NUCLEIC ACID HYBRIDIZATION STUDIES WITHIN THE GENUS CUCURBITA[1]

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ABSTRACT

The DNAs of Cucurbita species were examined by several methods. All Cucurbita DNAs have a similar CsCl isopycnic banding pattern consisting of a major band at 1.695 g/cc and a well separated satellite band at 1.707 g/cc. Compared to other plant and animal genera, Cucurbita species have a large genomic proportion of rDNA; this value ranging from 1.4% to 3.1%. The genomic proportion of rDNA was found not to be useful as a characteristic indicating degree of relatedness of the various Cucurbita species. However, Cucurbita DNAs can be distinguished by the extent to which their repetitive sequences cross-hybridize to each other and an assessment of species relationships can be made on this basis.

NUCLEIC acid hybridization studies have been used to estimate the degree of nucleotide sequence divergence that has taken place during evolution (see reviews by HOYER, MCCARTHY and BOLTON 1964; HOYER and ROBERTS 1967). The majority of these studies have compared organisms on broad taxonomic levels (kingdom, family, and genus) but, few have dealt with systematic relationships between members of a single genus (cf. BENDICH and MCCARTHY 1970a). The species of two animal genera, Drosophila and Mus have been compared by hybridization techniques. Species within the genus Drosophila can be distinguished both by DNA/DNA (LAIRD and MCCARTHY 1968; ENTINGH 1970) and DNA/RNA hybridization (ROBERTSON, CHIPCHASE and THI MAN 1969) and the same is true for Mus species (MCLAREN and WALKER (1969). Only recently have nucleic acid hybridization techniques been applied to the taxonomy of plant genera. BENDICH and MCCARTHY (1970b) revealed small differences between species in the genus Triticum (wheat) but CHOOI found no differences between the genomes of five of six examined species of the genus Vicia.

The purpose of the research reported here was to determine the applicability of nucleic acid hybridization techniques to an analysis of the relatedness of species in another genus of higher plants, Cucurbita (pumpkin, squash, and gourds), which has been well studied from an evolutionary point of view (WHITAKER and BEMIS 1965; BEMIS *et al.* 1970; HURD, LINSLEY and WHITAKER 1971).

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Research was designed to: (1) determine and compare the isopycnic banding patterns in CsCl of the DNAs of species in the genus; (2) determine whether the genomic content of rDNA (DNA complementary to ribosomal RNA) can be used to arrange species within the genus systematically; and, (3) ascertain whether and to what extent plant species can be distinguished from each other by DNA/DNA hybridization reactions.

The results show that DNAs of all the species have similar banding properties in CsCl and that genomic proportion of rDNA, although varying among the species, is not a useful taxonomic characteristic for this group of plants. DNA/DNA hybridization experiments, however, reveal both intra- and intergeneric differences and that phylogenetic relationships may be deduced from these differences.

MATERIALS AND METHODS

Leaf material from the following species of the genus Cucurbita was used: *C. pepo* L. cv. Small Sugar; *C. maxima* Dutch. cv. Banana; *C. moschata* Duch. ex Poir. cv. Butternut; *C. digitata* Bail.; *C. palmata* Wats.; *C. okeechobeensis* Bail.; *C. andreana* Naud.; *C. martinezii* Bail.; *C. ecuadorensis* Cut. and Whit.; *C. sororia* Bail.; *C. mixta* Pang.; *C. texana* Gr.; *C. pedatifolia* Bail.; *C. cylindrata* Bail.; *C. cordata* Wats.; *C. gracilor* Bail.; *C. palmeri* Bail.; *C. lundelliana* Bail.; *C. ficifolia* Bou.; *C. foetidissima* HBK. Leaf material from the following species of plants was also used: *Nicotiana tabacum* L. cv. Samsun (tobacco) and *Brassica pekinesis* Lour. Repr. cv. Wong Bok (Chinese cabbage).

Isotopic labeling and extraction of DNA: Plant leaf DNA was labeled with (methyl-^{3}H)-thymidine (Schwartz, 18 Ci/mM) according to a modification of the procedure of ZAITLIN, SPENCER and WHITFIELD (1968). Commonly 5 g of leaf material were washed with distilled water containing 2% sodium dodecyl sulfate (SDS). The washed leaves were placed under distilled water and slices approximately 1–2 mm apart were made with a razor blade, the slices extending from the midribs to the edge of the leaf. The leaves were then placed in Petri dishes containing 10 ml of 0.05 M 2-(N-morpholino) ethanesulfonic acid buffer (MES, Calbiochem), pH 5.8, 100 μg of Rimacidin (Pfizer), 3 mg cephaloridine (Eli Lilly) (FRANCKI, ZAITLIN and JENSEN 1971) and 250 μCi of ^{3}H-thymidine for 24–36 hr under a fluorescent light bank at room temperature. After the labeling period, the nuclear DNA was extracted according to the method of MATSUDA, SIEGEL and LIGHTFOOT (1970) and it was further purified by preparative isopycnic centrifugation in CsCl (FLAMM, BOND and BURR 1966). The concentration of DNA was estimated by determining absorbance at 260 nm and by assuming $A^{0.1\%}_{260nm} = 20$ (EIGNER and DOTY 1958).

One to two μg of each isotopically labeled DNA preparation was co-banded with 200 μg of unlabeled plant DNA in a preparative CsCl gradient (FLAMM, BOND and BURR 1966) in order to assess whether an appreciable proportion of the label might have been incorporated into bacterial rather than plant DNA. The labeled plant DNA was judged free of contaminating bacterial DNA if the radioactive profile was coincident with the absorbance profile. All labeled DNA's used in these experiments were free of bacterial nucleic acid by this criterion.

Isotopic labeling and extraction of ribosomal RNA: Tobacco leaf ribosomal RNA (rRNA) was labeled with uridine-5-^{3}H (Schwartz, 20 Ci/mM) according to ZAITLIN, SPENCER and WHITFELD (1968) as outlined in the previous section. Ribosomes were obtained from leaves exposed to label by griding in two volumes (w/v) of ribosome grinding buffer (0.05 M Tris-HCl, pH 7.5, 0.01 M KCl, 0.01 M $MgCl_2$, and 0.004 M mercaptoethanol) and filtering the macerate through two layers of Miracloth (Calbiochem). Triton X-100 (ROHM and HAAS) was added to 4% and the brei was centrifuged at 10,000 rpm in a Sorvall RC-2 centrifuge for 15 min. Ribosomes were pelleted from the supernatant solution by centrifugation at 45,000 rpm for one hour in the 50 Ti rotor of a Spinco, model L2, ultracentrifuge and they were then suspended in 2 ml of M/15 phosphate buffer, pH 7.0, containing 1% SDS.

The rRNA was extracted with SDS-phenol repeatedly until no protein appeared in the phenol-water interface. Residual phenol was removed from the aqueous layer by shaking with three volumes of cold ether several times and the remaining ether was removed by bubbling N_2 through the solution. The rRNA was precipitated by adding 2.5 volumes of cold 95% ethanol and a few drops of 3 M sodium acetate buffer, pH 4. The precipitated RNA was pelleted at 10,000 rpm for 10 min, and the pellet resuspended in 1 to 2 ml of 0.1 × SSC (SSC is 0.15 M NaCl, 0.015 M Na citrate).

High molecular weight rRNA was separated from low molecular weight RNAs and DNA by precipitation with 2 M LiCl (BALTIMORE 1966). The concentration of rRNA was estimated by determining its absorbance at 260 nm and by assuming that $A_{260nm}^{0.1\%} = 25$.

Isotopically labeled rRNA preparations were examined for contamination with bacterial RNA by polyacrylamide electrophoresis according to BISHOP, CLAYBROOK and SPIEGELMAN (1967). For all the rRNA preparations used in hybridization experiments, the radioactivity profile superimposed the absorbancy profile and this was the criterion used to judge that the preparations were free of bacterial RNA contamination. If there were such contamination, one would expect the specific activity of the 23 and 16S rRNA components to be considerably greater than that of the 25 and 18S components.

DNA/DNA hybridization: DNA preparations were denatured by adjusting the solution to pH 12–12.5 by addition of 0.1 volume M NaOH and, after 15 min, neutralizing by adding .15 volume of M $KH2PO_4$. Ten μg denatured DNA in 1 ml 2 × SSC were immobilized by vacuum filtration onto a 24 mm diameter nitrocellulose membrane (SCHLEICHER and SCHUELL, B-6) (GILLESPIE and SPIEGELMAN 1965) and four 6 mm discs were cut from the membrane with a paper punch so that each disc contained 14% of the total DNA in the membrane (MCCARTHY and MCCONAUGHY 1968). About 95% of the DNA remained imbedded in the membranes after a mock hybridization experiment.

Labeled DNA was sheared by passage through a 27 gauge needle ten times and was denatured as above. The single-stranded size of this DNA determined by analytical band sedimentation (STUDIER 1965) was 9×10^5 daltons. The DNA immobilized in nitrocellulose discs was annealed to the ^{3}H-DNA in 1-dram specimen vials that had been pretreated with siliclad (Clay-Adams) to prevent single-stranded DNA from sticking to the glass. Each vial contained 0.2 ml of 2 × SSC containing 0.1% SDS and 0.3 μg of labeled DNA in addition to a DNA membrane. The ratio of filter bound DNA to labeled DNA in solution was 20:1 in order to minimize self-annealing of the DNA in solution. The 2 × SSC was overlaid with mineral oil to prevent evaporation and the annealing reaction was carried out for 18 hr at 60° or 70°. After the annealing period the membranes were washed according to WARNAAR and COHEN (1966) to remove non-specific, unhybridized DNA from the membranes. This consisted of three washes in 0.003 M Tris-HCl buffer, pH 9.4. The membranes were dried and counted in a Packard Tri-Carb liquid scintillation spectrometer using a scintillation fluid consisting of 0.5% PPO and 0.01% POPOP in toluene.

DNA/rRNA hybridization: Annealing of rRNA to DNA was conducted by the procedure of GILLESPIE and SPIEGELMAN (1965). Usually 1 μg of DNA was annealed to 0.5–1.0 μg of rRNA at 68° in 2 × SSC for 18 hr. After the hybridization period the membranes were washed according to BIRNSTIEL *et al.* (1968), which included one wash with RNAase A. After washing, the membranes were dried and counted in a liquid scintillation spectrometer as noted in the previous section. Tobacco rRNA was used in all DNA/RNA hybridization experiments because of convenience and because dicot DNAs yield the same saturation values when hybridized with homologous or heterologous dicot rRNAs (MATSUDA and SIEGEL 1967).

Thermal stability of DNA/DNA hybrids: The thermal stability of DNA/DNA hybrids was determined by a modification of the procedure of CHURCH and MCCARTHY (1968). After hybridization and washing, membranes containing the DNA/DNA hybrids were placed in a scintillation vial containing 2 ml of 1 × SSC and put in a 60° water bath for 10 min. After this period the vials were placed on ice and the membranes removed to other vials containing 2 ml of 1 × SSC which were then incubated for 10 min at 65°. This operation was repeated at 5° increments until boiling was attained (97°–98° in Tucson). The radioactivity in each vial (representing the amount of hybrid dissociated at each temperature) was determined and the cumulative

percent counts plotted as a function of temperature. The mean thermal stability or T_m was that temperature at which 50% of the counts on the membranes were released. The radioactivity released was estimated after addition of scintillation fluid consisting of ⅓ Triton X-100, ⅔ toluene and the same fluors as previously noted.

Analytical CsCl density gradient centrifugation: A DNA solution consisting of 0.75 g CsCl and 0.60 ml of 1 SSC containing 3 μg of plant DNA and 1 μg of *Micrococcus luteus* DNA as marker ($\rho = 1.731$) was loaded into an analytical ultracentrifuge cell containing a 12 mm epon centerpiece and centrifuged at 44,000 rpm in the An-F rotor of a Spinco Model E analytical ultracentrifuge. Ultraviolet photographs of the banded DNA were taken after 20 hr of centrifugation and these were then analyzed with a Beckman Model RB Analytrol equipped with a film densitometer. The buoyant densities of the plant DNAs were calculated relative to that of the bacterial DNA marker according to the procedure of SUEOKA (1961).

RESULTS

Base composition of Cucurbita DNAs: DNAs from 20 Cucurbita species were banded in CsCl to determine whether differences might exist in their buoyant density and/or satellite content. The results are like those shown in Figure 1; all Cucurbita DNAs band at 1.695 g/cc and have a dense satellite component at 1.707 g/cc. The main band of the different species varied from 1.694–1.696 g/cc, while the satellite band varied from 1.705–1.708 g/cc. These differences in

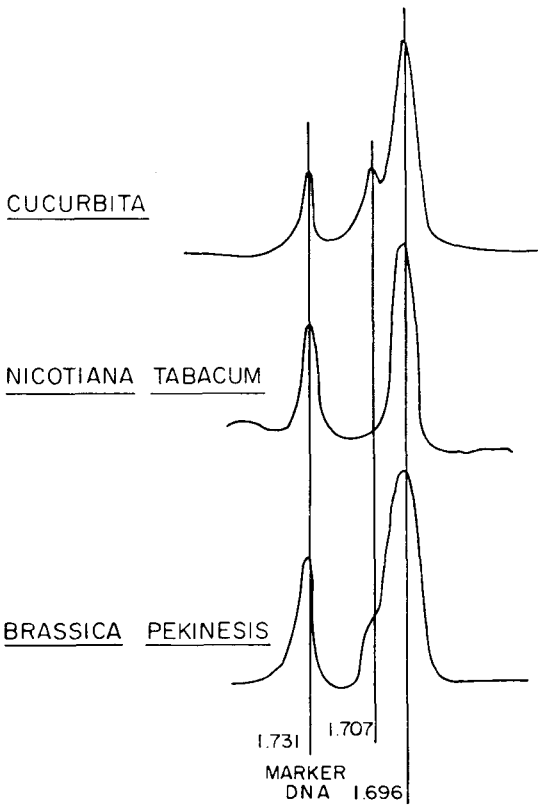

FIGURE 1.—Microdensitometer tracings of plant DNAs banded in analytical CsCl gradients.

buoyant density for each component are not considered significant because six determinations of the DNA of one of the species (*C. pepo*) revealed a similar range of values. The banding patterns of the DNAs of other plant species are also shown in Figure 1 to compare with that of Cucurbita DNA. Tobacco DNA bands at 1.696 g/cc with no detectable satellite and Chinese cabbage DNA bands at 1.696 g/cc and has a dense shoulder instead of a defined peak at 1.707 g/cc. The percent GC of Cucurbita DNAs, calculated from the buoyant densities, (SCHILDKRAUT, MARMUR and DOTY 1962) proves to be 36% for the main band and 47% for the dense satellite. That the DNAs of Cucurbita species have the same base composition is not surprising because plant DNAs, in general, do not differ appreciably from each other in base composition (BISWAS and SARKAR 1970). All species in the genus Cucurbita have a prominent satellite DNA and this is also true of *Cucumis sativus* L. (cucumber), another genus in the family Cucurbitaceae (A. JAWORSKI 1971, personal communication). The possibility arises, therefore, that all plants in the family Cucurbitaceae may have this dense satellite which has been shown to contain sequences complementary to the high molecular weight RNA components from both cytoplasmic and chloroplastic ribosomes (MATSUDA, SIEGEL and LIGHTFOOT 1970) and also to the low molecular weight 5s rRNA (W. THORNBURG 1971, personal communication).

Hybridization of Cucurbita DNA to rRNA: It has been shown that plant species differ greatly in the percentage of their genome which codes for rRNA (MATSUDA and SIEGEL 1967; MATSUDA *et al.* 1970; VODKIN and KATTERMAN 1971; INGLE and SINCLAIR 1972). Hybridization experiments were therefore performed to determine if differences exist among the various Cucurbita species in this regard. Results of a saturation experiment of *C. pepo* DNA with rRNA are shown in Figure 2. It is seen that in this case 2% of *C. pepo* DNA proves to be comple-

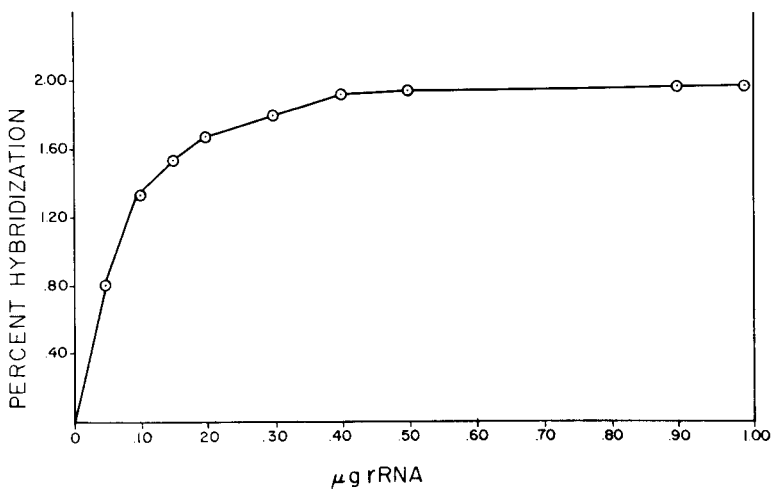

FIGURE 2.—Saturation curve of *C. pepo* DNA with tobacco ^{3}H-rRNA. Two μg of *C. pepo* DNA were annealed with increasing amounts of ^{3}H-*N. tabacum* rRNA (specific activity, 7200 cpm/μg).

TABLE 1

Percent homology of Cucurbita DNAs to rRNA

Species group	Species	Exp. 1*	Exp. 2	Exp. 3	Average
Sororia	*C. sororia*	1.7	—	1.7	1.7
	C. mixta	2.5	2.3	2.7	2.5
Pepo	*C. pepo*	2.6	2.0	2.5	2.4
	C. texana	1.7	—	1.7	1.7
Maxima	*C. maxima*	3.3	—	2.8	3.1
	C. andreana	1.7	1.5	2.0	1.7
Digitata	*C. palmata*	—	—	1.5	1.5
	C. digitata	1.6	1.6	—	1.6
Lundelliana	*C. okeechobeensis*	1.4	1.4	—	1.4
	C. martinezii	1.7	1.7	—	1.7
Ecuadorensis	*C. eduadorensis*	1.7	—	1.8	1.8
Moschata	*C. moschata*	1.4	—	1.3	1.4
Pedatifolia	*C. pedatifolia*	1.7	1.5	—	1.6

* 1 μg of membrane-fixed DNA was hybridized to 1 μg of ^{3}H-tobacco rRNA (specific activity of 54,300 cpm/μg) at 68° for 18 hr in 1 ml of 2 × SSC. Each value represents an average of three replicates within each experiment.

mentary to rRNA and that saturation is achieved with 0.5 μg of RNA when 1 μg DNA is imbedded in the membrane. Table 1 shows the results of hybridization of rRNA to DNAs of a number of Cucurbita species at saturation. It is evident that Cucurbita species have different proportions of DNA complementary to rRNA; the value ranging from 1.4 to 3.1%. S. KEENER (1972, personal communication) has estimated the DNA content of mature *C. pepo* leaf cells to be equivalent to 9.6×10^{11} daltons. On the assumption that the cell cycle is arrested in the G1 stage and that equal numbers of cistrons are present in the nucleus for the RNA in the chloroplast and cytoplasmic ribosomes (MATSUDA *et al.* 1971), one can calculate 3200 cistrons for each type of rRNA per haploid genome of this species.

Cucurbita species have been grouped according to their degree of relatedness as judged by a number of criteria (BEMIS *et al.* 1970) and thus, the species examined in these experiments have been listed according to their species group in the data tables where appropriate. An examination of Table 1 shows that there is probably no relationship between genomic proportion of rDNA and the phylogenetic classification of the genus. For example, although *C. andreana* and *C. maxima* are members of the same species group, Maxima, their DNAs differ considerably in rRNA saturation-hybridization value; 1.7% for *C. andreana* and 3.1% for *C. maxima*. On the other hand, species belonging to different groups may have the same genomic proportion of rDNA. An example of this situation is supplied by *C. sororia*, a member of the Sororia species group and *C. andreana*, a member of the Maxima species group, both of which have a value of 1.7%. These results are in agreement with those of LIGHTFOOT (1972) who found that the genomic proportion of rDNA could not be used to arrange species systematically within the genus Nicotiana.

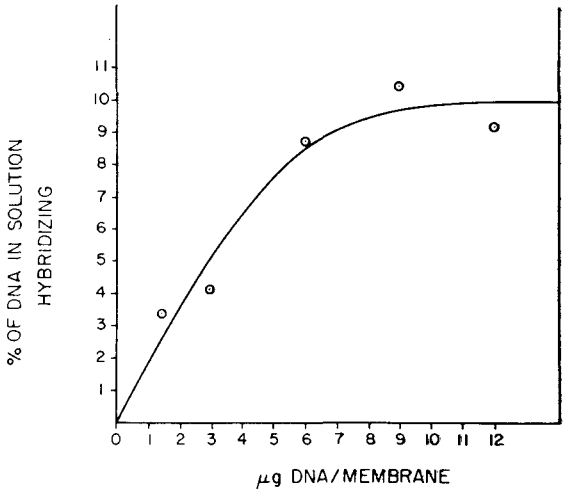

FIGURE 3.—Homologous hybridization of ^{3}H-labeled *C. palmata* DNA to unlabeled *C. palmata* DNA embedded in nitrocellulose membranes. Tritium labeled sheared DNA (0.3 μg, specific activity 920 cpm/μg) was annealed with increasing amounts of membrane-bound homologous DNA.

Nucleotide sequence homologies between DNAs of various plant genera: Comparative DNA hybridization experiments were performed to find out whether Cucurbita species could be distinguished from each other by this method. In order to determine appropriate conditions for these experiments, a saturation hybridization curve was obtained where a constant amount of labeled DNA in solution was incubated with increasing amounts of homologous membrane-immobilized DNA. Figure 3 shows that the amount of labeled DNA which hybridizes plateaus at a filter bound DNA/free DNA ratio of 20 (6 μg on the filter and 0.3 μg in the solution). Approximately 10% of the DNA in solution hybridizes at saturation; those DNA sequences which hybridize being repetitious (cf. MELLI and BISHOP 1969).

Before attempting to distinguish Cucurbita species, a test was made to determine whether and to what extent the DNA hybridization technique employed would distinguish plants belonging to different families.

These experiments were conducted using stringent annealing conditions (2 × SSC, 70°) which permit only closely related base sequences to anneal and which minimizes base sequence mispairing (CHURCH and MCCARTHY 1968). Table 2 shows that this technique does indeed permit distinction between plants of different families; considerably less tobacco and *C. maxima* labeled DNAs hybridize to heterologous DNAs from different plant families than to homologous DNA.

It is to be noted that the reciprocal hybridization values for *C. maxima* and tobacco DNAs are not the same. The lack of reciprocity of hybridization values has been observed by BENDICH and MCCARTHY (1970a) as well as by SEARCY (1970). SEARCY has postulated that lack of reciprocity in hybridization values

TABLE 2

Comparative DNA/DNA hybridizations with species from different families

Filter bound DNA*		^{3}H-DNA†	
Family	Species	*C. maxima*	Tobacco
Cucurbitaceae	*C. maxima*	100‡	28
Solanaceae	*N. tabacum*	13	100
Cruciferae	*B. pekinensis*	28	7
	E. coli	2	2

* Six μg of DNA embedded in nitrocellulose filters were annealed to 0.3 μg ^{3}H-DNA for 18 hr in 0.2 ml 2 × SSC.

† Specific activity of *C. maxima* and tobacco DNAs were 9300 and 2390 cpm/μg, respectively.

‡ Homologous hybridization values were 8.3% for *C. maxima* and 15% for tobacco. Each value represents an average of four replicates. A homologous hybridization range of 88–112% was typical.

will occur when the DNAs being compared have different genome sizes and/or extent of redundancy of nucleotide sequences held in common.

Nucleotide sequence homologies between DNAs of Cucurbita species: Comparisons were made between DNAs from several species in the genus Cucurbita in an attempt to determine whether this technique could be used to verify systematic relationships established by other methods. Cross-hybridization experiments were conducted using four labeled Cucurbita DNAs (*C. maxima*, *C. lundelliana*, *C. palmata*, and *C. pepo*) representative of four major species groups. Table 3 presents the extent of cross-hybridization of these DNAs with a number of unlabeled DNAs. It can be seen that: (1) DNAs of Cucurbita species can be distinguished from each other by this method and, (2) Reciprocal hybridization

TABLE 3

DNA/DNA hybridization of DNAs from species in the genus Cucurbita

Species group	Filter-bound DNA*	*C. palmata*		^{3}H-DNA† *C. maxima*	*C. pepo*	*C. lundelliana*
		Exp. 1	Exp. 2			
Digitata	*C. palmata*	100‡	100	74	68	72
	C. digitata	86	—	51	—	—
Maxima	*C. maxima*	75	—	100	87	105
	C. andreana	—	73	—	—	—
Pepo	*C. pepo*	—	—	82	100	99
Sororia	*C. sororia*	70	—	—	—	—
	C. mixta	—	66	—	—	—
Lundelliana	*C. lundelliana*	—	—	—	87	100
	C. okeechobeensis	—	75	—	—	—

* Six μg of unlabeled DNA were bound to nitrocellulose membranes and annealed to 0.3 μg of labeled DNA in 0.2 ml of 2 × SSC for 18 hr at 70° by the method of WARNAAR and COHEN (1966). The numbers are the average of three independent experiments.

† Specific activities of ^{3}H-DNAs were: *C. palmata* (Exp. 1), 920 cpm/μg; *C. palmata* (Exp. 2), 16,100 cpm/μg; *C. maxima*, 9,300 cpm/μg; *C. pepo*, 35,800 cpm/μg; and *C. lundelliana*, 11,500 cpm/μg.

‡ Homologous hybridization values were 18.6% for *C. maxima*, 10.9% for *C. palmata* (Exp. 1), 8.5% for *C. palmata* (Exp. 2), 11.2% for *C. pepo*, and 9.2% for *C. lundelliana*.

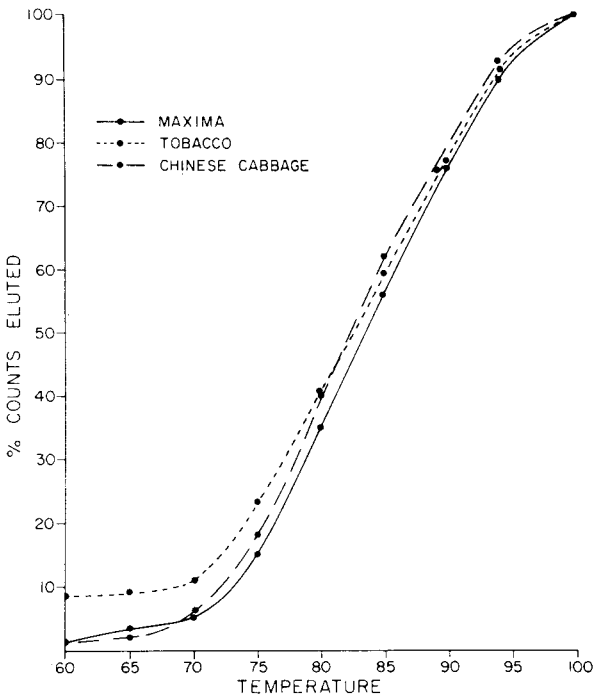

FIGURE 4.—Thermal elution profiles of intergeneric DNA/DNA duplexes formed under stringent hybridization conditions (70°C) with ^{3}H-*C. maxima* DNA (specific activity, 9300 cpm/μg). Three membranes containing each of the different duplexes were eluted in each experiment. They contained 833, 258 and 75 cpm, respectively, for *C. maxima*, Chinese cabbage and tobacco. At the termination of the experiment 9% of the counts remained on the membranes.

experiments give similar values indicating that genome sizes are probably not too different and that no large differences exist between species in extent of redundancy of nucleotide sequences held in common (SEARCY 1970).

Thermal stability measurements of hybrid DNA duplexes: Cross-hybridization experiments measure all base sequences sufficiently similar to hybridize under a particular set of annealing conditions, but they do not discriminate between duplexes of different degrees of mismatching (CHURCH and MCCARTHY 1968). The fidelity of base-pair matching in the intergeneric hybrid DNAs generated in the current series of experiments was assessed by determining their thermal stabilities (SHEARER and MCCARTHY 1970). The curves, Figure 4, when compared with the melting curve for native Cucurbita DNA (MATSUDA and SIEGEL 1967) show an increased temperature range and a reduced T_m indicating some mismatching, as expected (BRITTEN and KOHNE 1968), even with self-annealed DNA. The heterologous DNA duplexes have similar thermal stabilities to that of the homologous *C. maxima* reannealed DNA and, thus, there does not appear to be additional base sequence divergence in the heterologous as compared with the homologous hybrids.

The above experiment was performed with hybrids formed at 70°. SHEARER

TABLE 4

T_m values for interfamily DNA/DNA heteroduplexes formed with labeled C. maxima *DNA*

DNA	70°		60°	
	T_m	Reduction in T_m*	T_m	Reduction in T_m*
C. maxima	83.4	—	82.2	—
N. tabacum	82.4	1.0	77.8	4.4
B. pekinensis	82.2	1.2	79.6	2.6

* Homologous T_m minus heterologous T_m.

and McCarthy (1970) have pointed out that base sequence divergence between DNAs, as observed by decreased thermal stability, may be more obvious following less stringent annealing conditions. Thus, thermal stabilities of hybrids formed at 60° were determined and the resultant T_m values are presented in Table 4. It is evident that larger T_m differences between homologous and heterologous hybrids are observed when they are formed at 60° rather than at 70°, although the T_m obtained for the homologous duplex is approximately the same regardless of the temperature at which it was formed.

Hybrids formed at 60° reveal an additional 7% base mismatching in the *C. maxima*-tobacco duplex as compared to the reannealed *C. maxima* DNA on the assumption that a 1° lowering in T_m indicates 1.5% base mispairing (Laird, McConaughy and McCarthy 1969; Kohne, Chiscon and Hoyer 1970). A value of 4% additional divergence is obtained for Chinese cabbage–*C. maxima* duplexes. Thus, Chinese cabbage seems more closely related to Cucurbita than it is to tobacco. This is in agreement with the cross-hybridization data (see Table 2).

A similar set of experiments conducted with a series of interspecific Cucurbita DNA hybrids formed at 70° revealed little difference in T_m values between homologous and heterologous hybrids. In view of the results with inter-family DNA hybrid duplexes, it is no surprise that little difference in thermal stability was observed with the intrageneric hybrids.

DISCUSSION

The DNAs of a number of Cucurbita species have been compared by a number of methods to assess whether one or more of these might be suitable for determination of degree of relatedness of species within the genus. Isopycnic centrifugation in CsCl is not useful for this purpose because the DNAs of all of the Cucurbita species prove to have a similar banding pattern. This consists of a major band at 1.695 g/cc and a well separated rDNA containing satellite band at 1.707 g/cc. This is in contrast to species in the genus Nicotiana, only some of which have a visible rDNA satellite (Lightfoot 1972). Directly related to the presence of the satellite is the fact that a large percentage of the genome of each species is complementary to rRNA with lowest value being 1.4% and the highest 3.1%. Despite the range of difference, this characteristic is also not useful for

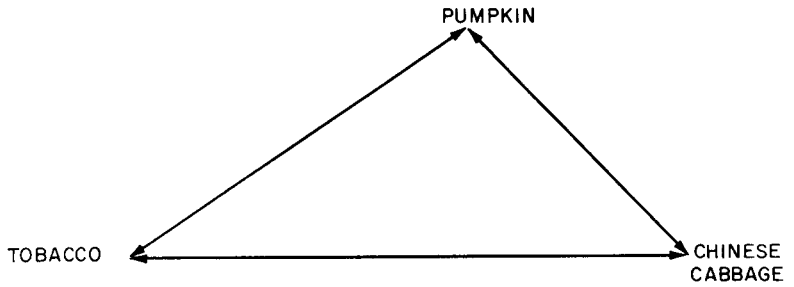

FIGURE 5.—Relationships between plants from three different families as inferred from DNA/DNA hybridization experiments. The longer the line between species, the lower the amount of DNA cross-hybridization, and hence a more distant relationship.

measuring degree of relatedness because no correlation is apparent between it and the taxonomic grouping of the Cucurbita species. Why this genus contains such a high proportion of its DNA as cistrons for rRNA and why the species within the genus differ over more than a two-fold range in this regard, remain matters for speculation. Perhaps plants cells require a minimum number of cistrons for rRNA and those species with a high proportion of rDNA have a relatively low amount of nuclear DNA. This hypothesis receives support from the observation of LIGHTFOOT (1972) that diploid species of Nicotiana have a greater genomic proportion of rDNA than do tetraploid species. INGLE and SINCLAIR (1972) have also presented evidence to support such a contention. The Cucurbita species all contain the same number of chromosomes but it is unknown whether they might differ in cellular DNA content.

The extent to which the repetitive fraction of genomic DNAs cross-hybridize may prove to be a useful criterion for determining both inter- and intrageneric phylogenetic relationships in the plant kingdom. Figure 5 presents a drawing of the relationships between tobacco, Chinese cabbage, and pumpkin (Cucurbita) inferred from the DNA cross-hybridization data in Table 2. It is seen that tobacco and Chinese cabbage are more closely related to pumpkin than they are to each other. These results disagree, somewhat, with more traditional taxonomic principles based on structure and morphology of floral parts which, according to HUTCHINSON (1964), would have tobacco and Chinese cabbage more closely related to each other than to pumpkin.

Figure 6 presents the relationships between species of Cucurbita as deduced from the cross-hybridization data shown in Table 3. It appears that *C. palmata*, a xerophytic species which is a member of the Digitata species group is distantly related to all other species. This is in agreement with the results of WHITAKER and BEMIS (1965) who concluded that the xerophytic species of Cucurbita have become isolated both genetically and geographically from the rest of the species in the genus. *Cucurbita lundelliana*, a tropical species which is a member of the Lundelliana species group, is closely related to *C. maxima* and *C. pepo*, their DNAs being difficult to distinguish by cross-hybridization. This is also in agreement with the observation of WHITAKER and BEMIS (1965) that *C. lundelliana* is genetically compatible with both *C. pepo* and *C. maxima*, implicating *C.*

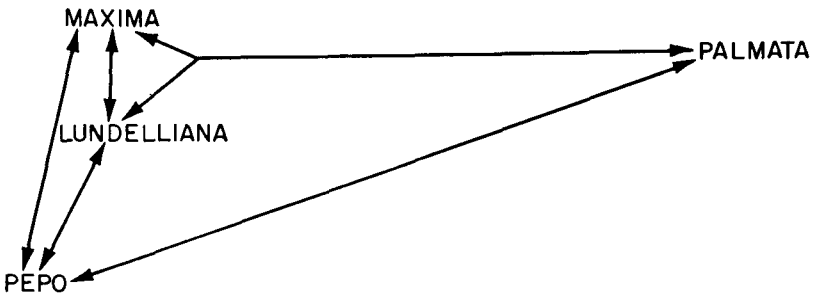

FIGURE 6.—Relationships between species of the genus Cucurbita inferred from DNA/DNA hybridization experiments.

lundelliana as a possible progenitor of these species. *Cucurbita pepo* and *C. maxima* prove to be closely related to each other, according to the cross-hybridization data, but not as closely as each is to their putative ancestor, *C. lundelliana.*

The results reported in this paper indicate that species within a higher plant genus are distinguishable when their repetitive DNA sequences are compared by cross-hybridization and that information concerning both inter- and intrageneric degrees of relationship can be deduced from such data.

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