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Cryptic invasion by non-native reed canary grass, *Phalaris arundinacea*, in Japan endangers native conspecific populations through displacement and hybridization

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Abstract

Invasions by non-native conspecific populations can result in genetic disturbance and even local extinction of native populations. In cases of intraspecific cryptic invasion, where distinction between native and non-native individuals by morphology is difficult, it is challenging to delineate the extent of invasion and assess its ecological impact. *Phalaris arundinacea* is a cosmopolitan grass species native to Eurasia and North America. Although it is also native to Japan, commercial strains from the USA have been introduced and grown for nearly 150 years. The cryptic spread of these non-native genotypes has raised concerns regarding their impact on native populations. Here, we investigated the distributions of native, non-native, and admixed *P. arundinacea* in eastern and northern Japan. We collected over 270 individuals in the Hokkaido, Tohoku, Kanto, and Koshinetsu regions and analyzed genome-wide single nucleotide polymorphisms and chloroplast sequences. Nearly all individuals north of 39.7°N were genetically identified as purely non-native, whereas admixed individuals were widespread south of this latitude. These findings suggest that the effect of non-native *P. arundinacea* on native individuals varies by region: native individuals have been largely displaced in the Hokkaido and northern Tohoku regions and genetically introgressed in the rest of the Tohoku region. Furthermore, in the Kanto region, pure native genotypes were detected only in a restricted area. Native populations in both eastern and northern Japan are at substantial risk of local extinction.

Keywords

Alien species, genetic disturbance, SNP, admixture analysis

Introduction

Invasive alien species are major drivers of biological extinctions (Bellard et al. 2016; Roy et al. 2024). They degrade native communities through mechanisms such as predation (Doherty et al. 2016; Loss et al. 2022), competitive exclusion (Porter and Savignano 1990), reproductive interference (Takakura

2013), and altered species interactions (Perkins and Wilson 2005; Spyreas et al. 2010). In addition, when reproductive isolation between native and introduced non-native species is incomplete, non-native genotypes may introgress into the native gene pool. This genetic disturbance can harm native populations by reducing hybrid offspring fitness (Keller et al. 2000; Muhlfeld et al. 2009; Ryan et al. 2009) and by facilitating the replacement of pure native populations with hybrid swarms (Kawamura et al. 2001; Prentis et al. 2007). Therefore, invasion by non-native species or populations with distinct phenotypic and genetic characteristics poses a substantial threat to the persistence of native taxa.

When an introduced species is cosmopolitan and conspecific native populations exist in the range to which it is introduced, detecting the establishment and subsequent spread of the non-native genotypes becomes challenging. This phenomenon is referred to as intraspecific cryptic invasion (Morais and Reichard 2018). This issue is particularly relevant when local strains possess traits of economic value, as such strains may have been transported intentionally across geographic barriers and subsequently become naturalized as invasive alien species. *Phalaris arundinacea*, a grass species native to both Eurasia and North America (Voshell and Hilu 2014), exemplifies this scenario. Agricultural and ornamental strains have been selectively bred from local populations and introduced into various regions. In the USA, *P. arundinacea* was introduced from Europe and has since become widely established as an invasive species across the country (Jakubowski et al. 2014).

In Japan, *P. arundinacea* is native to wetlands and riverbanks. However, commercial strains have been introduced from the USA to the northern regions of Japan, which contains 94% of the total area of pasture in Japan (Ministry of Agriculture, Forestry and Fisheries 2024). At present, non-native populations derived from the commercial strains are believed to be naturalized in Hokkaido (Hokkaido Government 2004) and other areas of northern Japan. Nevertheless, owing to the morphological similarity between native and non-native genotypes, distinguishing populations remain challenging.

Consequently, the geographic distribution of non-native *P. arundinacea* in Japan has yet to be clearly determined. The extent to which hybridization is occurring is also unknown; if it is progressing, measures will need to be taken promptly to conserve the populations of native strains.

Genetic markers, such as organelle haplotypes and single nucleotide polymorphisms (SNPs), have been employed to distinguish cryptic non-native individuals from native ones (Comtet et al. 2015; Morais and Reichard 2018). Analyses of organelle haplotypes have been used to reveal not only the presence and geographic distributions of non-native strains of various species (Saltonstall 2002; Mergeay et al. 2005; Mabuchi et al. 2008) but also the maternal parents of hybrid individuals (Li et al. 2017; Jeong et al. 2022). Furthermore, the analysis of codominant markers (e.g., microsatellites and SNPs) has provided insights into admixtures between native and non-native individuals (Hsu et al. 2024; Ironside et al. 2025; Rusterholz and Baur 2025). Thus, organelle and nuclear genomes provide different types of information. These molecular approaches have proven effective in characterizing cryptic invasions by non-native *P. arundinacea* in the USA (Lavergne and Molofsky 2007; Jakubowski et al. 2014). Analyzing both organelle and nuclear DNA together would help to assess the invasion status of non-native *P. arundinacea* in Japan.

Here, we analyzed the genome-wide SNP markers and chloroplast sequences obtained from three imported commercial strains (as non-native references), one domestically selected ornamental strain (as a native reference), and over 270 field-collected *P. arundinacea* individuals to assess (1) the geographic distribution of non-native individuals, and (2) the extent of hybridization between native and non-native individuals. In addition, we aimed to identify genomic regions that could be used reliably to distinguish native individuals from non-native and admixed individuals. Through these analyses, we characterize the cryptic invasion by non-native *P. arundinacea* in Japan and thus provide a foundation for the conservation of native Japanese *P. arundinacea* populations.

Materials and Methods

Study species

The study species, *P. arundinacea* (Poaceae) (Fig. 1), is a cool-season, perennial, rhizomatous geophyte widely distributed in the Northern Hemisphere (Apfelbaum and Sams 1987; Casler 2010; Voshell and Hilu 2014). In Japan, *P. arundinacea* was originally distributed on riverbanks, in wetlands, and around irrigation canals nationwide. It is a wind-pollinated species and has strong self-incompatibility, resulting in a high outcrossing rate (Casler 2010). Diploid and hexaploid *P. arundinacea* are known in some local populations, but tetraploid *P. arundinacea* is widespread throughout its entire species range worldwide (Voshell and Hilu 2014), and a flow cytometry study revealed that the individuals occurring in eastern and northern Japan are tetraploid (Akiyama et al. 2015). The species propagates via hydrochory of seeds and by rhizomes; the seeds can be dispersed along water channels for many kilometers (Casler 2010).

Phalaris arundinacea has been used globally for a variety of medicinal, agricultural, and environmental purposes. In Japan, artificially bred, commercial strains of *P. arundinacea* were first introduced most likely in the late 19th century from the USA for use in pastoral agriculture as forage for livestock (Tsuji 1905). Since its initial introduction, it has been grown widely, especially on poorly drained pastures in eastern and northern Japan, because of its high tolerance to anaerobic soils and its vigorous vegetative reproduction capacity. Several agricultural cultivars have been adopted, and some of them have been designated as “encouraged cultivars” for dissemination by several prefectural governments, including Niigata in Koshinetsu and Hokkaido (Agriculture, Forestry and Fisheries Research Council 1985). The species has also been used for erosion control (Utaka et al. 2023).

Although the industrial strains introduced from overseas have not yet been distinguished from the native Japanese strains, Akiyama et al. (2015) suggested that they are genetically different from the native strains originally occurring in Japan. Therefore, genetically distinct non-native strains may be

spreading in the natural environment and mixing with native strains, given that individuals that have escaped from cultivation have already become widely naturalized.

Samples analyzed

We analyzed 340 *P. arundinacea* individuals collected from eastern and northern Japan—namely from the Hokkaido, Tohoku, Koshinetsu, and Kanto regions (Fig. 2; Table S1). Most samples were collected in the field between 2016 and 2024, but others were obtained from the Genebank of the National Agriculture and Food Research Organization, Japan (accession numbers: JP41017–JP41024, JP41027, JP41028, JP41030–JP41037, JP41039–JP41043, JP236449, JP236454, JP236455, JP236458–JP236461). Field sampling was conducted along riversides and roadsides near paddies, which are the main habitats of this species. Collected samples were transported to the laboratory, where live individuals were either immediately used for DNA extraction or stored at –20 °C until DNA extraction. In addition to the field-collected samples, we analyzed four commercially distributed strains: Palaton, Vantage, Canada Common, and Towada-ashi. The first three are North American strains that were introduced into Japan for agronomic and soil management purposes. Towada-ashi is an ornamental strain selected from a native Japanese *P. arundinacea* population.

Chloroplast typing and restriction-site-associated DNA sequencing

Genomic DNA was extracted from two or three leaf segments (2 to 3 cm^2 each) per individual in DNAs-ici! S buffer (Rizo Inc., Tsukuba, Japan). The extracted DNA was used for both chloroplast genotyping and library preparation of double-digest restriction-site-associated DNA sequencing (ddRAD-seq).

ddRAD libraries were prepared by following the standard protocol described by Peterson et al. (2012), with minor modifications. *Eco*RI-HF and *Pst*I-HF (New England BioLabs, Ipswich, MA, USA) were used for double digestion. The resulting ddRAD libraries were sequenced on NovaSeq 6000, HiSeq X, or NextSeq 1000 sequencers (Illumina, San Diego, CA, USA). The sequencing reads were

deposited in the DNA Data Bank of Japan (DDBJ accessions DRR710785–DRR711088).

For the analysis of chloroplast sequences, partial sequences of the *trn*T–*trn*L region were amplified with primers CAT TAC AAA TGC GAT GCT CT (forward) and TCT ACC GAT TTC GCC ATA TC (reverse) (Taberlet et al. 1991). We amplified the region using EmeraldAmp PCR master mix (TaKaRa, Shiga, Japan). The reaction solution consisted of 10 μL of 2× premix, 1 μL of primer mix (5 nM each), 6 μL of pure water, and 3 μL of template DNA solution. The thermal conditions were 94 °C for 3 min; 30 cycles of 94 °C for 20 s, 55 °C for 20 s, and 72 °C for 40 s; and a final 72 °C for 7 min. To determine whether the region could be used to distinguish native from non-native individuals, we first constructed a genealogy from 74 selected samples. The PCR products were purified with NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany). Sequence reactions and sequencing were conducted at Fasmac Co., Ltd. (Atsugi, Japan) To infer the genealogy, the sequences obtained were aligned in SeaView 5 software (Gouy et al. 2009). We then removed sites at which at least one sample had a nucleotide insertion and deletion gap. A maximum likelihood tree was inferred in PhyML (Guindon et al. 2010) implemented in SeaView 5 software. We used GTR+I+G with optimization of nucleotide equilibrium frequencies as a substitution model. In this phylogenetic analysis, we included Palaton, Vantage, Canada Common, and Towada-ashi, as well as some possible native, non-native, and admixed samples suggested by the admixture analysis from the genome-wide SNP dataset (see below). We included *Torreyochloa pallida* (NCBI GenBank accession NC_027486.1) and *Calamagrostis epigejos* (DDBJ accession LC885479) as an outgroup. Because we found length differences between possible native and non-native individuals in the *trn*T–*trn*L region, we conducted chloroplast typing of the other samples by gel electrophoresis of the PCR products. We used *a priori* native and non-native types of amplicons as size standards in each batch of electrophoresis. Sequences were deposited in DDBJ (accessions LC885412–LC885487).

159 ***Variant calling and conversion to binary data***

160 We used the Stacks pipeline (Catchen et al. 2013) and Fastp (Chen et al. 2018) for demultiplexing,
161 quality control, clustering, and variant calling. Raw ddRAD-seq data were first demultiplexed by using
162 the process_radtags module of Stacks with the options -r, -c, and -q. Subsequently, stricter quality
163 filtering was applied in Fastp. In this step, 30 bases were trimmed from the 3′ end of each read. This was
164 followed by trimming of low-quality ends until the base quality score reached ≥20. Reads were
165 discarded if the average quality of four consecutive bases was ≤15, or if the length was <70 bp. Poly-G
166 tails were also removed. The filtered reads were then processed sequentially by using the Stacks
167 modules ustacks, cstacks, sstacks, tsv2bam, and gstacks for variant calling. Biallelic SNPs with a
168 missing data rate of ≤30% were exported as a VCF file from the populations module of Stacks.
169 Individuals with >30% missing SNP data were excluded from the dataset in PLINK v. 1.9 software
170 (Purcell et al. 2007). Finally, genotype data were converted to binary format (i.e., presence/absence of
171 the minor allele), as both native and non-native *P. arundinacea* populations in Japan are tetraploid
172 (Akiyama et al. 2015) and accurate recovery of tetraploid genotypes is challenging with our method.
173 This binary conversion approach was used in a study of North American *P. arundinacea* (Jakubowski et
174 al. 2013).

175 ***Genetic relationships among individuals***

176 To examine genetic relationships among individuals, we conducted a phylogenetic analysis based on the
177 SNP data (not binary data). The SNP dataset was first converted into DNA sequence format in
178 vcf2phylip software (DOI: 10.5281/zenodo.2540861), in which heterozygous sites were encoded as
179 degenerate bases. The ML tree was inferred in IQ-TREE v. 2.1.2 software(Minh et al. 2020). The best-
180 fit substitution model was selected in ModelFinder software (Kalyaanamoorthy et al. 2017) and
181 corrected for ascertainment bias. To assess the robustness of the inferred tree, we performed ultrafast

bootstrap approximation (Hoang et al. 2017) with 1000 replicates.

Admixture analysis

To examine the presence of individuals admixed between native and non-native strains, we estimated individual admixture proportions by using the binary dataset in STRUCTURE v. 2.3.4 software (Pritchard et al. 2000; Falush et al. 2007). The analysis used the admixture model with ploidy set to 4. We performed 300 000 Markov chain Monte Carlo iterations: 100 000 iterations for burn-in and 200 000 iterations for sampling. The analysis was performed five times for each value of K ranging from 1 to 9, and the results of these analytical replications were summarized in CLUMPP software (Jakobsson et al. 2007). The optimal number of clusters (K) was determined by using the Evanno method (Evanno et al. 2005), as implemented in STRUCTURE Harvester v. 0.6.91 software (Earl and von Holdt 2012). Some studies have suggested that the Evanno method is susceptible to evenness of samples (Evanno et al. 2005; Puechmaille 2016); the results of STRUCTURE based on the full dataset showed that >35% of our samples were classified to a cluster. To avoid this problem, we used the method of Puechmaille (2016): samples were grouped into four populations according to the region from which they were collected (i.e., Hokkaido, Tohoku, Kanto, and Koshinetsu). We also calculated ΔK by using a reduced dataset in which the estimated clusters were contained more evenly (Puechmaille 2016). In addition, we conducted a PCA (principal component analysis) based on the binary dataset in the R package pcaMethods (Stacklies et al. 2007).

Results

Variant calling from the ddRAD-seq data yielded SNP data comprising 5990 loci from 271 individuals. Insufficient RAD tag recovery prevented SNP calling in 34 out of the total of 305 individuals. The optimal number of genetic clusters (K) was estimated to be 2 by both Puechmaille's method (i.e., MedMed K, MedMean K, MaxMed K and MaxMean K) and the Evanno method (ΔK = 18.56). The

non-native strains (i.e., Canada Common, Palaton, and Vantage) were assigned to the same genetic cluster (cluster A), with Q-values exceeding 0.99, whereas the native strain Towada-ashi was assigned to the other cluster (cluster B), with a Q-value of 0.87 (Fig. 3). In addition we found a substantial number of individuals admixed between clusters A and B.

Pure and nearly pure cluster-A individuals ($Q > 0.8$ for cluster A; hereafter Q_A) formed a monophyletic group, with the exception of two individuals, although the bootstrap support for the basal node of this group was low (Fig. 4). Similarly, individuals assigned to cluster B were closely related to each other. These individuals of pure and nearly pure clusters A and B were genetically distant from each other (Fig. 4). Most of the individuals admixed among clusters A and B were placed intermediate between them (Fig. 4).

In the PCA plot, individuals of pure cluster A formed a dense cluster, whereas individuals with $Q > 0.8$ for cluster B (hereafter Q_B) were positioned on the opposite side of cluster A along the PC1 axis. The PC2 axis captured the variation within cluster B (Fig. 5). Individuals that had both cluster A and B ancestries were located in intermediate positions among these clusters (Fig. 5).

The majority of individuals analyzed in Hokkaido and northern Tohoku were assigned to pure or nearly pure cluster A. Additionally, some cluster A individuals were found in northern Koshinetsu and southern Tohoku (Fig. 6A). Notably, 87% of the 93 individuals sampled north of 39.7°N latitude had $Q_A > 0.8$. In contrast, individuals with $Q_B > 0.8$ were distributed primarily in the Kanto region, with a few also occurring in Hokkaido, Tohoku, and Koshinetsu. Admixed individuals covered a broad geographic range, though most were located south of 39.7°N.

Alignment of the sequences of the chloroplast *trn*T–*trn*L region revealed a relatively large indel (145 bp) and some short indels in the region. The longer sequences were 706–707 bp long, and the three non-native strains had these long sequences (“L type”). The shorter sequences were 560 bp long, and

Towada-ashi had a short sequence ("S type"). In the inferred genealogy, each L and S type of sequence was a monophyletic group (bootstrap values were 99 for long types and 90 for short ones) (Fig. 7). Most of the individuals in the Hokkaido and northern Tohoku regions were of the L type, whereas the S type was more common in the southern Tohoku, Koshinetsu, and Kanto regions (Fig. 6B). Among cluster A and B individuals, the chloroplast type of the field-collected individuals corresponded mostly with the genomic component suggested by the admixture analysis: Neally all individuals with $Q_A > 0.8$ had long sequences and nearly all individuals with $Q_B > 0.8$ had short sequences (Fig. 8). Among the admixed individuals, 29.8% had long sequences and the other 70.2% had short sequences (Fig. 8).

We identified 20 SNP markers that are potentially able to distinguish individuals with $Q_B > 0.8$ from individuals with $Q_A > 0.8$. Nucleotide substitutions of these markers were almost fixed differently in each group: > 90% individuals in the same group had the same allele. Furthermore, missing rates of these loci were not very high (0–14.9%) (Table S2).

Discussion

Our results show that (1) *P. arundinacea* individuals in eastern and northern Japan are genetically divided into two clusters, with non-native strains distributed especially in Hokkaido and Tohoku; and (2) large-scale hybridization is occurring between clusters. In the following sections, we summarize our results and discuss the invasion by non-native *P. arundinacea*.

Native and non-native strains revealed by genetic analyses

The admixture analysis revealed that the field-collected samples were derived from two ancestral populations. All of the estimators MedMed K, MedMean K, MaxMed K, and MaxMean K suggested K = 2 as the best K. Although the Evanno method suggested 7 as the best K when the whole dataset was used to perform the admixture analysis, this method may be vulnerable to unevenness of samples (Evanno et al. 2005; Puechmaille 2016). Indeed, when a subset dataset with adjusted samples was used,

the Evanno method selected K = 2 as the best K. In addition, these two clusters corresponded, respectively, to non-native populations originating from North America and a Japanese native population, because the reference non-native samples—Palaton, Vantage, and Canada Common—belonged to the same cluster (cluster A), whereas the native reference sample, Towada-ashi, had higher genomic contribution from the other cluster (cluster B; $Q_B = 0.87$). Our results also suggested the existence of individuals admixed between native and non-native *P. arundinacea*. As in our admixture analysis based on genome-wide SNPs, we found that the chloroplast *trn*T–*trn*L region also could be classified into native and non-native types. The non-native reference samples had long sequences in that region, whereas the native reference sample had a shorter sequence. Thus, the L and S types corresponded, respectively, to the non-native and native types of chloroplasts.

Widespread distribution of non-native populations in northern Japan

Our study reveals for the first time that non-native *P. arundinacea* is already widespread in Japan. Although the establishment of non-native individuals in wild habitats has been suspected (Hokkaido Government 2004), the actual extent of their spread has remained unclear owing to a lack of information on the morphological differences between native and non-native *P. arundinacea* in Japan. Our genetic analyses reveal the predominance of non-native individuals in northern Japan. Especially in Hokkaido, most of the individuals analyzed had a non-native type of chloroplast and purely non-native ancestry, and few pure native and admixed individuals were detected (Fig. 3). This situation is reminiscent of that in the USA, where non-native *P. arundinacea* introduced from Europe has also become widespread, with little evidence of hybridization with native populations (Jakubowski et al. 2014; Noyszewski et al. 2021). Given that the introduction of non-native *P. arundinacea* to Hokkaido began in the late 19th century, it appears that these populations have spread extensively over the past 150 years. The distribution of native populations prior to the invasion of non-native populations remains largely

unknown. Native individuals may have been widespread across Hokkaido or may have occurred only locally in a few areas. Consequently, non-native *P. arundinacea* may have either displaced native individuals or colonized areas where native populations were previously absent during its invasion process. In either case, non-native individuals are now widely distributed throughout Hokkaido and are genetically disturbing native populations, indicating a high level of invasiveness. Plant strains that have been artificially selected for commercial purposes can have invasive characteristics (Driscoll et al. 2014), such as prolific seed production and vigorous vegetative reproduction capacity (Casler 2010). In addition, the cool climate of Hokkaido may have been particularly suitable for the growth of non-native *P. arundinacea*, and repeated introductions may have facilitated the successful establishment of non-native populations in northern Japan.

Predominance of admixed individuals in central and southern Tohoku

Although most individuals in Hokkaido and the northern Tohoku region were identified as pure non-native, admixed individuals were more prevalent than pure non-native individuals south of 39.7°N (Fig. 6). There are three possible explanations for this geographic difference: (1) propagule pressure, (2) adaptive advantage of admixed individuals, (3) sampling bias, and (4) historical differences in the distribution of native *P. arundinacea* between regions. These four explanations are not mutually exclusive and may have worked simultaneously.

First, propagule pressure and introduction frequency facilitate the establishment of alien plant species (Sakai et al. 2001; Simberloff 2009). The land area of Hokkaido (77 984 km^2 on the main island) is comparable to that of the Tohoku region (66 889 km^2). However, the total pasture area in Hokkaido (496 400 ha) is ~9× that in Tohoku (55 400 ha) (Ministry of Agriculture, Forestry and Fisheries, 2024). Given this substantial difference, it is likely that both propagule pressure and the extent of the area of introduction of non-native *P. arundinacea* were higher in Hokkaido than in Tohoku. Moreover, there is

297 larger pasture area in the two prefectures in northern Tohoku (Aomori and Iwate; 39 400 ha in total),
298 where more non-native individuals were observed, than in the other prefectures in Tohoku (Akita,
299 Miyagi, Yamagata, and Fukushima; 16 350 ha), suggesting that there is relatively low propagule
300 pressure in central and southern Tohoku. Thus, low propagule pressure may have resulted in a higher
301 proportion of admixed individuals, rather than complete displacement by non-native genotypes.
302 Secondly, admixed strains may have spread in central and southern Tohoku because of the
303 ecological advantage of admixed individuals. The almost complete displacement by pure non-native *P.*
304 *arundinacea* in Hokkaido suggests that they are more adaptive to the cool environment of Hokkaido and
305 northern Tohoku than are native individuals. If this is true, then admixed individuals may be adaptive to
306 the warmer environment in central and southern Tohoku, because admixed individuals may have
307 ecological characteristics that are intermediate between those of native and non-native *P. arundinacea*.
308 Similar hypotheses have been discussed in invasion biology: numerous studies have suggested that
309 hybridization can enhance invasiveness (Ellstrand and Schierenbeck 2000; Lavergne and Molofsky
310 2007; Shi et al. 2018). The geographic distribution of non-native and admixed individuals may be
311 attributable to their ecological differences.
312 Thirdly, we may have missed pure non-native individuals in central and southern Tohoku
313 because of sampling bias. Given that, in theory, hybridization requires the presence of both native and
314 non-native parental individuals, non-native individuals should occur, and breed, with native or admixed
315 individuals. Even if the distribution of non-native individuals is restricted to pasture and managed
316 slopes, they may be able to crossbreed with native or admixed individuals occurring in natural habitats,
317 as grass pollen is generally dispersed over several hundreds of meters (Kanya et al., 2009; Frisk et al.,
318 2023). Indeed, many of the admixed individuals occurring in central and southern Tohoku had the S type
319 (i.e., native type) of chloroplast (Fig. 6), suggesting that non-native strains were the pollen parents of the

admixed individuals. In addition,as about 70% of admixed individuals had native-type chloroplasts (Fig. 8), non-native individuals may become pollen parents more frequently than do native individuals. This type of asymmetric mating pattern may eventually reduce the fitness of native individuals and decrease the population size (Konishi and Takata 2004; Takakura 2013). This may be why the density of native individuals was low in central and southern Tohoku.

Fourthly, geographic differences in the distribution of native populations prior to the invasion by non-native populations may have contributed to the observed geographic bias in the occurrence of admixed individuals. As mentioned above, if native populations had been restricted to a few local areas, non-native populations could have colonized the vacant regions without hybridizing with native individuals. Conversely, if native populations had been widely distributed in areas south of central Tohoku, the invasion of non-native individuals would likely have resulted in a widespread occurrence of admixed individuals.

In summary, we reveal here the genetic admixture between native and non-native *P. arundinacea.* The resulting genetic disturbance in Tohoku raises a critical and interesting question: Why does the status of invasion differ across geographic regions? The above hypotheses (i.e., propagule pressure, adaptive advantage of admixed individuals, sampling bias, and geographic difference in historical distribution) do not contradict each other, but their examination is important if we are to elucidate the invasiveness of non-native *P. arundinacea* in Japan.

Concluding remarks regarding the conservation of native *P. arundinacea* in Japan

Here, we show the cryptic invasion of non-native *P. arundinacea* in eastern and northern Japan. Native *P. arundinacea* populations are scattered across northern Japan and are confined to limited areas of the Kanto region. Moreover, admixed individuals were found close to these native populations (Fig. 6A). Given that *P. arundinacea* is an allogamous species, the remaining native populations are particularly

vulnerable to genetic invasion by non-native or admixed individuals. However, the difficulty in distinguishing native from non-native *P. arundinacea* poses a substantial challenge for conservation efforts. Although genetic analysis can play a crucial role in the identification of native individuals (Morais and Reichard 2018), the techniques required are generally not widely accessible. We identified several SNP loci that may be useful in distinguishing native individuals (i.e., $Q_B > 0.8$) from non-native individuals (i.e., $Q_A > 0.8$) (Table S2). The development of a simplified identification method based on these SNPs could greatly facilitate the conservation of native *P. arundinacea* in Japan.

Our findings suggest that there has been a substantial reduction in the area of distribution of native *P. arundinacea*. Occurring S type chloroplast and individuals of pure native cluster over the range we investigated suggests that native individuals were once widespread in eastern and northern Japan, but its present occurrence is largely confined to concentrated populations in the Kanto region. In Hokkaido, considering the prevalence of non-native individuals, it is even possible that some local populations may have already gone extinct. This reduction of native *P. arundinacea* is apparently attributable to the cryptic invasion of non-native *P. arundinacea*. Thus, non-native *P. arundinacea* exerts negative ecological impacts on native *P. arundinacea*, potentially leading to the loss of locally adapted genetic resources and evolutionary history. To balance the industrial and agricultural use of non-native *P. arundinacea* and the conservation of native *P. arundinacea* populations, it is ultimately important to raise growers' awareness of the invasiveness and risks of commercial non-native strains, thereby helping to prevent their escape from cultivation.

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367 Akiko Tomioka and Miyako Ohkubo for support with the molecular experiments.

368 **Data availability**

369 All sequence data used in the study are available through the DNA Data Bank of Japan
370 (https://www.ddbj.nig.ac.jp/index-e.html) (DRR710785–DRR711088 for sequencing reads obtained on
371 the Illumina platform; LC885412–LC885487 for chloroplast sequences). Chloroplast type data are
372 included in Table S1.

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377 **Competing interests**

378 The authors have no relevant financial or non-financial interests to disclose.

379 **Author contributions**

380 The study was conceptualized and designed by Hiroyuki Shibaike and Satoshi Yamamoto. Materials
381 were prepared by Chika Egawa, Yasuyuki Yoshimura, Hiroyuki Shibaike and Natsumi Ueda. Data were
382 collected and analyzed by Satoshi Yamamoto and Natsumi Ueda. The first draft of the manuscript was
383 written by Satoshi Yamamoto and Chika Egawa, and all authors commented on previous versions of the
384 manuscript. All authors read and approved the final manuscript.

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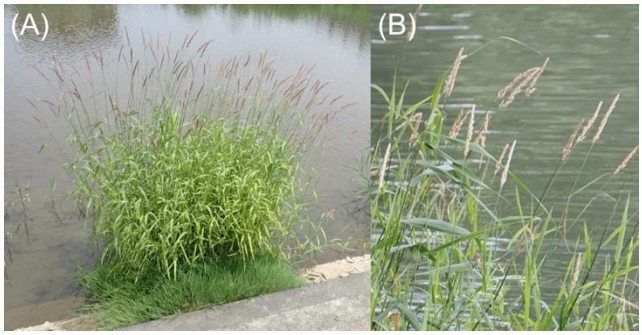


Fig. 1. *Phalaris arundinacea* (A) whole plant, (B) flower spikes.

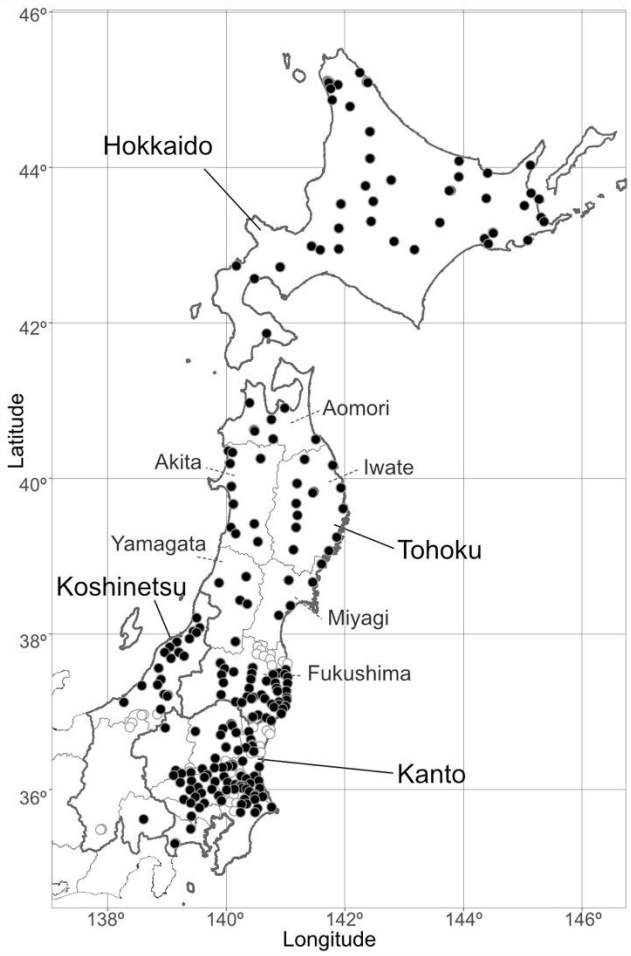


Fig. 2. Sampling sites in eastern and northern Japan. Each circle indicates a sampling position (● used for both admixture analysis and chloroplast typing; ○ used only for chloroplast typing).

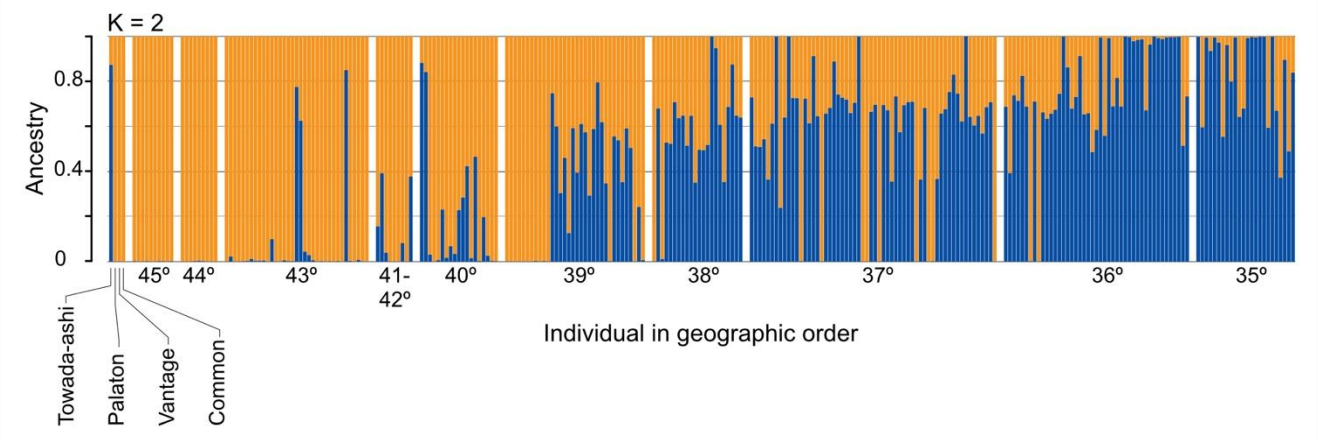
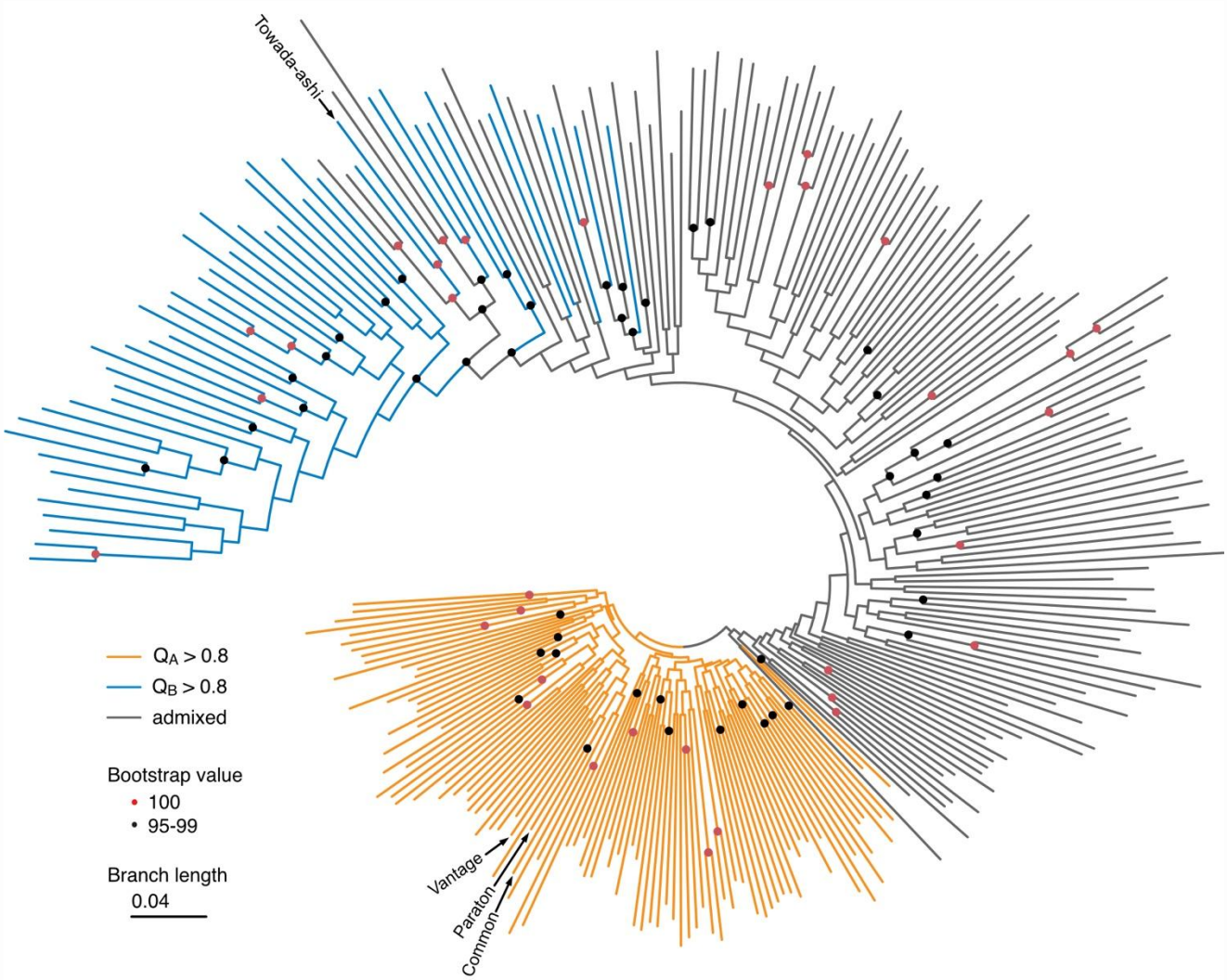


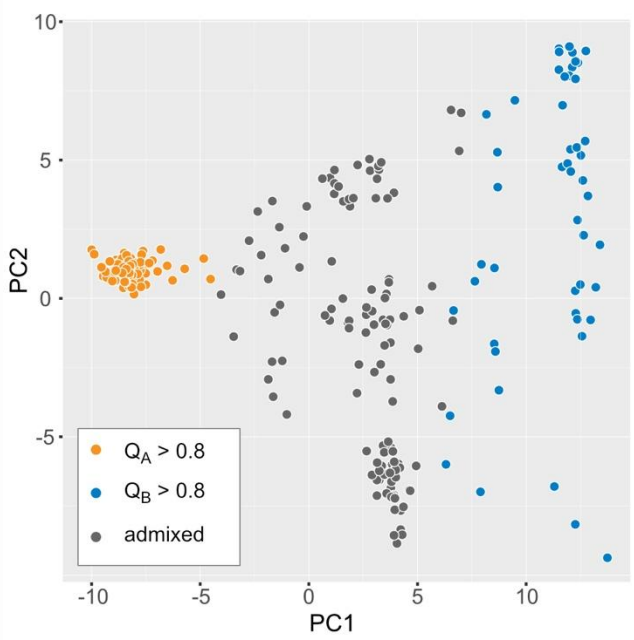
Fig. 3. Bar plot of admixture analysis. Each bar plot indicates an individual analyzed in this study. The first four individuals are of native (Towada-ashi) and non-native (Palaton, Vantage, and Canada Common) strains, and the other individuals are ordered by latitudinal position of sampling locality.



575

576 **Fig. 4.** Maximum likelihood tree inferred from genome-wide SNPs. Colors of branches indicate
577 individuals with $Q_A > 0.8$ (orange) or $Q_B > 0.8$ (blue) and admixed individuals (gray). Classification to
578 these clusters was based on admixture analysis using a whole genome-wide SNP dataset under the
579 assumption of K = 2. The bootstrap value at each node is indicated by a colored circle (red, bootstrap =
580 100; black, 95–99; bootstrap values of nodes without circles were <95).

581



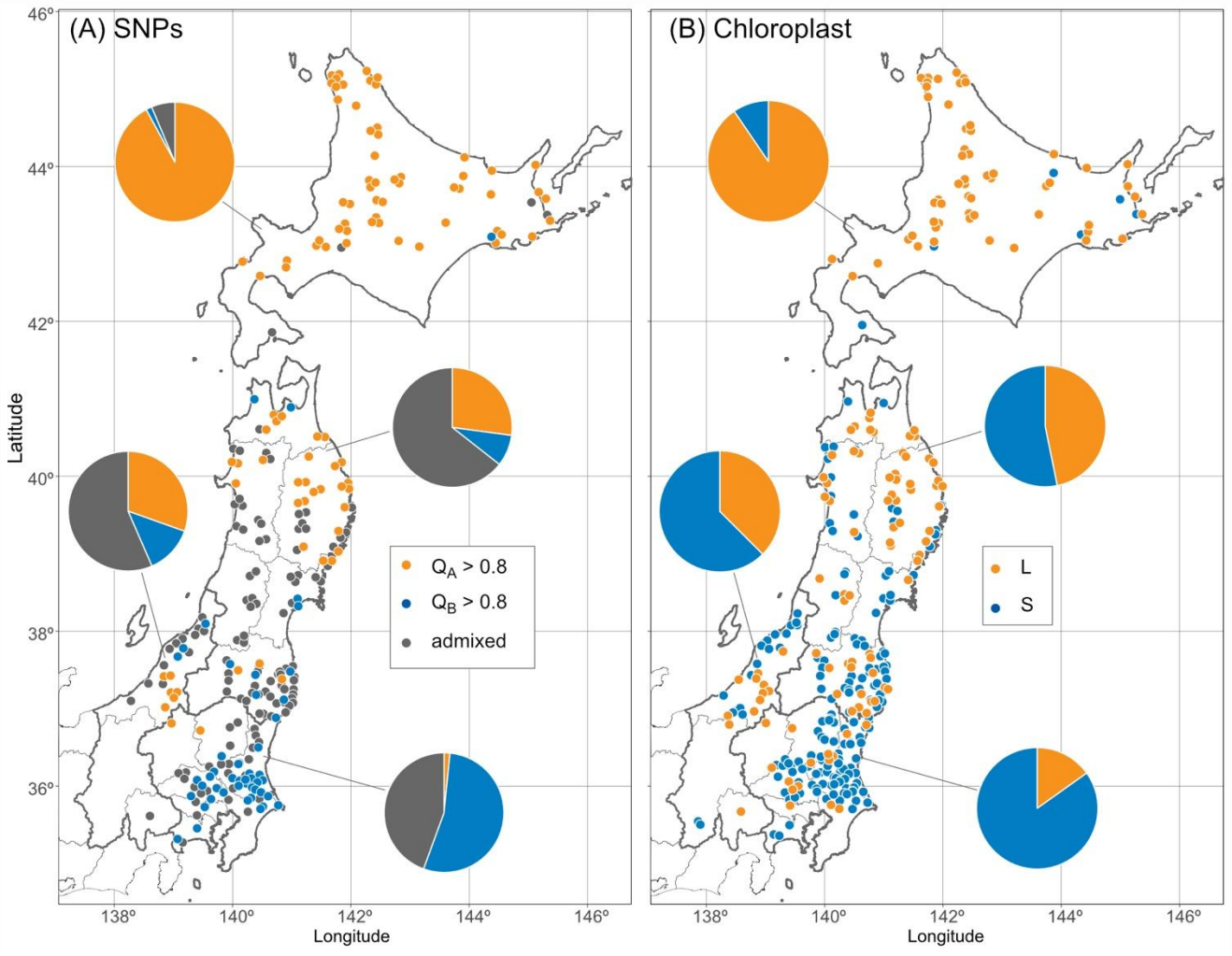
582

583 **Fig. 5.** Principal component analysis plot. Colors indicate Q-values (orange, cluster A; blue, cluster B;

584 gray, admixed individual). Classification to these clusters was based on an admixture analysis using a

585 whole genome-wide SNP dataset under the assumption of K = 2.

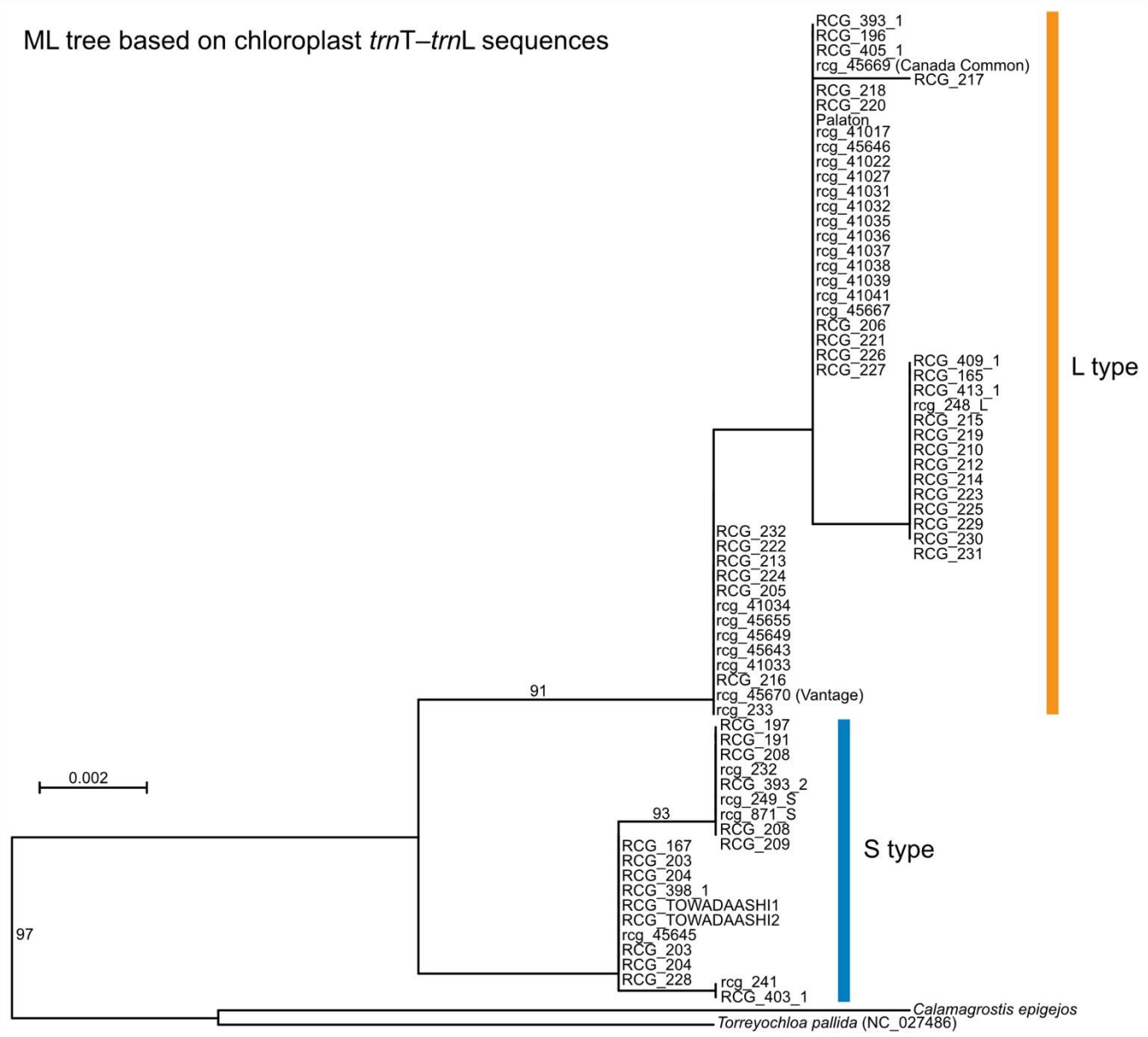
586



587

588 **Fig. 6.** Geographic distribution of genetic characteristics in eastern and northern Japan. (A) Distributions
589 of cluster A (orange), cluster B (blue), and admixed individuals (gray) are shown, and (B) distributions
590 of chloroplast types (L, orange; S, blue) are shown. Position of each circle indicates the sampling site of
591 that individual, although circles may have been moved slightly to show overlapping points.

592



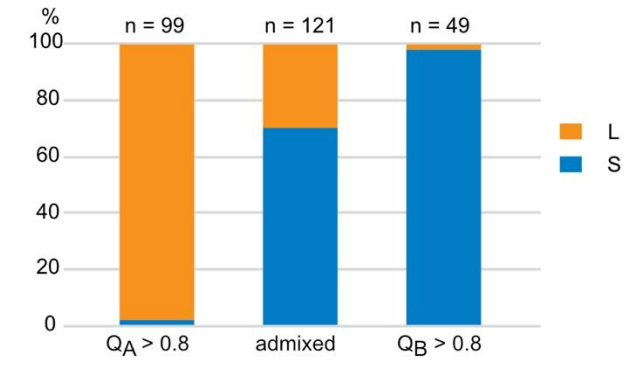
593

594 **Fig. 7.** Maximum likelihood tree based on chloroplast *trn*T–*trn*L sequences. Bootstrap values are

595 indicated on nodes if the value is ≥90. *Torreyochloa pallida* and *Calamagrostis epigejos* were used as an

596 outgroup.

597



598

599 **Fig. 8.** Proportions of chloroplast types (L, long; S, short) in cluster A, cluster B, and admixed

600 individuals.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS10713.xlsx
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