# Japanese Knotweed:
## Invasion of the Clones?

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## Introduction

Have you ever seen headlines like this one? "Watch for flying carp" from the *Toronto Sun,* May 24, 2009. Because of amazing photos of Asian carp flying through the air and YouTube videos of people fishing being struck by fish in their boats, this highly visible invasive species is hard to miss. Other introduced species are not so noticeable, and some invasive plants have beautiful flowers that we admire. High-profile or almost invisible, introduced species are a major threat to biodiversity. Many introduced species do not succeed in new environments. Others, however, are highly successful, and without predators or diseases, they can spread rapidly and disrupt natural communities.

Since the introduction of Japanese knotweed (*Fallopia japonica*) to North America about a century ago, it has gone from being a prize-winning horticulture plant to being labelled one of the world's top 10 invasive species. In England, Japanese knotweed is sterile and is considered a single, large female **clone**. In terms of biomass, it is the largest individual female on Earth. With each new plant genetically identical to its mother, it spreads by **fragmentation** (small pieces breaking off and starting new plants). Evidence indicates that in other parts of Europe and North America, in the absence of male partners of their own species, female Japanese knotweed plants are mating with males from other species. Female Japanese knotweed plants are being fertilized by pollen from giant knotweed (*Fallopia sachalinensis*), as well as a few other *Fallopia* species. The fertile hybrid Bohemian knotweed (*Fallopia xbohemica*) is the product of the cross between Japanese knotweed and giant knotweed, and has become quite common (see Photos A, B, and C).

**KEY CONCEPTS**

- Comparing genome sequences provides clues to evolution and development.
- Speciation can take place with or without geographic separation.
- Human activities threaten Earth's biodiversity.

**PHOTO A. Japanese knotweed.**
*Source: © Jason Smalley/Nature Picture Library.*

**PHOTO B. Giant knotweed.**
*Source: © John T. Fowler.*

**PHOTO C. Bohemian knotweed.**
*Source: King County Noxious Weed Control Program, Seattle, Washington.*

Should we consider the hybrid *F. xbohemica* to be the same species as *F. japonica*, or should we consider it a new species? This is a difficult question to answer. The concept of species is crucial to the classification system in biology; but, the literature contains different definitions of species. The biological species concept, which is based on all members within a species potentially being able to interbreed but being reproductively isolated from other species, is the most frequently used. An alternative model is the genetic species concept, where species is defined as being genetically isolated (different genes and/or chromosomes) from another species but not reproductively isolated.

A new species can evolve very quickly in plants through **polyploidy,** an increase in the normal set of chromosomes. Humans are diploid ($2n$), with two complete sets of chromosomes. Polyploid organisms can be triploid ($3n$), tetraploid ($4n$), pentaploid ($5n$), etc. Although rare, some animals, such as some goldfish and rats, are polyploids. In plants, however, this is much more common and has played an important role in plant evolution. Many agricultural crops, such as apples, bananas, and wheat, are polyploid plants.

A special case of polyploidy called **allopolyploidy** occurs when two different species interbreed and produce fertile hybrid offspring. Japanese knotweed is octoploid (88 chromosomes); giant knotweed is tetraploid (44 chromosomes); and many researchers have found varying numbers of chromosomes in the hybrids, with the most common being hexaploid (66 chromosomes). Is the hybrid *F. xbohemica* a new species—an example of **sympatric speciation** (the development of a new species occurring without geographic isolation from the parent species)? Are we witnessing ongoing speciation within genus *Fallopia* as hybrids interbreed with each other?

A **pioneer species** (a species capable of growing in disturbed and exposed environments) that colonizes volcanoes in Japan, Japanese knotweed is a shrub with extensive underground rhizomes. Whereas it can tolerate a variety of soil conditions, it prefers full or partial sun and moist soils. Combined with its aggressive growth habit, the plant has the ability to out-compete native flora. Japanese knotweed is now present in eight Canadian provinces. In British Columbia, it can sometimes even be seen out-competing another highly invasive plant, Armenian blackberry (*Rubus armeniacus*).

Antoine Lecerf from the University of British Columbia has shown that Japanese knotweed has a negative impact on stream ecology because it alters invertebrate populations and lowers stream nutrient levels. Research in the Pacific Northwest by Lauren Urgenson indicates that Japanese knotweed also has a negative impact on riparian forests (i.e., forests near waterways or lakes): it displaces native species and reduces the nutrient quality of litter inputs, thereby possibly causing long-term changes in the structure and functioning of riparian forests and the adjacent aquatic habitats.

The control or elimination of these invasive plants is difficult. Mechanical methods often must be repeated for years to be effective. Herbicides are not always effective because of the persistence of underground rhizomes. Current research is focused on developing or finding a biological control agent—a highly specific parasite, a disease, or a herbivore—that will attack or eat *F. japonica* but leave related native plants untouched. But will such a highly specific biological control agent be equally effective against hybrids? In order to predict the effectiveness of a biological control, we must know with what species and/or hybrids are present in an area.

**Research Question**

How do we determine if the plants invading British Columbia are Japanese knotweed clones, giant knotweed, or hybrid Bohemian knotweed?

I became interested in researching Japanese knotweed while teaching a biology course on native plants and animals of British Columbia. During field trips in 2006, my class observed an unfamiliar shrub at mid-elevation on Seymour Mountain and at low elevation riparian areas of the Fraser Valley. I later identified the plant as Japanese knotweed. I am now collaborating with Rob Bourchier, a research scientist from Agriculture and Agri-Food Canada in Lethbridge, Alberta, to determine the identity of the knotweed populations in Abbotsford, British Columbia, and to discover if they represent more than one species.

**Hypothesis**

Invasive knotweeds in the Abbotsford area of British Columbia are a mix of Japanese knotweed, giant knotweed, and hybrid Bohemian knotweed.

# Methods and Results

Morphological characteristics were compared: trichome size (the size of small hair-like outgrowths on veins on the underside of leaves), leaf shape, and leaf size. Figure 1 shows the different morphological characteristics helpful in identifying knotweeds. Japanese knotweed leaves tend to be small with no leaf-base indent and have either no obvious trichomes or a small bump; hybrids are intermediate in size, have a small base indent, and have trichomes made of one to four cells; giant knotweeds have large leaves with large leaf-base indents and have large trichomes composed of many cells.

It can be difficult to separate hybrids from the two-parent species using a single morphological trait because they can appear more like one parent or the other. Rhizome pieces were grown and fresh white roots were used to do chromosome counts. Our results were similar to those of other researchers: *F. japonica* plants had 88 chromosomes and *F. sachalinensis* plants had 44. Most of the hybrid *F. xbohemica* plants had 66, but researchers have found the ploidy levels in hybrids can vary from tetraploid to octoploid. A comparison of morphological characteristics and chromosome counts (see Table 1) indicated that sites in Abbotsford had Japanese knotweed and giant knotweed, but most sites appeared to have hybrid Bohemian knotweed.

There was a strong relationship between leaf length and leaf-base indent as a good morphological indicator of species and hybrids (see Figure 2). Growing fresh roots to

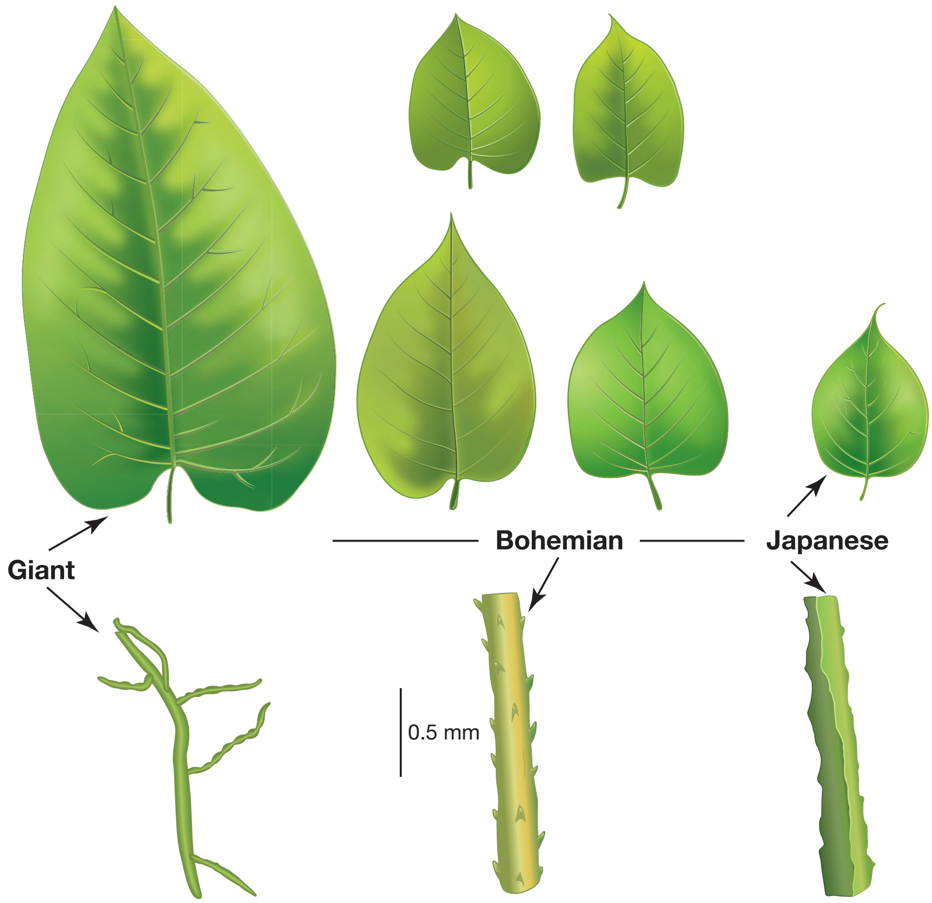


**FIGURE 1. Morphology of invasive knotweed species in British Columbia.**
Giant knotweed leaves are large (20–40 cm long); Japanese knotweed leaves are small (3–10 cm long); and hybrid Bohemian knotweed leaves are intermediate (5–30 cm long). Trichomes are large with many cells on the veins on the underside of giant knotweed leaves; they are not visible or are only small bumps on Japanese knotweed leaves; and they are intermediate in size on hybrid Bohemian knotweed leaves.

*Source: Based on Wilson, L.M. 2007. Key to Identification of Invasive Knotweeds in British Columbia. B.C. Ministry of Forests & Range, Forest Practices Branch, Kamloops, B.C. Adapted with permission.*

**TABLE 1. Relationship between Trichome Characteristics and Average Leaf Length and Chromosome Number**

| Trichome Characteristics | Average Leaf Length (cm) | Chromosome Number | |
|---|---|---|---|
| No obvious trichomes or single small bump | 14.56 ± 5.58 (SD) | 88 | *F. japonica*<br>Japanese knotweed |
| Trichome composed of one to four cells | 23.88 ± 6.18 (SD) | 44, 66* | *F. xbohemica*<br>Bohemian knotweed |
| Trichome composed of more than four cells | 42.3 ± 7.6 (SD) | 44 | *F. sachalinensis*<br>Giant knotweed |

*Most frequent chromosome number
*Source: S. Gillies, 2010.*

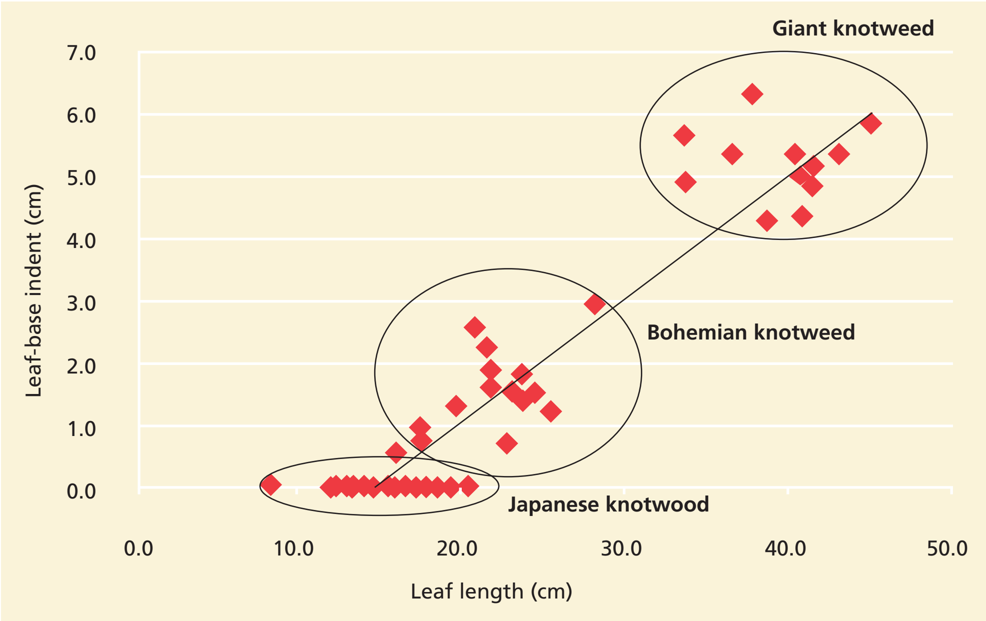


**FIGURE 2. Relationship between leaf length and leaf-base indent of Japanese knotweed from Abbotsford sites.**
The morphological characteristics of leaf length versus leaf base form three clusters that approximately relate to Japanese knotweed, giant knotweed, and hybrid Bohemian knotweed.
*Source: S. Gillies, 2010.*

determine chromosome number is time-consuming and can be done to confirm identification, but for most field studies using morphological criteria is suitable.

# Conclusions

Invasive knotweed in the Abbotsford area of British Columbia are a mix of Japanese knotweed, giant knotweed, and hybrid Bohemian knotweed. Hybrid knotweed in other areas of North America are producing viable seed. Sexual reproduction results in genetic variation in the offspring. No longer clones, hybrid knotweeds have the potential to further adapt to environmental conditions and increase their competitive advantage. With the predominance of hybrids in the Abbotsford area, it is likely that sexual reproduction is also taking place here and plants are dispersing by both fragmentation and seeds. What may have started as an invasion of Japanese knotweed clones has now become complicated by the creation of a new sexually reproducing invader hybrid, but is it a new species?

# Future Directions

The International Barcode of Life project began in Canada. At the Canadian Centre for DNA Barcoding (CCDB), the University of Guelph members Mehrad Hajibabaei and Sujeevan Ratnasingham have been actively involved in developing the barcode of life for plants. The goal is to use short DNA sequences from a standardized and agreed-upon position in the genome as a molecular diagnostic for species-level identification. The barcode of life is the practical application of genomic analysis and the comparison of many species. It will eventually provide biologists with a standard method that is reasonably quick and cheap for confirming the identification of a species. In August 2009, the barcode of life consortium temporarily approved two DNA sequences—*rbcL* and *matK*—as required barcode regions for land plants. These sequences are, however, only 72 percent successful in separating different species. This is much lower than the standard for animals, and research to improve the success rate continues.

A research project at the University of the Fraser Valley is currently testing several sections of DNA as well as the two DNA sequences selected from the barcode of life for plants to see if they can be used to distinguish Japanese knotweed and giant knotweed from the hybrids.

## Critical Thinking Questions

1. Why can Japanese knotweed out-compete many native plant species?
2. Why might a hybrid that can reproduce both sexually and asexually be more successful as an invasive species than a sterile plant that reproduces only asexually?
3. What criteria would you use to determine if the hybrid *Fallopia xbohemica* is a new species?

## Further Research Question

How will the barcode of life help biologists in their research?

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