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Predominance of Clonal Reproduction, but Recombinant Origins of New Genotypes in the Free-Floating Aquatic Bladderwort *Utricularia australis* f. *tenuicaulis* (Lentibulariaceae)

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Abstract

Aquatic plant is a biological group sharing several adaptations to aquatic conditions. The most striking evolutionary convergence in this group is the extensive reliance on clonal reproduction, which largely determine the patterns and process of evolution in aquatic plants. *Utricularia australis* f. *tenuicaulis* is a free-floating aquatic bladderwort that reproduces both sexually via seeds and clonally via turions and shoot fragments. Amplified fragment length polymorphism analysis was conducted on 267 ramets collected from 30 populations in Japan. The genotypic diversity within populations was extremely low regardless of the geographical distribution range: the mean number of genotypes per population (*G*) was 1.4 and the mean genotypic diversity (*D*), including monoclonal populations, was 0.17. In contrast to the predominance of a few clones within populations, many of the populations investigated had different genotypes; a large portion of the genetic variation was explained by variation among populations. Character compatibility analysis clearly revealed that somatic mutations did not contribute to the origin of genotypic diversity in this aquatic bladderwort; instead, rare to sporadic sexual reproduction likely generated new genotypes. Thus, future studies should examine the role of sexual reproduction in this species from the viewpoint of long-term evolutionary benefits.

Key words: *AFLP, aquatic plant, character compatibility analysis, genotypic diversity, sexual and clonal reproduction, somatic mutation,*

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Introduction

As a biological group, aquatic plants share several adaptations to aquatic conditions, such as a reduction in vascular tissue and highly polymorphic vegetative forms (Les and Philbrick 1993; Philbrick and Les 1996). One of the most striking cases of convergence in this group is the extensive reliance on clonal reproduction (Grace 1993; Les and Philbrick 1993). For example, some forms of clonal offspring, such as turions, winter buds, and shoot fragments are highly effective means of numerical increase, resource acquisition, and dispersal under aquatic conditions (Grace 1993; Les and Philbrick 1993), which largely determine the patterns and process of evolution in aquatic plants (Barrett et al. 1993).

Utricularia australis R. Br. (Lentibulariaceae) is a free-floating aquatic bladderwort widely distributed in temperate and tropical regions (Taylor 1989). In spite of its widespread distribution, this taxon displays almost complete sterility (Taylor 1989). A fertile group has been observed only in Japan, where *U. australis* is classified into two forma: sterile *U. australis* f. *australis* and fertile *U. australis* f. *tenuicaulis* (Komiya and Shibata 1980; Taylor 1989; but see also Kadono 1994). The taxonomic and phylogenetic relationships between *U. australis* and its two forma distributed in Japan, *U. australis* f. *australis* and *U. australis* f. *tenuicaulis*, remain unclear. However, it has been clearly demonstrated that (1) *U. australis* f. *australis* is a diploid hybrid that originated by asymmetric hybridization between *U. australis* f. *tenuicaulis* (mostly as the female parent) and its close relative *U. macrorhiza*; and (2) the absence of post-F_1 generation in natural hybrid population is confirmed by the additive patterns of AFLP bands (Kameyama et al. 2005).

Clonal reproduction plays a dominant role for the establishment of sterile *U. australis* f. *australis*, because propagation of this hybrid depends solely on clonal offspring in the form of many turions and shoot fragments. In contrast, the two parental species, *U. australis* f. *tenuicaulis* and *U. macrorhiza*, use both of these forms of clonal reproduction as well as sexual reproduction via seed. Thus, population genetic structure of these parental species is determined by the balance of the two reproductive modes: sexual and clonal reproduction.

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In flowering plants, the balance of sexual and clonal reproduction can vary widely across geographical range. For example, sexual reproduction in a clonal plant *Decodon verticillatus* at the northern periphery of the geographical range is severely reduced due to both ecological and genetic factors (Dorken and Eckert 2001; Eckert 2002). Population genetic structure of clonal plants, therefore, should be examined across the whole distribution range of the focal species. In addition, it is required to reveal whether genotypic diversity resulted from sexual recombination or somatic mutation. Recently, it became possible to determine the relative contribution of sexual recombination and somatic mutation on the origin of new genotypes by employing a new molecular genetic approach: character compatibility analysis (Mes 1998).

In the present study, we focus on the role of sexual and clonal reproduction in the free-floating aquatic bladderwort, *U. australis* f. *tenuicaulis*, addressing the following questions: (1) how much genotypic diversity is maintained within populations, (2) are the levels of genotypic diversity associated with geographical distribution range, and (3) whether genotypic differences result from sexual recombination or somatic mutation.

Materials and methods

Study sites and sample collection

Plant materials were sampled from 30 populations in Japan: 17 from the northern region and 13 from the southern region (Table 1; Fig. 1). In the northern region, most lakes and ponds are large, have a relatively scattered distribution, and originated naturally. In contrast, most lakes and ponds in the southern region are relatively small, densely distributed, and artificially managed for the irrigation of paddy fields. Stems of 1–13 samples were collected from each population (267 in total), cleaned with deionized water, and frozen at –80 °C for later DNA extraction.

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The extensive reliance on clonal reproduction combined with the strong dispersal ability of clonal offspring should largely determine the population genetic structure of aquatic plants. However, the importance of the two reproductive modes and their relationship to genetic variation have rarely been studied in aquatic plants, especially floating-leaved, submerged, or free-floating taxa (Barrett et al. ... [1]

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Study species

Utricularia australis f. *tenuicaulis* is a free-floating bladderwort that reproduces clonally via turions and shoot fragments and sexually via seeds. The taxonomic classification of this species has long remained unclear because of the existence of the morphologically similar taxa *U. australis* f. *australis* and *U. macrorhiza* (Taylor 1989; Kadono 1994; Komiya et al. 1997; see also Kameyama et al. 2005). Recently, it was revealed that *U. austra*... [2]

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AFLP analysis

Total genomic DNA was isolated from about 50 mg of tissue from each of the 267 frozen stems by means of the CTAB (hexadecyltrimethylammonium bromide) miniprep procedure (Stewart and Via 1993).

AFLP analysis was performed according to the method of Vos et al. (1995), with some modifications (see Kameyama et al. 2005). Selective amplifications were conducted with three combinations of primer pairs: *Mse*I-CC and *Eco*RI-ACT (FAM), *Mse*I-CA and *Eco*RI-ACG (VIC), and *Mse*I-CC and *Eco*RI-AGC (NED). The AFLP Amplification Core Mix (Applied Biosystems, Foster City, California, USA) and the GeneAmp PCR system thermal cycler (Applied Biosystems) were used for both amplifications. AFLP fragments were detected with an ABI Prism 3100 automated sequencer (Applied Biosystems) and the GENESCAN analysis software (Applied Biosystems).

Statistical analysis

The number of genotypes observed (G) and the genotypic diversity (D) within populations were calculated for each population. Genotypic diversity (D) was estimated as $D = 1 - \Sigma[n_i(n_i - 1)/n(n - 1)]$, where n is the number of ramets sampled and n_i is the number of ramets with genotype i. The value of D ranges from 0, when all ramets sampled have the same genotype, to 1, when each ramet sampled has a different genotype (Pielou 1969).

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Population genetic structure was assessed by analysis of molecular variance (AMOVA; Excoffier et al. 1992), which partitioned the total variance in the AFLP data into regional, among-population, and within-population components. Of the 267 ramets collected, 266 were used for this analysis because only one sample was available from population 1 (Table 1). The statistical significance of the variance components was determined by 999 random permutations. These analyses were performed with the GenAlEx 5.1 software (Peakall and Smouse 2001).

An UPGMA dendrogram of AFLP genotypes was constructed based on the standard genotypic similarity: $S_{ij} = 2N_{ij}/(N_i + N_j)$, where N_{ij} is the number of shared bands between genotypes i and j and N_i and N_j are the number of bands found in

genotypes *i* and *j*, respectively (Dice 1945). All calculations were conducted using the R Package 4.0 software (Casgrain and Legendre 1999).

Character compatibility analysis was conducted to reveal whether the genotypic diversity originated from sexual recombination or somatic mutations (Mes 1998; van der Hulst et al. 2000). In two binary character data, such as the presence or absence of AFLP bands at two loci, the presence of all four possible combinations of characters (0/0, 1/0, 0/1, 1/1) is more parsimoniously explained by recombination than by three mutation events. The presence of all four possible combinations is referred to as "incompatibility," and it can be used as a measure of recombination when summed over all pairwise comparisons (matrix incompatibility; Wilkinson 2001).

In the present study, the contribution of a particular genotype to matrix incompatibility was calculated by jackknifing using the JACTAX option in PICA (Wilkinson 2001), and then the genotypes with the highest contribution were successively removed from the dataset until the matrix incompatibility count became zero. The analyses were conducted for the genotypes distributed in the northern region (n = 17), southern region (n = 19), and whole region (n = 36). If only a few recombinant genotypes contribute to overall matrix incompatibility, there would be a sharp decrease of the incompatibility count upon deletion of these genotypes (van der Hulst et al. 2000). However, if the deletion of nearly all genotypes is required to remove matrix incompatibility, then the genotypic diversity originated mostly from sexual recombination.

削除: The relationship between genotypic similarity (S_{ij}) and geographic distance was estimated using the normalized Mantel statistic value (R_M) to examine: (1) the correlation between the genetic similarity matrix and geographic distance matrix (simple Mantel test; Mantel 1967), and (2) the degree of spatial autocorrelation (Mantel correlogram; Oden and Sokal 1986). The second approach is a modification of the simple Mantel test in which geographic distance is divided into several classes and the successive R_M values are calculated (Stehlik et al. 2001). In the present study, geographic distance was divided into 10 classes at 150–km intervals from class 0 (within populations, 0 km) to class 10 (1350–1500 km). To reveal the geographic distribution of sexually produced genotypes (genets), one representative was used for each genotype within populations and those genotypes that were distributed across multiple populations were excluded from the analysis. All calculations were conducted using the R Package 4.0 software (Casgrain and Legendre 1999) and tested for significance with 999 permutations.

Results

Genotypic diversity

A total of 97 polymorphic bands were identified by AFLP analysis, which discriminated 36 genotypes from the 267 ramets (Table 1). The number of genotypes in the northern and southern regions were 17 from 17 populations and 19 from 13 populations, respectively (Table 1). The mean number of genotypes within each population (G) was 1.29 (SE = 0.11) in the northern region, 1.54 (SE = 0.14) in the southern region, and 1.40 (SE = 0.09) in total. Mean genotypic diversity (D), including monoclonal

削除: Each population had only one or two genotypes that were occasionally observed in distant populations: genotype 1 in populations 1 and 5 (distance between the populations, 65.2 km); genotype 4 in populations 3 and 4 (5.7 km); genotype 8 in populations 8, 9, 10, and 11 (mean, 2.5 km; range, 0.4–4.2 km); and genotype 21 in populations 20 and 23 (11.2 km).

populations, was 0.12 (SE = 0.05) in the northern region, 0.23 (SE = 0.07) in the southern region, and 0.17 (SE = 0.04) in total.

Hierarchical AMOVA partitioned the overall genetic variation into three levels: between regions, among populations, and within populations (Table 2). Only 3.3% of the total variation was explained by the differences within populations (Table 2), reflecting the small number of genotypes within each population. A relatively small amount of the genetic variation was attributed to regional differences (15.2%), and a large portion was explained by the differences among populations (81.6%; Table 2).

The UPGMA dendrogram constructed for 36 genotypes showed that the genets distributed within the same populations were genetically similar with each other (Fig. 2). In contrast, no apparent group was detected among populations or between regions (Fig. 2).

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The simple Mantel test revealed a significant correlation between pairwise genotypic similarity and pairwise geographic distance (R_M = –0.274, P < 0.001). The Mantel correlogram, in which geographic distance was divided into 10 classes and the successive R_M values were calculated, clearly showed significant positive correlations at the first (within populations) and second (among populations within 150 km of one another) distance classes (Fig. 3).

Character compatibility

Results of the character compatibility analyses were shown as the pattern of matrix incompatibility count (MIC) after successive removal of the genotypes that contribute most to the overall MIC (Fig. 4). If the deletion of nearly all genotypes is required to remove matrix incompatibility (MIC = 0), then the genotypic diversity would have originated mostly from sexual recombination.

The analysis conducted for all genotypes (whole region) revealed that most genotypic diversity was derived from sexual recombination: 30 of 36 genotypes (83.3%) had to be deleted to obtain the strictly clonal genetic structure (MIC = 0; Fig. 4). When the analysis was conducted separately for the northern and southern regions, a relatively sharp decline of MIC was observed in the northern region compared with the southern region (Fig. 4). The sharp decline of MIC suggests that there are major groups of genotypes that are more incompatible with each other (as the measure of recombination) than with other individuals (van der Hulst et al. 2000). The total number of genotypes that needed to be deleted to eliminate all MIC, however, was similar between the two regions: 11 of 17 genotypes (64.7%) and 13 of 19 (68.4%) in the northern and southern regions, respectively (Fig. 4).

Discussion

Low genotypic diversity within populations

Clonal plants usually exhibit substantial genotypic diversity within populations, even if sexual recruitment is absent or extremely limited (Ellstrand and Roose 1987; Widén et al. 1994). For example, a broad comparison of 27 studies involving 21 clonal species (Ellstrand and Roose 1987) showed that the mean number of genotypes per population (*G*) was 16.1 (range, 1–167) and the mean genotypic diversity (*D*) of multiclonal populations was 0.62 (range, 0.1–1.0). It may be notable that most of these studies were conducted in small number of populations employing the markers such as allozyme.

In contrast, *Utricularia australis* f. *tenuicaulis* showed extremely low genotypic diversity within all surveyed populations, in spite of the use of highly polymorphic and reliable DNA marker (AFLP): the mean number of genotypes within populations (*G*) in the northern region, southern region and in total was 1.29, 1.54 and 1.40, the mean genotypic diversity (*D*), including monoclonal populations, was 0.12, 0.23 and 0.17, respectively.

Clonal reproduction in aquatic plants is highly effective for both numerical increase and expansion of the area of distribution (Grace 1993). The rapid expansion of a few founder genotypes via extensive clonal reproduction greatly impedes the subsequent establishment of seedling and clonal offspring. In addition, self-fertilization in *U. australis* f. *tenuicaulis* reduces the success of sexual reproduction; previous studies showed that the mean number of seeds per fruit in selfed and outcrossed fruit was 16 and 48 (Kameyama et al. 2005), and the mean seed set ratio was 7.6% and 45.7% in selfing and outcrossing treatments, respectively (Araki and Kadono 2003). Thus, the rapid and widespread expansion of a few founders may not only excludes the subsequent establishment of seedlings and clonal offspring, but also inhibits sexual reproduction because of reduced seed production caused by increased selfing.

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Spatial genetic structure and gene flow

Contrary to the low genotypic diversity within populations, genotypes across populations were highly diverse (Fig. 2) and the great extent of genetic variation was explained by the differences among populations (Table 2). This pattern is apparently because of the restricted gene flow via pollen and seeds. A significant relationship between pairwise genotypic similarity and pairwise geographic distance (that is, a pattern of isolation-by-distance) was found among genets at the whole region (simple Mantel test, $P < 0.001$). In addition, the Mantel correlogram demonstrated significant positive correlations only at the first (within populations) and second (among populations within 150 km of one another) distance classes (Fig. 3). Thus, in this aquatic bladderwort, gene flow among populations via pollen and seeds appears to be limited to less than 150 km.

The dispersal ability of clonal offspring is revealed by the existence of the same genotypes in distant populations: four genotypes (10 ramets in total) were distributed in multiple populations separated by several to several tens of kilometers. A wide distribution of a single clone was also found in *U. australis* f. *australis*: populations separated by about 250 km across the Tsugaru Straits in Japan were dominated by a single genotype (Y. Kameyama and M. Ohara, unpublished data). Thus, clonal offspring of aquatic bladderworts can disperse relatively long distances, possibly comparable with that of sexually produced seeds and pollen grains.

The origin of new genotypes: sexual recombination or somatic mutations?

The genotypic diversity within aquatic blatterworts is extremely low compared to any other clonal plants. Many of the populations investigated, however, had different and highly variable genotypes (Table 1, Fig. 2); a large portion of the genetic variation was explained by variation among populations (Table 2). How were these genotypes produced regardless of the predominance of clonal reproduction within populations?

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Somatic mutations can generate new genotypes without sexual reproduction. Recently, character compatibility analysis has made it possible to determine whether genotypic differences resulted from somatic mutations or sexual recombination (Mes 1998). In the terrestrial plant *Allium vineale* (wild garlic), in spite of extensive reliance on clonal reproduction, most genotypes originated by sexual recombination rather than by mutations within asexual lineages (Ceplitis 2001). Similarly, in a population of apomictic dandelions, somatic mutations did not contribute to the generation of new genotypes (van der Hulst et al. 2000, 2003, but see also Mes et al. 2002). In contrast, genotypes of the aquatic plant *Butomus umbellatus* largely originated by somatic mutations with almost no contribution of sexual recombination (Eckert et al. 2003).

In this study of the aquatic bladderwort *U. australis* f. *tenuicaulis*, the character compatibility analysis revealed that most genotypic diversity was derived from sexual recombination. Six genotypes showed a strictly clonal genetic structure (MIC = 0) in all analyses (Fig. 4). However, character compatibility analysis requires at least four genotypes to detect incompatibilities (van der Hulst et al. 2003), which is close to the number of genotypes that remained in the present study. Thus, we conclude that somatic mutations may not contribute the origin of genotypic diversity in this aquatic bladderwort; instead, rare to sporadic sexual reproduction likely generated new genotypes.

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Future studies should examine the role of sexual reproduction in *U. australis* f. *tenuicaulis* from the viewpoint of long-term evolutionary benefits, such as rapid adaptation by recombination (Crow and Kimura 1965), escape from coevolving pathogens (Maynard Smith 1978), and prevention of mutational meltdown (Lynch and Lande 1993).

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Figure legends

Fig. 1. Sampling locations of *Utricularia australis* f. *tenuicaulis*. The common name and geographic coordinates of each population are shown in Table 1.

Fig. 2. UPGMA dendrogram constructed for 36 genotypes of *Utricularia australis* f. *tenuicaulis*. The population ID numbers (1–30) and regions (N, northern; S, southern) are shown on the right side.

Fig. 3. Matrix incompatibility count (MIC) after successive removal of the genotypes that contribute most to the overall MIC.

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Fig. 3. Correlogram of Mantel's *r* (R_M) for each 150-km distance class. Distance class 0 represents comparisons within populations. One representative was used for each genotype within populations. Correlations were calculated for 32 genets, excluding the four genotypes distributed across multiple populations. Filled circles indicate R_M values that are significantly different from zero after Bonferroni correction ($\alpha = 0.05$).

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Although most terrestrial plants also have the ability to produce clonally, a trait that originated independently in numerous lineages (Klimeš et al. 1997), one of the most notable features of aquatic plants is the ability of clonal offspring to disperse over long distances (Grace 1993; Les and Philbrick 1993). In terrestrial plants, sexually produced seeds are usually smaller than clonal offspring and have various specialized dispersal mechanisms (Starfinger and Stöcklin 1996). Thus, sexual reproduction is indispensable for the persistence of most terrestrial plants at the metapopulation level, even if clonal reproduction is favored at the population level (Olivieri et al. 1995; Piquot et al. 1998; Stöcklin and Winkler 2004). Unlike terrestrial plants, however, many aquatic plants possess vegetative structures that can be dispersed exceptionally long distances, such as turions, winter buds, and shoot fragments (Grace 1993). Thus, the long-distance dispersal ability of clonal offspring may reduce the selective disadvantages of asexual reproduction (Les and Philbrick 1993) even at the metapopulation level.

The extensive reliance on clonal reproduction combined with the strong dispersal ability of clonal offspring should largely determine the population genetic structure of aquatic plants. However, the importance of the two reproductive modes and their relationship to genetic variation have rarely been studied in aquatic plants, especially floating-leaved, submerged, or free-floating taxa (Barrett et al. 1993). In the present study, we address the spatial genetic structure of the free-floating aquatic bladderwort *Utricularia australis* f. *tenuicaulis*, focusing on the roles of sexual and clonal reproduction. Amplified fragment length polymorphism (AFLP) analysis was conducted to examine the genotypic diversity within populations, the spatial genetic

structure at the geographical scale (0–1500 km), and the origin of new genotypes via sexual recombination and somatic mutation.

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Study species

Utricularia australis f. *tenuicaulis* is a free-floating bladderwort that reproduces clonally via turions and shoot fragments and sexually via seeds. The taxonomic classification of this species has long remained unclear because of the existence of the morphologically similar taxa *U. australis* f. *australis* and *U. macrorhiza* (Taylor 1989; Kadono 1994; Komiya et al. 1997; see also Kameyama et al. 2005). Recently, it was revealed that *U. australis* f. *tenuicaulis* and *U. macrorhiza* are strict species, whereas *U. australis* f. *australis* is a sterile F_1 hybrid between them (Kameyama et al. 2005). In the present study, we focus solely on the genetic structure of *U. australis* f. *tenuicaulis*, because of its wide distributional range across Japan.

Table 1. Sampling locations, population identities, and observed AFLP genotypes in *Utricularia australis* f. *tenuicaulis*.

Region	ID of population	ID of genotype (*N* of samples)	Locality	Common name[a]	Coordinates
Northern	1	1 (1)	Hokkaido Pref., Ishikari City	Ishikarihama	43°13'N, 141°20'E
	2	2 (4), 3 (1)	Hokkaido Pref., Ebetsu City	Higashinopporo Moor	43°02'N, 141°33'E
	3	4 (16)	Hokkaido Pref., Atsuma Town	Hamaatsuma	42°35'N, 141°51'E
	4	4 (9), 5 (4)	Hokkaido Pref., Atsuma Town	Irishikabetsu Pond	42°37'N, 141°55'E
	5	1 (12)	Hokkaido Pref., Atsuma Town	Matsunonuma Pond	42°41'N, 141°51'E
	6	6 (12)	Aomori Pref., Tsugaru City	(Tsugaru E)	40°51'N, 140°18'E
	7	7 (12)	Aomori Pref., Tsugaru City	(Tsugaru 26)	40°47'N, 140°18'E
	8	8 (12)	Aomori Pref., Tsugaru City	Otsutsumi Pond	40°50'N, 140°18'E
	9	8 (12)	Aomori Pref., Tsugaru City	(Otsutsumi-south Pond)	40°50'N, 140°18'E
	10	8 (12)	Aomori Pref., Tsugaru City	Kamisawabenuma Pond	40°53'N, 140°19'E
	11	8 (8)	Aomori Pref., Tsugaru City	(Tsugaru D)	40°51'N, 140°18'E
	12	9 (3)	Aomori Pref., Tsugaru City	(Tsugaru 6)	40°51'N, 140°20'E
	13	10 (5), 11 (1)	Akita Pref., Odate Clty	(Odate)	40°18'N, 140°38'E
	14	12 (4), 13 (2)	Yamagata Pref., Sagae City	(Hiranosann)	38°23'N, 140°13'E
	15	14 (6)	Fukushima Pref., Shimogo Town	Kannonnuma Pond	37°11'N, 139°55'E
	16	15 (6)	Niigata Pref., Joetsu City	Unoike Pond	37°13'N, 138°21'E
	17	16 (5), 17 (1)	Niigata Pref., Joetsu City	Asahiike Pond	37°13'N, 138°21'E
Southern	18	18 (4), 19 (1)	Shiga Pref., Moriyama CityShi	(Imahama)	35°04'N, 135°56'E
	19	20 (6)	Shiga Pref., Koka City	(Isoo)	34°53'N, 136°08'E
	20	21 (12)	Hyogo Pref., Kasai Clty	(Asazuma 1)	34°54'N, 134°52'E
	21	22 (7), 23 (3)	Hyogo Pref., Kasai Clty	(Asazuma 2)	34°54'N, 134°52'E
	22	24 (7), 25 (6)	Hyogo Pref., Kasai Clty	Nakaike Pond	34°55'N, 134°51'E
	23	21 (12), 26 (1)	Hyogo Pref., Ono City	(Kashitani 3)	34°52'N, 134°59'E
	24	27 (9), 28 (1)	Hyogo Pref., Ono City	(Kashitani 6)	34°52'N, 134°59'E
	25	29 (13)	Hyogo Pref., Kakogawa City	Nonoike Pond	34°46'N, 134°53'E
	26	30 (13)	Hyogo Pref., Akashi City	(Okubo)	34°40'N, 134°56'E
	27	31 (12)	Hyogo Pref., Yashiro Town	(Yashiro)	34°57'N, 135°02'E
	28	32 (3), 33 (2)	Hiroshima Pref., Higasni-Hiroshima Cit	Budoike Pond	34°24'N, 132°42'E
	29	34 (2), 35 (1)	Saga Pref., Nanayama Village	Kashibaru Moor	33°24'N, 130°08'E
	30	36 (4)	Saga Pref., Fuji Town	(Okushi)	33°24'N, 130°10'E

[a] Names in parentheses are arbitrarily given by the authors.

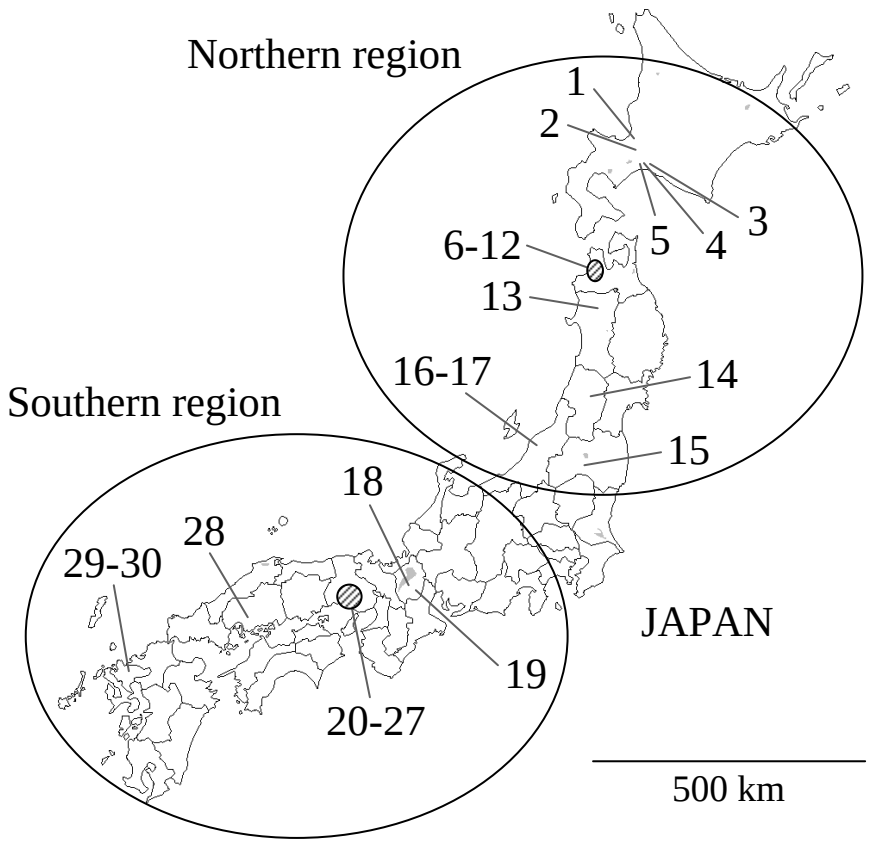

Fig. 1. Sampling locations of *Utricularia australis* f. *tenuicaulis*. The common name and geographic coordinates of each population are shown in Table 1.

Table 2. Hierarchical analysis of molecular variance (AMOVA) for *Utricularia australis* f. *tenuicaulis*. Population 1 was excluded for the analysis, because only one sample was available (Table 1). The significance value was determined from a 999 permutation test.

Source of variation	d.f.	Sum of squares	Variance component	% Total variation	*P*-value
Between regions	1	340.3	1.80	15.15	<0.001
Among populations	27	2378.7	9.68	81.58	<0.001
Within populations	237	92.1	0.39	3.28	<0.001

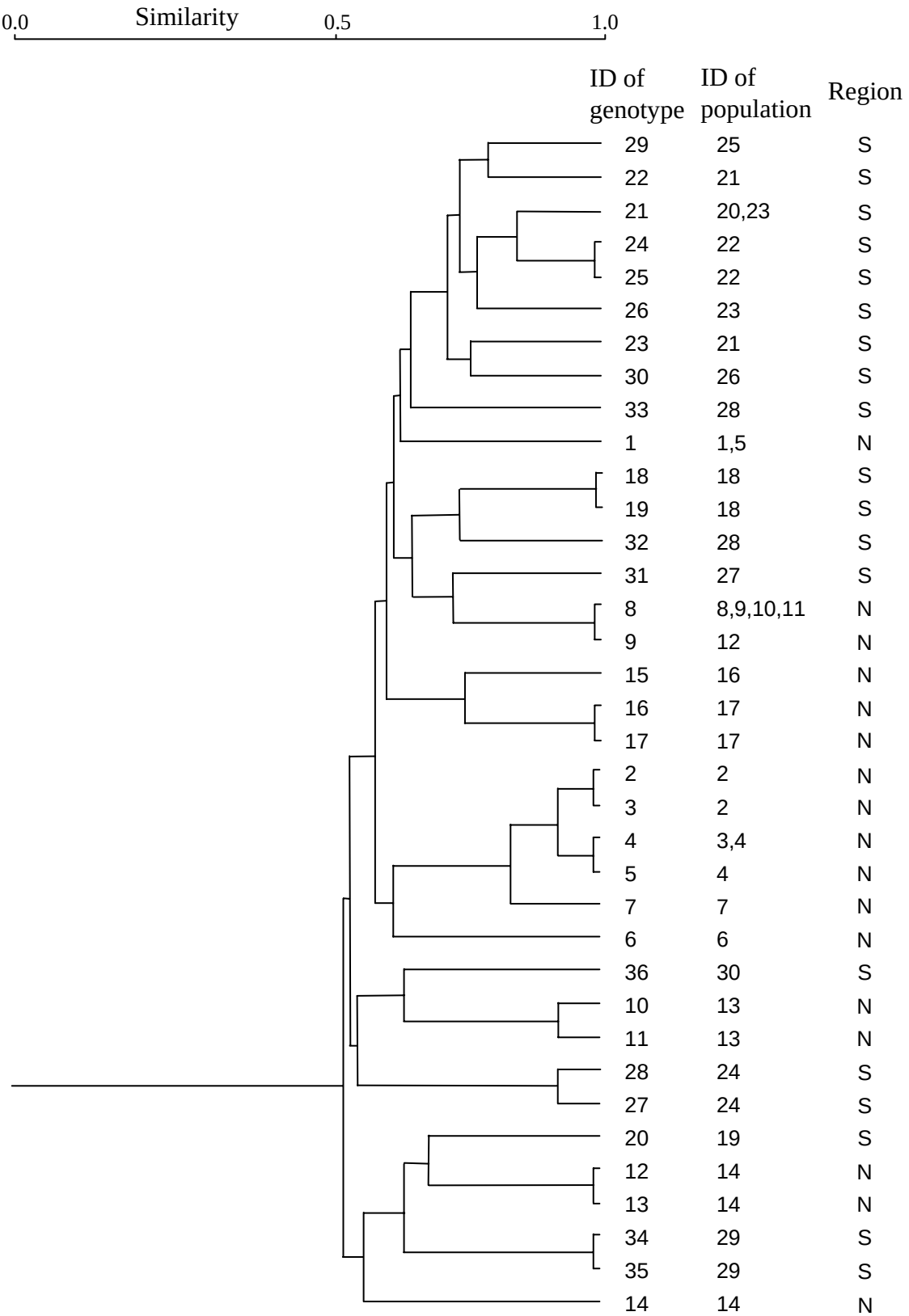

Fig. 2. UPGMA dendrogram constructed for 36 genotypes of *Utricularia australis* f. *tenuicaulis*. The population ID numbers (1-30) and regions (N: northern, S: southern) are shown on the right side.

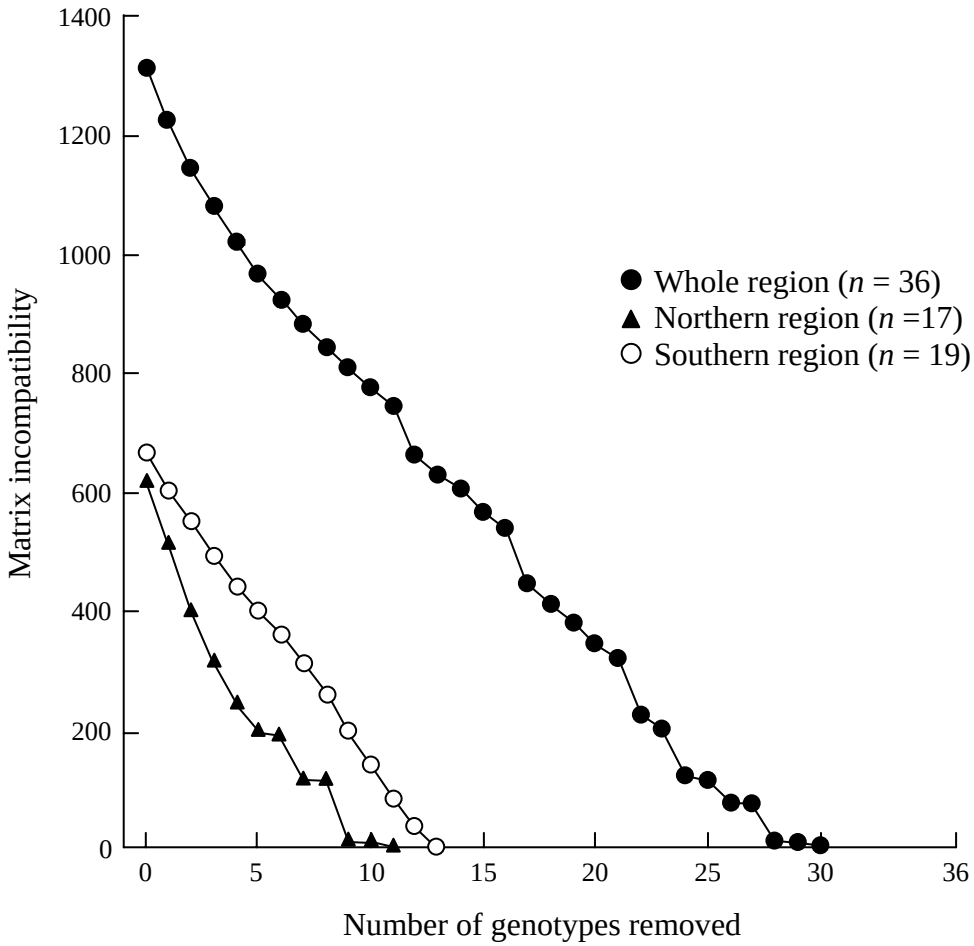

Fig. 3. Matrix incompatibility count (MIC) after successive removal of the genotypes that contribute most to the overall MIC.