

**An Analysis of Interspecific Hybrids
and Phylogenetic Implications¹
in *Cucumis* (*Cucurbitaceae*)**

By

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Key Words: Angiosperms, *Cucurbitaceae*, *Cucumis*.—Interspecific crossability, chromosome pairing, pollen fertility, phylogenetic relationship, karyomorphology.

Abstract: Investigations on interspecific crossability in 8 *Cucumis* species ($2n = 24$) and chromosome pairing and pollen fertility of their hybrids from 15 combinations have been utilized for tracing the phylogenetic relationships among these taxa and factors responsible for their differentiation. A collective evaluation of data suggests that there are three broad groups of species, one of the spiny fruited interfertile species, whose hybrids show varying degree of chromosome associations and low to high pollen fertility; the second of species with non-spiny fruits, which are completely incompatible with the former but weakly compatible with the cultivated species, *C. melo* L. to produce partly developed seeds, and the third group of *C. metuliferus* E. MEY. ex SCHRAD. and *C. melo* and its different botanical varieties. The species with spiny fruits can be further divided based on karyomorphological similarities and/or on relative genomic affinity, indicated by chromosome pairing and hybrid pollen fertility.

Morphological and karyomorphological investigations in the genus *Cucumis* L. indicated South African annual species as primitive and identified 5 distinct groups in taxa with $2n = 24$ (YADAV & SINGH 1984). The crossability studies, chromosome pairing and pollen fertility in F_1 hybrids are useful in assessing species relationships and phylogeny. The genus *Cucumis* has 30 species divided into two groups on base number (JEFFERY 1980). Hybridization between *C. hardwickii* ROYLE ($2n = 14$) and *C. sativus* L. ($2n = 14$) of Indian origin was reported by IMAZU & FUJISHITA (1956) and DEAKIN & al. (1971), who concluded a close phylogenetic relationship between the two. In the larger group of species

¹ Cytogenetics in *Cucumis* III.

with $2n = 24$ which are distributed in South and East Africa, a series of reports on interspecific hybridization are available without comprehensive and conclusive evidences on phylogeny (WHITAKER 1933; BATRA 1953; SMITH & VENKAT RAM 1954; DEAKIN & al. 1971; NORTON 1969; ANDRUS & FASSULIOTIS 1965; KROON & al. 1979; DANE & al. 1980; NIJS & OOST 1980; KHO & al. 1980). DEAKIN & al. (1971) were the first to produce a comprehensive and monographic account on relative cross compatibility between these species and pollen fertility of their F_1 hybrids, involving 14 species including cultivated *C. melo* L. Based on these data they grouped *Cucumis* species into four major groups. Recently DANE & al. (1980) have provided additional data on chromosome associations in F_1 hybrids of 6 diploid species ($2n = 24$) and discussed phylogeny of these species.

The present paper reports data on crossability between 8 diploid species ($2n = 24$) including *C. melo* in 33 combinations. Chromosome pairing and pollen fertility of 15 successful interspecific hybrids along with crossability have been utilized for tracing relative phylogenetic affinities among these taxa.

The phytogeographical, phytochemical and karyological data (REHM 1960, BROWN & al. 1969; YADAVA & SINGH 1984) in relation to the taxa studied, and others have also been discussed for comprehensive and rational conclusions.

Material and Methods

Identities and sources of *Cucumis* wild species used have been summarised in Table 1. The seeds were sown in small beds in the Botanical Garden, University of Jodhpur, Jodhpur, under normal rainy season.

Anthesis occurs around an hour before sunrise and anthers dehisce soon after. Stigma is receptive upto 4 hours after anthesis. Healthy female flower buds, a day before anthesis were bagged with butter paper. The following morning the stigmas were dusted with pollen from the desired male parent using sterilized mounted needles. Two days later bags were either removed or punched to facilitate proper development of the fruits.

For cytological analysis young flower buds were collected and fixed in (1:3) acetic alcohol containing a drop of 5% ferric chloride. Anthers were squashed in 1% acetocarmine. Temporary preparations were used for detailed analysis and photography. Pollen grains were stained in 1% acetocarmine and stained pollen grains were counted fertile.

The chromosome associations in F_1 hybrids were analysed statistically by one way analysis of variance programme over computer.

Results

Hybridizations were made in 33 combinations involving 8 taxa including the cultivated taxon *C. melo* var. *momordica* (Table 2). Hybrids could be established only in 15 combinations (Table 3). Except with the

Table 1. Taxonomic status and sources of *Cucumis* species. A = Annual. P = Perennial. DDR = Deutsche Demokratische Republik (= German Democratic Republic)

Species	Habit	Fruit Surface	Source	Coll. No.	Origin
<i>C. melo</i> var. <i>momordica</i> COGN.	A	Smooth	India	—	S. Africa
<i>C. dipsaceus</i> EHRENB. ex SPACH	A	Soft-Spined	DDR	CUC 14/76	Ethiopia
<i>C. anguria</i> L. var. <i>anguria</i> MEEUSE	A	Spined	DDR	CUC 9E 74	S. America Introduction
<i>C. anguria</i> L. var. <i>longipus</i> MEEUSE	A	Spined	DDR	CUC 27E 74	S. Rhodesia
<i>C. myriocarpus</i> NAUD.	A	Spined	DDR	CUC 31/74	S. W. Africa
<i>C. zeyheri</i> SOND.	P	Spined	DDR	CUCU 416/765	S. Africa
<i>C. prophetarum</i> L.f.	P	Spined	India	—	Sudan, Egypt
<i>C. species</i>	P	Spined	DDR	CUCU 44/74	

Table 2. Number of pollinations (A) and fruit set (B) in crosses between *Cucumis* species (2n = 24). NA = Not attempted. () = Figures in brackets indicate seeds inviable, hybrid not established

♂ Parent	1	2	3	4	5	6	7	8
♀ Parent	A/B	A/B	A/B	A/B	A/B	A/B	A/B	A/B
1. <i>C. dipsaceus</i>	—	12/5	6/3	16/0	NA	18/5	20/4	5/0
2. <i>C. myriocarpus</i>	11/0	—	7/0	24/2	NA	10/0	NA	NA
3. <i>C. zeyheri</i>	5/0	5/2	—	20/(3)	NA	4/2	NA	NA
4. <i>C. prophetarum</i>	15/6	NA	20/10	—	2/2	6/3	15/11	5/(3)
5. Accession CUCU 44/74	NA	NA	NA	8/0	—	NA	NA	NA
6. <i>C. anguria</i> var. <i>longipus</i>	31/0	30/13	11/5	42/4	NA	—	40/18	12/0
7. <i>C. anguria</i> var. <i>anguria</i>	12/0	NA	NA	3/0	NA	30/7	—	NA
8. <i>C. melo</i> var. <i>momordica</i>	20/0	NA	NA	10/0	NA	15/0	NA	—

Table 3. Comparative morphology of *Cucumis* species and their F₁ hybrids. L = Length, Ser = Serrate, Ent = Entire, SSSp. = Short Sparse Spine, LSSp. = Long Sparse Spine, Dense Spine, Str =

Species/Cross	Size in cm		Leaf	Apex	Margin
	L	W	Shape		
<i>C. dipsaceus</i>	8.5	8.1	Spathulate	Ob	Ser
<i>C. myriocarpus</i>	4.1	5.5	Pl, 5-7 lob	Ob	Ent
<i>C. zeyheri</i>	10.3	9.4	Pl, 5-7 lob	Ac	Ser
<i>C. prophetarum</i>	4.5	6.2	Pl, 5-7 lob	Acu	Ser
<i>C. anguria</i> var. <i>longipus</i>	9.7	10.9	Pl, 5-7 lob	Ac	Ser
<i>C. anguria</i> var. <i>anguria</i>	8.8	10.7	Pl, 5-7 lob	Acu	Ent
Collection CUCU 44/74	3.5	3.7	Pl, 5-7 lob	Ac	Ser
<i>C. dipsaceus</i> × <i>C. zeyheri</i>	5.5	6.8	Pl, 5-7 lob	Ac	Ser
<i>C. dipsaceus</i> × <i>C. myriocarpus</i>	6.6	9.2	Pl, 5 lob	Ob	Ser
<i>C. prophetarum</i> × <i>C. dipsaceus</i>	5.5	6.5	Pl, 3-5 lob	Acu	Ser
<i>C. dipsaceus</i> × <i>C. anguria</i> var. <i>longipus</i>	8.2	10.1	Pl, 3-5 lob	Ac	Ser
<i>C. dipsaceus</i> × <i>C. anguria</i> var. <i>anguria</i>	7.5	7.7	Pl, 5 lob	Acu	Ser
<i>C. myriocarpus</i> × <i>C. prophetarum</i>	4.2	4.8	Pl, 5-7 lob	Acu	Ent
<i>C. zeyheri</i> × <i>C. myriocarpus</i>	4.2	3.7	Pl, 3-5 lob	Ac	Ser
<i>C. prophetarum</i> × <i>C. zeyheri</i>	3.9	5.0	Pl, 3-5 lob	Acu	Ser
<i>C. zeyheri</i> × <i>C. anguria</i> var. <i>longipus</i>	7.3	8.3	Pl, 5-7 lob	Ac	Ser
<i>C. prophetarum</i> × Collection CUCU 44/74	5.3	5.2	Pl, 5 lob	Ac	Ser
<i>C. prophetarum</i> × <i>C. anguria</i> var. <i>longipus</i>	5.5	6.3	Pl, 5-7 lob	Ac	Ser
<i>C. anguria</i> var. <i>longipus</i> × <i>C. prophetarum</i>	6.3	7.2	Pl, 5-7 lob	Ac	Ser
<i>C. prophetarum</i> × <i>C. anguria</i> var. <i>anguria</i>	5.4	6.2	Pl, 5-7 lob	Acu	Ser
<i>C. anguria</i> var. <i>longipus</i> × <i>C. anguria</i> var. <i>anguria</i>	6.9	9.5	Pl, 5-7 lob	Ac	Ser
<i>C. anguria</i> var. <i>anguria</i> × <i>C. anguria</i> var. <i>longipus</i>	7.2	8.5	Pl, 5-7 lob	Acu	Ent

non-spiny fruited cultivated taxon, *C. melo* var. *momordica*, the spiny fruited species were cross compatible with each other in one or the other direction. There were differences in reciprocal crossability. *C. prophetarum* produced fruits with immature seeds when *C. melo* var. *momordica* used as a male parent. *C. anguria* var. *longipus* × *C. anguria* var. *anguria* and *C. anguria* var. *longipus* × *C. prophetarum* were the two combinations in which the hybrids could be established in both the directions (Table 3).

W = Width, Pl = Palmate, Lob = Lobed, Acu = Acuminate, Ac = Acute, Ob = Obtuse, LDSp. = Long Dense Spine, TSSSp. = Thin Soft Sparse Spine, TSDSp. = Thin Soft Striped, Unstr = Unstriped

Flower (size in cm)				Fruit		
Female		Male		Size in cm		Surface
L	W	L	W	L	W	
1.3	1.7	1.6	1.4	6.7	4.8	TSSSp., Unstr
1.2	1.3	1.5	1.0	2.3	2.0	SSSp., Str
1.1	2.0	2.2	1.3	5.0	3.5	LDSp., Str
1.3	1.2	2.1	1.2	4.1	3.3	SSSp., Str
2.0	1.7	0.9	1.6	4.2	3.6	LSSp., Unstr
2.2	1.3	1.1	1.4	5.5	4.3	SSSp., Unstr
1.1	0.9	1.7	1.1	3.8	3.1	SSSSp., Str
1.5	1.8	2.1	1.4	2.2	1.4	TSSSp., Str
0.8	1.0	1.3	1.5	1.8	1.4	TSDSp., Str
1.1	1.5	1.0	0.5	2.0	1.4	TSDSp., Unstr
1.0	1.6	2.0	1.8	2.0	1.6	TSDSp., Unstr
2.1	1.5	2.2	1.8	1.8	1.2	TSDSp., Unstr
0.9	0.7	1.5	1.3	3.2	2.0	LSSp., Unstr
1.0	1.3	1.0	0.9	2.0	1.7	SSSp., Str
1.1	0.8	1.2	0.8	3.3	2.4	LDSp., Str
0.7	1.2	1.8	1.3	4.8	3.1	LDSp., Unstr
0.9	0.4	2.0	1.8	2.0	1.1	SSSp., Str
0.6	0.8	1.7	1.2	2.1	1.6	SSSp., Unstr
1.1	1.0	1.3	1.2	1.8	1.4	LSSp., Unstr
0.7	0.3	1.5	1.0	1.8	1.3	SSSp., Str
1.9	1.1	1.8	1.2	1.9	1.3	LSSp., Unstr
2.0	1.2	1.2	1.3	1.5	1.1	LSSp., Unstr

Morphology. A comparative account of morphological features of parents and their F_1 hybrids have been summarised in Table 3. The characters such as lobing of leaves, acute leaf apex over acuminate, and acuminate leaf apex over obtuse; serrate margin of leaves, and long dense spines on the fruit surface wherever present in a cross combination were expressed in F_1 hybrids, and hence are dominant. Breeding behaviour of striping of fruits was inconsistent. In *C. dipsaceus* \times *C. myriocarpus* the

Table 4. Chromosomal association and chiasma frequency at MI in F_1 hybrids of *Cucumis* *C. dipsaceus* hybrids differ at 5% level of significance from rest. Mean ring bivalent

Cross	No. of univalents	
	Range	Mean
<i>C. dipsaceus</i> × <i>C. zeyheri</i>	0-24	3.04 ±0.95
<i>C. dipsaceus</i> × <i>C. myriocarpus</i>	0-12	4.86 ±0.49
<i>C. dipsaceus</i> × <i>C. anguria</i> var. <i>longipus</i>	0-9	3.88 ±0.39
<i>C. dipsaceus</i> × <i>C. anguria</i> var. <i>anguria</i>	0-24	3.06 ±0.53
<i>C. prophetarum</i> × <i>C. dipsaceus</i>	0-10	2.2 ±0.53
<i>C. prophetarum</i> × <i>C. zeyheri</i>	0-4	1.0 ±0.27
<i>C. prophetarum</i> × Collection CUCU 44/74	0-8	1.98 ±0.39
<i>C. prophetarum</i> × <i>C. anguria</i> var. <i>longipus</i>	0-6	2.0 ±0.39
<i>C. prophetarum</i> × <i>C. anguria</i> var. <i>anguria</i>	0-5	1.84 ±0.35
<i>C. myriocarpus</i> × <i>C. prophetarum</i>	0-8	1.32 ±0.35
<i>C. zeyheri</i> × <i>C. myriocarpus</i>	0-2	0.28 ±0.16
<i>C. zeyheri</i> × <i>C. anguria</i> var. <i>longipus</i>	0-8	2.96 ±0.45
<i>C. anguria</i> var. <i>longipus</i> × <i>C. prophetarum</i>	0	0
<i>C. anguria</i> var. <i>longipus</i> × <i>C. anguria</i> var. <i>anguria</i>	0-4	0.24 ±0.10
<i>C. anguria</i> var. <i>anguria</i> × <i>C. anguria</i> var. <i>longipus</i>	0	0

striping of fruits of the male parent was expressed in F_1 hybrid, but not in the crosses, *C. zeyheri* × *C. anguria* var. *longipus* and *C. prophetarum* × *C. anguria* var. *longipus* although it was a feature of the female parents. In certain crosses the maternal feature was expressed in the F_1 (Table 3). Quantitative characters such as leaf size and flower size were expressed as intermediate between the parents.

Cytology. All the parent species and 15 established F_1 hybrids were analysed cytologically for chromosome pairing, chiasma frequency,

species. 25 PMCs were analysed in each combination. Mean univalent and bivalent in *C. anguria* var. *longipus* hybrids differ at 5% level of significance from rest

Range	No. of bivalents		No. of multivalents		No. of chiasma/PMC		Chiasma Termination Coefficient
	Ring Mean	Rod Mean	Range	Mean	Range	Mean	
0-11	8.92 ±0.31	0-4 1.2 ±0.21	0-2	0.18 ±0.08	0-24	21.8 ±0.98	0.93
5-12	8.76 ±0.41	0-2 0.64 ±0.12	0-1	0.12 ±0.06	11-24	18.32 ±0.54	0.97
8-11	9.22 ±0.23	0-4 0.84 ±0.18	0	0	16-22	17.45 ±0.41	0.96
0-11	7.2 ±0.22	0-3 1.66 ±0.15	0-2	0.8 ±0.05	0-24	19.66 ±0.88	0.95
7-12	8.68 ±0.37	1-4 1.88 ±0.17	0-2	0.14 ±0.08	11-23	20.84 ±0.52	0.95
7-11	9.8 ±0.37	1-3 1.4 ±0.21	0-2	0.20 ±0.07	16-22	18.36 ±0.44	0.93
6-11	9.68 ±0.21	1-3 1.32 ±0.32	0	0	15-23	17.8 ±0.63	0.92
8-11	10.08 ±0.19	0-2 0.80 ±0.13	0-1	0.06 ±0.07	16-23	21.36 ±0.39	0.96
7-12	9.24 ±0.28	0-2 1.6 ±0.13	0-1	0.12 ±0.06	15-24	21.84 ±0.47	0.92
5-12	9.8 ±0.32	0-4 1.2 ±0.19	0-2	0.2 ±0.11	18-24	21.36 ±0.34	0.90
9-11	10.6 ±0.19	0-3 1 ±0.32	0-1	0.10 ±0.12	21-23	21.12 ±0.29	0.98
8-11	8.8 ±0.24	0-3 1.7 ±0.20	0	0	15-22	21.32 ±0.34	0.98
9-12	10.68 ±0.22	1-3 1.04 ±0.17	0-2	0.12 ±0.11	17-23	22.76 ±0.21	0.91
8-12	10.72 ±0.22	0-3 0.96 ±0.13	0-2	0.12 ±0.08	19-24	22.88 ±0.26	0.99
9-12	10.84 ±0.15	0-3 1.0 ±0.11	0-1	0.04 ±0.08	20-24	23.4 ±0.17	0.96

distribution of chromosomes at anaphase I (A I) and pollen fertility. The details for hybrids are summarised in Tables 4 and 5, whereas for parents that showed normal meiotic cycle elsewhere (YADAVA & SINGH 1984).

Hybrids from crosses with *C. dipsaceus* as one of the parents showed a higher mean of univalents and lower mean of bivalents at 5% level of significance than the hybrids of rest of the species (Table 4; Figs. 1-3, 6). The mean univalent number ranged from 4.86 in *C. dipsaceus* × *C. myriocarpus*, to a minimum of 2.2 in *C. prophetarum* × *C. dipsaceus*

(Table 4). Whereas the hybrids, between the two varieties of *C. anguria*, and between *C. anguria* var. *longipus* and *C. prophetarum* have higher ring bivalent associations at 5% level of significance than others (Table 4).

The chromosome associations in other species hybrids were not significantly different from each other. *C. myriocarpus* hybrids with *C. prophetarum* and *C. zeyheri* have 11.0 and 11.66 bivalents respectively compared to 9.40 recorded for *C. dipsaceus* × *C. myriocarpus* (Table 4, Fig. 5).

In crosses involving *C. prophetarum* highest mean number of bivalents were observed in *C. prophetarum* × *C. zeyheri* and *C. prophetarum* × accession CUCU 44/74 (Table 4). However the pollen fertility was less in the former (23.07%) than the latter (48.85%). In other crosses involving *C. prophetarum* and two varieties of *C. anguria*, the mean bivalent frequencies and pollen fertility were similar (Table 4, Fig. 4). However, the hybrid from reciprocal cross, *C. anguria* var. *longipus* × *C. prophetarum* showed near normal pairing and a high pollen fertility (81.74%) (Tables 4, 5). In *C. myriocarpus* × *C. prophetarum* the bivalent frequency was 11.0 with a pollen fertility of 21.6%.

Both the ring and the rod bivalents were recorded. The frequency of rod bivalents was low with the mean ranging from 0.64 to 1.88 (Table 4). The mean chiasma frequency in the hybrids was not significantly different from parent species (YADAVA & SINGH 1983). Chiasma frequency was maximum in the intraspecific cross of *C. anguria* and minimum in the hybrids involving *C. dipsaceus* as one of the parents (Table 4).

Majority of the hybrids showed multivalents but in a low frequency (Table 4; Figs. 1–8).

At AI hybrids often exhibited unequal distribution, laggards and chromosome bridges with or without fragments (Table 5; Figs. 9, 10). Chromosome bridge with a fragment was observed in 5 hybrid combinations, *C. dipsaceus* crossed with *C. myriocarpus*, *C. zeyheri* and *C. anguria* var. *anguria*, and in the crosses, between two varieties of *C. anguria* and *C. prophetarum*.

Figs. 1–10. Pollen mother cells at metaphase I in *Cucumis* species hybrids. — Fig. 1. 7II + 2III + 4I in *C. dipsaceus* × *C. anguria* var. *longipus*. — Fig. 2. 9II + 1IV + 2I. — Fig. 3. 6II + 2III + 6I, and — Fig. 4. 8II + 1IV + 4I in *C. prophetarum* × *C. anguria* var. *anguria*. — Fig. 5. 6II + 1IV + 8I in *C. myriocarpus* × *C. prophetarum*. — Fig. 6. 8II + 6I and — Fig. 7. 6II + 2IV + 4I in *C. dipsaceus* × *C. zeyheri*. — Fig. 8. 5II + 1III + 1I in *C. zeyheri* × *C. anguria* var. *anguria*; (multivalents arrows). — Fig. 9. A lagging II, and — Fig. 10. Bridge with fragment (arrow) in *C. prophetarum* × *C. anguria* var. *anguria*

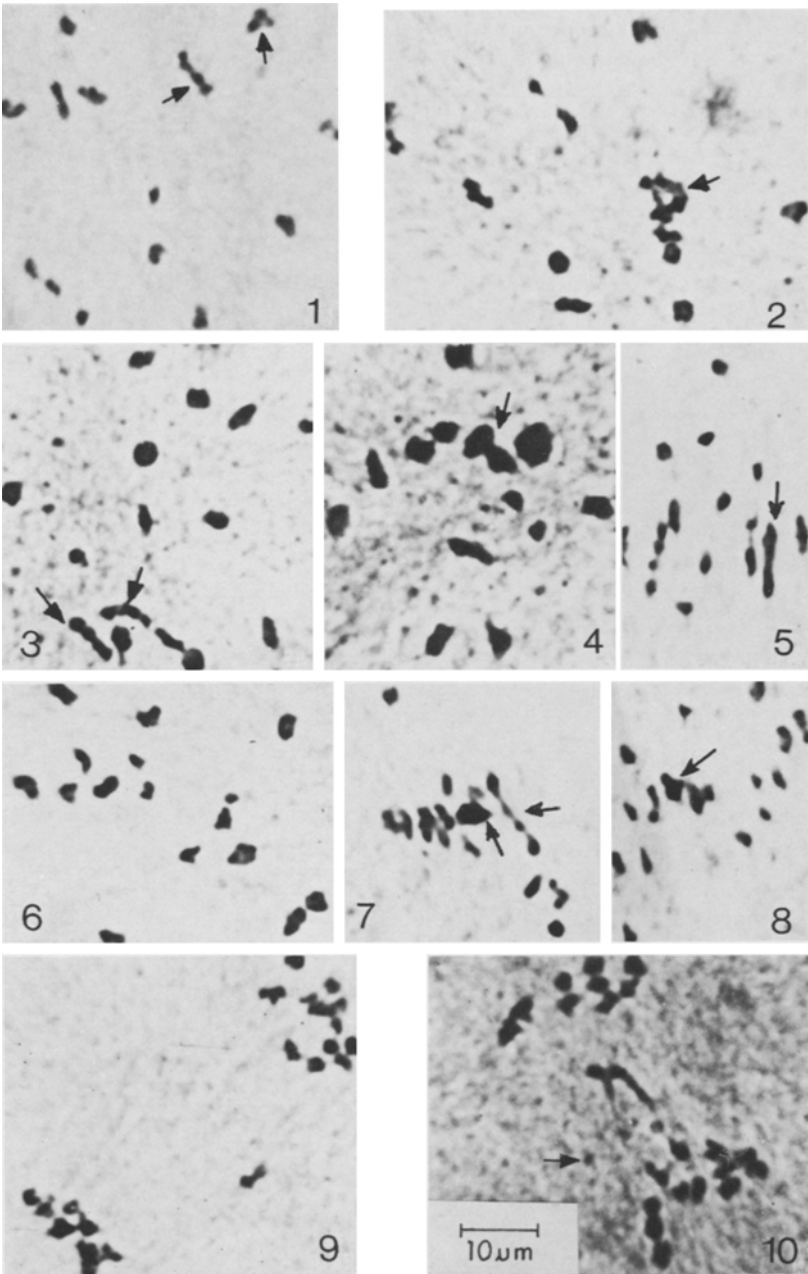


Table 5. Chromosome distribution at AI and pollen fertility in F_1 hybrids of *Cucumis* species. Pollen fertility in parent species ranged from 86–98%.
* Combination with a chromosome bridge and fragment

Cross	% Cells with regular distribution	% Cells with unequal distribution	% Cells with laggards	% Cells with bridge(s)	Pollen fertility %
<i>C. dipsaceus</i> × <i>zeyheri</i> *	32	34	10	24	22.5
<i>C. dipsaceus</i> × <i>myriocarpus</i> *	24	34	10	32	13.02
<i>C. dipsaceus</i> × <i>anguria</i> var. <i>longipus</i>	32	28	32	8	17.26
<i>C. dipsaceus</i> × <i>anguria</i> var. <i>anguria</i> *	40	25	25	10	19.75
<i>C. prophetarum</i> × <i>dipsaceus</i>	48	10	30	12	25.68
<i>C. prophetarum</i> × <i>zeyheri</i>	40	32	28	0	23.07
<i>C. prophetarum</i> × Coll. CUCU 44/74	24	36	40	0	48.85
<i>C. prophetarum</i> × <i>anguria</i> var. <i>longipus</i> *	40	32	20	8	19.71
<i>C. prophetarum</i> × <i>anguria</i> var. <i>anguria</i> *	72	4	8	16	17.41
<i>C. myriocarpus</i> × <i>prophetarum</i>	48	20	32	0	21.61
<i>C. zeyheri</i> × <i>myriocarpus</i>	40	20	40	0	22.36
<i>C. zeyheri</i> × <i>anguria</i> var. <i>longipus</i>	32	28	20	20	16.01
<i>C. anguria</i> var. <i>longipus</i> × <i>prophetarum</i>	88	5	7	0	81.74
<i>C. anguria</i> var. <i>longipus</i> × <i>anguria</i> var. <i>anguria</i>	60	10	14	16	79.60
<i>C. anguria</i> var. <i>anguria</i> × <i>anguria</i> var. <i>longipus</i>	100	0	0	0	80.00

Discussion

Cucumis comprises a large number of morphologically related, cross compatible and cross incompatible taxa. The wild species with $2n = 24$ are mainly distributed in East and South Africa, whereas the cultivated species *C. melo* ($2n = 24$) is widespread in arid and semi-arid regions of Africa and Asia. As Cucurbits have been known for photoperiod and temperature sensitivity the relative crossability data obtained in the present investigation under natural conditions may not be accurate for tracing relative affinities between the species at microevolutionary level. However at higher level studies of DEAKIN & al. (1971) did support morphological grouping of spiny fruited species into one, which are cross compatible with each other but cross incompatible with other non-spiny fruited species including the cultivated *C. melo*.

The species studied in the present investigation exhibited different degree of female cross compatibility. The species *C. dipsaceus* and *C. prophetarum* were found to be more compatible when used as female. Such differences are often associated with habit and ploidy, but in these species the former is an annual and the latter a perennial with similar ploidy levels; hence the differences in crossability (Table 2) may be because of different physiological, genetical or environmental factors.

The high degree of chromosome pairing observed in the hybrids of the presently investigated species, indicates genome homology. Based on karyotypic similarities, YADAVA & SINGH (1984) divided the 13 investigated *Cucumis* species ($2n = 24$) into 5 distinct groups. They are – (1) *C. leptodermis* SCHWEIK., *C. africanus* LINDLEY f. – (2) *C. ficifolius* A. RICH., *C. hookeri* NAUD., *C. dipsaceus* EHRENB. ex SPACH – (3) *C. myriocarpus* NAUD., *C. zeyheri* SOND., *C. prophetarum* L. f., *C. species* CUCU 44/74, *C. anguria* L. – (4) *C. metuliferus* E. MEY. ex SCHRAD. – (5) *C. sagittatus* PEYR., *C. melo* L. Species of the third karyomorphological group, *C. zeyheri*, *C. prophetarum*, *C. myriocarpus*, accessions CUCU 44/74 and two varieties of *C. anguria* appear to have strong genomic affinities as evidenced by the chromosome associations in their F_1 hybrids which do not differ significantly from each other (Table 4, 5). However, based on bivalent associations (11.04), which is an indicator of genetic homology and a high pollen fertility (48.85%) in this group, *C. prophetarum* is very close to the newly investigated accession CUCU 44/74, although the homology between the respective pairs of chromosomes has reduced as evidenced by univalents (Table 4). The hybrids of *C. prophetarum* with other taxa, *C. zeyheri*, *C. myriocarpus*, *C. anguria* var. *longipus* and *C. anguria* var. *anguria* have almost identical bivalent and univalent associations (Table 4) but unlike above combinations, they show multivalents and lower pollen fertility. This suggests structural differences between the chromo-

somes of *C. prophetarum* and these species. *C. prophetarum* may differ from *C. anguria* by an inversion as a bridge with fragment was observed at AI in a few PMCs of hybrids with both the varieties. Such differences may affect genetic segregation and pollen fertility in hybrids even after higher genomic homology between species. But the established reciprocal of *C. anguria* var. *longipus* \times *C. prophetarum* showed nearly normal bivalent association suppressing quadrivalent and AI abnormalities resulting in high pollen fertility (81.74%), probably a cytoplasmic influence.

It may be noted that in most of these crosses pollen fertility is low even after high bivalent associations (Tables 4, 5). This may be due to one or more of the following reasons—(1) structural differences between the chromosomes of parental species that are manifested as multivalent associations observed in majority of the hybrids but at a very low frequency,—(2) due to early terminalisation of chiasmata because of inadequate homology leading to non-coorientation of the participating chromosomes, and —(3) as a result of cryptic structural differences between chromosomes resulting in genic imbalance and reduction of pollen fertility. Therefore genomic affinity as expressed by pairing may not be expressed in terms of pollen fertility of the hybrid.

Higher bivalent associations and pollen fertility of hybrids between *C. anguria* var. *anguria* and *C. anguria* var. *longipus* suggest their close relationship. Their next close relatives are *C. prophetarum*, and *C. zeyheri* (Table 4). However, *C. zeyheri* is a little distinct evidenced by a higher mean of univalents and very low pollen fertility (Tables 4, 5). Among the rest of the species, *C. zeyheri* and *C. myriocarpus* appear closer to each other than they are to *C. prophetarum* based on high bivalent and low univalent and multivalent associations in the hybrids of former species than the latter (Table 4), although pollen fertility was uniformly low in all combinations (Table 5).

The crossability between two varieties of *C. anguria* in both directions, normal chromosomal pairing and high pollen fertility in their hybrid are in agreement with the earlier conclusions of MEEUSE (1958), DEAKIN & al. (1971) and DANE & al. (1980) on their varietal status. The differences in crossability and significantly higher mean of ring bivalents in the hybrids of *C. anguria* var. *longipus* suggest that *C. anguria* var. *longipus* is closer to these species than *C. anguria* var. *anguria* (Table 2, 4).

C. dipsaceus which was placed in a different group on karyomorphological grounds, crossed with other species of the preceding group in one or the other direction. The hybrids between *C. dipsaceus* and other species have significantly less bivalents and more univalents and low pollen fertility (Table 4, 5). Mean bivalent associations and pollen fertility ranged from 9.4 and 13% respectively in case of *C. dipsaceus* \times *C. myriocarpus* to 10.56 and 26% in case of *C. prophetarum* \times *C. dipsaceus*. These values are

less than those observed in intragroup hybrids of the previous group (Tables 4, 5). Therefore, the phylogenetic affinities of *C. dipsaceus* with other species are of distant nature and hence, this taxon may be recognised as a member of another subgroup. Regarding relative phylogenetic relationships between *C. dipsaceus* and other species, based on chromosome pairing and pollen fertility of the hybrids, *C. prophetarum* is the closest with lowest mean univalent and highest pollen fertility followed by *C. zeyheri*, *C. anguria* and *C. myriocarpus* (Table 4, 5). Observation of a bridge with a fragment in 3 hybrid combinations of *C. dipsaceus* out of 5 (Table 5) suggests a difference by an inversion between these three species and *C. dipsaceus*.

The present cytological analysis also revealed some factors responsible for speciation. The multivalent association observed in a majority of hybrids (Table 4) suggest that chromosomal alteration such as translocation and inversion has been one of the factors responsible for species differentiation. However, the frequencies across different combinations were low and not significantly different from each other. At AI along with other abnormalities in 5 combinations, observation of a chromosome bridge with fragment and high pollen sterility suggest that the involved species probably differ by a paracentric inversion.

A comprehensive look at morphology, karyotype, crossability, chromosome pairing and pollen fertility in these hybrids suggests that *C. dipsaceus*, *C. myriocarpus*, *C. zeyheri*, *C. prophetarum*, accession CUCU 44/74, *C. anguria* var. *longipus* and *C. anguria* var. *anguria* are members of a single group with varying degrees of cytological divergence. However, *C. dipsaceus*, karyomorphologically, and based on chromosome pairing in its hybrids with other species stands distinct from the rest.

The success in crossing between some other species belonging to different karyomorphological groups and some species studied in present investigation have been reported by DANE & al. (1980). DANE & al. (1980) observed a similar level of pairing in hybrids of *C. anguria* × *C. africanus*; *C. myriocarpus* × *C. leptodermis* besides *C. dipsaceus* × *C. zeyheri* and *C. anguria* × *C. dipsaceus* indicating that these species although falling in different karyomorphological groups have close genomic affinities. But the high univalent formation in *C. anguria* × *C. myriocarpus* suggests that the two species are far differentiated, contrary to the karyomorphological observations of YADAVA & SINGH (1984). On the other hand meiotic behaviour of *C. ficifolius* × *C. zeyheri* hybrid with high univalent frequency is in agreement for their placement into two karyomorphological subgroups (YADAVA & SINGH 1984). In brief, all these species should be recognised as members of one group, from the same gene pool containing primitive wild species, and are interfertile.

Hybridization between *C. melo* var. *momordica*, a widely adapted

cultivated taxon of the Indian subcontinent and other wild species failed except in *C. prophetarum* × *C. melo* var. *momordica*, where fruits with inviable seeds were obtained (Table 2). This is suggestive of reproductive isolation and/or separation of gene pools of cultivated *C. melo* and the wild spiny fruited species of *Cucumis*. From the biosystematic point of view, they should be regarded as members of distinct group. From the breeding point of view, the barriers between the species of these two groups appear to be postzygotic and may be overcome by the technique of embryo culture.

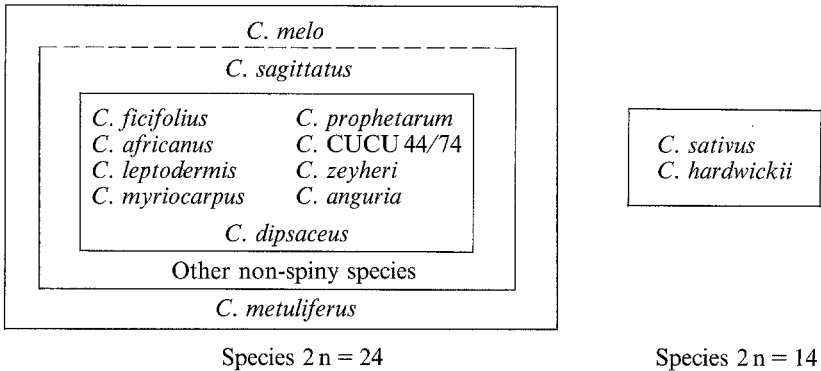


Fig. 11. Grouping by compatibility and chromosome pairing in *Cucumis*. ——— Incompatible, - - - - weak compatibility. *C. ficifolius* and *C. dipsaceus* are distinct by karyomorphology & pairing in F_1

Based on morphological similarity in fruit character and hybridization between *C. sagittatus* × *C. melo* yielding partly developed seeds DEAKIN & al. (1971) kept the two species and other related non-spiny taxa together in a group. These two species failed to cross with spiny fruited species, indicating that they have evolved along a separate line diverging from spiny fruited species, and should be recognised as separate groups. It is also evidenced by karyomorphological studies of YADAVA & SINGH (1984). However, *C. metuliferus* which was earlier recognised as the alone member of a separate group based on morphology and cross incompatibility between this species and other species (DEAKIN & al. 1971) and also falls in a separate karyomorphological group (YADAVA & SINGH 1984), have been reported to produce fertile hybrids with *C. melo* (NORTON 1969, NORTON & GRANBERRY 1980) concluding that *C. metuliferus* is evolutionarily close to *C. melo*.

The picture that emerges from the foregoing discussion is the existence of three broad groups in *Cucumis* species with $2n = 24$ (Fig. 11). The first

group is of wild spiny fruited species distributed in different regions of Africa. They are interfertile, have common ancestry and can further be divided into subgroups based on karyomorphological similarities and chromosome pairing in their F_1 hybrids. Among these species based on karyomorphological symmetry (YADAVA & SINGH 1984) and host-parasite relationship (LEPPIK 1966) the South African species are considered primitive. The second group is of the non-spiny fruited species which are cross incompatible with the spiny fruited species and hybridize with *C. melo* but rarely producing viable seeds. Therefore, according to HARLAN & DE WET (1971) the non-spiny fruited wild species can be recognised as a group of species with secondary gene pool, while *C. metuliferus*, the cultivated species, *C. melo* and its varieties which are completely isolated from the primary gene pool of spiny fruited species and have weak compatibility with the second group, can be recognised as the third group. The fourth group of species with *C. hardwickii* and *C. sativus*, has a different base number ($x = 7$), origin and distribution (YADAVA & SINGH 1984), and probably deviated at an early stage of evolutionary ladder from primary gene pool of spiny fruited species (Fig. 11).

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