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

C. H. PASHLEY[1]

Institute for Lung Health, Department of Infection Immunity and Inflammation, University of Leicester, Glenfield Hospital, Groby Road, Leicester, LE3 9QP

J. P. BAILEY[2] and C. FERRIS

Department of Biological Sciences, University of Leicester University of Leicester, University Road, Leicester, LE1 7RH

ABSTRACT

Fallopia sachalinensis (F. Schmidt) Ronse Decraene was introduced to Europe during the 19th century, and whilst not as invasive as the notorious *F. japonica* var. *japonica* (Houtt.) Ronse Decraene, it is still an invasive plant capable of both clonal and sexual reproduction. It is also a significant pollen source for the male-sterile *F. japonica* var. *japonica*, producing the highly invasive hybrid *F.* ×*bohemica* (Chrtek & Chrtkova) J. P. Bailey. Given the lack of diversity in *F. japonica* var. *japonica*, *F. sachalinensis* is a potentially important source for introducing novel variation into *F.* ×*bohemica*. The majority of British *F. sachalinensis* was found to be one of two widespread genotypes, either a male-fertile or a male-sterile clone. In contrast there was a much higher level of genetic variation detected in both the rest of the introduced range and in the native range. Evidence is given for two separate introductions of native material into Britain; one from Niigata, Honshu, directly into Britain, and the other from Northern Japan, via St. Petersburg (Russia) to Europe.

KEYWORDS: genetic variation, introduced plants, Giant knotweed, Polygonaceae.

INTRODUCTION

The introduction of plants to new environments often brings together species that may not have been sympatric in their native range. These factors can lead to interspecific hybridisation occurring between a native and an invading plant species, or two invading species. This hybridisation can have numerous effects, the extremes being the evolution of new plant taxa e.g. *Senecio cambrensis* Rosser, which arose as an allohexaploid hybrid between Groundsel, *S. vulgaris* L., and the introduced species, Oxford Ragwort, *S. squalidus* L. (Harris & Ingram 1992), or the extinction of the native species (Sakai *et al.* 2001).

Genetic variation may be lower in newly established introduced populations because either the number of plants introduced is often small or genetic drift during colonization may reduce genetic variation in the newly established population. Thus a newly established population is likely to be much less genetically diverse than the population from which it is derived (Sakai *et al.* 2001). When an introduced ornamental plant can be easily propagated by vegetative means, such as is the case with Japanese Knotweed *sensu lato* (*s.l.*) the likelihood is that very little material is introduced at any one time.

Clonal growth is a widespread phenomenon in the plant kingdom, which can also occur in lichens, fungi and some groups of lower animals. Plants can reproduce clonally in many ways, including the formation of ramets on creeping stems above or belowground, and the vegetative production of plantlets and bulbils on aerial plants (Stuefer *et al.* 2001). For the plants that comprise the group Japanese Knotweed *s.l.*, clonal spread is achieved through large underground rhizomes, and in the case of the most renowned member of the group,

[1]Corresponding author present address
e-mail:chp5@leicester.ac.uk
[2]e-mail: jpb@leicester.ac.uk

TABLE 1. LOCATION DETAILS OF *F. SACHALINENSIS*, SEX OF THE PLANT WHERE KNOWN, CHLOROPLAST HAPLOTYPE (MPH) AND GENOTYPE (G) RESULTS

Plant ID	MPH	G	Location	Grid reference/ location	Sex
B9	6	i1	Caerynwich, Water gardens, Wales	SH7518	male-fertile
B10	6	i1	Caerynwich, Wales	SH7518	male-fertile
B11	6	i1	Congerstone, Leicestershire, England	SK3605	male-fertile
B12	6	i4	Wanlip, Leicestershire, England	SK5919	
B13	6	i1	Amroth Beach, Wales	SN1608	male-fertile
B14	6	i1	Amroth, Wales	SN1608	male-fertile
B15	6	i4	Cwrt Newydd, Wales	SN4947	male-sterile
B16	6	i4	Howey village, Wales	SO0558	male-sterile
B17	6	i1	Nant Y Frith, Wales	SO2654	male-fertile
B18	6	i1	Cirencester Roundabout, England	SP0201	male-fertile
B19	6	i1	Cirencester Abbey grounds, England	SP0202	male-fertile
B20	6	i6	Guys Cliff, Warwick, England	SP2865	male-sterile
B21	6	i6	Guys Cliff, Warwick, England	SP2865	male-sterile
B22	6	i4	Epwell, Warwicks, England	SP3540	
B23	6	i4	Nuneaton, Warwickshire, England	SP3592	
B24	6	i4	Tiscott, Cornwall, England	SS2309	male-sterile
B25	6	i4	Coed-y-felin Woods, Wales	ST1882	male-sterile
B26	6	i4	Scarrier, Cornwall, England	SW7244	male-sterile
B27	6	i4	Polzeath, Cornwall, England	SW9378	male-sterile
B28	6	i13	Christchurch road, South Hampshire, England	SZ2392	male-fertile
B29	6	i1	Hempstead Old Rectory, England	TG4028	male-fertile
B30	1	i8	Colchester, Bunting's nursery, England	TM0025	male-sterile
B31	1	i16	Colchester, Bunting's nursery, England	TM0025	male-fertile
G1	6	i15	Aachen, Germany		male-fertile
G2	6	i14	Aachen, Stadtwald, Germany		male-fertile
G3	6	i9	Aachen, Stadtwald, Germany		male-sterile
G4	6	i3	Leverkusen, Germany		
G5	6	i10	Umminger, Germany		
C1	6	i5	Konopiste, (Benesov), Czech Republic		male-sterile
C2	6	i4	Kostelec, (Kolin,), Czech Republic		male-sterile
U1	6	i7	Aberdeen, Washington, USA		male-sterile
U2	6	i12	125th Street, Seattle, USA		male-fertile
J1	1	n2	Joetsu, Niigata, Honshu, Japan	N 37 06 E 138 15	
J2	1	n13	Joetsu, Niigata, Honshu, Japan	N 37 06 E 138 15	
J3	4	n10	Yonezawa Rd, Fukushima, Honshu, Japan	N 37 50.37 E 140 22.36	
J4	4	n11	Yonezawa Rd, Fukushima, Honshu, Japan	N 37 50.37 E 140 22.36	male-fertile
J5	5	n15	Zao Quasi National Park, Yamagata, Japan	N 38 07.73 E140 26.19	
J6	2	n8	Mt. Aoba-yama, Miyagi, Honshu, Japan	N 38 15.00 E 140 53.14	male-fertile
J7	5	n5	Yamagata, Japan	N 38 38.97 E 140 10.36	male-fertile
J8	2	n12	Eai River Valley, Miyagi, Honshu, Japan	N 38 44.05 E 140 46.12	
J9	3	n3	Hirosaki, Iwaki river, Aomori, Honshu, Japan	N 40 36.00 E 140 26.37	male-sterile
J10	3	n14	Hirosaki, Iwaki river, Aomori, Honshu, Japan	N 40 36.00 E 140 26.37	male-fertile

TABLE 1 CONTINUED

Plant ID	MPH	G	Location	Grid reference/ location	Sex
J11	6	n1	Memuro, Hokkaido, Japan	N 42 50.12 E 143 00.44	
J12	6	n7	Memuro, Hokkaido, Japan	N 42 50.12 E 143 00.44	
J13	6	n6	Memuro, Hokkaido, Japan	N 42 50.12 E 143 00.44	
J14	6	n4	Ishikari, Hokkaido, Japan	N 43 12.24 E 141 23.11	
J15	6	n9	Ishikari, Hokkaido, Japan	N 43 12.24 E 141 23.11	
J16	6	n16	Ishikari, Hokkaido, Japan	N 43 12.24 E 141 23.11	

i – genotype possessed by a plant from the introduced range
n – genotype possessed by a plant from the native range

Fallopia japonica var. *japonica* (Houtt.) Ronse Decraene, as little as 0·7g is required to give rise to a new plant (Brock & Wade 1992). Well-established plants develop woody stocks with a central taproot that penetrates vertically into the ground. Buds form on the stock and woody rhizomes between autumn and winter, and emerge to give vertical shoots the following spring. Woody stocks continue to increase in width by secondary thickening with age, and also produce lateral creeping rhizomes within their first year (Beerling *et al.* 1994). The rhizomes of a mature plant can extend up to 7m away from the parent plant (Child & Wade 2000).

Japanese Knotweed *s.l.* comprises taxa from the genus *Fallopia* Adans., section *Reynoutria* (Houtt.) Ronse Decr. This includes *F. japonica*, which in Britain can be found as both var. *japonica* and var. *compacta* (Houtt.) Ronse Decr. (Hook. f.) J. P. Bailey; *F. sachalinensis* (F. Schmidt) Ronse Decr., which is also commonly known as Giant Knotweed; the hybrid between *F. japonica* and *F. sachalinensis* called *F. ×bohemica* (Chrtek & Chrtková) J. P. Bailey; and any backcrosses these plants may form (Bailey & Conolly 2000). The different species were introduced to Britain from parts of Asia at various times during the 19th century whilst the hybrids are believed to have arisen since the introduction of the parental species.

Fallopia japonica var. *japonica*, the most common of the Japanese Knotweed *s.l.* plants in Europe, was shown to be represented in Britain by a single male-sterile octoploid (2n = 88) clone. This clone was also found in France, Germany, the Czech Republic and the U.S.A. (Hollingsworth & Bailey 2000a), and in Holland (Pashley 2003). In contrast there appeared to be higher levels of genetic variation within the UK in the related Giant Knotweed, *F. sachalinensis* (Hollingsworth 1998; 2000b), than in the other introduced *Fallopia* species.

Fallopia sachalinensis was first recorded in the wild in Germany in 1869, in the Czech republic in 1869, and in Great Britain in 1896 (Sukopp & Starfinger 1995). Examination of historical plant collection and distribution records suggest that there were at least two routes by which *F. sachalinensis* may have arrived in Europe. The first is via St. Petersburg, Russia, and the second route is via Kew Botanic Gardens, London (Bailey & Conolly 2000). *F. sachalinensis* has not spread to the same extent as *F. japonica* var. *japonica*. Instead, its occurrence appears to reflect many independent primary escapes (Conolly 1977). *F. sachalinensis* may not be as invasive as *F. japonica* var. *japonica*, but it can be a significant pollen source for the male-sterile *F. japonica* var. *japonica*, producing the hybrid *F. ×bohemica* (Chrtek & Chrtková) J. P. Bailey. *F. ×bohemica* is gaining in notoriety, and is thought to be at least as invasive as *F. japonica* var. *japonica* (Brabec & Pyšek 2000; Bímová *et al.* 2001). Given the lack of diversity in *F. japonica* var. *japonica*, and the rarity of *F. japonica* var. *compacta* (Hook. f.) J. P. Bailey

(the alternative *F. japonica* parent for *F.* ×*bohemica*), the genetic composition of *F. sachalinensis* is an important factor for potentially introducing novel variation into *F.* ×*bohemica*.

AIMS

The main aims of this study were to estimate levels of genetic variation among British *F. sachalinensis*, in order to determine whether sexual reproduction or clonal spread best explain the current distribution. We have compared genetic diversity within the British plants to a limited number of plants from other countries where *F. sachalinensis* has been introduced, namely Germany, the Czech Republic, and the USA, to see if the genetic diversity within Britain is similar or different from the rest of the introduced range. We have investigated the levels of genetic variation among native *F. sachalinensis* from Japan in order to compare levels of genetic diversity between the introduced and native range, and to potentially determine the region of Japan from which the introduced plants originated.

MATERIALS AND METHODS

PLANT MATERIAL

Leaf material was obtained from 56 *F. sachalinensis* accessions, 40 from the introduced range and 16 from the native, as detailed in Table 1. The introduced plants include 31 individuals representing 21 populations from Britain, five from Germany, two from the Czech Republic and two from the USA. The 16 native plants were chosen to represent the geographical distribution of the plants within Japan. Voucher specimens for each accession were collected and deposited at the University of Leicester herbarium (**LTR**).

DNA EXTRACTION

Total genomic DNA was isolated from approximately 100 mg of fresh leaf tissue using the DNeasy plant mini kit (QIAGEN, Valencia, CA). Duplicate extractions were prepared for each plant.

CHLOROPLAST RFLP ANALYSIS

The chloroplast regions *trn*C-*trn*D and *trn*F-*trn*V were amplified using the universal primers of Demesure *et al.* (1995) and Dumolin-Lapegue *et al.* (1997) respectively. PCR reactions were carried out in a total volume of 25 µL containing 2·5 µl 10 × NH_4 reaction buffer (TaKaRa), 2·5 µl dNTPs (2 mM), 0·5 µl of each primer (10 µM), 0·5 units of Ex taq polymerase (TaKaRa) and 2·5 µl of genomic DNA (1 ng/µl). Amplification was carried out using the following PCR profile: 1 cycle of 94 ºC for 4 min; 32 cycles of 45s at 94 °C, 45s at 58 °C, and 2 min 30s at 72 °C; a soak at 72 °C for 10 min; and finally held at 10 °C for 30 min.

Chloroplast PCR products were digested for a minimum of one hour at 37 °C, with the restriction enzyme *Hin*fI following the method of Ferris *et al*. (1993). 10 µl of the restriction digest products were then run on 3% MetaPhor XR agarose gel for *trn*C-*trn*D products or 1·6% multipurpose agarose gel for *trn*F-*trn*V. Fragments were visualized by staining with ethidium bromide (0·5 µg/ml).

INTER-SIMPLE-SEQUENCE REPEAT (ISSR) ANALYSIS

Thirty primers from the University of British Columbia (UBC) primer set nine and twelve from the laboratories of Professor Mike Wilkinson (University of Reading) were screened. A final set of seven polymorphic primers was selected on the basis of reproducibility between duplicate DNA extractions from the same plant, and between different PCR runs (details available on request). PCR reactions were carried out in a total volume of 25 µL containing 2·5 µl 10 × NH_4 reaction buffer (Bioline), 2·5 µl dNTPs (2 mM), 1·25 µl magnesium chloride (50 mM), 0·33 µl of primer (15 µM), 1 unit of BIOTAQ DNA polymerase (Bioline) and 10 µl of genomic DNA (1 ng/µl). Amplification was carried out using the following PCR profile: 1 cycle of 94 ºC for 4 min; 40 cycles of 20s at 94 °C, 30s at X °C, and 1 min at 72 °C; a soak at 72 °C for 7 min; and finally held at 4 °C for 30 min. X is primer specific (details available on request). Reactions were performed in duplicate using the independently extracted DNA, and 10 µl from each reaction, with duplicates placed in adjacent wells, were run on a 1·6% agarose gel with a 1 kb ladder in the outer wells. Products were visualized by staining with ethidium bromide (0·5 µg/ml). Bands were considered to be reproducible when the same DNA pattern was obtained using the two different DNA isolates. A table of presence/absence of ISSR fragments was constructed and is available from the corresponding author on request.

DATA ANALYSIS

RFLP bands were scored by eye and recorded in a binary data matrix table, as presence (1) and absence (0). Each combination of these bands for a given chloroplast product is referred to as a haplotype. The combination of these haplotypes is referred to as a multi-primer-haplotype (MPH) and is intended to represent the chloroplast type of the plants in which it was found.

Winboot (Yap & Nelson 1996) was used to bootstrap the ISSR binary data 1000 times using the Jaccard's coefficient to produce confidence values. The companion program WinDist was then used to produce a matrix of pair-wise genetic distances between all individuals using the complement of the Jaccard's similarity coefficient. A Neighbour-Joining tree was drawn from the resulting matrix using the NEIGHBOUR option in PHYLIP version 3.6a3 (Felsenstein 2002) and TreeView version 1.6.6 (Page 1996). Additionally, the number of differences between each ISSR genotype was calculated and analysed using MINSPNET (Excoffier & Smouse 1994) to produce a minimum spanning tree.

RESULTS

CHLOROPLAST HAPLOTYPES

Six different MPH were detected from the native plants, designated MPH 1–6. All 47 of the introduced *F. sachalinensis* accessions were found to have a MPH also found in the native samples, 44 having MPH 6 and the remaining three having MPH 1 (Table 1).

ISSR GENOTYPES

Each ISSR primer generated a banding pattern referred to as a phenotype. From five to thirteen bands were scored per primer, resulting in between five and twenty-eight phenotypes depending upon the primer. A total of fifty-nine bands was scored, of which 52 were polymorphic. A unique combination of these phenotypes is assumed to be representative of a genotype. A total of 32 genotypes were detected (Table 1). Sixteen genotypes were found in plants from the introduced range; these have been given the letter "i" followed by a unique number. The remaining sixteen genotypes were from native Japanese plants; these have been given the letter "n" followed by a number. No native genotypes were found in the introduced plants.

DISTRIBUTION OF GENOTYPES

Sixteen native *F. sachalinensis* plants were analysed. As seen in Fig. 1, each plant possessed a unique genotype, indicating a high level of genetic diversity among native plants. Plants from the same geographical area share the same chloroplast haplotype, however none from the same area shared a genotype. Whilst some plants within populations were genetically similar (Fig. 2, MPHs 1, 3, and 4), this was not true for all of them.

By contrast, in Britain only eight genotypes were found from the thirty-one plants analysed (Fig. 3). As can be seen there were two widespread clones, i1 shared by eleven accessions and i4 by thirteen. These represent a male-fertile and a male-sterile clone respectively, both with chloroplast MPH 6. The remainder of the genotypes were found at single localities, with a maximum of two plants sharing the genotype. The three accessions with MPH 1 were found to contain different ISSR genotypes. The other three genotypes with MPH 6 were represented by a male-fertile plant from South Hampshire (i13), two male-sterile stands from Warwick (i6), and a single stand from Merioneth (i2).

There were nine *F. sachalinensis* accessions analysed from the introduced range that were not from Britain. The two plants from the U.S.A had unique genotypes (i7 and i12). Five plants came from Germany, three of which were from the same area of Germany, Aachen, however all five were found to have unique genotypes (i3, i9, i10, i14 and i15). Two plants were analysed from the Czech Republic. Whilst one had a unique genotype, i5, the other possessed the male-sterile genotype, i4, which was common in Britain.

NEIGHBOUR JOINING TREE

The Neighbour Joining tree for all genotypes is shown in Fig. 4. The internal branches are short and the majority of the distances are confined to the terminal branches. Bootstrapping 1,000 times has shown that only four of the branches were supported over 50% of the time. In each case the support was for a group of two genotypes. There was support of 68·0% for the two genotypes with MPH 4, native plants from Fukushima (n10 and n11), and a bootstrap value of 91·5% for the two native genotypes with MPH 1, which came from Niigata (n2 and n13). The other support was for introduced plants. The genotypes i8 and i11 (76·9% bootstrap) consisted of two of the three British

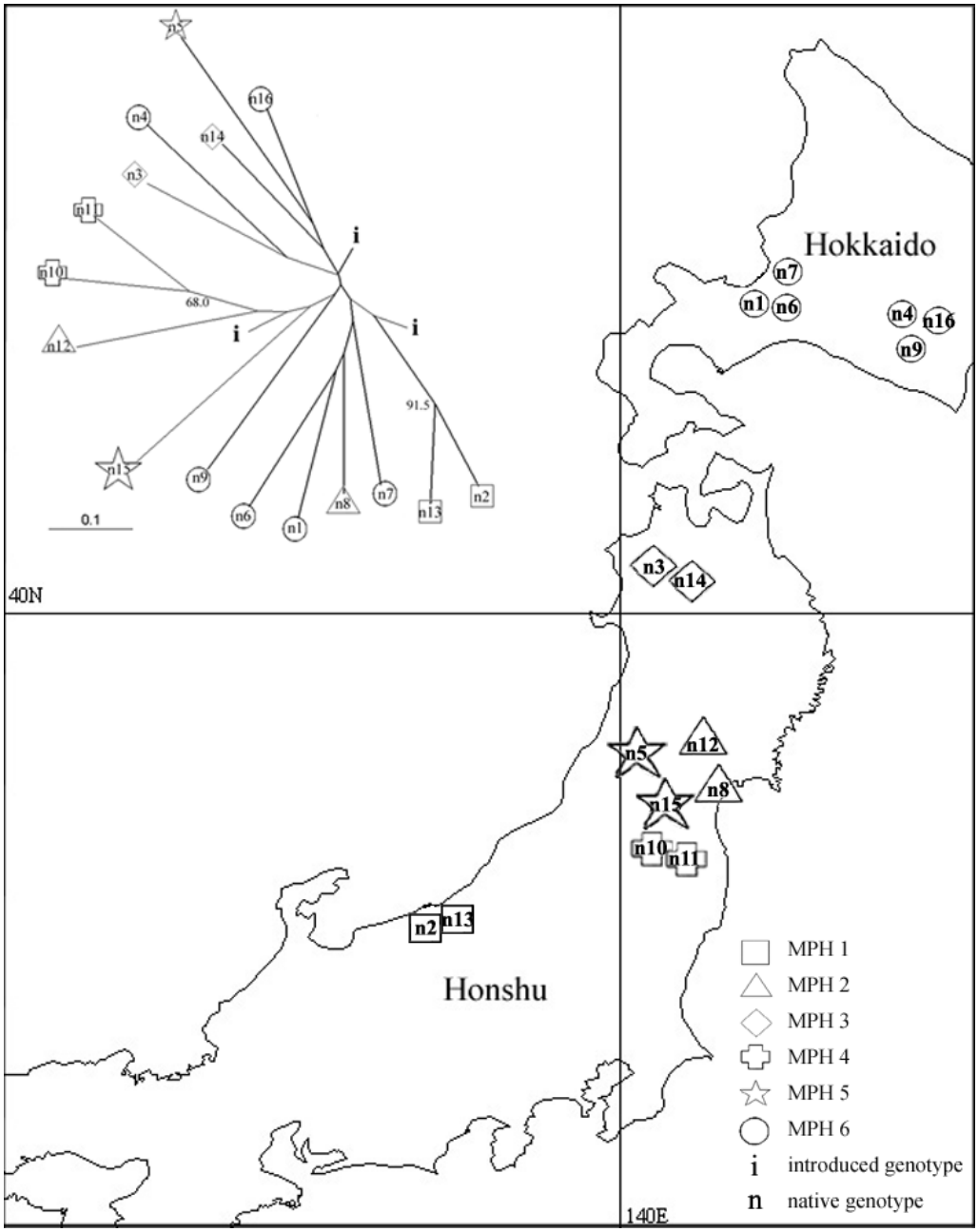

FIGURE 1. Localities of Japanese *F. sachalinensis* genotypes. The map was produced using Dr. A. J. Morton's DMAP program. A simplified version of the unrooted Neighbour-Joining tree (Fig. 4) has been included to show the relationship between the different native genotypes, and their relationship to the introduced plants.

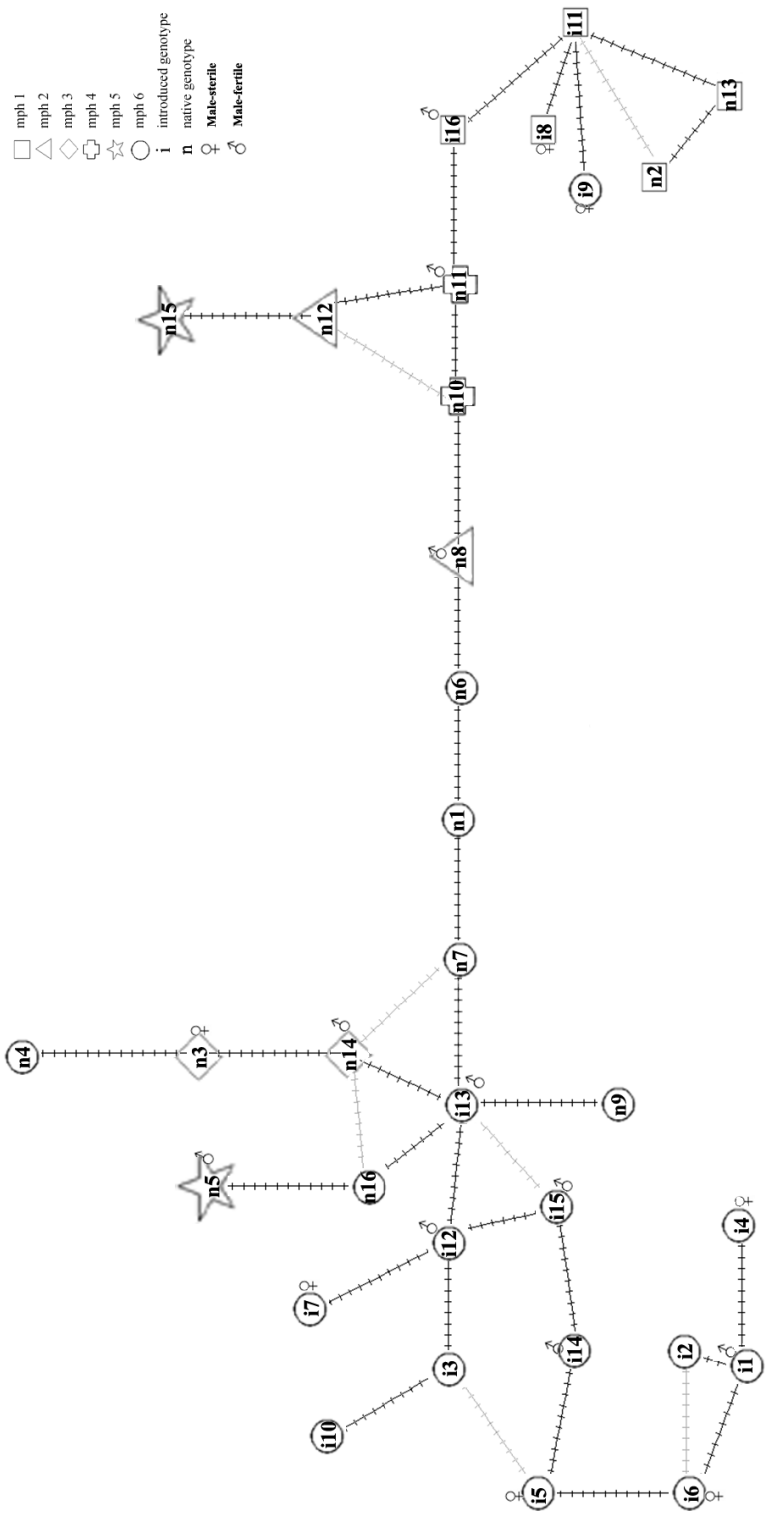

FIGURE 2. Minimum spanning network showing the number of differences between *F. sachalinensis* genotypes. The cross-links show the number of differences. The grey links are equally likely alternatives between genotypes. The shape superimposed over the genotype represents the chloroplast haplotype that corresponds with the genotype. Sex of the genotype is indicated where known.

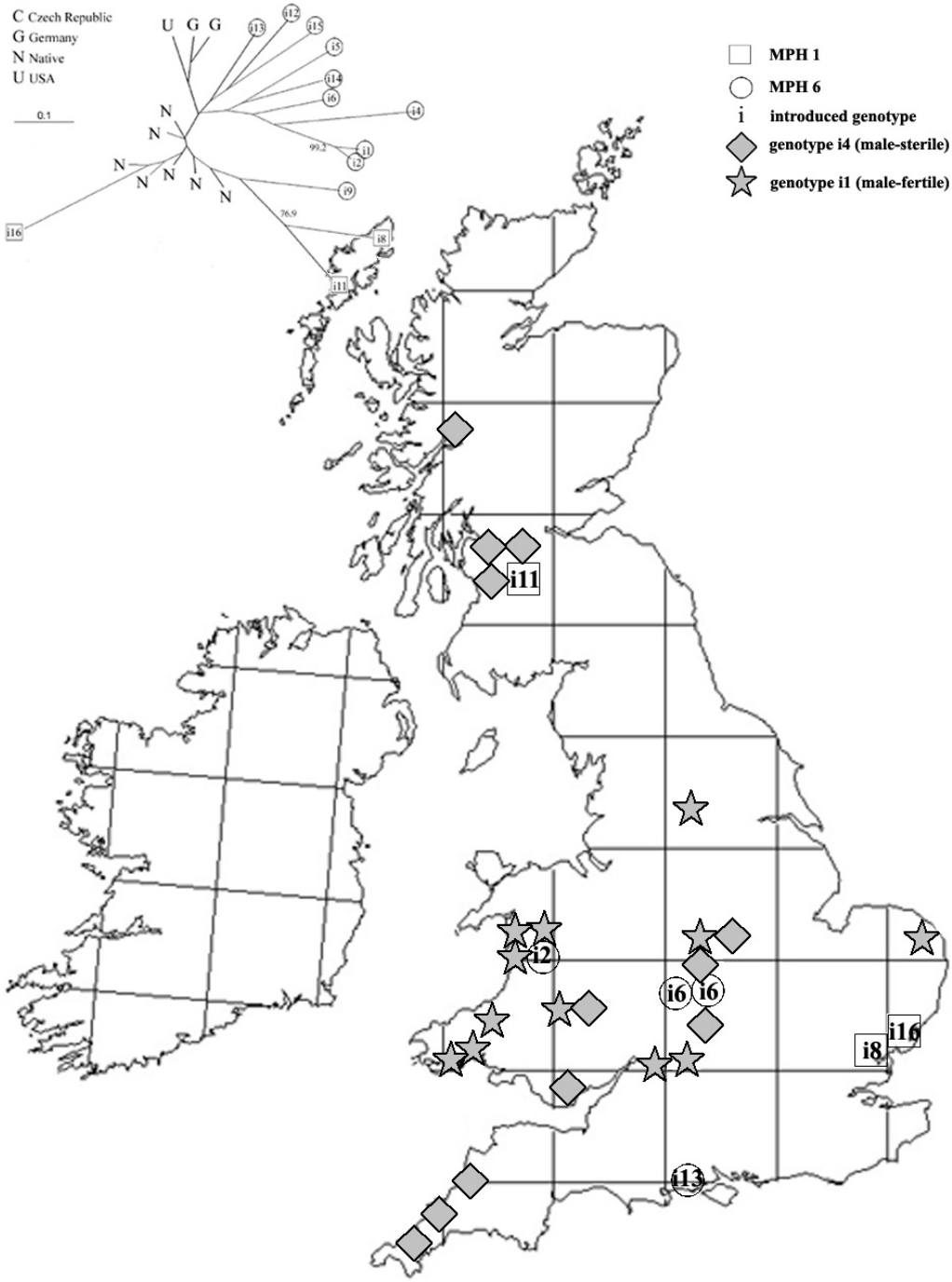

FIGURE 3. Localities of British *F. sachalinensis* genotypes. The map was produced using Dr. A. J. Morton's DMAP program. The position of some genotypes have been moved slightly, to allow all samples to be seen, due to multiple records in single recording 10 km squares. A simplified version of the unrooted Neighbour-Joining tree (Fig. 4) has been included to show the relationship between the British genotypes, and their relationship to the non-British introduced plants, and the native plants.

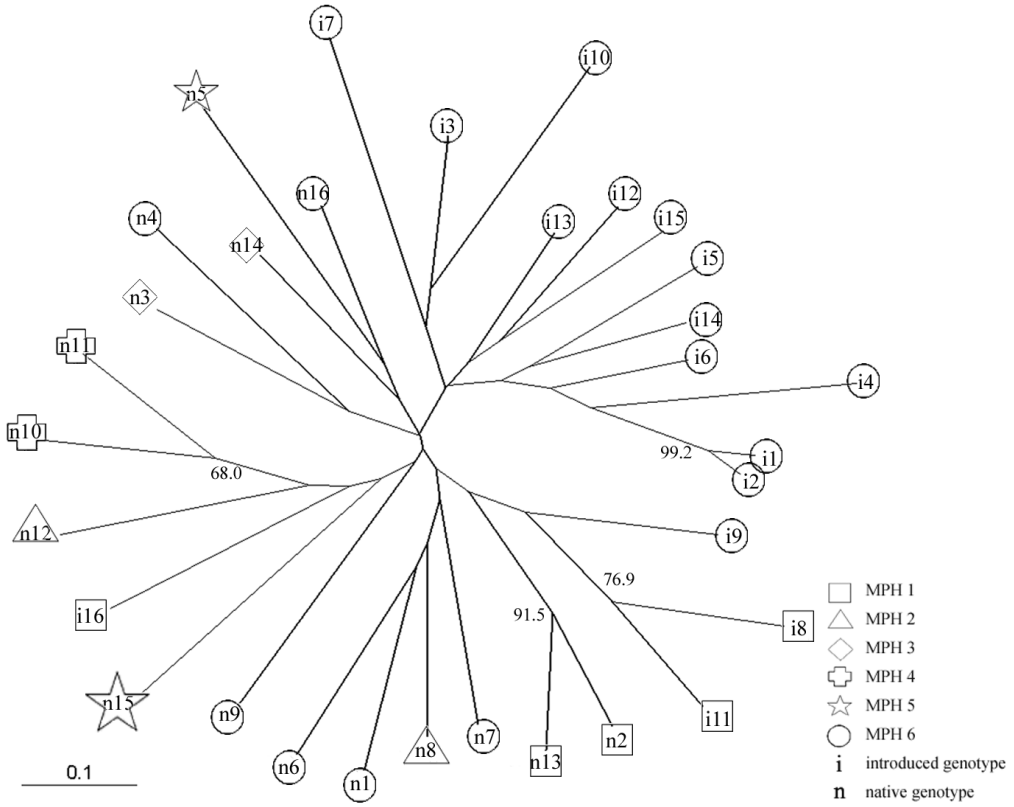

FIGURE 4. Unrooted Neighbour-Joining tree depicting relationships between the various ISSR genotypes, based on Jaccard's similarity coefficient. Numbers along branches are confidence values based on 1,000 bootstraps. Only bootstrap values greater than 50% are shown. The shape superimposed over the genotype represents the corresponding chloroplast haplotype.

plants with MPH 1: the male-sterile plant from Colchester and a plant of unknown sex from Glasgow. The other introduced genotypes that grouped together were i1 and i2 (99·2% bootstrap) representing the common male-fertile genotype (i1) and an individual stand from Merioneth.

The majority of the introduced genotypes, twelve of the 16, were all found to diverge from a single branch with no native genotypes forming part of the group, although this group is not statistically supported (Fig. 4). In contrast the native genotypes appeared to arise from four main branches, with two of the branches closer to the branch with the majority of the introduced genotypes than the other two. One of the four branches was further divided into two clear groups, one comprising n2 and n13, and the other made up of n1, n6, n7 and n8. The three introduced genotypes from plants with MPH 1 were found intermingled with native genotypes, two of them grouping with the Japanese plants with MPH 1 as well as a genotype, i9, that came from a plant with MPH 6. The other genotype that was found mixed in with the native genotypes was the British male-fertile genotype with MPH 1, i16.

MINIMUM SPANNING NETWORK

The minimum-spanning network (Fig. 2) is based on the number of band differences between each pair of genotypes. Next to the genotype is a symbol to represent whether the genotype is male-fertile or male-sterile. There was no obvious separation between male-fertile and male-sterile genotypes. The network is almost linear in shape, with only seven equally likely alternative links throughout the whole network. The genotypes found in the introduced plants occupy either end of the

network, with six native genotypes found between the nearest of the introduced genotypes at either end. These six genotypes represent 65 band differences between i13 and i16, which were the closest of the genotypes from the two ends. The two common British genotypes (i1 and i4) were closely related and were found to have only ten band differences. The group of twelve introduced genotypes to the left of the network were the same genotypes that came from the single branch in the Neighbour Joining analysis (Fig. 4). The minimum-spanning network showed a closer relationship between the four introduced genotypes that were not grouped with the other introduced genotypes than did the Neighbour Joining analysis, being interconnected by genotype i11.

DISCUSSION

DISTRIBUTION AND GENETIC DIVERSITY OF THE NATIVE PLANTS

Sixteen native *F. sachalinensis* plants were analysed, each of which possessed a unique genotype. The native plants harboured a much higher level of genetic diversity than the introduced plants, a common phenomenon found in many different species as a result of the genetic bottleneck experienced during the introduction process (Barrett & Richardson 1986; Barrett & Shore 1989). Whilst plants from the same geographical area shared the same chloroplast haplotype, there was less geographic structuring evident with the genotypes, although there does appear to be some correlation between genetic relationship and geographical distribution. Japanese individuals with MPH 1 came from Niigata, Honshu, and had genotypes n2 and n13, which form a supported group (91·5% bootstrap) on the Neighbour-Joining tree (Fig. 4), and are each others nearest neighbours on the minimum-spanning network (Fig. 2). Likewise the individuals with MPH 3 from Aomori, Honshu cluster together as do those with MPH 4 (68% bootstrap) from Fukushima, Honshu. In general, the taxa to the left of the minimum-spanning network as it is portrayed in Fig. 2 were found in the northernmost part of Honshu and on Hokkaido, and those to the right in the central and southernmost part of the native range (Fig. 1).

DISTRIBUTION AND GENETIC DIVERSITY OF THE INTRODUCED PLANTS

The majority of the introduced genotypes, twelve of the 16, were found to group together both on the Neighbour-Joining tree (Fig. 4) and the minimum-spanning network (Fig. 2). The four introduced genotypes not clustering with these being the three British genotypes with MPH 1 and a male-sterile German plant (G3) with genotype i9 and MPH 6.

It is interesting to note that, on both the Neighbour-Joining tree (Fig. 4) and the minimum-spanning network (Fig. 2), the non-British introduced genotypes appear to be more closely related to each other than they do the other genotypes found in Britain (genotypes i3, i5, i7, i10, i12, i14 and i15), the exception being genotype i9. Genotype i13, the British genotype most closely associated with the non-British introduced genotypes in both analyses being found in Britain in a plant from the closest collection point to mainland Europe. The lack of support for this observation could, however, imply that this is mere coincidence. The odd non-British (German) introduced genotype, i9, having the same chloroplast haplotype (MPH 6) as the majority of the introduced *F. sachalinensis*, yet has a genotype more closely related to the British introduced plants with the rarer MPH 1. The reason for this is unclear and further sampling and analysis would be required to better understand how this individual arose.

The sharing of the male-sterile genotype between Britain and the Czech Republic implies that the majority of the plants (those with MPH 6) were introduced from the same collection, presumably from St. Petersburg botanic garden. There also appears to be a higher level of genetic variation within the non-British introduced *F. sachalinensis* individuals, as demonstrated by each having a unique genotype, although further sampling of mainland Europe and USA would be required to support or refute this point.

Spread of two clones, a male-fertile and a male-sterile clone, appears to be the primary explanation for the current *F. sachalinensis* distribution within Britain (Fig. 3). The male-sterile clone, i4, being found as far north as Fort William, Scotland, and as far south as Cornwall, England. The male-fertile clone, i1, appears to have a more restricted distribution, being found predominantly in the centre of England, and Wales, but spreading from the

East to the west coast. The remainder of the British genotypes were found at single localities, with a maximum of two plants sharing the genotype. Unlike in Britain, the remainder of the introduced plants demonstrate no signs of clonal spread, although as noted above they do appear to be closely related to each other. This greater diversity could be the result of in situ sexual reproduction, but further sampling would be needed to support or refute such a claim.

A largely unpublished preliminary study cited by Hollingsworth & Bailey (2000b) also found more clonal diversity in *F. sachalinensis* (14 genotypes from 30 samples) than in the uniclonal *F. japonica* var. *japonica*. However, unlike the current study no shared genotypes were detected between populations. It is probable that in the previous study the lack of shared genotypes among at least some of the sites is erroneous and is attributable to PCR artefacts (a lack of time prevented duplicate DNA extractions being used to test the reliability of different genotypes; Hollingsworth 1998).

SOURCE OF THE INTRODUCED *FALLOPIA SACHALINENSIS*

Both of the MPHs found in introduced material were found in native plants. The most common in the introduced plants, MPH 6, was found only in individuals from Hokkaido. The rarer introduced chloroplast haplotype found in the introduced range only in Britain, MPH 1, was only found in individuals from Niigata, Honshu.

The low level of resolution and support found with the neighbour joining analysis means conclusive statements about the relationships between the native Japanese and introduced genotypes of *F. sachalinensis* cannot be made. Even so, some observations can be made, and tentative suggestions towards how the current distribution arose can be proposed.

Whether the genotypes found in the introduced *F. sachalinensis* were present in the native range before the plants were collected, or have arisen by sexual reproduction since their introduction is unclear. It is likely that the situation has arisen from a combination of the two factors. A limited number of genotypes were probably introduced from Japan, most likely Hokkaido or the Sakhalin Island, into St. Petersburg. The most plausible explanation is that a minimum of a male-fertile and a male-sterile clone were sent to Britain from St. Petersburg as established plants rather than seed. These were then distributed throughout Britain to various nursery gardens, and from there to many stately homes and manors, eventually leading to the escapes reflected in the distribution noted by Conolly (1977). Given how easy *F. sachalinensis* is to propagate from the rhizome, there would be little point in the gardeners germinating and growing seed for distribution.

At a similar time, or earlier, plants arising from the same original collection were probably being distributed to Germany, the Czech Republic and other European countries. *Fallopia sachalinensis* was recommended, especially on the continent, as a forage plant for cattle, as well as being introduced as a riverbank stabiliser (Bailey & Conolly 2000). For stabilisation of riverbanks, mature plants were most likely used, which would presumably originate from vegetative reproduction. In addition to its use as a forage plant, *F. sachalinensis* was also used on large estates for scenic plantings and cover for shoots (Bailey & Conolly 2000). As with riverbank stabilising, the plants introduced for scenic plantings were probably introduced as mature plants. However, seed may have been used to produce the plants for forage, which could explain why there was found to be higher levels of genetic variation on the continent, but these plants all cluster together away from the native, possibly indicating that they were produced from the genetically limited original introductions by sexual reproduction. Presumably material either directly from St. Petersburg, or from elsewhere on mainland Europe was sent to the USA.

Three different collections are presumed to have been housed at St. Petersburg: that of Dr H. Weyrich, from Sakhalin in September 1853; that of P. von Glehn in 1861, also from Sakhalin that arrived in St. Petersburg in early 1863; and material of Japanese origin collected by C. J. Maximovicz during 1859–1864 (Bailey & Conolly 2000). Whilst it is not possible, from this study, to determine whether the introduced plants came from Sakhalin or Japan due to a lack of material from the Sakhalin island, data would suggest that if the plants with MPH 6 came from Japan it would have been from the northern island Hokkaido.

The three genotypes found in Britain in plants that have MPH 1 were probably introduced directly from Niigata on Honshu,

Japan. Whilst it is possible that this chloroplast type could be found in non-sampled Japanese sites, a study of chloroplast variation in two hundred and forty Japanese Knotweed *s.l.* accessions from eighty seven sites spanning the four main islands that make up Japan, found this chloroplast type to be restricted to Niigata (Pashley 2003). This could explain why no plants with this chloroplast type have been found anywhere besides Niigata and Britain. The two sites in Britain where these were found were Glasgow and Colchester. The plants (both male-fertile and male-sterile) in Colchester shared MPH 1 and were found growing on the site of an old nursery garden reported to have sold *F. sachalinensis* (Bailey & Conolly 2000). The site in Glasgow where the individual with MPH 1 (B3) was found is close to a botanic garden, and the implication is that the plants at Colchester and the individual B3 from Glasgow came from a common source.

CONCLUSIONS

The majority of British *F. sachalinensis* were found to be one of two widespread genotypes, either a male-fertile or a male-sterile clone. The remaining British genotypes that came from plants that share the chloroplast MPH 6 were presumed to have arisen from the same introduction, as were most of the plants in the other introduced countries included in the study, most likely introduced via St. Petersburg. Only one genotype was found shared between Britain and any of the other countries to which *F. sachalinensis* was introduced. That genotype was the widespread male-sterile clone, and was found in a single accession from the Czech Republic.

The three genotypes found in Britain in plants that have chloroplast MPH 1 were probably introduced straight from Niigata on Honshu, in Japan, and were not found in any other country included in this study to which *F. sachalinensis* was introduced.

A high level of genetic diversity was found among native *F. sachalinensis*, with each of the sixteen plants analysed having a unique genotype. There was some correlation between genetic relationship and the geographical distribution of these native plants.

There is clearly a higher level of genetic diversity in *F. sachalinensis* than there is in *F. japonica* var. *japonica* (Hollingsworth & Bailey 2000a), however, there is strong evidence that the current British distribution of *F. sachalinensis* has resulted mainly from the spread of two clones rather than via sexual reproduction.

ACKNOWLEDGMENTS

We are very grateful to MAARA for the financial support to CHP, and to the British Ecological Society (BES), Botanical Society of the British Isles (BSBI), Botanical Research Fund, and Leicester University who provided funding to enable the collection trip to Japan. We also thank Mark Chapman who provided helpful comments on an earlier version of the manuscript. Our thanks also to Ann Conolly, for her support, encouragement, helpful conversations, and company on field trips. We are also grateful to the many people who aided in the plant collection in Britain, including Tony Leech, Jerry Heath, Peter Jepson, R. Hemming, Caroline Wilson, K. Pyne, James Partridge, G. Hutchinson, Peter Zika, James MacFarlene, Dick Shaw, Harry Evans. Finally we are particularly grateful to our Japanese friends and collaboraters Kazuhito Matsuo, Jun Suzuka, Hiroyuki Shibaike, Takashi Fujita, Teruo Sano and Tatuyoshi Morita, without whose generous assistance in the planning and execution of our visit to Japan, this work would have been impossible to achieve.

REFERENCES

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.

BARRETT, S. C. H. & RICHARDSON, B. J. (1986). Genetic attributes of invading species, in R. H. GROVES & J. J. BURDON eds. *Ecology of Biological Invasions*, pp. 21–33. Australian Academy of Science, Canberra, Australia.

BARRETT, S. C. H. & SHORE, J. S. (1989). Isozyme variation in colonizing plants, in D. E. SOLTIS & P. S. SOLTIS eds. *Isozymes in Plant Biology*, pp. 106–126. Dioscorides, Portland, Oregon, USA.

BEERLING, D. J., BAILEY, J. P. & CONOLLY, A. P. (1994). *Fallopia japonica* (Houtt) Ronse, Decraene (*Reynoutria japonica* Houtt, *Polygonum cuspidatum* Sieb. and Zucc). *Journal of Ecology* **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 G. BRUNDU, J. BROCK, I. CAMARDA, L. CHILD, *et al*. eds. *Plant invasions: Species Ecology and Ecosystem Management*, pp. 283–290. Backhuys Publishers, Leiden, The Netherlands.

BRABEC, J. & PYŠEK, P. (2000). Establishment and survival of three invasive taxa of the genus *Reynoutria* (Polygonaceae) in mesic mown meadows: A field experimental study. *Folia Geobotanica* **35**: 27–42.

BROCK, J. H. & WADE, P. M. (1992). Regeneration of *Fallopia japonica*, Japanese Knotweed, from rhizome and stems: Observation from greenhouse trials. *The 9th International Symposium on the biology of weeds*, Dijon, France: 85–94.

CHILD, L. & WADE, M. (2000). *The Japanese Knotweed Manual*. Packard Publishing Limited, Chichester.

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

DEMESURE, B., SODZI, N. & PETIT, R. J. (1995). A set of universal primers for amplification of polymorphic noncoding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology* **4**: 129–131.

DUMOLIN-LAPEGUE, S., PEMONGE, M. H. & PETIT, R. J. (1997). An enlarged set of consensus primers for the study of organelle DNA in plants. *Molecular Ecology* **6**: 393–397.

EXCOFFIER, L. & SMOUSE, P. E. (1994). Using allele frequencies and geographic subdivision to reconstruct gene trees within a species – molecular variance parsimony. *Genetics* **136**: 343–359.

FELSENSTEIN, J. (2002). PHYLIP (Phylogeny Inference Package). Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

FERRIS, C., OLIVER, R. P., DAVY, A. J. & HEWITT, G. M. (1993). Native oak chloroplasts reveal an ancient divide across Europe. *Molecular Ecology* **2**: 337–344.

HARRIS, S. A. & INGRAM, R. (1992). Molecular systematics of the genus *Senecio*-L .1. Hybridization in a British polyploid complex. *Heredity* **69**: 1–10.

HOLLINGSWORTH, M. L. (1998). Clonal growth and hybridisation in some invasive *Fallopia* spp. PhD thesis, University of Leicester, Leicester.

HOLLINGSWORTH, M. L. & BAILEY, J. P. (2000a). Evidence for massive clonal growth in the invasive weed *Fallopia japonica* (Japanese Knotweed). *Botanical Journal of the Linnean Society* **133**: 463–472.

HOLLINGSWORTH, M. L. & BAILEY, J. P. (2000b). Hybridisation and clonal diversity in some introduced *Fallopia* species (Polygonaceae). *Watsonia* **23**: 111–121.

HOLLINGSWORTH, M. L., HOLLINGSWORTH, P. M., JENKINS, G. I., BAILEY, J. P., *et al.* (1998). The use of molecular markers to study patterns of genotypic diversity in some invasive alien *Fallopia* spp. (Polygonaceae). *Molecular Ecology* **7**: 1681–1691.

PAGE, R. D. M. (1996). TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**: 357–358.

PASHLEY, C. H. (2003). The use of molecular markers in the study of the origin and evolution of Japanese Knotweed *sensu lato*. PhD thesis, University of Leicester, Leicester.

SAKAI, A. K., ALLENDORF, F. W., HOLT, J. S., LODGE, D. M., *et al*. (2001). The population biology of invasive species. *Annual Review of Ecology and Systematics* **32**: 305–332.

STUEFER, J. F., ERSCHBAMER, B., HUBER, H. & SUZUKI, J.-I. (2001). The ecology and evolutionary biology of clonal plants: An introduction to the proceedings of Clone-2000. *Evolutionary Ecology* **15**: 223–230.

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 P. PYŠEK, K. PRACH, M. REJMANEK, & M. WADE eds. *Plant Invasions, general aspects and social problems*, pp. 151–159. SPB Academic Publishing, Amsterdam.

YAP, I. V. & NELSON, R. J. (1996). WinBoot: A program for performing bootstrap analysis of binary data to determine the confidence limits of UPGMA-based dendrograms. International Rice Research Institute (IRRI), Philippines.

(*Accepted June 2006*)