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Different AT-rich satellite DNAs in *Cucurbita pepo* and *Cucurbita maxima*

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Summary. The AT-rich highly repeated satellite DNA of *Cucurbita pepo* (zucchini) and *Cucurbita maxima* (pumpkin) were cloned and their DNA structure was investigated. DNA sequencing revealed that the repeat length of satellite DNA in *Cucurbita pepo* is 349–352 base pairs. The percentage of AT-base pairs is about 61%. This satellite is highly conserved in restriction enzyme pattern and DNA sequence; sequence heterogeneity is about 10%. In contrast, the satellite DNA of *Cucurbita maxima* has a repeat length of 168–169 base pairs. This satellite is also rich in AT-base pairs (64%), existing in at least three different variants as revealed by restriction enzyme analysis and DNA sequencing. The sequence heterogeneity between these variants is about 15%. The two satellite DNAs showed no cross-hybridization to each other and sequence homology is only limited. Nevertheless, we found in the *C. pepo* genome a high amount of sequences resembling the satellite of *C. maxima*. In contrast, the satellite repeat of *C. pepo* is found in the *C. maxima* DNA only in a few copies. These observations were discussed with respect to satellite DNA evolution and compared to the data received from monocotyledonous species.

Key words: *Cucurbita pepo* – *Cucurbita maxima* – Satellite DNA – Genome evolution

Introduction

Highly repetitive satellite DNA is a common feature of most eukaryotic species.

The presence of these elements is independent of genome size and evolutionary relationships (Brutlag 1980; Miklos and Gill 1982; Singer 1982). Normally, satellite DNA is found to be located in heterochromatic regions and to be transcriptionally inactive although some exceptions do exist (Varley 1980). In animals the organization and evolution of satellite DNA has been extensively investigated (Miklos and Gill 1982; Miklos 1982) and sequences compared. The evolutionary mechanisms of dispersing such sequences within the genome have also been discussed (Smith 1976; Dover 1982; Arnheim 1983).

In plants only a limited number of satellite DNAs have been characterized (Bedbrook et al. 1980; Dennis et al. 1980; Deumling 1981; Peacock et al. 1981; Hemleben et al. 1982; Capesius 1983; Kato et al. 1984; Barnes et al. 1985; Ganal et al. 1986). In some monocotyledonous species evolutionary analyses have been performed (Flavell 1982; Flavell et al. 1983; Dennis and Peacock 1984) whereas in the dicotyledonous species no such detailed analysis of closely related species exists.

Plants in the family of *Cucurbitaceae* are characterized by an enormous amount of highly repetitive sequences (Ingle et al. 1975). Up to 30% of the total nuclear DNA is represented by satellite DNA. We have previously investigated the satellite DNA of two closely related *Cucumis* species (Hemleben et al. 1982; Brennicke and Hemleben 1983; Ganal et al. 1986).

Here we present the analysis of satellites from two closely related *Cucurbita* species (*Cucurbita pepo* and *Cucurbita maxima*). The data are discussed with respect to structure, function, and evolution of repetitive DNA in *Cucurbitaceae*.

Material and methods

Plant material

Seeds were purchased commercially (Hild, Marbach a.N., FRG). *Cucurbita pepo* (zucchini; cv. 'Cocozelle von Tripolis') and *Cucurbita maxima* (pumpkin; cv. 'Riesenmelonen', genetzte) were grown under sterile conditions at 25 °C in the dark. Seedlings were harvested after 7–10 days.

DNA isolation and restriction enzyme analysis

DNA from hypocotyls and cotyledons was purified as previously described (Hemleben et al. 1982). Total nuclear DNA

was used for further analysis. After restriction enzyme digestion DNA was separated on 5–8% polyacrylamide gels or 2% agarose gels. Standard methods were carried out as described by Maniatis et al. (1982).

Cloning of satellite DNA

Zucchini nuclear DNA was digested with Hind III or Hae III and the satellite DNA monomeric bands were eluted out of a preparative polyacrylamide gel using the procedure of Maxam and Gilbert (1977). These fragments were ligated into pUC 8 (Vieira and Messing 1982) using the Hind III or Sma I site. Clones were characterized by colony hybridization (Grunstein and Hogness 1975) using the ^{32}P-nick-translated monomeric satellite DNA band. Recombinant clones were further investigated by restriction enzyme analysis and purified by the alkaline lysis method (Birnboim 1983) and subsequent centrifugation in CsCl-ethidium bromide gradients.

Pumpkin nuclear DNA was digested with Rsa I and cloned into the Sma I site of pUC 8.

DNA sequencing

Cloned repeats were eluted from the respective plasmids and ligated into M13mp8 or M13mp9 (Messing and Vieira 1982). Sequencing was done according to Sanger et al. (1977). In addition some genomic satellite DNA repeats were cloned directly into M13mp8 and compared to the cloned and sequenced repeats. The analyzed repeats, therefore, represent typical repeats of the respective satellite DNA type.

Hybridization

For all hybridizations the eluted inserts were used. Nick-translation and hybridization was performed as described in Ganal et al. (1986). The stringent hybridization temperature was 70 °C; relaxed conditions included a hybridization temperature of 60 °C.

Results

The satellite DNA of *Cucurbita pepo* and *Cucurbita maxima* is cryptic in CsCl and CsCl-actinomycin D gradients. This is in contrast to the previously described satellite DNA of the closely related *Cucumis* species (Hemleben et al. 1982; Ganal et al. 1986). However, satellite DNA of zucchini and pumpkin could be identified by the digestion of total nuclear DNA due to their appearance as very prominent bands in the gels. A rough estimation of the amount of satellite DNA resulted in about 10% of nuclear DNA.

Restriction enzyme analysis of genomic DNA

Digestion of total nuclear DNA of *C. pepo* with Hind III, Alu I, and Taq I resulted in very prominent bands in polyacrylamide gels (Fig. 1 A). Digestion with Hind

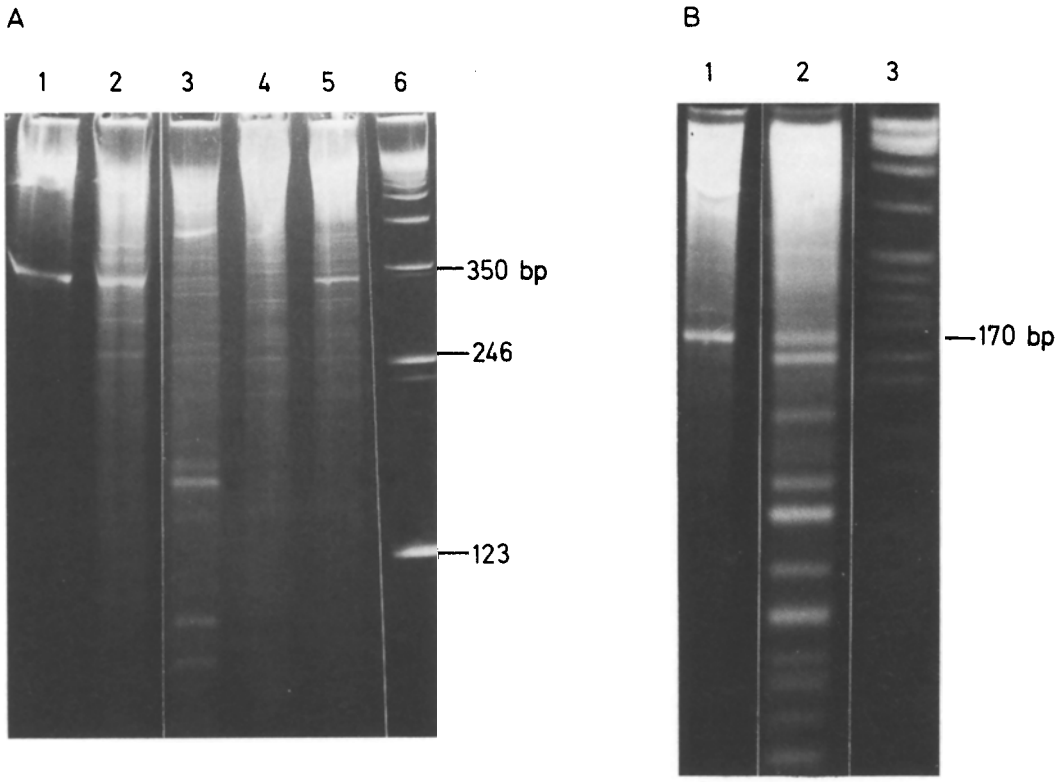

Fig. 1 A, B. Restriction enzyme analysis of genomic DNA. **A** Total nuclear DNA of *C. pepo* was digested with Hind III (*1*), Alu I (*2*), Taq I (*3*), Hpa II (*4*) and Msp I (*5*) and separated on a 6% polyacrylamide gel. **A** 123-bp ladder was used as marker (lane *6*). **B** Total nuclear DNA of *C. maxima* was digested with Rsa I (*1*) and Taq (*2*), and separated on an 8% polyacrylamide gel. pBR 322- Hpa II digested was used as marker (lane *3*)

III and Alu I showed one single band of about 350 bp. Analysis with Taq I resulted in three major bands which indicates that there are at least three sites for Taq I in a typical satellite repeat. Analysis with Hpa II and Msp I showed that the satellite DNA of *C. pepo* is only cut by Msp I into monomeric units, whereas in Hpa II digestions no significant degradation could be detected. This indicates a methylation of the inner cytosine at the single Hpa II/Msp I site in zucchini satellite DNA (see also Fig. 2). Therefore, the methylation pattern is differing from the pattern in the *Cucumis* satellites where both cytosine residues of a Hpa II/Msp I site are found to be methylated (Grisvard 1985; Ganal et al. 1986).

The nuclear DNA of *C. maxima* showed one prominent band after digestion with Rsa I (Fig. 1 B) of about 170 bp in length while digestion with Taq I indicated a large number of bands. At least ten different bands could be identified in a high-resolution polyacrylamide gel. The size of these bands is between 170 and 45 bp. This suggests that the satellite DNA of *C. maxima* contains a certain number of distinct variants.

Sequence and organization of the Cucurbita pepo satellite DNA

Satellite DNA repeats of *C. pepo* were cloned as described in "Methods". Since we used total nuclear DNA to elute the monomeric bands not all the clones contained satellite DNA. The clones containing highly repeated DNA were selected by colony hybridization using the same monomeric band eluted out of a preparative gel which we used for cloning. The fragments were ^{32}P-nick-translated and hybridized to the clones. Clones containing satellite DNA repeats gave a very strong signal after autoradiography because of the frequency of the satellite DNA repeats in the eluted band compared to sequences which are middle-repetitive or unique. The clones were further characterized by restriction enzyme analysis. Three cloned satellite repeats were ligated into M13 and sequenced. The repeat length (Z1– Z3) was determined with 349 (Z3), 351 (Z1), and 352 bp (Z2) (Fig. 2). Most restriction enzyme sites are the same as those found for the genomic repeats; however, in two repeats there are minor differences. In Z2 there is a second Alu I site present at position 53, whereas in Z3 a second Sau3A site is located at position 232. All sequenced repeats have a Hae III site, a Hpa II/Msp I site and four Taq I sites. Two of these Taq I sites are separated by only ten base pairs. The repeats are rich in AT-base pairs (about 61%). Due to their homology in DNA sequence and restriction enzyme patterns, the three sequenced repeats represent a single repeat type. They show a heterogeneity of 4% between Z1 and Z2, 9% between Z1 and Z3, and 10% between Z2 and Z3 (see Fig. 2).

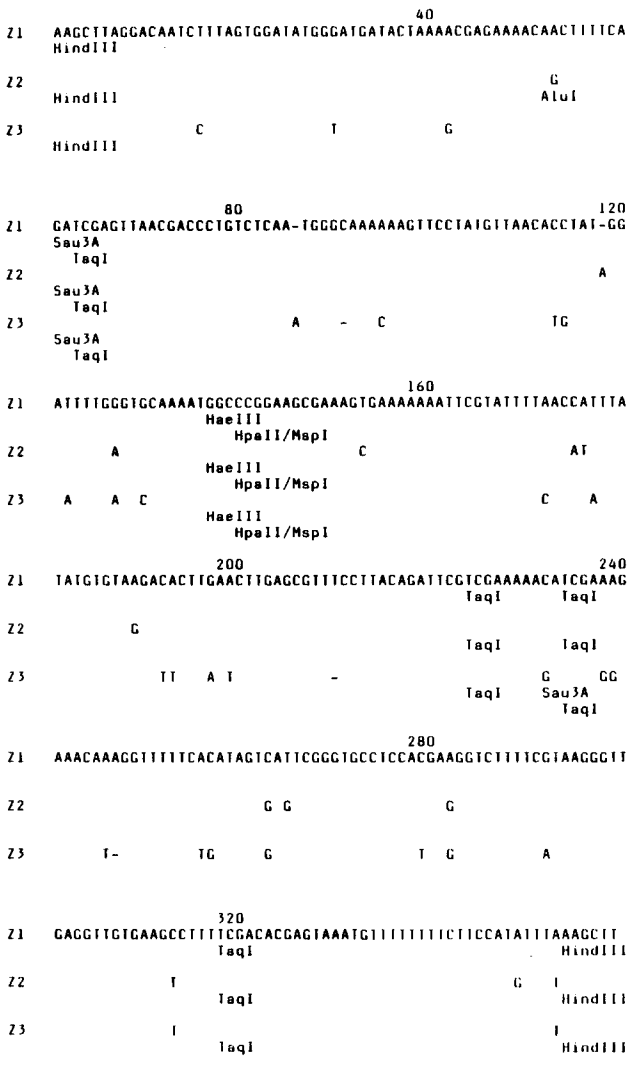

Fig. 2. DNA sequence of the *C. pepo* satellite. The sequences of three cloned repeats (Z1–Z3) were aligned to each other. For Z2 and Z3 only the differences in sequence are shown. Relevant restriction enzyme sites are marked

Hybridization at high stringency of genomic DNA digested with the respective enzymes with either Z1, Z2 or Z3 reveals a comparable pattern (Fig. 4; results are shown only for Z1) again suggesting that they represent only variants of a common repeat type. The hybridization pattern is typical for a repeated element arranged in tandem.

Sequence and organization of the Cucurbita maxima satellite DNA

The cloned repeats of *C. maxima* identified by their Taq I pattern let us suggest that there are at least three different variants, here called K1–K3, in the pumkin

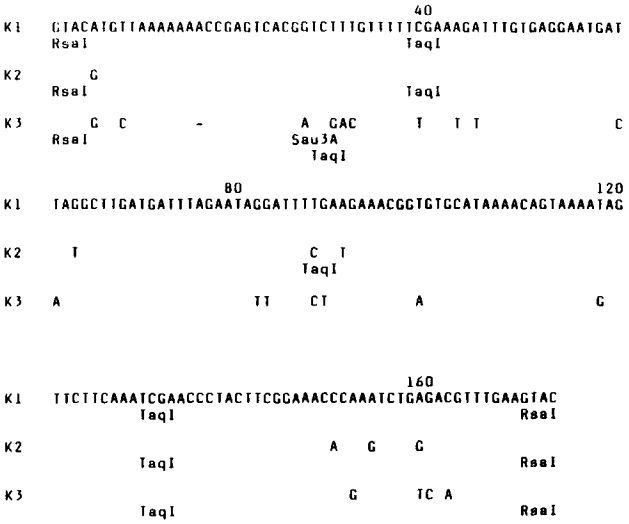

Fig. 3. DNA sequence of the *C. maxima* satellite. Sequence of three cloned repeats (K1–K3) were aligned to each other. For K2 and K3 only the differences are shown. The position of the relevant restriction enzyme sites is indicated

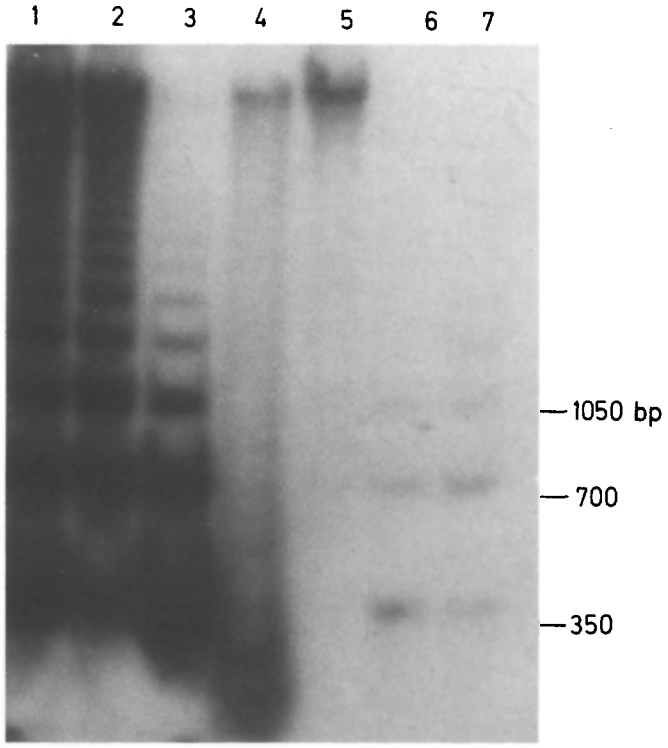

Fig. 4. Hybridization of genomic DNA of *C. pepo* and *C. maxima* with a cloned repeat of the *C. pepo* satellite (Z1). Total nuclear DNA of *C. pepo* (about 1 µg) was digested with Hind III (*1*), Alu I (*2*), Hae III (*3*), and Taq I (*4*). Total nuclear DNA of *C. maxima* (about 5 µg) was digested with Hind III (*5*), Alu I (*6*), and Hae III (*7*). DNA was separated on an 1.5% agarose gel and after transfer hybridized with the ^{32}P-nick-translated Z1 repeat at 60 °C. The film was highly overexposed to show the difference in intensity of hybridization

satellite DNA. The different variants were cloned into M13 and sequenced (Fig. 3). The sequence analysis revealed that they are 168–169 bp in length and highly AT-rich (64%). The polymorphism in the Taq I sites is clearly confirmed by the sequenced repeats. The heterogeneity in DNA sequence is 5% between K1 and K2, 13% between K1 and K3, and 15% between K2 and K3. Using these three repeats it is possible to explain at least seven of the Taq I bands in Fig. 1 B assuming a random distribution of the respective repeats. The bands which could not be explained by these three repeats may represent either some other variants of the satellite repeat not detected in our clones or they are other minor repetitive elements which are present in the nuclear DNA of *C. maxima*.

These three repeats were used to hybridize the digested nuclear DNA of pumpkin. A typical pattern for a tandemly repeated DNA element was observed here, too (Fig. 5). The hybridization pattern was identical with all three variants. In the Sau3A digestion of *C. maxima* DNA especially was no difference observed in the hybridization pattern between K1 (not shown), which has no Sau3A site and K3, which does have one, and 13% variation in DNA sequence compared to K1 (Fig. 3). This suggests an absence of clustering of K3 variants in the pumpkin satellite DNA but a more or less random distribution. The sequenced repeats revealed no Hae III site (Fig. 3); however, the hybridization pattern of genomic DNA indicates the presence of a Hae III site in some repeats (Fig. 5).

Sequence homologies and evolutionary aspects

We searched for homologies in satellite DNA sequences in the two closely related species. A detailed comparison of these two satellites revealed no extensive homology. The two sequences of *C. pepo* and *C. maxima* could be aligned in an order to maximum homology of about 48% (data not shown). Compared to other satellites in animals (Strachan et al. 1985) we do not believe that this observation is very significant. Perhaps this homology is caused by the high AT-content or other properties (like common motifs) of these two satellites, as observed in animals (Levinson et al. 1985).

Because of limited sequence homology between these two satellites they show no hybridization to each other even at reduced stringency, as revealed by the hybridization of cloned repeats to each other (data not shown). Therefore, we used these cloned repeats to probe the genomic DNA of the respective other *Cucurbita* species. The results are shown in Figs. 4 and 5. By hybridization with a cloned *C. pepo* satellite DNA repeat we

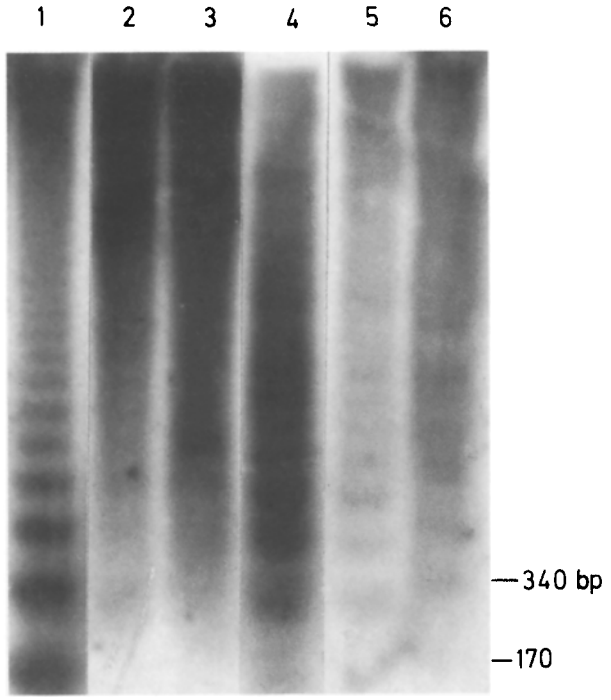

Fig. 5. Hybridization of genomic DNA of *C. maxima* and *C. pepo* with a cloned repeat of the *C. maxima* satellite (K3). Genomic DNA of *C. maxima* (about 0.5 μg) was digested with Rsa I (*1*), Sau3A (*2*), and Hae III (*3*). Digestion of the *C. pepo* DNA (about 5 μg) with Rsa I (*4*), Sau3A (*5*), and Hae III (*6*) was shown in the subsequent lanes. DNA was separated on an 1.5% agarose gel and hybridized with the K3 repeat at 60 °C

found homologous sequences in the *C. maxima* genome (Fig. 4). The hybridization pattern was similar to that of the *C. pepo* DNA with the exception that the satellite DNA repeat of *C. pepo* has a Hind III site whereas in the hybridizing *C. maxima* DNA no such site was detected. The Alu I and Hae III pattern of *C. maxima* DNA was identical to that of *C. pepo*. The difference in the Hind III digestion, furthermore, excludes the possibility that the *C. maxima* DNA was accidentally contaminated by a trace of *C. pepo* DNA. The hybridization data indicates a very low copy number of the *C. pepo* element in *C. maxima* DNA (less than 100 copies/genome; see Fig. 4).

In contrast, sequences with homology to the *C. maxima* satellite DNA repeat are represented in higher amounts in the *C. pepo* DNA (Fig. 5). Hybridization of digested genomic DNA of *C. pepo* with Rsa I, Sau3A and Hae III with satellite repeat K3 of *C. maxima* results in a strong hybridization signal (Fig. 5). The Rsa I and Hae III patterns of *C. pepo* are comparable to that of *C. maxima* DNA although no such fine ladder is observed in the Rsa I digestion. Differences are found in the Sau3A digestion: whereas the *C. maxima* DNA is not extensively degraded by Sau3A, a considerable part of the hybridizing *C. pepo* DNA is degraded into monomers or lower multimers. In *C. pepo* a typical pattern for a tandemly repetitive element is found. Probably the presence of a Sau3A site indicates a repeat which is similar to K3 in *C. pepo* since K3 is characterized by a Sau3A site (Fig. 3). The copy number in *C. pepo* is about 1/10 of the satellite DNA in *C. maxima* as revealed by the hybridization intensities.

Discussion

Cucurbitaceae are characterized by an enormous amount of tandemly repeated sequences. Up to 30% of the total nuclear DNA is satellite DNA. Further 5–10% of the genome consist of ribosomal RNA genes (Ingle et al. 1975). We have previously analyzed two species of the *Cucumis* group (*Cucumis melo* and *Cucumis sativus*) with respect to their highly repetitive elements (Hemleben et al. 1982; Brennicke and Hemleben 1983; Ganal et al. 1986) and found prominent satellites of either about 180 or 350 base pairs, respectively. The satellites of the *Cucurbita* species are cryptic in contrast to the satellites of the *Cucumis* species suggesting a different AT-content. The sequencing data confirm this observation, detecting an AT-content of 61% and 64% compared to 44–53% in the *Cucumis* satellite DNA.

The repeat length seems to be highly conserved. Up to now two groups have been identified (see Table 1): the first group shows a repeat length of about 350 base pairs. This group is represented by the satellites of *Cucumis melo, Cucurbita pepo,* and an element of the *Cucumis sativus* satellite DNA. The second group is consisting of the two satellites in *Cucumis sativus* and the *Cucurbita maxima* satellite. This suggests a selection onto the repeat length whereas base composition can vary considerably (from 44% to 64% AT-base pairs). It was found in many other species not related to these plants that satellite repeat length is also about 170–180 base pairs or a multimeric form of this number (Capesius 1983; Dennis and Peacock 1984; Barnes et al. 1985). This is in correlation to the nucleosomal repeat length in plants (Leber and Hemleben 1979).

The satellites in the *Cucurbita* species show a moderate level of variation in DNA sequence. Sequence heterogeneity is 10% for the zucchini satellite and 15% for the pumpkin satellite. The different types presumably are not clustered but equally distributed. A detailed analysis using restriction enzyme analysis indicates that a part of the variation in the zucchini and pumpkin satellite DNA could be interpreted as the development of new variants. In the pumpkin satellite DNA especially there is a considerable group of variants as shown by the Taq I digestion (Figs. 1 B and 3).

Table 1. Comparison of the characterized satellite DNAs in *Cucurbitaceae*

Species	Repeat length (bp)	AT-content	References
Cucumis melo	352	44%	Brennicke and Hemleben 1983
Cucumis sativus	182	53%	Ganal et al. 1986
	177	47%	Ganal et al. 1986
	360	53%	Unpubl. results
Cucurbita pepo	349–352	61%	This paper
Cucurbita maxima	168–169	64%	This paper

However, these variants are probably not organized as separated satellites as observed in *Cucumis sativus* (Ganal et al. 1986; unpublished results).

Interestingly, the basic repeat of the investigated *Cucurbitaceae* species showed no hybridization to the prominent repeat of the other species. A detailed analysis of the satellite DNA sequence indicates that there is no or only a very limited overall homology. That means that in this family of dicotyledonous plants there is a very rapid evolution of a specific highly repeated DNA sequence. One can speculate that these different satellites are either a reason or a consequence of the speciation event (Rose and Doolittle 1983).

A possibility to investigate the mechanism of amplification and rearrangement is to search and characterize sequences which are homologous to the prominent satellite of one plant in the genome of the other species of the same family. We have found sequences in the *Cucurbita* species as well as in the *Cucumis* species (Ganal et al. 1986) which showed hybridization to the satellite DNA of the respective other plants. The copy number of these relic-elements is very variable. In *Cucurbita pepo* a considerable part of the genome (0.5–1%) is represented by an element which shows hybridization to the *Cucurbita maxima* satellite. Vice versa, in *C. maxima,* only a few copies similar to the *C. pepo* satellite are present.

Similar observations were made in such monocotyledonous species as *Secale, Triticum,* and *Aegilops* (Bedbrook et al. 1980; Flavell 1982; Flavell et al. 1983; Flavell 1985). Plants in these genera can be discriminated by the presence of at least one group of specific repeats although they are present in a minor copy number in closely related species. A comparison between these elements revealed that complicated rearrangements and subsequent amplification steps have occurred (Flavell 1982). In *Cucurbitaceae* apparently no such complicated rearrangements happened but specific elements are amplified in the respective species. This may be due to their tandemly repeated structure, whereas in the monocotyledonous species these repetitive elements are also interspersed (Evans et al. 1983; Bedbrook et al. 1980). A common ancestor of the *Cucurbita* species may be characterized by the presence of both repetitive elements although these elements are not completely identical to the main satellite repeats of the two plants *C. pepo* and *C. maxima.*

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