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Phylogenetic relationships and gene flow between sympatric diploid and tetraploid plants of *Dactylis glomerata* (*Gramineae*)

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Abstract: Phylogenetic relationships between sympatric, morphologically indistinguishable diploid and tetraploid plants of *Dactylis glomerata* L. (*Gramineae*) in Galicia (Spain) were assessed using allozyme markers for 6 distinct systems. The study exploited recent introduction in Galicia and subsequent hybridization of an alien 4x *Dactylis* subspecies possessing distinct allozymes from those of all the native plants. Opportunities for gene exchanges between the ploidies were estimated from in situ observations of flowering, examination of progenies in 2x/4x natural and experimental crosses, and enzyme analyses. Results show a high genetic similarity between the Galician diploids and tetraploids, which possess peculiar alleles in common. Although the ploidy levels usually have distinct flowering periods, interploidal crosses do occasionally occur. Gene flow is likely much more important from the diploid to the tetraploid level. A good genetic intermixing occurs between the Galician and the alien tetraploid entities which have simultaneous flowering. Autopolyploidization of the diploids followed by various rates of hybridization is proposed as one very probable origin of natural tetraploids in *Dactylis*.

One of the more suitable situations in which to study the adaptive and evolutionary significance of autopolyploidy is when morphologically indistinguishable individuals with distinct ploidy levels (therefore assumed to be closely related) are found in sympatry. Such situations have been described in *Claytonia virginica* L. (*Portulacaceae*) (LEWIS 1976) and *Dactylis glomerata* L. (*Gramineae*) (BORRILL & LINDNER 1971; LUMARET 1981a; ARDOUIN & al. 1985, 1987). Recently, a new situation of sympatry between morphologically similar diploid ($2n = 14$) and tetraploid ($2n = 28$) plants of *Dactylis glomerata* was discovered in central Galicia (Spain) by CHORTON, YOUNG and TYLER of the Welsh Plant Breeding Station Aberystwyth, G.B. (CHORTON & YOUNG 1980, and TYLER, pers. comm.). The diploid and tetraploid plants could occasionally be found in the same sites but each cytotype showed a distinct habitat preference (LUMARET & al. 1987a).

Evidence that the Galician tetraploids are likely of autopolyploid origin has already been obtained from cytology (DELAY & LOUTFI, pers. comm.), flavonoid chemistry (ARDOUIN 1985, ARDOUIN & al. 1987), and chloroplast DNA restriction pattern variability (LUMARET & al. 1987b, and unpubl.). Further support for the autopolyploid origin of the Galician tetraploids is provided in this work from the study of enzyme polymorphism. Tetraploids produced by autopolyploidy are expected to show some genetic similarity with their related diploids. More specifically, the entities of both ploidies would possess several predominant alleles in common. However, as a direct consequence of tetrasomic inheritance, which slows down the segregation rate, autotetraploids are expected to maintain several additional alleles at low frequencies and to possess therefore a higher genetic diversity than their diploid counterparts (STEBBINS 1950, 1980; GRANT 1981).

Furthermore, in autopolyploid complexes, opportunities may occur for gene exchange in crosses between sympatric individuals of distinct ploidy level. An alien *D. glomerata* tetraploid entity possessing allozymes distinct from those of the Galician endemic *Dactylis* plants has recently been introduced into the study area, and these have been observed to hybridize (BARRIENTOS 1985, LUMARET & al. 1987a). Gene exchange between ploidy levels was therefore estimated using allozymes as markers.

Materials and methods

Plant material. Populations from 7 sites located mainly in central Galicia were scored for enzyme polymorphism. Location and environmental characteristics of these sites are given in a previous paper (LUMARET & al. 1987a). The relative frequencies of each ploidy level in each site, as well as the degree of hybridization between the Galician and alien (*D. g.* subsp. *glomerata*) types, are indicated diagrammatically in Table 1. Frequency of hybridization at each site was estimated from the relative frequencies of Galician, intermediate and "*glomerata*" morphotypes as defined previously from detailed data (LUMARET & al. 1987a).

Table 1. Location of the 7 study sites of *Dactylis* in C. Galicia. Diploid/tetraploid frequency (*H* high; *M* medium; *L* low) and degree of hybridization with subsp. *glomerata* (cos-Glo 4x) (high, medium, low, not detectable) in the respective populations

Site no.	Locality	Coordinate	Plant ploidy level		Hybridity
			2x	4x	
13	Forcarey	42 40′N 8 23′W	H	L	not detectable
3	Siador	42 40′N 8 16′W	H	L	low
12	Convite	43 15′W 7 28′W	M	M	medium
1a	Senra	42 42′N 8 19′W	L	H	high
1b	Senra	42 42′N 8 19′W	L	H	medium
2	Fraiz	42 41′N 8 22′W	–	H	not detectable
14	Villa de Cruzes	42 36′N 8 7′W	–	H	high

Biochemical methods. Allozyme assays were made from leaf tissue, generally from plants that had been collected in nature and grown in a greenhouse under uniform conditions for eight weeks. The number of plants analysed per sample ranged from 19 (site 3, tetraploid plants) to 134 (site 1a, tetraploid plants). Samples were scored for a maximum of 6 different enzyme systems after electrophoresis in either horizontal starch gels or vertical zonal polyacrylamide gels. Inheritance of allozymes and electrophoretic techniques used to detect them have already been reported in detail by LUMARET & VALDEYRON (1978) for glutamate oxaloacetate transaminases (locus *GOT1*), LUMARET (1981b) for acid phosphatases (*AcPH1*), LUMARET (1981a) for tetrazolium oxidases (*TO1*), LUMARET (1982) for peroxydases (*PX1*) and malate dehydrogenases (*MDH1*), and LUMARET (1986) for phosphoglucose isomerases (*PGI1* and *PGI2* were shown to be duplicated loci involving the same alleles).

Survey of plant phenology. Flowering periods were determined for two consecutive years from 150 plants labelled and mapped in sites 1a and 3. Observations were recorded on 3 or more dates throughout the flowering period. At each census, plants were classified into one of the following stages: 0 = no panicle; 1 = panicle formation; 2 = beginning of flowering (at least one panicle with dehiscent anthers); 3 = about half of the panicles flowering; 4 = most of the panicles finished flowering; 5 = flowering completely over (seeds maturing).

Experimental crosses. Diploid and tetraploid Galician plants were used for 17 bidirectional interploid crosses. *Dactylis* is mostly but not exclusively allogamous; therefore, parental genotypes of known constitution at the *GOT1* locus were crossed in order to aid identification of progeny obtained from selfing and/or cross-fertilization.

Because tetraploids were found to flower earlier than diploids, the following process was used to synchronize flowering of the two ploidies. At the time they were ready to flower, the tetraploids were placed in a cold room (+4 °C) under short day period (8 h) to stop their development at this stage. They were maintained under these conditions until the diploids began to flower.

Cytological survey. Meiosis was observed in anthers collected in situ from each studied plant in order to determine its chromosome number. The fixing and staining methods are described in detail in LUMARET (1986), and LUMARET & al. (1987a). The same methods were used to determine chromosome numbers of progenies obtained from interploidal crosses. Chromosome numbers were also determined from mitoses observed in root tip squashes of seedlings collected in situ from several open-pollinated mother plants of both diploid or tetraploid type by the modified FEULGEN technique described in LUMARET & al. (1987a).

Pollen fertility. For pollen fertility estimation, heads were collected from the studied plants and stored in ethanol: acetic acid, 3:1 (v/v); the pollen was stained in iodine solution. Two counts of approximately 200 grains were made from each plant.

Results

Enzyme polymorphism in the Galician populations. (1) Allele distributions. Allelic frequencies at the seven studied loci are indicated in Table 2 for three diploid samples (populations 13, 3, and 12) and six tetraploid samples (populations 2, 3, 12, 1b, 1a, 14). These samples are ordered according to ploidy level as well as degree of contact or/and hybridity between Galician and cosmopolitan "*glomerata*" morphotypes. In the three diploid samples, allele frequencies are generally very similar. Extra-alleles are in low frequencies in populations 3 and 12 relative to population 13. This difference may be attributable to the occurrence of direct gene exchange between diploids and tetraploids growing in syntopy in sites 3 and 12 (several of the extra alleles are present in both ploidy levels). Alternatively, the

Table 2. Allele frequency (%) at loci *GOT1*, *AcPH1*, *PX1*, *MDH1*, *TO1*, and *PGI1−2* in 9 diploid and tetraploid populations sampled in Galicia. In brackets, errors (p=0.05). ε < 1%. * Population no. ** Individual no.

Loci	allele	2x			4x					
		13* 26**	3 109	12 34	2 69	3 19	12 22	1b 31	1a 134	14 22
GOT1−	1.26	–	–	–	–	–	8	–	–	3
	1.00	54 (14)	54 (7)	59 (12)	55 (6)	50 (11)	71 (10)	65 (8)	50 (4)	55 (10)
	0.72	46 (14)	46 (7)	41 (12)	45 (6)	50 (11)	21 (8)	33 (8)	48 (4)	42 (10)
	0.50	–	ε	–	–	–	–	–	–	–
	0.38	–	–	–	ε	–	–	2	1	–
AcPH1−	1.06	52 (14)	34 (6)	56 (12)	43 (6)	19 (9)	9	21 (7)	32 (4)	17 (8)
	1.00	48 (14)	61 (7)	44 (12)	54 (6)	80 (9)	84 (8)	70 (8)	56 (4)	71 (10)
	0.95	–	–	–	–	–	6	3	2	8
	0.88	–	5	–	3	1	–	6	10 (3)	4
Px1	1.00	100	99	100	96	100	97	93	94 (2)	94
	0.93	–	ε	–	3	–	2	6	4 (2)	4
	0.90	–	–	–	ε	–	–	–	–	–
	null	–	–	–	ε	–	1	1	2	2
TO1	1.12	–	–	–	–	–	–	1	–	
	1.03	100	89 (4)	93	70 (5)	80 (9)	63 (10)	65 (8)	63 (4)	
	1.00	–	ε	–	1	5	–	2	3	no
	0.88	–	10 (4)	7	28 (5)	15 (8)	33 (10)	32 (8)	33 (4)	data
	0.80	–	–	–	1	–	4	ε	–	
	0.76	–	–	–	ε	–	–	ε	ε	
PGI1−2	1.75	–	–	–	ε	–	–	ε	1	–
	1.50	–	2	–	2	2	–	3	5 (2)	10
	1.25	35 (13)	18 (5)	41 (12)	25 (5)	24 (10)	36 (10)	28 (8)	27 (4)	32 (10)
	1.00	65 (13)	75 (6)	41 (12)	68 (6)	61 (11)	62 (10)	62 (9)	53 (4)	55 (10)
	0.75	–	4	10	2	2	2	4	13 (3)	3
	0.67	–	–	–	1	–	–	–	–	–
	0.50	–	1	8	2	1	–	2	1	–
MDH1	1.00	100	100	100	100	100	97	100	99	84 (8)
	0.88	–	–	–	–	–	3	–	1	16 (8)

Table 3. Allele distribution (%) at the seven studied loci in the Galician type (Gal 2x) and in the subsp. *glomerata* (cos 4x), both taken as reference (origin of the data in the text). Alleles used as diagnostic for either the * Galician (2x) or the ** "cos-*glomerata*" types. In brackets, errors (p = 0.05). ε < 1%

Loci	Alleles	Galician type Gal 2x	Subsp. *glomerata* cos-Glo 4x
GOT1	1.26	–	ε
	1.00	54 (5)	77 (3)
	0.72	45 (5)	23 (3)
	0.50	ε	–
	0.38	–	ε
AcPH1	1.06*	41 (5)	–
	1.00	56 (5)	67 (3)
	0.95	–	6 (2)
	0.88	3 (2)	27 (3)
PX1	1.00	100	70 (3)
	0.93**	ε	17 (2)
	0.90	–	5 (2)
	0.84	–	6 (2)
	null	–	2
TO1	1.12	–	2
	1.09	–	1
	1.03*	91 (3)	–
	1.00**	ε	56 (3)
	0.88	9 (3)	40 (3)
	0.80	–	1
PGI1–2	1.75	–	1
	1.50	1	3 (2)
	1.25	25 (5)	14 (2)
	1.00	68 (5)	67 (3)
	0.75	4 (2)	13 (2)
	0.67	–	1
	0.50	2	1
MDH1	1.00	100	99
	0.88	–	1
	others	–	ε

much greater sample size in site 3 may permit rare alleles to be detected more easily there. The occurrence of allele *GOT1* 0.50, peculiar to the diploids, which had never been found previously in *Dactylis*, would support the second hypothesis. The distribution of alleles was compiled using data from the three diploid samples (Table 3) so as to compare Galician morphotypes with cosmopolitan "*glomerata*" tetraploids (cos 4x), also included in Table 3. The latter data were obtained by studying enzyme polymorphism in 15 natural populations located throughout subsp. *glomerata*'s European distribution, Spain (but not Galicia) included (LUMARET 1981a). This distribution was also found to be very similar to the allelic

Table 4. Allele frequency (%) at the four diagnostic alleles for either Galician (Gal 2x) or subsp. *glomerata* (cos-Glo 4x) *Dactylis*, respectively in the Galician, intermediate, and "*glomerata*" type 4x *Dactylis* plants from site 1a. In brackets, errors ($p = 0.05$). $\varepsilon < 1\%$

Loci		Gal 4x 51 ind.	inter 4x 53 ind.	cos-Glo 4x 28 ind.
$AcPH1^{1.06}$	Gal 2x	35 (6)	32 (6)	23 (8)
$TO1^{1.03}$	Gal 2x	69 (6)	64 (6)	54 (9)
$TO1^{1.00}$	cos-Glo	ε	3	4
$PX1^{0.93}$	cos-Glo	5 (3)	4	4

composition usually observed in most commercial *D. g.* subsp. *glomerata* varieties introduced recently in central Galicia (LUMARET, unpubl.).

Information about the genetic specificity of each entity can be deduced from the comparison of the Galician and "*glomerata*" allelic distribution. Four alleles present in noticeable frequencies (more than 15%) in one entity are absent (or constitute less than 1%) in the other. Such alleles were "diagnostic" as they could be used to detect gene flow between the entities. *AcPH1 1.06* and *TO1 1.03* constitute such alleles for the Galician type, while *PX1 0.93* and *TO1 1.00* are diagnostic for the "*glomerata*" type. The frequency distributions of these four diagnostic alleles, among the three morphotypes of the tetraploid plants that grow together in site 1, are indicated in Table 4. Tables 3 and 4 show that both alleles characterizing the Galician type are present at high frequencies in the diploid as well as tetraploid individuals of Galicia whatever the site and the plant morphotype. However, a slight variation is evident in tetraploids from site 1, with a decrease of *AcPH1 1.06* and *TO1 1.03* frequency from the Galician to the "*glomerata*" morphotypes. It can also be noticed that the intermediate-type frequencies are closer to those of the Galician type than to those of the "*glomerata*" type. *PX1 0.93* and *TO1 1.00*, which are diagnostic for subsp. *glomerata*, do not seem to penetrate easily in the Galician populations regardless of ploidy level and morphotype. However, higher frequencies are regularly found in tetraploids than in diploids, in which only a low cryptopolymorphism is observed for each allele.

(2) Genetic similarity between the Galician populations. The genetic similarity between the Galician populations was quantified from their allelic distribution, by calculating the genetic identity statistic of NEI (1972). These statistics were used as the basis for a cluster analysis (UPGMA) using as the clustering criterion, the "average distance" that preserves the initial space (LEGENDRE & LEGENDRE 1979).

Results are indicated in Fig. 1 for the populations from sites 1a, 1b, 2, and 3 in which a sufficient number of plants were analysed, as well as for the Galician and "*glomerata*" reference types scored for the 7 loci. Data from site 1a were considered from three distinct groups defined by 4x plant morphotype. The scale in the diagram was modified so that NEI's identities (which ranged between 0.79 and 1.00) could correspond to a variation of the clustering level from 0 to 100.

A clear partition is observed between diploid and tetraploid populations, except in site 3 where the genetic composition of ploidies is similar. This similarity could indicate the occurrence of very close relationships between the diploids and their

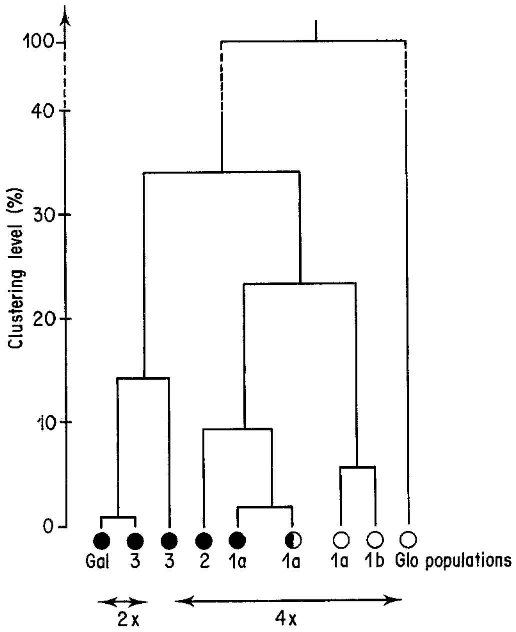

Fig. 1. Phenogram showing the relationships between several diploid and tetraploid populations (or sub-populations) from Galicia and their respective position compared to the Galician (Gal 2x) and cosmopolitan "*glomerata*" (cos-Glo 4x) references types. Hierarchical clustering is based on NEI's identities calculated from the 7 studied enzyme loci. Plant morphotype: ● Galician; ◐ intermediate; ○ "*glomerata*"

Table 5. Allelic diversity in 2x and 4x populations sampled from sites 1a, 1b, 2, and 3

Population	N	Average no. alleles per pop.	Shannon's index	Equitability
1a	134	4.2	0.98	0.48
1b	31	3.5	0.90	0.45
2	69	3.8	0.76	0.40
3 (4x)	19	2.5	0.70	0.45
3 (2x)	109	2.8	0.65	0.47

morphologically indistinguishable syntopic tetraploids. Among the tetraploids, one group constitutes populations composed of either Galician or intermediate morphotype plants. These plants from sites 2 and 1a, unlike the tetraploids from site 3, do not grow directly in contact with numerous diploid Galician individuals. A second group includes two populations constituted chiefly (site 1b) or exclusively (site 1a) of "*glomerata*" morphotypes. Lastly, the genetic composition of the "*glomerata*" reference type appears to be completely distinct from those of the populations from Galicia regardless of ploidy level or the phenotype. This is due to the occurrence of alleles, peculiar to Galicia, in all the populations of this region (even those which derive from recently introduced allochthonous type). This result may indicate that important gene mixing is happening within the region.

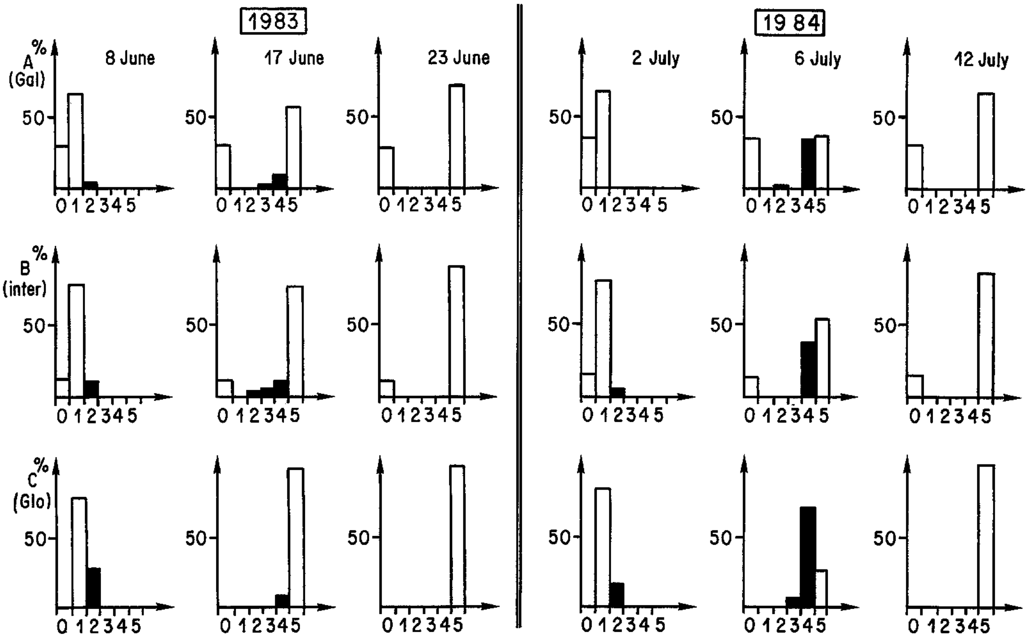

Fig. 2. Histograms showing the distribution pattern of Galician (*A*), intermediate (*B*), and "*glomerata*" (*C*) individuals from site 1a over 6 phenological classes ranging from 0 (no head) to 5 (seed maturation), at several times of the year and for two consecutive years (criteria used to define the classes are in the text). Period: ■ during flowering; □ apart from flowering

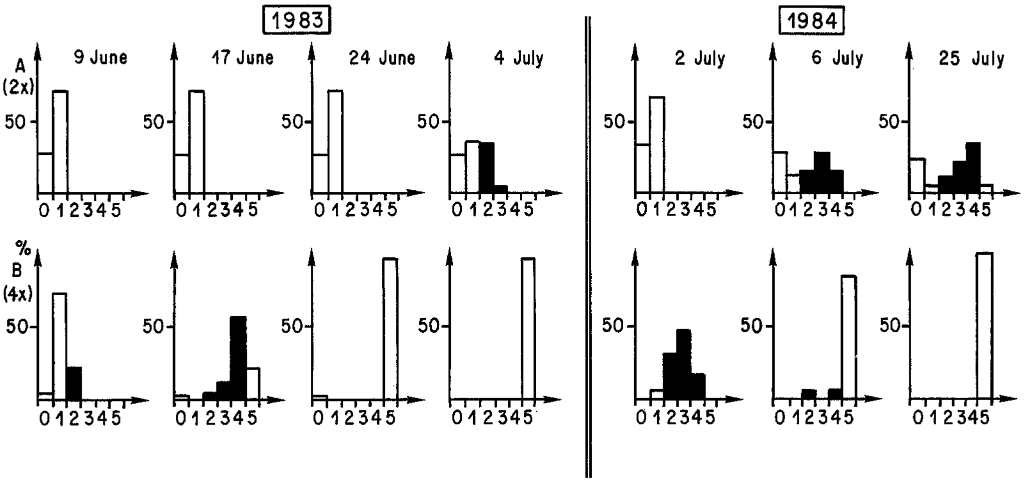

Fig. 3. The distribution of diploid (*A*) and tetraploid (*B*) individuals from site 3 over the 6 phenological classes (defined in the text), at several times of the year and for two consecutive years. Period: ■ during flowering; □ apart from flowering

(3) Allelic diversity in diploids and tetraploids. Allelic diversity in populations from sites 1a, 1b, 2, and 3 was estimated by the SHANNON-WEAVER index (1963) and was averaged over the 7 loci studied. The results are plotted in Table 5. The average number of alleles per population and equitability (the ratio

Table 6. Distribution of 2x and 4x seed-parent involved in interploidal crosses according to respective seed production

Seed no.	No. seed-parents	
	2x	4x
1 – 10	4	4
11 – 50	3	2
51 – 100	–	–
101 – 200	1	2
201 – 400	–	2

Table 7. Genotypic frequencies at the *GOT1* locus in progenies from 5 tetraploid and 2 diploid seed-parent involved in interploidal crosses. *N* Number of offspring in each progeny

Ploidy level	Seed parent no.	Parent genotype		N	Offspring genotypes						
					11	22	1 111	1 122	1 133	2 222	112
4x	1	1 113	22	1	–	–	1	–	–	–	–
	2	1 111	22	8	–	–	7	1	–	–	–
	3	1 113	44	2	–	–	1	–	1	–	–
	4	1 111	22	1	–	–	–	–	–	–	1
	5	2 222	11	66	–	–	–	–	–	66	–
2x	1	11	2 222	1	1	–	–	–	–	–	–
	2	22	1 111	1	–	1	–	–	–	–	–

of the Shannon index to its value when the alleles are equifrequent) are also indicated.

It is clear from the results that allele diversity is higher in tetraploids than in diploids. The difference is due essentially to the occurrence of several particularly rare alleles in the tetraploids, because the equitability is similar between ploidy levels. Among the tetraploids, the highest allele diversity values (0.98 and 0.90) are found in populations with the highest proportion of "*glomerata*" phenotype individuals (sites 1a and 1b), while the lowest values are observed in sites with exclusively (site 2) or predominantly (site 3) Galician morphotyped plants.

Flowering periods of diploids and tetraploids. The results of phenological censuses are indicated in Figs. 2 (site 1a) and 3 (site 3). Regardless of habitat (open in site 1a or shady in site 3) or morphotype (see the diagrams in Fig. 2), tetraploid plants flower simultaneously. Tetraploids consistently flower earlier and more briefly than the diploids, which spread panicle production and flowering time throughout summer. After flowering, the native tetraploids from both shady and open sites usually stop growing and begin to dry, their seeds maturing inside the panicles. In contrast, the "*glomerata*" and a part of the intermediate morphotyped tetraploid plants that originate from the introduced types (subsp. *glomerata*) drop their seeds, which can mature within only two or three weeks.

An important interannual variation in the flowering period also exists. This is not surprising, as most of the physiological cues for flowering in *Dactylis* are known to be directly dependent on climatic factors (REBISCHUNG 1962, NIQUEUX & ARNAUD 1981). As a consequence of such variations, no overlap is observed in some years (e.g., 1983) between the flowering period of tetraploids and diploids, whereas in other years (e.g., 1984) a short overlap occurs. In 1984, two tetraploid individuals of site 3 were observed to flower simultaneously with several neighbouring diploid plants. These were labelled so that chromosomal number in their progeny could be analysed.

Cytological survey of progenies from interploid crosses. (1) Seeds collected in situ from open-pollinated plants. Mitosis was examined in progenies from seeds collected in site 3 on two tetraploid and nine diploid plants that had been noticed to flower simultaneously. On average, 105 (range 74 – 146) offspring were studied per mother plant. Tetraploid progeny as obtained only from the two tetraploid mother plants. Six of the 9 diploid mother plants were found to produce exclusively diploid individuals: progeny of the other 3 diploid mothers included 5 – 22% aneuploid individuals (with several extra chromosome), but no triploid progenies were found.

(2) Progenies from controlled interploid crosses. Among the 17 bidirectional interploid crosses, 3 produced no seed regardless of the parent, 4 gave seeds only from the diploid parent, 6 only from the tetraploid parent and 4 provided seeds from both parents.

Seed production from the various crosses is indicated in Table 6. The mother plants can be divided into two classes, according to the either small (50) or large (100) number of seeds in their progeny. Most of the diploid parents occur in the first class, whereas a larger proportion of tetraploid parents occur in the second.

The germination rate was low (6.95%). Seedling mortality was 12%, so that 80 young plants were obtained finally from the 1 322 seeds originally produced by the 17 crosses. These 80 individuals were scored for their allele composition at the *GOT1* locus. In each progeny, offspring genotypes were compared to the genotypes of their putative parents. The results (Table 7) clearly showed that the offspring from two diploid mother plants originated exclusively from selfing, whereas, among the tetraploid progenies, two plants from two distinct progenies originated from cross-fertilization. Chromosome counts confirmed that one plant was tetraploid and the other triploid. Both were completely fertile and produced as much pollen stainable by iodine solution in anther as their parents (i.e., 62 – 87% fertile pollen).

Discussion

Phylogenetic relationships. Consistent information from several studies supports the very close phylogenetic relationships between the Galician plants of different ploidy levels. A high genetic similarity was observed indeed in this study between the native Galician diploid and tetraploid *Dactylis* plants which, furthermore, have identical morphology. The closer the Galician tetraploids are to the diploids and the further they are from the introduced cultivated tetraploids, the greater this similarity. The common possession of several peculiar alleles coding for allozymes is particularly relevant and reinforces several other results reported previously. These showed very similar composition of flavonoid compounds (ARDOUIN & al. 1987) and the occurrence of an identical specific molecular variant in the chloroplast DNA (LUMARET & al. 1987b, and unpubl.). Such similarity for so many traits, including those usually considered to be selectively neutral such as enzymes (KIMURA 1983), cannot be attributed to adaptive convergence. The possibility that the Galician diploids are derived from the tetraploids by polyhaploidization must also be ruled out. Even if polyhaploids (individuals derived from polyploids and having half their chromosome number) may occur spontaneously in low frequencies in natural autotetraploid populations such as those from Galicia, these diploids would not survive or be likely weak, sterile or both, and therefore would not become established successfully (STEBBINS 1971). Conversely, the possibility that the Galician tetraploids derived from autopolyploidization of the Galician diploids appears much more probable. Spontaneous occurrence of doubled sectors of somatic tissue or gametic non-reduction are the usual mechanisms of autopolyploidization. Although the spontaneous production of polyploid tillers in diploid grasses has not yet been observed, unreduced gametes are known to occur with some regularity in *Dactylis* (ZOHARY & NUR 1959, CARROLL & BORRILL 1965, BORRILL 1978, VAN SANTEN & CASLER 1986). Simultaneous chance occurrence of gamete non-reduction in pollen and ovules leading directly to autopolyploidy cannot be completely ruled out, although they are very likely quite rare events in nature. In previous experiments, autotetraploid *Dactylis* plants were obtained directly from diploids parents selected previously to produce either male or female non-reduced gametes (M. CASLER, pers. comm.). The tetraploid plant obtained in the present study from the progeny of a diploid × tetraploid cross very probably originated from an unreduced gamete in the diploid parent. Fertility of this "newly made" tetraploid plant was comparable to natural tetraploid individuals in controlled laboratory conditions. Finally, all the results suggest that Galician morphotype plants of both cytotypes belong to a single taxonomic entity. This has been recognized to be morphologically and genetically distinct from the 14 diploid and 5 tetraploid subspecies known in *Dactylis glomerata* and to be confined to central Galicia (LUMARET 1981a, LUMARET 1985, ARDOUIN & al. 1985, LUMARET 1988a). This entity probably constitutes a further subspecies which has not yet been formally described.

Although overall they possess a genetic composition very similar to the diploids, the Galician tetraploids differ slightly by possessing several extra alleles in very low frequencies. These alleles increase substantially the genetic diversity, and above all the level of heterozygosity (tetraploid individuals may possess up to three or four distinct alleles at several loci). The same results were obtained in a previous study concerning the indistinguishable diploid and tetraploid *Dactylis* plants growing intermixed in the gorges of Kerrata (Algeria) (LUMARET 1981a, 1985). In the same way, allelic diversity and heterozygosity in *Tolmiea menziesii* (*Saxifragaceae*) were found to be substantially higher in autotetraploids than in the morphologically indistinguishable diploid cytotpye (SOLTIS & RIESEBERG 1986). In both situations, the difference in genetic diversity between ploidy levels is probably due to two factors. One is the inertia of the tetraploid genetic structure, which very probably contributes to the retention of the alleles (originated from mutation or occasional migration) by slowing down segregation rate. The second reason is the occurrence of several different degrees of hybridity from direct contacts with other tetraploid entities (in the case, with plants of subsp. *glomerata*), followed by back crossing with plants of the native type. This process ends up probably in gradual infiltration of genes into the Galician tetraploids.

Gene flow. In general, the tetraploids from Galicia (what ever their morphotype) have an earlier flowering period than the diploids. This contrasts with earlier results from artificial and natural autopolyploids in other genera (Lewis 1976, Levin 1983), but confirms our previous findings in *Dactylis* from several areas around the Mediterranean (Lumaret & al. 1987a, Lumaret 1988c). We concluded that: (1) observed habitat differentiation and divergence in physiological requirements between natural autotetraploids of *Dactylis* and their sympatric diploid progenitors are probably related to the advantages of reduced crossing between the ploidy levels with maintenance of populations of both cytotypes: (2) tetraploids tend to dominate under environmental conditions that reduce the growing season and favour individuals with high growth rate and early flowering time. The ability of tetraploids to grow faster and flower earlier may result from larger cell size without proportionately slower cell division and more importantly, from increased tolerance of environmental variability. Subsequent hybridization among tetraploids very probably increases differentiation between the related cytotypes.

Because of divergence in flowering period, very restricted gene flow (if any) is expected to occur between plants of different ploidy levels. Evidence that interploidal crosses and gene flow do likely occur is shown in the present study by three results: (1) Unusually large numbers of aneuploid seedlings were produced in situ in progenies of diploids flowering simultaneously with neighbouring tetraploids: (2) A triploid seedling was found in the progeny of a tetraploid mother plant, which had been open-pollinated in situ. According to Tyler (pers. comm.), this seedling gave a vigorous and fertile adult plant in the laboratory. (3) When only a few experimental interploidal crosses are made, triploids as well as tetraploids (in case of unreduced gametes) were obtained in the progeny of several tetraploid parents. Results from previous studies of interploidal reciprocal crosses in a wide range of *Dactylis* taxonomic groups showed that the direction of cross has a marked effect. On average, the fertility was 5 times higher when the mother was a tetraploid rather than a diploid (Jones & Borrill 1962). However, viability in situ of the aneuploid and even of the triploid seedlings produced in Galicia may be low as neither aneuploid nor triploid individuals were found from numerous chromosome counts made during meiosis of adult plants in sites where individuals of different ploidy levels grow completely intermixed.

Results of a detailed study concerning the product of natural interploidal crosses in Israel (Zohary & Nur 1959), as well as numerous experimental interploidal crosses and triploid backcrosses with either the diploid or tetraploid parent (Jones & Borrill 1962), had led to the conclusion that the aneuploid plants were rarely viable. Among the euploid individuals, only the tetraploids were as fertile as wild plants, whereas the diploids were much less fertile. As a consequence, gene flow is expected to be more important from the diploid to the tetraploid level. This is probably the case in Galicia. The diagnostic alleles of the "*glomerata*" introduced type were in fact observed in the Galician type tetraploids but not in the diploids or, if so, in a very low frequency.

Gene exchange among tetraploids. A good genetic intermixing occurs between the Galician and the "*glomerata*" tetraploid types. It is favoured by their overlapping simultaneous flowering periods. Consequently, most of the tetraploid individuals classified previously according to their morphological traits were revealed by their allozyme structure to be hybrids. In fact, a continuum of hybridity is observed in several sites. This shows that the mixing has already been occurring for several successive generations. The average degree of hybridity in each site is determined by the amount of introduced commercial *Dactylis* varieties in the area, which depends on local cultural practice (LUMARET & al. 1987a). One of the more striking results is that the alleles characteristic of the native diploid type are found in high frequencies in tetraploids, even in the "*glomerata*" morphotype plants which are very similar to the introduced *Dactylis* varieties. Conversely, the alleles that characterize the subspecies *glomerata* and that are introduced with the cultivated varieties do not invade significantly the Galician tetraploid populations. This asymmetric gene flow can be explained from observation of the hybridization process itself. The "*glomerata*" type of the natural pastures (i.e., of site 1a) that were derived recently from the cultivated *Dactylis* varieties on the average form much larger plants that produce significantly more seeds than the Galician or even the intermediate tetraploid plants from the same site (LUMARET & al. 1987a). Variation in seed production between morphotypes was shown to be partly due and/or to be at least increased by the feeding behaviour of cattle: the "*glomerata*" morphotype plants and a part of the intermediates which possess rough leaves are consumed less than the native types which have much smoother leaves (LUMARET 1988b). Moreover, as *Dactylis glomerata* that possesses a self incompatibility system is essentially allogamous and is very sensitive to consanguinity, it is expected that these large "*glomerata*" plants would receive pollen chiefly from the native individuals which up to now, have been much more numerous in the natural pastures. The hybrid progenies produced by these "*glomerata*" individuals would contain therefore essentially "Galician alleles". Concerning cytoplasmic markers (studied in site 1a from chloroplast DNA restriction patterns), a very high proportion of the tetraploid hybrid plants (whatever the morphotype) were found to possess the "*glomerata*" cytoplasmic type, which is distinct from the Galician one (LUMARET & al. 1987b and unpubl.). This last result also suggests that large "*glomerata*" *Dactylis* plants may chiefly be seed producers (females) whereas the Galician type plants (native or close to the native type) would be chiefly pollen producers (males). If so, gene flow is expected to occur primarily from the native to the introduced type for nuclear markers such as enzymes, and to be more important in the reverse direction for cytoplasmic markers as is likely the case in the present study.

Origin of tetraploids. Researchers (e.g., STEBBINS & ZOHARY 1959, BORRILL 1978, LUMARET 1981a) agree that, in *Dactylis*, tetraploids arose from the diploids by chromosome doubling. The hypothesis supported previously by STEBBINS & ZOHARY (1959) and STEBBINS (1956, 1971) that intersubspecific hybridization in diploids preceded this chromosome doubling cannot be ruled out. Such opportunities could indeed easily have happened when diploids were substantially more widespread than now so that direct contacts between distinct diploid entities could occur. However, most of the diploid entities are, at present, geographically isolated and surrounded by tetraploids, which probably constitute more than 95% of the total individuals.

The results presented in this paper add to previous data to support clearly the idea of an intrasubspecific autopolyploid origin, by gamete non-reduction, for a substantial part of the tetraploids in *Dactylis*. These results were obtained from investigations in areas where morphologically indistinguishable diploid and tetraploid *Dactylis* individuals grew intermixed (BORRILL & LINDNER 1971, LUMARET 1981a, ARDOUIN & al. 1987, LUMARET & HANOTTE 1987, LUMARET & al. 1987b), as well as from experiments in which fertile and vigorous tetraploids were recovered from 2x/4x or even 2x/2x crosses involving diploid plants selected previously for production of male or female non-reduced gametes (CASLER & HUGESSEN 1988). Polyploidization very likely takes place in various localities. Subsequent hybridization between newly-made tetraploids and other neighbouring tetraploids would probably occur more or less rapidly according to the local opportunities, so that the polyploids would have different degrees of hybridity. In Galicia, for example, both geographical and environmental isolation have probably prevented hybridization until the recent massive introduction of commercial varieties.

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References

ARDOUIN, P., 1985: Analyse, par la biochimie flavonique, de la stucture génétique et de l'organisation évolutive d'un complexe polyploide, *Dactylis glomerata* L. (*Gramineae*). – Spe. Thesis, Université Claude Bernard (Lyon 1), No. 1652, Lyon, France.

– FIASSON, J. L., JAY, M., LUMARET, R., HUBAC, J. M., 1985: Chemical diversification within the *Dactylis glomerata* L. polyploid complex (*Gramineae*). – In JACQUARD, P., & al., (Eds.): Genetic differentiation and dispersal in plants. – NATO ASI Series G5: p. 3–15. – Berlin, Heidelberg, New York, Tokyo: Springer.

– JAY, M., LUMARET, R., 1987: Étude d'une situation de sympatrie entre diploides et tétraploides sur la base du polymorphisme enzymatique et phénolique. – Canad. J. Bot. **65**: 526–535.

BARRIENTOS, E., 1985: Evolution dans les complexes autopolyploides. Exemple de *Dactylis glomerata* L. – Étude comparative de la structure génétique des populations dans deux situations de contact entre diploides et tétraploides. – Thesis. Univ. Sci. Tech. Languedoc, Montpellier, France.

BORRILL, M., 1978: Evolution and genetic resources in cocksfoot. – Ann. Report Welsh Plant Breeding Station (Aberystwyth, G.B.), 1977: 190–210.

– LINDNER, R., 1971: Diploid-tetraploid sympatry in *Dactylis* (*Gramineae*). – New Phytol. **70**: 1111–1124.

CARROLL, C. P., BORRILL, M., 1965: Tetraploid hybrids from crosses between diploid and tetraploid (*Dactylis* and their significance. – Genetica **36**: 65–82.

CASLER, M. D., HUGESSEN, P. M., 1988: Performance of tetraploid progeny derived from 2x–4x inter-subspecific crosses in *Dactylis glomerata* L. – Genome **30**: 591–596.

CHORTON, K. H., YOUNG, N. R., 1980: List of grass ecotypes collected in July and August 1977 in Galicia, NW. Spain. – Report Welsh Plant Breeding Station (Aberystwyth, G.B.): 1–23.

GRANT, V., 1981: Plant speciation, 2nd edn. – New York: Columbia University Press.

JONES, K., BORRILL, M., 1962: Chromosomal status, gene exchange and evolution in *Dactylis*, 3. The role of the inter-ploid hybrids. – Genetica **32**: 296–322.

KIMURA, M., 1983: The neutral theory of molecular evolution. – In NEI, M., KOEHN, R. K., (Eds.): Evolution of genes and proteins, pp. 208–233. – Sunderland: Sinauer Associates.

LEGENDRE, L., LEGENDRE, P., 1979: Écologie numérique. 1, le traitement multiple de données écologiques. 2, la structure des données écologiques, Écologie **12** and **13**. – Paris: Masson.

LEVIN, D. A., 1983: Polyploidy and novelty in flowering plants. – Amer. Naturalist **122**: 1–25.

LEWIS, W. H., 1976: Temporal adaptation correlated with ploidy in *Claytonia virginica*. – Syst. Bot. **1**: 340–347.

LUMARET, R., 1981a: Structure génétique d'un complexe polyploïde: *Dactylis glomerata* L. (Fam. Graminacées). Relations entre le polymorphisme enzymatique et certains aspects de la biologie, de l'écologie et de l'évolution de l'espèce. – State D. Thesis, Université des Sciences et Techniques du Languedoc, Montpellier, France.

— 1981b: Étude de l'hérédité de phosphatases acides chez le Dactyle (*Dactylis glomerata* L.) diploïde et tétraploïde. – Canad. J. Genet. Cytol. **23**: 513–523.

– 1982: Protein variation in diploid and tetraploid orchard grass (*Dactylis glomerata* L.), formal genetics and population polymorphism of peroxidases and malate dehydrogenases. – Genetica **57**: 207–215.

– 1985: Phenotypic and genotypic variation within and between populations of the polyploid complex, *Dactylis glomerata* L. – In HAECK, J., WOLDENDORP, J. W., (Eds.): Phenotypic and genotypic variation in plant populations, pp. 343–345. – Amsterdam: North-Holland.

– 1986: Doubled duplication of the structural gene for cytosolic phosphoglucose isomerase in the *Dactylis glomerata* L. polyploid complex. – Mol. Biol. Evol. **3**: 499–521.

– 1988a: Cytology, genetics and evolution in the genus *Dactylis*. – C.R.C. Crit. Rev. Plant Sci. **7**: 55–91.

– 1988b: Invasion of natural pastures by a cultivated grass (*Dactylis glomerata* L.) in Galicia (Spain): process and consequence on plant cattle interactions. – In DI CASTRI, F., (Ed.): History and patterns of biological invasions, pp. 389–395. – Dortrecht: Kluwer.

– 1988c: Adaptive strategies and ploidy levels. – Acta Oecol., Oecol. Plant. **9**: 83–93.

– VALDEYRON, G., 1978: Les glutamate-oxaloacétate transaminases du dactyle (*Dactylis glomerata* L.), génétique formelle d'un locus. – Compt. Rend. Acad. Sci. Paris **287**: 705–708.

– HANOTTE, C., 1987: Mise en évidence d'un écotype de dactyle (*Dactylis glomerata* L.) de pelouses dolomitiques subalpines dans les Grisons (Suisse). Origine et échanges géniques avec des dactyles de prairies adjacentes. – Acta Oecol., Oecol. Plant. **8**: 3–208.

– GUILLERM, J. L., DELAY, J., LOUTFI, A., IZCO, J., JAY, M., 1987a: Polyploidy and habitat differentiation in *Dactylis glomerata* L. from Galicia (Spain). – Oecologia **73**: 436–446.

– – BARRIENTOS, E., JAY, M., FIASSON, J. L., ARDOUIN, P., DELAY, J., LOUTFI, A., IZCO, J., AMIGO, J., 1987b: Signification évolutive de la polyploidie infraspécifique: cas des dactyles diploides et tétraploides de Galice. – In LEGAY, J. J., (Ed.): Biologie des populations, évaluation et prospective, pp. 129–133. – Lyon: I.A.S.B.S.E. Université Claude Bernard (Lyon 1).

NEI, M., 1972: Genetic distance between populations. – Amer. Naturalist **106**: 283–292.

NIQUEUX, M., ARNAUD, K., 1981: Peut-on prévoir la date d'épiaison des variétés de graminées. – Fourrages **88**: 39–56.

REBISCHUNG, J., 1962: Études sur la croissance et le développement du dactyle (*Dactylis glomerata* L.). – Ann. Amélior. Plants **12**: 175–196.

SHANNON, C. E., WEAVER, W., 1963: The mathematical theory of communication. – Urbana: University of Illinois Press.

SOLTIS, D. E., RIESEBERG, L. H., 1986: Autopolyploidy in *Tolmiea menziesii* (*Saxifragaceae*): genetic insights from enzyme electrophoresis. – Amer. J. Bot. **73**: 310–318.

STEBBINS, G. L., 1950: Variation and evolution in plants. – New York: Columbia University Press.

– 1956: Cytogenetics and evolution of the grass family. – Amer. J. Bot. **43**: 890–905.

– 1971: Chromosomal evolution in higher plants. – Reading: Addison-Wesley.

– 1980: Polyploidy in plants: unsolved problems and prospects. – In LEWIS, W. H., (Ed.): Polyploidy, biological relevance, pp. 495–520. – New York: Plenum.

– ZOHARY, D., 1959: Cytogenetic and evolutionary studies in the genus *Dactylis*. 1. Morphology, distribution and interrelationships of the diploid subspecies. – Univ. California Bull. Bot. **31**: 1–40.

VAN SANTEN, E., CASLER, M. D., 1986: Evaluation of indirect ploidy indicators in *Dactylis* L. subspecies. – Crop Sci. **26**: 848–853.

ZOHARY, D., NUR, U., 1959: Natural triploids in orchard grass *Dactylis glomerata* L. polyploid complex and their significance for gene flow from diploid to tetraploid levels. – Evolution **13**: 311–317.

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