Rediscovery of the tetraploid mānuka cultivar *Leptospermum scoparium* 'Keatleyi'

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Leptospermum scoparium (common name mānuka) is endemic to south eastern mainland Australia, Tasmania, and New Zealand where it occurs in a range of habitats ranging from lowland forests to subalpine scrub (Allan, 1961; Thompson, 1989). Given its adaptability to different environments, it is perhaps not surprising that it is also a species noted for its diversity of forms, especially in New Zealand. In some regions plants grow into trees up to 5 m in height, whilst dwarf and/or prostrate types predominate in other areas. This diversity, coupled with the attractive floral display of *L. scoparium*, facilitated direct introductions from the wild and breeding of a wide range of ornamental forms (Dawson, 2009, 2010). Mānuka is now one of New Zealand's better known native ornamental species.

The remarkable diversity of this species is also reflected in its many known uses. Other than its use as an ornamental garden plant, *Leptospermum scoparium* has been used medicinally (Brooker et al., 1987); as a herbal tea; for the production of essential oils (Douglas et al., 2004); as a cut flower (Burge et al., 1996); a nursery species for the re-establishment of native forest; a ground-durable timber (Stephens et al., 2005); for firewood, as a charcoal source; to provide flavour when smoking fish or meat products; and as the source of a highly valued honey (Butz Huryn, 1995).

We are currently investigating the diversity of *L. scoparium* and part of that work is an exploration of the potential impact of ploidy on plant form, plant development and nectar biochemistry. Ploidy refers to the number of chromosome sets that an organism has. Like humans, many plants have two chromosome sets; one inherited from their mother and one from their father. Such plants are known as 'diploids', a term derived from the Greek terms for 'two' and 'fold (or form)'. Unlike animals, however, plants are also often quite tolerant of different ploidy states and may grow quite successfully as 'triploids' (three chromosome sets), 'tetraploids' (four sets), 'pentaploids' (five sets), 'hexaploids' (six sets) and so on. Higher ploidy often leads to the formation of larger organs such as leaves (Fig. 1A–B), flowers, and fruit. Polyploidy has been important in the development of many of our commercially cropped species. Bananas, for example, are triploid; potatoes, cotton and tobacco are tetraploids, and wheat is a hexaploid.

Fig. 1 Foliage of selections of *Leptospermum scoparium* demonstrating the influence of ploidy on organ size. **A**, diploid leaves. **B**, tetraploid leaves.

According to the most recent and comprehensive revision of the genus *Leptospermum* there are 79 species (Thompson, 1989), although several more have been described since then raising the total to 87 species (Dawson, 2009). Of the species that have been studied in sufficient depth, most have been recorded as diploid, with chromosome numbers of $2n = 22$, but some naturally occurring tetraploid species (with $2n = 44$ chromosomes) are known, such as *L. minutifolium*, *L. myrtifolium* and *L. parvifolium* (Smith-White, 1948; Dawson, 1987, 1990, 1995). Although *Leptospermum scoparium* is typically a diploid species, a single tetraploid selection is known in horticulture (Dawson, 1990). That selection, named *L. scoparium* var. *incanum* 'Keatleyi' (Fig. 2), was discovered in Northland by Captain Keatley in 1917 (Stevens, 1945; Dawson, 1990, 2009). The flowers of *L.* 'Keatleyi' are conspicuously larger than the typical flowers of diploid *L. scoparium* var. *incanum*, which predominates in the Northland region, and we speculate that it is this difference that prompted Captain Keatley to select the plant for propagation.

Fig. 2 Flowers of the tetraploid cultivar *Leptospermum scoparium* 'Keatleyi'.

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The origin of *L. scoparium* var. *incanum* 'Keatleyi'

All accounts place the site of origin of *L. scoparium* 'Keatleyi' in Northland. This is also consistent with its characters (pink flowers and young leaves with long silky hairs) that place it in *L. scoparium* var. *incanum*. There are, however, different reports regarding the exact location of its collection.

An early Duncan & Davies Nursery Catalogue (no. 15, 1928–30) claimed that it was found in the Hokianga Harbour region, a location repeated in several later references (e.g., Metcalf, 1972; Harrison, 1974).

Stevens (1945) interviewed Captain Keatley (then aged 70) and provided a credible account of it being discovered in 1917 between Parengarenga Harbour and North Cape (about 2.4 km from North Cape and near "Te Hupaua"[1]). Keatley took many cuttings and gave them to various friends to try to strike. Only Fred Walker, a notable plantsman of Whanganui was successful, and material was disseminated from there.

Fred Walker's grand-daughter Phillipa Christie provided another possible location for *L.* 'Keatleyi', namely from Mt Camel, near the Houhora Harbour, also in Northland. According to their family word-of-mouth (Phillipa Christie, pers. comm.):

> "Captain Keatley was standing off Mt Camel in his coastal ship. He looked up at the bush covered slopes, mostly tea tree, and noticed one plant with conspicuous pink flowers. He sent one of the young crew ashore to go and pick some branches for him to which he then passed on to Fred Walker in Whanganui for propagation. Although it is often cited that Captain Keatley passed the material on to Fred Walker to strike cuttings we suspect that Walker most likely used seed propagation to secure the variety. Given the nature of travel in 1917 the material would have taken some time to reach Fred Walker and *L.* 'Keatleyi' is difficult to propagate by cuttings even when the material is fresh. Furthermore, Walker apparently noted three different flower colours amongst the young plants he obtained, suggesting that they were seedlings. Fred Walker had several nurseryman friends, and was in the habit of sharing with them. One of whom was Mr Martin, also of Whanganui who was later to introduce *L.* 'Martinii' into cultivation."

[1] We do not know of a place-name called "Te Hupaua". The nearest match we found in the area is Te Hapua within the Parengarenga Harbour.

Three triploid *Leptospermum scoparium* cultivars; *L.* 'Martinii', *L.* 'Lambethii' and *L.* 'Helene Strybing' are reported, arising in cultivation from seedlings presumably derived from *L.* 'Keatleyi' following pollination by a nearby diploid plant (Dawson, 2010). Each triploid arose in a different country; *L.* 'Martinii' was raised in Whanganui, New Zealand; *L.* 'Lambethii' arose in Australia, and *L.* 'Helene Strybing' arose in the Strybing Arboretum, USA. *L.* 'Martinii' is still readily available in New Zealand where it is popular for its large pink and red flowers and floriferous nature (Fig. 3).

We were keen to obtain material of the triploid *L.* 'Martinii' and the tetraploid *L.* 'Keatleyi' for our study so explored a range of commercial and other sources. The *L.* 'Martinii' plants obtained were confirmed as triploids but all of those labelled *L.* 'Keatleyi' were also found to be triploid, suggesting they were not true-to-type. Counting chromosomes is a difficult, technical task and not one that is often repeated. Instead we used a flow cytometer, a machine that measures the DNA content of a cell to infer chromosome numbers. It's a lot faster and more efficient than counting chromosomes on a microscope slide.

Fig. 3 Flowers of the triploid cultivar *Leptospermum scoparium* 'Martinii'.

We also noted that most of the material we obtained labelled as *L.* 'Keatleyi' instead resembled *L.* 'Martinii'. The original error was probably due to something simple like a stock label being lost or misplaced in a production nursery. The flowers of *L.* 'Keatleyi' and *L.* 'Martinii' are superficially similar as both are large and pink, but they do have conspicuous differences (Fig. 4A–B).

The flowers of *L.* 'Keatleyi' are approximately 25 mm in diameter, and have petals and filaments of a soft pink colour (as is typical of *L. scoparium* var. *incanum*). Also, *L.* 'Keatleyi' often has one or two petals additional to the usual five.

In contrast, *L.* 'Martinii' has darker pink flowers that are typically 22 mm diameter, crimson filaments and sepals, and the usual number of five petals on each flower.

In our studies we've noticed that *L.* 'Martinii' is much easier to propagate by cuttings than *L.* 'Keatleyi'. This would have exacerbated the impact of a labelling error with the more easily propagated clone becoming the most common in the trade. The difficulty of multiplying *L.* 'Keatleyi' by cuttings was noted as far back as the time of Fred Walker of Whanganui. This likely prompted some propagators to substitute cuttings with seed propagation which is more convenient and produces many more plants, on the assumption that *L.* 'Keatleyi' breeds true from seed. Whilst this is often true, the presence of the triploids demonstrates that not all the seedlings of *L.* 'Keatleyi' are necessarily tetraploid, especially if the seed plant

Fig. 4 Flowering stems of *Leptospermum scoparium* showing the differences between cultivars. **A**, *L*. 'Martinii', a triploid cultivar. **B**, *L*. 'Keatleyi', a tetraploid cultivar.

is outcrossed by pollen from a nearby diploid plant. Another possibility for the origin of the mislabelled material, therefore, is that an unknown triploid seedling of *L.* 'Keatleyi' was a stock bed contaminant and the origin of the error.

After discovering that the material labelled *L.* 'Keatleyi' was not true-to-type, we then tried to obtain the correct cultivar by other means, spreading the word amongst people we knew with knowledge of mānuka cultivars. Finally, with the invaluable help of Graeme Platt and Dr Mike Clearwater of Waikato University, we located an old plant growing in an Auckland garden that suited the description of *L.* 'Keatleyi'. This material was subsequently confirmed as tetraploid by flow cytometry. The discovery of this plant is a gratifying outcome as it ensures that this cultivar remains available, as it was in danger of being lost from cultivation in New Zealand. Material is now under multiplication. Intriguingly, it is proving hard to propagate by cuttings, which further convinces us that it is the cultivar we were looking for.

We are persisting with its propagation, and numbers are increasing, albeit slowly. As soon as sufficient numbers are available, plants will be distributed to people maintaining botanical collections of New Zealand species and from there the material can be distributed more widely.

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