INHERITANCE OF ISOENZYMES IN SESSILE OAK (*QUERCUS PETRAEA* (MATT.) LIEBL.) AND OFFSPRING FROM INTERSPECIFIC CROSSES

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ABSTRACT

Inheritance studies were based on full sib families of sessile oak (*Quercus petraea* (Matt.) Liebl.) and offspring from interspecific crosses with inclusion of other species of the white oak section, *i.e.*, *Quercus robur* L., *Q. pubescens* Willd., *Q. toza* Bosc. Standard gel electrophoresis and isoelectric focusing were applied in the verification of the mode of inheritance of isoenzymes with respect to different tissues, *i.e.*, seeds, radicles of germinating seeds and bud tissue. Isoenzymes with tissue-specific expression were excluded from segregation studies. The remaining 14 enzyme systems were shown to be controlled genetically by at least 17 polymorphic gene loci. A nomenclature is suggested which includes polymorphism in oak populations in France and Germany. The analyses of two-locus segregations revealed non-random associations in 23 out of 88 combinations which could be tested. At least two groups of gene loci ("linkage groups") appear to be located on the same chromosome, *i.e.*, group 1 with *Aap–A, Acp–C, Lap–A, Pgi–B* and group 2 with at least *Idh–B* and *6Pgd–B*.

Our studies clearly demonstrate the great genetic similarity among oak species within the white oak section (*Lepidobalanus*). Results from previous studies on the inheritance of isoenzymes in pedunculate oak (*Quercus robur* L.) are confirmed. Ambiguities with respect to the present taxonomic status of the studied oak species are briefly addressed.

Key words: gene marker, alloenzymes, inheritance, linkage, full sibs, hybrids, *Quercus* species

INTRODUCTION

Sessile oak (*Quercus petraea* (Matt.) Liebl.) is widespread within Central Europe, Caucasus and adjacent areas. Its occurrence is regionally clustered. Sessile oak is often associated with pedunculate oak (*Quercus robur* L.). Generally, sessile oak is more frequent at higher elevations and on dryer sites than pendunculate oak.

Both oak species are significant deciduous tree species with carrier function in various forest ecosystems. They are outstandingly long-lived and are silviculturally managed within rotation cycles of up to 200 or even 300 years, *i.e.*, longer than any other species in European forestry. Consequently, both species are exposed to an extraordinary large variety of stress conditions in time which altogether challenge considerably the potential of oak populations to adapt to and to survive in present and future environmental heterogeneity.

Genetic variation determines the adaptive potential of species and is an essential component of the stability of ecosystems (*e.g.*, LEDIG 1986, SCHOLZ *et. al.* 1989, HAMRICK & GODT 1989, MÜLLER-STARCK & ZIEHE 1991a). Inventories on genetic, morphological and physiological characters revealed high levels of variation within temperate taxa of the genus *Quercus* (*e.g.*, KREMER *et. al.* 1993). Large intraspecific genetic variation is indicated in central European populations of oaks in contrast to other species (*e.g.*, KREMER *et. al.* 1991, MÜLLER-STARCK 1991, MÜLLER-STARCK & ZIEHE 1991b, DUCOUSSO *et. al.* 1992, 1993, BACILIERI *et. al.* 1993, MOREAU *et. al.* 1994, MÜLLER-STARCK *et. al.* 1993, ZANETTO *et. al.* 1993, SAMUEL *et. al.* 1995).

In contrast to extensive genetic variation within oak populations, genetic variation among populations within species and among species is not pronounced. It appears that within the white oak section (*Lepidobalanus*) genetic similarity is strongly indicated between *Quercus petraea, Q. pubescens,* and *Q. robur* (*e.g.*, KREMER *et.*

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al. 1993). Various controlled crosses among species have resulted in viable hybrids (RUSHTON 1977, AAS 1991, STEINHOFF 1993, BACILIERI *et. al.* 1993, ZANETTO *et. al.* 1994). Incompatibilities vary from individual to individual and between reciprocal crosses (*e.g., Q. robur* females are less incompatible with *Q. petrea* male than vice versa). KREMER & PETIT (1993) compiled data from genetic inventories in hybridizing *Quercus* species and concluded, that 74 % of the total genetic variation is evident within populations, 3 % between populations within species and 23 % between species. In the case of *Q. petraea* and *Q. robur* the corresponding percentages were 95 %, 1 % and 4 % (data taken from inventories in German oak populations, MÜLLER-STARCK *et. al.* 1993). Corresponding values for *Q. petraea, Q. pubescens,* and *Q. robur* were recently estimated to be 74 %, 13 % and 13 % (SAMUEL *et. al.* 1995).

Interspecific hybridization and introgression of hybrids especially with respect to *Q. petraea* and *Q. robur* is well known (*e g.,* RUSHTON 1993, BACILIERI *et. al.* in press). In contrast to efficient gene flow within and among populations, spatial substructuring within populations is obvious which is interpreted for the present to result from strongly localized fruit dispersal (*e.g.,* BACILIERI *et. al.* 1994).

In the comparison of results of genetic inventories, the problem arises that designation of loci and of alleles does not always correspond. In the case of the enzyme system GOT (=AAT), three loci are described by MÜLLER-STARCK *et. al.* (1993) and by LÖCHELT (1994), but one locus by BACILIERI *et. al.* (1993). The number of loci, which code for the enzyme systems LAP and AAP varies between one and three (further explanations see ZANETTO *et. al.* 1996).

In spite of the intensive use of isoenzymes in various genetic inventories in European oak populations, the verification of the genetic control and the mode of inheritance of these markers has been neglected in the past. The only genetic analyses that are based on the study of parents and offspring from controlled crosses are in *Q. robur* (ZANETTO *et. al.* 1996; see also MÜLLER-STARCK & HATTEMER 1990). Also in other deciduous tree species information on the mode of inheritance of isoenzymes is rare [*e.g.,* LINARES BENSIMON (1984) for red alder, FINESCHI *et. al.* (1990) for sweet chestnut, MÜLLER-STARCK (1992) for poplars, MÜLLER-STARCK & STARKE (1993) for European beech]. The present paper contributes further to the verification of the mode of inheritance of isoenzymes in oak species and continues the investigation of ZANETTO *et. al.* (1996). The main focus of this investigation is the study of full sib families of sessile oak. Because of the obvious genetic similarity among oak species within the white oak section (*Lepidobalanus*) offspring from interspecific crosses is included in our study.

MATERIALS AND METHODS

Controlled crosses

During 1987 and 1992, pollinations were performed in seed orchards of the Lower Saxony Forest Research Institute, Escherode, Germany (Forest District Diekholzen/Hildesheim) and on adult trees located close to the INRA Research Station at Pierroton/ Bordeaux (France). The pollination programme included self- and cross-fertilization, reciprocal crosses and crosses among species. Techniques for isolation of female flowers, collection of pollen and pollination are identical to those which were applied in controlled crosses of pedunculate oak (ZANETTO *et. al.* 1996). In Table 1 only those controlled crosses are surveyed which resulted in sample sizes of at least ten individuals per family. As in the case of pedunculate oak, controlled pollinations were very difficult to perform, so that sample sizes generally tend to be very small. This is true especially for offspring from self –fertilization which all revealed sample sizes of less than ten individuals. A total of fourteen full sib families are the subject of the present investigation. Nine of these crosses are intraspecific, the remaining five are interspecific, four of which are crosses between females of *Q. petraea* and males of *Q. pubescens* and one between a female of *Q. robur* and a male of *Q. pubescens.*

Electrophoretic methods

Methods for extraction of bud tissues, seeds and young radicles of germinating acorns and for separation of enzymes are indicated in ZANETTO *et. al.* (1996). Techniques used for visualization of isoenzymes in full sib families and populations of *Q. petraea, Q. pubescens* and *Q. toza* are surveyed in Table 2 (for *Q. robur* these are indicated in ZANETTO *et. al.* 1996).

Studied enzyme systems, metabolic category, structure and putative enzyme coding gene loci in *Q. petraea* and interspecific crosses are surveyed in Table 3. Enzyme systems which appear to be fully expressed environmentally-dependent, such as peroxidase, are not included. The same holds for systems which are weakly expressed in at least one of the studied tissues or reveal ambiguities in the interpretation of zymograms (*e.g.,* the systems of aconitase, NADH-dehydrogenase and phosphoenolpyruvate caboxylase).

DIA and MR are two tetrameric enzymes (Table 3) involved in the biochemical pathway of the quinones, that are stained with non-specific substrates. When migration is done on different buffers for the two

Table 1 Survey of the controlled crosses in *Quercus petraea* and interspecific crosses (*Q. pet.* = *Q. petraea; Q. rob.* = *Q. robur; Q. pub.*= *Q. pubescens; Q. toz.* = *Q. toza*) performed at Diekholzen, Germany (1) or Pierroton, France (2)

Location (Year)	Design of cross	Female parent	Species	Male parent	Species	N	Extracted tissue
1 (1987)	a	Trei 4	*Q. pet.*	Trei5	*Q. pet.*	19	seeds
1 (1988)	b	Lip85	*Q. pet.*	Erd50	*Q. pet.*	25	buds
	c	Lip85	*Q. pet.*	Rhe38(12/3)	*Q. pet.*	31	buds
	d	MarbS1	*Q. pet.*	Ka4	*Q. pet.*	48	buds
	e	MarbS1	*Q. pet.*	MarbS5	*Q. pet.*	48	buds
	f	Rhe38(11/1)	*Q. pet.*	Erd50	*Q. pet.*	24	buds
	g	Rhe38(11/1)	*Q. pet.*	Erd51	*Q. pet.*	24	buds
	h	Rhe38(12/3)	*Q. pet.*	Lip85	*Q. pet.*	66	buds
2 (1992)	i	3P	*Q. rob.*	PU5	*Q. pub.*	11	radicles
	j	OS2	*Q. pet.*	PU6	*Q. pub.*	12	radicles
	k	OS2	*Q. pet.*	PU7	*Q. pub.*	25	radicles
	l	OS2	*Q. pet.*	PU5	*Q. pub.*	16	radicles
	m	OS1	*Q. pet.*	PU3	*Q. pub.*	12	radicles
	n	T7	*Q. toz.*	T17	*Q. toz.*	38	radicles

Table 2 Survey of electrophoretic methods used for verification of isoenzymes. For designation of enzyme systems and E.C. Nos. See Table 3

Enzyme system	Buffer [1]	Enzyme system	Buffer [1]	Enzyme system	Buffer [1]
AAP	1 [4], 2	GOT [3]	2, 3	MR	2, 3
ACP	1, 2	IDH	4, 6	6PGD	4, 6
DIA [2]	6	LAP	1 [4], 2	PGI	1, 4, 6
GDH	3, 5	MDH	4, 6	PGM	5, 6
EST	1,3	SKDH	5		

[1] Electrode and gel buffers used for starch gel electrophoresis
[2] Isoelectric focusing recommended
[3] = Aspartate aminotransferase
[4] Gel buffer diluted (1:1)

No	Electrode buffer, pH	Gel buffer, pH
1	0.05 M LiOH–0.19 M boric acid, pH 8.1	0.05 M tris–0.01 M citric acid, pH 8.1*
2	0.04 M LiOH–0.19 M boric acid, pH 8.3	0.05 M tris–0.001 M citric acid, pH 8.3*
3	0.06 M NaOH–0.30 M boric acid, pH 8.0	0.07 M tris–0.02 M HCl, pH 8.7
4	0.14 M tris–0.04 M citric acid, pH 7.8	Electrode buffer : H_20 = 1:2.5, pH 7.8
5	0.14 M tris–0.04 M citric acid, pH 7.5	Electrode buffer : H_20 = 1:2.5, pH 7.0
6	0.04 M citric acid titrated with N(3-Aminopropyl)-Morpholine , pH 7.0	Electrode buffer : H_20 = 1:19, pH 7.0

* Addition of 10 % electrode buffer is recomended

enzymes, different electrophoretic profiles are observed on the same genotypes; however, when migration is done on the same buffers, similar patterns appear. It is therefore not sure if the two enzymes are the same enzymes, or if they are different and tightly linked as also observed in *Q. robur* (ZANETTO *et al.* 1996).

Segregation studies

The mode of inheritance of isoenzymes was verified by testing the hypothesis of conformity of the observed segregation among offspring from controlled crosses with the expected Mendelian segregations. An isoenzyme that followed Mendelian segregations was consid-

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ered to represent an allele (alloenzyme) and thus to be applicable as a species–specific gene marker. Full sibs with at least one double heterozygous parent were used to test recombination (MÜLLER-STARCK & STARKE 1993, ZANETTO *et. al.* 1996). Genetic map distances were calculated by using the Kosambi method (KOSAMBI 1944) in centimorgans (cM). For calculation of the standard deviation of genetic map distances, see ZANETTO *et. al.* (1996).

RESULTS AND DISCUSSION

Isoenzyme polymorphism in full-sib families and populations

In Figure 1 isoenzymes are compiled which are represented in both, full-sib families and various population studies in Europe (MÜLLER-STARCK & ZIEHE 1991a, MÜLLER-STARCK *et. al.* 1993, ZANETTO *et. al.* 1993, 1994, ZANETTO & KREMER 1995). In a similar way to the study on *Q.* robur (ZANETTO *et al.* 1996), genetic types were designated by letters for gene loci and by numbers for alleles. Succeeding letters designate additional zones of activity in decreasing order of relative mobility. Alleles which are represented in full-sibs, are indicated by arrows. The most frequent alleles in population studies are represented by thick lines. The nomenclature is the same as for *Q. robur*. Missing alleles were identified until now in *Q. robur* but not in *Q. petraea*.

For *Q. pubescens* and *Q. toza* results from genetic inventories in populations are not yet available. Alleles in full-sib families of these two species are included in Figure 1. Until now there are no species-specific alleles indicated for these two species.

For the present, 14 enzyme systems indicate the existence of a total of 27 putative loci (26 putative loci in *Q. robur*). The additional locus is *Mdh–D* which is expressed in both species but was not monitored in *Q. robur*. Out of these 27 suggested loci, 8 loci were not used in the segregation studies because of problems in the visualization in at least one of the studied tissues (*Lap–B, Acp–A, B, Got–A, Skdh–B, Idh–A, Pgi–A, Estα–A*). Except for *Got–A*, the corresponding position in the zymogram is marked by an empty box. The two ACP-loci and *Estα–A* revealed tissue-specific expression. *Mdh–A* was monomorphic in both full-sib families and populations.

The remaining 18 putative gene loci were used in the segregation studies and verified with respect to those alleles, which are represented in the full-sib families. The inheritance of the remaining alleles is suggested, because each of these isoenzymes appears in population studies in single bands or combinations of bands, which does not refute the hypothesis of a phenotypic representation of homozygotes (single bands) or heterozygotes (combination of bands according to the enzyme structure) at single loci. This is also true for combinations between the identified alleles and the suggested ones. There is no contradiction between the present interpretation and the inheritance of those enzyme systems in *Quercus robur* (ZANETTO *et. al.* 1996).

Segregation among full-sib families

Segregations with respect to 18 loci are surveyed in Table 4. Among 78 segregations analysed, four segregations revealed statistically significant deviations from the Mendelian expectations. Distortions were evident in *Aap–A, Got–C, Pgi–B* and *Pgm–A*. In all these cases there were other families which do not confirm these distortions at the same locus. For the present these distortions are interpreted as consequences of prezygotic or postzygotic viability selection. This hypothesis is strongly supported by the fact, that empty seed percentages varied considerably among full sib families.

All other segregations (95 %) do not reject the hypothesis of a one-locus control for those isoenzyme bands which appear within one and the same zone in the zymograms. For the present, there is no indication for null alleles, only with respect to *Aap–A* and *Lap–A*. All alleles appear to be expressed codominantly. It can be concluded that in all the studied enzyme systems homozygotes are presented in the zymograms by single band and heterozygotes by combinations of: double bands (monomeric systems), three-banded types (dimeric systems), five-banded types (tetrameric systems) or by multi-banded types (polymeric systems).

Recombination studies

A total of 88 two-locus combinations could be tested (see Table 5). Further segregations were available but could not be included due to small sample sizes. The analysis of two-locus segregations revealed non-random associations in 23 cases (see Table 5). Those full-sib families which revealed statistically significant one-locus distortions are not included in the recombination studies. The 23 cases of non-random two-locus segregations are surveyed in Table 6.

There are five cases of linkage: *Lap–A/Pgi–B, Aap–A/Lap–A, Acp–C/Lap–A, Acp–C/Aap–A* and *Idh–B/6pgd–B*.

The four loci *Aap–A, Acp–C, Lap–A, Pgi–B* appear to be located in one tight linkage group. The arrangement of these four loci is not unequivocal but the linkage relations which are described below support the hypothesis of a sequence from left to right of *Pgi–B, Lap–A, Aap–A* and *Acp–C*. Other loci like *Got–C* and

Table 3 Enzyme systems, metabolic category (I – primary, II – secondary), enzyme structures and putative enzyme coding gene loci in *Q. petraea, Q. pubescens, Q. robur and Q. toza*

Enzyme System (E.C. No.)	Metabolic category	Structure	Putative loci
Acid phosphatase (3.1.3.2)	II	monomeric	*Acp–A, Acp–B, Acp–C*
Alanine aminopeptidase (3.4.11.1)	II	monomeric	*Aap–A*
Diaphorase (1.6.4.3)	II	tetrameric	*Dia–A*
Esterase (3.1.1.1)	II	monomeric	*Est –A, Est –B*
Glutamate dehydrogenase (1.4.1.3)	I	polymeric	*Gdh–A*
Glutamate oxaloacetate transaminase (2.6.1.1)*	I	dimeric	*Got–A, Got–B, Got–C*
Isocitrate dehydrogenase (1.1.1.42)	I	dimeric	*Idh–A, Idh–B*
Leucine aminopeptidase (3.4.11.1)	II	monomeric	*Lap–A, Lap–B*
Menadione reductase (1.6.99.2)	II	tetrameric	*Mr–A*
Malate dehydrogenase (1.1.1.37)	I	dimeric	*Mdh–A, Mdh–B, Mdh–C*
6–Phosphogluconate dehydrogenase (1.1.1.44)	I	dimeric	*6Pgd–A, 6Pgd–B*
Phosphoglucose isomerase (5.3.1.9)	I	dimeric	*Pgi–A, Pgi–B*
Phosphoglucomutase (2.7.5.1)	I	monomeric	*Pgm–A*
Shikimate dehydrogenase (1.1.1.25)	II	monomeric	*Skdh–A, Skdh–B*

* = Aspartate-aminotransferase

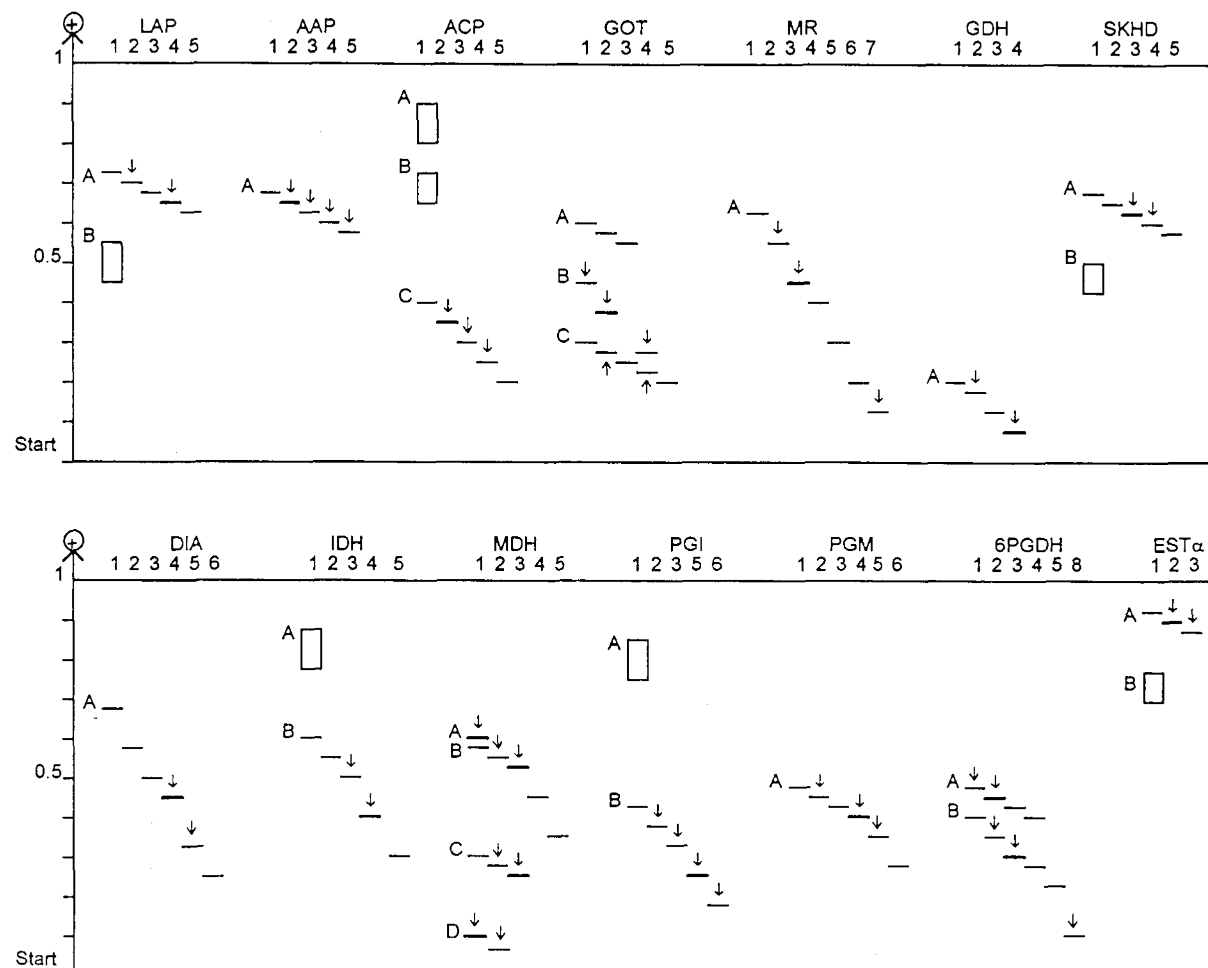

Figure 1 Banding patterns of isoenzymes from 19 polymorphic enzyme loci in *Quercus petraea*. The vertical scale represents the Rf values. The most frequent allele is represented by a thick line. The arrows represent the alleles present in the controlled crosses. The other alleles were found in population surveys across Europe.

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Table 4 Segregation among full sib families from *Quercus petraea* and from interspecific crosses (for designation see Table 1)

Locus	Tissue	Cross			Isoenzymes of parents (f, m) and offspring					χ^2
		f		m	f	m	Segregation	Frequency	Total	
Acp–C	R	3P	×	PU5	22	23	22:23	7:4	11	ns
Acp–C	R	OS2	×	PU7	22	24	22:24	3:9	12	ns
Aap–A	R	3P	×	PU5	24	22	22:24	6:5	11	ns
Aap–A	R	OS1	×	PU3	35	35	33:35/53:55	6:3:3	12	ns
Aap–A	R	OS2	×	PU7	44	24	24:44	7:4	11	ns
Aap–A	R	T7	×	T17	23	22	22:23	21:14	35	ns
Aap–A	B	Lip85	×	Erd50	04	24	02:24:40/44	5:5:15	25	ns
Aap–A	B	Rhe38(12/3)	×	Lip85	24	04	20:24:40/44	10:20:36	66	ns
Aap–A	B	Rhe38(11/1)	×	Erd50	24	34	23:24:34:44	7:9:4:4	24	ns
Aap–A	B	Rhe38(11/1)	×	Erd51	24	44	24:44	15:9	24	ns
Aap–A	B	MarbS1	×	Ka4	24	23	22:23:24:34	14:10:19:5	48	*
Aap–A	B	MarbS1	×	MarbS5	24	25	22:24:25:45	11:13:11:13	48	ns
Aap–A	S	Trei4	×	Trei15	25	22	22:25	7:12	19	ns
Lap–A	R	OS1	×	PU3	24	44	24:44	2:7	8	ns
Lap–A	R	OS2	×	PU7	22	24	22:24	4:7	11	ns
Lap–A	B	Lip85	×	Erd50	22	24	22:24	15:10	25	ns
Lap–A	B	Rhe38(11/1)	×	Erd50	24	24	22:24/42:44	8:10:6	24	ns
Lap–A	B	Rhe38(11/1)	×	Erd51	24	44	24:44	10:14	24	ns
Lap–A	B	MarbS1	×	Ka4	04	04	00:04/40/44	7:41	48	ns
Lap–A	S	Trei4	×	Trei15	24	44	24:44	13:6	19	ns
Dia–A	B	Lip85	×	Erd50	45	44	44:45	12:13	25	ns
Dia–A	B	Lip85	×	Rhe 38(12/3)	45	45	44:45/54:55	11:16:4	31	ns
Dia–A	B	Rhe38(12/3)	×	Lip85	45	45	44:45/54:55	16:34:16	66	ns
Dia–A	B	Rhe38(11/1)	×	Erd50	45	44	44:45	14:10	24	ns
Dia–A	B	Rhe38(11/1)	×	Erd51	45	44	44:45	8:16	24	ns
Estα–A	S	Trei4	×	Trei15	23	22	22:23	12:7	19	ns
			×							
Gdh–A	B	Lip85	×	Erd50	24	44	24:44	8:17	25	ns
Gdh–A	B	Lip85	×	Rhe38(12/3)	24	24	22:24/42:44	8:15:8	31	ns
Gdh–A	B	Rhe38(12/3)	×	Lip85	24	24	22:24/42:44	20:27:19	66	ns
Gdh–A	B	Rhe38(11/1)	×	Erd50	24	44	24:44	10:14	24	ns
Gdh–A	B	Rhe38(11/1)	×	Erd51	24	24	22:24/42:44	5:11:8	24	ns
Gdh–A	B	MarbS1	×	Ka4	24	24	22:24/42:44	14:21:13	48	ns
Gdh–A	B	MarbS1	×	MarbS5	24	24	22:24/42:44	13:22:13	48	ns
Got–B	R	OS2	×	PU5	12	22	12:22	3:8	11	ns
Got–B	R	OS2	×	PU6	12	22	12:22	9:16	25	ns
Got–B	R	OS2	×	PU7	12	22	12:22	4:7	11	ns
Got–B	R	T7	×	T17	22	24	22:24	18:20	38	ns
Got–B	B	MarbS1	×	MarbS15	22	12	12:22	29:19	48	ns
Got–C	B	Lip85	×	Rhe38(12/3)	22	24	22:24	10:21	31	*
Got–C	B	Rhe38(12/3)	×	Lip85	24	22	22:24	29:37	66	ns
Got–C	B	Rhe38(11/1)	×	Erd51	24	22	22:24	11:13	24	ns
Got–C	B	MarbS1	×	Ka4	24	22	22:24	24:24	48	ns

Table 4 (continued)

Locus	Tissue	Cross			Isoenzymes of parents (f, m) and offspring					χ^2
		f		m	f	m	Segregation	Frequency	Total	
Idh–B	B	Lip85	×	Erd50	44	34	34:44	14:11	25	ns
Idh–B	B	Rhe38(12/3)	×	Erd50	33	34	33:34	9:15	24	ns
Idh–B	B	MarbS1	×	Ka4	34	44	34:44	28:20	48	ns
Idh–B	B	MarbS1	×	MarbS5	34	44	34:44	25:23	48	ns
Mdh–B	B	MarbS1	×	MarbS5	33	23	23:33	22:26	48	ns
Mdh–C	B	MarbS1	×	MarbS5	33	23	23:33	22:26	48	ns
Mdh–D	B	Lip85	×	Erd50	12	22	12:22	9:16	25	ns
Mdh–D	B	Lip85	×	Rhe38(12/3)	12	22	12:22	16:15	31	ns
Mr–A	R	OS1	×	PU3	77	34	37:47	7:5	12	ns
6pgd–A	B	Lip85	×	Rhe38(12/3)	12	11	11:12	13:18	31	ns
6pgd–A	B	Rhe38(12/3)	×	Lip85	11	12	11:12	37:29	66	ns
6pgd–A	B	Rhe38(11/1)	×	Erd50	12	11	11:12	14:10	24	ns
6pgd–A	B	Rhe38(11/1)	×	Erd51	12	12	11:12/21:22	3:15:6	24	ns
6pgd–B	B	T7	×	T17	38	38	33:38/83:88	7:19:9	35	ns
6pgd–B	B	Lip85	×	Erd50	33	23	23:33	15:10	25	ns
6pgd–B	B	Rhe38(11/1)	×	Erd50	33	23	23:33	12:12	24	ns
6pgd–B	B	MarbS1	×	MarbS5	33	23	23:33	25:23	48	ns
			×							
Pgi–B	R	OS1	×	PU3	35	35	33:35/53:55	6:3:3	12	ns
Pgi–B	R	OS2	×	PU5	56	55	55:56	2:9:6:8	11	*
Pgi–B	R	OS2	×	PU6	56	25	25:26:55:56	4:7	25	ns
Pgi–B	R	OS2	×	PU7	56	55	55:56	6:6	12	ns
Pgi–B	B	Lip85	×	Erd50	55	25	25:55	10:15	25	ns
Pgi–B	B	Rhe38(11/1)	×	Erd50	55	25	25:55	13:11	24	ns
Pgi–B	B	MarbS1	×	Ka4	55	56	55:56	25:23	48	ns
Pgi–B	B	MarbS1	×	MarbS5	55	56	55:56	22:26	48	ns
Pgm–A	R	OS1	×	PU3	44	24	24:44	10:2	12	*
Pgm–A	R	OS2	×	PU&	44	24	24:44	7:5	12	ns
Pgm–A	R	T7	×	T17	24	45	24:44:25:45	8:8:11:11	38	ns
Pgm–A	B	Rhe38 (11/1)	×	Erd51	44	24	24:44	12:12	24	ns
Pgm–A	B	MarbS1	×	Ka4	45	24	24:44:45:45	9:16:9:14	48	ns
Pgm–A	B	MarbS1	×	MarbS5	44	45	44:45	24:24	48	ns
Skdh–A	B	Lip85	×	Rhe38(12/3)	33	34	33:34	18:13	31	ns
Skdh–A	B	Rhe38(12/3)	×	Lip85	34	33	33:34	30:36	66	ns
Skdh–A	B	Rhe38(11/1)	×	Erd50	34	33	33:34	13:11	25	ns
Skdh–A	B	Rhe38(11/1)	×	Erd51	34	33	33:34	9:15	24	ns
Skdh–A	S	Trei4	×	Tre15	34	33	33:34	12:7	19	ns

χ^2: $p < 0.05$; Tissue from which enzymes were extracted: S-- seed, R – radicle, B-- bud

also *Idh–B, 6pgd–B,* and *Mdh–C* are located quite close to this linkage group. For the present, these eight loci are suggested to belong to the same linkage group (see Figure 2).

In more detail, the following linkage relations between gene loci are evident (see also Tables 5 and 6): ***Lap–A/Pgi–B:*** There is complete linkage (0.0cM), however only one full-sib family is available for verifi-

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Table 5 Survey of recombination rates for statistically significant two-locus segregation (1% level of significance)

	Aap–A	*Acp–C*	*Est–A*	*Dia–A*	*Gdh–A*	*Got–B*	*Got–C*	*Idh–B*
Acp–A	k 0.09							
Dia–A	h ns b ns							
Est–A	a ns							
Gdh–A	h ns			b ns f ns h ns		e ns	d ns h ns	
Got–B	e ns							
Got–C	g 0.17			g ns h ns				
Idh–B	e ns				d ns e ns		d 0.21	
Lap–A	a 0.16 g 0.04 k 0.00	k ns m 0.09	a ns	g ns			g 0.13	b ns
Mdh–C	e 0.33				e ns	e 0.35		
Mdh–D				b ns	b ns			
6pgd–A	h ns			f ns h ns	f ns			
6pgd–B	e ns n ns				e ns	n ns		b 0.04 f 0.13
Pgi–B					d ns e ns	j ns k ns l ns	d 0.19	b ns d 0.31 e 0.23 f ns
Pgm–A	e ns k ns n ns	k ns			e ns	n ns	d 0.31	d 0.27 e 0.35
Skdh–A	a 0.37 g ns		a 0.31	f ns h ns	f ns h ns		c ns g ns h ns	

cation (25 individuals); ***Aap–A/Lap–A:*** Linkage is indicated in all three full-sib families (cM values are 0, 4, and 16 with an average of 6.7); ***Acp–A/Aap–A:*** Linkage is suggested (9cM), but only one full-sib (*Q. petraea* × *Q. pubescens*) is available for verification; ***Acp–A/Lap–A***: Recombination in one full-sib (9cM) and random segregation in the other (both *Q. petraea* × *Q. pubescens*). Linkage is suggested since *Acp–A* appears to be linked to *Aap–A* and this locus to *Lap–A;*

Idh–B/6Pgd–B: Linkage is suggested in both full sibs (cMvalues are 4 and 13 with an average of 8.5cM).

The following combinations reveal larger recombination rates. Linkage relations in a moderate form are still suggested but should be interpreted with care: ***Got–C/Lap–A:*** Linkage is suggested (13cM) but only one full-sib is available for verification; ***Got–C/Aap–A:*** 17cM (one full-sib family) confirms the results with respect to *Got–C/Lap–A;* ***Got–C/Pgi–B:*** 20cM (one

Table 5 (continued)

	Lap–A	*Mdh–C*	*Mdh–D*	*Mr–A*	*6pgd–A*	*6pgd–B*	*Pgi–B*
Acp–A							
Dia–A							
Est–A							
Gdh–A							
Got–B				m ns			
Got–C							
Idh–B							
Lap–A							
Mdh–C							
Mdh–D							
6pgd–A			c ns				
6pgd–B	b ns	e 0.19					
Pgi–B	b 0.00					b ns e ns	
Pgm–A	k ns			m ns			d 0.33 e ns
Skdh–A	g ns				f ns		

* For designation of the crosses see Table 1

full-sib family) may be interpreted to still confirm the localisation of *Got–C* quite close to the PGI-LAP-AAP-ACP-complex; ***Got–C/Idh–B**:* 22cM (one full-sib family) indicates increasing distances and thus uncer tainty of statements. The combination *Got–C/6Pgd–B* could not be tested in order to verify the localization of *Got–C* with respect to the IDH-6PGD-complex; ***Mdh–C/6Pgd–B**:* 20 cM (one full-sib family) may still be considered to indicate a moderate linkage relationship. The combination *Mdh–C/Idh–B* could not be tested in order to verify the localization of *Mdh–C* with respect to the IDH-6PGD-complex. cM-distances of *Mdh–C* to other loci are large (>40cM) and do not allow conclusive statements.

The remaining combinations between loci do not help to specify information on linkage relations. For instance, cMdistances are large and nearly identical between *Pgm–A* and *Pgi–B* (40cM), *Got–C* (37cM), and *Idh–B* (37cM) respectively. In addition, cases of random segregation are indicated such as between *Pgm–A* and *Pgi–B*. The cM distances between *Skdh–A* and other loci are large (37 for *Estα–A* and 47 for *Aap–A*) or random segregation is evident (*Skdh–A/Aap–A*) because of these ambiguities, the loci *Estα–A*, *Pgm–A*, *Got–B*, and *Skdh–A* are not included in Figure 2.

The present results confirm conclusions of recombination studies in *Quercus robur* (see ZANETTO *et al.* 1996), although also contradictions are evident. Generally, the comparison between *Q. petraea* and *Q. robur* suffers from the fact that pairs of loci which were subject to recombination studies in one species were not available for the other. In both species, the PGI-LAP-AAP-complex is indicated, although *Pgi–B* is only moderately linked to *Lap–A/Aap–A* in *Q. robur*. The IDH-6PGD-complex and also *Mdh–C* in *Q. petraea* could not be observed in *Q. robur* because of appropriate segregating progeny. The opposite is true for the DIA-MR-complex in *Q. robur*. The moderate linkage

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Table 6 Recombination frequencies and genetic map distances

Des.	Cross	Loci	r	V_r	D (cM)
k	OS2 × PU7	*Aap–A* × *Acp–C*	0.0909	0.0075	9
g	Rhe38(11/1) × Erd 51	*Aap–A* × *Got–C*	0.1667	0.0058	17
g	Rhe38(11/1) × Erd 51	*Aap–A* × *Lap–A*	0.0417	0.0017	4
a	Trei4 × Trei15	*Aap–A* × *Lap–A*	0.1579	0.0070	16
k	OS2 × PU7	*Aap–A* × *Lap–A*	0.0000	0.0000	0
e	MarbS1 × MarbS5	*Aap–A* × *Mdh–C*	0.3333	0.0039	40
a	Trei4 × Trei15	*Aap–A* × *Skdh–A*	0.3684	0.0122	47
k	OS2 × PU7	*Acp–C* × *Lap–A*	0.0909	0.0075	9
a	Trei4 × Trei15	*Estα–A* × *Skdh–A*	0.3158	0.0114	37
d	MarbS1 × Ka4	*Got–C* × *Idh–B*	0.2083	0.0034	22
g	Rhe38(11/1) × Erd 51	*Got–C* × *Lap–A*	0.1250	0.0046	13
e	MarbS1 × MarbS5	*Got–B* × *Mdh–C*	0.3542	0.0048	44
d	MarbS1 × Ka4	*Got–C* × *Pgi–B*	0.1875	0.0032	20
d	MarbS1 × Ka4	*Got–C* × *Pgm–A*	0.3125	0.0036	37
b	Lip85 × Erd 50	*Idh–B* × *6pgd–B*	0.0400	0.0015	4
f	Rhe38(11/1) × Erd 50	*Idh–B* × *6pgd–B*	0.1250	0.0046	13
d	MarbS1 × Ka4	*Idh–B* × *Pgi–B*	0.3125	0.0045	37
e	MarbS1 × MarbS5	*Idh–B* × *Pgi–B*	0.2292	0.0037	25
d	MarbS1 × Ka4	*Idh–B* × *Pgm–A*	0.2708	0.0031	30
e	MarbS1 × MarbS5	*Idh–B* × *Pgm–A*	0.3542	0.0048	44
b	Lip85 × Erd 50	*Lap–A* × *Pgi–B*	0.0000	0.0000	0
e	MarbS1 × MarbS5	*Mdh–C* × *6pgd–B*	0.1875	0.0032	20
d	MarbS1 × Ka4	*Pgi–B* × *Pgm–A*	0.3333	0.0039	40

Note: Recombination frequency: $r = n_l/(n_1 + n_3)$ with n = sample size
Variance of r: $V(r) = r(1-r)/(n_1 + n_3)$
Kosambi distance: $D(cM) = 25 \ln[(1+2r)/(1-2r)]$

relationship of *Idh–B* to the PGI-LAP-AAP-complex is the same in *Q. petraea* and *Q. robur*.

Contradictive are the statements with respect to *Acp–C:* In the interspecific full sib families of *Q. petraea* × *Q. pubescens*, *Acp–C* appears to be linked to the PGI-LAP-AAP complex, while in *Q. robur* a moderate linkage relationship may still be suggested but Acp is located on the other side of *Idh–B* (approx. 70cM apart from the PGI-LAP-AAP-complex). For clarification, further studies are required.

Generally, the present results reveal several cases of linkage which have not been observed in other deciduous tree species, particularly those which belong to the *Fagaceae*. For instance, no linkage was evident in recent recombination studies of European beech (*Fagus sylvatica* L.), although 10 loci are common to both studies (MÜLLER-STARCK & STARKE 1993). The same is true for *Castanea sativa* (FINESCHI *et al.* 1990).

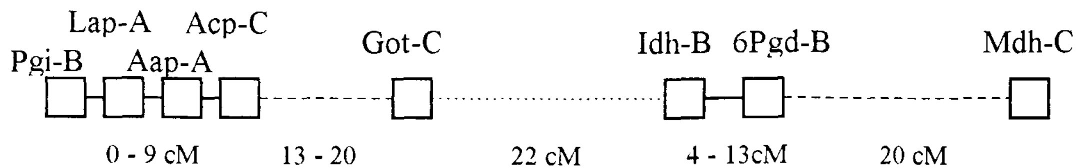

Figure 2 Estimated genetic map distances in centiMorgans (cM). Minimum and maximum values are indicated

RAJORA (1986) tested recombination between Mdh- and 6PGD-loci in *Populus nigra* and Idh- and 6PGD-loci in *Populus maximowiczii* but there was no evidence for linkage. The same conclusion was drawn by HYUN *et al.* (1987) who studied recombination between 6PGD- und PGI-loci in *Populus tremuloides*. In poplar clones of section *Tacamahaca*, MÜLLER-STARCK (1992) observed linkage between *Got–A/Got–B* and *Ndh–A/Pgm–A* and more moderate linkage relations between *Idh–B/6pgd–B* and *Idh–B/Pgi–B*. In *Alnus glutinosa* L, linkage was evident with respect to *Aap–A/Aap–B* (LINARES BENSIMON 1984). In coniferous species linkage is evident particularly between LAP- and GOT-loci (*e.g.*, STRAUSS & CONKLE 1986, RUDIN & EKBERG 1978). Such findings either confirm, or do not contradict, tendencies which are evident in oak species.

CONCLUDING REMARKS

The genetic similarity among white oak species which can be concluded from various investigations is fully confirmed by the rzesults of the present study. The mode of inheritance of isoenzymes is identical in *Quercus petraea* and *Q. robur*. Five full-sib families which result from interspecific crosses between *Q. petraea* and *Q. robur* respectively and *Q. pubescens*, strongly support the hypothesis of a great genetic similarity among these three oak species. The only available full-sib family of *Q. toza* allows the same conclusion, although this species has a narrow distribution in northern Spain and southern France and can be distinguished morphologically from the remaining three species.

Species-specific alleles are suggested (*e.g.*, *6pgd-*B_6 and B_7 for *Q. petraea*) but this hypothesis cannot be verified because these alleles are rare in population studies (in many cases frequencies close to 1% or smaller). It cannot be excluded that deviations between frequencies of rare alleles in different species account to such "species-specific alleles".

In the recombination studies, small sample sizes (especially with respect to four interspecific crosses) limit the utilization of the information potential of the studied gene loci. Consequently, further studies are required in order to include more pairs of loci and to better specify information on the chromosomal arrangement of enzyme-coding gene loci. In particular, overestimation of linkage relations will be avoided. In the present study, tight linkage and more moderate forms of non-random association of loci are clearly indicated. The existing information on *Quercus robur* could be considerably extended.

The fact that white oak species show substantial genetic similarities and that crosses between oak species result in viable offspring, suggest ambiguities with respect to the botanical classification into two distinct species, *i.e., Quercus robur* and *Quercus petraea*. The question arises whether or not this concept can still be maintained. It is self evident that the present study refer to a small set of isoenzyme gene markers but recent studies which utilize molecular markers suggest similar conclusions (*e.g.*, PETIT *et al.* 1993).

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