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The allopolyploid Arabidopsis kamchatica originated from multiple individuals of Arabidopsis lyrata and Arabidopsis halleri

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Abstract: Polyploidization, or genome duplication, has played a critical role in the diversification of animals, fungi and plants. Little is known about the population structure and multiple origins of polyploid species because of the difficulty in identifying multiple homeologous nuclear genes. The allotetraploid species Arabidopsis kamchatica is closely related to the model species Arabidopsis thaliana and is distributed in a broader climatic niche than its parental species. Here, we performed direct sequencing of homeologous pairs of the low-copy nuclear genes WER and CHS by designing homeolog-specific primers, and obtained also chloroplast and ribosomal internal transcribed spacer sequences. Phylogenetic analysis showed that 50 individuals covering the distribution range including North America are allopolyploids derived from Arabidopsis lyrata and Arabidopsis halleri. Three major clusters within A. kamchatica were detected using Bayesian clustering. One cluster has widespread distribution. The other two are restricted to the southern part of the distribution range including Japan, where the parent A. lyrata is not currently distributed. This suggests that the mountains in Central Honshu and surrounding areas in Japan served as refugia during glacial–interglacial cycles and retained this diversity. We also found that multiple haplotypes of nuclear and chloroplast sequences of A. kamchatica are identical to those of their parental species. This indicates that multiple diploid individuals contributed to the origin of A. kamchatica. The haplotypes of low-copy nuclear genes in Japan suggest independent polyploidization events rather than introgression. Our findings suggest that self-compatibility and gene silencing occurred independently in different origins.

1 **The allopolyploid *Arabidopsis kamchatica* originated from multiple individuals of *A.**
2 ***lyrata* and *A. halleri***
3

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38

38 **Abstract**

39

40 Polyploidization, or genome duplication, has played a critical role in the diversification

41 of animals, fungi, and plants. Little is known about the population structure and multiple

42 origins of polyploid species because of the difficulty identifying multiple homeologous

43 nuclear genes. The allotetraploid species *Arabidopsis kamchatica* is closely related to

44 the model species *A. thaliana* and is distributed in a broader climatic niche than its

45 parental species. Here, we performed direct sequencing of homeologous pairs of the

46 low-copy nuclear genes *WER* and *CHS* by designing homeolog-specific primers, and

47 obtained also chloroplast and ribosomal internal transcribed spacer (ITS) sequences.

48 Phylogenetic analysis showed that 50 individuals covering the distribution range

49 including North America are allopolyploids derived from *A. lyrata* and *A. halleri*. Three

50 major clusters within *A. kamchatica* were detected using Bayesian clustering. One

51 cluster has widespread distribution. The other two are restricted to the southern part of

52 the distribution range including Japan, where the parent *A. lyrata* is not currently

53 distributed. This suggests that the mountains in Central Honshu and surrounding areas

54 in Japan served as refugia during glacial–interglacial cycles and retained the diversity.

55 We also found that multiple haplotypes of nuclear and chloroplast sequences of *A.*

56 *kamchatica* are identical to those of their parental species. This indicates that multiple

57 diploid individuals contributed to the origin of *A. kamchatica*. The haplotypes of low-

58 copy nuclear genes in Japan suggest independent polyploidization events rather than

59 introgression. Our findings suggest that self-compatibility and gene silencing occurred

60 independently in different origins.

61

Introduction

Allopolyploidization, or genome-wide duplication with hybridization, has played an important role in evolution and diversification in plants (Stebbins 1950, 1971; Ohno 1970; Levin 2002; Comai 2005; Marhold & Lihová 2006; Otto 2007). It was suggested that nearly all angiosperms experienced polyploidization in the past and that 57% to 70% of them experienced polyploidy relatively recently (Otto 2007). Despite the prevalence of polyploids, the identification of the parental species of polyploids has been difficult. Common markers used in molecular phylogenetic studies include chloroplast DNA (cpDNA) sequences with uniparental inheritance and nuclear ribosomal internal transcribed spacer (ITS) sequences, which often retain only one parental unit because of concerted evolution. When the cpDNA and ITS are derived from different species, the incongruence can help identify the parents of an allotetraploid. However, when concerted evolution of ITS has resulted in maintenance of the haplotype from the same parent as that of cpDNA, only one of the parents can be identified. In addition, it is technically difficult to separate homeologs derived from multiple parental species because of their high degree of similarity with each other. Cloning of PCR products would result in false sequences because of PCR errors and artificial recombination between alleles and homeologs (Cronn *et al.* 2002; Lihová *et al.* 2006). To solve these problems, it is necessary to design homeolog-specific primers for 'low-copy nuclear genes'. Here, genes of a polyploid that are orthologous to a single-copy nuclear gene in the parental species are referred to as low-copy nuclear genes, and each copy is referred to as a homeolog.

We recently suggested that *Arabidopsis kamchatica* had an allotetraploid origin derived

3

85 from *A. lyrata* and *A. halleri* (Shimizu *et al.* 2005). *Arabidopsis kamchatica* has been

86 exploited as a model species to study the evolution of polyploids by using the extensive

87 genomic and genetic resources of *A. thaliana* and the ongoing whole genome

88 sequencing of its putative parent, *A. lyrata* (http://genome.jgi-

89 psf.org/Araly1/Araly1.home.html) (Shimizu 2002; Shimizu & Purugganan 2005). Self-

90 compatibility (Dart *et al.* 2004; Mable *et al.* 2004; Sugisaka & Kudoh 2008), flowering

91 time (under the nomenclature as *A. lyrata* from Alaska, Kuittinen *et al.* 2008), and the

92 epigenetically regulated *FWA* gene (Fujimoto *et al.* 2008) of *A. kamchatica* have been

93 studied recently. *Arabidopsis kamchatica* is distributed in East Asia and North America.

94 The overlap of *A. kamchatica, A. lyrata*, and *A. halleri* is limited only to Far East Russia

95 (Shimizu *et al.* 2005). While we suggested an allopolyploid origin based on a low-copy

96 nuclear gene in two individuals from Japan (Shimizu *et al.* 2005), an autopolyploid

97 origin of North American individuals was proposed based on cpDNA and ITS sequences

98 (Koch & Matschinger 2007).

99   Soltis *et al.* (2003) emphasized that recurrent origin of polyploid species is the

100 rule rather than the exception. The independent origin cases allow one to examine the

101 repeatability of evolution (Adams & Wendel 2005). Geographically independent origins

102 (or polytopic origins) have been documented in a few polyploid species that appeared

103 very recently. In *Tragopogon*, multiple origins during the 20th century were suggested

104 by a concordance between geographic variation patterns of the diploids and polyploids

105 (Tate *et al.* 2006). Levin (2002) noted that such a concordance is the best evidence for

106 multiple independent origins. However, unless the origin was very recent, geographic

107 variation may not be useful to identify independent origins of most polyploid species,

108 including *A. kamchatica*, because previous studies have suggested that the current

4

109   ranges of hybrids and parental species are poor predictors of the site of hybridization

110   and that polyploid species tend to expand their distribution range by shifting to new

111   ecological niches (Anderson & Stebbins 1954; Watanabe & Yahara 1984; Levin 2002;

112   Beck *et al.* 2008). Apart from geographic data, independent origins have been supported

113   strongly by multiple haplotypes (or alleles) shared by polyploid and parental species.

114   The sharing of a polyploid with more than one chloroplast haplotype with a parental

115   species, or more than two haplotypes of a nuclear homeolog indicates that multiple

116   haplotypes of parental species contributed to the polyploid species, and suggests the

117   independent origins of the polyploid species. A number of studies have shown the

118   sharing of multiple polymorphic markers such as isozyme and cpDNA among polyploid

119   and parental species, and have suggested that independent origins are widespread

120   (reviewed by Soltis & Soltis 1993, 1999). However, it has also been suggested that

121   haplotype sharing with parental species can result from both independent

122   polyploidization events and introgression from diploid parental species (Ramsey &

123   Schemske 1998; Husband 2004).

124   In this study, we address the population structure and polyploid origin of *A.*

125   *kamchatica* by examining multiple populations distributed across its distribution range.

126   In addition to cpDNA and ITS regions, we sequenced homeologous pairs of low-copy

127   nuclear genes. We focused on the contribution of multiple parental individuals rather

128   than the geographically independent origins. We discuss scenarios of independent

129   polyploidization events vs. introgression based on low-copy nuclear genes.

130

131

Materials and Methods

Sampling

For *A. kamchatica*, two subspecies are recognized based on morphology, life history, and habitats. The first subspecies, *A. kamchatica* subsp. *kamchatica*, is a perennial, described originally from Kamchatka, Russia. It is reported from East Asia (Far East Russia, China, Korea, Japan, and Taiwan) and North America (Alaska, Canada, and Pacific Northwest of the United States). The second subspecies, *A. kamchatica* subsp. *kawasakiana*, is an annual found in sandy open habitats along seashores or lakeshores in lowlands in western Japan. Tetraploid chromosome number counts ($2n = 32$ and $n = 16_{II}$) were reported from samples in Japan, Far East Russia, Alaska, and Canada, and represent both subspecies (see references in Mulligan 1995; Shimizu *et al.* 2005; Warwick & Al-Shehbaz 2006). *Arabidopsis kamchatica* is morphologically similar to *A. lyrata*, and the taxon has been treated either as an infraspecific taxon of *A. lyrata* (O'Kane & Al-Shehbaz 1997) or as a distinct species (see references in Mulligan 1995; Shimizu *et al.* 2005).

Altogether 45 populations of the tetraploid *A. kamchatica* (both subspecies) were sampled, including one or two individuals per locality, giving a total of 50 individuals (Table 1). The sample locations ranged from the southwestern (Taiwan) to the northeastern (Alaska and Washington state in the USA, Canada) areas of the species range. The emphasis was on Kamchatka, from which *A. kamchatica* was described originally, and on Japan, where *A. kamchatica* subsp. *kawasakiana* has been recognized (see Fig. 1, Table 1) (Mulligan 1995; Shimizu *et al.* 2005). To facilitate the reference to the areas sampled, they are denoted by letters A–I (Fig. 1, Table 1).

6

156   For the potential parental species, diploids *A. lyrata* and *A. halleri,* we collected at

157   least one sample from each subspecies described by O'Kane and Al-Shehbaz (1997) and

158   Kolník and Marhold (2006). Within *A. halleri*, subspecies *gemmifera* is distributed in

159   Eastern Asia, and the other four subspecies occur in Europe. Within *A. lyrata*,

160   subspecies *petraea* is reported from Eurasia and subspecies *lyrata* from North America.

161   Thus, the current distribution of *A. kamchatica* overlaps only partly with those of the

162   diploids. Because *A. lyrata* is not found in Taiwan and Japan and *A. halleri* does not

163   occur in North America, Far East Russia is the only area where the three species co-

164   occur. Here we sampled the diploids mainly from Eastern Asia (Far East Russia, Japan)

165   and from more remote areas (Europe and the USA). The samples represent 15

166   individuals of *A. halleri* from 13 populations and seven individuals of *A. lyrata* from

167   four populations (Table 1). Although our sampling did not represent species-wide

168   coverage (which was not the aim of the present study), we exploit here much of the

169   sequence data on these diploids published previously (Ramos-Onsins *et al.* 2004; Koch

170   & Matschinger 2007; Schmickl *et al.* 2008).

171

172   *Chromosome number counts*

173   Chromosome number was counted to check ploidy. Root tips were treated with cold

174   water at 0°C for 24 hours, fixed in 3:1 (vol:vol) ethanol–acetic acid at 5°C for 1 hour,

175   and stained in 1% acetic–orcein (see Shimizu *et al*. 2005). An individual from the

176   population of kamC11, from which *CHS*-hal was not amplified, and two individuals

177   from Kamchatka (the population of kamG39), where *A. kamchatica* was described

178   originally, were assayed. Ihara (1976) reported triploid plants (as *Arabis* sp.) from the

179   same site as kamC11 (Mt. Shikokutsurugi), but our chromosomal count showed a

180   tetraploid count.

181

182   *Primer designs and strategies for separating homeologs and alleles*

183   We sequenced two low-copy nuclear genes *WER (WEREWOLF)* and *CHS*

184   *(CHALCONE SYNTHASE)*, two cpDNA regions (the *trnL* intron and the *trnL–trnF*

185   intergenic spacer region, see Koch *et al.* 2005; Ansell *et al.* 2007), and the ITS region of

186   nuclear ribosomal DNA (ITS1-5.8S-ITS2) (Alvarez & Wendel 2003). The *WER* gene

187   encodes a protein with an myb DNA-binding domain and is involved in the root hair

188   development in *A. thaliana* (Lee & Schiefelbein 1999). A WU-BLAST search

189   (www.arabidopsis.org) showed that the *WER* coding sequence has only 62%–80%

190   identity with its closest homologs, *GL1* and *MYBRTF*, and is considered a single-copy

191   gene. The *CHS* gene forms multigene families in some taxa but has been reported to be

192   a single-copy gene in *A. thaliana* and other related diploid Brassicaceae taxa (Shimizu

193   *et al.* 2005; Lihová *et al.* 2006).

194   To infer a phylogeny based on nuclear sequences, natural and artificial

195   recombination between homeologs and between alleles should be excluded. To obtain

196   sequences of two homeologs separately for both *WER* and *CHS* genes from the

197   tetraploid *A. kamchatica*, we designed homeolog-specific primers using the methods

198   described by Lihová *et al.* (2006) (Supporting Fig. S1a, Table S1, and Text S1). To

199   obtain haplotypes (or allele sequences) within each homeolog, we applied the following

200   strategies while avoiding cloning.

201   1. Plants were self-fertilized in a growth chamber to obtain homozygous individuals.

202   This was feasible for *A. kamchatica* because selfing was possible in all 18 individuals

203   we tried (Table 1). In several individuals of the self-incompatible diploids *A. halleri* and

8

204   *A. lyrata* (Castric & Vekemans 2004), pollination at the flower bud stage often avoided

205   the self-incompatibility reaction and allowed self-fertilization.

206   2. When only one heterozygous site was found by direct sequencing, the haplotypes

207   were resolved (e.g., *CHS* sequences named haltat1a1 and haltat1a2 from the individual

208   haltat1, see Table 1).

209   3. When only one indel was found in direct sequencing, sequencing from both directions

210   resolved the haplotypes (e.g., *WER* sequences kamA3Ha1 and kamA3Ha2; Table 1).

211   4. We sequenced multiple individuals of *A. halleri* subsp. *halleri* and subsp. *dacica* to

212   find homozygotes.

213

214   *Sequence alignments, copy numbers, phylogenetic and population genetic analysis,*

215   *intrapopulation polymorphism, and Bayesian clustering*

216   The details are given in Supporting Text S1 and Tables S1–S3. All sequences obtained

217   in this study were deposited under GenBank accession numbers GQ303456–GQ303550.

218   The sequence assemblies and alignments were performed in BioLign version 4.0.6.2

219   (http://www2.maizegenetics.net/index.php?page=bioinformatics/index.html) and edited

220   manually using the program BioEdit version 7.0.4.1 (Hall 1999). After the alignments

221   were assembled, identical sequences were detected using MacClade 4.0 PPC (Maddison

222   & Maddison 2000) and merged, which reduced the alignments to comprise only the sets

223   of unique sequences.

224   The final alignments of the nuclear regions (*WER*, *CHS*, *CHS*-lyr and ITS) were

225   subjected to maximum-parsimony (MP) analysis (Swofford 2001) and to Bayesian

226   inference based on a Markov chain Monte Carlo algorithm (MCMC; Huelsenbeck &

227   Ronquist 2001). Bootstrap analyses (Felsenstein 1985) were performed using 100,000

9

228   resamplings with the fast-heuristic search as implemented in PAUP* (Swofford 2001).

229   Gaps were used as additional characters in the MP analyses (see also Text S1). Except

230   for short 1- or 2-bp gaps that appeared to be caused by slipped-strand mispairing, each

231   gap was scored, and the scoring was appended to the alignment. In the case of simple,

232   nonoverlapping gaps, these were coded as binary characters using the "simple gap-

233   coding" approach as suggested by Simmons and Ochoterena (2000). More complex

234   gaps (i.e., of different lengths and overlapping) were coded as multistate (up to four

235   states) characters.

236   The haplotype network of the *trnL* intron region was constructed by a minimum-

237   spanning network using the NETWORK program v. 4.5.1.0 (Bandelt *et al*. 1999; freely

238   available at www.fluxus-engineering.com). To survey the extent of polymorphism

239   within populations, sequences of the *trnL–trnF* region were obtained from additional

240   individuals of three populations (see Text S1 and Table S3). MEGA4 (Tamura *et al*.

241   2007) was used for neighbor-joining analysis. The minimum numbers of recombination

242   events were detected using the four-gamete test (Hudson & Kaplan 1985), and the levels

243   of silent-site nucleotide diversity of each homeolog were estimated as π (Tajima 1983)

244   (Table S2) as implemented in DnaSP version 4.10.7 (Rozas *et al*. 2003). To survey the

245   associations between different loci, the gametic disequilibrium D' (Hedrick 1987) was

246   calculated.

247   To infer the population structure of *A. kamchatica*, the Bayesian clustering

248   algorithm   implemented   in   the   program   *structure*   version   2.2

249   (http://pritch.bsd.uchicago.edu/structure.html) (Pritchard *et al*. 2000) was used. The data

250   were treated as haploid data as recommended for complete-selfing species (Gao *et al*.

251   2007) and as commonly done for predominantly-selfing species (e.g., Nordborg *et al*.

252   2005; Beck *et al*. 2008). The programs *CLUMPP* (Jakobsson & Rosenberg 2007),

253   *distruct* (http://rosenberglab.bioinformatics.med.umich.edu/distruct.html) (Rosenberg

254   2004), and ΔK statistic (Evanno *et al*. 2005) were used to summarize and interpret the

255   outputs.

256

257

258   **Results**

259

260   *Chromosome counts*

261   We counted chromosome numbers of two individuals from the population of kamG39 in

262   Kamchatka and one individual from the population of kamC11 in Japan (see Table 1 for

263   population origins). All were tetraploids with $2n = 32$ (Fig. 2).

264

265   *Homeologous pairs of* WER *and* CHS

266   Amplification of the nuclear genes *WER* and *CHS* in the tetraploid *A. kamchatica* using

267   homeolog-specific primers resulted in two homeologs in each gene, named here as

268   *WER*-hal*, WER*-lyr*, CHS*-hal, and *CHS*-lyr (Table 1). Among the 50 individuals

269   analyzed, *CHS*-hal was not amplified from three individuals from Central Honshu and

270   Shikoku (kamC11, kamC12, and kamD23), which suggests a large deletion or a

271   rearrangement (see Text S1 for details). The tetraploid count of one of them (kamC11)

272   indicates that it was not caused by the change in ploidy. These results were also

273   supported by the survey of copy numbers using PCR and restriction patterns (Fig. S1)

274   (following Lihová *et al.* 2006).

11

275　　　　No natural recombination was detected in either *WER* or *CHS* homeologs of *A.*

276　*kamchatica* using the four-gamete test (Hudson & Kaplan 1985). However, natural

277　recombination was detected in three of eight *CHS* sequences obtained from European

278　diploids (lyrpet2, haltat1a2, and halovi1) (Fig. S2a). We conducted a phylogenetic

279　analysis without these three sequences.

280　　　　Homeologous pairs of *A. kamchatica* were aligned with those of the diploid

281　species *A. lyrata* and *A. halleri,* and with *A. pedemontana* and *A. thaliana* as outgroups.

282　The phylogenetic trees of all *WER* and *CHS* sequences are shown in Figs 3 and 4. The

283　phylogenetic tree of the *lyrata*-originated *CHS* homeolog alone was also inferred

284　because we obtained longer sequences than for the *halleri*-originated homeolog, and

285　several unique haplotypes were identified (Fig. S3a). Maximum parsimony analysis and

286　Bayesian inference resulted in very similar tree topologies with slight differences only

287　in weakly supported clades (see also Fig. S3, Text S1 and Table S2 for more details).

288　　　　Three major clades with high bootstrap supports are resolved in the phylogenetic

289　tree of *WER* (Fig. 3): one clade comprised all individuals of *A. halleri* and the

290　corresponding homeolog from the tetraploids (*WER*-hal), another clade comprised three

291　individuals of *A. lyrata* and the other homeolog from the tetraploids (*WER*-lyr), and the

292　third clade included three individuals of *A. lyrata*. In the analysis of *CHS*, three major

293　clades were resolved similarly (Fig. 4). These results strongly support the allopolyploid

294　origin of *A. kamchatica* (including both subspecies) from the diploids *A. lyrata* and *A.*

295　*halleri.*

296　　　　Nucleotide diversity (π) of silent sites of the tetraploid *A. kamchatica* is in the

297　range of 0.0006–0.0026 among the four nuclear loci (average 0.0013) (Table S2). The

298　number of haplotypes resolved in the tetraploid is in the range of 8–11 among the four

299   loci when indel polymorphisms are included.

300   The geographic distribution of the nuclear haplotypes of *A. kamchatica* was not

301   random. We found a widespread and common haplotype within each locus (shown with

302   one or two black stars in Figs 3, 4; for *CHS*-lyr, see also Fig. S3a based on longer

303   sequences), which was observed mainly from the broad area F–I (Figs 1 and S4), along

304   with several geographically more restricted haplotypes. In particular, two regions

305   harbored one or more haplotypes that were geographically restricted in all four loci:

306   lowlands in Western Honshu (B), represented by *A. kamchatica* subsp. *kawasakiana*,

307   and mountains in Central Honshu together with mountains in Western Honshu and

308   Shikoku (C and D) (Figs 1 and S4). The division of these areas suggested by the

309   association between homeologs is also supported by the Bayesian clustering analysis

310   (see below).

311   Although our sampling of the parental diploid species was limited, we identified

312   one or two haplotype sequences that were identical to those of *A. kamchatica* in each of

313   the four loci (Figs 3 and 4, Text S1). First, in *WER*-hal and *CHS*-hal, two haplotypes

314   observed in *A. kamchatica* are identical to those of *A. halleri*. Second, in *WER*-lyr and

315   *CHS*-lyr, a haplotype found in North America is identical to a haplotype of *A. lyrata*

316   individuals from Far East Russia (lyrpet4 and 5). In addition, in *CHS*-lyr, an

317   intermediate frequency haplotype (kamCL,DL in Fig. S2a and S3a) is identical to a

318   haplotype of *A. lyrata* individuals from western Russia (lyrpet2) over more than 1 kb to

319   the left of the recombination breakpoint (Fig. S2a).

320   To increase the sequences of diploid taxa, 22 *CHS* sequences from *A. lyrata* and *A.*

321   *halleri* reported by Ramos-Onsins *et al.* (2004) were combined with our data. Critically,

322   Ramos-Onsins *et al.* (2004) used cloning, and the possibility of artificial recombination

13

323 cannot be excluded. Thus, we estimated the gene genealogy, in which the identity of the

324 haplotypes could be revealed but the branch pattern might not reflect the historical

325 phylogenetic relationship of the entire region (Fig. S5). We observed the same pattern of

326 haplotype sharing between polyploid and diploid parents with or without the sequences

327 reported by Ramos-Onsins *et al.* (2004); two haplotypes of *CHS*-hal were shared with *A.*

328 *halleri*, and a haplotype of *CHS*-lyr was shared with *A. lyrata* (Figs 4 and S5). The

329 haplotype of lyrpet2 mentioned above was identical to that from the same population

330 sequenced by Ramos-Onsins *et al.* (2004).

331   These results confirm further that *A. kamchatica* is allopolyploid derived from *A.*

332 *halleri* and *A. lyrata*, and that at least two distinct haplotypes of both *A. halleri* and *A.*

333 *lyrata* were incorporated into *A. kamchatica*.

334

335 *Chloroplast and ITS sequences*

336 Once allopolyploidy is confirmed by low-copy nuclear genes, cpDNA is highly suitable

337 to infer the independent origin of a polyploid because a unique uniparental haplotype is

338 transmitted in each hybridization event without recombination. Sequencing two cpDNA

339 regions (*trnL* intron and the *trnL-trnF* intergenic spacer region) resulted in 18 cpDNA

340 haplotypes in *A. kamchatica*, *A. lyrata*, and *A. halleri*. In 50 individuals of *A.*

341 *kamchatica*, we identified seven cpDNA haplotypes (cpHap1−7) (Table 1, Fig. S2c).

342 Four of them (cpHap1, 2, 3, 5) were also found in diploid *A. halleri* subsp. *gemmifera*

343 but not in any other diploid taxa. These results suggest that at least four individuals of *A.*

344 *halleri* contributed to the origin of the allopolyploid species *A. kamchatica*.

345   We further analyzed our data in the context of previously published data of the

346 two cpDNA regions (582 individuals from Koch & Matschinger 2007, Schmickl *et al.*

347   2008). Whereas the *trnL* intron region was alignable, the alignment of the *trnL–trnF*

348   intergenic spacer region was uncertain because of frequent and possibly parallel

349   mutations in tandemly duplicated copies (Fig. S2c). Thus, as the first step, the *trnL*

350   intron region was used to construct a haplotype network. In our 50 samples of *A.*

351   *kamchatica*, three haplotypes of the *trnL* intron (one in cpHap2, 3, 4, and 5, one in

352   cpHap6, one in cpHap1 and 7) were identified (Fig. 5a). The resolution of the *trnL*

353   intron region alone was not enough to infer the parental species because the same

354   haplotypes have been observed in the published data of *A. arenosa*, *A. halleri*, and *A.*

355   *lyrata*. Therefore, the identities of the *trnL–trnF* intergenic spacer region were

356   considered to distinguish the cpDNA haplotypes from different species (Figs 5b and 6).

357   Among seven haplotypes of *A. kamchatica* (cpHap1−7), we found that cpHap1, 2, 3,

358   and 5 were shared with *A. halleri* subsp. *gemmifera* and that they were not found in any

359   other diploid taxa. In turn, cpHap4, 6, and 7 were found exclusively in *A. kamchatica*

360   analyzed here. These results agree with our conclusions reported above, suggesting that

361   at least four cpDNA haplotypes of *A. halleri* subsp. *gemmifera* were incorporated into *A.*

362   *kamchatica*.

363   In the tree based on the ITS region (ITS1−5.8*S*−ITS2), *A. kamchatica*, except for

364   one individual (kamD17), formed a clade with diploid *A. lyrata*. In contrast, the ITS of

365   the single individual from Japan (kamD17) was distinct from all other allotetraploids

366   and formed a clade with *A. halleri* (Figs 7, S2b, and S3d).

367

368   *Population structure of* Arabidopsis kamchatica

369   We used a model-based Bayesian clustering method (Pritchard *et al.* 2000) to infer the

370   population structure of *A. kamchatica* integrating the information from the four nuclear

15

371 loci (*WER*-hal, *WER*-lyr, *CHS*-hal, *CHS*-lyr) and the cpDNA (Fig. 8 and Text S1). The

372 high values of the mean posterior probability of data Ln $P(X|K)$, ΔK and the symmetric

373 similarity coefficient (*SSC*) supported the clustering of $K = 3$ (Fig. 8b–d). The three

374 clusters correspond to those described above in the section of phylogeny. Cluster 1

375 (light green in Fig. 8a) covers a wide range of distribution including northern Japan,

376 Kamchatka, Alaska, Canada, and the Pacific Northwest of the USA (areas F–I). Cluster

377 2 (orange) includes *A. kamchatica* subsp. *kawasakiana* from lowlands in Western

378 Honshu (B) and three individuals from Taiwan (A). Cluster 3 (blue) comprises the

379 individuals from mountains in Central Honshu, Western Honshu, and Shikoku (C and

380 D) (Figs 1 and 8a).

381 Genetic admixtures between different clusters were suggested mostly in

382 geographic border regions. For example, one individual from the lowlands of Northern

383 Honshu (kamE26 in area E) had haplotypes characteristic of subsp. *kawasakiana* in

384 *WER*-hal and *CHS*-hal, whereas haplotypes typical for subsp. *kamchatica* were seen in

385 *CHS*-lyr and cpDNA, suggesting that it is a hybrid between these two subspecies. In

386 addition, individuals in area F had common haplotypes of low-copy nuclear genes and

387 belonged to cluster 1, but its cpDNA (cpHap3) was mainly found in cluster 3. Such

388 'plastid capture' is observed often in plant species (Okuyama *et al.* 2005).

389

390

391 **Discussion**

392

393 *Allopolyploid origin of* Arabidopsis kamchatica *from* A. lyrata *and* A. halleri

394 In contrast to the previous studies on *Arabidopsis* species, exploring mainly cpDNA and

16

395   ITS nuclear ribosomal data (Koch & Matschinger 2007), we focused the present study

396   on biparentally inherited low-copy nuclear genes. By designing homeolog-specific

397   primers, we targeted two genes (*WER* and *CHS*) in the tetraploid *A. kamchatica*, for

398   which both allo- and autopolyploidy were argued previously (Shimizu *et al.* 2005, Koch

399   & Matschinger 2007). Confounding factors in the analyses of low-copy nuclear genes

400   are artificial and natural recombination. In the present study, the former problem was

401   excluded by avoiding cloning procedure, and the latter was not detected by the four-

402   gamete test in our Asian and American materials. The subsequent phylogenetic analysis

403   of both *WER* and *CHS* genes revealed that one of the homeologs retrieved from *A.

404   kamchatica* clustered with *A. lyrata*, whereas the other clustered with *A. halleri*. We

405   obtained congruent results for the recently studied *FWA* genes for two individuals of *A.

406   kamchatica* (representing both subspecies), which showed homeologs corresponding to

407   *A. lyrata* and *A. halleri*, respectively (Fujimoto *et al.* 2008). These results provide strong

408   evidence that *A. kamchatica* (both subspecies recognized by Shimizu *et al.* 2005) is an

409   allopolyploid derived from the diploids *A. lyrata* and *A. halleri*.

410   Bayesian cluster analysis that integrated the nuclear and cpDNA haplotype data

411   identified three geographically defined clusters. Cluster 1 covered a broad range from

412   Northern Japan and Kamchatka to North America. Cluster 2 comprised mainly *A.

413   kamchatica* subsp. *kawasakiana* in lowlands of Western Honshu, Japan. Cluster 3

414   included the individuals from mountains in Central Honshu, in which a number of rare

415   haplotypes were found. In the next sections, we discuss the origin of polyploidy as a

416   possible explanation of this population structure.

417   Hybrid and allopolyploid origins have quite often been inferred from the

418   incongruence between cpDNA and ITS data. However, this approach can fail to detect

419   the hybrid origin in cases when both cpDNA and ITS represent only one of the parents

420   or when the sampling and/or the resolution is not adequate (Kim *et al.* 2008). Thus, it

421   may not be suitable for species-wide analysis of polyploid species. The cpDNA and ITS

422   of *A. kamchatica* (Koch & Matschinger 2007) did not suggest the hybrid origin of

423   American individuals, although these support the hybrid origin of Japanese individuals

424   that was shown previously using the low-copy nuclear gene *CHS* (Shimizu *et al.* 2005).

425   We suggest that, provided that the effects of natural and artificial recombination are

426   avoided, low-copy nuclear genes provide critical information for the study of hybrid and

427   allopolyploid origins, as well as the geographic organization of their genetic variation.

428

429   *The origin of the allopolyploid* Arabidopsis kamchatica *from multiple individuals of its*

430   *diploid parents*

431   Critical data to support the independent origins of polyploid species include the sharing

432   of multiple haplotypes between diploid and polyploid species (Soltis *et al.* 2003). Here

433   we found ample evidence in both cpDNA and low-copy nuclear DNA that multiple

434   haplotypes of the parental species (*A. lyrata* and *A. halleri*) contributed to the polyploid

435   *A. kamchatica*. Because ITS sequences displayed a low level of variation, we discuss

436   these only briefly.

437   Here we identified seven cpDNA haplotypes in the allopolyploid *A. kamchatica*,

438   and four of them were shared with the Asian diploid taxon, *A. halleri* subsp. *gemmifera*.

439   Even when we increased the sample size by incorporating the large-scale surveys of

440   cpDNA in the genus *Arabidopsis* by Koch and Matschinger (2007) and Schmickl *et al.*

441   (2008), the same pattern of haplotype sharing was found. These results suggest strongly

442   that at least four individuals of *A. halleri* subsp. *gemmifera* contributed to the origin of *A.*

443   *kamchatica* because a unique uniparental haplotype of cpDNA is usually transmitted at

444   each generation without recombination. This also indicates that *A. halleri* subsp.

445   *gemmifera* was always the maternal parent, although the possibility of shared

446   polymorphism caused by rare introgression or by incomplete lineage sorting between *A.*

447   *lyrata* and *A. halleri* cannot be excluded (Ramos-Onsins *et al.* 2004).

448   In *WER*-hal and *CHS*-hal homeologs, two haplotypes observed in *A. kamchatica*

449   were identical to those of *A. halleri*. In addition, two haplotypes of *CHS*-lyr were found

450   in *A. lyrata* over more than 1 kb. We increased the sample size by incorporating 22 *CHS*

451   sequences of *A. lyrata* and *A. halleri* reported by Ramos-Onsins *et al.* (2004). Again, the

452   same pattern of haplotype sharing was found. These results are consistent with

453   independent origins, although it is also possible that two haplotypes may have entered

454   the tetraploid *A. kamchatica* through an unreduced diploid gamete of a parent in a single

455   polyploidization event.

456   It is expected that many haplotypes of polyploid species cannot be identified from

457   diploid parents, possibly because the sampling may not be dense enough or because the

458   haplotypes may be derived in polyploids or lost in diploids. The loss in diploid would be

459   pronounced and complicated in low-copy nuclear genes because they are subjected to

460   recombination. Recombination was detected in our relatively long sequence length (~1

461   kb) in diploid parental species, which are self-incompatible (Castric & Vekemans 2004).

462   For example, over 1 kb of *CHS* was shared between diploid (lyrpet2) and polyploid

463   individuals (*CHS*-lyr of kamCL,DL in Fig. S2a), but a recombination breakpoint was

464   identified. It is possible that the haplotype sharing of low-copy nuclear genes is limited

465   only if gene flow occurred very recently or if a haplotype was common and maintained

466   for a long time. We suggest that cpDNA is useful for studying independent origins

19

467 because of the uniparental inheritance and the absence of recombination between

468 haplotypes, once allopolyploidy is confirmed by low-copy nuclear genes. Our data

469 suggest strongly that multiple individuals of parental species contributed to the origin of

470 the allopolyploid *A. kamchatica.*

471

472 *Independent polyploidization events vs. introgression*

473 Although a common interpretation of the sharing of multiple haplotypes has been

474 independent polyploidization events, it has been also noted that introgression from

475 diploid into polyploid can also result in haplotype sharing (Ramsey & Schemske 1998;

476 Husband 2004). Introgression is possible through a triploid bridge or through the

477 hybridization of polyploids with unreduced gametes or with autopolyploids of a parental

478 diploid, although the fertility of such hybrid individuals tends to be low. Distinguishing

479 these two scenarios is challenging because their effects would be similar. However, we

480 propose that the two scenarios would have different effects on low-copy nuclear genes

481 when combined with cpDNA (Fig. 9), although they cannot be distinguished with

482 certainty. In the introgression scenario, only the introgression parent would contribute

483 additional sequence diversity to the homeologous loci, whereas the loci from the other

484 parent would not receive any new haplotypes. Thus, homeologs derived from only one

485 parent should have distinct haplotypes compared with other polyploid individuals,

486 whereas the homeologs from other parents would be maintained. In contrast, in the

487 scenario of independent polyploidization events, homeologs derived from both parents

488 could be distinct from those of other polyploid individuals.

489 In our data, a few individuals in North America among the cluster 1 may represent

490 the scenario of introgression. Most of the individuals in cluster 1 (areas F–I) displayed a

20

491 single haplotype (most common and widespread among the tetraploids) in all four

492 nuclear loci as well as cpDNA (Fig. 9, represented by kamG34 individual). Its cpDNA

493 haplotype (cpHap1) was also found in diploid *A. halleri* subsp. *gemmifera*. Nevertheless,

494 a few individuals (including kamH46 and kamI48 in Fig. 9) showed different *lyrata*-

495 homeolog sequences (in both *WER* and *CHS* datasets; Figs 3, 4, and 9). These

496 homeologs are shared with two individuals of *A. lyrata* from Far East Russia (lyrpet4,

497 lyrpet5, see Table 1), suggesting a recent gene flow. Similarly, the ITS sequence of a

498 few individuals was consistent with the overlap of a common haplotype of *A.*

499 *kamchatica* and a haplotype of *A. lyrata* (lyrlyr1,2, lyrpet4,5; Fig. S2b)*,* suggesting

500 again a recent gene flow. These data might suggest introgression from *A. lyrata* rather

501 than independent polyploidization events. We note that it is difficult to exclude the

502 possibility that it represents another independent polyploidization event and that the

503 *halleri*-parent was nearly identical.

504   On the other hand, populations of *A. kamchatica* subsp. *kawasakiana* (area B in

505 cluster 2) are suggested to represent an independent polyploidization event. In both

506 *lyrata*- and *halleri*-originated homeologs (*WER*-lyr, *WER*-hal, *CHS*-lyr and *CHS*-hal),

507 subsp. *kawasakiana* exhibited rare (mostly unique to this group) haplotypes that are

508 distinct from other individuals (Fig. 9, represented by kamkwsB8). This is not consistent

509 with introgression from a parent. Its cpDNA haplotype (cpHap2) was shared between

510 the polyploid subsp. *kawasakiana* and the diploid *A. halleri* subsp. *gemmifera* (Fig. 5).

511 These results suggest strongly that *A. kamchatica* subsp. *kawasakiana* originated by an

512 independent polyploidization event compared with other polyploid individuals.

513 Although we cannot exclude formally the possibility of introgression from both parents

514 or of recurrent derived mutations, they would be much less parsimonious.

515   The most complex pattern appeared in cluster 3, which had a number of unique

516   haplotypes along with the widespread haplotype. Two cases in area D (mountains in

517   Central Honshu) fulfill the same criterion of independent origins as subspecies

518   *kawasakiana*. First, in many individuals with cpHap3 (represented by kamD16 in Fig.

519   9), both *lyrata-* and *halleri-*originated homeologs were distinct, and their cpDNA

520   haplotype was also found in *A. halleri* subsp. *gemmifera*. Second, a single individual

521   (kamD18 in Fig. 9) had unique haplotypes in both *lyrata-* and *halleri-*originated

522   homeologs (*CHS*-lyr and *CHS*-hal), and its cpDNA haplotype (cpHap5) was shared with

523   *A. halleri* subsp. *gemmifera*. In addition, kamD18 had a distinct ITS haplotype, which is

524   consistent with its unique history. These data suggest that those individuals had

525   independent origins from the other tetraploids analyzed, although more data from the

526   same population are needed to provide more details.

527   In summary, our data suggest that *A. kamchatica* comprises individuals with

528   independent origins. The independent origins of subspecies *kawasakiana* from other

529   individuals were strongly suggested, and two more independent origins of individuals in

530   the mountains in Central Honshu are also suggested. In the Bayesian clustering (Fig. 8),

531   three of the four suggested independent origins appeared as distinct clusters, except for

532   one represented by a single individual kamD18. These results suggest strongly that the

533   independent origins had a profound effect on the population structure of *A. kamchatica*.

534   In addition, introgression from *A. lyrata* into *A. kamchatica* would explain the distinct

535   haplotypes found in some North American populations.

536

537   *Genetic diversity in* Arabidopsis kamchatica

538   The nucleotide diversity was lower in the self-compatible species *A. kamchatica*

539 (average 0.0013) than in the outcrossing parental species (0.0150 in *A. halleri*, 0.0230 in

540 *A. lyrata* subsp. *petraea*, and 0.0031 in *A. lyrata* subsp. *lyrata*) (Ramos-Onsins *et al.*

541 2004). It was also lower than in the self-compatible *A. thaliana* (~0.0035–0.0055 at

542 synonymous sites and ~0.007 at intronic regions) (Nordborg *et al.* 2005). This low

543 nucleotide diversity of *A. kamchatica* might reflect its self-compatibility, and it might

544 also reflect a bottleneck in which only a few haplotypes of the parental species were

545 incorporated into *A. kamchatica* and a relatively recent origin of this species.

546 Consistently, many haplotypes of the *WER* and *CHS* genes and cpDNA of *A.*

547 *kamchatica* are identical to those of the parental species, supporting the idea that the

548 origin of *A. kamchatica* is relatively recent.

549 Interestingly, diverse haplotypes arising from independent origins of *A.*

550 *kamchatica* were found in the mountains in Central Honshu and surrounding areas in

551 Japan, where *A. lyrata* is not found currently. Thus, this diversity cannot be explained

552 solely by contemporary or very recent polyploidization and introgression. Because the

553 mountains of Central Honshu, Japan, are known to have acted as refugia for many plant

554 species (Fujii & Senni 2006), we suggest that *A. kamchatica* or its parental species

555 might have survived there during Pleistocene glacial periods. The independent origins

556 of *A. kamchatica* contrast with the single origin of *A. suecica* (Jakobsson *et al.* 2006),

557 which is distributed in Northern Europe and might not have originated in refugial areas.

558

559 Arabidopsis kamchatica *as a model to study the molecular basis of polyploid evolution*

560 Although polyploid species with very recent independent origins such as *Tragopogon*

561 and artificial polyploids provide insights into the immediate responses of

562   polyploidization (Comai 2005; Tate *et al.* 2006; Otto 2007), *A. kamchatica* offers a

563   different case, in which evolutionary changes occurred over a longer timescale. Our

564   previous report on the epigenetically regulated *FWA* gene showed that the homeolog

565   derived from *A. halleri* is silenced in both subspecies. In conjunction with the finding of

566   independent origins, this case represents an example of repeatable gene silencing after

567   polyploidization (Adams & Wendel 2005). We have also shown that *A. kamchatica* is

568   self-compatible (Table 1; Sugisaka & Kudoh 2008), whereas *A. halleri* and most *A.*

569   *lyrata* have been reported to be predominantly self-incompatible (Castric & Vekemans

570   2004; Mable *et al.* 2004). This suggests either that self-incompatibility was lost

571   independently, as reported in a few species (Okamoto *et al.* 2007; Shimizu *et al.* 2008),

572   or that self-compatible haplotypes spread beyond different populations. A biogeographic

573   study of the genus *Arabidopsis* (Hoffmann 2005) reported that *A. kamchatica* (as *A.*

574   *lyrata* subsp. *kamchatica*, most of which should correspond to *A. kamchatica*) grows in

575   a broader range of climate in terms of temperature and precipitation than other

576   subspecies of *A. lyrata* and *A. halleri*. *Arabidopsis kamchatica* will be a unique model to

577   understand the molecular basis of parallel evolution and habitat exploitation in

578   polyploid species.

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598

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758

759   Research interest of the authors

760   R.S.-I. is interested in the molecular and physiological basis of plant polyploidization.

761   J.L. and K.M. are interested in the taxonomy, phylogeny and phylogeography of

762   Brassicaceae. H.I. is interested in the nucleotide variation of a variety of organisms

763   to infer their histories. H.K. is working on the ecological genetics of adaptation in plants.

764   O.S. studies the genetics of adaptation in plants, in *Arabidopsis* species and Scots pine.

765   K.W. is interested in polyploid evolution. V.V.Y. studies the flora of the Far East. K.K.S.

766   is interested in evolutionary and ecological functional genomics of *Arabidopsis* relatives.

767

768

Figure legends

769

770 Fig. 1. Map showing the sample sites and geographic distribution of haplotypes of the

771 *CHS*-hal homeolog. Circles indicate *Arabidopsis kamchatica* subsp. *kamchatica* and

772 asterisks indicate subsp. *kawasakiana*. Eight haplotypes are depicted in different colors,

773 as shown also in Fig. 4. Heterozygotes are shown as half circles. Populations with

774 missing *CHS*-hal data are indicated by white circles. The Honshu and Shikoku islands

775 of the Japanese archipelago are magnified.

776

777 Fig. 2. Chromosome number count in *Arabidopsis kamchatica*. Mitotic metaphase

778 chromosomes ($2n = 32$) from the population named kamG32 in Kamchatka, Russia (A),

779 and from the population kamC11 in mountains in Western Honshu and Shikoku, Japan

780 (B). For population origin, see Table 1.

781

782 Fig. 3. Strict consensus tree of the 78 most-parsimonious trees based on nuclear *WER*

783 sequence data (955 aligned positions plus additional coding of 14 indels). Bootstrap

784 values above 50% are shown along the branches. The tree displays 24 unique sequences,

785 representing 120 sequences obtained from 72 individuals. Haplotype names follow

786 those in Table 1, and the numbers preceding the names indicate the number of

787 sequences represented by a given branch. *WER*-lyr of E26L and A1-3L are not included

788 in the tree because of the existence of long indels.

789

790 Fig. 4. Strict consensus tree of the four most-parsimonious trees based on nuclear *CHS*

791 sequence data (1314 aligned positions plus additional coding of six indels). Bootstrap

792   values above 50% are shown along the branches. The tree displays 22 unique sequences,

793   representing 118 sequences obtained from 68 individuals. Haplotype names follow

794   those in Table 1, and the numbers preceding the names indicate the number of

795   sequences represented by a given branch. The colored symbols correspond to those in

796   Fig. 1.

797

798   Fig. 5. Two cpDNA regions (*trnL* intron and *trnL–trnF* intergenic spacer) of

799   *Arabidopsis kamchatica* and other *Arabidopsis* species. **a.** Minimum spanning network

800   based on the *trnL* intron region of *Arabidopsis* species. The *trnL* intron sequences

801   obtained in this study and obtained from GenBank were used to construct the network.

802   GenBank data (Koch & Matschinger 2007; Schmickl *et al.* 2008) are marked with stars

803   and are categorized as explained in Text S1. The circle size indicates the number of

804   individuals. Crossbars on a branch represent unsampled or extinct haplotypes. **b.** Inset:

805   cpDNA haplotypes incorporating data from the *trnL–trnF* intergenic spacer region as

806   well as *trnL* intron region. cpHap2–5 have the same haplotype at the *trnL* intron and are

807   distinguishable in their deletion pattern in the *trnL–trnF* intergenic spacer region.

808   Likewise, cpHap1 and cpHap7 have the same haplotype at the *trnL* intron. The

809   haplotype cpHap6 represents another haplotype at the *trnL* intron. The haplotypes that

810   are not identical to those of *A. kamchatica* are represented as "others". The number

811   below each circle represents the number of individuals included.

812

813   Fig. 6. Map showing the geographic distribution of cpDNA haplotypes. Circles indicate

814   *Arabidopsis kamchatica* subsp. *kamchatica*, asterisks subsp. *kawasakiana*, triangles *A.*

815   *halleri*, and squares *A. lyrata*. Haplotypes are depicted in different colors: haplotype 1,

34

816   pink; 2, green; 3 orange; 4, sky blue; 5, dark blue; 6, yellow; and 11, red. Haplotype 7 is

817   not visible because of overlapping with 6, and the distributions of 8 to 10 and 12 to 18

818   are outside of the range of this map (see Table 1). The Honshu and Shikoku islands of

819   the Japanese archipelago are magnified. Seven haplotypes were found in *A. kamchatica*

820   (including both subspecies). Four of them (cpHap1, 2, 3, 5) were found both in diploid

821   *A. halleri*. subsp. *gemmifera* and *A. kamchatica*; cpHap1 was common and widespread

822   throughout *A. kamchatica*, cpHap2 was restricted mostly to subsp. *kawasakiana* (area

823   B), cpHap3 was found in Honshu and Hokkaido (areas C, D, and F), and cpHap5 was

824   found in one individual in Central Honshu (kamD18). Three other haplotypes of *A.*

825   *kamchatica* (cpHap4, 6, 7) were not shared with diploid taxa; cpHap4 was short and

826   found in *A. kamchatica* subsp. *kawasakiana* and may represent a deletion derivative,

827   and cpHap6 and cpHap7 were found in Taiwan. See also Fig. 8a.

828

829   Fig. 7. Fifty-percent majority-rule consensus tree of 74,944 most-parsimonious trees

830   based on nuclear ribosomal ITS sequence data. Values above the branches indicate the

831   percentage of the most-parsimonious trees bearing their respective clades. The values in

832   brackets below the branches are bootstrap values (above 50%). The tree displays 25

833   unique sequences, representing 75 individuals.

834

835   Fig. 8. Genetic structure of *Arabidopsis kamchatica* based on cpDNA and nuclear *WER*

836   and *CHS* data, as inferred by the Bayesian clustering algorithm implemented in

837   *structure* software.

838   **a.** Population structure of *A. kamchatica*. Each individual is shown as a thin vertical

839   column partitioned into *K* colored components representing inferred membership in *K*

840   genetic clusters. The regional origin of the individuals (A–I) is shown on the top. The

841   individual name (ID 1–50) and the cpDNA haplotype (cpHap1–7) of each individual are

842   shown below. **b.** Mean symmetric similarity coefficient (*SSC*) ± SD over 190 pairs of 20

843   runs for each *K* value. **c.** Mean posterior probability of data Ln $P(X|K)$ ± S over 20 runs

844   for each *K* value. **d.** Plot of ΔK for each *K*.

845

846   Fig. 9. Schematic diagram of haplotypes of *A. kamchatica* and two scenarios,

847   introgression from a diploid vs. independent polyploidization events.

848   The four haplotypes of cpDNA (cpHap1, 2, 3, and 5) are shared with diploid *A. halleri*

849   subsp. *gemmifera*. The haplotypes of six loci (cpDNA, ITS, *WER*-hal, *CHS*-hal, *WER*-

850   lyr, and *CHS*-lyr) of six representative individuals are shown. In each locus, different

851   haplotypes are shown by different shapes, and also by colors that correspond to those in

852   Figs 1, 4, 6, S3 and S4. The ITS sequence of the kamI48 individual was heterogeneous.

853   See text for details.

Table 1. Samples

Name of taxon	Area		Sample name	Population
Arabidopsis kamchatica subsp. *kamchatica*	A	Taiwan	kamA1	Taiwan, Taroko N.P., close to the entrance of the park, 2930 m
Arabidopsis kamchatica subsp. *kamchatica*	A	Taiwan	kamA2	Taiwan, Taroko N.P., close to the entrance of the park, 2930 m
Arabidopsis kamchatica subsp. *kamchatica*	A	Taiwan	kamA3	Taiwan, Taroko N.P., close to the high altitude experimental station, 3000 m
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB4	Japan, Mie, Meiwa, Fukiiura, 2 m
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB5	Japan, Mie, Meiwa, Fukiiura, 2 m
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB6	Japan, Shiga, Takashima, 85m
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB7	Japan, Shiga, Takashima, 85m
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB8	Japan, Shiga, Ohtsu, Ohmimaiko, 85 m
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB9	Japan, Shiga, Hikone, 85 m
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB10	Japan, Toyama, Hamakurosaki, 2 m
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC11	Japan, Tokushima, Mt. Shikokutsurugi, 1740 m
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC12	Japan, Tokushima, Mt. Shikokutsurugi, 1740 m
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC13	Japan, Tottori, Daisenji, 580 m
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC14	Japan, Tottori, Mt. Daisen, 1600 m
Arabidopsis kamchatica subsp. *kamchatica*	D	Mountains in Central Honshu, Japan†	kamD15	Japan, Toyama, along Jintsu River at Toyama airport, 30 m
Arabidopsis kamchatica subsp. *kamchatica*	D	Mountains in Central Honshu, Japan	kamD16	Japan, Ishikawa, Mt. Hakusan, Ichinose, 1080 m
Arabidopsis kamchatica subsp. *kamchatica*	D	Mountains in Central Honshu, Japan	kamD17	Japan, Toyama, Mt. Shirouma, 2800 m
Arabidopsis kamchatica subsp. *kamchatica*	D	Mountains in Central Honshu, Japan	kamD18	Japan, Toyama, Tsurugigozen, 2740 m
Arabidopsis kamchatica subsp. *kamchatica*	D	Mountains in Central Honshu, Japan	kamD19	Japan, Toyama, Tateyama, Mikurigaike, 2400 m
Arabidopsis kamchatica subsp. *kamchatica*	D	Mountains in Central Honshu, Japan	kamD20	Japan, Toyama, Tateyama, Midorigaike, 2400 m
Arabidopsis kamchatica subsp. *kamchatica*	D	Mountains in Central Honshu, Japan	kamD21	Japan, Toyama, Kurobe-dam, 1500 m
Arabidopsis kamchatica subsp. *kamchatica*	D	Mountains in Central Honshu, Japan	kamD22	Japan, Nagano, Kamikochi, Myojin, 1520 m
Arabidopsis kamchatica subsp. *kamchatica*	D	Mountains in Central Honshu, Japan	kamD23	Japan, Nagano, Kamikochi, Shimomatashirodani-deai, 1570 m
Arabidopsis kamchatica subsp. *kamchatica*	D	Mountains in Central Honshu, Japan	kamD24	Japan, Yamanashi, Mt. Kitadake, 3090 m
Arabidopsis kamchatica subsp. *kamchatica*	D	Mountains in Central Honshu, Japan	kamD25	Japan, Shizuoka, Mt. Fuji, Subashiri, 1300 m
Arabidopsis kamchatica subsp. *kamchatica*	E	Lowland in Northern Honshu, Japan	kamE26	Japan, Niigata, Tsugawa
Arabidopsis kamchatica subsp. *kamchatica*	F	Hokkaido, Japan	kamF27	Japan, Hokkaido, Takinoue
Arabidopsis kamchatica subsp. *kamchatica*	F	Hokkaido, Japan	kamF28	Japan, Hokkaido, Asahikawa, Sounkyo, 640 m
Arabidopsis kamchatica subsp. *kamchatica*	F	Hokkaido, Japan	kamF29	Japan, Hokkaido, Kushiro, Obirashike, 20 m
Arabidopsis kamchatica subsp. *kamchatica*	F	Hokkaido, Japan	kamF30	Japan, Hokkaido, Kushiro, Kombumori, 5 m
Arabidopsis kamchatica subsp. *kamchatica*	G	Far East Russia	kamG31	Russia, Kamchatskii krai, Nachiki, basin of the Nachikinskoe ozero lake, 400 m
Arabidopsis kamchatica subsp. *kamchatica*	G	Far East Russia	kamG32	Russia, Kamchatskii krai, near the road from Petropavlovsk Kamchatskii to Esso, 260 m
Arabidopsis kamchatica subsp. *kamchatica*	G	Far East Russia	kamG33	Russia, Kamchatskii krai, Ganaly, close to the bridge over the river Vaktan Malkinskii, 300 m
Arabidopsis kamchatica subsp. *kamchatica*	G	Far East Russia	kamG34	Russia, Kamchatskii krai, Pushchino, close to the bridge over the river Denokhonok, 265 m
Arabidopsis kamchatica subsp. *kamchatica*	G	Far East Russia	kamG35	Russia, Kamchatskii krai, Petropavlovsk Kamchatskii, Mishenaya gora, 10 m
Arabidopsis kamchatica subsp. *kamchatica*	G	Far East Russia	kamG36	Russia, Kamchatskii krai, Elizovo, 70 m
Arabidopsis kamchatica subsp. *kamchatica*	G	Far East Russia	kamG37	Russia, Kamchatskii krai, Nachiki, Mt. Nachikinskoe zerkaltse, 730 m
Arabidopsis kamchatica subsp. *kamchatica*	G	Far East Russia	kamG38	Russia, Kamchatskii krai, Petropavlovsk Kamchatskii, Avachinskaya sopka, 640 m
Arabidopsis kamchatica subsp. *kamchatica*	G	Far East Russia	kamG39	Russia, Kamchatskii krai, Ganaly, close to the bridge over the river Vaktan Ganal'skii, 320 m
Arabidopsis kamchatica subsp. *kamchatica*	G	Far East Russia	kamG40	Russia, Kamchatskii krai, Pushchino, close to the bridge over the river Pravaya Kamchatka,
Arabidopsis kamchatica subsp. *kamchatica*	G	Far East Russia	kamG41	Russia, Kamchatskii krai, Esso, Srednii kamchatskii khrebet, 910 m
Arabidopsis kamchatica subsp. *kamchatica*	H	Alaska	kamH42	USA, Alaska, Kenai, 300 m
Arabidopsis kamchatica subsp. *kamchatica*	H	Alaska	kamH43	USA, Alaska, Chugach State Park, Potter, 5 m
Arabidopsis kamchatica subsp. *kamchatica*	H	Alaska	kamH44	USA, Alaska, Healy
Arabidopsis kamchatica subsp. *kamchatica*	H	Alaska	kamH45	USA, Alaska, Chena River, Chena Hot Springs Rd.
Arabidopsis kamchatica subsp. *kamchatica*	H	Alaska	kamH46	USA, Alaska, Richardson Highway, South of Darling Creek bridge
Arabidopsis kamchatica subsp. *kamchatica*	H	Alaska	kamH47	USA, Alaska, Portage Bay Rd
Arabidopsis kamchatica subsp. *kamchatica*	I	Canada and Washington	kamI48	Canada, Yukon, Mush Lake, 670 m
Arabidopsis kamchatica subsp. *kamchatica*	I	Canada and Washington	kamI49	USA, Washington, Mt. Baker
Arabidopsis kamchatica subsp. *kamchatica*	I	Canada and Washington	kamI50	USA, Washington, Mt. Baker
Arabidopsis lyrata subsp. *lyrata*			lyrlyr1	USA, North Carolina, Pores Knob, ca. 780 m
Arabidopsis lyrata subsp. *lyrata*			lyrlyr2	USA, North Carolina, Pores Knob, ca. 780 m
Arabidopsis lyrata subsp. *petraea*			lyrpet1	Russia, Karhumaki
Arabidopsis lyrata subsp. *petraea*			lyrpet2	Russia, Karhumaki
Arabidopsis lyrata subsp. *petraea*			lyrpet3	Germany, Stolberg, 300 m
Arabidopsis lyrata subsp. *petraea*			lyrpet4	Russia, Yakutiya (Sakha Republic), alluvium of Kolyma, banks of Suharnaya river
Arabidopsis lyrata subsp. *petraea*			lyrpet5	Russia, Yakutiya (Sakha Republic), alluvium of Kolyma, banks of Suharnaya river
Arabidopsis halleri subsp. *gemmifera*			halgem1	Japan, Hyogo, Taka, Omoide River, 200 m
Arabidopsis halleri subsp. *gemmifera*			halgem2	Japan, Osaka, Inagawa, Tadaginzan, 140 m
Arabidopsis halleri subsp. *gemmifera*			halgem3	Russia, Kamchatskii krai, Esso, Srednii kamchatskii khrebet, 660 m
Arabidopsis halleri subsp. *gemmifera*			halgem4	Russia, Kamchatskii krai, Esso, Srednii kamchatskii khrebet, 520 m
Arabidopsis halleri subsp. *gemmifera*			halgem5	Russia, Kamchatskii krai, Esso, valley of the river Ulavkavchan, 470 m
Arabidopsis halleri subsp. *gemmifera*			halgem6	Russia, Kamchatskii krai, Esso, valley of the river Ulavkavchan, 470 m
Arabidopsis halleri subsp. *gemmifera*			halgem7	Russia, Kamchatskii krai, Esso, 580 m
Arabidopsis halleri subsp. *gemmifera*			halgem8	Japan, Nagano, Kamikochi, Shimomatashirodani-deai, 1570 m
Arabidopsis halleri subsp. *dacica*			haldac1	Romania, Fagaras, Mts., Saua Caprei glacial lake, 2270 m
Arabidopsis halleri subsp. *tatrica*			haltat1	Slovakia, Belianske Tatry, 1200 m
Arabidopsis halleri subsp. *tatrica*			haltat2	Slovakia, Vysoke Tatry, 1800 m
Arabidopsis halleri subsp. *tatrica*			haltat3	Slovakia, Slovensky Raj, 1000 m
Arabidopsis halleri subsp. *ovirensis*			halovi1	Austria, Carinthia, Ebriach, 1557 m
Arabidopsis halleri subsp. *halleri*			halhal1	Switzerland, Ticino, Giubiasco, 400 m
Arabidopsis halleri subsp. *halleri*			halhal2	Switzerland, Ticino, Giubiasco, 400 m
Arabidopsis pedemontana			ped	Italy, north of Valle Po, north of Crissolo, Colle delle Porte, 2260 m

*two ITS haplotypes are obtained from this sample because one-base pair indel was identified.
** At trnL intron: a difference in a simple repeat, that was not considred in the analyses.
† Mountains in Central Honshu include Japan Alps, Mt. Fuji and Mt. Hakusan.
Selfing: n.a. not assayed, y: successful
Voucher specimens are deposited in the herbaria KYO, SAV, Z, and SHO (herbaria of Shoei Junior College, Kobe, Japan) when available.

Collector	selfing	*WER*-lyr	*WER*-hal	*CHS*-lyr	*CHS*-hal	ITS	cpDNA haplotype
H. Tsukaya	n.a.	kamA1L	kamA1H	kamA1L	kamA1H	kamA1	cpHap6
H. Tsukaya	n.a.	kamA2L	kamA2H	kamA2L	kamA2H	kamA2	cpHap6
H. Tsukaya	n.a.	kamA3L	kamA3Ha1, a2	kamA3L	kamA3H	kamA3a1, a2*	cpHap7
HK	n.a.	kamkwsB4L	kamkwsB4H	kamkwsB4L	kamkwsB4H	kamkwsB4	cpHap2
HK, KKS	n.a.	kamkwsB5L	kamkwsB5H	kamkwsB5L	kamkwsB5H	kamkwsB5	cpHap2
S. Fujii, KKS	y	kamkwsB6L	kamkwsB6H	kamkwsB6L	kamkwsB6H	kamkwsB6	cpHap4
S. Fujii, KKS	y	kamkwsB7L	kamkwsB7H	kamkwsB7L	kamkwsB7H	kamkwsB7	cpHap4
S. Fujii, KKS	y	kamkwsB8L	kamkwsB8H	kamkwsB8L	kamkwsB8H	kamkwsB8	cpHap2
HK	n.a.	kamkwsB9L	kamkwsB9H	kamkwsB9L	kamkwsB9H	kamkwsB9	cpHap4
HK	y	kamkwsB10L	kamkwsB10H	kamkwsB10L	kamkwsB10H	kamkwsB10	cpHap1
M. Kanaoka, KKS	y	kamC11L	kamC11H	kamC11L	deletion	kamC11	cpHap1
M. Kanaoka, KKS	y	kamC12L	kamC12H	kamC12La1, a2	deletion	kamC12	cpHap1
KKS	y	kamC13L	kamC13H	kamC13L	kamC13H	kamC13	cpHap3
KKS	n.a.	kamC14L	kamC14H	kamC14L	kamC14H	kamC14	cpHap3
HK, J. Sugisaka	y	kamD15L	kamD15H	kamD15L	kamD15H	kamD15	cpHap3
KKS	n.a.	kamD16L	kamD16H	kamD16L	kamD16H	kamD16	cpHap3
M. Kanaoka, KKS	n.a.	kamD17L	kamD17H	kamD17La1, a2	kamD17H	kamD17	cpHap1
KKS	n.a.	kamD18L	kamD18H	kamD18L	kamD18H	kamD18	cpHap5
KKS	n.a.	kamD19L	kamD19H	kamD19L	kamD19H	kamD19	cpHap3
KKS	y	kamD20L	kamD20H	kamD20L	kamD20H	kamD20	cpHap3
KKS	n.a.	kamD21L	kamD21H	kamD21L	kamD21H	kamD21	cpHap3
KKS	y	kamD22L	kamD22H	kamD22L	kamD22H	kamD22	cpHap3
HK	n.a.	kamD23L	kamD23H	kamD23L	deletion	kamD23	cpHap2
KKS	n.a.	kamD24L	kamD24H	kamD24L	kamD24Ha1, a2	kamD24	cpHap3
HK	y	kamD25L	kamD25H	kamD25L	kamD25H	kamD25	cpHap3
A. Kawabe	y	kamE26L	kamE26H	kamE26L	kamE26H	kamE26	cpHap1
KKS	n.a.	kamF27L	kamF27H	kamF27L	kamF27H	kamF27	cpHap3
H. Nakai, KKS	y	kamF28L	kamF28H	kamF28L	kamF28H	kamF28	cpHap3
KKS	n.a.	kamF29L	kamF29H	kamF29L	kamF29H	kamF29	cpHap3
KKS	y	kamF30L	kamF30H	kamF30L	kamF30H	kamF30	cpHap3
KM, VVY	n.a.	kamG31L	kamG31H	kamG31L	kamG31H	kamG31	cpHap1
KM, VVY, HK, RSI, KKS	n.a.	kamG32L	kamG32H	kamG32L	kamG32H	kamG32	cpHap1
KM, VVY, HK, RSI, KKS	n.a.	kamG33L	kamG33H	kamG33L	kamG33H	kamG33	cpHap1
KM, VVY, HK, RSI, KKS	n.a.	kamG34L	kamG34H	kamG34L	kamG34H	kamG34	cpHap1
KM, VVY, HK, RSI, KKS	n.a.	kamG35L	kamG35H	kamG35L	kamG35H	kamG35	cpHap1
KM, VVY	n.a.	kamG36L	kamG36H	kamG36L	kamG36H	kamG36	cpHap1**
KM, VVY	n.a.	kamG37L	kamG37H	kamG37L	kamG37H	kamG37	cpHap1
KM, VVY	n.a.	kamG38L	kamG38H	kamG38L	kamG38H	kamG38	cpHap1
KM, VVY, HK, RSI, KKS	n.a.	kamG39L	kamG39H	kamG39L	kamG39H	kamG39	cpHap1
KM, VVY, HK, RSI, KKS	n.a.	kamG40L	kamG40H	kamG40L	kamG40H	kamG40	cpHap1
KM, VVY, HK, RSI, KKS	n.a.	kamG41L	kamG41H	kamG41L	kamG41H	kamG41	cpHap1
A. Caicedo	n.a.	kamH42L	kamH42H	kamH42L	kamH42H	kamH42	cpHap1
KKS	y	kamH43L	kamH43H	kamH43L	kamH43H	kamH43	cpHap1
KKS	n.a.	kamH44L	kamH44H	kamH44L	kamH44H	kamH44	cpHap1
OS	y	kamH45L	kamH45H	kamH45L	kamH45H	kamH45	cpHap1
OS	y	kamH46L	kamH46H	kamH46L	kamH46H	kamH46	cpHap1
OS	y	kamH47L	kamH47H	kamH47L	kamH47H	kamH47	cpHap1
H. Schöb	n.a.	kamI48L	kamI48H	kamI48L	kamI48H	kamI48	cpHap1
OS	n.a.	kamI49L	kamI49H	kamI49L	kamI49H	kamI49	cpHap1
OS	n.a.	kamI50L	kamI50H	kamI50L	kamI50H	kamI50	cpHap1
KKS	n.a.	lyrlyr1		lyrlyr1a1, a2		lyrlyr1	cpHap8
KKS	y	lyrlyr2		lyrlyr2		lyrlyr2	cpHap8
OS	n.a.	lyrpet1				lyrpet1	cpHap9
OS	n.a.			lyrpet2		lyrpet2	cpHap9
M. Clauss, KKS	n.a.	lyrpet3		lyrpet3		lyrpet3	cpHap10
VVY	n.a.	lyrpet4		lyrpet4		lyrpet4	cpHap11
VVY	n.a.	lyrpet5		lyrpet5		lyrpet5	cpHap11
T. Kawagoe, KKS	y		halgem1		halgem1	halgem1	cpHap1
KKS	y		halgem2		halgem2	halgem2	cpHap1
KM, VVY, HK, RSI, KKS	n.a.		halgem3		halgem3	halgem3	cpHap2
KM, VVY, HK, RSI, KKS	n.a.		halgem4		halgem4	halgem4	cpHap2
KM, VVY, HK, RSI, KKS	n.a.		halgem5		halgem5	halgem5	cpHap2
KM, VVY, HK, RSI, KKS	n.a.		halgem6		halgem6	halgem6	cpHap2
KM, VVY, HK, RSI, KKS	n.a.		halgem7		halgem7	halgem7	cpHap5
KM, HK	n.a.		halgem8		halgem8	halgem8	cpHap3
M. Kolnik	n.a.		haldac1			haldac1	cpHap12
KM, JL, HK, KKS	n.a.		haltat1		haltat1a1, a2	haltat1	cpHap13
KM, JL, HK, KKS	n.a.		haltat2		haltat2	haltat2	cpHap14
KM, JL, HK, KKS	n.a.		haltat3			haltat3	cpHap15
M. Kolnik	n.a.		halovi1		halovi1	halovi1	cpHap16
RSI, KKS, T. Tsuchimatsu, M. Helling	n.a.		halhal1a1,a2			halhal1	cpHap17
RSI, KKS, T. Tsuchimatsu, M. Helling	n.a.		halhal2			halhal2	cpHap18
KKS, KM, JL	n.a.		ped		ped	ped	
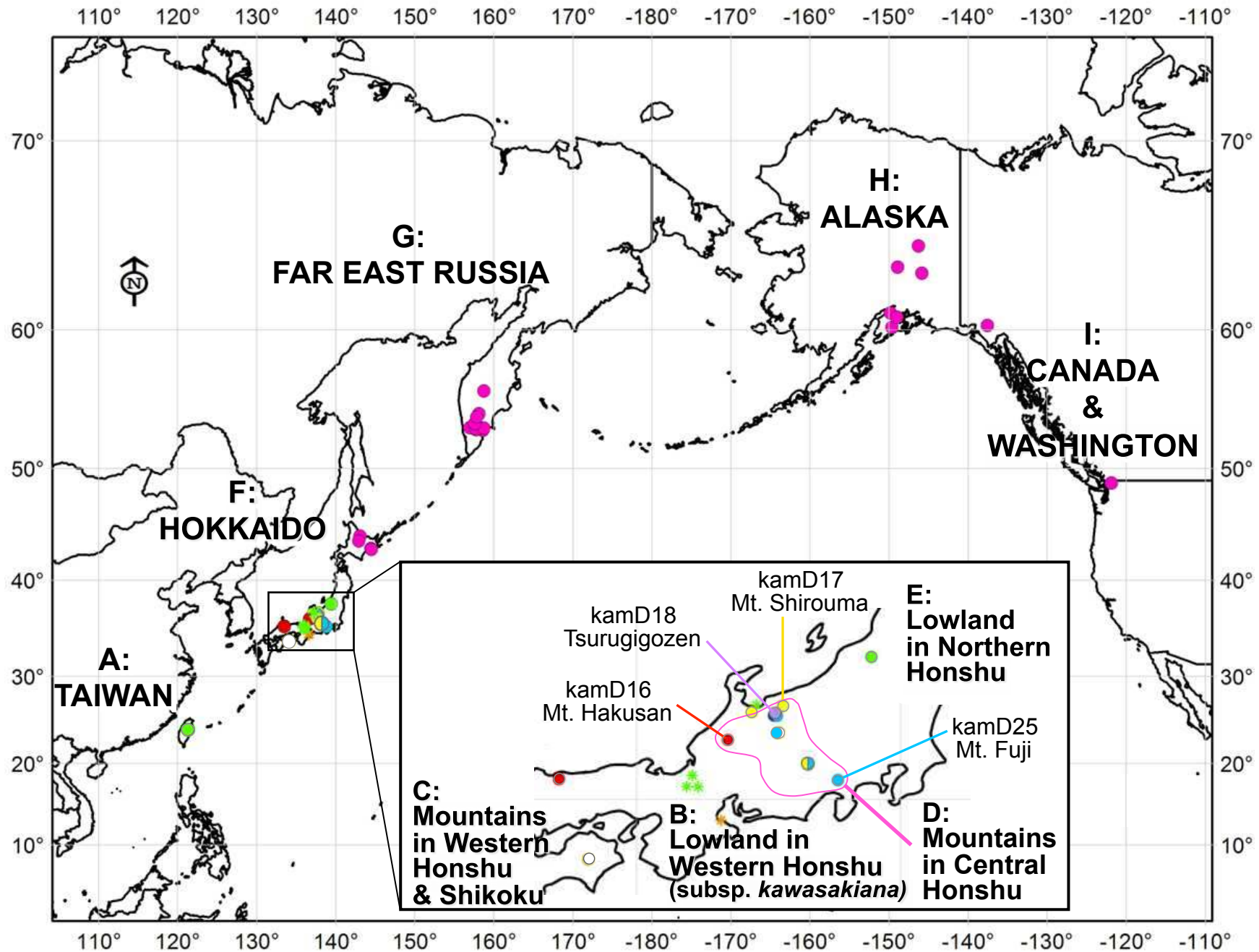
Fig.1 CHS-hal map
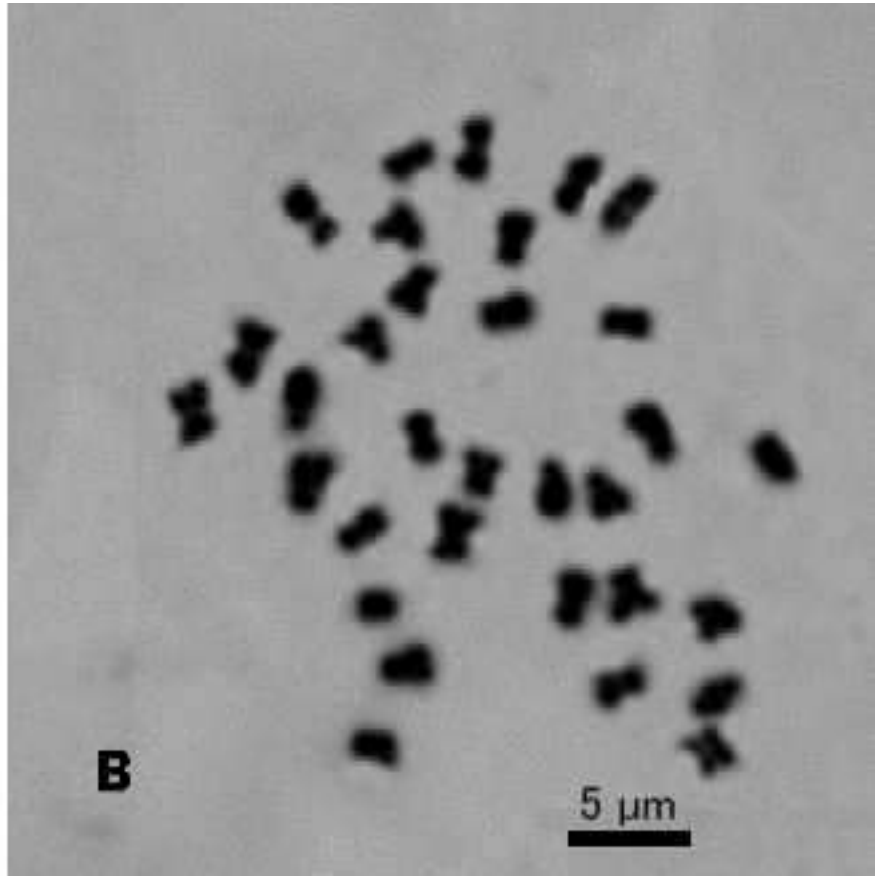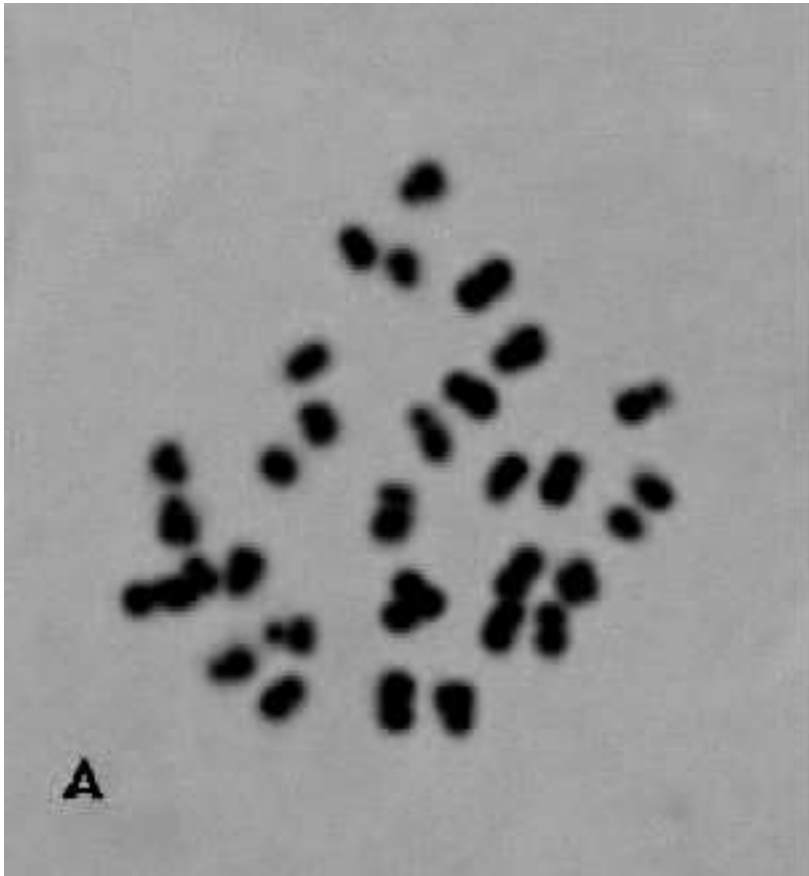
Fig. 2 chromosome
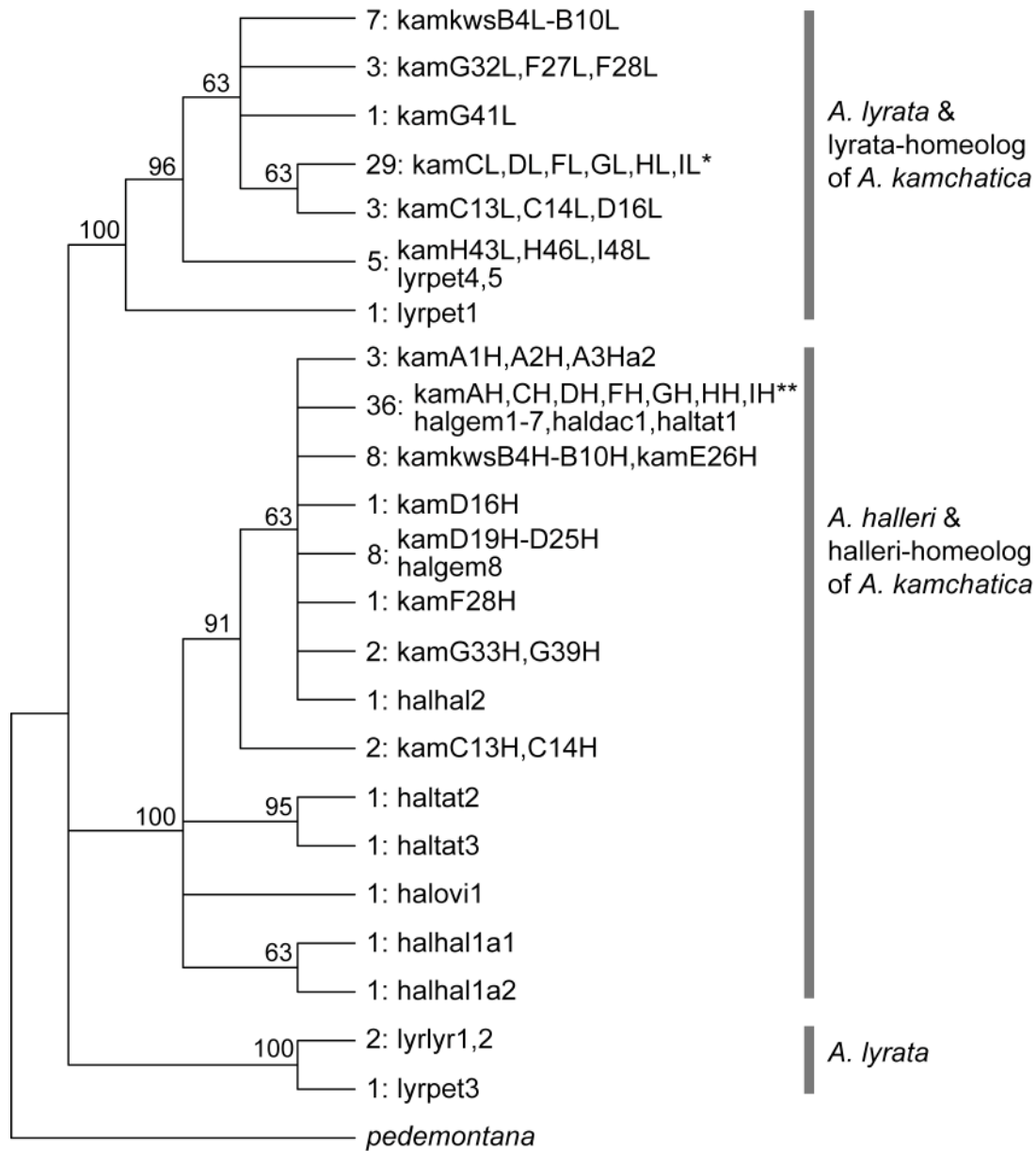
Fig.3 WER MP tree

*kamC11L, C12L, D15L, D17L-D25L, F29L, F30L, G31L, G33L-G40L, H42L, H44L, H45L, H47L, I49L, I50L

**kamA3Ha1, C11H, C12H, D15H, D17H, D18H, F27H, F29H, F30H, G31H, G32H, G34H-G38H, G40H, G41H, H42H-47H, I48H-I50H
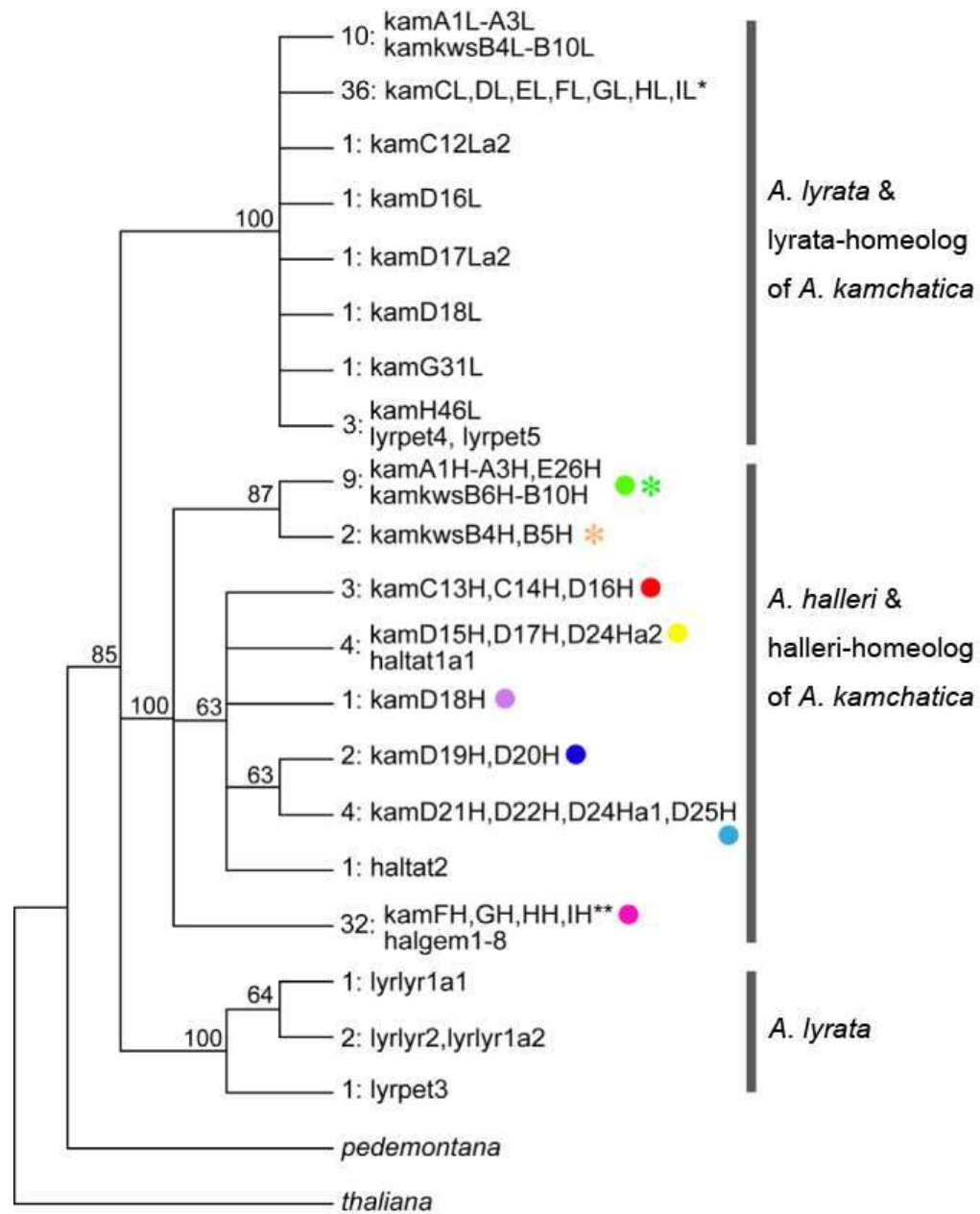
Fig.4 CHS MP tree

*kamC11L, C12La1, C13L, C14L, D15L, D17La1, D19L-D25L, E26L,
 F27L-F30L, G32L-G41L, H42L-H45L, H47L, I48L-I50L

**kamF27H-F30H, G31H-G41, H42H-H47H, I48H-I50H

a

- 🟥 *A. kamchatica* subsp. *kamchatica*, Asia and America
- ▨ *A. kamchatica* subsp. *kawasakiana*, Japan

- 🟪 *A. halleri* subsp. *gemmifera*, Asia
- ▦ *A. halleri* subsp. *gemmifera*, Asia *
- 🟦 *A. halleri*, Europe
- 🟦 *A. halleri*, Europe *

- ⬛ *A. lyrata*, Asia
- ⬜ *A. lyrata*, Asia *
- 🟥 *A. lyrata* subsp. *lyrata*, America
- 🟧 *A. lyrata "arenicola"*, America *
- 🟨 *A. lyrata*, Europe
- 🟧 *A. lyrata*, Europe *
- 🟪 *A. lyrata* or *A. kamchatica*, America and Asia *

- 🟩 *A. arenosa* *

b

cpHap2-5

cpHap6

cpHap1&7

cpHap2	cpHap3	cpHap4	cpHap5	others
8	23	3	2	1

cpHap6 — others — 2 — 40

cpHap1 — cpHap7 — others — 39 — 1 — 73

Haplotype including t*rnL-trnF*

A. pedemontana *

A. cebenensis *

A. croatica *

Haplotype network based on *trnL* intron

Fig.6 chloroplastHap map

kamA,C,D,F,G,H,I*
kamkwsB4-B10
kamA3a2
kamD15
kamD24
kamE26
kamG32
lyrpet1
lyrpet2,3
kamD18
kamH43,H47,I48
lyrlyr1,2,lyrpet4-5

A.lyrata &
A. kamchatica

kamD17
halgem1-4,8
halgem5
halgem6
halgem7
haltat1
haltat2
haltat3
halovi1
halhal1
halhal2
haldac1

A. halleri &
A. kamchatica D17

pedemontana
thaliana

75
100
(100)
100
(86)
100
(79)
100
(62)
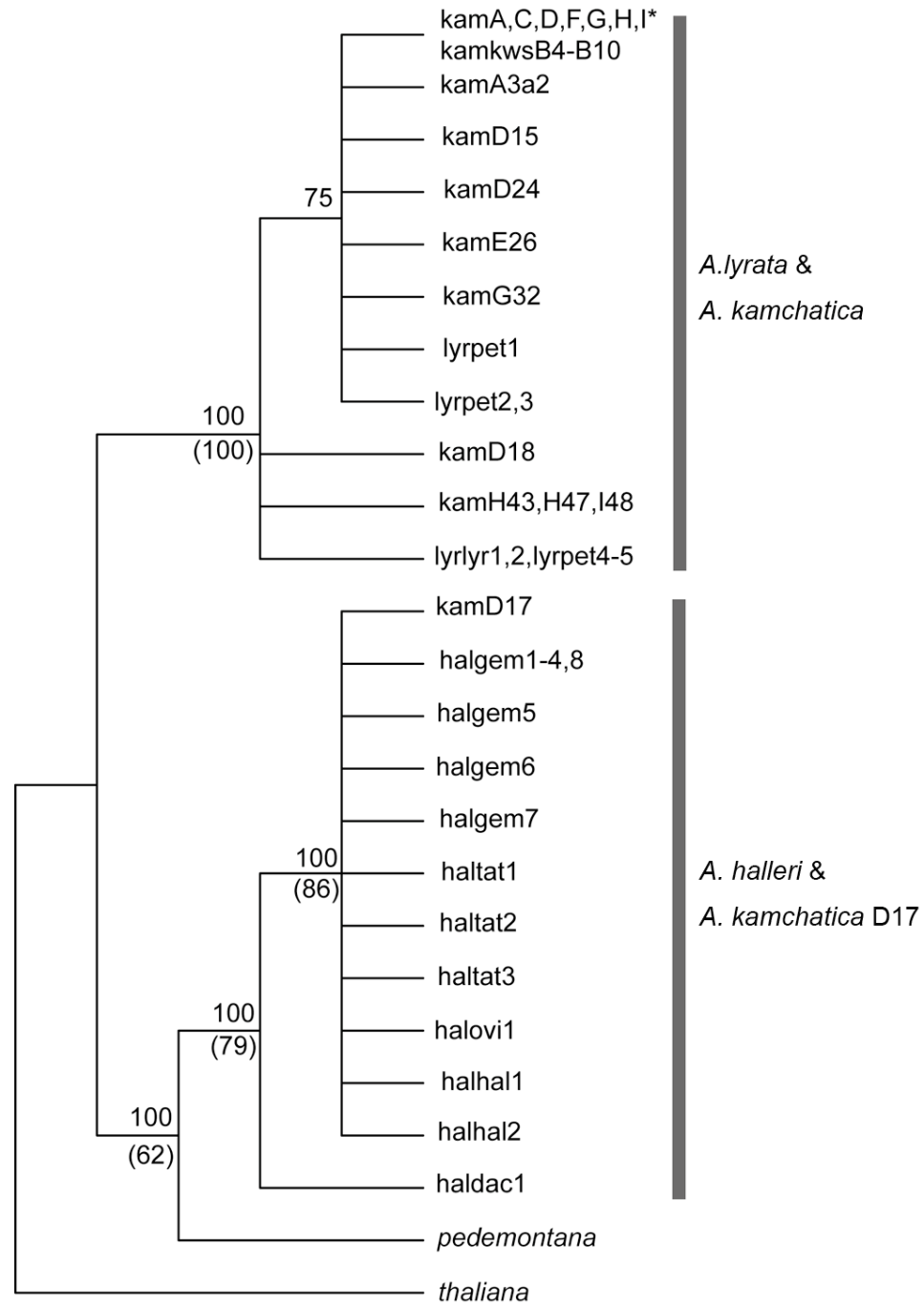
*kamA1, A2, A3a1, C11-C14, D16, D19-D23, D25, F27-F30, G31, G33-G41, H42, H44-H46, I49, I50

Fig. 7 ITS MP
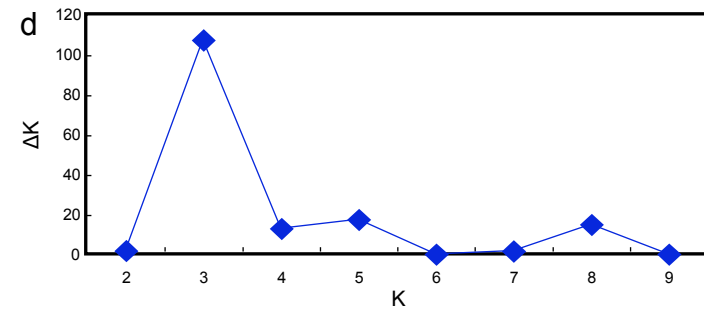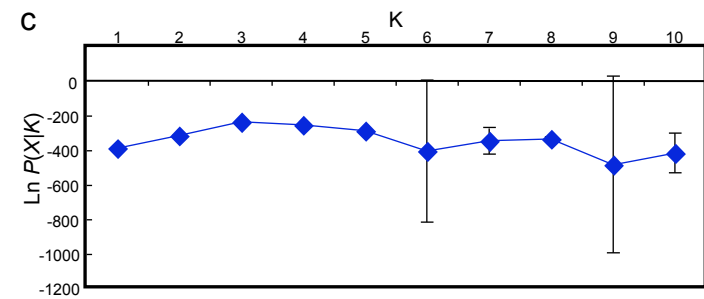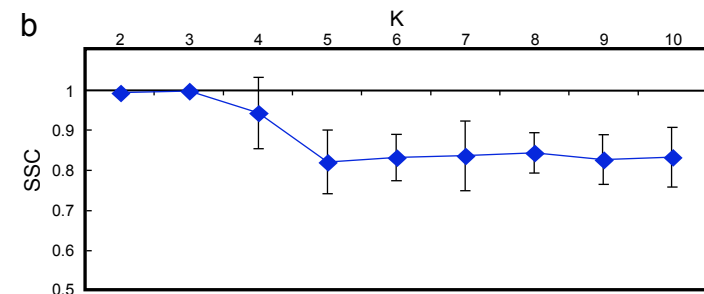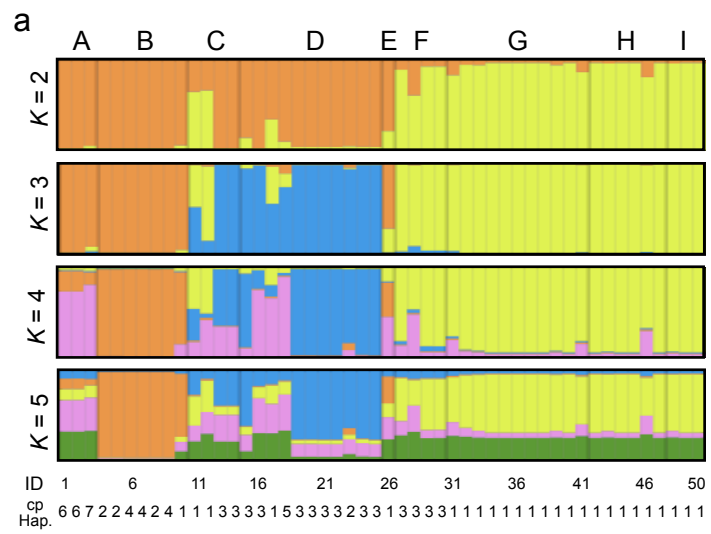
Fig.8 Structure
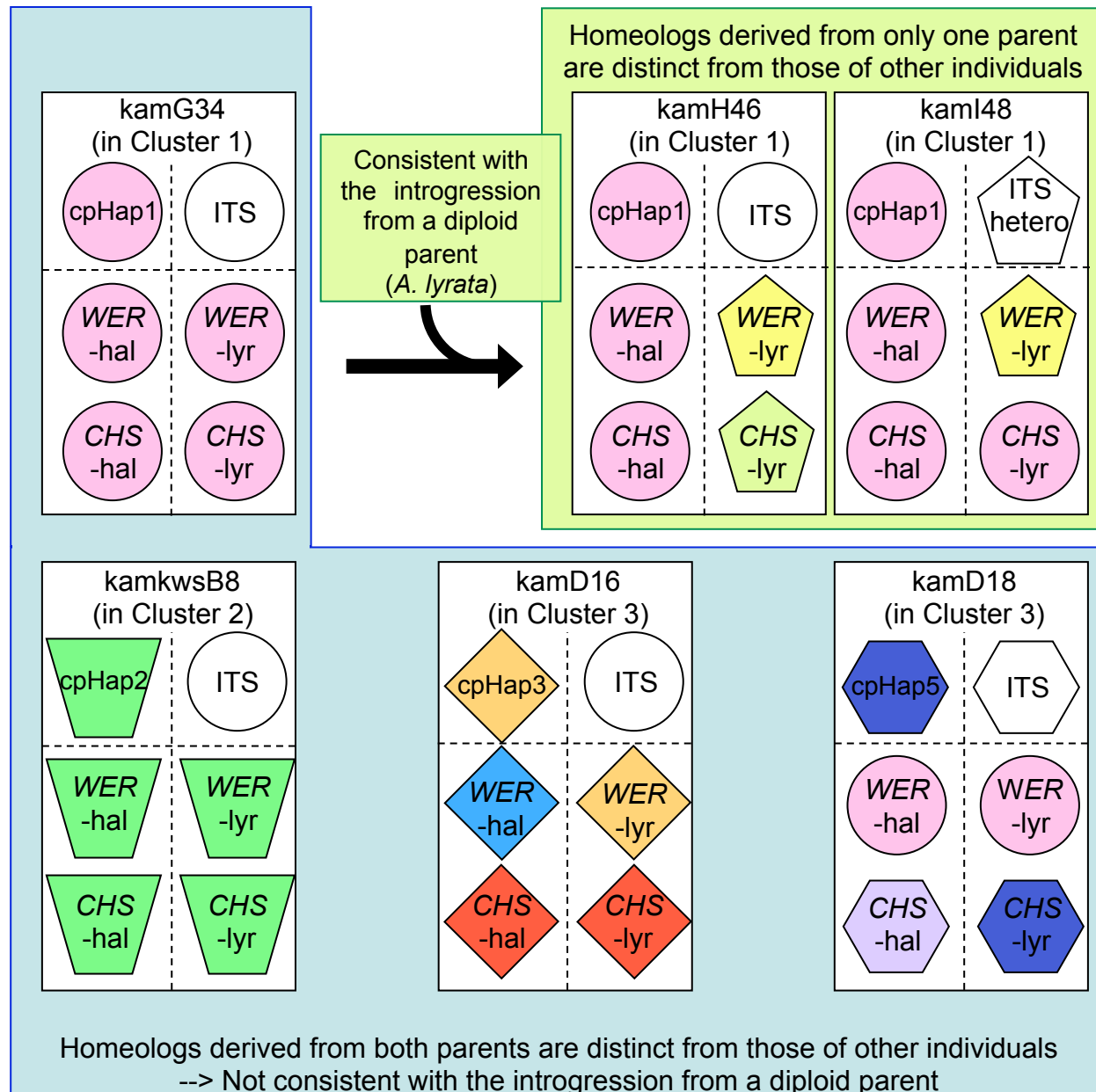
Fig. 9

Supporting Information (5 figures, 3 tables, 1 section of text)

Supporting Fig. S1. Primers used for amplification of *WER* and *CHS* genes, and the number of homeologs
a. Each arrow indicates the position of the primer and its direction. Gray boxes represent the coding region of each gene. See Supporting Table S1 for the details of the primers used.
b. Amplification of *WER*-hal and *WER*-lyr using primers WERF4-R3e1. The primers were designed in the conserved region so that they amplified both *WER*-hal and *WER*-lyr (e.g., E26, G31 and B4). Two homeologs of C13 and C14 had the same length, and see Text S1.
c. Digestion of PCR fragments to confirm the homeolog type. Each fragment was amplified with primers CHSkamF1-CHSR3 and digested by *Xba*I. The primers were designed in the conserved region so that they amplified both halleri- and lyrata-derived homeologs of *CHS*. *CHS*-hal but not *CHS*-lyr was digested, resulting in shorter bands.

Supporting Fig. S2. Segregating sites of the *CHS* gene and ITS region, and sequences of distinct cpDNA haplotypes
a, b. Summary of the segregating sites among the **a.** *CHS* gene and **b.** ITS region from *A. kamchatica*, *A. lyrata*, and *A. halleri*. Dots indicate the nucleotide identical to that of the upper row sequence, - indicates the deletion site and N indicates the site where the sequence was not obtained. The haplotype names correspond to those in Table 1. In ITS region, IUPAC ambiguity codes were used for coding polymorphic positions. In three individuals of North American *A. kamchatica* (kamH43, kamH47 and kamI48), three heterozygous sites were observed, which is consistent with the overlap of two haplotypes: a common haplotype in *A. kamchatica*, and a haplotype identical to the haplotype observed in four individuals of *A. lyrata*.
c. Sequences of the *trnL* intron and *trnL-trnF* intergenic spacer regions of distinct 18 cpDNA haplotypes (cpHap1–18) observed in *A. kamchatica*, *A. lyrata*, and *A. halleri*. The cpHap1* from the individual kamG36 has one additional nucleotide T at a polyT site of the *trnL* intron region, shown as * at the 300th site compared with cpHap1. This difference between cpHap1 and cpHap1* was ignored in all analyses. The alignment of *trnL-trnF* region was not clear due to tandem duplications. See Table 1 for the correspondence between individuals and haplotypes.

Supporting Fig. S3. Phylogenetic trees of *WER, CHS* and *ITS*
Accession abbreviations follow Table 1.
a. A single most-parsimonious tree obtained from the analysis based on nuclear *CHS* sequence data of the lyrata-clade. The data matrix includes 1589 aligned positions and additional coding of eight indels, i.e., it is longer than that of Fig. 4 because of inclusion of the longer promoter region, which was lacking or not amplified in the halleri clade. Bootstrap values above 50% are shown along the branches. The number to the right of each branch indicates how many sequences are included in the branch. The colored symbols correspond to that in Fig. S4c. The tree displays 14 unique sequences, representing 57 sequences obtained from 55 individuals.
b. Majority-rule consensus tree of the Bayesian inference based on nuclear *WER* sequence data (955 aligned positions). The posterior probability values of the nodes are indicated above the branches. The colored symbols correspond to that in either of Fig. S4a (for *WER*-lyr) or Fig. S4b (for *WER*-hal). *WER*-lyr of E26L (dark blue in Fig. S4a) and A1-3L (dark green in Fig. S4a) are not included in the tree because of the existence of long indels. The tree displays 24 unique sequences, representing 120 sequences obtained from 72 individuals.

c. Majority-rule consensus tree of the Bayesian inference based on nuclear *CHS* sequence data (1314 aligned positions). The posterior probability values of the nodes are indicated next to the branches. The tree displays 22 unique sequences, representing 118 sequences obtained from 68 individuals. Accession abbreviations follow Table 1.
d. Majority-rule consensus tree of the Bayesian inference based on nuclear ITS sequence data. The posterior probability values of the nodes are indicated above the branches. The tree displays 25 unique sequences, representing 75 individuals.

Supporting Fig. S4. Geographic distribution of *WER* and *CHS* haplotypes
Haplotype maps showing the geographic distribution of each haplotype of **a.** *WER*-lyr, **b.** *WER*-hal and **c.** *CHS*-lyr. Circles indicate *A. kamchatica* subsp. *kamchatica* and asterisks indicate subsp. *kawasakiana*. Heterozygotes are shown as half-circles. The upper map shows the Pacific Ocean rim, and the lower magnified map shows the Japanese archipelago. Different haplotypes of each homeolog are depicted in different colors. The color symbols in **a**, **b** and **c** correspond to those in the *WER*-lyr clade in Fig. S3b, *WER*-hal clade in Fig. S3b and *CHS*-lyr in Fig. S3a, respectively.

Supporting Fig. S5. Neighbor-joining tree of *CHS* including publicly available data
The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0.11702052 is shown. In addition to our data, published sequences, namely, seven sequences of *A. lyrata* subsp. *lyrata* from America (noted as lyrlyr AL), four sequences of *A. lyrata* subsp. *petraea* from Europe (as lyrpet AP), and eleven sequences of *A. halleri* from Europe (as hal CH) were included. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

Supporting Text 1. Sequencing, alignments, phylogenetic analyses, intrapopulation polymorphism, and Bayesian clustering

DNA extraction and sequencing
We conducted one round of selfing using 18 individuals to propagate, and repeated up to four rounds for subspecies *kawasakiana* with a short life-cycle. Genomic DNA was isolated from young leaves using the DNeasy Plant Mini kit (Qiagen). DNA sequencing was conducted at the Institute of Plant Biology, University of Zurich, with a Prism 3730 48-capillary automated sequencer (Applied Biosystems). The sequence alignments were done in Biolign version 4.0.6.2 (http://www2.maizegenetics.net/index.php?page=bioinformatics/index.html) and edited manually using the program BioEdit version 7.0.4.1 (Hall 1999). Ambiguous polymorphisms were rechecked with PCR reamplification and sequencing. The two cpDNA regions were concatenated and analyzed as a haplotype (called superhaplotype by Koch & Matschinger 2007).

Primer design
We designated the genes of polyploids, which are orthologs of "single-copy nuclear genes" in the parental species, as "low-copy nuclear genes" of polyploids. In contrast, the nuclear ribosomal ITS region is tandemly repeated and is not a low-copy nuclear gene. The PCR primers used in the study are listed in Table S1 and are shown schematically in Fig. S1. Design of the homeolog-specific primers and PCR amplification were conducted using the methods described by Lihova *et al.* (2006). Because of redundancy of genes in polyploid

species, homeologs often exhibit rearrangements or gene loss. In addition, the indels and SNPs used for the design of homeolog-specific primers are often polymorphic, even among individuals in the species. Thus, homeolog-specific primers designed based on a particular individual often yield nonspecific or unsuccessful amplification in other individuals, as we reported in the study of hexaploid *Cardamine asarifolia* (Lihova *et al.* 2006). In the present study, we designed multiple homeolog-specific primers to amplify the *WER* and *CHS* genes.

To amplify *WER* homeologs, we designed three primers in the 5′-upstream region and six primers in the 3′-downstream region based on the genome sequence of the closely related species *A. thaliana*. The PCR product by WERFU3 and WERRD6 turned out to be lyrata-homeolog specific. The PCR products by WERFU1 and WERRD1 included both homeologs. The product was sequenced directly using WERRD1 primer and yielded single peak sequences followed by double peaks. Using the methods described in Figure 2 of Lihova *et al.* (2006), we identified a 4-bp indel polymorphism and designed the halleri-homeolog specific primer WERgemRD1 at this position. Although sequencing of several individuals was successful using this primer, PCR amplification was weak in many individuals. Thus, based on the 3-bp indel polymorphism in the third exon, another specific primer, WERgemR3e2, was designed.

Three individuals from Taiwan did not yield a lyrata-homeolog of *WER* using primers WERFU3 and WERRD6. The forward primer WERlyrF1 in the second intron in combination with the reverse primer WERRD6 yielded specific amplification of a lyrata-homeolog, suggesting that rearrangements occurred in the former half of the gene.

To amplify *CHS* homeologs, Shimizu *et al.* (2005) designed the homeolog-specific primers CHSlyrFU1 and CHSgemFU1, which were designed at ~500 upstream of the start codon, and a common reverse primer CHSR1. In several individuals, these primers yielded low or no amplification, presumably because of rearrangements and mutations. To amplify the halleri-homeolog, the primer CHSgemFU15 was designed at ~150 upstream of the start codon. These primers amplified the halleri-homeolog in 47 of the 50 individuals; the other three individuals had presumable deletions or rearrangements (see below). The primer CHSlyrFU4 was designed to encompass two SNPs near the primer CHSlyrFU1, based on a comparison of the sequence of *A. lyrata* and *A. halleri* ssp. *gemmifera*.

Copy numbers

To verify the deletion and the copy numbers, primers were designed in the conserved sequences to amplify both homeologs together. As for the *WER* homeologs, WERF4 in exon 2 and WERR3e1 in exon 3 were designed to encompass the 54-bp insertion found in most of the halleri-homeologs. PCR yielded a longer band of the halleri-homeolog and a shorter band of lyrata-homeolog in all 50 individuals of *A. kamchatica* (Fig. S1b; e.g., G31 and B4), except for individuals with different indels (kamE26 from Tsugawa and kamC13 and kamC14 from Daisen). In kamE26, the lyrata-homeolog was longer than the halleri-homeolog (Fig. S1b). The PCR products of kamC13 and kamC14 were digested by *Pst*I and were confirmed to have two homeologs (data not shown). As for the *CHS* homeologs, CHSkamF1 in exon 1 and CHSR3 in the exon 2 were designed to encompass an SNP at the *Xba*I restriction site. By digesting the PCR products with *Xba*I, 47 of 50 individuals yielded bands of both lyrata and halleri-homeologs (Fig. S1c; e.g., G31 and B4). Three individuals (kamC11 and kamC12 from Shikokutsurugi and kamD23 from Shimomatashirodani-deai) yielded only one band that corresponded to the lyrata-homeolog. Direct sequencing (as described in Lihova *et al.* 2006) confirmed that only the lyrata-homeolog was amplified. In conjunction with the PCR failure of the halleri-homeolog described above, these data suggest strongly that the halleri-homeolog of *CHS* is deleted or rearranged in the three individuals. In contrast, diploid samples showed only one band (Fig. S1b, c and data not shown). In short, allopolyploidy was supported in all

50 individuals either by *WER* or *CHS*, and rearrangement or deletion occurred in several individuals.

Alignments and phylogenetic analyses

We aimed to achieve as much sample overlap between the individual data sets (*WER*, *CHS* and ITS) as possible, although this was not always possible for several reasons (sequence recombination, PCR failure, deletion, etc.).

MP analyses were conducted with PAUP* version 4.0b10 (Swofford 2001). Heuristic searches were made with the following settings: gaps treated as missing data, single-site polymorphisms as uncertainties, tree construction with stepwise addition, 1,000 replicates with random taxon addition, TBR branch swapping, no MAXTREES limits, and MULTREES option in effect. For character-state optimization, the ACCTRAN (accelerated character transformation) option was used. The most-parsimonious trees generated were summarized in the strict consensus and 50% majority-rule consensus trees. Bootstrap analyses (Felsenstein 1985) were performed using 100,000 resamplings with the fast-heuristic search as implemented in PAUP*.

The Bayesian inference was run using MrBayes version 3.0 beta4 (Huelsenbeck & Ronquist 2001). Four Markov chains were run for 20 million generations while adjusting the temperature difference between the cold and heated chains to achieve efficient swapping between the chains. Six substitution rates (nst = 6) and a gamma distribution (rates = gamma) were assumed. The trees were sampled every 100 generations and, finally, majority-rule consensus trees were computed that excluded the trees found in the burn-in phase (i.e., those generated before the likelihood values reached a plateau and fluctuated within a more or less stable range). The percentage of trees recovering an individual node is indicated on the consensus trees by the node's posterior probability.

WER homeologs

The alignment of *WER* homeologs (spanning from the middle of exon 1 to the middle of exon 3) comprised 120 sequences obtained from 72 individuals (summarized in Table S2). Only *A. pedemontana* was used as the outgroup species because inclusion of *A. thaliana* would introduce a high number of additional indels and complicate the sequence alignment and data analyses. The final alignment of *WER* comprised 24 unique haplotypes and included 955 aligned positions. Fourteen indels longer than 1 bp were introduced in the alignment, coded as additional binary to four-state characters, and included in the MP analyses. In total, 83 sites were variable, and 60 of them were parsimony informative.

MP analyses and Bayesian inferences resulted in very similar tree topologies, and in the following text, we report on the MP results only. The MP analysis of the *WER* data set resulted in a strict consensus tree (78 most-parsimonious trees, $L = 96$ steps, CI = 0.96, RI = 0.99), which displayed three main and well-supported (100% bootstrap) clades (Fig. 3). The *WER* sequences from the diploids *A. lyrata* and *A. halleri* were clearly differentiated from each other (placed in distinct clades). Two apparently different homeologs were retrieved from the tetraploids (*A. kamchatica*, including both subspecies) and placed in the respective clades of the diploids. Among the three clades resolved, one clade comprised all individuals of *A. halleri* and the corresponding homeolog from tetraploids (*WER*-hal), and the other clade comprised the Russian (both western and easternmost Russia) accessions of *A. lyrata* and the other homeolog from tetraploids (*WER*-lyr). Two accessions of *A. lyrata* from USA (North Carolina) and one from Germany of *A. lyrata* formed an additional clade.

Despite our limited sampling of the diploids, both *A. lyrata* and *A. halleri* were found to be more diverse than the tetraploids. We also identified haplotypes identical to those found in some tetraploids. Two accessions of *A. lyrata* from Far East Russia (Yakut, lyrpet4,

5) shared the same haplotype with *A. kamchatica* from Alaska and Yukon. Similarly, one accession of *A. halleri* subsp. *gemmifera* from Shimomatashirodani-deai (halgem8) shared the haplotype with *A. kamchatica* from the central mountains of Japan; and seven accessions of *A. halleri* subsp. *gemmifera* from Japan and Kamchatka (halgem1-7, along with two individuals of *A. halleri* from Europe) shared another, apparently widespread, haplotype with many individuals of *A. kamchatica* from nearly the entire area sampled.

Some geographic structure seems to be present among the *A. kamchatica* haplotypes. Within both the lyrata- and halleri-homeologs we found a widespread and common haplotype, which was observed in many accessions from the broad area sampled, and several haplotypes geographically restricted to one of the areas delimited (see Table 1 and area denoted as A–I). A specific haplotype was also found in the accessions of *A. kamchatica* subsp. *kawasakiana* (lowland in western Honshu). In the halleri-homeolog this haplotype was shared only with a single sample from the lowland of northern Honshu (kamE26H).

CHS homeologs

Two alignments of *CHS* homeologs were assembled (summarized in Table S2). Alignment 1 spanned from the promoter sequence to near the end of exon 2 and comprised 118 sequences obtained from 68 individuals. Alignment 2 included a longer region of the promoter sequence obtained only for the *lyrata*-clade (*A. lyrata* and lyrata-homeolog of tetraploids, see Results), i.e., 57 sequences obtained from 55 individuals. Alignment 2 was analyzed to determine whether better resolution can be seen within that clade. *Arabidopsis thaliana* and *A. pedemontana* were used as outgroups. Because of recombination, we excluded the *CHS* haplotypes of the three European diploid accessions from phylogenetic analyses. The recombinant sequences are shown in Fig. S2a. The all-accession-*CHS* alignment (Alignment 1) displayed 22 unique sequences. It had 1314 aligned positions and involved six indels longer than 1 bp, which were coded as additional binary to four-state characters in the MP analyses. One hundred and ten sites were variable, and 44 of them were parsimony informative.

The lyrata-*CHS* alignment (Alignment 2) comprised 14 unique sequences. It had 1589 aligned positions, and eight indels longer than 1 bp, which were coded as binary to four-state characters in the MP analyses. One hundred and seventeen sites were variable, and 25 of them were parsimony informative.

The MP analysis of the all-accession-CHS data set resulted in a strict consensus tree (four most-parsimonious trees, $L = 127$ steps, CI = 0.92, RI = 0.95), which displayed three main clades comprising: 1) all accessions of the diploid *A. halleri* and the respective homeolog from the tetraploids; 2) two accessions of *A. lyrata* (lyrpet4, 5) and the respective homeolog from the tetraploids; and 3) three accessions of *A. lyrata* (Fig. 4). Thus, as in the case of the *WER* data, the haplotypes from the diploids are clearly differentiated from each other, and two homeologs are proved to be present in the tetraploids.

We found considerable variation in *A. lyrata*. Two accessions of this species from Far East Russia (Yakut, lyrpet4, 5) shared their haplotype with *A. kamchatica* from Alaska (kamH46). All eight accessions of *A. halleri* subsp. *gemmifera* from Japan and Kamchatka (halgem1-8) had a haplotype identical to that found in *A. kamchatica* from Hokkaido, Far East Russia, Alaska, Yukon, and Washington state. Three accessions of *A. kamchatica* had a haplotype otherwise found in *A. halleri* in Europe (Slovakia, haltat1a1). In addition, *CHS* of lyrpet2 from western Russia was identical to the widespread haplotype of *A. kamchatica* along > 1 kb at the left side of the recombination breakpoint (Fig. S2a), whereas the 3′-side was identical to a few individuals of *A. lyrata*.

In both the lyrata- and halleri-homeologs, one widespread and common haplotype along with several restricted ones were found in *A. kamchatica*. Especially within the halleri-

homeolog, the haplotypes showed a geographic structure. Three subclades or groups can be recognized here: 1) accessions from lowland in western Honshu (= *A. kamchatica* subsp. *kawasakiana*), northern Honshu, and from Taiwan formed one subclade (areas A, B, and E); 2) those from the mountains of western and central Honshu (areas C and D) formed a second subclade; and 3) all the other accessions (i.e., Hokkaido, Far East Russia, Alaska, Yukon, Washington state; areas F–I) were characterized by another haplotype. The consensus tree based on the lyrata-*CHS* data set (Fig. S3a) showed a similar structure among the *A. kamchatica* haplotypes: those found in accessions from lowland in western Honshu, northern Honshu, from Taiwan (areas A, B, and E), and the common haplotype (areas F–I) formed a distinct clade, and were differentiated from the haplotypes from western and central Honshu (areas C and D).

Published *CHS* sequence data (Ramos-Onsins *et al.* 2004) were included in a Neighbor-Joining analysis using MEGA4 (Tamura *et al.* 2007) (Fig. S5). The Genbank accession numbers are AJ619886, AJ619888-619906, AJ619938, and AJ619939. They represent seven sequences of *A. lyrata* subsp. *lyrata* from America, four sequences of *A. lyrata* subsp. *petraea* from Europe, and eleven sequences of *A. halleri* from Europe (representing subsp. *halleri* as defined by Kolnik and Marhold 2006, judged from localities). The sequence AJ619887 was not used because the sequence was short. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1275 positions in the final dataset.

ITS region

The ITS alignment comprised sequences from 75 individuals, was 619 positions long, and included only a single 1bp-long indel, which was not coded separately. Those of *Arabidopsis thaliana* and *A. pedemontana* were obtained from GenBank (AC006837 and DQ914842) and used as the outgroups. The alignment comprised 25 unique sequences; 59 sites were variable, and 14 of them were parsimony informative.

Intraindividual polymorphic sites were present only scarcely, and IUPAC ambiguity codes were used for coding such polymorphic positions. These sites were found more frequently in the diploids than in the tetraploids, which might suggest that the ITS sequences in the polyploids have been largely homogenized towards one of the parental types possibly due to higher rate of selfing by self-compatibility (see Alvarez & Wendel 2003).

The MP analysis resulted in a 50% majority-rule consensus tree (74,944 most-parsimonious trees, $L = 64$ steps, CI = 0.98, RI = 0.99) with two main and relatively well-supported (100% and 79% bootstrap) clades (Fig. 7). The two clades corresponded to *A. lyrata* and *A. halleri*, respectively. All *A. kamchatica* accessions (with one exception, kamD17) were placed in the clade of *A. lyrata*. A single individual, kamD17, originating from the central mountains in Japan was found in the clade of *A. halleri*. This indicates clearly that concerted evolution of the ITS region was highly effective here and homogenized its sequences towards a repeat type of one parental species, *A. lyrata*. Very little resolution was found within the two main clades, precluding any further inferences (Figs. 7 and S3d).

cpDNA haplotype network and intrapopulation polymorphism

Sequences of two regions of cpDNA (*trnL* intron and *trnL-trnF* intergenic spacer region) were obtained from 50 individuals of *A. kamchatica*, seven individuals of *A. lyrata*, and 15 individuals of *A. halleri* (Table 1). The final alignment of the *trnL* intron was 494 bp long. The *trnL-trnF* sequences varied considerably in length (271–772 bp) because of multiple *trnF* gene duplications and subsequent pseudogene formation (Koch *et al.* 2005). The alignment

was extremely difficult because of the tandem duplications, although it was based on the analysis by Koch *et al.* (2005). Multiple overlapping gaps at the 3′-end of the spacer were identified after alignment. The final alignment comprised 850 aligned positions. Sequences of the two regions from each individual were combined, and a single cpDNA haplotype was produced. Distinct 18-cpDNA haplotypes (cpHap1–18) were observed from *A. kamchatica*, *A. lyrata*, and *A. halleri* (Table 1). The sequences of these cpDNA haplotypes are shown in Fig. S2c.

Seven haplotypes (cpHap1-7) were observed from *A. kamchatica* (including subsp. *kawasakiana*). Four of them (cpHap1, 2, 3, 5) were observed also from *A. halleri*. subsp. *gemmifera,* and their sequences were distinct with different duplicated structure of *trnF* pseudogenes (Fig. S2c). Three other haplotypes were found only in *A. kamchatica*. The cpHap4 was short and found in *A. kamchatica* subsp. *kawasakiana,* and may represent a deletion derivative. The cpHap6 was found in Taiwan. The cpHap7 was also found in Taiwan, and is close to the cpHap1 with a SNP.

The minimum spanning network was drawn based on the *trnL* intron region of *Arabidopsis* species. In total 27 *trnL* intron region haplotypes obtained in this study and in Koch and Matschinger 2007 and Schmickl *et al.* 2008 (GenBank accession numbers DQ313494-313502, DQ313504-313508, DQ313510-313520, DQ914841, DQ529016) were included in this analysis (Fig. 5). *Arabidopsis thaliana* and *A. suecica* were not included because their cpDNA haplotypes are highly divergent. Total 582 individuals from Koch & Matschinger 2007 (SI Table 1) and Schmickl *et al.* 2008 (Supplementary material Table 1) are categorized according to the rules described below:

Arabis umbrosa from East Russia is categorized into *A. lyrata*, Asia. Because Schmicle *et al.* (2008) noted "the difficulties in assigning herbarium vouchers from Canada to ssp. *lyrata* or ssp. *kamchatica*", and because the taxonomic treatment of *A. kamchatica* has been a matter of debates, the individuals designated as *A. kamchatica* or *lyrata* from America or Asia in these references are categorized into one category, *A. lyrata* or *kamchatica*, America and Asia. *Arabidopsis arenosa* and *A. neglecta* are categorized into *A. arenosa*. The individuals designated as hybrid or without the information of haplotype were excluded. Several individuals most possibly overlapping in these two references (marked with the same herbarium number) were counted only once.

The network was constructed using median-joining method ($\varepsilon = 0$), implemented in the NETWORK program v. 4.5.1.0 (Bandelt *et al.*1999; freely available at www.fluxus-engineering.com). Every insertion and deletion was scored as a single mutational event regardless of its length. The *trnL-trnF* intergenic spacer region was not included in the construction of the network, because frequent and possibly parallel mutations in tandemly-duplicated copies made the alignment uncertain (Koch *et al.* 2005). Instead, we used the *trnL-trnF* intergenic spacer region to assess if cpDNA haplotype between those of *A. kamchatica* observed in this study and those of other *Arabidopsis* species are identical. In some of the individuals reported by Koch and Matschinger 2007 and Schmickl *et al.* 2008, only *trnL* intron sequence was reported and *trnL-trnF* regions was not available. Those individuals were included in *trnL* intron network (Fig. 5b), but were not used to count the haplotype including the *trnL-trnF* intergenic spacer region (Fig. 5a).

To survey the extent of polymorphism within populations, sequences of the *trnL-trnF* region were obtained from additional individuals from three selected populations: two populations of subsp. *kamchatica* (eight individuals from the population kamC14, and six individuals from the population kamC11, respectively) and one population of subsp. *kawasakiana* (five individuals from the population kamkwsB6) (Table S3). Despite the high levels of variation among populations in the *trnL-trnF* region, no polymorphism in *trnL-trnF*

was found in any local populations. This suggests that most of the polymorphisms are distributed among populations.

Bayesian clustering

To detect the population structure of *A. kamchatica* and assign individuals to populations, we used the Bayesian clustering algorithm implemented in program *structure* version 2.2 (http://pritch.bsd.uchicago.edu/structure.html) (Pritchard *et al.* 2000) (Fig. 8). The algorithm uses individual multilocus genotypic data and attempts to assign individuals to clusters under the predefined model with a certain number of clusters (K). In this study, two homeologous pairs of nuclear *WER* and *CHS* genes (*WER*-lyr, *WER*-hal, *CHS*-lyr and *CHS*-hal), and the cpDNA haplotype were included in the *structure* analysis. A high rate of selfing in *A. kamchatica* (Sugisaka & Kudoh 2008) violates the assumption of the Hardy–Weinberg equilibrium within a population in the *structure* analysis. Therefore, as recommended for complete-selfing species (Gao *et al.* 2007) and as commonly done in predominantly selfing species (e.g., Nordborg *et al.* 2005; Beck *et al.* 2008), the data were treated as haploid data. To prepare the haploid data set, the cpDNA haplotype and one haplotype from each nuclear locus were selected randomly and scored for each individual. Both substitutions and indels were used as information to distinguish haplotypes. As described in Results, the locus *WER*-lyr in three individuals from Taiwan appeared to have rearrangement or indels in the 5′-half of the gene and was treated as a distinct haplotype. The locus *CHS*-hal in three individuals was not amplified and was treated as missing data for these three individuals. The genetic distances between haplotypes were not considered in this program.

The model used in the *structure* analysis assumes no association between alleles from different loci arising from physical linkage by chromosomal proximity. To survey the association between different loci, the degree of the gametic disequilibrium D' (average of the absolute value of D_{ij}/D_{max} over all pairs of alleles from different loci weighted by the frequencies of the gametes) (Hedrick 1987) was calculated for four nuclear loci (six pairs of loci). Four of the six pairs (*WER*-lyr–*WER*-hal; *CHS*-lyr–*CHS*-hal; *WER*-lyr–*CHS*-hal and *CHS*-lyr–*WER*-hal) must reside on different chromosomes because they are the pairs from different parents. The D' value between these four pairs of loci was 0.788, 0.937, 0.806, and 0.818, respectively. These high gametic disequilibria observed between the loci from different parents were probably not the result of physical linkage but arose because of other factors such as the population structure. The other two pairs could potentially be in physical linkage (*WER*-hal–*CHS*-hal and *WER*-lyr–*CHS*-lyr), which would result in a higher D' value because *WER* and *CHS* reside on the same chromosome in the related species *A. thaliana*. However, the D' values (0.892 and 0.719 for *WER*-hal–*CHS*-hal and *WER*-lyr–*CHS*-lyr, respectively) are similar or lower than the pairs from different parents compared with the range of D' value of the pairs from different chromosomes described above (0.788-0.937). This indicates that the two pairs derived from the same parents do not have an elevated level of gametic (linkage) disequilibrium because of physical linkage and were thus treated as independent loci in the *structure* analysis.

Twenty independent runs with 100,000 iterations for the burn-in phase and 100,000 iterations for the data collection phase were conducted for different numbers of clusters ranging from $K = 1$ to 10. For all runs, admixture and correlated allele frequency models were used. Using the program *CLUMPP* (Jakobsson & Rosenberg 2007), the optimal alignments of 20 replicate clustering estimates were found for each number of clusters K. The *Greedy* algorithm (for $K = 2$ to 7) and the *LargeKGreedy* algorithm (for $K = 8$ to 10) of the program with 1,000 random input orders of 20 replicates were used. The averages of cluster membership coefficients were taken for all runs of each K with the optimal alignment, and the outputs were graphically displayed by the program *distruct*

(http://rosenberglab.bioinformatics.med.umich.edu/distruct.html) (Rosenberg 2004). To investigate the similarity of clustering estimates between different runs, the symmetric similarity coefficient (*SSC*) (Jakobsson & Rosenberg 2007) was computed for all pairs of runs with a given *K* using the program *CLUMPP*. The optimal number of clusters (*K*) was inferred based on evaluation of the Δ*K* statistic (Evanno *et al.* 2005).

Supporting Table S1. Primers used for PCR

Gene	homeolog type	Primer name	Primer sequence (5' to 3')	annealing (˚C)
WER	lyrata	WERFU3 WERRD6	TATACATAAATATTCCACTAGGTTCTG AATTGAAGAAACATTTAAAACATT	57
	lyrata (Taiwan)	WERlyrF1 WERRD6	CTATTTCAAGAGAAGAAAAAACAGC AATTGAAGAAACATTTAAAACATT	53
	lyrata & halleri	WERFU1 WERRD1	TCTCTCGTTTTATGATCTCTCTCG AGCCAATCATACACTACCACATCA	57
	halleri	WERFU1 WERgemRD1	TCTCTCGTTTTATGATCTCTCTCG GTTTGATCAGCTTTGCATGCA	53
	halleri	WERFU1 WERgemR3e2	TCTCTCGTTTTATGATCTCTCTCG TGTTTGGTTTTCTCATGATCT	53
	lyrata & halleri	WERF4 WERR3e1	TGTAGATTGAGGTGGATGAA TGAACCCAAAGTGAACTCAAGTAG	53
CHS	lyrata	CHSlyrFU1 CHSR1	TGGGAAGTGAAATCTCCTTATGGTG AGAGGAACGCTGTGCAAGAC	57
	lyrata	CHSlyrFU4 CHSR1	GGTGGAGAAACTATACAACAAAT AGAGGAACGCTGTGCAAGAC	57
	halleri	CHSgemFU1 CHSR1	GAAATCTCCGTAGTCCGTATGGTG AGAGGAACGCTGTGCAAGAC	57
	halleri	CHSgemFU15 CHSR1	CTAACAACTAGCCACGTATATCTTC AGAGGAACGCTGTGCAAGAC	* 67.5
	lyrata & halleri	CHSkamF1 CHSR3	CTAACCCTGAGAACCATGTG TATGGCACCATCAGAGTCTG	53
ITS		ITSP1A ITSP4	GGAAGGAGAAGTCGTAACAAGG TCCTCCGCTTATTGATATGC	45
trnL_trnF		trnL/FIGSF trnL/FIGSR	GGTTCAAGTCCCTCTATCCC GATTTTCAGTCCTCTGCTCTAC	45
trnL intron		trnLintrF trnLintrR	CGAAATCGGTAGACGCTACG GGGGATAGAGGGACTTGAAC	38

*Temp. used for *kamchatica*, 55 for *halleri*

Supporting Table S2. Comparative information for nuclear DNA.

	nDNA			
	WER	**CHS-all-accessions**	**CHS-lyr-clade**	**ITS**
no. individuals	72	68	55	75
no. outgroup	1	2	2	2
no. sequences	120	118	57	75
sequence length (bp)	835-925	1286-1305	1551-1574	618-619
aligned length (bp)	955	1314	1589	619
no. coded indels	14	6	8	0
no. haplotypes	24	22	14	25
variable characters (% by aligned length, incl. indel	83 (8.6%)	110 (8.3%)	117 (7.4%)	59 (9.5%)
parsimony-informative characters (%,incl. indel coding)	60 (6.2%)	44 (3.3%)	25 (1.6%)	14 (2.3%)
no. MP trees	78	4	1	74,944
tree length (steps)	96	127	127	64
CI (consistency index)	0.96	0.92	0.96	0.98
RI (retention index)	0.99	0.95	0.85	0.99
no. haplotypes of halleri-homeolog of *A. kamchatica*	8	8		
nucleotide diversity π of halleri-homeolog of *A. kamchatica*	0.0008	0.0026		
no. haplotypes of lyrata-homeolog of *A. kamchatica*	8	11		
nucleotide diversity π of lyrata-homeolog of *A. kamchatica*	0.0006	0.0012		

One haplotype was used from each individual in the calculation of nucleotide diversity π (Tajima 1983)
WER-lyrata of kamA1-3 and kamE26 accessions were removed due to large indels

Table S3 Samples for intra-populaton variation analyses (only *trnL-trnF* of cpDNA was surveyed)

Name of taxon	Area		sample name	Population	Collector	*trnL-trnF* of cpDNA
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC101	Japan, Tokushima, Mt. Shikokutsurugi	M. Kanaoka, KKS	cpHap1
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC102	Japan, Tokushima, Mt. Shikokutsurugi	M. Kanaoka, KKS	cpHap1
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC103	Japan, Tokushima, Mt. Shikokutsurugi	M. Kanaoka, KKS	cpHap1
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC104	Japan, Tokushima, Mt. Shikokutsurugi	M. Kanaoka, KKS	cpHap1
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC105	Japan, Tokushima, Mt. Shikokutsurugi	M. Kanaoka, KKS	cpHap1
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC106	Japan, Tokushima, Mt. Shikokutsurugi	M. Kanaoka, KKS	cpHap1
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC107	Japan, Tottori, Mt. Daisen	KKS	cpHap3
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC108	Japan, Tottori, Mt. Daisen	KKS	cpHap3
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC109	Japan, Tottori, Mt. Daisen	KKS	cpHap3
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC110	Japan, Tottori, Mt. Daisen	KKS	cpHap3
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC111	Japan, Tottori, Mt. Daisen	KKS	cpHap3
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC112	Japan, Tottori, Mt. Daisen	KKS	cpHap3
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC113	Japan, Tottori, Mt. Daisen	KKS	cpHap3
Arabidopsis kamchatica subsp. *kamchatica*	C	Mountains in Western Honshu and Shikoku, Japan	kamC114	Japan, Tottori, Mt. Daisen	KKS	cpHap3
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB115	Japan, Shiga, Takashima	KKS	cpHap4
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB116	Japan, Shiga, Takashima	KKS	cpHap4
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB117	Japan, Shiga, Takashima	KKS	cpHap4
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB118	Japan, Shiga, Takashima	KKS	cpHap4
Arabidopsis kamchatica subsp. *kawasakiana*	B	Lowland in Western Honshu, Japan	kamkwsB119	Japan, Shiga, Takashima	KKS	cpHap4

a.

FU3 (L)   FU1 (H, L)   lyrF1 (L)   gemR3e2 (H)   gemRD1 (H)   RD1 (H, L)   RD6 (L)

WER

ATG   F4   R3e1   TGA

lyrFU1 (L)   lyrFU4 (L)

gemFU1 (H)   gemFU15 (H)

H: used to amplify halleri type
L: used to amplify lyrata type

R1 (H, L)

CHS

ATG   kamF1   R3   TAA

200 bp

b. WER fragment (F4-R3e1)

C13 C14 E26 G31 B4 hal-gem4 lyr-pet4

bp
1000
830
650

halleri type
lyrata type

c. CHS fragment (kamF1-R3) digested by *Xba*I

C11 C12 D23 G31 B4 hal-gem4 lyr-pet4

bp
930

420

lyrata type (not digested)

halleri type (digested)

Supporting Fig. S1 Primers and homeolog numbers

Haplotype name	Frequency	Segregating sites
kamA1L–A3L,kamkwsB10L	4	C T G C C A C T A T G C C C G – A C C G C T T C T T C T T C C C C A G A C C G C T C C T C A G C G T G
kamkwsB4L–B9L	6	. C . . . . – . . . .
kamCL,DL *	12	. . A . . . . T . . . .
kamC12La2	1	. . A . . . . C . . . .
kamD16L	1	. . A . . . . T . . . . G . . . .
kamD17La1	1	. . A T . . . . T . . . .
kamD17La2	1	. . A T . . . . T . . . . – . . . .
kamD18L	1	T . A . . . . T . T . . . .
kamEL,FL,GL,HL,IL **	23	. . . . T . . . .
kamG31L	1	. . . . T . . . . G . . . .
kamH46L,lyrpet4,lyrpet5	3	. . A . . . . . C . . . . . . T . . . .
lyrpet2	1	. . A . . . . T . . . . T . T A . T . T T A T . C A
lyrpet3	1	. . A . C . . . . A T . T A T A C C T C . . C G T . . . T T . . . T . T A . T . T T A T . C A
lyrlyr1a1	1	N N N N T . . . . A T . T A T A C C T C . . C G T . . T T . . . . T . T A . T . T T A T . C A
lyrlyr1a2,lyrlyr2	2	N N N N T . . . . A T . T A T A C C T C . . C G T . A T T . . . T . T A . T . T T A T . C A
kamA1H–A3H,E26H,kamkwsB6H–B10H	9	N N N N . . T . . G . A . . A T T T A T A C C T C . . . . . . T C C G . T . . . . . A . . . . . T . .
kamkwsB4H,B5H	2	N N N N . . T . . G . A . . A T T T A T A C C T C . A . . . . T C C G . T . . . . . A . . . . . T . .
kamC13H,C14H,D16H	3	N N N N . . . . . G . A G . A T . T A T A C C T C . . . . . . T C C G . T . . T . A . . . . . T . .
kamD15H,D17H,D24Ha2,haltat1a1	4	N N N N . . . . . G . A . . A T . T A T A C C T C . . . . . . T C C G . T . . T . A . . . . . T . .
kamD18H	1	N N N N . . . . . G . A . . A T . T A T A C C T C . . . . . . T C C G . T . . T . A . . . . . T . .
kamD19H,D20H	2	N N N N . . . . . G G C A . . A T . T A T A C C T C . . . . . T C C G . T . . T . A . . . . . T . .
kamD21H,D22H,D24Ha1,D25H	4	N N N N . . . . . G C A . . A T . T A T A C C T C . . . . . . T C C G . T . . T . A . . . . . T . .
kamFH,GH,HH,IH*,halgem1–8	32	N N N N . . . . . G . A . . A T . T A T A C C T C . . . . . . T C C G . T . . . . A . . . . . T . .
haltat1a2	1	N N N N . . . . . G . A . . A T T T A T A C C T C . . . . . . T C C G . T . . T . A . . . . . T . .
haltat2	1	N N N N . . . . . G . A . . A T . T A T A C C T C . . . . . . T C C G . A . . T . A . . . . . T . .
halovi1	1	N N N N . . . . . G . A . . A T T T A T A C C T C . . . . . . T C C G . A . . T . A . . . . . T . .

* kamC11L, C12La1, C13L, C14L, D15L, D19L–D25L

** kamE26L, F27L–F30L, G32L–G41L, H42L–H45L, H47L, I48L–I50L

Supporting Fig. S2a Segregating sites of *CHS*

Haplotype name	Frequency	Segregating sites
kamA,C,D,F,G,H,I*,kamkwsB4–B10	41	C C G C C T T C G C G C A G G A G G T G T A A C C C T T G G T G C T A C C G
kamA3a2	1	. . . . – . . . .
kamD15	1	G . . . . K . . . . R
kamD17	1	. . . T T C . . A T A . T T A G . A . A . T . . T T C C . . . . . C G . . .
kamD18	1	. . . . G . . . .
kamD24	1	. . . . K . . . .
kamE26	1	. . . . W . . . . . . Y . . . .
kamG32	1	. . . . R . . . . . . R . . . .
kamH43,H47,I48	3	. . . . Y . R S . .
lyrpet1	1	. . . . R . . . . W K . . . .
lyrpet2,3	2	. . . . A T . . . .
lyrlyr1,2,lyrpet4,5	4	. . . . T . G G . .
haldac1	1	. . . . . . C C . . . A . T . A G . A . . . . C C . . . . . C G . . .
halgem1–4, 8	5	. . . . . . C . . . T A . T . A G . A . A . T . . . . C C A . . . . C G . . .
halgem5	1	. . . . . . C . . . T A . T . A G . A . A . T . . . . Y C A . . . . C G . .
halgem6	1	. . . Y . C . . R T A . T . A G . A . A . T . . Y . Y C R K . . . C G . Y .
halgem7	1	. . . . . . C . . . T A . T . A G . A . A . T . . Y . Y C R K . . . C G . Y .
halhal1	1	. Y . . . C . . . T A . T . A G . A . A . . . . . . C C R . . . . C G . . .
halhal2	1	. Y R . . C . Y R T A Y T . A G . A . A . W . . . . C C R . . . . C G . . .
haltat1	1	. . . . . C . . . T A . T . A G . A . A . . . . . . C C R . . . . C G . .
haltat2	1	. . . . . C . . . T A . T . A G . A . A . . . . . . C C A . W . . C G . .
haltat3	1	. . . . . C . . . T A . T . A G . A . A . . . . . . Y C A . . S . C G . . .
halovi1	1	. T . . . C . . . T A . T . A G . A . A . . . . . . C C A . . . . C G . . .

* kamA1, A2, A3a1, C11–C14, D16, D19–D23, D25, F27–F30, G31, G33–G41, H42, H44–H46, I49, I50, kamkwsB4–B10

Supporting Fig. S2b Segregating sites of ITS

trnL intron

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                    10        20        30        40        50        60        70
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1   TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_2   TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_3   TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_4   TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_5   TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_6   TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_7   TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_8   TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_9   TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_10  TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_11  TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_12  TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_13  TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_14  TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_15  TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_16  TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_17  TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG
cpHap_18  TACTAAGTGATAACTTTCAAATTCAGAGAAACCCTGGAATTAACAATGGGCAATCCTGAGCCAAATCCTG


                    80        90       100       110       120       130       140
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1   GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_2   GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_3   GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_4   GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_5   GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_6   GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_7   GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_8   GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_9   GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_10  GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_11  GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_12  GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_13  GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_14  GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_15  GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_16  GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_17  GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG
cpHap_18  GTTTACGCGAACAAACCGGAGTTTACAAAGCGCGAAAAAAGGGATAGGTGCAGAGACTCAATGGAAGCTG


                   150       160       170       180       190       200       210
          ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1   TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_2   TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_3   TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_4   TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_5   TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_6   TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_7   TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_8   TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_9   TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_10  TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_11  TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_12  TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_13  TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_14  TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_15  TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_16  TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_17  TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
cpHap_18  TTCTAACAAATGGAGTTCACTACCTTGTGTTGATAAAGGAATCCTTCGATCGAAACTTCAAATCAAAAAG
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                    220       230       240       250       260       270       280
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1  GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_2  GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_3  GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_4  GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_5  GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_6  GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_7  GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_8  GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_9  GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_10 GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_11 GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_12 GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_13 GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_14 GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_15 GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_16 GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACGAAAACGATCTCAAAAATGACGACCTTAA
cpHap_17 GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA
cpHap_18 GATGAAGGAGAAAAACCTATATTGTATAAATTTAGGTAACACAAAACGATCTCAAAAATGACGACCTTAA

                    290       300       310       320       330       340       350
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1  TCTCGATTTCTATTTTTTTT*ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_2  TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_3  TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_4  TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_5  TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_6  TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_7  TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_8  TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_9  TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_10 TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_11 TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_12 TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_13 TCTCGATTTCTATTTTTTT--ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_14 TCTCGATTTCTATTTTTTT--ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_15 TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_16 TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_17 TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA
cpHap_18 TCTCGATTTCTATTTTTTTT-ATAAACAAAATCGAAATGTTGTGAATCAATTCGAAGTTTAAGAAATAATA

                    360       370       380       390       400       410       420
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1  TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_2  TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_3  TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_4  TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_5  TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_6  TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_7  TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_8  TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGAACTTAATTAATCGGACGAGAAT
cpHap_9  TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_10 TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_11 TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGAACTTAATTAATCGGACGAGAAT
cpHap_12 TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_13 TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_14 TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_15 TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_16 TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_17 TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
cpHap_18 TTTATTGATCAAATGATTCACTTCATAGTCTGATAGATCCTTGATGGA-----ATTAATCGGACGAGAAT
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                              430        440        450        460        470        480        490
                         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1    AAAGATAGAGTCCCATTTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_2    AAAGATAGAGTCCCATCTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGAAGAAAATCCGTTG
cpHap_3    AAAGATAGAGTCCCATCTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGAAGAAAATCCGTTG
cpHap_4    AAAGATAGAGTCCCATCTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGAAGAAAATCCGTTG
cpHap_5    AAAGATAGAGTCCCATCTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGAAGAAAATCCGTTG
cpHap_6    AAAGATAGAGTCCCATCTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_7    AAAGATAGAGTCCCATTTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_8    AAAGATAGAGTCCCATTTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_9    AAAGATAGAGTCCCATTTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_10   AAAGATAGAGTCCCATTTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_11   AAAGATAGAGTCCCATTTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_12   AAAGATAGAGTCCCATCTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGAAGAAAATCCGTTG
cpHap_13   AAAGATAGAGTCCCATTTTACATGTCAATGCTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_14   AAAGATAGAGTCCCATTTTACATGTCAATGCTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_15   AAAGATAGAGTCCCATCTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_16   AAAGATAGAGTCCCATTTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_17   AAAGATAGAGTCCCATTTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG
cpHap_18   AAAGATAGAGTCCCATTTTACATGTCAATACTGACAACAATGAAATTTATAGTAAGATGAAAATCCGTTG

                         ....
cpHap_1    ACTT
cpHap_2    ACTT
cpHap_3    ACTT
cpHap_4    ACTT
cpHap_5    ACTT
cpHap_6    ACTT
cpHap_7    ACTT
cpHap_8    ACTT
cpHap_9    ACTT
cpHap_10   ACTT
cpHap_11   ACTT
cpHap_12   ACTT
cpHap_13   ACTT
cpHap_14   ACTT
cpHap_15   ACTT
cpHap_16   ACTT
cpHap_17   ACTT
cpHap_18   ACTT
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trnL-trnF intergenic region

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                              10        20        30        40        50        60        70
                         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1    TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_2    TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_3    TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_4    TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_5    TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_6    TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_7    TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_8    TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_9    TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_10   TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_11   TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTACTTAGAA
cpHap_12   TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_13   TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_14   TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_15   TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_16   TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_17   TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
cpHap_18   TTTTTTTCGTTATTATTTATTTGAATTATTTAGAATCTATATCATTTTTCATTTTCAAACTTA----GAA
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                  80        90       100       110       120       130       140
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1  AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_2  AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_3  AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_4  AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_5  AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_6  AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_7  AGTCTTCTTTTATTTATAAAATCCAAGAAATGCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_8  AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_9  AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_10 AGTTTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_11 AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_12 AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_13 AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_14 AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_15 AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_16 AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_17 AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT
cpHap_18 AGTCTTCTTTTATTTATAAAATCCAAGAAATTCCCGGTCCACAACTTTTTGAATTTACTACTTTTGAGTT

                 150       160       170       180       190       200       210
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1  TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_2  TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_3  TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_4  TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_5  TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_6  TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_7  TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_8  TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_9  TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_10 TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_11 TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_12 TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_13 TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_14 TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_15 TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_16 TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_17 TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT
cpHap_18 TCTTTTCATTGACATAGACCTAAGTCATATATTAAAATGATACTGATACTTCAGTAGATGATACTTCGGT

                 220       230       240       250       260       270       280
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1  AATGGTAGACATAGCTTAATTGCGGGGGACTGAAAATCCTTGTGTCACCATTCGGAAAAGCAAGATGATA
cpHap_2  AATGGTAGACATAGCTTAATTGCGGGGGACTTAAAATCC----------------------------
cpHap_3  AATGGTAGACATAGCTTAATTGCGGGGGACTTAAAATCC----------------------------
cpHap_4  AATGGTAGACATAGCTTAATTGCGGGGGACT-----------------------------------
cpHap_5  AATGGTAGACATAGCTTAATTGCGGGGGACTTAAAATCC----------------------------
cpHap_6  AATGGTAGACATAGCTTAATTGCGGGGGACTGAAAATCC----------------------------
cpHap_7  AATGGTAGACATAGCTTAATTGCGGGGGACTGAAAATCCTTGTGTCACCATTCGGAAAAGCAAGATGATA
cpHap_8  AATGGTAGACATAGCTTAATTGCGGGGGACTGAAAATCC----------------------------
cpHap_9  AATGGTAGACATAGCTTAATTGCGGGGGACTGAAAATCCTTGTGTCACCATT------------------
cpHap_10 AATGGTAGACATAGCTTAATTGCGGGGGACTTAAAATCCTTGTGTCACCATTCGGAAAAGCAAGATGATA
cpHap_11 AATGGTAGACATAGCTTAATTGCGGGGGACTGAAAATCC----------------------------
cpHap_12 AATGGTAGACATAGCTTAATTGCGGGGGACTTAAAATCCTTGTGTCACCATTCGGAAAAGCAAGATGATA
cpHap_13 AATGGTAGACATAGCTTAATTGCGGGGGACTGAAAATCCTTGTGTCACCATTCGGAAAAGCAAGATGATA
cpHap_14 AATGGTAGACATAGCTTAATTGCGGGGGACTGAAAATCCTTGTGTCACCATTCGGAAAAGCAAGATGATA
cpHap_15 AATGGTAGACATAGCTTAATTGCGGGGGACTGAAAATCCTTGTGTCACCATTCGGAAAAGCAAGATGATA
cpHap_16 AATGGTAGACATAGCTTAATTGCGGGGGACTTAAAATCCTTGTGTCACCATTCGGAAAAGCAAGATGATA
cpHap_17 AATGGTAGACATAGCTTAATTGCGGGGGACTTAAAATCCTTGTGTCACCATTCGGAAAAGCAAGATGATA
cpHap_18 AATGGTAGACATAGCTTAATTGCGGGGGACTTAAAATCCTTGTGTCACCATTCGGAAAAGCAAGATGATA
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                    290       300       310       320       330       340       350
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1    CTTCGGTAATGGTCGGCATAGCTTTTTTTGCGGGGGGCTTAAAATCCTTGTGTCACCATTAGGAAATAGGA
cpHap_2    ----------------------------------------------------------------------
cpHap_3    ----------------------------------------------------------------------
cpHap_4    ----------------------------------------------------------------------
cpHap_5    ----------------------------------------------------------------------
cpHap_6    ----------------------------------------------------------------------
cpHap_7    CTTCGGTAATGGTCGGCATAGCTTTTTTTGCGGGGGGCTTAAAATCCTTGTGTCACCATTAGGAAATAGGA
cpHap_8    ----------------------------------------------------------------------
cpHap_9    -------------------------------------------------------AGGAAATAGGA
cpHap_10   CTTCGGTAATGGTCGGCATAGCTTTTTTTGCGGGGGGACTTAAAATCCTTGTGTCACCATTAGGAAATAGGA
cpHap_11   ----------------------------------------------------------------------
cpHap_12   CTTCGGTAATGGTCGGCATAGCTTTTTTTGCGGGGGGACTGAAAATCCTTGTGTCACCATTAGGAAATAGGA
cpHap_13   CTTCGGTAATGGTCGGCATAGCTTTTTTTGCGGGGGGACTGAAAATCCTTGTGTCACCATTAGGAAATAGGA
cpHap_14   CTTCGGTAATGGTCGGCATAGCTTTTTTTGCGGGGGGACTGAAAATCCTTGTGTCACCATTAGGAAATAGGA
cpHap_15   CTTCGGTAATGGTCGGCATAGCTTTTTTTGCGGGGGGACTTAAAATCCTTGTGTCACCATTAGGAAATAGGA
cpHap_16   CTTCGGTAATGGTCGGCATAGCTTTTTTTGCGGGGGGACTGAAAATCCTTGTGTCACCATTAGGAAATAGGA
cpHap_17   CTTCGGTAATGGTCGGCATAGCTTTTTTTGCGGGGGGACTGAAAATCCTTGTGTCACCATTAGGAAATAGGA
cpHap_18   CTTCGGTAATGGTCGGCATAGCTTTTTTTGCGGGGGGACTGAAAATCCTTGTGTCACCATTAGGAAATAGGA

                    360       370       380       390       400       410       420
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|...|
cpHap_1    AAAGCAAGATGATACTTCAGTAGATGATACCTCAGTAATGGTGGACATAGCTTTTTTTGCGGGGGACT---
cpHap_2    ----------------------------------------------------------------------
cpHap_3    ----------------------------------------------------------------------
cpHap_4    ----------------------------------------------------------------------
cpHap_5    ----------------------------------------------------------------------
cpHap_6    ----------------------------------------------------------------------
cpHap_7    AAAGCAAGATGATACTTCAGTAGATGATACCTCAGTAATGGTGGACATAGCTTTTTTTGCGGGGGACT---
cpHap_8    ----------------------------------------------------------------------
cpHap_9    AAAGCAAGACGATACTTCAGTAGATGATACCTCAGTAATGGTGGACATAGCTTTTTTTGCGGGGGACT---
cpHap_10   AAAGCAAGACGATACTTCAGTAGATGATACCTCAGTAATGGTGGACATAGCTTTTTTTGCGGGGGACT---
cpHap_11   ----------------------------------------------------------------------
cpHap_12   AAAGCAAGACGATACTTCAGTAGATGATACCTCAGTAATGGTGGACATAGCTTTTTTTGCGGGGGACT---
cpHap_13   AAAGCAAGATGATACTTCAGTAGATGATACCTCAGTAATGGTGGACATAGCTTTTTTTGCGGGGGACT---
cpHap_14   AAAGCAAGATGATACTTCAGTAGATGATACCTCAGTAATGGTGGACATAGCTTTTTTTGCGGGGGACT---
cpHap_15   AAAGCAAGATGATACTTCAGTAGATGATACCTCAGTAATGGTGGACATAGCTTTTTTTGCGGGGGACTGAA
cpHap_16   AAAGCAAGATGATACTTCAGTAGATGATACCTCAGTAATGGTGGACATAGCTTTTTTTGCGGGGGACT---
cpHap_17   AAAGCAAGATGATACTTCAGTAGATGATACCTCAGTAATGGTGGACATAGCTTTTTTTGCGGGGGACT---
cpHap_18   AAAGCAAGATGATACTTCAGTAGATGATACCTCAGTAATGGTGGACATAGCTTTTTTTGCGGGGGACT---

                    430       440       450       460       470       480       490
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1    ----------------------------------------------------------------------
cpHap_2    ----------------------------------------------------------------------
cpHap_3    ----------------------------------------------------------------------
cpHap_4    ----------------------------------------------------------------------
cpHap_5    ----------------------------------------------------------------------
cpHap_6    ----------------------------------------------------------------------
cpHap_7    ----------------------------------------------------------------------
cpHap_8    ----------------------------------------------------------------------
cpHap_9    ----------------------------------------------------------------------
cpHap_10   ----------------------------------------------------------------------
cpHap_11   ----------------------------------------------------------------------
cpHap_12   ----------------------------------------------------------------------
cpHap_13   ----------------------------------------------------------------------
cpHap_14   ----------------------------------------------------------------------
cpHap_15   AATCCTTGTGTCACCATTAGGAAATAGGAAAAGCAAGACGATACTTCAGTAGATGATACCTCAGTAATGG
cpHap_16   ----------------------------------------------------------------------
cpHap_17   ----------------------------------------------------------------------
cpHap_18   ----------------------------------------------------------------------
```

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                      500       510       520       530       540       550       560
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1         ------------------------------------------------------------------------
cpHap_2         ------------------------------------------------------------------------
cpHap_3         ------------------------------------------------------------------------
cpHap_4         ------------------------------------------------------------------------
cpHap_5         --------------------------------------CTGTGTCACATCATACCGATCCTTCAGTAATGGTAG
cpHap_6         ------------------------------------------------------------------------
cpHap_7         ------------------------------------------------------------------------
cpHap_8         ------------------------------------------------------------------------
cpHap_9         ------------------------------------------------------------------------
cpHap_10        ------------------------------------------------------------------------
cpHap_11        ------------------------------------------------------------------------
cpHap_12        -------------------------TAAAATCCCTGTGTCACATCATACCGATCCTTCAGTAATGGTAG
cpHap_13        -------------------------GAAAATCCCTGTGTCACATCATACCGATCCTTCAGTAATGGTAG
cpHap_14        -------------------------GAAAATCCCTGTGTCACATCATACCGATCCTTCAGTAATGGTAG
cpHap_15        TGGACATAGCTTTTTTTGCGGGGGGACTGAAAATCCCTGTGTCACATCATACCGATCCTTCAGTAATGGTAG
cpHap_16        -------------------------TAAAATCCCTGTGTCACATCATACCGATCCTTCAGTAATGGTAG
cpHap_17        ------------------------------------------------------------------------
cpHap_18        -------------------------TAAAATCCCTGTGTCACATCATACCGATCCTTCAGTAATGGTAG

                      570       580       590       600       610       620       630
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1         ------------------------------------------------------------------------
cpHap_2         ------------------------------------------------------------------------
cpHap_3         -------------------------------CTGTGTCACATCATACCGATCCTTCAGTAATGGTAGACA
cpHap_4         ------------------------------------------------------------------------
cpHap_5         ACATAGCTTAATTGCGGGGGGACTTAAAATCCCTGTGTCACATCATACCGATCCTTCAGTAATGGTAGACA
cpHap_6         ------------------------------------------------------------------------
cpHap_7         ------------------------------------------------------------------------
cpHap_8         ------------------------------------------------------------------------
cpHap_9         ----------------------GAAAATCCCTGTGTCACATCATACCAATCCTTCAGTAATGGTAGACA
cpHap_10        ------------------------------------------------------------------------
cpHap_11        ------------------------------------------------------------------------
cpHap_12        ACATAGCTTAATTGCGGGGGGACTTAAAATCCCTGTGTCATATG-------------------------
cpHap_13        ACATAGCTTAATTGCGGGGGGACTTAAAATCCCTGTGTCATATGATAATGATCCTTCAGTAATGGTAGACA
cpHap_14        ACATAGCTTAATTGCGGGGGGACTTAAAATCCCTGTGTCATATG-------------------------
cpHap_15        ACATAGCTTAATTGCGGGGGGACTGAAAATCCCTGTGTCATATG-------------------------
cpHap_16        ACATAGCTTAATTGCGGGGGGACTTAAAATCCCTGTGTCATATG-------------------------
cpHap_17        -------------------------TAAAATCCCTGTGTCATATG-------------------------
cpHap_18        ACATAGCTTAATTGCGGGGGGACTTAAAATCCCTGTGTCATATG-------------------------

                      640       650       660       670       680       690       700
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
cpHap_1         ------------------------------------------------------------------------
cpHap_2         -------------------------CTGTGTCATATGATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_3         TAGCTTAATTGCGGGGGGACTTAAAATCCCTGTGTCATATGATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_4         ------------------------------------------------------------------------
cpHap_5         TAGCTTAATTGCGGGGGGACTTAAAATCCCTGTGTCATATGATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_6         -------------------------CTGTGTCATATGATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_7         ------------------------------------------------------------------------
cpHap_8         -------------------------CTGTGTCATATGATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_9         TAGCTTTTTTTGCGGGGGGACTGAAAATCCCTGTGTCATATGATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_10        ------------------------------------------------------------------------
cpHap_11        -------------------------CTGTGTCATATGATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_12        -----------------------------------ATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_13        TAGCTTAATTGCGGGGGGACTTAAAATCCCTGTGTCATATGATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_14        -----------------------------------ATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_15        -----------------------------------ATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_16        -----------------------------------ATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_17        -----------------------------------ATAATGATCCTTCAGTAAAGGTAGACATAG
cpHap_18        -----------------------------------ATAATGATCCTTCAGTAAAGGTAGACATAG
```

Fig. S2c. cpHap

4: kamA1L-A3L ●✳
kamkwsB10L

6: kamkwsB4L-B9L ✳

23: kamEL,FL,GL,HL,IL** ●

1: kamG31L ●

12: kamCL,DL* ●

1: kamC12La2 ○

1: kamD16L ●

1: kamD17La1 ●

1: kamD17La2 ●

1: kamD18L ●
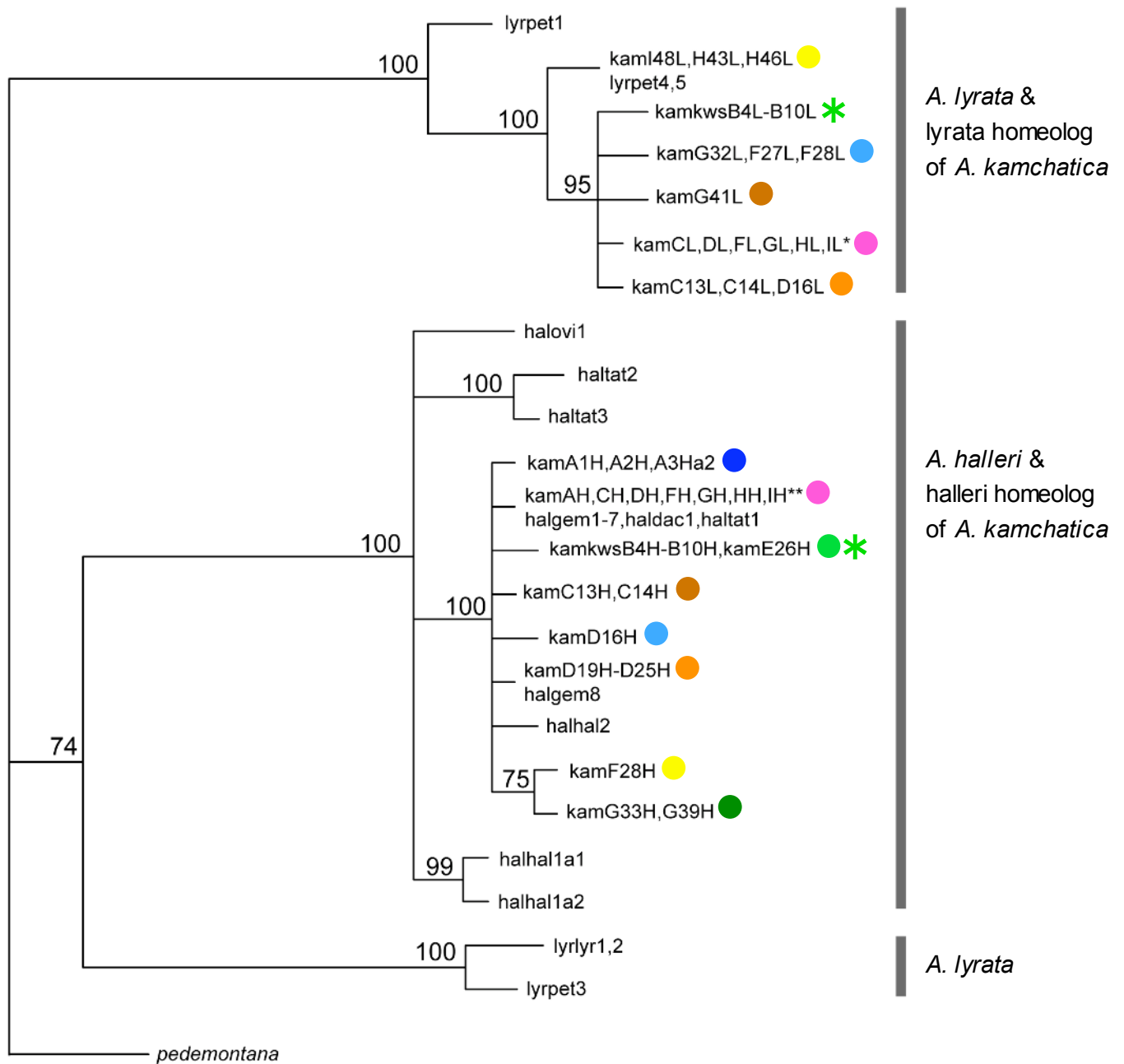
3: kamH46L ●
lyrpet4, lyrpet5

1: lyrpet3

pedemontana

thaliana

*kamC11L, C12La1, C13L, C14L, D15L, D19L-D25L
**kamE26L, F27L-F30L, G32L-G41L, H42L-H45L, H47L, I48L-I50L

Supporting Fig. S3a *CHS* most-parsimonious tree,
lyrata clade

*kamC11L, C12L, D15L, D17L-D25L, F29L, F30L, G31L, G33L-G40L, H42L, H44L, H45L, H47L, I49L, I50L

**kamA3Ha1, C11H, C12H, D15H, D17H, D18H, F27H, F29H, F30H, G31H, G32H, G34H-G38H, G40H, G41H, H42H-47H, I48H-I50H

Supporting Fig. S3b *WER* Bayesian tree

kamA1L-A3L
kamkwsB4L-B10L

kamCL,DL,EL,FL,GL,HL,IL*

kamC12La2

kamD16L

100

kamD17La2

kamD18L

kamG31L

kamH46L
lyrpet4, lyrpet5

lyrata-homeolog
of *A. kamchatica*

kamA1H-A3H,E26H
kamkwsB6H-B10H

100

kamkwsB4H,B5H

kamC13H,C14H,D16H

kamD15H,D17H,D24Ha2
haltat1a1

100

86

kamD18H

A. halleri &
halleri-homeolog
of *A. kamchatica*

100

kamD19H,D20H

99

kamD21H,D22H,D24Ha1,D25H

haltat2

85

kamFH,GH,HH,IH**
halgem1-8

83

lyrlyr1a1

100

lyrlyr2,lyrlyr1a2

A. lyrata

lyrpet3

pedemontana

thaliana

0.0070

*kamC11L, C12La1, C13L, C14L, D15L, D17La1, D19L-D25L, E26L, F27L-F30L, G32L-G41L,
 H42L-H45L, H47L, I48L-I50L

**kamF27H-F30H, G31H-G41, H42H-H47H, I48H-I50H

Supporting Fig. S3c *CHS* Bayesian tree

kamA,C,D,F,G,H,I*
kamkwsB4-B10
kamA3a2
kamD15
kamD24
kamE26
kamG32
kamH43,H47,I48
lyrpet1
lyrpet2,3
kamD18
lyrlyr1,2,lyrpet4-5

A. lyrata &
A. kamchatica

kamD17
halgem1-4,8
halgem5
halgem6
halgem7
haltat1
haltat2
haltat3
halovi1
halhal1
halhal2
haldac1

A. halleri &
A. kamchatica D17

pedemontana

thaliana

0.008
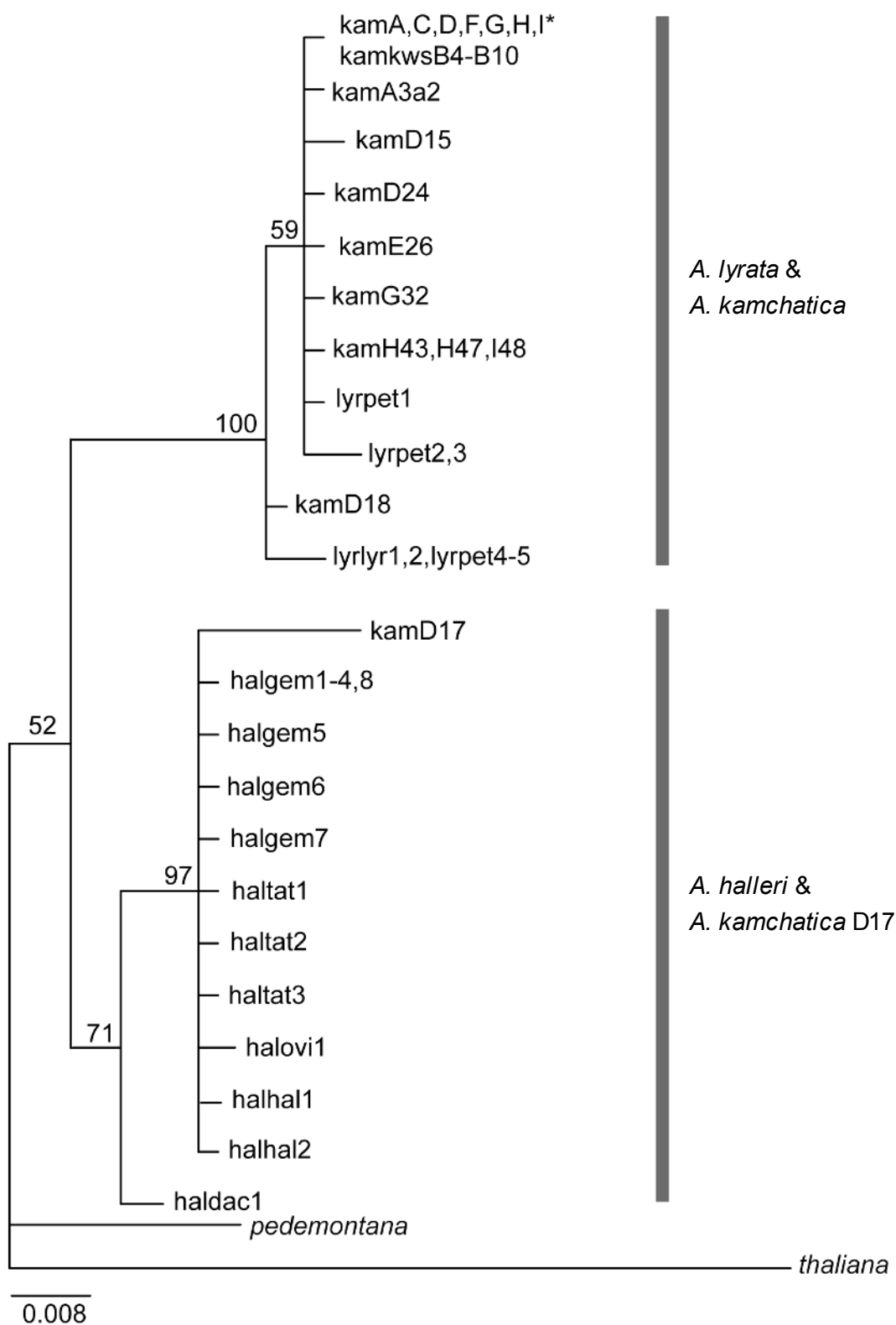
*kamA1, A2, A3a1, C11-C14, D16, D19-D23, D25, F27-F30, G31, G33-G41, H42, H44-H46, I49, I50
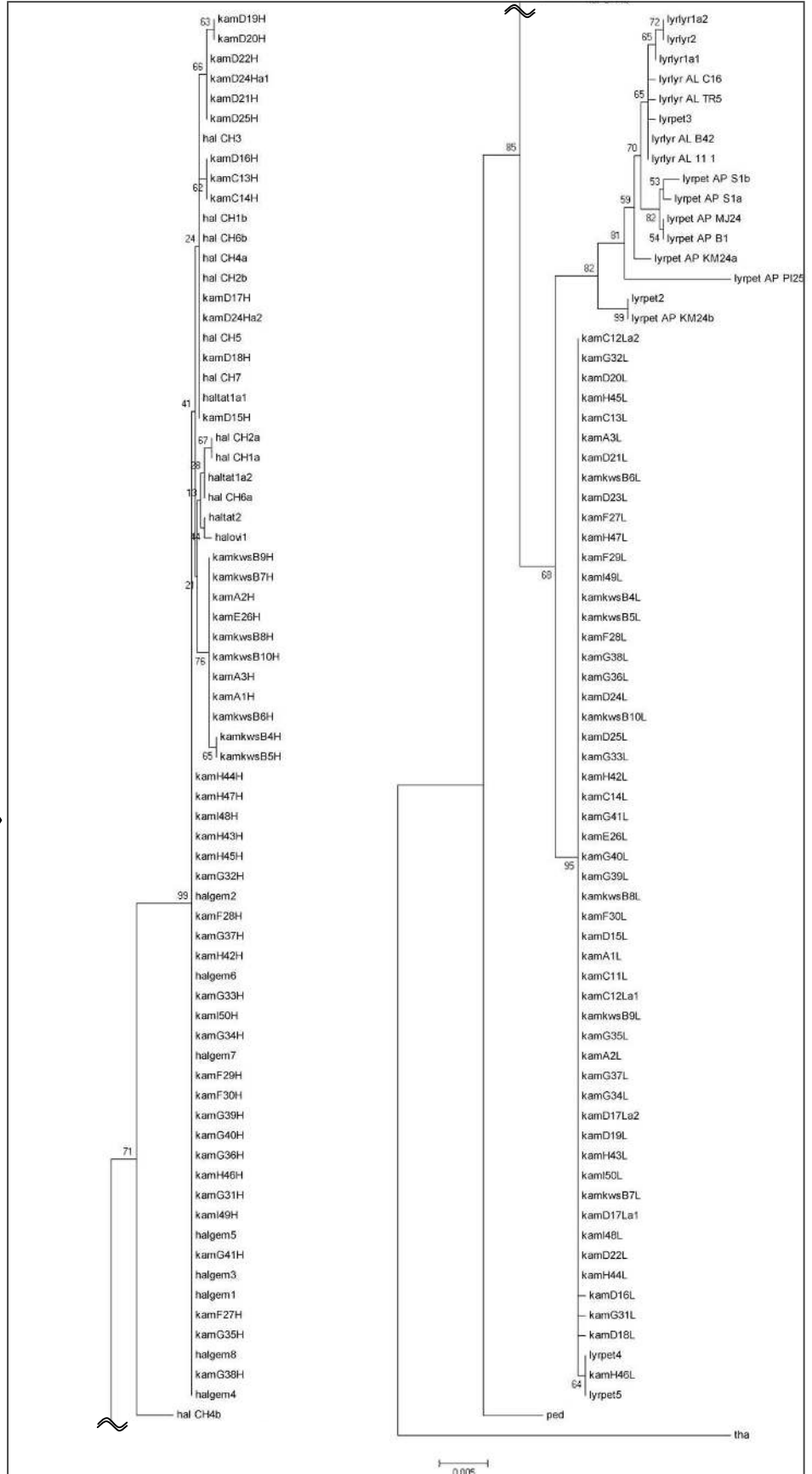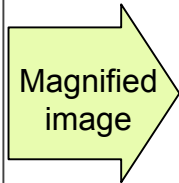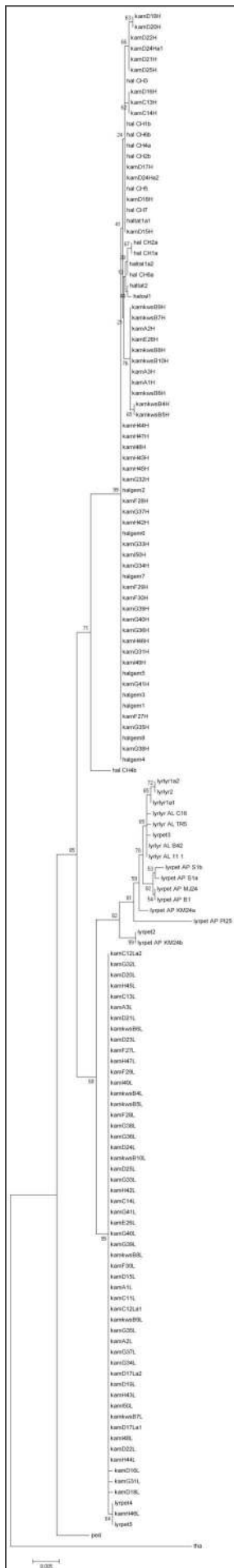
Supporting Fig. S3d ITS Bayesian tree
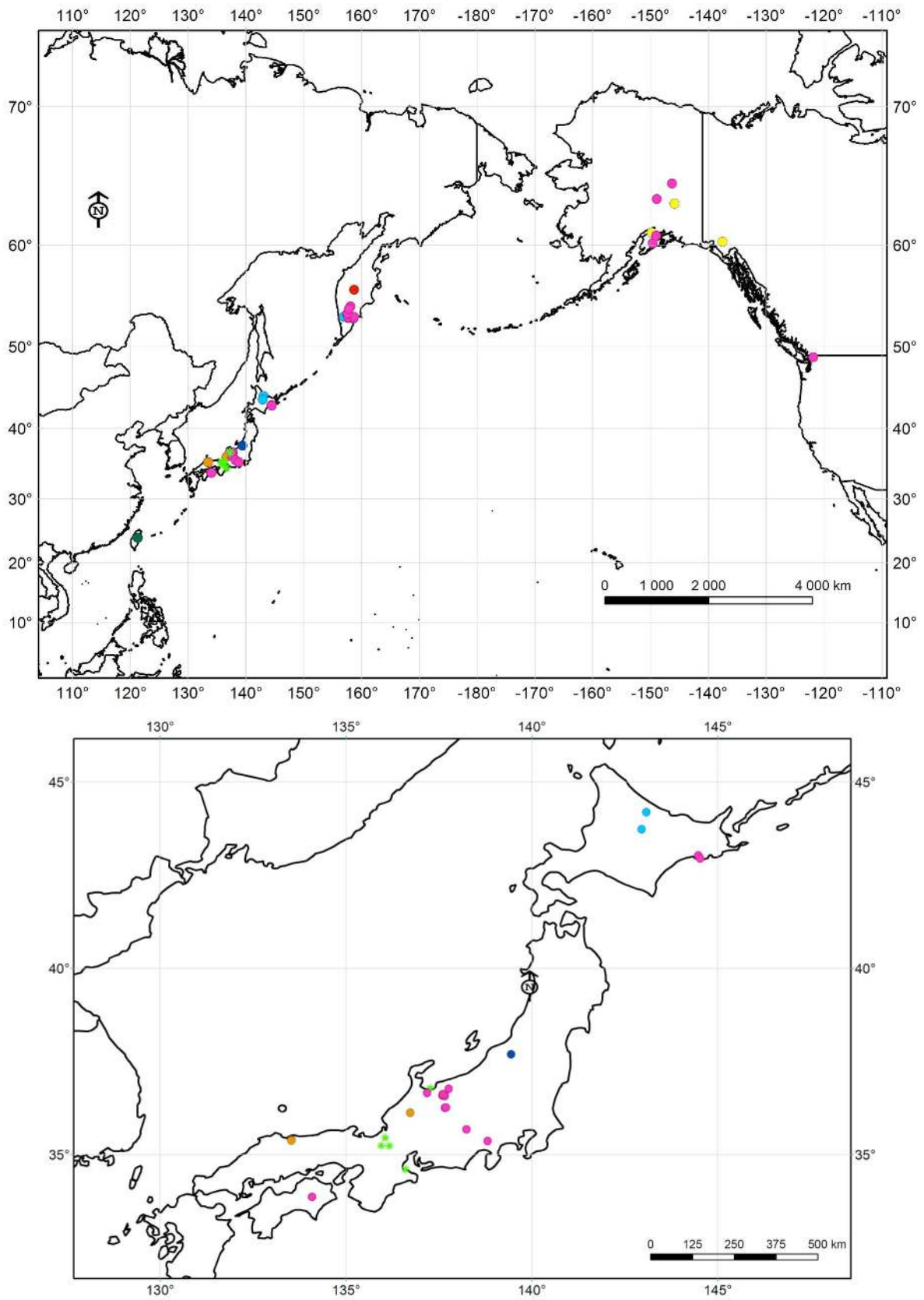
Supporting Fig. S4a *WER lyrata* type
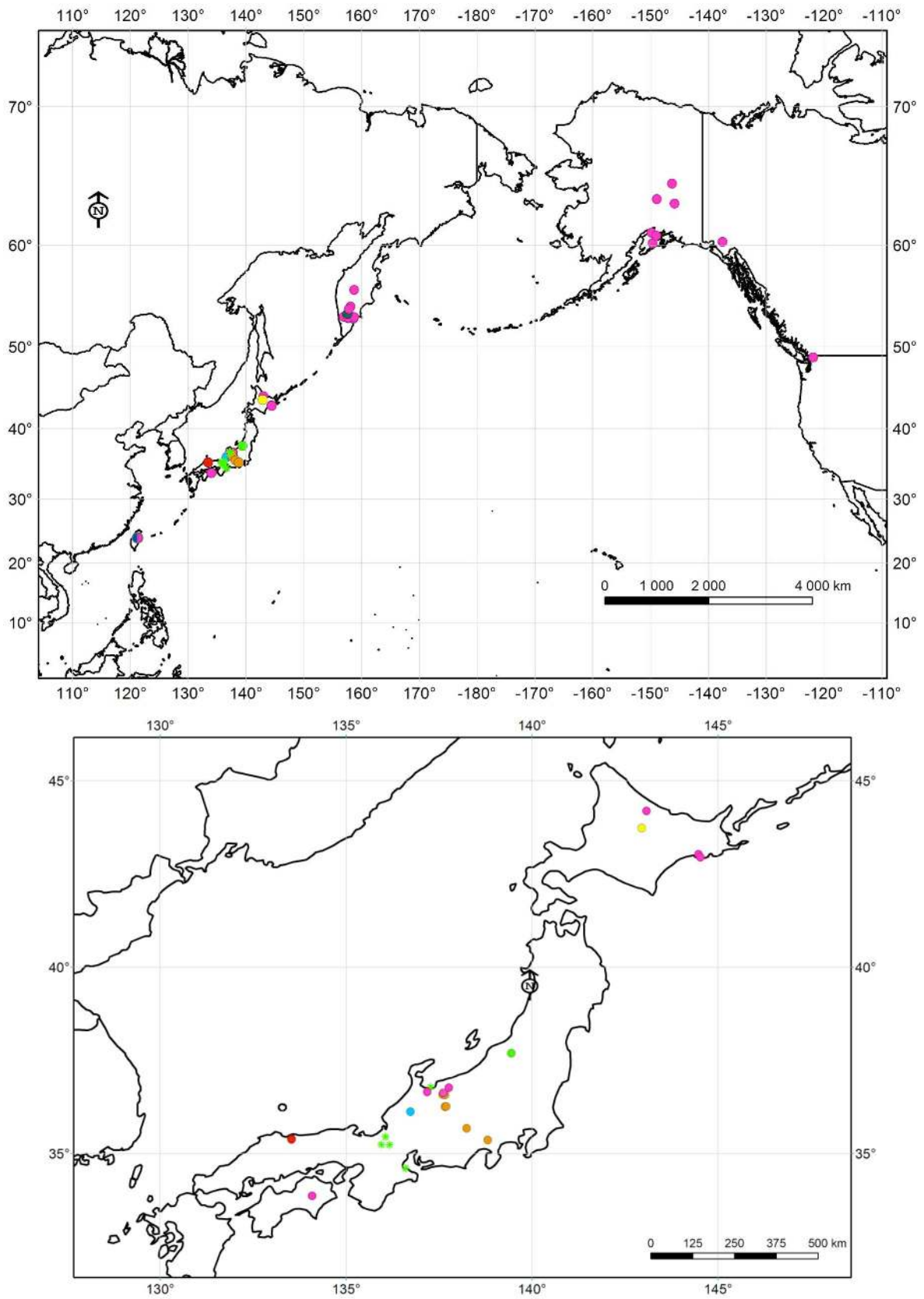
Supporting Fig. S4b *WER halleri* type
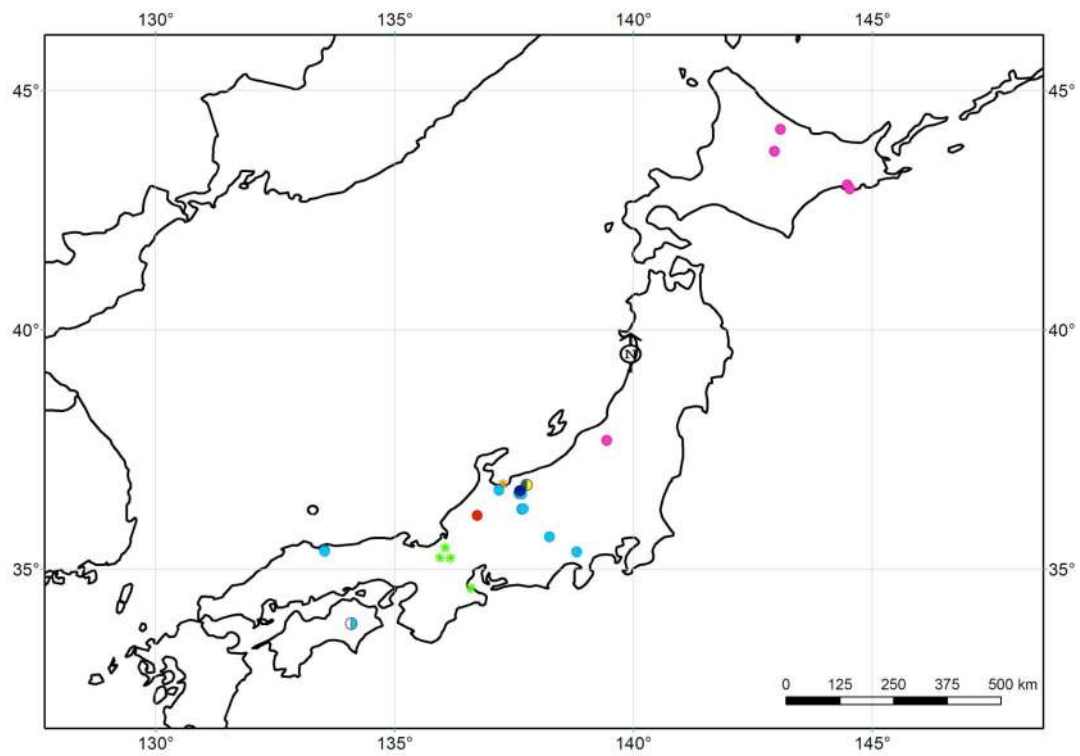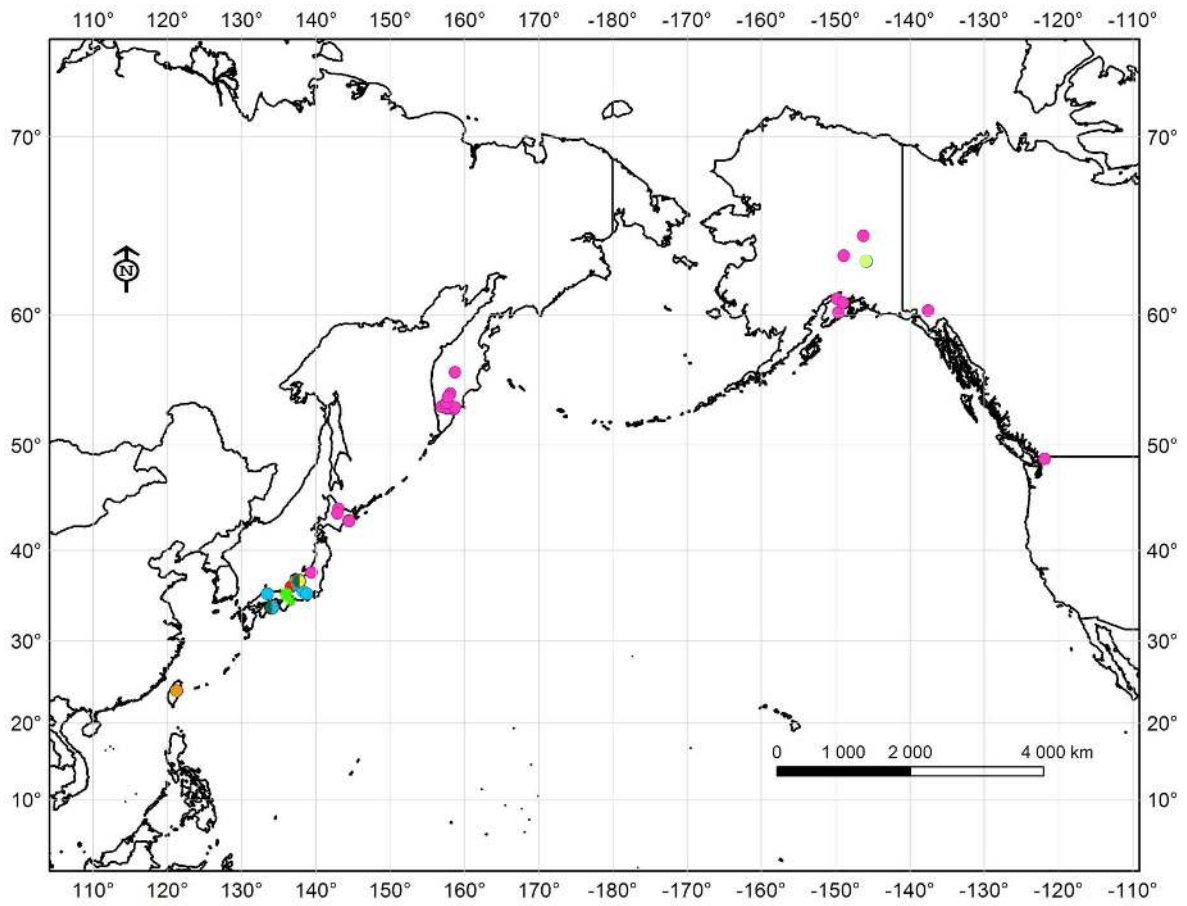
Supporting Fig. S4c *CHS lyrata* type

Supporting Fig. S5
Neighbor-joining tree of *CHS*
including publicly available data