

ISSR as a technique to distinguish *Lilium* species and cultivars: evaluation and applications.

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Abstract

ISSR markers are one of the most popular methods to distinguish plants; however, the ability of the method to distinguish *Lilium* species and cultivars had not yet been evaluated prior to this study. ISSR fingerprinting on seventeen closely and distantly related lilies revealed that the technique has the capacity to distinguish distantly related lilies, but not necessarily closely related lilies. In one such case, the three offspring of a cross between the cultivars 'Silk Road' and 'Ice Caves' had ISSR fingerprints indistinguishable from the pollen parent, 'Ice Caves', indicating that paternal apomixis may have occurred. ISSR fingerprinting was also used to evaluate the relatedness of 'Silk Road' and 'Northern Carillon', two cultivars that share a majority of their genome and look nearly identical, which has caused controversy over their distinctness. The ISSR fingerprints could not distinguish between the plants; however, they are not necessarily a single event clone as they may have arisen from maternal apomixis, meiotic reduction division (preferential loss of one parent's chromosomes) or another rare reproductive event. The current research sets a foundation for future work that will hopefully solve these genetic mysteries.

Introduction

Molecular marking techniques that use random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR), are commonly used to distinguish plants and verify hybrids within various genera. RAPD primers consist of random nucleotide sequences, usually 10 nucleotides in length. ISSR primer sequences are designed to amplify microsatellite regions, also approximately 10 nucleotides in length. ISSRs are thought to more accurately distinguish closely related plants, and so have largely replaced RAPDs.

ISSR markers provide a convenient method to evaluate *Lilium* hybrids, however, the sensitivity of the method to distinguish closely related plants had not yet been evaluated prior to this study. *Lilium* hybrids often undergo complex reproductive events that can lead to offspring that share a majority of their genome or even their entire genome. One such event is known as apomixis, during which the offspring receives all of its DNA from one parent. Apomixis generally clones the mother plant and is known to occur in *Lilium*; however, apomixis that clones the pollen parent can also occur and has been identified in the rare cypress, *Cupressus dupreziana* (Maâtaoui and Pichot, 2001).

The first aim of this study was to evaluate the ability of ISSR markers to distinguish *Lilium* species and cultivars using seventeen distinct plants. Results quickly indicated that a more detailed investigation into the genetics of three offspring from a cross between 'Silk Road' (Figure 1) and 'Ice Caves' (Figure 2) and was needed.



Figure 2: *Lilium* 'Ice Caves', photo by Judith Freeman.

The second aim of this study was to evaluate the relatedness of two *Lilium* cultivars, 'Silk Road' and 'Northern Carillon' (Figure 3). These two cultivars were independently bred; however, they share at least two thirds of their genome (Figure 4) and as a result look nearly identical. The popular opinion that these two cultivars are clones of one another has proved problematic for both breeders and it is important to resolve the issue.

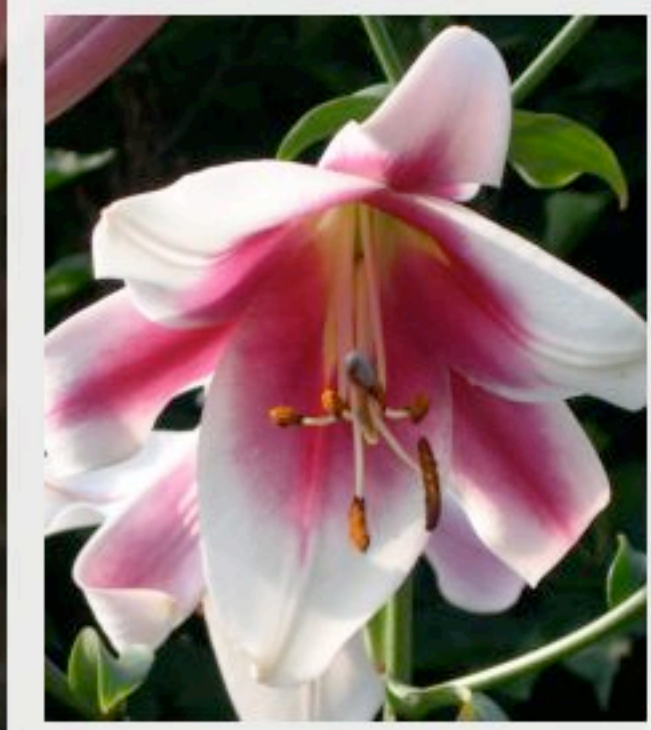


Figure 1: *Lilium* 'Silk Road', photo by Jim Ault.

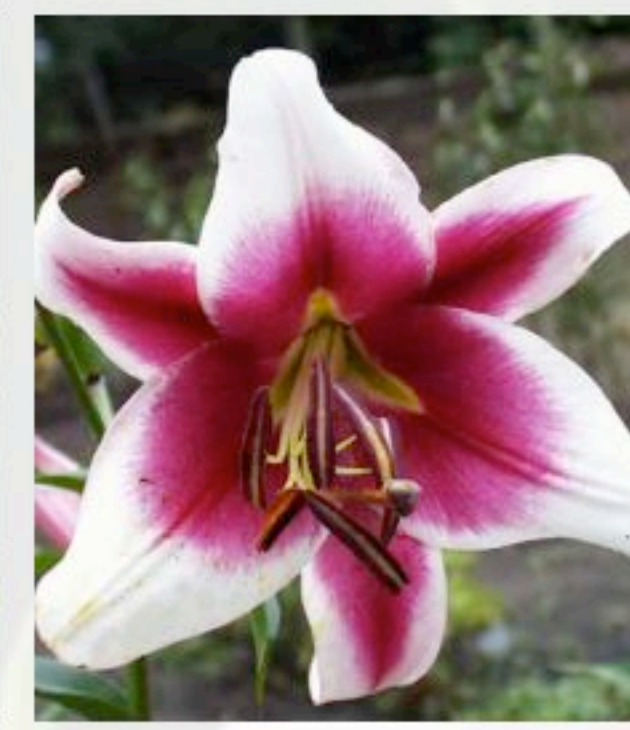


Figure 3: *Lilium* 'Northern Carillon', photo by Jim Ault.

Methods

- Leaf samples were obtained from plants in the Chicago Botanic Garden greenhouse or directly from breeders.
- DNA was extracted with the FastDNA kit.
- DNA was diluted to 1:20, 1:30, or 1:40, depending on the experiment.
- PCR was performed with ISSR primers 801-900 using the ISSR49 program.
- Loading dye with SYBR green dye was added to the samples before they were run on agarose gels.

Aim 1: Results and Discussion

ISSR fingerprinting of seventeen *Lilium* species and cultivars was performed with three primers (Figure 5). These experiments revealed that in many cases both closely and distantly related *Lilium* species and cultivars could be distinguished with the ISSR technique. Offspring from a cross of 'Silk Road' x 'Sarabande' could be distinguished; however, offspring from a cross of 'Silk Road' x 'Ice Caves' could not. This finding led to a more detailed investigation of 'Ice Caves' and three of its offspring.

ISSR fingerprinting on DNA from 'Ice Caves' and its offspring ('Silk Road x 'Ice Caves') was performed with 63 primers. Of these primers, 6 did not produce bands, 47 produced bands that were identical among the four plants, and 10 produced bands that could not be deciphered.

The great similarities between 'Ice Caves' and its offspring indicate that either 1) an unusual reproductive event occurred, such as apomixis via the pollen parent that resulted in clones of 'Ice Caves', or 2) the ISSR technique is not sensitive enough to differentiate these closely related *Lilium*. This second explanation seems unlikely because the ISSR technique could distinguish between the offspring of the 'Sarabande' x 'Silk Road' cross. Further studies will need to be performed to conclusively prove that apomixis via the pollen parent occurred, and if it is, this will only be the second plant known to perform such a reproductive event.

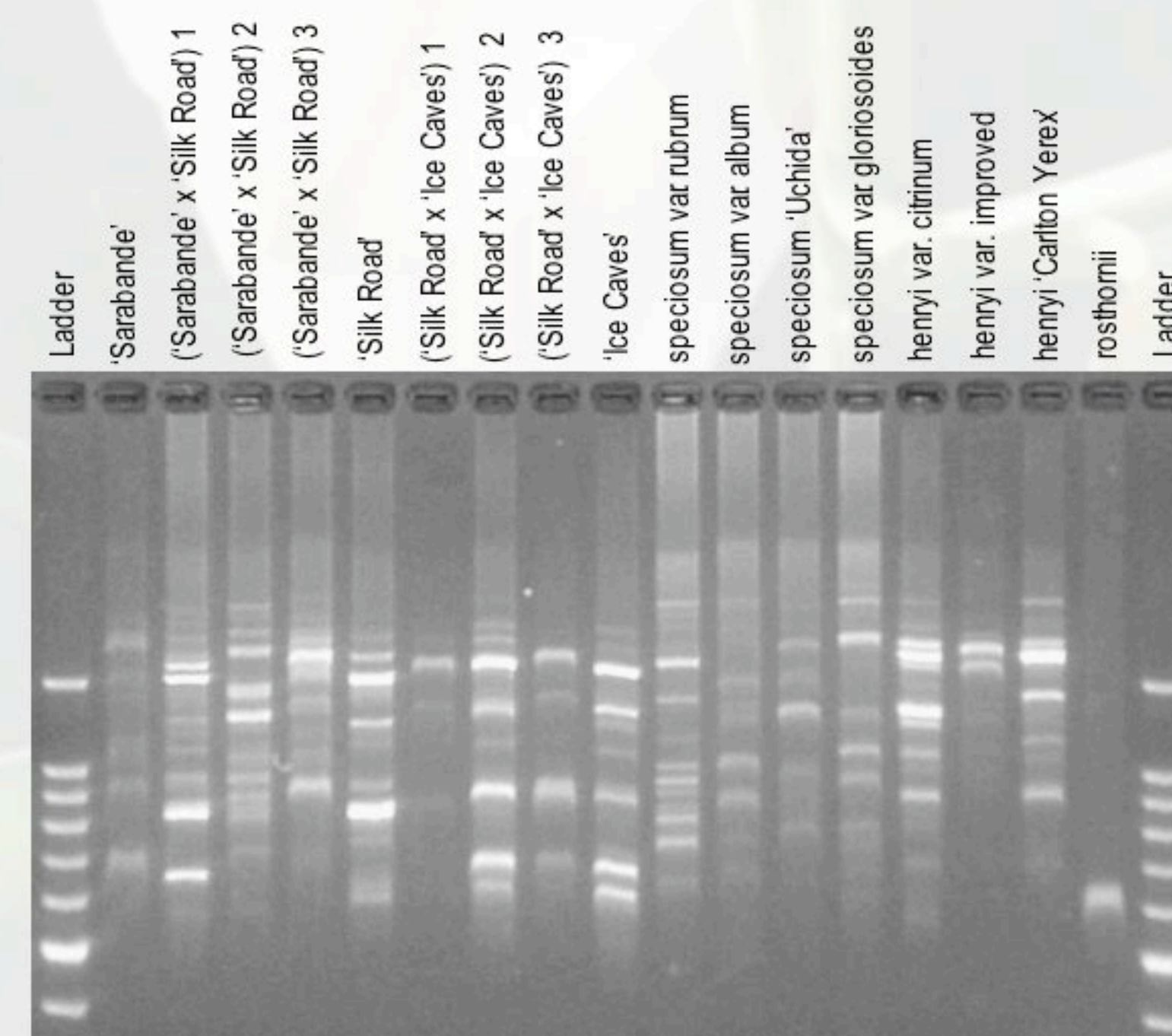


Figure 5: The ISSR fingerprint of 17 *Lilium* species and cultivars with primer 846. Lanes 'Sarabande' through 'Ice Caves' represent closely related cultivars. Lanes *speciosum* var. *rubrum* through *rosthornii* represent more distantly related species and cultivars. All plants show banding differences except for the 'Silk Road' x 'Ice Caves' offspring and 'Ice Caves'. Banding intensity differences are due to differences in DNA concentration pre- and post-PCR.

Aim 2: Results and Discussion

ISSR fingerprinting of 'Silk Road' and 'Northern Carillon' was performed with 96 primers, of which 32 did not produce bands and 64 produced bands identical for each plant (Figure 6).

These genetic similarities indicate that either 1) 'Silk Road' and 'Northern Carillon' are more closely related than previously thought, 2) 'Silk Road' and 'Northern Carillon' are indeed the same clone, representing a single cross, or 3) the ISSR technique is not sensitive enough to differentiate the two plants. Based on the history of the plants, the first explanation seems the most likely if ISSR is in fact sensitive. The plants share at least two-thirds of their genome due to an identical maternal parent (Figure 4), and the paternal parents could be closely related due to the vast inbreeding of *Lilium* cultivars. Or, it is possible the paternal genome was eliminated during meiosis, which has been reported in other allopolyploid plants. In conclusion, our research does not definitively prove or disprove that 'Silk Road' and 'Northern Carillon' are indeed separate plants. Further research is needed to finally resolve this horticultural dispute.

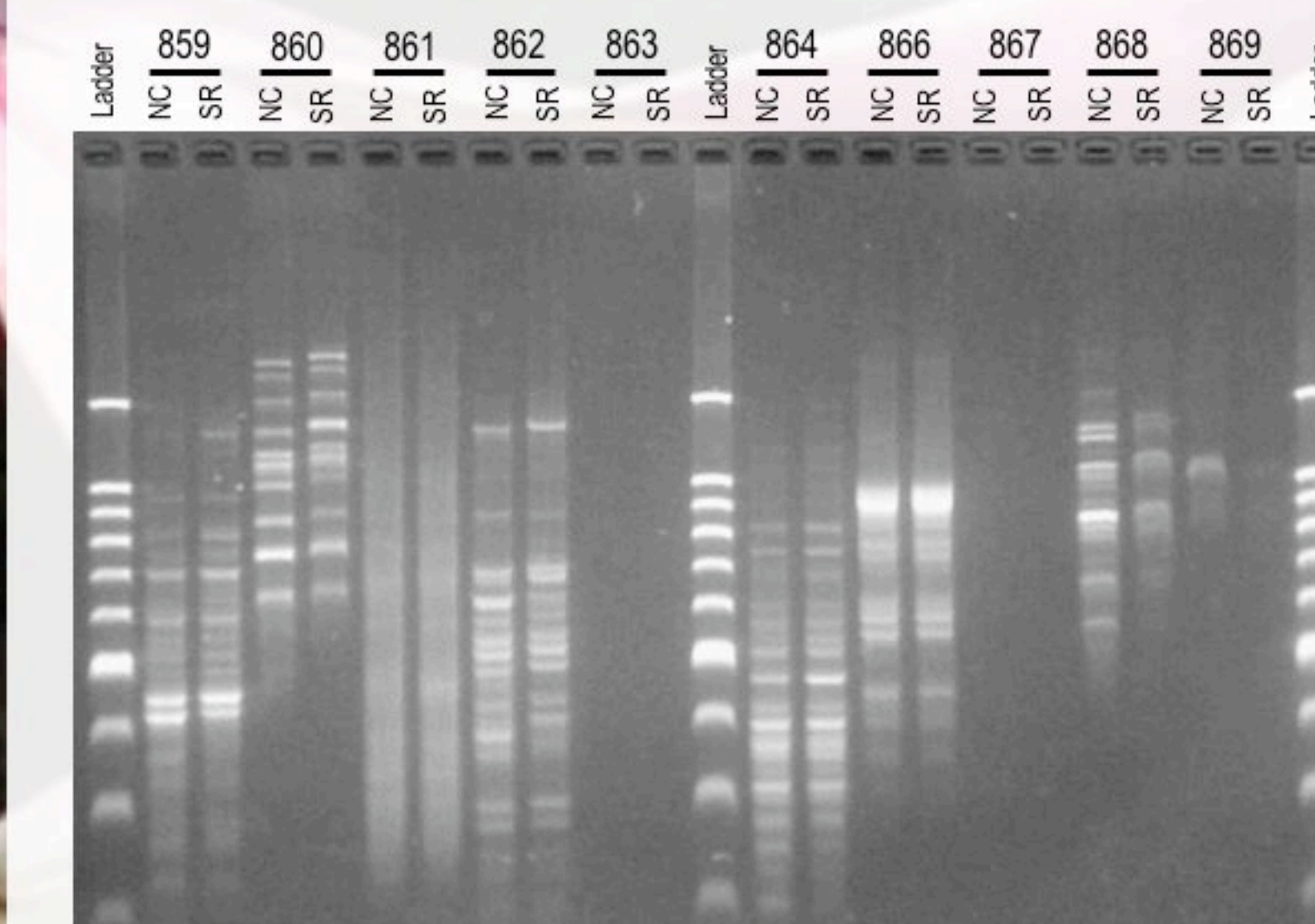


Figure 6: The ISSR fingerprint of 'Silk Road' and 'Northern Carillon' with primers 859 through 869. Primers 863 and 867 did not produce bands. Primers 859, 860, 861, 862, 864, and 866 clearly show identical banding patterns. Primers 868 and 869 were later retested and showed identical banding patterns.

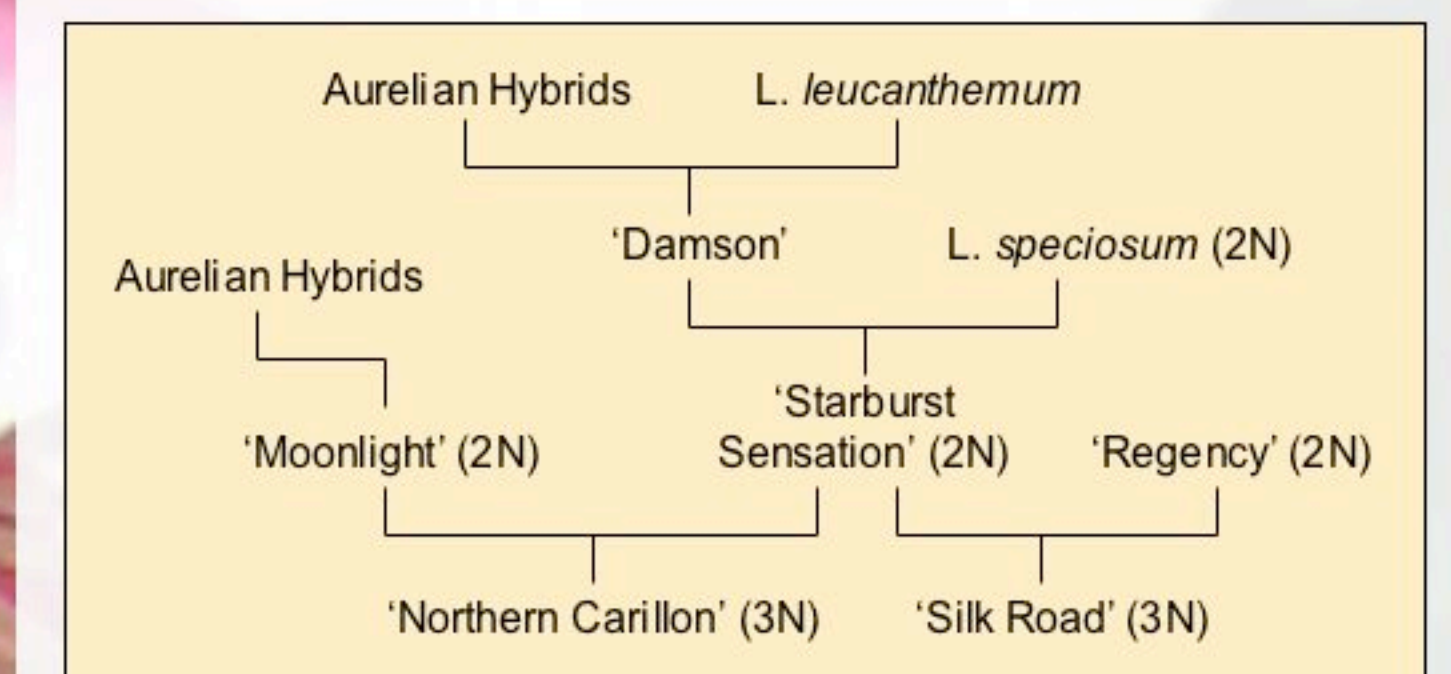


Figure 4: Genealogy of 'Silk Road' and 'Northern Carillon'. Both plants presumably received 2 sets of chromosomes from the mother, 'Starburst Sensation', and 1 set from the father, 'Regency' or 'Moonlight'.

References

- El Maâtaoui, M., and C. Pichot. 2001. Microsporogenesis in the endangered species *Cupressus dupreziana* A. Camus: evidence for meiotic defects yielding unreduced and abortive pollen. *Planta* 213: 543-549.