

Sterility in double-flowered *Prunus serrulata*

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Introduction

Auckland Council listed three cherry species in its Regional Pest Management Plan (RPMP) 2020–2030 (AC, 2020) for sustained control in the Auckland region: *Prunus campanulata* (Taiwan cherry), *P. serotina* (black or rum cherry) and *P. serrulata* (Japanese flowering cherry). *Prunus serotina* is also listed on the 2012 National Pest Plant Accord, meaning that it is banned nationally from propagation, sale and distribution. Under the RPMP, *P. serrulata* and *P. campanulata* are banned from further sale in the Auckland region, from 1 September 2022⁵. An exemption was provided for two cultivars of Taiwan cherry – ‘Mimosa’ and ‘Pink Clouds’ – both said to be sterile (seedless) cultivars.

We set out to investigate whether any *P. serrulata* cultivars in trade are also sterile, and could be exempted from the RPMP ban.

The general literature suggests that double and semi-double flowered cultivars are often recognised as being poorer pollen- and nectar-producing plants than the single-petalled types, yielding lower rewards for insect (and bird) visitors than single-flowered cultivars (Garbuzov and Ratnieks, 2014). Japanese cherry flowers naturally have five petals, and double flowers are not usually found in the wild. Cultivars bred for fuller blossoms have many more petals, e.g., the pink double blossoms of the cultivar ‘Kanzan’ have as many as 28 petals on each flower. ‘Chrysanthemum’ type flowering cherries have petal numbers ranging from 60 up to 380, and are usually considered to be sterile (Kuitert, 1999).

Two double-flowered cultivars of Japanese flowering cherry are noted as being female sterile (seedless) in the literature: ‘Shirofugen’ (syn. ‘Fugenzo’) and ‘Ichiyo’. These have distinctly modified pistils, forming leaf-like structures, making them sterile (Katsuki, 2015). There is anecdotal evidence that other double-flowered *Prunus* cultivars are seed sterile in New Zealand. A reasonable hypothesis is the additional rows of petals create other structural modifications to the flower, including to the anthers, which could lead to male sterility. Multi-petally could also limit physical access to the flower for pollinating insects.

In this study we investigated DNA variation and floral morphology of five *Prunus serrulata* cultivars and compared them with wilding-type material growing in Auckland.

Experimental methods

Up to 10 flowers of each cultivar and the wild comparator were examined to look at the:

- Number of stamens
- Presence of pollen in anthers and stainability (‘viability’) counts
- Number and morphology of pistils.

Flower samples from five wild-type *P. serrulata* trees and from individual trees of known commercial cultivars in Auckland were sent to The New Zealand Institute for Plant and Food Research Limited, collected from street trees (‘Amanogawa’, ‘Shimidsu Sakura’, ‘Shirotae’) or nursery stock (‘Kanzan’, ‘Shimidsu Sakura’, ‘Ukon’) of known identity in the Waikato region, between 7 and 29 October 2021. Flowers were collected prior to anther dehiscence, transported on ice, and allowed to dehisce before being stored in a -20°C freezer until assessment.

At least nine flowers from each cultivar were collected and assessed (Table 1).

Morphological observations were made of each flower, including counts of petals, styles, stamens and anthers. Any abnormality in the reproductive parts was noted.

Pollen viability was assessed using fluorescein diacetate (FDA) staining under a fluorescence microscope. Frozen anthers were allowed to thaw, and the mature pollen was mixed with stain. Pollen was considered viable if the FDA stain resulted in bright green pollen, and was recorded as non-viable if little to no fluorescence was detected.

For DNA analysis, leaf material from each of the cultivars and the wild-type *P. serrulata* were sent to EcoGene (Manaaki Whenua – Landcare Research) in Lincoln. DNA extraction of c. 3 mm² fresh leaf samples was performed using the Maxwell DNA Extraction Kit (Promega) following the manufacturer’s instructions. The DNA sample was amplified using the KAPA3G plant kit (Custom Science) and sequenced with M13F/R sequencing primers for the following possibly diagnostic regions:

- (i) Two internal transcribed spacers (ITS1 and ITS2) that flank the 5.8s nuclear ribosomal DNA region of plant genomes (White et al., 1990)
- (ii) One external transcribed spacer (ETS) and 18S ribosomal RNA gene partial sequence (Wright et al., 2001).

Edited DNA sequences were then compared against sequences from GenBank, administered by The National Center for Biotechnology Information (NCBI).

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⁵ The date stated in the RPMP is 1 April 2021, but an 18-month extension has been granted by Auckland Council to allow for a longer lead-in time.

The sequences were then aligned with the available *P. pseudocerasus* and *P. serrulata* sequences in GenBank based on the alignment of Cho et al. (2021) and used to construct a phylogenetic tree.

Results

Two of the five cultivars showed substantial abnormalities in the female reproductive parts, including lack of ovaries (Table 1).

Two cultivars showed substantial abnormalities in male reproductive parts, and one cultivar showed moderate abnormalities (Table 1).

Three accessions showed high pollen viability: wild-type *P. serrulata*, and the cultivars 'Amanogawa' and 'Shirotae' (Table 1). 'Kanzan' showed moderate pollen viability and the remaining two cultivars had very little or no pollen viability (Table 1).

Prunus 'Amanogawa' (Fig. 1) and 'Shirotae' both had more petals than wilding *P. serrulata*, and their pollen viability was similar. 'Kanzan' flowers had ovaries and styles replaced by a pair of leaflets (Fig. 2), many stamens without anthers, many anthers without pollen or with poor pollen viability, and a large number of petals limiting pollinator access to reproductive organs (also limiting pollen available for pollinating other

Table 1 Summary data for flower viability assessments of *Prunus serrulata* cultivars.

Cultivar	No. of trees	No. of flowers ¹	Abnormal style/ stigma ²	Abnormal stamen/ anther ³	Pollen viability [mean \pm SEM] ⁴	Preliminary designation ⁵
Wild-type	5	1, 6, 7, 9, 10	0%	0%	90 \pm 4%	Fertile
'Amanogawa'	2	3, 10	0%	0%	91 \pm 2%	Fertile
'Kanzan'	1	10	100%	100%	44 \pm 14%	Female infertile, partially male infertile
'Shimidsu Sakura'	1	10	100%	100%	3 \pm 2%	Infertile
'Shirotae'	1	10	0%	50%	86 \pm 2%	Partially male infertile
'Ukon'	2	9, 9	0%	100%	0%	Male infertile

¹Number of flowers sampled for each tree.

²Percentage of flowers observed to have abnormal or lacking styles, and missing ovaries.

³Percentage of flowers observed to have abnormal male reproductive structures that could negatively affect fertility.

⁴Mean percentage viability of pollen grains calculated by tree ('Ukon') or flower (all other cultivars).

⁵Summary remarks on the likely fertility status based on these data. Note that this study did not assess pollen tube growth, fertilisation, seed development or seed germination, so cultivars with designated "fertile" status need to be further assessed for other factors that might prevent viable seed production.



Fig. 1 *Prunus serrulata* 'Amanogawa' flowers, showing normal floral morphology. Photo: Melissa Broussard.



Fig. 3 *Prunus serrulata* 'Shimidsu Sakura' flower, showing a central pair of leaflets and anthers with attached filaments. Photo: Melissa Broussard.



Fig. 2 *Prunus serrulata* 'Kanzan' flower, showing a pair of green leaflets in the flower centre, as well as aberrant anthers with petaliform filaments. Photo: Melissa Broussard.



Fig. 4 *Prunus serrulata* 'Ukon' flower, showing a majority of stamens without anthers, or with reduced/aberrant anthers. Photo: Melissa Broussard.

cherries). ‘Shimidsu Sakura’ flowers had ovaries and styles replaced by a pair of leaflets (Fig. 3), and pollen viability was poor. ‘Ukon’ stamens were largely missing anthers (Fig. 4), and those with anthers contained inviable pollen (<1% viability).

Conclusions

This study suggests that structural floral modifications for double and semi-double flowers could contribute to a reduction in the number of or modification to anthers and stamens, as well as abnormality of stigmas and styles. These are likely to affect overall pollination and subsequent fruit set.

Based on our findings, we believe it is likely that *Prunus* ‘Kanzan’ and ‘Shimidsu Sakura’ are at least female infertile and therefore unlikely to pose a risk of producing viable seed. ‘Ukon’ exhibited male infertility, but further work is required to determine whether it also has female infertility.

Although the remaining cultivars did not show clear signs of female infertility and had limited evidence of inviable pollen, further work is required to establish whether these cultivars are capable of producing viable seed. Other factors preventing the production of viable seed might be present, such as incompatibility mechanisms, but we were unable to assess these in this initial trial.

A key limitation of this study is the small number of trees of each cultivar sampled. While the floral characteristics observed in flowers from these individuals are likely to be representative of the cultivars which are clonal, it would be prudent to conduct further assessment of more individuals of each cultivar initially designated as “infertile” to confirm this. Although we sampled only five trees, it is likely that most wilding *P. serrulata* are highly fertile.

Complex interspecific hybrid history

Prunus serrulata is native to Japan, Korea and China. The Japanese established two terms to differentiate the wild cherries from those of cultivated or garden origin. They are “Yama-zakura” (mountain cherries) for wild plants, and “Sato-zakura” (village cherries) for the cultivated selections.

The true unselected species *P. serrulata* is rarely sold commercially because it does not have the showy floral traits sought after in the highly bred cultivars.

Actively bred village cherries are thought to have come into cultivation around the beginning of the seventeenth century (Wybe, 1999), although some cultivars may date back as far as 794 and 1192 AD (Honda and Hayashi, 1974). Today, more than 200 cultivars with *P. serrulata* parentage are known in Japan, producing showy flowers in spring and good leaf colour in autumn. Their exact parentage remains quite uncertain. Wybe (1999) noted that most of the semi-double forms have some constant botanical characteristics which point

to the Oshima cherry (*P. speciosa*) as a parent. This species is thought to have a tendency to mutate into double-flowered cultivars.

Recent molecular analysis has confirmed the *Cerasus* Sato-zakura Group were produced by complex interspecific crosses between Oshima cherry *P. speciosa* (endemic to Japan) and many different wild species such as *P. apetala*, *P. campanulata*, *P. incisa*, *P. itosakura*, *P. leveilleana*, *P. sargentii* and *P. serrulata* (Kato et al., 2014; Katsuki, 2015).

The phylogenetic tree that was generated (Fig. 5) should be treated with caution because the real identity of many of the GenBank sequences is questionable and there are usually some errors associated with sequences retrieved from GenBank.

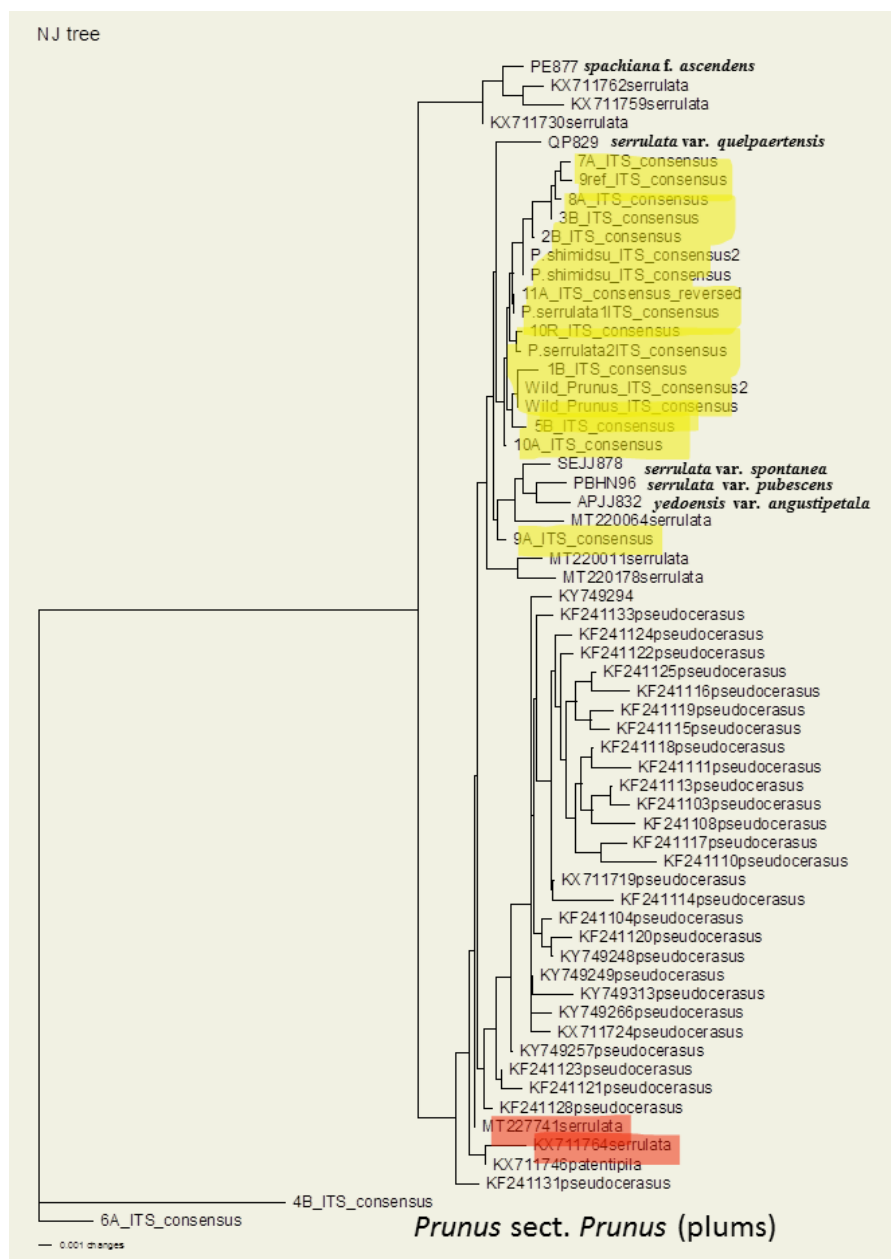


Fig. 5 Phylogenetic tree of *Prunus* ITS sequences. Image: Rob Smissen.

Acknowledging this limitation, in this tree, most of the samples (highlighted gold in Fig. 5) grouped with parts of *P. serrulata*, but two samples (highlighted pink) grouped with members of *Prunus* subg. *Prunus*, a subgenus that includes plums. Variation within *P. serrulata* (both the database sequences and the New Zealand ones) is complex and not amenable to drawing clear conclusions about relationships among the plants.

Genome sequencing projects and other genetic approaches to tackling *Prunus* diversity, as well as functional genetics, are being carried out around the world and it should be possible to use more sophisticated approaches to identify both wild and cultivated flowering cherries using genetic methods. Such methods could cope with the complex history of hybridisation that has produced many wild and cultivated taxa and also the presence of polyploid taxa. At this time, the major impediments to doing so are the lack of standardisation among the different research groups, and the fragmented nature of reference collections. However, this is starting to change and a more revealing study of New Zealand material may be practical in the near future.

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Prunus serrulata cultivar, Japan. Photo: © 阿橋 HQ (CC BY-SA 2.0).