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Using the nuclear *LEAFY* gene to reconstruct phylogenetic relationships among invasive knotweed (*Reynoutria*, Polygonaceae) populations

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Research Article

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Abstract

Knotweed species in the genus *Reynoutria* are native to eastern Asia but have become noxious weeds in Europe and North America. In the United States, invasive populations of Japanese knotweed (*Reynoutria japonica* Houtt.), giant knotweed [*Reynoutria sachalinensis* (F. Schmidt) Nakai], and their interspecific hybrid known as Bohemian knotweed (*R.* × *bohemica* Chrtek & Chrtková) continue to expand their ranges. Although these plants are among the most invasive terrestrial species, there are relatively few molecular tools for identifying the parental species, the F_1 hybrid, or subsequent hybrids or introgressed individuals. We studied *Reynoutria* populations in Wisconsin, a state where all three taxa grow, to determine whether molecular data would be useful for distinguishing species and identifying hybrids. We obtained DNA sequence data from the plastid *matK* gene and the nuclear *LEAFY* gene and compared these to previously published sequences. Data from the uniparentally inherited *matK* region included haplotypes attributable to *R. japonica* and *R. sachalinensis*. Nuclear data indicated that *R. sachalinensis* plants are most similar to native plants in Japan, with two Wisconsin accessions exhibiting a monomorphic genotype for the *LEAFY* gene. Three Wisconsin accessions of *R. japonica* were each characterized by having three distinct kinds of *LEAFY* sequence. Most plants in our study were found to possess two or three phylogenetically distinct copies of the *LEAFY* gene, with the copies being most closely related to *R. japonica* and *R. sachalinensis*, respectively, and these were inferred to be interspecific hybrids. Altogether, five kinds of interspecific hybrids were identified, reflecting various combinations of *LEAFY* sequence types from the parental species. The widespread existence of hybrid plants in Wisconsin, many of which are morphologically identifiable as *R. japonica*, indicates a cryptic genetic diversity that should be examined more broadly in North America using molecular tools.

Introduction

Knotweeds in the genus *Reynoutria*, native to eastern Asia, are noxious weeds in Europe and North America, where they continue to spread rapidly (Bailey 2003; Bailey and Wisskirchen 2006; Bailey et al. 2009; Freeman and Hinds 2005; Gaskin et al. 2014; Grimsby et al. 2007). Invasive *Reynoutria* plants are notorious for impacting native vegetation through allelopathy (Murrell et al. 2011; Parepa et al. 2012; Vrchotová and Šerá 2008) and for propagating readily through both sexual and asexual means (Bailey et al. 2009; Brock et al. 1995; Forman and Kesseli 2003; Hollingsworth and Bailey 2000; Pyšek et al. 2003). Molecular evidence strongly supports the phylogenetic independence of *Reynoutria* from related genera (Galasso et al. 2009; Sanchez et al. 2009; Schuster et al. 2011), thus we use nomenclature that regards *Reynoutria* as a distinct genus in the Polygonaceae. There are only five *Reynoutria* taxa, most with native geographic ranges that are rather narrow (Bailey 2003; Steward 1930). Three taxa have established as invasive plants in Europe and North America: Japanese knotweed (*Reynoutria japonica* Houtt.; syn. *Polygonum cuspidatum* Siebold & Zucc.) from China, Japan, Korea, and Russia; giant knotweed [*Reynoutria sachalinensis* (F. Schmidt) Nakai; syn. *Polygonum sachalinense* F. Schmidt ex Maxim.], native to Japan, Korea, and Sakhalin Island (Russia); and Bohemian knotweed (*R.* × *bohemica* Chrtek & Chrtková; syn. *Polygonum* × *bohemicum* (Chrtek & Chrtková) Zika and Jacobson). The last is a hybrid between *R. japonica* and *R. sachalinensis*, comprising natural hybrids in Japan and invasive populations in Europe and North America (Bailey 2003; Mandák et al. 2003). The invasive *Reynoutria* taxa are monophyletic and distinct from the other two species in the genus: *Reynoutria ciliinervis* (Nakai) Moldenke [syn. *Polygonum ciliinerve* (Nakai) Ohwi] and *Reynoutria multiflora* (Thunb.) Moldenke (syn. *Polygonum multiflorum* Thunb.) (Park et al. 2018).

Management Implications

Reynoutria (knotweeds) are targeted for herbicide treatment and mechanical control, and researchers are actively exploring biocontrol solutions to their rapid spread in Europe and North America. In published studies, *Reynoutria japonica* (syn. *Polygonum cuspidatum* Siebold & Zucc.; Japanese knotweed) has received the most attention, but *Reynoutria* invasions also include *Reynoutria sachalinensis* (syn. *Polygonum sachalinense* F. Schmidt ex Maxim.; giant knotweed) and a variety of interspecific hybrids. Management approaches can achieve differential success depending on the identity and genetic makeup of the species being targeted. It will be important to evaluate the effectiveness of treatment approaches at least by species, preferably taking into account the genetic makeup of hybrids, where applicable. Our study confirms that *Reynoutria* species and hybrids are genetically diverse in North America, and we offer a novel approach for identifying them in a meaningful way. We also demonstrate that morphological characters may be less useful in places where hybrid *Reynoutria* are diverse. Land managers who are interested in evaluating the effectiveness of their control strategies against the genetic identities of noxious *Reynoutria* plants may benefit from having their target material sequenced for the plastid *matK* gene and the nuclear *LEAFY* second intron. Our study was conducted on plants in Wisconsin, but one could reasonably use the same approach to identify *Reynoutria* plants across the native and introduced ranges.

Both *R. japonica* and *R. sachalinensis* are widespread in North America, as are F_1 hybrids (i.e., *R.* × *bohemica*) and later-generation hybrids or introgressed individuals (Bailey 2013; Forman and Kesseli 2003; Gaskin et al. 2014; Grimsby et al. 2007). Although the invasive taxa are closely related and have similar ecologies and morphologies, they may respond differently to management applications (e.g., Bímová et al. 2004; Clements et al. 2016; Fung et al. 2020). Additionally, hybridization and introgression may produce novel genetic combinations that present further management challenges (Bailey et al. 2007; Clements et al. 2016; Mitchell et al. 2019; Parepa et al. 2014). To implement effective management plans, it will be important to identify species and hybrids correctly. However, it can be difficult to know in the field whether individuals are hybrids or pure parental species because of their morphological similarity and ability to interbreed. Molecular tools can offer a valuable and independent source of characters for identification, and several strategies have been developed. Sequencing or restriction fragment analyses of plastid genes can produce data reliably; however, the uniparentally inherited plastid data can offer evidence of only one parental lineage per plant. A more thorough summary of genetic variation and hybrid parentage can be obtained using data from nuclear gene regions, which are biparentally inherited, may exist as allelic variants, and may be used to reconstruct polyploidization events (Small et al. 2004).

Phylogenetic studies of *Reynoutria* in the introduced range are relatively sparse, and many have relied upon plastid sequence data that cannot distinguish parental species from hybrids. In light of the fact that two species and their hybrid coexist throughout most of the introduced range, it is important to identify taxonomic entities correctly. Nuclear DNA sequence data are surprisingly rare for *Reynoutria* species and comprise few sequences of the internal transcribed spacer (ITS) region and *LEAFY* second intron, compared with the relative abundance of plastid sequence data (Gammon and Kesseli 2010; Grimsby and Kesseli 2010; Hollingsworth et al. 1999). Park et al. (2018) conducted an extensive study of genetic variation in native *Reynoutria* populations over much of Japan and Korea, as well as Sakhalin Island and mainland Russia, and their study also included introduced plants in the United Kingdom and United States. Combined plastid and nuclear data largely supported the reciprocal monophyly of *R. japonica* and *R. sachalinensis*, with the exception of plants collected from Korean islands, which resolved among *R. sachalinensis* on the *LEAFY* tree but within the clade of *R. japonica* on the plastid tree (Park et al. 2018). Additionally, a small number of samples were determined to have polymorphic *LEAFY* sequences, some reflecting a combination of *R. japonica* and *R. sachalinensis* genetic elements. Molecular data also supported the synonymy of *Fallopia forbesii* (Hance) Yonek. & H. Ohashi with *R. japonica*.

Hybridization and introgression are well documented among invasive *Reynoutria* taxa, and in North America there is a rich diversity of genotypes and morphological variation (Gaskin et al. 2014; Grimsby and Kesseli 2010; Grimsby et al. 2007). Nuclear genetic variation has been examined using a variety of methods that analyze sequence length polymorphisms (Bzdega et al. 2016; Gammon et al. 2007; Gaskin et al. 2014; Grimsby and Kesseli 2010; Grimsby et al. 2007; Hollingsworth et al. 1998), but for many applications these techniques may be prohibitively expensive or laborious. Moreover, the clustering and principal components analyses that are used to summarize sequence length polymorphism data are not phylogenetic and cannot reconstruct genetic inheritance with the same level of confidence as a phylogenetic analysis of nucleotide sequence data.

North American *Reynoutria* species are overdue for a phylogenetic analysis that combines plastid and nuclear sequence data and potentially reconstructs the parentage of hybrids. To this end, we investigated *Reynoutria* plants in Wisconsin, USA, using plastid *matK* and nuclear *LEAFY* sequence data to ascertain whether they represent parental species or hybrids and to infer the most closely related plants among those that have been sampled in eastern Asia. *Reynoutria* plants introduced to the East Coast continue to expand their range westward, and the surge of invasive *Reynoutria* in Wisconsin is relatively recent (Barney 2006; EDDMapS 2020). Both *R. japonica* and *R. sachalinensis* are reported from Wisconsin (Figure 1), as well as *R.* × *bohemica* and numerous plants that are difficult to identify, possibly as a result of mixed parentage (i.e., hybridization or introgression). The prospect of identifying *Reynoutria* species using a small number of nucleotide sequences holds the potential to make genetic identification more accessible to land managers (i.e., by decreasing cost and technical difficulty) and to increase the number of plants that are identified in this way, thus improving our understanding of the ecology and geographic distribution of these troublesome plants.

Materials and Methods

Reynoutria samples were collected from 37 localities across 14 Wisconsin counties (Table 1; Figure 1). Voucher specimens were deposited in the University of Wisconsin–Whitewater herbarium (UWW). Plants were identified initially using two morphological characters that were previously reported to be diagnostic (Bailey et al. 2009; Zika and Jacobson 2003). Leaf laminar bases were scored as truncate (*R. japonica*), with lobes >2 cm long (*R. sachalinensis*), or with lobes <2 cm long (*R.* × *bohemica*). Hairs on the abaxial leaf veins were scored as multicellular (*R. sachalinensis*),

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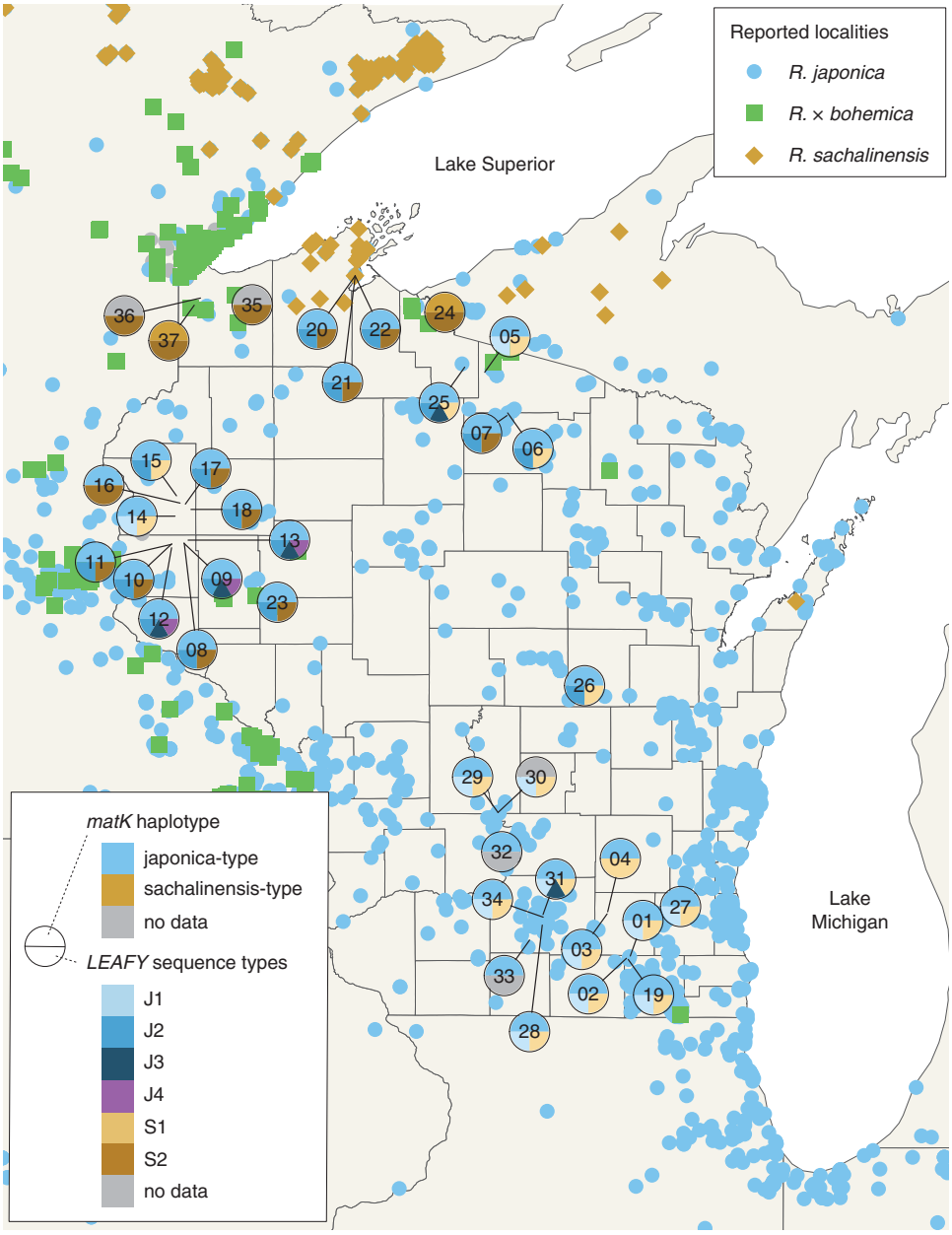

Figure 1. Sampling localities for *Reynoutria* in Wisconsin. Locality numbers are referenced in Table 1. Underlying shapes depict reported localities for *Reynoutria* species (EDDMapS 2020). Pie charts show genetic contributions from the plastid *matK* gene (top half of pie) and the nuclear *LEAFY* gene (bottom half of pie); plants with different *LEAFY* sequence types have different colors in the bottom half.

stout and unicellular (*R.* × *bohemica*), or reduced to rounded bumps, that is, scabrous (*R. japonica*). Secondary morphological characters included the laminar apex (scored as acuminate or acute) and inflorescence length relative to leaf length.

We also recorded the reproductive condition of plants, where possible. Authors variously have described *Reynoutria* plants as gynodioecious (Bailey 1994; Barney et al. 2006; Beerling et al. 1994; Freeman and Hinds 2005; Hollingsworth et al. 1998), subdioecious (Forman and Kesseli 2003; Grimsby et al. 2007), functionally dioecious (Kim and Park 2000), or dioecious (Decraene and Akeroyd 1988; Li and Park 2003). Although the technical definition of the reproductive system in *Reynoutria* may not be universally agreed upon (or consistent across native and adventive ranges), there is general consensus that individuals are either primarily staminate or fully pistillate. We recorded the reproductive system of collected plants as "male-fertile" if we observed stamens on flowers and as "male-sterile" if no stamens could be located (Bailey et al. 1996). By using these categories, we acknowledge that male-fertile plants may have functional gynoecia but potentially at low frequencies that escape detection. Male-fertile plants also were characterized by extensive post-anthetic flower abscission, although it should be noted that flower abscission also has been implicated in seed dispersal on male-sterile plants (Beerling et al. 1994).

Genomic DNA was extracted from dried or fresh leaf material using a standard CTAB protocol (Doyle and Doyle 1987), modified as described previously (Tippery et al. 2020). We used primers that other researchers had designed for the plastid *matK* protein-coding region (AF and 8R; Yan et al. 2008) and the second intron of the nuclear *LEAFY* gene (MLFYI2-1543F and MLFYI2-2385R;

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Table 1. Collection localities and morphological features of *Reynoutria* specimens used in this study.

ID	Latitude °N	Longitude °W	Laminar base	Abaxial vein hairs	Laminar apex	Inflorescence relative to leaf	Reproductive condition
R01	42.840252	88.732346	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R02	42.827315	88.762639	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R03	43.077214	88.914878	Truncate	Stout and unicellular	Acuminate	No data	Not observed
R04	43.089105	88.910258	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R05	46.1263	89.8795	Cordate <2 cm	Stout and unicellular	Acuminate	Shorter	Male-sterile
R06	45.8974	89.6989	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R07	45.8825	89.7041	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R08	45.16444	92.24758	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R09	45.16429	92.2507	Truncate	Scabrous	Acuminate	Shorter	Male-sterile
R10	45.16047	92.34542	Truncate	Stout and unicellular	Acuminate	Shorter	Male-fertile
R11	45.15784	92.34735	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R12	45.13858	92.34751	Truncate	Scabrous	Acuminate	Shorter	Male-sterile
R13	45.18125	92.21728	Truncate	Scabrous	Acuminate	Shorter	Male-sterile
R14	45.31609	92.32021	Truncate	Stout and unicellular	Acuminate	Shorter	Male-fertile
R15	45.43074	92.30874	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R16	45.38996	92.28060	Truncate	Stout and unicellular	Acuminate	Shorter	Male-fertile
R17	45.38432	92.23868	Cordate <2 cm	Scabrous	Acuminate	Shorter	Male-fertile
R18	45.35425	92.19483	Cordate <2 cm	Scabrous	Acuminate	Shorter	Male-fertile
R19	42.82099	88.74862	Cordate <2 cm	Scabrous	Acuminate	Shorter	Male-fertile
R20	46.66917	90.90000	Cordate <2 cm	Scabrous	Acuminate	Shorter	Male-fertile
R21	46.66972	90.90028	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R22	46.66972	90.90083	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R23	44.83191	91.51343	Cordate <2 cm	Stout and unicellular	Acuminate	No data	Not observed
R24	46.46387	90.19359	Cordate <2 cm	Scabrous	Acuminate	Shorter	Male-fertile
R25	46.15494	90.03720	Cordate <2 cm	Scabrous	Acuminate	No data	Not observed
R26	44.36191	89.09252	Truncate	Stout and unicellular	Acuminate	Shorter	Male-sterile
R27	43.11875	88.33576	Truncate	Stout and unicellular	Acuminate	Shorter	Male-fertile
R28	43.01445	89.42587	Truncate	Stout and unicellular	Acuminate	Shorter	Male-fertile
R29	43.64815	89.77477	Truncate	Stout and unicellular	Acuminate	Shorter	Male-fertile
R30	43.64815	89.77477	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R31	43.0739	89.4198	Truncate	Stout and unicellular	Acuminate	Shorter	Male-fertile
R32	43.42704	89.74496	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R33	42.93011	89.52551	Cordate <2 cm	Scabrous	Acute	Shorter	Male-fertile
R34	43.05899	89.41850	Truncate	Stout and unicellular	Acuminate	No data	Not observed
R35	46.50590	91.71190	Truncate	Scabrous	Acuminate	Shorter	Male-fertile
R36	46.54421	92.11927	Cordate <2 cm	Scabrous	Acuminate	Shorter	Male-fertile
R37	46.50377	92.16879	Cordate >2 cm	Long and multicellular	Acuminate	No data	Not observed

Schuster et al. 2011). We elected to target the *matK* and *LEAFY* genes so that our data could be evaluated in a phylogenetic context against the extensive data that were reported for plants in the native range (Park et al. 2018). After recovering polymorphic *LEAFY* sequences for several accessions, we designed two forward primers that would differentially amplify the two kinds of sequence: Japo1F (5′-GGTAAAGTGAGAGACGTATAAATG-3′) and Sach1F (5′-AGCTAGTTAGCTAGGTAGCTAC-3′), named for the similarity of amplicon sequences to reported sequences of *R. japonica* and *R. sachalinensis*, respectively (Park et al. 2018).

The polymerase chain reaction (PCR) was conducted in 10-µl reactions each containing 2 µl of 5× reaction buffer, 0.8 µl of 2.5 mM dNTPs, 0.5 µl of each 10 µM primer, and 0.2 µl of Phire™ Hot Start II DNA Polymerase (Thermo Fisher, Waltham, MA, USA). The thermal cycler protocol consisted of an initial denaturation at 98 C for 30 s, followed by 35 cycles of 90 C for 5 s, 52 C (*matK* primers) or 58 C (*LEAFY* primers) for 5 s, and 72 C for 20 s, ending with a final extension at 72 C for 60 s. Sanger sequencing was conducted remotely (Eurofins Genomics, Louisville, KY, USA) and employed the same primers used for PCR.

Sequence files were curated in 4Peaks v. 1.8 (Griekspoor and Groothuis 2006) and exported as text files. Any samples that produced polymorphic *LEAFY* sequences with the newly designed primers were subcloned into bacteria using the pGEM®-T Easy Vector System (Promega Corporation, Madison, WI, USA), then subjected to PCR and sequencing as described earlier. Outgroup sequences were obtained from GenBank; sequences of *R. multiflora* (EU024768, HM357918, KJ863098, KJ863100) were used to root the *matK* tree (Park et al. 2018; Yu et al. 2008), and the *LEAFY* tree was rooted with sequences from several *Muehlenbeckia* species (JF831223–JF831230, JF831348–JF831355; Schuster et al. 2011). Newly generated and outgroup sequences were combined with *Reynoutria* sequences obtained from GenBank and previously published *Reynoutria* sequences that are not yet deposited in GenBank (Park et al. 2018), then all sequences for each gene region were aligned manually in Mesquite v. 3.61 (Maddison and Maddison 2019). Insertions and deletions (indels) were encoded using simple indel coding (Simmons and Ochoterena 2000), implemented in the program SeqState v. 1.4.1 (Müller 2005).

Separate phylogenetic analyses were run for the plastid and nuclear data matrices, using maximum-likelihood criteria in IQ-TREE v. 2.0.5 (Nguyen et al. 2015) with separate partitions (Chernomor et al. 2016) for nucleotide and binary indel data. ModelFinder (Kalyaanamoorthy et al. 2017) selected the general time reversible (GTR) model for *LEAFY* and the transversion model (TVM) for *matK*, both with empirical frequencies and two rate categories. The indel data were analyzed using the GTR model for binary data with empirical frequencies, two rate categories, and ascertainment bias correction (Lewis 2001).

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Nodal support values for the maximum-likelihood analysis were obtained by running 1,000 bootstrap replicates. Bayesian phylogenetic analyses were conducted in MrBayes v. 3.2.7 (Ronquist et al. 2012) using the same models as for nucleotide data and the "standard" model for indel data. Nodal support values were calculated by summarizing the posterior distribution after running 2 million generations and discarding the first 25% of generations as burn-in.

Results and Discussion

On the basis of morphological characters, only one accession (R37) was unambiguously identified as *R. sachalinensis*, because it had leaves >20 cm long with distinct basal laminar lobes and multicellular abaxial vein hairs (Table 1). In all other accessions, the vein hairs were either stout and unicellular or lacking, as have been described for *R.* × *bohemica* and *R. japonica*, respectively (Zika and Jacobson 2003). Laminar apices were almost all acuminate, and laminar bases were predominantly truncate. On the basis of these characters, 16 specimens would be identified as *R. japonica* (i.e., with truncate laminar bases and scabrous abaxial veins), and 2 specimens (R05, R23) would be identified as *R.* × *bohemica* (i.e., with shallowly cordate laminar bases and stout, unicellular hairs). Another 18 specimens exhibited combinations of laminar base and abaxial vein morphologies that were inconsistent with published descriptions (Zika and Jacobson 2003). Flowering material was observed on most specimens, the majority of which were scored as male-fertile. Inflorescence length was consistently shorter than leaf length for all flowering specimens, although the available material was collected from apical stem regions, and these regions may not have the same leaf and inflorescence morphologies as midstem regions (Barney et al. 2006).

Plastid (*matK*) sequence data were obtained for 34 accessions, and nuclear (*LEAFY*) sequence data for 35 accessions. Newly generated sequences were deposited in GenBank under accession numbers MW770182–MW770215 (*matK*) and MW771206–MW771273 (*LEAFY*) (Table 1). PCR amplifications that used specific *LEAFY* primers produced products for 32 accessions with the Sach1F primer, and for 29 accessions with the Japo1F primer. Three accessions (R09, R12, R13) produced PCR products only for the Japo1F primer, and six accessions (R04, R16, R24, R35, R36, R37) produced products only for the Sach1F primer. The *matK* data matrix comprised 78 sequences and 1,602 nucleotide characters, with 68 (4.2%) of these being variable among accessions, and one indel character. The *LEAFY* data matrix included 126 sequences and 1,667 characters (256, or 15.4% variable), and 96 indels were scored. The phylogeny for *matK* (Figure 2) clearly differentiated two haplotypes for the specimens in our study. Most plants had the "*japonica*-type" *matK* sequence, which had been reported previously for plants in Canada and the United States, as well as native *R. japonica* plants in Japan and Russia. Plants with the "*sachalinensis*-type" *matK* sequence were similar to native *R. sachalinensis* plants from Japan and one accession from the adventive range in the United Kingdom (EF438009).

The *LEAFY* phylogeny (Figure 3) resolved six clades of sequence types in Wisconsin plants. Four of these, identified as J1 through J4, resolved within a larger clade of sequences from *R. japonica* (i.e., "*japonica*-type"), and two sequence types (S1 and S2) were more closely related to *R. sachalinensis* (i.e., "*sachalinensis*-type") (Park et al. 2018). Sequence types J1 and J2 were most similar to two sequences cloned from an *R. japonica* plant collected in Vladivostok, Russia (j_rs01).

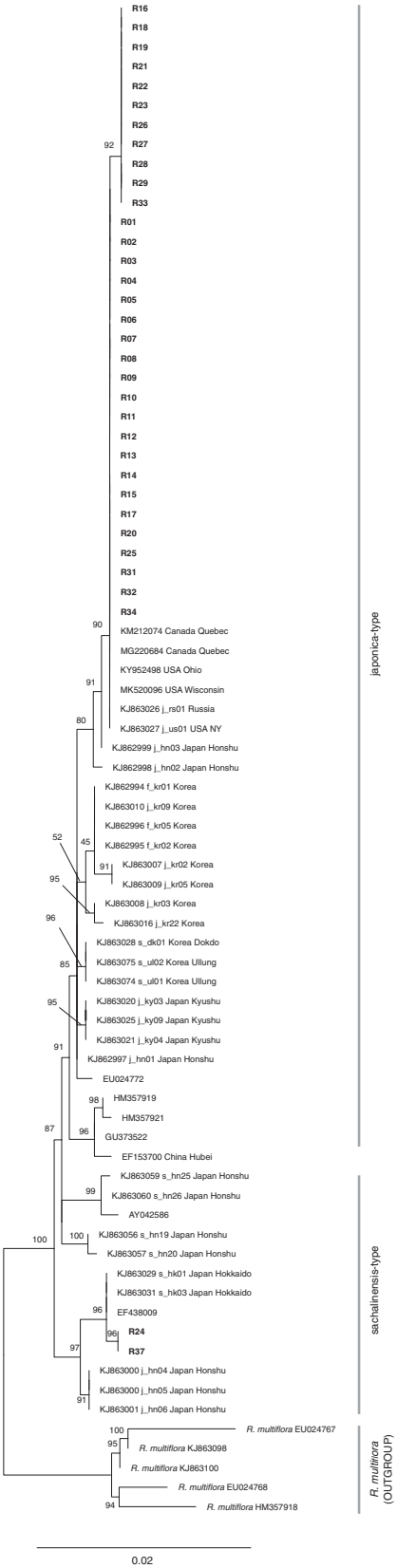

Figure 2. Phylogeny of *Reynoutria* species constructed using data from the plastid *matK* gene. Previously published sequences are shown with GenBank accession numbers and sample identifiers (Park et al. 2018). Newly generated sequences are shown in bold and referenced in Table 1. Nodal support values represent maximum-likelihood bootstrap percentage.

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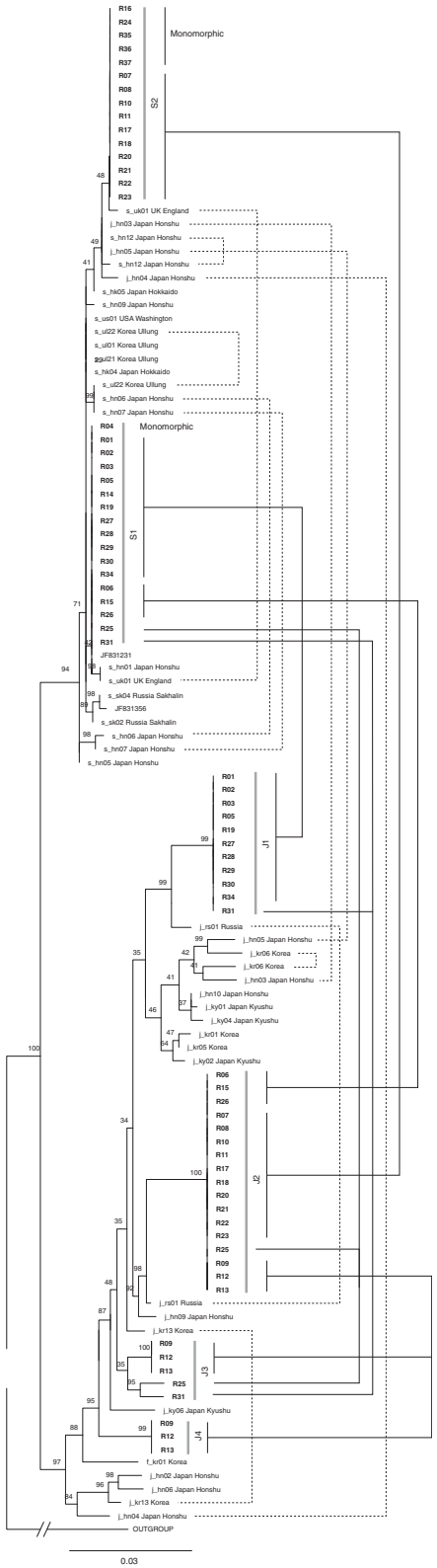

Figure 3. Phylogeny of *Reynoutria* species constructed using data from the nuclear *LEAFY* gene. Previously published sequences are shown with GenBank accession numbers or sample identifiers (Park et al. 2018). Newly generated sequences are shown in bold and referenced in Table 1. Nodal support values represent maximum-likelihood bootstrap percentage. Multiple sequences that were obtained from the same accession are connected by lines at right; dotted lines connect previously reported sequences, and solid lines connect newly reported sequences.

The relationships of sequence types J3 and J4 to native plants were less certain, and these sequences resolved among plants from Japan and Korea on the *LEAFY* phylogeny. Three accessions collected in northwestern Wisconsin (R09, R12, R13) were polymorphic for "*japonica*-type" *LEAFY* sequences, having a combination of J2/J3/J4 sequence types (Figure 1). Two other accessions also had three types of *LEAFY* sequence: R25 had the S1/J2/J3 combination, and R31 combined S1/J1/J3. No plants in our study were monomorphic for "*japonica*-type" leafy sequences, and no individuals had the J1 and J2 sequence types in the same plant.

Sequences falling into the *R. sachalinensis* clade were identified as S1 and S2, and no single individual from Wisconsin was found to have both kinds of "*sachalinensis*-type" *LEAFY* sequence. However, five accessions (R16, R24, R35, R36, R37) were monomorphic for the S2 sequence, including two accessions (R24, R37) that also had "*sachalinensis*-type" *matK* sequences. Interestingly, in a prior study, one *R. sachalinensis* plant from the adventive range in Leicester, UK (s_uk01), was found to contain both the S1 and S2 sequence types (Park et al. 2018). Among plants in the native range, the sequences most similar to S1 and S2 appear in plants from Japan. An invasive *R. sachalinensis* plant sequenced from Washington, USA (s_us01), was similar but not identical to the S1 sequence type (Park et al. 2018). Overall, there was less differentiation among the "*sachalinensis*-type" *LEAFY* sequences than among the "*japonica*-type" sequences: 43 nucleotide positions and 45 indels were variable among "*japonica*-type" sequences, whereas "*sachalinensis*-type" sequences had only 10 variable nucleotide positions and 5 indels.

In total, seven distinct multilocus genotypes were identified, when the *matK* and *LEAFY* data were considered together. Three accessions (R09, R12, R13) exhibited only "*japonica*-type" sequences, including three types of *LEAFY* sequence, and these were all recovered from Polk County in northwestern Wisconsin. Only "*sachalinensis*-type" sequences were recovered from two accessions from far northern Wisconsin (R24, R37), and two other accessions with missing *matK* data (R35, R37) may belong to this genotypic group as well. All other multilocus genotypic groups had the "*japonica*-type" *matK* or were missing *matK* data. Among these, the combination of "*japonica*-type 1" and "*sachalinensis*-type 1" *LEAFY* sequences was found in 11 individuals, mostly in southeastern Wisconsin (e.g., R01, R29). Ten individuals in northwestern and far northern Wisconsin (e.g., R17, R22) had the combination of "*japonica*-type 2" and "*sachalinensis*-type 2" *LEAFY* sequences. A small number of individuals (R06, R15, R26) had the "*japonica*-type 2" and "*sachalinensis*-type 1" *LEAFY* sequences, with no clear geographic pattern. Unique multilocus genotypes were recovered from R25 and R31, which each had the "*sachalinensis*-type 1" *LEAFY* sequence and two kinds of "*japonica*-type" *LEAFY* sequences.

Altogether, our data support the existence of genetically pure *R. sachalinensis* plants (i.e., without any evidence of *R. japonica* parentage) in northern Wisconsin, consistent with morphological identification of plants in that region. It is noteworthy, however, that not all of the plants with this genetic signature were identified as *R. sachalinensis* using morphological data, and the genetic composition and morphological variation of these plants merit further study. The *R. japonica* plants in our study with "*japonica*-type" *matK* sequences and only "*japonica*-type" *LEAFY* sequences are potentially genetically pure members of that species. *Reynoutria japonica* consists entirely of male-sterile individuals in the adventive range (Forman and Kesseli 2003; Grimsby et al. 2007;

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Hollingsworth and Bailey 2000), and the three accessions with exclusively "*japonica*-type" sequences (R09, R12, R13) were male-sterile. Although some genetically pure *R. japonica* or *R. sachalinensis* plants may exist in Wisconsin, the majority of plants we studied contained two kinds of *LEAFY* sequence, one kind attributable to *R. japonica* and one to *R. sachalinensis*. Five or more plants fell into each of the following categories. Plants with the J1 and S1 sequences were collected predominantly from southeastern Wisconsin (Figure 1). Plants with the J2 and S1 sequence combination were relatively rare but occurred evenly across the state. Northern and northwestern plants were largely characterized by the J2 and S2 combination. No plants were recovered with the J1 and S2 combination.

Plants with combinations of *R. japonica* and *R. sachalinensis* genetic elements are inferred to be hybrids. Hybrids with a male-sterile *R. japonica* ovule parent would be expected to have "*japonica*-type" *matK* sequences, and indeed we found this to be the case for suspected hybrid plants. The abundance of hybrid individuals in our study supports previous evidence that *Reynoutria* plants in North America have undergone extensive interbreeding (Gaskin et al. 2014; Grimsby et al. 2007). Similar genetic variability and phylogenetic reticulation have been reported for *Reynoutria* species in the native range (Park et al. 2018). For example, three accessions from Honshu, Japan (j_hn03, j_hn04, and j_hn05), were found to have two different kinds of *LEAFY* sequence belonging to the "*japonica*-type" and "*sachalinensis*-type," respectively (Figure 2), along with plastid parentage from either *R. sachalinensis* (j_h03) or *R. japonica* (j_h04 and j_h05; Figure 3). Therefore, the molecular tools have been established for verifying pure *R. japonica* or *R. sachalinensis* plants, as well as F_1 hybrids and plants with intraspecific sequence variation, using a combination of nuclear and plastid sequence data.

Reynoutria taxa have a distinct ploidy in Polygonaceae, and their base chromosome number of $x = 11$ sets them apart from the related genera *Fallopia* and *Muehlenbeckia* that both have $x = 10$ (Schuster et al. 2011). Moreover, in their native range, *Reynoutria* species have ploidy levels that range from tetraploid ($2n = 44$) to dodecaploid ($2n = 132$) (Park et al. 2018). Ploidy is an important component of genetic diversity in plants, as polyploids often result from hybridization and sometimes become novel species (Doyle et al. 2008; Grant 1981). Recently formed polyploids contain paralogous duplicates of nuclear genes, and over time the gene copies may be lost or become nonfunctional (Buggs et al. 2009). Park et al. (2018) identified plants with apparently monomorphic *LEAFY* sequences, as well as plants having two or more copies of the *LEAFY* gene, with some copies resolving to distantly related clades on the *LEAFY* phylogeny (Figure 3). Some of the recovered copies of *LEAFY* may be allelic, and others could be paralogous in *Reynoutria*. We determined that most of the plants in Wisconsin have two distinct kinds of *LEAFY* sequence, most similar to reported sequences for *R. japonica* and *R. sachalinensis*, respectively. This pattern is consistent with interspecific hybridization, whereby the "*japonica*-type" and "*sachalinensis*-type" sequences would reflect the original parentage. Adventive *Reynoutria* populations have been characterized as tetraploid, hexaploid, or octaploid (Bailey 2003; Bailey et al. 2007; Forman 2003; Mandák et al. 2003; Suda et al. 2010); thus, it might be possible to observe up to eight copies of the *LEAFY* gene in any one plant. We did not obtain chromosome numbers during this study, but the diversity of *LEAFY* genotypes we recovered is consistent with prior evidence that *R. sachalinensis* exists at lower ploidy levels than *R. japonica* and interspecific hybrids (Gammon et al. 2010). In future studies, it will be valuable to determine ploidy levels and compare these with genotypes to gain a more complete understanding of *Reynoutria* genetic diversity.

We collected morphological data for the plants that were studied, but we did not find a strong correlation between morphology and genotype, in contrast to what some studies have reported (e.g., Gaskin et al. 2014). Only one plant (R37) was unambiguously scored as *R. sachalinensis* using both morphological and molecular methods. A large number of accessions were morphologically identifiable as *R. japonica*, but only three of these (R09, R12, R13) contained only "*japonica*-type" *LEAFY* sequences, and curiously these three accessions all had three kinds of *LEAFY* sequences, including the "*japonica*-type 4" sequence that was unique to them. The existence of plants that morphologically match *R. japonica* but contain "*sachalinensis*-type" genetic elements may indicate a need to reevaluate plants formerly identified as *R. japonica*. In the native range, plants identified as *R. japonica* also were found to have *LEAFY* sequences of both the "*japonica*-type" and the "*sachalinensis*-type" (j_hn03, j_hn04, j_hn05; Park et al. 2018; Figure 3), confirming that genetic admixture between *R. japonica* and *R. sachalinensis* can complicate identification in both the native and invasive ranges. With updated knowledge about the widespread existence of hybridization and morphological variability, it will be important to evaluate the genetic and morphological diversity of populations in future studies.

It seems insufficient to characterize the invasive *Reynoutria* hybrids under a single taxon (i.e., *R.* × *bohemica*), because they contain such a wide array of genetic diversity and continue to produce later-generation hybrids. Many of the *Reynoutria* populations in Wisconsin, far from the original introduction sites for *Reynoutria* species in North America, likely represent later-generation hybrids, potentially with introgressed genetic material obtained through backcrosses with one or the other parental species. Our results are consistent with earlier evidence that *R.* × *bohemica* contains the greatest genetic diversity in the adventive range (Bailey 2013; Gaskin et al. 2014; Hollingsworth et al. 1998). High genetic diversity in invasive species could impact land management strategies, because diverse populations may be more capable of adapting to utilize novel habitats or evade control measures (Benoit et al. 2019; Crawford and Whitney 2010; Li et al. 2020; Tippery et al. 2020).

In this study, we determined that morphological characters may be insufficient for identifying *Reynoutria* plants in Wisconsin, and an ancillary study of molecular data would be advisable to help characterize individuals and populations. The DNA sequencing approach advocated here is relatively quick and inexpensive, and with the use of newly designed primers to distinguish "*japonica*-type" and "*sachalinensis*-type" *LEAFY* sequences, subcloning into bacterial vectors should not be required for most accessions. It will be valuable to expand the molecular evaluation of *Reynoutria* species across the adventive range to explore the possibility of multiple introductions from the native range, geographic patterns of diversity in North America, or other vital information that may help us to understand these pernicious plants.

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References

Bailey JP (1994) Reproductive biology and fertility of *Fallopia japonica* (Japanese knotweed) and its hybrids in the British Isles. Pages 141–159 *in* de Waal LC, Child LE, Wade PM, Brock JH, eds. Ecology and Management of Invasive Riverside Plants. Chichester, UK: Wiley

Bailey JP (2003) Japanese knotweed s.l. at home and abroad. Pages 183–196 *in* Child L, Brock JH, Prach K, Pysek P, Wade PM, Williamson M, eds. Plant Invasions: Ecological Threats and Management Solutions. Leiden, Netherlands: Backhuys

Bailey JP (2013) The Japanese knotweed invasion viewed as a vast unintentional hybridisation experiment. Heredity 110:105–110

Bailey JP, Bímová K, Mandák B (2007) The potential role of polyploidy and hybridisation in the further evolution of the highly invasive *Fallopia* taxa in Europe. Ecol Res 22:920–928

Bailey JP, Bímová K, Mandák B (2009) Asexual spread versus sexual reproduction and evolution in Japanese knotweed s.l. sets the stage for the "Battle of the Clones." Biol Invasions 11:1189–1203

Bailey JP, Child LE, Conolly AP (1996) A survey of the distribution of *Fallopia* × *bohemica* (Chrtek & Chrtková) J. Bailey (Polygonaceae) in the British Isles. Watsonia 21:187–198

Bailey JP, Wisskirchen R (2006) The distribution and origins of *Fallopia* × *bohemica* (Polygonaceae) in Europe. Nord J Bot 24:173–199

Barney JN (2006) North American history of two invasive plant species: phytogeographic distribution, dispersal vectors, and multiple introductions. Biol Invasions 8:703–717

Barney JN, Tharayil N, DiTommaso A, Bhowmik PC (2006) The biology of invasive alien plants in Canada. 5. *Polygonum cuspidatum* Sieb. & Zucc.[= *Fallopia japonica* (Houtt.) Ronse Decr.]. Can J Plant Sci 86:887–906

Beerling DJ, Bailey JP, Conolly AP (1994) *Fallopia japonica* (Houtt.) Ronse Decraene. J Ecol 82:959–979

Benoit LK, Les DH, King UM, Na HR, Chen L, Tippery NP (2019) Extensive interlineage hybridization in the predominantly clonal *Hydrilla verticillata*. Am J Bot 106:1622–1637

Bímová K, Mandák B, Kašparová I (2004) How does *Reynoutria* invasion fit the various theories of invasibility? J Veg Sci 15:495–504

Brock JH, Child LE, de Waal LC, Wade M (1995) The invasive nature of *Fallopia japonica* is enhanced by vegetative regeneration from stem tissues. Pages 131–139 *in* Pyšek P, Prach K, Rejmánek M, Wade M, eds. Plant Invasion—General Aspects and Special Problems. Amsterdam, Netherlands: SPB Academic

Buggs RJA, Doust AN, Tate JA, Koh J, Soltis K, Feltus FA, Paterson AH, Soltis PS, Soltis DE (2009) Gene loss and silencing in *Tragopogon miscellus* (Asteraceae): comparison of natural and synthetic allotetraploids. Heredity 103:73–81

Bzdega K, Janiak A, Książczyk T, Lewandowska A, Gancarek M, Sliwinska E, Tokarska-Guzik B (2016) A survey of genetic variation and genome evolution within the invasive *Fallopia* complex. PLoS ONE 11:e0161854

Chernomor O, von Haeseler A, Minh BQ (2016) Terrace aware data structure for phylogenomic inference from supermatrices. Syst Biol 65: 997–1008

Clements DR, Larsen T, Grenz J (2016) Knotweed management strategies in North America with the advent of widespread hybrid Bohemian knotweed, regional differences, and the potential for biocontrol via the psyllid *Aphalara itadori* Shinji. Invasive Plant Sci Manag 9:60–70

Crawford KM, Whitney KD (2010) Population genetic diversity influences colonization success. Mol Ecol 19:1253–1263

Decraene LPR, Akeroyd JR (1988) Generic limits in *Polygonum* and related genera (Polygonaceae) on the basis of floral characters. Bot J Linn Soc 98:321–371

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19:11–15

Doyle JJ, Flagel LE, Paterson AH, Rapp RA, Soltis DE, Soltis PS, Wendel JF (2008) Evolutionary genetics of genome merger and doubling in plants. Annu Rev Genet 42:443–461

[EDDMapS] Early Detection & Distribution Mapping System (2020) Home page. https://www.eddmaps.org. Accessed: December 28, 2020

Forman J (2003) Of Knotweed and Other Weeds: Invasive Plants at Home and Abroad. Ph.D dissertation, Boston: University of Massachusetts

Forman J, Kesseli RV (2003) Sexual reproduction in the invasive species *Fallopia japonica* (Polygonaceae). Am J Bot 90:586–592

Freeman CC, Hinds HR (2005) *Fallopia*. Pages 541–546 *in* Flora of North America Editorial Committee, ed. Flora of North America North of Mexico. Volume 5, Magnoliophyta: Caryophyllidae. Part 2. New York: Oxford University Press

Fung C, González-Moreno P, Pratt C, Oliver TH, Bourchier RS, González-Suárez M (2020) Effect of humidity and temperature on performance of *Aphalara itadori* as a biocontrol for Japanese knotweed. Biol Control 146:104269

Galasso G, Banfi E, De Mattia F, Grassi F, Sgorbati S, Labra M (2009) Molecular phylogeny of *Polygonum* L. s.l. (Polygonoideae, Polygonaceae), focusing on European taxa: preliminary results and systematic considerations based on *rbcL* plastidial sequence data. Atti Soc Ital Sci Nat Mus Civ Stor Nat Milano 150:113–148

Gammon MA, Baack E, Orth JF, Kesseli R (2010) Viability, growth, and fertility of knotweed cytotypes in North America. Invasive Plant Sci Manag 3:208–218

Gammon MA, Grimsby JL, Tsirelson D, Kesseli R (2007) Molecular and morphological evidence reveals introgression in swarms of the invasive taxa *Fallopia japonica*, *F. sachalinensis*, and *F.* × *bohemica* (Polygonaceae) in the United States. Am J Bot 94:948–956

Gammon MA, Kesseli R (2010) Haplotypes of *Fallopia* introduced into the US. Biol Invasions 12:421–427

Gaskin JF, Schwarzländer M, Grevstad FS, Haverhals MA, Bourchier RS, Miller TW (2014) Extreme differences in population structure and genetic diversity for three invasive congeners: knotweeds in western North America. Biol Invasions 16:2127–2136

Grant V (1981) Plant Speciation. 2nd ed. New York: Columbia University Press

Griekspoor A, Groothuis T (2005) 4Peaks. Version 1.7. https://nucleobytes.com

Grimsby JL, Kesseli R (2010) Genetic composition of invasive Japanese knotweed s.l. in the United States. Biol Invasions 12:1943–1946

Grimsby JL, Tsirelson D, Gammon MA, Kesseli R (2007) Genetic diversity and clonal vs. sexual reproduction in *Fallopia* spp. (Polygonaceae). Am J Bot 94:957–964

Hollingsworth ML, Bailey JP (2000) Evidence for massive clonal growth in the invasive weed *Fallopia japonica* (Japanese knotweed). Bot J Linn Soc 133:463–472

Hollingsworth ML, Bailey JP, Hollingsworth PM, Ferris C (1999) Chloroplast DNA variation and hybridization between invasive populations of Japanese knotweed and giant knotweed (*Fallopia*, Polygonaceae). Bot J Linn Soc 129:139–154

Hollingsworth ML, Hollingsworth PM, Jenkins GI, Bailey JP, Ferris C (1998) The use of molecular markers to study patterns of genotypic diversity in some invasive alien *Fallopia* spp. (Polygonaceae). Mol Ecol 7:1681–1691

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods 14:587–589

Kim JY, Park CW (2000) Morphological and chromosomal variation in *Fallopia* section *Reynoutria* (Polygonaceae) in Korea. Brittonia 52:34–48

Lewis PO (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. Syst Biol 50:913–925

Li A, Park CW (2003) *Reynoutria*. Page 319 *in* Wu, CY, Raven PH, Hong DY, eds. Flora of China. Volume 5. St Louis, MO: Missouri Botanical Garden Press

Li C, Ohadi S, Mesgaran MB (2020) Asymmetry in fitness-related traits of later-generation hybrids between two invasive species. Am J Bot 108:51–62

https://doi.org/10.1017/inp.2021.14 Published online by Cambridge University Press

Maddison WP, Maddison DR (2019) Mesquite: a modular system for evolutionary analysis. Version 3.61. http://www.mesquiteproject.org

Mandák B, Pyšek P, Lysák M, Suda J, Krahulcová A, Bímová K (2003) Variation in DNA-ploidy levels of *Reynoutria* taxa in the Czech Republic. Ann Bot 92:265–272

Mitchell N, Owens GL, Hovick SM, Rieseberg LH, Whitney KD (2019) Hybridization speeds adaptive evolution in an eight-year field experiment. Sci Reports 9:1–12

Müller K (2005) SeqState—primer design and sequence statistics for phylogenetic DNA data sets. Appl Bioinf 4:65–69

Murrell C, Gerber E, Krebs C, Parepa M, Schaffner U, Bossdorf O (2011) Invasive knotweed affects native plants through allelopathy. Am J Bot 98:38–43

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol Biol Evol 32:268–274

Parepa M, Fischer M, Krebs C, Bossdorf O (2014) Hybridization increases invasive knotweed success. Evol Appl 7:413–420

Parepa M, Schaffner U, Bossdorf O (2012) Sources and modes of action of invasive knotweed allelopathy: the effects of leaf litter and trained soil on the germination and growth of native plants. NeoBiota 13:15–30

Park CW, Bhandari GS, Won H, Park JH, Park DS (2018) Polyploidy and introgression in invasive giant knotweed (*Fallopia sachalinensis*) during the colonization of remote volcanic islands. Sci Rep 8:16021

Pyšek P, Brock JH, Bímová K, Mandák B, Jarošík V, Koukolíková I, Pergl J, Štěpánek J (2003) Vegetative regeneration in invasive *Reynoutria* (Polygonaceae) taxa: the determinant of invasibility at the genotype level. Am J Bot 90:1487–1495

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model selection across a large model space. Syst Biol 61:539–542

Sanchez A, Schuster TM, Kron KA (2009) A large-scale phylogeny of Polygonaceae based on molecular data. Int J Plant Sci 170:1044–1055

Schuster TM, Wilson KL, Kron KA (2011) Phylogenetic relationships of *Muehlenbeckia*, *Fallopia*, and *Reynoutria* (Polygonaceae) investigated with chloroplast and nuclear sequence data. Int J Plant Sci 172:1053–1066

Simmons MP, Ochoterena H (2000) Gaps as characters in sequence-based phylogenetic analyses. Syst Biol 49:369–381

Small RL, Cronn RC, Wendel JF (2004) Use of nuclear genes for phylogeny reconstruction in plants. Aust Syst Bot 17:145–170

Steward AN (1930) The Polygoneae of eastern Asia. Contrib Gray Herbarium Harvard U 88:1–129

Suda J, Trávníček P, Mandák B, Berchová-Bímová K (2010) Genome size as a marker for identifying the invasive alien taxa in *Fallopia* section *Reynoutria*. Preslia 82:97–106

Tippery NP, Pesch JD, Murphy BJ, Bautzmann RL (2020) Genetic diversity of native and introduced *Phragmites* (common reed) in Wisconsin. Genetica 148:165–172

Vrchotová N, Šerá B (2008) Allelopathic properties of knotweed rhizome extracts. Plant Soil Environ 54:301–303

Yu WG, Fa SJ, Xu CM, Zhu LT, Hou YT, Lin FY, Li FZ (2008) Systematic position of *Reynoutria* and *Polygonum sibiricum* inferred from sequences of chloroplast *trnL-F* and *matK*. J Syst Evol 46:676–681

Yan P, Pang QH, Jiao XW, Zhao A, Shen YJ, Zhao SJ (2008) Genetic variation and identification of cultivated *Fallopia multiflora* and its wild relative by using chloroplast *matK* and 18S rRNA gene sequences. Planta Med 74:1504–1509

Zika PF, Jacobson AL (2003) An overlooked hybrid Japanese knotweed (*Polygonum cuspidatum* × *sachalinense*; Polygonaceae) in North America. Rhodora 105:143–152

https://doi.org/10.1017/inp.2021.14 Published online by Cambridge University Press