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MOLECULAR AND MORPHOLOGICAL EVIDENCE REVEALS INTROGRESSION IN SWARMS OF THE INVASIVE TAXA *FALLOPIA JAPONICA*, *F. SACHALINENSIS*, AND *F. ×BOHEMICA* (POLYGONACEAE) IN THE UNITED STATES[1]

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Fallopia japonica (Japanese knotweed, Polygonaceae) is a well-known East Asian perennial that is established throughout the U.S. and Europe. Another congener, *F. sachalinensis*, and their hybrid, *F. ×bohemica,* also persist on both continents. Their invasive success is primarily attributed to their ability to spread via clonal growth. However, mounting evidence suggests invasion history and dynamics differ between continents and that sexual reproduction is more common than previously assumed. We used published morphological traits designed to distinguish the three taxa to characterize their distribution in 24 New England towns. We found continuous variation of all five traits, with 84% of our 81 individuals having at least one trait outside parental limits. Hierarchical cluster analysis, along with two chloroplast and one nuclear species-specific markers, suggests the presence of intercrossing, segregating hybrids, and likely introgression between F1 hybrids and *F. japonica*. Our markers also show the first evidence of bidirectional hybridization between parental taxa in the U.S., emphasizing the complex structure of populations in our region. This study is a first step toward unraveling the evolutionary forces that have made these taxa such aggressive invaders in the U.S. The data may also affect management strategies originally designed for largely monomorphic, clonal populations.

Key words: introgression; invasive species; Japanese knotweed; Polygonaceae; SNP; SSR; United States.

For researchers interested in witnessing "evolution in action," the dynamic nature of invasive species makes them ideal targets for experimental studies, but understanding invasive species dynamics and managing these species are major challenges (Mooney and Hobbs, 2000). Many correlates and mechanisms of plant invasions have been proposed, debated, and reviewed (Sher and Hyatt, 1999; Davis et al., 2000; Ellstrand and Schierenbeck, 2000; Mack, et al., 2000; Rejmánek, 2000; Keane and Crawley, 2002; Lee, 2002; Shea and Chesson, 2002; Levine et al., 2003; Colautti, 2004), yet the search for universal principles for predicting invasions has been complicated and at times contradictory (Scott and Panetta, 1993; Reichard and Hamilton, 1997; Londsdale, 1999; Kolar and Lodge, 2001; Muth, 2006). Hybridization, however, may be a key factor involved in the evolution of many invasive species. By introducing new genetic diversity, hybridization can rescue populations from bottleneck or drift-related problems. Hybridization and subsequent segregation can generate new recombinants that can be tested by natural selection (Ellstrand and Schierenbeck, 2000). Hybrid differences in growth rates, phenology, herbivory, or pathogen defense, for example, could contribute to establishment (Vila et al., 2000). Also, for perennial taxa, particularly those with extensive asexual reproduction, hybrids can be retained and vigorous genotypes can spread to fixation in populations. Finally, hybridization can conceivably produce very different invasion dynamics depending on the founding taxa and genotypes.

Although the perennial *Fallopia japonica* (Houtt.) Dcne. (*Polygonum cuspidatum*, *Reynoutria japonica*, Japanese knotweed) and *F. sachalinensis* (F. Schmidt & Maxim) Dcne. (*P. sachalinese, R. sachalinensis,* giant knotweed) are not new to invasion biology, their invasion dynamics are complex and not well understood. Both East Asian taxa were introduced into Europe and New England by the 19th century. The herbaceous *F. japonica* is a prolific invader of riparian and disturbed habitats throughout Canada, the U.S., and Europe, where it forms dense stands of bamboo-like ramets that crowd out native species (Wade and Child, 2001). *Fallopia sachalinensis* is found in similar habitats in Europe and the U.S. but is assumed to be less of a threat because of its lower frequency (Hollingsworth et al., 1998; Dukopp and Starfinger, 1995; Mandak et al., 2004). In New England, for example, only 31 *F. sachalinensis* herbarium specimens have been recorded in the Invasive Plant Atlas of New England database (Mehrhoff et al., 2003), with only 13 of these in Massachusetts. The two species, *F. japonica* and *F. sachalinensis,* are known to hybridize, producing *Fallopia ×bohemica* (Chrtek & Chrtkova) J. Bailey (*Polygonum ×bohemicum*, *Reynoutria ×bohemica*). While it has been argued that the *F. japonica* in Europe is one massive clone (Hollingsworth et al., 1998), genetic diversity has been detected in both *F. sachalinensis* and *F. ×bohemica* populations (Hollingsworth et al., 1998; Mandak et al., 2003; Pysek et al., 2003). This diversity may contribute to the rapidly expanding distribution of *F. ×bohemica* in some regions of Europe (Mandak et al., 2004). While no comparable study has been done in the U.S., the PLANTS database (USDA, 2007) shows that the parent species are sympatric in 28 states.

The composition, structure, and invasion dynamics of knotweed populations in the U.S. and Europe appear to differ. Remarkably, no male fertile *F. japonica* plants have been found in Europe, suggesting that sexual reproduction occurs only between congeneric taxa (Bailey and Stace, 1992; Hollingsworth and Bailey, 2000). The low genetic diversity, low viability of hybrid seeds, rarity of seedlings, and

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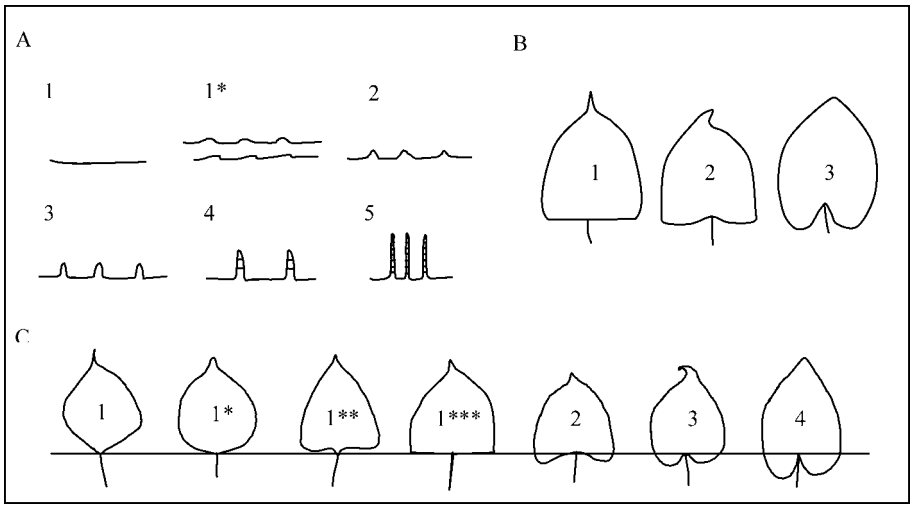


Fig. 1. Morphological traits and cluster analysis interval assignment for (A) subaxial trichome, (B) leaf tip, and (C) leaf base morphologies. Numbers represent values used in cluster analysis based on the variation of each trait from *Fallopia japonica*-typical to *F. sachalinensis*-typical morphologies. Morphologies are described from left to right, top to bottom. (A) Trichomes: glabrous, scabrous to tuburculate, muricate, papillate, minutely hirsute, and hirsute. Morphologies 1 and 1* have both been observed in *F. japonica* and are not considered obvious trichomes. Remaining morphologies are ranked in order of trichome length from most *F. japonica* like to the described *F. sachalinensis* trichomes with a rank of 5. (B) Leaf tip: the *F. japonica* cuspidate or acuminate, curved intermediate, and the *F. sachalinensis* acute to obtuse. (C) Leaf base: acuminate, ovate, nearly truncate, truncate, sagittate or mildly cordate, moderately cordate, and deeply cordate. Leaf bases 1, 1*, 1** and 1*** have all been described in *F. japonica* and reflect a positive angle of leaf attachment to the petiole, as can be seen by comparison to the horizon drawn under the leaves. The remaining morphologies have progressively more negative values in relation to the horizon. Photographic documentation of these morphologies is available at http://www.genetics.umb.edu.

impressive rhizomatous growth point to asexual reproduction as the primary mechanism of dispersal in Europe (Bailey, 1994; Hollingsworth et al., 1998; Pysek et al., 2003). However, there is no evidence that *F. japonica* in the U.S. is solely clonal; in fact recent work has detected genetic diversity (Grimsby et al., 2007). The taxa are subdioecious, and populations are almost always composed of both male and female individuals. Some males set small amounts of viable seed while others do not (Forman and Kesseli, 2003; personal observations). Recent work has found high rates of field and greenhouse germination in both parental and various hybrid crosses (Forman and Kesseli, 2003; Bram and McNair, 2004). Finally, morphological criteria that distinguish *F. ×bohemica* from *F. japonica* in Europe are not as robust in the U.S. (Forman, 2003). *Fallopia ×bohemica* has been reported to have several morphological characteristics that are intermediate to either parent species and distinguish these taxa in Europe (Bailey et al., 1995). In 2003, some of these criteria were modified for North America using herbarium and field specimens (Zika and Jacobson, 2003).

Here we examine and quantify the extent of introgression in the U.S. through morphology-based cluster analysis and molecular markers. We argue that existing differences between continents may reflect greater genetic diversity and extensive sexual reproduction within U.S. populations. The potential genetic diversity within these complex swarms should be considered in any management strategy.

With the heightened interest in biological control for these species in the U.S. (Shaw and Sieger, 2002), knowledge of the role of inter- and intraspecific sexual reproduction and hybridization may be critical, and the effectiveness of such control efforts may depend upon genotype-specific interactions (Nissen et al., 1995).

MATERIALS AND METHODS

Morphological markers—Bailey et al. (1995) noted that hybrids can be distinguished from parentals based on their intermediate leaf and stand size; a slightly cordate leaf base; slightly acuminate apex or tip; intermediate subaxial trichome length, shape, and density; and in most cases, intermediate ploidy values. Zika and Jacobson (2003) considered leaf and trichome size to be reliable indicators in North America, but rejected leaf base shape and tip morphology. They also described a new diagnostic trait; panicle lengths in *F. ×bohemica* and *F. sachalinensis* are shorter than subtending leaves. We used these guidelines to examine New England populations.

In 2003 and 2004 we surveyed vegetative and reproductive morphological traits for 81 individuals in 24 towns in CT, MA, ME, RI, and VT. Populations were chosen to include the diversity present in New England with some patches resembling *F. sachalinensis* or *F. japonica* and others that would likely be defined as hybrids. To control for developmental effects on morphology, only plants that were greater than 1.5-m tall were included. Three fully mature, mid-branch leaves and floral branches were collected from each plant. We scored leaf length and width as overall indicators of size on the collected leaves. Trichome morphology was scored with 20× magnification and was grouped into five classes representing trichome height (Fig. 1A). Bailey et al. (1995) and Zika and Jacobson's (2003) descriptions of parental morphology were used to help rank these classes. The glabrous to tuburculate surfaces of *F. japonica* were considered equivalent to one another and were given the same value of one for the cluster analysis. A subset of samples was used to measure trichome cell number to define categories. The number of cells per trichome ranged from one to five in category 4, and eight or greater in category 5. We measured panicle and subtending leaf lengths during or after peak flowering. Sex was scored when possible. Tip morphology was also ranked from *F. japonica* to *F. sachalinensis* typical morphology, representing estimates of parental expression for this trait (Fig. 1B). Seven leaf base shapes were found in our populations and were given ordinal values based on published observations of parental morphology (Bailey et al., 1995). The angle where the leaf base joins the petiole, as in Kim and Park (2000), was used as a measure of parental expression (Fig. 1C). Described *F. japonica* bases all attach at an angle ≥ 0, and were treated as equivalent in the cluster analysis. The remaining morphologies were ranked in order of the decreasing angle of attachment and were assigned values accordingly. Leaf tip morphology and leaf base shape were examined for all mature leaves for each plant. After observations were completed, we gave

TABLE 1. Criteria for taxonomic identification of *Fallopia* species.

Trait	*F. japonica*	*F. sachalinensis*
Leaf base	See Fig. 1, no. 1	See Fig. 1, no. 4
Length	≤180 cm	≥230 cm
Panicle	≥0 cm	≤−13.5 cm
Tip	See Fig. 1, no. 1	See Fig. 1, no. 3
Trichome	See Fig. 1, no. 1	See Fig. 1, no. 5

Note: Morphological limits for leaf base shape, length (average leaf length), panicle (average panicle length − average leaf length), tip of leaf shape, and trichome height. Bailey et al. (1995) is the source for the *Fallopia japonica* base and *F. sachalinensis* length value. The guidelines of Zika and Jacobson (2003) were used for all other criteria, with their fig. 3 serving as a lower limit for the *F. sachalinensis* panicle trait.

each sample a tentative species identifier, *F. sachalinensis*, *F. ×bohemica*, or *F. japonica,* based on published criteria (Table 1). When possible, North American limits were used. Individuals that did not satisfy all traits for either parental were labeled as *F. ×bohemica*. Hierarchical cluster analysis was performed on *z*-score transformed morphological traits using SPSS 14.0.1 (Chicago, Illinois, USA), with the Euclidean distance measure and UPGMA (between-groups linkage) clustering method selected. Leaf width was excluded because it was correlated to length and was as diagnostic as length. Elongated leaves in hybrids and *F. sachalinensis* are commonly cited anecdotally, and length : width values have been reported in Europe (Bailey et al., 1995). We examined this ratio in our populations to test for elongated leaves and possible differences in values between continents.

Molecular markers—DNA was extracted using Qiagen's Plant DNeasy Mini or Maxi Kit according to protocol (Valencia, California, USA). Several sets of universal intergenic chloroplast primers were screened to detect single nucleotide polymorphisms (SNPs) capable of distinguishing *F. japonica* from *F. sachalinensis*. Initially, each locus was amplified using polymerase chain reaction (PCR) and sequenced in five individuals from each parental species. PCR was performed in a 25 μL volume reaction with 5 μL DNA, 2.5 μL of 2.5 mM $MgCl_2$, 2.5 μL of 2 mM dNTPs, 2.5 μL of 10× reaction buffer, 1 μL of each primer (10 mM), and 0.2 μL of *Taq* polymerase (5 units/μL) using standard PCR cycles. Amplified fragments were sequenced using a 3100 Avant sequencer (Applied Biosystems, ABI, Foster City, California, USA). When a consistent polymorphism between the two taxa was found for a given marker, NEBcutter 2.0 (Vincze et al., 2003) was used to find single-cutting restriction endonucleases whose recognition sequence included the SNP, so that only one species' haplotype would be cut. Two primer pairs produced suitable polymorphisms. The *Trn*L(UGU)_TrnT(UAA) locus (Taberlet et al., 1991, primers a and b, AT = 50, ~600 bp) had four species-specific polymorphisms. One SNP is located within the 5′ GGCC3′ recognition sequence of several restriction enzymes that are unique cutters for this sequence. All *F. japonica* samples had 5′ GACC3′ at this site and were not cut by the chosen enzyme, *Hae*III The second locus, *rbcL-1_atpB-1* (Chiang et al., 1998, AT = 49, ~900 bp), contained four base-substitution SNPs, one single-base pair indel, and one eight-base pair indel. The SNP targeted for restriction digest was cut by the enzyme *Hpy*CH4III in all *F. japonica* individuals (5′ ACTGT3′ vs. 5′ ACTGG3′ in *F. sachalinensis*). For both loci, 10 μL of PCR product was digested with 2.5 units of enzyme for 1 h at 37°C. Results were visualized on a 1% agarose gel stained with ethidium bromide. Once the protocol was established, PCR was performed on 70 of 81 individuals. *Fallopia japonica* and *F. sachalinensis* GenBank accession numbers are EF017663 and EF017664 for the *Trn*L(UGU)_*Trn*T(UAA) locus, and EF017665 and EF017666 for the *rbcL-1_atpB-1* locus.

The nuclear, simple sequence repeat (SSR) marker, KW6, was discovered in a concurrent study (Grimsby et al., 2007). KW6 is a potentially diagnostic *F. sachalinensis*-specific marker. Upon PCR amplification of a subset of samples, this marker only amplified DNA from plants that were previously identified morphologically as *F. ×bohemica* or *F. sachalinensis*. *Fallopia japonica*

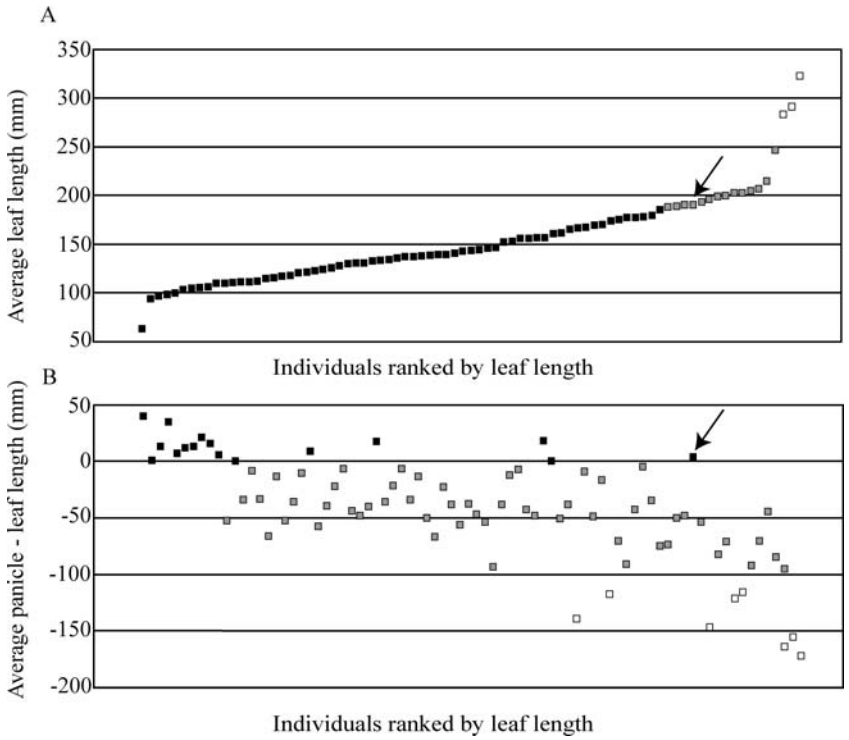


Fig. 2. Continuous variation for traits and subsequent taxon ambiguities as seen in (A) average leaf length and (B) average panicle-subtending leaf length. Accessions are ranked by average leaf length in both charts. Data points that fall within the published *Fallopia japonica* leaf length range are black, while those in the *F. sachalinensis* range are white. Putative *F. ×bohemica* are represented by gray squares. If the more conservative 150-mm limit for *F. japonica* had been used (Bailey, 1995), 19 additional individuals would be labeled as hybrid. (B) All accessions are still ranked by average leaf length, although the panicle trait is the *Y*-variable. Color coding of parental species is the same as in (A) but is based on the parental criterion for the panicle trait. Arrows in (A) and (B) point to individual no. 73, one of the largest hybrids that nonetheless showed a typical *F. japonica* value for the panicle trait.

TABLE 2. Summary of trait combinations found in swarms of *Fallopia japonica, Fallopia ×bohemica,* and *Fallopia sachalinensis*.

Base	Length	Panicle	Tip	Trichome	No. cases	% Total	Taxon
FJ	FJ	FJ	FJ	FJ	11	13.58	FJ
FS	FS	FS	FS	FS	3	3.70	FS
FXB	FXB	FXB	FXB	FXB	7	8.64	FXB
FJ	FJ	FJ	FJ	FXB	1	1.23	FXB
FJ	FJ	FJ	FXB	FXB	1	1.23	FXB
FJ	FJ	FXB	FJ	FJ	23	28.40	FXB
FJ	FJ	FXB	FXB	FJ	2	2.47	FXB
FJ	FJ	FXB	FJ	FXB	7	8.64	FXB
FJ	FJ	FXB	FXB	FXB	4	4.94	FXB
FJ	FXB	FJ	FXB	FXB	1	1.23	FXB
FJ	FXB	FXB	FXB	FJ	1	1.23	FXB
FJ	FXB	FXB	FXB	FXB	2	2.47	FXB
FS	FS	FXB	FS	FS	1	1.23	FXB
FXB	FJ	FJ	FJ	FXB	1	1.23	FXB
FXB	FJ	FS	FXB	FJ	1	1.23	FXB
FXB	FJ	FXB	FJ	FJ	1	1.23	FXB
FXB	FJ	FXB	FJ	FXB	2	2.47	FXB
FXB	FJ	FXB	FXB	FJ	2	2.47	FXB
FXB	FJ	FXB	FXB	FXB	6	7.41	FXB
FXB	FJ	FXB	FS	FXB	1	1.23	FXB
FXB	FXB	FS	FJ	FXB	1	1.23	FXB
FXB	FXB	FXB	FJ	FXB	2	2.47	FXB
				Totals	81	100.00	

Note: FJ = sample trait lies within the range of *F. japonica* as shown in Table 1; FXB = sample does not fit within range of either parent species and is assumed to be *F. ×bohemica*; FS = sample fits within the range of *F. sachalinensis*.

individuals produced no visible amplification product. It was suspected that a sequence variation that distinguished *F. japonica* from either hybrid or *F. sachalinensis* is located on the reverse primer site because a test PCR using the same forward primer and a new reverse primer resulted in amplification of all samples, not just *F. ×bohemica* and *F. sachalinensis*. The cloned fragment did not contain any additional downstream sequence for primer design that would allow for an examination of this variation. Accessions of each taxon were screened for amplification of this locus under the same PCR conditions as in Grimsby et al. (2007). All PCRs were carried out at least twice for verification. The presence of *F. sachalinensis* chloroplast and nuclear markers were superimposed on the cluster analysis.

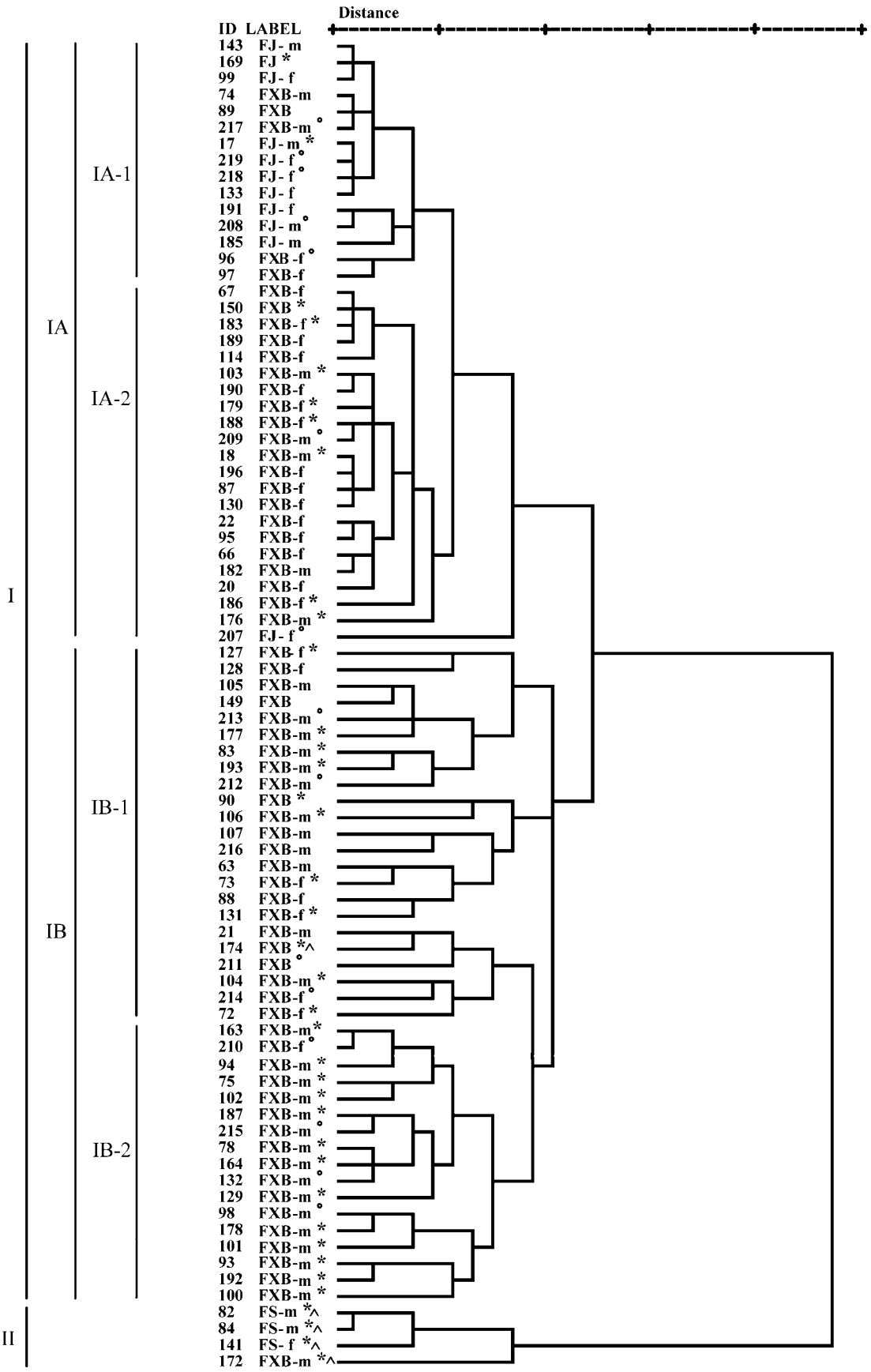
RESULTS

All of our traits had continuous variation, and diverse combinations could be found in different individuals (Figs. 1 and 2). Multiple morphologies were detected within individuals for the trichome and leaf base traits, though these had limited ranges within individuals and still appeared to be useful traits. In general, the range in variation for each trait was greater than has been reported in European accessions. For example, Bailey et al. (1995) reported leaf length : width ratios of 1.0–1.5 for *F. japonica,* 1.1–1.8 for *F. ×bohemica*, and 1.5 for *F. sachalinensis*. The values for this trait among our collections ranged from 0.78 to 2.43 and, when assigned to taxonomic categories based on the characters used in our cluster analysis, taxa had a greater overlap in range; values of 0.84–1.46 for *F. japonica*, 0.78–2.43 for *F. ×bohemica*, and 1.20–1.44 for *F. sachalinensis*.

Of the 81 individuals scored, three fit all morphological criteria for *F. sachalinensis*, 11 fit all criteria for *F. japonica*, and the remaining individuals were intermediate for one or more traits and were thus classified as *F. ×bohemica* (Table 2). The relative panicle length trait (hereafter referred to as "panicle") was the harshest criterion, excluding 23 individuals from the *F. japonica* and one from the *F. sachalinensis* classification. The cluster analysis using all traits (Fig. 3) divided the accessions into several groups. All these major groups possessed both male and female individuals, with male bias seen in the *F. ×bohemica* groups IB-1 and 1B-2, as is common in European populations (Bailey et al., 1996; Bailey, 1997). The deepest branch distinguishes a predominant *F. sachalinensis* cluster (Group II) from all other individuals. Most individuals (10 of 11) that fit all criteria for *F. japonica* were all embedded within Group IA-1. A failure to meet the criterion for the panicle trait is largely responsible for the clustering of *F. ×bohemica* into Group IA-1 and II. For example, if the panicle trait was excluded from the criteria for taxonomic identification, Group IA-1 would then contain three more *F. japonica* individuals, with only two individuals of the cluster (nos. 96 and 97) still violating established *F. japonica* criteria because of trichome morphology. Similarly, no. 172 in Group II would be reclassified as *F. sachalinensis*, creating a *F. sachalinensis* specific cluster. This individual was part of a young and small (1.7 m tall) stand, suggesting that age or habitat in addition to taxon may influence relative panicle size.

The likely *F. sachalinensis* specific Group II is formed from the deepest branch of the cluster analysis. This appears to reflect the morphological breaks for leaf length, leaf tip, base morphology, and trichome size that clearly separated this group from all other individuals (Fig. 4A, B). This morphological separation for multiple traits suggests that introgression into *F. sachalinensis* may be minimal and contrasts with the situation for *F. japonica*. A clear, morphologically distinct *F. japonica* is not apparent for any trait. A second cluster analysis using the two-step approach (SPSS 14.1) also failed to produce exclusively parental groups, irrespective of the inclusion of molecular markers as binary factors.

Molecular markers that appear to distinguish the genomes of *F. sachalinensis* from *F. japonica* (Fig. 5) were superimposed on the morphological analysis (Fig. 3) and appear to roughly


Distance
ID LABEL
143 FJ- m
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99 FJ- f
74 FXB-m
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17 FJ- m*
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218 FJ- f°
133 FJ- f
191 FJ- f
208 FJ- m°
185 FJ- m
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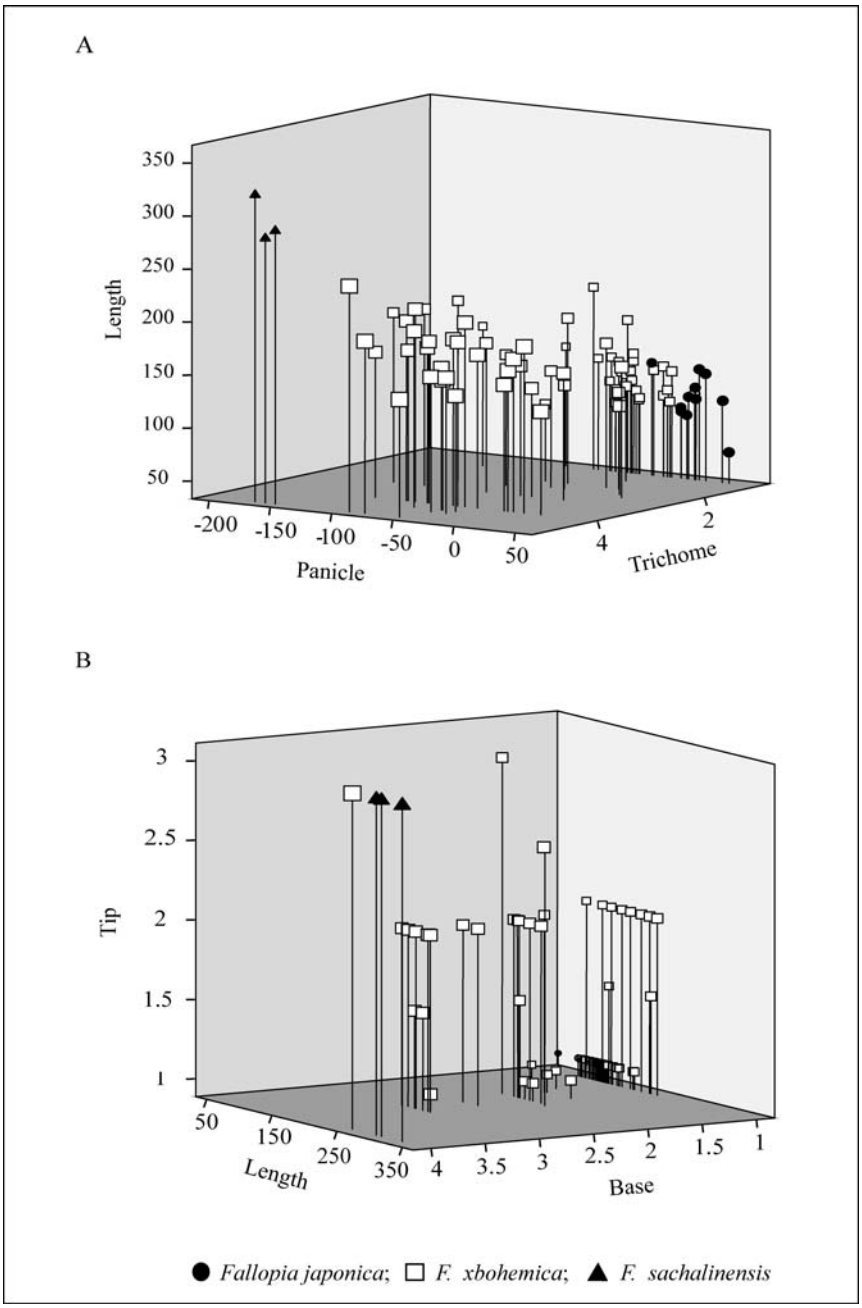

Fig. 4. Three-dimensional graphs of morphological data. (A) Length, panicle, and trichome values. (B) Length, tip, and base traits.

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Fig. 3. Hierarchical cluster analysis of morphological traits, with molecular marker data and putative taxonomic identification displayed. FJ, *Fallopia japonica*; FXB, *F.* ×*bohemica*; FS, *F. sachalinensis*; f, female; m, male; *, simple sequence repeat marker of *F. sachalinensis*, where absence of this notation represents a *F. japonica* genotype; ^, chloroplast single nucleotide polymorphisms of *F. sachalinensis*, where the absence of this notation represents a *F. japonica* haplotype; °, sample not tested with molecular markers.

support the taxonomic categories defined by the morphology. Specifically, the four individuals in Group II all have the chloroplast and nuclear genotypes suggestive of *F. sachalinensis*. In the largely *F. japonica* specific Group IA-1, seven of nine individuals scored had both the chloroplast and nuclear genotypes indicative of *F. japonica*. Interestingly, the only other group with consistent genotypes for all members was Group 1B-2, which showed the chloroplast haplotypes of *F. japonica* and the nuclear genotype of *F. sachalinensis*, consistent with the notion that these plants could be F1 hybrids (and would carry this dominant *F. sachalinensis* specific nuclear marker) of a cross between female *F. japonica* and male *F. sachalinensis*.

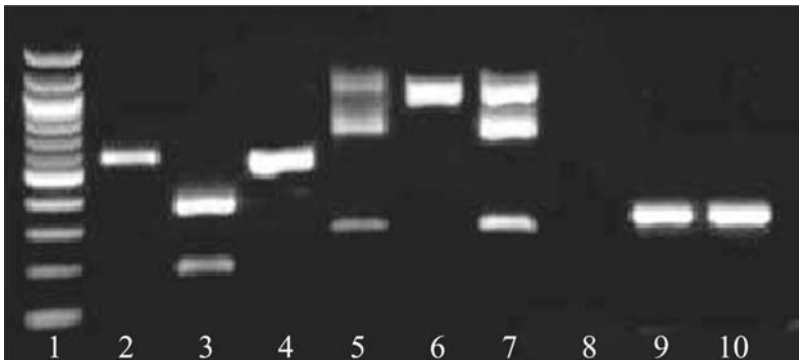

Fig. 5. Genotype scoring of the single nucleotide polymorphism and simple sequence repeat markers. Putative *Fallopia* ×*bohemica* individuals nos. 130, 174, and 75, from left to right. Hybrid individual no. 130 (lanes 2, 5, 8) shows a *F. japonica* nuclear and chloroplast genotype; no. 174 (lanes 3, 6, 9) shows both nuclear and chloroplast *F. sachalinensis* genotypes; and no. 75 (lanes 4, 7, 10) reflects the most common type of hybrid, where chloroplast markers show *F. japonica* maternity and *F. sachalinensis* paternity. Lane 1, 100-bp ladder; lanes 2, 3, and 4 show PCR products at the *Trn*L(UGU)_*Trn*T(UAA) cp locus, where lane 3 is the *F. sachalinensis*-specific sequence digested with *Hae*III; lanes 5, 6, and 7 show the PCR products at the cp locus *rbcL-1_atpB-1*, where lanes 5 and 7 are the *F. japonica*-specific sequences digested with *Hpy*CH4III; lanes 8, 9, and 10 are the KW6 nuclear marker, where the presence of amplification is *F. sachalinensis* specific.

The cpDNA markers that appear to distinguish *F. sachalinensis* were present in the four individuals of Group II and only one other individual (no. 174) in the remaining 61 individuals tested. The dominant nuclear marker that appears diagnostic for *F. sachalinensis* was detected in some individuals of all major groups. This distribution pattern suggests that *F. japonica* has generally been the female parent in hybridizations.

DISCUSSION

Post-invasion adaptation has been suggested and demonstrated in several plant species (Siemann and Rogers, 2001; Lee, 2002; Maron et al., 2004). Interspecific hybridization may often be responsible for this adaptation (Ellstrand and Schierenbeck, 2000; Bleeker, 2003). Our study shows that the range in variation for traits thought to be diagnostic for species identification in *Fallopia* is high in U.S. populations and that individuals often possess combinations of traits that preclude their characterization as either *F. sachalinensis* or *F. japonica*. Multiple introductions from Asia could contribute to this high level of morphological and genetic variation in the U.S., which has not been found in European studies (Bailey et al., 1995; Hollingsworth and Bailey, 2000; Mandak, et al., 2005). The analyses here, however, suggest that repeated hybridization and introgression are also likely and may be responsible for taxonomic ambiguities in the genus. For example, our *F. sachalinensis*-specific SSR marker is not always concordant with morphology-based taxon assignment, suggesting backcrossing or intermating among hybrids and segregation. This is not surprising, because both male and female individuals of *F. japonica* (Group IA-1), *F.* ×*bohemica* (Groups IB-1 and IB-2), and *F. sachalinensis* (Group II) are present in local populations, and such diversity would generate many more sexual combinations than may be possible in European populations. We have seen that both males and females of *F.* ×*bohemica* are fertile in the wild (M. Gammon, data not shown). There are indications from several studies that European populations may also have accessions that are backcrosses or later generation hybrids. Specifically, Hollingsworth et al. (1998) found five different hybrid genotypes yet only two of *F. sachalinensis* and one of *F. japonica*. Similarly, Pysek et al. (2003) identified nine hybrid genotypes but only five for *F. sachalinensis* and one for *F. japonica*. Both groups note that these novel hybrid genotypes could be caused by backcrossing to parents or by intercrossing among hybrids and subsequent segregation.

The *F. sachalinensis*-specific chloroplast haplotypes were detected in only one individual outside of the distinct Group II cluster, indicating that most hybridization events have involved *F. japonica* as the maternal parent. However, one likely hybrid individual (no. 174) had *F. sachalinensis* chloroplast and nuclear genotypes. Because chloroplasts are maternally inherited in these taxa (Hollingsworth et al., 1998), this is evidence of past *F. sachalinensis* maternity in this individual. While this kind of cross has not previously been documented in the U.S., four morphologically hybrid individuals collected for a different study and currently growing in our greenhouse also show this genetic pattern. One of these individuals, a Canadian female, has a ploidy estimate of $2n = 66$, typical of *F.* ×*bohemica* and suggesting the product of a *F. sachalinensis* × *F. japonica* var. *japonica* cross and further supporting its hybrid nature (data not shown). At collection, the no. 174 hybrid female was 3 m tall, and all its leaves were mildly to deeply cordate with acute or obtuse tips. It was found at a site on Nantucket, Massachusetts, USA, in a population with *F. sachalinensis*, *F. japonica*, and *F.* ×*bohemica* individuals.

Because New England has a more extensive history with the Old World than any other region of the U.S., it is a logical place to explore differences in species introductions and subsequent adaptations. We have shown morphological and molecular evidence that Japanese and giant knotweed have hybridized and produced complex swarms that appear to include backcrossed and later-generation hybrids. Continuous variation and character segregation patterns appear to make morphological criteria that distinguish *F.* ×*bohemica* and *F. japonica* in Europe and possibly other parts of North America unreliable in New England. Even the cluster most representative of a parental *F. japonica* group (Group IA-1) has individuals that appear to carry some *F. sachalinensis* traits; three of 15 appear to be outside the appropriate range for the panicle trait (nos. 74, 89, 217), two of 15 appear outside the

range for both panicle and trichome traits (nos. 96, 97), and two of the 10 scored carry a copy of the *F. sachalinensis* nuclear marker (nos. 17, 169). In total, 25 of 70 individuals have taxonomic labels that do not concur with genetic markers. The degree to which these ambiguities indicate a broader range of trait variation in *F. japonica* than originally believed, or extensive introgression of *F. sachalinensis* into the *F. japonica* gene pool, remains uncertain. However, the range and segregation of morphologies within sites, along with the recent detection of genetic diversity in New England (Grimsby et al., 2007) do not support the hypothesis that plasticity is responsible for the range of traits shown here.

Hybridization and extensive introgression into *F. japonica* seem evident and are likely potent forces generating the diversity and invasiveness seen in New England. While the perennial habit and capability for clonal growth are unquestionably major factors promoting the spread of these taxa, previous data showed that seeds are viable and seedlings establish in nature (Forman and Kesseli, 2003; Bram and McNair, 2004) and that stands are composed of multiple genotypes (Grimsby et al., 2007). The current study shows that interspecific hybridization and introgression are likely common occurrences. Together these studies demonstrate that sexual reproduction is important in these taxa, both for dispersal and for generating diversity. This reality highlights the need for a local as well as global approach when studying the spread and evolution of invasive species. For example, control of the often overlooked *F. sachalinensis* may aid in the prevention of further hybridization. In addition, as complete removal of vast stands is often impossible for management, the prevention of flowering or removal of seeds should be considered. Finally, biological control programs that may be successful in Europe may not yield equivalent control of U.S. swarms.

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