Origins of *Hosta* Cultivars Based on Sequence Variations in Chloroplast DNA

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We analyzed the sequence variations in chloroplast DNA (cpDNA) for 31 *Hosta* cultivars, which may have been derived from *Hosta sieboldiana* and *H. albomarginata* in Japan, in order to elucidate the population origins of *Hosta* cultivars in their native habitats. In a previous study, we identified 39 haplotypes of cpDNA with specific regional features in the native habitats (16 and 23 in *H. sieboldiana* and *H. albomarginata*, respectively). These regional features of cpDNA variations were used to explore the maternal population origins of *Hosta* cultivars. We found that multiple *Hosta* cultivars may have originated from *H. sieboldiana* and *H. albomarginata*. We also observed some additional haplotypes that had not been detected previously. It is possible that these haplotypes originated from populations that were not investigated in previous studies, or that mutations occurred in the cultivar sequences after breeding.

**Key Words:** cpDNA, cultivar origins, gardening, haplotype, *Hosta*.

**Introduction**

*Hosta* is a genus of the family Asparagaceae distributed in East Asia and Russia (Takahashi, 2002; Tamura and Fujita, 2013). There are two species distributed in China (Chen and Boufford, 2000), while Chung and Chung (1988) recognized 11 species and two varieties of the genus in Korea. On the other hand, Fujita (1976) recognized 18 species and seven varieties of the genus *Hosta* in Japan. The Japanese Archipelago may be the center of speciation of the genus *Hosta* since the majority of species are distributed in Japan.

Over 100 species, varieties and cultivars of the genus *Hosta* have been described globally to date. It is believed that the Chinese species, *H. plantaginea* and *H. ventricosa*, were introduced into Europe approximately 230 years ago, and these were the first *Hosta* species cultivated in Europe (Grenfell, 1981). The *Hosta* cultivars are popular for landscaping and gardening in many countries. In addition, many cultivars are being traded, especially in Japan. In Japan, horticulture and garden design of *Hosta* cultivars flourished during the Edo period (Schmid, 1991). At this time, many *Hosta* cultivars originating from Japan were bred and improved (Nakamura, 2010). Although there are many *Hosta* cultivars on the market, the origin of these species remains unclear. *Hosta sieboldiana* and *H. albomarginata* are the two most common and widespread herbaceous species in Japan and may be the origins of some cultivars of *Hosta* in the Japanese Archipelago (Fujita, 1976).

The origins of cultivars of *Primula sieboldii*, a popular species in Japan, were examined by analyzing chloroplast DNA (cpDNA) variations, suggesting that most cultivars were derived from wild populations (Honjo et al., 2008). From the *P. sieboldii* cultivars, 10 cpDNA haplotypes were identified, three of which were newly detected and not present in wild populations. Honjo et al. (2008) determined that the majority of the cultivars had been improved by intraspecific crossing among *P. sieboldii* originating from the Asama-Arakawa populations and other cultivars from other areas of Japan. Similar to cultivars of *P. sieboldii*, the *Hosta* cultivars may have originated from different regions of Japan.

cpDNA variations have often been used to trace cultivar origins (Honjo et al., 2008; Tanaka et al., 2013). We previously analyzed sequence variations in two noncoding regions of cpDNA in over 100 wild populations of *H. sieboldiana* and *H. albomarginata* in Japan (Lee and Maki, 2013), and identified 39 haplotypes of cpDNA with specific regional features in these two species (16 and 23 in *H. sieboldiana* and *H. albomarginata*, respectively). These regional cpDNA variations can be
used to explore the maternal origins of Hosta cultivars. In this study, we focused on Hosta cultivars that may be derived from the two species *H. sieboldiana* and *H. albomarginata*, which are native to Japan, and explored the population origins of these cultivars based on cpDNA haplotypes.

**Materials and Methods**

**Plant materials**

A total of 31 cultivars of the genus *Hosta* were obtained from a plant supplier, Touhou Botanical Gardens (Table 1). Although many cultivars are known for *Hosta*, we excluded the cultivars that are considered to have derived from species other than *H. sieboldiana* and *H. albomarginata* in this study. The horizontal to vertical ratios of the leaves in typical *H. sieboldiana* and *H. albomarginata* are 1.09–1.69 and 2.65–4.25, respectively. We tentatively categorized these cultivars into the two species, *H. sieboldiana* and *H. albomarginata*, based on the values of the horizontal to vertical ratio of the leaves and the base form (cordate or truncate) of the leaves (Fig. 1). Leaf materials were sampled from the cultivars in the spring and stored in an ultra-cold freezer (−70°C) in Ziplock plastic bags until DNA extraction.

**DNA extraction and sequencing**

Genomic DNA was extracted from the leaves accord-

![Fig. 1. Two types of Hosta cultivar based on the morphology of leaves. (a) Hosta ‘Guacamole’: *Hosta sieboldiana* type. (b) Hosta ‘Shiro-Kapitan’: *H. albomarginata* type.](image-url)

**Table 1.** The phenetic type, the cpDNA haplotype, and the estimated species of origin for each *Hosta* cultivar compared with the results of Lee and Maki (2013).

| Samples | Cultivar name          | cpDNA haplotype compared with the results by Lee and Maki (2013) | Phenetic type | Originated species |
|---------|------------------------|---------------------------------------------------------------|---------------|--------------------|
| HG1     | Hosta ‘Shuchunotama’   | Haplotype q                                                   | *H. albomarginata* | *H. albomarginata* |
| HG2     | Hosta ‘Kinsei obagiboushi’ | Haplotype H                                               | *H. sieboldiana* | *H. sieboldiana*   |
| HG3     | Hosta ‘Uzunomai’       | New haplotype                                                 | *H. sieboldiana* | *H. albomarginata* |
| HG4     | Hosta ‘Sagae’          | Haplotype F                                                   | *H. sieboldiana* | *H. sieboldiana*   |
| HG5     | Hosta ‘Gypsy Rose’     | New haplotype                                                 | *H. sieboldiana* | *H. albomarginata* |
| HG6     | Hosta ‘Summer Breeze’  | Haplotype b                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG7     | Hosta ‘Diana Remembered’ | Haplotype b                                               | *H. sieboldiana* | *H. albomarginata* |
| HG8     | Hosta ‘Great Escape’   | Haplotype c                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG9     | Hosta ‘Northern Exposure’ | Haplotype J                                           | *H. sieboldiana* | *H. albomarginata* |
| HG10    | Hosta ‘Francee’        | Haplotype b                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG11    | Hosta ‘Guacamole’      | Haplotype b                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG12    | Hosta ‘Kabitan’        | Haplotype f                                                   | *H. albomarginata* | *H. albomarginata* |
| HG13    | Hosta ‘Bunchoko’       | Haplotype c                                                   | *H. albomarginata* | *H. albomarginata* |
| HG14    | Hosta ‘Ougon’          | New haplotype                                                 | *H. sieboldiana* | *H. albomarginata* |
| HG15    | Hosta ‘Akagikin’       | Haplotype v                                                   | *H. albomarginata* | *H. albomarginata* |
| HG16    | Hosta ‘Reizan’         | Haplotype b                                                   | *H. albomarginata* | *H. albomarginata* |
| HG17    | Hosta ‘Shiro-Kapitan’  | Haplotype b                                                   | *H. albomarginata* | *H. albomarginata* |
| HG18    | Hosta ‘Silver Streak’  | Haplotype b                                                   | *H. albomarginata* | *H. albomarginata* |
| HG19    | Hosta ‘An’             | Haplotype b                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG20    | Hosta ‘Zounds’         | Haplotype j                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG21    | Hosta ‘Patriot’        | Haplotype b                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG22    | Hosta ‘Hadspen Blue’   | Haplotype c                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG23    | Hosta ‘Fortunei Ki-fukurin’ | Haplotype b                                               | *H. albomarginata* | *H. albomarginata* |
| HG24    | Hosta ‘Shirobukurin renge giboushi’ | New haplotype                                             | *H. albomarginata* | *H. albomarginata* |
| HG25    | Hosta ‘Brim Cup’       | Haplotype b                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG26    | Hosta ‘Francee’        | Haplotype b                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG27    | Hosta ‘Janet’          | Haplotype b                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG28    | Hosta ‘June’           | Haplotype c                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG29    | Hosta ‘Night before Christmas’ | Haplotype b                                           | *H. sieboldiana* | *H. albomarginata* |
| HG30    | Hosta ‘Shade Fanfare’  | Haplotype b                                                   | *H. sieboldiana* | *H. albomarginata* |
| HG31    | Hosta ‘Gold Standard’  | Haplotype b                                                   | *H. sieboldiana* | *H. albomarginata* |
Phylogenetic analyses

All sequences were aligned using ClustalW (Thompson et al., 1994), and indistinctly aligned regions caused by indels were corrected manually following the method of Lee and Maki (2013). The cpDNA haplotypes were determined based on these aligned sequences, and a haplotype network based on statistical parsimony was created to evaluate possible relationships among the haplotypes using TCS 1.06 (Clement et al., 2000). All indels were treated as point mutations and weighted evenly with other mutations. All haplotypes of the two Hosta species, *H. sieboldiana* and *H. albomarginata*, detected by Lee and Maki (2013), were also analyzed together with the haplotypes detected in this study.

Results

Chloroplast DNA haplotypes detected in Hosta cultivars

On the basis of approximately 1380 bp of the two noncoding regions of cpDNA, seven substitutions, three indels and 11 types of polyT tracts (repeated sequences of T) were detected among the examined sequences of the *Hosta* cultivars (Table 2). All of the sequences obtained were deposited in DDBJ (accession nos. AB987835–AB987896). A total of 13 haplotypes were detected among these cultivars. We compared the haplotypes of the cultivars to those in natural populations of *H. sieboldiana* and *H. albomarginata*, which were detected by Lee and Maki (2013). The genetic relationships among the haplotypes of the *Hosta* cultivars and two natural populations of both *Hosta* species were revealed using the parsimony network (Fig. 2). We identified two types of origin for the *Hosta* cultivars; some were the same as or similar to *H. sieboldiana* haplotypes, and the others were the same as or similar to

| Haplotype | trnL | rpl32F | trnG-trnS | Comparison with the previous results of Lee and Maki (2013) | Originated species |
|-----------|------|--------|-----------|------------------------------------------------|-------------------|
| HG1       | A A  | T T11' | G I1'     | T A  | T9  | Haplotype q | *H. albomarginata* |
| HG2       | A C  | T T15  | T T10  | G T  | T   | T12 | Haplotype H | *H. sieboldiana* |
| HG3       | C A  | T T10  | C T11  | G A  | T11 | New haplotype | *H. albomarginata* |
| HG4       | A C  | T T13  | T T10  | T T12 | A I3 | T12 | Haplotype F | *H. sieboldiana* |
| HG5       | A A  | T T12  | C T11  | G T  | A   | T11 | New haplotype | *H. albomarginata* |
| HG6       | A A  | T T12  | C T11  | G T  | A   | T10 | Haplotype b | *H. albomarginata* |
| HG8       | A A  | T T11  | C T11  | G T  | A   | T10 | Haplotype c | *H. albomarginata* |
| HG9       | A C  | T T14  | T T10  | G T  | T   | T11 | Haplotype J | *H. sieboldiana* |
| HG12      | A A  | T T11  | C T12  | G T  | A   | T10 | New haplotype | *H. albomarginata* |
| HG14      | A A  | T T14  | T T9   | G T  | A   | T11 | New haplotype | *H. sieboldiana* |
| HG15      | A A  | T T11  | C T11  | G I1  | T A  | T9  | Haplotype v | *H. albomarginata* |
| HG20      | A A  | T T10  | C T10  | G T  | A   | T10 | Haplotype j | *H. albomarginata* |
| HG24      | A A  | C T12  | C T11  | G T  | A   | T10 | New haplotype | *H. albomarginata* |

* T number means “the number of track”.

1, I1, I2, and I3 mean the deleted sequence. 11: deleted sequence=A, I2: deleted sequence=TTAAA, I3: deleted sequence=A.
Among these haplotypes, three and six were the same as *H. sieboldiana* and *H. albomarginata* respectively, while four haplotypes were detected for the first time in this study (Table 2). Among these unique haplotypes, one and three were phylogenetically similar to *H. sieboldiana* and *H. albomarginata*, respectively. Over half of the cultivars (27 out of 31) contained haplotypes of *H. albomarginata* (Table 1). The majority of cultivars had haplotype b, which is found in wild populations in Kinki, Chubu and Hokkaido. Four of the cultivars possessed haplotype c, which has been detected in the Tohoku region. Three other cultivars had haplotypes f, j, and q, which are distributed in the Shikoku, Chubu, and part of the Tohoku regions, respectively. Only three cultivars possessed the haplotypes of *H. sieboldiana* (Table 1); the samples HG2, HG4, and HG8 had haplotypes H, F, and J, respectively. Haplotype H was detected on the Pacific side of the Japanese Archipelago, and haplotype F was found only in Yamagata Prefecture in the Tohoku region. As inferred from the name *Hosta* ‘Sagae’ (HG4), this cultivar originated in Sagae, a city in Yamagata Prefecture. On the other hand, haplotype J was detected from the natural population in the Chubu region, Tokushima and Gifu Prefecture in Honshu. We also found that some of the cultivars contained unique haplotypes that were not detected in the natural populations examined in the previous study. Two cultivars (HG5 and HG24) showed haplotypes closely related to haplotype b and one cultivar (HG3) had a haplotype closely related to haplotype j of *H. albomarginata* (Fig. 2). The haplotype of HG5 was also genetically similar to haplotype t, which was detected only in Fukui Prefecture in Honshu. However, one cultivar (HG14) showed a haplotype closely related to haplotype J of *H. sieboldiana*.

**Comparison between phenetic types and cpDNA haplotype results**

Totals of 21 and 10 cultivars were categorized into *H. sieboldiana* type and *H. albomarginata* type, respectively, based on leaf morphology (Table 1). By contrast, according to the cpDNA haplotype results, only four of 31 cultivars possessed the haplotypes of *H. sieboldiana*. Only 12 cultivars coincided phenetically with the cpDNA haplotypes.

**Discussion**

**Geographic origin of cultivars**

We found that the majority of *Hosta* cultivars had the same haplotypes as found in natural populations of *H. albomarginata* (haplotypes b, c, f, v, and q) and some cultivars had the haplotypes found in natural populations of *H. sieboldiana* (haplotypes H, F, and J). These results suggest that the majority of *Hosta* cultivars investigated in this study were bred from plants that originated from several natural populations of *H. sieboldiana* or *H. albomarginata*.

Some of the haplotypes were not detected in previous phylogeographic studies (Lee and Maki, 2013). It is considered that natural populations with these haplotypes were not sampled in our previous study, or that the *Hosta* cultivars may retain genetic diversity lost from wild populations. Another possible explanation is that spontaneous mutations occurred in the ancestral cultivar and the mutations might have accumulated during breeding in the cultivars. The majority of *Hosta* cultivars contained cpDNA derived from *H. albomarginata*, and cultivars with...
Hosta cpDNA from *H. sieboldiana* were less common, suggesting that *H. albomarginata* may have been used more generally to breed cultivars than *H. sieboldiana*. However, several inconsistencies were observed between the morphological and molecular data. Although the majority of cultivars have cpDNA from *H. albomarginata*, the leaf morphology was more similar to that of *H. sieboldiana*. Using cpDNA results alone, it remains unclear why the majority of cultivars display the *H. sieboldiana* morphology. We could consider three possibilities. First, it is possible that these cultivars were generated through the artificial hybridization between *H. sieboldiana* and *H. albomarginata*, although there are few reports confirming that artificial interspecific hybridization has been practiced in *Hosta* cultivar formation. Second, it is possible that these cultivars hybridized with natural populations during their domestication. Lastly, we could consider the possibility that *H. sieboldiana* and *H. albomarginata* hybridize in nature, as a previous study showed (Takahashi, 2002). *Hosta* cultivars derived from hybrid origins might exhibit inconsistencies between morphological and molecular data, which were shown in this study.

In this study, because we investigated only cpDNA variations, our results reflected the genetic types of the maternal lineages. It is difficult to elucidate the exact origins of the cultivars. Some studies analyzing both cpDNA and nuclear DNA in the cultivars precisely elucidated their origins (Li et al., 2013; Renner et al., 2007; Roullier et al., 2013). Therefore, nuclear DNA variations should be investigated to determine the exact origins of the cultivated *Hosta* species in a future study. Such data may clarify the domestication process of the *Hosta* cultivars in Japan in more detail and explain the inconsistencies between morphological and cpDNA data. Our ongoing study using nuclear DNA data will shed light on these two points.

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