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DISCONTINUOUS DNA VARIATION IN THE EVOLUTION OF PLANT SPECIES: THE GENUS *LATHYRUS*

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The genus *Lathyrus* is closely related to *Vicia* and belongs to the family Leguminosae. Most species in this genus are diploids with $2n = 14$. The divergence and evolution within this genus is accompanied by a three-fold increase in chromosome size which is directly correlated with a four-fold increase in their nuclear DNA amounts. The fraction of DNA responsible for the variation in total amounts of DNA in this genus consists of repetitive and non-repetitive DNA in a consistent ratio of 4.1 to 1.0. Quantitative comparisons of the total DNA and of DNA constituents show discontinuity in the pattern of variation among the species of this genus. The constraints upon the composition of DNA among species and the discontinuity in the pattern of variation would suggest genome evolution by a succession of spasmodic changes rather than by a continuous progression. This investigation was carried out with two major objectives in mind, 1) to determine the nature of DNA variation accompanying the evolution of this genus and 2) to compare among species the relative changes in nuclear components which are closely correlated with DNA changes. Multivariate analysis was used to classify the data.

MATERIALS AND METHODS

The 21 diploid species investigated were *L. miniatis, L. angulatus, L. articulatus, L. nissolia, L. maritimus, L. clymenum, L. ochrus, L. setifolius, L. aphaca, L. cicera, L. sphaericus, L. pratensis, L. annus, L. sativus, L. odoratus, L. tuberosus, L. hirsutus, L. tingitanus, L. sylvestris, L. latifolius,* and *L. vestitis*. Seeds collected from at least two geographical regions were available for each species. The characters selected were as follows.

1. Chromosome Volume.—Chromosome volumes were measured using the method suggested by Hazarika (1969). Excised root tips from the germinating seeds were treated with 0.1% colchicine for 3 h and then fixed for 4 h in 1:3 acetic alcohol. The root tips were stained with Feulgen and then squashed out in acetocarmine. The lengths of the chromosomes and the width of the chromatids were measured at C-mitosis, at the stage just prior to the separation of the chromatids. Measurements were made under oil-immersion lens using the Vickers instrument eye piece attachment with a moving scale which gave a high degree of accuracy. The chromosomes in each cell were measured individually for length and five chromatids in each

cell taken at random to get the mean chromatid width. Volume was then calculated from total chromatid length (2× chromosome length) and average chromatid width assuming the chromatids to be cylindrical in form. The mean chromosome volumes were based on at least five cells from different plants.

2. Total Nuclear DNA.—Total amount of nuclear DNA was measured using Feulgen photometry. Root meristems from germinating seeds were fixed and Feulgen stained using the method first suggested by McLeish and Sunderland (1961). Steps involved in this method have been modified to minimize experimental error as reported by Teoh and Rees (1976). The DNA measurements were made on a Vickers M85 microdensitometer. Species were measured in batches of eight which included *Allium cepa* as control. Small variation between batches was standardized using the control species. *Allium cepa* (2C = 33.5 pg) was also used as a standard to convert the DNA estimates to absolute amounts. All DNA estimates were corrected to the second decimal place which was accurate under the present experimental conditions. All DNA measurement experiments consisted at least of two replications. Plants collected from different geographical regions were used as replicates. At least three plants were measured in each replication and 20, 2C nuclei were scored in each root tip. Analysis of variance showed no statistically significant difference among nuclei within root tips and among plants within replications. The mean DNA amount estimated for each replication appears in Table 2. Supernumerary (B) chromosomes in the chromosome complements of species could vitiate the DNA estimations. All species were therefore cytologically examined and none had B chromosomes in their complements.

3. DNA in Euchromatin and Heterochromatin. The area of interphase nuclei occupied by heterochromatin was measured microdensitometrically (Narayan and Rees, 1974, 1976). Feulgen stained interphase nuclei in root meristems were scanned at optical densities 0–1.3 in the Vickers M85 microdensitometer. The distribution of areas scanned at each optical density was plotted as a cumulative graph. Using the scanning dot, densities were measured for five heterochromatic sectors in each interphase nucleus in order to calculate the mean density of the heterochromatin. The areas scanned at this optical density and above represent the area composed of the heterochromatin. The area occupied by euchromatin was obtained by subtraction of the area of the heterochromatin from the total nuclear area measured at an optical density of 0. The average optical density for heterochromatin was found to be 1.6 times the density of euchromatin. Knowing the separate areas of heterochromatin and euchromatin and their densities it was possible to estimate how much of the total DNA in each species is located in each fraction. Independent measurements were made on three root tips from different plants. In each root tip a minimum of 15 nuclei were measured.

4. Repetitive and Non-repetitive DNA.—The melting profiles, buoyant densities in neutral CsCl and the average G+C contents were remarkably similar for all species compared. It was possible therefore to carry out Cot reassociations of DNA of all species under the same experimental conditions. The percentage reassociation was measured spectrophotometrically (Narayan and Rees, 1976). The estimate of the percentage reassociation at a given Cot value was the mean for three separate measurements. From the Cot reassociation curves the total DNA of the 12 *Lathyrus* species were classified into the following components based on their reassociation kinetics: 1) a fast fraction or highly repetitive DNA; 2) intermediate fraction or middle repetitive DNA; and 3) slow or single copy DNA. Between 5% and 13% of the total DNA belonged to the first category. In absolute amounts this component did not show significant variation among species. On the other hand, the intermediate fraction, consisting of be-

tween 56% and 70% of the total DNA in each species, accounted for most of the quantitative changes in nuclear DNA. The remainder of the genome is made up of non-repetitive DNA (Rees and Narayan, 1977). Independent estimates were obtained from two separate experiments of the middle and non-repetitive components of each species.

Statistical Analysis

Twelve of the 21 species were grouped on the basis of their differences in 12 characters which consisted of their ranked chromosomes, euchromatin, heterochromatin, non-repetitive DNA, middle repetitive DNA and the total DNA using multivariate analysis. The means for the 12 characters were obtained from a large number of measurements and the statistical analysis was based on their dispersion. The species were grouped using the Mahalanobis D^2 statistic as suggested by Rao (1952). The calculation of D values involved the following steps. 1) From the measurements for the 12 characters for all species the variance-covariance matrix (common dispersion matrix) was computed (Table 4). 2) The uncorrelated linear combinations were obtained by the pivotal condensation of the dispersion matrix and the mean values then converted into a set of uncorrelated means (Ys). 3) D square between the ith and the jth species from the 12 characters were calculated using the formula

$$D^2ij = \sum_{t=1}^{n} (Yit - Yjt)^2.$$

The D^2 for the 12 characters were calculated separately and added up to give D^2ij (Narayan and Macefield, 1976). The D^2 for each character for each combination of species was ranked in descending order of magnitude and the rank totals were obtained for all combinations (Murty and Arunachalam, 1967). The clustering of the species was by the Tochers method as illustrated by Rao (1952). After clustering, the inter- and intra-cluster relationships were studied and the generalized statistical distances between groups represented diagrammatically using the D values. All statistical analyses were programmed on an IBM computer.

TABLE 1. *Discontinuity in DNA variation between nine* Lathyrus *species.*

Species	Total DNA in picograms	Middle repetitive fraction: As absolute amount in picograms	Middle repetitive fraction: Average kinetic complexity in base pairs
Group A			
L. ochrus	13.63	7.39	0.53×10^6
L. aphaca	14.04	6.74	0.50×10^6
L. cicera	14.04	7.51	0.53×10^6
Mean	13.90	7.21	0.52×10^6
Group B			
B_1			
L. articulatus	12.15	6.35	1.55×10^6
L. nissolia	12.92	6.73	1.55×10^6
L. clymenum	13.43	7.01	1.15×10^6
Mean	12.83	6.70	1.42×10^6
B_2			
L. sativus	16.78	9.94	1.55×10^6
B_3			
L. hirsutus	20.21	12.56	1.55×10^6
L. tingitanus	22.08	13.19	1.55×10^6
Mean	21.15	12.90	1.55×10^6

From Narayan and Rees (1976). The data for *L. tingitanus* are revised estimates.

RESULTS

A) Nuclear DNA Variation in Lathyrus

The Cot reassociation data for the middle repetitive fraction of 12 species were normalized to 100% and the reassociation rate constant (Cot ½) was estimated. It ranged from 0.65×10^0 in *L. aphaca* to 2.0×10^0 in *L. hirsutus* (Narayan and Rees, 1976). Using *E. coli* (genome size, 4.6×10^6 base pairs) as standard, which by the same procedure had a Cot ½ of 6, it was possible to convert the rate constants to base sequence complexity expressed as number of base pairs. The base sequence complexity can be viewed as an index of divergence between repeated sequences and the nine species classified in two groups, A and B (Table 1). Group A contained three species with an average

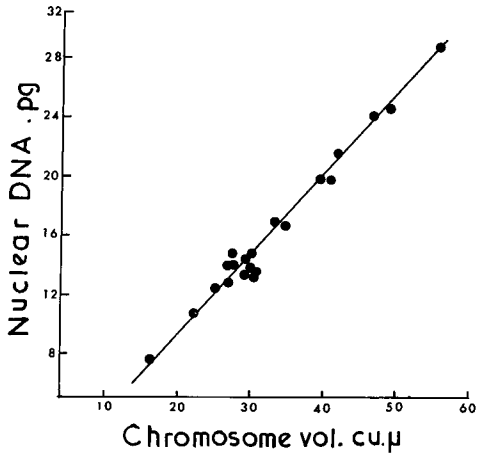

FIG. 1. Distribution of nuclear DNA within the genus *Lathyrus*. The total nuclear DNA amounts in picograms for 21 *Lathyrus* species are plotted against their chromosome volumes. The regression is significant at .1% level.

kinetic complexity for the middle repetitive sequences of 0.52×10^6 base pairs. The remaining six species grouped in B had an average kinetic complexity of 1.49×10^6 base pairs. A quantitative comparison of the middle repetitive DNA among species in group B showed that they in turn are separable into three subgroups B_1, B_2, B_3. Species in B_1 had low DNA amounts and are similar to species in A. In B_2 the average middle repetitive DNA amount was increased by 3.2 picograms. The species in B_3 had a further increase of 3.0 picograms of middle repetitive DNA which corresponds to an average total DNA of 3.90 picograms. To determine whether these observed quantum DNA changes among groups of species are fundamental to the DNA variation in *Lathyrus*, I have compared the total nuclear DNA amounts in the 21 *Lathyrus* species listed above. The species were not selected other than for the availability of their seed. Their range of total DNA was 22.4 picograms. If the difference in amounts of DNA between species is 3.90 picograms, then the 21 species should fall into six or seven groups. The mean nuclear DNA amounts plotted against the total chromosome volume in Figure 1 show in fact a discontinuous distribution and the species fall into the seven groups listed in Table 2.

An analysis of variance of the amounts of DNA shows significant differences between the species within the groups but by far the greater amount of variation, 98% in the component analysis, is due to the differences between groups which are highly significant when tested against the within-group variation. The average interval between group DNA means is 3.72 picograms. A more rigorous classification of 12 of the 21 species was made using multivariate analysis. These 12, whose DNA comprised about two thirds of the total range of DNA shown by the 21 species, were classified on the basis of their divergence in 12 nuclear characters.

B) Multivariate Analysis

As a measure of divergence between populations, statistical distances have been used for classification in anthropometry, psychometry, and biology (Hotelling, 1931; Rao, 1952; Nair and Mukherjee, 1960; Murty et al., 1965) using multivariate analysis. Among the various methods in multivariate analysis, the D^2 statistic of Mahalanobis (1936) is useful for the classification of biological populations. (Sokal and Rohlf, 1969; Wright, 1978).

The analyses of variance in Table 3 show that the 12 species differed significantly in all characters confirming that the 12 characters are meaningful for the classification of these species. The mean values of the 12 characters for the 12 species are given in Table 5. In Table 6 are given the average D values within and between clusters.

1) Grouping of Species into Clusters.— The 12 species were grouped into six sharply defined clusters on the basis of the D^2 values derived from pair-wise comparisons using Tocher's method as given in Rao (1952). The clusters and the generalized statistical distances between them are shown in Figure 2. The maximum intercluster distance (D = 1,523) was between clusters A and E which indicated a large measure of divergence between them.

TABLE 2. *Nuclear DNA amounts for 21* Lathyrus *species.*

Species/groups	DNA in picograms (2C values) Replication 1	Replication 2	Mean (standard error = 0.346)	Group means
Group 1				
L. miniatis	7.24	6.47	6.86	6.86
Group 2				
L. angulatus	10.90	10.61	10.76	10.76
Group 3				
L. articulatus	12.45	11.86	12.15	
L. nissolia	13.20	12.64	12.92	
L. maritimus	12.98	13.33	13.15	
L. clymenum	13.75	13.11	13.43	
L. ochrus	13.95	13.31	13.63	
L. setifolius	14.80	13.22	14.01	13.74
L. aphaca	13.97	14.10	14.04	
L. cicera	14.18	13.90	14.04	
L. sphaericus	14.06	14.29	14.18	
L. pratensis	15.44	14.00	14.72	
L. annus	14.95	14.90	14.93	
Group 4				
L. sativus	17.15	16.46	16.78	16.88
L. odoratus	16.75	17.16	16.96	
Group 5				
L. tuberosus	19.81	19.22	19.52	
L. hirsutus	20.27	19.58	19.93	20.51
L. tingitanus	22.18	21.98	22.08	
Group 6				
L. sylvestris	24.26	25.04	24.65	24.73
L. latifolius	24.58	24.98	24.78	
Group 7				
L. vistitis	29.65	28.78	29.22	29.22

Between species-within group analysis of variance

	Degrees of freedom	Mean squares	Variance ratio
Between groups	6	184.65	126.72***
Within groups	14	1.47	6.10***
Error	21	0.24	

*** Significant at .1% levels.

Estimated components of variation

Components	Estimate
(groups)	41.44
(species)	0.61
(error)	0.24

98% of the total variation is estimated to be due to differences between groups.

TABLE 3. *Analyses of variance on 12 characters in 12 species of* Lathyrus.

Characters	Error mean square (11 *d.f.*)	Species mean square (11 *d.f.*)
Chromosome volume		
Chromosome 1	0.036	3.015***
Chromosome 2	0.114	2.988***
Chromosome 3	0.035	2.255***
Chromosome 4	0.039	1.967***
Chromosome 5	0.051	2.338***
Chromosome 6	0.032	2.224***
Chromosome 7	0.043	2.475***
DNA		
In euchromatin	0.069	8.302***
In heterochromatin	0.070	9.890***
Non-repetitive	0.078	1.686***
Middle repetitive	0.166	20.913***
Total DNA	0.143	36.441***

*** Significant at .1% level.

Clusters A and E represented species *L. angulatus* and *L. sylvestris,* respectively. The three species (*L. ochrus, L. aphaca, L. cicera*) are included in cluster B_2. Clusters C and D contained two species each. *Lathyrus odoratus* and *L. sativus* together formed cluster C and *L. tingitanus* and *L. hirsutus* cluster D. The distance between clusters B_1 and B_2 is significantly smaller than the distances between other clusters. It is adduced from this that the species included in these clusters bear closer resemblance to each other than those grouped in other clusters. Cluster B_1 is therefore considered to be a subgroup of B_2. B_1 is made up of three species, viz. *L. articulatus, L. nissolia* and *L. clymenum.*

2) Divergence among Species within Clusters.—The *D* values in the leading diagonal in Table 6 give the magnitude of divergence among species within each cluster and show that the average *D* value within each cluster is consistently smaller than the *D* values between major clusters. The average intra-cluster distances for B_2 (38.5), B_1 (76.3), and C (13.5) show that the species grouped in each are markedly similar to each other for the 12 characters. The exceptionally large intra-cluster divergence of group *D* (215.0) suggests a large measure of divergence between the

TABLE 4. *The variance-covariance matrix for 12 characters in 12* Lathyrus *species.*

Species	Chromosome volume (cu μ)							DNA in picograms				
	1	2	3	4	5	6	7	In euchromatin	In heterochromatin	Non-repetitive DNA	Middle repetitive DNA	Total DNA
Angulatus	0.020	−0.024	0.001	0.005	0.016	0.012	0.011	−0.010	0.010	−0.019	−0.027	0.012
Articulatus		0.114	−0.001	0.024	0.007	−0.013	−0.009	0.036	−0.036	−0.010	0.003	−0.007
Nissolia			0.036	0.024	0.022	0.019	0.029	0.008	−0.008	−0.006	0.006	0.046
Clymenum				0.039	0.040	0.025	0.031	0.016	−0.016	−0.011	0.012	0.029
Ochrus					0.051	0.030	0.034	0.004	−0.004	−0.010	−0.018	0.032
Aphaca						0.030	0.026	0.002	−0.002	−0.006	0.006	0.030
Cicera							0.043	−0.006	0.006	−0.002	0.007	0.026
Sativus								0.070	−0.070	−0.005	0.005	0.035
Odoratus									0.070	0.006	−0.009	−0.035
Hirsutus										0.077	−0.107	0.012
Tingitanus											0.166	−0.030
Sylvestris												0.146

two species *L. tingitanus* and *L. hirsutus* for one or more characters. A comparison of the component D^2's (square of the difference between the transformed uncorrelated means for each character) showed that 88.2% of the total divergence between *L. tingitanus* and *L. hirsutus* was accounted for by changes in amounts of DNA in heterochromatin. In Figure 3, C-banded chromosome complements of *L. hirsutus* and *L. tingitanus* are compared. Whereas the banding pattern for *L. hirsutus* is typical for most of the *Lathyrus* species, the constitutive heterochromatin being confined to the centromeric regions in all chromosomes, chromosomes 1, 2 and 4 of *L. tingitanus* have additional intercalary bands on their short and long arms. 34.2% of the total chromosome volume in *L. tingitanus* is made up of constitutive heterochromatin, which is 10.72% higher than in *L. hirsutus*. Quantitative comparisons of the amounts of constitutive heterochromatin showed species were similar within each of the clusters B_2, B_1, and C.

The conclusions are that there are large differences between species in different clusters compared with the marked similarity of the species within the clusters for most characters and that the somewhat larger divergence in cluster D between *L. tingitanus* and *L. hirsutus* is mainly due to differences in amount of heterochromatin.

3) Discontinuity in Evolutionary Progression.—The close similarity of species within each cluster and the sharp separation of clusters themselves from one another suggest discontinuity in evolutionary progression. In Figure 2 the major clusters A, B_2, C, D and E fall in a straight line. When we compare the statistical distances between successive clusters (A & B_2, B_2 & C, C & D, and D & E) it is clear that the five clusters are separated from each other by a regular series of steps which make up the discontinuous series. The average inter-cluster distance for this series is 385.25. Although, however, the distances between A and B_2, B_2 and C and D and E are similar (mean D = 335.3),

TABLE 5. *Means for the 12 characters in 12* Lathyrus *species.*

Species	Chromosome volume (cu μ)							DNA in picograms				
	1	2	3	4	5	6	7	In euchromatin	In heterochromatin	Non-repetitive DNA	Middle repetitive DNA	Total DNA
Angulatus	4.62	3.25	3.09	2.99	2.77	2.75	2.52	9.01	1.89	4.29	4.88	10.76
Articulatus	4.89	4.26	4.02	3.46	2.92	2.80	2.48	10.26	2.20	5.19	6.64	12.15
Nissolia	4.87	5.06	3.95	3.64	3.43	3.25	3.10	10.66	2.55	5.26	6.88	12.92
Clymenum	5.76	5.17	4.55	4.45	3.36	2.87	2.64	10.10	2.76	5.24	7.00	13.43
Ochrus	5.60	5.03	4.85	4.52	4.33	3.77	2.62	10.32	3.63	5.59	7.38	13.63
Aphaca	5.15	4.61	4.35	3.98	3.20	2.85	2.72	10.07	3.91	5.04	7.00	14.04
Cicera	4.98	4.19	4.04	3.91	3.79	3.45	2.91	10.68	3.51	5.78	7.69	14.04
Sativus	6.18	5.21	5.04	4.89	4.68	4.51	4.33	12.50	4.66	5.07	10.42	16.78
Odoratus	6.07	4.95	4.90	4.76	4.34	4.21	4.07	11.96	5.20	5.71	9.73	16.96
Hirsutus	7.22	5.91	5.67	5.57	5.33	5.08	4.86	12.67	7.60	6.46	12.24	19.93
Tingitanus	7.37	6.70	6.39	6.12	5.73	5.52	5.19	15.05	7.64	7.51	13.19	22.08
Sylvestris	8.84	7.94	6.79	5.99	5.90	5.78	5.33	15.74	8.53	7.09	15.72	24.65
Standard error	0.110	0.240	0.135	0.139	0.160	0.122	0.159	0.186	0.186	0.199	0.200	0.267

TABLE 6. *Average* D *values within and between clusters.*

Clusters	A	B_1	B_2	C	D	E
A	—	229.4	356.8	709.0	1,175.0	1,523.0
B_1		76.3	141.1	488.3	953.0	1,300.0
B_2			38.5	356.2	822.0	1,169.0
C				13.5	116.6	813.9
D					215.0	371.0
E						—

the distance between C and D is higher (D = 446.0).

The regularity with which clusters are separated from one another becomes more striking when we compare the average DNA amounts for each cluster (Fig. 2). The successive clusters are separated from each other by a regular quantum change in their mean DNA amounts. The average quantum DNA change for the five clusters is 3.5 picograms.

4) Relative Role of Nuclear Characters in Species Divergence.—The component D^2's derived from the pairwise comparisons of species were ranked in their descending order of magnitude, where rank 1 was assigned to the highest value. The rank totals for the 12 characters, obtained from all possible pairwise combinations of species, give information about the order of priority of the 12 characters in divergence, the inference being that the character with the smallest rank total has made the most contribution to cluster divergence, while characters with increasing rank totals have made correspondingly less. The component D^2's were summed over all combinations for each of the 12 characters and the D values calculated from the totals. These, as well as rank totals, are given in Table 7, which shows that the order of priority of the characters in their contributions to cluster divergence was DNA in heterochromatin, non-repetitive DNA, total DNA, DNA in euchromatin, repetitive DNA and chromosome volume. Among the 12 characters, DNA in heterochromatin has contributed the maximum (57.42% of the total D^2s) to inter-cluster divergence followed by non-re-

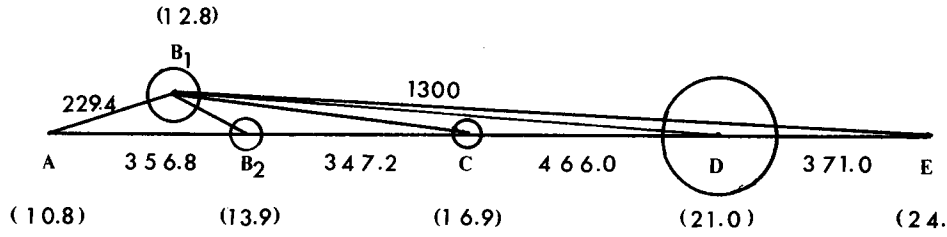

FIG. 2. The clusters and the generalized statistical distances between them. Clusters having more than one species are represented by circles. The diameter of the circle is drawn proportional to the average intracluster divergence. The figures in parentheses are the mean DNA amounts in picograms for each cluster.

petitive DNA (23.4%), total DNA (7.00%), repetitive DNA (2.6%), and DNA in euchromatin (2.46%). 7.48% of the total D^2 was accounted for by the sum of component D^2's from seven chromosomes.

5) Random Distribution of Evolutionary Changes in Chromosomes.—Table 7 shows that the sum of the component D values for the seven chromosomes is consistently small when compared with the other characters. The table also shows that, with the minor exception of chromosome number 3, they have closely similar D values (mean 94.36). Chromosome 3 has a lower D value of 61.12. The close similarity between D values (and their rank totals) implies that the total chromosome variation among the 12 species is distributed at random among chromosomes within each complement. Total chromosome volume is directly correlated with nuclear DNA amount in *Lathyrus* species, hence a direct comparison of the DNA contained in individual chromosomes would tell us about the DNA distribution within each chromosome complement and a comparison between species would also give us information about its changes during evolution. The chromosomes of four *Lathyrus* species were C-banded using giemsa stain and the volume of heterochromatin and euchromatin were mea-

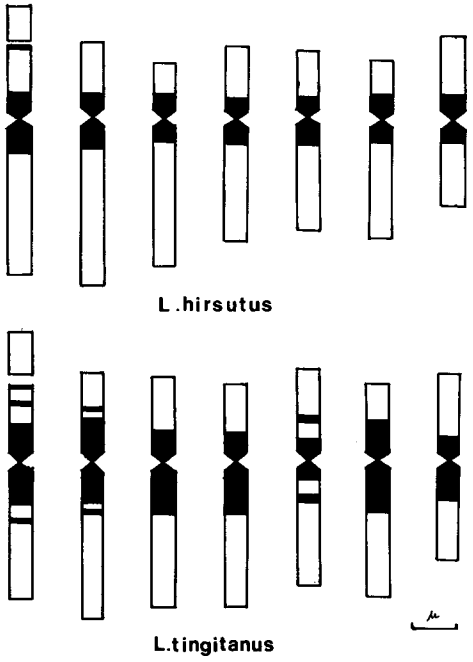

FIG. 3. C-banded chromosome complements of *L. tingitanus* and *L. hirsutus*.

TABLE 7. *Total* D *values and rank totals for 12 characters.*

	Total D value	Rank total
Chromosome volume		
Chromosome 1	102.9	538
Chromosome 2	106.7	509
Chromosome 3	61.12	679
Chromosome 4	103.3	594
Chromosome 5	94.0	538
Chromosome 6	99.5	563
Chromosome 7	92.4	556
DNA		
In euchromatin	213.9	344
In heterochromatin	5,071.2	84
Non-repetitive	2,034.6	153
Middle repetitive	232.8	348
Total DNA	618.64	242

sured separately for each chromosome. Using spot microdensitometry, it was established that heterochromatin is on average 1.6 times denser than euchromatin in the interphase nuclei of *Lathyrus* species from which it was possible to calculate the DNA content of each chromosome. The four species used in this analysis represented a two-fold variation in their nuclear DNA amounts. In Figure 4 the DNA amounts of the seven chromosomes, ranked according to DNA amounts of each species, are plotted against the mean amounts of all four species. The regression analysis shows that the slopes are strikingly similar for the four species, but as expected from the significant DNA variation between species, the differences between the means are highly significant. These results suggest that the quantitative DNA changes associated with speciation have affected all chromosomes within the *Lathyrus* complements. If we make the assumption that chromosomes which are assigned identical serial numbers in different species are homeologous to each other, then the joint regression would imply that the DNA changes were of the same magnitude in all chromosomes. No cytological evidence from interspecific hybrids is available at present to establish the homeologous relationship between different chromosomes, but the inference that quantitative DNA changes are accounted for by all chromosomes within each complement is supported by the results of molecular hybridization experiments (unpubl. results).

The experiment in brief was as follows. Purified DNA samples of *L. tingitanus* were chemically coupled with an antibiotic (actinomycin-D) and silver in separate experiments and DNA was centrifuged to equilibrium in CsCl or $CsSO_4$ density gradients. Using this method it was possible to isolate three separate DNA components (satellite sequences) from the total *L. tingitanus* DNA. The three satellite components together represented less than 7% of the total *tingitanus* DNA and could not be isolated from *L. hirsutus* included in group D. The low base sequence complexity of the satellite sequences and their relative absence in *L. hirsutus* would suggest that these sequences were amplified recently during the evolution of the genus. The satellite DNA retrieved using actinomycin-D was then transcribed into H^3 labelled C- RNA using the method of Jones (1973). The labelled C- RNA was hybridized in situ with the DNA in the metaphase chromosomes (Pardue and Gall, 1969) of *L. tingitanus*. The hybridization was repeated using six other *Lathyrus* species which contained less DNA in their

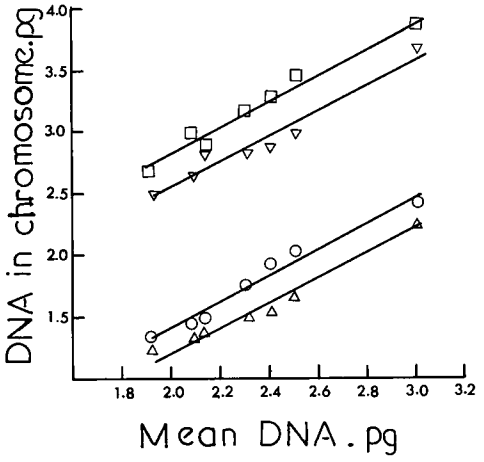

FIG. 4. Amounts of DNA in the seven chromosomes of four *Lathyrus* species plotted against the mean amounts of the four species: △ *L. angulatus*, ○ *L. articulatus*, ▽ *L. hirsutus*, □ *L. tingitanus*.

Regression analysis

	Degrees of freedom	Mean squares	Variance ratio	Probability
Heterogeneity—regression	3	0.0076	0.8837	>.05
Heterogeneity—means	3	4.48	520.93	<.001
Error	20	0.0086		

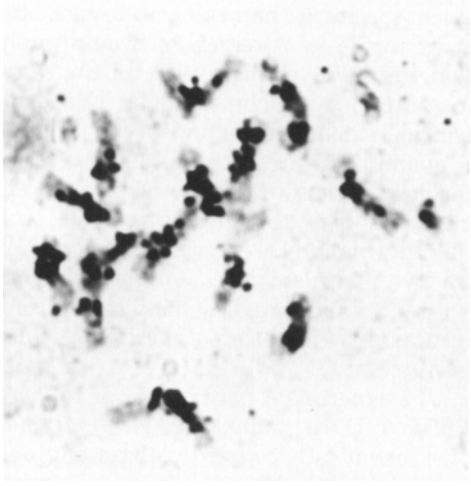

FIG. 5. Satellite DNA of *L. tingitanus* separated using actinomycin-D as ligand was transcribed into H^3 labelled C- RNA. It was hybridized in situ with the metaphase chromosomes of *L. tingitanus*. The figure shows that the satellite DNA is distributed in the heterochromatic regions of all chromosomes (magnification ×1000).

genome than *L. tingitanus*. Autoradiographs showed that the satellite sequences are in the heterochromatin at, or near, their centromere in all chromosomes of *L. tingitanus* (Fig. 5) whereas they are smaller and occur only in some of the chromosomes in each of the other species. The results imply that evolution and divergence of species are accompanied by discrete quantum jumps of DNA and that these amounts are distributed fairly evenly over all chromosomes.

C) DNA Groups and Taxonomical Classification in Lathyrus

The classification of *Lathyrus* species into a separate genus is well defined. Evolution within this genus was accompanied by large scale changes in chromosome size, structural rearrangements of chromosomes and genic changes. The constancy in chromosome number and similarity in chromosome morphology in many species have made cytotaxonomical classification of *Lathyrus* difficult. Moreover, interspecific crosses within *Lathyrus* have been rarely successful. A classification based on interspecific compatibilities and chromosome pairing in interspecific hybrids was therefore not possible. The taxonomical classifications of this genus are based mainly on general morphology, geographical distribution, plant habits and karyomorphology.

Senn (1936, 1938) has classified 42 *Lathyrus* species into seven major sections (*Aphaca, Nissolia, Clymenum, Orobus, Orobastrum, Cicercula, Eulathyrus*) and 17 of the 21 species listed in Table 2 are included in his classification. On the basis of general morphology, plant habits and geographical distribution, Senn suggested the section *Orobus* to be the oldest followed by section *Orobastrum*. The three highly specialized sections *Aphaca, Nissolia* and *Clymenum* are considered to have directly derived from the *Orobastrum* section. The section *Orobastrum* has also given rise to section *Eulathyrus* from which section *Cicercula* has evolved more recently.

Taking the 17 species included in this study, five major sections of *Lathyrus* are arranged in the order of increasing DNA amounts in Table 8. The eight species from the closely related sections *Aphaca, Nissolia, Clymenum,* and *Orobastrum* appear in the same DNA group (3). Species in sections *Eulathyrus* and *Cicercula* are not confined to a single DNA group. The four species included in *Cicercula* cluster into DNA groups 3, 4, and 5 with average DNA amounts 13.74, 16.88 and 20.51 pgs, respectively. Six species in *Eulathyrus* also fall into three DNA groups 4, 5, and 6 with average DNA values 16.88, 20.51 and 24.73 pgs, respectively. Gams (1926) has pointed out the close taxonomical relationship between *L. sylvestris* and *L. latifolius* which are in the same group. *Lathyrus pratensis, L. angulatus, L. miniatis* and *L. vestitis* are not included in Senn's classification. A precise cytotaxonomical classification of *Lathyrus* species is required to understand the interspecific affinities within each section and to find out whether such groups would correspond closely with the DNA groups.

Increase in total DNA, chromosome size

TABLE 8. *Nuclear DNA variation and taxonomical classification in* Lathyrus.

DNA groups	Taxonomical sections					
	Aphaca	*Nissolia*	*Clymenum*	*Orobastrum*	*Cicercula*	*Eulathyrus*
1 (6.86)						
2 (10.76)						
3 (13.74)	1	1	3	3	2	
4 (16.88)					1	1
5 (20.51)					1	2
6 (24.73)						2
7 (29.22)						

Figures in parentheses are the mean DNA amounts in picograms for each group. Sections *Aphaca* and *Nissolia* are monotypic and have *L. aphaca* and *L. nissolia*, respectively. Section *Clymenum* includes *L. clymenum, L. articulatus,* and *L. ochrus*. Section *Orobastrum* has species *L. sphaericus, L. maritimus* and *L. setifolius*. Section *Cicercula* has species, in order of increasing DNA, *L. cicera, L. annus, L. sativus* and *L. hirsutus* and section *Eulathyrus, L. odoratus, L. tuberosus, L. tingitanus, L. sylvestris* and *L. latifolius*.

and repetitive and non-repetitive DNA in a consistently regular ratio also suggests that the evolution of this genus is accompanied by an increase in nuclear DNA amounts. Therefore, the arrangement in Table 7 may be the evolutionary progression within this genus. A comparison of the base sequence divergence among *Lathyrus* species was used to assess the evolutionary divergence between them. The middle repetitive and non-repetitive fractions of *Lathyrus hirsutus* DNA were isolated, radioactively labelled and then cross reassociated with the total DNA of six other species (*L. tingitanus, L. odoratus, L. sphaericus, L. clymenum, L. articulatus* and *L. angulatus*) which showed a wide range of variation in their nuclear DNA amounts. The DNA homologies of the repetitive and the non-repetitive components of *L. hirsutus* with the total DNA of each of the six species were estimated from the percentage cross reassociation of their base sequences as well as from the thermal stability of the reassociated DNA duplexes (Narayan and Rees, 1977). The results showed that species differing most in DNA having the largest differences in base composition both in repetitive and non-repetitive components.

DISCUSSION

In *Lathyrus* up to 70% of the total DNA is made up of repetitive sequences which account for most of the DNA variation within this genus. The repetitive component ranges from almost identical satellite sequences to families of repetitive sequences which show substantial divergence between them. The remainder of the genome is made up of non-repetitive sequences.

Much of the non-repetitive and most of the repetitive sequences have no known genetic function. If we assume eukaryotic organisms on average contain 55,000 structural genes, this would correspond approximately to 55×10^6 base pairs, which in turn amount to less than 0.06 picograms. This comprises a mere 0.09% of the total genome of *L. miniatis* which has the smallest genome size. This would imply that more than 99% of the total *Lathyrus* DNA carries no obvious genetic function. The remarkable stringency with which the C values of plant species are conserved over countless cycles of cell divisions, on the other hand, shows that specificity in DNA amounts, apart from its genetic information content, is vital to the organization and stability of the genome. Base sequence amplifications and deletions together with interspersion and divergence are essential for the evolution of plant genomes and these are reflected in the changes in nuclear fractions.

The divergence and evolution in *Lathyrus* is accompanied by increase in both repetitive and non-repetitive components. Moreover the ratio between the two components is remarkably constant through the evolution of the genus (Rees and Narayan, 1977). As in *Lathyrus,* many other plant and animal genera show consistently reg-

ular ratios between repetitive and non-repetitive fractions in the extra DNA contributing to the DNA variation (Hutchinson et al., 1980). The increase in the repetitive component can be readily conceived as due to the proliferation of repetitive sequences. We cannot, however, account for large increases in the non-repetitive DNA by extensive amplification of base sequences because by this means they would become repetitive. A possible explanation would be that the DNA increase was initially restricted to repetitive fraction followed by the generation of single copy sequences by mutations, deletions, insertions and base sequence rearrangements. The large scale base sequence divergence observed among families of repetitive sequences is a proof of such events taking place during the evolution of higher organisms.

Thompson and Murray (1980) have also demonstrated a consistently regular quantitative relationship between single copy DNA and the total genome size in eukaryotes. In a comparison of the mung bean and pea genomes they have shown that most single copy sequences are in fact short interspersed elements from ancient repetitive families which have now diverged so that they no longer reassociate with one another under standard experimental conditions. However, some of the families could be detected by reassociation under low stringency conditions which tolerated more base pair mismatches. In their experiments almost all single copy DNA has reassociated with the kinetics expected for sequences present in one or two copies. Thermal denaturation profiles have also shown substantial amounts of base pair mismatches in the reassociated duplexes consistent with the assumption that they are obtained from divergent repeats.

Watson (1965) has given the estimate that the replication error at the nucleotide level is of the order of 10^{-9}. From the frequency of hemoglobin variants in man the mutation rate per nucleotide per generation is estimated to be 4.4×10^{-8}, which is close to Watson's estimate (Nei, 1975). If we assume that the overall base sequence divergence took place at a constant rate during evolution, the constant ratio between repetitive and non-repetitive sequences in the extra DNA would imply that the DNA increase in *Lathyrus* also took place at a constant rate.

The distribution of DNA within this genus, however, is not continuous. Species cluster into groups, members of each group having closely similar DNA amounts. It is reasonable to suppose, therefore, that species within each group share a similar equilibrium or balance in nuclear organization, despite their interspecific divergence and that the groups are "steady states" in genome evolution. Nevertheless, the small but significant variation within each group suggests that a certain amount of variation is permissible within each "steady state." Interspecific divergence within each group results from reproductive isolation, gene mutations, karyotype rearrangements or rapid change subsequent to random genetic drift.

Yet genome evolution is a discontinuous change giving a series of "steady states" into which the evolving species must fall. The constant increment in DNA of average 3.7 picograms implies that this is a property of the genome which has presumably arisen by selective forces in the range of environments it has experienced in the past. The DNA amounts which fall in between "steady states" have no selective advantage in terms of nuclear organization and are therefore eliminated. If DNA increase occurred at a constant rate as adduced above it would appear that the evolutionary transition from one "steady state" to another took place at approximately equal intervals of time.

The substantial divergence of *L. tingitanus* from *L. hirsutus* in cluster D and the divergence of subgroup B_1 from B_2 are significant. The nuclear DNA of *L. tingitanus* is approximately 2.1 picograms higher than that for *L. hirsutus*. Multivariate analysis showed that 88.2% of the total divergence between *hirsutus* and *tingitanus* is due to changes in the DNA contained in heterochromatin. It is also

significant that *L. tingitanus* contains 3 satellite sequences which are not amplified in *L. hirsutus* and five other *Lathyrus* species from clusters C, B_1, B_2 and A. In *L. tingitanus* perhaps we witness a species in transition from one "steady state" to another.

Group 3 in Table 2 contained 11 species of which six were used in the multivariate analysis. The six species classified into two subgroups B_2 (*ochrus, aphaca,* and *cicera*) and B_1 (*articulatus, nissolia,* and *clymenum*). Within each subgroup species are remarkably similar to each other for all characters compared. An independent classification of nine *Lathyrus* species, which included species in B_1 and B_2, based on the average kinetic complexity of their middle repetitive sequences, has shown that species in B_1 are different from those in B_2 (Table 1). The average kinetic complexity is a theoretical estimate for base sequence divergence which restricts in vitro reassociation of denatured DNA. In broad and general terms the observed differences in the average kinetic complexity would suggest that the middle repetitive DNA for species in B_2 results from the rapid turnover of a relatively smaller variety of repetitive sequences repeated to a greater degree. For species in B_1, however, their middle repetitive sequences are made up of a larger variety of sequences repeated to a limited degree. The average DNA amount for species in B_1 is approximately 1 picogram less than for species in B_2. Here again the indications are that species in B_1 represent a transitional stage moving from one "steady state" to another.

Discontinuous type of DNA variation is reported in other plant genera as for example in *Anemone* (Rothfels et al., 1966), *Vicia* (Martin and Shank, 1966) and several genera within the Gramineae (Sparrow and Nauman, 1973). In the genus *Clarkia,* Lewis (1962) suggested that catastrophic selection followed by inbreeding, extensive chromosome breakage and rearrangements, with or without numerical chromosome changes, have played a very prominent role in the evolution of this genus. A survey of the DNA variation within this genus has shown that the 26 species measured for their DNA amounts clustered into four distinct groups. The DNA differences between groups are similar, the average being 2.1 picograms. The genus *Nicotiana* comprises 62 recognized species. Geographical barriers, amphidiploidization and karyotype rearrangements have promoted rapid speciation within this genus and there is a large variation in their chromosome numbers among diploid species (Goodspeed, 1954). DNA measurements were made on 41 diploid and 10 amphidiploid species, falling into seven DNA groups. The DNA interval between groups is approximately 2 picograms, as in *Clarkia*. The DNA groups in *Clarkia* and *Nicotiana* can be shown to correspond broadly with their taxonomical classification by Lewis and Lewis (1955) and Goodspeed (1954). Twenty-four *Allium* species (20 diploid and 4 polyploid) formed six DNA groups with an average interval of 4.2 picograms which is closely similar to that of *Lathyrus*. What is remarkable with *Clarkia, Nicotiana* and *Allium* is that the discontinuous changes show no correlation with numerical chromosome changes or with levels of polyploidy. While each species can be assigned to its DNA group, polyploid and diploid species with different basic chromosome numbers also occurred in the same DNA group (Narayan and Durrant, unpubl.). The above observations show that the discontinuity in DNA variation is fundamental to the organization and evolution of the genome. It is not clear, however, whether such quantum DNA changes arise de novo or whether they arise in a continuously varying distribution formed by the steady accretion of DNA.

SUMMARY

A survey of the nuclear DNA variation among 21 diploid *Lathyrus* species showed that the distribution of total DNA in this genus is discontinuous. The 21 species clustered into seven separate DNA groups. The DNA interval between successive groups was similar, the average being 3.71 picograms. A rigorous classification of 12

of the 21 species was done using multivariate analysis. The 12 species classified into five major clusters. Successive clusters were separated from each other by a regular quantum change in their mean DNA amounts.

A comparison of the distribution of nuclear DNA within the chromosome complements of four *Lathyrus* species showed that the extra DNA responsible for total DNA variation is distributed in all chromosomes in each complement. It is suggested that species within each DNA group shared a similar equilibrium or balance in nuclear organization despite their interspecific divergence and that the groups are "steady states" in genome evolution. Evolution of the *Lathyrus* genome gave a series of "steady states" into which the evolving species clustered. Transition from one "steady state" to another during evolution involved a regular quantum change in nuclear DNA and these amounts were distributed fairly evenly on all chromosomes in each complement.

Survey of the nuclear DNA variation within three other plant genera, *Clarkia, Nicotiana* and *Allium,* has also shown that species within each genus occur naturally in groups at regular intervals of approximately 2 or 4 picograms. It is not clear however, whether such discontinuous changes arise de novo or whether they arise in a continuously varying distribution formed by the steady accretion of DNA.

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