

Hybridisation and clonal diversity in some introduced *Fallopia* species (Polygonaceae)

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ABSTRACT

Molecular markers have been used to examine the amounts of genotypic diversity and sample inter-relationships among introduced invasive *Fallopia* taxa in Britain. The highly invasive Japanese Knotweed (*Fallopia japonica* var. *japonica*) appears to be represented by a single clone in Britain. In contrast, the related species Giant Knotweed (*F. sachalinensis*) and their hybrid (*F. × bohemica*) show much higher levels of clonal diversity. The difficulties of distinguishing between recurrent origins and hybrid fertility are discussed in relation to the variability of *F. × bohemica*. Finally, a very preliminary survey of native Asian *F. japonica* has detected high levels of genetic diversity. This, coupled with some limited morphological and cytological observations, suggests that a taxonomic revision of *Fallopia* section *Reynoutria* is needed to clarify the status of British *F. japonica*.

INTRODUCTION

Many plant communities contain a large proportion of introduced species. Human activities have vastly accelerated the movement of plants, carrying thousands of species, either deliberately or accidentally, across natural barriers such as oceans and mountain ranges. Many of these introduced species have become naturalised in the wild and some have become invasive weeds. In Britain, alien plants form a significant component of the flora, with a recent account reporting 1391 aliens out of the 2963 species recorded (Stace 1997).

Hybridisation is a frequent and important component of plant evolution and is widespread amongst natural populations (Rieseberg & Ellstrand 1993). Occasionally, alien species hybridise with native or other introduced species. In the *New Flora of the British Isles*, Stace (1997) recognised 770 angiosperm hybrids; of these, 58 involve at least one non-native taxon and 12 are hybrids between two alien species.

Hybridisation involving an introduced species, either with a native species or another introduced taxon, can give rise to hybrid combinations that would be unlikely to occur naturally due to a lack of sympatry of native ranges. The evolutionary implications of such post-introduction interactions are well documented and provide some of the clearest and most unequivocal examples of speciation such as the allopolyploid origins of *Senecio cambrensis* Rosser (Ashton & Abbott 1992; Harris & Ingram 1992) and *Spartina anglica* C. E. Hubb. (Marchant 1967; Raybould *et al.* 1991).

Introduced plants make useful model systems for the study of evolutionary processes such as hybridisation and speciation. Long term historical factors can be eliminated as confounding variables if the introduction dates are known. We can study evolutionary events as they are happening (or at least very recently after they have happened) rather than attempting to make inferences about distant historical events. In addition, as some introduced plants show weedy tendencies, it is also informative to study the amounts and partitioning of their genetic variation, as this can provide information on the population genetic consequences of colonisation.

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INVASIVE *FALLOPIA* SPECIES IN THE BRITISH ISLES*FALLOPIA JAPONICA* VAR. *JAPONICA* - JAPANESE KNOTWEED

Perhaps the most notorious invasive plant in the British flora is Japanese Knotweed, *F. japonica* var. *japonica*. This species is native to Japan, Taiwan and Northern China where it grows along roadsides, river banks and managed pasture, and it is one of the dominant colonists of lava fields (I.C.O.L.E. 1997). It is insect-pollinated and visitors to its flowers include flies, honey bees and wasps (Tanaka 1966). *F. japonica* var. *japonica*, as recognised in Britain today, was introduced to the British Isles in the 1850s (Bailey & Conolly 2000). Since then it has spread rapidly. The first recorded escape from cultivation was in 1886 (Storrie 1886); by 1996 it had been recorded from 55% of the 2862 10-km squares of the Biological Records Centre mapping system of the British Isles (B.R.C. unpublished data). Its spread has been documented by Conolly (1977) and Bailey & Conolly (2000).

In Britain *F. japonica* var. *japonica* occurs in a variety of man-made habitats such as canal sides, road verges, railway embankments and cemeteries, as well as stream and river banks. The abundance of this plant in urban areas and the costs associated with its control have given it much bad publicity. Its shoots are able to push up through asphalt and damage pavements, car parks and other public facilities and the height to which the plants grow leads to reduced visibility along roadsides and railways. Along water courses, decaying shoots can cause blockages and dense stands on riverbanks can impede flow in high water, thus increasing the risk of flooding. Since 1981 it has been a criminal offence to knowingly introduce *F. japonica* var. *japonica* into the wild.

FALLOPIA SACHALINENSIS - GIANT KNOTWEED

A closely related species to *F. japonica* var. *japonica* is *F. sachalinensis* (Giant Knotweed). *Fallopia sachalinensis* occurs in Britain, again stemming from a horticultural introduction (Conolly 1977; Bailey & Conolly 2000). It is native to eastern Asia where it is known from the southern part of Sakhalin Island, the southern Kurile Islands, the Japanese islands of Hokkaido and Honshu, and Korea (Sukopp & Starfinger 1995). It occurs in disturbed habitats from sea level to c. 1050 m and can be found along roadsides, forest edges, cliff tops and river sides, and like *F. japonica* var. *japonica*, it is one of the pioneer colonists of the "lava deserts" caused by volcanic eruptions (Sukopp & Starfinger 1995). *Fallopia sachalinensis* shows less invasive tendencies than *F. japonica* var. *japonica* and is still a rather uncommon plant in much of the British Isles (Bailey 1997). The first account of this species growing outside cultivation was published in 1896 (Davies 1896).

OTHER INTRODUCED *FALLOPIA* SPECIES

In addition to *F. sachalinensis* and *F. japonica* var. *japonica*, other members of the genus which have been introduced to the British Isles are *F. japonica* var. *compacta* and *F. baldschuanica*. *Fallopia japonica* var. *compacta* is a dwarf variety of *F. japonica* which is only rarely found outside of cultivation and is known from only 30 10-km squares of the Biological Records Centre mapping system (B.R.C. unpublished data). *Fallopia baldschuanica* (Russian-vine) was introduced from central Asia and is a common garden plant. It frequently occurs as discarded material on waste ground, but is rarely well naturalised (Stace 1997). Although reference will be made to these two taxa in this paper, we primarily concentrate on *F. sachalinensis* and *F. japonica* var. *japonica*.

REPRODUCTIVE BIOLOGY OF *FALLOPIA JAPONICA* AND *F. SACHALINENSIS*

Both *F. japonica* and *F. sachalinensis* are gynodioecious and can occur as either male-sterile (female) or hermaphrodite plants. In Britain, both female and hermaphrodite forms of *F. sachalinensis* and *F. japonica* var. *compacta* have been recorded, but only female plants of *F. japonica* var. *japonica* are known (Bailey 1994). *Fallopia sachalinensis* and *F. japonica* var. *compacta* thus have the capacity to reproduce both sexually and asexually (via vegetative propagation). However, despite the remarkable spread and abundance of *F. japonica* var. *japonica*, as no male-fertile plants are known from Britain, it appears that seed dispersal is not responsible for its success (no evidence for apomictic seed production has been recorded). Rhizome fragments

as small as 0.7 g are capable of regenerating into new plants (Brock & Wade 1992) and the transport and tipping of earth containing plant fragments and water-borne dispersal along river systems are considered to be the major means of dispersal (Conolly 1977).

HYBRIDISATION

Morphological and cytological studies have provided convincing evidence that hybrids between *F. japonica s. l.* and *F. sachalinensis* occur in the British Isles (Bailey & Conolly 1985). The hybrid between *F. japonica* and *F. sachalinensis* - *F. × bohemica* (Chrtek & Chrtková) J. Bailey - was first described in 1983 in the Czech Republic (Chrtek & Chrtková 1983). Two taxonomic crosses have been detected in Britain: plants with $2n = 66$ stemming from a cross between *F. japonica* var. *japonica* ($2n = 88$) and *F. sachalinensis* ($2n = 44$); and plants with $2n = 44$ stemming from a cross between *F. japonica* var. *compacta* ($2n = 44$) and *F. sachalinensis*. Very rarely, hybrids have also been reported between *F. japonica* var. *japonica* and *F. japonica* var. *compacta*; these plants have $2n = 66$ (Bailey & Conolly 1991). Interestingly, the majority of seed on British *F. japonica* var. *japonica* appears to have *F. baldschuanica* as the pollen donor and these progeny are $2n = 54$ (Bailey 1988). However, only one such hybrid is known to have become established in the wild in Britain (Bailey 1988). In contrast the hybrid between *F. japonica* var. *japonica* and *F. sachalinensis* is relatively common in Britain (Bailey *et al.* 1996). This hybrid shows some of the invasive tendencies of *F. japonica* var. *japonica* and occurs as both female and hermaphrodite plants.

Although morphological and cytological data have provided much useful information about this complex in Britain, many questions regarding the biology of these plants require additional data. For instance, while there is very good evidence that hybrids between some *Fallopia* taxa are present in the wild, we have no information on how many times such hybrids may have arisen, and indeed to what extent hybrid fertility allows for generations beyond the F1. In addition, little is known about the amounts and partitioning of genetic diversity in the parental taxa. The lack of male fertile plants of *F. japonica* var. *japonica* in Britain suggests that this taxon may have an extremely narrow genetic base and, at an extreme, could consist of a single widespread clone. In *F. sachalinensis*, which is represented in Britain as both hermaphrodite and female plants, the possibility for both sexual and asexual reproduction exists, although the relative importance of either is unknown. In this paper, we summarise our published and unpublished data which have attempted to shed light on some of these issues. Particular attention is paid to the relative importance of sexual versus asexual reproduction, and the dynamics of inter-specific hybridisation, concentrating primarily on *F. japonica* var. *japonica*, *F. sachalinensis* and their hexaploid ($2n = 66$) hybrid *F. × bohemica*.

LEVELS OF CLONAL DIVERSITY IN THE PARENTAL TAXA

FALLOPIA JAPONICA VAR. *JAPONICA*

To assess the levels of clonal diversity of *F. japonica* var. *japonica* in the British Isles, we collected 150 samples from localities ranging from Land's End to Shetland (Hollingsworth & Bailey, submitted). These samples were assayed for genetic variability using the arbitrary DNA fingerprinting technique RAPDs: Randomly Amplified Polymorphic DNA (Williams *et al.* 1990). Using ten primers, we amplified a total of 108 reproducible bands. No genetic variation was detected between any of the 150 samples collected from across the British Isles (Hollingsworth & Bailey, submitted). These data, coupled with the absence of male fertile individuals in the British Isles, provide strong evidence to support the hypothesis that the entire British population of *F. japonica* var. *japonica* consists of a single extraordinarily vigorous clone (Hollingsworth & Bailey, submitted). Furthermore, preliminary studies on a few samples from Europe and North America also showed the same genotype (genetic individual), which suggests that the distribution of this genotype extends well beyond the British Isles (Hollingsworth & Bailey, submitted). It seems possible that the enormous social and economic costs associated with the Japanese Knotweed invasion can be attributed to a single, ill-advised introduction.

FALLOPIA SACHALINENSIS

To provide a comparison with the data from *F. japonica* var. *japonica*, we carried out a more limited study on the levels of genotypic diversity in *F. sachalinensis*. A total of 30 plants were collected from twelve localities. Single samples were collected from most populations, but 17 plants were collected from the River Kelvin, Glasgow (Lanark, v.c. 77), and three samples were collected from the River Clywedog, Caerynwch (Merioneth, v.c. 48). All samples were analysed for genetic variation using 10 RAPD primers. In contrast to the data from *F. japonica* var. *japonica*, high levels of clonal diversity were detected. A total of 14 genotypes were recovered, and no genotype was shared between sites (Hollingsworth 1998). Two genotypes were detected from the 17 plants collected from the River Kelvin, with one of these genotypes restricted to a single plant (Hollingsworth *et al.* 1998). Two genotypes were detected from the three plants sampled along the River Clywedog. In the absence of additional well-sampled populations such as the River Kelvin it is difficult to say much on the partitioning of genetic variability in this species in Britain. However, in contrast to the simple situation of a single widespread clone of *F. japonica* var. *japonica*, *F. sachalinensis* appears to be genetically variable in Britain. Bailey & Conolly (2000) suggest that multiple introductions of *F. sachalinensis* to Britain may be the source of the greater levels of genetic variability of *F. sachalinensis* compared to *F. japonica* var. *japonica*. However, while M.L.H. agrees that this may be an important factor, she believes that there are presently insufficient data to eliminate sexual reproduction as a source of at least some of the variability. *Fallopia sachalinensis* has plants producing both functional ovules and pollen, and sets viable seed. The potential for sexual reproduction in this taxon in Britain is clear, and the correlation of this with genetic variability may well be meaningful. Progeny segregating from only a single heterozygous individual can lead to a multitude of genetic combinations.

LEVELS OF GENETIC VARIABILITY IN $6 \times F. \times BOHEMICA$

Genetic diversity can occur among hybrid individuals due to multiple origins (defined here as multiple gamete fusions) or hybrid fertility. Although meiosis is irregular in $6 \times F. \times bohemica$ (J. P. Bailey, unpublished) some seed set does occur, and the possibility of F₂ (and beyond) hybrid plants exists. To investigate the amounts and partitioning of genetic variability in $6 \times F. \times bohemica$ we have investigated two populations in detail, the River Kelvin, Glasgow (23 plants), and the River Wnion system, Dolgellau (Merioneth, v.c. 48) (36 plants). In addition we also collected a total of 16 samples from 14 other sites across Britain.

The River Kelvin site in Glasgow is of interest as, despite the fact that both *F. japonica* var. *japonica* and *F. sachalinensis* grow close to the hybrid, only female plants of the parental taxa are present. In contrast, *F. \times bohemica* occurs as both hermaphrodite and female plants along the river (Hart *et al.* 1997). Their co-occurrence may be due to independent colonisation, rather than being indicative of *in situ* hybridisation. This situation provides an opportunity to examine levels of sexual reproduction within the hybrid population, with reduced probabilities of multiple origins as a confounding variable.

Using arbitrary fingerprinting techniques (RAPDs and Inter-SSRs (inter simple sequence repeats)), five genotypes were detected from 23 *F. \times bohemica* plants (Hollingsworth *et al.* 1998). Of the four female plants sampled, all had the same genotype. The 18 hermaphrodite plants sampled had one of three different genotypes, with a final genotype restricted to a plant which did not flower (and thus could not be sexed). Given that we detected five different genotypes, and there are no male fertile individuals of the parental taxa at this site, it is possible that some of this genetic variability is due to hybrid fertility, rather than recurrent origins of the hybrid (Hollingsworth *et al.* 1998). Clearly, one cannot rule out the possibility that male fertile individuals of the parental taxa previously occurred in the area (although a thorough search of surrounding localities and checks of herbarium specimens found no evidence that this is the case (Hart *et al.* 1997)), or that the different hybrid genotypes are independent colonists of the river. However, given that the highest levels of genetic variation were detected in the only male fertile taxon present along the river (see the above sections on *F. japonica* var. *japonica* and *F. sachalinensis*), perhaps the most parsimonious explanation of the data is that at least some of the genetic variation in *F. \times bohemica* is attributable to local sexual reproduction.

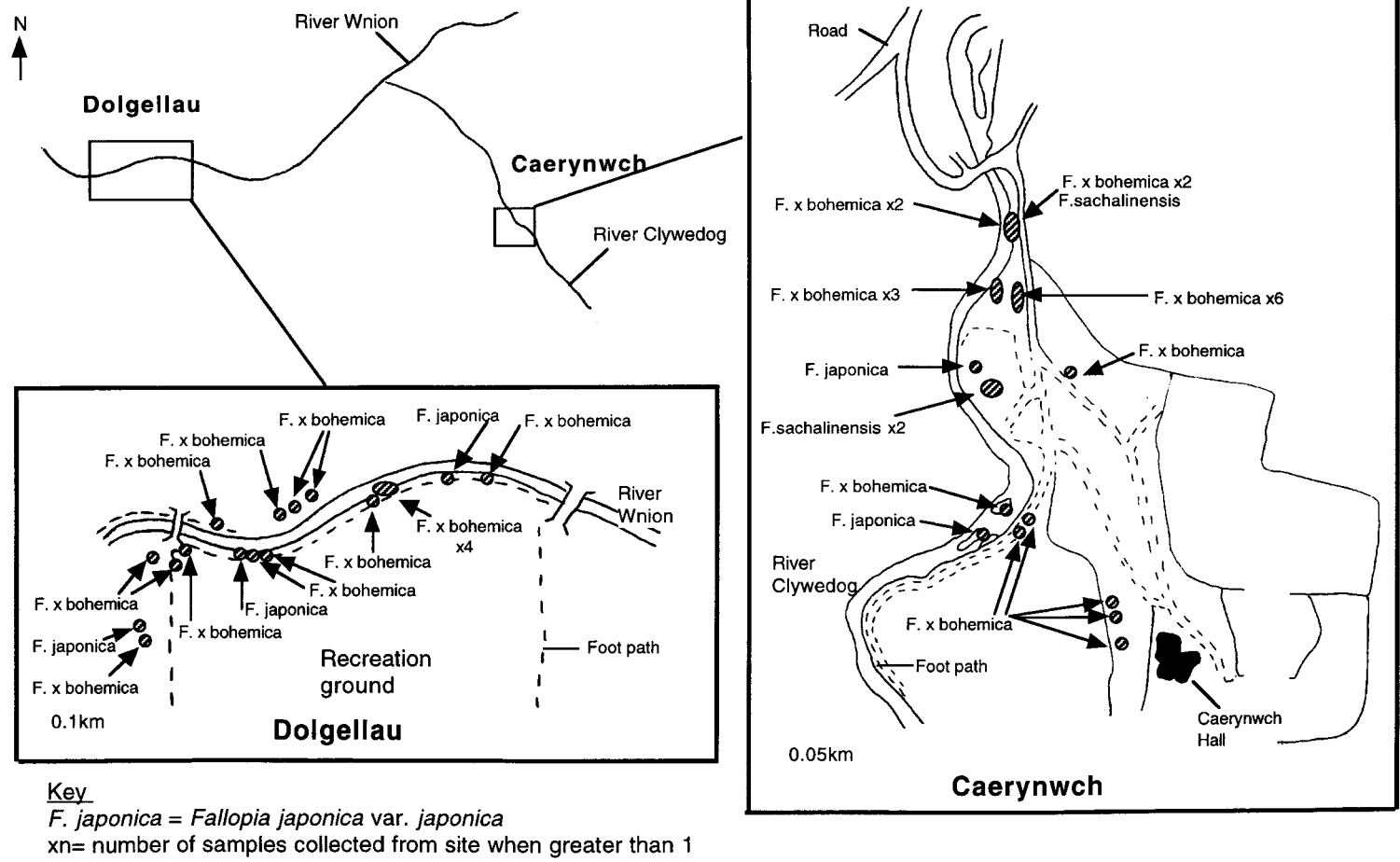


FIGURE 1. Location of the study population of *Fallopia* taxa along the River Wnion and the River Clywedog.

The second site we have examined in detail is a more complicated population along the River Wnion system (Fig. 1). *Fallopia japonica* var. *japonica* is common at many sites along this river system but there are only two known localities for *F. × bohemica* and one known locality for *F. sachalinensis*. At Caerynwch Hall on the River Clywedog (Fig. 1) female *F. japonica* var. *japonica*, hermaphrodite *F. sachalinensis* and both female and hermaphrodite *F. × bohemica* grow together along the edge of the river. The production of *Fallopia* seedlings has been recorded at this site (Bailey *et al.* 1996). The second area where *F. × bohemica* is found is 5 km downstream at Dolgellau (Fig. 1). Both female *F. japonica* var. *japonica* and female and hermaphrodite hybrids occur here, growing on the banks of the River Wnion and also in the surrounding field and recreational area; *F. sachalinensis* is not present at this site. Two samples of *F. japonica* var. *japonica*, three samples of *F. sachalinensis* and 20 samples of *F. × bohemica*, including six seedlings, were collected from Caerynwch Hall. Three samples of *F. japonica* var. *japonica* and 16 samples of *F. × bohemica* were collected from Dolgellau. All samples were examined for genetic variation using ten RAPD primers.

The five samples of *F. japonica* var. *japonica* all gave the standard genotype for this taxon in Britain, and are assumed to be ramets of the widespread genet. Two genotypes of *F. sachalinensis* were detected from the three samples from this site (see above). A total of 14 genotypes were detected from the 30 mature plants of *F. × bohemica*, with a further six genotypes detected from the six seedlings (Fig. 2a & b). The genetic structure of *F. × bohemica* along the river system suggests that both asexual and sexual reproduction have played a role in the history of this population. At some locations the same genotype was found in 2–5 ramets, with these samples being either adjacent to each other (i.e. genotype B5 Fig. 2b), or in two cases widely separated and found at both Caerynwch and Dolgellau (i.e. genotype B2 & B3; Figs 2a & 2b). This suggests the occurrence of both local (within site) and widespread (between site) dispersal via vegetative propagation. All six seedlings we examined had unique genotypes; only a single RAPD primer was required to discriminate between these samples, highlighting the sensitivity of the assay.

The presence of female *F. japonica* var. *japonica* with hermaphrodite *F. sachalinensis* at Caerynwch Hall, coupled with the high number of genotypes of *F. × bohemica*, suggest multiple hybridisation events as the source of this variability. However, other factors may be responsible. The presence of a large number of *F. × bohemica* genotypes found only at Dolgellau, where *F. sachalinensis* does not occur, implies the need for caution before multiple hybridisation events are invoked as the causal explanation for the genetic variability of *F. × bohemica* in this river system.

The private genotypes of *F. × bohemica* at Dolgellau could be explained by water-borne dispersal of seeds from Caerynwch Hall, or dispersal (vegetative or seed) from plants (unknown to us) along parts of the river system not sampled in this study. However, if multiple independent introductions to this site from outside of the river system (which seems unlikely given the large number of genotypes detected) are excluded, there is one other explanation for this variability: hybrid fertility.

Distinguishing between hybrid fertility and multiple origins as a source of genetic variability in *F. × bohemica* is difficult. Some insights may be gained from examining the chromosome numbers of the individuals in question. Although only a small sample, of the seven plants of *F. × bohemica* from the River Wnion system from which cytological data are available, all are $2n = 66$. Bailey (1997) suggested that the irregular meiosis of *F. × bohemica* would tend to lead to subsequent generations being aneuploids. Cytological analysis of seeds collected from isolated plants of *F. × bohemica* have shown a diverse array of chromosome numbers (Bailey 1990; Bailey *et al.* 1996). Thus one may suggest that if hybrid fertility was the source of the genetic variability in the *F. × bohemica* population from the River Wnion system, then many of these plants would be expected to be aneuploids. However, while the presence of aneuploids would presumably be good evidence that the plants were the result of an irregular meiosis, it should be stressed that the converse is not conclusive. Irregular meiosis could still give rise to $2n = 66$ individuals, and this number is presumably at least as likely as any other possible chromosome number from $2n = 66$ plants. As we have no information on the survival characteristics of aneuploid versus euploid *F. × bohemica* it is premature to place too strong an interpretation on the sole recovery of $2n = 66$ plants. It is possible that there is a disproportionate survival of euploid plants over aneuploids (but see J.P.B. opinion below).

From our limited survey of *F. × bohemica* from more widespread British localities (16 plants from 14 sites), no genotypes were shared between localities (Hollingsworth 1998). The only shared genotype was from Swansea, where, from our sample of three plants, two, growing 20 m apart, gave identical RAPD profiles.

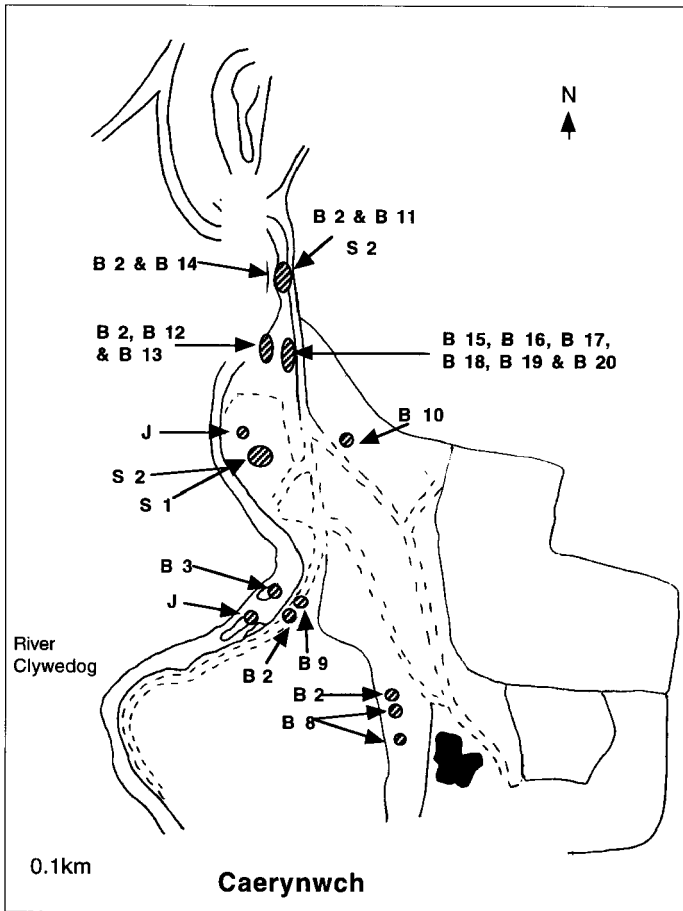


FIGURE 2a. Distribution of *Fallopia* genotypes along the River Clywedog, Caerynwch. J *Fallopia japonica* var. *japonica*; S n *Fallopia sachalinensis*, n refers to genotype code; B n *Fallopia* × *bohemica*, n refers to genotype code

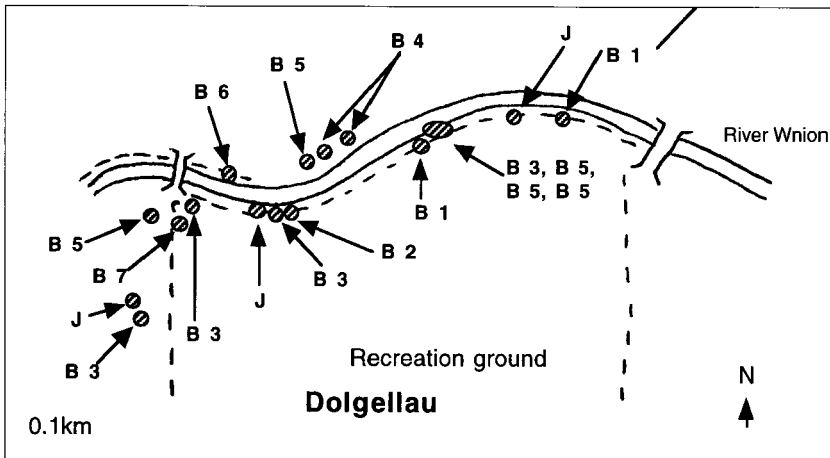


FIGURE 2b. Distribution of *Fallopia* genotypes along the River Wnion, Dolgellau. J *Fallopia japonica* var. *japonica*; B n *Fallopia* × *bohemica*, n refers to genotype code

Although further work is required (with more intensive within-site sampling), $6\times F. \times bohemica$ is a genetically variable taxon, and we conclude that sexual reproduction appears (in one way or another) to have been an important determinant of population structure. There remains, however, some question as to precisely how this has occurred, i.e. what is the relative importance of hybrid fertility versus multiple origins as the source of the genetic variability? This is one area where we as authors disagree. M.L.H. argues that the "jury is still out" and that the current data set (Hollingsworth, 1998; Hollingsworth *et al.* 1998) is *inadequate to distinguish between the two phenomena*. She does not believe the possibility of hybrid fertility giving rise to new genotypes can yet be excluded. In contrast, J.P.B. believes that the lack of aneuploids among those plants whose chromosome numbers have been counted is sufficient evidence against hybrid fertility contributing to the observed genotypic diversity. Unlike many biosystematic problems, this issue may be easily tractable. *Fallopia japonica* var. *japonica* appears to be represented in the British Isles by a single clone. Our RAPD and inter-SSR data have shown that there are numerous markers that distinguish this clone from our samples of *F. sachalinensis*. To distinguish between hybrid fertility and multiple origins as determinants of the genetic variability in $F. \times bohemica$ the following simple experiments need to be carried out. Controlled crosses should be made between *F. japonica* var. *japonica* and *F. sachalinensis*. The parents and large numbers of progeny should be examined for their RAPD and inter-SSR profiles (polyploid segregation tests require extensive progeny arrays). Although RAPDs and inter-SSRs are dominant markers and it is not possible to distinguish between homozygotes and heterozygotes for any given gene in individual samples, examination of the progeny of controlled crosses will allow homozygotes to be identified. Bands that are homozygous in *F. japonica* var. *japonica* should be present in all of its F1 hybrids. In contrast, those bands that are heterozygous in *F. japonica* var. *japonica* and segregate, will be present in some, but not all progeny. Following this line of reasoning, any wild-collected $6\times F. \times bohemica$ which lacks bands that are homozygous in *F. japonica* var. *japonica* (present in all of its controlled F1 progeny) may be considered to be of more complicated origin than F1 hybrids.

As a final point, however, it is important to note that there is one other potential confounding variable which is not so easily tested for, and this is whether the variability was generated *in situ* at all. It is not known to what extent material of $F. \times bohemica$ was originally distributed as seed for horticultural purposes (see Bailey & Conolly 2000). Although we feel that the evidence presented here for *in situ* sexual reproduction within Britain is virtually conclusive (as there are so many different genotypes present), it remains possible that some of the variability in this taxon in Britain could stem from horticulturally raised and distributed seed.

JAPANESE KNOTWEED IN ASIA

Although the results presented here provide insights into the reproductive biology and population structure of these invasive *Fallopia* taxa in Britain, there is a conspicuous absence of reference to native material. A detailed knowledge of the genotypic and genetic variability of these plants in their native range would be desirable for comparative purposes and may also serve to identify the geographic source of the introduced material. This is of value as *F. japonica* var. *japonica* is represented by only a single genotype in Britain and it may be particularly susceptible to an appropriate biological control. Identification of the source population may be a useful step in the search for such controls. However, not only is there a need for molecular work on the native material, there is also a need for more fundamental alpha taxonomic research. Whilst the morphological and cytological variation in British *Fallopia* taxa has received considerable attention (Bailey 1994; Bailey *et al.* 1996; Bailey *et al.* 1995; Bailey & Conolly, 1985; Bailey & Stace, 1992), the variability in the native range has only begun to be appreciated. Plants of *Fallopia japonica* collected from Japan and China show a high degree of intraspecific variability, in both cytological and morphological characters (Bailey 1999). Material of *F. japonica* var. *japonica* found in the British Isles today, although morphologically more similar to Japanese than Chinese material, is not typical of either (Bailey 1999). Japanese *F. japonica* are predominantly tetraploids ($2n = 44$), whilst the three Chinese and one Korean plant that have been counted so far are octoploid or decaploid ($2n = 88$ and $2n = 110$). With this in mind, in the following section we refer to native material as *F. japonica* var. '*japonica*' to distinguish it from British *F. japonica* var. *japonica*.

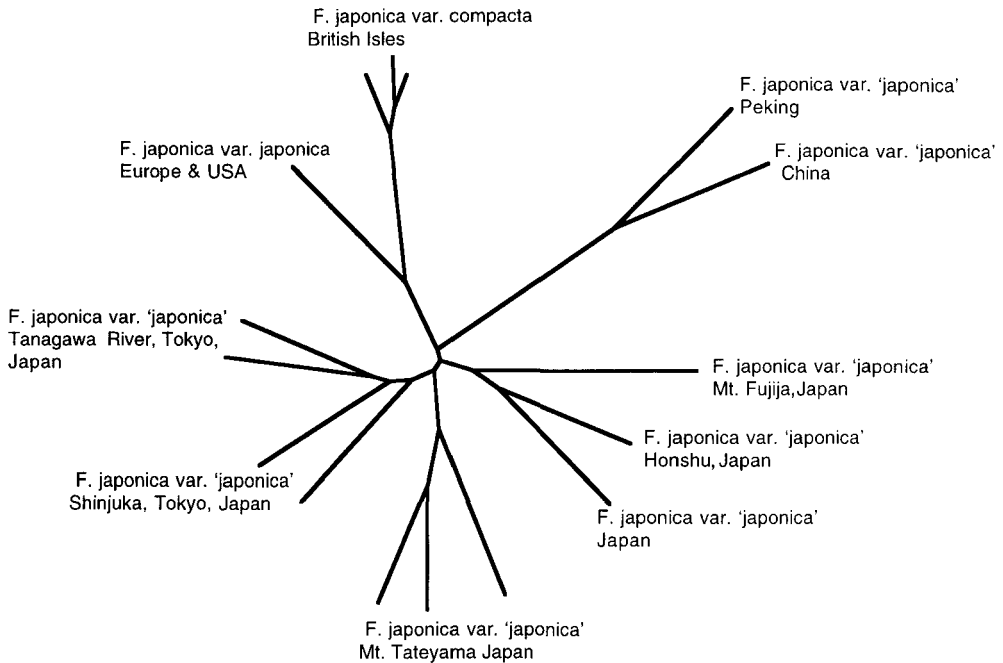


FIGURE 3. Neighbour Joining tree of *Fallopia japonica* s.l.

We carried out RAPD analysis using 10 primers on 14 native samples of *F. japonica* var. 'japonica' and compared them to the British genotype of *F. japonica* var. *japonica* and three British plants of *F. japonica* var. *compacta* (Table 1) (Hollingsworth 1998). The interrelationships of the samples are shown in Fig. 3. Although most of the genetic distance between samples in this data set is due to differences among individuals rather than groups of individuals (i.e. most of the genetic distance in the figure is on the terminal, rather than internal branches) a few points stand out. The three *F. japonica* var. *compacta* samples cluster together, and they together form a group with the introduced genotype of *F. japonica* var. *japonica*. The two Chinese samples cluster together, and there is a cluster containing the Japanese samples (although separated by only a very short branch from the other samples). A key point is that there is considerable diversity among the native samples, with much greater genetic distance between Japanese and Chinese samples of *F. japonica* var. 'japonica' than there is between the two different named varieties that occur in Britain.

This point is further emphasised by our studies on the chloroplast genome of these taxa. Using either sequence data from the chloroplast *trnL* intron or PCR RFLPs of the *trnK* intron, we are able to distinguish the chloroplast genomes of British material of *F. japonica* var. *japonica*, *F. japonica* var. *compacta* and *F. sachalinensis* (Hollingsworth *et al.* 1999). When we compared the *trnL* sequences of British *F. japonica* var. *japonica* and *F. japonica* var. *compacta* to five accessions of native *F. japonica* var. 'japonica' we did not detect either of the British chloroplast types among these native plants (Hollingsworth 1998). Instead, from the five native samples we detected five additional chloroplast types (all different), although relationships among these were poorly resolved in cladistic analyses, as most differences were autapomorphic (unique to individual samples). However, although there is little taxonomic information in these sequence data, the results corroborate those of morphology, cytology and RAPDs, i.e. that there is considerable diversity among native plants named as *F. japonica* var. *japonica*, and this exceeds the diversity between the British introduced varieties *F. japonica* var. *japonica* and var. *compacta*. Furthermore, no material which precisely matches the introduced and highly invasive *F. japonica* var. *japonica* is known to us from the native range.

TABLE 1 COLLECTION DETAILS OF *FALLOPIA* TAXA

Taxon	Locality	Chromosome No.
<i>F. japonica</i> var. ' <i>japonica</i> '	Peking Botanic Gardens, China	2n = 88
<i>F. japonica</i> var. ' <i>japonica</i> '	China (no further details known)	2n = 110
<i>F. japonica</i> var. ' <i>japonica</i> '	Honshu, Japan	2n = 44
<i>F. japonica</i> var. ' <i>japonica</i> '	Japan (no further details known)	
<i>F. japonica</i> var. ' <i>japonica</i> '	Mt. Fujija, Japan	2n = 44
<i>F. japonica</i> var. ' <i>japonica</i> '	Mt. Tateyama, Midagahara, Japan	2n = 44
<i>F. japonica</i> var. ' <i>japonica</i> '	Mt. Tateyama, Midagahara, Japan	
<i>F. japonica</i> var. ' <i>japonica</i> '	Shinjuku, Tokyo, Japan	
<i>F. japonica</i> var. ' <i>japonica</i> '	Shinjuku, Tokyo, Japan	
<i>F. japonica</i> var. ' <i>japonica</i> '	Tanagawa River, Tokyo, Japan	
<i>F. japonica</i> var. ' <i>japonica</i> '	Tanagawa River, Tokyo, Japan	2n = 44
<i>F. japonica</i> var. <i>japonica</i>	Leicester (v.c. 55), England	2n = 88
<i>F. japonica</i> var. <i>compacta</i>	Brecon (v.c. 42), Wales	2n = 44
<i>F. japonica</i> var. <i>compacta</i>	Bracken Hill, Surrey (v.c. 16), England	2n = 44
<i>F. japonica</i> var. <i>compacta</i>	Blarney Castle, Co. Cork (v.c. H4), Ireland	2n = 44

Thus there are different interpretations of what constitutes Japanese Knotweed (*F. japonica* var. *japonica*) in native (Asian) and introduced (Europe and North American) regions. To resolve and clarify these differences, a detailed monograph of section *Reynoutria* (*F. japonica* s.l. and *F. sachalinensis* s.l.) including morphological, cytological and molecular data is required. Widespread collections should be made from across the native range and the samples compared using both phenotypic and genotypic data. Only when an understanding of material in both introduced and native ranges is acquired will the most appropriate taxonomic designation for these samples become apparent. One safe prediction, however, is that there will undoubtedly be a more focused (narrow) consideration of what should be included under the name of *F. japonica* var. *japonica*.

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REFERENCES

- ASHTON, P. A. & ABBOTT, R. J. (1992). Multiple origins and genetic diversity in the newly arisen allopolyploid species, *Senecio cambrensis* Rosser (Compositae). *Heredity* **68**: 25–32.
- BAILEY, J. P. (1988). Putative *Reynoutria japonica* Houtt. × *Fallopia baldschuanica* (Regel) Holub hybrids discovered in Britain. *Watsonia* **17**: 163–164.
- BAILEY, J. P. (1990). Breeding behaviour and seed production in alien Giant Knotweeds in the British Isles, in ANON., ed. *The biology of invasive plants; a BES industrial ecology group symposium*, pp. 110–130. Richards, Moorehead and Laing, Ruthin.
- BAILEY, J. P. (1994). Reproductive biology and fertility of *Fallopia japonica* (Japanese Knotweed) and its hybrids in the British Isles., in DE WAAL, L. C., CHILD, L. E., WADE, P. M. & BROCK, J. H., eds. *Ecology and management of invasive riverside plants*, pp. 141–158. John Wiley & Sons, Chichester.
- BAILEY, J. P. (1999). The Japanese Knotweed invasion of Europe, the potential for further evolution in non-native regions, in YANO, E., MATSUO, K., SYIYOMI, M. & ANDOW, D. A., eds. *Biological invasions of ecosystems by pests and beneficial organisms*, pp. 27–37. Tsukuba: Institute of Agro-Environmental Sciences, Tsukuba, Japan.
- BAILEY, J. P., CHILD, L. E. & CONOLLY, A. P. (1996). A survey of the distribution of *Fallopia* × *bohemica* (Chrtek & Chrková) J. Bailey. *Watsonia* **21**: 187–198.

- BAILEY, J. P., CHILD, L. E. & WADE, M. (1995). Assessment of the genetic variation and spread of British populations of *Fallopia japonica* and its hybrid *Fallopia* × *bohemica*, in PYSEK, P., PRACH, K., REJMÁNEK, M. & WADE, M., eds. *Plant invasions - general aspects and special problems*, pp. 141–150. S.P.B. Academic Publishing, Amsterdam.
- BAILEY, J. P. & CONOLLY, A. P. (1985). Chromosome numbers of some alien *Reynoutria* species in the British Isles. *Watsonia* **15**: 270–271.
- BAILEY, J. P. & CONOLLY, A. P. (2000). Prize-winners to pariahs - A history of Japanese Knotweed *s.l.* (Polygonaceae) in the British Isles. *Watsonia* **23**: 93–110.
- BAILEY, J. P. & STACE, C. A. (1992). Chromosome number, morphology, pairing and DNA values of species and hybrids in the genus *Fallopia* (Polygonaceae). *Plant systematics and evolution* **180**: 29–52.
- BROCK, J. H. & WADE, M. (1992). Regeneration of Japanese Knotweed (*Fallopia japonica*) from rhizomes and stems: observations from greenhouse trials, in ANON., ed. *The 9th International Symposium on the biology of weeds*, pp. 85–94. (unpublished conference proceedings).
- CHRTEK, J. & CHRTEKOVÁ, A. (1983). *Reynoutria* × *bohemica*, nový krinzinec z celedi rdesnovitých. *Casopis národního musea. Oddíl Přírodovědy* **152**: 120.
- CONOLLY, A. P. (1977). The distribution and history in the British Isles of some alien species of *Polygonum* and *Reynoutria*. *Watsonia* **11**: 291–311.
- DAVIES, J. H. (1896). Notes on some casuals in Co. Antrim. *Irish naturalist* **5**: 309–312.
- HARRIS, S. A. & INGRAM, R. (1992). Molecular systematics of the genus *Senecio* L. 1: Hybridisation in a British polyploid complex. *Heredity* **69**: 1–10.
- HART, M. L., BAILEY, J. P., HOLLINGSWORTH, P. M. & WATSON, K. J. (1997). Sterile species and fertile hybrids of Japanese Knotweeds along the River Kelvin. *Glasgow naturalist* **23**: 18–22.
- HOLLINGSWORTH, M. L. (1998). *Clonal growth and hybridisation in some invasive Fallopia spp.* Ph.D. thesis, University of Leicester.
- HOLLINGSWORTH, M. L. & BAILEY, J. P. (submitted). Evidence for massive clonal growth in the invasive weed *Fallopia japonica* (Japanese Knotweed).
- HOLLINGSWORTH, M. L., BAILEY, J. P., HOLLINGSWORTH, P. M. & FERRIS, C. (1999). Hybridisation and chloroplast DNA variation amongst some invasive *Fallopia* (Polygonaceae) species. *Botanical journal of the Linnean Society* **129**: 139–154.
- HOLLINGSWORTH, M. L., HOLLINGSWORTH, P. M., JENKINS, G. I., BAILEY, J. P. & FERRIS, C. (1998). The use of molecular markers to study patterns of genotypic diversity in some invasive alien *Fallopia* spp. (Polygonaceae). *Molecular ecology* **17**: 1681–1692.
- I.C.O.L.E. (1997). *All you ever wanted to know about Japanese Knotweed but were afraid to ask*. Japanese Knotweed - a strategy for effective control. Loughborough University (unpublished workshop report).
- MARCHANT, C. J. (1967). Evolution in *Spartina* (Gramineae). 1. History and morphology of the genus in Britain. *Botanical journal of the Linnean Society* **60**: 1–24.
- RAYBOULD, A. F., GRAY, A. J., LAWRENCE, M. J. & MARSHALL, D. F. (1991). The evolution of *Spartina anglica* C. E. Hubbard (Gramineae): origin and genetic variability. *Biological journal of the Linnean Society* **43**: 111–126.
- RIESEBERG, L. H. & ELLSTRAND, N. C. (1993). What can molecular and morphological markers tell us about plant hybridisation? *Critical reviews in plant sciences* **12**: 213–241.
- STACE, C. A. (1997). *New Flora of the British Isles*, 2nd ed. Cambridge University Press, Cambridge.
- STORRIE, J. (1886). *The Flora of Cardiff*. Cardiff.
- 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 PYSEK, P., PRACH, K., REJMÁNEK, M. & WADE, M., eds. *Plant invasions - general aspects and special problems*, pp. 151–159. S.P.B. Academic Publishing, Amsterdam.
- TANAKA, H. (1966). The insect visitors of *Polygonum cuspidatum* Sieb. et Zucc. *Collecting and breeding* **28**: 141–143.
- WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. & TINGEY, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research* **18**: 6531–6535.

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