Studies on the Phylogenetic Differentiation in Taro, *Colocasia esculenta* Schott

by
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Contents

1. Introduction

Taro (Sato-imo in Japanese), *Colocasia esculenta* Schott, is a species in the family Araceae, which grows wild or is cultivated widely in the tropical, subtropical and temperate area. The family is a very large group and its classification systems are various to be confused.Engler (1889) set up 8 subfamilies, and Hooker (1894) did 6 tribes under 3 subfamilies, who wrote there were about 100 genera and about 1000 species. Engler and Krause (1920) reduced them to 3 tribes. Ohwi (1953) described the family as having more than 1500 species, while Kitamura et al. (1979) treated it as a group of 1800 species under 110 genera.

Limited only in taro, the species name is *C. antiquorum* Schott by Hooker and this has been used most frequently. However, Young (1924) proposed to divide it into 2 species: *C. antiquorum* and *C. esculenta.* He distinguished 1 variety of the former and 3 varieties of the latter. He mentioned the ratios of portions of the inflorescence but not as a key character to species or variety. According to him, the ratio of the staminate portion to the sterile appendage of the spadix appeared to be nearly constant in all the varieties of the true *C. antiquorum.*

Though other authors also noticed the presence of the sterile appendage and the ratio of portions of the inflorescence as Hooker (1894) had done, the significance of this character has been vague. Yet, it appears to have become an important key character in the classification of taro or other species without being examined.

Recently Hotta (1970) proposed an arranged classification system of this family in Japan and the adjacent areas. He set up 6 subfamilies and put the tribe Colocasieae under the subfamily Philodendroideae. Genera *Colocasia* and *Alocasia* were in this tribe together with other 4 genera:

4. Secondary veins started from primary lateral veins more or less parallely ascend and then are collected between the primaries forming reticulate venation; male flowers usually forming a typical synandrium ---------- Tribe Colocasieae
   11. Spadix without appendix; tuberous herbs. ---------- 12
      12. Parietal placentation ---------- *Remusatia*
      12. Basal Placentation. ---------- *Gonatanthus*
   11. Spadix usually with appendix or sterile apical part of male inflorescence -------- 13
      13. Ovary with an ovule ---------- *Hapaline*
      13. Overy with several ovules ---------- 14
         14. Parietal placentation; ovule anatropous or hemianatropous; seed many and small ---------- *Colocasia*
         14. Basal placentation; seed few (1-6), usually larger ---------- 15
            15. Ovule orthotropous or hemianatropous; leaf simple rarely pinnatifid ---------- *Alocasia*
            15. Ovule anatropous; leaf pinnatilobed or pinnatifid --------- *Shizocasia*

He gave *C. esculenta* Schott, *C. gigantea* Hook., *A. cucullata* (Lour.) Schott and *A. odora* (Roxb.)

C. Koch. Besides, *C. esculenta* was divided into 2 varieties: var. *aquatilis* and var. *esculenta*, and the latter contained 5 cultivar groups.

1. Plants with long stolons; appendage of the spadix long; diploids; mostly wild or naturalized, a few cultivated in tropics ---------- Var. *aquatilis*
1. Plants without stolons; appendages various; diploids or triploids; mostly cultivated ---------- Var. *esculenta*
   2. Chromosome number 2n=28 (diploid); lateral tuber with normal leaves; appendages short (?) ---------- 3
   2. Chromosome number 2n=42 (triploid) or unknown; lateral tubers often without normal leaves or with a few ones; appendages short or long ---------- 4

He commented that Bailey (1924) recognized two species of cultivated taro namely *C. esculenta* with short appendages and *C. antiquorum* with long appendages, but that many intermediate types exsisted.

Thus, the classification of this plant group including taro is very complicated and not established. The present author started this work at first on the point of view to study right- and left- handedness in taro. And after collecting taro in the Nepal Himalayas in 1974, he began to gather data of the inflorescence and to make crossing experiments with taro for the purpose of knowing the phylogenetic significance of the ratios of different portions of the inflorescence in this species.

However, these experiments could not bring a clear answer. The next step was necessary, so molecular-biological techniques were taken and the purpose of experiments naturally changed to investigate the phylogenetic differentiation in *C. esculenta* Schott and the relationships with other species.

2. General Materials

Almost all materials used in the present works were collected by the present author himself. His main travels for collection and field works were as follows;

1. 1973.2 –1973.6	Eastern Nepal, India, Sri-Lanka
2. 1981.8 –1981.9	Central Nepal
3. 1982.8	Ishigaki Island, Iriomote Island
4. 1983.6 –1983.7	Tokunoshima Island, Amami Island, Kikai Island
5. 1983.7 –1983.8	North-western Thailand
6. 1986.8	North-eastern Thailand
7. 1987.5 –1987.6	Amami-ohshima Island, Kakeroma Island
8. 1988.10	Okinawa Island

More than 400 strains were collected, but many of them were dead because of the difficulties to keep them during the winter season or because of diseases and insect damages. Of these collections, those from Nepal, Thailand, Ryukyu and other parts of Japan were used in the present experiments. Fig.1 shows some places they were collected.

Fig.2 and Table 1 and Table 2 are the travel route and the list of collections in 1973 (Yoshino, 1976). Most of the collections were transfered to the Plant Germ-plasm Institute of Kyoto University and to Tokyo Agricultural University. Only KUYE373, KUYE442 and KUYE474 have been left in Okayama. Fig.3 is a map of Central Nepal in 1981. Some strains collected through these two times travels were to be very important samples in the present work. Fig.4 shows travels in Thailand in 1983 and 1986. Two strains, TC8614 and TC83021.2, should have been noticed.

The present work consists of four parts, through which some strains were used almost in common, so they are listed in Table 3. Species names are generally after the systems by Hotta (1970) and partly after Engler and Krause (1920). As it is rather difficult to decide 'wild', 'natural', 'escape' or 'cultivated', the presence of long stolon is taken as a standard of wild types in the table. However, it was naturally considered to get informations from the native people as much as possible and to observe ecological conditions. Fig.5 and Fig.6 show an example of the habitats of wild types in Nepal and long stolons, respectively.

In these general materials, accession No.2 was a sterile wild diploid and accession No.17 was a wild triploid. Fig.7 shows that accession No.2 was completely male sterile while other two triploid (accessions No.22 and No.17) had partially fertile pollen grains with three nuclei, and that some pollen tubes were observed to be germinating. Besides, accession No.2 was female sterile, too. As shown in Fig.8, univalents were observed in MI stage of accession No.2. The sterility of this strain will have a special meaning later.

Table 1. Wild or escaped species of tribe Colocasieae on the caravan route

No.	Collection No.	Point: Name	Point: Environment	Point: Altitude	Wild or escaped	Domestic name	Chromosome No. (RTC)	Plant: Form	Plant: Height	Color: Petiole	Color: Leaf	Color: Vagina	Color: Leaf center	Leaf: Shape	Leaf: Angle	Lateral rhizome	Axial tuber	Species	Classification	Remarks
(District I)																				
1	473	①	C, s	72m	W (?)	Kiechú or Pindalú	42	S	T	p	G	p	(—)	P, u	<R	G-LL, t	G	*C. esculenta* Schott	a-2'	leaf edible
2	474	①	Y, h	72	W	—	28	E	T	pm-P	G	p-P	p	P	R	E-LL, t, b	Ob	*C. esculenta* Schott	a-2	leaf edible, flowered in Japan (June)
3	477	①	C, s	72	W	Kiechú or Pindalú	—	S	TT	G (pm)	G	G	P	P, u, l	R	LL	ht	*C. esculenta* Schott	a-1	leaf edible
4	478	①	R, h	72	W (?)	Kiechú	—	E	T	pm-G	G	p-G	(—)	p, u, r	<R	—	Ob	*C. esculenta* Schott	a-3	leaf edible
5	480	①	R, s	72	W	Darsãni	—	S	TT	pm-G	G, l	g (p)	(—)	S, La	R<	—	Ob, t	*A. macrorrhiza* Schott	d	not for use
6	—	①	C, s	72	W (?)	—	—	E	T	P	G	P	p	P, l	R	LL	ht	*C. esculenta* Schott	a-1	leaf edible, rather thin
(District II)																				
7	465b	②	K, s	350	W (?)	—	—	S	M	G (pm)	G	G	(—)	P	<R	E-LL	—	*C. esculenta* Schott	a-2	not for use, only one individual among maize
8	464a	③	B, s	700	—	—	28	S	M	g, w	g, w	g, w	(—)	P	R<	—	—	*C. gigantea* Hook	b	basal lobe and its sinus round
9	464b	③	B, s	700	E (?)	Piralú	28	S	M	G (pm)	G	G	(—)	P, c	R	—	Ob	*C. esculenta* Schott	a-4	leaf thick
10	466b	③	B, s	700	W	—	—	E	M	g (pm)	G	g	(—)	R	R	LL, t, g	ht	*C. esculenta* Schott	a-1	
11	—	③	S, s	660	E (?)	Piralú	—	S	TT	—	—	—	—	P	—	—	Ob	*C. esculenta* Schott	a-4	
12	—	③	Fd, s	400	—	—	—	S	M	—	—	—	—	P	<R	—	—	*C. esculenta* Schott	—	
13	61	⑤	Fp, s	360	W	Piralú	—	—	SS	g	pg	g	(—)	P	R	LL	lt	*C. esculenta* Schott (?)	a-1'	
14	64	⑤	P, d	340	W	Manié	—	E	TT	P	G	P	(—)	P	R	LL	ht	*Colocasia* sp.	a-1	small spadix observed in the vagina sheath (end of February)
15	65	⑤	Ps, d	340	W	Manié	28	S	M	g	g	g	(—)	P, r	R	LL, t, g	lt	*C. fallax* Schott	a-1	petiole fibrous, flowered in Japan (June-October)
16	410	⑪	B, s	650	W	—	—	S	T	pm-g	G	p-g	(—)	P	<R	LL, t, g	ht	*Colocasia* sp.	c	variegated purple between lateral nerves of upside of leaf
17	—	⑪	B, h	650	W	—	—	E	T	pm-g	G	p-g	(—)	P	R	LL, t, g	O-Ob	*C. esculenta* Schott	a-1	
18	—	⑲	F, h	700	E (?)	—	—	S	T	G (pm)	G	p-g	(—)	P, u	<R	—	Ob	*C. esculenta* Schott	a-4	
19	—	⑳	R-Fm ; s	850	W	—	—	S	T	—	—	—	—	P	<R	LL	—	*C. esculenta* Schott	a-1	some grew mixed with No. 41 in Table 2
(District IIIa)																				
20	373	⑯	F, h	1550	W	—	42	S	M	pm-G, tp	G	p	(—)	P, ss	<R	E-LL, t	Ob	*C. esculenta* Schott	a-2	eruciform lateral tuber branched, flowered in Japan (August)
21	376	⑰	R, s	1300	E (?)	—	—	S	T	G	G	G	(—)	S, Lr	R<	—	O-Ob, t	*A. cucullata* Schott (?)	d	
22	382	⑰	F, d	1250	W (?)	—	—	E	T	g	G	g	(p)	P	R	—	O-Ov	*C. esculenta* Schott	—	
23	381	⑱	F, d	1100	W	—	—	S	M	g (pm)	G	p-g	(—)	P, r	R	LL	Ob	*C. esculenta* Schott	a-1	
24	378	⑱	F, d	1200	W	—	—	E	M	G	G	G	(—)	P, r	R	E	Ob	*Colocasia* sp. (?)	a-2 (?)	leaf thin and soft
25	399	⑱	F, h	1100	W (?)	—	—	E	M	p-g, tp	G	p	P	P, r	R	LL	O-Ob	*Colocasia* sp.	a-1	petiole rather slender
26	400	⑱	Fp, s	1050	W	—	—	S	T	p-g, tp	G	p	P	P	R	LL	Ob	*C. esculenta* Schott	a-1	root and bud reddish brown
27	—	⑱	F, s	1200	W	Manié	—	—	S	G	G	G	(—)	P, c	R	—	G	—	f	leaf thick, flowered (June), spadix with very long appendage
(District IIIb)																				
28	460	④	F, h	1150	W (?)	—	—	S	M	G	G	G	(—)	P, r	R	LL (?)	Ob	*C. esculenta* Schott	a-1 (?)	
29	461	④	F, h	1200	W	—	—	E	M	G	G, l	G	(—)	P, r	R	LL, t	Ob	*Remusatia* sp.	e	terrestreal branch erect with many bulbils
30	462x	④	F, h	1200	W	—	—	E	M	—	—	—	—	P	—	LL	—	*C. esculenta* Schott	a-1	
31	—	⑥	Mw, h	1100	W	—	—	—	SS	g	g	g	(—)	P	—	—	—	*C. esculenta* Schott (?)	a-1'	probably same species as No. 13
32	—	⑨	Fp, s	1600	W	Manui	—	S	SS	g	g	g	(—)	P	—	LL	—	*C. esculenta* Schott (?)	a-1'	do.

Notes to Table 1

1) Point, Name : See Figure 1.
2) Point, Environment
   R : roadside
   Y : house yard
   C : creek
   B : river bed
   S : stream
   Fd : field
   Fm : farm
   F : forest
   Fp : footpath in the puddy field
   P : pond
   Ps : pondside
   K : kitchen garden
   s : sunny
   h : half-shady
   d : shady
3) Wild or escaped
   W : wild
   E : escaped
4) Plant, Form
   S : spread
   E : erect
   C : caulescent
   F : fasciculate
5) Plant, Height
   TT : very tall
   T : tall
   M : middle
   S : small
   SS : very small
6) Plant, Color
   Vagina : margin of vagina sheath
   Leaf center : junction point of costa and lateral nerves on the upside of leaf
   G : dark green
   g : light green
   V : bluish violet
   P : reddish purple
   p : light purple
   pp : pinky purple
   GP : dark green wearing blackish purple
   W : glaucous
   bp : base of petiole light purple
   uP : upper part of petiole reddish purple
   pm : petiole transversely light-purple-marbled
   tp : top of petiole light purple
   nV : nerves of backside of leaf bluish violet
   nP : nerves of backside of leaf reddish purple
   bv : backside of leaf more or less tinged violet
   mP : margin of leaf reddish purple
   (—) : without colored spot
7) Leaf, Shape
   P : peltate
   S : sagittate
   u : margin of leaf undulate
   l : relatively long
   r : nearly round
   c : typically cordate
   ss : sinus of basal lobe slight
   La : point of basal lobe relatively acute
   Lr : point of basal lobe relatively round
   Lw : nerve of basal lobe exposed without lamina near petiole insertion
   Le : lamina of basal lobe existing to petiole insertion
8) Leaf, Angle : to petiole
   R< : obtuse angle
   R : right angle
   <R : acute angle
9) Lateral rhizome
   E : eruciform
   LL : very long and slender
   L : long
   t : sometimes terrestreal
   b : brown-colored when terrestreal
   g : light-green when terrestreal
10) Axial tuber
   G : globular
   Ob : obovoid
   Ov : ovoid
   O-Ob : oblong-obovoid
   O-Ov : oblong-ovoid
   lt : thickened a little
   ht : hardly thickened
   t : terrestreal
11) Classification :
   a : *C. esculenta* Schott
   -1 : having slender and long lateral rhizome
   -1' : same as a-1, but plant very small (10-15cm high)
   -2 : having slender and long lateral rhizome and lateral rhizome thickened eruciform
   -2' : same as a-2, but having small and rather globular lateral tuber too
   -3 : not having slender and long lateral rhizome
   -4 : probaby cultivated species escaped
   b : *C. gigantea* Hook.
   c : *Colocasia* sp. except a. and b.
   d : *Alocasia* sp.
   e : *Remusatia* sp.
   f : Genus not identified
12) — : not collected or not confined.
13) (...) : having slightly the tendency of ...

Table 2. Cultivated species of tribe Colocasieae on the caravan route

No.	Collection No.	Point: Name	Point: Environment	Point: Altitude	Domestic name	Chromosome No. (RTC)	Plant: Form	Plant: Height	Color: Petiole	Color: Leaf	Color: Vagina	Color: Leaf center	Leaf: Shape	Leaf: Angle	Tuber: Lateral	Tuber: Axial	Species	Remarks
(District I)																		
33	479	①	K, s	72m	Dudh-ya-Kachú	—	S	T	g, w, bp	g	g	(—)	S, Le, Lr, w	R<	—	—	*X. sagittifolium* Schott	bud pinky purple, vagina sheath large and spread out
34	—	①	K, s	72	—	—	C	TT	G	G	G	(—)	P, l, u, s	R	—	t, e	*Colocasia* sp.	leaf robust and thick, edible, lateral nerves of leaf many, wild species (?)
35	—	①	K, h	72	Karukálo or Karukául	—	F	T	g	g	g	(—)	S	R<	—	—	*Alocasia* sp.	tuber edible.
(District II)																		
36	462	②	K, h	350	—	—	S	T	V, w	G, nV, bv	V	(—)	S, Le	R<	L, t	—	*X. violaceum* Schott	
37	463a	②	K, h	350	—	—	S	M	g	G	g	(—)	P, r	R	—	—	*C. esculenta* Schott	vagina sheath large and spread out
38	463b	②	K, h	350	Dudh Manié	—	S	M	g, w	g	g	(—)	S	R<	L-G	—	*Xanthosoma*.sp.	petiole not fibrous
39	136	⑩	K, h	900	Dudh Mané	26	S	T	V, w	G, nV, bv	V	(—)	S, Le, La, n	R<	L, t	—	*X. violaceum* Schott	
40	411	⑪	K, h	650	Piralú	—	S	TT	g, uP	G	—	p	P	<R	—	Ob	*C. esculenta* Schott	leaf thick
41	—	⑳	Fm, s	850	—	—	S	TT	g, uP	G	—	P	P	<R	—	—	*C. esculenta* Schott	probably same cultivar as No. 40
(District III)																		
42	458	④	Fm, h	ca. 1000	—	—	S	T	V, w	G, nV, bv	V	(—)	S, Le, La	R<	—	—	*X. violaceum* Schott	
43	78	⑥	Fm, s	1200	—	42	F	TT	G-pm, tp	G, bv	p	(—)	P, c	R	L	O-Ob	*C. esculenta* Schott	leaf thick
44	370	⑰	Fm, s	1300	—	—	S	T	V, w	G, nV, bv	V	(—)	S, Le, La	R<	t	—	*X. violaceum* Schott	
45	371	⑰	Fm, s	1300	—	39	S	M-T	g, w	G	pp	(—)	S, Le, Lr, w	R<	L, t	Ob, Ov	*Xanthosoma*.sp.	bud pinky purple, vagina sheath large and spread out
46	380	⑰	Fm, h	1300	Pindalú	—	E	M	g, tp	G	g	(—)	P, (u)	<R	G	Ov	*C. esculenta* Schott	lateral nerves of leaf many
47	382	⑰	K, h	1300	—	—	S	T	p, tp	G	P	(—)	P, r, s	<R	—	O-Ov	*C. esculenta* Schott	vagina sheath large and spread out
48	401	⑱	K, s	1050	—	—	S (F)	T	G	G	G	(—)	P	R	L	O-Ob	*C. esculenta* Schott	having a tendency to be fasciculate.
49	—	⑫	—	ca. 1000	Pindalú	—	—	—	—	—	—	—	—	—	L-G	Ob	*C. esculenta* Schott	tuber like insect abdomen.
(District IV)																		
50	442	⑦	Fm, s	2200	Piralú	42	S	TT	P, bp, tP	G, mP, nP	P	p	P, u, c, s	<R	L, t	O-Ob	*C. esculenta* Schott	leaf robust
51	443	⑧	Fm	ca. 2350	Piralú	—	—	—	—	—	—	—	—	—	—	Ov	*C. esculenta* Schott	
52	169	⑬	K, s	1940	Piralú	26	S	T	g, w	g	pp	(—)	S, Lw, Lr, w	R<	L, t	Ob	*Xanthosoma*.sp.	bud pinky purple, vagina sheath large and spread out, resemble to No. 45
53	333	⑭	Fm, h	2050	Pindalú	—	S	S-M	g	G	tp	p	P, r, s	<R	G	Ob	*C. esculenta* Schott	vagina sheath large and spread out
54	336	⑮	Fm, s	1800	Pindalú	—	S	S-M	g	G	tp	p	P, s	<R	G	Ob	*C. esculenta* Schott	same cultivar as No. 53

Notes to Table 2

1) Point, Name : See Figure 1.
2) Point, Environment
   R : roadside
   Y : house yard
   C : creek
   B : river bed
   S : stream
   Fd : field
   Fm : farm
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   La : point of basal lobe relatively acute
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8) Leaf, Angle : to petiole
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   E : eruciform
   LL : very long and slender
   L : long
   t : sometimes terrestreal
   b : brown-colored when terrestreal
   g : light-green when terrestreal
10) Axial tuber
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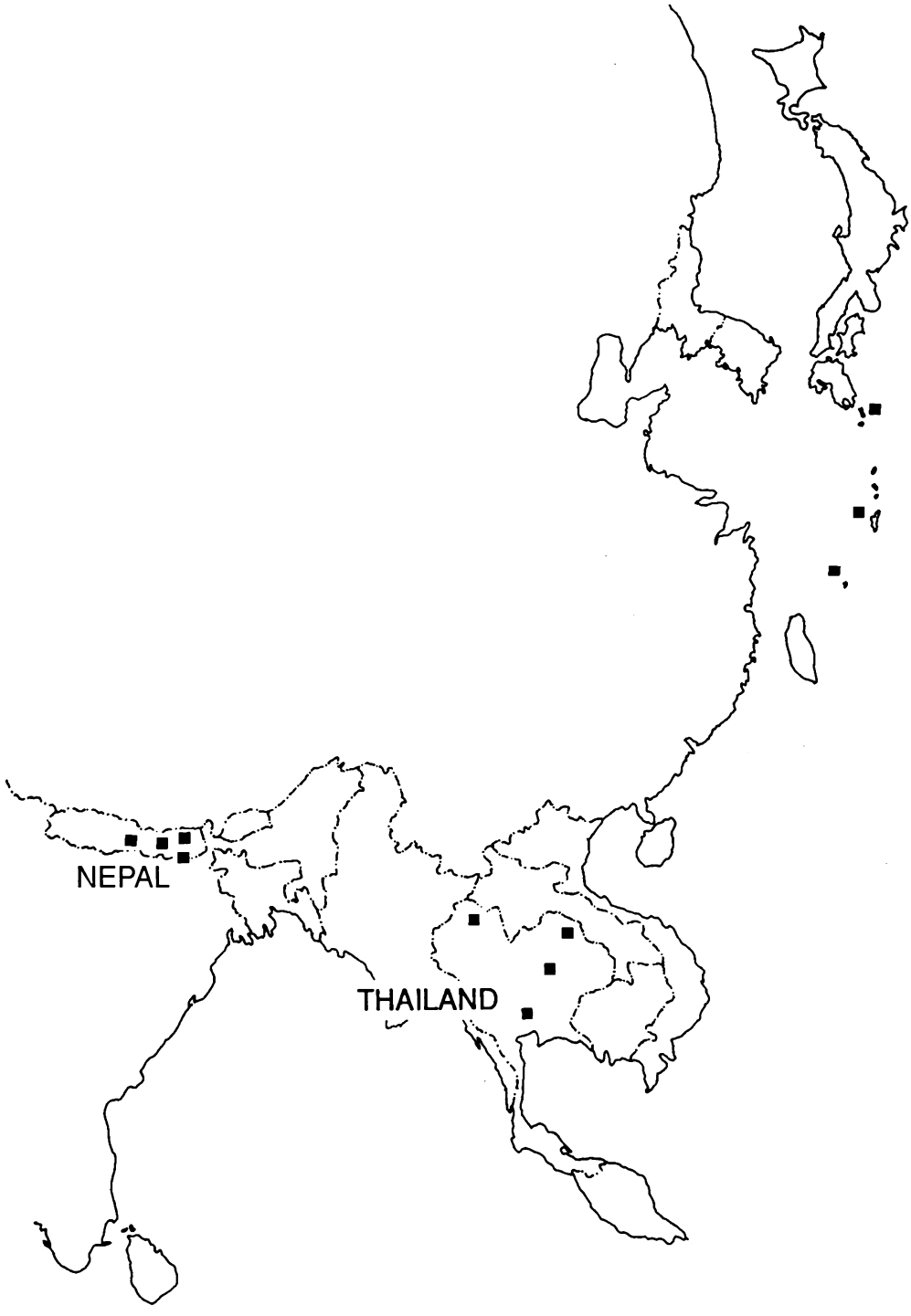


Fig. 1. Some of the sites where the wild types of *C. esculenta* were collected.

Table 3. Materials used mainly in the restriction endonuclease analysis

General No.	Strain	Species	Source	Habitat	Type	Remarks
* 1	C81019.2	Cea	N	h • r	2 • w	
* 2	KUYE474	Cei	N	l • m	2 • w• s	without long stolon
* 3	C81045.3	Cea	N	h • r	2 • w	
* 4	C8030	Cea	N	h • c	2 • w	donor:Higuchi
* 5	C81113	Cee	N	b	3 • c	stalk edible, round tuber
6	TC8611b-2	Cee	T	l • k	3 • c	
7	TC83001-3	Cea	T	l • m	2 • w	
* 8	C84001-3	Cea	B	?	2 • w	donor:Takamura
9	CL8203.1	Cea	R	l • c	2 • w	Iriomote Island
* 10	C7409-3CT	Cee	R	l • p	? • c	Okinawa Island,"Taumu"
* 11	Egu-imo	Cee	J		3	cultivar.
12	Takenoko-imo	Cee	J		2	cultivar.
13	Akame-imo	Cee	J		3	cultivar.
* 14	C86001	Cei	J		? • c	ornamental, donor:Ishida
15	Cg	Cg	T	l • k	? • c	
16	TC8614	Amv	T		2	ornamental
* 17	KUYE373	Ce	N	h • f	3	without long stolon
* 18	C81027.111	Cea	N	h • m	2 • w	
* 19	C81079.3	Cea	N	h • r	2 • w	
* 20	C81125.2-1	Cee	N	l • k	2 • c	
* 21	C81101	Cee	N	h • b	3 • c	
* 22	KUYE442	Cee	N	h • k	3 • c	in Kathmandu
23	C81114	Cee	N	h • b	? • w	in Kathmandu, stalk edible
24	C81123	Cea	N	h • m	2 • w	
* 25	CL83022	Cea	R	l • m	2 • w	Kikai Island
26	TC83021.2	S	T	h • f	? • w	
* 27	C81073	Ce	N	h • f	? • w	small plant,without long stolon
* 28	C81081	Cea	N	l • r	2 • w	

Abbreviation:

Species — Ce: *C. esculenta*, Cee: *C. esculenta* var. *esculenta*,
Cea: *C. esculenta* var. *aquatilis*, Cei: *C. esculenta* var. illustris,
Cg: *C. gigantea*, Amv: *A. macrorrhiza* var. *variegata*,
S: *Schismatoglottis* sp.

Source — N: Nepal, T: Thailand, B: Bali Island, R: Ryukyu Island, J:other of parts Jap

Habitat — h: hillside, l: lowland, m: marshland, r: riverside, c: creek,
p: puddy field, f: forest, k: kitchen garden, b: bazar.

Type — Arabic numerical: ploidy, c: cultivated type, w: wild type, s: sterile.

*: strain of which inflorescences were measured for analysis of inflorescence.

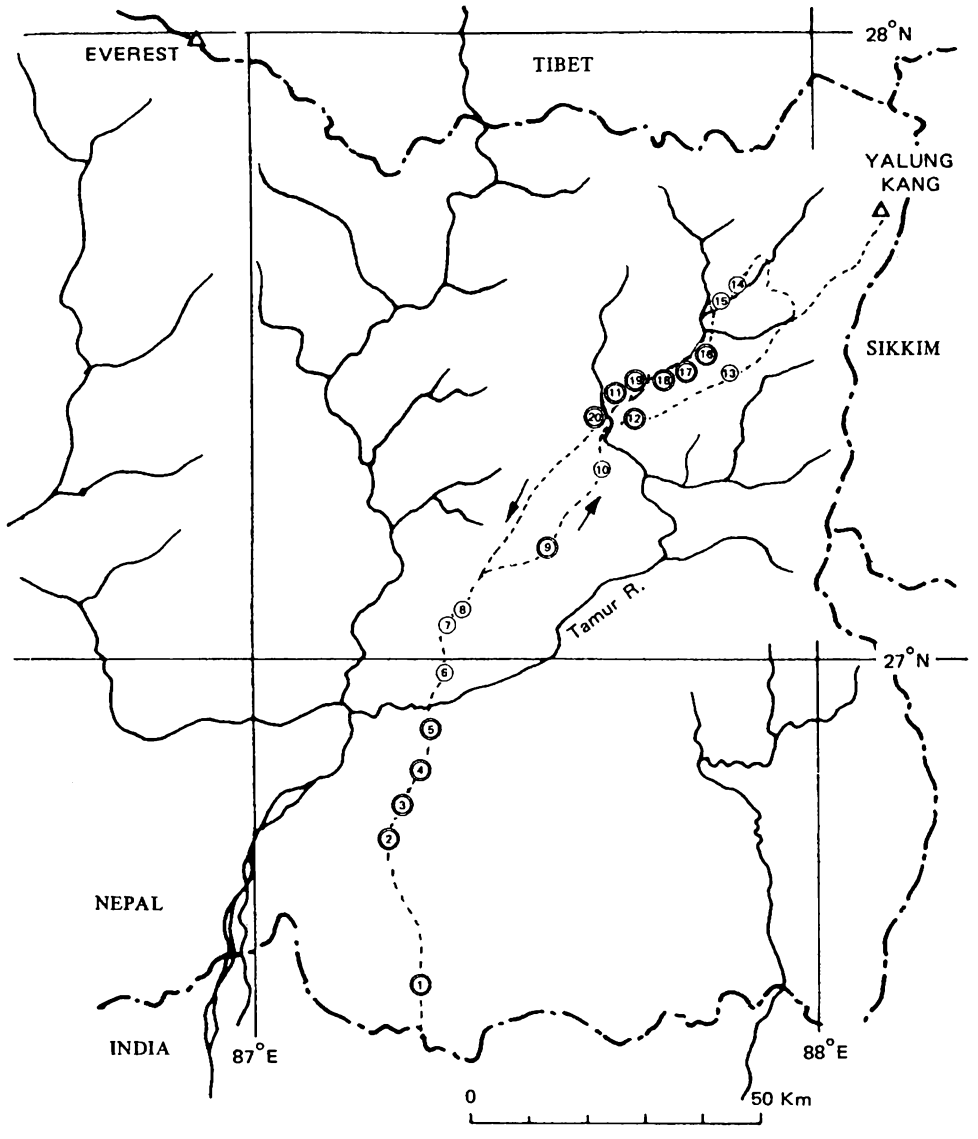


Fig. 2. Caravan route and the points where tribe Colocasieae grew spontaneously or were cultivated.

1)----- : caravan route

2) ◎ : wild or escaped species were observed

3) ○ : only cultivated species were observed

4) names of the points and their altitude

1 Biratnagar (72m)
2 Dharan (350m)
3 North of Dharan (500~700m)
4 North slope of Shangri La (ca. 1150~1200m)
5 Leuti Khola (340~360m)
6 Dhankuta Bazaar (1200m)
7 North of Hille (2200m)
8 Near Chitre (2350m)
9 North-east of Morang (1600m)
10 Tchanke (770m)
11 Doban (650m)
12 Near Taplejung (1000m)
13 Kesheba (1940m)
14 Gaiyabari (1900m)
15 Shadup (1800m)
16 Near Tapletok (ca. 1550m)
17 Near Tiwa, Dawa (ca. 1300m)
18 Near Siwa, Miterung (1050~1150m)
19 Handlung (ca. 700m)
20 East slope up from Doban (ca. 850m)

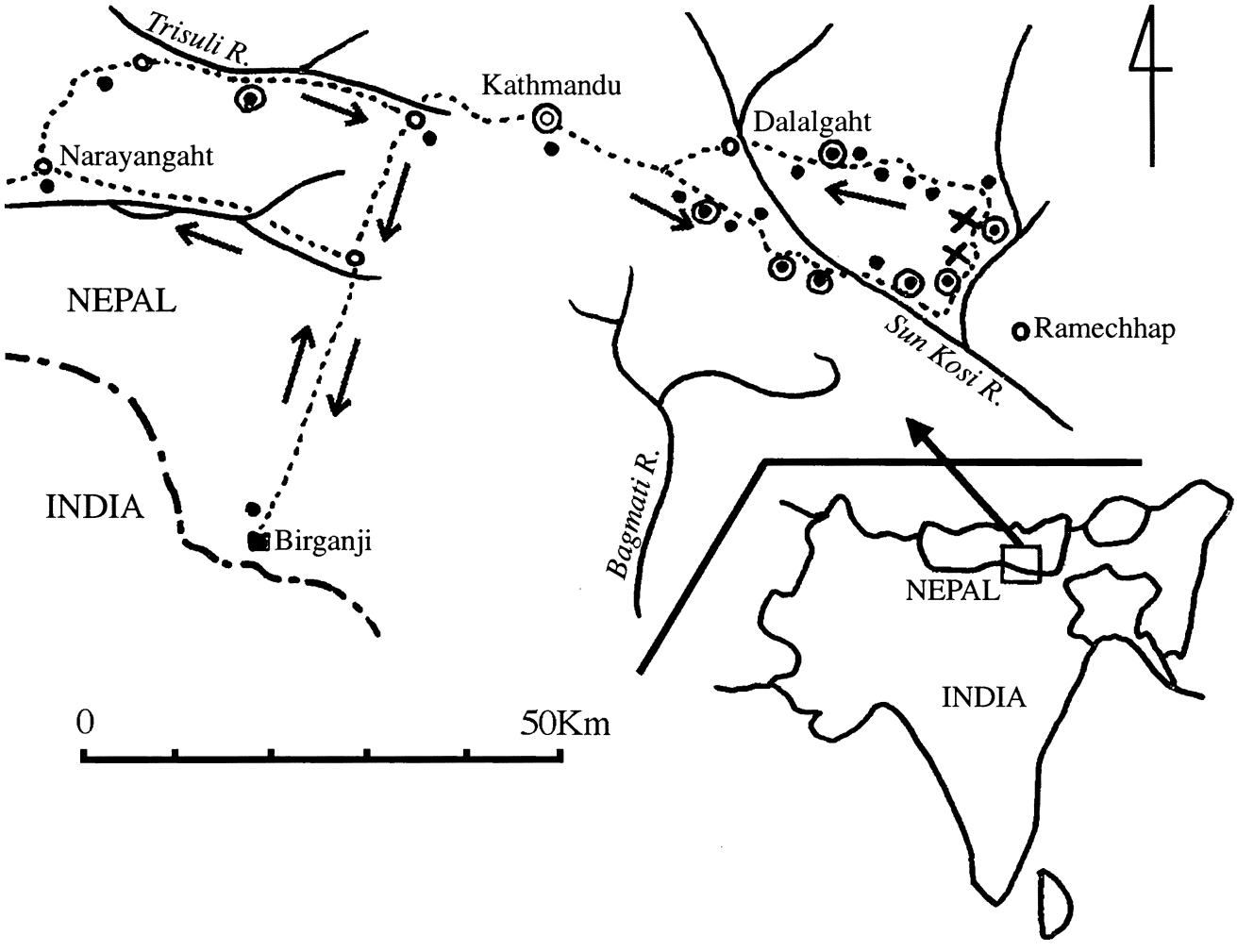


Fig. 3. Travel route in the central Nepal in 1981.

● : main collection site, × : site where wild triploid was collected, ⦿ : species not decided (*C. fallax* ?)

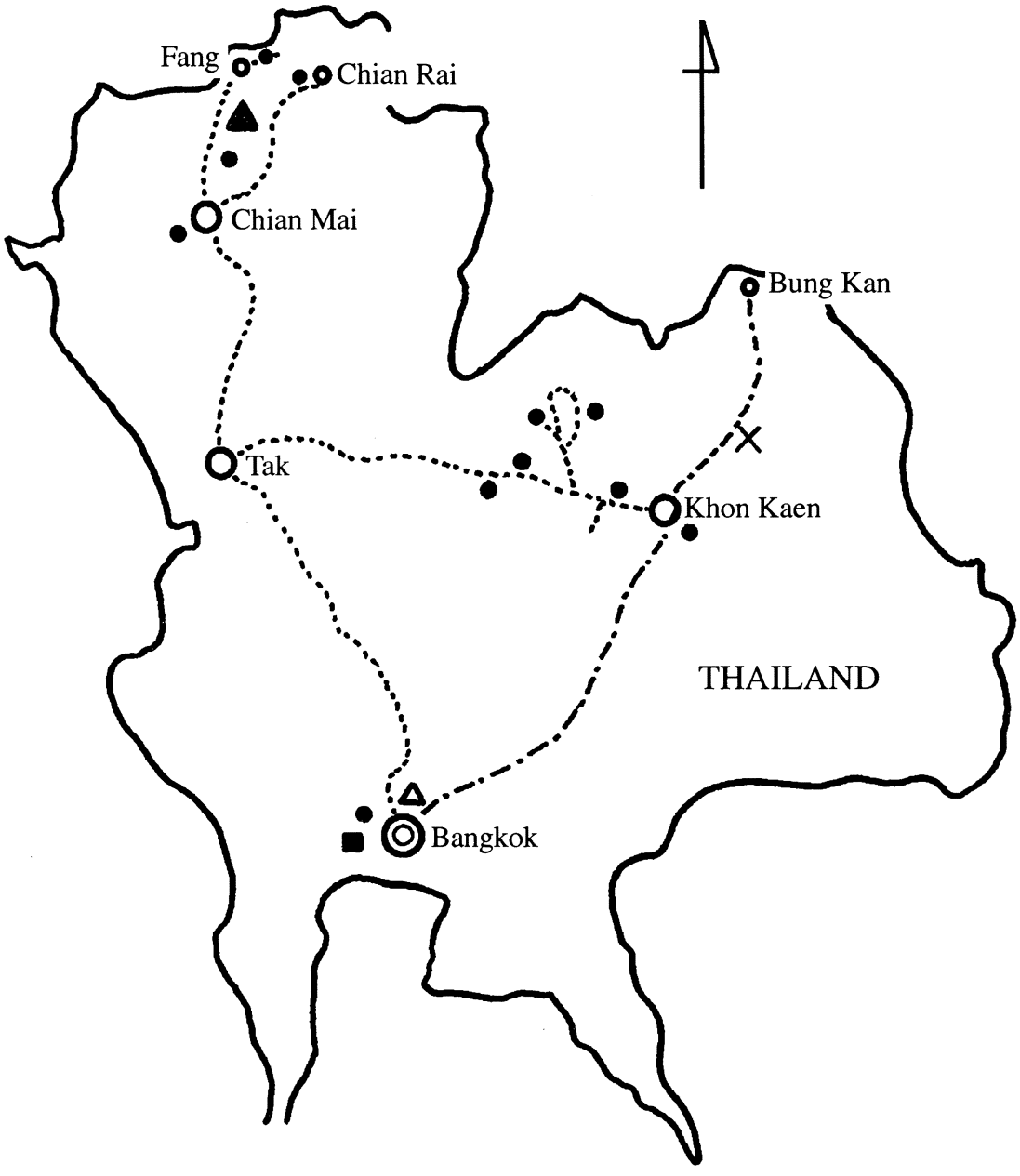


Fig. 4. Sites of collecting taro in Thailand.

----- : route in 1983, —·—· : route in 1986, ● : *Colocasia esculenta*, ■ : *Alocasia macrorrhiza*, × : *A. macrorrhiza* var. *variegata*, △ : *Cyrtosperma edule*, ▲ : *Schismatoglottis sp.*

Fig. 5. Wild type of taros grown in the marshland in Nepal.

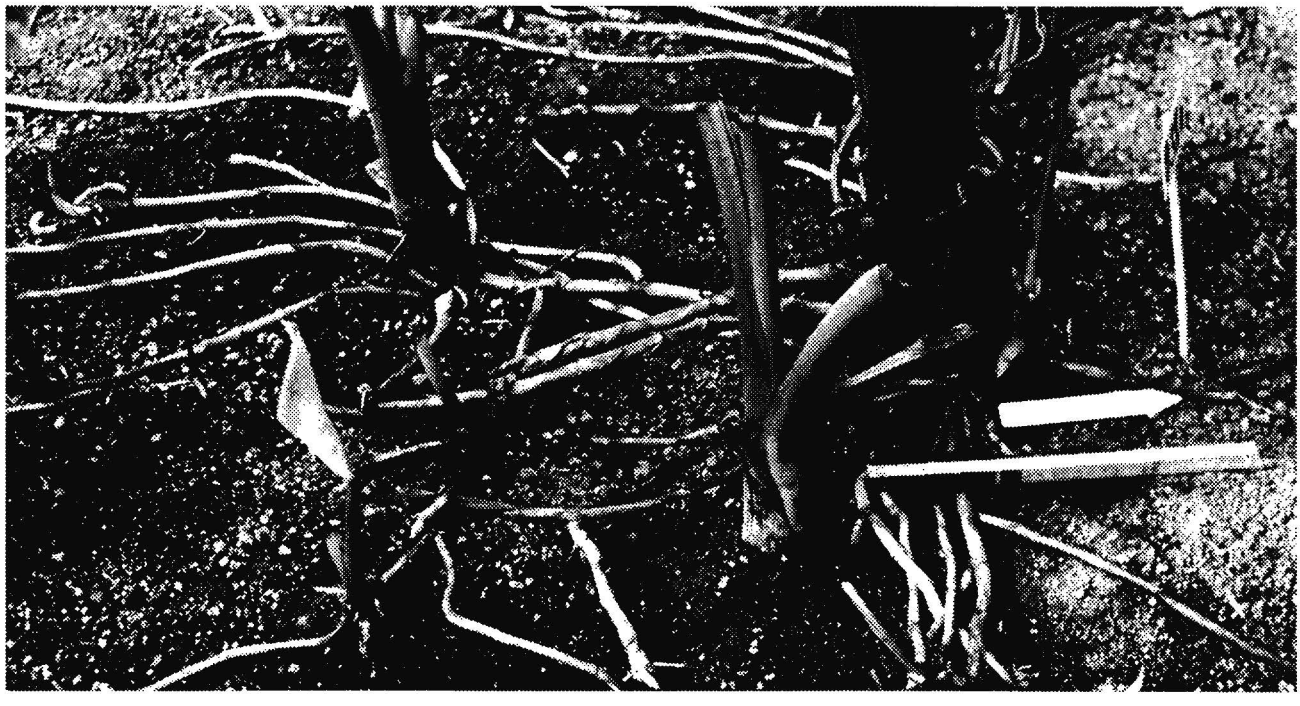
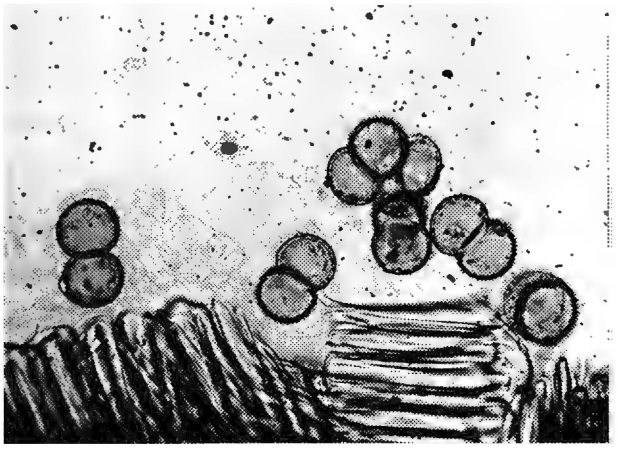
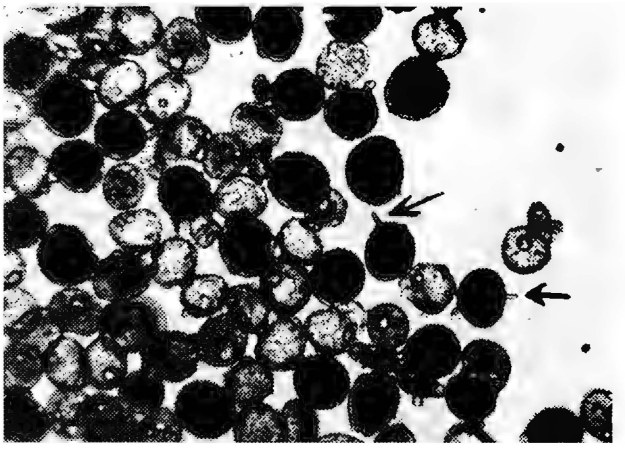


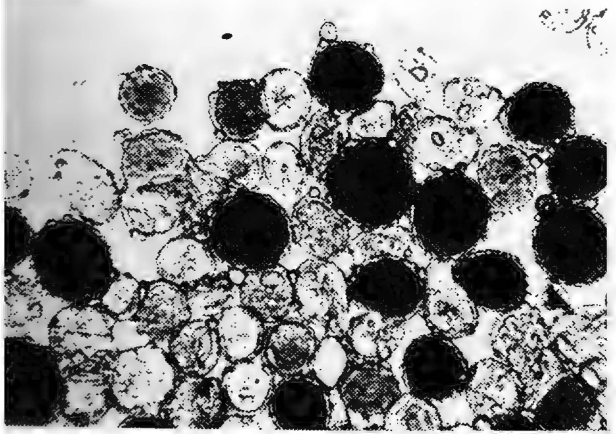
Fig. 6. Exposed long stolons of wild type, a 40cm scale center right.



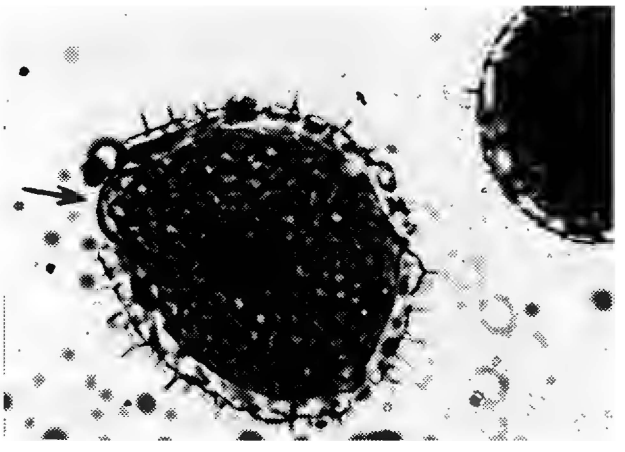
a



c



b



d

Fig. 7. Pollen grains of 3 strains.

a : accession No.2 (diploid), b : accession No.22 (triploid), c and d : accession No.17 (triploid), ↙ shows germinating pollen tubes.

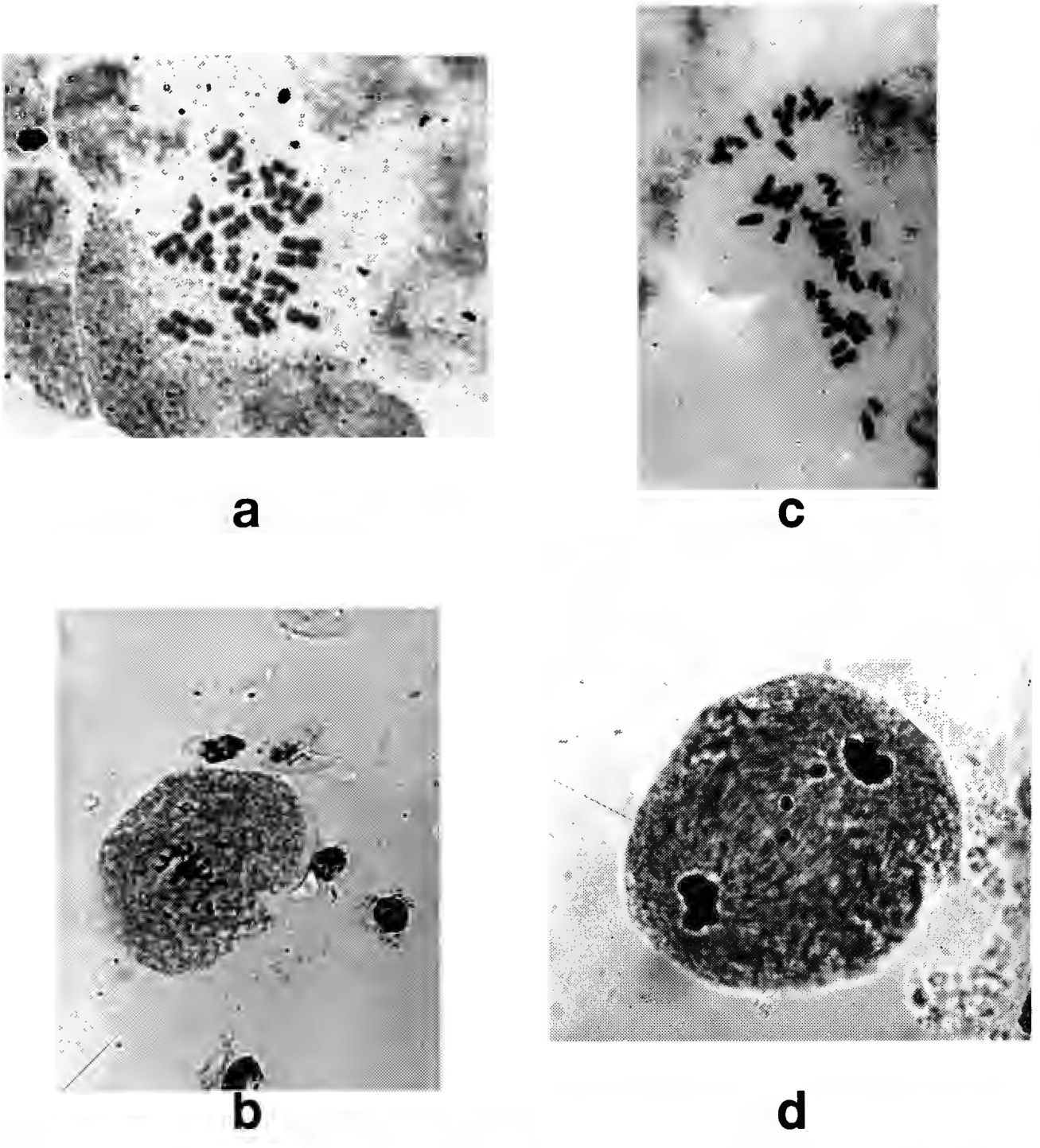


Fig. 8. Chromosomes of 3 strains.

a : RTC of accession No.2 (2n=28), b : PMC of accession No.2, c : RTC of accession No.17 (2n=42), d : PMC of accession No.22. Univalents are seen in b and d.

3-1. Composition of inflorescence

C. esculenta has a spadix wrapped with a spathe, which is divided into two parts by the constriction, and upper half is yellow while lower one is green. Fig.9 shows a flowering inflorenscence (a) and various types of spadicis (b). Fig.10 shows female, sterile and male flowers. Sterile white blocks among female flowers are said to be characteristic of *C. esculenta*. As shown in Fig.11, spadix has four parts; sterile appendage (χ_1), staminate part (χ_2), sterile part (or staminode, χ_3), and pistillate part (χ_4) from the top to the bottom. It has been noticed and described in the systems of classification of family Araceae as so and so species had a long one or a certain species a short one while others lacked it. The ratio of a sterile appendage to the total length or to that of staminate part and so on have been described and quoted many times without any criticizing manner. Considering that this plant group has such a unique inflorescence part as a spadix, it would be acceptable to describe and use these characters as key characters to classify the group. Certainly they seem to be determined genetically. But sometimes observed are deformed inflorescences as those of accession No.2. (KUYE 474) in Fig.12. Enviromental and/or physiological conditions may cause such deformities. So, measuring of inflorescence was made carefully considering the conditions of the plant.

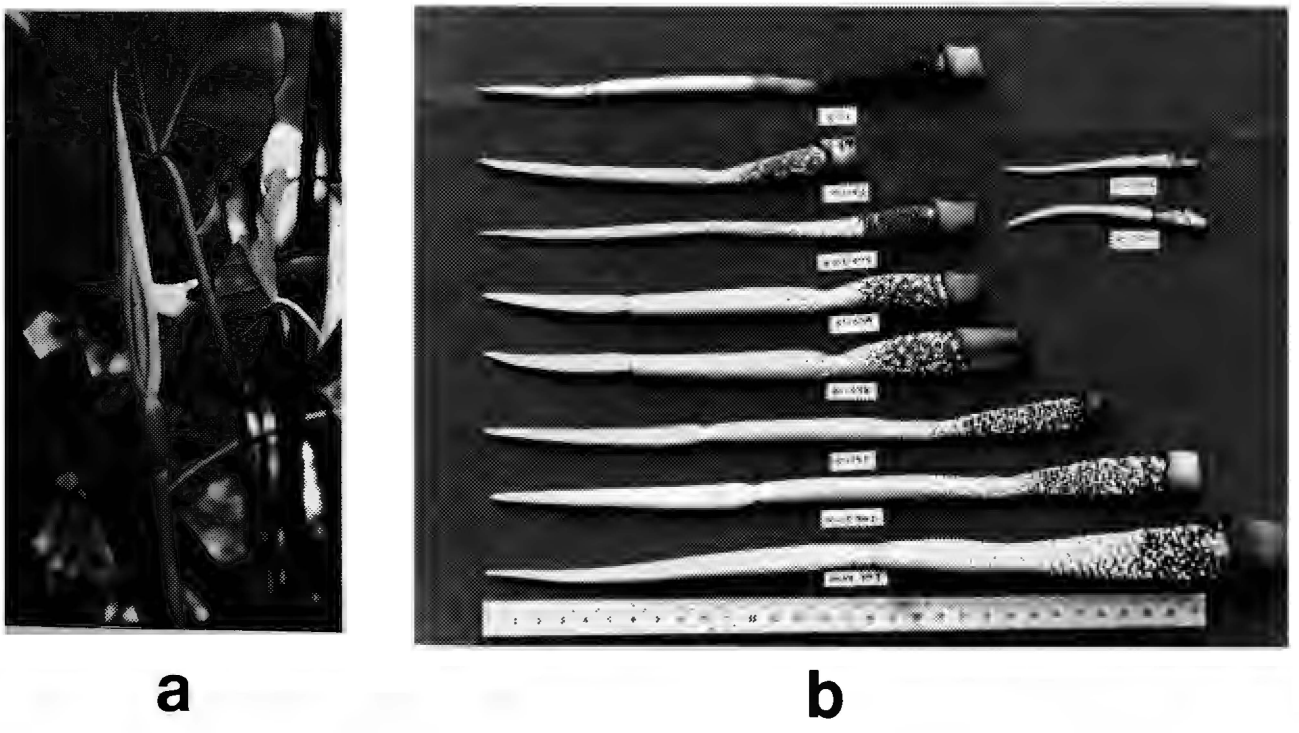
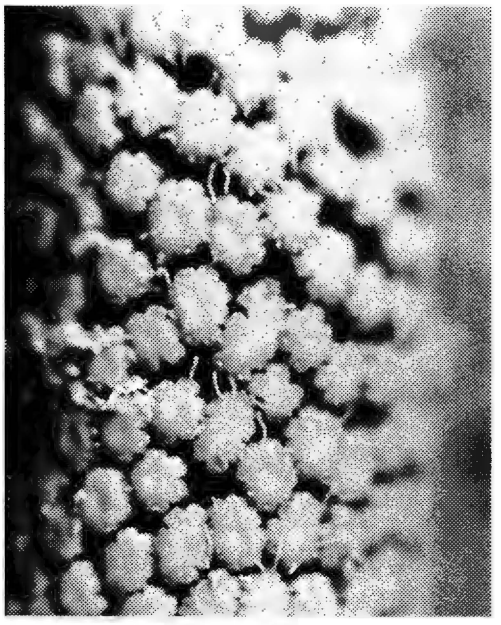
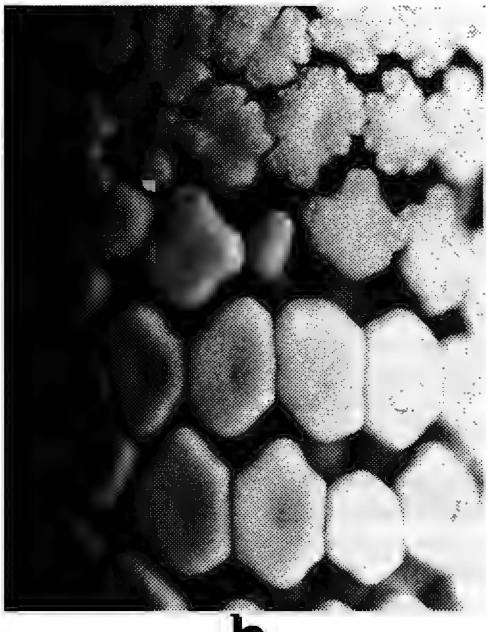


Fig. 9. Flowering (a) and various types of inflorescence (b) of *C. esculenta*. Two small spadicis upper right are probably of *C. fallax*.

a



c



b

Fig. 10. Some parts of inflorescence of *C. esculenta*.

a : female flowers and sterile ones (white blocks among female flowers),

b : male flowers (upper) and staminode (lower),

c : male flowers ; pollen grains are being pushed out.

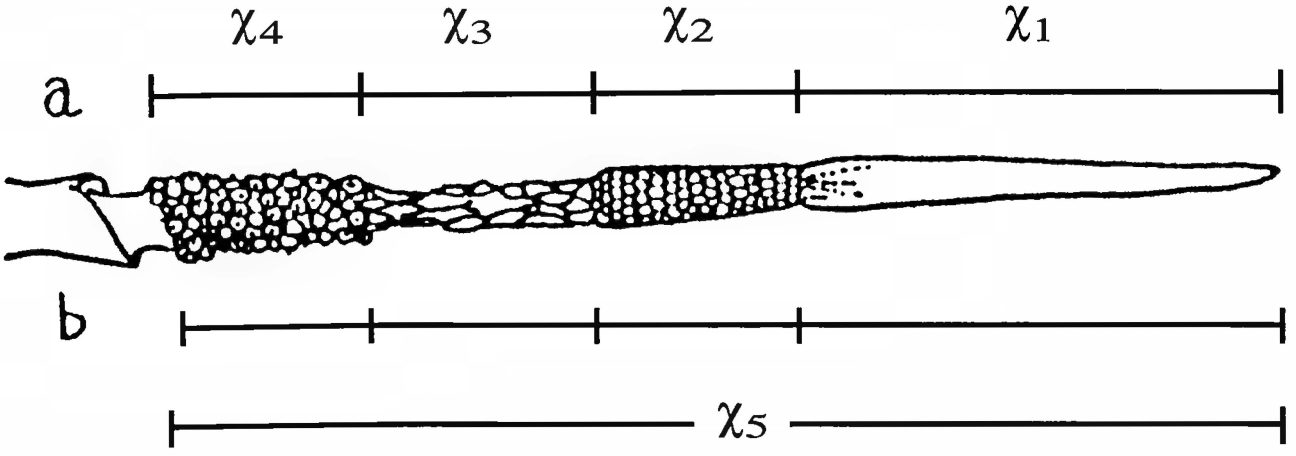


Fig. 11. Inflorescence of *C. esculenta*.

χ_1, χ_2, χ_3, χ_4 and χ_5 : sterile appendage, staminate part, sterile part, pistillate part and total length, respectively. a : abdominal side, b : dorsal side towards the center of shoot.

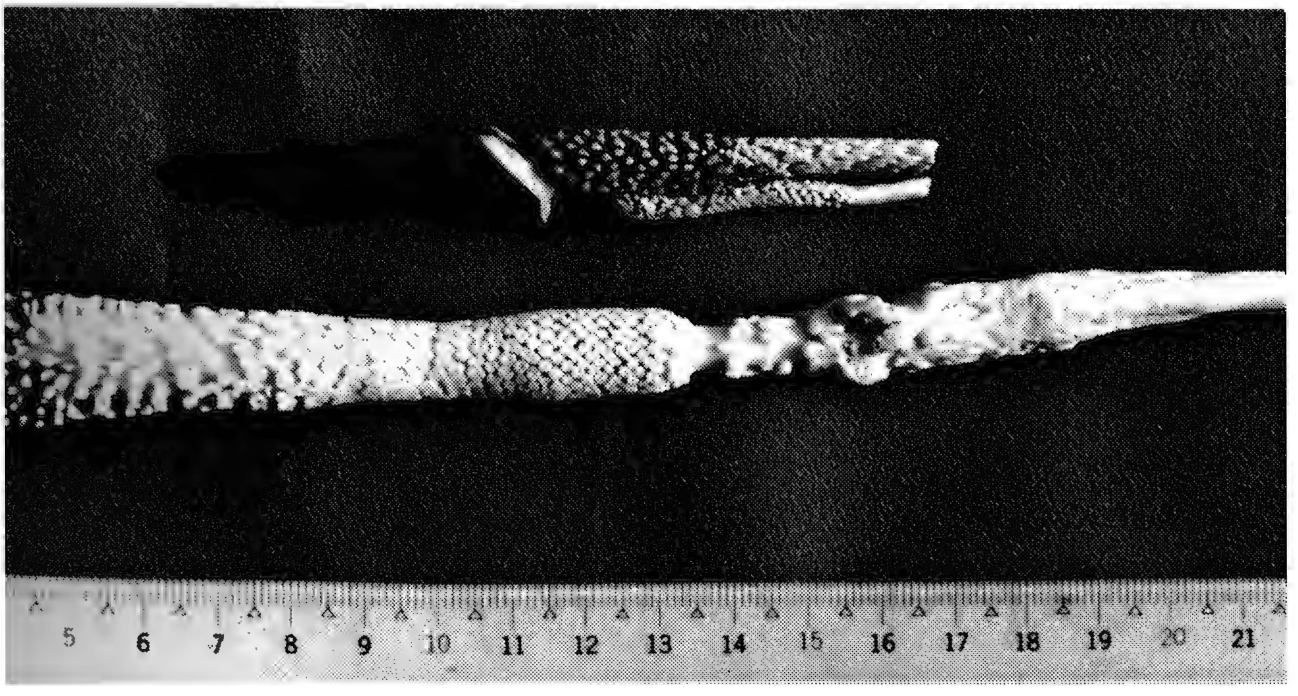


Fig. 12. Deformities of inflorescence.

3-2. Genetic stability of the parts of inflorescence

Only sound and fully grown inflorescences were measured in summer season (from July to september) for 6 years from 1976 to 1981. Four strains were selected because of having many inflorescences:

accession No.2	wild diploid from Nepal, sterile,
accession No.11	cultivated triploid from Japan,
accession No.17	wild triploid from Nepal,
accession No.22	cultivated triploid from Nepal.

The number of measured inflorescences were 292, 89, 140 and 97, respectively. As shown in Fig.11, length of each part was different when measured either at the abdominal side or at the dorsal side towards the center of shoot. In the present experiment, sum of the both sides of each part was used as measurements. Average lengths and ratios of the 4 strains are shown in Fig.13. Accessions No.2 and No.17 were closely resembled each other in the ratio. The coefficient of variation of the measurements was rather large as shown in Table 4 (CV.1). And the partial correlation coefficient was rather large as shown in Table 5. So the arcsin-converted value of the ratio (CV.2) was decided to be used for the calculation of principal component score. Real length may be said not to be so stable in the strain. Considering the long period during which the data had been collected, such rather large value might be expected. It may be said that these characters are relatively stable using arcsine conversion.

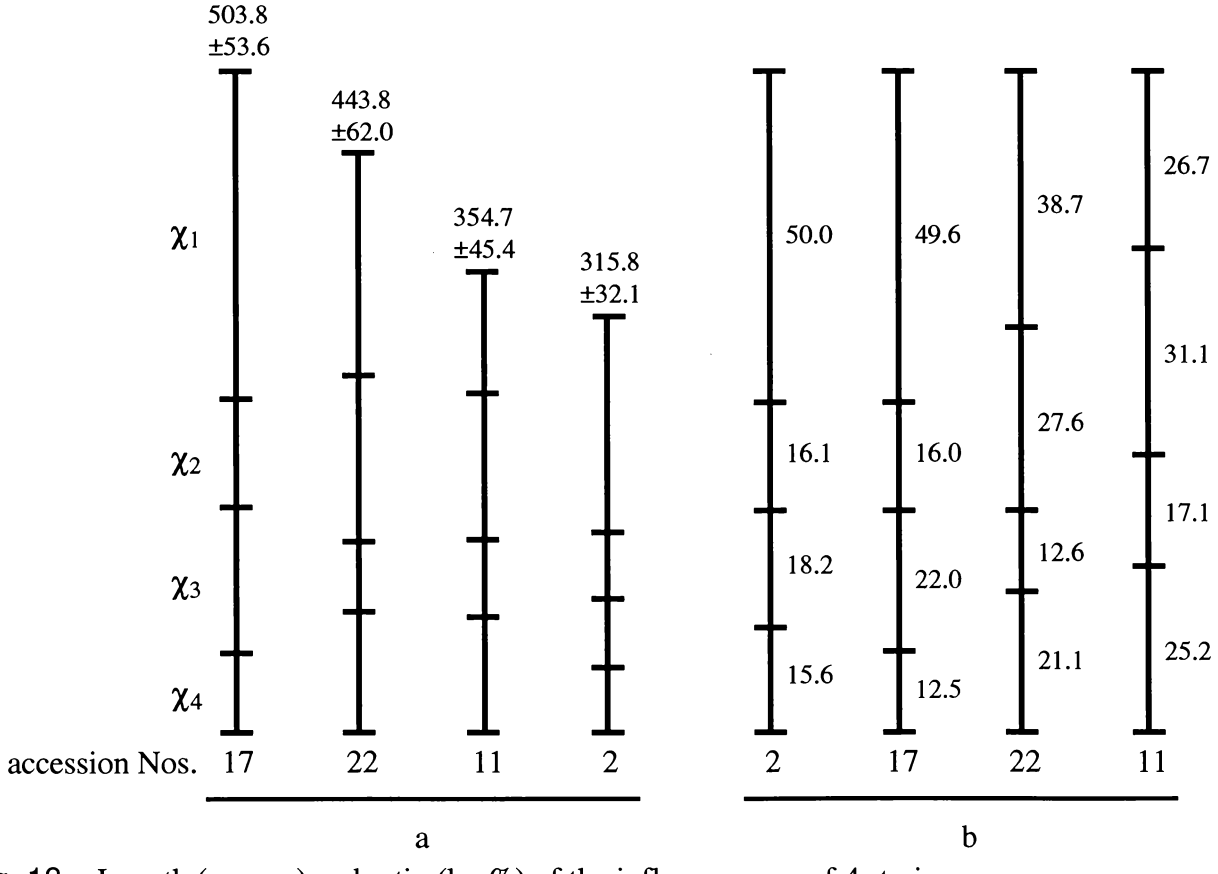


Fig. 13. Length (a : mm) and ratio (b : %) of the inflorescences of 4 strains.

Table 4. Coefficient of variation calculated from the length of each part of inflorescence (CV.1) and that from the arcsine-converted value of its ratio to the total length (CV.2)

Accession	CV.1				CV.2			
	χ_1	χ_2	χ_3	χ_4	χ_1	χ_2	χ_3	χ_4
No.2	.113	.145	.124	.154	.023	.056	.054	.053
No.11	.115	.166	.137	.201	.056	.040	.068	.051
No.17	.142	.096	.101	.135	.032	.054	.037	.043
No.22	.153	.166	.149	.173	.030	.049	.070	.038

Table 5. Partial correlation coefficient of the length of each part

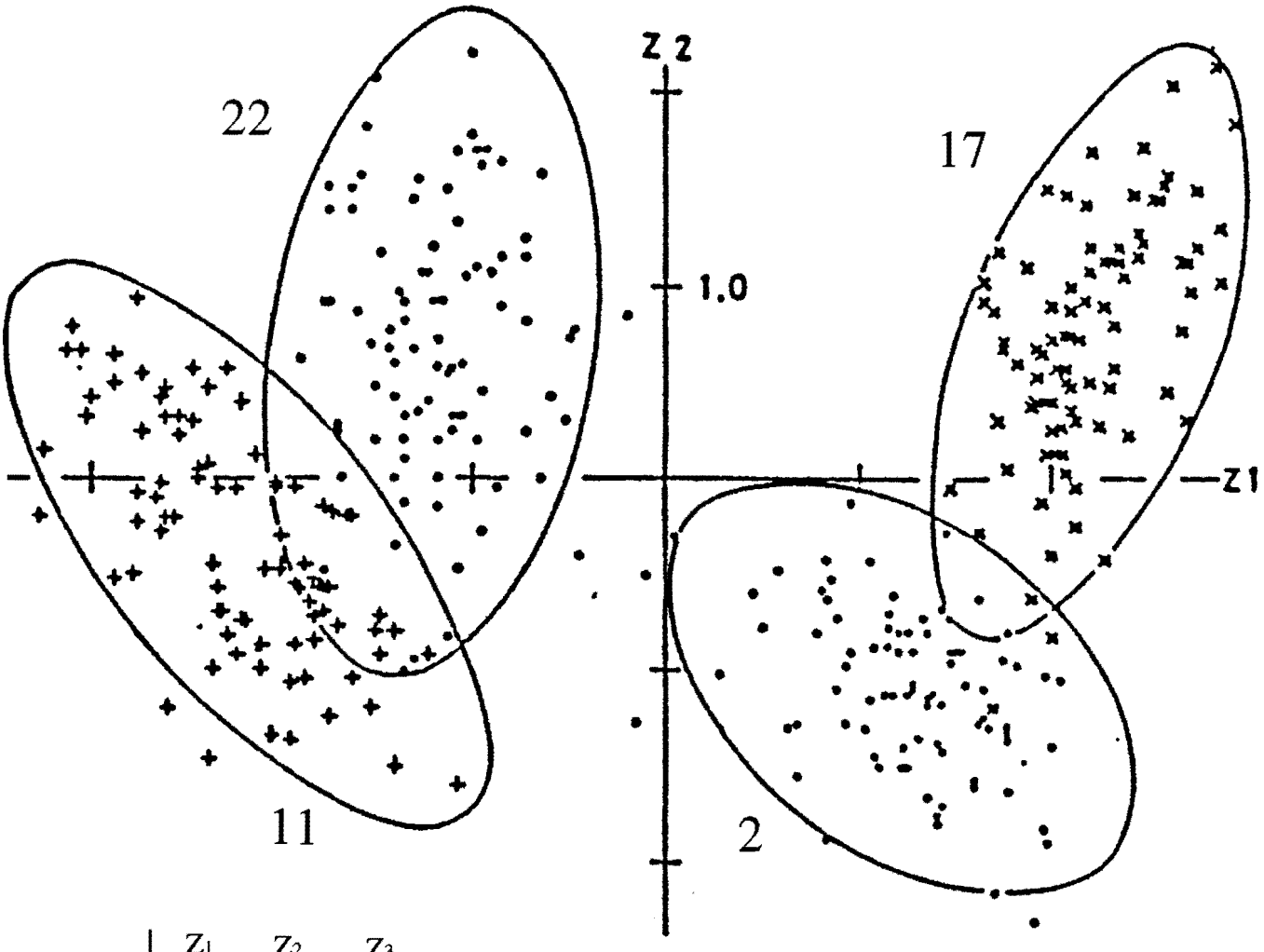
inflorescence part	Accession											
	No.2				No.11				No.17				No.22			
χ_1	—				—				—				—			
χ_2	.37	—			.05	—			.04	—			.28	—		
χ_3	.57	-.32	—		-.03	.06	—		.41	.16	—		.45	-.22	—	
χ_4	.39	.39	.01	—	.21	.82	.19	—	.49	.15	.23	—	.61	.38	-.02	—

3-3. Results of principal component analysis and cluster analysis

The result of principal component analysis is shown in Fig.14. It was enough satisfactory. Cumulative ratio from Z_1 component to Z_2 component was more than 85%. These 4 strains could be well separated on the scatter diagram. Z_1 component was a shape factor which has close correlation with the ratio of fertile parts (χ_2 and χ_4) to sterile parts (χ_1 and χ_3), and Z_2 component was a size factor. However, strictly speaking, this was not a 'size' factor because variances included the shape coefficients, from χ_1 to χ_4.

Next, 87 strains including F_1s, F_2s between accession No.19 (wild diploid) and, accession No.28 (wild diploid) and S_1s of accession No.28 were analysed. Newly added 70 strains are shown in Table 6 and Table 7, and the total inflorescence numbers were 391. When these sample data were added to those of the former experiments, the unbalance of sample numbers among strains might cause some difficulty to understand the results. So in every strain, the relative length, namely the ratio of the average part length to the total one was used as χ_1, χ_2, χ_3 and χ_4, and the variances of χ_1, χ_2, χ_3, χ_4,χ_5 were added as χ_6, χ_7, χ_8, χ_9, χ_{10}, respectively.

The result of principal component analysis is shown in Table 8. Cumulative ratio from Z_1 component to Z_3 component was more than 78%. Unlike the result of the former experiment, both components Z_1 and Z_2 were shape factors though Z_1 component has close negative correlation with the ratios of appendage (χ_1) and with the length and variance of total length (χ_5). Z_3 had closer correlation with the ratio and variance of staminode (χ_3). Principal component scores were calculated using these principal components and cluster analysis were made with Ward's method (Ward, 1963). Rotation of factor axis with Varimax method (Kaisar, 1958) did not bring much difference among cluster members, though the distances among the clusters were enlarged. Therefore, a dendrogram obtained without the rotation is shown in Fig.15. The dendrogram consisted of 7 main branches. Strains and hybrids included in each branch are in the columns from the top to the bottom.



	Z_1	Z_2	Z_3
χ_1	.925*	.004	-.380
χ_2	-.960**	.118	.114
χ_3	.714	-.268	.647
χ_4	-.964**	.012	.062
χ_5	.338	.924*	.174
CR	.666	.188	.122

Fig. 14. Factor loading matrix, contribution ratio (CR) and a scatter diagram of 4 strains of *Colocasia* : α of ellipse is 0.05.

** and * : significant at the 1% and the 5% level, respectively.

Table 6. Other materials of which inflorescences were measured

No.	Strain	Species	No. inflorescences	Source	Habitat	Type	Remarks
29	C81002	Cee	3	N	h	3·c	
30	C81023	Cea	2	N	c	2·w	
31	C81034	Ce	3	N	h·f	?·w·s	small plant, massive short stolon
32	C81037	Ce	4	N	h·f	?·w·s	ditto
33	C81038	Cea	13	N	h·r	2·w	
34	C81042	Cee	5	N	h·k	3·c	
35	C81043	Cea	11	N	h·r	2·w	
36	C81049	Cea	3	N	h·c	2·w	
37	C81059	Cee	2	N	h·k	·c	
38	C81063	Cee	7	N	h·k	·c	long tuber
39	C81080	Cea	11	N	h·c	2·w	
40	C81082	Cea	4	N	h·c	2·w	
41	C81085	Cee	3	N	h·k	3·c	stalk edible
42	C81089	Cea	8	N	h·c	2·w	
43	C81092	Cea	3	N	l·c	2·w	ants gathered inside spathe
44	C81096	Cee	2	N	h·k	3·c	stalk edible
45	C81106	Cee	2	N	h·b	3·c	
46	C81124	Cee	6	N	l·c	·w	short stolon (?) or long tuber
47	C81126	Cea	9	N	l·m	2·w	
48	C81135	Cea	3	N	l·r	2·w	stalk edible
49	C81140	Cee	14	N	l·k	·c	long tuber, stalk edible
50	C81143	Cea	4	T	l·c		
51	C81145	Cea	12	T	l·c		
52	C81146	Cea	3	T	l·c	2·w	
53	TC83006	Ce	2	T	l·m	·w	
54	TC83013	Cea	4	T	·c	·w	
55	TC83014b-1	Ce	6	T	·c	2·w	ants building a nest inside spathe
56	CL83019	Cea	4	J	·m	2·w	Amami Ohshima
57	CL83020	Cea	4	J	·m	2·w	ditto
58	CX001	Cee	7		·	·c	donor : Takayanagi, from Solomon, staminate colored pink

Table 7. Progenies of which inflorescences were measured

No.	Generation (Parents)	Cross No.	Individual No.	No. inflorescences
59	F_1 (No.4 x No.51)	CR82023b	— 2	3
60			— 4	3
61			— 5	3
62			— 101	9
63			— 301	4
64			— 05	6
65	F_1 (No.51 x No.4)	CR82024	— 2	2
66			— 6	4
67			— 10	8
68			— 14	7
69			— 20	2
70	F_1 (No.33 x No.51)	CR82051	— 01	3
71			— 02	3
72	F_1 (No.51 x No.19)	CR82052	— 001	2
73			— 01	13
74			— 02	2
75			— 111	6
76			— 112	5
77			— 301	2
78			— b.1	2
79	F_1 (No.19 x No.28)	CR83084	CT.1 — 1	16
80			— 2	10
81			CT.3 — 1	9
82			— 2	12
83			— 3	11
84			— 6	8
85			— 10	5
86			.3 — 201	5
87			2R.31	2
88		CR83092	.1 — 3	12
89			.2 — 1	9
90			— 2	3
91			.3 — 6	11
92	F_1 (No.28 x No.19)	CR83089	CT.3 — 51	7
93			3. — 100	2
94	F_2 (No.81)	CR86168	. 03	2
95	(No.91)	CR86163	. 40	3
96	S_1 (No.28)	CR83091	. 22	3
97			. 23	3
98			. 42	5

Table 8. Factor loading matrix and contribution ratio from the inflorescences of 87 strains

PC	χ_1	χ_2	χ_3	χ_4	χ_5	χ_6	χ_7	χ_8	χ_9	χ_{10}	CR
Z_1	-.84**	.77**	-.19	.76*	-.74*	-.88**	-.22	-.60	-.23	-.76*	.427
Z_2	-.32	.50	-.56	.45	.21	.10	.75*	.17	.79**	.55	.244
Z_3	-.37	-.18	.74*	.25	-.20	-.03	-.06	.46	.29	.09	.112

χ_1-χ_5: average value of each strain, χ_6-χ_{10}: variance of each strain,

PC : principal component, CR: contribution ratio,

** and * : significant at the 1% and the 5% level, respectively

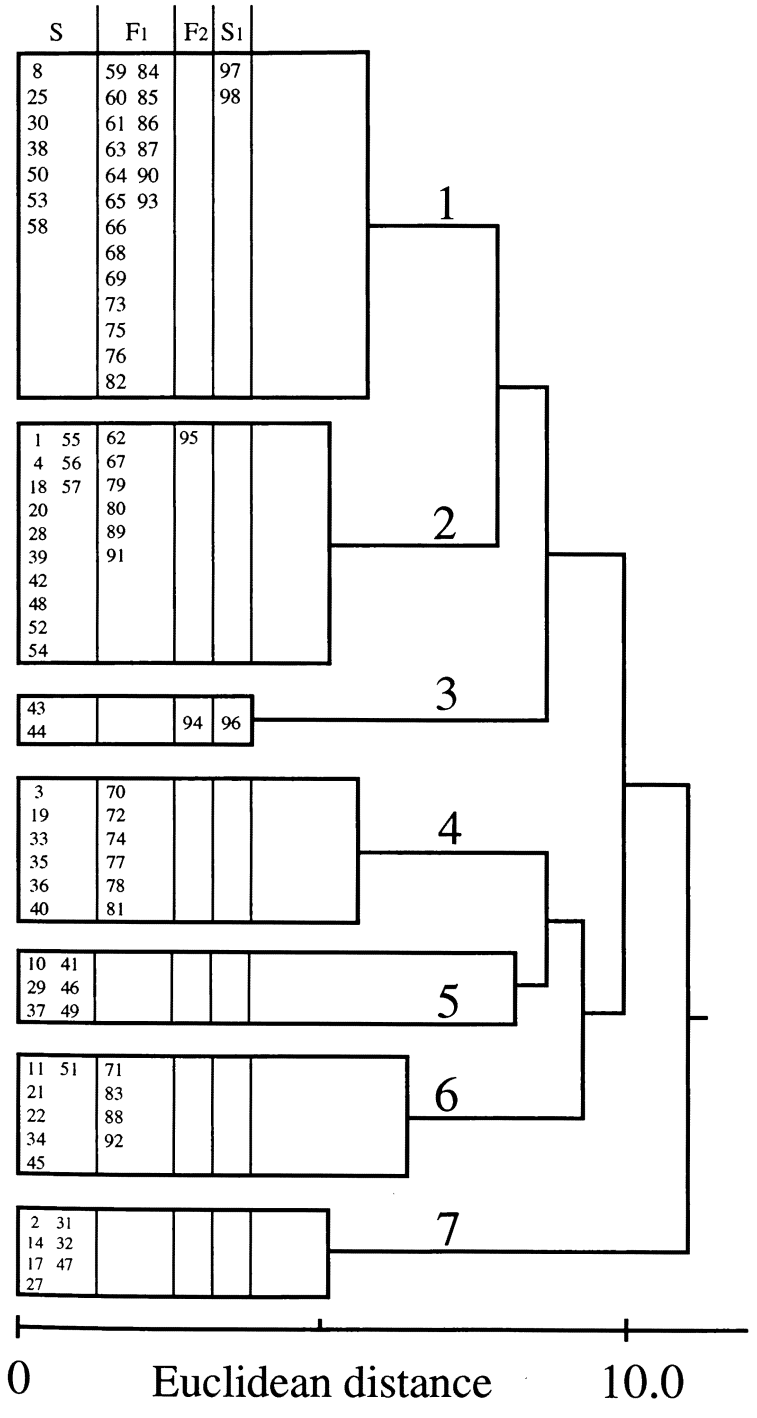


Fig. 15. A dendrogram from the inflorescence of 47 strains (Column S), 37 F_1 hybrids (Column F_1) and F_2 hybrids (Column F_2), and 3 S_1 progenies (Column S_1) based on their inflorescence parameters, χ_1–χ_{10}. Arabic numerical in each column is the accession Nos. in Tables 3, 4 and 5.

3-4. Discussion

In the first experiment using 4 strains, Z_1 component was found to be a shape factor and Z_2 component was a size factor. So accessions No.2 and No.17 which had long sterile appendages were well separated in the scatter diagram. The difference between accession No.11 and accession No.22 was also clear. This result was expected to some extent from their appearance of inflorescence. In the second experiment using the average value and the variance of 87 strains, though the treatment of sample data was naturally different from that of the former, the clusters were clearly divided into two groups. One of them, i. e., branch 7 in Fig.15, included accessions No.2, No.17, No.31, No.32 and No.47 having long sterile appendages. This cluster is characterized by large variances of sterile appendage (χ_6), staminate (χ_7) and pistillate part (χ_9) affecting the Z_1 and Z_2 components. The result of the second experiment also suggested that the ratio of the sterile parts (χ_1 and χ_3) to the fertile parts (χ_2 and χ_4) is a main factor to determine the shape of taro's spadix.

Engler (1920) described the ratio of χ_1 to χ_2 and others followed or quoted (Kitamura 1949, Hidaka 1977) his description. Seeing from the present results, lengths of the parts and shapes of inflorescences in family Aracea vary among and within species and certainly seem to be determined genetically. However, it seems unreasonable to use these characters as key characters to species at least in the case of the species which have such an inflorescence as that of *C. esculenta*, having a sterile appendage, a staminate part, a sterile part and a pistillate part in sequence from the top to the bottom of the inflorescence. In such species, only when supplemented with the data of other characters such as the presence of long stolon or tuber, ploidy or ecological adaptation, those characters can be used to classify varieties or subspecies.

4. Analysis of the Length and Angle of Leafvein

4-1. Leaf shape of taro

Leaf shape of *C. esculenta* is peltate or ovate and sometimes arrowhead-shaped. Its two basal lobes are connate. Fig.16a is a leaf of a wild diploid, accession No.2, and Fig.16b is that of a wild triploid, accession No.17. They resemble each other. Fig.16c is a leaf of *A. macrorrhiza*. Except for a depth of gap between the lobes, its leaf shape is alike that of accession No.17. But the appearance of these two is very different; *A. macrorrhiza* has more lustrous surface and much stouter than accession No.17, the difference of them being very remarkable. Number, running direction and way of curving of the leafveins are different. From the horticultural point of view, the leaf shape is very important which is determined genetically. The present author felt some difficulties in recording leaf characteristics. For the purpose of using leafveins for classifying taro strains, he started to investigate a few numerical parameters concerning the leafveins.

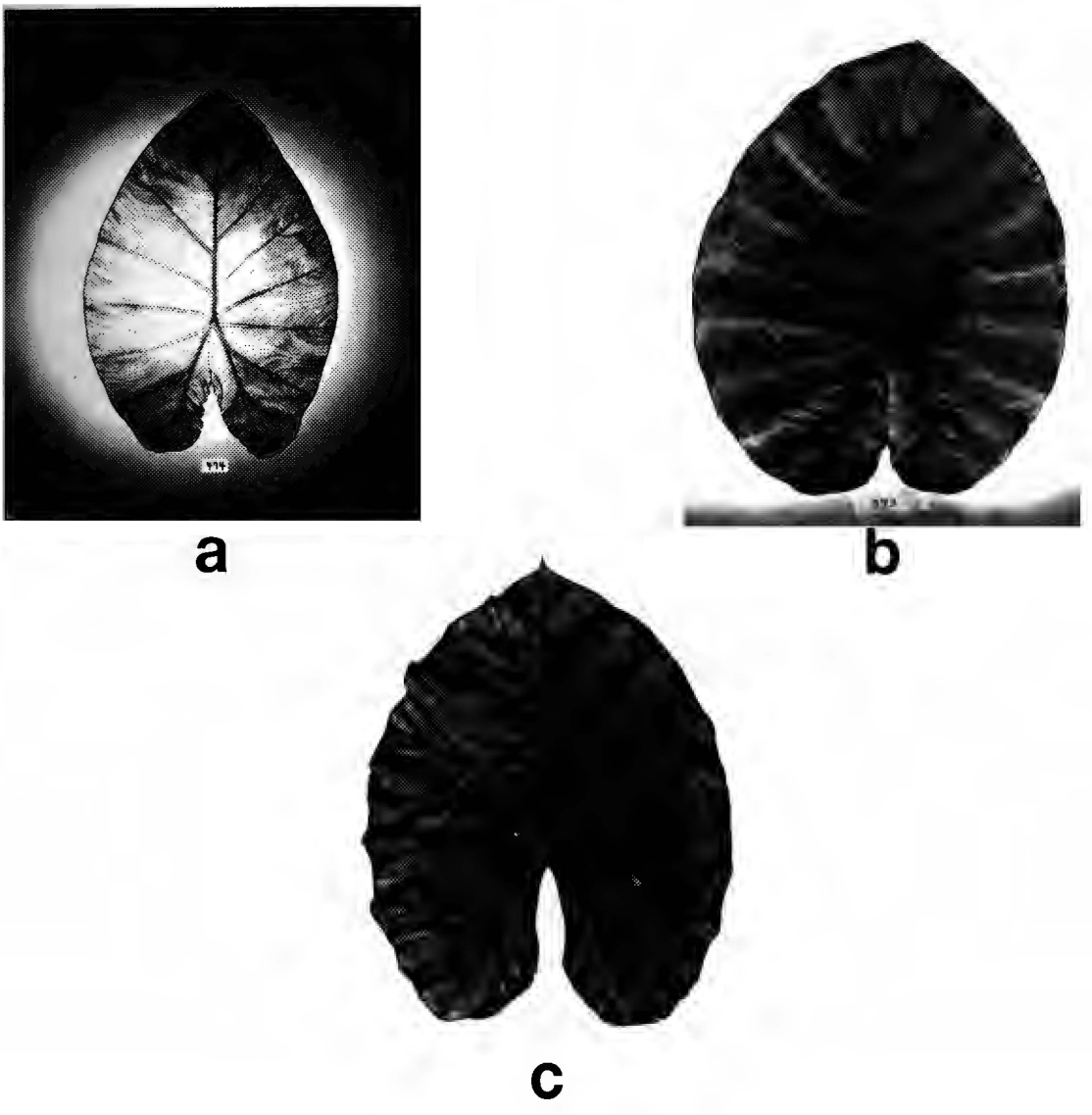


Fig. 16. Leafveins of two accessions (a : No.2, and b : No.17) of *C. esculenta* and an accession of *A. macrorrhiza*.

The young leaf appears outside from the vagina sheath of the preceding petiole with winded right or left. Fig.17a shows the condition, and as shown in Fig.17b the right- or left- handedness causes asymmetric leaf shape. A lamina is divided into right and left halves by the curved line of midrib. Right half lamina of a left-handed young leaf was expressed as LR. And LR, if turned over, would be RL. The handedness of right or left is usually decided by chance. For this reason, RR and LL were expected to bring almost the same result, and RL and LR also do the same. To assure this a preliminary analysis was made with accession No.2. Thirty-one leaves of RR and the same number of LL leaves were analyzed. This analysis revealed that there were some problems exsisting in treating RR in the same way as LL and RL as LR.

A grown-up leaf was cut off from the petiole and the lobes were cut apart along the connate line. The backside of this leaf was xerox-copied. The origin was set in the center of petiole-attached area, and the axis was set from the origin to the end point of midrib. The length and the angle to the axis of the secondary veins located closer to the origin were taken for measurements. Cosine value was used for the data of the angle. Actually digitizer was used to measure them. Measured veins are shown in Fig.17c. Measured points from 1 to 7 are on the RR/LL half lamine. The ratio of each length to the midrib length and the angle to the axis were normalized and analyzed by principal component analysis and Ward's cluster analysis. Five accessions, No.28, No2, No.17, No.22, No.11 and a Japanese cultivar Yatsugashira were studied. Number of their leaves measured were 50, 50, 48, 50, 50 and 50, respectively.

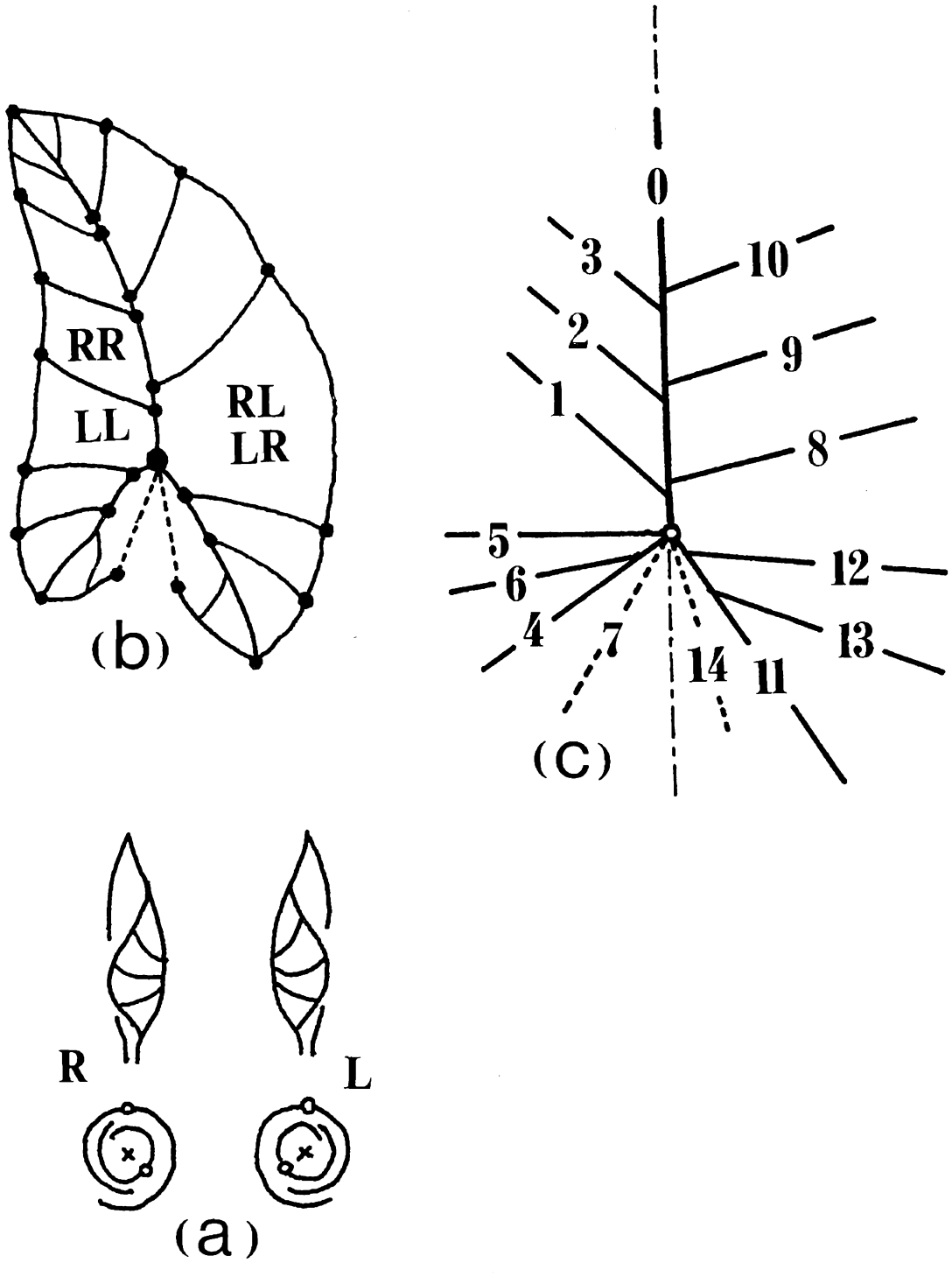


Fig. 17. Right- and left-handedness of a leaf.

(a) : wrapped leaves and their cross section of right-handed (R) and left-handed (L) leaves.

(b) : an unfolded leaf ; RL means left half lamina of a right-handed leaf • shows measured points.

(c) : leafveins, measured (1-14). ---- : cut line

4-3. Results

The result of a preliminary experiment was as follows. According to principal component analysis, Z_1 was found to be a size factor influenced by leafvein length in all of RR, LL, RL and LR. Both components Z_2 and Z_3 were shape factors and were a little different between RR and LL. It was thought to be caused by the small sample size. Cumulative ratio from components Z_1 to Z_3 were 75.8% (LL), 87.5% (RR) and 77.0% (LL+RR). By the way, taro is much weakened when its leaf is damaged because of having few leaves per shoot, so it was difficult to cut a large number of leaves for the experiments. Supposing that RR and LL show no difference, an additional experiment was conducted by increasing the sample size a little more.

Table 9 shows the correlation between angle and principal components from Z_1 to Z_3 of 6 accessions and the mixed of them. Z_1 component was a size factor in all accessions, which was closely correlated with the lengths of almost all the secondary veins measured, however, in some accessions, for example accession No.17, angles contributed considerably to the principal component score. As for Z_2 component, it was a shape factor expressing the degree of asymmetry between right- and left-half lamina. Z_3 was also a shape factor. As Table 9 shows, these 6 accessions had various principal components to express their leaf shapes. When they were analyzed together, the result was as shown in the column 'Mixed'. Cluster analysis was made using the principal component scores by the components from Z_1 to Z_4. Fig.18 shows the result. Accession No.11 was not so clearly characterized. However, 3 diploids (accessions No.28, No.2, Yatsugashira) and 2 diploids (accessions No.17, No.22) were cleary divided. The latter had large and relatively round and symmetric leaves.

Table 9. Correlation between the angles of the secondary veins (χ_1–χ_{14}) to the midrib axis and the components from Z_1 to Z_3 of 6 accessions and the mixed of them

Accession		No.28			No.2			Yatsugashira			No.17			No.22			No.11			Mixed		
		Z_1	Z_2	Z_3	Z_1	Z_2	Z_3	Z_1	Z_2	Z_3	Z_1	Z_2	Z_3	Z_1	Z_2	Z_3	Z_1	Z_2	Z_3	Z_1	Z_2	Z_3
RR LL	χ_1	*	*	(**)	(**)	**			**	(**)	**		**	*	**	(**)	(**)	(**)	(*)		**	
	χ_2		*	(**)	(**)	**	(*)		**	(**)	**		**	*	**	(**)	(**)	(**)	(*)		**	
	χ_3			(**)	(**)	**			**	(**)			**		**		(**)		(**)	(*)	**	
	χ_4		**			**	(**)		**		**			**	*			(**)			**	
	χ_5		**		(*)	**			**	(**)	**		**	**	**	(*)		(**)			**	
	χ_6		**			**	(**)		**		**		**	*	**	(*)		(**)	(*)		**	(*)
	χ_7		*			*	(**)	*			*	(**)			**			(**)	(*)	*		(**)
RL LR	χ_8	*	(**)	(**)	(*)	**	*		**	**		**			**	**	(*)	(**)			**	**
	χ_9		(**)	(*)	(**)	**	*		**	**		**			**	*	(*)	(**)			**	**
	χ_{10}		(**)	(**)	(**)	*			**	*		**			**		(**)		(*)	(*)		**
	χ_{11}		(**)				**		*	*	**			*	**			(**)	*			
	χ_{12}		(**)			*			**	**	**	(**)			**	**		(**)			**	
	χ_{13}		(**)			*	*			**	**				**	*		(**)	*		**	
	χ_{14}		(**)							**		(**)						(**)	**			(*)
CR		.481	.173	.110	.463	.220	.101	.465	.162	.111	.568	.163	.091	.403	.261	.100	.479	.209	.101	.460	.161	.087

** and * : significant at the 1% and the 5% level, respectively, ( ) : negative correlation, CR : contribution ratio.

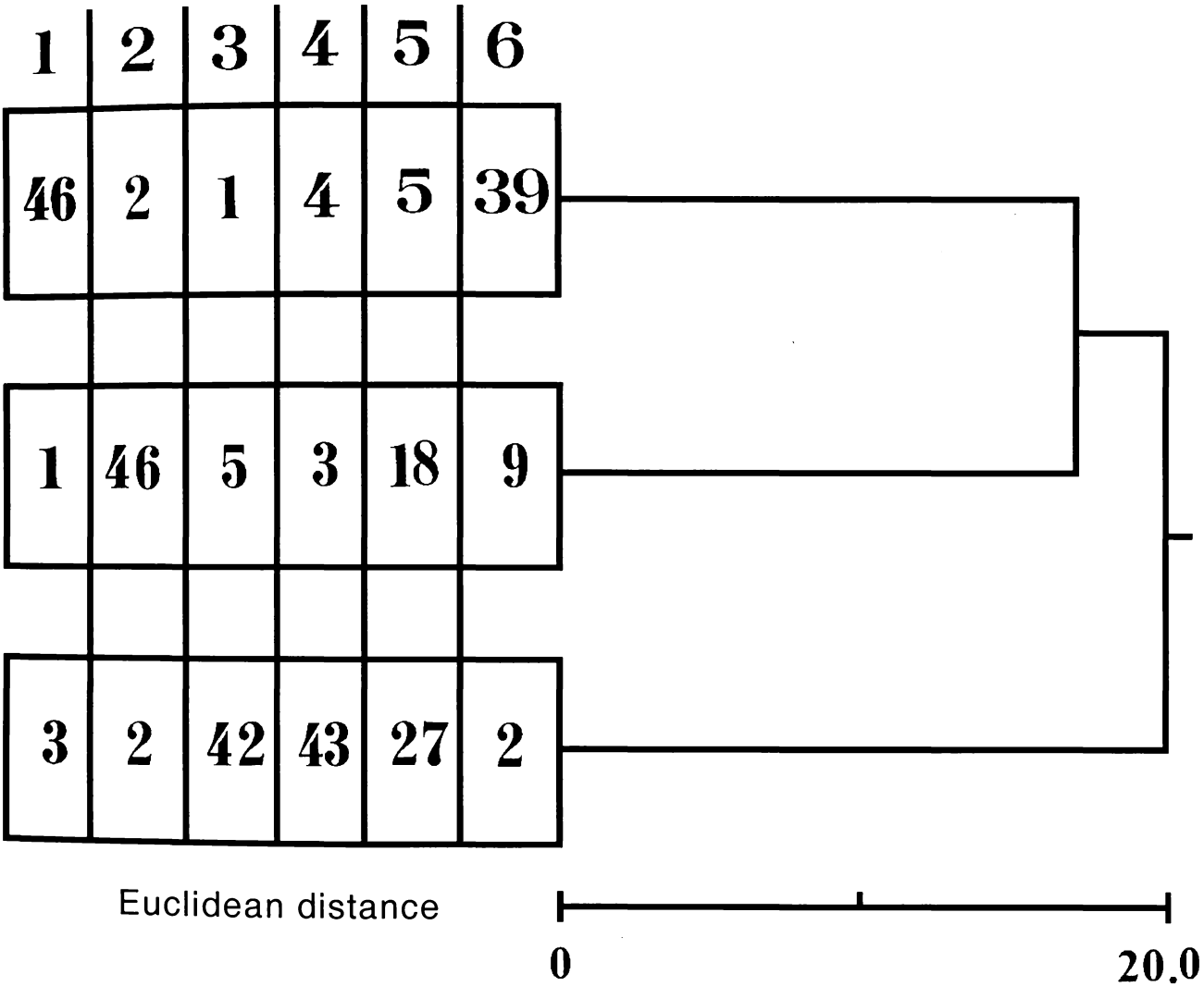


Fig. 18. A dendrogram from the length and angle of leafvein.

Column 1 : No.28 (2χ), 2 : No.2 (2χ), 3 : No.17 (3χ), 4 : No.22 (3χ), 5 : No.11 (3χ), 6 : Yatsugashira (2χ). Arabic numerical in the column shows the number of samples in each cluster.

To classify *C. esculenta* by leafvein characters seems to be a little difficult. In the present experiment, the diploid strains having relatively small leaves and the triploid ones of large round-shaped leaves were well divided because Z_1 was a size factor and the contribution ratio was largest in Z_1. Though the correlation among the principal components are calculated as to be minimum as possible, the present result will not be used to classify *C. esculenta* because of such a close correlation between the vein length and Z_1 component. And, of course, size factors can not be ignored to describe a leaf for the purpose of classification. If the length and the angle of the secondary leafveins is useful for this purpose, so much time-consuming search for the proper measuring points will be necessary. It is not practical for the present purpose.

5. Restriction Endonuclease Analysis

5-1. Plant materials

Plant materials used in the present experiments are listed in Table 3. *Alocasia macrorrhiza* var. *variegata*, *Colocasia gigantea*, *C. esculenta* var. *illustris* and *Schismatoglottis* sp. were added as outgroup species. Other 24 strains belonged to *C. esculenta*. They were originally collected by the present author except 3 strains in Nepal, Thailand and Japan.

5-2. Methods

5-2-1. Total DNA preparation

Extraction of DNA was made after a protocol informed by Juliana Ramser (1992) with some modifications.

Approximately 5g of fresh leaves were frozen with liquid N_2 and homogenized with 25ml of 2 × CTAB buffer containing 1% 2-mercaptoethanol for 30min at room temperature. Adding with 20ml of 24 : 1 chloroform iso-amylalcohol, the homogenate was shaken gently for 15min. After centrifugation at 5000rpm for 15min at room temperature, aquaous phase was taken, to which CTAB buffer was added again and the same steps were repeated. Then aquaous phase was mixed with 2/3 volume of iso-propanol gently to be centrifuged at 5000rpm for 20min. Pellet was washed with 76% ethanol containing 10mM ammonium acetate and centrifuged at 5000rpm for 15min. To dried pellet 2ml of TE was added and kept at 4°C for several days to be dissolved. DNA solution was added with 2.64g of CsCl to be dissolved, to which 20μl of 10mg/ml EtBr was added and centrifuged at 100000rpm for 4 hours. Red-colored zone was taken, from which EtBr was removed by shaking with TE-saturated 2-propanol 4 times until no color was visible. Solution was added with 2 volume of TE and next with 4 volume of 99.5% cold ethanol to 10ml, and kept at −20°C overnight. Solution was centrifuged at 5000rpm for 30min at −20°C. Pellet was dried, added with 100μl of TE and kept at 4°C to be dissolved.

5-2-2. DNA fingerprinting

After the opitical density was read using a spectrophotometer, a digestion mix was prepared, and treated overnight at 37°C with *Hin*fI. Agarose gel (Sea Kem, GTG agarose, 0.8%) with 1 × TBE was prepared, and digested DNA (4μg/lane) was loaded and electrophored at 40V for 48 hours. After gel was denaturated with 0.5M NaOH: 0.15M NaCl solution, washed once with H_2O and was neutralized with 0.5M Tris and 0.15M NaCl (pH8.0) and dried. A synthetic oligonucleotide of $(CA)_8$ was labelled with ^{32}P and used as a probe.

5-2-3. Southern hybridization

Probes were offered by Dr. R. Terauchi (Fig.19). They were DO#1, DO#2 and DO#3 clones derived from the *Sal*I fragments of chloroplast (ct) DNA of *Dioscorea opposita* Thunb. and the *abc* clone from the *BamH*I fragments of *D. bulbifera* L. ctDNA with which 2/3 of the total chloroplast genoms (ca. 152 kbp) were covered. Restriction endnucleases, *BamH*I, *Dra*I, *EcoR*I, *Hin*dIII, *Msp*I and *Hae*III were used in the present experiment. As for electrophoresis, 0.8% agarose gel with 1 × TAE was prepared and lμg of digested ctDNA per lane was loaded and electrophored at 20V for 30hrs. CtDNA was transfered to nylon membrane after Southern blotting (Southern, 1975). Hybridized ctDNA fragments were detected with Dig luminescent method (Boehringer Manheim Biochemica, 1991). Membrane was boiled in 0.5% (w/v) 2 × SDS for 10min for reprobing.

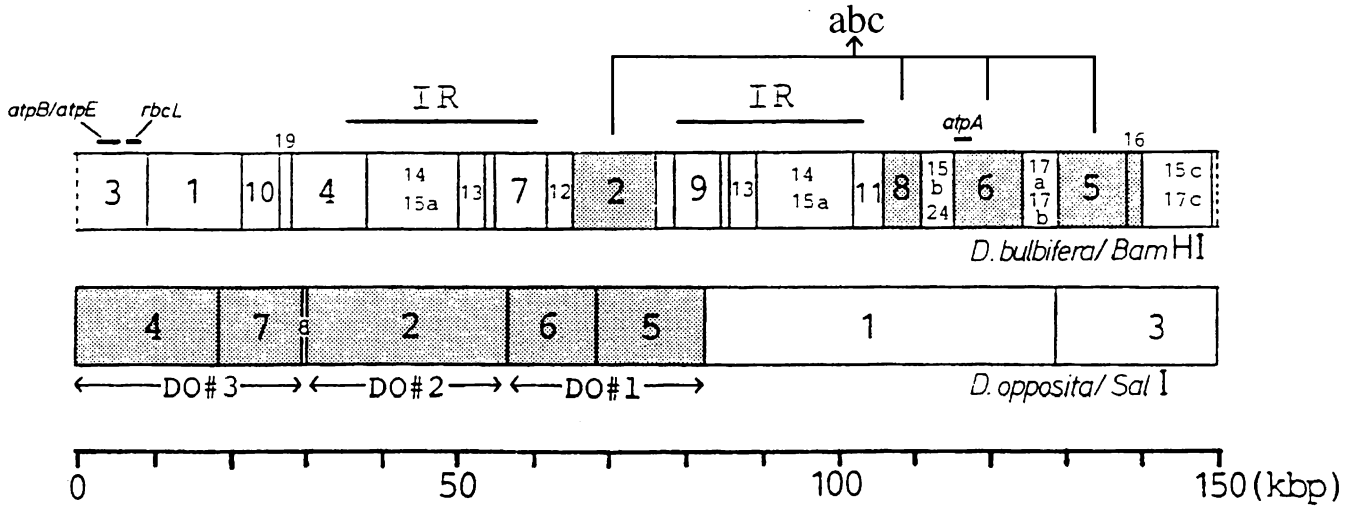


Fig. 19. Homologous and heterologous probes used for Southern-hybridization (after Terauchi et al., 1989).

5-3. Results

5-3-1. DNA fingerprinting

Fig.20 shows band pattern of 28 strains. It was very polymorphic and there were few bands in common among the strains. However, it was analized with UPGMA method (Sneath and Sokal 1973). The dendrogram obtained was too complex to be described and discussed in the relation to the phylogenetic relationships among the strains.

Fig.21 shows band pattern of 2 sets of the parents and the F1s, i. e. accessions No.20 × No.18 set and accessions No.19 × No.28 set. Bands of either parent were shared by the F1s. This result proved that the crosses were made successfully.

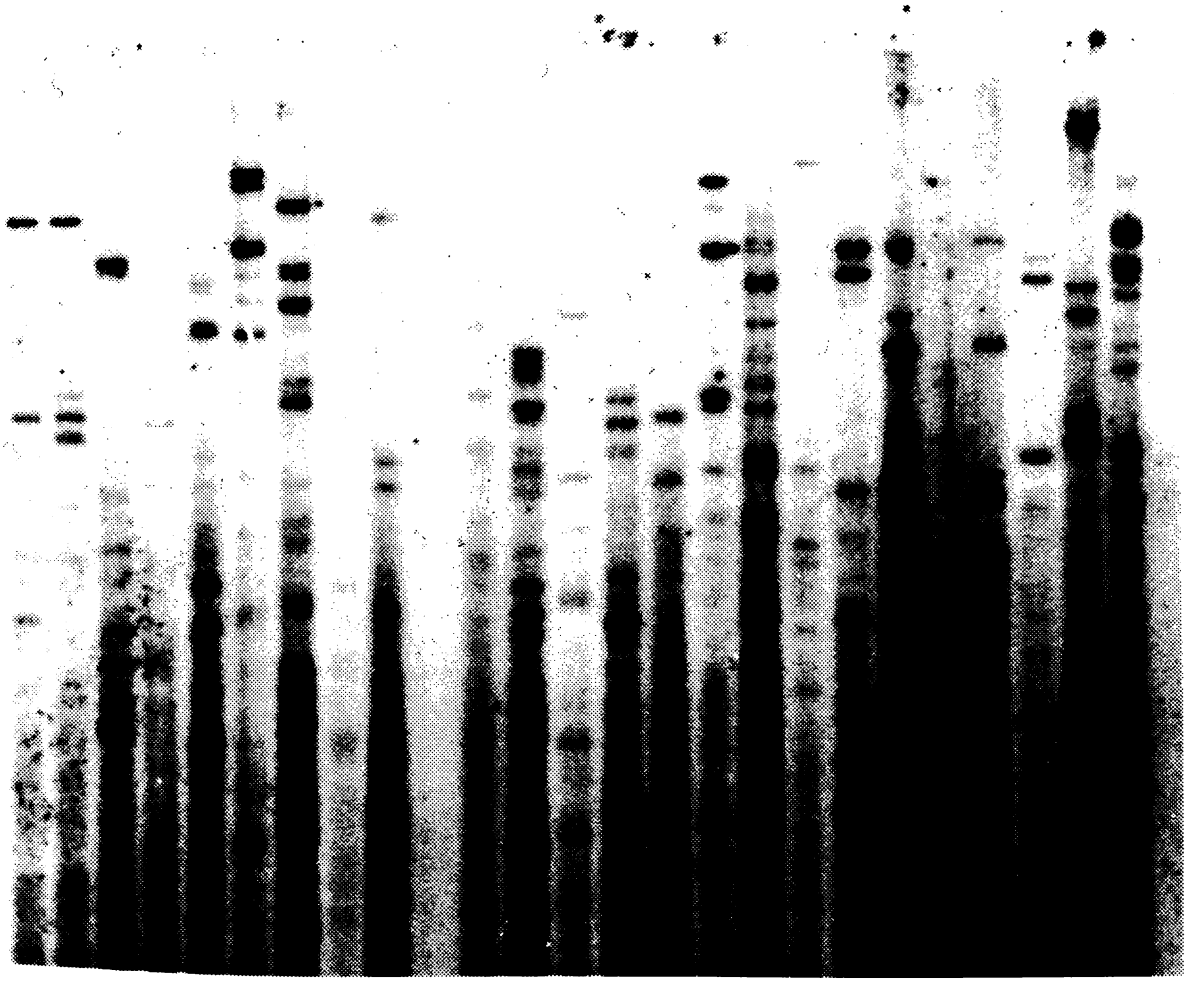


Fig. 20. DNA fingerprinting patterns of *C. esculenta* and other species from Nepal, Thailand and Japan.

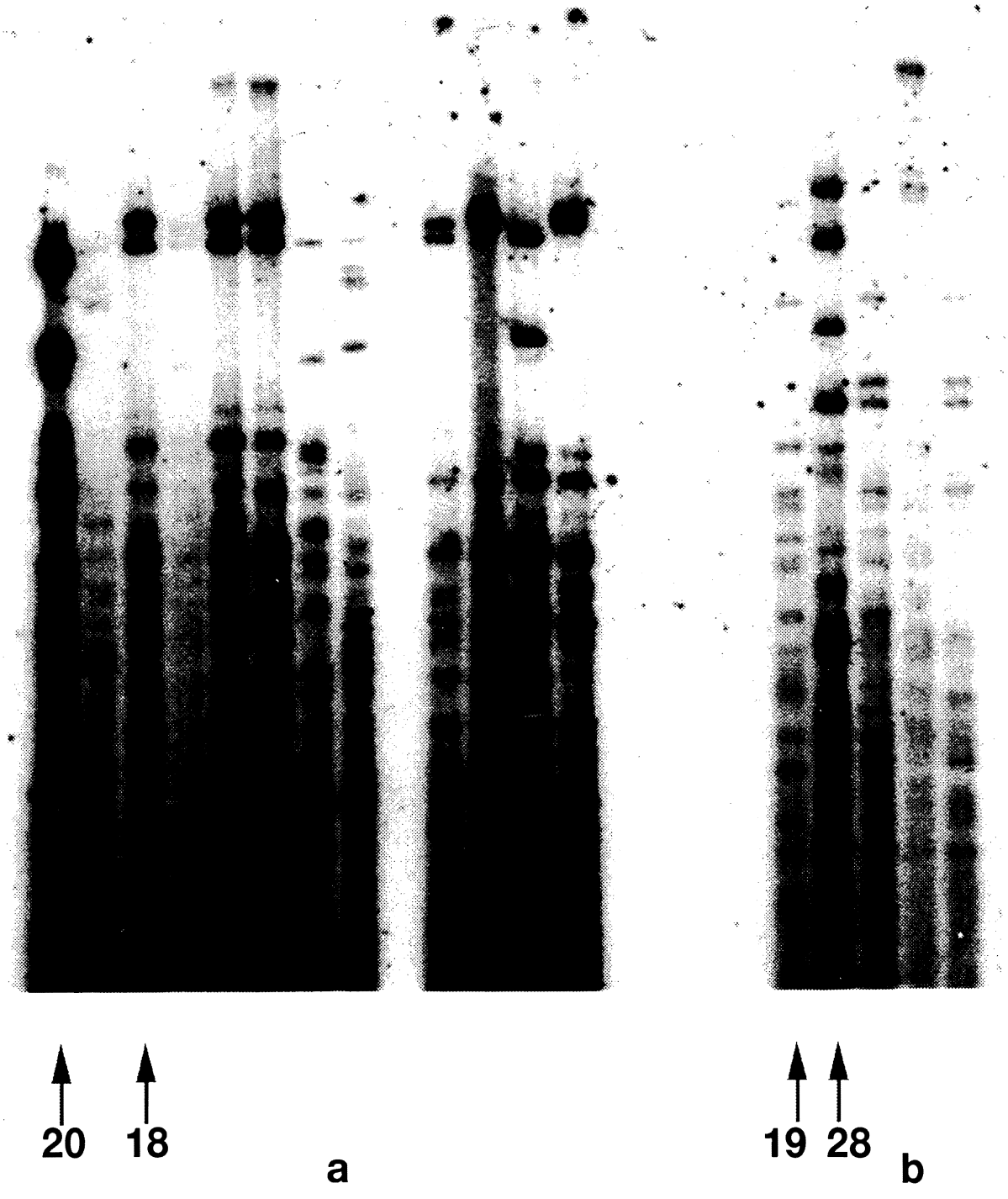


Fig. 21. DNA fingerprinting patterns of F1s and their parents. a : accessions No.20 and No.18 and their F1s, b : accessions No.19 and No.28 and their F1s.

5-3-2. Southen hybridization with ctDNA probes

Among the possible combinations between 4 probes and 6 enzymes, readable band patterns were obtained from 17 probe-enzyme combinations as shown with + sign in the following table.

	*Dra*I	*Eco*RI	*Bam*HI	*Hin*dIII	*Msp*I	*Hae*III
DO#1	+	+	+	+	+	+
DO#2	+	+	+			
DO#3	+	+		+	+	+
abc	+	+	+			

Some of them are shown in Figs.22 to 25. The most remarkable point was that these strains were divided into three groups;

A. accessions No.2, No.5, No.15, No.16
B. accession No.26
C. others

Above all, group A was definitely separated from the others. It did not share almost any bands with other groups. Group B was a little different from group C, but had some bands in common with group C. Group C had a unity as a whole though some strains were different as to one or two bands. Accessions No.2 and No.5 of group A could not be distinguished morphologically from group C, all of which belonged to *C. esculenta*. Two other strains of group A, accession No.15 belonging to *C. gigantea*, and accession No.16 to *Alocasia macrorrhiza* var. *variegata*. Accession No.26 of group C seemed to be classified as a species of genus *Schismatoglottis*, judging from the description by Hotta (1965) on the shape of leaf, spathe and flower. If this classification is correct, this strain belongs to tribe Philodendreae, while the two genera *Colocasia* and *Alocasia* belong to tribe Colocasiae.

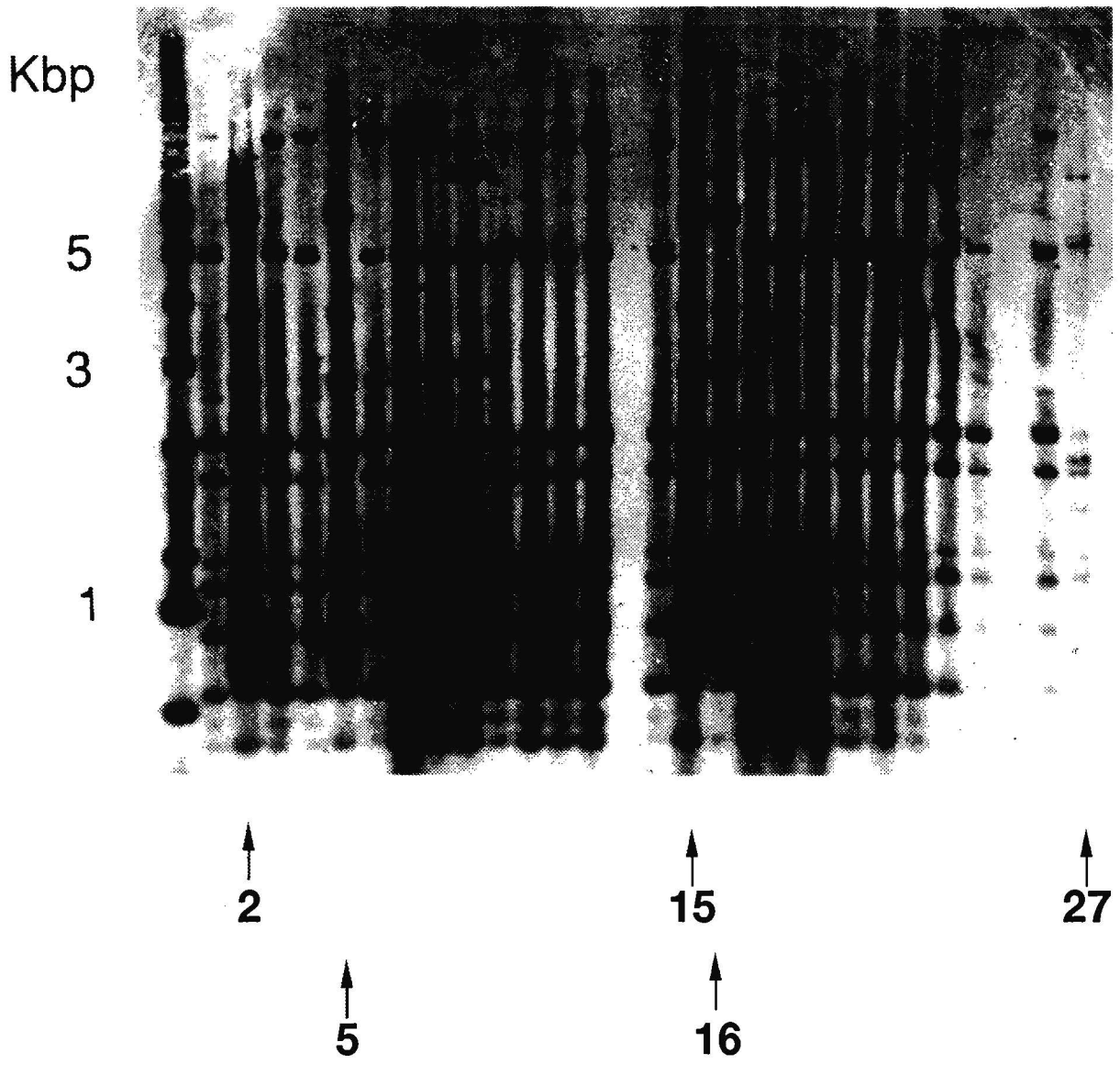


Fig. 22. *Bam*HI restriction fragment patterns of ctDNAs of *C. esculenta* and other species. Probe : Do#2 of *D. opposita*.

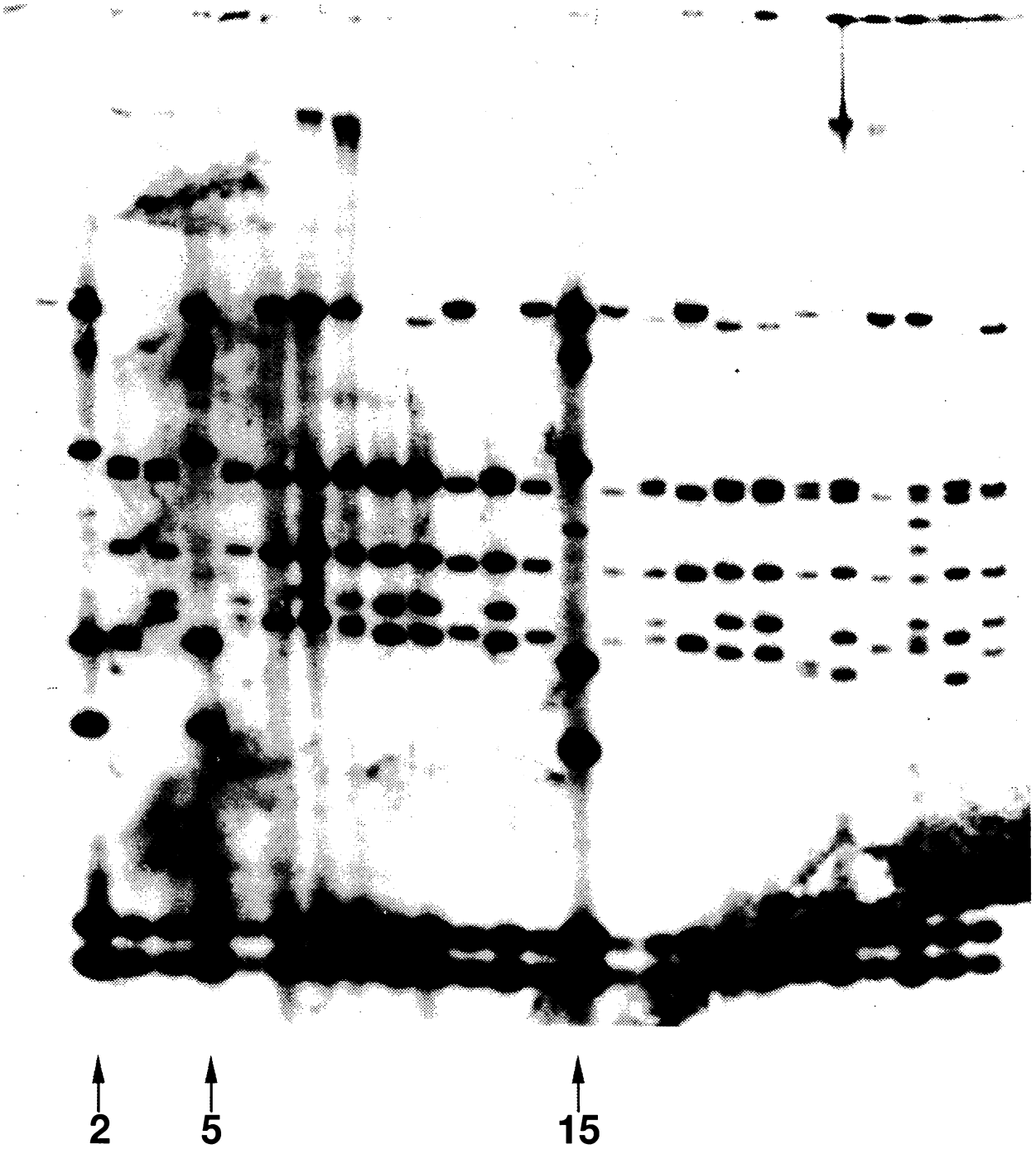


Fig. 23. *Hin*dIII restriction fragment patterns of ctDNAs of *C. esculenta* and other species. Probe : Do#1 of *D. opposita*.

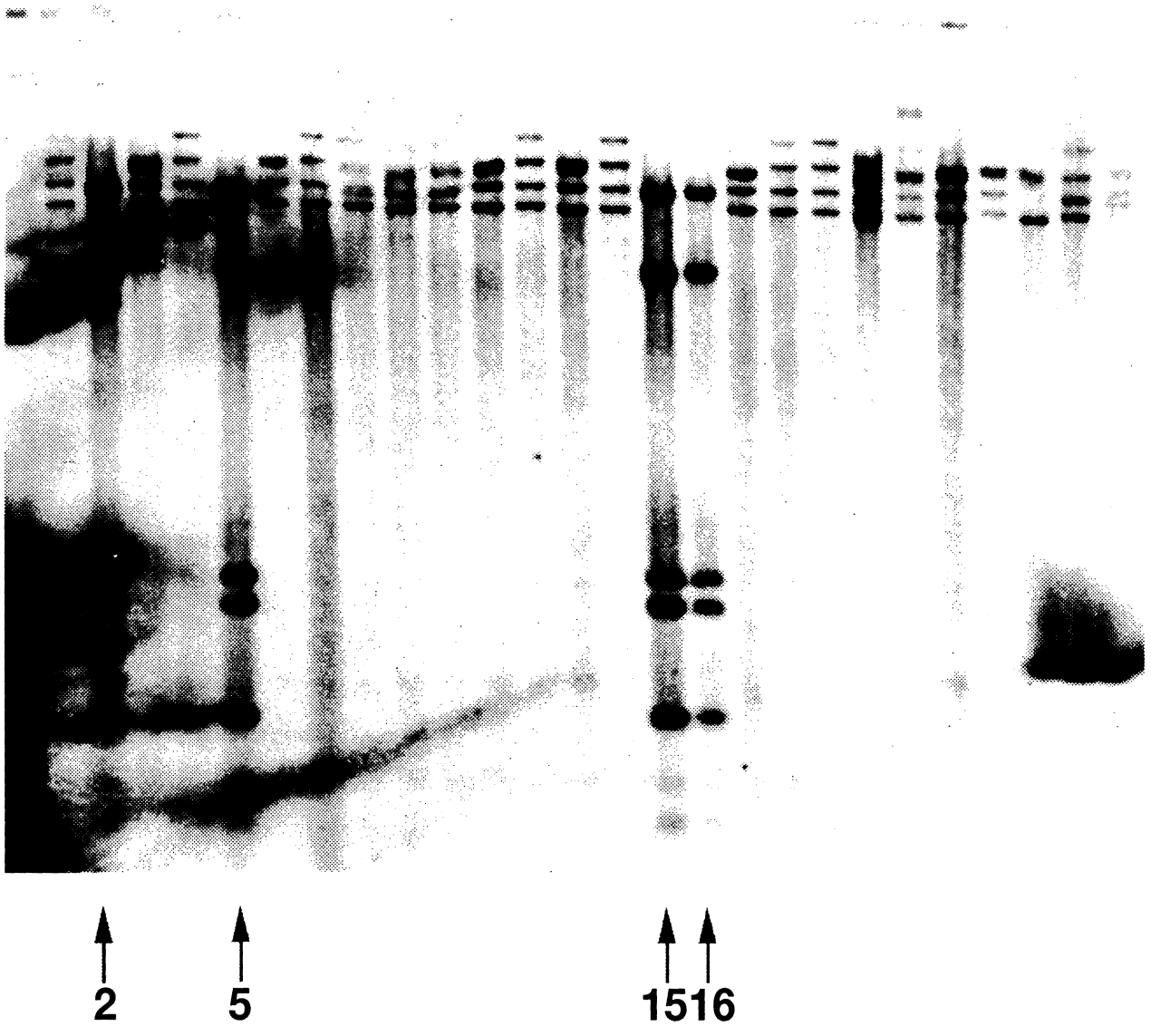
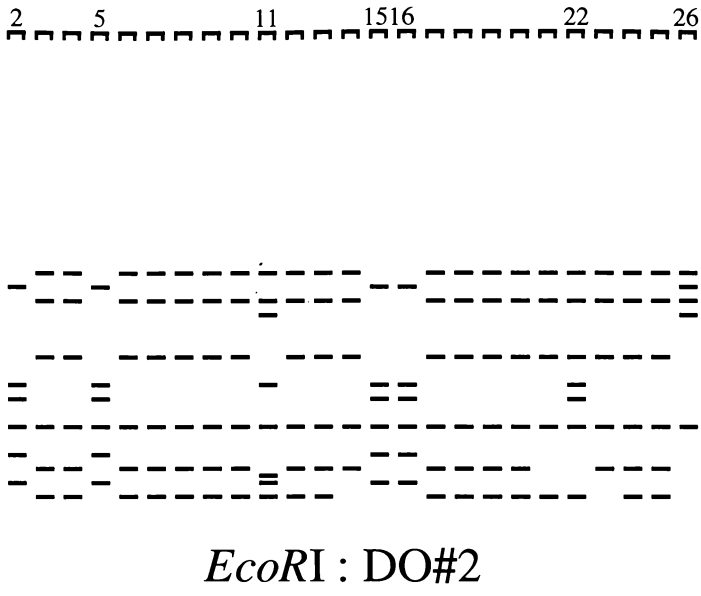
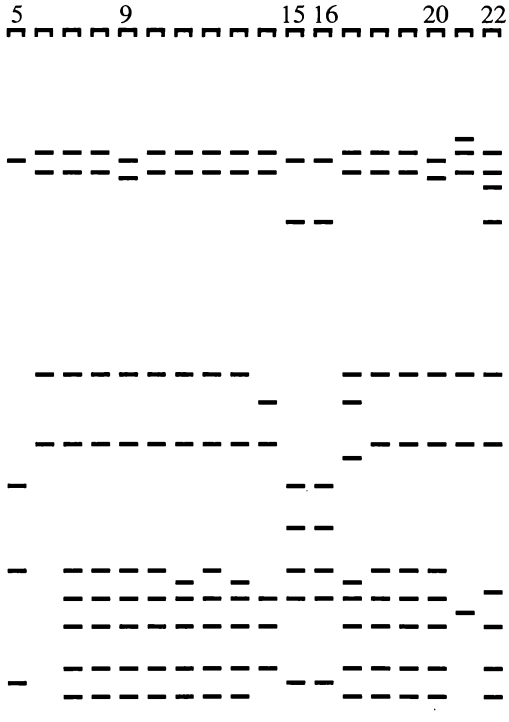


Fig. 24. *Dra*I restriction fragment patterns of ctDNAs of *C. esculenta* and other species. Probe : Do#2 of *D. opposita*.



*EcoR*I : DO#2



*Dra*I : DO#3

Fig. 25. Restriction fragment patterns of ctDNAs of *C. esculenta* and other species.
Upper : *EcoR*I / DO#2, lower : *Dra*I / Do#3, Arabic numericals on the top are accession numbers.

5-4. Discussion

The band patterns except accession No.28, of whitch clear band patterns could not be obtained, were analyzed with UPGMA method. Fig.26 shows the result. If all these strains divided into two clusters, the group A gathers in one big cluster. To another cluster, accession No.26 of different tribe belongs with many strains of *C. esculenta*. Even if divided into three clusters to separate a different tribe, it cannot be a solution as far as the group A exsists.

The results are very clear. *Alocasia*-type ctDNA exsists in genus *Colocasia*. There is no way left but to examine ctDNAs of genera *Colocasia* and *Alocasia*. The hybrid origin of No.2, No.5 and No.15 should be discussed in General discussion.

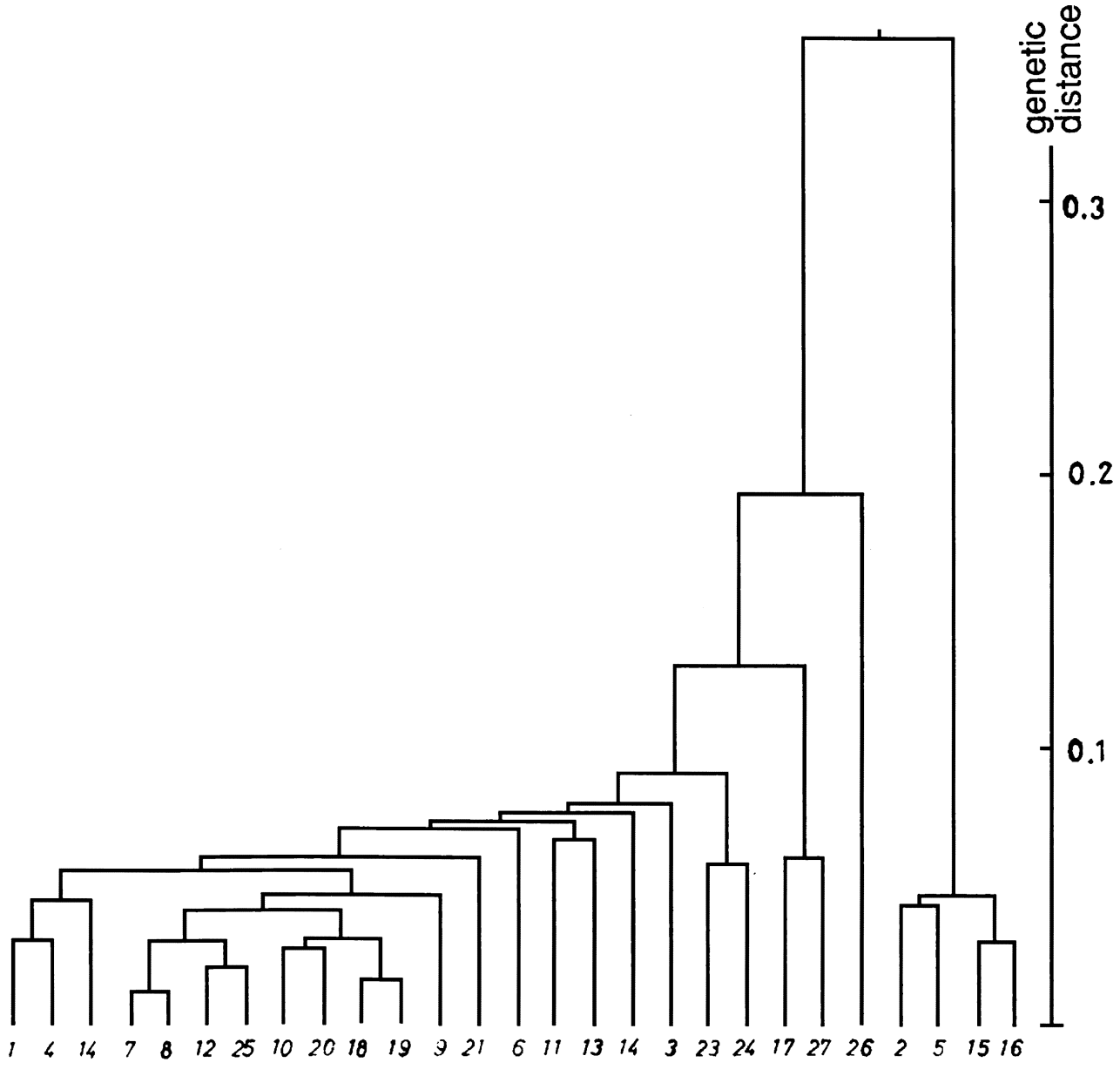


Fig. 26. A dendrogram from ctDNA showing phylogenetic relationships between ctDNAs of *C. esculenta* strains and other species as reference. Arabic numericals on the bottom are accession Nos.

6. Crossing Experiments

6-1. Flowering pattern of taro

In the subtropical area such as India or Thailand, taro flowers most in the summer. In Japan too, flowering period is usually from August to October. Under the good condition like a green house, flowering continues up to the next January. In the western Japan, from the end of April flowering begins if plants are vigorous. It has been often said or written that taro has self-incompatibility. Female flower is said to mature earlier than male flower.

In summer, several days after the inflorescence appears out of the vagina sheath, the color of upper half of the spathe begins to change from light green to yellow and lower half remains green. A spathe opens at a little upper part over the constriction. It begins to smell rather strong. On the next day, usually in the morning, the staminate flowers. But usually the pollen grains are blocked at the constriction and pollination is not fulfilled probably without the help of insects as flies or ants. The present author observed in Nepal that ants are gathering inside the spathe at the pistillate part. Pollination under the natural condition seems to take place rather frequently.

6-2. Crossing technique

When a spathe opens at above the constriction, spathe must be cut open and pollination is easily done. If an outcrossing is to be done, staminate part must be cut off after that treatment. In summer, about 30 or 40 days after pollination, the color of pistillate part changes from green to orange or yellow. That part is crashed in the water and seeds can be gathered. If they are dried at once, under the dry condition in the desiccater at the room temperature, even after one year, more than half of seeds can germinate. Fig.27a shows the germination of *C. esculenta* 7 days after sowing. A cotyledon is seen on the left bottom of the seed, of which longitudinal length is about 0.8mm. Seeds of *A. odora* are shown in Fig.27b for comparison. They are much larger than those of *C. esculenta*.

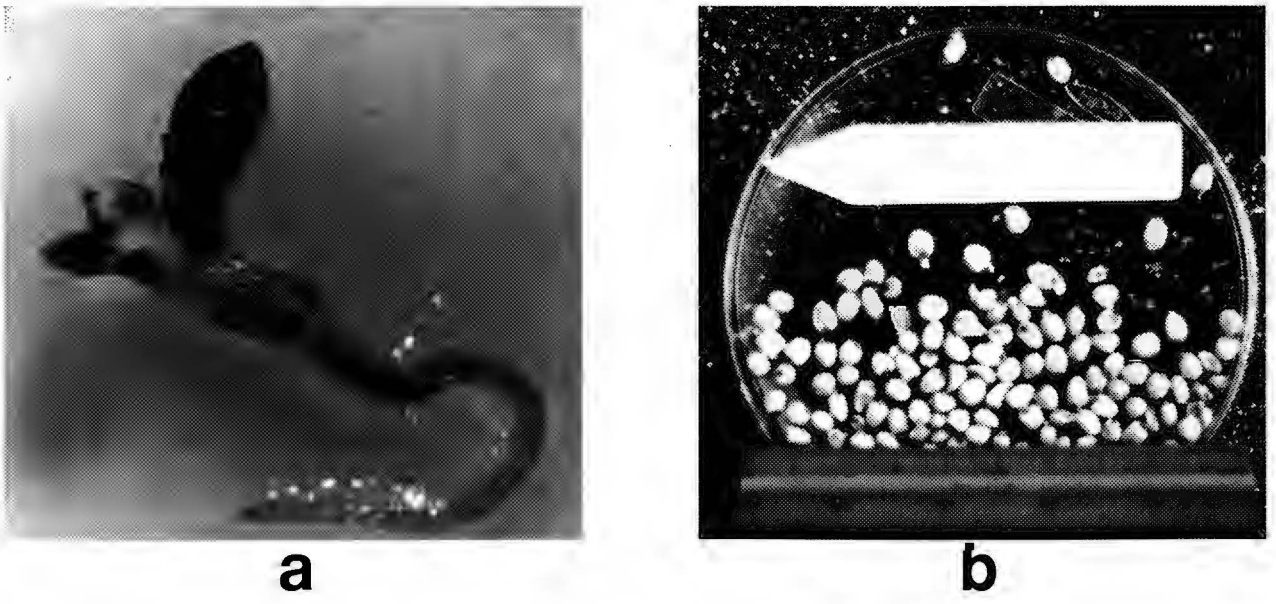


Fig. 27. A germinating seed of *C. esculenta* (a) ; left end is a cotyledon, and seeds of *A. odora* (b).

First, to know the possible period of fertilization before male flowering, the artifical pollination was done on different dates. Two wild diploid strains, accessions No.19 and No.28, were used as parents. Table 10 shows the result. Even 6 days before male flowering fertilization took place. And two or three days after male flowering fertilization was also possible, though the mucus exuded from the stigma. The result indicates that fertilization takes place by selfing as easily as by outcrossing. It is clear that the accessions used in this experiment are fully self-compatible.

Next, to know the relationships between parents and their progeny on the inflorescence length and relative length of each part, crossing were made between two accessions No.19 and No.28. The mean ratios and total length are shown in Table 11. The number of individuals studied were as follows, accession No.28 : 5, accession No.19 : 5, S_1 of accession No.28 : 6, accessions No.19 × No.28 F_1 hybrids : 16, accessions No.28 × No.19 F_1 hybrids : 2.

Using the mean values of individual plants, principal component analysis was worked out. The results after Varimax rotation are shown in Tables 12 and 13. To know the relationships between F_1s and the parents, S_1s and the parent, they were analysed separately.

In both the F_1 and S_1 progenies, F_1 component was commonly and strongly affected by a character χ_4 (relative length of pistillate part), whereas components F_2 and F_3 were mainly determined by different characters in the F_1 and S_1.

Based on the five infloresence parameters, χ_1-χ_5, the parents and the F_1 and S_1 progenies were clustered by Ward's method. The results are given in Fig.28. The S_1 progeny slightly differed from the parent accession No.28. No clear difference was found between the reciprocal F_1 hybrids, accessions No.19 × No.28 and accessions No.28 × No.19. About half of the F_1s were intermediate of the two parents, whereas the remaining half closely resembled one of the parents, accession No.19.

And other sets of crossing were made, i. e. accessions No.4 ×.No.51, No.51 × No.4, No.33 × No.51, No.51 × No.19, No.19 × No.28, No.28 × No.19, and two sets of F_2 were obtained. In Fig.29, they are shown in the same dendrogram that was used in Fig.15.

In taro, petiole color varied. As it is strongly influenced by environment factors, critical analysis is difficult. But the progeny of the almost green-colored strains are usually green. Here, petiole color of the parents and offspring recorded, i. e., top of petiole, vagina sheath and the upper and lower half of petiole. Four plant parts were selected for observation which were relatively stable in color. The result is shown in Table 14. All progenies of type B (almost green) were also type B, whereas those of type D and E segregated other types indicating their hybrid nature. But in other materials (indicated as 'source' in Table 14) too, different types segregated.

Table 10. Results of pollination

(a)	No. inflorescences		
	fertile	sterile	total
outcrossing	12	13	25
selfing	11	10	21

(b)		No. inflorescences							
days before male flowering		0	1	2	3	4	5	6	total
outcrossing	fertile	1	4	2	1	2	0	2	12
	sterile	1	5	2	3	2	0	0	13
selfing*	fertile	6	2	0	0	1	1	1	11
	sterile	5	4	0	0	0	1	0	10

(a) : summary of (b), (b) : pollinated on the different dates before male flowering, * : pollinated with a flower of the same individual.

Table 11. Mean ratios from χ_1 to χ_4 and mean length of χ_5

accessions	χ_1	χ_2	χ_3	χ_4	χ_5
No.28	20.9	34.9	15.9	28.3	233 ± 34.2
S1 of No.28	16.0	39.5	13.8	30.7	204 ± 29.4
No.19 x No.28 F1	27.8	34.0	14.1	24.1	266 ± 58.7
No.28 x No.19 F1	31.9	31.0	13.5	23.6	277 ± 46.3
No.19	35.8	35.3	11.0	17.9	297 ± 53.6

Table 12. Rotated factor loading matrix and correlation coefficients among the five factors of the five inflorescence characters of accessions No.19, No.28 and their F1 hybrids

a. Rotated factor loading matrix

	F1	F2	F3	F4
χ_1	-.707	.502	.387	.308
χ_2	.148	-.982**	.110	-.042
χ_3	.158	.094	-.968**	-.172
χ_4	.925*	-.116	-.105	-.347
χ_5	-.483	.074	.242	.838

b. Correlation coefficients among the five factors

	F1	F2	F3	F4	F5
F1	1.000				
F2	.371	1.000			
F3	-.432	-.124	1.000		
F4	-.764	-.378	.387	1.000	
F5	-.405	-.379	.433	.282	1.000

Table 13. Rotated factor loading matrix and correlation coefficients among the five factors of accession No.28 and its selfed progeny

	F1	F2	F3	F4
χ_1	-.239	.370	.269	-.857
χ_2	.228	.032	-.908*	.351
χ_3	-.628	-.400	.605	.280
χ_4	.925*	.029	-.212	.315
χ_5	.092	.965**	-.085	-.231

	F1	F2	F3	F4	F5
F1	1.000				
F2	.220	1.000			
F3	-.638	-.315	1.000		
F4	.310	-.546	-.357	1.000	
F5	-.354	-.427	.355	.461	1.000

Table 14. Classification of plants into five types (A–E) according to coloration in the four plant parts (a), and the segregation of those types in the progenies (b)

(a) Type	Part 1	2	3	4
A	−	−	−	−
B	±	±	±	−
C	±	+	±	+
D	+	+	+	+*
E	++	++	++	++

1 : top of petiole
2 : vagina sheath
3 : upper half
4 : lower half
− : completely green
± : almost green
+ : colored
+* : colored with stripes
++ : colored dark

(b) Type	Generation	Source	No. plants B	C	D	E	Total B	Others
B	S_1	C8030	21				21	
	S_1	C81145	48				48	
B x B	F_1	C8030 x C81145	42				42	
	F_2	〃	67				67	
D	S_1	C81135	4	13		2	4	15
	S_1	C81027	4		1	2	4	3
	S_1	C81019	6			3	6	3
D x A	F_1	C81027 x C81125	28*	2	6	1	28	9
B x E	F_1	C81079 x C81081	25	1	2	15	25	18
D x E	F_1	C81027 x C81081	6		4	8	6	12
E	S_1	C81081	2	2	1	2	2	5
E x E	F_2	C81079 x C81081	5	1		6	5	7
	F_2	〃	3	4		5	3	9

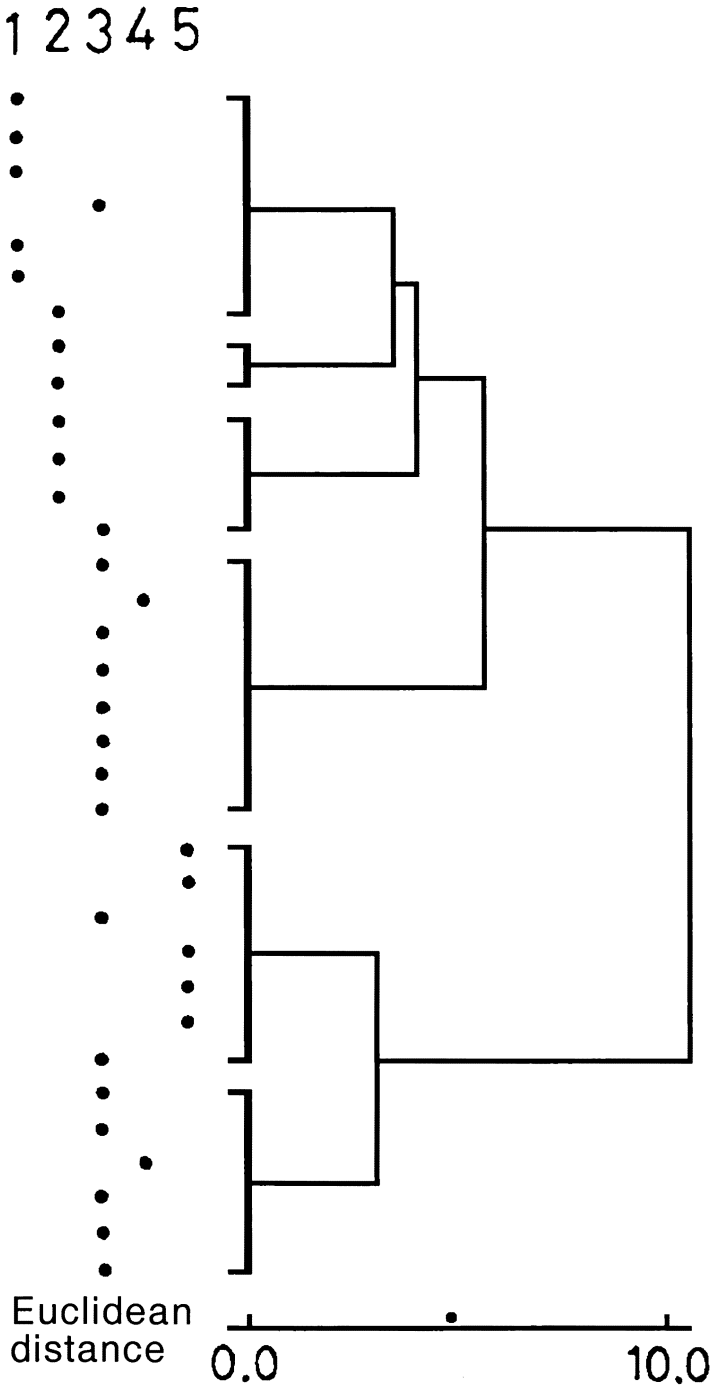


Fig. 28. A dendrogram from the inflorescence showing the relationships among the parents and their F_1 and S_1 progenies (after Ward's cluster analysis)

1 : parent accession No.28, 2 : S_1 progenies of accession No.28, 3 : accessions No.19 x No.28 F_1 hybrids, 4 : accessions No.28 x No.19 F_1 hybrids, 5 : parent accession No.19. • correspends to one individual

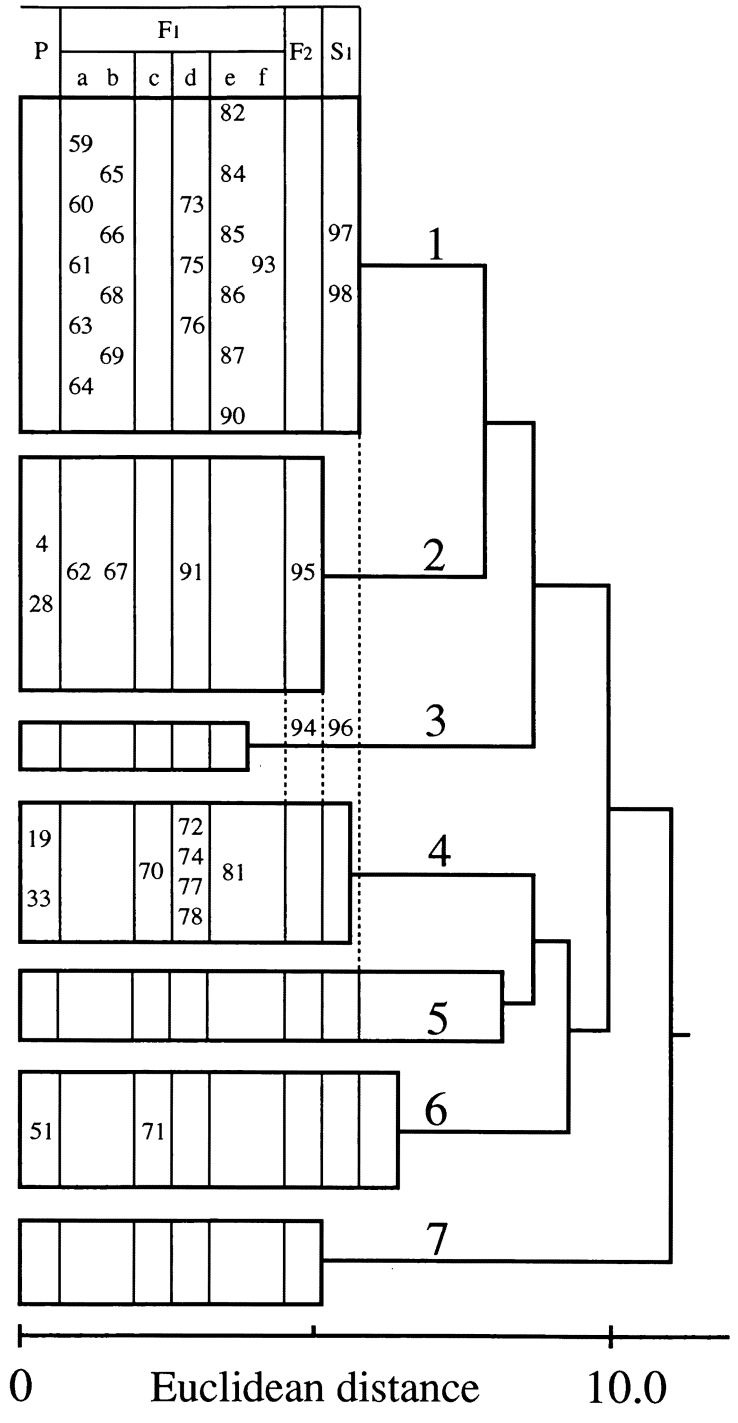


Fig. 29. A dendrogram from the inflorescence showing the relationships between parents (column P) and their progenies (column F_1, F_2, S_1), Arabic numerical in each column is accession No. (F_1) a : accessions No.4 x No.51, b : accessions No.51 x No.4, c : accessions No.33 x No.51, d : accessions No.51 x No.19, e : accessions No.19 x No.28, f : accessions No.28 x No.19, (F_2) accession No.94 : self of accession No.81, accession No.95 : self of accession No.91, (S_1) self of accession No.28.

6-4. Discussion

Contrary to general belief, taro was found to be fully self-compatible (Table 10b). However, S_2 progeny is a little difficult to be selfed, and some strains did not produce S_1 progenies. These facts indicate that some degree of self-incompatibility exsists in taro. To compensate, taro is usually reproduced by tuber or stolon.

As for the genetic control of the inflorescence characters, this inheritance is not so simple. The S_1 progeny of accession No.28 more or less differed from the latter (Table 11, Fig.29). Furthermore, the F_1 hybrids between accessions No.29 and No.19 showed wide variation, indicating possible involvement of polygenic system. It is reasonable because most adaptive characters of the plant are known to be quantitative characters under control of the polygenic system. Anthocyanin pigmentation in different plant parts showed segregation in the selfed progenies of types D and E, although type B (almost green type) appeared to be bred true. This fact suggests that most colored plants are heterozygous for the genes controlling this character. Because of this heterozygosity of the parents, it was not possible to test segregation ratios in different parental combinations against any specific theoretical ratio on a sound basis.

The result of cluster analysis based on five inflorescence characters are shown in Fig.15, in which the accession numbers of the strains used in the restriction endonuclease analysis are also given. Fig.30 and Fig.31 show the plants of some accessions which were used in the present investigation. *A. ordora* in Fig.30 bottom is shown for comparing with accession No.16 (*A. macrorrhiza* var. *variegata*). Accession No.3 in Fig.31 is a typical wild diploid from Nepal, classified as *C. esculenta* var. *aquatilis*. Fig.32 shows the inflorescence of accession No.26 (probably *Schismatoglottis* sp.) on top and that of *A. odora* on bottom. They show clear differences of their inflorescence components from those of *C. esculenta*. Other strains which were used in the restriction endonuclease analysis did not produce enough number of inflorescences necessary for the principal component analysis.

In the dendrogram of ctDNA (Fig.26), accessions No.17 and No.27 are located in one of the two clusters of the group C to which all other strains of *C. esculenta* belonged. The results from the inflorescence and those from ctDNA analysis coincide well. In the dendrogram of ctDNA, four accessions, No.2, No.5 (both wild *C. esculenta* from Nepal), No.15 (*C. gigantea*) and No.16 (*Alocasia macrorrhiza* var. *variegata*) are placed in group A, greatly differing from all other accessions. Three of them, mainly, accessions No.5, No.15 and No.16 are not included in the inflorescence analysis. Therefore, it is not clear whether these three accessions are located in the same cluster as accession No.2, when their inflorescences are studied. This point must be confirmed in a future experiment.

The group A of the ctDNA clusters raises a great challenge to the present classification of taro. As mentioned above, group A includes four accessions, i. e., two Nepalese accessions of *C. esculenta* (accessions No.2 and No.5), an accession of *C. gigantea* and an accession of *A. macrorrhiza*.

Accession No.2 is a diploid, showing abnormal meiosis, and complete pollen sterility. All attempts to pollinate it with other diploid *C. esculenta* accessions have been failed. From these facts accession No.2 might be assumed to have been originated as a hybrid between *Alocasia* (as a maternal parent) and *Colocasia*, thus having morphological similarity to *C. esculenta*, and ctDNA similarity to *A. macrorrhiza*. Its pollen sterility would be resulted from the nature of the intergeneric hybridity. As for accession No.5 that is triploid, it might have been produced from an unreduced gamete pollinated with normal *C. esculenta* pollen. Finaly accession No.15 that belong to *C. gigantea* is said to be diploid. The possibility of its hybrid origin seems to be large, because of its appearance is much more resemble to *Alocasia macrorrhiza*. than to *C. esculenta*. All these considerations favor to suppose that the three accessions, No2. and No.5 of *C.esculenta*, and No.15 of *C. gigantea* are hybrid origin, having an *A. macrorrhiza* as mother. On this point, another noteworthy report may support this hypothesis; Matthews (1990) compared mitochondrial (mt) DNA of three species, i. e. *C. esculenta*, *C. gigantea* and *Alocasia brisbanensis*, and similar *Bam*HI and *Hae*III fragments were detected for *C. gigantea* and *A. brisbanensis* but different from the fragments produced by *C. esculenta* with mtDNA probes from maize. Considering that ctDNA is said to have less variation than mtDNA, the present result that the same type of ctDNA exsists in

the three species will have considerable significance in the phylogeny of this family.

Accepting the above-mentioned hypothesis of their hybrid origin, an important question is left; the placenta of *Alocasia* is basal, but that of *Colocasia* is parietal. Plancentation is an important key character in the classic classification, in some cases, for example, a key character to distinguish genera *Remusatia* and *Gonatanthus* (Hotta, 1970). Then, a second hypothesis is proposed, that is, the parietal placenta is expressed epistatically over the basal placenta in the intergeneric hybrids between *Alocasia* and *Colocasia*. Furthermore, tha last unsolved question is whether their intercrosses will give viable hybrids or not. So far, no case of successful crosses between these two genera has been reported. The most important work left in future is to obtain the F1 hybrids between those genera, having *Alocasia* as mother, and to test the nature of placentation and other characters of taxonomical importance.

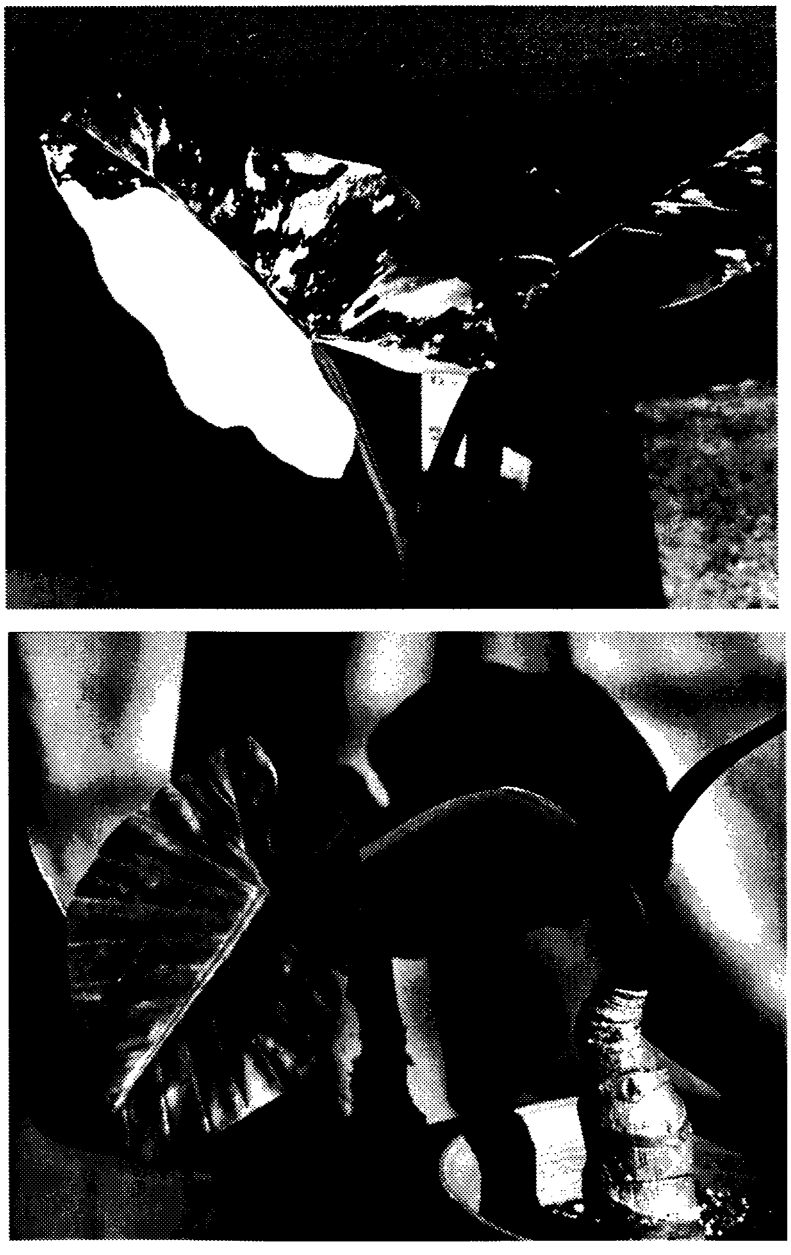


Fig. 30. Leaf, stalk and tuber of *Alocasia*. Top : *A. macrorrhiza* var. *variegata*, accession No.16 Bottom : *A. odora* (Roxb.) C. Koch.

2

5

15

17

3

26

Fig. 31. Six (accessions No.2, 3, 5, 15, 17 and 26) of the strains used in the restriction endonuclease analysis. Refer Table 3 for their informations.

a ; 26

b

Fig. 32. Inflorescence of an accession No.26 (*S. gamoandra* ?) (top) and that of *A. odora (bottom).*

8. Summary

The component of inflorescence is often described as a key character in the classification of family Araceae. The present work has aimed to examine the genetic stability, the differences and the phylogenetic significance of the inflorescence of *Colocasia esculenta* Schott and further to verify the usefulness of nuclear and chloroplast DNA in its phylogenetic study.

From the results obtained, the following conclusions are drawn:

1. The ratios of 4 inflorescence parts to the whole inflorescence length of *C. esculenta* are comparatively stable characters if they are normalized.
2. The ratios of the sterile parts, an appendage and a sterile part (staminode), to the fertile parts, a staminate and a pistillate, are useful indexes in the classification of *C. esculenta*.
3. The angles of the secondary veins to the midrib axis are useful parameter to indicate asymmetry of leaf shape.
4. From the leaf shape, 6 strains of *C. esculenta* have been divided into two clusters, diploid and triploid, but the usefulness of this character as a taxonomic key has not been claimed because of difficulty in its numeration.
5. From the total DNA fingerprinting using a synthetic oligonucleotide as a probe, *C. esculenta* showed extensive polymorphism making clustering of the accessions impossible.
6. The F_1s hybrids of two cross combinations have been proved to be true hybrids by DNA fingerprinting.
7. Two strains of *C. esculenta* and one strain of *C. gigantea* have been proved to have the same ctDNA as *Alocasia macrorrhiza* var. *variegata*. Their restriction fragment patterns are greatly different from those of other *C. esculenta* strains and 1 *Schismatoglottis* species.
8. Some *C. esculenta* strains have been proved to be self compatible. Cross pollination is successful 6 days before and 2 or 3 days after male flowering. Seeds can be alive and germinate after being stored in the desiccater at room temperature at shortest 1 year after seed harvest.
9. Most Strains are heterozygous for petiole color genes, segregating diffrent pigmentation types in the hybrid as well as selfed progenies. However, almost green individuals are found to be bred true.
10. Three *Colocasia* strains, two of *C. esculenta* and one of *C. gigantea*, are assumed to have been originated from intergeneric cross, *Alocasia* (mother) ×. *Clocasia*.

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