sistance. This study was partly funded by a B. C. Ministry of Forests and Lands contract [Section 88 (1)] to MacMillan Bloedel Limited.

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Genetic Control of Isozyme Systems and Heterogeneity of Pollen Contribution in Beech (Fagus sylvatica L.)

By D. MERZEAU[1]), F. DI GIUSTO[2]), B. COMPS[1]), B. THIÉBAUT[2]), J. LETOUZEY[1]) and J. CUGUEN[2])

(Received 10th June 1988)

Summary

The study of the alloenzymatic polymorphism and the genetic structure of beechwoods began some years ago, but until now, only few markers have principally been used. For common beech, several new loci, mostly polymorphous, are described in the present paper. The genetic control is carried out analysing the offspring of heterozygous mothers (in case of diallelic loci), assuming the codominance of the alleles for loci coding the MDH, 6PGD, PGI, and SOD systems and testing the segregation ratio 1:1. For the triallelic loci that control IDH and ACP isozyme variants, we only verified that the different genotypes observed in each offspring corresponded to the genotypes expected in the case of three codominant alleles. Additional some presumptions of the genetic control of PX3 and SKDH1 are given.

Some markers were used for studying the variations of the allelic frequencies of the paternal contribution within three populations. In all cases, the paternal contribution is heterogeneous. This heterogeneity presents an inter-group and an intra-group components with allelic frequencies different as well for neighbouring trees as for isolated trees several hundred meters apart.

Key words: allozymes, inheritance, pollen pool variations, beech.

Résumé

L'étude du polymorphisme enzymatique et de la structuration génétique des hêtraies a débuté il y a quelques années, mais jusqu'à présent, seuls quelques marqueurs ont été utilisés. Dans cet article, de nouveaux loci sont décrits chez le Hêtre, la plupart d'entre eux étant polymorphes. Le contrôle du déterminisme génétique est assuré par l'étude de la descendance de mères hétérozygotes (dans le cas de loci diallélіques) en postulant la codominance des allèles pour les loci codant les systèmes MDH, 6PGD, PGI et SOD et en testant le rapport de ségrégation 1:1. Dans le cas des loci triallélіques qui contrôlent les allozymes d'IDH et d'ACP, nous avons seulement pu vérifier que les différents génotypes observés dans chaque descendance correspondaient aux génotypes attendus sous l'hypothèse de trois allèles codominants. Des hypothèses sur le contrôle génétique de PX3 et SKDH1 ont également été avancées.

Plusieurs marqueurs ont été appliqués à l'étude de la variation des fréquences alléliques du pool génique paternel dans trois populations. Dans tous les cas, la contribution allélique paternelle est hétérogène. Cette hétérogénéité présente une composante inter-groupes et une composante intra-groupe avec des fréquences alléliques différentes aussi bien pour des arbres voisins que pour des arbres isolés, séparés les uns des autres de plusieurs centaines de mètres.

Mots-clés: allozymes, contribution paternelle, Hêtre.

Introduction

The isozyme technique offers a practical way of examining genetic variation in natural populations. At the present time, the number of biochemical studies on beech tree is very small: PAGANELLI *et al.* (1973) examined the variation of dehydrogenases in winter buds; KIM (1979) studied the genetics of two enzymatic markers in young leaves, one

[1]) Université de Bordeaux I, Département de Physiologie des Végétaux ligneux — Ecologie génétique, F-33405 Talence Cedex
[2]) Centre L. Emberger, C.N.R.S., Biologie des Populations et des Peuplements, B. P. 5051, F-34033 Montpellier Cedex

for leucine amino-peptidases (LAP) and the other for acid phosphatases (ACP). Then, THIEBAUT *et al.* (1982) established the formal genetics of three new markers in dormant buds: two in peroxidases (PX) and one in glutamate oxalo-acetate transaminases (GOT). Recently, MÜLLER-STARCK (1985) used several markers to determine genetic differences between tolerant and sensitive beeches in an environmental stress adult forest stand: the new markers have been named and the number of alleles at each locus given, but the analysis techniques and the genetic control have still not been published. At present, the beech tree populations have been studied mostly using 3 markers (PX1, PX2 and GOT1: THIEBAUT *et al.*, 1982; N'TSIBA, 1984; BARRIERE *et al.*, 1985; CUGUEN *et al.*, 1985; CUGUEN, 1986). These different studies prove that beechwood polymorphism varies a great deal according to the locality and that a population is more or less structured; this structure depends in particular on the mating system.

The main object of our research is to assess the mating system of beech. At present, the common model in forestry is the mixed mating model. One of the assumptions derived from this model is random outcrossing which supposes an homogeneous pollen pool. However, CUGUEN (1986) has shown the important role of neighbourhood within dense beechwoods; in this case, gene flow may be limited and pollen pool heterogeneous. Thus, it is worthwhile to estimate this heterogeneity. All these studies must be carried out, if possible, using a large number of markers. In this paper, we describe the methods used to detect several new enzyme markers and we present our first results relative to pollen pool variations among maternal trees using four loci.

Material and Methods

Sampling method for the detection of new loci and inheritance tests

For most enzyme systems, we analysed a great number of individuals coming from various regions, in order to identify all the presumed alleles; indeed, some loci may be monomorphic within some natural beechstands and polymorphic elsewhere. In each beechstand, 50 individuals were studied from vegetative material (dormant buds and cortical tissues of axes). Moreover, we examined the maternal offspring (40 beechnuts per tree) of 40 trees chosen from the three beech stands described below. If we assume allelic codominance and a 1:1 segregation ratio, thus, in the case of a diallelic locus, we can expect a 1:1 genotype ratio (heterozygotes vs homozygotes) among the progeny of each heterozygous maternal tree. A G-test for goodness of fit was then performed at the individual heterozygous tree level and on the combined data if G for heterogeneity revealed non significant values.

Procedure for estimating pollen pool variations

Estimations of pollen pools are based on offspring studies using beechnuts sampled within the tree canopy. Three large beech stands were chosen, all aged about 150 years old: two located on the Aigoual mountain (Serreyrede and Plo-du-Four) and one in the Pyrenees (Issaux). Serreyrede and Issaux are characterized by a high tree density (about 200 trees/ha). In each of the two Aigoual beechwoods, beechnuts were sampled from 10 mothers chosen at random and about 40 meters apart. In the Pyrenean beechwood, we selected 15 mothers which were divided into 3 equal groups: one group of neighbouring individuals arranged in a row (within the dense stand), a second one, also arranged in a row, but located at the edge of the same stand and a third group of isolated trees belonging to the same population. The choice of 3 groups located in very different situations within the studied population was aimed at increasing the probability of detecting a possible heterogeneity of the pollen pool.

Table 1. — Gel and electrode buffers and electrophoretic running conditions.

Gels	Buffer systems	Electrode buffer		Gel buffer		Described by	Running conditions
Starch 12%	A	Orthoboric acid NaOH pH 8.2	300.82 mM 60 mM	Tris Orthoboric acid pH 8.7	103.22 mM 42.46 mM	Poulik (1957) modified by Thiébaut el al. (1982)	Direct current 50 mA 250V during 6 hours.
	B	Citric acid Tris pH 8.0	95.20 mM 399.90 mM	Histidine pH 8.0	5.00 mM	Brewer (1970) modified by Second and Trouslot (1980)	Direct current 90 mA 140V during 5 hours.
Polyacrylamide 9%	C	Tris Glycine pH 8.3	7.08 mM 54.85 mM	Tris HCl RG* : Tris 377.80 mM HCl 60 mM pH 8.9 CG* : Tris 61.70 mM HCl 60 mM pH 6.7		Davis (1964)	Pulsed power : 300p/s (Gasquez - Compoint) CG* : 35 mA-300V during about 20 min. RG* : 70 mA-500 to 700V during 1 h.30
	D	Barbituric acid Tris pH 7.0	30.09 mM 8.26 mM	Tris HCl RG* : Tris 70.71 mM HCl 60 mM pH 7.5 CG* : Tris 51.09 mM HCl 60 mM pH 5.5		Williams and Reisfield (1964)	Pulsed power : 300p/s (Gasquez-Compoint modified). CG* : 35 mA - 200V during about 20 min. RG* : 70 mA-400V, 30 min. after tracking dye has reached anod.

*) RG: running gel
*) CG: concentration gel

Table 2. — Composition of used buffers and stains.

Enzyme name	Locus	Buffer system	Staining solution				
			Buffer	Substrate		Other chemical components	
Phosphoglucose isomerase						NADP	2.61 mM
PGI EC 5.3.1.9	PGI1	A	Tris 0.2 M pH 8	D-fructose-6-phosphate	3.29 mM	NBT	24.46 mM
						PMS	6.53 mM
						$MgCl_2$	20.00 mM
						G-6-PD	20 units/100ml
Malate dehydrogenase						NAD	0.26 mM
MDH EC 1.1.1.37	MDH1	A	Tris 0.2 M pH 8	Na-L-malate solution 0.2 M pH 7		MTT	4.83 mM
						PMS	0.65 mM
Isocitrate dehydrogenase						NADP	0.26 mM
IDH EC 1.1.1.42	IDH1	B	Tris 0.2 M pH 8	Isocitric acid	0.78 mM	MTT	4.83 mM
						PMS	0.65 mM
6-Phosphogluconate dehydrogenase	PGD1	C	Tris 0.1 M pH 8	6-Phosphogluconic acid		$MgCl_2$	2.00 mM
6PGD EC 1.1.1.44					1.25 mM		
Peroxidase						Gaiacol	10.8 mM
PER EC 1.11.1.7	PX3	C		H_2O_2 à 30%	10 ml/50 ml	Acetic acid	70.00 mM
Superoxide dismutase			SOD appears as colourless bands on gels stained for PGD				
SOD EC 1.15.1.1.	SOD1	C					
Acid phosphatase			Tris 0.03 M pH 5.2	α Naphtyl acid phosphate		FBK	3.49 mM
ACP EC 3.1.3.2.	ACP1	D	Malic acid 0.02 M		8.13 mM	$MgCl_2$	2.00 mM
Shikimate dehydrogenase						NADP	0.26 mM
SKDH EC 1.1.1.25	SKDH1	D	Tris 0.1 M pH 8	Shikimic acid	11.49 mM	MTT	4.83 mM
						PMS	0.65 mM

We calculated allelic frequencies from genotypic frequencies. Paternal allelic contributions (auto- + allopollen) were then estimated substrating the maternal contribution from the total allelic frequencies. In the case of heterozygous mothers, this estimation is possible only if we admit a 1:1 segregation ratio (cf results).

Extraction and electrophoresis techniques

The procedures used for extracting enzymes from vegetative material and beechnuts were those described by Thiebaut *et al.* (1982). These authors used a Tris-HCl buffer (pH 7.6) containing 0.1 M tris, 1% polyethylene glycol 20000, 4mM sodium thioglycolate and 10 mg of insoluble polyvinylpyrrolidone.

Specific buffer systems and running conditions are reported in *Table 1* and staining methods in *Table 2*. Capital letters refer to a specific enzyme; each gene locus coding for this enzyme is noted by capital letters followed by a numeral. When systems are controlled by multiple loci, these loci are numbered according to the chronological order of their genetic control. The most frequent allele of the locus numbered as 1 is assigned the value of 100; all the other alleles are named according to their mobility relative to the allele 100.

Results and Discussion

Description of the electrophoretic phenotypes

All assayed enzyme systems migrated anodally on gels. Seven new loci with clear and consistent band patterns were identified. A large number of analyses were carried out using extracts of vegetative organs and beechnuts together on the same gels: all observed banding patterns were absolutely identical in both types of organ. However, one of them (PX3) only appears in axis and bud tissues: consequently, the genetic control of allozymes may be postulated from the band pattern observed in seedlings. For some systems, several zones of activity appear on gels, but we only describe clear and consistent band patterns.

Isocitrate dehydrogenase, acid phosphate, phosphoglucose isomerase and 6-phosphogluconate dehydrogenase (*Fig. 1* to *4*)

For each of these four enzyme systems, only one staining zone is described. For IDH and ACP, 6 phenotypes were observed in each case: three one-banded phenotypes and three triple-banded ones. The intermediate band of the latter is always the darkest. The most likely hypothesis is that of dimeric enzymes specified by two loci (IDH1, ACP1)

Fig. 1. — Phenotypes showing segregation of allozymes at IDH1.

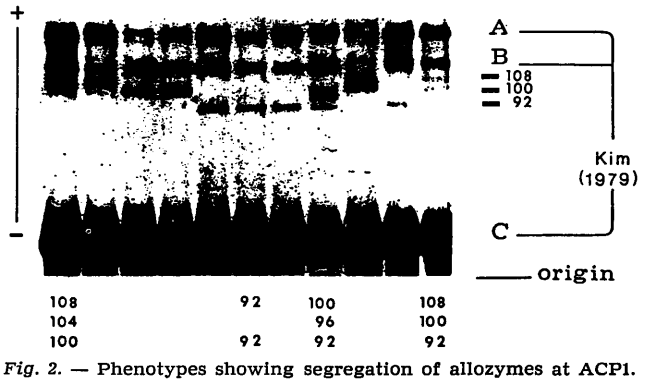

Fig. 2. — Phenotypes showing segregation of allozymes at ACP1.

with 3 codominant alleles. The same hypothesis can be suitable for the two other systems though we only observed 4 phenotypes (two one-banded ones and two with three bands), the fastest allele being extremely rare for PGI1 as well as for 6PGD1. These four systems have been reported to be dimeric in various species (Allen *et al.*, 1963; Grover and Byrne, 1975; Valizadeh, 1978; Mitton *et al.*, 1979; O'Malley *et al.*, 1979; Adams and Joly, 1980).

For the ACP system, four zones of activity occur. Three of them are blurred and often present unconsistent bands and could correspond to the active zones A, B and C described by Kim (1979).

Peroxidase (*Fig. 5*)

For peroxidase system, five active zones can be observed on zymograms. Four of them have been mentioned by Thiebaut *et al.* (1982), but only two have been genetically interpretated and are coded by two independent loci (PX1 and PX2). The fifth band pattern (PX3), the slowest, shows phenotypes with one band more or less stained according to the individuals and three others with two bands. Among the latter, we observed a phenotype with equally stained bands and the two others with one darker band. Several hypothesis can be proposed, but until now, our tests are insufficient to know what occurs really.

Malate dehydrogenase (*Fig. 6*)

There was one major dark staining zone (the fastest) and one lighter staining zone on starch gels for this system. The former was not included in our study because of its poor resolution. In the latter (MDH1), the grouped results of our analysis reveal the existence of 3 phenotypes: two with one band and the third with two bands. Consequently, we assume that it is one polymorphic locus with two codominant alleles which specify monomeric enzymes. MDH isozymes are reported to be either dimeric in some species (King and Dancik, 1983; Guries and Ledig, 1978) or monomeric (El-Kassaby *et al.*, 1982).

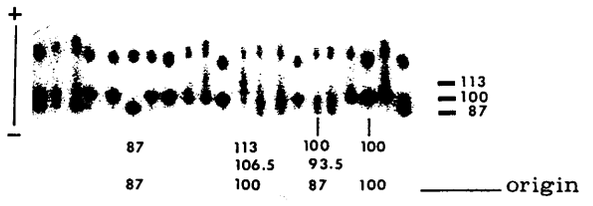

Fig. 3. — Phenotypes showing segregation of allozymes at PGI1.

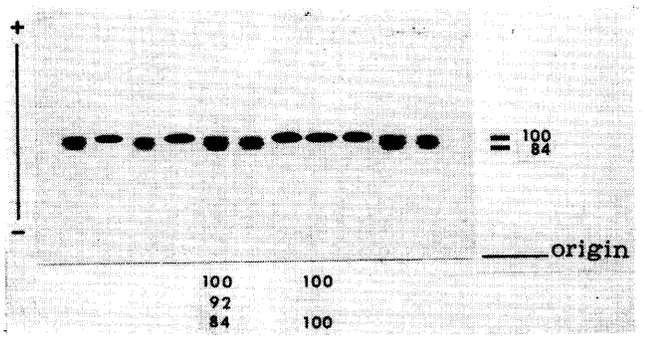

Fig. 4. — Phenotypes showing segregation of allozymes at 6PGD1.

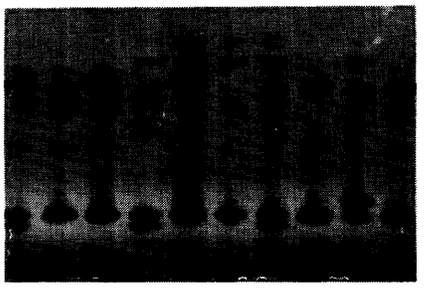

Fig. 5. — Phenotypes showing segregation of allozymes at PX system.

Fig. 6. — Phenotypes showing segregation of allozymes at MDH1.

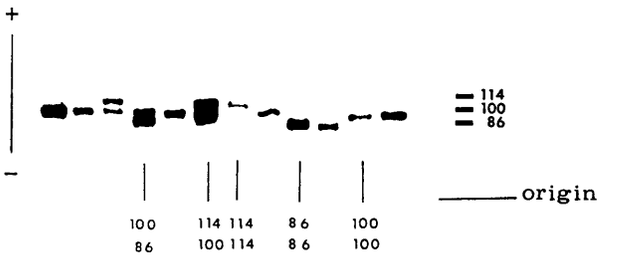

Fig. 7. — Phenotypes showing segregation of allozymes at SKDH1.

Superoxide dismutase

SOD appears as clear bands on polyacrylamide gels background-stained with tetrazolium dye. It is likely that there are several zones of activity, but only one is polymorphic and sufficiently visible to be scored reliably (SOD1); it is the most anodal. SOD1 displays 3 phenotypes: two one-banded phenotypes and a tripled-banded one. We have hypothesized that it is a locus with two codominant alleles coding for dimeric enzymes; this agrees with the SOD structure described in literature (Baur and Schorr, 1969; El-Kassaby *et al.*, 1982).

Shikimate dehydrogenase (*Fig. 7*)

There was a single, intensely staining zone (SKDH1). Only five phenotypes have been observed: three one-banded phenotypes and two with two bands. The most likely hypothesis is that of monomeric enzymes specified by one locus with 3 alleles; this agrees with the monomeric structure described by Neale *et al.* (1984) in Douglas-fir. The absence of one heterozygous phenotype in our observations may be due to the rarity of the two concerned alleles; moreover, this enzyme system has been until now studied only in a few populations.

Inheritance of the studied polymorphisms (Table 3)

Twenty one tests were performed on the studied diallelic loci, at the individual tree level: none of them shows significant deviation from the expected 1:1 ratio. These tests included PGI1 and 6PGD1, because only two alleles were found for these loci within the two studied regions. For the PX3 and SKDH1 loci, tests were not performed because the number of analysed seedlings was insufficient. Thus, except for these two last loci, all tests confirmed the genetic control of the observed phenotypes.

Table 3. — Observed genotypes in offsprings of heterozygous mother trees and the goodness-of-fit to 1:1 ratio.

					G - statistics								
	F	H	Ht	Total	G_t	df	P	G_p	df	P	G_h	df	P
Loci													
MDH1	10	418	435	853	10.08	10	.50-.75	.34	1	.50-.75	9.74	9	.50-.75
SOD1	10	181	184	365	7.41	10	.50-.75	.03	1	.75-.90	7.38	9	.50-.75
PGI1	2	36	43	79	1.64	2	.30-.50	.62	1	.50-.75	1.02	1	.30-.50
6PGD1	1	24	15	39	2.07	1	.10-.20						

F: family number — H: homozygote number — Ht: heterozygote number — G_t: Total G — G_p: pooled G — G_p: heterogeneity G. — df: degrees of freedom — P: probability of χ^2.

On the other hand, for the two tri-allelic loci (IDH1 and ACP1), the test could not be performed. However within the offspring of mothers homozygous for a given allele (even if it is rare), we never observed the homozygous genotypes for the two other alleles, nor the corresponding heterozygote. Likewise, the offspring of a mother heterozygous for two given alleles never presents the homozygous genotype for the third allele. Although it is a qualitative argument, it supports the hypothesis concerning the genetic control of these loci.

Variation of the pollen pool gene frequency

For the Issaux forest, results are presented in *Tables 4* and *5*. A global χ^2 test of heterogeneity was performed for the 15 maternal trees studied, using the absolute allelic frequency of paternal contribution. It was found to be significant for each of the four loci studied, allowing us to reject the hypothesis of homogeneity (*Table 5*). The heterogeneity of the pollen pool may be due to the rather large distance between the three groups of trees (a few hundred meters) and to their different situations (dense stand, edge and isolated trees).

Thus, an analysis of variance was performed in order to test the organization of the inter- and intra-group variations. The different values of F mentioned in *Table 5* are more or less significant for the three first loci: at the level of 0.01 for MDH1, 0.05 for GOT1 and only 0.10 for SOD1. Thus, the different groups do not receive the same pollen pool for the concerned loci. On the other hand, the total variation for the IDH1 locus does not present an inter-group component. The comparison by pairs (DUNCAN'S method) of the mean allelic frequencies of the paternal contribution, calculated for each group and for one determined allele, shows the originality of the G2 group (edge trees) for the GOT1 and MDH1 loci. For SOD1, the three groups are organized in two overlapping classes: G1—G2 and G1—G3.

The values of the coefficient of intraclass correlation show that MDH1 is the only locus for which most of the variation in the sample is among groups. However, for all four loci, there is an important intra-group variation, as shown by the values of χ^2 heterogeneity calculated for each group: from a total of 12 values, only 4 are non-significant, one of them becoming significant at the level of 0.10. Thus, even within a group, nearby trees received different pollen and this whatever the group. Moreover, BARTLETT's homogeneity test of intra-group variances shows that there is no significant heterogeneity from one group to another for the four loci.

We also carried out a discriminant analysis which defined 2 canonical variables that explain 100% of the total variance: the first one accounts for 93.5% and is dominated by a large loading from GOT1, SOD1 and MDH1; the second one accounts only for 6.5% and essentially refers to IDH1 locus. The means of each group on the first canonical axis are not significantly different ($0.05 < p\ \chi^2 < 0.10$) using BARTLETT's approximation (RAO, 1973). When the means are compared by pairs, again we observe the tendency of the second group G2 to be differentiated from the two others, the difference with the third group being significant.

For the two populations of the Aigoual mountain, we carried out a random sampling without distinguishing several groups of trees. Thus, only a global χ^2 heterogeneity test was performed for the ten mother trees in each stand. Moreover, only two loci (MDH1 and IDH1) were used.

As shown on *Table 6*, χ^2 values referring to the MDH1 locus are significant in both stations, but only one value is significant for the IDH1 locus. Hence, in most cases, the pollen pool is heterogeneous as in the Issaux forest.

Table 4. — Absolute allelic frequencies in paternal contribution — ISSAUX forest.

		GOT1		SOD1		MDH1		IDH1		
		100	105	100	112	100	120	84	100	116
Groups	Mother-trees									
	176	97	59	37	29	104	52	2	98	16
	177	107	47	78	54	98	54	2	107	5
G1	180	81	75	50	11	105	46	2	33	3
	181	100	60	56	18	112	40	3	68	9
	182	103	49	58	22	104	53	1	38	1
	Total T1	**488**	**290**	**279**	**134**	**123**	**245**	**10**	**344**	**34**
	L1	123	116	68	22	176	60	1	67	16
	L2	144	96	87	24	168	69	4	92	18
G2	L3	133	105	85	32	188	51	7	135	15
	L4	110	128	111	27	196	42	3	74	1
	L5	82	98	78	10	136	45	0	65	11
	Total T2	**592**	**543**	**429**	**115**	**864**	**267**	**15**	**468**	**61**
	110	65	55	39	25	66	50	1	26	3
	111	82	38			80	38	0	39	1
G3	112	86	34	21	17	81	34	0	37	2
	118	113	46	71	21	85	70	0	23	7
	122	73	47	36	26	75	42	0	28	7
	Total T3	**419**	**220**	**167**	**89**	**387**	**234**	**1**	**153**	**20**

G1: group of trees located within the dense forest
G2: edge-trees
G3: isolated trees

Table 5. — Analysis of the intra- and inter-group variation of the allelic paternal contribution — ISSAUX forest.

Loci		GOT1	SOD1	MDH1	IDH1
Global X^2 (15 mother-trees)		75.66 14 df p<0.001	59.04 13 df p<0.001	64.61 14 df p<0.001	19.41 10 df p<0.05
ANOVA $F_{[2,11]}$ using arcsine transformation		4.37 p<0.05	3.63 p<0.10	9.73 p<0.01	0.88 ——
Mean intra-group allelic frequency (for one allele*) using arcsine transformation +	G1	0.66	0.58	0.60	0.28
	G2	0.77	0.47	0.51	0.34
	G3	0.64	0.65	0.67	0.38
Coefficient of intraclass correlation		0.421	0.361	0.653	——
X^2 for intra-group heterogeneity	G1	12.46 4 df p<0.05	17.19 4 df p<0.01	3.64 4 df p<0.50	5.77 3 df p<0.25
	G2	13.79 4 df p<0.01	8.56 4 df p<0.10	9.94 4 df p<0.05	9.59 4 df p<0.05
	G3	12.62 4 df p<0.05	9.38 3 df p<0.05	10.04 4 df p<0.05	1.02 2 df p<0.05
BARTLETT's test for homogeneity of intra-group variances (2 df)		0.366 p>0.50	0.617 p>0.50	1.272 p#0.50	0.582 p>0.50

*) rarer allele in case of a diallelic locus and sum of the 2 rarer alleles in case of IDH1.
+) in radians.
df: degrees of freedom — p: probability of χ^2.

Conclusions

Whatever the analysis method, the results obtained for the IDH1 locus differ from those based on the three other markers. This is a triallelic locus, but two of its alleles are rare, which could explain an apparent homogeneity of the pollen pool. On the other hand, for the other loci, variations of the paternal allelic contribution have been revealed in the Issaux forest, corresponding to two heterogeneity components:

— an inter-group component;
— an intra-group component with allelic frequencies of pollen pools that are different independently of whether the trees are several hundred meters apart (G1 group) or close together (G2 and G3 groups).

The Issaux population is often exposed to strong winds, particularly in open places, while the trees located within the dense forest are more protected. Hence, it seemed likely that the wind could have a more homogenizing effect on the pollen around isolated trees than within the forest, the forest boundary trees constituting an intermediate situation, being not only exposed to wind effects, but also influenced by their close neighbours. The results suggest that the three groups of trees show the same degree of heterogeneity yet mean allelic frequencies of the paternal contribution are different for the boundary group (G2). This last result could be explained by the particular location of the G2 group along a ridge exposed to frequent whirlwinds.

Other authors have observed such a pollen pool heterogeneity, particularly in allopollen, in populations of various plant species: *Picea glauca* (Moench.) Voss (Cheliak *et al.*, 1985); *Pinus contorta* (Hamrick and Schnabel, 1985); *Pseudotsuga menziesii* (Mirb.) Franco (Yeh and Morgan, 1987); *Cynosurus cristatus* L. (Ennos, 1985); *Bidens menziesii* (Ritland and Ganders, 1985).

In fact, the results obtained refer to the total paternal contribution (allopollen + autopollen), so that the observed differences correspond to variations of the individual rate of self-fertilization and/or to actual variations of allopollen frequencies. The latter may be due to a spatial structure of the genotypes (limited flows even for a wind-pollinated species, mating between relatives: Cuguen, 1986) or to phenotypic differences inducing changes of the temporal pollen allelic frequencies.

Acknowledgements

We thank R. M. Guilbaud for her technical assistance which was invaluable in carrying out electrophoretic analysis.

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Table 6. — Absolute allelic frequencies in paternal contribution — SV: Serreyrede forest; PF: Plo-du-Four forest.

	MDH1		IDH1		
	100	120	84	100	116
Mother-trees					
SV1	120	31	8	153	11
SV2	59	23	7	63	12
SV3	60	7	3	60	6
SV4	14	12	0	21	7
SV5	21	4	3	18	4
SV6	16	6	1	18	2
SV7	16	8	1	23	0
SV8	21	8	0	25	4
SV9	16	9	1	17	7
SV10	20	11	0	31	0
Total T4	363	119	24	429	53
χ^2 = 21.51 (p<0.005)			$\chi^2_{(1)}$=14.55 (p<0.05)		
PF1	101	82	12	167	14
PF2	18	12	5	20	5
PF3	18	4	0	21	1
PF5	4	5	2	8	0
PF6	16	15	0	30	2
PF7	14	9	1	19	3
PF8	22	3	0	27	0
PF9	26	8	3	27	1
PF10	11	13	4	22	3
Total T5	230	138	27	341	29
χ^2 = 22.01 (p<0.01)			$\chi^2_{(1)}$ = 8.91 n.s.		

$\chi^2_{(1)}$: Calculation was carried out grouping the two rarest alleles and excluding classes whose size was smaller than 4.

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Within- and Between-Population Variation in Growth of Pinus contorta var. latifolia: A Combined Study of Growth-Chamber and Field-Trial Experiments

By I. B.-J. Jiang[1]), A. Jonsson and G. Eriksson

Department of Forest Genetics,
Swedish University of Agricultural Sciences,
Box 7027, S-750 07 Uppsala, Sweden

(Received 13th June 1988)

Summary

Thirty-four plus trees of *Pinus contorta* var. *latifolia* were selected from four stands at the same latitude (60° N) in Yukon, Canada. Single-tree families from wind pollination were sown and grown in the growth chamber for four growth periods. Two levels of nutrient treatment (ordinary and stress) were applied from the second growth period and onwards. Plant height after each of the four growth periods, final stem diameters, green and oven-dry weights of the

[1]) Current address: Department of Forest Science, University of Alberta, Edmonton Alberta T6G 2H1, Canada.