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Characterization of mixed disomic and polysomic inheritance in the octoploid strawberry (*Fragaria* × *ananassa*) using AFLP mapping

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Abstract A two-way pseudo-testcross strategy, combined with Single Dose Restriction Fragment (SDRF) marker analysis, was used for genetic mapping in the octoploid cultivated strawberry *Fragaria* × *ananassa* (2n = 8*x* = 56). Based on a 113 full-sib progeny from a cross between the variety Capitola and the clone CF1116, we generated two parental maps using Amplified Fragment Length Polymorphism (AFLP) markers. Ninety two percent of the markers (727 out of 789) showed ratios corresponding to simplex markers (the majority being SDRF markers), and 8% (62 out of 789) fitted a multiplex ratio. Linkage maps were first established using SDRF markers in coupling phase. The female map comprised 235 markers distributed among 43 co-segregation groups, giving a map size of 1,604 cM. On the male map, 280 markers were assigned to 43 co-segregation groups, yielding a map size of 1,496 cM. Once the co-segregation groups were established, their association was tested using repulsion-phase markers. In total, taking into account associations representing the same linkage groups, 30 linkage groups were detected on the female side and 28 on the male side. On the female map, 68.3% of the pairwise marker linkages were in coupling versus 31.7% in repulsion phase, and the corresponding figures on the male map were 72.2% and 27.8%, respectively. In addition, both groups linked only in the coupling phase and groups linked in the repulsion phase were characterized. The observations suggest that the meiotic behavior of the *F.* × *ananassa* genome is neither fully disomic nor fully polysomic, but rather mixed. The genome may not be as completely diploidized as previously assumed.

Keywords AFLPs · *Fragaria* × *ananassa* · Linkage map · Polyploidy · Strawberry

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Introduction

The cultivated strawberry *Fragaria* × *ananassa* (2n = 8*x* = 56) belongs to the Rosaceae family. It was described and named in 1766 by the French botanist Duchesne. The species originated from chance hybridizations that occurred in some European botanical gardens between two octoploid species, *Fragaria virginiana* and *Fragaria chiloensis*, imported from the New World at the end of the 16th century and the mid-18th century, respectively. The genomic constitution of the parental species remains unknown. Based on cytological experiments, several genomic formulas have been proposed for *F.* × *ananassa*. The first was AABBBBCC (Federova 1946), but it was later modified to AAA′A′BBBB (Senanayake and Bringhurst 1967) and more recently to AAA′A′BBB′B′ (Bringhurst 1990), based on data suggesting it has a highly diploidized genome. However, its composition is still being debated, and the type of meiotic behavior (poly- or di-somic) remains unclear, although determination of these characteristics is important for understanding the evolution of the genus and for developing appropriate breeding strategies.

The segregation pattern analysis of molecular markers is considered a reliable method for determining meiotic behavior (see, for instance, Qu and Hancock 1995). Two methods have been proposed to distinguish between disomy and polysomy: analysis of the ratio of single to multiple dose markers (Da Silva et al. 1993), and comparison of the number of loci linked in coupling versus the repulsion phase (Sorrells 1992; Wu et al. 1992). Low frequencies of multi-dose or the absence of repulsion-phase linked markers, are found in polysomy. Analysis of repulsion-phase markers through mapping has

been greatly simplified in polyploid species by the use of single-dose restriction fragments (SDRFs), as proposed by Wu et al. (1992). An SDRF marker is defined as a DNA fragment that is present on just one chromosome in one of the parents and can be identified by a 1:1 segregation, regardless of the organism's ploidy. Such markers have been used in several species, e.g. cassava (Fregene et al. 1997), alfalfa (Diwan et al. 2000) and rose (Rajapakse et al. 2001), and have generated interesting conclusions concerning genome behavior in various plants, e.g. sugar cane (Hoarau et al. 2001).

At the molecular level, most of the studies using molecular markers reported so far for the genus *Fragaria* have been carried out on the diploid *Fragaria vesca*. These studies have included segregation analyses (e.g. Arulsekar et al. 1981), punctual linkage-analysis between specific traits and isozymes (e.g. Williamson et al. 1995; Yu and Davis 1995), ISSR-PCR markers (Cekic et al. 2001) or genes (Deng and Davis 2001), and investigations leading to the first linkage map of the genus (Davis and Yu 1997). At the octoploid level, there has been only one such published study, reporting linkage between RAPD markers and the resistance gene to *Phytophthora fragariae Rpf1* (Haymes et al. 1997). The characterized region encompassed 22 cM and displayed disomic behavior.

In the study presented here, we developed linkage maps of the cultivated strawberry *F.* × *ananassa* using AFLPs as SDRF markers in order to improve our knowledge of the genome and our understanding of its meiotic behavior.

Materials and methods

Plant material

To develop the linkage map we used a progeny of 119 individuals from a single full-sib family. This population size was chosen to allow us to distinguish a 1:1 ratio from all other segregation ratios at the 99% confidence level (Wu et al. 1992). The female parent (Capitola: CA75.121-101 × Parker) is a selection from the University of Davis (California, USA) and the male, CF1116 [Pajaro × (Earlyglow × Chandler)], is a reference from the Inter-Regional Centre for Research and Experimentation on strawberry (Ciref, France). The cross was chosen for its suitability for disease resistance and fruit quality analysis. The mapping progeny was obtained in 1999.

AFLPs

DNA was extracted using a Dneasy Plant Mini Kit from Qiagen, starting with about 90 mg of young leaves ground in liquid nitrogen.

The digestion and ligation steps of the AFLP analysis were performed on 125 ng of DNA using an AFLP Core Reagent kit from Gibco BRL, Life Technologies. The following steps were adapted from Vos et al. (1995), following a two-step amplification strategy using *Eco*RI and *Mse*I primers. The first amplification was carried out with primers containing one selective nucleotide (*Eco*RI+A and *Mse*I+C) in a total volume of 50 μl with 1× reaction buffer (Sigma), 0.2 mM of each dNTP, 0.3 μM of each primer, 1.5 mM $MgCl_2$, 1 U of *Taq* DNA polymerase (Sigma) and 5 μl of the ligation product, diluted 10-fold. This amplification was carried out according to Vos et al. (1995), except that 28 cycles were used instead of 20. The 30-fold diluted products were then used as templates for the second amplification, using primers containing three selective nucleotides (*Eco*RI+3/*Mse*I+3), including one *Eco*RI+3 primer labeled with (γ-^{33}P) ATP. These steps were carried out according to Vos et al. (1995). Following the amplification, reaction products were mixed with an equal volume of 98% formamide, 10 mM of EDTA, bromophenol blue and xylene cyanol. After denaturation, 4 μl of each sample was loaded on a 4.5% denaturing polyacrylamide gel that had been pre-equilibrated in TBE (0.5 ×) buffer at a constant 95 W for 30 min. The reaction products were then separated, under the same conditions, for 110 min. Following the electrophoresis, the gel was dried and exposed to a Kodak Biomax MR X-ray film. Then, two independent readers visually recorded the presence (or absence) of clearly visible bands.

Other types of markers

In addition to the AFLP markers, two putative genes of *F.* × *ananassa* were mapped, alcohol transferase (AAT) and dihydroflavonol 4-reductase (DHFR), using the following pairs of primers:

–
AAT_FOR: 5′-GTACTATCCACTCTCTGGAAGG-3′ and
–
AAT_REV: 5′-TAAGTTCACTGCCTGGGC-3′ for AAT,
–
DHFR_FOR: 5′-GGCTCGTCATGAGACTCCTC-3′ and
–
DHFR_REV: 5′-GTGAATCGTGGCATCGTG-3′ for DHFR.

These primer pairs were based on sequences from GenBank accession numbers AF193789 (Aharoni et al. 2000) and AF029685 (Moyano et al. 1998), respectively.

Each amplification began with 4-min denaturation at 94 °C, followed by 35 cycles of 45 s at 94 °C, 1 min at 52 °C (for AAT; 1 min at 55 °C for DHFR) and 2 min at 72 °C, followed by a final elongation step of 10 min at 72 °C. The amplification products were separated on 2% agarose gels and recorded visually.

In addition, the *Prunus persica* microsatellite BPPCT028 was used to evaluate the possible presence of selfings among the progeny (see Dirlewanger et al. 2002 for the amplification conditions and sequence).

Linkage-map construction

Each polymorphic marker was tested by χ^2 analysis for goodness of fit ($\alpha \leq 0.01$) to the segregation ratios expected for simplex (single-dose) and multiplex markers under both disomic and polysomic inheritance (see Table 1). For simplex markers that differ between the two parents (i.e. presence in one and absence in the other), the expected segregation ratio is 1:1 (SDRF markers) whatever the chromosome pairing (Wu et al. 1992). For simplex markers present in both parents, the expected segregation ratio is 3:1 in both disomic and polysomic inheritance. For multiplex markers, several expected segregation ratios in disomic and polysomic inheritance deduced in the octoploid context from Da Silva and Sobral (1996) were tested.

The linkage-map development strategy combined the use of undistorted Single-dose restriction fragments (SDRFs) and the double pseudo test- cross strategy developed by Grattapaglia and Sederoff (1994). The pseudo test-cross configurations Aaaaaaaa × aaaaaaaa (presence of a band in Capitola and absence in CF1116) and aaaaaaaa × Aaaaaaaa (absence of a band in Capitola and presence in CF1116) were kept to develop the female and male linkage maps, respectively.

Due to the difficulty of accurately detecting repulsion-phase linkage in cases of polyploids with polysomic inheritance, as described in Wu et al. (1992) and detailed recently in Qu and Hancock (2001), a two-step mapping procedure was applied (Grivet

et al. 1996; Fregene et al. 1997). First, linkage analysis was performed with MAPMAKER/EXP 3.0 (Lander et al. 1987) using coupling markers with a LOD $\geq$ 5.0 and a recombination frequency of $\theta \leq 0.35$. The groups of linked markers in coupling phase are referred to below as co-segregation groups. The recombination frequency value was chosen according to Wu et al. (1992), who showed that the maximum detectable recombination fraction for detecting coupling linkage with a family size of 110 was 0.389. The LOD value was then decreased to 3.0 to test the association between co-segregation group ends. The linkage was accepted when one marker from a group was linked to more than one marker from another group. Possible genotyping errors were checked before map construction using the error detection system in MAPMAKER/EXP 3.0. In a second step, the linkage between the coupling- and repulsion-phase markers was tested by pairwise comparisons using MAPMAKER/EXP 3.0 with a LOD value of 5.0 and then with a LOD of 3.0, to evaluate the possibility of more linkages being present. Since the assignment of "1" or "0" to alternate alleles at a locus was arbitrary, the marker matrix had been previously recoded (1 to 0 and 0 to 1). Groups linked in repulsion phase were discerned from the linkages. The preferential chromosome pairing observed in disomic inheritance could be differentiated from the random pairing characteristically observed in polysomic inheritance and where groups remained in the coupling phase. The Kosambi mapping function (Kosambi 1944) was used to convert the recombination fractions into map distances.

The ratio of markers linked in coupling- versus repulsion-phases was tested by χ^2 analysis of goodness of fit ($\alpha \leq 0.01$) for the 1:1 ratio corresponding to disomy, and for the 1:0 ratio observed in cases of polysomy. This ratio was calculated first by pooling all the co-segregation groups and secondly by separately considering each group (in coupling or in the repulsion phase).

Finally, pairwise linkage between single-dose markers segregating in 1:1 and 3:1 ratios was tested using JOINMAP 2.0 (Stam 1993) at a LOD of 5.0 in order to detect associations between male and female co-segregation groups. The 3:1 segregating markers were added to the maps as accessory markers.

The expected genome length was estimated following Hulbert et al. (1988).

Results

Population screening for selfing

Based on a careful analysis of the AFLP profiles, we suspected the presence of contaminants amongst our progeny. This suspicion was later confirmed by segregation analysis of the *Prunus persica* microsatellite (BPPCT028) (data not shown), which revealed the presence of six individuals derived from selfing. Subsequent mapping was therefore carried out on 113 individuals instead of 119.

Repartition of the markers according to dose ratios

Forty AFLP primer combinations (E-AXX/M-CXX) were used for this study. Based on a sample of nine primer combinations, and considering all observed bands (including monomorphic bands), the percentage of bands segregating in the progeny between Capitola and CF1116 was estimated to be about 42% ± 7. The 40 primer combinations generated a total of 890 polymorphic bands with an average of 22 ± 6 polymorphic bands per combination (range, 10 to 40). In addition, two markers (AAT and DHFR) corresponding to the two studied genes were obtained.

The markers were tested for closeness to the various segregation ratios presented in Table 1. The number of AFLP markers in each class is summarized in Table 2. Among the 890 markers, 28 (3.2%) did not fit any of the tested ratios from Table 1 and can be regarded as distorted. For 73 (2, 2 and 69 when the parents were 1/0,

Table 1 Segregation ratios of disomic and polysomic octoploids[a]. 1/0 = presence of the marker on the female side, absence on the male side; 0/1 = absence of the marker on the female side, presence of the marker on the male side; 1/1 = presence of the marker on the female and male sides. ND = Null-dose marker, SD = Single-dose marker, DD = Double-dose marker, TD = Triple-dose marker, 4D = Four-dose marker

	Parental marker type	Di/octo-somic inheritance	Parental configuration	Segregation ratio
Simplex	1/0 or 0/1	Disomic	SD × ND	1:1
		Octosomic	SD × ND	1:1
	1/1	Disomic	SD × SD	3:1
		Octosomic	SD × SD	3:1
Multiplex	1/0 or 0/1	Disomic[b]	DD × ND[b]	3:1
			TD × ND[b]	7:1
			4D × ND[b]	15:1
		Octosomic	DD × ND	11:3
			TD × ND	13:1
			4D × ND	69:1
			>4D × ND	No segregation
	1/1	Disomic[c]	DD × SD	7:1
			TD × SD	15:1
			4D × SD	31:1
			DD × DD	15:1
			TD × DD	31:1
			4D × DD	63:1
			TD × TD	63:1
		Octosomic[c]	DD × SD	25:3
			TD × SD	27:1

[a] Deduced from Da Silva and Sobral 1996

[b] Only segregations with markers present on non-homologous chromosomes (e.g. Aa Aa aa aa) are reported. When the marker is present on homologous chromosomes (e.g. AA aa aa aa) no segregation is observed

[c] Segregation ratios higher than 63:1 are not considered

Table 2 Repartition of the markers to the different segregation ratios

Parental marker type[a]	Total no.	No fit to any segregation ratios[b]	Undefined dose[c]	Dose	
				Simplex	Multiplex[d]
				1:1	
1/0	276	7	2	257	10: 3 ratio **3:1** and 7 ratio **3:1** or 11:3
0/1	338	20	2	291	25: 9 ratio **3:1**, 2 ratio 69:1 and **14** several ratios
				3:1	
1/1	276	1	69	179	27: 20 ratio **7:1** or 25:3 and 7 ratio **31:1** or **63:1** or 27:1
Total	890	28	73	727	62

[a] 1/0 = presence of the marker on the female side, absence on the male side; 0/1 = absence of the marker on the female side, presence of the marker on the male side; 1/1 = presence of the marker on the female and male sides
[b] Markers fitting none of the tested ratios from Table 1
[c] Markers fitting both a simplex or a multiplex segregation ratio and for which the proper dose therefore remains undefined
[d] Ratios in bold correspond to disomic behavior and underlined ratios to polysomic behavior. Ratios that are both bold and underlined correspond to both types of behavior

0/1 and 1/1 respectively) of the 862 remaining markers, their segregation ratio cannot be precisely identified since they could correspond to both simplex and multiplex markers. Ninety two percent of the markers (727 out of 789) fitted simplex ratios and 8% (62 out of 789) fitted multiplex ratios. Among the 727 markers characterized as simplex, the majority (548) were in pseudo-test cross configurations and so were the SDRF markers (257 showing a band on the female side and 291 a band on the male side). They were therefore used to develop the female and male linkage-maps. The 179 remaining simplex markers were present in both parents and fitted a 3:1 ratio, which is observed regardless of whether the inheritance is disomic or polysomic.

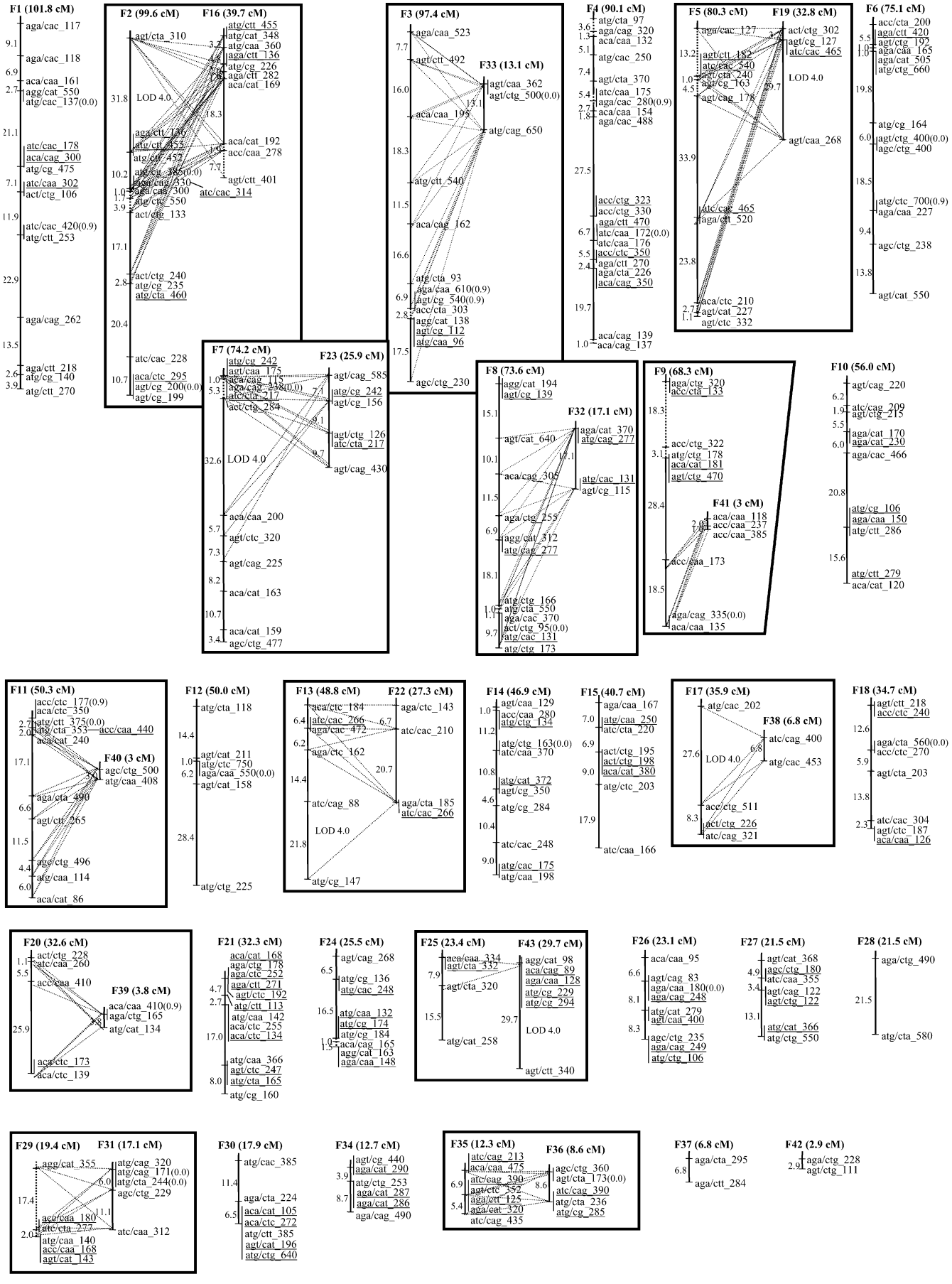
The multiplex markers (62) were divided into two classes according to their inheritance, disomic or polysomic (Table 2). However, since the size of the population was relatively low, most of the multiplex marker segregation-ratios fitted more than one ratio, so the multiplex markers were not further analyzed.

Linkage maps

Female and male linkage-maps were developed based on the SDRF markers in coupling-phase. Out of 257 markers displaying the Aaaaaaaa × aaaaaaaa configuration on the female side, 235 (91.4%) mapped onto 43 co-segregation groups, including four with a LOD value of 4.0. Seven (F28, F32, F37, F38, F40, F42 and F43) of these co-segregation groups comprised only two SDRF markers, but the average number of markers per group was 5.5. The co-segregation group size ranged from 2.9 to 101.8 cM, and the total map size equaled 1,604 cM (Fig. 1). The average marker density was 8.4 (±7.9 cM). The male map included 280 markers out of 293 (aaaaaaaa × Aaaaaaaa configuration; 291 AFLP and two putative genes; 95.9%), which were also assigned to 43 co-segregation groups (three at LOD > 4.0 and one at LOD > 3.0), with a size ranging from 1.0 to 149.2 cM (Fig. 2). Of these, seven groups (M28, M30, M35, M37, M38, M41 and M43) had only two SDRF associated markers whereas the average number of markers per group was 6.5. The experimental size reached 1,496 cM and the average marker density was 6.3 (±7.0).

Once the co-segregation groups had been established, their association was tested using repulsion-phase markers. The association was accepted when more than one linkage in the repulsion-phase was detected. The proportion of linkages between markers in coupling and in the repulsion-phase was then calculated. On the female side, 68.3% of the pairwise linkages were in coupling versus 31.7% in the repulsion-phase (LOD > 3.0), and on the male side 72.2% and 27.8% were in coupling and in the repulsion-phase, respectively. In both cases, the ratios were significantly different from a 1/1 ratio ($P < 0.0001$) and a 1/0 ratio ($P < 0.0001$), corresponding to the proportions of coupling and repulsion linkage expected in preferential pairing and random pairing, respectively. On the female map, 26 co-segregation groups were associated in pairs through repulsion linkages, resulting in 13 linkage groups (Fig. 1). Three co-segregation groups (F10, F24, F26) were in repulsion phase, each with one unlinked marker, and 14 co-segregation groups remained unassociated. Therefore, since one association between two co-segregation groups represented one linkage group, there were 30 linkage groups in total on the female side (13 + 14 + 3). The corresponding analysis for the male map

Fig. 1 Linkage map of the female parent (Capitola). Linkage groups were named by following their size. Uncertain marker orders in the co-segregation groups (alternate orders not ruled out at a LOD = 1) are represented by a *dotted line*. The 3:1 segregating markers are *underlined* and not included in the count of groups with only two markers. Linkage in the repulsion phase between two markers is represented by a *dashed line* (LOD = 3.0). *Boxes* represent linkage groups in the repulsion phase. The genetic distance, in centimorgans, of each marker to its closest neighboring marker is given *in parenthesis*


F1 (101.8 cM)
aga/cac_117
aga/cac_118
aca/caa_161
agg/cat_550
atg/cac_137(0.0)
atc/cac_178
aca/cag_300
atg/cg_475
atc/caa_302
act/ctg_106
atc/cac_420(0.9)
atg/ctt_253
aga/cag_262
aga/ctt_218
atg/cg_140
atg/ctt_270
F2 (99.6 cM)
F16 (39.7 cM)
agt/cta_310
LOD 4.0
aga/ctt_130
atg/ctt_455
atg/cat_348
atg/caa_360
aga/ctt_136
atg/cg_226
aga/ctt_282
aca/cat_169
aca/cat_192
acc/caa_278
agt/ctt_401
atc/cac_314
atg/ctt_453
atg/ctt_452
atg/cg_385(0.0)
aca/caa_330
aga/caa_300
atg/ctc_550
act/ctg_133
act/ctg_240
atg/cg_235
atg/cta_460
atc/cac_228
aca/ctc_295
agt/cg_200(0.0)
agt/cg_199
F3 (97.4 cM)
aga/caa_523
agt/ctt_492
F33 (13.1 cM)
agt/caa_362
agt/ctg_500(0.0)
aca/caa_195
atg/cag_650
atg/ctt_540
aca/cag_162
atg/cta_93
aga/caa_610(0.9)
agt/cg_540(0.9)
acc/cta_303
agg/cat_138
agt/cg_112
atg/caa_96
agc/ctg_230
F4 (90.1 cM)
atg/cta_97
aga/cag_320
aca/caa_132
atg/cac_250
atg/cta_370
atc/caa_175
aga/cac_280(0.9)
aca/caa_154
aga/cac_488
acc/ctg_323
acc/ctg_330
aga/ctt_470
atc/caa_172(0.0)
atc/caa_176
acc/ctc_350
aga/ctt_270
aga/cta_226
aca/cag_350
aca/cag_139
aca/cag_137
F5 (80.3 cM)
F19 (32.8 cM)
aga/cac_127
agt/ctt_182
atc/cac_540
agt/cta_240
aga/cg_163
agt/cag_178
LOD 4.0
act/ctg_302
agt/cg_127
atc/cac_465
agt/caa_268
atc/cac_465
aga/ctt_520
aca/ctc_210
agt/cat_227
agt/ctc_332
F6 (75.1 cM)
acc/cta_200
aga/ctt_420
agt/ctg_192
aga/caa_165
aga/cat_505
atg/ctg_660
atg/cg_164
agt/ctg_400(0.0)
agc/ctg_400
atg/ctc_700(0.9)
aga/caa_227
agc/ctg_238
agt/cat_550
F7 (74.2 cM)
F23 (25.9 cM)
atg/cg_242
agt/caa_175
aca/caa_115
aga/cag_338(0.0)
atc/cta_217
act/cta_284
LOD 4.0
aca/caa_200
agt/ctc_320
agt/cag_225
aca/cat_163
aca/cat_159
agc/ctg_477
atg/cg_242
agt/cg_156
agt/ctg_126
atc/cta_217
agt/cag_430
agt/cag_585
F8 (73.6 cM)
agg/cat_194
agt/cg_139
F32 (17.1 cM)
agt/cat_640
aga/cat_370
atg/cag_277
aca/cag_305
atg/cac_131
agt/cg_115
aga/ctg_255
agg/cat_312
atg/cag_277
atg/ctg_166
atg/cta_550
aga/cac_370
act/ctg_95(0.0)
atg/cac_131
atg/ctg_173
F9 (68.3 cM)
aga/ctg_320
acc/cta_133
acc/ctg_322
atg/ctg_178
aca/cat_181
agt/ctg_470
F41 (3 cM)
aca/caa_118
acc/caa_237
acc/caa_385
acc/caa_173
aga/cag_335(0.0)
aca/caa_135
F10 (56.0 cM)
agt/cag_220
atc/cag_209
agt/ctg_215
aga/cat_170
aga/cat_230
aga/cac_466
atg/cg_106
aga/caa_150
atg/ctt_286
atg/ctt_279
aca/cat_120
F11 (50.3 cM)
acc/ctc_177(0.9)
aca/ctc_350
atg/ctt_375(0.0)
atg/cta_353
acc/caa_440
aca/cat_240
F40 (3 cM)
agc/ctg_500
atg/caa_408
aga/cta_490
agt/ctt_265
agc/ctg_496
atg/caa_114
aca/cat_86
F12 (50.0 cM)
atg/cta_118
agt/cat_211
atg/ctc_750
aga/caa_550(0.0)
agt/cat_158
atg/ctg_225
F13 (48.8 cM)
F22 (27.3 cM)
aca/ctc_184
atc/cac_266
aga/cac_472
aga/ctc_162
atc/cag_88
LOD 4.0
atg/cg_147
aga/ctg_143
atc/cac_210
aga/cta_185
atc/cac_266
F14 (46.9 cM)
agt/caa_129
acc/caa_280
atg/ctg_134
atg/ctg_163(0.0)
atc/caa_370
atg/cat_372
agt/cg_350
atg/cg_284
atc/cac_248
atg/cac_175
atg/caa_198
F15 (40.7 cM)
aga/caa_167
atg/caa_250
atc/cta_220
act/ctg_195
act/ctg_198
aca/cat_380
atg/ctc_203
atc/caa_166
F17 (35.9 cM)
atg/cac_202
F38 (6.8 cM)
atc/cag_400
atg/cac_453
LOD 4.0
acc/ctg_511
act/ctg_226
atc/cag_321
F18 (34.7 cM)
agt/ctt_218
acc/ctc_240
aga/cta_560(0.0)
acc/ctc_270
agt/cta_203
atc/cac_304
agt/ctc_187
aca/caa_126
F20 (32.6 cM)
act/ctg_228
atc/caa_260
acc/caa_410
F39 (3.8 cM)
aca/caa_410(0.9)
aga/ctg_165
atg/cat_134
aca/ctc_173
aca/ctc_139
F21 (32.3 cM)
aca/cat_168
aga/ctg_178
aga/ctc_252
aga/ctt_271
agt/ctc_192
atg/ctt_113
atg/caa_142
aca/ctc_255
aca/ctc_134
atg/caa_366
agt/ctc_247
atg/cta_165
atg/cg_160
F24 (25.5 cM)
agt/cag_268
atg/cg_136
atg/cac_248
atg/caa_132
atg/cg_174
atg/cg_184
aca/cag_165
agg/cat_163
aga/caa_148
F25 (23.4 cM)
F43 (29.7 cM)
aca/caa_334
agt/cta_332
agt/cta_320
atg/cat_258
LOD 4.0
agg/cat_98
aca/cag_89
aga/caa_128
atg/cg_229
atg/cg_294
agt/ctt_340
F26 (23.1 cM)
aca/caa_95
agt/cag_83
aga/caa_180(0.0)
aga/cag_248
atg/cat_279
agt/caa_400
agc/ctg_235
aga/cag_249
atg/ctg_106
F27 (21.5 cM)
agt/cat_368
agc/ctg_180
atc/caa_355
agt/cag_122
agt/ctg_122
atg/cat_366
atg/ctg_550
F28 (21.5 cM)
aga/ctg_490
atg/cta_580
F29 (19.4 cM)
F31 (17.1 cM)
agg/cat_355
atg/cag_320
atg/cag_171(0.0)
atg/cta_244(0.0)
agc/ctg_229
aca/caa_180
atc/cta_277
atc/caa_312
atg/caa_140
acc/caa_168
agt/cat_143
F30 (17.9 cM)
atg/cac_385
aga/cta_224
aca/cat_105
aca/ctc_272
atg/ctt_385
agt/cat_196
atg/ctg_640
F34 (12.7 cM)
agt/cg_440
aga/cat_290
atg/ctg_253
aga/cat_287
aga/cat_286
aga/cag_490
F35 (12.3 cM)
F36 (8.6 cM)
atc/cag_213
aca/caa_475
atc/cag_390
agt/ctc_352
aga/ctt_125
aga/cat_320
atc/cag_435
agc/ctg_360
agt/cta_173(0.0)
atc/cag_390
atg/cta_236
atg/cg_285
F37 (6.8 cM)
aga/cta_295
aga/ctt_284
F42 (2.9 cM)
aga/ctg_228
agt/ctg_111

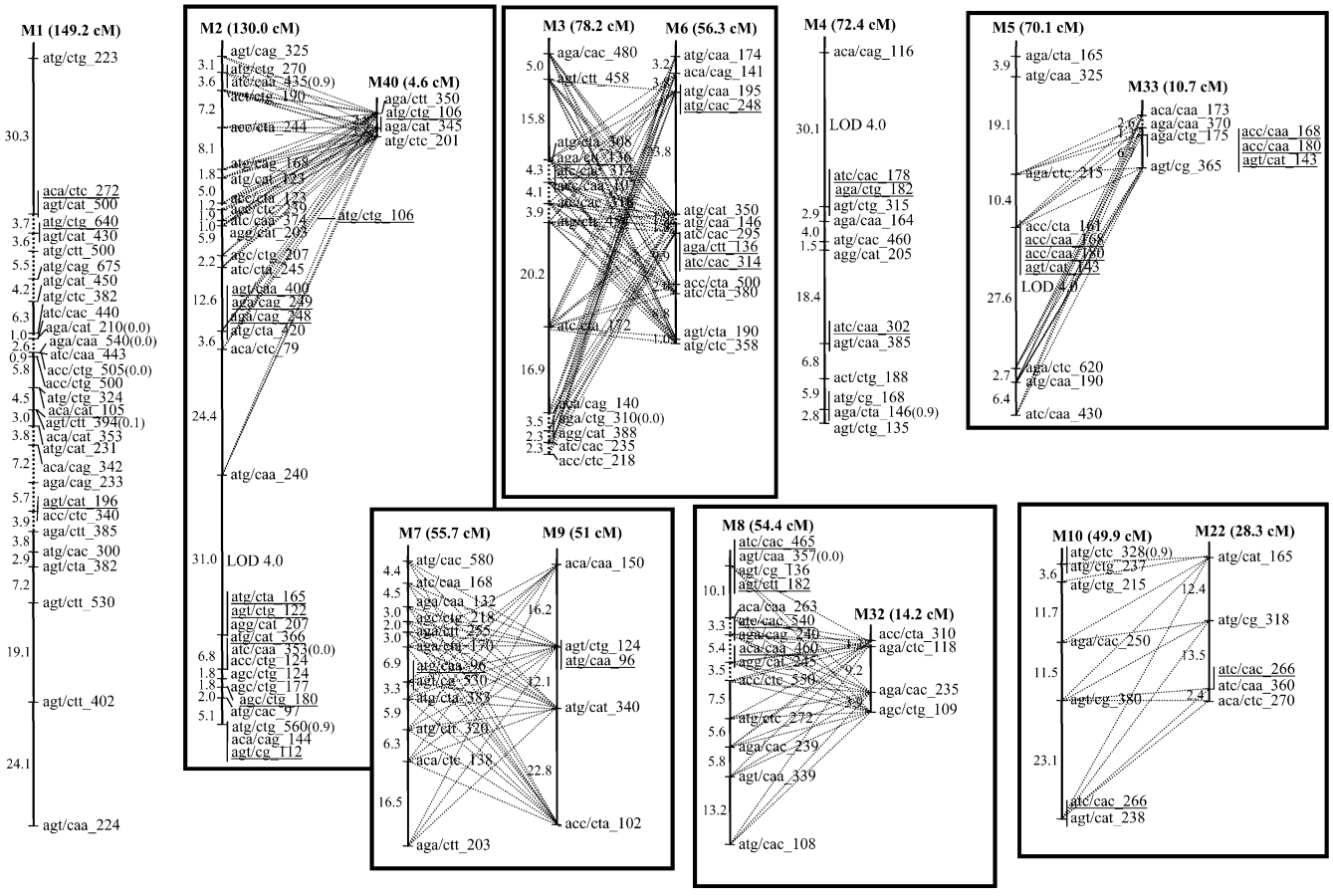


Fig. 2 Linkage map of the male parent (CF1116). Linkage groups were named by following their size. Uncertain marker orders in the co-segregation groups (alternate orders not ruled out at a LOD = 1) are represented by *dotted lines*. The 3:1 segregating markers are *underlined* and not included in the count of groups with only two markers. Linkage in the repulsion phase between pairs of markers is represented by a *dashed line* (LOD = 3.0). *Boxes* represent linkage groups in the repulsion phase. DHFR stands for dihydroflavonol 4-reductase and AAT for alcohol transferase. The genetic distance, in centimorgans, of each marker to its closest neighboring marker is given *in parenthesis*

revealed that 30 co-segregation groups were associated in pairs through repulsion linkages, forming 15 linkage groups, 11 co-segregation groups showed no association (Fig. 2) and two groups (M16, M43) were in the repulsion phase, each with an unlinked marker. Twenty eight linkage groups were finally deduced (15 + 11 + 2). In six out of eight cases, the repulsion linkage confirmed groups built at a LOD value of 4.0 (i.e. F2/F16, F5/F19, F17/F38, F7/F23, F13/F22, M5/M33).

Chi-square analysis based on the number of markers linked in the coupling phase and in the repulsion-phase, expected for disomic and polysomic inheritance performed separately on each linkage group, revealed several contrasting types of meiotic behavior. Among the 26 female and 30 male co-segregation groups for which at least two repulsion linkages were observed, this proportion fitted a 1/1 ratio for 20 (10 + 10) female and 26 (13 + 13) male linkage-groups, suggesting preferential chromosome pairings. Among the 14 female and 11 male unassociated groups, the proportion fitted a 1/0 ratio for nine and seven linkage-groups, respectively, including some large ones like F1, F4 and M1, suggesting random pairing (P = 0.05). For six female and four male co-segregation groups, no fit with either a 1/1 or a 1/0 ratio was observed, suggesting an intermediate behavioral type, between complete preferential chromosome pairing and random pairing. The remaining groups were either associated with one unlinked marker or insufficient markers were present to carry out a chi-square test.

Association between female and male maps

The 179 markers fitting strictly a 3:1 ratio were tested for linkage with the 1:1 markers and included in the maps as accessory markers. Ninety markers were mapped on the female side and 102 on the male side. Homology observed between the co-segregation groups of the two maps was considered real when more than one 3:1 segregating marker was found in both male and female co-segregation groups. Fifteen such associations were detected and are summarized in Table 3. For two other

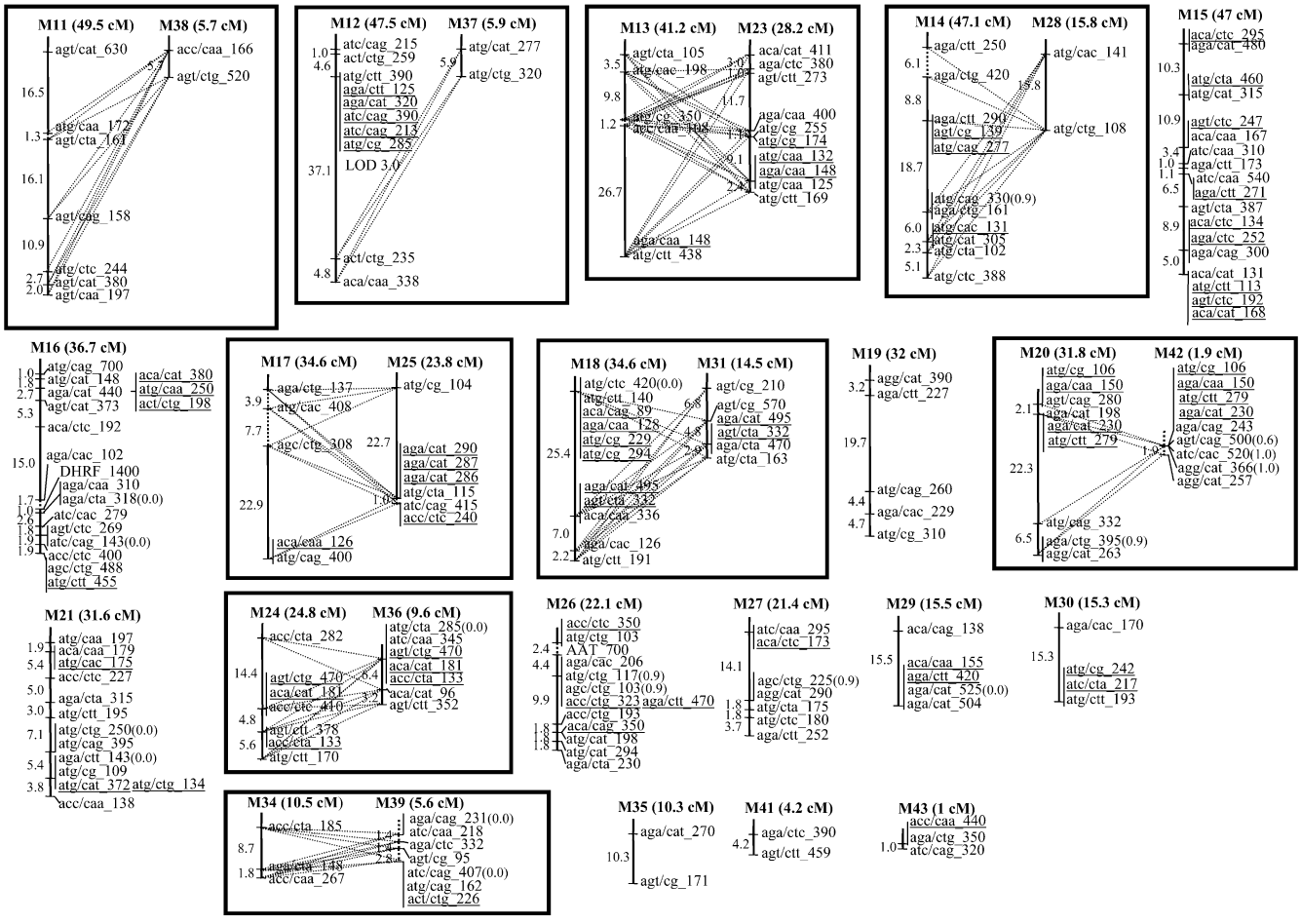


Fig. 2 (continued)

Table 3 Association between female and male linkage groups through 3:1 segregating markers

Female linkage groups[a]	Male linkage groups[a]	Number of 3:1 segregating markers
F1	M4	3
F4	M26	4
F5 + F19	M8	3
F7 + F23	M30	2
F8 + F32	M14	3
F9	M24 + M36	3
F10	M20 + M42	4
F14	M21	3
F15	M16	3
F34	M17 + M25	5
F24	M13 + M23	3
F25 + F43	M18 + M31	5
F29	M5 + M33	3
F30	M1	3
F35 + F36	M12	5

[a] (+) corresponds to linkage groups in the coupling/repulsion phase

associations, the homology between female and male groups could not be determined with certainty. In the first case, the co-segregation group M2 was associated with two different co-segregation groups, F26 and F27, with three markers for each association, whereas no linkage was detected between F26 and F27. In the second case, M15 was associated with F21 through seven markers and with F2 through two markers, whereas F2 was also associated with M3 + M6 through three markers. These results suggest that M15, M3 and M6 represent the same linkage group on the male side, and F2 and F21 the same linkage group on the female side, but these hypotheses need confirmation. In many cases, the mapping of 3:1 segregating markers on both the coupling and repulsion groups confirmed the linkage associations previously observed between these groups. For example, agt/cg_139 and atg/cag_277 were both mapped on F8 and F32 (Fig. 1), and agt/ctg_470, aca/cat_181 and acc/cta_133 were mapped on M24 and M36 (Fig. 2).

Genome size

Considering all the tested markers, we estimated the female genome length to be 2,870 cM (Z = 5) with a 95% confidence interval of 2,670–3,100 cM. The male genome length was estimated to be 1,861 cM (Z = 5) with a 95% confidence interval of 1,767–1,963 cM. Therefore, the percentage of the genome mapped so far amounts to

55.9% and 80.4% for the female and male parents, respectively.

Discussion

Linkage maps and genome size

This study demonstrated that AFLP markers can be useful for mapping the genome of octoploids. Large numbers of simplex markers in a pseudo test-cross configuration were detected, enabling them to be considered SDRF markers and for reliable linkage maps to be constructed. Analysis of the SDRF markers in coupling phase resulted in 43 female and male co-segregation groups, although 56 were expected since each chromosome (homologous or homoeologous) should correspond to a single co-segregation group in coupling phase amongst both the female and male linkage groups (Grivet et al. 1996). The genome is therefore not fully covered by markers and some parts are under-represented. A low number of SDRF markers remained unlinked, which suggests that the SDRFs are not evenly distributed across the whole genome.

The linkage groups resulting from the association of co-segregation groups through markers linked in the repulsion phase were clearly established and the risk of false association can be regarded as rather low. Indeed, the groupings were based on high numbers of markers linked in the repulsion phase (at least five), with the exception of the association between F25 and F43, where only two linkages were detected. In random pairing observed in polysomic inheritance, repulsion-phase linkage between two SDRFs on two particular homologous chromosomes is generally very difficult to demonstrate, since it requires both pairing of the chromosomes being considered, and observation of recombination between the loci (Sorrells 1992). The associated co-segregation groups were not integrated into a unique linkage group since such integration would bias the recombination distance between markers (Qu and Hancock 2001).

The observed map sizes (female and male) corresponding to the sum of the size of each co-segregation group were quite similar. However, the size is probably over-estimated since repulsion-linkage groups were represented by two co-segregation groups and were therefore counted twice. The *F.* × *ananassa* map we developed was about three to four times larger than the 445-cM RAPD map from the diploid *F. vesca* (Davis and Yu 1997). However, since none of the maps covered the entire genome, no conclusion could be drawn about the relationship between the two genomes. The diploid map included a high level of distorted markers with an excess of maternal alleles: skewing has not been observed in our cross.

The estimated genome size for the female parent was much higher than that calculated for the male parent, suggesting that the recombination frequency is higher in the female than in the male gametes. This difference could be artifactual, or it could reflect a genuine biological process, as reported in several other species [e.g. tomato (de Vicente and Tanksley 1991); barley (Graner et al. 1991)]. Similarly, male gametes reportedly recombine at a greater rate in certain cases too; for instance in the genus *Pinus* (Plomion and O'Malley 1996; Lerceteau et al. 2000) and *Manihot esculenta* (Fregene et al. 1997).

Genome behavior and composition

We have been able to characterize groups linked in coupling phase, and others linked in repulsion phase. Since the global ratio of markers linked in coupling phase to those linked in repulsion phase fitted neither a 1:1 ratio nor a 1:0 ratio, both completely disomic and completely polysomic behavior of the genome was rejected, according to Sorrells (1992) and Wu et al. (1992). The observed ratio was intermediate between these two extremes, which may reflect mixed meiotic behavior of the genome. Since the genome is not fully covered, we cannot exclude the possibility of bias, making the association between groups either impossible or partial. However, for large groups such as F1 or M1, the fact that no linkage between markers in repulsion phase was detected supports the existence of groups in the strictly coupling phase.

The proportions of multiplex (8%) and simplex (92%) markers did not correspond to any of the theoretical ratios described by Da Silva et al. (1993), in which a polysomic octoploid is characterized by multiplex and simplex proportions of 0.3 and 0.7, respectively, and a disomic octoploid by the proportions of 0.44 and 0.56. The high proportion of simplex markers might be related to the high heterozygosity of the *F.* × *ananassa* species observed in breeding (Hancock et al. 1991).

The mixed behavior of the genome of the cultivated strawberry suggested by our data has been reported in other polyploid species that have been subjected to extensive mapping efforts. In cassava, a species so far regarded as either a segmental allopolyploid or allopolyploid, a significant amount of random pairing has been observed (Fregene et al. 1997). Another example, given by Hoarau et al. (2001) is an interspecific cultivar of sugar cane (*Saccharum officinarum* × *Saccharum spontaneum*) for which complete polysomy was rejected and the possibility of complete local disomy was suggested.

This is the first study which suggests a mixed meiotic behavior of the genome of *F.* × *ananassa*. Most cytological studies of the species have demonstrated that bivalent-pairing occurs regularly in *F.* × *ananassa* (Ichijima 1926; Longley 1926; Powers 1944) with the exception of Mok and Evans (1971), where multivalents were observed, attributed to secondary associations of bivalents referred to as pseudo-multivalents (Ibrahim et al. 1981). Byrne and Jelenkovic (1976) confirmed in a larger study that all the chromosomes paired as bivalents, and concluded that the species displayed cytological diploidisation. However, homologues of autopolyploids often associate randomly into bivalents rather than

multivalents (see, for example, Qu et al. 1998). Consequently, diploidisation and disomy cannot be fully demonstrated purely on the basis of cytological studies such as those mentioned above. At the molecular level, disomy has been reported for punctual regions of the genome [e.g. PGI (Arulsekar et al. 1981)] and the resistance gene to *P. fragariae, Rpf1* (Haymes et al. 1997).

Several genome compositions have been proposed for the cultivated strawberry. Federova (1946) suggested the formula AABBBBCC, based on cytological observations. This was later modified to AAA′A′BBBB due to homologies between the A and C genomes (Senanayake and Bringhurst 1967). More recently, compiling cytological work of Byrne and Jelenkovic (1976) and the isozyme segregation patterns of Arulsekar et al. (1981), Bringhurst (1990) has suggested AAA′A′BBB′B′ to be the genomic formula of the octoploid strawberry (*F.* × *ananassa*, *Fragaria chiloensis* and *Fragaria virginiana*), which would reflect a high level of diploidisation in the genome. However, our results would rather support the formulas proposed by Federova (1946) and Senanayake and Bringhurst (1967): AACC or AAA′A′ for the disomic part (represented by the groups in repulsion phase) and BBBB for the polysomic part (represented by the groups in coupling phase).

In the present study, we suggested a di-polysomic behavior of the genome of *F.* × *ananassa* based on linkage maps. Such a mixed behavior cannot be extrapolated to any of the polyploid *Fragaria* species. In the genus *Vaccinium* the inheritance type (tetrasomy vs disomy) depends on the inter-specific cross studied and is a function of the homology between the species (Qu et al. 1998). Mixed behavior has been reported for the genome of several polyploid species. The development of linkage maps for species for which little molecular work has been carried out should give new insights into the complexity of polyploid species, including those considered the simplest, the allopolyploids. The addition of co-dominant markers, such as microsatellites, to our maps, or the mapping of multiplex markers, would greatly help further investigations into the characterization of homoeologous groups within and between species for a better understanding of the evolution of the genus.

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References

Aharoni A, Keizer LCP, Bouwmeester HJ, Sun Z, Alvarez-Huerta M, Verhoeven HA, Blaas J, van Houwelingen AMML, de Vos RCH, van der Voet H, Jansen RC, Guis M, Mol J, Davis RW, Schena M, van Tunen AJ, O'Connell AP (2000) Identification of the SAAT gene involved in strawberry flavor biogenesis by use of DNA microarrays. Plant Cell 12:647–661

Arulsekar S, Bringhurst RS, Voth V (1981) Inheritance of PGI and LAP isozymes in octoploid cultivated strawberries. J Am Soc Hortic Sci 106:679–683

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

Byrne D, Jelenkovic G (1976) Cytological diploidization in the cultivated octoploid strawberry *Fragaria* × *ananassa*. Can J Genet Cytol 18:653–659

Cekic C, Battey NH, Wilkinson MJ (2001) The potential of ISSR-PCR primer-pair combinations for genetic linkage analysis using the seasonal flowering locus in *Fragaria* as a model. Theor Appl Genet 103:540–546

Da Silva JAG, Sobral BWS (1996) Genetics of polyploids. In: Sobral BWS (ed) The impact of plant molecular genetics. Birkhauser Boston Inc, Cambridge, USA, pp 1–37

Da Silva JAG, Sorrells ME, Burnquist WL, Tanksley SD (1993) RFLP linkage map and genome analysis of *Saccharum spontaneum*. Genome 36:782–791

Davis TM, Yu H (1997) A linkage map of the diploid strawberry, *Fragaria vesca*. J Hered 88:215–221

Deng C, Davis TM (2001) Molecular identification of the yellow fruit color (*c*) locus in diploid strawberry: a candidate gene approach. Theor Appl Genet 103:316–322

Dirlewanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, Arus P, Laigret F (2002) Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). Theor Appl Genet 105:127–138

Diwan N, Bouton JH, Kochert G, Cregan PB (2000) Mapping of simple sequence repeat (SSR) DNA markers in diploid and tetraploid alfalfa. Theor Appl Genet 101:165–172

Federova NJ (1946) Crossability and phylogenetic relations in the main European species of *Fragaria*. C R (Dokl) Acad Sci USSR 52:545–547

Fregene M, Angel F, Gomez R, Rodriguez F, Chavarriaga P, Roca W, Tohme J, Bonierbale M (1997) A molecular genetic map of cassava (*Manihot esculenta* Crantz). Theor Appl Genet 95:431–441

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137:1121–1137

Grivet L, D'Hont A, Roques D, Feldmann P, Lanaud C, Glaszmann JC (1996) RFLP mapping in cultivated sugarcane (*Saccharum* spp.): genome organization in a highly polyploid and aneuploid interspecific hybrid. Genetics 142:987–1000

Hancock JF, Maas JL, Shanks CH, Breen PJ, Luby JJ (1991) Strawberries (*Fragaria*). Acta Hortic 290:491–548

Haymes KM, Henken B, Davis TM, van de Weg WE (1997) Identification of RAPD markers linked to a *Phytophthora fragariae* resistance gene (*Rpf1*) in the cultivated strawberry. Theor Appl Genet 94:1097–1101

Hoarau JY, Offmann B, D'Hont A, Risterucci AM, Roques D, Glaszmann JC, Grivet L (2001) Genetic dissection of a modern sugarcane cultivar (*Saccharum* spp.). I. Genome mapping with AFLP markers. Theor Appl Genet 103:84–97

Hulbert S, Ilott T, Legg EJ, Lincoln S, Lander E, Michelmore R (1988) Genetic analysis of the fungus, *Bremia lactucae*, using restriction length polymorphism. Genetics 120:947–958

Ibrahim AMF, Sadanaga K, Denisen EL (1981) Chromosomal behavior in octoploid strawberry progenies and their parental clones during meiosis. J Am Soc Hortic Sci 106:522–526

Ichijima K (1926) Cytological and genetic studies on *Fragaria*. Genetics 11:590–604

Kosambi D (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly M, Lincoln SE, Newburg L (1987) MAPMAKER: an interactive computer

package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lerceteau E, Plomion C, Andersson B (2000) AFLP mapping and detection of quantitative trait loci (QTLs) for economically important traits in *Pinus sylvestris*: a preliminary study. Mol Breed 6:451–458

Longley AE (1926) Chromosomes and their significance in strawberry classification. J Agric Res 32:559–568

Mok DWS, Evans WD (1971) Chromosome associations at diakinesis in the cultivated strawberry. Can J Genet Cytol 13:231–236

Moyano E, Portero-Robles I, Medina-Escobar N, Valpuesta V, Munoz-Blanco J, Caballero JL (1998) A fruit-specific putative dihydroflavonol 4-reductase gene is differentially expressed in strawberry during the ripening process. Plant Physiol 117:711-716

Plomion C, O'Malley DM (1996) Recombination rate differences for pollen parents and seed parents in *Pinus pinaster*. Heredity 77:341–350

Powers L (1944) Meiotic studies of crosses between *Fragaria ovalis* and *F. ananassa*. J Agric Res 69:435–448

Qu L, Hancock JF (1995) Nature of 2n gamete formation and mode of inheritance in interspecific hybrids of diploid *Vaccinium darrowii* and tetraploid *V. corymbosum*. Theor Appl Genet 91:1309–1315

Qu L, Hancock JF (2001) Detecting and mapping repulsion-phase linkage in polyploids with polysomic inheritance. Theor Appl Genet 103:136–143

Qu L, Hancock JF, Whallon JH (1998) Evolution in an autopolyploid group displaying predominantly bivalent pairing at meiosis: genomic similarity of diploid *Vaccinium darrowi* and autotetraploid *V. corymbosum* (Ericaceae). Am J Bot 85:698–703

Rajapakse S, Byrne DH, Zhang L, Anderson N, Arumuganathan K, Ballard RE (2001) Two genetic linkage maps of tetraploid roses. Theor Appl Genet 103:575–583

Senanayake YDA, Bringhurst RS (1967) Origin of *Fragaria* polyploids. I. Cytological analysis. Am J Bot 54:221–228

Sorrells ME (1992) Development and application of RFLPs in polyploids. Crop Sci 32:1086–1091

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Vicente MC de, Tanksley SD (1991) Genome-wide reduction in recombination of backcross progeny derived from male versus female gametes in an interspecific cross of tomato. Theor Appl Genet 83:173–178

Vos P, Hogers R, Bleeker M, Reijans M, Vandelee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Williamson SC, Yu H, Davis TM (1995) Shikimate dehydrogenase allozymes: inheritance and close linkage to fruit color in the diploid strawberry. J Hered 86:74–76

Wu KK, Burnquist W, Sorrells ME, Tew TL, Moore PH, Tanksley SD (1992) The detection and estimation of linkage in polyploids using single-dose restriction fragments. Theor Appl Genet 83:294–300

Yu H, Davis TM (1995) Genetic linkage between runnering and phosphoglucoisomerase allozymes, and systematic distortion of monogenic segregation ratios in diploid strawberry. J Am Soc Hortic Sci 120:687–690