Detection and Characterization of Genome-specific Microsatellite Markers in the Allotetraploid

Prunus serotina

Marie Pairon1 and Anne-Laure Jacquemart
Unité de Génétique, Biodiversity Research Center, Université Catholique de Louvain, Croix du Sud 2, Box 14, B-1348 Louvain-la-Neuve, Belgium

Daniel Potter
Department of Plant Sciences, Mail Stop 2, University of California, 1 Shields Avenue, Davis, CA 95616

ABSTRACT. The utility of microsatellite markers to characterize the genetic diversity of a polyploid species with disomic inheritance is often hampered by the impossibility of determining allele frequencies and the complexity of inheritance patterns. The objective of this study was to solve these problems in the allotetraploid Prunus serotina Ehrh. by finding genome-specific primers (i.e., primers that are specific to one or the two genomes that initially formed the species). Sixty-seven microsatellite primers described in cultivated Prunus L. species were tested for cross-amplification in P. serotina, and evidence that conserved markers were genome-specific was found by demonstrating their typical Mendelian diploid inheritance in embryos resulting from controlled crosses. Among the 67 microsatellite markers tested, 26 produced successful amplification and five were genome-specific. No linkage disequilibrium was detected for these loci, but evidence was found for the presence of a null allele at one locus. We found both a high number of alleles per locus (three to 12) and a high mean expected heterozygosity (0.71), which were nonsignificantly different from the number of alleles and estimates of expected heterozygosity calculated for three non-genome-specific markers in the same population. The potential use of these genome-specific markers in population genetic studies is discussed.

Prunus serotina is a wild, fast-growing tree native to North America (Marquis, 1990) which has been widely planted in European forests and has spread from plantations to invade several types of woodlands and open habitats on poor soils (Muys et al., 1992). Prunus serotina has been classified in subgenus Padus (Miller) Focke (Rehder, 1940), and molecular phylogenetic studies of Prunus place the species in a clade composed of members of that subgenus as well as subgenus Laurocerasus (Duh.) Rehder (Bortiri et al., 2006). Because P. serotina is economically important in North America for its timber quality (Marquis, 1990) and ecologically important in Europe owing to its invasive status, questions regarding fine-scale spatial genetic structure, mating system, or gene flow among populations in both its native and introduced ranges are of interest to foresters and ecologists. Over previous decades, microsatellites have emerged as markers of choice in such analyses because of their high level of polymorphism and their codominant inheritance (Powell et al., 1996). Analyses of polymorphic markers such as microsatellites, however, are hindered in allopolyploid species because of the presence of more than two alleles at each locus and the resulting complexity of the inheritance pattern (as reviewed in De Silva et al., 2005; Ronfort et al., 1998).

Allopolyploids have differentiated pairs of chromosomes (homeologous chromosomes) which form bivalents at meiosis, as in their diploid progenitors. Owing to the frequently high relatedness between the ancestral progenitors, multiple alleles are often present in more than one copy. The number of copies at microsatellite loci is, however, impossible to determine because allele dosage cannot be deduced with certainty from band intensities or electropherogram peak marker heights based on a nonquantitative exponential amplification process such as polymerase chain reaction (Markwith et al., 2006). For instance, a tetraploid individual displaying phenotype “ABC” can have any of the following genotypes: “aabc”, “abbc”, or “abcc”. Even if allele dosage were known, it would still be impossible to know at which of two or more duplicated loci a particular allele is actually segregating without performing controlled crosses (Obbard et al., 2006).

To analyze allopolyploid data, allelic phenotypes have therefore been used instead of allelic frequencies to calculate summary statistics (e.g., Becher et al., 2000; Brochmann et al., 1992; Chung et al., 1991). With this approach, phenotypes are treated only as being either different or identical, and it does not make use of all the information held by microsatellite markers. Recently, new estimates of genetic diversity were described to make use of all the information held by microsatellite markers. The journey and stay of Marie Pairon at UC Davis were supported by a grant of the Belgian National Fund of Scientific Research (FNRS). This research was financially supported by the Fonds Spécials de Recherche (FSR) of the Université Catholique de Louvain (UCL) (to A-L.J.) and by NSF Grant DEB-0515431 (to D.P.).

Received for publication 19 Dec. 2007. Accepted for publication 12 Feb. 2008. We thank Marie Jasienuik for facilities provided at UC Davis and Riaz Ahmad for technical assistance. The journey and stay of Marie Pairon at UC Davis were supported by a grant of the Belgian National Fund of Scientific Research (FNRS). This research was financially supported by the Fonds Spécials de Recherche (FSR) of the Université Catholique de Louvain (UCL) (to A-L.J.) and by NSF Grant DEB-0515431 (to D.P.). Marie Pairon is research fellow of the FRNS, and Anne-Laure Jacquemart is research associate in the same institution.

1Corresponding author. E-mail address: marie.pairon@uclouvain.be.
Materials and Methods

Detection of genome-specific markers

**Plant material and DNA extraction.** Five controlled crosses were performed on *P. serotina* using five pollen receivers and two pollen donors in June 2004 in an invasive population in Belgium (lat. 50°41′18″N, long. 4°43′33″E). Five to 10 twigs of pollen receiver trees, each bearing five up to 13 racemes, were enclosed in nylon mesh bags before anthesis, and crosses were performed by brushing stamens of the pollen donor flowers across the stigma of receptive flowers (details in Pairon and Jacquemart, 2005). Buds were removed when all stigmas of the enclosed racemes were no longer receptive, and fruit were collected unripe in early Aug. 2004 to prevent them from falling and being eaten by birds. DNA was extracted from buds of the seven trees used as pollen donors and receivers using a CTAB extraction protocol (Doyle and Doyle, 1990), and DNA from 30–36 embryos of seeds resulting from the five crosses was extracted using the extraction procedure of Cheung et al. (1993). DNA was extracted from embryos instead of leaves because going through germination would have led to considerable time and seedling loss as fruit had been collected unripe.

**PCR amplification and microsatellite analysis.** Sixty-seven microsatellite primer pairs originally designed in *Prunus persica* (L.) Batsch, *Prunus avium* L., and *Prunus cerasus* L. (Ahmad et al., 2004; Cipriani et al., 1999; Dirlewanger et al., 2002; Sosinski et al., 2000; Struss et al., 2003; Testolin et al., 2000) were tested for cross-species amplification in *P. serotina* on the seven individuals used as parents in controlled crosses. PCR reactions were performed in 15 μL containing 15 ng of template DNA, 2.5 μM MgCl₂, 0.2 mg·mL⁻¹ BSA, 0.1 μM of each dNTP, 0.5 μM of each primer, 0.6 U of *Taq* DNA polymerase (Roche Diagnostics, Basel, Switzerland), and 1.5 μL of the *Taq* buffer using a temperature of 94 °C for 4 min, then 35 cycles of (94 °C for 25 s, 56 °C for 45 s, and 72 °C for 45 s), and finally 72 °C for 7 min. Amplification products were electrophoretically separated on 2% agarose gels.

Forward primers of the 26 primer pairs that successfully produced amplified fragments in *P. serotina* were then fluorescently labeled with either 6-FAM or HEX (Sigma-Aldrich, St. Louis, MO). The same PCR conditions were used and the same seven individuals amplified, and PCR products were analyzed on an ABI-3100 Genetic Analyzer and sized using GeneMapper 3.5 (Applied Biosystems, Foster City, CA).

Because the aim was to find genome-specific markers, inheritance patterns were tested only for loci displaying a maximum number of two alleles in any of the seven parents by genotyping progenies of one controlled cross. The controlled cross to use for each locus was chosen in such a way that the genotypes of the two parents shared no more than one allele at the given locus (Table 1). In these cases, it is possible to determine the frequencies of alleles inherited from each of the two parents in the progenies. Thirty to 36 progenies were screened per marker to evaluate the frequencies of alleles inherited from each of the two parents separately. These observed values were then compared with allelic ratios expected under the diploid hypothesis using χ² goodness-of-fit tests (SAS version 8.2, SAS Institute, Cary, NC).

**Genome-specific markers genetic diversity and information content**

**Plant material and microsatellite analysis.** To characterize the overall genetic diversity of genome-specific markers and to compare the information content of the genome-specific markers found in this study to that of non-genome-specific markers previously described (pchpgms3, M4c, M12a) (Pairon and Jacquemart, 2005), leaves of 20 unrelated *P. serotina* individuals were collected in a second invasive population (lat. 50°79′03″N, long. 4°70′73″E), and DNA was extracted using the same CTAB protocol as that used for buds (Doyle and Doyle, 1990).

PCR conditions for amplification of the genome-specific markers were as described above, and the three non-genome-specific markers were as described in Pairon and Jacquemart (2005).

**Data analysis.** The genetic variability of the genome-specific markers was estimated by computing the number of alleles (Nₐ), the observed heterozygosity (Hₒ), and the expected heterozygosity (Hₑ) using GeneALEX (Peakall and Smouse, 2006). The fixation index (Fᵢₑ) of Weir and Cockeram was computed using GenePop 3.4 (Raymond and Rousset, 1995). Exact tests for departure from Hardy–Weinberg proportions and tests for linkage disequilibrium were performed using GenePop 3.4.

Because we wanted to test how informative the newly found genome-specific markers were compared with previously defined non-genome specific markers, we computed Nₐ, Hₒ,
Table 1. Phenotypes of seven *Prunus serotina* individuals used as pollen receivers (italic numbers 1–5) and pollen donors (underscored numbers 6 and 7) in the five controlled crosses performed (1×7, 2×6, 3×7, 4×6, 5×6) to study the inheritance pattern of five putatively genome-specific microsatellite markers.

| Locus        | Allele | Code | Individuals |
|--------------|--------|------|-------------|
|              | Size (bp) |      | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| UCD-CH14*    | 136     | ×    | × |
|              | 138     | A    |   |   |   |   |   |   |   |
|              | 142     |      |   |   |   |   |   |   |   |
|              | 148     | B    |   |   |   |   |   |   |   |
|              | 156     | C    |   |   |   |   |   |   |   |
|              | 158     |      |   |   |   |   |   |   |   |
|              | 160     |      |   |   |   |   |   |   |   |
| UCD-CH24*    | 78      |      |   |   |   |   |   |   |   |
|              | 80      | D    |   |   |   |   |   |   |   |
|              | 82      | F    |   |   |   |   |   |   |   |
|              | 84      | E    |   |   |   |   |   |   |   |
| UDP96-005w   | 82      | I    |   |   |   |   |   |   |   |
|              | 84      | G    |   |   |   |   |   |   |   |
|              | 86      |      |   |   |   |   |   |   |   |
|              | 88      | H    |   |   |   |   |   |   |   |
| UDP98-025v   | 109     | L    |   |   |   |   |   |   |   |
|              | 117     | J    |   |   |   |   |   |   |   |
|              | 119     | M    |   |   |   |   |   |   |   |
|              | 121     |      |   |   |   |   |   |   |   |
|              | 127     | K    |   |   |   |   |   |   |   |
| UDP98-405w   | 111     | P    |   |   |   |   |   |   |   |
|              | 113     | N    |   |   |   |   |   |   |   |
|              | 115     | O    |   |   |   |   |   |   |   |

*Allele sizes are given for the different loci; “×” symbols show the genotypes of the seven individuals. Shaded cells highlight the two individuals chosen as parents in the controlled crosses used to test the marker inheritance at each locus.

1Letter code used to characterize the expected and observed allelic ratios in Table 3.

2Struss et al. (2003).

3Testolin et al. (2000).

4Cipriani et al. (1999).

and an estimate of $H_e$ ($H_{PHEN}$) for the non-genome-specific markers. The observed heterozygosity ($H_o$) was computed assuming that an individual was heterozygote when it displayed a minimum of two different alleles at a locus. $H_{PHEN}$ was computed using allelic phenotypes instead of allelic frequencies as follows:

$$H_{PHEN} = (1 - \sum g_i^2)$$

where $g_i$ is the frequency of the $i$th phenotype. Note that this is equal to the equation of the discrimination power (e.g., Kloosterman et al., 1993). For comparison purposes, $H_{PHEN}$ was also evaluated for the genome-specific markers using Eq. [1].

Student's $t$ tests were performed to compare values of $N_o$, $H_o$, and $H_{PHEN}$ for the genome-specific and non-genome-specific markers (SAS version 8.2).

**Results**

**Detection of genome-specific markers.** PCR amplification was considered to be successful when an amplicon of sharp intensity and in the expected size range was visible on agarose gels. Using such a criterion, 26 out of 67 (39%) microsatellite loci gave apparently correct PCR amplification in *P. serotina*.

Of the 26 primer pairs that yielded specific bands of sharp intensity on agarose gels, five were monomorphic, three displayed unreliable PCR amplifications, 13 gave a maximum number of four alleles per individual (Table 2), and five (19%) displayed a maximum number of two alleles per individual (Table 3). The patterns observed were not correlated to the species in which the primer pairs were initially developed. Markers designed for *P. avium* and *P. persica* displayed all four patterns described above.

Inheritance patterns were tested for the five loci displaying a maximum of two alleles per individual. The alleles inherited from each of the two parents by 30–36 progenies per locus were compared with the allelic ratios expected in a diploid species (Table 3). There was no difference between expected and observed ratios as $P$ values of $\chi^2$ tests were all nonsignificant (Table 3). Locus UCD-CH24 was different from the four other loci because of a particular pattern observed in the allele segregation of one of the two parents. The pollen donor displayed a homozygote phenotype with a unique allele (F) at this locus. However, this unique allele was only found in 56% of the progenies, instead of being found in 100% of the progenies as would be expected from a homozygote genotype (ff). The only way to explain these proportions is to account for a null allele in the pollen donor genotype (–f) (Table 3).

**Genome-specific markers’ genetic diversity and information content.**

The genetic variability of these genome-specific markers was tested by screening 20 individuals in a second population. They all showed high information content, with a total number of alleles ranging from three to 12 and average observed and expected heterozygosities of 0.56 and 0.71, respectively (Table 4). The presence of a null allele at locus UCD-CH24 was further demonstrated by its significant departure from Hardy–Weinberg genotypic proportions ($P < 0.01$) in this population (Table 4). This test was not significant for the four other genome-specific loci (Table 4), and none of the locus pairs showed significant linkage disequilibrium ($P > 0.1$).

The genetic diversity of these genome-specific markers was then compared with that of the non-genome-specific markers on the same 20 individuals. The average number of alleles per locus and $H_{PHEN}$ were not significantly different from those of the non-genome-specific markers ($P = 0.12$, Table 4). The observed heterozygosity was significantly higher for the non-genome-specific markers because having a homozygous genotype is much more likely at genome-specific loci. In order for $H_o$ to be different from one in the case of non-genome-specific markers, both duplicated loci need indeed to be homozygous and have the same allele, which was never found in all three non-genome-specific loci in our study.
Table 2. Results of cross-amplification tests and level of polymorphism in *Prunus serotina* for 21 *Prunus persica*, *Prunus avium*, and *Prunus cerasus* microsatellite primers that were not genome-specific but showed good polymorphism (polymorphic), were monomorphic (monomorphic), or showed unreliable PCR amplification (poor amplification).

| Locus       | Origin         | Amplification in *P. serotina* | Size range (bp) | Amplification in *P. serotina* | Size range (bp) |
|-------------|----------------|--------------------------------|-----------------|--------------------------------|-----------------|
| BBPCT-002^a| *P. persica*   | Polymorphic                    | 179–191         | Polymorphic                    | 179–191         |
| BBPCT-017^a| *P. persica*   | Polymorphic                    | 316–364         | Polymorphic                    | 316–364         |
| BBPCT-024^a| *P. persica*   | Polymorphic                    | 92–96           | Polymorphic                    | 92–96           |
| BBPCT-025^a| *P. persica*   | Polymorphic                    | 188–206         | Polymorphic                    | 188–206         |
| BPCT-007^a | *P. persica*   | Monomorphic                    | —               | Polymorphic                    | 149             |
| BPCT-003^a | *P. persica*   | Monomorphic                    | —               | Polymorphic                    | 144             |
| UCD-CH11^a | *P. avium*     | Polymorphic                    | 78–118          | Polymorphic                    | 78–118          |
| UCD-CH13^a | *P. avium*     | Monomorphic                    | 120             | Polymorphic                    | 120             |
| UCD-CH15^a | *P. avium*     | Poor amplification            | —               | Polymorphic                    | —               |
| UCD-CH19^a | *P. avium*     | Poor amplification            | —               | Polymorphic                    | —               |
| PS01H03^a  | *P. cerasus*   | Poor amplification            | —               | Polymorphic                    | —               |
| PS08E08^a  | *P. cerasus*   | Monomorphic                    | 135             | Polymorphic                    | 135             |
| PS7A02^a   | *P. cerasus*   | Polymorphic                    | 66–92           | Polymorphic                    | 66–92           |
| UDP96-001^w| *P. persica*   | Polymorphic                    | 97–107          | Polymorphic                    | 97–107          |
| UDP96-019^w| *P. persica*   | Polymorphic                    | 202–236         | Polymorphic                    | 202–236         |
| UDP97-402^w| *P. persica*   | Polymorphic                    | 123–145         | Polymorphic                    | 123–145         |
| UDP98-022^w| *P. persica*   | Polymorphic                    | 112–138         | Polymorphic                    | 112–138         |
| UDP98-046^w| *P. persica*   | Polymorphic                    | 77–101          | Polymorphic                    | 77–101          |
| UDP98-048^w| *P. persica*   | Polymorphic                    | 65–406          | Polymorphic                    | 65–406          |
| UDP98-410^w| *P. persica*   | Monomorphic                    | 114             | Polymorphic                    | 114             |
| UDP98-416^w| *P. persica*   | Polymorphic                    | 102–106         | Polymorphic                    | 102–106         |

^aDirlewanger et al. (2002).
^bStruss et al. (2003).
^cSosinski et al. (2000).
^dTestolin et al. (2000).
^eCipriani et al. (1999).

Discussion

We demonstrated here that 39% of microsatellite flanking regions described in cultivated *Prunus* species were conserved in the wild *P. serotina*. Among the conserved primers that showed polymorphism, 50% amplified both duplicated loci and 19% were genome-specific.

The primer pairs that were found to amplify only a single locus from one of the two ancestral genomes present in *P. serotina* were derived from *P. persica* (UDP96-005, UDP98-025, UDP98-405) (Cipriani et al., 1999; Testolin et al., 2000) and *P. avium* (UCD-CH14, UCD-CH24) (Ahmad et al., 2004; Struss et al., 2003). UDP96-005 and UDP98-405 had previously been tested for cross-amplification in several cultivated species (Cipriani et al., 1999) classified in different subgenera of *Prunus* (e.g., Rehder, 1940). They were shown to be conserved in species of subgenera Amygdalus (L.) Focke [e.g., *P. persica, Prunus dulcis* (Miller) D.A. Webb], *Prunus* (e.g., *Prunus armeniaca* L.), and *Cerasus* (Miller) Focke (e.g., *P. avium* and *P. cerasus*), indicating a high degree of conservation across the genus, which was further confirmed by their utility in *P. serotina* (subgenus *Padus*). Recent phylogenetic studies (e.g., Bortiri et al., 2006) have suggested that species of subgenera *Padus* and Laurocerasus form a clade separated from the rest of the genus, implying greater divergence between the former species and the crop plants in which the microsatellite markers were developed than among the different crop species and cultivars. This provides a possible explanation as to why some of the primers were not conserved in one of the two duplicated loci in black cherry. A more detailed study of the subgenera is needed, however, to correctly infer the putative parental species.

All genome-specific markers except UCD-CH14 and UCD-CH24 have been placed on a map covering the *Prunus* genome by Aranzana et al. (2003). They do not seem to be restricted to one single region of the genome as locus UDP96-005 is found on G1, UDP98-025 on G2, and UDP98-405 on G7. Therefore, the presence of genome-specific microsatellite loci does not seem to be correlated to the locus position in the genome, which would be expected if part of the genome is becoming diplodized as is sometimes observed with polyploids (Bretagnolle, 1998).

The genetic variability of these new genome-specific markers was high (mean $H_e = 0.71$) when compared with values reported for cultivated diploid *Prunus* species [e.g.,

Table 3. Inheritance analysis results at the five microsatellite loci that displayed a maximum of two alleles in the seven *Prunus serotina* parental individuals.

| Locus   | Parent code | Parent phenotype^e | Putative parent genotype^e | Expected gamete ratios | Observed gamele genotype | $\chi^2$ | $P$  |
|---------|-------------|--------------------|----------------------------|------------------------|--------------------------|---------|------|
| UCD-CH14| 1           | AB                 | ab/–                       | 1a : 1b                | 17a : 15b                | 0.13    | 0.84 |
|         | 7           | C                  | cc/–                       | 1e                     | 32c                      | NA      | —    |
| UCD-CH24| 3           | DE                 | de/–                       | 1d : 1e                | 14d : 18e                | 0.5     | 0.6  |
|         | 7           | F                  | f/–                        | 1f : 1–                | 18f : 14–                | 0.5     | 0.6  |
| UDP96-005| 2           | GH                 | gh/–                       | 1g : 1h                | 18g : 18h                | 0       | 1    |
|         | 6           | I                  | ii/–                       | 1i                     | 36i                      | NA      | —    |
| UDP98-025| 2           | JK                 | jk/–                       | 1j : 1k                | 19j : 15k                | 0.47    | 0.61 |
|         | 6           | LM                 | lm/–                       | 1l : 1m                | 201 : 14m                | 1.06    | 0.39 |
| UDP98-405| 1           | NO                 | no/–                       | 1n : 1o                | 19n : 11o                | 2.13    | 0.2  |
|         | 7           | NP                 | np/–                       | 1p                     | 16n : 14p                | 0.13    | 0.84 |

^eThe phenotypes of the two parents used for the controlled crosses are given and the observed gamete genotypes are compared with the gamete ratios expected under the hypothesis of genome-specific marker.

^fLetter codes as described in Table 1. Note that cap letters represent phenotypes and lowercase letters represent genotypes.

^gThe ‘/’ represents separation between two duplicated loci that segregate disomically at meiosis and that come from each of the two ancestral genomes that initially formed the allotetraploid.

^h‘NA’ is a reminder that $\chi^2$ tests are not possible when there is only one possible expected value.
Table 4. Characterization of the five genome-specific loci described in this study and of the three non-genome-specific loci previously described in an invasive population of *Prunus serotina* (*n* = 20 individuals). *

| Marker          | Locus | Origin       | Size range (bp) | *N*<sub>a</sub> | *H*<sub>e</sub> | *H*<sub>PHEN</sub> | *H*<sub>s</sub> | *F*<sub>ST</sub> |
|-----------------|-------|--------------|-----------------|----------------|----------------|----------------|---------------|---------------|
| Genome-specific | UCD-CH14<sup>4</sup> | *P. avium* | 136–164         | 12             | 0.80           | 0.94           | 0.87          | 0.10          |
|                 | UCD-CH24<sup>4</sup> | *P. avium* | 80–106          | 8              | 0.32           | 0.88           | 0.84          | 0.64*         |
|                 | UDP96-005<sup>5</sup> | *P. persica* | 82–88           | 4              | 0.55           | 0.77           | 0.60          | 0.10          |
|                 | UDP98-025<sup>5</sup> | *P. persica* | 109–127         | 9              | 0.65           | 0.91           | 0.83          | 0.24          |
|                 | UDP98-405<sup>5</sup> | *P. persica* | 113–119         | 3              | 0.50           | 0.59           | 0.43          | –0.13         |
| Non-genome-specific | M4c<sup>6</sup> | *P. persica* | 65–85           | 8              | 1              | 0.94           | —            | —             |
|                 | pclpgms3<sup>6</sup> | *P. persica* | 182–232         | 14             | 1              | 0.94           | —            | —             |
|                 | Ps12a02<sup>6</sup> | *P. avium* | 145–187         | 14             | 1              | 0.94           | —            | —             |
|                 | Average |              |                 | 12             | 1              | 0.94           | —            | —             |

*Size range, number of alleles (*N*<sub>a</sub>), observed heterozygosity (*H*<sub>e</sub>), and estimate of expected heterozygosity following Eq. [1] described in Materials and Methods (*H*<sub>PHEN</sub>) are given for both types of markers. True expected heterozygosity (*H*<sub>e</sub>) and fixation index (*F*<sub>ST</sub>) were computed only for genome-specific markers.

*Struss et al. (2003).*  
*Cipriani et al. (1999).*  
*Cipriani et al. (1999).*  
*Yamamoto et al. (2002).*  
*Downey and Iezzoni (2000).*

mean *H*<sub>e</sub> = 0.47 in *P. persica* (Testolin et al., 2000) or mean *H*<sub>e</sub> = 0.60 in *P. avium* (Dirlewanger et al., 2002), or even for wild *P. avium* accessions [mean *H*<sub>e</sub> = 0.60 (Vaughan and Russell, 2004)].

Interpreting data from non-genome-specific markers as allelic phenotypes as we did when calculating *H*<sub>PHEN</sub>, has limited use when it comes to comparing estimates of genetic diversity between diploid and polyploid species. The estimate of expected heterozygosity, *H*<sub>PHEN</sub>, does not recognize the greater similarity of phenotypes that share more alleles over those that share fewer (e.g., Obbard et al., 2006). New estimates that try to take this particularity into account (e.g., Bruvo et al., 2004; Meirmans and Van Tienderen, 2004; Obbard et al., 2006) have not been compared with estimates of genetic diversity traditionally computed in diploid organisms [e.g., *F* statistics (Wright, 1951)] in real populations because, to date, no studies have found both genome-specific and non-genome-specific markers in the same species. We have shown here that these two types of markers can be used to generate both genome-specific genotypes that can be analyzed as diploid genotypes and non-genome-specific phenotypes for the same populations of the wild tree species *P. serotina*. It is therefore possible to test the difference between allopolyploid estimates of genetic diversity and differentiation, computed using non-genome-specific markers, and traditional *F*<sub>ST</sub> values or other diploid estimates of genetic diversity, computed using genome-specific markers on the same individuals. This could be helpful to assess the quality of estimates of heterozygosity and *F*<sub>ST</sub>-like statistics designed for polyploid organisms.

Aside from the potential interest this could have on the study of estimates of genetic diversity of polyploids in general, the newly found genome-specific primers can be used together with the two genome-specific primer previously described (pchpms2, PceGA34) (Piron and Jacquemart, 2005) and locus pchpms3 that amplified both loci with different allele ranges to study population genetic structure or perform assignment tests on *P. serotina*, with the same power as if the species were diploid. These will be useful, for instance, to predict and model the species establishment in new habitats and describe the overall genetic structure of the species in its native and invasive ranges to detect founder effects or describe historical patterns of introduction in Europe.

**Literature Cited**

Ahmad, R., D. Potter, and S.M. Southwick. 2004. Identification and characterization of plum and plum cultivars by microsatellite markers. J. Hort. Sci. Biotechnol. 79:164–169.

Aranzana, M., A. Pineda, P. Cosson, E. Dirlewanger, J. Ascasibar, G. Cipriani, C. Ryder, R. Testolin, A. Abbott, G. King, A. Iezzoni, and P. Arus. 2003. A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theor. Appl. Genet. 106:819–825.

Becher, S.A., K. Steinmetz, K. Weising, S. Bory, D. Peltier, J.-P. Renou, G. Kahl, and K. Wolff. 2000. Microsatellites for cultivar identification in *Pelargonium*. Theor. Appl. Genet. 101:643–651.

Bortiri, E., B. Vanden Heuvel, and D. Potter. 2006. Phylogenetic analysis of morphology in *Prunus* reveals extensive homoplasies. Plant Syst. Evol. 259:53–71.

Bretagnolle, F. 1998. La polyploïdie chez les plantes. Bot. Helvetica 108:5–37.

Brochmann, C., P.S. Soltis, and D.E. Soltis. 1992. Recurrent formation and polyphyly of nordic polyploids in *Draba* (Brassicaceae). Amer. J. Bot. 79:673–688.

Bruvo, R., N.K. Michielis, T.G. D’Souza, and H. Schulenburg. 2004. A simple method for the calculation of microsatellite genotype distances irrespective of ploidy level. Mol. Ecol. 13:2101–2106.

Cheung, W.Y., N. Hubert, and B.S. Landry. 1993. A simple and rapid DNA microextraction method for plant, animal, and insect suitable for RAPD and other PCR analyses. PCR Methods Appl. 3:69–70.

Chung, M.G., J.L. Hamrick, S.B. Jones, and G.S. Derda. 1991. Isozyme variation within and among populations of *Hosta* (Liliaceae) in Korea. Syst. Bot. 16:667–684.

Cipriani, G., G. Lot, W.G. Huang, M.T. Marrazzo, E. Peterlunger, and R. Testolin. 1999. AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: Isolation, characterization and cross-species amplification in *Prunus*. Theor. Appl. Genet. 99:65–72.
De Silva, H.N., A.J. Hall, E. Rikkerink, M.A. McNeilage, and L.G. Fraser. 2005. Estimation of allele frequencies in polyploids under certain patterns of inheritance. Heredity 95:327–334.

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

Downey, S.L. and A.F. Iezzoni. 2000. Polymorphic DNA markers in black cherry (Prunus serotina Ehrh.) are identified using sequences from sweet cherry, peach, and sour cherry. J. Amer. Soc. Hort. Sci. 125:76–80.

Doyle, J.J. and J.L. Doyle. 1990. Isolation of plant DNA from fresh tissue. Focus 12:13–15.

Fernandez-Fernandez, F., N.G. Harvey, and C.M. James. 2006. Isolation and characterization of polymorphic microsatellite markers from European pear (Pyrus communis L.). Mol. Ecol. Notes 6:1039–1041.

Kloosterman, A.D., B. Budowle, and P. Daselaar. 1993. PCR amplification and detection of the human D1S80 Vntr locus—Amplification conditions, population genetics and application in forensic analysis. Intl. J. Legal Med. 105:257–264.

Korzun, V., M.S. Roder, K. Wendehake, A. Pasqualone, C. Lotti, M.W. Ganal, and A. Blanco. 1999. Integration of dinucleotide microsatellites from hexaploid bread wheat into a genetic linkage map of durum wheat. Theor. Appl. Genet. 98:1202–1207.

Markwith, S.H., D.J. Stewart, and J.L. Dyer. 2006. TETRASAT: A program for the population analysis of allotetraploid microsatellite data. Mol. Ecol. Notes 6:586–589.

Marquis, D. 1990. Prunus serotina Ehrh. Black cherry, p. 594–602. In: Silvics of North America, Vol. 2: Hardwoods. U.S. Dept. Agr., For. Serv., Washington, D.C.

Meirmans, P.G. and P.H. Van Tienderen. 2004. GENOTYPE and GENODIVE: Two programs for the analysis of genetic diversity of asexual organisms. Mol. Ecol. Notes 4:792–794.

Muys, B., D. Maddelein, and N. Lust. 1992. Ecology, practice and policy of black cherry (Prunus serotina Ehrh.) management in Belgium. Silva Gandavensis 27:28–45.

Obbard, D.J., S.A. Harris, and J.R. Pannell. 2006. Simple allelic-phenotype diversity and differentiation statistics for allopolyploids. Heredity 97:296–303.

Pairol, M.C. and A.L. Jacquemart. 2005. Disomic segregation of microsatellites in the tetraploid Prunus serotina Ehrh. (Rosaceae). J. Amer. Soc. Hort. Sci. 130:729–734.

Peakall, R. and P.E. Smouse. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6:288–295.

Powell, W., G. Machray, and J. Provan. 1996. Polymorphism revealed by simple sequence repeat. Trends Plant Sci. 1:215–221.

Ronfort, J.L., E. Jenczewski, T. Bataillon, and F. Rousset. 1998. Analysis of population structure in autotetraploid species. Genetics 150:921–930.

Saa, B., J. Plieske, J. Hu, C.F. Quiros, and D. Struss. 2001. Microsatellite markers for genome analysis in Brassicaceae. II. Assignment of rapeseed microsatellites to the A and C genomes and genetic mapping in Brassica oleracea L. Theor. Appl. Genet. 102:695–699.

Rehder, A. 1940. Rosaceae Prunus, p. 452–481. In: A. Rehder (ed.). Manual of cultivated trees and shrubs hardy in North America. 2nd ed. Macmillan, New York.

Ronfort, J.L., E. Jenczewski, T. Bataillon, and F. Rousset. 1998. Analysis of population structure in autotetraploid species. Genetics 150:921–930.

Vaughan, S.P. and K. Russell. 2004. Characterization of novel microsatellites and development of multiplex PCR for large-scale population studies in wild cherry, Prunus avium. Mol. Ecol. Notes 4:429–431.

Wright, S. 1951. The genetical structure of populations. Ann. Eugenet. 15:223–254.

Yamamoto, T., K. Mochida, T. Imai, Y. Shi, I. Ogwara, and T. Hayashi. 2002. Microsatellite markers in peach (Prunus persica (L.) Batsch) derived from an enriched genomic and cDNA libraries. Mol. Ecol. Notes 2:298–301.