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High variability and disomic segregation of microsatellites in the octoploid *Fragaria virginiana* Mill. (Rosaceae)

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Abstract The objectives of the present study were to develop microsatellite markers for the wild strawberry, *Fragaria virginiana*, to evaluate segregation patterns of microsatellite alleles in this octoploid species, and assess genetic variability at microsatellite loci in a wild population. A genomic library was screened for microsatellite repeats and several PCR primers were designed and tested. We also tested the use of heterologous primers and found that *F. virginiana* primers amplified products in cultivated strawberry, *Fragaria* × *ananassa* Duch. and *Fragaria chiloensis*. Similarly, microsatellite loci developed from cultivated strawberry also successfully amplified *F. virginiana* loci. We investigated four microsatellite loci in detail, three developed from *F. virginiana* and one from cultivated strawberry. A survey of 100 individuals from a population of *F. virginiana* in Pennsylvania demonstrated high heterozygosities (H_e or gene diversity ranged from 0.80 to 0.88 per locus) and allelic diversity (12–17 alleles per locus), but individual plants had no more than two alleles per locus. Segregation patterns in parents and progeny of two controlled crosses at these four loci were consistent with disomic Mendelian inheritance. Together these findings suggest that the genome of *F. virginiana* is "highly diploidized" and at least a subset of microsatellite loci can be treated as codominant, diploid markers. Significant heterozygote deficiencies were found at three of the four loci for hermaphroditic individuals but for only one locus among females in this gynodioecious species.

Keywords *Fragaria virginiana* · Wild strawberry · Microsatellites · Polyploid · Octoploid · *Fragaria* × *ananassa*

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Introduction

Microsatellites or simple sequence repeats (SSRs) have become valuable genetic markers in plant studies, with applications that include linkage mapping (Akkaya et al. 1995; Röder et al. 1998; Cregan et al. 1999), cultivar identification (Rongwen et al. 1995; Guilford et al. 1997), and characterizing pollination (Chase et al. 1996; Dow and Ashley 1998a, b; Streiff et al. 1999, Lian et al. 2003) and seed dispersal patterns (Dow and Ashley 1996; Aldrich and Hamrick 1998; Lian et al. 2003). Other widely used markers, such as RAPDs and AFLPs, allow for more rapid development of large numbers of markers, but microsatellites retain several advantages. Both RAPD and AFLP markers are dominantly inherited, whereas microsatellites exhibit codominant inheritance, making many types of analyses more straightforward. RAPD markers often exhibit poor reproducibility and may exhibit other technical and analytical problems (Perez et al. 1998; Isabel et al. 1999; Rabouam et al. 1999). AFLP markers have been shown to have uneven distribution in some plant genomes (Ellis et al. 1997). Extremely high levels of polymorphism and random genomic distribution of microsatellites, together with codominant inheritance, combine to make microsatellites very powerful markers, especially for analysis of plant genetic diversity, popula-

tion structure, mating systems and genetic linkage studies (Morgante and Olivieri 1993; Ashley and Dow 1994; Gupta et al. 1996).

The development of microsatellite markers for polyploid plant species has lagged behind that of diploid species to some degree. This represents a serious limitation to widespread application in plants given that 30–50% of angiosperms are polyploid (Grant 1971; Stebbins 1971). Part of the problem is due to poorly understood and potentially complex polysomic inheritance patterns, particularly in autopolyploids (Lian et al. 2003). Compounding the problem of complex inheritance, problems with amplification and reproducibility are associated with polyploid plants. For example, low amplification success has been reported in allohexaploid wheat (Röder et al. 1995), tetraploid alfalfa (Diwan et al. 1997b) and hexaploid sweet potato (Buteler et al. 1999).

We developed microsatellite markers from the genome of wild strawberry, *Fragaria virginiana*, an octoploid species native to North America. Our aim was to produce markers for analyzing pollination patterns and male reproductive success in natural and experimental populations of *F. virginiana.* We were also interested in investigating the use of heterologous primers in *Fragaria*, including both those developed from *F. virginiana* but particularly those under development for the cultivated strawberry, *Fragaria* × *ananassa* (Nourse et al. 2002). The development of microsatellites for crop species could provide a valuable source of markers for population biologists studying natural populations of uncultivated plants if the regions flanking microsatellites are conserved between the cultivars and their wild relatives. For example, microsatellites developed for sweetpotato (Buteler et al. 1999), soybean (Peakall et al. 1998), grapevine (Di Gaspero et al. 2000) and sugarcane (Cordeiro et al. 2000) have been successfully transported to congeneric wild relatives. Some studies have indicated problems in cross-species amplification and relatively low rates of successful cross-species transfer (Whitton et al.1997; Peakall et al. 1998). Other studies have reported success with cross-species and cross-genera amplification and detection of polymorphisms (Dayanandan et al. 1997; Van Treuren et al. 1997). The cultivated strawberry, also an octoploid (2n=8*x*=56), resulted from hybridization between two New World species, *F. virginiana* and *Fragaria chiloensis* (L.) Duch., another octoploid species, sometime in the early 18th century (Staudt 1962). We therefore felt there was a high likelihood of transferability of markers from cultivated strawberry to *F. virginiana* and *F. chiloensis*.

The objectives of the present study were to: (1) develop microsatellite markers by screening a *F. virginiana* genomic library; (2) test cross-species amplification of *F. virginiana* primers as well as some recently developed for cultivated strawberry by amplification; (3) evaluate the inheritance patterns of a subset of markers in *F. virginiana* by examining parental and progeny genotypes from controlled crosses; and (4) characterize microsatellite variability in a wild population of *F. virginiana*.

Materials and methods

Study system

F. virginiana (Rosaceae), the Virginian wild strawberry, is a creeping perennial herb that is native to eastern North America and has a gynodioecious (co-occurrence of female and hermaphrodite plants) breeding system (Hancock 1999). Commonly, however, hermaphrodites fail to set fruit, and thus can be considered as functionally male (Staudt 1989; Ashman 1999). Sex determination is under nuclear control, with male sterility (femaleness) dominant to male fertility (Valleau 1923; Ahmadi and Bringhurst 1989). *F. virginiana* is octoploid (2n=56) and is believed to have arisen from an ancient allo-autopolyploidization. However, cytological and isozyme inheritance studies suggest that it currently behaves like a diploid (forming 28 bivalents at meiosis), and its genomic structure is proposed to be AAA′A′BBB′B′ (Arulsekar et al. 1981; Bringhurst 1990; Hancock 1999).

Plant material

Two groups of plant material originally derived from wild adult *F. virginiana* plants in Crawford Co., Pennsylvania, were used. The first group consisted of two families. Forty progeny were derived from each of two controlled crosses 79×204 and 259×197. Both sets of parents were collected from the 'PR' population, the location described in Ashman (1999). In both crosses, a female plant served as the seed parent and a hermaphrodite plant as the pollen donor. The two family groups were used to study transmission patterns for four microsatellite primer pairs described below and in Table 1.

The second group consisted of leaf material from 100 plants in a greenhouse-population that was originally collected from the same wild-population. This population material was used to examine variability, heterozygosity and other population genetic parameters in a natural population of *F. virginiana*.

Library construction and screening

A genomic library was constructed and screened for *F. virginiana* using a common protocol (Dow et al. 1995). Briefly, frozen leaf material was ground to a fine powder in liquid nitrogen for DNA extraction following Keim et al. (1989). *Sau*3AI fragments (200–1,000 bp) were size selected on 1.2% agarose gels, ligated into the pUC18 vector and transformed into *Escherichia coli* competent cells. White colonies were re-plated and lifted onto nylon membranes, then screened with a cocktail of chemiluminescently labeled di- and tri- nucleotides (ECL 3′-oligolabeling and detection system, Amersham Biosciences, Piscataway, New Jersey). Positive clones were grown overnight and plasmid DNA was sequenced using an ABI 373A automated sequencer. PCR primers flanking microsatellite loci were designed using MacVector software (International Biotechnologies Incorporated, New Haven Conn.).

A limited study of heterologous primers was conducted using the protocol described in Nourse et al. (2002). Primers derived from *F. virginiana* were used to amplify two cultivars of *F.* × *ananassa* ('Earliglow' and 'Sweet Charlie') and two genotypes of *F. chiloensis*. Several primers derived from cultivated strawberry were also used to amplify the *F. chiloensis* genotypes.

Microsatellite data collection

One primer of each pair was fluorescently labeled. PCR amplification was performed in reactions containing 200 μM of dNTPs, 0.3 μM of each primer, 1.0 μg/μl of bovine serum albumin, 1.5–

2.5 mM of $MgCl_2$, PCR buffer and 0.05 U of *Taq* polymerase (Promega Corporation, Madison Wis.). All PCR reactions consist of a 3-min pre-heat period at 95°C followed by 38 cycles of denaturing at 94°C for 60 s annealing at 57°C–64°C for 60 s, and extension at 72°C for 60 s and a final extension at 72°C for 2 min. All genotypes were scored on an MJ Research BaseStation automated sequencer (MJ Research, Inc., Waltham Mass.). Cartographer Analysis Software (MJ Research, Incorporated) was used to size and score genotypes. Genetic variation was assayed for 100 individuals from the PR population for *Fvi*6b, *Fvi*9, *Fvi*11 and ARSFL-4. A smaller sample of individuals was scored for loci *Fvi*20, ARSFL-7, ARSFL-8 and ARSFL-9.

Genetic analyses

Descriptive statistics such as allele frequencies, and observed and expected heterozygosities, were calculated using GDA Version 1.0 (d16c) (http://lewis.eeb.uconn.edu/lewishome/gda.html) (Lewis and Zaykin 2001). We used GENEPOP (Raymond and Rousset 1995) to calculate *F*-statistics (Weir and Cockerham 1984) and to test for deviations (heterozygote deficiency) from Hardy-Weinberg equilibrium. GENEPOP calculates unbiased estimates of *P* values using a Markov-chain method (Guo and Thompson 1992). GENEPOP was also used to test for linkage disequilibrium among loci.

Results

Approximately 3,000 colonies with plasmids having *F. virginiana* inserts were screened for microsatellite repeats, and 17 positive clones were obtained and sequenced. Of these, nine were discarded either because the repeat was too far from both cloning sites to be sequenced or too close to one end of the insert to design a suitable primer. The remaining eight clones had microsatellite loci with ten or more repeats. Seven were $(GA/CT)_n$ repeats and one was a $(CA/GT)_n$ repeat. Two of these repeats occurred in AT-rich regions that were unsuitable for designing primers. Primers were synthesized for six loci and tested on a subset of samples. Two primer sets failed to consistently yield scorable PCR products. The first four loci shown in Table 1 reliably amplified products in the predicted size range and the first three were chosen for more-extensive analysis, along with one from cultivated strawberry. One of these loci, *Fvi*11, exhibited a single fixed band in all individuals in addition to a variable set of alleles. This constant band was not scored.

A preliminary screening of primers developed for cultivated strawberry, *F.* × *ananassa* (Nourse et al. 2002), identified four that produced PCR products near the expected size, as scored on ethidium bromide-stained agarose gels. No effort was made to optimize the primers that failed to amplify in our preliminary screening. One (ARSFL-9) yielded multiple bands (up to four per individual) suggesting either polysomic inheritance or the presence of tandem flanking sequence repeats from the same chromosome. Three loci, ARSFL-4, ARSFL-7 and ARSFL-8, produced banding patterns indicating one or two alleles per locus per individual in the expected size range. ARSFL-4 was included in our survey for segregation patterns and population variation. Table 1 shows the genotyping results for all loci with a comparison of product sizes amplified from *F. virginiana, F.* × *ananassa* and *F. chiloensis*. All loci tested amplified PCR products of the expected size in all three species.

Analysis of crosses indicates that segregation of the alleles for seven of eight loci was consistent with disomic Mendelian inheritance (Table 2). The only exception was *Fvi* 6, where offspring of one of the two families (79×204) deviated significantly from expectations (χ^2=11.6, P<0.05). In addition, it is notable that expected classes for tetrasomic or octosomic inheritance were completely absent in the offspring for informative crosses. For example, in ab×cc crosses, the abc genotype never

Table 1 Description of *Fragaria* loci used in this study. *N* represents the number of individuals (for *F. virginiana*) or cultivars (for *F.* × *ananassa* and *F. chiloensis*) scored for each locus

Locus	Motif	Primers	Expected Product	# Alleles/N (size range) *F. virginiana*	# Alleles/N (size range) *F.* × *ananassa*	# Alleles/N (size range) *F. chiloensis*
*Fvi*6b	$(GA)_{15}$	*F: tcctgattcaaccacaagat* *R: gtaacactcattgcttcaggta*	282	17/63 (262–296)	2/2 (275, 277)	4/2 (272–281)
*Fvi*9	$(CA)_{11}$	*F: gggatctcaaagcaccaagaag* *R: tgttgacaaagccgattggg*	175	13/98 (169–199)	4/2 (155–180)	4/2 (154–172)
*Fvi*11	$(GA)_{16}$	*F: gcatcatcgtcataatgagtgc* *R: ggcttcatctctgcaattcaa*	137	12/99 (118–142)	5/2 (114–292)	5/2 (114–301)
*Fvi*20	$(GA)_{20}$	*F: gagtttgtcatcctcagacacc* *R: agtgacccagaaacccagaa*	162	20/89 (149–191)	6/2 (140–182)	7/2 (126–180)
ARSFL-4	$(GA)_{15}$	*F: gcggtcgcattgagttggaggata* *R: gcgtagccaaacaccgatctacc*	166	15/87 (162–190)	5/2 (165–186)	4/2 (166–188)
ARSFL-7	$(CT)_{14}(GA)_{13}$	*F: gcgcgcataaggcaacaaag* *R: gcgaatggcaatgacatcttctct*	256	7/24 (247–265)	5/2 (211–259)	6/2 (212–273)
ARSFL-8	$(GA)_7$ $(GA)_9$	*F: gcggacccaagatgacctcaccc* *R: gcgttagccgagaatgttctactg*	297	10/16 (302–318)	2/2 (290, 301)	1/2 (306)
ARSFL-9	$(GA)_{10}$	*F: gcgaggcgatcatggagaga* *R: gcgtagccaaacaccgatctacc*	222	14/11 (202–232)	6/2 (199–225)	5/2 (203–218)

Table 2 Tests for Mendelian segregation of alleles at four microsatellite loci in two families of wild strawberry

Family	Locus	Parental genotype		Progeny genotypes		$\chi 2$	*df*
		M	F	Observed	Expected		
79×204	ARSFL4	aa	bb	40	1	–	–
	*Fvi*6	ab	cd	9:4:7:18	1:1:1:1	11.600*	3
	*Fvi*9	ab	cc	23:17	1:1	0.900	1
	*Fvi*11	ab	cc	16:21	1:1	0.680	1
197×259	ARSFL4	ab	aa	23:16	1:1	1.310	2
	*Fvi*6	ab	bb	18:21	1:1	0.230	1
	*Fvi*9	aa	ab	19:18	1:1	0.027	1
	*Fvi*11	ab	cd	11:7:10:12	1:1:1:1	1.400	3

* Denotes significant deviation from expected at the $P<0.05$ level

Table 3 Genetic diversity and deviation of observed genotypes from Hardy-Weinberg equilibrium for four *F. virginiana* microsatellite loci

Locus[a]	N	A_o	H_e	H_o	F_{IS}	*P*
*Fvi*6b	63	17	0.864	0.762	0.119	0.0268
Females	13	9	0.797	0.846	–0.065	0.846
Herm.	50	16	0.877	0.740	0.158	0.023
*Fvi*9	98	13	0.808	0.776	0.041	0.076
Females	20	9	0.785	0.850	–0.086	0.885
Herm.	78	12	0.814	0.756	0.071	0.039
*Fvi*11	99	12	0.868	0.878	–0.013	0.674
Females	20	10	0.853	0.850	0.003	0.391
Herm.	79	12	0.873	0.886	–0.015	0.703
ARSFL4	87	15	0.867	0.632	0.272	<0.001
Females	17	11	0.898	0.647	0.286	0.009
Herm.	70	15	0.856	0.629	0.267	0.002
All loci	86.75	14.25	0.852	0.762	0.105	<0.001
Females	17.5	9.75	0.833	0.798	0.043	0.144
Herm.	69.25	13.75	0.855	0.753	0.120	<0.001

[a] For each locus the number of samples genotyped (N), the observed number of alleles (A_o), the expected (H_e) and observed (H_o) heterozygosities, and the fixation index (F_{IS}) calculated according to Weir and Cockerham (1984). For each locus, values for all individuals scored is shown on the first line, followed by females only then hermaphrodites only. For "All loci," A_o, H_e, and H_o are mean values across loci. A Markov-chain method, implemented in GenePop (Raymond and Rousset 1995) was used to estimate *P*-values for tests of heterozygote deficiency

appeared in the offspring. Similarly, for the ab×cd crosses, none of the offspring exhibited three or four parental alleles, as would be expected if transmission were polysomic.

Analysis of crosses provides strong evidence of diploidization of the octoploid *F. virginiana* genome. Expected classes for tetrasomic or octosomic inheritance were completely absent in the offspring of informative crosses. For example, in ab×cc crosses, the abc genotype, which should have been the predominant class, never appeared among the offspring. Similarly, for the ab×cd crosses, none of the offspring exhibited three or four alleles as would be expected if transmission were polysomic. Instead the phenotypic classes from these informative crosses were those that would be expected from diploid segregation: ac and bc in the first example; ac, ad, bc and bd in the second example.

For *Fvi* 6, offspring of one of the two families (79×204) deviated significantly from disomic expectations. In this case, segregation of the progeny classes, ac:ad:bc:bd, did not fit the expected 1:1:1:1 ratio ($\chi^2=11.6$, $P<0.05$), even though both the male gamete segregation, a-:b-, and the female gamete segregation, c-:d-, were not significantly different from the expected 1:1 ratio (male $P=0.052$, female $P=0.330$). Though not statistically different from expected, transmission of the male b-gamete was slightly more common than that of the a-gamete (13 a-: 25 b-).

A total of 100 individuals (80 hermaphrodites and 20 females) from a single population were assayed for variability for three loci developed from *F. virginiana* and for ARSFL-4, developed from the cultivated strawberry (Table 3). With the exception of a constant band present in all individuals for *Fvi* 11, all individuals had either one or two scorable bands for each of these loci, with no indication of ploidy levels higher than diploid. All loci were highly polymorphic, with at least 12 alleles per locus and gene diversity (H_e) of $\geq$0.80 (Table 3). Tests of linkage disequilibrium were made between each pair of loci; none showed significant associations (data not shown).

When all individuals from the PR population were included in the analysis, Hardy-Weinberg equilibrium was rejected for two loci, *Fvi*6b and ARSFL4. These loci showed significant heterozygote deficiencies, as indicated by positive F_{IS} values (Table 3). The heterozygote deficiency was significant when all loci were combined. Separation of the population into females and hermaphrodites revealed that hermaphrodites, but not females, had significant heterozygote deficiencies at *Fvi*6b and *Fvi*9 and over all loci. Assuming inbreeding equilibrium, selfing rate (S=2F/1-F, Awadalla and Ritland 1997) in hermaphrodites was estimated to be 0.27.

Discussion

We used a conventional microsatellite screening protocol for *F. virginiana* that did not involve an enriched library. This was, in part, because we were interested in characterizing a rather small set of microsatellite loci to be used for parentage assignment, rather than the large number needed for applications such as linkage mapping. Our yield of loci was low, with only eight microsatellites

identified of which four could be scored reliably. This was sufficient for our purposes, but widespread application of microsatellite markers in plants clearly will benefit from the use of enrichment protocols (Fischer and Bachmann 1998; Dutech et al. 2000; Zane et al. 2002). Once microsatellite loci were identified, our successful amplification rate was relatively high (four of six, 67%) comparable to 63.6% reported in the polyploid willow, *Salix reinii*, Lian et al. (2001). In contrast, studies of other polyploid species including sweetpotato (14.3%, Buteler et al. 1999) and alfalfa (5%, Diwan et al. 1997a) have reported much lower success. It may therefore be premature to draw general conclusions regarding amplification problems in polyploid plants. Although our library was screened for both di-, and tri-nucleotide repeats, only dinucleotide repeats were found. The relative higher abundance of (GA/CT) repeats than (CA/GT) repeats in the *F. virginiana* genome is consistent with many previous plant studies (Morgante and Olivieri 1993; Wang et al. 1994; Dow et al. 1995), but our sample size is small, and exceptions have been reported in other species (Butcher et al. 2000; Cordeiro et al. 2000).

Because of their utility in gene mapping and plant breeding, development of microsatellite loci for agriculturally important crop species is progressing very rapidly. This represents a potentially important molecular tool for ecological and evolutionary biologists, if microsatellites can be successfully transferred to the wild relatives of cultivated plants. To-date, results for transferring loci from crops to wild relatives have been mixed (reviewed in Peakall et al. 1998), with successful cross-species amplification generally restricted to congeners. Cultivated strawberry is of very recent origin, with *F. virginiana* and *F. chiloensis* being the parental species. Thus, the non-coding regions of the *F. virginiana* and *F. chiloensis* genomes most likely exist relatively unchanged in cultivated strawberries. It would therefore be expected that many microsatellite loci developed in cultivated strawberry would amplify *F. virginiana*. It is perhaps less predictable whether primers derived from the *F. chiloensis* component of the genome will amplify loci in *F. virginiana* or whether primers derived from *F. virginiana* would amplify *F. chiloensis*. However, our results provide preliminary evidence that the genomes of *F. virginiana* and *F. chiloensis* are similar enough that cross-amplification of microsatellites generally occurs. *Fvi*6b, *Fvi*11 and *Fvi*20 amplified products in both *F.* × *ananassa* and *F. chiloensis*. All four primers were derived from cultivated strawberry amplified products in *F. chiloensis* (Table 1).

We found evidence for polysomic banding for one locus derived from cultivated strawberry in *F. virginiana* (ARSFL-9) where individuals had two, three or four bands in the expected size range. For all other loci, only one or two bands were amplified from each individual in *F. virginiana*. Furthermore, in controlled crosses, seven of eight produced offspring genotypic ratios consistent with disomic inheritance, and offspring classes expected for polysomic inheritance were absent.

Understanding the transmission genetics in polyploid species is an essential element of plant genetic research. Given the potential complexities of polysomic inheritance and the wide range of segregation possibilities that might occur in an octoploid species such as *F. virginiana*, our finding that some microsatellite loci exhibit disomic inheritance may be somewhat surprising. To our knowledge, this is the first report of disomic inheritance at microsatellite loci for a wild polyploid plant. Lian et al. (2003) reports up to seven bands per locus in *Salix reinii*. Tetraploid genotypes of *Actinidia chinensis*, a relative of kiwifruit, have four or more alleles per locus (Huang et al. 1998), and more than two alleles are observed at microsatellite loci in polyploid sugarcane (Cordeiro et al. 2000). Although an additional non-variable band consistently amplified at one locus in our study, this phenomenon is also commonly observed in diploid species so may or may not indicate amplification of a homologous site on additional chromosomes.

Our finding of disomic segration for wild strawberry microsatellites supports the conclusion that the genome is highly diploidized (Arulsekar et al. 1981; Bringhurst 1990). The regions flanking the microsatellite loci that we screened apparently were present on a single pair of segregating chromosomes, and thus PCR resulted in the amplification of only one (for homozygotes) or two alleles (for heterozygotes) per genotype. Viruel et al. (2002) recently reported that disomic inheritance of RFLP markers also was "predominant" in cultivated strawberry. Interestingly, Nourse et al. (2002) reported that primers designed to amplify simple-sequence repeats in cultivated strawberry amplified more products than the one or two that might be expected from a diploid genome. However, since both progenitor species of cultivated strawberry are thought to have resulted from the doubling of a diploid genome (perhaps *Fragaria vesca*), and because cultivated strawberry is highly heterozygous due to the breeding strategies used, it may be theoretically possible to amplify up to eight products from a genome, two from each of four homologous loci.

Microsatellite loci are extremely variable in *F. virginiana*. In a single population, we observed at least 12 alleles per locus (Table 3, Fig. 1) and gene diversity (H_e) was above 0.80 for all loci. Our study supports the finding that relatively short repeats may show higher levels of polymorphism in plants than in animals (Peakall et al. 1998). Our research plan is to implement these microsatellite markers for determining paternity and male reproductive success of hermaphrodites using genetic exclusion. Because the probability that two unrelated individuals share an allele at every genetic locus is negatively correlated with the degree of variation at these loci, these markers will provide high exclusion probabilities. They will also be sensitive markers for studying population-level processes such as gene flow and fluctuations in population size.

We observed excess homozygosity in our study population for three of the four loci, and for all loci together (Table 3). Among the several factors that could

explain this pattern, two warrant careful consideration. The first is that, for PCR-based markers, null alleles can occur when mutations in the flanking primer regions prevent amplification. Re-designing primers can often resolve null alleles; however, the only primer pair that was re-designed in our study was *Fvi*6b because the original *Fvi*6 primer pair failed to amplify any product, not because null alleles were suspected. Null alleles can be identified when the progeny of putative homozygotes fail to show a parental allele. In our test crosses, however, all of the offspring of homozygous parents had parental alleles. We cannot rule out null alleles as an explanation for heterozygote deficiencies, although it seems unlikely that null alleles could be at frequencies high enough to explain the deficiency entirely.

Heterozygote deficiencies could also be explained by mating among relatives, partial selfing and the sex-determination system. As explained above, wild strawberries are gynodioecious, with the frequency of females varying from about 0.20 to 0.50 among populations (Ashman 1999). Although our sample was highly skewed towards hermaphrodites, we found that females did not show heterozygote deficiencies at *Fvi6b* or *Fvi9*, nor over all loci (Table 3). Excess homozygosity is much more pronounced in hermaphrodites and is therefore more likely to be related to the mating system of the species. Hermaphrodites are self-compatible and can self-pollinate. Females, on the other hand, are obligatorily outcrossed. So the population mating system is one of mixed outcrossing and selfing. Furthermore, the sex-determination system creates sex morphs with potentially different mating histories. Because hermaphrodites are homozygous at the sex-determining locus (e.g., hh) they produce only hermaphrodite offspring and all pollen they produce carries the recessive allele. Females are heterozygous (Fh), and when pollinated by a hermaphrodite, give rise to both female and hermaphrodite progeny. Thus, any female in the population must have come from an outcrossing event (although biparental inbreeding in females is possible), whereas hermaphrodites can be the product of selfing or outcrossing, depending on whether they had a hermaphrodite or female mother, respectively. Given the genetics of sex-determination in wild strawberries, hermaphrodites would be expected to exhibit greater heterozygote deficiency than females.

This is one of the first microsatellite studies involving a wild polyploid plant, and the first to assess both population variability and segregation patterns in controlled crosses. Wild strawberries exhibit high levels of variability at microsatellite loci (Table 3, Fig. 1) and the transmission of alleles at the loci we investigated was consistent with disomic inheritance (Table 2). Thus, the usefulness of these loci for population and parentage studies will be high, and the interpretation of results may be more straightforward than previously thought. If other wild polyploid plants are similarly "diploidized" at microsatellite loci, this may greatly enlarge the potential for applying microsatellites to studies of such species.

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