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Nuclear DNA Variation in *Lathyrus*

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Abstract. In the genus *Lathyrus* the divergence and evolution of species was accompanied by large scale changes in nuclear DNA amount. All the species are diploids with 14 chromosomes so that the DNA changes were the result of amplification or deletion of segments within chromosomes. Our evidence indicates that the quantitative changes involve mainly the repetitive, as distinct from the non-repetitive fraction of the chromosomal DNA and, on a cytological basis, mainly heterochromatin in contrast to euchromatin. There is an element of discontinuity in the distribution of DNA amounts among species which suggests that the DNA variation results from a series of separate, spasmodic events. The discontinuities may be viewed, also, as "steady states" from the standpoint of genetic balance and biological fitness.

Introduction

The divergence and evolution of species within the genus *Lathyrus* is accompanied by a three fold variation in the nuclear DNA amount (Rees and Hazarika, 1969). All the species investigated are diploids ($2n = 14$) so that the nuclear DNA variation stems from the amplification or deletion of DNA within chromosomes as distinct from numerical chromosome change. This view is supported by the increase in chromosome size associated with increasing nuclear DNA amount (Fig. 1). The following work was concerned with finding out which of the chromosome components are involved in the nuclear DNA variation. The questions posed are, (a) is the variation in the nuclear DNA amount associated with change in the overall base-ratio, (b) how much of the DNA in the different species is made up of, on the

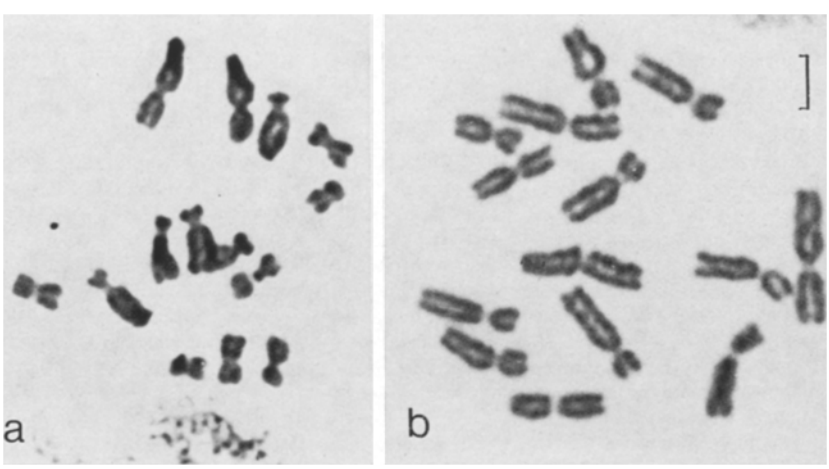

Fig. 1a and b. The chromosomes of (a) *L. articulatus* and (b) *L. hirsutus* at metaphase of mitosis. The bar scale represents 10 μm

one hand, repetitive base sequences and, on the other, of non-repetitive sequences. In particular, to what extent is the quantitative variation in DNA attributable to change in the repetitive in contrast to non-repetitive base sequences, (c) from a cytological standpoint, is the DNA variation associated with changes in heterochromatin or in euchromatin and, finally, (d), is the amount of heterochromatin correlated with variation in the repetitive or non repetitive fractions.

While there is a wealth of information from a wide range of organisms about those aspects of chromosome variation we have enumerated there have been few investigations dealing with groups of closely related species within the one genus. This kind of investigation is likely to provide direct and useful information about the events contributing to the evolution of chromosomes and their constituent parts.

Materials and Methods

9 diploid species were investigated. They are *L. articulatus*, *L. nissolia*, *L. cylmenum*, *L. ochrus*, *L. aphaca*, *L. cicera*, *L. sativus*. *L. tingitanus* and *L. hirsutus*. We also included information from *L. angulatus* with respect to heterochromatin.

(1) Heterochromatin. The amount of heterochromatin, expressed as the proportion of the total area of the interphase nucleus, was estimated as follows. Feulgen stained interphase nuclei in root meristems were scanned at optical densities 0–1.3 in the Vickers M 85 microdensitometer. The distribution of areas scanned at each optical density was plotted by a computer as a cumulative graph (Fig. 2). Using the scanning dot, densities were measured for five heterochromatic sectors in each interphase nucleus in order to calculate the mean optical density of the heterochromatin. The area scanned at this optical density and above represents the area composed of the heterochromatin, which is then converted to a percentage of the total nuclear area, measured at an optical density of 0.

(2) Extraction of the Nuclear DNA. Surface sterilised seeds (soaked in 2% sodium hypochlorite for 25 minutes) were washed in distilled water and sown in sterile vermiculite. The plants were grown under axenic conditions for 14 to 18 days. The method adopted for the extraction of the nuclear DNA was essentially the same as suggested by Bendich and Bolton (1967). After repeated deproteinisation of the aqueous extract with chloroform-octonol the DNA was precipitated in 95% ethyl alcohol. After washing in 70% ethyl alcohol it was dissolved in 0.1 × SSC (0.015 M sodium chloride, 0.0015 M sodium citrate, pH 7). It was further purified by treating with RNase (50 μg per ml) at 37° C for one hour and with self digested pronase (100 μg per ml) for 15 hours at 37° C. After shaking with chloroform-octonol until no visible interphase remained, the DNA was precipitated in iso-propyl alcohol in the presence of Acetate EDTA (0.3 M Na-acetate and 10^{-4} M EDTA; Marmur, 1961). The DNA was then dissolved in 0.12 M phosphate buffer (PB) (equimolar mixture of Na H_2PO_4 and Na_2HPO_4, pH 6.8) and also in 0.1 × SSC.

L. aphaca had pigments in the seedlings which coloured the DNA despite repeated precipitations. Hence it was passed through a column of Sepharose 4 B (45 × 2 cm) which separated the pigment from the DNA peak. Purified samples of DNA were dyalised extensively against the respective buffers and stored at —10° C.

E. coli strain K_{12}, used as a standard, was grown at 37° C in tris-glycerol media (Nomura *et al.*, 1962) and the DNA was extracted according to the method of Marmur (1961).

(3) DNA Melting Experiments. All DNA meltings were carried out in 0.1 × SSC at pH 7. DNA samples were diluted to an optical density of 0.4–0.5. In order to achieve equal ionic conditions, the different samples to be compared were simultaneously dialysed in 0.1 × SSC for 72 hours (3 changes of 24 hours each against 500 volumes of the buffer) at 4° C. All samples were degassed under vacuum.

Meltings were done in an SP 1800 B spectrophotometer having electrically heated cell blocks with accessories SP 1805 (programme controller) SP 876 (temperature programmer) and an ADCP-2 digital print out. The time-temperature linearity was further monitored using an AR-25 linear recorder connected to SP 876. The rate of temperature increase was adjusted

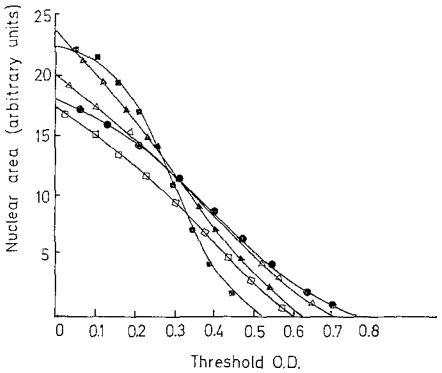

Fig. 2. The areas scanned at different threshold optical densities in the interphase nuclei of *Lathyrus* species: ▪ *L. articulatus*, ▫ *L. clymenum*, ▵ *L. sativus*, ▲ *L. tingitanus*, • *L. hirsutus*

to $^1/_4$° C per minute and the absorbance recorded every 2 minutes. At peak transition temperatures, however, the absorbance was automatically recorded every minute. The data were corrected for volume expansion using the table given by Mandel and Marmur (1968). The corrected absorbances were plotted as a ratio of AT/A_{25} against the temperature. The GC ratio was calculated using the equation GC = (T_m–53.99) 2.44. DNA from *Allium fistulosum*, which has a 37.9% GC content, (Kirk *et al.*, 1970) was used as a reference and check with each of the *Lathyrus* species. The compositional heterogeneity, as % GC, was calculated using the equation 25 = (ΔT–3.0) 2.44 (Mandel and Marmur, 1968). The heterogeneity refers to the spatial distribution of GC and AT pairs. Thus a high degree of heterogeneity implies concentration of one or the other base pair.

(4) Caesium Chloride Density Gradient Analysis. Analytical isopycnic centrifugation of the DNA samples was carried out in an MSE centriscan-75 ultra centrifuge. Neutral caesium chloride (ref. index 9°40′–9°50′) was prepared in 0.01 M tris pH 7.8 containing 5 μg of sample and 5 μl of marker DNA (500 μg per ml). The marker used was *Micrococcus lysodeikticus* DNA which has a buoyant density of 1.731 g/ml. The samples were centrifuged for 20 hours at 25° C. At equilibrium the density gradients were scanned and plotted using the built-in scanning device. The GC ratio was calculated from the buoyant density of the DNA peak using the equation GC = ϱ–1.66/0.098 (Mandel *et al.*, 1968).

(5) Preparation of the Sheared DNA. DNA samples in 0.12 M PB were chilled in an ice-bath and sonicated using an ultra sonic disintegrator (MSE 150 watt with rotate power control). The amplitude was maintained at 24 microns and the dosage was three one-minute blasts with one minute intervals for cooling. The mean molecular weight of the sheared DNA was determined by sucrose gradient analysis in an MSE–65 centrifuge, using a labelled marker DNA of known molecular weight. The sonicated double stranded DNA had an average molecular weight of 2.7×10^5 daltons, corresponding approximately to 450 nucleotides. The sonication did not cause any change from the initial optical density of the DNA samples.

(6) C_0t Reassociation of the DNA. Reassociation kinetics of the DNA samples were followed spectrophotometrically. The following conditions were adopted to follow the reassociation kinetics from $C_0t\ 10^{-2}$ to $C_0t\ 10^4$. The reaction mixture contained different concentrations of sheared DNA (0.05 mg to 5.6 mg) in 0.12 M PB (pH 6.8). The DNA was denatured at 99° C in the spectrophotometer heated cell blocks. A diluted sample (0.2–0.4 O.D.) in 0.12 M PB was kept in an adjacent cell to monitor the completion of denaturation as measured from its hyperchromicity. The DNA samples were then transferred to a waterbath maintained at 62° C. A layer of liquid paraffin was placed over the reaction mixture to prevent evaporation. For prolonged incubations, however, the reassociations were carried out in an incubator maintained at 62° C. Aliquots of the reaction mixture (5 μl to 1 ml depending on the initial concentrations) were pipetted out at time intervals corresponding to various C_0t values and diluted

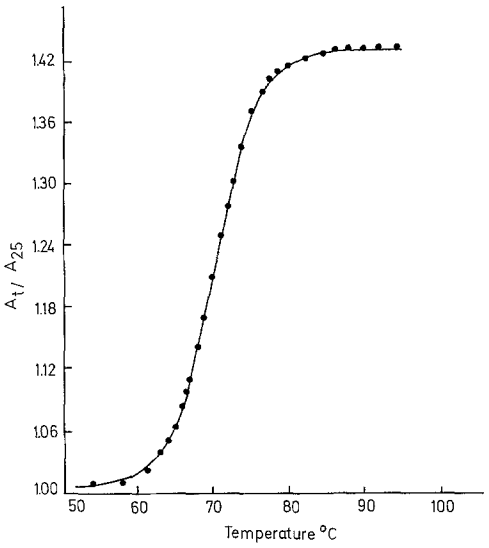

Fig. 3. The melting profile of the DNA of *L. tingitanus*

to 3 ml in 0.12 M PB. They were transferred to a spectrophotometer cell maintained at 62° C. After equilibrating the temperature to 62° C the optical density ($O.D.T._{62}$) was recorded. The temperature was suddenly raised to 99° C and another set of readings ($O.D.T_{99}$) was recorded. In the meantime the average hyperchromicity for a given sample of DNA was calculated from a series of DNA meltings in 0.12 M PB. This was expressed as a fraction $O.D.T_{62}/O.D.T_{99} = Hy$, which was not different from $O.D.T_{25}/O.D.T_{99}$ in the ionic concentrations of the PB once adjusted for the volume expansion. The optical density of the double stranded DNA in the sample was then determined as $O.D.T_{99} \times Hy$.

The percentage reassociation was calculated from, $O.D.T_{99}-O.D.T_{62}/O.D.T_{99}-O.D.T_{99} \times Hy \times 100$

The estimate of the % reassociation at a given C_0t value was the mean of three samples. The percentage reassociation was plotted against the corresponding log C_0t values.

For low C_0t values the reassociation could be followed in the spectrophotometer itself. In this case the DNA was denatured in a waterbath at 100° C. For rapid cooling to the criterion temperature it was passed through a water-jacketed syringe needle to the spectrophotometer cell, maintained at 62° C. The first readings could be taken after 30 seconds. Subsequent readings were recorded at regular intervals by switching the spectrophotometric system to the automatic mode.

Results

A. DNA Melting Profiles and the Base Ratio

The melting profile of the DNA, measured as the hyperchromicity, is a cumulative plot representing DNA segments differing in average base composition. When measured under strictly identical ionic conditions comparisons among the melting profiles of the DNA from different species give information not only about the base ratio (the proportion of the G+C content) but, as well, about any heterogeneity in the dispersion of the base pairs within the DNA from different species. The melting profile of *L. tingitanus*, which is typical, is given in Fig. 3. The curve

Table 1. The GC ratios of the DNA among the *Lathyrus* species

Species	From T_ms	From the buoyant densities
L. articulatus	38.06	37.76
L. nissolia	38.06	—
L. ochrus	40.50	—
L. clymenum	39.30	—
L. cicera	39.28	37.80
L. sativus	41.50	39.79
L. tingitanus	39.28	37.75
L. hirsutus	38.06	36.70

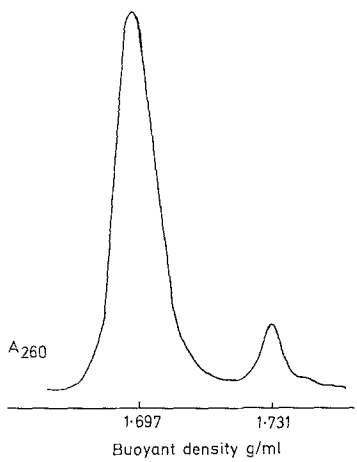

Fig. 4. The density gradient profile of the DNA of *L. tingitanus*. The marker DNA (*Micrococcus lysodeikticus*) has a buoyant density of 1.731 gm/ml

is steep and smooth indicating a large measure of homogeneity in the dispersion of the AT and GC base pairs within the complement.

From the T_ms (the temperature at which 50% of the DNA is rendered single stranded) the estimated GC ratios (expressed as % GC) are given in Table 1. As will be seen the eight species studied show virtually identical melting profiles with the T_m's falling within the range 69.5° C to 71° C and the GC ratios clustered closely around the mean value of 39.25%. The mean base compositional heterogeneity, which reflects the degree of dispersion, was 13.2% GC. There was no significant variation between species.

On grounds of base ratios and of base pair distribution, as adduced from the melting profiles, the fraction involved in the quantitative DNA changes associated with speciation is indistinguishable from that of the DNA complement as a whole.

B. Caesium Chloride Density Gradient Analysis

DNA from 5 of the *Lathyrus* species, representing a range of nuclear DNA amounts, was centrifuged to equilibrium on neutral caesium chloride. As could be expected from the melting profiles, the DNA main peak in each case was narrow

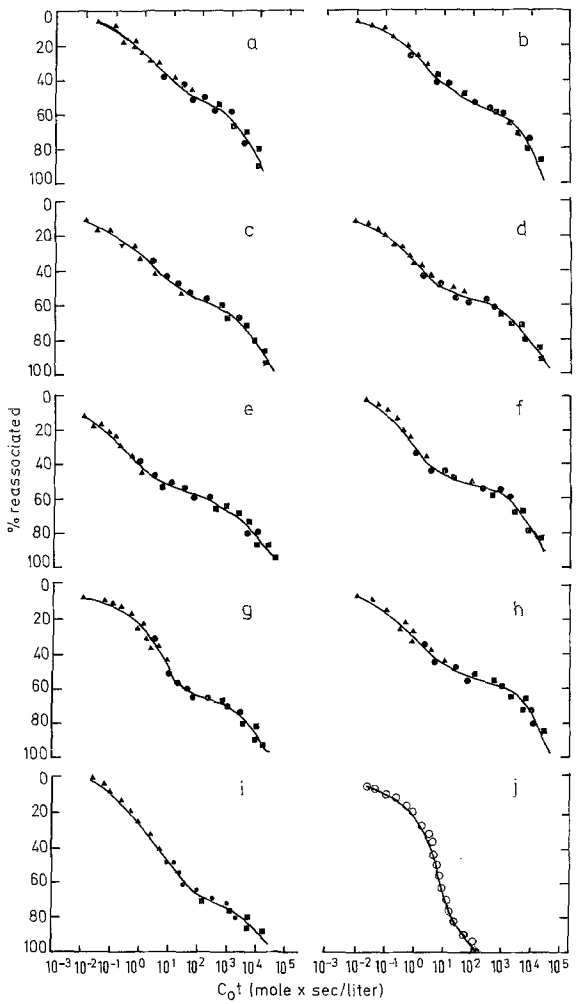

Fig. 5 a—j. The C_0t reassociation curves for the DNA of 9 *Lathyrus* species and for *E. coli*. (a) *L. articulatus*. (b) *L. nissolia*. (c) *L. clymenum*. (d) *L. ochrus*. (e) *L. aphaca*. (f) *L. cicera*. (g) *L. sativus*. (h) *L. tingitanus*. (i) *L. hirsutus*. (j) *E. coli*. Initial DNA concentrations were ▲ 5–200 μg/ml, • 0.5 to 1.2 mg/ml, ■ 2.8 to 5.6 mg/ml

and symmetric and without any suggestion of a satellite (Fig. 4). Buoyant densities were calculated with reference to the marker DNA (Mandel *et al.*, 1968). These provided alternative estimates of GC ratios (Table 1) which quite closely corresponded with those based on the T_m's.

C. The C_0t Reassociation of the DNA

The C_0t curves for each of the nine *Lathyrus* species along with *E. coli* for comparison are presented in Fig. 5. Between 5% and 13% of the total DNA is reassociated before C_0t 10^{-2}. This, the "fast" fraction, is made up of very highly

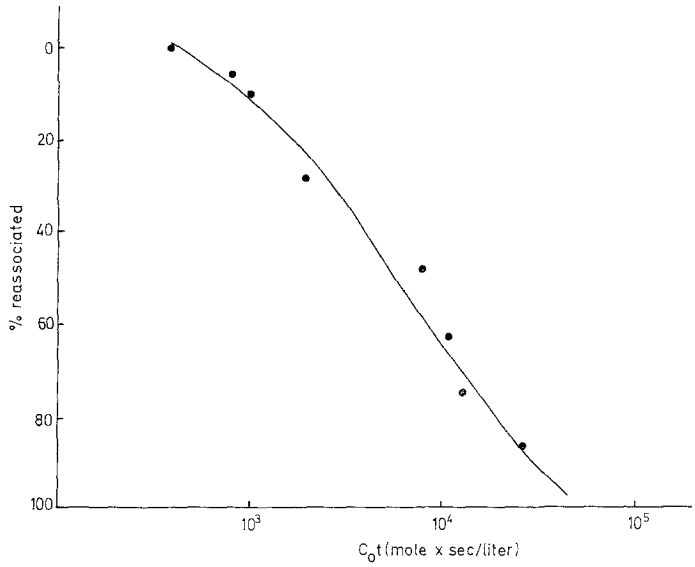

Fig. 6. The reassociation kinetics of the non repetitive fraction in *L. articulatus*. The last 44% in C_0t curve 5(a) was normalised to 100%. The $C_0t_{1/2}$ was 7,000 and the apparent rate constant $K_2 = 14.28 \times 10^{-5}$

repeated sequences-repeated to the order of a million or more. A moderately fast fraction reassociates between C_0t^{-2} to $C_0t\ 10^3$ although in all species the reassociation between $C_0t\ 4 \times 10^2$ and $C_0t\ 10^3$, involves only about 2% of the total DNA.

The non-repetitive component which reassociates at $C_0t\ 10^3$ and beyond follows a second order kinetics in its reassociation (see Fig. 6). The highest percentage reassociation achieved was 94.6. The C_0t plot was extrapolated on the theoretical assumption that under ideal conditions the reassociation would proceed to completion. The complexities of the non-repetitive components appear in Table 2. The average complexity for the nine species, which is an estimate of the total amount of the non-repetitive DNA, is 5.56×10^9 base pairs, equivalent to 5.25×10^{-12} g. This corresponds very closely with the 'analytical' estimate of non-repetitive DNA obtained directly by substracting the amount of repetitive DNA from the total. The ratio of the analytical to the kinetic estimates approximate to unity in all species (Table 2) and serves to confirm that the non-repetitive fraction estimated by the present technique is indeed made up of single copy DNA.

Variation Between Species

While the C_0t curves show, as might be expected, a general similarity there are nevertheless well defined differences in the composition of the DNA from the different species.

a) Repetitive and the Non-repetitive DNA. Table 2 and Fig. 7 show that in absolute terms the amount of repetitive DNA varies between species. The amount of non-repetitive DNA remains relatively constant. The data also show that the

Table 2. Repetitive ,non repetitive DNA and heterochromatin in *Lathyrus* species

Species	Total nuclear DNA $\times 10^{-12}$ g	Repetitive DNA		Non repetitive DNA			Heterochromatin as % of total nuclear area
		As % of the total DNA	As absolute amount $\times 10^{-12}$ g	Analytical estimate $\times 10^{-12}$g	Kinetic estimate $\times 10^{-12}$g[a]	Analytical estimate/ kinetic estimate	
L. articulatus	12.45	56	6.98	5.48	4.90	1.12	10.76
L. nissolia	13.20	59	7.79	5.41	5.11	1.06	—
L. clymenum	13.75	62	8.52	5.23	5.25	0.99	15.62
L. ochrus	13.95	60	8.37	5.58	5.60	0.99	—
L. aphaca	13.97	63	8.80	5.17	4.90	1.05	—
L. cicera	14.18	58	8.22	5.96	5.60	1.06	—
L. sativus	17.15	66	11.90	5.23	4.90	1.07	25.95
L. tingitanus	17.88	59.5	10.95	6.93	5.95	1.23	29.00
L. hirsutus	20.27	70.0	14.10	6.17	6.74	0.91	30.25

[a] Based on the $C_0t_{1/2}$ values for the non-repetitive fraction, using *E. coli* (genome size 4.6×10^6 base pairs) as a standard, which by the present procedure has a $C_0t_{1/2}$ of 6. Converted to picograms using the constant 0.913×10^9 base pairs per picogram (Britten and Davidson,1971).

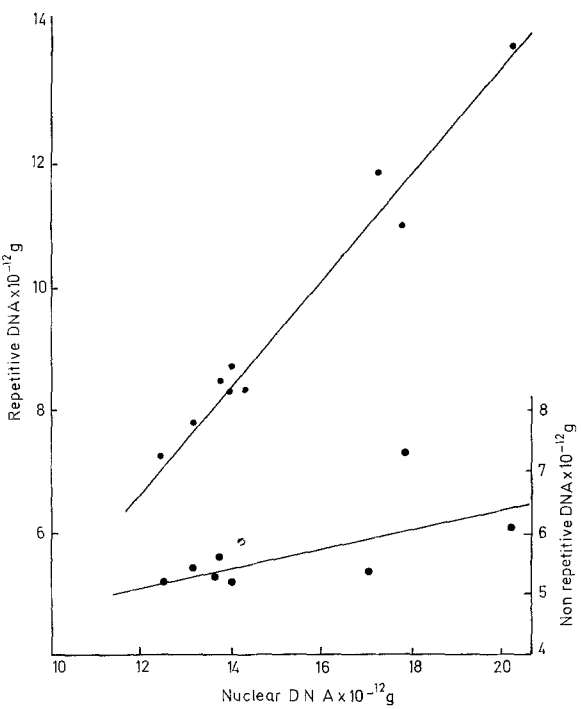

Fig. 7. The amount of repetitive and non repetitive DNA in *Lathyrus* species plotted against the total nuclear DNA amount

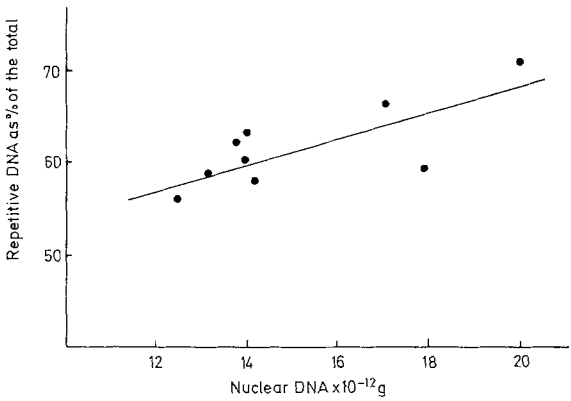

Fig. 8. The proportion of the repetitive DNA in 9 *Lathyrus* species plotted against the total nuclear DNA amount

absolute amount of repetitive DNA, and with it the proportion, increased with increasing total nuclear DNA amount (Fig. 8). The correlation is significant ($P = < 0.001$). In contrast the mass of the single copy DNA shows no significant correlation with total DNA amount although there is a slight increase in species with high nuclear DNA content. We conclude that the quantitative DNA variation associated with speciation in *Lathyrus* was mainly attributable to variation in the repetitive component.

b) Fast vs. Moderate Components. Table 3 shows a variation among species with respect to the relative amounts of fast and moderate components. The amount of the fast reassociating component, however, is unrelated to the variation in the total DNA amount, from which it may be inferred that the moderate fraction was chiefly implicated in the quantitative nuclear DNA changes.

c) The Moderate Component. Further information about the qualitative differences in the composition of the nuclear DNA from the different species comes from examining the rates of reassociation within the moderately fast reassociating DNA. In Table 3 are the $C_0t_{1/2}$ values for the DNA reassociating between $C_0t\ 10^{-2}$ and $C_0t\ 10^3$, *i.e.* omitting the fast and single copy DNA. These values were obtained by normalising to 100% the re-association data over the range $C_0t\ 10^{-2}$ to $C_0t\ 10^3$ (*cf.* Laird and MacCarthy, 1969). They range from $0.65 \times 10^\circ$ in *L. aphaca* to $2.0 \times 10^\circ$ in *L. hirsutus*. The higher value for *L. hirsutus* reflects a greater heterogeneity between the repetitive DNA sequences. The heterogeneity in turn is probably attributable to base substitutions or rearrangements following the divergence of species to a degree that engenders mismatching and hampers the reassociation of the denatured DNA (Walker, 1969; Southern, 1974). Translated to complexity as an index of divergence between the repeated sequences *L. hirsutus* shows, on average, a threefold increase in comparison with *L. aphaca*.

Put in another way, the results imply that the moderate fraction in *L. aphaca*, as compared with *L. hirsutus*, comprises a smaller variety of sequences repeated to a greater degree. More detailed information about the composition of the repeated sequences is obtained from fractionation on hydroxyapatite and the cross

Table 3. The variation in the repetitive DNA among *Lathyrus* species

Species	Fast fraction		Moderate fraction			Complexity as number of base pairs
	As % of the genome	As absolute amount $\times 10^{-12}$ g	As % of the genome	As absolute amount $\times 10^{-12}$ g	$C_0t_{1/2}$	
Group A						
L. ochrus	7	0.98	53	7.39	0.70×10^0	0.53×10^6
L. aphaca	13	1.81	50	6.74	0.65×10^0	0.50×10^6
L. cicera	5	0.71	53	7.51	0.70×10^0	0.53×10^6
Mean	8.33	1.17	52	7.21	0.68×10^0	0.52×10^6
Group B						
B_1						
L. articulatus	5	0.62	51	6.35	2.00×10^0	1.55×10^6
L. nissolia	8	1.06	51	6.73	2.00×10^0	1.55×10^6
L. clymenum	11	1.51	51	7.01	1.50×10^0	1.15×10^6
Mean	8.00	1.06	51	6.70	1.83×10^0	1.42×10^6
B_2						
L. sativus	8	1.37	58	9.94	2.00×10^0	1.55×10^6
L. tingitanus	8	1.43	52	9.29	2.00×10^0	1.55×10^6
Mean	8.00	1.40	55	9.62	2.00×10^0	1.55×10^6
B_3						
L. hirsutus	8	1.62	62	12.56	2.00×10^0	1.55×10^6

reassociation of the DNA fractions from the different species. These details will be discussed in a later publication. For the moment, it will suffice to emphasise the changes in DNA base composition, as well as quantity which accompany the divergence and evolution of *Lathyrus* species.

Discontinuity

It will be observed that the nine species fall into two sharply defined classes on the basis of the complexity of the moderately repetitive DNA fraction. The first (A) includes *L. ochrus*, *L. aphaca* and *L. cicera* with DNA of conspicuously similar low complexity. In the remaining six species (B) the DNA complexity is again remarkably uniform and almost exactly three times greater than that of the species in A. One species, *L. clymenum*, does in fact differ from the others in B in having a lower complexity, 1.15 as distinct from a consistent 1.55 in all others. This, however, does not detract from the element of discontinity. Indeed one might well be justified in putting *L. clymenum* in a separate group, having exactly twice as opposed to three times the DNA complexity of species in A. There are, in short, indications not only of discontinuity but, also, of a surprisingly regular series of short steps which make up the discontinuous series.

The six B species, those with high DNA complexity, are in turn separable on the basis of the quantity of the moderate fraction. Species in B_1 have low DNA amounts similar to species in A. In *L. sativus* and *L. tingitanus* (B_2) the

DNA amount is increased by 3×10^{-12} g. The DNA amount in *L. hirsutus* (B_3) is again increased by 3×10^{-12} g relative to B_2.

The regularity and the discontinuity in the pattern of variation in respect both of quantity and quality are, on the face of things, suggestive of evolution by a series of spasmodic changes as distinct from a continuing and continuous progression. Spasmodic changes in nuclear DNA, amount are readily conceivable (Bolton *et al.*, 1967; Britten and Kohne, 1968; Walker, 1971). Where these involve distinctive base sequences they will, at the same time, affect the overall composition of the chromosomal DNA. It is difficult, however, to envisage bursts of mutations causing sudden alteration in DNA base composition in the absence of change in DNA amount. It is worth considering an alternative approach to interpreting the discontinuities in respect of DNA complexity among the *Lathyrus* species with comparable DNA amounts, namely that they be viewed from the standpoint of "steady states" in terms of genetic balance within the nucleus and, in a wider sense, fitness in the context of selection and adaptation.

Progression

If we assume that increasing complexity is a consequence of mutational events in the form of base substitutions or rearrangements superimposed upon a common ancestral DNA complex we may tentatively conclude the species in B to derive from species of the kind represented in A.

It will be recalled that quantitative nuclear DNA increase is mainly accounted for by increase in this same moderate fraction under consideration (see Table 3). The fact that DNA increase from B_1 to B_3 is independent of changes in complexity, which remains more or less constant (Table 3), suggests proliferation of a random as distinct from a specific component of this moderate fraction.

It is of interest that a comparison based on a multivariate analysis of nuclear characters (in particular, the relative chromosome lengths within complements and the average chromatid width at metaphase) showed that species within each of the groups A, B_1, B_2 are closely related while the groups themselves are sharply distinguished from one another (Narayan unpublished). In this sense there is a parallel between the divergence in molecular terms and in terms of the nuclear phenotype. We have no evidence that the parallel extends to other morphological aspects of plant development.

D. Heterochromatin

The amount of heterochromatin in five species, expressed as a percentage of the total nuclear area, is given in Table 2. It varies substantially between species. Fig. 9 shows a positive correlation between heterochromatin and the total nuclear DNA content among the species. The regression is significant at the 0.1% level. Quantitative DNA change evidently involves disproportionate change in heterochromatin as compared with euchromatin. Fox (1972) showed the same to apply in the genus *Dermestes*.

Fig. 10 shows the proportion of heterochromatin plotted against the amount of repetitive and also of non-repetitive DNA in the different species. There is a positive correlation between heterochromatin and repetitive DNA but not with

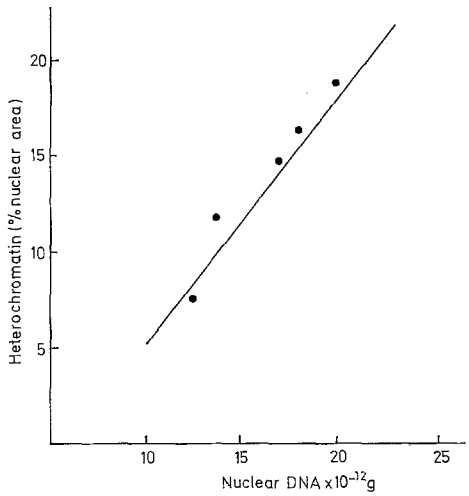

Fig. 9. The proportion of heterochromatin plotted against the nuclear DNA amounts

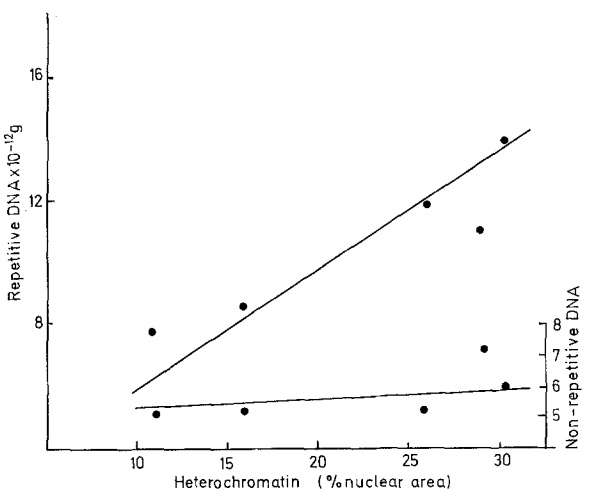

Fig. 10. The amount of repetitive and of non repetitive DNA in 5 *Lathyrus* species plotted against the proportion of heterochromatin at interphase in root meristem nuclei

the non-repetitive fraction. It appears that the repetitive DNA implicated in quantitative DNA change is at least largely located in heterochromatin.

Summary and Conclusions

(1) Increase in nuclear DNA within the genus *Lathyrus* is accompanied by an increase in the proportion of the total DNA made up of repetitive sequences, particularly of the moderately repetitive fraction. The amount of the non-repetitive DNA is more or less constant among species so that in proportional terms it decreases with increase in total DNA. These findings are in agreement or at least

compatible with the results of surveys among salamanders by Mizuno and McGregor (1974) and among flowering plant species drawn from various genera by Smith *et al.* (1974). They are compatible also with the view that the non-repetitive, single copy DNA incorporates at least a large proportion of the structural genes, in the sense that we would perhaps expect this fraction to be less tolerant of large scale quantitative change.

(2) Our survey indicates that while there is a wide range of variation in DNA amount and composition within *Lathyrus* species there is an element of discontinuity with respect to both aspects of the variation. The discontinuity may, on the one hand, reflect change due to sudden spasmodic events during the course of evolution or, on the other hand, denote 'steady states' to be viewed from the standpoint of genetic balance and of biological fitness.

(3) In cytological terms increase in the total DNA is associated with a disproportionate increase in heterochromatin as distinct from euchromatin. There are also indications that the heterochromatin is at least partly made up of repetitive DNA sequences although in *Lathyrus*, unlike mouse and *Drosophila* (Walker *et al.*, 1969; Travaglini *et al.*, 1972), such repetitive DNA is not distinguishable as a satellite on centrifugation in a neutral caesium chloride gradient.

It is clear, however, that while heterochromatin, or more strictly the constitutive heterochromatin, is associated with a repetitive DNA organisation, the reverse is not necessarily true, that is to say species with a very high degree of repetitive DNA, such as wheat (Bendich and McCarthy, 1970), do not necessarily show detectable heterochromatin. It may well be of course that the reason for this is a dispersion and dilution of the heterochromatin along the chromosomes. Another possibility, however, is that the intense coiling which is the basis of heterochromatinisation demands the juxtaposition of particular base pairs acting as recognition sites for the agents (histones ?) required for the promotion of coils, sites such as presumably exist in those chromosomes which are facultatively as distinct from constitutively heterochromatic like the X chromosomes or whole paternal complements in female mammals and male mealy bugs respectively (Lyon, 1970; Brown and Nur, 1964).

(4) The variation between species in the relative proportions of repetitive and non-repetitive fractions of the chromosomal DNA rules out the possibility of quantitative change due to a variation in the number of DNA strands within chromosomes *i.e.* a differential polynemy (Martin and Shanks, 1966). It also eliminates the possibility that, in *Lathyrus*, the change in DNA amount is caused by end to end duplication of each of the chromosomes within the complement (Sparrow and Nauman, 1973). This is not to say that Sparrow and Nauman's proposal may not be valid in other families *e.g.* in the Gramineae with which they were particularly concerned. The answer is, however, readily amenable to verification by surveys of the kind we have described.

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