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INVASION NOTE

Genetic diversity in the invasive *Rubus phoenicolasius* as compared to the native *Rubus argutus* using inter-simple sequence repeat (ISSR) markers

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Abstract Invasive species are one of most significant factors in human-influenced global change. Management actions that prevent the spread and impacts of invasive species require knowledge of their ecological and genetic characteristics. The genetic characteristics of the invasive wine raspberry, *Rubus phoenicolasius* Maxim. (Rosaceae) and the native sawtooth blackberry, *Rubus argutus* Link, were examined in two forest habitats on the Maryland Coastal Plain. Using inter-simple sequence repeat (ISSR) markers we quantified the genetic diversity of both species. We analyzed genetic diversity using analysis of molecular variance (AMOVA) and found less genetic diversity in the invasive species, *R. phoenicolasius,* with variation between sites was 0.418 between sites and 0.075 within sites as compared to the native, *R. argutus,* where the variation between sites was 1.538 and 0.370 within sites. The lower genetic diversity in the invasive may be due to a history of limited introductions or frequent self-fertilization and clonal reproduction.

Keywords *Rubus phoenicolasius* · *Rubus argutus* · Invasive · ISSR · Genetic diversity

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Introduction

Determining what characters make an exotic species a successful invader has proven difficult. The characteristics of weeds that Baker put forth in 1974 are still being examined to determine if they pertain to invasive species (Baker 1974; Parker 1997; Rambuda and Johnson 2004; Sutherland 2004; Hawkes 2007; van Kleunen et al. 2010). One approach to studying the characteristics of invasive species is to compare the relative performance of invasive species with that of closely related congeners (Parker 2000; Radford and Cousens 2000). This approach has the advantage of minimizing phylogenetic differences and allowing a closer examination of the basis of an invasive species' success in new communities (Sakai et al. 2001). We looked at the trait of genetic diversity in two *Rubus* congeners, the native sawtooth blackberry (*R. argutus*) and the invasive wine raspberry (*R. phoenicolasius*).

Methods

Research organisms: *Rubus argutus* is native to the United States ranging from Massachusetts to Florida and west to Missouri. *Rubus phoenicolasius* is native to Japan, Korea and China, and its US introduced range is similar to *R. argutus*. *R. phoenicolasius* was introduced in the 1890s possibly through John Lewis Childs, who ran a mail order seed company in Floral

Park, New York (Hummer 1995). *R. phoenicolasius* is listed by The United States Department of Agriculture, The National Park Service, National Biographical Information Infrastructure, The Nature Conservancy and The Maryland Department of Natural Resources as an invasive species. Both *Rubus* species are found in old fields and early to mid-successional forests.

Rubus phoenicolasius and *R. argutus* have similar life histories, producing biennial above ground shoots, hereafter called canes, from a perennial rootstalk or from underground rhizomes. The first year cane, primocane, is vegetative while the second year cane, floricane, undergoes lateral branching and produces flowers and fruit. Both species produce aggregate fruits, which ripen together (Ellis et al. 1997). Both species are able to reproduce clonally through underground rhizomes, but only *R. phoenicolasius* is capable of tip rooting from parts of the cane that touch the ground.

Study site: The study was conducted at the Smithsonian Environmental Research Center (SERC), Edgewater, MD, USA (~10 km SSE of Annapolis, 38°53′ N, 76°33′ W). The 1,000 hectares that are part of the SERC property include agricultural fields, abandoned fields, successional and mature forests that are typical of the region (Brush et al. 1980). Two non-contiguous forests were used. Three 1 m^2 plots were established in each forest for each species, for a total of six 1 m^2 plots per species. In each 1 m^2 plot, leaf samples were collected from five first year individuals. An individual was defined as a group of canes from one rootstock. In total we sampled 30 individuals of *R. phoenicolasius* and 30 individuals of *R. argutus.* After sampling, leaves were wrapped in wet paper towels and kept at 4°C. Within 48 h, DNA was extracted from a 1 cm^2 subsample of each leaf using the protocol from the DNeasy Plant Mini Kit.

We used inter-simple sequence repeat (ISSR) markers for our genetic analysis. For ISSR DNA amplification, the DNA elution was diluted to 1:10 with sterile, distilled H_2O. Each PCR tube had 2.5 µl of diluted DNA sample, 8.75 µl H_2O, 1.25 µl ISSR Primer, 1.25 µl *taq* RedMix™ Plus 12.0 Master Mix (GeneChoice Inc. PGC Scientific Corp.). The ISSR PCR cycle was 2 min at 96°C, 1 min at 94°C, 1 min at 44°C, 2 min at 72°C for 35 cycles and finished at 0°C (Smith et al. 2002).

Results

We screened 79 ISSR primers (University of British Columbia from UBC primer set #9) for *R. phoenicolasius* and found 4 to be polymorphic. For *R. argutus* we screened only 38 primers to find 4 polymorphic primers (Table 1). We screened more primers for *R. phoenicolasius* as there were fewer polymorphisms in *R. phoenicolasius*. For *R. argutus* we found four primers, three of which were polymorphic for at least 13 bands. But for *R. phoenicolasius* the four primers were less polymorphic and three of them were polymorphic for only 2 bands. We took subsamples of PCR product and ran them on a polyacrilimide gel to determine whether variation was masked using the lower-resolution agarose gel. We found no masked variation. To analyze genetic diversity, we used Analysis of Molecular Variance (AMOVA) using genetic analysis in excel (GenAlEx V5.1) (Peakall and Smouse 2001; Peakall et al. 2003). For *R. argutus* the variation between sites was 1.538 and within sites was 0.370. For *R. phoenicolasius* variation between sites was 0.418 between sites and 0.075 within sites. Combined with the greater number of primers required to find polymorphism in *R. phoenicolasius*, the successful primers for *R. phoenicolasius* having fewer polymorphisms, and the reduced variance as indicated by AMOVA combine to indicate that less genetic variation was present in *R. phoenicolasius* than in *R. argutus*.

Discussion

Invasive plants with a history of multiple introductions show high levels of genetic diversity (Genton et al. 2005; Ward et al. 2008). Low genetic diversity in the invasive *R. phoenicolasius* may be attributed to fewer introductions into their invaded habitat; this was shown for the invasive biennial garlic mustard, *Alliaria petiolata* (Meekins et al. 2001). In addition, many invasive species (including *R. phoenicolasius*) are clonal and/or self-compatible (Liu et al. 2006), which could also lead to a decrease in variation (Amsellem et al. 2000). The lack of genetic diversity in clonal invasives was demonstrated using ISSRs and there is the possibility that characteristics other than genetic diversity facilitate the invasive success (Ye et al. 2004; Poulin et al. 2005; Sun et al. 2005; Li

Table 1 Summary of primers used for *R. argutus* (*R. a.*) and *R. phoenicolasius* (*R. p.*), including notation if variable (y = yes) and number of polymorphs found per primer

Primer number	Primer	Variable for *R. a.*	Polymorphisms in *R. a.*	Variable for *R. p.*	Polymorphisms in *R. p.*
807	AGA GAG AGA GAG AGA GT			Y	2
825	ACA CAC ACA CAC ACA CT	Y	2	Y	2
827	ACA CAC ACA CAC ACA CG			Y	1
834	AGA GAG AGA GAG AGA GXT	Y	17		
856	ACA CAC ACA CAC ACA CXA	Y	13		
864	ATG ATG ATG ATG ATG ATG			Y	2
881	GGG TGG GGT GGG GTG	Y	15		

In primer sequences (X = C,T). Primers from University of British Columbia from UBC primer set #9

et al. 2006; Gutierrez-Ozunaa et al. 2009). Most likely *R. phoenicolasius* has had a history of few introductions in the study sites. In addition, possibly through more frequent clonal growth and self-fertilization, *R. phoenicolasius* displays less genetic diversity than the native *R. argutus*.

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