Allozyme polymorphism in diploid and polyploid populations of *Galium*

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Five polymorphic enzyme genes (*Est-1*, *Est-5*, *Got-2*, *Pgi-2* and *6-Pgdh-1*) were studied in Lower Austrian populations of the herbaceous, allogamous polyploid complex *Galium pusillum* agg. Allele frequencies in the relic *G. austriacum-2x* document a high amount of genetic differentiation between the isolated, rather uniform and stenoecious populations which are separable into an older mountain and a more recent foothill series. Genetic divergence from *G. austriacum-2x* is small in *G. austriacum-4x* and only somewhat greater in *G. pumilum-8x*. Allele frequencies in the $4x$ (and $8x$) suggest tetrasomic inheritance and autopolyploid behaviour in spite of nearly exclusive bivalent formation. The amount of genic variation (number of different alleles and average heterozygosity) appears uniform in diploids and polyploids. In contrast, the polyploids exhibit much increased genotypic variation as compared to the diploids. This can be linked to their more continuous, variable, euryoecious and expansive populations.

INTRODUCTION

Within the large Angiosperm genus *Galium* (Rubiaceae-Rubieae), the perennial herbaceous and outbreeding members of the European-Mediterranean sect. *Leptogalium* have long been the object of biosystematic studies (Ehrendorfer, 1949, 1953, and 1962). In particular, the *G. pusillum* aggregate has been shown to consist of a very polymorphic polyploid complex with several parallel series of diploids and polyploids ($x = 11$: $2x-4x-6x-8x-10x$) and a remarkable ecogeographical differentiation. In the foothills and lower mountains of the NE. Alps in Lower Austria, south of Vienna, this aggregate is represented by taxa exhibiting completely different variation patterns: *G. austriacum-2x* and $-4x$, with a micromosaic of strongly isolated and relictual populations, and *G. pumilum-8x* with a diffuse and more or less continuous distribution of very variable and more expansive populations (Ehrendorfer 1949, 1962).

In the present study the electrophoretic analysis of enzyme proteins was used to investigate intra- and interspecific variation at the gene level (cf. Gottlieb, 1977 and 1981; Hurka, 1980; Crawford, 1985). The paper concentrates on the following problems:

- (1) The regional differentiation pattern of *G. austriacum-2x*.
- (2) The relationships between *G. austriacum-2x*, $-4x$, and *G. pumilum-8x*.
- (3) The reasons for the relict or more expansive nature of these taxa.

MATERIALS AND METHODS

Plant Material

Population samples of about 30 individuals each were taken during 1988 from nine ecologically homogeneous sites in Lower Austria, south of Vienna (fig. 1): Seven diploid populations, $2n = 22$, $2x$ (A–G) and one tetraploid population, $2n = 44$, $4x$ (H) of *Galium austriacum* as well as one octoploid population of *G. pumilum*, $2n = 88$, $8x$ (I). Vouchers are preserved in the herbarium of the Institute of Botany, University of Vienna (WU).

Habitats of *G. austriacum* populations are always open SW-, S- or SE-exposed slopes with shallow rendzina soils on carbonate rock (limestone or dolomite). Sites of populations A–D and F–G are loose *Pinus nigra* relic forests, E and H have *Pinus sylvestris* admixed; *Sesleria caerulea* subsp. *varia*, *Polygala chamaebuxus*, *Calamintha*

Figure 1 Sites of *Galium pusillum* agg. populations from the mountains south of Vienna (=Wien) in Lower Austria: ● *G. austriacum*-2x, B-C-D foothill series = horizontal hatching, A-E-F-G mountain series = vertical hatching; ■ *G. austriacum*-4x; H; * *G. pumilum*-8x; I. Plains shown in white, mountains in grey, city boundaries of Vienna dark.

alpina, *Galium lucidum*, etc. are characteristic elements of the undergrowth. Sites of populations A-H are:

- (A) Triesting Valley, between Pottenstein and Fahrafeld, ca. 350 m.
- (B) Piesting Valley, between Waldegg and Oed, ca. 400 m.
- (C) Sierning Valley, Mt Gösing near Sieding, ca. 500 m.
- (D) Semmering, Adlitzgraben, S. of Klamm near Schottwien, ca. 600 m.
- (E) Schwarza Valley, Mt Schneeberg: Gahns, 2.5 km NE. of Payerbach, above Obertal, ca. 700 m.
- (F) Schwarza Valley: Höllental, ca. 0.5 km N. of Hirschwang, ca. 500 m.
- (G) Schwarza Valley: Höllental, ca. 0.5 km S. of Kaiserbrunn, ca. 530 m.
- (H) Puchberg/Schneeberg, Losenheim, ca. 750 m.

Galium pumilum usually grows on brown forest soils and extends from open deciduous oak and beech forests to nutrient-poor secondary grassland. Site of population:

- (I) Semmering, ca. 1 km NE. of Klamm, grassy road margins in mixed deciduous forest on schist, ca. 770 m.

Electrophoresis

Preliminary experiments with material collected at the natural habitat and brought to the laboratory for electrophoretic analysis showed that enzyme activity is decreasing rapidly in detached leaves with time. For that reason it became necessary to establish the living plants in the garden of the Institute of Botany (HBV). For electrophoresis 40 mg of actively growing young leaves were picked, cooled immediately on ice cubes and used for the investigations.

Leaf material was homogenized in 25 µl of extraction buffer (0.1 M Tris/HCl pH 7.1 with 3 mg/vol EDTA, 60 mg/vol PVP-40, 1 mg/vol Dithiothreitol) using 2-ml Eppendorf cups and a rotating plastic rod driven by an electric stirrer. Cooling was applied throughout homogenization. Horizontal starch gel electrophoresis was carried out according to Ayala *et al.* (1972). Two different buffer systems were used:

- (a) bridge: 0.04 M trisodium citrate/citric acid pH 7.5
gel: 0.005 M histidine/NaOH pH 7.5
run: 6 h at 100 V/50 mA
enzymes: 6-Pgdh, Pgi
- (b) bridge = gel: 0.087 M Tris/0.01 M boric acid/0.0014 M EDTA pH 8.9
run: 6 h at 300 V/30 mA
enzyme: Got

Vertical polyacrylamide gelelectrophoresis was carried out in a Hoefer Mighty Small chamber. A multiphasic buffer system (Tris/glycine, Tris/phosphate, Tris/HCl) according to Davis (1964) was used with an acrylamide concentration of 3.75 per cent in the stacking gel and 10 per cent in the resolving gel. The running time for the gel was 3 h at 75 V/30 mA. PAGE was applied for the study of esterases only.

Enzyme staining followed the standard procedures given by Harris and Hopkins (1976) with slight modifications. Alleles were designated according to the relative mobility of the allozymes relative to the most common variant (100).

RESULTS

Iso- and allozymes studied

In a preliminary survey (data not shown) a total number of 15 enzyme systems were checked for genetic variation. Some enzyme systems (e.g., *Pgm*

Figure 2 Zymogram for 6-Pgdh (dimeric) from 18 individuals of *Galium austriacum*-2x populations: 1-9 from D (Klamm) and 10-18 from B (Waldegg). 6-Pgdh-2 is monomorphic, 6-Pgdh-1 polymorphic with two alleles (88 and 100). Individuals 9, 12, 13 and 18 are homozygotes 88/88; 1, 3, 4, 5, 6, 8, 11, 16 and 17 heterozygotes 88/100; and 2, 7, 10, 14 and 15 homozygotes 100/100.

and *Adh*) were found to be monomorphic and thus were not analysed further. Others (e.g., *Acph*, *Mdh*) were variable but difficult to interpret genetically. In *Est*, 6-Pgdh, *Pgi* and *Got* loci highly polymorphic isozymes with clearly interpretable banding patterns were found and hence selected for our investigation. For *Est* five activity zones were rec-

ognized in the gel, representing five genetically independent isozymes. Three of them (*Est*-1, *Est*-2 and *Est*-5) were polymorphic. In *Est*-2 (a β -esterase according to substrate preference *in vitro*), however, enzyme activity appeared to be influenced by the physiological condition of the plant and changed from spring to autumn. Thus, only the

Figure 3 Zymogram for *Pgi* (dimeric) from *Galium austriacum*-2x (several populations, individuals 1-5, 10-12), *G. austriacum*-4x (population H: Losenheim, individuals 6-9) and *G. pumilum*-8x (population I: Klamm, individuals 13-16). *Pgi*-1 is monomorphic, *Pgi*-2 polymorphic with five alleles (55, 61, 85, 100, 115). The following genotypes are shown: In *G. austriacum*-2x individual 12 is 61/61, 1, 5 and 10 are 85/85, 2 is 85/100, 4 is 85/115, 3 and 11 are 100/100; in *G. austriacum*-4x 9 is 85/85/85/100, 8 is 85/85/100/100, 7 is 85/100/100/100 and 6 is 100/100/100/100; in *G. pumilum*-8x, with the alleles 55, 61, 85 and 100, all four individuals are genetically different.

Table 1 Allele frequencies (in per cent) in seven population samples of *Galium austriacum*-2x. *n* = number of genes examined; A-G = data from all populations pooled

Locus	Allele	Population							
		A	B	C	D	E	F	G	A-G
<i>Est-1</i>	82	—	—	—	—	—	6.3	—	1.0
	86	27.3	26.7	23.1	42.3	30.0	25.0	30.0	29.1
	100	18.2	13.3	23.1	—	16.7	37.5	26.7	19.9
	107	31.8	56.7	46.2	57.7	23.3	31.3	36.7	40.3
	120	22.7	3.3	7.7	—	30.0	—	6.7	9.7
	<i>n</i>	22	30	26	26	30	32	30	196
<i>Est-5</i>	90	45.8	56.7	26.9	29.2	60.7	85.3	40.0	51.0
	100	54.2	43.3	73.1	70.8	39.3	14.7	60.0	49.0
	<i>n</i>	24	30	26	24	28	34	30	196
<i>Got-2</i>	72	10.0	—	19.2	13.3	—	20.8	10.7	10.1
	100	90.0	100.0	80.8	86.7	100.0	79.2	89.3	89.9
	<i>n</i>	30	26	26	30	34	24	28	198
<i>Pgi-2</i>	61	7.1	9.4	3.6	2.6	21.9	13.3	3.6	8.8
	85	21.4	43.8	60.7	36.8	25.0	33.3	35.7	36.6
	100	71.4	31.3	32.1	60.5	53.1	53.3	60.7	51.8
	115	—	15.6	3.6	—	—	—	—	2.8
	<i>n</i>	28	32	28	38	32	30	28	216
6- <i>Pgdh-1</i>	88	—	56.3	10.0	39.5	18.8	21.9	—	22.3
	100	100.0	43.7	90.0	60.5	81.2	78.1	100.0	77.7
	<i>n</i>	28	32	30	38	32	32	28	220

two α -esterases *Est-1* (alleles 82, 86, 100, 107 and 120) and *Est-5* (alleles 90 and 100) were analysed in our study.

In each of the 6-*Pgdh*, *Pgi* and *Got* enzyme systems, two isozymes were obtained, one of them polymorphic (6-*Pgdh-1*, *Pgi-2*, *Got-2*), the other monomorphic (6-*Pgdh-2*, *Pgi-1*, *Got-1*). A zymogram for 6-*Pgdh* in *G. austriacum*-2x (fig. 2) shows the three genotypes in 6-*Pgdh-1* (alleles 88 and 100). Data for *Got-2* are closely comparable. In contrast, for the polymorphic *Pgi-2* (fig. 3, tables 1 and 5), six different alleles have been found (55, 61, 75, 85, 100 and 115). Consequently, genetic interpretation of *Pgi-2* zymograms is more complex in 2x and 4x, and quite difficult in 8x taxa. According to the banding pattern in the heterozygotes, *Est-1* and *Est-5* appear as monomeric enzymes. In 6-*Pgdh-1*, *Got-2* and *Pgi-2* the three bands of activity found in the heterozygotes suggest a dimeric structure of the enzymes.

Allele frequencies and ecogeographical differentiation of diploid populations

The allele frequencies of the five polymorphic genes in the seven *G. austriacum*-2x populations are shown in table 1. A χ^2 -test for heterogeneity (table 2) reveals ecogeographical variation

between populations. Significant differences are found for all five genes. This result indicates that these diploid populations do not belong to one large panmictic assembly but instead are partially isolated.

Observed and expected heterozygosity values are compared in table 3. At two loci (*Est-5* and 6-*Pgdh-1*) a significant deficiency of heterozygotes is observed. In *Got-2* and *Pgi-2* the deviation from the Hardy-Weinberg expectation points to the same direction, although here the differences are not significant. From the pooled data of the five loci the inbreeding coefficient was calculated as $F_{ST} = 0.171$. Because *G. austriacum* and its relatives are self-incompatible (Ehrendorfer, unpublished), the decrease in observed heterozygosity has to be ascribed to the fragmented population

Table 2 Test for heterogeneity of allele frequencies between the *Galium austriacum*-2x populations. df = degrees of freedom; *P* = probability

Locus	χ^2	df	<i>P</i>
<i>Est-1</i>	45.7	18	<0.001
<i>Est-5</i>	29.8	6	<0.001
<i>Got-2</i>	12.6	6	<0.05
<i>Pgi-2</i>	28.8	12	<0.01
6- <i>Pgdh-1</i>	46.7	6	<0.001

Table 3 Observed and expected heterozygosity per locus (H_L) and average heterozygosity ($\bar{H}$) for the five polymorphic genes in *Galium austriacum*-2x. N = number of genotypes tested; *, **, *** = significant at the 0.05, 0.01, 0.001 level; $df = 1$ in all tests.

Locus	H_L		N	χ^2	
	Obs.	Exp.			
<i>Est</i> -1	0.735	0.704	98	0.44	ns
<i>Est</i> -5	0.265	0.500	98	21.62	***
<i>Got</i> -2	0.141	0.182	99	1.09	ns
<i>Pgi</i> -2	0.537	0.589	108	1.20	ns
6- <i>Pgdh</i> -1	0.246	0.347	110	4.94	*
$\bar{H}$	0.385	0.464	513	13.23	***

structure and not to autogamy. Gene flow between the subpopulations appears to be inhibited by geographical barriers. But even between populations located in the same valley (e.g., E-F-G) and over a distance of only ca. 10 km, significant local differences in the allele frequencies can be observed at the five polymorphic loci (table 2).

Table 4 Genetic identity values (Nei's I) between the *Galium austriacum*-2x populations A-G, calculated from the allele frequencies at the five polymorphic loci

	B	C	D	E	F	G
A	0.8297	0.9236	0.9047	0.9671	0.9048	0.9879
B		0.8680	0.9385	0.8950	0.8739	0.8497
C			0.9258	0.8903	0.8435	0.9625
D				0.8912	0.8305	0.9222
E					0.9349	0.9488
F						0.9055

From the allele frequencies in *G. austriacum*-2x populations the coefficient of genetic identity (I) was calculated according to Nei (1972). An identity matrix is given in table 4, based on the data of the five polymorphic loci. From the identity matrix a dendrogram was constructed (fig. 4) by means of the UPGMA-method (Sneath and Sokal, 1973). The dendrogram clearly shows that the population samples fall into two distinct clusters. This clustering is consistent with the geographic distribution and ecological differences, and corresponds to a

Figure 4 UPGMA dendrogram of *Galium austriacum*-2x populations A-G calculated from the identity matrix (cf. table 4). The mountain series A-E-F-G stands apart from the foothill series B-C-D.

foothill series including the populations B-C-D and a mountain series with the populations A-E-F-G. At the level of allele frequencies the differences between the two clusters proved statistically significant at four of the five polymorphic loci (*Est-1*: $\chi^2 = 15.7$, $df = 3$, $P < 0.01$; *Est-3*: $\chi^2 = 8.14$, $df = 1$, $P < 0.01$; *Got-2*: ns; *Pgi-2*: $\chi^2 = 17.1$, $df = 3$, $P < 0.001$; *6Pgdh-1*: $\chi^2 = 21.2$, $df = 3$, $P < 0.001$).

Genic variation and relationships of diploid and polyploid populations

In order to assess the relationships of *G. austriacum-2x* within the *G. pusillum* aggregate, samples of a *G. austriacum-4x* (H) and *G. pumilum-8x* (I) were analysed in a comparable manner as for *Pgi* and *Est-1*.

An example of the observed electrophoretic phenotypes for *Pgi* is given in fig. 3. The *Pgi-1* isozyme is constantly monomorphic, and both diploids and polyploids, appear homozygous for the same electrophoretic allele. On the other hand, *Pgi-2* is polymorphic with multiple alleles in diploids and polyploids (tables 1 and 5). Some alleles, e.g., 85 and 100, are shared by all three taxa indicating an ancient polymorphism already present in the common ancestor. A few alleles may be more limited: 115 so far has been found only in *G. austriacum-2x* and 4x, 61 only in *G. austriacum-2x*, 75 only in *G. austriacum-4x*, and 55 only in *G. pumilum-8x*.

The interpretation of the electrophoretic phenotypes in *Pgi-2* (fig. 3) is readily achieved with the 2x and 4x *G. austriacum* populations. The 2x show the characteristic banding pattern of a dimeric enzyme in the heterozygotes. As expected with random association of the peptide subunits, the band representing the heterodimer is about twice as intense as each of the two homodimers. In the 4x, where each gene is present in four copies, three different heterozygotes are observed: the 2/2 heterozygotes with two copies of each allele and two different types of 3/1 heterozygotes. The electrophoretic phenotype of the 2/2 heterozygotes is the same as in heterozygous diploids. In the 3/1 heterozygotes, however, the ratio of the three bands is 9:6:1, and since the homodimer from the rare allele contributes only 6.25 per cent of the total enzyme activity, its staining is very faint. According to what can be estimated from the direct comparison in the gel, the amount of enzyme produced in 2x and 4x is about the same. That means, that all four copies of the gene in 4x contribute to the phenotype, but each with a reduced activity as compared to the 2x.

In *G. pumilum-8x* the *Pgi-2* banding patterns become even more complicated due to the fact that several different alleles can occur in a single genotype, and that heterodimers are formed between the different polypeptides. In fig. 3 two of the octoploids shown possess two alleles, yet the ratio of the two alleles could be either 4/4 or 5/3. The other two phenotypes apparently are produced by four different alleles, but it seems impossible to determine the copy numbers exactly. The expected staining intensity of a homodimer from a particular allele is 1.6 per cent of the total. This means that the band is too faint to be detected in the gel.

Table 5 Number of individuals observed for the different genotypes at *Est-1* and *Pgi-2* in *G. austriacum-2x* and -4x. G = number of different genotypes, N = number of individuals

<i>Galium austriacum</i>				
Locus	2x		4x	
	Genotype	N	Genotype	N
<i>Est-1</i>	86/86	6	—	—
	100/100	6	—	—
	107/107	14	—	—
	82/86	1	—	—
	82/100	1	—	—
	86/100	9	86/86/100/100	2
	86/107	27	—	—
	86/120	8	—	—
	100/107	15	—	—
	100/120	2	—	—
	107/120	9	—	—
	—	—	86/86/86/100	1
	—	—	86/100/100/100	1
	—	—	86/86/86/107	1
	—	—	80/80/100/107	1
	—	—	80/80/107/120	1
	—	—	80/107/107/120	1
	—	—	86/86/100/107	1
	—	—	86/100/107/107	1
	—	—	86/107/107/120	1
	—	—	100/107/120/120	1
<i>Pgi-2</i>	G = 11	98	G = 11	12
	61/61	2	—	—
	85/85	15	—	—
	100/100	33	100/100/100/100	1
	61/85	5	—	—
	61/100	9	—	—
	85/100	38	85/85/100/100	1
	85/115	6	85/85/115/115	1
	—	—	75/75/75/100	1
	—	—	75/100/100/100	1
	—	—	85/85/85/100	1
	—	—	85/100/100/100	4
	—	—	100/100/100/115	2
	G = 7	108	G = 8	12

Table 6 Allele frequencies (in per cent) for *Est-1* and *Pgi-2* in populations of *Galium austriacum*-2x (mountain series: A, E, F, G and foothill series: B, C, D) and -4x (H). *n* = number of genes examined

Locus	Allele	<i>Galium austriacum</i> -2x		<i>Galium austriacum</i> -4x (H)
		Mountains (A, E, F, G)	Foothills (B, C, D)	
<i>Est-1</i>	80	—	—	10.4
	82	1.8	—	—
	86	28.1	30.5	31.3
	100	25.4	12.2	25.0
	107	30.7	53.7	22.9
	120	14.0	3.7	10.4
	<i>n</i>	114	82	48
<i>Pgi-2</i>	61	11.9	5.1	—
	75	—	—	8.3
	85	28.8	45.9	22.9
	100	59.3	42.9	60.4
	115	—	6.1	8.3
	<i>n</i>	118	98	48

Because of these difficulties with reliable genotype determinations, we had to exclude the octoploids from our more detailed comparisons.

In table 5 the genotypes at *Est-1* and *Pgi-2* found in *G. austriacum*-2x and -4x are compared, and corresponding allele frequencies are given in table 6. Considering the amount of *genic variation*, no striking difference is observed between 2x and 4x. The number of different alleles is exactly the same at both genes. Two alleles (*Est-1*⁸² and *Pgi-2*⁶¹) were found only in the 2x, two others (*Est-1*⁸⁰ and *Pgi-2*⁷⁵) apparently are characteristic for 4x. Average heterozygosity calculated for the two loci is almost the same (0.647 vs. 0.667). In the 4x no fixed heterozygosity is apparent: inheritance follows the tetrasomic pattern of autopolyploids.

A clear difference, however, is found with respect to *genotypic variation*. In *G. austriacum*-2x 11 different genotypes among 98 individuals were found at the *Est-1* gene, 7 out of 108 at *Pgi-2*. The corresponding numbers for 4x are 11 out of 12 for *Est-1* and 8 out of 12 for *Pgi-2*. This means that almost every 4x individual carries its particular genotype. The main genetic difference between 2x and 4x is the much higher genotypic variation in the tetraploids due to their higher number of possible combinations, even if the genetic raw material in the form of different alleles is very similar in both.

In table 7 *genetic identity* I_N (Nei, 1972) and *genotypic identity* I_H (Hedrick, 1971) were calculated between the two series of *G. austriacum*-2x populations and the *G. austriacum*-4x population (H). Surprisingly, at both loci I_N was highest

Table 7 Genetic identity I_N , calculated from allele frequencies (Nei, 1972), and genotypic identity I_H , calculated from genotype frequencies (Hedrick, 1971), between populations of *G. austriacum*. The 2x-mountain series (2x-m), the 2x-foothill series (2x-f), and one 4x-population are compared

Locus		2x-m/2x-f	2x-m/4x	2x-f/4x
<i>Est-1</i>	I_N	0.8957	0.9613	0.8279
	I_H	0.6559	0.2340	0.0000
<i>Pgi-2</i>	I_N	0.9257	0.9643	0.8883
	I_H	0.8849	0.2575	0.2800

between 4x and the mountain series of 2x. This is remarkable because the 4x sample was taken from the same valley system as the 2x population C, a member of the foothill series.

At the genotypic level the highest I_H similarity is observed between the 2x foothill and the 2x mountain series. The 4x population is genotypically differentiated from both 2x clusters, but still closer to the mountain series. In one case (*Est-1* in the comparison with the 2x foothill series) no common genotype was found at all. Thus, *G. austriacum*-4x differs from *G. austriacum*-2x not only by its higher amount of genotypic variation within the population, but also by genotypic divergence.

DISCUSSION

Deviations of genotype frequencies for allozymes from Hardy-Weinberg expectations (F_{ST}) are clearly correlated to the reproductive system, i.e.,

they are lower in allogamous and higher in autogamous populations (Levin, 1978; Loveless and Hamrick, 1984; Hamrick, 1987). Our data on *Galium austriacum*-2x ($F = 0.171$) are in line with pollination experiments demonstrating self-incompatibility and obligatory allogamy (Ehrendorfer, unpublished), and comparable with F values for outbreeding (but not panmictic) species, e.g., of *Lupinus* (Babbal and Selander, 1974), *Stephanomeria* (Gottlieb, 1975), and *Phlox* (Levin, 1978). In contrast, for taxa of *Relbunium*, a genus related to *Galium*, allozyme data have given clear evidence for autogamy (Cavalli-Molina *et al.*, 1989).

The F_{ST} coefficient (being relatively high for an allogamous group) and the advanced genetic differentiation of the *G. austriacum*-2x populations clearly demonstrate strongly limited gene flow and lack of panmixis. As in *Coreopsis cyclocarpa* and *C. grandiflora* (Crawford and Bayer, 1981; Crawford and Smith, 1984), and in *Phlox drummondii* (Schwaegerle *et al.*, 1986), this can be linked to the relative inefficiency of long-distance pollen and seed transfer of *G. austriacum*. Furthermore, one has to consider the strongly isolated nature of its habitats: open and rocky *Pinus nigra* forests on steep limestone or dolomite slopes. Such habitats with their more ancient and relic vegetation appear as islands among the otherwise dominating postglacial climax formations of deciduous forests or secondary communities under human influence on deeper and better soils. This ecological isolation is reinforced by the strongly dissected orographic situation of the area. Thus, the populations of *G. austriacum*-2x studied, correspond to the "stepping-stone" pattern of gene flow as described by Hamrick (1987), comparable to species of *Quercus* subg. *Erythrobalanus* (Manos and Fairbrothers, 1987) or Hawaiian Islands *Tetramolopodium* (Lowrey and Crawford, 1985).

Many recent papers have shown the significance of iso- and allozyme data for elucidating the phylogenetic links between related diploid and polyploid taxa of vascular plants (Roose and Gottlieb, 1976; Werth *et al.*, 1985; Hurka *et al.*, 1989; Sanchez de Hoz and Fominaya, 1989).

The close relationship between the members of the polyploid complex *G. pusillum* agg. is documented by their similar iso- and allozymes (table 5, fig. 4), e.g., with the monomorphic *Pgi*-1 and the alleles for *Pgi*-2⁸⁵ and *Pgi*-2¹⁰⁰ occurring in *G. austriacum*-2x, -4x and *G. pumilum*-8x. Possible qualitative genetic differences among these taxa in respect to iso- and allozymes need confirmation.

But in respect to allele frequencies (table 7), *G. austriacum*-4x is closer to the mountain than to the foothill populations of *G. austriacum*-2x.

Genotype frequencies in *G. austriacum*-4x (table 5) prove an autopolyploid behaviour and tetrasomic inheritance. This is remarkable because meiotic pairing in PMC's is nearly exclusively in bivalents (Ehrendorfer 1949). Thus, with chromosome pairing evidently by chance among homologues of the four genomes, particular mechanisms strongly limiting multivalent formation have to be postulated. This situation corresponds well with observations on the auto-6x *Coreopsis grandiflora* var. *longipes* (Crawford and Smith, 1984) and on the auto-4x *Tolmiea menziesii* (Soltis and Riesenberger, 1986), both with freely segregating allozyme alleles in spite of "normal" meiotic behaviour. In auto-4x *Dactylis glomerata* Lumaret (1981, 1982) has shown the effect of genetic control of bivalent versus tetravalent formation on the tetrasomic inheritance of *Acph*-1, *Px*-1 and *Mdh*-1 alleles. In contrast, the recently established North American allo-4x hybrids *Tragopogon porrifolius* × *T. dubius* and *T. dubius* × *T. pratensis* (Roose and Gottlieb, 1976) or the world-wide 4x weed *Capsella bursa-pastoris* (Hurka *et al.*, 1989) are obvious allopolyploids with a high proportion of fixed heterozygosity and disomic inheritance.

Generally, the increase of genotypic variation and heterozygosity from 2x to 4x and 8x of *G. pusillum* agg. can be clearly documented from the allozyme data presented (fig. 3, table 5). There is evidence for somewhat reduced activities of multiple copies of enzyme loci in polyploids, but no suggestion of their complete "silencing". These data are in line with theoretical and factual evidence from several polyploid complexes (both auto- as well as allopolyploid) studied in a comparable way (Barber, 1970; Bingham, 1980; Lumaret, 1984).

As a consequence, polyploids are thought to exhibit a greater enzymatic diversity, more biochemical versatility, better buffering against stress factors, and broader metabolic amplitudes. This, in turn, often results in better adaptability, wider ecological niches, and successful colonization of a greater range of different habitats in polyploids. Populations of *Galium austriacum*-2x are rather uniform and stenoecious, those of *G. pumilum*-8x very variable, differentiated and euryoecious (Ehrendorfer, 1953). Similarly *Festuca microstachys*-6x has highly polymorphic enzyme genes and occupies a wide range of habitats (Adams and Allard, 1977).

Galium austriacum-4x has differentiated from *G. austriacum*-2x and successfully expanded into the more strongly glaciation effected Lower Austrian Alps. A comparable situation has been very well documented by enzyme analyses for *Dactylis glomerata*-2x and -4x populations in the north-western Iberian Peninsula (Lumaret *et al.*, 1987; Lumaret and Barrientos, 1990) or for *Tolmiea menziesii*-2x and -4x in western North America (Soltis and Riesenberger, 1986). The role of polyploidy thus is to contribute to both stability and change in evolution (Ehrendorfer, 1980, 1988).

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