Unravelling the Japanese Knotweed story

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WHAT'S MY AGENDA?

Impartial observer of a fascinating Biological Phenomenon

I don't only have a UK perspective - interested in JK worldwide

I'm more interested in the sex life of these plants than I am in killing them

Japanese Knotweed (*Fallopia japonica*) at home in Wales

Japanese Knotweed has gained much notoriety in the West as an invasive and persistent weed

There has been much press attention on Japanese Knotweed

Largest female on Earth could strangle Britain

Reports by Roger Highfield, Science Editor

A PLANT alien to Britain may soon be able to have sex, spread seed and tighten its stranglehold on the countryside, scientists believe.

Yesterday, botanists presented research on the extent and nature of the

far less invasive but both male and female fertile plants are found in Britain.

Researcher Catherine Pashley 14A/82268

ed (*Heracleum mantegazzianum*), bracken (*Pteridium aquilinum*), Himalayan lora), rhododendron (*Rhododendron ponticum*) and Japanese seaweed (*Sargassum muticum*)

Monster weed is tightening grip

may swamp countryside

By Roger Highfield, Science Editor

DAVID MONTGOMERY

Japanese knotweed, could be distributed large distances in

Largest female on Earth could strangle Britain

Reports by Roger Highfield, Science Editor

Edinburgh International Science Festival

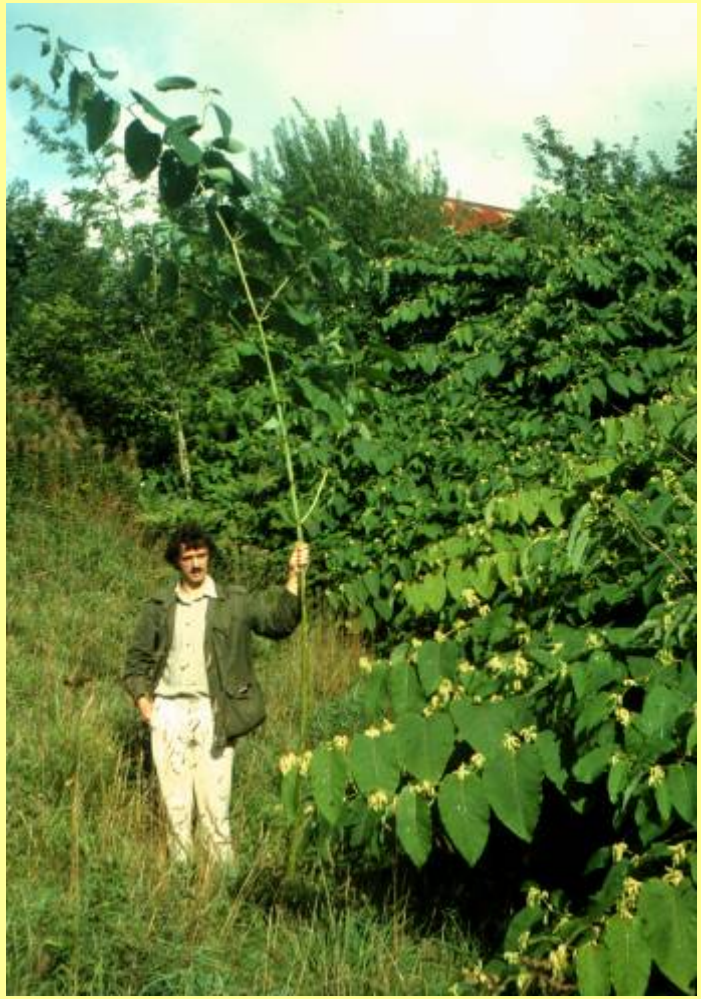
A PLANT alien to Britain may soon be able to have sex, spread seed and tighten its stranglehold on the countryside, scientists believe.

Yesterday, botanists presented research on the extent and nature of the far less invasive but both male and female fertile plants are found in Britain.

Now some of the hybrids between these species have been found to be fertile, creating another invasive weed.

The Japanese Knotweed

The press coverage isn't all 'hype' - make no mistake these plants are capable of prodigious growth



Lines of enquiry

1. Historical
2. Taxonomical
3. Morphological
4. Anatomical
5. Breeding behaviour
6. Artificial hybridisation
7. Cytological
8. Molecular

FALLOPIA ADANSON

Section Fallopia: annual climbers *F. convolvulus*, *F. dumetorum* and *F. scandens*

Section *Paragonum* HARALDSON: smaller perennial climbers *F. cilinodis* and *F. cynanchoides*

Section *Sarmentosae* (GRINTZ) HOLUB: the larger perennial climbers *F. multiflora* and *F. baldschuanica*

Section *Reynoutria* (HOUTT) RONSE DE CRAENE: herbaceous non-climbing perennials *F. japonica* and *F. sachalinensis*

Introduction to Europe

Phillip von Siebold imported a whole range of novel plant species from the East in the first half of the 19th Century.

This was a commercial enterprise and he produced regular catalogues

Siebold reported that in 1847 his **Japanese Knotweed** was awarded a gold medal by the Society of Agriculture & Horticulture at Utrecht for the

‘***most interesting new ornamental plant of the year’.***

Entry from the 1856 sales list of von Siebold & Co.

Polygonum filiforme *Thbg.*	c	id.	1	
— pictum *Sieb.*	c	id.	1	
— Sieboldii *Reinw.* [2])	c	Pl.'dor.etfour.		1
— — la douzaine				3
— — le cent.				25

2) Cette Renouée est une de nos introductions les plus importantes du Japon, une plante d'ornement vivace, inextirpable, d'un feuillage luisant et des fleurs en grappe très gracieuses, par laquelle on peut improviser des bosquets, abriter les jeunes plantations et fortifier les collines sabloneuses et les dunes. L'herbe qu'on peut faucher en printems à plusieurs reprises fournit un fourrage excellant pour l'engraissement des bestiaux qui la mangent de préférance; les fleurs, qui paraissent en automne, sont très mielleuses et donnent aux abeilles leur provision d'hiver; la racine amère et tonique est un médicament réputé chez les Chinois et les Japonais; enfin les tiges même qui meurent en hiver sont bonnes à bruler et pour en faire des allumettes.

Polygonum Sieboldii is Japanese Knotweed

The numbers on the right are prices in francs

Disembarkation in Britain

9 August 1850 - an unsolicited package of plants from von Siebold, including Japanese Knotweed reaches RBG Kew (Kew Inwards Book 1848-58)

26 April 1854 - '*Polygonum sieboldii*' arrives at RBG Edinburgh from Jackson's of Kingston - a stones throw from Kew (Edinburgh 'Plant Book' 1849-1855)

THE ARRIVAL IN ENGLAND

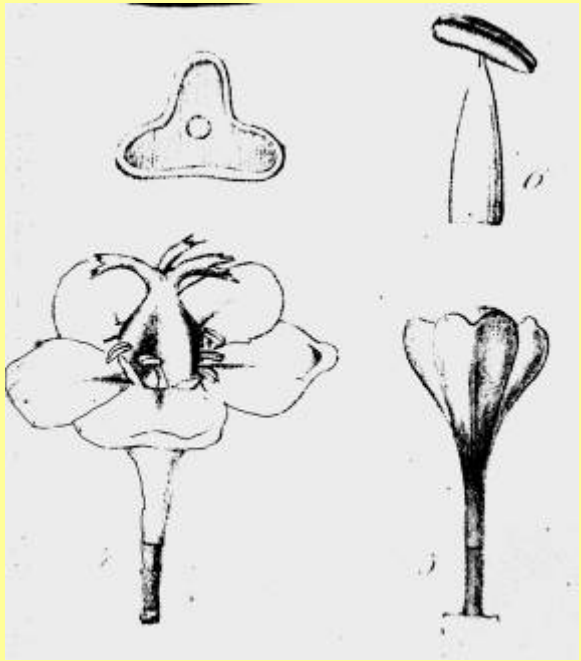
RBG Kew 'Inwards book" 1848-1858

Entry dated 9 August 1850 records that a large batch of plants was received from M. Siebold of Leiden including a certain ***Polygonum sieboldii*** (Japanese Knotweed)

'They are intended to be in exchange for new China and Japan plants. But on account of the bad selection he is written to, telling him that only 6 of them are probably new to us'

What do we know about Siebold's plant?

Fortunately some contemporary illustrations and herbarium material exist. They match the morphology and sex of our current plant.



De Vriese 1849

The role of Gardeners

William Robinson, proponent of the Wild Garden, recommended Japanese Knotweed under:

'***Plants with large or graceful foliage suitable for naturalisation'***

Mrs C.W Earle 1897

'..is the handsomest, easiest grown, hardiest, most useful plant for London gardens'

Favoured by Gertrude Jekyll, and included in several of her planting lists - though she did recant a little by 1900:

'We should not forget the quick growing ways of the great Japan Knotweeds growing fast and tall'

Nursery Gardens Stocking Japanese Knotweed *s.l.*

Nursery	Town	date or catalogue Number	japonica	compacta	sachalinensis	Comments
Backhouse, J. & Son	York	1895	1/-	-	1/6d	
Backhouse, J. & Son	York	1898	1/-	1/-	1/6d	
Bull, William	Chelsea	1869-70	-	-	7/6d	
Dickson's Nurseries	Chester	1901	6d	9d	6d	
Gauntlett, V. N.	Redruth	No. 88	1/-	-	9d	Japanese Nurseries
Haage & Schmidt	Erfurt	1878	15pf	40pf	-	
Jackson, T. & Son	Kingston	1854	+			Sent F. japonica to RBG Edin
Methven, & Sons	Edinburgh	1891	9d	-	1/9d	
Perry, Amos	Enfield	1936/7	9d	5/-	9d	
Siebold, von P.F.	Leiden	1848	26 plants/500F	2F		Sent F. japonica to RBG Kew 1850
Smith, Richard	Worcester	1880-81	9d	9d	1/-	
Smith, T.	Newry	1891 (No6)	9d	1/-	1/-	Daisy Hill Nurseries
Wallace & Co.	Colchester	1897	-	-	6d	Kilnfield Gardens
Wood, J.	Kirkstall,	Pre 1902?	6d	6d	9d	Wood's Hardy Plant Club

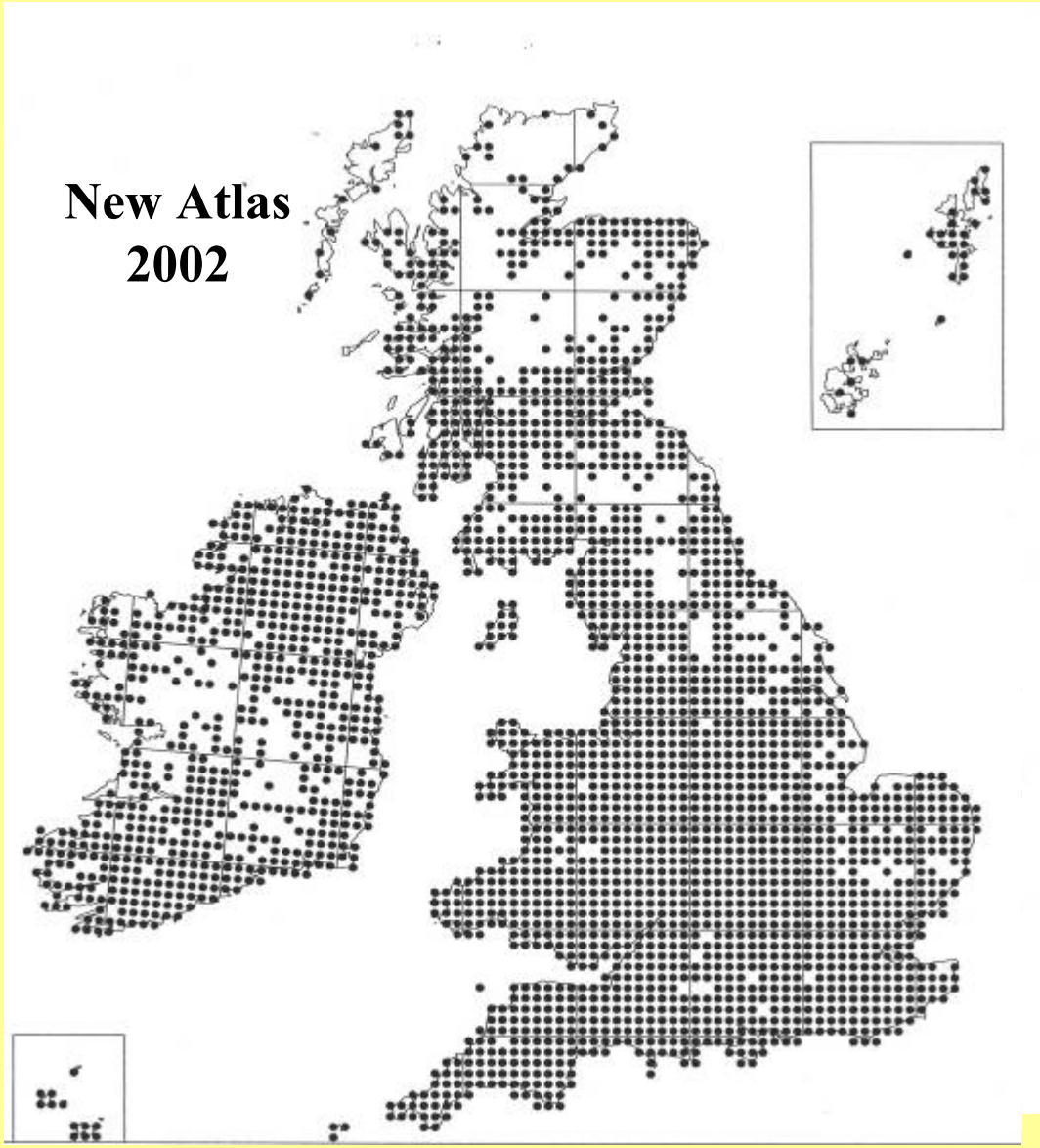
The invasion of the British Isles

It didn’t take long for this invited guest to outlive her welcome.

Gardeners acting in the time honoured manner dug up and discarded pieces of the rhizome, so promoting the invasion.

It is now recorded from 2761 of the 3859 10 x 10km recording squares in Britain

The spread of Japanese Knotweed



Data from Conolly 1977 & Preston et al 2002

Breeding Behaviour and Reproductive Biology

Early in my PhD in the 1980s I discovered that:

a. **These plants are gynodioecious i.e. they exist as male and female individuals**

b. **The octoploid *F. japonica* var. *japonica* here was all male sterile**

c. **As a consequence of the presence of only female Japanese Knotweed, extensive hybridization was occurring**

Japanese Knotweed *s.l* is gynodioecious

Female(male-sterile)

Hermaphrodite (male fertile)

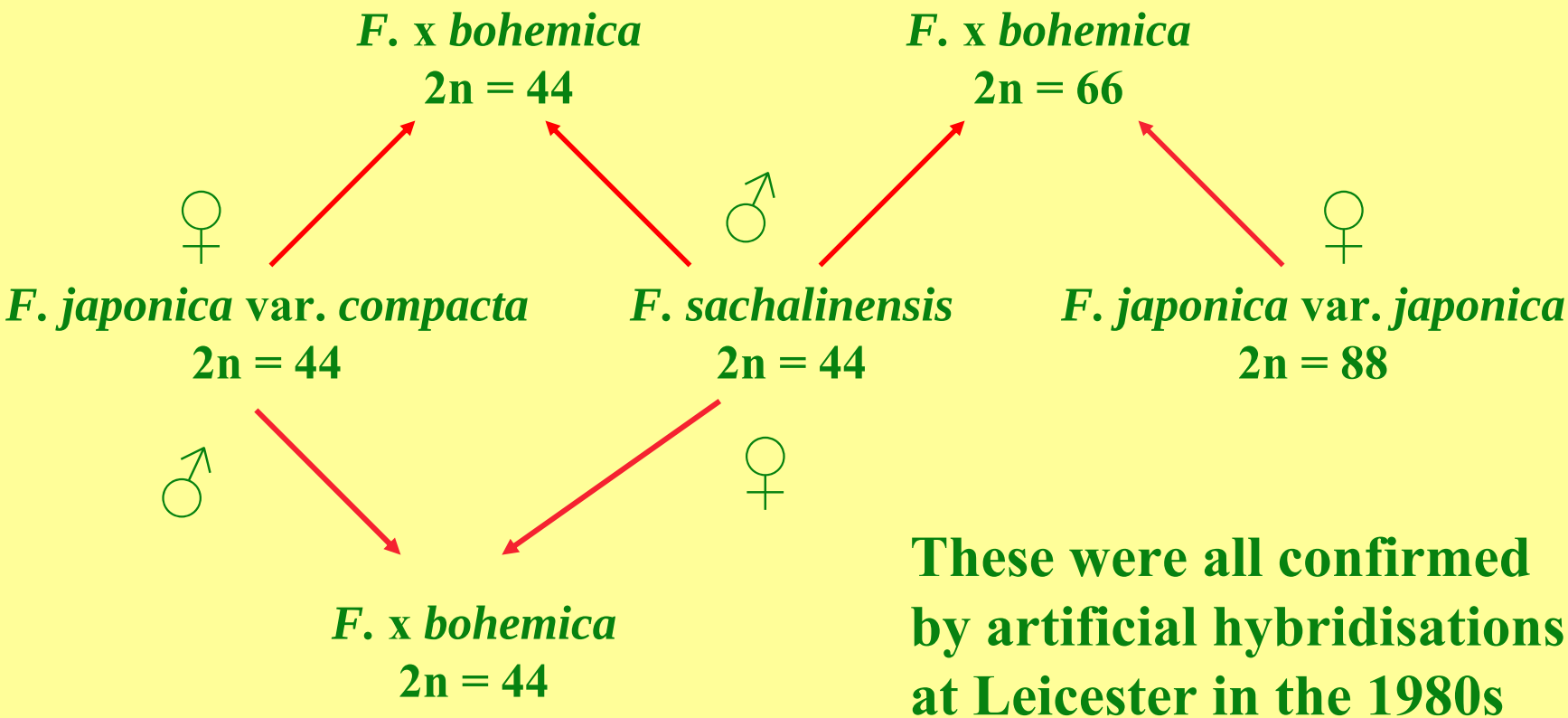
Flowers are ‘perfect’ so even females have tiny empty stamens

The role of Cytology

When I began my studies, the accepted word was that both *F. japonica* and *F. sachalinensis* were found as males and females, though male *japonica* was rather rare

The application of cytology changed all this and showed that only female japonica occurred and the males thought to be *japonica* were actually hybrids with *F. sachalinensis* - now known as *Fallopia* x *bohemica*

Cytological background of *F.* x *bohemica*

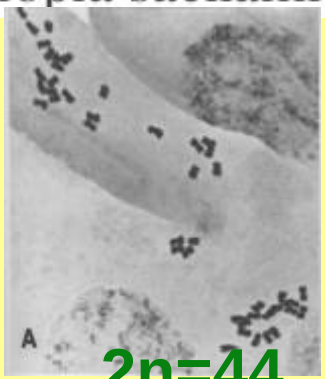


These were all confirmed by artificial hybridisations at Leicester in the 1980s

HOW DO YOU IDENTIFY *FALLOPIA* X *BOHEMICA*?

Leaf shape and chromosome number?

2n=66



2n=44

2n=88

THIS IS ALL VERY WELL, BUT CHROMOSOME COUNTS ARE HARD TO COME BY AND WHAT ABOUT DRIED SPECIMENS?

ANOTHER PROBLEM WAS THAT DRIED SPECIMENS RARELY HAD THE LARGER LEAVES WHICH SHOWED THE SHAPE CHARACTERS BEST

LED TO AN EXAMINATION OF EPIDERMAL CHARACTERS OF THE LEAF LOWER SURFACE

Morphology

& Anatomy

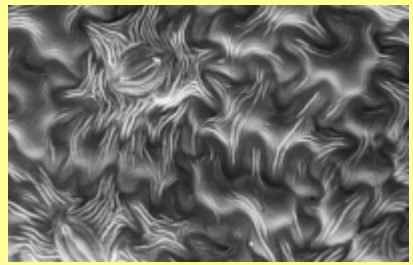
Leaf lower epidermis

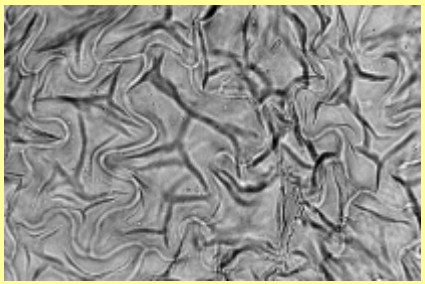
Epidermal peels
Light microscopy

Scanning electron microscopy

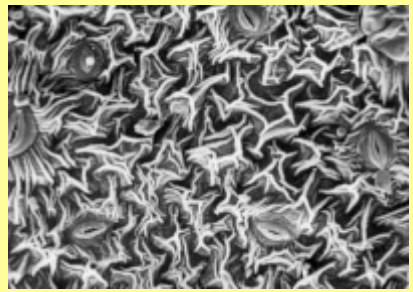
F. japonica

F. x *bohemica*





F. sachalinensis



Japanese Knotweed *s.l.*

Fallopia japonica var. *japonica*

Fallopia japonica var. *compacta*

Fallopia sachalinensis

Fallopia x *bohemica* (*F. japonica* x *F. sachalinensis*)

Fallopia x *conollyana* (*F. japonica* x *F. baldschuanica*)

Backcrossses between the hybrid and its parents and F2's from *F.* x *bohemica*

However this was not the whole answer - male *F. sachalinensis* was rather rare in the wild, and var. *compacta* was hardly ever naturalised in Britain *yet* isolated plants of *F. japonica* were still able to produce seed in the absence of these two taxa!

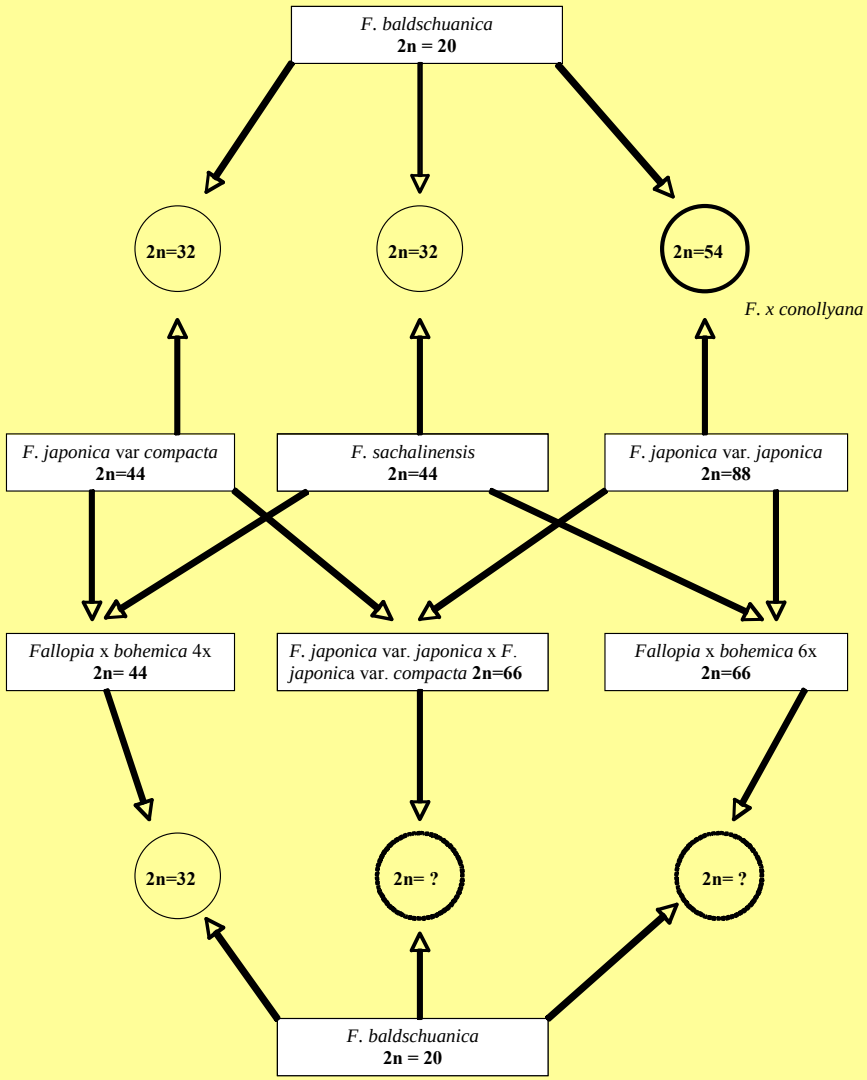
What was going on?

Most of the seed collected from *F. japonica* had 54 chromosomes and grew into a plant with a twining habit.

This was found to be a hybrid with *F. baldschauanica*, and has since been found naturalised in a few places in Europe.

Now named as *Fallopia* x *conollyana*

Pollinators of Japanese Knotweed



This figure from my 1989 thesis has needed little modification in the succeeding years

Circles: bold line = found as seed and established plants; normal line = found only as seed: dotted line = not yet found

THE ROLE OF REPRODUCTION BY SEED?

Since there is no male 8x var. *japonica* in Britain, *F. japonica* var. *japonica* cannot reproduce itself sexually - all the dramatic spread in the British Isles is by vegetative means

***F. japonica* var. *japonica* produces large amounts of viable seed in the UK and continental Europe - but this very very rarely becomes established spontaneously**

Japanese Knotweed seed set

This sample from Dolgellau (Wales) has a remarkable amount of seed. It has been pollinated by the hybrid *F.* x *bohemica*.

Seedling establishment?

River Ain near Lyon, France August 2006

An unusual mixture of 8x *F. japonica* and 8x *F.* x *bohemica*

4 seedlings were collected from the River Ain and grown on in Leicester

All were 8x - not known whether they were from the hermaphrodite 8x *F.* x *bohemica* or the 8x *F. japonica*

Ploidy levels of *F.* x *bohemica* in the wild

Country	Reference	4x	6x	8x	10x	Sample size
British Isles	Bailey & Wisskirchen 2006	**21%**	**75%**	**4%**	**0%**	**51**
Continental Europe (excl Czech Rep)	Bailey & Wisskirchen 2006	**0%**	**86.4%**	**13.6%**	**0%**	**42**
Czech Republic	Mandak et al 2003	**2.1%**	**92.5%**	**5.3%**	**0%**	**94**
USA	Bailey unpublished	**0%**	**96.4%**	**0%**	**3.6%**	**28**

Seed produced by *F*. x *bohemica* in Britain

Ploidy Level	No. seeds	Chr. Nos.	Putative male parent	Location
4x female	10	2n=32	*F. baldschuanica*	Surrey
	2	2n=44	*F. sachalinensis*	Cirencester
6x female	8	2n=ca73,74,75, 2 x 76, ca77, ca98, 110	*F*. x *bohemica*	Dolgellau
6x hermaphrodite	8	2n=69-71,ca 85,95-97, 96, **ca121**	*F*. x *bohemica*	Dolgellau
8x female	2?	2n=66**	*F*. sachalinensis	Dolgellau
8x hermaphrodite	2	2n=88	*F*. x *bohemica* self-pollinated	Dolgellau

THE ROLE OF SEED PRODUCTION?

***F. japonica* var. *japonica* has successfully invaded the British Isles by clonal reproduction alone - this means that there is no genetic variation present**

The hybrid *F.* x *bohemica* may also spread clonally, but there is much more genetic variation present

Back-crosses between the hybrid and its parents could potentially lead to the production of genotypes better suited to the British Isles

Need for a molecular approach

I eventually reached the point where I felt I had answered all the questions that I could with the resources available to me, and actually moved on to some other areas of research

However I was not to escape the embrace of the Knotweed for long!! A fortuitous series of events brought about the possibility of bringing DNA fingerprinting to bear on the question.

Molecular techniques allowed fundamental new questions to be asked about the invasion

Need for a molecular approach

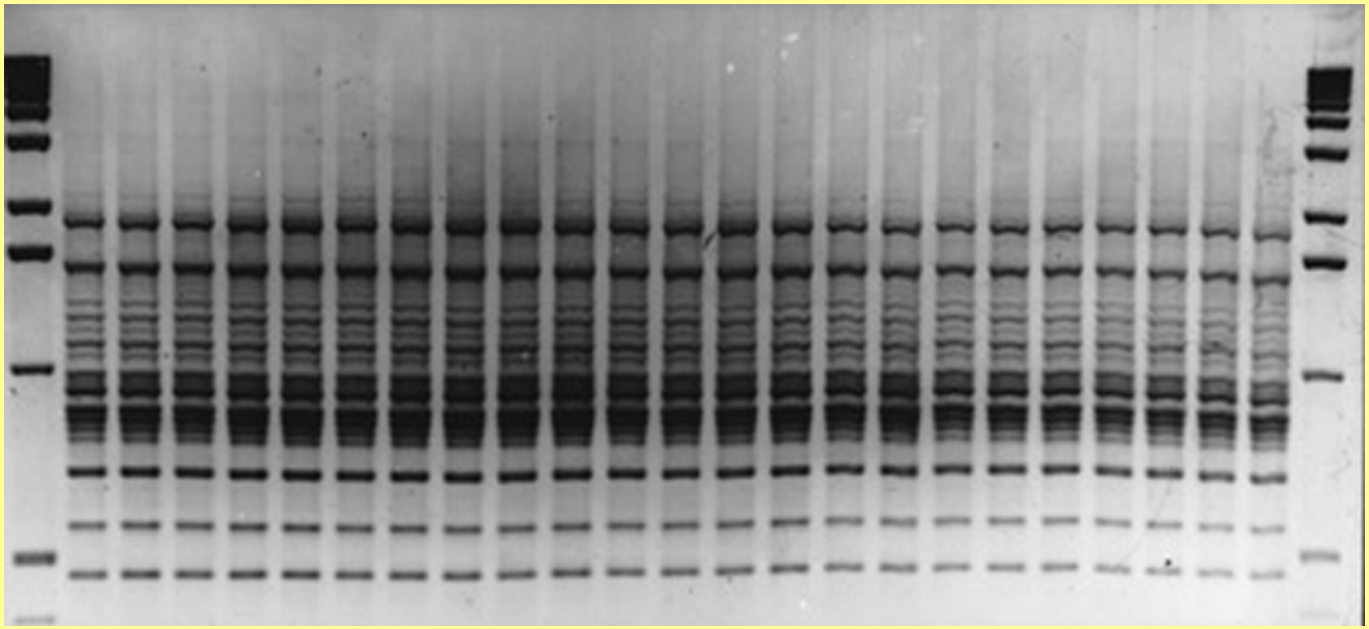
Was it possible that all the female *F. japonica* var. *japonica* in Britain was a single clone propagated vegetatively from the Von Siebold introduction?

Was the *Fallopia* x *bohemica* population made up of a few wide-spread clones, or had it arisen many times from seed?

Where did our introductions come from originally?

Invasion of the cloned female?

Hollingsworth & Bailey (2000) Bot J Linn Soc examined 150 accessions of Japanese Knotweed from around the British Isles, Continental Europe and the USA



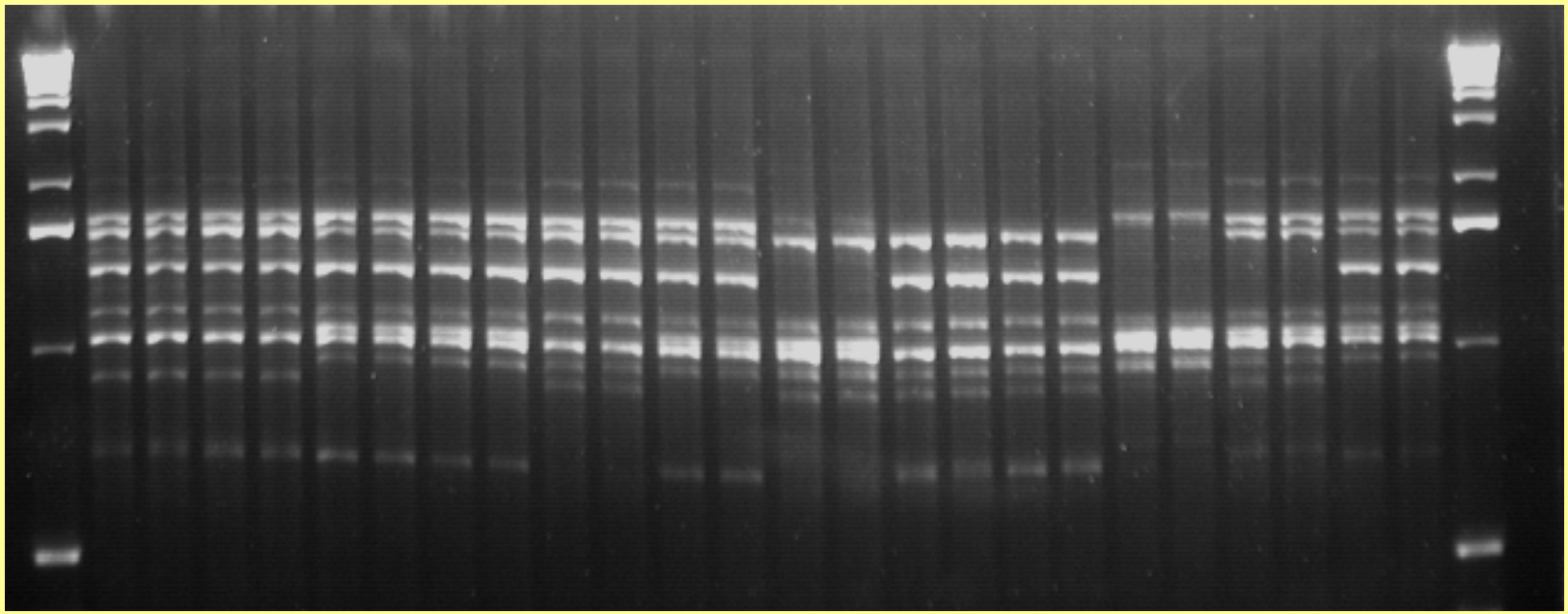
RAPD GELwith 23 accessions of Japanese Knotweed (Hollingsworth 1998)

Origins of *F*. x *bohemica*

Our research concentrated on certain Knotweed 'hotspots' where JK, hermaphrodite *F. sachalinensis* and *F*. x *bohemica* were known to grow together - surprisingly few in UK!

The most studied site was at Caerywnch Hall near Dolgellau in Wales, where the plants all grew together in an abandoned Victorian water garden, next to a small river. The larger river in Dolgllau town was unusually heavily infested with Knotweeds

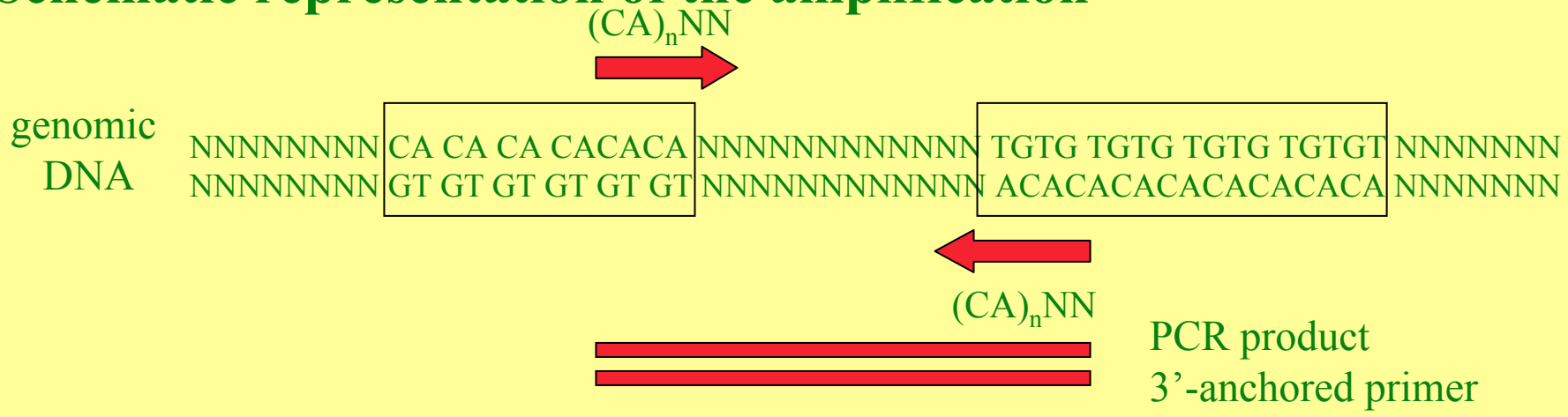
In the Dolgellau study RAPDs were replaced by the more reliable ISSRs



Identification of clones

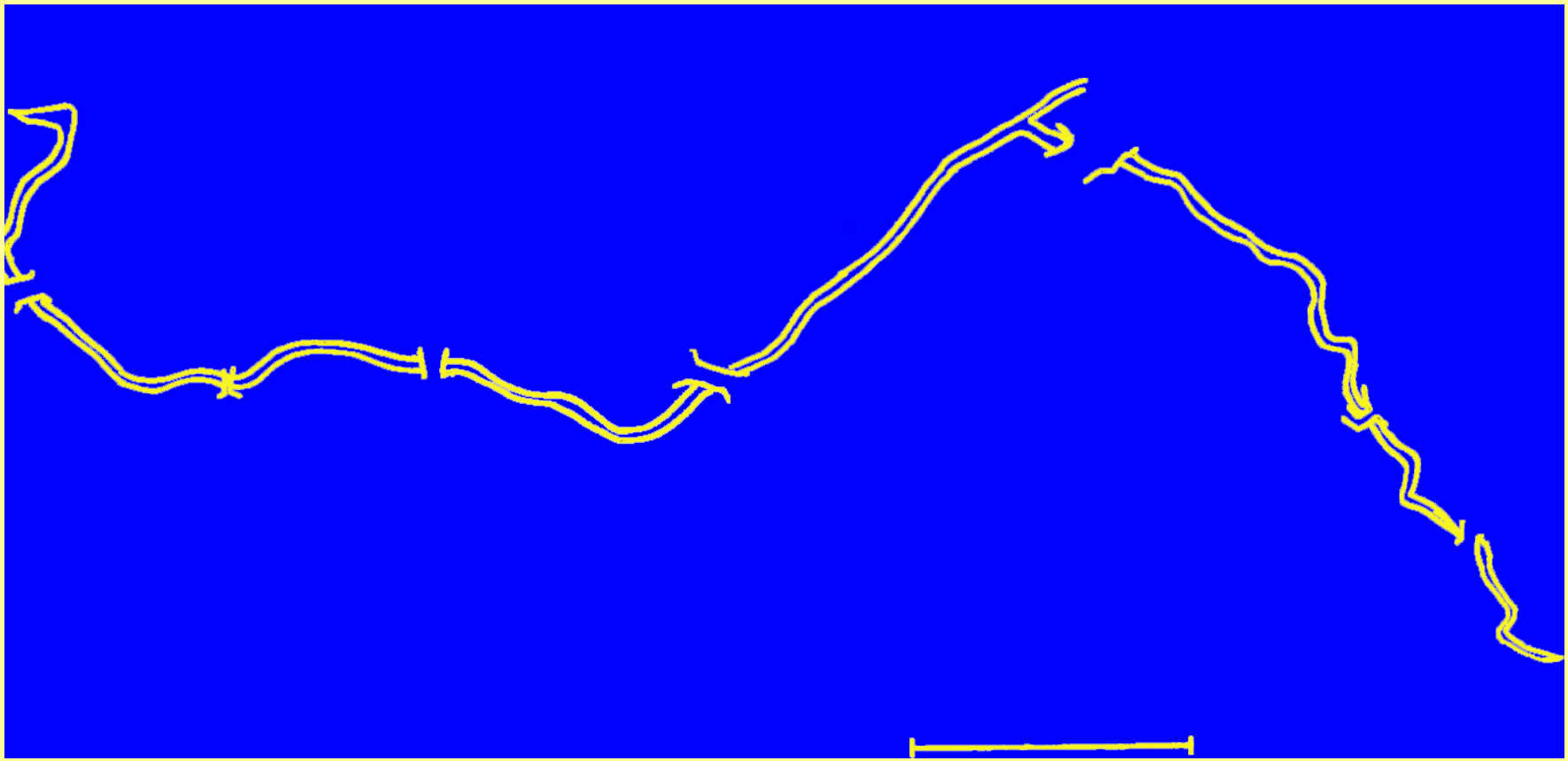
- Microsatellites / Simple Sequence Repeats (SSR)
  - Short, tandem repetitive DNA sequences
  - Repeat length of 1-5 base pairs
- Inter-SSR PCR / Anchored microsatellites
  - Anchored by extending outside the repeat into a unique sequence
  - Nucleotide sequence between two SSR priming sites orientated on opposite DNA strands
  - Reproducible multi-locus patterns

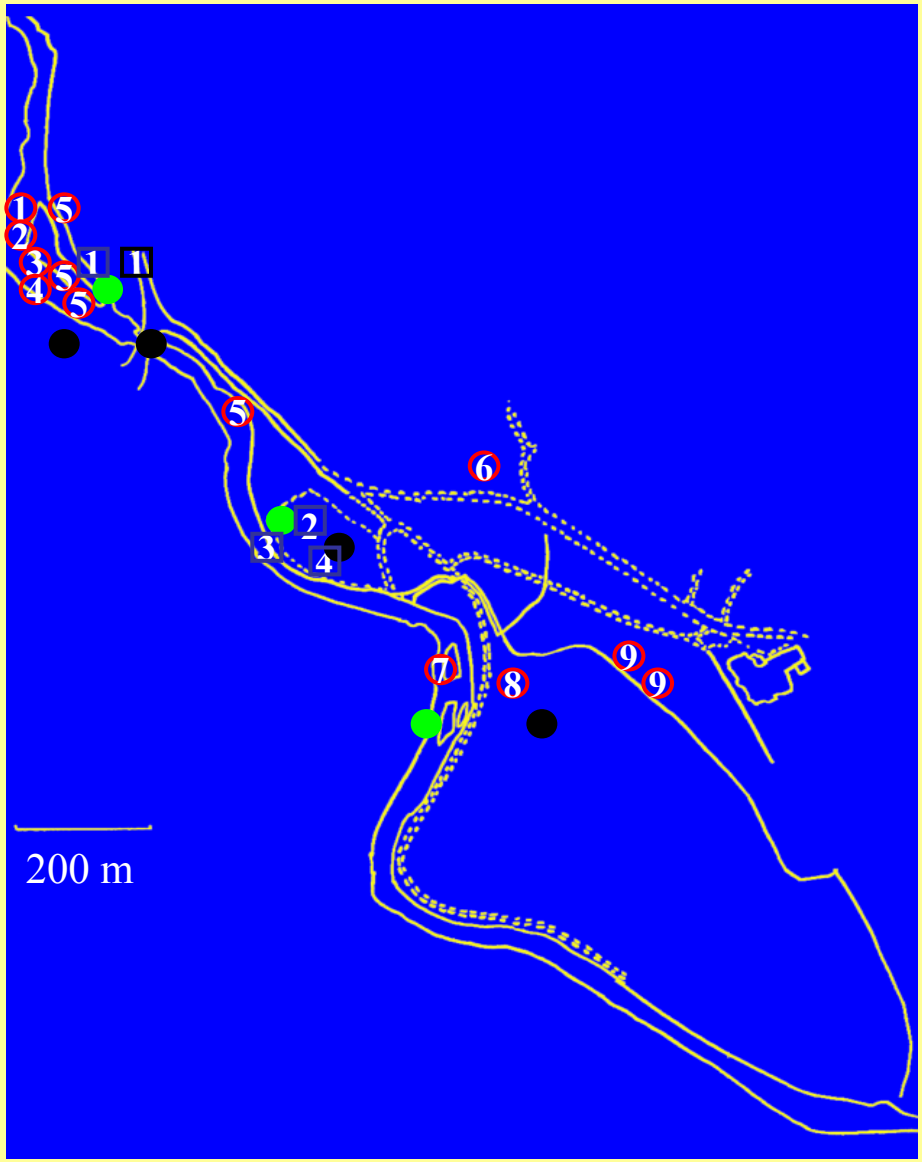
Schematic representation of the amplification



Dolgellau, Merioneth, Wales.

(Pashley *et al*., 2002)





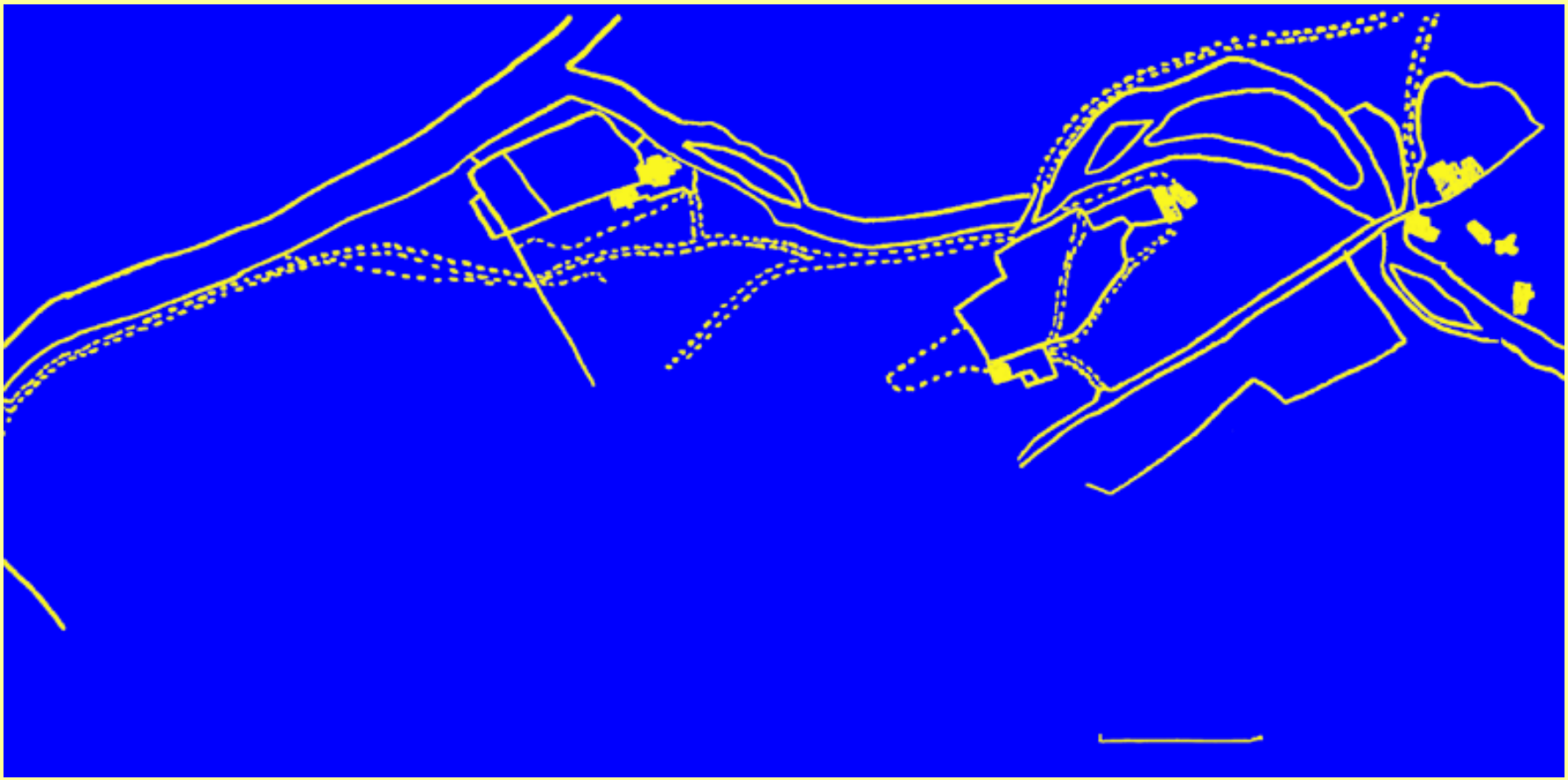
Genetic diversity of Japanese Knotweed *s.l.* at Caerynwch

(Pashley *et al.*, 2002)

- *F. japonica*
- *F. sachalinensis*
- *F.* x *bohemica*
- F1 *F.* x *bohemica* seedling
- Back-crossed *F.* x *bohemica* seedling

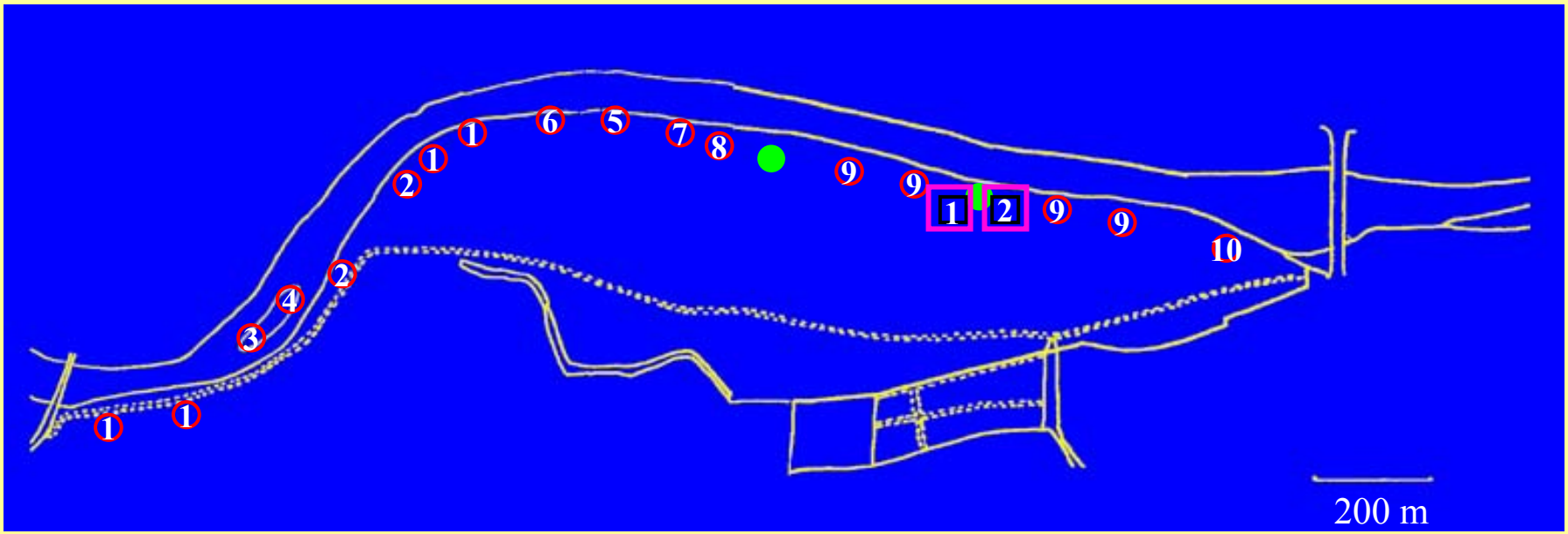
Torrent walk Dolgellau Wales

(Pashley *et al.*, 2002)



Dolgellau recreation ground

(Pashley *et al*., 2002)



○ *F*. x *bohemica*

● *F. japonica*

□ Backcrossed *F*. x *bohemica* seedling

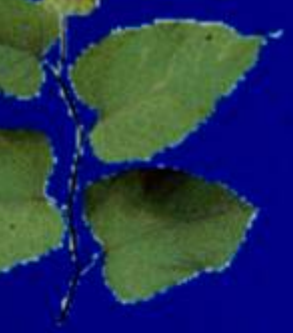
CHLOROPLAST HAPLOTYPES

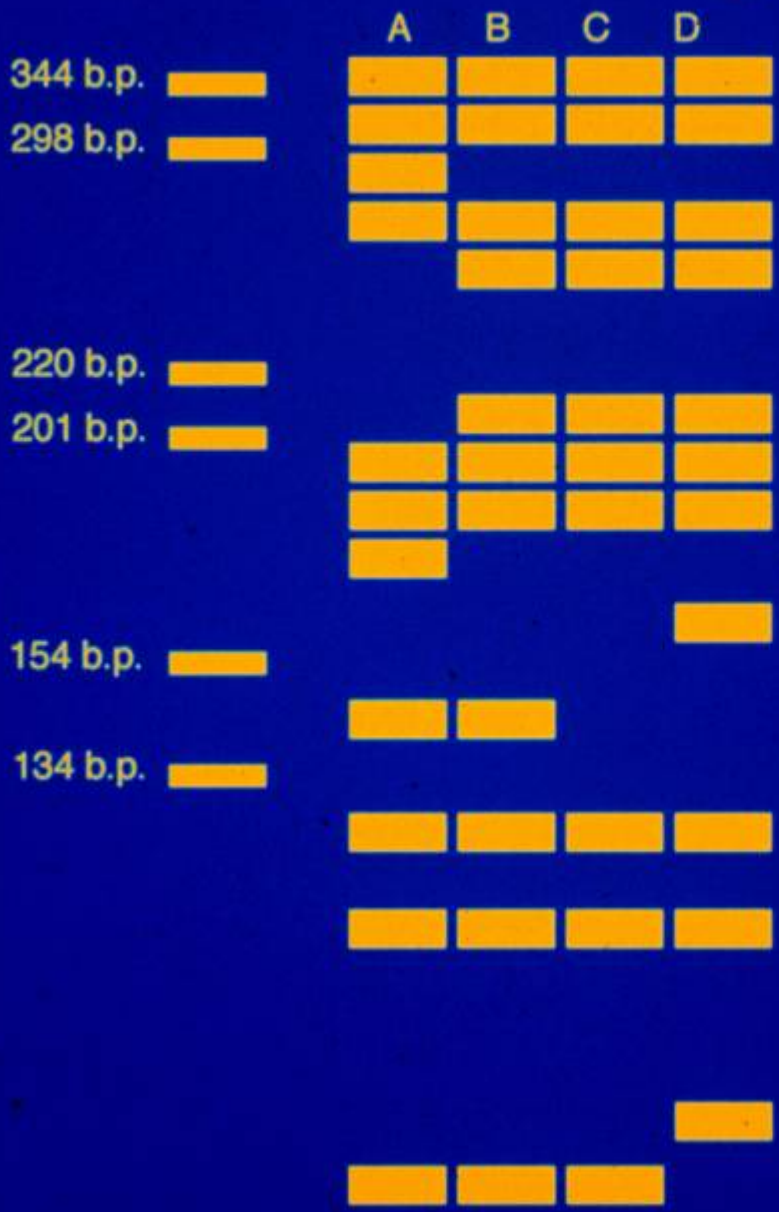
Mutations in certain non-coding parts of the chloroplast genome were discovered that were capable of distinguishing the 4 different types of Knotweed in Britain. This enabled us to produce chloroplast haplotypes which:

a. Allowed us to identify the female parent in any *F*. x *bohemica* hybrids

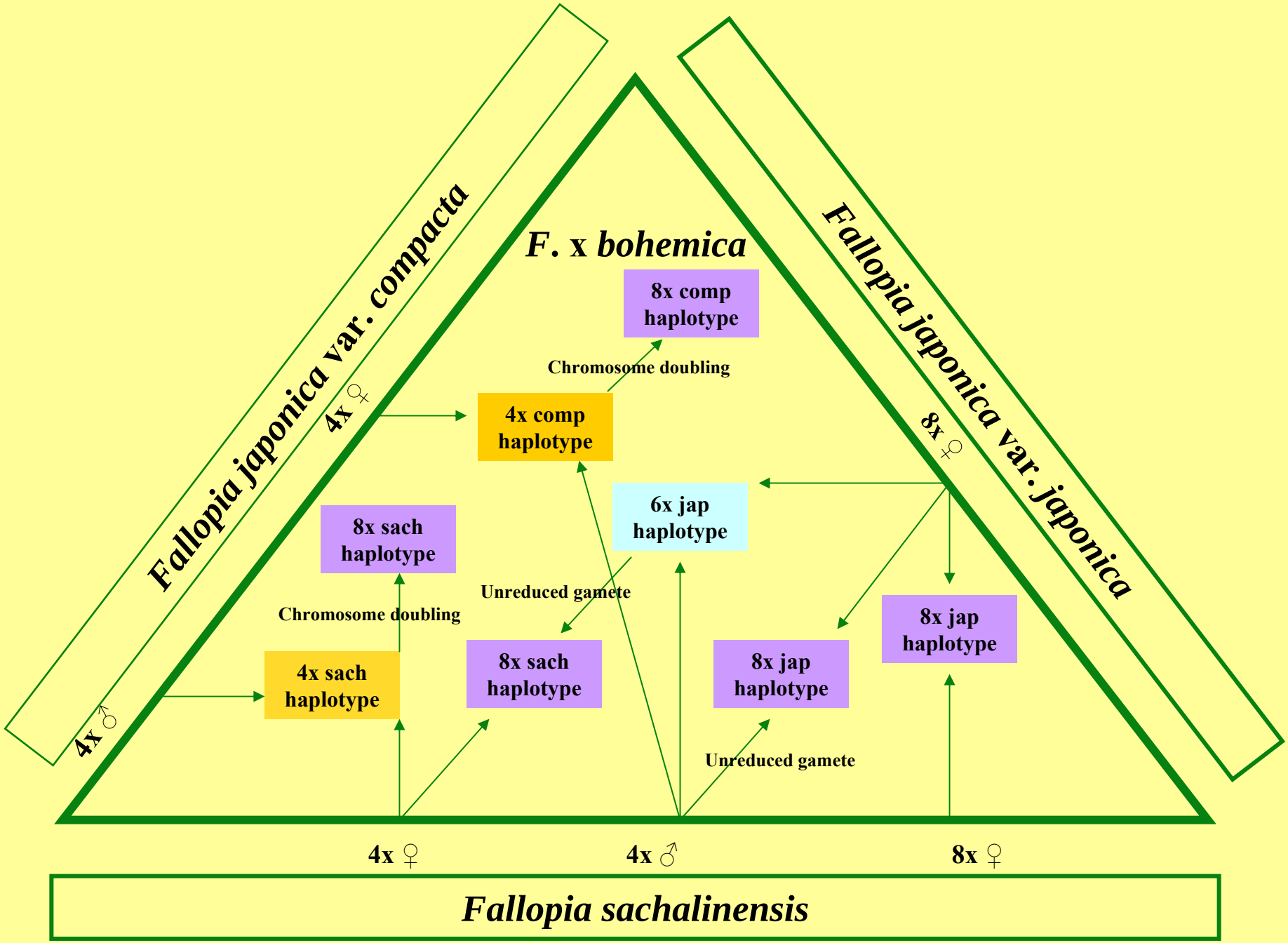
b. Gave us a geographical handle on the plants since this sort of variation is know to vary with location of origin

*Hinf*I RFLP fingerprint of chloroplast trnC-D region





KEY:
A: British *F. japonica* var. *compacta*
B: British *F. sachalinensis*
C: Chinese *F. japonica* var. *japonica*
D: British *F. japonica* var. *japonica*


Fallopia japonica var. compacta
Fallopia japonica var. japonica
F. x bohemica
8x comp haplotype
Chromosome doubling
4x ♀
4x comp haplotype
8x ♀
6x jap haplotype
8x sach haplotype
Unreduced gamete
Chromosome doubling
8x jap haplotype
4x sach haplotype
8x sach haplotype
8x jap haplotype
4x ♂
Unreduced gamete
4x ♀
4x ♂
8x ♀
Fallopia sachalinensis

Where do you come from?

Using herbarium specimens and floras it is possible to map the distribution.

However, Japanese Knotweed has a very wide distribution range

Origin of European Japanese Knotweed *s.l.?*

Historical evidence suggests Japan - the exploits of von Siebold are well documented. There was an earlier Chinese introduction in London but it did not thrive.

Morphological evidence suggests Japan - examination of herbarium specimens indicated characteristic differences in leaf shape between the Japanese and Chinese plants

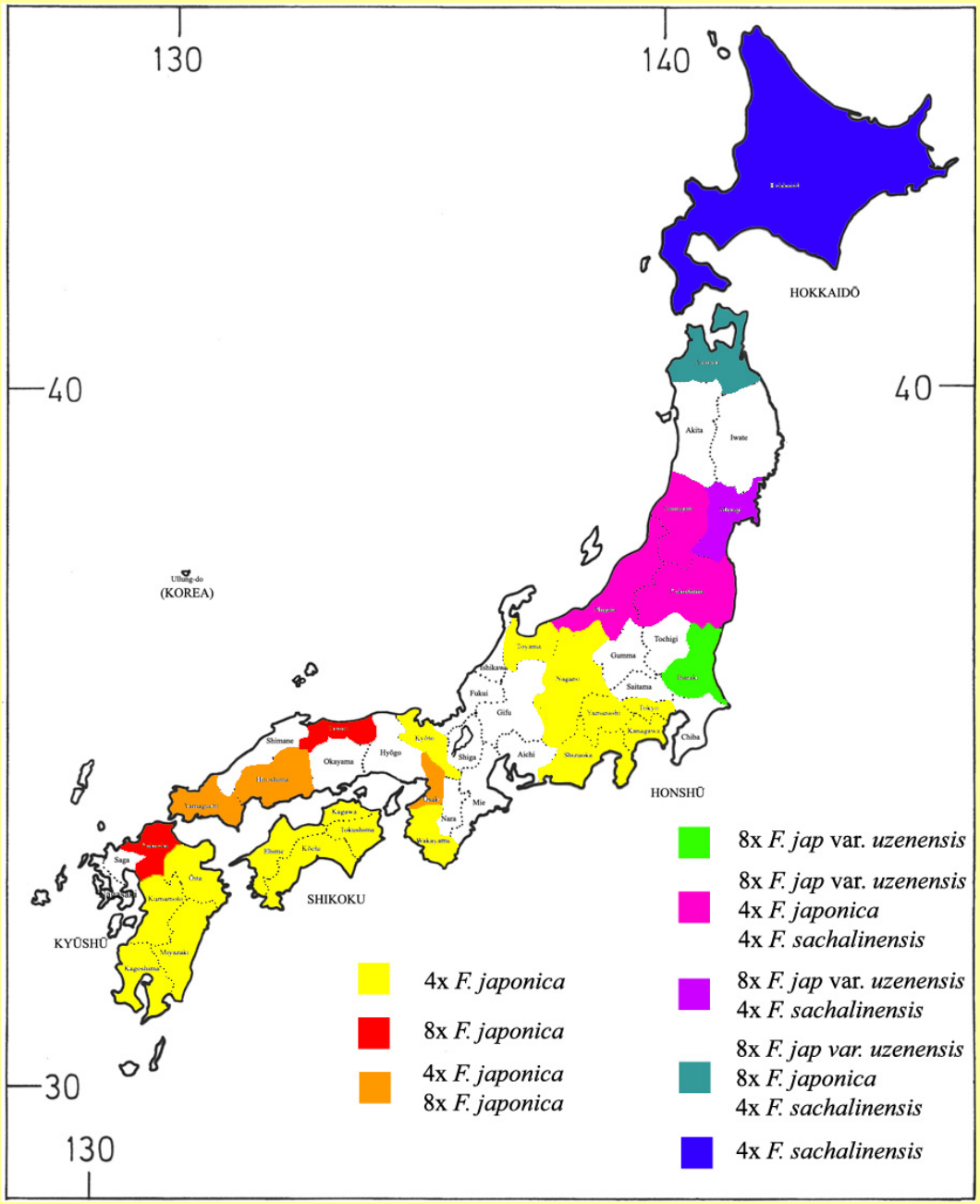
The commonly mentioned 1825 introduction date for London was of a Chinese plant - which is not the source of the invasion and has vanished without trace

The importance of origin

Due to problems with herbicide control a BIOLOGICAL CONTROL programme was launched by CABI Biosciences:

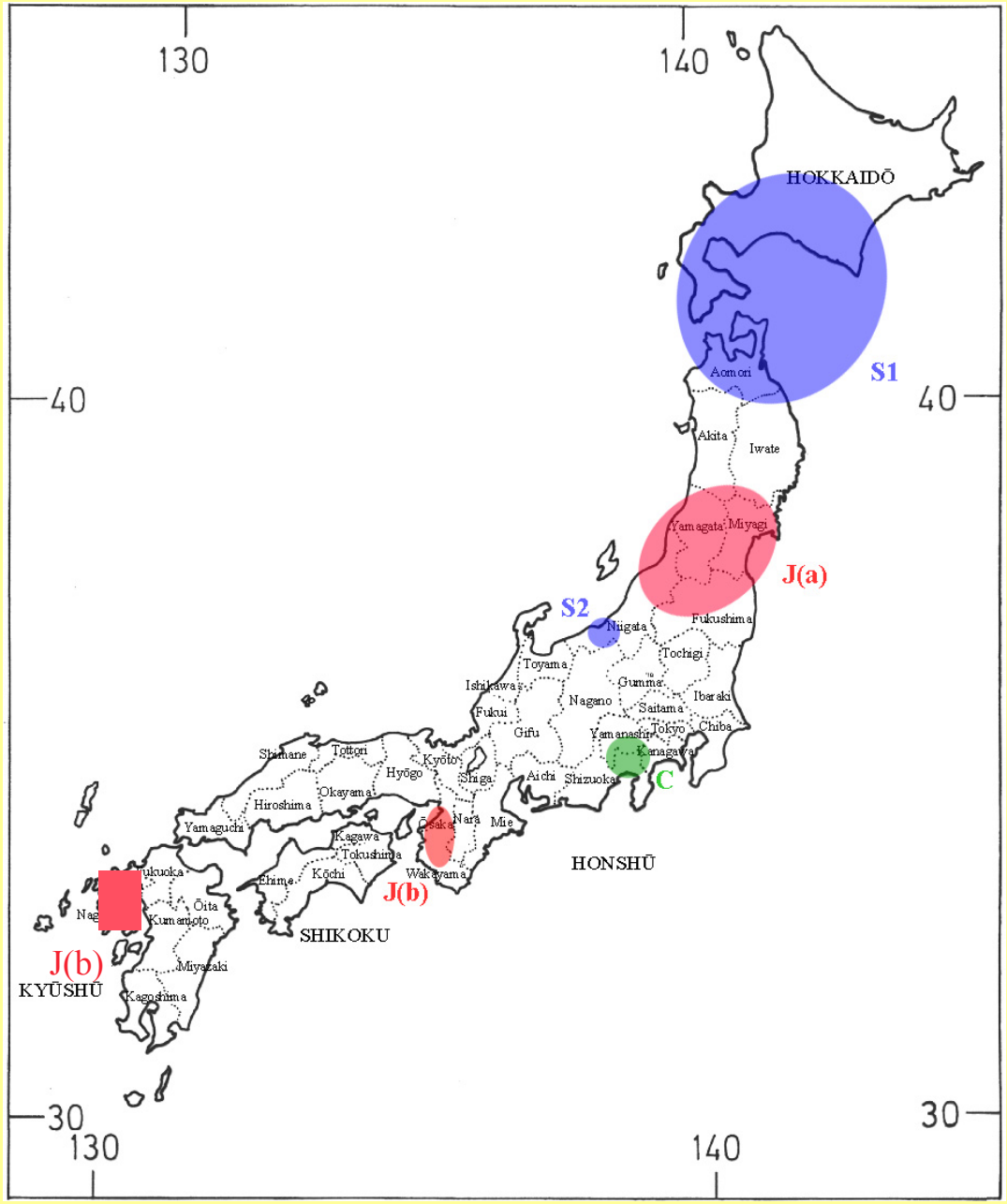
1. **a good understanding of the taxonomy and breeding behaviour of the target species**
2. **accurate knowledge of where the particular introduction originated**

Extensive underground rhizomes are essential on volcanoes



Map of Japan showing the different taxa sampled from each prefecture. Represents only the plants used in this study and is not a complete distribution map for the different taxa. Putative hybrids have been excluded.

Pashley 2003

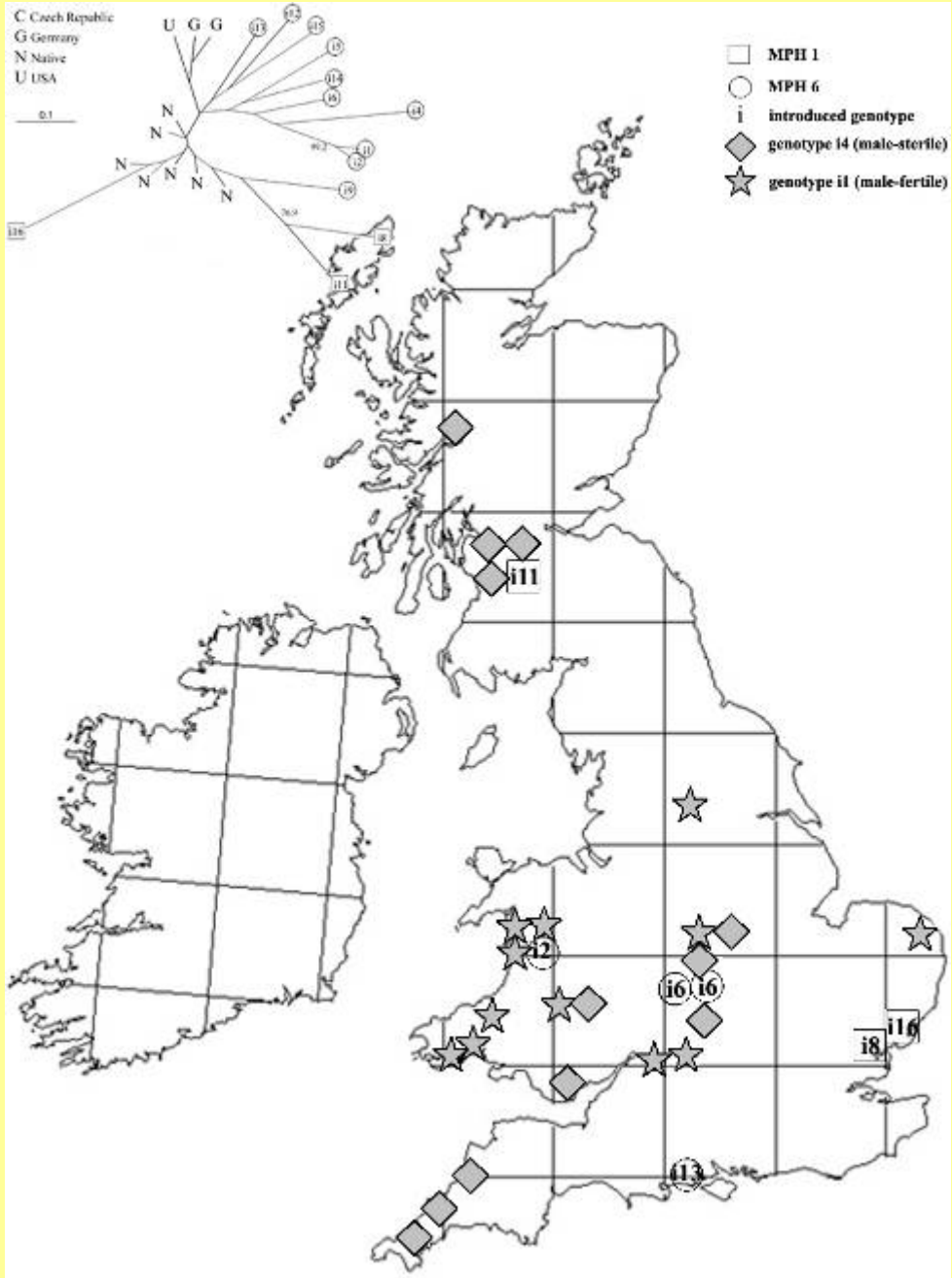


Chloroplast haplotype evidence for the origin of the British plants

S1 main *sachalinensis* type
S2 rare *sachalinensis* type
C The 2 *compacta* types
J(a) *F. japonica* var. *uzenensis* sharing British *japonica* haplotype

J(b) British *F. japonica* var. *japonica*

Pashley 2003



British genotypes of *Fallopia sachalinensis*

Chloroplast data indicated a widespread male and a widespread female genotype, with scattered rarer genotypes

64,000 DOLLAR QUESTIONS!

How frequent is *F*. x *bohemica* in the USA?

Do any of the native *Fallopia* taxa hybridise with Japanese Knotweed in the USA?

How important is reproduction by seed in the USA?

How many separate introductions of Japanese Knotweed have been made to the USA?

How can we identify the different mixtures of *japonica* and *sachalinensis* genomes in hybrids and backcrosses?

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