Identification of “Duplicate” Accessions within the USDA-ARS National Plant Germplasm System *Malus* Collection

Briana L. Gross, Gayle M. Volk, and Christopher M. Richards

USDA-ARS National Center for Genetic Resources Preservation, 1111 S. Mason Street, Fort Collins, CO 80521

Philip L. Forsline, Gennaro Fazio, and C. Thomas Chao

USDA-ARS Plant Genetic Resources Unit, Geneva, NY 14456-0462

**Additional Index Words.** apple, *Malus ×domestica*, simple sequence repeat, probability of identity, clonal crop, perennial crop

**Abstract.** The U.S. Department of Agriculture, Agricultural Research Service, National Plant Germplasm System (NPGS), Plant Genetic Resources Unit apple (*Malus*) collection in Geneva, NY, conserves over 2500 trees as grafted clones. We have compared the genotypes of 1131 diploid *Malus ×domestica* cultivars with a total of 1910 wild and domesticated samples representing 41 taxonomic designations in the NPGS collection to identify those that are genetically identical based on nine simple sequence repeat (SSR) loci. We calculated the probability of identity for samples in the data set based on allelic diversity and, where possible, use fruit images to qualitatively confirm similarities. A total of 237 alleles were amplified and the nine SSRs were deemed adequate to assess duplication within the collection with the caveat that “sport families” likely would not be differentiated. A total of 238 *M. ×domestica* and 10 samples of other taxonomic groups shared a genotype with at least one other *M. ×domestica* individual. In several cases, genotypes for cultivars matched genotypes of known rootstocks and indicated that these accessions may not accurately represent the indicated named clones. Sets of individuals with identical genotypes and similar cultivar names were assigned to sport families. These 23 sport families, comprised of 104 individuals, may have mutational differences that were not identified using the nine SSR loci. Five of the selected markers (CH01h01, CH02d08, CH01f02, G12, GD147) overlap with sets of markers that have been used to fingerprint European apple collections, thus making it possible to compare and coordinate collection inventories on a worldwide scale.

The global production of apples is threatened by disease, pest susceptibility, suboptimal cold-hardiness and heat tolerance, minimal resistance to drought and wet soils, undesirable storage and transport characteristics, and expensive production methods. The U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) National Plant Germplasm System, Plant Genetic Resources Unit (PGRU) apple collection in Geneva, NY, conserves key genetic resources useful for breeding and research programs that address threats to apple crop production. The *Malus* collection is maintained as grafted, clonally propagated trees and own-rooted seedling trees. The grafted orchards include 33 species of *Malus*, of which only one species, *Malus ×domestica*, is cultivated for fruit production, as well as some hybrid materials. These species are represented by between one and 1372 unique accessions each. The taxonomic and ecological breadth of this collection makes it a vital genetic resource for both the United States and the world.

The clonally propagated apple field collection is grafted on ‘East Malling 7’ (EMLA 7) rootstock (Wertheim, 1998), which was selected for its dwarfing and increased resistance to fireblight infection (Aldwinkle et al., 2004; Forsline et al., 2010). Currently, the grafted collection has ≈2500 clones acquired primarily from gene banks, breeders, and wild collection trips (Table 1). The field collection also includes several thousand *Malus* species seedling (non-grafted) trees, derived primarily from seeds collected during plant exploration trips. Field trees are expensive to maintain; the most recent estimated cost was more than $50 per tree each year (Hokanson et al., 1998), and this figure has almost certainly been exceeded in recent years. Thus, it is critical to understand the genetic constitution of the collection materials to ensure continued maintenance of high-priority genetic resources using limited financial resources.

Microsatellite markers have been used successfully to assess the diversity of wild and clonally propagated fruit collections (Gökirmak et al., 2009; Koehmstedt et al., 2011; Laucou et al., 2011; Njuguna et al., 2011; Urrestarazu et al., 2012; van Treuren et al., 2010). As a result of the high levels of allelic diversity within most *Malus* species, relatively few markers are required to differentiate among unique *M. ×domestica* cultivars (Hokanson et al., 1998) or among half-sib individuals from wild populations of *M. sieversii* and *M. orientalis* (Richards et al., 2009a, 2009b; Volk et al., 2005, 2008). Conversely, the very low genomic coverage of microsatellite data sets limits our ability to differentiate among individuals in “sport” families, because these individuals may exhibit distinct phenotypic traits that are based on one or a few mutations (Venturi et al., 2006; Wünsch and Hormaza, 2002).

In this work, we determined whether a set of nine SSR loci is sufficiently variable to differentiate among siblings and identify
Table 1. Taxonomic designation and number of individual inventories of *Malus* trees in the Geneva, NY, Plant Genetic Resources Unit (PGRU) grafted orchard, the number of those grafted trees that are known to be diploid, the number of individuals included in the nine simple sequence repeat (SSR) data set, and the number of seedling trees in the nine SSR data set.

| Species             | PGRU orchard | 9 SSR data set |
|---------------------|--------------|----------------|
|                      | Grafted clones (no.) | Diploid grafted clones (no.) | Grafted clones (no.) | Seedlings (no.) |
| Malus angustifolia  | 17           | 7              | 0                         |
| Malus asiatica      | 16           | 10             | 7                         |
| Malus baccata       | 51           | 47             | 42                        |
| Malus bhutanica     | 7            | 1              | 1                         |
| Malus brevipes      | 2            | 2              | 2                         |
| Malus coronaria     | 40           | 4              | 3                         |
| Malus × domestica   | 1372         | 1227           | 1131                      |
| Malus florentina    | 3            | 3              | 0                         |
| Malus floribunda    | 10           | 10             | 8                         |
| Malus fusa          | 40           | 40             | 8                         |
| Malus halliana      | 14           | 8              | 8                         |
| Malus hupehensis    | 16           | 3              | 2                         |
| Malus hybrid"      | 328          | 317            | 235                       |
| Malus ioensis       | 36           | 17             | 0                         |
| Malus kansuensis    | 6            | 6              | 3                         |
| Malus kirghisorum   | 15           | 15             | 7                         |
| Malus mandshurica   | 3            | 3              | 3                         |
| Malus micromalus    | 15           | 13             | 9                         |
| Malus ombrophila    | 3            | 3              | 0                         |
| Malus orientalis    | 16           | 16             | 4                         |
| Malus orthocarpa    | 1            | 1              | 1                         |
| Malus pratii        | 4            | 4              | 0                         |
| Malus prunifolia    | 36           | 36             | 29                        |
| Malus pumila        | 12           | 11             | 8                         |
| Malus sargentii     | 18           | 1              | 1                         |
| Malus sieversii     | 283          | 283            | 151                       |
| Malus sikkimensis   | 13           | 2              | 2                         |
| Malus sp."        | 29           | 23             | 17                        |
| Malus spectabilis   | 7            | 5              | 2                         |
| Malus sylvestris    | 22           | 21             | 17                        |
| Malus toringo       | 22           | 12             | 9                         |
| Malus transitoria   | 4            | 2              | 2                         |
| Malus tschonoskii  | 3            | 3              | 0                         |
| Malus ×"         | 51           | 44             | 36                        |
| Malus yunnanensis  | 15           | 13             | 5                         |
| Malus zhaojaoensis | 2            | 2              | 2                         |
| Total              | 2532         | 2214           | 1755                      | 155                        |

"Malus hybrid indicates that the accession appears to be a hybrid between two species, but its parentage is not known.

"Malus sp. indicates that the accessions are likely not *Malus × domestica* (based on phenotypic differences), but they have not been positively identified at the species level.

"Malus × includes *M. ×adstringens*, *M. ×arnoldiana*, *M. ×atrosanguinea*, *M. ×dawsoniana*, *M. ×hartwigii*, *M. ×magdebursensis*, *M. ×moerlandsii*, *M. ×platzycarpa*, *M. ×purpurea*, *M. ×robusta*, *M. ×scheideckeri*, *M. ×soulardii*, *M. ×subhombata*, and *M. ×sumi*.

identical accessions in the USDA-ARS *Malus* field collection based on the probability of identity (ProbI) for the data set. We identified genetically identical *M. ×domestica* cultivars maintained as clones in the field collection and, where possible, used fruit images to qualitatively confirm similarities.

**Materials and Methods**

**Plant Material.** Most of the trees in the grafted PGRU orchards were sampled for potential inclusion in the genetic analyses, and, in some cases, seedling trees were included as additional representatives of wild species. For both grafted and seedling trees, only diploid trees had microsatellite signatures that could be scored in a comparative manner, and only these were included in the final data set. The final data set included 1910 individuals, or accessions, each identified with a unique Plant Introduction number [PI# (for grafted trees)] or Geneva *Malus* number (for seedlings in most cases) identification numbers (Table 1). For the present analyses, we focus on 1131 diploid *M. × domestica* cultivars in the PGRU orchards. To identify duplicates, each *M. × domestica* genotype has been compared with all the other domesticated genotypes as well as 779 wild genotypes in the 1910 individual data set.

**Microsatellite Markers.** Genomic DNA was extracted from leaf tissue using DNeasy 96 plant kits (Qiagen, Valencia, CA). Nine previously published SSRs [GD12, GD15, GD96,
This research focuses on the *M. ×domestica* cultivars in the grafted collection but does include some seedling materials for comparison purposes. The *M. ×domestica* collection has 1372 individuals, of which 1240 (90%) are diploid, and thus produced amplification products that could be accurately scored. Individuals with missing data at more than one locus were not included in the data set, leaving 1131 *M. ×domestica* accessions that were compared with each other as well as the additional 779 diploid individuals from other species in the data set.

Nine SSRs were amplified in the 1910 individuals, and a total of 237 alleles (average of 26.3 alleles per locus) were obtained from GWG-Biotec (High Point, NC). Unlabeled reverse primers were purchased from IDT (Coralville, IA). All polymerase chain reactions (PCRs) were carried out in 15 μL total volume using previously published methods (Volk et al., 2005). PCR products were visualized and scored in one of two ways. Some were visualized on a slab sequencer (LI-COR 4200; LI-COR, Lincoln, NE); digital images were collected from the sequencer using LI-COR Saga Generation 2 software and were manually interpreted and scored using the Saga software. Others were visualized on a capillary sequencer (ABI 3730; Applied Biosystems, Foster City, CA); chromatograms were scored using the GeneMarker software (SoftGenetics, State College, PA). Peaks were scored automatically based on allelic bins created for each SSR locus and then corrected manually for cases of errors or nonsensical automatic scoring. When a single SSR was scored using both the LI-COR and ABI systems, a minimum of 10 individuals were run on each system to control for allele length differences resulting from instrumentation.

**ProbI**

| SSR locus | A | H_s | H_e | ProbI | D | PIC |
|-----------|---|-----|-----|-------|---|-----|
| GD12      | 31| 0.66| 0.89| 0.054 | 0.946| 0.788|
| GD15      | 5 | 0.37| 0.50| 0.601 | 0.399| 0.221|
| GD96      | 31| 0.79| 0.94| 0.016 | 0.984| 0.898|
| GD142     | 26| 0.81| 0.91| 0.018 | 0.982| 0.894|
| GD147     | 26| 0.55| 0.93| 0.027 | 0.973| 0.864|
| GD162     | 30| 0.66| 0.94| 0.026 | 0.974| 0.866|
| CH01h01   | 28| 0.74| 0.94| 0.020 | 0.980| 0.887|
| CH01f02   | 34| 0.86| 0.93| 0.013 | 0.987| 0.910|
| CH02d08   | 26| 0.67| 0.90| 0.030 | 0.970| 0.854|

**Phenotypes and fruit images.** Fruit images for each accession identified as a duplicate were downloaded from the Germplasm Resources Information Network database (USDA, 2012). Images were qualitatively compared for fruit size, fruit ground color, fruit over color, shape, and russet appearance.

**Results**

Table 2. Genetic parameters for each of the nine simple sequence repeat (SSR) loci used to determine duplicate genotypes in the *Malus* collection, including number of alleles (A), observed heterozygosity (H_s), expected heterozygosity (H_e), the probability of identity (ProbI), the discriminating power (D), and the polymorphic information content (PIC).

![Fig. 1. Probabilities of identity (ProbI) for increasing combination of the nine simple sequence repeat (SSR) loci arranged in decreasing order of ProbI for each locus. Dashed lines show the standard ProbI, and solid lines show the more conservative ProbIsibs.](image)
present across the nine SSRs. Levels of heterozygosity were high; \( H_o \) was greater than 0.50 and \( H_e \) was greater than 0.85 across all species for all loci except for GD15 (Table 2). In \( M. \times domestica \), \( H_o \) was greater than 0.70 for all loci except for GD15 (data not shown). \( \text{ProbI} \) and \( \text{ProbIsibs} \) were calculated for the data set to determine whether any empirically determined duplicated genotypes were likely the result of chance alone or whether they were the result of true identity at the genetic level. Although there is not a firm cutoff for when to interpret a level of \( \text{ProbI} \) as sufficient, one suggested standard (Peakall et al., 2006) is the reciprocal of the sample size \( (1/1910 = 0.00052) \). Thus, a value for \( \text{ProbI} \) or \( \text{ProbIsibs} < 0.00052 \) would indicate that when samples do share identical genotypes, it is the result of true identity between the individuals. The values of \( \text{ProbI} \) and \( \text{ProbIsibs} \) are plotted in Figure 1, and the required level of \( \text{ProbI} \) is achieved with two SSRs for \( \text{ProbI} \) and with seven SSRs for the more conservative \( \text{ProbIsibs} \). \( \text{ProbI} \) values for each SSR locus, representing a measure of the discriminatory power of a given marker, are given in Table 2.

Given that the number and variability at the loci used in this study are likely sufficient to discriminate between individuals and matches that represent true identity, another way to interpret the data are to consider the probability (however low) that two individuals would match by chance alone. For the standard value of \( \text{ProbI} \), assuming no relatedness, the probability is \( 4.8 \times 10^{-15} \), or one in 21 trillion. For the more conservative value of \( \text{ProbIsibs} \), it is \( 7.7 \times 10^{-5} \), or one in 13,000. The real value is likely somewhere in between these two extremes, because some of the individuals are undoubtedly related, but many are not, given the number of different species present in the data set.

The explicit possibility that full- or half-sibs might have the same genotype based on their close relatedness also must be considered, especially in the context of the sport families. The probability that two full-sibs will have the same genotype is

Fig. 2. Chromatograms of locus CH02d08 for two sets of duplicated \( Malus \times domestica \) genotypes. The first two are PI #437036 and PI #613830 (cultivars Aromat de Vara and Inducoa No. 11) with alleles of length 212 and 230 bps. The second two are PI #129820 and PI #249925 (cultivars Cole and King Cole) with alleles of length 214 and 230 bps.
Table 3. Plant Introduction (PI) or Geneva *Malus* (GMAL) number, cultivar name (followed by species name if not *Malus domestica*), and country of origin for matching genotypes or sets of genotypes, arranged horizontally.*

| PI/GMAL no. | Cultivar name | Country of origin | PI/GMAL no. | Cultivar name | Country of origin |
|------------|---------------|-------------------|------------|---------------|-------------------|
| Same genotype, similar images | | | | | |
| PI 344547  | Alkmene        | PI 105498         | Bella de Jardins       | France          |
| PI 187296  | Bouteille               | PI 158731         | Bramot                 | United Kingdom  |
| PI 162549  | Boche                      | PI 188524         | Storappel                      | The Netherlands |
| PI 162645  | Grosse Mouché              | PI 589288         | Not named (hybrid)              |                  |
| PI 589593  | Blenheim Orange             | PI 175551         | Twistbody Jersey               | United Kingdom  |
| PI 347268  | Koeko strain 2               | PI 437047         | Red Ralls                        | Poland          |
| PI 589690  | Le Bret                    | PI 589081         | Sweet Alford                   | United Kingdom  |
| PI 589196  | Crow Egg                    | PI 589123         | Geest.van Fanny                  | Australia       |
| PI 589555  | Joys                       | PI 589444         | Reinette Clochard                  | France          |
| PI 589354  | Cheal’s Weeping               | PI 78170          | Oekonomier Echter-meyer           | Germany         |
| PI 131215  | Nanny                       | PI 137056         | Surprise Reinette                  | Switzerland     |
| PI 161840  | Lorna Doone                | PI 205460         | Merton Beauty                     | United Kingdom  |
| PI 437036  | Aromat de Vara               | PI 175010         | King Harry                       | United Kingdom  |
| PI 613830  | Inducoa No. II             | PI 589153         | Magnolia Gold                     | United States   |
| PI 589135  | Golden Noble                   | PI 188525         | Vogelcaiville                     | The Netherlands  |
| PI 349324  | Coast Apple                  | PI 209939         | Subtropical Apple                  | South Africa    |
| PI 276567  | Orleans                      | PI 589303         | Quindell                           | United States   |
| PI 279643  | Ivette                       | PI 589502         | Z 71                               | The Netherlands  |
| PI 589254  | Doud Golden Delicious 2-4-4-4 | PI 283698        | Imperial Stayman                   | United States   |
| PI 383505  | Ernak                        | PI 589908         | Whitney Crab (hybrid)              | United States   |
| PI 589215  | Crimson Gold (pumila)        | PI 437064         | Yar Mohammadi                     | Pakistan        |
| PI 537000  | Drakenstein                  | PI 589342         | E 12-14                           | South Africa    |
| PI 589446  | Idagon                       | PI 588793         | Snow                               | Canada          |
| PI 589574  | Borowitsky                   | PI 588801         | Duchess                            | United Kingdom  |
| PI 588841  | Idared                       | PI 589553         | Mantuanskyoe                       | Italy           |
| PI 589150  | Lowry                        | PI 589001         | Winter Sweet Paradise              |                  |
| Same genotype, image, and name | | | | | |
| PI 588900  | Jubilee (hybrid)             | PI 589725         | Jubilee                           | Canada          |
| PI 279645  | Odin                         | PI 589921         | Odin*                              | The Netherlands  |
| PI 280029  | Pitmaston Pine Apple (hybrid) | PI 279323        | Pitmaston Pineapple × 692         | France          |
| PI 589457  | Rosu de Cluj                 | PI 613831         | Rosu de Cluj                      | Romania         |
| PI 588849  | Russian                      | PI 589312         | Russian sdlg.                      |                  |
| PI 589025  | Splendor (Stark)             | PI 437054         | Splendour (hybrid)                 | New Zealand     |
| PI 483256  | YP                           | PI 588827         | YP (MB4) (pumila)                   | Finland         |
| Same genotype, paired images not available | | | | | |
| PI 589540  | Alpinist                     | PI 589670         | Livadiyskoye                      | Ukraine         |
| PI 267399  | Ananas Berzienicki            | PI 262962         | Prof. Grebicka Renete              | Latvia          |
| PI 589885  | Beacon                       | PI 590171         | Northern Lights                    |                  |
| PI 589664  | Beauty of Bath               | PI 102140         | Gladstone                          | United Kingdom  |
| PI 126495  | Belle Imperiale              | PI 589001         | Winter Sweet Paradise              |                  |

| GMAL 4662 | Bisquet                | France | PI 589670 | Michelin* | France |
|           | Doux-AMR               |        |           |           |         |
| PI 122616 | C-13-30-88 (hybrid)     |        |           |           |         |
| PI 383503 | Chulanka               |        |           |           |         |
| GMAL 4660 | Clos Renaux            |        |           |           |         |
| PI 589191 | Collamer Twenty Ounce  |        |           |           |         |
| PI 589149 | Collins June           |        |           |           |         |
| PI 589293 | Early Banta            |        |           |           |         |
| PI 392132 | Edward VII             |        |           |           |         |
| PI 157207 | Gold Canel             |        |           |           |         |
| PI 589491 | Korichnove Polosatoje  |        |           |           |         |
| PI 590220 | Hardy Cumberland        |        |           |           |         |
| PI 136001 | Herring’s Pippin       |        |           |           |         |
| PI 589054 | Red Astrachan          |        |           |           |         |
| PI 589598 | La Paix                |        |           |           |         |

continued next page
mainly dependent on the level of homozygosity of the parents and ranges from 0.25 if the parents are heterozygous with four different alleles across a single locus to 1.0 if the parents are homozygous. There are a variety of ways that these probabilities can be combined across nine SSRs. However, even if one or the other parent is homozygous at each of the nine SSRs (although the other is heterozygous), the probability that the siblings would be genetically identical, had related cultivar names, and mostly source planting. When sets of individuals were identified as being genetically identical, had related cultivar names, and mostly similar fruit appearances, they were assigned to "sport" families. The USDA seeks to maintain a diverse collection of Malus genetic resources. Given the maintenance cost per tree and the limited space available, it is critical that new materials strategically expand the collection by capturing novel genetic diversity that has potential value to the user community. Effectively maintaining the genetic diversity of clonal crop collections such as the NPGS Malus orchard is particularly important in the context of modern production, in which the occasional infusions of genetic diversity through cross-pollination with wild relatives, once a major source of new variation, are rarely incorporated into crop germplasm (McKey et al., 2010). Indeed, the U.S. apple industry is based primarily on 11 apple cultivars (Dennis, 2008), and these modern commercial cultivars are derived from progeny of only four seedling parents: 'Cox’s Orange Pippin’, ‘Golden Delicious’, 'Jonathan’, and ‘McIntosh’.

### Discussion

The USDA seeks to maintain a diverse collection of *Malus* genetic resources. Given the maintenance cost per tree and the limited space available, it is critical that new materials strategically expand the collection by capturing novel genetic diversity that has potential value to the user community. Effectively maintaining the genetic diversity of clonal crop collections such as the NPGS *Malus* orchard is particularly important in the context of modern production, in which the occasional infusions of genetic diversity through cross-pollination with wild relatives, once a major source of new variation, are rarely incorporated into crop germplasm (McKey et al., 2010). Indeed, the U.S. apple industry is based primarily on 11 apple cultivars (Dennis, 2008), and these modern commercial cultivars are derived from progeny of only four seedling parents: ‘Cox’s Orange Pippin’, ‘Golden Delicious’, ‘Jonathan’, and ‘McIntosh’.
Diverse, well-characterized apple collections of both *M. domestica* as well as wild *Malus* species provide breeding programs with novel alleles that have the potential to increase yield, value, and quality as well as decrease susceptibility to biotic and abiotic stresses. Essentially, these collections serve as a resource to maintain a high overall genetic diversity for a crop despite very low diversity in the field.

Genetic assessments of the USDA-ARS-NCGRP apple collection allow for a more in-depth understanding of diversity further at the phenotypic level to determine if the cultivars possess unique traits or if they might be similar enough to be considered synonyms of genotypes in the collection. This approach can also be taken with the sets of sport families, which can be characterized for phenotypic or physiological traits of interest. Sport family members may have unique, desirable traits that are based on simple mutations (potentially as a result of retrotransposon activity) compared with other family members (Kobayashi et al., 2004; Sun et al., 2010; Venturi et al., 2006). The cultivar Wijcik McIntosh is an example of a cultivar that may appear to be unique but is actually a sport of Golden Delicious.
Table 4. Sport family name, PI number, cultivar name, and country of origin for matching genotypes or sets of genotypes, arranged horizontally.

| Sport family        | PI no.     | Cultivar name               | Country of origin  |
|---------------------|------------|-----------------------------|--------------------|
| Antonovka           | PI 588784  | Antonovka                   | Russia             |
| Antonovka           | PI 589837  | Antonovka Monasir           |                    |
| Antonovka           | PI 589329  | Antonovka Polutorafuntovaya | Former Soviet Union|
| Antonovka           | PI 322715  | Tayshnoe                    | Former Soviet Union|
| Belflower           | PI 589577  | Brabant Belfleur            | The Netherlands    |
| Belflower           | PI 264692  | Red Belflower               | Serbia             |
| Ben Davis           | PI 590025  | Anisim                      | Former Soviet Union|
| Ben Davis           | PI 588953  | Ben Davis                   | United States      |
| Ben Davis           | PI 589178  | Black Ben Davis             | United States      |
| Cheddar             | PI 199100  | Cheddar                      | United Kingdom     |
| Cheddar             | PI 589656  | Cheddar Cross                | United Kingdom     |
| Cole                | PI 129820  | Cole                        | United Kingdom     |
| Cole                | PI 249925  | King Cole                   | Australia          |
| Court Pendu         | PI 589601  | Court Pendu                 | Belgium            |
| Court Pendu         | PI 589602  | Court Pendu Gris'           | France             |
| Cox                 | PI 247022  | Cox’s Orange Cherry         | Denmark            |
| Cox                 | PI 247023  | Cox’s Orange Otago          |                    |
| Cox                 | PI 588853  | Cox’s Orange Pippin         | United Kingdom     |
| Cox                 | PI 589127  | Potter Cox                  | United Kingdom     |
| Golden Delicious    | PI 589139  | Badami Golden Delicious     | United States      |
| Golden Delicious    | PI 589224  | Clear Gold                  | United States      |
| Golden Delicious    | PI 589316  | Empress Spur Golden Delicious| United States     |
| Golden Delicious    | PI 590184  | Golden Delicious            | United States      |
| Golden Delicious    | PI 589041  | Golden Delicious (Smoothee)  | United States      |
| Golden Delicious    | PI 589535  | Golden Delicious SE-69      | Czech Republic     |
| Golden Delicious    | PI 589448  | Golden Precoc              |                    |
| Golden Delicious    | PI 644190  | Goldspur Golden Delicious   | United States      |
| Golden Delicious    | PI 589966  | Puregold                    | United States      |
| Golden Delicious    | PI 589136  | Razor Golden Delicious      | United States      |
| Golden Delicious    | PI 589125  | Sergeant Russet Golden Delicious| United States   |
| Golden Delicious    | PI 589904  | Smoothgold                  |                    |
| Golden Delicious    | PI 589210  | Starkspur Golden Delicious  | United States      |
| Golden Delicious    | PI 589528  | Woodward                    | United States      |
| Golden Delicious    | PI 589856  | Yellow Delicious            | United States      |
| Jersey              | PI 161845  | Red Jersey                  | United Kingdom     |
| Jersey              | PI 175545  | Royal Jersey                | United Kingdom     |
| Jonathan            | PI 194199  | Bowden Seedling             | United Kingdom     |
| Jonathan            | PI 589160  | Conkle Jonathan 2-4-4-4     | United States      |
| Jonathan            | PI 588940  | Jonared                     | United States      |
| Jonathan            | PI 590185  | Jonathan                    | United States      |
| Jonathan            | PI 590150  | Kingjon                    | United States      |
| Jonathan            | PI 589097  | Welday Jonathan 2-2-4-4     | United States      |
| Jonathan            | PI 589144  | Welday Jonathan 4X          | United States      |
| Lamb Abbey Pearmain | PI 199418  | Lamb Abbey Pearmain-A       | United Kingdom     |
| Lamb Abbey Pearmain | PI 199419  | Lamb Abbey Pearmain-B       | United Kingdom     |
| McIntosh            | PI 588961  | Boller McIntosh             | United States      |
| McIntosh            | PI 589557  | Calvil Crymski              | Former Soviet Union|
| McIntosh            | PI 589183  | Cornwall McIntosh 2-2-2-4   | United States      |
| McIntosh            | PI 589069  | Green Peak McIntosh 2-2-4-4 | United States      |
| McIntosh            | PI 589507  | Hamilton (hybrid)           | Canada             |
| McIntosh            | PI 589206  | Johnson McIntosh 2-4-4-4   | United States      |
| McIntosh            | PI 589122  | Kimball McIntosh 2-4-4-4   | United States      |
| McIntosh            | PI 588999  | MacSpur                    | Canada             |
| McIntosh            | PI 588998  | Marshall McIntosh           | United States      |
| McIntosh            | PI 588817  | McIntosh Summerland Red     | Canada             |
| McIntosh            | PI 588968  | Rogers McIntosh             | United States      |
| McIntosh            | PI 590186  | Wijcik McIntosh             | Canada             |
| Merton              | PI 589849  | Merton Ace                  | United Kingdom     |

continued next page
excellent example of such a sport; it exhibits a unique, heritable
columnar growth habit. Sports like ‘Wijcik McIntosh’ with
desirable traits could be selected for further genetic analyses to
identify new loci controlling traits of interest or new alleles at
known loci that could be useful for future breeding efforts.

This research presented data from nine SSR loci that are
sufficient to determine potential duplicates among 1910 acces-
sions in the USDA Malus collection and likely from other large
collections of Malus as well. These markers overlap those
previously used to assess diversity within wild species
(Richards et al., 2009a, 2009b; Volk et al., 2005, 2008, 2009)
and among M. ×domestica, M. orientalis, M. sieversii, and
M. sylvestris in the USDA-ARS-NPGS collection (Gross et al.,
2012). Five of the markers (CH01h01, CH02d08, CH01f02,

| Sport family | PI no.    | Cultivar name                | Country of origin |
|--------------|-----------|------------------------------|-------------------|
| Merton       | PI 589429 | Merton Knave                 | United Kingdom    |
| Muscadet     | PI 162544 | Belle de Crollon             | France            |
| Muscadet     | PI 200780 | Muscadet Bernay              | France            |
| Muscadet     | PI 173985 | Muscadet de Lense            | France            |
| Orange       | PI 589757 | Ellison’s Orange             | United Kingdom    |
| Orange       | PI 392307 | Finson’s Orange              | Australia         |
| Paragon      | PI 589193 | Dermen Paragon 3-6-6         | United States     |
| Paragon      | PI 589148 | Dermen Paragon 6-3-3         | United States     |
| Paragon      | PI 589039 | Paragon                      | United States     |
| Red Delicious| PI 589841 | Delicious                    | United States     |
| Red Delicious| PI 589110 | Dermen Delicious 4X          | United States     |
| Red Delicious| PI 589068 | Earlhilite Delicious         | United States     |
| Red Delicious| PI 589064 | Fruitland Delicious          | United States     |
| Red Delicious| PI 590026 | Hawkeye                      | United States     |
| Red Delicious| PI 589037 | Idaho Spur Delicious         | United States     |
| Red Delicious| PI 589255 | Redspur Delicious            | United States     |
| Red Delicious| PI 589151 | Richared Delicious           | United States     |
| Red Delicious| PI 589137 | Rose Red Delicious           | United States     |
| Red Delicious| PI 589190 | Starking                     | United States     |
| Red Delicious| PI 589192 | Yellow Red Delicious         | United States     |
| Rome         | PI 589201 | Barkley Rome                 | United States     |
| Rome         | PI 589103 | Gallia Beauty                | United States     |
| Rome         | PI 588986 | Hotle Rome                   | United States     |
| Rome         | PI 589033 | Loop Rome 2-2-2-4            | United States     |
| Rome         | PI 588956 | Milton                       | United States     |
| Rome         | PI 588850 | Rome Beauty Law              | United States     |
| Spartan      | PI 589231 | Hunter Spartan 2-4-4         | Canada            |
| Spartan      | PI 588871 | Spartan                      | Canada            |
| Spartan      | PI 589204 | Sweden Spartan               | Sweden            |
| Spitzenburg  | PI 588785 | Esopus Spitzenburg           | United States     |
| Spitzenburg  | PI 589100 | Red Spitzenburg              | United States     |
| Spy          | PI 589047 | Farmer Spy                   | United States     |
| Spy          | PI 589055 | Field Spy                    | United States     |
| Spy          | PI 589132 | Green Peak Spy #1            | United States     |
| Spy          | PI 589203 | Hunter Spy 2-4-4             | Canada            |
| Spy          | PI 589207 | Loop Giant Spy               | United States     |
| Spy          | PI 589096 | Loop Red Spy                 | United States     |
| Spy          | PI 589027 | Loop Spy 2-2-4               | United States     |
| Spy          | PI 588872 | Northern Spy                 | United States     |
| Spy          | PI 589842 | Red Spy                      | United States     |
| Spy          | PI 588969 | Scholarie Spy                | United States     |
| Stayman      | PI 589152 | Dermen Black Stayman         | United States     |
| Stayman      | PI 589236 | Dermen Stayman 6-3-3         | United States     |
| Stayman      | PI 588975 | Stayman                      | United States     |
| Wealthy      | PI 589188 | Coombs Wealthy               | United States     |
| Wealthy      | PI 588788 | Wealthy                      | United States     |
| Wealthy      | PI 588779 | Wealthy Double Red PC-310    | United States     |

*Country of origin is based on information in the Germplasm Resources Information Network (GRIN) database (U.S. Department of Agriculture, 2012) and sometimes refers to the country from which the accession was received.
\(^{1}\)Unavailable in 2012.
G12, GD147) overlap with the markers that have been selected within the European community for collection comparison purposes (F. Laurens, personal communication; M. Ordidge, personal communication), so it will be possible to extend this type of analysis across collections in the future. Ultimately, studies of this type can be used to lay the groundwork for genomic analyses of apple diversity and functional loci.

**Literature Cited**

Aldwinckle, H.S., N. LoGiudice, G. Fazio, J.L. Norelli, T.L. Robinson, H.T. Holleran, and W.C. Johnson. 2004. Resistance of apple rootstocks to fire blight infection caused by internal movement of *Erwinia amylovora* from scion infection. Acta Hort. 663:229–233.

Botstein, D., R.L. White, M. Skolnick, and R.W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Amer. J. Hum. Genet. 32:314–331.

Brown, S. 2012. Apple, p. 329–368. In: Badenes, M.L. and D.H. Byrne (eds.). Fruit breeding. Springer, New York, NY.

Dennis, F.G., Jr. 2008. *Malus × domestica*: Apple, p. 661–674. In: Janick, J. and R.E. Paull (eds.). The encyclopedia of fruits and nuts. CABI Publishing, Cambridge, MA.

Forsline, P.L., H.S. Aldwinckle, E.E. Dickson, J.J. Luby, and S.C. Malus. 2008. Genetic diversity and structure of local apple cultivars from northeastern Spain assessed by microsatellite markers. Tree Genet. Genomes 5:339–347.

Gökirmak, T., S. Mehlenbacher, and N. Bassil. 2009. Characterization of European hazelnut (*Corylus avellana*) cultivars using SSR markers. Genet. Resources Crop Evol. 56:147–172.

Gross, B.L., A.D. Henk, P.L. Forsline, C.M. Richards, and G.M. Volk. 2012. Identification of interspecific hybrids among domesticated apple and its wild relatives. Tree Genet. Genomes (in press).

Hokanson, S.C., A.K. Szewc-McFadden, W.F. Lamboy, and J.R. McFerson. 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus × domestica* Borkh. core subset collection. Theor. Appl. Genet. 97:671–683.

Kalinowski, S.T., M.L. Taper, and T.C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol. Ecol. 16:1099–1106.

Kobayashi, S., N. Goto-Yamamoto, and H. Hirochika. 2004. Retrotransposon-induced mutations in grape skin color. Science 304:982.

Koehmstedt, A., M. Aradhya, D. Soleri, J. Smith, and V. Polito. 2011. Molecular characterization of genetic diversity, structure, and differentiation in the olive (*Olea europaea* L.) germplasm collection of the United States Department of Agriculture. Genet. Resources Crop Evol. 58:519–531.

Laucou, V., T. Lacombe, F. Dechesne, R. Siret, J.P. Bruno, M. Dessup, T. Dessup, P. Ortigosa, P. Parra, C. Roux, S. Santoni, D. Varès, J.P. Péros, J.M. Boursiquot, and P. This. 2011. High throughput analysis of grape genetic diversity as a tool for germplasm collection management. Theor. Appl. Genet. 122:1233–1245.

Liebhard, R., L. Gianfranceschi, B. Koller, C.D. Ryder, R. Tarchini, E. Van De Weg, and C. Gessler. 2002. Development and characterisation of 140 new microsatellites in apple (*Malus × domestica* Borkh.). Mol. Breed. 10:217–241.

Luby, J.J. 2003. Taxonomic classification and brief history, p. 1–14. In: Ferree, D.C. and I.J. Warrington (eds.). Apples: Botany, production, and uses. CABI Publishing, Cambridge, MA.

McKey, D., M. Elias, B. Pujol, and A. Duputié. 2010. The evolutionary ecology of clonally propagated domesticated plants. New Phytol. 186:318–332.

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.

Njuguna, W., K. Hummer, C. Richards, T. Davis, and N. Bassil. 2011. Genetic diversity of diploid Japanese strawberry species based on microsatellite markers. Genet. Resources Crop Evol. 58:1187–1198.

Noitom, D.A.M. and P.A. Alspach. 1996. Founding clones, inbreeding, coancestry, and status number of modern apple cultivars. J. Amer. Soc. Hort. Sci. 121:773–782.

Peakall, R., D. Ebert, R. Cunningham, and D. Lindenmayer. 2006. Mark-recapture by genetic tagging reveals restricted movements by bush rats (*Rattus fuscipes*) in a fragmented landscape. J. Zool. 268:207–216.

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.

Richards, C.M., G.M. Volk, P.A. Reeves, A.A. Reilley, A.D. Henk, P.L. Forsline, and H.S. Aldwinckle. 2009a. Selection of stratified core sets representing wild apple (*Malus sieversii*). J. Amer. Soc. Hort. Sci. 134:228–235.

Richards, C.M., G.M. Volk, A.A. Reilley, A.D. Henk, D. Lockwood, P.A. Reeves, and P.L. Forsline. 2009b. Genetic diversity and population structure in *Malus sieversii*, a wild progenitor species of domesticated apple. Tree Genet. Genomes 5:339–347.

Sun, J., J.G. Fang, F. Wang, Q.B. Sun, and Z. Zhang. 2010. Characterisation of RNaSeq-LTR sections of Tyl-copia retrotransposons in apple and fingerprinting of four apple clones by S-SAP analysis. J. Hort. Sci. Biotechnol. 85:53–58.

Taberlet, P. and G. Luikart. 1999. Non-invasive genetic sampling and individual identification. Biol. J. Linn. Soc. Lond. 68:41–55.

Tesser, C., J. David, P. This, J.M. Boursiquot, and A. Charrier. 1999. Optimization of the choice of molecular markers for varietal identification in *Vitis vinifera* L. Theor. Appl. Genet. 98:171–177.

Urrestarazu, J., C. Miranda, L. Santesteban, and J. Royo. 2012. Genetic diversity and structure of local apple cultivars from northeastern Spain assessed by microsatellite markers. Tree Genet. Genomes in press.

U.S. Department of Agriculture. 2012. Germplasm Resources Information Network (GRIN). 1 May 2012. <http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl>.

van Treuren, R., H. Kemp, G. Ernsting, B. Jongejan, H. Houtman, and L. Visser. 2010. Microsatellite genotyping of apple (*Malus × domestica* Borkh.) genetic resources in the Netherlands: Application in collection management and variety identification. Genet. Resources Crop Evol. 57:853–865.

Venturi, S., L. Dondini, P. Donini, and S. Sansavini. 2006. Retrotransposon characterisation and fingerprinting of apple clones by S-SAP markers. Theor. Appl. Genet. 112:440–444.

Volk, G.M., C.M. Richards, A.D. Henk, A.A. Reilley, P.A. Reeves, P.L. Forsline, and H.S. Aldwinckle. 2009. Capturing the diversity of wild *Malus orientalis* from Georgia, Armenia, Russia, and Turkey. J. Amer. Soc. Hort. Sci. 134:453–459.

Volk, G.M., C.M. Richards, A.A. Reilley, A.D. Henk, P.L. Forsline, and H.S. Aldwinckle. 2005. Ex situ conservation of vegetatively propagated species: Development of a seed-based core collection for *Malus sieversii*. J. Amer. Soc. Hort. Sci. 130:203–210.

Wertheim, S.J. 1998. Rootstock guide: Apple, pear, cherry, european plum. Proefstation voor de fruitteelt, Wilhelminadorp, The Netherlands.

Wünsch, A. and J.I. Hormaza. 2002. Cultivar identification and genetic fingerprinting of temperate fruit tree species using DNA markers. Euphytica 125:59–67.