Genetic interpretation of enzyme variation in sexual and agamospermous taxa of Taraxacum sections Vulgaria and Mongolica

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Genetic interpretation of enzyme variation in sexual and agamospermous taxa of *Taraxacum* sections *Vulgaria* and *Mongolica*

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Abstract

The inheritance of 15 enzymes, comprising at least 22 genetic loci, was investigated in crosses between sexual diploid individuals of *Taraxacum* sections *Vulgaria* and *Mongolica*. Patterns were consistent with simple Mendelian segregation. From the inheritance information isozyme phenotypes in agamospermous plants from natural populations were inferred. In some crosses part or all of the progeny originated from self-fertilization, sofar a very rare phenomenon in the sections *Vulgaria* and *Mongolica*. It is possible that the probability of self-fertilization increases after pollination by triploid pollen, affecting the cohabitation dynamics of the various ploidy components in mixed natural stands.

Introduction

A multitude of breeding systems characterize the Plant Kingdom (see *e.g.*, Brown, 1979; Richards, 1986). The genus *Taraxacum* (dandelions) exhibits a wide range of strategies such as obligate and facultative agamospermy, obligate outcrossing, and self-fertilization (Richards, 1970, 1986; Müller, 1972; Jenniskens, 1984). Obligate agamospermy, where variation is organized among polyploid (mostly triploid) clones, was thought to be the most common breeding system. However, variation within clones seems to be generated at a rate greater than would be expected on background mutation level alone by a variety of mechanisms (Richards, 1986). In addition, variation can be generated in certain areas where diploid sexual and triploid asexual plants occur together and where swarms of intermediate types may arise through hybridization (Fürnkranz, 1961, 1966; Richards, 1970).

The incidental recordings of sexual diploid specimens have long been seen as an indication of a relic distribution pattern (Fürnkranz, 1966; Richards, 1970; Müller, 1972). This point of view has recently been challenged by Den Nijs and Sterk (1980; 1984a and b), Jenniskens (1984), Elzinga *et al.* (1987), and Den Nijs and Van der Hulst (1988). Apparently, diploids of several sections of the genus *Taraxacum* (*e.g.*, *Vulgaria* and *Erythrosperma*) are widely distributed throughout the western and southeastern parts of Europe. A similar pattern exists in Japan and parts of South East Asia (section *Mongolica*; Morita, 1976 and 1980). Diploids rarely form pure stands: in nearly all populations they grow in mixed stands with triploids. Both greenhouse and field experiments have shown that di- and triploids can hybridize, giving rise to diploid as well as triploid offspring (Jenniskens, 1984; Sterk, 1987). Thus gene flow breaks down at least partly the boundaries between the (agamosper-

mous) microspecies (Richards, 1970; Jenniskens, 1984). The presence of this mechanism prompted some authors to the hypothesis that in several *Taraxacum* sections a cyclic di-polyploid evolutionary development occurs in which the adaptive advantages of the respective reproductive strategies play a critical role (Den Nijs & Sterk, 1980, 1984b; Den Nijs & Van der Hulst, 1988).

This and forthcoming publications are aimed at elucidating the relative importance and dynamics of various breeding systems and the population structure of *Taraxacum* species in Western and Central Europe (including the disjunct distribution pattern of diploid *Vulgaria*) and Japan by means of electrophoresis of enzymes as markers.

Iso- and allozymes have frequently and successfully been used to study breeding system dynamics, population genetical structure and their taxonomic implications (*e.g.*, in *Taraxacum*: Lyman & Ellstrand, 1984; Ford & Richards, 1985; Hughes & Richards, 1985; Mogie, 1985; Van Oostrum *et al.*, 1985).

Reliable models of inheritance have to be established before one can embark on the aforementioned types of study. Apomictic plants do not allow formal genetic analysis. The present investigation shows that the genetic basis of isozyme phenotypes in apomictic plants can be understood from the inheritance information of sexual diploids.

Materials and methods

Plants from two *Taraxacum* sections were used, *viz. Vulgaria* from Western Europe and *Mongolica* from Japan (species *hondoense, japonicum*, and *longeappendiculatum*).

Inheritance patterns of allozymes were studied by reciprocally crossing sexual self-incompatible dandelions and electrophoretically analyzing parents and offspring. In some crosses one parent died before its genotype was scored. In these cases, the genotype was inferred from the F1 and

Table 1. Enzyme specifications of *Taraxacum* with gel systems providing best resolution (P, polyacrylamide; S, starch), locus designations and subunit structure (M, monomer; D, dimer; –, unknown).

Enzyme (EC number)	Gel	Locus abbreviation	Subunit structure
Alcohol dehydrogenase (1.1.1.1)	P, S	*Adh-1*	D
	P	*Adh-2*	D
Shikimate dehydrogenase (1.1.1.25)	P	*Shdh*	M
Malate dehydrogenase (1.1.1.37)	P	*Mdh*	D
Malic enzyme (1.1.1.40)	P	*Me*	–
6-Phosphogluconate dehydrogenase (1.1.1.43)	P	*6Pgdh-1*	D
		6Pgdh-2	D
Octanol dehydrogenase (1.1.1.73)	P, S	*Odh*	M
Xanthine dehydrogenase (1.1.1.204)	P	*Xdh*	–
Glutamate dehydrogenase (1.4.1.2)	P	*Gdh*	–
NADH dehydrogenase (1.6.99.3)	P	*Nadh. dh*	M
Catalase (1.11.1.6)	P	*Cat*	–
Aspertate aminotransferase (2.6.1.1)	P	*Aat*	D
Amylase (3.2.1.1)	P	*Amy*	–
Leucine aminopeptidase (3.4.11.1)	S	*Lap-1*	M
		Lap-2	M
Glucose-6-phosphate isomerase (5.3.1.9)	S	*Gpi-1*	D
		Gpi-2	–
Phosphoglucomutase (5.4.2.2)	S	*Pgm-1*	M
		Pgm-2	M
		Pgm-3	–
		Pgm-4	M

in all cases results agreed well with Mendelian segregation. Identical crosses, including reciprocals, were lumped after being tested for homogeneity (G-test, Sokal & Rohlf, 1981) and found homogeneous before we determined their fit to expected Mendelian ratios. G-tests for goodness of fit were adjusted by Williams' correction (Williams, 1976) resulting in a conservative test as recommended by Sokal and Rohlf (1981) for sample size under 200.

Genotypes of agamospermous plants were inferred from the inheritance data of the sexual crosses.

Sample preparations and electrophoresis was carried out below 5 °C. Enzymes were extracted from young leaves by homogenizing 70 milligram in 450 milliliter of a grinding buffer containing 0.14 M NaCl, 0.02 M NaNO3 and some sea sand. To avoid denaturing of the enzymes by protease activity, 1% (w/v) Dowex Cl^-, 0.12% (w/v) dithioerythritol and 0.1% (v/v) 2-mercaptoethanol were added. Extracts were centrifuged for 5–10 min at 10,000 rpm in order to get a clear supernatant. The enzymes listed in Table 1 were assayed in discontinuous non-dissociating buffers.

LAP, PGM and PGI were studied on 12% Connaught/Sigma (1 : 1) starch gels, prepared according to Ayala *et al.* (1972), in the buffer system described by Ridgway *et al.* (1970) with the following modifications: the gel buffer contained 10% electrode buffer and the pH was adjusted to 8.2. All other enzymes were investigated on 7% polyacrylamide gels following the protocol of Shields *et al.* (1983). Electrophoresis was stopped after the Bromocresol Green front had migrated 6 cm (starch) or the Bromophenol Blue front 8.5 cm (polyacrylamide) from the origin.

Enzymes were specifically stained following the usual recipes (see *e.g.* Vallejos, 1983) with minor modifications to suit the material. In the following capitals refer to a specific enzyme, while italics refer to the gene coding for that enzyme.

Isozymes (different genes coding for enzymes that catalyze the same reaction) were numbered according to their relative migration, with the locus specifying the least anodally migrating form designated 1. At each locus alleles were numbered alphabetically with increasing migration from the origin.

Many plant enzymes are known to be duplicated, especially those functioning in glycolysis and the pentose phosphate cycle, one form being active in the cytoplasm, the other in the plastids (Gottlieb, 1982). The subcellular organization of some of the enzymes (*viz.*, 6PGDH, ADH, PGI, PGM and LAP) was studied following the method of Weeden and Gottlieb (1980) by soaking approximately 20 mg of *Taraxacum* pollen in extraction buffer for 2.5 h at room temperature. The mixture was shaken by hand every 15 min.

Results and discussion

A total of 15 enzymes, representing at least 22 loci were resolved in specimens of *Taraxacum* with the electrophoretic techniques used. Representative examples of banding patterns are presented in Figure 1. All crosses assayed for GDH, XDH, CAT, and AMY produced a single zone of activity and were interpreted as monomorphic single-gene traits. ME showed a monomorphic three banded pattern, that might represent one to three loci.

Segregation data for the polymorphic loci are given in Table 2. Cases of non-segregating progenies have not been included for space consideration. Observed segregations did not deviate significantly from those expected under Mendelian ratios except for two (Table 2). This lies within the distribution expected by chance. The observation of simple Mendelian inheritance and the similarity in the ratio between reciprocal crosses indicate that all loci are encoded by nuclear genes.

6-Phosphogluconate dehydrogenase (6PGDH, Fig. 1a) consisted of two variable zones of activity. In each zone homozygotes appeared as single bands, heterozygotes showed three bands with a 1AA : 2AB : 1BB allozyme activity ratio. This pattern is consistent with a dimeric enzyme with random dimer formation between the pro-

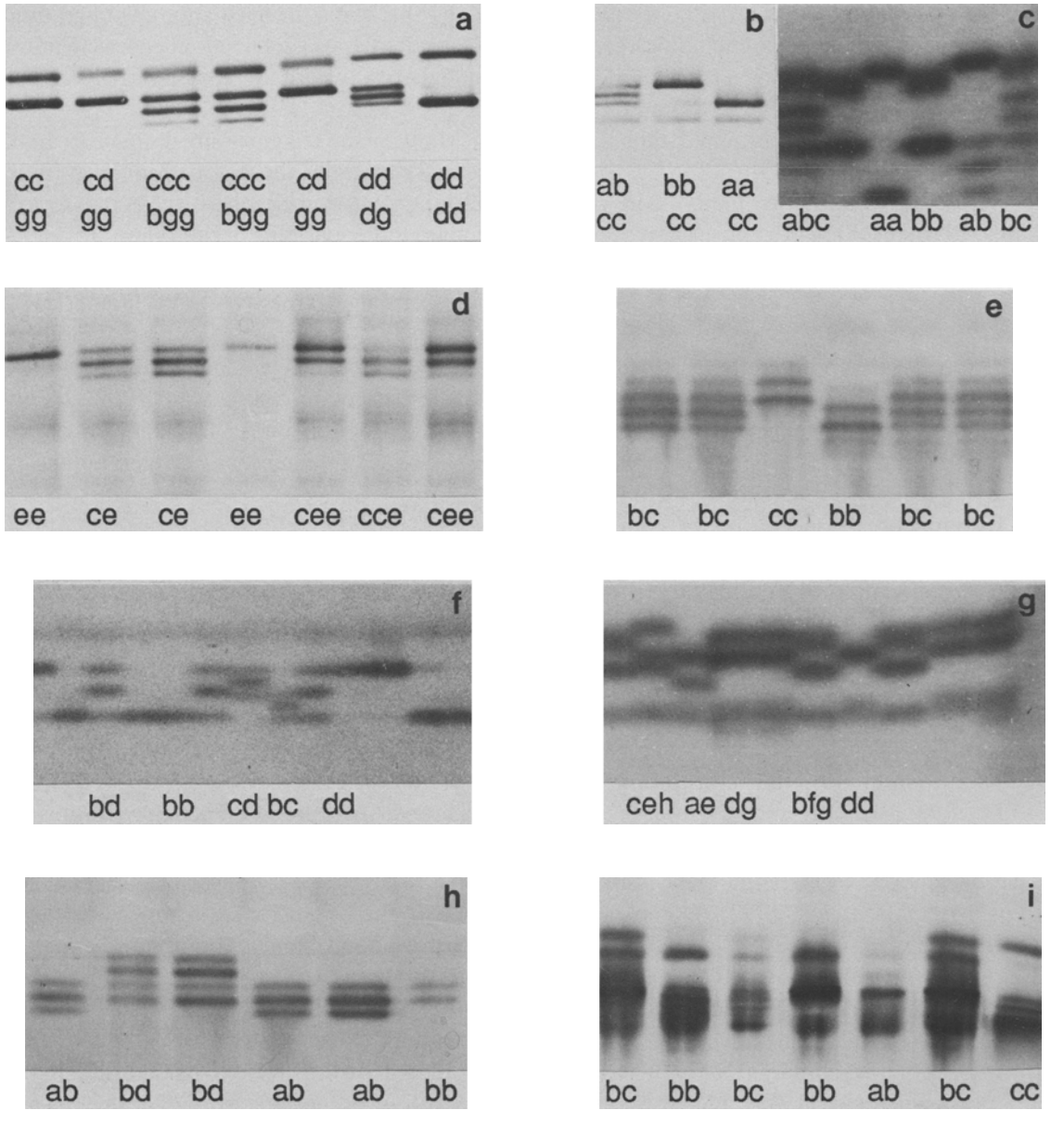

Fig. 1. Zymograms for eight enzyme systems from diploid and triploid *Taraxacum* with genotype indications; (a) 6-phosphogluconate dehydrogenase; (b) alcohol dehydrogenase on polyacrylamide; (c) alcohol dehydrogenase locus 1 on starch; (d) malate dehydrogenase; (e) aspertate aminotransferase; (f) glucose-6-phosphate isomerase locus 1; (g) leucine aminopeptidase locus 2; (h) shikimate dehydrogenase; (i) NADH dehydrogenase.

ducts of both equally expressed structural alleles, producing a heterodimer in addition to both parental homodimers.

Triploid agamospermous plants showed one, three or five banded patterns in various activity ratios. The three banded patterns had either a 1AA : 4AB : 4BB or a 4AA : 4AB : 1BB allozyme activity ratio. In plants polyploidy usually does not alter the level of expression per gene so that gene dosage is directly correlated with quantity of gene product (see *e.g.*, Ciferri *et al.*, 1969; DeMaggio & Lambrukos, 1974; Roose & Gottlieb, 1980). Thus, the occurrence of both kinds of asymmetrical heterozygotes can be inter-

Table 2. Inheritance data and goodness of fit G test for single locus segregation at 12 loci in *Taraxacum*, with 1, 2 and 3 degrees of freedom for 1:1, 1:2:1, and 1:1:1:1 ratios, respectively.

Enzyme locus	Parental genotypes	Offspring genotypes	Expected ratio	G
6Pgdh-1	bb × dd	bd : 20	–	–
	bd × bd	bb : 20 bd : 30 dd : 15	1 : 2 : 1	1.091
	dd × df	dd : 17 df : 29	1 : 1	3.133
	dd × fi	df : 12 di : 15	1 : 1	0.328
	dd × gi	dg : 9 di : 9	1 : 1	0.000
6Pgdh-2	bc × bc	bb : 7 bc : 19 cc : 13	1 : 2 : 1	1.822
	bc × cc	bc : 16 cc : 4	1 : 1	7.522*
	ce × cc	cc : 10 ce : 8	1 : 1	0.217
Adh-2	aa × ab	aa : 43 ab : 36	1 : 1	0.617
	ab × ab	aa : 23 ab : 51 bb : 30	1 : 2 : 1	0.959
	ab × bb	ab : 9 bb : 11	1 : 1	0.195
Mdh	be × be	bb : 8 be : 21 ee : 9	1 : 2 : 1	0.427
	ce × ee	cc : 18 ee : 20	1 : 1	0.104
Aat	aa × dd	ad : 30	–	–
	ac × cc	ac : 10 cc : 6	1 : 1	0.980
	ac × dd	ad : 11 cd : 7	1 : 1	0.872
	cc × dd	cd : 20	–	–
Gpi-1	aa × ab	aa : 21 ab : 16	1 : 1	0.669
Gpi-2	ac × bc	ab : 7 ac : 13 bc : 12 cc : 7	1 : 1 : 1 : 1	3.118
Pgm-1	bb × bc	bb : 11 bc : 13	1 : 1	0.163
Pgm-2	ab × ab	aa : 6 ab : 10 bb : 8	1 : 2 : 1	0.931
Pgm-4	ac × bc	ab : 7 ac : 7 bc : 7 cc : 6	1 : 1 : 1 : 1	1.037
	cc × bc	bc : 19 cc : 21	1 : 1	0.099
Lap-1	aa × ab	aa : 19 ab : 23	1 : 1	0.377
	ab × ab	aa : 4 ab : 11 bb : 6	1 : 2 : 1	0.436
	ab × bb	ab : 19 bb : 18	1 : 1	0.027
	ab × bo	ao : 9 ab : 11 bb + bo : 22	1 : 1 : (1 + 1)	0.291
Lap-2	ab × bb	ab : 5 bb : 8	1 : 1	0.673
	ab × bc	ab : 5 ac : 5 bb : 17 bc : 11	1 : 1 : 1 : 1	9.955*
	ac × bc	ab : 9 ac : 7 bc : 14 cc : 12	1 : 1 : 1 : 1	2.754
	bc × aa	ab : 15 ac : 9	1 : 1	1.485
Sdh	ab × bc	ab : 4 ac : 7 bb : 3 bc : 9	1 : 1 : 1 : 1	3.871
	bb × bc	bb : 14 bc : 17	1 : 1	0.286
	bc × cc	bc : 20 ce : 26	1 : 1	0.776

* Significant at P = 0.05

preted as triallelic genotypes, with allele B and A present twice, respectively.

Five banded patterns occasionally occurred in a 1AA : 2AB : 3[= 1BB + 2AC] : 2BC : 1CC ratio. These too were interpreted as triallelic heterozygotes. As the AA and CC products migrated equidistant from BB, BB and AC dimers overlapped, resulting in a five banded symmetrical phenotype.

In the crosses no null alleles were observed. However, some 3× plants from the field showed regular diploid heterozygous patterns, which we

interpreted as oab heterozygotes. The extraction procedure was too crude to ascertain the genotypic state of single-banded phenotypes, especially if null alleles were involved.

No intergenic heterodimers were found. This could be caused either by substantial structure differences between the subunits that precluded dimerization and/or physical separation. As for the latter, 6PGDH-2 seemed to be localized in organelles, as soaked pollen (initially not releasing organelle enzymes: Weeden & Gottlieb, 1980) gave off 6PGDH-1 only.

Patterns similar to those in 6PGDH were observed for the next two enzymes.

Alcohol dehydrogenase (ADH, Fig. 1b) comprised two loci, each coding for a dimer. *Adh-1* was usually monomorphic and produced less active enzymes than *Adh-2*. ADH activity is often absent from leaf tissue, but this was not the case in *Taraxacum*, although it was best assayed during winter time. ADH stained better on starch gels, but due to a poorer resolution heterozygotes often exhibited broad single bands, especially where the parental homodimers had small differences in migration.

Figure 1c illustrates this effect together with some well resolved variation at *Adh-1*. The five banded *Adh-1* genotype was most easily explained by a tetraploid plant with genotype abcc. However, cytogenetic analysis revealed a normal 3× individual. Unequally active alleles or a duplication might explain the pattern. As for the former possibility alleles in diploid plants too do not always exhibit equal levels of activity. Again intergenic dimerization was not observed. Both enzymes were absent from soaked and crushed pollen; instead, a single faster band was observed.

Malate dehydrogenase (MDH, Fig. 1d) was represented by at least one locus. A fast locus was well resolved and coded for a highly variable dimeric enzyme. Additional, mainly less anodal, activity zones that appeared inconsistently were excluded.

The main part of the complex pattern shown by aspartate aminotransferase (AAT, Fig. 1e) was consistent with one locus, coding for a dimeric enzyme. In addition, secondary fainter bands, running 2–3 millimeters ahead of the main band, and sometimes even tertiary bands occurred. Such bands can be produced by post-translational modification in the plant or by the extraction procedure.

The main bands (AA and BB) formed the usual heterodimer AB and so did the secondary bands (A′A′, B′B′, and A′B′ respectively). No heterodimers, however, were produced between a main and a secondary band. Due to suboptimal resolution bands easily fused on the gels (*e.g.*, BB and A′B′). In some plants additional slower migrating bands appeared in an unpredictable manner and were not included. Moreover, there is at least one, weakly staining, faster variable *Aat* locus which was not consistently scorable.

Glucose-6-phosphate isomerase (GPI, Fig. 1f) occurred in two regions on gels run under the conditions given. Both loci were variable, but allozymes of *Gpi-2* were poorly resolved and had a lower activity than those of *Gpi-1*. GPI-1 appeared to be located in the cytosol.

All enzymes described hereafter showed monomeric behaviour.

A total of four isozymes have been resolved for phosphoglucose mutase (PGM, not shown). Generally, diploid plants contain two isozymes, one cytosolic and one plastid (Mühlbach & Schnarrenberg, 1978: Simcox & Dennis, 1978; Gottlieb, 1987). *Pgm* duplications, however, have previously been described in a number of species (Soltis *et al.*, 1987 and references therein). Moreover, polymorphism for isozyme number was found in *Helianthus* species (Riesenberg & Soltis, 1987). So far all inheritance studies documented monomeric proteins encoded by nuclear genes. In *Taraxacum Pgm-1* had the highest activity, *Pgm-4* the second highest. *Pgm-2* was usually faint, and *Pgm-3* often did not produce sufficiently clear bands to score. Only *Pgm-3* was monomorphic. Soaked pollen gave off merely considerable amounts of PGM-1.

Leucine aminopeptidase (LAP, Fig. 1g) exhibited two variable loci, with *Lap-2* having the highest activity. Some triploid specimens were fully heterozygous at *Lap-2*, showing a three-banded pattern. Occasionally abb and aab

triploids could be identified from band intensities. In most triploid cases, however, intensities varied too much to draw clear conclusions about their genotypic status. The same problematic interpretation of triploid patterns holds for PGM and SHDH. Moreover, null alleles, which appeared in some crosses (Table 2), rendered this locus less attractive for population studies. LAP-2 seemed to be localized in the organelles as soaked pollen exhibited a drop in LAP-2 activity in favour of LAP-1.

Shikimate dehydrogenase (SHDH, Fig. 1b) was a monomeric enzyme with all allozymes showing a lighter staining secondary band which migrated some three millimeters ahead of the main band. As the next allozyme often migrated also about three millimeters further to the anode, bands often overlapped. Consequently, heterozygotes showed either four or three banded patterns. Although octanol dehydrogenase (not shown) and NADH dehydrogenase (NADH.DH, Fig. 1i) were not studied in crosses, zymograms could be easily interpreted as simple Mendelian inheritance for a monomeric enzyme. ODH was not consistently scorable for unknown reasons. ADH activity usually appeared on the same gel, because the substrate octanol-1 was dissolved in ethanol. MDH patterns always came up on gels stained for NADH.DH as well as some of the additional slower activity zones. Tyrosinase (TYR) and acid phosphatase (ACPH: both not shown) zymograms were also compatible with monomeric enzymes.

Superoxide dismutase (SOD, not shown) activity appeared as achromatic bands on all gels stained for dehydrogenases or dehydrogenase-coupled enzymes. The system represented at least two loci, the slowest of which (*Sod-1*) was variable, encoding a dimeric enzyme.

Gels stained for esterase (EST) showed highly complex activity patterns, representing at least five loci. Their complexity did not allow conclusions as for their inheritance (compare Ford & Richards, 1985; Hughes & Richards, 1985).

Analysis of joint segregation ratios for deviations from random assortment did not reveal any linkage group among the loci studied.

All enzymes corroborated data on other plant species concerning their active states as mono- or dimers. Comparisons with the inheritance information of Hughes and Richards (1985), the most extensive *Taraxacum* data set available, revealed the following picture. MDH, 6PGDH, CAT, and GDH were similar as were TYR and ACPH for which we do not have inheritance data. For ME they found one invariable band instead of three. Three SOD isozymes appeared in their samples. The fastest locus coded for a monomer, whereas we found one and three banded patterns at *Sod-1*, indicating a dimer as active state for this slow locus. Finally, their AAT patterns were totally incompatible with ours. The other loci have not been studied before.

Table 3 lists three crosses in which expected genotypic classes were absent and/or not expected classes appeared.

Table 3. Aberrant crosses in *Taraxacum.*

Enzyme locus	Parental genotypes	Offspring genotypes	Expected ratio	G
Aat	dd × cc	cd : 31 dd : 3	all cd	–
	if dd × co	cd : 31 do : 3	1 : 1	26.451**
Sdh	de × dd	dd : 10 de : 16 ee : 2	1 : 1	–
	if all from selfing		1 : 2 : 1	6.247*
	if de × do	dd + do : 10 de : 16 eo : 2	(1 + 1) : 1 : 1	14.371**
6Pgdh-1	df × bb	dd : 2 df : 9 ff : 8	1 : 1	–
	if all from selfing		1 : 2 : 1	3.775
6Pgdh-2	ce × bcc	cc : 5 ce : 11 ee : 3	1 : 1 : 1 : 1	–

* Significant at P = 0.05, ** significant at P = 0.001

First, in the *Aat* cross three plants were not true heterozygous cd hybrids while all other progeny number were according to expectation. Partial selfing fits the pattern (strictly speaking, however, diplosporous parthenogenesis cannot be excluded). The alternative explanation of a null allele in the pollen donor is highly unlikely ($G = 26.451$, $P < 0.001$). A null allele in the mother is also possible (with phenotype dd comprising dd and do genotypes), but then class co is missing. Partial selfing was confirmed by the random combination of mother alleles at the diagnostic *Shdh* locus. The reciprocal cross produced heterozygotes only, as expected (Table 2).

Furthermore, at the *Shdh* locus class ee seemed to exemplify another instance of selfing. However, the probability that all progeny came from selfing was low ($G = 6.247$, $P < 0.05$). A nul allele in the father was even less likely ($G = 14.371$, $P < 0.001$). Probably this cross again comprised a mixed offspring, originating from both cross- and self-fertilization. Unfortunately, a multilocus estimate of selfing to check this was not effective as both parents were identically heterozygous at all four remaining loci that were polymorphic.

Finally, genotypes bd and bf did not show up among the progeny, in the *6Pgdh-1* cross. Instead three unforeseen classes appeared. The supposedly diploid pollen donor (diagnosed by pollen diameter analysis; Den Nijs & Sterk, 1980) appeared to be triploid after cytogenetic analysis (genotype bbb) and the offspring appeared to be the result of self-fertilization of the maternal plant (for details see Mortia *et al.*, in press). Moreover, only diploid progeny were observed, while diploids and triploids were expected as triploid plants produce haploid and diploid pollen capable of fertilization (see Introduction). The observed pattern can indeed be interpreted as originating entirely from selfing ($G = 3.775$, n.s.). This was corroborated by data from *6Pgdh-2* (Table 3). However, locus *6Pgdh-2* screened independently is less informative as cc and ce genotypes also could originate from cross-fertilization by haploid c pollen.

If under natural conditions diploids pollinated by triploids (merely) produce offspring by selfing, gene flow between di- and triploids might be severely reduced. Earlier experiments by Jenniskens (1984) and Sterk (1987), however, showed massive hybridization between diploids and triploids. These differences could be explained by variation within and among populations in fertilizing capacity of pollen from triploids and/or amount of self-compatibility of diploids. So far self-compatibility is a rare phenomenon in the genus *Taraxacum*. Only the small section *Leptocephala* comprises species which normally can reproduce by self-pollination. Moreover, rare self-compatibility has recently been recorded in the section *Vulgaria* in some plants from a mixed population from Western France (Jenniskens, 1984). These plants set a fairly high percentage of seed in selfing experiments. The study of Morita *et al.* (in press) suggests that self-fertilization is facilitated after pollination by triploids. This process will have consequences for the cohabitation dynamics of the various ploidy components in mixed stands.

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