Isoenzymes as an aid to clarify the taxonomy of French elms

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Isoenzymes were used to assess the genetic variability of the three French species of elms: *Ulmus laevis* Pall. from section *Blepharocarpus*, *Ulmus minor* Mill. and *Ulmus glabra* Huds. from section *Ulmus*. Three main results were obtained. The first was that these species are segmental tetraploids, i.e. they behave as tetraploids for part of the genome and as diploids for the rest of it. Secondly, we found that there exists a large amount of polymorphism in the French species of elm. Thirdly, *Ulmus laevis* produces isozyme patterns which differentiate it from species in section *Ulmus*. These results contribute to a clarification of the taxonomy of elms.

Keywords: genetic diversity, isoenzymes, polymorphism, taxonomy of elms, tetraploidy, *Ulmus* sp.

Introduction

The taxonomy of European elms is notoriously difficult. There are many reasons for this situation. Firstly, there are very few floral characters which discriminate between taxa. The fruits provide some useful traits to discriminate between major groups but they are not sufficient for more detailed discrimination. Also they are available for a short time only on mature trees and they are not found in many herbarium collections. Therefore, vegetative characters such as leaf shape, bark and cork features, and tree habit, are the main characters used to discriminate between the taxa (e.g. Jobling & Mitchell, 1974). These traits are very plastic; they vary even on a single tree during an individual life-time depending on age and environmental conditions. Herbarium samples do not supply information on the shape of the tree. Moreover, these traits can only be used with mature and nonpollarded trees (which, with Dutch Elm disease, are becoming scarce). Juvenile trees, which are in the majority in many populations, are very difficult to classify.

In addition to these technical difficulties, there has been extensive hybridization between taxa, which followed a phase of differentiation in several presumed refugia during the last glacial period (Richens, 1983, chap. 3). Moreover, some elms, especially field elms (*Ulmus minor* Mill. *sensu* Richens) and hybrid elms

(*U. × hollandica* Mill. *sensu lato*), have been extensively propagated by humans since prehistoric times (Richens, 1983). Cuttings were sometimes transported over large distances resulting in an intricate geographical pattern of many distinctive interlocked clones of varying size (see Richens, 1983 for a review of English populations).

Finally, botanists have burdened themselves with the rules of botanical nomenclature and the problems in applying them to recalcitrant material such as elm. More than 70 binomial names have been used for European elms, and there are many more intraspecific combinations. The same binomial is often used with a different meaning from one author to another.

In modern times, European botanists have used various taxonomic systems which lie between two extremes based on studies of English populations. One extreme position assumes the existence of many species and interspecific hybrids as described by Melville (1975, 1978). For British elms of the section *Ulmus*, he assumed the existence of six species, some 'almost hybridized out of existence', and 11 interspecific hybrids, involving up to four species. He carried out less investigation into continental European elms but recognized another species in the eastern Mediterranean region (*U. canescens* Melville). According to Richens (1983, p. 86), 'If the same approach were applied to the European elms as a whole, it would be difficult, without inconsistency of treatment, to get away with less than 20 species'. At the

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other extreme, Richens (1968) pooled all the European elms of the section *Ulmus* in two species: *U. glabra* Huds. and *U. minor* Mill. *sensu latissimo*, with one interspecific hybrid (*U. × hollandica* Mill. *s.l.*). Discussions concerning these two taxonomic treatments have been presented by Melville (1978) and Richens (1980).

For 50 years, very few studies have been concerned with the variability of French elm populations. Richens & Jeffers (1975, 1978) made a biometrical survey of elms of northern France. A taxonomic revision of elms in Anjou was carried out by Corillion (1991), using classical taxonomic methods; with a narrow-species concept, he assumed five species in this region (including the *U. laevis* Pallas species, which is quite different from the others and which belongs to the section *Blepharocarpus* Dumort.). In recent times, most French Floras and catalogues (e.g. Guinochet & Vilmorin, 1973; Grenier, 1992; Kerguelen, 1993) merely follow the taxonomic treatment of Tutin in *Flora Europaea* (1st edn, 1964), assuming four species in western Europe: *U. laevis* Pall. from the section *Blepharocarpus*, and *U. glabra* Huds., *U. minor* Mill. and *U. procera* Salisb. (= *U. minor* var. *vulgaris* (Aiton) Richens, see Richens, 1968) from the section *Ulmus*; but there is considerable disagreement and problems concerning the presence and frequency of this last species in France.

During the years 1970–80, the second epidemic of Dutch elm disease destroyed most mature trees in France as in other countries. The French Ministry for Agriculture has initiated a programme for the conservation of genetic resources including the search for and collection of healthy French elms and research. In the framework of this programme, we studied the genetic diversity of the elm collection. In this article, we present results obtained on allozyme diversity and we discuss implications for the systematics of French elms. Isoenzymatic data have been previously reported by Pearce & Richens (1977) and Richens & Pearce (1984) for European elms, with only one system, and by Sherman-Broyles *et al.* (1992) for the American species *U. crassifolia* Nutt. Wiegrefe (1992) and Wiegrefe *et al.* (1993) used molecular tools to study the genetic diversity of the genus *Ulmus*. In this paper, the taxonomic treatment of Richens is adopted (assuming four taxa: *U. laevis*, *U. glabra*, *U. minor* and the hybrids between *U. glabra* and *U. minor*: *U. × hollandica*) because our vegetative material, consisting mainly of young trees from cuttings, did not permit us to distinguish more precise species.

Materials and methods

In the framework of the programme led by the CEMAGREF (French Institute of Agricultural and Environmental Engineering Research), aimed at preserving the genetic diversity of French elms, a thorough survey was carried out in five regions (Table 1) to list every mature and healthy elm tree (diameter of the trunk more than 15 cm and height more than 1.3 m). Most trees were cloned by cuttings which were planted in a conservatory plantation at Nogent-sur-Vernisson (Loiret, France) comprising about 270 trees in 1993.

The plant material used for our study consisted of samples taken from all the trees in the conservatory plantation and other samples taken from other trees in their natural environment. Field determinations of species were made by the persons who collected the samples. We worked on 298 trees: 151 *U. minor* Mill., 48 *U. glabra* Huds., 74 *U. laevis* Pall. and 25 trees which have been recorded as putative hybrids between *U. minor* and *U. glabra*, (*U. × hollandica* Mill. *sensu lato*). Additionally, seedlings of offspring of three *U.*

Table 1 Sample sizes (*N*) for each region and species of elm

Regions	<i>N</i>	Species	<i>N</i>
Nord-pas de Calais	26	<i>U. minor</i>	20
		<i>U. glabra</i>	0
		<i>U. laevis</i>	6
		<i>U. × hollandica</i>	0
Ile de France	30	<i>U. minor</i>	18
		<i>U. glabra</i>	2
		<i>U. laevis</i>	8
		<i>U. × hollandica</i>	2
Normandie	65	<i>U. minor</i>	43
		<i>U. glabra</i>	2
		<i>U. laevis</i>	0
		<i>U. × hollandica</i>	20
Poitou	64	<i>U. minor</i>	59
		<i>U. glabra</i>	0
		<i>U. laevis</i>	5
		<i>U. × hollandica</i>	0
East of France	113	<i>U. minor</i>	11
		<i>U. glabra</i>	44
		<i>U. laevis</i>	55
		<i>U. × hollandica</i>	3
Total	298	<i>U. minor</i>	151
		<i>U. glabra</i>	48
		<i>U. laevis</i>	74
		<i>U. × hollandica</i>	25

minor trees (open pollinated) were checked to confirm the inheritance of the electrophoretic patterns we obtained. As the trees of the conservatory plantation were immature, this study was conducted on trees from Orsay (Essonne, France).

An American Elm (*U. americana* L. sect. *Blepharocarpus*) from the 'Arboretum des Barres' (Loiret, France) was also studied.

For the enzyme extraction, young leaf tissue was crushed with a mortar and pestle in liquid nitrogen and enzymes were extracted with a cysteine buffer: 1 g of leaf was ground with 3 mL of 10 mM Tris Cl⁻ pH 7.2, 4 mM dithioerythritol, 5 mM cysteine and 10 per cent polyvinylpyrrolidone (soluble PVP). After centrifugation (3000 g for 20 min) extracts were stored at -70°C until used for electrophoresis. Horizontal starch gels were composed of 13 per cent starch. Catalase, CAT (E.C. 1.11.1.6), anodic peroxidase, PRXA (E.C. 1.11.1.7) and phosphoglucose isomerase, PGI (E.C. 5.3.1.9) were resolved using a lithium-borate electrode and gel buffer adapted from Soltis & Soltis (1990). Malate dehydrogenase, MDH (E.C. 1.1.1.37), phosphoglucose mutase, PGM (E.C. 5.4.2.2) and 6-phosphogluconate dehydrogenase, 6PGD (E.C. 1.1.1.44) were resolved on an histidine gel and electrode buffer also adapted from Soltis & Soltis (1990) and cathodic peroxidase, PRXC (E.C. 1.11.1.7) was resolved using a Tris maleic gel and electrode buffer adapted from Shaw & Prasad (1970).

Measures of allozyme diversity were estimated by the software 'BIOSYS-1' (Swofford & Selander, 1981). Nei's genetic diversity H_T (Nei, 1973) was calculated

from all the loci but Nei's genetic distances (Nei, 1972) were only calculated from the systems whose behaviour was disomic.

Results

The seven systems we studied revealed eight loci. For some of the systems (CAT, 6PGD, PRXC and PGI), the patterns we obtained suggest disomic inheritance. The 6PGD system, for example (Fig. 1), shows typical dimeric patterns (one or three bands) assuming a disomic system.

For the other systems (PGM, PRXA and MDH), the number of bands and the variability of their intensity seem to be typical of a tetrasomic system. PGM patterns, for example (Fig. 2), consist of one to four bands of variable intensity. All the patterns found for this system may be explained on the basis of tetrasomic inheritance. The resolution of the PRXA gels was unclear but the patterns suggest that this system has the same behaviour as the PGM system. For MDH, the dimeric banding patterns (Fig. 3) showed variable intensity, which is easily explained by tetrasomic inheritance.

Table 2 gives the results for the three trees from Orsay and their offspring. The patterns are often monomorphic and provide little evidence to elucidate the inheritance system in these species.

The trees sampled for this study were very polymorphic for isoenzyme patterns. In particular, *U. minor* presented a high mean number of alleles per locus (Table 3) whereas *U. glabra* seemed to be mono-

Fig. 1 Photograph and zymogram of 6PGD phenotypes.

Fig. 2 Photograph and zymogram of PGM phenotypes.

morphic for the 6PGD system. *U. laevis* showed the least polymorphism (a mean of 1.9 alleles per locus) and was monomorphic for PRXA slow and fast loci and for the catalase system.

On the basis of these banding patterns, Nei's genetic diversity is higher for *U. glabra*, *U. minor* and *U. × hollandica* compared with *U. laevis*.

Allele frequencies are shown in Table 4 and the results of a χ^2 test to compare the allele frequencies

between the different species are presented in Table 5. The distributions of alleles were completely different between *U. minor* and *U. glabra*. We did find, however, similarities between *U. minor* and the intermediate forms and also between *U. glabra* and the intermediate forms.

A large difference was found between *U. laevis* and the other species for the PRXA system (Fig. 4). For the species *U. glabra* and *U. minor*, this system is interpreted as two independent tetraploid loci, both presenting four different alleles. *U. laevis* did not show any of the bands found in the other elm species at the slower locus. At the faster locus, all the *U. laevis* plants only presented a thin band at the level of the third allele in the other elms and, additionally, a characteristic thick band below those found in the other elms. The same phenotype was also observed for an elm from the same section: the *U. americana* tree from the 'Arbore-

Fig. 3 Zymogram of MDH phenotypes.

Table 2 Analysis by electrophoresis of the offspring of three *Ulmus minor* mother trees for PGM, MDH and PRXA fast loci

Locus	Genotype of the mother tree	Size of the offspring (seedlings)	Phenotype of the offspring
PGM	Tree no. 1: undetermined	8	Same phenotype for all the seedlings.
	Tree no. 2: undetermined	12	One band at the B level and one band at the C level, both having same intensity.
	Tree no. 3: undetermined	13	
MDH	Tree no. 1: undetermined	8	Same phenotype for all the seedlings.
	Tree no. 2: undetermined	12	One thin band at the A level, one weak band at the B level.
	Tree no. 3: undetermined	13	
PRXA fast locus	Tree no. 1: AACC	8	
		= 5	One band A, one band D, same intensity.
		and 3	One band C, one band D, same intensity.
	Tree no. 2: AADD	12	
		= 2	One band A, one band C, same intensity.
		4	One band D.
		4	One band A, one band D, same intensity.
		1	One band A, one band B, same intensity.
	Tree no. 3: DDDD	and 1	One band C, one band D, same intensity.
		13	
		= 6	One band A, one band D, same intensity.
		and 7	One band C, one band D, same intensity.

Table 3 Nei's genetic diversity for eight loci in the different species of elm

Species	Mean sample size per locus	Mean no. of alleles per locus	Polymorphic loci (%)	Nei's genetic diversity H_T
<i>U. minor</i>	114.8	3.0 (0.3)	100	0.330 (0.066)
<i>U. glabra</i>	34.9	2.6 (0.4)	75.0	0.395 (0.089)
<i>U. laevis</i>	54.9	1.9 (0.2)	75.0	0.247 (0.077)
<i>U. × hollandica</i>	19.9	2.9 (0.4)	87.5	0.366 (0.097)

Standard errors are in parentheses.

Table 4 Allele frequencies for eight polymorphic loci for each species of elm

Allele		<i>U. minor</i>	<i>U. × hollandica</i>	<i>U. glabra</i>	<i>U. laevis</i>
<i>PRXA</i> s.	<i>N</i>	0	0	0	1
	1	0.05	0.06	0.15	0
	2	0.12	0.19	0.59	0
	3	0.69	0.56	0.24	0
	4	0.14	0.19	0.02	0
No.		132	18	26	74
<i>PRXA</i> f.	1	0	0	0	0.75
	2	0.01	0.16	0.06	0
	3	0.50	0.32	0.39	0
	4	0.47	0.41	0.36	0.25
	5	0.02	0.11	0.19	0
No.		131	23	48	33
<i>6PGD</i>	1	0.91	0.98	1	0.93
	2	0.08	0.02	0	0.07
	3	0.01	0	0	0
No.		92	21	33	51
<i>PGM</i>	1	0.06	0.17	0.10	0
	2	0.45	0.34	0.15	0.09
	3	0.45	0.32	0.20	0.52
	4	0.04	0.17	0.55	0.39
No.		137	23	44	71
<i>PGI</i>	1	0.10	0.05	0.04	0.86
	2	0.87	0.86	0.96	0.14
	3	0.03	0.09	0	0
No.		134	22	46	71
<i>PRXC</i>	1	0.05	0.11	0.18	0.92
	2	0.95	0.89	0.82	0.08
No.		124	19	22	24
<i>CAT</i>	1	0.89	0.93	0.76	1
	2	0.11	0.07	0.24	0
No.		59	14	27	45
<i>MDH</i>	1	0.20	0.13	0.57	0.59
	2	0.80	0.87	0.43	0.41
No.		109	19	33	29

No. is the sample size. We assume that *Ulmus laevis* trees have a null allele (*N*) at the slower locus of the *PRXA* system and that the thin band is allele number 1 of the second locus (i.e. the genotypes are always *AAAD*, see Fig. 4).

tum des Barres' (Nogent-sur-Vernisson, France) but never for the other European elms (*U. glabra* and *U. minor*). No intermediate form was observed between the characteristics of *U. laevis* and those of the others.

The phenogram for species based on the calculations for disomic loci is shown in Fig. 5. One cluster was composed of *U. glabra*, *U. minor*, and

U. × hollandica which was intermediate between *U. glabra* and *U. minor*. The genetic distances within this cluster are very low. The other cluster only consisted of the *U. laevis* group and was separated at a distance of 0.71 unit.

Discussion and conclusion

Inheritance and tetraploidy

The electrophoretic patterns that we obtained suggest disomic inheritance for four loci in our study (*6PGD*, *PGI*, *PRXC* and *CAT*) and tetrasomic inheritance for the other four loci (*PRXA* *slow* and *fast*, *PGM* and *MDH*) based on observations from 298 trees.

In the *PGM* system, for example (Fig. 2), individuals can produce one to four bands. Three arguments lead us to the conclusion that inheritance is tetrasomic. (i) If many individuals possess three or four bands, it is possible that two or more loci are involved. However, as many individuals produce only one band, this would assume that null alleles exist at the loci in the homozygous state. A more simple explanation is that only one tetrasomic locus exists. (ii) When one individual possesses three bands, one of them is always more intense than the two others, indicating that the genotype could be *AABC* type. (iii) Almost all the combinations of the four alleles are found in our sample (Table 6).

For the *PRXA* loci, the arguments for tetrasomic inheritance are almost the same but the phenotypes are not well defined and it is difficult to ascertain the exact genotypes and patterns of inheritance. We assume that they are like the *PGM* system.

For the *MDH* system (Fig. 3), the arguments are different. This enzyme is usually dimeric. The trees of our sample possess one or three bands with variable intensity. A hypothesis of tetrasomic inheritance would explain all the zymograms obtained: (i) homozygous trees give only one spot, and (ii) heterozygous trees give three more or less intense spots depending on whether they are simplex digenic (*ABBB* or *AAAB*) or duplex digenic (*AABB*).

Our interpretation of the patterns we obtained suggests that elm trees are segmental allotetraploids. These species could derive from doubling the chromosomes of an interspecific hybrid whose parents were genetically close so that their chromosomes retain a high degree of homology, but which may differ by small structural changes. The doubling could also originate in an autotetraploidization of an *Ulmus* ancestor. We may assume that this occurred a long time ago followed by incomplete diploidization. This phenomenon has already been described in animal groups (Waples,

Table 5 Results of χ^2 tests comparing allele frequencies between the different species of elm

Locus		<i>U. m.</i> and <i>U. g.</i>	<i>U. m.</i> and <i>U. l.</i>	<i>U. g.</i> and <i>U. l.</i>	<i>U. m.</i> and <i>U. × hollandica</i>	<i>U. g.</i> and <i>U. × hollandica</i>
<i>PRXA s.</i>	d.f.	3	4	4	3	3
	χ^2	152	824	400	6.02	45
	<i>P</i>	0.0001	0.0001	0.0001	0.1105	0.0001
<i>PRXA f.</i>	d.f.	3	4	4	3	3
	χ^2	95	573	338	67	11
	<i>P</i>	0.0001	0.0001	0.0001	0.0001	0.0142
<i>6PGD</i>	d.f.	2	2	1	2	1
	χ^2	12	1.44	9	3.97	3.17
	<i>P</i>	0.0022	0.4867	0.0021	0.1375	0.0749
<i>PGM</i>	d.f.	3	3	3	3	3
	χ^2	263	238	65	41	36
	<i>P</i>	0.0001	0.0001	0.0001	0.0001	0.0001
<i>PGI</i>	d.f.	2	2	1	2	2
	χ^2	12	462	299	10	17
	<i>P</i>	0.0026	0.0001	0.0001	0.0074	0.0002
<i>PRXC</i>	d.f.	1	1	1	1	1
	χ^2	19	391	101	3.41	1.91
	<i>P</i>	0.0001	0.0001	0.0001	0.0649	0.1666
<i>CAT</i>	d.f.	1	1	1	1	1
	χ^2	10	21	48	0.74	7
	<i>P</i>	0.0017	0.0001	0.0001	0.3907	0.0078
<i>MDH</i>	d.f.	1	1	1	1	1
	χ^2	67	68	0.08	1.95	38
	<i>P</i>	0.0001	0.0001	0.7744	0.1629	0.0001

U. g., *Ulmus glabra*; *U. l.*, *U. laevis*; *U. m.*, *U. minor*. Bold indicates nonsignificance.

Fig. 4 Photograph of isoenzyme phenotypes for the PRXA system. Arrows show the *Ulmus laevis* phenotypes among all the French species of elm.

1988) and also in many plant groups (Stebbins, 1971). Wild olive trees (*Olea europaea* L.) for example seem to have the same behaviour (Ouazzani *et al.*, 1993).

The results (Table 2) for the offspring of the three mother trees from Orsay do not produce clear-cut evidence about the inheritance of the systems for many reasons. (i) For PGM and MDH, the patterns were monomorphic and the genotypes of the mother trees were missing; and (ii) for all the systems tested on the offspring, electrophoresis was performed on seedlings. It is possible that the expressions of genes are different in young plantlets and in the leaves of an adult tree. The tetraploid patterns could be the result of the expression of two loci (isoloci) placed on two homeologous pairs of chromosomes with each locus showing independent disomic inheritance as in the case of *Lotus* (Raelson *et al.*, 1989). It is possible that for the elm trees, one of these two loci does not express in young seedlings, producing disomic inheritance. The answer to this problem would be to study the adult offspring of selected genotypes, but we do not have such material.

Fig. 5 Phenogram of UPGMA cluster analysis based on Nei's genetic distances between the different French species of elm.

Table 6 Number (*N*) of elms with the different genotypes found for the *PGM* system

Genotypes	<i>N</i>	Genotypes	<i>N</i>	Genotypes	<i>N</i>	Genotypes	<i>N</i>
AAAA	1	ABBD	1	BBBB	13	BCCD	2
AABB	8	ABCC	6	BBBC	15	BCDD	5
AABC	9	ABCD	3	BBBD	2	CCCC	14
AACC	8	ABDD	1	BBCC	71	CCDD	4
AADD	2	ACDD	1	BBDD	15	CDDD	1
ABBC	15	ADDD	3	BCCC	29	DDDD	21

Few karyological studies have been carried out on these elm species. It is known that they all possess $2n = 28$ chromosomes except *U. americana* which has $2n = 56$ (Fedorov, 1974) while no species in the *Ulmaceae* family possesses 14 chromosomes. Leliveld (1934), observing meiosis of elm trees, saw only bivalents. Nevertheless, this does not preclude tetrasomic inheritance in some systems because Dawson (1941) demonstrated tetrasomic inheritance of cyanogenesis in *Lotus* when only bivalents were observed.

Comparison of the different species

The allele frequencies obtained indicate that each species possesses its own characteristic distribution of alleles. In our sample, *U. minor* and *U. glabra* had the same alleles, although with different distributions, and the genetic distances between them were very low. Intermediate forms (putative *U. x hollandica*) had an intermediate distribution. This indicates that the morphological continuum existing between the two extreme species is the expression of a true genetic continuity. Numerous authors, in the framework of selection of clones resistant to Dutch Elm disease, have published results of interspecific crosses (Townsend, 1975; Mittempergher & La Porta, 1991; and others reviewed by Heybroek, 1992). They showed that *U. glabra* and *U. minor* can crossbreed freely and that the hybrids are completely fertile. Backcrossing can give a range of possible intermediates which probably occur in overlapping distribution zones.

The results we obtained for *U. laevis* trees showed that this species is very close to the other elm species in many respects: they have the same number of chromosomes, they possess the same tetraploid structure, with the same diploidized systems, and for several systems, the difference found in the allele frequencies between *U. laevis* and the other species is not higher than that observed between *U. minor* and *U. glabra*. On the other hand, differences in the expression of the PRXA system set them apart. The results we obtained for the PRXA system of *U. laevis* can be interpreted in different ways depending on whether the characteristic thick band is attributed to one or the other locus existing in the other species of elms. *U. laevis* possesses a specific allele and/or a null allele for the slower locus. As long as no variability is found for this system it is impossible to determine the right interpretation. Nevertheless, the genetic distance calculated (Fig. 5) reflects the large difference existing between this elm species and the others. Opinions are divided, however, about the ability of *U. laevis* to crossbreed with the elms of the *Ulmus* section. Heybroek (1992) notes that Klotzsch (1855) obtained 'extremely vigorous hybrids' between *U. campestris* (syn. *minor*) and *U. effusa* (syn. *laevis*). More recent authors (Townsend, 1975; Mittempergher & La Porta, 1991) have not been able to obtain such hybrids in controlled crosses. The results we obtained could be a consequence of a genetic divergence between the different sections and sexual isolation of *U. laevis* in relation to the other French species.

Genetic diversity

Generally, elm trees are more diverse than other tree species in spite of the Dutch Elm disease which has severely restricted the population size: calculations for Nei's genetic diversity gave 0.258 for *Castanea sativa* Mill. (personal observation) and 0.275 for *Quercus petraea* (Kremer & Petit, 1993) in similar studies.

In our sample, the genetic diversity was higher for *U. glabra* than for the other French elm species. This result is in agreement with those found by Richens & Pearce (1984) concerning isoperoxidase variation. It can be explained by the fact that *U. glabra* is a sexually reproducing tree, native to western Europe, whereas *U. minor* has always been mainly asexually propagated.

U. laevis showed a lower genetic diversity than the other species. This result probably reflects its sexual isolation from the other French elms and possible drift caused by the disastrous effects of Dutch Elm disease.

In conclusion, this paper reports the first analysis of genetic diversity in French elms. At the isozymic level, the variability is rather high compared with other tree species. Because of the paucity of taxonomic characters, other methods have to be used to clarify the systematics of the genus *Ulmus*. Even though the number of markers used is low, the results of our study clearly demonstrate the unambiguous genetic distinction of the two sections but the differences between *U. minor* and *U. glabra* and the intermediate *U. × hollandica* are so small that they should be regarded as belonging to a common species.

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