Theor Appl Genet (1996) 93:1274–1281

A. Arcade · P. Faivre-Rampant · B. Le Guerroué
L. E. Pâques · D. Prat

Heterozygosity and hybrid performance in larch

Received: 16 April 1996 / Accepted: 10 May 1996

Abstract Random Amplified Polymorphic DNAs (RAPD) were used for estimating genetic distances between 12 European larches (*Larix decidua*) and 12 Japanese larches (*L. kaempferi*) that were the parents in a factorial mating design. One hundred and eleven fragments were used for establishing genetic distances based on Jaccard's coefficient between parents. Thirteen fragments differentiated the larch species. The genetic distance between individuals of the same species ($D_J = 0.39$ in the Japanese larch and 0.45 in the European larch) was lower than the genetic distance between species ($D_J = 0.72$). A UPGMA dendrogram based on genetic distances clearly clustered each larch species, confirming the speciation at a molecular level. Correlations between genetic distances of the parents and performances of the hybrid families were established for various quantitative traits. Significant values were found for growth characters and branch insertion angle, which suggested an effect of general heterozygosity level on hybrid traits. These correlations also evolved with tree age: the maximal correlation was noticed on 6-year-old trees for height. The lack of correlation between parental genetic distances and hybrid performances for the other quantitative traits suggested that these characters were controlled by fewer genes. The results of this study show that crosses between genetically distant parents produce hybrids with excellent growth performances; this represents a potential selection criterion of the genitors.

Key words *Larix* · RAPD · Genetic distance
Hybrid performance · Heterosis · Quantitative traits

Communicated by P. M. A. Tigerstedt

A. Arcade · P. Faivre-Rampant · B. Le Guerroué ·
L. E. Pâques · D. Prat(✉)
Station d'Amélioration des Arbres Forestiers, INRA, F-45160 Ardon, France

Introduction

Many observations and studies have consistently provided evidence that crosses between genetically dissimilar parents often produce superior progeny. This phenomenon, termed heterosis (Shull 1914), contrasts with inbreeding depression, i.e. crosses between related individuals tend to generate lower performing offspring. While the genetic basis of heterosis has been investigated for many decades, it is still non-resolved. The two major hypotheses put forward to explain the phenomenon are the dominance hypothesis and the overdominance hypothesis. As they are not mutually exclusive, their occurrence and relative importance are still debated. The dominance hypothesis (Jones 1917) assumes that the increased vigor of highly heterozygous individuals can be attributed to dominant alleles, i.e. heterosis is due to reduced homozygosity for deleterious recessive alleles. Indeed, many recessive alleles can be conserved in an heterozygous state (genetic load) and be revealed in an homozygous state by inbreeding (Gallais 1989). The overdominance hypothesis (East 1936; Shull 1952) supposes that the heterozygote state at single loci is superior to either of the homozygote states.

The implication of heterozygosity in the hybrid vigor phenomenon suggests a potential prediction of heterosis based on the measure of genetic distance between parents, as assessed in particular by isozyme data and molecular markers [random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP)] (Tsaftaris, 1995).

The prediction of hybrid performance is of primary interest to crop breeders and tree breeders as field-testing is expensive and labor-consuming. Moreover, with respect to forest trees, delays before the assessment of individual performances are long (Neale and Williams 1991), and a strategy for predicting heterosis before making the crosses and thereby reducing the number of combinations to be tested would be particularly desirable.

The *Larix* genus presents many attractive features, such as fast juvenile growth, relatively short rotation and high quality of timber, and a short-term objective is the supply of hybrid material for reforestation (Pâques 1989). The tree improvement programs for larch in France are expecially concerned with the hybrid between the European and Japanese larch (*Larix* × *eurolepis* Henry), which was advocated for its growth performance (Bastien and Keller 1980). According to Pâques (1989), the interest in hybridization lies in three main areas: heterosis for a given trait, combination of favorable traits leading to a higher degree of fitness and transfer of a favorable character specific to one species. Progeny field testing gives access to reliable information about genetic parameters but is expensive and time- and space-comsuming. Thus, a better knowledge of the genetic factors underlying hybrid performance represents an attractive goal and could provide criteria for choosing the best parents to be involved in a mating scheme.

Our goal in the study presented here was to investigate the relationships between genetic dissimilarity of the parents and progeny performance. We chose RAPD markers because of the high number of markers that can be obtained in a short time and because technically they are easy to use. The different steps of the study were (1) assessment of genetic variability among the European and Japanese parental trees involved in a factorial mating design, (2) study of the relationships between heterozygosity and performance of the hybrids for various quantitative traits and (3) the evaluation of the potential of marker-based genetic distance in predicting the performance of hybrid larches.

Materials and methods

Plant materials

The plant material of this study consisted of a factorial mating design involving 12 European larches (*Larix decidua* Mill.) as female parents and 12 Japanese larches (*Larix kaempferi* Lamb. Carr.) as male parents. The parents originated from various provenances (Table 1). Seven of the Japanese parents were related. The crosses were carried out in March 1982 at the INRA station in Orléans. Fifty-nine crosses were completed, and the seed lots were sown in May 1983 at the INRA nursery. Two-year-old plants of 53 families were established in the forest trial of Beaumont-du-Lac (Haute-Vienne, France) (elevation: 540 m). An incomplete randomized block design was used with eight replications, five blocks per replication and 1-tree plots (Pâques 1992).

Quantitative traits

A total of 1081 hybrid larches were evaluated in the nursery and in the field for all analyzed traits (Pâques 1992). The number of plants per full-sib family ranged from 3 to 38 individuals, with an average of 21 individuals per family. Traits could be distributed in three different groups:

- growth: total height at 2, 4, 5, 6, 8, 9, 10, 11 years from sowing (at 2 years in the nursery, then in the forest); height increment between ages 6 and 11; number of branches on the whorl of the 6th year (measured at 8 years); diameter of the two thickest branches on the whorl of the 6th year (measured at 8 years); breast-height girth at 11 years; individuals stem volume at 11 years.
- architecture: stem straightness at 6 and 11 years (scored according to a 5-point scale: 1 = crooked; 5 = straight); taper (total height/breast-height diameter ratio) at 11 years; average angle between the two thickest branches of the whorl of the 6th year and the stem (scored at 8 years according to the scale: 1 = 0°–30°; 2 = 30°–60°; 3 = 60°–90°).
- wood quality: wood specific gravity (estimated by pilodyn) at 11 years.

Table 1 Provenances of genitors of the mating design

Clone	Country	Source	Forest stand
European larch			
35	Austria (T)	Tyrol	Mittelwald
45	Poland (PL)	Polish Sudetan mountains	Przysiecz
92			Borowina
93			Borowina
97			Ozdrzychowice
102	Czech Republic (CZ)	Czech Sudetan mountains	Zabreh Dubicko
104			Ruda Nad Moravou
107			Olomouc
108			Olomouc
109			Olomouc
113			Hradec Nad O Pavou
212			Güglingen
Japanese larch			
3041	Denmark (DK)	Full-sibs from full-sibs	
3061			
3076			
3077			
3078			
3085			
3101			
3207	Japan (J)		Nishidake
3211			Mont Fuji
3212			Ina
3213			Kamikoshi
3217			Kamikoshi

RAPD markers

Total DNA was extracted from needles of the parental trees according to Greenwood et al. (1989) with modifications. Ten grams of needles were frozen in liquid N_2 and ground at 4°C in a Waring Blendor for 30 s with 200 ml 50 m*M* Tris (pH 8.0), 5 m*M* EDTA (ethylene diamine tetraacetic acid), 350 m*M* sorbitol, 0.1% bovine serum albumine, 10% polyethylene glycol 8000, 20 m*M* sodium bisulfite. The homogenate was filtered through a cheesecloth and pelleted by centrifugation at 2500 *g* for 5 min at 4°C. The pellet was resuspended in 15 ml 50 m*M* Tris (pH 8.0), 25 mM EDTA, 350 m*M* sorbitol, 700 m*M* NaCl, 0.1% cethyl trimethyl ammonium bromide, 1% sarkosyl, 20 m*M* sodium bisulfite and incubated for 10 min at 60°C. The solution was extracted with 20 ml of chloroform-isoamyl alcohol (24:1). The layers were separated by centrifugation at 13 000 *g* for 15 min at 20°C. The aqueous layer was re-extracted with 1 volume of chloroform-isoamyl alcohol (24:1) and then centrifuged at 13 000 *g* for 15 min at 20°C. The aqueous layer was removed, and the DNA was precipitated by adding a two-thirds volume of ice-cold isopropanol. The DNA was recovered and redissolved in 6 ml Tris-EDTA buffer (TE: 10 m*M* Tris (pH 8.0), 1 m*M* EDTA). Three milliliters of 7.5 *M* ammonium acetate was added to the solution, which was then placed in ice for 20 min and then centrifuged at 13 000 *g* for 30 min at 4°C. The DNA in the supernatant was precipitated by the addition of 6 ml of ice-cold isopropanol and washed with 76% ethanol for 30 min. After removal of the ethanol, the DNA was redissolved in 600 µl of TE.

The DNA of each parent was assayed for random amplified polymorphic DNA (RAPD) markers (Welsh and McClelland 1990; Williams et al. 1990). Amplification reactions (12 µl final volume) contained 1 ng DNA, 83 µ*M* each of dATP, dCTP, dGTP, dTTP, 167 n*M* of primer (Operon Technologies, Alameda, Calif.), 1X *Taq* polymerase buffer, 0.1 U *Taq* polymerase (Appligene). The reaction mixtures were overlaid with mineral oil. DNA amplifications were performed in a DNA thermocycler (MJ Research PTC100) with the following program: 35 cycles of 1 min at 94°C (denaturation), 1 min at 36°C (annealation) and 2 min at 72°C (extension). Amplification products were loaded on 1% agarose gels with ethidium bromide and run at 3.3 V/cm. Bands were scored on the gels.

Statistical analyses

As RAPD markers are dominant markers, presence and absence represent the two allelic forms at a locus. The presence and absence of bands were recorded for each parent, and the genetic similarity S between parents was calculated, using different indices:

- Jaccard's coefficient (Jaccard 1908): $S_J = \frac{a}{a+b+c}$
- Sokal and Michener's coefficient (Sokal and Michener 1958): $S_{SM} = \frac{a+d}{a+b+c+d}$
- Nei and Li's F statistics (Nei and Li 1979): $S_{NL} = \frac{2a}{e+f}$

assuming that:

- a is the number of bands shared by both parents P_1 and P_2
- b is the number of bands present in parent P_1, absent in parent P_2
- c is the number of bands present in parent P_2, absent in parent P_1
- d is the number of bands absent in both parents
- e is the total number of bands presented by parent P_1
- f is the total number of bands presented by parent P_2.

The genetic distance D was deduced from genetic similarity as $D = 1 - S$. A UPGMA dendrogram was performed on the genetic distance matrix using the computer program PHYLIP (Felsenstein 1993).

Correlations between family performance and genetic distance of the respective parents were calculated for each quantitative trait.

Significant variations among families were recorded for all of the measured traits (Pâques 1992). General hybridization abilities (GHA) and specific hybridization abilities (SHA) were determined for each quantitative trait (Pâques 1992). SHA values were significant for height at 2, 4 and 5 years, taper and stem straightness at 6 and 11 years. Correlations between these SHA values and genetic distances were calculated.

Results

RAPD data

Two hundred and ten primers were assayed on the 24 parental trees of the mating design. Of these, 84% produced amplification profiles. A single primer produced an average of 10 amplification products, and the size of the fragments ranged from 300 bp to 3 kbp. One hundred and eleven fragments with clear-cut polymorphism among the individuals tested were retained for this study, which represents 6.3% of the total number of fragments examined. The other bands were monomorphic for all individuals or were not clearly scorable. Thirteen fragments generated by 13 different primers differentiated completely the species (monomorphic present in all individuals of 1 species and monomorphic absent in all individuals of the other species). Ninety-eight fragments produced by 52 different primers showed intraspecific polymorphism. Most of these were polymorphic in 1 species and monomorphic in the other species, with only 8 fragments being polymorphic in both species. A total of 60 fragments polymorphic in European larches and 46 fragments polymorphic in Japanese larches were scored.

Genetic distance between parents

Genetic distances between trees from different species were higher than those between trees from the same species (Table 2). Since the three coefficients were highly correlated ($r \geq 0.87$), only Jaccard's distance was then considered.

Mean genetic distances within European larches (0.45) and within Japanese larches (0.39) were close when the related Japanese individuals were excluded. The related Japanese larches showed more genetic similarity among themselves than did the other Japanese parents.

Genetic distances between and within Tyrolian, Polish Sudetan and Czech Sudetan groups of European larch were very similar (0.44–0.48 for Jaccard's distance between the groups; 0.40–0.47 within the groups) and did not reflect any level of differentiation between these geographic origins. Even when focusing at the forest provenance level, genetic distances were not lower within stands (0.46–0.49 within the stands of Borowina and Olomouc) than between stands (0.38–0.53 between the stands of Borowina and Olomouc).

Table 2 Intraspecific and interspecific mean genetic distances between parents based on Jaccard (D_J), Sokal and Michener (D_{SM}) and Nei and Li (D_{NL}) coefficients

	Japanese larch			European larch	European -Japanese larch
Distance	Total	Related	Unrelated		
D_J	0.34	0.25	0.39	0.45	0.72
D_{SM}	0.19	0.12	0.21	0.24	0.48
D_{NL}	0.21	0.13	0.25	0.30	0.57

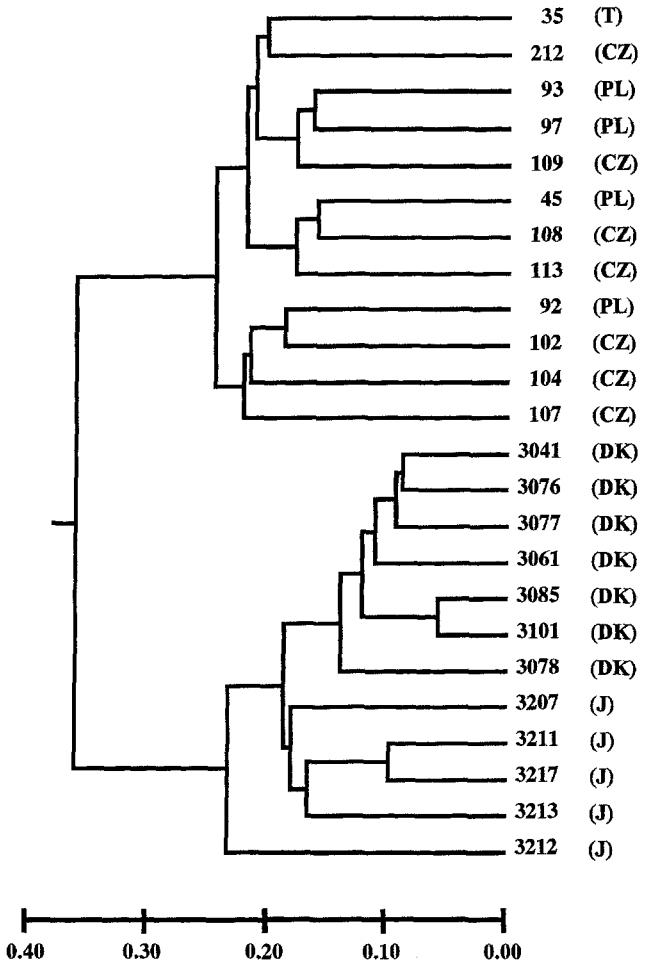


Fig. 1 UPGMA dendrogram based on Jaccard's genetic distance between the 24 parents of the mating design. Provenances: *T* Tyrol, *CZ* Czech Republic, *PL* Poland, *DK* Denmark, *J* Japan

The UPGMA dendrogram based on Jaccard's genetic distances (Fig. 1) showed two clusters: one comprising the European larches and the other the Japanese larches. The main portion of the genetic variability between individuals could be attributed to the genetic distance between species. The 7 related Japanese larches (DK) consisted of a single group. For European as for Japanese parents, trees originating from the same stand did not necessarily cluster together.

Genetic distance and hybrid performance

Significant correlations ($P > 0.999$) were found between genetic distances of the parents and performances of the hybrids for some quantitative traits (Table 3). Total height at 6, 8, 9, 10 and 11 years, 6th-year stem increment, B.H. girth and individual stem volume of the hybrids were positively correlated with the genetic distance between parents. A high level of heterozygosity appeared to be associated with a more vigorous growth, while branch insertion angle showed a negative correlation with genetic distance and decreased when genetic distance increased.

Table 3 Correlations between parental genetic distances based on Jaccard's coefficient and hybrid performances for quantitative traits

Character	Correlation
Total height at 2 years	0.17
Total height at 4 years	0.31
Total height at 5 years	0.43
Total height at 6 years	0.55***
Total height at 8 years	0.50***
Total height at 9 years	0.48***
Total height at 10 years	0.50***
Total height at 11 years	0.45***
5th-year stem increment	0.42
6th-year stem increment	0.62***
7th- and 8th-year stem increment	0.30
9th-year stem increment	0.28
10th-year stem increment	0.41
11th-year stem increment	0.30
Height increment between 6 and 11 years	0.42
B.H. girth at 11 years	0.52***
Individual stem volume at 11 years	0.52***
Taper at 11 years	−0.39
Number of branches on the 6th whorl	0.01
Diameter of branches on the 6th whorl	−0.41
Branch insertion angle on the 6th whorl	−0.45***
Stem straightness at 6 years	−0.06
Stem straightness at 11 years	−0.22
Wood specific gravity at 11 years	0.04

*** Significant at $P \geqslant 0.999$

The correlation between genetic distance and height evolved over time: it increased and reached a maximum value at 6 years (0.55) and then decreased but remained significant. However, the decomposition of height growth into annual stem increments showed a significant and positive correlation between genetic distance and 6th-year stem increment exclusively. The significant correlations found between genetic distance and height growth after 6 years represented in fact a remnant of the significant effect in the 6th year. Thus, heterozygosity seemed to be essentially effective on height growth between 5 and 6 years.

The distribution of families according to height at 6 years and genetic distance between parents exhibited a significant correlation (Fig. 2). The general tendency was an increase of total height at 6 years when the genetic distance between the parents increased. Some groups, including at least 6 families issued from the same parent, were individualized. Each group showed a small range of variability in genetic distances, and no significant correlation was established at the group level. Family groups including many crosses between genetically distant parents performed better than the others.

Genetic distance and specific hybridization ability (SHA)

Correlations between genetic distance and SHA for height at 4 and 5 years, taper and stem straightness at 6

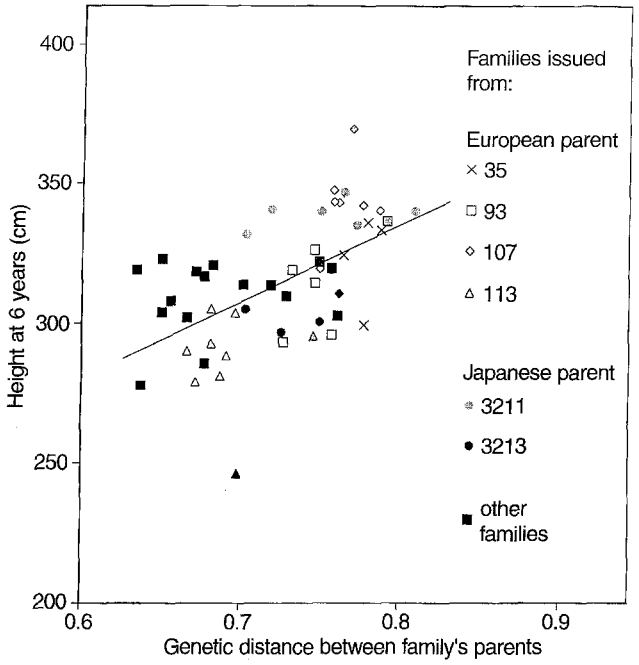
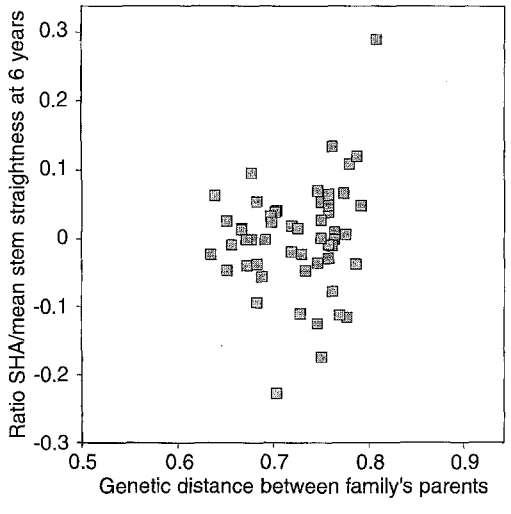


Fig. 2 Distribution of families according to height at 6 years and Jaccard's genetic distance between parents ($r = 0.55$***). Sets of families issued from a same parent are grouped. Combinations of shape and intensity of symbols are families issued from two parents simultaneously represented

and 11 years were not significant. However, a significant correlation ($r = 0.32$, $P > 0.95$) was found between SHAs in absolute value for stem straightness at 6 years and genetic distances.

For stem straightness at 6 years, the variation in SHA was greatest for the highest genetic distances (Fig. 3): the strongest SHA effects, whether they were positive or negative, were observed when the genetic distance between parents was high. The same trend was noticed for stem straightness at 11 years. No such observation could be made for height and taper.

Fig. 3 Distribution of families according to the ratio SHA on mean stem straightness at 6 years, and Jaccard's genetic distance between parents. A significant correlation ($r = 0.32$) was noticed only with SHA in absolute value



Discussion

Genetic distances were greater between species than within species and could be used to genetically differentiate between *Larix decidua* and *L. kaempferi*: 12% of the polymorphic fragments allowed the identification of species on the basis of the sample assessed. Most of the other fragments also revealed differences between the larch species since polymorphism was observed in 1 species only. However, Nkongolo and Klimaszewska (1995) have suggested a close phylogenetic relationship between European and Japanese larch, firstly because their natural or artificial hybridization occurs easily and secondly because of a high sequence homology between these two species. Likewise, Ennos and Qian (1994) have observed that very few enzymatic loci (SKDH and NDH) differentiate the two species. Conversely, on the basis of features of the seed cones, fossil records and past and present distribution patterns, LePage and Basinger (1995) suggested that the European and the Japanese larch belong to distinct groups that diverged early (Eocene time). Shiraishi et al. (unpublished) have detected RAPD markers that differentiate European and Japanese larches; this corroborates our results. The type of markers used may be decisive in determining the relationships between species. Indeed, the RAPD technique allows the amplification of any site of the genome, especially in non-coding regions that are more likely to accumulate mutations and to generate greater polymorphism between individuals or between species than coding regions such as isozyme loci. Finally, the 2 species of larch with disjoined range areas could be genetically differentiated in spite of the few species-related alleles detected by isozyme analyses.

We revealed a significant positive correlation between genetic distances of parents and performances of the hybrids for growth characters: total height, 6th-year stem increment, B.H. girth and stem volume. All these traits were highly correlated. This suggests that high levels of heterozygosity provide advantages to hybrid trees for these traits. This result is original since attempts to predict hybrid performance from parental genetic distance have often failed, as revealed by the literature. A decrease in progeny performance was usually observed when crosses involved close – generally related – parents, but only a slight increase in progeny performance was obtained when the genetic distance between the parents increased. For crop species, the predictive value of molecular-based genetic distance is often restricted to crosses between parents from the same heterotic group and not extended to crosses between different heterotic groups (e.g. oat: Moser and Lee 1994; maize; Melchinger et al. 1990a, b; Godshalk et al. 1990; Boppenmaier et al. 1993). Likewise, in studies involving forest trees, a lack of

association between genetic distance and various traits has generally been observed (*Pinus attenuata*: Strauss 1986; *Eucalyptus globulus*: Vaillancourt et al. 1995); this also holds for studies with interspecific hybrids (*Eucalyptus urophylla* × *E. grandis*: Verhaegen et al. 1995). Many hypothesis have been put forward to explain this lack of relationship. Moser and Lee (1994) suggested that markers involved in the estimation of genetic distance but unlinked to implicated quantitative trait loci (QTLs) might reduce the correlation and fail to predict heterosis. Thus, an alternative would be the preselection of specific markers linked to loci that affect the quantitative trait (Melchinger et al. 1990a, b). According to Charcosset and Essioux (1994), the linkage disequilibrium between a marker and a QTL should differ between groups, more especially between species, and prediction of hybrid performance based on marker heterozygosity would not be effective. However, in the present study on larch hybrids, the RAPD markers we used also detected some QTLs for height growth, stem straightness and wood quality (Arcade et al. 1995), and no obvious change of linkage disequilibrium between marker and QTL was detected within species. In this condition, a relationship between the genetic distance of the parents and progeny performance could be obtained. Inadequate genome coverage and different levels of dominance among hybrids are other reasons suggested for the low correlation between genetic distance and hybrid performance (Melchinger et al. 1990b; Bernardo 1992).

The sample size of the parental lines and progenies assessed might be another explanation as to why genetic distance failed in the literature to have a predictive value. When the crosses involve few parents, the genetic distance variation is restricted, especially in interspecific hybridization where interspecific distance represents the main part of the total distance. Intraspecific distance remains limited and does not influence to any great extent the total variation. Indeed, as shown in our results (Fig. 2), no correlation could be observed within groups of families issued from a single parent. These groups displayed a narrow range of genetic distances, since the greater part of the distance was attributable to the differentiation between species, while intraspecific polymorphism remained limited. Finally, a positive correlation between genetic distance and total height could be observed thanks to parent multiplicity, which allowed the range of variation of the genetic distance to broaden. Likewise, increasing the number of lines, progenies and markers analyzed has resulted in significant relationships between parental genetic distance and hybrid performance (Tsaftaris 1995). Thus, Smith et al. (1990) found a significant relation between RFLP diversity and hybrid grain yield.

No correlation between genetic distance and other traits such as taper, number or diameter of branches, stem straightness or wood specific gravity was noticed; this implies that the general level of heterozygosity had no direct effect on these characters. Such traits are likely controlled by a few genes with major effects on the phenotype, as has been shown for wood specific gravity in loblolly pine (Neale and Williams 1991; Neale et al. 1992). In this context, as pointed out by Leonardi et al. (1991), predicting performance from genetic distance based on markers would be ineffective if heterosis was determined by only a few QTLs. The identification of these individual loci that code for quantitative traits would be a more suitable approach to an understanding of their way of expression and to predicting hybrid performance. The only conclusion that can be drawn is that there is a heterozygote advantage for growth traits, which suggests that this kind of character is under polygenic control.

Our results have shown an evolution in the correlation between genetic distance and height with age; it reached a maximum value at 6 years. However, there was no significant correlation between genetic distance and SHAs for height from and after age 4, or any tendency of a correlation. This lack of correlation suggested that height is essentially controlled by additive effects. In this case, the influence of genetic distance would not have resulted from the cumulative effects of single-locus heterozygosity but mainly from the accumulation of different and favorable alleles provided by parents. It implies that the more distant the parents, the higher the probability to associate different and favorable alleles in the progeny. Pâques (1992) pointed out that the SHA effects for height turned from being significant at age 2 to being non-significant at age 6, while general hybridization ability effects for both parents were increasing during this period. Moreover, the markers linked to QTLs involved in height up to 6 years were no longer detected after this stage, while other QTLs for height were found from this age till the last measures at 11 years (Arcade et al. 1995). Thus, the 6th year represented a turning point, and changes in genetic expression occurred at this particular step.

No significant correlation could be established between genetic distances and SHAs for stem straightness. This result obscures the fact that the correlation between genetic distances and SHA in absolute values for stem straightness became significant, which is reflected by the observation that the most heterozygous families exhibited the strongest positive or negative SHA values. Although its effect was statistically non-significant, genetic distance tended to be involved in a part of dominance or epistasis of stem straightness variation. In this case, a higher genetic distance between parents increased the importance of favorable or unfavorable effects, depending on the combination and interaction of alleles at the same locus or at different loci (epistasis). This resulted in a lack of correlation between genetic distance and stem straightness because of variations in positive and negative ways with genetic distances.

In conclusion, the analysis of the relationships between genetic distances of the parents and hybrid performances in a factorial mating design using RAPD markers has provided us with important results. First,

intraspecific and interspecific genetic distances were assessed on the basis of the polymorphism of numerous loci using RAPD. We believe that other techniques would not have been likely to produce as many markers within the same amount of time. This observation is in agreement with Jain et al. (1994) and Vaillancourt et al. (1995) who have already used this technique for the same purpose. Likewise, Dos Santos et al. (1994) have compared the relative efficiency of RAPD and RFLP genetic markers in establishing genetic similarity among genotypes; they concluded that both methods were efficient but had a preference for the RAPD technique because it was more simple and less costly. However, as shown by Quiros et al. (1995), it should be kept in mind that bands of similar size are not necessarily homologous and that their sequence homology should be checked either by hybridization or sequencing. We have tested sequence homology for the single fragment polymorphic in both larch species: Southern hybridization confirmed the homology. RAPD markers are, however, dominant markers and an imprecision always remains regarding the genotype of the parents with respect to homozygosity or heterozygosity for the marker allele.

Secondly, we have shown a significant and positive correlation between genetic distance of the parents and growth performance of the hybrid. This result represents a potential selection criterion in a breeding program if growth is the requested character. Crosses should then be carried out in order to ensure a maximal genetic distance between parents.

Concerning other traits such as stem straightness, branch angle or wood density, investigations should focus on the identification of markers linked to QTLs involved in the expression of the character and could lead to a marker-assisted selection scheme in the larch breeding program.

Acknowledgements The authors are grateful to the staff of the Station d'Amélioration des Arbres Forestiers and especially to the technicians for their support in this study and the measurements of trees. They would like to thank M. Greenwood and K. Hutchison for their encouragement in this study.

References

Arcade A, Faivre Rampant P, Le Guerroué B, Pâques LE, Prat D (1995) Quantitative traits and genetic markers: analysis of a factorial mating design in larch. In: Boerjan W, Ahuja MR, Neale DB (eds) Somatic cell genetics and molecular genetics of trees. Kluwer Academic Publ, Dordrecht (in press)

Bastien JC, Keller R (1980) Intérêts comparés du mélèze hybride (*Larix* × *eurolepis* Henry) avec les deux espèces parentes. Rev For Fr 32:521–530

Bernardo R (1992) Relationship between single-cross performance and molecular marker heterozygosity. Theor Appl Genet 83:628–634

Boppenmaier J, Melchinger AE, Seitz G, Geiger HH, Herrmann RG (1993) Genetic diversity for RFLPs in European maize inbreds. III. Performance of crosses within versus between heterotic groups for grain traits. Plant Breed 111:217–226

Charcosset A, Essioux L (1994) The effect of population structure on the relationship between heterosis and heterozygosity at marker loci. Theor Appl Genet 89:336–343

Dos Santos JB, Nienhuis J, Skroch P, Tivang J, Slocum MK (1994) Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L. genotypes. Theor Appl Genet 87:909–915

East EM (1936) Heterosis. Genetics 21:375–397

Ennos RA, Qian T (1994) Monitoring the output of a hybrid larch seed orchard using isozyme markers. Forestry 67:63–73

Felsenstein (1993) PHYLIP (Phylogeny Inference Package) version 3.5c. Distributed by the author. Department of Genetics, University of Washington, Seattle, Wash.

Gallais A (1989) Théorie de la sélection en amélioration des plantes. Masson, Paris

Godshalk EB, Lee M, Lamkey KR (1990) Relationships of restriction fragment length polymorphism to single-cross hybrid performance of maize. Theor Appl Genet 80:273–280

Greenwood MS, Hopper CA, Hutchison KW (1989) Maturation in larch. 1. Effect of age on shoot growth, foliar characteristics and DNA methylation. Plant Physiol 90:406–412

Jaccard P (1908) Nouvelles recherches sur la distribution florale. Bull Soc Vaud Sci Nat 44:223–270

Jain A, Bhatia S, Banga SS, Prakash S, Lakshmikumaran M (1994) Potential use of random amplified polymorphic DNA (RAPD) technique to study the genetic diversity in Indian mustard (*Brassica juncea*) and its relationship to heterosis. Theor Appl Genet 88:116–122

Jones DF (1917) Dominance of linked factors as a means of accounting for heterosis. Proc Natl Acad Sci USA 3:310–317

Leonardi A, Damerval C, Hebert Y, Gallais A, de Vienne D (1991) Association of protein amount polymorphism (PAP) among maize lines with performances of their hybrids. Theor Appl Genet 82: 552–560

LePage BA, Basinger JF (1995) The evolutionary history of the genus *Larix* (*Pinaceae*). Symposium on ecology and management of *Larix* forests: a look ahead. USDA, Forest Service, Intermountain Research Station, General Technical Report GTR-INT-319, pp 19–29

Melchinger AE, Lee M, Lamkey KR, Hallauer AR, Woodman WL (1990a) Genetic diversity for restriction fragment length polymorphisms and heterosis for two diallel sets of maize inbreds. Theor Appl Genet 80:488–496

Melchinger AE, Lee M, Lamkey KR, Woodman WL (1990b) Genetic diversity for restriction fragment length polymorphisms: relation to estimated genetic effects in maize inbreds. Crop Sci 30:1033–1040

Moser H, Lee M (1994) RFLP variation and genealogical distance, multivariate distance, heterosis, and genetic variance in oats. Theor Appl Genet 87:947–956

Neale DB, Williams CG (1991) Restriction fragment length polymorphism mapping in conifers and applications to forest genetics and tree improvement. Can J For Res 21:545–554

Neale DB, Devey ME, Jermstad KD, Ahuja MR, Alosi MC, Marshall KA (1992) Use of DNA markers in forest tree improvement research. New For 6:391–407

Nei M, Li W (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Nkongolo KK, Klimaszewska K (1995) Cytological and molecular relationships between *Larix decidua*, *L. leptolepis* and *Larix* × *eurolepis*: identification of species-specific chromosomes and synchronisation of mitotic cells. Theor Appl Genet 90:827–834

Pâques LE (1989) A critical review of larch hybridization and its incidence on breeding strategies. Ann Sci For 46:141–153

Pâques LE (1992) First evaluation of genetic parameters in a factorial mating design with hybrid larch (*Larix decidua* × *Larix kaempferi*). In: Weisgerber H (ed) Results and future trends in larch breeding on the basis of provenance research. IUFRO Centennial Meeting of the IUFRO Working Party, S2.02–07, pp 136–145

Quiros CF, This P, Laudie M, Benet A, Chevre AM, Delseny M (1995) Analysis of a set of RAPD markers by hybridization and sequencing in *Brassica*: a note of caution. Plant Cell Rep 14:630–634

Shull GH (1914) Duplicate genes for capsule form in *Bursa capsula pastoris*. J Indian Abstr Vererb 12: 97–149

Shull GH (1952) Beginnings of the heterosis concept. In: Gowen JW (ed) Heterosis. Iowa State College Press, Ames pp 14–48

Smith OS, Smith JSC, Bowen SL, Tenborg RA, Wall SJ (1990) Similarities among a group of elite maize inbreds as measured by pedigree, F_1 grain yield, grain yield heterosis and RFLPs. Theor Appl Genet 80:833–840

Sokal RR, Michener CD (1958) A statistical method for evaluating systematic relationships. Univ Kan Sci Bull 38:1409–1438

Strauss SH (1986) Heterosis at allozyme loci under inbreeding and crossbreeding in *Pinus attenuata*. Genetics 113:115–134

Tsaftaris SA (1995) Molecular aspects of heterosis in plants. Physiol Plant 94:362–370

Vaillancourt RE, Potts BM, Watson M, Volker PW, Hodge GR, Reid JB, West AK (1995) Detection and prediction of heterosis in *Eucalyptus globulus*. For Genet 2:11–19

Verhaegen D, Kremer A, Vigneron P (1995) Relationships between heterosis and molecular polymorphism in interspecific crosses of *Eucalyptus urophylla* × *E. grandis*. In: Potts BM, Borralho NMG, Reid RJ, Cromer RN, Tibbits WN, Raymond CA (eds) Eucalypt plantations: improving fibre yield and quality. (Proc CRCTHF-IUFRO Conf.) CRC for temperate hardwood forestry, Hobart, pp 434–437

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535