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Unexpected genetic diversity of *Fallopia japonica* from Central Europe revealed after AFLP analysis

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ABSTRACT

Recently much attention has been paid to genetic aspects of invasive success in Japanese knotweed s.l. One hypothesis to explain the invasive spread of these species is a multiple introduction, which leads to a higher level of genetic diversity in the invaded range. *Fallopia japonica* is considered to be genetically uniform in Europe, introduced as a single female clone. However, there is some evidence suggesting that invasion history and dynamics differ between Western and Central-Eastern Europe. We used AFLP markers to characterize genetic diversity of three *Fallopia* taxa that occur in Poland: *F. japonica*, *F. sachalinensis* and their hybrid *Fallopia* × *bohemica*, growing in so-called 'homogeneous' populations, consisting of one taxon and 'heterogeneous' populations, composed of the three taxa cohabiting together. No polymorphism, resp. an insignificantly low variability was observed in the 'homogeneous' populations. In the 'heterogeneous' stands polymorphism was detected within each taxa, with one exception that concerns individuals of *F. sachalinensis* from a riparian habitat. The highest level of polymorphism was found among individuals of *F.* × *bohemica.* The most striking result of our study is the observation of polymorphism between individuals of *F. japonica*. The AFLP data also showed that *F.* × *bohemica* is most diverse when occurring in a heterogeneous configuration with *F. japonica* and *F. sachalinensis* in the same habitat. Our results are the first evidence of genetic diversity in *F. japonica* populations in Central Europe and can implicate the possibility of its multiple introduction in this region or the existence of sexual reproduction of this species.

Introduction

The species of knotweed s.l., involving *Fallopia japonica* var. *japonica*, *F. sachalinensis*, and the hybrid between the two – *F.* × *bohemica*, are recognized as most troublesome alien invasive species introduced outside their natural range to different continents and are spreading very rapidly in both Europe and North America (Bailey and Conolly, 2000; Bailey, 1999, 2003; Fojcik and Tokarska-Guzik, 2000; Seiger, 1997; Tiébré et al., 2007a,b). Populations of *Fallopia* taxa occupy a wide range of habitats, both anthropogenic and natural ones, typically spreading along river banks (Mandák et al., 2004; Pyšek et al., 2003; Richards et al., 2008; Tokarska-Guzik, 2005), railways and roads, and are causing growing concern because they pose a serious threat to native biodiversity, particularly in riparian ecosystems (Gerber et al., 2007; Pyšek and Prach, 1993; Tokarska-Guzik et al., 2006). Concern over not only significant ecological impact but also increasing economic costs connected with control and management, has generated wide theoretical and practical interest in the biology of these species (Bailey et al., 2009; Bímová et al., 2001, 2003, 2004; Child and Wade, 2000; Richards et al., 2008; Shaw et al., 2009).

In recent decades, much attention has been paid to genetic aspects of invasive success in Japanese knotweed s.l. populations. There are several hypotheses to explain the invasive spread of certain species. The first one assumes that the act of invasion occurs without any adaptation to a new environment and that the invading species does not develop any evolutionary changes (Bailey et al., 1995; Hollingsworth and Bailey, 2000). The second one presupposes that the invaders loose the genetic diversity due to founding effect and bottleneck events, but this does not preclude the rapid evolution of life history among invading lineages (Dlugosch and Parker, 2008). Another important factor promoting genetic diversity is polyploidy and hybridization as the recombination in hybrids generates novel variation. Higher genetic diversity in hybrid-derived populations may be responsible for the evolutionary success resp. invasiveness of the plant (Ellstrand and Schierenbeck, 2000). The invasive characteristics of *F.* × *bohemica* present an example for the hypothesis of an invasion based on

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hybridization. Spread of the alien plant is explained in this case as superiority of hybrids above parental species, ensuring invasive success in the non-native range (Weber et al., 1998). Finally, multiple introductions can help to generate a higher level of genetic diversity in the invaded range than in the native one (Genton et al., 2005; Rosenthal et al., 2008).

The knotweed taxa were introduced to Europe from Asia in the 19th century as ornamental garden plants and soon spread into natural habitats, becoming a serious threat to native species (Bailey and Conolly, 2000; Bailey et al., 2007; Beerling et al., 1994; Fojcik and Tokarska-Guzik, 2000; Mandák et al., 2004; Tokarska-Guzik, 2005). *Fallopia japonica* is considered to be genetically uniform in Europe, introduced as a single female clone. Nevertheless, it can produce seeds as a result of pollination by related *Fallopia* species, mainly *F. sachaliensis*, for which both hermaphrodite and female clones are found throughout Europe (Bailey et al., 2009). Hybridization in *Fallopia* is very common in Europe and it explains the wide occurrence of the *F.* × *bohemica* hybrid. The possible main hybridization routes of closely related *Fallopia* taxa are reviewed in Bailey et al. (2007, 2009). The history of the introduction of the alien invasive perennial taxa *F. japonica* and *F. sachalinensis* to Europe and their subsequent hybridization to produce *F.* × *bohemica* has been described by various authors (Bailey and Conolly, 2000; Bailey and Wisskirchen, 2004; Bailey et al., 1995; Chrtek and Chrtková, 1983; Fojcik and Tokarska-Guzik, 2000; Hollingsworth and Bailey, 2000; Tokarska-Guzik, 2005). The role and importance of polyploidy and hybridization in their invasion and the implication of these factors for the potential further evolution of the group were discussed recently by Bailey et al. (2007, 2009) and Tiébré et al. (2007a,b).

The genetic diversity of species has consequences for different processes such as colonization, many life history traits (e.g. mode of reproduction – sexual vs. clonal, seed dispersal and life cycle), population history, impact of environmental factors and anthropogenic disturbances as well as hybridization and polyploidization events. These factors have been frequently mentioned by many authors in relation to genetic diversity in the species studied (Dlugosch and Hays, 2008; Hamrick and Godt, 1989; Lembicz et al., 2011; Loveless and Hamrick, 1984; Novak and Mack, 2005; Nybom and Bartish, 2000; Nybom, 2004).

Several attempts to evaluate genotypic diversity in the *Fallopia* complex were made using morphological analysis and cytogenetic and molecular marker techniques, including RAPDs, ISSRs (Hollingsworth and Bailey, 2000; Hollingsworth et al., 1998; Tiébré et al., 2007a), SNPs, SSRs (Gammon et al., 2007; Grimsby et al., 2007) as well as isoenzyme analysis (Mandák et al., 2005). Genetic diversity in *Fallopia* taxa has been addressed in many papers. However, there is still a lack of detailed studies considering the spatial pattern of genetic diversity and distribution of the populations in Central Europe. In Poland increasing participation of the hybrid *Fallopia* × *bohemica* in local populations is observed, which is not so common for the majority of Western Europe occurrences. DNA markers such as AFLP have, to our best knowledge, not been used to assess the genetic diversity in *Fallopia* taxa.

The AFLP method is a firmly established molecular marker technique, with broad applications in population genetics, shallow-structure phylogenetics, linkage mapping, parentage analyses, and – to some extend – single-locus PCR marker development (Meudt and Clark, 2007). This technique is especially suitable for detection of genetic differences in populations exhibiting clonal growth (Eckert, 2002). Together with relatively low cost and high sensitivity and replicability, AFLP markers have proven to be very efficient in studying genetic diversity among and within populations, in spite of its dominant nature. There are many recent examples of their successful application in population studies of various species, e.g. in *Magnolia officinalis* subsp. *biloba*/Magnoliaceae (He et al., 2009), in *Suaeda maritima*/Chenopodiaceae (Prinz et al., 2009), in *Ranunculus kuepferi*/Ranunculaceae (Burnier et al., 2009), in *Astragalus bibullatus*/Fabaceae (Baskauf and Burke, 2009), and in *Gagea spathacea*/Liliaceae (Pfeiffer et al., 2012). Multilocus DNA markers with arbitrarily designed primers (AFLP, ISSR) have also been utilized for characterization of genetic diversity among population of species exhibiting different propagation strategies, e.g. clonal and sexual. A good example is *Leersia hexandra*, a perennial clonal aquatic weed studied with ISSR markers (Song et al., 2006). The high level of genetic diversity detected in this species was interpreted by authors as a consequence of occasional sexual recruitments in some habitats, wide distribution and perennial life type.

In the present study we used AFLP markers to characterize genetic diversity of selected populations of three *Fallopia* taxa in relation to (i) taxon constitution, i.e. populations consisted of one taxon (here called 'homogeneous') or composed of three taxa cohabiting in the same area (here called 'heterogeneous'), (ii) their mode of spread – with or without human influence and (iii) the number of genetic individuals (genets) in the populations.

Materials and methods

Plant material

The following knotweed taxa were used in the study: *Fallopia japonica*, *F. sachalinensis* and *F.* × *bohemica*. Knotweed leaves were collected from three 'homogeneous' stands consisted of one taxon and two 'heterogeneous' stands which were composed of three taxa cohabiting in the same area (Table 1 and Fig. 1). The studied populations were located in southern Poland. All homogeneous stands and one heterogeneous one were found in anthropogenic sites. The homogenous population from Pradnik Korzkiewski grows at the roadside close to the Pradnik River, the population from Dabrowa Gornicza was settled along the highway on the edge of a forest, the population from Bukowno was found at wasteland near a road, close to a *Robinia* forest (Chelidonio-Robinietum). The heterogeneous population from Katowice-Muchowiec was located in a derelict area of a public park. The remaining heterogeneous population was growing in a natural, riparian habitat (Wapienica River at Czechowice-Dziedzice).

The number of shoot clumps (the basic unit of the rhizome system; after Bailey et al., 2009) selected for the analysis varied from 5 to 12 for each population studied. Fifty-seven individual shoots, here called ramets, collected from selected shoot clumps, were examined using AFLP markers: 5 ramets from Pradnik Korzkiewski (P), 5 from Dabrowa Gornicza (D), 5 from Bukowno (B), 12 from Katowice-Muchowiec (KM) and 30 from the Wapienica River at Czechowice-Dziedzice (CD). All samples from the three 'homogeneous' populations (P, D and B) came from individuals from a single patch, and the material collected from the heterogeneous populations came from individuals from all patches in the respective area (Table 1). For the heterogeneous population from Czechowice-Dziedzice material was collected from 5 ramets from a single shoot clump and 2 shoot clumps were selected for each taxon. For the remaining populations each ramet was collected from a different clump. For each examined knotweed population, types of flowers and their fertility was recorded.

DNA extraction and AFLP procedure

Young leaves from each ramet were collected and dried on silica gel (Sigma) for 24 h. After grinding, total DNA was extracted using the CTAB method (Doyle and Doyle, 1987) with some modifications.

The AFLP method was performed in accordance with Vos et al. (1995) with modifications introduced in the Department of Genetics, University of Silesia. For the visualization of amplified fragments, the Li-Cor automated sequencer, model 4300 was