INHERITANCE ANALYSIS OF ISOZYME PHENOTYPES IN TETRAPLOID SPECIES USING SINGLE PLANT PROGENIES. AN EXAMPLE IN BLACK THORN (*PRUNUS SPINOSA* L.)

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ABSTRACT

A new method of inheritance analysis for tetraploid species with tetrasomic inheritance is proposed. This method consists of the formulation of necessary quantitative conditions between offspring genotype frequencies in open-pollinated single-individual progenies. Based on this method, an example of genetic analysis for three enzyme systems (IDH, 6-PGDH, PGI) in black thorn was conducted.

Key words: inheritance analysis, tetraploidy, isozymes, *Prunus spinosa*.

INTRODUCTION

Isozyme phenotypes are widely used as genetic markers in forest genetics. A prerequisite of investigations based on these markers is the inheritance analysis of the observed isozyme phenotypes. As was mentioned by GILLET and HATTEMER (1989), a couple of complexities like overlapping of zones or intergenic heterodimers can arise in the interpretation of enzyme phenotypes.

These complexities increase if tetraploid species are investigated, especially if there is evidence for tetrasomic inheritance of the observed isozyme phenotypes. In this case the effect of gene dosage on electrophoretic phenotype appear to be a multiplicity of heterozygote banding patterns (KREBS & HANCOCK 1989). Hence, the interpretation of zymograms is not restricted to the presence or absence of bands in putative heterozygous individuals, because gene dosage effects must be considered and one must decide whether a single band represents one, two or three quantities of a gene product. Individuals which are diallelic for the same alleles exhibit either a "balanced" tri-banded phenotype in which the two homodimers were equal in staining or "unbalanced" phenotypes in which one homodimer and one heterodimer had greater staining intensity than the remaining homodimer. In the literature, results of tetrasomic inheritance were reported for example from KREBS and HANCOCK (1989), MARTINEZ-ZAPATER and OLIVER (1984), QUIROS and MCHALE (1985), QUIROS (1982), ROOSE and GOTTLIEB (1976).

Inheritance analysis is usually conducted on the basis of crossings between two parents with certain phenotypes and the comparison of the segregation ratio among the progeny with the ratio expected on the basis of a hypothesis. However these experiments are often time-consuming and require a lot of effort.

In this study, a formal approach is proposed in order to conduct inheritance analysis for tetraploid species with tetrasomic inheritance on the basis of open-pollinated single-individual progenies.

METHOD OF INHERITANCE ANALYSIS IN TETRAPLOID SPECIES WITH TETRASOMIC INHERITANCE

This approach consists of the formulation of necessary quantitative conditions between the offspring genotype frequencies which must be fulfilled in each progeny-set under the given hypothesis and the basic assumptions on inheritance.

The method proposed here is restricted to putatively diallelic, triplex-heterozygous seed parents ($A_iA_iA_iA_j$ or $A_jA_jA_jA_i$). This is advantageous, because these seed parents segregate only two types of egg cells (A_iA_i) and (A_iA_j) with the frequencies $P(A\,♀_{ii}) = P(A\,♀_{ij}) = 1/2$, $(i \neq j)$, if chromosome segregation is assumed.

The hypothesis is that the observed isozyme phenotypes are under complete control of a single gene-locus with tetrasomically inherited codominant alleles and regular Mendelian segregation of the egg cell gametes.

The assumptions are:

a) chromosome segregation,

b) regular meiotic segregation (1:1) during egg production,

c) random fertilisation of the eggs by each pollen type, and

d) absence of viability selection prior to investigation.

Fertilisation of the egg cells with pollen possessing only the maternal alleles *i* and *j* ($i \neq j$).

Possible pollen contributions are then: A_iA_i, A_iA_j and A_jA_j.

Therefore, six different pairings between egg cells and pollen cells can be expected. For egg cells of type ($A♀_{ii}$) these are the pairings

$(A♀_{ii}) \cup (A♂_{ii}) = A_iA_iA_iA_i$
$(A♀_{ii}) \cup (A♂_{ij}) = A_iA_iA_jA_j$
$(A♀_{ii}) \cup (A♂_{ij}) = A_iA_iA_iA_j$

and for egg cells of type ($A♀_{ij}$) we have

$(A♀_{ij}) \cup (A♂_{ii}) = A_iA_iA_iA_j$
$(A♀_{ij}) \cup (A♂_{jj}) = A_iA_jA_jA_j$
$(A♀_{ij}) \cup (A♂_{ij}) = A_iA_iA_jA_j$.

These pairings are expressed in four different banding phenotypes. Two of these four are based on ordered genotypes in which male and female contributions are identifiable. These are the phenotype $A_iA_iA_iA_i$ and the phenotype $A_iA_jA_jA_j$. In the remaining two phenotypes $A_iA_iA_jA_j$ consisting of the combinations $(A♀_{ii}) \cup (A♂_{jj})$ or $(A♀_{ij}) \cup (A♂_{ij})$, and $A_iA_iA_iA_j$ as the result of pairings $(A♀_{ii}) \cup (A♂_{ij})$ or $(A♀_{ij}) \cup (A♂_{ii})$, male and female contributions are unknown. For triallelic seed parents ($A_iA_iA_iA_j$ or $A_jA_jA_jA_i$) a pairing resulting in the homozygous genotype $A_jA_jA_jA_j$ or $A_iA_iA_iA_i$ is not possible because in the case of a seed parent $A_iA_iA_iA_j$ each egg cell must contain at least one time the allele A_i or the allele A_j in the case of a seed parent $A_jA_jA_jA_i$.

Independently of the allele frequencies in the pollen contributions and due to random fertilisation of the egg cells by each pollen type (assumption c) the following equations hold:

$$P(A♀_{ii}) \times P(A♂_{ii}) = P(A♀_{ij}) \times P(A♂_{ii})$$
$$P(A♀_{ii}) \times P(A♂_{ij}) = P(A♀_{ij}) \times P(A♂_{jj})$$
$$P(A♀_{ii}) \times P(A♂_{ij}) = P(A♀_{ij}) \times P(A♂_{ij})$$

and therefore

$P(A♀_{ij}) \times P(A♂_{jj}) - P(A♀_{ii}) \times P(A♂_{ii}) = [P(A♀_{ii}) \times P(A♂_{jj}) + P(A♀_{ij}) \times P(A♂_{ij})] - [P(A♀_{ii}) \times P(A♂_{ij}) + P(A♀_{ij}) \times P(A♂_{ii})]$.

This is equivalent to

$P(A♀_{ij}) \times P(A♂_{jj}) + [P(A♀_{ii}) \times P(A♂_{ij}) + P(A♀_{ij}) \times P(A♂_{ii})] = P(A♀_{ii}) \times P(A♂_{ii}) + [P(A♀_{ii}) \times P(A♂_{jj}) + P(A♀_{ij}) \times P(A♂_{ij})]$

and for the expected number of genotypes for a single progeny set it holds that:

$$N_{iiij} + N_{ijjj} = N_{iiii} + N_{iijj} \qquad [1]$$

Pollen contributions which contain one non-maternal allele $k \neq i \neq j$

Possible pollen contributions are A_iA_k, A_jA_k and four combinations between egg cells and pollen cells are possible. For egg cells of type ($A♀_{ii}$) these pairings are:

$(A♀_{ii}) \cup (A♂_{ik}) = A_iA_iA_iA_k$
$(A♀_{ii}) \cup (A♂_{jk}) = A_iA_iA_jA_k$

and for egg cells of type ($A♀_{ij}$):

$(A♀_{ij}) \cup (A♂_{ik}) = A_iA_iA_jA_k$
$(A♀_{ij}) \cup (A♂_{jk}) = A_iA_jA_jA_k$.

In one of the resulting three phenotypes ($A_iA_iA_jA_k$), maternal and paternal contributions are not identifiable. This phenotype represents the sum of the combinations $(A♀_{ii}) \cup (A♂_{jk})$ and $(A♀_{ij}) \cup (A♂_{ik})$. The contribution of each combination to the phenotype $A_iA_iA_jA_k$ depends on the frequency of the pollentype $(A♂_{ik})$ and $(A♂_{jk})$ in the effective pollen pool. Only if these types posses the same frequencies, the contributions of each pairing $(A♀_{ii}) \cup (A♂_{jk})$, $(A♀_{ij}) \cup (A♂_{ik})$ to the phenotype $A_iA_iA_jA_k$ are equally frequent. However, if one assumes that $P(A♂_{jk}) \neq (P♂_{ik})$, the contribution of the former pollentype to the pairings of phenotype $A_iA_iA_jA_k$ still equals the frequency of the ordered phenotype $A_iA_jA_jA_k$.

Thus, the following conditions must be fulfilled in each progeny set for pollentype contributions with only one maternal allele:

$P(A♀_{ii}) \times P(A♂_{jk}) + P(A♀_{ij}) \times P(A♂_{ik}) = P(A♀_{ii}) \times P(A♂_{ik}) + P(A♀_{ij}) \times P(A♂_{jk})$.

For the expected frequencies of the observed genotypes we have

$$N_{iijk} = N_{iiik} + N_{ijjk} \qquad [2]$$

Pollen contributions possessing none of the maternal alleles

Possible pollen contributions are A_kA_k, $k \neq i \neq j$.

In this case each pairing results in a phenotype where maternal and paternal contributions are identifiable and the expected probability of pairings between each type of egg cells and a pollen type must be equal. Thus it holds that:

$$P(A♀_{ii}) \times P(A♂_{kk}) = P(A♀_{ij}) \times P(A♂_{kk})$$

and $N_{iikk} = N_{ijkk}$ [3]

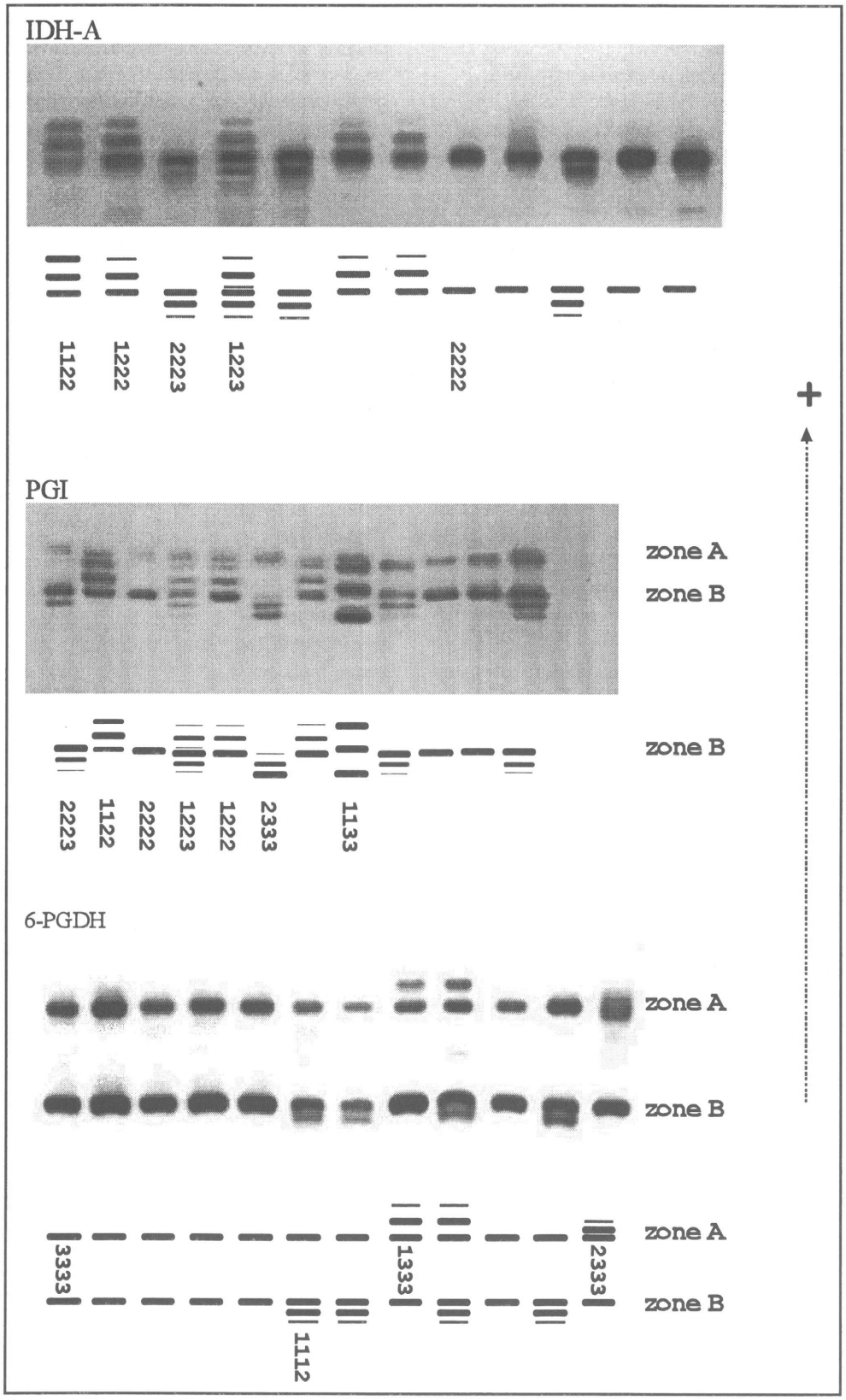

Figure 1. Examples of zymograms from bud tissue of black thorn. Digital photos and schematic illustrations. Putative genotypes at a gene locus are given below the schematic illustrations.

RESULTS

An example in black thorn (*Prunus spinosa* L.)

Black thorn is a common widespread European shrub and belongs to the genus *Prunus* subgenus *Prunus*. Black thorn is polyploid ($2n = 4x$) (HANELT 1997).

On the basis of cytological studies (SALESSES & BONNET 1993; SALESSES *et al.* 1994) it is assumed that black thorn is allopolyploid. As is shown in Figure 1, the isozyme patterns of black thorn give evidence for tetrasomic inheritance which supported the hypothesis of an autopolyploid origin of a species (KREBS & HANCOCK 1989). Similar results were observed by KAURISCH *et al.* (1988) in *Prunus cerasus* and *Prunus fruticosa*. But the origin of this polyploidy, i.e. is it autopolyploid due to chromosome doubling or alloploid due to hybridisation events, is not subject of this study. Whether isozymes are an appropriate tool for this kind of investigations at all, is discussed by STEBBINS (1971).

Figure 1 shows zymograms of buds of black thorn and schematic illustrations of the enzyme systems IDH (E.C. 1.1.1.42), PGI (E.C. 5.3.1.9) and 6-PGDH (E.C. 1.1.1.44). These three enzyme-systems are usually dimeric and heterozygous individuals showed intragenic hybrid bands.

IDH – This enzyme showed one zone (A) with high activity and three clearly separated variants. A second zone appeared after staining for several hours but the patterns in this zone B are not reliable. It is evident that the activity of the banding patterns showed strong influence of gene dosage as it is expected for species with tetrasomic inheritance. In figure 1, from left to right, the zymograms no. 2, 6 and 7 showed typical patterns of triplex-heterozygous individuals, whereas no. 1 reveals staining activity like a duplex-heterozygous individual comparable to heterozygous individuals of diploid species. Under the hypothesis of tetrasomic inheritance the occurrence of three putative alleles in zymogram no. 4 is striking.

PGI – Two zones occurred after staining for PGI, but only the variants in zone B are sufficiently well separated for reliable interpretation. Three different variants occur. The fastest one is near to zone A. As for IDH, the patterns are influenced by gene dosage effects. For example, zymogram no. 2 showed the same activity for the fastest variant 1 and for the most frequent variant 2 in a putatively duplex-heterozygous individual whereas in no. 5 and 7 the activity of variant 1 is much lower than the activity of variant 2. Again one zymogram (no. 4) shows patterns indicating an putative triallelic individual.

6-PGDH – This enzyme revealed two clearly separated zones with three variants in zone A and two variants in zone B. The most frequent variant in zone A is A_3 and in zone B variant B_1. Up to now, only triplex heterozygous individuals occurred in zone A such as in zymograms no. 8 and 9 ($A_1A_3A_3A_3$) and for no. 12 ($A_2A_3A_3A_3$) and in zone B for No. 6, 7, 9 and 11 ($B_1B_1B_1B_2$).

Table 1 shows the results of inheritance analysis for different progeny sets. As has been mentioned above, only progenies of putatively triplex-heterozygous seed parents were investigated. In most progeny sets, contributions with variants $k \neq i \neq j$ appeared only in low frequencies thus only equation (1) was tested. Only for

Table 1. Inheritance analysis of isozymes in black thorn. On the left, the proposed genotypes of maternal trees are grouped for each enzyme system. *N* is the size of the seed samples and P refers to the test equations (1) and (2). *P* represents the probability of equally or less like samples.

Gene locus	Type	Shrub #	*N*	Progenies				Summed values			$P_{(1)}$	$P_{(2)}$
				$N_{i \neq j \neq k}$	N_{ijjj}	N_{iiij}	N_{iiii}	N_{iijj}	$N_{ijjj}+N_{iiij}$	$N_{iiii}+N_{iijj}$		
Pgi–B	$B_2B_2B_2B_3$	S-2	28	3	2	8	9	6	10	15	0.424	–
	$B_2B_2B_2B_3$	S-23	30	–	4	8	3	15	12	18	0.362	–
	$B_2B_2B_2B_3$	S-24	30	2	4	7	5	12	11	17	0.345	–
	$B_2B_2B_2B_3$	S-26	30	4	2	10	7	7	12	14	0.845	–
6-Pgdh–A	$A_1A_3A_3A_3$	S-21	30	–	16	–	13	1	16	14	0.855	–
	$A_1A_3A_3A_3$	S-22	30	–	17	–	13	–	17	13	0.584	–
	$A_2A_3A_3A_3$	S-24	30	2	11	–	17	–	11	17	0.345	–
6-Pgdh–B	$B_1B_1B_1B_2$	S-7	30	–	–	15	15	–	15	15	1.000	–
	$B_1B_1B_1B_2$	S-8	30	1	–	11	17	1	11	18	0.261	–
Idh–A	$A_1A_2A_2A_2$	M-3	45	13	13	5	9	5	18	14	0.600	1.000
	$A_1A_2A_2A_2$	M-4	45	14	12	3	8	8	15	16	1.000	0.791

IDH there are numerous pollen contributions with a variant k = A_3. Therefore, also equation (2) could be tested.

The results are in good agreement with the test equations (1) and (2) and no significant deviation between expected and observed frequencies occurred. The complete absence of the homozygous individuals $A_jA_jA_jA_j$ in progenies of seed parents with the genotype $A_iA_iA_iA_j$ in all progenies further supports the hypothesis of a tetrasomic single locus mode of inheritance.

CONCLUSIONS

The interpretation of gene dosage effects is a permanent source of errors. Thus, especially in tetraploid species with tetrasomic inheritance genetic analysis is a necessity prior to further investigations. In this study an approach is proposed for inheritance analysis on the basis of progenies from open-pollinated triplex-heterozygous seed parents in order to confirm the hypothesis of tetrasomic inheritance. This approach refers to the method derived by GILLET and HATTEMER (1989) for diploid species. The results of inheritance analysis are in agreement with this hypothesis. No significant deviation from the expected genotype frequencies are observed. Five banded zymograms occured which are indications of triallelic genotypes. Considering the dimeric substructure of the observed allozymes, this provides additional support of the hypothesis of tetrasomic inheritance. Finally no homozygous individuals with genotype $A_jA_jA_jA_j$ occurred in the progeny sets of any investigated seed parent with genotype $A_iA_iA_iA_j$. Thus, the isozymes investigated in this study can be used as genetic markers.

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