MICROSATELLITE MARKER DEVELOPMENT FOR THE COASTAL DUNE SHRUB *Prunus maritima* (Rosaceae)**1**

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• *Premise of the study:* Microsatellite primers were developed in the beach plum, *Prunus maritima*, to investigate the genetic composition of remaining populations in need of conservation and, in future studies, to determine its relation to *P. maritima* var. *gravesii.*

• *Methods and Results:* Fourteen primer pairs were identified and tested in four populations throughout the species’ geographic range. Of these 14 loci, 12 were shown to be polymorphic among a total of 60 *P. maritima* individuals sampled (15 individuals sampled from four populations). Among the polymorphic loci, the number of alleles ranged from two to 10 and observed heterozygosity of loci ranged from 0.07 to 0.93 among specimens tested.

• *Conclusions:* These microsatellites will be useful in evaluating the population genetic composition of *P. maritima* and in developing approaches for further conservation and management of this species within the endangered coastal dune ecosystem of the northeastern United States.

Key words: coastal dune ecosystem; conservation genetics; microsatellites; plum; population genetics; *Prunus maritima*; Rosaceae.

The endangered coastal dune ecosystem of the northeastern United States consists of extreme abiotic conditions including frequent exposure to high levels of salinity, wind, erosion, and broad temperature fluctuations (Mclachlan, 1991). This unique ecosystem provides niches for highly specialized organisms such as *Prunus maritima* Marshall (beach plum; Rosaceae), which have adapted to thrive in this harsh environment. Throughout the past century, human-mediated habitat destruction and fragmentation of coastal lands has resulted in a significant decline of highly endemic species, such as the beach plum (Feagin et al., 2005). Today, *P. maritima* is listed as endangered in three states within its limited geographic range including Maine, Maryland, and Pennsylvania (USDA, NRCS, 2015). The beach plum is a long-lived shrub 3–4 m tall, typically possessing lanceolate leaves, although the shrub varies greatly in habit, fruit color, and size. Reproduction occurs in mid-May, at which time white, five-petaled, generalist-pollinated flowers are produced. The subsequent fruits develop over the summer months, ripening in late August and September and functioning as an important food resource for migrating bird species (Uva, 2003).

Previous research has revealed that *P. maritima* is a sister taxon to *P. geniculata* R. M. Harper (Shaw and Small, 2005), a federally listed species endemic to the central Florida scrublands. In light of this established evolutionary relationship, Germain-Aubrey et al. (2011) developed eight microsatellite loci in *P. geniculata* as a tool for investigating the conservation genetics of this rare lineage. These loci were further tested on samples of *P. maritima* collected from Massachusetts and Delaware to assess preliminary levels of polymorphism. All loci were polymorphic at all locations sampled, rendering these loci potentially useful for future conservation genetics research of both taxa.

The goal of this study was to generate an additional suite of microsatellite markers specifically developed for *P. maritima* and tailored to generate a robust evaluation of the genetic composition of remaining populations. To this end, 14 microsatellite markers were developed to assess levels of genetic variation and the genetic structure of populations of *P. maritima* along the northeastern coast of the United States. In future studies, we will also use these microsatellite markers to determine the relatedness of *P. maritima* and *P. maritima* var. *gravesii* (Grave’s beach plum), which is now considered to be extinct in the wild (Anderson, 1980).

**METHODS AND RESULTS**

Leaf samples of 15 *P. maritima* plants were collected from each of the following populations in the summer and fall of 2011 and 2012: Rachel Carson National Wildlife Refuge, Biddeford, Maine (43.4469, −70.3741); Milford...
Fluorescently labeled primers (6-FAM, VIC, PET, NED; Applied Biosystems) were ordered for 18 of the forward primers that consistently amplified single-banded PCR products. Fluorescent primers were tested both alone and in 10-μL multiplex mixtures consisting of 5 μL of Multiplex PCR Master Mix (QIAGEN), 3.5 μL of water, 1 μL of 2 μM forward and reverse primers, and 0.5 μL of DNA template. PCR was performed using the optimal conditions of 10 min at 95°C, followed by 28 cycles of 30 s at 95°C, 45 s at 55°C, and 45 s at 72°C; with a final elongation step of 10 min at 72°C. PCR products were stored at −4°C. GeneScan 500 LIZ Size Standard (Applied Biosystems) was mixed with the PCR product, separated on an ABI 3730xl capillary electrophoresis instrument, then analyzed using GeneMapper version 4.0 software (Applied Biosystems). Based on the results of the initial fragment analysis, 14 primer pairs produced easily discernable, amplified products that could be accurately and consistently genotyped using electropherograms. For the remainder of genotyping analyses, these 14 microsatellites were amplified in four sets based on variation in fragment size produced by each primer pair and the results of different multiplex mixtures tested: (1) PM1, PM9, PM14, PM21; (2) PM2, PM8, PM13, PM18; (3) PM7, PM11, PM16, PM22; (4) PM3, PM20.

All 14 of the primer pairs in these sets consistently amplified *Prunus maritima* DNA in four populations sampled from across the species range, and 12 primer pairs revealed polymorphic loci. Only PM8 and PM22 were determined to be monomorphic across the four populations and the 60 samples analyzed for this study. Primer sequence, repeat type, fragment size, ideal annealing temperature (calculated as 5°C below the lowest melting temperature of the primer pair), fluorescent label, and GenBank accession number are shown in Table 1. Ten of the 14 loci investigated amplified perfect repeat units.

Characteristics of each microsatellite region, such as the number of alleles and observed and expected heterozygosity values, were calculated as 5°C below the lowest melting temperature of the primer pair, fluorescent label, and GenBank accession number are shown in Table 1. Ten of the 14 loci investigated amplified perfect repeat units.

Characteristics of each microsatellite region, such as the number of alleles and observed and expected heterozygosity values, were calculated as

| Locus | Primer sequence (5′-3′) | Repeat motif | Allele size range (bp) | Fluorescent label | GenBank accession no. |
|-------|------------------------|--------------|------------------------|------------------|----------------------|
| PM1   | F: AAAGTGGCTTTTACACTTTGCTT | (CA)$_3$ | 147–149 | 6-FAM | KM013816 |
|       | R: GCATTGGAGGTTAATGGAG | | | | |
| PM2   | F: ATATACGGGTCACCAATGGAG | (CA)$_4$ | 189–193 | NED | KM013817 |
|       | R: TTATGTTTTTCTACAAAGGAATTCGC | | | | |
| PM3   | F: CCAAAGGCCAGGTCTTCCTT | (TC)$_3$ | 236–250 | VIC | KM013818 |
|       | R: ATGCGTCCGACCAAGTCTAC | | | | |
| PM7   | F: TTTAGACACGCATCAGAAA | (GA)$_{10}$ | 208–264 | VIC | KM013819 |
|       | R: CTGCTTAACGCTTCAGACGC | | | | |
| PM8   | F: AAGATTTGGAGCTCGATTGC | (GA)$_3$A(GA)$_3$ | 195 | PET | KM013820 |
|       | R: TCTCCTGTTAATGGTTTGGAG | | | | |
| PM9   | F: TGATGCTGTTAACCCTCCTTTCCTT | (AG)$_b$ | 157–177 | PET | KM013821 |
|       | R: TCCTCTGAGCTCACCAGAA | | | | |
| PM11  | F: AAACCTAGCTGCTTCTTATG | (CT)$_{11}$ | 219–248 | NED | KM013822 |
|       | R: TGGGCAAGGAGAAGGAAAAACC | | | | |
| PM13  | F: ATTTTATTTTGGATTGAG | (GA)$_3$(GG)(GA)$_2$ | 164–208 | VIC | KM013823 |
|       | R: GATGGGACCCACAGCCTAC | | | | |
| PM14  | F: AGGTATTGTTGGGGACAAT | (CT)$_{10}$ | 125–152 | VIC | KM013824 |
|       | R: GAGGCTATTGGGGAACAGG | | | | |
| PM16  | F: GTGGCCTACTTTCAATTTCCA | (TG)$_b$(AG)$_{13}$ | 195–226 | 6-FAM | KM013825 |
|       | R: TCGATGGTAAAGCAAATG | | | | |
| PM18  | F: TGATGGTAAATTTGCCACTGAGA | (TC)$_3$TT(TC)$_3$ | 168–190 | 6-FAM | KM013826 |
|       | R: TCGCGATGTTGGTGAAGAAC | | | | |
| PM20  | F: CTGGCGATCTTTACACATT | (CT)$_b$ | 228–233 | 6-FAM | KM013827 |
|       | R: CTTGGCATGTTTTGGAAT | | | | |
| PM21  | F: AATTGCTGACGAGCAGACGAC | (GT)$_{10}$ | 176–182 | NED | KM013828 |
|       | R: GGTGGTGGTTTTACAGGCA | | | | |
| PM22  | F: CAGAAGCGATTTTTTCCTTTC | (CT)$_b$ | 216 | PET | KM013829 |
|       | R: CCCCCACCTCTTTTCATT | | | | |

*The ideal annealing temperature for all primers is 55°C.*

http://www.bioone.org/loi/apps
TABLE 2. Results of initial primer screening and genotyping in four populations (15 individuals per population) of *Prunus maritima*.

| Locus | Rachel Carson Refuge, Biddeford, ME | Milford Point, Milford, CT | West Meadow Beach, Long Island, NY | Island Beach, Seaside Park, NJ |
|-------|-----------------------------------|-----------------------------|-----------------------------------|---------------------------------|
| PM1   | 2                                 | 0.07 0.07                   | 2                                 | 0.40 0.33                       |
| PM2   | 1                                 | 0.00 0.00                   | 1                                 | 0.00 0.00                       |
| PM3   | 2                                 | 0.60 0.43                   | 3                                 | 0.47 0.66                       |
| PM7   | 6                                 | 0.60 0.68                   | 7                                 | 0.93 0.81                       |
| PM8   | 1                                 | 0.00 0.00                   | 1                                 | 0.00 0.00                       |
| PM9   | 1                                 | 0.00 0.00                   | 1                                 | 0.00 0.00                       |
| PM11  | 7                                 | 0.93 0.82                   | 7                                 | 0.73 0.75                       |
| PM13  | 4                                 | 0.80 0.69                   | 6                                 | 0.93 0.80                       |
| PM14  | 4                                 | 0.47 0.60                   | 4                                 | 0.27 0.70                       |
| PM16  | 6                                 | 0.60 0.58                   | 4                                 | 0.60 0.56                       |
| PM18  | 6                                 | 0.73 0.78                   | 2                                 | 0.53 0.40                       |
| PM20  | 2                                 | 0.47 0.52                   | 3                                 | 0.27 0.25                       |
| PM21  | 4                                 | 0.80 0.64                   | 4                                 | 0.93 0.68                       |
| PM22  | 1                                 | 0.00 0.00                   | 1                                 | 0.00 0.00                       |
| Mean  | 3.36                              | 0.43 0.41                   | 3.29                              | 0.43 0.42                       |

Note: \( A \) = number of alleles; \( H_e \) = expected heterozygosity; \( H_o \) = observed heterozygosity.

CONCLUSIONS

The developed primers were found to successfully amplify 14 microsatellite loci in *P. maritima*. These microsatellites are reliably amplified in populations across the species range and are sufficiently variable for studying the population genetics of this species to evaluate the need for further conservation efforts and population management. In future studies, we will use these loci to assess the genetic composition of the closely related *P. maritima* var. *gravesii*. Using *P. maritima* as a model organism, these microsatellite loci are also helpful in providing a genetic context for interpreting the effects of habitat loss and fragmentation on flora within the coastal dune ecosystem.

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