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MICROSATELLITE LETTERS

# Cross amplification of 15 EST-SSR markers in the genus *Fraxinus*

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**Abstract** Ash (*Fraxinus*, Oleaceae) species occur on most continents, within a wide range of forest tree communities. Emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), introduced into the U.S. from Asia in the late twentieth century, has caused widespread mortality, primarily in green ash, *Fraxinus pennsylvanica* Marsh. (Section: Melioides) and now impacts other North American ash species. The development and successful reintroduction of resistant trees requires genetic tools to evaluate population dynamics and aid in species identification. Here, we report 15 novel EST-SSR markers developed in green ash, most of which amplify and are polymorphic across 18 species of *Fraxinus*, including six species native to North America. The high average polymorphism information content (0.741) and allelic richness (15.3) revealed in six disparate populations of green ash indicate that these markers also have utility for investigating population dynamics in this species.

**Keywords** *Fraxinus pennsylvanica* · EST-SSR · Heterologous markers · Green ash

The genus *Fraxinus* (ash), native in Asia, Africa, Europe, and the Americas, exhibits considerable variability in morphology, ploidy, and biogeography, both within and between species. Ash provides food and shelter for an array of arthropod, mammalian, and bird life and contributes to forest ecosystem services and canopy structure. Traditionally valued for furniture, tools, and weapons, ash is now favored in many cities as a street tree due to uniform canopy and attractive fall coloring (Kovacs et al. 2010).

Emerald ash borer (EAB), *Agrilus planipennis* Fairmarie (Coleoptera: Buprestidae) poses an acute threat to all 16 species of *Fraxinus* in North American forests. Native to Asia, EAB has killed more than 150 million ash trees since its initial introduction near Detroit in the 1990s and is now found in 20 states and two Canadian provinces (Liang and Fei 2014). EAB larvae generate long serpentine galleries that kill the tree once the cambium is girdled. The fungus *Hymenoscyphus pseudoalbidus*, discovered in the 1990s in Poland, causes ash dieback, resulting in severe mortality in European ash, *F. excelsior* L. (section Fraxinus). The disease has spread quickly throughout Europe (McKinney et al. 2012). In this study, we identify 15 highly heterologous EST-SSR markers that can used in breeding and

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conservation genetics programs targeted to these and other threats.

We collected an average of 29.6 individuals from each of six natural populations of green ash located in Ohio, Quebec, and Nova Scotia and from the *Fraxinus* species collection at Ohio State (18 additional *Fraxinus* species, 1–19 per species). We isolated DNA from leaves using a modified CTAB protocol (Hoban et al. 2010). We tested 211 primer pairs from novel green ash EST-SSR sequences for amplifiability, specificity, and polymorphism. PCR reactions contained 20 ng of template DNA, 20 pM of each forward and reverse primers, 15 mM $MgCl_2$, 2.5 mM dNTP, 1 μL 10× PCR Buffer, 0.5 U Taq polymerase, and double distilled $H_2O$ to a total volume of 10 μL. Our screening PCR protocol included denaturation at 94 °C for 2 min, 35 cycles (94 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s), followed by 60 °C for 30 min and a final 10 min extension at 72 °C. We optimized annealing temperatures via gradient PCR for selected primer pairs.

We sized fluorescently labeled amplicons with an ABI 3730xl (Applied Biosystems, Foster City, CA) and scored genotypes with GeneMapper version 4.1 (Applied Biosystems). For the six natural populations, we utilized GENEPOPONTHEWEB (Raymond and Rousset 1995) to calculate observed and expected heterozygosity under Hardy–Weinberg expectation and check for evidence of null alleles.

Of the primer pairs producing an amplicon, 21 generated multiple bands, 42 were monomorphic, and 13 did not reliably amplify across populations. Allelic richness in the remaining 15 markers ranged from 6 to 28 across 187 green ash individuals in six populations (Online Resource 1). All 15 markers had observed heterozygosity values consistent with random mating in at least three of the six populations and no population had more than four markers that deviated from this expectation. We did not find consistent evidence for null alleles for any of the 15 markers.

All 15 markers amplified and were polymorphic in four of five species in section Melioides while 10 of the 15 markers amplified in sections Fraxinus and Ornus, indicating that these EST-SSR perform well as heterologous markers within and among these three *Fraxinus* sections (Online Resource 2). We are continuing to evaluate these and other EST-SSR for parentage analysis studies and for the construction of a genetic map for green ash.

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