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Genotyping-by-sequencing enables linkage mapping in three octoploid cultivated strawberry families

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With the goal of evaluating genotyping-by-sequencing (GBS) in a species with a complex octoploid genome, GBS was used to survey genome-wide single-nucleotide polymorphisms (SNPs) in three biparental strawberry (*Fragaria ×ananassa*) populations. GBS sequence data were aligned to the *F. vesca* '*Fvb*' reference genome in order to call SNPs. Numbers of polymorphic SNPs per population ranged from 1,163 to 3,190. Linkage maps consisting of 30-65 linkage groups were produced from the SNP sets derived from each parent. The linkage groups covered 99% of the *Fvb* reference genome, with three to seven linkage groups from a given parent aligned to any particular chromosome. A phylogenetic analysis performed using the POLiMAPS pipeline revealed linkage groups that were most similar to ancestral species *F. vesca* for each chromosome. Linkage groups that were most similar to a second ancestral species, *F. iinumae*, were only resolved for *Fvb* 4. The quantity of missing data and heterogeneity in genome coverage inherent in GBS complicated the analysis, but POLiMAPS resolved *F. ×ananassa* chromosomal regions derived from diploid ancestor *F. vesca*.

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1 **Genotyping-by-sequencing enables linkage mapping in three octoploid cultivated**
2 **strawberry families**

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15

16 **ABSTRACT**

17 With the goal of evaluating genotyping-by-sequencing (GBS) in a species with a complex
18 octoploid genome, GBS was used to survey genome-wide single-nucleotide polymorphisms
19 (SNPs) in three biparental strawberry (*Fragaria ×ananassa*) populations. GBS sequence data
20 were aligned to the *F. vesca* '*Fvb*' reference genome in order to call SNPs. Numbers of
21 polymorphic SNPs per population ranged from 1,163 to 3,190. Linkage maps consisting of 30-
22 65 linkage groups were produced from the SNP sets derived from each parent. The linkage
23 groups covered 99% of the *Fvb* reference genome, with three to seven linkage groups from a
24 given parent aligned to any particular chromosome. A phylogenetic analysis performed using the
25 POLiMAPS pipeline revealed linkage groups that were most similar to ancestral species *F. vesca*
26 for each chromosome. Linkage groups that were most similar to a second ancestral species, *F.*
27 *iinumae*, were only resolved for *Fvb* 4. The quantity of missing data and heterogeneity in
28 genome coverage inherent in GBS complicated the analysis, but POLiMAPS resolved *F.*
29 *×ananassa* chromosomal regions derived from diploid ancestor *F. vesca*.

30 **BACKGROUND**

31 Genotyping-by-sequencing (GBS) is a powerful, cost-effective method for identifying
32 single-nucleotide polymorphisms (SNPs) on a whole-genome scale. The GBS technique
33 commonly used involves a form of reduced representation genome sequencing based on partial
34 restriction enzyme digestion, usually with a methylation-sensitive restriction enzyme, followed
35 by barcoded adaptor ligation and next-generation sequencing of highly multiplexed samples,
36 typically 48, 96, or 384 samples per lane (Elshire et al., 2011; Davey et al., 2011). Applications
37 of GBS range from germplasm diversity and population structure assessment to molecular
38 marker discovery. The high throughput and low per-sample cost of GBS makes it an attractive
39 option for plant breeding populations, as it can be used to saturate genetic maps (Russell et al.,
40 2014, Ward et al., 2013), perform QTL mapping and genome-wide association analyses (GWAS)
41 for traits of interest (Spindel et al., 2013), and enable genomic selection (Spindel et al., 2015) .
42 GBS has been applied to polyploid crop plants, including oat (Huang & Han, 2014), blueberry
43 (McCallum et al., 2016), and wheat (Poland et al., 2012; Saintenac et al., 2013).

44 The genus *Fragaria* consists of 20 species that range in ploidy from diploid to decaploid
45 (Liston et al. 2014). The polyploid species have complex evolutionary histories, including
46 hybridization events and chromosomal rearrangements (Njuguna et al., 2013; Liston, Cronn &
47 Ashman, 2014; Tennessen et al., 2014). The cultivated strawberry, *Fragaria ×ananassa* Duch.
48 ex Rozier, has 28 pairs of chromosomes and is a recent allo-octoploid ($2n = 8x = 56$), having
49 arisen in Europe in the 18th century from hybridization between two octoploids: North American
50 *F. virginiana* Mill. and South American *F. chiloensis* (L.) Duchesne ex Weston (Liston, Cronn &
51 Ashman, 2014). Phylogenetic analysis of octoploid *Fragaria* species have been conducted using
52 nuclear genes (Rousseau-Gueutin et al., 2009; DiMeglio et al., 2014), almost complete
53 chloroplast genomes (Njuguna et al., 2013; Govindarajulu et al., 2015), and genome-wide
54 markers (Tennessen et al., 2014; Govindarajulu et al., 2015; Qiao et al., 2016). These studies
55 support a model in which the octoploid *Fragaria* genome contains four ancestral sub-genomes.
56 One of the four sub-genomes appears to have originated from *F. vesca* L., one from *F. iinumae*
57 Makino, and two from an unknown ancestor phylogenetically close to *F. iinumae* (Tennessen et
58 al., 2014; Sargent et al., 2016). Two high-throughput genotyping platforms have been developed
59 for *F.× ananassa*: a 90K Affymetrix Axiom array containing 95,062 marker loci (Bassil et al.,
60 2015), and two microarrays based on Diversity Array Technology (DArT) markers (Sánchez-
61 Sevilla et al., 2015).

62 Linkage mapping has proved to be a useful tool for *Fragaria* genomics. The genome of
63 the diploid *F. vesca* was assembled using an SSR-based map (Sargent et al., 2012) and
64 subsequently improved with dense targeted capture maps comprising over 9,000 polymorphisms
65 (Tennessen et al., 2014). These diploid linkage maps have allowed traits such as sex
66 determination to be mapped (Ashman et al., 2015; Tennessen et al., 2016). While the similarity
67 of *Fragaria* sub-genomes has presented challenges for genetic linkage mapping in breeding
68 populations of octoploid *F.* ×*ananassa*, linkage maps have been applied to resolve genomic
69 structure and identify chromosomal rearrangements (Sargent et al., 2012, 2016; Isobe et al.,
70 2013; Tennessen et al., 2014; Davik et al., 2015; Sánchez-Sevilla et al., 2015), as well as to map
71 traits (Spigler & Ashman, 2011; Zorrilla-Fontanesi et al., 2011; Molina-Hidalgo et al., 2013;
72 Tennessen et al., 2016). Despite their recent polyploid origin, inheritance in the octoploid
73 *Fragaria* species is primarily disomic (Bringhurst, 1990; Lerceteau-Köhler et al., 2003;
74 Rousseau-Gueutin et al., 2008), allowing distinct linkage groups to be constructed and assessed.

75 The objectives of this study were to 1) evaluate the utility of GBS by developing linkage
76 maps for three bi-parental *F.* ×*ananassa* populations using SNP markers derived from GBS; and
77 2) test the efficacy of the POLiMAPS pipeline in resolving sub-genome contributions from the
78 ancestral diploid *Fragaria* species.

79

80 **MATERIALS & METHODS**

81 *Plant Material, DNA Extraction and Quantitation*

82 The strawberry samples analyzed in this study consisted of: parents and 24 offspring
83 from the 'Holiday' × 'Korona' population from the Netherlands (van Dijk et al., 2014); parents
84 and 60 seedlings from the 'Tribute' × 'Honeoye' population from Michigan State University
85 (MSU) (Castro et al., 2015; Sooriyapathirana et al., 2015); parents and 51 offspring from the
86 'Redgauntlet' × 'Hapil' population from East Malling Research, UK (Sargent et al., 2012;
87 Supplemental Table 1). DNA was extracted from actively growing leaf tissue with the E-Z 96®
88 Plant DNA extraction kit (Omega BioTek, Norcross, GA, USA) as previously described
89 (Gilmore, Bassil & Hummer, 2011). The resulting genomic DNA was quantitated with the
90 Quant-iT™ Picogreen® Assay (Invitrogen, Eugene, OR, USA) according to the manufacturer's

91 recommendations using a Victor^{3}V 1420 Multilabel Counter (Perkin Elmer, Downers Grove, IL,
92 USA). The DNA concentration was adjusted to 100 ng/μL per sample for subsequent genotyping
93 by sequencing (GBS) library preparation.

94

95 *GBS Library Preparation*

96 Three GBS libraries were constructed at the USDA-ARS National Clonal Germplasm
97 Repository (NCGR) and one was constructed at Clemson University according to the procedure
98 previously described (Elshire et al., 2011) for 96 samples using DNA (100 ng per sample)
99 digested with 4 U of ApeKI (New England Biolabs, Ipswich, MA, USA). The annealed and
100 normalized unique and four barcoded adaptors were obtained from Clemson University
101 Genomics Institute (CUGI) and from the Oregon State University (OSU) Center for Genome
102 Research and Biocomputing (CGRB) core facility. Two libraries were sequenced at the CGRB,
103 one at CUGI, and one at the North Carolina State University Genomic Sciences Laboratory
104 (Supplemental Table 1). At each of these labs, libraries were quantitated with a Qubit®
105 fluorometer (Invitrogen, Carlsbad, CA, USA), checked for adequate size distribution (150 – 350
106 bp) with the Bioanalyzer 2100 HS-DNA chip (Agilent Technologies, Santa Clara, CA, USA),
107 and sequenced with the Illumina HiSeq2000 (101 bp, single-end).

108

109 *Genotyping*

110 SNPs were called using the POLiMAPS pipeline (Tennessen et al., 2014). In brief,
111 sequence reads were aligned to the *Fvb* genome assembly (Tennessen et al., 2014) using BWA
112 version 0.7.12 with parameter -n 0.001 (Li and Durbin 2009). SAMtools version1.1 was used (Li
113 et al., 2009) to generate a pileup format file for each of the three crosses and a custom Perl script
114 was used to call polymorphisms (available at https://github.com/listonlab/POLiMAPS).
115 POLiMAPS identifies markers with approximately Mendelian segregation by requiring a
116 minimum number of offspring displaying each of the two possible genotypes (parameter –o,
117 default = 8). It also sets a maximum value for number of offspring with missing genotypes
118 (parameter –m, default = 1). Default parameters were used with the following exceptions.
119 Because there were relatively few offspring in the ‘Holiday’ × ‘Korona’ cross (24), we decreased

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120 –o to 6. Conversely, because there were more offspring in 'Redgauntlet' × 'Hapil' (51) and
121 'Tribute' × 'Honeoye' (63), we increased –m to 4 for 'Redgauntlet' × 'Hapil' and to 5 for
122 'Tribute' × 'Honeoye'.

123

124 *Linkage Mapping*

125 SNPs that were segregating in both parents were excluded from linkage mapping, as tri-
126 or quad-allelic markers were expected to be rare, and difficult to distinguish from sequencing
127 errors.. Segregating loci were organized into parental sets, which were subjected to linear
128 regression mapping using JoinMap® v. 4.1 (Van Ooijen, 2006). A minimum Independence
129 likelihood of odds (LOD) threshold of 3 was used for establishing the linkage groups (LG).

130

131 *Phylogenetic Analysis*

132 Dendrograms were constructed for each linkage group using the genetic information for
133 each cultivar and the diploid congeners following the previously described POLiMAPS approach
134 for octoploid *Fragaria* (Tennessen et. al. 2014). This method identified Illumina reads containing
135 markers associated with a linkage group, which were then assigned to that chromosome, and
136 treated all other sites on those reads as potential phylogenetic characters. For each of the seven
137 haploid *Fragaria* chromosomes, a phylogeny was generated with each parent expected to provide
138 distinct linkage groups representing the four sub-genomes. The *Fvb* reference genome of *F. vesca*
139 was used along with whole genome data from the same diploid samples as in Tennessen et al.
140 (2014): *F. mandshurica*, *F. bucharica*, *F. viridis*, *F. nipponica*, *F. iinumae*, and *Rubus coreanus*
141 as an outgroup. RAxML was used with -N autoMRE and -m GTRCAT and 100 bootstrap
142 replicates to estimate separate phylogenies for each of the seven haploid *Fragaria* chromosomes
143 (Stamatakis, 2006). Two rounds of analysis using the same protocol were performed. In the first
144 round, chromosome information from four diploids (*F. vesca*, *F. mandshurica*, *F. viridis*, and *F.*
145 *iinumae*) and the *F. ×ananassa* linkage groups which aligned to the homologous chromosome and
146 had at least 300 phylogenetic sites were included. The objective of this analysis was to identify the
147 LG from each population that belonged to each of the sub-genomes. When more than four linkage
148 groups per parental map were found, an attempt was made to merge some of the linkage groups in

149 each parental map using the following criteria: 1) continuous marker position on the *Fvb* reference;
150 2) a Chi squared (χ^2) test supporting linkage between the last SNP at the end of one linkage group
151 and the first SNP at the beginning of another linkage group ($P < 0.05$); 3) similar phylogenetic
152 position of the two linkage groups; and 4) strongest cross-link (SCL) metric between JoinMap
153 groups.

154 Once some of the LGs were merged, the phylogenetic analysis was repeated, with the
155 additional *Fragaria* comparators *F. nipponica* and *F. bucharica*, and with *Rubus coreanus* to serve
156 as anoutgroup. The minimum number of phylogenetic sites per JoinMap linkage group was
157 lowered to 150 for all parents and sites were allowed to be missing in any of the diploids.

158

159 *'Holiday' Chromosome 6 Comparison between GBS and Axiom Array Data*

160 An integrated linkage map for 'Holiday' chromosome 6D was created utilizing the 90K
161 Axiom data (Bassil et al., 2015) and the GBS data from 'Holiday' groups 2 and 3 via a graphical
162 mapping approach to further assess the quality of the GBS data. Bowtie2 version 2.2.9 (Langmead
163 et al., 2009) and SAMtools version 1.3.1 (Li et al., 2009) were used to align the Axiom 'Holiday'
164 chromosome 6D map and the integrated 'Holiday' 6D map to the *Fvb* assembly to visualize genetic
165 rearrangements between *F. vesca* ssp. *bracteata* and *F. ×ananassa*.

166

167 **RESULTS**

168 *Genotyping*

169 The yield of high-quality GBS data obtained from the parents, defined as Illumina reads
170 containing both the expected barcode sequence and ApeKI cut site remnant, ranged from 98 Mb
171 to 185 Mb (Table 1). Numbers of reads that aligned to the *Fvb* reference genome ranged from
172 992,053 ('Hapil') to 1,754,838 ('Honeoye'; Table 1). Numbers of SNPs, which were identified
173 by POLiMAPS from reads aligning to the *Fvb* reference genome, are also listed in Table 1. The
174 greatest number of SNPs was found in the 'Redgauntlet' × 'Hapil' population (3,190). In the
175 'Holiday' × 'Korona' population, the number of SNPs was 2,136. The fewest SNPs were found
176 in the 'Tribute' × 'Honeoye' population (1,163).

177 Progeny plants were categorized as having a high quantity of missing data when the
178 number of SNP sites lacking a defined genotype was greater than or equal to 10%. The smallest

179 population, 'Holiday' × 'Korona', had only one plant with 10% missing SNPs. In the
180 'Redgauntlet' × 'Hapil' population, two offsprings had greater than or equal to 10% missing
181 data: 11% and 27%, the latter of which was excluded from JoinMap analysis. In the largest
182 population, 'Tribute' × 'Honeoye', seven plants had greater than or equal to 10% missing data,
183 and four of these were excluded from further analysis because they had greater than or equal to
184 30% missing data.

185 *Linkage Mapping and Homolog Assignment*

186 The number of JoinMap linkage groups obtained from parental genotypes ranged from 30
187 ('Tribute') to 65 ('Holiday'). Over all three populations, there were 178 JoinMap groups, with 46
188 (26%) consisting of fewer than ten SNPs, and 25 with gaps of 15 cM or larger. For a given
189 chromosome, the number of aligned linkage map groups ranged from three to seven (Figure 1,
190 Table 2). For the 'Tribute' × 'Honeoye' cross, a total of 29 chromosome-aligned groups were
191 constructed from 'Tribute'-derived SNPs, and 33 were constructed from 'Honeoye'-derived
192 SNPs. For the 'Holiday' × 'Korona' cross, 38 groups were constructed from 'Holiday'-derived
193 SNPs, and 49 from 'Korona'-derived SNPs. For the 'Redgauntlet' × 'Hapil' cross, 39 groups
194 were constructed from 'Redgauntlet'-derived SNPs, and 37 were from 'Hapil'-derived SNPs.
195 The number of SNPs on chromosome-aligned linkage groups ranged from 21 to 169 (Fig. 3).
196 Altogether, the linkage groups covered 99% of the *Fvb* genome.

197 *Sub-genome Assignment*

198 A phylogenetic analysis was performed in order to identify distinct linkage groups
199 representing ancestral sub-genomes. The resulting trees – one for each chromosome – were
200 examined to identify JoinMap groups that could potentially be merged according to proximity on
201 a tree. Merging reduced the overall number of chromosome-aligned groups by six or fewer for
202 'Tribute' (from 29 to 27), 'Honeoye' (from 33 to 28), 'Redgauntlet' (from 39 to 34), and 'Hapil'
203 (from 37 to 31; Table 2). The number of 'Holiday' groups was reduced by nine (from 38 to 29),
204 and the number of 'Korona' groups was reduced by 17 (from 49 to 32).

205 Once JoinMap groups were merged, a second round of phylogenetic analysis was
206 performed to produce a final set of trees, with one representing each Fvb chromosome (Fig. 2).
207 Most linkage groups aligned uniquely to Fvb chromosomes. However, 'Holiday'-derived linkage

208 group 8, which consisted of 32 SNPs and had a length of 77.1 cM, aligned to two Fvb
209 chromosomes: Fvb3 and Fvb 6. Group 8 encompassed the entire Fvb3 chromosome, and also the
210 last approximately 3.7 Mb of Fvb6.

211 Clear *F. vesca*-like clades were distinguishable on just two of the chromosomal
212 cladograms: Fvb1 and Fvb2. All six parental genotypes were represented in the *F. vesca* clade on
213 Fvb1, and only 'Holiday' was absent from the *F. vesca* clade on Fvb 2 (Table 3). On the Fvb7
214 cladogram, *F. vesca* and the other *Fragaria* species were not clearly differentiated. An *F.*
215 *iinumae*-like clade was only distinguishable in the Fvb4 cladogram.

216 *'Holiday' Chromosome 6 Comparison between GBS and Axiom Array Data*

217 An integrated map for 'Holiday' chromosome 6D was created using marker data from the
218 90 K Axiom array for the 'Holiday' × 'Korona' population (Bassil et al., 2015). Three
219 individuals of the 23 used in the initial mapping in the present study were excluded either due to
220 a large amount of missing Axiom data (H-02552) or inconsistencies between the Axiom data and
221 GBS data for the samples (H-02572 and H-02637). When the GBS and Axiom markers were
222 mapped together, an 83 cM linkage group was produced, where markers co-segregated into 15
223 bins (Fig. 4, Supplementary table 1). The graphical mapping approach was able to show the
224 integration of the GBS data and recombination events were easy to visualize (Supplementary
225 table 1). Moreover, the total length of the integrated map is very similar to the 95.6 cM map
226 produced by Bassil et al. (2015) and the shared marker order did not vary, demonstrating the
227 quality of the GBS data. Map resolution was considerably lower in the integrated map due to the
228 reduced population size.

229 Many of the same major chromosomal rearrangements between *F. vesca* ssp. *bracteata*
230 and *F. ×ananassa* were observed between the integrated GBS map and the 90 K Axiom-derived
231 map (Fig. 4). The Axiom-derived map was able to identify a few more micro rearrangements
232 than the integrated map. This is to be expected as the population size used to construct the
233 Axiom-derived map was much larger than the integrated map. Many of the rearrangements
234 observed were a few markers rather than large blocks (Fig. 4). As such it is unknown if the
235 rearrangements observed are mapping or assembly errors or if the rearrangements are due to
236 evolutionary differences between *F. vesca* ssp. *bracteata* and *F. ×ananassa*

237

238 DISCUSSION

239 GBS and related reduced-representation sequencing methods have recently been used to
240 study *F. iinumae* (Mahoney et al., 2016), and *F. ×ananassa* (Davik et al., 2015). Both of those
241 studies employed protocols using two different restriction enzymes. The use of two restriction
242 enzymes further reduces the sequenced fraction of the genome because only genome fragments
243 containing the two different restriction enzyme sites in proximity are selected. This has the effect
244 of increasing overall sequencing depth and likelihood of SNP detection over covered regions. In
245 the present study, high-quality SNP data was obtained for three octoploid, biparental *F.*
246 *×ananassa* using a single restriction enzyme, ApeKI as previously done in many plant species
247 including blueberry (McCallum et al., 2016) and red raspberry (Ward et al., 2013). The
248 POLiMAPS pipeline (Tennessen et al., 2014b)was used to identify sub-genomes derived from *F.*
249 *vesca* (Av), *F. iinumae* (Bi). The remaining two sub-genomes, B1 and B2, could not be
250 distinguished and were un-assigned and referred to as B12. The Av sub-genome corresponds to
251 sub-genome A of van Dijk et al., (2014) who first denoted the sub-genomes based on their
252 divergence from *F. vesca* as A, B, C and D, in order of most to least divergence. .

253 The numbers of polymorphic SNPs obtained from 'Redgauntlet' × 'Hapil' and 'Holiday'
254 × 'Korona' were in the range of those obtained for *F. iinumae* and *F. ×ananassa* in the
255 aforementioned experiments. However, the numbers of polymorphic SNPs used for mapping
256 varied widely among the three analyzed populations. The 'Redgauntlet' × 'Hapil' population had
257 by far the greatest number of polymorphic SNPs, with 1,253 and 1,096 derived from each
258 parental genotype, while the 'Holiday' × 'Korona' population had 735 and 1,096, and the
259 'Tribute' × 'Honeoye' population had 358 and 468 SNPs. Relative levels of homozygosity may
260 account for this variability. The number of restriction enzymes employed, choice of restriction
261 enzymes, and sequence coverage depth per individual over the sampled portion of the genome
262 can also affect the number of polymorphic SNPs detected (Glaubitz et al., 2014). Interestingly,
263 the 'Tribute' × 'Honeoye' population had the highest number of reads aligned to the *F. vesca*
264 reference genome, yet it had the lowest number of SNP calls and the highest quantity of missing
265 data. The lower number of SNP calls may indicate low genetic variation within the parents.

266 Many of the JoinMap groups that aligned to the *Fvb* chromosomes were small, consisting
267 of fewer than ten SNPs. Others were longer, but had large gaps (> 15 cM). Homozygous genome

268 regions can account for large gaps in linkage maps, and the fragmented linkage groups can be
269 attributed to heterogeneity in genome coverage of GBS data, which results in an overall high
270 quantity of missing data. Low marker density and large gaps can also result from conserved
271 chromosomal regions with high homozygosity. 'Holiday' and 'Korona' have common ancestors
272 in their pedigrees, making regions of shared homozygosity more likely in that population (van
273 Dijk et al., 2014). A study of simple sequence repeat (SSR) markers in the 'Redgauntlet' ×
274 'Hapil' population reported six regions on five linkage groups with >20cM gaps (Sargent et al.,
275 2012), however, marker density in that study was overall much lower than in any of the datasets
276 analyzed here and the power to bridge these gaps was much larger due to the increased family
277 sizes used.

278 The goal of the POLiMAPS phylogenetic analysis was two-fold: First, to resolve
279 homoeologs in cases where more than four JoinMap linkage groups from any crossing parent
280 aligned to the same haploid reference chromosome; and second, to identify ancestral diploid sub-
281 genome contributions. Comparative genomic mapping between octoploid and diploid *Fragaria*
282 species has reported synteny and high colinearity along chromosomes (Davik et al., 2015; van
283 Dijk et al., 2014). The inclusion of more than one diploid *Fragaria* species in the phylogenetic
284 analysis increased the robustness of the *F. vesca* clade. We expected all sub-genomes to have
285 numerous regions sufficiently similar to the reference genome to produce correctly-aligning
286 reads, given the previous success of this alignment pipeline (Tennessen et al., 2014). However it
287 is likely that a higher proportion of reads from the *F. vesca* sub-genome aligned given its higher
288 similarity to the reference genome, leading to an enrichment for *F. vesca* sub-genome markers in
289 our analysis. Because one of our goals was to connect linkage groups with *Fvb* chromosomes,
290 we prioritized aligning reads with high confidence to the reference genome, and discarded reads
291 that could not align given the BWA -n 0.001 parameter. Other analyses of GBS data in Fragaria
292 may seek to maximize the number of markers even if their reference genome position is less
293 certain. When high-quality assemblies of the *F.* × *ananassa* subgenomes, and of other *Fragaria*
294 diploids, become available, the issue of aligning divergent reads will be less of a concern.
295 Overall, the greatest number of SNPs were assigned to the *F. vesca* sub-genome. Each parental
296 genotype had linkage groups that could be assigned to the *F. vesca* sub-genome on chromosome
297 Fvb1, and all parental genotypes except for 'Holiday' had *F. vesca*-like groups on chromosomes
298 Fvb2 and Fvb7. Fvb4 was the only chromosome for which all parental genotypes had linkage

299 groups that could be assigned to the *F. iinumae* sub-genomsub-genome, however, most of the
300 linkage groups from parental genotypes could not be assigned to any sub-genome. These results
301 are consistent with the findings of Hirakawa et al. (2013), who aligned 57% of the scaffolds in an
302 *F. ×ananassa* assembly to *F. vesca* pseudomolecules, and concluded that approximately 20% of
303 the scaffolds were *F. ×ananassa*-specific.

304 One linkage group derived from 'Holiday' aligned to both chromosome 3 and
305 chromosome 6. This region may represent a translocation event. Markers from the distal end of
306 Fvb6, comprising over 3Mb and over 700 genes, are included in a linkage group containing
307 markers from across Fvb3. This gene-dense region of Fvb6 contains genes and QTLs linked to
308 important traits, such as sex phenotype (Goldberg, Spigler & Ashman, 2010; Ashman et al.,
309 2015). However, given the small sample size of the 'Holiday' × 'Korona' cross, this could also
310 be a spurious association.

311 CONCLUSIONS

312 In summary, POLiMAPS was employed with genotyping-by-sequencing data in three
313 small families to resolve *F. ananassa* chromosomal regions derived from the diploid *F. vesca.*
314 However, the large number of missing data in GBS experiments, combined with the complex
315 relationships among homoeologs in polyploid plants, complicate such analyses and may limit the
316 usefulness of GBS in these plants. Use of an *F. ×ananassa* reference sequence for SNP detection
317 and higher coverage of GBS libraries developed after cutting with two restriction endonucleases
318 may address the challenges observed in this study and require further study.

319 The data is available via the NCBI Sequence Read Archive, BioProject PRJNA385347.

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323 Rosaceae' (2009-51181-05808).

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327 **References**

328 Ashman T-L., Tennessen JA., Dalton RM., Govindarajulu R., Koski MH., Liston A. 2015.
329 Multilocus Sex Determination Revealed in Two Populations of Gynodioecious Wild
330 Strawberry, *Fragaria vesca* subsp. bracteata. *G3: Genes|Genomes|Genetics* 5:2759–
331 2773. DOI: 10.1534/g3.115.023358.

332 Bassil NV., Davis TM., Zhang H., Ficklin S., Mittmann M., Webster T., Mahoney L., Wood D.,
333 Alperin ES., Rosyara UR., Koehorst-vanc Putten H., Monfort A., Sargent DJ., Amaya I.,
334 Denoyes B., Bianco L., van Dijk T., Pirani A., Iezzoni A., Main D., Peace C., Yang Y.,
335 Whitaker V., Verma S., Bellon L., Brew F., Herrera R., van de Weg E. 2015.
336 Development and preliminary evaluation of a 90 K Axiom® SNP array for the allo-
337 octoploid cultivated strawberry *Fragaria ×ananassa*. *BMC Genomics* 16:155. DOI:
338 10.1186/s12864-015-1310-1.

339 Bringhurst RS. 1990. Cytogenetics and evolution in American *Fragaria*. *HortScience* 25:879–
340 881.

341 Davey JW., Hohenlohe PA., Etter PD., Boone JQ., Catchen JM., Blaxter ML. 2011. Genome-
342 wide genetic marker discovery and genotyping using next-generation sequencing. *Nature*
343 *Reviews Genetics* 12:499–510. DOI: 10.1038/nrg3012.

344 Davik J., Sargent DJ., Brurberg MB., Lien S., Kent M., Alsheikh M. 2015. A ddRAD Based
345 Linkage Map of the Cultivated Strawberry, *Fragaria ×ananassa*. *PLOS ONE*
346 10:e0137746. DOI: 10.1371/journal.pone.0137746.

347 van Dijk T., Pagliarani G., Pikunova A., Noordijk Y., Yilmaz-Temel H., Meulenbroek B., Visser
348 RG., van de Weg E. 2014. Genomic rearrangements and signatures of breeding in the
349 allo-octoploid strawberry as revealed through an allele dose based SSR linkage map.
350 *BMC Plant Biology* 14:55. DOI: 10.1186/1471-2229-14-55.

351 DiMeglio LM., Staudt G., Yu H., Davis TM. 2014. A Phylogenetic Analysis of the Genus
352 *Fragaria* (Strawberry) Using Intron-Containing Sequence from the ADH -1 Gene. *PLOS*
353 *ONE* 9:e102237. DOI: 10.1371/journal.pone.0102237.
354 Elshire RJ., Glaubitz JC., Sun Q., Poland JA., Kawamoto K., Buckler ES., Mitchell SE. 2011. A
355 Robust, Simple Genotyping-by-Sequencing (GBS) Approach for High Diversity Species.
356 *PLoS ONE* 6:e19379. DOI: 10.1371/journal.pone.0019379.
357 Gilmore BS., Bassil NV., Hummer KE. 2011. DNA Extraction Protocols from Dormant Buds of
358 Twelve Woody Plant Genera. *Journal of the American Pomological Society* 65:201–206.
359 Glaubitz JC., Casstevens TM., Lu F., Harriman J., Elshire RJ., Sun Q., Buckler ES. 2014.
360 TASSEL-GBS: A High Capacity Genotyping by Sequencing Analysis Pipeline. *PLoS*
361 *ONE* 9:e90346. DOI: 10.1371/journal.pone.0090346.
362 Goldberg MT., Spigler RB., Ashman T-L. 2010. Comparative genetic mapping points to
363 different sex chromosomes in sibling species of wild strawberry (*Fragaria*). *Genetics*
364 186:1425–1433. DOI: 10.1534/genetics.110.122911.
365 Govindarajulu R., Parks M., Tennessen JA., Liston A., Ashman T-L. 2015. Comparison of
366 nuclear, plastid, and mitochondrial phylogenies and the origin of wild octoploid
367 strawberry species. *American Journal of Botany* 102:544–554. DOI:
368 10.3732/ajb.1500026.
369 Hirakawa H., Shirasawa K., Kosugi S., Tashiro K., Nakayama S., Yamada M., Kohara M.,
370 Watanabe A., Kishida Y., Fujishiro T., Tsuruoka H., Minami C., Sasamoto S., Kato M.,
371 Nanri K., Komaki A., Yanagi T., Guoxin Q., Maeda F., Ishikawa M., Kuhara S., Sato S.,
372 Tabata S., Isobe SN. 2013. Dissection of the Octoploid Strawberry Genome by Deep

373 Sequencing of the Genomes of *Fragaria* Species. *DNA Research*:dst049. DOI:
374 10.1093/dnares/dst049.
375 Huang X., Han B. 2014. Natural Variations and Genome-Wide Association Studies in Crop
376 Plants. *Annual Review of Plant Biology* 65:531–551. DOI: 10.1146/annurev-arplant-
377 050213-035715.
378 Isobe SN., Hirakawa H., Sato S., Maeda F., Ishikawa M., Mori T., Yamamoto Y., Shirasawa K.,
379 Kimura M., Fukami M., Hashizume F., Tsuji T., Sasamoto S., Kato M., Nanri K.,
380 Tsuruoka H., Minami C., Takahashi C., Wada T., Ono A., Kawashima K., Nakazaki N.,
381 Kishida Y., Kohara M., Nakayama S., Yamada M., Fujishiro T., Watanabe A., Tabata S.
382 2013. Construction of an integrated high density simple sequence repeat linkage map in
383 cultivated strawberry (*Fragaria ×ananassa*) and its applicability. *DNA research: an*
384 *international journal for rapid publication of reports on genes and genomes* 20:79–92.
385 DOI: 10.1093/dnares/dss035.
386 Langmead B., Trapnell C., Pop M., Salzberg SL. 2009. Ultrafast and memory-efficient alignment
387 of short DNA sequences to the human genome. *Genome Biology* 10:R25. DOI:
388 10.1186/gb-2009-10-3-r25.
389 Lerceteau-Köhler E., Guérin G., Laigret F., Denoyes-Rothan B. 2003. Characterization of mixed
390 disomic and polysomic inheritance in the octoploid strawberry (*Fragaria ×ananassa*)
391 using AFLP mapping. *Theoretical and Applied Genetics* 107:619–628. DOI:
392 10.1007/s00122-003-1300-6.
393 Li H., Handsaker B., Wysoker A., Fennell T., Ruan J., Homer N., Marth G., Abecasis G., Durbin
394 R. 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25:2078–
395 2079. DOI: 10.1093/bioinformatics/btp352.

396 Liston A., Cronn R., Ashman T-L. 2014. *Fragaria*: A genus with deep historical roots and ripe
397 for evolutionary and ecological insights. *American Journal of Botany* 101:1686–1699.
398 DOI: 10.3732/ajb.1400140.
399 Mahoney LL., Sargent DJ., Abebe-Akele F., Wood DJ., Ward JA., Bassil NV., Hancock JF.,
400 Folta KM., Davis TM. 2016. A High-Density Linkage Map of the Ancestral Diploid
401 Strawberry Constructed with Single Nucleotide Polymorphism Markers from the
402 IStraw90 Array and Genotyping by Sequencing. *The Plant Genome* 9:0. DOI:
403 10.3835/plantgenome2015.08.0071.
404 McCallum S., Graham J., Jorgensen L., Rowland LJ., Bassil NV., Hancock JF., Wheeler EJ.,
405 Vining K., Poland JA., Olmstead JW., Buck E., Wiedow C., Jackson E., Brown A.,
406 Hackett CA. 2016. Construction of a SNP and SSR linkage map in autotetraploid
407 blueberry using genotyping by sequencing. *Molecular Breeding* 36:1–24. DOI:
408 10.1007/s11032-016-0443-5.
409 Molina-Hidalgo FJ., Franco AR., Villatoro C., Medina-Puche L., Mercado JA., Hidalgo MA.,
410 Monfort A., Caballero JL., Muñoz-Blanco J., Blanco-Portales R. 2013. The strawberry
411 (Fragaria ×*ananassa*) fruit-specific rhamnogalacturonate lyase 1 (FaRGLyase1) gene
412 encodes an enzyme involved in the degradation of cell-wall middle lamellae. *Journal of*
413 *Experimental Botany* 64:1471–1483. DOI: 10.1093/jxb/ers386.
414 Njuguna W., Liston A., Cronn R., Ashman T-L., Bassil N. 2013. Insights into phylogeny, sex
415 function and age of *Fragaria* based on whole chloroplast genome sequencing. *Molecular*
416 *Phylogenetics and Evolution* 66:17–29. DOI: 10.1016/j.ympev.2012.08.026.

417 Poland JA., Brown PJ., Sorrells ME., Jannink J-L. 2012. Development of High-Density Genetic
418 Maps for Barley and Wheat Using a Novel Two-Enzyme Genotyping-by-Sequencing
419 Approach. *PLOS ONE* 7:e32253. DOI: 10.1371/journal.pone.0032253.
420 Qiao Q., Xue L., Wang Q., Sun H., Zhong Y., Huang J., Lei J., Zhang T. 2016. Comparative
421 Transcriptomics of Strawberries (*Fragaria* spp.) Provides Insights into Evolutionary
422 Patterns. *Frontiers in Plant Science* 7. DOI: 10.3389/fpls.2016.01839.
423 Rousseau-Gueutin M., Gaston A., Aïnouche A., Aïnouche ML., Olbricht K., Staudt G., Richard
424 L., Denoyes-Rothan B. 2009. Tracking the evolutionary history of polyploidy in
425 *Fragaria* L. (strawberry): New insights from phylogenetic analyses of low-copy nuclear
426 genes. *Molecular Phylogenetics and Evolution* 51:515–530. DOI:
427 10.1016/j.ympev.2008.12.024.
428 Rousseau-Gueutin M., Lerceteau-Köhler E., Barrot L., Sargent DJ., Monfort A., Simpson D.,
429 Arús P., Guérin G., Denoyes-Rothan B. 2008. Comparative Genetic Mapping Between
430 Octoploid and Diploid *Fragaria* Species Reveals a High Level of Colinearity Between
431 Their Genomes and the Essentially Disomic Behavior of the Cultivated Octoploid
432 Strawberry. *Genetics* 179:2045–2060. DOI: 10.1534/genetics.107.083840.
433 Russell J., Hackett C., Hedley P., Liu H., Milne L., Bayer M., Marshall D., Jorgensen L., Gordon
434 S., Brennan R. 2014. The use of genotyping by sequencing in blackcurrant (*Ribes*
435 *nigrum*): developing high-resolution linkage maps in species without reference genome
436 sequences. *Molecular Breeding* 33:835–849. DOI: 10.1007/s11032-013-9996-8.
437 Saintenac C., Jiang D., Wang S., Akhunov E. 2013. Sequence-Based Mapping of the Polyploid
438 Wheat Genome. *G3 Genes|Genomes|Genetics* 3:1105–1114. DOI:
439 10.1534/g3.113.005819.

440 Sánchez-Sevilla JF., Horvath A., Botella MA., Gaston A., Folta K., Kilian A., Denoyes B.,
441 Amaya I. 2015. Diversity Arrays Technology (DArT) Marker Platforms for Diversity
442 Analysis and Linkage Mapping in a Complex Crop, the Octoploid Cultivated Strawberry
443 (*Fragaria ×ananassa*). *PLoS ONE* 10. DOI: 10.1371/journal.pone.0144960.
444 Sargent DJ., Passey T., Šurbanovski N., Girona EL., Kuchta P., Davik J., Harrison R., Passey A.,
445 Whitehouse AB., Simpson DW. 2012. A microsatellite linkage map for the cultivated
446 strawberry (*Fragaria ×ananassa*) suggests extensive regions of homozygosity in the
447 genome that may have resulted from breeding and selection. *Theoretical and Applied*
448 *Genetics* 124:1229–1240. DOI: 10.1007/s00122-011-1782-6.
449 Sargent DJ., Yang Y., Šurbanovski N., Bianco L., Buti M., Velasco R., Giongo L., Davis TM.
450 2016. HaploSNP affinities and linkage map positions illuminate subgenome composition
451 in the octoploid, cultivated strawberry (Fragaria ×*ananassa*). *Plant Science: An*
452 *International Journal of Experimental Plant Biology* 242:140–150. DOI:
453 10.1016/j.plantsci.2015.07.004.
454 Spigler RB., Ashman T-L. 2011. Sex ratio and subdioecy in *Fragaria virginiana*: the roles of
455 plasticity and gene flow examined. *New Phytologist* 190:1058–1068. DOI:
456 10.1111/j.1469-8137.2011.03657.x.
457 Spindel J., Begum H., Akdemir D., Virk P., Collard B., Redoña E., Atlin G., Jannink J-L.,
458 McCouch SR. 2015. Genomic selection and association mapping in rice (*Oryza sativa*):
459 effect of trait genetic architecture, training population composition, marker number and
460 statistical model on accuracy of rice genomic selection in elite, tropical rice breeding
461 lines. *PLoS genetics* 11:e1004982. DOI: 10.1371/journal.pgen.1004982.

462 Spindel J., Wright M., Chen C., Cobb J., Gage J., Harrington S., Lorieux M., Ahmadi N.,
463 McCouch S. 2013. Bridging the genotyping gap: using genotyping by sequencing (GBS)
464 to add high-density SNP markers and new value to traditional bi-parental mapping and
465 breeding populations. *Theoretical and Applied Genetics* 126:2699–2716. DOI:
466 10.1007/s00122-013-2166-x.
467 Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with
468 thousands of taxa and mixed models. *Bioinformatics (Oxford, England)* 22:2688–2690.
469 DOI: 10.1093/bioinformatics/btl446.
470 Tennessen JA., Govindarajulu R., Ashman T-L., Liston A. 2014. Evolutionary origins and
471 dynamics of octoploid strawberry subgenomes revealed by dense targeted capture linkage
472 maps. *Genome Biology and Evolution*:evu261. DOI: 10.1093/gbe/evu261.
473 Tennessen JA., Govindarajulu R., Liston A., Ashman T-L. 2016. Homomorphic ZW
474 chromosomes in a wild strawberry show distinctive recombination heterogeneity but a
475 small sex-determining region. *New Phytologist* 211:1412–1423. DOI:
476 10.1111/nph.13983.
477 Van Ooijen. 2006. JoinMap ® 4, Software for the calculation of genetic linkage maps in
478 experimental populations. *Kyazma B.V., Wageningen, Netherlands*.
479 Ward JA., Bhangoo J., Fernández-Fernández F., Moore P., Swanson J., Viola R., Velasco R.,
480 Bassil N., Weber CA., Sargent DJ. 2013. Saturated linkage map construction in *Rubus*
481 *idaeus* using genotyping by sequencing and genome-independent imputation. *BMC*
482 *Genomics* 14:2. DOI: 10.1186/1471-2164-14-2.
483 Zorrilla-Fontanesi Y., Cabeza A., Domínguez P., Medina JJ., Valpuesta V., Denoyes-Rothan B.,
484 Sánchez-Sevilla JF., Amaya I. 2011. Quantitative trait loci and underlying candidate

485 genes controlling agronomical and fruit quality traits in octoploid strawberry (*Fragaria* ×
486 *ananassa*). *Theoretical and Applied Genetics* 123:755–778. DOI: 10.1007/s00122-011-
487 1624-6.

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498 **Tables**
499
500 Table 1. Sequence data obtained from the parents on the Illumina HiSeq2000. Numbers of
501 sequence reads aligning to the *Fragaria vesca* ssp. *bracteata* genome assembly, and the SNPs
502 called from those reads by the POLiMAPS pipeline, are indicated

Parent	No. Reads with Barcode and ApeKI cut site	Total Basepairs	Reads aligning to Fvb	Polymorphic SNPs per Parent	Polymorphic SNPs per Cross (common to both parents/total)
'Holiday'	2,113,031	135,233,984	1,304,324	735	
'Korona'	2,892,457	185,117,248	1,709,640	1,096	305/2136
'Tribute'	1,996,412	127,770,368	1,194,954	358	
'Honeoye'	2,892,682	185,131,648	1,754,838	468	337/1163
'Redgauntlet'	2,495,364	159,703,296	1,474,896	1,253	
'Hapil'	1,536,347	98,326,208	992,053	1,215	722/3190

503
504 Table 2. Numbers of JoinMap groups per parent, first round (before phylogenetic analysis) and
505 second round (after group merging).
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	Fvb1		Fvb2		Fvb3		Fvb4		Fvb5		Fvb6		Fvb7	
Parent	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Tribute	5	4	4	4	6	5	4	3	4	4	4	4	2	3
Honeoye	4	4	4	5	5	4	5	4	5	4	6	4	4	4
Holiday	4	4	6	4	5	5	4	4	6	4	9	6	4	2
Korona	6	4	9	5	9	5	6	4	9	6	6	4	4	3
Redgauntlet	5	4	8	6	5	4	5	4	5	6	5	5	6	5
Hapil	5	4	5	4	5	4	5	4	5	5	7	6	5	4

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508 Table 3. JoinMap groups sub-genome assignment:*F. vesca*-like (v), *F. iinumae*-like (i), unassigned
509 (u), for each parent.

	Fvb1	Fvb2	Fvb3	Fvb4	Fvb5	Fvb6	Fvb7
Parent	**Av, Bi, B12**	**Av, Bi, B12**	**Av, Bi, B12**	**Av, Bi, B12**	**Av, Bi, B12**	**Av, Bi, B12**	**Av, Bi, B12**
Tribute	1,0,3	2,0,2	1,0,4	1,1,1	0,0,4	0,0,4	1,0,2
Honeoye	1,0,3	1,0,4	0,0,4	1,2,1	2,0,2	0,0,4	1,1,2
Holiday	1,0,3	0,1,3	5,0,0	0,1,3	1,0,3	0,0,6	0,0,2
Korona	1,0,3	1,0,4	0,2,3	0,1,3	1,0,5	0,0,4	1,1,1
Redgauntlet	1,0,3	1,0,5	0,1,3	1,1,2	1,0,5	0,0,5	3,1,1
Hapil	1,0,3	1,0,3	0,1,3	0,1,3	1,0,4	1,0,5	1,1,2

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514 **Figures**
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516 Figure 1. Linkage group relative chromosome positions on the *Fragaria vesca* reference genome.
517 Separate graphs are shown for crossing parents ‘Holiday’ and ‘Korona.’ Linkage groups are color-
518 coded to reflect their relative proximity to ancestral species on phylogenetic trees. Undetermined
519 groups did not clearly cluster with any ancestral species.

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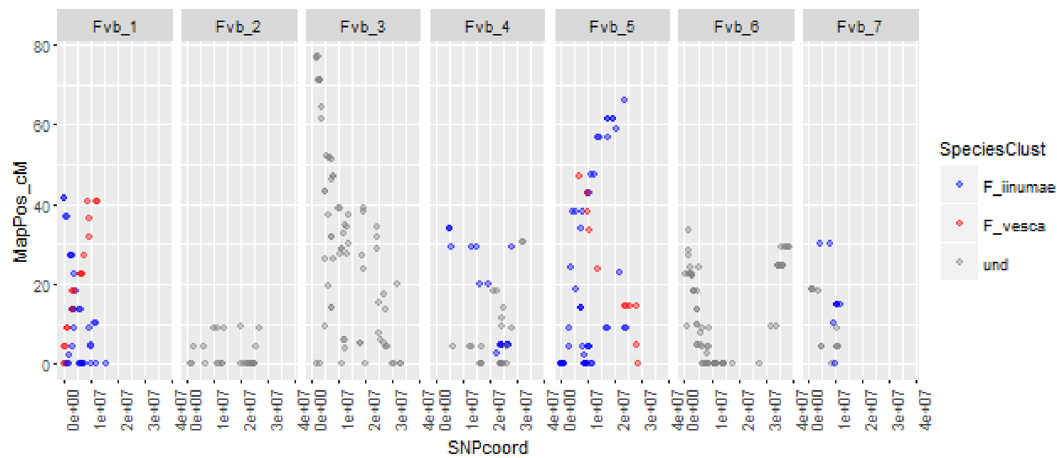

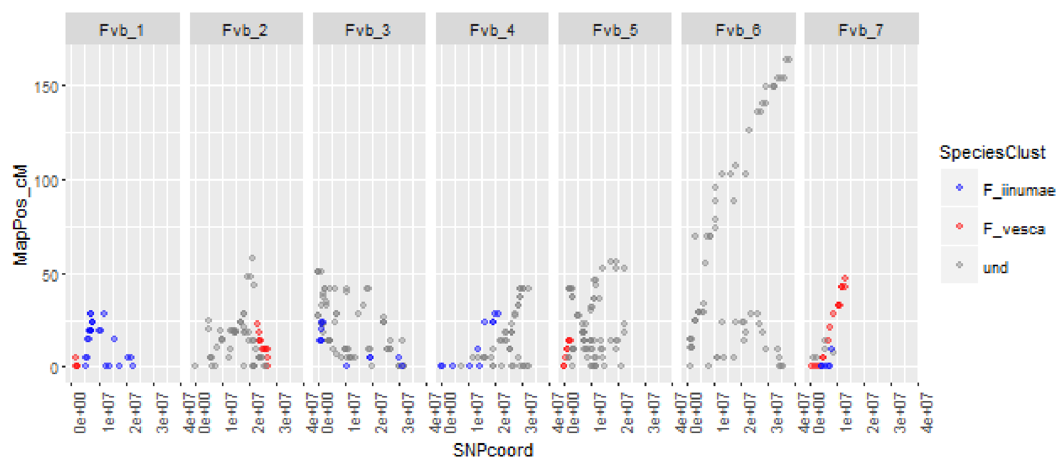

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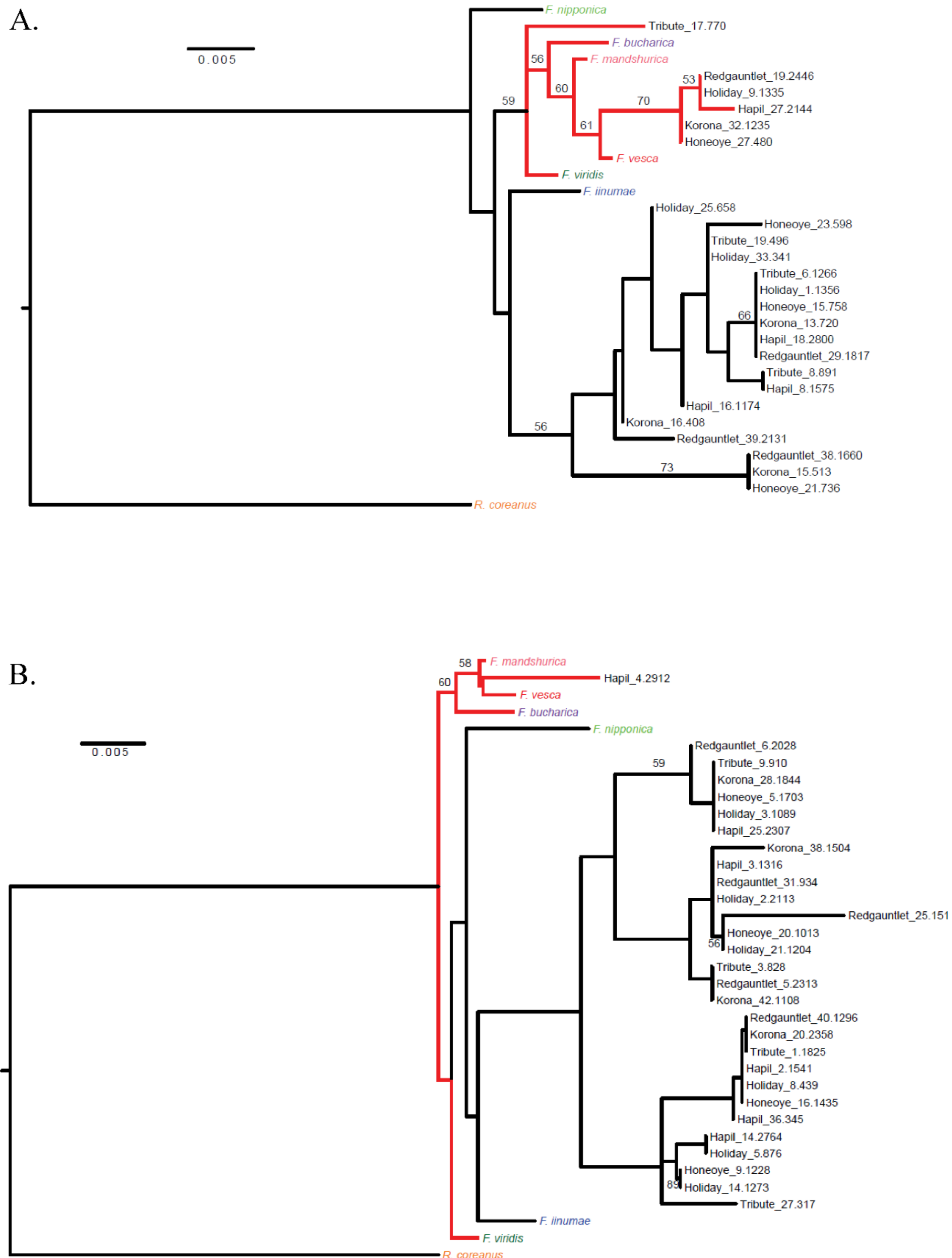
535 Figure 2. Cladograms representing linkage groups that aligned to *F. vesca* chromosomes Fvb1 (A)
536 and Fvb6 (B). Fvb1 shows a clear *F. vesca* –like clade (A), while Fvb6 lacks a distinct *F. vesca* –
537 like clade (B).



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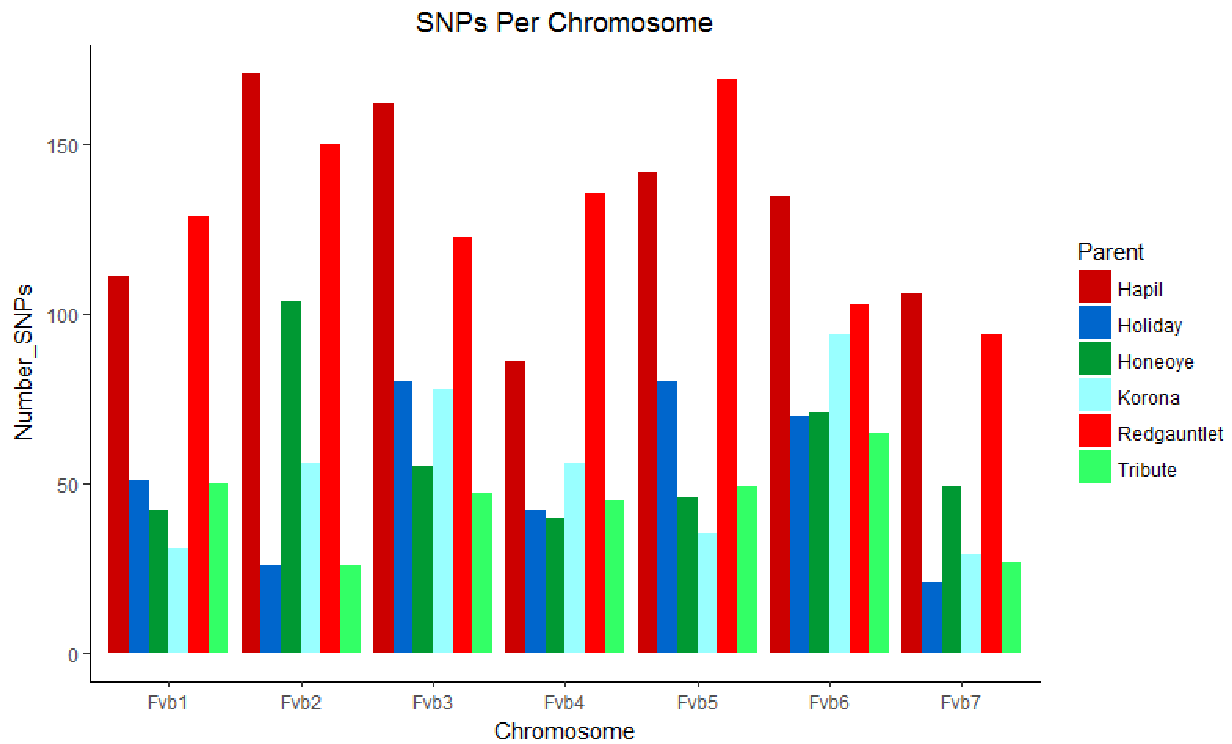
541 Figure 3. Numbers of SNPs from each parent represented on linkage groups aligning to *Fragaria*
542 *vesca* chromosomes.



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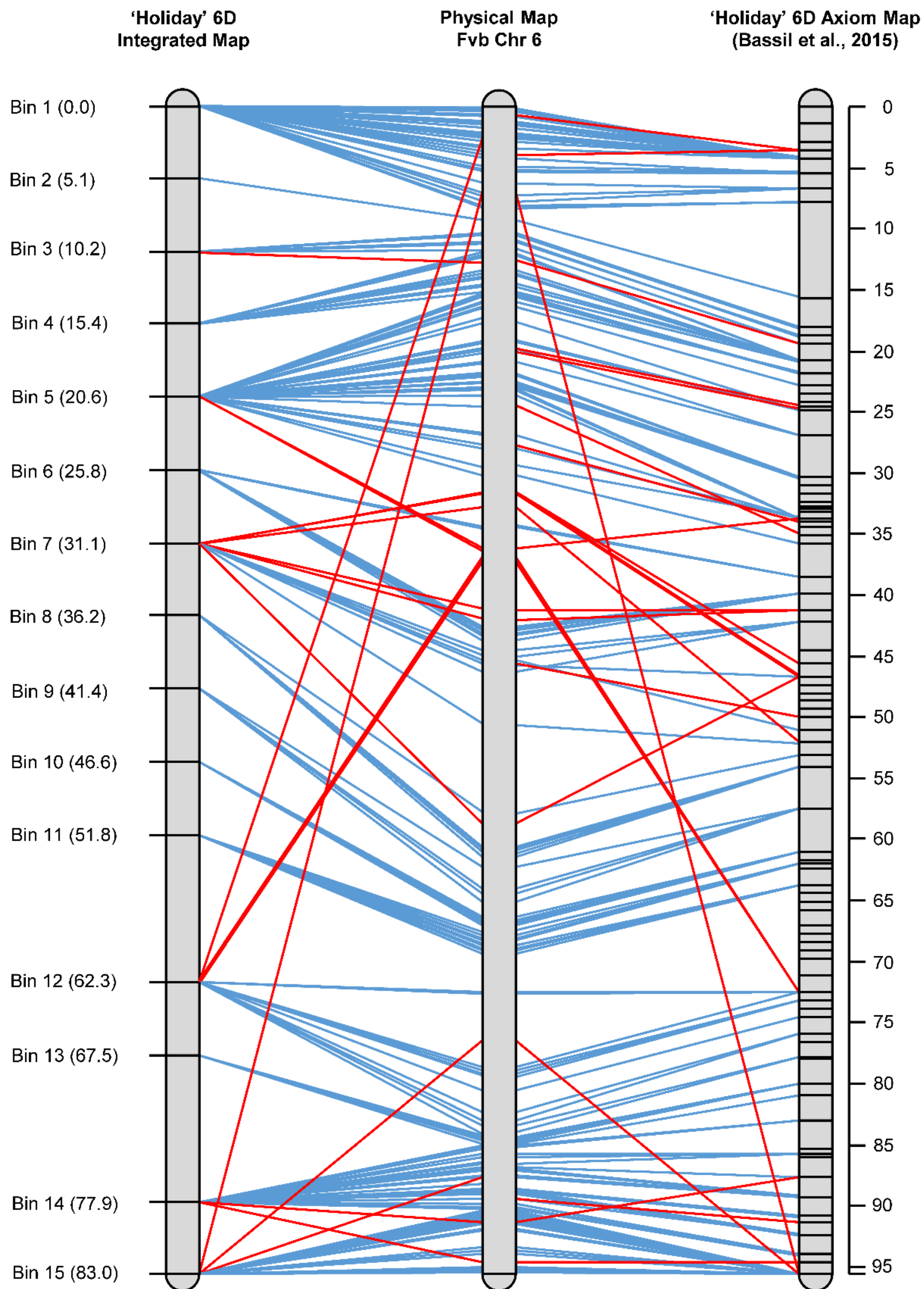
567 Figure 4. Alignment of the integrated map of 'Holiday' LG 6 to the physical map for that
568 chromosome from the Fvb assembly to LG 6 based on SNP data from the 90K Axiom array.



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