



Investigation the possibility of hybridization in breeding lineages of *Victoria cruziana* and *V. amazonica*

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Background

South America is home to a number of rare and unique species, many of which we are losing to habitat destruction. Water lilies have been referred to as one of the most beautiful flowers on earth. The largest species are found in South America and belong to the genus *Victoria*, so named to honor Queen Victoria. (She never visited there) The genus comprises of two species *Victoria cruziana* and *Victoria amazonica*. The two species can be difficult to distinguish, except they are native to different areas of South America. *Victoria cruziana* is found in Paraguay, Uruguay, and Argentina, while *Victoria amazonica* is found more in Peru, Columbia, Guyana, and Brazil. In 1961 Longwood Gardens, Patrick Nutt created the first hybrid cross between *V. cruziana*, as the parent pod and *V. amazonica* as the pollen parent. The *V. 'Longwood Hybrid'* could survive cooler temperature and bloomed much earlier in the year; this allowed many other botanical gardens to include them in their collections. Dr. Ben Knotts and Kit Knotts are trying to produce a breeding population of both *V. cruziana* and *V. amazonica*. However, it has proven hard to determine if some of the specimens are pure lines or hybrids.

Difference in Victoria Species

Victoria cruziana and *amazonica* have many similar characteristics, making them difficult to tell apart. The younger the lily, the more challenging it is to distinguish the two species. Still there are a number of characteristics, which separate the two species.

Victoria cruziana

- Pads are a bright green with tall rims
- Petals have a pointed shape
- Buds have no thorns and a pointed shape (Fig 1a)
- Flowers are white on the first day (Fig 2a)
- Flowers change to a light pink on the second day (Fig 3a)



Figure 1a: *V. cruziana* bud



Figure 2a: *V. cruziana* first day flower



Figure 3a: *V. cruziana* second day flower

Victoria amazonica

- Pads have a bronze green color with low rims
- Petals have a rounded shape
- Buds have thorns and a maroon color (Fig 1b)
- Flowers are white on the first day (Fig 2b)
- Flowers change to a pink, red or maroon on the second day (Fig 3b)



Figure 1b: *V. amazonica* bud



Figure 2b: *V. amazonica* first day flower



Figure 3b: *V. amazonica* second day flower

Methods

Samples were collected from a variety of sites (table 1). The DNA was extracted using a Fastprep DNA kit. A Polymerase Chain Reaction (PCR) was done testing ISSR primers. Spermine was used to purify the DNA. Of the hundred primers tested five were selected and tested on the samples, 807, 808, 809n 840, and 861. References

References

Knotts, Kit. (1998). *Victoria's history*. <www.victoria-adventure.org/victoria/victoria_history.html>

Table 1: *Victoria cruziana* and *Victoria amazonica* data on collection sites and breeding history

Country	Abbreviation	Collected site	Wild/Cultivated	Primer 808			Primer 809			Primer 861		
				A	B	C	A	B	C	A	B	C
<i>V. amazonica</i>												
Brazil	A16	Near Manaus	Wild	O	O	O	O	O	O	X	X	O
Brazil	A11	Near Silves	Wild		O	O	O	O	O	?	?	?
Brazil	A12	Near Manaus	Wild	X	O	O	O	O	O	X	X	O
Brazil	A18	Unknown	Cultivated	X	O	O	O	O	O	?	?	?
Brazil	A27	Unknown	Cultivated	X	O	O	O	O	O	?	?	?
Brazil	A11-B	Near Silves	Wild	X	O	O	O	O	O	X	X	O
Brazil	A13	Near Manaus	Cultivated	?	O	O	O	O	O	X	X	O
Brazil	A16-B	Near Manaus	Cultivated	X	O	O	O	O	O	X	X	O
Brazil	A11-B	Near Silves	Cultivated	X	O	O	O	O	O	X	X	O
Brazil	A19	Unknown	Cultivated	X	O	O	O	O	O	?	?	?
Brazil	A22	Unknown	Cultivated	X	O	O	O	O	O	X	X	O
Brazil	A11-C	Near Silves	Wild	X	O	O	O	O	O	X	X	O
<i>V. cruziana</i>												
Paraguay	C46-B	Lake 1	Wild	O	X	X	X	X	X	O	O	X
Paraguay	C47-L1	Lake 2	Wild	O	X	X	X	X	X	O	O	X
Paraguay	C47-L2	Lake 2	Wild	O	X	X	X	X	X	O	O	X
Domestic	C43-4	Unknown	Cultivated	O	X	X	X	X	X	O	O	X
Domestic	C43-2	Unknown	Cultivated	O	X	X	X	X	X	O	O	X
Domestic	C38	Belgium	Cultivated	O	X	X	X	X	X	O	O	X
Paraguay	C49	Unknown	Cultivated	O	?	?	?	?	?	O	O	X
Argentina	C49-6	Unknown	Wild	O	X	X	X	X	X	X	O	X

Results and Discussion

We screened individuals which we knew to be true species, using 100 ISSR primers. We selected five primers which gave banding patterns with clear and repeatable differences between the two species of water lilies. All individuals were scored for the present and absence of up to 15 bands, 3 per primer. When we scored the breeding lines of *Victoria amazonica* and *V. cruziana* we found no evidence of hybridization, i.e. all individuals showed banding patterns which clearly belonged to one or the other species (fig. 5). This is strong evidence that the breeding lines are true species and no hybridization has occurred in these lines.

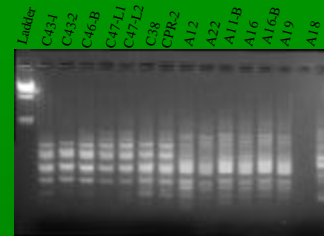


Figure 5a: ISSR primer 809

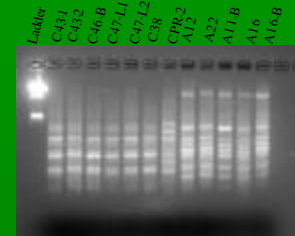


Figure 5b: ISSR primer 861

Conclusion

In order to breed these two different species of water lilies we should know if they are a pure breed or hybrid. By performing PCR we have successfully found a way to distinguish the difference between the two species. Using these ISSR primers we were able to show that breeding lines had no evidence of hybridization and did represent pure species lines. This technique could prove useful for future breeding programs.