

Natural Variation of Chromosome Length and a Critical Assessment of the Karyotype in Sweet Pea

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That the different horticultural strains of sweet pea (*Lathyrus odoratus* Linn.) have evolved through structural alteration of chromosomes and they represent different chromosomal biotypes have become evident by applying improved techniques (Sharma and Datta 1959). Reports of the natural occurrence of variation of length and diameter of chromosomes (Baetcke *et al.* 1967, Bennett and Rees 1969, Bennett 1970) necessitate a more critical and careful judgement on the analysis of karyotype. The present investigation is with a few more strains of sweet pea with special care and attention on natural variations in chromosome lengths.

Material and methods

Pure horticultural strains of *Lathyrus odoratus* L. were collected from Sutton and Sons Ltd., Calcutta and grown in the University garden in a mixture of sand and earth. Root tips for somatic study were pretreated with aesculin (Sharma and Sarkar 1955) and fixed, dyed and smeared in the aceto-orcein technique. Chromosomes were drawn with camera lucida at a magnification of 3000. Because the chromosomes of each strain vary considerably in length and proportion of segments between constrictions, even within the plates of the same root-tip, 10 plates from each strain were drawn. Measurements of the segments of each type of chromosomes of each strain from 10 plates were statistically analysed. Mean values of 20 chromosomes of each type have been considered for karyotype analysis.

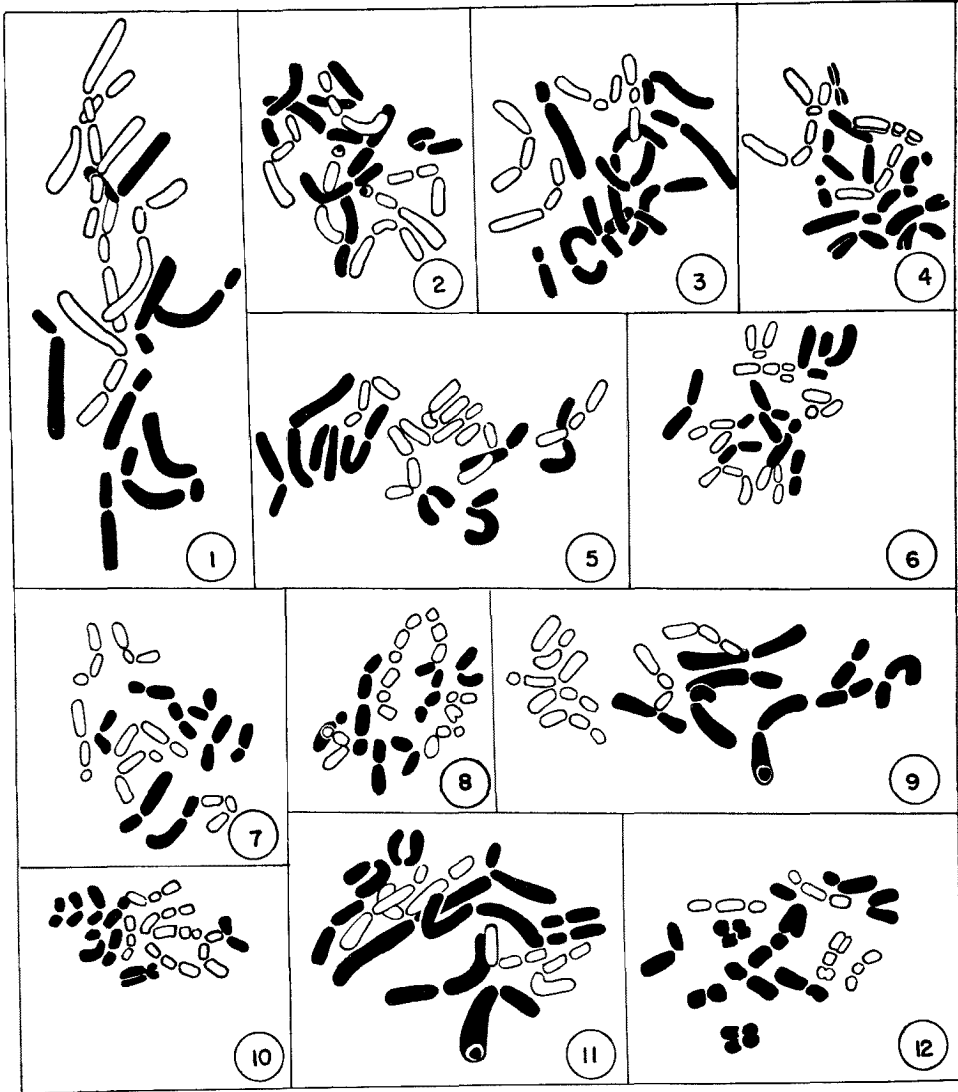
Results and conclusions

The chromosomes of *L. odoratus* is in general quite long (2-12 μ). Somatic metaphase plates of each strain show considerable variation in chromosome length and in the proportions of arms or segments between constrictions (Figs. 1-12). For this reason, 10 clear plates of each strain were drawn with camera lucida.

In Fig. 13, each narrow line represent the average length of each chromosome pair (of a particular chromosome type) found in a single plate. The broad lines represent the mean values of lengths. Fig. 14 shows all chromosome types encountered in the present karyological study. Following are the types:

A—With two constrictions forming three segments, one terminal remarkably long, middle slightly shorter than the other terminal. Mean lengths of segments, 3.4: 1.6: 1.8 μ .

B—With two constrictions forming three segments, one terminal remarkably long, middle slightly longer than the other terminal. Mean lengths of segments, 3.2: 2.1: 1.8 μ .



Figs. 1-12. Camera lucida drawings ($\times 3000$) of the somatic metaphase plates from different strains of *L. odoratus* Linn. 1 and 2, "Black Prince"; 3 and 4, "Emperor"; 5 and 6, "Grenadier"; 7 and 8, "Madonna"; 9 and 10, "Orange King"; 11 and 12, "Othello". Plates show variation in chromosome size and in the proportion of chromosome segment length.

C—With two constrictions forming three segments, the two terminal ones subequal, middle distinctly shorter. Mean lengths of segments 2.8: 1.2: 2.0 μ .

D—With only a subterminal primary constriction, one segment considerably shorter than half of the other. Mean lengths of segments, 3.9: 1.6 μ .

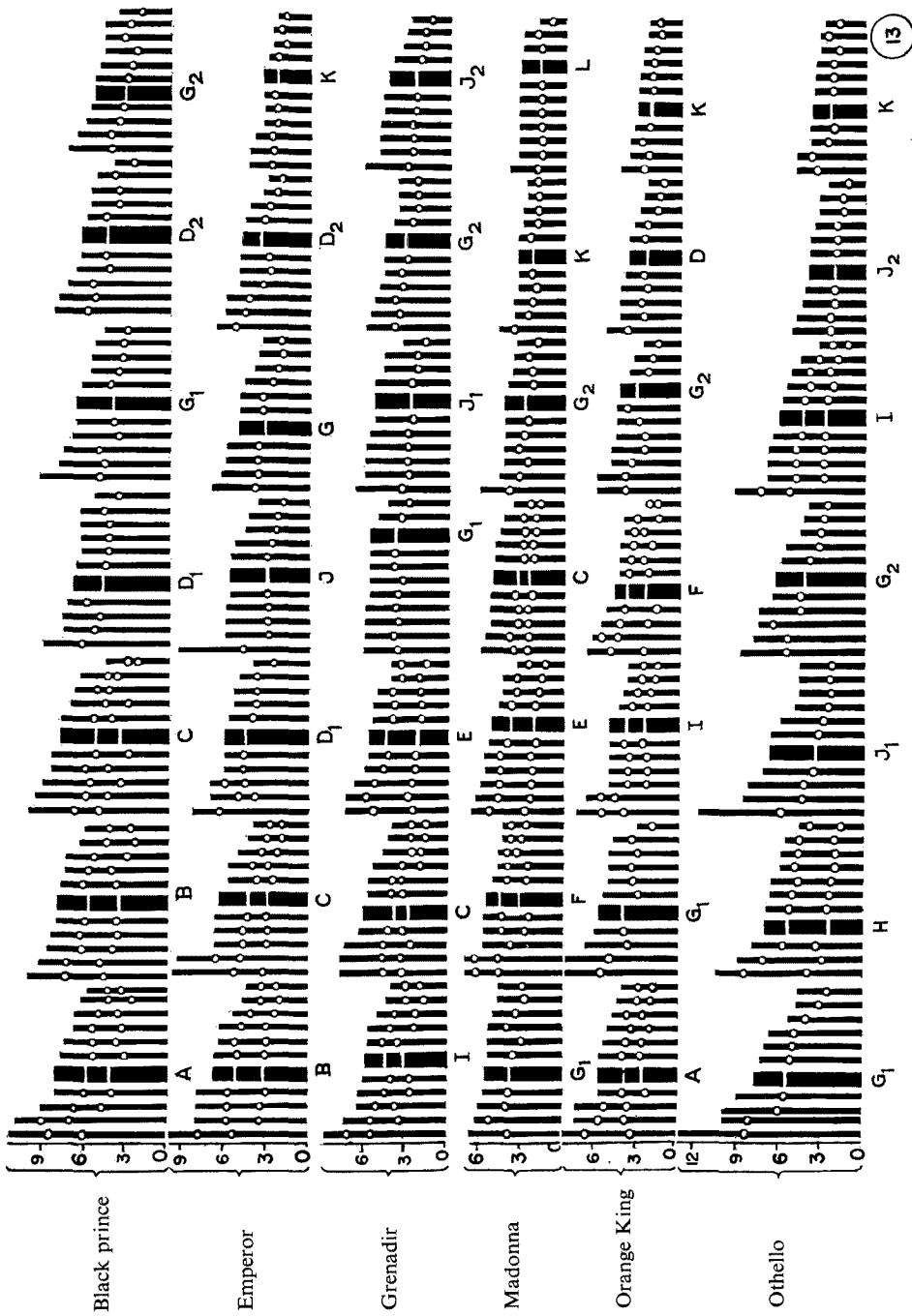


Fig. 13. Idiogram showing variation of chromosome length and of segmental proportion of different types of chromosomes found in 10 plates drawn (narrow lines) from each strain and a representation of the mean (broad line) for each type in each strain.

- E—With two constrictions forming three segments, two adjacent longer and subequal, other terminal short. Mean lengths of segments, 2.5: 2.4: 1.1 μ .
- F—With two constrictions forming three segments, one terminal distinctly longer and the other distinctly shorter than the middle. Mean lengths of segments, 2.8: 1.3: 0.9 μ .

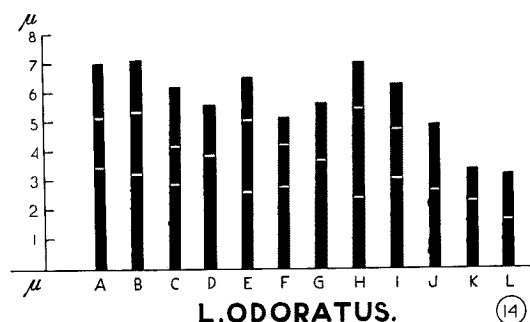


Fig. 14. Chromosome types observed altogether in different strains of *Lathyrus odoratus*.

- G—With only a primary constriction forming two segments, one longer than half of the other. Mean lengths of segments, 3.6: 1.9 μ .
- H—With two constrictions forming three segments, middle longest, one terminal distinctly shorter than the other terminal; 2.5: 2.9: 1.6 μ .
- I—With two constrictions forming three segments, two adjacent shorter and equal or almost equal, the other (one terminal) distinctly longer. Mean length of segments, 3.0: 1.7: 1.6 μ .
- J—With an almost median primary constriction. Mean lengths of arms, 2.5: 2.4 μ .
- K—Similar to G, but distinctly shorter. Mean lengths of arms, 2.3: 1.0 μ .
- L—Similar to J, but distinctly shorter. Mean lengths of arms, 1.6: 1.5 μ .

On this basis the salient features of the karyotype study of the strains of *L. odoratus* may be represented in Table 1.

Table 1. Brief representation of the karyotype analysis

Strains	Black Prince	Emperor	Grenadier	Madonna	Orange King	Othello
Chromosome types	2A, 2B, 2C, 4D, 4G	2B, 2C, 4D, 2G, 2J, 2K	2C, 2E, 4G, 2I, 4J	2C, 2E, 2F, 4G, 2K, 2L	2A, 2D, 2F, 4G, 2I, 2K	4G, 2H, 2I, 4J, 2K
Total chromosome length (n), μ . (mean value)	48.9	38.1	37.4	31.9	32.7	42.2
Position of primary constriction	2M, 10SM, 2ST	4M, 6SM, 4ST	6M, 8SM	2M, 10SM, 2ST	2M, 10SM, 2ST	4M, 10SM
No. of chromosomes with secondary constrictions (2n)	6	4	6	6	6	4
Range of chromosome length, μ .	3.5–11.5	2.0–10.0	2.5–9.0	2.0–7.0	2.0–9.0	3.0–14.0

M = Median, SM = Submedian, ST = Subterminal.

Table 2. Mean Lengths of chromosomes and segments

Type	Strains		Emperor	Grenadier	Madonna	Orange King	Othello
	Black Prince	Segment					
A	S. ter.	2.07 (± 0.15)				1.60 (± 0.09)	
	Mi.	1.80 (± 0.13)				1.52 (± 0.22)	
	L. ter.	4.13 (± 0.25)				2.70 (± 0.24)	
	Total	8.05 (± 0.60)				5.82 (± 0.45)	
B	S. ter.	2.03 (± 0.10)	1.73 (± 0.17)				
	Mi.	2.23 (± 0.11)	2.00 (± 0.13)				
	L. ter.	3.67 (± 0.26)	3.07 (± 0.28)				
	Total	7.93 (± 0.40)	6.80 (± 0.55)				
C	S. ter.	2.57 (± 0.26)	2.12 (± 0.27)	2.12 (± 0.18)	1.80 (± 0.32)		
	Mi.	1.47 (± 0.20)	1.30 (± 0.13)	1.10 (± 0.13)	0.90 (± 0.05)		
	L. ter.	3.73 (± 0.25)	2.93 (± 0.27)	2.67 (± 0.23)	2.33 (± 0.11)		
	Total	7.77 (± 0.51)	6.36 (± 0.60)	5.90 (± 0.43)	5.03 (± 0.21)		
D	S. A.	(2.03 (± 0.42))	(1.53 (± 0.10))			1.30 (± 0.10)	
		(1.87 (± 0.15))	(1.47 (± 0.11))			2.43 (± 0.24)	
	L. A.	(4.87 (± 0.25))	(4.43 (± 0.35))			3.73 (± 0.28)	
	Total	(4.47 (± 0.32))	(3.43 (± 0.32))				
E	S. ter.	(6.90 (± 0.32))	(5.96 (± 0.38))	1.27 (± 0.12)	1.13 (± 0.08)		
	Mi.	(6.34 (± 0.41))	(4.90 (± 0.36))	2.17 (± 0.15)	2.03 (± 0.13)		
	L. ter.			2.27 (± 0.12)	2.06 (± 0.12)		
	Total			5.71 (± 0.37)	5.22 (± 0.43)		
F	S. ter.				0.87 (± 0.08)	0.93 (± 0.12)	
	Mi.				1.30 (± 0.04)	1.42 (± 0.18)	
	L. ter.				3.23 (± 0.26)	2.40 (± 0.27)	
	Total				5.40 (± 0.31)	4.75 (± 0.39)	
G	S. A.	2.63 (± 0.26)	2.03 (± 0.16)	1.93 (± 0.10)	1.80 (± 0.15)	1.97 (± 0.23)	2.50 (± 0.41)
		2.17 (± 0.15)		1.53 (± 0.08)	1.43 (± 0.10)	1.60 (± 0.16)	2.00 (± 0.24)

Mean lengths in μ of chromosome types

Table 2 (Contd.)

Type	Strains		Black Prince	Emperor	Grenadier	Madonna	Orange King	Othello
	Segment							
L.	L. A.		4.00 (± 0.22)	3.07 (± 0.23)	3.67 (± 0.11)	3.70 (± 0.24)	3.80 (± 0.33)	5.33 (± 0.58)
			3.20 (± 0.24)		3.10 (± 0.25)	2.83 (± 0.18)	2.97 (± 0.26)	4.37 (± 0.42)
	Total		6.63 (± 0.44)	5.10 (± 0.38)	5.60 (± 0.17)	5.50 (± 0.21)	5.77 (± 0.54)	7.88 (± 0.90)
H	S. ter.		5.37 (± 0.20)		4.63 (± 0.33)	4.26 (± 0.23)	4.57 (± 0.34)	6.37 (± 0.54)
	Mi.							1.63 (± 0.12)
	L. ter.							2.90 (± 0.26)
I	Total							2.53 (± 0.24)
	S. ter.							7.06 (± 0.58)
	Mi.				1.50 (± 0.11)		1.17 (± 0.07)	1.70 (± 0.11)
	L. ter.				1.47 (± 0.10)		1.20 (± 0.07)	1.70 (± 0.11)
	Total				2.97 (± 0.33)		2.60 (± 0.30)	2.83 (± 0.32)
	S. A.			2.77 (± 0.25)	5.94 (± 0.43)		4.97 (± 0.38)	6.23 (± 0.51)
J	S. A.				2.67 (± 0.14)			3.40 (± 0.37)
	L. A.				2.10 (± 0.16)			1.97 (± 0.12)
	Total				2.67 (± 0.16)			3.43 (± 0.37)
K	S. A.				2.23 (± 0.17)			2.06 (± 0.13)
	L. A.				5.34 (± 0.31)			6.83 (± 0.75)
	Total				4.33 (± 0.33)			4.03 (± 0.26)
L	S. A.					0.97 (± 0.07)	1.00 (± 0.09)	1.27 (± 0.09)
	L. A.					2.37 (± 0.16)	2.10 (± 0.15)	2.60 (± 0.18)
	Total					3.34 (± 0.18)	3.10 (± 0.20)	3.87 (± 0.21)
L	S. A.					1.53 (± 0.10)		
	L. A.					1.67 (± 0.08)		
	Total					3.20 (± 0.50)		

Mean lengths in μ of chromosome types

Abbreviations: S. ter. = Shorter terminal; L. ter. = Longer terminal; S. A. = Shorter arm; L. A. = Longer arm; Mi. = Middle segment.

Table 2 represents the mean lengths of each chromosome and segments in each strain. The standard errors for each segment and total lengths of chromosome types have been shown in parentheses after each mean value. For the great variability of lengths of chromosome and their segments of such long chromosomes, calculation of such mean values appears necessary for obtaining correct values of the lengths.

Such changes in ratios and lengths of arms may be due to difference in spiraling and condensation of chromonemata in different plates. Gershoy (1934) found chromosomes in vacuolated cells and flower bud tissues of "violets" to be smaller than in root tip cells. Baetcke *et al.* (1967) found large difference in volume of root and shoot chromosomes in 30 plant species. Bennett and Rees (1969) found similar variation in different ages of plants. Bennett (1970) found similar volume difference in primary and lateral root meristem. Therefore, the other possibility is the occurrence of structural difference in relation to tissue environment or tissue differentiation. Further study is required for testing this possibility.

Correlation between chromosome volume and DNA amount may be positive (Rees *et al.* 1966, Rees and Jones 1968, Fox 1969), or the DNA amount may be constant and independent of the changes in chromosome volume (Bennett 1970). Differential hydration due to change in cell cycle time (Lyndon 1968), particularly of G₁ (Avanzi and Deri 1969) may be an important factor. Growth rate, cell cycle time, nuclear RNA and total nuclear protein content have been related to change in chromosome size (Bennett 1970). All these facts may be related to tissue environment. But the variation of the segmental length ratio of chromosomes is a more difficult question to answer.

From the mean values obtained, it is apparent that the chromosome types of strains are distinct in morphology and length. "Othello" can be isolated from other strains by the absence of chromosome types A to F. "Orange King" can be differentiated from other strains by the absence of B, C, E, J and L. Particularly C is common in all the remaining strains. "Grenadier" and "Madonna" have both C and E. (D absent), while "Black Prince" and "Emperor" have B, C and D (E absent). Type A is common in "Black Prince" and "Orange King" only. These differences have come about by structural alteration of chromosomes by fragmental interchange, deletion, etc., which has no doubt a role in evolution.

Chromosomes are considerably longer in "Black Prince" and "Othello" among all. "Othello" seems to be least evolved for its median and submedian primary constrictions and only two pairs of secondary constrictions. In "Black Prince", subterminal centromere and higher number of secondary constrictions are found. "Emperor" and "Grenadier" show comparatively reduced chromosome length, latter having higher number of secondary constrictions. The shortest chromosomes are found in "Madonna" and "Orange King," both having high number of secondary constrictions, generally subterminal centromeres. So they appear to be the most evolved strains.

Summary

Chromosomes being long, vary considerably within different metaphase plates,

in length and proportion of arms or segments. Therefore mean values of segmental lengths of 20 chromosomes of each type of each strain has been considered in preparing the idiogram. Occurrence of a large number of chromosomal biotypes of *L. odoratus*, each having distinct chromosome morphology, suggests an important role of structural alteration by deletion, translocation, etc., in the evolution of different strains.

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