a reciprocal recurrent selection frame, molecular dominance likely accumulates within the statistically additive values inherited by the hybrids.

1.2.2. Hybrids' stability

Homeostasis has been defined at two levels. First, developmental homeostasis is the ability of an organism to buffer its inner and outer environments in order to stabilize itself (Cannon 1932; *in* Lerner 1954). Second, genetic homeostasis is defined at the level of a Mendelian population, that is, a population of cross-fertile individuals sharing a common gene pool. Genetic homeostasis is the ability of the Mendelian population to balance its genetic composition and to resist sudden changes (Lerner 1954). Lerner (1954) proposed both practical illustrations and a cohesive theory that emphasizes the role of heterozygosity in both developmental and genetic homeostasis. Displaying greater homeostatic properties than the homozygous, the heterozygous are expected to be more canalized *i.e.* to have a better ability to produce a definite phenotype independently of minor variations during the course of their development. Given the close connection between heterosis and heterozygosity, this theory appears as a convenient perspective from which to consider heterosis.

The assumption of a developmental homeostasis of hybrids is generally well supported by hybrid field crops (Gallais 2009). These hybrids are produced as crosses between inbred lines. Gallais suggested that such hybrids display a higher stability in their performances, and especially perform better than their parents in unfavorable environments. As a result, homeostasis can be a full-fledged component of heterosis.

Knight (1973) demonstrated that the purely additive inheritance of reaction norms is sufficient to lead to the construction of heterosis, as shown in Fig. 2. In this illustration of a theoretical scenario and for a given X_1 (Fig. 2 b), the hybrid only produces best-parent heterosis in intermediate conditions of X_2; while in extreme conditions hybrids perform better than the average of its two parents. The hybrid is thus more stable than its parents. We have to complete this simplified representation with the mechanisms that were developed previously. Indeed, heterozygosity is also expected to generate heterosis from non-additive pathways, especially in extreme conditions (Gallais 2009). Practical and emblematic examples of the link between homeostasis and heterosis are provided by the cereal literature. Corn hybrids' performances are more stable across sites, in the sense that the interactions between genotypes and environment (G×E interactions) are reduced, especially in response to stress (Janick 1999). Also wheat, barley and triticale hybrids yields are more stable across sites leading to a decrease in the variance of G×E (Mühleisen *et al.* 2014).

Two major criticisms arise from this representation. First, in Mendelian (panmictic, naturally occurring) populations, the heterozygous homeostasis is a consequence of natural selection in the sense that it is a component contributing to fitness (Lerner 1954). Schlichting and Pigliucci (1998) make a sound distinction between the notions of homeostasis and phenotypic plasticity. The optimal shape of a 'fitness reaction norm' is the one that maximizes the fitness in any condition, and developmental homeostasis has been selected for stabilizing at its maximum the fitness across the environments. The plasticity of some traits can be a tool to achieve the overall homeostasis. Therefore, the stability of some traits, such as yield, should only be considered as the possible by-product of a phenomenon that primarily intends to maximize the fitness; but all traits are not expected to be equally stable. The second criticism concerns a central assumption in Lerner's theory: the context of a Mendelian population. Fridman (2015) detailed how the deviation from this assumption can affect the genetic homeostasis of naturally autogamous species, that are *de facto* non-Mendelian populations. Hybridization between different species, as it is done to produce inter-specific hybrid trees, also constitutes a major deviation from this assumption. The genetic homeostasis is not warranted when too different genetic pools are assembled. It can be illustrated by the sterility of some inter-specific hybrids, such as the mule. Hybrid larch also shows a lot of difficulty to reproduce, producing seeds that are mostly sterile (Pâques *et al.*, in prep). As an antonymous to heterosis, hybridization between too distant gene pools can lead to a phenomenon of hybrid depression.

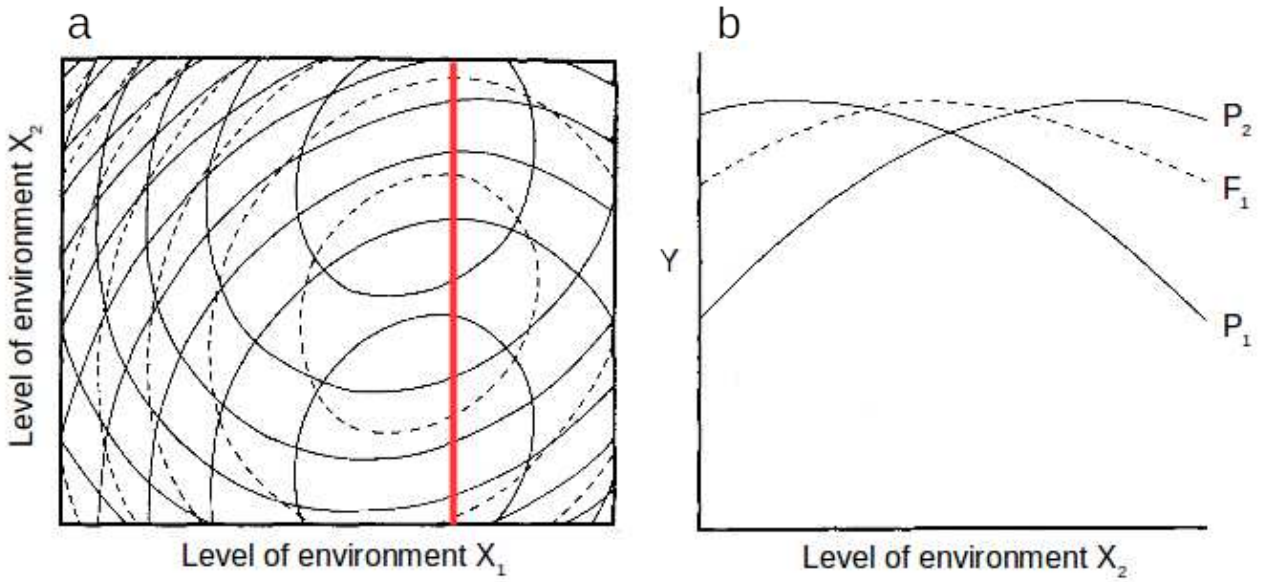

Figure 2: The additive transmission of bi-dimensional reaction norms, from Knight (1973). a) The contour lines of the reaction norms of the parents (plain lines) and of the hybrid (dashed line), in the plan formed by the two environmental variables X_1 and X_2. The red bold line is a section at a given value of X_1. b) The reaction norms of the parents (plain lines) and of the hybrid (dashed line) along X_2, for X_1 as fixed in (a).

1.3. Phenotypic plasticity

The distinction between genotype and phenotype is a pillar of genetics. Genotype and phenotype were defined by Johansen (1911), introducing by the way the idea that the phenotype was sensitive to the environment. The role of the environment on the expression of genotypes has been the topic of a rich literature, for which extensive reviews exist today (Schlichting and Pigliucci 1998; DeWitt and Scheiner 2004). These, and some of the excellent presentations I had the opportunity to attend thanks to the reflection group 'PlasPhen' (Debat 2017; Gibert and Till-Bottraud 2017), constitute a support for the current section.

1.3.1. The concept

Phenotypic plasticity is the 'environmentally sensitive production of alternative phenotypes by given genotypes' (Stearns 1989). As the concept of 'phenotype' is broad, the concept of phenotypic plasticity is also broad and as a consequence there is still some uncertainty on its borders. For instance, one can legitimately wonder if plants' stomatal closure under drought should be or not considered a manifestation of phenotypic plasticity. Indeed, as far as I understand, the answer to this question is not trivial. Some scientists keep the definition at its broadest scopes, whereas others are concerned that including such rapid and reversible features may finally dilute the initial idea. Indeed, at the extreme, any motion including eye blinking could finally fall into the broadest definition of phenotypic plasticity. I don't claim to defend a position here, but one should keep in mind that the question of phenotypic plasticity is a broad and complex field, whose boundaries are not consensually fixed up to date. Moreover, this notion includes an incredibly wide variety of manifestations, in all biological kingdoms, at short or at long term, in reversible traits or engraved for life.

The topic of phenotypic plasticity is at the junction of genetics, development, ecology and evolutionary biology. However, the neo-Darwinian synthesis that was proposed in the 1930s and 1940s (Dobzhansky, Huxley, Mayr, Simpson, reviewed by Schlichting and Pigliucci (1998)) completely neglected the role of developmental biology and of the environment on evolution. As a reaction, several authors proposed an alternative synthesis that emphasized these components, according more importance to phenotypic plasticity (Goldschmidt, Schmalhausen, Waddington, reviewed by Schlichting and Pigliucci (1998)).

The question with the alternative synthesis was absolutely not to put Lamarckism back on the agenda, although there has been some revival of this old debate (Debat 2017). For recall, at the same time Lysenko was still condemning genetics as a 'bourgeois pseudoscience' in Soviet Union. Evolutionary biology has been and, unfortunately, is still subject to many critics and detractors, for philosophical, religious and political reasons. Therefore it seems important to stress that giving some importance to the role of phenotypic plasticity does not challenge the modern view of Darwinism, but it rather brings key elements to complete this vast and complex picture.

We can classify plastic responses into two main categories of mechanisms (Windig *et al.* 2004):

- The 'phenotypic modulation' happens without an active involvement from the organism. For instance, an enzyme activity is directly affected by the temperature, according to Arrhenius equation, and this may have direct consequences on ectotherms' metabolism.
- The 'developmental conversion' implies an active involvement from the organism. The most extreme examples are the organisms that display contrasted phenotypes, such as polyphenic insects (*e.g.* small-horned *vs.* long-horned forms of dung beetles, spring *vs.* summer forms of butterflies, solitary *vs.* gregarious forms of locusts, etc., reviewed by Debat (2017)). However, one must not confuse polyphenism with ontogeny, illustrated for instance with larval *vs.* imaginal stages of insects.

From this, it is intuitive that developmental conversion leads to phenotypes that have been optimized by natural selection to thrive in certain environmental conditions, or to perform specific tasks. On the other hand, phenotypic modulation is often deleterious (*e.g.* resources shortage, suboptimal conditions for enzymatic functioning). Nevertheless, the products of phenotypic modulation occurring in a natural range of environments are also exposed to natural selection, and therefore, the 'optimal' of these degraded phenotypes is still expected.

The mechanics of phenotypic plasticity involve several steps: an environmental cue is detected by the organism, leading to a signal, that drives the alteration of the phenotype. This is especially true for developmental conversion, as in the case of pure phenotypic modulation the environmental cue may directly affect the expression of the phenotype. Gaps in space and time may exist between the detection of the environmental cue and the expression of the phenotype, meaning that the signal can be transported (spatial gap)

and/or stored (temporal gap) by the organism. In plants, the signal can be mediated by hormones such as auxins, gibberellins, cytokinins and brassinosteroids (Windig *et al.* 2004).

Schlichting and Pigliucci (1998) reviewed 4 attributes for plasticity: the amount, that is the magnitude of the contrast between phenotypes depending on the environment; the pattern, that is the shape of the relationship between the trait and the environment; the rapidity, that is the speed of the plastic response; and the reversibility, that is the ability of the phenotype to switch from one state to another. Schlichting and Pigliucci (1998) also proposed a 5th attribute, the competence, defined as the capability of an organism to both perceive and respond to an environmental signal at certain windows during its development.

1.3.2. Genetics of plasticity

Bradshaw (1965) suggested plasticity to be under its own genetic control. This idea was latter defended by Scheiner (and Lyman 1989; 1993), among others, but Via (1993) proposed an alternative theory. This led to two distinct visions of the genetics of phenotypic plasticity, each one coming with its own evolutionary implication, leading more intuitively to some mechanics and analytical methods, as summarized in Table 1.

Via's (1993) theory is that plasticity is a side-effect phenomenon, given by the fact that the alleles coding for the focal trait can also have the additional particularity of having effects that vary across environments. As a particular case, some alleles can express only in some environments and be shut down in others. Thus, the expression of one trait in two environments can be considered as two different traits, and the loci that affect these two traits are thus seen as pleiotropic. An across-environment additive correlation r_A can be computed between them, this is the character-step approach. Across-environment additive correlations have exactly the same properties as across-traits additive correlations, and in the same way, they can be used to compute a correlated response to selection, that is the response to selection for the trait in environment 2 while the parents have been selected for this trait in environment 1 (Falconer and Mackay 1996). The character-state approach can be extended to the case where the environment is a continuous gradient. In this case, the across-environment genetic correlation becomes a continuous covariance function (Kirkpatrick and Heckman 1989).

On the other hand, Bradshaw (1965) and later Scheiner (and Lyman 1989; 1993) defended the view that some loci directly control the shape of the function linking the trait to the environment. Such a function is called a reaction norm, a concept that was first introduced by Baldwin (1902). Reaction norms can be modeled as polynomials, so that different parameters, each one under its own genetic control, set the mean of the trait, the slope along the environmental gradient, the curvature, and even more complex shape parameters. Schlichting and Pigliucci (1993) emphasize the importance of environmentally driven regulatory genes, *i.e.* genes that control the expression of other subsets of genes in an epistatic way. Unlike the pleiotropy theory, the epistatic theory allow natural selection to act directly on plasticity, for instance controlling the rapidity of responses, or their competence. The latter is illustrated at best by anticipatory plastic responses.

Both theories are now recognized as valid (Via *et al.* 1995), and are mutually compatible. Intuitively, we are inclined to associate pleiotropy to phenotypic modulation, and epistasis to developmental conversion. However, this shortcut may be misleading to some extent. Indeed, some pleiotropic loci may affect a latent trait, leading to a threshold response on another set of traits. On the other hand, phenotypic modulation may be offset, or supported by, regulatory genes. Finally, an important point is that the character-state and the reaction norm approaches are both mathematically equivalent (Meyer 1998; Windig *et al.* 2004). Therefore, both approaches can be used interchangeably to analyze a case of plastic response, without the need for priors on its mechanisms.

Table 1: Theories for "plasticity genes": pleiotropy and epistasis (reviewed from Schlichting and Pigliucci 1998; DeWitt and Scheiner 2004; Gibert and Till-Bottraud 2017)

Theory	Pleiotropy	Epistasis
Definition	• Loci whose allelic effect varies across environments • Loci that express only in some environments	• Loci that determine the shape of the reaction norm • Regulatory genes
Evolutionary implication	Phenotypes are selected for in each environment, plasticity is a by-product	Natural selection acts directly on reaction norms
Intuitive mechanics	Phenotypic modulation	Developmental conversion
Analytical method	Character-state approach	Polynomial reaction norm
Historical defender(s)	Via (1993)	Scheiner (1993) Schlichting & Pigliucci (1993)

1.3.3. Dendroplasticity

In a tree, the flow of water is driven by the leaves transpiration, and guaranteed by a water column constituting a continuum from the soil to the leaves. Under drought, the dry air increases the transpiration water demand whereas the soil water offer decreases; such a tension exposes the hydraulic system to risks of embolism, that can lead to irreversible damages or even to the death of the tree (Bréda *et al.* 2006; Rennenberg *et al.* 2006).

Short term responses, such as stomatal closure, and longer term responses, such as phenotypic plasticity of several functional traits, are available for the tree to react to this stress. Plastic responses include leaf-shedding; allocation of more resources to the root system; and modifications of the sapwood, the tissue that drives water through the trunk, in order to control the flow and to increase its resistance to embolism. The latter solution includes an enhanced heartwood formation (duraminisation), that is a sealing of the conductive wood, and a diminished radial growth accompanied by alternative properties of the new wood - that is made of narrower cells, with larger wall, thus reducing its water conductivity (Bréda *et al.* 2006; Bryukhanova and Fonti 2013). In addition to these plastic responses, drought and any concurrent heat stress have direct negative effects on photosynthetic efficiency thus limiting the resources that may be allocated to radial growth (Rennenberg *et al.* 2006).

Under a temperate climate, the season usually switches from an abundance of water in spring to a drier weather in summer. This alternation is therefore responsible for a cyclic pattern in the wood formation. First in the season, the cambium produces an early wood made of large cells with thin walls, that are very conductive to water. Then, it is followed by a late wood built with narrow cells with large walls, that are less conductive. Between them lays a transition wood. These modifications of the cellular structure of the wood reflects in its density, that varies from year to year from a low level (early wood) to a peak (late wood). At the end of the season, the annual radial growth remains visible in the wood in the form of a ring. Thus, for a given tree, the succession of wood rings reflects its plastic responses to the succession of past climatic environments (though other factors, such as pests and disease attacks, also play a role in the annual radial growth). The phenotypic plasticity of the tree rings characteristics is often referred to as 'dendroplasticity', and it has been demonstrated to be under genetic control (Sánchez-Vargas *et al.* 2007; Fallour-Rubio *et al.* 2009; Martinez-Meier *et al.* 2009).

1.4. Fundamentals of quantitative genetics

In this section, we propose to review the basics of quantitative genetics (most of this was directly extracted from Falconer and Mackay (1996)) especially in a view to the study of phenotypic plasticity and in application to hybrid populations. Quantitative genetics and statistical modeling have a long, common history (Fisher 1918), and we are going to present them together in order to expose how and in which situation the parameters are estimated and needed.

1.4.1. Genetic variance and covariance

In quantitative genetics, the value taken by a quantitative trait (or 'phenotype') P is considered to result from both a genetic and an environmental influence. This can be formalized as follows (Falconer and Mackay 1996):

$$P = G + E \text{ (eq. 1)}$$

where G is the genotypic value and E is the environmental deviation. At the population level, the average phenotype is equal to the average genotype, and the mean environmental deviation is assumed to be null. From this arises the decomposition of the variance:

$$\sigma^2_P = \sigma^2_G + \sigma^2_E \text{ (eq. 2)}$$

where σ^2 will refer to a variance, from now and subsequently. Thus, the phenotypic variance is the sum of the genetic and the environmental variances. Let's consider an experimental design where a given number of genotypes are randomly field tested. The basic model is:

$$y_{ij} = \mu + c_i + r_{ij} \text{ (mod. 1)}$$

where y_{ij} is the phenotypic value of the trait from individual of genotype i and repetition j, μ is the population mean, c_i is the genotypic effect, and r_{ij} the residual. The two latter terms are considered as random samples of normal distributions (Henderson 1975), with variances σ^2_G and σ^2_E respectively. From this we can compute the broad-sense heritability $H^2 = \sigma^2_G / \sigma^2_P$. Let's go further in the decomposition of the phenotypic variance (eq. 2). Indeed, the genotypic value decomposes as follows (Falconer and Mackay 1996):

$$G = A + D + I \text{ (eq. 3)}$$

where A is the additive value, D is the dominance value, and I is the epistasis. These values can be interpreted mechanically as gene actions. The additive value A arises from

additive allele effects, *D* arises from the interaction between alleles at the same locus, and *I* arises from all the other allelic interactions notably those between alleles at different loci. This molecular definitions imply a statistical meaning. The additive value *A* also called 'breeding value' is additively transmitted, meaning that offspring inherits, on average, the mean of their parents breeding values. At the individual level, each sib is characterized by a Mendelian sampling term that comes from the segregation of different maternal and paternal alleles, resulting in a breeding value that deviates from that of other sibs, and from the mean parental value. The dominance value *D* expresses in particular the statistical interaction between the parents, *i.e.* in the full-sib family effects. All the genetic effects that are neither *A* nor *D* are considered to constitute the epistasis value *I*. Just like eq. 1, eq. 3 leads to the variance decomposition:

$$\sigma^2_P = \sigma^2_A + \sigma^2_D + \sigma^2_I + \sigma^2_E$$

Let's consider an experimental design where a pedigree is available, that is, where the relatedness between individuals is known. Relatedness in a pedigree induces resemblance in inherited traits by the sharing of alleles, and this resemblance can be modeled by a covariation function linking all members in the pedigree. The model, called the 'animal model', is (Henderson 1975; Mrode and Thompson 2005):

$$y_{ij} = \mu + a_i + d_i + r_{ij}$$

The difference with the previous model (mod. 1) is that the genetic effect decomposes into an additive component a_i and a dominance component d_i. This components are considered as random samples of normal distributions:

- $a \sim N(0, \mathbf{A}\ \sigma^2_A)$, where $\mathbf{A}$ is the additive relationship matrix, such as $\mathbf{A} = (2\ f_{xy})$ with f_{xy} the Malécot's coefficient of coancestry (Malécot 1948). This matrix describes resemblances by probabilistic allele sharing in the pedigree. The coefficient of coancestry f_{xy} is defined as the probability that a random allele sampled at a given neutral, autosomal locus in both individuals *x* and *y* are identical by descent. As a consequence, the coancestry between an individual *x* and itself is $f_{xx} = ½\ (1 + F_x)$ where F_x is the inbreeding coefficient of *x*, that is, the probability that both alleles are identical by descent at a random locus of *x*.
- $d \sim N(0, \mathbf{D}\ \sigma^2_D)$, where $\mathbf{D}$ is the dominance relationship matrix, such as $\mathbf{D} = (d_{xy})$ with d_{xy} the dominance relationship. The dominance relationship is the probability that at a given neutral, autosomal locus, both alleles of individual *x* are identical by descent to both alleles of individual *y* (Mrode and Thompson 2005).

- $r \sim N(0, \mathbf{I}\,\sigma^2_R)$, with $\mathbf{I}$ an identity matrix. Here $\sigma^2_R = \sigma^2_E$, meaning that we make no distinction between the residual and the environmental variances.

This matrix specification builds the bridge between the 'statistical' and the 'molecular' definitions of each genetic component, and thereby these definitions should be similar. However, in practice the molecular architecture of a trait cannot be inferred from the animal model and the consequent variance structure (Schlichting and Pigliucci 1998; Huang and Mackay 2016). Despite the limitations, the animal model has been widely used in the genetics and breeding literature and it has been shown to be robust, especially to estimate the breeding values and the variance parameters (Cf all the quantitative genetics literature that will be quoted in this dissertation). As the model stands, the epistasis is neglected and therefore its variance is captured partly by the residual component. However, if clonal repetitions are available, a clonal effect can be added to the model in order to capture the epistasis variance. It is also possible to neglect the dominance term and the associated variance.

A quantitative genetics parameter of great interest that can be estimated using the animal model is the narrow-sense heritability $h^2 = \sigma^2_A / \sigma^2_P$. This parameter can be used in the breeder's equation, to estimate a genetic gain in selection (R) from a differential of selection (S): $R = S \times h^2$ (Falconer and Mackay 1996). The differential of selection can be decomposed as $S = i \times \sigma_P$ so that an unit-less quantity i, the intensity of selection, is introduced. Considering that the phenotypic value follows a normal distribution, i is computed from the standard normal distribution. For instance, after a selection of the top 5% individuals for next generation, $i_{5\%}$ is the mean of the top 5% of a standard normal distribution, so $i_{5\%} \approx 2.06$. The breeder's equation develops as:

$$\begin{aligned} R &= S \times h^2 \\ &= (i \times \sigma_P) \times (h \times \sigma_A / \sigma_P) \\ &= i \times h \times \sigma_A \end{aligned}$$

from which we can see σ_A as a potential for breeding, and the square root of the narrow-sense heritability h as the accuracy of mass selection.

From eq. 1, we have been considering that a phenotype was a trait. Actually, organisms display as many traits as we can measure. These traits are correlated to some extent, and part of this correlation may have a genetic determinism. The genetic correlation between traits arises from two mechanisms, that are mutually compatible: pleiotropy and linkage disequilibrium. Pleiotropy is the ability for a locus or a gene to affect simultaneously several traits. An example is an 'albinos' allele, that affects both skin, hair

and eye colors, thus contributing to the genetic correlation between them. Linkage disequilibrium is the deviation from the independence in the segregation between alleles at different loci, in particular alleles that affect different traits. Genetic linkage between loci favors linkage disequilibrium, but divergent selection, by natural selection or by artificial breeding, and non-random mating can also produce strong linkage disequilibrium (Falconer and Mackay 1996).

The animal model can be extended to account for several (*p*) traits simultaneously. Let's consider the following multi-trait animal model (neglecting dominance and epistasis) (Mrode and Thompson 2005):

$$\begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_p \end{pmatrix}_{ij} = \begin{pmatrix} \mu_1 \\ \mu_2 \\ \vdots \\ \mu_p \end{pmatrix} + \begin{pmatrix} a_1 \\ a_2 \\ \vdots \\ a_p \end{pmatrix}_{i} + \begin{pmatrix} r_1 \\ r_2 \\ \vdots \\ r_p \end{pmatrix}_{ij}$$

The means are not random, so there is no stochastic dependence between them. However, the additive effects and the residuals are bound across-traits by their respective covariance matrices:

- $\begin{pmatrix} a_1 \\ a_2 \\ \vdots \\ a_p \end{pmatrix}_i \sim N\left(\begin{pmatrix} 0 \\ 0 \\ \vdots \\ 0 \end{pmatrix}, \boldsymbol{A} \otimes \begin{pmatrix} \sigma^2_{A1} & r_{A:12}\sigma_{A1}\sigma_{A2} & \cdots & r_{A:1p}\sigma_{A1}\sigma_{Ap} \\ r_{A:12}\sigma_{A1}\sigma_{A2} & \sigma^2_{A2} & \cdots & r_{A:2p}\sigma_{A2}\sigma_{Ap} \\ \vdots & \vdots & \ddots & \vdots \\ r_{A:1p}\sigma_{A1}\sigma_{Ap} & r_{A:2p}\sigma_{A2}\sigma_{Ap} & \cdots & \sigma^2_{Ap} \end{pmatrix}\right)$, with r_A the additive correlations between traits, sometimes improperly referred to as genetic correlations.
- $\begin{pmatrix} r_1 \\ r_2 \\ \vdots \\ r_p \end{pmatrix}_i \sim N\left(\begin{pmatrix} 0 \\ 0 \\ \vdots \\ 0 \end{pmatrix}, \boldsymbol{I} \otimes \begin{pmatrix} \sigma^2_{R1} & r_{R:12}\sigma_{A1}\sigma_{R2} & \cdots & r_{R:1p}\sigma_{R1}\sigma_{Rp} \\ r_{R:12}\sigma_{R1}\sigma_{R2} & \sigma^2_{R2} & \cdots & r_{R:2p}\sigma_{R2}\sigma_{Rp} \\ \vdots & \vdots & \ddots & \vdots \\ r_{R:1p}\sigma_{R1}\sigma_{Rp} & r_{R:2p}\sigma_{R2}\sigma_{Rp} & \cdots & \sigma^2_{Rp} \end{pmatrix}\right)$, with r_R the residual correlations between traits. Just like for variances, here we don't make a distinction between r_R and r_E, the environmental correlation.

The concept of genetic correlation is central to breeding, as breeding is often (if not always) performed on multiple traits simultaneously. However, antagonistic correlations between traits can make the simultaneous selection for those traits of interest much trickier. Indeed, selecting for a trait 1 correlated to a trait 2 produces a correlated response to selection on trait 2 that can be opposed to the desired selection goal. As we have seen previously, the response to selection for trait 1 is $R_1 = i \times h^2_1 \times \sigma_{P1}$. For trait 2, the correlated response to selection is:

$$CR_2 = i \times (h_1 \times h_2 \times r_A) \times \sigma_{P:2}$$

where r_A is the additive correlation between traits 1 and 2. The term $(h_1 \times h_2 \times r_A)$ is called coheritability (Falconer and Mackay 1996) or coefficient of genetic prediction (Fins *et al.* 1992) between traits 1 and 2, and it can be seen as a multi-trait generalization of the heritability. As we previously stated, the traits 1 and 2 can actually be the same trait observed in different environments.

1.4.2. Environment and the variance decomposition

The variance decomposition as presented in eq. 2 assumes two hypothesis. First, it assumes that genotypes and environments are independent, so that there is no structure in the distribution of the genotypes within the environment. Second, that genotypes and environment are not interactive. In other words, this means that the phenotypic expression of genotypes is conditioned by the environment to which they are exposed in an additive way only, *i.e.* the reaction norms are parallel. Taking into account the deviations from both hypothesis, eq. 2 becomes (Falconer and Mackay 1996):

$$\sigma^2_P = \sigma^2_G + \sigma^2_E + 2\,cov(G, E) + \sigma^2_{G\times E}$$

where $cov(G, E)$ is the covariance between genotype and environment, and $\sigma^2_{G\times E}$ is the variance due to genotype-by-environment interactions (G×E). In the wild, due to natural selection, we can expect the genotypes to be distributed according to the environmental constraints. However, this is not necessarily true in breeding experiments such as progeny tests, where genotypes are purposely randomly distributed in controlled trials, within given environments. For this reason, in practice, it is common to accept the hypothesis of independence between *G* and *E* and to neglect the $cov(G, E)$ term. On the other hand, the question of G×E is important in breeding and central in this dissertation. Indeed, G×E is the statistical manifestation of genetic variance of plasticity (Primer 2004).

In order to illustrate the importance of G×E in quantitative genetics and especially in breeding, let's consider two genotypes G1 and G2 whose phenotypes are expressed in two environments E1 and E2, as seen in Fig. 3. In this example, the ranking of the genotype on their ability to produce a high phenotypic value changes depending on the environment. The G×E interaction is therefore critical in this example, and it makes the breeder task trickier as none of the two genotypes is globally better than the other. Beside the effect of G×E on the relative performances between genotypes, there is also an effect of G×E on the variance components. Let's consider that G1 and G2 are representative

samples from a larger set of genotypes. To the extent of the representativity of G1 and G2, the genetic variance (σ^2_G) expressed in E1 is much lower than the genetic variance expressed in E2. This is symbolized on the left of Fig. 3 by the much narrower normal distribution of genotypic values in E1 (upper normal distribution) than in E2 (lower normal distribution). Beside the effect of G×E on the genetic variance, it is also intuitive that the environment may affect directly the environmental variance (σ^2_E). Therefore, all components of the heritability can be affected by the environment.

For simplicity sake, the term G×E is used whereas, often, additive-by-environment interactions are actually meant (this is the same commonly accepted mistake as using the term 'genetic correlation' when meaning r_A). In this dissertation, G×E will always implicitly refer to additive-by-environment interactions.

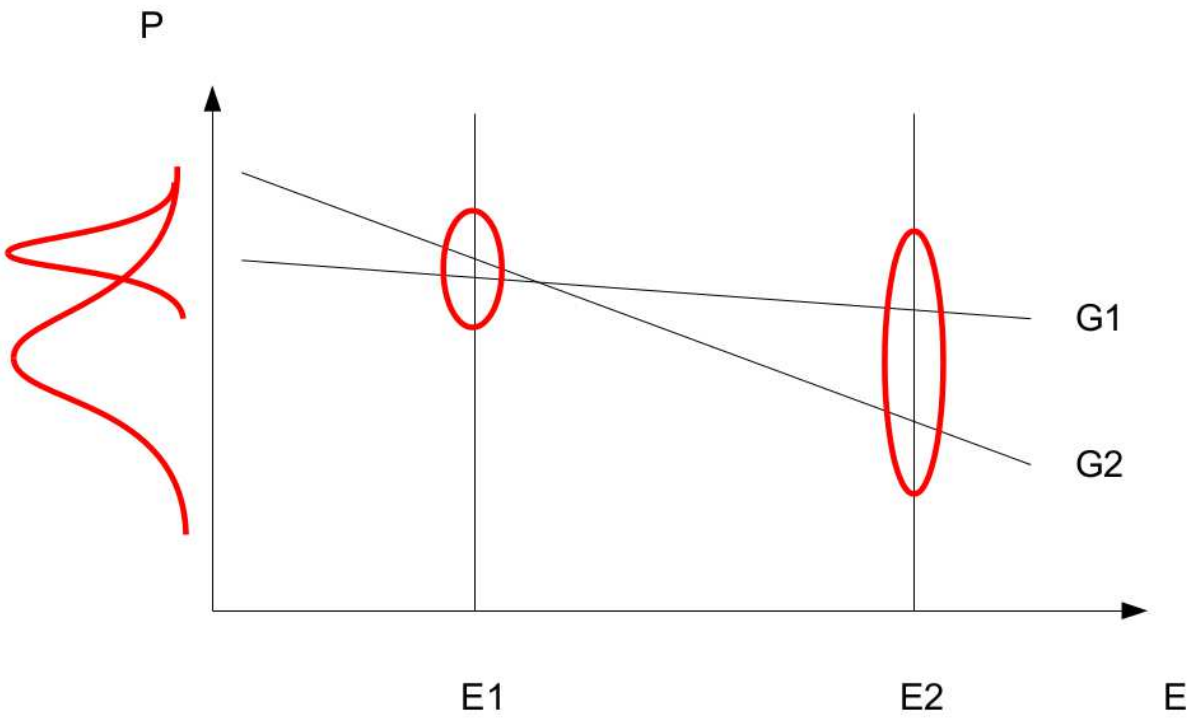

Figure 3: Illustration of G×E, for two hypothetical genotypes G1 and G2 exposed to two environments E1 and E2. Black lines: genetic performances expressed by the genotypes. Red distributions on the left side: hypothetical distributions from which the genetic performances are sampled, thus showing different genetic variances depending on the environment. Courtesy of Vincent Debat (2017)

1.4.3. Hybrids' genetic variance

Neglecting epistasis and G×E, hybrid phenotypic variance on a quantitative trait decomposes as follows:

$$\sigma^2_P = \sigma^2_{A1} + \sigma^2_{A2} + \sigma^2_D + \sigma^2_E \text{ (eq. 4)}$$

where σ^2_{A1} is the additive variance inherited from the genetic pool 1 and σ^2_{A2} the additive variance from the genetic pool 2. A statistical model has been proposed by Stuber and Cockerham (1966; see also Cros 2014) for hybrids genetic performances:

$$y_{ijk} = \mu + g_{1:i} + g_{2:j} + f_{ij} + r_{ijk} \text{ (eq. 5)}$$

where $g_{1:i}$ is the general combination ability (GCA) of the parent *i* from the genetic pool 1, and $g_{2:j}$ is the GCA of the parent *j* from the genetic pool 2. The interaction between the parents, f_{ij} or $i \times j$ family effect, is called the specific combination ability (SCA). Noteworthily, this model is defined at the family level. In other words, for two full-sibs the estimated genetic component ($\mu + g_{1:i} + g_{2:j} + f_{ij}$) is the same, therefore, the Mendelian segregation component is captured by the residual. As a consequence, not all the genetic variance is captured by the genetic components of the model. The variance specification is as follows:

- $g_1 \sim N(0, ½ \boldsymbol{A_1} \sigma^2_{A1})$, where $\boldsymbol{A_1}$ is the additive relationship matrix of the parents from genetic pool 1. This is the same specification for $g_2 \sim N(0, ½ \boldsymbol{A_2} \sigma^2_{A2})$. For non-inbred parents, the diagonal of $\boldsymbol{A_1}$ is 1. Therefore, is is straightforward that the variance of GCA is:

$$\sigma^2_{GCA\,1} = ½ \sigma^2_{A1} \text{ (eq. 6)}$$

- $f_{ij} \sim N(0, \boldsymbol{F} \sigma^2_D)$, where $\boldsymbol{F}$ is the relationship matrix for full-sib family effects (Hoeschele and VanRaden 1991; Lo *et al.* 1997). As such, the $(x, y)^{th}$ element of $\boldsymbol{F}$ is the dominance relationship between one random sibling from the family x and another random sibling from the family y. If the families are not inbred, the diagonal of $\boldsymbol{F}$ is ¼.
- $r \sim N(0, \boldsymbol{I} \sigma^2_R)$. Contrarily to the animal model, we make a distinction between the residual and the environmental variances. Indeed, the residual variance captures some genetic variance from the between-sibs Mendelian segregation (Lo *et al.* 1997; Cros 2014):

$$\sigma^2_R = \sigma^2_E + ½ \sigma^2_{A1} + ½ \sigma^2_{A2} + ¾ \sigma^2_D \text{ (eq. 7)}$$

Therefore, the phenotypic variance can be calculated from eq. 4 and 7:

$$\sigma^2_P = ½ \sigma^2_{A1} + ½ \sigma^2_{A2} + ¼ \sigma^2_D + \sigma^2_R$$

It is convenient for hybrids to estimate two narrow-sense heritabilities, one on each parental side, in order to understand how each of the parental genetic pool contributes to the hybrid variance, and thus to know which leverages are available for the breeder to improve the hybrid performances. These heritabilities are expressed as $h^2_1 = \sigma^2_{A1} / \sigma^2_P$ and $h^2_2 = \sigma^2_{A2} / \sigma^2_P$. We have seen previously that $h = \sqrt{h^2}$, and from the animal model it could be seen as the accuracy of mass selection, thus pinpointing its direct applicability to breeding. In the same way, the heritabilities in hybridization inform on our ability to predict the additive values in hybridization of parents from their hybrids' phenotype, as demonstrated by Cros (2014):

$$cor(P_H, g_1) = cov(P_H, g_1) / (\sigma_P \sigma_{GCA\,1}) \text{ (eq. 8)}$$

From eq. 5:

$$cov(P_H, g_1) = cov(\mu + g_1 + g_2 + f + r, g_1) = cov(g_1, g_1) = \sigma^2_{GCA\,1}$$

Therefore eq. 8 becomes:

$$cor(P_H, g_1) = \sigma^2_{GCA\,1} / (\sigma_P \sigma_{GCA\,1}) = \sigma_{GCA\,1} / \sigma_P$$

From eq. 6 we can deduct:

$$cor(P_H, g_1) = \sqrt{(\sigma^2_{A1}/2)}/\sigma_P = h_1/\sqrt{2}$$

Thus $h^2_1/2$ (or $h^2_2/2$) can be seen as the heritability at the cross level, and $h_1/\sqrt{2}$ as the accuracy of selection when selecting parents based on the performances of their hybrids. Heritability at the cross level is interesting because of its direct applicability in breeding, however in this dissertation we are more interested in the heritabilities at the individual level h^2_1 because of their comparability with heritabilities from the animal model (eq. 4). From this, the breeder's equation in hybridization allows the estimation of the theoretical response to selection for a hybrid if both its parents have been 'mass selected' from the observation of the phenotypes of a hybrid population (Cros 2014):

$$R = cor(P_H, g_1)\, i_1\, \sigma_{GCA\,1} + cor(P_H, g_2)\, i_2\, \sigma_{GCA\,2}$$

$$= \frac{h_1}{\sqrt{2}}\, i_1\, \frac{\sigma_{A1}}{\sqrt{2}} + \frac{h_2}{\sqrt{2}}\, i_2\, \frac{\sigma_{A2}}{\sqrt{2}}$$

$$= ½ (h_1\, i_1\, \sigma_{A1} + h_2\, i_2\, \sigma_{A2})$$

$$= ½ (h^2_1\, i_1 + h^2_2\, i_2)\, \sigma_P$$

with i_1 the intensity of selection in the population or species 1 and i_2 the intensity of selection in the population or species 2. The ½ that arises in this equation can be interpreted as the Mendelian sampling term, as parents only transmit half their genes to the hybrid.

1.5. Scientific questions

1.5.1. Questions of Chapter 1

A few European and Japanese larches were sampled from the INRA breeding population, and crossed in a diallel to produce pure species and hybrid progenies. From this almost complete and balanced mating design we aimed to describe the *sensu stricto* heterosis, *i.e.* the increase in vigor expressed by the hybrids with respect to their parental genetic material. Therefore, the first task of this thesis was to identify the traits expressing heterosis, and to quantify their level of heterotic response. More specifically, we characterized the dynamics of heterosis along age, and the environmental conditions of its expression.

Knowledge on the genetic variances and covariances of the components of the hybrid phenotype is also required in order to guide the hybrid larch breeding program. On the first hand, the variances information can indicate the possible genetic control or level of expected improvement of the hybrids performances: are there leverages at the taxon, at the family, or at the individual parent level ? On the second hand, the genetic covariances between these performances are also critical. Genetic correlations between traits may lead to the identification of antagonisms impairing the breeding effort, or on the contrary facilitating synergies. The correlations between performances across sites answers the question of the generalization of the gain in selection obtained in a specific location: will it also apply elsewhere ? We finally addressed the question of the correlation between the performances in pure species and in hybridization, in order to assess to what extent evaluation within pure species impacts improvement at the level of hybrids and helps to devise the breeding strategy for hybridization.

1.5.2. Questions of Chapter 2

The 2nd Chapter is a focus on the specific question of the environmental triggers behind hybrid larch heterosis. Indeed, our goal here was to contribute to the understanding of the role of the varying climatic environment on the construction and the modulation of the hybrid vigor that is eventually observed on mature trees. Given that the mature stem is constructed as a sum of annual growth increments, then the superiority observed in the mature stem necessarily arises from a sum of superiorities expressed in the annual growth increments. But, annual growth is plastic and it varies depending on environmental factors, such as the soil water availability. Therefore, phenotypic plasticity could be involved in the construction of the hybrid larch heterosis in several ways: is the hybrid able to maintain its productivity in unfavorable climatic conditions ? Or, is it able to take advantage from the most favorable weather to outperform its parental species ?

We suspected phenotypic plasticity not to only affect the contrasts between taxa, but more broadly the contrasts between genotypes. For this reason, we also questioned the role of phenotypic plasticity on the genetic variances and covariance expressed for the main wood formation traits, namely, the radial growth and the wood density. This aimed to contribute to the understanding of how the differences in performances between mature trees are built-up, and to the comprehension of the ultimate set-up of a negative genetic correlation between circumference and wood density.

This study was conducted with a random regression framework, and we questioned the generalization of this method for plasticity studies with a complementary simulation analysis.

2. Materials and methods

2.1. Experimental set-up

2.1.1. Mating design

The ability of some elite hybrid varieties to outperform elite varieties from the parental species, that we can define as the 'hybrid superiority', is the core reason for raising hybrids and of obvious interest for the forest managers. However, geneticists are more interested in *sensu stricto* heterosis such as we previously defined it, that is, the superiority of the hybrid in comparison to its parental genetic material (average-parent heterosis or best-parent heterosis). The parental genetic material could be represented directly, by cloning, or by its pure species offspring. A clonal set-up would have been particularly difficult to set-up for larch due to the recalcitrance of these species to this methods of propagation (except using grafting, but this involves the use of an exogenous rootstock). So, clearly, progeny testing is the only real possibility so far. An orthogonal experimental plan, appropriate for this question, is the diallel mating design (Hinkelmann 1974). An extra interest of such a design is that the performances of the parents in hybridization are assessed along with their performances in pure species crosses, with a comparable method (offspring *vs.* offspring). Moreover, up to now, seedling is the privileged way to deploy larch varieties: the performances and parameters as expressed in such a progeny are homogeneous with what can be expected from this genetic material if it were used to produce commercial seed lots.

The experimental population consists in a full diallel between 9 European larches (EL, *Larix decidua*) from the Sudetan mountains and 9 Japanese larches (JL, *L. kaempferi*). Originally, a second origin of EL was also intended to be included in the set-up: the Polish origin. A population of hybrid trees mixing a Polish EL mother and a JL father is represented in one of the experimental sites. Moreover, some trees are derived from polycrosses involving one tree from the diallel and a mixture of pollen lots. The whole mating design as available in each site is detailed in the Appendix 1 of Chapter 1. Finally, some trees that were not related to the diallel at all (*i.e.* commercial references, and even some Douglas-firs) were also present in the field but excluded from the analyses.

The mating campaign to produce the diallel began in 1988, and several years were necessary to collect enough seeds, from almost all the possible combinations, to produce

an almost full and balanced design. The seeds were sown in spring 1995 in the INRA nursery and raised for one additional year in the nursery before being transferred to their definitive sites when the seedlings were 2 years old.

2.1.2. Sites and set-up

Three ecologically contrasted sites were originally considered for plantation in France: Saint-Appolinaire (SA, 45°58'N 4°26'E, 784 m a.s.l.), Saint-Saud (SS, 45°31'N 0°48'E, 307 m a.s.l.) and Cumières, a site in the Meuse department (Fig. 4). The plantation took place in spring 1997, a drastically dry spring which obliged an exceptional watering of suitable sites: Saint-Saud and Cumières; this operation was not possible at Saint-Appolinaire due to the steep slope. This operation was mostly successful excepted at Cumières, which recorded severe losses and was discarded from the present dissertation. To compensate this loss, a clonal copy of SS was produced. To do so, three scions were collected during winter 2002 from a large sample of trees in SS (41% of SS trees were successfully cloned), grafted on rootstocks (2 scions on a HL rootstock and 1 scion on a JL rootstock), grown 2 years at the administrative nursery Peyrat-le-Château, and then set-up in a third site within INRA Peyrat-le-Château estate (PC, 45°49'N 1°44'E, 461 m a.s.l.) before 2004 spring (Fig. 4). Grafting on rootstocks was a necessity because of the difficulty to multiply larch by cutting. The choice of 2 HL and 1 JL rootstocks was driven by the wish to test a possible rootstock species effect. The sites are described in Table 2.

Figure 4: Map of the sites included in the study (St Saud, Peyrat-le-Château, St Appolinaire). Picture from Google Earth©

The experimental design in SA and SS was single-tree plot randomized incomplete blocks, while in PC the 3 scions were planted together in 3-tree row plots randomized incomplete blocks. Thereby, we selected the option to mix up all taxa instead of growing them separately in larger plots. The advantage of doing so is a better account for the micro-environment (spatial) heterogeneity and a better randomization across the field. Moreover, there are less boundary trees to discard, that would have reduced the effective size of the set-up. The counterpart of this option is that the different taxa, that have different growth dynamics, could compete with each other. Up to now, only SS was thinned in 2004 and in 2010.

Table 2: Description of the environmental conditions in each site (Saint Appolinaire 'SA', Saint Saud 'SS', Peyrat-le-Château 'PC'). An asterisk (*) indicates that the record concerns the period from the 1st of April to the 31th of October. Computed from Météo-France climatic data.

Site	Temp.: mean (°C)*	Temp.: s.d. (°C)*	Total rain (mm)*	Proportion of rainy days (%)*	Number of freezing days	Average wind (m/s at 10 m)	Altitude (m a.s.l.)	Slope (%)	Previous crop	Spacing of plantation
SA	14	5.2	595.4	45.9	80	5.4	784	22.3	Forest	3 m × 3 m
SS	16.3	4.5	619.4	37.1	33.4	2.8	307	4.7	Pasture	4 m × 2.5 m
PC	15.5	4.5	632	49.8	43.6	2.4	461	5.2	Forest	3 m × 2.5 m

2.2. Data collection

2.2.1. Field measurements

The three plantation sites have been measured regularly from the age of plantation by INRA technical staff (with my modest contribution for the 2014 campaign). Measurement included growth traits, namely height (HT) and breast-height circumference (BHC) as shown on Table 3. Total height was measured firstly with a pole and later using Vertex IV, a product of the Haglöf company (www.haglofcg.com). In most situations, the height of previous years (up to 2 years ago) could be retrospectively assessed from the stem increment growth delimited by pseudo-verticils.

Table 3: Growth traits measurement campaigns in each of the 3 sites (Saint Appolinaire 'SA', Saint Saud 'SS', Peyrat-le-Château 'PC'). SA and SS were planted in 1997 and PC in 2004. 'BHC': breast-height circumference and 'HT': height.

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
SA				BHC HT					BHC HT					BHC HT				
SS		HT		BHC HT			BHC HT	BHC HT	BHC HT				BHC HT					BHC HT
PC										HT								BHC HT

Aside growth, other traits were assessed in the field. Stem flexuosity (FLX) has been observed during almost all campaigns. Stem flexuosity was assessed with a 1 to 5 scoring scale drawn and adapted from Keiding and Olsen (1965) as shown on Fig. 5. The Keiding and Olsen scale was intentionally developed with the goal to favor the isolation of the genetic signal of stem flexuosity (*i.e.* to maximize its heritability) in application to larch.

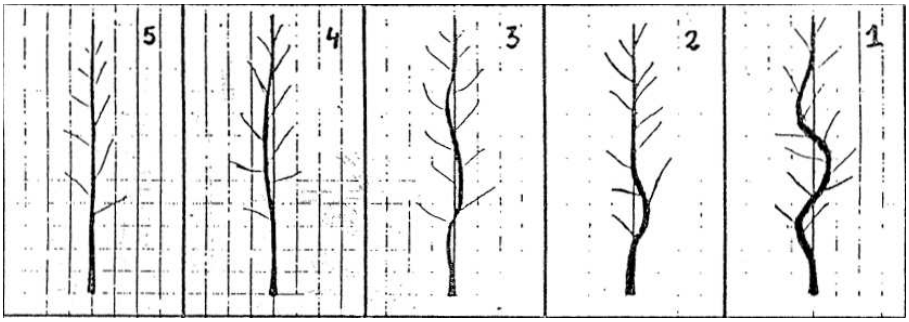

Figure 5: Stem flexuosity on a 1-5 scale, adapted from Keiding and Olsen (1965).

Bud phenology was also assessed one to two times at each site. The record consisted in a notation from a 0 to 5 score according to Gauchat and Pâques (2011) (Fig. 6), plus an extra score: 6, stem elongation. Phenology was assessed on the terminal bud.

Figure 6: Bud flush phenology on a 0-5 scale. From Gauchat and Pâques (2011)

From the measurements and the follow-up of the 3 sites, we could provide a better insight on their 'broad-sense fertility' of each site, that is, the ability of the forest stands to thrive therein. On the Table 4, we propose an overview of the forest stand in each site. The

most mountainous station SA was the poorest (the trees were the lowest there) and SS the most 'fertile'. The previous crop in SS, a fertilized pasture, may be involved in this performance. Within each site, the mortality underwent by each taxon often correlated to the height, except in SS where the mortality was overall due to the thinnings. European larch only outperformed JL (in height and survivability) in SA.

*Table 4: Description of the forest stand in each site (Saint Appolinaire 'SA', Saint Saud 'SS', Peyrat-le-Château 'PC'). The losses are recorded on a period whose duration is indicated, and the height were measured at a given age. The number of trees corresponds to the number of trees alive at the first field measurement (*i.e. *the trees that survived at the plantation).*

Site	Taxon	Losses (%)	Period (losses)	Height (m)	Age (height)	Number of trees	Number of blocks
SA	EL	6.1	12 yrs	11.44	14 y/o	544	66
	HL	6.2		12.69		2260	
	JL	24.2		10.41		945	
SS	EL	87.6	16 yrs	15.49	13 y/o	396	35
	HL	53.6		17.33		1361	
	JL	69		15.72		918	
PC	EL	10.6	8 yrs	10.81	11 y/o	517	44
	HL	6.7		13.19		1824	
	JL	4		12.42		865	

2.2.2. Increment cores data

Increment cores were collected in all three sites. In SA and in SS, all trees were sampled. This implied a special effort in SS to harvest the cores from trees before they were felled. In PC, only 1 tree per plot was randomly sampled (*i.e.* all ortets were represented by a single ramet). A summary of the age at sampling, and the size of the samples that were finally used are provided in the Appendix 1, Table 7 of Marchal et al. (2017).

On each diameter core, only the radius with the fewest defects was kept for further analysis. The half cores were sawn to 2 mm thick board, sanded, X-rayed to produce microdensitometric profiles that were treated with WinDENDRO (Regent Instruments Canada Inc. 2008). All this work was performed by the staff of the platform INRA-GENOBOIS. An example of microdensitometric profile is provided in Fig. 7. As seen on this illustrative sample, the succession of early and late wood allowed the reconstruction of

a the tree ring chronology. A consequent part of my work consisted in a manual check of the chronologies in order to avoid possible mislabeling of the rings. For instance, due to the 2003 heat wave (Bréda *et al.* 2006; Rennenberg *et al.* 2006), the corresponding ring is very narrow and can be confounded with a false ring, that is, a peak in wood density occurring in the middle of the growing season and that does not delimit a new year (*e.g.* on Fig. 7, the 2004 ring presents 2 false rings).

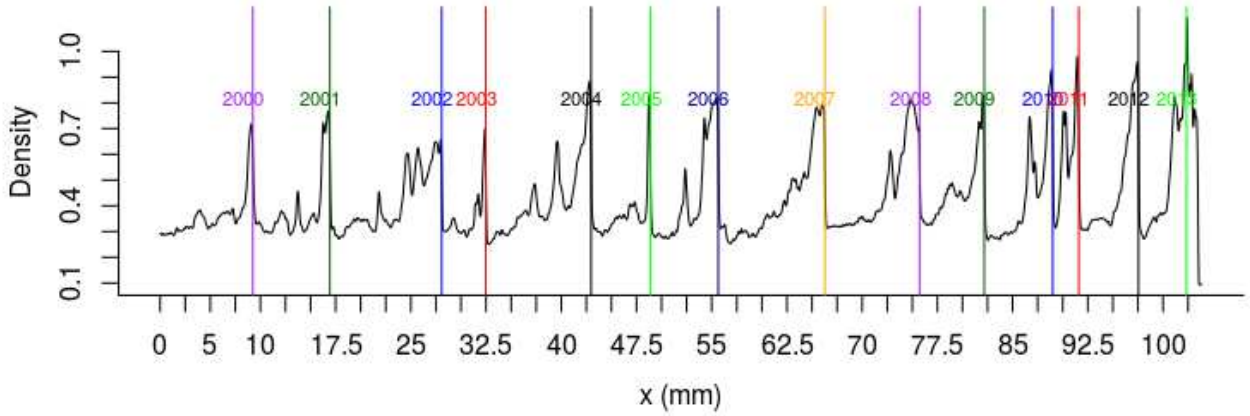

Figure 7: Microdensitometric profile from Saint-Appolinaire. The pith is on the left and the bark on the right, though they do not appear on the profile.

We noticed a particularity in SA cores, for which we did not have an explanation. For 129 half cores (6 % of the cores collection in this site), the ring corresponding to year 2012 was absent and for 60 other cores (2.8 % of the collection) the 2012 ring was extremely narrow. For the 129 cores for which the 2012 ring was absent, 93 had an exploitable opposite radius. On these opposite radius, 46 rings corresponding to the year 2012 were absent, 19 were extremely narrow, and 28 were normal. Up to now, we are still unable to explain this phenomenon, as it was evenly distributed in the site and across the taxa. As a possible explanation, this could be the effect of the frost events that occurred at the beginning of the season, as shown on the Fig. 8. The February frost was extremely harsh but the buds were supposedly dormant, and the April frost was lighter but the buds could have already started to flush. Alternatively, some pests (*e.g*. leaf-eating Lepidoptera such as *Coleophora laricella* or *Zeiraphera diniana*, (Nageleisen 2001)) could have prevented these trees from growing a normal ring this specific year, but yet we do not have experimental support for such hypothesis. In the subsequent analysis, the missing rings were considered as having a null width and an unknown density.

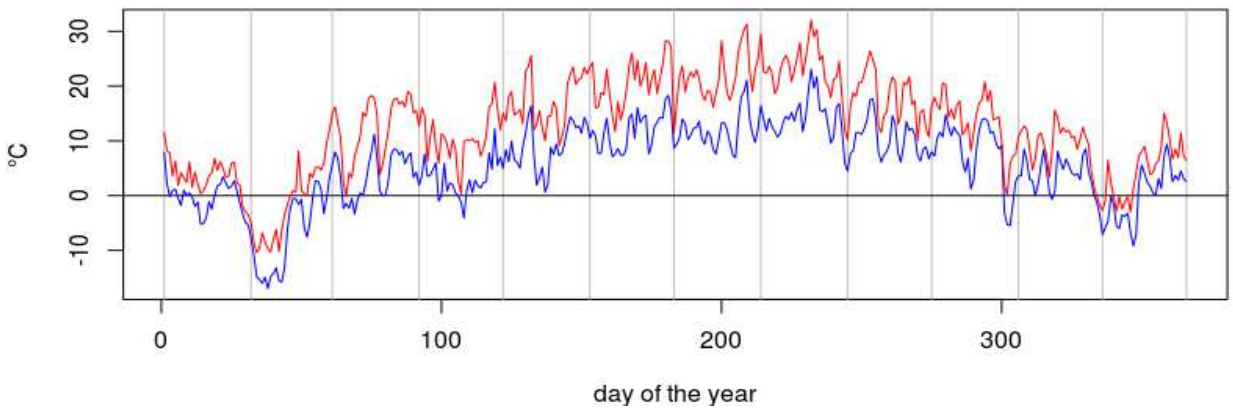

Figure 8: Temperature (blue: daily minimum and red: daily maximum) in St Appolinaire in 2012

The microdensitometric information was used for two different purposes. On one hand, it contributed to the overall phenotyping of trees for their genetic evaluation and to the assessment of their growth dynamics. Ring width (RW) was used to infer BHC whenever the latter measure was not field-evaluated. From the ring mean density (RMD), we could estimate the stem overall density (DEN), year after year. Finally, at the same time the tree ring chronologies were checked, the sapwood / heartwood delimitation was visually assessed on the core and translated into a surface radio using the RW information; the corresponding trait was thus the heartwood proportion (HWP) expressed in percentage. On the other hand, the microdensitometric profiles gave an access to the incremental growth information, at the annual scale. The corresponding traits were only RW and RMD. This information was leveraged to address the question of the role of the climatic environment on the annual growth and wood properties.

2.2.3. Environmental data

In a preliminary analytical step, all 3 sites were independently analyzed with a model that included a spatial component (see Chapter 1). The structure of the spatial effects in the field, especially on trees height, was interpreted as a proxy of the repartition of the fertility (in the broadest definition) within each site, the best growing trees being on the most fertile spots. We excavated 2 pits in each site (3 in SS) in the most contrasting areas, *i.e.* the areas where the spatial effects were the lowest and the highest. In Fig. 9, we present pictures of the 2 pits excavated in SA, and their position on the spatial effects map. Noteworthily, the pits corresponding to the highest spatial effect showed the deepest

horizons at any of the three sites. Contrarily, the pits with lowest spatial effects were the ones with shallow soils. We described the pits (depth of the horizons, stoniness, roots organization) (an example of a pit observation sheet is provided in Appendix 1) and sampled some soil from each horizon for further analysis, performed at INRA-Arras (for texture, chemical composition). Each pit in each site was identified by a code (A1, A2... C3) as seen on Table 5. From this soil information we computed the available water capacity (AWC) at each pit (Bruand *et al.* 2004).

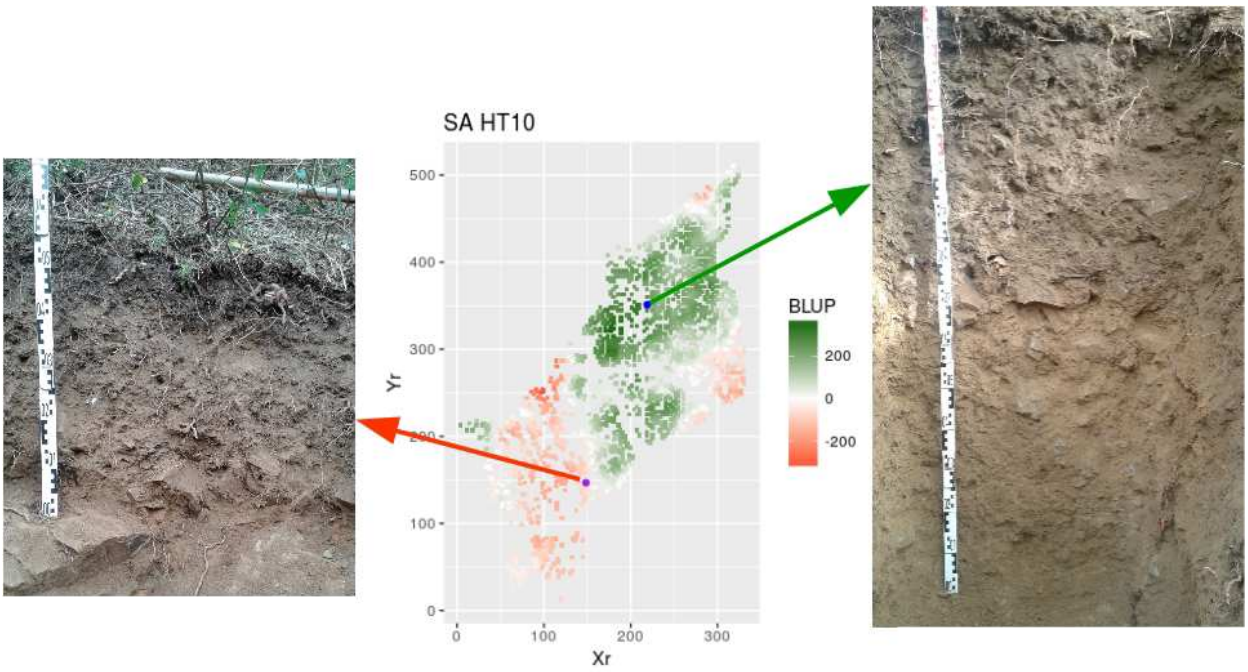

Figure 9: Pits excavated from Saint-Appolinaire (left and right) and position of the pits on the spatial effect map (center). The spatial effects are relative to the height in 2010 ('SA HT10'). 'BLUP' indicates that the spatial effects were estimated as best linear unbiased predictors. The scale indicates that a positive spatial effect on height was colored in green and a negative effect was colored in red.

We recovered daily climatic data provided by Météo-France via the platform INRA-CLIMATIK. The data came from the weather stations being the closest to the sites, with a special care in the choice for the similarity of the station to the site with respect to the altitude. Using a water balance model (Granier *et al.* 1999), we estimated daily variations of the relative extractable water (REW), varying from 0 (permanent wilting point) and 1 (field capacity).

Using BILJOU© (https://appgeodb.nancy.inra.fr/biljou/), the official implementation of Granier et al. (1999)'s water balance model, we described the climatic environment in each pit from each site and for each year, with an arbitrary leaf area index (LAI = 3). The

results are recorded in the Table 5. The most important pattern we see is that there is more variability within the sites than between. In any site, the 'fertile' pit showed no to a very few drought events (pits A1, B2, C1), and this was very contrasted with what happened in the 'harsh' pit (pits B1, A2, C3).

Table 5: Description of the climatic environment in each of the pits dug within each of the 3 sites: (a) number of days of stress (REW < 0.4) and (b) first day of stress (if there was a stress). The colors thresholds are in (a): no water stress (green), between 1 and 35 days of stress (yellow) and 36 or more days of stress (red); and in (b) the stress occurred before the day 152 (1st of June) (yellow) or the day 152 or later (red). The daily REW was computed using BILJOU©, assuming a constant LAI of 3.

a

		year																	
site	pit	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
SA	A1		0	0	0	0	0	28	0	9	3	0	0	2	0	0	0	0	
SA	A2		19	6	18	28	4	61	24	49	35	0	4	53	9	15	5	13	
SS	C1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SS	C2	4	40	4	31	17	11	18	15	48	23	0	4	25	27	27	40	29	0
SS	C3	52	57	39	53	41	26	33	29	74	42	13	18	62	49	51	63	42	22
PC	B1								45	44	36	10	24	69	40	44	51	38	29
PC	B2								0	0	0	0	0	0	0	0	0	0	0

b

		year																	
site	pit	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
SA	A1							175		221	211			230					
SA	A2		137	174	173	152	170	161	168	171	161		179	151	183	138	205	197	
SS	C1																		
SS	C2	213	141	175	176	151	177	171	166	161	160		213	155	189	147	214	195	
SS	C3	114	133	149	170	145	169	151	139	145	154	216	181	149	141	118	138	163	139
PC	B1								137	131	153	196	174	143	140	128	138	157	138
PC	B2																		

From Table 5, we can also draw some general lines about the particular weather of each year. The heat wave of 2003 had a strong effect in both pits in SA, whereas it was not a special year in SS. The years 2005 and 2009 were also constantly among the driest ones in all 3 sites. Likely due to their spatial proximity, the most oceanic stations SS and PC had comparable patterns and the whole period from 2009 to 2013 was notedly dry within these sites, with drought events happening early in the season.

These results should be considered with care, as (i) a constant LAI over years in such young and fast growing stations is unrealistic; (ii) these pits are not representative of the whole sites, but they are somehow their extremes; (iii) several factors were not accounted for in the model: the slope and the south exposition in SA, the fertilizer remainder from the previous crop (a pasture) in SS.

2.3. Inference

Throughout the two chapters of this dissertation, we tested a couple of hypothesis and we estimated several parameters. The hypothesis testing (*e.g.*, 'does the hybrid outperform its parental references for a given trait ?') was done in a frequentist way, that is, proposing a null hypothesis ('all taxa performed similarly for this trait') and evaluating the likelihood (or the unlikelihood) of this null hypothesis by the mean of a p-value. On the other hand, the parameters estimation was performed in a Bayesian frame that we will briefly describe here. All this information can be found in the Bayesian literature (*e.g.* Gelman and Hill 2007; Sorensen and Gianola 2007).

2.3.1. Bayesian inference

In classical frequentist statistics, the data (X) are considered as random samples from a distribution having fixed but unknown parameters (θ). The best estimate for the parameters is considered to be the one that maximizes the likelihood of the data, *i.e.* the probability of the data given such parameters P(X|θ). Bayesian statistics tackle the problem the other way around, considering that the best estimate of the parameters is the one that maximizes P(θ|X), *i.e.* the probability of the parameters given such data. The parameters are thus seen as random. To obtain P(θ|X) from P(X|θ) we use the Bayes' theorem:

$$P(\theta|X) = \frac{P(X|\theta)P(\theta)}{P(X)}$$

Two new terms appear here. First, P(θ) is the distribution of the parameters independently of the data: this is the prior, that is, the *a priori* idea we have about the possible distribution of the parameters. Then, P(X) is the distribution of the data independently of the parameters. In other words, P(X) is the integral of all the possible values that can take the data on the whole parametric space. That plays no role in the estimation of the parameters θ, so it can be neglected:

$$P(\theta|X) \propto P(X|\theta)P(\theta)$$

From this perspective, we can name P(θ|X) the 'posterior', that is, the distribution of the parameters that results *a posteriori* from the confrontation of our prior to the reality of our data. Bayesian statistics are therefore subjective, to the extent it uses prior information that is independent from the data under analysis. Bayesian statistics have both advocates

and detractors, and there are some philosophical consideration for or against the use of subjectivity in the analytical process (Gelman 2008).

2.3.2. Monte-Carlo Markov Chain

Philosophical considerations aside, our core motivation for using Bayesian statistics was the possibility to leverage Monte-Carlo Markov Chain (MCMC) algorithms, that are very convenient for several reasons. On the first hand, some MCMC algorithms are very robust solvers. We performed the multivariate analysis of up to 9 traits simultaneously, with highly structured variance-covariance matrices. I am sure that this analysis could have been done with some of the most efficient frequentist solvers as well, but it seems that, maybe due to their relative ease of implementation, performing MCMC software are blooming much faster than their maximum-of-likelihood, frequentist counterparts.

Another very important advantage of the MCMC algorithms, probably the most important, is to give access to the posterior distribution of each parameter, and therefore, to allow a great control of the uncertainty by means of credible intervals (the interval in which the parameter has *e.g.* 95% chance to be). Moreover, it is possible to build iteration by iteration the Markov Chain of derived parameters, such as correlations and heritabilities, and therefore to assess their uncertainty without the need for further assumptions about their parametric distribution.

The principle of Monte-Carlo is to describe the posterior distribution by sampling it. The construction of a Markov Chain is an efficient way to perform this random sampling. Several methods exist to build Markov Chains, Gibbs sampling being a famous and common one. Therefore at the end, the model is not solved with a point estimation of the parameter vector but with *n* samples of the parameter vectors instead. The first step is to ensure that the Markov Chain has well converged, as shown on Fig. 10.

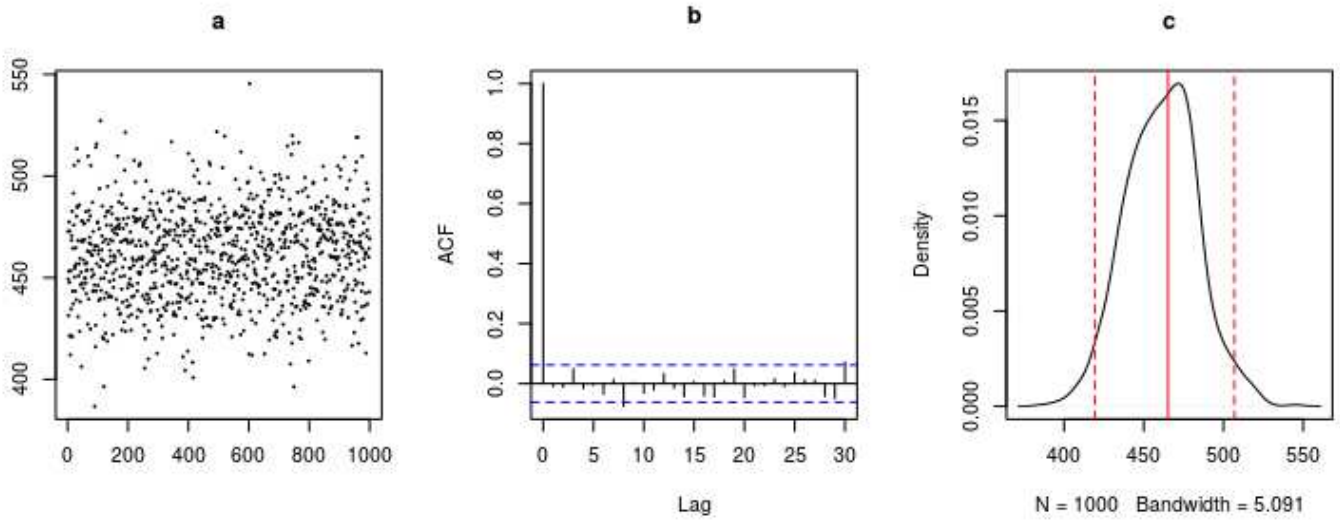

Figure 10: Convergence check for the Markov Chain of the mean (μ) of European larch in Peyrat-le-Château for circumference 11 years after plantation. (a): the row Markov Chain of μ in the order of the sampling. (b): autocorrelation function of the Markov Chain. The autocorrelation of lag 0 is by definition 1. In blue, the threshold of significance of the autocorrelation. (c): The marginal posterior distribution of μ, as estimated from the Markov Chain. In red, plain line: the mode of the posterior or maximum a posteriori; ashed line: the 95% credibility interval of μ.

2.3.3. Priors

The choice of priors in Bayesian statistics is a critical step, as it eventually impacts the posteriors and thus the estimates of the parameters. A common strategy is to select flat priors, *i.e.* priors that distribute the information evenly along the parametric space, in order to isolate the expression of the data information P(X|θ). This is an objectionable strategy, especially when dealing with small datasets as in such case the data contain few information to override the uncertainty brought by the prior (McNeish 2016). However, we assumed that our large datasets were informative enough to justify the use of flat priors.

We used the software (Hadfield 2010) default flat priors for the fixed effects of the models, that were normal distributions with null means and high variances. Defining priors for the variance-covariance matrices is a more tricky task, as the information that is given for the variance has an impact on the covariance and reciprocally. We used inverse-Wishart as priors for the variance-covariance matrices. The strategy we adopted was to use flat improper priors for the residual and environmental variance-covariance matrices, that is, priors that do not integrate to 1 (as should any distribution) (Hadfield 2016); and to use parameter expansion (PX) for the genetic variance-covariance matrices (Liu *et al.*

1998; Hadfield 2016). We checked that the correlations marginal priors under PX were uniform in the interval [-1, 1] as shown in Fig. 11. To do so, we proceeded to a Monte-Carlo sampling in a parameter-expanded inverse-Wishart and sampled 10^5 independent realizations from this distribution. Moreover, the variances marginal priors were informative close-to-zero, allowing very low genetic variance estimation; and they had a long and regular tail, allowing also the estimation of high values of variances (Fig. 11). We selected this option so that the genetic variances of different traits, expressed in different units, and with different magnitude, could be estimated with the same marginal variance prior.

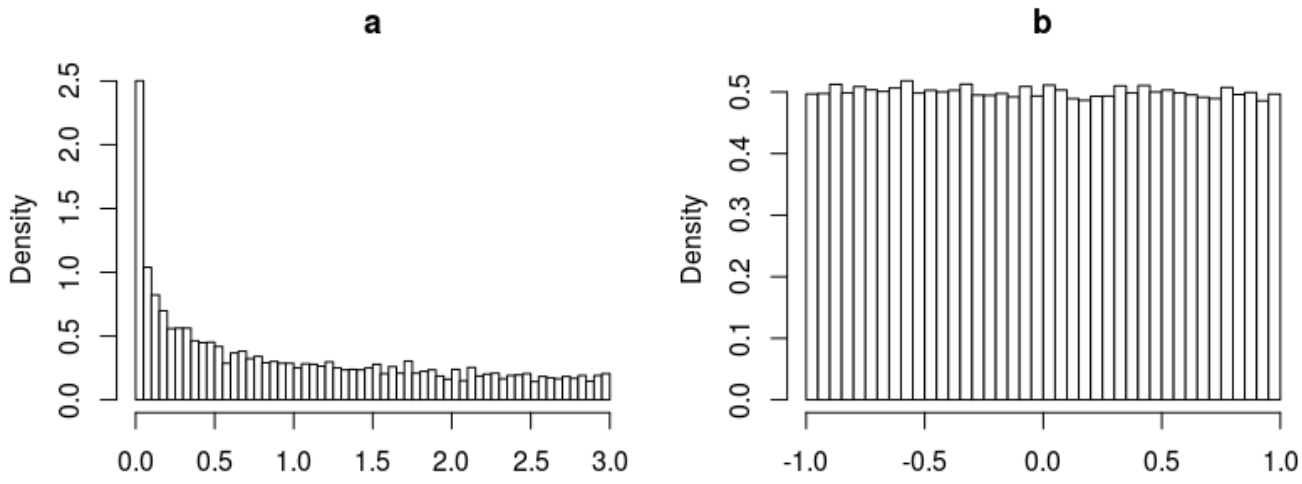

Figure 11: Marginal prior for the variances (a) and the correlations (b), from a parameter-expanded inverse Wishart (p = 6 dimensions, $\boldsymbol{V} = \boldsymbol{I_6}$ *(identity matrix of dimension 6),* $\nu = p + 1 = 7$, $V_\alpha = 1000\,\boldsymbol{I_6}$, $\mu_\alpha = 0$, *Cf Hadfield (2016) for more information).*

3. Chapter I. Hybrid larch heterosis: for which traits and under which genetic control?

The goal of this 1st Chapter was to describe the genetic determinism of 6 traits of major interest: height (HT) and circumference (BHC), stem flexuosity (FLX), wood density (DEN), bud flush phenology (BUD), and heartwood proportion (HWP). We addressed the question of the existence of heterosis for each of these traits, and we quantified its magnitude. The dynamics of heterosis over years, and the effect of the sites in its expression, were also investigated. Finally, we dissected the variance-covariance structure of these traits in pure species and in hybridization. The correlations between the traits, between their expression in the different sites, and between their expression in pure species and in hybrid offspring, were estimated and discussed.

3.1. Analytical considerations

3.1.1. The two-step approach

To achieve this goal, we considered a two-step approach through 2 different models as summarized in Table 6. The main difference between the two steps (namely models M1 and M2) was that M1 included the information of all taxa together. Therefore, M1 was the most appropriate model to:

- Compare the taxa and test the hypothesis of their dissimilarities. Thus, best-parent heterosis (*i.e.* the contrast between the population mean of the hybrid and the population mean of the best performing parental species) could be directly tested for.

- Predict spatial effects using the whole information from each site. Polycross progenies, and offspring from the Polish origin, were therefore included in order to increase the representativity of the spatial effects.

Model M1 was run for each combination of sites, traits, and available ages. Thus, it provided temporal series from which the dynamics of the contrasts between taxa, including best-parent heterosis, could be assessed.

On the contrary, the model M2 was fitted for each taxon separately. Only the progeny from the diallel was included (*i.e.* we excluded the offspring from the Polish parents and from the polycrosses), so that the genetic parameters estimates were only related to the diallel parents genetic material. Indeed, the purpose of the model M2 was to extract the

quantitative genetics information (variance parameters, breeding values) out from the diallel. This justified the use of a multivariate (in order to estimate the across-traits covariance) Bayesian (in order to control the uncertainty around the parameters estimates using MCMC) framework for model M2. However, this framework being computationally costly, M2 was only fitted for a given age in each site.

Table 6: Comparison between the models M1 and M2 from Marchal et al. *(2017)*

	Model (M1)	Model (M2)
taxa analysis	all together	separately
data	all available	pure diallel only
focus (estimations)	mean parameters	variance parameters
focus (predictions)	spatial effects	breeding values
response	phenotype	phenotype – spatial effects
additive effects	yes	yes
dominance effects	yes	yes
number of responses	univariate	multivariate
solution	frequentist	Bayesian

3.1.2. Account for the competition

In order to 'clean' the genetic signal for model M2, we subtracted from the phenotypes the spatial effects that were predicted by the model M1. In a preliminary research, we also tried to extract from the data the competition considered here as a noise. The competition noise was modeled as a competition term c_L (the lower case 'l' was changed to an upper case 'L' for readability) to be added to the term (M1c) in Marchal *et al.* (2017) (Muir 2005; Muñoz and Sánchez 2015):

$$c_L = w_L \sum_{m=1}^{M(max=8)} \frac{1}{dist^2(L,m)} (ca_m + ce_m)$$

with ca_m and ce_m respectively the additive competition effect and the permanent environment competition effect from the neighbor *m*, separated from *L* by a distance *dist(L, m)*. As the trees were planted according to a grid, there were up to 8 direct neighbors (each side plus each diagonal) that were accounted for in the model. Finally, w_L was a weighting such as $\sum_{m=1}^{M} \left(w_L \frac{1}{dist^2(L,m)} \right)^2 = 1$ so that the competition variances

(the variance of the 'inflicted' effects) could be interpreted as components of the phenotypic variance (the variance of the 'suffered' effects) (Cappa and Cantet 2008). Additive competition effects were structured $ca \sim N(0, \sigma^2_{CA}\,\boldsymbol{A})$ and permanent environment effects were not structured $ce \sim N(0, \sigma^2_{CE}\,\boldsymbol{I})$.

In this preliminary research, the model M1 did not have exactly the same structure than presented in Marchal *et al.* (2017) as the additive variance in hybridization on one parental side was forced to be half the additive variance in pure species from this parent. The core question, however, was the possible impact on variance partition of non-informative ('dumb') individuals in the spatial variance matrix ***S*** (model (M1c) in Marchal et al. (2017)). Indeed, the inclusion of a regular grid made of dumb individuals in ***S***, for which a spatial effect is predicted, allows the simple representation of the spatial effect map as a plain raster. The inclusion of dumb individuals in ***S*** is not supposed to alter the variances estimates as they do not participate to the likelihood. However, fitting the model with breedR (Muñoz and Sánchez 2015) and the expectation-maximization algorithm, led to a surprising pattern: adding dumb individuals in ***S*** altered the variance partition when (and only when) the permanent-environment competition effect was present in the model (Fig. 12). The permanent-environment competition variance σ^2_{CE} was particularly unstable. This unexpected result led us to conclude that the model was likely over-parametrized. For this reason, from here, we did not continue the investigations on the competition model, though we acknowledge competition as a potentially major factor of disturbance in our data.

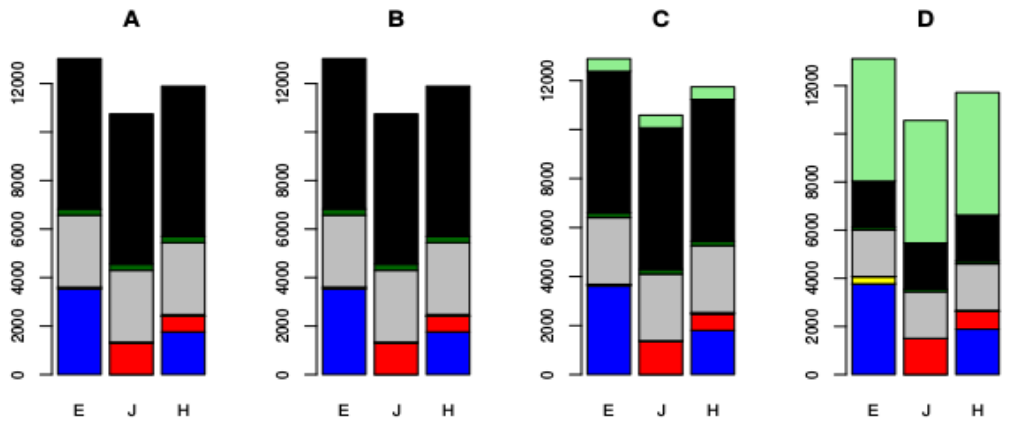

Figure 12: Variance structure estimated for breast-height circumference observed in 2010 in SA; for each taxon: European larch ('E'), hybrid ('H') and Japanese larch ('J'); depending on the model specification: with the inclusion of permanent environment competition effects (C, D) or without (A, B), and with the inclusion of dumb individuals in the spatial variance matrix (A, C) or without (B, D). Blue: additive variance from the European larch side; red: additive variance from the Japanese larch side; yellow: taxon-specific dominance variance; grey: spatial variance; black: residual variance; dark green: additive competition variance; light green: permanent environment competition variance.

3.1.3. Genetic effects

Throughout the preliminary phases of the analysis, we considered several paths that were finally not conserved for the final picture. Noteworthily, we finally chose to estimate the genetic additive variance and performances (*i.e.* the breeding values and general hybridization ability) independently in pure species breeding and in hybridization (unlike what we presented in Fig. 12), so that we could compare them in these two situations and assess the stability of the performances from one situation to the other.

We used the same method for the stability of the performances across the 3 sites of the study: the performances were independently estimated and we then addressed the question of their across-site stability. This was overall led by our current inability to fit a single multi-site multi-trait model. Indeed, if the pattern of the genetic variance-covariance matrix in a multi-site multi-trait model does not pose conceptual problems (as we could estimate across-traits across-sites genetic covariance), this is not equally applicable to the residual variance-covariance matrix, for which there is no across-site covariance. We could however have fit multi-site models with a single trait, trait by trait. This latter option was discarded because we prioritized the use of the across-trait information for the overall quality of the model M2 (Wei and Borralho 1998; Marchal *et al.* 2016). Ideally, a software allowing a fine-tuning of the residual variance-covariance matrix (*i.e.* allowing the estimation of across-traits within-sites covariances, but fixing null across-sites covariances) would have been the best option.

Finally, we also investigated for possible maternal and paternal effects. For instance, in larch, mitochondria are inherited from the mother and chloroplasts from the father (Acheré *et al.* 2004). The corresponding variances we estimated were extremely low and therefore we neglected these parental factors.

3.1.4. Modeling of ordinal categorical traits

Bud flush and stem flexuosity may be seen as categorical traits, *i.e.* traits for which the phenotype can only fall into discrete categories. These categories were ordinal, *i.e.* we could order them from a 'smallest' to a 'largest'. However, the quantitative genetics framework that we have been defining up to now only applies to continuous phenotypes, for which the concepts of means, variance, and continuous variation make sense. A way to

deal with ordinal categorical traits is the use of a latent variable model. In such a model, the phenotype (*y*) is replaced by a latent variable (*θ*) that has a continuous variation. For *n* categories of phenotypes, *n-1* thresholds have to be estimated, defining *n* ranges of values that can take *θ* (Fig. 13), to which corresponds one categorical phenotype. The latent variable is unknown, and must be predicted as the model is fitted.

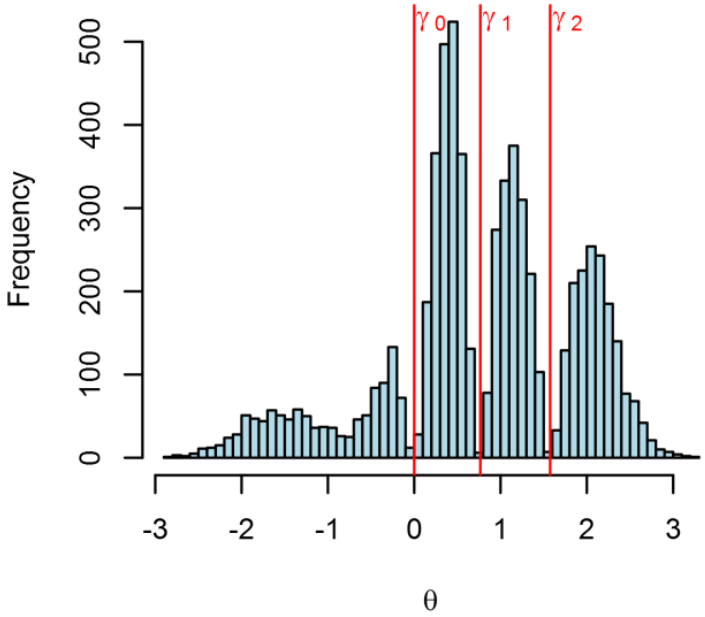

Figure 13: Illustration of the distribution of a latent variable (θ) for a 4 categories trait (the data were the 4 categories of color of the seedlings before they were distributed into their definitive sites). In red: the thresholds (γ). Modeled using MCMCglmm (Hadfield 2010, 2016)

The use of latent variable, though explored, was finally discarded for two reasons. On one hand, it was computationally very heavy. On the second hand, bud flush and stem flexuosity may be seen as continuous phenotypes as well, though we measured them with imprecision due to our inability to give, with a naked eye, a continuous mark to such traits. However, estimating floating point numbers for these traits (for instance, for spatial effects or breeding values) was not a problem and the concepts of means and variances applied. This was even the original motivation for defining the 1-5 scale for stem flexuosity (Keiding and Olsen 1965).

3.2. Article

Hybrid larch heterosis: for which traits and under which genetic control?*

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December 4, 2017

Abstract

Despite the interest foresters have for interspecific hybrid trees, still little is known about their quantitative genetics. This is especially true for the hybrid (HL) between *Larix decidua* (EL) and *L. kaempferi* (JL). Long-term, well-designed, multi-site experiments are necessary to estimate the parameters required for HL breeding programs. This paper presents the results from a diallel mating trial between 9 EL and 9 JL, set up in 3 contrasted sites. Growth traits (height, circumference), quality traits (wood density, stem form, heartwood proportion), and bud flush were measured from plantation to up to 18 years after plantation. Wood density and heartwood proportion were assessed using increment cores. We did a spatial analysis to take into account environmental heterogeneity at the tree level, and we fitted a multi-trait, Bayesian MCMC (Markov Chain Monte Carlo) genetic model. Our study confirmed, in most situations, that HL expressed heterosis over its best parent for growth traits taking advantage of an early faster growth, with no loss in wood density. However, growth traits showed low levels of heritability. On the other hand, bud flush and stem flexuosity had high heritabilities, and wood density was clearly under JL control. Site-dependent heritabilities were expressed by EL. Additive genetic correlations were presented. The traits with high heritabilities showed high correlation between their performances in pure species and in hybridization, as well as high across-site correlations. The discussion focused on the interest of these genetic parameters for the hybrid larch breeding programs.

Keywords: interspecific hybrid; diallel mating design; multi-site progeny test; growth; wood density; multi-trait model; Bayesian inference

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1 Introduction

Inter-specific hybridization is a common breeding strategy used in forest tree improvement. Commercial hybrids are produced for several species including poplars and aspens (Li et al, 1993), eucalypts, sub-tropical pines (reviewed by Nikles and Griffin, 1991), and larches (reviewed by Pâques, 2013). For foresters, hybrids are often interesting because of their superiority over their parental species for several traits of ecological and economic importance (Zobel and Talbert, 1984).

Although there is an abundant literature on the topic of heterosis, this term remains somewhat ambiguous. Some authors prefer to associate heterosis to non-additive gene effects expressed in hybrids, and sometimes add the complementarity between parental traits under additive control to the definition of hybrid vigor (Nikles and Griffin, 1991). Nevertheless, 'heterosis' was originally defined as a free-of-hypothesis concept, synonymous of 'hybrid vigor' (Shull, 1948). For a given quantitative trait, heterosis is considered as the difference between the hybrid and the mid-parent value (Falconer and Mackay, 1996). The best-parent heterosis, i.e. the difference between the hybrid and its best parent, is often preferred because it highlights perspectives in terms of breeding (Kearsey and Pooni, 1996; Dungey, 2001). In the present paper, heterosis will also be assumed a free-of-hypothesis concept, and will refer by default to best-parent heterosis. An extra ambiguity comes from the definition of 'hybrid'. Indeed, a 'hybrid' may refer to the cross between inbred lines, between populations, or between different species. Mechanisms underlying the heterosis of hybrids between inbred lines (e.g. maize lines) start to be well understood (Gallais, 2009; Baranwal et al, 2012; Chen, 2013); whereas the mechanisms behind the heterosis of inter-population and inter-species hybrids, i.e. hybrids between heterozygous parents, remain poorly documented (Perron, 2008)(see Li and Wu, 1996, though).

Despite the sparse information about the mechanisms, forest tree geneticists have well documented the superiority of some inter-populations and interspecific forest trees hybrids (Nikles and Griffin, 1991; Li et al, 1993; Pâques, 2013). However, this superiority over some commercial references, which is the final aim of a hybrid breeding program, does not provide information on the genetic architecture of the performances of the hybrid. At best, the study of heterosis by means of a proper mating design, and therefore proper parental references, allows the estimation of genetic parameters such as variance components (Hinkelmann, 1974) which can in turn be used to choose and develop a breeding strategy (Falconer and Mackay, 1996). In addition, the expression of heterosis may change with time and environmental conditions and its study, therefore, would require long-term multi-site experimentation. As an illustration, it can be demonstrated that genotype-by-environment interactions (G×E) alone can theoretically lead to heterosis including best-parent heterosis (Knight, 1973). Practical examples of the link between heterosis and G×E are also available. Thus, heterosis may be conditional to the site as shown by Li and Wu (1997) for aspen and by Weng et al (2014) for *Eucalyptus urophylla* × *E. tereticornis*. The G×E may not only manifest on the means but also on the components of variance. For instance, the parental contribution of *Pinus caribaea var. hondurensis* and *P. tecunumanii* to their hybrid heritability depends on the testing site (Mutete et al, 2015). Unfortunately, the creation of proper mating designs and the establishment of multi-site field trials over contrasted environments are often complicated by the species biology (crossing of 2 different species), expensive and time-consuming. Therefore such assays of true heterosis are rare in the forestry literature.

Hybridization between several species of larches (*Larix decidua* Mill., *L. kaempferi* (Lamb.) Carr. and *L. laricina* (Du Roi) K. Koch) is possible and the hybrid between European (*L. decidua*) and Japanese (*L. kaempferi*) larches (respectively EL and JL) particularly shows promising prospects for forestry in Europe and North-America (Pâques, 1992b; Baltunis et al, 1998; Greenwood et al, 2015). Hereafter, 'hybrid larch' (HL) will exclusively refer to *L. decidua* × *L. kaempferi* or its reciprocal *L. kaempferi* × *L. decidua*. The superiority of HL for height over its parents has been described in the literature, as reviewed by Pâques (1989, 2013). Depending on the site, HL can outperform either EL, JL, or both. This superiority justifies the existence of several hybrid larch breeding programs, in Europe and abroad (Perron, 2008). In addition to the heterosis on growth traits, HL inherits canker resistance from JL (Sylvestre-Guinot et al, 1999), which is of critical importance for lowland cultivation of EL in Western Europe.

However, the comparison of hybrid to its true parental references, i.e. heterosis sensu stricto, is poorly documented for larches (Pâques, 1989, 2013). Heterosis on growth traits was confirmed for HL at a juvenile stage (Baltunis et al, 1998; Pâques, 2002) and, more recently, on 22-years-old trees (Greenwood et al, 2015). In a breeding perspective, there is also a need for identifying the role of each parent species in the genetic control of the hybrid traits. Using 2 factorial mating designs, Pâques (2004) could identify the parental contribution to the phenotype of 16-years-old hybrid larches. He distinguished traits whose variability was more under Japanese parent control, such as stem volume and flexuosity.

In summary, while hybrid superiority has been shown for hybrid larch, true heterosis is still poorly docu-

mented because of the absence of proper experiments, as discussed before. Multisite, long-term, well designed experiments are of primary importance in order to gather reliable estimations of the genetic parameters needed to plan a hybrid larch breeding strategy (Pâques, 1989). To our knowledge, hybrid larch heterosis has never been studied with such an experiment yet. The first part of this study attempted to show for which trait and which amplitude one can expect heterosis from hybrid larch. True heterosis was assessed by the mean of a diallel mating design. The question of whether heterosis would be a juvenile character was addressed together with that of the impact of the environment on its expression. A better knowledge of the quantitative genetics of the traits should help us to better define a breeding strategy, and to define where and how efforts should be emphasized. For this reason, we investigated the importance of additive and non-additive genetic variances, the magnitude of genetic correlations, as well as the relative contribution of parental species in the genetic control of hybrid traits. We made a choice of solving such a complex genetic model in a Bayesian framework using MCMC techniques. Indeed, this framework brings the desirable feature of integrating estimation, prediction, and decision into a single analytical process (Gianola et al, 1989). Finally, the hypothesis of a better buffering of environmental constraints by hybrids vs. parental species was tested through a preliminary study of stability of species and hybrids across environments (G×E interaction).

2 Materials and Methods

2.1 Experimental population

Nine (9) EL parents from Sudetan Mountains (Czech Republic) and 9 JL parents from various Hondo Island origins (Japan) were sampled from INRA (Institut National de la Recherche Agronomique) EL and JL breeding populations and crossed following a full diallel mating design producing pure species and hybrid full-sib progenies. Parents involved in the experiment were selected at random among trees able to flower and to produce cones. Out of the 171 possible families (that is 324 crosses, a family being a cross plus its reciprocal), self-crosses mostly failed and none of them were field tested, and for 8 families (7 EL families and 1 HL family) no offspring were available. The remaining 145 families were represented by one cross and/or its reciprocal. In each site where the diallel was set up there were between 18 and 29 EL families, between 25 and 28 JL families, and between 56 and 71 HL families; the families were represented by 1 to 63 trees per site, with a mean of 23.2 trees. Thirty-six (36) extra full-sib and half-sib families, each having one of the parents of the diallel, were added to the main design. Details of available crosses are summarized in Appendix 1, Table 3-5. All seeds were sown at INRA nursery in Orléans (France) in spring 1995. Then 2-years-old bare-root seedlings were planted in 3 ecologically contrasted sites in spring 1997: Saint-Appolinaire (abbreviated SA), Saint-Saud (SS) and Cumières (not considered in this study because of a too high mortality), following a single-tree randomized incomplete blocks (RIB) design. To replace the Cumières site, 1311 trees were randomly sampled in SS, and 3 scions were collected from each of these trees and grafted on 2-year-old seedling. Because of the scarcity of available rootstocks, 2 scions were grafted on HL rootstocks (half-sib EL×JL hybrids) and the last one was grafted on a JL rootstock (from a commercial seedlot). The grafted trees were grown for 2 years in PNRGF (PÃťle National des Ressources Génétiques Forestières) nursery at Peyrat-le-Château (abbreviated PC). Resulting material was planted in spring 2004 in PC vicinity, following a RIB design with 3-tree row plots (the 3 scions from the same ortet together). On all 3 sites, progenies from the 3 taxa were randomly allocated to each block. We favored this option in order to better take into account micro-environmental effects.

The site of SA is established at the eastern edge of the Massif Central range in a mountainous area on a steep southern-aspect slope. On the contrary, the sites of SS and PC are located at lower elevations on the western edge of Massif Central on mostly flat areas, under oceanic influence. The site of SS was a former grassland, frequently fertilized, whereas the other sites were already forest lands in the past. Description of the sites is provided in Appendix 1, Table 6. Two thinning treatments were applied to SS: in early 2004 and in 2010. During the first thinning, 30% of the poorest performing trees (based on a selection index combining volume and flexuosity) within each family were removed. The second thinning favored an even spatial distribution of remaining trees, like in forest production. No thinning was applied to the 2 other sites. Counts of trees per family and per site, as well as the evolution of the number of trees due to mortality and thinning, is provided in Appendix 1, Fig. 4.

2.2 Measurements

Trees were measured several times from plantation to winter 2010 in SA, and to winter 2014 in SS and in PC. Measurements included breast-height circumference (BHC), total height (HT), stem flexuosity (FLX) and bud flush (BUD), and concerned all available trees at each assessment (except for SA in 2005). Stem flexuosity and terminal bud flushing were visually assessed using subjective scoring scales, following Keiding and Olsen (1965)(see Pâques, 1992b) for flexuosity (from 5:

straight stem to 1: stems with several severe crooks) and Gauchat and Pâques (2011)(Fig. 1) for bud flush (from 0: dormant bud to 5: elongated needles, 1-2 cm in open rosette; plus an extra class 6: stem elongation). Each observation of bud flush at any given site was done in a single day. Diameter increment cores were harvested at breast height in the 3 sites over two or three collecting periods depending on site (Appendix 1, Table 7). All individuals in SA and SS trials were sampled, with a special effort in SS for harvesting increment cores from trees before being thinned. In PC, only 1 ramet per ortet was randomly sampled. Increment cores were dried off, sawn in 2mm-thick axial boards and then X-rayed on negative films. Out of the two, only the pith to bark radius with the least defects (resin pockets, knots, compression wood) was kept for subsequent analysis. Microdensitometric profiles were produced from high resolution scans of the X-rayed films using WinDENDRO^{TM} (Regent Instruments Canada Inc., 2008). From microdensitometric profiles we visually delimited annual ring increments, which were used to estimate yearly BHC values. Inferred BHC from annual ring increments (Δ) for year $n+1$ was:

$$BHC_{n+1} = BHC_n + 2\pi \times \Delta_{n+1} \times \delta$$

with δ being an individual correction term, calculated as the ratio between difference in radius calculated from BHC measures and difference in radius calculated from ring increments, assuming a circular stem section and a constant bark thickness. Overall wood density (DEN_n) over years was calculated from the ring mean densities (d) weighted by the corresponding ring surfaces (S): we used as the first ring the one for which enough observations were available ($i_0 = 2000$ in SA and SS and $i_0 = 2007$ in PC, that is constantly 4 years after plantation, for which at least 50% of the ring observations were available in each site).

$$DEN_n = \frac{\sum_{i=i_1}^{n} d_i \times S_i}{\sum_{i=i_1}^{n} S_i} \text{ with } S_i = \frac{BHC_i^2 - BHC_{i-1}^2}{4\pi}$$

Delimitation between sapwood and heartwood was visually assessed on the boards based on color differences, and heartwood surface proportion (HWP) was calculated for 2009 and 2011 in SS, for 2013 in SA, and for 2014 in PC. All the traits (BHC, HT, DEN, HWP, FLX and BUD) were analyzed without any transformation.

2.3 Models

The data were analysed using a two-step approach. Firstly, we fitted a model M1 that aimed to estimate the taxa means, from which we assessed best-parent heterosis at the species level. Model M1 was fitted for each combination of site, age and trait; it took into account genetic and spatial environmental sources of variation. All 4 taxa (EL, JL, EL×JL and JL×EL) were analyzed simultaneously, including all available genetic material, at the tree level, so that (i) the contrasts between taxa were directly estimated and (ii) the spatial variance estimation used all available information within each trial. Estimating simultaneously genetic and spatial effects was computationally costly. For this reason M1 was a uni-trait model, meaning it ignored information across traits. However, traits are usually correlated both at genetic and environmental levels. This can be leveraged with a multi-trait analysis, gaining more insight on the genetic merits for each individual trait (e.g. Marchal et al, 2016), but also allowing for inference on the genetic cross-correlations. For these reasons, as a second step, we fitted a genetic model M2 that was multi-trait. The spatial effects estimated in M1 were subtracted from the phenotype before processing to the second step.

Model M2 was fitted for each combination of site and taxon; with the goal to estimate the genetic variance parameters such as heritabilities and genetic correlations between traits. Model M2 was also used to predict the genetic performances of the 18 parents of the diallel. The stability of these performances across sites, as well as their stability in pure species vs. in hybridization, were assessed by the mean of Pearson correlations. At this second step of M2, we used a Bayesian framework in order to infer directly the genetic parameters using posterior distributions. Attention must be drawn here on the terminology and concepts attached to the use of Bayesian approaches that differ from those in M1 that followed a frequentist approach. While the former makes decisions about the magnitude and relevance of estimates based on posterior distributions, the latter uses hypothesis testing and confidence intervals. With the choice of a Bayesian framework in M2, we focused on the magnitude of the genetic parameters and the uncertainty around their estimations, because these are the pieces of information that ultimately can guide breeders in their selection decisions.

2.4 Model M1 - heterosis estimation

Each trait at each age in each site (y) was analyzed with the model M1 that took the form:

$$y_{trijkl} = fixed_{tr} + genet_{tijk} + envt_l \qquad \text{(M1)}$$

with $fixed$ the fixed effects at the taxon level, $genet$ the individual random genetic effects, and $envt$ the individual random environmental effects. The fixed effects took the form:

$$fixed_{tr} = \begin{cases} \beta_0 + \beta_E + \iota_{PC}\gamma_{E:r} \text{ if t = 'EL'} \\ \beta_0 + \beta_J + \iota_{PC}\gamma_{J:r} \text{ if t = 'JL'} \\ \beta_0 + \iota_{PC}\gamma_{H:r} \text{ if t = 'EL×JL'} \\ \beta_0 + \beta_{J\times E} + \iota_{PC}\gamma_{H:r} \text{ if t = 'JL×EL'} \end{cases} \quad \text{(M1a)}$$

The fixed effects of M1 were β_0, the EL×JL taxon mean used as a reference; and β_E, β_J, $\beta_{J\times E}$, the contrasts between each taxon t and the reference EL×JL. The indicative variable ι_{PC} was defined such as the hybrid rootstock effect $\gamma_{t:r}$ were only accounted in the site PC. The hybrid rootstock effects $\gamma_{t:r}$ were calculated as contrasts between the trees whose rootstock r was HL compared to the trees whose rootstock r was JL; this effect included an interaction between rootstock species and scion species. The rootstock effects estimates in PC were presented in Appendix 2, Fig. 5. The genetic effects took the form:

$$genet_{tijk} = \begin{cases} a_{E:i} + d_{E:i} + \iota_{PC}c_{E:i} \text{ if t = 'EL'} \\ a_{J:i} + d_{J:i} + \iota_{PC}c_{J:i} \text{ if t = 'JL'} \\ h_{E:j} + h_{J:k} + f_{jk} + c_{H:i} \text{ if t = 'EL×JL'} \\ h_{E:k} + h_{J:j} + f_{kj} + c_{H:i} \text{ if t = 'JL×EL'} \end{cases} \quad \text{(M1b)}$$

For pure species, the additive effect or breeding value (BV) of the genotype i (that is, an individual tree in SA and SS and a clonal genotype in PC) was $a_E \sim N(0, \sigma^2_{AE}\mathbf{A_E})$ or $a_J \sim N(0, \sigma^2_{AJ}\mathbf{A_J})$ for EL or JL larches respectively, with $\mathbf{A_E}$ and $\mathbf{A_J}$ the additive relationship matrices. Dominance effects were $d_E \sim N(0, \sigma^2_{DE}\mathbf{D_E})$ or $d_J \sim N(0, \sigma^2_{DJ}\mathbf{D_J})$ for EL or JL larches respectively, with $\mathbf{D_E}$ and $\mathbf{D_J}$ the dominance relationship matrices. For hybrids, we used a Stuber and Cockerham (1966) model. The general hybridization abilities (GHA) from the mother j and the father k were $h_E \sim N(0, \sigma^2_{HE}\mathbf{I_{HE}})$ and $h_J \sim N(0, \sigma^2_{HJ}\mathbf{I_{HJ}})$, assuming unrelated, non-inbred parents (coancestry matrices for parents were half-identity matrices). This model also assumed that *L. kaempferi* (2n = 24) and *L. decidua* (2n = 24) contributed to the same extent to the hybrid nuclear genome, as Nkongolo and Klimaszewska (1995) showed in in vitro embryogenic lines of EL×JL karyotypes ranging between 2n = 24 and 2n = 25. The dominance variance was captured using a hybrid family effect, or specific hybridization ability (SCA): $f \sim N(0, \sigma^2_{DH}\mathbf{F})$ (Lo et al, 1997; Cros, 2014). As self-crosses were absent and diallel parents were assumed to be unrelated and non-inbred, $\mathbf{F} = \frac{1}{4}\mathbf{I_F}$. A random clonal effect c was added (i) in PC for all taxa, in order to capture the epistasis and the non-genetic effects associated with each ortet (except for traits measured from the increment cores in PC, as no clonal repetition was available for them) and (ii) for all sites with HL only, in order to capture the within-family Mendelian segregation genetic variance and thus to separate it from the residual. The clonal effects had taxon specific variances.

$$envt_l = s_l + e_l \quad \text{(M1c)}$$

Environmental heterogeneity was assumed to be spatially structured, so that the spatial effect at location l was $s \sim N(0, \sigma^2_S\mathbf{S})$ with $\mathbf{S} = \{\rho^{distance(a,b)}\}$ where ρ had to be estimated for each combination of trait, age and site. Spatial correlation matrices $\mathbf{S}$ were built using the R package fields (Nychka et al, 2015; R Core Team, 2015). Finally $e \sim N(0, \sigma^2_E\mathbf{I_R})$ were the unstructured residuals, common to the 4 taxa; its variance was σ^2_E. All single-trait models were fitted using restricted ML (REML) and the R package breedR (Muñoz and Sánchez, 2015).

For computational reasons ρ was estimated by Maximum Likelihood (ML) before the genetic effects were included in the model. Once ρ estimated, the spatial variance σ^2_S was obtained at the same estimation step as the other variance components. Estimates of ρ and spatial variance proportion (SVP) that we defined as SVP $= \sigma^2_S/(\sigma^2_E + \sigma^2_S)$ were presented in Appendix 2, Table 9. For 2 traits in PC (FLX at 11-years-old and BUD at 3-years-old), ρ was estimated to be low (0.69 and 0.63 respectively), correspondingly with very high SVP over 99% in both cases (results not shown). Such a parameterization of ρ may lead to a confusion between σ^2_S and the residual variance σ^2_E. Therefore, for these traits in PC we did not use the ML estimation for ρ but we fixed $\rho = 0.99$ instead.

2.5 Taxa means and heterosis

Using model M1 (including all taxa simultaneously), we estimated directly the contrast between each taxon mean and the reference EL×JL. Doing this way, we measured the best-parent heterosis where appropriate, or the parental superiority otherwise; and we obtained a standard error for each contrast estimation. We used the standard errors of these contrasts to estimate confidence intervals and we computed p-values (null hypothesis: âĂIJthe contrast is nullâĂİ) under Gaussian distribution assumption.

2.6 Model M2 - variance parameters estimation

Model M2 was independently fitted for each combination of site and taxon: EL, JL, and HL (EL×JL and JL×EL pooled together, assuming no reciprocal effects), on data previously adjusted for spatial heterogeneity. The response variables y of M2 were the observations of all p traits from which were subtracted the spatial effects predicted with model M1. In PC, we also subtracted the rootstock species effect γ estimated with M1. Multi-trait analysis is also known to account

properly for individuals that are censored (the case in SS due to thinning; and non-sampled individuals for increment cores traits in PC) in the estimation of genetic parameters (Wei and Borralho, 1998). Therefore in SS some traits (BHC, HT, DEN and FLX) were also included at a young stage in order to take into account the thinning censure; they were then considered as new traits. All included BHC were field measured, not inferred from ring increments. Exact ages for all traits involved in M2 are given in Appendix 1, Table 8. For pure species EL and JL, model M2 took the form:

$$\begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_p \end{pmatrix}_{il} = \begin{pmatrix} m_1 \\ m_2 \\ \vdots \\ m_p \end{pmatrix} + \begin{pmatrix} a_1 \\ a_2 \\ \vdots \\ a_p \end{pmatrix}_{i} + \begin{pmatrix} d_1 \\ d_2 \\ \vdots \\ d_p \end{pmatrix}_{i} + \iota_{PC} \begin{pmatrix} c_1 \\ c_2 \\ \vdots \\ c_p \end{pmatrix}_{i} + \begin{pmatrix} e_1 \\ e_2 \\ \vdots \\ e_p \end{pmatrix}_{l} = m + a_i + d_i + \iota_{PC} c_i + e_l \qquad \text{(M2a)}$$

The overall means were m. A complete variance-covariance matrix was used to structure the additive effects distribution, that is $a \sim N(0, \mathbf{\Sigma_A} \otimes \mathbf{A})$ with:

$$\mathbf{\Sigma_A} = \begin{pmatrix} \sigma^2_{A1} & r_{A:12}\sigma_{A1}\sigma_{A2} & \dots & r_{A:1p}\sigma_{A1}\sigma_{Ap} \\ r_{A:12}\sigma_{A1}\sigma_{A2} & \sigma^2_{A2} & \dots & r_{A:2p}\sigma_{A2}\sigma_{Ap} \\ \vdots & \vdots & \ddots & \vdots \\ r_{A:1p}\sigma_{A1}\sigma_{Ap} & r_{A:2p}\sigma_{A2}\sigma_{Ap} & \dots & \sigma^2_{Ap} \end{pmatrix}$$

The additive genetic correlation between traits r_A were thus estimated as the model was fitted. Dominance effects were independent between traits, that is $d \sim N(0, \mathbf{\Sigma_D} \otimes \mathbf{D})$ with:

$$\mathbf{\Sigma_D} = \begin{pmatrix} \sigma^2_{D1} & 0 & \dots & 0 \\ 0 & \sigma^2_{D2} & \dots & 0 \\ \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & \dots & \sigma^2_{Dp} \end{pmatrix}$$

Properly conditioned inverse matrices $\mathbf{A^{-1}}$ and $\mathbf{D^{-1}}$ were built directly using R packages MCMCglmm (Hadfield, 2010) and nadiv (Wolak, 2012). A complete variance-covariance matrix was also used to structure the residual effects distribution $e \sim N(0, \mathbf{\Sigma_R} \otimes \mathbf{I_R})$ so that residual correlations between traits r_R were estimated. In PC only, we added an unstructured, independent between traits, clonal effect $c \sim N(0, \mathbf{\Sigma_C} \otimes \mathbf{I_C})$.
Again, Stuber and Cockerham (1966) model was applied for hybrids, following model M2b:

$$\begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_p \end{pmatrix}_{ijkl} = \begin{pmatrix} m_1 \\ m_2 \\ \vdots \\ m_p \end{pmatrix} + \begin{pmatrix} h_{E:1} \\ h_{E:2} \\ \vdots \\ h_{E:p} \end{pmatrix}_{j} + \begin{pmatrix} h_{J:1} \\ h_{J:2} \\ \vdots \\ h_{J:p} \end{pmatrix}_{k} + \begin{pmatrix} f_1 \\ f_2 \\ \vdots \\ f_p \end{pmatrix}_{jk} + \iota_{PC} \begin{pmatrix} c_1 \\ c_2 \\ \vdots \\ c_p \end{pmatrix}_{i} + \begin{pmatrix} r_1 \\ r_2 \\ \vdots \\ r_p \end{pmatrix}_{l} = m + h_{E:j} + h_{J:k} + f_{jk} + \iota_{PC} c_i + r_l \qquad \text{(M2b)}$$

Additive variances in hybridization were structured using complete variance-covariance matrices $h_E \sim N(0, \mathbf{\Sigma_{HE}} \otimes \mathbf{I_{HE}})$ and $h_J \sim N(0, \mathbf{\Sigma_{HJ}} \otimes \mathbf{I_{HJ}})$ so that r_{HE} and r_{HJ}, the components of additive genetic correlation in the hybrids due to each of the parental species, were estimated. The family effects were independent $f \sim N(0, \mathbf{\Sigma_D} \otimes \mathbf{F})$ Similarly to M2a, the residual effects distribution was $r \sim N(0, \mathbf{\Sigma_R} \otimes \mathbf{I_R})$ Once again, we added an unstructured, independent between traits, clonal effect in PC.

The models were fitted using Markov Chain Monte Carlo (MCMC) with the R package MCMCglmm (Hadfield, 2010). Means priors were vague with $m \sim N(0, 10^{10}\mathbf{I_p})$. Variance-covariance matrices priors were inverse-Wishart (W^{-1}) distributions. We used parameter expansion (PX) (Liu et al, 1998) for the additive components: $\mathbf{\Sigma_A} = diag(\alpha_A)\mathbf{\Sigma_{A_\alpha}}diag(\alpha_A)$, with $\mathbf{\Sigma_{A_\alpha}} \sim W^{-1}(\mathbf{V} = \mathbf{I_p}, \nu = p + 1)$ and working parameters $\alpha_A \sim N(0, 10^3\mathbf{I_p})$. For hybrids, we used PX for $\mathbf{\Sigma_{HE}}$ and $\mathbf{\Sigma_{HJ}}$ with the same hyperparameters. On one hand, PX allows a better mixing of the chains. On the other hand, it avoids the modal shape of W^{-1} and it allows close-to-zero estimation for marginal variances (Gelman, 2006; Hadfield, 2010). The hyperparameters $\mathbf{V} = \mathbf{I_p}$ and $\nu = p + 1$ were set following Gelman and Hill (2007)(p. 286) in order to set marginal correlations priors to be uniform on $[-1; 1]$. To our knowledge, the effect of PX on the marginal correlations

priors remains undocumented. For this reason and to check any eventual impact we sampled 10^5 values in the parameter expanded inverse-Wishart distribution, with the same parameterization, and we used Monte-Carlo to draw the marginal correlation prior distribution. We visually assessed that it remained uniform on $[-1, 1]$ (result not shown). Parameter expansion was also set for dominance variances so that for each trait $\sigma^2_{D\alpha} \sim W^{-1}(V = 1, \nu = 2)$ with working parameters $\alpha_D \sim N(0, 10^3\mathbf{I_p})$. We used Sorensen and Gianola (2007)(p. 579) improper uniform prior for the residuals $\Sigma_R \sim W^{-1}(\mathbf{V} = 10^{-10}\mathbf{I_p}, \nu = -(p+1))$. Marginal residual correlations estimations are provided in Appendix 2, Fig. 7. In PC and for the hybrids, the clonal effect variance prior was also set improper uniform so that for each trait $\sigma^2_C \sim W^{-1}(V = 10^{-10}, \nu = -2)$. All chains were at least 1.5×10^6 iterations long (up to 10.5×10^6 long, depending on the taxon and site combination) with 5×10^5 iteration burn-in, and a regular thinning so that 10^3 uncorrelated samples from each chain were finally kept for inference.

2.7 Variance parameters

The genetic variance parameters were estimated from M2 only. For pure species, the phenotypic variance was $\sigma^2_P = \sigma^2_A + \sigma^2_D + \sigma^2_R$ as in this case σ^2_R the residual variance from the model was equal to σ^2_E the environmental variance, i.e. the variance of non-genetic effects (epistasis was neglected). The narrow-sense heritability was $h^2 = \sigma^2_A/\sigma^2_P$. For hybrids we fitted a model at the family level, therefore due to Mendelian segregation part of the genetic variance expressed at the individual level was captured by the residual variance σ^2_R. The individual level phenotypic variance was $\sigma^2_P = \sigma^2_{GHA:E} + \sigma^2_{GHA:J} + \sigma^2_{SCA} + \sigma^2_R = \frac{1}{2}\sigma^2_{HE} + \frac{1}{2}\sigma^2_{HJ} + \frac{1}{4}\sigma^2_D + \sigma^2_R$. Given that $\sigma^2_R = \sigma^2_E + \frac{1}{2}\sigma^2_{HE} + \frac{1}{2}\sigma^2_{HJ} + \frac{3}{4}\sigma^2_D$ (Lo et al, 1997), $\sigma^2_P = \sigma^2_{HE} + \sigma^2_{HJ} + \sigma^2_D + \sigma^2_E$ showing an analogy with pure species variance decomposition. In PC, we included the clonal variance $\sigma^2_P = \sigma^2_A + \sigma^2_D + \sigma^2_C + \sigma^2_R$ for pure species and $\sigma^2_P = \frac{1}{2}\sigma^2_{HE} + \frac{1}{2}\sigma^2_{HJ} + \frac{1}{4}\sigma^2_D + \sigma^2_C + \sigma^2_R$ for hybrids. We defined the hybrid heritabilities as $h^2_E = \sigma^2_{HE}/\sigma^2_P$ and $h^2_J = \sigma^2_{HJ}/\sigma^2_P$ (Stuber and Cockerham, 1966; Cros, 2014), these heritabilities being analogous to half their pure species counterparts. Degree of dominance $d^2 = \sigma^2_D/\sigma^2_P$, residual correlations (r_R) and coefficients of variations $CV = \sigma_P/m$ along with taxa means (m) were provided for all taxa in Appendix 2 (Figure 6, Figure 7 and Table 9 respectively). We used the variance components Markov chains to produce Markov chains of h^2, d^2 and the genetic and residual correlations and we computed 95% credibility intervals (CIs) of these parameters.

2.8 Parents performances

For each trait and site, we predicted BV and GHA for the 18 parents of the diallel using model M2. Individual performances of both intra- and inter-specific crosses of the 9 EL and the 9 JL parents are presented with 95% CIs in Appendix 3, Fig. 8-10 for the most heritable traits (bud flush, flexuosity and density). We assessed the across-sites stability of BV and GHA by the mean of Pearson correlations between the parents' performances in each site; moreover, a visual representation of the G×E stability of the 18 parents is provided for the most heritable traits in Appendix 3, Fig. 11. In the same way, we calculated the Pearson correlations between BV and GHA ($r_{BV,GHA}$) in order to assess the stability of performances in pure species vs. in hybridization. For both the across-site stability and the stability in hybridization, we used the parents' performances Markov chains to produce Markov chains of the Pearson correlations and we checked if their 95% CIs exceeded some given thresholds (0, 0.3, 0.6 and 0.9).

3 Results

3.1 Importance of reciprocal effects and rootstock effects in the model M1

Although some reciprocal effects ($\beta_{J\times E}$, designed as a contrast between JL×EL and EL×JL, Cf equation M1a) were significant (density in SS, 2 and 3 year height in SA) ($p < 0.05$ to $p < 0.01$), their magnitude was low (up to âĂŞ8.29 kg.m-3 for density in SS and up to +6.23 cm for height in SA) (Fig 1, d, g, h). For all other traits (BHC (Fig 1, a-c), height and density in the other sites (Fig 1, e-f, g, i), flexuosity (Fig 1, j-l), bud flush (results not shown) and HWP (results not shown)) reciprocal effects were very small and not significant. Therefore, we took EL×JL as the reference (HL) for heterosis assessment, with negative contrasts (β_J and β_E in equation M1a) indicating heterosis, and positive contrasts parental superiority.

In the particular case of PC, we found strong, positive, significant rootstock species effects (γ in equation M1a) for growth traits (Appendix 2, Fig. 5 a-b) ($p < 10^{-5}$ at the older age, for both BHC and height). Depending on the scion species, the effect of hybrid rootstock compared to Japanese rootstock varied between 7.3 cm and 5.5 cm for BHC at age 11. For height, there might be an interaction between the scion species and the rootstock species, JL scions seemed to undergo a lower HL rootstock effect. Thus for height the rootstock effect varied between 90.4 cm for EL scions and 48.3 cm for JL scions at age 11. Rootstock effects for the other traits were low though sparsely significant (Appendix 2, Fig. 5 c-d, and unpresented

results). These rootstock effects were accounted in the model M1.

3.2 Heterosis

Growth traits (height and BHC) showed a clear best-parent heterosis (Fig 1, a-f) with the exception of BHC in PC. In SA, the best parental reference for growth traits was EL. In this site, at age 14, HL exceeded the best parental reference EL by 112.6 cm for height and 7.2 cm for BHC. Circumference contrasts evolved very slowly after age 14. In SS, at age 18, EL and JL were not significantly different for growth traits; the contrast between HL and JL (respectively HL and EL) was 12.2 cm (respectively 17.3 cm) for BHC and 185.5 cm (respectively 197.7 cm) for height. Still in SS, growth traits contrasts showed a plateau after age 13. In both SA and SS, heterosis for growth traits was highly significant ($p < 10^{-3}$) at the oldest age. In PC, the best parental reference for growth traits was JL. For trait BHC at age 11, JL significantly exceeded HL of 2.8 cm ($p < 0.05$) and HL significantly exceeded EL of 13.0 cm ($p < 10^{-5}$). Hybrid larch height exceeded both JL height of 55.6 cm ($p < 0.05$) and EL height of 254.8 cm ($p < 10^{-5}$).

Japanese larch was straighter (higher flexuosity score) than the hybrid in all sites and ages (Fig 1, j-l). This difference was significant at age 14 in SA (difference of 0.41, $p < 0.05$), at any age from age 4 in SS (maximum difference of 0.58, $p < 0.01$), and at age 3 in PC (difference of 0.43, $p < 0.01$). We also found a significantly higher straightness for EL than for HL in SS at age 4 (difference of 0.31, $p < 0.05$). Density pattern was consistent across sites: EL was the densest, JL the least dense, and HL was intermediate (Fig 1, g-i). These results were significant in SA ($p < 0.01$), in SS for JL vs. HL ($p < 10^{-3}$) and EL vs. HL at age 5 then from age 8 onwards ($p < 0.01$), and in PC only for JL vs. HL until age 8 ($p < 0.05$). There was no clear trend with age for density contrasts, except in PC where differences between taxa diminished with time.

Bud flush occurred slightly but significantly earlier for HL than for EL at age 12 in SS (difference of 0.39, $p < 0.001$) and later for HL than for JL at age 3 in SA (difference of 0.32, $p < 0.05$). The other differences between taxa for this trait were small and non-significant (results not shown). In the same way, we found no clear pattern for HWP but a few contrasts were significant: HL had a higher HWP than EL in PC (+7.16%, $p < 0.05$) and than JL in SA (+3.55%, $p < 0.05$); these were the highest contrasts for this trait.

In summary, best-parent heterosis was found in most sites for growth traits (HT and BHC). In PC, for BHC, we only showed average-parent heterosis. Most of these heterotic advantages were attained at early ages (usually before 15), reaching a plateau in subsequent later ages. Hybrids looked intermediate for density, and poorer than the parental references for flexuosity.

3.3 Heritabilities

As previously detailed, genetic parameters were estimated in a Bayesian framework (no p-values and test of significance). We considered heritabilities lower than 0.2 as low, between 0.2 and 0.4 as moderate, and higher than 0.4 as high. European larch pure species heritabilities pattern strongly depended on the site (Fig 2, a-c). In SA, all EL heritabilities were moderate to high ($\hat{h}^2_E \in [0.232; 0.440]$) ($\hat{h}^2_E$: maximum a posteriori estimate for h^2_E); in SS only bud flush heritability was high ($\hat{h}^2_E = 0.660$), all the other heritabilities were low ($\hat{h}^2_E \leq 0.189$); in PC heritability was high ($\hat{h}^2_E = 0.471$) for flexuosity and moderate for height and bud flush ($\hat{h}^2_E = 0.288$ and 0.310 respectively). On the contrary, JL pure species heritabilities pattern was better preserved across sites compared to EL (Fig 2, d-f). Indeed, for this taxon, flexuosity, bud flush and density heritabilities were high in SA and in SS ($\hat{h}^2_J \in [0.412; 0.609]$) and intermediate in PC ($\hat{h}^2_J \in [0.190; 0.532]$); height heritabilities were low ($\hat{h}^2_J \in [0.151; 0.195]$); and BHC and HWP heritabilities were very low ($\hat{h}^2_J \leq 0.094$).

Hybrid heritability was high for bud flush ($\hat{h}^2_{HE} + \hat{h}^2_{HJ} \in [0.458; 0.651]$) though parental contributions to hybrid heritability for this trait depended on the site: EL and JL contributions were almost equal in SA ($\hat{h}^2_{HE} = 0.330$ and $\hat{h}^2_{HJ} = 0.322$) whereas EL parent contributed almost solely in SS ($\hat{h}^2_{HE} = 0.516$ and $\hat{h}^2_{HJ} = 0.135$) (Fig 2, a-f). Hybrid heritability was moderate to high for flexuosity ($\hat{h}^2_{HE} + \hat{h}^2_{HJ} \in [0.326; 0.546]$) with an important EL contribution in PC ($\hat{h}^2_{HE} = 0.378$). Hybrid total heritability was also moderate to high for density ($\hat{h}^2_{HE} + \hat{h}^2_{HJ} \in [0.294; 0.502]$), only due to JL contributions ($\hat{h}^2_{HJ} \in [0.250; 0.410]$) as EL contributions were negligible for this trait ($\hat{h}^2_{HE} \leq 0.092$). European larch contributed only a little to height in SA ($\hat{h}^2_{HE} = 0.203$) and in SS ($\hat{h}^2_{HE} = 0.197$). There was also a weak contribution from JL to HWP heritability in SS ($\hat{h}^2_{HJ} = 0.160$). All other HL heritabilities (excluding young stage heritabilities in SS) were low (Fig 2, a-f).

Therefore, bud flush and flexuosity resulted in the highest heritabilities across sites and taxa. Density heritability was high for JL and was under JL control in hybridization. Growth traits and HWP attained generally the lowest levels of heritability. The ranking in heritabilities among traits was better preserved in

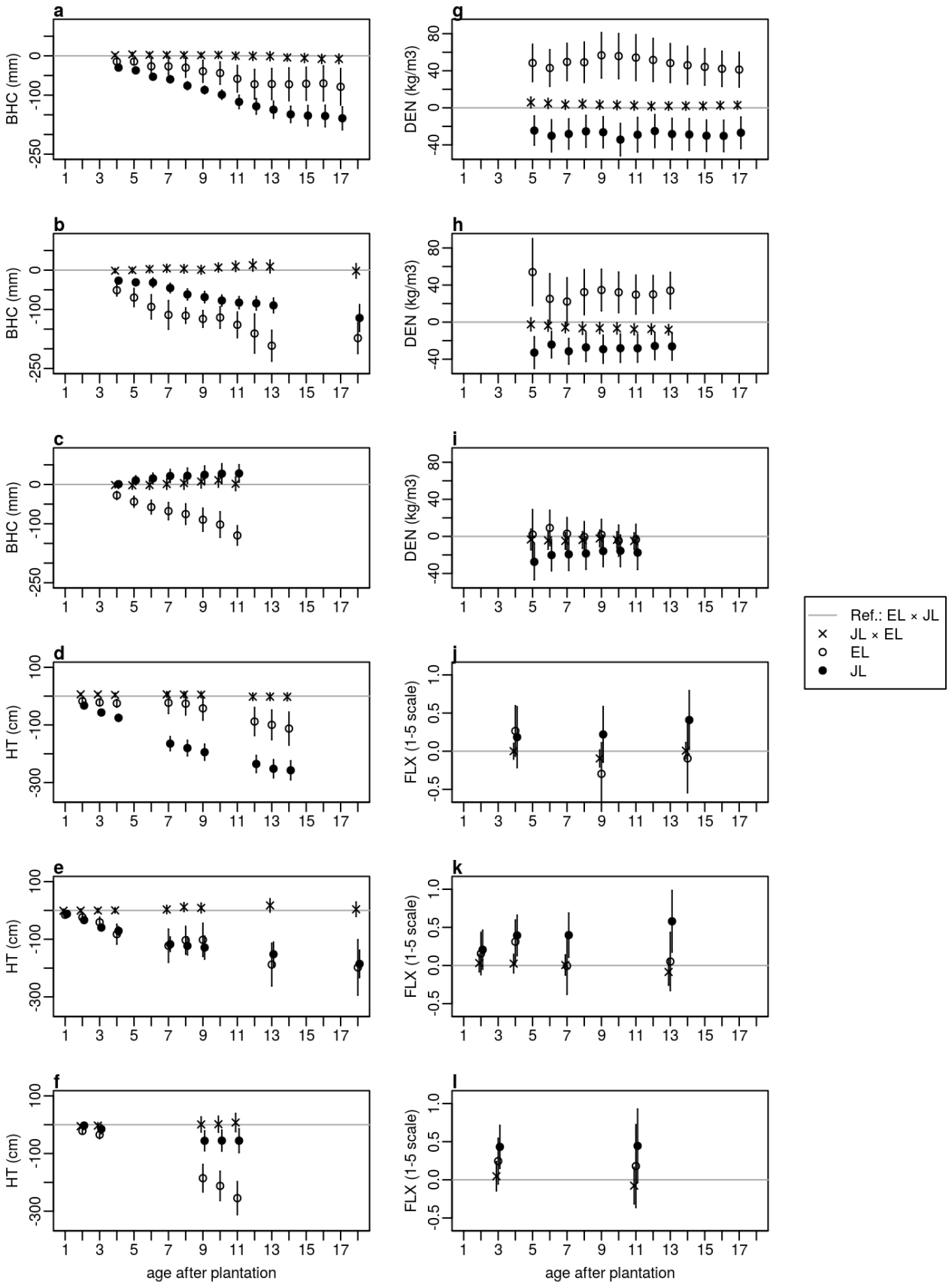

Figure 1: Contrasts between taxa means and European larch × Japanese larch (EL×JL) means (horizontal bar: $y = 0$), for breast-height circumference (BHC) (a-c), height (HT) (d-f), wood density (DEN) (g-i) and stem flexuosity (FLX) (j-l) over age, in Saint-Appolinaire (a, d, g, j), Saint-Saud (b, e, h, k) and Peyrat-le-Château (c, f, i, l). White dot: EL; black dot: JL; ×-shaped dot: reciprocal hybrid (JL×EL). All vertical bars: 95% confidence intervals under normal distribution assumption

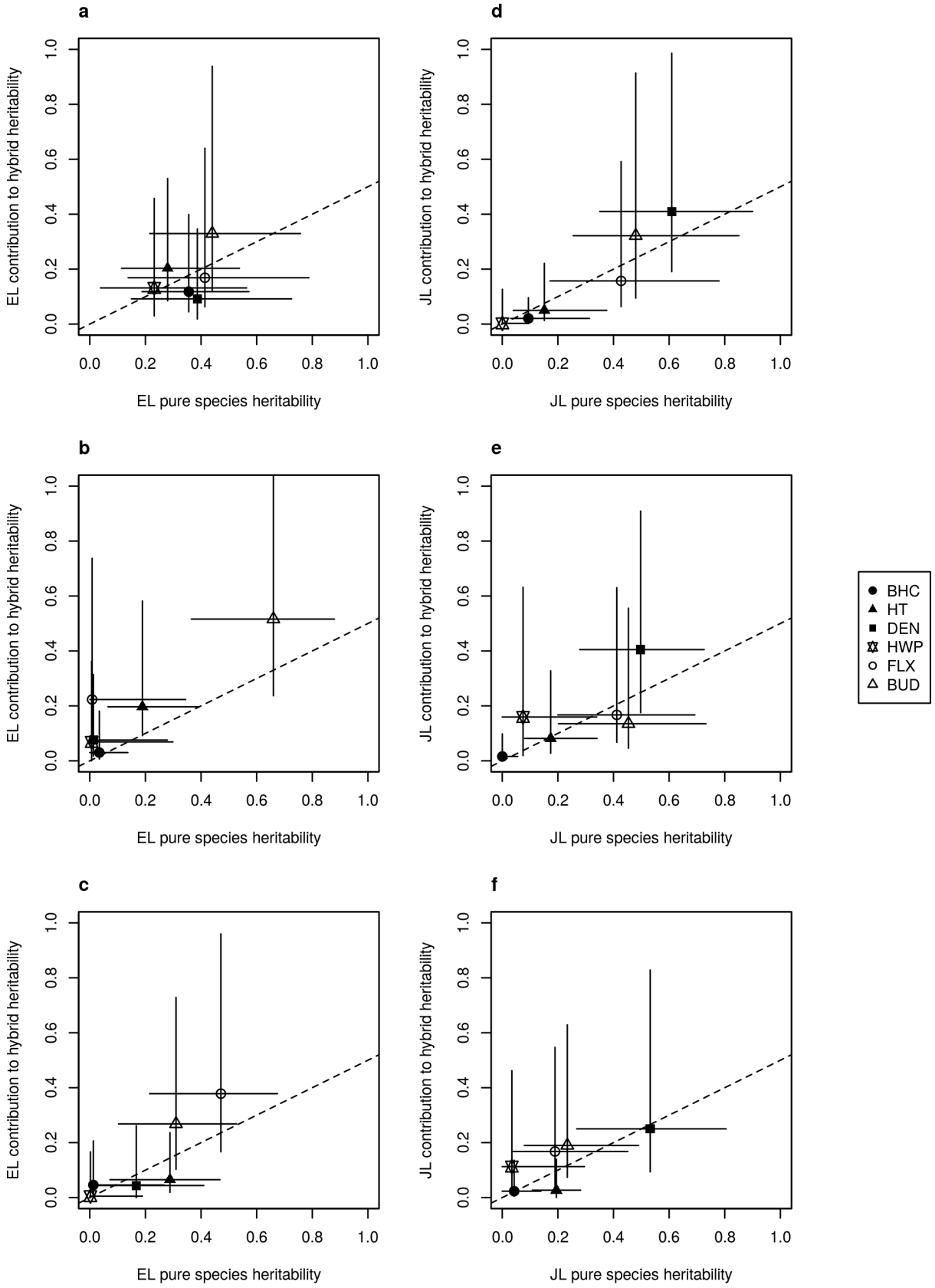

Figure 2: Pure species narrow-sense individual heritabilities vs. contributions to hybrid narrow-sense heritabilities, for European larch (a-c) and Japanese larch (d-f), in Saint-Appolinaire (a, d), Saint-Saud (b, e), and Peyrat-le-Château (c, f); for breast-height circumference (BHC), total height (HT), wood density (DEN), heartwood proportion (HWP), stem flexuosity (FLX), bud flush (BUD). The legend is provided on the side. Horizontal and vertical lines: 95% CIs. (y) = young stage. Dotted line: $y = \frac{1}{2}x$

JL across sites compared to EL. In particular, EL heritabilities both in pure species and in hybridization were generally higher in SA than in the other sites, even for growth traits. In general, EL had higher contributions to hybrid heritabilities for flexuosity and bud flush than JL.

3.4 Degrees of dominance

Degrees of dominance were very low for all traits for all taxa at all sites (Appendix 2 Fig. 6). The highest degree of dominance we estimated was 0.136 for wood density of HL in PC; any other degree of dominance was under 0.089.

3.5 Additive correlations

Only a few additive genetic correlations CIs excluded 0 (Fig. 3), whereas a lot of residual correlations CIs were above or below 0 (Appendix 2, Fig. 7). Additive correlation between bud flush and flexuosity was negative across sites and taxa (ranging from -0.581 to -0.073), meaning the later the bud flush, the straighter was the stem. This correlation was markedly negative in PC for pure EL species, with most of the posterior distribution below 0 ($\hat{r}_{AE} = -0.396$, 95% CI below 0 meaning $P(r_{AE} > 0) < 2.5\%$) and similarly for pure JL species ($\hat{r}_{AJ} = -0.380$, 90% CI below 0 meaning $P(r_{AJ} > 0) < 5\%$). On the other hand, in SA, bud flush and BHC additive correlation was positive for EL pure species ($\hat{r}_{AE} = 0.501$, $P(r_{AE} < 0) < 5\%$), meaning the earlier the bud flush, the larger was the stem.

In PC, additive correlation between height and density was positive for the pure EL and the pure JL species ($\hat{r}_{AE} = 0.862$, $P(r_{AE} < 0) < 2.5\%$ and $\hat{r}_{AJ} = 0.390$, $P(r_{AJ} < 0) < 2.5\%$ respectively). In the same way, in SS, we found a positive additive correlation between height and density in the pure JL species ($\hat{r}_{AJ} = 0.514$, $P(r_{AJ} < 0) < 5\%$). We also estimated positive additive correlations, though with some more uncertainty, between height and density for the pure EL species ($\hat{r}_{AE} = 0.404$) and both species contributions to hybridization ($\hat{r}_{HJ} = 0.356$ and $\hat{r}_{HE} = 0.292$). For JL in pure species as well as in hybridization, we always found negative additive correlation between BHC and density (ranging between -0.066 and -0.671). On the contrary, a positive correlation between BHC and density was found on the pure species EL side in PC ($\hat{r}_{AE} = 0.769$, $P(r_{AE} < 0) < 5\%$). Finally, additive correlation between BHC and height was positive for both pure species in SA ($\hat{r}_{AE} = 0.712$, $P(r_{AE} < 0) < 2.5\%$ and $\hat{r}_{AJ} = 0.484$, $P(r_{AJ} < 0) < 5\%$); as well as in PC ($\hat{r}_{AE} = 0.888$, $P(r_{AE} < 0) < 2.5\%$ and $\hat{r}_{AJ} = 0.488$, $P(r_{AJ} < 0) < 5\%$).

3.6 Stability of performances in hybridization

Stability (assessed as a Pearson correlation) between performances in pure species (BV) and in hybridization (GHA) showed a similar pattern to that of the heritability. For European larch, $r_{BV,GHA}$ were strongly site-dependent (Table 1). Indeed, for EL, $r_{BV,GHA}$ were moderate to high for all traits in SA, from 0.592 for height to 0.809 for HWP, 0.819 for flexuosity and 0.909 for bud flush. In PC, $r_{BV,GHA}$ was high for flexuosity (0.825) and for bud flush (0.722). Only bud flush had a high correlation in SS (0.801). For JL, still following the same trend as heritabilities, $r_{BV,GHA}$ were high for bud flush, flexuosity and density in any site (between 0.807 and 0.918), except for flexuosity in PC where $r_{BV,GHA}$ was only moderate (0.596). A graphical representation of performances correlations between BV and GHA, including performances CI, for bud flush, density and flexuosity is provided in Appendix 3, Fig 8-10. In short, BV appeared to be a good proxy of GHA for the highly heritable traits: bud flush and flexuosity, and density for JL. For the other less heritable traits, correlations were weak or, for EL, site-dependent.

3.7 Stability of performances across sites

Like the stability in hybridization, the stability of parents performances across sites (also assessed as Pearson correlations) were linked to the heritability of the traits; the most heritable traits were often the most stable across sites (Table 2). In pure species, EL showed high stability across sites for bud flush (between 0.763 and 0.918) only. However, the across-site stability of EL performance in hybridization was high for bud flush (between 0.894 and 0.911) and also for flexuosity (between 0.726 and 0.854), for HWP and for density in SA vs. SS (respectively 0.785 and 0.772), and for height in SS vs. PC (0.834). Following heritabilities trend, JL pure species performances were highly stable across sites for bud flush, flexuosity and density (between 0.687 and 0.955); across-site stability for height was also high in SS vs. PC (0.738). Across-site stabilities of JL performances in hybridization were high for density (between 0.817 and 0.899), but they showed a surprising pattern for flexuosity and bud flush: although they were high in SA vs. SS (respectively 0.927 and 0.923), they were only moderate in the comparisons involving PC (i.e. SA vs. PC and SS vs. PC) (between 0.643 and 0.681). Finally, JL across-site stability for height was moderate to high in hybridization in SA vs. SS (0.805) and SS vs. PC (0.672). A graphical representation of the across-site stability of the performances in hybridization is provided in Appendix 3, Fig. 11.

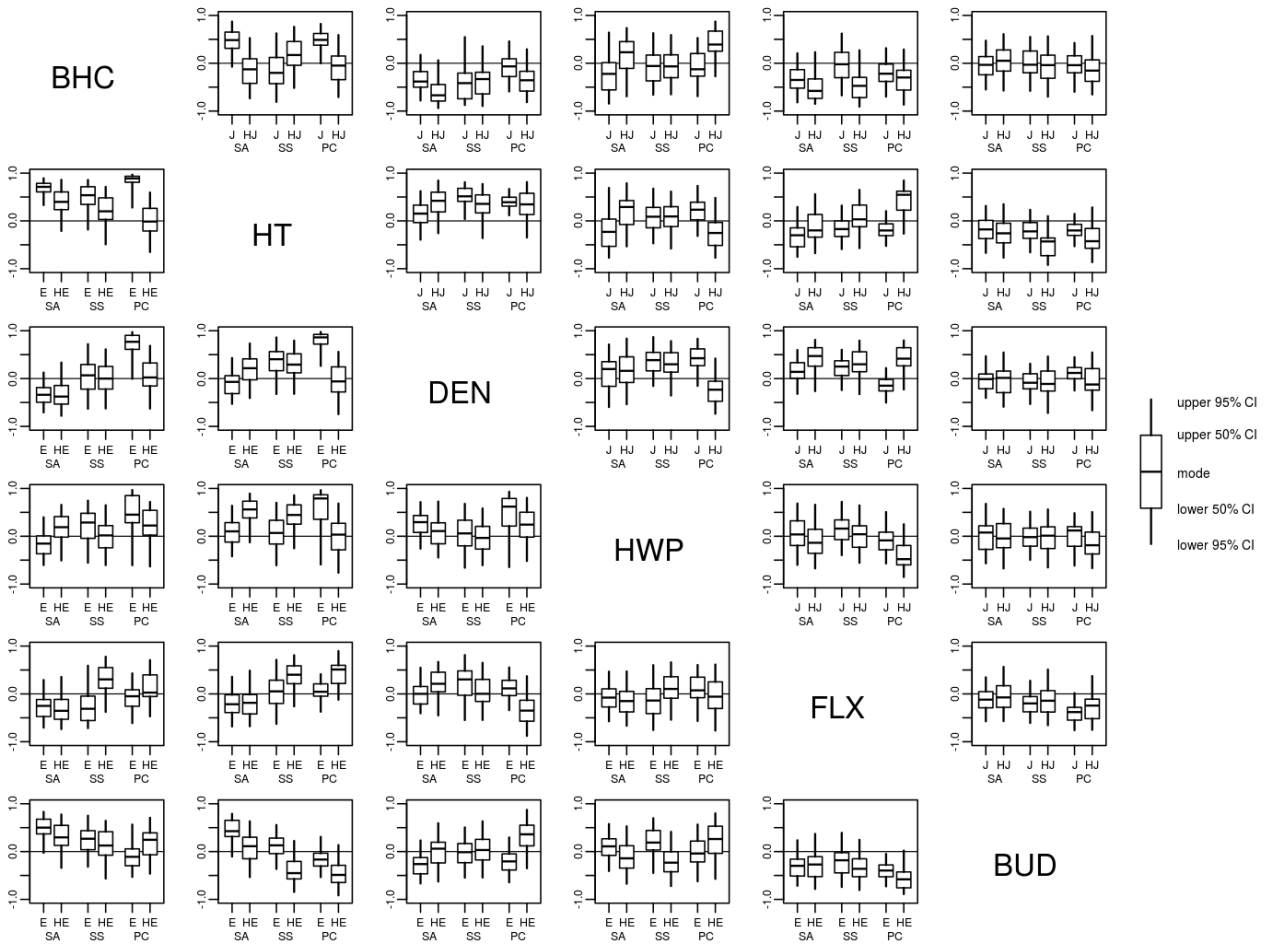

Figure 3: Additive genetic correlations between the traits: breast-height circumference (BHC), total height (HT), wood density (DEN), heartwood proportion (HWP), stem flexuosity (FLX), bud flush (BUD); on the European side (suffix 'E', bottom-left half-matrix) and on the Japanese side (suffix 'J', top-right half-matrix), in pure species (no prefix) or in hybridization (prefix 'H'); in each site: Saint- Appolinaire (SA), Saint-Saud (SS) and Peyrat-le-Château (PC). Whiskers: 95% CIs; box: 50% CIs; middle-line: mode (i.e. maximum a posteriori)

Briefly, JL showed more stable performances across sites than the EL counterpart for most of the assessed traits. Across-site correlations were particularly high in JL for highly heritable traits: bud flush, flexuosity and density. This trend of JL was paralleled in its contribution to hybrid performance across sites, with similar correlations and for the same set of stable traits. European larch, with less stable performances except for bud flush, showed a higher across-site stability in its contribution to the hybrid performances.

4 Discussion

Interspecific hybrids between European and Japanese larches present many desirable features in terms of growth and stability that are not observed simultaneously at parental level. Many hybrid varieties exist, but hybrid larch as many other hybrids lacks to a large extent recurrent breeding efforts, notably by the absence of a formal breeding program. The first steps towards fulfilling this absence are the production of quantitative genetic knowledge on hybrid larch performance. Our study was based on one of the very few intra-/inter-specific diallel mating designs existing for forest trees.

Our study showed clear heterosis for growth traits (height and BHC). This advantage occurred already at a juvenile stage, with apparently little or no extra heterosis gained after age 15-16. Those traits that benefited the most from heterosis tended to show low to medium heritabilities, and were among those in the study being less stable across sites and with relatively low correlation between performances in pure and hybrid crosses. Heterosis over the best parent was absent from the traits showing the highest heritabilities

Table 1: Pearson correlations between performances in hybridization (GHA) and in pure species breeding (BV), in Saint Appolinaire (SA), Saint-Saud (SS) and Peyrat-le-Château (PC). EL vs. HL: comparison between European larch and hybrid larch and JL vs. HL: comparison between Japanese larch and hybrid larch. Exceedance probability: ': CI (95%) above 0; *: CI (95%) above 0.3; **: CI (95%) above 0.6

site	trait	EL vs. HL	JL vs. HL
SA	BHC	0.654*	0.331
	HT	0.592'	0.475'
	DEN	0.690*	0.870**
	HWP	0.809*	0.151
	FLX	0.819*	0.807**
	BUD	0.909**	0.918**
SS	BHC	0.271	-0.028
	HT	0.452'	0.544'
	DEN	0.44	0.875**
	HWP	0.397	0.475
	FLX	0.593	0.887**
	BUD	0.801*	0.842**
PC	BHC	0.026	0.362
	HT	0.201	0.453'
	DEN	0.547	0.911**
	HWP	0.121	-0.027
	FLX	0.825*	0.596'
	BUD	0.722*	0.809*

Table 2: Pearson correlations between performances in the 3 sites: Saint Appolinaire (SA), Saint-Saud (SS) and Peyrat-le-Château (PC). Performances in pure species: 'EL' and 'JL'; Performances in hybridization: 'HE' and 'HJ' respectively. Exceedance probability: ': CI (95%) above 0; *: CI (95%) above 0.3; **: CI (95%) above 0.6

taxon	trait	SA vs. SS	SA vs. PC	SS vs. PC
EL	BHC	0.577	0.471	0.317
	HT	0.614'	0.489'	0.477'
	DEN	0.359	0.529'	0.183
	HWP	0.381	0.195	0.15
	FLX	0.719	0.684'	0.673
	BUD	0.918**	0.829*	0.763*
HE	BHC	0.114	0.142	0.630'
	HT	0.534*	0.424'	0.834**
	DEN	0.772*	0.514	0.457
	HWP	0.785*	0.344	0.142
	FLX	0.854**	0.726*	0.836**
	BUD	0.910**	0.911**	0.894**
JL	BHC	0.263	0.314	0.372
	HT	0.682*	0.567'	0.738*
	DEN	0.955**	0.926**	0.911**
	HWP	0.43	0.057	0.346
	FLX	0.912**	0.687*	0.759*
	BUD	0.895**	0.802*	0.844**
HJ	BHC	0.55	0.639'	0.396
	HT	0.805*	0.536'	0.672*
	DEN	0.899**	0.818**	0.817**
	HWP	0.559	0.436	0.454'
	FLX	0.927**	0.669*	0.661*
	BUD	0.923**	0.643*	0.681*

in the study, namely bud flush, stem flexuosity and wood density. These traits were stable across sites and showed generally high correlation between performances in pure and hybrid crosses. These results, although based on a relatively narrow base population, are of key importance when planning hybrid breeding for larch.

4.1 Hybrid larch heterosis

Best-parent heterosis in growth traits (BHC and total height) was validated in all sites, except in PC for BHC where HL almost reached its best parent (JL), though it overpassed it for height. As a result, regardless if the site was more favorable to EL or to JL, the hybrid yielded more than or as much as the best parent species for growth traits. For BHC, the relative best-parent global heterosis was 15.7% in SA (best parent: EL), the least 'fertile' site and 12.0% in SS (best parent: JL), the most 'fertile' site. In PC (similar 'fertility' as SS), the hybrid BHC was 6.3% lower than that of JL and 25.8% higher than that of EL (Fig. 1 and Appendix 2, Table 9). Using the multi-site diallel mating design trial we described here (though, including Cumière site instead of PC), heterosis was already shown for growth traits at a juvenile stage (age 6) (Pâques, 2002). Near-rotation age (at year 22) heterosis between EL and JL has also been shown using a diallel mating design trial, in a single site in Central Maine, USA (Greenwood et al. 2015). Like us, Greenwood et al (2015) found no reciprocal effect between JL×EL and EL×JL, and they demonstrated best-parent heterosis at the taxon level for height, BHC and volume over proper parental references. The novelty of our study was to show heterosis at near-rotation age in several contrasted sites, using a constant genetic material.

In SA and in SS, after age 15-16, hybrid superiority over its parental species reached a plateau for growth traits and more clearly for BHC. In SS only, the last thinning campaign could play a role in this phenomenon. Nevertheless, this result comforts the hypothesis that heterosis would be expressed at a juvenile stage, and that the acquired superiority would be conserved along the tree's life. Indeed, it is now well-known by foresters that hybrid larch in pure plantations reaches its maximum mean annual increment

for stem volume 5 to 20 years earlier than its parental species (Rondeux, 2003). Also, in trials where taxa are closely inter-mixed as in our study, one can expect that hybrid with its rapid growth could have overgrown its parental counterparts in the neighborhood and prevented them from catching up at later ages. As an illustration, a link between genetic performances and competitiveness has been shown by Cappa et al (2015) in loblolly pine, with a high correlation (CI: 0.77 to 0.92) between BHC breeding value and competition ability. We failed to fit such a competition model (Muir, 2005; Muñoz and Sánchez, 2015) to our data. The model led to unreliable results, likely due to the mix of species and the over-parametrization, and it was thereby disregarded (result not shown). Presumably, competition could have interfered with the phenomenon of heterosis for growth traits, notably for BHC which is known to be affected by competition.

We did not find a clear pattern of best-parent heterosis for the other investigated traits. Hybrid wood density was intermediate between both parental values; this trend was clear and significant in SA and SS but more confused in PC. Similar results for hybrid wood density were also found in Pinus hybrids (Dungey, 2001). In larch, Charron et al (2003) already reported a lower hybrid wood density when compared to EL counterparts. Nevertheless, the fact that faster growing HL still produced higher density than its worst parent for this trait (JL) is promising for further breeding and for the sustainability of larch wood products quality. Concerning the other traits, hybrid seemed to be less straight than its parents, notably when compared against JL. For bud flush and HWP, the ranking of taxa was not conclusive (result not shown).

Some singularities affected PC site, namely the absence of best-parent heterosis for BHC, and the confusion of density ranking that was otherwise clear in the other sites. Given that PC was the only site with grafted trees, one can suspect complex interactions between rootstocks and scions to be one of the underlying causes of this singularity. Rootstock species effects were found for growth traits (Appendix 2, Fig. 5). This finding indicates that the use of grafted trees may introduce an additional unwanted source of variation that should be taken into account in any case. On the other hand, we showed for growth traits that any species grafted on a hybrid rootstock may express some heterosis that led to a gain in height and in BHC after 11 years. This finding suggests thus that heterosis results not only from favorable functional properties of the aerial part but also from the below-ground part of the tree.

4.2 Heritabilities

Some traits showed high heritabilities: bud flush, stem flexuosity and, for JL only, wood density. The gain in selection relies on high heritabilities as well as on phenotypic variances (Falconer and Mackay, 1996). Across sites and taxa, the estimated phenotypic standard deviations ranged from 0.79 to 1.22 for flexuosity on the 1-5 scale; from 0.66 to 1.18 for bud flush on the 0-6 scale (Appendix 2, Table 9). Therefore, it is possible to improve these traits through breeding. However, wood density standard deviation was quite low (ranging from 28.6 to 42.0 $kg.m^{-3}$) so the perspectives of gain for this trait remain low.

For all taxa, height heritability was often intermediate, and for BHC and HWP, heritabilities were mostly low. The general trend was not perfectly followed by EL however, showing a site-conditional expression of its heritabilities. In particular, BHC heritability was moderate for EL in SA ($\hat{h}^2_E = 0.356$) and accompanied by a phenotypic standard deviation of 101.9 mm. We also found a very low EL heritability for stem flexuosity in SS. This may be explained by the thinning that occurred in this site. Indeed, thinning censure could not be recovered from young stage observations because of the quite low additive correlation between young and old stage flexuosity for EL in SS ($\hat{r}_{AE} = 0.455$). On the other hand, JL heritabilities were generally better preserved across sites, with high magnitudes for density, bud flush and flexuosity. Interestingly, considering all taxa, the most heterotic traits (BHC and height) were associated with low to moderate heritabilities, whereas non-heterotic traits such as bud flush, stem flexuosity and wood density were highly heritable. This suggests that biomass related traits (BHC and height) could mostly be improved at the hybridization stage, whereas bud flush, density and flexuosity could be improved by recurrent selection within the pure species. Indeed, these later traits showed generally high stability of the performances in hybridization. Hybrid wood density was clearly under JL additive control. Only HWP showed a difficult path towards genetic improvement, with no heterosis and a very low level of heritability. However, as absolute heartwood size follows stem size (Pâques and Charpentier, 2015), total heartwood size may be improved along with BHC through hybridization.

We compared our narrow-sense heritabilities estimates (Fig. 2) to the ones from the literature which were reviewed by Pâques (2013) (Appendix 4, Fig. 12). Only EL and HL heritabilities were available for a few traits in this review. The EL heritabilities (h2E) that we estimated were well within the range of variation of the other authors' estimates for bud flush, height and flexuosity, except for flexuosity in SS for which our estimation was noticeably low. On

the other hand, BHC heritabilities were lower than the ones found in the literature, except in SA where it was consistent with published values. Hybrid heritabilities ($h^2_{HE}+h^2_{HJ}$) were close to the literature references for flexuosity and density, but our study obtained lower estimates for height and BHC than those already published. Only Hering (1990, in Pâques, 2013) found also low heritabilities for these growth traits. More generally, moderate heritabilities for height and high ones for wood density could be expected for tree species (e.g. see Cornelius, 1994; Kain, 2003), but according to these studies our estimated flexuosity heritabilities were unusually high and the BHC ones were unusually low.

Heritability underestimation, notably for growth traits, could be due to the inter-specific competition in our experimental design. Indeed, a potential limitation of our study came from our single- (or in PC three-) tree plot design, which may have enhanced competition between taxa. Some authors found a link between competition and depletion of heritability. Lin et al (2013) showed a depletion on breast-height diameter heritability at age 5 in *Pinus radiata* under competition induced by a high plantation density. Also at high plantation density, Bouvet et al (2003) observed a depletion of both BHC and height heritabilities in *Eucalyptus* hybrids. Although this latter depletion was not significant, this effect remained stable from age 4 onwards and it was validated in 3 independent trials. The question remains nevertheless on how far inter-species vs. intra-species competition affects heritability estimates.

Some of our results had no correspondence to those in Pâques (2004). The wood density, which we found mostly under Japanese larch control, was estimated to be under European larch control by Pâques (2004). Unlike this latter publication, we found little heritability for growth traits (height and BHC), and stem flexuosity being under European or both parents control. Several reasons may contribute to explain this. On one hand, our findings highlight the need to assess these parameters in several environments, while Pâques (2004) results come from a single site, which was different from ours. On the second hand, the mating design was more unbalanced in Pâques (2004) and included different genetic basis with EL from the Alps.

Pâques and Charpentier (2015) found HL HWP heritability (full-sib family mean h^2) to be lower (0.49) than heartwood diameter heritability (0.68), but still higher than our narrow-sense heritability estimates ($\hat{h}^2_E$, $\hat{h}^2_J$ and $\hat{h}^2_{HE}+\hat{h}^2_{HJ}$ ranging between near 0 and 0.232). This could suggest that for this trait there was either little genetic variance in our sampling, or lack of accuracy in our measurement method.

4.3 Dominance

The dominance variances and the subsequent degrees of dominance we estimated were very low ($\hat{d}^2 \leq 0.136$), even for traits showing heterosis. Kain (2003) also found very low d^2 (between 0 and 0.1) for 17 traits in the 2 *Pinus pure* species he investigated, however he obtained high dominance to additive variance ratio in *Pinus elliottii var. elliottii* × *Pinus caribaea var. hondurensis* hybrid for some traits including breast height diameter and volume. Dungey (2001) pinpointed that in several species the relative importance of dominance variance tends to diminish with age. This trend was also found in hybrid larch (Pâques, 1992a). In the present article, we presented the analysis of data collected on old trees, generally older than in many of the reviewed articles in Pâques (2013), which overall leads to particularly reliable estimations of the additive genetic performances.

4.4 Additive correlations

Bud flush was genetically correlated with flexuosity. We found a negative additive correlation between these two traits, meaning the later the bud flush, the straighter the stem was. We also found a positive additive correlation between bud flush and BHC in SA, pinpointing to the need for a trade-off between BHC and flexuosity if selecting on bud flush. We also showed positive additive genetic correlation between height and density in SS and in PC. This could be an effect of the competition within these 2 sites. Indeed, though regularly thinned, SS is from far the most fertile site, and PC is also more fertile than SA (see Appendix 1, Table 6, and taxa means ranking in Appendix 2, Table 9). Therefore we could expect competition to be stronger in SS and PC than in the somehow poorer SA site. However, the hypothesis that competition leads to positive correlations between height and density would deserve further investigation. Wood density variability was under JL control, and for this taxon we found a negative correlation between density and BHC, in pure species as well as in hybridization. This trend was only supported by moderate statistical evidence in hybridization in SA; but it might pinpoint at the possible trade-off between wood volume and wood quality, an issue raised by Kain (2003) with hybrid pines. However, on one hand, gain in wood productivity using best-parent heterosis at the taxon scale was not accompanied by a loss of density, as hybrid density was intermediate between its parental species. On the other hand, based on heritability differences alone, a genetic improvement of wood density would be more effective than that of wood volume.

4.5 Stability of performances

Correlations between BV and GHA and across-sites correlations followed the same pattern as heritabilities. This could be expected as low heritability traits lack the genetic information that is needed to properly assess these correlations. Conversely, we found high correlations between BV and GHA for high heritability traits. This result was in line with Dieters and Dungey (2000)'s suggestion that the correlation between BV and GHA may be positively related to additive to dominance variance ratio, as we found very low d^2 and $\frac{h^2}{d^2} = \frac{\sigma^2_A/\sigma^2_P}{\sigma^2_D/\sigma^2_P} = \frac{\sigma^2_A}{\sigma^2_D}$. Correlations between BV and GHA and across-site correlations are both valuable parameters for guiding the choice of breeding strategies. On one hand, the high correlations between pure species and hybrid performances indicate that breeding efforts at the parental species level can be easily translated into comparable progress at the hybrid stage. On the other hand, we expect the genetic gains to be stable across sites, especially for flexuosity and bud flush in hybridization. Moreover, the high proportionality between JL density performances in pure species and in hybridization together with the stability of JL density performances across sites are very valuable; they allow a field screening of JL trees for their genetic value for this trait in hybridization.

4.6 About the method

The MCMC algorithms used in this study are rarely used in quantitative genetic studies of forest tree species. However, these algorithms present many computational advantages. Overall, they tend to be robust solvers even when low level of genetic information is available. Using PX prior, we could handle close-to-zero variances, an issue sometimes met with average-information REML algorithm (e.g. see Mutete et al, 2015). The prior parametrization we used was flexible enough for variances as well as for covariances components. Heritabilities estimates ranged from almost 0 to 0.660, showing that phenotypic variance could be properly partitioned according to data information. In the same way, correlations estimates ranged between close-to-zero and close-to-one (plus or minus one, $max(|\hat{r}_A|) = 0.888$ and $max(|\hat{r}_R|) = 0.895$); for the PX (genetic) posteriors (Fig. 3) as well as for the improper (residual) posteriors (Appendix 2, Fig. 7). The possibility to make direct inference on derived parameters such as heritabilities and genetic correlations was very powerful. We could also measure the reliability of our correlations between BV and GHA, an issue raised by Dungey (2001).

In order to get rid from the inter-taxa competition interference, it could be interesting to re-think our experimental design for further study of such contrasted taxa. Pure taxa micro-plot design might be an alternative solution. However, border trees would still be exposed to inter-taxa competition, so they would have to be excluded - raising an extra-cost. Moreover, the spatial effect would be in confusion with the taxon effect. Spatial analysis methods that are less susceptible to high frequency effects, such as splines (Muñoz and Sánchez, 2015), could be a solution for the analysis of such an experimental design. Another solution would be the use of large pure taxa macro-plot design. However, taxa and environment effects would be confounded; using clonal repetitions as control in each macro-plot would produce an extra-cost because of border trees; and an extra-difficulty because of the potential interactions between the genetic material and the environment.

4.7 Conclusion

Hybrid outperformed, or at least equaled, both of its parents and this occurred in all three testing sites, without degrading wood density, one key wood property. This highlights the interest to develop in priority hybrid larch varieties. Although our results here are based on a relatively small sample of parents from both species, we could layout the broad outlines of a strategy for hybrid larch breeding. Basically, traits felt into two contrasting behaviors: those showing relatively high heritabilities, high across-site stabilities and high stabilities of performances in hybridization, and those with relatively low heritabilities, low across-site stabilities and low stabilities of performances in hybridization. The former group included bud flush, stem flexuosity and wood density, which could be easily improved by selection at the parental stage and be evaluated interchangeably by the mean of inter- or intra-specific progeny tests. The prospects for improving straightness at the parental level could somehow compensate the medium to poor performances of the hybrid for this trait in comparison to its parents. The additive correlation between bud flush and flexuosity would facilitate the selection on these 2 traits simultaneously. Hybrid larch wood density was intermediate between its parents, but it can be improved by selection on the JL side. Hybrids in a reciprocal recurrent selection could recover improvement made at the parental stage for these traits. Growth traits quantitative genetics was shown to be more complex, resulting in less heritable patterns and presumably with phenotypic expressions prone to be affected by inter-specific competition. However, these traits were also the ones benefiting the most from hybridization. The fact that growth traits in our study presented performances that were site dependent could indicate the need for strategies where selection is tailored depending on hybrid deployment. In that sense, our study

further supports the hypothesis of hybrid gaining site stability with respect to its parents, which is valuable in the handling of G×E and in the context of current environmental instability.

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Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest.

Data Archiving Statement

Our datasets are being submitted to GnpIS INRA Repository and the accession numbers will be supplied once available. We acknowledge that the final acceptance of a manuscript into TGG is contingent on all relevant accession numbers being made available in text.

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Appendix 1: Description of the set-up and data

Table 3: Mating design and number of trees per combination in Saint-Appolinaire. EL: European larch, JL: Japanese larch

		Diallel EL									Diallel JL									Polycrosses	
		104	106	109	166	214	221	222	242	284	3179	3180	3183	3190	3193	3194	3200	3203	3217	EL	JL
Diallel EL	104		20	21	12	11	33	11	5	26	33	29	38	28	29	31	9	18	33		16
	106	29		36	18		12	32	1	8	31	29	22	23	29	33	13	30	14		24
	109		13		16	2	16	8	4	4	16	8	2	9	9	21	17	13	10		
	166										14	11	1				10				
	214										7	14	5		4	4	9		11	25	
	221	5		10	8	9		12		16	10	4	6	7	24	17	7	5	6		
	222				1						25	11		21	23	9	18	11			
	242		33	9	5		9	11			20	18	8	9	29	9	24		34	26	
	284	11	12		4						7	21	3	6	14	12	10	18	4		
Diallel JL	3179	9	3	33	19	33	11	38	15			19	14	20	26	24	13	19	17		2
	3180	12		20	15	8	11		9		26		5	9	11	12	9	21	12		10
	3183	14	34	17	14	7	10	9	4	14	27	8		10	21	14	12	13			2
	3190	1	13	9	15	12	12	11	11	12	5	6	3		9	2	1	23	8		6
	3193	7	8	7	16	6	11		11		4	7	22	6		4	24	25	18		16
	3194				8				12		16	10	6		13		1	28	9		4
	3200						8					13	9	4	6	2					7
	3203	13	14	29	8	14	17		13	2	13	36	2	7	4	8	3		7		
	3217	39	17	18	15		49		11		36	32	13	2	16	24	19	20			11
Extra EL	78												8		8	8					
	108										11	12	22	17							
	129										11	7	30	7	14						
	147										1										
	151										14	29	20	4	19		4				
	229										7	9	23	7	4						

Table 4: Mating design and number of trees per combination in Saint-Saud. EL: European larch, JL: Japanese larch

		Diallel EL									Diallel JL									Polycrosses	
		104	106	109	166	214	221	222	242	284	3179	3180	3183	3190	3193	3194	3200	3203	3217	EL	JL
Diallel EL	104		25	25		11	26			20	27	28	22	23	30	27	19	20	30		
	106	24		26	27		10	29		9	22	29	23	17	28	24	12	27			24
	109				12		13	9						11		22	17		4		
	166										6	11					16				
	214																11		8	27	
	221			5						19				9	28	11	9				
	222										28	12		27	20	11	25	12			
	242		29				10	12			22	12		11	22		27		28	29	
	284											14			13	11	10	15			
Diallel JL	3179			27	12	25		29				20	19	24	25	23	12	21	21		25
	3180										21			5	14	23	11				25
	3183		27	21	20		9	10			27	10		17	19	21	15	14			18
	3190		12		21	11	11	10			10				8			21	9		21
	3193		9		14								19	5			19	27	12		26
	3194										9				13			29	10		21
	3200																		6		21
	3203	11	14	26		11	22		12			27					10				
	3217	30		13	13		28				22	29	12	10	6	28	21	19			21

Table 5: Mating design and number of trees per combination in Peyrat-le-Château. EL: European larch, JL: Japanese larch

		Diallel EL									Diallel JL									Polycrosses	
		104	106	109	166	214	221	222	242	284	3179	3180	3183	3190	3193	3194	3200	3203	3217	EL	JL
Diallel EL	104		32	30		29	31			26	31	32	32	28	32	34	33	31	26		
	106			25	28		21	25		21	29	28	29	31	27	31	25	33			
	109				30		25	15						25		34	29				
	166											25					26				
	214																26		21	35	
	221									30				3	31	24	23				
	222											32		30	28	24	31	28			
	242		31				24	28			32	32		25	32		29		29	31	
	284											31			26	27	27	34			
Diallel JL	3179			31	27	33		29				28	30	28	25	26	27	27	30		
	3180													8	18	21	16				
	3183			28	32		22	27				20		33	28	26	26	28			
	3190				31	29	24								19			26	20		
	3193				32									8			32	32	25		
	3194														27			26	23		
	3200																				
	3203			32		26	25		28			28					28				
	3217			34	30		31					30	25	7		3	32	31			

Table 6: Description of the environment of the 3 sites

Site	Coordinates	Altitude (m)	Slope (%)	Mean Temp[1] (°C)	Rain[1] (mm)	Previous crop	Spacing of plantation
SA (Saint-Appolinaire)	45°58'N 4°26'E	784	22.3	14	649	Forest	3m×3m
SS (Saint-Saud)	45°31'N 0°48'E	307	4.7	16.3	620	Pasture	4m×2.5m
PC (Peyrat-le-Château)	45°49'N 1°44'E	461	5.2	15.4	651	Forest	3m×2.5m

[1] from 04/01 to 10/31

Table 7: Age of the trees (years after plantation) during the increment cores samplings, in Saint Appolinaire (SA), Saint-Saud (SS) and Peyrat-le-Château (PC)

site	last year	age	sample size
SA	2015	19	33
	2013	17	2081
SS	2011	15	1241
	2009	13	693
	2003	7	915
PC	2015	12	200
	2014	11	1094

Table 8: Traits involved in multi-trait analysis, with indication of age (years after plantation); for each site: Saint Appolinaire (SA), Saint-Saud (SS) and Peyrat-le-Château (PC). Traits at a young stage, indicated '(y)', were introduced in SS in order to account for thinning censure

Trait		Site		
		SA	SS	PC
Breast height circumference	BHC	14	13	11
	BHC (y)		7	
Total height	HT	14	13	11
	HT (y)		7	
Wood density	DEN	14	13	11
	DEN (y)		7	
Heartwood proportion	HWP	17	13 to 15	11
Stem flexuosity	FLX	14	13	11
	FLX (y)		7	
Bud flush	BUD	2	2	3

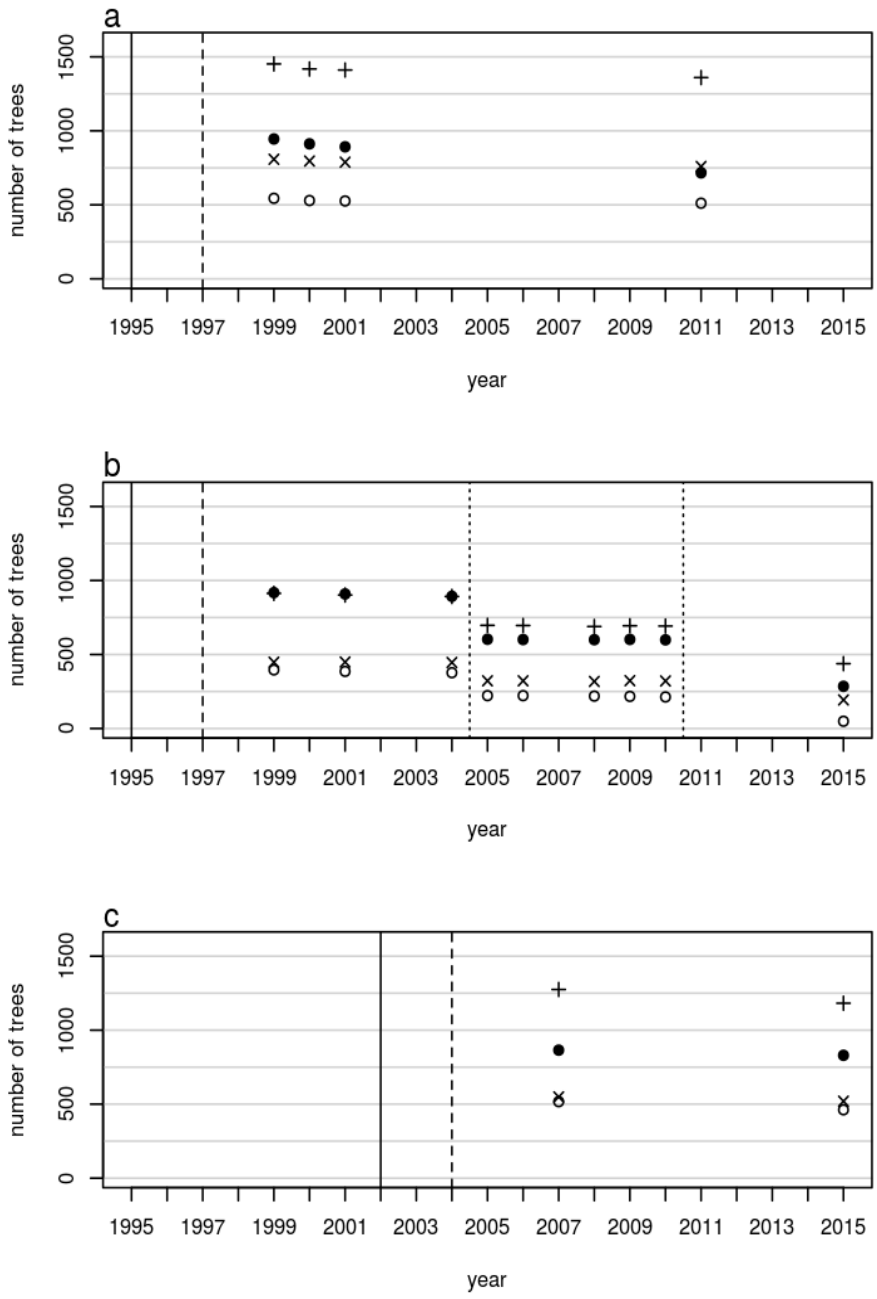

Figure 4: Evolution of the size of the set-up, in each site (a: Saint-Appolinaire, b: Saint-Saud, c: Peyrat-le-Château) and for each taxon (black dot: Japanese larch, white dot: European larch, +-shaped dot: EL×JL hybrid, ×-shaped dot: JL×EL hybrid). Vertical bars indicate the main operations. Plain bar: sowing (in SA and SS) or grafting (in PC), dashed line: plantation, dotted line: thinnings (in Saint-Saud only)

Appendix 2: Extra results

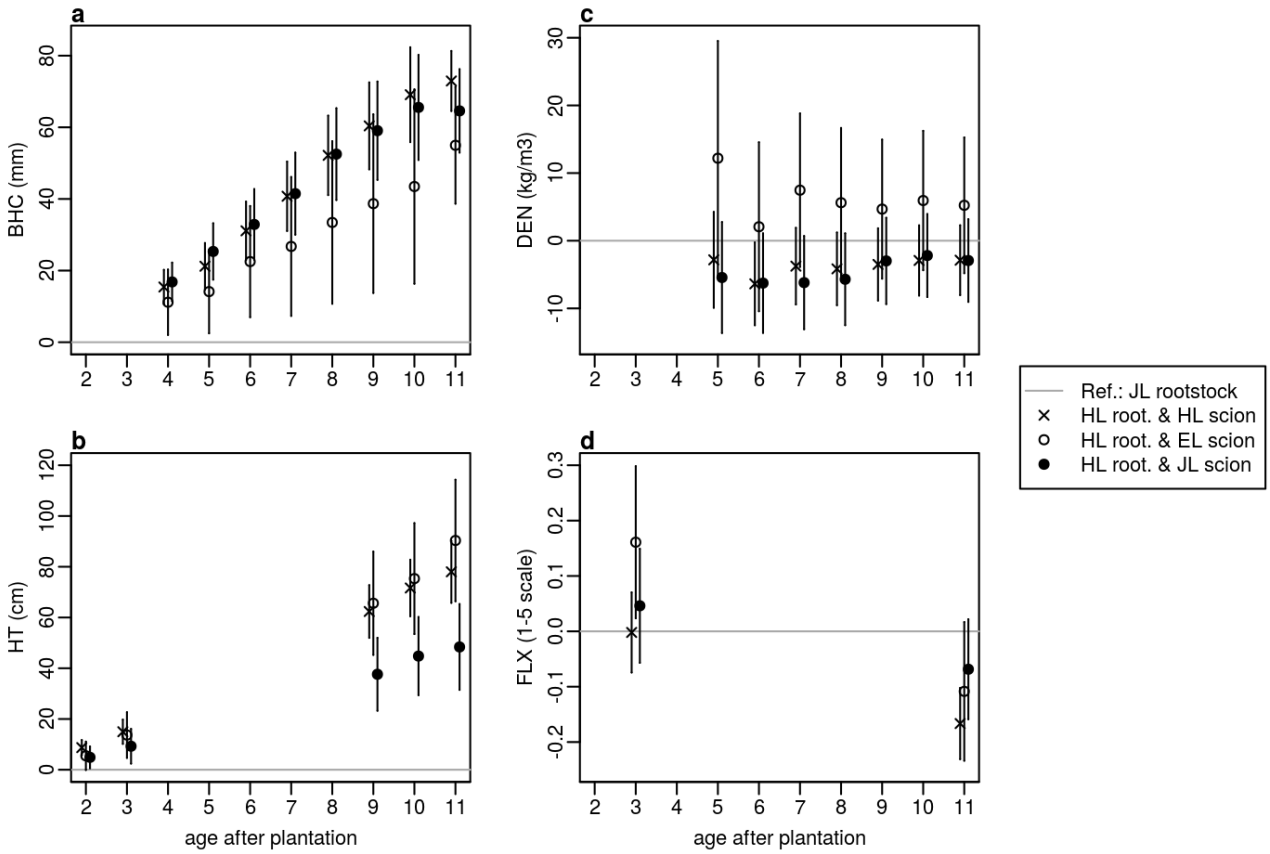

Figure 5: Hybrid rootstock effect in Peyrat-le-Château, defined, for each scion taxon, as the contrast between trees whose rootstock was HL (hybrid larch) and trees whose rootstock was JL (Japanese larch), for breast height circumference (a), height (b), wood density (c) and stem flexuosity (d). White dot: European larch scions; black dot: JL scions; ×-shaped dot: HL scions. All vertical bars: 95% confidence intervals under normal distribution assumption

Table 9: Means (m) and coefficients of variation (CV) from model M2, for European larch (EL), Japanese larch (JL) and their hybrid (HL), in Saint-Appolinaire (SA), Saint-Saud (SS) and Peyrat-le-Château (PC), for the 6 traits under study; and spatial variance proportions (SVP) and spatial autoregressive parameters (ρ) from model M1. The asterisk (*) indicates that ρ has been arbitrarily fixed to 0.99. Breast-height circumference (BHC) was expressed in mm, total height (HT) was expressed in cm, wood density (DEN) was expressed in kg.m$^-$3, heartwood proportion (HWP) was expressed in %, stem flexuosity (FLX) was a 1 to 5 mark, terminal bud flush was a 0 to 6 mark; the CVs and SVPs were expressed in %

		Model M2						Model M1	
		EL		JL		HL			
site	trait	m	CV	m	CV	m	CV	ρ	SVP
SA	BHC	465.21	21.9	406.47	18.6	552.09	16.4	0.99	44.6
	HT	1038.26	12.7	897.68	11.3	1154.2	11.2	0.99	68.3
	DEN	475.56	8.4	398.48	9.2	428.06	9.8	0.99	7.1
	HWP	56.56	22.4	55.61	23.3	58.46	20.6	0.99	14.6
	FLX	3.23	30	3.66	26	3.39	34.9	0.99	5.5
	BUD	2.42	39.1	2.39	39.4	2.35	48.4	0.99	7.3
SS	BHC	553.26	22.3	672.15	11.8	763.88	13.8	0.96	22
	HT	1476.3	10.5	1503.84	6.3	1670.89	8.1	0.96	48
	DEN	425.86	9	369.55	9.6	393.09	10.1	0.94	6.8
	HWP	59.87	18	62.84	15	63.35	17.9	0.96	8.9
	FLX	3.23	29.2	3.6	24.8	3.22	35.4	0.83	2.2
	BUD	2.75	35.8	2.38	35.3	2.02	58.1	0.98	5.7
P	BHC	335.65	24.9	481	15.9	452.54	21.5	0.95	23.8
	HT	1030.21	14.2	1196.4	7.4	1255.6	11.7	0.92	52.5
	DEN	401.81	7.2	386.59	7.4	402.63	10.2	0.99	7.2
	HWP	36.03	34.9	44.91	20.7	43.54	29.3	0.87	11.6
	FLX	3.91	20.1	4.12	20.4	3.64	33.5	0.99*	87.5
	BUD	3.61	18.6	3.87	17	3.63	24	0.99*	46.4

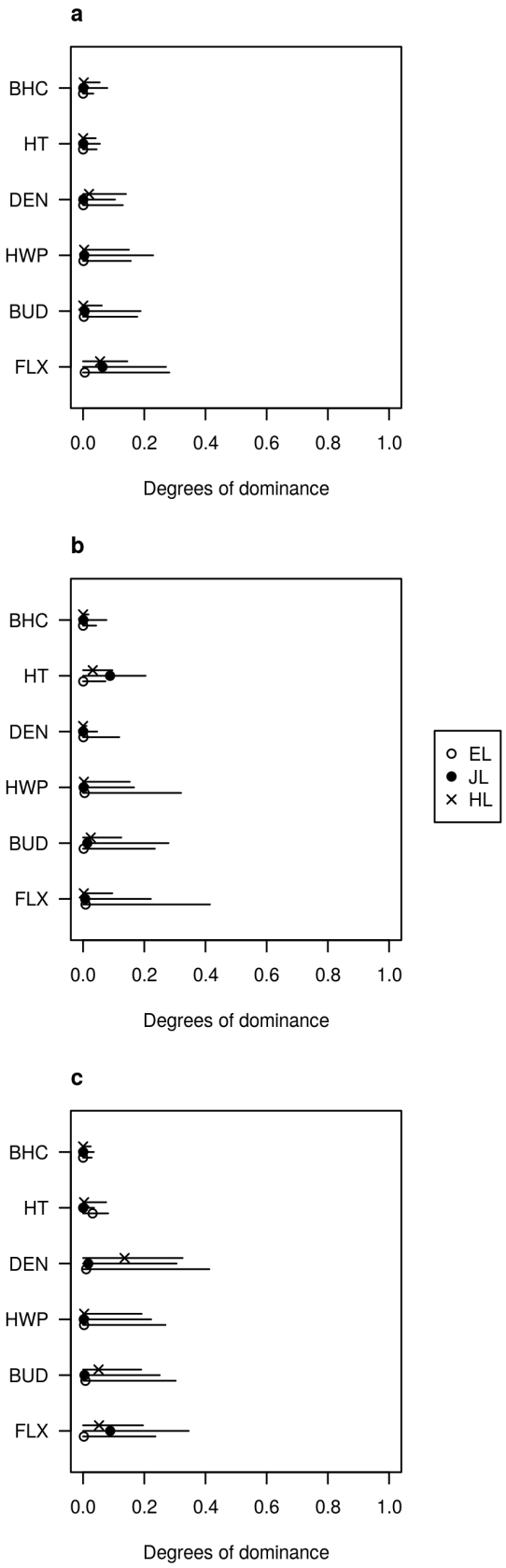

Figure 6: Degrees of dominance, in Saint-Appolinaire (a), Saint-Saud (b), and Peyrat-le-Château (c). White dot: European larch; black dot: Japanese larch; ×-shaped dot: hybrid larch. Horizontal lines: 95% CIs

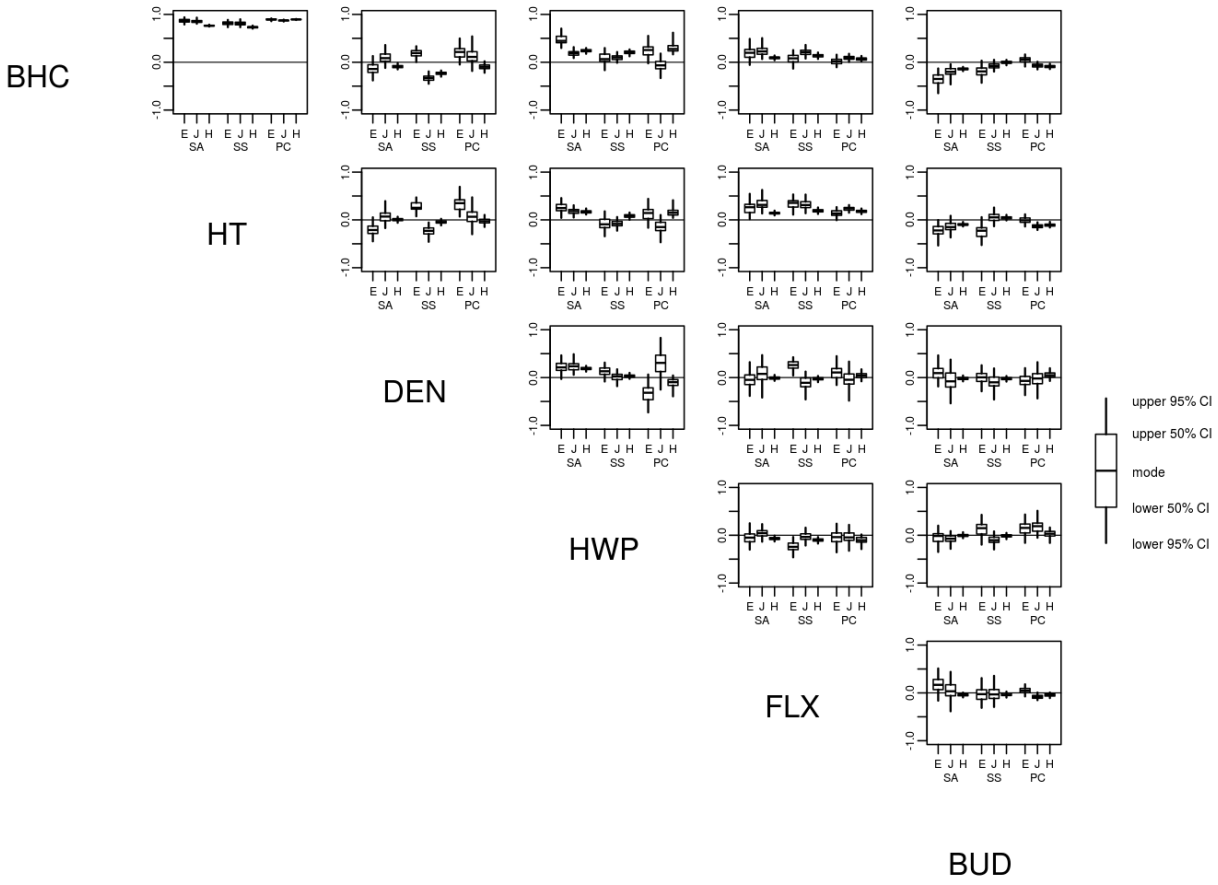

Figure 7: Residual correlations between the traits: breast-height circumference (BHC), total height (HT), wood density (DEN), heartwood proportion (HWP), stem flexuosity (FLX), bud flush (BUD); for each taxon ('E': European larch, 'J': Japanese larch, 'H': hybrid larch); in each site: Saint-Appolinaire (SA), Saint-Saud (SS) and Peyrat-le-Château (PC). Whiskers: 95% CIs; box: 50% CIs; middle-line: mode (i.e. maximum a posteriori)

Appendix 3: Performances of the parents predicted by model M2

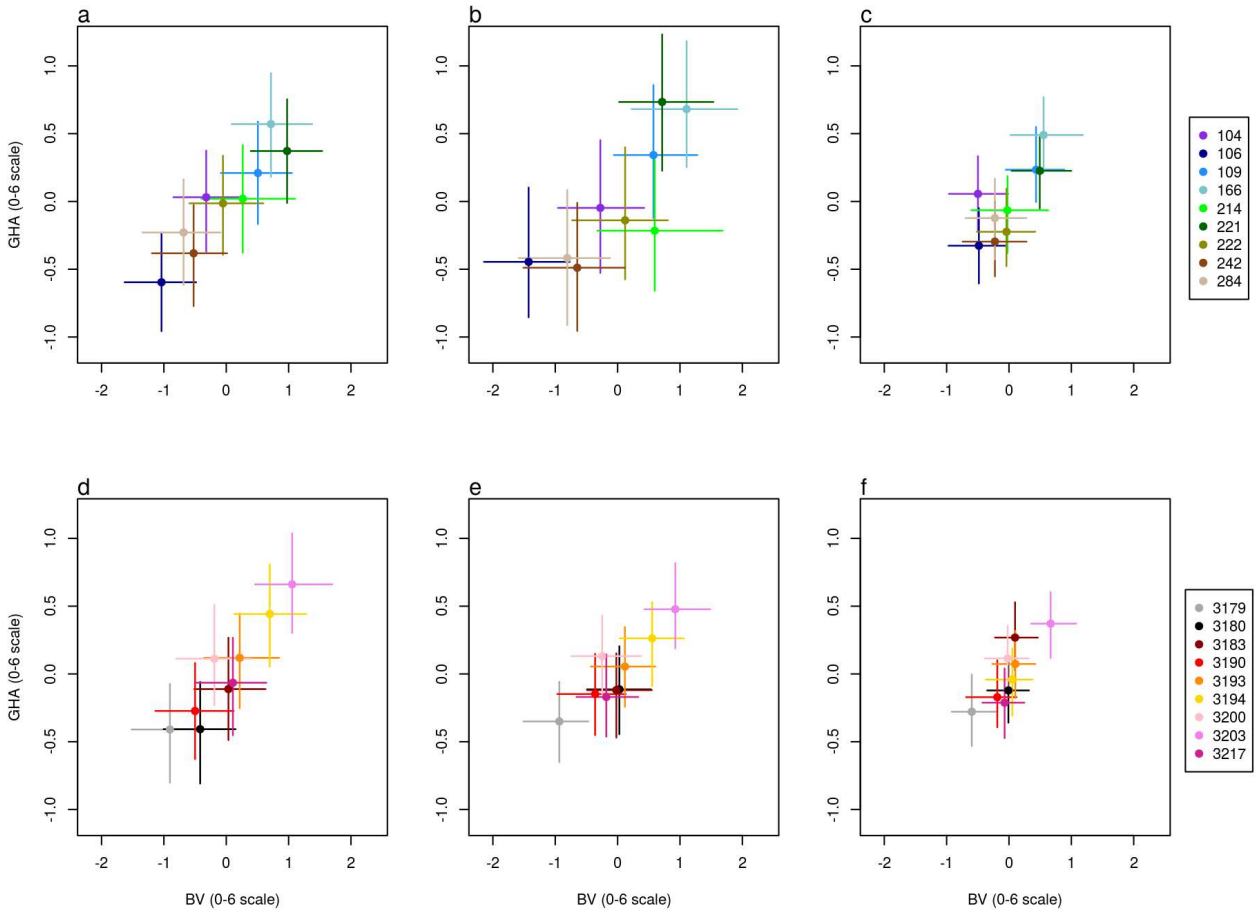

Figure 8: Performances of the 9 European larch parents (a-c) and the 9 Japanese larch parents (d-f) for bud flush, in pure species breeding (BV: breeding value) and in hybridization (GHA: general hybridization ability), in each of the 3 sites: Saint-Appolinaire (a, d), Saint-Saud (b, e) and Peyrat-le-Château (c, f). For each species, the labels of the trees associated with each dot are provided on the side. The horizontal and vertical grey bars are 95% CIs. Higher marks mean earlier bud flushes

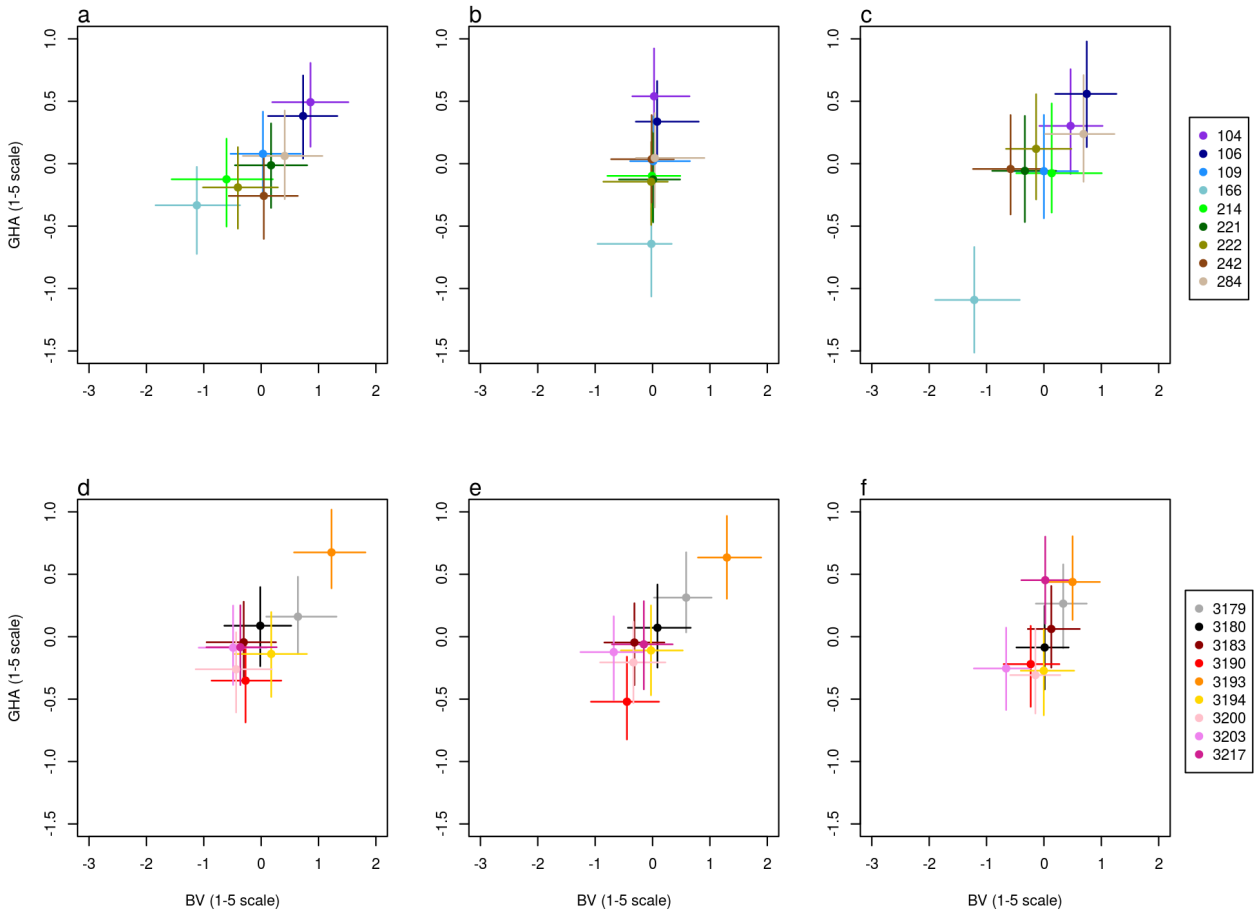

Figure 9: Performances of the 9 European larch parents (a-c) and the 9 Japanese larch parents (d-f) for stem flexuosity, in pure species breeding (BV: breeding value) and in hybridization (GHA: general hybridization ability), in each of the 3 sites: Saint-Appolinaire (a, d), Saint-Saud (b, e) and Peyrat-le-Château (c, f). For each species, the labels of the trees associated with each dot are provided on the side. The horizontal and vertical grey bars are 95% CIs. Higher marks mean earlier bud flushes

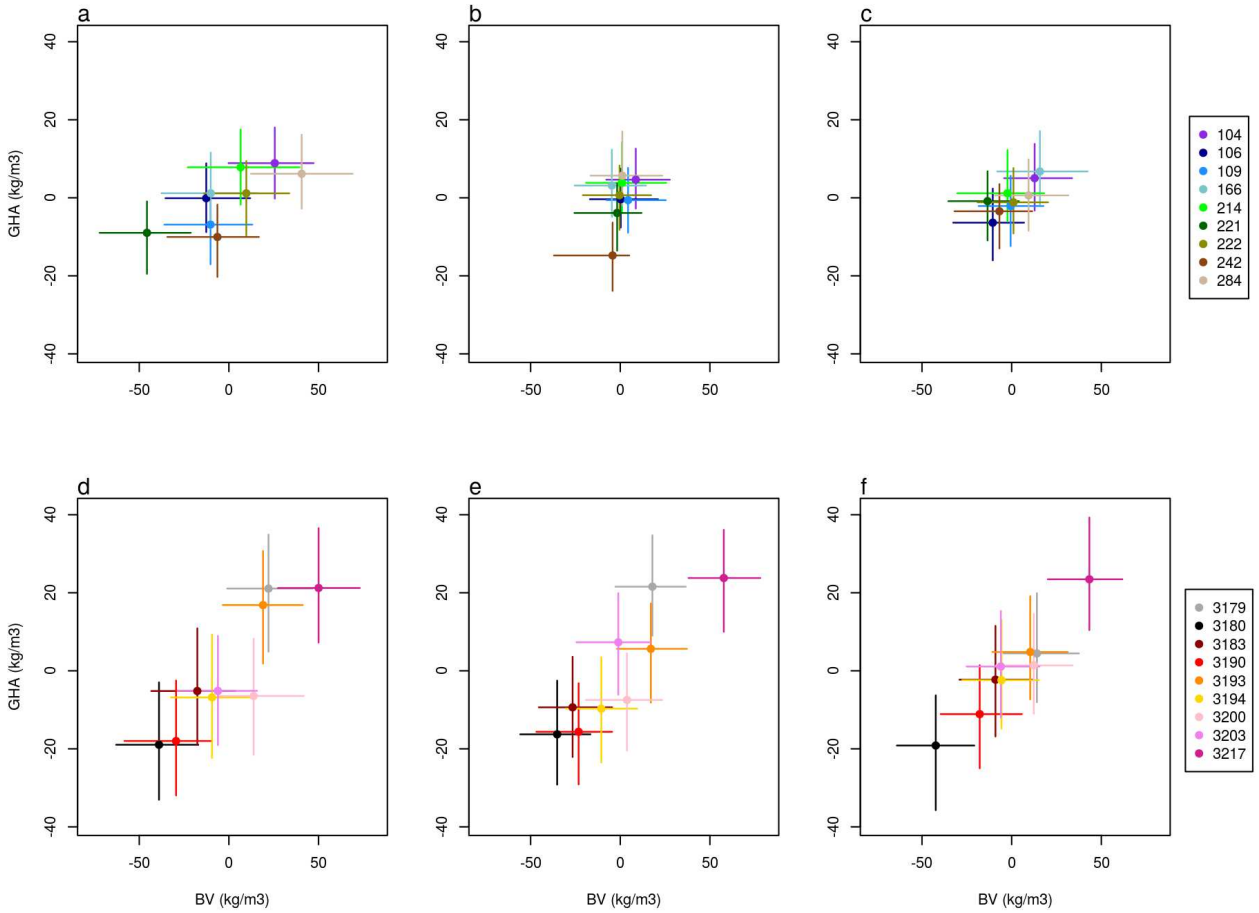

Figure 10: Performances of the 9 European larch parents (a-c) and the 9 Japanese larch parents (d-f) for wood density, in pure species breeding (BV: breeding value) and in hybridization (GHA: general hybridization ability), in each of the 3 sites: Saint-Appolinaire (a, d), Saint-Saud (b, e) and Peyrat-le-Château (c, f). For each species, the labels of the trees associated with each dot are provided on the side. The horizontal and vertical grey bars are 95% CIs. Higher marks mean earlier bud flushes

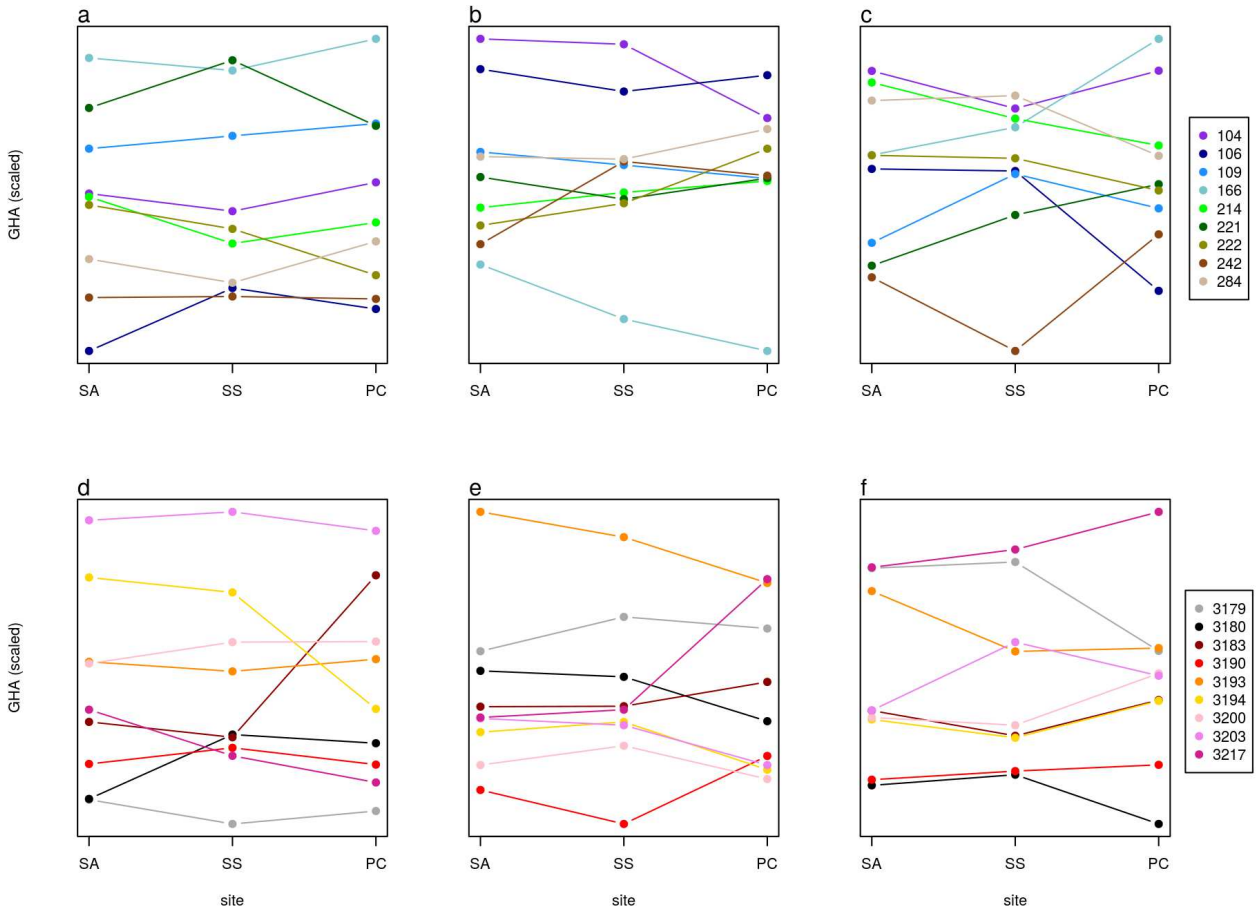

Figure 11: Changes in hybrid larch performances ranking caused by G×E interactions, for European larch GHA (general hybridization ability) (a-c) and Japanese larch GHA (d-f), for bud flush (a, d), stem flexuosity (b, e) and wood density (c, f). All traits were scaled. Test sites were SA: Saint-Appolinaire, SS: Saint-Saud and PC: Peyrat-le-Château

Appendix 4: Comparison between the heritabilities estimated in this study and the heritabilities of the literature reviewed by Pâques (2013)

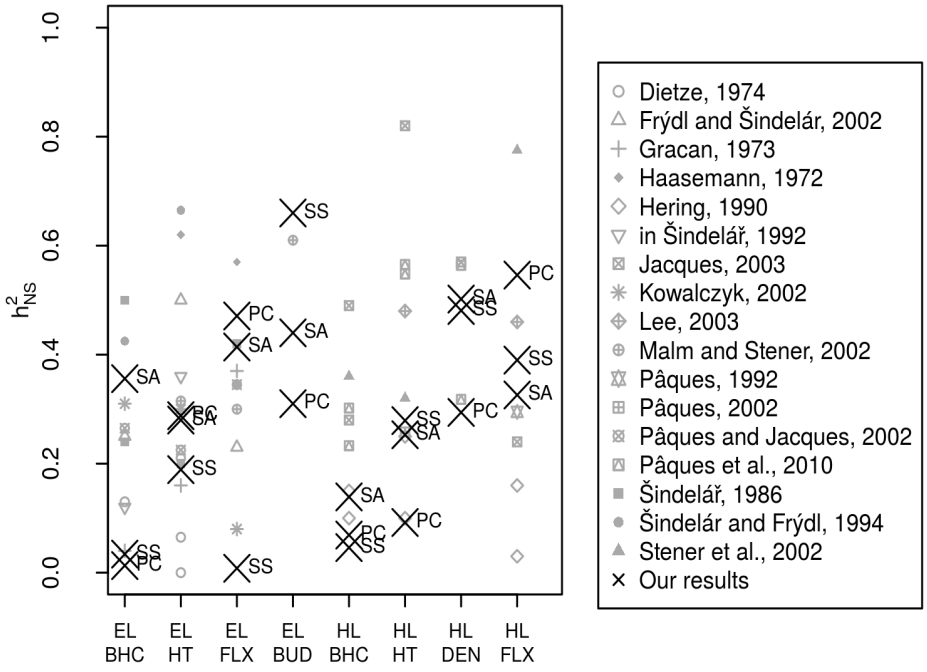

Figure 12: Heritabilities from Fig. 2 (×-shaped black dots), plotted together with heritabilities from literature (all other grey dots) reviewed in Pâques (2013); for European larch (EL) and Japanese larch (JL); and for the traits breast-height circumference (BHC), total height (HT), wood density (DEN), stem flexuosity (FLX), bud flush (BUD)

4. Chapter II. Deciphering hybrid larch reaction norms using random regression

One of the main results from the 1st Chapter was the evidence for best-parent heterosis on circumference in two sites of the study (SA and SS). The heterosis started to manifest form an early age, and increased almost linearly until it seemed to reach a plateau. The core motivation for the second chapter was to understand the role of the climatic environment on the construction of this radial heterosis. Circumference is an integrative trait, built as the sum of multiple years of growth. But, radial growth is variable from year to year due to dendroplasticity. For this reason, we aimed to determine the specific dendroplasticity of each taxon, and to understand the pattern displayed by the hybrid that could ultimately contribute to explain the overall heterosis.

4.1. Analytical considerations

In this chapter, we focused on two tree ring traits: the ring width (RW) and the ring mean density (RMD). We chose these traits because RW sums to the total stem circumference (BHC), and RMD averages to the overall wood density (DEN). Therefore, these traits contribute to the wood yield and quality described in the first chapter. We estimated the reaction norms of these traits *vs.* the climatic environment using random regressions. This choice was motivated by two reasons:

- Using Legendre polynomial base functions, we allowed the estimated reaction norms to take complex shapes.
- This framework accounts for the genetic structure of the data. Therefore, genetic parameters (heritability and additive correlations) and predictions (breeding values and general hybridization ability) were estimated and viewed as functions of the climatic environment.

4.1.1. Lessons from Chapter 1

Two lessons were drawn from the 1st Chapter. On one hand, we did not consider further the dominance variance as it appeared to be extremely low. On the second hand, special results were observed in the third site PC: no best-parent heterosis was observed for circumference, and the mid-parent position of the hybrid for wood density, confirmed in SA and SS, was not observed in PC. These results may be related to the particular experimental design in PC, as the trees there were grafted on rootstocks from arbitrary species. Indeed, we showed in Chapter 1 that the rootstock species effect was strong and significant. For the reason of this special grafting design in PC, we excluded it from the phenotypic plasticity analysis of Chapter 2. The motivation for doing so was that in Chapter 2, all sites (SA and SS) were analyzed together (unlike in Chapter 1) and there was the risk of a noise brought from one site blurring the whole picture.

The site PC was actually included in preliminary analyses and we found no real differences on the estimates of the across-site genetic parameters. We though confirmed the difficulty of interpretation of the results in this site, as shown in Fig. 14. Like in other sites, all taxa responded plastically to the water availability index (D1rew) for RW but not for RMD; however the ranking of the taxa was inconsistent with what was shown in SA and SS (especially for the density RMD) and we can't rule out the possibility of a rootstock effect on these reaction norms, especially given the critical fact that the rootstock is the bridge between the ground water availability and the wood properties (RW and RMD) expressed in the aerial parts of the tree.

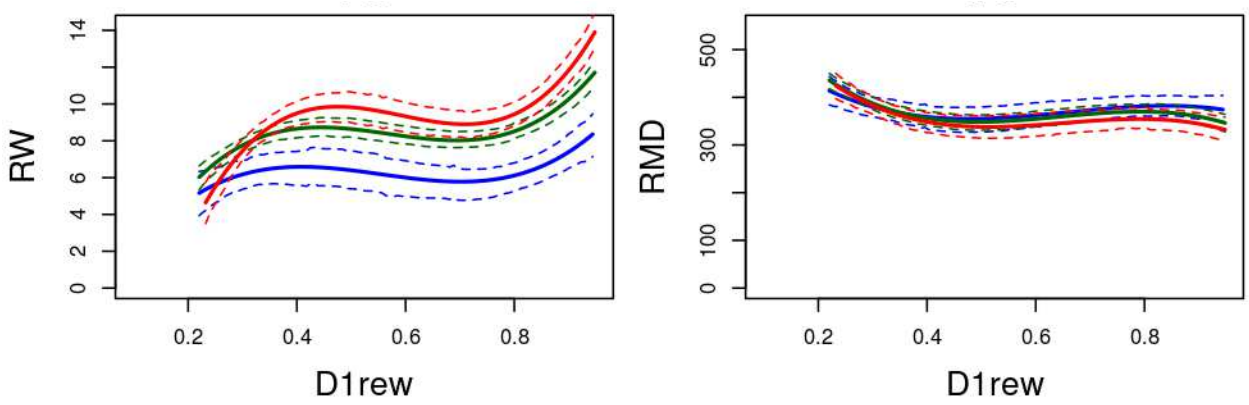

Figure 14: Reaction norms from Peyrat-le-Château, for ring width (RW, mm) and ring mean density (RMD, kg/m³), along the water availability index ('D1rew'). Green line: hybrid larch, blue line: European larch, red line: Japanese larch. Plain lines: point estimates and dotted lines: 95% credibility intervals

4.1.2. Series autocorrelation

Tree ring data are autocorrelated, as likely most biological increments growth data. This autocorrelation, especially in RW, arises from several factors:

- Ontogeny. As the tree grows older, its architecture changes: the roots explore new horizons, the crown develops and increases the transpiration, so the whole organism and its balances change and that necessarily affects the radial growth. Especially, the stem grows bigger each year, and 1 mm of radial growth does not represent the same surface, neither the same volume of wood, depending on the previous year stem circumference: this component of the problem is purely geometrical. Finally, the cambium gets older and its properties also change with age.
- Set-up of the competition with neighboring trees. Indeed, not only the tree but also its neighbors grow simultaneously. Basically, during the first decade following plantation (depending on many factors, including the site broad-sense fertility), the trees are in free growth. Then the competition start to settle: the crowns touch each other and so compete for light, and the roots compete for room and water. Finally, a hierarchy between the trees establishes. After a thinning, the remaining trees can experience a few years of free growth again, before they compete with the next neighbors. All these effects impact the radial growth and are involved in the tree rings autocorrelation.
- The multi-year effect of climatic events. In particular, extreme climatic events such as drought and heat waves may not only affect the ring of the current year, but also cause an aftereffect on the following rings. Reciprocally, trees that benefited from a favorable year could be better prepared for the following year.

We investigated the intensity of autocorrelation via ARIMA (autoregressive, integrative, moving average) models. The ARIMA models were fitted independently for each series of rings, *i.e.* for each core. The formulation ARIMA(1,0,0) and ARIMA(0,1,0), *i.e.* the pure autoregressive of order 1 and the pure moving average of order 1, yielded satisfactory results (Fig. 15). Autoregressive model takes the form:

$$y_t = \mu + \varphi\, y_{t-1} + e_t$$

where y_t is the observation at a given year t, μ the overall mean, e_t the residual, and φ an autoregressive parameter to be estimated.

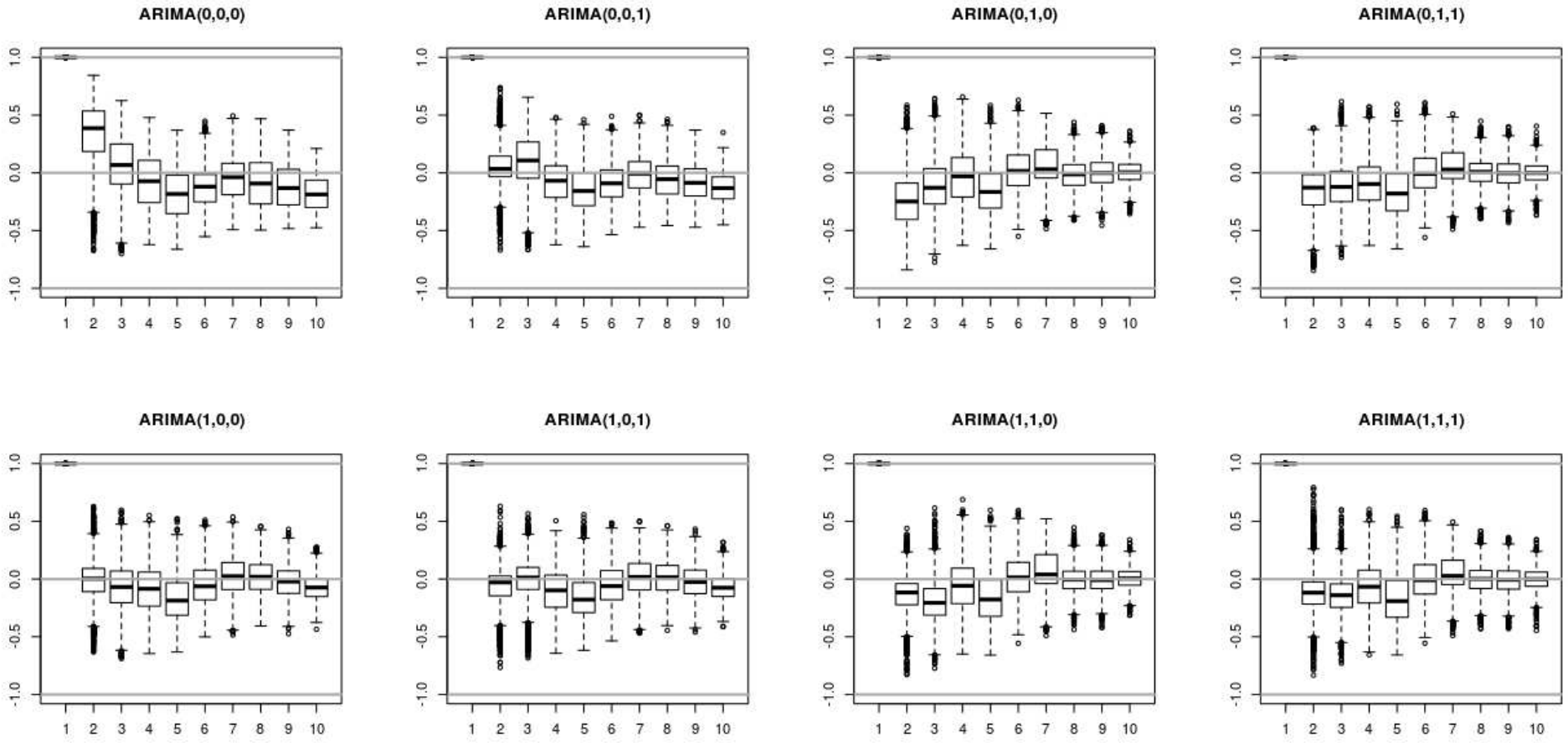

Figure 15: Autocorrelation functions of ring width series depending on the ARIMA model parametrization. Each boxplot summarizes the autocorrelation for a given lag, pulling together all the different series (i.e. the different cores) collected on hybrid larches in Saint-Appolinaire

Fitting an autoregressive model per core meant that we had to estimate as many autoregressive parameters (φ) than the number of cores, that was not a parsimonious solution. For this reason, our next step was to consider φ as random, such as $\varphi \sim N(\mu_{\varphi}, \sigma^2_{\varphi})$ so that only two parameters (μ_{φ} and σ^2_{φ}) had to be estimated and the autoregressive parameter φ was simply predicted for each core. This was possible using the flexible possibility to specify random effects in breedR (Muñoz and Sánchez 2015), and some key results are presented in Fig. 16. The underlying idea of this preliminary analysis was to subtract the autocorrelation term ($\varphi\, y_{t-1}$) from the data, in order to better isolate the plastic responses to the current climatic environment. Indeed, the high frequency signal that we are interested in is all contained within the residual (e_t).

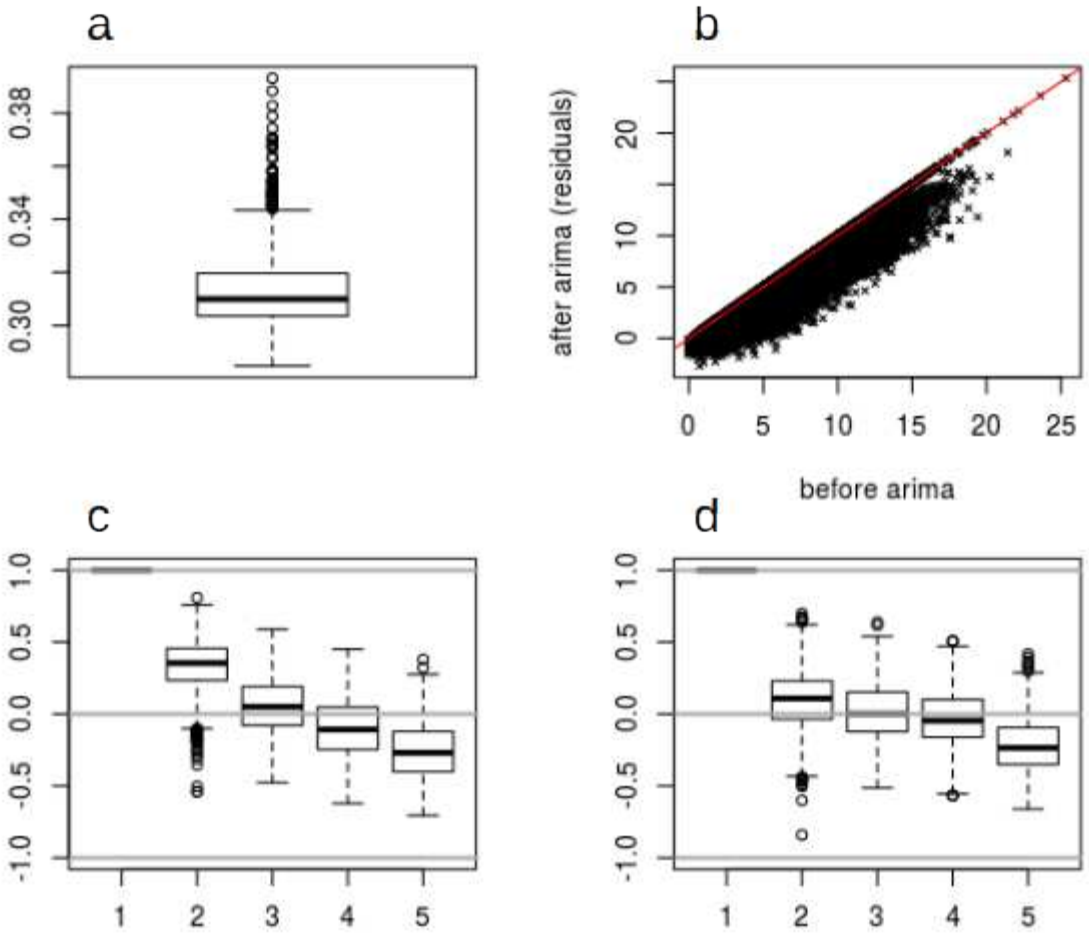

*Figure 16: Results from the autoregressive model with a random autoregressive parameter φ, applied to ring width data from all taxa pulled together in Saint-Appolinaire: (a) the distribution of the predictions of φ for each series (*i.e. *each increment core); (b) data minus the autocorrelation term ('after arima')* vs. *raw data ('before arima') plot; (c) autocorrelation function in the raw data; (d) autocorrelation function of the data after removal of the autocorrelation term*

We identified two limitations to this method. First, it is impossible to predict an autocorrelation term for the first ring (y_0) of each series. Here we simply assumed that the previous year ring (the hypothetical ring y_{-1}) had a null length, so that the autocorrelation term ($\varphi\ y_{t-1}$) was null. As seen in Fig. 16 (b), there was nothing to subtract from this first rings and therefore they remained identical before and after the subtraction. However, this method is not correct because the first rings, from which nothing is subtracted, become non-homogeneous with the other rings. The right way to do it is to rule out all the first ring from each series, this operation representing a cost of 7.6% of the dataset in SA and 10.3% in SS. Second, the main reason for which we did not go further in our attempt to account for the autocorrelation was the difficulty to interpret the results. For this reason, we favored the option to work with raw phenotypic data.

Several other options were more or less superficially explored though they would deserve deeper investigations:

- The inclusion of the autocorrelation term in the random regression model in a 1-step approach. Though conceptually more convenient, it is computationally very heavy and it still requires the sacrifice of all first rings.
- Alternatively, the use of an age effect in the model, that would absorb some low-frequency trends in the data. This would also come with a huge computational cost, especially if (and that would be the more realistic option) the age and the plasticity would be in interaction.
- Finally, it would have been possible to work with incremental surfaces instead of incremental width. However, on one hand that only solves one part of the problem (the geometry of radial growth), and on the other hand, it also requires the sacrifice the first ring of each series (as a surface is defined between 2 rings). Moreover, calculation of surfaces requires the information from field-measured BHC in addition to the tree ring data, and therefore it cumulates the uncertainty from both these pools of information.

4.1.3. Selection of an environmental gradient

As we previously stated, this Chapter implied the drawing of reaction norms of annual growth performances (RW and RMD) along a climatic environmental gradient. Therefore, a critical step inherent in this chapter was to summarize the climatic environment information into a pertinent index, indeed playing a role on the stem radial growth. Dendroplasticity is known to be affected by water availability, therefore we concentrated our efforts on this factor. We can divide the prospected climatic variables into two categories: the simple or direct ones, *i.e.* the ones that derived from raw climatic information; and the more sophisticated ones that derived from a mechanistic water balance model.

The first category of climatic variables included sums of rain, average temperatures, or naive water availability indexes such as De Martonne (1926) index, basically defined as a precipitation-by-temperature ratio. The most critical part was to define the period of the year that affected the most the tree ring growth, and in this sense the data information was very flat and did not match our prior expectations. Overall, the correlations between tree ring growth and such naive indexes remained incredibly low. Alternatively, we computed an index a bit more complex, but with a strong prior on the triggering period for ring growth: 'MJJA', inspired from Fallour-Rubio *et al.* (2009), that we defined as the sum of precipitation from May to August (both included). We also subtracted to the daily precipitations the potential evapo-transpiration (PET) before summing it, so that MJJA represented the water available for trees each year, modeled in a basic way though. We compared the coefficient of determination R^2 of the reaction norms along MJJA to the R^2 of the reaction norms from Marchal et al. (submitted), and concluded that this naive index was much less explicative than the index derived from a mechanistic water balance model ('D1rew').

The confluence of a better explicative ability (R^2) with an easier interpretability of the results led us to consider the use of a mechanistic water balance model as an obvious solution for the definition of our climatic index. Granier *et al.* (1999)'s water balance model was perfectly adapted for our needs and its implementation BILJOU© (https://appgeodb.nancy.inra.fr/biljou/) is authoritative in forest ecology. However, there were two major limitations to BILJOU© for an application to our particular case:

- We intended to estimate retrospectively the water balance of evolving stands, starting from very young trees (a few years after plantations). In the water balance model, the trees' age expresses by means of the leaf area index (LAI) that

influences notably the trees transpiration, but also the interception, and the inhibition of the understorey. Unfortunately, BILJOU© was not able to account for a varying LAI.

- As we previously showed using BILJOU©, the intra-site variability for the water availability was extremely high, due to the variability of the soil ability to store the water. This ability of the soil to store water is identified in the water balance model as the available water capacity (AWC). We did not want to neglect such an important intra-site information, but BILJOU© could not account for a spatial variation of AWC.

Because of our inability to allow both LAI to vary temporally and AWC to vary spatially, we turned towards an alternative implementation of Granier *et al.* (1999)'s water balance model that we described as the Supplementary material 2 of Marchal *et al.* (submitted). We insist on the important fact that this alternative implementation does not intend to compete with BILJOU©. Indeed, this alternative implementation (i) has not been field-calibrated, (ii) is designed for use on a young larch forest with no aspiration to generalizability, and overall (iii) has been roughly simplified.

The output of the water balance model was a daily relative extractable water (REW), from which we had to extract an annual index characterizing the water availability over the year. We proposed the first decile of each annual series of REW ('D1rew'), corresponding to the ~35 driest days of the year as an explicative climatic environment.

4.2. Article

Deciphering hybrid larch reaction norms using random regression *

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April 18, 2018

Abstract

The link between phenotypic plasticity and heterosis is a broad fundamental question, with stakes in breeding. We report a case-study evaluating temporal series of wood ring traits of hybrid larch (*Larix decidua* × *L. kaempferi* and reciprocal) in relation to soil water availability. Growth rings record the tree plastic responses to past environmental conditions, and we used random regressions to estimate the reaction norms of ring width and wood density with respect to water availability. We investigated the role of phenotypic plasticity on the construction of hybrid larch heterosis and on the expression of its quantitative genetic parameters. The data came from an intra-/interspecific diallel mating design between both parental species. Progenies were grown in two environmentally contrasted sites, in France. Ring width plasticity with respect to water availability was confirmed, as all three taxa produced narrower rings under the lowest water availability. Hybrid larch appeared to be the most plastic taxon as its superiority over its parental species increased with increasing water availability. Despite the low heritabilities of the investigated traits, we found that the quantitative genetic parameters varied along the water availability gradient. Finally, by means of a complementary simulation, we demonstrated that random regression can be applied to model the reaction norms of non-repeated records of phenotypic plasticity bound by a family structure. Random regression is a powerful tool for the modeling of reaction norms in various contexts, especially perennial species.

Keywords: phenotypic plasticity; heterosis; tree rings traits; soil water availability; multi-trait model

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1 Introduction

Heterosis in plants is a phenomenon that usually arises in unfavorable environments, as observed in a wide range of crop hybrids, like corn (Janick, 1999; Gallais, 2009). The relative stability of hybrids when the parents' performances drop is called 'hybrid homeostasis' and, as a consequence, the hybrids are often expected to perform better than their parents in most environmental conditions. The stability of heterosis across sites has recently been highlighted for hybrid larch (*Larix decidua* Mill. × *L. kaempferi* (Lamb.) Carr., and the reciprocal cross), a highly productive conifer cultivated for wood in Western Europe and North America (Marchal *et al.*, 2017). For this reason, phenotypic plasticity is suspected to play a key role in the construction of hybrid larch (HL) heterosis.

Phenotypic plasticity, in its narrow-sense definition, is the ability of a genotype to produce several phenotypes depending on the environmental conditions. It can be studied by means of reaction norms (Schlichting and Pigliucci, 1998). A reaction norm is an equation, or simply a graphical representation, of the value taken by a phenotype along an environmental gradient. Estimating reaction norms is not a trivial task. In particular, exposing the same genotype to different environments may be experimentally challenging, depending on the biological model. In some cases, the only solution is to expose related individuals to the different environments, relying on their genetic relationship to draw the common, additively inherited, component of the reaction norms (*e.g.* Gibert *et al.*, 2004; Valladares *et al.*, 2006). The random regression model is an extension to the classical quantitative genetics model (Kirkpatrick and Heckman, 1989), and as such it can predict the additive component of reaction norms, and give access to causal components of population variation. In addition, Murren *et al.* (2014) demonstrated with a large meta-analysis that when comparing reaction norms of close species or populations, changes in shapes (*i.e.* slope, curvature) were generally higher than the changes in the intercepts (*i.e.* the taxon means), suggesting the need of high-order modeling. In that view, random regression modeling has also the valuable capability to fit complex curves for reaction norms.

Random regression is a special case of covariance function (Meyer, 1998). Covariance functions present a particular interest in quantitative genetics, as they allow the representation of quantitative genetics parameters as functions. For instance, the cattle breeding literature is rich in illustrations of heritabilities and genetic correlations estimated as functions of time in the milk production context (*e.g.* Miglior *et al.*, 2007; Muir *et al.*, 2007; Jamrozik *et al.*, 2010). Although covariance functions and random regression have been often suggested for the modeling of reaction norms (Kirkpatrick and Heckman, 1989; De Jong and Bijma, 2002; Schaeffer, 2004), their application is still rare for most taxa (Morrissey and Liefting, 2016). For instance, in the forestry context, random regression has been used to model growth over time (Apiolaza and Garrick, 2001; Wang *et al.*, 2009), but not tree growth reaction norms over environmental gradients until very recently (Carnwath and Nelson, 2016; Marcatti *et al.*, 2017). Li *et al.* (2017) advocated for the use of random regression in forest trees, but the growth reaction norms they reviewed were only performed at the population scale, with fixed effects, without covariance functions. Nevertheless, because they are sessile, long-living, and because they record radial growth increments in the form of annual rings, trees are remarkable biological models for the study of phenotypic plasticity. The use of random regression has been suggested for the study of tree rings plasticity (Sánchez *et al.*, 2013).

For a given tree, the succession of wood annual rings constitutes an archive of its plastic responses to the succession of past climatic environments. Indeed, trees growth is conditioned by solar radiation, temperature and precipitation. They are particularly sensitive to drought, as this can eventually lead to lethal embolism of their hydraulic system. As a response to water stress, trees have a range of plastic responses including modification of the cambial activity to control the sap flow rate and xylem resistance to embolism (Bréda *et al.*, 2006; Rennenberg *et al.*, 2006). Under a temperate climate, water availability is usually high in spring with cool temperatures and lower in summer with higher temperatures. Trees respond to this seasonal succession by producing an early wood, made of large cells with thin walls, progressively followed by a late wood, made of narrow cells with thick walls, allowing a rapid change in the trunk of the water conductance along the season (Sánchez-Vargas *et al.*, 2007; Martinez-Meier *et al.*, 2009; Bryukhanova and Fonti, 2013). This succession of early and late wood is recorded in the wood as an annual growth ring.

In summary, trees present the remarkable feature of recording long repeated series of phenotypes that are known to respond plastically to the climatic environment, and differentially among individuals. This provides a gold mine of information for the study of phenotypic plasticity. First, based on a random regression approach, we leveraged this information to address the question of the role of phenotypic plasticity in the construction of HL heterosis for radial growth. Indeed, integrative heterosis as observed for HL stem circumference necessarily arises from the cumulation of heterosis occurring at the annual ring scale: this might be expressed differentially with respect to parental genotypes depending on water availability. Secondly,

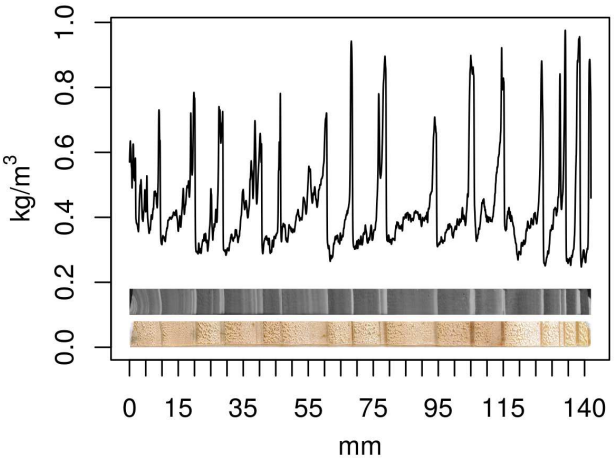

Figure 1: Microdensitometric profile from one wood core sample in Saint-Appolinaire. Bottom: wood core sample (pith on the left). Middle: X-ray radiography of the sample. Top: wood density variation (kg/m^3) along the core (mm). The peaks correspond to late wood and delimit the annual growth rings

we described how the water availability affected the quantitative genetic parameters of HL and of its parental species. Finally, since most biological models do not archive repeated series of phenotypic plasticity records, we addressed the question of the generalizability of the method. Using simulations, we evaluated the robustness of the random regression approach for the modeling of reaction norms in the case where no repeated series of phenotypes per individual are available.

2 Materials and Methods

The data are part of a multi-site progeny trial established in early 1997 on two environmentally contrasted sites at Saint-Appolinaire (SA, 45°58'N 4°26'E, 784 m a.s.l.) and Saint-Saud (SS, 45°31'N 0°48'E, 307 m a.s.l.) in central France. The site of SA is a relatively high-elevation site, on a steep slope with a southern aspect formerly planted with Douglas-fir; whereas the site of SS is a low elevation site on a former meadow, under oceanic influences. Progenies were produced by control-crossing in the frame of a diallel mating design between 9 European larch (*Larix decidua*, EL) parents and 9 Japanese larch (*L. kaempferi*, JL) parents, producing pure species and HL full-sib progenies. The set-up is described further in Marchal *et al.* (2017).

2.1 Phenotypic data: wood formation records

One breast-height diameter increment core was collected from each tree from each site. For each diameter increment core, only one radius (the one exhibiting the fewest defects) was kept for further analysis. These radial increment cores were sawed in 2 mm thick boards and X-rayed to obtain microdensitometric profiles (Fig. 1). From the alternating of early wood and late wood, the year of formation for each ring was identified (Regent Instruments Canada Inc., 2008). Ring width (RW) and ring mean density (RMD) were measured from the microdensitometric profiles. A total of 1998 increment cores in SA and 2278 increment cores in SS were collected. In SS, the increment cores were collected at three different periods: before the first thinning in 2003 from trees to be felled, and two later ones before and after the second thinning, that is in 2009 and in 2011. In SA, the collection was done in 2013. The average number of rings available per core was 13.2 in SA and 9.7 in SS (Supplementary 1, Fig. AS 1).

2.2 Environmental data: soil water availability

The soil daily relative extractable water (REW) content was estimated using a water balance model. The REW varies between 1 (field capacity) and 0 (permanent wilting point, meaning the water is no longer available for the plants), and it was estimated for each combination of site and year for which ring data were available. We used an adapted, simplified implementation of Granier *et al.* (1999)'s daily water balance model. This implementation allowed us to account for temporal evolution of the stand, and to run the model at the individual scale. Indeed, during the growth of the trees, the canopy leaf area increases and subsequently increases the transpiration, whereas understorey shrubs and grass vegetation decrease. Also, the intra-site spatial heterogeneity of the soil storage capacity causes variation in the water availability at the individual tree scale. The water balance model is described in Supplementary 2. The output of this model is the daily REW available at the tree level.

To summarize a year of fluctuation of REW, we selected the lowest decile of the whole distribution of the year's daily REW (D1rew). This index was considered because of its easy interpretation (the REW below which lay the ~35 driest days of the year), because of its likelihood to carry the information of drought events and therefore to affect the growth, and finally because it ensures a proper coverage of the environmental gradient (Supplementary 1, Fig. AS 2), unlike *e.g.* indexes based on a drought threshold that may not be reached some humid years. The environmental gradient coverage guarantees the stability of the regression parameters and the quality of the subsequent analyses.

The index D1rew, derived from a complex water

balance model, was compared to a simpler index MJJA defined as the sum of rain from May to August (inspired from Fallour-Rubio *et al.* (2009)), from which was subtracted the daily potential evapotranspiration (Supplementary 1, Fig. AS 2). Moreover, Bryukhanova and Fonti (2013) showed that for European larch, several traits including RW were more strongly correlated to the soil water deficit of year $t-1$ than to the deficit of the current year t. Therefore, we used D1rew for year t, for year $t-1$, and MJJA for year t as three separate environmental gradients, and we modeled the reaction norms along these environmental gradients separately.

2.3 Modeling of reaction norms by random regression model

Reaction norms were modeled using orthogonal Legendre polynomials (Kirkpatrick *et al.*, 1990; Schaeffer, 2004). Let $L_m(x')$ be the m^{th} order Legendre polynomial of x', with x' the standardization of x on [-1, 1], that is $x' = 2 * [x - min(x)]/[max(x) - min(x)] - 1$. We fitted the following model for each taxon (EL, HL or JL):

$$y_{ijkl}(x) = \sum_{m=0}^{M_S} s_{im} L_m(x') + \sum_{m=0}^{M_A} a_{jm} L_m(x') + \sum_{m=0}^{M_P} p_{km} L_m(x') + r_{ijkl}$$

where $y_{ijkl}(x)$ was the l^{th} observation of individual k, of genotype j, from site i, with environment x. The site's effect s was fixed. The additive effects a were random, and depended on the species. For EL and JL pure species, respectively, $a_E \sim N(0, \mathbf{\Sigma_{AE}} \otimes \mathbf{A_E})$ and $a_J \sim N(0, \mathbf{\Sigma_{AJ}} \otimes \mathbf{A_J})$; and for the hybrid $a_H = g_E + g_J$ with $g_E \sim N(0, \mathbf{\Sigma_{HE}} \otimes \frac{1}{2}\mathbf{A_{HE}})$ on the EL side and $g_J \sim N(0, \mathbf{\Sigma_{HJ}} \otimes \frac{1}{2}\mathbf{A_{HJ}})$ on the JL side (Stuber and Cockerham, 1966). Given that the parents were supposed outbred and unrelated, $\mathbf{A_{HE}}$ and $\mathbf{A_{HE}}$ reduced to identity matrices. The permanent environment was $p \sim N(0, \mathbf{\Sigma_P} \otimes \mathbf{I_P})$. The residual was unstructured $r \sim N(0, \sigma^2_R \mathbf{I_R})$. We chose to fix $M_S = M_A = M_P = M$ so that an 'order M' applied to the whole model. Models of different orders are characterized by how much information on plastic responses is included: order 0 does not estimate any plasticity, order 1 calculates slopes, order 2 additionally fits parabolas, and so on.

This model was fitted for both traits RW and RMD, and also for RW and RMD simultaneously in a multivariate approach. In this latter case, the effects s, a and p were estimated for each trait; the variance-covariance matrices $\mathbf{\Sigma}$ gathered the variances of each combination of trait and order and the covariances between them all; the residual variance σ^2_R was independent for each trait.

The model was fitted by Markov chain Monte Carlo (MCMC) with the same priors as in Marchal *et al.* (2017) (Hadfield, 2010; R Core Team, 2017): parameter expansion was used on the genetic variance-covariance matrices, and flat improper priors were set on the permanent environment variance-covariance matrices as well as on the residual variance. All chains were 5.5×10^6 iterations long, with 5×10^5 iterations burn-in and a thinning of 5×10^3. Point estimations from chains were maximum *a posteriori*, and 95% credible intervals (CIs) were computed where appropriate. The quality of fitting was assessed using coefficients of determination R^2 (Nakagawa and Schielzeth, 2013; Johnson, 2014).

2.4 Estimation of genetic parameters

The additive variances ($\sigma^2_{A:\text{RW}}(x)$ and $\sigma^2_{A:\text{RMD}}(x)$) and covariance between RW and RMD ($c_A(x)$) were calculated from the multivariate random regression model as functions of the environmental gradient x. The additive variance-covariance matrix decomposes into $\mathbf{\Sigma_A} = \begin{bmatrix} \mathbf{S_{A:RW}} & \mathbf{C_A} \\ {}^t\mathbf{C_A} & \mathbf{S_{A:RMD}} \end{bmatrix}$ where $\mathbf{S_{A:RW}}$ and $\mathbf{S_{A:RMD}}$ are the sub-matrices for each trait and $\mathbf{C_A}$ is the across-traits covariance sub-matrix. The additive variance of a trait T is the variance of the additive performances within the population for this trait, that is the variance of a linear combination:

$$\sigma^2_{A:T}(x) = var(\sum_{m=0}^{M_A} a_{jm:T} L_m(x'))$$

$$= \sum_{m_1=0}^{M_A} \sum_{m_2=0}^{M_A} L_{m_1}(x') L_{m_2}(x') \mathbf{S_{A:T}}\{m_1+1, m_2+1\}$$

The permanent environment variance $\sigma^2_{P:T}(x)$ was computed in the same way. Similarly, the additive covariance between the 2 traits was computed as:

$$c_A(x) = cov(\sum_{m=0}^{M_A} a_{jm:\text{RW}} L_m(x'), \sum_{m=0}^{M_A} a_{jm:\text{RMD}} L_m(x'))$$

$$= \sum_{m_1=0}^{M_A} \sum_{m_2=0}^{M_A} L_{m_1}(x') L_{m_2}(x') \mathbf{C_A}\{m_1+1, m_2+1\}$$

Finally, the permanent environment covariance $c_P(x)$ was computed in a similar way. Narrow-sense heritabilities and additive correlation were then computed respectively:

$$h^2_T(x) = \frac{\sigma^2_{A:T}(x)}{\sigma^2_{A:T}(x) + \sigma^2_{P:T}(x) + \sigma^2_{R:T}}$$

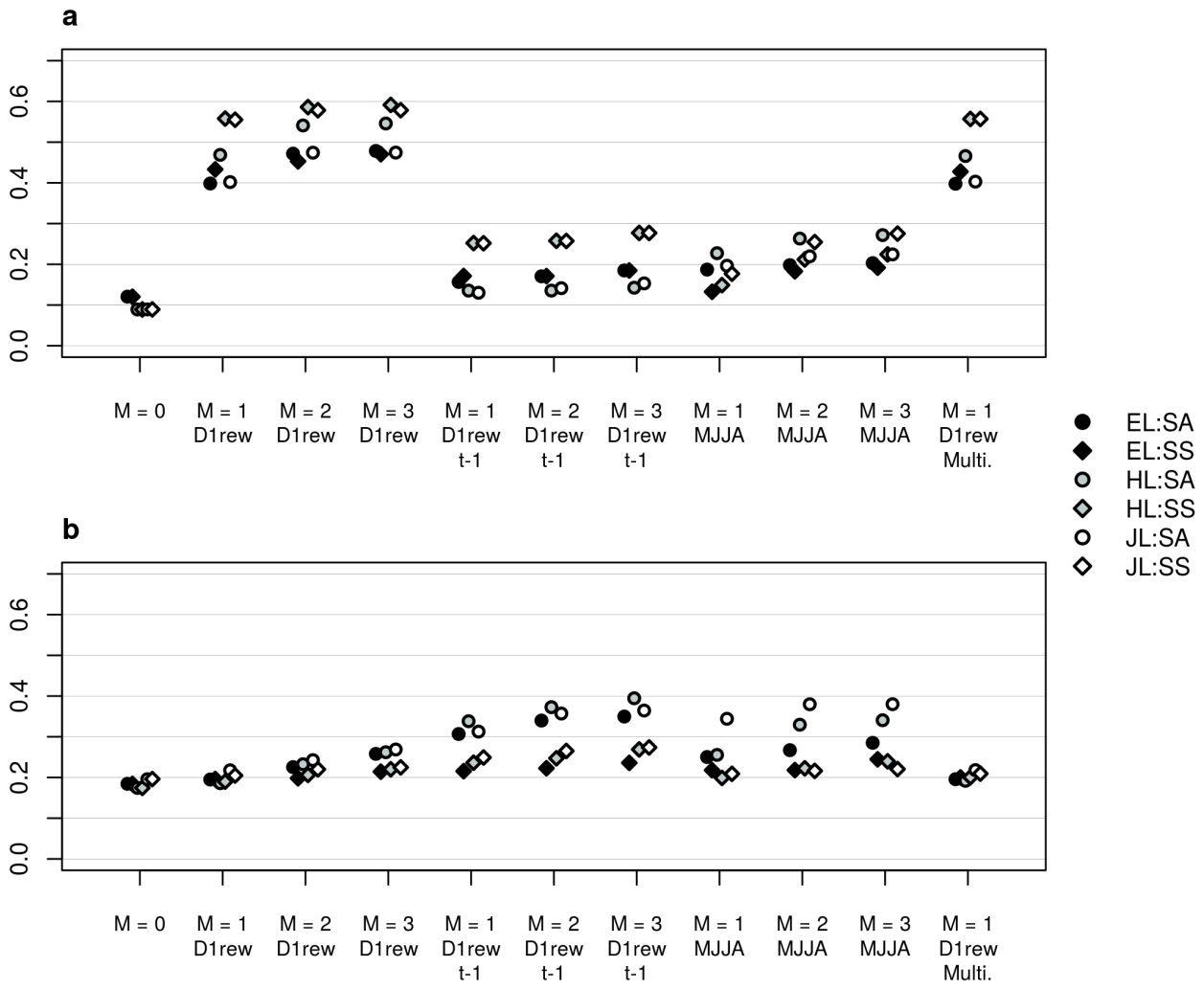

Figure 2: Coefficients of determination (R^2) for ring width (RW) (a) and ring mean density (RMD) (b), depending on the model specification. The polynomial order (M) varied from 0 to 3. Three covariates were compared: the first decile of the soil daily relative extractable water (D1rew) for the current year (year t, implicit) and for the previous year (year $t-1$, specified), and the sum of daily differences between precipitation and potential evapotranspiration from May to July (MJJA) for the current year. The last model specification ('Multi.') corresponds to 1$^{\text{st}}$ order regression with the covariate D1rew of the current year t, but in this case the model was multivariate and RW and RMD were analyzed simultaneously

and:

$$r_A(x) = \frac{c_A(x)}{\sqrt{\sigma^2_{A:\text{RW}}(x)\sigma^2_{A:\text{RMD}}(x)}}$$

For hybrids, narrow-sense heritabilities and additive correlation were computed for each of the parental contributions g_E and g_J. Therefore, on the EL side:

$$h^2_{HE:T}(x) = \frac{\sigma^2_{HE:T}(x)}{\frac{1}{2}\sigma^2_{HE:T}(x) + \frac{1}{2}\sigma^2_{HJ:T}(x) + \sigma^2_{P:T}(x) + \sigma^2_{R:T}}$$

and:

$$r_{HE}(x) = \frac{c_{HE}(x)}{\sqrt{\sigma^2_{HE:\text{RW}}(x)\sigma^2_{HE:\text{RMD}}(x)}}$$

and idem on the JL side.

2.5 Simulation: evaluation of the random regression model with single record per individual

We used the software Metagene to simulate data. The functioning of this simulator to derive *in silico* populations with phenotypic plasticity records is detailed

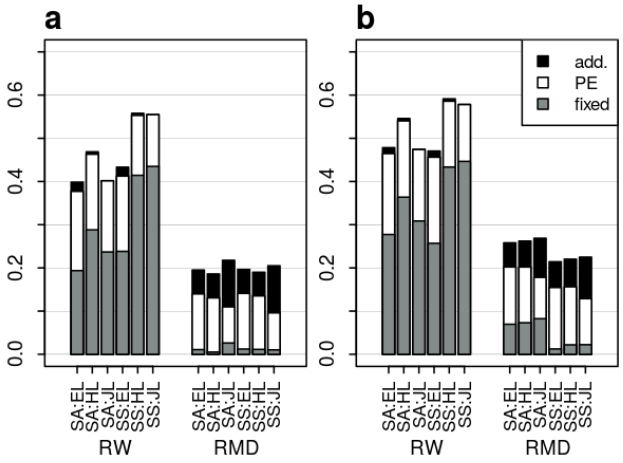

Figure 3: Decomposition of the coefficients of determination (R^2) obtained with the order 1 model (a) and with the order 3 model (b), for each trait: ring width (RW) and ring mean density (RMD), and for each combination of site (SA or SS) and taxon (EL, HL or JL). The environmental gradient was the first decile of the daily relative extractable water for the current year. The proportion of variance explained by each component is presented: genetic additive effects (in black), permanent environment (in white), and fixed terms (in grey)

in Supplementary 3. Basically, the genotypic effect at a given locus was set as a function of the environment $\alpha(x) = \alpha_0 + \alpha_1(x+\delta) + \alpha_2(x+\delta)^2$, where parameters α_0, α_1, α_2 and δ defined the parabola that was associated to each genotype in a set of X diallelic loci constituting the genome. Then, for each independent simulation, the simulator randomly sampled a genome for each founder, produced the mating between founders, the new offspring genomes, and returned their phenotypic plasticity in the form of longitudinal records over Y environments. We parameterized the allelic effects in such a way that the additive reaction norms were very interactive, that is, the ranking of the parents varied along the environmental gradient due to slopes and parabolas.

The mating design consisted of a full diallel between 10 monoecious founders, excluding selfs. Each combination of parents (A×B) produced $n/2$ sibs, so that the size of a full-sib family (A×B + B×A) was n. The environmental gradient was divided in n random positions, each position being defined by an environmental value x. Each of these environments hosted one sib per family, and as many individuals as families. The progenies' phenotypes, their pedigree, and the environmental values x were included in the analysis.

We tested 4 different scenarios, resulting from the combination of low (0.1) and high (0.6) heritabilities with small (20) and large (120) family sizes. Thus, these scenarios measured the importance of the quantity (*i.e.* the number of progenies) and quality (*i.e.* the heritability) of information for genetic inference. For each scenario, 100 independent simulations were run and analyzed.

We used the pure species univariate random regression model previously described, without the permanent environment component (as no permanent environment was simulated) and without the site effect (thus the fixed part of the model was a common linear combination of Legendre polynomials of order M). The chains were 1.5×10^5 iterations long, with 5×10^4 iterations burn-in and a thinning of 10^3. We measured the ability of the random regression to infer additive components of the parental reaction norms. To do so, we defined the accuracy of the model as the correlation between the parents' predicted additive performances and their true simulated additive performances at each point of the environmental gradient. The reaction norms of the parents were only inferred for the ranges on which the progenies were tested.

3 Results

3.1 Quality of model fitting to real data

The order 0 model (fixed and random intercepts) explained between 8.9% and 12.1% of RW variance depending on taxa and site combination (Fig. 2). The addition of fixed and random slopes (1st order) along D1rew of the current year (t) greatly improved the model, allowing it to account for 39.9% (EL in SA) to 55.8% (HL in SS) of the variance. Analyzing higher orders (order 2 and order 3 along D1rew of the current year) slightly increased the R^2 (by 5.1% on average compared to order 1). Such high R^2 were not reached using D1rew of the previous year, nor using the simpler index MJJA. For RMD, on the contrary, order 0 or higher orders led to very close R^2 (Fig. 2). Using D1rew of the previous year instead of that of the current year led to a marginal improvement of R^2 that happened only in SA, reaching up to 39.4% for HL with order 3.

Heterosis expressed mostly in radial growth, that was better explained by D1rew of the current year. For this reason, we focused on this environmental gradient only. The gain in R^2 with increasing orders was overall due to more variance explained by the fixed component of the models, as shown in Fig. 3. Therefore, we present in Fig. 4 order 3 reaction norms at the taxon scale (*i.e.* the fixed component of the model), but for parsimony consideration, the multi-trait random regression model (RW and RMD simultaneously), from which the genetic variance parameters were estimated, was fit with order 1. The R^2s of the multi-trait

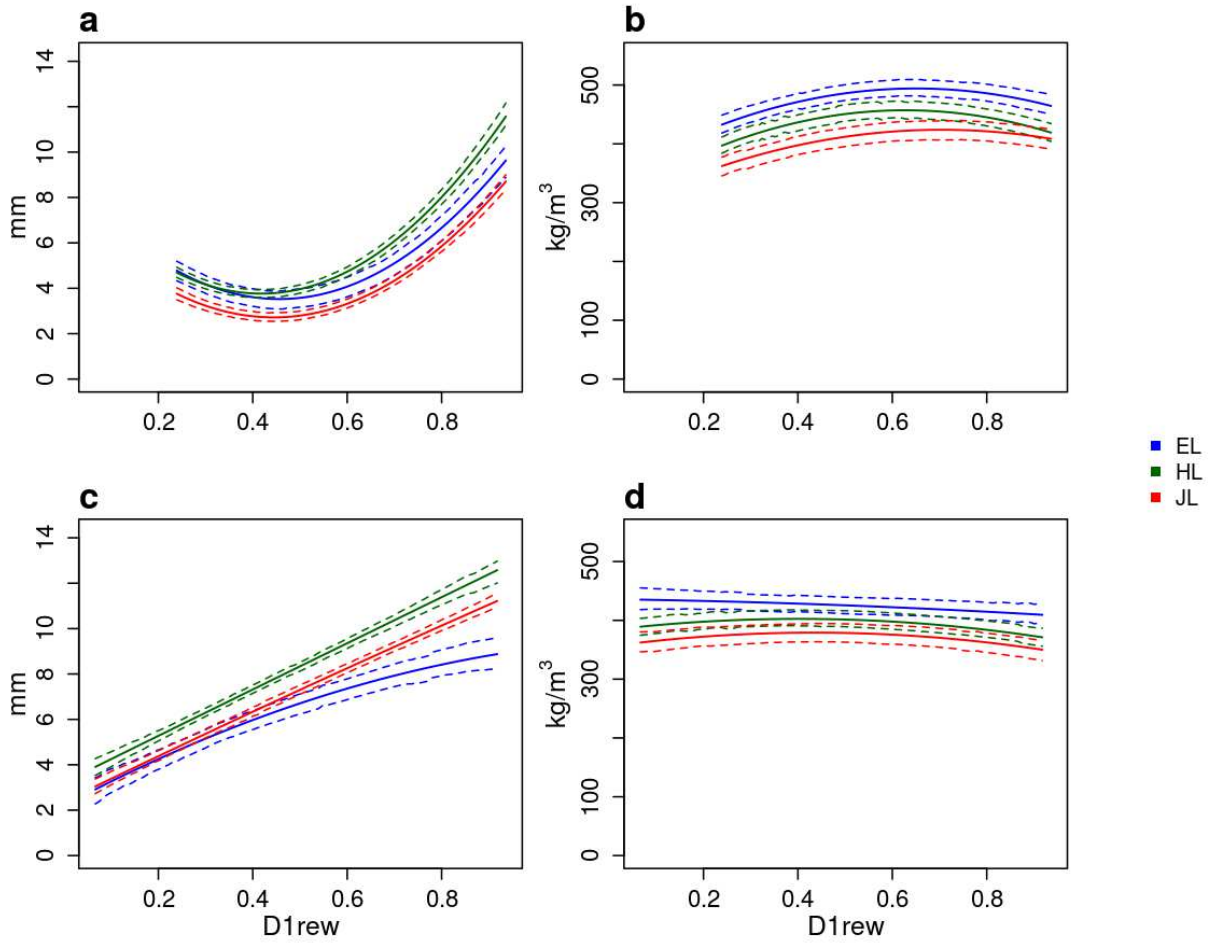

Figure 4: Reaction norms of ring width (a, c) and ring mean density (b, d) along the first decile of the daily relative extractable water (D1rew), in the sites SA (a-b) and SS (c-d), for each taxon: European larch (in blue, EL), Japanese larch (in red, JL) and their hybrid (in green, HL). These average reaction norms at the taxon level represent the fixed components of the order 3 random regressions. Dashed lines: 95% credible intervals

model for each trait were also presented in Fig. 2 and were very similar to their univariate counterparts.

3.2 Stability of the heterosis and its dependence on the water availability

The ranking in RW performance between EL and JL varied depending on the site: EL performed better in SA while JL did better in SS (Fig. 4). However, the superiority of the hybrid over both its parental references occurred in the two sites, and over the whole range of D1rew.

For any taxon and in any site, RW was plastic as it increased with increasing water availability (Fig. 4). The three taxa showed however different curves along the D1rew, being close to each other when water availability was minimal (D1rew close to 0), and splitting apart with increasing water availability, where the superiority of HL over its parental references was the highest. The gain in superiority for RW of HL over its parental references due to increasing D1rew ranged between +1.04 mm (HL *vs.* JL in SS) and +3.26 mm (HL *vs.* EL in SA). For high D1rew, the CIs of the HL reaction norm were not overlapping with those of the parental species.

On the opposite, the trait RMD showed neither heterosis nor plasticity. The hybrid ranged between both its parents, and all the norms of reaction were almost flat for this trait, showing no conspicuous pattern of variation along the water availability gradient D1rew.

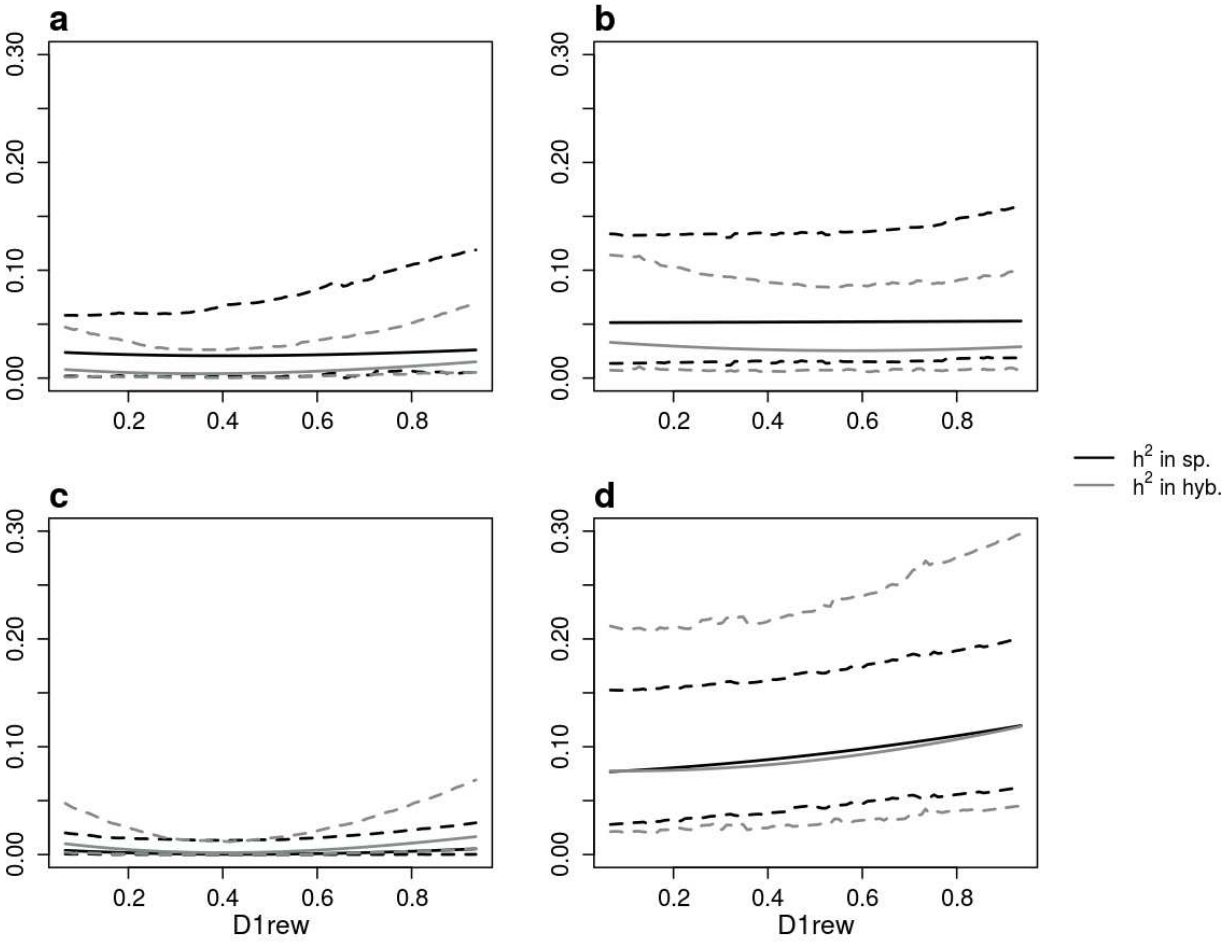

Figure 5: Narrow-sense heritabilities along the first decile of the daily relative extractable water (D1rew), for European larch (a-b) and Japanese larch (c-d), for the traits: ring width (a, c) and ring mean density (b, d). Black line: heritability in pure species crosses; and grey line: heritability in hybridization. Dashed lines: 95% credible intervals

3.3 Heritabilities and genetic performances along the water availability gradient

All the narrow-sense heritabilities we estimated were very low (Fig. 5). Low heritabilities were overall due to high residual variances in comparison to the lower additive and permanent environment variances (Fig. 3). Despite this residual noise, we could distinguish parental performances for both traits, and some extreme performances of contrasting genotypes were different with statistical credibility (Supplementary 4, Fig. DS 6-DS 7).

Ring width heritabilities were close to 0 (Fig. 5). The signal for performance contrasts for RW in pure species was also very weak, but both species showed contrasted performances in hybridization as the water availability increased; some of these contrasts were supported by non-overlapping 95% CIs when D1rew was high (Supplementary 4, Fig. DS 7).

Heritabilities for RMD were higher than those of RW, especially on the JL side for which they varied between 0.08 and 0.12 both in pure species and in hybridization (Fig. 5). Pure species heritability and heritability in hybridization were very close for JL, and they both increased with water availability. The increase in heritability for RMD on the JL side reflected an increase in the contrasts between individual performances (Supplementary 4, Fig DS 6), supported by some non-overlapping 95% CIs for high D1rew (Supplementary 4, Fig DS 7). Moreover, the ranking of the 9 JL parents' performances for RMD was consistent in pure species and in hybridization (Supplementary 4, Fig DS 6).

3.4 Correlations along the water availability gradient

The additive genetic correlation between RMD and RW showed similar patterns between pure species and

their respective contributions in hybridization (Fig. 6). The differences were marked when comparing EL versus JL correlation patterns across the environmental gradient. The additive correlation decreased slightly along the gradient from around 0 to -0.41 in EL pure species (-0.35 in hybridization). On the JL side, it started from a positive correlation of 0.48 in pure species (0.14 in hybridization) and it steeply switched to a negative correlation of -0.60 (-0.41 in hybridization). The change in sign occurred at D1rew between 0.4 and 0.5. Still on the JL side, 95% CI excluded 0 in pure species at high D1rew (around 0.65). Due to the higher genetic variance for RW for JL in hybridization than in pure species, the additive correlation pattern was especially visible in the JL performances in hybridization: for the highest D1rew, the ranking between RW and RMD was almost inverted (Supplementary 4, Fig DS 6).

The permanent environment correlation between RW and RMD was negative for HL and JL, and did not vary along the environmental gradient. It was also null to negative for EL (Supplementary 4, Fig DS 8). This means that the sum of effects that the model did not explicitly account for (*i.e.* micro-environment, competition between trees, non-additive genetic effects, etc.) tended to induce a negative correlation between radial growth and wood density.

3.5 Simulation: accuracy of the random regression model with single record per individual

Using simulated data, we evaluated the ability of the random regression model to predict the additive component of reaction norms from family series of single observations per environment. The accuracy of the model depended on the scenario and on the order of the random regression (Fig. 7). The 1st scenario ($n = 20$ progenies, $h^2 = 0.1$) showed very poor predictive abilities, independently of the order. When a larger number of progenies ($n = 120$) or a higher heritability ($h^2 = 0.6$) was available, the accuracy was still low for order 0 (fixed and random intercept model) but it greatly increased with order 1 (addition of fixed and random slopes). However, in each case ($n = 120$ or $h^2 = 0.6$) no further accuracy was gained from order 1 to order 2. Only the accuracy for the last scenario (both $n = 120$ and $h^2 = 0.6$) increased with order 2 (addition of fixed and random parabolas). The accuracy for the last scenario analyzed with order 2 model ranged between 0.905 and 1 depending on the simulation and on the position along the environmental gradient.

The order 0 models were not able to estimate the additive variance properly (Supplementary 4, Fig. DS 9). From order 1 and over, the ability of the model to estimate the additive variances appeared overall dependent on the heritability. Indeed, the estimated additive variance were close to the true ones in scenarios 3 and 4 (both $h^2 = 0.6$) with order 1 or 2. With lower heritability ($h^2 = 0.1$) and order 1 or 2, the additive variance were overestimated.

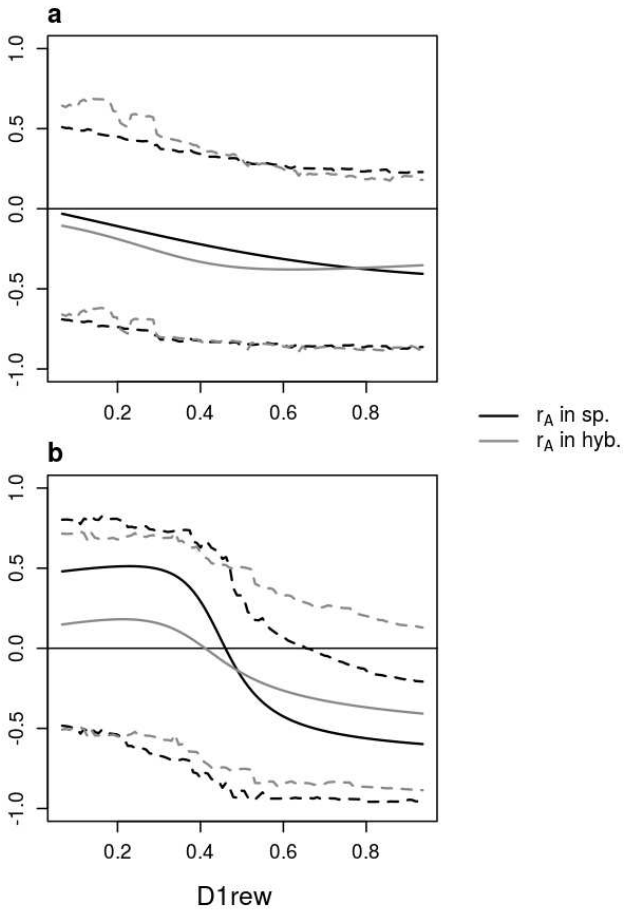

Figure 6: Additive correlation between ring width and ring mean density along the first decile of the daily relative extractable water (D1rew), for European larch (a) and Japanese larch (b). Black line: correlation in pure species crosses; and grey line: correlation in hybridization. Dashed lines: 95% credible intervals

4 Discussion

In this study, we constructed the reaction norms of annual wood-formation traits along a water availability gradient in a larch multi-site diallel mating experiment. The reaction norms were fitted using random regression modeling, allowing to assess the changes in heritability and genetic correlations along the gradient. Our study was complemented by using simulations involving the same analytical approach, where we evaluated the ability of the random regression model

to estimate the additive component in a frequent phenotypic plasticity experimental setting: that of parental reaction norms from non-repeated observations of their progenies as data.

The annual ring width was plastic and as expected increasing water availability allowed a higher radial growth (Fig. 4). At the taxon level, the hybrids performed better than their parents in each of the two sites of the study. This alternative modeling confirmed our previous findings (Marchal *et al.*, 2017), in that hybrid larch had a stable superiority across sites. However, within each site, HL demonstrated more plasticity than its parental references: indeed, under water stress all taxa produced a similarly narrow ring, whereas in favorable conditions of higher water availability the HL expressed superiority over its parental references. In other words, the HL reaction norms were steeper than those of the parental counterparts over the same gradient.

On the contrary, the second trait under study, RMD, was not plastic for this water availability gradient and expressed no heterosis in any site. Although RMD displayed higher narrow-sense heritabilities (h^2) than RW, it did not reach high values, with a global maximum of only 0.12. Despite the weak genetic signal, the performance functions of the 18 parents of the diallel could be represented distinctively across the water availability gradient, as well as the functions corresponding to the genetic parameters (h^2 and r_A, the additive correlation between RW and RMD). Interestingly, on the Japanese larch side, r_A switched from positive to negative values as water availability increased, with the highest negative values being different from zero with statistical credibility (Fig. 6). The emergence of this negative additive correlation might be explained by an increase in the early wood / late wood ratio with increasing water availability, with early (spring) wood being generally less dense than that of late (summer) wood (Fig. 1). However, we need to look more carefully to other ring traits (as did, *e.g.*, Bryukhanova and Fonti, 2013) and their respective correlations before proposing any causal explanation, with the goal to better understand the structure of the genetic variability of larch wood plasticity.

We obtained fairly high R^2 for the reaction norms models, suggesting that water plays an important role in the tree ring phenotypic plasticity. We evaluated a simpler environmental factor (sum of daily rain minus potential evapotranspiration from May to August) but it showed a lower R^2, highlighting the relevance of our water availability index 'D1rew'. It should be noted, however, that our water balance model has not been field-calibrated, and it should then be considered with care if generalizations are to be made. Moreover, the index D1rew gives no indication on the distribution of the driest days along the year. The timing of a water deficit, in spring or in summer, could have more or less effect on different ring traits; for instance, those relative to early or late wood, or to the transition between the two. It also has to be said that the relation between water balance and radial growth that we showed in the present study does not necessarily imply a direct causality. Indeed, other factors may play a role in the observed plastic response. For instance, heat affects directly the photosynthetic efficiency and the resources that may be allocated to growth (Rennenberg *et al.*, 2006). Heat and drought being highly correlated, their effects could well be confounded to some extent. More broadly, the environment has a multivariate nature. Soil, climate, but also competition with neighboring trees are known to affect the tree's growth. We isolated what we expected to be one of the most important environmental factor for radial growth, yet the existence of an important site effect pinpoints the fact that some other environmental factors might be involved in the tree ring phenotypic plasticity. Identifying relevant environmental factors of plant plastic reactions is an open area of research, notably in the context of global warming. The present study did not aim explicitly at the identification of relevant environmental triggers, rather it presented an approach that could help in such identification.

We studied phenotypic plasticity at two levels. The first level was spatial, at the across-site scale, and the second level was longitudinal, at the individual scale. At the across-site scale, heterosis was shown to be stable, supporting the common statement that hybrids are more stable across macro-environmental sites than their parental counterparts (Gallais, 2009). Specifically, the ranking of the parents species varied across sites whereas hybrid was invariably the highest performing taxon, in what could be qualified as hybrid homeostasis according to the theory developed by Knight (1973). The spatial plasticity is generally, and historically, the one that interests breeders the most because of the operational implications for the deployment of varieties. Using non-linear random regressions, Marcatti *et al.* (2017) fitted eucalyptus growth reaction norms along a gradient of spatial environments. The spatially distributed climatic environment was described with principal component analysis to account for its multivariate nature. This method is very appealing in order to deal with spatial phenotypic plasticity in tree breeding. However, Marcatti *et al.* (2017)'s approach could be further improved by accounting for pedigree information such as a mating design, from which quantitative genetic parameters could be estimated (Hinkelmann, 1974; Falconer and Mackay, 1996). In this context, the use of random regressions as defined by Kirkpatrick and Heckman (1989) and presented in this paper, or other covariance functions, would be relevant.

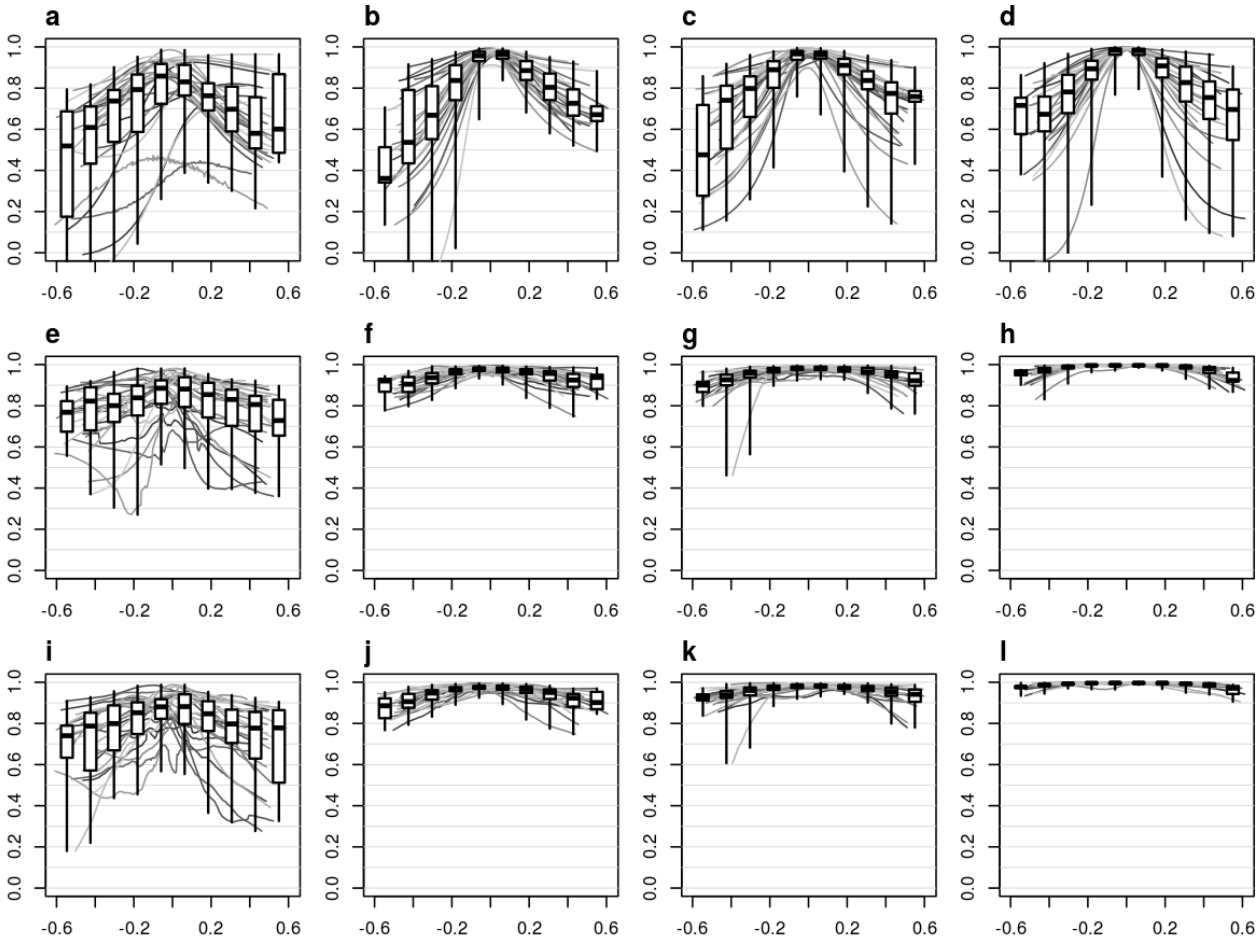

Figure 7: Accuracy of the predictions of parents' additive reaction norms in each scenario: (1) $h^2 = 0.1$ and $n = 20$ (a, e, i), (2) $h^2 = 0.1$ and $n = 120$ (b, f, j), (3) $h^2 = 0.6$ and $n = 20$ (c, g, k), and (4) $h^2 = 0.6$ and $n = 120$ (d, h, l); for 100 simulations in each scenario, and for each order of random regression: order 0 (a-d), order 1 (e-h) and order 2 (i-l). Each grey curve is the accuracy of 1 simulation; boxplots summarize all the point accuracies in segments of a 10^{th} of the simulated environmental gradient

The second level of phenotypic plasticity that we addressed was longitudinal, along the year-to-year water availability gradient. The resulting reaction norms were strongly influenced by the sites. Indeed, our study steps in the direction that tree ring longitudinal phenotypic plasticity should be seen as a plastic trait in itself, varying spatially (De Luis *et al.*, 2013; Natalini *et al.*, 2016), varying with long-term trends such as global warming (Natalini *et al.*, 2016), and varying with the level of competition between neighboring trees in wet years (Carnwath and Nelson, 2016). Growth recovery, the ability for trees to produce large rings the years following a drought event, is also site-dependent (Gazol *et al.*, 2017). Besides the site effect, a substantial part of the individual variation was shown to occur between taxa, with hybrid larch being more plastic and at a higher mean level than its parental counterparts. Therefore, this increase in longitudinal plasticity appears as a broader picture of the heterosis that was observed on an integrative scale across years, namely in the total circumference (Marchal *et al.*, 2017).

The molecular and physiological mechanisms behind the increased plasticity of hybrid larch remain open questions. Longitudinal plasticity as shown here is still a novel approach, with less immediate application to current plant breeding, unlike spatial plasticity. It certainly opens up new possibilities whenever long-time series are available, where extreme events, such as the 2003 drought in France (Bréda *et al.*, 2006; Rennenberg *et al.*, 2006), are recorded. Some initiatives, for instance, compared dead trees *vs.* alive neighbors immediately after extreme climatic events for their past wood records (Martinez-Meier *et al.*, 2008), finding that both classes had long-term distinctive patterns of reaction. This kind of study could well be un-

dertaken with a random regression approach to gain insight in the quantitative genetics of such longitudinal patterns and their environmental drivers, and be of potential use ultimately for breeders.

One eventual problem with longitudinal data is autocorrelation. In order to minimize its effects, several authors propose an extra step that consists in fitting an autocorrelation model on the tree ring series. The resulting residuals, that are more independent than the raw data, are then used as the response variable in the subsequent phenotypic plasticity models (Bryukhanova and Fonti, 2013; De Luis *et al.*, 2013). In this study, we did not do so because the chronology was somehow already involved in the environmental variable. Indeed, as the trees grew older, the stand LAI increased, and so did the transpiration, making water generally less available. Though this trend was not so strong (rain and potential evapotranspiration during the growing season were the main drivers of D1rew, as seen on Supplementary 1, Fig. AS 2), we did not want to account twice for the same chronological effect, and therefore we chose to work with raw data instead. The much weaker explicative power of D1rew of the previous year compared to that of D1rew of the current year supports our decision. We acknowledge though that there remains a risk of non-controlled autocorrelation in the data, in particular due to the trees' ontogeny and to the onset of competition between trees (Sánchez *et al.*, 2013).

The simulations showed that it was possible to estimate the additive component of reaction norms using only singular observations of related individuals. However, the quantity and the quality of information (*i.e.* respectively, the number of related individuals and the heritability) were key factors to estimate properly the additive components of reaction norms. Although the simulation was not meant to mimic the real case in the genomic layout of effects, it pinpointed the eventuality of potential biases in the estimation of the genetic variances (Supplementary 4, Fig. DS 9). As emphasized by Misztal *et al.* (2000), a limitation of the model used in the present study is the lack of covariance function for the residuals. This limitation could be a source of bias in the estimation of variance components and of heritabilities. Unfortunately, this feature was not available yet with the software we used. Indeed, we fitted linear mixed models in which the covariance functions were implicit and computed from the covariance between the regression coefficients. On the other hand, in the simulation, the additive reaction norms were properly estimated.

Random regression is already used for the modeling of reaction norms, especially in dairy cattle for which industry produces a large flow of longitudinal data (*e.g.* Kolmodin *et al.*, 2002; Windig *et al.*, 2006; Santana *et al.*, 2017). However, plasticity has been suggested (Bradshaw, 1965) and demonstrated (Murren *et al.*, 2014) to be of special importance in plants. Indeed, because they are sessile, plants have to face their environment in a different way than animals that are capable of behavioral responses and locomotion. Manifestations of phenotypic plasticity have been reported in several perennial crops. For instance, grape vine manifests phenotypic plasticity in terms of fruit weight and chemical composition (Dai *et al.*, 2011). Even in equatorial regions, oil palm is able to react to subtle variations in photoperiod and drought events by changing its bunch productivity (Legros *et al.*, 2009). Cherry tree phenology reacts promptly to climate, notably heat, with global warming expected to bring flowering a month forward (Allen *et al.*, 2014). Ismaili *et al.* (2016) showed a significant genotype-by-year interaction in apricot tree, and recommend the use of mixed models for the analysis of perennial plants' longitudinal data. All these examples could be good candidates for analysis based on covariance functions. Indeed, the possibility to define quantitative genetic parameters as functions of the environment and to model the additive contributions to phenotypic plasticity opens wide perspectives in terms of selection (De Jong and Bijma, 2002), especially in a global warming context (Koski, 1996).

Like trees, other organisms naturally cumulate growth records that reflect their reaction to past environmental conditions, etched in hard organs that grow incrementally: for instance, fish otoliths, mollusc shells, corals, whale ear plugs, ibex horns, *etc.* (reviewed by Morrongiello and Thresher, 2015). Morrongiello and Thresher (2015) advocate for the use of random regression for the analysis of such natural records of longitudinal data, especially with regards to these species' phenotypic plasticity. The random regression framework as developed by Kirkpatrick and Heckman (1989) exhibits two particular strengths that deserve to be emphasized once more. First, the use of orthogonal base functions, such as Legendre polynomials, allows the fit of virtually any shape of growth curves or reaction norms. Although the example we presented here did not illustrate this need, neglecting curvature when studying evolutionary divergence in reaction norms leads to a risk of missing critically important information (Murren *et al.*, 2014). The traditional use of x and x^2 as covariates should be avoided in any case, given the high correlation that binds the identity and the square (and any power) functions.

A second point of interest is the fact that genetic information can be taken into consideration in the model, which can be of relevance not only for breeding but also in ecology studies looking for drivers and patterns of natural selection (Brommer *et al.*, 2005). As we illustrated with the results of our simulations, this possibility opens up the use of a random regres-

sion framework to species whose individuals do not cumulate in any known form longitudinal records of their plastic responses. In this sense, our simulation provided an example of random regression being an alternative to traditional methods: related individuals can indeed give access to the additive component of the reaction norms, and this can likely be extrapolated to isofemale lines (Gibert *et al.*, 2004) or half-sib families (Valladares *et al.*, 2006). Finally, the pedigree information can be conveniently replaced by molecular information (*e.g.* Ly *et al.*, 2018), extending the potential of the random regression framework beyond the limitation of our capability to realize time-consuming, sometimes impossible, artificial mating.

4.1 Data archiving

Our datasets are being submitted to the INRA repository GnpIS (https://urgi.versailles.inra.fr/Tools/GnpIS). Until then, the data are available upon request.

The Metagene simulator is available on the NOVELTREE project page (http://www.igv.fi.cnr.it/noveltree/) and the code is being submitted to GitHub (https://github.com).

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A Supplementary material 1 Complementary information about the data structure

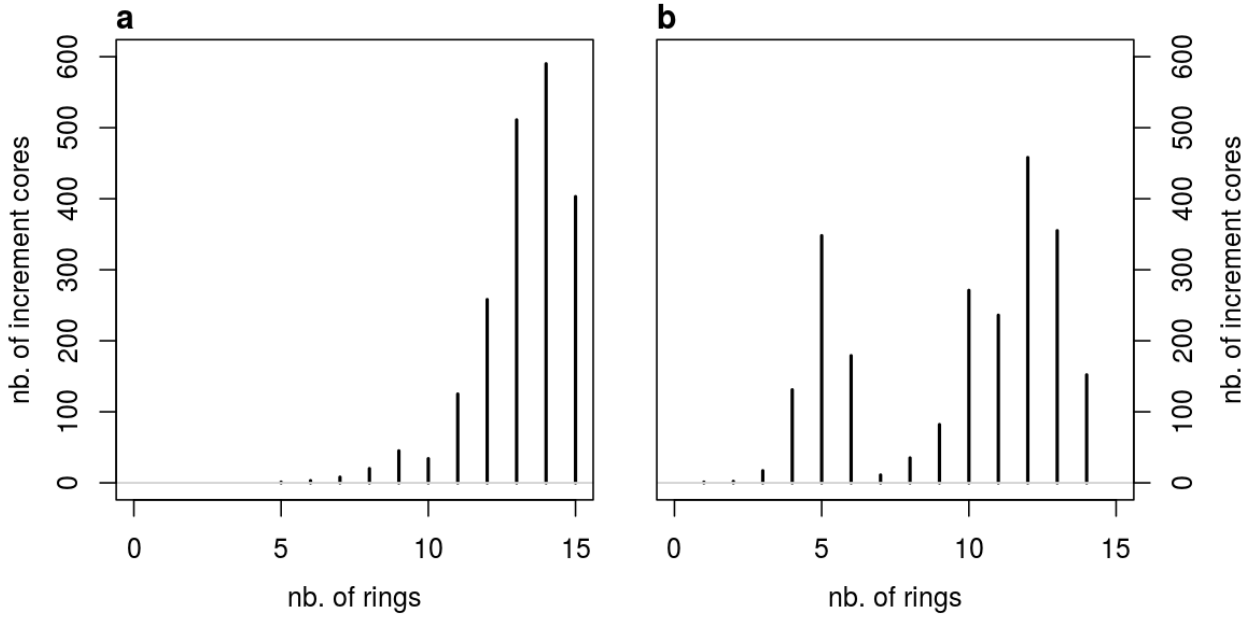

Figure AS 1: Number of rings per increment cores from Saint-Appolinaire (a) and from Saint-Saud (b)

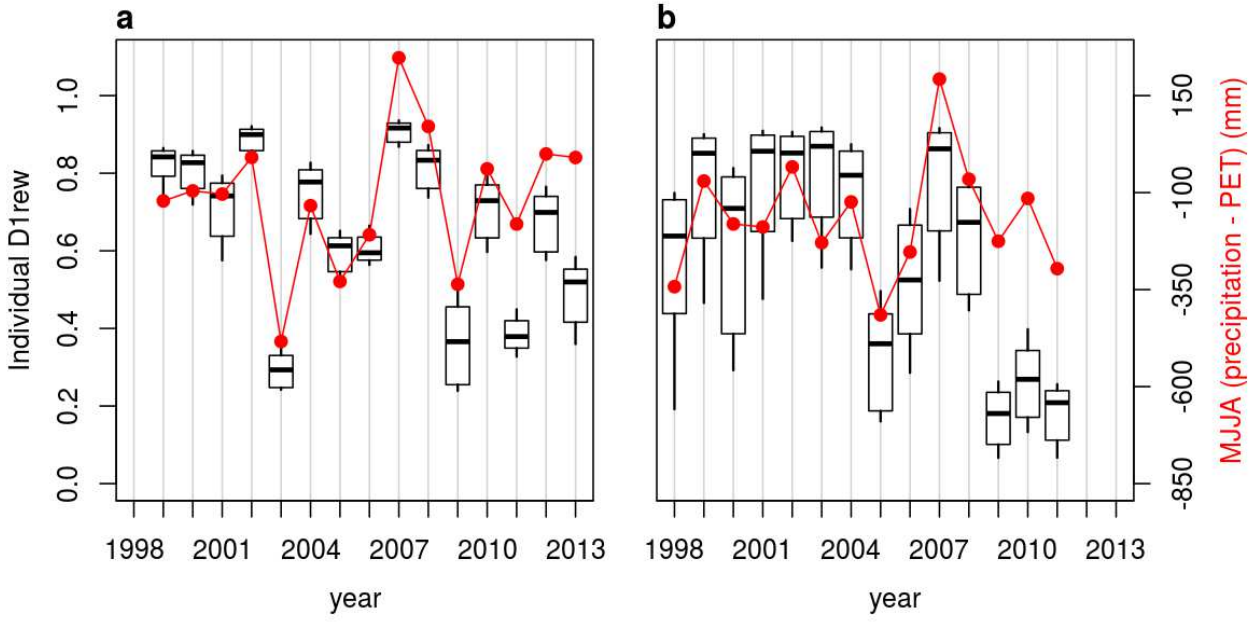

Figure AS 2: In black: boxplots of the distribution of individual D1rew (first decile of the daily relative extractable water) for each year in each site SA (a) and SS (b). In red: the simpler index MJJA, defined as the sum of the daily differences between precipitation and potential evapotranspiration from May to July

B Supplementary material 2 Water balance model

B.1 Leaf area index

The leaf area index (LAI) is the ratio of leave surface per ground surface. Thus in a growing stand the LAI is expected to increase, and this plays an important role in the water balance model, as detailed further. The LAI can be calculated from the basal area, that is, the surface of cross-section of tree stems per ground surface. Basal area was calculated in each site at each age for which we had a breast-height circumference (BHC) measurement. We estimated transmittance from basal area and age using Sonohat *et al.* (2004) ('Model 2', $R^2 = 0.867$):

$$\tau = exp(-b_{max}(\frac{age}{age_{max}}exp(1 - \frac{age}{age_{max}}))^p G)$$

where G was the basal area, and age_{max}, p, and b_{max} the model parameters available in Sonohat *et al.* (2004). From transmittance we could estimate the LAI as $\text{LAI}_{max} = -ln(\tau)/k$ with the value $k = 0.6$ for larch (Takeda *et al.*, 2008). The index 'max' in LAI_{max} means: the LAI when the vegetation is maximal in the season. Then, we linearly inferred the LAI_{max} at any age for which we had ring observations. The LAI_{max} was inferred at the site scale. We present the calculated and the inferred LAI_{max} in Fig. BS 3.

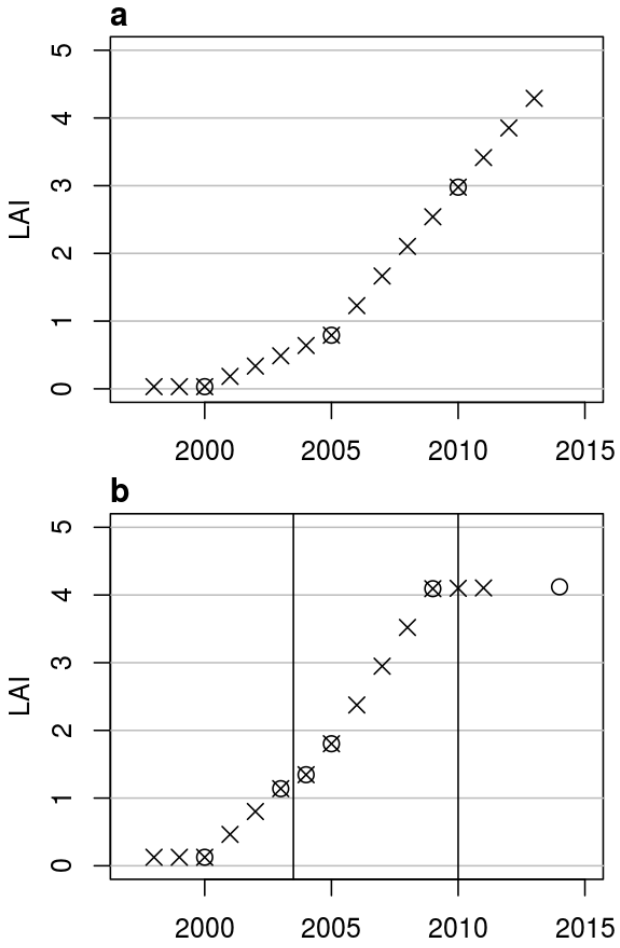

Figure BS 3: LAI_{max} evolution in each site: SA (a) and SS (b). Circles: LAI_{max} estimated from basal area. Non-circled crosses: LAI_{max} linearly inferred. All crosses: LAI_{max} used in the water balance model

B.2 Soil available water capacity

We excavated 2 pits in SA and 3 pits in SS, in the most contrasted areas. The contrasted areas were assessed using tree height (at the last age available) spatial effect maps. The spatial effects were predicted as best linear unbiased predictions (BLUPs) from model 'M1' in Marchal *et al.* (2017). In each pit, we measured soil horizon thickness, stone content, and we collected samples to assess the soil texture. We estimated the available water capacity (AWC) for each pit (Bruand *et al.*, 2004), that is, the maximal amount of water available for plants that the soil can store. We assumed that height spatial effect informed on AWC, as empirically supported in Fig. BS 4. Within each site, we considered a linear relation between tree height spatial effects (BLUPs) and AWC in order to infer AWC at the tree level. Nevertheless, for each site, we prevented the individual trees AWC from being lower (or higher) than the lowest (or highest) pits' AWCs, resulting in an inverse-M shaped distribution.

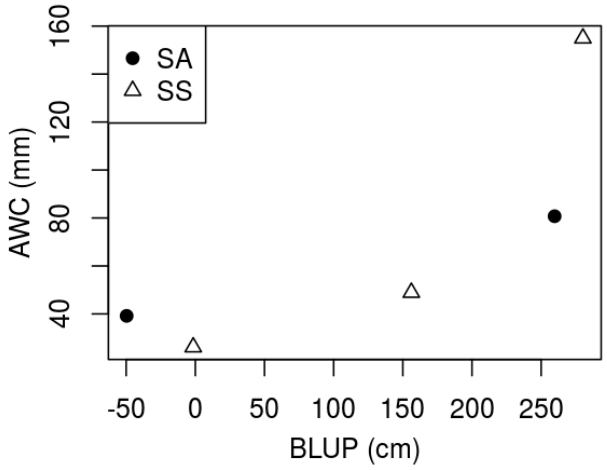

Figure BS 4: Link between height spatial effect (estimated as a best linear unbiased predictor, BLUP) and the available water capacity (AWC) in each site: Saint-Appolinaire (SA, black dots) and Saint-Saud (SS, white triangles). Each dot represents a pit

B.3 Climatic data

The daily climate information we used were the precipitations (P) and the potential evapotranspiration (PET). Raw climatic data were from Météo-France, via the platform INRA CLIMATIK which computed the PET with Penman-Montheith method. Ten PET data points were missing in SS, we imputed them using an autoregressive model (**?**) with an autocorrelation parameter $\rho = 0.95$.

B.4 Water balance model and water availability indexes

We used an adapted, simplified implementation of Granier *et al.* (1999)'s daily water balance model. We simplified the model as follows: (i) we proposed a simplified formulation for the understorey evapotranspiration; (ii) we proposed a simplified formulation for the rainfall interception; (iii) we ignored soil stratification and distribution of the roots, so only the overall AWC described the subsoil. The daily water balance was:

$$\text{(Granier } et\ al.\text{, 1999, eq. 1) } \Delta W = P - In - T - Eu - D$$

with W: the soil water content and ΔW its daily variation; P: the precipitation; In: the rainfall interception; T: the overstorey transpiration; Eu: the understorey evapotranspiration; and D: the drainage; all expressed in mm. The relative extractable water (REW) content in soil was calculated as REW $= W/$AWC. The LAI was 0 until day 105 (15^{th} of April for a non-leap year), then increased linearly in 30 days, stayed at LAI_{max} a while and finally decreased linearly to 0 in 30 days, finishing the decrease at day 288 (15^{th} of October). The canopy transpiration T was calculated as following:

$$\text{(Granier } et\ al.\text{, 1999, eq. 2) } T_{max} = r_T * PET \text{ with } \begin{cases} r_T = 0.125 * \text{LAI if LAI } < 6 \\ r_T = 0.75 \text{ otherwise} \end{cases}$$

If REW was above 0.4 (considered as a drought threshold), T was T_{max}. Otherwise, T decreased linearly with REW. We used no intercept to the linear relation between T and REW, so that the transpiration was null if no water was available. The drainage D was such as REW was never above 1. We proposed the following method for Eu:

$$Eu_{max} = r_{Eu} * PET * exp(-k * \mathrm{LAI})$$

with $k = 0.6$ for larch (Takeda *et al.*, 2008); then Eu was calculated from Eu_{max} the same way T was calculated from T_{max}. Using a model derived from Penman-Montheith equations, Kelliher *et al.* (1995) represented the relation between Gs/Gc and the LAI; with Gs the understorey surface conductance and Gc the tree canopy conductance. This relation is plotted in Fig. BS 5. We also present in Fig. BS 5 the ratio $(Eu+T)/T$ depending on r_{Eu}. It arises from this comparison that $r_{Eu} = 0.375$ provides water flow ratios that are consistent with the conductance ratios from Kelliher *et al.* (1995), especially in the condition of low LAI. Therefore, we used $r_{Eu} = 0.375$ to parameterize the model during the growth season. In order to account for the decrease in the overstorey biological activity in winter, r_{Eu} was set to 0.125 between the days 288 and 105 with 30 days of linear transition (just as the trees LAI varying from LAI_{max} to 0).

In order to model the water losses by rainfall interception we introduced a new compartment, the tree canopy water storage S. This compartment was limited by S_{max}, the total amount of water that the canopy could store, which was set as a function of the LAI: $S_{max} = 3\ \mathrm{mm} * (1 - exp(-k * \mathrm{LAI}_{max}))$. The value 3 mm and the absence of seasonal variation for the canopy water storage capacity were specific to larch (Reynolds and Henderson, 1967). The interception algorithm was unsophisticated: S was filled first, then when it was full the extra water dropped to the ground. We applied the following rules adapted from Granier *et al.* (1999): (i) The canopy transpiration T was reduced by 20% of S and (ii) the sum of T, Eu and the evaporation from S was limited to 1.2 times the PET. Finally, the remaining water on the leaves was transferred to the next day.

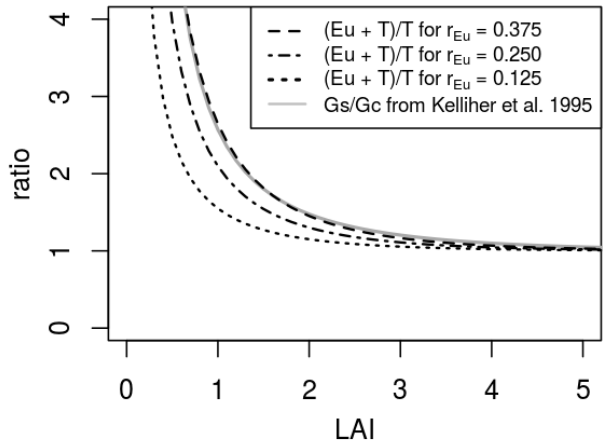

Figure BS 5: Ratio between the water transpired by tree canopy (T) and evapotranspired by the understorey vegetation (Eu), and ratio between understorey vegetation conductance (Gs) and tree canopy conductance (Gc) as modeled by Kelliher *et al.* (1995), depending on the leaf area index (LAI)

C Supplementary material 3 Simulator

A locus-based simulation software (Metagene) using a finite loci approach was previously developed at INRA (Sánchez *et al.*, 2008, http://www.igv.fi.cnr.it/noveltree). The initial software was adapted to the study of forest tree breeding strategies dealing with adverse genetic correlations (Hallingbäck *et al.*, 2014), or long-term genetic diversity issues (Wu *et al.*, 2016). It was further expanded for the present study to simulate heritable traits responding longitudinally to environmental variations. According to the phenotypic plasticity literature (Windig *et al.*, 2004; Pigliucci, 2005), two nonexclusive genetic mechanisms can be assumed for modeling a plastic response: 'epistatic' plasticity and 'pleiotropic' plasticity. Briefly, while epistatic plasticity denotes mainly the causal mechanism by which regulatory genes serve as environmentally operated signal boxes for switching between alternative genetic pathways, the pleiotropic plasticity concept refers to genes that have pleiotropic effects on a given character expressed in different environments. Both mechanisms were coded as available gene actions in the simulator, but only the latter was used in this study for simplicity. Thus, the effect of a locus was set as a function of a given environmental gradient x. Rather than prospecting the effects of underlying genetic architectures and mechanisms on the plastic response, our main objective here was to produce with a reasonably simple setup heritable reaction norms.

Therefore, our modeling of plastic responses relied on loci with alleles showing environmental sensibility in their genic effects. For this, genotypic values were modeled by a quadratic function such as $\alpha_(x) = \alpha_0 + \alpha_1(x+\delta) + \alpha_2(x+\delta)^2$, where parameters α_0, α_1, α_2 and δ defined a genotypic reaction norm with a certain parabolic shape over the range of the environment x. Each locus required two of these functions, one for the favorable homozygote (AA) and another for the unfavorable homozygote (aa), with heterozygote (Aa) being always intermediate (*i.e.* no dominance). Averaged allele effects were further computed following Falconer and Mackay (1996). Underlying the plastic trait, we considered 30 diallelic loci with alternating quadratic functions across the genome between two possible parametric sets (first set, AA: $\alpha_0 = 0$, $\alpha_1 = 2$, $\alpha_2 = 0$; aa: $\alpha_0 = 0$, $\alpha_1 = -1$, $\alpha_2 = 0$; and second set, AA: $\alpha_0 = 0$, $\alpha_1 = 0$, $\alpha_2 = 2$; aa: $\alpha_0 = 0$, $\alpha_1 = 0$, $\alpha_2 = -3$). The δ parameter was locus specific and varied between -0.7 and 0.75 across the genome, in such a way that a certain level of heterogeneity in $\alpha_(x)$ when $x \to 0$ was produced. The environmental deviate, x, was randomly sampled from a normal distribution (mean = 0 and standard deviation = 0.2) to obtain a set of n environments, equal to the number of sibs per mating.

All loci were considered to be evenly spaced across the genome, and recombination occurred without interference considering an arbitrary genome size of 600 cM. The initial sample of alleles that made up founder genotypes was randomly drawn from a distribution where allelic frequencies could be set randomly across loci within a range between 0.2 and 0.8. Individual genotypic values per environment were the result of the sum of all loci's genotypic contributions for the corresponding environment. The corresponding phenotypic value, expressed in a given environment x, was the sum of the genotypic value and a residual deviation which was sampled from N(0, $\sigma^2_R(x) = \sigma^2_A(x)(\frac{1}{h^2} - 1)$) where $\sigma^2_A(x)$ was the additive variance at the x environment, and h^2 the initial narrow-sense heritability being constant along the environmental gradient. Note that the residual deviation was not correlated to the environmental cue x.

D Supplementary material 4 Further results

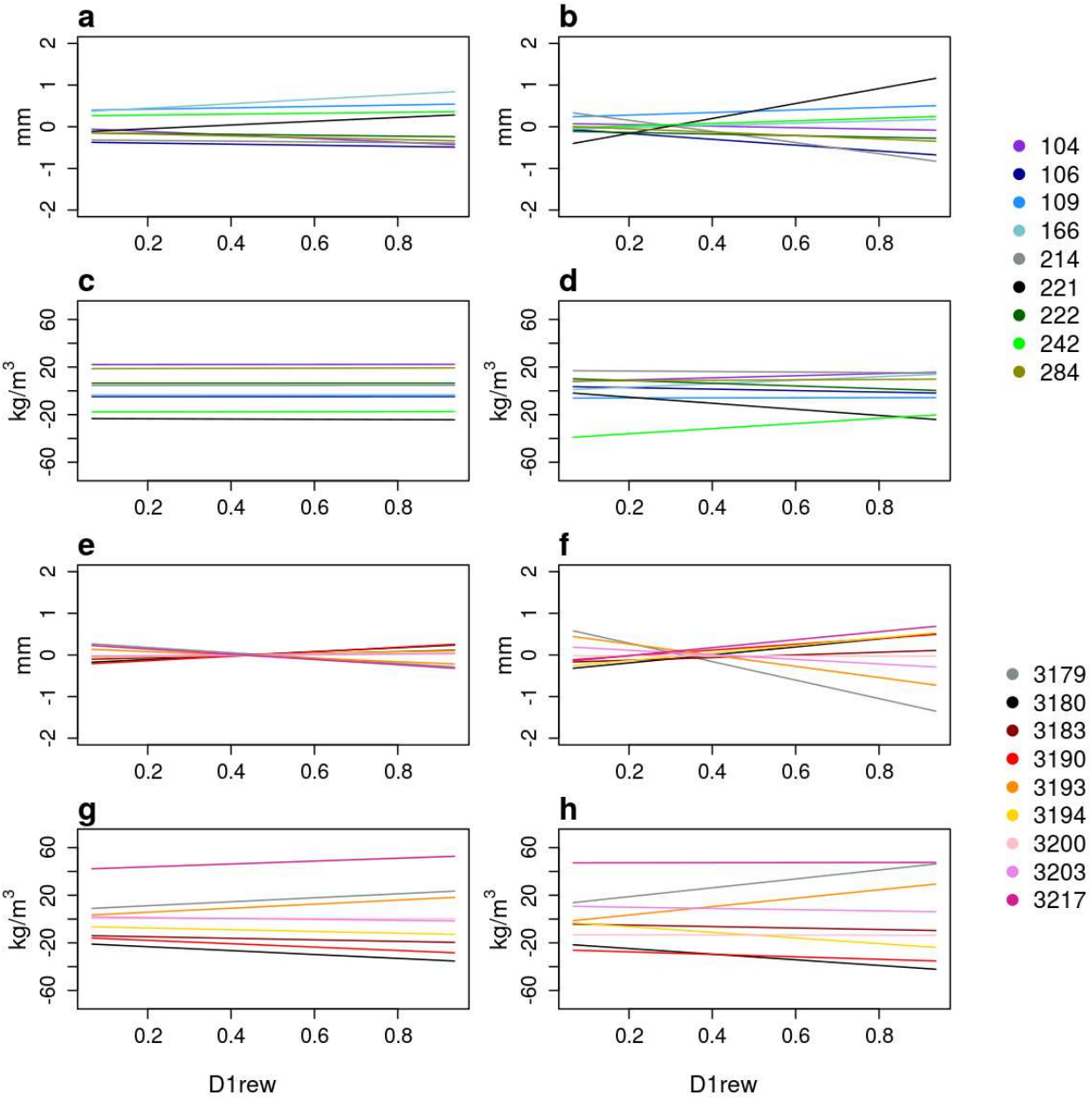

Figure DS 6: Genetic performances for the 9 European larch parents (a-d) and the 9 Japanese larch parents (e-h) for ring width (a-b, e-f) and ring mean density (c-d, g-h), in pure species (breeding value) (a, c, e, g) and in hybridization (twice the general hybridization ability) (b, d, f, h), along the first decile of the daily relative extractable water (D1rew). Each color represents one genotype, labeled in the legend on the right

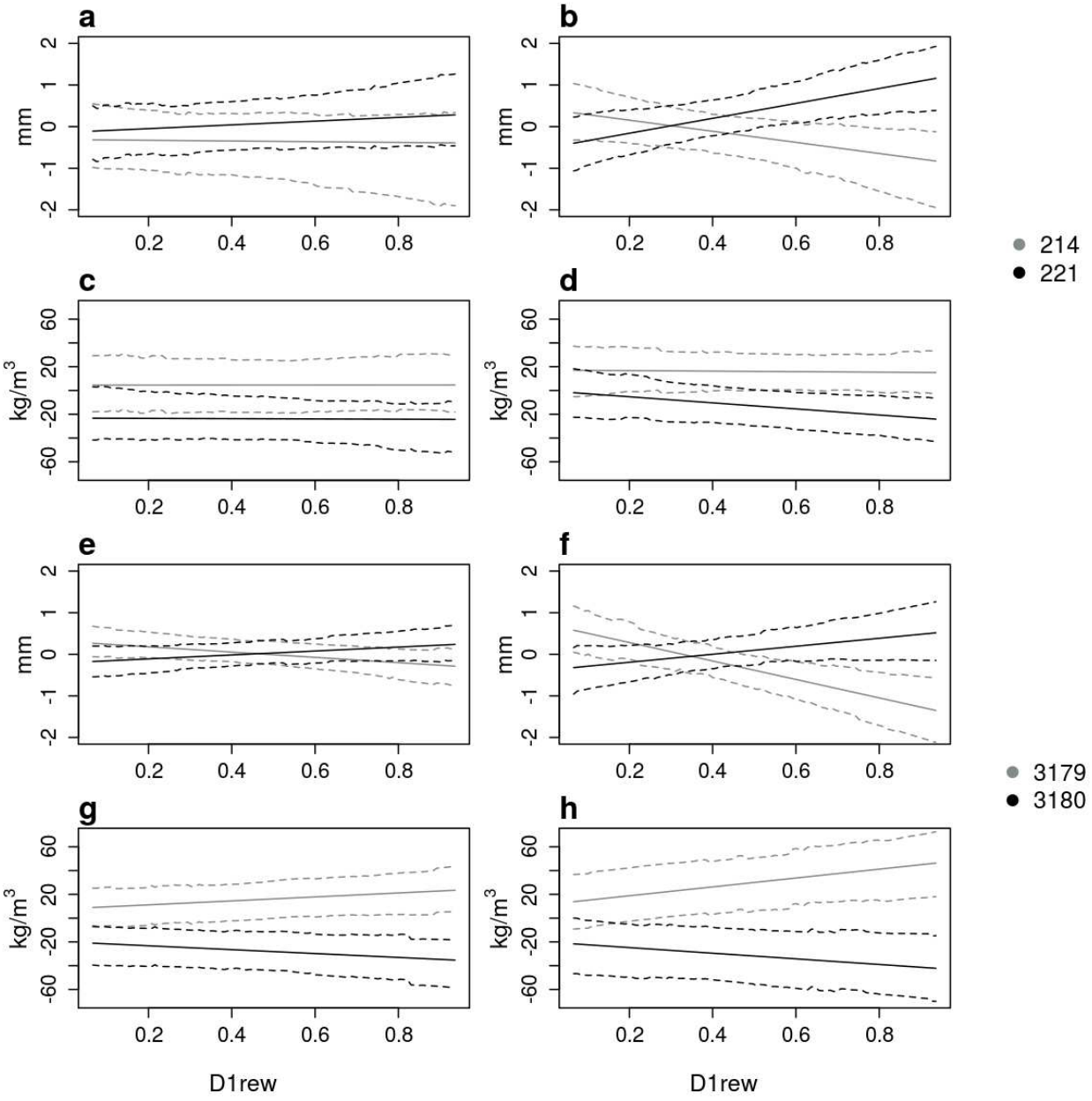

Figure DS 7: Genetic performances with 95% CIs (dashed lines) for some contrasted European larch parents (a-d) and Japanese larch parents (e-h) for ring width (a-b, e-f) and ring mean density (c-d, g-h), in pure species (breeding value) (a, c, e, g) and in hybridization (twice the general hybridization ability) (b, d, f, h), along the first decile of the daily relative extractable water (D1rew). Each color represents one genotype, labeled in the legend on the right

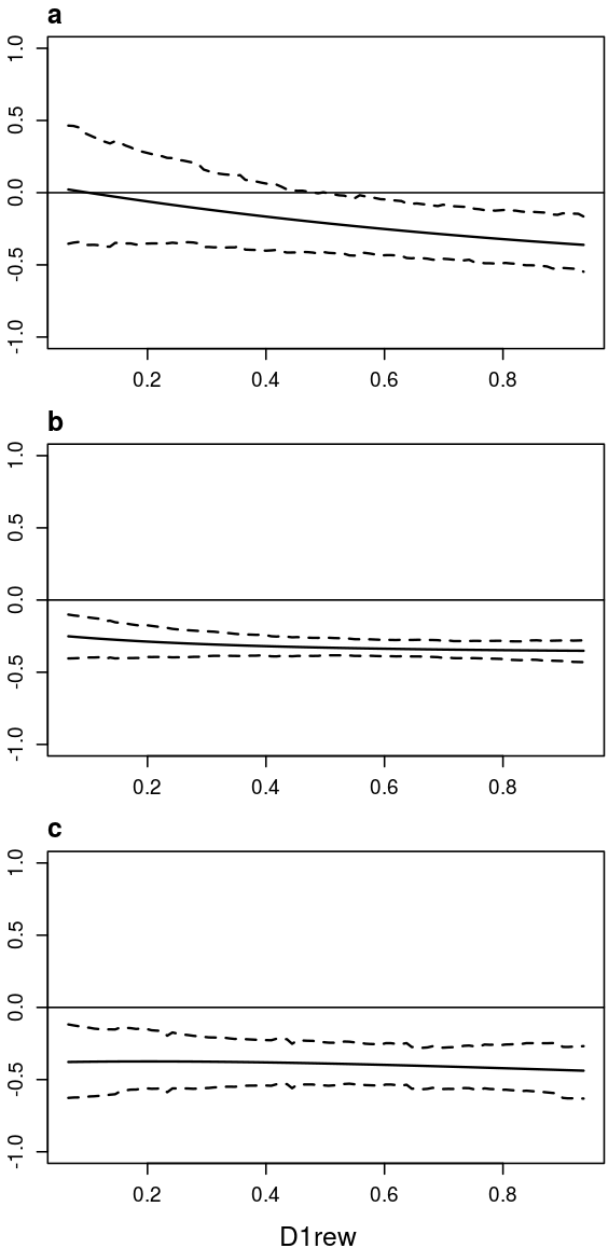

Figure DS 8: Permanent environment correlations between ring width and ring mean density along the first decile of the daily relative extractable water (D1rew), for European larch (a), hybrid larch (b) and Japanese larch (c). Dashed lines: 95% CIs

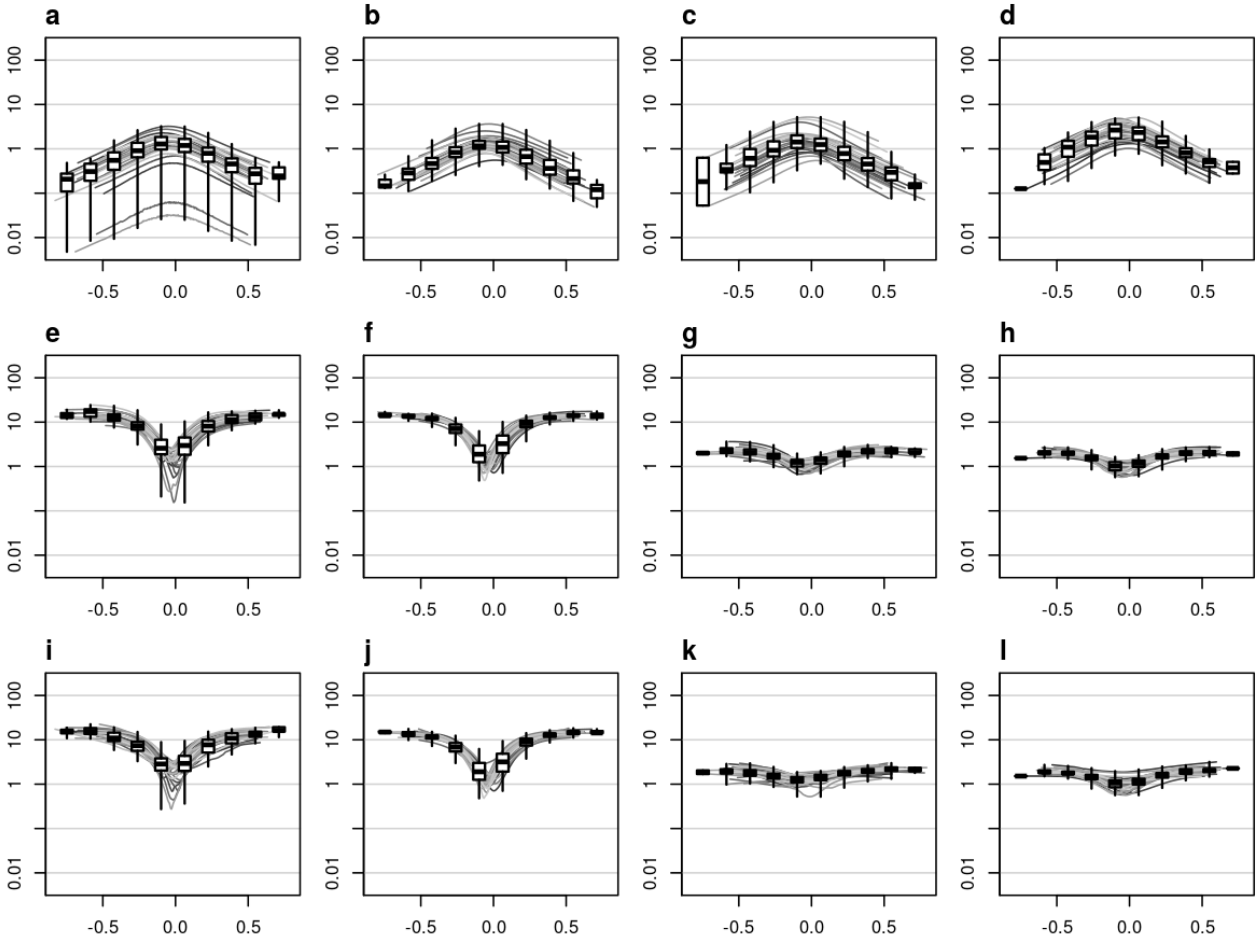

Figure DS 9: Ratio between the estimated additive variances and the true variances for each scenario: (1) $h^2 = 0.1$ and $n = 20$ (a, e, i), (2) $h^2 = 0.1$ and $n = 120$ (b, f, j), (3) $h^2 = 0.6$ and $n = 20$ (c, g, k), and (4) $h^2 = 0.6$ and $n = 120$ (d, h, l); for 100 simulations in each scenario, and for each order of random regression: order 0 (a-d), order 1 (e-h) and order 2 (i-l). Each grey curve is the ratio for 1 simulation; boxplots summarize all the point ratios in segments of a 10^{th} of the environmental gradient. The additive variances refer to the progeny population

5. Discussion

5.1. Main results

5.1.1. From Chapter 1

In the 1st Chapter, we analyzed simultaneously the performances of the 3 taxa (the hybrid and its parental species) for 6 traits expressed in 3 contrasted sites. Here is a summary of the main results.

- Hybrid larch heterosis was confirmed for growth traits. Indeed, best-parent heterosis was detected for both height (HT) and breast-height circumference (BHC) in each site, except at Peyrat-le-Château for BHC where the hybrid only reached the performance of its best parent.
- The heterosis came with no loss in stem wood density (DEN), as the hybrid density was intermediate between the densities of the two parental species. For another trait of major importance associated with wood quality: stem flexuosity (FLX), the hybrid showed levels comparable to those of the worst parental species (the European larch, EL).
- The hybrid showed a consistently higher performance compared to the parental counterparts, and that occurred across sites suggesting that the hybrid was the most stable taxon. On the other hand, the relative ranking of the parental species varied: EL performed better in the poorest and most mountainous site (SA), and JL thrived more in the lowland, most oceanic stations (PC and SS).
- Though not a core investigation in this study, we showed in the single grafted site that the rootstock species (hybrid larch, HL *vs.* Japanese larch, JL) had a significant effect on the growth of the grafted trees. The superiority of the trees grafted on hybrid rootstock over the trees grafted on a rootstock from the parental species (JL) can be interpreted as a component of the heterosis, a phenomenon that should not be seen as restricted to the aerial part of the trees.
- The narrow-sense heritabilities (h^2) were site-dependent on the EL side, while they showed a constant pattern on the JL side, and such pattern was consistent both in pure species and in hybridization.
- The dominance variances were all very low, even negligible in most cases.

- One trait, the heartwood proportion (HWP) did not show neither heterosis nor heritability.
- Some lessons could be drawn from the additive correlation matrices, though their biological interpretation is not trivial:
  - BUD and FLX were always (*i.e.* for each combination of site and taxon) negatively correlated. On the other hand, BUD and BHC were positively correlated in most cases on the EL side. This picture is completed with a negative correlation between BHC and FLX, on both the EL and the JL side. There is, therefore, a possible trade-off between two traits of major importance: BHC, the wood productivity, and FLX, that plays a central role in the quality and in the marketability of the stems.
  - DEN and HT were almost always positively correlated, while DEN and BHC were negatively correlated on the JL side. The correlation between the two growth traits, BHC and HT, was generally positive on the EL side, while on the JL side the pattern was unclear.
- The stability of the additive performances was assessed from two angles: across sites, and in pure species *vs.* in hybridization. The lesson was the same in the two cases, namely, the traits with the highest heritabilities (BUD, to a lesser extent FLX, and on the JL side only DEN) were generally the most stable.

This study was actually a small reading window of a larger and complex reality, as only 9 EL and 9 JL parents from the whole breeding populations were included, and representing for EL only the Sudetan origin (known for its fast growth but poor stem form). For this reason, some results should be considered with care. Notably, the magnitude of the variance components are specific to this genetic basis. For instance, heritability could have been higher with the inclusion of contrasting parents representing a wider population. For this reason, it may also be misleading to conclude that dominance had no role in the genetic architecture of traits given the negligible levels of variances that were detected. The dominance variance is involved in the family effect, and it is conceivable that low variation might be due to the random sample of particularly non-interactive parents.

Despite the narrowness of the genetic basis, this 1st Chapter contributes to the understanding of the construction of the hybrid larch phenotype and the way the genetic control builds up in the hybrid. First, we showed that the performances of the hybrid were more stable than the parental counterparts across the investigated sites. Moreover, heterosis and stability of hybrids did not penalized their wood quality. The stability of the

additive performances between pure species and hybrids, as well as across sites, and the genetic correlations that bind all these traits, are also important elements to guide the breeding program for hybrid larch.

5.1.2. From Chapter 2

In the second chapter, we compared the reaction norms of each taxon for wood formation traits along a soil water availability gradient. We summarize here the main results based on random regression, an original analytical approach in forestry.

- As expected, the annual radial growth (ring width, RW) was plastic across a gradient of soil water availability, and about half of the variance of RW could be explained by a model accounting for this environmental covariate. On the contrary, the wood density averaged per ring (ring mean density, RMD) did not show plasticity across the same water availability gradient.
- Overall, heterosis was expressed in favorable climatic conditions, *i.e.*, when soil water was the most available. As in Chapter 1, we confirmed that the best performing of the parental species varied across sites, but the hybrid always performed better as long as the water availability was high enough.
- All narrow-sense heritabilities along the gradient were very low, due to a very high residual variance. The highest h^2 was found for RMD on the JL side, and it increased with increasing water availability. In the same way, the contrasts between GHA for RW increased with increasing water availability on both parental sides.
- The additive correlation between RW and RMD varied sharply in reaction to the water availability: from around 0 (on the EL side) or a positive value (on the JL side) under dry conditions, it became markedly negative when more water (and thus faster growth) was available.
- Using a complementary analysis, we demonstrated the possibility to obtain reaction norms from non-repeated individual records bound by a family structure. However, this possibility was conditional to the quantity (number of offspring per family) and the quality (the heritability) of information in the data.

The Chapter 2 pinpoints the link between the annual climatic environment and the expression of hybrid larch heterosis. Heterosis was built during the years of water availability, while at the driest years all taxa performed similarly with low radial growth.

Interestingly, the taxa reaction norms of RW *vs.* water availability were different from one site to the other, indicating a hierarchy between temporal and spatial plasticities. This could result from other factors, apart from water availability, affecting plasticity at some particular sites and not conveniently addressed in our modeling.

5.2. Towards the architecture of larch heterosis

5.2.1. Synthesis

Because of the absence of molecular information, this dissertation has not been designed to infer explicitly the genetic architecture of hybrid larch heterosis. Keeping this in mind, we may however propose some ideas on the mechanisms behind larch heterosis that would explain such results as the ones that we exposed here.

From our 9 EL and 9 JL samples, heterosis on growth traits was defined at the taxon level without dominance variance. The absence of dominance variance does not rule out the possibility of the involvement of molecular dominance in heterosis; rather, we can state that there were no interactions between the parents into specific families. It leads to the idea that heterosis may be due to groups of alleles - possibly only few alleles - fixed in each population, whose interactive effects are neutral toward heterosis. The fixation could be due to natural selection or genetic drift in the respective environment of each of the two species. An interesting path to deepen our understanding of the genetic architecture of heterosis would be the study of second generation (F_2) hybrids and the resulting segregation patterns. As we previously reviewed, heterosis could arise from dominance complementation, but also from gene regulation and epistasis. Aside the molecular mechanisms, heterosis could be caused to some extent by the multiplicative complementarity between latent traits, and for this reason, it seems worth to keep studying the morphological and physiological characterization of each taxon. In this sense, the tree rings constitute a gold mine of information that we barely scratched throughout this dissertation.

Hybrid larch is an artificial taxon that has never been submitted to long-term natural selection. Embryo viability, natural mortality in the field (and artificial thinning in Saint-Saud) were the only sources of direct selection on the hybrid material we observed, without the possibility of advanced segregation for perfecting performances and fitness. Despite of this lack of long-term natural selection optimization, the hybrid performed better

than its parental species. As concluded by Ledig (1986, quoted in Nikles and Griffin 1991): 'heterosis is not a general phenomenon in interspecific hybrids, nor is it common in wide inter-population crosses'. Indeed, it is difficult to take as granted *a priori* the superiority of the hybrid from such distant gene pools. Such hybrids deviate from a Mendelian population and therefore have no special reason for being in genetic homeostasis.

Hybrid larch benefited from favorable water availability to build its heterosis, displaying an increased plasticity in comparison to the parental species. As we showed at Peyrat-le-Château, hybrid vigor seemed to be present through the roots as well. It is conceivable that a better developed root system could contribute to the higher resilience of the hybrid towards drought with respect to its parents, and this might ultimately lead to the increased plasticity that was observed. On the other hand, the fact that under dry conditions, the hybrid radial growth was similarly limited as that of its parents might indicate that the mechanisms that are responsible of this drought-sensitive growth limitation are equally present and preserved in both distant larch species. The hybrid would eventually have inherited a functional version of this feature due to the preservation of the genetic homeostasis in the diverging species for this specific trait. The major hypothesis behind this picture is that the ability to limit radial growth under drought could be interpreted as a survival trait, but that still needs further research.

5.2.2. Perspective: systemic approach

It may seem subjective coming from me to advocate that hybrid larch is an outstanding model for the study of hybrids homeostasis - but it truly is. The magnitude of heterosis and its persistence across sites are remarkable features that make this biological model worth of further investigations.

Heterosis is a global phenomenon, and we only assessed it on growth traits, that is the most integrative level of phenome. Behind this result, unknown intermediate mechanics have been at work and it is hard not to be keen to know more about that. The first, most intuitive scale to investigate heterosis would be to keep studying and comparing the ecophysiological functioning of both three taxa. As stated in the conclusion of Lippman and Zamir (2007)'s review, 'the phenotypes of heterosis are our best clues to its molecular basis'. An intensive phenotyping step, covering properly related individuals as the ones

involved in the present dissertation, might unravel complementarities between elementary traits that, together, build the heterosis that ultimately manifests on growth.

Gene expression and regulation would also be an interesting perspective from which to consider the question. In particular, we have previously seen that epigenetics has been documented to be involved in the heterosis of some plant models (Chen 2013). Yet, epigenetics is also pointed at as an underlying mechanism of forest tree phenotypic plasticity (Bräutigam *et al.* 2013; Plomion *et al.* 2016). Therefore, it may be rewarding to include this dimension when addressing further questions about the higher dendroplasticity of hybrid larch.

5.3. Implications for hybrid larch breeding

5.3.1. Synthesis

Throughout this dissertation, we reported the results from the first multi-site, mid-rotation (18 years old from plantation), properly designed (with a diallel) experimental set-up involving both hybrid larches and their parental controls. They confirmed the heterosis for volume-related growth traits (height and circumference) that had already been demonstrated elsewhere. The hybrid superiority for growth came with no counterpart in terms of wood quality, no matter whether we look at wood density or stem flexuosity. However, the volume-related traits did not show substantial potential for selection as their narrow-sense heritabilities were low, or at best site-dependent. It could be worth to investigate a bigger sample from the breeding population, and maybe to keep prospecting the natural variability in order to handle more genetic variance and therefore more potential for breeding for these growth traits. From the window of the present study, the only leverage that appears to improve growth is the hybridization step, and the release of heterosis at the taxon level.

A second category of traits that showed a higher level of additive control were: bud flush and stem flexuosity, and wood density on the Japanese larch side only. The additive variances of stem flexuosity and bud flush were high enough to conceive a management of these traits by selection, and they were also negatively correlated: the later bud-flushing trees were also the ones more likely to show stem straightness. This additive correlation is actually favorable, because late bud-flushing is a protection against exceptional late frosts. It may also be seen as a tool for breeding, because bud flushing of young saplings could

be used as a proxy to infer their mature stem straightness. On the other hand, our data also indicated adverse additive correlations between bud-flushing and stem circumference, and between stem flexuosity and stem circumference. Given the low additive variance for circumference, this adverse correlation was not of relevance in our sample; however it should be kept in mind and checked further if the circumference additive variance was inflated by the exploration of new genotypes.

Sudetan population, as used in this study, is known to be of moderate stem form quality among larch populations in contrast to straight alpine larch. Current investigations implying polycrosses with the tree main populations ('sudetica', 'polonica' and 'alpine') aim at determining the impact of these genetic backgrounds on stem straightness.

Finally, our study indicated that the additive performances of the heritable traits were stable in pure species *vs.* in hybridization. This information is valuable as it indicates the possibility of overlapping between the pure species and the hybrid breeding programs. Also, the hybrid breeding program could benefit from mass selection of pure-species parents, especially for bud flush and stem flexuosity. In particular, the prospection for natural variability may consider *in situ* stem straightness as an indicator of the straightness of the progeny in hybridization.

High correlations between GHA (general hybridization ability) and GCA (general combination ability, in pure species crosses) have been shown for some traits and for some *Pinus* hybrid combinations (reviewed by Nikles and Griffin 1991; Dungey 2001). Nikles and Griffin (1991) conclude that the pure species breeding of *Pinus elliottii* var. *elliottii* for stem straightness and wind firmness in Queensland was a fertile ground for the performances observed in hybridization with *P. caribaea* var. *hondurensis* for these traits. As detailed by Dungey (2001), high GHA/GCA correlations (as the ones we estimated in our study) may have direct implication for the breeding program. For instance, in a reciprocal recurrent selection frame, it would be possible to add a pure species selection step prior to hybrid progeny testing. This extra-step comes with a very low cost (in comparison to hybrid progeny testing), and yields fast and relatively easy genetic gains.

5.3.2. Perspective: molecular information

A way to improve progeny test set-up and analysis is the use of molecular markers. Twelve microsatellite markers have already been designed for both EL, JL, and HL

(Wagner *et al.* 2012). Firstly, microsatellite markers can be used to reconstruct *post-hoc* the pedigree of polymix progenies, avoiding the need for time-consuming controlled crosses (Lambeth *et al.* 2001), while reducing the risk of paternity mistakes: this is an option that is currently being implemented at INRA in order to avoid laborious controlled crosses with species such as *Pinus pinaster*.

Additionally, replacing pedigree-based genetic matrices in the mixed models by matrices estimated from molecular markers (*i.e.* G-BLUP model) may improve the accuracy of the analysis, as shown by Klápště *et al*. (2014) with *Larix occidentalis* using 8 microsatellite markers. Moreover, deciphering differences in relatedness at the within-family level by the use of markers opens up the possibility to exploit effectively the Mendelian sampling variation, which is hardly accessible with pedigree-based evaluation. The interest for the Mendelian sampling comes at any depth of pedigrees, but notably at early generations when within family variation tends to be larger. The G-BLUP model can be adapted to hybrids (*e.g.* Marchal *et al.* 2016) as well.

Genomic selection is also a promising prospect for long-living, perennial plants such as forest trees, mainly because of the possibility to shorten the selection cycles. Genomic selection has been shown to be successful for oil palm using dense genotyping with microsatellites (Cros 2014) and it is currently investigated in black poplar, another forest tree species (Pégard *et al.*, in prep.).

5.3.3. Perspective: in-depth hybridization

The interest for in-depth hybrid breeding, notably second generation (F_2) hybrids, arises from a practical consideration. Indeed, the commercial deployment of F_1 hybrids is limited by our ability to produce pure hybrid seeds lots on a large scale at a competitive price when supplemental pollination is required. A possible option is to set-up a F_1 orchard that, simply from open-pollination, produces F_2 seeds. Thereby, this process does not require additional costs for pollen supplementation. One issue with this latter option is, as we have previously stated, the low yield in viable seeds due to the hybrid larch breakdown in fertility. Another concern is the stability of heterosis across the generations: what can be expected from the F_2 in terms of average performances ? How do the variances express in F_2 due to recombination ? A dedicated mating plan (a diallel/factorial mating design involving a total of 18 F_1s) has been set up by INRA in 2000 to specifically study these

questions. Finally, it may be conceivable if hybrids keep their superiority with respect to the parental species to deepen further the hybridization (F_3, F_4...), to ultimately deal with a single breeding population, thus avoiding the complexity inherent in breeding and producing F_1 hybrids.

Some results from F_2 hybrids are available in the *Pinus* literature. Cappa *et al.* (2013) presented the results from pure species, F_1s, F_2s and back-crosses of *Pinus elliottii* var. *elliottii* (PEE) and *P. caribeae* var. *hondurensis* (PCH), and pointed at the role of the environment to determine the relative ranking of the taxa. Though the results were not statistically significant, the F_1 hybrid was always among the most performing taxa for volume-related traits, and the performances of the F_2 were more irregular. However, the tested genetic materials were actually unrelated (or of unknown kinship) seed lots, making it difficult to perform a quantitative evaluation of the heterosis in F_1, like the one presented in this thesis, or on the preservation of this heterosis through F_2. Dieters and Brawner (2007) presented the results from pure species, F_1s and F_2s data (from the same *Pinus* species) with a genetic structure. The founder individuals were crossed both in a pure-species manner and in hybridization factorials, and a balanced sample of these F_1s were used to produce the F_2 factorial. Under the conditions of this study, the best parent was PCH. The ranking between the F_1s and the PCH varied across sites, and the performances of the F_2s were often lower, but in any case above the mid-parent value. Therefore, in this study, the F_2s manifested average-parent heterosis for the volume related traits.

It would be extremely interesting to follow the deepening of the hybridization and the subsequent Mendelian segregation with molecular markers. Indeed, if the genome coverage is dense enough and if enough phenotypic information is available, it would be possible to identify loci involved in the hybrid larch heterosis. The research for major loci involved in heterosis could also be attempted from the pedigree information only (*e.g.* Li and Wu 1996; Kadarmideen and Janss 2005) but such results are surrounded with much more uncertainty (as they mobilize less data) and do not lead to the same operational implications.

5.4. Phenotypic plasticity and hybrid larch breeding

5.4.1. Synthesis

This dissertation particularly emphasized the role of environment on the construction of the hybrid larch phenotype: hybrid larch was both more stable in terms of ranking and more plastic than the parental species. More stable, because heterosis expressed at any site and it guaranteed the constant top-ranking of hybrid larch with respect to its parents. The higher plasticity of hybrids expressed at a different scale than that of stability. Indeed, without being surpassed by its parents in radial growth, hybrids showed a steeper reaction norm for this trait when conditions of water availability changed. Therefore, the higher plasticity at the annual scale can be seen as a component of the higher across-site stability of the hybrid.

Phenotypic plasticity *vs.* annual soil water availability did not only play a role on the manifestation of heterosis, but also on the expression of within-taxa genetic contrasts. Indeed, the wood density heritability (that was low but non-negligible on the Japanese larch side) increased with increasing annual soil water availability. In the same way, in dry conditions there were almost no contrasts between the genotypes for radial growth, but as the water availability increased the contrasts showed up. This was confirmed from both sides (European and Japanese larches) but only in hybridization. Finally, the negative additive correlation that we showed between the stem circumference and wood density (especially on the Japanese larch side, as the European larch correlation pattern for these traits was less clear) was built during the years of abundant water availability. In other words, the genotypes that benefited the most from the favorable conditions for their radial growth actually tended to produce a less dense wood these specific years than the other genotypes. These results bring preliminary insights on the role of climatic environment on the construction of the hybrid larch genetic variance architecture.

Despite the impact of phenotypic plasticity on the genetic variance at the inter-annual scale, we found the additive performances of the most heritable traits to be stable across sites, and this is beneficial to the hybrid larch breeding program. On one hand, we saw that volume-related traits can be improved at the hybridization step and that this gain was stable across sites. On the other hand, the gain that can be achieved by selection for the heritable, non-heterotic traits (*i.e.* bud flush, stem flexuosity and wood density on the

Japanese larch side) also appears to be transposable to other sites, suggesting that G×E should not be a major concern in the design of a hybrid larch breeding program.

5.4.2. Perspective: adaptability of the dendroplasticity

Our study suggested a hierarchy in the triggers of wood formation phenotypic plasticity. Thus, the dendroplastic response of annual radial growth *vs.* the soil available water was conditioned by the actual site within which it occurred. This dependency was made evident by the change in the ranking of the parental species reaction norms from one site to the other. Moreover, it remains likely that the annual growth reaction norms also depended on other factors that we did not account for. Notably, we previously demonstrated that heterosis of growth trait was built in the earliest ages, and subsequently preserved with no further increase. Trees ontogeny is therefore expected to have an effect on dendroplasticity, and the discrepancy between the reaction norms of the different taxa is supposed to diminish with time.

Most of the genetic variance, at the inter- and intra- taxa levels, was compressed under the driest conditions. This canalization would be consistent with an historical selective pressure (on the parental species) involving a single optimum: under drought, all larches shall produce similarly narrow rings to survive. Nevertheless, as far as I know, the adaptive nature of the radial growth reduction under growth as not been formally demonstrated - though it may be considered as trivial, and consistent with what we know about the hydraulic functioning of the tree. Such a demonstration would be difficult to carry on given that, due to the very canalization one would like to explain, the variance for ring width under drought is weak to null, leaving little room for experiments in this regards.

The relation between adaptivity and some micro-densitometric traits has been studied on the Douglas fir model (*Pseudotsuga menziesii*). The extreme heat wave of 2003 and the subsequent drought event have killed many Douglas firs in France. Wood records of dead and surviving trees were retrospectively compared by Martinez-Meier *et al.* (2008) and Britez *et al.* (2014). In all 3 contrasted sites of these studies, wood density correlated positively with survival to the extreme event. Britez *et al.* (2014) also showed that the number of cross-points between the annual microdensitometric profiles and a given density threshold (*i.e.* an indicative of the number of false rings, each false ring producing 2 cross-points, see Fig. 7 of the Material and Methods section) were more

important in surviving trees than in dead ones. This finding indicates that this specific form of dendroplasticity - the ability to produce false rings - may be involved in the Douglas fir adaptability to drought. Comparison of the reaction norms between dead and survivor trees to a given extreme event could be carried on using the random regression framework we presented in the 2nd Chapter, in order to specifically assess the adaptive role of wood formation plasticity with respect to the annual climatic environment.

It may also be rewarding to address the question of the adaptive nature of dendroplasticity at the intra-annual scale, as most of the densitometric information (intra-annual micro-variations of the density) lays in this compartment. Such approaches have already been started (Sánchez-Vargas *et al.* 2007; Martinez-Meier *et al.* 2009). The most important step from these studies is the set-up of a synchronization function, *i.e.* a function that assigns the intra-annual density variations to the corresponding intra-annual climatic events. Investigating the role of wood formation phenotypic plasticity in the adaptivity of forest trees, including but not limited to hybrid larch, may ultimately lead to operational outcomes for the breeding of these species. Indeed, such studies could contribute to identify survival-related traits, whose importance in the global warming context may be more and more critical.

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Appendices

Appendix 1: Observation sheet for the pit 'B2' in Peyrat-le-Château

B2

Description de l'environnement du sondage et/ou de la fosse

Informations générales

N° dispositif : 3308 Date : 16/12/15 Auteur : LEP

Commune : Peyrat Béthadeyre

Coordonnées Géographique : (X) 475 (Y) 895 Position

Situation dans la parcelle _____ N° Sondage [ ] N° Fosse associée [B2]

Antécédents climatiques

Nature [1]
1 _ pluie
2 _ neige
3 _ humidité
4 _ temps ensoleillé
5 _ temps sec
6 _ sécheresse
7 _ gel
8 _ vent
9 _ temps variable

Durée [1]
1 _ les jours précédents
2 _ les semaines précédentes

Intensité [2]
1 _ d'intensité faible
2 _ d'intensité moyenne
3 _ d'intensité forte

Conditions du jour [4]
(code Nature):

Préparation du sol

1_Labour 2_Terrasse 3_Andain 4_Billon 5_Fossé 6_Autre [ ]

Matériaux parentaux

Organisation Géologique [ ]
1 Profil monolithique
2 Profil bilithique
3 Profil polylitique

Désagrégation [ ]
0 Non désagrégée
1 Peu désagrégée
2 Désagrégée
3 Tres désagrégée

Nom roches dominantes: [ ] [ ] [ ]

Profondeurs d'apparition : [ ] [ ] [ ]

Géomorphologie

Pente (en %) [ ] Exposition [W]

Morphologie locale [ ]
1 Sur une bosse
2 Dans un creux
3 Sur une pente régulière
4 Sur un replat

Situation dans le versant [2]
1 Au bas du versant
2 Au tiers inférieur du versant
3 A mi-hauteur du versant
4 Au tiers supérieur du versant
5 Au sommet du versant

Description du sondage et/ou de la fosse

Profondeurs de l'horizon en cm

N° de l'horizon	Min	Max
1	60	
2	40	
3		
4		
5		
6		

Humidité

1	2
2	2
3	
4	
5	
6	

1 Sec
2 Frais
3 Humide
4 Très humide
5 Saturé
6 Noyé

Texture

1	S/L
2	S
3	
4	
5	
6	

Effervescence

Intensité

1	
2	
3	
4	
5	
6	

0_Nulle
1_Faible
2_Modérée
3_Forte
4_Extrêmement forte

Localisation

1	
2	
3	
4	
5	
6	

1_généralisée
2_localisée à la matrice
3_localisée au squelette
4_localisée aux éléments secondaires

pH

1	4
2	5
3	
4	
5	
6	

Matières organiques

Abondance

1	3
2	2
3	
4	
5	
6	

0_Absente
1_Indéterminée
2_Faible (<1 %)
3_Moyenne (1 à 4 %)
4_Assez forte (4 à 10 %)
5_Forte (10 à 20 %)
6_Très forte (20 à 30 %)
7_Extrêmement forte (>30 %)

Tâches

Abondance (A ,B et C)

	A	B	C
1			
2			
3			
4			
5			
6			

0_Pas de taches
1_Très peu nombreuses (<2 %)
2_Peu nombreuses (2 à 5 %)
3_Assez nombreuses (5 à 15 %)
4_Nombreuses (15 à 40 %)
5_Très nombreuses (40 à 80 %)
6_Dominantes (plus de 80 %)

A = oxydation

B = réduction

C = dégradation ou autres

Eléments grossiers 1/2

Abondance %

1	<1
2	<1
3	
4	
5	
6	

Taille (A et B)

	A	B
1	1	2
2		
3		
4		
5		
6		

1_graviers (0.2-2cm)
2_cailloux (2-6cm)
3_pierres (6-20cm)
4_blocs (>20cm)

Forme (A et B)

	A	B
1	4	
2	4	
3		
4		
5		
6		

1_arrondis
2_allongés anguleux
3_allongés émoussés
4_aplatis anguleux
5_aplatis émoussés
6_irréguliers anguleux
7_irréguliers émoussés
8_de formes diverses

Nature (A et B)

	A	B
1		
2		
3		
4		
5		
6		

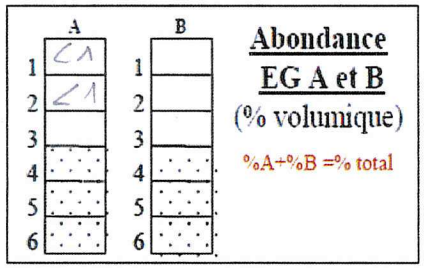
Extrait de STIPA 2000 – U.F.R Science du sol – INRA Montpellier

Description supplémentaire à partir de la fosse uniquement

N° Fosse B2 N° Sondages associés

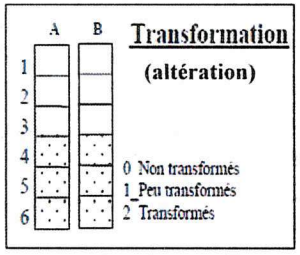
(Facultatif) Nom de sol _____ Séquence d'horizons(codés) _____

Eléments grossiers 2/2



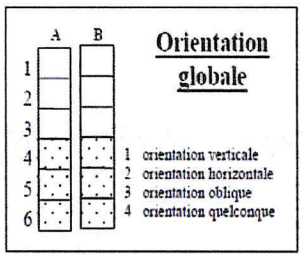
Abondance EG A et B (% volumique)

%A+%B =% total



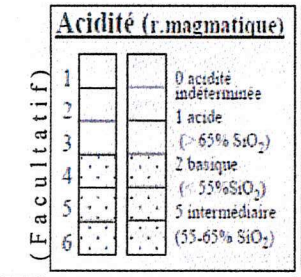
Transformation (altération)

0 Non transformés
1 Peu transformés
2 Transformés



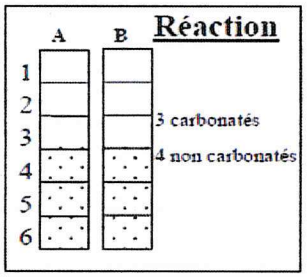
Orientation globale

1 orientation verticale
2 orientation horizontale
3 orientation oblique
4 orientation quelconque



Acidité (r.magmatique) (Facultatif)

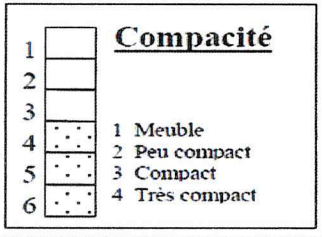
0 acidité indéterminée
1 acide (> 65% SiO_2)
2 basique (< 55% SiO_2)
3 intermédiaire (55-65% SiO_2)



Réaction

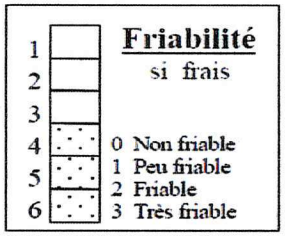
3 carbonatés
4 non carbonatés

Propriétés mécaniques



Compacité

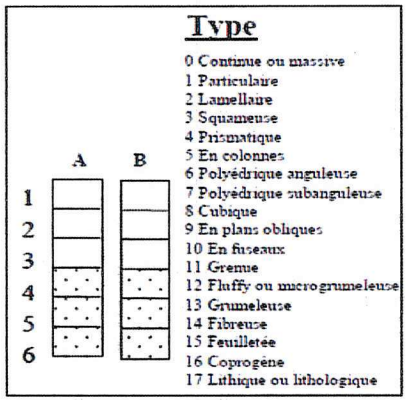
1 Meuble
2 Peu compact
3 Compact
4 Très compact



Friabilité si frais

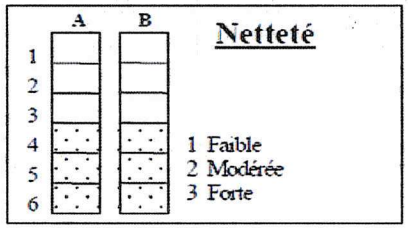
0 Non friable
1 Peu friable
2 Friable
3 Très friable

Structure



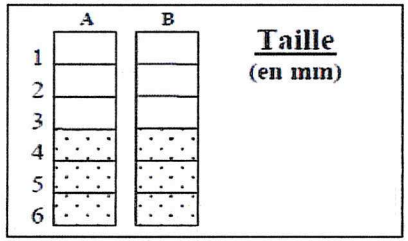
Type

0 Continue ou massive
1 Particulaire
2 Lamellaire
3 Squameuse
4 Prismatique
5 En colonnes
6 Polyédrique anguleuse
7 Polyédrique subanguleuse
8 Cubique
9 En plans obliques
10 En fuseaux
11 Grenue
12 Fluffy ou microgrumeleuse
13 Grumeleuse
14 Fibreuse
15 Feuilletée
16 Coprogène
17 Lithique ou lithologique

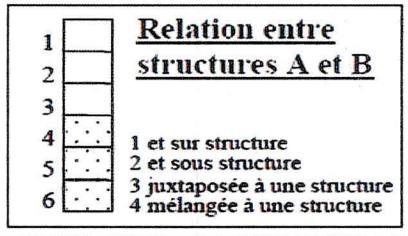


Netteté

1 Faible
2 Modérée
3 Forte



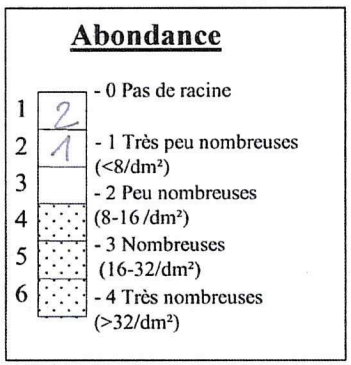
Taille (en mm)



Relation entre structures A et B

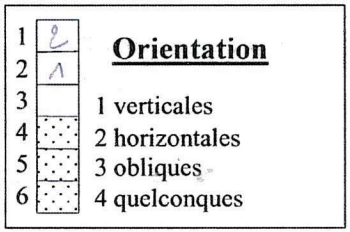
1 et sur structure
2 et sous structure
3 juxtaposée à une structure
4 mélangée à une structure

Racine



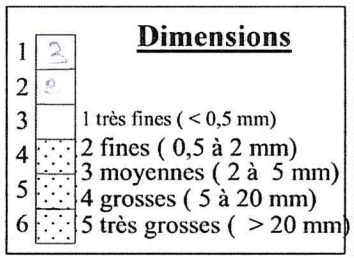
Abondance

- 0 Pas de racine
- 1 Très peu nombreuses (<8/dm²)
- 2 Peu nombreuses (8-16 /dm²)
- 3 Nombreuses (16-32/dm²)
- 4 Très nombreuses (>32/dm²)



Orientation

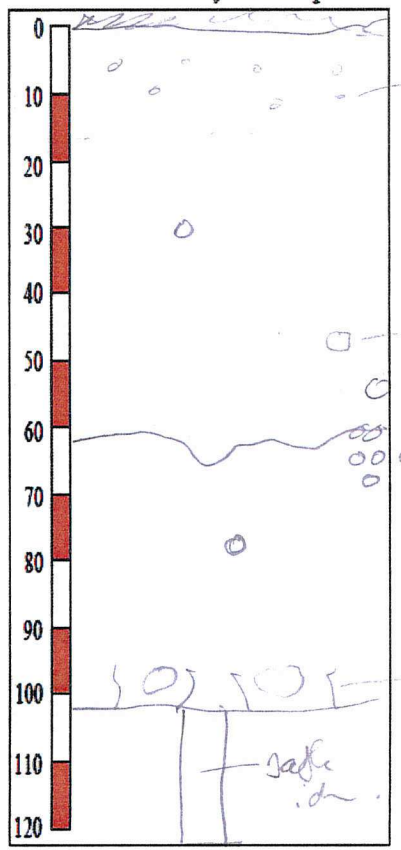
1 verticales
2 horizontales
3 obliques
4 quelconques



Dimensions

1 très fines (< 0,5 mm)
2 fines (0,5 à 2 mm)
3 moyennes (2 à 5 mm)
4 grosses (5 à 20 mm)
5 très grosses (> 20 mm)

Schéma synthétique



Commentaires : _____

Extrait de STIPA 2000 – U.F.R Science du sol – INRA Montpellier

Alexandre MARCHAL

Étude de la vigueur hybride chez le mélèze et rôle de la plasticité phénotypique dans la construction de l'hétérosis

Le mélèze d'Europe (*Larix decidua*) est traditionnellement exploité pour son bois de qualité. Malheureusement, la culture de cette essence hors de son aire de distribution a été un échec. L'hybridation avec le mélèze du Japon (*L. kaempferi*) est une voie prometteuse, en particulier grâce à l'hétérosis manifesté par cet hybride. Au cours de cette dissertation, nous valorisons les données d'un diallèle multi-site intra- et inter-spécifique d'âge avancé. Le 1er chapitre présente l'analyse de traits de production et de qualité du bois. Nous confirmons ainsi l'hétérosis pour les traits liés au volume. Cet hétérosis n'entraîne pas de contrepartie en termes de qualité, et se montre stable d'un site à l'autre. Au contraire, d'autres traits ne présentent pas d'hétérosis, mais davantage d'héritabilité. Les performances additives pour ces traits sont stables d'un site à l'autre, et en espèce pure *vs.* en hybridation. Au cours du 2ème chapitre, nous nous intéressons au rôle de la plasticité phénotypique de traits de formation du bois dans la construction de l'hétérosis. Le mélèze hybride apparaît comme le taxon le plus plastique : tout comme ses espèces parentes, il produit un cerne de croissance étroit en conditions hydriques limitantes, mais sa croissance radiale surpasse celle de ses parents quand l'eau est abondante. Ce 2ème chapitre est également un premier pas vers la compréhension du rôle de la plasticité phénotypique dans la construction de l'architecture de la variance génétique entre la circonférence et la densité des troncs. Cette thèse se termine sur une synthèse, au cours de laquelle nous discutons les retombées de nos résultats pour l'amélioration du mélèze hybride.

Mots clefs: mélèze (*Larix*), hétérosis, plasticité phénotypique, normes de réaction, homéostasis, génétique forestière, cernes de bois

Alexandre MARCHAL

Larch hybrid vigor and role of the phenotypic plasticity in the construction of heterosis

European larch (*Larix decidua*) has been historically exploited within its natural range for its high quality wood, but the attempt to grow this species outside its native range was a failure. Hybridization with Japanese larch (*L. kaempferi*) is a promising path, in particular because of the heterosis this hybrid manifests. In this dissertation, we took advantage of an old-enough, multi-site experiment with an inter-/intra-specific mating design. The first chapter presents the analysis of several traits involved in wood quality and productivity. We confirmed heterosis for volume related traits. The heterosis came with no counterpart in wood quality, and it was stable across sites. Contrarily, some other traits showed no heterosis but higher heritabilities, and the additive performances for these traits were stable across sites and in pure species *vs.* in hybridization. In the second chapter, we investigated the role of phenotypic plasticity of some wood formation traits in the construction of the heterosis. Hybrid larch appeared as the most plastic taxon: it equaled the parental controls in producing narrow growth increments under drought, but it produced the largest rings in favorable water availability conditions. This second chapter was also a first step towards a better understanding of the role of phenotypic plasticity on the construction of the genetic variance architecture between larch stem circumference and density. The dissertation ends with a synthesis in which we discussed the implication of our findings for the breeding of hybrid larch.

Key words: larch (*Larix*), heterosis, phenotypic plasticity, reaction norms, homeostasis, forest genetics, tree rings

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