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Reassociation Kinetics and Cytophotometric Characterization of Peanut (*Arachis hypogaea* L.) DNA[1]

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ABSTRACT

The base composition of peanut (var. NC-17) DNA determined from thermal denaturation profiles showed an average guanine plus cystosine content of 34% which was in close approximation to 36% guanine plus cytosine calculated from the buoyant density. Buoyant density also indicated the absence of satellite DNA. The genome size, 2.0 × 10⁹ base pairs, as determined by reassociation kinetics of the single copy DNA was close to the genome size determined by cytophotometry, 2.1 × 10⁹ base pairs. Peanut DNA averaging 450 to 600 base pairs long, reassociated in phosphate buffer and fractionated by hydroxylapatite, indicated a DNA genome composition of 36% nonrepetitive or single copy DNA; reassociation in formamide and followed by optical methods indicated the repetitive DNA possesses highly repeated, intermediately repeated and rarely repeated components of DNA with DNA sequences repeated on the average about 38,000, 6,700, and 200 times each. Different criteria of reassociation in formamide revealed further subdivisions of these four separate components of DNA. The DNA of above mentioned NC-17 variety compared to Florigiant variety showed no differences in thermal denaturation profiles, buoyant density, or in genome size.

Most eukaryotic organisms possess more nuclear DNA than is apparently required for genetic functions (35). Some of this DNA is repetitive or repeated DNA. The amounts of repetitive DNA in higher plants generally vary between 50 and 80% (13, 17, 29, 46). In spite of the postulated important role of repetitive DNA in gene regulation (6, 10), the genomes of only about 20 plants are characterized with the use of reassociation kinetics (47).

The reassociation kinetics are often performed without cytophotometric estimation of amounts of cellular nuclear DNA. For example, studies performed with the use of reassociation kinetics indicated over 1.4-fold difference in repetitive fractions as well as in the genome size of soybean (*Glycine max*) DNA (17, 20) and unbelievable difference of 9.2-fold in the genome size of pea (*Pisum sativum*) DNA (31, 32); these large differences suggest that estimation of genome size by reassociation kinetics should be accompanied by another technique such as cytophotometry.

The present study incorporates cytophotometric and reassociation kinetic characterization of peanut DNA (*Arachis hypogaea* L.), a plant whose DNA characterization has not been previously studied to our knowledge. Attempts are also made to compare the DNA of two varieties (NC-17 and Florigiant) which differ in external characters such as leaf size and time of maturity.

MATERIALS AND METHODS

The DNA was extracted from mature leaves of either greenhouse or field-grown plants of peanut (*A. hypogaea* L.) var. NC-17 and Florigiant. The cytophotometric study was performed on germinating root or shoot meristems of 4-day-old embryos, as well as on mature leaf tissue.

DNA Extraction. DNA was extracted from leaves by a modification of the urea phosphate procedure (9) which considerably improved the yield and purity of the DNA. One hundred g of leaves were frozen and ground to a powder in the presence of dry ice using a cold mortar and pestle. The finely ground powder was thawed with the gradual addition of 100 ml of urea phosphate (8 M urea + 0.24 M phosphate [pH 6.9]) and 10 ml (0.1 of urea phosphate volume) each of 20% SLS[3] and 0.1 M Na EDTA (pH 4.4). The homogenate was centrifuged and the supernatant containing the DNA saved. The tissue was refrozen, reground and reextracted with urea phosphate, SLS, and EDTA. This procedure was repeated four times to obtain complete lysis of cells. The supernatants from all four extractions were pooled and an equal volume of phenol and chloroform (1:2 mixture) was added to denature and precipitate the proteins. The DNA was selectively removed from the extracted cellular lysate with HAP by gently shaking and centrifuging. The amount of HAP (0.25–0.20 of the volume of lysate) used was critical to assure binding of all the DNA to HAP. The HAP which now contained the DNA was washed with urea phosphate to elute RNA, denatured proteins, polysaccharides, nucleotides, etc. The urea phosphate was washed from the HAP with washes of 30 mM PB (pH 6.9). The DNA was finally eluted from the HAP with washes of 0.42 M PB (pH 6.9). The DNA was dialyzed against 0.6 M Na-acetate and concentrated by precipitation with two volumes of ethanol.

The DNA pellet was resuspended in 0.1 × SSC (1 × SSC is 0.15 M NaCl, 15 mM sodium citrate [pH 7.0]). DNA purity was tested by its 260/280 nm *A* ratio and its hyperchromicity. The above extraction procedure routinely yielded about 0.15 mg of purified peanut DNA/g tissue (260/280 nm ratio of 1.9 or higher, thermal hyperchromicity of 25% or more [9]).

The chloroplast DNA did not appear to present a serious problem as our preliminary studies indicated roughly a 4% contribution from chloroplast DNA which was measured in a chloroplast fraction. Also, the chloroplast DNA is largely in repetitious fractions and would not appreciably affect genome size as determined from single copy DNA kinetics. This supposition is sup-

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[3] Abbreviations: SLS: sodium lauryl sulfate; HAP: hydroxylapatite; PB: phosphate buffer; SSC: standard saline citrate; Cot: initial concentration of DNA in mol of nucleotide per liter multiplied by time in s; Tm: melting temperature for half-strand separation; RBC: red blood cells.

ported by the cytophotometrically measured genome size which is virtually the same as the genome size determined by reassociation kinetics.

ANALYSIS OF DNA COMPOSITION

Thermal Denaturation. Thermal denaturation or melting profiles were obtained according to the method of Mandel and Marmur (27). DNA samples in 0.1 × SSC, within the concentration range of 25–50 μg DNA/ml (9), were denatured in standard silica cuvettes of 1-cm light path. The hyperchromicity due to the denaturation was monitored in a Shimadzu double-beam spectrophotometer model UV-210A. The hyperchromicity at 260 nm was determined at each temperature rise of 1 C. The per cent hyperchromicity *versus* temperature was plotted on normal probability paper. Use of normal probability paper facilitated rapid determination of the midpoints (Tm) of the hyperchromatic rise due to thermal denaturation (24). Calculation of base composition was made from Tm values using the equation of Mandel and Marmur (27).

Buoyant Density Determination. DNA (~50 μg) was dissolved in 0.5 ml CsCl (0.1 × concentration of lightest density 1.4969 g/ml) buffered with Tris-HCl (pH 8.5), and laid on the top of density gradient prepared by layering 2 ml each of four concentrations of CsCl (densities 1.4969–1.8310 g/ml) in Beckman plastic centrifuge tubes (⅝ × 3 inch). Samples were centrifuged to equilibrium in CsCl solution using a Beckman, L2-65B preparative ultracentrifuge with a fixed angle rotor at 40,000 rpm for 66 h at 25 C. Fractions were collected for 260 nm *A* readings and refractive indices were read from every fifth tube to calculate density values (Fig. 1) (21). The guanine plus cytosine content of the DNA sample was calculated according to the method of Szybalski (41).

SHEARING AND SIZING OF DNA

The DNA was sheared at 50,000 rpm for 50 min with a VirTis 60 homogenizer according to the method of Britten *et al.* (9). Electrophoretic migration compared to markers of known molecular size indicated that this procedure resulted in fairly uniform fragment sizes of 450–600 nucleotide pairs long. It was important to purify the DNA after shearing. Severely damaged DNA did not bind to HAP and could represent well over 25% of the total material present; this was found particularly to be the case when large amounts of DNA were sheared at one time. Less than 1 mg of total DNA is suggested for 10 ml of 0.6 M Na-acetate DNA solution. Twenty ml glycerine was added and the DNA sheared in the VirTis 16-117 cup, cooled with a dry ice-ethanol bath. The sheared DNA was purified by binding to and elution from a HAP column. After such purification, the DNA showed thermal denaturation characteristics similar to unsheared native peanut DNA indicating the absence of denatured or severely damaged fragments.

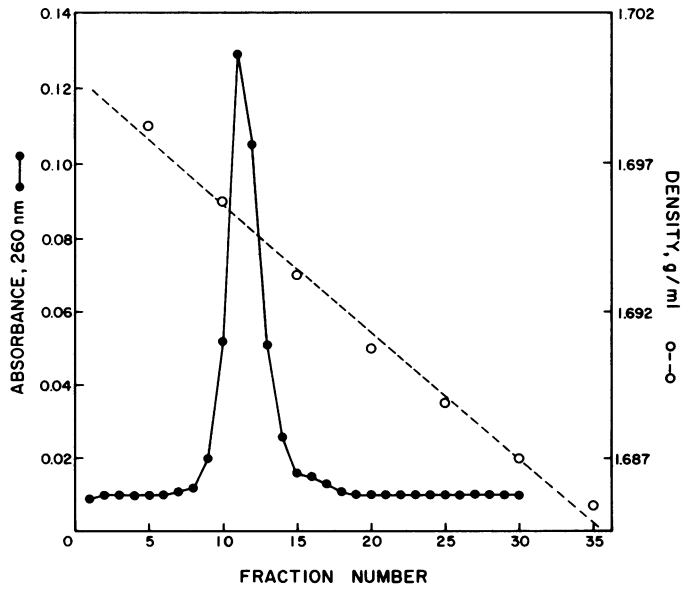

FIG. 1. The cesium chloride buoyant density of a sample of peanut (NC-17) DNA. The bulk DNA peak corresponds to a density of 1.695.

DNA REASSOCIATION

Reassociation in Formamide. Samples of DNA were denatured in 5 × SSC 50% formamide at 80 C for 2.5 min. The Tm of peanut DNA in this mixture was 60 C. The reassociation was carried out in a Shamadzu digital double-beam spectrophotometer model UV-210A equipped with a water-jacketed sample compartment. The denatured DNA was immediately loaded into a cuvette whose temperature was controlled at 31, 35, or 40 C by the water-jacketed compartment. The extent of reassociation was followed by the hypochromicity at 270 nm (28, 34). The DNA concentrations usually varied between 20 and 90 μg/ml and silica cuvettes used were of 1.0 cm light path.

The calculation of the extent of reassociation depends critically upon the estimation of the initial or completely denatured DNA optical density and the estimation of the final or completely reassociated DNA optical density. The initial values are directly determined by thermal denaturation of the DNA and its subsequent rapid (<2 min) cooling to the reassociation reaction temperature. Upon cooling there is a rapid decrease in *A* due to reformation of strand base stacking. The base stacking decrease in *A* occurs before the DNA sample reaches within 10 C of the reassociation reaction temperature. Thus, the true initial *A* is established before measurable reassociation (<1%) has a chance to occur. In practice one sees a rapid drop in *A* followed by a plateau in *A* as the temperature drops further toward the reassociation reaction temperature. Finally there follows a drop in *A* due to DNA reassociation. Bendich and Anderson (2) noted the same *A* decrease and subsequent plateau.

The final reassociation *A* is the starting *A* of the native DNA before denaturation is assumed and represents the completely reassociated state. A change in the *A*, from this starting point to the initial reassociation *A* described above, occurs upon heating and rapid cooling. The change in *A* is considered the maximum decrease in *A* that could occur if the single-stranded DNA ever completely reassociated. The ratio of *A* decrease at any time after the initial reassociation *A* to the maximum possible decrease in *A* is a measure of the extent of reassociation. All reassociations we could observe after the initial reassociation *A* were DNA concentration dependent.

Reassociation in Phosphate Buffer. The DNA samples 450–600 nucleotide base pairs long were denatured (100 C, 4 min) and reassociated in screw top vials in 0.14 M PB at 50 C (9), which was 29 C below the Tm in this buffer. *Escherichia coli* DNA of the same fragment size and in the same buffer had a half reassociation value of Cot = 2.7 when it was incubated at 60 C, or 29 C below its Tm in this buffer. The DNA concentrations varied between 45 and 2,350 μg/ml. The samples were collected at various times, diluted to 30 mM PB and passed over a HAP column at 50 C. The concentration of 30 mM PB for the samples containing DNA was found to be critical and provided the best compromise between binding and release of DNA from the HAP column. The unreassociated single-stranded DNA and the reassociated duplexes were eluted with 0.14 M PB and 0.42 M PB solutions, respectively (9). With the above procedure using 450–600 base-pair-long fragments most of the DNA (95 ± 1%) was recovered from the HAP column.

Therefore, aggregate formation was a very minor problem if at all. With the fragment size used, tails did not appear to affect appreciably our reassociation results, because the presence of tails should lead to aggregate formation which in pea contributed 30–40% aggregates and in calf thymus 10% aggregates (43). The DNA content of each fraction was determined spectrophotometrically at 260 nm and the percent reassociation of the DNA calculated (9).

The data for the reassociation was plotted as the log of the ratio of the concentration of unreassociated DNA to the concentration of reassociated DNA (Cu/Cr) against the log of the Cot value (8). The application of the log-log plot to the Cot curve data yields distinct straight lines (Fig. 2) (33, 34, 36) and offers more precise analysis than the conventional semi-log plot (Fig. 3) (34).

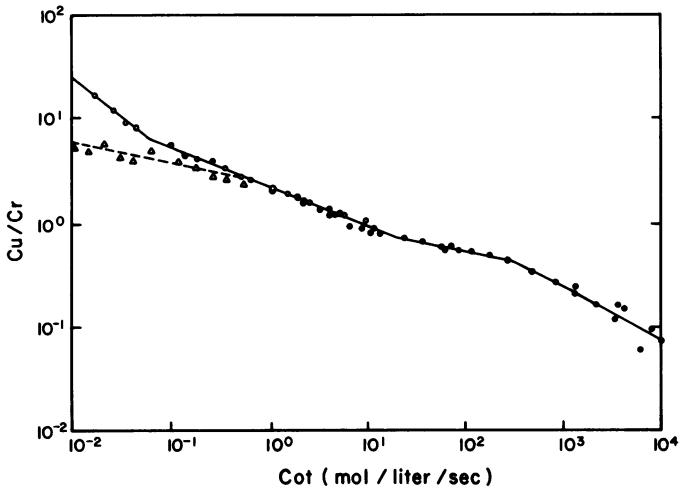

FIG. 2. Reassociation kinetics of peanut DNA sheared to 450–600 nucleotide fragment length. Reassociation at 29 C below the Tm. Optically monitored reassociation (○); HAP monitored reassociation (●). The line drawn is the best fit to the points by the power curve best fit method (34). The correlation coefficient of each line is listed in Table II. The broken line representing HAP monitored reassociation at very low Cot values had 11 points (▲) and correlation coefficient of 0.92; the large differences from optically monitored reassociation, not obvious at higher Cot values are discussed in the text.

The Cu/Cr plotting is very powerful in the rapid determination of incorrectly assigned initial or final reassociation values. Error as low as one part in 500 in estimation of the initial reassociation A will form a badly but characteristically skewed reassociation curve. The skewness is observed in the first 2% of the reassociation but has little effect on the percentage values for later reassociation. The same sensitivity is found in the estimation of the final reassociation A on the last 2% of the reassociation. Of course, larger per cent errors add greater effects, *i.e.* a 10% error in estimating the final A will skew the reassociation curve beyond the point of 50% reassociation of the DNA but has little effect on the first 50% reassociation values. The skewness of the reassociation lines is so characteristic by deviating at right angles from the straight line and occurs so abruptly that misinterpretation is difficult. Thus, in effect, reassociation of greater than 2% or less than 90% of the DNA can be precisely determined.

The best straight line fit to the data points was determined by a power curve fit to the data points. The correlation coefficient and its significance level for each of the straight lines was also calculated. The resulting series of lines depicting the reassociation of the DNA is in reality the result of a series of concurrent reassociations of the different fractions of the DNA. Each straight line generated by the data points does not represent a pure separate fraction of the DNA that is so reassociating; rather, because all components of DNA are reassociating all the time, the apparent values are weighted averages of all reassociations. Some experimentation will allow the determination of the number of fractions of the DNA and the characteristics of each fraction (per cent of the total DNA and one-half reassociation, ½ Cot) such that these hypothetical fractions all together will approximate the straight lines generated by the data. Such approximations allow assignment of rough estimations of the size and repetition of the various fractions of DNA.

A measure of how well the proposed theoretical DNA fractions generate the experimental data can be calculated. One can determine within what confidence limit to the experimental line does the theoretical line fall. The Student t value is calculated from the equation:

$$t = \frac{\ln Cu/Cr_0 \text{ theoretical} - \ln Cu/Cr_0 \text{ experimental}}{\sqrt{\frac{(1-r^2)\sum y^2}{n-2}\left(\frac{1}{n} + \frac{(\ln Cot_0 - Cot)^2}{\sum x^2}\right)}}$$

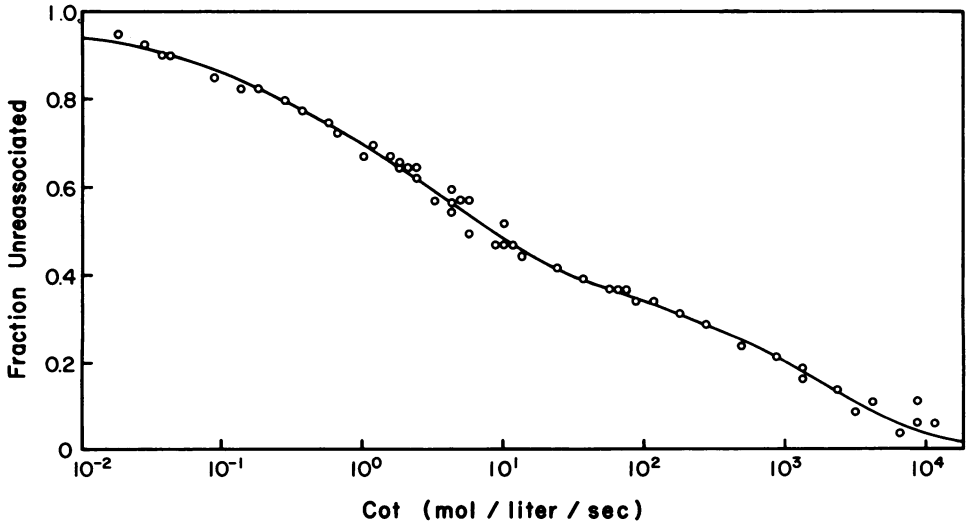

FIG. 3. Reassociation kinetic data of Figure 2 (used to depict DNA components) drawn in a semi-log plot. The line drawn is the best least-squares fit to the data.

where n = number of points used to generate experimental line; r^2 = correlation coefficient; $\overline{\text{Cot}} = \frac{\sum \ln \text{Cot}}{n}$; $\sum x^2 = \sum \ln \text{Cot}^2 - \frac{(\sum \ln \text{Cot})^2}{n}$; $\sum y^2 = \sum \ln \frac{Cu}{Cr} \exp^2 - \frac{\left(\sum \ln \frac{Cu}{Cr} \exp\right)^2}{n}$.

The confidence limit describes the area where one can expect experimental points to lie. Thus, if a point is very close to the experimental line, it may lie within the 50% confidence limit—*i.e.* 50% of the points will be closer to the line. If the point is at a greater distance, the confidence limit may rise to 99%; *i.e.* only 1% of the experimental points can be expected to fall at a greater distance from the experimental line.

This measure of closeness of fit is thus a function of two values, actual closeness of the theoretical line to the experimental line as well as the dispersion of the experimental data (correlation coefficient, r). Thus lines can fit very closely one to another but because the r value is very high, the theoretical line can lie at a very high confidence limit. As reported here, all the theoretical lines fall within the 80% or better confidence limit to the experimental lines over most of the course of reassociation (34).

Although the optical reassociation is reported to deviate from second-order kinetics (7, 39), the reassociation of *E. coli* DNA under similar conditions made it possible to superimpose the results of optical to HAP chromatography data. Inasmuch as *E. coli* DNA reassociated at 29 C below its Tm in phosphate buffer, analyzed by HAP chromatography, with a ½ Cot of 2.7 and in 5 × SSC 50% formamide, analyzed by hypochromicity, with ½ Cot of 2.5, the two reassociation kinetic patterns of peanut DNA could be superimposed by shifting the formamide results (multiplying the Cot values) by a factor of 1.08 (2.7/2.5). The closeness of superposition may be seen in Figure 2. All other formamide reassociation kinetic patterns were modified by the same factor so as to be comparable, one to the other.

FEULGEN CYTOPHOTOMETRY

The DNA content per nucleus was measured from germinating root- and shoot-meristem nuclei, and leaf nuclei of peanut. Fixation was in Carnoy's No. 2 (ethyl alcohol-chloroform-glacial acetic acid, 6:3:1) for 1 h. The material was then transferred to 70% ethyl alcohol and kept in a refrigerator. As described previously (4), the peanut nuclei were spread gently on the slides that also contained chicken (female Red Jungle Fowl, *Gallus gallus*) RBC which served as an internal standard to reduce staining variation and to facilitate the calculation of the amount of DNA in pg (11).

The internal standard dry mass DNA quantitation was determined by interference microscopy using a Vickers M86 scanning microdensitometer-interferometer which is equipped with helium neon laser, a motor-driven compensator, and reference voltage to automatically integrate electrical pulses and display optical path differences. The optical path differences were measured before and after DNA extractions of the same RBC nuclei. The DNA was extracted using 0.5 N $HClO_4$ (11.7 ml 70% $HClO_4$ in 238.3 ml water) at 70 C for 40 min. $HClO_4$ extraction revealed similar results to DNase extraction. For DNase treatment blood smear slides were incubated at 37 C for 4 h in 0.4 mg of DNase (Sigma, lot 127C-0346) per 1 ml of 30 mM $MgSO_4$, adjusted to pH 6.0 with 0.1 N NaOH. After recording the differences (δ) in optical path difference of original and extracted readings, the actual dry mass in pg was calculated from the following equation: $M = \delta A/x$, where A = area of nucleus and x is a constant (0.18). The DNA dry mass/RBC nucleus was 3.22 pg, which falls into the reported average value of 2.5–3.5 pg (1, 38).

For the Feulgen method, Schiff's reagent was prepared according to Berlyn and Miksche (4) and the Feulgen staining procedures were essentially those of Leuchtenberger (26) except for the use of 5 N HCl at 25 C for hydrolysis. A 25-min hydrolysis time was established.

The measurements of Feulgen stained DNA were made with Vickers M86 scanning microdensitometer-interferometer as described previously (30). The absolute amounts of DNA per peanut nucleus in pg were calculated from the Feulgen values as follows:

$$\text{peanut DNA (pg)} = \frac{\text{RBC DNA (3.22 pg)}}{\text{RBC DNA (Feulgen units)}} \times \text{peanut DNA (Feulgen units)}$$

RESULTS AND DISCUSSION

DNA COMPOSITION

The density of the peanut DNA averaged about 1.695 g/ml and this value falls within the range of 1.69–1.71 g/ml densities for most nuclear DNAs from higher plants (19). There was no indication of satellite DNA (Fig. 1), because even the widest part of the 260-nm absorption curve was within the density range of 1.693–1.697; all the satellite DNAs detected by CsCl fractionation

Table I. *Per Cent Hyperchromicity, Tm Values, and Base Composition in Two Varieties of A. hypogaea as Determined by Thermal Denaturation Technique*

Values represent mean ± SE (n = 6) and are not significantly different between the two varieties at 1% level by Tukey's honestly significant difference test. The average % G + C calculated from buoyant density was 36.07 and 36.11 for NC-17 and Florigiant, respectively.

Variety	Hyperchromicity	Tm	G + C
	%	C	%
NC-17	24.58 ± 0.35	66.19 ± 0.45	32.71 ± 0.91
Florigiant	25.39 ± 0.44	67.35 ± 0.48	35.03 ± 0.96
Average	24.99	66.77	33.87

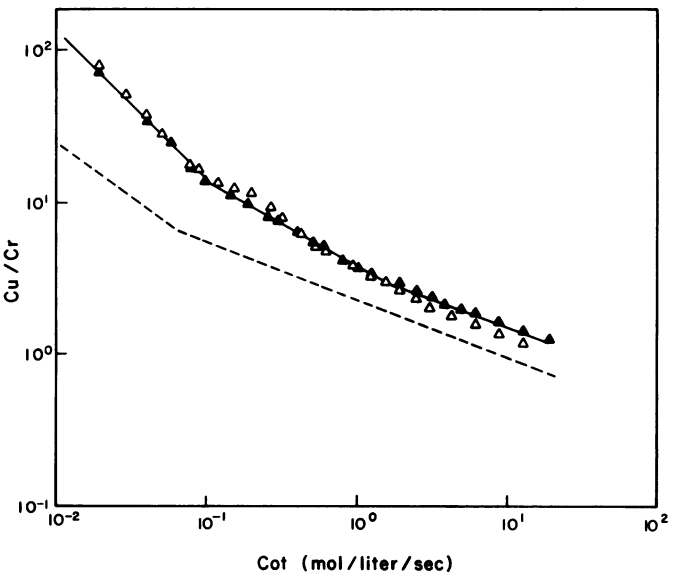

FIG. 4. Reassociation kinetics of peanut DNA. Reassociation at 29 C below Tm (- - -) and 20 C below Tm (——). Reassociation monitored optically. The data for the 29 C reassociation taken from Figure 2. The 20 C reassociation shown at two DNA concentrations, 45 μg/ml (△) and 91 μg/ml (▲). The correlation coefficient (r) for each of the 20 C reassociations are highly repeated DNA 0.99, intermediately repeated DNA 0.95, rarely repeated DNA 0.88.

Table II. *Reassociation Kinetics of Peanut DNA*

Sum of the theoretical DNA components with the listed characteristics of size and ½ Cot value will generate the experimental line depicted in Figure 2. Reassociation at 29 C below Tm.

DNA Component	DNA	½ Cot[a]	Number of Copies[b]	Correlation Coefficients[c]
	%			*r*
Highly repeated	11.9	0.031	3.8×10^4	0.996
Intermediately repeated	14.8	0.176	6.7×10^3	0.993
Rarely repeated	37.4	5.76	2.0×10^2	0.971
Single copy	35.9	1180	1	0.950

[a] ½ Cot value of the fraction within the reaction mixture; it is not the ½ Cot value of the fraction if it were purified.

[b] Derived from the ½ Cot value of the unique DNA/½ Cot value of that DNA fraction.

[c] Significant at the 1% level.

fall well outside this range; *i.e.* densities < 1.69 or > 1.70 (19). This buoyant density indicated an average G + C content of 36.1%; NC-17 had a GC content of 36.05% and Florigiant a content of 36.11%.

The base composition of peanut DNA analyzed by acid hydrolysis is reported to contain 35.6% G+C which includes 5.7% 5-methylcytosine (19). The G+C report of 35.6% is very close to the present findings of 36.1%. Although the presence of 5-methylcytosine is reported to decrease buoyant density (23, 42) and to increase Tm (16), the correction of the apparent peanut DNA GC ratio due to the presence of 5-methylcytosine does not appear necessary. In a similar fashion, Shah and Levings (37) analyzed the GC ratio in *Zea mays* using acid hydrolysis, buoyant density and thermal denaturation. They found GC ratios of 43.2, 42.9, and 44.5%, respectively. The acid hydrolysis method also indicated the presence of 6.8% 5-methylcytosine. However, the presence of the methylcytosine obviously did not require correction of the buoyant density or thermal denaturation values.

The results of thermal denaturation indicated that the two varieties, NC-17 and Florigiant, did not show significant difference in percent hyperchromicity and Tm of the DNA, although these values had a tendency to be higher in Florigiant variety (Table I). The average hyperchromicity value of 25% was in close approximation to the value suggested for good DNA preparation (9). The average 34% G+C calculated from the Tm values is close to 36% G+C calculated from the buoyant density.

DNA base composition, *per se*, has not proven to be as meaningful to study genetic relationships in eukaryotic organisms as in prokaryotes (5, 12, 18). The variation in base composition is very narrow in higher plants and it is not surprising to find similar GC ratios in the two peanut varieties. It appears that evolution of the two varieties within a species is determined by factors other than the base compositions of their DNAs.

DNA REASSOCIATION KINETICS

The optical reassociation in 5 × SSC 50% formamide did not indicate differences in reassociation kinetics of the two varieties, NC-17 and Florigiant. Therefore, further reassociation kinetics were completed only with NC-17. Figure 2 shows the complete reassociation kinetics of peanut DNA (averaging 450–600 base pairs long) which was measured by optical methods or by HAP chromatography.

There were large differences between the optical and HAP measured reassociation at very low Cot values. Thereafter, above a Cot of 1, the two measurements coincided. The early differences between the optical and HAP measured reactions could be due to both self reassociation of foldback DNA and reassociation and subsequent concatenation of stretches of highly repeated DNA. We do not mean to imply that at the low Cot values that one of the straight lines is due to one and the other due to the other cause. Rather it is probable that self reassociation is not visible in the optical reassociation, because it would occur faster than optical reassociation could measure. Foldback DNA would reassociate with itself some 2,000 times faster than the most rapidly reassociating fraction would reassociate at the concentrations used here. Further, both of the two fastest reassociating fractions show concentration-dependent reassociation kinetics, which foldback DNA does not demonstrate. As an example note that at 20 C below the Tm (Fig. 4) two different concentrations of DNA have the same kinetics of reaction on a Cot plot. Foldback DNA at different concentrations would not be superimposable on a Cot plot but would be on a simple time plot. That the initial hypochromicity we measured is not due to foldback reassociation or reformation of base stacking is indicated in that at the lower concentration of DNA described in Figure 4, the initial component of reassociation took some 20 min to reassociate after the establishment of the initial reassociation *A*. Such a time period is much too long for foldback reassociation or base stacking.

The highly repeated DNA could reassociate in short stretches, leaving long single-stranded tails. All these reassociated pieces would be removed by HAP, while only the reassociated regions would be registered by the optical methods. With further incubation, the continued rapid reassociation of one partially reassociated piece to another would continue, *i.e.* concatenation. The optical measurement of reassociation would continue to increase while the HAP measurement would not show any increase; the reassociated pieces would have all been removed in the early incubation. Thus, the early HAP measured reassociation would appear to be larger than the optical measurement and further, it would not increase over a period of time, as is demonstrated here. Obviously, at later times, concatenation is not a factor, since optical and HAP measurements coincide.

As described under "Materials and Methods," a group of theoretical DNA components that would generate the experimental line shown in Figure 2 are listed in Table II. The size and half reassociation values (½ Cot) are listed, as well as the number of copies present in each fraction (calculated from the apparent ½ Cot). The results indicate at least four distinct DNA components in the peanut genome, each with a different reiteration frequency. The line furthest to the left of Figure 2 is the highly repeated DNA. The line second from the left is composed of both the intermediately and rarely repeated DNA components. The line second from the right of Figure 2 is apparently not due to the reassociation of a distinct DNA fraction, but is the composite of results of the end of the rarely repeated DNA reassociation and the start of the reassociation of the last DNA component, the single copy or unique DNA (the line furthest to the right).

Comparison of the observed ½ Cot value (1,180) for the unique DNA sequences of peanut with the rate constant (½ Cot of 2.7) and complexity of *E. coli* DNA (4.5×10^6 nucleotide pairs) reassociated under identical conditions provides a kinetic estimate of 2.0×10^9 nucleotide pairs or 2.05 pg of DNA per haploid content of the peanut genome.

Reassociation kinetics indicate that approximately 64% or 1.28×10^9 nucleotide pairs of the DNA consist of reiterative DNA sequences. The high content of repetitive DNA in peanut exceeds that which is normally present in animal genomes with approximately the same genome size (15) but is consistent with the large quantity of repetitive DNA found in the genomes of most higher plants (14, 17, 48). Soybean (*G. max*) DNA has been reported to have 60 (17) or 47–36% (20) repetitive fractions, and the haploid genome size of 1.97 (17) or 2.78 pg (20). Similarly pea (*P. sativum*) DNA has been reported to have 85 (31) and 70% (32) repetitive

Table III. *Microspectrophotometric Determination of DNA Amounts per Nucleus in Germinating Root- and Shoot-meristem Nuclei of Peanut (NC-17)*

Mean ± SE (number of observations).

Seedling Part	Mitotic Figure	Peanut Feulgen Absorption Units	Chicken Erythrocyte Feulgen Absorption Units	DNA Amount	DNA C-levels
				pg	
Germinating root apex	Pro-metaphase	107.85 ± 2.63(63)	37.64 ± 0.15(30)	9.23 ± 0.22	4
	Ana-telophase	49.95 ± 1.41(100)	37.64 ± 0.15(30)	4.28 ± 0.12	2
Germinating shoot apex	Pro-metaphase	102.40 ± 7.61(16)	39.54 ± 0.42(20)	8.34 ± 0.62	4
	Ana-telophase	51.33 ± 2.89(18)	39.54 ± 0.42(20)	4.18 ± 0.24	2

Table IV. *DNA Amount (pg) per Leaf Nucleus in Two Varieties of A. hypogaea*

Mean ± SE for slides (n = 25) within a variety as well as between varieties are not significantly different at 1% level by Tukey's honestly significant difference test (40).

Variety	Slide No.			Mean ± SE
	1	2	3	
NC-17	7.74 ± 0.11	7.88 ± 0.08	8.01 ± 0.09	7.88 ± 0.08
Florigiant	7.73 ± 0.09	7.89 ± 0.12	7.87 ± 0.11	7.82 ± 0.05

fractions, and the haploid genome size of 4.6 (31) or 0.5 pg (32). The DNA amount of 4.6 pg for pea, however, is closer to the previous cytophotometric estimation (45). The large differences in repetitive DNA as well as genome size for the same species in the above reports strongly suggests the use of cytophotometry along with reassociation kinetics.

CYTOPHOTOMETRIC ESTIMATION OF GENOME SIZE

Chemical DNA estimations, such as use of diphenylamine assay (20, 29) are not reliable methods to determine the genome size or to confirm reassociation kinetics determination because of problems of inaccurate cell counts and the inclusion of cells with 2 C, 4 C, and intermediate amounts of DNA. Feulgen cytophotometry avoids such errors by measuring nuclei at specific C levels of the cell cycle; although precision depends on successful elimination of various errors (3, 11, 30).

The results of DNA determinations in the germinating seeds are given in Table III. The measurements of pro-metaphase and ana-telophase chromosome masses in both root- and shoot-meristems indicate 2:1 ratios. The average cytophotometrically determined 2 C DNA amount for the peanut genome is 4.31 pg or 4.2 $\times 10^9$ nucleotide pairs, which is in close approximation to the above described reassociation estimation of 4.11 pg or 4.0 $\times 10^9$ nucleotide pairs for diploid cells. The DNA amounts in the leaf tissues of the two varieties as determined from interphase nuclei are not significantly different from each other (Table IV). However, the DNA amounts in leaf tissue are close to 4 C DNA levels, indicating that the cells are in G_2 phase after DNA synthesis in leaf tissue.

The presence of 2 C DNA in excess of 3 pg and the absence of satellite DNA in peanut genome supports a suggestion of Ingle *et al.* (22). Comparing the data on the basic nuclear DNA content and the existence of satellite DNA these authors found that only plant species with less than 3 pg displayed a DNA satellite upon CsCl gradient ultracentrifugation. The cause of the negative relationship between nuclear DNA content and satellite DNA is not yet understood. However, at the cytological level, low nuclear DNA amounts are accompanied by the appearance of chromocenters (heterochromatin) (25), and in a number of animal and plant species relationship exists between the 2 C nuclear DNA content and the appearance of both satellite DNA and heterochromatin (44). The determination of heterochromatin seems to be an important part of genome characterization and is under investigation for peanut DNA.

COMPARISON OF REASSOCIATION KINETICS AT DIFFERENT CRITERIA

Peanut DNA was reassociated at two different temperatures, 29 C and 20 C below the melting point of the DNA (60 C in 50% formamide 5 × SSC). The reassociation at 20 C below the Tm is at a higher criterion than the 29 C below Tm reassociation; meaning that fewer errors in the matching of the DNA sequences are tolerated at 20 C (8). Reassociation at 25 C below the Tm gave results similar to those at 29 C below the Tm. Less DNA reassociated at the higher criterion (Fig. 4). The characteristics of theoretical DNA components that, reassociating together, would regenerate the total reassociation kinetics at each criterion are listed in Table V. The number of copies is also given. Although there is some question about the precision of such calculations, these results are meant only for comparison. The rarely repeated and unique DNA of the reassociation at 20 C below the Tm could only be estimated. The unique ½ Cot and size were considered to be the same in both reactions (somewhat larger size in the 20 C below Tm so as to keep the total DNA at 100%). The rarely repeated DNA characteristics could be estimated since the early part of its course of reassociation did occur in the time of observations of reassociation. Inasmuch as its total course was not followed, the estimation is, by necessity, crude.

It is evident that the number of copies of repetitious DNA that would match under the more rigorous criterion (20 C below the Tm) is less than the number of copies that would match under the relaxed criterion (29 C below Tm). Thus there are almost twice as many copies for the intermediately repeated DNA and the rarely repeated DNA at 29 C below the Tm as there are at 20 C below the Tm. Similarly in the highly repeated DNA there were about five times as many copies. The thermal stability data to estimate mismatch effects is under investigation but may not explain values as large as 5-fold shift. The increase in the number of copies in intermediately and rarely repeated DNA occurred without any increase in the amount of DNA within that fraction. On the other hand, the increase in copies of the highly repeated DNA accompanied a 2-fold increase in DNA present in that fraction. At present we do not know where the increased fraction in the amount of highly repeated DNA came from; *i.e.* from the intermediately, rarely or unique repeated DNA fractions. Nor do we know the source of the increase in number of copies of DNA of any fraction. It could be due to increase in recognition at a lower criterion solely within that fraction of DNA. On the other hand, it could be due to a movement of a part of DNA from one fraction to another, *i.e.* some part of rarely repeated DNA at a high criterion appears as intermediately repeated DNA at a lower criterion. In any case it is obvious that each of the four fractions of DNA must be composed of various subfractions of DNA. Further work will be necessary to determine the source of such size or copy variations that accompany changes in reassociation

Table V. *Reassociation Kinetics of Peanut DNA*

Reassociation at 29 C and 20 C below Tm. Sum of the theoretical DNA components with the listed characteristics of size and ½ Cot value will generate the experimental lines depicted in Figure 4.

DNA Component	29 C below Tm[a]			20 C below Tm		
	% DNA	½ Cot[b]	Number of Copies[c]	% DNA	½ Cot[b]	Number of Copies[c]
Highly repeated	11.9	0.031	3.8×10^4	6.3	0.154	7.7×10^3
Intermediately repeated	14.8	0.176	6.7×10^3	15.1	0.36	3.2×10^3
Rarely repeated	37.4	5.76	2.0×10^2	(37.4)[d]	(10.76)	(1.1×10^2)
Unique DNA	35.9	1180	1	(42.2)	(1,180)	(1)

[a] Data taken from Table II.

[b] ½ Cot value of the fraction within the reaction mixture.

[c] Derived from the ½ Cot value of the unique DNA/½ Cot value of that DNA fraction.

[d] Estimated values.

criteria.

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