Original article

Title

Drastic shift in flowering phenology, an instant reproductive isolation mechanism, explains the population structure of *Imperata cylindrica* in Japan

Running title

Drastic phenology shift of F_1 hybrids

Authors/Affiliations

Yasuyuki Nomura[1,2], Yoshiko Shimono[2], Nobuyuki Mizuno[2], Ikuya Miyoshi[2], Satoshi Iwakami[2], Kazuhiro Sato[3], Tohru Tominaga[2]

[1] Present address: Research Institute for Food and Agriculture, Ryukoku University, 1-5 Yokoya, Seta Oe-cho, Otsu, Shiga 520-2194, Japan

[2] Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo, Kyoto 606-8502, Japan

[3] Institute of Plant Science and Resources, Okayama University, 2-20-1 Chuo, Kurashiki, Okayama 710-0046, Japan

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20 Correspondence: carniplant8931@yahoo.co.jp

21

22 **Author contributions**

23 Y.N., Y.S., S.I. and T.T. designed experiments. T.T. provided plant materials that he has
24 maintained since the 1980s. Y.N., Y.S. and I.M. further collected plant materials. Y.N. performed
25 most of the experiments. Y.N, N.M. and K.S. and. performed RNA-Seq analysis. Y.N., Y.S.,
26 N.M., S.I. K.S. and T.T. wrote the paper.

27

28 **Acknowledgement**

29 Partly supported by the Japan Society for the Promotion of Science (21380015) and ESPEC
30 Foundation for Global Environment Research and Technology to T.T.

31

32 **Data accessibility statement**

33 The RNA-Seq and Sanger sequence data reported in this paper have been deposited in the DNA
34 Data Bank of Japan (DDBJ; accession numbers XXXXXX [RNA-Seq], XXXXXX [Sanger
35 sequences]).

36

Declaration of Interests

The authors declare no competing interests.

41 **Title**

42 Drastic shift in flowering phenology, an instant reproductive isolation mechanism, explains the

43 population structure of *Imperata cylindrica* in Japan

44

45 **Abstract**

46 Reproductive isolation plays an important role in population differentiation and speciation, thus

47 enhancing biodiversity in wild plants. Hybridisation sometimes involves rapid reproductive

48 isolation between parents and their hybrids through the novel traits of hybrids derived from a

49 new combination of genomes. Here, we report how a hybrids' new phenotype contributes to

50 rapid reproductive isolation between two ecotypes of *Imperata cylindrica*. The two ecotypes

51 differ in their flowering phenology and habitats. An analysis with genetic markers revealed that

52 hybrid populations consisted of only F_1 individuals. Both parental ecotypes flowered in spring,

53 but F_1s flowered in fall. This drastic shift in flowering phenology prevented backcrossing

54 parental ecotypes to F_1. F_1s flowered in fall and dispersed seeds in winter. The germination

55 percentage of seeds set on F_1 was extremely low in their habitats, and seedlings did not survive

56 due to the low temperatures in winter, resulting in the absence of a F_2 generation. In conclusion,

57 flowering phenology mismatch promotes reproductive isolation between parents and F_1,

58 resulting in a hybrid population consisting of only F_1s.

59

60 **Keywords**: hybridisation, flowering phenology shift, ecotype, instant reproductive isolation,

61 *Imperata cylindrica*, population genetic structure

62

Introduction

Around 50% of flowering plants are estimated to have experienced polyploid speciation. Nearly all such polyploid individuals are considered to be derived from hybridization (Soltis and Soltis 2009; Wood et al. 2009; Soltis et al. 2015). Hybridisation is a major source of phenotypic variation (Grant and Grant 1994) and a driving force of evolution (Coyne and Orr 2004). One of the evolutional consequences of hybridisation is hybrid speciation, which cause species diversification (Mallet 2007; Soltis and Soltis 2009; Wood et al. 2009). The resultant hybrids are sometimes prevented from crossing with their parental lineages due to an altered trait related to reproduction, resulting in the change of population structure and ultimately species diversification (Buerkle et al. 2000; Duenez-Guzman et al. 2009; Kagawa and Takimoto 2018).

Previous studies have demonstrated that novel traits derived from hybridization directly contribute to reproductive isolation. Mating between individuals that have evolved independently results in a novel combination of genomes or gene sets; this process can generate a wide range of phenotypes, including some of which that exceed or interpose variations among the parental lineages by overdominance or transgressive phenotype (Johansen-Morris and Latta 2006). For example, floral color in *Iris* species (Taylor et al. 2013) and floral odor for *Narcissus* species (Marques et al. 2016) differ among hybrids from those

of their parents, resulting in recruitment of novel pollinators for hybrids and reproductive isolation between hybrids and parents. Similarly, new hybrid phenotypes related to beak morphology and mating songs in Darwin's finch (Lamichhaney et al. 2018), wing color patterns in *Heliconius* butterfly (Mavárez et al. 2006), and behavioral mate choice in cichlids (Selz et al. 2014) have caused reproductive isolations from parents. Thus, novel hybrid traits can generate genetic and evolutionary changes.

In this study, we report a novel trait in hybrids of two *Imperata cylindrica* (L.) Raeusch (cogongrass) ecotypes . *Imperata cylindrica* is a perennial rhizomatous grass with a self-incompatible and wind-pollinated reproduction system; it is native to tropical and subtropical areas of the Northern and Southern Hemispheres, including Japan (Holm et al. 1977). Japanese cogongrass populations consist of two ecotypes: common type (C-type) and early flowering type (E-type) (Matumura and Yukimura 1980; Tominaga et al. 1989; Mizuguti et al. 2003). These ecotypes are typically distinguished by their morphology; the E-type has a glabrous culm, whereas the C-type has a hairy culm (Figure 1). They also differ in terms of habitat and flowering phenology: the C-type mainly lives in dry habitats (*e.g.* roadside, levee of paddy fields), whereas the E-type often lives in wet habitats (*e.g.* marshy area, moist fallow fields). In addition, the E-type flowers approximately 1 month earlier than the C-type. These differences in habitat and flowering phenology isolate the two ecotypes, although the existence

of hybrids between the two ecotypes was initially recognized through allozyme analysis using a single marker (Mizuguti et al. 2004; Tominaga et al. 2007). Since then, the distribution and genetic population structure of the hybrids remained unknown.

In this study, we analysed 183 populations of cogongrass collected from the 1980s to the 2010s throughout Japan using newly established molecular markers. We performed field surveys and common garden experiments to determine the outcomes of backcrossing and crossing among the F1 generation to evaluate the contribution of hybrid flowering phenology to their genetic population structure. Our findings will provide insight into the reproductive isolation resulting from hybridisation.

Materials and methods

Accession and study site

Three hundred fifty accessions of cogongrass were collected from throughout Japan, from 1980s to 2010s, and maintained at an experimental farm of Kyoto University, Kyoto, Japan (35°01'54.5"N, 135°46'59.5"E) (Figure 2; Table S1). The chloroplast DNA (cpDNA) haplotypes of 33 out of the 350 accessions were previously determined by Nomura et al. (2015). Field survey and material collection were conducted in north sites (Aomori, Akita, Iwate, Yamagata, Miyagi, Fukushima and Tochigi Prefecture; 36°54'41"N – 40°32'07"N, 139°48'34"E –

117 141°40'10"E) and south sites (Wakayama Prefecture; 33°29'01"N–33°39'39"N, 135°46'39"–
118 135°57'59") regions, Japan in 2016–2018 (Figure S1).

119

120 *Evaluation of morphological traits*

121 Morphological traits were evaluated for a total of 46 accessions: 17 accessions of the C-type, 15
122 accessions of the E-type, and 14 accessions of putative hybrids (Table S1). The existence of
123 hairs on culm nodes (1, absence; 2, partial presence; 3, presence), hairs on leaf sheathes (1,
124 absence; 2, partial presence; 3, presence), wax on leaf sheathes (1, absence; 2, presence), the
125 ratio of aerenchyma diameter to midrib diameter, and the ratio of aerenchyma diameter in a pith
126 to rhizome diameter were recorded (Figure 1).

127

128 *Nucleic acid extraction*

129 DNA was extracted from leaves of cogongrass using the modified cetyltrimethylammonium
130 bromide method (Murray and Thompson 1980). Total RNA was extracted from leaves using a
131 RNeasy Plant Mini kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions.

132

133 *Development of nuclear markers*

Total RNA extracted from 22 accessions of the E-type throughout Japan (Table S1) was bulked, and a total of 4 μg of total RNA was used to construct paired-end libraries using the TruSeq RNA Sample Preparation Kit v3 (Illumina, Inc., San Diego, CA, USA) according to the manufacturer's instructions. The libraries were sequenced on MiSeq (Illumina) with 300-bp paired-end reads. Filtering of low-quality bases (Phred score < 20) and adapter sequences, *de novo* transcriptome assembly, and detection of polymorphisms were conducted using CLC Genomics Workbench software version 8.0 (CLC bio Japan, Inc., Tokyo, Japan).

One hundred seven primer sets were designed to amplify DNA of 300–900 bp in which polymorphisms were detected in the above RNA-Seq analysis. PCR was conducted using DNA from two C-type and six E-type accessions selected at random. PCR amplification was performed in a 20-μL volume containing 2.5–25 ng of extracted DNA, 0.2 mM each dNTP, 0.5 μM each forward and reverse primer, 2.0 μL of 10× ThermoPol™ Buffer, and 0.5 U of *Taq* DNA polymerase (New England Biolabs Japan Inc., Tokyo, Japan). The PCR programme consisted of an initial denaturation at 95.0°C for 3 min followed by 45 cycles at 95.0°C for 15 s, annealing 55°C for 30 s, and extension at 68.0°C for 1 min; with a final extension at 68.0°C for 3 min. The PCR products were subjected to agarose gel electrophoresis. The PCR products that contained a single band of the expected size were purified using ExoSAP-IT (USB Corporation, Cleveland, OH, USA) and directly sequenced using an ABI 3130xl Genetic Analyzer (Applied

Biosystems, Foster City, CA, USA) with BigDye™ Terminator v. 3.1 (Applied Biosystems). Fifty seven PCR products were sequenced. Among the 57 primer sets that resulted in PCR products with a clear chromatogram in direct sequencing, 10 were selected for investigation of population structure (Table S2).

In addition to the 10 primer sets, 2 primer sets were designed based on the sequences of cogongrass in GenBank: *ppc-C4* (AM690231) and the ITS region (JN407507). The primers are listed in Table S2.

Development of CAPS markers to distinguish ecotypes

Cleaved amplified polymorphic sequences (CAPS) markers in nuclear DNA were developed to distinguish ecotypes and their hybrids. Primers were designed for the ITS region and EST104 (Table S2, ITS_CAPS and EST104_CAPS), and PCR was performed as described above. The PCR products of ITS and EST104 were digested with *Dde*I and *Rsa*I (New England Biolabs Japan, Inc., Tokyo, Japan), respectively, and resolved on an agarose gel (Figure S2).

A CAPS marker on cpDNA was also developed to distinguish maternal ecotypes. The *psbA–matK* region was amplified by PCR using the primer set (Yasuda and Shibayama 2006): forward PSA-F, 5′-CAGGCTTGTACTTTCGCGTC-3′; reverse MTK-R, 5′-CAATGTCCTGGTGAATCCTC-3′. PCR was carried out as described (Nomura et al. 2015).

170 The PCR products were incubated with *Dra*I (TaKaRa Bio, Inc., Shiga, Japan) and subjected to
171 gel electrophoresis.
172
173 *Analysis of population structure*
174 SNPs with a low frequency (<10% in all accessions) were excluded from haplotype analysis
175 using PHASE 2.1 (Stephens et al. 2001). Principal coordinate analysis (PCoA) was conducted
176 using GENALEX 6.502 (Peakall and Smouse 2006, 2012). The population genetic structure was
177 inferred using the Markov chain Monte Carlo (MCMC) and the Bayesian clustering algorithms
178 implemented in STRUCTURE v. 2.3.1 (Pritchard et al. 2000) with 1,000,000 MCMC steps
179 following 100,000 burn-in MCMC steps. The number of clusters (K) was tested from 1 to 10
180 with 10 replicates. The appropriate number of clusters was estimated using the Evanno ΔK
181 parameter (Evanno et al. 2005).
182 Distinction of hybrids from parental ecotypes and identification of generation of
183 hybrids were conducted using NEWHYBRIDS v. 1.1 (Anderson and Thompson 2002). Ten
184 independent runs were conducted with 1,000,000 MCMC steps following 100,000 burn-in
185 MCMC steps, and the posterior probability was computed for each of the six classes: the
186 two-parental ecotypes, F_1 and F_2 generations, and backcrosses to each parental class. The 10

187 independent results of STRUCTURE and NEWHYBRIDS were amalgamated using CLUMPP v.
188 1.1.2 (Jakobsson and Rosenberg 2007).
189
190 *Genotyping of putative F_2s*
191 Seven F_1 plants were open-pollinated in 15 m x 15 m plot of an experimental farm of Kyoto
192 when only F_1 plants were in bloom. The seeds collected from the plants were germinated on
193 filter paper in a Petri dish at 30/20°C day/night with a 12-h photoperiod in an incubator and
194 seedlings were transplanted into plastic pots (11.3-cm diameter × 14.0-cm height). The genotype
195 of each putative F_2 was evaluated using the CAPS marker in the ITS region and EST104, and
196 direct sequencing using ppc-C4 and EST72 primer sets (Table S2) described above.
197
198 *Percentage of hybridisation between the C- and E-types in natural habitats*
199 Seeds were collected during May to July in 2017 and 2018 from cogongrass individuals in north
200 sites and south sites, where C- and E-type occur next to each other (1–20 m). The seeds from
201 each seed parent were germinated at 30/20°C. The genotype of each ramet and each seedling
202 was evaluated using the CAPS marker in the ITS region described above.
203
204 *Investigation of flowering phenology*

Flowering periods of cogongrass were investigated in spring and fall in the natural habitats of north and south sites during 2016–2018 (Figure S2). In north sites, a total of 1,092 ramets were investigated in spring (July 2017 and May–June 2018) and 1,467 ramets in fall (November–December 2016 and October 2017) from 58 populations. In south sites, a total of 251 ramets were investigated in spring (April–June 2017) and 210 ramets in fall (November–December 2016 and October 2017) from six populations. Each population consisted of 2 to 92 ramets. Ramets in the flowering and non-flowering phases were sampled at >1-m intervals. The genotype of each ramet was evaluated using the CAPS marker in the ITS region described above.

Flowering phenology was monitored for C-, E-type, and artificially crossed F_1 plants cultivated from 1 April to 30 November 2018 at the experimental farm of Kyoto University. The experimental farm was located between two hybrid zone, north and south sites. The survey was conducted for 141 accessions from 122 populations of the C-type and 83 accessions from 54 populations of the E-type and artificially generated F_1 hybrids. The age of these plants were not controlled. The artificial F_1s were generated by hand pollination between two ecotypes collected from the same prefecture, namely populations from Miyagi, Ibaraki, Ishikawa, Fukui, Shizuoka, Aichi, Osaka, and Wakayama, in 2010 (Miyoshi and Tominaga 2017) and 2017. Success of hybridisation was evaluated using the CAPS marker in the ITS region. Two hundred twenty-one

223 individuals in which hybridisation was successful were used for the observation of flowering
224 phenology. The plants were grown in plastic pots (15.9-cm diameter × 19.0-cm height) or clay
225 pots (21.8-cm diameter × 17.5-cm height) containing paddy soil. The date on which the top of
226 the first panicle emerged from a leaf sheath was defined as the flowering date.
227
228 *Investigation of seed sets*
229 Together with the survey of flowering phenology in natural habitats in 2016–2018, panicles in
230 the seed dispersal phase were collected in May to July for the C-type (21 populations) and
231 E-type (23 populations) and in October to December for F_1 (25 populations) in 2016–2018
232 (Figure S1). One hundred twenty-two panicles of C-type and 183 panicles of E-type were
233 collected in spring and 228 panicles of F_1 in fall. Seed set was evaluated by counting the
234 spikelets (flowers) and seeds of each panicle. The genotype of each panicle was evaluated using
235 the CAPS marker in the ITS region described above.
236 Hand pollinations were conducted between different accessions from the same
237 ecotype: C-type x C-type, E-type x E-type, and F_1 x F_1. The crossing accession pairs were
238 isolated in a mesh-covered cubic frame and their panicles were rubbed when their anthers and
239 stigmas were matured. The seed sets of hand-pollinated C-type, E-type, and F_1 plants in the
240 experimental farm were evaluated (Table S3). Two accessions of the C-type were from south

241 sites. Two accessions of the E-type were from north sites, and two from south sites. Four
242 accessions of F_1 were from north sites, and four from south sites. Pairs consisted of accessions
243 from the same region and each accession was collected from different populations. These plants
244 were crossed within a pair during April–May 2017 for the C- and E-types and during
245 September–October 2018 for F_1. Panicles were collected during May–June 2017 for the C- and
246 E-types and during November–December 2018 for F_1. The spikelets and seeds of each panicle
247 were counted.
248
249 *Germinability tests*
250 In total, 1055, 958, and 99 seeds from 16 populations of the C-type, 22 populations of the
251 E-type, and 14 populations of F_1, respectively, were subjected to germination test. The seeds
252 were collected during the survey of seed sets in 2016–2018 (Figure S1). Seeds of cogongrass
253 are non-dormant, and seedling emergence occurs soon after seed dispersal (Matumura et al.
254 1983; Shilling et al. 1997); therefore, seed germination tests were conducted immediately
255 (within 2 months) after seed collection.
256 Under the outside condition, 271 seeds of the C-type and 433 seeds of the E-type were
257 sown on 23 May and 13 June 2017 and on 20 June 2018. Fifty-eight seeds set on F_1 were sown
258 on 19 December 2016 and 4 December 2017. Germination tests were conducted on 4 December

2017 using 177 seeds of the C-type, as seeds set on F_1 were too few to check the germinability of those sown in winter. The C-type seeds were collected during June–July 2017 in north and south sites and were stored at 4°C in a refrigerator. Seeds were sown on the surface of potting soil (Takii & Co., Ltd., Kyoto, Japan) in a plastic pot (23.2-cm diameter × 29.7-cm height) in the experimental farm of Kyoto University. Seed germination was observed daily for 1 month. For seeds set on F_1, seed germination was observed daily until the following June.

Under controlled conditions, 784 seeds of the C-type and 525 seeds of the E-type were sown on 24 May, 16 June, and 21 August 2017 and on 20 June 2018 in a growth chamber (Biorton NC-220S, NK system, Japan). Forty-one seeds set on F_1 were sown on 19 December 2016 and 4 December 2017. In addition to F_1, germination tests were conducted on 4 December 2017 using 160 seeds of the C-type collected during June–July 2017 in north and south sites and stored at 4°C in a refrigerator. Seed germination tests were conducted on filter paper in Petri dishes under 12 h light/dark and 30/20°C, the optimal conditions for seed germination of cogongrass (Matumura et al. 1983; Mizuguti et al. 2002). In addition to natural seeds, seed germination tests were conducted using seeds produced by artificial crossing of the above-mentioned four pairs of F_1 plants (Table S3). Seed germination tests were conducted on filter paper in Petri dishes under 12 h light/dark at 30/20°C, 25/15°C, 20/10°C, and 15/5°C

using 103, 90, 90, and 109 seeds, respectively. Seed germination was observed daily for 1 month.

Statistical analysis

All tests were two-tailed, and the significance level was set at $P < 0.05$. Hybridisation percentage (hybridised: 1/no: 0), flowering percentage (flowered: 1/no: 0), seed set percentage (set: 1/no: 0), and germination percentage (germinated: 1/no: 0) in the natural habitats were compared among genotypes (ecotypes and F_1 hybrids) using generalised linear mixed models (GLMM) constructed using the package 'lme4' (Bates et al. 2015) in R 3.5.1 software (R Core Team 2018). The model assumed a binominal distribution and a logit link function. The genotypes were treated as fixed effects. The origin of the populations was treated as a random effect. Pairwise significant differences were identified by *post hoc* tests for GLMM (Tukey honestly significant difference test) using the package 'multcomp' (Hothorn et al. 2008) in R 3.5.1.

Results

Population genetic structure of cogongrass

293 To investigate the genetic structure of cogongrass in Japan, we developed 12 nuclear and one
294 CAPS chloroplast markers (Figure S3; Table S4). Fifty-three SNPs were detected using the 12
295 nuclear markers from 223 accessions of cogongrass. Haplotypes in four regions, *ppc-C4*, ITS,
296 EST72, and EST104, were not shared by the C- and E-types in Japan (Figure S3; Table S4),
297 supporting the previously suggested genetic isolation of the two ecotypes (Mizuguti et al. 2004;
298 Nomura et al. 2015). F_2 genotypes were investigated using four molecular markers that can
299 distinguish ecotypes and hybrids: ppc-C4, EST72, ITS_CAPS, and EST104_CAPS (Table S2).
300 These four regions showed very low coefficients of linkage disequilibrium in an analysis of 28
301 putative F_2 s, suggesting that they are located on different chromosomes (Table S5).
302 PCoA based on the 12 nuclear markers separated 223 accessions into three clusters
303 along PCo1 (Figure 3B). The chloroplast CAPS marker, which is used to distinguish maternal
304 ecotypes, revealed that the clusters at both sides of PCo1 corresponded to the C- and E-types
305 (Figure 3B). The cluster in the middle of PCo1 was considered putative hybrids between
306 ecotypes. In a STRUCTURE analysis, the number of optimum clusters was estimated to be K =
307 2 by Evanno ΔK (Figure 3C), implying that Japanese cogongrass populations are composed of
308 two genetic clusters. All of the putative hybrids showed q values of around 0.5, suggesting that
309 they had half of the genomes of the C-type and half of the E-type. A NEWPHYBRIDS analysis
310 assigned the two ecotypes and putative hybrids to parental classes and the F_1 hybrid class,

311 respectively (Figure 3C). There was no putative F_2 hybrid or backcross individual, except for
312 one accession that belonged to the E-type cluster but carried the C-type haplotype of cpDNA. In
313 the first survey of 223 populations using 12 nuclear markers, F_1s were found in a few
314 geographic locations, namely the north and south sites. We further investigated 127 accessions
315 of F_1 populations using the ITS CAPS marker, which was developed to discriminate the C-type,
316 E-type, and F_1. The distribution pattern of F_1s was similar to the first survey. These F_1 plants
317 were found where C- and E-types share habitats. The majority (85%) of the F_1 hybrids exhibited
318 the E-type cpDNA haplotype, indicating that the E-type tends to be the maternal parent of
319 hybrids, as supported by the hybridisation percentage in natural habitats (Figure 3D).

320 The occurrence and direction of hybridisation events between the C- and E-types were
321 investigated in natural habitats sympatrically colonised by the C- and E-types (north and south
322 sites). Five hundred ninety-nine seeds set on 50 panicles of the C-type, and 481 seeds set on 50
323 panicles of the E-type were collected from 10 and 14 populations in natural habitats,
324 respectively. CAPS analysis of the ITS region of the progeny revealed a low percentage (~1.2%)
325 of hybridisation in south sites (Figure 3D). In contrast, north site populations exhibited a higher
326 percentage of hybridisation (~40%). The direction of hybridisation was asymmetric in both
327 regions: significantly more F_1 plants were observed among the seedlings from E-type seed

328 parents (GLMM, $P < 0.01$). This result is consistent with the observation that the majority of F_1

329 hybrids in the wild exhibit E-type cpDNA haplotypes (Figure 3C).

330

331 *Morphological traits of the ecotypes and F_1*

332 Culms, leaf sheathes (Tominaga et al. 1989; Mizuguti et al. 2003), and midrib aerenchyma (A.

333 Nishiwaki & A. Mizuguti unpublished data) are different between the C- and E-types. The

334 C-type had hairs on culm nodes and leaf sheathes and no wax on leaf sheaths, whereas the

335 E-type had no hairs on culm nodes or leaf sheathes and had wax on the leaf sheaths (Figure 1,

336 S4). The C-type also had a smaller ratio of midrib aerenchyma to midrib diameter than the

337 E-type had. In addition to the previously identified ecotype-specific characteristics, we

338 investigated rhizome aerenchyma based on the assumption that the habitat of soil moisture

339 contents of the ecotypes may be associated with their rhizome structures. We found that the size

340 of rhizome aerenchyma differed between ecotypes; the C-type had small aerenchyma, and the

341 E-type had large aerenchyma.

342 F_1 showed a wide range of variation in morphology; various numbers of hairs on culm,

343 no or few hairs and little wax on sheathes, and an intermediate ratio of aerenchyma. As a result,

344 F_1 was located in an intermediated position between parental ecotypes on axis 1 of PCA (Figure

345 3A). The C- and E-types and F_1 were divided into three groups by PCA. The axis 1 was

346 associated with hairs on culms, sheath was, sheath hairs, and rhizome aerenchyma, indicating
347 that they are the most diagnostic of hybridisation. Therefore, the C- and E-types and F_1 were
348 distinct, not only genetically but also morphologically.
349
350 *Flowering phenology and reproduction of F_1*
351 Population genetic analyses revealed that hybrid individuals were F_1s, suggesting reproductive
352 isolation of F_1 plants from their parents (backcross) and prevention of F_1 x F_1 crossing. We first
353 investigated the flowering period of the C- and E-types and F_1 in the fields to assess the
354 occurrence of reproductive isolation between F_1 and the two ecotypes. The number of ramets at
355 the flowering stage was counted in 64 populations. The genotype of each ramet was later
356 investigated using the CAPS marker in the ITS region. Three hundred one (66.9%) of 450
357 ramets of the C-type and 345 (77.9%) of 443 ramets of the E-type were in the flowering stage in
358 spring, in agreement with previous reports (Matumura and Yukimura 1980; Tominaga et al.
359 1989) (Figure 4A) (Tukey HSD, $P < 0.001$). In contrast, only 11 (2.0%) of 552 ramets of F_1
360 flowered in spring. In fall, up to 48.0% of F_1 ramets (581/1211) flowered, compared to only
361 5.3% of C-type and 1.6% of E-type ramets (Tukey HSD, $P < 0.001$). The flowering phenology
362 of F_1 plants was not affected by cpDNA type (Figure S5).

363 To validate the late flowering of F_1 in natural habitats, we generated artificial F_1 plants
364 of eight reciprocal sets and monitored their flowering phenology from April to November in
365 2018 at the experimental farm. During mid-April, flowering of E-type accessions peaked
366 (Figure 4B). Approximately 1 month later, flowering of the C-type peaked. During this period,
367 none of the F_1 plants flowered. In contrast, F_1 sporadically flowered from September to
368 November, as observed for wild F_1 populations. The results indicate that hybridisation between
369 the C- and E-types of cogongrass delays the flowering of F_1 progeny to fall, which prevent F_1s
370 from crossing with the parental ecotypes.

371 Next, to clarify the mechanism of prevention of F_1 x F_1 crossing, we surveyed seed
372 sets on F_1 plants in natural habitats from 25 populations in fall. The C-type produced more
373 flowers than the E-type (359.1 and 246.1 on average, respectively), as reported previously
374 (Matumura et al. 1983). F_1 plants produced 368.6 flowers on average, suggesting that they had
375 the same ability to produce flowers as the C-type. The average seed set, however, was 0.12%
376 (0.0–2.5%); thus, most of the F_1 panicles in natural habitats did not carry mature seeds (Figure
377 5A). In contrast, our spring survey showed that the average seed set of the C- and E-types in
378 natural habitats was approximately 70-fold higher than that of F_1 plants (Tukey HSD, $P <$
379 0.001): 7.1% (0.0–70.6%) for the C-type and 9.0% (0.0–60.7%) for the E-type. A similar seed
380 set percentage, 8.7% (0.6–24.7%), was observed in F_1 plants when different populations were

381 artificially crossed (Figure 5A), suggesting that F_1 plants have an extremely low seed set in
382 natural habitats, although they can produce offspring at rates similar to the C- and E-types.

383 We also investigated the viability of seeds derived from F_1 x F_1 crosses. Seeds
384 collected from artificial F_1 x F_1 crosses were subjected to germination tests at various
385 temperatures. As reported for the C- and E-types (Matumura et al. 1983; Mizuguti et al. 2002),
386 the frequency of germination was high at 30/20°C, although none of the seeds germinated at
387 15/5°C (Figure 5B). Under the optimal conditions, mature seeds on F_1 plants in natural habitats
388 were also tested for germination immediately after seed collection, as seeds of cogongrass do
389 not exhibit dormancy (Matumura et al. 1983; Shilling et al. 1997). A significantly lower and
390 similar germination percentage compared to seeds of C- and E-type plants from natural habitats
391 were observed in F_1 plants, respectively (C-type, Tukey HSD, $P < 0.05$; E-type, Tukey HSD, P
392 $= 0.64$) (Figure 5B). When the seeds were sown outside, however, the pattern of germination
393 differed between F_1 and the parental ecotypes (C-type, Tukey HSD, $P < 0.001$; E-type, Tukey
394 HSD, $P < 0.05$). Seeds of C- and E-types that were sown in May to June had a higher
395 germination percentage: 64.9% (176 seeds) for the C-type and 40.4% (179 seeds) for the E-type.
396 In contrast, seeds from F_1 plants in the natural habitats exhibited a low germination percentage
397 when sown in December. Only 1 of the 58 seeds used for the experiment emerged by the
398 following June. That seed germinated in April, when the average temperature was 16.4°C. We

399 also used C-type seeds for the overwinter experiment due to the limited number of seeds from
400 F_1 plants. In total, 160 C-type seeds from six panicles (likely from different ramets), collected in
401 June to July and stored at 4°C, were sown in the following December. Consistent with the result
402 of seeds from F_1 plants, a low germination percentage (7 of 177 seeds) was observed in C-type
403 seeds sown in winter compared to in spring–summer: 0% germination in 117 seeds from five
404 panicles and 11.7% germination in 60 seeds from one panicle (Figure S6) (GLMM, $P < 0.001$).
405

406 **Discussion**

407 *Drastic shift in flowering phenology shapes population structures*

408 In the genetic population structure of cogongrass in Japan, we found that hybrids between C-
409 and E-type comprised only F_1 generation except for one accession (Figure 3C). Four or five
410 markers are enough to distinguish F_1 from F_2 or backcross individuals (Boecklen and Howard
411 1997). Therefore, used markers are a few in this study but classification by NEWHYBRIDS is
412 appropriate. Origin of one accession with mismatched nuclear and chloroplast DNA types is
413 unclear. It may be a chloroplast capture or an incomplete lineage sorting (Rieseberg and Soltis
414 1991; Kuritzin et al. 2016). The fact that F_1 plants were identified in materials collected 30 years
415 ago indicates that backcrossing and F_1 x F_1 crossing events have been prevented for at least 30
416 years. Investigation of F_1 phenology revealed that they flowered in fall, whereas the parental

417 ecotypes flowered in spring (Figure 4), which acts as a reproductive barrier to backcrossing. Our
418 survey of the seed setting of F_1 plants in natural habitats in fall suggests that the success rate of
419 F_1 x F_1 crossing is 70-fold lower than that of the C- and E-types in spring (Figure 5A). Also, the
420 germination percentage of the seeds set on F_1 under the outside condition was markedly lower
421 than that of the C- and E-types (Figure 5B). Notably, F_1 plants possessed the potential for seed
422 propagation, as did the C- and E-types. A high level of seed setting by F_1 x F_1 crossing was
423 observed in hand pollination between plants from distinct populations (Figure 5A); the
424 germination percentage was higher under warmer conditions (Figure 5B). Thus, the lower seed
425 set and germination under natural conditions were likely caused by interactions with
426 environmental parameters, such as temperature and population structure, which F_1 plants
427 confront as a result of drastic shifts in flowering phenology. Previous study demonstrated that
428 some accessions of artificial F_1 showed higher performance for biomass production than
429 parental ecotypes under dry and wet conditions (Miyoshi and Tominaga 2017). Together, our
430 data indicate that the peculiar F_1 population structure of cogongrass is caused by loss of seed
431 propagation, mediated by the drastic shift in flowering phenology, and it has been maintained by
432 vigorous clonal reproduction via rhizomes (Figure 6).

433 Normal growth of artificially generated pseudo- F_2 individuals (data not shown)
434 indicates the existence of extrinsic mechanism(s) of excluding F_2 plants in nature. Our data

435 suggest that two events likely explain the absence of F_2 plants: the exceptionally low
436 seed-setting percentage of F_1 plants and the low germination percentage of seeds dispersed in
437 winter. The former mechanism(s) under natural conditions is unknown but is unlikely to be an
438 environmental parameter such as temperature, based on the higher seed-setting percentage in
439 our artificial crossing experiment in fall (Figure 5B) and the normal germination of F_1 pollen in
440 fall (T. Tominaga, unpublished data). The following are more plausible hypotheses: (1) Genetic
441 diversity within/among F_1 populations may affect seed setting percentage. Cogongrass can have
442 a diverse seed setting percentages, which, due to its self-incompatible and outcrossing nature,
443 would depend on the population genetic diversity (Shilling et al. 1997). F_1 populations may hold
444 insufficient genetic diversity to have a higher seed-setting percentage. (2) Non-synchronous
445 flowering of F_1 plants may affect the seed setting percentage. Contrary to the C- and E-types,
446 flowering of F_1 individuals occurred sporadically, and the flowering period was roughly 2
447 months, twofold longer than that of the C- and E-types (Figure 4B). Synchronisation of
448 flowering would have a marked impact on the seed setting percentage of cogongrass. Further
449 studies of the genetic population structures within/among F_1 populations and the flowering
450 period under natural conditions are needed to assess the low seed-setting percentage of F_1
451 plants.

452 The exceptionally low germination percentage of the seeds dispersed in winter would
453 also prevent the existence of F_2 plants within hybrid populations. The germination percentage of
454 the seeds sown in winter was 30-fold lower than that of seeds sown in spring. Given the low
455 seed-setting percentage of F_1 plants, roughly 0.05% that of the C- and E-types, an extremely low
456 percentage of seedling establishment would be expected. In addition, our data may overestimate
457 the germination percentage. In this study, we sowed the field-collected seeds in December,
458 when cogongrass germination is suppressed by low temperature. In nature, however, seeds set
459 and dispersed in early fall (such as September to early October) may germinate before winter.
460 The seedlings are unlikely to overwinter because of insufficient rhizome development due to
461 low temperature. Further investigation of seed dynamics, especially in nature, is required to
462 understand the mechanism that prevents successful F_1 x F_1 crossing.
463
464 *Role of instant reproductive isolation on evolution*
465 We identified a mechanism of instant reproductive isolation, in which almost complete
466 reproductive isolation is established in one generation due to the self-incompatible and
467 perennial nature of cogongrass. The former prevents generation of F_2 plants, and the latter
468 allowed the late-flowering hybrids to remain in the habitat by clonal reproduction (Figure 6).
469 Furthermore, these characteristics have led to the population structure of hybrids with only F_1

plants (Milne and Abbott 2008). Similarly, hybrid populations comprising only the F_1 generation have been reported in perennial plants including herbaceous plants and woods (Nason et al. 1992; Kuehn et al. 1999; Milne et al. 2003; Kameyama et al. 2008; Milne and Abbott 2008; Zha et al. 2010; Nagano et al. 2015). These previous studies have proposed two major hypotheses on the absence of F_2 and backcross hybrids: one is an extrinsic factor and the other is an intrinsic factor (Nason et al. 1992; Kuehn et al. 1999; Milne et al. 2003; Kameyama et al. 2008; Milne and Abbott 2008; Kameyama and Kudo 2011). The extrinsic factor hypothesis explains that a parental environment is unsuitable for F_2 and backcross hybrids (Nason et al. 1992; Milne et al. 2003; Milne and Abbott 2008), while the intrinsic factor hypothesis explains that hybrid breakdown occurs (Kuehn et al. 1999; Kameyama et al. 2008; Kameyama and Kudo 2011). However, no direct factor determining population structure has been identified in above mentioned studies.

F_1 progeny showed an intermediate morphology between the C- and E-types (Figure 3A, S4). The ecological roles of the traits investigated in this study are unknown, even in the parental ecotypes. However, the difference in morphology, particularly in rhizome aerenchyma, among the C-type, E-type, and F_1 may influence the preference of habitat. In our observations, F_1 plants were found in a wider range of soil moisture conditions than their parents (Nomura et al., in prep.), whereas the C- and E-types preferred relatively dry and wet habitats, respectively.

488 This species is a worldwide noxious weed and has a wide range of distribution (Holm et al.
489 1977; MacDonald 2004; Burrell et al. 2015). This species is intentionally introduced as an
490 ornament or forage grass and escapes unintentionally to natural habitats, expanding its
491 distribution (MacDonald 2004; Cseke and Talley 2012; Burrell et al. 2015). If
492 intentional/unintentional introduction provide F_1 plants a chance to be distributed to a warm
493 region where F_1 can sexually reproduce, F_1 will evolve into a new invasive taxon without
494 backcrossing with parental species. Further studies of the role of rhizome aerenchyma in
495 environmental preference may shed further light on the distribution of cogongrass in Japan.
496
497 *Regulation of flowering phenology*
498 To ensure reproductive success, plants must regulate flowering to synchronise with optimal
499 environmental conditions for seed production. A large number of molecular players are involved
500 in the regulation of flowering in plants (Hill and Li 2016), which allows plants to fine-tune the
501 flowering period. These players often function in an additive manner (Martin and Willis 2007;
502 Martin et al. 2007; Buckler et al. 2009); therefore, crossing between individuals with different
503 flowering periods often results in an intermediate flowering period. In contrast, marked delay of
504 flowering has been observed in F_1 hybrids of particular ecotypes/lines of *Arabidopsis thaliana*
505 (L.) Heynh. (Koornneef et al. 1994; Henderson and Dean 2004) and *Sorghum bicolor* (L.)

506 Moench (Murphy et al. 2014; Yang et al. 2014). In both cases, the parental ecotypes/lines have a
507 disrupted form of a floral repressor or its activator in the floral regulatory pathway, which lifts
508 the repression of flowering. Hybridisation of the two ecotypes/lines restores the flowering
509 repression pathway because the hybrids carry functional alleles of each gene. The genes
510 involved in the delayed phenology differ between *A. thaliana* and *S. bicolor* although their basic
511 relationship as a floral repressor and its activator is identical. The genes related to the delayed
512 phenology of F_1 in cogongrass are unknown. We speculate that similar molecular mechanisms
513 as in *S. bicolor* are involved in the delayed phenology in cogongrass because the two species are
514 evolutionarily similar—both are in the tribe Andropogoneae. Genomic and transcriptomic
515 approaches will enhance our understanding of the dynamic flowering shift in cogongrass.
516
517 *Conclusion*
518 A novel phenotype derived from hybridisation between two ecotypes has major effects on the
519 population structure of cogongrass. The fact that hybrid populations consist of almost of all F_1
520 plants implies that the F_1 progeny of C- and E-type cogongrass lose their sexual reproduction
521 system and only reproduce asexually. Namely, hybridisation of the two ecotypes altered not
522 only the flowering phenology but also the reproductive strategy of cogongrass. Considering that
523 similar delays in flowering phenology are observed in other plant lineages (*e.g. A. thaliana* and

524 *S. bicolor*), it is reasonable to speculate that hybridisations between independently evolved
525 ecotypes may cause a drastic shift of flowering phenology even in other plants. Our findings
526 will facilitate investigation of the ecological role of hybridisation in flowering phenology shifts
527 in other species. Also, the molecular basis of the flowering shift is an exciting challenge for the
528 future.
529
530
531

Reference

Anderson, E. C., and E. A. Thompson. 2002. A model-based method for identifying species hybrids using multilocus data. Genetics 160:1217–1229.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67:1–48.

Boecklen, W. J., and D. J. Howard. 1997. Genetic analysis of hybrid zones : Numbers of markers and power of resolution. Ecology 78:2611–2616.

Buckler, E. S., J. B. Holland, P. J. Bradbury, C. B. Acharya, P. J. Brown, C. Browne, E. Ersoz, S. Flint-Garcia, A. Garcia, J. C. Glaubitz, M. M. Goodman, C. Harjes, K. Guill, D. E. Kroon, S. Larsson, N. K. Lepak, H. Li, S. E. Mitchell, G. Pressoir, J. A. Peiffer, M. O. Rosas, T. R. Rocheford, M. C. Romay, S. Romero, S. Salvo, H. S. Villeda, H. Sofia da Silva, Q. Sun, F. Tian, N. Upadyayula, D. Ware, H. Yates, J. Yu, Z. Zhang, S. Kresovich, and M. D. McMullen. 2009. The genetic architecture of maize flowering time. Science 325:714–718.

Buerkle, C. A., R. J. Morris, M. A. Asmussen, and L. H. Rieseberg. 2000. The likelihood of homoploid hybrid speciation. Heredity 84:441–451.

548 Burrell, A. M., A. E. Pepper, G. Hodnett, J. A. Goolsby, W. A. Overholt, A. E. Racelis, R. Diaz,
549 and P. E. Klein. 2015. Exploring origins, invasion history and genetic diversity of
550 *Imperata cylindrica* (L.) P. Beauv. (Cogongrass) in the United States using genotyping by
551 sequencing. Mol. Ecol. 24:2177–2193.

552 Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer, Sunderland, MA U.S.A.

553 Cseke, L. J., and S. M. Talley. 2012. A PCR-based genotyping method to distinguish between
554 wild-type and ornamental varieties of *Imperata cylindrica*. J. Vis. Exp. 60:e3265.

555 Duenez-Guzman, E. A., J. Mavárez, M. D. Vose, and S. Gavrilets. 2009. Case studies and
556 mathematical models of ecological speciation. 4. hybrid speciation in butterflies in a
557 jungle. Evolution 63:2611–2626.

558 Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals
559 using the software STRUCTURE: a simulation study. Mol. Ecol. 14:2611–2620.

560 Grant, P. R., and B. R. Grant. 1994. Phenotypic and genetic effects of hybridization in Darwin's
561 finches. Evolution 48:297–316.

562 Henderson, I. R., and C. Dean. 2004. Control of *Arabidopsis* flowering: the chill before the
563 bloom. Development 131:3829–3838.

564 Hill, C. B., and C. Li. 2016. Genetic architecture of flowering phenology in cereals and
565 opportunities for crop improvement. Front. Plant Sci. 7:1906.

566 Holm, L. G., D. L. Plucknett, J. V. Pancho, and J. P. Herberger. 1977. *Imperata cylindrica* (L.)
567 Beauv. POACEAE (also GRAMINEAE). GRASS FAMILY. Pp. 62–71 *in* L. G. Holm, D.
568 L. Plucknett, J. V. Pancho, and J. P. Herberger, eds. The World's Worst Weeds,
569 Distribution and Biology. University Press of Hawaii, Honolulu, USA.

570 Hothorn, T., F. Bretz, and P. Westfall. 2008. Simultaneous inference in general parametric
571 models. Biometrical J. 50:346–363.

572 Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation
573 program for dealing with label switching and multimodality in analysis of population
574 structure. Bioinformatics 23:1801–1806.

575 Johansen-Morris, A. D., and R. G. Latta. 2006. Fitness consequences of hybridization between
576 ecotypes of *Avena barbata*: hybrid breakdown, hybrid vigor, and transgressive segregation.
577 Evolution 60:1585–1595.

578 Kagawa, K., and G. Takimoto. 2018. Hybridization can promote adaptive radiation by means of
579 transgressive segregation. Ecol. Lett. 21:264–274.

580 Kameyama, Y., T. Kasagi, and G. Kudo. 2008. A hybrid zone dominated by fertile F1s of two
581 alpine shrub species, *Phyllodoce caerulea* and *Phyllodoce aleutica*, along a snowmelt
582 gradient. J. Evol. Biol. 21:588–597.

583 Kameyama, Y., and G. Kudo. 2011. Clarification of the genetic component of hybrids between
584 *Phyllodoce caerulea* and *Phyllodoce aleutica* (Ericaceae) in Hokkaido, northern Japan.
585 Plant Species Biol. 26:93–98.

586 Koornneef, M., H. Blankestijn-de Vries, C. Hanhart, W. Soppe, and T. Peters. 1994. The
587 phenotype of some late-flowering mutants is enhance by a locus on chromosome 5 that is
588 not effetive in the Landsberg erecta wild-type. Plant J. 6:911–919.

589 Kuehn, M. M., J. E. Minor, and B. N. White. 1999. An examination of hybridization between
590 the cattail species *Typha latifolia* and *Typha angustifolia* using random amplified
591 polymorphic DNA and chloroplast DNA markers. Mol. Ecol. 8:1981–1990.

592 Kuritzin, A., T. Kischka, J. Schmitz, and G. Churakov. 2016. Incomplete lineage sorting and
593 hybridization statistics for large-scale retroposon insertion data. PLoS Comput. Biol. 12:1–
594 20.

595 Lamichhaney, S., F. Han, M. T. Webster, L. Andersson, B. R. Grant, and P. R. Grant. 2018.
596 Rapid hybrid speciation in Darwin's finches. Science 359:224–228.

597 MacDonald, G. E. 2004. Cogongrass (*Imperata cylindrica*) -Biology, Ecology, and
598 Management. CRC. Crit. Rev. Plant Sci. 23:367–380.

599 Mallet, J. 2007. Hybrid speciation. Nature 446:279–283.

600 Marques, I., A. Jürgens, J. F. Aguilar, and G. N. Feliner. 2016. Convergent recruitment of new
601 pollinators is triggered by independent hybridization events in *Narcissus*. New Phytol.
602 210:731–742.

603 Martin, N. H., A. C. Bouck, and M. L. Arnold. 2007. The genetic architecture of reproductive
604 isolation in louisiana irises: flowering phenology. Genetics 175:1803–1812.

605 Martin, N. H., and J. H. Willis. 2007. Ecological divergence associated with mating system
606 causes nearly complete reproductive isolation between sympatric *Mimulus* species.
607 Evolution 61:68–82.

608 Matumura, M., and T. Yukimura. 1980. The comparative ecology of intraspecific variants of the
609 Chigaya, *Imperata cylindrica* var. *koenigii* (Alang-alang) . (1) Habitats of the common and

610 early flowering types of the Chigaya based on the vegetation characteristics. Res. Bull. Fac.
611 Coll. Agric. Gifu Univ. 43:233–248. [In Japanese with English abstract]

612 Matumura, M., T. Yukimura, and S. Shinoda. 1983. Fundamental studies on artificial
613 propagation by seeding useful wild grasses in Japan IX. Seed fertility and germinability of
614 the intraspecific two types of Chigaya (Alang-alang), *Imperata cylindrica* var. *koenigii*. J.
615 Japanese Grassl. Sci. 28:395–404.

616 Mavárez, J., C. A. Salazar, E. Bermingham, C. Salcedo, C. D. Jiggins, and M. Linares. 2006.
617 Speciation by hybridization in *Heliconius* butterflies. Nature 441:868–871.

618 Milne, R. I., and R. J. Abbott. 2008. Reproductive isolation among two interfertile
619 *Rhododendron* species: low frequency of post-F1 hybrid genotypes in alpine hybrid zones.
620 Mol. Ecol. 17:1108–1121.

621 Milne, R. I., S. Terzioglu, and R. J. Abbott. 2003. A hybrid zone dominated by fertile F1s:
622 maintenance of species barriers in *Rhododendron*. Mol. Ecol. 12:2719–2729.

623 Miyoshi, I., and T. Tominaga. 2017. Growth of hybrids between the common and early
624 ecotypes of *Imperata cylindrica*. Grassl. Sci. 63:128–131.

625 Mizuguti, A., A. Nishiwaki, and M. Oyamada. 2002. Differences of seed germination characters
626 between two types of *Imperata cylindrica* (L.) Beauv. characterized by flowering
627 phenology. Grassl. Sci. 48:216–220. [In Japanese with English abstract]

628 Mizuguti, A., A. Nishiwaki, and Y. Sugimoto. 2004. Genetic difference between two types of
629 *Imperata cylindrica* (L.) Beauv. characterized by flowering phenology. Grassl. Sci. 50:9–
630 14.

631 Mizuguti, A., A. Nishiwaki, and Y. Sugimoto. 2003. Morphological differences between two
632 types of *Imperata cylindrica* (L.) BEAUV. characterized by flowering phenology. Grassl.
633 Sci. 49:324–329. [In Japanese with English abstract]

634 Murphy, R. L., D. T. Morishige, J. A. Brady, W. L. Rooney, S. Yang, P. E. Klein, and J. E.
635 Mullet. 2014. *Ghd7* (*Ma6*) represses Sorghum flowering in long days: alleles enhance
636 biomass accumulation and grain production. Plant Genome 7:1–10.

637 Murray, M. G., and W. F. Thompson. 1980. Rapid isolation of high molecular weight plant
638 DNA. Nucleic Acids Res. 8:4321–4326.

639 Nagano, Y., A. S. Hirao, and T. Itino. 2015. Genetic structure of a hybrid zone between two
640 violets, *Viola rossii* Hemsl. and *V. bissetii* Maxim.: dominance of F1 individuals in a
641 narrow contact range. Plant Species Biol. 30:237–243.

642 Nason, J. D., N. C. Ellstrand, and M. L. Arnold. 1992. Patterns of hybridization and
643 introgression in populations of oaks, manzanitas, and irises. Am. J. Bot. 79:101–111.

644 Nomura, Y., Y. Shimono, and T. Tominaga. 2015. Development of chloroplast DNA markers in
645 Japanese *Imperata cylindrica*. Weed Res. 55:329–333.

646 Peakall, R., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population
647 genetic software for teaching and research. Mol. Ecol. Notes 6:288–295.

648 Peakall, R., and P. E. Smouse. 2012. GenALEx 6.5: genetic analysis in Excel. Population
649 genetic software for teaching and research-an update. Bioinformatics 28:2537–2539.

650 Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using
651 multilocus genotype data. Genetics 155:945–959.

652 R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for
653 Statistical Computing, Vienna, Austria.

654 Rieseberg, L. H., and D. E. Soltis. 1991. Phylogenetic consequences of cytoplasmic gene flow
655 in plants. Evol. Trends Plants 5:65–84.

656 Selz, O. M., R. Thommen, M. E. Maan, and O. Seehausen. 2014. Behavioural isolation may
657 facilitate homoploid hybrid speciation in cichlid fish. J. Evol. Biol. 27:275–289.

658 Shilling, D. G., T. A. Bewick, J. F. Gaffney, S. K. McDonald, C. A. Chase, and E. R. R. L.
659 Johnson. 1997. Ecology, Physiology, and Management of Cogongrass (*Imperata*
660 *Cylindrica*): Final Report. Florida Institute of Phosphate Research. Institute of Food and
661 Agricultural Sciences, University of Florida, Gainesville, Florida.

662 Soltis, P. S., D. B. Marchant, Y. Van de Peer, and D. E. Soltis. 2015. Polyploidy and genome
663 evolution in plants. Curr. Opin. Genet. Dev. 35:119–125.

664 Soltis, P. S., and D. E. Soltis. 2009. The role of hybridization in plant speciation. Annu. Rev.
665 Plant Biol. 60:561–588.

666 Stephens, M., N. J. Smith, and P. Donnelly. 2001. A new statistical method for haplotype
667 reconstruction from population data. Am. J. Hum. Genet. 68:978–989.

668 Taylor, S. J., L. D. Rojas, S. W. Ho, and N. H. Martin. 2013. Genomic collinearity and the
669 genetic architecture of floral differences between the homoploid hybrid species *Iris*
670 *nelsonii* and one of its progenitors, *Iris hexagona*. Heredity (Edinb). 110:63–70.

671 Tominaga, T., H. Kobayashi, and K. Ueki. 1989. Geographical variation of *Imperata cylindrica*
672 (L.) Beauv. in Japan. J. Japanese Grassl. Sci. 35:164–171.

673 Tominaga, T., A. Nishiwaki, A. Mizuguti, and T. Ezaki. 2007. Weed Monograph 5. *Imperata*
674 *cylindrica* (L.) Beauv. J. Weed Sci. Technol. 52:17–27 [In Japanese].

675 Wood, T. E., N. Takebayashi, M. S. Barker, I. Mayrose, P. B. Greenspoon, and L. H. Rieseberg.
676 2009. The frequency of polyploid speciation in vascular plants. Proc. Natl. Acad. Sci.
677 106:13875–13879.

678 Yang, S., R. L. Murphy, D. T. Morishige, P. E. Klein, W. L. Rooney, and J. E. Mullet. 2014.
679 Sorghum phytochrome B inhibits flowering in long days by activating expression of
680 *SbPRR37* and *SbGHD7*, repressors of *SbEHD1*, *SbCN8* and *SbCN12*. PLoS One
681 9:e105352.

682 Yasuda, K., and H. Shibayama. 2006. Primer sets for DNA amplification of the noncoding
683 regions of the chloroplast genome in the grass family. J. Weed Sci. Technol. 51:146–151.

684 Zha, H. G., R. I. Milne, and H. Sun. 2010. Asymmetric hybridization in *Rhododendron*

685 *agastum*: a hybrid taxon comprising mainly F1s in Yunnan, China. Ann. Bot. 105:89–100.

686

687

688 Figure 1

689 Title: Morphology of C-, E-type and hybrids

690 Legends: Bars in right bottom of photos are scale bars. Scale bars mean 1 cm, 1 cm, 0.5 cm and

691 250 μm in rhizomes, leaf sheathes, culms and leaf midribs, respectively.

692

693 Figure 2

694 Title: Sampling location and distribution of each ecotype

695 Legends: Ecotypes were determined using CAPS marker in ITS region.

696

697 Figure 3

698 Title: Differentiation between C- and E-type and hybridisation percentage

699 Legends: Results of PCA for morphological traits (A). Results of PCoA for 12 markers in

700 nuclear DNA (B). Results of analysis for cpDNA and STRUCTURE and NEWHYBRIDS for

701 12 markers in nuclear DNA (C). Top, middle and bottom figures are results of analysis for

702 cpDNA, STRUCTURE and NEWHYBRIDS. Hybridisation percentage in natural habitats (D).

703 X-axis means genotypes of panicles (C- or E-type) and study sites (north and south sites). Points

704 means hybridisation percentage per each population (surveyed panicles in north sites: C-type, n

705 = 377; E-type, n = 395; south sites: C-type, n = 222; E-type, n = 86). Hybridisation percentage

706 of E-type is significantly higher than that of C-type (GLMM, $P < 0.01$).

707

708 Figure 4

709 Title: Flowering phenology of C-, E-type and F_1

710 Legends: Flowering phenology in natural populations (A). Flowering percentage means

711 (flowering ramets/all ramets) x 100 (surveyed ramets in spring: C-type, n = 407; E-type, n =

712 404; F_1, n = 532; fall: C-type, n = 264; E-type, n = 252; F_1, n = 1161). Values and error bars

713 represent means ± s.e.m. Flowering percentage of F_1 is significantly lower than that of C-type

714 and E-type in spring while that of F_1 is significantly higher than that of C-type and E-type in fall

715 (Tukey HSD, $P < 0.001$). Flowering phenology in Kyoto experimental farm (B).

716

717 Figure 5

718 Title: Viability in sexual reproduction of C-, E-type and F_1

719 Legends: Seed set analysis in natural habitats and an experimental field (A). Symbols mean a

720 year in which an experiment was conducted in natural habitats (surveyed panicles: C-type, n =

721 122; E-type, n = 183; F_1, n = 228). Seed setting of F_1 is significantly lower than that of C-type

722 and E-type (Tukey HSD, $P < 0.001$). Seed set percentage of hand-pollinated ecotypes and F_1

723 was measured in an experimental field of Kyoto University (surveyed panicles: C-type, n = 7;
724 E-type, n = 9; F_1, n = 20). Seed setting of F_1 is not significantly differ from that of C-type
725 (Tukey HSD, $P = 0.996$) and significantly lower than that of E-type (Tukey HSD, $P < 0.01$).
726 Germination tests of seeds from natural habitats or artificial pollination (B). Left panel shows
727 germination test of natural seeds under an incubator and an outside condition. Germination tests
728 in incubator were conducted under 30/20°C, light/dark condition. C- and E-type seeds were
729 sown in summer (May-August), while seeds set on F_1 were sown in winter (December)
730 (surveyed seeds under incubator: C-type, n = 784; E-type, n = 525; F_1, n = 41; outside: C-type, n
731 = 271; E-type, n = 443; F_1, n = 58). Germination percentage of F_1 is not significantly differ from
732 that of E-type (Tukey HSD, $P = 0.64$) and significantly lower than that of C-type (Tukey HSD,
733 $P < 0.05$) under the incubator condition. On the other hands, germination percentage of F_1 is
734 significantly lower than that of C-type (Tukey HSD, $P < 0.001$) and E-type (Tukey HSD, $P <$
735 0.05) under the outside condition. Right panel shows germination percentage of seeds produced
736 from F_1 x F_1 cross under four temperature conditions (surveyed seeds: 5–15°C, n = 109; 10–
737 20°C, n = 90; 15–25°C, n = 90; 20–30°C, n = 103). All points in three panels means seed set or
738 germination percentage per each population or cross treatment.
739
740 Figure 6

741 Title: Mechanism of F_1 domination in hybrid population

742 Legends: C- and E-type flower in spring and disperse their seeds in summer. Seedlings are
743 likely to be establish, because seeds of C- and E-type are dispersed in a warm season. In
744 contrast, F_1 flowers in fall and disperse their seeds in winter. This suggests that seeds set on F_1
745 are not able to germinate and are dead, because they are dispersed in a cold season.

746

747 **Supporting information**

748 Figure S1

749 Title: Sampling location of each survey

750 Legends: Sampling location of flowering phenology survey (A) and sampling location of samples
751 used for seed set (B) and germination test (C). The left panel is spring survey and the right panel is
752 fall survey. Symbols mean a year in which a survey was conducted.

753

754 Figure S2

755 Title: CAPS markers in nuclear DNA

756 Legends: Complete length of ITS in cogongrass and a recognition site (A). Arrows and a
757 triangle mean primers and a recognition site of *Dde*I, respectively. Grey bars mean
758 not-deposited sequences to Genbank. Fragment length polymorphisms of ITS (B). ITS regions

759 were amplified using ITS CAPS primer set and PCR products were digested by *Dde*I. Complete
760 length of EST104 in cogongrass and a recognition site (C). Arrows and a triangle mean primers
761 and a recognition site of *Rsa*I, respectively. Grey bars mean non-deposited sequences to
762 Genbank. Fragment length polymorphisms of ITS (D). EST104 were amplified using EST104
763 CAPS primer set and PCR products were digested by *Rsa*I.
764
765 Figure S3
766 Title: Estimated haplotypes in C-, E-type and F_1 and SNP sites in each region
767 Legends: The IDs correspond to haplotype IDs in each region (Table S4). Filled color means
768 haplotypes found in C-, E-type and F_1.
769
770 Figure S4
771 Title: Morphological traits of both ecotype and F_1
772 Legends: Culm hairs (A), wax on leaf sheaves (B), hairs on leaf sheaves (C), ratio of
773 aerenchyma diameter in a rhizome pith to rhizome diameter (D) and ratio of aerenchyma
774 diameter to leaf midrib diameter (E).
775
776 Figure S5

777 Title: CpDNA type of flowering ramets in natural habitats

778 Legends: Filled and hatched areas mean flowering and non-flowering ramets, respectively.

779 Orange and blue colors mean cpDNA of C- and E-type, respectively.

780

781 Figure S6

782 Title: Germination percentage of C-type sown in December under an incubator condition and an

783 outside condition

784 Legends: Germination tests in an incubator were conducted under 30/20°C, light/dark condition

785 (surveyed seeds under incubator, n = 160; outside, n = 177). Germination percentage under the

786 incubator condition is significantly higher than that under the outside condition ($P < 0.001$).

787

788 Table S1. Used accessions

789

790 Table S2 Primer sets

791

792 Table S3 Seed set of hand-pollinated panicles

793

794 Table S4. SNPs in haplotypes of each region

795

796 Table S5 Linkage disequilibrium r^2 for four regions

797

798

799 The English in this document has been checked by at least two professional editors, both native
800 speakers of English. For a certificate, please see: http://www.textcheck.com/certificate/LIBpy9
801 and http://www.textcheck.com/certificate/ZPfIvS

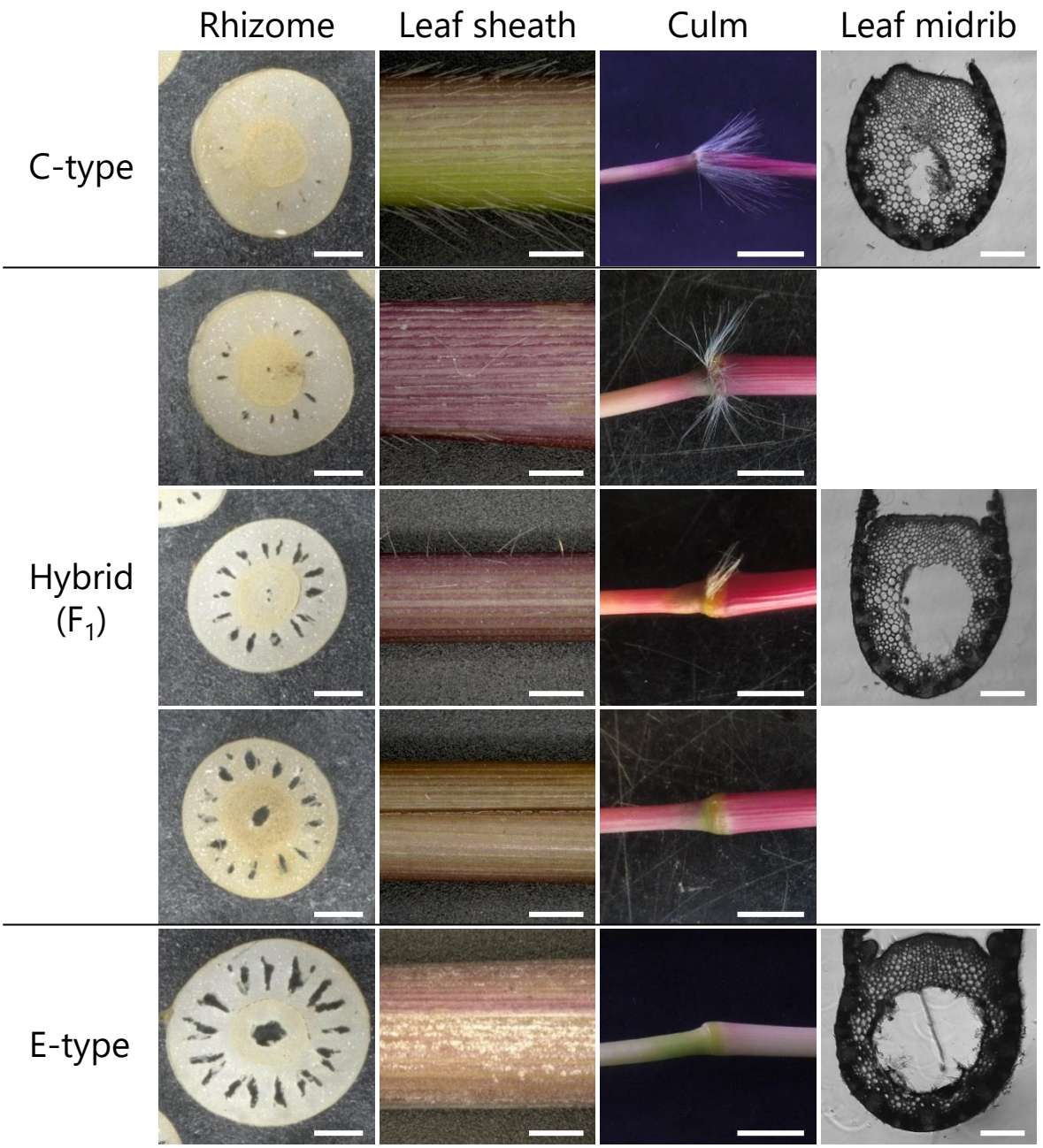

Figure 1
Title: Morphology of C-, E-type and hybrids
Legends: Bars in right bottom of photos are scale bars. Scale bars mean 1 cm, 1 cm, 0.5 cm and 250 μm in rhizomes, leaf sheathes, culms and leaf midribs, respectively.

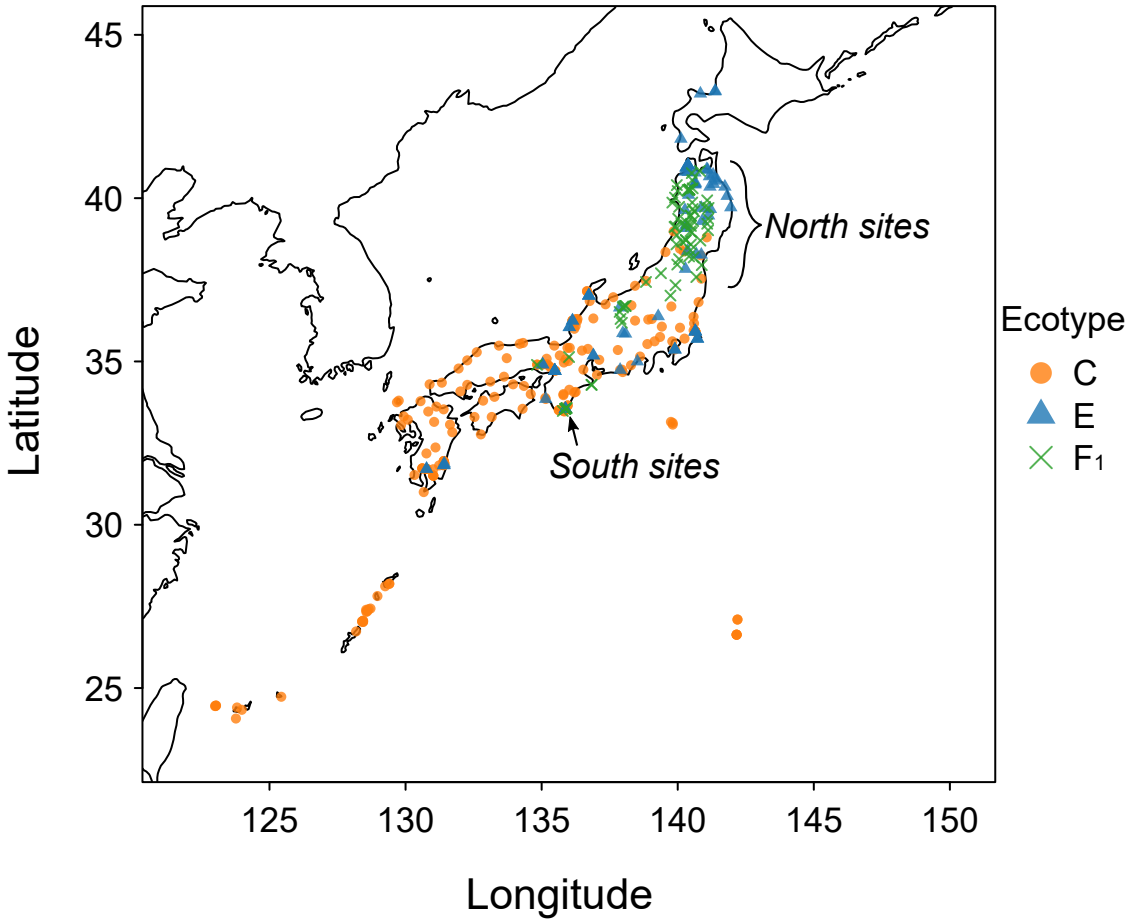

Figure 2
Title: Sampling location and distribution of each ecotype
Legends: Ecotypes were determined using CAPS marker in ITS region.

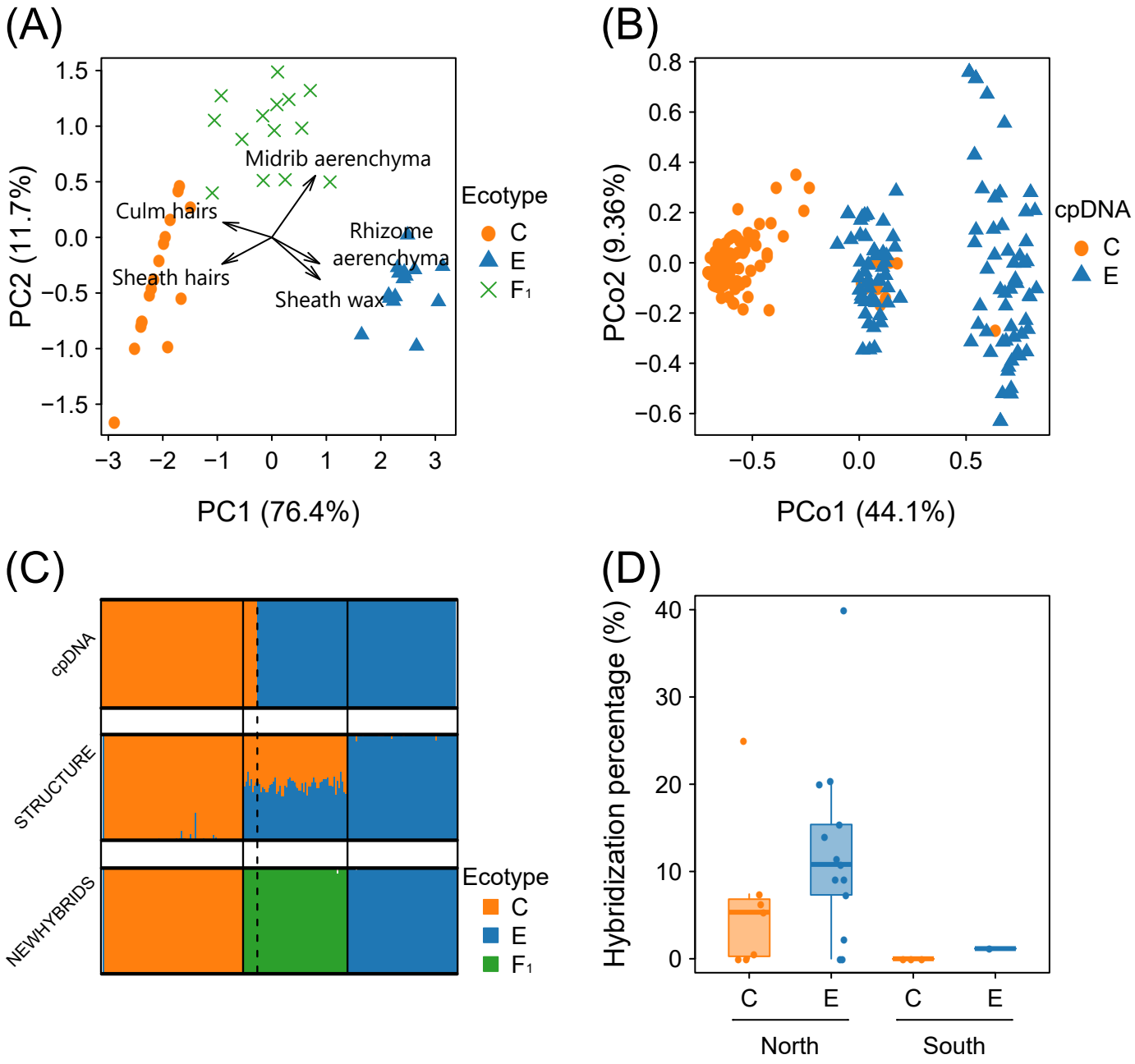

Figure 3

Title: Differentiation between C- and E-type and hybridisation percentage

Legends: Results of PCA for morphological traits (A). Results of PCoA for 12 markers in nuclear DNA (B). Results of analysis for cpDNA and STRUCTURE and NEWHYBRIDS for 12 markers in nuclear DNA (C). Top, middle and bottom figures are results of analysis for cpDNA, STRUCTURE and NEWHYBRIDS. Hybridisation percentage in natural habitats (D). X-axis means genotypes of panicles (C- or E-type) and study sites (north and south sites). Points means hybridisation percentage per each population (surveyed panicles in north sites: C-type, n = 377; E-type, n = 395; south sites: C-type, n = 222; E-type, n = 86). Hybridisation percentage of E-type is significantly higher than that of C-type (GLMM, $P < 0.01$).

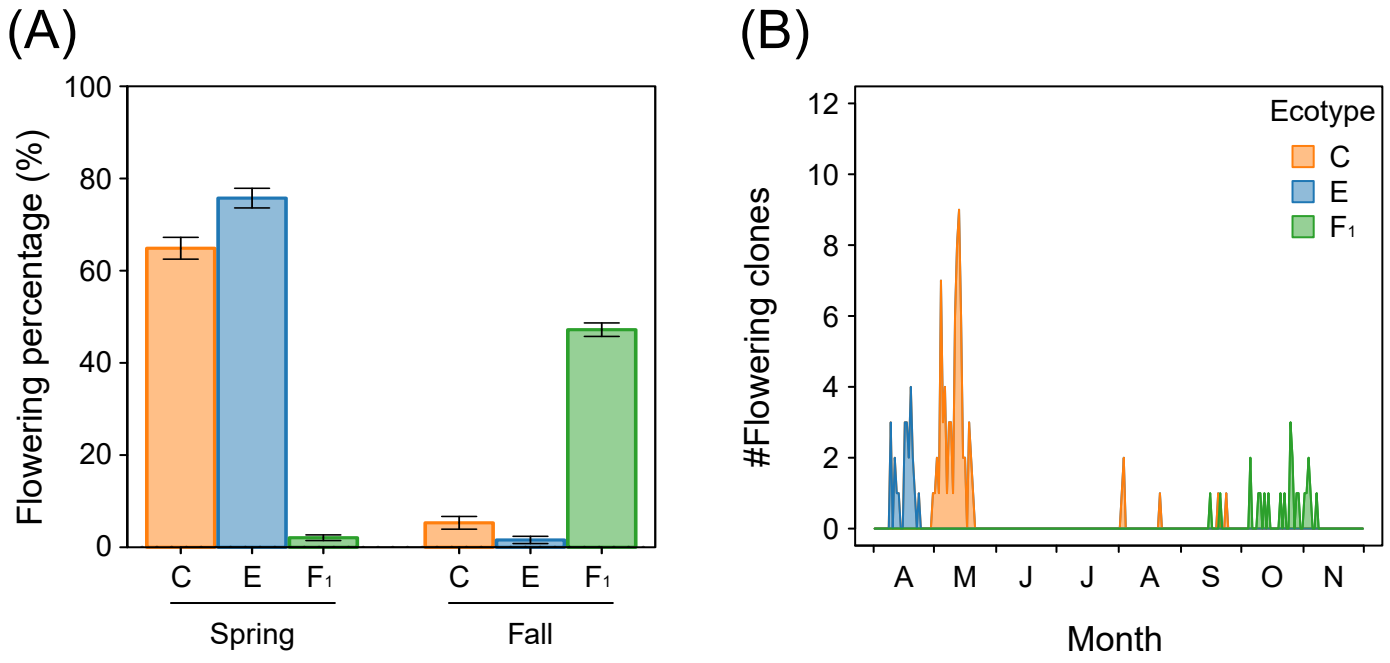

Figure 4
Title: Flowering phenology of C-, E-type and F1
Legends: Flowering phenology in natural populations (A). Flowering percentage means (flowering ramets/all ramets) x 100 (surveyed ramets in spring: C-type, n = 407; E-type, n = 404; F1, n = 532; fall: C-type, n = 264; E-type, n = 252; F1, n = 1161). Values and error bars represent means ± s.e.m. Flowering percentage of F1 is significantly lower than that of C-type and E-type in spring while that of F1 is significantly higher than that of C-type and E-type in fall (Tukey HSD, $P < 0.001$). Flowering phenology in Kyoto experimental farm (B).

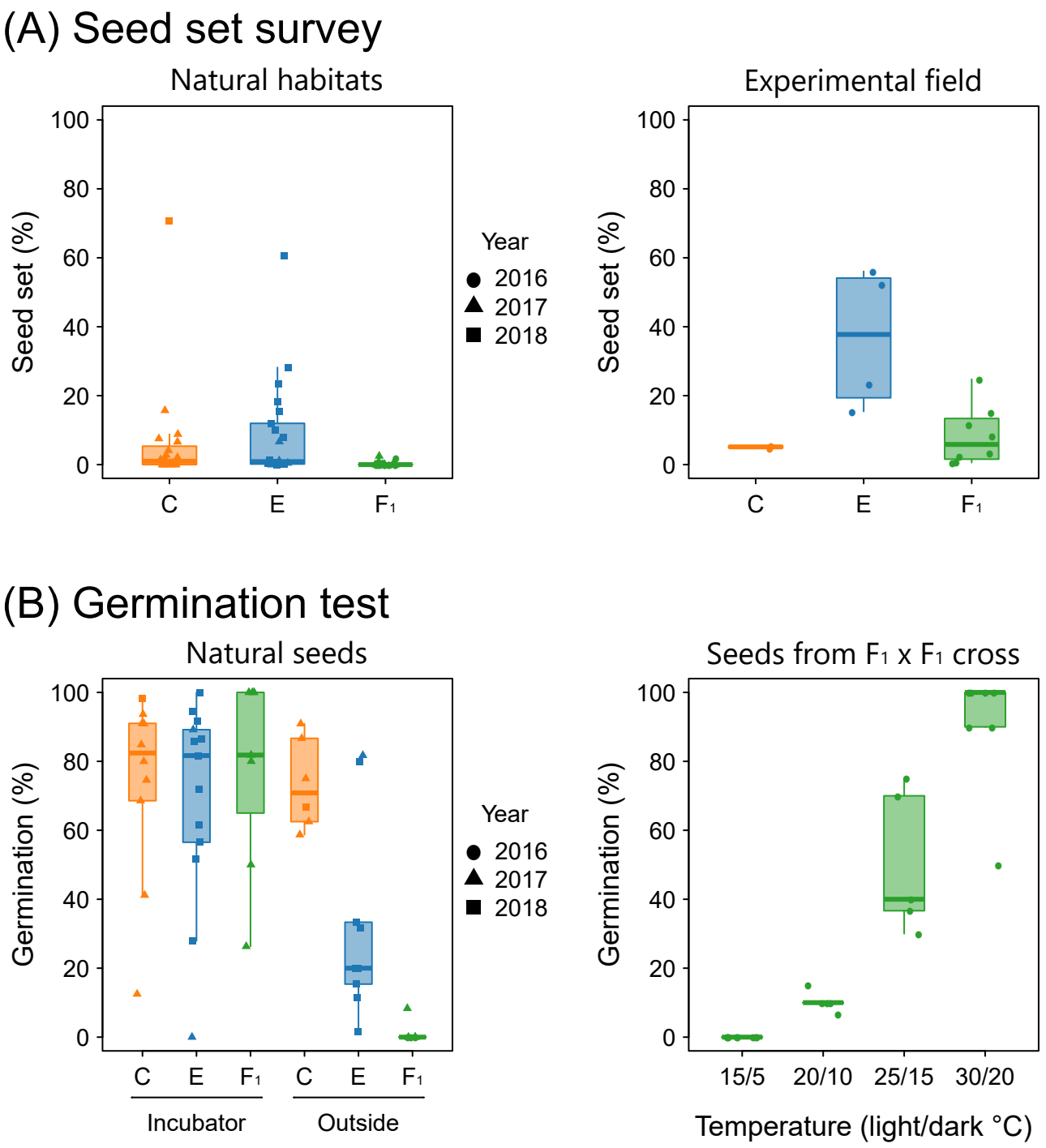

Figure 5

Title: Viability in sexual reproduction of C-, E-type and F1

Legends: Seed set analysis in natural habitats and an experimental field (A). Symbols mean a year in which an experiment was conducted in natural habitats (surveyed panicles: C-type, n = 122; E-type, n = 183; F1, n = 228). Seed setting of F1 is significantly lower than that of C-type and E-type (Tukey HSD, $P < 0.001$). Seed set percentage of hand-pollinated ecotypes and F1 was measured in an experimental field of Kyoto University (surveyed panicles: C-type, n = 7; E-type, n = 9; F1, n = 20). Seed setting of F1 is not significantly differ from that of C-type (Tukey HSD, $P = 0.996$) and significantly lower than that of E-type (Tukey HSD, $P < 0.01$). Germination tests of seeds from natural habitats or artificial pollination (B). Left panel shows germination test of natural seeds under an incubator and an outside condition. Germination tests in incubator were conducted under 30/20°C, light/dark condition. C- and E-type seeds were sown in summer (May-August), while seeds set on F1 were sown in winter (December) (surveyed seeds under incubator: C-type, n = 784; E-type, n = 525; F1, n = 41; outside: C-type, n = 271; E-type, n = 443; F1, n = 58). Germination percentage of F1 is not significantly differ from that of E-type (Tukey HSD, $P = 0.64$) and significantly lower than that of C-type (Tukey HSD, $P < 0.05$) under the incubator condition. On the other hands, germination percentage of F1 is significantly lower than that of C-type (Tukey HSD, $P < 0.001$) and E-type (Tukey HSD, $P < 0.05$) under the outside condition. Right panel shows germination percentage of seeds produced from F1 x F1 cross under four temperature conditions (surveyed seeds: 5–15°C, n = 109; 10–20°C, n = 90; 15–25°C, n = 90; 20–30°C, n = 103). All points in three panels means seed set or germination percentage per each population or cross treatment.

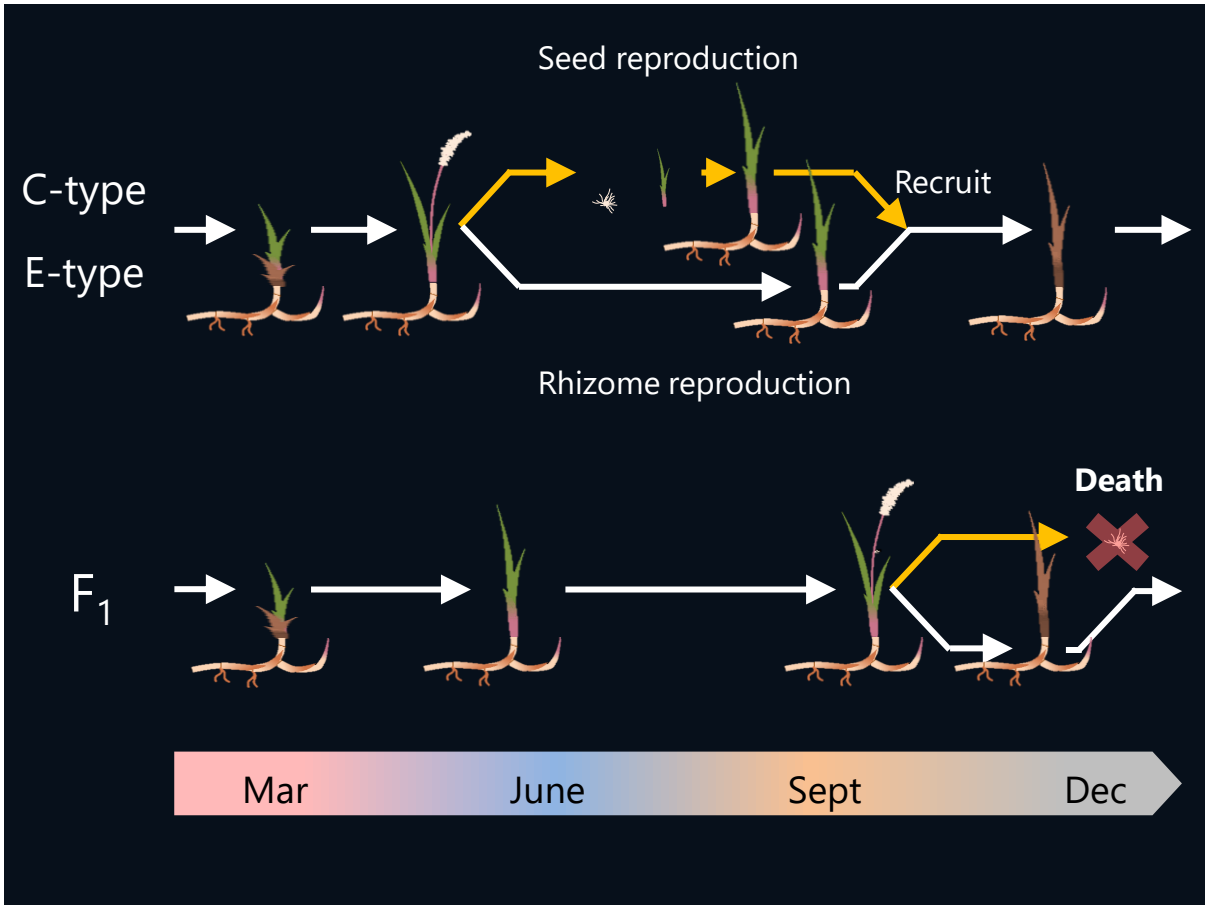

Figure 6
Title: Mechanism of F_1 domination in hybrid population
Legends: C- and E-type flower in spring and disperse their seeds in summer. Seedlings are likely to be establish, because seeds of C- and E-type are dispersed in a warm season. In contrast, F_1 flowers in fall and disperse their seeds in winter. This suggests that seeds set on F_1 are not able to germinate and are dead, because they are dispersed in a cold season.