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Assessment of SSR Marker Transfer from the Cultivated Strawberry to Diploid Strawberry Species: Functionality, Linkage Group Assignment, and Use in Diversity Analysis

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Abstract. **The cultivated strawberry, *Fragaria ×ananassa* Duchesne ex Rozier, originated *via* hybridization between octoploids *F. chiloensis* (L.) Mill. and *F. virginiana* Mill. These three octoploid species are thought to share a putative genome composition of AAA'A'BBB'B'. Diploid *F. vesca* L., is considered to have donated the A genome. Current attention to the development of a diploid model system for strawberry genomics warrants the assessment of simple sequence repeat (SSR) marker transferability between the octoploid and diploid species in *Fragaria* L. In the present study, 23 SSR primer pairs derived from *F. ×ananassa* 'Earliglow' by genomic library screening were evaluated for their utility in six diploid *Fragaria* species, including eight representatives of *F. vesca*, four of *F. viridis* Weston, and one each of *F. nubicola* (Hook. f.) Lindl. ex Lacaita, *F. mandshurica* Staudt, *F. iinumae* Makino, and *F. nilgerrensis* Schltdl. ex J. Gay. SSR primer pair functionality, as measured by amplification success rate (= 100% - failure rate) in each species, was ranked (from highest to lowest) as follows: *F. vesca* (98.4%) > *F. iinumae* (93.8%) = *F. nubicola* (93.8%) > *F. mandshurica* (87.5%) > *F. nilgerrensis* (75%) > *F. viridis* (73.4%). The extent to which these octoploid-derived SSR primer pairs generated markers that could be added to the *F. vesca* linkage map also was assessed. Of the 13 *F. ×ananassa* SSR markers that segregated codominantly in the *F. vesca* mapping population, 11 were assigned to linkage groups based upon close linkages to previously mapped loci. These markers were distributed over six of the seven *F. vesca* linkage groups, and can serve as anchor loci defining these six groups for purposes of comparative mapping between *F. vesca* and *F. ×ananassa.***

Simple sequence repeat (SSR) or microsatellite markers are well-suited for the purpose of comparative linkage mapping between diploid and octoploid strawberry (*Fragaria* spp.). SSRs are more likely to be transferable among populations than are anonymous markers such as the randomly amplified polymorphic DNA (RAPD) and amplification fragment length polymorphism (AFLP) markers employed in the first generation linkage maps of the diploid model species, *F. vesca* (Davis and Yu, 1997), and octoploid, cultivated strawberry, *F. ×ananassa* (Lerceteau-Kohler et al., 2003), respectively. The potential of SSRs for having multiple, codominant alleles is a feature of particular relevance to linkage mapping in an octoploid, in which a single SSR primer pair has the potential to detect as many as eight SSR variants in a single individual. Moreover, costly genomic library screening—the source of the initial wave of *Fragaria* SSRs (Ashley et al., 2003; Cipriani and Testolin, 2004; Hadonou et al., 2004; James et al., 2003; Nourse et al., 2002; Sargent et al., 2003)—is being supplanted by expressed sequence tag (EST) database mining as a means of discovering new SSR loci (Lewers et al., 2005).

The evident potential for SSR marker transferability between *F. vesca* and the commercially cultivated strawberry, *F. ×ananassa* (Cipriani and Testolin, 2004; Hadonou et al., 2004), is among the features that make *F. vesca* an attractive and appropriate diploid model species for strawberry genomics (Sargent et al., 2004). Highly self-fertile inbred lines and useful mutant forms of *F. vesca* are already available as genetic tools, due in part to the long history of cultivation of European *F. vesca* ssp. *vesca* L. forma *semperflorens* (Duchesne) Staudt (Darrow, 1966), the so-called 'Alpine', everbearing form of *F. vesca*. The first *F. vesca* linkage map was based on the F_2 generation of a cross between *F. vesca* ssp. *vesca* L. forma *semperflorens* cultivar Baron Solemacher and *F. vesca* ssp. *americana* (Porter) Staudt accession WC6 (Davis and Yu, 1997). 'Baron Solemacher' carries two independently inherited, recessive, single gene traits: runnerless (*r*) and everbearing (*s*) (Brown and Wareing, 1965).

Ancestry and genome composition of *F. ×ananassa*. The cultivated strawberry, *F. ×ananassa,* is known to have originated via hybridization between octoploids *F. chiloensis* and *F. virginiana* (Darrow, 1966). R. Bringhurst and collaborators have proposed that the latter two species and *F. ×ananassa* share a common

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genome composition: expressed initially as AAA'A'BBBB (Senanayake and Bringhurst, 1967) and later revised to AAA'A'BBB'B' (Bringhurst, 1990). Based upon cytological evidence, Senanayake and Bringhurst (1967) inferred a correspondence between the *F. vesca* genome and the A genome of the octoploids. *F. vesca* has by far the widest geographic distribution of any strawberry species (Hancock, 1999), and is widely regarded as a likely genome donor to the octoploid species. Therefore, the potential for SSR marker transfer between the octoploid *Fragaria* species, *F. vesca*, and other *Fragaria* diploids is of significant interest.

In the present study, SSR primer pairs derived by screening a library derived from *F. ×ananassa* 'Earliglow' (Lewers et al., 2005; Nourse et al., 2002) were evaluated for their capacities to amplify products and detect polymorphisms in a diverse sampling of diploid *Fragaria* germplasm, including representatives of six species. The extent to which these octoploid-derived SSR primer pairs generated markers that could be added to the *F. vesca* linkage map (Davis and Yu, 1997) was also assessed.

Materials and Methods

SSR genotyping. Twenty-three SSR primer pairs were identified by screening a genomic library of *F. ×ananassa* 'Earliglow', as described elsewhere (Lewers et al., 2005; Nourse et al., 2002). Primer sequences for the primer pairs with Agricultural Research Service Fruit Lab (ARSFL) numbers employed here are reported in Lewers et al. (2005). Genotyping of SSR PCR products was performed in the manner previously described (Lewers et al., 2005).

Primer pair functionality and diversity analysis. Initially, 23 SSR primer pairs were screened for their ability to amplify products (as manifested by the presence of a chromatographic peak, or peaks) and detect polymorphisms among 16 diploid accessions (Table 1) representing six species. A subset of 16 primer pairs was then selected for closer scrutiny, providing 256 accession × primer pair combinations used for examination of germplasm diversity and primer pair functionality. The PCR amplification failure rate (percentage) for each species was calculated as the number of failed amplifications in that species (× 100), divided by the total number of amplifications attempted in that species, where the denominator equals the number of representatives from the species in question times 16 (the number of primer pairs evaluated). The potential usefulness of 16 of these SSR primer pairs for detecting genetic relationships was assessed by subjecting the respective data set to a phenetic analysis. A data matrix was constructed, scoring the presence of PCR product as 1 and its absence as 0. The computer program MVSP (Multi-Variate Statistical Package, version 3.13; Kovach Computing Services, Pentraeth, U.K.) was used to create a similarity matrix based on Nei and Li's (1979) similarity coefficient and to construct a dendrogram using the unweighted pair group method with arithmetic mean (UPGMA) cluster analysis. Bootstrap support values were obtained from 1000 replicates using the program Treecon [version 1.3b for Windows (Van de Peer and De Wachter, 1994)].

The numbers of PCR product variants in three self-compatible species were compared to those in three self-incompatible species using a data subset chosen to minimize the number of missing data points. For this test, a square root transformation was applied to the data set, on the basis (Zar, 1999) that it consisted of counts (i.e., number of variants for each accession).

Mapping. Twenty-two SSR primer pairs, including most of those used in the diversity analysis, were evaluated for their ability to detect polymorphism between mapping parents 'Baron Solemacher' (BS) and WC6 (Davis and Yu, 1997). The BS and WC6 SSR data set was not added into the diversity analysis data set because the two data sets were generated in separate assays that may have produced alternate measures of "size" for the same SSR variant, or identical measures of size for similar but distinct variants.

Following the minimal mapping population concept of Joyeux et al. (1999), an informative subset of 35 F_2 generation plants from the original 80-plant mapping population of Davis and Yu (1997) was manually selected to maximize the number of detected crossovers and thus facilitate linkage group assignment and

Table 1. Diploid *Fragaria* germplasm used to assess the functionality of SSR primer pairs derived from genomic sequences of *F. ×ananassa* 'Earliglow'. The species, subspecies, and name or accession number are provided with the location from which each originated and the source from whom the plants or seeds were obtained.

Germplasm identification	Site of origin	Source[z]
F. vesca ssp. *vesca*		
'Yellow Wonder' (YW)	European cultivar	Burpee
F. vesca ssp. *americana*		
'Pawtuckaway' (PAWT)	New Hampshire (Mt. Pawtuckaway)	T. Davis
F. vesca (ssp. *bracteata*)[y]		
DN1C	California (Del Norte Co.)	T. Davis
HP6A	California (Humboldt Co.)	T. Davis
BC23	British Columbia (South of Prince George)	T. Davis
BC29	British Columbia (East of Prince George)	T. Davis
BC35	British Columbia (near McBride)	T. Davis
GS2C	British Columbia (near McBride)	G. Staudt
F. mandshurica		
GS99	Jakutsk, Russia	G. Staudt
F. nubicola		
CFRA 520 (PI 551851)	Pakistan	NCGR
F. iinumae		
CFRA 377 (PI 551751)	Japan	NCGR
F. nilgerrensis		
CFRA 1358 (PI 616672)	Yunnan, China	NCGR
F. viridis		
CFRA 333 (PI 551741)	Germany	NCGR
CFRA 341 (PI 551742)	Germany	NCGR
SIB3	Siberia	UNH
NOV3A	Siberia	UNH

[z]Accessions with CFRA prefixes were obtained from the USDA National Clonal Germplasm Repository (NCGR) in Corvallis, Ore. *F. vesca* 'Yellow Wonder' was initially purchased as seed from W. Atlee Burpee and Co., Warminster, Pa., and was subsequently seed propagated through natural self pollination at the University of New Hampshire. GS2C and GS99 were obtained as seed from Guenter Staudt, Merzhauzen, Germany. SIB3 and NOV3A were obtained as seed from, respectively, Garrett Crow (University of New Hampshire, Durham) and Ivan Krasnoborov (Central Siberian Botanical Garden, Novosibirsk, Russia). Accessions DN1C, HP6A, PAWT, BC23, and BC29 were collected from the wild as runner plants by T.M. Davis.

[y]The accessions listed as *F. vesca* ssp. *bracteata* were collected within the range of this subspecies, as delineated by Staudt (1999), but have not been definitively typed.

rough approximation of map distance using a minimum number of genotyping assays. In the present study, genotypic data from the mapping population subset for all of 14 previously mapped codominant RAPD markers, the DNA sequence-based marker *Adh* (alcohol dehydrogenase), and isozyme markers *Sdh* (shikimate dehydrogenase) and *Pgi-2* (phosphoglucose isomerase-2) were used to construct seven framework linkage groups in the program Map Manager (Manly and Olson, 1999). The SSR genotyping data set for this population subset was then entered into a single "new chromosome" file and mapped to the framework linkage groups using the "Distribute" function. Linkage relationships within each linkage group were then refined using the "Ripple" function. Map distances were expressed as "Morgan" map units (i.e., as recombination percentage). Any linkages detected by Map Manager based upon recombination percentages of greater than 20% were subjected to confirmation or rejection on the basis of chi-squared contingency testing.

Results

SSR primer pair functionality and diversity analysis. Of 23 SSR primer pairs evaluated on the 16 germplasm accessions, six primer pairs (ARSFL numbers 9, 15, 16, 20, 25, and 26) failed to give satisfactory results, either due to inconsistent amplification, or (for ARSFL_9 only) an excessive number of chromatographic peaks. One other primer pair, ARSFL_28, reproducibly amplified a product in only one accession, *F. iinumae*. No further data are presented for the aforementioned seven primer pairs.

The remaining 16 primer pairs evaluated on 16 germplasm accessions provided 256 accession × primer pair combinations used for examination of germplasm diversity and SSR primer pair functionality. Successful amplification of a PCR product is a necessary prerequisite to marker transfer. Among the 256 accession × primer pair combinations under consideration, 27 combinations reproducibly failed to produce a detectable chromatographic peak (Table 2). The failure rate in *F. viridis*, in which four accessions (CFRA 333, CFRA 341, NOV3A, and SIB3) were tested and 17 failed amplifications occurred, was $100 \times 17/(4 \times 16) = 26.6\%$, the highest failure rate observed. At least one amplification failure occurred for 11 out of 16 primer pairs evaluated in *F. viridis* (Table 2). The comparable failure rates (in parentheses) for the other species tested, in descending order, were *F. nilgerrensis* (25%), *F. mandshurica* (12.5%), *F. nubicola* (6.2%), *F. iinumae* (6.2%), and *F. vesca* (1.6%).

Germplasm diversity. The numbers and sizes of amplification products (detected as chromatographic peaks) generated from each accession with each of the 16 primer pairs under consideration are presented in Table 2. Of the 256 accession × primer pair combinations assayed, 174 generated single peaks, 52 generated two peaks, and three generated three peaks (Table 2). When summed over all plant accessions, the number of SSR variants detected per primer pair ranged from six (ARSFL_19) to 19 (ARSFL_13), with a mean of 11.1. ARSFL_19 was the only primer pair that did not detect multiple variants in at least one accession, while at the opposite extreme, primer pair ARSFL_4 detected two variants in each of eight accessions.

The differences in the number of representatives per species and the non-random distribution of amplification failures among species present obstacles to interspecies comparisons of SSR PCR product diversity in the total data set. However, if *F. viridis* accession CFRA 341 is excluded from consideration because of its apparently low sequence similarity with 'Earliglow', the source of the SSR primer pairs, there were eight primer pairs (ARSFL numbers 4, 7, 10, 17, 19, 22, 24, and 27) that amplified one or more products in each of the other 15 accessions. Considering only this defined data subset (shaded data, Table 2), and summing SSR PCR product diversity for each accession over the eight respective primer pairs, the total number of SSR PCR product size variants per accession ranged from a low of eight (the minimum possible for eight primer pairs) in *F. vesca* accessions GS2C, PAWT, and YW, to 13 in *F. viridis* accession NOV3A. When the known breeding system characteristics (Staudt, 1989) of the species are used to group the species, the mean number of variants per accession was 9.1 in the group of self-compatible (SC) species (*F. vesca*, *F. iinumae*, and *F. nilgerrensis*), and 11.0 in the group of self-incompatible (SI) species (*F. viridis*, *F. nubicola*, and *F. mandshurica*). The difference in mean number of variants between SC and SI species is statistically significant ($P = 0.047$) based on a two-tailed t test.

Phenetic analysis. The results of the cluster analyses are displayed graphically in Figure 1. Only four nodes in the UPGMA dendrogram have bootstrap support above 50%. All of the *F. vesca* accessions, including seven North American accessions and one European accession (YW), form a cluster with 52% bootstrap support. Within the *F. vesca* cluster, two of the four accessions from British Columbia (BC23 and BC29) form a subcluster with 51% bootstrap support; however, the other two accessions from British Columbia (BC35 and GS2C) fall just outside this subcluster. The four *F. viridis* accessions comprised another cluster, which has the highest bootstrap value (72%) of any cluster in the tree.

SSR mapping in *F. vesca*. Of 22 SSR primer pairs evaluated in the BS and WC6 mapping parents, 13 primer pairs detected single band mobility polymorphisms that distinguished the parents and that segregated as codominant markers in the informative mapping population subset. Identities of all evaluated primer pairs, and the allele sizes of polymorphic markers, are reported in Table 3. For the segregating markers, four phenotypic categories were recognized and encoded for linkage analysis: parental (BS), heterozygous, parental (WC6), and no band detected. The latter category was likely due to amplification failure for technical reasons: such reactions were not repeated. Because the informative mapping population subset was not a randomly chosen sample of the original mapping population (Davis and Yu, 1997), goodness-of-fit tests to a monohybrid expectation were not appropriate and were not performed.

Eleven of the SSR primer pairs detected single loci that were assigned to linkage groups on the basis of close (<15% recombination frequency) linkages to previously mapped markers (Table 3). Three loci each were located on linkage groups II and III; two were located on group I; and one each was located on groups V, VI, and VII. No SSR marker was assigned to group IV. The three codominant SSR markers assigned to group II clustered into two unlinked subgroups, one of which was anchored by the previously mapped codominant markers *Adh* and BC103AB, and the other by previously mapped codominant marker *Pgi-2* (Table 3). When the recessive linkage group II marker *r* (= runnerless) was included in the analysis, the two subgroups were loosely connected (results not shown), in agreement with the linkage relationships of markers *Adh*, BC103AB, *r*, and *Pgi-2* in the original *F. vesca* map (Davis and Yu, 1997). SSR markers ARSFL_11 and ARSFL_15 were not linked to any of the previously mapped anchor loci, and so could not be assigned to any linkage group.

Table 2. Size (base pairs) of amplification products from PCR reactions using 16 primer pairs derived from genomic sequences of *Fragaria* ×*ananassa* 'Earliglow', an octoploid strawberry cultivar, to assay 16 diploid strawberry germplasm accessions maintained at the University of New Hampshire. For each SSR primer pair, the total number of PCR size variants amplified from this collection of diploid germplasm representatives is summed in the far right column

	Diploid strawberry germplasm accessions evaluated																
SSR primer pair names	BC23	BC29	BC35	GS2C	DN1C	HP6A	PAWT	YW	CFRA 333	CFRA 341	NOV3A	SIB3	GS99	CFRA 520	CFRA 377	CFRA 358	Unique PCR size variants (no.)
ARSFL_1	247[z]	247	264	Ø[y]	254	283	250	263	Ø	Ø	Ø	264	240 252	279	269	Ø	10
ARSFL_2	238	275	240 246	242	279	281	238 256	282	Ø	235	245	235	Ø	245	Ø	246	12
ARSFL_3	318 322	318	318 325	328	328	322	312 316	325	Ø	311	294 312	311	314	Ø	314	Ø	9
ARSFL_4[x]	170 174	170 174	170 174	174	170 186	170	174	196	177 197	177 197	205	191 197	172	180 184	240	172	12
ARSFL_7	218	218	216	216	216	218	216	216	213	213 222 248	213	206 213	225	219 223	231	253 265	12
ARSFL_8	302 309	302 309	311	302	304	293	304	293	Ø	Ø	306	Ø	304 310	302	304	Ø	7
ARSFL_10	223	223 227	235	223	233	215	223	231	230	239 264	231 244	232	233	227 239	254	231	10
ARSFL_11	277	261 265	275	271	271	267	247 255	255	296	Ø	306	308	Ø	271 279	241 252	252 271	14
ARSFL_12	261	261	259 261	261	261 263	263	259	261	257	261	Ø	Ø	259 261	320	177 257	218 268	8
ARSFL_13	302	206 352	285	299	280	278	211 339	167	Ø	Ø	243	253	208	197	167 193	271 275	19
ARSFL_14	215	232	215	214	217	234	231	Ø	279	Ø	280	247 293	222	231 246	229	Ø	13
ARSFL_17	216	218	216	216	218	218	216	220	218	Ø	213 220	216	217	219	257	222	8
ARSFL_19	314	328	314	328	328	328	328	328	342	Ø	342	342	330	326	342	341	6
ARSFL_22	195	205	189 193	191	197	176 212	168	270	173	197	189 197	189	150 154	167 204	263	254	17
ARSFL_24	258 264	256	256	272	245 282	258	244	286	203 219	203	203 219	203	250	242	162 242	238	15
ARSFL_27	162	162	162	162	162	162	162	162	186 193	Ø	197 207	203	162	164	162	157	8
Total number of PCR products per accession	20	21	21	15	19	17	21	15	14	12	20	17	18	21	20	16	

[z]Size of amplified PCR product in base pairs (bp).
[y]Ø = No amplification with this primer pair × accession combination.
[x]Shaded boxes indicate primer pairs that amplified at least one product from every accession (excluding CFRA 341 from which few primer pairs could amplify a product, indicating little sequence similarity to 'Earliglow').

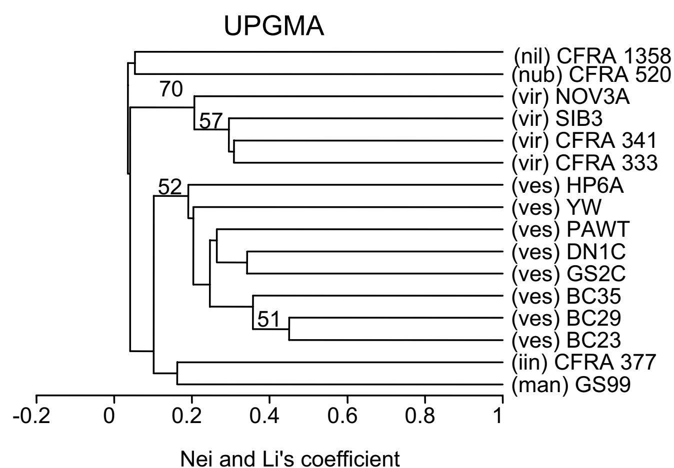

Fig. 1. Dendogram of *Fragaria* accessions representing six diploid species characterized with 16 SSR primer pairs. The six species are *F. vesca* (ves), *F. viridis* (vir), *F. nubicola* (nub), *F. mandshurica* (man), *F. iinumae* (iin), and *F. nilgerrensis* (nig). The SSR primer pairs were derived from genomic sequences of *F.* ×ananassa 'Earliglow'. The unrooted dendrogram was constructed using UPGMA cluster analysis of a similarity matrix generated using Nei and Li's coefficient. Bootstrap values over 50% are shown and were generated using UPGMA with 1000 replicates.

Discussion

Three criteria are applicable to the assessment of SSR marker transferability among taxa. First, an SSR primer pair based on DNA sequence from the source taxon or population must amplify a PCR product (i.e., must be functional) in one or more of the sampled members of the recipient taxon or population. Second, to be useful as a genetic marker in the recipient taxon or population, polymorphism must be detected between/among the population members of interest. Finally, marker codominance, as associated with band mobility polymorphism, is generally more useful than marker dominance, as associated with presence/absence polymorphism.

The transferability of SSR primer pairs among cultivars, populations, and subspecies within *F. vesca* has proven to be quite good (Cipriani and Testolin, 2004; Hadonou et al., 2004; James et al., 2003). When Hadonou et al. (2004) evaluated 31 primer pairs derived from *F. vesca* ssp. *vesca* on a sampling of diploid *Fragaria* germplasm that included all four *F. vesca* subspecies [*vesca*, *americana*, *bracteata* (A. Heller) Staudt, and *californica* (Cham. & Schltdl.) Staudt], PCR amplifications were successful with all 15 of the *F. vesca* accessions, and 26 of the 31 primer pairs detected band mobility polymorphism in the sampled germplasm. When the latter 26 primer pairs were evaluated on F_2 plants from a cross between *F. vesca* ssp. *vesca* L. forma *semperflorens* cultivar Yellow Wonder x *F. vesca* ssp. *americana* 'Pawtuckaway', 20 of the primer pairs detected band mobility polymorphism and codominant marker segregation, while the remaining six markers were monomorphic in this intraspecific cross.

In comparison, when the interspecific cross *F. vesca* ssp. *vesca* FDP815 x *F. nubicola* FDP601 was used for map construction by Sargent et al. (2004), 14 out of 45 *F. vesca*-derived SSR primer pairs failed to amplify a product in the *F. nubicola* parent. In addition, one of five *F. nubicola*-derived and two of 12 *F. viridis*-derived SSR primer pairs failed to amplify a product in the *F. vesca* parent. Both *F. nubicola* and *F. viridis* are diploids. Thus, the advantage of SSR marker codominance was partially sacrificed in this interspecific cross due to amplification failure for one or the other parental allele at each of 17 SSR loci. It was still possible to map the latter 17 loci as dominant markers, and 51 codominant SSRs also were mapped.

Table 3. Assignment of SSR markers derived from cultivated octoploid strawberry *Fragaria* ×*ananassa* 'Earliglow' genomic sequences to diploid *F. vesca* linkage groups, as defined by linkages to markers (marked by asterisks) previously located on the *F. vesca* map (Davis and Yu, 1997). SSR primer pairs that failed to detect polymorphism between the mapping parents were: ARSFL numbers 7, 11, 16, 18, 19, 22, 23, 26, 28.

Linkage group	SSR marker	Allele sizes (bp) BS	WC6	Closest linkages (recombination %)
I	ARSFL_10	241	229	ARSFL_25 ------------------ ARSFL_10 -------------------- SDH* (1.6) (2.9)
	ARSFL_25	187	181	
II	ARSFL_3	326	314	*Adh** // ARSFL_12 --------------- ARSFL_3 --------------- BC103AB* (0.0) (3.5) (12.2)
	ARSFL_12	261	258	
	ARSFL_17	218	214	ARSFL_17 -----------------*Pgi*-2* (3.0)
III	ARSFL_8	291	302	ARSFL_8 --------------------------- ARSFL_9//ARSFL_14//PZ14CD* (9.8) (0.0) (0.0)
	ARSFL_9	221	227	
	ARSFL_14	245	229	
V	ARSFL_1	261	244	ARSFL_1 ----------------- PZ04CD* (3.2)
VI	ARSFL_2	280	235	ARSFL_2 ----------------- PX18AB* (2.3)
VII	ARSFL_4	194	174	ARSFL_4 ----------------- BC194BD* (3.1)

SSR marker transfer between *F. viridis* and *F. vesca* is also partially restricted. Sargent et al. (2003) developed 22 SSR primer pairs by screening a microsatellite-enriched *F. viridis* genome library, of which 21 primer pairs detected polymorphisms while one was monomorphic in a sampling of six *F. viridis* accessions. When these primer pairs were evaluated on *F. vesca* mapping parents 'Yellow Wonder' and 'Pawtuckaway', seven of 22 *F. viridis* primer pairs (32%) failed to consistently amplify a product in either parent (Sargent et al., 2003); thus, these *F. viridis* SSR loci were not transferable to the *F. vesca* mapping population. The remaining 15 *F. viridis* primer pairs detected product length polymorphisms, and thus could be mapped in the *F. vesca* population as codominant markers.

SSR primer pair transferability from diploid to octoploid strawberry germplasm has been assessed in three studies. All 31 of the *F. vesca*-derived primer pairs described by Hadonou et al. (2004) amplified one or more products in three *F.* ×*ananassa* cultivars, although three of the primer pairs produced only smears or multiple products of larger than the expected size. Of 20 SSR primer pairs developed from *F. vesca* by Cipriani and Testolin (2004), 19 amplified single or multiple products in *F.* ×*ananassa* cultivar Queen Elisa, while 16 of the 20 amplified single or multiple products in an unidentified accession of octoploid *F. chiloensis*. Lewers et al. (2005) evaluated the functionality in octoploid *Fragaria* species of 10 SSRs developed from *F. vesca* genomic DNA sequences (James et al., 2003) and four developed from *F. vesca* EST sequences. Of the SSRs that amplified a product from any *Fragaria* template DNA, all amplified one or more products from *F. virginiana, F. chiloensis*, and 13 of 14 amplified products from *F.* ×*ananassa*. The observed high levels of shared SSR primer pair functionality among *F. vesca* and the octoploids *F.* ×*ananassa* and *F. chiloensis* is consistent with the hypothesis that *F. vesca* is a genome contributor to the octoploids, but leaves open the question of whether the shared sequences are specific to the particular subgenome in the octoploids that was putatively contributed by *F. vesca*.

Octoploid-derived SSR primer pair functionality in diploid species was reported by Lewers et al. (2005). Of the 62 *F.* ×*ananassa*-designed SSR primer pairs that amplified a product from any of the genotypes tested, 58 amplified a product from at least one of the diploid strawberry species tested, and all four SSR primer pairs designed from *F. virginiana* amplified a product from a diploid strawberry. Functionality of octoploid-derived SSR primer pairs in other octoploid species also was examined. Of the 62 *F.* ×*ananassa*-derived primer pairs, 57 amplified a product from *F. chiloensis,* 57 from *F. virginiana*, and 58 from other representatives of *F.* ×*ananassa*. All four SSR primer pairs designed from *F. virginiana* were functional in *F. virginiana*, *F. chiloensis,* and *F.* ×*ananassa* (Ashley et al., 2003; Lewers et al., 2005).

In the present study, SSR primer pairs based upon DNA sequence from octoploid *F.* ×*ananassa* were evaluated in representatives of six diploid *Fragaria* species, including eight representatives of *F. vesca*, four of *F. viridis*, and one each of *F. nubicola*, *F. mandshurica*, *F. iinumae*, and *F. nilgerrensis*. Because of the sampling imbalance within species in this exploratory survey, care must be taken in drawing conclusions about functionality and polymorphism detection in the test taxa. Nevertheless, the results reported here, particularly when considered in conjunction with those of previous studies of *Fragaria* SSRs, provide useful insight into the patterns of SSR primer pair transferability among *Fragaria* species.

Fragaria ×*ananassa* SSR primer pair functionality, as measured by amplification success rate (= 100% – failure rate) in the test species, was ranked (from highest to lowest) as follows: *F. vesca* (98.4%) > *F. iinumae* (93.8%) = *F. nubicola* (93.8%) > *F. mandshurica* (87.5%) > *F. nilgerrensis* (75%) > *F. viridis* (73.4%). Even the lowest of these amplification success percentages (to *F. viridis*) is quite good compared with the poor to zero rates of *Fragaria* SSR primer pair functionality in other Rosoideae genera, as reported by Lewers et al (2005). Nevertheless, the observed disparity in amplification failure rates between *F. vesca* (1.6%) and *F. viridis* (26.6%) is sufficient to prompt closer examination of the assertion (Lewers et al. 2005) that sequences from all *Fragaria* species are equally useful in designing SSR primers for any *Fragaria* species.

SSR primer pair transfer between *F. vesca* and *F.* ×*ananassa* has encountered little to no restriction. In two prior studies that examined a combined total of 51 *F. vesca*-derived SSR primer pairs (Cipriani and Testolin, 2004; Hadonou et al., 2004), over 90% were functional in *F.* ×*ananassa*. Similarly, the functionality in *F. vesca* of *F.* ×*ananassa* SSR primer pairs was virtually unrestricted (98.4%) in the present study. In contrast, *F. viridis* SSR marker function in *F. vesca* was restricted to 68% (15 out of 22) of *F. viridis* primer pairs that consistently amplified a product in either parent (YW and PAWT) of an *F. vesca* mapping population (Sargent et al., 2003). In the present study, *F.* ×*ananassa* primer pair function in *F. viridis* was similarly restricted (73.4%). The reverse marker function test, from *F. viridis* to *F.* ×*ananassa*, was not examined in the present study. However, the foregoing discussion justifies the expectation that *F. viridis* primer pair function might be restricted, as compared to *F. vesca* primer pair function, in *F.* ×*ananassa*.

The absence of strong bootstrap support for the phenetic dendrogram provides no justification for an attempt to draw phylogenetic inferences from this data set. Nevertheless, the dendrogram does contain aspects of interest, particularly when considered in comparison to a similar dendrogram of *F. vesca* accessions characterized with 26 SSR primer pairs designed from genomic sequences of *F. vesca* 'Ruegen' (Hadonou et al., 2004). In the latter study, excluding two *F. vesca* accessions that did not cluster as expected based upon their assigned subspecies, a major clade comprised of European *F. vesca* ssp. *vesca* accessions, including 'Yellow Wonder', was distinguished (with 60% bootstrap support) from the second major clade comprised of North American subspecies *americana*, *bracteata*, and *californica* accessions. In the present study, 'Yellow Wonder' did not separate from the North American *F. vesca* accessions, perhaps because too few SSR primer pairs were used, or because the source of SSR primer sequences is *F.* ×*ananassa* instead of *F. vesca*. In both studies the North American *F. vesca* representatives fell into a common cluster, providing no encouragement for the use of SSR primer pairs to differentiate the three North American *F. vesca* subspecies: *americana* (represented by PAWT in the present study and two accessions in the prior study); *bracteata* (represented by one accession in the prior study and six tentatively classified accessions in the present study); and *californica* (represented by two accessions in the prior study). This lack of subspecies resolution was unexpected, because SSR primer pairs are commonly used to distinguish crop cultivars. The resolution of *F. vesca* from *F. viridis* in the present UPGMA tree does point to the potential for using SSR primer pairs to assess possible gene flow between *F. viridis* and *F. vesca* ssp. *vesca*, which are sympatric over much of their common range of western Europe to Lake Baikal (Staudt, 1989) and form natural unilateral (*F. vesca* x *F. viridis*) hybrids

that have been recognized as a unique taxon, *Fragaria ×bifera* Duchesne (Staudt et al., 2003).

The mean number of SSR variants per accession was higher in the group of three self-incompatible (SI) species (11.0 variants per primer accession) than in the group of three self-compatible species (9.1 variants per accession). This outcome is not surprising, given the small sample size and the expectation of a higher level of heterozygosity in self-incompatible vs. self-compatible species. Nevertheless, we have refrained from assuming that variants detected by any given primer pair in a single individual are necessarily allelic. The alternate possibility—that two variants detected in any individual by a particular primer pair may result from monomorphic character states at two distinct, co-amplified loci—must be taken into account. The assumption of allelism must be validated by genetic data, such as a monogenic segregation pattern observed for putative alleles in the segregating progeny of a genetic cross.

Of the 13 *F. ×ananassa* SSR markers that segregated as codominant markers in the *F. vesca* mapping population, 11 were assignable to linkage groups based upon close linkages to previously mapped anchor loci. These markers were distributed over six of the seven *F. vesca* linkage groups, and can serve as anchor loci defining these six groups for purposes of comparative mapping. These results contribute to the continuing development of the *F. vesca* map, and constitute a significant step toward defining the correspondences between linkage groups in *F. vesca* and their counterparts in *F. ×ananassa*.

It will now be of interest to determine whether the close linkages between/among SSR markers detected, respectively, on *F. vesca* linkage group I (ARSFL_10 and ARSFL_25), linkage group II (ARSFL_3 and ARSFL_12), and linkage group III (ARSFL_8, ARSFL_9, and ARFSL_14) are conserved in *F. ×ananassa*. Similarly, it will be of interest to determine whether the close linkage detected in *F. vesca* between SSR marker ARSFL_17 and the *Pgi*-2 isozyme locus is conserved in the octoploids. An answer to the latter question would provide an informative bridge between SSRs and isozymes as the latest and the first generations of molecular marker types in strawberry, because PGI (phosphoglucoisomerase) is the best characterized isozyme system in *Fragaria*, and its variants and inheritance patterns have been extensively characterized in the cultivated species (Arulsekar et al., 1981).

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