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Enzyme analysis of genetic variation and relationships in diploid and polyploid taxa of *Galium* (*Rubiaceae*)

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Abstract: Allozyme variation at 11 loci (with 37 alleles) was studied electrophoretically in seven outbreeding, closely related diploid and tetraploid taxa, seven from sect. *Leptogalium* and two from sect. *Leiogalium.* Whereas the sections are clearly distinct by several different alleles, aggregates, species and subspecies differ only in the frequency or presence/absence of common alleles. The resulting dendrogram suggests phylogenetic relationships and is supported by other multidisciplinary evidence. Tetraploids have originated independently in several groups, and there is evidence for tetrasomic inheritance and thus for autopolyploidy in spite of normal meiotic bivalent pairing and partly suspected hybrid origin. Tetraploids differ from related diploids only little in number of alleles and expected heterozygosity within populations, but clearly exhibit higher numbers of genotypes. This often corresponds to their greater morphological variability, increased adaptive flexibility, and better colonizing capacity compared to related diploids.

Gel electrophoresis of proteins has become a standard research tool in systematic biology because the distribution of allozyme variation within and among species is of key interest to both systematists and evolutionary geneticists (SOLTIS & SOLTIS 1990). Allozyme studies provide information on the amount of variation, population structure, breeding system, effective gene flow (LOVELESS & HAMRICK 1984, HAMRICK 1990, HAMRICK & GODT 1989), and phylogenetic divergence (CRAWFORD 1983, 1985, 1990). The genus *Galium* has been extensively used for biosystematic studies and considerable information has been accumulated on morphological, karyological and eco-geographical differentiation over the last decades (EHRENDORFER 1949, 1962, 1976; KRENDL 1967, 1993); recently, also molecular (cpDNA) data have become available (MANEN & al. 1994, NATALI & al. 1995). Therefore, taxa of *Galium* offer an excellent test system for hypotheses concerning population genetics, speciation processes and mechanisms underlying evolutionary differentiation.

The present study deals with several perennial, herbaceous, insect-pollinated and outbreeding taxa of the large and world-wide genus *Galium* from tribe

Table 1. *Galium* taxa and samples studied. Bold numbers characterize the sections (1, 2), letters the species groups (in the ranks of aggregates or series): *A G. pumilum* group, *B G. baldense* group, *C G. saxatile* group. *Abbr.* abbreviations, *Pop.* number of populations, *L. Austria* Lower Austria. Numbers in brackets mark individual populations (see Table 6)

Taxon	Abbr.	Ploidy level	Pop.	Provenance
Sect. *Leptogalium* **(1)**				
G. valdepilosum H. Braun in Form. (A)	val	2x	2	L. Austria: Senftenberg (1); Weißenkirchen (2)
G. austriacum Jacq. subsp. *austriacum* (A)	aus	2x	7	L. Austria: see Samuel & al. (1990) for 5 pop.: Sierning Valley (1), Schwarza Valley (2)
– subsp. *vindobonense* Ehrend. (A)	aus	4x	2	L. Austria: Losenheim near Puchberg (1); Perchtoldsdorf (2)
G. anisophyllon Vill. (A)	ani	4x	2	L. Austria: Schneeberg (1); Rax (2)
"*G. bellatulum* Klokov" (A)	bel	4x	1	Slovakia: Velka Fatra
G. noricum Ehrend. (B)	nor	4x	2	L. Austria: Rax
G. saxatile L. (C) (= *G. harcynicum* Weigel)	sax	4x	1	L. Austria: Waldviertel, Karlsstift
Sect. *Leiogalium* **(2)**				
G. mollugo L.	mol	2x	1	L. Austria: Stopfenreuth
G. album Miller	alb	4x	1	L. Austria: Stopfenreuth

Rubieae (*Rubiaceae*). Seven taxa studied are members of sect. *Leptogalium,* two of sect. *Leiogalium.* Both sections have a European-Mediterranean distribution, their representatives mostly occur in various grassland habitats and open forests, from the lowland to arctic-alpine areas (EHREDORFER 1976, MEUSEL & JÄGER 1992). The samples (Table 1) cover a wide taxonomic range from populations, subspecies and species, to groups of closely related species (aggregates), series and sections. Our intention is to analyse the genetic variation and relationships of these diploid (2x) and tetraploid (4x) *Galium* taxa, to quantify their genetic differentiation, and to obtain genetic markers for their identification. Previously, infraspecific differentiation and correlation between ploidy levels and variation has been investigated in only one of the species (SAMUEL & al. 1990): *G. austriacum* exhibits a group of foothill and another of mountain populations; in *G. austriacum*-4x allele frequencies suggest tetrasomic inheritance, and the genotypic diversity is higher in the 4x- compared to the 2x-populations. Using more enzyme systems, comparable problems are now investigated in other closely related diploid and tetraploid taxa. A general question addressed in the discussion concerns the various factors influencing genetic variation at the population level.

Material and methods

Plant material. Populations were represented by 10–26 individuals each. The plants were collected from their natural habitats, potted and established in the garden of the Institute of Botany, University of Vienna (HBV). Taxa and their taxonomic grouping, ploidy levels, number of populations studied and geographic provenances are given in Table 1. Vouchers are preserved in the herbarium WU of the Institute.

Electrophoresis. The following enzymes were studied: esterase (EST), glutamate oxalacetate transaminase (GOT), isocitrate dehydrogenase (IDH), leucine amino peptidase (LAP), 6-phosphogluconate dehydrogenase (6-PGDH), phosphoglucose isomerase (PGI), and phosphoglucomutase (PGM). Enzyme staining followed standard procedures given by HARRIS & HOPKINSON (1976). The most common allele at each locus was designated as 100, the others were named according to the relative electrophoretic mobility (in %) of the enzymes. In systems with different, genetically independent isozymes, the loci were labelled sequentially, the fastest isozyme was assigned the number 1. Horizontal starch gel electrophoresis was carried out according to AYALA & al. (1972). Extracts were prepared from leaf material stored at –80 °C. For the details of homogenization and buffer systems used for electrophoresis of 6-PGDH, PGI and GOT see SAMUEL & al. (1990). For the enzymes LAP, IDH and PGM the following buffer systems were applied:

A: bridge: 0.2 M Trisma base/0.002 M EDTA/0.15 M boric acid pH 8.5, gel: 0.015 M Tris/0.002 M EDTA/0.037 M boric acid pH 8.0, run: 7 h at 300 V/50 mA, enzyme: LAP.

B: bridge: 0.135 M Tris/citric acid pH 7.1, gel: 1 : 10 dilution of bridge buffer, run; 6 h at 120 V/35 mA, enzymes: IDH and PGM. For the investigation of EST vertical polyacrylamide gel electrophoresis (PAGE) was carried out as described previously by SAMUEL & al. (1990).

Results

Enzyme analysis. Seven enzyme systems were studied in the seven taxa of sect. *Leptogalium* (1) and two of sect. *Leiogalium* (2). The enzymes EST, GOT, 6-PGDH and PGI are each represented by two genetically independent isozymes, whereas only one activity zone was detected in the zymograms of IDH, LAP, and

Table 2. Allele frequencies at 11 loci in 9 taxa of *Galium*. Abbreviations as in Table 1. *n* number of genes examined

Locus	Allele	Section *Leptogalium*							Section *Leiogalium*	
		val 2x	aus 2x	aus 4x	ani 4x	bel 4x	nor 4x	sax 4x	mol 2x	alb 4x
Est-1	82	–	0.010	–	–	–	–	–	–	–
	86	0.263	0.286	0.221	0.044	0.300	0.454	0.375	–	–
	100	0.053	0.199	0.294	0.478	0.400	0.409	0.625	–	–
	107	0.368	0.408	0.235	0.369	0.300	0.091	–	–	–
	114	–	–	–	–	–	–	–	0.500	0.182
	120	0.316	0.097	0.250	0.109	–	0.046	–	–	–
	121	–	–	–	–	–	–	–	0.150	0.182
	132	–	–	–	–	–	–	–	0.350	0.500
	136	–	–	–	–	–	–	–	–	0.136
	n	38	196	68	92	40	44	48	20	44
Est-5	86	–	–	–	–	0.050	–	–	–	–
	90	0.658	0.516	0.309	0.413	0.350	0.273	–	–	0.227
	100	0.342	0.484	0.426	0.424	0.550	0.386	1.000	1.000	0.773
	105	–	–	0.265	0.163	0.050	0.341	–	–	–
	n	38	194	68	92	40	44	48	20	44
Got-1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	n	34	198	92	108	44	44	48	24	40
Got-2	72	0.206	0.101	0.130	0.435	0.341	0.364	0.542	0.417	–
	100	0.706	0.899	0.794	0.565	0.659	0.636	0.458	0.583	1.000
	128	0.088	–	0.076	–	–	–	–	–	–
	n	34	198	92	108	44	44	48	24	40
Idh	83	–	0.109	0.019	0.017	0.050	–	–	–	–
	88	–	–	–	–	–	–	–	1.000	1.000
	100	1.000	0.891	0.962	0.931	0.950	1.000	1.000	–	–
	120	–	–	0.019	0.052	–	–	–	–	–
	n	34	46	104	116	40	40	52	26	44
Lap	94	–	–	–	–	–	–	–	0.038	0.071
	100	0.794	0.694	0.727	0.702	0.589	0.133	0.133	0.577	0.393
	115	0.206	0.306	0.273	0.298	0.411	0.867	0.867	0.385	0.536
	n	34	36	88	104	56	60	60	26	56
6-Pgdh-1	88	0.318	0.223	0.337	0.440	0.350	–	–	–	–
	100	0.682	0.777	0.663	0.560	0.650	1.000	1.000	1.000	1.000
	n	44	220	80	116	40	64	60	28	60
6-Pgdh-2	88	–	–	–	–	–	–	0.217	–	–
	100	1.000	1.000	1.000	1.000	1.000	1.000	0.783	1.000	1.000
	n	44	220	80	116	40	64	60	28	60
Pgi-1	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	n	46	216	104	116	44	64	60	28	60
Pgi-2	25	–	–	–	–	–	–	–	–	0.033
	55	–	–	–	–	–	–	–	0.786	0.483
	61	–	0.083	0.058	0.009	0.341	–	–	0.214	0.450
	85	0.239	0.366	0.336	0.431	0.068	1.000	0.383	–	–
	100	0.696	0.523	0.606	0.560	0.591	–	0.617	–	0.033
	119	0.065	0.028	–	–	–	–	–	–	–
	n	46	216	104	116	44	64	60	28	60
Pgm	90	–	–	–	–	–	–	–	1.000	1.000
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	–	–
	n	38	84	38	32	40	32	52	26	44

PGM, respectively. At these 11 loci a total of 37 alleles can be electrophoretically distinguished; their frequencies are shown in Table 2. According to our present knowledge, only 13 of these alleles have been found in members of both sections. Of these, two correspond to completely uniform loci: *Got-1*, *Pgi-1*; nearly uniform is *6-Pgdh-2*^{100}; the others are polymorphic: *Est-5*^{90,100}, *Got-2*^{72,100}, *Lap*^{100,115}, *6-Pgdh-1*^{100} (uniform in sect. 2), *Pgi-2*^{55} (common in sect. 2, found only once in *G. pumilum* Murr.-8x of sect. 1; cf. Samuel & al. 1990), and *Pgi-2*^{61,100}. As no electrophoretic differences are detectable between these shared polymorphic alleles, they also indicate relationships between the two sections. Differences between taxa of sect. *Leptogalium* (1) and sect. *Leiogalium* (2) concern *Pgm*, which has different but uniform alleles in sect. 1 and 2. Of the more common alleles *Est-1*^{86,100,107,120}, *Est-5*^{105}, *Idh*^{83,100}, *6-Pgdh-1*^{88} and *Pgi-2*^{85} are found only in sect. 1, in contrast to *Est-1*^{114,121,132}, *Idh*^{88} and *Lap*^{94} reported so far only for sect. 2. Other alleles are too rare to suggest a group specific distribution.

Taxa within sect. *Leptogalium* (1) are held together by several common alleles which differ mainly in respect to their frequencies: *Est-1*^{86,100,107,120}, *Est-5*^{90,100,105}, *Got-2*^{72,100}, *Idh*^{83,100}, *Lap*^{100,115}, *6-Pgdh-1*^{88,100}, and *Pgi-2*^{61,85,100}. The presence or absence of rare alleles, within sect. 1, apparently is less significant. Nevertheless, that *6-Pgdh-2*^{88}, occurs rather frequently in the sample of *G. saxatile* but has not yet been found in any other taxon, is remarkable. Furthermore, the lack of *6-Pgdh-1*^{88} in *G. noricum* (1B) and *G. saxatile* (1C), and of *Est-5*^{90} in 1C is noteworthy. In the two species of sect. *Leiogalium* (2, each represented by only one population) one can point to the relatively common occurrence of *Got-2*^{72} in *G. mollugo*-2x and of *Est-5*^{90} in *G. album*-4x, alleles which were not observed in the respective sister taxon.

Phylogenetic analysis. Genetic identity and genetic distance (Table 3) were calculated from the allelic frequencies (Nei 1972) and the UPGMA dendrogram (Sneath & Sokal 1973) was constructed (Fig. 1). According to the dendrogram,

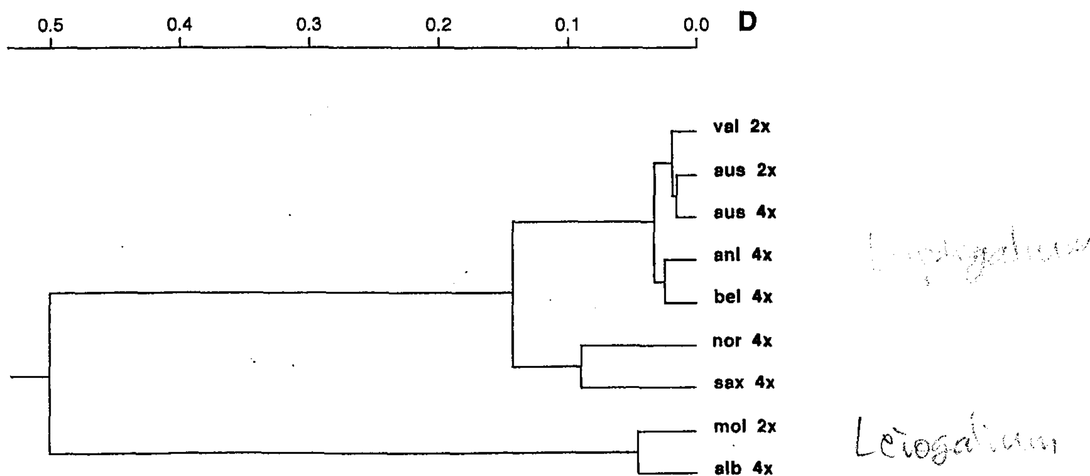


Fig. 1. UPGMA-dendrogram showing the genetic relationships between the nine taxa of *Galium* studied, based on the genetic distance values (D) given in Table 3. Abbreviations as in Table 1. Further explanations in the text

the two sections *Leptogalium* and *Leiogalium* are clearly separated ($\bar{D}$ = 0.48). Sect. *Leptogalium* (1) comprises a cluster of close relatives with $\bar{D}$-values between 0.02 and 0.04: *G. valdepilosum*-2x, *G. austriacum* subsp. *austriacum*-2x and subsp. *vindobonense*-4x, *G. anisophyllon*-4x, and "*G. bellatulum*"-4x which are combined as group 1A. The two species *G. noricum* and *G. saxatile* share less similarities with the rest ($\bar{D}$ = 0.14), are only distantly related ($\bar{D}$ = 0.09), and designated as groups 1B and 1C, respectively. In sect. *Leiogalium* (2) the two species *G. mollugo*-2x and *G. album*-4x appear as close relatives with a $\bar{D}$-value of 0.04.

Allozyme variation, genotypes, heterozygosity and polyploidy. The genetic interpretation of the electrophoretic phenotypes is readily achieved with the 2x- and 4x-*Galium* taxa studied, as is shown for *G. mollugo*-2x and *G. album*-4x, and the polymorphic loci *Est-1* and *Pgi-2* in Table 4; comparable results have been published already for *G. austriacum*-2x/4x and *Pgi-2* by SAMUEL & al. (1990). The 2x-taxa exhibit the characteristic banding pattern of heterozygotes (2 bands of equal intensity in monomeric enzymes, 3 bands with intensities 1 : 2 : 1 in dimeric enzymes). In the 4x-taxa, where each gene is present in four copies, three different heterozygotes (with two alleles) can be observed. In highly variable genes like *Est-1*, up to four different alleles can be expected in a single genotype. In the *Galium* populations studied, genotypes with 3 different alleles were found at this locus (Table 4). The presence of more than two alleles in one individual as well as the lack of fixed heterozygosity suggest tetrasomic inheritance.

The numbers of alleles and genotypes detected in the diploid and tetraploid taxa of both sections, *Leptogalium* (groups 1A, 1B, and 1C) and *Leiogalium* (group 2), are shown in Tables 2 and 5. In groups 1A and 2 which contain closely related 2x- and 4x-taxa, there are very few alleles from polymorphic loci in the 4x which are not also found in the 2x: only 1 of 23 in group 1A, and 4 of 15 in group 2. Within the closely related taxa of group 1A, in the diploids (*val* 2x and *aus* 2x) the mean number of different genotypes found was 4.1, in the tetraploids (*aus* 4x, *bel* 4x and *ani* 4x) 5.4, within the close *Leiogalium* taxa 2.3 (*mol* 2x) and 3.9 (*alb* 4x). Due to the tetrasomic pattern of inheritance, the number of different genotypes is significantly increased in the tetraploids as compared to the closely related diploids. An example for this phenomenon is shown in Table 4. Although the number of individuals is about the same, the number of observed genotypes at the loci *Est-1* and *Pgi-2* is much higher in *G. album*-4x than in *G. mollugo*-2x. In contrast to the genotypes, allele numbers exhibit no significant increase when the corresponding 2x/4x-pairs are compared.

A standardized measure of genetic variation is $\bar{H}_e$, the expected heterozygosity, calculated from allelic frequencies according to the Hardy-Weinberg law. This measure can be applied to compare the amount of genetic variation between diploids and tetraploids (Table 6). In closely related pairs or groups of diploid and tetraploid populations, average heterozygosity apparently is slightly higher in the polyploids of sect. *Leptogalium*, group 1A (*aus* 2x and *val* 2x: $\bar{H}_e$ = 24.3 versus *aus* 4x, *ani* 4x, *bel* 4x: $\bar{H}_e$ = 29.0), and sect. *Leiogalium*, group 2 (*mol* 2x: $\bar{H}_e$ = 17.7 versus *alb* 4x: $\bar{H}_e$ = 19.4). Nevertheless, it is obvious that the phylogenetically more remote tetraploid *Leptogalia G. noricum* (group 1B): $\bar{H}_e$ = 17.9 and *G. saxatile* (group 1C): $\bar{H}_e$ = 18.3 (for which data from closely allied 2x are still lacking) exhibit lower values than the diploids in group 1A from the same section. Thus, in

Table 3. Genetic identities ($\bar{I}$, above diagonal) and genetic distances ($\bar{D}$, below diagonal) between 9 taxa of *Galium*. Abbreviations as in Table 1

	val 2x	aus 2x	aus 4x	ani 4x	bel 4x	nor 4x	sax 4x	mol 2x	alb 4x
val 2x	–	0.983	0.980	0.965	0.960	0.841	0.837	0.587	0.616
aus 2x	0.018	–	0.984	0.967	0.973	0.888	0.875	0.628	0.669
aus 4x	0.021	0.016	–	0.980	0.974	0.885	0.879	0.611	0.641
ani 4x	0.035	0.033	0.020	–	0.972	0.884	0.885	0.600	0.605
bel 4x	0.041	0.028	0.027	0.029	–	0.872	0.911	0.632	0.656
nor 4x	0.174	0.119	0.122	0.124	0.137	–	0.914	0.588	0.616
sax 4x	0.179	0.133	0.129	0.123	0.093	0.090	–	0.630	0.622
mol 2x	0.532	0.466	0.492	0.511	0.459	0.531	0.462	–	0.955
alb 4x	0.485	0.402	0.445	0.503	0.422	0.484	0.475	0.046	–

Table 4. Number of individuals observed for the different genotypes at *Est-1* and *Pgi-2* in *G. mollugo*-2x and *G. album*-4x of sect. *Leiogalium*. *N* number of individuals, *G* number of genotypes

Locus	*G. mollugo*-2x		*G. album*-4x	
	genotype	N	genotype	N
Est-1				
homozygotes	114/114	3	–	–
	121/121	1	121/121/121/121	1
	132/132	1	132/132/132/132	2
1 : 1 heterozygotes	114/132	4	114/114/132/132	1
	121/132	1	121/121/132/132	2
1 : 3 heterozygotes	–	–	114/132/132/132	1
	–	–	114/114/114/132	1
	–	–	114/114/136/136	1
			132/136/136/136	1
	–	–	132/132/132/136	1
	G = 5	10	G = 9	11
Pgi-2				
homozygotes	55/55	8	55/55/55/55	2
	–	–	61/61/61/61	1
1 : 1 heterozygotes	–	–	25/25/55/55	1
	55/61	6	55/55/61/61	1
	–	–	55/55/100/100	1
1 : 3 heterozygotes	–	–	55/61/61/61	6
	–	–	55/55/55/61	3
	G = 2	14	G = 7	15

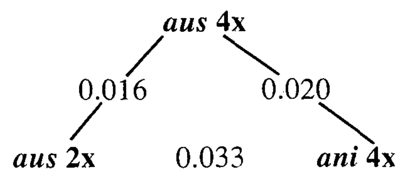
Galium there is no general correlation between average heterozygosity and ploidy level; but such a correlation may exist in closely related 2x and 4x.

Discussion

Phylogeny and genetic distances. The wide range of phylogenetic divergence represented by the *Galium* samples studied allows to test enzymes against morphological, karyological (Ehrendorfer 1949, 1962, 1976; Krendl 1967, 1993), and cpDNA (Manen & al. 1994, Natali & al. 1995) markers of evolutionary divergence. The UPGMA dendrogram (Fig. 1) clearly shows the distinctness of the sections *Leptogalium* and *Leiogalium* ($\bar{D}$ = 0.48). This is supported by the well established separation of the two sections by morphological features, and is paralleled by the complete lack of hybrids between them. Also, according to the cladistic analysis of a long noncoding cpDNA intergenic spacer, sect. *Leptogalium* (represented by *G. corsicum* Sprengel) and sect. *Leiogalium* (represented by *G. mollugo, G. album* and others) appear as two independent branches within the more comprehensive *Galium* sect. *Galium* clade (which corresponds to subgeneric rank: Natali & al. 1995).

Within the sections $\bar{D}$-values drop below 0.13 (Table 3) which conforms well with a decrease in morphological distinctness. In sect. *Leptogalium* morphological similarities and considerable hybrid links have been decisive to place *G. valdepilosum, G. austriacum, G. anisophyllon* and others into a *G. pusillum* L. group (1A), whereas reliable differential characters and genetic isolation justify the classification of *G. noricum* in the *G. baldense* Sprengel group (1B), and of *G. saxatile* in a group (1C) by itself. This is well substantiated by our enzyme data. The two sibling species of *G. mollugo* and *G. album* within sect. *Leiogalium* are even closer ($\bar{D}$ = 0.04), and have been separated taxonomically only recently, with the diploid *G. mollugo* obviously involved in the origin of the tetraploid *G. album* (Krendl 1967).

The taxonomic problems concerning the intricate and polymorphic *Galium pusillum* polyploid complex can be resolved only in part with the available enzyme data. That *G. valdepilosum*-2x and *G. austriacum* subsp. *austriacum*-2x are morphologically very close allopatric taxa which can be crossed to produce fully fertile F_1 (Ehrendorfer 1962, and unpubl.) is well in line with their very low genetic distance ($\bar{D}$ = 0.02). That *G. austriacum* subsp. *vindobonense*-4x has originated from *G. austriacum* subsp. *austriacum*-2x and *G. anisophyllon*-4x, as suggested by its intermediate morphology, ecology and distribution, is supported by their genetic distances:



Furthermore, enzyme data are not in conflict with our suggestion that "*G. bellatulum*", described as a local taxon from the Ukrainian Carpathians and believed

Table 5. Number of alleles (A) and genotypes (G) in different taxa of *Galium*. Abbreviations as in Table 1

	Est-1		*Est-5*		*Got-2*		*Idh-1*		*Lap-1*		*Pgi-2*		*6-Pgdh-1*		mean (7 loci)	
	A	G	A	G	A	G	A	G	A	G	A	G	A	G	A	G
Group 1A																
val 2x	4	8	2	5	3	4	1	1	2	3	3	4	2	3	2.4	3.7
aus 2x	5	11	2	3	2	3	2	2	2	2	4	7	2	3	2.7	4.4
aus 4x	4	13	3	9	3	6	3	3	2	4	3	6	2	4	2.9	6.1
bel 4x	3	6	4	6	2	4	2	2	2	4	3	6	2	3	2.6	4.4
ani 4x	4	10	3	7	2	5	3	3	2	4	3	6	2	5	2.7	5.7
Group 1B																
sax 4x	2	4	1	1	2	4	1	1	2	3	2	4	1	1	1.6	2.6
Group 1C																
nor 4x	4	7	3	5	2	4	1	1	2	2	1	1	1	1	2.0	3.0
Group 2																
mol 2x	3	4	1	1	2	3	1	1	3	4	2	2	1	1	1.9	2.3
alb 4x	4	9	2	3	1	1	1	1	3	5	4	7	1	1	2.3	3.9

Table 6. Expected heterozygosity (in %) at 11 loci in 13 related diploid and tetraploid *Galium* populations. Abbreviations and numbers in brackets as in Table 1. $\bar{H}_e$ average expected heterozygosity

Locus	Section *Leptogalium*											Section *Leiogalium*	
	val (1) 2x	val (2) 2x	aus (1) 2x	aus (2) 2x	aus (1) 4x	aus (2) 4x	ani (1) 4x	ani (2) 4x	bel 4x	nor 4x	sax 4x	mol 2x	alb 4x
Est-1	65.6	62.5	67.8	70.1	73.8	74.7	50.0	68.3	66.0	61.6	46.9	60.5	66.5
Est-5	49.6	34.6	38.4	27.3	64.7	62.4	57.0	65.5	57.0	66.0	0.0	0.0	35.1
Got-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Got-2	44.6	49.1	34.0	24.3	37.4	31.7	49.4	45.5	44.9	46.3	49.7	48.6	0.0
Idh	0.0	0.0	26.3	9.6	8.0	6.0	0.0	25.4	9.5	0.0	0.0	0.0	0.0
Lap	34.7	30.9	37.6	48.0	32.6	50.0	48.8	28.6	48.4	23.1	23.1	51.8	55.4
6-Pgdh-1	23.0	50.0	18.0	39.4	32.8	0.0	49.5	49.0	45.5	0.0	0.0	0.0	0.0
6-Pgdh-2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.9	0.0	0.0
Pgi-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pgi-2	37.0	52.0	52.7	58.9	66.0	47.0	50.0	48.4	53.0	0.0	47.3	33.7	56.2
Pgm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$\bar{H}_e$	25.6	22.5	24.9	24.4	27.8	28.9	27.7	30.0	29.5	17.9	18.3	17.7	19.4

to be widespread in the SE European mountains (ŠIPOŠOVÁ 1987), is in reality conspecific with *G. anisophyllon*. In particular, it appears inseparable from the polymorphic 4x-cytotype which occupies an extensive area from the E Alps through the Carpathian and Dinaric Mts. Considering only the polymorphic loci *Est-1*, *Est-5*, *Got-2*, *Pgi-2*, and *6-Pgdh-1* (used by SAMUEL & al. 1990), the three populations of *ani*-4x and *bel*-4x exhibit a high identity value ($\bar{I}$ = 0.92), and thus are less different than the two local races of *G. austriacum*-2x (SAMUEL & al. 1990). In view of strong morphological affinities and transitions, hybrid links, and the low $\bar{D}$-values existing between many of the (micro)species of the *G. pusillum* group, one could regard it as a single biological species. This situation is comparable, e.g., to the relatively young species divergence within the *Compositae* genus *Tetramolopium* on the Hawaiian Islands (LOWREY & CRAWFORD 1985).

A comparison with the extensive literature shows that the range of genetic distances demonstrated for different sections, species, infraspecific taxa and populations in *Galium* is well in line with enzyme data from other angiosperm groups, e.g., *Capsicum* (MCLEOD & al. 1983), *Quercus* (SAMUEL & al. 1995, and literature cited therein) and the numerous examples listed by HAMRICK & al. (1979), HAMRICK (1990), HAMRICK & GODT (1989), and CRAWFORD (1983, 1985, 1990). Just as in all these parallel studies, also in our case of *Galium,* the hierarchical relationships between the taxa, as documented by thorough morphological and karyological studies, are well supported by the available enzyme (and also by some DNA) data.

Origin of polyploids. Multidisciplinary evidence (EHRENDORFER 1976; MEUSEL & JÄGER 1992; KRENDL 1967, 1993) and the enzyme data presented here demonstrate the multiple origin of the polyploid *Galium* taxa studied. Within sect. *Leptogalium*, the isolated *G. saxatile* (1C) has a regional 2x-cytotype in the NW Iberian Peninsula, whereas the very similar 4x-cytotype is widespread from W to C Europe. Among the vicarious and disjunct alpine species of the *G. baldense* group (1B), *G. noricum*-4x occurs in the eastern limestone Alps. It is the only 4x-member of this otherwise diploid series, and particularly close to *G. baldense*-2x, an endemic of the central southern Alps. As a contrast *G. anisophyllon* from the *G. pusillum* group (1A) has a wide and continuous area throughout the mountain systems of C and S Europe. It is extremely polymorphic and made up of numerous, very similar 2x-, 4x-, 6x-, 8x-, and 10x-cytotypes. The 4x-populations of *G. anisophyllon* in the E Alps are linked at lower montane elevations by *G. austriacum*-4x to *G. austriacum*-2x which occurs in the warmer limestone foothills S of Vienna (SAMUEL & al. 1990). Within sect. *Leiogalium* (2), not only *G. mollugo*-2x, centred in warmer W and C Europe, but also other 2x-members of this series from SE Europe evidently have participated in the origin of the very much more widespread and polymorphic *G. album*-4x.

This multiple origin of polyploids and their relationships to diploids in *Galium* corresponds well with reports on other angiosperm groups (e.g., *Dactylis glomerata* L. agg., *Turnera ulmifolia* L. agg., *Lasthenia californica* DC.: see below), and with the dynamic nature of polyploidy outlined recently by SOLTIS & SOLTIS (1993). In all these groups (including *Galium*) polyploids have very few new alleles, exhibit tetrasomic inheritance and lack fixed heterozygosity, decisive criteria for autopolyploidy and the homology of polyploid genomes. In spite of this, such autopolyploids usually have ± normal meiotic bivalent pairing (because of genet-

ically controlled multivalent reduction), and are often of hybrid origin. This can be demonstrated by the following examples, ranging from auto- to allopolyploidy: In the 2x-, 4x-, (6x-)species *Coreopsis grandiflora* NUTT. (CRAWFORD & SMITH 1984), *Tolmiea menziesii* TORR. & GRAY (SOLTIS & SOLTIS 1988) and *Lasthenia californica* (DESROCHERS & BOHM 1995) we find straightforward allogamous, non-hybrid autopolyploids with tetrasomic inheritance and bivalent formation, similar to *G. saxatile, G. baldense-noricum*, and *G. anisophyllon. Turnera ulmifolia* (SHORE 1991), *Haplopappus spinulosus* (PURSH) DC. (HAUBER 1986), *Dactylis glomerata* agg. (LUMARET 1985, LUMARET & HANOTTE 1987, LUMARET & BARRIENTOS 1990), the *Stellaria longifolia* MUHL. ex WILLD.-*S. porsildii* CHINAPPA-*S. longipes* GOLDIE group (MACDONALD & CHINAPPA 1988, CAI & al. 1990), and the *Lotus alpinus* SCHLEICH.-*L. japonicus* REGEL-*L. corniculatus* L. group (RAELSON & GRANT 1988) are comparable to *G. austriacum, G. mollugo*, and *G. album,* and include intra- and interspecific hybrid autopolyploids, partly with ± autogamy and some multivalent formation. Finally, tendencies towards allopolyploidy, disomic inheritance, and fixed heterozygosity become predominant in autogamous and perennial arctic *Draba* species (BROCHMANN & al. 1992), and the annual and predominantly selfing *Hordeum marinum* HUDS. agg. (JAASKA 1994) and *Capsella bursa-pastoris* L. (HURKA & al. 1989, HURKA 1993).

Genetic variation. The broad range of genetic variation as revealed by our enzyme analysis within taxa and populations of *Galium* is shown in Table 5 for number of alleles and genotypes, and in Table 6 for heterozygosity percentages. There is no clear evidence for the silencing of duplicate alleles in the polyploids, as reported, e.g., for *Chenopodium* (WILSON & al. 1983). In the following paragraphs we will discuss briefly some of the factors which apparently influence this variation.

It is well known that the breeding system, in particular out- versus inbreeding, has a decisive influence on the variation patterns in angiosperms (see, e.g., LOVELESS & HAMRICK 1984, HAMRICK 1990). The allogamous mating system demonstrated experimentally for members of sections *Leptogalium* and *Leiogalium* is very well paralleled by the relatively high heterozygosity values found in this study and already published for *G. austriacum* (SAMUEL & al. 1990). In contrast, for a neotropical close ally of *Galium*, i.e., *Relbunium*, autogamy has been proven by enzyme analysis for the perennial *R. hypocarpium* (L.) HEMSL.-2x (CAVALLI-MOLINA & al. 1989). Otherwise, selfing and inbreeding is particularly common and predominant in annual members of *Galium* and related genera in the Mediterranean and Near East.

Auto- and allopolyploidy are known generally to increase genetic variation (see, e.g., CRAWFORD 1990, SOLTIS & SOLTIS 1993). From Tables 5 and 6 it is obvious that this cannot be maintained as a general statement in *Galium*, because the 4x-taxa of groups 1B, 1C and 2 are less variable than the 2x-taxa of group 1A. A correlation between polyploidy and higher variation becomes apparent only, when the closely related di- and tetraploids within groups 1A and 2 are compared. There, the number of alleles in the 4x-taxa is slightly (but not significantly) higher than in the 2x. But there is a considerable increase in the number of genotypes observed, with means of 4.1 (2x) versus 5.4 (4x) for group 1A, and 2.3 (2x) versus 3.9 (4x) for group 2. Furthermore, the corresponding heterozygosity % values

for the populations apparently are also higher in the tetraploids, with means of $\bar{H}_e$ = 24.3 (2x) versus 29.0 (4x) for group 1A, and 17.7 (2x) versus 19.4 (4x) for group 2. This increase in number of genotypes and in heterozygosity has to be ascribed to the suspected tetrasomic inheritance in the tetraploids. It appears reasonable to regard this relative increase of genetic variation in the polyploids as one of the reasons for their often greater morphological variability, increased adaptive flexibility, and better colonizing capacity as compared to their diploid ancestors.

Nevertheless, the *Galium* tetraploids studied also include populations with obviously reduced genetic variation, as *G. noricum*-4x (1B) and *G. saxatile*-4x (1C) in sect. *Leptogalium*. Both have much fewer alleles (2.0 and 1.6), fewer genotypes (3.0 and 2.6), and lower heterozygosity (17.9 and 18.3) than the 2x-members of group 1A from the same section with mean values for alleles (2.6), genotypes (4.1), and heterozygosity (24.4). It is likely that the low level of genetic variation in the enzyme loci of these two populations, which is paralleled by their very low morphological variation, is linked to their particular phylogenetic status: *G. noricum* is fragmented into a number of strongly isolated populations in the NE and SE Alps (MEUSEL & JÄGER 1992). This situation apparently is due to the Pleistocene glaciations after which the taxa of the *G. baldense* group have hardly managed to reinvade lost territory from their ice age refugia. In contrast, *G. saxatile*-4x is a W European cytotype which has (and still is) expanding into C (and E) Europe. The population studied from Lower Austria is a recent founder population with no contacts to the main centre of the species. There are several examples in the literature, where a reduction of allozyme variation has been documented under similar conditions, e.g., quite uniform island founder populations in *Turnera ulmifolia* var. *intermedia*-4x (SHORE 1991) or in *Androcymbium* (PEDROLA-MONFORT & CAUJAPÉ-CASTELLS 1994), marginal populations in *Menziesia ferruginea* A. GRAY (WELLS & BOHM 1994) or depauperate taxa of *Magnolia*, apparently stressed by cold periods of the Pleistocene (QIU & PARKS 1994).

Hybrid contacts among genetically differentiated taxa and populations will naturally increase the amount of variation in enzyme loci (see, e.g., CRAWFORD 1983, 1985, 1990). In the present study this applies, e.g., to all 2x- and 4x-taxa of the *G. pusillum* group (1A) and to the *G. mollugo* group (2), which had (and partly still have) opportunities for genetic contacts and reciprocal gene flow. In contrast, such opportunities seem to be very reduced in the groups of *G. noricum* (1B) or *G. saxatile* (1C), mentioned before. An excellent example for the strong positive influence of hybridization on allozyme variation is afforded by the genus *Quercus* (see, e.g., SAMUEL & al. 1995 and literature cited therein).

It is obvious that genetic variation depends on the number of monomorphic versus polymorphic enzyme loci studied. From the 11 loci analysed in *Galium* (Table 2), only 4 are monomorphic in the *G. pusillum* group of sect. *Leptogalium*, contrasting with 6 in the *G. mollugo* group of sect. *Leiogalium*. This is also reflected in the lower number of alleles, genotypes and heterozygosity values in the latter group, a remarkable difference in view of the much wider and more continuous distribution of the members of sect. *Leiogalium* studied. As no other differential factors can be recognized, one could assume that group specific genetic differences in respect to the potential for variation of enzyme loci are involved. There are comparable cases, e.g., the remarkably uniform (but morphologically very

diverse) genus *Erigeron* (HUBER & LEUCHTMANN 1992) as compared to other *Compositae* with very polymorphic enzyme loci; or the morphologically quite homogeneous genus *Capsicum*, where a nearly ten-fold difference exists in the same enzyme loci between the rather uniform *C. baccatum* L. as compared to the highly polymorphic *C. frutescens* L. (MCLEOD & al. 1983).

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