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Asexual spread versus sexual reproduction and evolution in Japanese Knotweed *s.l.* sets the stage for the "Battle of the Clones"

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Abstract The invasion of NW Europe by a single clone of male-sterile *Fallopia japonica* var. *japonica* by purely vegetative reproduction has demonstrated that reproduction by seed and the genetic diversity associated with it may not be an essential feature of plant invasions, at least in the short term. What is less well known is that a significant proportion of the Japanese Knotweed *s.l.* involved is not *F. japonica* var. *japonica,* but the hybrid between it and *F. sachalinensis–F.* × *bohemica*. This hybrid is able to backcross to either parent with the potential to replace the missing male *F. japonica;* by the same process, the hybrid is generating the genetic diversity so conspicuously lacking in *F. japonica*. In terms of understanding the population structure in a particular country, it is important to be able to identify hybrids and putative back-crosses. We bring together a mixture of published and unpublished information to provide a comprehensive section on morphological and anatomical aids to identification. Regeneration of plants from stem and rhizome fragments varies by taxon, as do responses to control techniques, underlining the importance of correct identification of these plants. In this paper we look closely at this group of taxa, with special emphasis on the role played by hybridization, with an examination of the genetic make up of seedlings produced in the wild or by artificial hybridizations, and the implications that this will have on the future directions of the invasion process.

Keywords Clonality · *Fallopia* · Hybridisation · Invasibility · *Polygonum* · Polyploidy · *Reynoutria*

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Introduction

Hybridisation is now recognized as an important evolutionary aspect that can significantly influence alien species and increase their invasibility (Rieseberg 1997; Ellstrand and Schierenbeck 2000; Hänfling and Kollmann 2002; Lee 2002). Many biologists see this point as a wonderful opportunity to study "evolution in action", which can generate important information for evolutionary biology (Lee 2002) as well as help to explain the invasibility of individual taxa (Abbott 1992; Ferris et al. 1997; Bleeker 2003; Mandák et al. 2004). In certain cases, hybridisation of two alien or alien and native species can bring into being new

hybrid taxa that are more invasive than their parents (Mandák et al. 2004) and can even threaten native species with extinction (Rhymer and Simberloff 1996). Hence, the effect of either interspecific (between species) or intraspecific (between previously isolated populations after multiple introductions to a new area—Kolbe et al. 2004) hybridisation may provide the raw material for adaptive evolution in rapidly changing environments and should be considered as an important speciation mechanism (Rieseberg 1997). For these reasons, understanding the evolution of invasive species is of basic importance not just for evolutionary biologists but also for anyone involved with invasive species.

The phenomenon of the Japanese Knotweed invasion of Europe has been well-studied and is well-understood (Bailey 1994; Mandák et al. 2003, 2004, 2005). Basically, a male sterile clone of *F. japonica* var. *japonica* became widely distributed and now occupies large areas of generally riparian or ruderal habitat. It was discovered early on (Bailey 1994) that in the absence of the male plant in this gynodioecious species, that reproduction of *F. japonica* var. *japonica* by seed was not possible. The plants did, however, produce hybrid seed, and this paper will concentrate on the extent and impact of this seed production. The situation in America, although less well-understood, will also be discussed.

In this paper we will be reviewing the different taxa that comprise Japanese Knotweed *s.l.*, in terms of their history, distribution, genetics, cytology, regeneration abilities, ecology and their overall impact on their adventive range. We will also consider the threats that they may pose, and how those threats can be countered.

Taxonomy of the group

The term Japanese Knotweed *s.l.* is used to cover *Fallopia japonica*, *F. sachalinensis*, hybrids between the two (*F. × bohemica*), backcrosses, and crosses involving any of the former with *F. baldschuanica* and other related taxa. Tables 1 and 2 give an overview of the more important of these taxa with ploidy levels and distribution in their native and adventive ranges.

Whilst these taxa have been (and still are) known under a range of different genera we use the *Fallopia* combinations, in agreement with the work of Ronse Decraene and Akeroyd (1988). All taxa possess the same suite of morphological characters that distinguish them from the rest of *Polygonum s.l.* Additional support for *Fallopia* comes from the work of Frye and Kron (2003) on the *rbcL* phylogeny of the family. Whilst their paper only includes *F. sachalinensis* and *F. japonica*, in later work *F. cilinodis*, *F. scandens* and *F. convolulus* also cluster with *F. sachalinensis* and *F. japonica* (Frye A.S.L. 2005, personal communication). All Japanese Knotweed *s.l.* taxa, with the exception of *F. baldschuanica*, are gynodioecious (i.e. male sterile and hermaphrodite individuals).

The Japanese Knotweed *s.l.* taxa

Fallopia japonica (Houtt.) Ronse Decraene var. *japonica* [syn.: *Polygonum cuspidatum* Sieb. et Zucc., *Reynoutria japonica* Houtt. var. *japonica*]

The history of the introduction of *Fallopia* taxa to Europe was described in detail by Bailey and Conolly

Table 1 Sectional treatment of the genus *Fallopia*

Section	Species	Life form
Fallopia Adanson	*F. convolvulus* (L.) Löve	Annual climbers
	F. dumetorum (L.) Holub	
	F. scandens (L.) Holub	
Paragonum Haraldson	*F. cilinodis* (Michaux) Holub	Smaller perennial climbers
	F. cynanchoides (Hemsl.) Haraldson	
Sarmentosae (Grintz) Holub	*F. multiflora* (Thunb.) Haraldson	The larger perennial climbers
	F. baldschuanica (Regel) Holub [Syn. *F. aubertii* (L. Henry) Holub]	
Reynoutria (Houtt.) Ronse Decraene	*F. japonica* (Houtt.) Ronse Decraene	Herbaceous non-climbing perennials
	F. sachalinensis (F. Schmidt) Ronse Decraene	

Table 2 Records of chromosome numbers found in *Fallopia* taxa in both native and adventive distribution ranges (Bailey 1999, 2003; Bailey and Stace 1992; Mandák et al. 2003)

Taxa	Native	Adventive
F. japonica var. *japonica*	44, 66, 88	**88**
F. japonica var. *compacta*	44	**44**
F. sachalinensis	44 (102, 103, 132)[a]	**44**, 66, 88
F. × bohemica	66	44, **66**, 77, 88, 105, 110

The most common chromosome numbers in adventive distribution range are highlighted

[a] Restricted to Ullong-do—an island between Korea and Japan (see Kim and Park 2000)

(2000). The introduced *F. japonica* var. *japonica* is cytologically and genetically uniform (Table 2). The octoploid ($2n = 8x = 88$) female clone recorded in Europe (Bailey and Stace 1992; Mandák et al. 2003) belongs to the same genotype that is present in the whole of Europe (Bailey et al. 1995; Hollingsworth and Bailey 2000; Mandák et al. 2005). Since only a female clone is known in Europe, the pollen of *F. japonica* var. *japonica* is absent. Nevertheless, plants do produce seed because they are fertilized by the pollen of related taxa. Although no hermaphrodite plants are known in Europe or Australasia, they have been reported from the East Coast of America by Forman and Kesseli (2003).

Fallopia japonica var. *compacta* (Hooker fil.) J. P. Bailey [syn.: *Reynoutria japonica* var. *compacta* (Hooker fil.) Moldenke]

Introduction of a dwarf mountain variant of *F. japonica* referred to as var. *compacta*, dates back to 1841 (Bailey and Conolly 2000). In spite of its early introduction, *F. japonica* var. *compacta* achieved neither the popularity nor distribution of the nominate variety. However, *F. japonica* var. *compacta* was not recognized within its native distribution range, where high morphological variation creates a continuum between *F. japonica* var. *japonica* and *F. japonica* var. *compacta* (Shiosaka and Shibata 1993).

F. japonica var. *compacta* is tetraploid ($2n = 4x = 44$) (Table 2) (Mandák et al. 2003), rarely naturalizes, and has low genetic variability (only two hermaphrodite genotypes known in the Czech Republic—Mandák et al. 2005), though both sexes are found in the UK. Although infrequently naturalized, historically it has played an important role in the production of tetraploid *F. × bohemica*.

Fallopia sachalinensis (F. Schmidt) Ronse Decraene [syn.: *Polygonum sachalinense* F. Schmidt, *Reynoutria sachalinensis* (F. Schmidt) Nakai]

Fallopia sachalinensis, native to Northern Japan, Sakhalin and Ullung-do, arrived in Europe in the late 1850s (Bailey and Conolly 2000). Both hermaphrodite and female tetraploid clones are found throughout Europe (Bailey and Stace 1992; Mandák et al. 2003). However, hexaploid ($2n = 6x = 66$) and octoploid ($2n = 8x = 88$) clones have recently been found in the Czech Republic (Table 2) (Mandák et al. 2003). *F. sachalinensis* has limited genetic variation, with a few widespread clones and additional rarer and more restricted genotypes in its adventive range (Pashley et al. 2007; Mandák et al. 2005).

Fallopia × bohemica (Chrtek et Chrtková) J. P. Bailey [syn.: *Polygonum × bohemicum* (Chrtek et Chrtková) P. F. Zika et A. L. Jacobson, *Reynoutria × bohemica* Chrtek et Chrtková]

F. × bohemica has been grown in English gardens since at least 1872; the oldest herbarium record comes from the Manchester Botanic Garden (Bailey and Conolly 2000). The hybrid *F. × bohemica* is mainly hexaploid ($2n = 6x = 66$), but tetraploid, octoploid, aneuploid (Bailey and Stace 1992; Mandák et al. 2003) and a single decaploid (J.P. Bailey, unpublished result) clone have also been found (Table 2). This taxon exhibits the highest genetic variation of all Japanese Knotweed *s.l.* taxa (Mandák et al. 2005), due to its occurrence at three ploidy levels (with both sexes present at each) and the fact that the F1 hybrid has been produced a number of times at different places (Pyšek et al. 2003; Mandák

et al. 2005). In spite of its ability to be produced sexually, it is thought that its spread in Europe has been mostly vegetative. Surprisingly, the occurrence of *F. × bohemica* in its native Japan was only confirmed quite recently and described as *Reynoutria × mizushimae* Yokouchi (Bailey 2003).

Fallopia × conollyana J. P. Bailey

The hybrid between *F. japonica* var. *japonica* and *F. baldschuanica* has only been described recently (Bailey 2001). It has rarely become established spontaneously in NW Europe, but it is the most frequently produced seed on Japanese Knotweed *s.l.* plants in the UK and parts of mainland NW Europe.

Distribution of Japanese Knotweed *s.l.* in Europe

The European distributions of *F. japonica* and *F. sachalinensis* are given in Jalas and Suominen (1979; maps 422 and 423), and discussed in Beerling et al. (1994). More recent distribution data is available for *F. × bohemica*, and discussion of its world-wide distribution is found in Bailey and Wisskirchen (2006), see Fig. 1.

Identification of Japanese Knotweed *s.l.* taxa

The most obvious characters for identification are the leaf shape and size (Fig. 2; Table 3). Those of *F. sachalinensis* are by far the largest and have a deeply cordate base. The leaves of *F. japonica* var. *japonica* are much smaller, and always have a truncate base. Leaves of *F. × bohemica* are intermediate in shape and size. When looking at these features, it is important to use only the largest most fully developed stem leaves, since leaves associated with the inflorescences are remarkably similar in shape and size in all taxa.

Important additional characters are provided by an examination of the leaf surface. The abaxial leaf surfaces of *F. japonica* and *F. sachalinensis* differ both in their cuticle surfaces and their trichomes, with *F. × bohemica* being clearly intermediate. The cuticle may be studied using a simple peel technique in
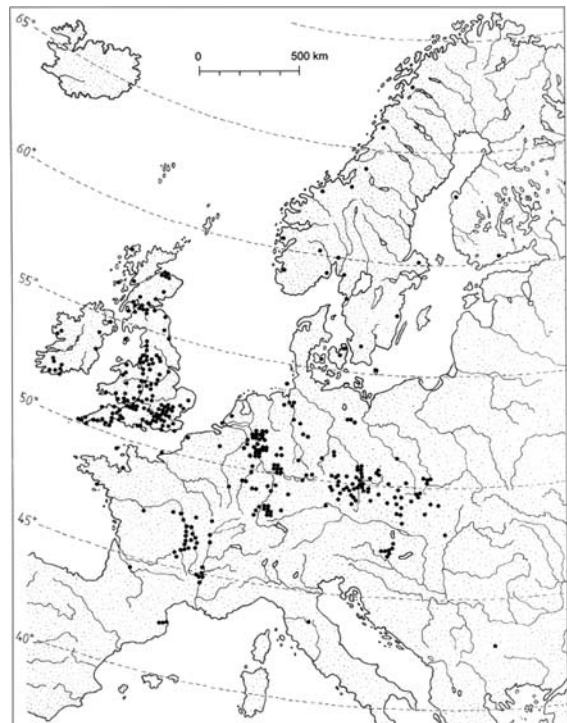

Fig. 1 Distribution of *F. × bohemica* in Europe (Bailey and Wisskirchen 2006; reproduced with the permission of Nordic Journal of Botany)

live material, or by using nail varnish replicas on dried material. *F. japonica* of both varieties has a very smooth cuticle surface with only a few striations (Fig. 3a, b and h) whereas that of *F. sachalinensis* has a very wrinkled cuticular surface (Fig. 3c, j). The cuticle of *F. × bohemica* is of intermediate appearance, Fig. 3d, f, g and i illustrate the range of striation found in different artificial and wild hybrids. Although the SEMs show these differences in spectacular detail, leaf epidermal peels are just as effective and much easier to carry out. A comparison of Fig. 3h *F. japonica* var. *japonica* and Fig. 3j *F. sachalinensis*, shows that the outline of the leaf epidermal cells which is so clear in *F. japonica*, is completely covered in *F. sachalinensis* by the heavily folded layer of cuticle. The *F. × bohemica* (Fig. 3i) is clearly intermediate.

The leaf abaxial trichomes are an even better means of distinguishing *F. × bohemica* from its parents. Whilst *F. japonica* has only heavily striate single celled 'bumps' along its veins and veinlets, *F. sachalinensis* often has long flimsy uniseriate hairs
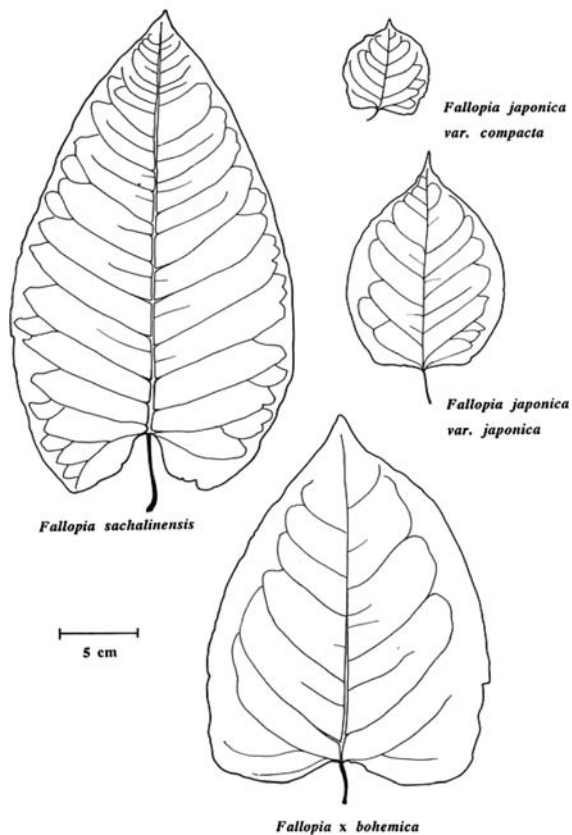
Fig. 2 Japanese Knotweed *s.l.* leaf shapes (reproduced by permission of Watsonia)

up to 14 cells long (Fig. 4a and b, respectively). The hybrids have stout ornamented hairs two to four cells long, which are satisfyingly intermediate (Fig. 4c, d), and generally visible with a good hand lens.

Thus a combination of leaf shape, abaxial trichomes and epidermal striation should identify these taxa. What they cannot distinguish are the standard *F.* × *bohemica* hybrids from back-crosses, which are generally aneuploid, and for which chromosome counts or flow cytometry remain the only means of identification.

Hybridisation

The presence in NW Europe, the USA and elsewhere of thousands of hectares of male-sterile *F. japonica* var. *japonica* can be viewed as an enormous inadvertent crossing experiment, since any related plant, native or alien, capable of pollinating it will have done so.

In Fig. 5 the four taxa in the first round of hybridisation, *F. japonica* var. *japonica*, *F. japonica* var. *compacta*, *F. sachalinensis* and *F. baldschuanica*, are shown with their chromosome numbers in boxes with bold unbroken lines. In the center are the initial hybridisations giving rise to the 4x and 6x *F.* × *bohemica* and the hybrid between the two varieties of *F. japonica*. These have all been

Table 3 Key characters for distinguishing *F.* × *bohemica* from its parents

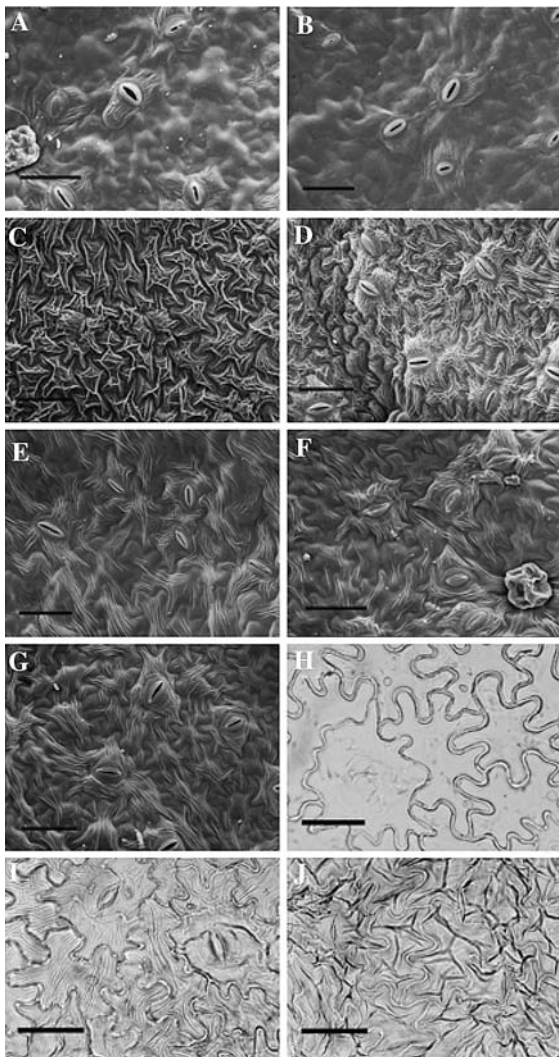|	*F. sachalinensis*	*F.* × *bohemica*	*F. japonica* var. *japonica*
Habit	Striking large plant—up to 4 m in height	2.5–4 m in height	2–3 m in height
Lower leaf size	Up to 40 × 22 cm	Up to 23 × 19 cm	10–15 cm long
	Length:width ratio 1.5–1.7	Length:width ratio 1.1–1.6	Length:width ratio 1–1.5
Lower leaf shape	Ovate to oblong, base cordate	Intermediate in shape, with a weak to moderately cordate base	Ovate, base truncate, apex acuminate
Leaf texture and appearance	Leaves thin with crumpled surface, somewhat glaucous abaxial surface	Leaves of intermediate texture and appearance	Leaves thicker, smooth with a leathery feel to them
Trichomes on lower leaf abaxial surface	Usually with scattered long flexuous hairs	Very distinct stout (1)2–3(5) celled pointed hairs	Single celled 'bumps' on leaf veins—sometime elongated, but always blunt
Epidermis of lower leaf abaxial surface (epidermal peels)	Cuticle highly 'crumpled', epidermal cell outlines barely visible	Clearly intermediate	Outline of epidermal cells clearly visible, striations restricted to edges of stomata

Fig. 3 Leaf abaxial surfaces (**a–g**) SEM, (**h–i**) light microscopy. Scale bar = 50 μm. **a** *F. japonica* var. *japonica*, **b** *F. japonica* var. *compacta*, **c** *F. sachalinensis* (2n = 44), **d** artificial hybrid between female *F. japonica* var. *compacta* and male *F. sachalinensis* (2n = 44), **e** artificial hybrid between female *F. sachalinensis*, and male *F. japonica* var. *compacta* (2n = 44), **f** artificial hybrid between female *F. japonica* var. *japonica* and male *F. sachalinensis* (2n = 66), **g** wild 6x *F.* × *bohemica* from Lye Green (2n = 66), **h** *F. japonica* var. *japonica*, **i** artificial hybrid between female *F. japonica* var. *japonica* and male *F. sachalinensis* (2n = 66), **j** *F. sachalinensis* (2n = 44)

confirmed by the production of artificial hybrids at University of Leicester, UK in the 1980s. Note also the interaction of *F. baldschuanica* with the other taxa. *F.* × *conollyana* has also been found as seed collected from *F. japonica* var. *japonica* in the Czech Republic (J.P. Bailey, unpublished result). *F. baldsch-uanica* has also crossed with *F. sachalinensis*, *F. japonica* var. *compacta* and 4x *F.* × *bohemica* to give highly sterile plants with 2n = 32.

At the bottom right of Fig. 5 the bold hatched box indicates backcrosses between the *F.* × *bohemica* and its parents as well as F2s produced by the *F.* × *bohemica*. This set of genotypes is something of a 'black box' since an enormous range of aneuploid plants is produced here. The intervarietal *F. japonica* hybrid is also able to back-cross, but is omitted so as not to further complicate the diagram.

Seed production

In order to investigate the true extent of the hybridisation, seed has been collected from the different taxa of Japanese Knotweed *s.l.*, germinated, and the chromosomes counted of both the parents and the seedlings. This work is ongoing and has been in progress for more than 25 years. The data presented here are a compilation of previously published work (Bailey 1994, 1999) and more recent unpublished data; all follow the technique in Bailey and Stace (1992). Careful note was taken of any suitable local pollen sources. Artificial pollinations were carried out in a cold greenhouse on potted plants, inflorescences being enclosed in a viskine bag, the opening plugged with non-absorbent cotton wool, and held in place with a wire tie. Although these data have not been collected in a systematic manner, they are the only relevant data available at this time. We hope their publication will stimulate a wider examination of this phenomenon.

It is interesting to note that, at least in Britain, Japanese Knotweed has not always been the regular seed producer that it is today. That Conolly (1977), was able to state "Fruit is set apparently only very occasionally and then only after a 'hot dry summer'…" implies that something has changed drastically over the last 30 years, since a *F. japonica* var. *japonica* plant without seed in the Autumn is now something of a novelty. We can identify three factors at work here. Firstly, *F. japonica* var. *japonica* as a late summer flowerer is particularly vulnerable to an early frost, the risk of which has greatly diminished since Conolly was writing. Secondly, there have been observable changes in

Fig. 4 Trichomes from the leaf abaxial surface, light microscopy of epidermal peels. Scale bar = 100 μm. **a** *F. japonica* var. *japonica*, **b** *F. sachalinensis*, **c** wild 8x *F.* × *bohemica*, Dolgellau (2n = 88), **d** wild *F.* × *bohemica*, Maam (2n = 66)
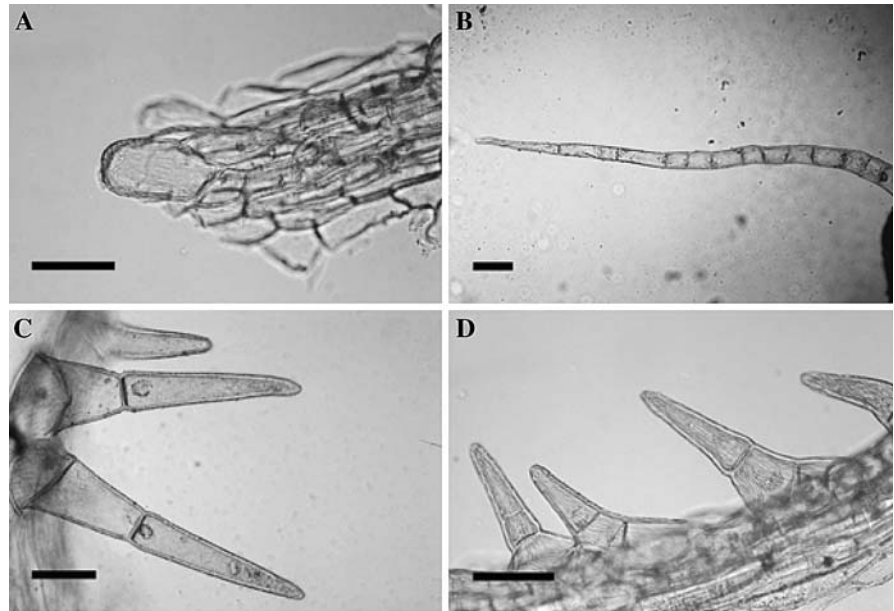
pollinator behavior, in the late seventies and early eighties, although very attractive to insects, the flowers did not appear to attract honeybees. Thirdly, there has been a great increase of garden planting of *F. baldschuanica*—the chief pollen source in Britain and parts of mainland Europe. It is suggested that the more recent attraction of honey bees, with their more rigorous gathering activities, the increasing availability of a suitable pollen source (*F. baldschuanica*), and the generally increasingly warm climate have combined to give the high levels of seed production that we see today. Seed set varies according to the weather, availability of suitable pollen and the fertility and sex expression of the individual clone (Bailey 1994). Plants of *F. japonica* var. *japonica* at Dolgellau (Wales), adjacent to hermaphrodite 6x *F.* × *bohemica*, have been observed with practically every flower fertilized.

In places where *F. japonica* var. *japonica* and male-fertile *F. sachalinensis* co-occur (extremely rarely in UK), the F1 *F.* × *bohemica* is formed and established spontaneously (Pashley et al. 2003). Seed production from rare hybridisation and from F1 hybrid plants cannot account for the widespread distribution of *F.* × *bohemica* in the UK, which is thought to be predominantly clonal. Viable seed produced by female 6x *F.* × *bohemica*, has only been found in the last 10 years in the wild in the UK, in spite of actively searching for it. 2006 marks the first

year that viable seed has ever been found on the hermaphrodite 6x *F.* × *bohemica* in the wild (J.P. Bailey, personal observation).

Seed on *F. japonica* var. *japonica* in the UK is generally the result of pollination by *F. baldschuanica*, to form the hybrid *F.* × *conollyana*. Whilst this is of great interest in itself, *F.* × *conollyana* very rarely becomes established, and would require chromosomal doubling to regain fertility. Seed produced on *F. japonica* var. *japonica* by pollination with *F.* × *bohemica* (Table 4) or F2 seed produced by *F.* × *bohemica* itself (Table 5) are much more significant. Table 4 shows that of the seed collected from *F. japonica* and studied at Leicester, euploid seed is only produced by pollination by *F. baldschuanica* (hybrid 2n = 5x = 54) and *F. sachalinensis* (hybrid 2n = 6x = 66) and the very occasional unreduced gamete from an *F.* × *bohemica* (hybrid 2n = 10x = 110), everything else is aneuploid with numbers between 68 and 108. Table 5 shows the same data for seed produced by both sexes of *F.* × *bohemica*. Pollination of 4x *F.* × *bohemica* by *F. baldschuanica* gives euploid and viable triploid progeny (2n = 3x = 32). Hexaploid *F.* × *bohemica* plants of both sexes give mostly aneuploid progeny, but even with the apparently euploid progeny (55, 77, 99, 110 and 121) it is not known whether or not they are genomically rather than numerically euploid. The 8x plants are more or less completely fertile,

Fig. 5 Simplified scheme showing hybridisation of Japanese Knotweed *s.l.* in the UK
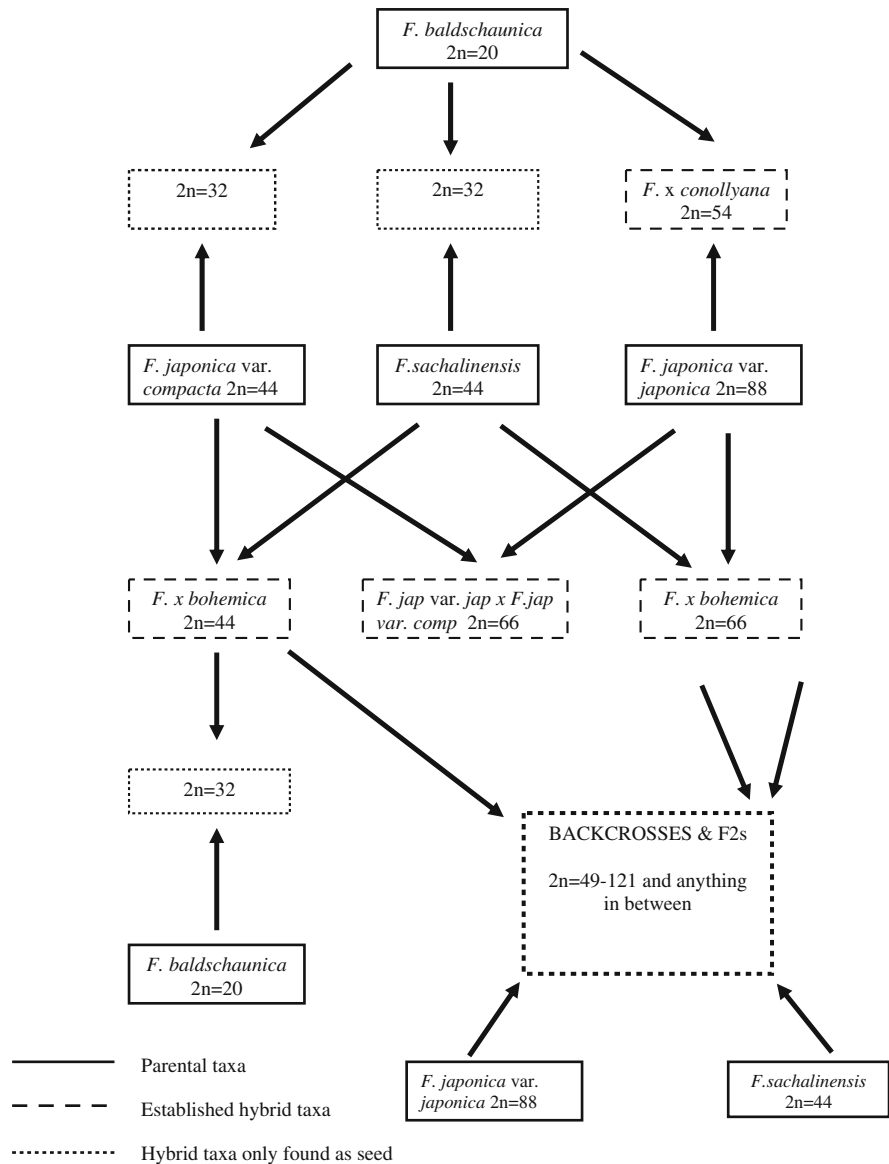
producing 6*x* or 8*x* progeny. The open pollinated 8*x* from Dolgellau, pollinated by *F. sachalinensis*, is of particular interest as it is a 6*x* *F.* × *bohemica* but with the proportions of *F. sachalinensis* and *F. japonica* genomes reversed compared to the normal 6*x* *F.* × *bohemica*.

It is a fundamental feature of these plants as studied in Britain, the Czech Republic and the USA, that the range of chromosome numbers of the plants produced from seed bear absolutely no resemblance to the established plants growing in the wild. Plants found growing in the wild are almost exclusively euploid with 2*n* = 44, 66, 88 and rarely 110—indeed

we know of only a handful of cases where this is not the case. This rather implies that in the areas studied so far, recruitment from seed is the exception rather than the rule.

Ecology

In Japan *F. japonica* (var. *compacta*) is a component of communities inhabiting extreme lava and ash sheets at high altitudes. *F. japonica* serves there as pioneer species facilitating the recruitment of other plant species (Adachi et al. 1996). *F. japonica* var.

Table 4 Chromosome numbers of seedlings produced in the UK by female *F. japonica* var. *japonica* ($2n = 8x = 88$)

Site	Open pollinated or artificial hybrid	Chromosome numbers ($2n$)	Nearest suitable pollen source/pollen used	Ploidy
Eleven sites in England and Wales	Open pollinated	$37 \times 2n = 54$	None apparent	Pentaploid
Leicester (England)	Artificial hybrid	$3 \times 2n = 54$	*F. baldschuanica*	Pentaploid
Brithdir (Wales)	Open pollinated	$5 \times 2n = 66$	*F. sachalinensis*	Hexaploid
Dolgellau (Wales)	Open pollinated	54, c68, 71, 76, 2×100, 101, 4×105, 107, 2×108, 2×110	$6x$ *F. × bohemica*	Aneuploid and decaploid
Albury Heath (England)	Open pollinated	54, 110	$6x$ *F. × bohemica*	Pentaploid and decaploid
Prior's Mesme (England)	Open pollinated	96	$6x$ *F. × bohemica*	Aneuploid
Leicester (England)	Artificial hybrid	110	$6x$ *F. × bohemica*	Decaploid
Buryas Bridge Cornwall (England)	Open pollinated	76, 3×78, 80, 81, 87, 106	$6x$ *F. japonica* var. *japonica* × *F. japonica* var. *compacta*	Aneuploid

Table 5 Chromosome numbers of seedlings produced in the UK by *F. × bohemica* plants of various ploidy levels and different sexes

Ploidy level of parental plant, gender and site	Open pollinated or artificial hybrid	Chromosome numbers ($2n$)	Nearest suitable pollen source/pollen used	Comments
$4x$ Preston (UK)	Open pollinated	$1 \times 2n = 44$	$4x$ *F. × bohemica*	Euploid
$4x$ Cirencester (UK)	Open pollinated	3×32, 6×44	*F. sachalinensis*	Euploid
$4x$ Cheshunt (UK)	Open pollinated	5×32	None apparent	Euploid
$4x$ Gomshall (UK)	Open pollinated	2×32	None apparent	Euploid
$6x$ ♀Dolgellau (UK)	Open pollinated	c74, 75, 2×77, 83, c88, 92, 94	$6x$ *F. × bohemica*	Aneuploid F2 plants
$6x$ ♂Dolgellau (UK)	Open pollinated	c85, 91, c95, c96, 98, 99, 121, c128	$6x$ *F. × bohemica*	F2 plants
$6x$ ♀Freshfield (UK)	Open pollinated	52, 2×53, c56	*F. sachalinensis*	Backcrosses
$6x$ ♀ Leicester plant 1	Open pollinated	c73, 76, c110	Many male plants	
$6x$ ♀ Leicester plant 2	Open pollinated	2×55	Many male plants	Could be pentaploids
$6x$ ♀ Leicester	Artificial hybrid	41	*F. baldschuanica*	Very weak dwarf plant
$8x$ ♀Dolgellau (UK)	Open pollinated	4×66	*F. sachalinensis*	Different type of $6x$
$8x$ ♀Dolgellau (UK)	Artificial hybrid	2×88	$8x$ ♂ *F. × bohemica*	Euploid

japonica spreads to man-made habitats, pastures and roadsides where it is one of the most difficult weeds in Japan (Nashiki et al. 1986). *F. sachalinensis* occurs in natural vegetation of coastal cliffs and mountain riversides, both of which are subject to natural disturbance, and on road banks and other places with man-induced disturbance. There are also pure stands of *F. sachalinensis* as pioneer vegetation on bare soils in human settlements (Sukopp and Starfinger 1995).

In Europe *Fallopia* taxa show a strong preference for man-made habitats (Mandák et al. 2004). However, among them, the hybrid *F. × bohemica* shows the highest proportion of localities outside human settlements (Mandák et al. 2004). Localities along roads and watercourses are the most frequent habitat for all taxa. A comparison of all three taxa in the Czech Republic (Mandák et al. 2004) showed that while *F. × bohemica* was the most common along water-courses, *F. japonica* var. *japonica* was most

often found along roads. Compared to other taxa, *F. sachalinensis* was more confined to gardens and parks where it was frequently planted as an ornamental. *F. japonica* var. *japonica* invaded more habitat types than *F. sachalinensis* and *F. × bohemica*, whilst *F. × bohemica* out competes the parental taxa at sites where both taxa co-occur (Bímová et al. 2004).

Fallopia species create dense monospecific stands, which almost entirely exclude native species, as stands expand through growth of an extensive rhizome system. Rapid stem growth (up to 15 cm/day) (Pergl 2001), dense stands and large leaves form a compact canopy, allowing little light to reach the ground, from early spring until the end of the season.

Plants contain high concentrations of phenolic compounds (e.g. *trans*-resveratrol) particularly in the rhizomes (Vastano et al. 2000; Vaher and Koel 2003) and thus can influence the presence of native species by allelopathy.

Bímová et al. (2004) showed that decrease in native species number is higher in species-rich communities (Fig. 6) due to the high competitive ability of *Fallopia* taxa. There are few other species in *Fallopia* stands, mainly spring geophytes (i.e. *Ficaria bulbifera*) or nitrophilous ruderals such as *Urtica dioica*, *Geranium robertianum* or *Aegopodium podagraria*. Field experiments have established that

no single mechanism is responsible for this exclusion of native taxa. Whilst shading appears to have a dramatic impact on juvenile plants, many seedlings are simply unable to establish in the dense *Fallopia* stands (Siemens and Blossey 2007).

Rhizome growth pattern, clonality and vegetative regeneration

All *Fallopia* Section *Reynoutria* taxa exhibit vigorous clonal growth that allows them to both outcompete surrounding species and rapidly colonize new areas. The basic unit of the rhizome system is a shoot clump that is of different sizes in the various *Fallopia* taxa (Fig. 7 shows the shoot clump structure). In general, the apex of the rhizome branch eventually becomes an aerial shoot. The annual aerial shoots produce a number of subterranean winter buds at the base by the end of the growth period, though small shoots and those which die during the growing period fail to produce such buds. One or more winter buds at the aerial shoot base sprout the following spring to form new aerial shoots close to the mother shoot. Aerial shoots are produced in almost the same position for several years, forming a small cluster of shoots (a shoot clump). When the shoot clump ceases to produce new aerial shoots and dies, some lateral
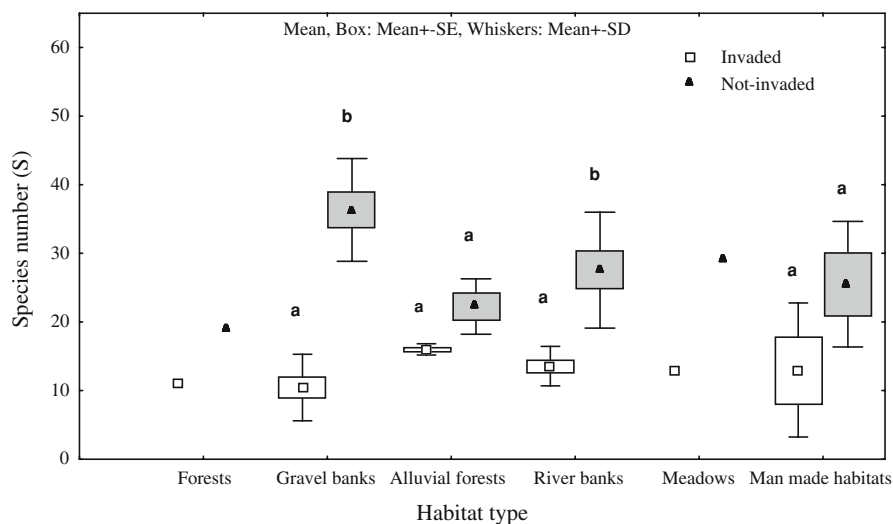

Fig. 6 Species number of pairs of invaded and non-invaded vegetation in particular habitat types (based on results of study located to North Bohemia, for details see Bímová et al. 2004). The same letters on boxes indicate non-significance differences

in paired *t*-test; forests: $n = 1$, gravel banks: $n = 9$, $df = 9$, $P = 0.00079$, alluvial forests: $n = 5$, $df = 4$, $P = 0.07961$, river banks: $n = 10$, $df = 8$, $P = 0.00111$, meadows: $n = 1$, man made habitats: $n = 4$, $df = 3$, $P = 0.03047$

Fig. 7 Structure of shoot clump with rhizome branches (according to Adachi et al. 1996, slightly modified). *SC* shoot clump, *AS* annual aerial shoot, *DS* dead shoot, *R* rhizome, *RBr* rhizome branch, *SB* stem bud, *WB* winter bud, *RBd* rhizome bud, *Rt* root
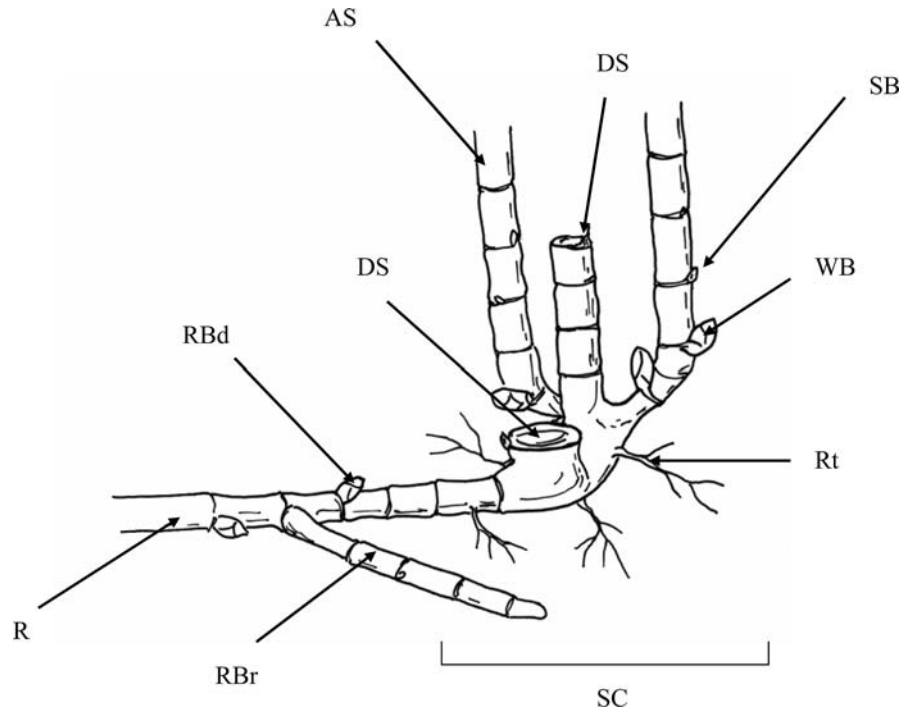
buds break dormancy and begin to grow horizontally as new rhizome branches. New rhizome branches sometimes extend more than 1 m. The apex of the new rhizome develops into a new aerial shoot and forms a new shoot clump (Adachi et al. 1996).

While *F. japonica* var. *japonica* has quite large shoot clumps connected by long thin rhizomes, *F. sachalinensis* produces smaller shoot clumps more closely connected and growing in rows. *F. × bohemica* combines the characteristics of both parents and possesses an intermediate patch structure with smaller shoot clumps than *F. japonica* and longer rhizome connections between individual shoot clumps than *F. sachalinensis*.

Fragmentation and dispersal of rhizomes by floods or human activity are the main means of spread, rhizome fragments of 1 cm length and 0.7 g weight being able to regenerate.

Fallopia taxa can also regenerate from stem fragments, though with lower regeneration rates. Fragmentation and subsequent regeneration may significantly increase their overall regenerative potential in aquatic or damp habitats. Bímová et al. (2003) found differences in the ability and speed of regeneration, as well as in the quality of shoots produced. Regeneration from stems was less efficient than from rhizomes in all taxa except *F. sachalinensis*.

F. × bohemica had the highest regeneration rate of all taxa (61%) and is the most successful in terms of regeneration and establishment of new shoots (Fig. 8). *F. japonica* var. *compacta* also showed high regeneration rates (52%). Other taxa showed generally lower regeneration rates (*F. japonica* var. *japonica* 39% and *F. sachalinensis* 21%), but under some treatments the percentage of regenerated segments was also high. *F. japonica* var. *japonica* rhizomes regenerated successfully in all three soil treatments but not in the water. The opposite pattern was found for its stems: they regenerated well in water but not in soil. *F. sachalinensis* and *F. × bohemica* both regenerated better in loam while the opposite was true for *F. japonica* var. *japonica*. *F. japonica* var. *compacta* produced the tallest and *F. × bohemica* the heaviest and most robust shoots.

Eradication

It is a fact that eradication of well-established Knotweed clones is extremely difficult, and can take several seasons of intensive treatment. There are several more or less effective treatments, including mechanical, chemical and their use in combination.

Fig. 8 Cumulative percentage regeneration for pooled rhizome and stem segment data. Percentage of the total number of segments, which regenerated up to the given date is shown on the *y*-axis (Bímová et al. 2003). *B = Fallopia × bohemica*, *C = F. japonica* var. *compacta*, *J = F. japonica* var. *japonica* and *S = F. sachalinensis*

Regeneration from stems and rhizomes (pooled data)
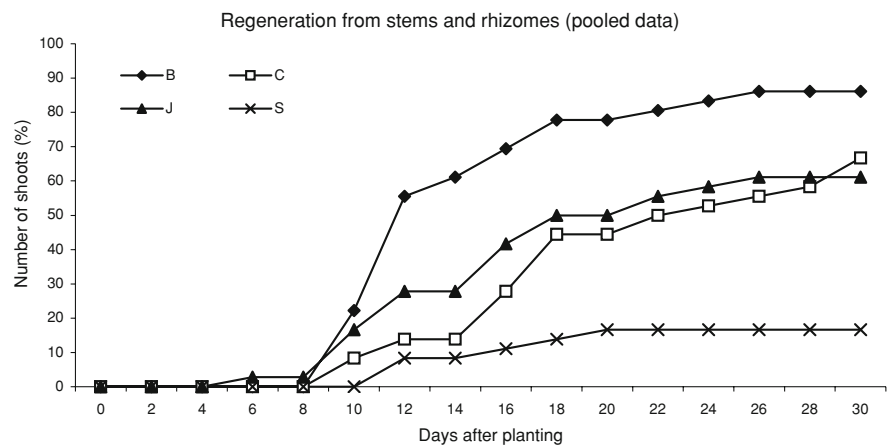
Mechanical treatments, such as cutting the above ground growth or digging the belowground parts, are insufficient. Combined "digging + glyphosate spraying" is the most efficient and least time consuming of all methods listed above (Child and Wade 2000; Bímová et al. 2001). Moreover, current work shows that the application of glyphosate at the flowering stage not only kills above ground growth but also gets translocated better into the rhizomes (B. Mandák, unpublished data).

Different *Fallopia* taxa respond differently to the various treatments (Bímová et al. 2001). *F. japonica* var. *japonica* was successfully controlled by a combination of digging and herbicide application. *F. sachalinensis* was easiest to control with both combined treatments, and the mechanical disturbance of rhizomes alone was almost sufficient to control it. The hybrid *F. × bohemica* was the most resistant and none of these treatments controlled it successfully. Bearing in mind the much greater genetic diversity of *F. × bohemica*, it is clear that this diversity should be represented in any future control trials.

Discussion

In this paper we show that, in spite of the limited genetic diversity of the original introductions, hybridisations in both the wide and narrow senses are rapidly making up for this deficit. The high vegetative regeneration rates of the different taxa greatly facilitates their spread. This combination of hybridization and vigorous vegetative spread resulted first in the production of the more invasive *F. × bohemica* at 3 (4 counting the extremely rare

decaploid) different ploidy levels, each capable of back-crossing with either parent or with each other. Given that the parent taxa exist at mixed ploidy levels (4*x* and 8*x F. japonica*, 4*x F. sachalinensis*) the stage is set for practically any combination/admixture of the *F. sachalinensis* and *F. japonica* genomes. It should of course be born in mind that these two species have very different areas of natural distribution, *F. sachalinensis* extending much further north than *F. japonica*, and *F. japonica* extending much further south than *F. sachalinensis*. In addition the 4*x* /Emphasis> *F. japonica* var. *compacta* (one parent in the 4*x F. × bohemica*) is a high altitude species. Studies of seed production (Tables 4, 5) indicate that massive rearrangements may be occurring, the hybrids and back crosses can exist at any number between 44 and 128, and apart from ploidy level, can also vary in the make up of their genomes in terms of the numbers and proportions of the *F. japonica* var. *japonica*, *F. japonica* var. *compacta* and *F. sachalinensis* chromosomes. Aneuploidy is common, and in this vast natural breeding experiment, is it too fanciful to suggest that nullisomic and polysomic lines are being continuously produced for the different adventive environments to screen?

Much of our basic knowledge of the Japanese Knotweed invasion has been gathered from research in Britain, the Czech Republic, and, more recently, Belgium (Bailey et al. 1996; Mandák et al. 2003, 2004, 2005; Tiébré et al. 2007a, b). But whilst we know much about the European populations, the North American ones are less well known, although they have recently attracted research attention (Forman and Kesseli 2003; Gammon et al. 2007; Grimsby et al. 2007). It is not yet possible to elucidate the

pattern of North American invasion without a combination of morphological, cytological and molecular data. Also, it is not clear whether the same patterns of invasion are occurring on the East and West coasts of the continent. Whilst Forman and Kesseli (2003) clearly demonstrate that sexual reproduction occurs in these plants in Massachusetts, they have not made a cytological analysis, so it is unclear whether male-fertile 8*x F. japonica* var. *japonica* is present and responsible or that the same pattern of hybridisation and backcrossing typical of Europe is occurring. One important question then, is has there really been a separate introduction of hermaphrodite 8*x F. japonica* var. *japonica* into the US? In the presence of the perfectly fertile 8*x F.* × *bohemica* (Bailey and Stace 1992) and a climate suitable for seed survival and germination, it can be conjectured that a cross between these two taxa followed by a series of back-crosses to the var. *japonica* could recreate the missing hermaphrodite. Seiger (1993; as cited in Forman and Kesseli 2003) found that seed collected from the wild in Washington DC appeared to be mostly hybrid with Russian Vine *F. baldschaunica* (several dozen chromosome counts have been made on rhizomes collected from the East and West Coasts of North America—J.P. Bailey, unpublished data) and with one exception they are a mixture of 6*x F.* × *bohemica* and its parents. At a recent meeting of the Western Society of Weed Research in Portland it became apparent that the majority of Knotweed in the Western US was actually *F.* × *bohemica*.

The extensive hybridisations detailed above all involve other alien taxa of varying degrees of relatedness, but what about crosses with native taxa? In the UK we have two native *Fallopia* taxa, *F. convolvulus* and *F. dumetorum*. The first of these is practically cleistogamous with minute anthers, whilst the other is now extremely rare in the UK. In continental Europe *F. dumetorum* is much more widespread. In the North America there are *F. scandens* and *F. cilinodis*, and in Australasia various *Muhlenbeckia* species, which the molecular phylogeny of Frye and Kron (2003) indicates are close relatives of *Fallopia*. Such hybridisations, if possible, would add another dimension to the Japanese Knotweed story, since some of the most significant amphiploid speciation events have involved an alien and a native, i.e. *Spartina anglica* and *Senecio cambrensis* (Raybould et al. 1991; Abbott and Lowe 2004). It should be born in mind that the

hybrid between *F. japonica* and *F. baldschuanica* is a most unlikely 'marriage', occurring as it does between an octoploid, herbaceous, rhizomatous perennial with a chromosome base number of 11 and a diploid woody perennial climber with base of 10, that have been geographically isolated from each other for millions of years. In this context there is no way of predicting what other related taxa around the world may be able to cross with *F. japonica*.

Another critical question, is: why, when so much viable and genetically diverse seed is produced, does so little of it ever germinate in situ in the wild in Europe? One theory is that the winters in many parts of the adventive range are not cold enough or too damp and that the seed rots in the soil. The USA with its great climatic range may be expected to contain regions suitable for the survival and germination of these ubiquitous seeds. Looking at the chromosome numbers of seedlings germinated from open pollinated wild British seed (Tables 4, 5), it can be seen that aneuploidy has little impact on the viability of these plants. Since the vast majority examined are fast growing and fit, it can safely be assumed that once established, they would have no problem surviving and spreading vegetatively, just like their euploid parents. Excessively high chromosome number also appears no bar to survival and dispersal in this group, since once established as a mature plant, with such strong powers of vegetative reproduction, further spread is possible. Currently, much of the area occupied by Japanese Knotweed *s.l.* is made up of a relatively small number of successful clones, but waiting in the wings to do battle may be armies of seeds with radically remixed genomes—the battle of the clones?

References

Abbott RJ (1992) Plant invasions, interspecific hybridization and the evolution of new plant taxa. Trends Ecol Evol 7:401–405

Abbott RJ, Lowe AJ (2004) Origins, establishment and evolution of new polyploid species: *Senecio cambrensis* and *S. eboracensis* in the British Isles. Biol J Linn Soc 82:467–474

Adachi N, Terashima I, Takahashi M (1996) Central die-back of monoclonal stands of *Reynoutria japonica* in an early stage of primary succession on Mount Fuji. Ann Bot 77:477–486

Bailey JP (1994) The reproductive biology and fertility of *Fallopia japonica* (Japanese Knotweed) and its hybrids in the British Isles. In: de Waal C, Child LE, Wade M, Brock JH (eds) Ecology and management of invasive riparian plants. Wiley, Chichester, pp 141–158

Bailey JP (1999) The Japanese Knotweed invasion of Europe; the potential for further evolution in non-native regions. In: Yano E, Matsuo K, Syiyomi M, Andow DA (eds) Biological invasions of ecosystem by pests and beneficial organisms. National Institute of Agro-Environmental Sciences, Tsukuba, pp 27–37

Bailey JP (2001) *Fallopia × conollyana* the railway-yard Knotweed. Watsonia 23:539–541

Bailey JP (2003) Japanese Knotweed *s.l.* at home and abroad. In: Child L, Brock JH, Prach K, Pysek P, Wade PM, Williamson M (eds) Plant invasions—ecological threats and management solutions. Backhuys, Leiden, pp 183–196

Bailey JP, Conolly AP (2000) Prize-winners to pariahs—a history of Japanese Knotweed s.l. (Polygonaceae) in the British Isles. Watsonia 23:93–110

Bailey JP, Stace CA (1992) Chromosome number, morphology, pairing, and DNA values of species and hybrids in the genus *Fallopia* (Polygonaceae). Plant Syst Evol 180:29–52

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

Bailey JP, Child LE, Wade M (1995) Assessment of the genetics variation of British populations of *Fallopia japonica* and its hybrid *Fallopia × bohemica*. In: Pyšek P, Prach K, Rejmánek M, Wade M (eds) Plant invasions: general aspects and special problems. SPB Academic, Amsterdam, pp 141–150

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

Beerling DJ, Bailey JP, Conolly AP (1994) Biological flora of the British Isles No.183 *Fallopia japonica* (Houtt.) Ronse Decraene (*Reynoutria japonica* Houtt.; *Polygonum cuspidatum* Sieb. & Zucc.). J Ecol 82:959–979

Bímová K, Mandák B, Pyšek P (2001) Experimental control of *Reynoutria* congeners: a comparative study of a hybrid and its parents. In: Brundu G, Brock J, Camarda I, Child L, Wade M (eds) Plant invasions: species ecology and ecosystem management. Backhuys, Leiden, pp 283–290

Bímová K, Mandák B, Pyšek P (2003) Experimental study of vegetative regeneration in four invasive *Reynoutria* taxa (Polygonaceae). Plant Ecol 166:1–11

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

Bleeker W (2003) Hybridization and *Rorippa austriaca* (Brassicaceae) in vision in Germany. Mol Ecol 12:1831–1841

Child L, Wade M (2000) The Japanese Knotweed manual. Packard, Chichester

Conolly AP (1977) The distribution and history in the British Isles of some alien species of *Polygonum* and *Reynoutria*. Watsonia 11:291–311

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus of the evolution of invasiveness in plants? Proc Natl Acad Sci USA 97:7043–7050

Ferris C, King RA, Gray AJ (1997) Molecular evidence for the maternal parentage in the hybrid origin of *Spartina anglica* C. E. Hubbard. Mol Ecol 6:185–187

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

Frye ASL, Kron KA (2003) *rbcL* phlyogeny and character evolution in Polygonaceae. Syst Bot 28:326–332

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

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

Hänfling B, Kollmann J (2002) An evolutionary perspective of biological invasions. Trends Ecol Evol 17:545–546

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

Jalas J, Suominen J (eds) (1979) Atlas Florae Europeae vol. 4 Polygonaceae. The committee for mapping the Flora of Europe, Helsinki

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

Kolbe JJ, Glor RE, Rodriguez-Schettino L, Chamizo-Lara A, Larson A, Losos JB (2004) Genetic variation increases during biological invasion by a Cuban lizard. Nature 431:177–181

Lee CE (2002) Evolutionary genetics of invasive species. Trends Ecol Evol 17:386–391

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

Mandák B, Pyšek P, Bímová K (2004) History of the invasion and distribution of *Reynoutria* taxa in the Czech Republic: a hybrid spreading faster than its parents. Preslia 76:15–64

Mandák B, Bímová K, Pyšek P, Štěpánek J, Plačková I (2005) Isoenzyme diversity in *Reynoutria* taxa: escape from sterility by hybridization. Plant Syst Evol 253:219–230

Nashiki M, Nomoto T, Meguro R, Sato K (1986) Effect of natural conditions and management of pastures on weed invasion in cooperative livestock farms in Japan. Weed Res 31:221–227

Pashley CH, Bailey JP, Ferris C (2003) Further evidence of the role of Dolgellau in the production and dispersal of Japanese Knotweed s.l. In: Child L, Brock JH, Prach K, Pyšek P, Wade PM, Williamson M (eds) Plant invasions—ecological threats and management solutions. Backhuys, Leiden, pp 197–211

Pashley CH, Bailey JP, Ferris C (2007) Clonal diversity in British populations of the alien invasive giant Knotweed, *Fallopia sachalinensis* (F. Schmidt) Ronse Decraene, in the context of European and Japanese plants. Watsonia 26:359–371

Pergl J (2001) Biometrická studie druhů rodu Reynoutria (Biometric study of Reynoutria taxa). Thesis, Czech Agriculture University, Prague

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

Raybould AF, Gray AJ, Lawrence MJ, Marshall DF (1991) The evolution of *Spartina anglica* CE Hubbard (Gramineae): origin and genetic variability. Biol J Linn Soc 43:111–126

Rhymer JM, Simberloff DS (1996) Extinction by hybridization and introgression. Ann Rev Ecol Syst 27:83–109

Rieseberg LH (1997) Hybrid origin of plant species. Ann Rev Ecol Syst 27:359–389

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

Seiger LA (1993) The ecology and control of *Reynoutria japonica* (*Polygonum cuspidatum*). Unpublished PhD thesis, The George Washington University, USA

Shiosaka H, Shibata O (1993) Morphological changes in *Polygonum cuspidatum* Sieb. et Zucc. reciprocally transplanted among different altitudes. Jpn J Ecol 43:31–37

Siemens TJ, Blossey B (2007) An evaluation of mechanisms preventing growth and survival of two native species in invasive Bohemian Knotweed (Fallopia × bohemica, Polygonaceae). Am J Bot 94:776–783

Sukopp H, Starfinger U (1995) *Reynoutria sachalinensis* in Europe and in the Far East: a comparison of the species ecology in its native and adventive distribution range. In: Pyšek P, Prach K, Rejmánek M, Wade M (eds) Plant invasions—general aspects and special problems. SPB Academic, Amsterdam, pp 151–159

Tiébré M-S, Bizoux J-P, Olivier JH, Bailey JP, Mahy G (2007a) Hybridization and morphogenetic variation in the invasive alien Fallopia (Polygonaceae) complex in Belgium (Western Europe). Am J Bot 94(11):1900–1910

Tiébré M-S, Vanderhoeven S, Saad L, Mahy G (2007b) Hybridization and sexual reproduction in the invasive alien *Fallopia* (Polygonaceae) complex in Belgium. Ann Bot 99:193–203

Vaher M, Koel M (2003) Separation of polyphenolic compounds extracted from plant matrices using capillary electrophoresis. J Chromatogr A 990:225–230

Vastano BC, Chen Y, Zhu NQ, Ho CT, Zhou ZY, Rosen RT (2000) Isolation and identification of stilbenes in two varieties of *Polygonum cuspidatum*. J Agr Food Chem 48:253–256