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Differences in the genetic diversity and genome size between two ecotypes of *Imperata cylindrica* in Japan

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Abstract

Understanding genomic characteristics, such as genetic diversity and genome size, is important because they relate to species and ecotype performance. *Imperata cylindrica* has two ecotypes, the common type (C-type) and the early-flowering type (E-type), which differ in their ecological characteristics and distribution ranges in Japan. This study aimed to elucidate the genetic diversity of the C-type and E-type ecotypes of *I. cylindrica* throughout Japan, using the multiplexed inter-simple sequence repeat genotyping by sequencing (MIG-seq) method. Genome-wide single-nucleotide polymorphism data analysis revealed that the C-type had greater genetic diversity and clearer isolation by distance than the E-type. Additionally, the C-type exhibited clear genetic differentiation between the southern part of Amami Oshima and other populations, consistent with differences in its life history. The C-type also had a smaller genome than the E-type, which may contribute to faster plant growth and smaller seed mass, as compared with the larger genome of the E-type. These phenomena were consistent with the characteristics of the C-type. These results showed that the two ecotypes differ genetically, highlighting the necessity for different guidelines for each ecotype in their conservation and use for revegetation.

KEYWORDS

ecotype, genetic diversity, genome size, *Imperata cylindrica*, MIG-seq

1 | INTRODUCTION

Understanding plant genomic characteristics, such as genetic diversity and genome size, is essential because they are related to species and ecotype performance (e.g., niche, spatial distribution, and local adaptation) (Dubin et al., 2015; Kawakatsu et al., 2016; Suda et al., 2015). Population genetic diversity can be used to estimate population dynamics and local adaptation (Nomura et al., 2022; Pfeilsticker et al., 2023). Genome

size variation can also be used to estimate the ecological and life history strategy of a species or a population because genome size is correlated with cell size, cell cycle duration, stomatal density, seed mass, and relative growth rate (Beaulieu et al., 2008; Francis et al., 2008; Suda et al., 2015). For example, species or lineages with smaller genome sizes tend to be more invasive (Meyerson et al., 2020; Pyšek et al., 2018; Schmidt et al., 2017; Suda et al., 2015) because a small genome may promote rapid growth and development. Revealing the genetic diversity

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and genome size variation in lineages (Dubin et al., 2015; Kawakatsu et al., 2016; Suda et al., 2015) with widespread and limited distributions can provide insights into the factors causing differences in spatial distributions over a landscape in plants.

In this study, we focused on *Imperata cylindrica* (L.) Raeusch (cogongrass). This species is a perennial rhizomatous grass with a self-incompatible and wind-pollinated reproduction system that is native to the tropical and subtropical areas of the Northern and Southern Hemispheres, including Japan (Holm et al., 1977).

Imperata cylindrica populations in Japan consist of two ecotypes: the common type (C-type) and the early-flowering type (E-type) (Matumura & Yukimura, 1980; Mizuguti et al., 2003; Tominaga et al., 1989). These ecotypes have $2n = 20$ or 40 chromosomes (Tominaga et al., 2007). The C-type and E-type are sometimes referred to by their variety, var. *koenigii* and var. *cylindrica*, respectively (Tominaga et al., 2007). The C-type has hairy culms, hairy leaf sheathes, small aerenchyma leaf midribs, and small aerenchyma rhizomes, and the E-type has hairless culms, waxy leaf sheathes, large aerenchyma leaf midribs, and large aerenchyma rhizomes (Nomura et al., 2024; Tominaga et al., 2007). The distribution of these two ecotypes outside of Japan is unknown, but they are probably distributed at least in Taiwan, based on morphological evidence (Cheng & Chou, 1997). The E-type flowers appear approximately 1 month earlier than the C-type flowers. Therefore, these two ecotypes are reproductively isolated from each other owing to flowering phenology (i.e., prezygotic isolation).

The two ecotypes are distributed in different habitats: the C-type is mainly found in dry habitats (e.g., roadsides and levees of paddy fields), and the E-type is mainly found in wet habitats (e.g., marshy areas and moist fallow fields). Consistent with the habitats and aerenchyma size, the C-type performs better than the E-type in dry conditions, and the E-type performs better than the C-type in wet conditions (Miyoshi & Tominaga, 2017; Nomura et al., 2024). The E-type grows exclusively in relatively narrow and moist habitats and shows more obvious local adaptations than the C-type, which grows in extensive and continuous habitats (Nomura et al., 2018). Additionally, the C-type shows greater biomass and a faster relative growth rate than the E-type under dry conditions (Matumura et al., 1984; Nomura et al., 2024), and the C-type performs better than the E-type in a wide range of temperature environments under dry conditions (Nomura et al., 2018). Based on these characteristics, the C-type appears to be more invasive than the E-type under dry conditions.

Despite the different flowering times of the ecotypes, F1 hybrids between the C-type and E-type are

occasionally produced (Nomura et al., 2022). The F1 hybrids are distributed mainly in the Tohoku region and form some small populations in the Kinki region (Nomura et al., 2022) (Figure S1). The flowering time of the F1 hybrids is in autumn, 5–6 months later than the parental ecotypes, resulting in reproductive isolation between the F1 hybrids and parental ecotypes. The F1 hybrids are distributed in a wider range of soil moisture conditions than the two ecotypes, which was suggested to be due to the large plasticity in the rhizome aerenchyma formation of F1 hybrids (Nomura et al., 2024).

These two ecotypes have been reported to have various ecological differences, but evolutionary knowledge is scarce, including genetic diversity, population genetic structure, and genome size. Investigating genetic diversity using individuals collected over a wide range is essential to elucidate the evolutionary history. Allozymes (Mizuguti et al., 2004), chloroplast DNA (Nomura et al., 2015; Yasuda & Shibayama, 2003), and 12 nuclear markers (Nomura et al., 2022) have shown genetic differentiation between the two ecotypes. However, genetic differentiation between regions in each ecotype has not been clarified. The widespread use of next-generation sequencing (NGS) has made obtaining large amounts of genetic diversity data at relatively low cost possible (Peterson et al., 2012; Suyama et al., 2022; Suyama & Matsuki, 2015). Multiplexed inter-simple sequence repeat genotyping by sequencing (MIG-seq), which is a polymerase chain reaction (PCR)-based NGS method for constructing highly reduced representation libraries without restriction enzyme digestion steps, can be applied to field samples containing low-quality and/or small amounts of DNA (Suyama & Matsuki, 2015).

In this study, the genetic diversity of *I. cylindrica* was investigated throughout Japan by using the MIG-seq method. Owing to the difference in spatial distribution between the two ecotypes, the C-type is predicted to exhibit greater genetic diversity than the E-type and spatial genetic cline. Because of the difference in distribution breadth and invasiveness, the C-type may have a smaller genome size than the E-type. Therefore, we also measured the genome size of the two ecotypes. This study will clarify genetic diversity and genome size differences between ecotypes.

2 | MATERIALS AND METHODS

2.1 | Plant materials, DNA extraction, library preparation, and sequencing

A total of 365 accessions of cogongrass (C-type, 194 accessions; E-type, 95 accessions; and F1 hybrids,

76 accessions) were collected throughout Japan from the 1980s to the 2010s (Table S1). Genomic DNA was extracted from the leaves by using the modified cetyltrimethylammonium bromide method (Murray & Thompson, 1980). Approximately 50–100 mg of fresh leaf per sample was used, and approximately 5–20 µg DNA was extracted from the samples.

The MIG-seq method was used for library preparation for NGS (Suyama & Matsuki, 2015). The first PCR amplifications were performed in a 7-µL solution containing 1 µL extracted DNA (2.5–25 ng), 2.24 µL 16 multiplexing primers (10 µM) listed in a previous study (Suyama & Matsuki, 2015), 0.225 µL nuclease-free water, 3.5 µL 2 × Multiplex PCR buffer (Mg2+, dNTP plus), and 0.035 µL Multiplex PCR Enzyme Mix in the Multiplex PCR Assay Kit ver.2 (TAKARA Bio Co. Ltd., Japan). The first PCR program consisted of an initial denaturation at 94°C for 1 min, followed by 25 cycles at 94°C for 30 s, 38°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min.

The second PCR amplifications were performed in a 12-µL solution containing 2.5 µL diluted 50-fold first PCR products, 1.2 µL forward (Set-F1 and F2) and reverse (Set-R2 and R3) second PCR primers (2 µM) listed in a previous study (Suyama et al., 2022), 0.96 µL dNTP (2.5 mM each), 3.5 µL distilled water, 2.5 µL 5 × PrimeSTAR GXL Buffer (Mg2+ plus), and 0.24 µL PrimeSTAR GXL DNA Polymerase (TAKARA Bio Co. Ltd., Japan). The second PCR program consisted of 12 cycles at 98°C for 10 s, 54°C for 15 s, and 68°C for 1 min.

Polymerase chain reaction products from the second amplifications were pooled and purified by mixing with an equal volume of AMPure XP (Beckman Coulter, Inc., USA). Short (< 300 bp) and long (> 800 bp) fragments were removed from the purified second PCR products by using an E-Gel SizeSelect II (Thermo Fisher Scientific, Inc., Japan). The size-selected library was concentrated by mixing 1.8 times the volume of AMPure XP into the library.

The size-selected library was quantified using KAPA HiFi HotStart ReadyMix (Roche Diagnostics, Inc., Japan) and LightCycler 480 II (Roche Diagnostics, Inc., Japan). Paired-end sequencing was conducted on a mixture of 8 pM library and 2 pM PhiX by using MiSeq (Illumina, Illumina K.K. Inc., Japan) with the MiSeq Reagent Kit v3 (150 cycles, Illumina, Illumina K.K. Inc., Japan).

2.2 | Preprocessing of NGS data and single-nucleotide polymorphism (SNP) calling

Illumina adapter removal, low-quality filtering ($Q < 20$), and cutting to the same length (70 bp) of the NGS data

were performed using Trimmomatic-0.33 (Bolger et al., 2014). By using all accessions, SNP calling was performed using Stacks v.2.60 (Rochette et al., 2019). Loci were assembled de novo by using the *denovo_map.pl* pipeline with the following parameters: The minimum depth of coverage (m) and number of mismatches allowed between stacks within individuals (M) were set to 3 and 2, respectively. The number of mismatches allowed between stacks of individuals (n) was set to 1. Loci were extracted using a *populations* pipeline with the following parameters: Minimum percentage of individuals in a population required to process a locus for that population (r), minimum number of populations in which a locus must be present to process a locus (p), and minimum minor allele frequency required to process a nucleotide site at a locus (*min-maf*) were set to 0.8, 1, and 0.01, respectively. The minimum percentage of individuals across populations required to process a locus (R) was set to 0.8 or 0.5. The number of populations was set to 3, corresponding to the C-type, E-type, and F1. One SNP was selected for all loci using the parameter *-write-single-snp*. Single-nucleotide polymorphism calling was also performed under the aforementioned settings by using only the C-type or E-type accessions.

2.3 | Statistical analysis of NGS data

The genetic distance matrix was calculated using R 4.1.0 (R Core Team, 2021) based on SNP data. The calculations used were from a previous study (Smouse & Peakall, 1999) and were identical with the method used in GenAlEx (Peakall & Smouse, 2012). A distance matrix was used to perform a principal coordinate analysis (PCoA) for all accessions. Additionally, accessions with a proportion of null loci >30% were excluded from the analysis when $R = 0.8$. When $R = 0.5$, accessions with a proportion > 70% were excluded from the analysis. In analyses other than PCoA, accessions with a high proportion of null loci were excluded. Principal coordinate analysis was also performed using SNP calling data for only the C-type or E-type.

The optimal number of clusters (K) was estimated using the software STRUCTURE 2.3.4 (Pritchard et al., 2000). Ten independent runs were performed for each K from 1 to 10 and were conducted with 25 000 Markov chain Monte Carlo (MCMC) steps, following the burn-in 10 000 MCMC steps. The optimal K was estimated using log likelihood ($\ln(P(X|K))$) and Evanno ΔK (Evanno et al., 2005). For the two ecotypes and F1, except for the null-rich accessions, the observed heterozygosity (H_o), expected heterozygosity (H_s), Wright's fixation index (F_{is}), averaged allelic richness (AR), and rate of polymorphic

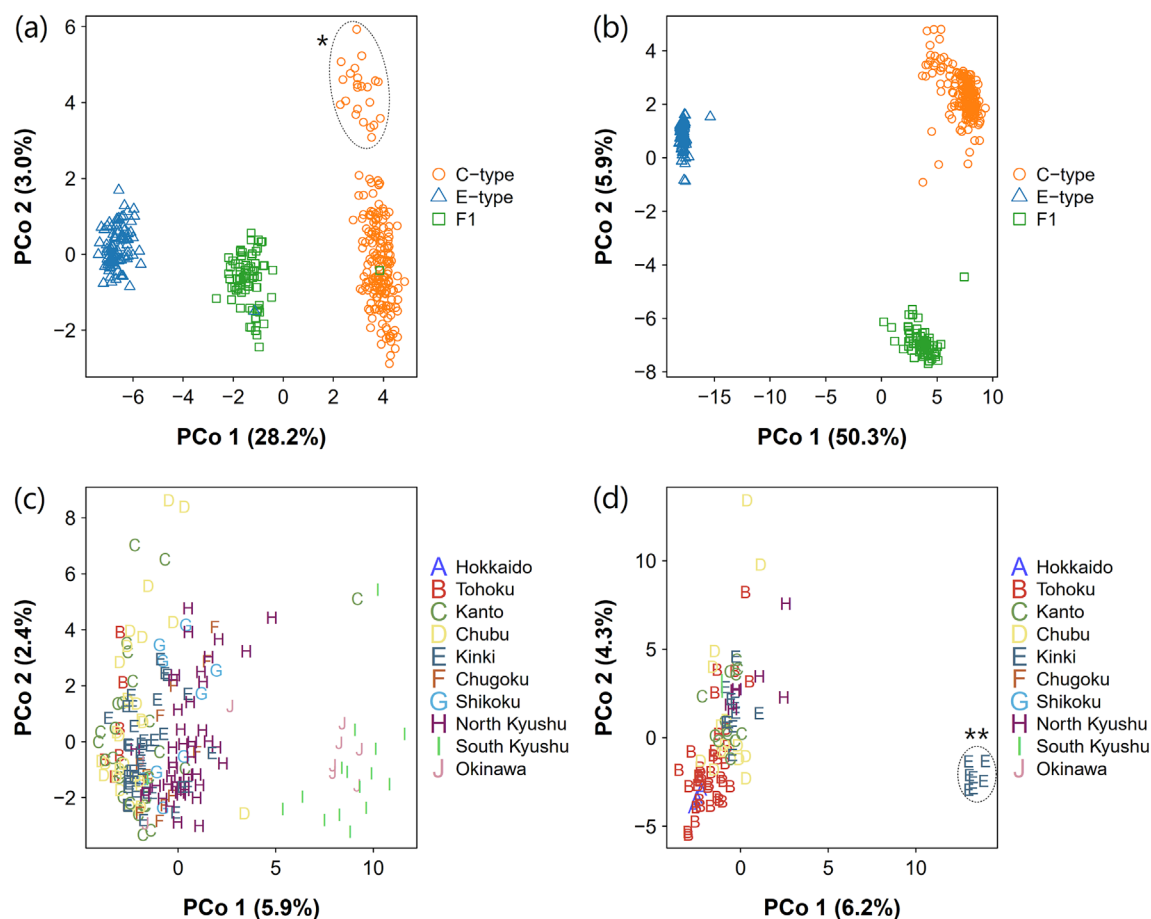


FIGURE 1 Principal coordinate analyses (PCoA) show genetic differentiation of *Imperata cylindrica*. (a) All accessions, except for accessions with a percentage of null loci over 30%. The minimum percentage of individuals across populations required to process a locus (R) was set to 0.8. The asterisk represents the Nansei population. (b) All accessions, except for accessions with a percentage of null loci over 70%. R was set to 0.5. (c) The C-type, except for the C-type with a percentage of null loci over 30%. R was set to 0.8. (d) The E-type, except for the E-type with a percentage of null loci over 30%. R was set to 0.8. Double asterisks represent the Yodogawa River accessions.

sites (RPS) were calculated using R package “hierfstat” (version 0.5–10) (Goudet, 2005; Goudet & Jombart, 2021). The “spdep” (version 1.2–4) and “spatialreg” (version 1.2–3) packages (Bivand, 2022; Bivand et al., 2021) were used for latitudinal and spatial autocorrelation effects, respectively, on H_o , H_s , F_{is} , AR , and RPS . For this analysis, the prefectures whose borders touched each other were considered contiguous. Analysis of molecular variance (AMOVA) was conducted for each ecotype by using “poppr” (version 2.9.3) (Kamvar et al., 2014, 2015). The hierarchical structure for estimating genetic diversity consisted of islands (Hokkaido, North Honshu, Izu-Ogasawara, South Honshu, Shikoku, Kyushu, and Nansei), regions (Hokkaido, Tohoku, Kanto, Izu, Ogasawara, Chubu, Kinki, Chugoku, Shikoku, Kyushu, Amami, and Ryukyu), and prefectures (Table S1). Mantel tests between genetic and geographic distances were performed using “ade4” (version 1.7-18) (Dray & Dufour, 2007) with 9999 permutations. The genetic distance was defined as it is in GenAlEx (Peakall &

Smouse, 2012). The geographic distance between sampling points was calculated using the “geosphere” (Hijmans et al., 2005) based on latitude and longitude.

2.4 | Genome size estimation and statistical analysis

The DNA content of *I. cylindrica* was estimated using a Quantum P flow cytometer (Quantum Analysis GmbH). The C-type, E-type, and F1 genotypes were tested in 27, 18, and 17 accessions, respectively. Young leaf tissues ($\sim 1 \text{ cm}^2$ of each) from *I. cylindrica* and *Oryza sativa* (“Nipponbare” cultivar, 389 Mbp) (Kawahara et al., 2013), used as an internal standard, were chopped together with a razor in an extraction buffer (Quantum Stain NA UV 2 component A, Quantum Analysis GmbH, Germany) on a glass petri dish. The lysates were stained using a staining reagent containing DAPI (Quantum Stain NA UV

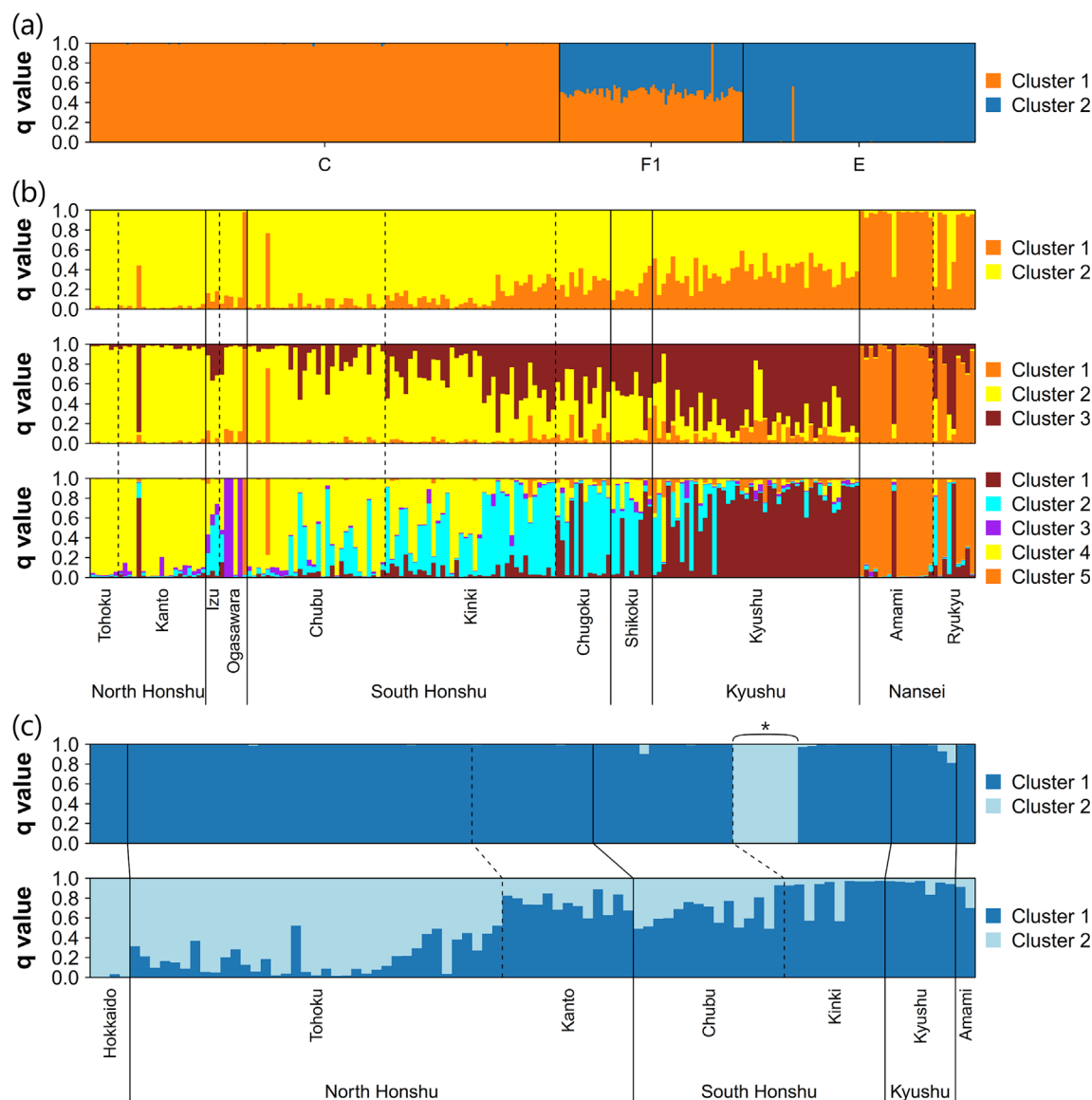


FIGURE 2 STRUCTURE analyses show genetic differentiation of *Imperata cylindrica*. (a) All accessions, except for those with a percentage of null loci over 30%. The minimum percentage of individuals across populations required to process a locus (R) was set to 0.8. (b) The C-type, except for those with a percentage of null loci over 30%. R was set to 0.8. The top, middle, and bottom are at $K = 2$, $K = 3$, and $K = 5$, respectively. (c) The E-type, except for those with a percentage of null loci over 30%. R was set to 0.8. The top includes the Yodogawa River accessions. An asterisk represents the Yodogawa River accessions. The bottom excludes the Yodogawa River accessions.

2 component B, Quantum Analysis GmbH, Germany). The number of nuclei was counted based on fluorescence intensity. Because the genome size of *I. cylindrica* is larger than that of *O. sativa* (Burrell et al., 2015), the peak with the relatively small genome size was designated as *O. sativa*. The genome size of *I. cylindrica* was estimated from the ratio of the mean values of each peak.

A linear model was used to test whether the genome size differed significantly among the groups (C-type, E-type, and F1 hybrids). Significant pairwise differences between ecotypes were identified using post hoc tests for the linear model (Tukey's Honestly Significant Difference

[HSD] test). All analyses were performed using R 4.1.0 (R Core Team, 2021). Tukey's HSD test was performed using the package "multcomp" (Hothorn et al., 2008).

3 | RESULTS

3.1 | MIG-seq

The sequencing of 364 accessions resulted in an output of 41.5 M reads. In the *denovo_map.pl* pipeline, 319 SNPs were obtained when $n = 1$, $r = 0.8$, and $R = 0.5$, and

152 SNPs were obtained when $R = 0.8$. The same pipeline was run for the C-type and E-types alone. For $n = 1$, $r = 0.8$, and $R = 0.8$, 460 and 532 SNPs were obtained for the C-type and E-type, respectively.

3.2 | Genetic differentiation among ecotypes and F1

The optimal number of clusters for STRUCTURE was estimated as $K = 2$, based on the two criteria $\text{Ln}(P(X|K))$ and Evanno ΔK (Figure S2). PCoA and STRUCTURE showed clear genetic differentiation between the C-type and E-type (Figures 1, 2, S3). The hybrids between the C-type and E-type were likely to be F1 hybrids because the posterior probability q of assignment to parental ecotypes was close to 0.5. These results were consistent with those reported by Nomura et al. (2022) and supported the results using over 100 molecular markers. Analysis using 152 SNPs at $R = 0.8$ showed two accessions whose genotypes were inconsistent with the previous study (Nomura et al., 2022) (Figure 1a). These accessions were consistent with the previous study that analyzed 319 SNPs at $R = 0.5$ (Figure 1b). Distinct differentiation was detected along PCo 1 at $R = 0.5$ between the E-type and the other groups (Figure 1b). When $R = 0.5$, loci present within an ecotype but not shared among ecotypes can be detected. Of the 319 loci, the C-type had all loci, and the E-type had 159 loci (i.e., the remaining 160 loci were null loci). F1 had 299 of the 319 loci. The difference in the number of null loci may reflect an imbalance in the number of C-type and E-type accessions.

All genetic diversity indices calculated using 152 SNPs indicated that the C-type had higher genetic diversity than the E-type (Table 1). In addition, F1 showed the highest genetic diversity among the groups. The reason for the negative value of F_{is} in F1 is that F1 is heterozygous at the locus where the C-type and E-type are genetically differentiated.

3.3 | Genetic differentiation within each ecotype

The optimal number of clusters was set to $K = 2$ based on Evanno ΔK for the 460 SNPs in C-type (Figure S4). PCoA and STRUCTURE revealed distinct genetic differentiation between the Nansei and other populations (Figures 1a,c, 2b, 3a–c). STRUCTURE also suggested a latitudinal genetic cline in the C-type. Furthermore, consistent results were obtained across the 10 runs at $K = 3$ and $K = 5$ (Figures 2b, 3b,c). The Nansei population was shown as a distinct genetic cluster from the other populations at $K = 3$ and $K = 5$. At $K = 5$, the Kyushu population was shown as a distinct genetic cluster from other

TABLE 1 Summary of genetic diversity on average of each ecotype for 152 SNP sites.

Ecotype	N	Ho	Hs	Fis	AR	RPS
E	95	0.052	0.066	0.209	1.492	0.572
C	194	0.118	0.140	0.158	1.687	0.724
F1	76	0.253	0.190	−0.327	1.857	0.868

Abbreviations: AR, allelic richness; F_{is} , Wright's fixation index within populations; H_o , observed heterozygosity; H_s , expected heterozygosity; N, number of accessions; RPS, rate of polymorphic sites.

populations. The Ogasawara population was also likely differentiated from the other populations. Latitudinal genetic cline was found at $K = 3$ and $K = 5$, such as at $K = 2$ in North and South Honshu regions. Within the cline, genetic differences existed between eastern and western populations, with the Chubu and Kinki regions as the boundaries. Consistent with this result, the pairwise geographic and genetic distances were significantly correlated (Figure 4; Mantel test, $p < 0.001$).

For the C-type, we calculated the genetic diversity indices for each prefecture and considered their relationship with latitude (Table S2). The effects of latitude were significant for H_s , H_o , AR, and RPS (Figures 5, S5, Table S3; $p < 0.05$). The effect of spatial autocorrelation was also significant for H_s (Figure S5, $\rho = 0.351$, $p < 0.05$). These results indicated that genetic diversity was low in the northern populations. When we hierarchically calculated fixation indices, genetic differentiation was greater among islands than among regions or prefectures (Table 2). Analysis of molecular variance showed a significant genetic differentiation in all hierarchies (Table 3, $p < 0.05$).

The optimal number of clusters was set to $K = 2$ based on Evanno ΔK for the 532 SNPs in the E-type (Figure S6). Principal coordinate analysis and STRUCTURE indicated distinct genetic differentiation between the Yodogawa River population and other populations (Figures 1d, 2c). Excluding the Yodogawa River population, the optimal number of clusters was also set to $K = 2$ based on Evanno ΔK (Figure S7), and STRUCTURE suggested a latitudinal genetic cline of the E-type (Figures 2c, 3d). Within the cline, genetic differences existed between northern and southern populations, with the Kanto and Chubu regions as the boundaries. At $K = 3$ –10, no consistent results were obtained across the 10 runs. Similar to the C-type, the E-type showed a significant correlation between geographic and genetic distances (Figure 4; Mantel test, $p < 0.01$).

Genetic diversity indices for the E-type were calculated for each prefecture, and their relationship with latitude was determined. The effect of spatial

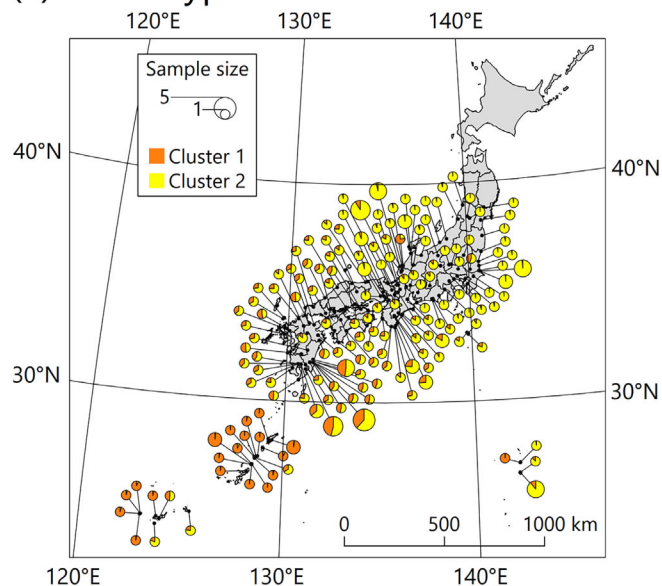
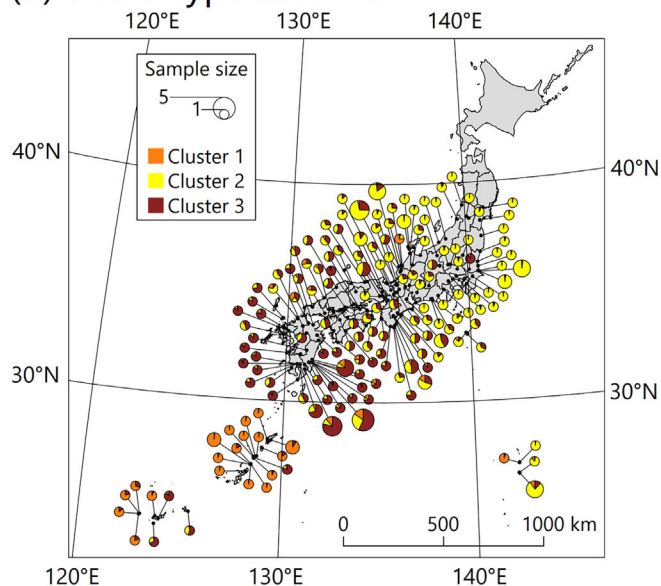
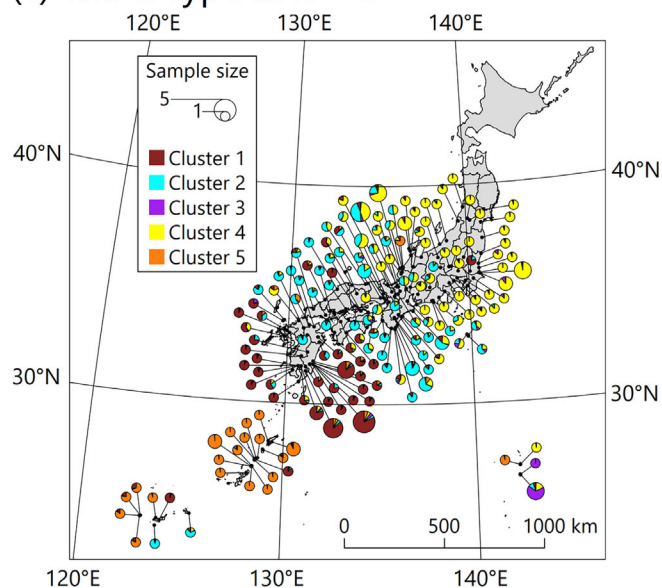
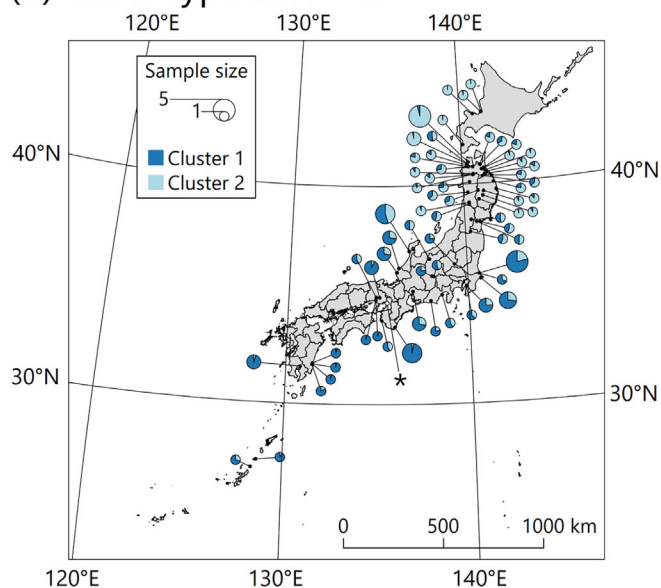
(a) The C-type at $K = 2$ (b) The C-type at $K = 3$ (c) The C-type at $K = 5$ (d) The E-type at $K = 2$ 

FIGURE 3 Distribution of each cluster in the STRUCTURE. (a–c) The C-type. The percentages of clusters in (a), (b), and (c) correspond to the STRUCTURE results in the top, middle, and bottom in Figure 2b, respectively. (d) The E-type. The percentage of clusters corresponds to the STRUCTURE results in Figure 2c. An asterisk represents the Yodogawa River population. The pie chart size represents the sample size.

autocorrelation was not significant for any index (Figures 5, S5; $p > 0.05$). The effect of latitude was also not significant for any of the indices (Figures 5, S5; $p > 0.05$). For the E-type, the hierarchical fixation indices showed greater genetic differentiation among the prefectures than among the islands or regions (Table 2). Analysis of molecular variance detected significant genetic differentiation in the inter-island and inter-prefecture hierarchies (Table 3, $p < 0.005$). This result was similar

when the Yodogawa River population was excluded from the analysis.

3.4 | Genome size

The genome sizes of the C-type, E-type, and F1 hybrids were estimated to be 514.8 (± 7.2 standard deviation [SD]), 562.5 (± 9.8 SD), and 538.2 (± 6.9 SD) Mbp,

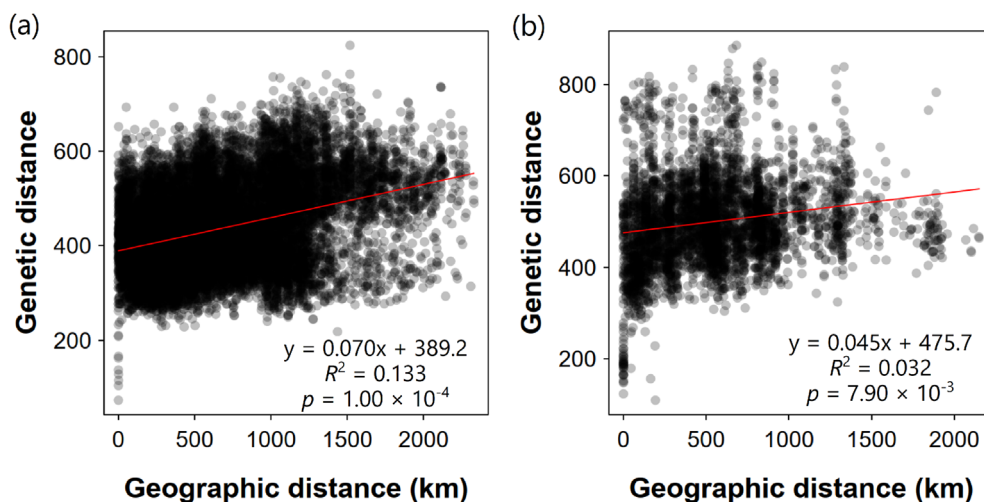


FIGURE 4 Mantel tests show the isolation by distance of two ecotypes of *Imperata cylindrica*. Mantel tests were conducted between genetic distance calculated using the GenAlEx algorithm and geographic distances for the (a) C-type and (b) E-type.

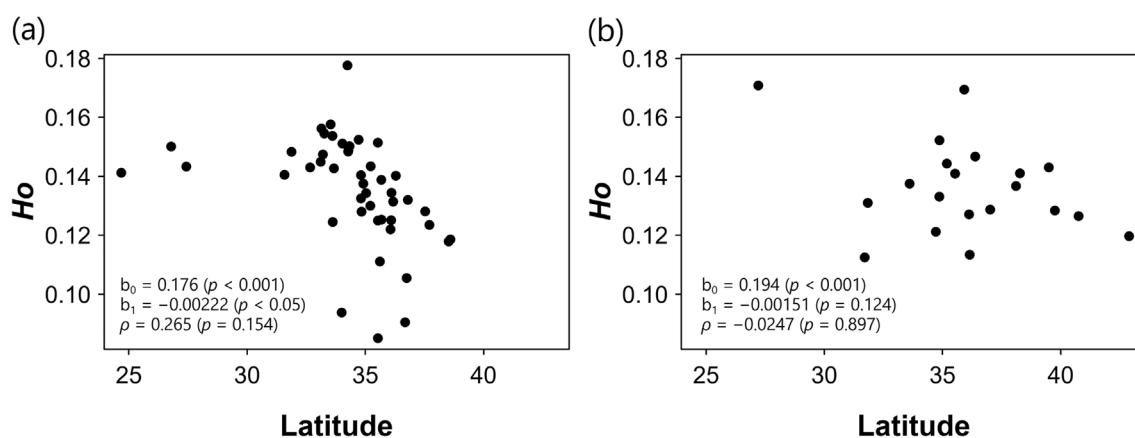


FIGURE 5 Spatial autocorrelation analyses on observed heterozygosity. (a) C-type and (b) E-type. b_0 (intercept) and b_1 (slope) represent coefficients of linear regression between latitude and expected heterozygosity considering spatial autocorrelation. ρ represents the magnitude of spatial autocorrelation. The exact values are shown in Table S3.

respectively (Figure 6). The genome sizes of the two ecotypes and F1 differed significantly (Table S4; Tukey's HSD, all pairs, $p < 0.001$).

4 | DISCUSSION

4.1 | Distribution width was associated with differences in genetic diversity

We observed that the C-type, the E-type, and the F1 hybrid between the two ecotypes showed a clear genetic differentiation from each other, as reported previously (Nomura et al., 2022). Additionally, other plant species also exhibit ecotypes adapted to distinct environments, which sometimes show significant genetic differentiation and reproductive isolation (Sugai et al., 2023; Tsuneki et al., 2014). In addition, the genome size differed significantly between the C-type, E-type, and F1 hybrid

(Figure 6). Genome size is also known to vary by the region of origin, even within the same species (Pyšek et al., 2018, 2023).

Generally, species with wide distribution ranges and large population sizes have higher genetic diversity than species with narrow distribution ranges and small population sizes because a large effective population size can reduce stochastic allele loss (genetic drift) (Ellstrand & Elam, 1993). The C-type *I. cylindrica* inhabits a wide range of environments, ranging from mesic to relatively dry (Matumura & Yukimura, 1980). *Imperata cylindrica* is a representative species of seminatural grasslands and commonly occurs in environments created by human activities, such as roadsides, median strips, and agricultural lands (Hattori et al., 1994; Holm et al., 1977; Nomura et al., 2022). Hence, the C-type patches are continuous and widespread. As expected from the distribution, the C-type had greater genetic diversity than the E-type, indicated by the five genetic diversity indices

TABLE 2 Hierarchical fixed index. Rows indicate upper hierarchy; columns indicate lower hierarchy.

	Island	Region	Prefecture	Individual
C-type				
Total	0.0472	0.0636	0.0711	0.1862
Island		0.0172	0.0250	0.1459
Region			0.0080	0.1309
Prefecture				0.1240
E-type				
Total	0.0209	0.0546	0.1926	0.2234
Island		0.0344	0.1753	0.2068
Region			0.1459	0.1785
Prefecture				0.0381
E-type without Yodogawa Pop.				
Total	0.0232	0.0688	0.1457	0.2077
Island		0.0467	0.1255	0.1889
Region			0.0826	0.1492
Prefecture				0.0726

(Table 1). Pairwise geographic versus genetic distance showed isolation by distance (Wright, 1943) for the C-type (Figure 4).

Conversely, the E-type grows mostly in wetlands and floodplains south of the southern Tohoku region (Matumura & Yukimura, 1980; Nomura et al., 2022), while populations in Hokkaido and the central and northern Tohoku regions are distributed in seminatural grasslands. The wet environments are isolated from each other. Hence, the E-type is likely to have lower genetic diversity than the C-type. As with the C-type, the pairwise geographic distance versus genetic distance indicated that isolation by distance existed for the E-type (Figure 4). However, the slope and R^2 value were smaller than those for the C-type, which reflects the smaller genetic differentiation within the E-type than within the C-type, which is also supported by the indices of genetic diversity (Table 1).

4.2 | The C-type showed continuous genetic variation along latitudes

The C-type had a continuous population genetic structure from north to south, except for the Nansei populations (Figures 1–3), indicating that the C-type had a large population, as supported by the discussions in this article. The large population and high genetic diversity of the C-type are also consistent with the observed significant genetic differentiation at various geographical

scales (islands, regions, and prefectures), especially the greatest genetic differentiation between islands (Tables 2 and 3). Genetic diversity is related to the location of the distribution range, with the distribution edges having lower genetic diversity than the center (Eckert et al., 2008). This information can help clarify the history of the distributional expansion. The C-type had lower genetic diversity in the northern part than in the southern part of Japan (Figures 5 and S5). Based on the sampling location, the C-type was not distributed north of Aomori Prefecture, the northernmost prefecture in Honshu. These findings suggested that the C-type expanded its distribution from the Kyushu region in southern Japan to the central Tohoku region in northern Japan.

Nansei and other populations showed different population genetic structures (Figures 1–3). The boundary separating the Nansei population from the remainder of Japan roughly coincides with the Watase Line (the Tokara gap), which is one of the most important biogeographic boundaries in Japan. The Tokara gap is more than 1000 m deep, and the flora and fauna change around the Watase Line (Kubota et al., 2011; Nakamura, 2012). The strait is believed to have existed during the last glacial period (Kimura, 1996; Ota, 1998), and the islands have never been connected across this boundary since then. Previous studies have suggested that the population fragmentation over tens of thousands of years led to genetic differentiation among C-type populations.

The ecological differentiation of *I. cylindrica* according to latitudinal clines has been previously described (Tominaga et al., 1989). The C-type with hairs on the nodes of the culms (Matumura & Yukimura, 1980) was divided into two ecotypes (Tominaga et al., 1989): the type that flowers only once a year and whose aboveground parts die in winter (hereafter, temperate type), and the other type that flowers multiple times a year and whose aboveground parts are evergreen in winter (hereafter, subtropical type). The subtropical type is distributed south of Amami Oshima Island, and the temperate type is distributed north of the island. The distribution boundary coincided with the Watase Line. Our results support the existence of subecotypes within the C-type.

In this study, we further showed that the Kyushu and the Ogasawara populations are genetically differentiated from other populations (Figures 2b and 3a–c). These populations, such as the Nansei population, are separated from other populations by seas. However, the ecological differences among these populations are unknown. Further research is necessary to clarify if these populations are ecologically different.

TABLE 3 Results of analysis of molecular variance for C-type and E-type.

	Df	Sum of square	Mean of square	Variance components	Percentage of variance	Fixed index	p-Value
C-type							
Between islands	5	533.0	106.6	1.138	4.50	0.0472	0.001
Between regions within an island	5	193.9	38.8	0.450	1.78	0.0172	0.001
Between prefectures within a region	35	893.8	25.5	0.027	0.11	0.0080	0.009
Between samples within a prefecture	146	3700.7	25.3	1.663	6.58	0.1240	0.004
Within samples	192	4227.9	22.0	22.020	87.04	0.1862	0.003
Total	383	9549.3	24.9	25.298	100		
E-type							
Between islands	4	380.6	95.1	0.078	0.28	0.0209	0.001
Between regions within an island	2	274.4	137.2	2.009	7.12	0.0344	0.497
Between prefectures within a region	13	679.2	52.2	3.516	12.46	0.1459	0.001
Between samples within a prefecture	75	1683.0	22.4	−0.183	−0.65	0.0381	0.002
Within samples	95	2166.7	22.8	22.807	80.80	0.2234	0.301
Total	189	5183.9	27.4	28.227	100		
E-type without Yodogawa population							
Between islands	4	347.6	86.9	0.179	0.61	0.0232	0.001
Between regions within an island	2	237.8	118.9	2.092	7.09	0.0467	0.121
Between prefectures within a region	12	529.4	44.1	2.232	7.56	0.0826	0.001
Between samples within a prefecture	69	1787.9	25.9	0.887	3.00	0.0726	0.002
Within samples	88	2124.0	24.1	24.137	81.74	0.2077	0.265
Total	175	5026.6	28.7	29.528	100		

4.3 | The E-type showed low genetic diversity

A previous study suggested that the E-type performs worse than the C-type in relatively dry conditions (Miyoshi & Tominaga, 2017). Therefore, the E-type habitats are probably fragmented in relatively dry environments, resulting in reduced gene flow among habitats. The low gene flow and fragmented populations may have resulted in lower genetic diversity and a higher fixation index for the E-type than the C-type (Table 1). The overall genetic diversity was low, but the E-type showed genetic variation with latitude (Figures 2c and 3d). Analysis of molecular variance also revealed significant genetic differentiation between islands and between prefectures (Tables 2 and 3). The absence of a significant genetic differentiation between regions may be due to the low genetic diversity of the E-type (Table 1). Despite the smaller genetic differentiation of the E-type than of the C-type, a previous study found that the E-type had

clearer latitude-related local adaptations than the C-type (Nomura et al., 2018).

The relationship between genetic diversity and latitude was not clear for the E-type, suggesting that the C-type and E-type have different histories of range expansion. The E-type had lower genetic diversity than the C-type (Table 1); therefore, the genetic diversity of the E-type may have decreased due to population fragmentation, resulting in the loss of its association with latitude (Figures 4, 5, S5). Alternatively, the E-type may have expanded its distribution in Japan earlier than the C-type, and genetic diversity may have increased sufficiently to the distribution edges. The history of the E-type range expansion should be clarified by further increasing the number of molecular markers.

In this study, the Yodogawa River population in Osaka Prefecture was phylogenetically distinct from the other E-types (Figures 1d and 2c). No similar accessions were found in other areas, and the origins of these accessions are unknown. *Imperata cylindrica* has been used as

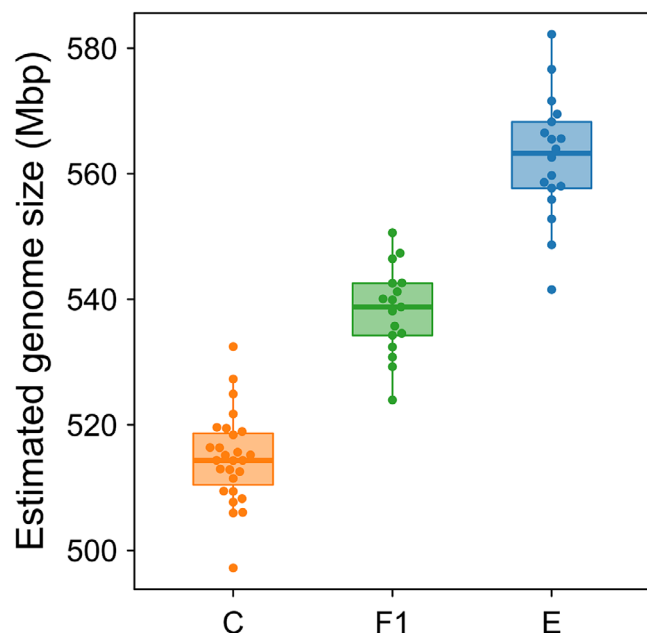


FIGURE 6 Genome sizes of the two ecotypes and F1 hybrid. Genome size was calculated as a relative value to the rice genome size (389 Mbp). The results of multiple comparisons are shown in Table S4. [Correction added on 19 December 2024, after first online publication: Figure 6 has been replaced in this version.]

a revegetation plant (Hattori et al., 1994; Iwakiri et al., 2010; Yamada & Nemoto, 2016), and its seeds have been imported from outside Japan (Ministry of Land, Infrastructure, Transport and Tourism, 2007). These foreign strains may have been established in the Yodogawa River. In further research, the inclusion of strains from outside Japan in analyses may help in understanding the origin of the Yodogawa River accessions.

4.4 | Genome size may be related to differences in distribution width between two ecotypes

This study found that the C-type had a smaller genome size than the E-type, with no overlap in the genome size range between the two ecotypes, and an average difference of approximately 50 Mbp (Figure 6). Factors contributing to genome size include a difference in the number of chromosomes (Nishiwaki et al., 2011; Rayburn et al., 2009), genome size variation in each chromosome derived from large deletions and insertions in chromosomes (Benson et al., 2023), or gene amplification through extrachromosomal circular DNA (Pereira & Dunning, 2023). The chromosome-level genome assembly results showed that the two ecotypes are $2n = 20$ (K. Sato et al., unpublished data); thus, the genome size per chromosome is probably larger in the E-type than in

the C-type. Intraecotype variation in karyotype is unknown and is a topic for further research. Additionally, whether the genome size was responsible for the differences in habitat and spatial distribution between the two ecotypes remains unclear.

However, previous studies have suggested a relationship between the differences in genome size and distribution width (Meyerson et al., 2020; Schmidt et al., 2017; Suda et al., 2015). A small genome may result in faster plant growth and development, larger phenotypic plasticity, smaller seed mass, shorter cell cycles, faster rates of cell division, and a lower cost of nutrients (e.g., N and P) for nucleic acid production than a large genome (Beaulieu et al., 2008; Francis et al., 2008; Meyerson et al., 2020; Suda et al., 2015). These phenomena are consistent with the characteristics of the C-type: a smaller seedling biomass (Matumura & Akikawa, 1989; Matumura et al., 1984), smaller seed mass (Matumura et al., 1983), and faster relative growth rate (Matumura et al., 1984) than those of the E-type. Small seed mass may have a positive effect on dispersal distance in wind dispersal plants (Augspurger & Franson, 1987; Skarpaas et al., 2011), which allows invasion into open patches. Furthermore, relatively fast growth rates are thought to promote establishment in the patches. The advantages of small genome sizes are consistent with the C-type being more invasive than the E-type. Further research in transcriptomics may reveal physiological differences between the two ecotypes, helping to determine the relationship between genome size and ecological differences.

The genus *Imperata* includes nine species, of which *I. cylindrica* has large intraspecific variation in morphology and ecology and is a cosmopolitan species (Gabel, 1982; MacDonald, 2004). Several varieties (Holm et al., 1977) and ecotypes (Al-Juboory & Hassawy, 1980; Cheng & Chou, 1997) have been reported for this species, but their taxonomic status, including the two ecotypes used in this study, remains unclear. Holm et al. (1977) reported five major varieties, var. *africana*, var. *condensata*, var. *europa*, var. *latifolia*, and var. *major*, based on their morphology and distribution. The culm morphology of the C-type is similar to that of var. *major* ($2n = 20$) (Holm et al., 1977; MacDonald, 2004). The C-type (var. *koenigii*) and var. *major* are often treated as the same varieties (Hattori et al., 1994; Tominaga et al., 2007). Because var. *major* is thought to be distributed in East Asia, including Japan (Holm et al., 1977), there is no inconsistency in treating the C-type and var. *major* as identical. *Imperata cylindrica* var. *major* is considered invasive (Holm et al., 1977), and the C-type also appears to be more widely distributed and invasive than the E-type.

The E-type is similar to var. *africana* ($2n = 60$) or var. *europa* ($2n = 40$) when only the culm morphology is considered (Holm et al., 1977; MacDonald, 2004). However, chromosome number and distribution differ between the E-type and var. *africana* or var. *europa*. *Imperata cylindrica* var. *africana* is distributed in southern, central, and western Africa, and var. *europa* is distributed from southern Europe to the arid regions of central Asia (Holm et al., 1977). Therefore, the E-type may be an ecotype endemic to East Asia that adapted to wet environments. A phylogenetic analysis that includes the worldwide *Imperata* accessions is necessary to clarify the origin of the E-types.

Genome size may be related to var. *major* (the C-type), which is the least polyploid and the most invasive of these varieties (Meyerson et al., 2020; Schmidt et al., 2017; Suda et al., 2015). However, ploidy seems to have a complex effect on invasiveness apart from genome size (Pyšek et al., 2023). Further research could clarify taxonomic studies and the relationship between invasiveness and genome size or polyploidy in *I. cylindrica*.

4.5 | Conclusions

Clarifying genomic information helps to understand the differing performances of the two ecotypes of *I. cylindrica*. Sequence data and statistical analyses showed that the two ecotypes have different geographic genetic population structures, suggesting different range expansion histories. Currently, these two types are considered intraspecific polymorphisms. However, genetic differentiation, genome size differences, pre- and postzygotic reproductive isolation, and different ecology highlight the necessity for different guidelines between each ecotype in their conservation and use for revegetation. Future analysis should include worldwide *Imperata* accessions to understand the origin of each ecotype.

AUTHOR CONTRIBUTIONS

Y.N. and Y.S. designed experiments. Y.N. performed all of the experiments and created R script. T.T. provided the plant materials. Y.S., A.J.N., and J.I. provided the reagents and equipment necessary for the experiment. Y.N. wrote a draft. Y.N., A.J.N., J.I., and Y.S. revised this paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

The MIG-seq data were submitted to the NCBI Sequence Read Archive repository under the BioProject number PRJNA976071 (<http://www.ncbi.nlm.nih.gov/bioproject/976071>).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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