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The use of discriminant function analysis to study diploid and tetraploid cytotypes of *Lathyrus pratensis* L. (Fabaceae: Faboideae)

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SUMMARY

Univariate and multivariate analyses of 28 morphological traits were carried out on seven tetraploid and 16 diploid accessions of *Lathyrus pratensis* L. grown under uniform conditions. More than 80% of the plants from diploid populations but less than 50% from the tetraploids flowered the first growing season. Cluster analysis (Wards' method) and principal component analysis did not provide clear differences between these cytotypes. Discriminant function analysis based on ploidy level identified those characters which can provide a reasonably good separation. The results confirmed that *L. pratensis* can still be regarded as a semi-cryptic species with two cytological races in which tetraploids have arisen through autopolyploidy.

Key-words: cytology, experimental taxonomy, fodder legume, morphology, semi-cryptic species, variation.

INTRODUCTION

The genus *Lathyrus* (Fabaceae: Fabaoideae) comprises about 150 species of annual and perennial herbs which are widespread in the temperate regions of Europe, Asia and North America and in tropical East Africa and South America (Kupicha 1983). The most recent taxonomic revision of *Lathyrus* was carried out by Kupicha (1983) who divided the genus into 13 sections. *L. pratensis* is taxonomically situated within the section *Pratensis* Bässler (Bässler 1966; Kupicha 1983). She also included five other species within this section, namely *L. binatus* Pančić, *L. czeczottianus* Bässler, *L. hallersteinii* Baumg., *L. layardii* Ball ex Boiss. and *L. laxiflorus* (Desf.) O. Kuntze *L. pratensis* is found throughout Europe, Asia and Africa (Ball 1968; Davis 1970; Brunsberg 1977).

Following the studies of Larsen (1953, 1954, 1957) several researchers have recorded $2n=2x=14$ or $2n=4x=28$ as chromosome numbers for *L. pratensis* (e.g. Simola 1964; Brunsberg 1965, 1977; Cartier & Blaise 1981; Reynaud *et al.* 1981). The occurrence of triploid and hexaploid cytotypes is apparently rare (Brunsberg 1977). Populations with triploid individuals were recorded however by Simola (1964) in Sweden and Finland and one hexaploid cytotype was found by Brunsberg (1977) in France.

According to Brunsberg (1977) and Cartier & Blaise (1981) tetraploid cytotypes ($2n=4x=28$) are autopolyploid and are distributed in western Europe. Diploids ($2n=2x=14$) are more widespread towards eastern Europe. The cytotypes are sympatric in a zone between France, Belgium, The Netherlands and Italy.

Brunsberg (1977) carried out a biosystematic study within the section *Pratensis*. According to her survey *L. pratensis* can be clearly distinguished from the other five species of the section, but she stated that diploid and tetraploid cytotypes cannot be distinguished from each other on a morphological basis. However, her study was based on univariate analyses of morphological traits and no multivariate analysis techniques were utilized.

In this paper we shall present the results of a study of *L. pratensis* using univariate and multivariate analyses of morphological data in an attempt to understand better the differentiation of diploid and tetraploid cytotypes. Recent reports from Clausen & Crisci (1989), Ingrouille *et al.* (1990) and Moret *et al.* (1991) indicate that multivariate analysis can be used effectively to reveal such differentiation between cytotypes within a single species.

MATERIALS AND METHODS

The 23 *L. pratensis* accessions analysed in this study were obtained from 17 botanical gardens and research institutes in Europe and represent just a sample from the species, which cannot be related to actual natural populations. A list of accessions is given in Table 1.

Cytology

Somatic chromosomes were counted from root tips of each of 10 plants per accession grown in vermiculite. Root tips were pretreated in water at 0°C for 8 h. They were fixed in 9:2:1 absolute ethanol:chloroform:acetic acid for 12 h, hydrolysed in 1 N HCl for 8–9 min at 60°C and stained in 1% acetocarmine for 12 h.

Univariate and multivariate analyses

Between three and 12 plants, depending upon germination from each of the 23 accessions, were grown in a fully randomized block experiment in a glasshouse. A total of 169 plants were studied. Seeds were sown in early February 1988. In early June flowering commenced and 28 traits were recorded (Table 2). Flower characters were recorded on the oldest flower of the oldest inflorescence. Leaf and stipule characters were taken from the node below the oldest inflorescence. Pod and seed characters were recorded on a randomly selected pod from each individual.

The mean, standard deviation and coefficient of variation were determined for each character by ploidy for all accessions. Analyses of variance were also carried out for each trait by ploidy.

Data were standardized prior to all multivariate analyses. Euclidean distance values were established between each individual plant (OTU). Cluster analysis (CA) was carried out using Ward's minimum variance cluster method (Ward 1963). Both principal component analysis (PCA) and CA were accomplished using the CLUSTAN 3 package (Wishart 1987). Discriminant function analyses (DFA) using cluster groups from CA and ploidy level as classification criteria were carried out in order to assess morphological differention between plants in clusters and cytotypes. Both univariate analyses and DFA were carried out using the SPSS 3·1 package (Norusis 1988).

RESULTS

The most obvious difference between diploids and tetraploids was in the time to flowering. More than 80% of the diploid plants produced flowers in the first growing season.

Table 1. *Lathyrus pratensis* accessions used in this study. Numbers of plants which flowered the first growing season and chromosome counts are also given

Access number	Donor	Donor number	Ploidy	Total plants	Flowering plants
1	Reading University	(317)	4x	12	6
2	Oxford Bot. Gard.	(628)	4x	6	3
3	Liege Bot. Gard.	(3358)	2x	11	11
4	Liege Bot. Gard.	(3357)	2x	7	7
5	Liege Bot. Gard.	(3356)	2x	5	1
6	Bordeaux Bot. Gard.	—	4x	3	1
7	Paris Bot. Gard.	(333)	4x	7	3
8	Hamburg Bot. Gard.	(193)	2x	8	8
9	Hamburg Bot. Gard.	(194)	2x	10	4
10	Brussels Bot. Gard.	—	4x	9	5
11	Liege Bot. Gard.	(3355)	2x	5	5
12	Kaunus Bot. Gard.	(1303)	2x	6	6
13	Bremen Bot. Gard.	(362)	2x	11	5
14	Gatersleben Inst.	(14/77)	2x	3	3
15	Prague Bot. Gard.	(224)	2x	6	6
16	Nantes Bot. Gard.	(583)	2x	7	7
17	Berlin Bot. Gard.	(856)	2x	5	5
18	Geneve Bot. Gard.	(1770)	4x	10	4
19	Geneve Bot. Gard.	(755)	4x	6	2
20	Paris Bot. Gard.	(85/215)	2x	8	5
21	Leningrad Bot. Gard.	(1913)	2x	8	8
22	Jena Bot. Gard.	(412)	2x	4	1
23	Dijon Bot. Gard.	—	2x	12	12

Number of diploid plants which flowered = 94.
Number of diploid plants which did not flower = 22.
Number of tetraploid plants which flowered = 24.
Number of tetraploid plants which did not flower = 29.

However, less than 50% of the plants from the tetraploid accessions flowered. Ploidy level and number of plants which flowered per accession are summarized in Table 1. However, one-way analyses of variance showed that between accession variation was significantly greater ($P = 0{\cdot}05$) than within accession variation for 25 of the 28 characters scored (Table 2).

Cluster analysis

The dendrogram obtained after hierarchical classification is shown in Figure 1. Two almost equal clusters were formed at a dissimilarity coefficient of 29·05. However, diploid and tetraploid individuals were not separated into different clusters. Analysis of the traits responsible for separation between these two clusters was carried out by means of DFA. As none of the characters had very high Pearson's correlation coefficients (the traits with the highest correlation were style length and petal standard length with a correlation coefficient of 0·75), it was assumed that there was no indirect weighting in the CA. Results from this DFA are shown in Table 3. Wilk's lambda values indicated that there were significant differences between cluster means for only 12 traits. Cluster 1 was formed by small, narrow-leaved plants with short petioles and narrow stipules. They tended also to

Table 2. Morphological characters of *Lathyrus pratensis*, including flowering time and between-accession y within-accession variances of 28 characters studied

Character code	Character	Variance: Within accessions (22 d.f.)	Variance: Between accessions (95 d.f.)	*F*-ratio
PHEIGH	Plant height	1703	998	**
LLENGT	Leaflet length	0·930	0·448	***
LWIDTH	Leaflet width	0·082	0·022	***
PLENGT	Petiole length	0·723	0·392	***
STLENG	Stipule length	0·121	0·127	NS
STWIDT	Stipule width	0·033	0·022	NS
NUTEND	Number of tendrils	1·198	0·745	NS
FLTIME	Number of days between sowing and flowering	232	137	**
FNUMBE	Number of flowers per inflorescence	5·070	2·980	**
FLENGT	Flower length	0·023	0·008	***
FPETLE	Peduncle length	19·350	6·300	***
SLENGT	Standard petal length	0·045	0·012	***
SWIDTH	Standard petal width	0·026	0·012	***
OLENGT	Ovary length	0·002	0·001	**
STYLEN	Style length	0·011	0·004	***
ONUMBE	Number of ovules	6·810	1·290	***
CATET1	Uppermost calyx tooth length	0·006	0·001	***
CATET2	Distance between the uppermost calyx teeth	0·005	0·002	***
CABRET	Lowest calyx tooth length	0·002	0·001	**
NVEINS	Number of violet veins on upper part of standard petal	42	13·4	***
POLENG	Pod length	0·487	0·122	***
POWIDT	Pod width	0·006	0·002	***
SEEDLE	Seed length	0·004	0·001	***
SEEDBR	Seed width	0·002	0·001	**
SEEDWI	Seed breadth	0·002	0·001	**
LL&LW	Leaf length/leaf width ratio	3·050	1·130	***
STL&W	Stipule length/stipule width ratio	1·252	0·626	**
SL&SW	Standard petal length/standard petal width ratio	0·020	0·011	**

NS = Not significant; ** = significant at 5% level; *** = significant at 1% level.

have short leaves and standard petals. When results from CA were considered together with accession identity, it was found first, that only rarely did individuals from the same accession group in the same cluster, and secondly that there was no clear differentiation between accessions.

Principal component analysis

Results from PCA are summarized in Table 4. The first eight components had eigenvalues greater than one and accounted for almost 70% of the total variance. On factor 1 were the characters mainly related with leaf and stipule size such as petiole length, stipule width, leaf length and leaf width. The second factor was mostly concerned with floral characters, namely petal standard length, style length, flower length, ovary length. A scatter diagram

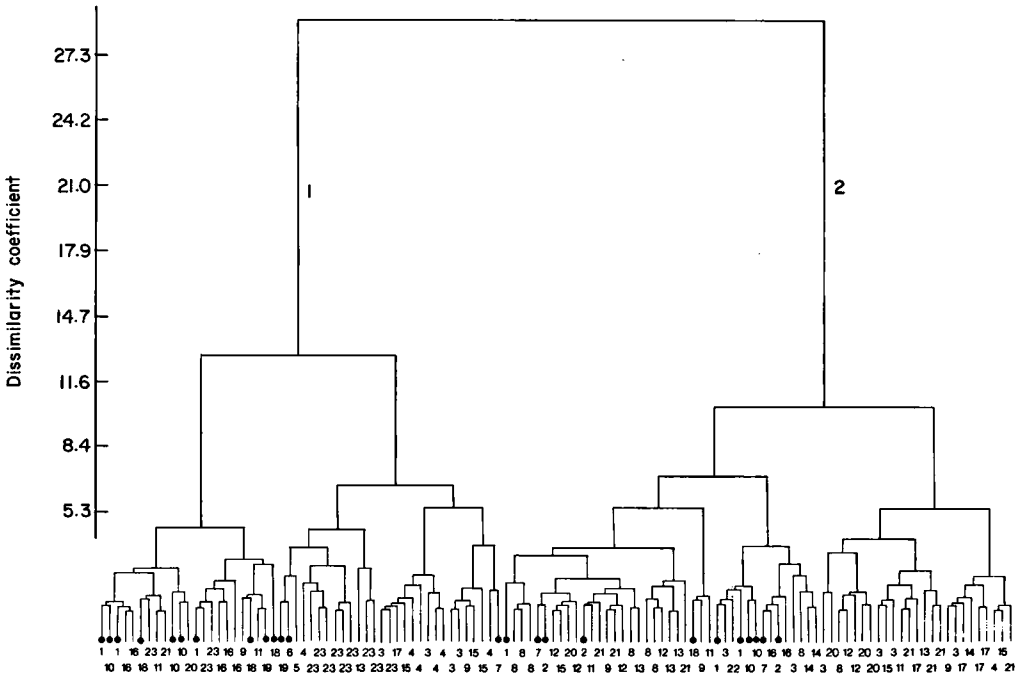

Fig. 1. The dendrogram from cluster analysis (Ward's method) of 118 diploid and tetraploid plants of *Lathyrus pratensis*. Tetraploid individuals are indicated by closed circles. Labels refer to accession number.

for the first two components is illustrated in Figure 2. Individuals which grouped together in Cluster 1 from the hierarchical classification also tended to appear in close proximity on the left region of this diagram. Their patterns of distribution were in agreement with results from CA as OTUs from Cluster 1 had low values along the first component. There was a considerable overlapping between scores for diploids and tetraploids along the first two factors. However, tetraploids seldom had negative values on both components.

Discriminant function analysis by ploidy

The only multivariate technique which enabled a better separation between diploids and tetraploids was DFA. Using ploidy level as a classification criterion of the morphological data, DFA differentiated individuals with a common chromosome number. The correct classification of plants into their two 'cytotype groups' was almost 90% (Table 5). There was a slight degree of overlapping between the frequency diagrams of cytotypes along the discriminant function (Fig. 3). Traits that separated the cytotypes along the discriminant function are given in Table 6. They were mainly flower characters, such as style length, standard petal length, flower length and standard petal length:standard petal width ratio. However, when the total range of variation of the seven traits which contributed the most to the separation was drawn in bar diagrams (Fig. 4), there were no sharp discontinuities between the two cytotypes, even though the Wilk's lambda values had indicated that there were statistically significant differences between these characters. It was only when DFA was carried out that morphological differences were identified.

This trend was also confirmed for these seven characters in the one-way analyses of variance by ploidy. Simple statistical analyses by ploidy of the morphological characters

Table 3. Wilk's lambda values and correlation coefficients between original and discriminant variables in a discriminant function analysis using morphology cluster membership as a classification criterion. Mean cluster values, with coefficient of variation in brackets are also given

Character	Wilk's lambda	Correlation coefficient	Cluster statistics	
			Cluster 1	Cluster 2
PLENGT**	0·619	0·435	1·77 (27·1)	2·60 (21·9)
STWIDT**	0·631	0·424	0·41 (26·8)	0·65 (21·5)
LWIDTH**	0·689	0·372	0·56 (23·2)	0·79 (21·5)
PHEIGH**	0·726	0·343	85·90 (33·2)	121·20 (23·8)
LLENGT**	0·756	0·315	2·74 (23·3)	3·47 (18·4)
STLENG**	0·759	0·313	1·51 (21·2)	1·86 (16·1)
FNUMBE**	0·768	0·313	7 (21·1)	8 (21·3)
ONUMBE**	0·800	0·277	10 (14·5)	11 (11·8)
POLENG**	0·826	0·254	2·96 (13·5)	3·32 (12·1)
FPETLE**	0·837	0·244	7·03 (40·2)	9·43 (27·8)
STL&W**	0·852	−0·232	3·83 (27·4)	3·17 (15·8)
NUTEND**	0·878	0·207	1 (65·0)	2 (63·0)
FLENGT*	0·913	0·172	1·23 (8·1)	1·29 (7·8)
CATET2*	0·918	0·166	0·32 (15·6)	0·35 (14·3)
STYLEN*	0·919	0·164	0·88 (6·8)	0·92 (7·6)
SLENGT*	0·928	0·154	1·46 (8·9)	1·54 (8·4)
SWIDTH*	0·932	0·151	1·06 (10·4)	1·13 (9·7)
POWIDT	0·949	0·127	0·54 (9·2)	0·56 (10·7)
CATET1	0·938	0·116	0·24 (20·8)	0·26 (15·1)
LL&LW	0·962	−0·111	5·32 (25·7)	4·85 (21·6)
SEEDLE	0·972	−0·094	0·31 (12·9)	0·29 (10·3)
FLTIME	0·977	−0·084	22 (60·0)	20 (55·0)
OLENGT	0·978	0·082	0·45 (6·7)	0·46 (6·5)
SEEDBR	0·979	−0·079	0·22 (9·1)	0·21 (14·2)
SEEDWI	0·996	−0·033	0·27 (11·1)	0·27 (7·4)
SL&SW	0·997	−0·031	1·38 (8·7)	1·37 (8·1)
NVEINS	0·998	−0·025	8 (66·8)	8 (41·7)
CABRET	0·999	−0·006	0·22 (13·6)	0·22 (13·6)

**Wilk's lambda values significant at 1% level.
*Wilk's lambda values significant at 5% level.

are also given in Table 6. Coefficients of variation ranged between 6·7% for style and stipule length and 59% for flowering time. Each cytotype had similar coefficients of variation for each single character. Nine of the characters had a coefficient of variation greater than 25%, a fact that indicated that the accessions were rather variable for almost one third of the analysed traits.

DISCUSSION

Using both PCA and CA, which attempt to identify distinct groups independently of any prior classification criterion, it was not possible to identify distinct morphological subgroups which were related either with accessions or with ploidy level. This situation was improved however when DFA was used. Nevertheless, this method relies on prior classification criteria. Even so, in the case of *L. pratensis* a clear separation between diploids and

Table 4. Six characters which had the highest absolute eigenvector values along the first two factors in a principal component analysis of 28 characters in 23 accessions of *Lathyrus pratensis*

	Factor 1		Factor 2	
	PLENGT	0·75	SLENGT	0·65
	STWIDT	0·71	STYLEN	0·60
	LLENGT	0·64	FLENGT	0·56
	LWIDTH	0·62	OLENGT	0·54
	PHEIGH	0·61	SWIDTH	0·52
	FPETLE	0·61	STWIDT	−0·52
Eigenvalue		5·70		3·40
Percentage variance		20·30		12·10

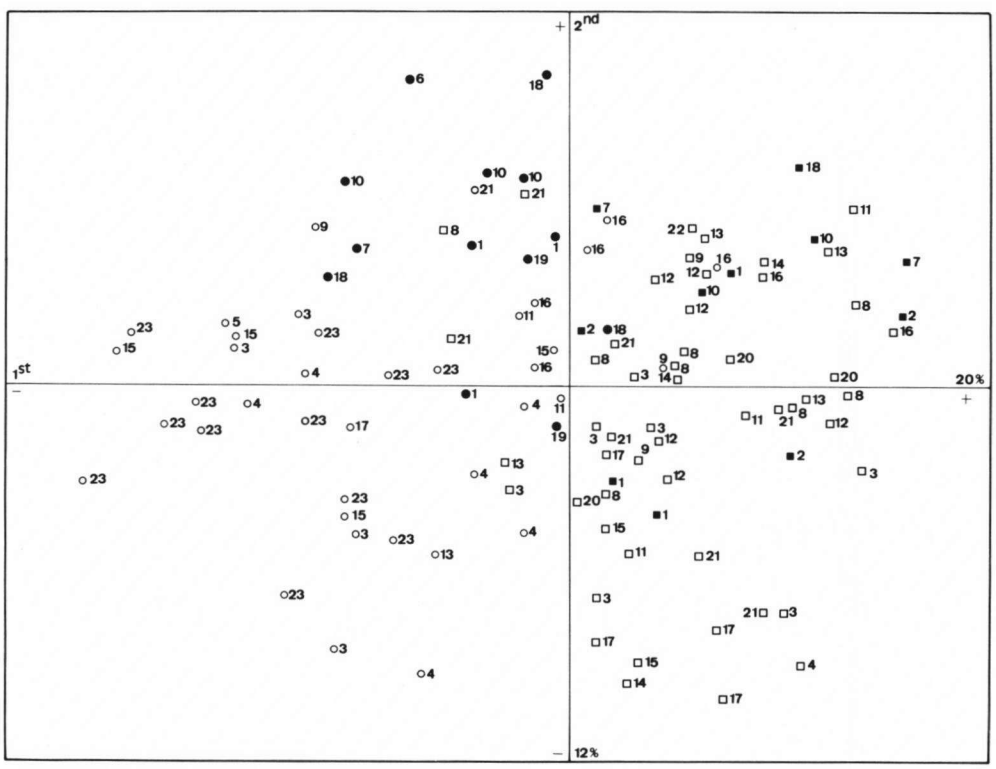

Fig. 2. Scatter diagram of 118 individuals (OTUs) of 23 populations of *Lathyrus pratensis* along the first two factors of principal component analysis. Tetraploid individuals are indicated by closed symbols. OTUs which grouped in morphology cluster 1 are indicated with circles. Squares refer to OTUs which grouped in morphology cluster 2.

tetraploids is only obtained if chromosome counts are made before carrying out DFA. The other univariate and multivariate techniques utilized in this paper did not have this

Table 5. Classification results after discriminant function analysis using ploidy level of individuals of *Lathyrus pratensis* as a classification criterion

Actual cytotype	Number of cases	Predicted cytotype membership	
		Diploid	Tetraploid
Diploid	94	82 87·2%	12 12·8%
Tetraploid	24	0 0%	24 100%

89·3% of individuals were correctly classified.

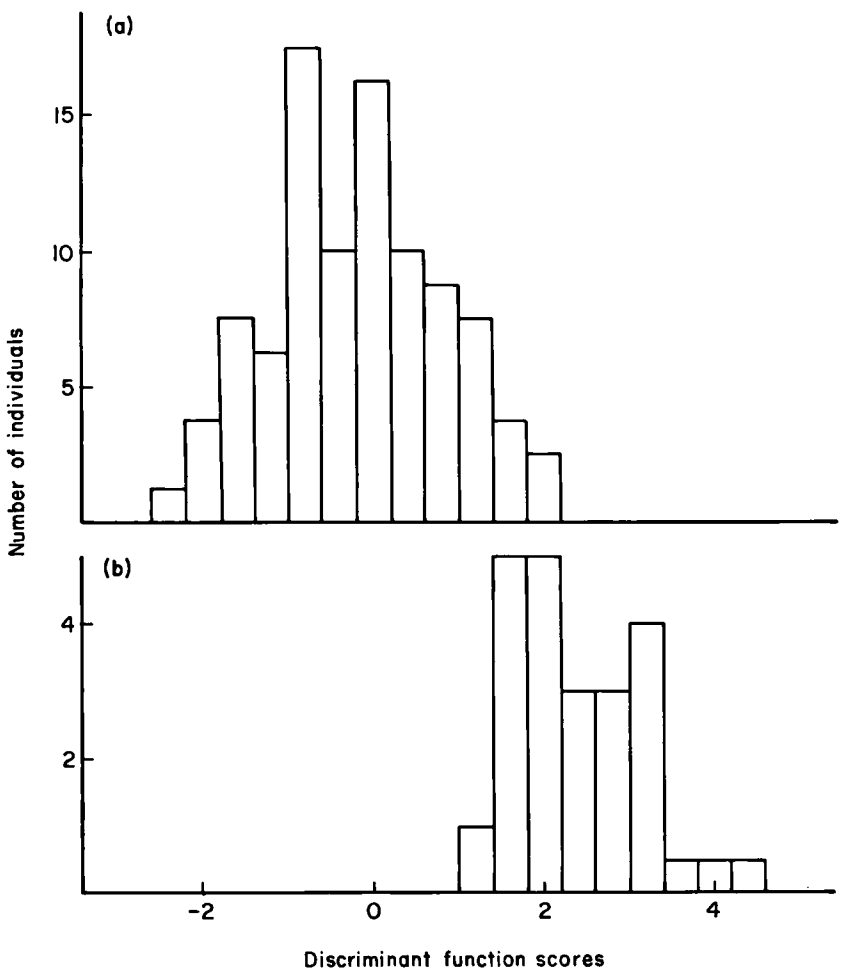

Fig. 3. Frequency diagram from discriminant function analysis using ploidy level as a classification criterion. (a): Diploid individuals; (b): Tetraploid individuals.

Table 6. Wilk's lambda values and correlation coefficients between original and discriminant variables in a discriminant function analysis using ploidy level as a classification criterion. Mean values, with coefficient of variation in brackets are given for each cytotype

Character	Wilk's lambda	Correlation coefficient	Cytotype statistics	
			Diploid	Tetraploid
STYLEN**	0·86	0·38	0·89 (7·6)	0·96 (6·7)
SLENGT**	0·87	0·37	1·48 (6·7)	1·60 (7·2)
FLENGT**	0·90	0·31	1·24 (7·9)	1·33 (7·2)
SL&SW**	0·91	0·30	1·35 (7·9)	1·44 (8·4)
SEEDBR**	0·92	0·28	0·21 (12·5)	0·23 (13·9)
POLENG**	0·92	−0·28	3·22 (13·4)	2·93 (12·6)
CABRET**	0·94	0·23	0·22 (14·8)	0·23 (16·5)
CATET1*	0·95	0·22	0·24 (18·6)	0·27 (20·7)
SEEDWI*	0·96	0·21	0·26 (10·3)	0·27 (8·0)
FPETLE*	0·96	0·19	8·08 (35·9)	9·40 (31·1)
SEEDLE*	0·96	0·18	0·31 (12·4)	0·32 (12·4)
PLENGT	0·97	−0·16	2·29 (23·0)	2·05 (28·7)
OLENGT	0·98	0·14	0·45 (7·8)	0·46 (6·2)
CATET2	0·98	0·13	0·33 (16·4)	0·34 (13·1)
LWIDTH	0·98	−0·13	0·66 (28·3)	0·60 (27·3)
LL&LW	0·98	0·13	4·98 (24·2)	5·37 (23·5)
POWIDT	0·98	0·11	0·55 (10·9)	0·56 (8·1)
NVEINS	0·99	−0·09	9 (51·7)	8 (45·8)
STL&W	0·99	0·07	3·43 (24·5)	3·59 (26·5)
SWIDTH	0·99	0·06	1·10 (11·1)	1·12 (9·8)
STWIDT	1·00	−0·06	0·52 (29·7)	0·50 (32·3)
PHEIGH	1·00	−0·05	106·60 (32·8)	101·90 (27·7)
FNUMBE	1·00	−0·04	8 (24·0)	8 (23·4)
ONUMBE	1·00	0·04	11 (14·0)	11 (14·0)
STLENG	1·00	−0·03	1·71 (21·2)	1·68 (19·9)
LLENGT	1·00	−0·03	3·16 (24·0)	3·11 (20·0)
NUTEND	1·00	−0·02	2 (47·0)	2 (50·0)
FLTIME	1·00	0·01	21 (56·0)	22 (59·0)

**Wilk's lambda values significant at 1% level.
*Wilk's lambda values significant at 5% level.

a priori weighting and they did not give such clear differences between the cytotypes. However, there is a tendency for tetraploid plants to be different from diploids in some flower, seed and pod traits. This trend was amplified by the DFA, as this multivariate technique maximizes separation between groups and identifies those characters of discriminant value.

The lack of agreement between PCA and CA with DFA appears to confirm previous reports from Abbott *et al.* (1985) about the value of these three numeric methods in taxonomy. These authors reported that, in some instances, DFA is the most appropriate technique for analysing patterns of variation at or below the species level. At these two levels, classification is often difficult to achieve as there is considerable overlapping for each single character. DFA overcomes this obstacle by minimizing the overlapping among previously designated groups. In this present case, DFA provides a reduced one-dimensional model which identifies measurable differences between cytotypes. However,

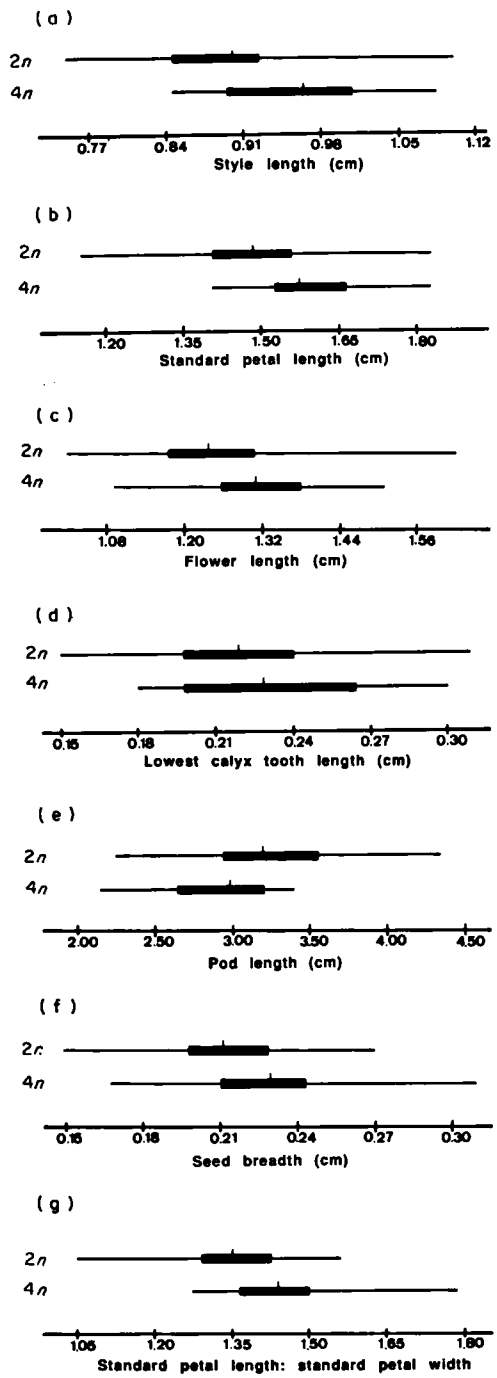

Fig. 4. Bar diagrams for the seven traits which had the highest correlation coefficients between original and discriminant variables in a discriminant function analysis using ploidy levels as a classification criterion. The median is marked. The heavy bar represents the values between the first and third quartiles. ($2n$) = diploid and ($4n$) = tetraploid cytotypes. (a) Style length; (b) standard petal length; (c) flower length; (d) lowest calyx tooth length; (e) pod length; (f) seed breadth; (g) standard petal length/standard petal width ratio.

morphological differentiation between these cytotypes was not so sharp as to yield two distinct groups when the other models were utilized. As we found considerable overlapping for each of the morphological traits in diploid and tetraploid cytotypes, and as only DFA could provide some criteria to distinguish them, it is not appropriate to assign formal taxonomic rank to them.

The evolutionary significance of polyploidy in this species may be related to several physiological traits. Relationships between physiology and chromosome numbers in *L. pratensis* were also suggested by Toulemonde & Vartanian (1986), who found that diploids had stomatal regulatory mechanisms which allowed them to withstand dehydration during a drought period whereas tetraploids lacked these mechanisms. Brunsberg (1977) indicated that flowering shoots were not usually observed until the second vegetative year. Results from this paper do not entirely coincide with her observations as there was some relationship between the production of flowers during the first vegetative period and ploidy level. This relationship could be another example of how polyploidy in this species has led to differences which are physiological rather than morphological.

The fact that there are similar patterns of morphological variation in both cytotypes suggests that tetraploids can be regarded as having an autopolyploid origin, and that *L. pratensis* should be regarded as a semi-cryptic species.

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