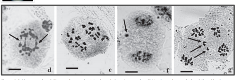
Introduction and Literature Review

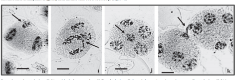
Sarcococca, or sweetbox, is a genus of 16-20 species of evergreen woody shrubs native primarily to Asia (eFloras, 2008). As members of the Buxaceae, they are close relatives to common ornamental plants including boxwoods (*Buxus*) and *Pachysandra*. In the United States, sweetbox has seen limited use as a landscape ornamental, even though they are reputed for their intensely fragrant winter flowers and shade adaptability (Dirr, 2009). In my personal experience, few species besides *S. confusa*, *S. hookeriana*, *S. humilis*, and *S. ruscifolia* are seen in American commerce.

Cytogenetics

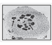
Sarcococca has been under-explored cytologically. Of the 16-20 species, only four have reported chromosome number counts, *S. brevifolia*, *S. pruniformis*, *S. saligna*, and *S. trinervia*, of which all are unresolved taxa and may be synonyms (The Plant List, 2013). All have a base chromosome number of n = 14, and S. pruniformis and S. saligna have been reported to occur at 2n = 2x = 28 (Chatha and Bir, 1987; Love, 1984; Saggoo et al, 2011). Aside from the determination of chromosome numbers in few species, the only other cytological work is a 2011 paper in which the authors observed meiosis in *S. pruniformis* and *S. saligna* and related it to pollen viability (Saggoo et al., 2011). In this paper, the authors found several instances of meiotic abnormalities, some of which are presented below.



Chromatin bridges at anaphase I (d),unequal segregation (16:12 instead of 14:14) at metaphase II (e), micronucleus at telophase I (f), and lagging chromosomes at anaphase II (g) in pollen mother cells of *Sarcococca pruniformis*



Extra chromatin at telophase II (h), multipolarity at anaphase II (i) and telophase II (j), and chromatin exchange between cells at telophase II (k) in pollen mother cells of *Sarcococca pruniformis*



Similar abnormalities were found in *S. saligna*, as well as B chromosomes, as seen on the right. Notice the lack of pairing of the two chromosomes.

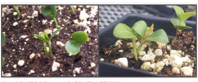
Saggoo et al. (2011) found that the two species studied had reduced pollen viability, as indicated in the table below. This viability was found to be as low as 28.78%. They also observed cytomixis, the phenomenon in which chromatin moves from one cell to another through intercellular channels. The exact role of cytomixis is not well understood, but it is thought to possibly lead to the development of 2n or polyploid gametes, the elimination of DNA, or an increase in genetic diversity (Mursalimov et al., 2013). Lastly, it was determined that both *S. pruniformis* and *S. saligna* have bivalents that vary greatly in size. In *S. pruniformis* they found 10 large bivalents and 4 small bivalents at metaphase I, while they found three exceptionally small bivalents of 14 total in *S. saligna*

	Cytomixis			Pollen grains		
	PMCs %	No. of PMCs involved	Meiotic stage/stages	Apparent fertility (%)	Size (μm)	RF
Sarcococca pruniformis Lindl.						
Kangra, Sali	—	—	—	28.78	35.44×32.85	02.5
					28.18×26.25	97.5
Kangra, enrute Triund	1.80	2	T-II	87.51	35.44×33.55	00.4
					29.46×27.82	99.6
S. saligna (D. Don) Muell-Arg. Poonch, Sailina	—	—	—	71.05	28.50×26.66	98.3
					20.45×14.60	01.7

Pollen viability and cytomixis in *Sarcococca pruniformis* and *S. saligna*. PMC - pollen mother cell, RF - relative frequency of small and large pollen grains

Apomixis

Considering the amount of meiotic abnormalities and the low pollen viability found in both *Sarcococca* species, it may not be surprising that at least one species in the genus has been reported to be apomictic. *Sarcococca humilis* is a confirmed apomict, reproducing asexually via nucellar polyembryony (Naumova, 1992). Other species in the genus are suspected apomicts, including *S. ruscifolia* and *S. confusa* (See photos below). In class, we discussed apomixis as a method of escape from sterility. It is a common feature in polyploids (Carman, 1997) and is thought to be useful for overcoming improper pairing in alloploids and insufficient chromosomes for pairing in odd ploidies. However, the only reported apomict in *Sarcococca* does not have a known ploidy level, and the only known ploidies are diploid.



Two seedlings from a single seed in both *Sarcococca confusa*(left) and *S. ruscifolia*(right).

Objective

As stated in the introduction, apomixis appears to be a common occurrence in *Sarcococca*. However, because apomixis is more common in higher ploidies and the only known ploidy in Sarcococca is diploid, I wanted to investigate chromosome number in a species with unreported chromosome number. This could provide insight into the evolutionary history of the genus. For example, it may be possible that apomixis developed in a polyploid ancestor (especially an autoploid), with members of the genus eventually undergoing a reduction in chromosome number but retaining apomictic gene expression. It may also help explain some of the meiotic abnormalities and the drastic size difference in bivalents found by Sagoo et al. (2011).

Sarcococca orientalis is a worthwhile candidate for study. It has an unknown chromosome number and larger, thicker leaves than many *Sarcococca* species. While not a true indicator, larger leaves is a common feature of polyploids (Dhawan and Lavania, 1996)

Materials and Methods

Species Propagation

On January 14, 2017, cuttings approximately six inches in length were taken and dipped in Hormodin 1 (Olympic Horticultural Products, 1000 ppm indole-3-butyric acid) before being stuck. The cuttings were placed under mist running for 20 seconds every 20 minutes during the day. One hundred percent of the cuttings successfully rooted (see photos on right).

Root Tip Harvest

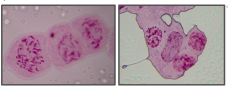
In early April 2017, root tips were harvested on a sunny morning following a sunny day. The root tips selected were white in color and appeared to be actively growing, as in the photo on the left. Immediately after being harvested, the tips were placed in 2 mM 8-hydroxyquinoline pre-fixative at 4 degrees C for approximately 5 hours.

Root Tip Squashes

The root tips were squashed immediately following the five hours in the prefixative. No fixative was used. Anecdotally, it seemed like 24 hours in Farmer's Fixative (3 100% ethanol: 1 glacial acetic acid by volume) made the root tips harder to soften with hydrochloric acid. To soften the unfixed root tips for squashing, they were placed in 5 M hydrochloric acid for 5 minutes. The root tips were squashed using the sliding razor method and carbol fuchsin stain from Carolina Biological Supply Company. Slides were sealed with clear nail polish and observed under a light microscope at 400-1000x magnification.

Stages of Mitosis

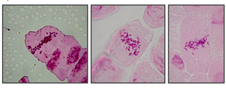
Prophase



Above left: Various stages of chromosome condensation during prophase.

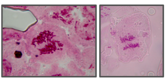
Above right: The cell on the far left is clearly in prophase, while others are in various stages from interphase (center cell) to early prophase.

Metaphase



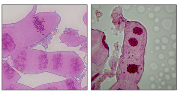
Above: Chromosomes lined up at metaphase. A few chromosomes are slightly lagging. The photots on the left and right show chromosomes more densely aligned on the metaphase plate than in the center photo.

Anaphase



Above: Chromatids being pulled apart during anaphase. In the photo on the left, some chromatids are still in the process of being separated, while in the photo on the right, all chromatids have clearly been separated.

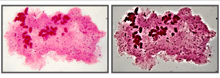
Telophase



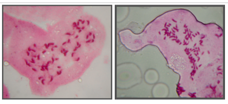
Above left: The cell on the left has undergone anaphase, chromosomes have mostly decondensed, and a cell plate has formed. The cell on the right has undergone anaphase, and the chromosomes have decondensed. A cell plate has yet to form, however. Also notice the metaphase at the top of the photo.

Above right: The binuclear cell has undergone anaphase and chromosomes have mostly decondensed. A cell plate has yet to form.

Chromosome Number



Above: Two pictures of a cell with varying contrast to better show chromosomes. 2n = 2x = 28



Above: Other cells that are countable or close to countable.

Discussion

Chromosome number in *Sarcococca orientalis* appears to be the same or similar to other species with reported chromosome numbers (2n = 2x = 28). Thus, I did not find evidence of polyploidy in this species to support a hypothesis of ploidy reduction while retaining apomixis gene expression. This is the first report of chromosome number in this species, though it should be confirmed by further root tip squashing. Additionally, a relative genome size (pg) should be determined for this and other species using flow cytometry to fully understand ploidy level(s) in the genus.

References

Carman JG (1997) Asynchronous expression of duplicate genes in angiosperms may cause apomixis, bispory, tetraspory, and polyembryony. *Biol J Linnean Soc* **61**: 51-94

Chatha GS, Bir SS (1987) Population analysis of some woody species from Palni Hills, south India. *J Cytol Genet* **22**: 83-94

Dirr M (2009) *Manual of woody landscape plants: their identification, ornamental characteristics, culture, propagation and uses.* Champaign, Illinois: Stipes Publishing

Dhawan OP, Lavania UC (1996) Enhancing the productivity of secondary metabolites via induced polyploidy: a review. *Euphytica* **87**: 81-89

eFloras (2008) *Sarcococca*. *Flora of China* http://www.efloras.org

Love A (1984) *Chromosome number reports LXXXII*. *Taxon* **33**: 126-134

Mursalimov SR, Sidorchuk YV, Deineko EV (2013) New insights into cytomixis: specific cellular features and prevalence in higher plants. *Planta* **238**: 415-423

Naumova TN (1993) *Apomixis in angiosperms: nucellar and integumentary embryony*. Boca Raton, Florida: CRC Press

Saggoo MIS, Farooq U, Lovleen (2011) Meiotic studies in *Sarcococca* species (Buxaceae) from western Himalayas. Cytologia **76**: 329-335

The Plant List (2013) *Sarcococca*. Version 1.1. http://www.theplantlist.org/1.1/browse/A/Buxaceae/Sarcococca/org/

This website contains my final project for PBGG 8890 Plant Cytogenetics at the University of Georgia. *Sarcococca* is a genus of 16-20 evergreen shrubs native primarily to Asia. It is poorly explored cytologically, with only four disputed species having reported chromosome numbers. Of particular interest is the presence of apomictic species in the genus. In this website, I explore mitosis and ploidy in oriental sweetbox, *Sarcococca orientalis*.

Photo: hereisabee on flickr