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Allozyme analysis of the hybrid origin of *Arisaema ehimense* (Araceae)

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Allozyme diversity was examined in the Japanese jack-in-the pulpit species, *Arisaema serratum* and *A. tosaense*, and their putative hybrid species, *A. ehimense* (all diploid). *Arisaeme ehimense* contains mostly alleles found in one or both of the putative parent species but few unique alleles, partially supporting the hypothesis that *A. ehimense* is of hybrid origin from the two species, although the possibility that *A. ehimense* arose via divergent speciation cannot be excluded. Because only limited information was gained from the survey of allozyme variation, additional taxon-specific markers from *A. serratum* and *A. tosaense* are required to test rigorously the hybrid origin of *A. ehimense*. A phenogram based on allele frequencies suggested that introgression occurs between *A. serratum* and *A. tosaense* where these species are sympatric.

Keywords: allozyme, *Arisaema ehimense*, diploid species, genetic diversity, genetic identity, homoploid hybrid speciation.

Introduction

Natural hybridization is considered to play a major role in plant speciation (Arnold, 1997), although this has not always been recognized (Wagner, 1970; Gottlieb, 1972; Heiser, 1973). Undoubtedly, natural hybridization is important as a first step for allopolyploid speciation, which is a major process in plant speciation (Grant, 1981). However, it remains uncertain to what extent hybridization contributes to speciation at the diploid level, even though many species have been considered to have homoploid hybrid origin in studies of native floras (e.g. Stace, 1975).

Morphological analysis of putative homoploid hybrid species can lead to false conclusions because morphological resemblance does not always indicate a close phylogenetic relationship. Therefore, morphological intermediacy of a species is not sufficient evidence that it is of hybrid origin. More exact evidence is obtained from additional characters such as molecular markers.

To date, several examples of homoploid hybrid plant species have been unambiguously confirmed by molecular markers (Rieseberg, 1997). Conversely, some species morphologically presumed to be of diploid hybrid origin were concluded not to be so (Rieseberg *et al*., 1990; Spooner *et al*., 1991; Wolfe & Elisens, 1993; Dubouzet & Shinoda, 1999) or their origin remained ambiguous, based on molecular marker evidence (Crawford & Ornduff, 1989; Wendel *et al*., 1991; Allan *et al*., 1997; Harris & Abbott, 1997).

One of the necessary conditions under which diploid hybrid speciation occurs is rapid formation of reproductive isolation between hybrid and parental individuals. However, this condition is not expected to be satisfied easily, possibly explaining why homoploid hybrid speciation is relatively rare (Rieseberg, 1997). Some investigators have proposed that diploid hybrid species have greater evolutionary potential than that of parent species because they combine the alleles of the two parents (Anderson, 1949; Stebbins, 1950; Grant, 1958). Currently, this hypothesis is not supported by the findings of previous studies (Rieseberg, 1997), although few detailed analyses have been conducted.

Arisaema ehimense J. Murata & Ohno (Araceae) is distributed only in the north-west part of the Shikoku Island of Japan. This species is morphologically intermediate between *A. serratum* (Thunb.) Schott and *A. tosaense* Makino, and is diploid ($2n = 28$) (Murata & Ohno, 1989). Artificial hybrids between *A. serratum* and *A. tosaense* are morphologically very similar to *A. ehimense* (Murata & Ohno, 1989). The pollen stainability of *A. ehimense* is similar to that of *A. serratum* and *A. tosaense*, and seeds from the natural populations germinate well (Murata & Ohno, 1989). *Arisaema ehimense* forms populations allopatrically with *A. serratum* and *A. tosaense*. These facts indicate

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that *A. ehimense* is not a simple F_1, but rather an established breeding species, and therefore a putative diploid hybrid species (Murata & Ohno, 1989).

At present, *A. ehimense* is isolated from its putative parental species geographically and phenologically (Murata & Ohno, 1989). Generally, *A. tosaense* occurs at higher altitudes than *A. ehimense*, although both species occasionally occur parapatrically. *Arisaema serratum* has not been found in or proximate to populations of *A. ehimense*. Although *A. ehimense* flowers almost simultaneously with *A. serratum*, these species flower approximately three weeks earlier than *A. tosaense*.

In this study, we address the following questions using allozyme markers. Do allozyme data support the hypothesis that *A. ehimense* is a putative diploid hybrid species? If so, does *A. ehimense* have greater genetic diversity than that of the putative parent species?

Materials and methods

Plant materials

Arisaema ehimense is a perennial herb that occurs in the understorey of temperate forests and planted *Cryptomeria japonica* D. Don afforestation, and is restricted to the north-western part of the Shikoku Island of Japan. To date, fewer than 10 localities of *A. ehimense* have been identified. In contrast, *A. serratum* is a variable species and is widely distributed in Japan, and occupies similar habitat to *A. ehimense*. *Arisaema tosaense* is distributed on Shikoku and the contiguous islands, and also occurs in the understorey of temperate forests, though on Shikoku it is found mainly at higher altitudes than *A. ehimense* and *A. serratum*. All three species exhibit sequential dioecy; i.e. individuals change gender through the growing season.

Five populations (E1–E5) of *A. ehimense* were sampled and selected to represent the geographical range of the species (Fig. 1). Although *A. serratum* is distributed widely, only those populations proximate to the distribution of *A. tosaense* may have been involved in the origin of *A. ehimense*. Consequently five populations (S1–S5) of *A. serratum* were sampled from Shikoku and the adjacent areas. Although *A. serratum* is a very variable species and many local races have been recognized by taxonomists, only *A. serratum sensu stricto* has been invoked as a putative parent of *A. ehimense* (Murata, unpubl. data). Thus, only populations with this morphology were sampled. Six populations (T1–T5) of *A. tosaense* were also sampled. Some individuals of *A. tosaense* were found within a few hundred metres of a *A. ehimense* population (E3). Populations S5 and T6 were located on the same part of a hill, where a few intermediate individuals to *Arisaema serratum* and *A. tosaense* were also found (Masuda and Maki, unpubl. data). Mature leaves from individuals within each population were collected and transported on ice to the laboratory. Samples were kept in a refrigerator for up to three weeks until electrophoresis was carried out; enzyme activity did not decrease during this period.

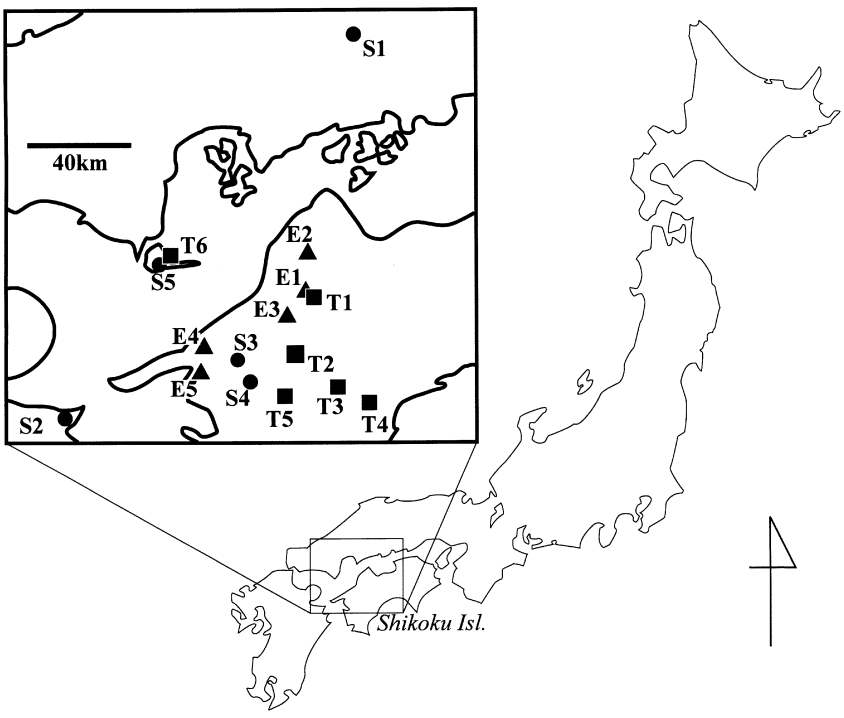

Fig. 1 Distribution of the populations examined. The letters preceeding the number indicate the species names (S, *Arisaema serratum*; T, *A. tosaense*; E, *A. ehimense*).

Enzyme electrophoresis

One hundred mg of leaf sample per individual was homogenized in 1 mL of extraction buffer (Tsumura *et al*., 1990). Homogenates were centrifuged at 20, 000 g at 4°C for 30 min and supernatant was used as a crude enzyme extract.

Polyacrylamide vertical slab gel electrophoresis was carried out according to procedures described by Tsumura *et al*. (1990). Ten μL of crude extract per individual were examined per gel. The following 12 enzyme systems were examined: alanine aminopeptidase (AAP; EC 3.4.11.1), alcohol dehydrogenase (ADH; EC 1.1.1.1), aspartate aminotransferase (AAT; EC 2.6.1.1), colometric esterase (EST; EC 3.1.1), glucose-6-phosphate isomerase (GPI; EC 5.3.1.9), glutamate dehydrogenase (GDH; EC 1.4.1.2), leucine aminopeptidase (LAP; EC 3.4.11.1), menadione reductase (MNR; EC 1.6.99.2), phosphoglucomutase (PGM; EC 5.4.2.2), 6-phosphogluconate dehydrogenase (6PGDH; EC 1.1.1.44), superoxide dismutase (SOD; EC 1.15.1.1), and triose-phosphate isomerase (TPI; EC 5.3.1.1) using established staining protocols (Tsumura *et al*., 1990).

Results

A total of 15 enzyme loci were consistently resolved. Among these loci, two (*Sod-1* and *Sod-2*) were monomorphic in all populations examined. Fifty-three putative alleles were identified at 15 presumed loci (Table 1). Fourteen alleles were identified which could potentially serve as marker alleles, i.e. they were found in one of the putative parent species, *A. serratum* or *A. tosaense*, but not in both (Table 1). These marker alleles did not occur at high frequency in either putative parent species. Three marker alleles ($Tpi\text{-}1^d$, $Aat\text{-}2^d$, and Gdh^e) were found in at least one of the putative hybrid populations. The allele $Aat\text{-}2^d$ occurred in all five populations of *A. ehimense* and four populations of *A. tosaense*. None of the marker alleles found in *A. serratum* were present in *A. ehimense*. Only two alleles (Lap^a and Lap^f) were unique to the putative hybrid, *A. ehimense*, and occurred at very low frequencies (0.020 and 0.040, respectively) in a single population (E-4).

Genetic identities (Nei, 1972) among populations within a species were very high in *A. ehimense* and *A. tosaense*, and lower in *A. serratum* (Table 2). Genetic identities between species were relatively high (Table 2). These levels of divergence are lower than the average for congeneric species, and are similar to those of an intraspecific comparison (Crawford, 1990). Genetically, *A. ehimense* is slightly closer to *A. tosaense* ($I = 0.928$) than to *A. serratum* ($I = 0.887$).

The phenogram using the NJ-method based on standard genetic distance (Saitou & Nei, 1987) is shown in Fig. 2. The support for individual nodes was assessed using 1000 bootstrap replicates in PHYLIP (Felsenstein, 1995). Populations of *A. ehimense* are clustered together, suggesting that the species has a single origin. By contrast, *A. serratum* does not show such a pattern; populations S1 and S2 are clustered together, although the bootstrap value is low, whilst the other populations are remote from them. This result is consistent with previous documentation of the multiple origin of *A. serratum* (Kawahara & Murata, 1995). However, this result may also be explained by the variability of *A. serratum*. It is noteworthy that the populations of *A. serratum* and *A. tosaense* (S5 and T6) that cluster together are sympatric.

At the population level, the proportion of polymorphic loci and the expected heterozygosities were lower in *A. ehimense* ($P = 57.3$, $h = 0.190$) than in the putative parent species, *A. serratum* ($P = 58.7$, $h = 0.259$) and *A. tosaense* ($P = 61.3$, $h = 0.230$). The number of alleles per locus of *A. ehimense* ($A = 2.08$) was slightly higher than that in *A. serratum* ($A = 1.97$), but lower than that in *A. tosaense* ($A = 2.31$; Table 3). Genetic diversity indices within a population for each species showed the same trend as those within a species (Table 3).

Inbreeding coefficients for all polymorphic loci were calculated for each population and their statistical significance was examined using a χ^2-test (Li & Horvitz, 1953). Most of the values are not significant (Table 4), coinciding with the fact that all species exhibit sequential dioecy and obligate outcrossing. Genetic differentiation among populations (G_{ST}; Nei, (1973) are 0.245, 0.052, and 0.128 in *A. serratum*, *A. ehimense*, and *A. tosaense*, respectively. This indicates that genetic differentiation among populations is small in *A. ehimense* and *A. tosaense* and gene flow among populations would be large, while interpopulation genetic differentiation is somewhat large in *A. serratum*.

Discussion

Origin of Arisaema ehimense

Additivity of marker alleles and a scarcity of unique alleles in the putative hybrid species are necessary (Rieseberg, 1997) to unambiguously document homoploid hybridity. In this study, no alleles showing additivity were found, a result that does not strongly support the hybridity of *A. ehimense*. In this case, the low frequencies of marker alleles and the genetically close relationship between the proposed parent species make unambiguous documentation of hybridity difficult. However, considering that alleles unique to *A. ehimense* are very rare (only

Table 1 Allele frequencies for 15 loci in the putative hybrid, *Arisaema ehimense*, and its parents, *A. serratum* and *A. tosaense*. *N* for each population is the mean sample size per locus. Alleles unique to the proposed hybrid are in italics. Marker alleles that distinguish the putative parents are shown in boldface. Marker alleles that also occur in the putative hybrid (additive alleles) are followed by an asterisk

		S1	S2	S3	S4	S5	E1	E2	E3	E4	E5	T1	T2	T3	T4	T5	T6
	N	32.8	29.1	30.3	25.0	29.6	28.5	23.9	23.1	23.7	23.3	29.3	29.3	20.0	23.3	29.9	28.7
Adh	*a*	0.829	0.167	0.234	0.000	0.267	0.048	0.075	0.140	0.033	0.174	0.100	0.110	0.137	0.060	0.100	0.000
	b	**0.028**	0.000	**0.250**	**0.020**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	c	0.143	0.833	0.516	0.980	0.733	0.952	0.925	0.860	0.967	0.827	0.900	0.883	0.864	0.940	0.900	0.983
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.017**
Gpi-1	*a*	0.015	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000
	b	0.691	0.533	0.283	0.480	0.258	0.083	0.100	0.080	0.280	0.167	0.017	0.033	0.000	0.854	0.133	0.000
	c	0.294	0.467	0.719	0.520	0.742	0.917	0.900	0.920	0.720	0.833	0.983	0.900	0.775	0.125	0.850	0.667
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.225**	0.000	0.000	0.000
	e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.000	**0.017**	**0.333**
Gpi-2	*a*	0.000	0.000	0.000	0.000	0.016	0.033	0.000	0.000	0.000	0.000	0.033	0.093	0.023	0.000	0.000	0.067
	b	0.338	0.383	0.531	0.220	0.419	0.350	0.240	0.280	0.520	0.230	0.333	0.333	0.114	0.220	0.117	0.500
	c	0.544	0.600	0.469	0.600	0.565	0.555	0.720	0.540	0.460	0.729	0.600	0.481	0.818	0.620	0.600	0.333
	d	0.118	0.017	0.000	0.180	0.000	0.067	0.040	0.180	0.020	0.042	0.033	0.093	0.045	0.160	0.283	0.100
Tpi-1	*a*	**0.257**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	b	0.000	**0.033**	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	c	0.743	0.967	1.000	1.000	1.000	1.000	1.000	0.980	0.900	1.000	0.967	0.967	0.955	1.000	1.000	1.000
	d	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020*	0.100*	0.000	0.033	**0.033**	**0.045**	0.000	0.000	0.000
Tpi-2	*a*	0.880	0.000	0.000	0.000	0.097	0.000	0.000	0.000	0.100	0.021	0.050	0.140	0.286	0.182	0.050	0.050
	b	0.120	1.000	1.000	1.000	0.903	1.000	1.000	1.000	0.900	0.979	0.933	0.860	0.643	0.818	0.950	0.950
	c	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.017**	0.000	**0.071**	0.000	0.000	0.000
Aat-1	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.955	1.000	1.000	0.983
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.045**	0.000	0.000	**0.017**
Aat-2	*a*	0.086	0.328	0.000	0.000	0.017	0.017	0.060	0.021	0.152	0.125	0.056	0.000	0.075	0.077	0.052	0.196
	b	0.500	0.587	1.000	1.000	0.983	0.950	0.860	0.938	0.783	0.854	0.796	0.933	0.775	0.808	0.862	0.786
	c	0.414	0.086	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.130	0.067	0.100	0.058	0.069	0.018
	d	0.000	0.000	0.000	0.000	0.000	0.033*	0.080*	0.042*	0.065*	0.021*	**0.019**	0.000	**0.050**	**0.058**	**0.017**	0.000
6Pdgh	*a*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.036**	0.000	0.000	0.000	0.000
	b	0.137	0.034	0.293	0.180	0.259	0.060	0.140	0.063	0.120	0.125	0.037	0.125	0.136	0.060	0.100	0.121
	c	0.833	0.828	0.569	0.780	0.552	0.740	0.800	0.938	0.860	0.875	0.796	0.821	0.727	0.840	0.850	0.759
	d	0.030	0.138	0.138	0.040	0.000	0.200	0.060	0.000	0.000	0.000	0.167	0.018	0.136	0.100	0.050	0.052
	e	0.000	0.000	0.000	0.000	0.190	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.069
Aap	*a*	0.630	1.000	1.000	0.560	0.963	1.000	1.000	0.980	0.900	1.000	0.983	0.733	0.909	0.818	0.867	0.860
	b	0.370	0.000	0.000	0.440	0.037	0.000	0.000	0.020	0.100	0.000	0.017	0.267	0.091	0.182	0.133	0.140

Gdh	*a*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.183**	**0.167**	**0.119**	**0.167**	**0.086**	**0.161**	
	b	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	**0.054**	
	c	0.043	0.350	0.323	0.700	0.549	0.500	0.720	0.478	0.304	0.563	0.283	0.200	0.095	0.083	0.328	0.214
	d	0.957	0.650	0.677	0.300	0.452	0.500	0.280	0.522	0.609	0.396	0.533	0.633	0.786	0.688	0.586	0.571
	e	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.087*	0.042*	0.000	0.000	0.000	**0.063**	0.000	0.000
Mnr	*a*	0.069	0.583	0.905	0.760	0.925	0.883	0.900	1.000	0.980	0.821	1.000	0.900	0.781	1.000	0.983	0.975
	b	0.000	0.050	0.024	0.240	0.075	0.067	0.100	0.000	0.000	0.179	0.000	0.000	0.125	0.000	0.017	0.025
	c	0.931	0.367	0.071	0.000	0.000	0.050	0.000	0.000	0.020	0.000	0.000	0.100	0.094	0.000	0.000	0.000
Pgm	*a*	0.000	0.000	0.000	0.000	0.274	0.083	0.083	0.040	0.000	0.021	0.033	0.000	0.024	0.000	0.000	0.050
	b	0.043	0.017	0.422	0.540	0.645	0.283	0.125	0.360	0.200	0.229	0.467	0.200	0.143	0.100	0.100	0.767
	c	0.829	0.655	0.531	0.460	0.081	0.500	0.705	0.600	0.720	0.750	0.500	0.800	0.738	0.560	0.900	0.183
	d	0.129	0.328	0.047	0.000	0.000	0.133	0.042	0.000	0.080	0.000	0.000	0.000	0.095	0.340	0.000	0.000
Lap	*a*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.020*	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	b	0.014	0.052	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.021	0.033	0.000	0.000	0.000	0.017	0.000
	c	0.485	0.552	0.797	0.640	0.617	0.133	0.040	0.020	0.120	0.229	0.950	0.914	0.763	0.846	0.600	0.467
	d	0.000	0.379	0.203	0.360	0.383	0.750	0.960	0.980	0.640	0.667	0.000	0.086	0.184	0.154	0.250	0.450
	e	0.500	0.017	0.000	0.000	0.000	0.117	0.000	0.000	0.100	0.083	0.017	0.000	0.053	0.000	0.133	0.083
	f	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	*0.040*	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Sod-1	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Sod-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 2 Summary values for Nei's genetic identity for the pairwise comparison between populations and species of *Arisaema serratum*, *A. ehimense*, and *A. tosaense*. Values in parentheses are standard errors

	A. serratum	*A. ehimense*	*A. tosaense*
A. serratum	0.857 (0.042)		
A. ehimense	0.887 (0.025)	0.984 (0.002)	
A. tosaense	0.899 (0.019)	0.928 (0.005)	0.947 (0.007)

Table 3 Genetic diversity at the population and species level of *Arisaema serratum*, *A. ehimense*, and *A. tosaense*. P = percentage of polymorphic loci at 95% level. A = mean number of alleles per locus; h = expected heterozygosity

	P	A	h
Each population			
S1	66.7	2.13	0.283
S2	60.0	2.13	0.277
S3	53.3	1.80	0.238
S4	53.3	1.73	0.246
S5	60.0	1.93	0.246
E1	53.3	2.13	0.205
E2	60.0	1.93	0.160
E3	46.7	1.87	0.156
E4	66.7	2.40	0.246
E5	60.0	2.07	0.213
T1	46.7	2.33	0.192
T2	66.7	2.20	0.223
T3	73.3	2.60	0.272
T4	53.3	2.20	0.229
T5	60.0	2.13	0.209
T6	53.3	2.40	0.254
Average across populations			
A. serratum	58.7	1.97	0.259
A. ehimense	57.3	2.08	0.190
A. tosaense	61.3	2.31	0.230
Species level			
A. serratum	80.0	2.67	0.343
A. ehimense	80.0	2.67	0.207
A. tosaense	86.7	3.20	0.264

two in one population), the hypothetical hybridity of *A. ehimense* cannot be rejected, although this fact *per se* does not support the hypothesis. Because of genetic similarity, the same alleles tended to be present in both putative parent species, and marker alleles that distinguished them were relatively few. The distribution of *A. ehimense* overlaps that of both *A. serratum* and *A. tosaense* and a phenogram based on genetic distance (Fig. 2) indicated the possibility of gene exchange

Table 4 Fixation indices for individual loci and the mean values for all polymorphic loci for each population

	S1	S2	S3	S4	S5	E1	E2	E3	E4	E5	T1	T2	T3	T4	T5	T6
Adh	−0.21	0.04	0.31	—	0.15	−0.05	0.64**	0.24	—	−0.01	—	0.51**	0.23	−0.06	0.26	—
Gpi-1	0.23	−0.09	0.03	0.04	−0.01	0.35	−0.09	−0.09	0.52**	0.32	—	−0.11	0.35	0.17	−0.18	0.55
Gpi-2	0.11	0.17	0.25	−0.17	0.15	0.26	−0.10	0.28	0.36	0.05	0.20	−0.11	0.30	0.25	−0.25	0.19
Tpi-1	−0.08	—	—	—	0.26	—	—	—	−0.11	—	—	—	—	—	—	—
Tpi-2	−0.13	—	—	—	0.26	—	—	—	−0.11	—	−0.07	−0.16	−0.40	−0.22	—	—
Aat-2	0.26	0.00	—	—	—	—	−0.05	−0.02	0.23	0.16	−0.03	−0.07	0.00	−0.23	−0.16	−0.27
6Pdgh	0.18	−0.21	−0.12	−0.28	−0.28	−0.17	0.50*	0.64**	−0.16	−0.14	−0.29	−0.22	−0.38	0.11	0.08	0.10
Aap	−0.31	—	—	0.18	—	—	—	—	−0.11	—	—	0.17	−0.10	−0.69*	−0.13	−0.19
Gdh	—	0.25	0.11	−0.43*	0.05	0.04	−0.39	0.20	0.49*	−0.10	0.33	0.14	0.07	0.13	0.32	0.45*
Mnr	−0.07	0.31	0.45*	0.12	0.77***	−0.13	0.63*	—	—	0.76**	—	0.26	−0.28	—	—	—
Pgm	0.00	−0.18	0.18	−0.05	−0.22	−0.10	0.06	−0.29	−0.19	0.11	0.15	0.38*	0.29	−0.14	0.25	0.07
Lap	0.08	0.16	−0.08	−0.22	0.18	−0.33	—	—	0.41*	0.11	−0.05	0.26	0.19	0.41*	0.34	−0.19
Mean	0.01	0.05	0.14	−0.10	0.13	−0.02	0.15	0.14	0.13	0.14	0.03	0.10	0.02	−0.03	0.06	0.09

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

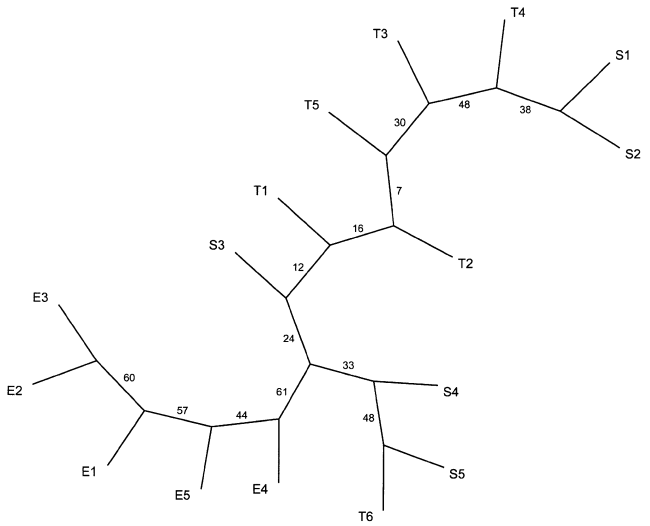

Fig. 2 Phenogram for three *Arisaema* species examined using the neighbour-joining method on Nei's (1972) standard genetic distance. The number on each branch indicates a confidence value estimated by 1000 times bootstrap resampling.

between *A. serratum* and *A. tosaense* (populations S5 and T6).

An alternative hypothesis, that the evolution of *A. ehimense* occurred without hybridization, is possible. In this scenario, the three species may have diverged in recent times, and *A. ehimense* differentiated from *A. tosaense* more recently, because only *A. tosaense* marker alleles were found in *A. ehimense* and the genetic identity between these species is greater than that between *A. ehimense* and *A. serratum*. However, this seems less plausible than that of a derivation following hybridization. Individuals that result from artificial hybridization of *A. serratum* and *A. tosaense* are morphologically very similar to those of *A. ehimense* (Murata & Ohno, 1989). Additionally, the morphological characteristics of *A. ehimense* show a mosaic of patterns found in the proposed parental species. This is a typical morphological feature of interspecific hybrids of *Arisaema*. More than a dozen such interspecific hybrids have been reported and examined in Japan (Murata & Ohno, 1989); however, morphological intermediacy can be found even in divergent evolution (Wilson, 1992). In order to test unambiguously between hybrid origin and divergence speciation of *A. ehimense*, more markers are needed which clearly distinguish the two parents.

Genetic diversity of the populations of three Arisaema *species*

Genetic differentiation among populations was not large in all three species. Most *Arisaema* species are pollinated

by fungus gnats, and their seeds are dispersed by birds. In addition, the species examined in this study are sequentially dioecious and obligately outcrossing. These factors would promote gene flow among populations of species and probably hybridization between co-occurring species.

The genetic diversity of *A. ehimense* is roughly equivalent to or lower than that of its putative parent species. Although hybrid taxa have been suggested to be more genetically variable than their parents (Anderson, 1949; Grant, 1958), it is not found be the case (Rieseberg, 1997). If *A. ehimense* was of hybrid origin, only a small number of parental individuals would be expected to have been involved in its origin.

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