STAGES OF MITOSIS AND CHROMOSOME COUNT IN PAPERWHITE (*NARCISSUS PAPYRACEUS*)

INTRODUCTION
MATERIALS & METHODS
RESULTS
DISCUSSION
REFERENCES
ACKNOWLEDGMENTS

Introduction

I. *Narcissus* Nomenclature and Taxonomy

In the United Kingdom, large-cup flower varieties of *Narcissus* are referred to as ‘daffodils’ and smaller flower varieties are referred to as ‘narcissi’ (Hanks, 2002). There is a lot of debate about the taxonomy of the genus *Narcissus* due to the ease in which hybridization and speciation occurs (Webb, 1980). The *Narcissus* genus is in the Monocotyledon family Amaryllidaceae, in which it contributes 80 species to the total 850 species (Meerow and Snijman, 1998). *Narcissus papyraceus* is strictly related to *Narcissus tazetta*, but they have distinguishing karyological differences such as different chromosome numbers and sizes (D’Amato, 2004).

II. Etymology

In Greek mythology, Narcissus was the son of the river god Cephissus and a nymph Liriope (Britannica, 2015). He fell in love with his own reflection in a spring and died staring at himself. A flower sprung up where he died, and this flower was given his name (Britannica, 2015). *Narcissus* flowers commonly grow near river banks and often bend towards the water. This makes them appear as if they are looking at themselves and may attribute to the name *Narcissus*, since it is similar to word narcissism.

III. *Narcissus* Breeding Objectives

Narcissus cultivars are mostly bred for high bulb and flower production, but sturdy leaves and stems, reliable bud opening, long-lasting flowers, fragrance, and tolerances or resistances to different diseases are also considered (De Hertogh and Kamp, 1986). One of the most important resistances is base rot, found in *Narcissus jonquilla* (Linfield, 1988). Future breeding programs may focus on alkaloid or essential oil content (Hanks, 2002).

IV. Paperwhite Species Description and Origin

Paperwhites (*N. papyraceus* [Ker-Gawler]) have compressed scapes up to 50 cm long and flowers about 2.5 to 4 cm in diameter in umbels with up to 20 flowers (Fig. 1) (Hanks, 2002). The flowers are very fragrant, and its segments and corona are entirely white. The corona is cup-shaped and usually 204 mm deep (Hanks, 2002). *N. papyraceus* is the oldest and most widely distributed of the *Narcissus* genus. It originated in the Mediterranean and in adjacent areas of central Asia (Hanks, 2002).

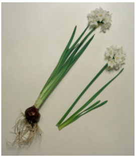

Figure 1. Paperwhite plant with an umbel of small, white flowers, a long scape, and a dark brown bulb.
Image from: http://www.gettyimages.com/

V. Cytology

A considerable amount of cytogenetic work has been done on the genus *Narcissus*; most species have unambiguous chromosome counts and karyotypes (Kamari et al., 2013). This is likely due its economic importance in the ornamental sector and due to the variability in its species (Sharma and Sharma, 1960). This variability includes various ploidy levels and even the presence of B chromosomes in some species (Wylie, 1952). There are two sub-genera of *Narcissus*: *Hermione* with a base haploid number of 5, 10 or 11 chromosomes, and *Narcissus* with 7 or 13 chromosomes (Hanks, 2002). Crosses between the two genera result in progeny with a wide range of chromosome numbers; there are numerous *Narcissus* aneuploids and ployploids. Most *narcissus* cultivars are tetraploid, but diploids, triploids and higher ploidy levels such as hexaploids are also common (Brandham and West, 1993).

Although a lot of cytogenetic work has been done in *Narcissus*, extensive work has not been done in the one of its most famous species *N. papyraceus*. There are about 15 published papers that include a chromosome count for *N. papyraceus*, yet a majority of these papers are in Spanish and Italian. However, it helps that its closest relative *N. tazetta* is well characterized. *N. papyraceus* ($2n = 22$) and *N. tazetta* ($2n = 2x = 20, 33$) are both in the *Herminone* sub-genus with a haploid base chromosome number of 11 and sometimes 10 in *N. tazetta* (Fig. 2 and 3) (Suzzi et al., 2004; Kamari et al., 2013; D’Amato, 2004). The ploidy level of *N. papyraceus* is often omitted in literature, but has been reported as 2x and 4x in different papers (Suzzi et al., 1997; Zonneveld, 2008). While Kamari et al. (2013) stated the karyotype was dipolid, Zonneveld stated it was tetraploid in a study using flow cytometry (2008). The karyotype of *N. papyraceus* is asymmetrical and comprised of mostly acrocentric and submetacentric chromosomes as well as two telocentric pairs and one metacentric pair. The sizes of the chromosomes range from 3.5 to 11.3 µm (Kamari et al., 2013). The nuclear organization regions (NORs) are generally located near the centromeres of acrocentric chromosomes (Weitz and Feinbrun, 1977). Although a unambiguous chromosome count has been published for *N. papyraceus* and quality photos of the stages of both mitosis and meiosis have been published for *N. tazetta* (Karihaloo and Koul, 1985), there is an opportunity to contribute mitotic and meiotic images of *N. papyraceus*. Additionally, all chromosome counts in *N. papyraceus* have been done in somatic cells, so a gametophytic chromosome count would be novel. In this experiment, I aim to contribute images of the stages of mitosis.

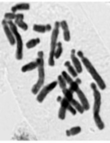

Figure 2. *N. papyraceus* squash revealing a clear chromosome count of 22 (Kamari et al., 2013).

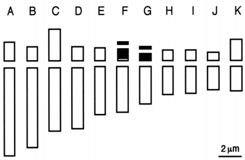

Figure 3. Ideogram of haploid set of *N. papyraceus* (D’Amato, 2004).

Chandler Maddox
Ph.D. Candidate
University of Georgia
Center for Applied Genetic Technologies
Email: cbm46672@uga.edu

This website was created in April, 2017 for the purposes of the PBGG 8890 Plant Cytogenetics final project.

INTRODUCTION
MATERIALS & METHODS
RESULTS
DISCUSSION
REFERENCES
ACKNOWLEDGMENTS

Materials and Methods

Root Tip Squash:

1. Root Collection
Bulbs were grown in a greenhouse on top of river stone in a solid container with a consistent water level held at the bottom of the bulbs (Fig. 1). Roots were collected and immediately placed in 8-hydroxyquinoline on ice.

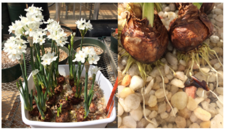

Figure 1. The left half shows the environment and container the bulbs were grown in. The right shows a close-up of the bulbs and their roots. The lack of soil made for clean slides to view under the microscope.

2. Root Pretreatment
After 5 hours in the 8-hydroxyquinoline at 4°C, the roots were placed in Farmer's fixative overnight at 22°C. Alternatively, a fixative containing 6 ml of methanol, 3 ml of chloroform, 2 ml of distilled water, .1 g of acetic acid, and .1 g of mercuric chloride as well as a fixative containing these ingredients plus .01 g of colchicine were used in an attempt to further compact the chromosomes; these were modified from Obstergren and Heneen (1962). In the morning, the roots were transferred into 70% EtOH and stored in a refrigerator for at least ten minutes until use.

3. Stain and Squash
The root tips were hydrolyzed in 1M HCl for 20 minutes at 60°C and then rinsed in distilled water. On a slide, all but the bottom 2 mm of the root tip was removed with a scalpel. A drop of modified carbol fuchsin stain was added. A razor blade was placed next to the stain and a cover slip was slowly dropped on top of the stain and razor blade. While holding the cover slip and razor blade firmly, the root tip was tapped five times with a pointed dissection needle. Holding the cover slip in place, the razor was moved 1 mm from the root tip and then the root tip was tapped five more times with the dissection needle. This was repeated until the razor blade was completely removed from under the cover slip. The slide was placed on a paper towel with the cover slip facedown and pressed firmly with the thumb. Lastly, the cells were viewed with a Olympus BH-2 microscope. Photos were taken with an Olympus DP25 digital color camera with the Olympus DP2-TWAIN program.

Chromosome Count in a Cell in an Early Stage of Microgametogensis:

1. Bud Collection
Buds of all sizes were collect by peeling away the protective tissue enclosing the umbel and placing the buds in Carnoy's fixative for 24 hours. Then, the buds were stored in 70% EtOH until use.

2. Stain and Squash
The anthers were dissected from the flowers and macerated in a drop of aceto-carmine on a microscope slide. A drop of Hoyer's was mixed into the slide contents. A coverslip was added and the edges were sealed with nail polish. Lastly, the cells were viewed with a Olympus BH-2 microscope. Photos were taken with an Olympus DP25 digital color camera with the Olympus DP2-TWAIN program.

Chandler Maddox
Ph.D. Candidate
University of Georgia
Center for Applied Genetic Technologies
Email: cbm46672@uga.edu

This website was created in April, 2017 for the purposes of the PBGG 8890 Plant Cytogenetics final project.

STAGES OF MITOSIS AND CHROMOSOME COUNT IN PAPERWHITE (*NARCISSUS PAPYRACEUS*)

INTRODUCTION
MATERIALS & METHODS
RESULTS
DISCUSSION
REFERENCES
ACKNOWLEDGMENTS

Results

Stages of Mitosis:

Interphase

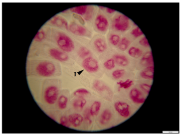

Figure 1. Interphase: Cells are most commonly found in interphase, a part of the cell cycle not involved in mitosis. During interphase, the chromosomes are not condensed and are not distinguishable in the nucleus. In this figure, the nucleolus is the unstained region in the cell. Scale = 500 µm.

Prophase

In this first stage of mitosis, the nucleolus disappears and the chromatin in the nucleus begins to condense and become visible as chromosomes.

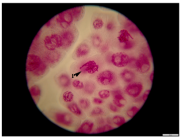

Figure 2. The cell indicated by the black arrow is in prophase, identifiable by the condensed chromatid that appears dark pink due to the stain. Scale = 500 µm.

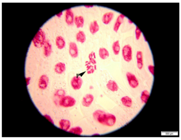

Figure 3. The cell indicated by the black arrow is in prophase. This cell is further along in prophase than the cell in Fig. 2, given that the chromosomes are more condensed in this figure. Scale = 500 µm.

Series of prophase photos:

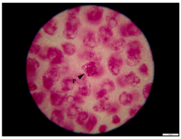

Figure 4. A cell in prophase. Scale = 500 µm.

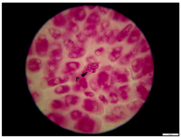

Figure 5. A cell in prophase. Scale = 500 µm.

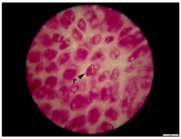

Figure 6. A cell in prophase. Scale = 500 µm.

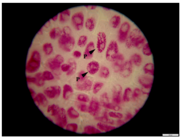

Figure 7. Two cells in prophase; the top cell is further along in this stage. Scale = 500 µm.

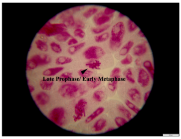

Figure 8. A cell in late prophase/ early metaphase. Scale = 500 µm.

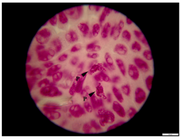

Figure 9. Two cells in prophase. Scale = 500 µm.

Metaphase

During metaphase, the chromosomes line up along the middle of the cell at the metaphase plate.

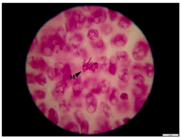

Figure 10. The cell indicated by the black arrow is in metaphase, indicated by the chromosomes aligned at the metaphase plate in the middle of the cell. Scale = 500 µm.

Series of metaphase photos:

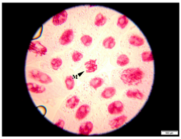

Figure 11. A cell in metaphase. Scale = 500 µm.

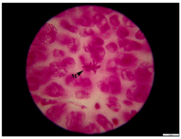

Figure 12. A cell in metaphase. Scale = 500 µm.

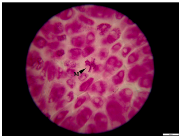

Figure 13. A cell in metaphase. Scale = 500 µm.

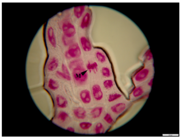

Figure 14. A cell in metaphase. Scale = 500 µm.

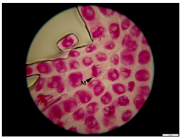

Figure 15. A cell in metaphase. Scale = 500 µm.

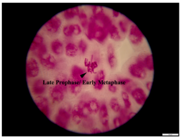

Figure 16. A cell in early prophase/ late metaphase. Scale = 500 µm.

Anaphase

In this stage, the paired sister chromatids are separated at their centromeres by mitotic spindles and pulled to opposite sides of the cell.

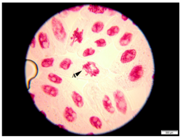

Figure 17. The cell indicated by the black arrow is in anaphase, indicated by the sister chromatid being separated and pulled to opposite poles. Scale = 500 µm.

Series of anaphase photos:

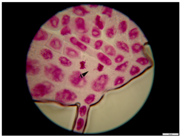

Figure 18. A cell in anaphase. Scale = 500 µm.

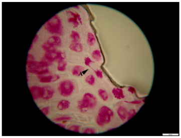

Figure 19. A cell in anaphase. Scale = 500 µm.

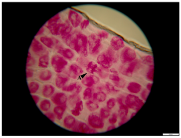

Figure 20. A cell in anaphase. Scale = 500 µm.

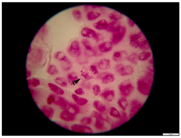

Figure 21. A cell in anaphase. Scale = 500 µm.

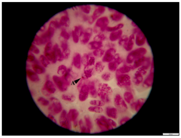

Figure 22. A cell in anaphase. Scale = 500 µm.

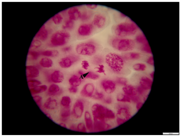

Figure 23. A cell in anaphase. Scale = 500 µm.

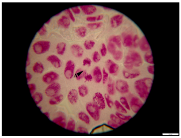

Figure 24. A cell in anaphase. Scale = 500 µm.

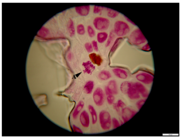

Figure 25. A cell in anaphase. Scale = 500 µm.

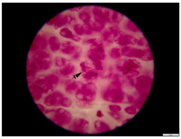

Figure 26. A cell in anaphase. Scale = 500 µm.

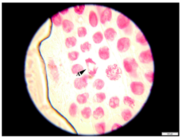

Figure 27. A cell in anaphase. Scale = 500 µm.

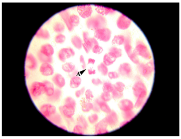

Figure 28. A cell in anaphase. Scale = 500 µm.

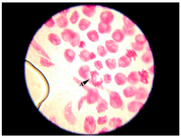

Figure 29. A cell in anaphase. Scale = 500 µm.

Telophase

The last stage of mitosis begins once the replicated DNA has been completely pulled to opposite sides of the cell. During telophase, a nuclear membrane forms around each set of chromosomes, which separates them from the cytoplasm, and the chromosomes begin to uncoil.

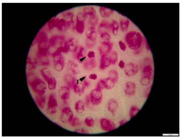

Figure 30. A cell undergoing telophase, which is indicated by the two circular sets of chromosomes that are beginning to uncoil and become less condensed. The black arrows are pointing at each set of chromosomes. Scale = 500 µm.

Series of telophase photos:

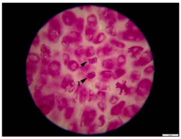

Figure 31. A cell in telophase; the black arrows are pointing at each set of chromosomes. Scale = 500 µm.

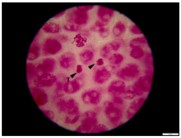

Figure 32. A cell in telophase; the black arrows are pointing at each set of chromosomes. Scale = 500 µm.

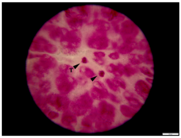

Figure 33. A cell in telophase; the black arrows are pointing at each set of chromosomes. Scale = 500 µm.

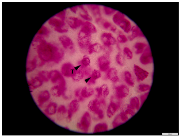

Figure 34. A cell in telophase; the black arrows are pointing at each set of chromosomes. Scale = 500 µm.

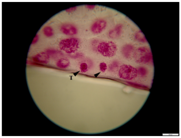

Figure 35. A cell in telophase; the black arrows are pointing at each set of chromosomes. Scale = 500 µm.

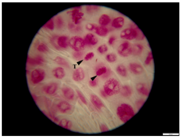

Figure 36. A cell in telophase; the black arrows are pointing at each set of chromosomes. Interestingly, the small, dark pink stain between the two sets may be a lost fragment caused by a breakage or it may be from a cell in a different plane. Given that it is in focus, it is more likely a lost fragment of DNA. Scale = 500 µm.

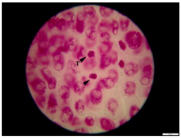

Figure 37. A cell in telophase; the black arrows are pointing at each set of chromosomes. Scale = 500 µm.

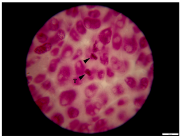

Figure 38. A cell in telophase; the black arrows are pointing at each set of chromosomes. Scale = 500 µm.

Chromosome Count:

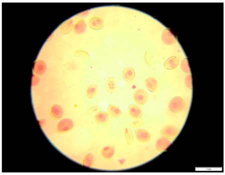

Figure 39. Photo at 40x magnification of a cell in an early stage of microgametogensis with condensed chromosomes; this cell is undergoing prophase in mitosis. This magnification is crisper than 100x magnification, and zooming in on this photo yields a higher quality photo than 100x. The following photos will show a photo at 100x and a zoomed in version of this one. Given *N. papyraceus* is 2n = 22, there should be 11 chromosomes. Scale = 1 mm.

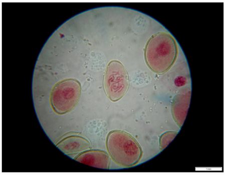

Figure 40. Photo at 100x magnification of a cell undergoing an early stage of microgametogensis; this cell is in prophase in mitosis. This photo is less crisp than the photo at 40x magnification. Given *N. papyraceus* is 2n = 22, there should be 11 chromosomes. Scale = 1 mm.

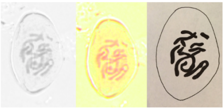

Figure 41. The first two sections of the figure are zoomed in photos of Fig. 38, which was an image of a cell undergoing an early stage of microgametogensis at 40x magnification. The last section is an interpretive drawing of the chromosomes, showing 11 chromosomes as expected. Ideally, more cells with tightly condensed chromosomes need to be found to confirm this count.

Chandler Maddox
Ph.D. Candidate
University of Georgia
Center for Applied Genetic Technologies
Email: cbm46672@uga.edu

This website was created in April, 2017 for the purposes of the PBGG 8890 Plant Cytogenetics final project.

STAGES OF MITOSIS AND CHROMOSOME COUNT IN PAPERWHITE (*NARCISSUS PAPYRACEUS*)

INTRODUCTION
MATERIALS & METHODS
RESULTS
DISCUSSION
REFERENCES
ACKNOWLEDGMENTS

Discussion

Producing photos of the stages of mitosis was relatively easy compared to producing a photo of an unambiguous chromosome count. In the results page, many of the photos contain multiple stages of mitosis, indicating that the mitotic index was high. One of the photos not included in the results page (due to suboptimal lighting) had three cells in anaphase and one in telophase at 100x magnification. This high mitotic index is not species specific but environmental. I first attempted this project with bulbs grown in small pots of dirt set on top of sand. Roots were to be collected when they came out of the bottom of the pot and into the sand that is easily washed off. However, the roots grew much slower in soil than they did on river stone in water. The partially clean root tips taken from the soil showed no stages of mitosis in the microscope slides I made, and the dirt degraded the quality of the slides. However, the roots grown on the river stone were vigorous with a high mitotic index. Furthermore, growing the bulbs on river stone enabled collection of newly germinating roots, which would also explain the high mitotic index.

Another finding of making slides with *N. papyraceus* root tips was that they do not digest well in 1:3 concentrated HCl to 95% EtOH. I tried submerging them for 5, 7, 10, 15, 20, 30, 45, and 60 seconds, but the root tips were never digested enough to produce quality slides. I found that the best method was hydrolyzing them in 1M HCl for 20 minutes at 60°C. This technique does not exactly match any of the published studies in *Narcissus*, but it appears many methods work given the diversity of digestion protocols in the literature (Kamari et al., 2013; D'Amato, 2004; Sharma and Sharma, 1961). Although, none of these protocols included a short submersion in 1:3 concentrated HCl to EtOH for digestion. It would be interesting to find out why this technique is not sufficient in *N. papyraceus* but is sufficient in other species such as garlic and onion, among others.

Attaining an unambiguous chromosome count in the root tip cells was unsuccessful with all three fixatives used. The Farmer's fixative, which is 3:1 100% EtOH to glacial acetic acid, was the closest in producing a chromosome count (Fig. 3 on the Results page). The chromosomes needed to be more condensed and more spread to achieve an accurate count. Therefore, a fixative containing 6 ml of methanol, 3 ml of chloroform, 2 ml of distilled water, .1 g of acetic acid, .1 g of mercuric chloride and a fixative containing these ingredients plus .01 g of colchicine were used in an attempt to further compact the chromosomes. These fixatives were modified from Obstergren and Heneen (1962), who produced some spectacular photos of unambiguous chromosomes counts in various species. As they had warned, the mercuric chloride left a lot of crystalline particles of mercury, which caused the slides to be covered in dark purple specks. These deposits essentially ruined any chance of producing a clear chromosome count. The authors recommended washing the roots in iodine mixed with 45-50% acetic acid to remove the mercury deposits. I had forgotten to add iodine to the wash; this result emphasizes the importance of the iodine wash when using this technique.

Unable to condense and spread the 22 chromosomes enough for an accurate count, I switched to pollen mother cells with the idea that 11 chromosomes would be easier to count. I collected and fixed about 50 buds, yet only one of them was young enough to contain cells that had not fully developed into pollen grains. I was only able to make one slide from this bud. Since I found a cell undergoing prophase in an early stage of microgametogenesis that showed a clear chromosome count, I concluded that pollen mother cells and cells undergoing microgametogenesis may be easier to find chromosome counts in, especially for *N. papyraceus*. There are less steps to fix pollen mother cells- eliminating the digestion stage and the need to spread the chromosomes makes it easier than somatic cells. Also, pollen mother cells are smaller, so more fit on the microscope slide, which increases the chance of finding a cell with a clear chromosome count. The photograph of the cell in an early stage of microgametogenesis in this study (Fig. 41 on the Results page) was not perfectly clear, but by comparing it to photos of *N. papyraceus* chromosomes in previous studies (Kamari et al., 2013; D'Amato, 2004) and a previous ideogram (D'Amato, 2004), it is possible to count the 11 chromosomes present. Ideally, more photos of chromosome counts would be found in both pollen mother cells and root tips cells to confirm this count.

Chandler Maddox
Ph.D. Candidate
University of Georgia
Center for Applied Genetic Technologies
Email: cbm46672@uga.edu

This website was created in April, 2017 for the purposes of the PBGG 8890 Plant Cytogenetics final project.

STAGES OF MITOSIS AND CHROMOSOME COUNT IN PAPERWHITE (*NARCISSUS PAPYRACEUS*)

INTRODUCTION
MATERIALS & METHODS
RESULTS
DISCUSSION
REFERENCES
ACKNOWLEDGMENTS

References

Brandham, P., and J. West. 1993. Correlation between nuclear-DNA values and differing optimal ploidy levels in *Narcissus*, *Hyacinthus*, and *Tulipa* cultivars. Genetica 90(1):1-8. doi: 10.1007/BF01435172

D'Amato, G. 2004. Karyotype and heterochromatin characterization in some species of *Narcissus* (Amaryllidaceae), Caryologia 57(1):99-105. doi: 10.1080/00087114.2004.10589377

De Hertogh, A. and M. Kamp. 1986. Commercial forcing and marketing requirements for flower bulbs in the U.S. and Canada. Acta. Hort. 177:267-272. doi: 10.17660/ActaHortic.1986.177.37

The Editors of Encyclopaedia Britannica. 2015. Narcissus: Greek Mythology. In: Encyclopaedia Britannica. https://www.britannica.com/ (accessed 20 Mar. 2017).

Hanks, G. 2002. *Narcissus* and daffodil: The genus *Narcissus*. In: Medicinal and aromatic plants – Industrial profiles. Taylor & Francis, London, France.

Kamari, G., Blanché, C., and S. Siljak-Yakovlev. 2013. Mediterranean chromosome number reports – 23. Fl. Medit. 23:255-291. doi: 10.7320/FlMedit23.255

Karihaloo, J.L., and A.K. Koul. 1985. Cytogenetic studies in the genus *Narcissus* L. IV. Cytology of a naturalized variety of *N. tazetta* L. from Kashmir. Cytologia 50:265-274. doi: 10.1508/cytologia.50.265

Linfield, C. 1992. Resistance of wild *Narcissus* species to *Fusarium oxysporum* F. Sp. *Narcissi*. Arta. Hort. 325:683-688. doi:10.17660/ActaHortic.1992.325.98

Meerow, A., and D. Snijman. 1998. Amaryllidaceae. In: Families and genera of vascular plants. Vol. 3. Springer-Verlag, Berlin, Germany. p. 83-110.

Obstergren, G., and W.K. Heneen. 1962. A squash technique for chromosome morphological studies. Hereditas 48:332-341. doi: 10.1111/j.1601-5223.1962.tb01817.x

Sharma, A.K., and A Sharma. 1961. Chromosome studies of some varieties of *Narcissus tazetta* L. Caryologia 14(1):97-106. doi: 10.1080/00087114.1961.10796014

Suzzi, S., Vosa, C.G., and P. Bassi. 1997. Repetitive DNA as marker for the study of male gametogenesis in higher plants. I. Analysis in some species of *Tradescantia* and *Narcissus*, Caryologia 50(1):67-76. doi: 10.1080/00087114.1997.10797385

Webb, D. 1980. *Narcissus*. In: Flora Europaea. Cambridge University Press, Cambridge, UK. p. 78-84.

Weitz, S., and N. Feinbrun. 1977. Cytology and systematics of *Narcissus tazetta* in Israel. Israel J. Bot. 21:9-20.

Wylie, A.P. 1952. The history of the garden *Narcissi*. Heredity 6:137-156. doi:10.1038/hdy.1952.16

Zonneveld, B.J.M. 2008. The systematic value of nuclear DNA content for all species of *Narcissus* L. (Amaryllidaceae). Plant Syst. Evol. 275:109-132. doi: 10.1007/s00606-008-0015-1

Chandler Maddox
Ph.D. Candidate
University of Georgia
Center for Applied Genetic Technologies
Email: cbm46672@uga.edu
This website was created in April, 2017 for the purposes of the PBGG 8890 Plant Cytogenetics final project.

STAGES OF MITOSIS AND CHROMOSOME COUNT IN PAPERWHITE (*NARCISSUS PAPYRACEUS*)

INTRODUCTION
MATERIALS & METHODS
RESULTS
DISCUSSION
REFERENCES
ACKNOWLEDGMENTS

Acknowledgments

A special thank you to Dr. Joann Conner for taking a full day out of your busy schedule to teach me cytogenetic techniques and pitfalls. Thank you, Dr. Wayne Parrott, Dr. Edward McAssey, Dr. Peggy Ozias-Akins, and the Scott Jackson lab for lab materials, support, and insight during this project. Also, thank you Lokesh Dhakar for making the Javascript for the lightbox click-to-enlarge image effect available and thank you David Higgins for making it easier to understand.

Chandler Maddox
Ph.D. Candidate
University of Georgia
Center for Applied Genetic Technologies
Email: cbm46672@uga.edu
This website was created in April, 2017 for the purposes of the PBGG 8890 Plant Cytogenetics final project.