Allozyme Differentiation in Arisaema (Araceae): (1) Sections Clavata and Tortuosa, with Special Reference to the Systematic Position of A. negishii

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Jin Murata* and Takayuki Kawahara** : Allozyme Differentiation in *Arisaema* (Araceae) (1) Sections *Clavata* and *Tortuosa*, with Special Reference to the Systematic Position of *A. negishii*

邑田　仁*・河原孝行**：テンナンショウ属のアロザイム分化（1）アマミテンナンショウ節とマイヅルテンナンショウ節における分化およびシマテンナンショウの所属

Abstract

To provide genetic background for the systematics of *Arisaema*, allozyme differentiation in *Arisaema* was intended to study. In this study, five species of sects. *Clavata* and *Tortuosa* were examined by electrophoresis with 12 enzyme systems. Among the species examined, genetic distance is smallest between *A. negishii* and *A. heterocephalum* (D=0.35), which is a little smaller than the mean value between congeneric species in the flowering plants. This result supports placement of *A. negishii* and *A. heterocephalum* in the same section. In sects. *Clavata* and *Tortuosa*, species are well differentiated genetically as well as morphologically.

Key words: allozyme, *Arisaema*, systematics.

The genus *Arisaema* comprises about 150 species and occurs mainly in warm to temperate regions in most of Asia, and disjunctively in northeastern Africa, Saudi Arabia and Oman, and eastern North America southward to Mexico. According to the most recent taxonomic system of *Arisaema* proposed by Murata (1984) and emended by Murata (1991), three sections are recognized in Japan on the basis of morphological and cytological studies ; i.e. *Pedatisecta* Schott ex Engler, *Clavata* Engler, and *Tortuosa* Engler which is further subdivided into two subsections *Tortuosa* and *Flagellarisaema* (Nakai) Ohashi et J. Murata. The species of section *Pedatisecta* are generally similar to each other and there has been repeated argument about their taxonomic treatment. In contrast, the species of the latter two sections are morphologically well differentiated and easy to discriminate, but include several species whose systematic positions are still uncertain. *Arisaema negishii* which was included in sect. *Tortuosa* in Hara (1971) and Ohashi and Murata (1980), then moved to sect. *Clavata* in Murata (1984), is one of the species that needs reconsideration for the systematic position. Because this species possesses characteristics not only of the sect. *Clavata* (i.e. sterile flowers above fertile flowers and accessory buds) but also of the sect. *Tortuosa* (i.e. elongate and slender spadix appendage). This study aims to examine allozyme differentiation between the species of sections *Tortuosa* and *Clavata*, as examples of morphologically well differentiated species of the genus. Systematic position of *A. negishii* is commented based on the data.

Materials and Methods

Three species of sect. *Tortuosa* (*A. thunbergii* Blume, *A. tortuosum* Schott and *A. heterophyllum* Blume) and two species of sect. *Clavata* (*A. negishii* Makino and *A. heterocephalum* Koidzumi) were examined. Plants from nine natural populations (Table 1) were used for electrophoretic analysis. Plants of *A. tortuosum* and *A. hetero-*

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Table 1. A list of populations examined

Population name	Sample number	Locality (voucher specimen)
A. thunbergii ssp. *thunbergii*	30	Hiroshima Pref., Miyajima Is. (Ohno, Apr. 20, 1990)
A. thunbergii ssp. *urashima*(A)	30	Kyoto Pref., Yawata-shi (Murata, Apr. 15, 1990)
A. thunbergii ssp. *urashima*(B)	33	Tokyo Pref., Botanical Gardens, University of Tokyo (Murata, Apr. 16, 1990)
A. thunbergii ssp. *urashima*(C)	38	Chiba Pref., Awa-gun, Maruyama-machi (Murata, Apr. 20, 1990)
A. tortuosum	25	Nepal (Murata, 12303, 12304)
A. heterophyllum	26	Taiwan, Taipei Co., Jilung (Murata, 27016)
A. negishii	44	Tokyo Pref., Hachijo Is., Mt. Miharayama (Murata, Mar. 12, 1991)
A. heterocephalum ssp. *heterocephalum*	31	Kagoshima Pref., Amami-oshima, Mt. Yuwandake (Tabata, Apr. 20, 1990)
A. heterocephalum ssp. *okinawaense*	20	Okinawa Pref., Okinawa-shima, Mt. Awadake (Nakajima, Apr. 22, 1990)

phyllum were grown from seeds that were sampled at random from a mixture of the seeds from different three infructescences. Other materials were sampled directly from wild populations. Chromosome numbers of the sample populations were previously determined as diploid (2n=28). Voucher specimens are preserved in the Herbarium, University of Tokyo (TI).

Horizontal starch gel electrophoresis was conducted with 12 different enzyme systems; alcohol dehydrogenase (ADH), aldolase (ALD), fluorescent esterase (FE), glutamate dehydrogenase (GDH), glucose-3-phosphate isomerase (G3P), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), shikimate dehydrogenase (SKD), superoxide dismutase (SOD), triose phosphate isomerase (TPI). The extraction buffer was 0.1 M tris HCl, pH 7.5 (50ml) containing KCl (40mg), $MgCl_2$ (100mg), EDTA (tetrasodium salt), triton x 100 (0.1%v/v) and 2-mercaptoethanol (0.3%v/v) (Odrzykoski & Gottlieb, 1984). Two buffer systems were employed for starch gels. ADH, ALD, FE, GDH, LAP, PGI, PGM, SOD, TPI were resolved using a tris citrate gel buffer system (0.042 M tris, 0.007 M citric acid, 0.004M LiOH, 0.025M bolic acid, pH7.6) and an electrode buffer consisting of lithium-borate (0.039M LiOH, 0.263M boric acid) (Soltis *et al.*, 1983). G3P, MDH and SKD were resolved in a system with a gel buffer of 1:3 dilution of the following electrode buffer and an electrode buffer of 0.065M L-histidine (free base) and 0.007M citric acid (pH 6.5) (Cardy *et al.*, 1981). A starch gel was prepared in an acryl frame (200 x 200 x 12 mm).

Small pieces (about 100mg) of young leaves were crushed in cold extraction buffer (500μl) and PVP (20mg) using a ceramic rod and a small mortar. The extracts were centrifuged and the supernatant was soaked onto filter paper wicks for insertion into 11 % starch gels. Electrophoresis was conducted at 5°C at 100 volts for 15 minutes, then the wicks were removed. The electrophoresis was continued under constant current (at 35mA for tris citrate system and 30 mA for the L-histidine system) for 12 to 15 hours. Gels for PGM were soaked in 1.0M tris HCl (pH 8.0) about 20 minutes before staining. Staining methods for enzymes analyzed followed Vallejos (1983).

Isozyme loci were numbered consecutively beginning with the most anodal locus when more than one isozyme was present for an enzyme. Allozymes were named in alphabetical order sequentially starting with the most anodal form observed.

Genetic distance (D) were calculated using Nei's (1972) method. Proportion of polymorphic loci (P), numbers of alleles per locus (A) and gene diversity (H) for each population were also calculated using Nei's statistics on gene diversity (1973). The genetic distance matrix was used to construct a phenogram by the neighbor-joining method (Saitou and Nei, 1987).

Table 2. Allele frequences at 17 loci in the examined populations

Locus	Allele	*A. thunbergii* ssp. *urashima* (A)	(B)	(C)	ssp. *thunbergii*	*A. tortuosum*	*A. heterophyllum*	*A. negishii*	*A. heterocephalum* ssp. *okinawaense*	ssp. *heterocephalum*
Pgi-2	a						0.98	0.07		
	b							0.15		0.15
	c					0.04				
	d						0.02	0.78		
	e				0.63				0.28	
	f	0.95							0.05	0.39
	g								0.03	
	h	0.05	1.00	1.00	0.22	0.02			0.60	0.47
	i				0.15	0.62			0.05	
	j					0.32				
Tpi-1	a	0.03			0.17	0.98				
	b								0.33	
	c	0.97	0.92	1.00	0.83	0.02	1.00	1.00	0.67	0.73
	d		0.08							0.27
Tpi-2	a								1.00	
	b				0.32	1.00				1.00
	c	1.00	1.00	0.80	0.68		0.02			
	d			0.20						
	e						0.02			
	f						0.96	1.00		
Tpi-3	a					1.00				
	b						1.00	1.00	1.00	1.00
	c	1.00	1.00	1.00	0.27					
	d				0.73					
Gdh	a						1.00			
	b							0.11		
	c				0.43			0.89	0.73	0.53
	d	0.97	0.91	0.84	0.57	1.00			0.27	0.47
	e	0.33	0.09	0.16						
Pgm	a								0.08	0.16
	b					1.00	0.48		0.03	0.02
	c	1.00	1.00	1.00	0.90					
	d						0.52			
	e				0.10			0.43	0.13	
	f							0.57	0.78	0.82
Mdh-1	a		0.12	0.08						
	b	1.00	0.88	0.92	1.00	1.00	1.00	1.00	1.00	1.00
Mdh-2		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mdh-3	a	0.05			0.02					
	b			0.13			1.00	1.00	0.92	0.88
	c				0.45	1.00				
	d								0.05	
	e	0.95	1.00	0.87	0.45					0.12
	f				0.12				0.03	
Lap	a					1.00				
	b		0.38	0.51						
	c		0.62	0.49	1.00		1.00	1.00		
	d	1.00							1.00	1.00
Skd	a					1.00	0.52	1.00		
	b	1.00							1.00	1.00
	c						0.48			
	d		0.67	0.78						
	e		0.09	0.09	0.73					
	f		0.24	0.13	0.27					
Fe-1	a		0.90	1.00		1.00	0.50			
	b						0.50			
	c		0.10							
	d								0.10	
	e	1.00							0.90	1.00
	f							1.00		
Fe-2	a					0.62				
	b	1.00				0.32	1.00			
	c							1.00	1.00	1.00
	d		1.00	1.00						
	e					0.06				
Sod	a	1.00	1.00	1.00	0.54					
	b				0.46		1.00	1.00	1.00	1.00
	c					1.00				
G3p	a					1.00				
	b							1.00	1.00	1.00
	c	1.00	1.00	1.00						
	d				1.00		1.00			
Ald	a						0.77			
	b	1.00	1.00	1.00	1.00	0.78		1.00		0.05
	c					0.22			1.00	0.94
	d									0.02
Adh	a						1.00			1.00
	b	0.06	0.67	0.86	1.00	0.98		1.00	0.75	
	c		0.08							
	d	0.94	0.25	0.14		0.02			0.25	

Table 3. Gene diversity statistics for the populations examined

	P(%)	A	H
A. thunbergii ssp. *urashima*(A)	29	1.29	0.026
A. thunbergii ssp. *urashima*(B)	41	1.53	0.124
A. thunbergii ssp. *urashima*(C)	41	1.47	0.130
A. thunbergii ssp. *thunbergii*	60	1.59	0.256
A. tortuosum	24	1.47	0.087
A. heterophyllum	30	1.35	0.121
A. negishii	18	1.24	0.063
A. heterocephalum ssp. *okinawaense*	41	1.76	0.149
A. heterocephalum ssp. *heterocephalum*	35	1.53	0.127

P=proportion of polymorphic loci; A=number of alleles per locus; H=gene diversity.

Results and Discussion

A total of 17 loci, *Adh, Ald, Fe-1, Fe-2, Gdh, G3p, Lap, Mdh-1, Mdh-2, Mdh-3, Pgi-2, Pgm, Skd, Sod, Tpi-1, Tpi-2,* and *Tpi-3,* were used for genetic analysis. Table 2 shows the frequencies of alleles at the 17 loci in the populations examined. *Mdh-2* is fixed for the same allele in all plants examined. *Mdh-1*^{b} was found to be major in all populations examined. No other alleles were commonly found in all populations.

Gene diversity statistics (Table 3) were calculated from the values in Table 2. Proportion of polymorphic loci (P) and gene diversity within populations (H) are, comparing with the mean value of the monocotyledonous species (P=40.3, H=0.144; Hamrick and Godt, 1990), distinctly higher in *A. thunbergii* ssp. *thunbergii* and much lower in *A. thunbergii* ssp. *urashima* (A), *A. negishii* and *A. tortuosum*. In other populations, the value of the statistics is comparable to the mean value.

The low gene diversity (H=0.063) of *A. negishii* might be due to the effect of human activities for this species. This species is endemic to Izu Isls. and forms large populations at present. It is well known, however, that the tuber of *A. negishii* is edible and has been utilized as famine relief. Collections by man when starved might work as strong bottle-necks for the species. In contrast, the two subspecies of *A. heterocephalum* endemic to Ryukyu Isls. have higher gene diversities (H=0.138 on average) in spite of their small population size. They grow on restricted limestone hills and their populations are much smaller than *A. negishii.*

The values of Nei's (1972) genetic distance (D) between sample populations (Table 4) were calculated using allelic frequencies shown in Table 2. A phenogram (Fig. 1) was constructed by the neighbor-joining method (Saitou and Nei, 1987) based on the genetic distances. *Arisaema thunbergii* is a common species widely distributed in Japan from southern Hokkaido westward to Kyushu (Yakushima Is.) This species is divided into two subspecies, ssp. *thunbergii* of western Japan and ssp. *urashima* of eastern Japan. Their geographical ranges overlap in Shikoku and Chugoku Districts, but they are rarely found side by side. Although *A. thunbergii* ssp. *urashima* is morphologically invariable throughout its geographical range, the genetic distance between consubspecific populations is as large when geographically distant (D=0.40 between (A) and (C) which are about 500km apart) as the mean value between congeneric species of the flowering plants (Gottlieb, 1977; Crawford, 1983). The genetic distance is smaller between less distant populations (D=0.06 between *A. thunbergii* (B) and (C) which are about 100km apart). The genetic distance between ssp. *thunbergii* and ssp. *urashima* (D=0.33~0.57) is not much exceeding the value in ssp. *urashima*. The genetic distance between the two subspecies of *A. heterocephalum* (D=0.14) is comparable to the lowest value normally found between congeneric species (Crawford, 1983).

It is notable that *A. negisii* is far closer to *A. heterocephalum* than to any other species examined. This fact supports systematic treatment by Murata (1984) where both *A. negishii* and *A. heterocephalum* were included in sect. *Clavata*. The distances between other species well exceed the mean value between congeneric species (D=0.40; Crawford, 1983), which suggests that the species in sects. *Clavata* and *Tortuosa* are well differentiated genetically as well as morphologically. Notwithstanding their morphological similarity, *A. tortuosum* and *A. heterophyllum* are genetically very distant from each other (D=1.15) as well

Table 4. Mean genetic identities (upper triangle) and genetic distances (lower triangle) for populations examined

	1	2	3	4	5	6	7	8	9
1. *A. thunbergii* ssp. *urashima*(A)	×	0.70	0.67	0.57	0.26	0.25	0.19	0.40	0.41
2. *A. thunbergii* ssp. *urashima*(B)	0.36	×	0.99	0.72	0.34	0.26	0.27	0.27	0.25
3. *A. thunbergii* ssp. *urashima*(C)	0.40	0.06	×	0.70	0.36	0.27	0.28	0.29	0.25
4. *A. thunbergii* ssp. *thunbergii*	0.57	0.33	0.35	×	0.41	0.43	0.50	0.38	0.33
5. *A. tortuosum*	1.34	1.06	1.02	0.89	×	0.32	0.33	0.22	0.24
6. *A. heterophyllum*	1.37	1.34	1.30	0.83	1.15	×	0.55	0.38	0.51
7. *A. negishii*	1.67	1.32	1.25	0.70	1.10	0.59	×	0.70	0.70
8. *A. heterocephalum* ssp. *okinawaense*	0.92	1.32	1.24	0.96	1.51	0.96	0.35	×	0.87
9. *A. heterocephalum* ssp. *heterocephalum*	0.90	1.40	1.37	1.10	1.40	0.68	0.35	0.14	×

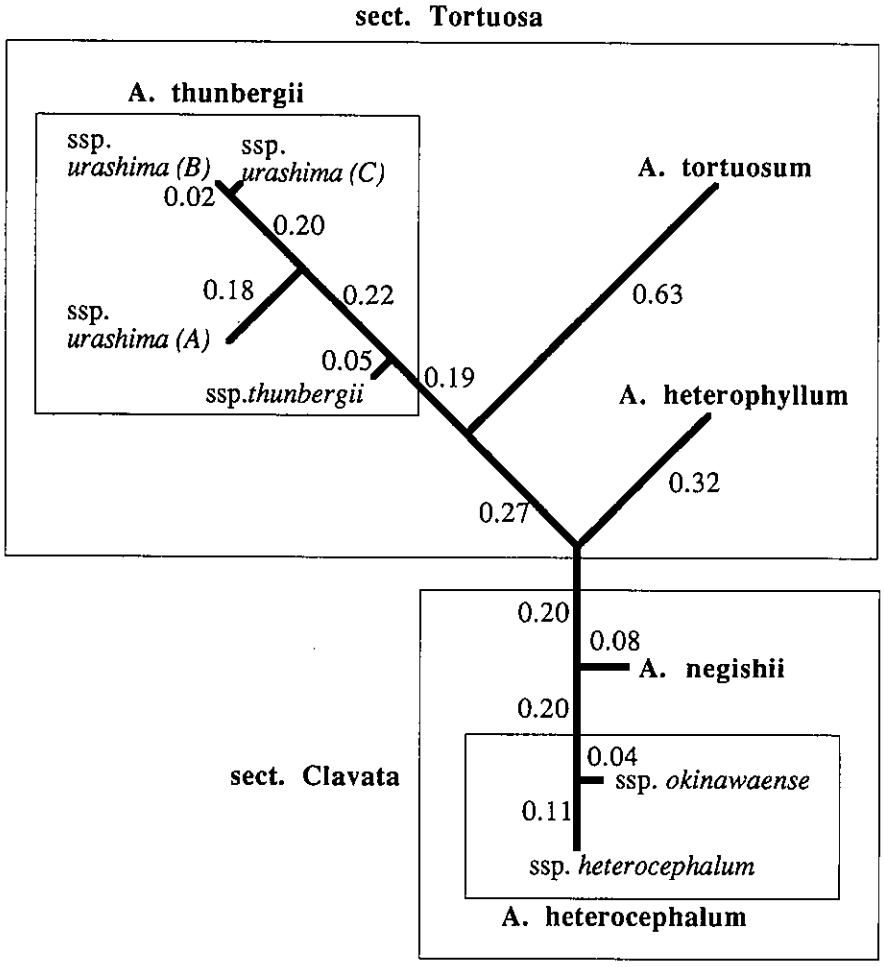

Fig. 1. A genetic distance phenogram of examined populations by neighbour-joining method. Values are standard genetic distances.

as from any other species.

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摘　要

テンナンショウ属の分類はこれまで形態的，細胞学的情報に基づいており，遺伝的な根拠が与えられてこなかった。そこで電気泳動法によりアロザイムの分化を調べ，現行の分類の妥当性を検討する。テンナンショウ属には形態的によく分化した種も，分化が十分ではなく識別が困難な種もあるが，本報では，形態的な分化が著しいマイヅルテンナンショウ節とアマミテンナンショウ節の計5種2亜種9集団を対象とした。この結果，種間ではシマテンナンショウとアマミテンナンショウの遺伝的距離が最も小さく（D＝0.35），シマテンナンショウをアマミテンナンショウ節に含める現行の分類体系が支持された。また，これ以外の種間の遺伝的距離は一般に知られる属内種間の平均的な値（D＝0.40）を超えており，遺伝的にもよく分化した種であることが明らかとなった。広い分布域を持つウラシマソウでは形態的には一様であるにもかかわらず，集団間に距離に関連すると思われるかなりの分化がみられ，京都府と東京都の集団間ではD＝0.40に達していた。なお，八丈島のシマテンナンショウでは調べた集団の遺伝的多様度が著しく小さかったが，これは救荒食糧として利用されたという人為的な瓶首効果によるものかも知れない。これに比べ集団のサイズがはるかに小さいと思われる奄美大島のアマミテンナンショウや沖縄島に固有のオキナワテンナンショウでは通常の種の集団と同程度の多様度を保有していた。

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