1 Sex-determining chromosomes and sexual dimorphism: insights from genetic
2 mapping of sex expression in a natural hybrid *Fragaria ×ananassa* subsp.
3 *cuneifolia*.

4 Rajanikanth Govindarajulu[1], Aaron Liston[2] and Tia-Lynn Ashman[1]*
5
6 [1]Department of Biological Sciences, University of Pittsburgh, Pittsburgh,
7 Pennsylvania, 15260, USA
8 [2]Department of Botany and Plant Pathology, Oregon State University, Cordley
9 Hall 2082, Corvallis, Oregon, 97331, USA.
10 *Author for correspondence
11 tia1@pitt.edu, 412-624-0984
12
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21 **Abstract –**

22 We studied the natural hybrid (*Fragaria ×ananassa* subsp. *cuneifolia*) between
23 two sexually dimorphic octoploid strawberry species (*F. virginiana* and *F.*
24 *chiloensis*) to gain insight into the dynamics of sex chromosomes and the
25 genesis of sexual dimorphism. Male sterility is dominant in both the parental
26 species and thus will be inherited maternally, but the chromosome that houses
27 the sex-determining region differs. Thus, we asked whether 1) the cytotypic
28 composition of hybrid populations represents one or both maternal species, 2)
29 the sex-determining chromosome of the hybrid reflects the location of male
30 sterility within the maternal donor species, and 3) crosses from the hybrid
31 species show less sexual dimorphism than the parental species. We found that
32 *F. ×ananassa* subsp. *cuneifolia* populations consisted of both parental
33 cytotypes but one predominated within each population. Genetic linkage
34 mapping of two crosses showed dominance of male sterility similar to the
35 parental species, however, the map location of male sterility reflected the
36 maternal donor in one cross, but not the other. Moreover, female function
37 mapped to a single region in the first cross, but to two regions in the second
38 cross. Aside from components of female function (fruit set and seed set), other
39 traits that have been found to be significantly sexually dimorphic in the pure
40 species were either not dimorphic or were dimorphic in the opposite direction

41 to the parental species. These results suggest that hybrids experience some
42 disruption of dimorphism in secondary sexual traits, as well as novel location
43 and number of QTL affecting sex function.

44 **Keywords**: *Fragaria*, hybrid, sexual dimorphism, sex chromosome, male
45 sterility

46

## Introduction

Natural hybrids can form when two species with incomplete reproductive isolation come into secondary contact (Rieseberg and Carney, 1998). Once viewed as 'evolutionary noise' (Wagner, 1970), hybrid zones are now being viewed as natural laboratories for ecological and evolutionary studies in speciation and diversification (for example, Rieseberg and Wendel, 1993; Sweigart, 2009) as they provide insight into the prevailing direction of gene flow, gene introgression and adaptation (for example, Nolte *et al.,* 2009; Wallace *et al.,* 2011). It also has been noted that natural hybrid zones are an underexploited source of information on reproductive isolation and mating (reviewed in Rieseberg and Blackman, 2010), early sex chromosome evolution (Veltsos *et al.,* 2008) and the genetics of sexual dimorphism (Coyne *et al.,* 2008). Hybrid zones may be where new sterility alleles are expressed (reviewed in Rieseberg and Blackman, 2010) or old ones rearranged (for example, Petit *et al.,* 2010). Such novelty could lead to the evolution and spread of new sex-determining chromosomes (Pannell and Pujol, 2009; Veltsos *et al.,* 2008). Moreover, because sexual dimorphism (or sex limitation) that evolved separately in the two species can breakdown in the hybrids (Parker and Partridge, 1998), the pattern of dissolution or re-expression of

66 male traits in females can provide information on the origin and type of genetic
67 control underlying sexual dimorphism (Coyne *et al.,* 2008).

68 While there are a handful of well-studied plant hybrid zones that
69 involve gender dimorphic (i.e., dioecious [males and females], or
70 gynodioecious [females and hermaphrodites]) parental species; for example,
71 Buggs and Pannell, 2007; Lexer *et al.,* 2010; Minder *et al.,* 2007; Wallace *et*
72 *al.,* 2011), there are few where we also have explicit knowledge of the location
73 of sex-determining genes in the parental species. In fact, we have very few
74 studies that compare genetic maps of sex determination in hybrids to their
75 parent species (but see, Macaya-Sanz *et al.,* 2011; Paolucci *et al.,* 2010), or the
76 level of sexual dimorphism in the hybrid to that of its parental species. Yet, it
77 is in these systems where we will be most readily able to address questions of
78 novel locations of sex-determining genes and of the effect of hybridization on
79 sexual dimorphism.

80 As a first step in addressing these gaps, we investigated the level of
81 population admixture and the location of the sex-determining region in in two
82 populations of *Fragaria* × *ananassa* subsp. *cuneifolia*, a natural hybrid of two
83 octoploid species, *F. chiloensis* and *F. virginiana.* These are the same two
84 species that were cultivated in Europe in the 1700s, and hybridized to produce

85 the cultivated strawberry *Fragaria* × *ananassa* subsp. *ananassa* (Darrow,
86 1966).

87 Both parental species show gender dimorphism and sexual dimorphism
88 in secondary traits (Ashman, 2003; Ashman, 2005; Ashman *et al.,* 2011;
89 Spigler *et al.,* 2011) but they differ in the chromosome that houses the sex-
90 determining region (Goldberg *et al.,* 2010). *Fragaria chiloensis* is
91 predominantly dioecious (Hancock and Bringhurst, 1979b) and recent mapping
92 studies have revealed that sex is determined by a dominant sterility allele (*A*) at
93 the male function 'locus' and recessive sterility allele (*g*) at the female function
94 'locus' and that these 'loci' co-localize on linkage group (LG) VI.A (Goldberg
95 *et al.,* 2010). *Fragaria virginiana* is subdioecious, and sex expression is also
96 controlled by a dominant male-sterility allele and a recessive female-sterility
97 allele but here linkage between the two sex function 'loci' is less complete
98 (that is, recombination can lead to hermaphrodites and neuters), and these
99 major sex-determining loci are on LG VI.C (Spigler *et al.,* 2010). The
100 dominance and different chromosomal locations of male sterility means that,
101 barring rearrangements, the location of sex determining genes in the hybrid
102 should reflect that of the maternal donor species. That is, when *F. chiloensis* is
103 the donor we expect male sterility to map to linkage group VI.A but when *F.*
104 *virginiana* is the maternal donor it will map to VI.C. One can use species-

105 specific plastome markers to identify the maternal donor of individuals and the
106 populations they derive from (i.e., fixed cytotype or admixture) (Arnold *et al.,*
107 2010; Minder *et al.,* 2007) to facilitate testing this hypothesis. In addition,
108 since several of the sexually dimorphic secondary traits have been shown to be
109 controlled, in part, by loci colocalizing with the sex-determining region
110 (Ashman *et al.,* 2011; Spigler *et al.,* 2011), we predicted that dimorphism in
111 these traits would be less in the hybrid than found in the pure species crosses.

112 Specifically, we sought to determine whether 1) two populations of *F.*
113 ×*ananassa* subsp. *cuneifolia* reflect a single parental species or a mixture of
114 the two parental species as maternal donors; 2) the sex-determining
115 chromosome of the hybrid reflects the location of male sterility within the
116 maternal donor species; and 3) crosses from the hybrid species show less
117 sexual dimorphism than published reports for the parental species.

118 **Material and Methods**

119 ***Species description and study populations***

120 *Fragaria* ×*ananassa* subsp. *cuneifolia* is a perennial stoloniferous herb
121 that inhabits edges of pastures, pine forests, roadways and the interfaces of
122 woods and back dunes (Hancock and Bringhurst, 1979a; Staudt, 1999). It is
123 morphologically intermediate to the parental species (Salamone *et al*.,

124 unpublished; Staudt, 1999) and currently exists in a narrow hybrid zone that
125 stretches from southern British Columbia to northern California (Staudt, 1999).
126 While the two progenitor species last shared a common ancestor 400,000-
127 1,770,000 years ago based on the 95% highest posterior density of a Bayesian
128 dating analysis (Njuguna *et al*., submitted), the timing of secondary contact is
129 unknown. Staudt (1989) speculated that hybridization occurred after glaciation
130 in the Fraser River valley of British Columbia receded. Evidence from
131 diagnostic plastome SNPs indicates that either species can act as the maternal
132 donor (Salamone *et al*., in prep). Cytological and genetic evidence also
133 suggests that both wild octoploid species and the cultivated hybrid *F.*
134 ×*ananassa* subsp. *ananassa* have a genomic structure of AAA'A'BBB'B'
135 (Bringhurst, 1990), and exhibit disomic inheritance ($2n = 8x = 56$) (Ashley *et*
136 *al.,* 2003; Lerceteau-Köhler *et al.,* 2003; Rousseau-Gueutin *et al.,* 2008).
137 *Fragaria* ×*ananassa* subsp. *cuneifolia* is subdioecious with three sexual
138 morphs (hermaphrodites, males and females) co-occurring within a population
139 (Ashman personal obs; Staudt, 1999), similar to one of its progenitor species
140 *F. virginiana* (Ashman and Hitchens, 2000).

141 We collected 32 *F.* ×*ananassa* subsp. *cuneifolia* plants along transects
142 through each of two populations in Benton Co, Oregon (Wren [44.5878 N,
143 123.4272 W, 133 m] and Mary's Peak [44.5044 N, 123.55 W, 1203 m]). All

144 three sexual phenotypes were observed in these populations and females
145 represented ~50% in each population (Wren: 47%, Mary's Peak: 53%). Plants
146 were grown in 200 ml pots filled with a 2:1 mixture of Fafard #4 (Conrad
147 Fafard) and sand in the greenhouse at the University of Pittsburgh. Plants
148 received fertilizer and protection from pests as needed.
149

150 ***Creation and cultivation of F. ×ananassa* subsp. *cuneifolia mapping***
151 ***populations***

152 To map sex determination we created two mapping populations by
153 crossing plants derived from Wren (WREN) and Mary's Peak (MP)
154 populations. In MP12×WREN2 cross a MP female (67% fruit set) was
155 pollinated with pollen from a WREN individual that was male fertile but set no
156 fruit. In the WREN7×MP10 cross, a WREN female with 96% fruit set was
157 pollinated with pollen from a MP hermaphrodite (32% fruit set). We pollinated
158 female parents with pollen collected from the male parents during February
159 through April 2010 and planted 144 seeds from each cross in May 2010. Seeds
160 were planted in 72-well trays with a custom germination mix (Sunshine
161 germination mix: Fafard #4: sand), and exposed to 14-hr days and 15°C/20°C
162 night/day temperatures in a growth chamber. Germination was high (both
163 crosses ~ 95%), and after two months of growth seedlings were transplanted

164 into 200 ml pots filled with a 2:1 mixture of Fafard #4 and sand. At this time
165 we also produced two clones of each parent. All plants were exposed to
166 $12^{\circ}C/22^{\circ}C$ night/day and 12-hr day light for four months prior to a two-month
167 dark treatment at $4^{\circ}C$. Growth conditions during flowering were 11-hr day
168 light at $12^{\circ}C/18^{\circ}C$ night/day. We hand-pollinated each flower on all plants
169 three times per week with outcross pollen to ensure full potential fruit and seed
170 set. During the entire course of study all plants received 7 beads of granular
171 nutricote 13:13:13 N: P: K fertilizer (Chisso-Ashai fertilizer) and were
172 protected from pests as needed.
173

174 ***Sex expression and phenotype data***

175 Sex expression was scored on each plant at least twice during
176 flowering. As in previous studies (Goldberg *et al.,* 2010; Spigler *et al.,* 2010)
177 we scored male function qualitatively based on the presence or absence of
178 pollen production and this was assessed in at least two flowers per plant.
179 Individuals with yellow anthers visibly releasing pollen were scored as 'male-
180 fertile', whereas plants that produced white vestigial stamens and whose anther
181 sacs lacked pollen were scored as 'male-sterile'. Female function was
182 quantitatively estimated as the percentage of flowers that produced fruit ('fruit
183 set'). To be consistent with the previous qualitative mapping of female

184 function in *F. virginiana* (Spigler *et al.,* 2008) and *F. chiloensis* (Goldberg *et*
185 *al.,* 2010), we considered plants with ≥5% fruit set as 'female fertile' and those
186 with ≤5% fruit set as 'female sterile'. We scored sex expression on all the
187 flowering plants in the mapping populations and present the data for the
188 representative subset that were genotyped. In addition, we scored several
189 phenotypic traits that have been shown to be sexually dimorphic in *F.*
190 *virginiana* and/or *F. chiloensis* (proportion seed set, anther number per flower,
191 flowers per plant, leaf number per plant and runner number per plant)
192 following the protocols described in Spigler *et al*. (2011).

193 For each F1 mapping population we determined whether there was
194 sexual dimorphism between male-sterile and male-fertile morphs using *t*–tests.
195 To facilitate comparisons with published indices of sexual dimorphism in
196 parental species (Ashman *et al.,* 2011; Spigler *et al.,* 2011), we calculated a
197 sexual dimorphism index following McDaniel (2005) as $|(x_{MS} - x_{MF})|/ [(SE_{MS} +$
198 $SE_{MF})/2]$, where $x$ and SE are the mean and standard error respectively, for
199 each trait for male-sterile (MS) and male-fertile (MF) morphs.

200

201 ***DNA extraction***

202 DNA was extracted from 10-15 mg of silica-dried young leaf tissue
203 from the 32 plants per population for cytotyping and from progeny (85 from
204 MP12×WREN2, 90 from WREN7×MP10) and two replicates of the parents of
205 the two crosses for genetic mapping. We used a CTAB extraction protocol
206 (Doyle and Doyle, 1987) modified to accommodate a 96-well high-throughput
207 format. DNA was quantified using a Spectromax 190 spectrophotometer
208 (Molecular devices, Sunnyvale, California, USA) and diluted to 0.3ng/µl with
209 deionized sterile water for PCR reactions.
210

211 ***Cytotyping***

212 To distinguish the parental donor of *F.* ×*ananassa* subsp. *cuneifolia*
213 individuals, we screened each individual for two SNPs that differentiate *F.*
214 *virginiana* and *F. chiloensis* chloroplast genomes (Njuguna *et al*., submitted;
215 Salamone *et al*., in prep). SNPs within the intron of *pet*D and an exon of *ndh*F
216 were screened in 32 plants per population using the dCAPs technique (Neff *et*
217 *al.,* 1998).

218 Specifically, we amplified the two chloroplast regions *pet*D (primers
219 petDintron-77875F [5′ GGATAGGCTGGTTCGTTTGA 3′ ], petDintron-
220 78409R [5′ GCTCGAGCATGAATCAACAG 3′]) and *ndh*F (ndhF-113272F

221 [5′ AAAATCCCCGACACGATTAG 3′], ndhF-113799R [5′
222 ACCGTTCATTCCACTTCCAG 3′]). PCR reactions included 1X PCR buffer
223 (Qiagen 10x buffer with $MgCl_2$), 100 µM of each dNTP, 0.5 µM of each
224 forward and reverse primer, 1.5 units of *Taq* polymerase and 1 µl of genomic
225 DNA in a 20 µl reaction. PCR amplification began with a hot start of 95°C for
226 2 min to activate the *Taq* polymerase (New England Biolabs, Beverly,
227 Massachusetts, USA.) followed by 94°C for a 45 sec denaturation step,
228 followed by 35 cycles of: 1) 45 sec. denaturation at 94°C; 2) 30 sec. annealing
229 at 51°C; 3) 60 sec. extension at 72°C, and a final extension for 8 min at 72°C.
230 The amplified PCR products were purified using Qiagen PCR purification kit.
231 To validate the SNPs for cytotyping we sequenced purified products on ABI
232 3730XL DNA analyzer (Applied Biosystems, UK). For dCAPs, we digested 6
233 µl of purified *ndh*F product from each sample with 5 units of the restriction
234 enzyme *Msl*I for 2 hrs at 37°C, and 6 µl of purified *pet*D product with 5 units
235 of *Taq*$^{\alpha}$I at 65°C for 2 hrs. The recognition site of *Msl*I includes the underlined
236 variable site (CATTG^AAGT$\underline{\text{A}}$/CATTGAAGT$\underline{\text{G}}$) within *ndh*F and that of
237 *Taq*$^{\alpha}$I includes the underlined variable site (T^C$\underline{\text{G}}$A/TC$\underline{\text{A}}$A) in *pet*D. Products
238 were assayed on agarose gels and species specific cytotypes identified as
239 follows: 1) *ndh*F locus, a 500 base pair (uncut) product identifies *F. virginiana*
240 whereas two fragments (336 and 166 base pair product) corresponds to the *F.*

241 *chiloensis* cytotype; 2) two *pet*D fragments (56 and 44 bp) differentiate the *F.*
242 *chiloensis* cytotype from the *F. virginiana* (uncut) cytotype..

243

244 ***Nuclear marker analysis and genotyping***

245 Because we were interested in determining the location of sex
246 determining region and assessing the homology of the sex-determining
247 chromosome in *F. ×ananassa* subsp. *cuneifolia* to its progenitors, we
248 genotyped the mapping populations using primer pairs that have been shown to
249 amplify DNA markers (SSRs or genes) on LG within the homoeologous group
250 (HG) that houses the sex determining chromosomes in *F. virginiana* and *F.*
251 *chiloensis* (that is, HG VI, Goldberg *et al.,* 2010; Spigler *et al.,* 2010). Thirty-
252 nine primer pairs were used to construct genetic map of HG VI and map the
253 sex determining region in *F. ×ananassa* subsp. *cuneifolia* in the
254 WREN7×MP10 cross and 10 of these were used to construct genetic map for
255 the MP12×WREN2 cross.

256 For nuclear markers, PCR reactions were performed using the Poor
257 Man's PCR protocol as previously described (Goldberg *et al.,* 2010; Spigler *et*
258 *al.,* 2008). We multiplexed PCR products from 2 - 4 primers by mixing 1.3µl
259 aliquots from each reaction with 0.2 µl LIZ500 standard and 10.5µl Hi-Di
260 formamide (Applied Biosystems). Fragment analysis and genotyping were

261 conducted using ABI 3730XL DNA analyzer and GeneMapper (Applied
262 Biosystems).
263
264 ***Construction of genetic maps***

265 We used the single-dose restriction fragment marker analysis and a
266 pseudo test-cross strategy to construct genetic linkage maps for *F.* ×*ananassa*
267 subsp. *cuneifolia*, as is customary in polyploids (Garcia *et al.,* 2006; Wu *et al.,*
268 1992). Since a single primer pair can amplify multiple PCR products as a result
269 of paralogs in the octoploid genome, we considered each PCR product a
270 single-dose marker and scored it as present (1) and absent (0) in the progeny.
271 Using $\chi 2$ tests, we evaluated each polymorphic marker for goodness of fit
272 according to the expected Mendelian segregation ratio of either 1:1 if present
273 in only one parent or 3:1 if present in both parents. In each cross, we discarded
274 markers that deviated from the expected segregation ratios at $P \geq 0.0001$ and
275 retained all other markers for mapping. We mapped the marker data from each
276 primer pair in JoinMap 4.0 (Van Ooijen, 2006) to determine whether markers
277 from a given primer pair represented co-segregating alleles at a single locus.
278 The PCR products from single primer were considered to be allelic at a locus if
279 they mapped in the same location and were in repulsion. For these, we retained
280 one member of each pair to be consistent with the single-dose marker approach

281 (Spigler *et al.,* 2010; Wu *et al.,* 1992). For PCR products that were linked but
282 did not map to the same location, we checked the raw data to reevaluate the
283 genotypes and reconfirm scores as in past work (Spigler *et al*., 2010).
284 We constructed separate maps of HG VI for maternal and paternal
285 parents by considering them as CP (cross-pollinator) population type (outbred
286 full-sib family cross). Before mapping we excluded markers or individuals that
287 were missing data for >25%. This resulted in the exclusion of only two
288 individuals from one cross (MP12×WREN2). Initial linkage groups were
289 inferred at LOD ≥6. Additional ungrouped markers were assigned at LOD >4
290 threshold using strongest cross link (SCL) values. Marker order and map
291 distance were determined using the Kosambi mapping function with default
292 parameter settings (minimum LOD threshold of 1.0, recombination threshold
293 of 0.40, and jump threshold of 5.0) to include maximum number of markers to
294 identify homoeologs within HG VI. We also applied strict mapping parameters
295 (minimum LOD threshold of 3.0, recombination threshold of 0.35 and jump
296 threshold of 3.0) to test stability of linkage between markers within each
297 homoeolog and found no major differences from the less strict mapping
298 parameter settings. We considered genetic maps derived from default mapping
299 parameter for further analyses. Graphical maps were constructed in MapChart
300 2.1 (Voorrips, 2002).

The LGs within each parental HG VI map were assembled on the basis of SCL values of LOD $\geq$4 between markers on the LGs or by comparing the LG with a putative homoeolog in the other parent (based on shared markers fitting 3:1 segregation ratio). Linkage groups were named VI.A, VI.B, VI.C and VI.D based on the LG-specific markers on homoeologous LGs in *F. virginiana* and *F. chiloensis* genetic maps (Goldberg *et al.,* 2010; Spigler *et al.,* 2010). Mapped markers that deviated in expected segregation ratios at $0.0001 < P < 0.01$ were identified as skewed and are denoted along with the direction of skew (that is, under or over representation of the heterozygote).

***Qualitative and quantitative mapping of sex expression***

To qualitatively map sex function traits, we considered male sterility and female sterility as single-dose markers and tested for fit to Mendelian segregation ratios (1:1 and 3:1) as in past work (Goldberg *et al.,* 2010; Spigler *et al.,* 2008).

To quantitatively map female function the mapping populations were treated as ‘doubled haploid populations’ which can evaluate QTLs more efficiently (Van Ooijen, 2004), but require the exclusion of markers found in both parents that segregate 3:1 (that is, “hk×hk” segregation types in JoinMap)

320 from the genetic map (Van Ooijen, 2006). We conducted QTL analysis on
321 each parent map separately using MapQTL®5 (Van Ooijen, 2004). As in past
322 work (Spigler *et al.,* 2010, Ashman *et al.,* 2011) we employed Kruskal-
323 Wallace analysis followed by interval mapping and composite interval
324 mapping (multiple QTL model, "MQM", in MapQTL) to identify potential
325 single marker associations and QTL for fruit set. Only the markers identified
326 as significant in the MQM were also significant in the single marker tests after
327 Bonferroni correction, thus we only report the results of the more conservative
328 MQM results. The QTLs (delineated by 2-LOD intervals) detected through
329 MQM are depicted on the original linkage group map for simplicity using an
330 autoqtl function in MapChart (Voorrips, 2002). Results were unchanged when
331 QTL analysis was performed without the 'marker' male sterility (data not
332 shown). We tested for epistatic effects between male sterility and female
333 function QTL when they did not overlap, as well as between multiple female
334 function QTL when present, following the ANOVA approach of Spigler *et al*.
335 (2011).

336 To assess macrosynteny of the sex-determining chromosomes in *F.*
337 ×*ananassa* subsp. *cuneifolia* with the parental species, we aligned the LGs
338 carrying male sterility found here with those published for *F. virginiana* and *F.*
339 *chiloensis* (Goldberg *et al.,* 2010; Spigler *et al.,* 2010). We also included the

340 homoeolog from a cross between two diploid hermaphrodite species, *F. vesca*
341 and *F. nubicola* ('Fv×Fn', Sargent *et al.,* 2009) for reference.
342

343 **Results**

344 ***Cytotyping***

345 Both *F.* ×*ananassa* subsp. *cuneifolia* populations contained *F. chiloensis* and
346 *F. virginiana* cytotypes, but they differed dramatically in the frequency of the
347 two types (Table 1). The *F. chiloensis* cytotype was predominant in the
348 Mary's Peak population (93.5%) whereas the *F. virginiana* one was at Wren
349 (93.4%). The parents of the mapping populations had the majority cytotypes
350 of their respective populations.

351 ***Genetic maps of HG-VI***

352 **MP12×WREN2 --**Ten primer pairs amplified 75 PCR products, 57 of which
353 met our criteria for map construction. Five of the 25 markers (19 [1:1 markers]
354 and 6 [3:1 markers]) used for the maternal map co-segregated so 20 markers
355 were retained for mapping. In the paternal map, 12 of the 36 markers (30 [1:1
356 markers] and 6 [3:1 markers]) co-segregated, thus 24 were retained for
357 mapping. From these, 7 and 3 markers (maternal and paternal, respectively)
358 were unlinked and not included in the final map.

359 We assembled the LGs of the maternal map (Supplementary Figure 1
360 top) into two homoeologous chromosomes (VI.A and VI.B) based on synteny
361 with the progenitor species and the WREN map (see below). The size of
362 linkage groups ranged from 20cM to 66.2cM, with 4 to 7 markers per group.

363 The paternal map (Supplementary Figure 1 bottom) was resolved into
364 five LGs that were assembled into three homoeologous chromosomes (VI.A,
365 VI.B and VI.C). The size of the LGs ranged from 1.2cM to 41.1cM, with 2 to 8
366 markers per group. In the paternal map only, 14% of the 21 mapped markers
367 were skewed. There were three single markers (SCAR2, CFCVT017, and
368 UFFxa01E03) that showed moderate segregation distortion ($P$<0.01) and over-
369 representation of heterozygous gametes among genotypes.

370 **WREN7×MP10 --**Thirty-nine primer pairs amplified 225 products. From
371 these, 142 met our criteria for genetic map construction. Thirteen of the 79
372 markers (54 [1:1 markers] and 25 [3:1 markers]) used for maternal map co-
373 segregated, so 66 were retained for final mapping. In the paternal map, 16 of
374 the 88 markers (63 [1:1 markers] and 25 [3:1 markers]) co-segregated and thus
375 72 were retained for mapping. Twenty-five and 30 markers of the final sets
376 were unlinked (maternal and paternal, respectively), thus are not included in
377 the final maps.

378 The maternal map of HG VI (Supplementary Figure 2 top) was
379 comprised of 41 markers that resolved into eight LGs. We assembled six of
380 these LGs into four homoeologous chromosomes and identified LGs VI.A
381 through VI.D based on the presence of LG-specific markers in comparison
382 with the progenitor genetic maps. For instance, SCAR2 on VI.A, F.v.a108 on
383 VI.C, F.v.B119 on VI.D in both *F. virginiana* and *F. chiloensis* maps
384 (Goldberg *et al.,* 2010; Spigler *et al.,* 2010) and tandemly duplicated EMFv104
385 markers along with other markers as LG VI.B in *F. chiloensis* (Goldberg *et al.,*
386 2010). These LGs ranged in size from 13.4 cM to 82.1 cM, with 3 to 17
387 markers per group. Two small LGs (2 markers) were not assigned to a
388 homoeologous chromosome. Only one confirmed case of a duplicated marker
389 was found (CFVCT006 on LG VI.A).
390
391 The paternal map of HG VI (Supplementary Figure 2 bottom) included
392 42 markers and resolved into seven LGs, that we assembled into four LGs
393 homologous to the maternal map. Three small LGs (2 markers each) were not
394 assigned to homoeologous chromosomes. LGs had 5 to 17 markers per group
395 and ranged from 25.7 cM to 90 cM.
396

397 In this cross, 16 (19%) of the mapped markers did not fit the expected
398 Mendelian ratios. Of these 69% where paternal markers whereas 31% where
399 maternal ones. These were most often single markers (Supplementary Figure 2
400 bottom). One potentially interesting case, however, is the two products of
401 CFCVT017 on LG VI.B close to the QTL for fruit set in both maternal and
402 paternal maps that showed a modest ($P$< 0.01) under-representation of
403 heterozygous gametes among genotypes.
404
405 ***Phenotypic sex expression: variation and mapping***

406 **Phenotypic sex expression --** Of the 85 genotyped progeny from
407 MP12×WREN2 cross, 40 progeny were male sterile and 45 were male fertile,
408 thus male function segregated 1:1 (Figure 2a; $\chi^2 = 0.30$, $P = 0.59$). In this
409 cross, female function also segregated 1:1 ($\chi^2 = 0.42$, $P = 0.51$) as 46 were
410 female fertile and 39 were female sterile. The majority (92%) of progeny were
411 either female (male sterile and female fertile; 47%), or male (male fertile and
412 female sterile; 45%), and six were hermaphrodites. Six females had fruit set
413 lower than 75% (Figure 2a).

414 In the WREN7×MP10 cross, 47 of the genotyped progeny were male
415 sterile and 43 were male fertile, thus male function segregated 1:1 (Figure 2b;
416 $\chi^2 = 0.17$, $P = 0.67$) in this cross as well. When female function was scored

417 qualitatively, 92% of progeny were female fertile and female fertility deviated
418 significantly from both 1:1 and 3:1(both $P < 0.0001$), precluding qualitative
419 mapping (see below). Overall, 36 (40%) progeny were hermaphrodite, 47
420 (52%) female, and seven (0.07%) male (Figure 2b).

421 **Male function mapping --**The results from both the crosses confirm the
422 dominance of male sterility over male fertility, and in both map crosses male
423 sterility mapped to one of the homoeologs within HG VI (Figure 1;
424 Supplementary Figures 1, 2). In MP12×WREN2, male sterility mapped to the
425 bottom of VI.A with tight linkage to two markers, PSContig6115 (LOD 10.0)
426 and EMFn153 (LOD 5.1). In WREN7×MP10, male sterility was linked in
427 coupling to EMFv104_143 at a very high LOD (21.4). This marker is one of
428 three tandemly duplicated products of the EMFv104 primer pair on LG VI.B
429 (for example, EMFv104_143, EMFv104_135, EMFv104_133).

430 **Female function mapping --** Qualitative mapping of female function in
431 MP12×WREN2 indicated that female function was linked to male sterility
432 (Figure 1a). Similar to that seen in *F. chiloensis* (Goldberg *et al.,* 2010), female
433 fertility was dominant to female sterility and mapped in coupling with male
434 sterility in the maternal parent. The QTL analysis of proportion fruit set is
435 consistent with the qualitative mapping in this cross. A major QTL for
436 proportion fruit set was found on LG VI.A in maternal map with a LOD score

437 of 46.8 in MQM (Supplementary Figure 1). This QTL explained 93.2% of the
438 variation in fruit set and its peak was within 1.27 cM of male sterility.

439 In the WREN7×MP10 cross only a quantitative approach to mapping
440 female function was possible. In the maternal map, a QTL that explained 91.3
441 % variation in fruit setting ability co-localized with male sterility on LG VI.B
442 with a LOD score of 47.2 (Supplementary Figure 2). In the paternal map a
443 QTL explaining 10.6% variation for fruit set was linked to CFVCT017 with
444 LOD score of 1.89 and this QTL had a negative additive effect on fruit set.
445 However, no significant epistatic interaction was found between this QTL and
446 the female function QTL in the maternal parent when tested using ANOVA
447 ($P$> 0.05).

448 ***Macrosynteny of sex-determining chromosomes***

449 We compared the linkage map of the male sterility-determining chromosomes
450 to their homoeologs in *F. virginiana* and *F. chiloensis* and in hermaphrodite
451 diploid cross (dLG 6) (Figure 1). This revealed macrosynteny and only small
452 differences in colinearity among these LGs. For instance, while the linkage
453 map of the sex-determining chromosomes from both *F.× ananassa* subsp.
454 *cuneifolia* crosses share 3 to 5 markers with LG 6 in diploid Fv×Fn cross, the
455 order of EMFv160AD and EMFn153 in the MP12×WREN2 cross agrees with

456 the order in *F. chiloensis* but not Fv×Fn (Figure 1a). The synteny with *F.*
457 *chiloensis* is in accord with fact that the maternal parent (MP12) has the *F.*
458 *chiloensis* cytotype. A notable difference, however, is the greater estimated
459 distance (30 cM vs.7 cM) between the marker EMFn153 and male sterility in
460 the *F.*× *ananassa* subsp. *cuneifolia* cross than in the *F. chiloensis* cross
461 (Goldberg *et al.,* 2010). In WREN7×MP10, male sterility mapped above the
462 three markers (ARSFL022, CFVCT017 and EMFn153) that subtended male
463 sterility in MP12 and very close to a set of tandemly duplicated EMFv104
464 markers on LG VI.B. This location is not in agreement with the expected
465 location of male sterility (on the tip of LG VI.C) given the *F. virginiana*
466 cytotype of the maternal parent (WREN7). The tandemly duplicated set of
467 EMFv104 markers, however, is similar to their arrangement on LG VI.B in *F.*
468 *chiloensis* (Figure 1b; Goldberg *et al.,* 2010) an alignment not observed in *F.*
469 *virginiana* (Figure 1b; Spigler *et al.,* 2010). Two markers located below male
470 sterility (ARSFL022, CFVCT017) are shared by LG VI.B in *F. chiloensis* and
471 two (ARSFL022, CO816667) by LG VI.B in *F. virginiana* (Figure 1b).

472 ***Sexual dimorphism***

473 Proportion fruit set and proportion seed set were strongly sexually dimorphic
474 in the progeny of both *F.* ×*ananassa* subsp. *cuneifolia* crosses while ovule
475 number was dimorphic in one cross (Table 2). In contrast, sexual dimorphism

476 was nonexistent in anther number, runner number and leaf number, and was
477 only weakly ($P < 0.08$) dimorphic for flower number in the WREN7× MP10
478 cross (Table 2). The direction of dimorphism for flower number (male-sterile >
479 male-fertile morphs) was opposite of that published for either of the pure
480 species crosses (male-sterile < male-fertile morphs; Ashman *et al.,* 2011;
481 Spigler *et al.,* 2011), as was the direction of dimorphism for ovule number in
482 the WREN7× MP10 cross (male-sterile < male-fertile morphs; Ashman and
483 Hitchens, 2000; Spigler *et al.,* 2011).

484

485 **Discussion**

486

487 ***Cytotypic composition of F. ×ananassa* subsp. *cuneifolia hybrid populations***

488 Our results show for the first time that natural hybrid populations of *F.*
489 *×ananassa* subsp. *cuneifolia* contain cytotypes from both maternal species but
490 that individual populations show a strong bias towards one cytotype or the
491 other. The presence of both cytotypes suggests that both species contributed as
492 maternal parents to the studied populations, and that admixture is still
493 occurring, or that there was paternal leakage, although the latter is very rare in
494 flowering plants (e.g., Mc Cauley *et al* 2007) and was not detected in our

495 crosses (unpubl. data). Extreme bias in cytotypes is not uncommon in hybrid
496 species, and can reflect the signature of the historical range of the dominant
497 maternal species or of selective forces that favor one cytotype (Arnold *et al.,*
498 2010; Minder *et al.,* 2007). The two populations here are 13km apart and are
499 both near the margin of *F. virginiana*'s range, although the Mary's Peak
500 population is closer to the coast. On the other hand, the Mary's Peak
501 population is on the highest peak (1249 m) in the Oregon Coast Range, an
502 unusual habitat for *F. chiloensis* subsp. *lucida* and *pacifica*, and thus could
503 reflect a remnant population, or a long distance migrant, which was then
504 subject to gene flow from *F. virginiana*. Broad sampling is underway to
505 determine whether there is a geographic or edaphic pattern to the dominance of
506 maternal cytotype and to assess the extent and direction of admixture in the
507 nuclear genome across the hybrid zone. Such work will provide a landscape
508 assessment of introgressive hybridization between *F. chiloensis* and *F.*
509 *virginiana*, as well as indicate the potential for spread of new sterility alleles
510 across the hybrid zone to contribute to turnover in sex-determining
511 chromosomes in the two parental octoploid species (Veltsos *et al.,* 2008).
512 Reciprocal transplant and selection analyses will also provide powerful means
513 to assess fertility selection on male and female function (for example, Spigler

514 and Ashman, 2011) in the context of the sex expression variation provided by
515 the hybrid zone (see below).

516

517 ***Variation in sex determination regions of F. ×ananassa* subsp. *cuneifolia***
518 ***crosses***

519 Although there was variation in the location of sex function loci, major
520 QTL were found in HG VI, confirming the important role of this HG in sex
521 determination in the octoploid *Fragaria* species. A similar consistent
522 involvement of a specific LG has been seen among *Populus* species, that is, all
523 sex-containing *Populus* linkage maps show sex determination on LG XIX,
524 although in different positions and sometimes segregating in different parental
525 genders (Pakull *et al.,* 2009).

526 *Fragaria ×ananassa* subsp. *cuneifolia* is similar to both its parental
527 species in that male sterility mapped in the female parent and was dominant to
528 male fertility in both crosses. In one cross (MP12×WREN2) male sterility
529 mapped to the location predicted by maternal cytotype (*F. chiloensis*), but in
530 the other cross (WREN7×MP10) male sterility mapped to a novel location.
531 The novel location could be the result of transposition during hybrid formation
532 as such rearrangements can be common in some hybrids (for example, Lai *et*

533 *al.,* 2005). Alternately, it is possible that male sterility has a different location
534 in the western subspecies of *F. virginiana* subsp. *platypetala* than in the
535 eastern *F. virginiana* subsp. *virginiana*. Current hypotheses for the
536 biogeographic history of *F. virginiana* suggest that it, like many species in
537 North America, may have been separated into two vicarious groups as the
538 result of uplifting of the Rocky Mountains (Staudt, 1999). Using
539 morphological and RAPD data, Harrison *et al*. (1997) concluded that *F.*
540 *virginiana* subsp. *platypetala* was substantially differentiated from the rest of
541 *F. virginiana,* and was more closely related to *F. chiloensis* than to *F.*
542 *virginiana* subsp. *virginiana*. Staudt (1999) also suggested that *F. virginiana*
543 subsp. *platypetala* may itself been derived from recent hybrids of *F. virginiana*
544 and *F. chiloensis.* While there is little consensus regarding these designations
545 (Hancock *et al.,* 2004), data to date do fuel speculation that gene flow between
546 *F. virginiana* and *F. chiloensis* could be responsible for the dynamic nature of
547 the sex-determining region. Future work mapping sex determination within *F.*
548 *virginiana* subsp. *platypetala* and work underway characterizing the extent of
549 admixture in the nuclear genome across the hybrid zone will help resolve these
550 issues.

551 Female function was found to map to a single region (and female
552 sterility was recessive to female fertility) in one cross but mapped to two

553 regions in the other cross. A single major QTL for female function in
554 MP12×WREN2 was linked to male function on VI.A, consistent with the
555 location and linkage phase in the genetic map of *F. chiloensis* (male sterility in
556 coupling with female fertility; Goldberg *et al.,* 2010), but the linkage between
557 the two was not as tight and recombinants were formed. In this cross, we can
558 infer the genotypes of the parents as *AG*|*ag* for the maternal parent and *ag*|*ag*
559 for the paternal parent, creating a majority of non-recombinant male and
560 female progeny, and a smaller fraction of recombinant progeny, that is, the low
561 fruit-setting females and hermaphrodites (Figure. 2a; Supplementary Table
562 1A). This also conforms to Charlesworth and Charlesworth's (1978) two-locus
563 model for sex determination (also see Spigler *et al.* 2008).

564 Not only was the location of male sterility in WREN7×MP10 not as
565 predicted, but the finding of two QTL for female sterility was unexpected.
566 Multiple QTL affecting female function could indicate that 1) rearrangements
567 of loci in the hybrid has led to novel placements of existing loci or 2) the
568 hybrid has unique female-sterility alleles. We discuss the present results in the
569 context of these two possibilities.

570 The QTL for female function linked to EMFv104 on VI.B clearly
571 represents a new location relative to published maps of parental species and
572 coincides with the QTL for male sterility, a location that could reflect either a

573 transposition or new sterility genes as the result of hybridization, or as
574 mentioned above a difference in the location of the sex-determining genes
575 derived from the western subspecies of *F. virginiana*. If transposition
576 occurred, it may have involved linked genes or a single gene that has
577 pleiotropic effect on both male and female function. Determining this would
578 require finer mapping and a larger population size, as there is no clear evidence
579 in our current map for any other transposed markers in this region.

580 The QTL for fruit set overlapping CVCT017 (and EMFn153) on LG
581 VI.B in the paternal parent, could also represent a transposition of a QTL,
582 possibly from VI.A, as the paternal parent has *F. chiloensis* cytotype, or given
583 the allopolyploid origin (for example, AA A'A'BB B'B') of the parental
584 species could represent an orthologous QTL affecting fruit set on VI.B, that is,
585 from a different genome donor, or a novel sterility locus. An orthologous QTL
586 is a viable hypothesis because a recent study of the cultivated hybrid
587 strawberry (*F.* ×*ananassa* subsp. *ananassa*) concluded that ~25% of QTL for
588 fruit traits were at orthologous positions on a different homoeologous LGs, that
589 is, were putative 'homoeo-QTL' (Lerceteau-Köhler *et al.,* 2012), and these
590 may be segregating in the natural hybrid species as well. Moreover, there is no
591 evidence for transposition of other markers which might be expected if
592 nonhomologous recombination triggered the QTL at this position. In fact, there

593 was only one confirmed case of novel duplication (CFVCT006 on LG VI.A) in
594 our map of the natural hybrid.

595 However, sterility can directly result from hybridization (reviewed in
596 Maheshwari and Barbash, 2011). Of the many possible mechanisms, two have
597 some support in plants: 1) negative genetic interactions between two or more
598 loci fixed in the two parental species (for example, Dobzhansky-Muller
599 Incompatibilities; Fishman and Willis, 2001; Maheshwari and Barbash, 2011;
600 Moyle and Nakazato, 2010), and 2) rearrangements (for example, pollen
601 sterility QTL were located near rearrangement breakpoints in artificial
602 *Helianthus* hybrids; Lai *et al.,* 2005). Both are possibilities here because the
603 characteristics of sex chromosomes, in particular, are thought to make them
604 'hotspots' for speciation genes (Qvarnstrom and Bailey, 2008), and
605 rearrangements have been found in the octoploid species that involve LGs in
606 HG VI (Sargent *et al.,* 2012; Spigler *et al.,* 2010). The negative additive
607 effect of the fruit set QTL in paternal parent (reduces fruit set by 10%) and the
608 skewed segregation of the nearest marker (Supplementary Figure 1) might
609 indicate the involvement of a Dobzhansky-Muller Incompatibility. Further
610 crosses are required to resolve whether female sterility at this location is the
611 manifestation of different genes related to speciation in this region.

612 In the context of past models (Goldberg *et al.,* 2010; Spigler *et al.,*
613 2008), the diversity of sexual phenotypes in the WREN (Figure 2), including
614 low fruiting females and moderately fruiting hermaphrodites, can be accounted
615 for by recombination between the two female function QTL on LG VI.B
616 (Supplementary Table 1B).
617

618 ***Sexual dimorphism in hybrid F. ×ananassa* subsp. *cuneifolia***

619 Aside from components of female function (fruit set and seed set), other traits
620 that have been found to be significantly sexually dimorphic in the pure species
621 were either not dimorphic in the hybrid crosses (anther number, runner number
622 and leaf number, flower number; Ashman *et al.,* 2011; Spigler *et al.,* 2011;
623 Staudt, 1999) or if they were dimorphic the direction of dimorphism was in the
624 opposite to what has been observed in the pure species (ovule number and
625 flower number in the WREN7×MP10 cross; Table 2). These results suggest
626 that hybrids experience some disruption of dimorphism in secondary sexual
627 traits. Loss of dimorphism in hybrids has been interpreted as reflecting
628 breakdown of modifiers or regulatory elements that are responsible for
629 dimorphism and suggests that these traits were originally expressed in both
630 sexes but that modifier evolved afterwards (Coyne *et al.,* 2008). This

631 interpretation may also apply to the traits studied here because all are
632 expressed in both sexes to some degree and modifiers may be linked to sex
633 determining region (Spigler *et al.,* 2011). In one of the few studies to assess
634 dimorphism after hybridization, Zluvova *et al*. (2005) found females from
635 crosses between dioecious *S. latifolia* and hermaphrodite *S. viscosa* had anthers
636 that developed beyond the stage characteristic for *S. latifolia* females. Thus,
637 the hybrid had less dimorphism in anther size than the pure species, and they
638 interpreted this as evidence that the suppression of anthers was brought about
639 by a recessive allele that could be rescued by the genome of *S. viscosa*. They
640 did not find the same effect for fruit set in the males, however. Our work is far
641 from conclusive, but does suggest that studies of the effects of hybridization on
642 sexual dimorphism in plants will be useful for gaining insight into the genetic
643 underpinnings and evolutionary processes (Coyne *et al.,* 2008) and will be
644 exemplary complements to studies of early sex chromosome evolution in
645 plants.

646

647 ***Conclusion***

648 The work presented here suggests that hybrid zones of
649 dioecious/subdioecious plants are valuable and underutilized resource for

650 studying the ecology and evolution of sex chromosomes and sexual
651 dimorphism. Ecological work will be especially valuable to test hypotheses
652 for the spread of new chromosomal sex determination systems across hybrid
653 populations (Veltsos *et al.,* 2008). In addition, surveys of sexual dimorphism in
654 natural hybrid zones, along with the creation of experimental hybrids will
655 provide novel insight into the evolution and control of sexual dimorphism
656 during the evolution of sex chromosomes.

657 **Data archiving**

658 Sequence data have been submitted to GenBank: JX064433- JX064440;
659 JX064449-JX064456

660 Genotype data have been submitted to Dryad: doi: xxx

661 **Conflict of interest**

662 The authors declare no conflict of interest.

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669

670 Supplementary Information accompanies the paper on Heredity website
671 (http://www.nature.com/hdy)

672 Table 1 Frequency of *F. chiloensis* and *F. virginiana* cytotypes found in two

673 *Fragaria ×ananassa* subsp. *cuneifolia* populations.

| | | Gene | | Consensus | |
|---|---|---|---|---|---|
| Population | Cytotype | *pet*D | *ndh*F | Total | % |
| MP | *F. chiloensis* | 29 | 29 | 29 | 93.5 |
| | *F. virginiana* | 2 | 2 | 2 | 6.5 |
| WREN | *F. chiloensis* | 2 | 2 | 2 | 6.6 |
| | *F. virginiana* | 30 | 27 | 30 | 93.4 |

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681 Table 2 Trait means (± s.e.) for male-sterile and male-fertile F1 progeny in the *Fragaria ×ananassa* subsp. *cuneifolia*
682 map crosses and sexual dimorphism (SD) indices.

683

| | **MP12 × WREN2** | | | | **WREN7 × MP10** | | | |
|---|---|---|---|---|---|---|---|---|
| Trait | Male sterile | Male fertile | $P$[1] | SD index[2] | Male sterile | Male fertile | $P$[1] | SD index[2] |
| Proportion fruit set | 0.89 (0.20) | 0.04 (0.11) | **<0.0001** | 35.81 | 0.84 (0.11) | 0.16 (0.11) | **<0.0001** | 43.25 |
| Proportion seed set | 0.74 (0.25) | 0.13 (0.30) | **<0.0001** | 14.59 | 0.88 (0.10) | 0.64 (0.34) | **<0.0001** | 7.43 |
| Flower number | 7.0 (3.6) | 8.2 (3.8) | 0.16 | 2.02 | 16.0 (5.3) | 14.0 (5.2) | **0.08** | 2.55 |
| Ovule number | 52.0 (11.9) | 50.4 (15.0) | 0.58 | 0.79 | 55.0 (11.6) | 64.7 (14.2) | **<0.005** | 5.06 |
| Anther number | 20.7 (4.1) | 20.2 (3.7) | 0.53 | 0.89 | 20.2 (1.8) | 20.2 (1.3) | 0.96 | 0.08 |
| Leaf number | 10.8 (4.4) | 11.7 (4.6) | 0.35 | 1.33 | 18.0 (7.1) | 18.5 (7.7) | 0.75 | 0.46 |
| Runner number | 6.3 (2.1) | 6.4 (2.2) | 0.72 | 0.51 | 5.0 (2.2) | 5.1 (2.3) | 0.88 | 0.21 |

684
685 [1] Progeny means were evaluated using *t*-test and $P$ <0.05 indicated in bold.
686 [2] SD index = $(x_{MS} - x_{MF})$|/ [($SE_{MS} + SE_{MF}$)/2], where $x$ and SE are the mean and standard error respectively, for each
687 trait male-sterile (MS) and male-fertile (MF) progeny.

688 **Titles and legends to figures**

689

690 **Figure 1** Comparison of male sterility carrying sex determining chromosomes
691 from both cross (a & b) to the corresponding homoeologs of *F. virginiana*
692 (Spigler *et al.,* 2010) and *F. chiloensis* (Goldberg *et al.,* 2010) and the
693 homoeologous LG6 in hermaphroditic diploid cross (Fv x Fn, adapted from
694 Sargent *et al.,* 2009) from previously published maps. SSRs on the
695 corresponding homoeologs in octoploids are connected by lines and are
696 highlighted in bold to indicate synteny with the diploid reference
697 homoeologous linkage group (dLG6). Phenotypic trait marker representing the
698 putative determining sex loci (male sterility/female fertility) are indicated in
699 bold and italicized. Markers denoted by asterisk had skewed segregation ratios
700 ($0.0001 < P < 0.01$).

701 **Figure 2** Frequency histograms of proportion fruit set (female function) for
702 male sterile (MS) and male fertile (MF) progeny of *F. ×ananassa* subsp.
703 *cuneifolia* crosses. a) MP12× WREN2. b) WREN7 × MP10 cross.

704

705 **Supplementary Figure 1** Parental linkage map of homoeologous group VI
706 for MP12 × WREN2 map cross. Linkage groups identified as distinct from one
707 another (see results) are given names following Spigler *et al.,* 2010. The LGs
708 are named according to their homoeologous group using Roman numeral VI

709 and a letter (A–C). Associations between pairs of linkage groups within the
710 parental maps were identified either through SCL values between markers in
711 the two groups at LOD >4 (indicated by dashed line) or through comparison to
712 the putative homoeolog in the other parent map. Additional linkage groups that
713 did not group with any of the four major groups are named using Arabic
714 numbers. Lower case "m" and "p" are used to indicate the maternal and
715 paternal maps respectively. The location of QTL for fruit set is denoted as a
716 black bar. A major QTL for fruit set is mapped in the maternal parent LG
717 VI.A-m. The location of the qualitative trait marker "Male sterility" is
718 indicated in bold. Markers that had co-segregating products are denoted with
719 'T' tick mark rather than a simple horizontal tick mark. Marker names denoted
720 by asterisk showed segregation ratios that deviated from the expected
721 Mendelian ratio ($0.0001 < P < 0.01$), and the up and down arrow denote an
722 under or overrepresentation of heterozygous gametes among genotypes in the
723 mapping population.
724
725 **Supplementary Figure 2** Parental linkage map of homoeologous group VI
726 for WREN7 × MP10 map cross. Linkage groups identified as distinct from one
727 another (see results) are given names following Spigler *et al*., 2010. The LGs
728 are named according to their homoeologous group using Roman numeral VI

and a letter (A–D), and the names were assigned first in the maternal map according to largest to smallest linkage group (in cM) within the HG VI then accordingly to the corresponding homologs in the paternal map. Associations between pairs of linkage groups within the parental maps were identified either through SCL values between markers in the two groups at LOD >4 (indicated by dashed line) or through comparison to the putative homoeolog in the other parent map. Additional linkage groups that did not group with any of the four major groups are named using Arabic numbers. Lower case "m" and "p" are used to indicate the maternal and paternal maps respectively. The location of QTL for fruit set is indicated using a black bar in the maternal parent on LG VI.B-m, similarly a minor QTL for fruit set is located in the paternal parent on LG VI.B-p. The location of the qualitative trait marker "Male sterility" is indicated in bold. Markers that had co-segregating products are denoted with 'T' tick mark rather than a simple horizontal tick mark. Marker names denoted by asterisk showed segregation ratios that deviated from the expected Mendelian ratio ($0.0001 < P < 0.01$), and the up and down arrow denote an under or overrepresentation of heterozygous gametes among genotypes in the mapping population.

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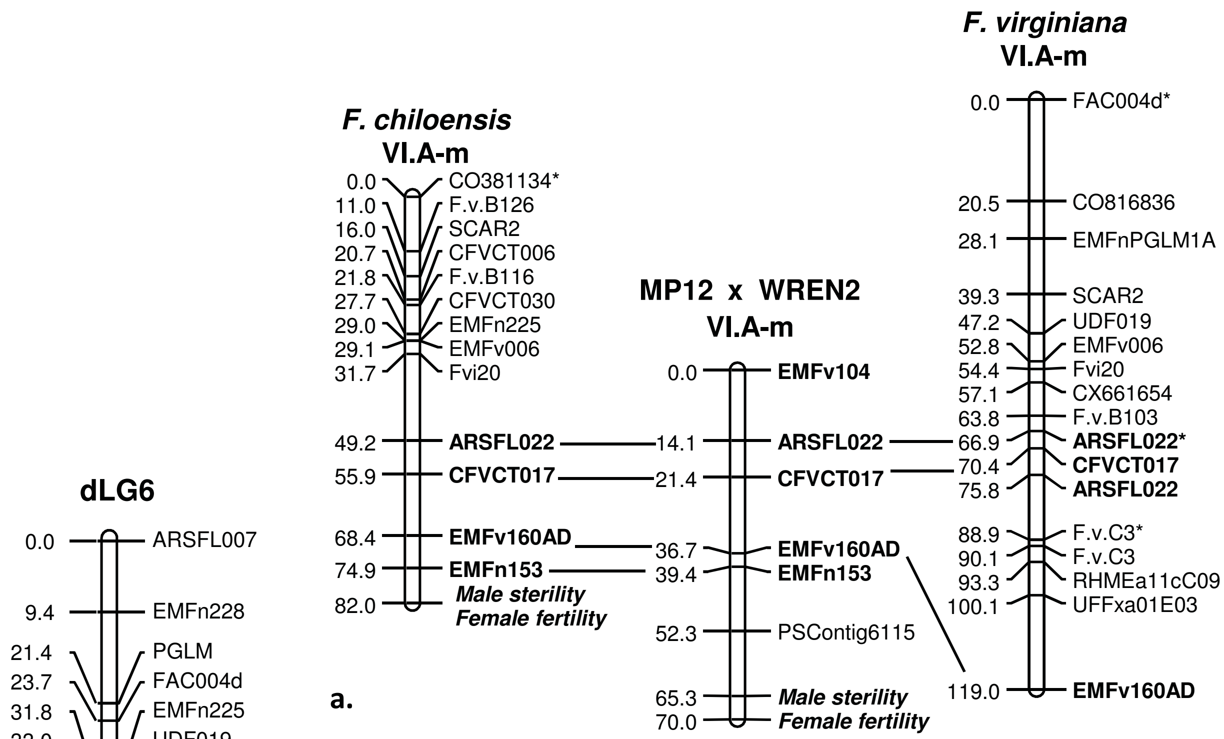
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9.4 EMFn228
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23.7 FAC004d
31.8 EMFn225
33.0 UDF019
34.8 CFVCT030
35.1 Fvi20
35.5 EMFv104
35.8 EMFv006
40.6 ARSFL022
42.4 CFVCT017
50.1 EMFn153
50.9 EMFv160AD
64.7 UFFxa01E03
(Fv x Fn)
F. chiloensis
VI.A-m
0.0 CO381134*
11.0 F.v.B126
16.0 SCAR2
20.7 CFVCT006
21.8 F.v.B116
27.7 CFVCT030
29.0 EMFn225
29.1 EMFv006
31.7 Fvi20
49.2 ARSFL022
55.9 CFVCT017
68.4 EMFv160AD
74.9 EMFn153
82.0 Male sterility
Female fertility
MP12 x WREN2
VI.A-m
0.0 EMFv104
14.1 ARSFL022
21.4 CFVCT017
36.7 EMFv160AD
39.4 EMFn153
52.3 PSContig6115
65.3 Male sterility
70.0 Female fertility
F. virginiana
VI.A-m
0.0 FAC004d*
20.5 CO816836
28.1 EMFnPGLM1A
39.3 SCAR2
47.2 UDF019
52.8 EMFv006
54.4 Fvi20
57.1 CX661654
63.8 F.v.B103
66.9 ARSFL022*
70.4 CFVCT017
75.8 ARSFL022
88.9 F.v.C3*
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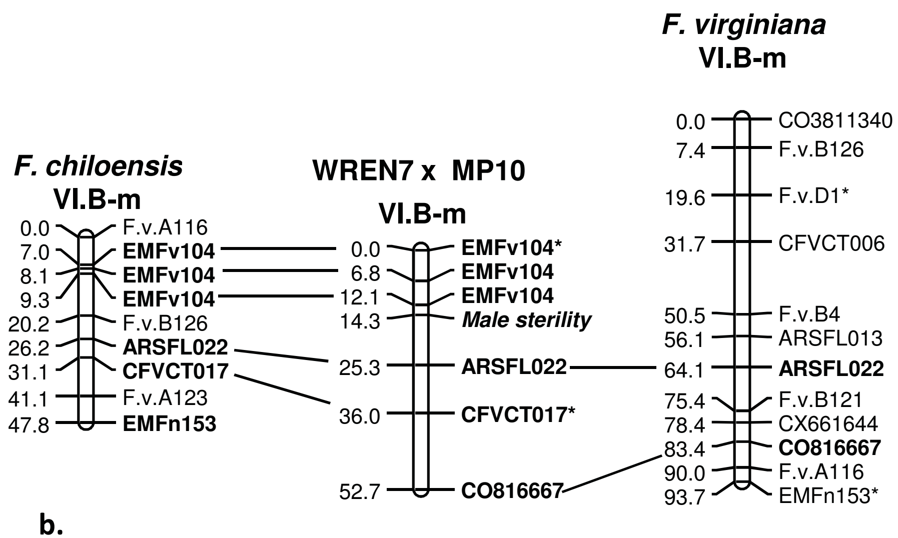
100.1 UFFxa01E03
119.0 EMFv160AD
a.


F. chiloensis
VI.B-m
0.0 F.v.A116
7.0 EMFv104
8.1 EMFv104
9.3 EMFv104
20.2 F.v.B126
26.2 ARSFL022
31.1 CFVCT017
41.1 F.v.A123
47.8 EMFn153
WREN7 x MP10
VI.B-m
0.0 EMFv104*
6.8 EMFv104
12.1 EMFv104
14.3 Male sterility
25.3 ARSFL022
36.0 CFVCT017*
52.7 CO816667
F. virginiana
VI.B-m
0.0 CO3811340
7.4 F.v.B126
19.6 F.v.D1*
31.7 CFVCT006
50.5 F.v.B4
56.1 ARSFL013
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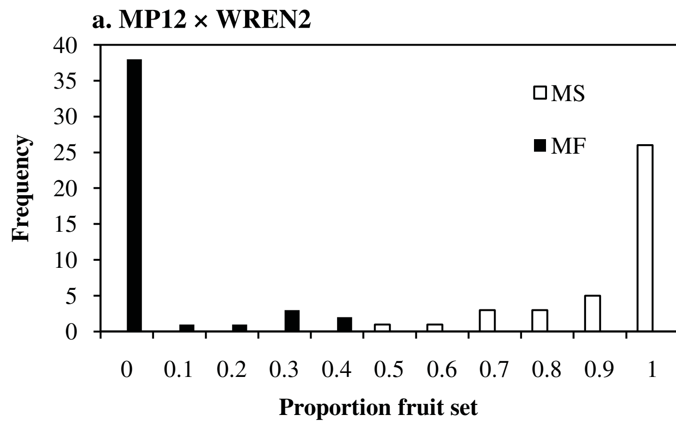
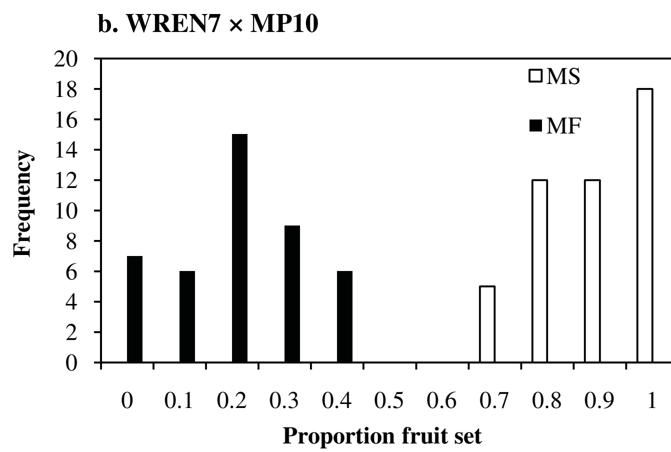
b.


a. MP12 × WREN2
Frequency
Proportion fruit set
MS
MF
0
5
10
15
20
25
30
35
40
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1


b. WREN7 × MP10
Frequency
Proportion fruit set
MS
MF
0
2
4
6
8
10
12
14
16
18
20
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1