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Comparative Genetic Mapping Between Octoploid and Diploid Fragaria Species Reveals a High Level of Colinearity Between Their Genomes and the Essentially Disomic Behavior of the Cultivated Octoploid Strawberry

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ABSTRACT

Macrosynteny and colinearity between Fragaria (strawberry) species showing extreme levels of ploidy have been studied through comparative genetic mapping between the octoploid cultivated strawberry (*F.* ×*ananassa*) and its diploid relatives. A comprehensive map of the octoploid strawberry, in which almost all linkage groups are ranged into the seven expected homoeologous groups was obtained, thus providing the first reference map for the octoploid Fragaria. High levels of conserved macrosynteny and colinearity were observed between homo(eo)logous linkage groups and between the octoploid homoeologous groups and their corresponding diploid linkage groups. These results reveal that the polyploidization events that took place along the evolution of the Fragaria genus and the more recent juxtaposition of two octoploid strawberry genomes in the cultivated strawberry did not trigger any major chromosomal rearrangements in genomes involved in *F.* ×*ananassa*. They further suggest the existence of a close relationship between the diploid Fragaria genomes. In addition, despite the possible existence of residual levels of polysomic segregation suggested by the observation of large linkage groups in coupling phase only, the prevalence of linkage groups in coupling/repulsion phase clearly demonstrates that the meiotic behavior is mainly disomic in the cultivated strawberry.

POLYPLOIDY is especially prevalent in plants, where at least 30 to 80% of angiosperms have experienced one or more polyploidization events in their evolutionary history (Bennett 2004). Polyploidization is usually followed by a process of diploidization whereby gene redundancy is reduced via gene silencing, sequence elimination and rearrangement, demethylation of retroelements, and relaxation of imprinting (see reviews in Osborn *et al.* 2003; Chen 2007). The corresponding evolution of genome structure and of associated rearrangements can be studied through comparative genetic mapping between polyploid species and their diploid relatives. When high degrees of macrosynteny and colinearity are revealed, genetic information can then be transferred from diploids to polyploids (Sorrells 1992). Phenotypic traits can also be compared in polyploids and diploids. This approach allows not only a better understanding of the genetic origin of complex agronomical traits (*e.g.*, the fibers in cotton, Jiang *et al.* 1998; Rong *et al.* 2007) but also the identification of homoeologous QTL in the polyploid (Paterson 2005) which is a prerequisite for map-based cloning of the gene responsible for the trait. In addition, knowledge of chromosome organization of a polyploid species permits the determination of its meiotic behavior (Jannoo *et al.* 2004; Aitken *et al.* 2005). To date, comparative mapping studies in plants have been mostly focused on model plants or on diploid crop species (for reviews, see Paterson *et al.* 2000; Schmidt 2002). Despite their crucial importance for establishing effective breeding programs and germplasm conservation strategies, only few linkage map comparisons between complex polyploids and their diploid relatives are available. Few crop polyploid species of major economical importance were studied, *e.g.*, the Brassicaceae family (Cheung *et al.* 1997), the Poaceae family (Ming *et al.* 1998), and the Gossypium genus (Brubaker *et al.* 1999; Desai *et al.* 2006). This can be largely attributed to the complexity of the genomes studied.

Strawberry (Fragaria sp.) belongs to the large Rosaceae family, which includes many economically important species such as the apple, peach, and plum and to the Rosodeae supertribe (Potter *et al.* 2007), which includes various edible and cultivated berries (*e.g.*, Rubus and Fragaria) and ornamental plants (*e.g.*, Rosa and Poten-

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tilla). Many of them have a polyploid origin. Because diploid Fragaria species such as *F. vesca* have a very small genome (164 Mb C-value reported by Akiyama *et al.* 2001) and that reverse genetics strategies can be easily used to study gene function in both diploid and cultivated strawberry (Hoffmann *et al.* 2006; Oosumi *et al.* 2006), strawberry is becoming a model of choice for functional genomics approaches in the studying of Rosaceae genetics. In addition, the Fragaria genus includes a fairly small number (21) of species ranging from diploids ($2n = 2x = 14$) to octoploids ($2n = 8x = 56$), with a large world repartition, mostly in the northern hemisphere. The main cultivated species is the octoploid strawberry *F.* ×*ananassa*, a hybrid species that originated by chance following the hybridization of the two New World strawberry species, *F. chiloensis* and *F. virginiana*, in the botanical gardens of Europe in the mid-18th century (Darrow 1966). The relationships between the 11 Fragaria diploid species and the parental genomes of the polyploid species are still not clear despite phylogenetic analyses (Potter *et al.* 2000) and hypotheses on genome composition (Federova 1946; Senanayake and Bringhurst 1967; Bringhurst 1990). Three genome formulas have been enunciated for the octoploid Fragaria. Federova (1946) suggested the formula AABBBBCC, on the basis of cytological observations. This was later modified to AAA′A′BBBB due to homologies between the A and C genomes (Senanayake and Bringhurst 1967), and then to AAA′A′BBB′B′ (Bringhurst 1990) to take into account the cytological work of Byrne and Jelenkovic (1976) and disomic segregation of isozymes (Arulsekar *et al.* 1981). However, its composition is still being debated, and its type of meiotic behavior (poly- or disomic) remains unclear. Until now, current studies of the Fragaria genus at the genetic level have been mainly limited to the diploid species *F. vesca*, with the recent exception of an incomplete map in the cultivated octoploid using AFLP markers (Lerceteau-Köhler *et al.* 2003). The recent development of microsatellite markers (SSRs) that can be transferred within the Fragaria genus (Davis *et al.* 2007) now allows the comparative mapping of Fragaria species showing highly different levels of ploidy.

Strategy in the construction of linkage maps in polyploid species depends upon the extent of genome redundancy created by polyploidization, and therefore depends on chromosome pairing behavior at meiosis. Two different chromosome pairings can be observed, *i.e.*, preferential pairing or disomic behavior as usually observed in allopolyploids or random pairing or polysomic behavior as observed in autopolyploids (Osborn *et al.* 2003). Between these two extremes, polyploids can exhibit a continuous range of pairing affinities between chromosomes, as has been observed in the auto-allopolyploid sugarcane (Jannoo *et al.* 2004). When polyploids display a disomic behavior similar to that of diploids, linkage map construction is similar to the genetic mapping of diploid species, *e.g.*, cotton (Desai *et al.* 2006). When polyploids display complete polysomic behavior, it is necessary to take double-reduction into account as done in the cultivated tetraploid alfalfa map (Julier *et al.* 2003). When genome constitution and pairing at meiosis of polyploidy species is unclear, strategy of construction of genetic maps is conducted according to a multistep process developed by Wu *et al.* (1992) and refined by Ripol *et al.* (1999) and Qu and Hancock (2001). The segregation of each allele is analyzed and only single-dose (SD) alleles are used for construction of the linkage maps. Linkages in coupling, followed by those in repulsion phases are subsequently identified. This approach has been demonstrated in sugar cane (Da Silva *et al.* 1995; Grivet *et al.* 1996; Aitken *et al.* 2005). Disomy can thus be distinguished from polysomy by the comparison of the number of loci or linkage groups linked in coupling *vs.* those in repulsion phase (Sorrells 1992; Wu *et al.* 1992) or through the analysis of the ratio of single- to multiple-dose markers (Da Silva *et al.* 1993).

In this work, we report the first comparative mapping of the cultivated octoploid strawberry (*F.* ×*ananassa* Duch., $2n = 8x = 56$) with two of its diploid relatives *F. vesca* and *F. bucharica* (formerly identified as *F. nubicola*, M. Rousseau-Gueutin, unpublished results), allowing large-scale structural study of the patterns of chromosomal evolution following polyploidization events and juxtaposition of genomes in interspecific hybridization. The ordered map obtained with microsatellite (SSR) markers (i) provides a map of the octoploid *F.* ×*ananassa* that can be considered as a reference linkage map for the cultivated strawberry, (ii) gives new insights into the behavior of the 56 chromosomes at meiosis, and (iii) highlights the extent of diploidization that has occurred in the octoploid *F.* ×*ananassa* genome. This work will contribute to a better understanding of the evolution of the Fragaria genome, to the development of map-based cloning approaches for identifying QTL controlling agronomical traits in Fragaria species, and to the gaining of new insights into the genetics of the Rosaceae polyploids.

MATERIALS AND METHODS

Plant material: Two mapping populations from the two levels of ploidy, diploid, and octoploid, were used in this study. The octoploid mapping population used in this investigation was an intraspecific F_1 progeny derived from a cross between two heterozygous parents of the cultivated octoploid strawberry: "*Capitola*" ("*CA75.121-101*" × "*Parker*," University of California, Davis) and "CF1116" ["*Pajaro*" × ("*Earliglow*" × "*Chandler*"), Ciref, France] with contrasting fruit quality traits (Moing *et al.* 2001). One-hundred nineteen F_1 progeny were previously used for the first linkage map of the cultivated strawberry (Lerceteau-Köhler *et al.* 2003). This population was extended to $N = 213$ in this study and is maintained in France at INRA-Bordeaux and at CIREF-CV-Douville. The diploid Fragaria population used was the F_2 ($N = 76$) progeny of the interspecific cross between *F. vesca* "*815*" and *F. bucharica* "*601*" (formerly identified as *F. nubicola*), used by Sargent *et al.* (2006) to construct the reference map for the diploid strawberry and is maintained in the United Kingdom at East Malling Research, in France at Institut National de la

TABLE 1

List of the 148 SSRs tested in the mapping procedure according to their origin and their polymorphism

Species origin of SSRs	References	No. of tested SSRs	No. of polymorphic SSR	Names of the mapped SSRs
		Fragaria species		
F. vesca (2×)	James *et al.* (2003); Hadonou *et al.* (2004); Cipriani and Testolin (2004)	41	29	EMFv004, 006–008, 010, 012, 013, 016–024, 026, 028–029, 2c8ii UDF001–004, 006–009, 016
F. viridis (2×)	Sargent *et al.* (2003)	31	13	EMFvi008, 018, 022, 072, 075, 079, 092, 102, 108, 136, 146, 175, 179
F. nubicola (2×)	Sargent *et al.* (2004)	2	2	EMFn017, 049
F. ×*ananassa* (8×)	This article and Lewers *et al.* (2005)	54	30	BFACT002, 003, . . . , 050 ARSFL4, ARSFL7
F. virginiana (8×)	Ashley *et al.* (2003)	2	2	Fvi11, Fvi20
		Other Rosaceae species		
Malus domestica		1	0	
Prunus persica	Dirlewanger *et al.* (2002)	3	1	BPPCT028
Rosa hybrida	Hibrand-Saint Oyant *et al.* (2008)	14	2	Rw55E12, Rw5G14
Total		148	79	

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Genotyping: The DNA extraction procedures have been previously described in Lerceteau-Köhler *et al.* (2003) and Sargent *et al.* (2004) for the octoploid and diploid populations, respectively. The octoploid map was developed using AFLP, SSR, STS, and SCAR markers, while the diploid reference map was developed using SSR, STS, and SCAR markers. The 40 AFLP primer combinations previously reported (Lerceteau-Köhler *et al.* 2003) were used to extend the population size to 213 individuals. Microsatellite marker analysis was performed using primers obtained from various sources (Table 1). First, the SSRs developed in this study were derived from the CT/AC-enriched library (P. Arús, personal communication) obtained from the octoploid cultivar, "Tudla," using the same method followed by Monfort *et al.* (2006). Briefly, primer pairs were designed using PRIMER 3 (http://bioweb.pasteur.fr/seqanal/interfaces/eprimer3.html) to be 20–24 bp long with an annealing temperature ~60°, and to give an expected product size of 150–300 nt. Second, a total of 98 SSRs derived variously from the octoploid *F. virginiana* (Ashley *et al.* 2003), the diploids *F. vesca* (James *et al.* 2003; Cipriani and Testolin 2004; Hadonou *et al.* 2004), *F. viridis* (Sargent *et al.* 2003), and *F. nubicola* (Sargent *et al.* 2004), as well as from other Rosaceous species such as Rosa (Hibrand-Saint Oyant *et al.* 2008), Malus and Prunus (Dirlewanger *et al.* 2002) were used. In the parental octoploid maps, the nomenclature of AFLP loci is indicated as previously described (Lerceteau-Köhler *et al.* 2003). Names of SSR loci were as previously published (Table 1), and for those developed here as indicated in Table 1S (see supplemental data at http://www.genetics.org/supplemental/ for details).

PCR was performed on DNA from the diploid and *F.* ×*ananassa* parents and progeny in a total volume of 12 μl with 1× reaction buffer (Sigma-Aldrich, St. Quentin Fallavier, France), 0.2 mM of each dNTP, 0.2 μM, of each primer, 1.5–2.5 mM $MgCl_2$, 0.8 units *Taq* polymerase (Sigma-Aldrich) and 8 ng of genomic DNA. The reaction consisted of 3 min denaturation at 94°, 35 cycles of 45 sec at 95°, 45 sec at 52–62°, 2 min at 72°, and a final extension step of 4 min at 72°. Polymorphic bands were visualized following separation on a 6% denaturing polyacrylamide gel for 2 hr 30 min to 3 hr at 80 W by silver-staining according to Cho *et al.* (1996). Microsatellite profiles were scored visually as presence/absence of each band by two persons, independently.

Multistep process to construct the octoploid parental linkage maps of the cultivated strawberry: Data analysis and map construction for each parent of the cultivated octoploid strawberry progeny were conducted according to a multistep process developed by Wu *et al.* (1992) and refined by Ripol *et al.* (1999) and Qu and Hancock (2001). Briefly, only SD markers that were in a backcross configuration and segregated 1:1 (Wu *et al.* 1992) were used. The 1:1 markers that had the presence of a band (+) in the female (F) parent and the absence of a band (−) in the male (M) parent were separated from the 1:1 markers with − in F and + in M and the data were analyzed separately to produce individual F and M maps, conforming to the double pseudo testcross strategy described previously by Grattapaglia and Sederoff (1994). A two-step mapping procedure developed for polyploids by constructing alternatively the parental linkage maps using markers in coupling and then markers in coupling/repulsion phases was employed (Grivet *et al.* 1996; Fregene *et al.* 1997) using MapMaker/Exp v3.0 (Lander *et al.* 1987).

We distinguished single-dose markers from all other dose markers in the testcross configuration by testing the null hypothesis H_0 presence:absence = 1:1 against the alternative hypothesis, presence:absence ≥ 3:1, because whatever the genome behavior exhibited (auto- or allopolysomy), non-single-dose marker ratios will segregate either 3:1 or greater (Wu *et al.* 1992). The size of our population (213) allowed a high level of confidence for the results of the χ^2 tests ($P > 0.01$). Individuals with different phases for two linked loci (coupling ++/−− and repulsion +−/−+) cannot be distinguished by their phenotype, nor can MapMaker distinguish them from their segregation patterns. Therefore the raw data, as they were originally scored and analyzed with MapMaker, could be misleading, as markers that are linked but in repulsion would be declared as unlinked. To determine linkage between markers linked in repulsion, we produced a

raw data file that included the original data plus the reciprocal of this file (*i.e.*, substituting the + with − and the − with + for all data). This permitted the discovery of linkage between all linked loci in repulsion that displayed disomic behavior. Thus a final data set was constructed for linkage analysis using all data that had had their genotypes corrected, so that they could be analyzed by MapMaker as if they were in the same phase.

For linkage analysis, we used the parameters previously described by Lerceteau-Köhler *et al.* (2003), employing regression analysis using the Kosambi mapping function (Kosambi 1944) and the "group" command of MapMaker with a LOD (logarithm of odds) score ≥5.0 and a recombination fraction of $r \leq 0.35$, for establishment of linkage groups using only markers with nondistorted segregation ratios. The LOD value was then decreased to 3.0 to test the association between linkage group ends. Markers were ordered by multipoint analysis with the "order" command, and "ripple" was employed to assess the robustness of the marker order. Then, SD markers with distorted ratios ($0.01 \geq P \geq 0.001$) were integrated into the pre-established linkage groups using the same parameters when they did not disturb the marker order. Possible genotyping errors were checked after map construction using the "error detection" feature.

Integration of parental octoploid linkage maps: Genetic information of the cultivated octoploid strawberry F_1 progeny was summarized by merging the linkage maps of the two parents using JoinMap 3.0 (Van Ooijen and Voorrips 2001). First, parental linkage maps were separately constructed by using all segregation data, including markers heterozygous in both parents: 3:1 SD (χ^2 test, $P > 0.01$). This step was conducted using the software JoinMap using the CP population type. Linkage groups of the parental maps were determined using regression mapping using the Kosambi mapping function with a LOD threshold of ≥4.0, a recombination frequency of 0.35, and a jump threshold of 5.0. When conflicting orders were observed between the MapMaker and JoinMap maps, the order of the markers was fixed to be that of MapMaker. Markers, and in particularly those segregating 3:1, were discarded during the mapping step when their presence caused inconsistencies in the map, *e.g.*, insufficient linkage, conflict with other markers that led to a disruption of marker order.

Second, integrated linkage groups were built up by merging the data from homologous parental linkage groups of the F and M maps using, as anchor markers, those dominant markers that were heterozygous in both parents and codominant markers. Codominant markers segregating in both parents were identified among the SSRs and AFLP markers tested by examining their banding patterns. Markers were deemed to be codominant when the map positions of the two alleles segregating in the same parent were identical and when markers generated by the same SSR or AFLP primer pairs located to similar positions on the homologous M and F linkage groups. The merging of F and M linkage groups was conducted using the parameters described above.

Diploid linkage map construction: Compared to the published diploid reference map of Sargent *et al.* (2006), which already included 30 SSRs used in this study, 19 SSRs out of the SSRs mapped in the octoploid linkage map were placed. Goodness-of-fit to expected 1:2:1 or 3:1 ratios was determined using the χ^2 test. Linkage analysis with all markers was performed first using MapMaker and then with JoinMap. We used the same parameters and commands as described above, and the "fixed order" option of JoinMap was used to maintain the order of MapMaker when conflicting orders occurred between the maps constructed using the different programs.

Identification of homoeologous groups in the octoploid map: Linkage groups of the integrated octoploid map were ordered according to their belonging to one homoeologous group (HG). The identification of HGs was first conducted within the octoploid species and then confirmed by comparing diploid and octoploid linkage maps. First, HGs were assembled in the integrated octoploid map when at least two SSRs were common to two linkage groups (Ming *et al.* 1998). Second, HGs were declared as homoeologous to linkage groups of the diploid on the basis of at least four microsatellites common between the diploid linkage group and the ensemble of the octoploid LGs that constituted each HG. The nomenclature of HGs I–VII was consistent with that of the diploid map (Sargent *et al.* 2006).

Study of the diploidization of the octoploid genome: The extent to which the *F.* ×*ananassa* genome has been diploidized was investigated on the basis of the ratio of SD markers linked in repulsion phase to markers linked in coupling phase for each linkage group (Sorrells 1992; Wu *et al.* 1992; Al-Janabi *et al.* 1993). A 1:1 ratio of markers linked in coupling/repulsion corresponds to disomic behavior, a 1:0 ratio corresponds to polysomic behavior. These ratios were tested by χ^2 analysis of goodness of fit ($\alpha \leq 0.01$). We analyzed LGs with a minimum number of eight markers, to have a probability <0.005 that they were by chance in the same phase. Additional evidence for diploidization in specific chromosomal regions was derived from codominant SSRs that were heterozygous in one of the parents and were mapped as SD markers to the same position. Also relevant were codominant SSRs that were heterozygous in both parents (1:1:1:1 or 1:2:1 segregations) and that, when scored as SD markers, located to homologous positions on the F and M maps.

RESULTS

A genetic map of the cultivated octoploid Fragaria for comparative mapping with its diploid relatives: *Development of microsatellite markers for improving map coverage:* To increase the number of markers transferable within the Fragaria genus, we developed microsatellite markers. An enriched SSR library of *F.* ×*ananassa* was constructed (see materials and methods), and 148 clones were sequenced, out of which 50 contained an SSR motif that could be used for primer design. Of these 50 primer pairs, 28 amplified polymorphic products between the *F.* ×*ananassa* parents used and were scored in the octoploid mapping population (see Table 1S supplemental information).

Mapping single-dose markers for construction of unambiguous polyploid map: SD markers are particularly useful for constructing genetic maps of polyploids when meiosis behavior is unclear (Da Silva and Sobral 1996), which is the case in the strawberry. SD markers have the advantage over multidose markers to be clearly assigned to a single LG (Qu and Hancock 2001). In this work, we scored a total of 1135 SD markers, which were ranged according to their backcross or F_2 configuration (Table 2). All SDs produced by AFLPs (824), STS, and SCAR markers (5) were already integrated into the genetic linkage map of *F.* ×*ananassa* reported by Lerceteau-Köhler *et al.* (2003). Here, we have extended the AFLP data set to 94 additional progeny of the cross previously mapped (to a final total plant number of 213) and added SSR data for the entire population. In total, 148 SSR primer pairs were tested from which 50 were obtained from the enriched SSR library and 98 from other origins

TABLE 2

Segregation types of markers according to their configuration

		Configuration of single-dose markers distorted (d)[a] or not					
		Backcross configuration				F_2 configuration:	
		Female		Male		Heterozygous	
Class of markers	Total markers	1:1	1:1 (d)	1:1	1:1 (d)	3:1	3:1 (d)
AFLP	824	259	6	285	26	208	40
SSR	306	97	2	115	12	62	18
STS and SCAR	5	3	0	2	0	0	0
Total	1135	359	8	402	38	270	58

[a] Markers are considered as distorted when probability is composed of between 0.001 and 0.01.

(Table 1). From these 148 SSRs, 79 derived from different sources of Rosaceous species were polymorphic and revealed clear segregation profiles with polymorphic bands, while 32 revealed no polymorphism, and a further 37 gave no or inconsistent amplification (Table 1).

Detecting SD markers in coupling or in coupling/repulsion phase within each linkage group using the multistep process: To further study the meiotic behavior of the octoploid strawberry, distribution of SD markers in coupling and in coupling/repulsion phases within each linkage group has to be determined. For this purpose, we used the multistep process on the basis of the SD markers in backcross configuration (see MATERIALS AND METHODS). Almost all these 807 markers were included in the F and M octoploid linkage maps, which consisted of a total of 2582 cM and 2165 cM, respectively (see Table 3 for a summary of the data of the parental linkage maps and Figure 1 for their chromosomal representations). For the F and M maps, respectively, among the 28 F and 26 M LGs, 21 LGs for both linkage maps contained markers in coupling/repulsion phase, while the remaining 7 F and 5 M LGs contained only markers in coupling phase. Markers with distorted segregation ratios ($P < 0.01$) were assigned preferentially to 5 male linkage groups IVa-m, IVb-m, IVd-m, VIIa-m, and VIIb-m (Figure 1).

Merging genetic information into an integrated map of the cultivated octoploid Fragaria: To compare the genome macrostructure of the polyploid cultivated strawberry with that of its diploid relatives, we compiled the genetic information of the parental octoploid maps into an integrated map using JoinMap. The female and male maps were merged using the codominant loci and the markers segregating 3:1 (F_2 configuration) as anchor markers. Twenty-five codominant loci (23 SSRs and two AFLPs) were identified following 1:1:1:1 (15) or 1:2:1 (10) segregation ratios. Of the 328 markers in an F_2 configuration, 10 were already recorded as codominant markers and the remaining 318 markers were used for merging the parental maps. Among them, 166 were mapped and the remaining152 markers were discarded since they caused inconsistencies in the maps. In total, 191 anchor markers were obtained.

All LGs of MapMaker parental maps were used for constructing the integrated map, except the LG M1 since no microsatellite or anchor markers was mapped on this group. The merged map maintained the same marker order as the parental maps. The final outcome was a map with 32 LGs, which spanned a total distance of 2195 cM, with linkage groups ranging from 37 cM to 126 cM (Figure 2). Twenty-three (72%) of the 32 LGs were constructed from the merging of LGs from the previous F and M MapMaker maps including small linkage groups (named as f or m in Ib-f/m, f/IId-m*, VIc-f/m). One out of the 23 resulted from the merging of 3 LGs previously found in the F and M MapMaker maps, since links between 2 female or male LGs were found as a consequence of the addition of the 3:1 segregating markers (IIIc-f/IIIc1&2-m). Four pairs of LGs were identified as parental homologs but not integrated since only one or two markers were present for merging parental LGs (*i.e.*, Ia-f/Ia-m).

TABLE 3

Parental octoploid linkage maps obtained with single-dose markers in F_2 segregation configuration and using MapMaker software

Description of the linkage maps	Female map	Male map
Total no. of markers	367	440
Distorted markers	8	38
Unlinked markers	15	23
Total map size (cM)	2582	2165
Total no. of linkage groups	28	26
No. of linkage groups in coupling/repulsion	21	21
No. of linkage groups in coupling only (>10 markers)	7 (5)	5 (5)
No. of markers per group (mean) (and SD)	12.6 (± 5.3)	16.0 (± 5.5)
Range of marker no. per group	6–25	8–28
Mean size per group (cM) (and SD)	92.2 (± 37.7)	83.3 (± 35.6)
Range of size per group	23.8–155.8	19.5–159.9

A diploid genetic map in Fragaria: For the comparison of diploid and octoploid maps, the diploid map was constructed using the same parameters and commands used for the construction of the octoploid map. The addition of the 19 SSR markers in this study to the 182 of the previous map (Sargent *et al.* 2006) led to a linkage map covering a total length of 396 cM. The addition of 19 markers improved the marker density from 1 marker every 2.3 cM to 1 marker every 1.9 cM and reduced the number of gaps over 10 cM in length from eight to six. Moreover, there were a few minor rearrangements in the placement of some loci due to the addition of the novel SSRs.

The octoploid genome is organized in seven homoeologous groups and shows colinearity with the diploid genome: The construction of the polyploidy map organized by homoeologous groups is an important step before undertaking more detailed genetic analyses. Among the linkage groups of the octoploid map, we identified the homoeologous linkage groups on the basis of their common markers amplified by the same primers. We declared these homoeologous linkage groups of the integrated octoploid map homolog to a corresponding diploid LG when they shared at least five common markers. This study was based on 51 SSRs and one SCAR locus developed for anthracnose resistance (Lerceteau-Köhler *et al.* 2005), which all generated more than one locus in the octoploid map (average of 2.4 loci per SSR) (Figure 2). The 28 integrated or related LGs of the octoploid linkage map were assigned to one of the seven HGs expected in Fragaria ($x = 7$). Among the SSRs mapped in the octoploid progeny, 46 anchored the diploid map. This allowed a second round of synteny analysis comparing each HG of the octoploid map to the diploid reference LGs, as well as the order of the anchor markers between diploid and octoploid LGs. The number of anchor markers per LG in the diploid map ranged from four to nine, with an average of 6.6 per LG.

The map comparison, shown in Figure 2, confirmed the identification of the HGs defined at the octoploid level. Each HG contained four LGs of the octoploid genome (Table 4). Each HG has only one LG counterpart in the diploid genome and none contains anchor markers from more than one LG. The order of the majority of the markers presented was conserved within each HG. The only discrepancies in marker order between LGs of the same HG within the octoploid species were EMFv004 in HG III and EMFv007 in HG IV.

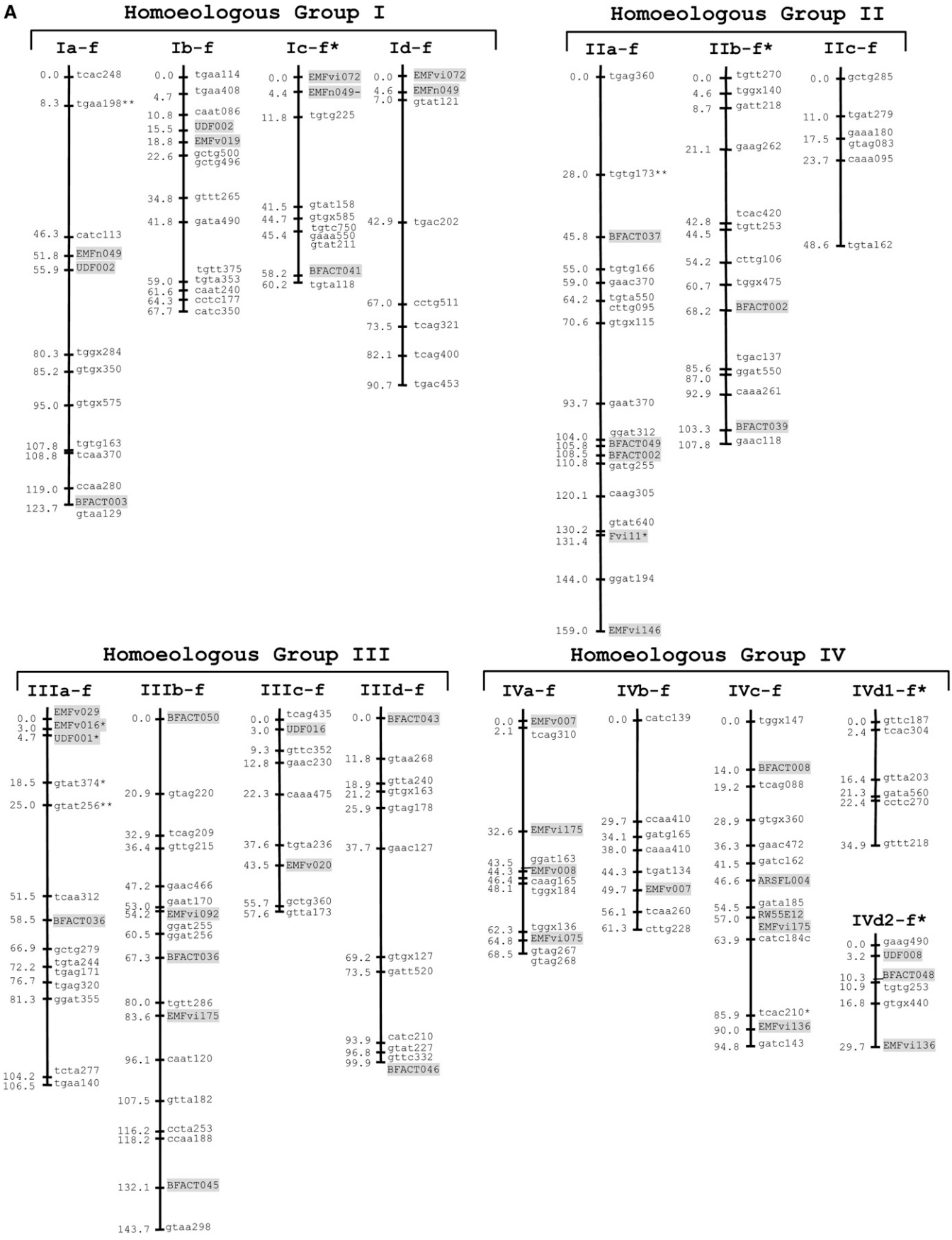
Disomic behavior is prevalent in the cultivated strawberry: We investigated the disomic *vs.* polysomic chromosome behavior by analyzing the number of single-dose markers linked in coupling and repulsion phases within each linkage group using the parental MapMaker maps. As mentioned above, among the 28 female LGs, 21 contained both markers linked in coupling and repulsion phases and their ratios (loci linked in coupling *vs.* repulsion phase) fitted the 1:1 ratio ($P > 0.01$), as expected for disomic behavior (Wu *et al.* 1992; Qu and Hancock 2001). For the remaining 7 linkage groups, Ic-f*, IIb-f*, IVd1&2-f* (counted as 2 LGs), Va-f*, Vd-f*, and VIIb-f*, all markers were in coupling phase and were thus in agreement with the 1:0 ratio, as expected under polysomic segregation. The analyses of ratios for the 26 male linkage groups gave similar results. For the 21 LGs containing both markers linked in coupling and repulsion phases, ratios fitted 1:1 ratio ($P > 0.01$), as was expected for disomic behavior. For the remaining 5 linkage groups including only markers in coupling phase, IIb-m*, IId-m*, VId-m*, VIIb-m*, and M1 (not attributed to an HG group), ratios fitted the 1:0 ratio.

Considering the overall integrated map, only 7% (2) of the merged LGs were composed of 2 parental LGs that segregated only in coupling (IIb-f*/IIb-m* and VIIb-f*/VIIb-m*), whereas 75% had either one (3) or both (18) LGs in coupling/repulsion (Table 4). The segregation phase of the remaining 18% of merged LGs could not be determined. The distribution of the pairs of homologous linkage groups on the basis of the presence or absence of repulsion phase markers was not significantly different from random ($\chi^2 = 5.35$; 3 d.f.; $P = 0.15$), although there was an excess of LGs that were both in coupling/repulsion (15 expected *vs.* 18 observed) or both in coupling (0.9 expected *vs.* 2 observed).

DISCUSSION

We established the most comprehensive genetic map produced in the cultivated octoploid strawberry, *F.* ×*ananassa* ($8x = 56$) by classifying almost all linkage groups into the seven HGs expected in Fragaria, which displays a base chromosome number of $x = 7$ as other Rosodeae. The map was constructed using SSR markers and the approach of the multistep process developed when the pairing of polyploids at meiosis is unclear (Wu *et al.* 1992; Ripol *et al.* 1999; Qu and Hancock 2001). It provides thus a reference map for further mapping projects at the octoploid level and

Figure 1.—Parental linkage maps of *F.* ×*ananassa* using a F_1 segregating progeny. The linkage groups (LGs) include only markers that segregated in a backcross configuration and were constructed using MapMaker software. A and B show, respectively, the female and male maps. Microsatellites (SSRs) are shown in shaded boxes. Distorted markers are noted with asterisks at the end of their name (*$0.01 \geq P \geq 0.001$ and **$P < 0.001$). Clusters of distorted markers are visualized by a gray oval on the chromosome bar. The LGs are grouped by homeologous groups (HGs) based on common SSR markers and on anchor markers with the diploid Fragaria genome. The name of each LG includes the number of their HG (I–VII), followed by a letter (a, b, c, or d) to identify arbitrarily groups within the same HG, and a dash with an f or m for LGs of the female or male map, respectively. Linkage groups noted with an asterisk at the end of their name contain exclusively markers segregating in coupling phase. M1 is a linkage group from the male map that has no anchor loci with any other linkage group from this parent.



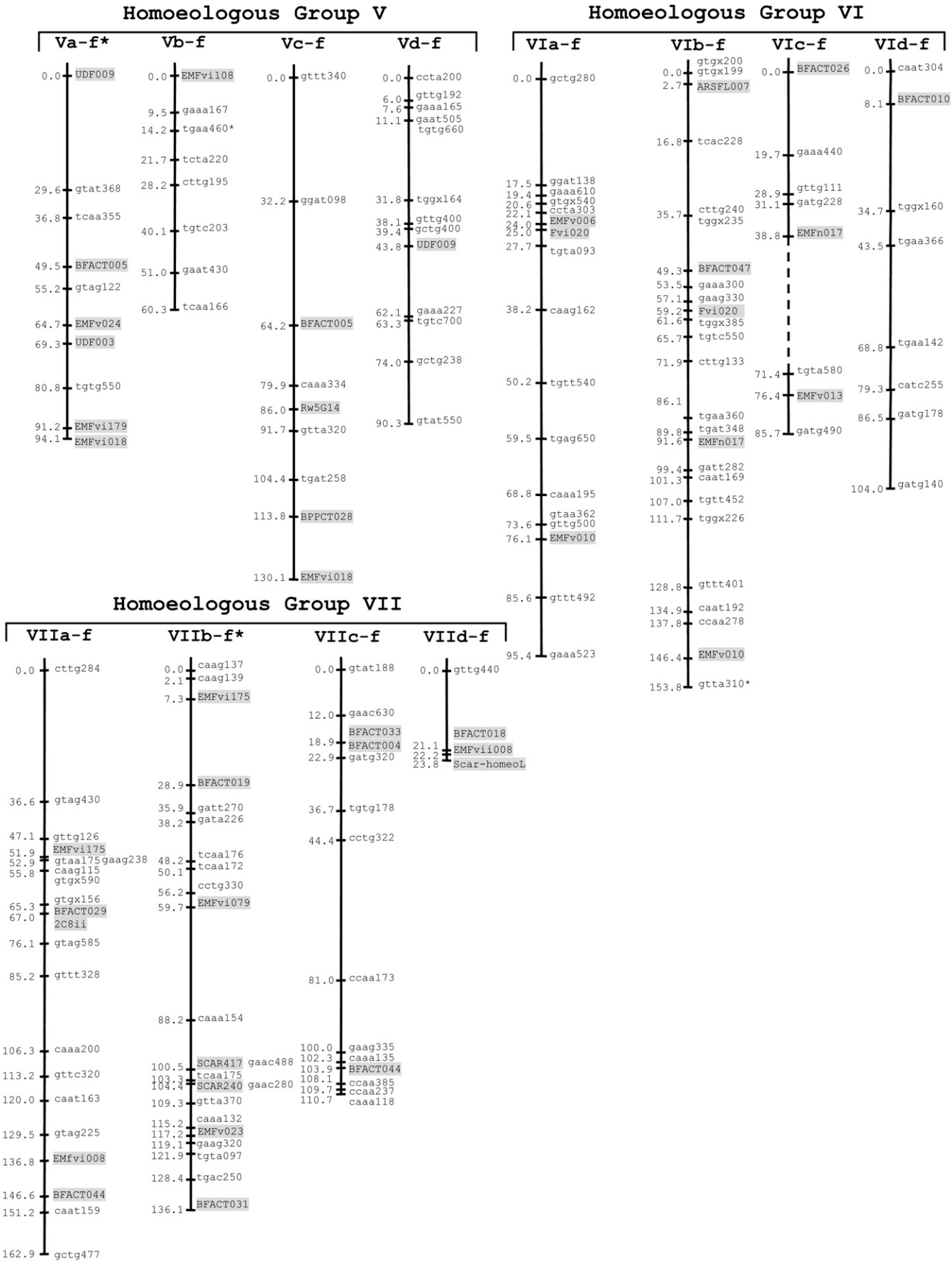


FIGURE 1.—*Continued.*

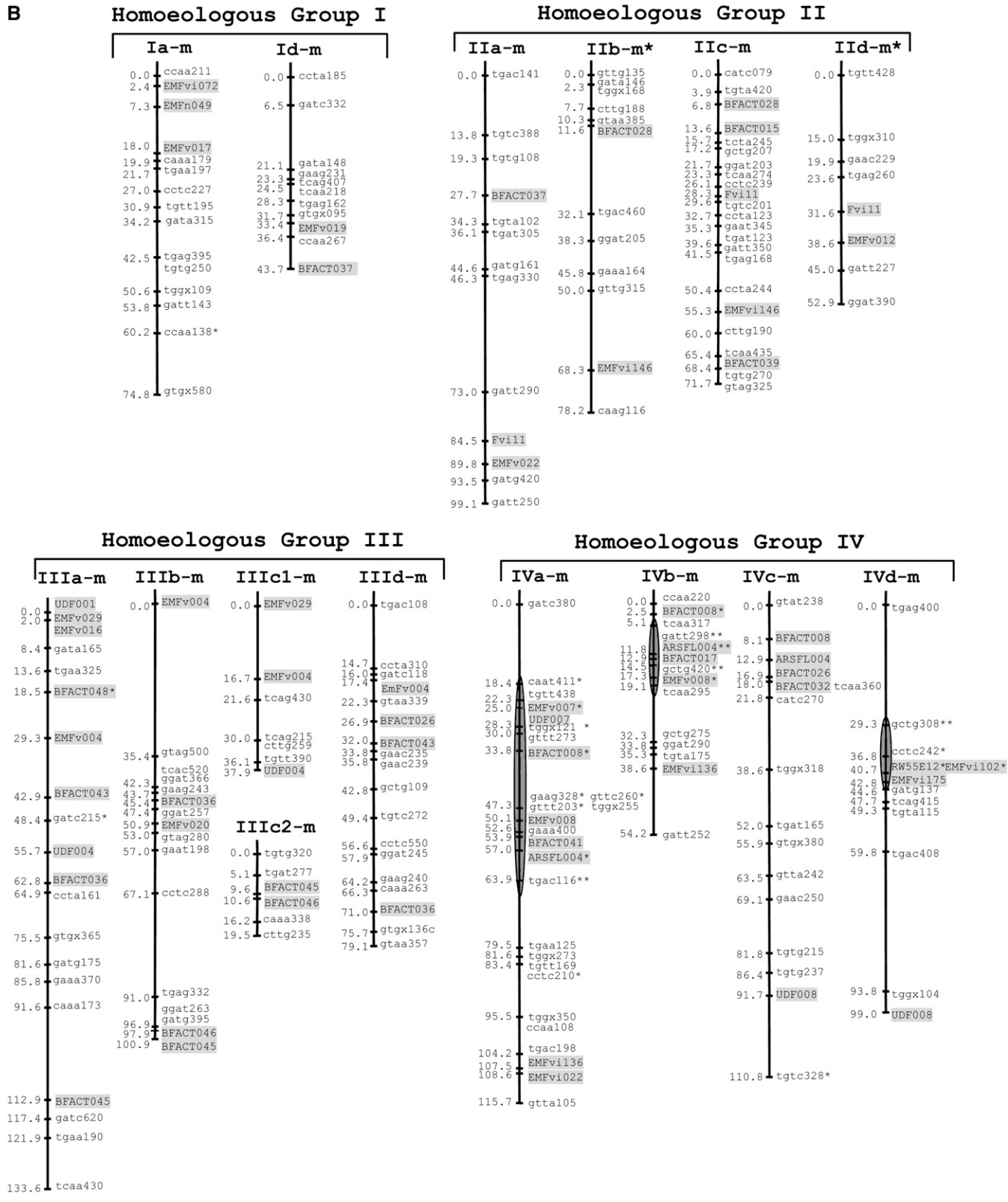


FIGURE 1.—*Continued.*

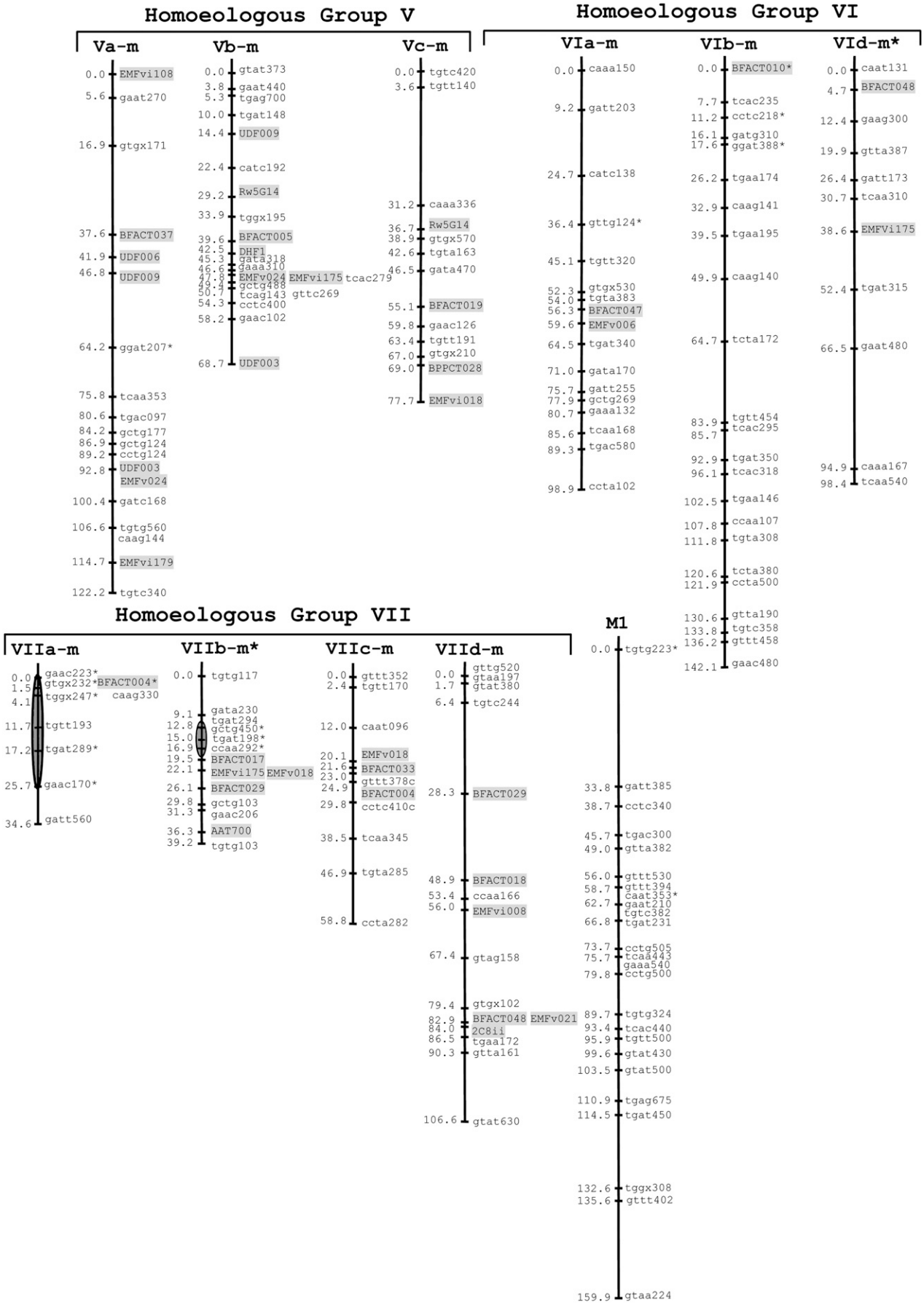


FIGURE 1.—*Continued.*

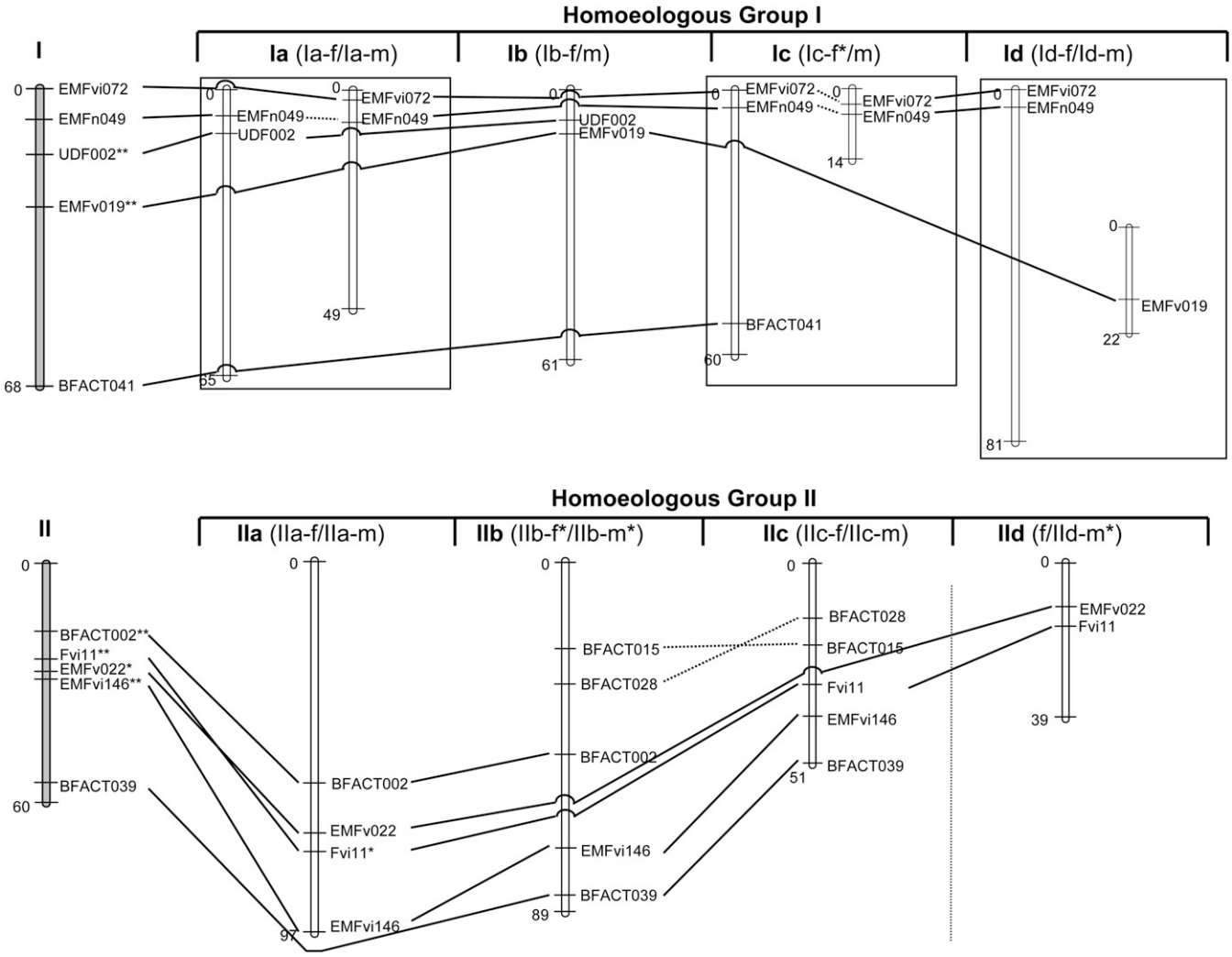


FIGURE 2.—An integrated linkage map of the octoploid *F.* ×*ananassa*, based on an F_1 segregating population, and its comparison with the diploid Fragaria F_2 reference map generated from the cross between *F. vesca* "*815*" and *F. bucharica* "*601*." Only microsatellite (SSR) and SCAR markers that anchored at least two linkage groups (LGs) are shown. LGs were integrated using JoinMap software. The names of the LGs of the diploid map are those used by SARGENT *et al.* (2006) (from I to VII, chromosome bars shaded). Integrated homoeologous groups (HG) for the octoploid map are notated with the same number (from I to VII) of the corresponding diploid LG. Four pairs of linkage groups identified as homologs were not integrated due to the limited number of anchor common markers and are arranged in a rectangle. Linkage group names for the octoploid consist of the number of the HG to which they belong followed by a letter (a, b, c, or d). The name of the two linkage groups (male and female) that merged to constitute the integrated linkage group is given in parentheses. These groups are named with the same name of the LG followed by a dash and a letter that indicates their origin (f for the female and m for the male maps). An asterisk is added when the LG included only markers in coupling phase.

constitutes a great improvement compared to the previous octoploid strawberry map (LERCETEAU-KÖHLER *et al.* 2003), which included mostly AFLP markers.

The strawberry comparative map reveals a high level of colinearity between diploid and octoploid Fragaria species: Comparative mapping between species with different levels of ploidy is a powerful tool for studying macrorearrangement events after the formation of polyploid species (LAGERCRANTZ and LYDIATE 1996). In this study we developed a comparative genetic map between species showing two extreme levels of ploidy in the Fragaria genus to study the evolution of macrostuctural organization in the Fragaria genome along the species evolution and polyploidization. The comparison was performed between the cultivated octoploid strawberry, *F.* ×*ananassa* and the diploid interspecific cross between *F. vesca* with *F. bucharica*. The cultivated octoploid *F.* ×*ananassa* results from the interspecific hybridization between two octoploid species, *F. virginiana* and *F. chiloensis* (DARROW 1966). Therefore, the macrostructure of the genomes analyzed in this study derives from two successive polyploid contexts. First, the genomes evolved separately within two distinct highly polyploid species, *i.e.*, *F. virginiana* and *F. chiloensis*, which are both of monophyletic origin and have different geographical repartition (POTTER *et al.* 2000). Then, two and half centuries ago, these genomes were juxtaposed within the actual cultivated strawberry, *F.* ×*ananassa* through interspecific hybridiza-

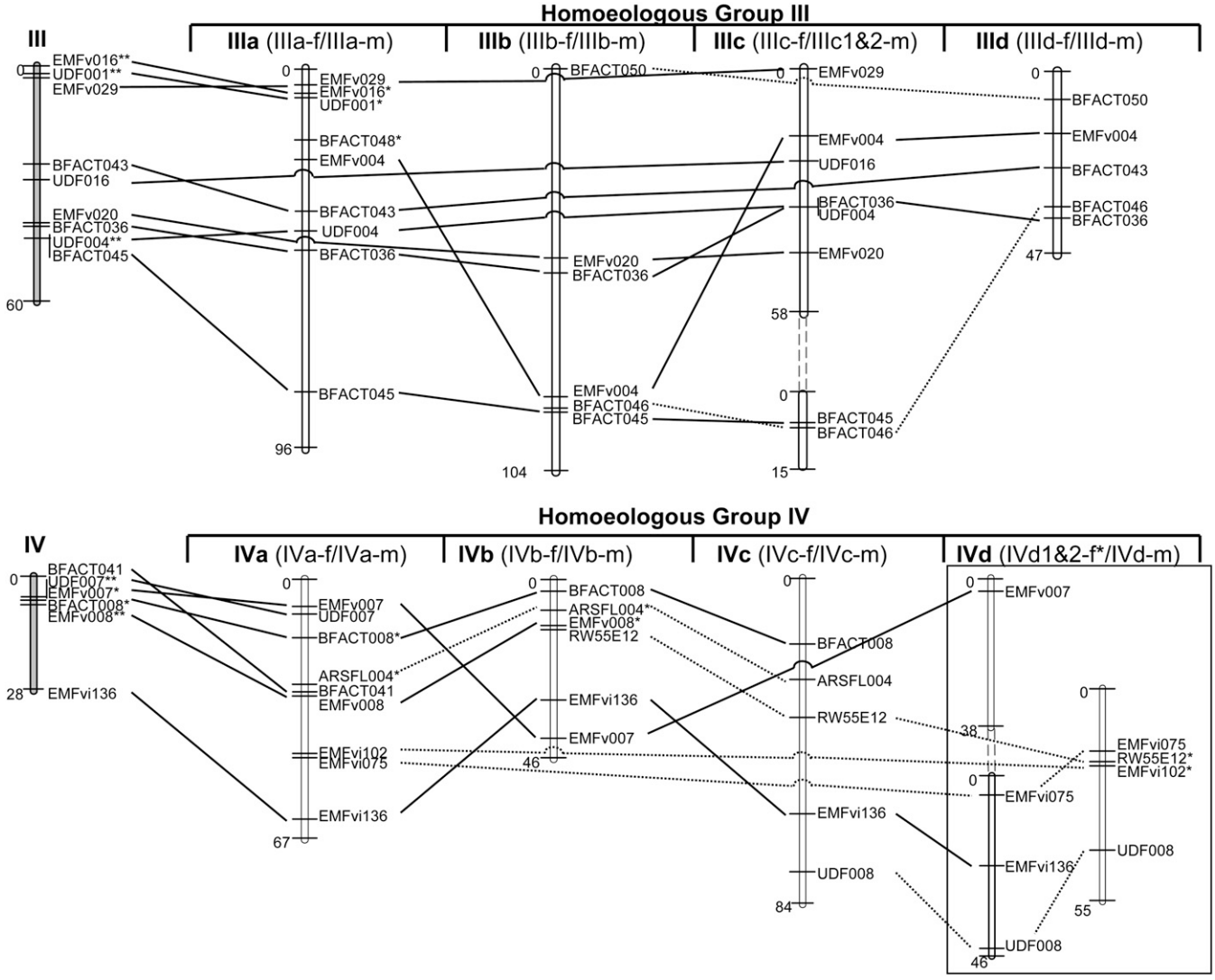


FIGURE 2.—*Continued.*

tion. Regarding the cytological formula of the Fragaria octoploids, AAA′A′BBB′B′ (BRINGHURST 1990), it is clear that at least two genomes are involved in their origin. The A genome is thought to have been contributed by *F. vesca* ($2x = 14$) or its ancestor (BRINGHURST and GILL 1970), and the B genome is thought to be *F. iinumae* ($2x = 14$) or its ancestor (M. ROUSSEAU-GUEUTIN, unpublished results). As a consequence, the study of the macrosynteny and colinearity between homoeologous linkage groups within homoeologous groups of the octoploid Fragaria map can be regarded as the study of the colinearity between diploid genomes involved in the origin of the Fragaria octoploids.

The marker alignment between diploid and octoploid Fragaria species reveals extremely high levels of macrosynteny and colinearity between these genomes with the exception of two putative inversions (identified through the mapping of single SSR loci). This result supports the hypothesis that major chromosomal rearrangements have not been frequent throughout the evolution of species in the Fragaria genus. Therefore, the higher genetic value of the octoploid species compared to their diploid relatives should arise from intergenomic heterosis or epigenetic phenomena such as neofunctionalization rather than major chromosomal rearrangements (ADAMS and WENDEL 2005; COMAI 2005). Similar observations have also been made between the allotetraploid cotton and its diploid relatives (BRUBAKER *et al.* 1999) or between the allotetraploid *Brassica napus* and the diploid *B. oleracea* (CHEUNG *et al.* 1997). Moreover, comparative mapping between the sugarcane cultivars of high ploidy levels and *Sorghum bicolor*, which can be considered as their diploid relative in the absence of known extant taxa (GUIMARAES *et al.* 1997), revealed a high level of colinearity between these species (MING *et al.* 1998), later confirmed by microsynteny analyses (JANNOO *et al.* 2007).

The discrepancies of colinearity observed between homo(eo)logous linkage groups in two HGs of the *F.* ×*ananassa* map can be due either to chromosomal

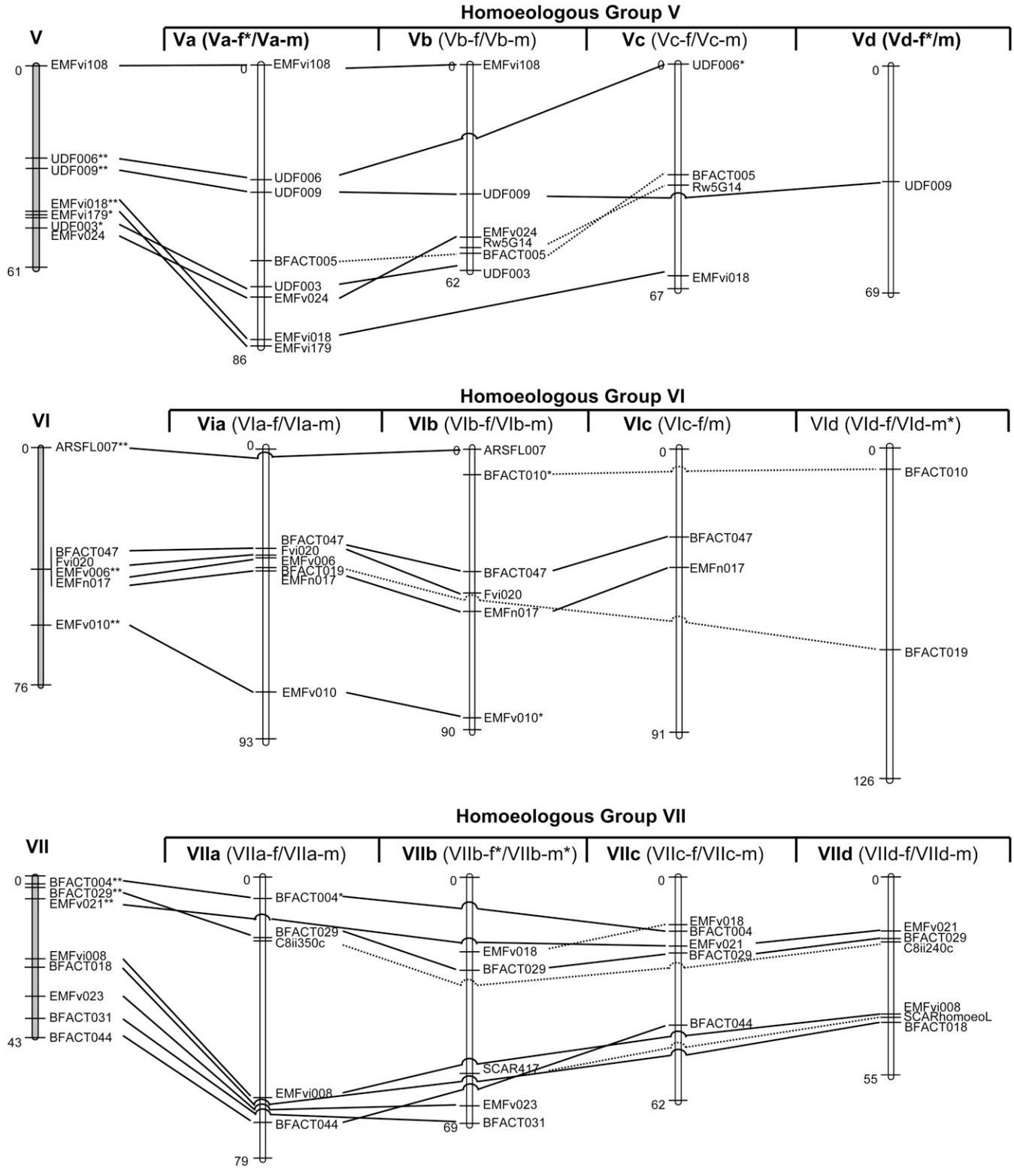


FIGURE 2.—*Continued.*

structural polymorphism of the different genomes within the cultivated strawberry, as observed in the polyploid Saccharum (MING *et al.* 1998) or to the evolution within the octoploid species after the initial hybridization of the ancestral genomes, as observed in cotton (BRUBAKER *et al.* 1999; DESAI *et al.* 2006). Due to the relative low covering of the comparative map, large-scale rearrangements could be readily detected, whereas those involving small chromosomal segments are likely to go undetected (BURKE *et al.* 2004).

TABLE 4

Number of integrated linkage groups according to the phase (coupling/repulsion or coupling alone) of their markers and to the homoeology group (HG) to which they belong

	No. of integrated linkage groups[a]			
HG	Both coupling/repulsion	Both coupling	One coupling: one coupling/repulsion	Undetermined
I	2	—	—	2
II	2	1		1
III	4	—	—	—
IV	3	—	1	—
V	2	—	1	1
VI	2	—	1	1
VII	3	1	—	—
Total observed	18	2	3	5
Expected[b]	15.00	0.86	7.29	4.86

[a] The integrated linkage groups were obtained by merging the female and male homologous linkage groups using JoinMap software.
[b] Assuming random distribution.

The high level of colinearity between linkage groups of the octoploid with their corresponding diploid homologs suggests a close relationship between genomes in Fragaria. This hypothesis is supported by the difficulty to separate clearly Fragaria species in phylogenetic analyses using cpDNA and rDNA (Harrison and Luby 1997; Potter *et al.* 2000). The elucidation of the relationships between the diploid strawberry genomes and further insights into how they have been involved in the construction of the different Fragaria polyploids will probably await phylogenetic studies of nuclear genes.

Markers exhibiting distorted segregation ratios are preferentially distributed on homoeologous regions: Markers exhibiting distorted segregation ratios on the current octoploid maps were preferentially distributed in only two HGs grouping a total of five male groups, IVa-m, IVb-m, IVd-m, VIIa-m, and VIIb-m* (Figure 1). The clustering of genetic markers showing distorted segregation ratios suggests that they may be linked to viability genes that would affect embryonic development or fitness (Tani *et al.* 2003; Sledge *et al.* 2005). Such genes could be located on the characterized homoeologous regions HGs IV or VII, which can be further studied by adding markers in these regions. The level of inbreeding, which is likely to be present in the cultivated strawberry (Sjulin and Dale 1987; Dale and Sjulin 1990; Luby *et al.* 1991), may have played a role in the exposure of deleterious alleles (Remington and O'malley 2000).

Low levels of segregation distortion have been observed to date in the construction of the octoploid linkage maps. However, by selecting only single-dose markers for use in the mapping procedure, we may have underestimated the extent of segregation distortion (Julier *et al.* 2003). In our study, by excluding markers that did not clearly segregate 1:1, as expected for single-dose markers, we may have excluded distorted markers that segregate 3:1 and proposed that they were double-dose alleles. This hypothesis could be confirmed through the mapping of these potential double-dose markers, as described by Da Silva *et al.* (1995).

Disomic behavior is predominant in the cultivated strawberry *F.* ×*ananassa*: The determination of the type of inheritance displayed by *F.* ×*ananassa* is critical for genetic analyses, such as elucidating the phylogeny in the Fragaria genus to clarify the origin of the cultivated strawberry and developing molecular-assisted breeding strategies. Bivalents are usually observed in Fragaria during meiosis (Ichijima 1926; Longley 1926; Powers 1944), with the exception of multivalents mentioned by Mok and Evans (1971) but rejected by Ibrahim *et al.* (1981) since chromosomes have been observed during diakinesis. Finally, the type of segregation that occurs in octoploid Fragaria (*i.e.*, disomic or polysomic) has remained unclear.

On the basis of the majority of linkage groups segregated in coupling/repulsion, we were able to demonstrate that disomic behavior is predominant in the cultivated strawberry *F.* ×*ananassa*. The discrepancy with previous results that suggested a mixed behavior with disomy and partial polysomy (Lerceteau-Köhler *et al.* 2003) can be attributed to the use in this study of codominant microsatellite markers, the higher number of progeny used here, and the greater marker coverage of the octoploid maps. Microsatellite markers allow finer genetic analyses to be performed compared to the almost exclusive use of dominant AFLPs in the study of Lerceteau-Köhler *et al.* (2003). This is the first report of genetic analyses of genomewide meiotic behavior of the octoploid Fragaria since previous works reported segregation of a limited number of markers (Ashley *et al.* 2003, Kunihisa *et al.* 2005).

However, the observation of large LGs, *i.e.*, IIb-f*/IIb-m*, and VIIb-f*/VIIb-m*, which were composed exclusively of markers in the coupling phase, is inconsistent

with normal disomy and may suggest that partial residual polysomic behavior occurs. Preliminary results from a comparison between the octoploid genetic map presented here with another one (A. Monfort, unpublished results) has shown that this other map contains the same LGs segregating only in coupling. Therefore, residual polysomic behavior is likely to suggest that diploidization is currently an ongoing process in the cultivated strawberry.

Establishing Fragaria as a model system: Extensive conservation of long-range genome organization in Fragaria supports the use of the diploid Fragaria as a model system for studying genomics and molecular dissection of the much more complex octoploid *F.* × *ananassa* genome (Davis and Yu 1997; Sargent *et al.* 2004). Complementary to the use of diploid Fragaria, the dense and reliable parental maps of the octoploid cultivated strawberry presented here provide a valuable reference tool for further genetic analysis in the octoploid strawberry. They will facilitate QTL analyses by identifying QTL located on homo(eo)logous linkage groups that were duplicated through polyploidization (Paterson 2005). Moreover, it will also facilitate the information transfer for gene cloning in either the diploid or the octoploid Fragaria species and, beyond, in other Rosaceae species. Finally, considering the small size of diploid Fragaria genomes (164 Mbp/C), and the high level of synteny with the cultivated octoploid strawberry, it would be timely to sequence the genome of a species such as one progenitor of the cultivated strawberry genome. Such a sequence would provide a scaffold for fine mapping and positional cloning in the cultivated octoploid strawberry and in other Rosaceae species.

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