Theor Appl Genet (1992) 84:859–865

Chloroplast DNA diversity among wild and cultivated members of *Cucurbita* (Cucurbitaceae)

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Received November 30, 1991; Accepted February 26, 1992
Communicated by G. Wenzel

Summary. Cladistic analysis of 86 chloroplast DNA restriction-site mutations among 30 samples representing 15 species of *Cucurbita* indicates that annual species of the genus are derived from perennials. The Malabar Gourd, *C. ficifolia*, is placed as a basal, sister taxon relative to other domesticated species and allied wild-types. The pattern of variation supports three species groups as monophyletic: (1) *C. fraterna*, *C. pepo*, and *C. texana*, (2) *C. lundelliana*, *C. martinezii*, *C. mixta*, *C. moschata* and *C. sororia*, and (3) *C. foetidissima* and *C. pedatifolia*. Domesticated samples representing subspecies of *C. pepo* are divided into two concordant groups, one of which is allied to wild-types referable to *C. texana* and *C. fraterna*. The data failed to resolve relationships among cultivars of *C. moschata* and *C. mixta* and their association to the wild *C. sororia*. The South American domesticate, *C. maxima*, and its companion weed, *C. andreana*, show close affinity and alliance to *C. equadorensis*.

Key words: *Cucurbita* – Phylogeny – cpDNA – Domestication – Evolution

Introduction

Cucurbita, one of approximately 90 genera of the Cucurbitaceae, is a relatively well-defined assemblage of herbaceous vines, all native to the Americas. The genus includes five domesticated species: *C. ficifolia*, a high-elevation landrace cultigen of Mexico and South America; *C. maxima*, the only domesticate with a native range of distribution restricted to South America (commerical cultivars: 'Turk's Turban', 'Hubbard', 'Banana Squash' and 'Big Max' pumpkins); *C. moschata*, the common landrace squash of lower elevations in both Mexico and South America (commerical cultivars: 'Butternut' and 'Kentucky Field'); *C. mixta*, grown at various elevations in Mexico, often for seed (commerical cultivar: 'Cushaw'); and *C. pepo*, native to higher elevations throughout Mexico and, possibly, northern Central America, and the most common commercial squash (Zucchini, Acorn and Scallop, Jack-O'Lantern, Vegetable Spaghetti, Crookneck). Prior research, using artificial hybridization (Whitaker and Bemis 1964), morphometric analyses (Bemis et al. 1970), and association with oligolectic pollinators (Hurd et al. 1971), has produced a relatively coherent picture of phylogenetic and systematic relationships (Whitaker and Bemis 1975). Recent studies (Andres 1990; Merrick 1990; Decker-Walters 1990; Nee 1990; Wilson 1990), however, suggested significant changes in perspective with regard to both systematic and phylogenetic relationships within several crop/weed complexes. We examined chloroplast DNA (cpDNA) restriction-site variation among 15 species of the genus. Our principal goal was to test existing phylogenetic models using restriction-site variation in the chloroplast genome.

Materials and methods

Thirty accessions were chosen to represent five cultivated and ten wild species of *Cucurbita* (Table 1). Two other members of the Cucurbitaceae, *Lagenaria siceraria* and *Luffa aegyptiaca*, were selected as outgroups. Voucher specimens for these accessions are deposited in the TAMU herbarium. Total cellular DNA was isolated from a single plant of each accession. Leaves were ground to a fine powder in liquid nitrogen and the powder suspended in grinding buffer (50 mM Tris-HCL, 25 mM EDTA, 0.35 M sorbitol, 5% PVP-40, 1% sodium bisulfite, 0.2% 2-mercaptoethanol, ph 8.0) at a ratio of 5 ml per gram fresh weight of

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Table 1. List of samples

No.	Species[a]	Origin	Locality	Collection[b]	(kb)[c]
1.	*Lagenaria siceraria* L.	Stokes Seeds	Commerical	HW 5981	–
2.	*Luffa aegyptiaca* Miller	Park Seeds	Commerical	HW 5980	–
3.	*Cucurbita digitata* Gray	Mexico	Chihuahua	HW 5792	3.70
4.	*C. foetidissima* H.B.K.	Mexico	Jalisco	TW 1779	3.24
5.	*C. pedatifolia* Bailey	Mexico	Oaxaca	HW 5463	3.23
6.	*C. lundelliana* Bailey	U.S.D.A.	Belize	PI 438543	3.70
7.	*C. ecuadorensis* Cutler & Whitaker	U.S.D.A.	Ecuador	PI 432445	3.80
8.	*C. ecuadorensis*	U.S.D.A.	Ecuador	PI 432443	3.80
9.	*C. ficifolia* Bouché	Mexico	Guerrero	RB 13452	3.80
10.	*C. ficifolia*	Mexico	Guauajuato	CD 007	3.65
11.	*C. martinezii* Bailey	Mexico	Tamaulipas	HW 3748	3.65
12.	*C. martinezii*	Mexico	Veracruz	MN 27080	3.65
13.	*C. sororia* Bailey[d]	Mexico	Veracruz	MN 29664	3.97
14.	*C. sororia*	Mexico	Jalisco	HI 29459	3.50
15.	*C. sororia*	Mexico	Oaxaca	HW 5448	4.10
16.	*C. sororia*	Mexico	Guerrero	HW 5400	3.80
17.	*C. moschata* (Lam.) Poir.	Mexico	Puebla	HW 5375	4.00
18.	*C. moschata* 'butternut'	Letherman's	Commercial	HW 5985	4.00
19.	*C. moschata*	Colombia	Cali	MH 34	4.00
20.	*C. mixta* Pang.[e] 'striped cushaw'	Redwood City	Commercial	HW 5984	3.90
21.	*C. fraterna* Bailey	Mexico	Tamaulipas	HW 5532	3.65
22.	*C. fraterna*	Mexico	Tamaulipas	HW 5531	3.65
23.	*C. fraterna*	Mexico	Tamaulipas	HW 5529	3.65
24.	*C. pepo* L.[f]	Mexico	Oaxaca	HW 5457	3.90
25.	*C. pepo*[f] 'dark green zucchini'	Ferry Morse	Commercial	HW 5466	3.55
26.	*C. pepo*[g] 'early summer crookneck'	Redwood City	Commercial	HW 5986	3.75
27.	*C. pepo*[g] 'small spoon gourd'	Letherman's	Commercial	HW 5988	3.75
28.	*C. pepo*[g] 'crown of thorns'	Letherman's	Commercial	HW 5987	3.75
29.	*C. texana* (Scheele) Gray[h]	Texas	Gonzales	HW 3173	3.75
30.	*C. maxima* Lam. 'big max pumpkin'	Park Seed	Commercial	HW 5982	3.55
31.	*C. maxima* 'jumbo pumbo pumpkin'	Burgess Seed	Commercial	HW 5983	3.55
32.	*C. andreana* Naud.	Argentina	Buenos Aires	HW 5602	3.70

[a] Recent nomenclatural changes (d–e: Merrick and Bates 1989; f, g, h: Decker 1988)
[b] RB = Robert Bye, CD = Cliff Dixon, HI = Hugh Iltis, PI = U.S.D.A. Plant Introduction, MH = Miguel Holle, MN-Michael Nee, TW = Terrence Walters
[c] *Nsi*I – P3 fragment size
[d] *C. argyrosperma* Huber subsp. *sororia* (L. H. Bailey) Merrick and Bates
[e] *C. argyrosperma* Huber subsp. *argyrosperma* var. *callicarpa* Merrick and Bates
[f] subsp. *pepo*
[g] subsp. *ovifera* (L.) Decker var. *ovifera*
[h] subsp. *ovifera* (L.) Decker var. *texana* (Scheele) Decker

leaves. This mixture was centrifuged at 2,000 g for 10 mm. The pellet was resuspended in extraction buffer (100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% mixed alkyltrimethylammonium bromide, 1% 2-mercaptoethanol) and processed as outlined by Saghai-Maroof et al. (1984). Twenty-one restriction enzymes (*Bam*HI, *Bgl*I, *Bgl*II, *Bst*EII, *Cla*I, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Kpn*I, *Nco*I, *Nru*I, *Nsi*I, *Pst*I, *Pvu*II, *Sac*I, *Sac*II, *Sal*I, *Ssp*I, *Stu*I and *Xmn*I) were purchased from Bethesda Research Laboratories or New England Biolabs and used to digest the DNAs in accordance with manufacturer's recommendations. Restricted DNAs were electrophoresed in 0.8% agarose gels with a running buffer of 100 mM Tris-Acetate, 1 mM EDTA, pH 8.1. DNA fragments in the gels were denatured and transferred to a nylon hybridization membrane (Gene Screen Plus®) according to manufacturer's (New England Nuclear) recommendations. Nylon filters were prehybridized overnight in a hybridization buffer of 10% dextran sulfate, 1 M NaCl and 1% SDS. Cloned chloroplast DNA fragments were labeled with dATP-[α-^{32}P] by nick translation (Maniatis et al. 1982) or with dCTP-[α-^{32}P] by random primer labelling (Feinberg and Vogelstein 1983). Labelled probes were separated from unincorporated dATP- or dCTP-[α-^{32}P] on spun columns (Maniatis et al. 1982), heat denatured, and then added to the hybridization buffer. Hybridizations were carried out at 65 °C overnight. Hybridization membranes were washed according to manufacturer's instructions and exposed to X-ray film (Kodak XAR-5®) at −80 °C. Radiolabelled probes included the *Petunia* cpDNA clones P1, P3, P4, P6, P8, P10, P12, P14, P16, P18, S6 and S8 described by Sytsma and Gottlieb (1986) and a *Lactuca* cpDNA clone *Sac*I-1 (S1) described by Jansen and Palmer (1987). Pairs of smaller probes that are adjacent in the chloroplast genome were pooled for labelling and hybridizations as follows: P8/P10, P12/P14, S6/P16 and S8/P18.

A Wagner parsimony phylogenetic tree for the taxa was constructed with the MIX program of the Phylogenetic Inference Package (PHYLIP version 3.1) written by Felsenstein (Department of Genetics, University of Washington, SK-50, Seattle, Wash. 98195). The BOOT program of PHYLIP was used to

place bootstrap-based confidence limits on branching points in the parsimony tree (Felsenstein 1985). In this analysis, 100 bootstrap-samples were drawn. The Phylogenetic Analysis Using Parsimony (PAUP version 2.4.1) package written by Swofford (1985) was used with the MULPARS option to find multiple equally parsimonious trees.

Results

Hybridization of the 13 cpDNA probes to Southern blots for each of the 21 enzymes revealed a total of 867 restric-

Table 2. Changes in the numbers and sizes of cpDNA fragments caused by loss/gain of a restriction site

No.[a]	Enzyme	Probe	Loss (kb)[b]	Gain (kb)	Mutated samples
1	*Bam*HI	P12/14	0.8	0.6+[0.2]	3
2	*Bam*HI	P1	2.7+2.2	4.8	9–10
3	*Bam*HI	S1	2.4	2.0+[0.4]	4–32
4	*Bam*HI	S6/S8	13.6	9.5+3.2	4–5
5	*Bam*HI	S6/P16	1.6	1.0+0.6	32
6	*Bam*HI	P8/P10	2.6+1.2	3.8	4–32
7	*Bam*HI	S8/P18	2.3+[0.4]	2.7	13–16
8	*Bgl*II	S1	1.8+0.6	2.4	7–8
9	*Bgl*II	S6/P16	6.4+4.5	10.9	24–25
10	*Bgl*II	S6/P16	4.6+2.7	7.3	21–29
11	*Bgl*II	S6/P16	2.9	2.6+[0.3]	6–8, 11–32
12	*Bgl*II	P1/S1	1.4+[0.7]	2.1	3
13	*Bgl*II	S8/P18	1.7	1.0+0.8	9–10
14	*Bgl*II	P3	6.3+3.9	10.5	24–25
15	*Bst*EII	S1	2.4	2.3+[0.1]	21–23, 26–29
16	*Cla*I	S1	4.0+1.5	5.7	3
17	*Cla*I	P12/P14	1.2	0.7+0.5	3
18	*Cla*I	P6	3.9+3.7	7.7	21–29
19	*Cla*I	S8/P18	22.3	13.8+8.5	3
20	*Cla*I	P3	3.7	3.2+[0.5]	13–17, 20
21	*Cla*I	P3	8.0	6.7+1.0	7–8
22	*Cla*I	P8/P10	4.3	3.5+[0.7]	9–10
23	*Cla*I	P8/P10	4.8+[0.7]	5.4	9–10
24	*Cla*I	P8/P10	2.5	1.4+(1.1)	3
25	*Cla*I	P8/P10	4.3+(1.1)	5.4	3
26	*Dra*I	P6	10.2+(4.3)	14.3	21–29
27	*Dra*I	P6	2.6+1.7	(4.3)	21–29
28	*Dra*I	P6	1.7	1.1+0.6	5, 30–32
29	*Dra*I	P8/P10	3.6	2.4+1.2	3
30	*Dra*I	P8/P10	3.6	2.6+0.9	6, 11–17, 20
31	*Dra*I	P8/P10	4.3+3.6	7.9	24–25
32	*Dra*I	S8/P18	1.8	1.0+0.8	11–12
33	*Dra*I	S8/P18	1.8	1.2+0.6	6
34	*Dra*I	S8/P18	5.6+2.6	8.2	9–10
35	*Dra*I	S8/P18	5.6	4.9+0.8	3
36	*Dra*I	S6/P16	1.8	1.4+[0.4]	6–8, 11–32
37	*Dra*I	P3	2.2+[0.3]	2.5	5, 21–23, 30–32
38	*Dra*I	P3	6.4	6.1+0.7	13–16
39	*Dra*I	S1	5.1	3.5+1.7	20
40	*Eco*RI	P1/S1	4.5+2.3	6.8	6, 11–20
41	*Eco*RI	P1/S1	2.3+1.4	3.7	3
42	*Eco*RI	P1/S1	1.4	1.2+[0.2]	6–8, 11–32
43	*Eco*RI	P1/S1	1.4	1.3+[0.1]	21–23, 26–29
44	*Eco*RI	P12/P14	1.6	1.3+[0.3]	3
45	*Eco*RI	S6/S8	4.9	4.3+[0.6]	5
46	*Eco*RI	S6/S8	4.9	2.6+2.2	9–10
47	*Eco*RI	S8/P18	6.0+0.7	6.6	4–5
48	*Eco*RI	S8/P18	1.0+[0.3]	1.3	3
49	*Eco*RI	S8/P18	6.0	5.3+0.9	24–25
50	*Eco*RI	P3	21.5+5.1	26.6	18–19
51	*Eco*RV	P6	4.4+2.3	6.7	6, 11–20
52	*Eco*RV	P12/P14	2.3	2.0+[0.4]	3
53	*Eco*RV	P8/10	8.0	6.2+1.6	4
54	*Eco*RV	S8/P18	16.7+1.6	18.9	4–32
55	*Hin*dIII	S8/P18	4.6	3.3+1.3	9–10
56	*Hin*dIII	P8/P10	4.7+1.9	6.7	3
57	*Kpn*I	P1/P4	17.6+2.9	20.5	10
58	*Kpn*I	S1	22.8	16.0+6.7	4–5
59	*Kpn*I	P6	28.6	27.4+1.2	30, 31
60	*Nco*I	P8/P10	10.0	7.0+2.8	6, 11–12
61	*Nru*I	P6	1.6	0.9+0.6	3
62	*Nsi*I	P1/S1	1.8+1.6	3.4	4–5
63	*Nsi*I	P4/S1	2.4+2.4	4.6	21–29
64	*Nsi*I	P8/P10	1.9+0.7	2.6	6–32
65	*Nsi*I	P12/P14	3.0	2.5+0.5	3
66	*Nsi*I	P3	6.6	4.3+2.3	3
67	*Pst*I	P6/P8	6.7+9.2	16.0	9–10, 12
68	*Pst*I	S6/P16	29.3	24.4+4.4	3
69	*Sac*I	P3/P16	7.6+1.9	9.5	5, 7–10, 21–32
70	*Sac*II	P1/P4	13.2+5.0	18.1	5
71	*Sac*II	P4	1.9	1.8+[0.1]	6, 11–12
72	*Sac*II	P6	9.3+0.4	9.6	6–8, 11–32
73	*Sal*I	P3/P6	25.3	15.0+10.1	9, 10
74	*Ssp*I	P12/P14	4.2	3.6+0.9	3
75	*Ssp*I	P6	1.5	1.0+0.8	18–19
76	*Ssp*I	S6/P16	1.8	1.0+0.9	7–8, 30–32
77	*Ssp*I	S6/P16	1.2+0.7	1.8	6–8, 11–32
78	*Stu*I	S1	25.6	21.2+5.2	3
79	*Stu*I	P6	11.2	6.3+4.6	9, 10
80	*Xmn*I	P4	3.4	1.8+1.4	25
81	*Xmn*I	P4	4.4	3.7+0.8	13–16
82	*Xmn*I	S1	2.7+[0.5]	3.2	7–8, 21–29
83	*Xmn*I	P8/P10	3.6	2.2+1.3	6–8, 11–32
84	*Xmn*I	P8/P10	2.3+2.1	4.4	3
85	*Xmn*I	P6	4.3+1.9	6.4	6–8, 11–32
86	*Xmn*I	P3	6.3	3.8+2.6	5–8, 11–20, 30–32

[a] The identification number is underscored if we were able to polarize the mutation with the outgroups *Luffa* and *Lagenaria*

[b] Fragment in brackets [ ] were not visualized probably because of their small sizes. Fragments in parentheses ( ) were not visualized probably because they were affected by two restriction site changes

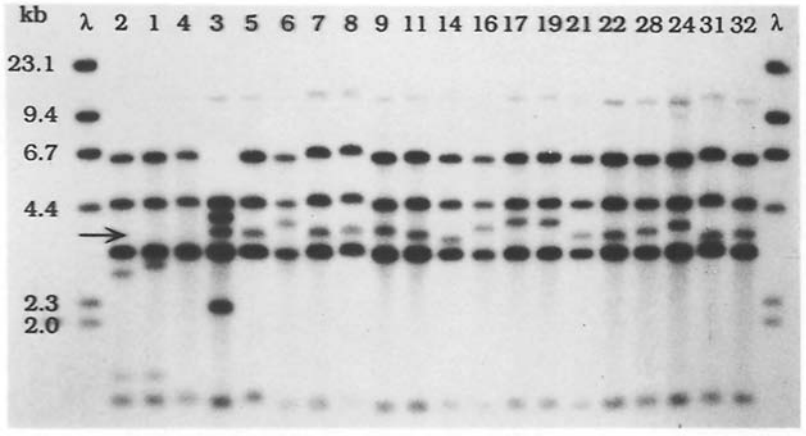

Fig. 1. Autoradiograph showing cpDNAs digested with the restriction endonuclease *Nsi*I and probed with the *Petunia* cpDNA clone P3. The figure shows a restriction fragment that varies in size from 3.2 to 4.1 kb among the cpDNAs. The sample number of the cpDNAs (Table 1) is shown at the top of each lane. The two outermost lanes contain λ-DNA digested with *Hin*dIII

tion fragments. After correcting for the fact that adjacent and overlapping probes hybridize to many of the same fragments, it was estimated that a total of 626 restriction sites had been assayed, representing approximately 2.5% of the genome. Of 626 sites, 86 varied among the *Cucurbita* cpDNAs (Table 2). Fifty-nine of the variable sites are in the large single-copy region of the genome. Of the remaining 27 variable sites, 19 appear to be in the small single-copy region and eight in the inverted repeats. The probes represent approximately 84 kb of the large single-copy region, 19 kb of the small single-copy region and 24 kb of the inverted repeats. Accordingly, the small single-copy region appears most variable with 0.91 variable sites per kb, followed by the large single-copy region at 0.65 and the inverted repeats at 0.25.

In addition to restriction-site variants, we observed several insertion/deletion mutations among the cpDNAs. These were not studied in detail nor used in our phylogenetic analyses. The reason for this is that the region of the genome possessing most of these variants (probe P3) was highly polymorphic for insertion/deletion mutations. This situation is best illustrated with *Nsi*I digests for which one restriction fragment varies in size from 3.23 to 4.10 kb with at least seven size variants (Fig.1; Table 1). Similar results were seen with several other enzymes. Our interpretation is that this region has undergone multiple independent insertion/deletion mutations. As a result, these insertion/deletion mutations cannot be polarized for use in phylogenetic analysis. Within some species, such as *C. sororia* and *C. pepo*, several size variants for the *Nsi*I fragment are found (Table 1).

For eight of the 15 species, we have analyzed more than a single accession (Table 1). In choosing multiple accessions of a species to study, we attempted to maximize the geographic and taxonomic diversity. *Cucurbita ecuadorensis*, *C. maxima*, *C. fraterna* and *C. sororia* possessed no intraspecific site variation. *Cucurbita ficifolia*, *C. martinezii*, *C. moschata*, and *C. pepo* all showed intraspecific restriction site variation, despite the fact that we examined only two to five accessions of each. Accessions of *C. pepo* differed from one another by as many as seven restriction-site changes, and accessions of *C. moschata* by as many as four site changes.

The restriction-site variants (Table 2) were used to construct a Wagner parsimony phylogenetic tree for the *Cucurbita* cpDNAs (Fig. 2). We were able to polarize 67 of the 86 mutations using *Luffa* and *Lagenaria* as outgroups, and thereby specify the ancestral states of these mutations in our phylogenetic analyses. Once the tree was constructed, it was apparent that *C. digitata* was the basal species within *Cucurbita*. This enabled us to polarize all but six of the remaining 19 sites (Fig. 2).

Phylogenetic analyses produced a single most parsimonious tree which required 95 steps to account for the 86 site changes (Fig. 2). This tree has a consistency index of 0.91 and includes 24 autapomorphies, 56 synapomorphies and six characters that could not be polarized. The tree contains two convergent site gains (site changes 28 and 86) and seven convergent site losses (site changes 37, 67, 69 and 82). The most parsimonious tree requires three independent losses for mutation 37 and four independent losses for mutation 69. While this appears unusual, both of these sites are located in the region of the genome covered by probe P3. As noted above, this region experiences frequent insertion/deletion events that could result it convergent site losses. If the site changes covered by probe P3 are removed from the analysis, the resulting tree requires 79 steps to account for 76 characters with a consistency index of 0.96.

Bootstrap-based confidence limits were calculated with the BOOT program of PHYLIP (Fig. 2). This analysis supported the monophyletic nature of several species groups including: (1) *C. fraterna*, *C. pepo*, and *C. texana*, (2) *C. lundelliana*, *C. martinezii*, *C. mixta*, *C. moschata* and *C. sororia*, and (3) *C. foetidissima* and *C. pedatifolia*. The tree clearly indicates that *C. pepo* is divided into two groups, one of which is allied to *C. texana* and *C. fraterna*. The tree also indicates that the xerophytic, perennial

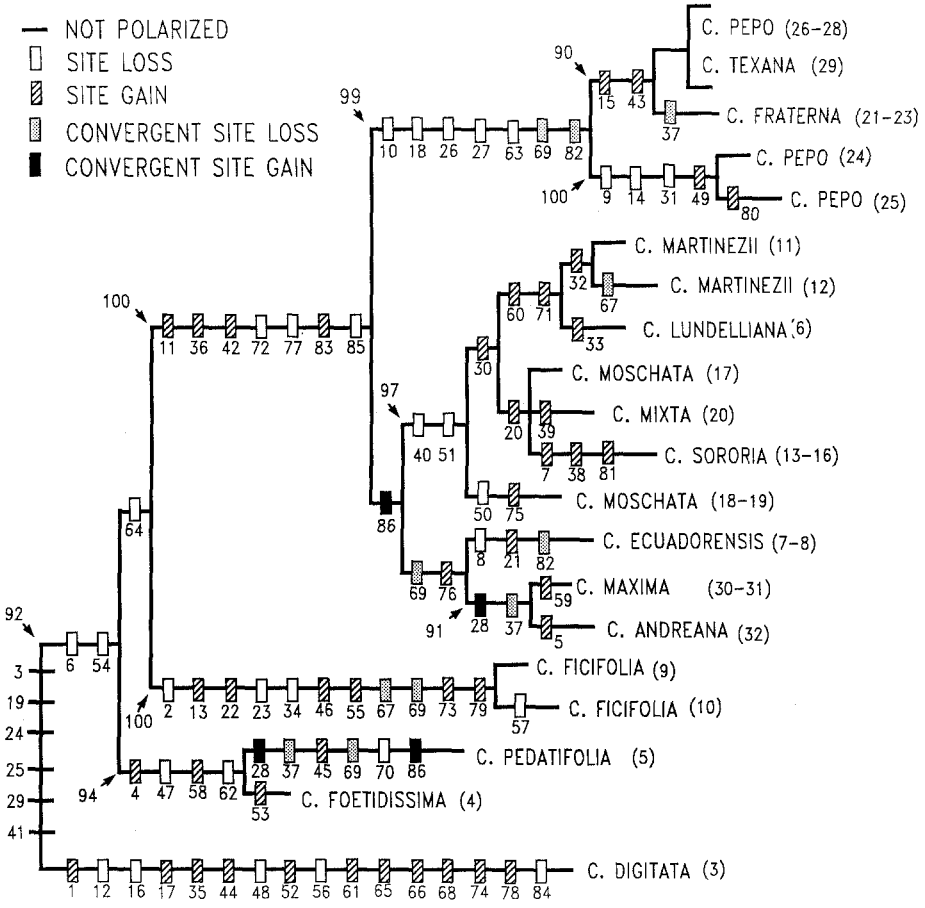

Fig. 2. Wagner parsimony phylogenetic tree for 15 species of *Cucurbita*. The species names and the sample numbers from Table 1 (*in parentheses*) are indicated at the ends of the branches. The identification number for each restriction-site change (Table 2) is indicated along the branches of the tree. The types of changes are indicated by the *shading* of the cross-bars (see key in figure). If a group of species was monophyletic in 90 or more of the 100 bootstrap-samples, the number of bootstrap-samples in which these species formed a monophyletic group is indicated by an *arrow* pointing to the node at the base of the group

species (*C. digitata*, *C. foetidissima*, and *C. pedatifolia*) are all basal within the genus and, with the exception of *C. ficifolia*, the annual species (the other 11 species) form a monophyletic group. The data failed to resolve the relationship among *C. moschata*, *C. mixta* and *C. sororia*, which resulted in a trichotomy.

Discussion

The basal position of perennial species (*C. digitata*, *C. foetidissima*, *C. pedatifolia*), while consistent with general phyletic trends in angiosperms, is a direct reversal of prior phylogenetic alignments of *Cucurbita* species (Whitaker and Bemis 1975). Xerophytic perennials have been positioned (Whitaker and Bemis 1964) as specialized derivatives from a mesophytic, annual ancestral type allied to the *C. lundelliana*/*C. moschata* lineage. However, placement of *C. lundelliana* as a basal type (Whitaker and Bemis 1964) was based on possible misinterpretations of crossing data (Merrick 1990). Chloroplast DNA data from other genera of the tribe Cucurbiteae tribe, which includes both annual and perennial taxa, would further clarify the maternal lineage leading to *Cucurbita* and thereby resolve this fundamental question of polarity between xerophytic and mesophytic species groups.

While the placement of *C. foetidissima*/*C. pedatifolia* as ancestral sister taxa of the domesticated annuals is not an element of previous phylogenetic treatments of the genus, separation of *C. foetidissima* from other xerophytic perennials (as here exemplified by *C. digitata*) and alliance of this species to the domesticated taxa is congruent with patterns of relationships based on other data (Whitaker and Bemis 1975; Singh 1990). The position of *C. pedatifolia* within a monophyletic lineage that includes *C. foetidissima* is informative in that the accession here identified as *C. pedatifolia* is unusual and problematic. This accession originated from fruits collected near the type locality of *C. galeottii* in the highlands (2200 m) of Oaxaca, well south of the known range of *C. pedatifolia*

and well above the elevation limit of most free-living *Cucurbita* species in Mexico. Initially identified as *C. galeottii* (Wilson 1989) in the absence of foliar and floral characters, plants subsequently grown from seed produced leaves roughly comparable to those of *C. pedatifolia* and unlike those of the single specimen representing *C. galeottii* (Bailey 1943). Alignment of this sample with *C. foetidissima*, while not providing a firm identification, clearly places this accession of uncertain identity in one of two species groups of xerophytic *Cucurbita*.

Prior research has consistently placed *C. ficifolia* as an outlier relative to other domesticated annuals and their free-living relatives. While the data presented here are consistent with that perspective, they also place *C. ficifolia* in a basal position that is usually occupied by *C. moschata* (Decker-Walters et al. 1990). The range of distribution of *C. ficifolia* exceeds that of all other *Cucurbita* species. It shows firm morphological connections, in the form of pubescent filaments, to xerophytic perennials allied to *C. foetidissima* and, as pistillate parent, will hybridize with both *C. foetidissima* and *C. pedatifolia* (Andres 1990). This species is also unique among the domesticates in that, like free-living mesophytic taxa, it is a strong climber with a tendency to perennate. On the other hand, some cultivars of essentially all domesticated taxa express structural features, such as leaf shape, stem trichomes, peduncle morphology, and fruit characteristics, that are typical of *C. ficifolia* (Andres 1990). Using embryo culture, F_1 hybrids can be obtained from artificial hybridizations involving *C. ficifolia* and *C. maxima*, *C. moschata*, and *C. pepo*. Thus, placement of *C. ficifolia* as a basal sister group of the mesophytic annuals, with linkage to xerophytic elements that are most closely allied to the annuals, is at least partially congruent with available comparative information.

Samples representing mesophytic annual species, both domesticated and free-living, form a well-defined lineage with three terminal branches composed of (1) North American samples taken from high elevations or latitudes (*C. pepo* complex), (2) North American and South American samples from low elevations or latitudes (*C. moschata*/*C. mixta*), and (3) South American elements of the *C. maxima* complex.

The distinctive lineage representing the *C. pepo* complex bifurcates to form subgroups that correspond to those defined by prior work (Whitaker and Carter 1946; Decker 1985; Wilson 1990) and formalized by Decker (1988) as subsps. *ovifera* (samples 26–28) and *pepo* (samples 24–25). The two lineages of *C. pepo*, separated by six site changes, could represent independent domestication events as proposed by Whitaker and Carter (1946) and Decker-Walters (1990), although this interpretation is complicated by the position of the free-living taxa *C. texana* and *C. fraterna*. These taxa could be placed as ancestral, free-living associates for subsp. *ovifera* cultivars; however, there are no wild forms closely allied with subsp. *pepo*. One must consider the possibilities that, (1) additional wild forms of *C. pepo* exist in nature, or (2) that the ancestor of the second subgroup is now extinct, or (3) that ancestral, free-living types have co-evolved with cultivars of the subsp. *ovifera* lineage.

While relationships between Mexican *C. mixta*, *C. moschata*, and *C. sororia* are unresolved, the three site-gains shared by samples representing *C. sororia* are informative. This free-living taxon has been placed as both progenitor (Merrick 1990) and interactive 'companion weed' (Wilson 1990) of *C. mixta*. The site changes carried by these samples suggest that *C. sororia* is a distinct element of the Mexican complex. However, because our sample of *C. mixta* is from a commercial source, it may not be representative of diversity in landraces of *C. mixta*. Thus, additional material of *C. mixta* will need to be examined to test the presumed status of *C. sororia* as the progenitor of *C. mixta*.

Our data reveal a South American group composed of *C. ecuadorensis*, *C. andreana* and *C. maxima*. The phylogenetic position of *C. ecuadorensis* has been the subject of debate (Nee 1990). Data presented here, while limited, suggest separation from *C. moschata* and alliance, as a basal type, with other South American elements representing the *C. maxima* complex. This pattern of relationships is also indicated by esterase and peroxidase phenotypes (Puchalski and Robinson 1990) and pollinator associations (Hurd et al. 1971). As terminal branches of a lineage derived from a common ancestor with *C. ecuadorensis*, the phylogenetic relationship between the free-living *C. andreana* and domesticated *C. maxima* is unresolved. The South American *C. maxima*/*C. andreana* clade has been placed as a distinctive outlier relative to the primarily North American group composed of *C. pepo*, *C. moschata*, and *C. mixta* (Whitaker and Bemis 1975; Decker-Walters et al. 1990). Data presented here indicate that the *C. pepo* complex is relatively distinct; however, the topology of our tree in this regard is dependent upon a single convergent site-gain (No. 86) and should not be considered robust.

It should be noted that phylogenetic analysis of cpDNA restriction-site mutations produces an estimated phylogeny, in this case a first estimate. Resolution of the phylogenetic reconstruction presented in Fig. 2 will increase as samples representing additional taxa are added. Increased resolution can be expected to result in changes of tree topology, especially in those areas that are not marked with high bootstrap-based confidence values. Phylogenetic analyses employed here also assume a phylogenetic continuity of the chloroplast genome that might not be the case in *Cucurbita*. Evidence derived from cytological study (Singh 1990) and isozyme analysis (Weeden and Robinson 1990) indicate that *Cucurbita* is an allotetraploid genus. If the genomic constitution of all

species is not uniform, as suggested by the work of Weiling (1959), and different ancestral maternal diploid types have been involved, then basic assumptions of the analytical procedure are not valid.

Acknowledgements. We wish to thank those providing germplasm (Table 1) and acknowledge assistance and expertise provided by the Mexican National Botanical Garden, Institute of Biology, UNAM for field collection of experimental material. This research was supported in part by grants from the U.S. National Science Foundation (BSR-8508490) and the U.S. Department of Agriculture (83-CRCR-1-1267).

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