Common milkweed, *Asclepias syriaca* L. (Apocynaceae), is one of the most common and widely distributed of approximately 100 North American milkweed species. *Asclepias syriaca* can be found throughout the Great Plains from southern Canada south to northeastern Oklahoma, northwestern Georgia, and Texas, and east from North Carolina to Maine. Its range continues to move south; in the last two decades specimens have been collected from Georgia and Louisiana (Wyatt et al., 1993; Wyatt, 1996), and it has become naturalized in the Western U.S. and invasive in parts of Europe. Although *A. syriaca* produces milky latex with toxic steroid glycosides, it hosts several specialist insect herbivores, including the monarch butterfly (*Danaus plexippus*), the milkweed beetle (*Tetraopes tetraophtalmus*), large milkweed bug (*Oncopeltus fasciatus*), small milkweed bug (*Lygaeus kalmii*), and milkweed leaf beetle (*Labidomera clivicollis*). Its broad geographic range and specialized ecological interactions makes *A. syriaca* an ideal species with which to examine geographic patterns of coevolution.

*Asclepias syriaca* reproduces both asexually and sexually. It is self-incompatible (Morse and Fritz, 1983), and, during sexual reproduction, pollen grains are packaged in discrete units called pollinia, which contain enough pollen to ensure full seed-set of a single flower (Ivey et al., 2003). The wind-dispersed seeds are attached to long, white fleshy hairs and encased in large follicles. Asexual reproduction occurs by the elaboration of underground rhizomes. Accordingly, sexual reproduction gives rise to new genets, whereas asexual reproduction can produce multiple ramets per genet. Assigning ramets to genets is challenging under field conditions, and microsatellite markers have been used to differentiate among genets in a variety of systems [e.g., quaking aspen (*Populus tremuloides*) (Namroud et al., 2005)]. Using molecular markers to distinguish among genets of *A. syriaca* would facilitate studies of its ecology and evolutionary biology (Helms et al., 2004; Van Zandt and Agrawal, 2004). Previous microsatellite markers have been isolated from *A. syriaca* (O’Quinn and Fishbein, 2009), and here we describe additional markers that will increase resolution of clonality and genetic structure in natural populations.

**METHODS AND RESULTS**

DNA was extracted (DNeasy Plant Kit, Qiagen, Valencia, CA) from one *A. syriaca* ramet collected at the University of Michigan Biological Station (UMBS), Pellston, Michigan (45°33’S, 84°40’W). DNA was enriched twice for simple sequence repeats using the Oligomix 2 mixture of repeat units [(AG)\(_{12}\), (TG)\(_{12}\), (AAC)\(_{6}\), (AAG)\(_{8}\), (AAT)\(_{12}\), (ACT)\(_{12}\), (ATC)\(_{8}\)] and protocol of Glenn and Schable (2005). Polymerase chain reaction (PCR) products were ligated to a plasmid vector using the TOPO TA Cloning Kit (Invitrogen Corporation, Carlsbad, CA). Plasmid inserts were amplified and sequenced (BI Model 3730 Sequencer). Thirty-four of the 105 sequenced clones (32%) contained microsatellites. Primers were designed using the software OligoCalc (Köhbe, 2007). Polymorphism was screened in 30 *A. syriaca* genets from the University of Michigan Biological Station for 12 loci.

PCR was carried out in a volume of 10 µL containing ~30 ng of template DNA, 2 µL 10× PCR buffer, 1.5 mM MgCl\(_2\), 0.2 mM of each dNTP, 1 U Taq polymerase and 0.2 µM of each primer. Hot Start Taq Polymerase (Qiagen, Valencia, CA) was used for loci AS94 and ASF2, whereas GoTaq (Promega Corporation, Madison, WI) was used for all other loci. PCR for ASC5 and AGS5 included 25 µg/mL of BSA. The thermal cycle began with a 4-min denaturation step at 94°C, followed by 45 cycles of 30 s at 94°C, 30 s at 52°C, and 60 s at 72°C, and a final extension at 72°C for 10 min. A 15-min denaturation step was used for loci AS94 and ASF2.

**Key words:** Apocynaceae, *Asclepias syriaca*, microsatellite, milkweed.

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Eight of the 12 loci were found to be polymorphic and generated consistent and easily scored amplification products of the expected size range (Table 1). Amplified products were genotyped on an ABI 3730 Sequencer and analyzed using GeneMarker v 1.8 (SoftGenetics LLC, State College, PA).

The loci contained 3 to 13 alleles in a sample of 30 individuals from the UMBS, with observed heterozygosity ranging from 0.33 to 0.83 (Table 2). Three loci (ASG6, ASB5, ASG5) showed significant excess of heterozygotes. Significant LD was detected between loci ASF9 and ASH8. Probability of identity (PI) using all loci showed a significant excess of homozygotes. Significant LD and deviations from Hardy-Weinberg (HW) were assessed in the UMBS and other natural populations of A. syriaca. These markers will also be useful in assessing population genetic structure and gene flow at local and regional spatial scales.

**CONCLUSIONS**

The excesses in heterozygosity in three loci may reflect biological properties particular to the UMBS population, such as the level of clonality, while the excess homozygosity in two loci may be caused by null alleles. Even though A. syriaca can reproduce asexually, there is no indication that the samples used in this study came from repeated genets. However due to the relative isolation from other populations of A. syriaca, it is possible that inbreeding could cause some of the homozygote excess. Inbreeding would be expected to affect all loci equally, however, yielding homozygote excess at all loci, not just two of five examined. The high probability of identity indicates that these markers will provide clear resolution of genet and ramet structure in the UMBS and other natural populations of A. syriaca. These markers will also be useful in assessing population genetic structure and gene flow at local and regional spatial scales.

**LITERATURE CITED**


