Meiotic chromosome analysis in tropical orchid genus *Sobralia*

José MORA[1], **Stefano ALBERTAZZI, Pablo BOLAÑOS-VILLEGAS**[1,*]

[1] Fabio Baudrit Agricultural Research Station, University of Costa Rica, La Garita de Alajuela, Alajuela 20102, Costa Rica
[*] **pablo.bolanosvillegas@ucr.ac.cr**

Abstract – Tropical orchid genus *Sobralia* comprises terrestrial plants with elongated, cane-like stems and large symmetrical, yet ephemeral flowers. This genus is distributed throughout Central and South America and may hold horticultural potential. Nonetheless, little is known about the reproductive viability of species that comprise this genus. In this project male meiocytes have been examined by light microscopy to determine chromosome numbers, frequency of chromosome segregation defects and the frequency of normal tetrads at the end of meiosis II. The species sampled belong to an *ex situ* collection managed by the Lankester Botanical Garden of the University of Costa Rica. The species were: *S. amparoae, S. artropubescens, S. boucheri, S. bradeorum, S. carazoi, S. crispissima, S. danjanzenii, S. fenzliana, S. geminata, S. helleri* and *S. rosea.* Our results indicate that meiocytes from these species have a variable diploid (2n) chromosome number of 24, 30 and 32, that segregation defects are rare and that tetrad formation rates exceed 80%, suggesting efficient meiotic progression and high pollen viability. Taken together our results suggest that at least in Costa Rica the populations sampled are reproductively healthy and amenable to horticultural breeding.

Keywords: *Sobralia*, orchid, meiosis, chromosome analysis, Costa Rica.

INTRODUCTION

Little is known about how geographical isolation and the absence of pollinators impacts the reproduction of tropical orchids (Waterman and Bidartondo, 2008), Nonetheless it has been hypothesized that in Central America at any given time the number of fertile orchid plants is low, seed production is limited, and that therefore gene flow is also severely restricted (Tremblay and Ackerman, 2001). This set of conditions may lead to high selection pressures and the creation of new species (Tremblay *et al*., 2005).

To test these hypotheses experimental comparisons of populations are required, but usually this is not possible due to slow reproductive cycles, few flowers and little or no divergence in the genome of the individuals sampled (Lahaye *et al*., 2008). One approach to tackle these problems is to study chromosomes (Kao *et al*., 2007). Chromosomes are complexes of nucleic acids and proteins whose number and morphology varies across species thus allowing for evolutionary studies (Kao *et al.*, 2007; Lee *et al.*, 2011). Fortunately, the University of Costa Rica runs a dedicated orchid *ex situ* collection called the Lankester Botanical Garden. Within the garden a greenhouse is used to grow and study plants from orchid tribe *Sobraliae.*

Sobraliae is polyphyletic neotropical orchid tribe from the Americas that comprises about 200 species from genera *Elleanthus*, *Epilyna*, *Sertifera* and *Sobralia* (Neubig *et al*., 2011). These plants are often terrestrial plants with cane like stems and in the case of genus *Sobralia,* large flowers (Neubig *et al*., 2011). Flowers are symmetrical and beautiful, however little to no plant breeding has been performed in Costa Rica using *Sobralia,* a situation that makes little commercial sense considering the availability of native species, and its ease of cultivation and propagation, factors that have been determinant in the success of breeding programs elsewhere (Kamemoto and Kuehnle, 1996).

For this study *Sobralia* plants from *ex situ* collection of the Lankester Botanical Garden were sampled and pollinia were collected to determine the chromosome number and to observe and record chromosome segregation patterns during meiosis, including the formation of tetrads, which is the end stage of meiosis (Mercier *et al*., 2015).

MATERIALS AND METHODS

Pollinia from flower buds before anthesis were collected from the *ex situ* orchid collection located at the Lankester Botanical Garden, University of Costa Rica. Pollinia were processed according to Lee and Chung (2010). Briefly, they are placed in 2 mM 8-hydroquinoline (Sigma-Aldrich) solution for 5 hrs at 25° C, fixated in ethanol/glacial acetic acid solution (3:1, v/v) for 12 hrs and then frozen at -20° C. Samples were digested enzymatically with 6% cellulase and pectinase solution (Sigma-Aldrich) dissolved in 75 mM KCl at a pH of 4.0, for 1 hr at 37° C.

Digested samples were macerated in a drop of 40% acetic acid solution and then stained with fluorescent DNA stain 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI, Sigma-Aldrich). Images were obtained with a BX53 epifluorescence microscope (Olympus, Tokyo) connected to a ColorQ5 CCD camera (Olympus, Tokyo) and a Dell Precision Tower T7810 computer (Dell, Round Rock, TX). Images were analyzed with Adobe Photoshop CS5 (Adobe Systems, San José, CA).

RESULTS AND DISCUSSION

Analysis of meiotic cell cycle progression across all species suggested that there are no apparent defects during synapsis and pairing (zygotene and pachytene) (Mercier *et al*., 2015), and no defects during the alignment and segregation of bivalents (metaphase, anaphase and telophase I and II) (Mercier *et al*., 2015), as observed in Figure 1.

Counts of normal tetrads (regular tetrads) suggest that formation is normal (Table 1), suggesting normal chromosome segregation, and possibly formation of viable pollen after mitosis I and II. Preliminary chromosome counts during metaphase I and anaphase I suggest a variable chromosome number of 24, 30 and 32. Sampling will continue with frozen samples.

These preliminary results indicate that unlike in Puerto Rican *Lepanthes* (Tremblay and Ackerman, 2001), sexual viability in Costa Rican *Sobralia* is normal, suggesting reproductive success, effective population sizes, competitive ability, or ecological tolerance (Levin, 2002). For instance, sizeable groups of *Sobralias* are observed in disturbed habitats across Costa Rica (personal communication, Dr. Robert Dressler). It is also known that most *Sobralias* are pollinated by *Euglossine* bees and hummigbirds, which are common pollinators of Central American orchids (personal communication, Dr. Mario Blanco), and that these plants show synchronous gregarious flowering (Dressler, 1990). Therefore, availability of either a pollinator or a flowering partner does not appear to be a problem in these plants.

Table 1. Formation of tetrads is normal in *Sobralia* species, suggesting high pollen viability. Results are the mean of three biological and three technical samples, *n*=100 plus the standard deviation, nd: not determined.

Species	Normal Tetrads (%)	Chromosome number (2n)
S. amparoae	89,3±8,5	30
S. artropubescens	80,0±6,6	nd
S. boucheri	88,4±5,3	nd
S. bradeorum	89,7±1,8	nd
S. carazoi	85,7±5,1	nd
S. crispissima	83,1±10,2	nd
S. danjanzenii	93,5±0,7	32
S. fenzliana	87,5±1,0	nd
S. geminata	89,5±1,0	32
S. helleri	86,0±2,1	nd
S. rosea	93,1±2,1	24

Our results also indicate that wild individuals of *Sobralia* may be amenable to hybridization and plant breeding. Work is scheduled to continue until 2019 and may involve propagation by tissue culture.

Acknowledgements

This work was possible thanks to grant B5A49 from the office of the Vice Chancellor for Research and a travel permit to attend the EEOCE Paris 2018 from the Chancellor of the University of Costa Rica. The authors would like to thank the following students for their contribution to this project: Y. Corrales, M.A. Vega, K. Scott, K. Scott, and S. Castro-Pacheco. The authors also thank the scientific

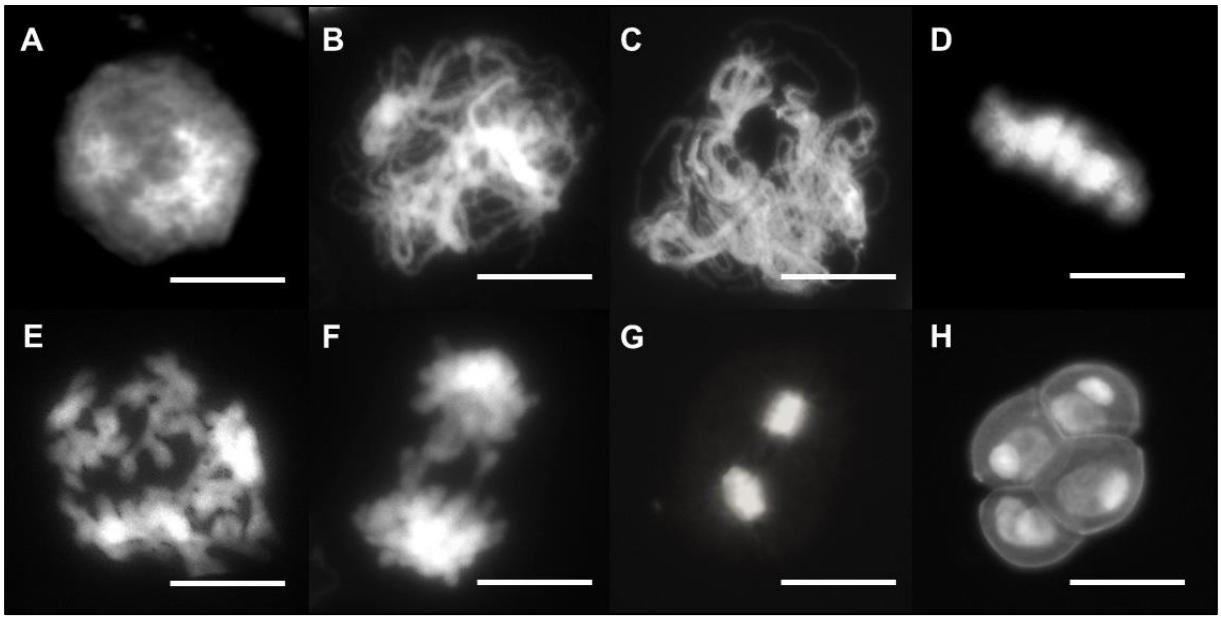

Figure 1. Cell division during male meiosis is normal in *Sobralia* species. Image is a representative composite from meiocytes across different species. A, leptotene; B, zygotene, C, pachytene; D, metaphase I; E, anaphase I; F, late anaphase I/early telophase I; G, metaphase II, H, tetrad stage. Scale bars, A-C, 20 µm, D-H, 10 µm. Meiocytes were stained with DAPI.

personnel of the Lankester Botanical Garden including Robert Dressler, Mario Blanco, Franco Pupulin and Jorge Warner.

References

Dressler R.L. 1990. The orchids: natural history and classification. Harvard University Press, Cambridge.

Kamemoto H., Amore T.D., Kuehnle A. 1996. Breeding *Dendrobium* orchids in Hawaii. University Hawaii Press, Honolulu.

Kao Y.Y., Lin C.C., Huang C.H., Li Y.H. 2007. The cytogenetic of *Phalaenopsis* orchids. *In: Orchid Biotechnology*. W.H. Chen and H.W. Chen (Eds.). World Scientific Publishing, Singapore. pp. 115-128.

Lahaye R., Van der Bank M., Bogarin D., Warner J., Pupulin F., Gigot G., Maurin O., Duthoit S., Barraclough T.G., Savolainen V. 2008 DNA barcoding the floras of biodiversity hotspots. *Proc. Natl; Acad; Sci; USA*, 105: 2923-2928.

Lee Y.I., Chang F.C., Chung M.C. 2011. Chromosome pairing affinities in interspecific hybrids reflect phylogenetic distances among lady's slipper orchids (*Paphiopedilum*). *Ann. Bot.*, 108: 113-121.

Lee Y.I., Chung M.C. 2010. Karyomorphological observation on some *Paphiopedilum* hybrids. *Acta Hort.*, 878: 99-106.

Levin D.A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, New York.

Mercier R., Mézard C., Jenczewski E., Macaisne N., Grelon M. 2015. The molecular biology of meiosis in plants. *Annu. Rev. Plant Biol.*, 66: 297-327.

Neubig K.M., Whitten M., Blanco M.A., Endara L., Williams N.H., Koehler S. 2011. Preliminary molecular phylogenetics of *Sobralia* and relatives (*Orchidaceae: Sobralieae*). *Lankesteriana*, 11: 307-317.

Tremblay R.L., Ackerman J.D. 2001. Gene flow and effective population size in *Lepanthes* (*Orchidaceae*): a case for genetic drift. *Biol. J. Linn. Soc.*, 72: 47-62.

Tremblay R.L., Ackerman J.D., Zimmerman J.K., Calvo R.N. 2005. Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biol. J. Linn. Soc.*, 84: 1-54.

Waterman R.J., Bidartondo M.I. 2008. Deception above, deception below: linking pollination and mycorrhizal biology of orchids. *J. Exp. Bot.*, 59: 1085-1096.