Clonal Growth and Hybridisation in Some Invasive *Fallopia* spp.

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Abstract

Molecular markers have been used to examine the population biology and dynamics of hybridisation in Britain, of some invasive weeds from the genus Fallopia: F. japonica var. japonica and F. sachalinensis. Both species are gynodioecious, but in Britain, only female plants of F. japonica var. japonica are present. Molecular studies using RAPDs (randomly amplified polymorphic DNA) suggest that the entire British population of this taxon consists of a single genet. The same genotype has also been recovered in all samples examined from Europe and North America. F. sachalinensis, on the other hand, showed higher levels of clonal diversity consistent with sexual reproduction.

The two species hybridise in Britain, and the hybrid (Fallopia x bohemica) shows partial to full fertility. High levels of clonal diversity were detected using RAPDs and inter-SSRs (inter-simple sequence repeats), probably reflecting a combination of multiple origins and hybrid fertility.

A study of chloroplast RFLPs (restriction fragment length polymorphisms) has shown that the mode of chloroplast inheritance appears to be predominantly maternal, and Fallopia japonica var. japonica is the female parent of all of its hybrids.

Based on RAPD data, evidence for backcrossing between Fallopia x bohemica and F. japonica var. japonica was detected at two sites. Flowering material at one site showed the putative backcross to be male fertile. Such plants are of interest as they are possible intermediate steps in the acquisition of genes restoring male fertility to F. japonica var. japonica. The ability of an already invasive species to reproduce and disperse by seed as well as by vegetative propagation, may serve to enhance its invasive capabilities.

Molecular data based on chloroplast trnL intron sequences and RAPDs support previous morphological evidence that introduced material of at least Fallopia japonica var. japonica is different from Asiatic stock.

Dedication

To Pete: thanks for your help and guidance with the thesis,

for your love and support,

and for making me laugh when things are tough. QAL

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Preface

This thesis describes an investigation into the population genetics and biosystematics of some invasive weeds from the genus Fallopia. The thesis has been written as a series of papers, rather than in the traditional format. This inevitably causes an element of repetition due to the 'stand alone' nature of the individual papers, and a flux of styles depending on the target audience of the journal. Chapter 1 is an introduction, outlining the study taxa and the background to the molecular approaches. Chapter 2 is a distribution survey of invasive Fallopia taxa along a single river system. Chapter 3 describes a molecular investigation of this population, investigating clonal growth and hybridisation. Chapter 4 is a molecular investigation of invasive Fallopia taxa along a second river system, again investigating clonal growth and hybridisation, but in a situation complicated by multiple male fertile taxa. Chapter 5 outlines the use of chloroplast markers to study hybridisation and the mode of organelle inheritance in Fallopia. Chapter 6 is an assessment of the extent of genetic variability in F. japonica var. japonica in the British Isles. Chapter 7 is an investigation into the levels and partitioning of clonal diversity in F. sachalinensis and F. x bohemica in Britain. Chapter 8 describes preliminary data from the native range of these Fallopia taxa and related species. The final chapter highlights areas for future research.

The following chapters have already been published, or are accepted for publication:

Chapter 2: Hart ML, Bailey JP, Hollingsworth PM, Watson KJ (1997) Sterile species and fertile hybrids of Japanese Knotweeds along the River Kelvin. Glasgow Naturalist **23**, 18-22.

Chapter 3: Hollingsworth ML, Hollingsworth PM, Jenkins GI, Bailey JP, Ferris C (1998a - in press) The use of molecular markers to study patterns of genotypic diversity in some invasive alien Fallopia spp. (Polygonaceae). Molecular Ecology

Chapter 5: Hollingsworth ML, Bailey JP, Hollingsworth PM, Ferris C (1998b - in press) Hybridisation and chloroplast DNA variation amongst some invasive Fallopia (Polygonaceae) species. Botanical Journal of the Linnean Society

Getting married during my thesis write up has resulted in a name change from Hart (chapter 2 - Hart et al., 1997) to Hollingsworth (all other papers/chapters).

Chapter 1
Introduction

The study genus: Fallopia

Fallopia Adans. is one of 46 genera within the Polygonaceae (Mabberley, 1997). Bailey & Stace (1992) divided the genus into four sections : section Fallopia containing the annual climbers F. convolvulus (L.) Löve, F. dumetorum (L.) Holub and F. scandens (L.) Holub; section Parogonum Haraldson containing the perennial climbers F. cilinoides (Michaux) Holub. and F. cynanchoides (Hemsl.) Haraldson; section Sarmentosae (Grintz) Holub containing the woody climbers F. multiflora (Thunb.) Haraldson and F. baldschuanica (Regel) Holub; and section Reynoutria (Houtt.) Ronse Decraene containing the robust rhizomatous perennials F. japonica (Houtt.) Ronse Decraene and F. sachalinensis (F. Schmidt ex Maxim.) Ronse Decraene.

Five species of Fallopia occur in the British Isles, although only two, F. convolvulus (Black-bindweed) and F. dumetorum (Copse-bindweed) are considered natives (Stace, 1997). F. baldschuanica (Russian-vine) is a common garden plant, introduced from central Asia and frequently occurs as discarded material on waste ground, but is rarely well naturalised (Stace, 1997). F. japonica (Japanese Knotweed) and F. sachalinensis (Giant Knotweed) are again horticultural introductions from Asia, and both of these species have become naturalised in Britain. It is the latter two taxa which are the focus of this study.

Fallopia japonica is native to Japan, Taiwan and Northern China where it grows along roadsides, river banks and managed pasture and is one of the dominant colonists of larva fields (ICOLE, 1997). It has been recorded up to an altitude of 2600m on Mt. Fuji, where it plays a significant role in ecosystem development by stabilising soil (Maruta, 1983). It is insect pollinated and visitors to its flowers include flies, honey bees and wasps (Tanaka, 1966 - cited by Bailey, 1994).

Fallopia sachalinensis 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 ca. 1050m and can be found along roadsides, forest edges, cliff tops and river sides, and like F. japonica it is one of the pioneer colonists of the 'larva desserts' caused by volcanic eruptions (Sukopp & Starfinger, 1995).

Fallopia japonica and F. sachalinensis were introduced to the British Isles in ca.1841 (Beerling et al., 1994) and 1869 respectively (Conolly, 1977). The history of the invasion of F. japonica and F. sachalinensis in the British Isles has been documented by Conolly (1977). Painstaking examination of herbarium specimens from all over the country and careful collation of collection dates and grid references resulted in a series of maps depicting the spread of these plants through both space and time. The first report of naturalisation of F. japonica in the British Isles was in 1886 (Storrie, 1886). Since this date it has spread throughout the British Isles, most rapidly in the west of Britain (Conolly, 1977). F. sachalinensis shows less invasive tendencies 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).

Fallopia japonica generally occurs in a variety of man made habitats such as canal, stream and river banks, road verges, railway embankments, in cemeteries and various other urban habitats. The concentration of F. japonica in urban areas is a reflection of both its horticultural origin and the effects of ground disturbance (I.C.O.L.E, 1997). Fragments as small as 0.7g are capable of regenerating into new plants (Brock & Wade, 1992) and the cartage of earth and water-transport are the usual means of dispersal (Conolly, 1977). F. sachalinensis also grows along river banks and roadsides but is often found as isolated stands in places such as

estate grounds, where it was presumably planted for ornamental purposes. Stands range in size from individual plants to clumps of more than 500 square metres (Palmer, 1994)

The abundance of these taxa, in particular Fallopia japonica in urban areas, and the costs associated with their control, have given these plants much bad publicity. F. japonica shoots are able to push up through asphalt, damaging pavements, car parks and other public facilities and the height to which these Knotweed taxa grow leads to reduced visibility on roadsides and railways. Along water courses problems include decaying aerial shoots creating blockages and dense stands on riverbanks impeding flow in high water, increasing the risk of flooding.

Detailed investigations of these taxa by John Bailey, Ann Conolly and co-workers have lead to series of papers on their morphology, cytology and reproductive biology (Bailey, 1994; Bailey et al., 1996; Bailey et al., 1995; Bailey & Conolly, 1985; Bailey & Stace, 1992; Beerling et al., 1994; Conolly, 1977). With the application of cytological data (Bailey & Conolly, 1985) it became apparent that the Knotweed invasion was more complex than first imagined. Plants were found at three different ploidy levels, 2n=44, 66 & 88 (tetraploid, hexaploid and octoploid respectively). Comprehensive cytological studies (Bailey & Conolly, 1985; Bailey & Stace, 1992) showed the typical Fallopia japonica, namely F. japonica var. japonica is octoploid and F. sachalinensis is tetraploid. A tetraploid dwarf variety of F. japonica, var. compacta (Hook.f.) J. Bailey, also occurs in the British Isles. Morphological and cytological comparisons with a range of artificial hybrids also strongly support the suggestion that hybrids between F. japonica s.l. and F. sachalinensis exist in the British Isles (Bailey & Conolly, 1985). These hybrids are given the binomial F. x bohemica (Chrtek & Chrtková) J. Bailey. Two different ploidy levels have been detected in the hybrids: hexaploid plants (2n=66) that are thought to stem from a cross between F. japonica var. japonica (2n=88) and F.

sachalinensis (2n=44) and tetraploid plants (2n=44) thought to stem from a cross between F. japonica var. compacta (2n=44) and F. sachalinensis.

Bailey & Conolly (1985) also noted that all individuals of Fallopia japonica var. japonica are morphologically very similar to each other and they are all male sterile. Both F. japonica and F. sachalinensis are gynodioecious and can occur as either male sterile (female) or hermaphrodite plants (Bailey, 1994). In Britain both male sterile and hermaphrodite forms of F. japonica var. compacta, F. sachalinensis and both 4x and 6x F. x bohemica have been recorded, but only male sterile plants of F. japonica var. japonica are known (Bailey, 1994). The implication of this is that F. japonica var. japonica is not capable of reproduction by seed in Britain, and that its spread has been purely by vegetative propagation. In addition it was found that the 4x and 6x F. x bohemica plants show some pollen fertility (Bailey, 1994) and are thus potentially capable of backcrossing with the parental taxa.

The fertility of Fallopia x bohemica is interesting. The 4x hybrid appears to show greater fertility than the 6x hybrid, with pollen stainability values of 61% and 25% respectively (Bailey, 1994). The pollen stainability of 4x F. x bohemica is within the range detected for F. sachalinensis and F. japonica var. compacta whose pollen showed 54.2% and 98.7% stainability respectively (no data is available from the male sterile F. japonica var. japonica). The high levels of pollen stainability of the 4x hybrid are supported by data on meiotic pairing, and regular bivalent formation has been detected, similar to that found in F. sachalinensis and F. japonica var. compacta (Bailey & Stace, 1992). In the 6x F. x bohemica, however, meiosis is extremely irregular with large numbers of univalents and multivalents formed (Bailey & Stace, 1992). Nevertheless, despite this irregular meiosis and low pollen stainability these 6x F. x bohemica do show some fertility and seed can be found on these hybrids (J. P . Bailey pers. comm. 1998).

The aim of this thesis is to extend these studies by using molecular techniques to gain a deeper insight into the evolutionary biology of these taxa, and in particular to investigate the relative importance of sexual versus asexual reproduction, and the dynamics of inter-specific hybridisation.

The approach: molecular markers

The estimation of relationships amongst individuals, populations and species is of key importance to the understanding of evolutionary patterns and processes. The amounts, dynamics and the spatial partitioning of genetic variability largely determines the evolutionary potential of a species. At higher taxonomic levels, understanding the relationships between species can provide a retrospective window on how they evolved.

The study of relationships among plants has been revolutionised over the last 50 years or so due to advances in molecular genetic techniques. Classic problems that have been addressed include teasing out environmentally induced variability from genetic variability, finding markers to characterise individuals, the estimation of breeding systems, assessing levels of gene flow, studying hybridisation and establishing phylogenetic relationships.

Two major breakthroughs in genetics research in the 1950s can perhaps be pinpointed as the start of the molecular revolution in systematics. Most famously was Watson and Crick's (1953) report of the structure of DNA. This short, two page paper describing the structure, and suggesting a replication mechanism for the molecule, has formed the basis for perhaps the majority of current biological research. More subtly, but perhaps with a more immediate effect on our understanding of natural populations, was the discovery of starch gel electrophoresis and the histochemical staining of enzymes on gels (Smithies, 1955; Hunter & Markert, 1957). Although it was a further 10 years before the

simple, single locus co-dominant nature of allozyme polymorphisms was understood (Harris, 1966; Lewontin & Hubby, 1966), from the late 1960s to the present there have been tens of thousands of papers published using starch gel electrophoresis of isozymes to assess levels of genetic variability amongst populations.

The popularity of isozymes is due mainly to the speed, ease and cost effective way in which polymorphisms in protein encoding genes can be (albeit indirectly) assessed. However, the technique is not without its limitations and concerns have been raised as to whether different allelic variants are selectively equivalent (Watt, 1983; Karl & Avise, 1992) and as to whether the genetic variation at a few protein encoding genes is representative of the genome as a whole. While allozyme studies continue to provide an enormous contribution to our understanding of plant evolutionary biology, since the 1970s there has been a constant search for improved molecular techniques which can access DNA variability directly, instead of looking at the products of gene expression.

One of the major limitations to the study of DNA is the methodology for visualising polymorphisms. Put simply, DNA is very small and to detect variation in any one region there is a need to either utilise a sensitive detection mechanism, or alternatively, to amplify the region in question to make it big enough to see. A major breakthrough came in 1975 with the development of the Southern blot (Southern, 1975). Southern's technique of visualising DNA fragments by hybridisation to radioactively labelled DNA probes, enables the effective magnification of DNA. However, while the use of Southern blotting has become standard practice in molecular biology laboratories, the technique carries limitations. In particular, large amounts of relatively clean DNA are required for most assays, as well as the use of hazardous radiochemicals. Although recent developments in nonradioactive labelling (Isaac, 1997) can to some extent circumvent the latter problem, the technique remains labour intensive and often

requires more sample tissue than is available. The development of the Polymerise Chain Reaction (Mullis et al., 1986) provided the key step which truly freed molecular systematists from many of the technical limits they had been facing. Given appropriate primers, the technique allows the amplification of DNA from minute starting quantities and the resulting PCR products can be surveyed for genetic polymorphisms by examining either sequence composition and/ or length variation.

Given these developments in accessing molecular data, it is important to consider the characteristics and attributes of the genomes from which the data is derived. In plants, information can be obtained from three genomes, chloroplast DNA, mitochondrial DNA and nuclear DNA. Mitochondrial DNA has become the molecule of choice for many zoologists, due to its relatively conserved gene order, the availability of universal primers and its rapid substitution rate which has lead to high resolution assays of population structure (Avise, 1987). In plants, however, mitochondrial DNA is far less amenable to study. Large scale genomic rearrangements are common, with master and daughter molecules existing within individual plants (Palmer, 1992), and the substitution rate is the slowest of all eukaryotic genomes (Wolfe et al., 1987, 1989). These characteristics lead to both technical and genetic limitations in the application of this molecule to study relationships among plants (Palmer, 1992). Chloroplast DNA, on the other hand, has been far more widely used (Olmstead & Palmer, 1994). It has a conserved gene order facilitating the design of universal primers (e.g. Demesure et al., 1995; Taberlet et al., 1991) and has a more rapid substitution rate than plant mitochondrial DNA (Wolfe et al., 1987). RFLP analysis, and more recently sequence analysis of cpDNA, has been widely applied to phylogenetic studies assessing the relationships of species and higher taxonomic groups (Olmstead & Palmer, 1994). However, despite the suitability of this molecule for phylogenetic systematics, its rates of substitution are still too low to provide a universally useful abundant source of markers for intra-specific studies and studies involving closely

related species (Soltis et al., 1992). The main area of progress that has been made using chloroplast DNA in studies within and among closely related species has been either conventional RFLP analysis or a combination of PCR, RFLPs and/or sequencing. Using such methodologies, different haplotypes have been identified in a range of species (Dijk & Bakx-Schotman, 1997; Ferris et al., 1995; Levy et al., 1996; McCauley et al., 1996). The predominantly uniparentally (maternal in most angiosperms) inherited nature of chloroplast DNA (Harris & Ingram, 1991) provides a useful means of identifying the male and female parents in crosses (Arnold et al., 1991) and investigating seed versus pollen flow (Ennos, 1994). However whilst recent improvements in the speed and cost efficiency of screening for polymorphisms have increased the use of cpDNA in population genetics (McCauley, 1995) the molecule's slow substitution rate and small effective population size causes a tendency towards low levels of intra specific variability (Ennos et al., in press). Recently, highly polymorphic simple sequence repeats have been discovered in the chloroplast genome (cpSSRs) and these can show high levels of variability even within populations (Powell et al., 1995). This has lead to suggestions that these may provide a source of markers with similar information content to animal mitochondrial DNA. However, the mutational processes at these loci are not fully understood and the structure of available data sets suggests high levels of homoplasy (P.M. Hollingsworth, pers. comm. 1998). Coupled with the lack of universal cpSSR primers, it seems unlikely that these markers will have wide scale applicability, at least in the short term.

In contrast with the difficulties in finding markers from the organelle genomes, the nuclear genome offers a virtually unlimited supply of markers. It is orders of magnitude larger than the organelle genomes, and its bi-parental inheritance provides information from both parents. The large and complex array of coding and non-coding nucleotides means that there are segments of DNA evolving at vastly different rates. Nuclear DNA has been used to address questions in plant evolutionary biology ranging from the identification of individuals (Besse et al.,

1993) to the evolution of green plants (van de Peer et al., 1990). However, the large and complicated nature of the nuclear genome raises technical problems in characterising markers. Universal primers for true single copy gene loci are not available. Investigations of variability at single loci have typically required intensive molecular studies aimed at developing primers or probes for specific loci (e.g. White & Powell, 1997). Universal primers are available for repetitive gene families such as ribosomal DNA, but the evolutionary mechanisms of such multi-gene families are still poorly understood, and levels of intra specific variability typically low (Baldwin, 1992).

The most commonly applied DNA techniques for studying nuclear DNA variation in plants at the intra-specific level and between closely related species are arbitrary fingerprinting techniques (Weising et al., 1995). These encompass a range of related methodologies which aim to produce complex 'fingerprints' such as RAPDs (Randomly Amplified Polymorhpic DNA) (Williams et al., 1990), AFLPs (Amplified Fragment Length Polymorphisms) (Vos et al., 1995) and inter-SSRs (inter-Simple Sequence Repeats) (Zietkiewicz et al., 1994). Although differences exist in detail between these techniques, they have certain characteristics in common. Firstly, they do not require sequence information from the samples in question. Secondly they produce markers that are typically dominant (i.e. - each band is interpreted as a locus, with presence or absence being the allelic states). Thirdly, no information is available as to the genomic location of the bands in question. In essence an arbitrary bar code is generated from different samples, and some inference on the similarities or differences between individuals is made based on the number of bands shared between them.

The first of these techniques to be utilised widely (and still the most commonly used) is RAPDs - Randomly Amplified Polymorphic DNA. This technique uses short 10-mer primers of arbitrary sequence. The principal is that these primers will bind to sites scattered throughout the genome. Where primer binding sites occur

on opposite strands within 3-4kb of each other, a product (band) can be generated using PCR. Different individuals, with different sequences of genomic DNA, will have different priming sites. The more closely related individuals are, the more priming sites they will share in common and hence the greater the similarity in their banding profiles.

The simplicity of the technique is responsible for its widespread application, costs are relatively low and the equipment and training required are not particularly sophisticated. However, the technique does have its limitations. One of the foremost criticisms of RAPDs has been with regards to reproducibility. Given the nature of the technique, binding short primers at low stringency to complex templates, it is perhaps not surprising that reproducibility can be difficult. There are well publicised examples of the failure of separate laboratories to produce the same (or even similar) banding profiles from the same template DNA (Karp et al., 1998). These reports have lead to widespread scepticism of the technique. However, much of this criticism seems unfair and is perhaps driven by the molecular one-up-manship common to a rapidly moving field. Techniques are introduced with claims of widespread benefits, partly to aid publication and grant application success. After a few years, a more sophisticated technique is introduced and to aid its acceptance, criticisms of previous methodologies are inevitable. Many of the criticisms are genuine and valid, but some seem unnecessarily harsh. In the case of RAPDs, it seems important to stress that reproducibility is a technical, rather than a genetic problem and is easily tested for. If in a given experiment, identical profiles are achieved in repeat experiments, some confidence can be had in the results. It is worth stressing that although different laboratories may generate different profiles from a given sample, biological interpretations of the data often remain unchanged (P. M. Hollingsworth, pers. comm. 1998). Thus in those studies that have compared sets of samples in different laboratories, the inferred relationships among taxa have remained constant; within each laboratory the same conclusions are reached. It is

simply that different experimental conditions in different laboratories favour the amplification of different fragments. The most extreme example of this would be in clonal identification. Here the question is do all samples share the same banding pattern? If they do, the finer details of the banding patterns from different laboratories are not the issue, it is simply how many genotypes are detected with how many primers within each laboratory.

One genuine and more troubling criticism of the technique (and other arbitrary fingerprinting methods) is fragment homology. Just because two fragments comigrate to the same point on a gel does not mean that they are homologous. Detailed studies on Helianthus by Rieseberg (1996) showed that in comparisons of 220 pairs of comigrating fragments among three wild sunflower species, 91% were homologous (as tested by restriction digests or Southern hybridisation), although 13% of these mapped to different genomic locations suggestive of paralogous rather than orthologous relationships. Homology of fragments can be tested, but testing (particularly if positional homology is under scrutiny) is labour intensive and removes one of the main advantages of the technique, namely speed. Fragment homology thus remains something of an Achilles heel for many RAPD (and other arbitrary fingerprinting) based studies. Its impact on resulting biological interpretation is obviously dependent on the question being addressed. For clone identification, where the basic question is whether samples have an identical banding pattern or not over a range of primers, then the issue is largely irrelevant. If, however, some form of distance between different genets is being assessed, then fragment homology becomes a more critical question, and with increasing divergence the probability of false homologies will increase. In the current study, which attempts to use RAPDs for both the identification of clones and to look at relationships of closely related taxa, I accept that assumptions are being made about band homology that may be erroneous. However, the analytical approaches to examine inter-genet relationships have been chosen explicitly for relatively insensitivity to this type of problem. It seems a reasonable

working assumption, that at the taxonomic levels of the current study, the amount of noise introduced by non-homologous comigrating fragments is likely to be small and overidable by true signal in the data. The possible implications of violations of this assumption are raised in the relevant sections. Likewise, it is clear that the information content from dominant markers is reduced compared to their co-dominant counter parts due to our inability to distinguish between the AA and Aa genotypic states. Although explicit population genetic statistics have been developed to accommodate RAPD data (e.g. Lynch & Milligan, 1994), the underlying assumptions on which these statistical frameworks are based are in fact the end goal of many studies. The extreme violations of the assumptions of the basic Hardy-Weinberg model by the organisms in the current study (particularly polyploidy and clonal growth) preclude its application here.

Chapter 2

Sterile species and fertile hybrids - Japanese Knotweeds along the River Kelvin

Abstract

Fallopia japonica var. japonica, F. sachalinensis and their hybrid F. x bohemica occur along the River Kelvin in Glasgow. Their distribution along the river and in the surrounding area are described here. All three taxa are gynodioecious, and in the Glasgow area only male sterile plants of F. japonica var. japonica and F. sachalinensis were found, although both male sterile and male fertile plants of F. x bohemica were present. Due to the absence of male fertile individuals of the parental taxa it is unlikely that the hybrid has arisen in situ.

Introduction

A wide range of plant species have been introduced (either deliberately or accidentally) to the British Isles, and have now become a significant component of our flora. While many of these introduced species occur in geographically restricted areas, others have become well established and widespread. Riparian habitats in particular have changed dramatically due to colonisation by some invasive alien weeds. One such species that has received considerable attention is the Japanese Knotweed - Fallopia japonica (Houtt.) Ronse Decraene var. japonica.

Fallopia japonica var. japonica was introduced to Britain from Japan in the early half of the 19th century. There is some debate as to the precise date, but the plants most probably came from a nursery in Leiden in ca. 1841 (Beerling et al., 1994). Since its introduction it has spread to virtually all parts of the British Isles and its rapid spread coupled with the destructive capabilities of its rhizomes have led to it gaining notoriety as a troublesome weed. A closely related species, the Giant Knotweed - Fallopia sachalinensis (F. Schmidt ex Maxim.) Ronse Decraene also occurs in Britain, and is again thought to be the result of a horticultural introduction in the 19th century, probably in the 1860s (Conolly, 1977).

Both Fallopia japonica var. japonica and F. sachalinensis are gynodioecious and can occur as either male-sterile (female) or hermaphrodite plants. In the hermaphrodite flowers, the stamens are (usually) longer than the perianth and the anthers are large, swollen and full of pollen. In the male sterile flowers the stamens are shorter than the perianth and the anthers are small, flat and empty. In Britain both female and hermaphrodite forms of F. sachalinensis have been recorded, but only male sterile plants of F. japonica var. japonica are known (Bailey, 1994). The implication of this is that F. japonica var. japonica is not capable of reproduction by seed in Britain, and that its spread is purely by vegetative propagation. Seed can be found on British plants of F. japonica var. japonica but all seed examined so far is the result of hybridisation with closely related species such as F. baldschuanica (Regel) Holub, the Russian-vine, and F. sachalinensis. Although the majority of this seed appears to have F. baldschuanica as the pollen donor (Bailey, 1988), this hybrid very rarely becomes established in the wild. Only one such hybrid is known in Britain, in Haringey, Middlesex (Bailey, 1988). The hybrid between F. japonica var. japonica and F. sachalinensis - F. x bohemica (Chrtek & Chrtková) J. Bailey is, however, more widespread (Bailey et al., 1996). In Glasgow all three of these taxa (F. japonica var. japonica, F. sachalinensis and F. x bohemica) are known to occur along the banks of the River Kelvin and I describe here their distribution and reproductive biology along the watercourse.

Description of taxa

Fallopia japonica var. japonica grows annually to a height of about 3 m and forms dense clumps with stout, erect and often red spotted stems arising from strong rhizomes. The leaf blades are 5-12 cm long, 5-8 cm wide, broadly ovate, cuspidate at the apex and truncate at the base, coarse in texture and glabrous (Beerling et al., 1994).

Fallopia sachalinensis, like F. japonica var. japonica, is rhizomatous with stout, erect stems, but this species is somewhat larger and can grow up to 4 m, and the stems tend to lack the red spotted appearance of F. japonica var. japonica. The basal leaves are much larger (28-40 cm x 18-24 cm) and are ovate to oblong with a distinctive cordate base. The undersides of the leaves are scattered with long flexuous hairs (trichomes) (Bailey et al., 1996).

The hybrid, Fallopia x bohemica, has an intermediate habit, growing from 2.5 - 4 m and its leaves are intermediate in both shape and size. The leaf tip is acuminate and the base is weakly to moderately cordate. The undersides of the leaves have numerous short hairs which are easily visible with a hand lens on the larger leaves (Bailey & Conolly, 1991). The stems show an intermediate level of spotting to the parental species. On morphological grounds, plants with red flecks on the stems, short hairs on the underside of the leaves, along with weakly cordate leaf bases are likely to be the hybrid. Some caution should be applied when using stem spotting as a character, as discoloration of F. sachalinensis stems (the taxon with which F. x bohemica is most likely to be confused) towards the end of the growing season can cause confusion.

Chromosome numbers

Chromosome counts for a number of individuals of all three taxa are given by Bailey & Stace (1992) and those from the British Isles are summarised in Table 1. The difference in chromosome number of all three taxa is useful as it provides additional information to confirm identifications based on morphological characters.

Table 1 Chromosome numbers in selected Fallopia species[1]

Fallopia japonica var. japonica	2n=88	Octoploid
Fallopia sachalinensis	2n=44	Tetraploid
Fallopia x bohemica[2]	2n=66	Hexaploid

[1] Taken from Bailey & Stace (1992)

[2] A chromosome number of 2n=44 has also been recorded for F. x bohemica. This is from a cross between F. sachalinensis and F. japonica var. compacta (Hook. f.) J. P. Bailey (which has 2n=44). Neither F. japonica var. compacta, or the hybrid between F. japonica var. compacta and F. sachalinensis are known from the Glasgow area.

Distribution and ecology of Fallopia japonica var. japonica, F. sachalinensis and F. x bohemica along the River Kelvin, Glasgow.

In the Glasgow area Fallopia japonica var. japonica has been recorded from the majority of the tetrads (Dickson, 1991) and is very likely present in all 90. It is found forming small or large stands in a variety of habitats such as parks, gardens, railway banks, waste ground and along riverbanks. Its spread is remarkable given the lack of seed production, and its current distribution reflects disturbance and transport by human activities, although along water courses, dispersal by floating rhizome fragments seems likely.

Fallopia japonica var. japonica is well represented along the River Kelvin from the Killermont Golf Course (NS/551.702) south to the confluence with the Clyde (NS/556.660). In 1996, during a walk from the Kelvingrove Museum (NS/55.66) along the riverside to the Garscube Estate (NS/55.70) I rarely walked a distance of 100m without noting a stand of this species. Further north, near Summerston (NS/562.709) and Balmore (NS/565.709), the open and grazed banks of the Kelvin provide a less suitable habitat. The Kelvin downstream of the Killermont Golf Course provides a mosaic of habitats, but all have been more or less disturbed in recent history, and the river water, although cleaner now than in previous times, is still enriched by agricultural drainage and treated sewage. F. japonica var. japonica can form particularly large stands on the alluvial influenced river banks and it can also extend up the banks above the reaches of even the highest floods.

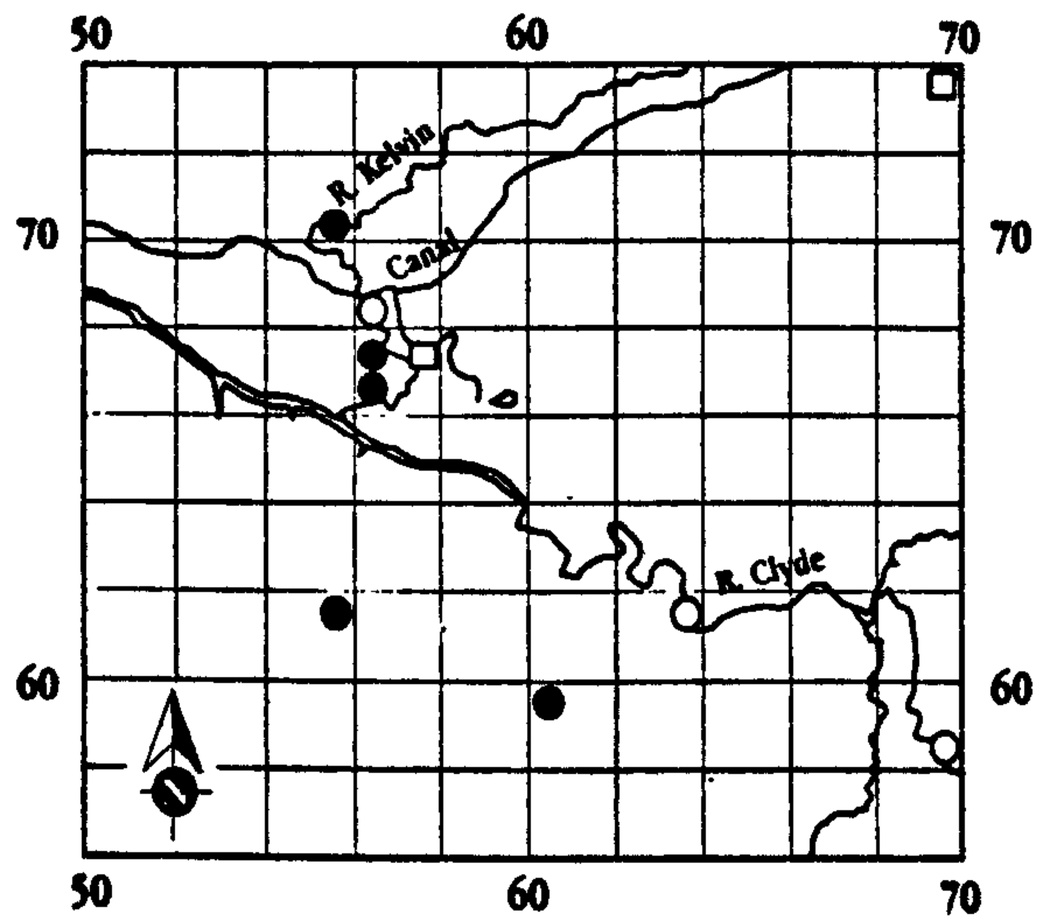
The localities of Fallopia japonica var. japonica along the Kelvin can be rich in associated species, reflecting the complex mixing of habitats. Native plants of the shaded river edges include Wood Stitchwort (Stellaria

nemorum L.), Comfreys (Symphytum L. spp.), Ramsons (Allium ursinum L.), Garlic Mustard (Alliaria petiolata (M. Bieb.) Cavara & Grande), Lesser Celandine (Ranunculus ficaria L.), Rough Meadow-grass (Poa trivialis L.), Giant Fescue (Festuca gigantea (L.) Villars) and Bistort (Polygonum bistorta (L.) Samp.). Alien species can be particularly well represented and typically include Few-flowered Garlic (Allium paradoxum (M. Bieb.) Don), Pink Purslane (Claytonia sibirica L.), Dame's-Violet (Hesperis matronalis L.), Sweet Cicely (Myrrhis odorata (L.) Scop.), Daffodils (Narcissus L. Heist ex. Fabr. spp.), Hybrid or Spanish Bluebells (Hyacinthoides spp.) and the other notorious 'troublesome weeds' Indian Balsam (Impatiens glandulifera Royle) and Giant Hogweed (Heracleum mantegazzianum Sommier & Levier). Additional diversity is provided by a range of often ephemeral colonisers of alluvial deposits, and the more ubiquitous aggressive species such as Nettle (Urtica dioica L.), Creeping Thistle (Cirsium arvense (L.) Scop.), Cleavers (Galium aparine L.), Common Couch (Elymus repens (L.) Desv. ex Nevski) and Ground Elder (Aegopodium podagraria L.).

Fallopia sachalinensis and the hybrid, in contrast to F. japonica var. japonica, are rare in the local Glasgow area. Their distributions are summarised in Fig. 1. The localities of F. sachalinensis are associated with the landscaped gardens of former Policy Estates, where it was presumably first introduced, although the plants have usually spread to the adjacent water courses where they may form large stands. It occurs in similar situations to, and shares associates with, F. japonica var. japonica, and can out compete it on alluvial influenced sandy river banks. The hybrid is also associated with old estates, where it may well have been introduced from nursery stock, but it also appears to show some of the invasive

characteristics of F. japonica var. japonica as at Eastfield Burn (NS/63.61) and South Johnstone (NS/41.62) where it grows in disturbed areas, well away from an obvious local source.

Fig. 1 Distribution map of Fallopia x bohemica and F. sachalinensis in the Glasgow area



Each square is a 4 square kilometre (= tetrad) block based on the Ordnance Survey National Grid. A sample of grid lines (50, 60, 70) is labelled for reference.

Topographical features include the Rivers Clyde and Kelvin and the Forth and Clyde Canal.

Black circles = Fallopia sachalinensis
White Circle = F. x bohemica
White Squares = Both taxa

Three records from outlying area are:

F. x bohemica	NS/41.62	NS/54.78
F. sachalinensis	NS/72.62	

Along the Kelvin, Fallopia sachalinensis occurs in three large but very localised stands. The furthest upstream is at the Garscube Estate (NS/551.702), where a dense stand approximately 20m long is present on the south bank of the river. The second stand is situated some 3.5km downstream on the north bank by the Queen Margaret Drive road bridge (NS/57.67). This is the largest stand of F. sachalinensis on the river, and stretches from the Botanic Gardens footbridge at (NS/569.675) to the road bridge (NS/571.675). This stand continues downstream to the weir at NS/571.674, although here it is less dense, being interspersed with other Knotweed taxa. The third stand is on the north bank of the river at the Kelvin Way road bridge at NS/569.663. F. sachalinensis grows here in a few small discrete clumps along with F. japonica var. japonica. At this site the abundance of F. sachalinensis has recently been much reduced due to erosion of the river bank and recent chemical spraying.

Fallopia x bohemica was first recorded on the River Kelvin in 1993, immediately upstream of the footbridge at NS/568.680. At this site there is a stand approximately 15m long on a steep embankment on the west side of the river. During the course of this survey two further sites were identified, although one of these was only a small isolated clump on the west side of the river, downstream of the footbridge at NS/569.680. The most extensive stand of this hybrid, and perhaps the easiest place to view it, is by the Queen Margaret Drive road bridge. Here, on the south bank of the river there are three large patches of F. x bohemica growing between the footbridge and the road bridge (NS/570.675) along with large stands of F. japonica var. japonica. On the north bank of the river, immediately downstream of Queen Margaret Drive road bridge, the hybrid grows over a 100m stretch of the river

bank down to the weir (NS/571.674-5). F. x bohemica grows here with both F. sachalinensis and F. japonica var. japonica and the differences in morphology between the three taxa can clearly be seen.

Sex expression

The sex of the plants was obtained by examining all flowering plants at a locality. All Fallopia japonica var. japonica and F. sachalinensis plants examined were male sterile. Both hermaphrodite and male sterile plants of F. x bohemica were found growing along the River Kelvin. The location of the plants examined and their sex is given in Table 2.

Chromosome counts

Chromosomes counts of all three taxa were made from mitotic root tip preparations as described by Bailey & Stace (1992), and the results are presented in Table 2 and Fig. 2. The chromosome numbers obtained in all cases are in agreement with the identifications based on morphological characters and help confirm the identity of all three taxa.

Table 2. Locality, sex and chromosome numbers of Fallopia taxa from the River Kelvin

Locality	Grid Reference	Sex	Chro. no
F. x *bohemica*			
Botanic Gardens (west bank)	NS/ 568.680	F	2n= 66
Botanic Gardens (west bank)	NS/ 569.680	FM	2n= 66
Botanic Gardens (north bank)	NS/ 571.674-5	FM	2n= 66
Botanic Gardens (south bank)	NS/ 570.675	FM	2n= 66
F. sachalinensis			
Botanic Gardens (north bank)	NS/ 569-71.674-5	F	2n= 44
Kelvin Way (north bank)	NS/569.663	F	2n= 44
Garscube (south bank)	NS/551.702	F	2n= 44
F. japonica var. *japonica*[1]			
Botanic Gardens (north bank)	NS/ 571.674	F	2n= 88
Botanic Gardens (south bank)	NS/ 570.675	F	2n= 88

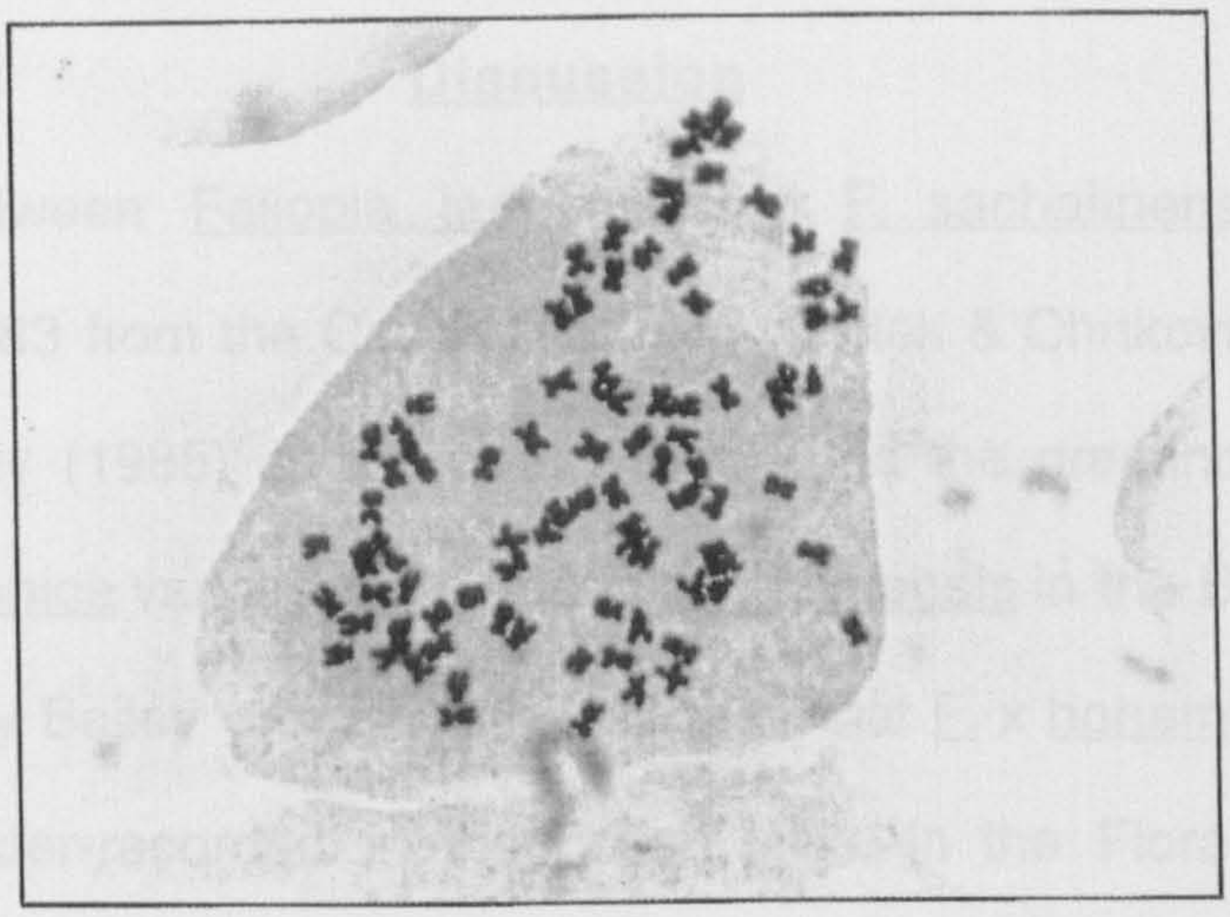
Chro. no = chromsome number

F = male sterile

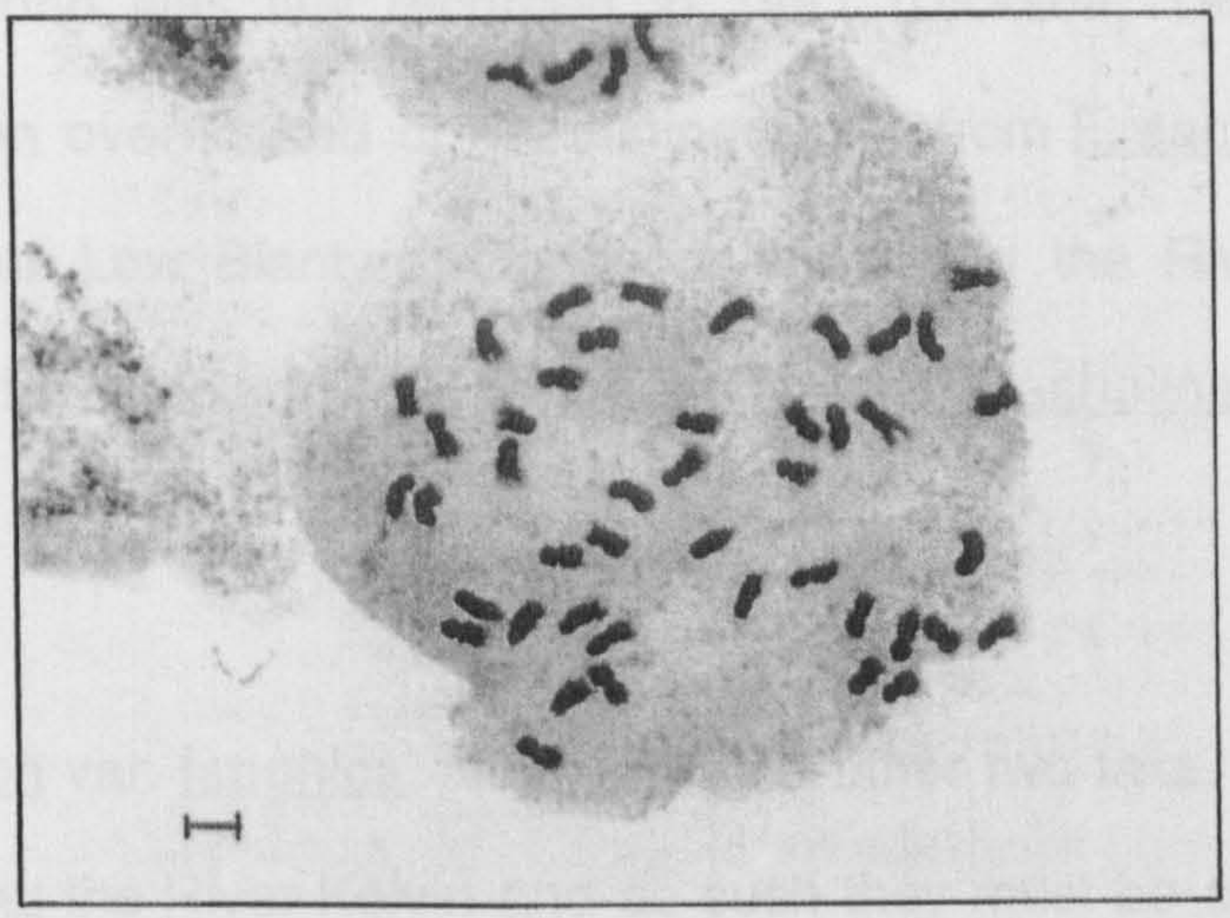
FM = hermaphrodite

[1] *F. japonica* var. *japonica* is abundant along the river, I include here only those localites from which I have cytological data

a)



b)



c)

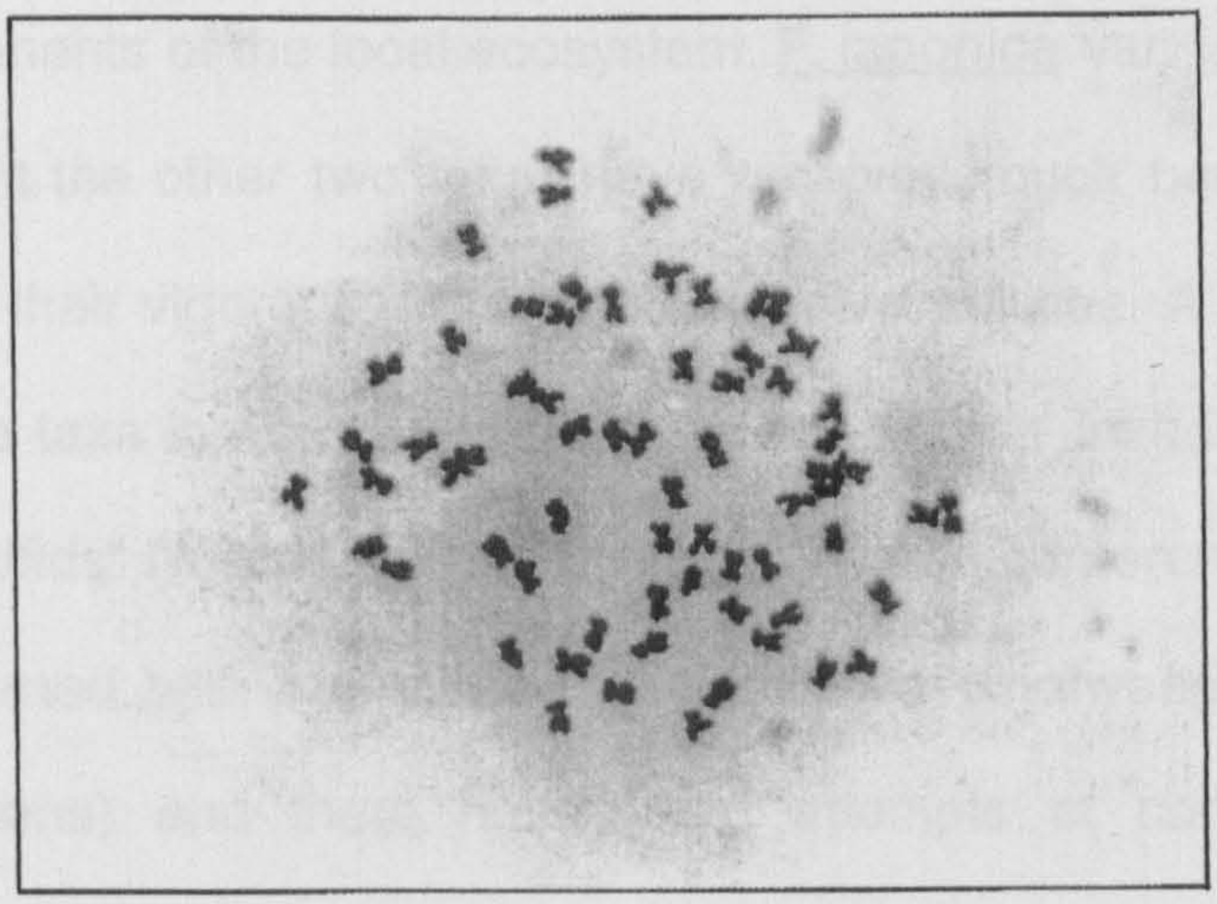


Fig. 2 Mitotic chromosome preparations of a) Fallopia japonica var. japonica, b) F. sachalinensis and their hybrid c) F. x bohemica.

Scale bar = 3μm

Discussion

The hybrid between Fallopia japonica and F. sachalinensis was first described in 1983 from the Czech Republic (Chrtek & Chrtková, 1983) and Bailey & Conolly (1985) subsequently suggested the presence of hybrids between F. japonica var. japonica and F. sachalinensis in the British Isles. A recent survey by Bailey et al. (1996) indicates that F. x bohemica has been significantly under-recorded in the British Isles. In the Flora of Glasgow surveys, the hybrid was first recorded in 1991 (Dickson, 1991) and has presumably been over-looked or not distinguished from F. sachalinensis at locations such as Low Blantyre, Gartshore and along the River Kelvin. A summary of the local records for F. x bohemica and F. sachalinensis is given in Table 3.

Fallopia japonica var. japonica, along with the other two taxa, is now firmly established along the River Kelvin and as such they must be considered as important components of the local ecosystem. F. japonica var. japonica (and to a lesser extent the other two taxa) have received much bad press as a consequence of their vigorous growth and invasive abilities. A recent article describing these taxa in Britain was entitled "Japanese invader poised for the day of the triffids" (Nuttall, 1996). There have been conferences and local meetings concerned with the spread of Japanese Knotweeds (and other troublesome aliens) and there have been attempts at control, through chemical spraying, along the lower reaches of the River Kelvin.

In recent years, however, some ecologists have called for a more long-term and dispassionate consideration of the ecological role of Knotweeds. Gilbert (1994) argued that Fallopia japonica var. japonica is "starting to play a

valuable role in the ecology of urban areas". This view is supported by other authors and Dickson (1996) argued that Knotweeds and other alien plants should be seen as welcome additions to the British flora. F. japonica var. japonica, especially in its periodically disturbed riverside situations, is potentially an important component of climax vegetation. Winter flooding as well as providing additional nutrients, can remove dead stems and other debris, leaving open ground which can host a diverse array of vernal 'woodland' species; these are well adapted to early season growth before the Fallopia canopy shade becomes suppressive by mid-summer.

Fallopia japonica var. japonica is undoubtedly a very aggressive and damaging weed in many places, and often proves extremely difficult to control and eradicate, and is most unwelcome in parks and gardens. However I consider that along certain urban riverbanks, the plant could be viewed as providing valuable ground cover and does not necessarily constitute a serious problem, and in fact may be beneficial to the local ecology. Rivers, such as the urban stretch of the Kelvin, have suffered much disturbance in the past, but they are naturally dynamic habitats, and the current development of the rich and varied flora along the Kelvin, both native and alien, should be welcomed.

Fallopia sachalinensis, F. japonica var. japonica and F. x bohemica form an unusual hybrid situation on the River Kelvin in Glasgow with the only male fertile taxon being the hybrid. Although I was not expecting to find any male fertile plants of F. japonica var. japonica, it is surprising, given the presence of F. x bohemica, that no male fertile plants of F. sachalinensis were found. It seems likely that on the River Kelvin, F. sachalinensis, like F. japonica var.

japonica, perpetuates by purely vegetative propagation. This raises the question as to how the F. x bohemica plants originated, as hybridisation between two effectively female populations of the parental species is impossible. Two possible explanations are suggested. Firstly that there either is, or once was, a male fertile F. sachalinensis in the area and this served as the pollen donor to form the hybrid. A second possibility is that the hybrid has arisen elsewhere and its distribution along the River Kelvin with its parents is coincidental. All Glasgow records of F. sachalinensis have been checked and as yet no male fertile plants have been found (Table 3). I also examined all F. x bohemica populations in the Glasgow area, and the distribution of the sexes is shown in Table 3.

Given the absence of male fertile Fallopia sachalinensis in the region, I have no evidence to suggest that F. x bohemica has arisen locally, and it seems likely that the hybrid has been introduced to the Glasgow area. Furthermore, as I have found evidence for at least two clones of F. x bohemica, (one hermaphrodite and one male sterile), it is possible that it has been introduced on multiple occasions. The association of this taxon with the Botanic Gardens could well be far from coincidental, and it is quite possible that different clones have been introduced here. It should be borne in mind, however, that as the genetic basis of the difference between the male sterile and hermaphrodite forms is still unknown, I cannot rule out that the male sterile F. x bohemica plants are second generation hybrids that have arisen in situ from the hermaphrodite plants of F. x bohemica.

Table 3: Local Records of Fallopia sachalinensis and F. x bohemica

Location	Grid Ref.	VC	1st Recorder	Date	Sex
F. sachalinensis					
Pollok Park	NS/55.61	76	Dickson	1982	F
Castlemilk Glen	NS/60.59	77	Macpherson	1985	F
Botanic Gardens	NS/57.67	77	?	?	F
Kelvin Way	NS/56.66	77	?	?	F
Mill Bank	NS/72.62	77	Watson	1989	F
Gartshore Estate	NS/69.73	86	Dickson & GNHS	1987	F
Garscube Estate	NS/55.70	99	Watson	1988	F
F. x bohemica					
South Johnstone	NS/41.62	76	Watson	1994	FM
Eastfield Burn	NS/63.61	77	Macpherson	1993	F
Low Blantyre	NS/69.58	77	Macpherson & Lindsay	1991	F
Botanic Gardens	NS/56.68	77	Watson	1993	F
Botanic Gardens	NS/56.68	77	Hart, Hollingsworth & Watson	1996	FM
Botanic Gardens	NS/57.67	77	Hart & Hollingsworth	1996	FM
Dumbrock Loch	NS/54.78	86	Dickson	1994	FM
Gartshore Estate	NS/69.73	86	Hart, Hollingsworth & Watson	1996	FM

F = male sterile

FM= hermaphrodite

GNHS = Glasgow Natural History Society

Chapter 3

The use of molecular markers to study patterns of genotypic diversity in some invasive alien Fallopia spp. (Polygonaceae)

Abstract

RAPDs and inter-SSRs have been used to study clonal growth and hybridisation in some non-native, gynodioecious, invasive weeds from the genus Fallopia (Polygonaceae). At the study site (the River Kelvin, Glasgow, Scotland) a single genotype of Japanese Knotweed (F. japonica) was detected, consistent with all the individuals sampled being ramets of a single clone. Two genotypes of Giant Knotweed (F. sachalinensis) were detected, with one genotype accounting for all but one of the samples, again indicative of widespread clonal growth. Five genotypes of the hybrid between Japanese and Giant Knotweed (F. x bohemica) were recovered. F. x bohemica is the only male fertile taxon present at the site and it seems likely that at least some of this genetic variation is attributable to hybrid fertility. A single plant identified on morphological grounds as a backcross between F. japonica and F. x bohemica was analysed, and the molecular data were consistent with this theory. A comparison of RAPDs and inter-SSRs showed that the two techniques gave data that is broadly congruent, and both showed a similar sensitivity in the number of genotypes detected.

Introduction

Throughout the world, many plant communities contain a large proportion of introduced species. In a recent account of the flora of the British Isles, Stace (1997) judged 1391 out of the 2963 species described to be alien. Many of these introduced species have become naturalised in the wild, and some have become invasive weeds. Occasionally these alien species hybridise with native or other introduced species and this can lead to speciation or introgression and the evolution of new biodiversity (Abbott, 1992). This chapter describes the genetic structure of a population of two introduced weed species and their hybrid, and discusses the consequences of post-introduction interactions between them.

Japanese Knotweed, Fallopia japonica (Houtt.) Ronse Decraene var. japonica (Polygonaceae), was introduced to Europe from Japan in ca.1841 as an ornamental garden plant (Beerling et al., 1994). It has subsequently spread from the parks and gardens where it was originally planted and has now become a widespread and troublesome invasive weed, present throughout the British Isles (Fig. 3a). Its colonising ability is facilitated by vigorous rhizomatous growth and it is capable of regenerating from minute fragments of rhizome (Brock & Wade, 1992). The transport and tipping of soil containing plant fragments, coupled with water mediated dispersal in riparian habitats, provides an efficient means of dispersal.

A dwarf variety of Fallopia japonica, F. japonica var. compacta (Hook. f.) J. Bailey also occurs in the British Isles although it is much rarer than var. japonica. As it is not found in the river system covered in this chapter, it is not considered further and for brevity, in the remainder of this paper I refer to F. japonica var. japonica as F. japonica.

A second alien Fallopia species, Giant Knotweed (F. sachalinensis (F.Schmidt ex Maxim.) Ronse Decraene) also occurs in Britain, again stemming from a 19th century horticultural introduction from eastern Asia (Conolly, 1977). F. sachalinensis has subsequently colonised new habitats, although this secondary spread has been more restricted than that of F. japonica (Fig.3b).

Both species are gynodioecious, occurring as hermaphrodite and male sterile plants (Bailey, 1994); insect visitors to their flowers include houseflies, hoverflies and bees (Beerling et al., 1994). In Britain, all Fallopia japonica examined so far has been male sterile (effectively female), the implication being that all reproduction is by vegetative spread (Bailey, 1994). No true bred seed has been found on British plants of F. japonica (Beerling et al., 1994). F. sachalinensis, however, occurs as both hermaphrodite and male sterile plants, pure bred seed is formed in Britain, and reproduction can potentially occur both sexually and asexually (Bailey, 1994). The two species hybridise in Britain and the hybrid F. x bohemica (Chrtek & Chrtková) J. Bailey occurs as both male sterile and hermaphrodite individuals and shows partial to full fertility (Bailey et al., 1996). F. x bohemica occurs where the parental taxa are sympatric, but is most commonly found at sites where one or both parents are absent; its distribution in Britain is summarised in Fig. 3c.

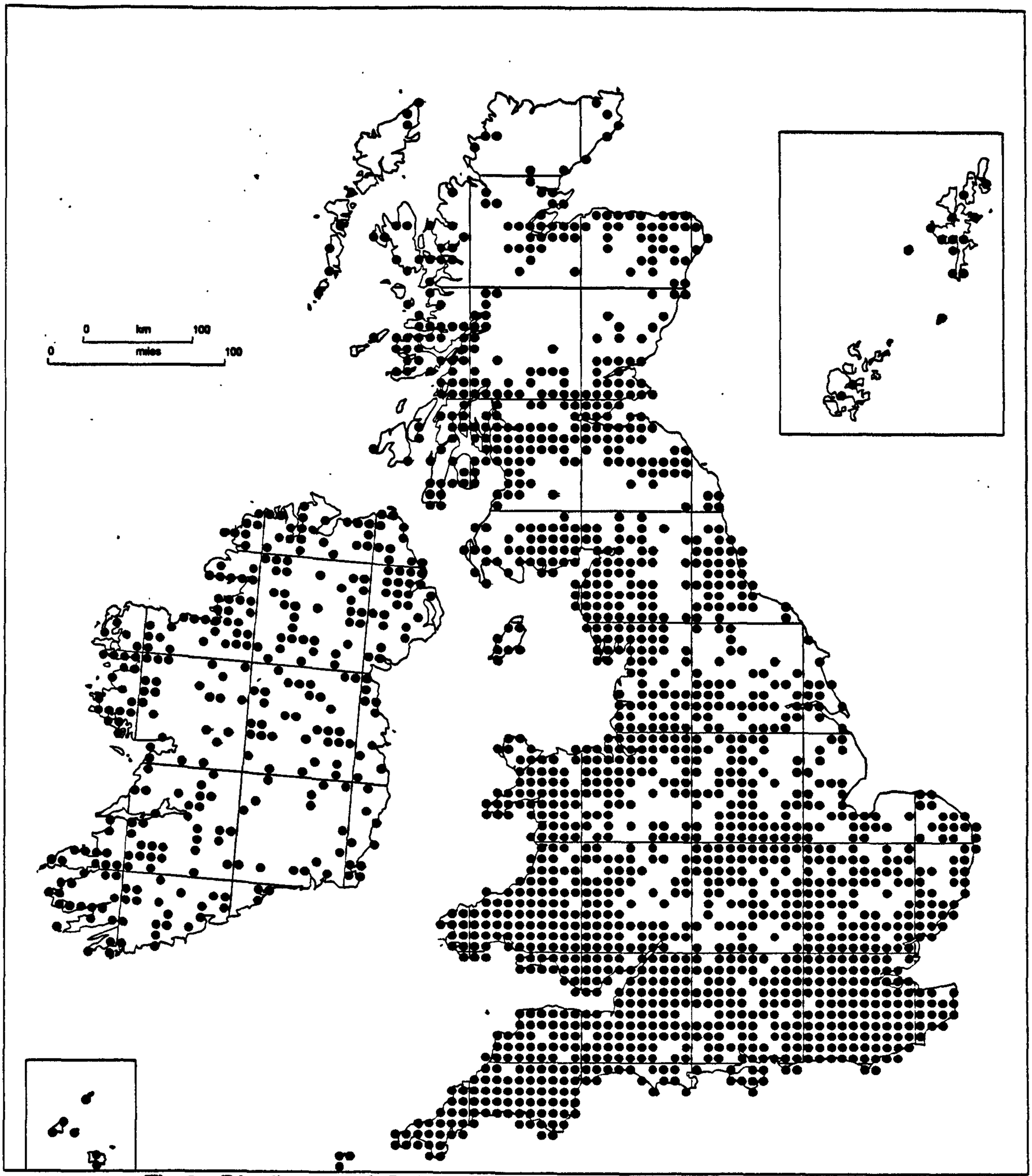


Fig.3a Distribution of Fallopia japonica var japonica in the British Isles

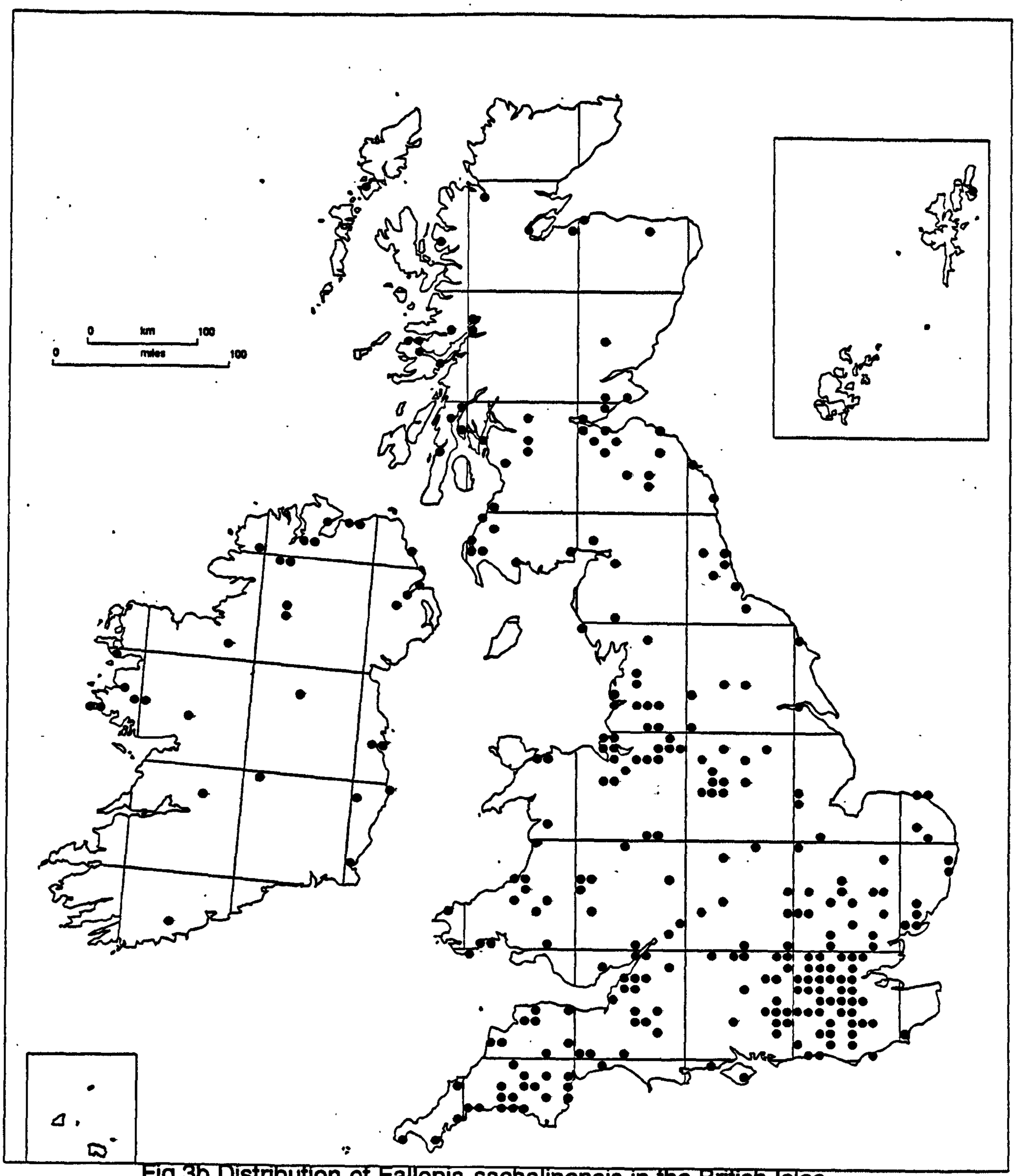


Fig.3b Distribution of Fallopia sachalinensis in the British Isles

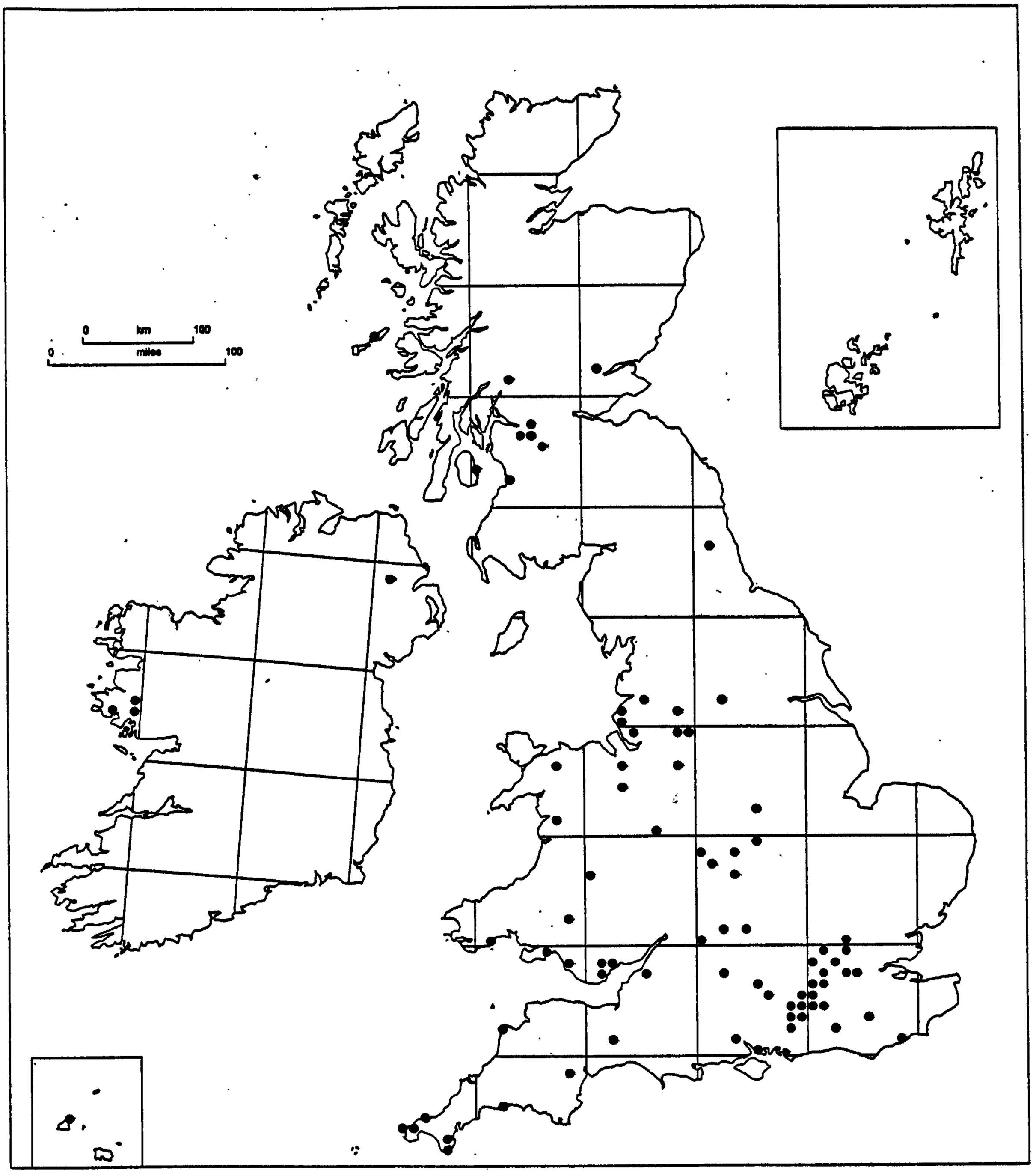


Fig.3c Distribution of Fallopia x bohemica in the British Isles

Sites where both parents and the hybrid are found are of interest, as there is the potential for genetic exchange between taxa. Hybridisation between Fallopia japonica and F. sachalinensis, if followed by backcrossing, could lead to the evolution of genotypes with enhanced invasive abilities. While F. sachalinensis is less invasive than F. japonica, some of its British genotypes do have the ability to produce viable pollen, and the introgression of genes which confer this ability to F. japonica is a cause for concern. The acquisition of the ability for seed production and dispersal in an already invasive plant is likely to be problematical for control and eradication programmes.

The River Kelvin in Glasgow is one of only a few sites in Britain where Fallopia japonica, F. sachalinensis and their hybrid co-occur. In light of the potential for introgressive hybridisation, I have investigated these plants along the water course in some detail.

Fallopia japonica is abundant along the course of the River Kelvin and is found in both large and small stands. In contrast, F. sachalinensis and the hybrid are less common, with the former being restricted to three sites and the latter to two sites, where they form large and dense clumps. One of the hybrid sites was discovered during the course of this study and first impressions suggested that it represented an in situ origin, as the hybrid was found growing in a large, mixed stand in intimate contact with both of the parental taxa. However, all of the individuals of the parental taxa along the river I have seen, have been females and the only functional hermaphrodite taxon present is the hybrid (male sterile F. x bohemica plants are also present along the river (Hart et al., 1997 - chapter 2)). Although one might not expect to find any male fertile plants of F. japonica as none are known in Britain, it is surprising, given the presence of the hybrid, that no male fertile plants of F. sachalinensis have been found. The absence of functional

males of the parental taxa raises the question as to how the F. x bohemica plants originated. Ruling out residual fertility, of which I have found no evidence, there are two possible explanations. First, that there is, or once was, a male fertile F. sachalinensis in the area, and this served as the pollen donor to form the hybrid. Second, that the hybrid has arisen elsewhere and its distribution along the River Kelvin with the parental taxa is coincidental.

As part of a broader study into the population genetics and biosystematics of this weed complex in Britain, I have carried out a genetic survey of these plants from the River Kelvin. The primary aim has been to investigate the levels of clonal diversity and inter-relationships of the genotypes. This river provides an ideal system to assess the extent of reproduction by seed in the hybrid, with reduced probability of multiple origins as a confounding variable. Furthermore, it allows for the study of backcrosses with the controlling factor of only one possible pollen donor. A second aim of this study is to compare the efficiency of RAPDs (randomly amplified polymorphic DNA) and inter-SSRs (simple sequence repeats) in assessing the patterns of genetic variation in these taxa. Although these techniques are conceptually similar in that they both aim to produce arbitrary fingerprints using single primers, it has been suggested that inter-SSRs, which use longer primers targeted to inverted microsatellite repeats, are more polymorphic than RAPDs (Zietkiewicz et al., 1994).

Materials and Methods

Plant Material

To assess the patterns of genetic variation in these Fallopia taxa, a total of 22 F. japonica, 17 F. sachalinensis and 23 F. x bohemica individuals was sampled along the course of the River Kelvin. A plant tentatively identified as a backcross between F. x bohemica and F. japonica was also sampled. Voucher specimens of all taxa have been deposited at LTR. Full details of the sampling localities and the sexes (where known) of the plants are given in Table 4. Chromosome numbers differ between the taxa, with F. japonica 2n=88 (octoploid), F. sachalinensis 2n=44 (tetraploid) and F. x bohemica 2n=66 (hexaploid) (Bailey & Stace, 1992). Chromosome counts from all three taxa from the River Kelvin, taken from Hart et al. (1997 - chapter 2) are summarised in Table 4.

Table 4. Sample localities of Fallopia taxa from the River Kelvin, Glasgow, UK

Taxon	Grid reference	Sex	n	Chro. No.[1]
F. japonica				
Kelvinside Conference Centre	NS/555.698	MS	1	
Kelvindale Bridge	NS/563.685	MS	1	
Kelvingrove Museum	NS/565.664	MS	2	
River Kelvin Walk	NS/568.681	MS	1	
View point	NS/568.677	MS	2	
Kelvin Way bridge	NS/569.663	MS	2	
Nr Kelvin Way bridge	NS/569.664	MS	1	
Queen Margaret Bridge, S. bank	NS/570.675	MS	2	2n=88
Queen Margaret Bridge, N. bank	NS/571.674	MS	2	2n=88
Queen Margaret Bridge, N. bank	NS/571.675	MS	2	
Ruin	NS/572.675	MS	1	
River Kelvin Walk	NS/573.668	MS	1	
River Kelvin Walk	NS/574.669	MS	1	
Great Western Road Bridge	NS/575.670	MS	2	
River Kelvin Walk	NS/575.671	MS	1	
F. sachalinensis				
Garscube Estate	NS/551.702	MS	1	2n=44
Kelvin Way Bridge	NS/569.663	MS	3	2n=44
Queen Margaret Bridge, N. bank	NS/569-71.674-5	MS	13	2n=44
F. x bohemica				
Arboretum footbridge	NS/568.680	MS	4	2n=66
E. of Arboretum footbridge	NS/569.680	?	1	2n=66
Queen Margaret Bridge, S. bank	NS/570.675	H	7	2n=66
Queen Margaret Bridge, N. bank	NS/571.674-5	H	11	2n=66
F. x bohemica x F. japonica (tentative identification)				
Botanic Gardens	NS/690.675	?	1	2n=88

[1]Taken from Hart et al. 1997. A single plant was counted from each of the localities where a count is given.

Chro. No. = Chromosome number, n = sample size for molecular data (minimum sample interval between plants was 2 m)

MS = male sterile, H = hermaphrodite (male fertile), ? = sex unknown.

Grid reference is the UK Ordnance Survey mapping locality to the nearest 100m.

Isolation of Genomic DNA

Genomic DNA was isolated using a protocol based on the CTAB method of Doyle & Doyle (1990). This technique was slightly modified to include a washing step with 7.5 M ammonium acetate in order to remove the large amounts of carbohydrates that were co-isolated with the Knotweed DNA. Quality and concentration of the DNA was assessed by running the samples alongside concentration markers on a 1% agarose gel.

Molecular Analyses

Sixty 10-mer oligonucleotide RAPD primers from the Operon A, F and P kits and forty inter-SSR primers from the University of British Columbia Set 9 were screened for a subset of the samples, and from these, ten RAPD and five inter-SSR primers were chosen which gave clear, reproducible banding patterns. Primer sequences are given in Table 5. Three of the inter-SSR primers are sequences of 2-base repeats. The repeat sequence is interrupted at the 3' end by bases that are out of phase with the repeat and thus act as anchors. The remaining two primers are 3- and 5-base repeats without anchors.

RAPD Analysis

PCR reactions of 25 µl contained 2.5 µl 10x reaction buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris HCl, 0.1% Tween 20, pH8.8), 2.5 mM $MgCl_2$, 200 µM dNTPs, 0.5 µM primer, 5 ng template DNA, 1 Unit of Taq polymerase (Bioline UK). Amplification was carried out using the following PCR profile: 2 min at 95 °C, 2 cycles of 30 s at 95 °C, 1 min at 37 °C, 2 min at 72 °C, 2 cycles of 30 s at 95 °C, 1 min at 35 °C, 2 min at 72 °C, 41 cycles of 30 s at 94 °C, 1 min at 35 °C, 2 min at 72 °C, followed by a final 5 min extension at 72 °C.

Inter-SSR Analysis

PCR reactions were as above, but using 0.2 µM primer, 10 ng template DNA and 1.25 Units of Taq polymerase (Bioline UK). Amplification was carried out using the following PCR profile: 40 cycles of 20 s at 93 °C, 1 min at 55 °C and 20 s at 72 °C, followed by a final 6 min extension at 72 °C.

For both the RAPD and inter-SSR analyses, PCR products were separated by gel electrophoresis on 1.6% agarose gels in TBE buffer and visualised using ethidium bromide staining (0.1 µg/ml). Negative controls, lacking template DNA were included in each PCR run and all sample / primer combinations were repeated at least once. Replicate DNA extractions were carried out on a subset of the samples and the arbitrary 'fingerprint' of these were compared with the original. In all cases an identical profile was obtained.

Table 5. Primer sequences for RAPD and inter-SSR analyses

Primer	Sequence 5'-3'
RAPD:	
OPA-06	GGTCCCTGAC
OPA-08	GTGACGTAGG
OPA-16	AGCCAGCGAA
OPF-08	GGGATATCGG
OPF-14	TGCTGCAGGT
OPF-16	GGAGTACTGG
OPF-17	AACCCGGGAA
OPF-20	GGTCTAGAGG
OPP-03	CTGATACGCC
OPP-20	GACCCTAGTC
Inter-SSR:	
UBC-840	GAGAGAGAGAGAGAGAYT
UBC-853	TCTCTCTCTCTCTCTCRT
UBC-855	ACACACACACACACACYT
UBC-868	GAAGAAGAAGAAGAAGAA
UBC-881	GGGTGGGGTGGGGTG

Key: OP = Operon; UBC = University of British Columbia

Single letter abbreviations for mixed base positions: Y = (C,T); R = (A, G)

Data Analysis

The presence or absence of the different bands was scored by eye, with only strong and reproducible bands considered in the analysis. The computer package R (Legendre & Vaudor, 1991) was used to calculate Jaccard's Coefficient: $D_J=2n_{xy}/n_x+ny$ where n_x is the number of bands present within accession x, n_y is the number of bands present within accession y, and n_{xy} is the number of bands shared by accessions x and y (Jaccard, 1908). The complement of the similarity values were then calculated and the resulting distance matrix was converted to a planar splits graph using the computer package SplitsTree 2 (Huson, 1997). SplitsTree 2 (available from ftp://ftp.uni-bielefeld.de/pub/math/splits) implements the split-decomposition algorithm (Bandelt & Dress, 1992) to create a network allowing the visualisation of the first and second most strongly supported groupings among each quartet. This method is useful as it does not restrict the data to a rigid tree-like model, and can be used to identify and visualise the character conflict expected in a reticulate system. Effectively, the technique works by decomposing a distance matrix into a number of splits, which represent opposing groupings (Bandelt & Dress, 1992). These are represented on a splits graph as parallel edges. The residual indecomposable portion of the data is termed the split prime residue and is typically not visualised on the graphs (Bandelt and Dress, 1992, D. H. Huson pers. comm. 1997). The split prime residue is expected to contain much of the random noise in the data (Bandelt & Dress, 1992) and the advantages of effectively removing this noise from the network in the context of RAPD data sets, where co-migrating fragments may not be homologous and thus create noise, was highlighted by Rieseberg (1996).

Results

Comparison of Techniques

A total of 142 reproducible polymorphic bands were produced from the 15 primers employed in this study (96 from the RAPD primers and 46 from the inter-SSR primers). The distribution of these bands among taxa is shown in Figs. 4 & 5 and described below. A mean of 9.6 RAPD bands (range 3-14) and 9.2 inter-SSR bands (range 5-13) per primer were reproducibly amplified and found to be polymorphic either within or between taxa. The RAPD primers detected a mean of 6.7 genotypes per primer (range 4-9). The inter-SSR primers detected a mean of 5.6 genotypes per primer (range 4-7). This difference was not significant (Mann-Whitney U test, $p=0.218$). Only one primer detected all nine of the genotypes identified in this study (RAPD primer OPF08, Fig. 6).

Genetic Variability Within and Among Taxa

Based on multi-primer genotypes, a single genotype was detected in *Fallopia japonica* and two genotypes were detected in *F. sachalinensis* (Figs. 4 & 6). Five genotypes were detected amongst the *F.* x *bohemica* plants, one genotype representing the male sterile plants, three representing the hermaphrodite plants, with the plant B? (of unknown sex) having a fifth genotype (Figs. 4 & 6).

Fig.4 Presence and absence of arbitrary fingerprinting bands in Fallopia taxa from the River Kelvin, Glasgow, UK

Genotypes detected	OPP03	OPF14	OPP20	OPF16	OPF17
F. japonica JMS	1 1 1 1 1 1 0 1	0 0 1 0 0 1 1 0 0 0 0 1 0 1	1 0 0 0 1 0 1	0 1 1 0 1 0 0	1 0 0 1 1 1 1 0
F. sachalinensis SMS1	0 0 1 1 1 0 1 0	0 1 1 1 1 0 0 1 1 0 0 0 1 0	1 0 1 1 0 1 0	0 1 0 1 0 1 1	0 1 1 0 0 0 1 1
F. sachalinensis SMS2	0 0 1 0 1 0 1 0	1 1 0 1 0 0 0 1 1 0 0 1 1 0	0 0 1 1 0 1 0	0 1 0 1 0 1 1	0 1 1 0 0 0 0 1
F. x bohemica BMS	0 1 1 0 1 1 1 0	0 1 1 1 0 1 1 0 1 0 0 1 1 1	1 1 1 1 1 0 1	0 1 1 0 1 0 0	0 1 1 1 1 0 1 0
F. x bohemica BH1	1 1 1 0 1 0 1 0	0 1 0 0 0 1 1 0 1 1 0 1 1 1	1 1 1 0 1 0 1	0 1 1 1 0 1 0	0 1 1 0 1 0 1 1
F. x bohemica BH2	1 1 1 0 1 0 1 0	0 1 0 0 0 1 1 0 1 1 1 1 1 1	1 1 1 0 1 0 1	0 1 1 1 0 1 0	0 1 1 0 0 0 1 0
F. x bohemica BH3	1 1 1 0 1 0 1 0	0 1 0 0 0 1 1 0 1 1 0 1 1 1	1 1 1 0 1 0 1	0 1 1 1 0 1 0	0 1 1 0 0 0 1 0
F. x bohemica B?	0 0 0 0 0 0 1 0	0 1 0 0 0 1 1 0 1 1 1 1 1 1	1 1 1 0 1 0 1	1 1 1 1 0 0 0	0 1 1 0 1 0 0 0
F. x bohemica x F. japonica BJ?	1 1 1 1 1 1 0 1	0 0 1 0 0 1 1 0 0 0 1 1 0 1	1 1 1 0 1 0 1	0 0 1 0 1 0 0	1 0 0 1 1 1 1 0

Genotypes detected	OPF08	OPA08	OPF20	OPA16	OPA06
F. japonica JMS	1 0 1 1 1 0 1 1 0 0 1 0 0 1	1 1 1 0 1 0 0 0 0 1 0 1	1 0 0 1 1 0 0 1 1 1 0 1	0 0 1 0 1 1 0 1 0 0 0	1 1 1
F. sachalinensis SMS1	0 1 0 0 0 1 0 0 1 1 1 1 1 0	0 0 0 1 0 1 0 1 1 0 1 0	1 1 1 1 1 1 1 0 0 0 1 0	1 1 1 1 0 1 1 0 1 0 1	0 0 0
F. sachalinensis SMS2	0 1 0 0 0 1 0 0 1 1 0 1 1 0	0 0 0 1 0 1 0 1 1 0 1 0	1 1 1 1 1 0 1 0 0 0 1 0	1 0 0 1 0 0 1 0 1 0 1	0 0 0
F. x bohemica BMS	1 1 0 1 1 0 1 1 0 1 0 1 1 0	1 0 0 0 1 0 1 0 1 1 0 1	1 1 0 1 1 0 1 1 1 1 0 1	0 0 1 0 1 1 1 1 1 0 0	1 0 1
F. x bohemica BH1	1 1 1 0 1 0 1 1 0 1 1 0 1 0	0 0 0 0 0 1 0 0 1 1 0 1	1 1 0 1 1 0 1 0 1 1 0 1	0 0 1 1 1 1 0 1 1 0 0	0 0 1
F. x bohemica BH2	1 1 1 1 1 0 1 1 1 0 1 0 1 0	0 1 0 1 1 1 0 0 1 1 0 1	0 1 0 1 1 0 1 0 1 1 0 1	0 0 1 0 1 1 0 1 1 0 0	0 1 1
F. x bohemica BH3	1 1 1 0 1 0 1 1 0 0 1 0 1 0	0 1 0 1 1 1 0 0 1 1 0 1	0 1 0 1 1 0 1 0 1 1 0 1	0 0 1 0 1 1 0 1 1 0 0	0 0 1
F. x bohemica B?	1 1 1 1 1 0 0 1 1 0 0 0 1 0	0 1 1 0 0 0 0 0 1 1 0 1	1 1 0 0 0 0 0 1 1 0 0 1	0 0 1 0 1 1 1 1 1 1 0	0 0 1
F. x bohemica x F. japonica BJ?	1 0 1 1 1 0 1 1 0 0 0 0 0 1	1 1 0 1 0 0 1 0 0 1 0 1	1 0 0 1 1 0 0 1 1 1 0 1	0 0 1 0 1 1 0 0 1 0 0	1 1 1

Genotypes detected	UBC840	UBC855	UBC881	UBC853	UBC868
F. japonica JMS	0 1 0 0 0 1 1 1 0 0 0 0 1	1 1 0 0 0 1 0 1	1 0 1 0 1 0 1 1 0	0 1 1 0 1	0 1 0 0 0 0 1 1 1 0 0
F. sachalinensis SMS1	0 0 1 1 1 0 0 0 1 0 1 1 0	0 0 1 1 1 0 1 0	0 1 0 1 0 1 0 0 0	1 0 0 0 0	1 0 0 1 1 1 0 0 0 1 1
F. sachalinensis SMS2	0 0 1 1 1 0 0 0 1 0 1 1 0	0 0 1 1 1 0 1 0	0 1 0 1 0 1 0 0 0	1 0 0 0 0	1 0 0 1 1 0 0 0 0 1 1
F. x bohemica BMS	0 0 1 1 1 0 1 1 1 1 1 1 1	1 0 1 0 1 1 1 1	1 0 0 1 1 0 0 1 1	1 1 1 1 0	0 1 0 0 1 0 1 1 1 0 1
F. x bohemica BH1	0 0 1 1 1 0 1 0 0 0 0 1 1	1 1 1 0 1 1 1 1	1 0 1 1 1 1 1 1 1	0 1 1 1 0	0 1 0 0 1 0 0 1 0 1 0
F. x bohemica BH2	0 0 1 1 0 1 1 0 0 0 1 1 1	1 1 1 0 1 1 1 1	1 0 1 1 1 1 1 1 1	1 1 1 1 0	0 1 1 0 1 0 0 1 0 1 0
F. x bohemica BH3	0 0 1 1 0 1 1 0 0 0 0 1 1	1 1 1 0 1 1 1 1	1 0 1 1 1 1 1 1 1	1 1 1 1 0	0 1 0 0 1 0 0 1 0 1 0
F. x bohemica B?	0 0 1 1 0 1 1 0 0 0 0 1 1	1 1 1 0 1 1 1 1	1 0 1 1 1 1 1 1 1	1 1 1 1 0	0 1 0 0 1 0 0 1 0 1 0
F. x bohemica x F. japonica BJ?	1 1 0 0 0 1 1 1 1 1 1 0 1	1 1 0 0 1 1 1 1	1 0 1 1 1 1 1 1 1	1 1 1 1 1	0 1 0 0 0 0 1 1 1 1 0

The table shows the presence or absence of all scored bands for all genotypes detected in this study. The distribution of these genotypes amongst plants is shown in Fig. 5. Where these bands are refered to in the text they are identified by the primer name followed by a band number, the band numbers are derived from left to right in ascending order, starting from one for each primer.
Key: JMS = F. japonica male sterile; SMS = F. sachalinensis male sterile; BMS = F. x bohemica male sterile; BH = F. x bohemica hermaphrodite (male fertile); B? = F. x bohemica sex unknown; BJ = F. x bohemica x F. japonica sex unknown. The number following the taxon code is an arbitrary code to distinguish between different genotypes of the same taxon and sex.

Fig. 5. Location of the study populations of Fallopia taxa along the River Kelvin, Glasgow, UK

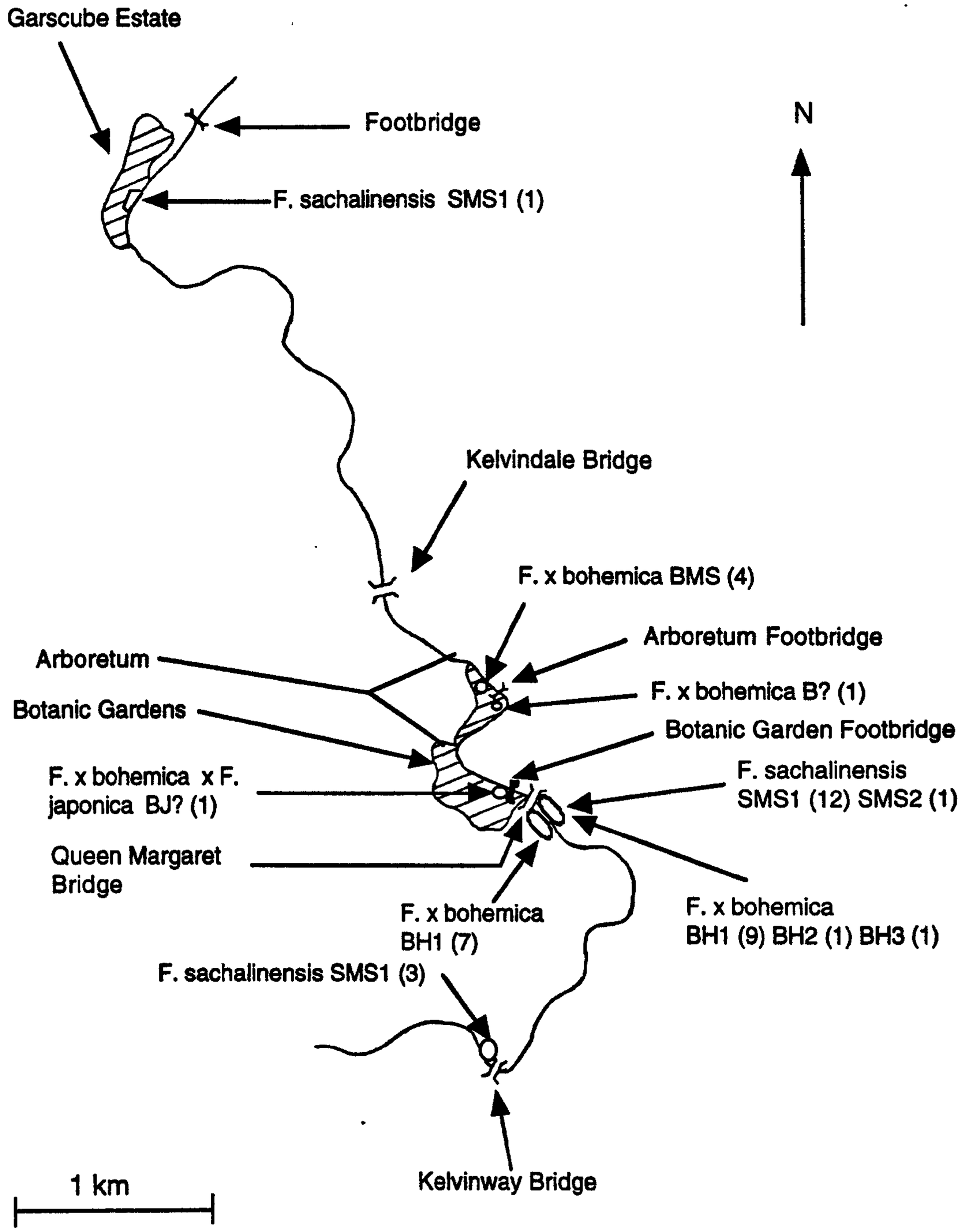
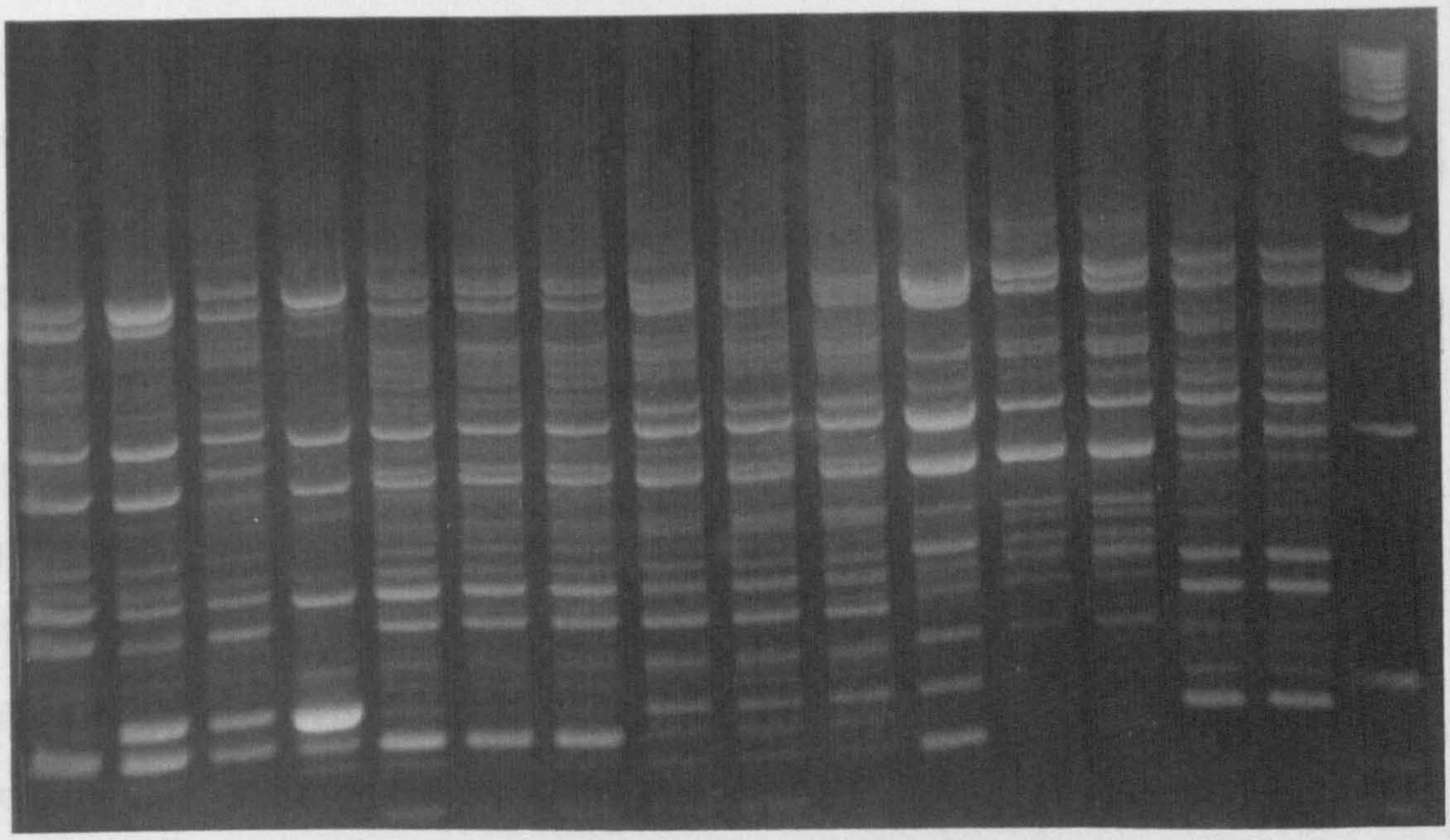


Fig. 6 RAPD photograph showing all the genotypes detected in this study (primer OPF08)



From left to right the genotypes are: lane 1 F. x bohemica BH3 (hermaphrodite); lane 2 F. x bohemica BH2 (hermaphrodite); lane 3 F. x bohemica x F. japonica BJ? (sex unknown); lane 4 F. x bohemica B? (sex unknown); lane 5 F. x bohemica BH1 (hermaphrodite); lane 6 F. x bohemica BH1 (hermaphrodite); lane 7 F. x bohemica BH1 (hermaphrodite); lane 8 F. x bohemica BMS (male sterile); lane 9 F. x bohemica BMS (male sterile); lane 10 F. x bohemica BMS (male sterile); lane 11 F. sachalinensis SMS2 (male sterile); lane 12 F. sachalinensis SMS1 (male sterile); lane 13 F. sachalinensis SMS1 (male sterile); lane 14 F. japonica JMS (male-sterile); lane 15 F. japonica JMS (male-sterile); lane 16 1 kbp marker.

When these data are explored using split decomposition (Figs. 7a, b & c), Fallopia japonica and F. sachalinensis are widely separated and the hybrids form a group between the parental taxa. The chromosomal contributions from the parental taxa are almost perfectly represented by distance on the graph, with the hybrids (2n=66), being approximately 2/3 closer to F. japonica (2n=88), than to F. sachalinensis (2n=44).

Split decomposition analysis of the inter-SSR data for Fallopia x bohemica shows a major split between the male sterile plants and the hermaphrodite plants along with the individual B? (unknown sex) (Fig. 7a). The RAPD data groups the hermaphrodite individuals together, separate from the male sterile plants, with the B? plant separated on a long branch, with no clear affiliation to either of these two groupings (Fig. 7b). The combined analysis, reflects the expected fusion of the two data sets (Fig. 7c), maintaining the separation of the hermaphrodite plants from the male sterile plants, with the B? plant showing a minor affinity with the hermaphrodite group. This difference in graph topology in the placement of the F. x bohemica B? plant, is the major difference between the inter-SSR and the RAPD data (Figs. 7a & b). The inter-SSRs do not differentiate this plant from the hermaphrodite genotype F. x bohemica BH3. The RAPD data clearly differentiates the B? plant from all of the other F. x bohemica, by 4 novel absent bands (OPP3.3, OPP3.5, OPF20.4 & OPF20.5) and 2 unique RAPD bands (OPF16.1 & OPA16.10).

The putative backcross (Fallopia x bohemica x F. japonica) shows a greater affinity to F. japonica than to F. x bohemica based on the RAPD data (Fig. 7b). A more intermediate position between the putative parental taxa is obtained from the inter-SSR data set (Fig. 7a). The combined analysis reflects the expected fusion of the data (Fig. 7c) with the backcross

occupying a position between that obtained with the separate data sets. The backcross has a single inter-SSR band (UBC-840.1) that is unique, and there are two RAPD bands (OPF16.2, OPA16.8) which are fixed present in F. japonica and F. x bohemica, but absent from the backcross. One of these RAPD bands is a unique absence (OPF16.2) the other (OPA16.8) is also absent in F. sachalinensis.

All of the split graphs equally well represent the data matrix from which they were derived, with fit values (the sum of the approximated distances divided by the sum of all original distances) of 87.1% for the inter-SSRs, 88.0% for the RAPDs and 88.7% for the combined data. The remaining 11.3-12.9% of the original distances have been transferred to the split prime residue (the indecomposable portion of the data) and effectively removed from the network.

Fig. 7a Split decomposition graph of inter-SSR data of Fallopia taxa from the River Kelvin

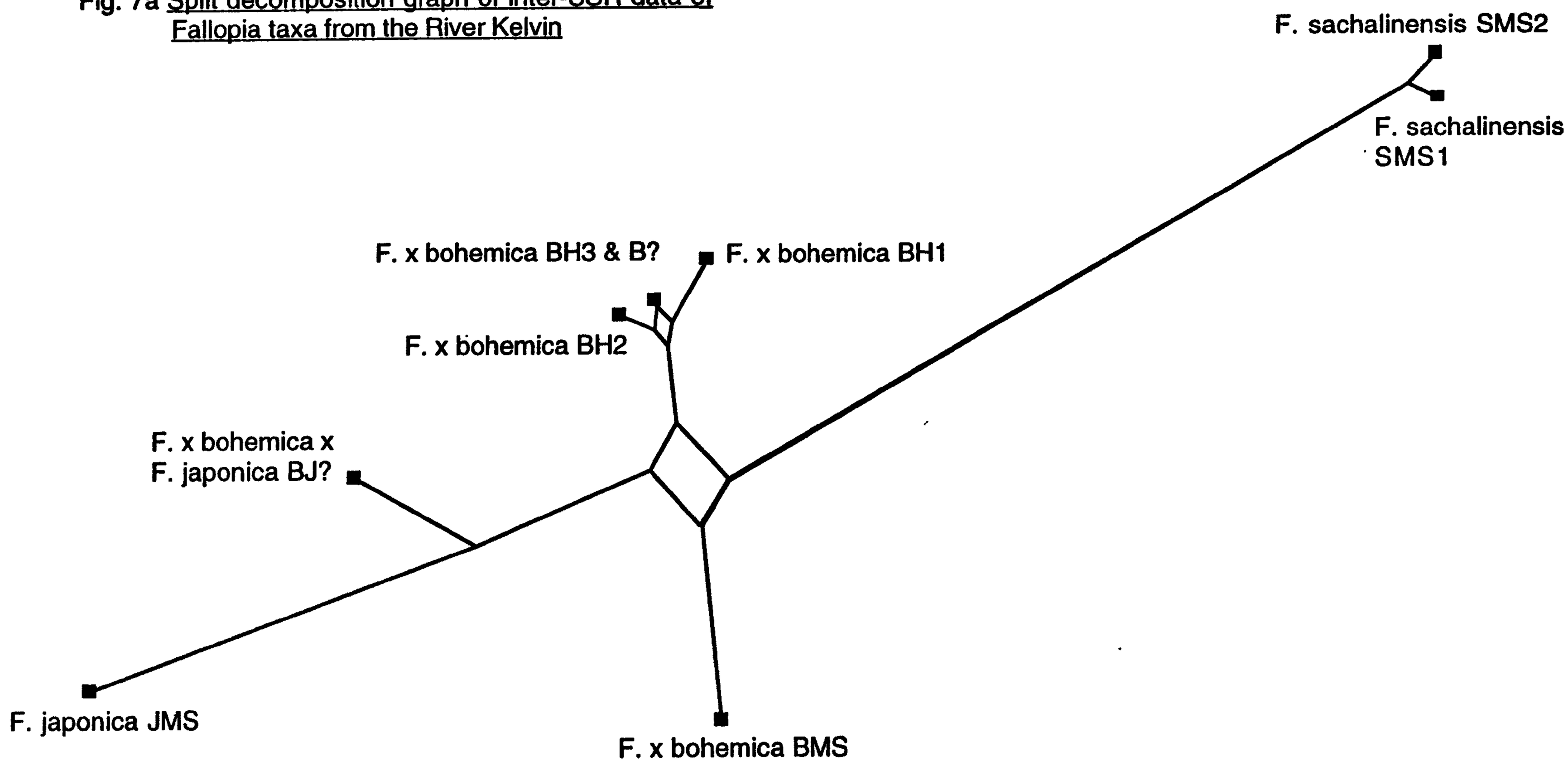


Fig. 7b Split decomposition graph of RAPD data of Fallopia taxa from the River Kelvin

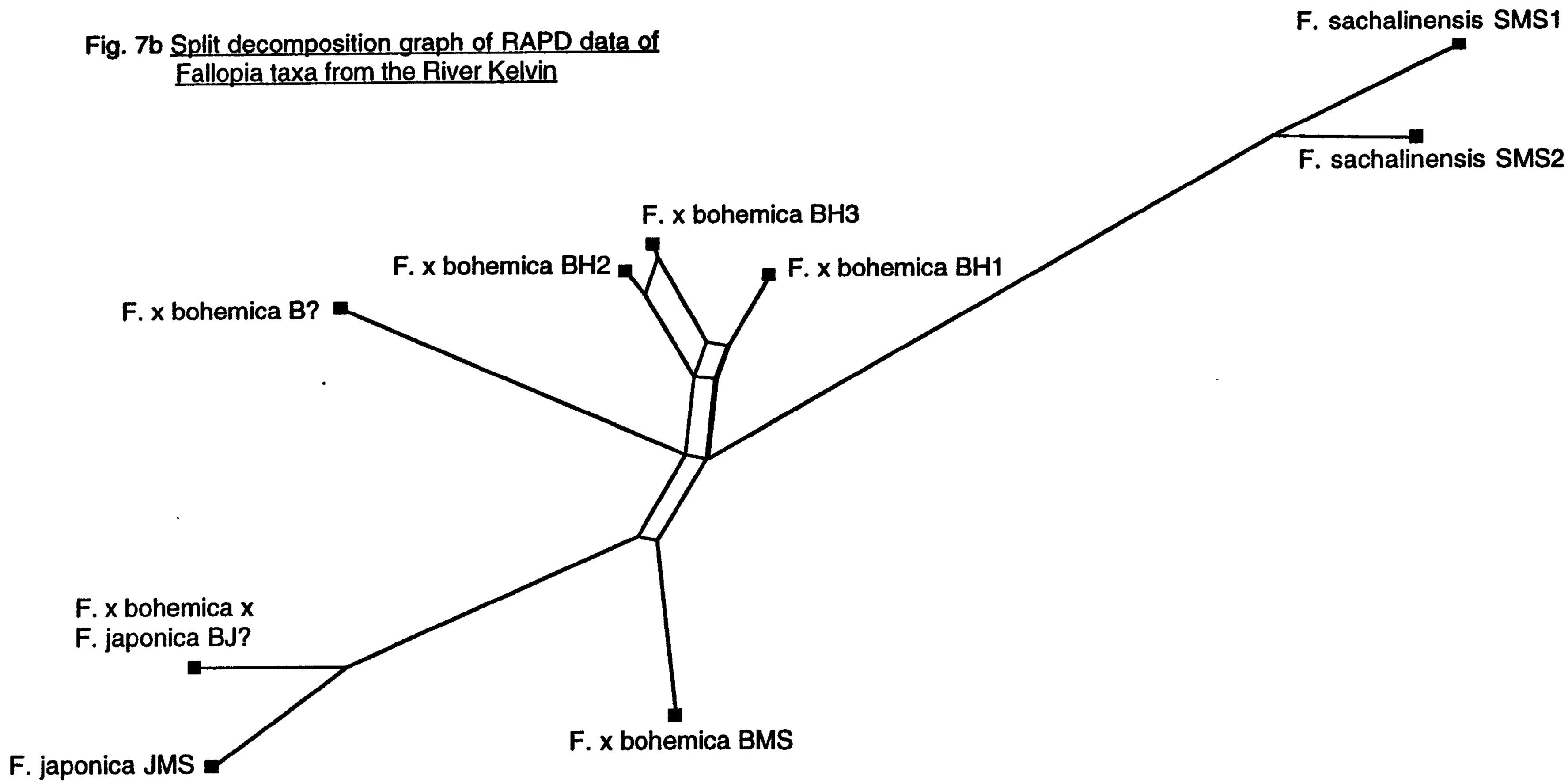
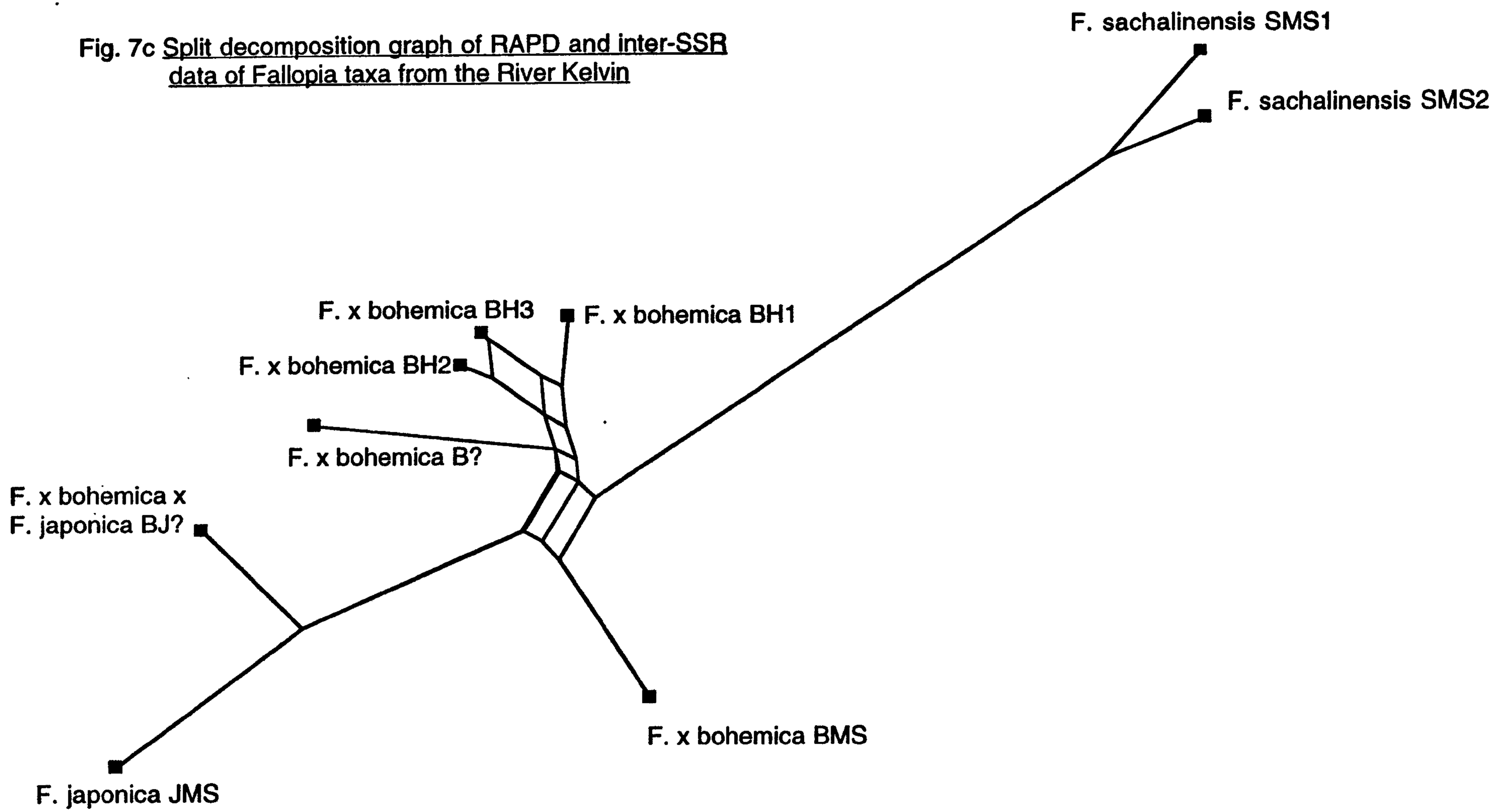


Fig. 7c Split decomposition graph of RAPD and inter-SSR data of Fallopia taxa from the River Kelvin



Discussion

Technique Sensitivity

This survey has used inter-SSRs and RAPDs to investigate genotypic diversity in some invasive Fallopia taxa along the River Kelvin in Glasgow. The data obtained from both techniques appear to be essentially congruent with the exception of the F. x bohemica B? plant (discussed below). Greater resolution was obtained from the RAPD data set than from the inter-SSRs, although this reflects the fact that twice as many RAPD than inter-SSR primers were used. On a per-primer basis, no significant difference between the resolving power of the different techniques was detected. This contrasts with the findings of Zietkiewicz et al. (1994) who concluded that inter-SSRs generate more polymorphic markers per single experiment than RAPDs. However, Zietkiewicz et al. (1994) resolved inter-SSRs using polyacrylamide gels, whereas I have used agarose gels. Aside from inherent confounding variables within these techniques such as primer choice, as a general comment, it seems worth stressing that the sensitivity of arbitrary fingerprinting techniques is as much dependent on the resolution of the gel separation system as the method and the class of fragments that are being amplified. Comparisons of techniques, if they are to be informative, should be between the latter and not the former. Variables such as different gel separation systems and different scoring procedures should be standardised if comparisons are to be meaningful. With regards to technique reproducibility, as no problems were experienced with either technique, I cannot comment on whether inter-SSRs are more robust than RAPDs, as might be predicted given their employment of longer primers and higher annealing temperatures.

Genetic Variability

The combined molecular data detected only a single genotype of Fallopia japonica from 22 individuals, and two genotypes of F. sachalinensis from 17 individuals, along the River Kelvin. I interpret this as the presence of a single clone of F. japonica and two clones of F. sachalinensis. Although a lack of genetic diversity between samples measured by arbitrary fingerprinting with 15 primers is not necessarily indicative of genet identity (e.g. Nusser et al., 1996), I believe this is the most likely explanation here, given the gynodioecious sexual system, the lack of male fertile individuals along the river system and the plants' propensity for clonal spread and efficient water mediated dispersal. Furthermore, using the same primers, I have been able to uniquely genotype individual seedlings in progeny arrays (M. L. Hollingsworth, unpublished data). Arbitrary fingerprinting has been widely used in studies of genetic variation within populations (Chalmers et al., 1992; Dawson et al., 1993) and RAPDs have been shown to be useful in the identification of clones and for distinguishing between vegetative and sexual reproduction (e.g. Kuhl & Neuhaus, 1993; Stiller & Denton, 1995). Indeed, the use of arbitrary fingerprinting techniques for the study of clonal diversity perhaps reflects the optimum use of such techniques. RAPDs and inter-SSRs seem ideally suited to estimating whether two samples are genetically identical or not. Perhaps the most pertinent criticism of the techniques, namely fragment homology, only becomes an issue when one attempts to quantify differences between genets.

The data suggest that all reproduction of Fallopia japonica along the River Kelvin is by vegetative propagation. The abundance of this plant along the river system, where it forms extensive dense clumps is a testament to the regenerative capacity of its rhizomes. The extent of this clone outside of the

River Kelvin, and the levels of clonal diversity across Britain are currently under investigation.

Clonal growth also appears to be the major mode of reproduction of Fallopia sachalinensis. Of the two genotypes detected (which differ by twelve bands) one was by far the most abundant and represented 16 of the 17 plants sampled. This common genotype was present at all of the localities of the plant along the river. The second genotype was detected in a single plant in the largest stand of the species on the river, at the Queen Margaret Bridge site (Fig. 5). As all plants of F. sachalinensis along the River Kelvin are male sterile, a separate introduction would seem the most obvious source of the second genotype. The Glasgow Botanic Gardens borders the river at this site and could well be the source of such an introduction, although I have been unable to trace any records to either support or refute this.

A total of five genotypes of Fallopia x bohemica were detected. In the absence of male fertile individuals of the parental taxa, multiple in situ hybridisation events are unlikely to be the source of this variation. The question remains as to whether this variation is the result of in situ sexual reproduction amongst the hybrids or the result of multiple introductions. Little is known of the history of this population and I have been unable to trace any horticultural records regarding the propagation of these plants at the Glasgow Botanic Gardens. Given that the highest levels of genetic variation were detected in the only male fertile taxon present along the river, perhaps the most parsimonious explanation of the data is that at least some of the genetic variation in F. x bohemica is attributable to local sexual reproduction. The high levels of genetic similarity among the three hermaphrodite genotypes detected here (Fig. 7) could perhaps best be ascribed to hybrid fertility. On the other hand, the large number of genetic

differences between the male sterile plants and the male fertile plants (Fig. 7) implies that representatives of the two different floral types may stem from separate introductions (and ultimately separate hybridisation events). Distinguishing between in situ sexual reproduction and separate introduction requires a knowledge of the population structure of this taxon from other localities, and until these data are available, interpretations remain speculative. A widespread survey of the genetic variability of this taxon in Britain, assessing the extent of the distribution of individual clones would be informative.

Sex Markers

As the male sterile plants of Fallopia x bohemica appear to be represented by a single clone, it is not clear to what extent marker differences separating the floral types relate to clone markers or sex markers. Only a single band (OPF14.10) was found that was uniquely present in all of the male fertile individuals of F. x bohemica and absent from all of the male sterile individuals in this study. This is the only sex correlated marker in the data set. This band (OPF14.10) was also present in the F. x bohemica B? plant, indicating that if this is a reliable sex marker, this plant may be male fertile. However, if the control of sex expression stems from cyto-nuclear conflict between a mitochondrial sterility gene and a nuclear restorer gene (Saumitou-Laprade et al., 1994) then a single band is unlikely to be a robust predictor of sex expression. Based on split decomposition analysis of the inter-SSR data, the B? plant clusters with the hermaphrodite (male fertile) plants with an identical multi-primer genotype to genotype BH3 (Fig. 7a). The RAPD data, however, separates this plant from both the male sterile and the male fertile individuals, and places it on a long branch with no clear affinity to either (Fig. 7b). The discrepancy between the two data sets requires some explanation. Although the failure of the inter-SSRs to distinguish this

genotype may reflect the lower number of primers used (five inter-SSR compared to ten RAPD primers) this may not in itself be a sufficient explanation. Eight out of the ten RAPD primers were able to distinguish this genotype and based on this one might expect that at least one of the inter-SSR primers would detect a difference. The two techniques may be targeting different regions of DNA due to the bias in the distribution of simple sequence repeats in the nuclear, over the organelle genomes (Wang et al., 1994). However, if this is the case, I have no explanation for why such a discrepancy should be apparent for only this sample. I also note that if the RAPDs were amplifying a mixture of nuclear and organelle DNA (c.f. Aagaard et al., 1995) and the inter-SSRs were amplifying predominantly nuclear DNA, one would expect the hybrid group to be differentially placed on the split decomposition graphs due to the skewing effect of the uniparentally inherited organelle genomes.

Interactions Between Taxa

As both Fallopia japonica and F. sachalinensis are male sterile along the River Kelvin, the only potential source of genetic interactions between the taxa at this site is pollen mediated backcrossing from F. x bohemica to either parent. That this has occurred on at least one occasion is supported by the molecular data. The putative backcross between F. japonica (2n=88) and F. x bohemica (2n=66) is placed between these taxa on the split decomposition graphs. This plant has 2n=88 chromosomes which presumably stem from the chance production of an n=44 gamete from F. x bohemica. The putative backcross is intermediate between F. japonica and F. x bohemica on the inter-SSR split decomposition graph (Fig. 7a), whereas based on RAPD data, it is closer to F. japonica (Fig. 7b). If the RAPD data were used in isolation one could suggest that this plant is just a different genotype of F. japonica, however, the inter-SSR data and the intermediate morphology

(occasional, short leaf hairs and a slightly cordate leaf base) provide good evidence for this plant being a backcross. Furthermore, although F. japonica was sampled from a number of sites along the river, the fact that this genotype was found at the only site where male fertile F. x bohemica grow in intimate proximity with F. japonica, adds at least circumstantial evidence to the backcross theory.

The ecological consequences of introgressive hybridisation between Fallopia japonica and F. sachalinensis may be far reaching. As well as producing new genotypes, the possibility of restored male fertility and seed production in F. japonica in Britain could further enhance its invasive abilities, although regrettably I have no information on the sex expression of the backcrossed plant in this study. The post introduction interactions between alien taxa with other introduced or native species is a well documented mode of the evolution of invasive weeds (Abbott, 1992) and further studies investigating hybridisation between alien Fallopia taxa in Britain will be informative in determining the extent and consequences of such interactions.

Chapter 4

Hybridisation, genetic diversity and spatial structure within a mixed population of Knotweed species.

Abstract

Patterns of clonal diversity and genotype inter-relationships were assessed in a mixed population of invasive Fallopia taxa along the River Wnion system in Wales. The population consisted of male sterile F. japonica var. japonica, hermaphrodite F. sachalinensis, and both male sterile and hermaphrodite F. x bohemica. A single genotype was detected from five samples of F. japonica var. japonica, two genotypes from three samples of F. sachalinensis and 20 genotypes from 36 samples of F. x bohemica. Recurrent in situ origins of the hybrid are invoked at least in part, as the explanation for the high levels of genotypic diversity in F. x bohemica.

Introduction.

Hybridisation is a frequent and important component of plant evolution and is widespread amongst natural populations (Rieseberg & Ellstrand, 1993). The frequency of hybridisation varies along a continuum (Avise, 1994). At one extreme, hybridisation may be rare and confined solely to the production of one or a few F1 hybrids, such as the hybrid grass Catapodium marinum (L.) C.E. Hubb. x C. rigidum (L.) C. E. Hubb., which has been found only once as a single plant (Stace, 1987). At the other extreme, hybridisation can be extensive and the taxa involved can merge completely into one panmictic gene pool.

Despite the widespread occurrence of hybridisation, there have been few attempts to quantify its extent on a global scale. A recent review by Ellstrand et al. (1996) compared the frequency of natural hybrids from five biosystematic Floras. The occurrence of hybrids when expressed as a percentage of the total number of species in the flora, ranged from approximately 22% for the British flora to 5.8% for the intermountain flora of North America, with an average of 11% over all five floras. Assuming a similar frequency of natural hybrids world-wide, Ellstrand et al. (1996) extrapolated an estimate of at least 27,500 hybrid combinations from the 250,000 plant species that have been described. On a regional basis, in the New Flora of the British Isles, Stace (1997) recognised 770 angiosperm hybrids as being present. 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. Episodes of hybridisation can lead to the origin of new fertile homoploid hybrid species, such as Helianthus anomalus Blake, H deserticola Heiser, H. paradoxus Heiser (Rieseberg et al., 1990; Rieseberg, 1991) and Iris nelsonii Randolph (Arnold et al., 1990) or to the production of new allopolyploid species e.g. Senecio cambrensis Rosser (Ashton & Abbott, 1992; Harris & Ingram, 1992) and Spartina anglica C. E. Hubb. (Marchant, 1967; Raybould et al., 1991). In many of these examples the recently evolved taxa have shown invasive weedy tendencies.

Hybridisation between the two alien invasive weeds Japanese Knotweed (Fallopia japonica (Houtt.) Ronse Decraene) var. japonica and Giant Knotweed (F. sachalinensis (F.Schmidt ex Maxim.) Ronse Decraene) was first recorded from Britain in 1985 (Bailey & Conolly, 1985). Despite the massive expenditure associated with the control measures for these taxa (particularly for F. japonica var. japonica) and the recent discovery of how widespread the hybrid F. x bohemica (Chrtek & Chrtková) J. Bailey, has become (Bailey et al., 1996) there is little information on the number of times the hybrid has arisen and what the levels of genetic diversity are within and between hybrid populations. Furthermore, little is known as to whether these hybrids are F1 plants or whether hybrid fertility has resulted in sexual reproduction amongst the hybrids, or indeed to what extent the hybrids are reproductively isolated from the parental taxa.

These questions, aside from pure academic interest, are particularly important from the perspective of the evolution of the weed complex. All of these taxa are gynodioecious and can occur as either male sterile or hermaphrodite plants. So far only male sterile plants of Fallopia japonica var. japonica have been found in Britain (Bailey, 1994). This completely asymmetric sex ratio

precludes perpetuation by sexual reproduction of this taxon in Britain, and it is thought to persist purely by vegetative propagation. F. sachalinensis and F. x bohemica, on the other hand, occur as male sterile and hermaphrodite plants and thus there exists the potential for reproduction by seed in these taxa (the hybrid, F. x bohemica shows partial to full fertility (Bailey, 1994)).

Hybridisation between Fallopia japonica var. japonica and F. sachalinensis is of cause for concern for two reasons. Firstly, the combination of the already invasive genotype of F. japonica var. japonica with genes from F. sachalinensis may simply lead to the production of novel genotypes that are more suited to the prevailing environmental conditions and show even more vigorous growth characteristics. Secondly, there exists the possibility that hybridisation between F. japonica var. japonica and F. sachalinensis could result in the introgression of genes conferring male function from F. sachalinensis to British material of F. japonica var. japonica. This would lead to the ability of seed production and dispersal which would further enhance the colonising ability of this species.

Given this potential for further evolution in this complex, I have addressed questions regarding the levels of genetic variability amongst the hybrids by using molecular markers to study a population of these taxa along the River Kelvin in Glasgow (chapter 3). The Glasgow site was explicitly chosen to provide a simple first level assessment to address levels of genetic variation in these taxa in one river system. A complete absence of male fertile individuals of both parental taxa at this site reduced the likelihood of recurrent origins of the hybrid. Five genotypes were detected amongst the Fallopia x bohemica plants, one genotype representing male sterile plants, three representing hermaphrodite plants, with a plant of unknown sex having a fifth genotype. Of the three hermaphrodite genotypes, one was representative of

the majority of samples (85%). Given the absence of male fertile individuals of the parental taxa it seems likely that at least some of this genetic variation is attributable to hybrid fertility, rather than recurrent origins of the hybrid. Molecular data also strongly supported the morphological and cytological identification of a putative backcross between F. japonica var. japonica and the hybrid.

The aim of the current study is to examine levels of genetic variability of the hybrid in a second river system. This site differs from the Glasgow site (chapter 3) by the presence of male fertile Fallopia sachalinensis. The co-existence of male sterile F. japonica var. japonica and male fertile F. sachalinensis at this site, as well as both male sterile and male fertile hybrids, potentially represents an increased dimension of complexity. An investigation of this population has been carried out to establish whether the presence of male fertile F. sachalinensis, and thus the possibility of recurrent in situ origins of the hybrid, has resulted in greater genetic variability among the F. x bohemica plants than in the Glasgow population.

The river system examined in this study is the River Wnion in North Wales. Fallopia japonica var. japonica is common at many sites along the river but there are only two known localities for the other taxa. The first of these is Caerynwch Hall on the River Clywedog, a tributary of the River Wnion. At this site male sterile F. japonica var. japonica, male fertile F. sachalinensis and both male sterile and male fertile F. x bohemica grow together along the edge of the river where it runs through the hall grounds (see Fig. 8). The production of Fallopia seedlings has been recorded at this site (Bailey et al., 1996). Small stands of F. japonica var. japonica grow along the course of the river and also in the surrounding ornamental water gardens and adjacent fields. F. sachalinensis is restricted to two areas, one large stand in the garden area

and one small isolated clump on the river's edge. F. x bohemica is common throughout the grounds, with stands at different stages of maturity frequent along the river's edge and isolated stands scattered throughout the gardens.

The second area where Fallopia x bohemica is found along the river system is 5 km downstream at Dolgellau (Fig. 8). Both male sterile F. japonica var. japonica and male sterile and male fertile 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. Dense stands of F. japonica var. japonica and F. x bohemica line the water's edge for a distance of 0.75 km on the south side of the river and both taxa also grow in scattered clumps on the north bank.

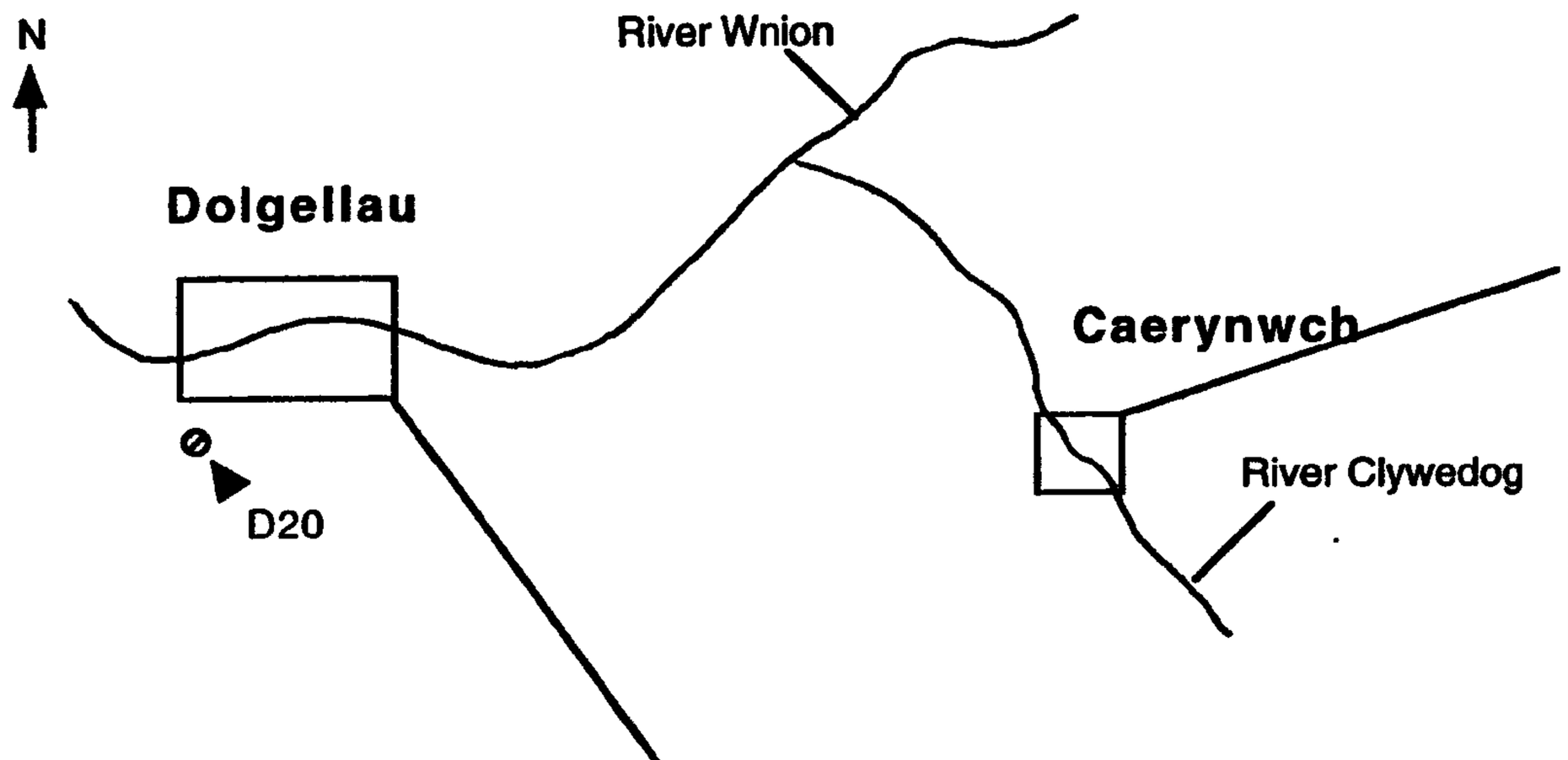
Material and methods

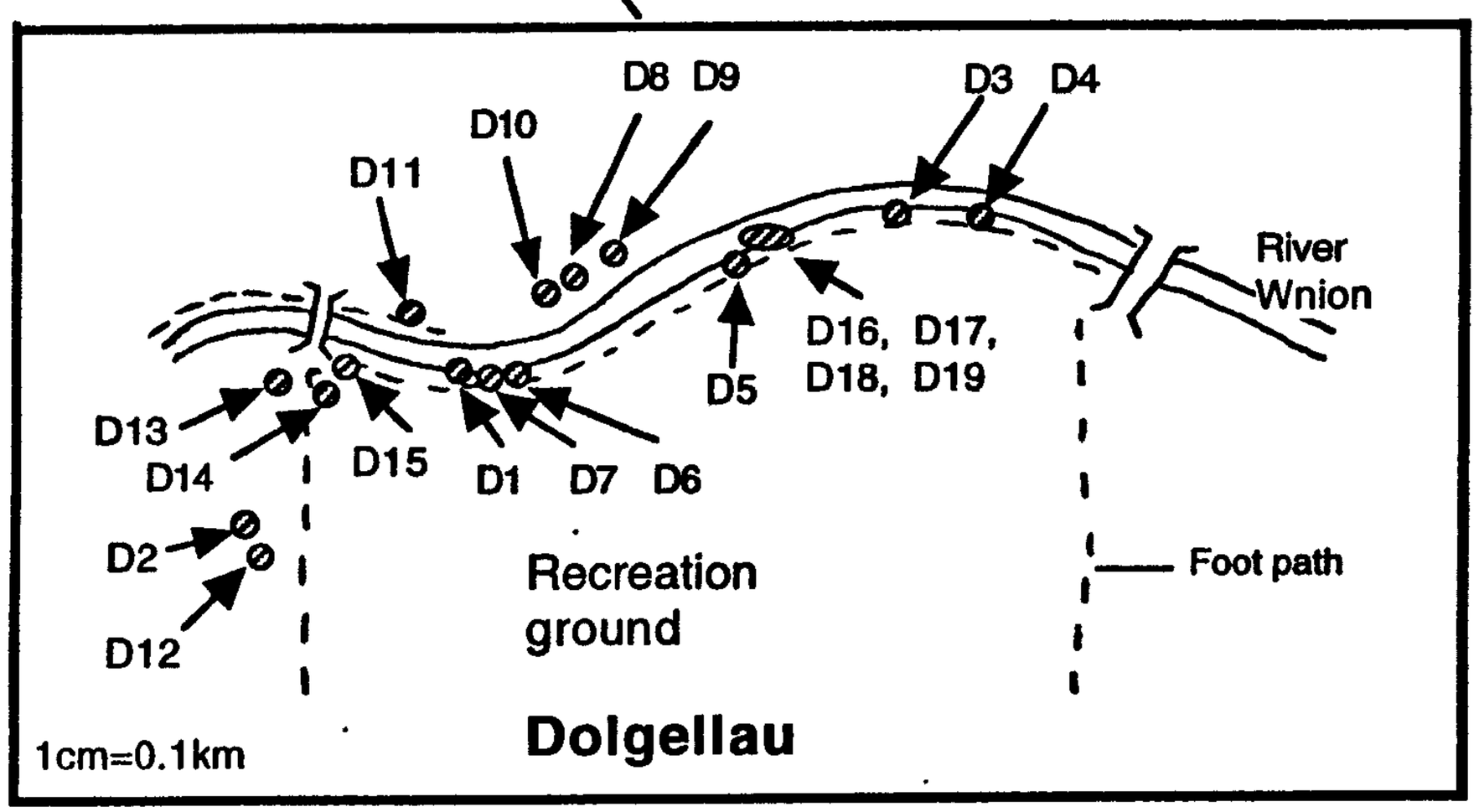
Plant Material

A map of the river system showing the sample localities is given in Fig. 8. Hybrid plants were identified by leaf shape and epidermal trichome characters (Bailey & Conolly, 1991). Two samples of Fallopia japonica var. japonica (C1 & C2), three samples of F. sachalinensis (sample codes C3, C4 & C5) and 20 samples of F. x bohemica, including six seedlings (C6 - C25) were collected from Caerynwch Hall. Three samples of F. japonica var. japonica (D1, D2 & D3) and 16 samples of F. x bohemica (D4 - D19) were collected from Dolgellau. A plant tentatively identified as a backcross between F. x bohemica and F. japonica (D20) was also sampled from Dolgellau. British individuals of these taxa typically have the following chromosome numbers: F. japonica var. japonica 2n=88, F. sachalinensis 2n=44 and F. x bohemica (F. japonica var. japonica x F. sachalinensis) 2n=66. Chromosome counts for material collected at both sites are given in Table 6. Voucher specimens of all taxa have been deposited at LTR.

Table 6. Chromosome counts of Fallopia taxa collected from the River Wnion system

Code	Taxon	Chromosome number
D1	Fallopia japonica	2n=88
C9	Fallopia japonica	2n=88
C3	Fallopia sachalinensis	2n=44
C4	Fallopia sachalinensis	2n=44
D4	Fallopia x bohemica	2n=66
D5	Fallopia x bohemica	2n=66
D6	Fallopia x bohemica	2n=66
C6	Fallopia x bohemica	2n=66
C20	Fallopia x bohemica	2n=66
C21	Fallopia x bohemica	2n=66
C25	Fallopia x bohemica	2n=66
D20	Putative backcross	2n=88





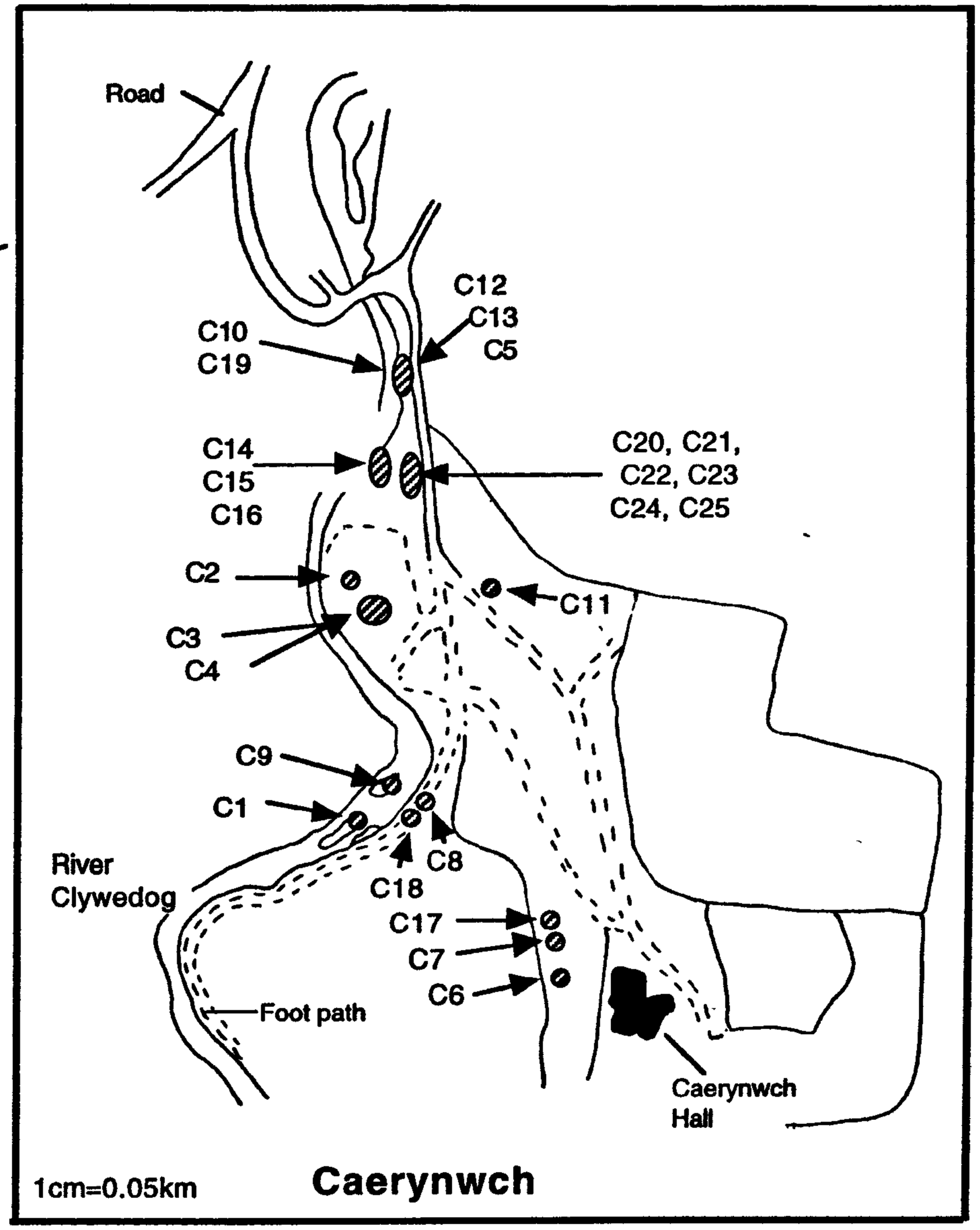


Fig. 8 Location of the study populations of Fallopia taxa along the River Wnion and the River Clywedog

Methods

DNA extraction and RAPD analysis were carried out as described in chapter 3.

Data Analysis

The presence or absence of the different bands was scored by eye, with only strong and reproducible bands considered in the analysis. The complement of Jaccard's Coefficient was calculated as described in Chapter 3. Although split decomposition analysis was a valuable tool for the exploration of the data set from the River Kelvin population, this method of analysis does not cope well with more than ca. 15 genotypes (Huson, 1997), and when larger data sets are analysed, the resolution decreases and relationships collapse into polytomies. As an alternative, principal coordinates analysis (PCO) and neighbour joining (Saitou & Nei, 1987) have been used to represent the similarities between samples.

Principal coordinates analysis (PCO) was performed using the computer package R (Legendre & Vaudor, 1991). Effectively the technique aims to describe the variance of the hyperellipsoid of inter-individual similarities in terms of separate, independent factors and visualise these as trends along a small number of axes. The analysis works by firstly identifying the single axis that accounts for the maximum variance of the cloud of points in multidimensional space that represent the individual samples. Once the first axis (principal coordinate) is found, a second independent axis that accounts for the next highest amount of variance is identified. The procedure then continues, with each subsequent axis accounting for progressively less of the variance. As the percentage of variance accounted for by subsequent axes asymptotes, the assumption is that much of the variance at this level is noise. To represent the relationships among samples, the principal coordinates that

account for the majority of the variance can be plotted against each other as two or three dimensional graphs (Manly, 1994). This technique is useful for the visualisation of data when the working assumption is one of reticulation rather than hierarchical structure.

Neighbour joining (Saitou & Nei, 1987) is a method of representing distances between samples on a tree like diagram. The technique is conceptually similar to UPGMA (unweighted pair group method using unweighted averages) in that OTU's (operational taxonomic units) are grouped together on the basis of overall similarity. The main difference of neighbour joining from traditional cluster analysis is that the assumption of ultrametricity is removed, i.e. the method does not assume that all units are equally divergent (Swofford et al., 1996). Although this technique constrains the data into the dimensions of single tree which may mask underlying conflicting signal, the overall representation of the data using an unrooted tree can still serve to illustrate major relationships.

Results

Distribution of genotypes amongst samples.

Of the 87 bands scored, 65 were polymorphic, giving a total of 24 multiprimer genotypes from the three taxa of Fallopia. The distribution of bands amongst genotypes is given in Fig. 9, and the distribution of genotypes amongst the samples is shown in Table 7 and Figs. 10a & 10b.

Based on multi-primer genotypes, a single genotype was detected in Fallopia japonica var. japonica. Two genotypes were detected from the three samples of F. sachalinensis collected from Caerynwch (Table 7 and Fig. 10a). Both F. sachalinensis genotypes (B and C) were found in the garden area and genotype C was also found in the riverside sample. The five band differences between F. sachalinensis genotypes B and C were detected with four out of the ten primers (OPP3.4, OPP3.5, OPP20.1, OPF20.2 and OPA16.1).

In total, 21 multiprimer genotypes were detected amongst the Fallopia x bohemica plants collected from Dolgellau and Caerynwch. Genotypes E and F were found in samples at both sites. One sample from Dolgellau and five samples from Caerynwch had genotype E and four samples from Dolgellau and one sample from Caerynwch had genotype F. Six genotypes of F. x bohemica were found only at Dolgellau. Three of these genotypes (I, J & K) were unique to individual samples (D11 ,D14 and D20 respectively). Genotype D was found in two samples (D4 & D5) as was genotype G (D8 & D9). Genotype H was found in the remaining five samples (D10, D13, D16, D18 & D19). Thirteen genotypes were found only at Caerynwch. Six of these (S, T, U, V, W & X) are unique to the six individual seedlings (C20, C21, C22, C23, C24 & C25). Of the seven remaining genotypes, six (M, N, O, P, Q & R) are unique to individual samples (C8, C11, C13, C14, C16 & C19) and one, genotype L was shared by two samples (C6 & C7). The hybrids show a

varying degree of genetic differentiation. Aside from unique combinations, sample D20, the 8x F. x bohemica has two unique RAPD bands (OPF20.5 & OPA16.4). Sample C23 has one unique RAPD band (OPF8.2) and sample C14 has three unique RAPD bands (OPF8.1, OPF20.1 and OPF20.6). Sample C20 has one novel absence (OPF16.2) as does C16 (OPA8.5).

When these data are explored using PCO (Fig 11) the first principal coordinate, which represents 26% of the variance, clearly separates Fallopia japonica var. japonica from F. sachalinensis. The hybrids form a cluster in a intermediate position between the parental taxa, albeit closer to F. japonica var. japonica than to F. sachalinensis. The second and third principal coordinates, which account for 9% and 7% of the variance respectively, mainly serve to show differences within the hybrid cluster. However, whilst genotypes G and F show an affiliation to each other when the first and second principal coordinates are plotted against each other, no obvious consistent groupings are evident when subsequent principal coordinates are examined. This lack of substructure amongst the hybrid samples is also evident when the data are explored using neighbour joining (Fig 12). The internal branches of the tree are short, with the majority of the branch lengths being restricted to the terminal branches. This tree topology is indicative of a lack of definable groupings within the data.

Fig. 9 Presence and absence of arbitrary fingerprinting bands in Fallopia taxa from the River Wnion system.

The table shows the presence (1) and absence (0) of all scored bands for all samples, together with missing data (9). The spatial distribution of these genotypes is shown in Figs. 10a & 10b. When these bands are referred to in the text they are identified by the primer name followed by a band number, the band numbers are derived from left to right in ascending order, starting from one for each primer.

Code	OPF8	OPF16	OPF14	OPP3	OPP20
D1	0 0 0 0 1 1 1 1 0 0 0 1 1 1 1	1 1 1	0 1 0 1 1 1 1	1 1 1 0 0 1 0 0 1	1 1 1 1 0 1 1 0
D2	0 0 0 0 1 1 1 1 0 0 0 1 1 1 1	1 1 1	0 1 0 1 1 1 1	1 1 1 0 0 1 0 0 1	1 1 1 1 0 1 1 0
D3	0 0 0 0 1 1 1 1 0 0 0 1 1 1 1	1 1 1	0 1 0 1 1 1 1	1 1 1 0 0 1 0 0 1	1 1 1 1 0 1 1 0
D4	0 0 0 0 1 1 1 1 1 0 1 0 1 1 1	1 1 1	1 1 1 1 1 1 1	1 1 1 0 0 1 1 1 1	1 1 1 1 0 1 0 1
D5	0 0 0 0 1 1 1 1 1 0 1 0 1 1 1	1 1 1	1 1 1 1 1 1 1	1 1 1 0 0 1 1 1 1	1 1 1 1 0 1 0 1
D6	0 0 0 0 1 0 1 1 1 0 1 0 1 0 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 1 1 1 0	1 1 1 1 1 1 1 1
D7	0 0 0 0 1 0 1 0 1 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 0	1 1 0 1 0 1 1 1
D8	0 0 0 1 1 1 1 1 1 0 1 0 1 0 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 0	1 1 0 1 0 1 1 1
D9	0 0 0 1 1 1 1 1 1 0 1 0 1 0 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 0	1 1 0 1 0 1 1 1
D10	0 0 0 1 1 1 1 0 1 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 1	1 1 0 1 1 1 0 0
D11	0 0 0 1 1 0 1 0 1 0 1 0 1 1 1	1 1 1	1 1 0 1 1 1 1	1 1 1 0 0 1 1 1 1	1 1 1 1 0 1 1 0
D12	0 0 0 0 1 0 1 0 1 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 0	1 1 0 1 0 1 1 1
D13	0 0 0 1 1 1 1 0 1 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 1	1 1 0 1 1 1 0 0
D14	0 0 0 0 1 1 1 1 1 0 1 0 1 1 1	1 1 1	1 1 1 1 1 1 1	1 1 1 0 0 1 1 1 1	1 1 0 1 0 1 1 1
D15	0 0 0 0 1 0 1 0 1 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 0	1 1 0 1 0 1 1 1
D16	0 0 0 1 1 1 1 0 1 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 1	1 1 0 1 1 1 0 0
D17	0 0 0 0 1 0 1 0 1 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 0	1 1 0 1 0 1 1 1
D18	0 0 0 1 1 1 1 0 1 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 1	1 1 0 1 1 1 0 0
D19	0 0 0 1 1 1 1 0 1 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 1	1 1 0 1 1 1 0 0
D20	0 0 1 0 1 1 1 1 0 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 1 1 1 1	1 1 0 1 0 1 0 0

Fig. 9 cont.

Code	OPF8	OPF16	OPF14	OPP3	OPP20
C1	0 0 0 0 1 1 1 1 0 0 0 1 1 1 1	1 1 1	0 1 0 1 1 1 1	1 1 1 0 0 1 0 0 1	1 1 1 1 0 1 1 0
C2	0 0 0 0 1 1 1 1 0 0 0 1 1 1 1	1 1 1	0 1 0 1 1 1 1	1 1 1 0 0 1 0 0 1	1 1 1 1 0 1 1 0
C3	0 0 0 1 0 0 0 0 1 1 1 0 1 0 1	1 1 1	1 0 1 0 0 1 1	1 1 1 0 0 0 1 1 0	0 1 0 0 1 1 0 1
C4	0 0 0 1 0 0 0 0 1 1 1 0 1 0 1	1 1 1	1 0 1 0 0 1 1	1 1 1 1 1 0 1 1 0	1 1 0 0 1 1 0 1
C5	0 0 0 1 0 0 0 0 1 1 1 0 1 0 1	1 1 1	1 0 1 0 0 1 1	1 1 1 1 1 0 1 1 0	1 1 0 0 1 1 0 1
C6	0 0 0 0 1 0 1 1 1 1 1 0 1 1 1	1 1 1	1 1 0 1 1 1 1	1 1 1 0 0 0 1 1 1	1 1 1 1 0 1 0 1
C7	0 0 0 0 1 0 1 1 1 1 1 0 1 1 1	1 1 1	1 1 0 1 1 1 1	1 1 1 0 0 0 1 1 1	1 1 1 1 0 1 0 1
C8	0 0 0 0 1 0 1 1 1 0 1 0 1 0 1	1 1 1	1 0 1 1 1 1 1	1 1 1 0 0 1 1 1 1	1 1 1 1 1 1 0 1
C9	0 0 0 1 1 1 1 0 1 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 1 1 1	1 1 0 1 1 1 0 0
C10	0 0 0 0 1 0 1 1 1 0 1 0 1 0 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 1 1 1 0	1 1 1 1 1 1 1 1
C11	0 0 0 0 1 1 1 1 1 0 1 0 1 1 1	1 1 1	1 0 0 0 1 1 1	1 1 1 0 0 1 1 1 1	9 9 9 9 9 9 9 9
C12	0 0 0 0 1 0 1 1 1 0 1 0 1 0 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 1 1 1 0	1 1 1 1 1 1 1 1
C13	0 0 0 1 1 1 1 1 1 1 1 0 1 1 1	1 1 1	1 0 1 1 1 1 1	1 1 1 0 0 0 1 1 0	1 1 1 1 0 1 1 0
C14	1 0 1 1 1 1 1 1 1 0 1 0 1 0 1	1 1 1	1 0 1 1 1 1 1	1 1 1 0 1 0 1 1 1	1 1 1 1 1 1 1 1
C15	0 0 0 0 1 0 1 1 1 0 1 0 1 0 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 1 1 1 0	1 1 1 1 1 1 1 1
C16	0 0 0 1 1 1 1 1 1 0 1 0 1 1 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 1 1 1 1	1 1 0 1 1 1 0 1
C17	0 0 0 0 1 0 1 1 1 0 1 0 1 0 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 1 1 1 0	1 1 1 1 1 1 1 1
C18	0 0 0 0 1 0 1 1 1 0 1 0 1 0 1	1 1 1	1 0 0 1 1 1 1	1 1 1 0 0 1 1 1 0	1 1 1 1 1 1 1 1
C19	0 0 0 1 1 1 1 1 1 0 1 0 1 0 1	1 1 1	1 0 1 1 1 1 1	1 1 1 0 0 1 1 1 1	1 1 1 1 1 1 0 0
C20	0 0 0 0 1 1 1 1 1 1 1 0 1 1 1	1 0 1	1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1	1 1 1 1 0 0 0 0
C21	0 0 0 1 1 1 1 0 1 1 1 0 1 1 1	0 1 1	1 0 0 0 1 1 1	1 1 1 0 1 1 1 1 1	1 1 1 1 1 1 1 1
C22	0 0 0 0 1 0 1 1 1 1 1 0 1 1 1	0 1 1	1 1 0 1 1 1 1	1 1 1 0 0 0 1 1 0	1 1 1 1 1 1 0 1
C23	0 1 0 0 0 0 0 0 1 1 1 0 1 1 1	0 1 1	0 0 0 1 1 1 1	1 1 1 1 0 0 1 1 1	1 1 1 1 0 0 1 0
C24	0 0 0 1 0 1 1 1 1 1 1 0 1 1 1	1 1 1	1 1 0 1 1 1 1	1 1 1 0 0 0 1 1 1	9 9 9 9 9 9 9 9
C25	0 0 0 1 1 1 1 1 1 0 1 0 1 1 1	0 1 1	1 0 0 1 1 1 1	1 1 1 0 0 0 0 0 1	9 9 9 9 9 9 9 9

Fig. 9 cont.

Code	OPA8	OPA6	OPF20	OPF17	OPA16
D1	0 1 1 1 1 1 1 1 1 0 1 1 1	1 1 0 1 1	0 1 1 0 0 0 1 1 1 1 1	1 1 1 1 1	0 1 0 0 1 1 0 1 1 1 0
D2	0 1 1 1 1 1 1 1 1 0 1 1 1	1 1 0 1 1	0 1 1 0 0 0 1 1 1 1 1	1 1 1 1 1	0 1 0 0 1 1 0 1 1 1 0
D3	0 1 1 1 1 1 1 1 1 0 1 1 1	1 1 0 1 1	0 1 1 0 0 0 1 1 1 1 1	1 1 1 1 1	0 1 0 0 1 1 0 1 1 1 0
D4	0 1 1 1 1 1 1 1 0 0 1 1 1	1 0 0 1 1	0 1 1 1 0 0 1 1 1 0 1	1 0 1 1 1	0 1 1 0 1 1 0 1 1 1 1
D5	0 1 1 1 1 1 1 1 0 0 1 1 1	1 0 0 1 1	0 1 1 1 0 0 1 1 1 0 1	1 0 1 1 1	0 1 1 0 1 1 0 1 1 1 1
D6	0 1 0 1 1 1 1 1 0 1 1 1 1	1 1 0 1 1	0 1 1 1 0 0 1 1 1 1 1	1 1 1 1 1	0 1 1 0 1 1 0 1 1 1 1
D7	0 1 0 1 1 1 1 1 1 0 0 1 1	1 1 0 1 1	0 1 0 1 0 0 1 1 1 1 1	1 0 1 0 1	0 0 0 0 1 1 0 1 1 0 1
D8	0 1 0 1 1 1 1 1 1 0 0 1 1	1 1 0 1 1	0 0 0 1 0 0 1 1 1 1 1	1 0 1 0 1	1 0 0 0 1 1 0 1 1 0 1
D9	0 1 0 1 1 1 1 1 1 0 0 1 1	1 1 0 1 1	0 0 0 1 0 0 1 1 1 1 1	1 0 1 0 1	1 0 0 0 1 1 0 1 1 0 1
D10	0 1 0 1 1 1 1 1 1 1 1 0 1	0 1 0 1 1	0 0 1 1 0 0 1 1 1 1 1	1 1 1 0 1	0 1 1 0 1 1 0 1 1 1 1
D11	0 1 0 1 1 1 1 1 1 0 1 1 1	1 1 0 1 1	0 0 0 1 0 0 1 1 1 1 1	1 1 1 0 1	0 1 1 0 1 1 1 1 1 1 1
D12	0 1 0 1 1 1 1 1 1 0 0 1 1	1 1 0 1 1	0 1 0 1 0 0 1 1 1 1 1	1 0 1 0 1	0 0 0 0 1 1 0 1 1 0 1
D13	0 1 0 1 1 1 1 1 1 1 1 0 1	0 1 0 1 1	0 0 1 1 0 0 1 1 1 1 1	1 1 1 0 1	0 1 1 0 1 1 0 1 1 1 1
D14	0 0 0 1 1 1 1 1 1 1 0 1 1	0 1 0 1 1	0 0 0 1 0 0 1 1 1 1 1	1 0 1 1 1	0 1 1 0 1 1 0 1 1 1 1
D15	0 1 0 1 1 1 1 1 1 0 0 1 1	1 1 0 1 1	0 1 0 1 0 0 1 1 1 1 1	1 0 1 0 1	0 0 0 0 1 1 0 1 1 0 1
D16	0 1 0 1 1 1 1 1 1 1 1 0 1	0 1 0 1 1	0 0 1 1 0 0 1 1 1 1 1	1 1 1 0 1	0 1 1 0 1 1 0 1 1 1 1
D17	0 1 0 1 1 1 1 1 1 0 0 1 1	1 1 0 1 1	0 1 0 1 0 0 1 1 1 1 1	1 0 1 0 1	0 0 0 0 1 1 0 1 1 0 1
D18	0 1 0 1 1 1 1 1 1 1 1 0 1	0 1 0 1 1	0 0 1 1 0 0 1 1 1 1 1	1 1 1 0 1	0 1 1 0 1 1 0 1 1 1 1
D19	0 1 0 1 1 1 1 1 1 1 1 0 1	0 1 0 1 1	0 0 1 1 0 0 1 1 1 1 1	1 1 1 0 1	0 1 1 0 1 1 0 1 1 1 1
D20	1 1 1 1 1 1 1 1 1 0 1 1 1	0 0 0 1 0	0 1 0 1 1 0 1 1 0 1 1	1 0 1 1 1	0 1 0 1 1 1 0 1 1 0 0

Fig. 9 cont.

Code	OPA8	OPA6	OPF20	OPF17	OPA16
C1	0 1 1 1 1 1 1 1 1 0 1 1 1	1 1 0 1 1	0 1 1 0 0 0 1 1 1 1 1	1 1 1 1 1	0 1 0 0 1 1 0 1 1 1 0
C2	0 1 1 1 1 1 1 1 1 0 1 1 1	1 1 0 1 1	0 1 1 0 0 0 1 1 1 1 1	1 1 1 1 1	0 1 0 0 1 1 0 1 1 1 0
C3	0 0 0 1 1 1 0 1 0 1 0 0 1	0 0 1 1 1	0 0 0 1 0 0 1 1 1 0 1	1 1 0 0 1	0 0 1 0 1 1 0 0 1 0 1
C4	0 0 0 1 1 1 0 1 0 1 0 0 1	0 0 1 1 1	0 1 0 1 0 0 1 1 1 0 1	1 1 0 0 1	1 0 1 0 1 1 0 0 1 0 1
C5.	0 0 0 1 1 1 0 1 0 1 0 0 1	0 0 1 1 1	0 1 0 1 0 0 1 1 1 0 1	1 1 0 0 1	1 0 1 0 1 1 0 0 1 0 1
C6	0 1 1 1 1 1 0 1 1 0 1 1 1	1 0 1 1 1	0 1 1 1 0 0 1 1 1 1 1	1 1 1 0 1	0 0 0 0 1 1 0 1 1 1 1
C7	0 1 1 1 1 1 0 1 1 0 1 1 1	1 0 1 1 1	0 1 1 1 0 0 1 1 1 1 1	1 1 1 0 1	0 0 0 0 1 1 0 1 1 1 1
C8	0 1 0 1 1 1 1 1 1 0 0 0 1	1 0 1 1 0	0 1 0 1 0 0 1 1 1 1 1	1 1 1 1 1	0 1 1 0 1 1 0 1 1 0 1
C9	0 1 0 1 1 1 1 1 1 1 1 0 1	0 1 0 1 1	0 0 1 1 0 0 1 1 1 1 1	1 1 1 0 1	0 1 1 0 1 1 0 1 1 1 1
C10	0 1 0 1 1 1 1 1 0 1 1 1 1	1 1 0 1 1	0 1 1 1 0 0 1 1 1 1 1	1 1 1 1 1	0 1 1 0 1 1 0 1 1 1 1
C11	1 1 1 1 1 1 0 1 1 0 1 0 1	1 0 0 1 1	0 0 1 1 0 0 1 1 1 1 1	1 0 1 1 1	0 1 1 0 1 1 0 1 1 1 0
C12	0 1 0 1 1 1 1 1 0 1 1 1 1	1 1 0 1 1	0 1 1 1 0 0 1 1 1 1 1	1 1 1 1 1	0 1 1 0 1 1 0 1 1 1 1
C13	9 9 9 9 9 9 9 9 9 9 9 9 9	1 1 1 1 1	0 0 0 1 0 0 1 1 1 1 1	1 1 0 0 1	0 0 0 0 1 1 1 1 1 1 1
C14	0 1 0 1 1 1 1 1 1 1 1 0 1	0 1 0 1 0	1 0 1 1 0 1 1 1 0 1 1	1 0 1 0 1	1 0 0 0 1 1 0 1 1 1 1
C15	0 1 0 1 1 1 1 1 0 1 1 1 1	1 1 0 1 1	0 1 1 1 0 0 1 1 1 1 1	1 1 1 1 1	0 1 1 0 1 1 0 1 1 1 1
C16	0 1 1 1 0 1 0 1 1 0 1 0 1	1 1 1 1 0	0 0 1 1 0 0 1 1 1 1 1	1 1 1 0 1	0 1 1 0 1 1 0 1 1 1 1
C17	0 1 0 1 1 1 1 1 0 1 1 1 1	1 1 0 1 1	0 1 1 1 0 0 1 1 1 1 1	1 1 1 1 1	0 1 1 0 1 1 0 1 1 1 1
C18	0 1 0 1 1 1 1 1 0 1 1 1 1	1 1 0 1 1	0 1 1 1 0 0 1 1 1 1 1	1 1 1 1 1	0 1 1 0 1 1 0 1 1 1 1
C19	0 1 0 1 1 1 1 1 1 0 0 0 1	0 1 1 1 1	0 1 0 1 0 0 1 1 1 1 1	1 0 1 1 1	1 0 1 0 1 1 0 1 1 1 1
C20	0 1 0 1 1 1 0 1 1 1 1 1 1	1 1 0 1 1	0 1 1 1 0 0 1 1 1 1 1	1 0 1 1 1	0 1 1 0 1 1 0 1 1 1 0
C21	0 0 0 1 1 1 1 1 1 1 1 1 1	0 0 0 1 0	0 1 1 1 0 0 1 1 1 0 1	1 1 1 0 1	1 0 0 0 1 1 0 1 1 1 1
C22	0 0 0 1 1 1 1 1 1 1 1 1 1	1 1 0 1 1	0 1 1 1 0 0 1 1 1 0 1	1 0 1 1 1	0 1 1 0 1 1 0 1 1 1 1
C23	9 9 9 9 9 9 9 9 9 9 9 9 9	9 9 9 9 9	9 9 9 9 9 9 9 9 9 9 9	1 1 1 0 1	1 0 1 0 1 1 0 1 1 0 1
C24	0 1 1 1 1 1 0 1 0 0 1 1 1	9 9 9 9 9	0 1 1 1 0 0 1 1 1 1 1	1 1 1 0 1	0 1 1 0 1 1 0 1 1 1 1
C25	0 1 0 1 1 1 0 1 1 0 1 1 1	9 9 9 9 9	0 1 1 1 0 0 1 1 1 0 1	1 1 1 1 1	0 1 1 0 1 1 0 1 1 1 0

Table 7. Distribution of Fallopia genotypes among samples collected from the River Wnion system

Sample code	Taxa	Genotype
D1	F. japonica	A
D2	F. japonica	A
D3	F. japonica	A
C1	F. japonica	A
C2	F. japonica	A
C3	F. sachalinensis	B
C4	F. sachalinensis	C
C5	F. sachalinensis	C
D4	F. x bohemica	D
D5	F. x bohemica	D
D6	F. x bohemica	E
C10	F. x bohemica	E
C12	F. x bohemica	E
C15	F. x bohemica	E
C17	F. x bohemica	E
C18	F. x bohemica	E
D7	F. x bohemica	F
D12	F. x bohemica	F
D15	F. x bohemica	F
D17	F. x bohemica	F
C9	F. x bohemica	F
D8	F. x bohemica	G
D9	F. x bohemica	G
D10	F. x bohemica	H
D13	F. x bohemica	H
D16	F. x bohemica	H
D18	F. x bohemica	H
D19	F. x bohemica	H
D11	F. x bohemica	I
D14	F. x bohemica	J
D20	F. x bohemica	K
C6	F. x bohemica	L
C7	F. x bohemica	L
C8	F. x bohemica	M
C11	F. x bohemica	N
C13	F. x bohemica	O
C14	F. x bohemica	P
C16	F. x bohemica	Q
C19	F. x bohemica	R
C20	F. x bohemica	S
C21	F. x bohemica	T
C22	F. x bohemica	U
C23	F. x bohemica	V
C24	F. x bohemica	W
C25	F. x bohemica	X

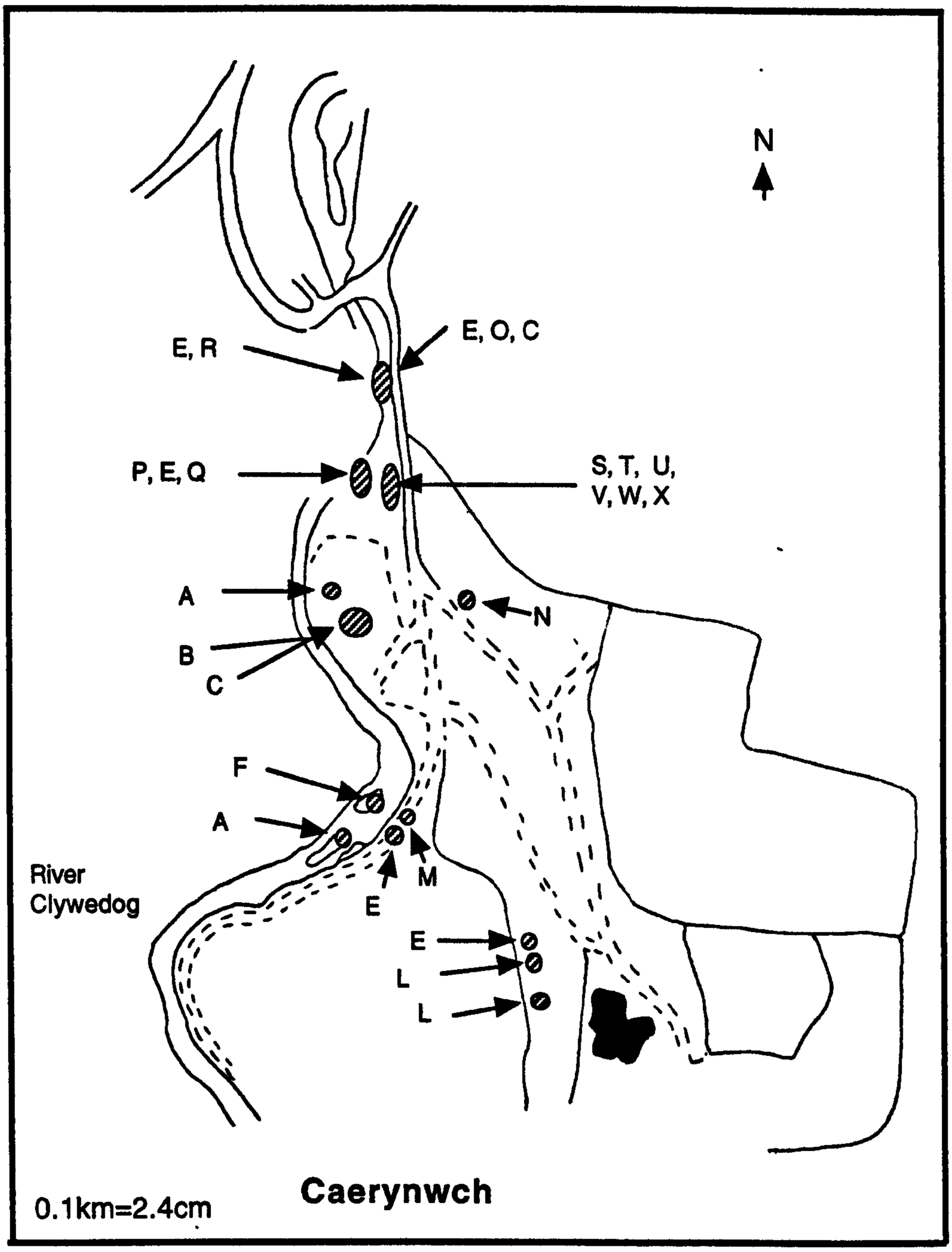


Fig. 10a Distribution of Fallopia genotypes along the River Clywedog, Caerynwch

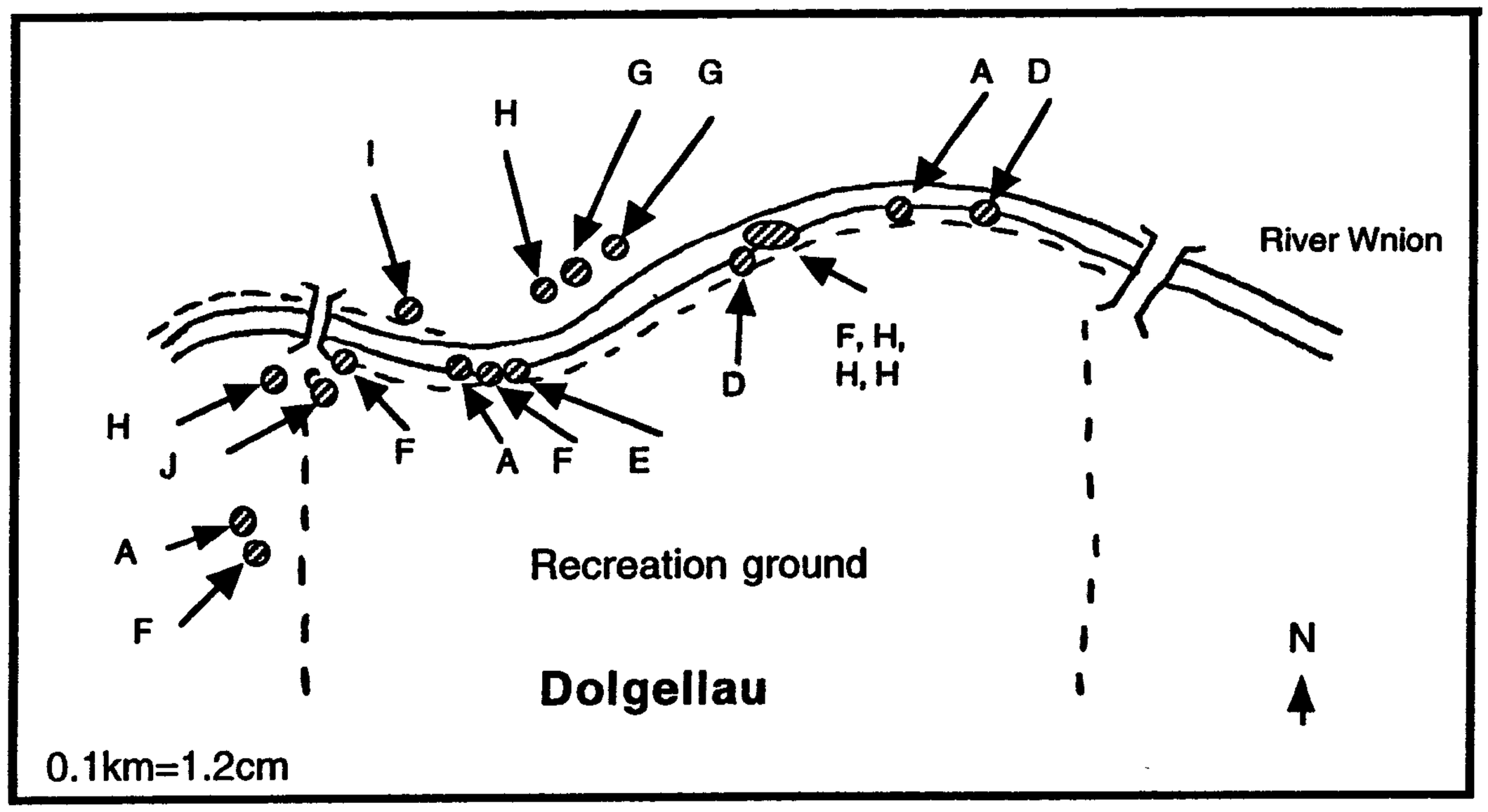


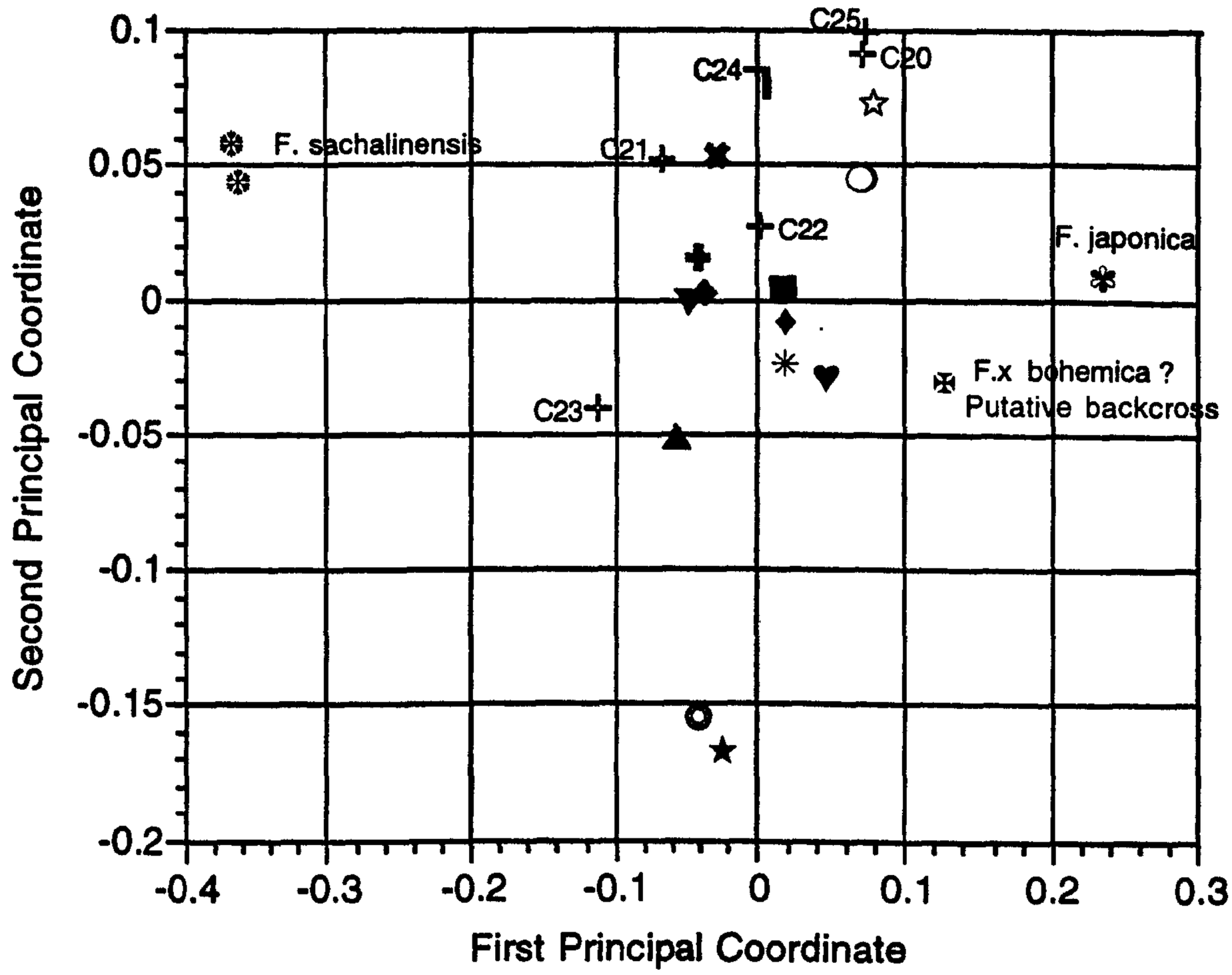
Fig. 10b Distribution of Fallopia genotypes along the River Wnion, Dolgellau

Fig. 11

KEY

	Sample code	Genotype & Taxon
✽	D1, D2, D3, C1 & C2	A. F. japonica
○	D4 & D5	D. F. x bohemica
■	D6, C10, C12, C15, C17 & C18	E. F. x bohemica
★	D7, D12, D15 ,D17 & C9	F. F. x bohemica
❂	D8 & D9	G. F. x bohemica
✖	D10, D13, D16, D18 & D19	H. F. x bohemica
♥	D11	I. F. x bohemica
✳	D14	J. F. x bohemica
✠	D20	K. F. x bohemica ? Putative backcross
❄	C3, C4 & C5	B & C. F. sachalinensis
♦	C6 & C7	L. F. x bohemica
◆	C8	M. F. x bohemica
☆	C11	N. F. x bohemica
▲	C13	O. F. x bohemica
▼	C14	P. F. x bohemica
❙	C16	Q. F. x bohemica
✚	C19	R. F. x bohemica
✢	C20,C21, C22, C23, C24 & C25	S,T,U,V,W & X. F. x bohemica seedlings

Principal coordinate	% of Variance
1	26.283
2	8.754
3	7.273



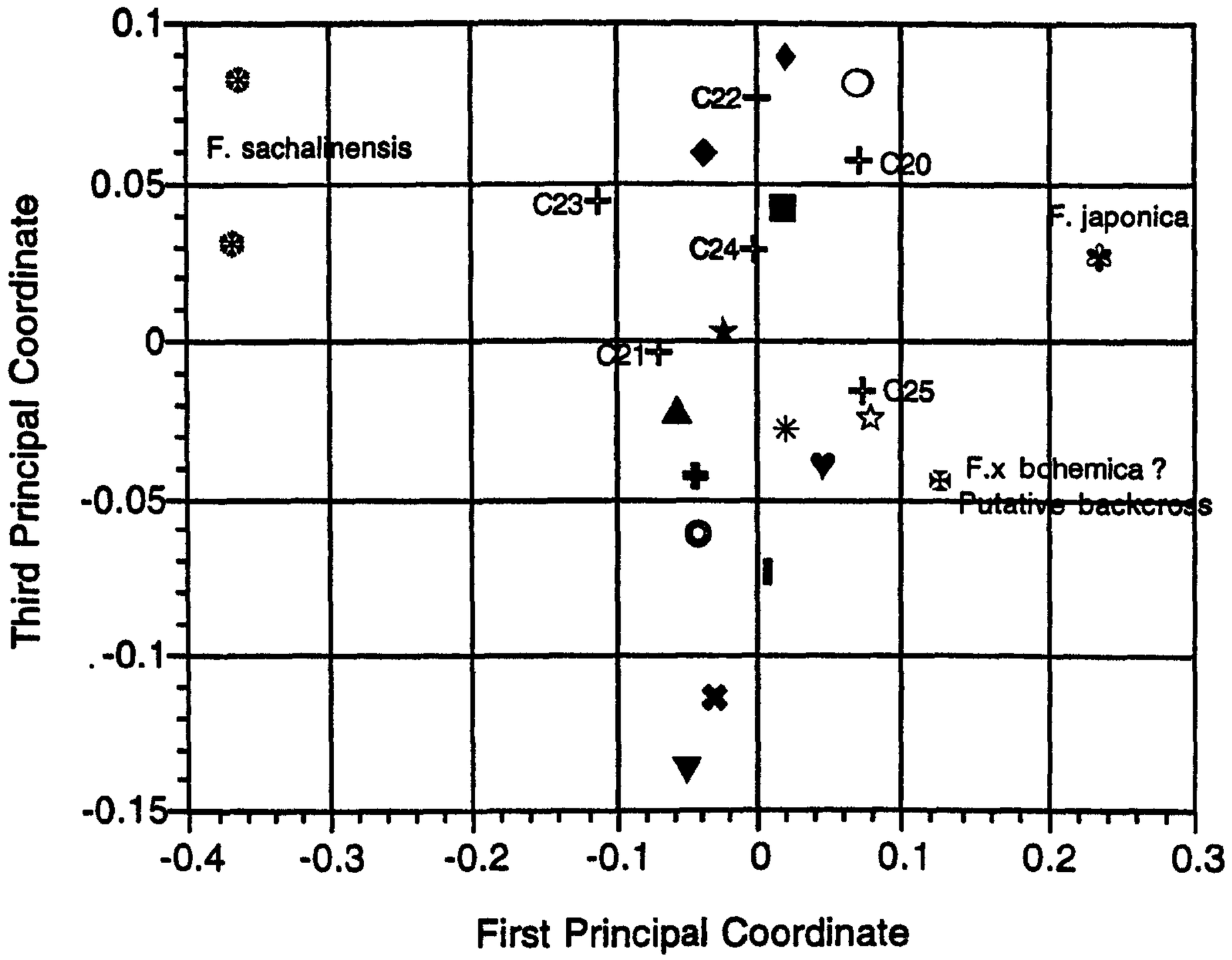
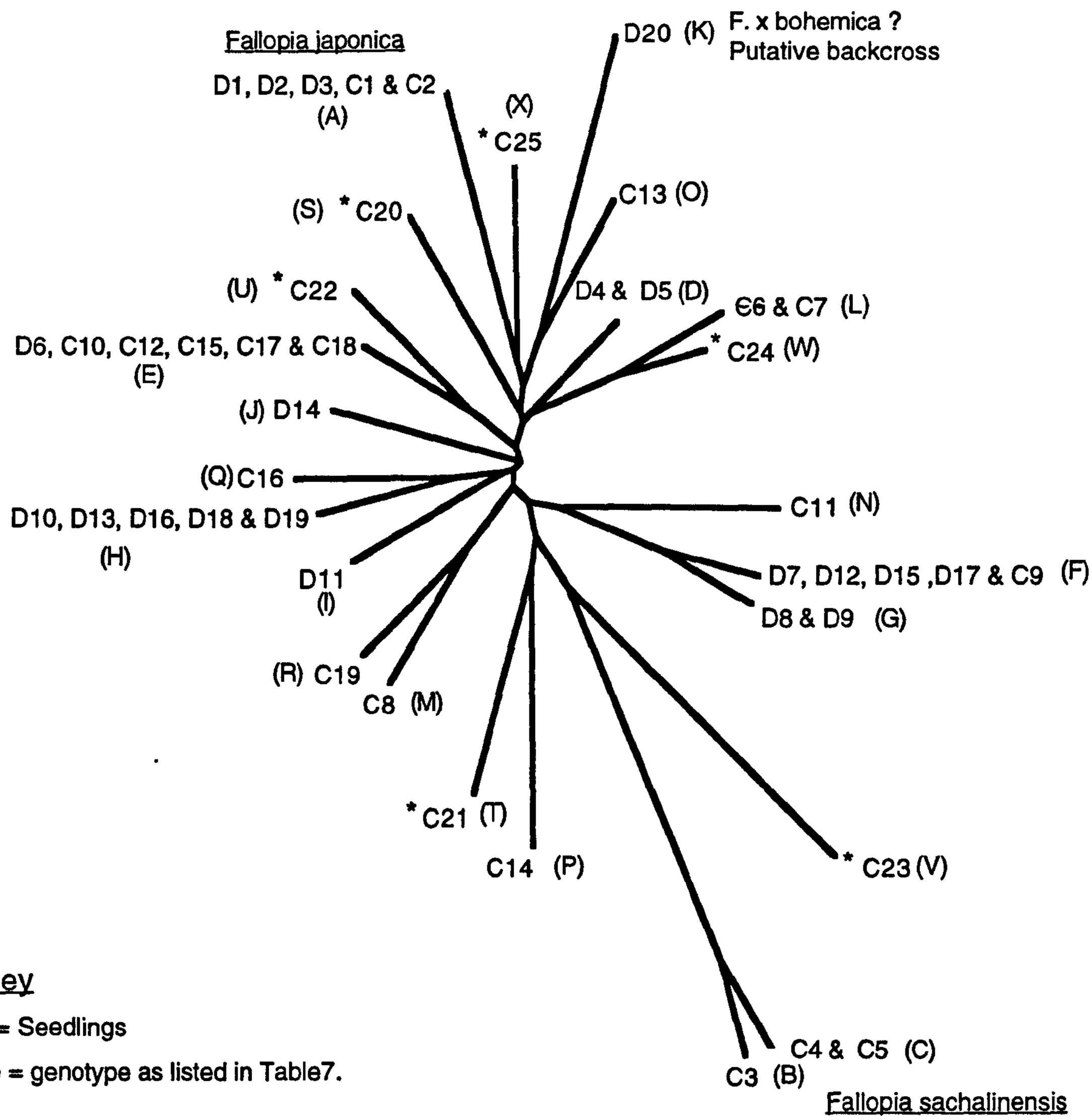


Fig. 11 Principal Coordinate Analysis of Fallopia taxa from the River Wnion system



Key

* = Seedlings

( ) = genotype as listed in Table7.

Fig.12 Neighbour Joining Tree of Fallopia taxa from the River Wnion system

Discussion

Genetic variation in Fallopia japonica var. japonica and F. sachalinensis.

No variation was detected between the five samples of Fallopia japonica var. japonica. The multiprimer genotype detected here was identical to that found in all 22 plants of F. japonica var. japonica examined from the River Kelvin population in Glasgow. I interpret this as the widespread distribution of a single vigorous clone, and these data are consistent with the hypothesis of Bailey (1990) that all British material of this plant may stem from the introduction of a single female genotype.

Two genotypes of Fallopia sachalinensis were detected from the River Clywedog, at Caerynwch Hall. These genotypes differ by five bands and are different from those genotypes detected in the Glasgow population. The limited sample size from the River Clywedog precludes estimations about the extent of clonal growth at this site being made, although I stress the sample size reflects the local abundance of this taxon (material was analysed from the only two stands present). Regardless, I remain ignorant as to the source of the two detected genotypes. A previous owner of the estate is known to have made plant collections during visits overseas (A. P. Conolly pers. comm. 1996) and it is possible that she introduced at least one of these genotypes to the hall grounds. A second explanation is that sexual reproduction is occurring at the site, and that one of the genotypes represents the progeny of the other. Although F. sachalinensis is considered to be self-incompatible, small numbers of seed thought to be from self pollination suggest that the barrier is not absolute (Bailey, 1990).

Reproduction of Fallopia x bohemica

A total of 14 genotypes of Fallopia x bohemica were detected from the 30 mature plants with a further six genotypes detected from the six seedlings.

The genetic structure of F. x bohemica along the river system suggests that both asexual and sexual reproduction have played an important 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. genotypes H & L) or in two cases widely separated and found at both Caerynwch and Dolgellau (genotypes E & F) (See Figs. 10a & 10b). If these genotypes equate to clones, this suggests the occurrence of both local (within site) and widespread (between site) dispersal via vegetative propagation. Evidence that the RAPD genotypes do indeed equate to clones can be gleaned from the analysis of the F. x bohemica seedlings. All six seedlings had unique genotypes; only a single primer is required to discriminate between these samples, highlighting the sensitivity of the assay.

The levels of genetic variation in the Fallopia x bohemica population in this river system is high when compared to the genetic variability detected along the River Kelvin (Chapter 3). At Caerynwch Hall, nine genotypes were detected from 14 mature F. x bohemica plants. At Dolgellau (where only one of the parental taxa is present) seven genotypes were detected, with five of these being unique to this location. None of the F. x bohemica genotypes in this study were the same as those detected from the River Kelvin population in Glasgow.

That higher levels of genetic variability were detected in Fallopia x bohemica from the River Wnion system, where male fertile individuals of F. sachalinensis are present, than in the River Kelvin population, where they are not, can perhaps be attributed to recurrent in situ origins of the hybrid along the River Wnion system. There is good evidence that at Caerynwch Hall, F. x bohemica becomes established directly from seed; on two separate visits to the site, seedlings have been found (Bailey et al., 1996; Bailey & M. L.

Hollingsworth, unpublished data). These seedlings grow well in cultivation and show all the vigorous growth characteristics of the parental taxa and wild collected mature hybrids. Although the survival of seedlings of F. japonica var. japonica in Japan is low, those seedlings that do become established have a high probability of survival as mature plants (Hirose & Tateno, 1984).

While the correlation between the presence of the male fertile Fallopia sachalinensis and the high number of genotypes of F. x bohemica points to multiple hybridisation events, other factors may be responsible. The presence of a large number of private genotypes of F. x bohemica at Dolgellau where F. sachalinensis does not occur, implies the need for caution before multiple hybridisation events are invoked as the conclusive explanation for the genetic variability of F. x bohemica in this river system.

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

As stressed in chapter 3, distinguishing between hybrid fertility and multiple origins as a source of genetic variability in Fallopia x bohemica is difficult. However, some insights may be gained from examining the chromosome numbers of the individuals in question. Of the seven plants of F. x bohemica from the River Wnion system where cytological data are available, all are 2n=66. Bailey (1997) suggested that the irregular meiosis of F. x bohemica would tend to lead to subsequent generations being aneuploids. Cytological

analysis of seeds collected from isolated plants of F. x bohemica (assumed to be self pollinated) 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. x 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 (as might be expected from F. x bohemica gametes) it should be stressed that the converse is not conclusive. The probability of irregular meiosis giving rise to a 2n=66 individual is presumably as likely as any other possible aneuploid from a 2n=66 plant. As I have no information on the survival characteristics of aneuploid versus euploid F. x bohemica it is premature to place too strong an interpretation on the sole occurrence of 2n=66 plants. It is possible that there is a disproportionate survival of euploid plants over aneuploids.

The cytological data from the River Kelvin population is unfortunately not particularly informative in this respect. Chromosome counts for three Fallopia x bohemica genotypes were made from this population, the male sterile genotype, the common hermaphrodite genotype and one of the three rare hermaphrodite genotypes (chapter 3). All of these had 2n=66. At least two of these three F. x bohemica genotypes are suspected to be due to separate introductions (chapter 3). I am therefore left with a single sample of 2n=66 which I have interpreted as stemming from a F. x bohemica x F. x bohemica cross. Aside from the unprovable possibility that this plant represents a third introduction to the river system, a sample size of one plant with 2n=66 is hardly enough to make sound conclusions.

There thus remains some doubt as to the relative importance of hybrid fertility versus multiple origins as a source the genetic variation in Fallopia x

bohemica. Comparing the River Kelvin and the River Wnion systems, it seems likely that both need considering. Perhaps the simplest explanation is that hybrid fertility can result in the production of some new genotypes, as the Glasgow data suggest, but where male fertile F. sachalinensis occurs with F. japonica var. japonica, multiple hybridisation events can generate much higher levels of genetic variability, as is evident from the River Wnion.

The putative backcross (Fallopia x bohemica ? D20) collected from Dolgellau, like the backcross from the River Kelvin (chapter 3), is an octoploid (2n=88). The principal coordinate analysis supports its identification as a backcross and places it between F. x bohemica and F. japonica var. japonica based on the 1st principal coordinate (Fig. 11). The neighbour joining tree (Fig. 12) also groups this plant close to F. japonica var. japonica, albeit with genotype C25 (a 2n=66 seedling) between them. However, the internal branches causing this apparently erroneous grouping are very short compared to the terminal branches. All things considered the data are broadly consistent with this plant being a backcross. The possibility that it arose by the hybridisation of an unreduced (n=44) male gamete from F. sachalinensis (2n=44) x F. japonica var. japonica (2n=88) seems unlikely, as a hybrid with that genomic constitution would be expected to show greater affinities with F. sachalinensis. It seems more likely, that as suggested for the River Kelvin plant, that a chance n=44 gamete was produced by F. x bohemica and crossed with F. japonica var. japonica. Of key interest, is that unlike the Glasgow backcross, this individual appears to be male fertile and produces anthers full of pollen. This plant, with 2n=88 chromosomes, could represent a possible intermediate step in the restoration of male fertility to British samples of F. japonica var. japonica.

Chapter 5

Chloroplast DNA variation and hybridisation between invasive populations of Japanese Knotweed and Giant Knotweed (Fallopia, Polygonaceae)

Abstract

Chloroplast inheritance and the direction of hybridisation have been investigated in some invasive weeds from the genus Fallopia. PCR RFLP analysis of the tmK intron has been used to identify markers that distinguish between the chloroplast genomes of British samples of F. japonica var. japonica, F. japonica var. compacta and F. sachalinensis. Maternal inheritance of chloroplast DNA has been observed in controlled crosses and in hybrid seed from known maternal parents. PCR RFLP analysis of wild hybrids between F. japonica var. japonica and either F. japonica var. compacta or F. sachalinensis, indicates that F. japonica var. japonica is the maternal parent of all of its hybrids, consistent with the apparent male-sterility of this taxon in Britain. Hybrids between F. japonica var. compacta and F. sachalinensis show the chloroplast haplotype of one or other of the parents, consistent with bi-directional hybridisation.

Introduction

Chloroplast DNA is the best characterised of all plant genomes and has been completely sequenced in a number of widely separated taxa such as Epifagus virginiana (L.) Barton (Wolfe, et al., 1992), Marchantia polymorpha L. (Ohyama et al., 1986), Nicotiana tabacum L. (Shinozaki et al., 1986), Oryza sativa L. (Hiratsuka et al., 1989), Pinus thunbergii Franco (Wakasugi et al., 1994) and Zea mays L. (Maier et al., 1995). It is a circular molecule, ranging from 120 - 217 kilobase pairs in photosynthetic land plants and shows a generally conserved gene order (Palmer, 1987). Its slow rate of substitutions, uniparental inheritance, lack of recombination and predictable arrangement of genes (Clegg et al., 1991; Harris & Ingram, 1991; Palmer, 1987; Wolfe et al., 1987) have led to it becoming the molecule of choice for many plant phylogenetic studies (Olmstead & Palmer, 1994). The design of universal primers flanking intergenic spacers and introns (Demesure et al., 1995; Dumolin-Lapegue et al., 1997; Taberlet et al., 1991), coupled with theoretical advances in the population genetic analysis of data from organelle genomes (Asmussen & Schnabel, 1991; Ennos, 1994; Hu & Ennos, 1997; Petit et al., 1993), have led to an increase in the number of studies aimed at lower taxonomic levels, examining the amounts and partitioning of chloroplast (cp) DNA variability within and between closely related species.

Empirical data gathered so far, bears out theoretical predictions (based on the small effective population size and slow mutation rate of the molecule) that levels of intra-specific genetic variability in cpDNA are typically lower than those detected in the nuclear genome. There are, however, a large number of studies that have successfully identified and utilised cpDNA variation in population genetic and biosystematic studies (Dawson et al., 1995; Demesure et al., 1996; Dijk & Bakx-Schotman, 1997; Ferris et al., 1995; Ferris et al., 1997; Levy et al., 1996; McCauley, 1994 & 1995; McCauley et al., 1996;

Mousadik & Petit, 1996; Powell et al., 1995; Soltis et al., 1989; Wolf et al., 1997). One area which has benefited in particular from the use of cpDNA markers, is the study of plant hybridisation (Rieseberg, 1995). By examining the distribution of chloroplast haplotypes within and between taxa, it is often possible to test hypotheses regarding the hybrid status of particular populations, as well as revealing cases of previously unsuspected introgression (Rieseberg & Soltis, 1991). Furthermore, given that cpDNA is typically uniparentally inherited and non-recombinant, some insights can be gained into the direction of hybridisation, i.e. identifying the maternal or paternal parent (Arnold et al., 1991; Ferris et al., 1997).

In the majority of angiosperms, cpDNA is inherited from the female, this contrasts with gymnosperms where chloroplast inheritance is more commonly from the male (Birky, 1995; Dong & Wagner, 1994; Dong et al., 1992; Harris & Ingram, 1991; Lloyd-Mogensen, 1996). There have, however, been sufficient exceptions to these rules to warn against blind acceptance of stereotypical modes of inheritance for a particular taxonomic group (Lee et al., 1988; Metzlaff et al., 1981; Sewell et al. 1993).

In the current study, the distribution of chloroplast haplotypes amongst taxa and in the progeny of controlled crosses is examined to establish the direction of hybridisation and the mode of chloroplast inheritance in some invasive weeds from the genus Fallopia (Polygonaceae). A number of Fallopia taxa have been introduced to Britain from eastern Asia and have since become established as naturalised aliens (Bailey & Conolly, 1985; Conolly, 1977). In particular, Japanese Knotweed (F. japonica (Houtt.) Ronse Decraene) var. japonica has gained considerable notoriety as a troublesome weed (Bailey, 1994; Hart et al., 1997; Nuttall, 1996). Other introduced taxa from this genus include Giant Knotweed, F. sachalinensis (F. Schmidt ex Maxim.) Ronse

Decraene and F. japonica var. compacta (Hook. f.) J. Bailey although their secondary spread has been much more limited (Bailey, 1994). F. japonica var. japonica, for instance, has been recorded from 1584 of the 2862 10km squares of the Biological Record Centre mapping system of Britain, whereas F. sachalinensis and F. japonica var. compacta are known from 274 and 25 10 km squares respectively.

All of these taxa are gynodioecious and occur as either hermaphrodite or male sterile (effectively female) plants. In Britain, however, only male sterile plants of Fallopia japonica var. japonica are known (Bailey, 1994), and a high proportion of populations of F. sachalinensis and F. japonica var. compacta are of male sterile only plants. Vegetative propagation by rhizomatous growth is a major mode of reproduction for these taxa and they are capable of regeneration from minute fragments of rhizome (Brock & Wade, 1992). Indeed, the colonising success of F. japonica var. japonica is largely attributable to the transportation and tipping of soil containing plant fragments, and water borne dispersal of vegetative material along river systems.

Hybrids between all three taxa (Fallopia japonica var. japonica, F. sachalinensis and F. japonica var. compacta) have been recorded. The hybrid between F. japonica var. japonica and F. sachalinensis is by far the most common and is widespread throughout the British Isles and shows evidence of being every bit as invasive as F. japonica var. japonica (Bailey et al., 1996). In contrast, hybrids involving F. japonica var. compacta as one parent are much rarer.

The hybrid between Fallopia japonica var. japonica and F. sachalinensis shares the binomial F. x bohemica with the hybrid between F. japonica var. compacta and F. sachalinensis. Additionally, as the hybrid

between F. japonica var. japonica and F. japonica var. compacta has not been typified, to avoid confusion, in this paper I refer to the hybrids directly by their parental combinations.

A study investigating the nature and direction of hybridisation in these taxa is important from both an evolutionary biology, and a weed control, perspective. Hybridisation involving introduced species is a well documented mode of plant evolution (Abbott, 1992). Species which do not occur in sympatry in their native ranges, may co-occur and hybridise in sites where they are introduced. These hybridisation events may lead to hybrid speciation (either homoploid or polyploid) and such interactions provide an environment for speciation to be directly observed, without the need for assumptions to be made regarding past events. Additionally, these hybridisation events can lead to the production of novel genotypes which may show invasive tendencies and become problematical weeds.

As all individuals of Fallopia japonica var. japonica examined so far in Britain are male sterile, one would predict that it would be the female parent of most, if not all of its hybrids. In contrast, hybridisation between F. japonica var. compacta and F. sachalinensis could potentially occur in either direction. If chloroplast inheritance is maternal in Fallopia, all hybrids involving F. japonica var. japonica would be expected to contain its cpDNA haplotype. The presence of the chloroplast type of the other parent would indicate either paternal chloroplast transmission or residual male fertility of F. japonica var. japonica. Although I have no evidence of any male fertility in British plants of this taxon, the possibility of the occasional production of fertile pollen cannot be excluded. The sporadic occurrence of functional gametes in apparently highly sterile taxa is well documented (Jefferies & Gottlieb, 1983; Stace,

1987) as is the importance of such rare events in plant evolution (Stace, 1987; 1993).

The overall aim of this study is to investigate the population genetic structure of Fallopia japonica var. japonica, F. japonica var. compacta and F. sachalinensis using organelle DNA, to gain insights into the dynamics of hybridisation. Specifically, I wish to a) establish whether chloroplast DNA is predominantly maternally inherited in crosses of F. japonica var. japonica, F. japonica var. compacta and F. sachalinensis, b) assess whether there is any evidence for F. japonica var. japonica being the male parent of any of its hybrids and c) to see whether hybridisation between F. japonica var. compacta and F. sachalinensis is bi-directional.

Materials and Methods

Plant Material

A total of 158 samples of Fallopia taxa was examined for cpDNA variation (Tables 8 & 9; Figs. 13a, b, c & d). 76 individuals of the three parental taxa were examined, as well as 56 wild hybrid plants (45 F. japonica var. japonica x F. sachalinensis, ten F. japonica var. compacta x F. sachalinensis, and one F. japonica var. japonica and F. japonica var. compacta; Table 8). A total of 13 individuals, grown from seed collected from four wild F. japonica var. japonica plants and later identified as being hybrids with F. sachalinensis, was also included in the analysis. In addition, I have also examined a limited number of progeny (13) available from controlled crosses.

Samples were identified using the characters given in Table 10.

Representative voucher specimens of all taxa have been deposited at LTR.

Table 8. Collection localities of Fallopia samples

F. japonica var. japonica

Vice county	v.c. No.
West Cornwall (2)	1
East Cornwall	2
Isle of Wight	10
East Kent (2)	15
Surrey (2)	17
Middlesex	21
Oxfordshire	23
Buckinghamshire	24
West Norfolk	28
Cambridgeshire	29
Northamptonshire	32
Warwickshire (2)	38
Glamorganshire (9)	41
Breconshire	42
Merionethshire (3)	48
Caernarvonshire (2)	49
North Lincolnshire	54
Leicestershire (4)	55
Nottinghamshire	56
Derbyshire	57
South Lancashire (4)	59
South-west Yorkshire	63
Westmorland	69
Renfrewshire	76
Lanarkshire	77
Fife	85
Stirlingshire	86
West Perthshire	87
Westerness	97
Dunbartonshire (2)	99
West Ross (2)	105
Shetland (2)	112
Co. Waterford	H6
Co. Cavan	H30

F. sachalinensis

Vice county	v.c. No.
East Gloucestershire	33
Radnorshire (2)	43
Pembrokeshire	45
Cardiganshire	46
Merionethshire (2)	48
Leicestershire	55
Westmorland	69
Lanarkshire (3)	77
Westerness	97
West Ross	105

F. japonica var. compacta

Vice county	v.c. No.
West Kent	16
Breconshire	42
Glamorgan	41
Mid Cork	H4

F. japonica var. japonica x F. sachalinensis

Vice county	v.c. No.
East Cornwall	2
Surrey (4)	17
East Suffolk	25
East Gloucestershire	33
West Gloucestershire	34
Warwickshire	38
Glamorganshire (3)	41
Pembrokeshire	45
Merionethshire (20)	48
Cheviotland	68
Renfrewshire	76
Lanarkshire (7)	77
West Perthshire	87
Morayshire	95
W. Galway	H16
Co. Londonderry	H40

F. japonica var. compacta x F. sachalinensis

Vice county	v.c. No.
Surrey (3)	17
Hertfordshire	20
East Gloucestershire (4)	33
West Lancashire	60
Mid-west Yorkshire	64

F. japonica var. japonica x F. japonica var. compacta

Vice county	v.c. No.
Middlesex	21

Key: v.c. No. = vice county number.
Sample sizes greater than one are given in parentheses.

Table 9. Details of individual artifical crosses and hybrid seed collected from known maternal parents.

	Collection locality	N
Wild collected hybrid seed		
F. japonica var. japonica x F. sachalinensis	v.c.33	1
F. japonica var. japonica x F. sachalinensis	v.c.33	7
F. japonica var. japonica x F. sachalinensis	v.c.33	2
F. japonica var. japonica x F. sachalinensis	v.c.17	3
Artificial hybrids		
F. japonica var. japonica x F. sachalinensis	-	3
F. japonica var. japonica x F.japonica var. compacta	-	1
F. japonica var. compacta x F. sachalinensis	-	1
F. sachalinensis x F.japonica var. compacta	-	2
Artificial backcross		
F. sachalinensis x (F. japonica var. compacta x F. sachalinensis)	-	6

Key: v.c. 17 = Surrey, v.c.33 = East Gloucestershire. N = number of progeny examined per maternal plant. All crosses in this table are given with the maternal parent first.

Table 10. Identification characters for Fallopia taxa [1]

TAXON	PLANT HEIGHT	LEAF CHARACTERS (applicable only to largest cauline lvs)	HAIRS ON LOWER LEAF EPIDERMIS	CHRO. NO.
F. japonica var. japonica	2 - 3m	5-12cm long x 5-8cm wide, broadly ovate, cuspidate apex, truncate base.	Absent	2n=88
F. japonica var. compacta	Usually less than 70cm	7-8cm long x 6-10cm wide, squarish, cuspidate apex, truncate base, conspicuously undulate margins and red petioles	Absent	2n=44
F.sachalinensis	2.5 - 4m	Up to 40cm long x 22cm wide, heart shaped, base highly cordate generally thinner than the other taxa	Abundant long thin-walled many celled flexuous hairs, which collapse readily	2n=44
F. japonica var. japonica x F. sachalinensis	2 - 3.5m	Up to 25cm long x 17cm wide, broadly heart shaped with cordate base. Intermediate in texture and appearance to parental taxa	Numerous short stout trichomes 1-4 cells long	2n=66
F. japonica var. compacta x F.sachalinensis	2 - 2.5m	Up to 25cm long x 17cm wide, but sometimes with undulate margins	Numerous short stout trichomes 1-4 cells long	2n=44
F. japonica var. japonica x F. japonica var. compacta	Up to 2m	5-12cm long x 5-8cm wide, broadly ovate, cuspidate apex, truncate base, sometimes with the red petioles and crimped margins of var. compacta	Absent	2n=66

[1] Based on information presented by Bailey, 1989.

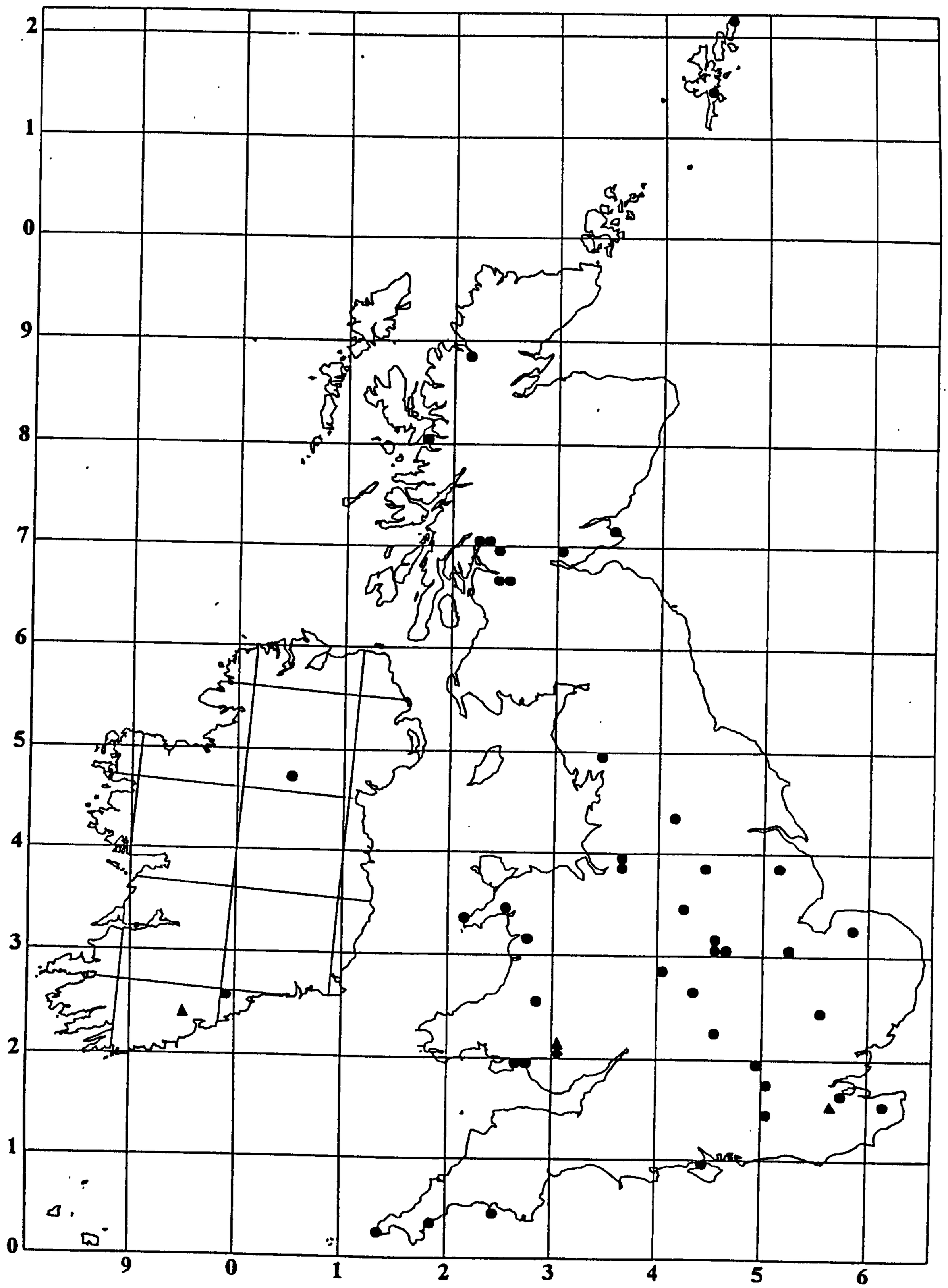


Fig.13a Sample localities of Fallopia japonica var. japonica ● Fallopia japonica var. compacta ▲ Both taxa ◆

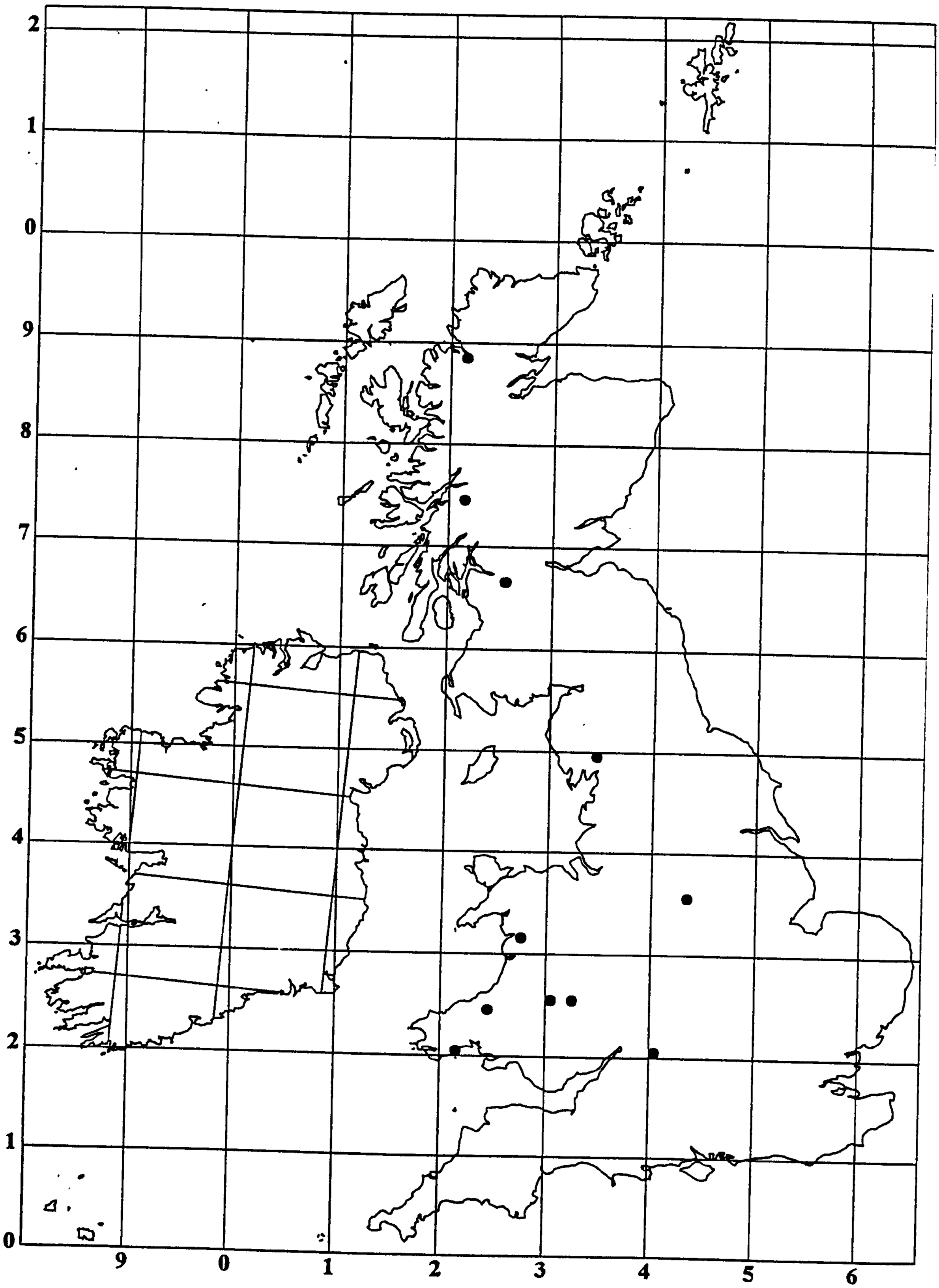


Fig.13b Sample localities of Fallopia sachalinensis

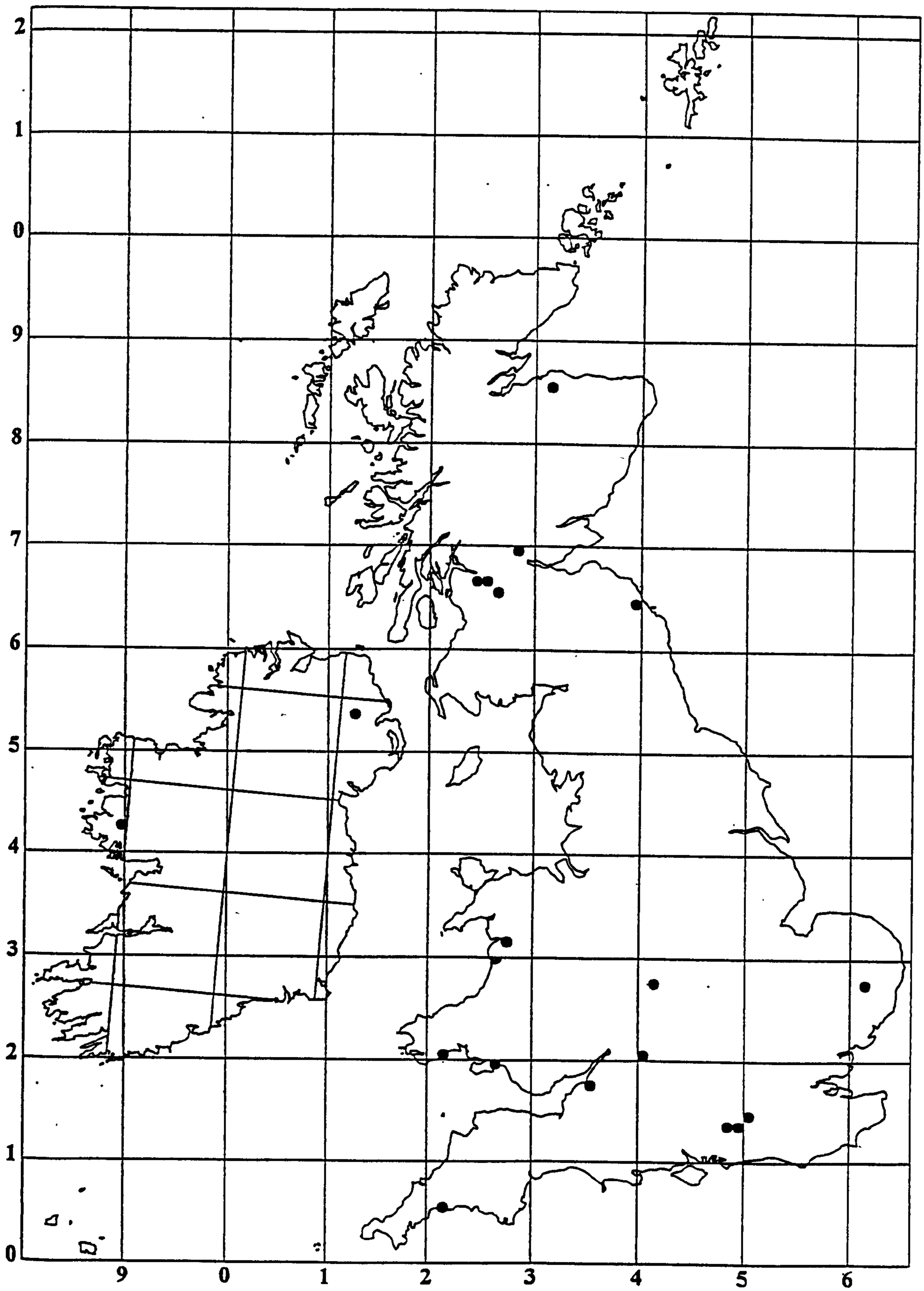


Fig.13c Sample localities of Fallopia japonica var. japonica
x Fallopia sachalinensis

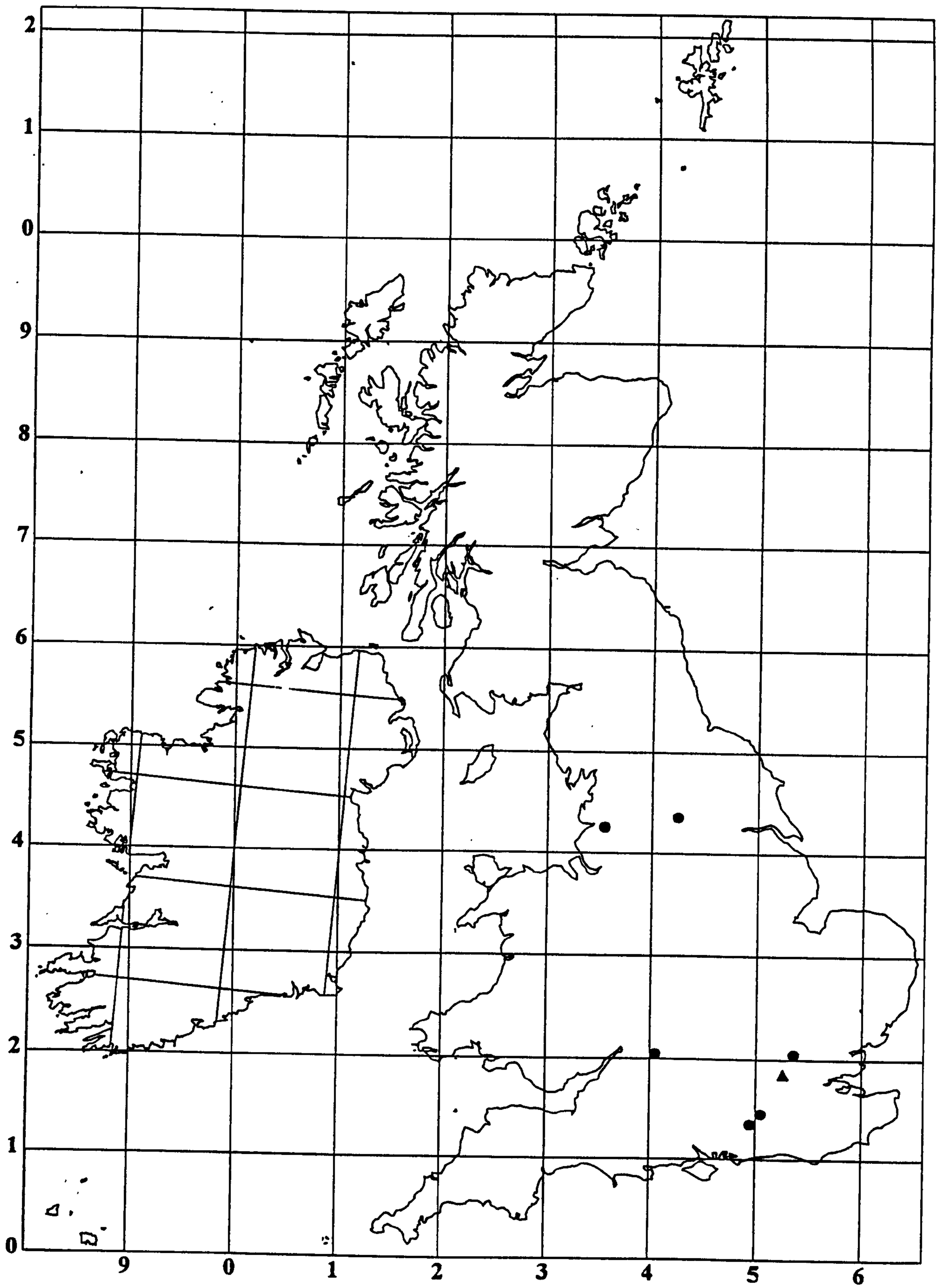


Fig.13d Sample localities of Fallopia japonica var. compacta x Fallopia sachalinensis ● and Fallopia japonica var. japonica x Fallopia japonica var. compacta ▲

Identification of chloroplast DNA variation

To identify polymorphisms in the chloroplast genome of Fallopia species, I instigated two approaches, namely sequencing of the tmL intron and PCR-RFLP analysis of the tmK intron. Initially, six individuals of F. japonica var. japonica, three individuals of F. japonica var. compacta, four individuals of F. sachalinensis, four individuals of F. japonica var. japonica x F. sachalinensis and one individual of F. japonica var. compacta x F. sachalinensis were assayed using both techniques (M.L. Hollingsworth, unpublished data). As the same number of haplotypes were identified by RFLP analysis of the tmK intron as by sequencing the tmL intron, for speed and cost efficiency, the samples in this study were assayed using restriction fragment analysis alone.

DNA extraction

Total genomic DNA was extracted from leaf material using the CTAB protocol of Doyle & Doyle (1990) with the addition of an ammonium acetate wash to remove excess carbohydrates.

RFLP analysis of the tmK intron

The tmK intron was amplified using the universal primers described by Demesure et al., (1995). PCRs (50µl) contained: ~ 0.1µg genomic DNA, 100µM of each dNTP, 0.3 µM of each primer, 2 Units Taq polymerase (Bioline), 2mM $MgCl_2$ and 5µl reaction buffer (160 mM $(NH_4)_2SO_4$, 670 mM Tris HCl, 0.1% Tween 20, pH8.8). The PCR profile was: 1 cycle of 94°C for 4 min followed by 30 cycles of 45 s at 92°C, 45 s at 53°C, and 3 min at 72°C and finally 1 cycle at 72°C for 10 min. The PCR products were then digested with the restriction enzymes Alu1, Cfo1 and Rsa1. All restriction digests were carried out according to the manufacturers instructions (Gibco BRL). The fragments were separated on 1.6% agarose gels (1xTBE buffer) and visualised with ethidium bromide and ultraviolet light.

Results

Chloroplast DNA variation between the parental taxa

Amplification of the tmK intron resulted in a product of approximately 2700 bp in all taxa. Restriction digests using Alu1, Cfo1 and Rsa1, identified three haplotypes. Using Cfo1, Fallopia japonica var. compacta and F. japonica var. japonica are distinguishable from F. sachalinensis by the presence of a single restriction site (Fig. 14). With Alu1, F. japonica var. japonica is distinguishable from F. japonica var. compacta and F. sachalinensis by the presence of an additional restriction site (Fig. 14). With Rsa1, an additional restriction site distinguishes F. japonica var. compacta from F. sachalinensis and F. japonica var. japonica (Fig. 14). Thus using Cfo1 and Alu1 in combination, it is possible to identify all three haplotypes. No variation was detected within any of these taxa.

Chloroplast DNA inheritance and variation in the hybrids

Restriction enzyme analysis of the tmK intron did not identify any novel haplotypes in the hybrid plants. The distribution of the parental haplotypes amongst the hybrids is given in Table 11. In all cases where the maternal parent is known (either controlled crosses or hybrid seed collected directly from plants of Fallopia japonica var. japonica) the hybrid has inherited the chloroplast haplotype of the maternal parent (Fig. 15). In all of the wild hybrids examined in which F. japonica var. japonica was one of the parents, the hybrids have the F. japonica var. japonica haplotype (Fig. 16). The wild hybrids of F. sachalinensis and F. japonica var. compacta contain either the F. sachalinensis or the F. japonica var. compacta haplotypes (Table 11).

Figure 14. Legend

Diagrammatic representation of the Fallopia chloroplast restriction fragment profiles of the trnK intron after digestion with Cfo1, Alu1 and Rsa1. The numbers adjacent to the bands are approximate sizes of the fragments. Codes are given at the top of the figure to identify each haplotype. These codes are referred to in Table 11.

Cfo1: Fallopia japonica var. japonica and var. compacta have a restriction site which cleaves the ca. 2700bp band (not cut in F. sachalinensis) to give two fragments of ca. 1600bp and ca. 1100bp.

Alu1: F. japonica var. japonica has an additional restriction site to var. compacta and F. sachalinensis which cuts the ca. 1460bp band to give two fragments of ca. 975bp and ca. 485bp.

Rsa1: F. japonica var. compacta lacks the ca. 400bp fragment found in var. japonica and F. sachalinensis. Instead, it has an additional fragment of ca. 330bp which +/- co-migrates with the ca. 340bp fragment. The fragment lengths of the different profiles for this enzyme do not add up to ca. 2700bp; I attribute this to the presence of additional small fragments that are not resolved on the gel system used.

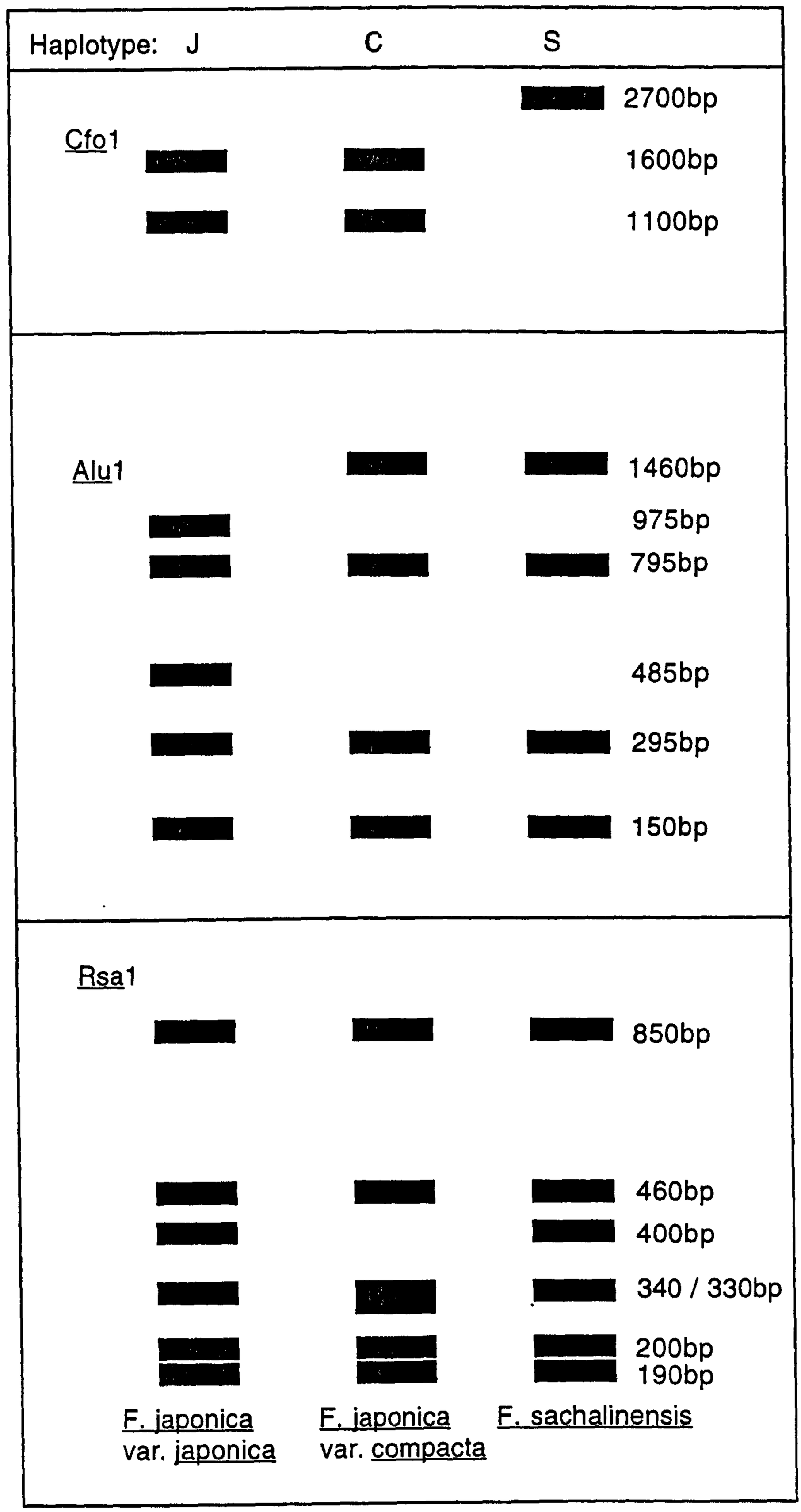


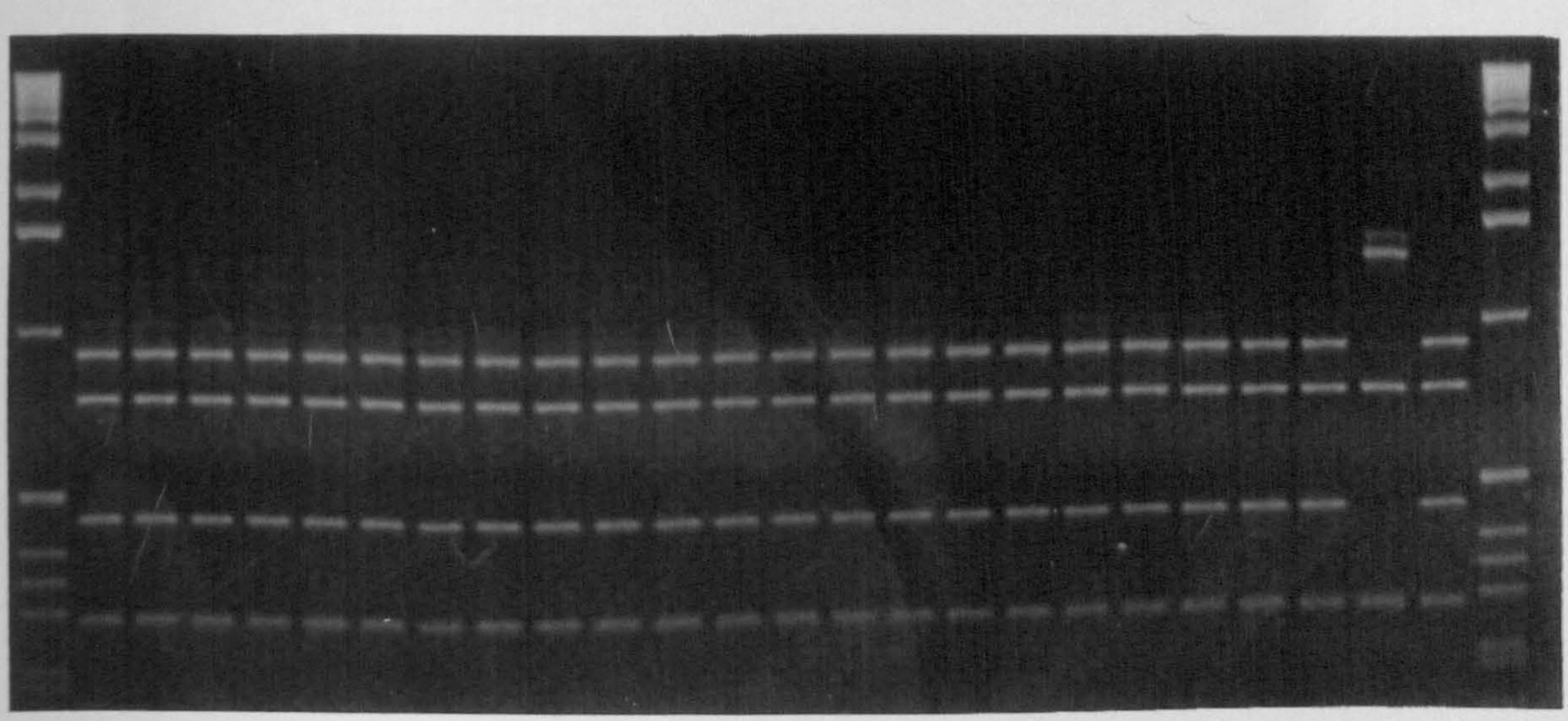
Figure 14. Chloroplast (trnK) DNA restriction fragment profiles of Fallopia taxa

Table 11. Distribution of cpDNA haplotypes amongst Fallopia taxa

TAXON	HAPLOTYPE[1]	N
Parental Taxa		
F. japonica var. japonica	J	58
F. japonica var. compacta	C	4
F. sachalinensis	S	14
Controlled crosses		
F. japonica var. japonica x F. sachalinensis	J	3
F. japonica var. japonica x F. japonica var. compacta	J	1
F. japonica var. compacta x F. sachalinensis	C	1
F. sachalinensis x F. japonica var. compacta	S	2
F. sachalinensis x (F. japonica var. compacta x F. sachalinensis)	S	6
Hybrid seed from known maternal parents		
F. japonica var. japonica x F. sachalinensis	J	13
Wild hybrids		
F. japonica var. japonica x F. sachalinensis	J	45
F. japonica var. japonica x F. japonica var. compacta	J	1
F. japonica var. compacta x F. sachalinensis	C	7
	S	3

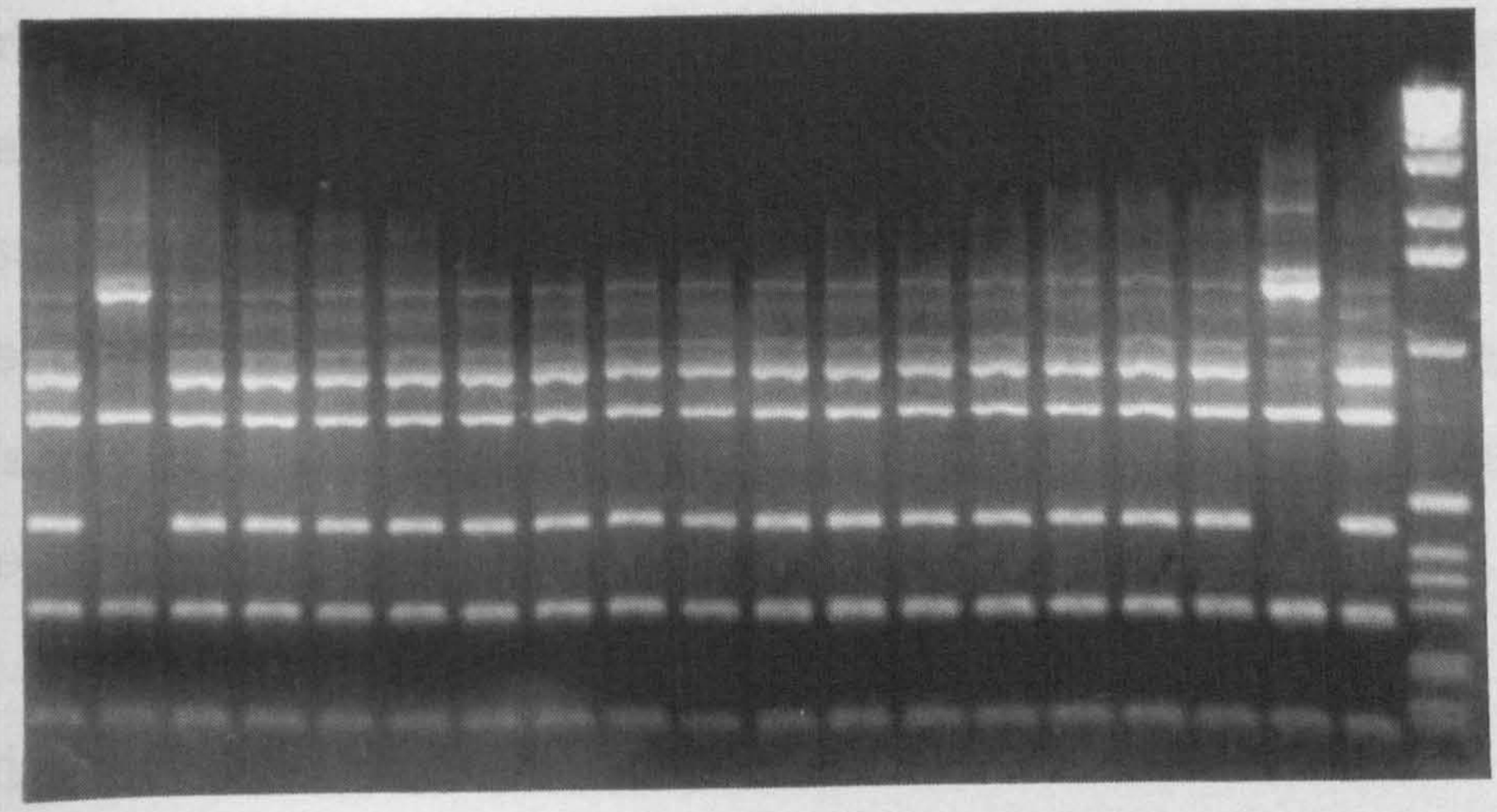
[1]Haplotype codes refer to those in Fig. 14.
N = number of samples examined.

Figure 15. Maternal inheritance in controlled crosses in Fallopia



Chloroplast (trnk) DNA of parental taxa and wild hybrids (Fallopia japonica var. japonica x F. sachalinensis) digested with the restriction enzyme Alu1. From left to right lane: lane 1 1Kb size marker; lanes 2-23 F. japonica var. japonica x F. sachalinensis wild collected hybrids; lane 24 F. sachalinensis; lane 25 F. japonica var. japonica; lane 26 1Kb size marker.

Figure 16. RFLP evidence for uni-directional hybridisation between Fallopia japonica var. japonica x F. sachalinensis



Gel showing maternal inheritance of the chloroplast genome in Fallopia. Chloroplast (trnK) DNA of parents, artificial hybrids, and hybrids of known maternal parentage digested with Alu1.

From left to right the lanes are: 1 artificial hybrid F. japonica var. japonica x F. japonica var. compacta; 2 F. japonica var. compacta; 3 F. japonica var. japonica; lanes 4 -14 wild collected hybrid seed (F. japonica var. japonica x F. sachalinensis); lanes 15 -17 artificial hybrids F. japonica var. japonica x F. sachalinensis; 18 F. sachalinensis; 19 F. japonica var. japonica; 20 1Kb size marker.

Discussion

Chloroplast inheritance in controlled crosses

RFLP analysis of the tmK intron has allowed the differentiation of the chloroplast genomes of Fallopia japonica var. japonica, F. japonica var. compacta and F. sachalinensis. In the progeny of controlled crosses, and in hybrids with a known maternal parent, only the chloroplast haplotype of the female parent (Fig. 15) was observed. This is consistent with chloroplast DNA inheritance being predominantly maternal. I stress the data does not allow sweeping conclusions that exclude the possibility of occasional male transmission of the chloroplast genome in these taxa to be made, as the power of the experiments to distinguish between strictly maternal inheritance and some trace level of paternal or bi-parental inheritance is low, based on these sample sizes (Milligan, 1992; Reboud & Zeyl, 1994).

Hybrids of Fallopia japonica var. japonica

When the chloroplast haplotypes of the 45 wild hybrids of Fallopia japonica var. japonica x F. sachalinensis were examined, it was found that in all cases, they had the chloroplast haplotype of F. japonica var. japonica (Fig. 16). Given the maternal inheritance in all of the controlled crosses discussed above, and the apparent male sterility of F. japonica var. japonica in Britain, this provides strong evidence that hybridisation between F. japonica var. japonica and F. sachalinensis is unidirectional, with F. japonica var. japonica as the female parent. I detected no evidence of either residual male fertility in F. japonica var. japonica, nor paternal chloroplast leakage of F. sachalinensis (in the sense that no F. sachalinensis chloroplast haplotypes were found in these hybrids). The single sample examined of the hybrid between F. japonica var. japonica and F. japonica var. compacta, had the F. japonica var. japonica haplotype. This is again consistent with F. japonica var. japonica being the female parent.

Attempting to address the question of the direction of hybridisation, as well as the mode of chloroplast DNA inheritance in wild hybrids, using the same data set, potentially runs a risk of circularity. However, the results from the controlled crosses, coupled with the apparent male sterility of Fallopia japonica var. japonica, provides strong additional evidence that a) cpDNA inheritance is predominantly maternal and b) F. japonica var. japonica is the female parent of its hybrids. The only other possible explanation of this data set (i.e. the presence of the F. japonica var. japonica cpDNA haplotype in all of its hybrids) would be, that in direct contrast to the results of the controlled crosses and hybrids with known mothers, all inheritance of cpDNA in the wild hybrids was completely paternal, and that the apparently male sterile F. japonica var. japonica was the sole pollen donor in the hybrid crosses. Although I accept that this is theoretically possible, I feel that as an explanation, it is both contrived and unlikely.

One area where some caution is required in the interpretation of this data, relates to the possible lack of independence between the samples of the wild hybrids. Hybrids of Fallopia japonica var. japonica x F. sachalinensis do show some fertility (Bailey, 1994; chapter 3). If some of the samples in this study are not first generation hybrids, but instead, are products of sexual reproduction amongst these hybrids, they will not represent independent samples for assessing the mode of chloroplast inheritance and the direction of hybridisation (i.e. a cross between two hybrids with the same chloroplast haplotype tells us nothing about inheritance or direction). Unpublished RAPD data shows that all of the hybrids used in this study have different genotypes, however, I cannot unambiguously distinguish between multiple origins or hybrid fertility as the source of this variation. The chromosome numbers of the different taxa offer some evidence to suggest the former. F. japonica var.

japonica has 2n=88 chromosomes and F. sachalinensis has 2n=44. First generation hybrids are 2n=66 (Bailey & Stace, 1992). Of the 16 out of the 45 individuals of the hybrid examined here for which chromosome counts are available, all were 2n=66; in contrast chromosome counts from seed from second generation hybrids are typically aneuploid (Bailey, 1997). However, I add the caveat that irregular meiosis in a 2n=66 taxon can still generate n=33 gametes, and as there is no information as to whether there is any preferential survival of euploid plants, it seems premature to comment further. Suffice it to say, I note that the examination of the chloroplast haplotypes of 45 wild hybrids of F. japonica var. japonica x F. sachalinensis, may not necessarily represent the products of 45 separate hybridisation events (gamete fusions).

Hybrids between Fallopia japonica var. compacta and F. sachalinensis

Of the wild hybrids between Fallopia japonica var. compacta and F. sachalinensis, seven had the F. japonica var. compacta haplotype and three had the F. sachalinensis type. Although greater sample sizes are required to give statistical confidence, in the light of the data presented above, the most obvious interpretation of this result is that hybridisation between F. japonica var. compacta and F. sachalinensis occurs in both directions. This is fully consistent with the sex expression of these taxa in Britain, as both occur as male sterile and hermaphrodite plants. This provides a clear contrast to the unidirectional hybridisation noted for hybrids of the male sterile F. japonica var. japonica. The hybrids between F. japonica var. compacta and F. sachalinensis show the highest fertility of all British hybrids of invasive Fallopia taxa, this is perhaps attributable to this being a homoploid cross (both parents are 2n=44 (Bailey & Stace, 1992)). A detailed investigation of mixed populations containing male fertile F. sachalinensis and F. japonica var. compacta would be informative, and the organelle markers

identified in this study will be useful tools in the study of introgressive hybridisation amongst these plants.

This chapter has provided new information on the actively evolving populations of Fallopia species in Britain. In particular, given the importance of the F. japonica var. japonica x F. sachalinensis hybrid, which appears to be as invasive as F. japonica var. japonica, any information on its biology and origins is of great interest. The dynamic evolution of populations of introduced weeds such as these, give rise to useful model systems to investigate processes such as hybrid speciation and introgression, and a knowledge of the time scale since their introduction can allow the elimination of long term historical factors as confounding variables in the interpretation of present day pattern.

Chapter 6

Evidence for massive clonal growth in the invasive weed *Fallopia japonica* (Japanese Knotweed)

Abstract

Clonal growth in introduced populations of Japanese Knotweed (*Fallopia japonica*) in Britain was assessed using RAPDs (Randomly Amplified Polymorphic DNA). A total of 150 British samples was analysed for genetic variation using ten arbitrary decamer primers, and compared with data from 16 samples of other introduced populations from Europe and the USA. All samples produced an identical multi-primer RAPD profile consistent with the presence of a single, exceptionally widespread clone. The enormous biomass of this clone may represent one of the world's largest genets.

Introduction

Episodes of colonisation are important determinants of the genetic structure of populations (Brown & Marshall, 1981). The colonisation of new territory, by one or a few individuals, can lead to marked population differentiation. Migrants are not necessarily representative of the genetic diversity of the source population from which they were drawn, and this sampling effect of individuals from populations has been invoked as a key evolutionary step in speciation models such as genetic revolutions (Mayr, 1954), the founder-flush (Carson, 1968), and genetic transilience (Templeton, 1980), and at a more subtle level, as a cause of population sub-structure and differentiation (Barrett & Shore, 1989; Barrett & Husband, 1990).

Perhaps some of the clearest examples of the effects of colonisation on population genetic structure are those involving the introduction of organisms to areas outside their present native range. Comparisons of the genetic variability of species in native and introduced populations can show a marked asymmetry in the distribution of variability, with genetic depauperacy evident in introduced populations, compared with native populations. One example of this phenomenom is the genetic depauperacy of introduced populations of Echinochloa microstachya (Wiegand) Rydb. in Australia compared with native North American progenitor populations (Barrett & Shore, 1989). Other studies, however, have been more equivocal and shown no clear differences between native and introduced populations (e.g. Warwick et al., 1987). Predictions of the genetic effects of colonisation are thus difficult, and the genetic diversity and structure of introduced populations are dependent on several factors including the number of founding individuals, the rate of increase in population size following colonisation, the nature of the breeding system, the mode of reproduction

and the ploidy level (Barrett & Shore, 1989; Haldane, 1930; Hamrick et al., 1991; Nei et al., 1975).

One particularly notorious introduced colonising species is Japanese Knotweed (Fallopia japonica (Houtt.) Ronse Decraene) var. japonica. Japanese Knotweed is native to Japan, Korea and China and was introduced to Britain in the 1840s (Beerling et al., 1994). Its exotic appearance and ease of growth made it appear an ideal ornamental garden plant. It has since, however, proved to be a problematical invasive species (Conolly, 1977). The first recorded escape from cultivation was in 1886 (Storrie, 1886); by 1996 it had been recorded from 1584 of the 2862 10 km squares of the Biological Records Centre mapping system of the British Isles (B.R.C. unpublished data). It is regarded as the most pernicious alien weed in the British Isles (Mabey, 1996) and it is a criminal offence to knowingly introduce Japanese Knotweed into the wild. Once established this plant is almost impossible to eradicate and thousands, if not millions, of pounds are spent each year on its control.

Remarkably, this rapid and aggressive invasion appears to have been purely by vegetative propagation. Japanese Knotweed is gynodioecious, occurring as hermaphrodite and male sterile plants. Although both floral morphs occur in its native range, in Britain only male sterile (female) plants are known (Bailey, 1994). This implies that the British populations of Japanese Knotweed have no capacity for perpetuation by seed. Thus, the genetic base of these introduced populations may be severely limited, and at an extreme, all individuals may be ramets of the same vigorous clone.

The aim of the present study is to examine the amount of genetic diversity of Japanese Knotweed in Britain and thereby establish whether populations

are multi-clonal. Available material from introduced populations from continental Europe and the USA has also been included. I have not yet, however, attempted a formal comparison of the levels of genetic variability with native populations. Preliminary morphological, cytological and molecular analyses of native populations have revealed a large amount of diversity. However, none of the native samples of Japanese Knotweed I have examined precisely match the introduced plants in Europe and the USA; all show differences in morphology, chromosome number, chloroplast sequence and RAPD profiles (Bailey, 1997; chapter 8). Until further samples are collected and the taxonomy of this complex clarified, comparisons of native and introduced populations remain difficult to place in context.

To assess the levels of clonal diversity in Japanese Knotweed in the British Isles, the approach has been to genotype the samples using RAPDs: Randomly Amplified Polymorphic DNA (Williams et al., 1990). Although the technique has been criticised primarily due to concerns about reproducibility and possible non homology of co-migrating fragments, it is ideally suited for studies whose aim is to investigate levels of clonal diversity. Here the issue is simply whether samples have the same or different RAPD profiles, and concerns of fragment homology are minimal. Although reproducibility is a concern for all applications, it is a technical rather than a genetic problem, and careful experimental design can be employed to assess its impact. In the current study, negative controls lacking template DNA were included in each PCR run and all samples/primer combinations were repeated at least once. In addition, replicate DNA extractions were carried out on a subset of the samples, and their RAPD profiles compared with the original extraction. Repeating DNA extractions on samples in arbitrary fingerprinting studies, rather than just carrying out multiple PCRs on the same sample is important. Artifactual genotypes can be temporarily generated by instability of the

experimental conditions or errors in PCR preparation. However, contamination of the original sample and/or the presence of partial inhibitors in the extracted DNA can result in consistently aberrant results, even in a stable and error free system. The repetition of RAPD PCRs on individual samples carries with it an element of false security, and the incorporation of some repeated extractions is required to fully assess the reliability of the results.

Materials and Methods

Plant material

A total of 150 samples of Japanese Knotweed was collected from the British Isles. The sampling has been both coarse, and fine scaled, with a broad geographical representation, as well as intensive collections in three areas: Glasgow, Leicester and Swansea (24, 28 and 23 samples respectively). An additional 16 samples from other introduced populations of this taxon from the USA and continental Europe (France, Germany and the Czech Republic) were also included for comparison. Details of the sample localities are given in Tables 12 & 13 and Fig. 17.

Molecular analyses

DNA extraction and RAPD analyses were carried out according to the methods described in chapter 3. From an initial screen of 60 RAPD primers, ten were chosen that gave clear and reproducible banding patterns. All 150 samples were screened for genetic variation using these primers, along with replicate DNA extractions from a subset of the samples. RAPD products were separated on 1.6% agarose gels in TBE buffer and visualised using ethidium bromide under ultraviolet light.

Table 12. Sample localities of British material of Fallopia japonica var. japonica

VC	Location
1	Lands End, West Cornwall
1	Falmouth, West Cornwall
1	D Point, West Cornwall
?	Chienhall, Cornwall
2	Rame, East Cornwall
6	Arno's Castle, Somerset
10	Isle of Wight (4)
12	Froxsfield, West Sussex
15	Chatham, East Kent
15	Bekesbourne, East Kent
17	Albury Heath, Surrey (2)
19	Broomfield, Chelmsford, North Essex
21	Staines, London
21	Hornsey, London
23	B430 Oxfordshire
24	Beaconsfield, Buckinghamshire
28	East Rudham, West Norfolk
29	Langwood hill, Cambridgeshire
29	Linton, Cambridgeshire
29	Waterbeach, Cambridgeshire
32	Eye, Northamptonshire
38	Leamington Spa, Warwickshire
38	Birmingham University, Warwickshire
41	Maesteg, Glamorgan
41	Neath Abbey, Glamorgan
41	Swansea, Glamorgan (23)
42	Abergwesyn, Breconshire
42	Beulah, Breconshire
44	Llanelli, Breconshire
45	Amroth, Pembrokeshire
46	New Quay, Cardiganshire
46	Devils bridge, Cardiganshire
48	Caerynwich, Merionethshire (2)

VC	Location
48	Dolgellau, Merionethshire
49	Bryn Villa, Caernarvonshire
49	Tremadog, Caernarvonshire
54	Lissington, North Lincolnshire
55	Snell's Nook, Leicestershire
55	Loughborough, Leicestershire
55	Leicester (28)
56	Kirkby in Ashfield, Nottinghamshire
57	Brassington, Derbyshire
57	Millington Green, Derbyshire
59	Leigh, Manchester
59	Warrington, South Lancaster
59	Liverpool, South Lancaster (2)
61	Hull, South-east Yorkshire
63	Bradford, South-west Yorkshire
64	Harrogate, Mid-west Yorkshire
69	Windermere, Westmorland
76	Kilbarchan, Renfrewshire
77	Bothwell castle, Lanarkshire
77	Glasgow, Lanarkshire(24)
85	St Andrews, Fife
86	Balmaha, Stirlingshire
87	Menstrie, West Perth
87	Rumblingbridge, West Perth
93	North Auquharney, Aberdeen
97	Inverie, Westerness
99	Tarbet, Dumbarton
99	Arrochar, Dumbarton
105	Ullapool, West Ross (2)
108	Kerracher, West Sutherland
112	Shetland (3)
H3	Lismore, Ireland,
H30	County Cavan (4)

Sample sizes greater than one are given in parentheses VC=Vice county

Table 13. Collection details of Fallopia japonica var. japonica samples from continental Europe and the USA

Locality	Country
Cernosico	Czech Republic
Karlstejn	Czech Republic
Ceske Budejovice	Czech Republic (4)
Prague	Czech Republic (2)
Langendreer	Germany
Thyssenwerk	Germany
Oberwinter	Germany
Montceau les Mines	France
Tournus	France
Massachussetts	USA
Conetticut	USA
Ohio	USA

Sample sizes greater than one are given in parentheses

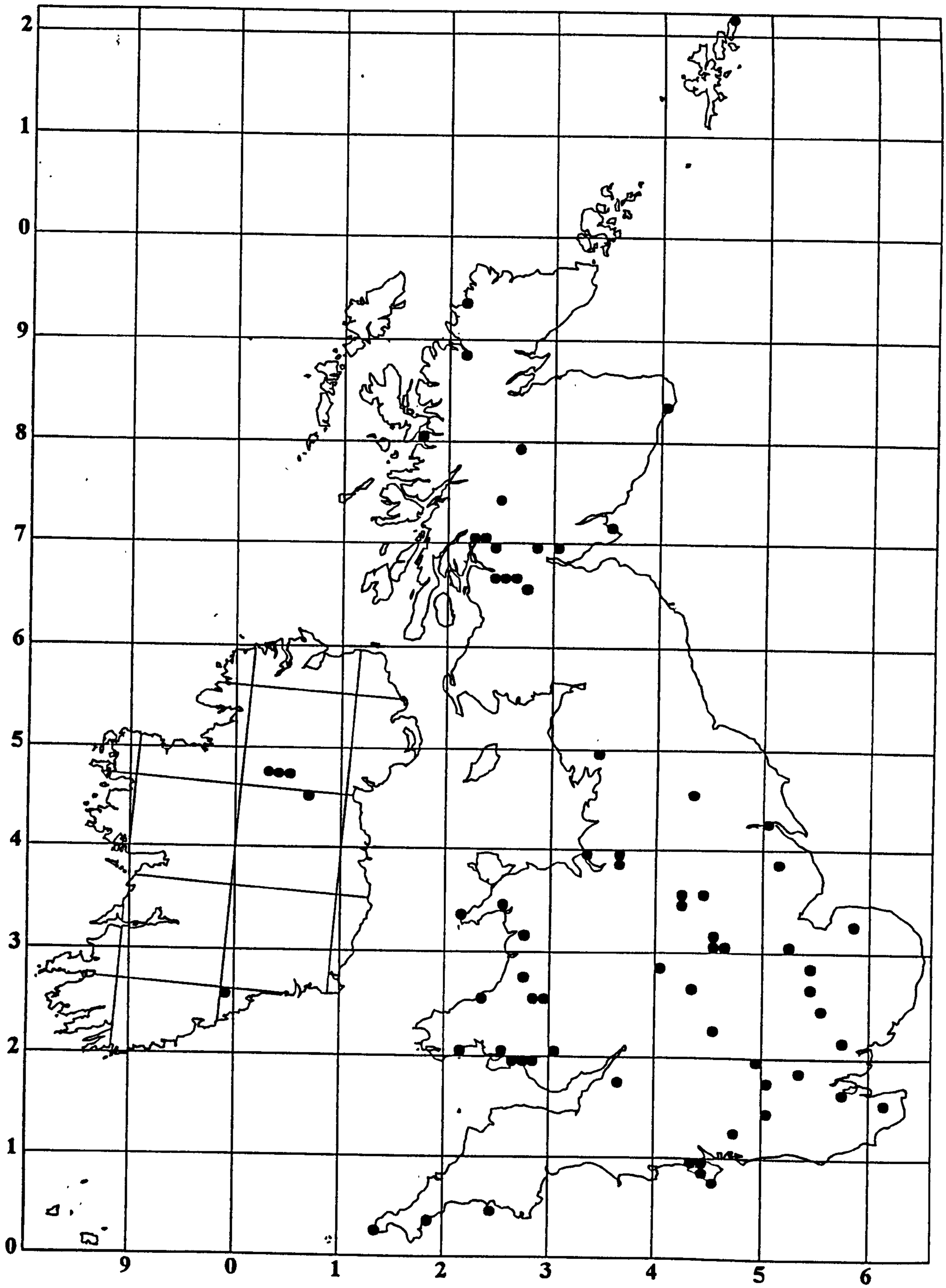
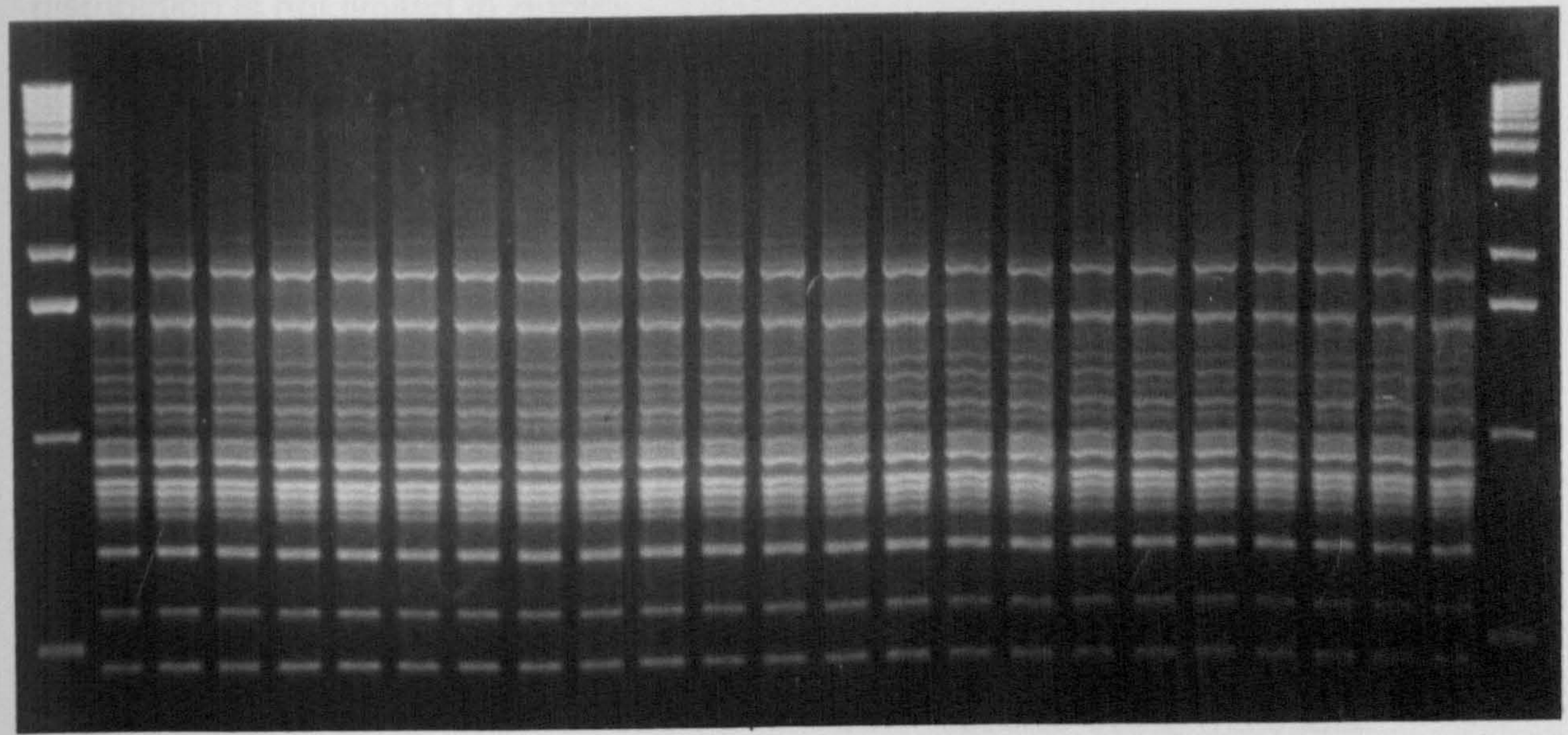


Fig.17 Sample localities of Fallopia japonica var. japonica

Results

Using ten RAPD primers, 108 reproducible fragments were amplified, which given the dominant nature of RAPDs (Williams et al., 1990), I interpret as 108 loci. No genetic variation was detected among 144 out of the 150 samples of Fallopia japonica var. japonica collected from the British Isles. For the remaining British samples, polymorphisms were observed in up to six of the ten primers. Repeated DNA extractions of these samples were analysed to investigate the reliability of this variation. In all cases, the re-extracted DNA produced identical RAPD profiles to the other 144 samples, indicating the initial differences were artifactual in nature. No variation was detected in the samples of F. japonica var. japonica from continental Europe and the USA; these samples produced identical RAPD profiles to the British material.

Fig 18. RAPD photograph showing the Fallopia japonica var japonica genotype detected in this study (primer OPF08).



From left to right the samples are: Lane 1 1Kbp marker; Lane 2 Chienhall; Lane 3 Amroth; Lane 4 Rame; Lane 5 Chatham; Lane 6 Albury Heath; Lane 7 Ballyconnel; Lane 8 Staines; Lane 9 Leamington; Lane 10 Swansea Swan 13; Lane 11 Abergwesyn; Lane 12 Caerynwich; Lane 13 Leicester Leic5; Lane 14 Liverpool; Lane 15 Albury Heath; Lane 16 Killarchan; Lane 17 Bothwell castle; Lane 18 Glasgow Glas 38; Lane 19 Balmaha; Lane 20 Red Carr; Lane 21 Inverie; Lane 22 Kerracher; Lane 23 Swansea Swan 8; Lane 24 Alloa; Lane 25 Lismore; Lane 26 1Kbp marker

Discussion

The molecular data generated in this study, coupled with the absence of male fertile individuals, provide strong evidence to support the hypothesis that the entire British population of Japanese Knotweed consists of a single clone. Furthermore, the identical RAPD profile detected in samples of this taxon from France, Germany, Czech Republic and the USA indicate that its distribution is not limited to Britain.

I am confident that complete uniformity across samples for 108 bands from ten primers reflects genet identity rather than assay insensitivity. In parallel studies of other introduced Fallopia taxa, I have demonstrated the ability of these RAPD primers to detect intra-population variation (chapters 3 & 4) and to distinguish different genotypes in progeny arrays (M. L. Hollingsworth, unpublished data).

Extensive clonal growth has been detected in other species using molecular genetic techniques. Perhaps one of the best publicised examples is that of the fungus Armillaria bulbosa (Barla) Kile & Watling (= Armillaria gallica Marxmüller & Romagnesi). Smith et al. (1992) used RAPDs and nuclear RFLPs to study clone size in this species and concluded that a single clone occupied a minimum of 15 hectares and weighed in excess of 10,000 kg. Grant et al. (1992) responded to the claim that this represents one of the world's largest and oldest organisms by highlighting the presence of a putative clone of Quaking Aspen (Populus tremuloides Michx.).occupying 43 hectares with an estimated mass of 6,000,000 kg, although I am not aware of any detailed molecular studies to confirm that this is a single genet. The size of the largest organism on earth is difficult to quantify, not least due to the problem of defining the unit to be measured. Clearly there is an important distinction to be made between the total mass of a genet and those

modules that maintain physical connections. Although there is undoubted physiological importance in determining the extent of interconnected shoots, as well as the intrinsic fascination with the size of a single ramet, many clonal plants defy easy quantification due to the difficulties of establishing continuous connectivity of underground root systems. In this respect, a consideration of genet size is perhaps more practical (at least as a minimum estimate) and of potentially greater evolutionary significance. The widespread clone of Japanese Knotweed detected in this study, must (using the criterion of the genet) be one of the world's largest organisms.

An interesting parallel to the case of Japanese Knotweed is that of Canadian Pondweed (Elodea canadensis Michx.). Introduced to Britain from Canada in ca 1836 it rapidly spread through canal and river systems (Simpson, 1984). Like Japanese Knotweed, all individuals of Canadian pondweed present in Britain are female. I am not aware of any molecular studies to test the genetic variability of this taxon, but it would seem a strong possibility that the entire British population of this taxon also consists of ramets of a single clone. Other examples of postulated extensive clonal growth in unisexual populations of sexual dimorphic taxa include Petasites fragans (Vill.) C. Presl (Richards, 1986), Lagarosiphon major (Ridley) Moss (Cook, 1987) and Populus alba L. (Richards, 1986). However, it is worth stressing that unisexuality does not necessarily equate to uniclonality. Unisexual populations of Stratiotes aloides L. presumed to be uniclonal were found to consist of several clones when assayed with RAPDs (J. Ouborg pers. comm. 1998).

The perpetuation of organisms purely by clonal propagation is considered to carry three major disadvantages: 'Müller's ratchet' (Müller, 1964), failure to keep pace with the 'Red Queen' (Van Valen, 1973) and viral accumulation

(Richards, 1986). 'Müllers ratchet' is the irreversible accumulation of deleterious mutations that cannot be eliminated in the absence of meiosis. The 'Red Queen' hypothesis refers to the need for an organism to evolve and respond to biotic and abiotic changes in its environment (a species has to keep running to stay in the same place). The accumulation of viruses occurs due to the absence of the cleansing step of gametogenesis, as many plant viruses are not transmitted from parents to offspring. Thus solely clonally propagated taxa can develop an ever increasing viral load. The ability to remove deleterious mutations, to respond to environmental change, and eliminate viruses is of fundamental importance to an organism, but the time scale over which these factors become important is unclear. In the short term at least, the advantages of the rapid mass production of a single successful genotype can temporarily outweigh the benefits of sex. Certainly Japanese Knotweed in Britain does not appear to be disadvantaged by its solely asexual propagation. Its long term fate, however, remains open to question. Although it is difficult to highlight unambiguous cases of the cost of asexuality in natural populations due to the difficulties of establishing controls, the potential fragility of temporarily successful clones is well exemplified by horticultural examples, such as the rapid die back of Primula x 'scapeosa' documented by Richards (1986). This hybrid was synthesised in 1949 and clonally propagated and distributed to nurseries around the world from 1972. Between 1978 and 1982 a world-wide die back, attributed to viral accumulation eliminated most individuals of this taxon. Alternatively clones of other species have been postulated as surviving for many years, such as up to 10,000 years for Potamogeton x suecicus K. Richt. (Hollingsworth et al., 1996) and Armillaria bulbosa (Smith et al., 1992), with an estimated age of to 1 million years suggested for genets of Populus tremuloides (Mitton & Grant, 1996).

The future success and longevity of introduced plants of Japanese Knotweed are difficult to predict. Polyploidy may offer some evolutionary reprieve from the lack of inter-individual variability, as high levels of allelic richness can be maintained within individuals. Japanese Knotweed is an octoploid (2n=88), and it is noteworthy that polyploidy is a common to all 18 of the 'worst weeds in the world' listed by Holm (1977). It is worth qualifying this, however, with the comment that polyploidy per se is not a prerequisite to successful plant invasions, and Gray (1986) noted that of the 20 most successful alien plants in Britain (Crawley, 1987), nine are diploid. Regardless, one way in which the invasive capabilities of Japanese Knotweed may increase and the current weed control problems become exacerbated, is via hybridisation (Abbott, 1992). Hybrids between Japanese Knotweed and a related introduced species Giant Knotweed (Fallopia sachalinensis (F. Schmidt ex Maxim.) Ronse Decraene) are now common throughout the British Isles and share the invasive attributes of Japanese Knotweed (Bailey et al., 1996). These hybrids (F. x bohemica Chrtek & Chrtková) show partial to full fertility and backcrosses with Japanese Knotweed have recently been documented (chapters 3 & 4). Hybridisation thus appears to offer a potential escape from the obligate asexuality caused by the absence of functional males of Japanese Knotweed in Britain and the production of sexually derived genotypes may serve to enhance the long term survival and success of this weed invasion.

Chapter 7

Genetic Diversity in Fallopia sachalinensis and F. x bohemica in Britain

Abstract

Levels of clonal diversity in Fallopia sachalinensis and F. x bohemica in Britain have been examined using RAPDs. High levels of clonal diversity have been detected with no genotypes shared between populations. When the data were analysed using principal coordinate analysis, neighbour joining and split decomposition, two distinct clusters of genotypes of F. sachalinensis were detected, showing some correlation with sex expression. The genetic basis of gynodioecy is discussed and questions are raised as to how sex linked clustering can occur. In F. x bohemica no distinct clusters were obtained, with most of the inter-genotype distances separating individuals, rather than groups.

Introduction

The success of the Japanese Knotweed invasion of the British Isles is well documented (Conolly, 1977) and hundreds of miles of roadsides, railways, and riversides have been colonised. Recent molecular studies (chapter 6) suggest that despite the widespread occurrence of Fallopia japonica (Houtt.) Ronse Decraene var. japonica, all British individuals of this taxon are ramets of a single clone. This simple situation of a single widespread clone propagated by purely asexual means is contrasted by the related invasive species Giant Knotweed (F. sachalinensis (F. Schmidt ex Maxim.) Ronse Decraene) and its hybrid with F. japonica var. japonica (F. x bohemica (Chrtek & Chrtková) J. Bailey). Both F. sachalinensis and F. x bohemica occur as male sterile and male fertile plants in Britain. Studies of two river systems have detected intra-population genetic diversity within these taxa indicating that sexual events may occur. However, the extent of sexual versus asexual reproduction in these taxa remains open to question. A sample of 17 plants of F. sachalinensis and 23 plants of F. x bohemica from the River Kelvin in Glasgow revealed the presence of two and five clones respectively (chapter 3). Likewise, a sample of three F. sachalinensis and 36 F. x bohemica from the River Wnion system in Wales revealed the presence of two and 20 clones respectively (chapter 4). No clones were shared between populations. There are two possible extreme explanations of these results. Firstly, localised sexual reproduction accounts for the presence of the different genotypes at the different sites. Alternatively, widespread dispersal of different clones is responsible for the polymorphisms, with the difference between the River Wnion and the River Kelvin populations reflecting a sampling artefact (either methodological or biological). These two extremes lead to rather different predictions as to the constitution of the British

populations of these taxa. If sexual reproduction is an important determinant of population structure, then one would expect that samples from different areas would show unique genotypes. If vegetative propagation and subsequent dispersal is the predominant mode of reproduction, then one would expect further sampling to recover the same genotypes at different sites.

To establish which of these hypotheses best describes the British populations of Fallopia sachalinensis and F. x bohemica, I have sampled individuals of these taxa from additional localities in Britain and screened them for polymorphisms using RAPDs.

Materials and Methods

Plant material was collected from the localities listed in Table 14 and shown in Figs. 19 & 20. In contrast to the fine scale sampling of the River Wnion and River Kelvin studies, the sampling was coarse. Single samples were taken from each locality, with the exception of F. x bohemica at Swansea, where three samples were collected. Leaf material was snap frozen in liquid nitrogen and the DNA extracted following the protocol described in chapter 3. All samples of F. sachalinensis were analysed for RAPD variability using the same ten decamer primers as described in chapter 3. All samples of F. x bohemica were analysed for variation using five of these primers (OPF08, OPF20, OPP20, OPA08 & OPA16). Time prevented them being screened for the full set of primers. All samples were run against the samples from the River Kelvin and the River Wnion for comparison (chapters 3 & 4). As comparing banding patterns across gels is difficult, the samples from the River Kelvin and the River Wnion were rerun together with the samples in this study. Unfortunately degradation of the DNA of the following samples meant they could not be included in the comparisons: genotype B? from the River Kelvin and genotypes R, T and V from the River Wnion (T, and V were two of the six seedlings from the Wnion).

Relationships among genotypes were examined using principal coordinate analysis (PCO), neighbour joining and split decomposition. Methodological details are as described in chapters 3 and 4.

Table 14. Collection details of Fallopia sachalinensis and F. x bohemica

Thesis code	Taxon	Locality	VC
Nant	F. sachalinensis	Nant Y Frith	43
Howey	F. sachalinensis	Howey	43
Amroth	F. sachalinensis	Amroth	45
Wnion C3	F. sachalinensis	Caerynwich	48
Ciren	F. sachalinensis	Cirencester	33
Wnion C4	F. sachalinensis	Caerynwich	48
Ulla	F. sachalinensis	Ullapool	105
Cwrt	F. sachalinensis	Cwrt Newydd	46
Cong	F. sachalinensis	Congerstone	55
Gart	F. sachalinensis	Gartshore Est, Glasgow	77
Fort	F. sachalinensis	Fort William	97
Wind	F. sachalinensis	Windermere	69
KelvinSMS1	F. sachalinensis	Glasgow	77
KelvinSMS2	F. sachalinensis	Glasgow	77
Fowey	F. x bohemica	Fowey, Cornwall	2
Holmbury	F. x bohemica	Holmbury	17
L Hang	F. x bohemica	Lower Hanger Shotter	17
Buckingham	F. x bohemica	Buckingham Palace	21
Hoxne	F. x bohemica	Hoxne	25
Swan1	F. x bohemica	Blackpill, Swansea	41
Swan2	F. x bohemica	Blackpill, Swansea	41
Swan3	F. x bohemica	Blackpill, Swansea	41
Amroth	F. x bohemica	Amroth	45
WnionD20	F. x bohemica	Dolgellau	48
WnionD4	F. x bohemica	Dolgellau	48
WnionD6	F. x bohemica	Dolgellau	48
WnionD7	F. x bohemica	Dolgellau	48
WnionD8	F. x bohemica	Dolgellau	48
WnionD11	F. x bohemica	Dolgellau	48
WnionD14	F. x bohemica	Dolgellau	48
WnionD10	F. x bohemica	Dolgellau	48
WnionD18	F. x bohemica	Dolgellau	48
WnionC6	F. x bohemica	Caerynwch	48
WnionC8	F. x bohemica	Caerynwch	48

Thesis code	Taxon	Locality	VC
WnionC11	F. x bohemica	Caerynwch	48
WnionC13	F. x bohemica	Caerynwch	48
WnionC14	F. x bohemica	Caerynwch	48
WnionC16	F. x bohemica	Caerynwch	48
WnionC20	F. x bohemica	Caerynwch	48
WnionC22	F. x bohemica	Caerynwch	48
WnionC24	F. x bohemica	Caerynwch	48
WnionC25	F. x bohemica	Caerynwch	48
Horncliffe	F. x bohemica	Horncliffe	68
Kilarchan	F. x bohemica	Kilarchan	76
KelvinBH1	F. x bohemica	River Kelvin, Glasgow	77
KelvinBH2	F. x bohemica	River Kelvin, Glasgow	77
KelvinBMS	F. x bohemica	River Kelvin, Glasgow	77
KelvinBJ?	F. x bohemica	River Kelvin, Glasgow	77
Gart	F. x bohemica	Gartshore, Glasgow	77
Alloa	F. x bohemica	Alloa	87
Kellas	F. x bohemica	Kellas, Morayshire	95
Maam	F. x bohemica	Maam Galway	H16
Lough Neagh	F. x bohemica	Lough Neagh N.Ireland	H40

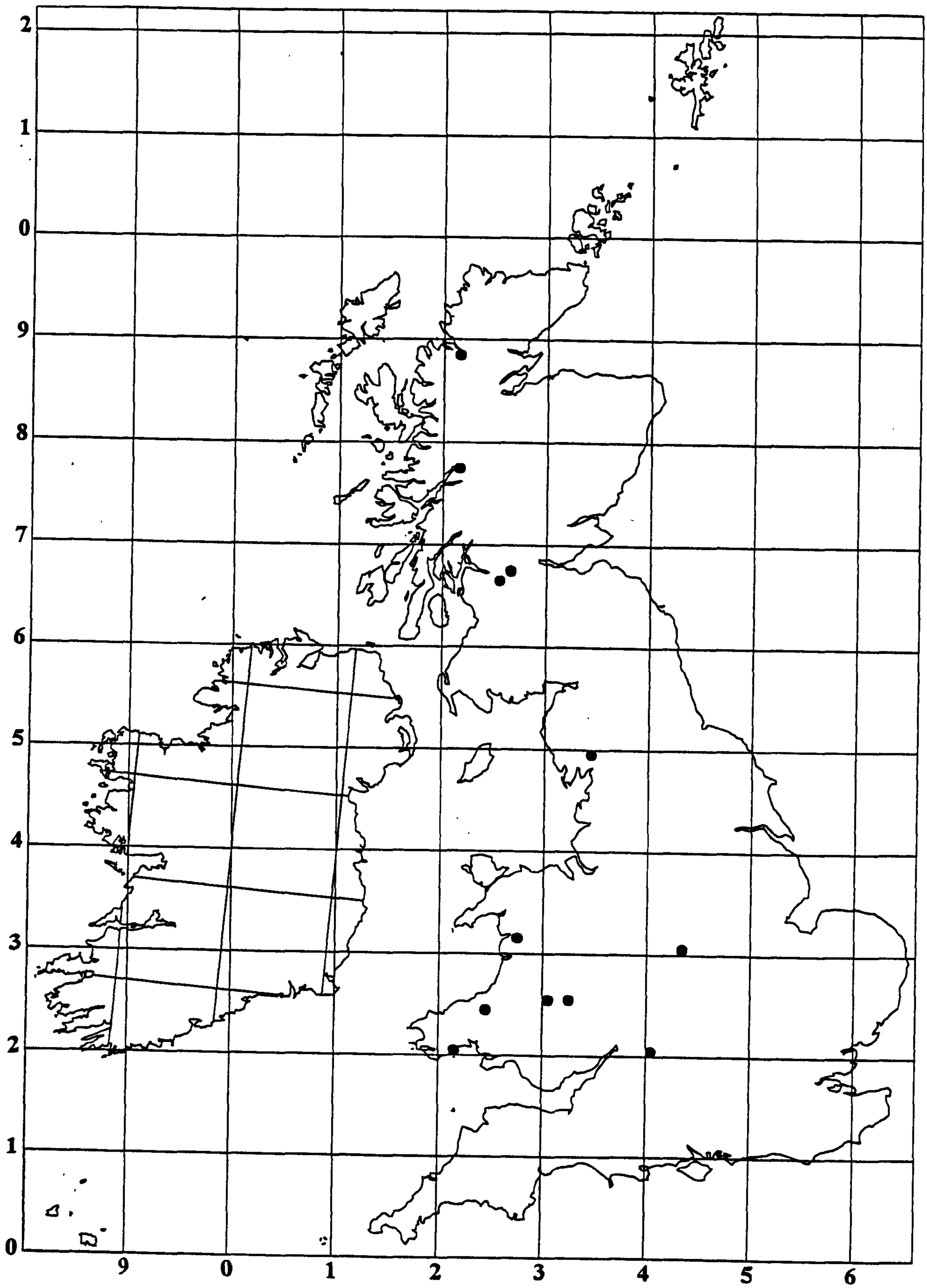


Fig.19 Sample localities of Fallopia sachalinensis

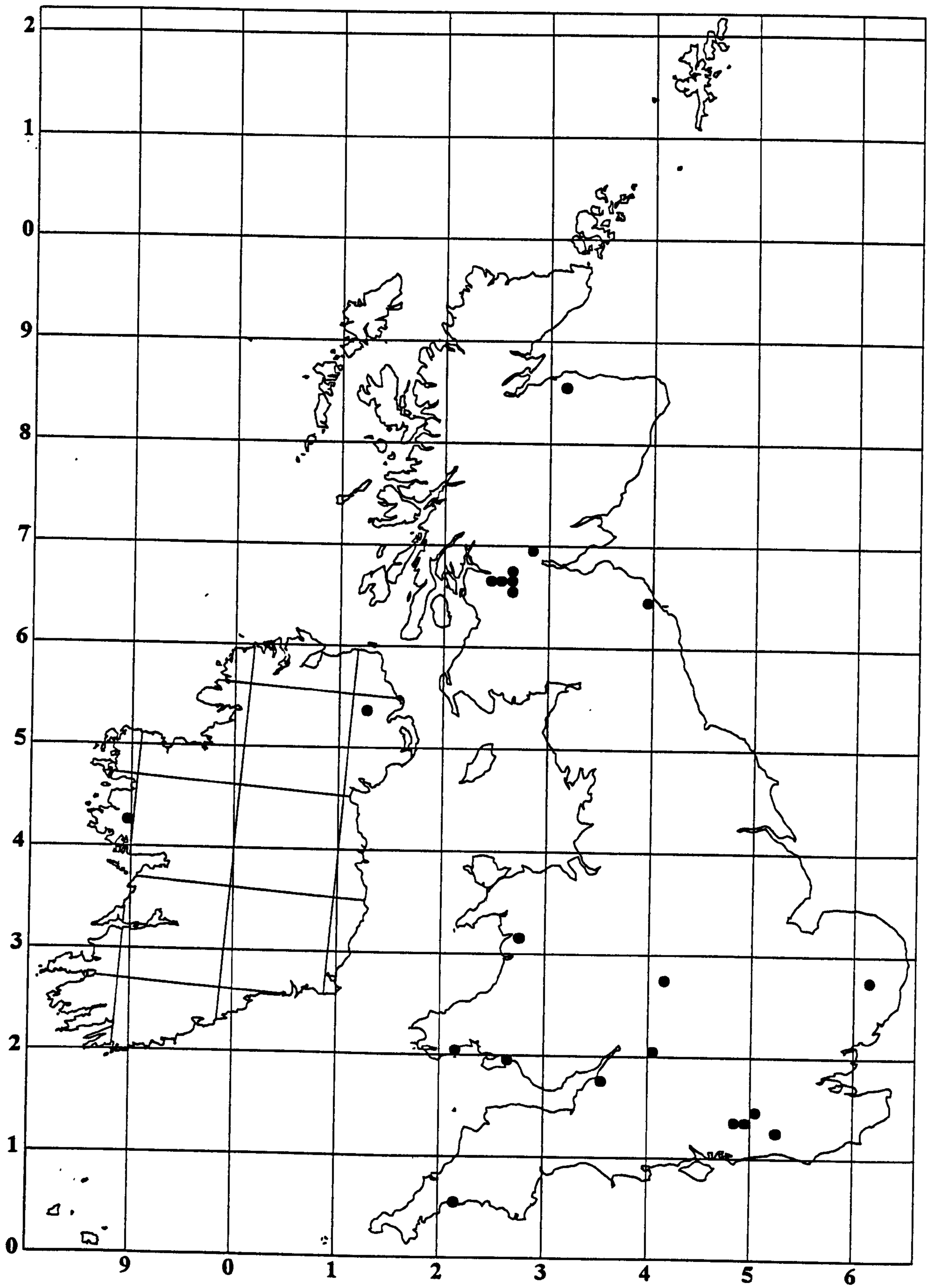


Fig.20 Sample localities of Fallopia x bohemica
(F. japonica var. japonica x F. sachalinensis)

Results

RAPD analysis of Fallopia x bohemica and F. sachalinensis detected high levels of variability. All samples of F. sachalinensis showed different genotypes (Figs. 21 & 22) and from the 39 samples of F. x bohemica 38 genotypes were detected (Fig. 23). The only shared genotype was from two plants collected ca. 20m apart in Swansea. No other genotypes were shared either within or between taxa. No genotypes were shared with the samples from the River Wnion and the River Kelvin populations (chapters 3 & 4).

In F. sachalinensis, two distinct groupings were detected by all methods of data analysis (Figs. 24, 25 & 26). One cluster of samples (cluster A) consisted of plants from Scotland and the North of England, along with two plants from Wales. The second group (cluster B) contained plants from England and Wales. Although the sex of only seven out of the 13 genotypes is known, there is some correlation between sex and genetic relationships. All of the male sterile plants occur in cluster A (along with some unsexed plants) and the hermaphrodite plants occur in cluster B (along with some unsexed plants). The River Kelvin and the River Wnion samples, however, did not show population specific clustering.

Data analysis of the Fallopia x bohemica samples failed to detect any clearly identifiable groupings of genotypes, regardless of the method of data analysis used (Figs. 27, 28, 29 & 30). The amounts of variance described by the PCO plots is low (the first principal coordinate accounted for only 8% of the variance). The fit of the split decomposition graph is remarkably low (21.9%) and the graph is effectively a star-like polytomy. The neighbour joining tree shows greater resolution than the split decomposition graph, but even here,

the internal branches are short and the majority of the distances are confined to the terminal branches. Samples from the River Wnion were scattered amongst samples from other sites rather than clustering together (Figs. 27, 29 & 30). The only vaguely explicable clustering was that of the three hermaphrodite genotypes from the River Kelvin which clustered together, albeit with a sample from Cornwall (Fowey) nested between them.

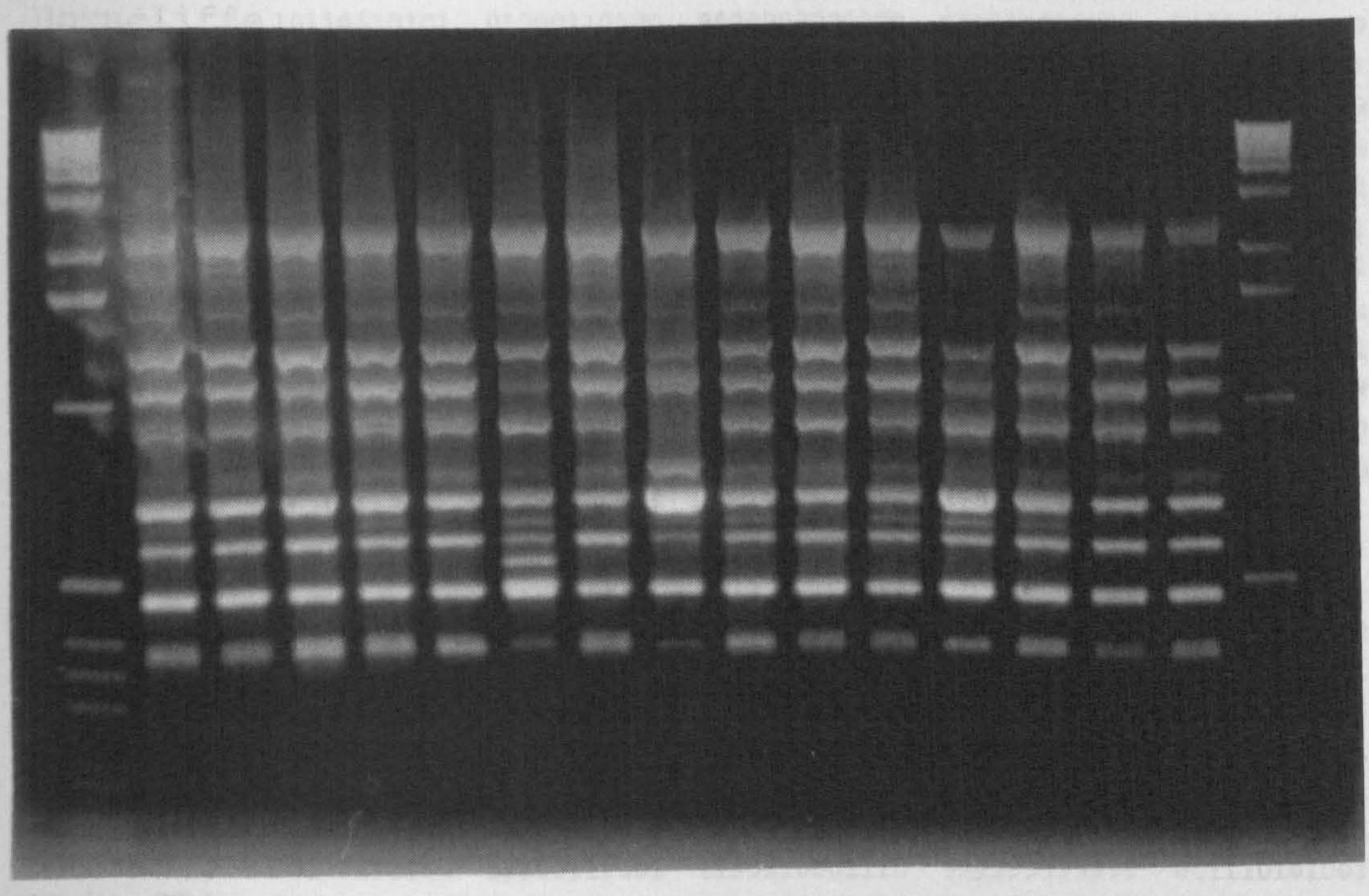
Fig. 21 Presence and absence of arbitrary fingerprinting bands in Fallopia sachalinensis from the British Isles

	OPF08	OPF14	OPF16	OPF17	OPF20
Nant	1 1 1 1 1 1 1 1	1 0 1 0 1 1 0 0 1 1 1 1	1 0 1 1 0 1 1 1	0 0 1 1 1 1	1 0 1 1 1 1 1 0 1 1 1 0
Howey	1 1 1 1 1 1 1 1	1 0 1 0 1 0 0 0 1 1 0 1	1 0 0 1 0 1 0 1	0 0 1 1 1 1	1 0 1 1 1 1 1 1 1 1 1 0
Fort	1 1 1 1 1 1 1 1	1 0 1 0 1 0 0 1 1 1 1 1	1 0 1 1 0 1 0 1	0 0 1 0 1 1	1 0 1 1 1 1 1 1 1 1 1 0
Ciren	1 1 1 1 1 1 1 1	1 0 1 0 1 1 0 0 1 1 1 1	1 0 1 1 0 1 1 1	0 0 1 1 1 1	1 0 1 1 1 1 1 1 1 1 1 0
Ulla	1 1 1 1 1 1 1 1	1 0 1 0 1 0 0 1 1 1 1 1	1 0 1 1 0 1 0 1	0 0 1 0 1 1	1 0 1 1 1 1 1 1 1 1 1 0
Amroth	0 1 1 0 1 1 1 1	1 0 1 0 1 0 0 0 1 1 0 1	1 1 1 1 0 1 0 1	0 0 1 1 1 0	1 0 1 1 1 1 1 1 1 1 1 0
Wind	1 1 1 1 1 1 1 1	1 0 1 0 1 0 0 0 1 1 0 1	1 0 1 1 0 1 0 1	0 0 1 1 1 1	1 0 1 1 1 1 0 1 1 1 1 0
Gart	1 1 1 0 1 1 0 1	1 0 1 0 1 0 1 0 1 1 0 1	1 0 0 1 0 0 0 1	0 0 1 0 1 1	1 0 1 1 1 1 0 1 0 0 1 0
Cong	1 1 1 0 1 1 1 1	1 0 1 0 1 1 0 0 1 1 0 1	1 0 1 1 0 1 0 1	0 0 1 1 1 1	1 0 1 1 1 1 0 0 0 1 1 0
Cwrt	1 1 1 0 1 1 1 1	1 0 1 0 1 0 0 0 0 1 0 1	1 0 1 1 1 1 0 1	0 0 1 1 1 1	1 1 1 1 1 1 0 1 0 1 1 1
KelvinSMS1	0 1 1 0 1 1 1 1	1 1 1 1 1 0 0 0 0 1 1 1	1 0 1 1 0 1 0 1	1 1 1 1 1 1	1 0 1 1 1 1 1 1 1 1 1 0
KelvinSMS2	1 1 1 1 1 1 1 1	1 0 1 0 1 0 0 1 1 1 1 1	1 0 1 1 0 1 0 1	0 0 1 0 1 1	1 0 1 1 1 1 1 1 1 1 1 0
WnionC3	0 1 1 0 1 1 1 1	1 0 1 0 1 1 0 0 1 1 1 1	1 0 1 1 0 1 1 1	0 0 1 1 1 1	1 0 1 1 1 1 1 1 1 1 1 0
WnionC4	1 1 1 0 1 1 1 1	1 0 1 0 1 1 0 0 1 1 0 1	1 0 1 1 0 1 0 1	0 1 1 1 1 0	1 0 1 1 1 1 1 1 1 1 1 0

	OPP03	OPP20	OPA06	OPA08	OPA16
Nant	1 1 1 0 1 1 0 1	1 1 1 1 0 0 0 1 1 0 1	0 0 1 1 1 1	1 1 1 1 1 0 1	0 0 1 0 0 1 1
Howey	0 1 1 0 1 1 0 1	1 1 1 0 0 1 0 1 1 1 1	0 0 1 1 1 1	1 1 1 1 1 0 1	1 0 0 0 0 1 1
Fort	0 1 1 0 1 1 0 1	1 1 1 0 0 1 0 1 1 1 1	0 0 1 1 1 1	1 1 1 1 1 0 1	1 0 0 0 0 1 1
Ciren	1 1 1 0 1 1 0 1	1 1 1 1 0 0 0 1 1 0 1	0 1 1 1 1 1	1 1 1 1 1 0 1	0 0 0 0 0 1 1
Ulla	0 1 1 0 1 1 0 1	1 1 1 1 0 1 0 1 1 1 1	0 1 1 1 1 1	1 1 1 1 1 0 1	1 0 0 0 0 1 1
Amroth	0 1 0 0 1 1 1 1	1 1 1 1 0 0 0 0 1 0 1	1 0 1 1 1 1	1 1 1 1 1 0 1	0 0 1 0 0 1 1
Wind	0 1 1 1 1 1 0 1	1 1 1 0 0 1 0 1 1 1 1	0 1 1 1 1 1	1 1 1 1 1 0 1	1 0 0 0 0 1 1
Gart	1 1 1 0 0 1 0 1	1 1 1 0 1 1 1 0 1 1 1	0 0 1 1 1 1	1 1 1 1 1 0 1	0 0 0 0 0 0 1
Cong	0 1 1 1 1 1 0 1	1 1 1 1 0 0 0 1 1 0 1	0 1 1 1 1 1	1 1 1 1 1 0 1	0 0 1 0 1 1 1
Cwrt	0 1 1 1 1 1 0 1	1 1 1 0 0 1 0 1 1 0 1	0 1 1 1 1 1	1 1 1 1 1 0 1	1 1 1 1 0 0 1
KelvinSMS1	0 1 1 1 1 1 0 1	1 1 1 0 0 1 0 1 1 1 1	0 0 1 1 1 1	1 1 1 1 1 0 1	1 0 0 1 0 1 1
KelvinSMS2	0 1 1 0 1 1 0 1	1 1 1 0 0 1 0 1 1 1 1	0 1 1 1 1 1	1 1 1 1 1 0 1	1 0 0 0 1 1 1
WnionC3	0 1 1 0 1 1 0 1	1 1 1 1 0 0 0 1 1 0 1	0 1 1 1 1 1	1 1 1 1 1 0 1	0 0 0 0 0 1 1
WnionC4	0 1 1 1 1 1 0 1	1 1 1 1 0 0 0 1 1 0 1	0 1 1 1 1 1	1 1 1 1 1 1 1	0 0 0 0 1 1 1

1 = presence of a band, 0 = absence of a band, 9 = missing data

Fig. 22 RAPD photograph showing the Fallopia sachalinensis genotypes detected in this study (primer OPF16).



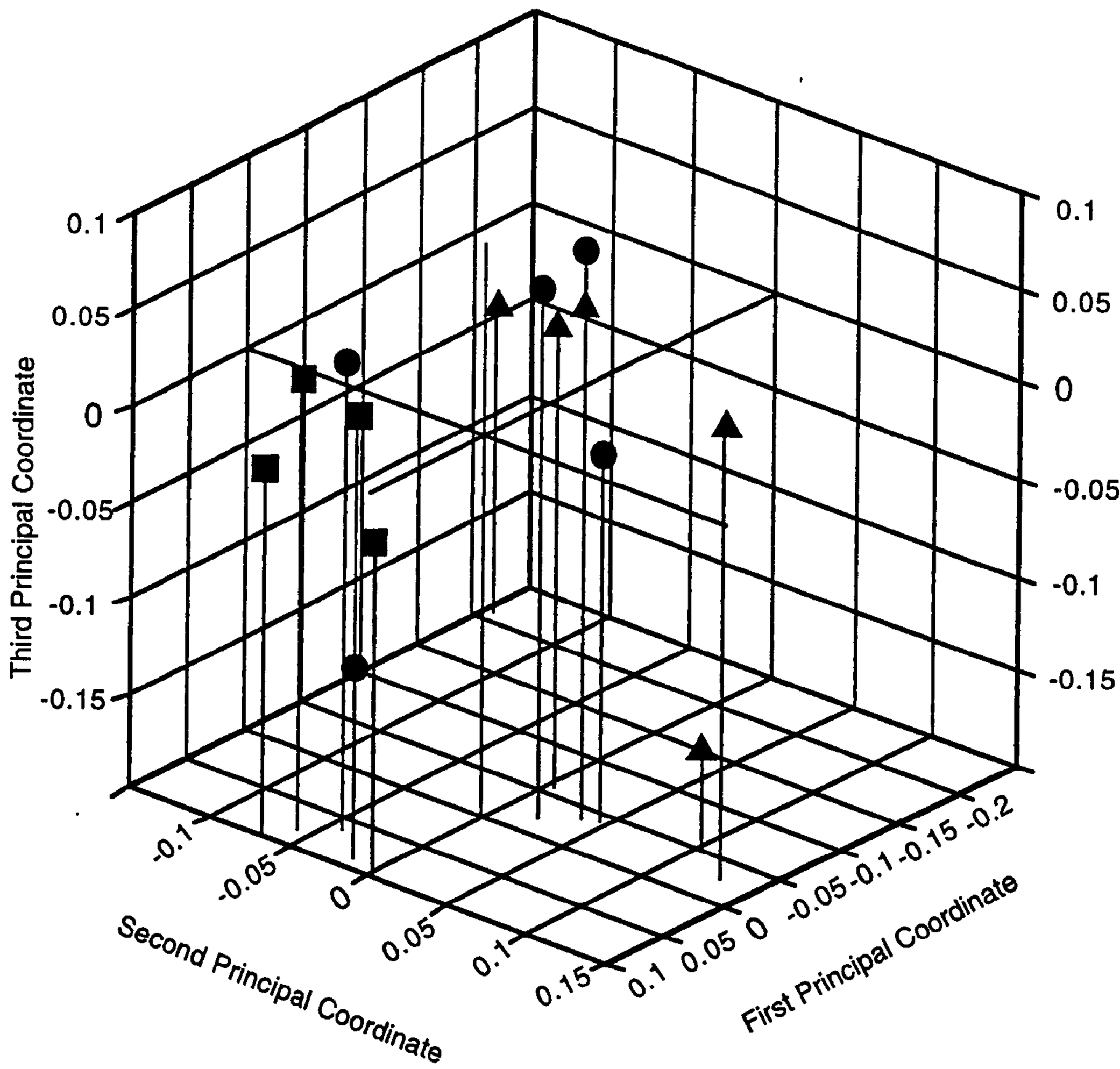
From left to right the samples are: lane 1 1Kbp marker; lane2 Nant; lane 3 Howey; lane 4 Fort; lane 5 Ciren; lane 6 Ulla; lane 7 Amroth; lane 8 Wind; lane 9 Gart; lane 10 Cong; lane 11 Cwrt; lane 12 C3; lane 13 C4; lane 14 C5; lane 15 KelvinSMS1; lane 16 KelvinSMS2.

Fig. 23 Presence and absence of arbitrary fingerprinting bands in Fallopia x bohemica from the British Isles.

Primer	OPF08	OPF20	OPP20	OPA08	OPA16
Maam	10110110101	01110001001	9999999999999	011011010111	999999999999
WnionD20	10111110101	01111100100	1110110000000	111111010011	999999999999
Amroth	10111110101	01110110100	9999999999999	111111011111	100011000001
Holmbury	10110110101	11110101001	1101010101010	010011010110	000100010101
Horncliffe	10110110101	01000110100	9999999999999	011111010011	000111101010
Alloa	10111111101	11100101101	1111110011110	010111000111	001111101001
Kilarchan	10111101101	11100101101	1111110011110	010111000111	999999999999
Hoxne	10110101101	01100100101	1110110001010	010111000110	001101001001
KelvinBH3	00001010011	11010111101	1110110000011	000011000111	001111000001
Fowey	01111111101	11010111101	1110110001011	000111000111	001111101001
Kellas	10110110101	11100101101	1111110001010	010111000111	001111011001
Lough n	00111111101	11010101101	0110110111010	010111000111	000110101001
Witley	10111111101	01010101001	1011001000010	010011110110	001100010101
Gartshore	10110110101	99999999999	9999999999999	100011000110	999999999999
Swan1	10111111101	11110111111	1010010010000	011111010111	010111101110
Swan2	10111111101	11110111111	1010010010000	011111010111	010111101110
Swan3	00011111101	11100101101	1111110111010	010111010111	001111111001
KelvinBH1	10110101101	11100111101	1110110000011	000011010111	001101100001
KelvinBMS	10111110101	11100111101	1111110101011	010111011111	000111101001
KelvinBJ?	10111110101	11100111101	1101110011111	011111010111	000111101010
KelvinBH2	01101011101	11100111101	1110110000011	000011010111	000101000001
B' Palace	10111110101	11100111001	1110110011110	010111000111	010111101001
WnionC14	10110111101	01000110101	1011110011011	010011011101	001110011001
WnionC16	10110110101	01100111100	1011110101010	010011011111	001111101001
WnionC20	10111111101	11100110001	1110110000111	011011010111	001110101001
WnionC22	10110110101	11100111001	1110110001110	999999999999	001110101001
WnionC24	00111110101	11000100100	1111110000010	010011010110	999999999999
WnionC25	10110110101	99999999999	1110110011010	010111010100	001111010001
WnionC13	10111111101	11100111100	1110110011011	011011011111	000110101001
WnionC6	10111111101	11100111101	1111110011011	010011011110	001110101010
WnionC11	10111101101	11100111101	1101110011111	010011011110	000111101110
WnionC8	10110110101	11100111101	1110110111010	010011011111	001111101001
WnionD4	10110111101	11100101101	1110110011110	010111010111	000111101001
WnionD6	10110110101	11110101001	0110110111110	010111011110	001111101001
WnionD7	10110111101	11100111101	1110110101111	000011011111	000110111001
WnionD10	10111111101	11110111101	1110110011010	011011011111	001111101001
WnionD11	10111111101	01110111101	1111110101111	010011011110	000111101001
WnionD8	10111111101	01100110001	1110110011010	010011011110	999999999999
WnionD14	10111111101	11010111101	1110110101111	010011011110	000111101101

1 = presence of a band, 0 = absence of a band, 9 = missing data

Fig 24. Principal Coordinate Analysis of Fallopia sachalinensis



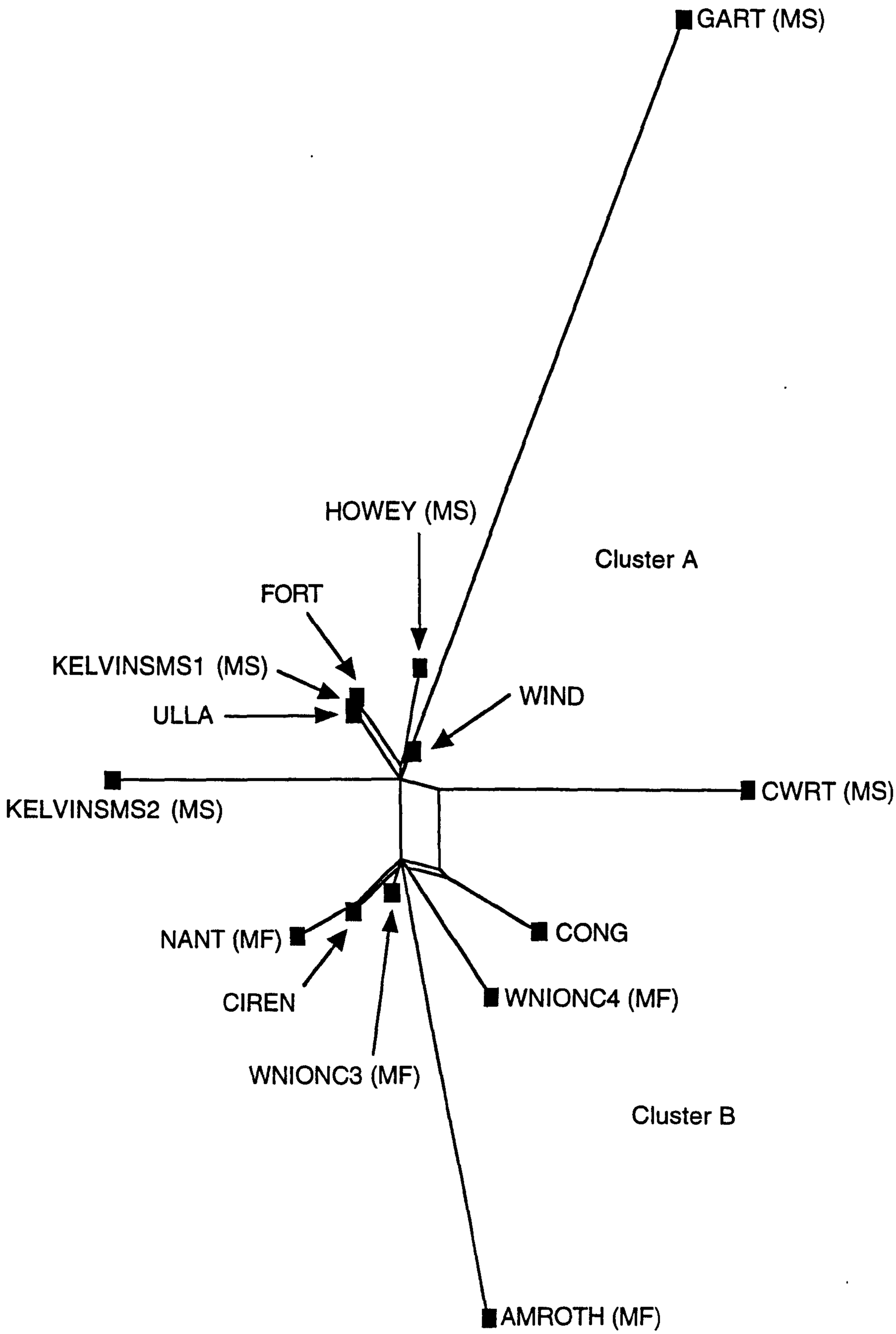
■ Fallopia sachalinensis Male Fertile

▲ Fallopia sachalinensis Male sterile

● Fallopia sachalinensis Sex Unknown

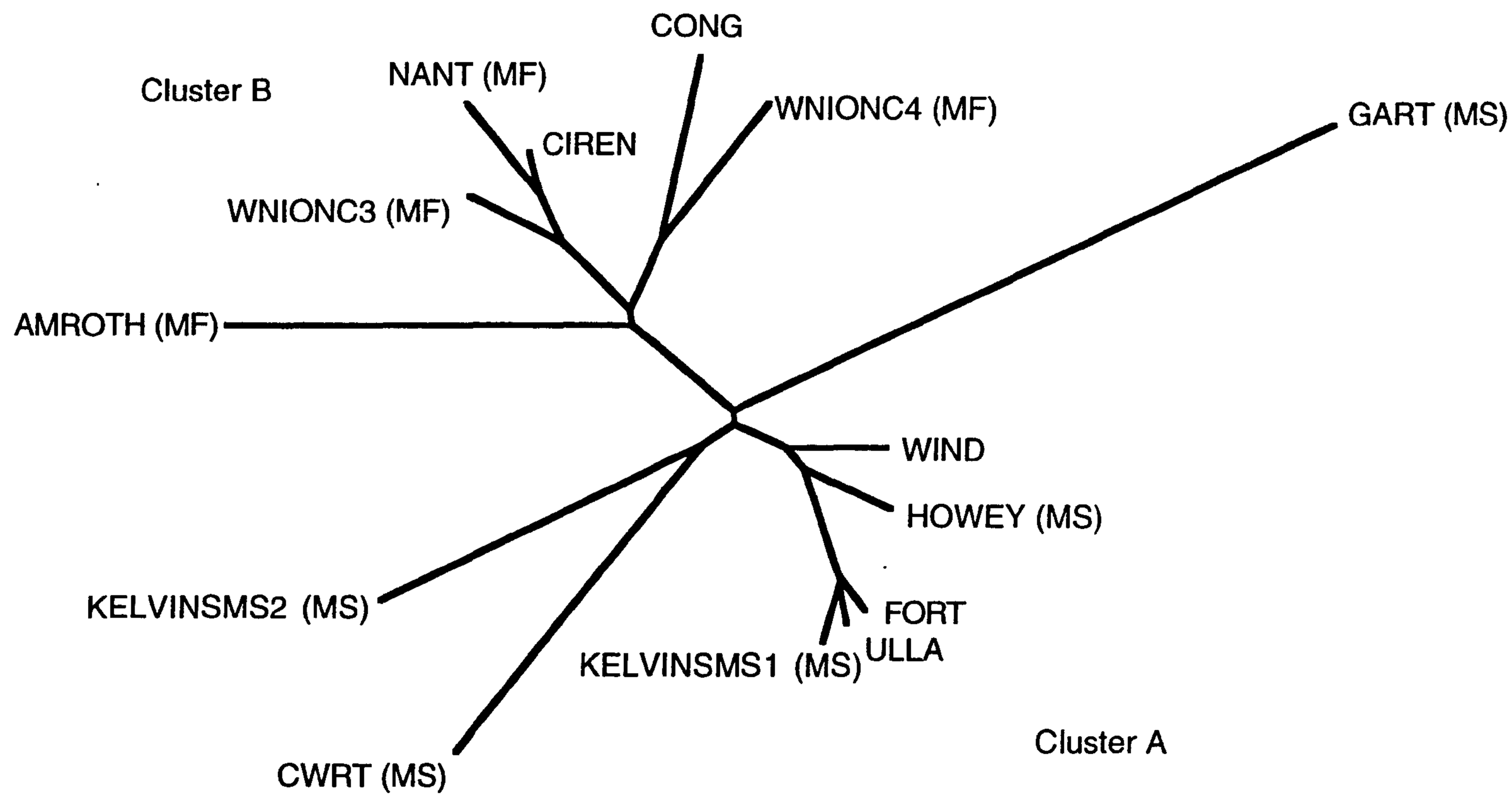
Principal coordinate	% of Variance
1	28.379
2	18.582
3	17.178

Fig. 25 Split decomposition graph of RAPD data f rom Fallopia sachalinensis from the British Isles



Fit 50.8 Sex (where known) is given in parentheses (MF = hermaphrodite, MS = male sterile)

Fig.26 Neighbour joining tree of RAPD data of Fallopia sachalinensis from the British Isles



Sex (where known) is given in parentheses
(MF = hermaphrodite, MS = male sterile

Legend Fig. 27

KEY

- ● Hoxne
- ✢ Wnion
- ■ Amroth
- ▲ Buckingham Palace
- ✻ Alloa
- ❙ Swansea
- ✚ Fowey
- ○ Kellas
- ✠ Lough Neagh
- ❄ Witley
- ★ Kilarchan
- ✖ Holmbury
- ▼ Horncliffe
- ♥ Kelvin
- ☆ Gartshore
- ➢ Maam

Principal coordinate	% of variance
1	8.917
2	7.534
3	6.478

Fig. 27 Principal coordinate graph of RAPD data of Fallopia x bohemica (F. japonica var. japonica x F. sachalinensis) from the British Isles

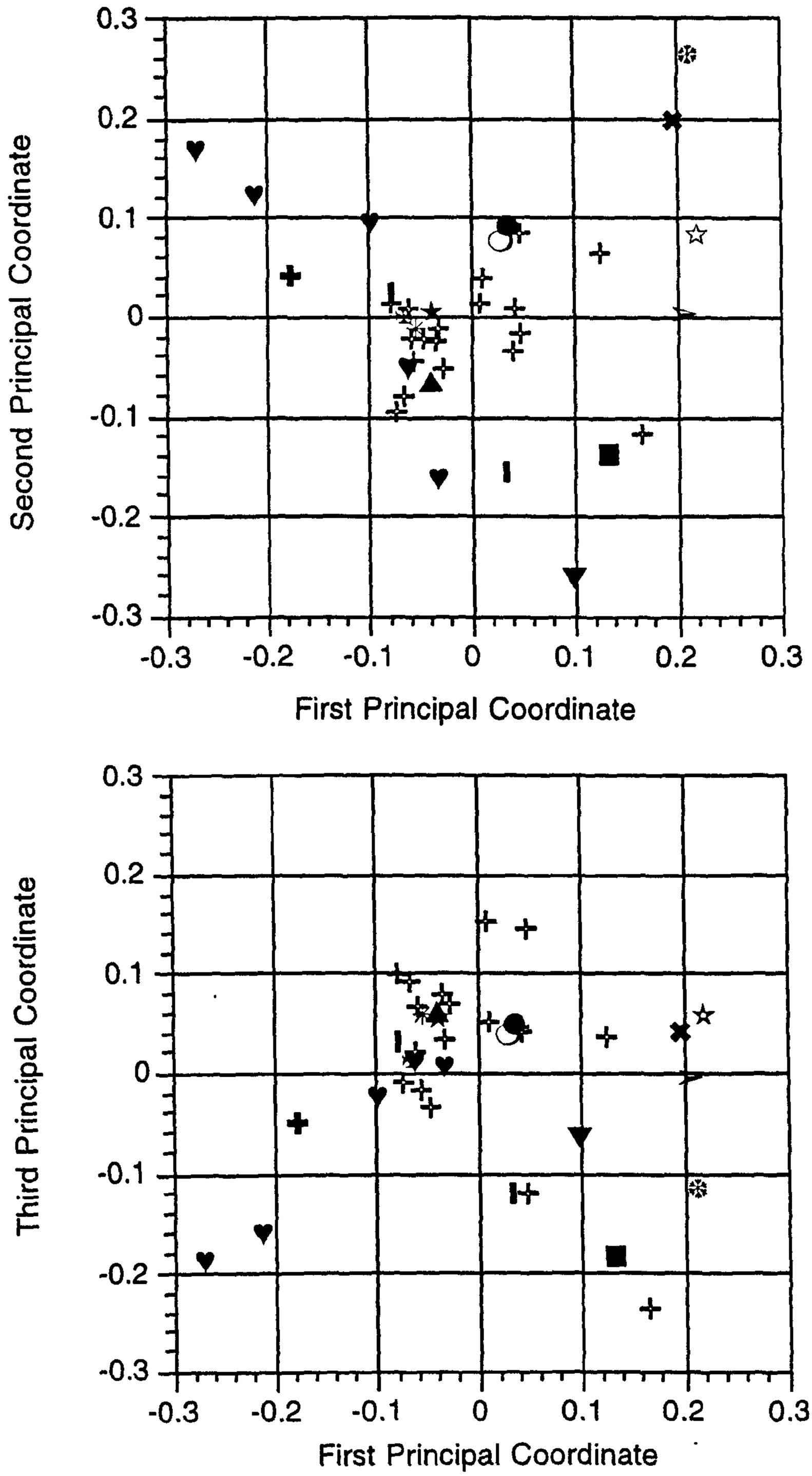


Fig. 28

Samples from the Wnion have been excluded from this plot in order for the distribution of the remaining samples to become clearer

KEY

- ● Hoxne
- ■ Amroth
- ▲ Buckingham Palace
- ✳ Alloa
- I Swansea
- ✚ Fowey
- ○ Kellas
- ✠ Lough Neagh
- ❄ Witley
- ★ Kilarchan
- ✖ Holmbury
- ▼ Horncliffe
- ♥ Kelvin
- ☆ Gartshore
- ➢ Maam

Fig. 28 Principal coordinate graph of RAPD data of Fallopia x bohemica (F. japonica var. japonica x F. sachalinensis) from the British Isles

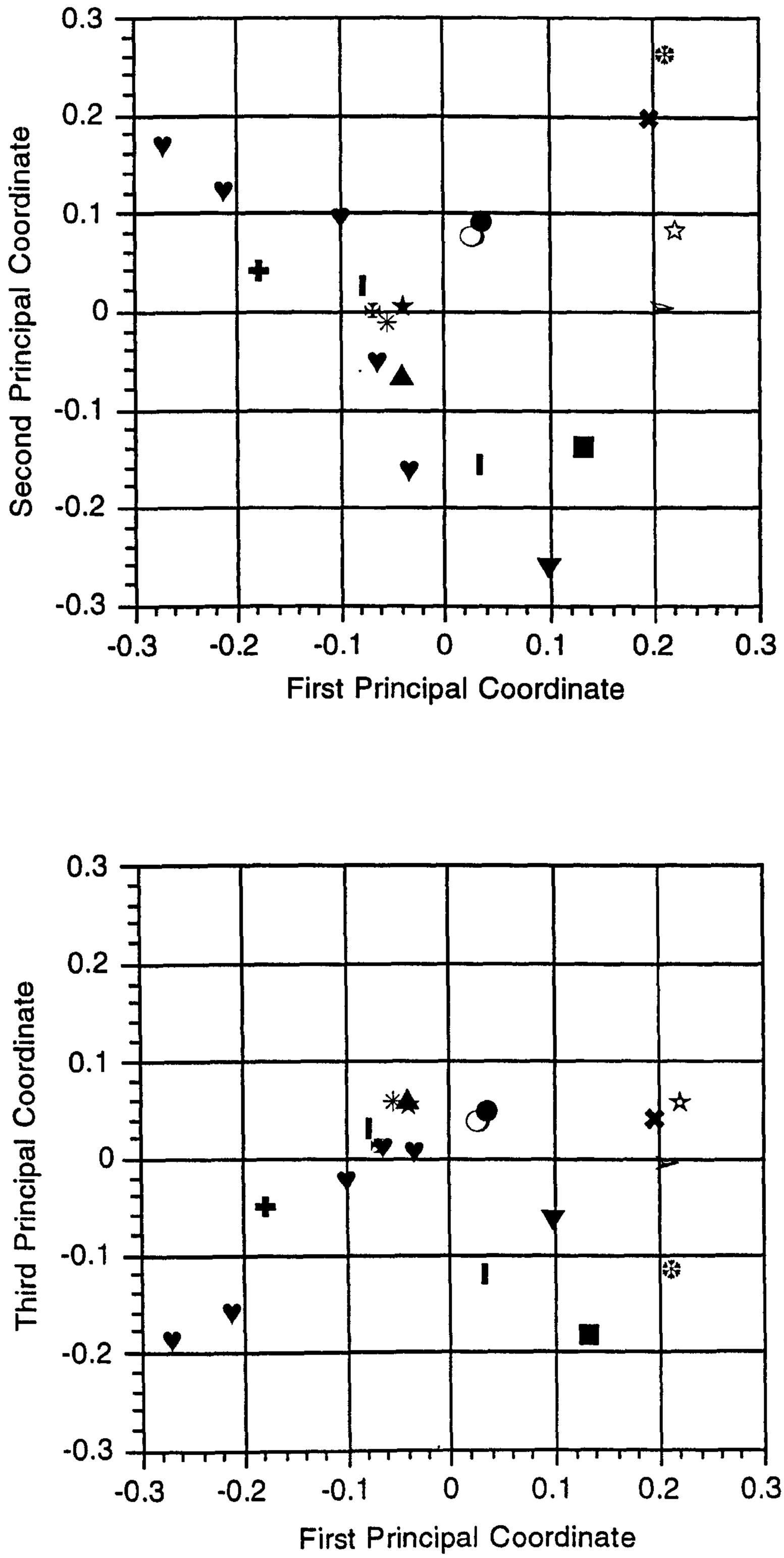


Fig. 29 Split decomposition graph of RAPD data of Fallopia x bohemica (F. japonica var. japonica x F. sachalinensis) from the British Isles

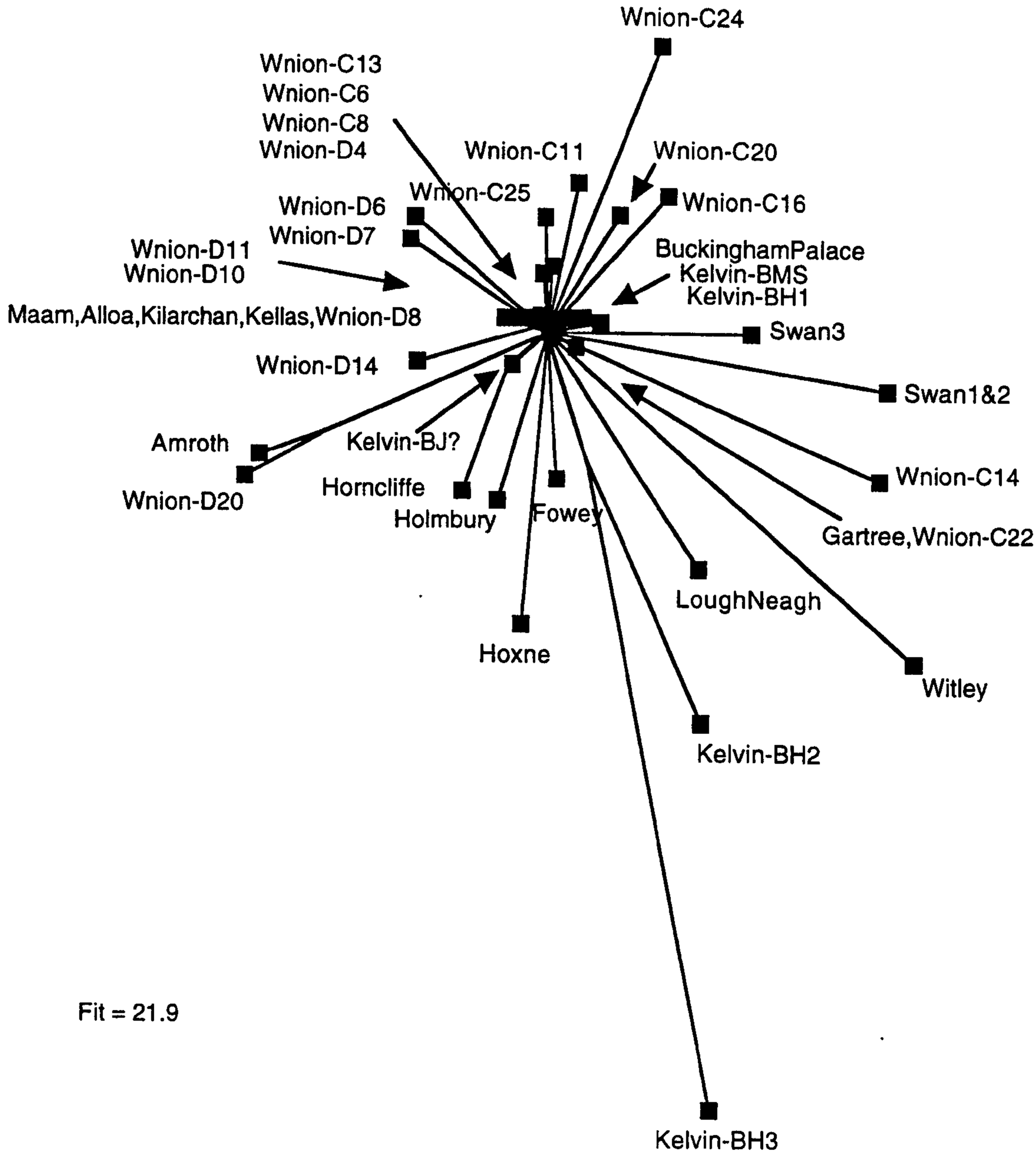
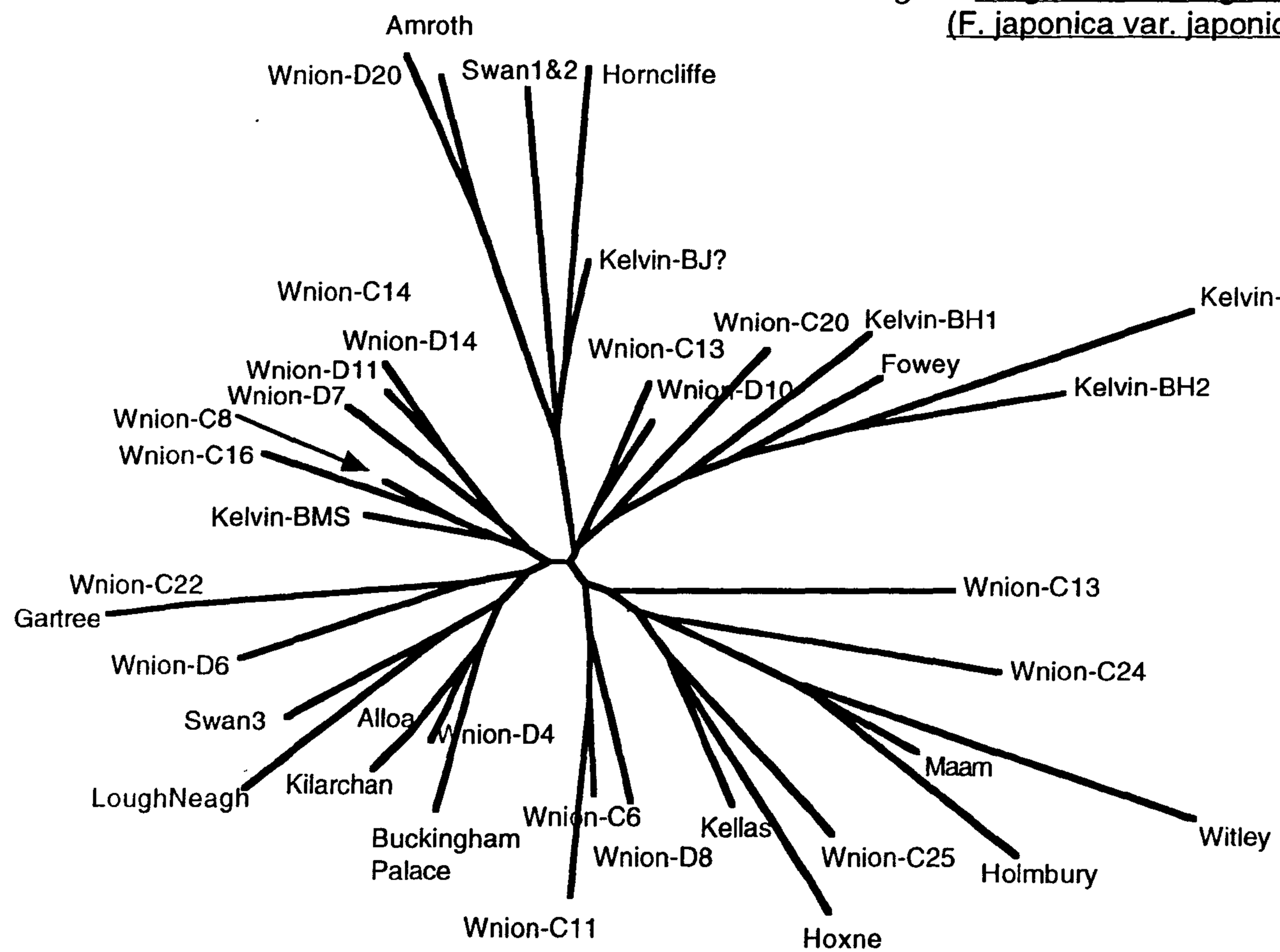


Fig. 30 Neighbour Joining tree of RAPD data of Fallopia x bohemica (F. japonica var. japonica x F. sachalinensis) from the British Isles



Discussion

The preceding results offer some interpretational challenges, due in part to the paucity of both samples, and data on their sex expression. Regardless, it seems some general points can be made. Most obviously, levels of genotypic diversity are high compared to those detected in British Fallopia japonica var. japonica (chapter 6). In F. x bohemica and F. sachalinensis, no genotypes have been detected at more than one locality. The only multiple representatives of genotypes are those detected at single sites (e.g. the River Wnion, the River Kelvin and Swansea). While this clearly points to a different mode of population structure to F. japonica var. japonica, there are two factors worthy of consideration before conclusions are made on the relative roles of sexual versus asexual reproduction in F. x bohemica and F. sachalinensis.

Firstly, due to time constraints and material availability, no repeat DNA extractions were carried out in the current study. Repeat PCRs were carried out on individual samples, but as stressed in chapter 6, care is required in relying solely on repeated amplification as evidence of results reliability (the age old distinction between accuracy and precision). Thus there is the possibility that the large number of genotypes detected is in part attributable to PCR artifacts. However, based on past experience, I believe that this is unlikely. In Fallopia japonica var. japonica only six out of 150 samples (4%) showed errant banding patterns. If this is extrapolated to the current study (which based on the identical procedures employed seems reasonable) one would predict that at most, a single sample would show an artifactual genotype. A second consideration, and perhaps of greater concern, is the degree to which our sampling represents the populations of these taxa in

Britain. In all cases where I have sampled multiple individuals from an area (F. sachalinensis = 2, F. x bohemica = 3), I have detected multiple genotypes. Thus the implication is that where I have only single samples from a site, all of the genetic diversity is not being represented. In practice the extent of this problem will vary from site to site. For some localities such as Cwrt Newyd, where only one clump of F. sachalinensis was present, it is likely that the sample is representative. In others, such as Fort William, where the stands of F. sachalinensis extend for hundreds of meters, there is a strong possibility that additional undetected genotypes are present. With this in mind, care is needed in distinguishing between the two ends of the hypothesis continuum raised in the introduction: namely extensive sexual reproduction versus widespread vegetative propagation and dispersal. The results of the current study are consistent with sexual reproduction being a significant factor in the population biology of these taxa. The extent to which individual clones occur at multiple sites requires further sampling. It may be that individual genets occur at multiple populations, and that our small samples have not detected them. Regardless, given the clear contrast with the clonal uniformity of the male sterile F. japonica var. japonica in Britain, it seems safe to conclude that sexual reproduction has been a significant determinant of population genetic structure in F. x bohemica and F. sachalinensis.

To gain further insights into the population biology of these taxa in Britain it is informative to consider them individually. Starting with Fallopia sachalinensis, the most notable result is the recovery of two main genetic clusters of the plants (Figs. 24, 25 & 26). The most striking correlation with these groupings and the plant's biology is sex expression. All of the male sterile plants (along with some unsexed plants) cluster together (cluster A) and all of the

hermaphrodite plants (along with some unsexed plants) cluster together (cluster B). It is tempting to speculate that some of the RAPDs bands are detecting sex linked markers. However, before reading too much into this, it is worth digressing momentarily to consider the underlying genetic base of gynodioecy.

The maintenance of male sterile plants in populations of hermaphrodites has been the subject of considerable debate dating back at least to the writings of Darwin (1877). The genetic control of gynodioecy can vary. In many cases, it appears to be a result of interactions between mitochondrial sterility genes and dominant or recessive nuclear restorer genes (Saumitou-Laprade et al., 1994; Richards, 1997). The maternally inherited mitochondrion presumably gains from increased resources allocation to female, over male function, in male sterile plants. The bi-parentally inherited nuclear genome, however, has conflicting requirements, and male-function-restorer-genes can serve to counteract the mitochondrial sterility genes. Alternatively, gynodioecy may be under purely nuclear control, although this appears to be less common as a stable polymorphism (Saumitou-Laprade et al., 1994; Richards, 1997).

In Fallopia the genetic control of gynodioecy may be the result of cytonuclear interactions or it may be that some nuclear genes simply produce an impaired male function. Although the genetic basis of Fallopia's sexual system is far from understood, it is worth making the following comments / observations. Firstly, selection pressures on sexual systems (such as female versus hermaphrodite plants) are presumably greatly reduced in rhizomatous perennials capable of vigorous clonal spread (compared to species where reproduction is entirely sexual). Secondly, introduced gynodioecious species

may behave differently in their introduced ranges compared to their native ranges. Even where gynodioecy is caused by cytonuclear conflict, founder effects can lead to only one mitochondrial haplotype being present in the introduced range. This effectively shifts the control of gynodioecy to the nuclear genome. The reciprocal of this is also possible, although perhaps less likely given the smaller effective population size for organelle versus nuclear genes. Thirdly, whatever the mechanism is that restores male function in Fallopia, it is not recessive. Hybrids between male sterile F. japonica var. japonica and male fertile F. sachalinensis can be either male fertile or male sterile. As male sterile F. japonica var. japonica is the maternal parent in all of these crosses, it seems that male fertility is dependant on the inheritance of a dominant, nuclear restorer gene or genes from F. sachalinensis.

So how does this help in interpreting the results of the apparent sexual correlation of the two genetic clusters in F. sachalinensis in Britain? Being blunt, it probably doesn't. Finding a convincing explanation for the clustering of the different genotypes that relates to sex is difficult. It requires that the markers relating to sex override all other relationships. It seems difficult to imagine that sufficiently large portions of the genome (or genomes) are involved in encoding sex expression to cause such clustering. In this respect it is noteworthy that there is no one marker band that is present in all individuals of one sexual type (Figs. 21 & 23).

An alternative explanation is that the sex correlation is a misleading distraction and that the two separate clusters contain the descendants of two different lineages, possibly stemming from two separate introductions. Under this hypothesis it is necessary to assume that the cluster containing the male

sterile plants also contains some male fertile plants (strong genetic clustering of multiple genotypes cannot easily be achieved by female plants alone). That some male fertile plants may be present in cluster A is not inconceivable. My assumption that this is a unisexual cluster is based on the fact that five of the eight genotypes are male sterile. I have no information on the sex of the other three genotypes. Whether these, or other genotypes of this cluster (as yet unsampled) are male fertile remains for now pure speculation.

In conclusion, for F. sachalinensis I have no clear understanding as to the biological phenomenon behind the two genetic clusters. The data is fully consistent with them being related to sex expression, but for the reasons outlined above, I find this is difficult to believe. The alternative explanation, of two genetically distinct introductions is also not entirely satisfactory, given the sole recovery of females in cluster A.

In Fallopia x bohemica there is considerably less structure in the data (Figs. 27, 28, 29 & 30). The split decomposition graph (Fig. 29) is effectively an unresolved polytomy. However, as split decomposition often struggles to provide resolved graphs for greater than 15 genotypes (Daniel Huson pers. comm. 1998), caution is required in making biological interpretations from this. Of more significance are the PCO plots and the neighbour joining tree. In the PCO analysis (Fig. 27), the first principal coordinate accounted for only 8.9% of the variance and the steady gradual tail off in values for subsequent principal coordinates suggests that much of what is being considered is noise. In the neighbour joining tree (Fig. 30) the internal branches are typically short compared to the terminal branches, indicating that the majority of the signal is differentiating individuals rather than supporting groupings. Although I have

little data on the sex expression of the plants in this study, there does not appear to be any clear groupings relating to sex expression.

Some associations of genotypes make some sense, such as the co-occurrence of the three hermaphrodite genotypes from the River Kelvin (most clearly seen on the neighbour joining tree). However, the overwhelming picture is of a lack of geographical structure. Even where the three hermaphrodite genotypes from the River Kelvin cluster together, they do so with a sample from Fowey in Cornwall. This lack of structure is perhaps exemplified by the strongly differentiated genotypes Wnion C20 & C22. These two genotypes are from seeds collected within a few meters of each other, and yet they are remarkably separate on both the neighbour joining tree and the PCO plots (Figs. 27 & 30). Likewise, the other pair of seedlings from the River Wnion population that were collected together (genotypes Wnion C24 & C25) also fail to form a discrete cluster, although their separation is less marked than the preceding pair (Fig. 30). The failure of these seedlings to cluster together is interesting, as they have almost certainly not been introduced to the sites where they were collected and are a reflection of the local genetic diversity. Although I cannot unambiguously distinguish between these plants being F1 hybrids or being of more complex origin, it is clear that there are high levels of genetic heterogeneity within this site. This potential for highly divergent genotypes to be thrown up within individual sites inevitably causes problems for the interpretation of the population structure of this taxon in Britain. Different genotypes can result from multiple crosses between the same two heterozygote genotypes, between different genotypes and from later generation hybrids. Disentangling the relative roles of each of these is not possible without detailed examination of controlled crosses and their

progenies. Furthermore, and potentially untestable, is the extent to which material of these taxa was originally distributed as seed for horticultural purposes. Although the evidence presented here for sexual reproduction within populations is virtually conclusive, it remains that some of the variability in this taxon in Britain could stem from horticulturally raised and / or distributed seed.

Chapter 8

Inter-relationships of introduced and native material of invasive Japanese Knotweeds

Abstract

Chloroplast DNA sequences of the tmL intron and RAPD data have been used to estimate the genealogical relationships of invasive genotypes of Fallopia japonica var. japonica, var. compacta and F. sachalinensis with material from their native ranges. Parsimony analysis of tmL sequences using F. baldschuanica, F. convolvulus, F. scandens var. dentatoalatum and F. multiflora as outgroups, provided strong support for the monophyly of section Reynoutria. Little resolution was obtained within section Reynoutria, however, due to low levels of sequence divergence and high levels of homoplasy. RAPD analysis of F. japonica s.l. showed that introduced populations of F. japonica var. japonica are more similar to introduced populations of F. japonica var. compacta than to any of the native genotypes sampled. No evidence pinpointing the source of the introduced invasive genotypes was found, and the need for a basic alpha taxonomic revision of section Reynoutria is clear.

Introduction

This thesis discusses the population structure and the interactions of three Fallopia Adans. taxa, namely F. japonica (Houtt.) Ronse Decraene var. japonica, F. sachalinensis (F. Schmidt ex Maxim.) Ronse Decraene and their hybrid, F. x bohemica (Chrtek & Chrtková) J. Bailey. An assessment of the genetic variability of these taxa using arbitrary fingerprinting techniques demonstrates that they perpetuate by either vegetative and/or sexual reproduction. No genetic variation was detected in 150 samples of F. japonica var. japonica collected from across the British Isles, consistent with this plant being represented by a single female clone with all reproduction being asexual (chapter 6). Asexual reproduction via vegetative propagation is also important for F. sachalinensis and F . x bohemica. Population studies show that F. sachalinensis and F . x bohemica form large clonal stands (chapters 3 & 4). However, in contrast to F. japonica var. japonica, genetic variability has been detected both within and between populations (chapters 3, 4 & 7). The distribution of this molecular variability among individuals has allowed some inference to be made on the dynamics of hybridisation and reproduction. This information has greatly enhanced our understanding of the evolutionary biology of these plants in the British Isles. Conspicuously absent, however, is reference to native material.

It is clearly desirable to place the results of the current study in the context of section Reynoutria (Houtt.) Ronse Decraene as a whole. Herein lies one of the classical problems associated with the interpretation of invasive species research. Often the research into a species in its introduced range where it is problematical, is far more intense and focused than in its native range where it may be benign. This is particularly evident when the introduced and native sites are found in the first and third world respectively. There is a danger of gross misinterpretation of comparisons of genetic variability in introduced

versus native populations if the taxonomic treatments are not standardised. Alluded to in chapter 6 was the problem of relating British taxa, particularly Fallopia japonica var. japonica, to native material.

Whilst the morphological and cytological variation in British Fallopia taxa has received considerable attention, the variability in the native range remains largely unexplored. Samples of F. japonica s.l. collected from Japan and China show a high degree of cytological and morphological variability (Bailey, 1997). Material of F. japonica var. japonica found in the British Isles today, although morphologically more similar to Japanese than to Chinese material, is not typical of either (Bailey, 1997). Japanese F. japonica var. japonica are predominantly tetraploids (2n=44), whilst Chinese and Korean plants are octoploid or decaploid (2n=88 & 2n=110). British material of F. japonica var. japonica is octoploid (2n=88).

Clearly a detailed knowledge of the variability of these plants in their native range is desirable. Introduced populations of plants can provide useful model systems for evolutionary biologists, as long term historical factors can effectively be eliminated as determinants of present day patterns. However, an understanding of the biology of invasive weeds in their natural habitat is often required to place these results in context. In addition, studies of native populations of introduced species can provide the opportunity of pinpointing the geographic source of the introduced material.

In this final chapter I describe the genealogical relationships among all the native material I could obtain, paying particular attention to F. japonica var. japonica. It must be stressed that the goal was not to produce representative species phylogenies, merely to estimate the relationships among these

samples and the British taxa. The wide diversity and limited number of native samples that I have managed to obtain precludes any wider conclusions.

Before describing the methodological approach and discussing the implications of the data, it is worth briefly considering some of the background issues to the estimation of these relationships. Implicit in the evolutionary study of relationships is the search for natural groupings, thereby finding entities that are more closely related with other, than to other entities. This search for hierarchical groupings has been a preoccupation for systematists throughout the history of evolutionary classification. Such hierarchical approaches have varied from informal simplistic observations based on crude similarities to intensive studies aimed at categorising diversity based on rigorous scientific methodology. Few biologists would question that a hierarchical classification is desirable and that an understanding of evolutionary patterns is important. How this should be achieved, however, is the subject of much heated and often acrimonious debate.

At the forefront of the argument over the use of numerical analytical methods for determining relationships has been the phenetics versus cladistics debate. Pheneticists argue that overall similarity is the most appropriate method of grouping taxa (e.g. Sneath & Sokal, 1973). Cladists, in contrast, seek to base relationships explicitly using shared derived characters (e.g. Hennig, 1966; Swofford et al., 1996). The relative merits of the two approaches have been widely discussed in the literature. Although the underlying philosophical approach of the cladistic methodology has received wide support and seems conceptually sound, it is important to consider that it is explicitly aimed at producing natural hierarchical groupings. Approaches based on overall similarity, however, are applicable where relationships are reticulate (most population genetic statistics are essentially phenetic). When relationships are

divergent, phenetics and cladistics differ primarily in the treatment of pleisiomorphic character states, uninformative in cladistic, but not phenetic methodologies.

Despite these differences, there are a number of problems common to both cladistic and phenetic approaches.

1. Firstly can homologous characters be identified? This apparently simple requirement is a stumbling block of many studies. Although some superficially similar characters are clearly not homologous, such as the leaves of a bryophyte and the leaves of an angiosperm (Stace, 1989), in many cases it can be difficult to determine character homology. This is as true for molecular data as morphological data. Co-migrating products of equal molecular weight in arbitrary fingerprinting studies may be non-homologous (Rieseberg, 1996) and gene duplications and sequence alignment problems can lead to incorrect homology assessments even when direct comparisons of sequences are made (Doyle & Doyle, in press).

2. A second problem lies in the interpretation of character states. Identity in state does not necessarily equate with homology (identity by descent) and recurrent origins of the same phenotype (and even genotype) are possible. In molecular data, repeated mutations at single sites giving rise to identity in state are likely, given that only four possible character states exist (Li, 1997). Furthermore, when the studies involve closely related taxa, mutational hotspots such as simple sequence repeats are often targeted for analyses. The recurrent homoplastic origins of allelic variants of a given length are inevitable when studying simple sequence motifs. The classic example of this is the vast majority of published studies that have used chloroplast microsatellites, where studies of linkage disequilibrium shows that homoplasy

is not just present but is rife throughout the data (P. M. Hollingsworth, unpublished data).

3. A third main area for concern is whether the history of the characters reflect the history of the biological entity that houses them. This is often referred to as the gene tree/species tree problem and it is well known that hybridisation and lineage sorting can result in seriously misleading estimates of relationships (Doyle, 1997; Maddison, 1997; Page & Charleston, 1997). Using a phylogenetic tree constructed from a single gene to infer the phylogenetic relationships of a group of taxa can be likened to using a phylogeny of parasites to estimate the phylogenetic relationships of their hosts. There are many cases where they will agree and there are many cases where they will not.

4. Finally, following on naturally from the above, the assumption that the relationships being estimated are tree-like is of course dependent on the biology of the study samples. Although saltation undoubtedly occurs, all evolution does not leap straight from panmixia to hierarchical divergence and the interface of population genetics and phylogenetics contains a grey area where choosing how best to analyse data can be difficult (Davis, 1996; Doyle, 1995).

The above concerns raise a host of questions regarding both character choice and method of data analysis for a given problem. Approaches to study relationships at one taxonomic level, are often inappropriate at another. Furthermore, given the inequality of taxonomic ranks across groups, generalisations about appropriate approaches at particular taxonomic levels are often difficult.

In the current study I have obtained data from both the nuclear and the chloroplast genomes. For chloroplast DNA, I have chosen sequencing as the method of data gathering. DNA sequences are well suited to phylogenetic analysis, providing a gene evolving at an appropriate rate can be found. The beauty of chloroplast DNA is that it is uniparentally inherited and non-recombinant (Clegg & Zurawski, 1992). In theory at least, there is only one true phylogeny for the molecule (providing the study group is monophyletic). Whether it is recoverable or not (due to homoplasy) and whether it reflects the organismal phylogeny is of course another issue. The nuclear genome, in contrast, is more complicated as recombination can result in a myriad histories for any one gene (Doyle, 1995). I have thus taken a different approach for the nuclear genome, and instead of concentrating on detailed information from a single gene, have sampled coarsely many areas of the genome using arbitrary fingerprinting.

The application of these markers in the current study have been at somewhat different hierarchical levels. To illustrate broad relationships I have used chloroplast sequence data analysed using cladistic methodology. At lower taxonomic levels where chloroplast DNA divergence is low and reticulation is likely, I have used RAPDs to study relationships among samples of F. japonica s.l., and analysed the data based on overall similarities.

Materials and Methods

Nomenclature

The nomenclature and taxonomy of section Reynoutria are confused. The change of genus from Polygonum L. to Reynoutria to Fallopia (Bailey, 1989) coupled with different treatments of intra-specific variation by different authors, and different use of synonyms in different floras, has resulted in the existence of a rather chaotic assemblage of names. As many of the combinations of varieties of F. japonica have not been formally published, the application of their names is difficult. For the introduced taxa, the nomenclature in this chapter (as for the rest of the thesis) follows Stace (1997). For the native species, I have adopted a pragmatic approach. Those taxa that have only been named under the now redundant generic name Reynoutria are transferred here for convenience to Fallopia. Their authorities under the genus Reynoutria are given in Table 15. As there is some question as to whether F. japonica var. japonica in Europe and North America is the same taxon as material found so far in the native range (Bailey, 1997), in this chapter, the native material is refered to as F. japonica var. 'japonica' to distinguish it from the introduced F. japonica var. japonica.

Sequence data of the tmL intron was obtained from five samples of Fallopia japonica var. 'japonica' (three from Japan and two from China); single samples each of F. japonica var. terminalis (Japan), F. japonica var. uzenensis (Japan), F. japonica var. elata (Japan) and F. elliptica (Korea). Sequences were also obtained from single representative samples of introduced F. japonica var japonica (Britain), F. japonica var. compacta (Britain) and F. sachalinensis (Britain) (chapter 5), and from a single sample of F. sachalinenesis from Korea. In addition, single samples of F.

baldschuanica (Regel) Holub, F. convolvulus (L.) Löve, F. scandens L. and F. multiflora (Thunb.) Haraldson were obtained to serve as outgoups.

RAPD analysis was carried out on 20 samples of F. japonica s.l., collected from both native and introduced regions. Ten of these samples were included in the cpDNA analysis.

Collection details of all samples and nomenclatural notes are given in Table 15.

DNA extraction and RAPD analysis

Extraction of genomic DNA and RAPD analysis was carried out as described in chapter 3.

Sequence analysis

PCR amplification of the tRNA ^{LEU1} intron was performed using the universal chloroplast primers described by Taberlet et al. (1991).

50µl PCRs were performed containing ~ 0.1µg genomic DNA, 200µM of each dATP, dCTP, dGTP, dTTP, 0.3 µM of each primer, 2 Units Taq polymerase, 2.5mM $MgCl_2$ and 5µl reaction buffer. PCR was performed using the following programme: 1 cycle of 94°C for 4 min followed by 30 cycles of 30 s at 94°C, 30s at 55°C, and 1 min at 72°C and finally 1 cycle at 72°C for 10 min.

Double stranded PCR products were purified using Qiagen columns and sequenced as double stranded template. Sequencing was performed using the dideoxy-chain termination method with a fluorescent primer and the T7 sequencing kit on an Automated Laser Fluorescence sequencing machine

Table 15 Collection details of Fallopia taxa

Accession No. and Taxon	Locality	RAPDs	Chloroplast Data	Chromosome No.
Section Reynoutria (Houtt.) Ronse Decraene				
P105 F. japonica var. uzenensis 1	Nasu Mountain, Japan	Y	Y	2n=88
P113 F. japonica var. 'japonica' 2	Peking Botanic Gardens, China	Y	Y	2n=88
P114 F. japonica var. 'japonica' 2	Tokyo, Japan	N	Y	2n=44
P134 F. japonica var. terminalis 3	Hachijo Islands, Japan	N	Y	2n=44
P166 F. japonica var. elata 4	Japan	Y	Y	
P583 F. japonica var. 'japonica' 2	China	Y	Y	2n=110
P584 F. japonica var. 'japonica' 2	China	Y	N	2n=110
P594 F. japonica var. 'japonica' 2	Honshu Mt, Japan	Y	N	2n=44
P595 F. japonica var. 'japonica' 2	Japan	Y	N	
P673 F. japonica var. 'japonica' 2	Fujija, Japan	Y	N	2n=44
P726 F. japonica var. 'japonica' 2	Mt Tateyama, Midagahara, Japan	Y	Y	2n=44
P727 F. japonica var. 'japonica' 2	Mt Tateyama, Midagahara, Japan	Y	N	
P728 F. japonica var. 'japonica' 2	Mt Tateyama, Midagahara, Japan	Y	N	
P730 F. japonica var. 'japonica' 2	Shinjuku, Tokyo, Japan	Y	N	
P732 F. japonica var. 'japonica' 2	Shinjuku, Tokyo, Japan	Y	Y	
P733 F. japonica var. 'japonica' 2	Tanagawa River, Tokyo, Japan	Y	N	

Table 15`cont.

Accession No. and Taxon	Locality	RAPDs	Chloroplast Data	Chromosome No.
Section Reynoutria (Houtt.) Ronse Decraene				
P734 F. japonica var. 'japonica' 2	Tanagawa River, Tokyo, Japan	Y	N	2n=44
P012 F. japonica var. japonica 5	Leicester, England	Y	Y	2n=88
P002 F. japonica var. compacta 6	Brecon, Wales	Y	Y	2n=44
P174 F. japonica var. compacta 6	Bracken Hill, Surrey, England	Y	Y	2n=44
P193 F. japonica var. compacta 6	Heads of Valley, Wales	Y	Y	2n=44
P413 F. japonica var. compacta 6	Blarney Castle, Ireland	Y	N	2n=44
P057 F. sachalinensis 7	Howey, Wales	N	Y	2n=44
P476 F. sachalinensis 7	Korea	N	Y	2n=102
P555 F. elliptica 8	Korea	N	Y	2n=88
Section Fallopia Adans.				
P729 F. scandens var. dentatoalatum 9	Korea	N	Y	
F. convolvulus 10	Britain	N	Y	2n=40
Section Sarmentosae (Grintz) Holub				
F. baldschuanica 11	Cultivated origin	N	Y	2n=20
F. multiflora 12	China	N	Y	2n=22

Table 15. Legend

The number following each taxon name in Table 15 refers to the authorities described below. For taxa in the British flora, the nomenclature follows Stace (1997). For taxa not described in that work (Asian material that has not been introduced to the British Isles), I have given the equivalent designations under Reynoutria, the genus in which these plants are most commonly placed in the majority of the Asian Floras.

1 F. japonica var. uzenensis (=Reynoutria japonica var. uzenensis Honda)

2 F. japonica var. 'japonica' (=Reynoutria japonica Houtt.),

3 F. japonica var. terminalis (=Reynoutria japonica var. terminalis Honda)

4 F. japonica var. elata (=Reynoutria japonica var. elata Hiyama)

5 F. japonica (Houtt.) Ronse Decraene var. japonica

6 F. japonica var. compacta (Hook. f.) J. P. Bailey

7 F. sachalinensis (F. Schmidt ex Maxim.) Ronse Decraene

8 F. japonica var. eliptica (=Reynoutria elliptica (Koidzumi) Migo)

9 F. scandens var. dentatoalatum (=Reynoutria scandens var. dentatoalatum (F. Schmidt) Maxim.)

10 F. sachalinensis (F. Schmidt ex Maxim.) Ronse Decraene

11 F. conolvulus (L.) Á Löve

12 F. baldschuanica (Regel) Holub

Data analysis

Sequence data were edited using the computer software Sequence Navigator™ and aligned using the Megalign™ program, with manual modification to minimise the number of gaps. A gap matrix was constructed by scoring indels (insertion deletion events) that were homologous in length and position as single binary presence or absence characters. Indels with jagged edges were broken down to separate characters to 'straighten' the edges. This has the undesirable effect of weighting long indels, as single evolutionary events (insertion or deletion) may be scored as several characters. In addition, the homology of overlapping indels is open to question. Although the method of scoring indels described above is far from ideal, it remains that there is no objective way of dealing with jagged indels. After scoring, the gap matrix was added to the sequence matrix. Separate analyses were carried out for sequence data alone and sequence data + indels.

Phylogenetic relationships among samples were estimated under the parsimony criterion using PAUP* (D. L. Swofford, unpublished). The taxa specified for the outgroup were Fallopia multiflora, F. baldschuanica, F. scandens var. dentatoalatum and F. convolvulus. The search strategy was heuristic, based on 1000 replicates of random sequence addition, with tree bisection -reconnection (TBR) branch swapping under the Fitch criterion (unordered states and equal weights) (Fitch, 1971). Strict and semi strict consensus trees were calculated to summarise nodes that did not conflict in alternative trees (Swofford, 1993). Confidence was assessed using 1000 'fast bootstrap' replicates (Felsenstein, 1985).

The relationships among samples based on the RAPD data were estimated using split decomposition, principal coordinate analysis and neighbour joining as described in chapters 3 & 4.

Results

cpDNA analysis

Sequence analysis of the tmL intron detected 13 haplotypes from 17 samples. The size of the intron varies between 535bp in Fallopia convolvulus to 597bp in F. japonica var. 'japonica' (accession P114) (see Table 16).The complete nucleotide sequences of the tmL intron for all the Fallopia taxa studied are given in Fig. 31.

Parsimony analysis of the sequence data (with indels treated as missing data), using Fallopia multiflora, F. baldschuanica, F. scandens var. dentatoalatum and F. convolvulus as the outgroup resulted in six shortest trees, with a tree length of 45 (Fig. 32). The ingroup is monophyletic with respect to the outgroup and eight site mutations characterise section Reynoutria. The strict and the semi strict consensus trees are identical, but resolution is poor (Fig. 33a). F. japonica var. uzenensis (P105) and F. japonica var. elata (P166) have identical sequences and are grouped together on the basis of a single synapomorphic change (G-T) at base pair (bp) 202. F. japonica var. 'japonica' (P113) and F. elliptica (P555) also have identical sequences and share one synapomorphic change (A-C) at bp 266. These are the only groupings evident within section Reynoutria. The two F. sachalinensis samples and F. japonica var. terminalis have identical haplotypes, although this similarity is apparently pleisiomorphic. All other sequence changes are autapomorphic, with F. japonica var. 'japonica' from Mount Tateyama having two autapomorphic mutations (A-T at bp 358, A-C at bp 487) and F. japonica var. 'japonica' from Shinjuku also having two autapomorphic mutations (T-A at bp 323, G-T at bp 348).

Greater resolution is obtained when indels are considered. When the gap matrix is included, the number of haplotypes detected within section

Reynoutria rises from five to nine from the 13 different samples. Parsimony analysis recovered ten equally most parsimonious trees of a length of 60 steps (Fig. 34). The groupings from the sequence data alone were maintained. In addition, in the semi strict consensus tree, the indel data supported a clade containing Fallopia japonica var. compacta as sister to a Chinese F. japonica var. 'japonica' (P583) and a Japanese F. japonica var. 'japonica' (P114) (Fig. 35a). In addition, the British F. japonica var. japonica joined the 'F. japonica var. elata - F. japonica var. uzenensis' clade. In the strict consensus, however, and the bootstrap consensus, this grouping was not robust enough to persist (Fig. 35 a &b).

Table 16. Chloroplast haplotypes and size of the trnL intron in Fallopia taxa

Accession No and Taxon	Locality	Chloroplast haplotype	TmL intron size (base pairs)
P105 F. japonica var. uzenensis	Nasu Mountain, Japan	tmLA	577
P113 F. japonica var. 'japonica'	Peking Botanic Gardens, China	trnL B	581
P114 F. japonica var. 'japonica'	Tokyo, Japan	tmL C	597
P134 F. japonica var. terminalis	Hachijo Islands, Japan	tmL D	579
P166 F. japonica var. elata	Japan	trnL A	577
P584 F. japonica var. 'japonica'	China	trnL E	588
P726 F. japonica var. 'japonica'	Mt Tateyama, Midagahara, Japan	trnL F	570
P732 F. japonica var. 'japonica'	Shinjuku, Tokyo, Japan	trnL G	579
P012 F. japonica var. japonica	Leicester, England	tmL H	577
P174 F. japonica var. compacta	Bracken Hill, Surrey, England	trnL I	580
P057 F. sachalinensis	Howey, Wales	trnL D	579
P476 F. sachalinensis	Korea	trnL D	579
P555 F. elliptica	Korea	trnL B	581
P729 F. scandens var. dentatoalatum	Korea	trnL I	545
F. baldschuanica	Britain	trnL J	537
F. convolvulus	Britain	trnL K	535
F. multiflora	China	tmL L	567

Fig. 31 Sequence data of the trnL intron of Fallopia taxa

```
                10         20         30         40         50         60         70         80         90
P114      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P113      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P583      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P555      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P134      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P726      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P732      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P057      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P476      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P174      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P166      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P105      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
P012      AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
F conv    AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAA-TGGG CAATCCTGAG
F dent    AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAA-TGGG CAATCCTGAG
F bald    AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
F mult    AATTGGTTTG AGCCTTAGTA TGGAAACCTA CTAAGTGAGA ACTTTCAAAT TCAGAGAAAC CCTGGAATTA AAAAAATGGG CAATCCTGAG
```

Fig. 31 cont.

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                100        110        120        130        140        150        160        170        180
P114      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P113      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P583      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P555      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P134      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P726      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P732      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P057      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P476      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P174      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P166      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P105      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
P012      CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGCTG TTCTAACAAA TGGAGTTGAT GGTCTTGCGT
F conv    CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGTTG TTCTAACAAA TGGAGTTGAC GGTCTTGCGT
F dent    CCAACTCCTT CTTTCCAAAA GGAAGACAAA AGGATAGGTG CAGAGACTCA ATGGAAGTTG TTCTAACAAA TGGAGTTGAC GGTCTTGCGT
F bald    CCAACTCCTT CTTTCCAAAA GGAAGACAAA AGGATAGGTG CAGAGACTCA ATGGAAGTTG TTCTAACAAA TGGAGTTGAC GGTCTTGCGT
F mult    CCAACTCCTT CTTTCCAAAA GGAAGAAAAA GGGATAGGTG CAGAGACTCA ATGGAAGTTG TTCTAACAAA TGGAGTTGAC GGTCTTGCGT
```

Fig. 31 cont.

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                190        200        210        220        230        240        250        260        270
P114      TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AAATATA
P113      TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AACTATA
P583      TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AAATATA
P555      TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AACTATA
P134      TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AAATATA
P726      TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AAATATA
P732      TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AAATATA
P057      TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AAATATA
P476      TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AAATATA
P174      TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AAATATA
P166      TGTTATAAGA ATTCTTCCAT CTAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AAATATA
P105      TGTTATAAGA ATTCTTCCAT CTAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AAATATA
P012      TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- ---------- ---AAATATA
F conv    TGTTATAAGA ATTCTTCCAT CGAAACTTCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- --------AA ATTAAATATA
F dent    TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- --------AA CTTAAATATA
F bald    TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAAGGATGA AGAATAAACC TAGACGCATA CGTACTT--- --------TA ATTTTATATA
F mult    TGTTATAAGA ATTCTTCCAT CGAAACTCCA AAAGGGATGA AGAATAAATC TAGACGCATA CGTACTTAAA TGAAATTTAA ATTAAATATA
```

Fig. 31 cont.

```
                 280        290        300        310        320        330        340        350        360
P114     TATATAACTT AAATATATAT ATAATACAAT ACTTTATTTA -----TATAT TATATATATA AAA------T ATATAAAGAA AAATTTTATA
P113     TATATA---- ---------- -TAATACAAT ACTTTATTTA -----TATAT TATATATATA AA-------T ATATAAAGAA AAATTTTATA
P583     TATATA---- ---------- -TAATACAAT ACTTTATTTA -----TATAT TATATATATA AAATATAAAT ATATAAAGAA AAATTTTATA
P555     TATATA---- ---------- -TAATACAAT ACTTTATTTA -----TATAT TATATATATA AA-------T ATATAAAGAA AAATTTTATA
P134     TATA------ ---------- -TAATACAAT ACTTTATTTA -----TATAT TATATATATA AA-------T ATATAAAGAA AAATTTTATA
P726     TATA------ ---------- -TAATACAAT ACTTTATTTA -----TATA- TATATATATA AA-------- -------GAA AAATTTTTTA
P732     TATA------ ---------- -TAATACAAT ACTTTATTTA -----TATAT TAAATATATA AA-------T ATATAAATAA AAATTTTATA
P057     TATA------ ---------- -TAATACAAT ACTTTATTTA -----TATAT TATATATATA AA-------T ATATAAAGAA AAATTTTATA
P476     TATA------ ---------- -TAATACAAT ACTTTATTTA -----TATAT TATATATATA AA-------T ATATAAAGAA AAATTTTATA
p174     TATA------ ---------- -TAATACAAT ACTTTATTTA -----TATAT TATATATATA AAA------T ATATAAAGAA AAATTTTATA
P166     TA-------- ---------- -TAATACAAT ACTTTATTTA -----TATAT TATATATATA AA-------T ATATAAAGAA AAATTTTATA
P105     TA-------- ---------- -TAATACAAT ACTTTATTTA -----TATAT TATATATATA AA-------T ATATAAAGAA AAATTTTATA
P012     TA-------- ---------- -TAATACAAT ACTTTATTTA -----TATAT TATATATATA AA-------T ATATAAAGAA AAATTTTATA
F conv   AA-------- ---------- -TAATAC--- --TCTATTTA -----TATAA AAT------- ---------- ---TAAACTA AAATTTT---
F dent   AA-------- ---------- -TAATAC--- --TCTATTTA -----TATAA AAT------- ---------- ---TAAACTA AAATTTT---
F bald   AA-------- ---------- -TAATAC--- --TCTATTTA -----TATAA AAT------- ---------- ---TAAACTA AAATATT---
F mult   AA-------- ---------- -TAATAC--- --TCTATTTA ATTTATATAA ---------- ---------- ---------- ----TTTATA
```

Fig. 31 cont.

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                370        380        390        400        410        420        430        440        450
P114     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P113     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P583     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P555     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P134     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P726     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P732     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P057     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P476     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P174     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P166     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P105     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
P012     TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
Fconv    ---------- --TAGAATAT TCAAATTAAA TGAAAAA-TA TGGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
Fdent    --TTAAAATT TTTAGAATAT TAAAAT-AAA TAAAAAAATA TGGATGACGA CCCGAATCTT TATTTTATGA TTATGAAAAA GTGGAAGAAT
Fbald    ---------- --TAGAATAT TCAAATTAAA TGAAAAAATA TGGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
Fmult    TATTCTATAA AATAGAATAT TCAAATTAAA TGAAAAAATA TTGATGACGA CCCGAATCTT TATTTTATGA CTATGAAAAA GTGGAAGAAT
```

Fig. 31 cont.

```
                460        470        480        490        500        510        520        530        540
P114      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P113      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P583      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P555      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P134      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P726      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGACTAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P732      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P057      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P476      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P174      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P166      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P105      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
P012      TGTTGTGAAT CGATTTCAAG TTTAAGAAAG AATCGAATAT TCGTTTTT-A GTAAATTAAA GCATTTATTT ACCAGTCCGA TAGATCTTTT
F.conv    TGTTGTGAAT TGATTCCAAG TTTAAGAAAG AATCGAATAT TCGTTTTTTA GTAAATGAAA ----CTAGTC CCCAGTCCGA TAGATCT---
F.dent    TGTTGTGAAT TGATTCCAAG TTTAAGAAAG AATCGAATAT TCGTTTTTTA GTAAATGAAA ----CTATTC CCCAGTCCGA TAGATCT---
F.bald    TGTTGTGAAT TGATTCCAAG TTTAAGAAAG AATCGAATAT TCGTTTTTTA GTAAATTAAA ----TTATTC CCCAGTCCGA TAGATCT---
F.mult    TGTTGTGAAT CGATTCCAAG TTTAAGAAAG AATCGAATAT TCGTTTTTTA GTAAATTAAA GCATTTATTC CCCAGTCCGA TAGATCTTTT
```

Fig. 31 cont.

```
                550       5600        570        580        590        600        610        620
P114     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P113     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P583     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P555     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P134     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P726     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P732     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P057     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P476     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P174     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P166     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P105     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
P012     GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
F.conv   ------CCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC ATTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
F.dent   ------CCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC ATTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
F.bald   ------CCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC ATTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
F.mult   GAAAACCCGA CCCATCGGAT GAGAATGAAG ATAGAGTCCC GTTCTACGTG TCAATCCTGA CAACAATGAA ATTTATAGTA AGAGG
```

Fig. 32 The Six Most Parsimonious Trees From Sequence Data

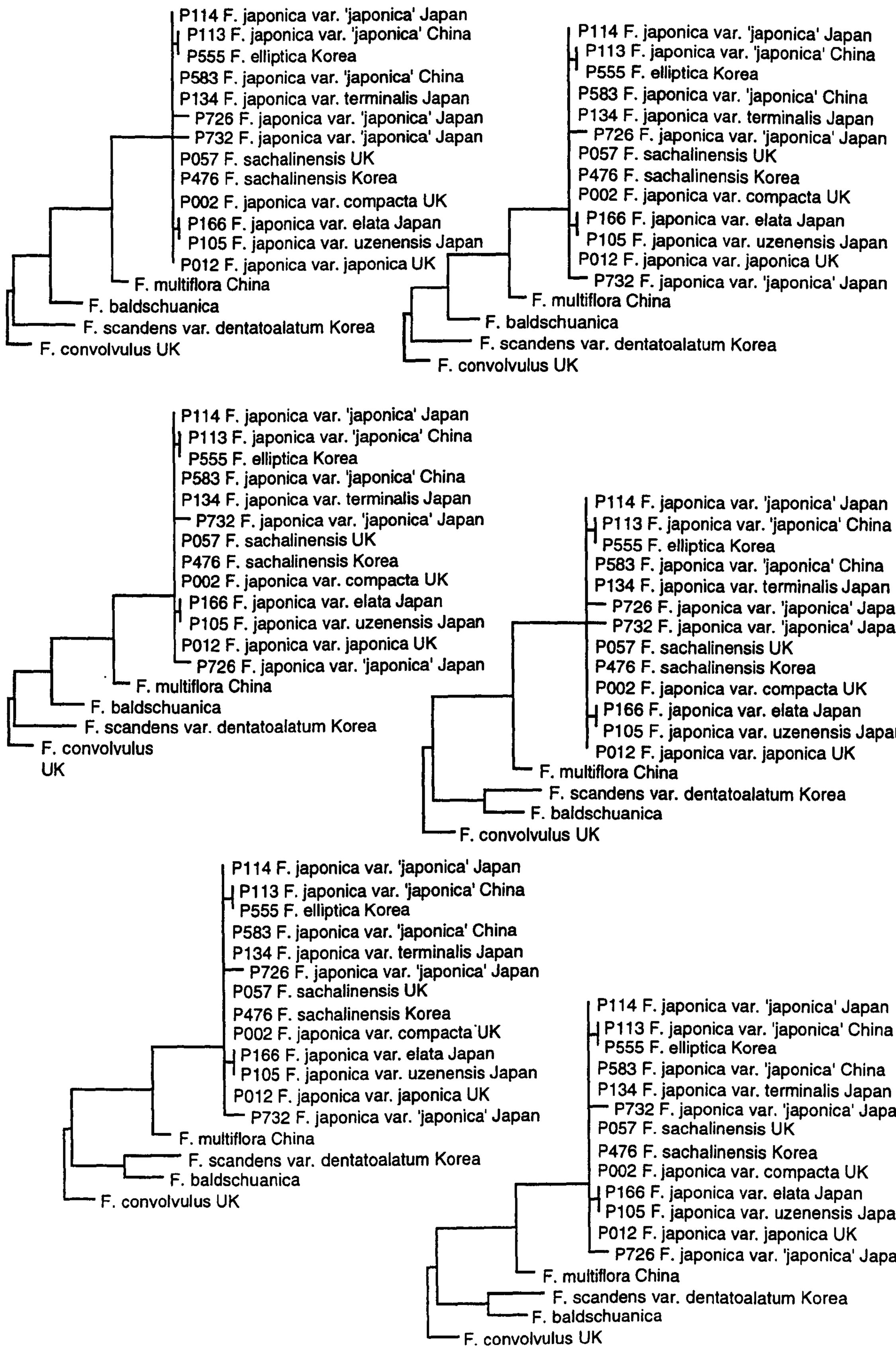
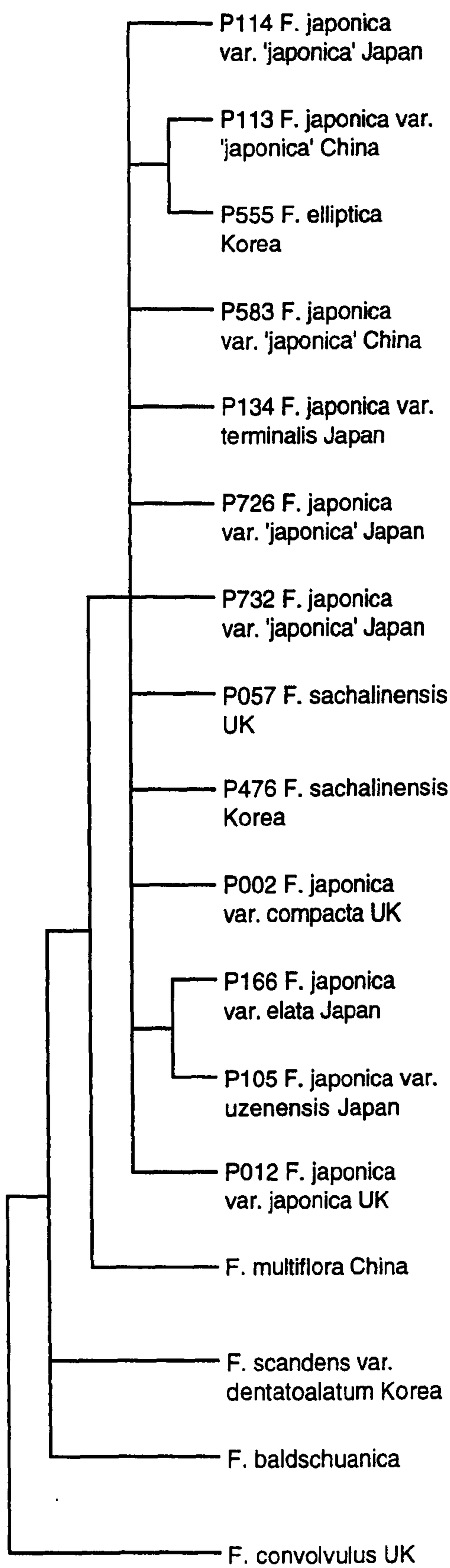


Fig. 33a Sequence Data Consensus Trees



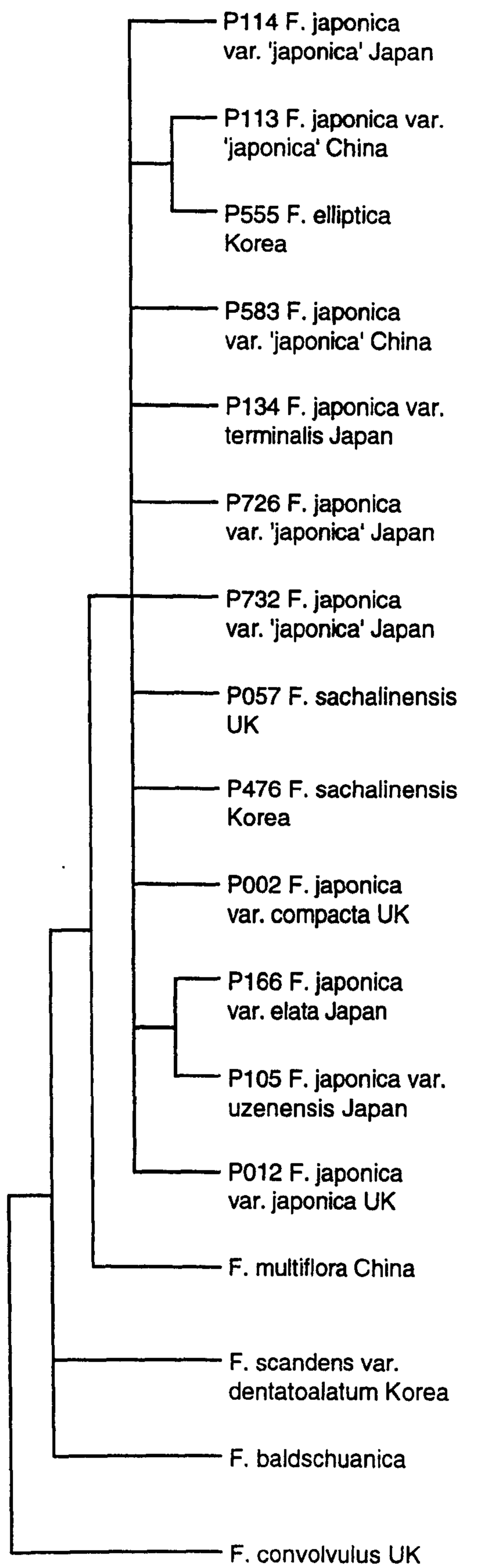
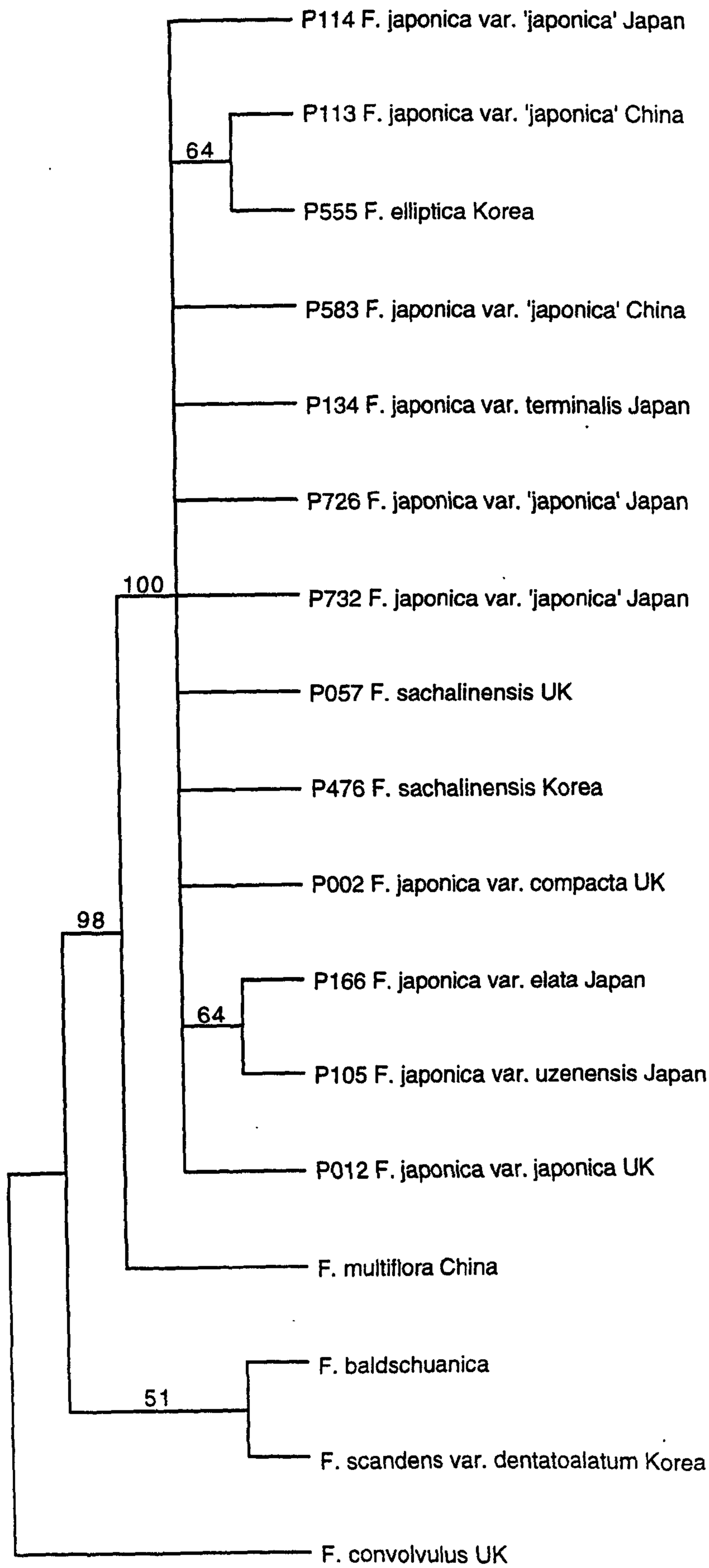


Fig. 33b Sequence Data Bootstrap Consensus Tree



Bootstrap values greater than 50 % are given above the branches

Fig. 34 The Ten Most Parsimonious Trees From Sequence and Indel Data

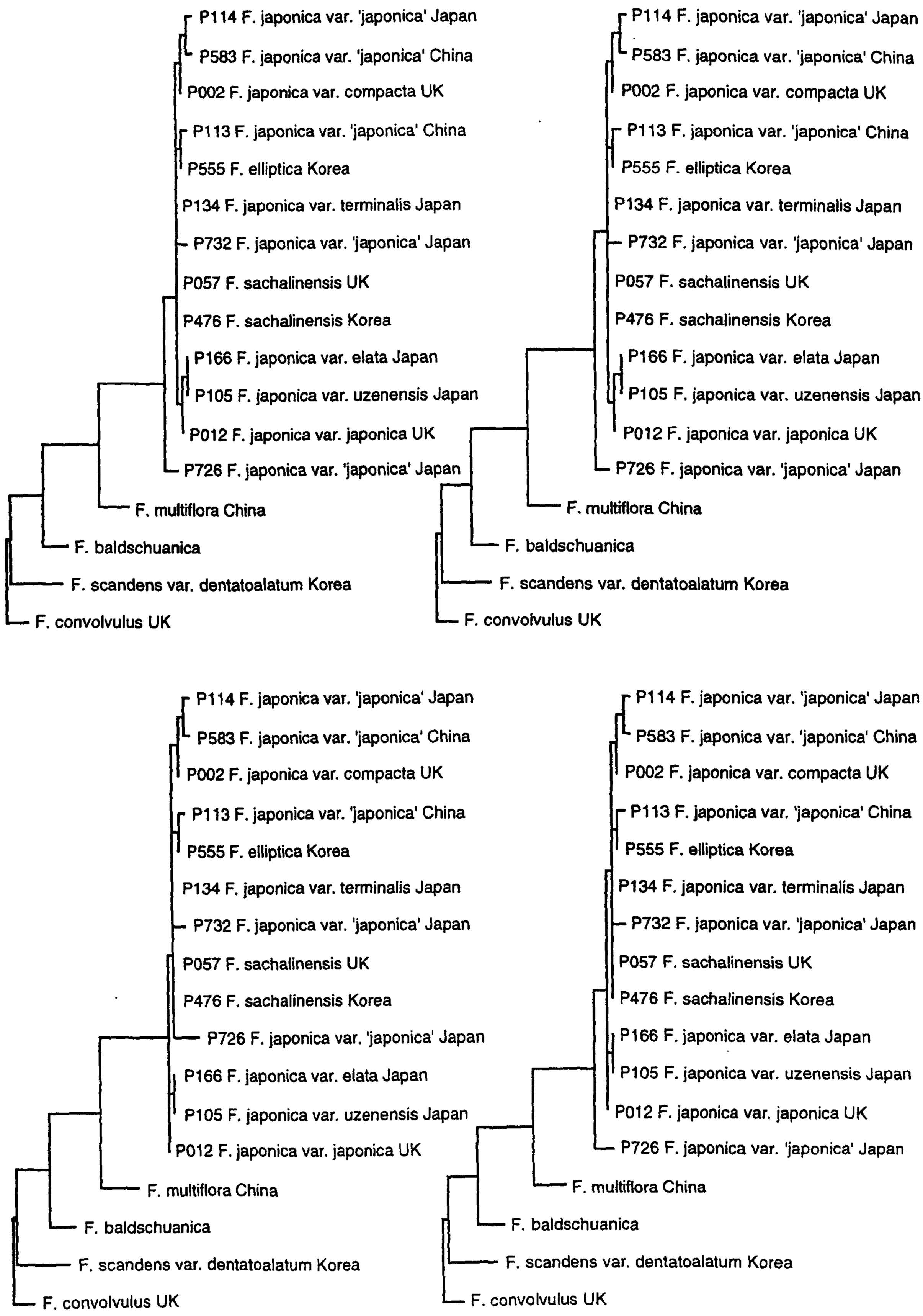


Fig. 34 cont. The Ten Most Parsimonious Trees From Sequence and Indel Data

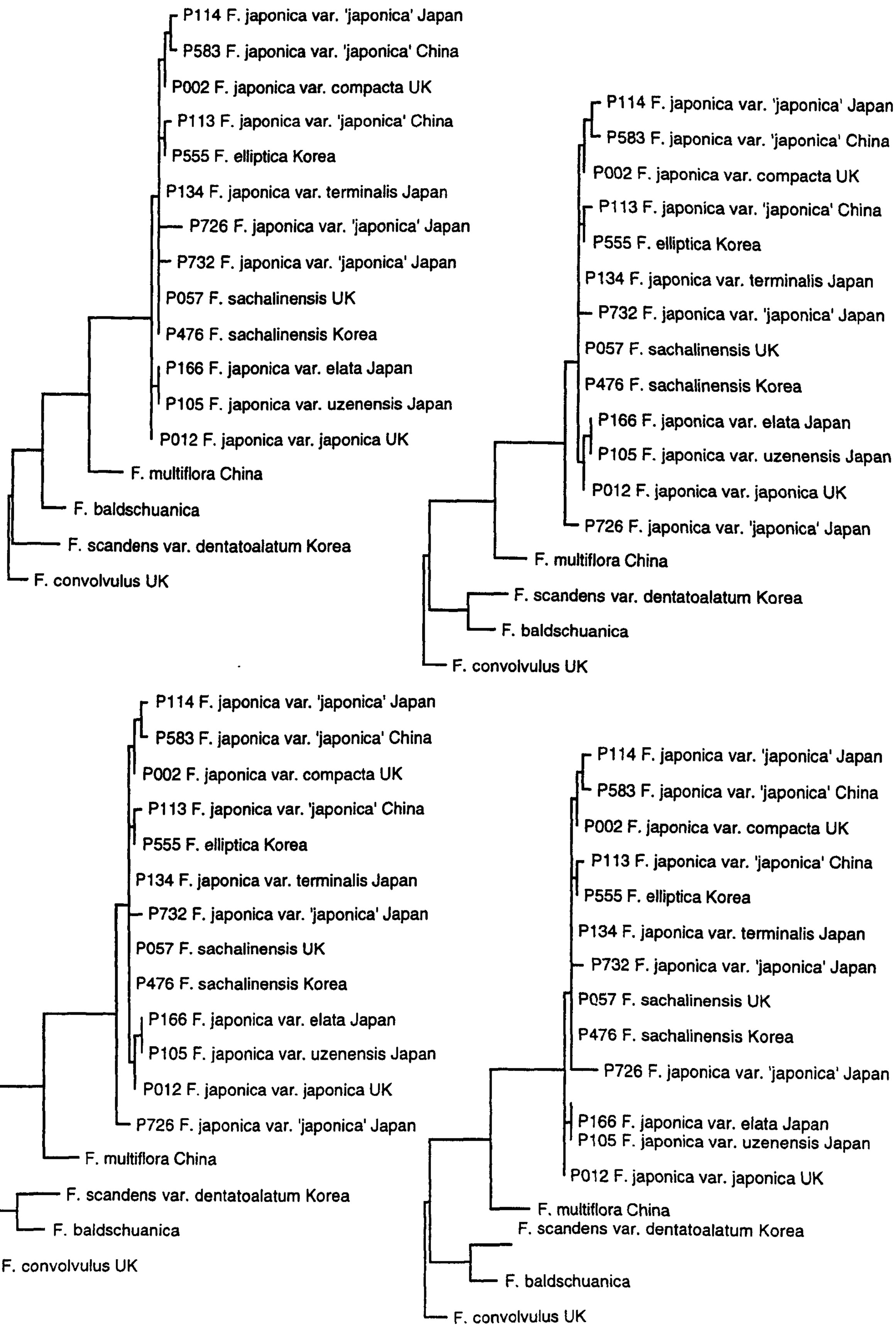


Fig. 34 cont. The Ten Most Parsimonious Trees From Sequence and Indel Data

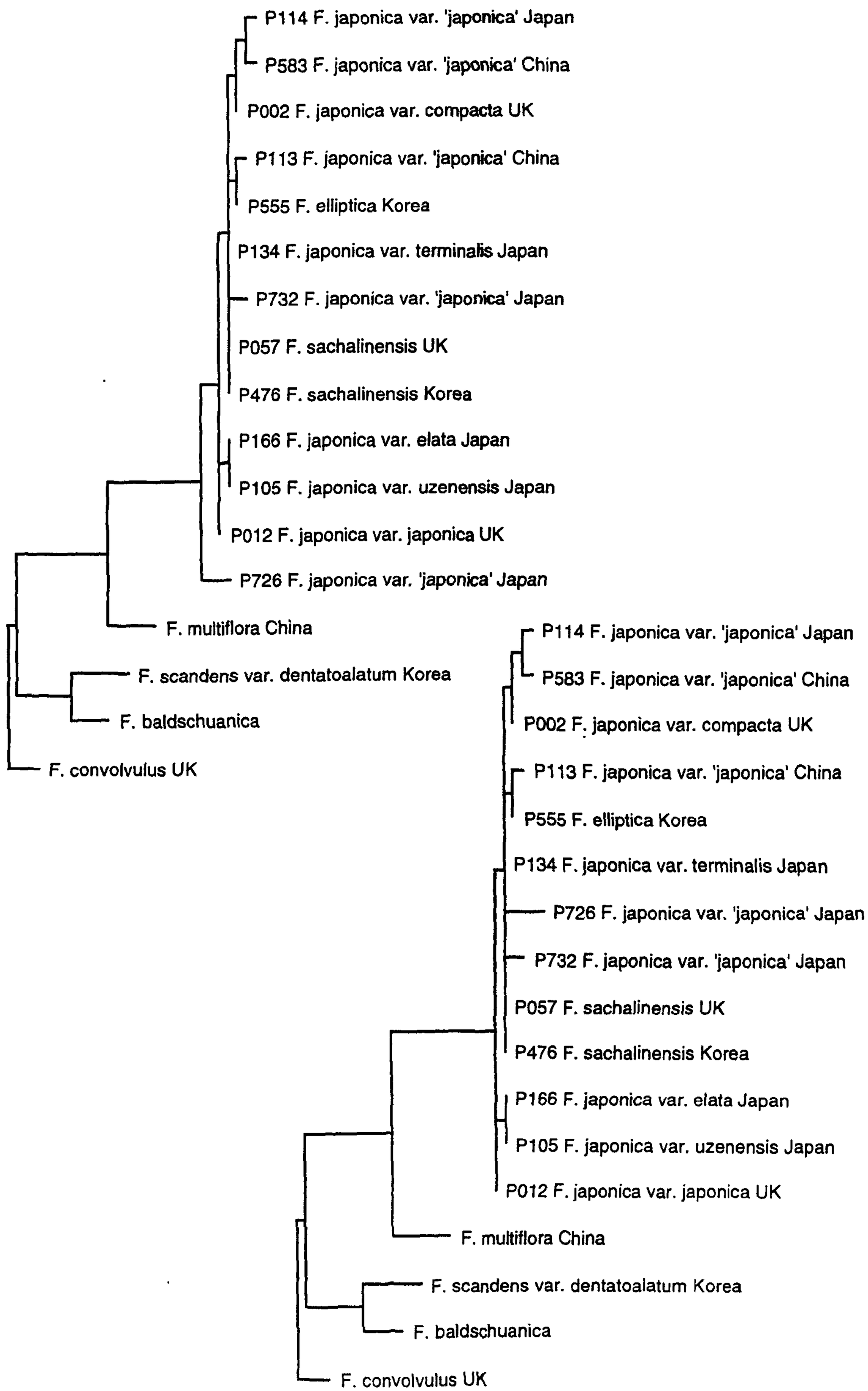


Fig. 35a Sequence and Indel Data Consensus Trees

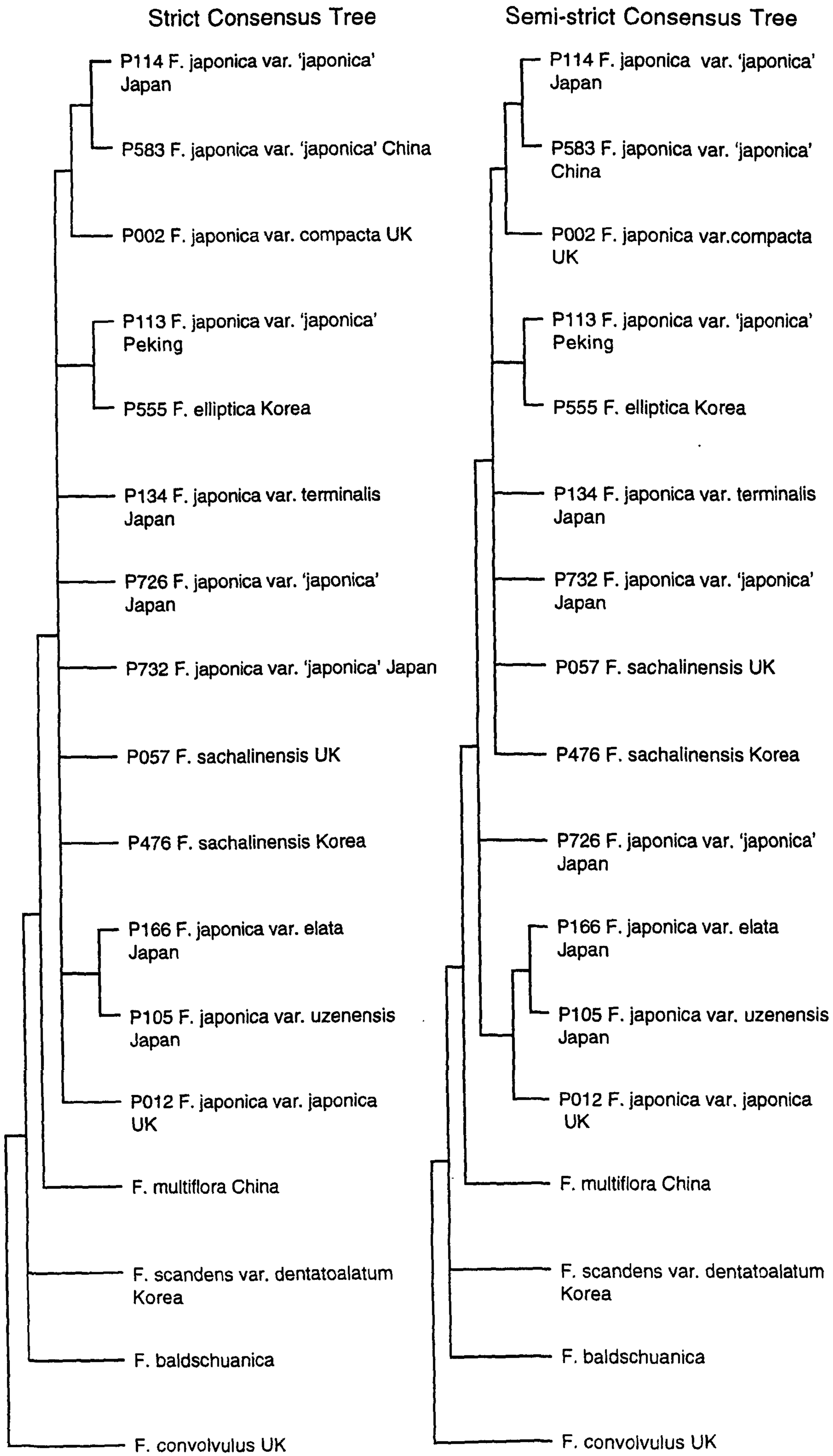
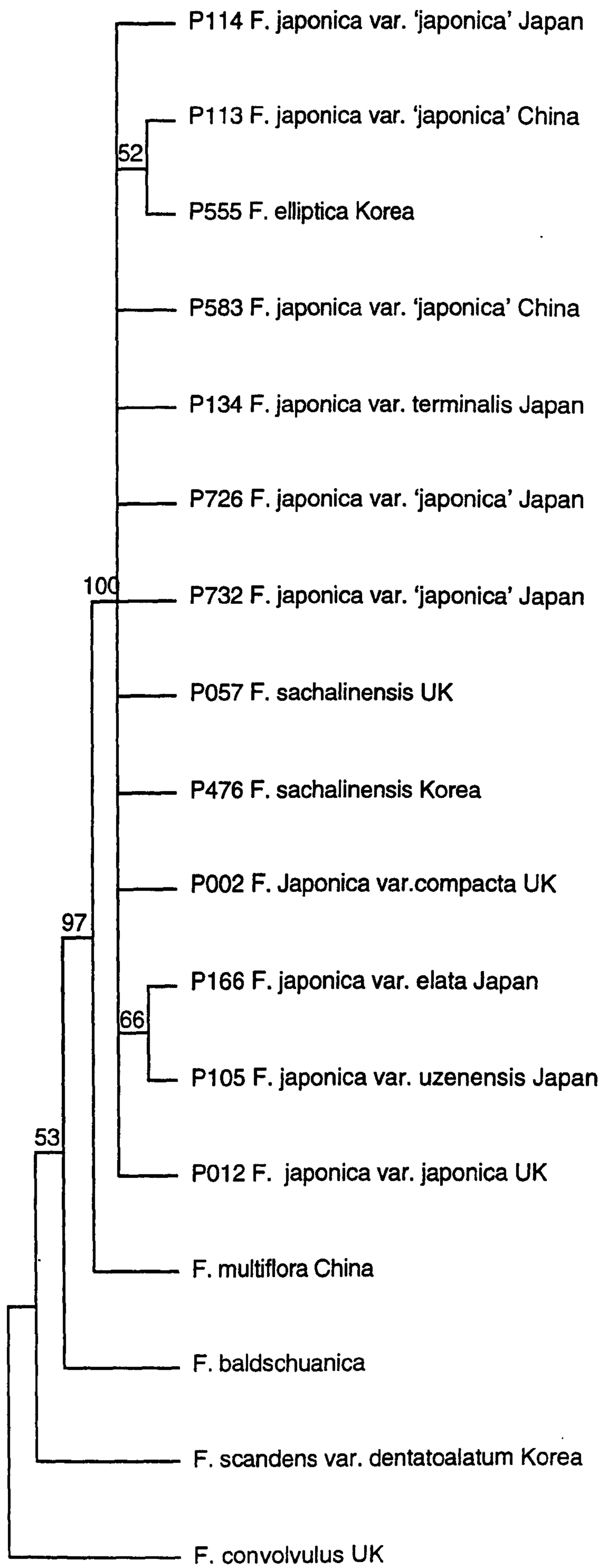


Fig. 35b Sequence and Indel Data
Bootstrap Consensus Tree



Bootstrap values greater than 50 % are given above the branches

RAPD analysis

A total of 158 reproducible polymorphic bands was produced from the ten RAPD primers employed in this study. The distribution of these RAPD bands amongst the samples is shown in Figs. 36 & 37. A total of 18 multiprimer genotypes was detected in the 20 samples of Fallopia japonica s.l. examined.

Samples P583 & P584, collected from China share the same multiprimer genotype. Three genotypes were detected from the four British samples of F. japonica var. compacta, with samples P193 and P174 sharing the same multiprimer genotype.

The neighbour joining tree (Fig. 38) and the split decomposition tree (Fig. 39) are congruent, although greater resolution is obtained from the former. The introduced genotype of F. japonica var. japonica groups together with the British samples of F. japonica var. compacta. The three samples of F. japonica var. 'japonica' from Mount Tateyama also group together as part of a broader grouping containing all of the other F. japonica var. 'japonica' from Japan. The two other Japanese samples (F. japonica var. uzenensis and F. japonica var. elata) group together, and are joined (all be it on a short branch) with the two Chinese samples of F. japonica var. 'japonica'.

These groupings are broadly repeated in the principal coordinate analysis (Fig. 40) where the first three principal coordinates account for 26, 19 and 10% of the variance respectively. The first principal coordinate clearly separates the introduced samples from the native material and the second principal coordinate clearly separates the Chinese samples from all others. The co-occurrence of F. japonica var. uzenensis with F. japonica var. elata is noticeable when the first and second principal coordinates are plotted against

each other. The grouping of three samples from Mount Tatyama is less clear, although the samples remain close to one another.

Fig. 36 Presence and absence of arbitrary fingerprinting bands in Fallopia japonica s.l.

	OPF08	OPF14	OPF16	OPF17	OPF 20
P105 F. japonica var.uzenensis	0001011101011011	0000000100	010010111000000010	0000000001011	0010111001001001110
P113 F. japonica var. 'japonica'	1001011000011110	1000001100	010100110100111100	1001011001011	1110110110101010001
P166 F. japonica var. elata	0111001000111011	0001001101	010010110010001000	0000001001011	1100110001001001010
P583 F. japonica var. 'japonica'	0101011000110111	0000101100	010100110100111100	1001111000110	1110110110101001001
P594 F. japonica var. 'japonica'	0100011000110011	0101000101	010010100000010000	1000001001010	0010100110001001001
P595 F. japonica var. 'japonica'	0001001011110001	0001000101	010010101000110000	1000001001010	0010100100001001010
P673 F. japonica var. 'japonica'	0000100010000010	0001000101	010100101000110010	1001011001001	0010100101010101010
P726 F. japonica var. 'japonica'	0011011001010000	0100000101	010010100000000101	0000001001000	0010100000001101010
P727 F. japonica var. 'japonica'	0011011001110110	0000000101	010010100000010001	0000001001000	0010100001000001000
P728 F. japonica var. 'japonica'	0000011001010100	0010000101	010000100100010101	0001001001011	0001100001001100010
P730 F. japonica var. 'japonica'	0100011000010011	0001001101	010010110000010000	1000011001011	0011110100001101010
P732 F. japonica var. 'japonica'	0101010001110011	0011000101	010010100000010100	0011001001011	0000100001001100010
P733 F. japonica var. 'japonica'	0100011000010011	0100000101	010000100000010100	0001001001011	0011100000001100010
P734 F. japonica var. 'japonica'	0000001001110001	0101000101	010010100000010010	0010001001011	0011100000001101010
P012 F. japonica var. japonica	0100011001010011	0001001101	010010100000010001	1001111001011	1010100001010011101
P174 F. japonica var. compacta	0100111001010011	0011001100	010011101000110011	1000101011111	0010100011010010100
P002 F. japonica var. compacta	0100111001010011	0001001100	010011101000000011	1000001011110	0010100001010010100
P413 F. japonica var. compacta	0100111001010011	0001001100	010010101000010011	1000001011110	0010100001010010100

Fig. 36 cont.

	OPP03	OPP20	OPA08	OPA06	OA16
P105 F. japonica var. uzenensis	0111110001000100	00110110010000	000110000001010010110010110000001	0010	0000100101110010
P113 F. japonica var. 'japonica'	0111111010100000	00111010011001	110110000000000101000010100000001	0011	0000100101000000
P166 F. japonica var. elata	0111110001010101	01011010010000	000100101000000010110010110010001	0010	0000101000000011
P583 F. japonica var. 'japonica'	0111001000111001	00111010011001	110100000010000011000010110100001	0011	0011000101100000
P594 F. japonica var. 'japonica'	0111110000100100	00010110010101	000100010100010000110110110011101	0011	1100101001100110
P595 F. japonica var. 'japonica'	0111110000110100	00010110010111	000100010100000000110101110011101	0011	0000100001010010
P673 F. japonica var. 'japonica'	0111110000110100	00010110110111	000101010100000000110100100011101	0010	0101001001110010
P726 F. japonica var. 'japonica'	0111110010110100	00010110010001	001100010100000000110010000010111	0011	0100100001010011
P727 F. japonica var. 'japonica'	0111110010100100	00010010010000	001100010100010000110010010010111	0011	0100100001110011
P728 F. japonica var. 'japonica'	0111010000110100	00010010010000	001100010100000000010001011001011	0011	0101100001010111
P730 F. japonica var. 'japonica'	0111010000110000	01010110110000	000101010100010000110010110010111	0011	0111000011110010
P732 F. japonica var. 'japonica'	0111110000110000	01010110110000	000101000100000000010001000001011	0011	0111000001110110
P733 F. japonica var. 'japonica'	0111110000110100	00010110110000	000100000100000000110010000010111	0010	0011001001111000
P734 F. japonica var. 'japonica'	0111110000110100	00010110110000	000100010000010000110010000001101	0010	0111001001110010
P012 F. japonica var. japonica	0111110010000101	00010110110000	000110110100010000110011101011101	1111	0001010011110011
P174 F. japonica var. compacta	0110110001000001	00010100010100	000101110000010000110010101011011	0011	0111100011111111
P002 F. japonica var. compacta	0110110001000001	00010100010000	000101111000010000110010101011011	0011	0111110011111011
P413 F. japonica var. compacta	0110110001000001	00010100000100	000101110100010000110010101011011	0011	0111110011111011

1= presence of a band, 0 = absence of band, 9 = missing data

Fig. 37 RAPD photograph showing the genotypes of Fallopia taxa detected in this study (primer OPF20).

From left to right the samples are: lane 1 F. japonica var. 'japonica' P734; lane2 F. japonica var. 'japonica' P733; lane 3 F. japonica var. 'japonica' P732; lane 4 F. japonica var. 'japonica' P730; lane 5 F. japonica var. 'japonica' P728; lane 6 F. japonica var. 'japonica' P727; lane 7 F. japonica var. 'japonica' P726; lane 8 F. japonica var. 'japonica' P595; lane 9 F. japonica var. 'japonica' P673; lane 10 F. japonica var. 'japonica' P594; lane 11 F. japonica var. 'japonica' P583; lane 12 F. japonica var. 'japonica' P584; lane 13 F. japonica var. elata P166; lane 14 F. japonica var. 'japonica' P113; lane 15 F. japonica var. uzenensis P105; lane 16 F. scandens var. dentatoalatum P729; lane 17 1Kbp marker.

Table 17. Distribution of RAPD genotypes detected in Falllopia japonica s.l.

Accession Number and Taxon		Country	RAPD Genotype
P105	F. japonica var. uzenensis	Japan	RAPDPHY A
P113	F. japonica var. 'japonica'	Peking	RAPDPHY B
P166	F. japonica var. elata	Japan	RAPDPHY C
P583	F. japonica var. 'japonica'	China	RAPDPHY D
P584	F. japonica var. 'japonica'	China	RAPDPHY D
P594	F. japonica var. 'japonica'	Mt. Honshu Japan	RAPDPHY E
P595	F. japonica var. 'japonica'	Japan	RAPDPHY F
P673	F. japonica var. 'japonica'	Mt. Fujija, Japan	RAPDPHY G
P726	F. japonica var. 'japonica'	Mt Tateyama, Japan	RAPDPHY H
P727	F. japonica var. 'japonica'	Mt Tateyama, Japan	RAPDPHY I
P728	F. japonica var. 'japonica'	Mt Tateyama, Japan	RAPDPHY J
P730	F. japonica var. 'japonica'	Shinjuku, Tokyo, Japan	RAPDPHY K
P732	F. japonica var. 'japonica'	Shinjuku, Tokyo, Japan	RAPDPHY L
P733	F. japonica var. 'japonica'	Tanagawa River, Tokyo, Japan	RAPDPHY M
P734	F. japonica var. 'japonica'	Tanagawa River, Tokyo, Japan	RAPDPHY N
P012	F. japonica var. japonica	Leicester, England	RAPDPHY O
P002	F. japonica var compacta	Brecon, Wales	RAPDPHY P
P174	F. japonica var compacta	Bracken Hill, Surrey, England	RAPDPHY Q
P193	F. japonica var compacta	Heads of Valley, Wales	RAPDPHY Q
P413	F. japonica var compacta	Blarney Castle, Ireland	RAPDPHY R

Fig. 38 Neighbour Joining Tree of Fallopia japonica s.l.

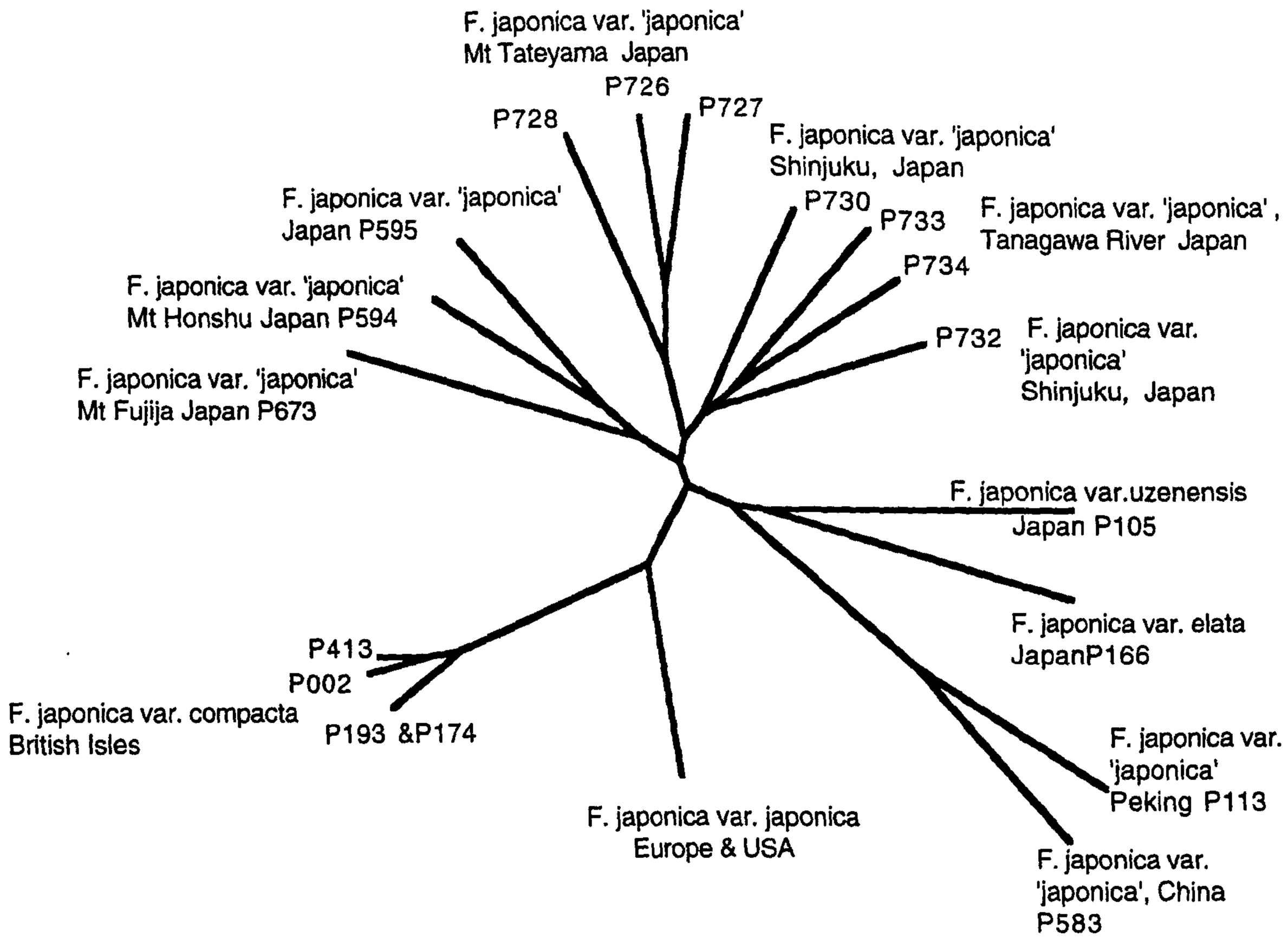


Fig. 39 Split decomposition of RAPD data of Fallopia japonica s.l.

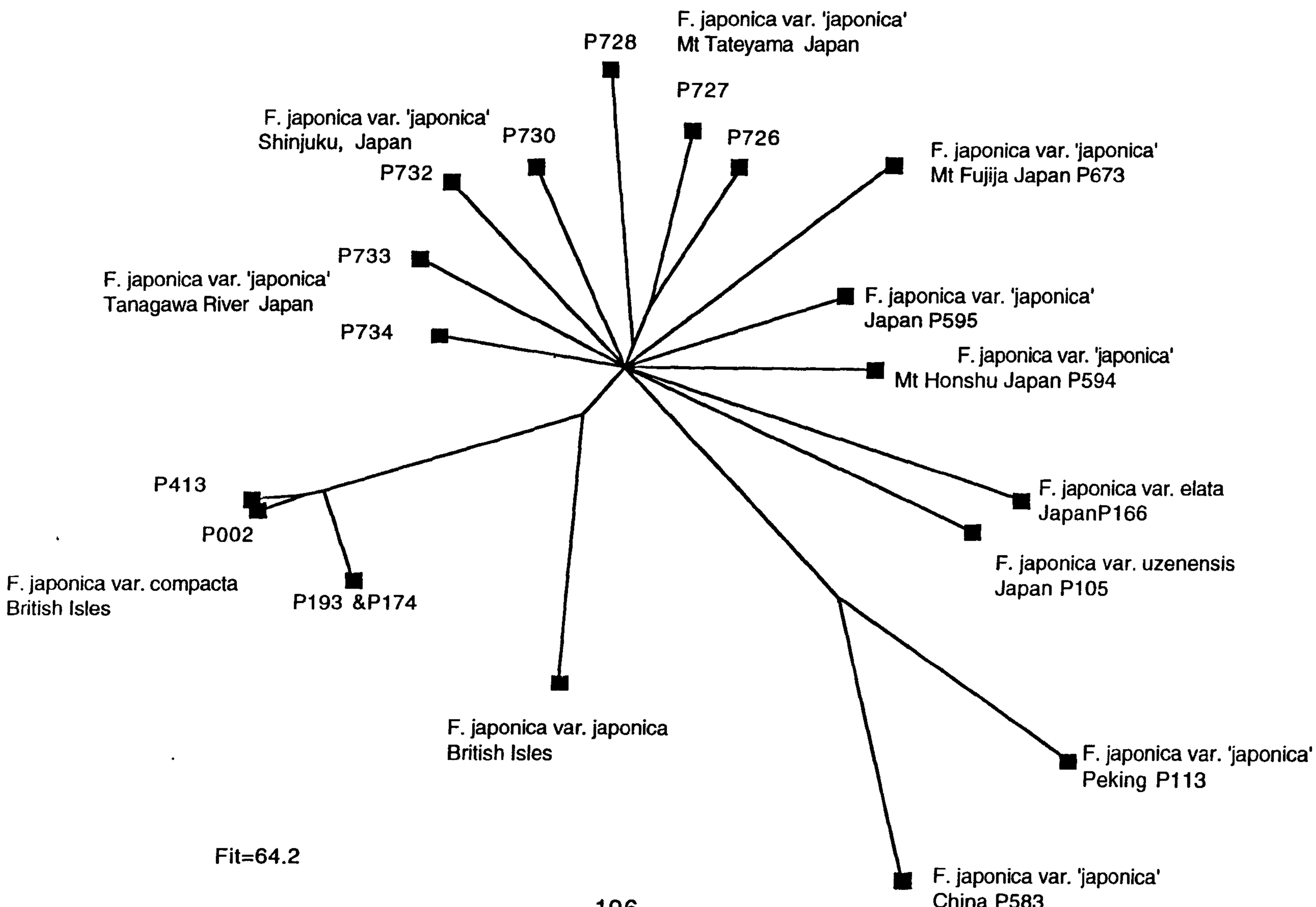
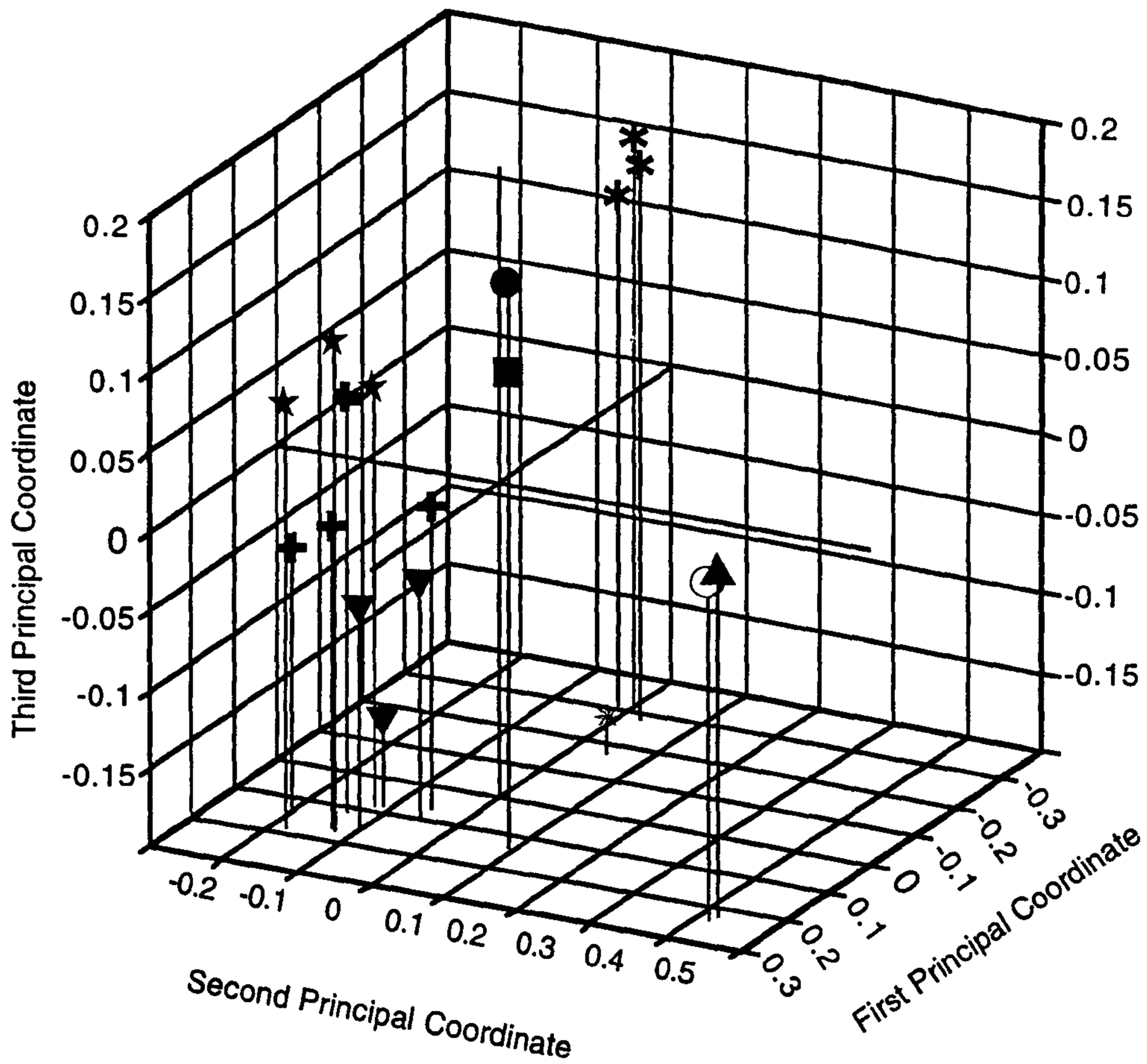


Fig. 40 Principal coordinate graph of RAPD data from Fallopia japonica s.l.



● F. japonica var. uzenensis, Japan P105

○ F. japonica var. 'japonica', Peking P113

■ F. japonica var. elata, JapanP166

▲ F. japonica var. 'japonica', China P583

▼ F. japonica var. 'japonica' , Mt Honshu Japan P594, Mt Fujija Japan P673 & Japan P595

★ F. japonica var. 'japonica' , Mt Tateyama Japan P726, P727 & P728

✳ F. japonica var. japonica British Isles

✱ F. japonica var. compacta British Isles

✚ F. japonica var. 'japonica' , Shinjuku, Japan P730 & P732
F. japonica var. 'japonica' , Tanagawa River Japan P733 & P734

Discussion

The chloroplast tmL sequences showed low levels of divergence within the study group. DNA sequence analysis has been used to study variation in the tmL intron in a number of different taxa (Gielly & Taberlet, 1994). It appears that the value of this region depends on the taxa being studied. No sequence divergence was found between the tmL introns of the genera Fraxinus L. and Alnus Mill. but 6% sequence divergence was found within the genus Gentiana L. (Gielly & Taberlet, 1994). The tmL intron is a group 1 intron and is conserved in primary sequence, secondary structure and position (Kuhsel et al., 1990). The secondary structure of the Fallopia consensus sequence, based on lowest free energies is shown in Fig. 41. The intron of the RNA folds to form the active site required for splicing (Davies et al., 1982). This structure contains regions of complementary sequences that form nine stem loop structures (Cech, 1988). These stem regions include four short domains, P, Q, R and S, that are conserved in primary sequence among all group I introns. In addition, the tmL intron possess an unusually high degree of sequence similarity in the regions surrounding these four domains (Kuhsel et al., 1990). Approximately half of the cpDNA mutations detected in this study are short indels (1-15 bases). Almost all of these indels, together with the majority of base substitutions, are located in the large loop structure, outside of the constraints of the core region (Fig. 41). Of the four remaining polymorphisms, none were found in the conserved primary sequence (P,Q,R and S) and only one was found in one of the nine stem regions, although as this polymorphism was a G-A transition, the overall structure of the stem is not changed.

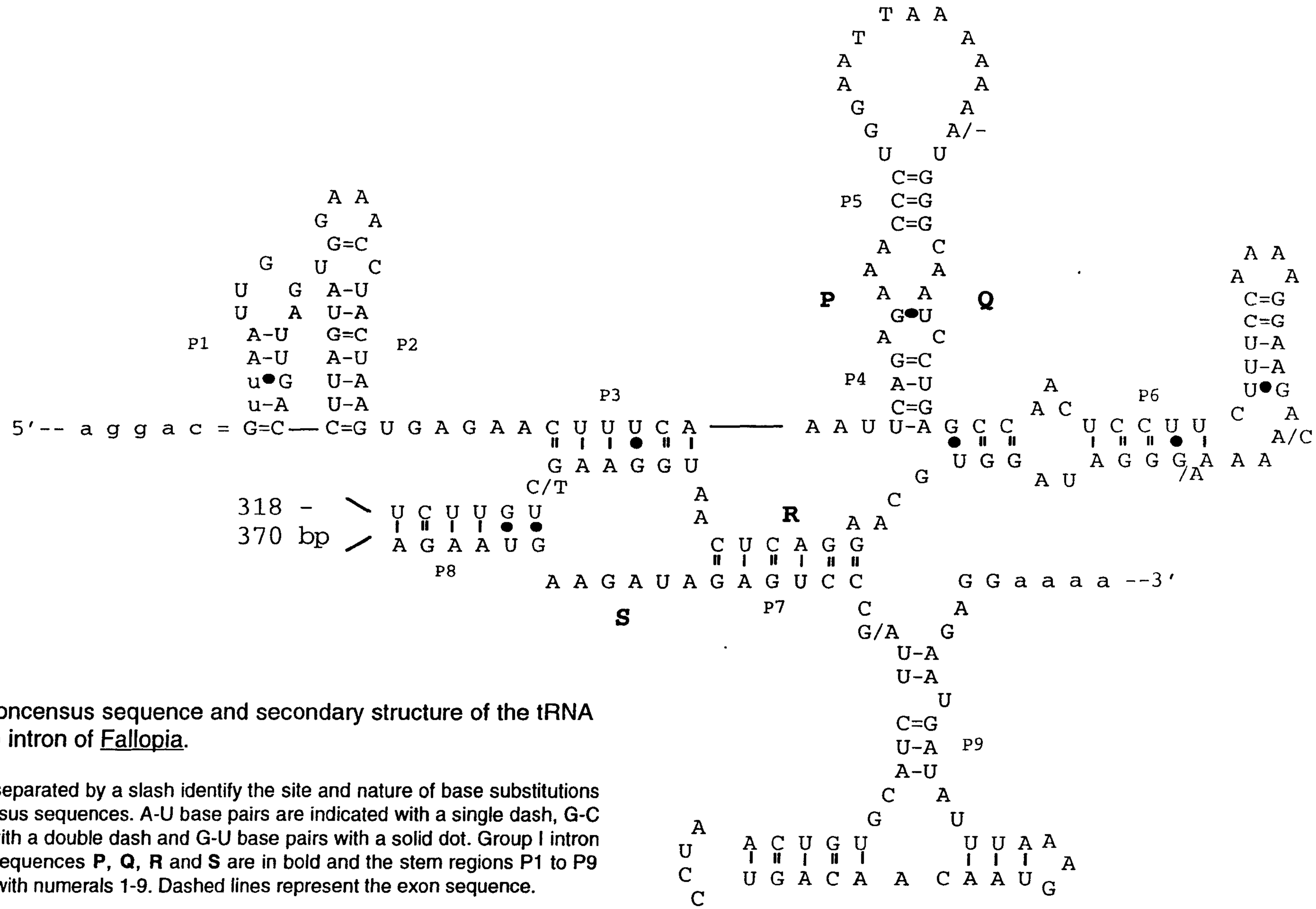


Fig. 41 Concensus sequence and secondary structure of the tRNA Leu (UAA) intron of Fallopia.

Two bases separated by a slash identify the site and nature of base substitutions from consensus sequences. A-U base pairs are indicated with a single dash, G-C base pairs with a double dash and G-U base pairs with a solid dot. Group I intron conserved sequences **P**, **Q**, **R** and **S** are in bold and the stem regions P1 to P9 are marked with numerals 1-9. Dashed lines represent the exon sequence.

When the sequence data are analysed to study relationships, parsimony analysis provides strong support for the monophyly of the ingroup with respect to the outgroup (Figs. 33 a,b & 35 a,b). The rhizomatous perennials of section Reynoutria form a monophyletic cluster, with Fallopia multiflora being the sister taxon to this clade. This grouping is interesting as F. multiflora is the most likely of the extant Fallopia taxa to be the progenitor (or a descendant of the progenitor) of section Reynoutria. F. multiflora is a diploid (2n=22) with the same base number as the section Reynoutria taxa and is a herbaceous, rhizomatous perennial with flowers, stigmata and seed closely resembling F. japonica, with which it occurs in sympatry in its native range. However, although the levels of sequence divergence detected here suggest that trnL may be informative for examining sectional relationships and origins, further sampling and outgroups from outside of the genus are required before anything further can be said.

The low level of sequence divergence within the ingroup, and the high levels of homoplasy of those changes that were detected, hampers any real insight into the relationships of the chloroplast haplotypes (and by extension, the taxa that house them). For instance, although seven haplotypes were detected from the nine individuals of native Fallopia japonica s.l. and F. elliptica, the phylogenetic signal is extremely low. Effectively all that can really be said, is that some taxa share haplotypes and some have unique haplotypes. Those taxa that share haplotypes do not do so in any obvious taxonomic or geographical sense. The chloroplast haplotypes of introduced F. japonica s.l. were not found in the native material examined in this study. The most closely related samples to the introduced F. japonica var. japonica were those of F. japonica var. uzenensis (P105) and F. japonica var. elata (P166), both collected from Japan. The haplotype of these samples differed from that of introduced F. japonica var. japonica by a single site mutation. However whilst

F. japonica var. uzenensis has the same chromosome number as introduced material of F. japonica var. japonica (2n=88), it is morphologically distinct (var. uzenensis has hairy stems and leaves).

At lower taxonomic levels the RAPD data show some meaningful associations (Fig. 38). The samples of Fallopia japonica var. compacta cluster together, and form a group with the introduced genotype of F. japonica var. japonica. Thus all of the introduced material forms a group relative to the native material. In addition the RAPD data shows geographical structure among the native samples. The Chinese samples group together, and all of the F. japonica var. 'japonica' from Japan group together, with samples from Mount Tatyama forming one cluster and the samples from Tokyo another. Beyond this it is important, however, to reiterate the fact that all of the Japanese F. japonica var. 'japonica' have 2n=44 chromosomes and are much smaller (2 m) than the 2n=88 European and American introduced populations (3 m). It is clear that there are two variables that need considering in the interpretations of the tree, namely taxonomy and geography. The group containing the Japanese F. japonica var. 'japonica' clearly represent a separate variety to the introduced genotype (and possibly to the Chinese material?), and it is noteworthy that the introduced genotype of F. japonica var. japonica is more closely related to F. japonica var. compacta than to native material. This reinforces the decision made in chapter 6 not to attempt a formal comparison of introduced F. japonica var. japonica with native genotypes to confirm technique sensitivity and estimate levels of variability in the native range. Any measure of technique sensitivity using the current taxonomic framework would be scientifically flawed, and no meaningful comparisons within taxa can be made until the taxa have been correctly circumscribed. It is clear that what is needed is a detailed morphological and cytological monograph of section Reynoutria. Until this has been carried out,

all attempts to use molecular data to study the placement of invasive populations of Fallopia taxa will be in vain. The importance of having a sound taxonomic framework on which to base molecular hypotheses is absolute.

Chapter 9

Summary and Future Work

Despite the technical difficulties of working with Fallopia Adans. due to high concentrations of polysaccharides co-isolated with the DNA, molecular markers have shown great promise as tools for investigating the evolutionary biology of Fallopia species. The protocols developed and applied to Fallopia during the course of this research have allowed the investigation of the distribution of polymorphisms in the nuclear and chloroplast genomes. RAPDs, inter-SSRs, chloroplast RFLPs and sequencing have been used to investigate the population biology and relationships of species of Fallopia section Reynoutria Houtt. (chapters 3-8). From the outset the aim of this research program was to use molecular markers to gain a deeper insight into the evolutionary biology of these taxa and in particular to investigate the relative importance of sexual versus asexual reproduction and the dynamics of inter-specific hybridisation.

The molecular data have been very useful in addressing these issues. Arbitrary fingerprinting data supports the hypothesis that Fallopia japonica var. japonica is represented in Britain as a single clone (chapter 6), whereas higher levels of genotypic diversity have been detected in F. sachalinensis and F. x bohemica, consistent with at least some sexual reproduction (chapters 3, 4, & 7). In-depth studies focusing on 6x F. x bohemica from two river systems (chapter 3 - the River Kelvin, Glasgow, and chapter 4 - the River Wnion, North Wales) suggest that a combination of recurrent origins of the hybrid and hybrid fertility is responsible for the high levels of clonal diversity detected in this taxon. Furthermore, the abitrary fingerprinting data has provided evidence that backcrossing between F. x bohemica and F. japonica var. japonica has occurred on at least two occasions. This possibly represents an intermediate step in the restoration of male fertility to the British population of F. japonica var. japonica.

Using chloroplast RFLPs it has been possible to unambiguously distinguish the chloroplast DNAs of Fallopia japonica var. japonica, F. japonica var. compacta and F. sachalinensis in Britain (chapter 5). By examining the distribution of these haplotypes amongst natural and artificial hybrids, I have been able to conclude that the mode of chloroplast inheritance is predominantly maternal and that F. japonica var. japonica is the female parent of all of its hybrids. The most parsimonious explanation of the data I obtained on the distribution of chloroplast DNA haplotypes among hybrids of F. japonica var. compacta and F. sachalinensis is that hybridisation of these two taxa is bi-directional, consistent with them both having male fertile plants present in Britain.

Investigations into the relationships of the introduced plants of Fallopia japonica var. japonica, F. japonica var. compacta and F. sachalinensis in Britain with material from the native range has been confounded by the absence of a comparable taxonomic framework (chapter 8). Chloroplast sequencing and RAPDs have shown that there is greater genetic variability among native material than introduced plants, although the extent to which this is an inter-specific rather than intra-specific observation remains for now unclear. Nevertheless, one interesting finding has been that using RAPDs, British material of F. japonica s.l. forms a group relative to native material. This is perhaps surprising as one may have predicted that F. japonica var. japonica and F. japonica var. compacta represent separate independent introductions from Asia, and would fall scattered within the broader pattern of variability of native genotypes.

Whilst the molecular data has been very useful and has enhanced our understanding of the evolutionary biology of these taxa, as many questions have been raised as have been answered. As the research on these plants is ongoing at the University of Leicester, it is worth considering potential directions for future work. For convenience invasive populations and native populations will be discussed separately.

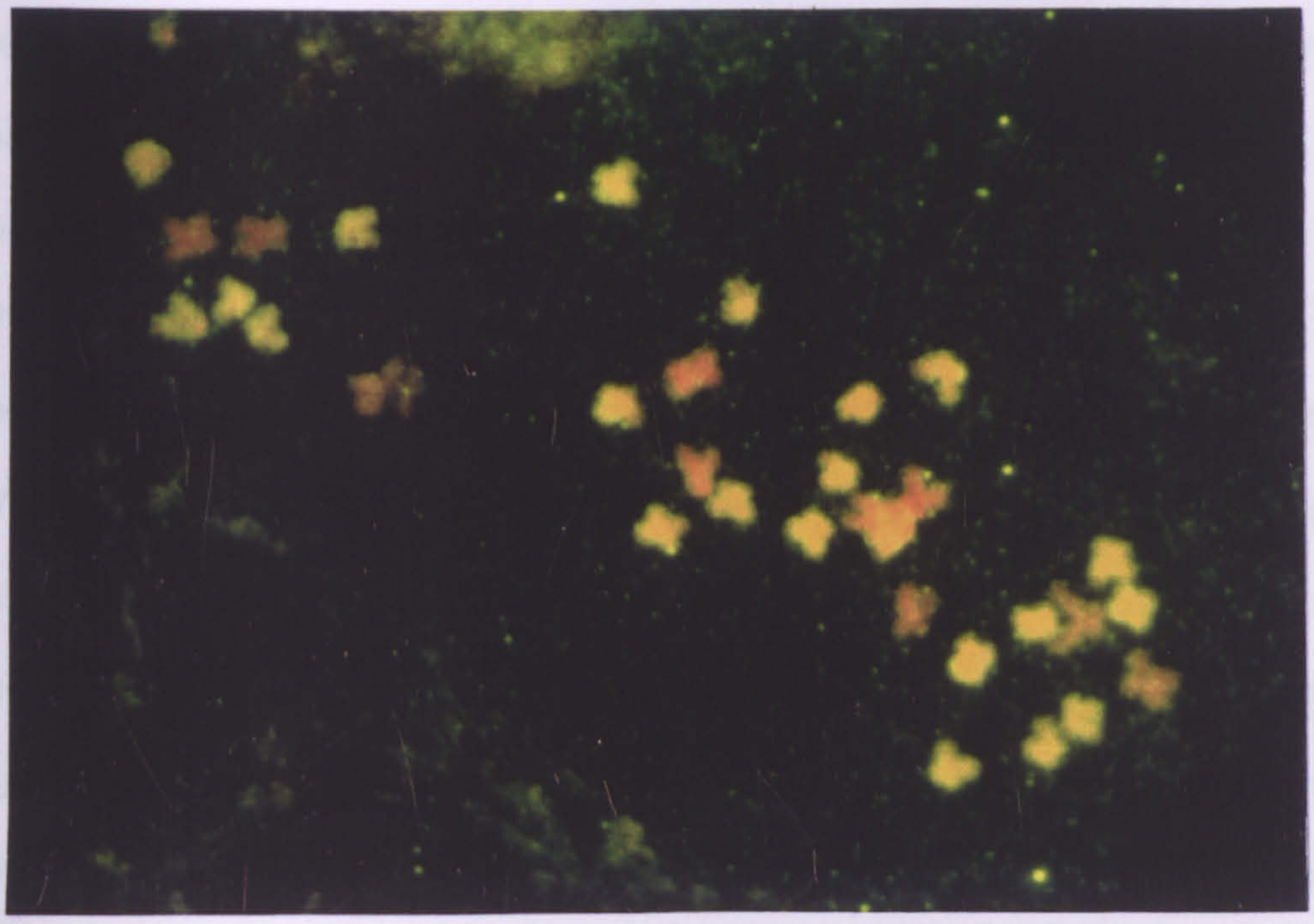
Invasive populations

Long term funded research programs into a particular species or group of species are uncommon under the current 'rapid return' research funding structure. However, when such programmes exist, the importance of long term planning and making investments into thoroughly characterising the genetic system of the study organisms is high. Making controlled crosses and raising progeny arrays of large perennials is labour intensive, requires the allocation of considerable space to maintain the plants, and considerable time for future generations to reach maturity and flower. In many short term projects (e.g. three year PhDs) it is impossible to obtain useful results from many generations of crosses. At Leicester, however, the continuing nature of the research makes such an investment worthwhile. Examination of the progenies from controlled crosses can provide information on inheritance patterns of morphological traits and allow greater information to be gleaned from arbitrary fingerprinting data. A knowledge of the sex ratios of progeny from controlled crosses will help in the estimation of the genetic control of sex expression and assist in the interpretation of sex ratios in natural populations. Likewise, screening these controlled crosses for marker inheritance can provide estimates of levels of segregational heterozygosity. As Fallopia japonica (Houtt.) Ronse Decraene var. japonica appears to be represented by a single clone in Britain (and possibly Europe and North America?) thoroughly characterising this genotype would be informative. Controlled crosses with F. sachalinensis (F. Schmidt ex Maxim.) Ronse Decraene and examination of segregation ratios will allow one to distinguish those bands that are homozygous and should be present in all F1 hybrids, and those that are heterozygous and occur in some, but not all, progeny. This information can then be utilised directly in wild populations, and the absence in hybrids of bands that are homozygous in F. japonica var. japonica can be used as evidence of sexual recruitment from hybrids.

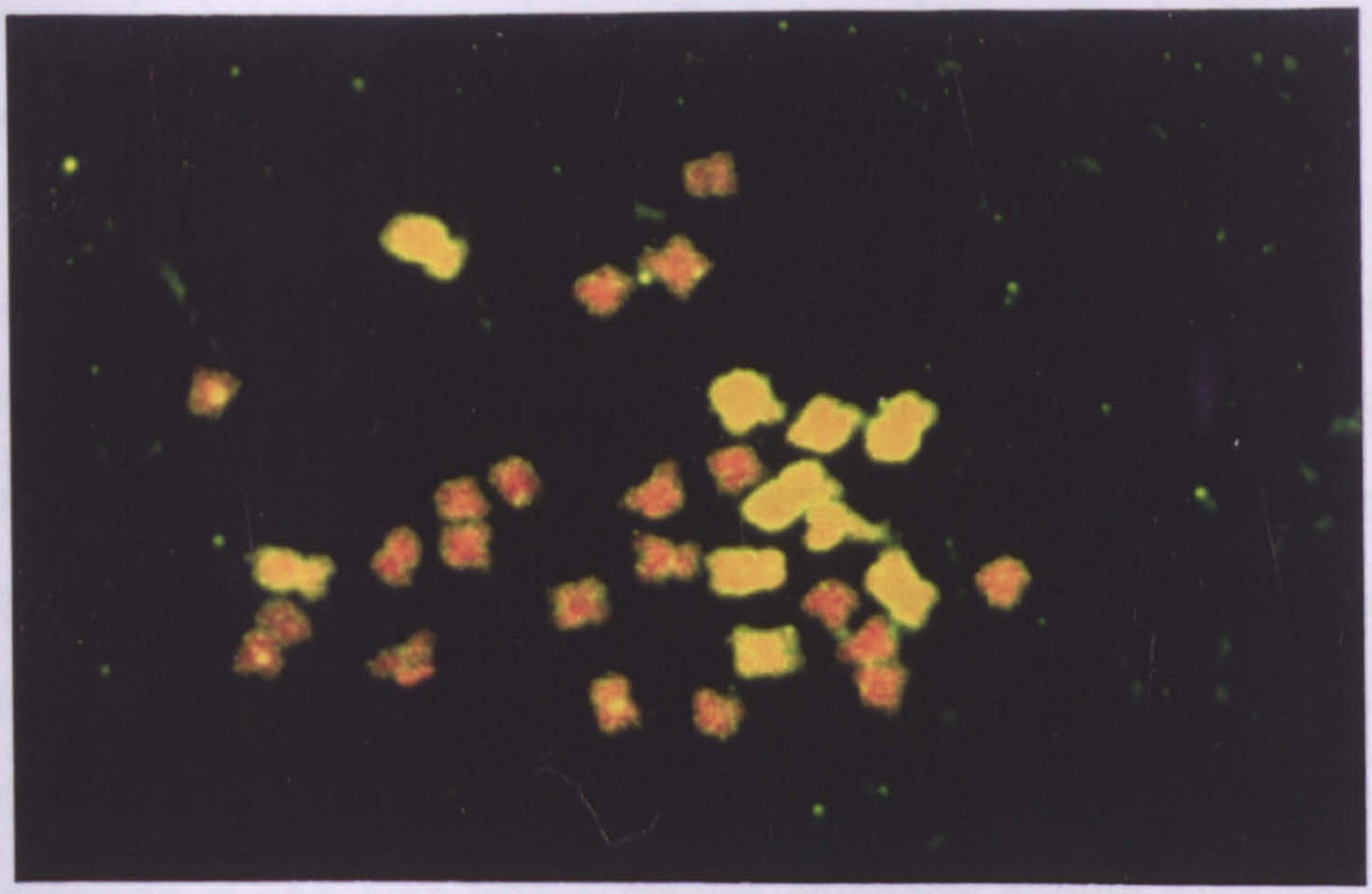
This issue of distinguishing between recurrent origins and hybrid fertility as the explanation for the high levels of genetic variability in British populations of Fallopia x

bohemica (Chrtek & Chrtková) J. Bailey is important. The extent to which F. x bohemica can perpetuate as an independent sexual 'species' is interesting both from the academic perspective of hybrid speciation and with regards to the success of the weed invasion. As well as investigating segregation patterns of arbitrary fingerprinting markers from F. japonica var. japonica, one possible direction for future research is genomic in situ hybridisation (GISH). GISH involves the differential labelling of genomes or parts of genomes in chromosome preparations (Leitch et al., 1994; Stace & Bailey, in press). The technique allows the identification of the genomic constituency of hybrids and interactions between different chromosomal complements. Time prevented the inclusion of GISH data in the current study; the technique is complicated and requires extensive optimisation experiments. I did, however, carry out some preliminary experiments, and the technique shows promise. Fig. 42 shows metaphase preparations of a hybrid between F. x bohemica (F. japonica var. compacta (Hook. f.) J. Bailey (2n=44) x F. sachalinensis (2n=44)) and F. baldschuanica (Regel) Holub (2n=20).

Fig. 42 Genomic in situ hybridisation of Fallopia



a. Mitotic chromosome preparation of Fallopia x bohemica (F. sachalinensis x F. japonica var. compacta) x F. baldschuanica. Chromosomes from the F. x bohemica parent fluoresce yellow due to hybridisation with the F. sachalinensis probe. Unprobed chromosomes (F. baldschuanica) are coloured red due to counterstaining with propidium iodide.



b. Mitotic chromosome preparation of Fallopia x bohemica (F. sachalinensis x F. japonica var. compacta) x F. baldschuanica. Chromosomes from the F. baldschuanica parent fluoresce yellow due to hybridisation with the F. baldschuanica probe. Unprobed chromosomes (F. x bohemica) are coloured red due to counterstaining with propidium iodide.

The yellow chromosomes are those to which the probe (total genomic DNA labelled with fluorescein isothiocyanate) has bound in competition with blocking DNA. The red/orange chromosomes are those which the probe has not bound to. The red/orange colour is produced by counterstaining with propidium iodide. Fig. 42a shows a preparation in which the probe was F. sachalinensis genomic DNA and the block was F. baldschuanica genomic DNA. The F. sachalinensis probe has bound to the 22 chromosomes of the F. x bohemica parent. Figure 42b shows the reciprocal probing and block; the F. baldschuanica probe has labelled the ten chromosomes from the F. baldschuanica parent. The F. x bohemica and F. baldschuanica genomes are clearly differentiated using the technique. Whether with increased stringency, it is possible to distinguish between the genomes of F. japonica var. compacta and F. sachalinensis in F. x bohemica remains to be seen. Stace & Bailey (in press) point out that GISH is a relative, not an absolute technique, and the lower limits of its discriminatory powers remain to be determined. Certainly, there are many published studies where GISH has been used successfully to identify different genomes in inter-specific hybrids (e.g. Bailey et al., 1993; Bennett et al., 1992), but the techniques success is obviously dependent on the nature of the genomic differences between the species in question.

In addition to the application of GISH to Fallopia x bohemica and an increased understanding of the inheritance of arbitrary fingerprinting markers, increased sampling of F. x bohemica, F. sachalinensis and F. japonica var. compacta in Britain would be informative. Although high levels of genetic variability have been detected in these taxa, an intensive survey of the levels and distribution of clonal diversity is still required, preferably in conjunction with data on sex expression. Data on sex expression is one area where greater emphasis should have been placed in the current study. In mitigation, obtaining this data is difficult, as it involves making all of the collections when the plants are in flower, which effectively condenses the field season to ca. four weeks in late summer when the plants are flowering and leaf tissue

is still healthy enough to collect. Collecting early season and marking the plants for sexing at a later date is not practical, given the public urban habitats in which the plants grow. Furthermore, not all plants flower in any one year. However, practical problems aside, knowledge of the presence or absence of male fertile plants is of such key importance in the interpretation of population structure that it should be considered a priority.

Native populations

The variability of section Reynoutria in its native range is poorly understood. Given the enormous amount of attention afforded to European populations of Fallopia japonica var. japonica, it is interesting that native material matching this taxon has not yet been found. It seems that the introduced invasive populations of F. japonica var. japonica may represent a different taxon to Japanese Japanese Knotweed. As concluded in chapter 8, a taxonomic revision of the section is needed. Only when the basic alpha taxonomy has been clarified, will it be worthwhile using more costly molecular techniques. When a taxonomic framework is available, the next step will be to estimate the phylogenetic relationships of the species. Regarding an appropriate gene for a molecular phylogenetic study, it is clear that tmL evolves too slowly to offer information on intra-sectional relationships. The internal transcribed spacers (ITS) of the 18S-5.8S-28S ribosomal repeat array may be informative, as sequence data from ITS often provides greater insights into relationships among closely related species than tmL (e.g. Möller et al., in press). An alternative would be the use of introns of low-copy-number protein encoding genes (Doyle et al., 1996; Doyle & Doyle, in press). Although still early days in the development of such approaches, these introns often provide sufficient signal to resolve the phylogenies of closely related species. Not withstanding the problems of disentangling orthologous and paralogous copies of genes and the difficulties of dealing with heterozygosity (Doyle, 1994), such regions may be more informative than the technically easier chloroplast introns and the spacers in repetitive gene families such as ribosomal DNA.

Appendices

Table 18. Collection details of Fallopia samples analysed in Chapter 3

DNA code given is their DNA code. The thesis code is how they are refered to in this thesis

DNA / Thesis code	Taxon	Locality	Grid reference
Glas 1 / KelvinJMS	F. japonica	R. Kelvin	NS/568.681
Glas 2 / KelvinJMS	F. japonica	R. Kelvin Walk	NS/573.668
Glas 4 / KelvinJMS	F. japonica	R. Kelvin, view point	NS/568.677
Glas 5 / KelvinJMS	F. japonica	Kelvin Way Bridge	NS/569.663
Glas 6 / KelvinJMS	F. japonica	Kelvin Way Bridge	NS/569.663
Glas 7 / KelvinJMS	F. japonica	Nr Kelvin Way Rd Bridge	NS/569.664
Glas 8 / KelvinJMS	F. japonica	R. Kelvin, Museum	NS/565.664
Glas 9 / KelvinJMS	F. japonica	R. Kelvin, Museum, Lister memorial	NS/57.66
Glas 14 / KelvinJMS	F. japonica	Queen Margarets Bridge S Bank	NS/570.675
Glas 16 / KelvinJMS	F. japonica	Queen Margarets Bridge S Bank	NS/570.675
Glas 18 / KelvinJMS	F. japonica	R. Kelvin, Gt Western Rd Bridge	NS/575.670
Glas 20 / KelvinJMS	F. japonica	R. Kelvin, Gt Western Rd Bridge	NS/575.670
Glas 21 / KelvinJMS	F. japonica	R. Kelvin Walk	NS/574.669
Glas 23 / KelvinJMS	F. japonica	R. Kelvin Walk	NS/575.671
Glas 25 / KelvinJMS	F. japonica	R. Kelvin, Ruin	NS/572.675
Glas 26 / KelvinJMS	F. japonica	Queen Margarets Bridge N Bank	NS/571.674
Glas 27 / KelvinJMS	F. japonica	Queen Margarets Bridge N Bank	NS/571.674
Glas 28 / KelvinJMS	F. japonica	Queen Margarets Bridge N Bank	NS/571.675
Glas 29 / KelvinJMS	F. japonica	Queen Margarets Bridge N Bank	NS/571.675
Glas 30 / KelvinJMS	F. japonica	R. Kelvin, view point	NS/568.677
Glas 33 / KelvinJMS	F. japonica	Garrioch Road	NS/569.567
Glas 34 / KelvinJMS	F. japonica	Kelvindale Road Bridge	NS/563.685
Glas 35 / KelvinJMS	F. japonica	Kelvinside Conference centre	NS/555.698
Glas 36 / KelvinJMS	F. japonica	Cowal road wasteground	NS/653.692
Glas 37 / KelvinJMS	F. japonica	Possil cemetery	NS/583.698
Glas 38 / KelvinJMS	F. japonica	Cowcaddons	NS/58.66
Glas 42 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge S Bank	NS/570.675
Glas 43 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge S Bank	NS/570.675
Glas 44 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge S Bank	NS/570.675
Glas 45 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge S Bank	NS/570.675
Glas 46 / KelvinBH1	F. x bohemica	R. kelvin, Queen Margarets Bridge N Bank	NS/571.674-5
Glas 47 / KelvinBH1	F. x bohemica	R. kelvin, Queen Margarets Bridge N Bank	NS/571.674-5
Glas 48 / KelvinBH1	F. x bohemica	R. kelvin, Queen Margarets Bridge N Bank	NS/571.674-5
Glas 49 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge N Bank	NS/571.674-5
Glas 50 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge N Bank	NS/571.674-5
Glas 51 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge N Bank	NS/571.674-5

DNA / Thesis code	Taxon	Locality	Grid reference
Glas 52 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge N Bank	NS/571.674-5
Glas 53 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge N Bank	NS/571.674-5
Glas 54 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge N Bank	NS/571.674-5
Glas 56 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge S Bank	NS/570.675
Glas 57 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge S Bank	NS/570.675
Glas 58 / KelvinBH1	F. x bohemica	R. Kelvin, Queen Margarets Bridge S Bank	NS/570.675
Glas 60 / KelvinBMS	F. x bohemica	R. Kelvin, bohemica bridge	NS/568.680
Glas 61 / KelvinBMS	F. x bohemica	R. Kelvin, bohemica bridge	NS/568.680
Glas 62 / KelvinBMS	F. x bohemica	R. Kelvin, bohemica bridge	NS/568.680
Glas 64 / KelvinB?	F. x bohemica	R. Kelvin, 50 yds DS of bohemica bridge	NS/569.680
Glas 65 / KelvinB?	F. x bohemica	R. Kelvin, 50 yds DS of bohemica bridge	NS/569.680
QM1 / KelvinBH2	F. x bohemica	R. Kelvin, Queen Margarets Bridge S Bank	NS/571.674-5
QM2 / KelvinBH3	F. x bohemica	R. Kelvin, Queen Margarets Bridge S Bank	NS/571.674-5
New Bohem Bridge / KelvinB?	F. x bohemica	R. Kelvin, downstream of bohemica bridge	NS/569.680
New BBC / KelvinBJ?	F. x bohemica	R. Kelvin, steep slope next to foot path before bridge at BBC site	NS/690.675
Glas 67 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 68 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 69 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 70 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 71 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 72 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 73 / KelvinSMS2	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 74 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 75 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 76 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 78 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 79 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 80 / KelvinSMS1	F. sachalinensis	R. Kelvin, Queen Margarets Bridge	NS/569-71.674-5
Glas 84 / KelvinSMS1	F. sachalinensis	Kelvin Way Bridge	NS/569.663
Glas 85 / KelvinSMS1	F. sachalinensis	Kelvin Way Bridge	NS/569.663
Glas 86 / KelvinSMS1	F. sachalinensis	Kelvin Way Bridge	NS/569.663
Glas 88 / KelvinSMS1	F. sachalinensis	Garscube Estate	NS/551.702

Table 19. Collection details of Fallopia samples analysed in Chapter 4

Accession code given is their cultivation/DNA code. The thesis code is how they are refered to in this thesis

Dolgellau

Accession code	Thesis code	Taxon	Collection comments
D1	WnionD1	F. japonica	River bank Left
P426	WnionD2	F. japonica	Playing field
D3	WnionD3	F. japonica	River bank Left
P421	WnionD4	F. x bohemica	River bank Left
P422	WnionD5	F. x bohemica	River bank Left
P423	WnionD6	F. x bohemica	River bank Left
P424	WnionD7	F. x bohemica	River bank Left
D8	WnionD8	F. x bohemica	River bank Right
D9	WnionD9	F. x bohemica	River bank Right
D10	WnionD10	F. x bohemica	River bank Right
D11	WnionD11	F. x bohemica	River bank Right
D12	WnionD12	F. x bohemica	Playing field
D13	WnionD13	F. x bohemica	Footbridge
D14	WnionD14	F. x bohemica	River bank Right
D15	WnionD15	F. x bohemica	River bank Right
D16	WnionD16	F. x bohemica	Shingle bank
P619	WnionD17	F. x bohemica	Shingle bank
P620	WnionD18	F. x bohemica	Shingle bank
P622	WnionD19	F. x bohemica	Shingle bank
P051	WnionD20	Backcross?	

Caerynwch

Accession code	Thesis code	Taxon	Collection comments
P334	WnionC1	F. japonica	River island
P428	WnionC2	F. japonica	Hall Gardens
P326	WnionC3	F. sachalinensis	Water gardens
C4	WnionC4	F. sachalinensis	Hall Gardens Main clump
C5	WnionC5	F. sachalinensis	Hall Gardens Main clump
P328	WnionC6	F. x bohemica	Water gardens
P329	WnionC7	F. x bohemica	Water gardens
P330	WnionC8	F. x bohemica	Riverside
P331	WnionC9	F. x bohemica	Riverside
P332	WnionC10	F. x bohemica	Riverside
P333	WnionC11	F. x bohemica	Riverside
P429	WnionC12	F. x bohemica	Riverside
P430	WnionC13	F. x bohemica	Riverside
C14	WnionC14	F. x bohemica	Riverside
C15	WnionC15	F. x bohemica	Riverside
C16	WnionC16	F. x bohemica	Riverside
C17	WnionC17	F. x bohemica	Water gardens
C18	WnionC18	F. x bohemica	Riverside
C19	WnionC19	F. x bohemica	Riverside
P389a	WnionC20	F. x bohemica	93 Seedling 1
P389b	WnionC21	F. x bohemica	93 Seedling 2
P389c	WnionC22	F. x bohemica	93 Seedling 3
P389d	WnionC23	F. x bohemica	93 Seedling 4
P645	WnionC24	F. x bohemica	96 Seedling 2
P646	WnionC25	F. x bohemica	96 Seedling 3

Table 20. Collection details of Fallopia japonica var. japonica

The table shows collection details of Fallopia japonica var. japonica used in chapters 5 & 6. The accession number given is their cultivation / DNA code. The thesis code is how they are referred to in this thesis

cpDNA = samples were studied in Chapter 5; RAPDs = samples were studied in Chapter 6.

Accession No	/Thesis code	cpDNA / RAPDs	VC	Location	Grid reference
Lands End	Lands End	cpDNA / RAPDs	1	Lands End	SW/34.25
Falmouth	Falmouth	cpDNA / RAPDs	1	Falmouth	SW/80.32
D-Point	D-Point	RAPDs	1	D-Point	
P195	Chienhall	cpDNA / RAPDs	?	Chienhall	
P198	Rame	cpDNA / RAPDs	2	Rame	SX/425.495
P715	Arno's Castle	RAPDS	6	Arno's Castle	ST/6.7
P607	Isle of Wight	RAPDs	10	Buddle Inn, Isle of Wight	SZ/502.757
P608	Isle of Wight	RAPDs	10	Hamstead, Isle of Wight	SZ/390.906
P609	Isle of Wight	RAPDs	10	Carisbrooke, Isle of Wight	SZ/479885
P610	Isle of Wight	cpDNA / RAPDs	10	Dodnor lane, Isle of Wight	SZ/498908
P586	Froxsfield	RAPDs	12	Froxsfield	SU/726.267
P453	Chatham	cpDNA / RAPDs	15	Chatham	TQ/75.67
P633	Bekesbourne	cpDNA / RAPDs	15	Bekesbourne	TR/189.560
P240	Albury Heath	cpDNA / RAPDs	17	Albury Heath	TQ/061 465
P241	Albury Heath	cpDNA / RAPDs	17	Albury heath	TQ/061 465
P602	Broomfield	RAPDs	19	Broomfield	TL/70.10
P491	Staines	cpDNA / RAPDs	21	Staines	TQ/03.71
P639	Hornsey	RAPDs	21	Hornsey	TQ/30.89

Table 20 cont.

Accession No	/Thesis code	cpDNA / RAPDs	VC	Location	Grid reference
P590		cpDNA / RAPDs	23	B430 Oxfordshire	SP/53.21
Beaconsfield	Beaconsfield s	cpDNA / RAPDs	24	Beaconsfield	SU/94.90
Rudham	Rudham	cpDNA / RAPDs	28	East Rudham	TF/82.28
P493	Langwood Hill	RAPDs	29	Langwood Hill	TL/420.857
P494	Linton	cpDNA / RAPDs	29	Linton	TL/559.465
P636	Waterbeach	RAPDs	29	Waterbeach	TL/49.65
P612	Eye	cpDNA / RAPDs	32	A47, Eye	TF/22.02
P414	Leamington	cpDNA / RAPDs	38	Leamington Spa	SP/31.65
P450	Birmingham	cpDNA / RAPDs	38	Birmingham University	SP/05.85
P375	Maesteg	RAPDs	41	Maesteg	SS/85.90
P605	Neath Abbey	RAPDs	41	Neath Abbey, West glamorgan	SS/75.97
Tawe	Tawe	cpDNA / RAPDs	41	River Tawe, Swansea	SS/665.964
P196	Caersalem	cpDNA / RAPDs	41	Caersalem, Swansea	SS/65.93
Swan 1	Swan 1	RAPDs	41	Gorse Avenue, Swansea	SS/65.93
P435	Swan 6	RAPDs	41	Marina, Swansea	SS/665.975
P436	Swan 7	RAPDs	41	A483, Swansea	SS/679.931
P437	Swan 8	RAPDs	41	Kilvey Rd, Swansea	SS/664.942
P438	Swan 9	cpDNA / RAPDs	41	Kilvey Rd, Swansea	SS/664.943
P439a	Swan 10	cpDNA / RAPDs	41	B4489, Swansea,	SS/655.949
P493b	Swan 11	RAPDs	41	B4489, Swansea,	SS/655.95O
P439c	Swan 12	cpDNA / RAPDs	41	B4489, Swansea,	SS/655.951
P440	Swan 13	cpDNA / RAPDs	41	B4489 ruin, Swansea,	SS/654.961

Table 20 cont.

Accession No	/Thesis code	cpDNA / RAPDs	VC	Location	Grid reference
P441	Swan 14	cpDNA / RAPDs	41	Landore, Swansea	SS/659.959
P442	Swan 15	cpDNA / RAPDs	41	Rose hill quarry, Swansea,	SS/645.934
P443	Swan 16	RAPDs	41	Winch Wen, Swansea,	SS/685.964
P444	Swan 17	RAPDs	41	Carmel Rd, Swansea,	SS/691.963
P445	Swan 18	cpDNA / RAPDs	41	R Tawe, Wychtree St, Swansea	SS/671.973
Swan 19	Swan 19	RAPDs	41	Carmarthan Street PO.	SS/621.965
Swan 20	Swan 20	RAPDs	41	Heol Y Gors Left	SS/649.946
Swan 21	Swan 21	RAPDs	41	Carmarthan Rd, Goole Rd junc	SS/629.960
Swan 22	Swan 22	RAPDs	41	Caersalem Graveyard	SS/652.973
Swan 23	Swan 23	RAPDs	41	Caersalem Graveyard	SS/652.973
Swan 24	Swan 24	RAPDs	41	Pentrebach	SO/06.04
Swan 27	Swan 27	RAPDs	41	Carmarthan Street	SS/621.966
P452	Abergwesyn	cpDNA / RAPDs	42	River Irfon, Abergwesyn	SN/852.525
Beulah	Beulah	RAPDs	42	Beulah	SN/92.51
Llanelli	Llanelli	RAPDs	44	Llanelli	SN/50.00
P312	Amroth	RAPDs	45	Amroth	SN/16.07
P631	New Quay	RAPDs	46	New Quay, Wales	SN/39.59
Swan 25	Swan 25	RAPDs	46	Devils bridge	SN/73.76
P334	WnionC1	cpDNA / RAPDs	48	Caerynwch	SH/76.17
P428	WnionC2	cpDNA / RAPDs	48	Caerynwch	SH/76.17
P426	WnionD2	cpDNA / RAPDs	48	Dolgellau	SH/71.18
P585	Tremadog	cpDNA / RAPDs	49	Tremadog	SH/563.401

Table 20 cont.

Accession No	/Thesis code	cpDNA / RAPDs	VC	Location	Grid reference
P589	Bryn Villa	cpDNA / RAPDs	49	Bryn Villa	SH/196.326
P492	Lissington	cpDNA / RAPDs	54	Manor farm, Lissington	TF/112.837
Snells	Snells	RAPDs	55	Snells Nook	SK/50.18
Lough	Lough	cpDNA / RAPDs	55	Loughborough	SK/53.19
P629	Stoughton	RAPDs	55	Stoughton, Leicester	SK/643024
P012	Leic 1	cpDNA / RAPDs	55	Race course, Leicester	SK/617.013
Leic 2	Leic 2	RAPDs	55	Groby, Leicester	SK/53.07
Leic 3	Leic 3	RAPDs	55	Claredon Road, Leicester	SK/596.023
Leic 4	Leic 4	RAPDs	55	Howard Road, Leicester	SK/594.024
Leic 5	Leic 5	RAPDs	55	Little Holme Street, Leicester	SK/580.042
Leic 6	Leic 6	RAPDs	55	Evington Road, Leicester	SK/600.035
P625	Leic 7	cpDNA / RAPDs	55	Stoughton, Leicester	SK/63.01
Leic 8	Leic 8	RAPDs	55	Stoughton, Leicester	SK/63.01
Leic 9	Leic 9	RAPDs	55	A6, Leicester	SK/60.03
Leic 10	Leic 10	RAPDs	55	Western Boulevard, Leicester	SK/578.038
Leic 11	Leic 11	RAPDs	55	Western Boulevard, Leicester	SK/578.038
Leic 12	Leic 12	RAPDs	55	Western Boulevard, Leicester	SK/581.036
Leic 13	Leic 13	RAPDs	55	Western Boulevard, Leicester	SK/581.036
Leic 14	Leic 14	RAPDs	55	Western Boulevard, Leicester	SK/579.035
Leic 15	Leic 15	RAPDs	55	Western Boulevard, Leicester	SK/579.035
Leic 16	Leic 16	RAPDs	55	Western Boulevard, Leicester	SK/579.035
Leic 17	Leic 17	RAPDs	55	Western Boulevard, Leicester	SK/579.035

Table 20 cont.

Accession No	/Thesis code	cpDNA / RAPDs	VC	Location	Grid reference
Leic 18	Leic 18	RAPDs	55	Mere Road, Leicester	SK/604.036
Leic 19	Leic 19	RAPDs	55	Princess Road East, Leicester	SK/595.035
Leic 20	Leic 20	RAPDs	55	St Georges Retail Park, Leicester	SK/595.047
Leic 21	Leic 21	RAPDs	55	Conduit Street, Leicester	SK/595.041
Leic 22	Leic 22	RAPDs	55	Conduit Street, Leicester	SK/596.042
P634	Leic 25	RAPDs	55	Victoria Park Rd, Leicester	SK/59.02
P635	Leic 26	RAPDs	55	Leicester Botanic Gardens	SK/61.01
Leic 28	Leic 28	RAPDs	55	De Monfort Gardens	SK/594.033
Leic 29	Leic 29	RAPDs	55	Mount Street, Leicester	SK/602.047
Leic 30	Leic 30	RAPDs	55	Western Boulevard, Leicester	SK/578.038
P601	Kirkby	cpDNA / RAPDs	56	Kirkby in Ashfield	SK/48.56
P628	Brassington	cpDNA / RAPDs	57	Brassington, Derbyshire	SK/234.546
P630	Millington green	RAPDs	57	Millington green, Derbyshire	SK/262477
P195	Leigh	cpDNA / RAPDs	59	Leigh, Manchester	SJ/65.99
P415	Warrington	cpDNA / RAPDs	59	Santey station, Warrington	SJ/60.88
P457	Liverpool	cpDNA / RAPDs	59	Botanic gardens, Liverpool	SJ/3.9
P587	Liverpool	cpDNA / RAPDs	59	Liverpool	SJ/3.9
P628	Hull	RAPDs	61	Beverly Road, Hull	TA/08.29
P499	Bradford	RAPDs	63	Bradford	SE/16.32
P643	Harrogate	RAPDs	64	Harrogate	SE/30.54
P611	Windermere	cpDNA / RAPDs	69	Windermere	SD/40.98
P515	Killarchan	cpDNA / RAPDs	76	Killarchan, nr Paisley	NS/40.63

Table 20 cont.

Accession No	/Thesis code	cpDNA / RAPDs	VC	Location	Grid reference
	Bothwell	RAPDs	77	Bothwell castle,	NS/70.58
Glas 1	Glas 1KELVIN JMS	cpDNA / RAPDs	77	River Kelvin, Glasgow	NS/568.681
Glas 2	Glas 2KELVIN JMS	RAPDs	77	River Kelvin, Glasgow	NS/573.668
Glas 3	Glas 3KELVIN JMS	RAPDs	77	River Kelvin, Glasgow	NS/57.67
Glas 4	Glas 4KELVIN JMS	RAPDs	77	River Kelvin view point, Glasgow	NS/568.677
Glas 5	Glas 5KELVIN JMS	RAPDs	77	Kelvin Way Bridge, Glasgow	NS/569.663
Glas 6	Glas6 KELVIN JMS	RAPDs	77	Kelvin Way Bridge, Glasgow	NS/569.663
Glas 7	Glas7 KELVIN JMS	RAPDs	77	Nr Kelvin Way Bridge, Glasgow	NS/569.664
Glas 8	Glas 8 KELVIN JMS	RAPDs	77	River kelvin, Museum, Glasgow	NS/565.664
Glas 9	Glas 9 KELVIN JMS	RAPDs	77	River Kelvin, Lister memorial, Glasgow	NS/57.66
Glas 14	Glas14 KELVIN JMS	RAPDs	77	Queen Margarets Bridge S Bank, Glas	NS/570.675
Glas 16	Glas 16 KELVIN JMS	RAPDs	77	Queen Margarets Bridge S Bank, Glas	NS/570.675
Glas 18	Glas 18 KELVIN JMS	RAPDs	77	Gt Westn Road bridge, Glasgow	NS/575.670
Glas 20	Glas 20 KELVIN JMS	RAPDs	77	Gt Westn Road bridge, Glasgow	NS/575.670
Glas 21	Glas 21 KELVIN JMS	RAPDs	77	River kelvin Walk, Glasgow	NS/574.669
Glas 23	Glas 23 KELVIN JMS	RAPDs	77	River kelvin walk, Glasgow	NS/575.671
Glas 25	Glas 25 KELVIN JMS	RAPDs	77	River kelvin ruin, Glasgow	NS/572.675
Glas 27	Glas 27 KELVIN JMS	RAPDs	77	Queen Margarets Bridge N Bank, Glas	NS/571.675
Glas 30	Glas 30 KELVIN JMS	RAPDs	77	River kelvin view point, Glasgow	NS/568.677
Glas 33	Glas 33 KELVIN JMS	RAPDs	77	Garrioch Road, Glasgow	NS/569.567
Glas 34	Glas 34 KELVIN JMS	RAPDs	77	Kelvindale Road bridge, Glasgow	NS/56.69
Glas 35	Glas 35 KELVIN JMS	RAPDs	77	Kelvinside conference centre, Glasgow	NS/555.698
Glas 36	Glas 36 KELVIN JMS	RAPDs	77	Cowal Road , Glasgow	NS/653.692

Table 20 cont.

Accession No	/Thesis code	cpDNA / RAPDs	VC	Location	Grid reference
Glas 37	Glas 37 KELVIN JMS RAPDs		77	Possil cemetery, Glasgow	NS/583.698
Glas 38	Glas 38 KELVIN JMS RAPDs		77	Cowcaddons, Glasgow	NS/58.66
St Andrews	St Andrews	cpDNA / RAPDs	85	St Andrews	NO/51.16
P495	Balmaha	cpDNA / RAPDs	86	Balmaha, Loch Lomond,	NS/419.906
P496	Menstrie	RAPDs	87	Red Carr, Menstrie	NS/85.97
P498	Rumbling B	cpDNA / RAPDs	87	Rumbling Bridge	NT/01.99
P600	Auquharney	RAPDs	93	North Auquharney	NK/05.37
Inverie	Inverie	cpDNA / RAPDs	97	Inverie	NG/76.00
P449	Tarbet	cpDNA / RAPDs	99	Tarbet	NN/31.04
P454	Arrochar	cpDNA / RAPDs	99	Arrochar	NN/29.04
P558	Ullapool	cpDNA / RAPDs	105	Ullapool	NH/185.836,
P559	Ullapool	cpDNA / RAPDs	105	Ullapool	NH/192.819
P556	Kerracher	RAPDs	108	Kerracher	NC/179.348
P647	Shetland	cpDNA / RAPDs	112	Loch of Clickimin, Lerwick, Shetland	HV/462.409
P648	Shetland	cpDNA / RAPDs	112	Skeggie, Unst, Shetland	HP/644.130
P649	Shetland	RAPDs	112	Aithbank gardens, Fetlar, Shetland	HV/642.899
P447	Lismore	cpDNA / RAPDs	H3	Tallow turn nr Lismore, Ireland	X/09
P597	Co Cavan	cpDNA / RAPDs	H30	Ballyconnel House, Co Cavan.	H/273.185
P598	Co Cavan	RAPDs	H30	Baileborough, Co Cavan.	N/67.96
P599	Co Cavan	RAPDs	H30	Cloverhill, Co Cavan.	H/399.151
P596	Co Cavan	RAPDs	H30	Ballyhaise, Co Cavan.	H/44.10

Table 21. Collection details of Fallopia japonica var. compacta and F. sachalinenis

The table shows collection details of the samples analysed in chapters 5, 7 & 8. The accession number given is their cultivation/DNA code. The thesis code is how they are refered to in this thesis.

Access No	/ Thesis code	Taxon	Location	Vice county	Grid Reference	Sex
P002		F. japonica var. compacta	Bracken Hill	16	TQ/616.572	
P174		F. japonica var. compacta	Brecon, Breconshire	42	SO/007.127	
P193		F. japonica var. compacta	Heads of Valley	41	SO/0.0	
P413		F. japonica var. compacta	Blarney castle	H4	W/6.7	
P055	Nant	F. sachalinensis	Nant Y Frith	43	SO/265:542	Male fertile
P057	Howey	F. sachalinensis	Howey	43	SO/051.587	Male sterile
P310	Amroth	F. sachalinensis	Amroth	45	SN/166.071	Male fertile
P326	Wnion C3	F. sachalinensis	Caerynwich	48	SH/761.177	Male fertile
P335	Ciren	F. sachalinensis	Cirencester	33	SP/01.02	
P427	Wnion C4	F. sachalinensis	Caerynwich	48	SH/761.177	Male fertile
P560	Ulla	F. sachalinensis	Ullapool	105	NH/192.819	
P618	Cwrt	F. sachalinensis	Cwrt Newydd	46	SN/493.477	Male sterile
P626	Cong	F. sachalinensis	Congerstone	55	SK/36.05	
P710	Gart	F. sachalinensis	Gartshore Est, Glasgow	77	NS/69.73	Male sterile
Fort	Fort	F. sachalinensis	Fort William	97	NN/11.73	
Wind	Wind	F. sachalinensis	Windermere	69	SD/41.98	
Glas88	KelvinSMS1	F. sachalinensis	Glasgow	77	NS/571.674	Male sterile
Glas73	KelvinSMS2	F. sachalinensis	Glasgow	77	NS/571.674	Male sterile

Table 22.Collection details of Fallopia x Bohemica

The table shows collection details of the samples analysed in chapters 5 & 7.The accession number is their cultivation/DNA code. The thesis code is how they are refered to in this thesis.

cpDNA = samples were analysed in chapter 5; RAPDs = samples were analysed in chapters 7

6x Fallopia x bohemica (F. japonica var. japonica x F. sachalinensis)

Accession /	Thesis	cpDNA / RAPDs	Location	VC	Gird reference
P627	Fowey	cpDNA / RAPDs	Fowey, Cornwall	2	SX/12.51
P217		cpDNA	Holmbury Hill	17	TQ/099.430
P382	Holmbury	cpDNA / RAPDs	Holmbury	17	TQ/099.430
P388		cpDNA	Witley	17	SU/94.39
P458	L Hang	cpDNA / RAPDs	Lower Hanger Shotter	17	SU/88.32
P487	Buckingham	RAPDs	Buckingham Palace	21	TQ/29.27
P613	Hoxne	cpDNA / RAPDs	Hoxne	25	TM/181.768
P462		cpDNA	Cirencester	33	SP/02.01
P380		cpDNA	Bristol	34	ST/5.7
P346		cpDNA	Shirley, W. Midlands	38	SP/1.7
P431	Swan1	cpDNA / RAPDs	Blackpill, Swansea	41	SS/619.907
P432	Swan2	RAPDs	Blackpill, Swansea	41	SS/619.908
P434	Swan3	cpDNA / RAPDs	Blackpill, Swansea	41	SS/617.908
P309	Amroth	cpDNA / RAPDs	Amroth	45	SN/167.071
P051b	D20	RAPDs	Dolgellau	48	SH/71.18
P421	D4	cpDNA / RAPDs	Dolgellau	48	SH/71.18
P423	D6	cpDNA / RAPDs	Dolgellau	48	SH/71.18
P424	D7	cpDNA / RAPDs	Dolgellau	48	SH/71.18
D8	D8	cpDNA / RAPDs	Dolgellau	48	SH/71.18
D11	D11	cpDNA / RAPDs	Dolgellau	48	SH/71.18
D14	D14	cpDNA / RAPDs	Dolgellau	48	SH/71.18
P620	D18	cpDNA / RAPDs	Dolgellau	48	SH/71.18
P328	C6	cpDNA / RAPDs	Caerynwch	48	SH/76.17
P330	C8	cpDNA / RAPDs	Caerynwch	48	SH/76.17

6x Fallopia x bohemica (F. japonica var. japonica x F. sachalinensis) cont.

Accession /	Thesis	cpDNA / RAPDs	Location	VC	Gird reference
P333	C11	cpDNA / RAPDs	Caerynwch	48	SH/76.17
P430	C13	cpDNA / RAPDs	Caerynwch	48	SH/76.17
C14	C14	cpDNA / RAPDs	Caerynwch	48	SH/76.17
C16	C16	cpDNA / RAPDs	Caerynwch	48	SH/76.17
C19	C19	cpDNA / RAPDs	Caerynwch	48	SH/76.17
P389a	C20	cpDNA / RAPDs	Caerynwch	48	SH/76.17
P389b	C21	cpDNA / RAPDs	Caerynwch	48	SH/76.17
P389c	C22	cpDNA / RAPDs	Caerynwch	48	SH/76.17
P389d	C23	cpDNA / RAPDs	Caerynwch	48	SH/76.17
P645	C24	cpDNA / RAPDs	Caerynwch	48	SH/76.17
P646	C25	cpDNA / RAPDs	Caerynwch	48	SH/76.17
P446	Horncliffe	cpDNA / RAPDs	Horncliffe	68	NT/923.495
P514	Kilarchan	cpDNA / RAPDs	Kilarchan	76	NS/40.63
P417		cpDNA	Bothwell castle	77	NS/69.59
P615	KelvinBH1	cpDNA / RAPDs	River Kelvin, Glasgow	77	NS/571.674
Glas QM1	KelvinBH2	cpDNA / RAPDs	River Kelvin, Glasgow	77	NS/570.675
Glas QM2	KelvinBH3	cpDNA / RAPDs	River Kelvin, Glasgow	77	NS/571.674
New Bohem Bridge	KelvinBMS	cpDNA / RAPDs	River Kelvin, Glasgow	77	NS/569.680
New BBC	KelvinBJ?	RAPDs	River Kelvin, Glasgow	77	NS/690.675
P711	Gart	cpDNA / RAPDs	Gartshore, Glasgow	77	NS/69.73
P497	Alloa	cpDNA / RAPDs	Alloa	87	NS/88.92
P712	Kellas	cpDNA / RAPDs	Kellas, Morayshire	95	NJ/17.54
P031	Maam	cpDNA / RAPDs	Maam Galway	H16	L/963.533
P455	Lough Neagh	cpDNA / RAPDs	Lough Neagh N.Ireland	H40	J/13.86

4x Fallopia x bohemica (F. japonica var. compacta x F. sachalinensis)

Accession / Thesis	cpDNA / RAPDs	Location	VC	Gird reference
P343	cpDNA	Barrow Hills Whitley	17	SU/940.398
P459	cpDNA	Shalford common	17	TQ/01.46
P465	cpDNA	Pitch Hill	17	TQ/08.43
P125	cpDNA	Cheshunt	20	TL/368.028
P301	cpDNA	Cirencester	33	SP/025.023
P381	cpDNA	Cirencester	33	SP/025.023
P462	cpDNA	Cirencester	33	SP/039.033
P464	cpDNA	Cirencester	33	SP/025.023
P461	cpDNA	Preston	60	SD/5.2
P723	cpDNA	Kirkstall Abbey, Leeds	64	SE/26.35

Table 23. Collection details of artificial crosses and hybrid seed studied in chapter 5.

Artificial F1 hybrids

Accession No	Taxon	Cross details
P075b	F. x bohemica	F. japonica var. japonica x F. sachalinensis
P075c	F. x bohemica	F. japonica var. japonica x F. sachalinensis
P075d	F. x bohemica	F. japonica var. japonica x F. sachalinensis
P076b	F. japonica var. japonica x F.japonica var. compacta	F. japonica var. japonica x F.japonica var. compacta
P078a	F. x bohemica	F. japonica var. compacta x F. sachalinensis
P079a	F. x bohemica	F. sachalinensis x F.japonica var. compacta
P079c	F. x bohemica	F. sachalinensis x F.japonica var. compacta

Artificial Backcrosses

Accession No	Taxon	Cross details
P316	F. sachalinensis x F. x bohemica	F. sachalinensis x (F. japonica var. compacta x F. sachalinensis)
P318	F. sachalinensis x F. x bohemica	F. sachalinensis x (F. japonica var. compacta x F. sachalinensis)
P319	F. sachalinensis x F. x bohemica	F. sachalinensis x (F. japonica var. compacta x F. sachalinensis)
P320	F. sachalinensis x F. x bohemica	F. sachalinensis x (F. japonica var. compacta x F. sachalinensis)
P321	F. sachalinensis x F. x bohemica	F. sachalinensis x (F. japonica var. compacta x F. sachalinensis)
P322	F. sachalinensis x F. x bohemica	F. sachalinensis x (F. japonica var. compacta x F. sachalinensis)

Seed collected from known maternal parent

Accession No	Taxon	Collection details	Locality details	VC
P221	F. x bohemica	Seed collected from F. japonica var. japonica. Plant1	Cirencester	33
P230	F. x bohemica	Seed collected from F. japonica var. japonica. Plant2	Cirencester	33
P230	F. x bohemica	Seed collected from F. japonica var. japonica. Plant2	Cirencester	33
P230	F. x bohemica	Seed collected from F. japonica var. japonica. Plant2	Cirencester	33
P230	F. x bohemica	Seed collected from F. japonica var. japonica. Plant2	Cirencester	33
P230	F. x bohemica	Seed collected from F. japonica var. japonica. Plant2	Cirencester	33
P230	F. x bohemica	Seed collected from F. japonica var. japonica. Plant2	Cirencester	33
P230	F. x bohemica	Seed collected from F. japonica var. japonica. Plant2	Cirencester	33
P232	F. x bohemica	Seed collected from F. japonica var. japonica. Plant3	Cirencester	33
P232	F. x bohemica	Seed collected from F. japonica var. japonica. Plant3	Cirencester	33
P241a	F. x bohemica	Seed collected from F. japonica var. japonica. Plant1	Albury Heath	17
P241b	F. x bohemica	Seed collected from F. japonica var. japonica. Plant1	Albury Heath	17
P241c	F. x bohemica	Seed collected from F. japonica var. japonica. Plant1	Albury Heath	17

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