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Genomic constancy during the development of *Lathyrus odoratus* cultivars

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The sweet pea, *Lathyrus odoratus*, has a well-documented history of the development of its cultivars in various parts of the world. Measurements of genome size, using flow cytometry, and observations on the karyotypes, as well as the analysis of meiotic pairing of F_1 hybrids (between the wild sweet pea and two modern cultivars), all show that this development has not been accompanied by changes in karyotype or genome size. However, the cultivars do differ in pollen size and guard cell chloroplast number, both characteristics that have been suggested to be controlled by the nucleotype; however, this is clearly not the case in *L. odoratus*.

Keywords: cultivars, flow cytometry, genomic constancy, *Lathyrus odoratus*.

Introduction

The differences between cultivated plants and their wild ancestors are usually striking and come about through selection for specific characters and as correlated responses to this selection. Many different character changes have been catalogued and include obvious features such as an increase in the size of specific organs, loss of dispersal mechanisms and changes in the breeding system (Pickersgill & Heiser, 1976). In addition there can be cryptic changes such as the alteration of chromosome structure and number. To some extent, however, this sort of change is constrained by the mode of reproduction of the crop; vegetative reproduction facilitates evolution via chromosome changes, such as aneuploidy, that reduce fertility. In *Hyacinthus orientalis* Darlington *et al.* (1949) were able to show a progressive increase in chromosome number with time and a similar story has been described by Brandham (1986) in *Narcissus* subgenus *Narcissus*. In both these exaples there is an increase in chromosome number/genome size correlated with the development of new cultivars. Nevertheless, as Brandham (1986) points out, other genera with many similar features (e.g. *Tulipa*) have not followed this pattern. An alternative mechanism for increasing genome size without a change in chromosome number has been found in North American populations of *Zea mays*. Both Laurie & Bennett (1985) and Rayburn *et al* (1989) have found a significant variation in nuclear DNA content, of the order of 38 per cent, without any change in chromosome number.

L. odoratus has a limited natural distribution in Sicily and Sardinia (Dicks, 1900). It is known to have been introduced into cultivation in England in 1699 and to Holland at about the same time. Variation in the cultivated sweet pea has arisen entirely by gene mutation and subsequent hybridization within the species (Crane & Lawrence, 1938). Mutations that affect flower colour, flower shape, plant habit and time of flowering have occurred, giving rise to many distinct cultivars.

These mutations have been developed into a very large numer of cultivars in diverse parts of the world, such as Britain, U.S.A. and Australia. In the different areas selection has been for different characteristics yielding the huge diversity in cultivars available today.

Feulgen microdensitometry has been the most frequently used technique to measure genome size in plants but in recent years there has been an increase in the use of flow cytometry together with fluorescent dyes such as propidium iodide, mythramicin and DAPI (4′,6-diamidino-2-phenylindole), for this purpose. This has followed the publication of a simple protocol showing that plant nuclei, isolated by mechanical chopping of plant tissue, can readily be used for the flow cytometric determination of plant genome size (Galbraith *et al.*, 1983). These techniques allow the rapid and accurate determination of plant nuclear DNA amounts based on a large number of nuclei (usually 10,000). There appear to be only two reports of the use of flow cytometry to measure intraspecific variation in nuclear DNA amount (Rayburn *et al.*, 1989; Rayurn & Auger, 1990). Both of these involve

Zea mays and show that the technique can readily be used to detect this sort of variation. Within the genus *Lathyrus* there have been several reports of large interspecific differences in DNA content (see Rees & Hazarika, 1969 and Narayan, 1982). In addition, Sharma & Datta (1959) and Roy & Singh (1967), for example, have reported considerable karyotype variation within the cultigen *L. odoratus* including large differences in total chromosome length.

Many changes in plant cell size have been attributed to the nucleotypic as opposed to the genotypic effects of DNA amount (Bennett, 1972, 1987) including pollen size and chloroplast number in guard cells. In many genera these parameters have been shown to be correlated with increases in genome size (Butterfass, 1979) but a causal relationship has not been demonstrated. Recently several observations have led us to question such a causal relationship as we have found that different chromosomes have specific effects on guard cell chloroplast number in the genus *Cyphomandra* (Standring *et al.*, 1990). Trisomic addition lines of *C. betacea* were found to have chloroplast numbers that ranged from the value of the diploid through to that of induced triploids, despite the fact that the different chromosomes that were added were of very similar size. In *Lolium perenne*, Francis *et al.* (1990) have shown that colchicine can induce heritable changes in cell size and chloroplast number per cell without any alteration to chromosome number. Thus care must be taken with the use of these sorts of characters to predict differences in genome size in plants.

As part of a wider study of the genus *Lathyrus*, it was seen that the well-recorded history of the development of the sweet pea and the large number of cultivars available, offered the opportunity to investigate the cytological changes associated with the development of the cultivars.

Materials and methods

The cultivars used in this study were selected from a much larger collection of authenticated cultivars so as to provide representative genotypes developed at different times and in different places (Table 1).

The methods for preparing karyotypes, meiotic spreads and the production of hybrids are outlined in Murray & Hammett (1989). Nucleolar organizer regions were identified by silver staining of air-dried chromosome preparations (Geber & Schweizer, 1988) following the staining schedule of Hizume *et al.* (1980).

To measure nuclear DNA amounts, isolated nuclei were prepared by chopping leaves with razor blades (Galbraith *et al.*, 1983) in the extraction buffer of de Laat & Blaas (1984) modified by the substitution of 10 mM sodium dithionite for dithiothreitol as this was found to be more effective in controlling oxidative processes. Leaf material (0.1 g) was used with 3 ml of the extraction buffer. The chopped material was filtered through 63- and 16-μm nylon mesh filters and stained with DAPI (Sigma) to give a final stain concentration of 10 μg/ml. Measurements were made on a Becton-Dickinson FACS440 flow cytometer equipped with a Coherent Innova Argon laser using a 488 nm laser line operating at 275 mW. Ten thousand nuclei were measured from each sample and each cultivar was examined on at least two separate occasions. In order to calibrate the measurements from the flow cytometer and minimize variation between runs, two standards [*Triticum aestivum* cv. Chinese Spring and *Hordeum vulgare* cv. Sultan with 34.63 pg and 11.12 pg DNA per 2C nucleus respectively (Bennett, *et al.*, 1982)] were included along with the wild type *L. odoratus* ('Original') with each replicate. A typical graph for one of the *L. odoratus* cultivars is shown in Fig. 1. The coefficient of variation (cv) of the 2C peak, calculated using the formula of Thornthwaite *et al.* (1980) is 0.69 per cent and in our material the cv was typically less than 1 per cent and never more than 2 per cent.

Chroloplast numbers were counted in 40 guard cells from the lower epidermis of fully expanded leaves. A preliminary study showed that the variation in chloroplast number in different mature leaves along a stem was not significant when analysed by ANOVA ($F = 4.982$, $0.0001 < P \leq 0.005$). Epidermal strips were mounted in a 1 per cent aqueous solution of silver nitrate and the chloroplasts stain brown after a short period of time. The polar diameter of 30 pollen grains per cultivar was measured after mounting fresh pollen in cotton blue in lacto-phenol (Darlington & LaCour, 1976).

Results

The karyotypes of the 20 cultivars examined all appear similar and consist of one pair of small metacentric chromosomes, one pair of large sub-metacentric chromosomes and five pairs of acrocentric chromosomes. Secondary constrictions were not observed in any of the chromosomes but the sites of the nucleolar organizer region have been established, using silver staining, as terminal on two of the acrocentric pairs. In the F_1 hybrids between 'Original' and the two modern cultivars 'Hunters Moon' and 'Gloria', there were no obvious differences in the size of homologous chromosomes. The meiotic behaviour of both these hybrids was completely regular. At pachytene it was not possible to trace all the chromosomes in any one nucleus,

Table 1 List of cultivars of *L. odoratus* used in this paper with their origin, number of chloroplasts/guard cell, pollen diameter, 2C nuclear DNA amount and whether they have been karyotyped

Number	Cultivar	Introduction	G/S	W/S/L	Origin	Chloroplast number	Pollen diameter (μm)	2C DNA content (pg)	Karyotype
1	Original	1699*	G	L	Med	9.11	82.90	15.93	+
2	Chiltern Matucana	C18?	G	L	SA∞	8.55			
3	Maishman Matucana	C18?	G	L	SA∞	9.15	90.00		
4	Painted Lady	C18?	G	S	SA∞	8.63			
5	Quito	C18?	G	L	SA∞	9.05		16.04	+
6	Lady Serena James	1868	G	L	B	8.03			
7	Pink Cupid	1895	G	S	USA				+
8	Prima Donna	1896	G	L	B	8.48			
9	America	1898	G	L	USA	9.65	87.40	15.93	+
10	Miss Willmott	1901	G	L	B	10.43			+
11	Dorothy Eckford	1903	G	L	B	9.15	94.80	15.54	
12	Janet Scott	1903	G	L	B	9.40			
13	King Edward VII	1903	G	L	B	10.08	89.40		
14	Lord Nelson	1907	G	L	B	8.95			
15	Mrs Collier	1907	G	L	B	9.70			
16	American Beauty	1920s?	S	W	USA	10.70			
17	Fantasy	1931	S	L	B	10.03			
18	Mrs Douglas MacArthur	1940s	S	W	USA	9.60		16.43	
19	Gaiety	1946	S	L	B	9.00	91.80		
20	Pixie	1952	S	L	B	10.05	85.10		
21	Coquette	1953	S	L	B	11.30			
22	Jester	1953	S	L	B	11.23		16.16	+
23	Twink	1953	S	L	B	9.68			
24	Ink Splash	1956	S	L	B	9.83	85.60		+
25	Chigasaki #11	1960s	S	W	J	10.10	85.70	15.93	
26	Diana	1960s	S	W	USA	9.28		16.19	
27	Gloria	1960s	S	W	USA	10.63	89.10	15.70	
28	Marilyn	1960s	S	W	USA	12.48		15.94	
29	Ballerina	1960s?	S	W	A	9.78		15.96	+
30	Beverly Kay	1960s?	S	W	A	9.70			
31	Christine Martin	1960s?	S	W	A	10.68			+
32	Fragrance	1960s?	S	W	A	10.85			+
33	Piralilla Cream	1960s?	S	W	A				+
34	White Gem	1960s?	S	W	A	10.36	83.30		+
35	Herald	1962	S	L	B	10.30			
36	Gipsy Queen	1965	S	L	B	12.15			
37	Hunters' Moon	1968	S	L	B	11.20	86.30	16.21	
38	Apricot Queen	1972	S	L	B	12.10		15.97	+
39	Southbourne	1972	S	L	B	10.00			+
40	Eva Bridger	1978	S	L	NZ				+
41	Mrs B. Jones	1978	S	L	B	10.38			+
42	Karen Reeve	1980	S	L	B	10.75			
43	Lady Diana	1981	S	L	B	11.30			+
44	North Shore	1982	S	L	B				+
45	Mammoth Lavender	1982	S	S	USA	10.35			
46	Mammoth Mid Blue	1982	S	S	USA	10.40		16.49	+
47	Mammoth Salmon Pink	1982	S	S	USA	9.98			+
48	Bridgewater Boy	1984	S	L	B	10.95			
49	Duchess of Roxburghe	1984	S	L	B	11.55			
50	Anniversary	1985	S	L	B	11.03			
51	Just William	1988	S	L	B	11.90	88.90	16.36	
52	E8B	•	S	S	NZ	9.95	91.80		

Table 1 *Continued*

Number	Cultivar	Introduction	G/S	W/S/L	Origin	Chloroplast number	Pollen diameter (μm)	2C DNA content (pg)	Karyotype
53	Purple Flake	•	S	L	NZ	9.15	81.40	16.32	
54	E8B × Gloria F_1		S	W	NZ		87.40		
55	Gloria × Original F_1		G	L	NZ	8.38		16.10	
56	Original × Hunters' Moon F_1		G	L	NZ			15.82	

* Introduced to Britain 1699, but natural species.
? Date of introduction to decade or century.
• Recent hybrid involving ancestral and more recent cultivars.
G Grandiflora flower form with clamped keel.
S Spencer flower form with open keel.
W Winter flowering.
S Spring flowering.
L Late or summer flowering.
A Australia.
B Britain.
J Japan, but selection from USA cultivars.
Med Mediterranean.
NZ New Zealand.
SA∞ South America. These cultivars are purportedly early variants of the original species and may have been taken to South American by Spanish colonists.
USA United States of America.

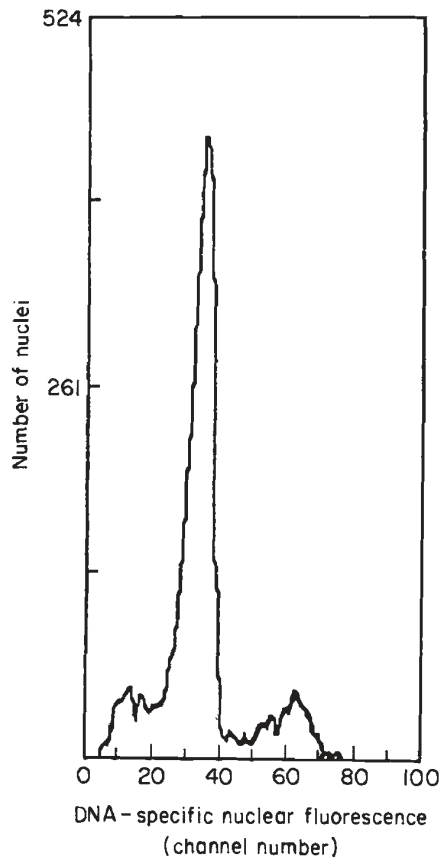

Fig. 1. Flow cytometric analysis of the fluorescence of DAPI stained nuclei from leaves of *L. odoratus* cv. Quito

but where the chromosomes could be clearly seen there was no sign of buckles, unpaired regions or any other irregularity (Fig. 2a). At metaphase I seven bivalents were formed in all cells and their structure appeared completely normal (Fig. 2b).

Figure 1 shows the flow cytometric analysis of leaf nuclei from the cultivar 'Quito'. There are clear 2C and 4C peaks, the latter being at double the channel number of the former. The majority of nuclei are at the 2C stage and typically 7–8 per cent of the nuclei are found in the 2C peak channel. The mean value for all the cultivars is 16.11 pg/2C nucleus with a range of 15.54 to 16.43 (Table 1). The variation between runs for any one cultivar was usually small and less than 5 per cent. The measurements of nuclear DNA amounts confirm the above observations on the karyotypes with no differences being observed between the cultivars.

Unlike the measurements of DNA amount, those of pollen diameter showed considerable variation between cultivars (Table 1) ranging from 81.4 μm in 'Purple Flake' to 94.8 μm in 'Dorothy Eckford'. An analysis of variance shows that the differences between cultivars are highly significant ($F = 50.52$, $P \leq 0.0001$), however the correlation between pollen size and date of first release or appearance of the cultivars is not significant. It is interesting that the hybrid between 'E8B' and 'Gloria' has a smaller pollen diameter than either of its parents. There is also considerable variation in guard cell chloroplast number between cult-

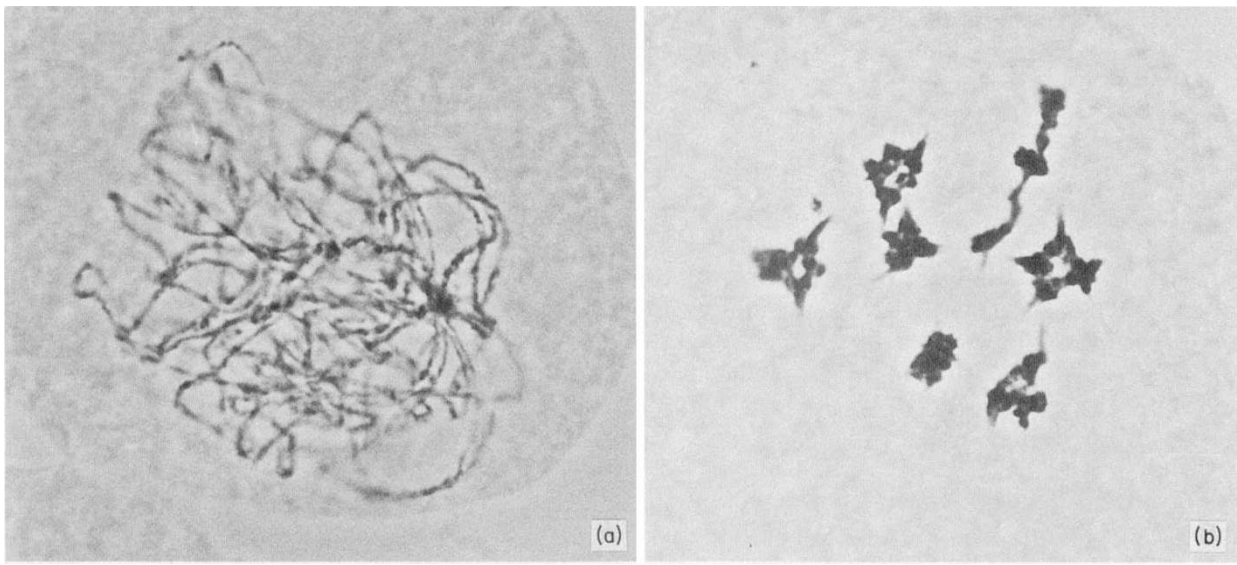

Fig. 2 Meiosis in the F_1 hybrid between *L. odoratus* cv. Gloria and cv. Original, (a) pachytene and (b) metaphase I.

ivars, with the lowest value (8.03) being found in 'Lady Serena James' and the highest (12.48) in 'Marilyn'. Unlike the pollen size variation, there is a clearer pattern to the variation in guard cell chloroplast number in relation to the chronological age of the cultivars. In that the development of the sweet pea has essentially taken place since 1868, it is most relevant to restrict the comparison of chloroplast number with the date of release or first appearance of the cultivar to this time frame. The correlation between these two parameters is positive and significant ($n = 42$, $r = 0.647$, $P < 0.001$).

Discussion

Cultivated plants provide a useful resource to observe evolutionary change over a short period of time, since their history, as is the case in *L. odoratus*, is often well documented. In *L. odoratus* there have been striking changes in plant morphology, particularly in the size, shape and colour of flowers but we have not found any evidence of changes in chromosome structure or genome size associated with this diversity of floral form. Our observations do not support those of Sharma & Datta (1959) who claimed large differences in total chromosome length between a range of cultivars. These differences appear difficult to reconcile. Of their 14 cultivars, two, 'Mariner' and 'Oriental', had larger chromosome complements than the others, and in addition they were recorded as having 'deep blue' and 'deep primrose' coloured flowers respectively (Sharma & Datta, 1959). Although long desired, these colours have not been recorded in *L. odoratus* cultivars (Lawrence, 1939). Sharma & Datta (1959) also reported that their cultivars had a range of different karyotypes with eight different types of chromosome with secondary constrictions. Our cultivars show no secondary constrictions as the nucleolar organizer regions are located at or very near to the ends of their chromosomes, however, we have found a wide range of NOR chromosome types in a study of almost 50 other *Lathyrus* species (B. G. Murray and K. R. W. Hammett, unpublished data). Thus, in the absence of voucher herbarium specimens, there must be doubt as to the identity of at least some of the material studied by Sharma & Datta (1959).

Roy & Singh (1967) compared the karyotypes of an unnamed cultivar from India with that of another unnamed cultivar from Britain. They claim that the Indian and British cultivars are karyotypically similar although they do differ in chromosome size. In addition the Indian material is reported to be very similar in karyotype to *L. sativus*, a blue-flowered species. We have examined several accessions of *L. sativus* all of which are clearly different to our *L. odoratus* cultivars. As our range of cultivars does not include any from India, we cannot exclude the possibility that those cultivars represent a separate line of development that is karyotypically different. We have, however, examined a wide range of cultivars from Europe, North America, Australia and New Zealand and have demonstrated that they are karyotypically very similar.

Our results indicate that the modern sweet pea is a species that has, in cultivation, been modified to show considerable morphological and physiological variation without any change in chromosome structure or genome size. In this respect *L. odoratus* differs greatly from *Hyacinthus* (Darlington *et al.*, 1949) and *Narcissus* (Brandham, 1986), which show clear

patterns of increasing chromosome number with the development of new cultivars or from *Zea mays*, which had developed with an accompanying amplification of its genome without a change in chromosome number (Rayburn & Auger, 1990). Differences in the breeding system can, at least in part, explain the difference in cultivar evolution between *Hyacinthus*, etc., and *Lathyrus* in that the former group is perennial and frequently propagated vegetatively whereas *Lathyrus* is annual and seed propagated. It does not, however, offer any explanation for the difference from *Zea* and the karyotype stability in *L. odoratus* is also interesting in the light of recent evidence for rapid genomic change at the molecular level in plants (Walbot & Cullis, 1985; Dover, 1988; John & Miklos, 1988). It remains to be seen how much change at the molecular level has occurred during the short period of development of this species as a crop.

Despite the uniformity of karyotype and nuclear DNA amount in these sweet pea cultivars, they do differ in several other cellular parameters such as pollen size and guard cell chloroplast number. It has been suggested that both of these parameters are under nucleotypic control (Bennett, 1972; Butterfass, 1973) and therefore should not vary between cultivars with uniform karyotypes. The observation that they do vary, clearly suggests that this relationship cannot hold for all cases and there are probably several inter-related control factors. For example, Francis *et al.* (1990) have shown that colchicine-treated plants of *Lolium perenne*, which remain diploid after treatment, can show heritable changes in both cell size and chloroplast number. Furthermore, John & Miklos (1988) point out the example of the moss, *Bryum caespiticium*, which underwent a 50 per cent decrease in cell volume in an 11-year period following a doubling of its chromosome number, thus illustrating that cell size can alter quite independently of genome size. We have also found that primary trisomics of *Cyphomandra betacea* can have very different guard cell chloroplast numbers (Standring *et al.*, 1990). Some trisomics have chloroplast numbers that are not significantly different from the diploid whereas others are not significantly different from the autotriploid, clearly showing that there can be chromosome-specific effects on cellular parameters such as chloroplast number.

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