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An isoenzyme study in the genus *Lotus* (Fabaceae).

Segregation of isoenzyme alleles in synthetic allo- and autotetraploids, and in *L. corniculatus*

J. V. Raelson, P. C. Lemaître, K. M. Starkie and W. F. Grant

Department of Plant Science, P.O. Box 4000, Macdonald College of McGill University, Ste. Anne de Bellevue, Quebec H9X 1CO, Canada

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Summary. Segregation of the cytosolic *Pgi2* locus was studied among progeny of the synthetic allotetraploid (*L. japonicus* × *L. alpinus*)^2, the synthetic autotetraploid (*L. alpinus*)^2, and the cultivated tetraploid species *L. corniculatus* L. Evidence of an original diploid duplication found within the interspecific hybrid *L. japonicus* × *L. alpinus* was also found within the synthetic allotetraploid (quadruplication of loci). Evidence suggesting quadruplication of loci was also found in the tetraploid *L. corniculatus*, but not in the synthetic autotetraploid (*L. alpinus*)^2. It is suggested that the original duplication resulted from unequal crossing-over between homoeologues and that it provides evidence that *L. corniculatus* is a segmental allotetraploid. Quadruplication of loci in *L. corniculatus* could explain previously reported distorted tetrasomic ratios for segregation of qualitative characters in this species.

Key words: *Lotus corniculatus* – Fabaceae – Autopolyploidy – Allopolyploidy – Isoenzymes

Introduction

This paper is the third in a series to report on an isoenzyme study of several species in the genus *Lotus* (Raelson and Grant 1988, 1989). One of the purposes of the study was to test several conflicting theories on the phylogenetic origin of the cultivated tetraploid species *Lotus corniculatus* L. (Birdsfoot Trefoil). It has been proposed that *L. corniculatus* is an allotetraploid involving hybridization between two diploid species. Somaroo and Grant (1972) proposed *L. japonicus* (Regel) Larsen and *L. alpinus* Schleich. as likely ancestral species, whereas Ross and Jones (1985) suggested that *L. uliginosus* Schkuhr was the pollen parent and that either *L. tenuis* Waldst. et Kit. or *L. alpinus* was the female parent of the original hybrid. We have provided isoenzyme evidence against the likelihood of *L. uliginosus* being involved in the ancestry of *L. corniculatus* (Raelson and Grant 1988).

Dawson (1941) observed tetrasomic inheritance of cyanogenesis in *L. corniculatus* from the putative cross *AAaa* × *aaaa* (duplex cyanogenic × nulliplex acyanogenic). He observed a 5:1 cyanogenic to acyanogenic ratio among the progeny, which would correspond to a 1 *AA*:4 *Aa*:1 *aa* ratio for gamete segregation in the duplex parent. On the basis of these and cytological results, he proposed that *L. corniculatus* was an autotetraploid of *L. tenuis*.

Because isoenzyme alleles are codominant, more powerful tests of segregation are possible than are available with analyses of segregation of morphological characters for which dominance prevents the distinction between heterozygotes and homozygous dominants (Gottlieb 1977). Analyses of isoenzyme segregation can be expected to provide new insights into the reported tetrasomic nature of segregation in *L. corniculatus* and the controversy concerning the allo-versus autotetraploid origin of this species.

We have previously reported the segregation of alleles for various isoenzyme loci (*Pgi2,3, Mdh3, Idh1,2,* and *6-Pgdh1,2*) among progeny of the diploid interspecific hybrid *L. japonicus* × *L. alpinus*, and have noted the unexpected duplication of loci at the diploid level that occurred in the hybrid but not in the diploid parental species (Raelson and Grant 1989). It is the purpose of this paper to report results of an investigation of segregation of alleles of the *Pgi2* locus at the tetraploid level for the synthetic allotetraploid (*L. japonicus* × *L. alpinus*)^2, the synthetic autotetraploid (*L. alpinus*)^2, and the tetraploid *L. corniculatus*.

Table 1. Disomic-digenic and tetrasomic models of allele segregation for selfed genotypes with various duplicated loci. F = fast, most anodal, S = slow, most cathodal, M = medium

Disomic-digenic, 2 alleles

F F / S S	1 FF	2 FS	1 SS
1 FF	1 FFFF	2 FFFS	1 FFSS
2 FS	2 FFFS	4 FFSS	2 FSSS
1 SS	1 FFSS	2 FSSS	1 SSSS

Disomic-digenic, 3 alleles

M M / F S	1 FF	2 FM	1 MM
1 SS	1 FFSS	2 FMSS	1 MMSS
2 MS	2 FFMS	4 FMMS	2 MMMS
1 MM	1 FFMM	2 FMMM	1 MMMM

Tetrasomic chromosome segregation

Duplex model *FFSS*

	1 FF	4 FS	1 SS
1 FF	1 FFFF	4 FFFS	1 FFSS
2 FS	2 FFFS	16 FFSS	2 FSSS
1 SS	1 FFSS	4 FSSS	1 SSSS

Triplex model *FFFS*

	1 FF	1 FS
1 FF	1 FFFF	1 FFFS
1 FS	1 FFFS	1 FFSS

Tetrasomic chromosome segregation

3 alleles *FFMS*

	1 FF	2 FM	2 FS	1 MS
1 FF	1 FFFF	2 FFFM	2 FFFS	1 FFMS
2 FM	2 FFFM	4 FFMM	4 FFMS	2 FMMS
1 FS	2 FFFS	4 FFMS	4 FFSS	2 FMSS
1 MS	1 FFMS	2 FMMS	2 FMSS	1 MMSS

Tetrasomic chromatid segregation

Duplex model *FFSS*

	3 FF	8 FS	3 SS
3 FF	9 FFFF	24 FFFS	9 FFSS
8 FS	24 FFFS	64 FFSS	24 FSSS
3 SS	9 FFSS	24 FSSS	9 SSSS

Materials and methods

Plant material

All taxa of *Lotus* used in this study were obtained from the world *Lotus* collection maintained by W. F. Grant at Macdonald College of McGill University. The diploids *Lotus uliginosus* (193-52; accession number-individual) and *L. tenuis* (109-21) were included in all electrophoretic gels as standards to which the banding pattern of other samples could be compared. This allowed comparison of patterns among different gels. The synthetic amphidiploid (*L. japonicus* × *L. alpinus*)^2 (28) and the synthetic autotetraploid (*L. alpinus*)^2 (774*x*-5) were also analyzed, as were the progeny obtained from selfing these individuals. The synthetic allo- and autotetraploids were descendant from the original material produced by Somaroo (1970).

Plants were selfed by tripping the flower keels with the tip of a toothpick to which a small piece of sandpaper was attached. Self-fertilized plants were protected within a netted cage against contamination by foreign pollen carried by insects. Seed was collected when pods became brown (approximately 35 days after pollination). From each selfed individual, 125 seeds were planted in flats in the greenhouse. Electrophoresis was performed when the plants were approximately 2 months old. A cross was also made between two plants from different accessions of the self-infertile *L. corniculatus*. Flowers of the female parent (554-5) were emasculated by removing the stamens and keels with forceps, and the plants were then sprayed with 10 ppm of 2,4,5-trichlorophenoxy propionic acid to discourage dropping of the injured flowers. Two days after emasculation, pollen from the male parent (Leo-1) was applied to the stigmas of the emasculated flowers. Seed collection was the same as that described above.

Table 2. Expected distribution of progeny genotypes for selfed plants with various levels of independently segregating duplicated digenic loci. F = fast, most anodal, S = slow, most cathodal, M = medium, ⊗ selfed

	Parental genotype		No. of genotypes
	$\frac{F}{S}$ ⊗	1 locus, 2 alleles	3
Ratio	(11)	1 2 1	
Genotype (FS)		20 11 02	
	$\frac{F}{S}\frac{F}{S}$ ⊗	2 independent loci, 2 alleles	5
Ratio	(22)	1 4 6 4 1	
Genotype (FS)		40 31 22 13 04	
	$\frac{F}{S}\frac{F}{S}\frac{F}{S}$ ⊗	3 independent loci, 2 alleles	7
Ratio	(33)	1 6 15 20 15 6 1	
Genotype (FS)		60 51 42 33 24 15 06	
	$\frac{F}{S}\frac{F}{S}\frac{F}{S}\frac{F}{S}$ ⊗	4 independent loci, 2 alleles	9
Ratio	(44)	1 8 28 56 70 56 28 8 1	
Genotype (FS)		80 71 62 53 44 35 26 17 08	
	$\frac{F}{M}\frac{F}{S}$ ⊗	2 independent loci, 3 alleles	9
Ratio	(211)	1 2 1 4 2 1 2 1	
Genotype (FMS)		400 301 202 211 112 220 121 022	
	$\frac{M}{F}\frac{M}{F}\frac{M}{S}\frac{M}{S}$ ⊗	4 independent loci, 3 alleles	25
Ratio	(242)	1 4 4 6 16 6 4 24 24 4 1	
Genotype (FMS)		800 710 701 620 611 602 530 521 512 503 440	
Ratio		16 36 16 1 4 24 24 4 6 16 6 4 4 1	
Genotype (FMS)		431 422 413 404 341 332 323 314 242 233 224 143 134 044	

Table 3. Expected distribution of progeny genotypes for selfed plants with various types of segregating tetrasomic loci. F = fast, most anodal, S = slow, most cathodal, M = medium, ⊗ selfed

	Parental genotype		No. of genotypes
	FFSS ⊗	1 duplex tetrasomic locus, 2 alleles, chromosome segregation	5
Ratio	(22)	1 8 18 8 1	
Genotype (FS)		40 31 22 13 04	
	FFSS ⊗	1 duplex tetrasomic locus, 2 alleles, chromatid segregation	5
Ratio	(22)	9 48 64 48 9	
Genotype (FS)		40 31 22 13 04	
	FFFS ⊗	1 triplex locus, 2 alleles, chromosome segregation	3
Ratio	(31)	1 2 1	
Genotype (FS)		40 31 22	
	FFFS ⊗	1 triplex tetrasomic locus, 2 alleles, chromatid segregation	4 frequent 1 rare
Ratio	(31)	225 360 174 24 1	
Genotype (FS)		40 31 22 13 04	
	FFSS ⊗ FFSS	2 independent duplex tetrasomic loci, 2 alleles, chromosome segregation	9
Ratio	(44)	1 16 100 304 454 304 100 16 1	
Genotype (FS)		80 71 62 53 44 35 26 17 08	
	FFMS ⊗	1 tetrasomic locus, 3 alleles, chromosome segregation	9
Ratio	(211)	1 4 4 4 10 4 4 4 1	
Genotype (FMS)		400 310 301 220 211 202 121 112 022	

Electrophoresis

The horizontal starch gel electrophoresis technique employed has been described previously (Raelson and Grant 1989). Phosphoglucose isomerase (PGI, E.C. 5.3.1.9) was electrophoresed on a LiOH-borate system, pH 8.1/8.4 (Ridgway et al. 1970).

Genetic models

Electrophoretic bands of the polymorphic *Pgi2,3* loci are denoted by their mobility relative to the consistently occurring band of the anodal locus *Pgi1*. An allele of these loci is designated by the relative mobility of the band that is produced when the locus is homozygous for that allele, e.g. *Pgi2-60*. These formal designations are often abbreviated to fast (F) and slow (S) for the more anodal or cathodal allelic bands, when it is obvious to which locus we are referring. A third allele that migrates between fast and slow will be called middle (M). A further abbreviation that is used in the tables and figures is to number the alleles when referring to a genotype. If two alleles are present, the genotype is described by two numbers; when three alleles are present, it is described by three numbers, which denote the number of copies of F, M, S, that are present. For example, the genotype *FFMS* is coded as 211, and the genotype *FSSS* as 13.

The expected electrophoretic phenotypes of individuals with various doses of distinct alleles at duplicated loci have been given previously (Raelson and Grant 1989). In Table 1 are presented Punnett squares, which illustrate the method for calculating the expected numbers of the various phenotypes among progeny of selfed heterozygotes, when independent disomic-digenic inheritance and tetrasomic inheritance are assumed. The essential difference between the two models is that for a tetrasomic locus, any chromosome can pair with any other chromosome, giving a gametic ratio of 1:4:1 (*FF:FS:SS*) for an individual that is duplex (*FFSS*) for two distinct alleles, whereas a given chromosome can only pair with its homologue for duplicated disomic loci, so that the gametic ratio is 1:2:1 (*FF:FS:SS*) (Schulz-Schaefer 1980).

Complications of the tetrasomic model arise for a triplex (*FFFS*) or simplex (*FSSS*) heterozygote and also when the tetrasomic locus is sufficiently distant from the centromere, so that crossing-over results in double reduction and chromatid segregation. A simplex or duplex individual produces only two types of gametes that recombine to give a 1:2:1 (*FFFF:FFFS:FFSS* or *FFSS:FSSS:SSSS*) ratio of progeny phenotypes, which is indistinguishable from the segregation ratio of a single disomic locus. When chromatid tetrasomic segregation is in effect, any chromatid can pair with any other, so that the gametic ratio becomes 3:8:3 (*FF:FS:SS*) for duplex heterozygotes.

When three alleles are present, the pattern of segregation becomes complex. However, the basic distinctions in chromosome pairing between tetrasomic and disomic segregation remain and can be used to construct various models, some of which are presented in Tables 2 and 3.

Examples of the number of different genotypes and their expected frequencies are presented in Table 2 for disomic inheritance. The expected frequencies of each progeny genotype is based upon the assumption of independent unlinked loci. We have already shown that the duplicated *Pgi2* loci in the interspecific diploid hybrid *L. japonicus* × *L. alpinus* do not segregate independently (Raelson and Grant 1989). Linkage will undoubtably give distortions in genotype frequency, generally producing more heterozygous individuals than would be expected under the assumption of non-linkage. The number of progeny genotypes, on the other hand, is not affected by linkage and is, thus, more reliable for testing models.

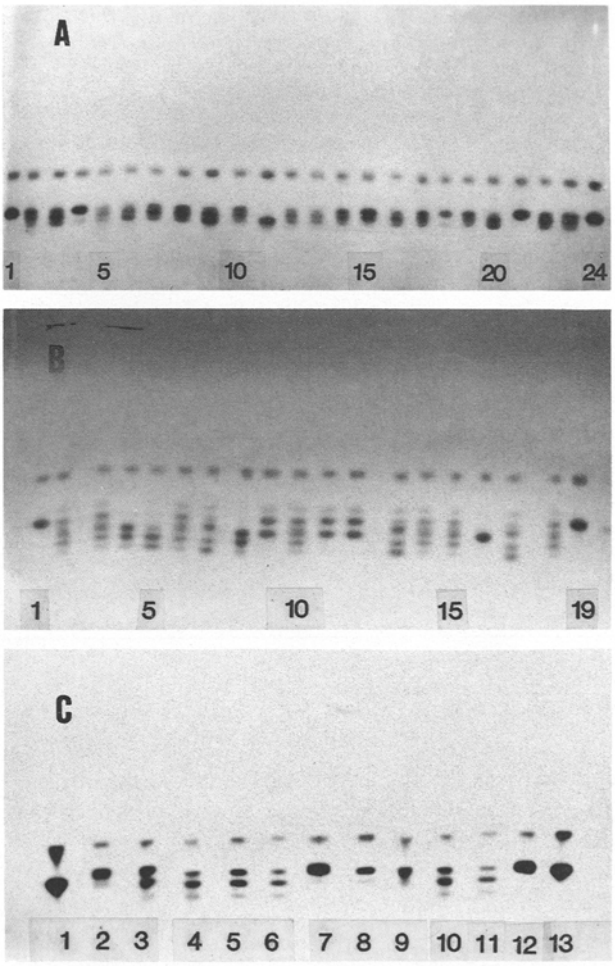

Fig. 1 A–C. Representative zymograms showing phenotypic variation among progeny of various crosses for the polymorphic isozyme *Pgi2,3*. **A** Progeny of selfed synthetic allotetraploid (*L. japonicus* × *L. alpinus*)^2 (28). *Lanes 2* and *23* parental phenotype, *lanes 3–22* progeny phenotypes. **B** Progeny of selfed synthetic autotetraploid (774*x*-5). *Lanes 2* and *18* parental phenotype, *lanes 3–17* progeny phenotypes. **C** Progeny of *L. corniculatus* cross (554-5) × (Leo-1). *Lanes 2* and *12* (Leo-1) phenotype, *lanes 3–11* progeny phenotypes. Individual zymograms do not contain all observed phenotypes

The parental hybrids used in this study had been previously synthesized by former researchers. It was not known whether different heterozygous alleles for different loci were in the *cis* or *trans* orientation in the parent. Because this information was not known, we could not determine the actual linkage intensity between duplicated loci by maximum likelihood analyses such as those described by Tanksley and Rick (1980) and Allard (1956).

Results

Representative zymograms for the cathodal isozymes of PGI are shown in Fig. 1 for the three crosses examined. The individual gels (10–20 of 100 individuals) do not

Table 4. Genetic models for segregation of PGI loci in the artificial allotetraploid (*L. japonicus* × *L. alpinus*)^2. All expected categories less than 5 (underscored) have been lumped with adjacent category for Chi^2 calculation

Phenotype no.	1	2	3	4	5	6	7
Observed	2	8	22	33	26	15	7
Expected for							
Three independent disomic loci	1.8	10.6	26.5	35.3	26.5	10.6	1.8
	Chi^2 4 df = 8.91 NS						
One duplex locus chromosome segregation	5 phenotypes						
One duplex and one triplex locus, chromosome segregation	0.7	7.3	25.7	45.5	25.7	7.3	0.7
	Chi^2 4 df = 28.9**						
Duplex and simplex loci, chromatid segregation	9 phenotypes						
Two duplex loci	9 phenotypes						

NS: Not statistically significant
** Significant at $P < 0.01$

contain all of the phenotypes found among the progeny. The different phenotypes found among all progeny for the various crosses are shown in Fig. 2.

Synthetic allotetraploid (L. japonicus × L. alpinus)^2 (28)

The PGI progeny phenotypes are complex and it is difficult to ascertain an exact genotype for each. However, seven phenotypes were observed among the progeny; the parental phenotype is unbalanced with the upper band being of greater density. These observations suggest the presence of one homozygous locus and three heterozygous loci for this tetraploid individual. Moreover, the observed progeny phenotypic ratio is not significantly different from the genotypic ratio that is predicted by a model for three segregating disomic loci (Table 4). The only tetrasomic model that would provide for seven phenotypes would be the independent segregation of one simplex and one duplex locus. The phenotypic ratios predicted by such a model do not fit the observed values (Table 4). It is of interest to note that the best-fit model provides for quadruplication of *Pgi2* loci, which could result from the chromosome doubling of the duplicated *Pgi2* loci found in the diploid interspecific hybrid *L. japonicus* × *L. alpinus* (Raelson and Grant 1989).

Synthetic autotetraploid (L. alpinus)^2 (774x-5)

The parental phenotype has five bands and is balanced with respect to band density. This fact suggests a genotype with three distinct alleles present and with two doses

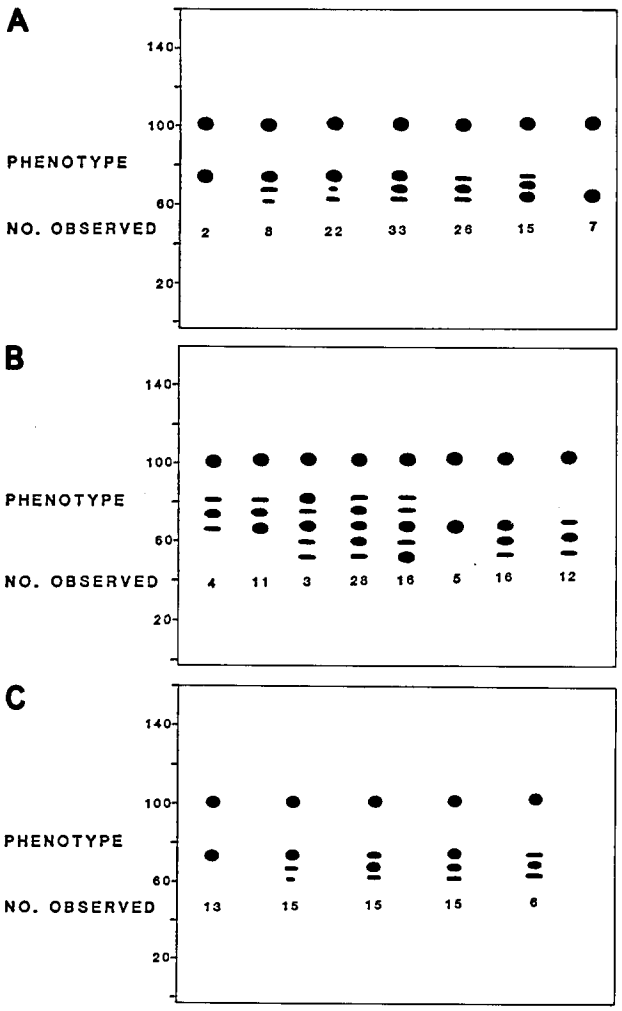

Fig. 2A–C. Observed isoenzyme banding phenotypes for *Pgi2,3* among progeny of various crosses. **A** Progeny of selfed synthetic allotetraploid (*L. japonicus* × *L. alpinus*)^2 (28). **B** Progeny of selfed synthetic autotetraploid (*L. alpinus*)^2 (774*x*-5). **C** Progeny of *L. corniculatus* cross (554-5) × (Leo-1)

of the middle allele (relative mobility 62). The genotype could be (*FMMS*) for a tetrasomic model, or (*F/M M/S*) for a digenic, disomic model. Eight progeny phenotypes were observed which could be produced by the segregation of one tetrasomic locus or two disomic loci, if it is assumed that one of the rarer genotypes (202) did not occur within our sample. Observed and expected frequencies of progeny phenotypes for disomic, digenic and tetrasomic models are shown in Table 5. All Chi-square values were significant, although the model with tetrasomic chromosome segregation provided the best fit to observed data.

It should be noted that the presence of only four allelic doses need be hypothesized to explain segregation in the synthetic autotetraploid, unlike the eight allelic doses needed for segregation in the amphidiploid. These

Table 5. Genetic models for duplicated PGI loci in artificial autotetraploid (*L. alpinus*)[2]. Categories with expected values less than five (underscored) have been grouped together for calculation of Chi^2

Genotype (FMS)	202	130	220	211	121	112	031	022	040	Other
Observed	0	11	4	3	28	16	16	12	5	0
Expected for										
Two independent disomic loci	6.0	11.8	6.0	11.8	23.8	11.8	6.0	11.8	6.0	0
	Chi^2 8 df = 32.24***									
Tetrasomic locus chromosome segregation	2.7	10.5	10.5	10.5	26.6	10.5	10.5	10.5	2.7	0
	Chi^2 7 df = 15.62*									
Tetrasomic locus chromatid segregation	2.2	11.6	9.2	9.7	21.3	9.7	11.6	9.2	4.4	5.3
	Chi^2 8 df = 26.79***									

* Significant at $P < 0.05$
*** Significant at $P < 0.001$

Table 6. Genetic models for segregation of PGI loci in progeny of cross *L. corniculatus* (acc. no. 554-5) × cultivar 'Leo'-1. Categories with expected values less than 5 (underscored) have been grouped together for calculation of Chi^2

554-5 gametes (F M)	40	31	22	13	04
Leo-1 gametes (F M)	40	40	40	40	40
Progeny genotype (F M)	80	71	62	53	44
Observed	13	15	15	15	6
Expected for					
Four independent disomic loci	4	16	24	16	4
	Chi^2 3 df = 18.63***				
Two tetrasomic duplex loci, chromosome segregation	1.8	14.2	32	14.2	1.8
	Chi^2 3 df = 75.00***				
Two tetrasomic duplex loci, chromatid segregation	2.9	15.7	26.8	15.7	2.9
	Chi^2 3 df = 35.29***				

*** Significant at $P < 0.001$

would be produced simply as the result of chromosome duplication of the diploid *L. alpinus* for which only one, nonduplicated *Pgi2* locus has been observed.

Lotus corniculatus L. (554-5) × (Leo-1)

This species is not self-fertile, thus, it was necessary to perform the more difficult task of artificial cross-pollination. Since an outcross was required in any case, the crosses were chosen so that a heterozygote was crossed with a homozygote, the equivalent of the test cross. The test cross is more precise than selfing because segregation is confined to the heterozygote for which the gamete frequency can be directly observed. The phenotypes for PGI for the parents of this cross are shown in Fig. 3.

Five progeny phenotypes were observed from this cross which indicates the presence of five gamete types in

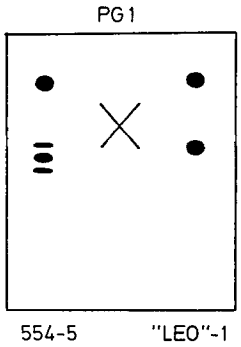

Fig. 3. Parental phenotypes for *L. corniculatus* cross (554-5) × (Leo-1) for PGI

the heterozygote (554-4). This number of gametes is consistent with a model for four segregating disomic loci (*F/M, F/M, F/M, F/M*) or, alternatively, two duplex tetrasomic loci (*FFMM, FFMM*). Results of goodness-of-fit for the alternative models are shown in Table 6. All Chi-square values were significant, indicating lack of fit. Again, linkage among some of the four loci could distort disomic ratios. The model for four independent disomic loci produced the best fit of the various models tested. The quadruplication as opposed to duplication of loci is again noted for *L. corniculatus*.

Summary

The results of the segregation studies are presented in Table 7. The best-fit model for the ratio of progeny phenotypic segregation was only statistically significant for the synthetic allotetraploid (*L. japonicus* × *L. alpinus*)[2] (28). Again, it is emphasized that our digenic/disomic models required the assumption of independence of loci, whereas we have shown the duplicated PGI loci to be linked in the synthetic diploid hybrid *L. japonicus* × *L. alpinus*. Thus, these models provide less reliable data. The indication of quadruplication of loci for the synthetic allotetraploid and for *L. corniculatus*, as opposed to

Table 7. Best-fit models for genetic segregation of *Pgi2,3* loci among progeny of various crosses

Cross	Implied no. of replicated loci	Best-fit model
Selfed (*L. japonicus* × *L. alpinus*)[2]	4	disomic[ns]
Selfed (*L. alpinus*)[2]	2	tetrasomic
(554-5) × (Leo-1) *L. corniculatus*	4	disomic

[ns] Chi-square not significant at the 0.05 probability level

simple duplication (expected from chromosome duplication) that is provided by the number of different progeny phenotypes (as opposed to their ratio of occurrence), is more dependable and logically arises from the presence of the duplication in the diploid hybrid.

Discussion

Duplication of *Pgi2* loci in the diploid interspecific hybrid *L. japonicus* × *L. alpinus* was observed (Raelson and Grant 1989) and it was suggested that this doubling could have resulted from unequal crossing-over between structurally distinct homoeologues. It is interesting that we have also observed evidence for the initial duplication not only in the diploid hybrid (2 *Pgi2* loci), but also for the synthetic amphidiploid (*L. japonicus*)^2 (28) (4 *pgi2* loci) and in tetraploid *L. corniculatus* (4 *Pgi2* loci), while in the synthetic autopolyploid (*L. alpinus*)^2 (774*x*-5), segregation could be explained by a model with only two PGI loci, which would result simply from chromosome duplication. We suggest here that these quadruplications provide evidence that *L. corniculatus* is a segmental allotetraploid, resulting from the duplication of the chromosome number of an interspecific hybrid between two taxa with homoeologous chromosomes, as was first proposed by Stebbins (1950).

In order to develop this argument, it is necessary to recall the various models of autotetraploidy, allotetraploidy, and segmental allotetraploidy. The classical concepts of auto- and allopolyploidy were perhaps best expressed by Darlington (1937). These models are illustrated in Fig. 4A and B. Doubling the homologous chromosomes of a diploid species results in four homologues and a tetrasomic locus in the autotetraploid. Doubling the chromosomes of an interspecific hybrid whose parental species are sufficiently different, so that bivalent formation between structurally heterologous chromosomes is greatly reduced, results in two distinct sets of homologues and fixed heterozygosity in the genomic allotetraploid. Stebbins (1947) elaborated on these classical concepts by introducing the idea of segmental allopolyploidy. A segmental allotetraploid is derived from doubling the chromosomes of an interspecific hybrid whose parents are phylogenetically close, so that their chromosomes retain a high degree of homology, but which may differ by small structural changes (homoeology). Upon chromosome-doubling, such polyploids may display tetrasomic or disomic inheritance, depending on whether pairing is homogenic or heterogenic.

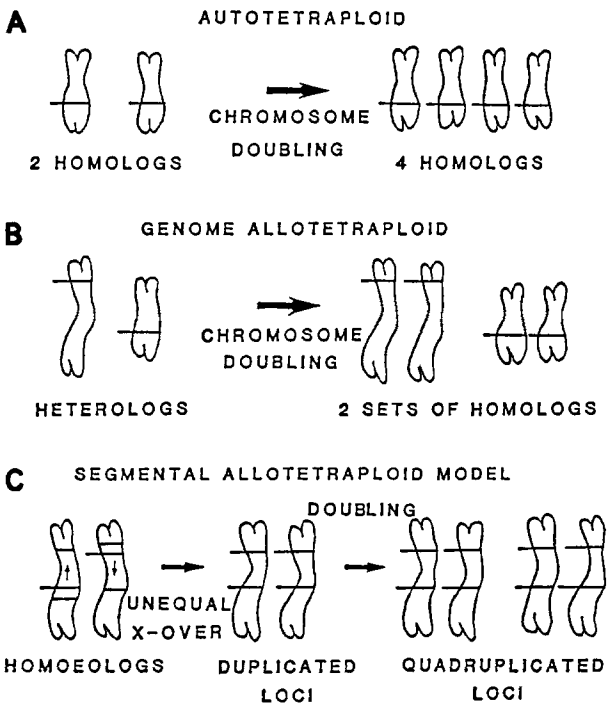

Fig. 4A–C. Models for various types of tetraploidy. **A** The classical concept of autotetraploidy producing a tetrasomic locus. **B** The classical model of allotetraploidy producing duplicated disomic loci. **C** One possible model for segmental allotetraploidy. Chromosomal rearrangement such as a pericentric inversion could lead to displaced loci in homoeologues of diploid hybrid. Unequal crossing-over could result in a duplication deficieny. Deficient chromosomes are inviable so that viable progeny contain homologous duplicated chromosomes. Subsequent doubling would result in eight allelic doses of the locus in an allotetraploid. Genetic factors may determine whether loci segregate in a disomic or tetrasomic manner

An example of one possible mechanism that can explain how duplicated loci in the interspecific hybrid could be associated with segmental allotetraploidy is illustrated in Fig. 4C. Chromosomal rearrangement associated with speciation may have resulted in structural differences between chromosomes of *L. japonicus* and *L. alpinus*. These differences are not enough to prevent pairing of homoeologues as the fertility of the hybrid attests. A pericentric inversion, e.g., would produce duplicated as well as inviable deficient chromosomes. Recombination would produce viable progeny with two duplication chromosomes

Table 8. Meiotic regularity of various taxa used in this study

Taxon	Accession no.	Diakinesis and MI (%)			Anaphase I, II's as			Pollen stainability (%)	Source
		II's	I's	IV's	II's	Bridges	Laggards		
L. alpinus	77	98.38	1.62	0.0	97.58	0.0	3.42	67.19	1[1]
(*L. alpinus*)[2]	774x	74.03	13.68	10.83	66.00	0.0	33.00	44.57	1, 2
L. corniculatus L.	554	91.98	3.93	3.62	87.27	–	12.73	78.81	1, 2
L. japonicus	129	99.02	0.98	0.0	99.64	0.0	0.36	96.43	1
L. japonicus × *L. alpinus*	129 × 77	96.13	2.53	0.93	91.11	3.7	5.19	26.77	1, 2
(*L. japonicus* × *L. alpinus*)[2]	(129 × 77)[2]	81.75	8.83	9.13	54.29	16.43	29.99	44.57	1, 2
L. tenuis	109	99.23	0.77	0.0	96.91	0.0	3.08	82.41	1

[1] Somaroo (1970)
[2] Somaroo and Grant (1971)

(and duplicated *Pgi2* loci) if the gametes with deficient chromosomes were inviable (PGI is a major "housekeeping" enzyme). The doubling of the chromosome number of these individuals would produce the segmental allotetraploid with four loci (either two tetrasomic or four disomic loci). Subsequent diploidization of the allotetraploid would involve genetic factors such as the *Ph* locus in wheat that would lead to bivalent formation and disomic inheritance (Jackson 1982, 1984). This model applies by extension to *L. corniculatus,* since it also displays duplicated *Pgi2* loci, though we do not mean to imply that *L. alpinus* and *L. japonicus* are necessarily the diploid ancestors.

Data concerning the meiotic regularity of various taxa (Somaroo 1970; Somaroo and Grant 1971) are presented in Table 8. The diploid species are all meiotically regular with no multivalents at diakinesis and MI, and with high pollen stainability. The interspecific hybrid has a much lower pollen viability but retains a high level of bivalent formation, which implies a high degree of chromosome homology. Doubling of the chromosome number of the hybrid resulted in increased fertility in the amphidiploid, but also resulted in the formation of more multivalents. In this respect the amphidiploid is similar to the autotetraploid (*L. alpinus*)[2]. Tetrasomic loci within the amphidiploid are not unlikely. *Lotus corniculatus* has a much greater level of pollen stainability and fewer multivalents. This could reflect genetic diploidization since its formation. Therrien and Grant (1984) found the frequency of quadrivalent formation to be less than previously reported for this species, suggesting some selection for increased diploidization had occurred. The meiotic data support the segmental allotetraploid model.

The evidence for eight allelic doses or four loci of *Pgi2* in *L. corniculatus* is interesting with respect to previous reports of tetrasomic inheritance in this species. As mentioned above, Dawson (1941) proposed tetrasomic inheritance of cyanogenesis based upon observations of a 5:1 ratio of cyanogenic to acyanogenic progeny from the putative duplex × nulliplex cross (*AAaa* × *aaaa*). He proposed that the nulliplex represented the acyanogenic genotype. The ratio that Dawson examined, assuming the dominance of cyanogenesis, is analogous to examining the ratio of all other genotypes to either of the homozygous genotypes for the codominant *Pgi2* alleles for the *L. corniculatus* cross (554-5) × Leo-1. These homozygous genotypes are 80 or 08 in Table 8. For two tetrasomic loci undergoing random chromosome segregation, the expected ratio to either homozygote would be 36:1, for four independent disomic loci, the expected ratio would be 16:1, while the ratios with respect to the observed homozygote 80 is essentially (and probably coincidentally) the 5:1 ratio found by Dawson.

Other workers have also reported tetrasomic inheritance for other characters in *L. corniculatus*. Perhaps the most studied of these is the brown versus yellow color of the keel tips. Several studies determined that the character was inherited in a tetrasomic manner based upon the observation of a low frequency of homozygotes to heterozygotes. However, in some crosses the frequency of homozygotes was even lower than that expected from tetrasomic inheritance. Hart and Wilsie (1959) proposed a second locus, besides that controlling keel-tip color, that was lethal in the nulliplex condition to explain the unexpectedly low number of homozygotes. Buzzell and Wilsie (1963) tested this hypothesis and found it to be incorrect. They suggested that meiotic irregularities were the explanation for the lower-than-expected homozygote frequency, as did Bubar and Miri (1965).

It is interesting to note that linkage among disomic loci would be sufficient to explain the distortion of expected ratios if these loci are present in four copies, as is the *Pgi2* locus, rather than in the previously assumed two copies. In this respect, the evidence for quadruplication may answer the objection that tetrasomic inheritance has been established for *L. corniculatus* which has been made to oppose the hypothesis of an allopolyploid origin for this species.

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