Genetic Differentiation, Molecular Phylogenetic Analysis, and Ethnobotanical Study of *Eutrema japonicum* and *E. tenue* in Japan and *E. yunnanense* in China

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This paper reports the level of genetic differentiation between two Japanese and one Chinese species of *Eutrema*: *E. japonicum*, “wasabi”; its wild relative in Japan, *E. tenue*; and their wild relative in China, *E. yunnanense*. Phylogenetic analyses were based on the DNA sequence of the chloroplast *trnK/matK* region of 16 Brassicaceae and an outgroup species. Neighbor joining (NJ) and maximum parsimony (MP) trees were constructed, revealing that the three *Eutrema* species form a single clade clearly separated from other Brassicaceae species. The two Japanese *Eutrema* species are highly differentiated from Chinese *E. yunnanense*, and it is estimated that they diverged from *E. yunnanense* approximately 5 million years ago. An ethnobotanical survey was conducted among ethnic Chinese in Yunnan Province, and the results indicate that *E. yunnanense* is not perceived as “hot” in taste, while a pungent flavor is associated with wasabi; in addition, no evidence was found for the domestication of *E. yunnanense*. On the basis of the present molecular phylogenetic study and the ethnobotanical survey, we conclude that wasabi acquired its specific pungent flavor during its long botanical history in Japan, and that its subsequent domestication in Japan was because of this acquired pungent flavor. The culinary habit of using wasabi with raw fish has since become an important feature of Japanese cuisine and culture.

Key Words: Brassicaceae, chloroplast DNA, divergence time, Japanese cuisine, *trnK/matK*.

Introduction

Wasabi [Japanese horseradish: *Eutrema japonicum* (Sieb.) Maxim., syn. *Wasabia japonica* (Miq.) Matsum.] is a perennial herb that plays an important role in traditional Japanese cuisine and culture. Historical studies suggest that the earliest record of wasabi as an herb was in the 6th or early 7th century CE in Japan

(Higuchi, 2002), while no historical reference to the use of wasabi in other countries has been noted. Given that wasabi is endemic to Japan, it is assumed that the first cultivation/domestication of wasabi occurred there. Until recently, wasabi remained unknown in Western cultures. In 1902, David Fairchild introduced the plant to the United States of America (Fairchild, 1903), and now, in the early 21st century, wasabi is cultivated in many parts of the world, including the People’s Republic of China (China), Commonwealth of Australia (Australia), New Zealand, Republic of Ecuador (Ecuador), Kingdom of Belgium (Belgique), Socialist Republic of Viet Nam (Viet Nam), on the Pacific Coast of the United States of America, Canada, Kingdom of Thailand (Thailand), Democratic People’s Republic of

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Korea, French Polynesia (Tahiti), Republic of Chile (Chile), Republic of Indonesia (Indonesia), Republic of the Philippines (Philippines), and United Mexican States (Mexico) (Yamane, 2010a, b).

Today, wasabi is customarily consumed as a condiment with raw sliced fish (sushi or sashimi) or cold buckwheat noodles (soba). The pungently flavored condiment is obtained by grating the fresh enlarged rhizome (see Fig. 1). The root of horseradish, *Armoracia rusticana*, is used in a similar manner in Europe. However, excluding Hokkaido, European horseradish is rarely cultivated in Japan. The pungent flavor of both European horseradish and wasabi and their purported beneficial effects come from the hydrolysis of precursor glucosinolates, which releases volatile and biomedically active isothiocyanates. Sultana et al. (2003) reported that the concentration of several isothiocyanates differs between *A. rusticana* and wasabi. Many studies have analyzed the biochemical and pharmaceutical properties

of wasabi, including its pungency (e.g., Hara et al., 2001a, b, 2003; Hou et al., 2000), flavor (Depree et al., 1998), and anti-carcinogenic and anti-inflammatory effects (Kinae et al., 2000; Morimitsu et al., 2000); however, the genetics of the wasabi plant is much less well studied.

Wasabi belongs to the genus *Eutrema*, which is found mainly in East Asia (Al-Shehbaz and Warwick, 2005). *Eutrema* comprises approximately 26 species (Al-Shehbaz and Warwick, 2005), including *Eutrema japonicum* (Fig. 2B and C) and *E. tenue* (Miq.) Makino “yuriwasabi” in Japanese (lily in Japanese is “yuri”, see Fig. 2E). *Eutrema japonicum* and *E. tenue* have been recognized as plants endemic to Japan, and the natural habitat of these two species covers much of the islands of Shikoku and Kyushu. On the basis of field data collected since 2005 from Hokkaido south to Kumamoto, the pungency of wild wasabi varies considerably. *Eutrema tenue* also has similar pungency to wasabi (Yamane, 2010a). However, no evidence has suggested that *E. tenue* was grown domestically for its pungent taste. This could reflect the different morphologies of the rhizomes of *E. japonicum* and *E. tenue* (Fig. 2). In particular, the rhizome of *E. tenue* is neither enlarged nor “tenuous”.

Recently, the ribosomal DNA sequences of *E. japonicum*, *E. tenue*, and several Brassicaceae species were determined and used to analyze the phylogenetic relationships among *Eutrema* species (Warwick et al., 2006). The results showed that *E. japonicum* and *E. tenue* were the closest phylogenetic neighbors of *E. yunnanense*, with whom they formed a single clade. *Eutrema yunnanense* is found in China and it is morphologically very similar to *E. japonicum* (see Fig. 2A and B). Actually, in 2006, the first author checked of all the *Eutrema* herbarium specimens at Beijing and Kunming Institutes of Botany, Chinese Academy of

Fig. 1. At a specialty shop, wasabi was sold in Nagano Province, Japan, for 2000 yen for three rhizomes.

Fig. 2. Three *Eutrema* species in China and Japan: (A) *Eutrema yunnanense* in Xinzhong at Lijiang, Yunnan, China, (B) wild *E. japonicum* in Niigata Prefecture, Japan, (C) cultivated *E. japonicum* ‘Masamidori’ in Shizuoka Prefecture, Japan, (D) enlarged rhizome of cultivated *E. japonicum*, and (E) *E. tenue* in Shimane Prefecture, Japan.

Sciences. It was confirmed that *E. yunnanense* is more similar to wasabi than other *Eutrema* species. However, no data have been published about the level of differentiation between wasabi and *E. yunnanense*, or their divergence time.

The goal of this study was thus to investigate the level of differentiation and time since the divergence of *E. japonicum*, *E. tenue*, and *E. yunnanense*. To this end, the DNA sequences were compared in the *trnK/matK* region of the chloroplast genome (cpDNA) of *E. japonicum*, *E. tenue*, *E. yunnanense*, and 19 Brassicaceae species including European horseradish, *Armoracia rusticana*. The construction of plant phylogenetic trees using chloroplast DNA regions has been described previously (reviewed by Olmstead and Palmer, 1994). In particular, the *trnK/matK* region is very useful because it is easily co-amplified with the flanking non-coding *trnK* intron, which is known to have a high mutation rate. As a consequence, the data allowed phylogenetic analysis at the inter- and intra-species levels. The results presented here describe the origin and the specific domestication of wasabi in Japan. However, it remains unclear why *E. japonicum*, wasabi, is the only *Eutrema* species cultivated domestically in Japan. The results were verified by conducting an ethnobotanical survey of residents of Yunnan

Province, China, in which information was gathered on the use and characteristics of *E. yunnanense*.

Materials and Methods

Plant materials

The strains analyzed in this study include 19 Brassicaceae, identified as economically important species by Koch et al. (2003) (Table 1). The species include: *E. japonicum* (wasabi or Japanese horseradish), *E. tenue* (yuriwasabi), *E. yunnanense*, and species from the genus *Brassica* (e.g. cabbage, black mustard, rape), *Lepidium sativum* L. (cress, peppergrass), *Armoracia rusticana* (horseradish), *Raphanus sativus* L. (radish), *Sinapis alba* L. (white mustard), *Nasturtium officinale* R. Br. (watercress), *Cochlearia officinalis* L. (spoonwort), and *Eruca vesicaria* (Mill.) Cav. (rocketsalad). Seed samples of 12 species were from the Seed Bank of the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany (Table 1). The two major cultivars of wasabi, 'Mazuma' and 'Fujidaruma', were selected from the preservation lines at Izu Agricultural Research Center, Shizuoka Prefectural Research Institute of Agriculture and Forestry. *Eutrema tenue* (Miq.) Makino, yuriwasabi in Japanese, was collected from its natural populations by the first author, and *Armoracia rusticana* from a weedy population in Japan,

Table 1. Plant materials of wasabi and other Brassicaceae species used in this study.

Species	Accession No.	Country ("Locality" or 'Cultivar')	Source
<i>Eutrema japonicum</i>	EJ/2006/Mazuma_a	Japan, 'Mazuma'	Izu Agricultural Research Center, Shizuoka Prefectural Research Institute of Agriculture and Forestry
	EJ/2006/Fujidaruma_a	Japan, 'Fujidaruma'	Izu Agricultural Research Center, Shizuoka Prefectural Research Institute of Agriculture and Forestry
<i>Eutrema tenue</i>	ET/2006/Takao_a	Japan, "Tokyo"	Native to Japan
	ET/2006/Hirao_a	Japan, "Fukuoka"	Native to Japan
<i>Eutrema yunnanense</i>	EY/2007/Xinzhu_a	China, "Yunnan_a"	Native to China
	EY/2007/Ludian_a	China, "Yunnan_b"	Native to China
<i>Armoracia rusticana</i>	AR/2006/Kazuno_a	Japan, "Akita"	Naturalized population in Japan
<i>B. rapa</i>	CR1021/95	Unknown	IPK ^z
<i>B. oleracea</i>	BRA530/95	England	IPK
<i>B. nigra</i>	CR1203/97	Unknown	IPK
<i>B. juncea</i>	CR76/90	Unknown	IPK
<i>B. napus</i>	CR162/95	Unknown	IPK
<i>B. carinata</i>	BRA927/00	Ethiopia	IPK
<i>Sinapis alba</i>	CR2165/97	Unknown	IPK
<i>Isatis tinctoria</i>	ISA 23/03	Tajikistan	IPK
<i>Lepidium sativum</i>	LEP44/92	Georgia	IPK
<i>Nasturtium officinale</i>	NAS/100	Unknown	IPK
<i>Cochlearia officinalis</i>	COCH7/03	Unknown	IPK
<i>Eruca vesicaria</i>	ERU17/81	Italy	IPK
<i>Raphanus sativus</i>	—	—	GenBank accession No. AB354257
<i>Aethionema coridifolium</i>	—	—	GenBank accession No. AP009366
<i>A. grandiflorum</i>	—	—	GenBank accession No. AP009367

^z Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany.

in 2006 (Table 1). In this study, wild wasabi was not investigated because it is very difficult to predict a “true” wild population of wasabi. Young leaves were harvested from an arbitrarily chosen individual plant grown in a greenhouse, and total DNA was extracted from the leaves by the CTAB method, as described by Escaravage et al. (1998), with some modifications. Voucher specimens were deposited at the herbarium of Gifu University. *Aethionema cordifolium* DC. and *Ae. grandiflorum* Boiss. & Hohen. were used as outgroup species, based on previous phylogenetic studies (Bailey et al., 2006; Beilstein et al., 2006; Koch et al., 2001).

The trnK/matK sequence analyses

The *trnK/matK* region was amplified using universal primers *trnK*/3914F and *trnK*/2R (Johnson and Soltis, 1995). PCR was carried out with *ExTaq* polymerase (Takara, Shiga, Japan). PCR-amplified DNA fragments were analyzed by direct DNA sequencing. The sequencing primers were as follows: *trnK*677L/R (5'-CAT-AGT-GCG-ATA-CAG-TCA-AA), *matK*209L/R (5'-CAC-AAC-AAA-GAC-GAA-GTA-TA), *matK*1460L/F (5'-ATT-TCC-CTT-TTT-AGA-AGA-CA), *matK*615L/F (5'-CAT-TCT-GGC-AAC-AAA-GGA-TA), *matK*144L/F (5'-AAA-AAG-GTT-GGG-CTC-TGG-TT), *matK*1480K/R (5'-TGT-TGA-TCG-TAA-ATG-AGA-GG), *trnK*1626L/F (5'-GAG-CTT-CCA-TTC-GTA-ATT-TGA), *matK*1466K/R (5'-TCA-TAA-GAT-GTC-GAT-CGT), and *trnK*5' 601F (5'-CCT-ATT-AAA-GAT-AGA-GAA-TCC). Products of the DNA sequencing reaction were analyzed on a 3100 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The sequence data of outgroup species and *Raphanus sativus* were cited from GenBank (sequence identification numbers) of DNA Data Bank of Japan (DDBJ): *A. cordifolium* (GenBank. AP009366), *Ae. grandiflorum* (GenBank. AP009367), and *R. sativus* (GenBank. AB354257). Nucleotide sequences were aligned manually using DNASIS version 3.0 (Hitachi Software Engineering, Tokyo, Japan) with manual modifications to minimize the number of gaps. DnaSP version 3.00 was used to estimate the net number of nucleotide substitutions between two populations (D_A) (Nei, 1987). All insertions and deletions (indels) were treated as the result of missing characters, and therefore excluded from the analysis. After excluding all indels, phylogenetic relationships were analyzed using the neighbor joining (NJ) (Saitou and Nei, 1987) and maximum parsimony (MP) methods with PAUP* version 4.0b10 (Swofford, 2002). The number of nucleotide substitutions per site was estimated by Kimura's two-parameter method (Kimura, 1980) and used to estimate genetic distance between species. For MP analyses, all sites were weighted equally and unordered character states were used (i.e., Fitch parsimony). The heuristic search option was used with a stepwise addition sequence set at random; branch-swapping method tree

bisection-reconnection (TBR) and MAXTREES were set to autoincrease. The reliability of the clades on the shortest tree(s) was assessed on 1000 replicates using bootstrapping with PAUP (Felsenstein, 1985). Nucleotide sequences were deposited in DDBJ/EMBL/GenBank databases under the accession numbers GBAN-LC064384 to GBAN-LC64402.

The following two methods were used to estimate time of lineage divergence: 1) Nei's 1987 equation ($T = D_A/2\lambda$), where λ is the rate of nucleotide substitution per site per year and D_A is the number of net nucleotide substitutions for the *matK*-gene-coding region between two given taxa; and 2) Bayesian inference framework as implemented in the program BEAST v. 1.8.0 (Drummond and Rambaut, 2007). Xml files for the BEAST analyses were constructed using BEAUti v. 1.8.0. The BEAST analyses rate variation as described in Drummond et al. (2006) under an uncorrelated relaxed clock drawn from a lognormal distribution that allows different rates to be optimized independently on each branch of the phylogenetic tree (Drummond and Rambaut, 2007). Calculations were based on 5×10^7 generations, with parameters sampled every 1000 generations using a Yule tree prior and a general time-reversible (GTR) evolutionary model. Tracer v. 1.6 (Rambaut and Drummond, 2009) was used to evaluate and ensure the convergence and the effective sample size (ESS) values, density plots, and trace plots. Model comparison was conducted by calculation of Bayes factor on the basis of the relative marginal likelihoods of the models under comparison (Suchard et al., 2001). The program TreeAnnotator v. 1.8.0 was used to obtain the maximum clade credibility tree from the postburn-in trees to determine the 95% posterior density of ages for all nodes in the tree (Arias et al., 2014).

Field survey

Interviews with ethnic minorities about the cultivation and culinary use of *E. yunnanense* in China were conducted in 1997. The ethnobotanical study sampled 35 individuals in 4 (self-identified) ethnic minorities (Yi, Bai, Naxi, and Lisu) at 5 villages, namely, “Xinzhu” at Lijiang, “Ludian” at Lijiang, “Yongchun” at Weixi, “Xiyuchun” at Heqing, and “Wuwei” at Dali in Yunnan Province (Fig. 3). These locations are selected based on the information of whole herbarium specimens of *Eutrema* in Beijing (PEK) and Kunming (KUN), at the Institutes of Botany, Chinese Academy of Sciences. As a result, the locations are close to natural populations of *E. yunnanense*.

Results

The *trnK/matK* region was analyzed in 19 Brassicaceae accessions, excluding *Raphanus sativus* and two outgroup taxa. The length of the analyzed region varied from 2188 to 2224 bp, and included 29 indels of variable length (1–20 bp). A total of 424

variable sites were identified, 240 of which were phylogenetically informative. The DNA sequences of *trnK/matK* in *E. japonicum* and *E. tenue* were identical, with the exception of one nucleotide position (= singleton at one accession in *E. tenue*), where a base substitution was observed. The DNA sequences of *E. yunnanense*-a and -b *trnK/matK* were also identical, with the exception of one nucleotide position, where a base substitution was also observed. In contrast, twenty-six autapomorphic nucleotide substitutions exist between

Fig. 3. The location of our field survey regarding *Eutrema yunnanense* in Yunnan Province, China. Black circles represent the location of hearing investigations.

the three *Eutrema* species and other Brassicaceae species. Moreover, *trnK/matK* in *E. japonicum* and *E. tenue* differed from *trnK/matK* in *E. yunnanense* at 13 nucleotide positions, suggesting greater genetic distance between *E. yunnanense* (from China) and the other two *Eutrema* species (from Japan), than between *E. japonicum* and *E. tenue*.

The phylogenetic relationships of three *Eutrema* species and several Brassicaceae species were analyzed, and the results are depicted in a neighbor joining (NJ) tree (Fig. 4). The maximum parsimony (MP) method was also used, generating a single shortest tree (data not shown) with relatively high consistency (CI = 0.8682) and retention indices (RI = 0.9038). This result suggests low homoplasy and high reliability (data not shown). Comparing NJ and MP trees, the only incongruence was a slight difference in position of *L. sativum*, but this had no impact on the phylogenetic position of wasabi (data not shown).

The phylogenetic relationships among Brassicaceae plants reported here, excluding *Eutrema*, are consistent with previous chloroplast DNA sequence-based phylogenetic studies. In particular, *Brassica* species were divided into two groups, *B. nigra* was closer to *Sinapis alba* than to other Brassicaceae, *Raphanus sativus* was close to *Brassica* species (Warwick and Black, 1997; Warwick et al., 1992; Yang et al., 2002), and *Armoracia rusticana* was phylogenetically close to *Nasturtium officinale* (Bailey et al., 2006). Consequently, the phylogenetic tree deduced here is highly plausible, and we present it with confidence. The phylogenetic distance (i.e., genetic divergence) between the two proposed *Eutrema* lineages was estimated using two methods. The first method employed Nei's equation, $T = D_A / 2\lambda$

Fig. 4. Neighbor joining tree based on Kimura 2-parameter distances using the *trnK/matK* sequences of three *Eutrema* species and Brassicaceae species, as indicated. Bootstrap values (%) for 1000 replicates are shown above the branch; values less than 50% are not shown.

(Nei, 1987), where λ is the rate of nucleotide substitutions per site per year and D_A is the number of net nucleotide substitutions for the *matK*-gene-coding region between the two taxa. Here, D_A of *Eutrema japonicum* and *E. tenue* is 0.0087, relative to *E. yunnanense*, and λ was reported to be 1.3×10^{-9} /synonymous site per year (Chase et al., 1993; Zurawski and Clegg, 1987), based on the *rbcL* region, and an adjusted K_s ratio (0.528 for *matK* vs. 0.372 for *rbcL*) (Drouin et al., 2008). Using this method, the estimated time since the *Eutrema* species in Japan diverged from *E. yunnanense* was estimated to be 4.7 million years ago (Mya), in the Late Tertiary. The second method, based on Bayesian inference, estimated that 5.9 million years have elapsed since the Japanese *Eutrema* diverged from *E. yunnanense*.

Ethnobotanical studies were conducted among ethnic minority Chinese in Yunnan Province, China, concerning their use and knowledge of *E. yunnanense*. The study involved interviews with 35 individuals in 4 (self-identified) ethnic minorities (Yi, Bai, Naxi, and Lisu) residing in 5 villages. Awareness of *E. yunnanense* was high (94%) among these individuals. The most common names for *E. yunnanense* were “Shan yu cai” or “Shan bai cai”, and less common names included “O mu ca” (Yi), “O kua che” (Lisu), “O kua” (Lisu), “Bong ning ang” (Yi), “Su ku zhuo” (Bai), “Su Na tu” (Bai), or “Wu sheng cai” (Bai). In contrast, only 7 names for wasabi are known in Japan (Yamane, 2010a). Ethnic minority Chinese collect and eat *E. yunnanense* from wild (i.e., undomesticated) mountain sites at ≥ 2500 meters above sea level. No evidence was found for the domestication of *E. yunnanense* in China. The interviewees stated that aerial portions of *E. yunnanense* are eaten, but not the rhizome of *E. yunnanense*. Consistent with this, the stem and leaf of *E. yunnanense* were seen on sale at a morning market in “Yongchun” at Weixi (Yunnan Province, China, see Fig. 5), at a price of 0.8 yuan per bunch, approximately the cost of one 500 mL bottle of water (cf. 0.8 yuan was equivalent to ≈ 12 Japanese yen in 2007, and 500 mL of bottled water was about 100 yen in 2007). The market value of the rhizome of wasabi in Japan is approximately 50-fold higher, i.e., 600–1000 Japanese yen per purchase (Fig. 1), and even for Japanese, this is an expensive and desirable food item. It is also noteworthy that the leaves, stems, and flowers of wasabi, which share the same hot flavor as wasabi rhizome, are consumed routinely, and these parts of the plant are considerably less expensive than the rhizome (Yamane, 2011). Pickled *E. yunnanense* at “Wuwei” at Dali market in Yunnan Province, China, closely resembles Japanese pickles, and *E. yunnanense* is frequently sautéed or used in broth or soup, which are not common ways to prepare wasabi in Japan. Lastly, we asked ethnic minority Chinese in Yunnan Province “Is *E. yunnanense* hot or not?” The answer to this question was uniformly “No”.

Fig. 5. At a morning market in Yongchun at Weixi, in Yunnan Province, China, the stem and leaf of *E. yunnanense* were sold by Lisu people for 0.8 yuan per bunch. *Eutrema yunnanense* is the left-innermost plant.

(The latter result was confirmed by the first four authors of this study, who are Japanese and Chinese). Therefore, we conclude that Chinese *E. yunnanense* lacks the pungent character of Japanese wasabi, independent of the ethnic background and historical culinary experience of the interviewees.

Discussion

The phylogenetic position of wasabi in Brassicaceae species

Wasabi is a member of the *Eutrema* genus in the Brassicaceae family, which includes > 300 genera and > 3000 species (Al-Shehbaz, 1984). Many Brassicaceae species are agriculturally and economically important, being the sources of spices and condiments, and including horseradish, *Armoracia rusticana* G. Gaertn., B. Mey. and Scherb, black mustard, *B. juncea* (L.) Czern. et Coss., and white mustard, *Sinapis alba*. The comprehensive interspecific phylogeny of the Brassicaceae has been analyzed using molecular approaches (Inaba and Nishio, 2002; Pradhan et al., 1992; Warwick and Black, 1991; Yang et al., 1999). However, only two phylogenetic studies have included *Eutrema* species (*E. halophilum* C. A. Mey. in the work of Bailey et al., 2006; and *E. heterophyllum* W. W. Sm. in that of Beilstein et al., 2006), the phylogenetic positions of *Eutrema* species are poorly characterized. The present study addresses this gap in knowledge, presenting an NJ tree based on chloroplast DNA sequences in which the cluster of three *Eutrema* species and its “sister” group (including *Brassica*, *Sinapis*, *Raphanus*, *Eruca*, and *Isatis*) have bootstrap values = 100%. Furthermore, twenty-six autapomorphic nucleotide substitutions were identified among the three *Eutrema* species and the other Brassicaceae species, and the *Eutrema* branch in the NJ/MP trees is longer than other branches in the tree

(Fig. 4 and data not shown). The results suggest that 1) *Eutrema* species diverged from other Brassicaceae species including European horseradish; 2) the two Japanese *Eutrema* species, *E. japonicum* and *E. tenue* form a single clade with a bootstrap value = 100%; and 3) *E. yunnanense* diverged from the clade of two Japanese *Eutrema* species.

Genetic divergence of E. japonicum, E. tenue, and E. yunnanense

The two Japanese *Eutrema* species are highly differentiated from Chinese *E. yunnanense*, and it is estimated that the Japanese *Eutrema* species diverged from *E. yunnanense* 4.7 Mya (using Nei's equation) or 5.9 Mya (using Bayesian inference). Because these two estimates of divergence time are similar, the data and its interpretation, as presented here, are credible. Although the estimated time since the divergence of these species may be overestimated by DNA sequence-based methods (Pulquério and Nichols, 2007), it is likely to be accepted that wasabi has occupied a unique genetic background for an extended period of time. This is in turn consistent with the fact that wasabi and its wild relative are endemic to Japan. In contrast, the low number of DNA sequence differences identified in *trnK/matK* in *E. japonicum* and *E. tenue* suggest a much shorter time since the divergence of these two *Eutrema* species. This result is consistent with the idea that *E. tenue* could be an important genetic resource for wasabi breeding.

Ethnobotanical viewpoint of three Eutrema species in China and Japan

Historical studies suggest that the earliest record of wasabi in Japan is from the 6th to early 7th century CE, the Asuka period (Higuchi, 2002). In an old city in Nara Prefecture, an archeologist found a buried wood strip described as “wasabi”, indicating that the term “wasabi” is at least 1400 years old. The earliest culinary use of wasabi may have been as an ingredient in soup in the latter half of the Kamakura period (13th century). Wasabi has been known as a condiment for sushi or soba in Japan since the Edo period (18th and 19th centuries). With this background, the domestication and subsequent cultivation of native wasabi in Japan might have begun in the first half of the Edo period at Utogi (Shizuoka Prefecture), given its abundance of spring water, which made it possible to mass-produce wasabi products for commercial use in Edo City. Eventually, wasabi became an essential ingredient in Japanese cuisine.

Here, one question arises: Why is *E. japonicum*, wasabi, the only *Eutrema* species cultivated for agricultural purposes in Japan? As mentioned above, the earliest utilization of wasabi is dated at > 1400 years ago. Hodge (1974) and Chadwick et al. (1993) reported on the ethnobotany of wasabi in England, but very little is

known about the present and historical use of *E. yunnanense* in China.

On the basis of our interviews with 35 ethnic minority Chinese in Yunnan Province, China, we conclude that the level of awareness of *E. yunnanense* is extremely high. However, no evidence was found for the domestication of *E. yunnanense*, suggesting that *E. yunnanense* is harvested by ethnic minority Chinese in Yunnan Province from its natural habitat in the mountains there.

It is clear that the rhizome of wasabi was a prime target for domestication, and that the freshly grated enlarged rhizome of wasabi was valued for its pungent flavor. It seems remarkable that *E. japonicum*, wasabi, is widely recognized as “hot”, in Japanese and Western cultures, where it has become very popular in the context of Japanese cuisine. These observations are consistent with the hypothesis that the pungent flavor and property of Japanese *Eutrema* correlates with the presence of genetic traits that are not present in Chinese *Eutrema* (i.e., *E. yunnanense*). Interestingly, it is also conceivable that genetic differences between the two species, which may have evolved over many millions of years, are partly responsible for the culinary and other cultural differences between China and Japan.

In future studies, we would like to identify specific genetic traits that are linked to the prized culinary properties of wasabi, including the pungent flavor and “hot”-ness of the wasabi rhizome. In parallel, we will investigate the more general relationships between regional plant diversification and the cultures and cuisines of China and Japan.

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