GENETIC DIFFERENTIATION OF ELM (*ULMUS MINOR* MILL., *ULMUS LAEVIS* PALLAS) IN MIXED STANDS FROM THE ELBE FLOOD-PLAINS

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ABSTRACT

As many as 238 white elms (*Ulmus laevis* Miller) and 109 field elms (*Ulmus minor* Pallas) stocking in hardwood-floodplain relicts upon Elbe river middle reaches, Sachsen-Anhalt, Germany, were characterised genetically using starch gel electrophoresis. The genetic analysis on the basis of 344 single-plant progenies from open pollination gave no reason to reject the hypothesis for the genetic control of the investigated isoenzymes neither qualitatively nor quantitatively. Due to qualitative, interspecific differences of the zymograms between field and white elm, starch gel electrophoresis offers a helpful tool to classify the samples into either species.

Key words: flood-plains, genetic differentiation, isoenzymes, *Ulmus laevis*, *Ulmus minor*

INTRODUCTION

Preliminary investigations dealing with electrophoretic analysis of isoenzymes within the genus *Ulmus* were conducted already during the 80s. FERET and STAIRS (1971), FERET (1972) as well as PEARCE and RICHENS (1977) and RICHENS and PEARCE (1984) studied peroxidase from tissue of single individuals, classified into morphologically different species. For instance, RICHENS and PEARCE (1984) ascertained clear positional differences between *U. pumila*, *U. rubra*, and *U. laevis* and only individual, additional variants between the species *U. americana*, *U. glabra* and *U. minor*. The plant material under investigation originated both from hedges and from the Royal Botanic Garden in Kew, UK. The aim of these preliminary studies was a taxonomic classification of the individual elm species.

The principal goal of the present investigation is a preliminary genetic characterisation of field elm (*Ulmus minor* MILL.) and white elm (*Ulmus laevis* PALLAS) growing in floodplain forests upon middle reaches of the Elbe river, near Dessau, Sachsen-Anhalt, Germany. In this area there are relict occurrences of the two species which represent a characteristic species of the hardwood-floodplain (*Querco-Ulmetum* ISSLER 24, see HÄRDTLE *et al.* 1996). Usually the elm is strongly pushed back in favour of oak, or has succumbed to the Dutch elm disease (MINCKWITZ 1954, SCHAUER 1970, WAGNER 2000). The relict stands represent elms with diverse phenotypes on account of their different ages from roughly 20 up to 80 years.

MATERIALS AND METHODS

In most cases field elms occur sociologically suppressed in small groups of e. g. up to 20 adult trees and their natural revegetation. Twelve of such spatially isolated groups have been involved in the present investigation (Fig. 1). In contrast to this, white elms occur either as solitary trees, or in terms of a sympatric distribution together with field elm within predominant oak and ash stands. Three such solitary groups have

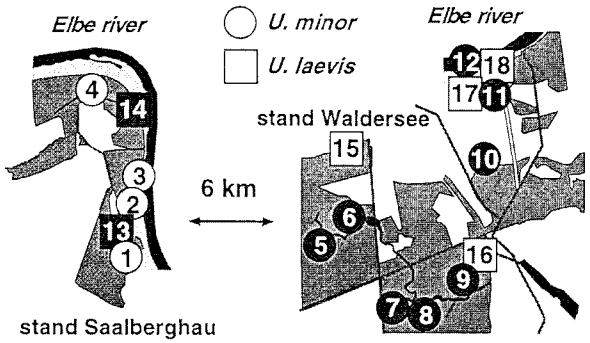

Figure 1. Localities of the 12 relict field elm groups (circle) and six white elm groups (square), including the three plantatins No. 15, 16, and 18. Having a clear ocular separation between the both flood-plains Saalberghau and Waldersee the white elm groups in Saalbergau and - vice versa - the field elm groups in Waldersee are black marked.

been completely examined. Juvenile white elms have so far not been found in the area under study. Hence, of three plantations, originated from the local flood-plains and aged between 20 and 40 years, each 50 trees were sampled. The area under study refers to two localities of which one has been a nature preserve since 1926 (stand Saalberghau, Fig. 1), the other being a floodplain forest complex located six kilometres away (stand Waldersee, Fig. 1).

One tree known morphologically as *U. laevis* and five other elms known as *U. minor* from the Botanical Garden and Arboretum Tharandt, Germany, as well as a progeny of a field elm from Rhine floodplain, and one specimen of whych elm (*Ulmus glabra* Hudson) from the Arboretum at Göttingen, Germany, were available as references for discrimination between species.

Furthermore, five morphological *U. laevis* from urban area of Göttingen and 10 *U. glabra*-types from Rabenauer Grund, Sachsen, were also included to the species differentiation.

Six isoenzymes of juvenile leaves material of a total of 109 field elms and 238 white elms were examined by electrophoresis (Tab. 1). The components of the extraction buffer are described in Table 2. The methods of starch gel electrophoresis, tested and adapted during the routine investigations on *Ulmus*, are outlined in Table 3.

Staining was slightly modified according to CHELIAK and PITEL (1984) and was carried out during incubation at 38 °C for about 15–30 minutes.

Table 1. Enzyme systems and corresponding gene loci.

Enzyme system	E. C. - number[1]	Quartenary structure	Gene loci
MDH	1.1.1.38	dimeric	*Mdh-A, B*
IDH	1.1.1.42	dimeric	*Idh-A*
6-PGD	1.1.1.44	dimeric	*6-Ppd-A, B*
PGM	2.7.5.1	monodimeric	*Pgm-A, B*
PGI	5.3.1.9	dimeric	*Pgi-A, B*
GOT	2.6.1.1	dimeric	*Got-A, B*

[1] E. C. = Enzyme Commission

Table 2. Components of extraction buffer.

132.1	mmol/l TRIS/HCl (1 mol/l) pH 7.3 (Tris(hydroxymethyl)aminomethane, $C_4H_{11}NO_3$)
3.4	mmol/l DTT 1,4-dithiotrietol, $C_4H_{10}O_2S_2$)
4.1	mmol/l EDTA Titriplex II (Ethylen dinitrilotetra acetic acid, $C_{18}H_{16}N_2O_8$)
3.6	mmol/l PVP "15"(polyvinylpyrolidone 15)
1%	[v/v] 2- mercaptoethanol 1 g DTE, C_2H_6OS

Table 3. Conditions of starch gel electrophoresis.

Proteins	Gel buffer	Gel components	Electrode buffer	Amperage	Time
IDH, MDH, 6-PGD	0.05/0.014 mol/l Tris[1]/citrate[2] pH7.4	11 % [g/100 ml] starch 75.6 mmol/l urea[4]	0.05/0.014 mol/l Tris[1]/citrate[2] pH7.4	180mA	5.5 h
PGI	0.05/0.012 mol/l Tris/citrate pH8.1 +10% [v/v] electrode buffer	11 % [g/100 ml] starch 66.4 mmol/l saccharose[5]	0.19/0.025 mol/l boric acid[3]/LiOH pH 8.1	80mA	4 h
PGM, GOT	0.08 mol/l Tris/HCl pH8.7 + 3 % electrode buffer	11 % [g/100 ml] starch 66.4 mmol/l saccharose[5]	0.30/0.062 mol/l boric acid[3]/NaOH pH 8.0	80 mA	4 h

[1] (Tris(hydroxymethyl)aminomethane, $C_4H_{11}NO_3$); [2] citric acid ($C_6H_8O_7 \cdot H_2O$; [3] H_3BO_8; [4] CH_4N_2O; [5] $C_{12}H_{22}O_{11}$

RESULTS AND DISCUSSION

Description of zymograms

Single-tree inheritance analysis on five strongly seed producing white elms from the village Flechtingen and six field elms from stand Waldersee (Fig. 1), Sachsen-Anhalt, Germany, did not reveal any contradiction to the interpretation of the zymograms for a total of 344 examined progenies. The results of the analysis according to the hypothesis by GILLET (1997) are shown in Tables 4 and 5 ($H_0: p_i = p_j = 0.5$, with p = observed frequency in the progeny of the alleles i and j). In this context, the fact has to be verified that the self-type alleles i and j are inherited by a heterozygotic mother tree of the genotype N_{ij} to its progeny at a ratio of 1:1 supposing random fusion of the female gametes.

The variation of the isoenzyme phenotypes allows a clear differentiation between white elm (*U. laevis*) and field elm (*U. minor*). For example, the enzyme system Got shows two zones for *U. minor* and *U. laevis*, of which the faster one is monomorphic in *U. laevis*. Conversely, the slower B zone in *U. minor* does not reveal any variation, but the A zone does with currently six isoenzyme phenotypes.

Compared to GOT, two zones of different migration ratio have been found in the enzyme system MDH as well (Fig. 2). Whereas the faster A zone regarding *U. minor* varies with five phenotypes, *U. laevis* phenotypes do not indicate any variation even for both zones. In this context, the large positional differences in A and B zone between *U. minor* and *U. laevis* phenotypes show a clear discrimination between the two elm species.

The enzyme system PGM shows also similar differences (Fig. 2). The zymogram reveals two zones each for the two species. Although the faster A zone shows a migration ratio identical for the two species, there are considerable differences in the position of the B zone between the two species. *U. minor* phenotypes of B zone directly migrate to the A zone, whereas the types of *U. laevis* tissue always remain distinctly below the *U. minor* B zone. Figure 2 shows clear the differences in zone positions for 6-PGD analogously to PGM and MDH. Only PGI phenotypes show an identical position for all isoenzyme variants between both tree species, whereas the single zone observed for IDH shows slight position changes. From that point of view for all enzyme systems except of GOT and PGI a genetic control by different but similar gene loci between *U. laevis* and *U. minor* has to be assumed. This assumption is supported by the zymorgams of 13 *U. minor* and *U. laevis* trees as well as 10 *U. glabra* types which are used as references (cf. Fig. 2).

Differentiation within and between the elm species

In comparison with similar genetic inventories on *Fagaceae* (TUROK 1996, MÜLLER-STARCK 1996, HERZOG 1998, KRABEL & HERZOG 1999, GEHLE 1999) polymorphic gene loci of the sampled field elm and white elm appear to be characterised by a high genetic variation in the area under study (Tab. 6). Field elm seems to be strongly differentiated between its smallest

Table 4. Results of the genetic analysis of *Ulmus laevis*. N = sample size. Test of fit to the χ^2 - distribution with statistic $G, df = 2$ und $P < \alpha = 0.05$ *, $\alpha = 0.01$ **, $\alpha = 0.001$ *. The indices i and j are defined as the self-type alleles of the mother tree genotype. Index $k \neq i, j$ describe foreign-type alleles.**

Gene locus	N	genotype mother tree N_{IJ}	P	N_{ik}	N_{jk}
Got-B	73	12	0.572	0	0
Pgi-B	88	45	0.228	0	0
6-Pgd-A	121	23	0.157	0	3
Pgm-A	123	12	0.819	0	1
Pgm-B	54	23	0.549	0	0

Table 5. Results of the genetic analysis of *Ulmus minor*. Text see table 4.

Gene locus	N	genotype mother tree N_{IJ}	P	N_{ik}	N_{jk}
Got-A	124	23	0.995	10	11
Pgm-B	153	23	0.803	5	4
Mdh-A	104	13	0.886	0	1
Idh-A	65	12	0.846	10	7

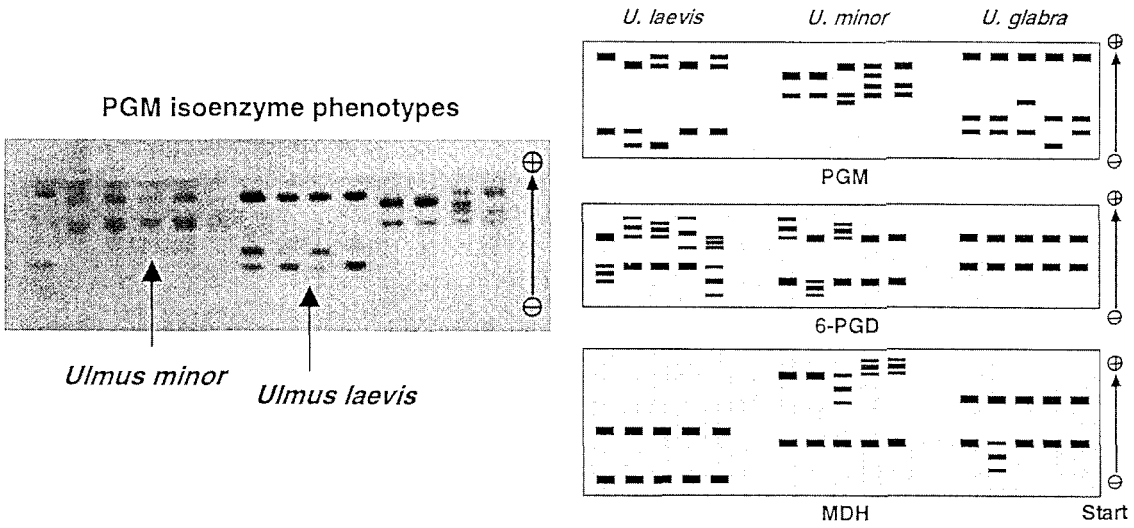

Figure 2. Differences between selected isoenzyme phenotypes of the three elm species. The scanned photo on the left shows a gel staining for PGM. On the right the pattern scheme of PGM, 6-PGD and MDH are shown.

Table 6. Mean values of some variation parameters of *U. minor* and *U. laevis*.

Variation parameter		*U. minor* $N = 109$	*U. laevis* $N = 238$
alleles per gene locus	A/L	3.3	2.1
diversity	v [1), 2)]	1.542	1.486
total population differentiation (%)	δ_T [1)] (%)	35.5	32.8
proportion of heterozygosity (%)	H_{ab} (%)	21.6	29.8

[1)] GREGORIUS (1978, 1987), [2)] ROUTLEDGE (1979)

demes (subpopulation differentiation δ = 23.5 %, GREGORIUS 1985). In contrast to this, white elm shows values with δ = 7.3 % which were likewise measured between demes of *Fagaceae*.

The actual high proportion of heterozygosity (*U. minor* H_a = 0.216, *U. laevis* H_a = 0.298) is striking in the total variation of the two tree species. If comparing the actual proportions of heterozygosity between the two localities Saalberghau and Waldersee, both interspecific and intraspecific differences become apparent. Regarding *U. minor*, H_a shows counter-rotating values at each gene locus, i.e. if the value for H_a in Saalberghau is high, it seems to be low in Waldersee, and vice versa. The actual proportions of heterozygosity at the gene loci *Pgm-A* and *Pgm-B* in Saalberghau values of 2.7 %, whereas in Waldersee H_a values come up to 21 % (*Pgm-A*) and 28 % (*Pgm-B*). Gene locus *6-Pgd-B* is monomorphic (fixation on allele B_2), whereas in Saalberghau the heterozygotes constitute a proportion of 27 % (genotype B_2B_3). In contrast, the H_a values referring to *U. laevis* appear to be specific for each gene locus. However, H_a is higher for trees of the plantations in Waldersee than for the solitary trees in Saalberghau. It is remarkable, that those demes of both species with the highest and lowest proportions of heterozygosity each are adjacent. (Fig. 3).

Figure 4 shows the result of a cluster analysis (UPGMA dendrogram, ROHLF 1997) with the pairwise genetic distance d_0 (GREGORIUS 1974). Genetically, the elm groups appear to be more similar each within the two localities Saalberghau and Waldersee, being about 6 km apart from one another (Fig. 1), than the elms between the two localities. Field elm from Saalberghau and field elm from Waldersee do not originate from one parent population (test of homogeneity to the χ^2 –distribution with statistic G, WOOLF 1957). This result has to be confirmed for both their genetic and genotypic structures and for the distribution of the degree of heterozygosity across all gene loci. The degree of heterozygosity H describes the proportion of investigated gene loci at which an individual is heterozygous. Except groups No. 1, 5 and 6, the single occurrences also cluster into the two locality groups. In contrast to this result, the solitary trees (group No. 14) and the trees standing on an avenue in Saalberghau (group 13) white elm do hardly differ from the three plantations

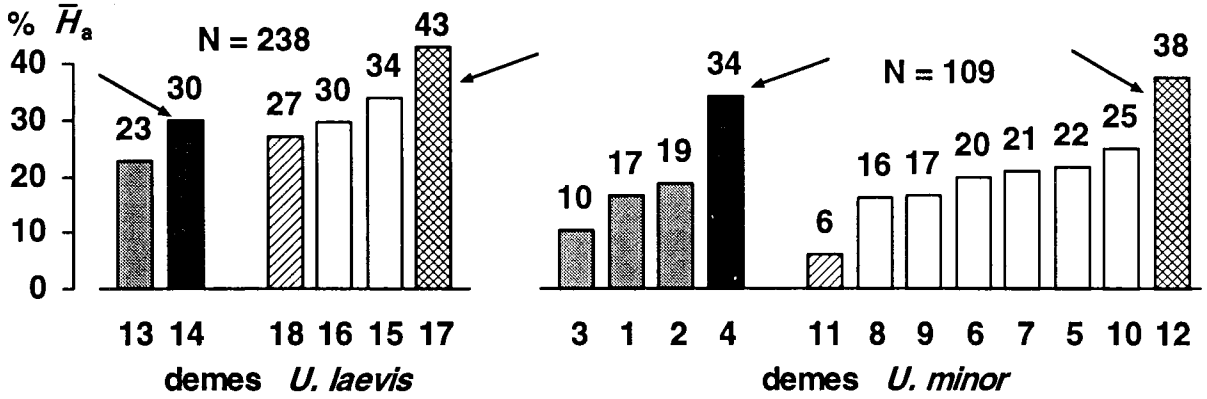

Figure 3. Average proportion of heterozygosity $\bar{H}_a$ of the elm groups of Fig. 1, sorted by size and subdivided by localities Saalberghau (left) and Waldersee (right). Group No. 13 is neighbouring the groups No. 1, 2 and 3. Group No. 14 is adjacent to group No. 4, and so is group No. 18 to group No. 11, and group No. 17 to group No. 12. The highest degrees of heterozygosity are encountered in interspecifically adjacent elm groups, respectively (see arrows, cf. Fig. 1).

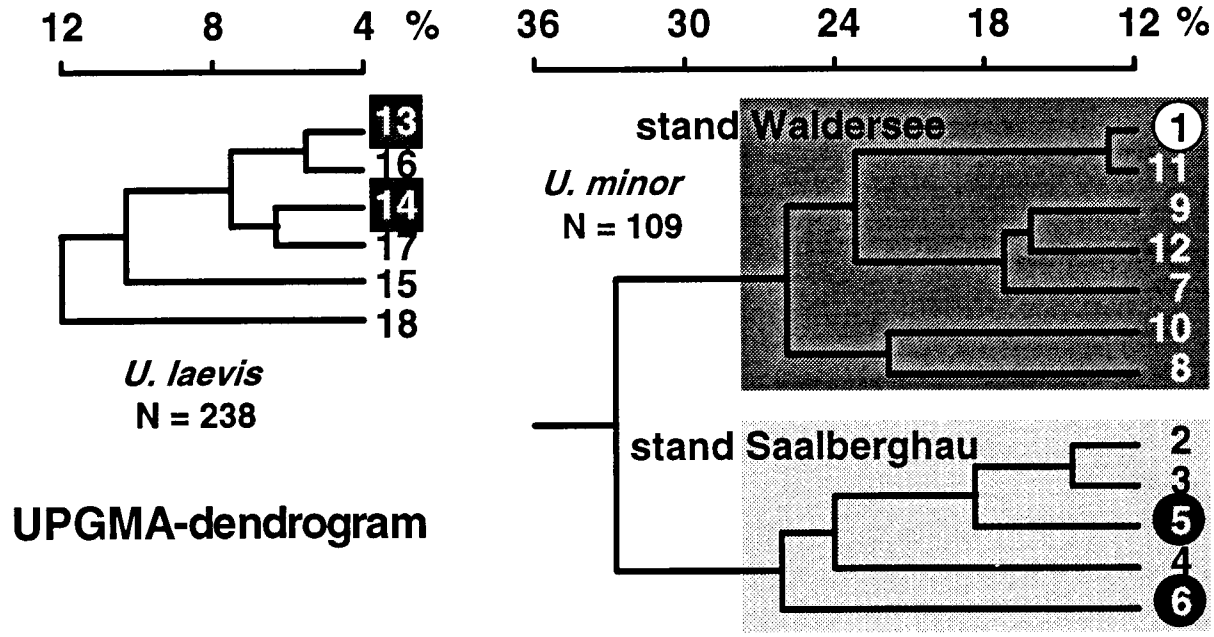

Figure 4. UPGMA-dendrogram of field and white elm groups. The groups appear to cluster corresponding to their genetic similarity. A cluster is computed based on the mean value for each of the smallest pairwise genetic distances d_0. The groups are analogously marked to the system in Fig. 1. For example, the field elm groups No. 5 and 6 are marked as black circles because they occur in the Waldersee floodplains except group No. 1 which was sampled in Saalbergau.

(Group No. 15, 16, 18) in Waldersee. No statistical significance concerning the distribution of genic and genotypic structures could be find, except for the distributions at gene locus *6-Pgd-A* (test of homogeneity to the c^2 –distribution with statistic *G*). Except the two younger plantations No. 16 and 18, analogously compared distributions of the degree of heterozygosity originate also from one parent population ($P = 0.5310$), but for the distributions of all the six white elm groups significance is observed.

The accumulation of identical multilocus-genotypes among field elm within one group has been stated as principal cause for the obvious genetic differences of the two species. So as much as 33 % of the total field elms appear to have an individual multilocus genotype, whereas among the white elm sample this proportion is approx. 70 %. The distribution of the multilocus genotypes suggests that about 50 % of the total field elms investigated originate from vegetative reproduction. For white elm, the proportion of generatively reproduced individuals exceeds 70 %, because the identical multilocus genotypes do not occur among immediate

laevis neighbours, and their combination in the given sample is being likely (GEHLE & KRABEL 2001).

SHERMAN-BROYLES & BROYLES (1992) studied for 319 cedar elms (*Ulmus crassifolia*) of seven demes in the USA on the basis of 10 enzyme systems. For the isoenzyme variants they determined a comparatively high mean degree of heterozygosity of 20 %. Hints to a genetic analysis are however not given by the authors. A more recent study conducted by MACHON *et al.* (1995) and MACHON *et al.* (1997) of *U. laevis*, *U. minor* and *U. glabra* as well as of alleged hybrids (*U. hollandica*) from five regions of France ($n = 351$) based on a similar set of assessed isoenzymes and a genetic analysis of *U. minor* showed likewise extremely high actual proportions of heterozygosity, above all in *U. minor* for the enzymes phosphoglucomutase (PGM) and malate dehydrogenase (MDH) with values of H_a = 0.8. MACHON *et al.* (1997) suggest that reproduction of elm being almost exclusively vegetative rather than generative in France. The authors interprete a low genetic differentiation between the demes and an obviously typical high genetic variation within the demes from their data. They investigated as many as 165 *U. minor*, 52 *U. glabra*, 75 *U. laevis* and 59 possible hybrids of *U. minor* and *U. glabra* from five regions of France by means of starch gel electrophoresis. Among others the enzyme systems PGI, 6-PGD, PGM and MDH were studied. In stands utilised by forestry, MACHON *et al.* (1997) found *U. minor* very scarce, but more often in hedges.

Already in 1995 MACHON *et al.* investigated - by morphological classification into the respective botanical species - 151 *U. minor*, 48 *U. glabra*, 74 *U. laevis* and 25 possible hybrids of *U. minor* and *U. glabra* from five regions of France. By means of three single-plant progenies from open pollination of *U. minor* (N = 8, 12 and 13) the discrimination among the seedling into PGM and MDH phenotypes was examined, without being aware of the respective genotype of the mother tree. As for PGM they interpreted a gene locus based on electrophoretic isolation of the enzyme by means of a histidine buffer system. In contrast to this, the present investigation shows two gene loci for both species (see Tab. 1 and 6, Fig. 2). For this reason, an immediate comparison of the both investigations is not possible. MACHON *et al.* (1995) described for *Ulmus minor* at the gene locus *Pgm* a major polymorphism referring to four alleles, with variant 4 being very rare (4 %). Whereas within 39 % in *U. laevis* it is of frequent occurrence.

The enzyme systems 6-PGD, PGI and MDH were separated electrophoretically in the same way by MACHON *et al.* (1995) as we did for the present investigation. Concerning gene loci *PGI* and *6-PGD,* for both elm species the authors describe a minor polymorphism for the same variants (both loci triallelic). In contrast, at gene locus *Mdh U. laevis* shows a major polymorphism, which in *U. minor* shifts to a minor polymorphism in favour of variant 2. In comparison to this results, clear differences could be observed between *U. laevis* and *U. minor* from Elbe river (Tab. 7). *Pgi-B* concerning *U. minor* is characterised by a minor polymorphism with five alleles, whereas for *U. laevis* a triallelic major polymorphism is obvious. Compared with the french investigations we found for the two gene loci of the 6-Pgd more variants among white elm. For white elm Mdh as well as the interpreted gene loci *Got-A* and for field elm *Got-B* remain monomorphic.

The aim of a genetic characterization with isoenzyme gene markers by COGOLLUDO-AUGUSTÍN *et al.* (2000) between 104 Spanish field elms originate from a local gene bank, 116 allochthonous Siberian elms (*U. pumila* L.) collected from urban areas and several regions of China and 83 morphological determinated hybrids of the both taxa, also sampled in urban areas of Spain towns, was to discriminate the three elm types in

Table 7. Allele frequencies for the polymorphic loci for each species of elm.

Gene locus	*Ulmus minor* $N = 109$					Gene locus	*Ulmus laevis* $N = 238$			
	Allele						Allele			
	1	2	3	4	5		1	2	3	4
Mdh-A	0.008	0.843	0.119			*Idh-A*	0.889	0.111		
Idh-A	0.826	0.037	0.138			*6-Pgd-A*	0.153	0.153	0.609	0.048
6-Pgd-A	0.014	0.005	0.981			*6-Pgd-B*	0.996	0.002	0.002	
6-PGgd-B	0.954	0.046				*Pgm-A*	0.395	0.605		
Pgm-A	0.023	0.394	0.555	0.028		*Pgm-B*	0.004	0.943	0.053	
Pgm-B	0.014	0.060	0.926			*Pgi-B*	0.002	0.645	0.353	
Pgi-B	0.005	0.119	0.055	0.688	0.133	*Got-B*	0.635	0.365		
Got-A	0.032	0.078	0.157	0.028						

a genetically way before using the plant material for gene conservation programs. The authors studied the genetic control of nine enzyme systems taken from leaf tissue (AAT, PRX, LAP, PGI, CAT, ACPH, IDH, MDH, 6-PGD). The enzymes MDH and 6-PGD of *U. minor* zymograms show invariant zones instead of observed variation in *U. pumila* samples. Therefore, COGOLLUDO-AUGUSTÍN *et al.* (2000) assume the occurence of species-specific alleles which allows them to distinguish between native elms and their hybrids. The occurence of species-specific alleles observed in the same zone position in the zymogram between *U. pumila* and *U. minor* points to the fact that the discrimination between the taxa is quantitative. This result supports the hypothesis of a close relationship between *U. minor* and *U. pumila*. In contrast, the biochemical differences which are observed in the present study between *U. laevis* and *U. minor* are partial qualitative and probable larger than between the interfertile taxa *U. minor*, *U. glabra* and *U. pumila*. The zone positions, for example, for the enzyme system Mdh show completely different proportions (cf. Fig. 2).

MACHON *et al.* (1997) postulate, that determination of differentiation on the basis of isoenzyme gene markers is more difficult than it is by metric trait measurements or other quantitative data. Examples for such classification are given by RAMISCH (1999a,b,c) for *U. glabra* and *U. minor* and MACKENTHUN (2000) for *U. glabra*, *U. minor*, *U. laevis* and *U. ´ hollandica*. However, just the opposite can be demonstrated by the present investigation. In contrast to white elm, field elm is genetically extremely differentiated (Fig. 4) and can be designated as a separate species. The high genetic variation of field elm, as well as its ability to reproduce both in a vegetative and generative manner causes not only a high degree of heterozygosity, but has maintained authochtonous structures as well. Such results could not be confirmed for white elm. In the hardwood floodplains natural regeneration is almost completely missing in white elm stands, but genetic structures are apparent just among neighbouring mature trees which virtually exclude vegetative reproduction at all (GEHLE & KRABEL 2001).

In this regard, for *U. minor* the previous opinion of MITTEMPERGHER (1996) that isoenzymes are not helpful in discriminating between morphologically similar taxa, could not be confirmed for the field elm and white elm from the middle reaches of the Elbe river. In contrast, not only do the zymograms point to qualitative differences between field elm and white elm, but also the genetic systems of the two tree species seem to be essentially different from one another.

It remains an open question to what an extent just hybrid swarms of *U. minor* and *U. glabra* allow an individual allocation due to specific isoenzyme variants. *U. glabra* types, as they were investigated here, could not be found in the area under investigation. For the enzyme system e.g. PGM the isoenzyme phenotypes of *U. glabra* appear to be more similar to those of *U. laevis* than to those of *U. minor*, for MDH the situation turns back (Fig. 1). As far as is known, whych elm does not occur in the hardwood-floodplains.

Furthermore, MACHON *et al.* (1995) discuss partial polyploidy because of the isoenzyme variants of Pgm and Mdh without doing any cytological studies. In contrast to MACHON *et al.* (1995), COGOLLUDO-AUGUSTÍN *et al.* (2000) who investigated Pgm and Mdh among seven other isoenzymes stated that *U. minor* and *U. pumila* were diploid. This hypothesis supports our present results (see Fig. 2). Already KRAUSE (1931), SAX (1933) and LELIVELD (1933) who studied *U. minor*, *U. laevis* and *U. glabra* microscopically counted a chromosome set of only $2n = 28$. However, within the genus *Ulmus* (e. g. *U. americana*) polyploidy is a well known phenomenon (SAX 1933, see DARLINGTON and JANAKI AMMAL 1945). Aneuploidy and hybridisation are described by SANTAMOUR 1970, 1971.

The present investigation clearly points out for the enzyme systems PGM, 6-PGD and MDH that a clear discrimination of *U. minor* and *U. glabra* from *U. laevis* is possible (Fig. 2). In addition, this way of discrimination between the field elm and white elm is described for the first time. Neither by MACHON *et al.* (1995) nor by COGOLLUDO-AUGUSTÍN *et al.* (2000) such differences were observed.

Our study also confirms high proportions of heterozygosity as well as a high degree of genetic diversity within the groups (see Tab. 6). This holds true for field elm and white elm. In this respect, the proportion of vegetative reproduction in field elm which is 50% higher than in white elm does not necessarily lead to a lower degree of genetic variation. Possibly this genetic condition reflects certain properties that might have facilitated the survival of the species with respect to Dutch elm disease until now, or an adaptation to the floodplain localities, or both altogether. For answering these questions more research needs to be done.

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