Theor Appl Genet (1993) 86:322–328

© Springer-Verlag 1993

Inheritance and linkage relationships of ten isozyme genes in hazelnut

M. Rovira [1], N. Aletà [1], E. Germain [2], and P. Arús [3]

[1] Department of Mediterranean Arboriculture, IRTA, Apartat 415, 43280 Reus (Tarragona), Spain
[2] Research Station on Fruit Trees, INRA, BP 81, 33883 Villenave d'Ornon CEDEX, France
[3] Department of Plant Genetics, IRTA, Carretera de Cabrils s/n, 08348 Cabrils (Barcelona), Spain

Received August 30, 1992; Accepted September 19, 1992
Communicated by H. F. Linskens

Summary. The inheritance of 6-phosphogluconate dehydrogenase (6PGD), malate dehydrogenase (MHD), aconitase (ACO), phosphoglucomutase (PGM), phosphoglucoisomerase (PGI), and glutamate-oxalacetate transaminase (GOT) polymorphic isozymes was studied in leaf extracts of nine hazelnut progenies using horizontal starch gel electrophoresis. Evidence of Mendelian inheritance was obtained for ten loci: *6-Pgd-2, Mdh-1, Aco-1, Aco-2, Pgm-1, Pgm-2, Pgm-3, Pgi-2, Pgi-3,* and *Got-2,* which permitted the analysis of 28 alleles (2.8 per locus). The presence of null alleles was detected in *Pgm-1* and *Pgm-3.* Joint segregation analysis of pairs of isozymes revealed four linkages: *Mdh-1–Pgi-2, Aco-2–Pgm-2, Pgm-1–Pgm-3,* and *6Pdg-2–Pgm-2.*

Key words: *Corylus avellana* – Hazelnut – Electrophoresis – Isozymes – Linkage

Introduction

Our knowledge of tree fruits at the genetic level is very poor. Long intergeneration periods and asexual reproduction as a commercial method of propagation are just two of the factors that have determined this situation.

A major breakthrough in this area has been the development and widespread use of isozyme electrophoresis as a simple and inexpensive method to uncover marker loci that are codominant, expressed early in the development of the plant, and environmentally stable. Isozyme genes have been studied in numerous fruit crops (Torres 1990). The process has been facilitated by the typical long life span of trees, which has allowed geneticists and breeders to make use of established populations segregating for marker loci as well as other morphological and agronomic characters of interest. Investigations on isozyme genes have generated information that has proven extremely useful in the areas of orchard design and tree breeding; for example, the detailed study of pollination in orchards (Jackson and Clarke 1991), the investigation on the ancestry of cultivars (Chyi and Weeden 1984), or the detection of linkage between markers and important agricultural characters (Manganaris and Alston 1987).

Electrophoretic analysis has been utilized in the genus *Corylus* to identify species (Ahmed et al. 1987; Todorovic 1989) or cultivars within *C. avellana* (Loukas et al. 1984; Truco et al. 1989; Cheng et al. 1990). Although no genetic studies had been previously conducted in hazelnut isozymes, researchers agree that a high level of polymorphism was present in most of the enzyme systems analyzed. In this report, we present results from our investigations on the inheritance and linkage relationships between the isozyme genes polymorphic in *C. avellana* for the 6PGD, MDH, ACO, PGM, PGI, and GOT enzyme systems.

Correspondence to: M. Rovira

Materials and methods

Nine unselected progenies (Table 1) from the hazelnut breeding program at INRA (Bordeaux, France) were utilized in this investigation. These progenies were chosen on the basis of their size and the isozymic phenotype of their parents (Table 2).

About 0.02 g of young developing leaves was crushed in 100 µl of extraction buffer (Tous et al. 1992). The extract was absorbed into 3 × 8 mm paper wicks (Whatman no. 3) and loaded into a horizontal starch gel (11.5% Connaught hydrolyzed starch).

Staining assays for the enzymes studied were prepared as follows. For 6PGD, PGI, PGM, and ACO we used 100 ml 0.1 *M* TRIS (pH 8.0), 6 mg PMS, 20 mg MTT and 8 mg NADP. Specific chemicals for each of these enzyme systems were: 25 mg 6-phosphogluconic acid for 6PGD; 60 mg *cis*-aconitic acid, 4 ml 10% $MgCl_2$ (w/v) and 50 units isocitrate dehydrogenase for ACO; 20 mg fructose-6-phosphate and 20 units glucose-6-phosphate dehydrogenase for PGI; 20 mg glucose-1-phosphate, 20 units glucose-6-phosphate dehydrogenase, and 2 ml 10% $MgCl_2$ (w/v) for PGM. For MDH, 20 mg NAD, 20 mg MTT, and 6 mg

PMS were added to a solution of 75 ml 0.1 *M* TRIS, pH 8.0, and 25 ml malate solution (0.1 *M* L-malic acid adjusted to pH 8.0 with Na_2Co_3). GOT was stained as described in Vallejos (1983).

The gel/electrode buffers used were: morpholine-citrate pH 6.1 (MC 6.1) for 6PGD, histidine-citrate pH 5.7 (HC 5.7) for MDH, histidine pH 7.0 (H 7.0) for ACO, PGM and PGI and TRIS-citrate, pH 8.3, (TC 8.3) for GOT. MC 6.1 and H 7.0 correspond respectively to buffers G and E of Shields et al. (1983). The gel buffer for HC 5.7 consisted of 1.40 g/l L-histidine adjusted to pH 5.7 with citric acid, and the electrode buffer was the same as in H 7.0. The TC 8.3 gel buffer was made with 6.25 ml 0.61 *M* TRIS adjusted to pH 8.3 with citric acid, and the electrode buffer was 0.3 *M* boric acid adjusted to pH 8.2 with NaOH.

Chi-square tests of goodness of fit to the expected single gene segregations and of independence between gene pairs were performed with the LINKAGE 1 software program (Suiter et al. 1983). The estimation of the recombination values, their variances, and joint linkage determinations when more than one progeny was available were done with the maximum likelihood methods described by Allard (1956).

Results

Differences in banding patterns were observed among the parental genotypes and in the progenies. Segregations of isozyme phenotypes and their adjustment to predicted Mendelian ratios are summarized in Table 3. The main characteristics of the variability and a genetic interpretation of each enzyme system are described in the following paragraphs.

6PGD

Activity was detected in two zones of the zymogram of this enzyme, 6PGD-1 and 6PGD-2 (Fig. 1). Only the most cathodal region 6PGD-2 was polymorphic. Segregation data were consistent with the presence of one gene (*6Pgd-2*) with three alleles (*a*, *b*, and *c*). In all cases we found the expected phenotypic classes, but departures from the predicted proportions were significant in three of the seven progenies analyzed.

MDH

Only the most anodal (MDH-1) of the three zones of activity detected was polymorphic (Fig. 2). Results indicate that variability at this region is encoded by a single locus *Mdh-1* with two alleles (*a* and *b*). In only one of the four progenies studied was there a significant departure from expected frequencies.

Table 1. Progenies between hazelnut genotypes and their size

Cross	Reference number	No. of sdls.
Butler × Ennis	H-268	32
Ennis × Butler	H-313	53
Santa Maria di Gesu × Butler	H-364	44
Tonda Romana × Tonda Gentile delle Langhe	H-368	17
Tonda Romana × Segorbe	H-370	34
Ennis × Merveille de Bolwiler	H-383	46
Romai × Butler	H-491	45
Fertile de Coutard × Cosford	H-501	86
Corabel × Tonda di Giffoni	H-510	54

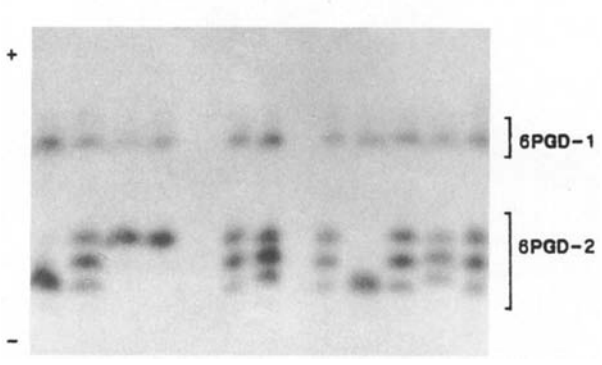

Fig. 1. 6PGD zymogram of progeny from the cross H-491. Genotypes for *6Pgd-2* are (from *left to right*): *bc, ac, aa, aa, ac, ab, ac, bc, ac, ab,* and *ac*

Table 2. Isozymic genotypes of the parents used in the inheritance studies

	6Pgd-2	*Mdh-1*	*Aco-1*	*Aco-2*	*Pgm-1*	*Pgm-2*	*Pgm-3*	*Pgi-2*	*Pgi-3*	*Got-2*
Butler	*ab*	*bb*	*dd*	*bb*	*nn*	*bb*	*bb*	*bb*	*aa*	*bb*
Corabel	*ab*	*bb*	*bc*	*bb*	*nn*	*ab*	*bb*	*bb*	*ac*	*bb*
Cosford	*bb*	*bb*	*dd*	*bb*	*nn*	*bb*	*ab*	*bb*	*aa*	*bb*
Ennis	*ab*	*bb*	*bd*	*bb*	*an*	*bb*	*bn*	*bb*	*ac*	*bb*
Fertile de Coutard	*aa*	*bb*	*bd*	*bb*	*an*	*ab*	*bn*	*bb*	*ac*	*bb*
Merveille de Bolwiler	*aa*	*bb*	*bb*	*bb*	*nn*	*ab*	*aa*	*bb*	*aa*	*bb*
Romai	*ac*	*ab*	*bc*	*bc*	*an*	*ab*	*cn*	*bb*	*aa*	*bb*
Santa Maria di Gesu	*aa*	*bb*	*bd*	*bb*	*an*	*ab*	*bb*	*bb*	*aa*	*bb*
Segorbe	*ab*	*bb*	*dd*	*bb*	*an*	*bb*	*bn*	*ab*	*ab*	*bb*
Tonda di Giffoni	*ab*	*ab*	*bd*	*bb*	*an*	*ab*	*bb*	*ab*	*ac*	*ab*
Tonda Gentile delle Langhe	*aa*	*bb*	*ad*	*bb*	*an*	*ab*	*dn*	*bb*	*aa*	*bb*
Tonda Romana	*aa*	*ab*	*dd*	*ab*	*nn*	*ab*	*ab*	*bb*	*aa*	*bb*

Table 3. Single locus segregation and chi-square goodness of fit analysis for ten-variable isozymes in hazelnut

Locus	Cross	No. of sdls.	Parental genotypes	Offspring genotypes	Expected ratio	χ^2
6Pgd-2	H-268	32	*ab* × *ab*	*aa*: 9 *ab*:14 *bb*: 9	1:2:1	0.50
	H-313	50	*ab* × *ab*	*aa*:15 *ab*:25 *bb*:10	1:2:1	1.00
	H-364	44	*aa* × *ab*	*aa*:25 *ab*:19	1:1	0.81
	H-370	33	*aa* × *ab*	*aa*:11 *ab*:22	1:1	3.66*
	H-383	46	*ab* × *aa*	*aa*:26 *ab*:20	1:1	0.78
	H-491	45	*ac* × *ab*	*aa*: 5 *ab*:16 *ac*:14 *bc*:10	1:1:1:1	6.28*
	H-510	53	*ab* × *ab*	*aa*: 9 *ab*:36 *bb*: 8	1:2:1	6.84*
Mdh-1	H-368	17	*ab* × *bb*	*ab*: 3 *bb*:14	1:1	7.11*
	H-370	34	*ab* × *bb*	*ab*:15 *bb*:19	1:1	0.47
	H-491	45	*ab* × *bb*	*ab*:28 *bb*:17	1:1	2.68
	H-510	54	*bb* × *ab*	*bb*:29 *ab*:25	1:1	0.29
Aco-1	H-268	31	*dd* × *bd*	*bd*:21 *dd*:10	1:1	3.90
	H-313	48	*bd* × *dd*	*bd*:22 *dd*:26	1:1	0.33
	H-364	44	*bd* × *dd*	*bd*:24 *dd*:20	1:1	0.36
	H-368	17	*dd* × *ad*	*ad*:11 *dd*: 6	1:1	1.47
	H-383	42	*bd* × *bb*	*bb*:19 *bd*:23	1:1	0.38
	H-491	45	*bc* × *dd*	*bd*:21 *cd*:24	1:1	0.20
	H-501	85	*bd* × *dd*	*bd*:38 *dd*:47	1:1	0.95
	H-510	54	*bc* × *bd*	*bb*:14 *bc*: 2 *bd*:17 *cd*:21	1:1:1:1	14.88*
Aco-2	H-368	17	*ab* × *bb*	*ab*: 1 *bb*:16	1:1	13.23*
	H-370	34	*ab* × *bb*	*ab*: 9 *bb*:25	1:1	7.52*
	H-491	45	*bc* × *bb*	*bb*:21 *bc*:24	1:1	0.20
Pgm-1	H-268	31	*nn* × *an*	*an*:18 *nn*:13	1:1	0.80
	H-313	53	*an* × *nn*	*an*:26 *nn*:27	1:1	0.01
	H-364	44	*an* × *nn*	*an*:22 *nn*:22	1:1	0.00
	H-368	17	*nn* × *an*	*an*: 7 *nn*:10	1:1	0.52
	H-370	34	*nn* × *an*	*an*:12 *nn*:22	1:1	2.94*
	H-383	46	*an* × *nn*	*an*:24 *nn*:22	1:1	0.08
	H-491	45	*an* × *nn*	*an*:23 *nn*:22	1:1	0.02
	H-501	86	*an* × *nn*	*an*:42 *nn*:44	1:1	0.04
	H-510	53	*nn* × *an*	*an*:25 *nn*:28	1:1	0.16
Pgm-2	H-364	44	*ab* × *bb*	*ab*:26 *bb*:18	1:1	1.45
	H-491	45	*ab* × *bb*	*ab*:26 *bb*:19	1:1	1.08
	H-510	50	*ab* × *ab*	*aa*:11 *ab*:30 *bb*: 9	1:2:1	2.16
Pgm-3	H-368	17	*ab* × *dn*	*ad*: 1 *an*: 3 *bd*: 6 *bn*: 7	1:1:1:1	5.35
	H-383	46	*bn* × *aa*	*ab*:26 *an*:20	1:1	0.78
	H-491	45	*cn* × *bb*	*bc*:23 *bn*:22	1:1	0.02
Pgi-2	H-370	33	*bb* × *ab*	*ab*:16 *bb*:17	1:1	0.03
	H-510	54	*bb* × *ab*	*ab*:27 *bb*:27	1:1	0.00
Pgi-3	H-268	32	*aa* × *ac*	*aa*:14 *ac*:18	1:1	0.50
	H-313	52	*ac* × *aa*	*aa*:20 *ac*:32	1:1	2.76
	H-370	34	*aa* × *ab*	*aa*:18 *ab*:16	1:1	0.11
	H-383	36	*ac* × *aa*	*aa*:17 *ac*:19	1:1	3.13*
	H-501	86	*aa* × *ac*	*aa*:41 *ac*:45	1:1	0.18
	H-510	54	*ac* × *ac*	*aa*:21 *ac*:23 *cc*:10	1:2:1	5.66*
Got-2	H-510	54	*bb* × *ab*	*ab*:29 *bb*:25	1:1	0.29

* $P<0.05$

ACO

Variability was detected for this enzyme in two regions: ACO-1 and ACO-2 (Fig. 3). Progeny tests support the hypothesis that this enzyme is controlled by two polymorphic loci: *Aco-1* with four alleles, and *Aco-2* with three. Observed and expected segregation ratios were in agreement in seven out of eight cases for *Aco-1*, whereas in *Aco-2* an excess of homozygous *bb* individuals was observed in two *ab* × *bb* crosses, while the remaining progeny fit the expected proportions.

PGM

Three polymorphic regions of activity, PGM-1, PGM-2, and PGM-3, were present in gels stained for this enzyme (Fig. 4). A single band was either present or absent at the most anodal region (PGM-1). Segregation data were

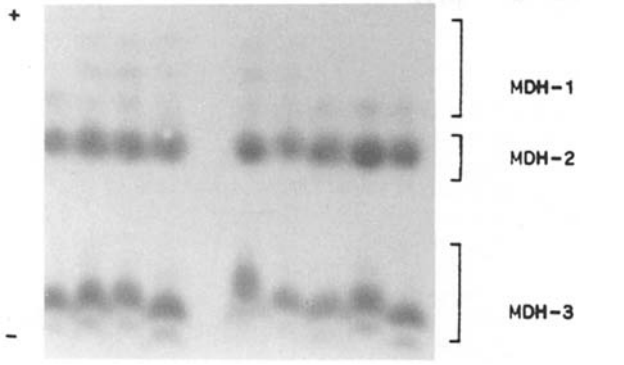

Fig. 2. MDH zymogram of progeny from the cross H-510. Genotypes for *Mdh-1* are (from *left to right*): *bb, ab, ab, ab, ab, ab, bb, bb,* and *bb*

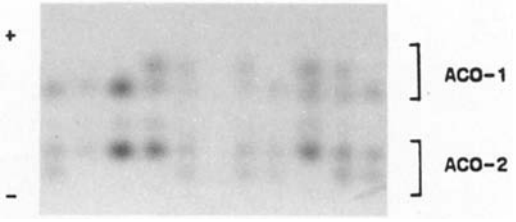

Fig. 3. ACO zymogram of progeny from the cross H-491. Genotypes are (from *left to right*): *Aco-1 cd, cd, cd, bd, bd, bd, cd, bd, bd,* and *cd*; for *Aco-2 bc, bb, bb, bb, bc, bc, bc, bb, bc,* and *bc*. Alleles *c* and *d* of *Aco-1* migrate to slightly different positions, and *cd* heterozygotes are distinguished because they produce thicker bands than either homozygote

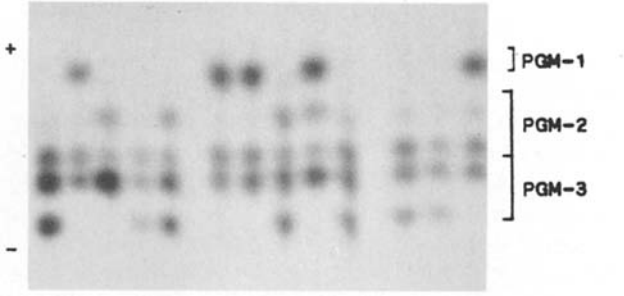

Fig. 4. PGM zymogram of progeny from the cross H-491. Genotypes are (from *left to right*): for *Pgm-1 nn, an, nn, nn, nn, an, an, nn, an, nn, nn, nn,* and *an*; for *Pgm-2 bb, bb, ab, bb, ab, bb, bb, ab, ab, bb, bb, bb,* and *bb*; for *Pgm-3 bc, bn, bn, bc, bc, bn, bn, bc, bn, bc, bc, bc,* and *bn*

consistent with the expected phenotypic proportions of one gene (*Pgm-1*) with two alleles, one active (*a*) and one null (*n*). PGM-2 presented phenotypes with either one or two bands. Observed progeny ratios were in agreement with the existence of gene *Pgm-2* with two alleles (*a* and *b*). The segregation pattern of bands at PGM-3 in three crosses suggested that a gene (*Pgm-3*) with four active

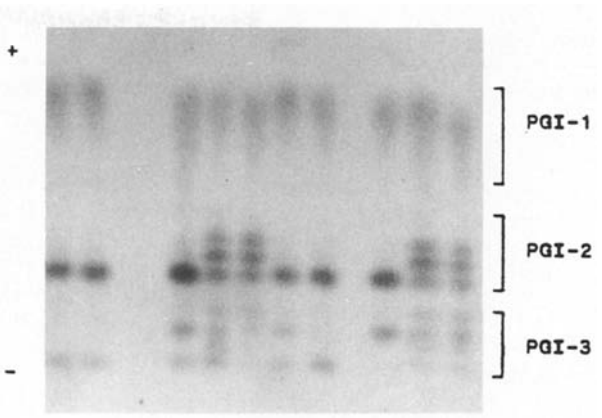

Fig. 5. PGI zymogram of progeny from the cross H-510. Genotypes are (from *left to right*): for *Pgi-2 bb, bb, bb, ab, ab, bb, bb, bb, ab,* and *ab*; for *Pgi-3 cc, cc, ac, ac, ac, ac, cc, aa, ac,* and *ac*

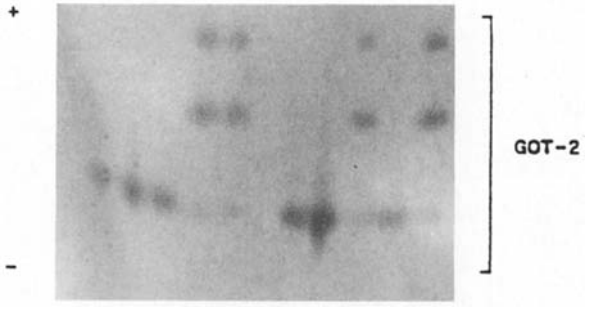

Fig. 6. GOT zymogram of progeny from the cross H-510. Genotypes for *Got-2* are (from *left to right*): *bb, bb, bb, ab, ab, bb, bb, ab, bb,* and *ab*

alleles (*a, b, c,* and *d*), and a null (*n*) allele explained the observed variation.

The interpretation of PGM isozymes was complex given the presence of null alleles for *Pgm-1* and *Pgm-3* and overlapping between the products of the two *a* alleles of *Pgm-2* and *Pgm-3*. Thus, the genotype of the parents for the three PGM genes could only be inferred knowing both their phenotypes and that of their progeny in one or more crosses. Moreover, the simultaneous presence of the two overlapping alleles precluded genetic interpretation of progenies segregating for *Pgm-2, Pgm-3*, or both, as in crosses H-368, H-383, H-370 and H-501.

PGI

This enzyme showed three zones of activity (Fig. 5). The most anodal (PGI-1) was monomorphic. PGI-2 had zymotypes that were either single banded or three banded. Two of the progenies analyzed gave phenotype ratios consistent with the existence of one gene (*Pgi-2*) that was responsible for variation at this region. Individuals with one, two, three, and four bands were observed at PGI-3. These banding patterns were in agreement with those expected considering that PGI-3 is the region of migra-

Table 4. Pairs of genes with segregations indicating linkage in hazelnut crosses

Gene pair	Cross	Parent genotypes	Offspring genotypes	χ^2	Distance (cM)	Distance SE	Pooled distance	Pooled SE	χ^2_h [a]
Mdh-1–Pgi-2	H-510	*bb/bb* × *ab/ab*	0 *ab/ab*: 25 *ab/bb*: 27 *bb/ab*: 2 *bb/bb*	46.55*	4	2.6			
Aco-2–Pgm-2	H-491	*bc/ab* × *bb/bb*	20 *bb/ab*: 1 *bb/bb*: 6 *bc/ab*: 18 *bc/bb*	22.65*	16	5.4			
Pgm-1–Pgm-3	H-383	*an/bn* × *nn/aa*	5 *an/ab*: 19 *an/an*: 21 *nn/ab*: 1 *nn/an*	26.00*	13	5.0			
	H-491	*an/cn* × *nn/bb*	2 *an/bc*: 21 *an/bn*: 21 *nn/bc*: 1 *nn/bn*	33.87*	7	3.8			
	H-368	*nn/ab* × *an/dn*	0 *an/ad*: 3 *an/an*: 0 *an/bd*: 4 *an/bn*: 1 *nn/ad*: 0 *nn/an*: 6 *nn/bd*: 3 *nn/bn*	9.92*	18	9.2			
							11	3.0	1.82
6Pgd-2–Pgm-2	H-491	*ac/ab* × *ab/bb*	4 *aa/ab*: 1 *aa/bb*: 12 *ab/ab*: 4 *ab/bb*: 5 *ac/ab*: 9 *ac/bb*: 5 *bc/ab*: 5 *bc/bb*	6.00	33	7.0			
	H-510	*ab/ab* × *ab/ab*	3 *aa/aa*: 3 *aa/ab*: 3 *aa/bb*: 6 *ab/aa*: 24 *ab/ab*: 2 *ab/bb*: 1 *bb/aa*: 3 *bb/ab*: 4 *bb/bb*	11.91*	30	5.8			
							32	4.5	0.69

* $P<0.05$

[a] Chi-square test of homogeneity

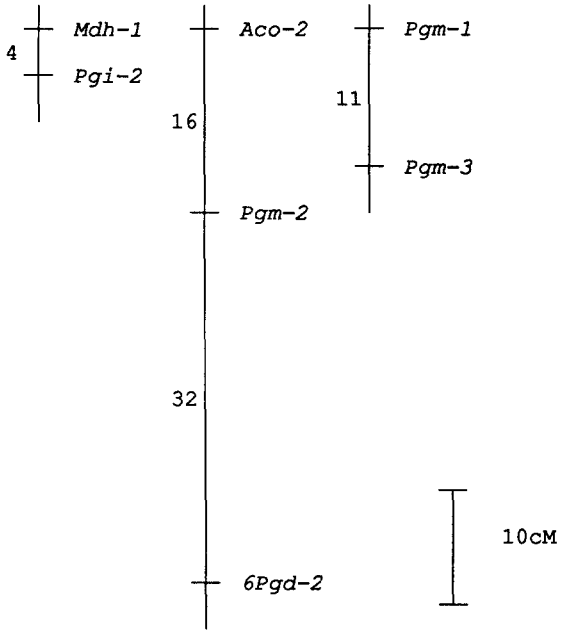

Fig. 7. Schematic linkage map of *Corylus avellana*

tion of the intergenic heterodimers formed by the monomers encoded by *Pgi-2* and those of another locus (*Pgi-3*), whose intragenic products were either inactive or not detected under our staining conditions. Single-banded individuals would correspond to homozygous genotypes for both genes; double-banded to heterozygous-homozygous combinations; and four-banded to heterozygotes for both genes. Three alleles were found for *Pgi-3* (*a*, *b*, and *c*). One of them (*b*) produced a double heterozygote (*Pgi-2^{ab}–Pgi-3^{ab}*) pattern with three bands due to overlapping between the two heterodimers with intermediate mobility.

GOT

Two zones of activity were found (Fig. 6), of which the most cathodal (*Got-2*) was variable. The segregating progeny analyzed supported the hypothesis that the enzymes of this region are encoded by a single gene (*Got-2*) with two alleles (*a* and *b*).

Linkage

Of the 45 possible combinations between pairs of the ten variable genes 40 were examined for linkage (all but *Pgi-2–Aco-2, Pgi-2–Pgm-3, Pgi-3–Aco-2, Got-2–Aco-2* and *Got-2–Pgm-3*). Significant departures from independence were observed in 10 cases involving 8 pairs of genes. Lack of independent assortment was caused by linkage in 4 pairs: *Mdh-1–Pgi-2, Aco-2–Pgm-2, Pgm-1–Pgm-3* and *6Pgd-2–Pgm-2* (Table 4). *Pgm-2* was linked to *Aco-2* and *6Pgd-2*, but the two latter loci were independent, suggesting that they flank *Pgm-2* but at some distance in the chromosome (Fig. 7). Three more cases deviated significantly from independence in one cross (*Pgm-1–Pgm-2* in H-510, *Aco-1–6Pgd-2* in H-268, and *6Pgd-2–Pgm-1* in H-383), but linkage was not confirmed in others (*Pgm-1–Pgm-2* in H-364 and H-491; *Aco-1–Pgd-2* in H-383, H-313, H-491 and H-510; and *6Pgd-2–Pgm-1* in H-370, H-313, H-268, H-510 and H-491), suggesting either loose linkage or that positive results were artifactual. Lack of independence was also found for *6Pgd-2–Got-2* in cross H-510, but it was not caused by linkage since the calculated recombination fraction did not deviate from 0.5.

Discussion

Our results provide information on the genetic basis of ten variable isozyme genes in hazelnut. Five additional

regions of activity (MDH-2, MDH-3, PGI-1, GOT-1, and 6PGD-1) are apparently monomorphic. The enzymes present in these regions may correspond to the products of five more loci that are fixed in the parentals used. Distorted segregations occurred in 10 of the 46 cases studied (22%).

Deviations from Mendelian expectations have been frequently observed in plant genes. Using data from *Lens, Capsicum* and *Lycopersicon*, Zamir and Tadmor (1986) estimated that 13% of genes segregate unequally in intraspecific crosses and 54% in interspecific crosses. Selection at various stages from pollination to seed germination were proposed as the cause of such deviations. Given that we have used intraspecific crosses, the proportion of significant deviations was higher than that calculated by Zamir and Tadmor, suggesting that selection at these life cycle stages may have been stronger in *C. avellana*.

The heterozygous phenotypes of four isozyme genes (*Mdh-1, 6Pgd-2, Pgi-2,* and *Got-2*) were three banded, whereas they were two banded in five other loci (*Aco-1, Aco-2, Pgm-1, Pgm-2* and *Pgm-3*), suggesting that the corresponding enzymes are dimeric in the former case and monomeric in the latter. We also interpreted the proteins encoded by *Pgi-3* to be subunits of a dimeric enzyme, but in this case the genotype for this gene was inferred from the banding patterns of the zone of intergenic dimers between the products of *Pgi-2* and *Pgi-3* since the region of intragenic dimers of *Pgi-3* was not present. Similar situations have been described in maize (Schwartz 1969) for alcohol dehydrogenase and in *Brassica oleracea* (Arús and Orton 1983) for alcohol dehydrogenase and acid phosphatase.

The number of isozymes of certain enzyme systems is constant in diploid species and can be used as a criterion for ploidy level determinations, since duplicated isozymes in most of these enzymes would indicate polyploidy (Gottlieb 1982). The chromosome number of *C. avellana* is n = 11 (Salesses 1973), but other species of the genus have n = 14 (Duke 1989). Species with a haploid complement of 14 or more were considered by Grant (1971) to be possible polyploids. Evidence based on isozymes favors the hypothesis that the hazelnut is a diploid species. GOT and 6PGD, with two isozymes each, and MDH with three are well within the expected number of diploids. Moreover, these three enzymes are dimeric, and we have not observed the presence of intergenic bands, which would indicate the duplication of enzymes active in the same subcellular compartment. Duplications did occur in PGI and PGM, both of which have three isozymes whereas two are commonly found in diploid species. For PGI, the existence of intergenic enzymes suggests that *Pgi-2* and *Pgi-3* are duplicated, but an inference such as that for the monomeric PGM would require the determination of which of the PGM isozymes are in the same subcellular compartment. Unequal crossing-over appears to be unlikely to be the cause of these duplications, since none of the PGI or PGM loci are tightly linked; *Pgm-1–Pgm-3* are linked, but are not sufficiently close (11 cM apart). Thus, other sources of gene duplication like those originating from insertional translocations or overlapping reciprocal translocations (Gottlieb 1983), or from duplications of chromosome arms or entire chromosomes may explain these events.

Linkage analysis between isozyme loci has provided the first results to date on hazelnut gene mapping. Three linkage groups with more than one locus have been detected, and the remaining three unlinked genes may correspond to a maximum of three more groups. This information can be extended with the addition of other isozymes known to be variable (Truco et al. 1989; Cheng et al. 1990) or with other sources of molecular polymorphisms. These markers may be useful tools for enhancing the efficiency of plant breeding in the hazelnut, either as unmapped markers, or by their linkage to loci responsible for useful qualitative or quantitative variation.

Acknowledgements. We wish to thank I. Batlle and E. J. Legg for their valuable suggestions. This research was supported in part by funds of project PA86-0125 from Comisión Interdepartamental de Ciencia y Tecnología.

References

Ahmad Z, Daley LS, Menendez MA, Lagerstedt HB (1987) Characterization of filbert (*Corylus*) species and cultivars using gradient polyacrylamide gel electrophoresis. J Environ Hortio 5:11–16

Allard RW (1956) Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia 24:235–278

Arús P, Orton TJ (1983) Inheritance and linkage relationships of isozyme loci in *Brassica oleracea*. J Hered 74:405–412

Cheng S, Mehlenbacher SA, Chen T (1990) Identification of hazelnut cultivars by isozyme analysis. In: XXIII Int Hortic Congr Florence, Italy, Abstr no. 3011

Chyi YS, Weeden NF (1984) Relative isozyme band intensities permit the identification of the 2n gamete parent in triploid apple cultivars. HortScience 19:818–819

Duke JA (1989) *Corylus avellana*. In: Duke JA (ed) Handbook of nuts. CRC Press, Boca Raton Flo., pp 419–470

Gottlieb LD (1982) Conservation and duplication of isozymes in plants. Science 216:373–380

Gottlieb LD (1983) Isozyme number and phylogeny. In: Jensen U, Fairbrothers DE (eds) Proteins and nucleic acids in plant systematics. Springer, Berlin Heidelberg New York, pp 209–221

Grant V (1971) Plant speciation. Columbia University Press, New York

Jackson JE, Clarke GR (1991) Gene flow in an almond orchard. Theor Appl Genet 82:169–173

Loukas M, Pontikis K, Vergini I, Papalexandris K (1984) Identification of walnut and filbert cultivars by isozyme banding patterns. Agric Res 8:31–44

Manganaris AG, Alston FH (1987) Inheritance and linkage relationships of glutamate oxaloacetate transaminase isoenzymes in apple. 1. The gene *GOT-1* a marker for the *S* incompatibility locus. Theor Appl Genet 674:154–161

Salesses G (1973) Etude cytologique du genre *Corylus*, mise en évidence d'une translocation hétérozygote chez quelques varietés de noisetier cultivé (*C. avellana*), a fertilité pollinique réduite. Ann Amelior Plant 23:59–66

Schwartz D (1969) Alcohol dehydrogenase in maize: Genetic basis for multiple enzymes. Science 164:585–586

Shields CR, Orton TJ, Stuber CW (1983) An outline of general resource needs and procedures for the electrophoretic separation of active enzymes from plant tissues. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding, part A. Elsevier Sci Publ, Amsterdam, pp 443–468

Suiter KA, Wendel JF, Case JS (1983) LINKAGE-1: a Pascal computer program for the detection and analysis of genetic linkage. J Hered 74:203–204

Todorovic R (1989) Investigation of filbert (*Corylus* L.) isozymes: Adv Hortic Sci 3:38–39

Torres AM (1990) Isozyme analysis of tree fruits. In: Soltis DE, Soltis PS (eds) Isozymes in plant biology. Chapman and Hall, London, pp 192–205

Tous J, Olarte C, Truco MJ, Arús P (1992) Isozyme polymorphisms in carob cultivars. HortScience 27:257–258

Truco MJ, Rovira M, Arús P, Pérez AM (1989) Application de l'électrophorèse isoenzymatique pour l'idéntification varietale chez le noisetier (*Corylus* sp.). Commission Communautés Européennes, Rapport EUR 12005, pp 119–130

Vallejos CE (1983) Enzyme activity staining. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding, part A. Elsevier Sci Publ, Amsterdam, pp 469–516

Zamir D, Tadmor Y (1986) Unequal segregation of nuclear genes in plants. Bot Gaz 147:355–358