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Allozyme Evidence for Intersectional and Intergeneric Hybridization in the Genus *Sasa* and Its Related Genera (Poaceae; Bambusoideae)

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ササ属とその近縁属（イネ科タケ亜科）における節間および属間雑種形成 — アロザイムによる証拠

Abstract

To confirm the hypothesized hybrid origin of morphological intermediates in the genus *Sasa* and its related genera, 10 enzymes were assayed using polyacrylamide gel electrophoresis. Allozyme phenotypes support the hypotheses that species in "*Sasa cernua* group" are intersectional hybrids between species in *Sasa* sect. *Sasa* and *S. kurilensis* (sect. *Macrochlamys*); species in *Sasa* sect. *Lasioderma* are intergeneric hybrids between species in the genera *Sasa* and *Sasamorpha*; and species in the genus *Sasaella* are intergeneric hybrids between species in the genera *Sasa* and *Pleioblastus*. Allozyme phenotypes also suggest that the plants of "*S. cernua* group" and sect. *Lasioderma* are not only F_1 hybrids, but backcrosses or advanced generation segregates.

Key words: allozyme, hybrid origin, *Sasa cernua* group, *Sasa* sect. *Lasioderma*, *Sasaella*.

The taxonomy of the Japanese bamboos has been much confused. In addition to problems of species recognition, the classification of the species into sections and genera is still unreliable (Suzuki, 1978; Murata, 1989). Taxonomic confusions are partly due to the presence of the plants that show morphologically intermediate features between sections or even genera. Based on this morphological intermediacy and evidence from distributional patterns, several authors have suggested that such intermediates were derived through intersectional or intergeneric hybridization (Tatewaki, 1940; Maekawa, 1960; Maruyama *et al.*, 1979; Kobayashi, 1985). Furthermore, recent studies have shown that artificial hybrids can be readily produced between sections or even genera in Japanese Bamboos (Muramatsu, 1972a, b, 1991; Hatakeyama *et al.*, 1987; Nishiwaki, 1989).

Suzuki (1978) recognized six genera in the Japanese "Sasa group" (the genus *Sasa* and its related genera): *Sasa* Makino et Shibata, *Sasaella* Makino, *Sasamorpha* Nakai, *Pseudosasa* Makino *ex* Nakai, *Pleioblastus* Nakai and *Chimonobambusa* Makino. He recognized five sections in the genus *Sasa* (sects. *Sasa*, *Crassinodi* Nakai, *Monilicladae* Nakai, *Macrochlamys* Nakai and *Lasioderma* Nakai), and three sections in the genus *Pleioblastus* (sects. *Caespitosae* Koidz., *Medakea* Koidz. and *Nezasa* Koidz.). The species in the genus *Sasaella* have been considered to be of hybrid origin between species in the two genera *Sasa* and *Pleioblastus* (Maekawa, 1960; Suzuki, 1987; Watanabe *et al.*, 1991). In addition, the species of *Sasa* sect. *Lasioderma* have been postulated to be intergeneric hybrids between *Sasa* and *Sasamorpha* (Tatewaki, 1940; Suzuki, 1978). Kawabata and Ito (1983, 1992) recognized the plants "com-

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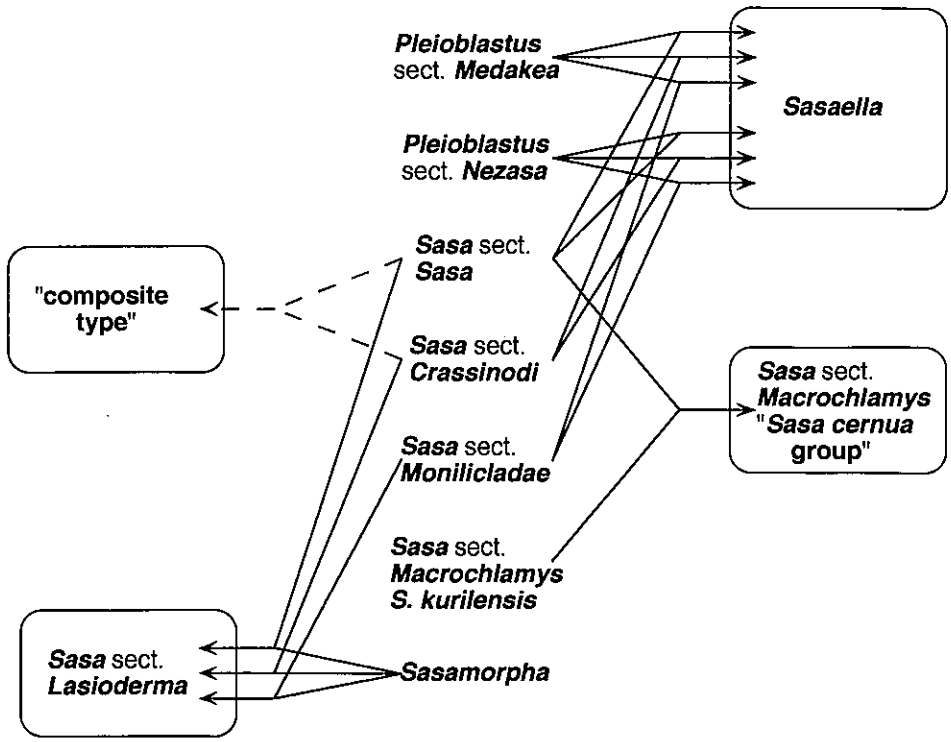


Fig. 1. The four putative hybrid taxa in the Japanese "Sasa group" investigated in the present study and their hypothesized parentages. The broken lines are hypothesized parentage failed to support by allozyme phenotypes but not rejected, and the solid lines allozymatically supported.

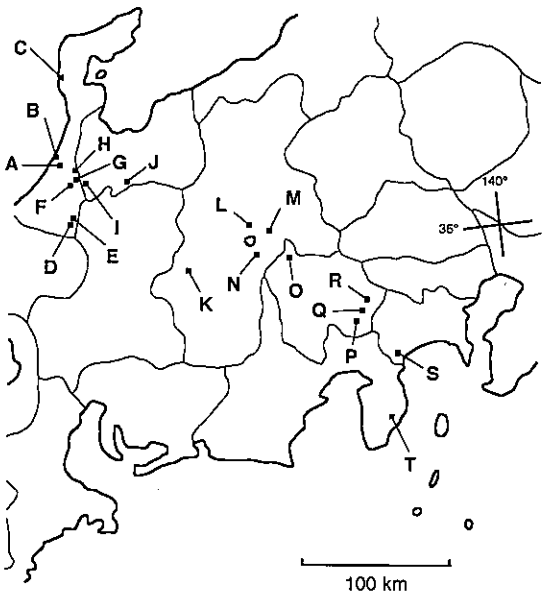


Fig. 2. Collection localities. A: Kanazawajôshi and Mt. Utatsuyama (alt. 30-140m), Kanazawa-shi, Ishikawa Pref.; B: Onebu and Nisiaraya (alt. 10-60m), Uchinada-cho, Isikawa Pref.; C: Fukuura (alt. 20m), Togi-cho, Isikawa Pref.; D: Chiburione (alt. 1400-2400m), Mt. Hakusan, Ishikawa Pref.; E: Midagahara (alt. 2340m), Mt. Hakusan, Ishikawa Pref.; F: Saigawa Dam (alt. 200-400m), Kanazawa-shi, Ishikawa Pref.; G: Yokotani (alt. 200-350m), Kanazawa-shi, Ishikawa Pref.; Mt. Iozen (alt. 400-900m), Kanazawa-shi, Ishikawa Pref.; I: Oyabe River (alt. 400-800m), Fukumitsu-cho, Toyama Pref.; J: Mt. Shirakimine (alt. 400-1400m), Yatsuo-cho, Toyama Pref.; K: Sachizawa River (alt. 800-1100m), Kisofukusima-cho, Nagano Pref.; L: Toyohashi (alt. 1000-1500m), Shimosuwa-cho, Nagano Pref.; M: Tateshina (alt. 1200-1870m), Chino-shi, Nagano Pref.; N: Nishichino (alt. 800-1400m), Chino-shi, Nagano Pref.; O: Mt. Tennyosan (alt. 1000-2000m), Oizumi-mura, Yamanashi Pref.; P: Mt. Hyakushidake (alt. 800-1300m), Fujiyoshida-shi, Yamanashi Pref.; Q: Kawadana, Mt. Shiroyama and Mt. Shirakiyama (alt. 400-700m), Tsuru-shi, Yamanashi Pref.; R: Mt. Kukisan (alt. 400-600m), Tsuru-shi, Yamanashi Pref.; S: Mt. Daikanyama (alt. 500-1000m), Yugawara-cho, Kanagawa Pref.; T: Mt. Amagisan (alt. 1000-1400m), Nakaizu-cho, Shizuoka Pref.

Table 1. Species used for allozyme electrophoresis

Species	Source (locality* and collection number**)
(Parental species)	
Sasa Makino et Shibata	
sect. *Sasa*	
S. palmata (Marl.) Nakai	A 10101-10110, B 10201, D 10401-10410, G 10701-10704, H 10801-10806
S. senanensis (Fr. et Sav.)Rehd.	A 20101, 20102, H 20801, 20802, K 21101-21107, L 21201-21204, M 21301-21306
S megalophylla Makino et Uchida	A 30101, 30102
S. veitchii (Carr.) Rehd. var. *hirsuta* (Koidz.) S. Suzuki	A 40101-40104, D 40401-40406
S. fugeshiensis Koidz.	A 50101, G 50701
sect. *Crassinodi* Nakai	
S. nipponica Makino et Shibata	M 61301, 61302, N 61401, 61402, O 61501-61506, Q 61701
S. chartacea Makino	N 71401
sect. *Monilicladae* Nakai	
S. tokugawana Makino	T 82001, 82002
S. hayatae Makino	Q 91701, S 91901, T 92001-92008
sect. *Macrochlamys* Nakai	
S. kurilensis (Rupr.)Makino et Shibata	D 100401-100414, F 100601-100606, G 100701-100703, H 100801-100810, I 100901-100906, J 101001-101003
Sasamorpha Nakai	
S. borealis (Hack.) Nakai	K 111101-111108, L 111201-111204, P 111601-111603 Q 111701, S 111901, T 112001
S. mollis Nakai	K 121101, 121102
Pleioblastus Nakai	
sect. *Medakea* Koidz.	
P. simonii (Carr.) Nakai	A 130101-130110, B 130201-130203
sect. *Nezasa* Koidz.	
P chino (Fr. et Sav.) Makino var. *chino*	Q 141701-141704, R 1418011
P. chino var. *viridis* (Makino) S. Suzuki	A 150101-150103, C 150301
P. chino var. *vaginatus* (Hack.) S. Suzuki	S 161901-161904
(Putative hybrids)	
Sasa	
"composite type" (sect. *Crassinodi* × sect. *Sasa)*	
S. nipponica × *S. senanensis*	M 171301, N 171401, O 171501-171503
"*Sasa cernua* group" (sect. *Macrochlamys*)	
S. cernua Makino	D 180401-180435, E 180501-180502, F 180601-180607, G 180701, H 180801-180807, I 180901, 180902, J 181001
S. suzukii Nakai	H 190801
sect. *Lasioderma* Nakai	
S. tsukubensis Nakai subsp. *tsukubensis*	L 201201-201212, M 201301, S 201901-201906
S. shimidzuana Makino subsp. *shimidzuana*	L 211201, 211202
S. shimidzuana subsp. *kashidensis* (Makino et Koidz.) S. Suzuki	Q 221701-221706
Sasaella Makino	
S. masamuneana (Makino) Hatus. et Muroi	A 230101-230107, B 230201, 230202
S. sawadae (Makino) Makino ex Koidz.	B 240201, Q 241701-241704, R 241801, S 241901-241909

*The alphabetical codes of localities correspond with those in Fig. 2.
**Collection number of K. Takahashi.

posite type", morphological intermediates between sects. *Crassinodi* and *Sasa*, which were regarded by Nishiwaki (1989) as intersectional hybrids. Kawabata and Ito (1992) also pointed out that *Sasa cernua* Makino, which is considered to be species in sect. *Macrochlamys*, is rather intermediate between sects. *Macrochlamys* and *Sasa* in the bud performance. The above cases of morphological intermediacy are illustrated with their hypothesized parents in Fig. 1.

In order to confirm the hypothesized reticulate relationships among species in the "Sasa group", we adopted allozymes as taxon-specific markers proven to be useful for the investigation of hybrid complex (Gallez and Gottlieb, 1982; Soltis and Soltis, 1986; Kato, 1987; Crawford, 1990).

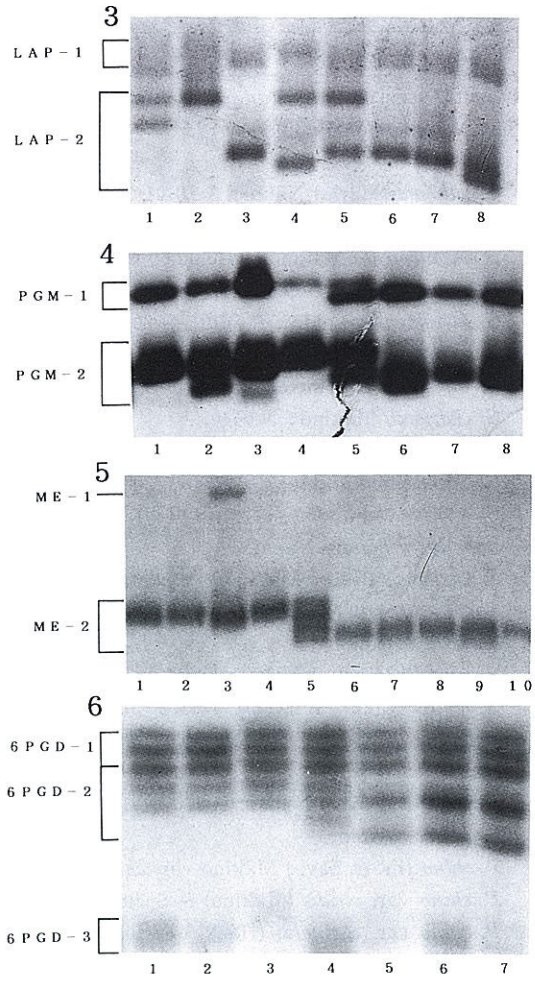
Materials and Methods

Plant materials

Culms with newly expanded leaves were collected from April to November in 1991 and 1992 from 20 localities in Central Japan given in Fig. 2. Since all plants are rhizomatous and capable of forming large clones, culms were collected at more than 100m intervals in any case. Allozymes were assayed for the species in following four putative hybrid taxa: 1) "composite type" (morphological intermediates between *Sasa* sects. *Crassinodi* and *Sasa*), 2) "*Sasa cernua* group" (species in *Sasa* sect. *Macrochlamys* except *S. kurilensis* (Rupr.) Makino et Shibata), 3) *Sasa* sect. *Lasioderma* and 4) *Sasaella*; and for the species in their hypothesized parental taxa: *Sasa* (sects. *Sasa, Crassinodi, Monilicladae* and *Macrochlamys* (*Sasa kurilensis*)), *Sasamorpha* and *Pleioblastus* (sects. *Medakea* and *Nezasa*). In addition, *Pseudosasa japonica* (Sieb. et Zucc.) Makino, occurred sympatrically with the plants under consideration, was also investigated. Species studied are shown in Table 1. The plants were identified by K. Takahashi based on Suzuki (1978). The voucher specimens are deposited in the herbarium of Kanazawa University (KANA).

Allozyme electrophoresis

Leaf blades of newly expanded leaves were used for electrophoresis. The following ten enzymes were assayed: aspartate aminotransferase (AAT), alcohol dehydrogenase (ADH), esterase (EST), gultamate dehydrogenase (GDH),



Figs. 3-6. Examples of zymograms. 3. LAP of *Sasa* sect. *Sasa* (lanes 1-2), *Sasa cernua* (lanes 3-5) and *Sasa kurilensis* (lanes 6-8). 4. PGM of *Sasa* sect. *Sasa* (lanes 6-8), *Sasa cernua* (lane 5) and *Sasa kurilensis* (lanes 1-4). 5. ME of *Sasa* sect. *Sasa* (lanes 1-3), sect. *Crassinodi* (lane 4), sect. *Lasioderma* (lane 5) and *Sasamorpha* (lanes 6-10). 6. 6PGD of *Sasa* sect. *Sasa* (lanes 1-2), sect. *Crassinodi* (lane 3), sect. *Lasioderma* (lane 4) and *Sasamorpha* (lanes 5-7).

leucine aminopeptidase (LAP), malic enzyme (ME), 6-phosphogluconate dehydrogenase (6PGD), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), triosephosphate isomerase (TPI). For enzyme extraction, 250 mg of leaf was cut into small pieces and then ground in 1.0 ml of ice-cold extraction buffer with a small quantity of quartz sand powder. Extraction buffer was 0.1M tris-HCl pH 7.5, 5% PVP, 0.5% sodium

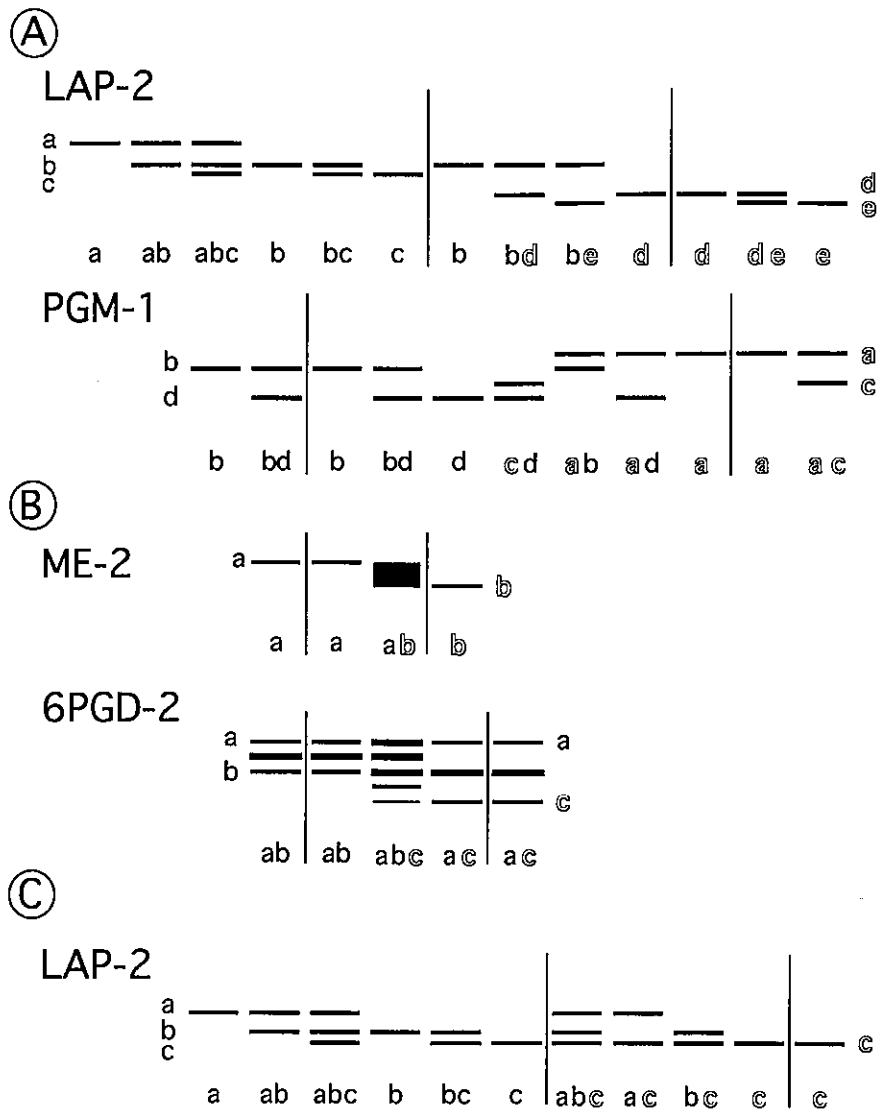


Fig. 7. Schematic illustrations of electrophoretic phenotypes in hybrid complex of "Sasa group". Phenotypes expressed by putative hybrids are shown in the middle, hypothesized parents in either side. (A) Phenotypes for LAP-2 (monomer) and PGM-1 (monomer). The left: *Sasa* sect. *Sasa*; the middle: "*Sasa cernua* group"; the right: *Sasa kurilensis*. (B) Phenotypes for ME-2 (tetramer) and 6PGD-2 (dimer). The left: *Sasa* sects. *Sasa, Crassinodi* and *Monilicladae*; the middle: *Sasa* sect. *Lasioderma*; the right: *Sasamorpha*. (C) Phenotypes for LAP-2 (monomer). The left: *Sasa* sects. *Sasa, Crassinodi* and *Monilicladae*; the middle: *Sasaella*; the right: *Pleioblastus* sects. *Medakea* and *Nezasa*.

metabisulfate, 0.5% sodium ascorbate, 10 mM magnesium chloride, 1 mM EDTA (tetrasodium salt), 0.5% 2-mercaptoethanol (Watano, 1988). The resulting slurry was centrifuged, and the supernatant was condensed up to two-fold through dry powder of Sephadex G-25 following the method of Kato (1987). The condensed extraction was applied on thin-layer horizontal polyacrylamide gel (0.1 × 9 × 17 cm; T=5%, C=5%. But T=7% for PGM). Three kinds of gel and electrode buffer systems were used: (system 1) 100 mM tris, 23.7 mM citric acid, 52.6 mM boric acid, 7.80 mM lithium hydroxide as gel buffer, 263 mM boric acid, 39.0 mM lithium hydroxide as electrode buffer (a modification of system 7 of Soltis *et al.* (1983) by Watano (1988)); (system 2)

Table 2. Summary of allozymes detected in parental taxa and putative hybrids in "Sasa group"

Taxa*	AAT-2	AAT-3	ADH-3	EST-3	EST-4	GDH
Sasa						
Sasa	b, *n2***	b	b, *c*	*c*,*d*,*e*,*g*,*h*,*i*	*a*,*d*,*f*,*g*,*h*	b,*c*
	(60)***	(60)	(15)	(42)	(42)	(39)
Crassinodi	*a*,b,*n2*,*d*	b	b	*c*,*d*,*e*,*g*,*h*	*a*,*b*,*d*,*f*,*g*,*h*	b,*c*
	(12)	(12)	(2)	(12)	(12)	(12)
Monilicladae	*a*,b,*d*	b	b,*c*	*c*,*d*,*g*,*h*	*a*,*d*,*f*,*g*,*h*	b,*c*
	(12)	(12)	(12)	(12)	(12)	(12)
Macrochlamys *S. kurilensis*	*n1*,b,*d*	a,b	b	*c*,d,*e*	*a*,e	b
	(29)	(29)	(5)	(19)	(19)	(19)
Sasamorpha	*b*,*c*	b	*a*,*b*	*a*,*b*,*c*	*b*,*c*,*d*,*f*,*g*	*a*,b
	(15)	(15)	(13)	(18)	(18)	(13)
Pleioblastus						
Medakea	b,*n2*,*n3*	b	b	f,*h*	*d*,h	b,*c*
	(13)	(13)	(5)	(8)	(8)	(11)
Nezasa	b,*n2*,*n3*	b	b,*c*	*c*,*e*,f,*e*,*h*	*d*,*f*,*g*,*h*	b,*c*
	(13)	(13)	(9)	(13)	(13)	(13)
Pseudosasa	b	b	a,b	b,f	d.f	b,c
	(12)	(12)	(10)	(12)	(12)	(12)
Sasa						
"composite type"	*a*,b,*n2*	b	b	*d*,g,*h*	a,*d*,*f*,*g*,*h*	b,*c*
	(5)	(5)	(2)	(5)	(5)	(5)
"*S. cernua* group"	b,*n2*	*a*,b	b	*c*,*d*,*e*,*g*,*h*,*i*	*a*,*d*,*e*,*g*,*h*	b,*c*
	(45)	(45)	(1)	(38)	(38)	(36)
Lasioderma	b,*c*,*n2*	b	*a*,b	*a*,*b*,*c*,*d*,*g*,*h*	*a*,*b*,*c*,*d*,*f*,*g*,*h*	*a*,b,*c*
	(13)	(13)	(18)	(28)	(28)	(29)
Sasaella	b,*n2*,*n3*	b	b	*c*,*d*,*e*,*f*,*g*,*h*	*a*,*d*,*f*,*g*,*h*	b,*c*
	(24)	(24)	(13)	(20)	(20)	(23)

*Species examined in each section are referable to Table 1.
**Allozymes in italic type were present but not fixed, others were consistently present.
***Sample sizes.

40.0 mM Tris, 12.8 mM citric acid as gel buffer, electrode buffer was the same as system 1; (system 3) 45.0 mM tris, 7.0 mM citric acid, 4.0 mM lithium hydroxide, 19.0 mM boric acid as gel buffer, 38.0 mM lithium hydroxide, 188.0 mM boric acid as electrode buffer (system 7 of Soltis *et al.* (1983)). AAT, ADH, EST, LAP, 6PGD, PGI and TPI were resolved on system 1. System 2 was used for PGM, and system 3 for GDH and ME. In the case of ME and 6PGD, NADP was added to gel and cathodal electrode buffers at the concentration of 0.05 mM. Electrophoresis was performed at 300 V for 4 hours (system 1 and 2), and at 180 V for 4 hours (system 3). Staining of enzymes was conducted following the method of Soltis *et al.* (1983).

Genetic interpretations of banding patterns were made by the known subunit structure of the enzymes and the number of isozymes typical for each enzyme in diploid species (Gottlieb, 1982). When more than one enzymatic activities were observed for an enzyme, the most anodally migrating zone was designated as zone 1, the next 2, and so on. Allozymes at individual zone were coded alphabetically with the most anodally migrating one designated "a", but inactive ones (null allozymes) were designated "n1", the next "n2". Since species in "Sasa group" are known to be tetraploid with 2n=48 chromosomes (Yamaura, 1933; Uchikawa, 1933, 1935; Tateoka, 1954, 1955; Namikawa and Imakita, 1992), two loci are expected to concern with individual zone as a result of gene duplication caused by polyploidization.

Pollen stainability

Pollen stainability was examined for the

Table 2. Continued

Taxa	LAP-2	ME-2	6PGD-1	6PGD-2	PGI-2	PGM-1	PGM-2	TPI-1
Sasa								
Sasa	*a*,*b*,*c*	a	a,b	a,b	*a*,*c*,*e*,f	b,*d*	b,*c*	a,b
	(64)	(25)	(66)	(66)	(43)	(62)	(66)	(38)
Crassinodi	*a*,*b*	a	a,b	a,b	c,f,*g*	b	b,*c*	a,b
	(11)	(7)	(8)	(8)	(6)	(12)	(12)	(8)
Monilicladae	*a*,*b*	a	a,b	a,b	*a*,*c*,*e*,f	*b*,*d*	b	a,b
	(12)	(12)	(12)	(12)	(10)	(12)	(12)	(12)
Macrochlamys *S. kurilensis*	*d*,*e*	a	a,b	a	c,f	a,*c*	*a*,*b*	a,b
	(40)	(5)	(35)	(35)	(30)	(40)	(40)	(22)
Sasamorpha	*b*,*c*	b	*a*,b,*c*	a,c	c,f	b	*a*,*b*,*c*	a,b
	(11)	(20)	(18)	(18)	(5)	(8)	(14)	(11)
Pleioblastus								
Medakea	c	a	a	a,b	c,*e*,f	b	b,*c*	a,*b*
	(11)	(11)	(11)	(11)	(5)	(11)	(11)	(11)
Nezasa	c	a	a,*b*	a,b	c,*e*,f	b	b,*c*	a,*b*
	(13)	(13)	(13)	(13)	(8)	(13)	(13)	(13)
Pseudosasa	b,c	a,b	a,b,c	a,b,c	b,c,d,f	b	a,c	a,b
	(12)	(12)	(12)	(12)	(7)	(12)	(12)	(12)
Sasa								
"composite type"	*a*,b	a	a,b	a,b	*a*,c,f	b	b,*c*	a,b
	(5)	(5)	(5)	(5)	(5)	(5)	(5)	(5)
"S. cernua group"	*b*,*d*,*e*	a	a,b	a,b	*a*,*c*,f	*a*,*b*,*c*,*d*	*a*,b	a,b
	(54)	(11)	(43)	(43)	(39)	(49)	(34)	(25)
Lasioderma	*a*,b	a,*b*	a,b,*c*	a,*b*,*c*	c,*e*,f	b	*a*,*b*,*c*	a,b
	(13)	(22)	(28)	(28)	(5)	(17)	(26)	(9)
Sasaella	*a*,*b*,c	a	a,*b*	a,b	*a*,*c*,*e*,f	b	b	a.b
	(23)	(23)	(23)	(23)	(13)	(23)	(12)	(23)

species in three putative hybrid taxa : "composite type", "*Sasa cernua* group" and *Sasaella*. Pollen stainability was also examined for the species in three of their hypothesized parental taxa (*Sasa* sects. *Sasa* and *Macrochlamys* (*S. kurilensis*) and *Pleioblastus* sect. *Medakea*) and *Pseudosasa japonica*. Pollen grains from mature anthers were stained with aniline blue-lactophenol solution. Pollen stainability was calculated as the percentage of stained, normalshaped pollen grains per 300 grains.

Results

Allozyme electrophoresis

Fourteen zones for 10 enzymes were analyzed: AAT-2, AAT-3, ADH-3, EST-3, EST-4, GDH, LAP-2, ME-2, 6PGD-1, 6PGD-2, PGI-2, PGM-1, PGM-2 and TPI-1. Although additional zones were observed (AAT-1, ADH-1, ADH-2, EST-1, EST-2, LAP-1, ME-1, 6PGD-3 and PGI-1), they were not well resolved or their enzymatic activities were varied according to seasons, and therefore excluded from the analysis. Allozymes detected in each taxon are summarized in Table 2.

1) "Composite type" : The species in *Sasa* sects. *Crassinodi, Monilicladae* and *Sasa* shared most allozymes in common (Table 2), and therefore the hypothesized parents could not be distinguished from each other by electrophoretic phenotypes.

2) "*Sasa cernua* group" : The species in sects. *Sasa* and *Sasa kurilensis*, hypothesized parents of "*Sasa cernua* group", were distinguished from each other by electrophoretic phenotypes of LAP-2 and PGM-1. Most plants of "*Sasa cernua* group" expressed additive banding patternes of the parents (Figs. 3, 4, 7A and Table 3). Since LAP and PGM are monomeric, alleles can be assumed for

Table 3. Electrophoretic expressions of LAP-2 and PGM-1 in *Sasa* sect. *Sasa* "*Sasa cernua* group" (sect. *Macrochlamys*) and *Sasa kurilensis* (sect. *Macrochlamys*)

	sect. *Sasa*	"*S. cernua* group"	*S. kurilensis*
LAP-2			
phenotype a	2	0	0
ab	3	0	0
abc	1	0	0
b	50	18	0
bc	5	0	0
c	3	0	0
bd	0	23	0
be	0	8	0
d	0	5	27
de	0	0	12
e	0	0	1
Total	64	54	40
PGM-1			
phenotype b	52	10	0
bd	10	3	0
d	0	2	0
cd	0	1	0
ab	0	28	0
ad	0	2	0
a	0	3	35
ac	0	0	5
Total	62	49	40

every one of the bands observed. For LAP-2, species in sect. *Sasa* expressed electrophoretic phenotypes composed of allozymes "a", "b" and "c", whereas *S. kurilensis* allozymes "d" and "e". Phenotypes "bd" and "be" were observed in "*Sasa cernua* group". For PGM-1, species in sect. *Sasa* expressed phenotypes composed of allozymes "b" and "d", whereas *S. kurilensis* allozymes "a" and "c". Phenotypes "ab", "ad" and "cd" were observed in "*Sasa cernua* group". The combinations of LAP-2 and PGM-1 phenotypes observed in "*Sasa cernua* group" are shown in Table 4. In addition to plants that expressed additive banding patterns for both zones (for example, phenotype "ab" for PGM-1 and phenotype "bd" for LAP-2), plants that expressed additive patterns for one of the two zones but were like species in sect. *Sasa* or *S. kurilensis* for the other zone were observed (for example, phenotype "ab" for PGM-1 and phenotype "b" for LAP-2).

Table 4. Observed number of individuals for each combination of LAP-2 and PGM-1 phenotypes in "*Sasa cernua* group"

		PGM-1						
		b	bd	d	cd	ab	ad	a
LAP-2	b	4	2	0	1	8	1	1
	bd	6	1	1	0	11	1	0
	be	0	0	1	0	6	0	1
	d	0	0	0	0	2	0	0

Phenotypes enclosed by broken line are additive banding patterns of species in sect. *Sasa* and *S. kurilensis* for both zones.

3) *Sasa* sect. *Lasioderma*: The species in *Sasa* (sects. *Sasa, Crassinodi* and *Monilicladae*) and *Sasamorpha*, hypothesized parents of *Sasa* sect. *Lasioderma*, were distinguished from each other by electrophoretic phenotypes of ME-2 and 6PGD-2. Most plants of sect. *Lasioderma* expressed additive banding patterns of the parents (Figs. 5, 6, Fig. 7B and Table 5). For ME-2, three kinds of phenotypes were observed. Since ME is tetrameric, heterozygotes can be distinguished by a five-

Table 5. Electrophoretic expressions of ME-2 and 6PGD-2 in *Sasa* (sects. *Sasa, Crassinodi* and *Monilicladae*), *Sasa* sect. *Lasioderma* and *Sasamorpha*

	sects. *Sasa, Crassinodi, Monilicladae*	sect. *Lasioderma*	*Sasamorpha*
ME-2			
phenotype a	44	4	0
ab	0	18	0
b	0	0	20
Total	44	22	20
6PGD-2			
phenotype ab	86	15	0
abc	0	12	0
ac	0	1	18
Total	86	28	18

Table 6. Observed number of individuals for each combination of ME-2 and 6PGD-2 phenotypes in *Sasa* sect. *Lasioderma*

		6PGD-2		
		ab	abc	ac
ME-2	a	2	2	0
	ab	9	8	1
	b	0	0	0

Phenotype enclosed by broken line is additive banding patterns of parental *Sasa* species and *Sasamorpha* for both zones.

Table 7. Electrophoretic expressions of LAP-2 in *Sasa* (sects. *Sasa, Crassinodi* and *Monilicladae*), *Sasaella* and *Pleioblastus*

	Sasa	*Sasaella*	*Pleioblastus*
LAP-2			
phenotype a	17	0	0
ab	5	0	0
abc	1	5	0
b	56	0	0
bc	5	13	0
ac	0	1	0
c	3	4	23
Total	87	23	23

banded phenotype. Although separation of bands is not clear, phenotype "ab" is considered to be heterozygous. All plants of parental *Sasa* species expressed phenotype "a", whereas all plants of *Sasamorpha* phenotype "b". Phenotype "ab" was observed in sect. *Lasioderma*. Since 6PGD is dimeric, a three-banded phenotype is expected for heterozygotes. For 6PGD-2, all plants of parental *Sasa* species expressed phenotype "ab", while all plants of *Sasamorpha* phenotype "ac". Phenotype "abc" was observed in sect. *Lasioderma*. The combinations of ME-2 and 6PGD-2 phenotypes observed in sect. *Lasioderma* are shown in Table 6. Plants that expressed additive banding pattern at only one of the two zones were also present in this case (for example, phenotype "ab" for ME-2 and phenotype "ab" for 6PGD-2).

4) *Sasaella*

The species in *Sasa* (sects. *Sasa, Crassinodi* and *Monilicladae*) and *Pleioblastus*, hypothesized parents of *Sasaella*, were nearly distinguished from each other by electrophoretic phenotypes of LAP-2. Most plants of *Sasaella* expressed additive banding patterns (Fig. 7C and Table 7). But allozyme "c", characteristic of *Pleioblastus*, was found rarely in *Sasa*, and thus the two parental taxa did not exhibit mutually exclusive allozymes in this case.

In each of the four cases, all allozymes observed in the putative hybrid taxa were present in both or one of their hypothesized parents, and therefore allozymes unique to putative hybrid taxa were not detected (Table 2). Allozymes characteristic of parental taxa were also detected in their putative hybrid taxa (Table 2).

Pollen stainability

The results of examinations of pollen stainability are shown in Table 8. All parental species exhibited high pollen stainabilities (more than 80.0%), exept *Pseudosasa japonica*. Reduced pollen stainabilities were observed in species of hybrid taxa, *Sasa cernua* and *Sasaella masa-*

Table 8. Pollen stainabilities of parental species and putative hybrids in "Sasa group"

Species	Locality*	N**	% Pollen stainability (S.D.)
Parental species			
Sasa megalophylla	A	5	92.4 (1.8)
S. palmata	A	5	96.2 (1.0)
	H	5	84.7 (2.2)
S. senanensis	H	5	93.3 (3.9)
S. kurilensis	D	5	87.7 (1.9)
	F	5	91.5 (2.5)
	H	5	95.3 (0.9)
Pleioblastus simonii	A	4	91.4 (3.1)
Pseudosasa japonica	B	6	41.1 (6.2)
Putative hybrids			
Sasa nipponica×*S. senanensis*	O	5	95.8 (1.7)
S. cernua	D	5	15.9 (3.3)
	D	5	24.2 (2.0)
	E	5	86.1 (3.4)
	E	5	90.2 (4.5)
	F	5	84.7 (5.1)
	F	5	41.3 (2.5)
	F	5	28.6 (2.6)
	F	5	17.5 (2.9)
Sasaella masamuneana	A	3	44.9 (3.7)
	A	5	62.0 (5.7)

*The alphabetical codes of localities correspond with those in Fig. 2.
**number of anthers examined.

muneana, although *Sasa nipponica* × *S. senanensis* and three plants of *S. cernua* exhibited high stainabilities.

Discussion

The hypothesized parental taxa of hybrid complex in the Japanese "Sasa group" were distinguished by allozyme phenotypes except among *Sasa* sects. *Sasa, Crassinodi* and *Monilicladae*. The plants of "*Sasa cernua* group", *Sasa* sect. *Lasioderma* and *Sasaella* expressed additive banding patterns of hypothesized parental taxa, and have no unique allozymes. The results indicate that the species in "*Sasa cernua* group" (sect. *Macrochlamys*) are intersectional hybrids between species in sect. *Sasa* and *S. kurilensis* (sect. *Macrochlamys*); the species in *Sasa* sect. *Lasioderma* are intergeneric hybrids between species in the two genera *Sasa* (sects. *Sasa, Crassinodi* and *Monilicladae*) and *Sasamorpha*; and the species in the genus *Sasaella* are intergeneric hybrids between species in the two genera *Sasa* (sects. *Sasa, Crassinodi* and *Monilicladae*) and *Pleioblastus*. Thus the hypotheses suggested by Tatewaki (1940) and Maekawa (1960) were supported allozymatically. Since marker allozymes for sect. *Macrochlamys* were not detected in sect. *Lasioderma* and *Sasaella*, parental species in *Sasa* for these hybrids may not include the species in sect. *Macrochlamys*. Similarly, allozymic data eliminated the possibilities that all taxa other than hypothesized parents were concerned with hybridization, as far as the plants examined in the present study. This is because taxa other than hypothesized parents possessed unique allozymes which were not detected in putative hybrids and lacked allozymes present in putative hybrids. Although parental taxa were not characterized allozymatically, proposed hybrid origin of "Composite type" was not rejected.

Reduced pollen stainabilities observed in *Sasa cernua* and *Sasaella masamuneana* also indicate the hybrid nature of these two species.

Grant and Grant (1971) reported an example of "clonal microspecies": vegetative multiplication of interspecific hybrids in the Cholla Cacti in south-central Arizona (*Opuntia fulgida* × *O. spinosior*). Furthermore, Grant (1981) referred to

the case that two or more vagetatively reproducing hybrid species arize from three or more original parental species as "clonal complex". The hybrid complex in the Japanese "Sasa group", reticulate relationships of which were confirmed electrophoretically in the present study, is considered to be an instance of "clonal complex".

In the case of "*Sasa cernua* group" and sect. *Lasioderma*, hypothesized parents were characterized by two isozyme zones. If all plants of "*Sasa cernua* group" and sect. *Lasioderma* were F_1 hybrids, the expected allozyme phenotypes would probably be restricted to additive patterns at both zones. However, the observed phenotypes include a considerable number of plants expressed additive patterns at only one of the two zones. Thus, it is suggested that the species in "*S. cernua* group" and sect. *Lasioderma* consist of not only simple F_1 hybrids, but also the plants that have undergone some genetic recombinations. Although pollen stainabilities of a part of *S. cernua* were reduced, considerably low value close to 0% was not observed and some plants exhibited high pollen stainabilities. Therefore it is suggested that *S. cernua* is not completely sterile and can yield progeny.

The present study provides electrophoretic evidence for intersectional and intergeneric hybridization in the Japanese "Sasa group". As stated by Nishiwaki (1989), it is likely that the complicated morphological variations via hybridization have been caused for taxonomic confusions and difficulties in identification of this group.

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摘　要

タケ・ササ類には，形態的に節や属のあいだの中間性を示す植物の存在が知られている。それらの中間型の分類学的取扱いは研究者のあいだで一致をみていないが，何人かの研究者はそれらが雑種起源である可能性を指摘している。本研究では，そのような中間型が雑種起源であるという仮説を検証することを目的として，本州中部で採集した材料を用いてポリアクリルアミドゲル電気泳動法により10酵素種についてアロザイムの解析を行った。今回とりあげたのは，以下に示す4つの中間型とその推定両親である。

(1) ササ属ミヤコザサ節植物とチマキザサ節植物の中間型
(2) ササ属チマキザサ節植物とチシマザサ節チシマザサの中間型である，チシマザサ節オクヤマザサ類
(3) ササ属植物とスズダケ属植物の中間型である，ササ属ナンブスズ節植物
(4) ササ属植物とメダケ属植物の中間型である，アズマザサ属植物

その結果，(1)では推定両親を特徴づけるアロザイムが存在しなかったが，(2)ではLAPとPGMにおいて，(3)ではMEと6PGDにおいて，(4)ではLAPにおいて，推定両親が相互に異なったアロザイムをもつ酵素活性のゾーンが存在し，中間型の多くは雑種において期待される両親のマーカー・アロザイムをあわせもつ表現型を示した。また，他のいずれのゾーンにおいても，中間型のみに固有なアロザイムは検出されなかった。さらに，(2)および(3)において，一方のゾーンで両親のマーカーをあわせもつにもかかわらず，もう一方のゾーンでは片親のマーカーのみからなる表現型を示す個体が観察された。以上の結果は，オクヤマザサ類がチマキザサ節植物とチシマザサのあいだの雑種起源であること，ナンブスズ節植物がササ属植物とスズダケ属植物のあいだの雑種起源であること，アズマザサ属植物がササ属植物とメダケ属植物のあいだの雑種起源であること，を支持するとともに，少なくともオクヤマザサ類とナンブスズ節植物には単純なF_1雑種以外の雑種起源の個体が存在していることを示唆している。

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