

Zeitschrift: Botanica Helvetica
Herausgeber: Schweizerische Botanische Gesellschaft
Band: 109 (1999)
Heft: 2

Artikel: Evidence from isozyme analysis of autopolyploidy in *Anthoxanthum alpinum* Å. & D. Löve
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DOI: <https://doi.org/10.5169/seals-73297>

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Evidence from isozyme analysis of autopolyploidy in *Anthoxanthum alpinum* Á. & D. Löve

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Manuscript accepted August 19, 1999

Abstract

Zeroual-Humbert-Droz C. and Felber F. 1999. Evidence from isozyme analysis of autopolyploidy in *Anthoxanthum alpinum* Á. & D. Löve. Bot. Helv. 109: 217–227.

To test the hypothesis of the autopolyploidy of *Anthoxanthum alpinum*, an analysis of isozymes by electrophoresis of two diploid and two tetraploid populations was conducted. Twenty-seven loci were found of which eighteen were polymorphic. Alleles were present in both cytotypes, with the exception of two rare alleles that were specific to tetraploid *A. alpinum*. The close genetic similarity between both cytotypes confirms the autopolyploid origin of the tetraploid. The proportion of polymorphic loci did not differ between the diploid (42.9% and 52.3%) and the tetraploid (47.6% and 52.4%) cytotypes. The mean number of alleles per locus was significantly higher in the tetraploid (1.29 and 1.31) than in the diploid (1.23 and 1.25). The genetic diversity of diploid and tetraploid *A. alpinum* is discussed in comparison to that of other autopolyploid complexes.

Key words: Isozymes, *Anthoxanthum alpinum*, autopolyploidy, genetic variation

Introduction

Autopolyploidy is the multiplication of the same genome in an organism. Genetic analysis of autopolyploids has often been carried out with isozymes because they are codominant and have a high polymorphism rate. Because of their monophyletic origin, autopolyploids generally display a high degree of genetic similarity with their diploid ancestor by sharing predominant alleles (Crawford 1989; Soltis and Soltis 1989; Lumaret and Barrentios 1990). Autopolyploids often exhibit specific rare alleles resulting in an increase in allelic diversity compared to their diploid ancestor (Stebbins 1957; Soltis and Rieseberg 1986; Thompson and Lumaret 1992; Soltis and Soltis, 1993).

Anthoxanthum alpinum Á. & D. Löve may be either diploid ($2n=10$) or tetraploid ($2n=20$). Diploid *A. alpinum* is a common perennial grass in Europe that grows in most parts of the Alps, in the northern part of the Jura, and in the Massif Central from the subalpine to the alpine belts. It also occurs in northern Scandinavia and Iceland, where it is equally present at both low and high altitudes. Tetraploid *A. alpinum* has a more restricted range. It replaces the diploid in the

southern Jura, on the western part of the Alps and in the Massif Central (Felber 1986 and 1988a). The autopolyploid origin of tetraploid *A. alpinum* was first suggested on the basis of the analysis of its karyotype by Teppner (1970), and by Hedberg (1970) who named it *A. odoratum* karyotype II and described the formation of multivalents during meiosis (Hedberg 1970).

In addition, investigations on flowering phenology (Felber 1988b), on morphology of the fertile lemma (Felber 1987a), and on the sensitivity to a specific rust in the two cytotypes of *A. alpinum*, as well as in its related species, diploid and tetraploid *A. odoratum* (Felber 1987b), corroborated the autopolyploid origin of *A. alpinum*. While the former analyses indicated close genetic relationships between diploid and tetraploid *A. alpinum*, levels and patterns of genetic variation of both cytotypes was never documented.

The aim of the present study was to test with isozymes the autopolyploid origin of tetraploid *A. alpinum*, and to compare the genetic diversities of both cytotypes. The isozyme patterns and their genetic interpretation are described, followed by the analysis of two diploid-pure and two tetraploid-pure populations.

Material and methods

Material

Pure populations of *Anthoxanthum alpinum* were sampled in zones where only one cytotype was previously identified (Felber 1986). Pure-diploid populations were collected in Moléson (alt. 2002 m, Fribourg, 86 plants) and Videmanette (alt. 2050 m, Vaud, 87 plants). Pure-tetraploid populations were sampled in Dent-de-Lys (alt 2014 m, 97 plants) and Rochers de Naye (alt. 2042 m, 92 plants), both located in Vaud. All the populations had a south-eastern exposition.

Methods

Soluble proteins were extracted from leaves following Lumaret (1981a) and separated on either polyacrylamide or starch gels. The enzymes tetrazolium oxydases (TO) and glutamate-oxaloacetate-transaminase (GOT) were assayed on polyacrylamide electrophoresis following the method of Gasquez and Compoin (1976), modified by Lumaret (1981a). Starch electrophoresis was conducted following Pasteur et al. (1987) and Wendel and Weeden (1989). Alcohol dehydrogenase (ADH), malate dehydrogenase (MDH) and isocitrate dehydrogenase (IDH) were separated using the histidine-citrate buffer system (Cardy et al. 1980). The Krisjansson (1963) migration system was used for acid phosphatase (AcPH), malic enzyme (ME), leucine-amino-peptidase (LAP) and peroxidase (aPX, cPX).

As the interpretation of heterozygotes tetraploids requires to consider the relative intensities of the bands (Fig 1), each sample was analysed at least 3 times for each enzyme system.

AcPH, LAP, PX and TO were stained following Lumaret (1981a). For ADH, the staining method of Lumaret (1981a) and Pasteur et al. (1987) was modified by incubating the gel for 30 min in the dark at 37°C in the reaction mix (12.5 ml of 0.2 M tris buffer pH 8.5, 0.75 ml 95% ethanol, 0.5 ml 1% NAD⁺). The staining solution (composed of 12.5 ml 0.2 M tris buffer pH 8.5, 0.5ml 1% MTT and 0.13ml 1% NBT) was then added, and after 15 min, 0.13 ml 1% PMS was added.

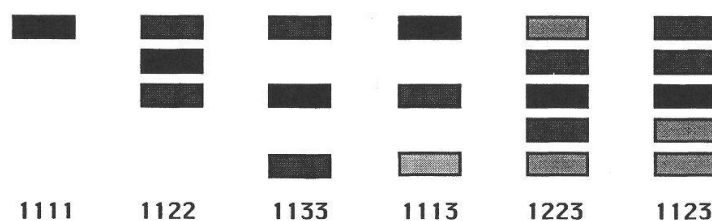


Figure 1. Example of allelic dosages for tetraploids, in the case of a dimeric enzyme.

For ME, the method of Pasteur et al. (1987) was modified by adding the reaction mix (10 ml 0.2M tris buffer pH 8.0, 1 ml 0.5M MgCl_2 , 1 ml 2 M malic acid and 0.1 ml 1% NADP) to the gel with 0.4 ml 1% MTT and 0.1 ml 1% NBT. The gel slice was incubated in this solution in the dark at 37°C for about 20 min, followed by the addition of 0.1 ml 1% PMS.

GOT was stained by a modification of the method of Lumaret (1981a). The gel was immersed in a half-volume of reaction mix (100 ml 0.2 M tris buffer pH 8.0, 0.3 g α -ketoglutaric acid, 0.6 g aspartic acid, pH adjusted to 8.0 to which a small amount of pyridoxal-5'-phosphate was added. The gel was incubated at 37°C in the dark for 10 minutes and a half-volume of the staining solution (constituted of 100 ml 0.2 M tris buffer, pH 8.0, 0.2 g Fast blue BB) was added. The staining solution was replaced several times.

For IDH gel, we modified the staining method of Trigui (1984). The gel slice was incubated 10 min in the reaction mix (10 ml 0.1 M tris buffer pH 8.5, 0.2 ml 0.5 M MgCl_2 , 0.2 ml 1% NADP⁺, 60 mg polyvinylpyrrolidone and 20 mg sodium isocitrate). Then the staining solution (1.0 ml MTT 1%, 0.25 ml 1% NBT, 0.15 ml 1% PMS) was added and the gel incubated at 37°C in the dark until the appearance of the bands.

Analysis of zymograms in terms of loci and alleles was based on the expectation of a conserved number of isozymes along with their quaternary structure, their subcellular compartmentation and the genetic interpretation of other grass species (Gottlieb 1981, Weeden and Wendel 1989).

Statistical analyses

For each locus for which genotypic determination was possible, the allelic frequencies (p_i) were calculated. The deviation from Hardy-Weinberg equilibrium was determined by a χ^2 test. When a significant difference appeared, the genotypic frequencies of heterozygotes were pooled to determine if the observed deviation resulted from a homozygote excess or deficit.

For each sample, genetic diversity was evaluated by the percentage of polymorphic loci and by the mean number of alleles per locus. A locus was considered polymorphic when the frequency of the most-frequent allele was below 95% (Hedrick, 1985). Both indexes were compared by Mann-Whitney's tests. In case of multiple comparisons, corrections of Bonferroni were used (Rice 1987).

Results

Description of enzyme systems

The AcPH enzyme system showed four loci, among which two were monomorphic (loci A and C), and two were polymorphic (loci B and D) (Fig 2). As in *Dactylis* (Lumaret 1981a, 1981b), wheat (Hart 1983) and rye (Jaaska 1983), the zymogram of AcPH corresponded to a monomeric enzyme. The locus B corresponded to three alleles, with alleles 2 and 3 predominant. The locus D exhibited two alleles. The AcPH-D-2 allele was absent in both the pure populations of Videmanette (2X) and Dent-de-Lys (4X), (Table 1).

ADH exhibited a single locus (Fig 2). The dimeric structure of ADH that we observed for *A. alpinum* corresponded to that described by Schwartz (1966, 1969), Freeling and Schwartz (1973), Freeling (1974) in Goodmann and Stuber (1983) for maize, Lumaret (1981a) for *Dactylis*, Jaaska (1983) for rye, and Trigui (1984) for *Pennisetum*. The ADH-A-2 was the most frequent allele for all the populations, whereas ADH-A-3 appeared at low frequency in only three samples (Table 1).

ME exhibited four loci, among which the three that migrated the most were monomorphic (ME-A, ME-B, ME-C), and the one that migrated the least was polymorphic (ME-D) (Fig 2). According to the zymogram, the structure of the enzymes was monomeric. ME-D had two alleles which were found in all the samples (Table 1).

For GDH, a single band appeared for each individual. This isozyme was consequently monomorphic for all our populations.

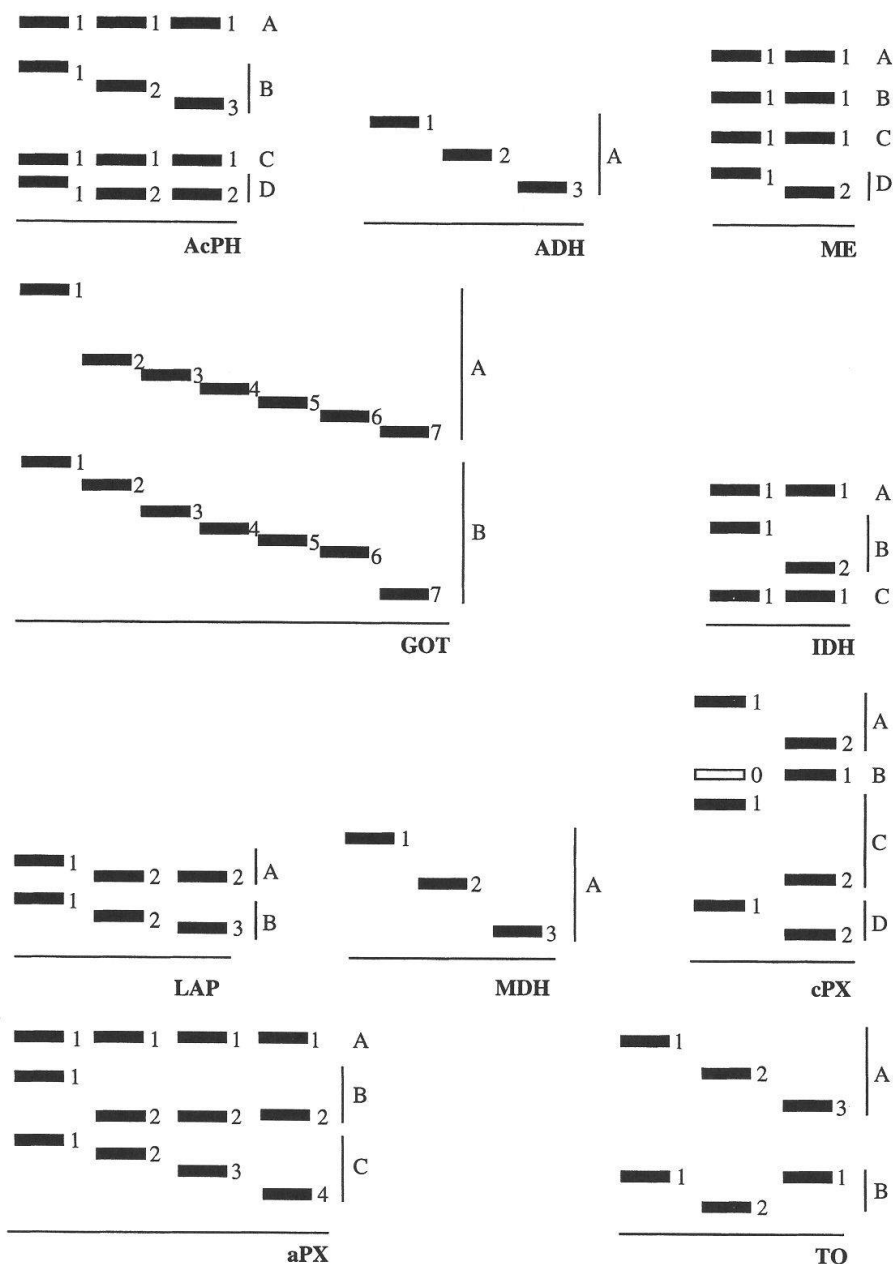


Figure 2. Isozyme banding patterns of *Anthoxanthum alpinum*. Only homozygotes genotypes are represented. Numbers to the right of each band indicate the alleles, and letters show the locus.

GOT presented two polymorphic loci (Fig 2). The allelic associations demonstrated the dimeric structure of this enzyme system for *A. alpinum*. The same genetic determinism was found for maize (Scandalios et al. 1975 in Goodman and Stuber 1983), wheat (Hart and Langston 1977b in Hart 1983), *Dactylis* (Lumaret 1981a) and rye (Humphreys 1992). Both loci were specified by seven alleles. GOT-A-3 and GOT-B-3 were predominant (Table 1). The other alleles were present at lower frequencies.

IDH exhibited three loci (Fig 2). IDH-A and IDH-C were monomorphic while IDH-B was polymorphic. Similar to maize (Stuber and Goodman 1980), the genetic determinism of this enzyme system was dimeric. For the locus B, the heterodimeric band always showed less

Table 1. Allelic frequencies per population of polymorphic loci.

Locus	Alleles	2X Videmanette	2X Moléson	4X Dent-de-Lys	4X Rochers de Naye
AcPH-B	1	0.105	0.042	0.043	0.043
	2	0.448	0.452	0.470	0.443
	3	0.448	0.506	0.486	0.514
AcPH-D	1	1.000	0.994	1.000	0.988
	2	0.000	0.006	0.000	0.012
ADH-A	1	0.114	0.019	0.139	0.023
	2	0.861	0.981	0.849	0.936
	3	0.025	0.000	0.011	0.041
ME-D	1	0.277	0.366	0.327	0.284
	2	0.722	0.634	0.673	0.716
GOT-A	1	0.000	0.012	0.035	0.000
	2	0.000	0.012	0.019	0.039
	3	0.902	0.768	0.788	0.660
	4	0.024	0.018	0.035	0.067
	5	0.073	0.173	0.121	0.211
	6	0.000	0.006	0.000	0.006
	7	0.000	0.012	0.003	0.017
GOT-B	1	0.029	0.072	0.183	0.060
	2	0.007	0.000	0.042	0.017
	3	0.645	0.754	0.492	0.724
	4	0.065	0.174	0.048	0.017
	5	0.210	0.174	0.197	0.172
	6	0.022	0.000	0.000	0.000
	7	0.022	0.000	0.039	0.009
LAP-A	1	0.988	0.987	0.973	1.000
	2	0.012	0.013	0.027	0.000
LAP-B	1	0.000	0.000	0.000	0.033
	2	1.000	1.000	1.000	0.949
	3	0.000	0.000	0.000	0.018
cPX-A	1	0.443	0.460	0.610	0.491
	2	0.557	0.540	0.390	0.509
cPX-C	1	0.524	0.413	0.503	0.483
	2	0.476	0.587	0.497	0.517
cPX-D	1	0.553	0.519	0.543	0.466
	2	0.447	0.481	0.457	0.534
aPX-B	1	0.444	0.500	0.407	0.358
	2	0.555	0.500	0.593	0.641
aPX-C	1	0.013	0.041	0.079	0.045
	2	0.959	0.946	0.904	0.923
	3	0.000	0.000	0.000	0.010
	4	0.027	0.013	0.018	0.022
TO-A	1	0.969	0.860	0.970	0.956
	2	0.031	0.140	0.027	0.044
	3	0.000	0.000	0.003	0.000
TO-B	1	0.975	0.860	0.970	0.956
	2	0.025	0.140	0.030	0.044

intensive coloration than the homodimeric bands. This was interpreted as the heterodimer being less active than the two other molecules. As the bands of this locus were often not clearly stained, only the presence or the absence of the bands were noted.

LAP had two polymorphic loci (LAP-A and LAP-B) (Fig 2). The analysis of zymograms suggested a monomeric structure. The same genetic determinism was found for wild barley (Gottlieb 1981). The first locus showed two alleles. The allele LAP-A-1 was predominant with a frequency that was higher than 90% (Table 1). The second locus showed three alleles. LAP-B-2 appeared in a frequency higher than 94%. The two other alleles were rare, as they only appeared in the tetraploid population of Rochers de Naye.

For MDH, one polymorphic locus could be interpreted (MDH-A) (Fig 2). According to the zymograms, MDH had a dimeric genetic structure, like maize (Longo and Scandalios 1969, in Goodmann et Stuber 1983) and *Dactylis* (Lumaret 1981a). For *A. alpinum*, this enzyme was duplicated: the diploid genotype corresponded to four alleles and the tetraploid genotype to eight alleles. This characteristic was also found for MDH in tetraploid *Deschampsia cespitosa* (Purdy and Bayer 1995). Because of the difficulty of comparing the relative intensity of bands in the determination of the tetraploid genotypes, only the presence or the absence of the bands was noted.

cPX from the cathode showed 4 loci, among which three were polymorphic. The genetic determinism corresponded to that of maize (Brewbaker and Hasegawa, 1975, in Goodmann and Stuber 1983) and *Dactylis* (Lumaret 1981a). The first locus had two alleles showing bands which were clearer than those of other loci. cPX-A-2 was generally more frequent than cPX-A-1, except for the population from Dent-de-Lys (Table 1). Since some individuals didn't show any band at locus B, this suggests the presence of a null allele. Genotypic identification was consequently not possible for this locus, which is not included in the following analyses. The third locus had two alleles. cPX-C-2 was the most frequent, apart from the populations of Dent-de-Lys et Videmanette. The locus D also showed two alleles. cPX-D-1 was more frequent than cPX-D-2 except for Rochers de Naye.

aPX showed three loci, which also had a monomeric genetic structure (Fig 2). aPX-A was monomorphic, while aPX-B and aPX-C were polymorphic. In case of oxydation of the proteins extracts, some additional bands appeared at both ends of the zymogram. In the rapid migration part, some bands could arise forming a pseudo-locus, whereas in the slow migration part, a large band appeared at the place of the third locus. The first locus had two alleles. For all the populations, the aPX-B-2 allele was the most frequent (Table 1). The second locus was characterized by four alleles. All the alleles except for the second, were rare with a frequency of less than 10%. aPX-C-3 was only found in the population of Rochers de Naye.

The TO showed two polymorphic loci that were dimeric (TO-A and TO-B) (Fig 2). The same genetic determinism was found for wheat (Beauchamps et Fridovich 1973, in Hart

Table 2. Percentage of polymorphic loci (P) and mean number of alleles (A) per locus for each population.

		P	A
2X	Videmanette	42.9	1.25
	Molésou	52.3	1.23
4X	Dent-de-Lys	47.6	1.31
	Rochers de Naye	52.4	1.29

1983), wild barley (Brown et al. 1978 in Gottlieb 1981) and *Dactylis* (Lumaret 1981a). The first locus had three alleles, while the second one showed two alleles. These two loci were linked as evidenced by the joint expression of homozygotes and heterozygotes. Moreover, the more frequent alleles for each locus (TO-A-3 and TO-B-1), were always more intensely stained, even if they were present in the same proportion as the other alleles. The TO-A-3 allele was extremely rare, because it was only found in the population of Dent-de-Lys (Table 1).

In summary, twenty-seven loci were identified of which nine were monomorphic (AcPH-A, AcPH-C, GDH-A, ME-A, ME-B, ME-C, IDH-A, IDH-C, aPX-A) and eighteen were polymorphic (APH-B, APH-D, ADH-A, GOT-A, GOT-B, ME-D, IDH-B, MDH-A, LAP-A, LAP-B, cPX-A, cPX-B, cPX-C, cPX-D, aPX-B, aPX-C, TO-A, TO-B).

Genetic analysis of the populations

Diploid and tetraploid *A. alpinum* shared the same alleles, with the exception of two rare alleles (TO-A-3 and aPX-C-3) that were specific to the tetraploid.

Deviation from the expected Hardy-Weinberg equilibrium was evaluated for the polymorphic loci for which the genotype was determined. Significant deviation occurred repeatedly among the tetraploid samples and generally resulted from an excess of homozygotes (α corrected according to Bonferroni). That was the case for GOT-B of the Dent-de-Lys, for LAP-B of Rochers de Naye and for TO-A and TO-B for both tetraploid samples. The last two loci showed the only significant homozygote excess concerning a diploid sample (population of Moléson). The GOT-A locus from the tetraploid sample of the population of Rochers de Naye was the only locus which displayed heterozygotes excess.

The proportion of polymorphic loci and the mean number of alleles per locus were calculated for all the loci that were described. As these indexes are independent of gene dosage, MDH was also included in the analysis.

Table 3. Probability of divergence from the Hardy-Weinberg equilibrium (*) shows that the divergence to the Hardy-Weinberg law is due to homozygote excess, (ns) points out the non-significant comparisons.

Locus	Videmanette	Moléson	Dent-de-Lys	Rochers de Naye
AcPH-B	ns	ns	ns	ns
AcPH-D	monomorphic	ns	monomorphic	ns
ADH-A	ns	ns	p<0.001	p<0.001
ME-D	ns	ns	ns	ns
GOT-A	ns	ns	p<0.001	p<0.001
GOT-B	ns	ns	p<0.001	p<0.001
LAP-A	ns	ns	ns	monomorphic
LAP-B	monomorphic	monomorphic	monomorphic	p<0.001
cPX-A	ns	ns	ns	ns
cPX-C	ns	ns	ns	ns
cPX-D	ns	ns	ns	ns
aPX-B	ns	ns	ns	ns
aPX-C	ns	ns	p<0.001	p<0.001
TO-A	ns	p<0.001	p<0.001	p<0.001
TO-B	ns	p<0.001	p<0.001	p<0.001

The proportion of polymorphic loci did not differ between the diploid and the tetraploid cytotypes (Mann-Whitney's test) (Table 2). The mean number of alleles per locus was significantly higher for the tetraploids (1.29 and 1.31) than for the diploids (1.23 and 1.25) (Mann-Whitney's test, $P < 0.01$).

Discussion

According to Lumaret and Barrentios (1990) and Soltis and Soltis (1989), a recent autopolyploid has several predominant alleles that are identical to those of the diploid ancestor and consequently shows low differentiation from the diploid. The genetic structure of tetraploid *A. alpinum* qualitatively offers numerous similarities to that of the diploid and thus confirms the autopolyploid origin of the tetraploid cytotype, as already assessed on the basis of karyotypes and biosystematics (Teppner, 1970; Hedberg, 1970; Felber, 1987a, 1987b, 1988a, 1988b).

Significant excess of homozygotes was observed in six cases in tetraploid populations, and twice in diploid samples. Excess of heterozygotes was exceptional and occurred only for one isozyme system in one tetraploid population. Excess of homozygotes can be interpreted as the result of inbreeding which may be more frequent in tetraploids than in diploids. Inbreeding may be favored either by the spatial structure of the population causing mating among relatives or by partial self-compatibility.

Evaluation of genetic diversity has been usually carried out with the proportion of polymorphic loci, the mean number of alleles per locus and the mean observed heterozygosity. Mean observed heterozygosity is not reported here, because that of diploids may not be compared with that of polyploids since diploids have only one type of heterozygote, while several types of heterozygotes are present in tetraploids. Nevertheless, proper comparison between diploids and tetraploids can be carried out for the tetraploids by calculating the probability of obtaining heterozygote gametes from the different heterozygote types following Lumaret (1981a) and Ducousso et al. (1990). This was unfortunately not possible from the data in the literature.

Comparison of indexes between species is difficult because values depend on the set of enzyme systems which have been used, based either exclusively on polymorphic enzymes or also including monomorphic ones. Therefore, the comparison of genetic diversity between diploids and of tetraploids of the same species with the same set of loci was the most reliable.

Tetraploid *A. alpinum* exhibited a similar proportion of polymorphic loci but a slightly higher mean number of alleles than diploids. The higher diversity of tetraploid *A. alpinum* resulted from the presence of private alleles.

Higher genetic diversity of tetraploids when compared to diploids has been noticed by Soltis and Soltis (1993) in five of six cases. They correspond to the upper part of Table 4 in which values may differ from Soltis and Soltis (1993) because we focused on the mean number of alleles per locus which was more uniformly used than the mean number of alleles per polymorphic locus. This table also includes recently published data which modify the picture of the highest variability of the polyploid. Thus, no difference between the genetic diversities of the diploid and of the tetraploid was found in the *Lotus alpinus/corniculatus* complex in the Alps (Gauthier et al. 1998). Moreover, the restricted tetraploid endemic *Deschampsia mackenziana* exhibited a lower genetic diversity than its widespread diploid progenitor (Purdy and Bayer 1995). The case of *Turnera ulmifolia* (Shore 1991), cited by Soltis and Soltis (1993) is particularly interesting. This complex includes one diploid (*Turnera ulmifolia* var.

Table 4. Proportion of polymorphic loci (P) and mean number of alleles per locus (A) for several polyploid complexes. (*) designates significant differences between diploids and tetraploids, while (ns) designates non-significant ones.

	P			A			reference
	diploid	tetra- ploid	P	diploid	tetra- ploid	P	
<i>Tolmiea menziessi</i>	24.0	40.8	*	—	—	—	Soltis and Soltis (1989)
<i>Heuchera micrantha</i>	24.1	41.7	*	1.41	1.71	*	Ness et al. (1989)
<i>Heuchera grossulariifolia</i>	23.8	31.1	ns	1.35	1.55	ns	
<i>Dactylis glomerata</i>	38.0	60.0	*	1.15	1.46	*	Lumaret (1981)
<i>Turnera ulmifolia</i> var. <i>intermedia</i>	45.9	20.1	*	1.55	1.20	*	Shore (1991)
<i>Turnera ulmifolia</i> var. <i>elegans</i>		65.3	*		2.03	*	
<i>Lotus alpinus/corniculatus</i>	80.0	86.0	ns	2.80	2.70	ns	Gauthier et al. (1998)
<i>Deschampsia cespitosa/ mackenziana</i>	30.5	18.9	*	2.70	2.17	*	Purdy and Bayer (1995)
<i>Anthoxanthum alpinum</i>	47.6	50.0	ns	1.24	1.30	*	present study

intermedia) and two tetraploids (*T. ulmifolia* var. *intermedia* and *T. ulmifolia* var. *elegans*). Both tetraploid taxa are highly interfertile and are considered autopolyploids of the diploid. Tetraploid *T. ulmifolia* var. *intermedia* had a lower genetic diversity than the diploid while *T. ulmifolia* var. *elegans* was more diverse. These differences were explained as the result of the history of the population and of gene flow between cytotypes. Thus, diploid *T. ulmifolia* var. *intermedia* and tetraploid *T. ulmifolia* var. *elegans* are sympatric on part of their range, which allows the possibility of gene flow between the cytotypes. On the contrary, tetraploid *T. ulmifolia* var. *intermedia* was collected on islands and the putative low number of founders and the isolation from the diploid cytotype might explain its low genetic diversity.

Genetic diversity of autopolyploids is not necessarily higher than that of their progenitors. The genetics of polyploids differs strikingly of that of diploids (see Bever and Felber 1992 for review). Nevertheless, the number of founders, and gene flow greatly influence the genetic diversity of populations of both diploids and polyploids. *Anthoxanthum alpinum* occurs at the subalpine belt in the Alps and its distribution at the regional level corresponds to patches of relatively small size that are separated from each other by valleys. Limited gene flow and migration could be the cause of its relatively low genetic variability.

We are grateful to Jason R. Grant for his linguistic corrections to this manuscript.

This paper belongs to the thesis of Corinne Zeroual-Humbert-Droz.

Résumé

Dans le but de tester l'hypothèse de l'autopolyploïdie chez *Anthoxanthum alpinum*, les isozymes de deux populations diploïdes et de deux populations tétraploïdes ont été analysés. Parmi les vingt-sept loci trouvés, dix-huit d'entre eux étaient polymorphes. Tous les allèles étaient présents dans les deux cytotypes, à l'exception de deux allèles rares qui étaient

spécifiques à *A. alpinum* tétraploïde. La similarité génétique entre les deux cytotypes confirme l'origine autopolyploïde du tétraploïde. La proportion de loci polymorphes ne diffère pas entre le cytotype diploïde (42,9% et 52,3%) et le cytotype tétraploïde (47,6% et 52,4%). Le nombre moyen d'allèles par locus était significativement plus élevé chez le tétraploïde (1,29 et 1,31) que chez le diploïde (1,23 et 1,25). La diversité génétique d'*A. alpinum* diploïde et tétraploïde est comparée à celle d'autres complexes autopolyploïdes.

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