Original article

Estimation of genetic parameters in eastern cottonwood (*Populus deltoïdes* Bartr.). Consequence for the breeding strategy

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Summary — Poplars bred for the future by INRA include two North American species : eastern and black cottonwood and the European black poplar. The breeding strategy, now under discussion, needs to be based on the biological and genetic properties of the species. The present study aims to estimate the genetic parameters in the eastern cottonwood (*Populus deltoïdes* Bartr.). A factorial crossing design involving 6 females and 6 males was carried out between 1971 and 1980. Observations on copies of the parents and of their offsprings were made in 1985 and 1986 in a design laid out in the INRA, experimental nursery near Orléans, France. Observations concerned phenology, growth and wood quality. Firstly, the results showed a significant variability of most traits, whether among parents, among families or among cloned full-sibs; but this variability was greater among parents than among families which can be explained by the assumption that allelic fixation occurred in the natural stands the parents originated from. This allelic fixation was high for phenologic traits, moderate for growth traits and absent for wood quality traits. Secondly, heritabilities were estimated. Broad sense heritabilities were generally high and significant for all types of traits. Narrow sense heritabilities estimated from parent-offspring regression are extremely low for wood quality traits. Thirdly, additive genetic correlations between traits were estimated. Significant values were found between phenology and growth traits and between growth termination and wood basic density, which means that fast growing genotypes should be looked for among early starters rather than among late growers. The latter will tend to produce wood with a low basic density which is not suitable for poplar.

Populus deltoïdes – variability – allelic fixation – heritability – genetic correlation – phenology – growth – wood quality

Résumé — **Estimation des paramètres génétiques chez le peuplier noir américain (*Populus deltoïdes* Bartr.). Conséquence pour la stratégie d'amélioration.** *Le programme d'amélioration des peupliers, en France, est basé sur 3 espèces principales. Deux sont nord-américaines, un peuplier noir,* Populus deltoïdes *Bartr. et un peuplier baumier,* Populus trichocarpa *Torr. et Gray. La troisième est européenne,* Populus nigra *L. Le choix de la stratégie d'amélioration est basé sur une connaissance biologique et génétique des espèces. Une publication récente des mêmes auteurs (Pichot & Teissier du Cros, 1988) a apporté une information génétique sur P. Nigra obtenue grâce à*

l'analyse de descendances issues d'un plan de croisement factoriel 4 x 6. La présente étude se propose d'apporter une information analogue sur P. deltoïdes.

Jusqu'à maintenant, chez les peupliers, l'information génétique sur les espèces a été obtenue dans des plantations comparatives de clones, ou mieux, grâce à des comparaisons de descendances maternelles issues de pollinisation libre. Les paramètres génétiques sont estimés ici au moyen d'un plan de croisement factoriel à partir de 6 mères et de 6 pères d'origine américaine et ayant fait partie de la collection de Vineuil, près de Blois, France (Tableau II). Le dispositif expérimental installé dans la pépinière du centre INRA d'Orléans, France, comprenait, dans chacune de ses 3 répétitions, 3 copies végétatives de 10 des 12 parents et 1 copie d'en général 30 descendants pour chacune des 33 familles qui ont été obtenues après 10 années de croisements (Tableau I). Dans ce dispositif cohabitaient les parents et leurs descendants dans des conditions physiologiques et environnementales en tous points comparables.

Les observations ont porté sur des caractères phénologiques – débourrement végétatif, arrêt de croissance, défeuillaison, – sur des caractères de croissance – hauteur en première et deuxième année, diamètre – et sur des caractères liés à la qualité du bois – angle des branches et densité du bois. Pour éviter une méthode destructive, la densité du bois a été mesurée sur les branches apparues en deuxième année, elle présente une corrélation de 0,55 avec la densité du bois des tiges.

Les analyses ont porté sur les clones parentaux, sur les descendances (demi-frères et pleins frères) et sur les pleins frères clonés. Elles ont d'abord permis de connaître la variabilité des caractères et le niveau de signification de cette variabilité (Tableaux V et VI). Mais une observation détaillée des résultats, confirmée par la valeur, de rapports de coefficients de variation et de variances additives entre familles de demi-frères et descendants clorés (Tableaux VII et VIII), a montré que les parents du plan de croisement avaient une variabilité plus élevée que la moyenne de leurs descendances. Ce fait peut être attribué à un taux de fixation allélique, relativement élevé pour les caractères phénologiques, moyen pour les caractères de croissance et faible pour les caractères liés à la qualité du bois (Tableau IX).

La deuxième phase de l'analyse a porté sur l'estimation des héritabilités au sens large sur les parents et les copies des descendants et sur les héritabilités au sens strict, tenant compte ou non des coefficients de fixation allélique, estimées à partir des familles et au moyen des régressions parents-descendants (Tableau X). Comme on peut s'y attendre, compte tenu des résultats obtenus jusqu'alors, des valeurs en général élevées sont trouvées pour les critères phénologiques, des valeurs moyennes pour les caractères de croissance et de qualité du bois. Une particularité importante apparaît, c'est la distorsion entre les valeurs d'héritabilité de la densité du bois suivant le mode d'estimation.

La troisième phase de l'analyse porte sur les corrélations génétiques entre caractères (Tableau XI). Les corrélations sont significatives entre caractères de croissance. De même, des corrélations élevées sont rencontrées entre caractères phénologiques et croissance, un débourrement précoce et un arrêt de croissance tardif sont en corrélation positive avec la croissance en hauteur. Mais plus difficile à prendre en compte est la corrélation entre l'arrêt de croissance et la densité du bois qui conduirait à diminuer la densité de génotypes à arrêt de croissance tardif, ce qui n'est pas concevable chez les peupliers. L'effet de cette dernière corrélation est, bien entendu, à nuancer compte tenu du fait qu'elle a été obtenue à partir d'une densité du bois de branche. Prises dans leur ensemble, ces corrélations conduiraient à choisir des génotypes à débourrement précoce (si cela n'induit pas une sensibilité aux gelées tardives), plutôt qu'à arrêt de croissance tardif.

La quatrième phase de l'analyse est la simulation de l'évolution des paramètres et de leur niveau de signification lorsqu'on réduit le plan de croisement pour tenter d'optimiser le coût d'une telle opération. Cette simulation montre que l'on peut réduire à 10 ou 15, au lieu de 30, le nombre de descendants par famille, mais que, au contraire, le nombre de 6 parents par sexe semble une limite inférieure (Tableaux XII et XIII).

Les conséquences de ces résultats sur la stratégie d'amélioration sont nombreuses. Dans un schéma d'amélioration faisant intervenir plusieurs espèces, une phase intraspécifique est indispensable. Elle débute par le rassemblement d'un matériel de base représentatif de l'aire de l'espèce. Elle se poursuit par une sélection clonale pour laquelle la connaissance génétique des espèces est indispensable. Cette étude montre que les héritabilités au sens large sont élevées pour tous les caractères étudiés, et que les corrélations génétiques entre caractères sont significatives, notamment pour ce qui concerne la liaison entre les critères phénologiques et la croissance, d'une part,

et l'arrêt de croissance et la densité du bois, d'autre part. La sélection clonale devient ensuite sélection parentale pour laquelle la connaissance des héritabilités permettra de déterminer le gain que l'on pourra obtenir d'une génération à l'autre. D'une manière générale, dans notre échantillon, l'additivité est plus élevée que la dominance. La sélection parentale conduit à un gain génétique chez les descendants. Ce gain est marqué pour les critères phénologiques, notamment le débourrement; il est moyen pour les critères de croissance, mais celle-ci, rappelons-le, faible pour les critères de qualité du bois.

Les résultats étant basés sur des observations de deux années de pépinière, nécessiteront confirmation, grâce au transfert en peupleraie des parents et descendants de ce plan de croisement. Ce transfert a eu lieu au printemps 1988. Un certain nombre d'années sera donc nécessaire avant d'entreprendre une nouvelle analyse de ce plan de croisement.

Populus deltoïdes – *variabilité – fixation allélique – héritabilité – corrélation génétique – phénologie – croissance – qualité du bois*

INTRODUCTION

The role of poplar in French wood production has recently been recalled by Pichot & Teissier du Cros, 1988. Emphasis has also been placed on the main selection criteria which are connected to the biology of poplar and to its culture. The need for founding poplar culture on a broad genetic basis has also been stressed.

As in several European countries, poplars bred for the future by INRA, France, include the European cottonwood, *Populus nigra* L. and two North American species, the black cottonwood (*P. trichocarpa* Torr. and Gray) and the eastern cottonwood (*P. deltoïdes* Bartr.).

The objective of the breeding programme is to provide poplar growers with a permanently renewed set of clones so as to avoid a narrow genetic basis (Teissier du Cros, 1984) and cope with new needs such as improved propagation capacities, resistance to unexpected diseases or the ability to produce a woody raw material adapted to new trends in economy and industry. The main stages of the programme, which was initiated by INRA in the mid-1960s, are the collection of base populations of the species, followed by provenance and clonal comparative tests, followed by the selection of clones for direct use in poplar growing or of parents for the next breeding generation.

A breeding strategy has now to be defined. The definition will be based on the biological and genetic properties of the species.

The present paper aims to estimate the genetic parameters in a population of *P. deltoïdes*. The results will be compared with information in the literature. Proposals will be made for the breeding strategy of that species and for its role in the general improvement programme run by INRA.

MATERIALS AND METHODS

Crossing design and nursery test

Genetic parameters of poplars have generally been estimated in trials involving clones or open pollinated progenies of trees chosen randomly from natural stands (Avanzo, 1974;

Table I. Mating design and year of crossing (in brackets number of progeny per family in the nursery test when not 30)

Female	Male 493	1656	36-50-1	MRP1	MRP6	TR
533	1977	1977	74, 78, 79	71, 78, 79	1977	1979
17-51-2	77, 80	1977	79, 80	1977	73, 79	1980
33-51-1	78, 79	1979 (29)	1974	78, 79	78, 79	79, 80
MRP 5B	78, 79	1980 (11)	78, 79	79, 80 (10)	1978	1979
484	1977	79, 80 (14)	1978	73, 80	1977	79, 80
21-50-1	1980 (2)	(0)	1980 (26)	(0)	(0)	1980

Table II. Parent material used (1)

Number	Sex	Code	Name	Geographic origin
1	Male	493	Scott Pauley 493	Erie Cy (New York)
2	"	1656	1656	(Illinois)
3	"	36-50-1	36-50-1	Jackson Cy (Illinois)
4	"	MRP 1	Murphysboro 1	Murphysboro (Illinois)
5	"	MRP 6	6	–
6	"	TR	Tittabawasse River	(Michigan)
7	Female	533	Scott Pauley 533	Sandusky Cy (Ohio)
8	"	17-51-2	17-51-2	Richland Cy (N. Dakota)
9	"	33-50-1		Pedria Cy (Illinois)
10	"	MRP 5B	Murphysboro 5B	Murphysboro (Illinois)
11	"	484	Scott Pauley 484	Erie Cy (New York)
12	"	21-50-1	21-50-1	Washington Cy (Missouri)

(1) Sent by Dr Scott Pauley to Pr Pourtet in the late 1940s.

Herpka, 1979; Olson *et al.*, 1985; Wilcox & Farmer, 1967). We propose a complementary parameter estimation in an artificial population. A factorial crossing design with 6 females and 6 males (poplars are dioecious) was made. It took 10 years to be completed but 3 combinations appeared to be incompatible (Table I). Copies of all parents, which originated from the United States (Table II), had been gathered at the beginning of the 1950s at the Vineuil populetum near Blois, in the Loire river valley, France. For the mating design, flower-bud bearing branches were collected from the tree canopy. Pollen was extracted in February from cut branches dipped in fresh water. It was air-dried and stored at 2°C in small vials until pollination. Female branches were bottle-grafted in a greenhouse until flower receptivity and pollination. Pollination usually took place in March. During receptivity and pollination, the grafted branches were isolated in individual transparent plastic cages. For maturation, the branches were kept in the greenhouse at a minimum temperature of 20 °C. The branch base, dipped in water (bottle), was frequently shortened with hand shears, in order to allow sufficient water suction. Seed maturation took 2 to 3 months according to the years and crossings. Seed was released from its cotton by slight rubbing in a sieve with 2 mm mesh. According to the years it was sown immediately after cleaning or stored at cold temperature under partial vacuum in sealed vials until sowing time (Muller & Teissier du Cros, 1982).

The seedlings were transplanted in nursery stool beds after one vegetation period.

The nursery design was laid out in the spring of 1985 in the INRA experimental nursery, Orléans, France. The trial included 3 complete replications of the 33 families obtained. Each replication consisted of one vegetative copy of

a maximum of 30 sibs per family (Table I shows the exact number of sibs present in the nursery trial for each combination). The trial also included copies of the parents (3 copies per replication). Unfortunately 2 of the 12 parents (1656 and TR) could not be propagated and therefore were missing from the experiment.

In order to homogenize the planting material which had been stored in stool beds for periods ranging from 2 to 12 years, each sib was vegetatively propagated in 1984. Cuttings for the trial were therefore collected from this second generation stool bed. Five cuttings of each sib (and 12 of each parent) were prepared and planted in the test : 3 in the three replications and 2 in border rows for possible refills after one growing season (respectively 9 and 3 for the parents).

Cuttings were planted in May 1985 under black polythene soil covering. This technique was used to lower soil water evaporation, to increase soil temperature, to reduce herbicide treatments and therefore to increase the rooting ability of the cuttings. It worked efficiently. Planting distance was 1.2 x 0.5 m.

Trees were grown for 2 years under nursery conditions and were irrigated during both growing seasons. Refills were made at the end of the first growing season. Such trees were not included in the analysis.

Observations

These concerned :

Phenologic traits

Budburst was measured at the beginning of the second growing season (1986). Four surveys were made between 28 April and 5 May. At each survey, each tree received a mark according to the following scale :

0 : dormant bud

1 : dormant terminal bud; leaf tips (1 to 5 millimeters) appearing on at least one lateral bud

2 : leaves of terminal bud appearing but closely stuck together. Bud size from 5 to 10 millimeters (mm)

3 : leaf tips of terminal bud separated. Bud size from 10 to 18 mm; leaves of lateral buds stuck together

4 : leaf tips of lateral buds separated

5 : external leaves of lateral buds starting to separate, leaves still folded; shoot length from 2.5 to 3.5 centimeters (cm)

6 : ratio of lateral buds with 2 unfolded leaves below 0.5; shoot length from 3.5 to 4.5 cm

7 : ratio of lateral buds with 2 unfolded leaves between 0.5 and 1.0. Shoot length from 4 to 4.5 cm

8 : all lateral buds have at least 2 unfolded leaves. Shoot length from 4 to 4.5 cm

9 : shoot length over 5 cm.

For analysis, budburst (BB) was the sum of the four marks given to each tree, but to give equal importance to each mark (M_i), their sum has been weighted by their own standard error (σ_i^2). Therefore budburst became :

$$BB = \frac{\sigma_4}{\sigma_1} M_1 + \frac{\sigma_4}{\sigma_2} M_2 + \frac{\sigma_4}{\sigma_3} M_3 + M_4$$

(1 to 4 refer to the rank of the survey).

Tests have proved BB statistical normality.

Growth termination was measured during the first and the second growing season. It is defined as a ratio :

$$\frac{\text{Terminal shoot elongation between August and October}}{\text{annual shoot length in October}}$$

The August observation was made when all trees were still elongating (20 Aug.). The October observation was made when elongation had stopped for all trees (22 Oct.) This ratio is highly related to growth termination.

Leaf fall is the ratio of terminal shoot defoliated length on total shoot length on 22 October.

Vigour

Observations concerned total height in year 1 and in year 2, shoot growth of year 2 and stem diameter at 1 meter height, on 22 July of the second growing season.

Wood characteristics

Branch angle is of great importance in poplars because, for a given branch diameter, the scar surface after pruning is smaller when the branch angle is larger. It has also been noticed by Teissier du Cros (1969) that the more horizontal a branch is, the thinner it tends to be (a strong clonal and environmental correlation). Furthermore, we have observed a high juvenile-mature correlation for this trait between 2-year-old and mature poplar clones. Branch angle was measured on 24 July, 1986 on one branch per tree chosen at a constant distance beneath the limit of 1985 and 1986 shoots.

Density is a major characteristic of wood. It is strongly related to its mechanical resistance. Basic density, which is usually used as an internationally reliable reference, is the ratio of the oven dry weight and of the water saturated volume. Polge (1963) adapted Keyworth's measurement technique by taking into account the oven dry weigh (ODW) and the water saturated weight (WSW).

$$\text{Basic density}: \quad 1/\left(\frac{ODV}{WSW} - 0.347\right)$$

where $0.347 = 1 - (1/1.53)$, in which 1.53 is the density of the ligneous substance.

Furthermore Nepveu et al. (1978) found a strong clonal juvenile-mature correlation of wood basic density between one-year-old stem wood and mature wood of Populus nigra and Populus euramericana. But our experiment could not be destroyed to measure stem basic density; therefore it was replaced by the measurement of the branch basic density after finding a 0.55 correlation (confidence interval : 0.30 and 0.79) between the density of one-year-old branch wood and stem wood (sample of 30 trees cut in border rows of our experiment). Measurements were made on 6-cm long branch samples.

All the observations concerned all trees of the 3 replications.

Variance analysis

All data were processed by a multivariate variance analysis (Anvarm) according to the following models for each trait (Bachacou et al., 1981, Tables III and IV).

As Anvarm does not allow a nested structure within interaction (clones in full-sib families), the second statistical model has to be split into two sub-models.

(1) $X_{ijkl} = \mu + R_i + M_j + F_k + (M \times F)_{jk} + e_{ijkl}$

(2) $X_{ijk} = \mu + FS_i + C_{ij} + e_{ijk}$

Table III. First statistical model : parent clones

Value expression	X_{ijk}	$=$	μ	$+$	R_i	$+$	C_j	$+$	e_{ijk}
FACTOR	General		Mean		Replicate		Clone		Error
EFFECT					Fixed (F)		Random (R)		(R)
VARIANCE	σ_p^2						σ_c^2		σ_w^2

Table IV. Second statistical model : cloned-sibs

Value expression	X_{ijk} =	μ	+	R_i	+	M_j	+	F_k	+	$(M \times F)_{jk}$	+	C_{jkl}	+	e_{ijklm}
FACTOR	General	Mean		Replicate	Male		Female		Interaction		Cloned sibs		Error
EFFECT				(F)	(R)		(R)		(R)		(R)		(R)
VARIANCE					σ_M^2		σ_F^2		σ_{MxF}^2		σ_c^2		σ_w^2

X_{ijk} is derived from X_{ijkl} after adjustment on replication, male and female effects;

FS = full-sibs regardless of their pedigree;

C = ramets of full-sibs (clones) regardless of the 3 replications.

First genetic model : parent clone

$$X''_{ijk} = G_{ijk} + E_{ijk}$$

where X''_{ijk} = phenotypic value adjusted to replication effect;

G_{ijk} = genotypic effect;

E_{ijk} = environmental effect.

Second genetic model : cloned-sibs

$$X''_{ijkl} = A_{ijkl} + D_{ijkl} + E_{ijkl}$$

where : X''_{ijkl} = phenotypic value adjusted to replication effect;
A_{ijkl} = additive effect;
D_{ijkl} = dominance effect (epistatic effect is ignored);
E_{ijkl} = environmental effect.

Estimation of genetic parameters

The first statistical model allows one to estimate the genotypic variance, broad sense heritability $h^2_{bs} = \sigma^2_{bs} / (\sigma^2_c + \sigma^2_w)$ and clonal correlation. The second statistical model gives estimates of additive and dominance variance, broad sense and narrow sense heritability and combined genetic variance. Mid-parent/full-sib covariance gives one more estimate of narrow sense heritability.

In a factorial mating design, in which sibs have been cloned there are several possibilities to estimate the additive genetic variance (σ^2_A) :

$$\sigma^2_A = 4\,\sigma^2_M \qquad (1)$$
$$\sigma^2_A = 4\,\sigma^2_F \qquad (2)$$

$$\sigma^2_A = 4\,\frac{(m-1)\,\sigma^2_M + (f-1)\,\sigma^2_F}{m+f-2}$$

where σ^2_M and σ^2_F respectively are the variance of the half-sib families of the m male parents and of the half-sib families of the f female parents. The third estimate is called the combined additive genetic variance. It is used in the estimation of the narrow and broad sense heritability (Table X) calculated in the progeny test. Due to the balance of the mating design (6 males and 6 females), this third σ^2_A estimate becomes :

$$\sigma^2_A = 2\,(\sigma^2_M + \sigma^2_F) \qquad (3)$$

The fourth possibility of estimating the additive variance is from the break-down of the genetic variance :

$$\sigma^2_{\text{cloned FS}} = \frac{1}{2}\,\sigma^2_A + \frac{3}{4}\,\sigma^2_D$$

The dominance variance is estimated from the variance of full sib families : $\sigma^2_D = 4\,\sigma^2_{P\times M}$. Thus the additive variance is :

$$\sigma^2_A = 2\sigma^2_{\text{cloned FS}} - 6\,\sigma^2_{P\times M} \qquad (4)$$

Optimum mating design

To help breeders to optimize the amount of information from a given number of crossings, the effect of a reduction of the number of parents on the one hand and of a reduction in the number of offsprings per family on the other on the accuracy of parameter estimates was tested.

RESULTS

Reliability of the mating design

Enzymatic analysis of the parents and siblings on 10 polymorphic systems has proved that apart from two sibs no mistake could be detected in the mating design (Malvolti *et al.*, 1989).

Trait variation (Tables V and VI)

To be generally applicable, this study should have been based on a large number of parent clones sampled in all parts of the eastern cottonwood natural range. For technical reasons (lack of flowering clones in our collections, the time and space needed for a larger mating design), mating was limited to a 6 male/6 female factorial design. Furthermore, some of the clones may have resulted from phenotypic selection (vigour, bole

Table V. Variation of the different traits.

	10 parents			33 full-sib families		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum
Budburst (Scale)	4.2	16.2	25.9	6.5	14.9	24.0
Growth termination year 1 (%)	21.6	28.2	39.3	19.2	26.7	41.6
Growth termination year 2 (%)	2.1	6.2	13.4	2.4	5.6	9.9
Leaf fall year 1 (%)	28.6	54.8	77.0	40.0	57.2	76.3
Total height year 1 (m)	0.93	1.53	2.11	1.42	1.79	2.18
Total height year 2 (m)	2.51	3.20	3.94	3.06	3.61	4.01
Terminal shoot growth year 2 (m)	1.09	1.64	2.17	1.64	1.83	2.04
Stem diameter (cm)	0.87	1.73	1.35	1.84	2.25	2.74
Branch angle (degrees)	48.1	54.2	63.0	46.4	54.2	60.8
Branch wood density (kg.m^{-3})	278	330	368	305	331	359

Table VI. Significance level of the variability (F) and variation coefficient (σ/μ) of each trait. **, *, NS : F of variance analysis significant at 1%, 5%, not significant respectively.

Variation of	Parent clones				Families				Cloned full-sibs	
			Half-sib families of male parent		Half-sib families of female parent		Full-sib families			
	F	σ_c/μ_c	F	σ_M/μ_M	F	σ_F/μ_F	F σ_{MxF}/μ_{MxF}		F $\left(\dfrac{MS_c - \sigma_c^2}{n}\right)^{1/2}/\mu_{cFS}$	
Degrees freedom	9		5		5		22		824	(1)
Budburst	**	0.45	**	0.25	**	0.25	*	0.03	**	0.26
Growth termination, year 1	**	0.22	**	0.12	**	0.15	**	0.04	**	0.12
Growth termination, year 2	NS	0.00	**	0.23	**	0.20	**	0.09	*	0.25
Leaf fall, year 1	**	0.24	**	0.08	**	0.13	**	0.04	**	0.10
Total height, year 1	**	0.19	**	0.09	**	0.05	**	0.03	**	0.08
Total height, year 2	*	0.11	**	0.05	**	0.04	**	0.02	**	0.06
Terminal shoot growth, year 2	*	0.14	NS	0.01	**	0.04	**	0.03	*	0.07
Stem diameter	**	0.23	**	0.09	**	0.05	**	0.03	**	0.09
Branch angle	**	0.07	**	0.05	**	0.04	**	0.03	**	0.07
Branch-wood density	*	0.06	**	0.03	**	0.02	**	0.02	**	0.04

(1) MS : mean square; σ_c^2 : variance of error; n : mean number of ramets per cloned full-sib; μ : average of the genotype concerned.

straightness) which may have limited the variability for these traits. Therefore it is first necessary to observe how much variability exists between parent clones before estimating genetic parameters involving their offsprings.

Genotypic variation among parent-clones

Table V gives information on the trait value and variation of the different genotypes. The range of variation, whether high or low, is confirmed in Table VI, by the significance level of the variance analysis and by the variation coefficient of each trait. Among the phenologic traits, budburst, growth termination in year 1 and leaf-fall in year 1 showed significant variability. Growth termination in year 2 showed no variability and was therefore ignored in the rest of the study. The loss of variability in growth termination in year 2 when compared to year 1 was due to a 7-fold decrease in genotypic variance and a 3-fold increase in the variance of error. The biological significance of these values is mostly due to an early growth termination in 1986 which flattened out the variability. Among the growth traits, variability of total height was less important in year 2 than in year 1, due to a high intraclonal variability of terminal shoot growth in year 2. Among wood characteristics, branch angle ranged around 54 degrees with a strong clonal variability. Conversely, the variation in branch wood density, is low with values of around 330 kg/m^3.

Genetic variation among families

Among phenologic traits, bud burst and growth termination in year 1 have a strong variability, particularly in half-sib families (strong additivity). Among growth traits, the only non-significant F value was found for the terminal shoot growth of year 2 among the male-parent half-sib families. Furthermore, families reached higher values than parents (plus 30% for stem diameter). The wood traits, branch angle and branch wood density had the same average values as the parents, and their variability was low. Finally, the variation among full-sib families was low. This probably reflects low dominance effects.

Variation among cloned full-sibs

The last columns of Table VI provide information on the variation among cloned full-sibs. All traits are variable with a lower intensity for shoot growth and growth termination in year 2, but this had already been noticed in parents and families. Phenologic traits tend to have high variation coefficients in comparison with growth traits and wood characteristics.

A comparison of the range of variation among parents and families

Phenotypic and genotypic variation

A careful observation of Table V showing mean values and limits of each trait for parents and full-sib families, and of Table VII showing the parent/offspring ratio of variation coefficient shows that except for branch angle, all traits appeared to have a greater phenotypic variation among parents than among families. This was partly due to site effect, but a similar tendency was also found with genotypic

Table VII. Parent/offspring variation coefficient ratio

| | Variation coefficient ratio | |
	Phenotypic	Genotypic
Budburst	1.06	1.03
Growth termination year 1	1.00	0.88
Leaf fall year 1	1.31	1.24
Total height year 1	1.47	1.46
Total height year 2	1.57	1.22
Terminal shoot growth year 2	1.45	1.15
Stem diameter	1.60	1.66
Branch angle	0.92	0.80
Branch-wood density	1.11	0.96

variation. Variability has thus been reduced from one generation to the next.

Two hypotheses were proposed :

– certain parents were the result of a strong dominance effect. Therefore, they did not represent the mean genetic variation for the corresponding trait;

– parents were partly homozygous and their offsprings, because of their stronger heterozygosity (with dominance effects and phenotype buffering), lost part of the genetic variability.

Furthermore, except for terminal shoot growth during year 2 and wood density, the additivity estimate was much higher in the half sib families than in the cloned offsprings (Table VIII) therefore the statistical model applied in our study did not fit with the genetic reality. So we returned to the genetic model in which two assumptions were made for the parameter estimation : no epistasis, no inbreeding in parents; and analyzed these assumptions.

– *Epistasis.* When epistasis is considered, additive variance estimates are :

• for the parent sample :

$$\sigma_A^2 = \sigma_{parent}^2 - 1/14\ \sigma_{AA}^2 - \ldots$$

• for the full-sib families :

$$\sigma_A^2 = 2\ \sigma_{cloned\ FS}^2 - 3/2\ \sigma_D^2 - 3/2\ \sigma_{AA}^2 - \ldots$$

Therefore neglecting epistasis will increase the additive variance estimated in cloned full-sibs with a term, $3/2\ \sigma_{AA}^2$, which is much higher than in parent clones : $1/14\ \sigma_{AA}^2$. This is contradictory with our results and epistasis may actually be neglected.

– *Inbreeding.* Except for the 3 Murphysboro clones which originate from the same county, all parents are from geographically distant origins. Inbreeding between them is difficult to assume. On the other hand allelic fixation may have taken place within the populations from which these clones originated. Allelic fixation is due to the genetic drift and to mating of inbred trees as mentioned by Wright (1976) and also observed by Weber & Stettler (1981) on black cottonwood. As with the inbreeding coefficient, the introduction of the fixation index (F) in the variance estimation (Becker, 1984) leads to :

$$\sigma_A^2 = 2\ (\sigma_M^2 + \sigma_F^2)\ /\ (1 + F) \tag{1}$$

$$\sigma_D^2 = 4\ \sigma_{MxF}^2\ /\ (1 + F)^2 \tag{2}$$

$$\sigma_{cloned\ FS}^2 = (1 - F)\ \sigma_A^2\ /\ 2 + [4 - (1 + F)^2]\ .\ \sigma_D^2\ /\ 4 \tag{3}$$

Table VIII. Ratio of two additivity estimates.

	$\sigma^2_{A\ parents} / \sigma^2_{A\ cloned\ FS}$
Budburst	1.75
Growth termination year	3.81
Leaf fall year 1	4.24
Total height year 1	2.40
Total height year 2	1.61
Terminal shoot growth year 2	0.53
Stem diameter	1.93
Branch angle	1.42
Branch-wood density	1.06

(*) $\sigma^2_{A\ parents}$ is the combined additive variance whose estimation is proposed in § *Estimation of genetic parameters*; $\sigma^2_{A\ cloned\ FS}$ is the fourth estimate of the additive variance also shown in § *Estimation of genetic parameters*.

Table IX. Allelic fixation coefficient.

Budburst	0.31
Growth termination year 1	0.49
Leaf fall year 1	0.45
Total height year 1	0.35
Total height year 2	0.21
Terminal shoot growth year 2	0.00
Stem diameter	0.28
Branch angle	0.12
Branch-wood density	0.05

Allelic fixation in parents reduces the clonal variability in the next generation whereas it increases the male x female interaction.

Estimation of the fixation index

Formulas (1), (2) and (3) given above permit the estimation of F with gradual approximation (Table IX). Phenologic traits have the highest fixation coefficient (0.31 to 0.49), then come growth traits (0.21 to 0.35) with the exception of the terminal shoot growth in year 2, and, finally wood characteristics (0.05 to 0.12). The main effect of these coefficients is an overestimation of narrow sense heritabilities calculated in the progeny test.

Narrow sense heritability

In Table X, values which take into account the allelic fixation coefficient (1) or ignore it (2) and (3) for comparison are given. Two

Table X. Heritability (**, *, NS : value respectively significant at 1 or 5 percent level, not significant).

Heritability	Narrow sense			From regression		Broad sense	
Traits	Families	Parents	Cloned sibs	Parent	Mid-parent	Parent clones	Cloned sibs
	(1)	(2)	(3)	half-sibs	full-sibs		
Budburst	0.89 **	1.17 **	0.60 **	0.96	0.79	0.86 *	0.90 **
Growth termination year	0.63 *	0.93 *	0.21 **	0.44	0.55	0.56 *	0.67 **
Leaf fall year 1	0.49 *	0.72 *	0.15 NS	0.26	0.36	0.48 NS	0.55 **
Total height year 1	0.54 *	0.73 *	0.26 **	0.55	0.54	0.59 *	0.59 **
Total height year 2	0.31 *	0.33 *	0.16 *	0.40	0.34	0.27 NS	0.34 **
Stem diameter	0.45 *	0.56 *	0.26 **	0.39	0.39	0.52 *	0.59 **
Branch angle	0.41 *	0.46 *	0.30 **	0.09	0.01	0.43 NS	0.55 **
Branch-wood density	0.30 *	0.31 *	0.26 **	0.00	0.00	0.33 *	0.41 **

(1) Values which takes into account the fixation coefficient. (2) and (3) do not.

Table XI. Additive genetic correlations from parent/offspring covariance and in the progeny test (values with * and ** are significant at 5 and 1% respectively)

| | ... in the cloned full-sibs | | | ... in families | | | | |
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) Budburst		0.47	0.07	0.57	0.69*	0.79*	0.12	−0.25
(2) Growth termination year	−0.16**		−0.44	0.64	0.55	0.70*	0.41	−0.78*
(3) Leaf fall year 1	0.09	−0.53**		0.41	−0.20	−0.25	−0.20	0.24
(4) Total height year 1	−0.02	0.15*	−0.11		0.95*	0.85*	0.33	−0.23
(5) Total height year 2	0.11	−0.12	0.18	0.84**		0.86*	0.09	−0.21
(6) Stem diameter	0.13	−0.03	0.07	0.78**	0.82**		0.38	−0.41
(7) Branch angle	0.03	0.08	0.04	−0.03	0.02	−0.07		−0.53
(8) Branch-wood density	0.13	−0.23*	−0.16	0.13	0.06	−0.02	−0.06	

other heritabilities are also shown. They are estimated from the parent/offspring regression. Most values are high and reflect the high additive genetic variance. As usual, high values are observed for phenologic traits even with heritability estimated from parent/offspring regression. One exception appears for the heritability of leaf fall estimated from the cloned sibs (0.15 NS). Growth traits have medium to high heritability values, and so do wood caracteristics except when estimated from parent/offspring regression.

Broad-sense heritability

The values are fairly high and most of them are significant. The size of the sample used for the estimation (10 parent clones or 824 cloned sibs) does not change these values markedly, but it affects their significance level. Nevertheless, insignificant values are observed among parent clones for leaf-fall (already mentioned for narrow sense heritability), total height in year 2 (whereas the same trait observed one year earlier had a higher and significant heritability) and branch angle (this trait had a low variation coefficient. (See Table VI.)

Correlation between traits

Among the relationships between the different traits two additive genetic correlation matrices are shown in Table XI. The upper part of the table gives an estimation of the progeny test using combined estimates of additive variance and covariance. The lower part of the table gives an estimation of the cloned full-sibs.

In families, high and generally significant correlations appear between growth traits. A significant value is also found between bud-burst and growth as well as between growth termination and diameter, meaning that the longer the vegetation period, the greater the height and diameter growth. Finally, a negative correlation appears between growth termination and branch wood density, meaning that late growing families will not have the densest wood.

In cloned full sibs, a strong relationship appears between growth traits. Phenologic traits are also interlinked. Budburst has a slight but significant negative correlation with growth termination, meaning that an early budburst corresponds with an early growth termination. Thus the vegetation period appears fairly stable

among full-sibs. A strong negative correlation is also shown between growth termination and leaf fall. It means that late growing genotypes bear their leaves late in the season. Furthermore, a greater height growth in year 1 is observed in late growing genotypes which appears to support this but which has to be compared with the absence of relationship between budburst and height or diameter growth. This is in contrast to observations made in families. Finally, as observed in families, late growing full-sibs will not have the densest wood.

DISCUSSION

Before discussing these results is should be reiterated that this study is limited to a 6 females x 6 males mating design which does not represent all the variability of eastern cottonwood. Therefore results are representative of this artificial population and will be compared with the literature. Generalization and application to a breeding strategy will be suggested only if a good level of agreement between different sources of information is found. Furthermore, traits were measured in one nursery trial, and the reader knows that such conditions may not be representative of all growing sites, particularly for site interactive traits. Finally, during a 2-year observation period it is not possible to estimate juvenile-mature relationships.

Phenologic traits

A fast growing species like the eastern cottonwood must be adapted to local climatic conditions, particularly those which may hinder its growth or kill its shoots. Budburst, growth termination and leaf-fall are usually considered to be good predictors of this adaptation in connection with late and early frost risk.

In our experiment, all phenologic traits except growth termination in year 2 are variable. Their allelic fixation coefficients seem high, their heritabilities are also generally high, and finally, they show different levels of correlation either among themselves or with other traits.

The high variability and the strong genetic control of these traits permit selection either among clones or among parents and their offsprings. The flushing period of eastern cottonwood in the Orléans climatic conditions is fairly late in the spring : the end of April, beginning of May. During this period frost risk declines rapidly, therefore it may not be useful to choose late flushing genotypes as for other more tender species. An early budburst will result in a greater height and diameter growth for families. Growth termination and leaf fall appear closely related in cloned full-sibs. Late growing will increase height and diameter, which is in slight contradiction with the choice of an early budburst in spring. Furthermore, a late growth termination may decrease wood density which is certainly not a favorable result for poplar wood utilization.

It has been observed that phenologic traits had a higher level of allelic fixation in comparison with other traits. This fact may have resulted from selection pressure exerted in natural stands.

Studies on the genetic control of phenologic traits of poplar have been made by different authors. Teissier du Cros (1968) observed in eastern cottonwood provenances that budburst is highly variable but cannot be connected to general information regarding origin (geographic coordinates, for instance). Conversely, frost damage in the spring

was directly connected to early budburst. The vegetation period varies from 137 days for an Indiana provenance to 163 days for South Ohio provenances. Some early flushing provenances tend to stop growing early but this observation is not general, since a northeast Ohio provenance had a late budburst and an early growth termination (144 days vegetation period). In contrast to budburst, growth termination seems to be closely linked to the latitude of the original stand as shown by Pauley & Perry (1954) on black and eastern cottonwood. Therefore it appears that the strong genetic control of phenologic traits, as also shown in eastern cottonwood by Farmer (1970) and Ying & Bagley (1976), may partly be due to environmental pressure such as temperature extremes (inducing allelic fixation) and photoperiod (connected to latitude).

Growth traits

Height and diameter are less variable than phenologic traits. Their allelic fixation coefficient, except for shoot growth, ranges between 0.28 and 0.35. Although still significant, the narrow sense heritability whether estimated in families or from the parent-offspring regression, are slightly lower than for phenologic traits, particularly for budburst (0.16 to 0.73). Broad sense heritability is also generally significant and fairly high (0.27 NS to 0.59). It has already been shown how phenologic traits can genetically influence growth, and the absence of a significant correlation between growth traits and wood quality traits tends to show that the latter will not be influenced by the former.

In his study on open pollinated progeny of eastern cottonwood, Farmer (1970) reports that since a *relatively small*

amount of variance was associated with family differences in growth (...), response to selection for this character will be much less than for others. This is further demonstrated by the fact that field selection of parents for growth was completely ineffective in terms of juvenile progeny performance. Our study comes to a slightly different conclusion, since the comparative design permitted the estimation of genetic heritability from genotypic information on the parents and on the families.

Therefore, while it seems ineffective to select phenotypically superior trees in natural stands to improve the vigour of their offsprings, it appears much more effective to do so through the selection of parents in clonal tests, at least for juvenile traits.

Wood quality traits

Branch angle and branchwood basic density, as a predictor of stemwood density, were observed. Although variability is low and although our heritability values reach lower values than those of Herpka (1979) and Olson et al. (1985), the genetic control of these traits is generally high except for parent-offspring heritability and broad sense heritability of parent clones. Therefore clonal or family or parent selection will probably sleghtley improve the wood quality. The allelic fixation of these traits seems to be very low. This is not surprising, since no environmental factor such as snow or wind tends to select highly adapted ecotypes with shorter branches or denser wood.

In contrast to *Populus nigra* for which a high parent-offspring additive correlation was found for branch angle (Pichot and Teissier du Cros, 1988), no similar result is found in our *P. deltoïdes* sample.

Table XII. Variation of value and significance level of different genetic parameters with a reduction of sibs per family.

Number of sibs per family	5	10	15	20	25	30
Narrow sense heritability of parents						
Budburst	1.13*	1.02*	1.07*	1.13**	1.17**	1.17**
Total height year 2	0.17NS	0.29*	0.30*	0.31*	0.31*	0.33*
Branch angle	0.53*	0.49*	0.49*	0.49*	0.47*	0.46*
Narrow sense heritability of cloned sibs						
Budburst	0.31NS	0.44**	0.55**	0.53**	0.57**	0.60**
Total height year 2	0.03NS	0.14NS	0.20NS	0.20*	0.16*	0.16*
Branch angle	0.12NS	0.27*	0.40**	0.36**	0.25*	0.30**
Additive correlation in families						
Height year 1 x diameter	0.70NS	0.82*	0.83*	0.84*	0.85*	0.85*
Additive correlation in cloned full-sibs						
Height year 1 x diameter	0.83NS	0.83NS	0.82*	0.82**	0.78**	0.78**

Finally, one must remember that late growing genotypes tend to produce wood with low density. Therefore, although this result needs confirmation since it is based on a 0.55 correlation between branchwood and stemwood densities, the final consequence of a different correlation between traits would be as follows. A high wood density will be obtained with genotypes and with a rather early growth termination. A longer vegetation period which is needed for increasing height growth will be obtained in early flushing genotypes.

The average wood basic density of poplar is fairly low and any method to increase it will result in a higher wood resistance which is of the greatest importance for its use as timber and veneer. Our observations only concerned very young branch wood, for which values ranged around 330 kilogrammes per cubic meter. A very important genetic parameter which could not be estimated in our experiment is the juvenile–mature correlation of wood density. This parameter will certainly have to be estimated in the future. Similar estimates have been made by Nepveu *et*

al., in 1978 on other poplar species. They found high juvenile-mature genetic correlations of wood density for clones *P. nigra* and *P.* x *euramericana.*

Optimization of mating designs

A 6 x 6 mating design with 30 offsprings per family may not have been the best factorial design to estimate the genetic parameters of this study. In particular, it is quite possible, although not demonstrated here, that more parents, representing a greater part of the natural range might have brought in more variability and might have given different parameter values. However, as in all research, manpower and money are limited, and it is important to optimize the scientific output obtained from a given technical and financial input. One optimization method is to study the evolution of parameters with a reduction in the number of parents and of sibs per family. This method was applied to heritability and additive correlation for a few traits. Table XII gives the effect of

Table XIII. Variation of value and significance level of different genetic parameters with a reduction of the male parent number

Number of male parents	3	4	5	6
Narrow sense heritability of parents				
Budburst	1.22NS	1.14*	1.14*	1.17**
Total height year 2	0.29*	0.23*	0.30*	0.33*
Branch angle	0.37NS	0.38*	0.36*	0.46*
Narrow sense heritability of cloned sibs				
Budburst	0.82**	0.71*	0.65**	0.60**
Total height year 2	0.18*	0.11NS	0.12NS	0.16*
Branch angle	0.26NS	0.32*	0.39**	0.30**
Additive correlation in families				
Height year 1 x diameter	0.74NS	0.90*	0.82NS	0.85*
Additive correlation in cloned full-sibs				
Height year 1 x diameter	0.79NS	0.77NS	0.77**	0.78**

reducing the number of sibs per family. It shows that a severe change in value and an important drop in significance does not occur before 15 or 10 sibs per family, whereas we have usually based our estimations on 30 sibs. Table XIII gives the effect of a reduction in the male parent number. It shows that although the values are not drastically changed, the significance level falls rapidly. In such a design 6 males appear to be a safe limit below which chance will play too important role in the estimation values. As little or no sex effect was observed in the parameter values, it may be assumed that 6 would also be the lower limit for the number of female parents. Therefore, only the number of sibs per family seems to be able to be reduced (for instance to 20 or 15, to be safe) without any detectable effect on the parameter value and significance.

Consequence for improvement

Current poplar improvement programmes of the West European institutes – Belgium,

Italy, France – are now based on short- and long-term strategies to fulfil the requirements of poplar growers in the near and distant future.

In the short-term, clonal selection within pure species for direct application to culture is still considered with some interest in regions which long have been using eastern cottonwood, as in south-western France or in Italy. The present study confirms the biologic and genetic knowledge which has already been gathered on clones, either empirically or scientifically.

In the long-term, recurrent breeding within pure species before interspecific hybridization is now considered compulsory, either to combine traits existing in distant geographical parts of the range or to purge deleterious genes (Kang, 1982).

The eastern cottonwood is included in the French poplar improvement scheme because of its vigour, its high wood quality and its ability to hybridize with the black cottonwood (*P. trichocarpa*) and the European black poplar (*P. nigra*).

Its breeding started in 1964, with the construction of base populations which

have been established in three French locations : the northeast, the centre and the southwest.

Meanwhile older collections, thanks to which this study was possible, have permitted the estimation of genetic parameters. The selection of parents for the production of the next intraspecific generation will now be initiated. The effect of this selection on the genetic nature of the new generation will be predictable.

As a result of this study, clonal selection will be effective on all analyzed traits. Multigeneration breeding will be highly efficient for phenologic traits, moderately efficient for growth traits and less efficient for wood quality traits. One difficulty will be the adaptation of the material to climatic extremes, particularly in the autumn. Since late growing clones tend to produce wood with low basic density, breeders' efforts will rather concentrate on early flushing genotypes.

Future development

This study is based on juvenile observations. Results definitely need to be extended to field conditions to permit observation on older trees. Parents and sibs were therefore planted in the spring of 1988 in a field trial which includes copies of the 10 parents and of 15 sibs per family. Later on the trial will be vegetatively replicated in the Orléans nursery for observations of leaf diseases after control-led inoculation. This development will permit the study of the evolution of genetic parameters with time and the environment. It will also allow the estimation of these parameters for new traits.

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