

1 **Genome sequencing and analysis of two early-flowering cherry (*Cerasus* × *kanzakura*) varieties,**

2 **'Kawazu-zakura' and 'Atami-zakura'**

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15

16 **Running title:** Genomes of early-flowering cherry varieties

17

18 **Abstract**

19 To gain genetic insights into the early-flowering phenotype of ornamental cherry, also known as sakura, we  
20 determined the genome sequences of two early-flowering cherry (*Cerasus* × *kanzakura*) varieties,  
21 'Kawazu-zakura' and 'Atami-zakura'. Since the two varieties are interspecific hybrids, likely derived from  
22 crosses between *Cerasus campanulata* (early-flowering species) and *Cerasus speciosa*, we employed the  
23 haplotype-resolved sequence assembly strategy. Genome sequence reads obtained from each variety by  
24 single molecule real-time sequencing (SMRT) were split into two subsets, based on the genome sequence  
25 information of the two probable ancestors, and assembled to obtain haplotype-phased genome sequences.  
26 The resultant genome assembly of 'Kawazu-zakura' spanned 519.8 Mb with 1,544 contigs and an N50  
27 value of 1,220.5 kb, while that of 'Atami-zakura' totaled 509.6 Mb with 2,180 contigs and an N50 value of  
28 709.1 kb. A total of 72,702 and 72,528 potential protein-coding genes were predicted in the genome  
29 assemblies of 'Kawazu-zakura' and 'Atami-zakura', respectively. Gene clustering analysis identified 2,634  
30 clusters uniquely presented in the *C. campanulata* haplotype sequences, which might contribute to its  
31 early-flowering phenotype. Genome sequences determined in this study provide fundamental information  
32 for elucidating the molecular and genetic mechanisms underlying the early-flowering phenotype of  
33 ornamental cherry tree varieties and their relatives.

34

35 **Keywords:** early-flowering, genome assembly, haplotype-phased genome sequence, long-read sequencing,  
36 sakura

37

38 **Introduction**

39 Flowering cherry, called sakura in Japanese, is an ornamental plant popular worldwide. A major *Cerasus* ×  
40 *yedoensis* cultivar 'Somei-Yoshino', which is an interspecific hybrid of *Cerasus spachiana* and *Cerasus*  
41 *speciosa*<sup>1</sup>, usually blooms from March to April in Japan. In addition, early-flowering sakura species, such  
42 as *Cerasus campanulata*, usually bloom 1–2 months earlier than 'Somei-Yoshino', and its interspecific  
43 hybrids such as *Cerasus* × *kanzakura* also exhibit early flowering. *C.* × *kanzakura* is considered a hybrid

44 between *C. campanulata* and *Cerasus speciosa* and/or *Cerasus jamasakura*<sup>2</sup>, but its origin is still debated.  
45 Two *C. × kanzakura* cultivars, ‘Kawazu-zakura’ and ‘Atami-zakura’, also bloom early (January and  
46 February, respectively); however, the molecular mechanisms underlying their early-flowering phenotype  
47 remain unknown. Although the mechanisms of early flowering in Rosaceae family members, Japanese  
48 plum (*Prunus mume*) and peach (*Prunus persica*), which flower in February and March, respectively, are  
49 well known<sup>3</sup>, it remains unclear whether these mechanisms are common between *Cerasus* and *Prunus*.

50 Genome sequence analysis provides information on nucleotide polymorphisms and gene copy  
51 number variation, which can lead to phenotypic differences among individuals and cultivars<sup>4</sup>.  
52 Pan-genomics, which involves de novo genome sequencing of multiple lines within a species, is conducted  
53 to obtain information on variation in all genes within a species to understand the origin of the organism  
54 under study<sup>5,6</sup>. In ‘Somei-Yoshino’, haplotype-phased genome sequences have been reported, and  
55 comprehensive changes in gene expression during floral bud development that contribute toward flowering  
56 have been revealed by time-course transcriptome analysis<sup>7</sup>. Therefore, comparative genomics of multiple  
57 lines of flowering cherry varieties, such as ‘Kawazu-zakura’, ‘Atami-zakura’, and ‘Somei-Yoshino’, could  
58 provide genetic insights into their early-flowering phenotypes.

59 A trio-binning strategy<sup>8</sup>, previously used in a bovine F1 hybrid to resolve two haplotype-phased  
60 genome sequences, was recently applied to ‘Somei-Yoshino’<sup>7</sup>. Genes associated with the early-flowering  
61 phenotype of ‘Kawazu-zakura’ and ‘Atami-zakura’ were assumed to be encoded by the *C. campanulata*  
62 haplotype sequences. Therefore, in this study, we used the trio-binning strategy to determine the  
63 haplotype-phased sequences of ‘Kawazu-zakura’ and ‘Atami-zakura’. Comparative analysis of three  
64 sakura genomes (‘Kawazu-zakura’, ‘Atami-zakura’, and ‘Somei-Yoshino’) facilitated the identification of  
65 genes unique to the *C. campanulata* haplotype sequences of ‘Kawazu-zakura’ and ‘Atami-zakura’ as  
66 candidates responsible for the early-flowering phenotype of these varieties.

67

## 68 **Materials and methods**

### 69 *Plant materials and DNA extraction*

70 Two early-flowering cherry (*Cerasus × kanzakura*) varieties, 'Kawazu-zakura' and 'Atami-zakura', were  
71 used in this study. Both varieties were planted at the orchard of Kyoto Prefectural University (Kyoto,  
72 Japan). Genome DNA was extracted from young leaves by a modified sodium dodecyl sulfate (SDS)  
73 method<sup>9</sup>.

74

#### 75 *Genome size estimation*

76 Software tools used for data analyses are listed in Supplementary Table S1. Genome libraries for short-read  
77 sequencing were prepared with the TruSeq DNA PCR-Free Sample Prep Kit (Illumina, San Diego, CA,  
78 USA), and sequenced on the NextSeq 500 platform (Illumina, San Diego, CA, USA) in paired-end, 150 bp  
79 mode. The genome size was estimated with Jellyfish.

80

#### 81 *De novo genome sequence assembly and reference-guided contig ordering and orientation*

82 Genomes of the two cherry varieties were sequenced using the single molecule real-time (SMRT)  
83 sequencing technology. Long-read DNA libraries were constructed using the SMRTbell Express Template  
84 Prep Kit 2.0 (PacBio, Menlo Park, CA, USA) and sequenced on SMRT cells (1M v3 LR) in a PacBio  
85 Sequel system (PacBio). Raw sequence reads of each variety were divided into two subsets with the  
86 trio-binning strategy<sup>8</sup> using the short-read data of *C. campanulata* ('Kanhi-zakura') and *C. speciosa*  
87 ('Ohshima-zakura') (DDBJ sequence archive accession no.: DRA008096)<sup>7</sup>. The sequence read subsets  
88 were assembled separately with Falcon or Canu to build haplotype-phased diploid genome sequences.  
89 Sequence errors in the contigs were corrected twice using long reads with ARROW. Potential  
90 contaminating sequence reads from organelle genomes were identified by alignments with the chloroplast  
91 and mitochondrial genome sequences of *Prunus avium* (GenBank accession no.: MK622380 and  
92 MK816392) with Minimap2, and then removed from the final assemblies. Haplotype-phased sequences,  
93 based on binning with *C. campanulata* and *C. speciosa*, were aligned against the *C. spachiana* and *C.*  
94 *speciosa* haplotype sequences, respectively, of the 'Somei-Yoshino' genome using Ragoos to build  
95 pseudomolecule sequences. Genome sequences were compared with D-Genies.

96

### 97 *Gene prediction and repetitive sequence analysis*

98 Potential protein-coding genes were predicted with the MAKER pipeline, which was based on peptide  
99 sequences predicted from the genome sequences of sweet cherry (PAV\_r1.0)<sup>10</sup>, peach (v2.0.a1)<sup>11</sup>, and  
100 Japanese plum<sup>12</sup>. Short genes (<300 bp) as well as genes predicted with an annotation edit distance (AED)  
101 greater than 0.5, which is proposed as a threshold for good annotations in the MAKER protocol, were  
102 removed to facilitate the selection of high-confidence (HC) genes. Functional annotation of the predicted  
103 genes was performed with Hayai-Annotation Plants. Gene clustering was performed with OrthoFinder.

104 Repetitive sequences in the pseudomolecules were identified with RepeatMasker using repeat  
105 sequences registered in Repbase and a *de novo* repeat library built with RepeatModeler. The identified  
106 repetitive sequences were classified into nine types, in accordance with RepeatMasker: short interspersed  
107 nuclear elements (SINEs), long interspersed nuclear elements (LINEs), long terminal repeat (LTR)  
108 elements, DNA elements, small RNAs, satellites, simple repeats, low complexity repeats, and unclassified.

109

## 110 **Results and data description**

### 111 *De novo assembly of 'Kawazu-zakura' and 'Atami-zakura' genomes*

112 Short reads amounting to 64.0 and 127.7 Gb were obtained for 'Kawazu-zakura' and 'Atami-zakura',  
113 respectively. The genome sizes of 'Kawazu-zakura' and 'Atami-zakura' were estimated at 672.7 and 675.2  
114 Mb, respectively (Figure 1). Since 'Kawazu-zakura' and 'Atami-zakura' are interspecific hybrids, we used  
115 a trio-binning strategy to establish haplotype-resolved genome assemblies representing each parental  
116 genome sequence.

117 Long-read data (34.9 Gb) of 'Kawazu-zakura' obtained from two SMRT cells were divided into two  
118 subsets (17.2 and 17.6 Gb), in accordance with the short-read data of potential parental species, *C.*  
119 *campanulata* and *C. speciosa*<sup>7</sup>, respectively. Reads in each subset were independently assembled with  
120 Falcon to construct contigs representing the two haplotype sequences. Potential errors in the haplotype  
121 sequences were corrected with long reads, and sequences of organelle genomes were removed to obtain the

122 final assembly of the diploid genome of ‘Kawazu-zakura’. The resulting assemblies consisted of *C.*  
123 *campanulata* (262.2 Mb, N50 = 1.4 Mb) and *C. speciosa* (257.6 Mb, N50 = 1.1 Mb) haplotypes (Table 1),  
124 and were designated as KWZcam\_r1.0 and KWZspe\_r1.0, respectively. Although the total assembly size  
125 was shorter than the estimated size, the complete BUSCO scores of KWZcam\_r1.0 and KWZspe\_r1.0  
126 were 93.1% and 96.7%, respectively, indicating that the assemblies were complete (Table 1). The two  
127 assemblies were merged to generate KWZ\_r1.0, with a complete BUSCO score of 98.0%.

128 The ‘Atami-zakura’ genome was sequenced in parallel with the ‘Kawazu-zakura’ genome.  
129 Long-read data of ‘Atami-zakura’ (14.3 Gb) were obtained from two SMRT cells, and divided into two  
130 subsets (7.4 and 6.8 Gb) using the short-read data of *C. campanulata* and *C. speciosa*<sup>7</sup>, respectively. The  
131 reads were assembled with Falcon to generate two haplotype contig sequences. However, the sizes of the  
132 two assemblies (139.9 and 110.0 Mb) were much smaller than the estimated sizes. Therefore, we used  
133 Canu to obtain long assemblies. This was followed by potential sequence error correction and organelle  
134 genome sequence removal. The sizes of the resultant assemblies were improved to 267.4 Mb (N50 = 853.5  
135 kb) and 242.2 Mb (N50 = 569.4 Mb) for the *C. campanulata* and *C. speciosa* haplotypes, respectively  
136 (Table 1), and the assemblies were designated as ATMcam\_r1.0 and ATMspe\_r1.0, respectively. The  
137 complete BUSCO scores were 93.4% and 93.5% for ATMcam\_r1.0 and ATMspe\_r1.0, respectively (Table  
138 1), and 98.2% for the merged assembly (ATM\_r1.0).

139

#### 140 *Reference-guided pseudomolecule sequence construction*

141 Since the genome structures are well conserved across the *Cerasus* and *Prunus* species<sup>7</sup>, we used the two  
142 haplotype pseudomolecule sequences of the ‘Somei-Yoshino’ genome, CYEspachiana\_r3.1 and  
143 CYEspeciosa\_r3.1, as references to establish the pseudomolecule sequences of ‘Kawazu-zakura’ and  
144 ‘Atami-zakura’. A total of 777 and 746 contigs of KWZcam\_r1.0 and KWZspe\_r1.0, respectively, were  
145 aligned against CYEspachiana\_r3.1 and CYEspeciosa\_r3.1 sequences, respectively. The lengths of the  
146 resultant ‘Kawazu-zakura’ pseudomolecule sequences were 256.7 Mb (KWZcam\_r1.0) and 246.5 Mb  
147 (KWZspe\_r1.0) (Table 2). On the other hand, 1,110 ATMcam\_r1.0 and 1,041 ATMspe\_r1.0 contigs were

148 aligned with the CYEspachiana\_r3.1 and CYEspeciosa\_r3.1 sequences, respectively, and the lengths of the  
149 ‘Atami-zakura’ pseudomolecule sequences obtained were 261.5 Mb (ATMcam\_r1.0) and 238.9 Mb  
150 (ATMspe\_r1.0) (Table 2). The pseudomolecule sequences of ‘Kawazu-zakura’ and ‘Atami-zakura’  
151 genomes covered the entire genome sequence of ‘Somei-Yoshino’ (Figure 2).

152

### 153 *Gene and repetitive sequence predictions*

154 A total of 36,264 and 36,264 HC protein-coding genes were predicted in KWZcam\_r1.0 and KWZspe\_r1.0  
155 assemblies, respectively (Table 2). The complete BUSCO scores of genes in the KWZcam\_r1.0 and  
156 KWZspe\_r1.0 were 88.3% and 86.6%, respectively, while the BUSCO score of all 72,582 genes was  
157 97.0%. Functional gene annotation revealed that 9,430, 17,907, and 12,603 sequences were assigned to  
158 Gene Ontology (GO) slim terms in the biological process, cellular component, and molecular function  
159 categories, respectively, and 2,264 genes had enzyme commission numbers.

160 On the other hand, 36,281 and 36,421 HC genes were predicted in ATMcam\_r1.0 and ATMspe\_r1.0  
161 assemblies, respectively (Table 2). Complete BUSCOs of genes in ATMcam\_r1.0 and ATMspe\_r1.0 were  
162 88.3% and 86.6%, respectively, while that of all 72,702 genes was 96.8%. According to the functional  
163 gene annotation, 9,836, 18,586, and 13,020 sequences were assigned to GO slim terms in the biological  
164 process, cellular component, and molecular function categories, respectively, and 2,301 genes had enzyme  
165 commission numbers.

166 Repeat sequences occupied varying proportions of the different genome assemblies: 48.0%  
167 (KWZcam\_r1.0), 45.7% (KWZspe\_r1.0), 47.7% (ATMcam\_r1.0), and 43.2% (ATMspe\_r1.0). LTR  
168 elements were the most abundant repetitive sequences (15.1–17.7%), followed by unclassified repeats  
169 (12.7–13.7%) and DNA transposons (11.1–13.2%) (Table 3).

170

### 171 *Clustering analysis of flowering time related genes in cherry varieties*

172 Four sets of genes predicted in the haplotype-phased genomes of ‘Kawazu-zakura’ and ‘Atami-zakura’  
173 clustered with two sets of genes in the two haploid sequences of ‘Somei-Yoshino’. A total of 35,226

174 clusters were obtained, of which 10,702 were common across all six gene sets. The early-flowering  
175 phenotype of *C. × kanzakura* could be explained by genes uniquely present in the *C. campanulata*  
176 haplotype sequences. In the *C. campanulata* haplotype sequences of ‘Kawazu-zakura’ and ‘Atami-zakura’  
177 genomes, a total of 2,634 clusters were found to include 3,113 and 3,123 genes, respectively, suggesting  
178 that these genes might be associated with the early-flowering phenotype of ‘Kawazu-zakura’ and  
179 ‘Atami-zakura’.

180

### 181 **Conclusion and future perspectives**

182 Here, we report haplotype-phased genome assemblies of two early-flowering cherry (*C. × kanzakura*)  
183 cultivars, ‘Kawazu-zakura’ and ‘Atami-zakura’, both of which are interspecific hybrids derived from *C.*  
184 *campanulata* and *C. speciosa*. Although the origin of *C. × kanzakura* remains unclear, *C. campanulata* and  
185 *C. speciosa* and/or *C. jamasakura* are considered as its potential parents<sup>2</sup>. Another possibility is that  
186 ‘Atami-zakura’ originated from *C. jamasakura* and *C. campanulata*<sup>13</sup>. This is supported by the fact that our  
187 attempt to divide the long reads of ‘Atami-zakura’ into two subsets using short-read data of *C. serrulata*  
188 (closely related to *C. jamasakura*<sup>7</sup>) and *C. campanulata* failed (data not shown). Therefore, we used short  
189 reads of *C. campanulata* and *C. speciosa* for both ‘Kawazu-zakura’ and ‘Atami-zakura’. This result  
190 suggests that both ‘Kawazu-zakura’ and ‘Atami-zakura’ are closely related to *C. campanulata* and *C.*  
191 *speciosa*.

192 Clustering analysis of genes predicted in the genomes of ‘Kawazu-zakura’ and ‘Atami-zakura’  
193 together with those of ‘Somei-Yoshino’ revealed that 2,634 gene clusters were uniquely present in the  
194 genome of *C. campanulata* but absent from the genomes of *C. spachiana* and *C. speciosa*. Such copy  
195 number variation (or presence/absence variation) of genes could explain the early-flowering phenotype of  
196 ‘Kawazu-zakura’ and ‘Atami-zakura’. Previously, we performed a time-course transcriptome analysis of  
197 the floral buds and flowers of ‘Somei-Yoshino’ to clarify gene expression patterns during flowering<sup>7</sup>. A  
198 similar time-course transcriptome analysis could be applied to ‘Kawazu-zakura’ and ‘Atami-zakura’.  
199 Comparative transcriptome analysis of three cultivars could identify the genes responsible for the



200 early-flowering phenotype of sakura. Furthermore, comparative transcriptome analysis of Japanese apricot  
201 and peach<sup>3</sup> could reveal the genetic mechanisms controlling flowering time across all *Prunus* and *Cerasus*  
202 species.

203 Although several flowering cherry cultivars are known to bloom in late-spring, fall, and winter  
204 seasons<sup>7</sup>, genome sequences of only a few of these cultivars are publicly available<sup>7,14,15</sup>. Comparative  
205 genomics and transcriptomics, also known as pan-genomics<sup>4-6</sup>, of sakura would provide insights into the  
206 origins of these cultivars and their flowering mechanisms, which could facilitate the development of new  
207 cultivars with attractive flower characteristics and provide us with the ability to forecast the date of sakura  
208 blooming.

209

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212 technical assistance.

213

#### 214 **Data availability**

215 Sequence reads are available from the DNA Data Bank of Japan (DDBJ) Sequence Read Archive (DRA)  
216 database (accession no.: DRA012553). The DDBJ accession numbers of assembled sequences are  
217 BPUM01000001–BPUM01000783 (KWZcam\_r1.0), BPUM01000784–BPUM01001544 (KWZspe\_r1.0),  
218 BPUL01000001–BPUL01001124 (ATMcam\_r1.0), and BPUL01001125–BPUL01002180 (ATMspe\_r1.0).

219 The genome sequence information generated in this study is available at Plant GARDEN  
220 (<https://plantgarden.jp>).

221

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224

#### 225 **Conflict of interest**

226 None declared.

227

228 **Supporting information**

229 **Supplementary Table S1** Software tools used for genome assembly and gene prediction.

230

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270

**Table 1** Statistics of the contig sequences of two flowering cherry (*Cerasus × kanzakura*) cultivars, ‘Kawazu-zakura’ and ‘Atami-zakura’

	<b>KWZ_r1.0</b>	KWZcam_r1.0	KWZspe_r1.0	<b>ATM_r1.0</b>	ATMcam_r1.0	ATMspe_r1.0
Total contig size (bases)	<b>519,843,677</b>	262,196,010	257,647,667	<b>509,633,549</b>	267,393,285	242,240,264
Number of contigs	<b>1,544</b>	783	761	<b>2,180</b>	1,124	1,056
Contig N50 length (bases)	<b>1,220,495</b>	1,445,144	1,108,133	<b>709,113</b>	853,547	569,444
Longest contig size (bases)	<b>8,019,066</b>	5,955,677	8,019,066	<b>5,799,312</b>	5,799,312	3,381,444
Gap (bases)	<b>0</b>	0	0	<b>0</b>	0	0
Complete BUSCOs	<b>98.2%</b>	93.1%	96.7%	<b>98.0%</b>	93.4%	93.5%
Single-copy BUSCOs	<b>7.5%</b>	86.7%	89.0%	<b>16.0%</b>	86.5%	87.8%
Duplicated BUSCOs	<b>90.7%</b>	6.4%	7.7%	<b>82.0%</b>	6.9%	5.7%
Fragmented BUSCOs	<b>0.3%</b>	0.7%	0.4%	<b>0.4%</b>	0.7%	1.6%
Missing BUSCOs	<b>1.5%</b>	6.2%	2.9%	<b>1.6%</b>	5.9%	4.9%
#Genes	<b>72,702</b>	36,281	36,421	<b>72,528</b>	36,264	36,264

**Table 2** Statistics of the pseudomolecule sequences of flowering cherry (*C. × kanzakura*) cultivars, ‘Kawazu-zakura’ and ‘Atami-zakura’

	Chrom.	‘Kawazu-zakura’						‘Atami-zakura’					
		Total length	%	Number of contigs	%	Number of genes	%	Total length	%	Number of contigs	%	Number of genes	%
<i>C. campanulata</i> haplotype	1	38,834,322	14.8	86	11.0	5,173	14.3	37,943,013	14.2	123	10.9	5,180	14.3
	2	45,216,402	17.2	216	27.6	6,881	19.0	49,279,926	18.4	289	25.7	6,710	18.5
	3	28,286,294	10.8	74	9.5	3,837	10.6	29,043,603	10.9	108	9.6	3,885	10.7
	4	31,150,796	11.9	110	14.0	3,747	10.3	33,258,136	12.4	157	14.0	4,442	12.2
	5	28,296,805	10.8	82	10.5	3,852	10.6	26,412,617	9.9	83	7.4	3,625	10.0
	6	33,630,106	12.8	83	10.6	4,908	13.5	34,947,855	13.1	132	11.7	4,871	13.4
	7	20,603,721	7.9	61	7.8	2,702	7.4	21,052,878	7.9	92	8.2	3,003	8.3
	8	30,708,646	11.7	65	8.3	4,559	12.6	29,538,870	11.0	126	11.2	3,745	10.3
	Unassigned	5,546,318	2.1	6	0.8	622	1.7	6,027,887	2.3	14	1.2	803	2.2
<b>Total</b>	<b>262,273,410</b>	<b>100.0</b>	<b>783</b>	<b>100.0</b>	<b>36,281</b>	<b>100.0</b>	<b>267,504,785</b>	<b>100.0</b>	<b>1,124</b>	<b>100.0</b>	<b>36,264</b>	<b>100.0</b>	
<i>C. speciosa</i> haplotype	1	42,661,824	16.6	82	10.8	5,912	16.2	38,473,692	15.9	136	12.9	5,644	17.0
	2	33,947,397	13.2	125	16.4	4,804	13.2	32,131,565	13.3	179	17.0	4,495	13.5
	3	29,126,079	11.3	81	10.6	4,367	12.0	30,781,260	12.7	111	10.5	4,269	12.8
	4	34,989,196	13.6	162	21.3	4,668	12.8	35,466,104	14.6	214	20.3	4,532	13.6
	5	25,691,272	10.0	99	13.0	3,729	10.2	23,383,776	9.6	100	9.5	3,062	9.2
	6	29,111,560	11.3	56	7.4	4,240	11.6	32,639,480	13.5	120	11.4	4,532	13.6
	7	16,872,426	6.5	20	2.6	2,328	6.4	16,033,168	6.6	40	3.8	2,174	6.5
	8	34,141,902	13.2	121	15.9	5,125	14.1	30,022,323	12.4	141	13.4	4,075	12.3
	Unassigned	11,181,211	4.3	15	2.0	1,248	3.4	3,413,596	1.4	15	1.4	481	1.4
<b>Total</b>	<b>257,722,867</b>	<b>100.0</b>	<b>761</b>	<b>100.0</b>	<b>36,421</b>	<b>100.0</b>	<b>242,344,964</b>	<b>100.0</b>	<b>1,056</b>	<b>100.0</b>	<b>33,264</b>	<b>100.0</b>	

**Table 3** Repetitive sequences in two flowering cherry (*C. × kanzakura*) cultivars, ‘Kawazu-zakura’ and ‘Atami-zakura’

Repeat type	‘Kawazu-zakura’						‘Atami-zakura’					
	<i>C. campanulata</i> haplotype			<i>C. speciosa</i> haplotype			<i>C. campanulata</i> haplotype			<i>C. speciosa</i> haplotype		
	Number of elements	Length occupied (bp)	%	Number of elements	Length occupied (bp)	%	Number of elements	Length occupied (bp)	%	Number of elements	Length occupied (bp)	%
SINEs	5,278	495,207	0.2	7,013	665,451	0.3	8,832	896,223	0.3	6,537	608,541	0.3
LINEs	9,358	3,548,357	1.4	9,980	3,635,040	1.4	9,242	3,175,048	1.2	9,285	3,460,432	1.4
LTR elements	63,025	45,423,275	17.3	57,175	42,749,594	16.6	61,503	47,443,444	17.7	52,221	36,517,551	15.1
DNA transposons	85,647	33,936,563	12.9	84,151	30,984,015	12.0	88,999	35,176,601	13.2	77,636	26,829,236	11.1
Unclassified	131,199	36,041,455	13.7	116,201	32,825,941	12.7	130,209	34,407,194	12.9	112,663	31,370,494	12.9
Small RNA	5,384	657,326	0.3	7,211	1,536,541	0.6	6,949	828,201	0.3	2,598	503,911	0.2
Satellites	1,072	277,222	0.1	297	53,425	0.0	1,083	399,307	0.2	342	75,860	0.0
Simple repeats	75,567	3,104,558	1.2	77,082	3,144,046	1.2	77,750	3,266,196	1.2	74,414	3,048,442	1.3
Low complexity	14,265	706,271	0.3	14,754	717,629	0.3	14,352	695,481	0.3	14,137	693,339	0.3

276 **Figure legends**

277 **Figure 1** Estimation of the genome size of two flowering cherry (*Cerasus × kanzakura*) varieties,  
278 ‘Kawazu-zakura’ and ‘Atami-zakura’, based on *k*-mer analysis ( $k = 17$ ), with the given multiplicity  
279 values.

280

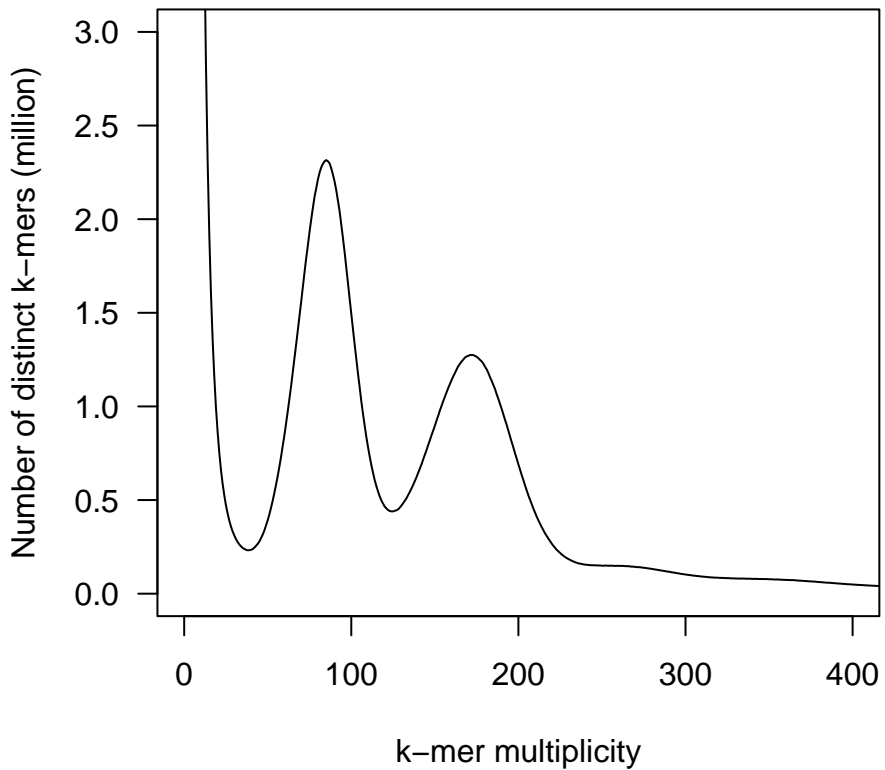
281 **Figure 2** Comparative analysis of the genome sequence and structure of flowering cherry varieties,  
282 ‘Atami-zakura’, ‘Kawazu-zakura’, and ‘Somei-Yoshino’.

283 Chromosome numbers are indicated above the x-axis and on the right side of the y-axis. Genome  
284 sizes (Mb) are below the x-axis and on the left side of the y-axis.

285



**'Kawazu-zakura'**



**'Atami-zakura'**

