Evolution and development of orchid flowers and fruits

Dirks, A.

Chapter 6

General discussion and conclusions

In vitro propagation of *Erycina pusilla*

During this PhD project, several innovative insights were obtained about the genetic basis of the development of floral organs and fruits of the orchid species *Erycina pusilla*. First, though, several basic skills had to be developed at Naturalis Biodiversity Center. One of the first challenges was to establish an efficient *in vitro* propagation protocol for *E. pusilla*, which could easily be implemented in other laboratories as well. We started in 2014 using an existing protocol developed by the Dutch orchid breeder Johan Keus. One of the biggest challenges that we encountered was contamination of the medium. Only after we started to use Sodium dichloroisocyanurate (NaDCC) as sterilant and the antimicrobial and Plant Preservative Mixture (PPM) as antimicrobial substance (Kendon *et al*., 2017), our *in vitro* cultures of *E. pusilla* could be completed from seed to fruiting stage without loss of plant material due to undesirable fungal and bacterial infections.

Life cycle of *Erycina pusilla*

Once a growing protocol had been established, a second necessity was to characterize the full development of the flowers and fruits of *E. pusilla* by studying the full ontogeny using the microscopy facilities present at Naturalis Biodiversity Center and Leiden University Medical Centre. Floral morphology and ontogeny of *E. pusilla* flowers was unraveled in **chapter 3**, in which the development of *E. pusilla* flowers was subdivided into five stages based on macro-morphological changes from the first emergence of an inflorescence stalk up to a fully open flower. *Erycina pusilla* fruits on the other hand were divided into four stages based on different macro- and micro-morphological changes as described in **chapter 4**.

Erycina pusilla is a common twig epiphyte in the meso-American tropics; our laboratory strain came from Surinam. In nature, *Centris* bees pollinate the yellow non-rewarding flowers. During floral visits, these bees cling to the stelidia (wings along the gynostemium) and the callus on the lip with their forelegs while searching for floral oils that are not present. Flowers of *E. pusilla* are mimicking the rewarding flowers of Malpighiaceae, which do have rewarding oil glands. The stelidia were detected to be the remnants of the six stamens, which are still fertile in a few basal orchids with rather primitive pollination syndromes such as *Apostasia wallichii* but reduced to sterile organs in most derived lineages with highly specialized pollination syndromes such as *E. pusilla*.

In the lab, we pollinated flowers of *E. pusilla* ourselves by removing the pollinarium with a forceps and placing the pollinia into the stigmatic cavity of the gynostemium. After a flower was successfully pollinated it wilted after a few days and the stelidia folded together (**Figure 1**). We actually postulate that this process, either active or an effect of senescence once pollination is achieved, protects the stigma with the developing pollen tubes against UV radiation and herbivores.

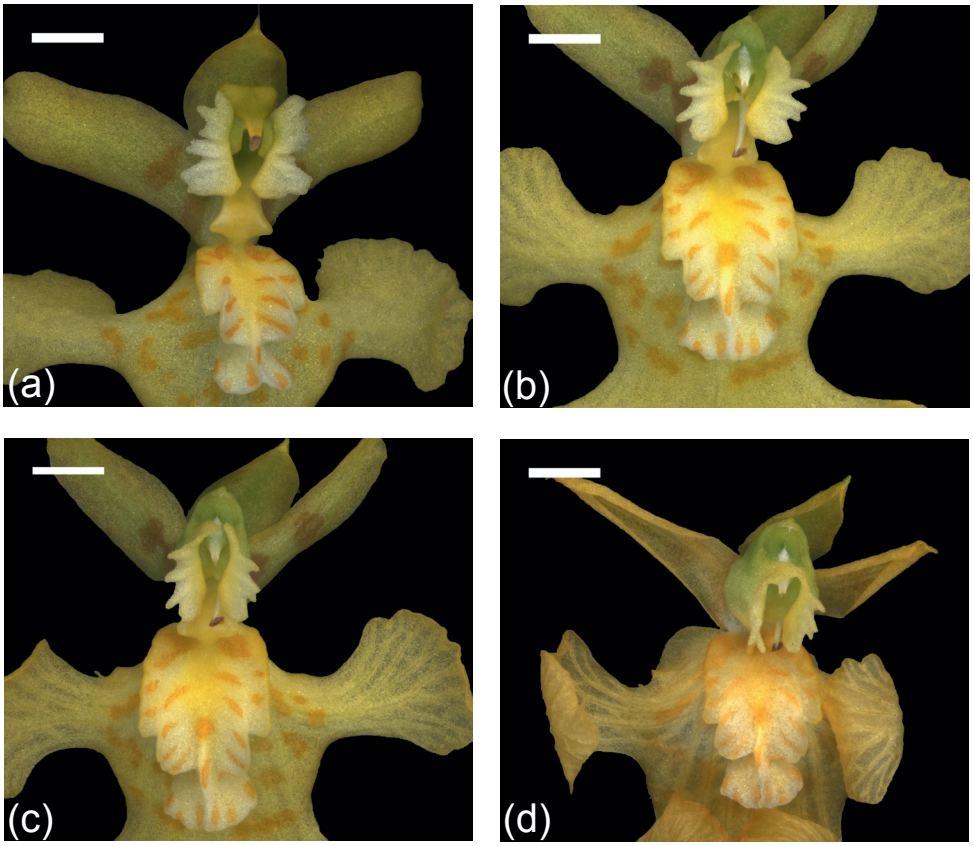

Figure 1. Macro-morphology of E. pusilla prior to and after pollination. (a) Fresh flower prior to pollination. **(b)** Flower one day after pollination. **(c)** Flower two days after pollination. **(d)** Flower five days after pollination. Scale bar=2 mm.

Pollen tubes were macroscopically visible in developing fruits until 7-8 weeks after pollination (WAP) suggesting that ovules were fertilized. After 16 WAP the fruit was ripe and opened to release the seeds. The seeds germinated in ~3-4 months from protocorms into protocorm-like bodies (PLBs). Once the first roots and leaves had developed from the PLBs, the sterile plantlets were transferred to individual tubes, in which they developed into full plants with inflorescences in ~ 3-4 months. One aspect that still needs to be optimized for our *E. pusilla* Surinam strain is cryopreservation of seeds, or embryos, for short- and long-term storage. Up until now the strain has been propagated by continuous tissue culture, which is time consuming, expensive and risky. Different attempts were made in the beginning of this PhD project, including dry storage at 4 °C and -20 °C but unfortunately these attempts all failed due to infected plates and lack of germination. Various articles were published over the last few years (Popova *et al*., 2016;Cerna *et al*., 2018;Magrini *et al*., 2018;Schofield *et al*., 2018) with instructions to set up a simple cryopreservation protocol for *E. pusilla* seeds and use triphenyl tetrazolium chloride

(TTC) staining to test seed viability before sowing the seeds for future attempts.

MADS-box genes involved in flower and fruit development

The shape of orchid flowers is very complicated due to gene duplications and sub-functionalizations that evolved over millions of years. MADS-box genes play an important role in flower development so to find out what the role is of these different genes was, we conducted an expression study of 20 different MADS-box genes isolated from floral organs of early and late developing floral buds. We then constructed gene lineage trees for every MADS-box gene class to investigate to which clades all duplicates belonged and whether they evolved at the same rate or not. Combining these data with micro-morphological data of developing floral organs and epidermal cell structures using SEM, the Oncidiinae model was developed in **chapter 3.** This model describes how different copies of MADS-box genes *AP3* (*EpMADS14*) and *AGL6* (*EpMADS4*) code for the shape of the lateral sepals. When these gene copies are expressed in the perianth, the lateral sepals have a sepaloid appearance. Different developmental MADS-box gene copies determine the various shapes of orchid sepals, lips and stelidia. Following the perianth-code model by Hsu *et al*. (2015), the development of the lip compared with the sepals and petals is based on the interaction of *PI* with two different *AP3* and *AGL6* copies. We found for *E. pusilla* flowers that (i) the Lip-complex is fully expressed in the lip and the callus on the lip, (ii) the SP-complex is fully expressed in the sepals and petals and (iii) *AG* (*EpMADS20*) and *STK* (*EpMADS23*) copies shape the stelidia and a copy of *AG* (*EpMADS22*) and *SEP* (*EpMADS6*) shape the stamen.

We concluded that the enlarged median sepal, incised lip, callus and stelidia of *E. pusilla* evolved to mimic the shape of the petals and oil glands of flowers of Malpighiaceae in order to attract oil-collecting bees for pollination. All these organs evolved for a perfect fit with bodies of specific pollinators to optimize pollination success.

Fruit-associated MADS-box proteins were studied in **chapter 4** by performing a yeast-two-hybrid assay to study protein-protein interactions. Together with an expression study on a series of developing fruits of *E. pusilla*, we characterized orthologs of fruit-associated MADS-domain transcription factors and of the *Arabidopsis thaliana* dehiscence-related genes *INDEHISCENT* (*IND*)/*HECATE3* (*HEC3*), *REPLUMLESS* (*RPL*) and *SPATULA* (*SPT*)/*ALCATRAZ* (*ALC*). We found that the key players of the eudicot fruit regulatory network appear well conserved in monocots. Protein-protein interaction studies revealed that MADS-domain complexes comprised of *FRUITFULL* (*FUL*), *SEPALLATA* (*SEP*) and *AGAMOUS* (*AG*) /*SHATTERPROOF* (*SHP*) orthologs can also be formed in *E. pusilla*, and that the expression of *HEC3*, *RPL* and *SPT* can be associated with dehiscence zone development similar to Arabidopsis. Our gene expression analysis also indicates differences, however, which may underlie fruit divergence.

Massive parallel sequencing of short RNA molecules added important additional insights in the genetic basis of the orchid sepals, petals and lip, as described in the Perianth Code model (Gravendeel and Dirks-Mulder, 2015;Hsu *et al*., 2015)

and Oncidiinae model. It also provided the first glimpses of the genetic basis of other orchid organs in the third and fourth floral whorls such as the stamen and stelidia, and fruit dehiscence. A third challenge was to start up transcriptome analyses. For this, initially, existing data in Orchidstra (Su *et al*., 2013;Chao *et al*., 2017) were mined. This was sufficient for the first expression studies on floral and fruit organs as presented in **chapter 3 and 4**, but we could not find any *E. pusilla HEC3* homologue in this database. This meant that we had to produce our own fruit transcriptomic data.

Orchid transcriptome analysis

It became apparent that we needed to generate transcriptome data ourselves to be able to detect additional genes involved in fruit development besides the MADS-box genes. This meant that we needed a full suite of tailor-made bioinformatics pipelines. Bioinformaticians of Naturalis Biodiversity Center and many bioinformatics students, staff and lecturers of the University of Applied Sciences Leiden were vital for developing the orchid genomic toolkit for generating a *de novo* transcriptome using Blast2GO (Gotz *et al*., 2008). This bioinformatics platform was used for analysis of the orchid transcriptomes generated. Vital for the annotating process as well was the creation of a custom made Orchid-Blast-Database to run local Blasts. This database turned out to be dominated by floral transcriptomes, though, and did not contain that many genes yet involved in fruit formation. By mapping reads against the assembled and annotated reference genome of *P. equestris*, though, several fruit transcriptomes of *E. pusilla* could be analyzed with various bioinformatics tools as presented in **chapter 5**. This resulted in the identification of a first group of candidate genes involved in seed formation, pollen tube development and lignification, that are either up- or down-regulated during development of the fruits of *E. pusilla.*

Currently, genome sequences are published for a total of seven orchid species: *Dendrobium officinale* and *D. catenatum* (Yan *et al*., 2015;Zhang *et al*., 2016), *Phalaenopsis equestris* and *P. aphrodite* (Cai *et al*., 2015;Chao *et al*., 2018) (all subfamily Epidendroideae), *Apostasia shenzhenica* (subfamily Apostasioideae) (Zhang *et al*., 2017), *Gastrodia elata* (Yuan *et al*., 2018) (subfamily Epidendroideae) and a draft genome of *Vanilla planifolia* (Hu *et al*., 2019) (subfamily Vanilloideae). Comparing these orchid genomes gave important first insights into the existence of new gene families, and how these gene families either expanded or contracted during the evolution of this plant family. This was done by comparing the genome of the rather basal orchid species *A. shenzhenica* with the genomes of the more derived species *Phalaenopsis equestris* and *D. catenatum* (Zhang *et al*., 2017). The authors found gains and losses of certain MADS-box and other genes (e.g. Myb factors) controlling a diverse suite of processes, e.g. the development of the lip and gynostemium, pollinia, and seeds without endosperm.

During this PhD project, the first attempts were made for full genome sequencing and hybrid assembly of the *E. pusilla* genome. We combined second

generation Illumina HiSeq sequencing, creating short (50-100 bp long) reads with high (35x) coverage, and PacBio analysis, creating longer (3-5 kb long) reads, sequenced with low (5x) coverage. Using different assembly methods, a first *de novo* hybrid genome assembly was created, consisting of more than 450,000 scaffolds with only 2% of the total data used. Ongoing developments in third generation sequencing technologies will soon enable retrieval of even higher quality genomes and also transcriptomes by sequencing longer reads that are expected to ultimately encompass full-length transcripts. An important emerging research tool is the MinION Oxford Nanopore DNA/RNA sequencing technology to generate such long (up to 50 kb) sequence reads, which can be used to read entire plant transcriptomes. This technique has already been used for whole genome sequencing of other plant species, also in combination with optical mapping (Belser *et al*., 2018;Deschamps *et al*., 2018). Plant genomes are large, often polyploid, and may contain over 90% of repetitive DNA (Mehrotra and Goyal, 2014) so sequencing the relatively small and diploid orchid genome of *E. pusilla* is feasible. For future studies, I therefore recommend completing the sequencing and assembly of the full genome of *E. pusilla*. Anonymous (short) reads, either genomic or transcriptomic, can then be matched to this reference genome to be able to carry out future gene identification and editing studies.

Genome editing

For *E. pusilla* to become an established research model for evolutionary, developmental and genetic studies, not only genomic data have to become available but also ways to genetically manipulate plants by e.g. transformation to either overexpress, down-regulate or knock-out genes. Lee *et al*. (2015) published an *Agrobacterium*-mediated genetic transformation protocol for *E. pusilla* using three-month-old protocorms for expressing *Arabidopsis thaliana* genes within 14 months. Unfortunately for every transformation experiment, ca. 2,500 protocorms have to be used and screened for more than ten rounds on selective media. For research purposes this is not a practical approach, although some adjustments can be made. Hsing *et al*. (2016) published a similar method for *Phalaenopsis* orchids using only three selection rounds and then transferring the transformed plants to non-selective media, which speeds up the protocol considerably.

To study gene functions of orchids, genes can either be transiently silenced or inhibited using RNA-interference (RNAi). RNA interference refers to suppression of gene expression of sequence-specific, homologous RNA molecules and is triggered by a double-stranded RNA (dsRNA) mediator to generate small interfering RNA, which then results in sequence-specific RNA degradation.

Different approaches have been used over the last two decades among which Virus Induced Gene Silencing (VIGS). Different *Cymbidium mosaic virus*-based VIGS vectors (pCymMV) have been used to induce gene silencing in orchids of which the pCymMV-Gateway is, in my opinion, the most convenient vector (Lu

et al., 2012), because of its Gateway cassette, which is optimal for easy and high-throughput cloning and screening of genes of interest. Before the start of my PhD in 2013, we requested this vector to suppress gene expression in *E. pusilla*. At that time, sequence information for *E. pusilla* was scarce and gene expression unknown so knocking-down genes of interest had to wait until the first expression studies had been carried out. Despite the fact that time did not permit me to use this technique during my PhD study, I advise to give VIGS a go with *E. pusilla* and first use the homologous MADS-box genes that Hsieh *et al*. (2013) used in *Phalaenopsis*, which are *PI* (*EpMADS16*) and *AP3*, clade 2 (*EpMADS14*). Down regulation can be monitored using RT-qPCR and SEM as carried out in **Chapter 3**. The VIGS technique has been used for examining knockdown phenotypes in non-model plants and is less labor intensive and more rapid than stable transformation approaches. Some considerations have to be taken into account, such as the low transformation efficiency and difficulty to assay the specific knockdown of a gene of interest if multiple copies exist.

Delivery of dsRNA in plants can also be achieved by directly rubbing the dsRNA into the plant. For this dsRNA is produced by the RNase III-deficient *E. coli* strain HT115 (DE3) containing the pL4440 plasmid, which carries the gene of interest. With this method, orchid plants were successfully protected against CymMV infection using dsRNA from the viral coat protein (Lau *et al*., 2015a) and the shape of epidermal cells of a *Dendrobium* orchid was changed using R2R3MYB transcription factor dsRNA (Lau *et al*., 2015b). For *E. pusilla* we cloned a *MYB* homologue and *PI*, two genes involved in floral color, in pL4440 and tried to downregulate these genes once by applying the dsRNA directly on young flower buds. No phenotypic effects were observed and due to time constraints and priority given to other research, this part of this PhD project could not be completed. Because this method seems so easy and does show an effect in other orchids, I definitely recommend spending more time on this when studying gene functions of orchid genes. I recommend treating not only young flower buds with dsRNA but also PLBs, use multiple treatments, apply purified dsRNA instead of crude lysate, and use vacuum to penetrate the dsRNA as possible options for *E. pusilla* to knock down gene expression.

Another method to edit genomes is CRISPR/Cas9 and with an increasing number of scientific papers published over the past five years in plants, this seems a very promising technique for editing orchid genomes as well. In 2017, Kui *et al.* published for the first time a method using *Agrobacterium* to deliver a CRISPR/Cas9 construct into *Dendrobium officinale* and showed that insertions, deletions and substitutions for a given gene target could be made. During the writing of this PhD thesis, no other CRISPR/Cas9 study on orchids was published.

Ancestral character state analysis of orchid fruits

In **chapter 5**, character evolutionary analysis of general orchid fruit traits was carried out to find possible patterns of co-evolution. In **chapter 4**, I showed that *E. pusilla* fruits dehisce without lignification at or near the dehiscence zone. We found evidence for the formation of a cuticle-like layer in the fruits of *E. pusilla*, using TEM and micro-CT scanning, causing the fruit to dehisce. Such a layer is not present in the fruits of two other orchid species, *Epipactis helleborine* and *Cynorkis fastigiata,* where lignification does play a role in fruit dehiscense. When examining dehiscence zone development in these fruits, no cuticle-like layer was observed. Examining more fruits from orchid species from all subfamilies for lignification patterns and combining this information with other characteristics such as ripening time, orientation dehiscence type, and number of slits revealed that an epiphytic or lianaceous habit, longer fruit ripening period and smaller number of opening slits clearly co-evolved in orchids. Similarly, pendant orchid fruits co-evolved with a preference for growing at intermediate to high temperatures and an epiphytic or lianaceous habit. All the methods discussed above now enable addressing fundamental evolutionary questions for any orchid species within a relatively short timeframe. An integrated toolbox can now be used for tracing character evolution to unravel the full genetic basis of the highly specialized organs that make orchids such fascinating subjects for evolutionary studies.

References

Belser, C., Istace, B., Denis, E., Dubarry, M., Baurens, F.C., Falentin, C., Genete, M., Berrabah, W., Chevre, A.M., Delourme, R., Deniot, G., Denoeud, F., Duffe, P., Engelen, S., Lemainque, A., Manzanares-Dauleux, M., Martin, G., Morice, J., Noel, B., Vekemans, X., D'hont, A., Rousseau-Gueutin, M., Barbe, V., Cruaud, C., Wincker, P., and Aury, J.M. (2018). Chromosome-scale assemblies of plant genomes using nanopore long reads and optical maps. *Nat Plants* 4**,** 879-887.

Cai, J., Liu, X., Vanneste, K., Proost, S., Tsai, W.-C., Liu, K.-W., Chen, L.-J., He, Y., Xu, Q., Bian, C., Zheng, Z., Sun, F., Liu, W., Hsiao, Y.-Y., Pan, Z.-J., Hsu, C.-C., Yang, Y.-P., Hsu, Y.-C., Chuang, Y.-C., Dievart, A., Dufayard, J.-F., Xu, X., Wang, J.-Y., Wang, J., Xiao, X.-J., Zhao, X.-M., Du, R., Zhang, G.-Q., Wang, M., Su, Y.-Y., Xie, G.-C., Liu, G.-H., Li, L.-Q., Huang, L.-Q., Luo, Y.-B., Chen, H.-H., De Peer, Y.V., and Liu, Z.-J. (2015). The genome sequence of the orchid Phalaenopsis equestris. *Nature Genetics* 47**,** 65-72.

Cerna, M., Valdivieso, P., Cella, R., Matyas, B., and Aucapina, C. (2018). Cryopreservation of orchid seeds through rapid and step freezing methods. *F1000Res* 7**,** 209.

Chao, Y.T., Chen, W.C., Chen, C.Y., Ho, H.Y., Yeh, C.H., Kuo, Y.T., Su, C.L., Yen, S.H., Hsueh, H.Y., Yeh, J.H., Hsu, H.L., Tsai, Y.H., Kuo, T.Y., Chang, S.B., Chen, K.Y., and Shih, M.C. (2018). Chromosome-level assembly, genetic and physical mapping of Phalaenopsis aphrodite genome provides new insights into species adaptation and resources for orchid breeding. *Plant Biotechnol J* 16**,** 2027-2041.

Chao, Y.T., Yen, S.H., Yeh, J.H., Chen, W.C., and Shih, M.C. (2017). Orchidstra 2.0-A Transcriptomics Resource for the Orchid Family. *Plant Cell Physiol* 58**,** e9.

Deschamps, S., Zhang, Y., Llaca, V., Ye, L., Sanyal, A., King, M., May, G., and Lin, H. (2018). A chromosome-scale assembly of the sorghum genome using nanopore sequencing and optical mapping.

Nat Commun 9**,** 4844.

Gotz, S., Garcia-Gomez, J.M., Terol, J., Williams, T.D., Nagaraj, S.H., Nueda, M.J., Robles, M., Talon, M., Dopazo, J., and Conesa, A. (2008). High-throughput functional annotation and data mining with the Blast2GO suite. *Nucleic Acids Res* 36**,** 3420-3435.

Gravendeel, B., and Dirks-Mulder, A. (2015). Floral development: Lip formation in orchids unravelled. *Nat Plants* 1**,** 15056.

Hsieh, M.H., Lu, H.C., Pan, Z.J., Yeh, H.H., Wang, S.S., Chen, W.H., and Chen, H.H. (2013). Optimizing virus-induced gene silencing efficiency with Cymbidium mosaic virus in Phalaenopsis flower. *Plant Sci* 201-202**,** 25-41.

Hsing, H.X., Lin, Y.J., Tong, C.G., Li, M.J., Chen, Y.J., and Ko, S.S. (2016). Efficient and heritable transformation of Phalaenopsis orchids. *Bot Stud* 57**,** 30.

Hsu, H.-F., Hsu, W.-H., Lee, Y.-I., Mao, W.-T., Yang, J.-Y., Li, J.-Y., and Yang, C.-H. (2015). Model for perianth formation in orchids. *Nature Plants* 1**,** 15046.

Hu, Y., Resende, M.F.R., Jr., Bombarely, A., Brym, M., Bassil, E., and Chambers, A.H. (2019). Genomics-based diversity analysis of Vanilla species using a Vanilla planifolia draft genome and Genotyping-By-Sequencing. *Sci Rep* 9**,** 3416.

Kendon, J.P., Rajaovelona, L., Sandford, H., Fang, R., Bell, J., and Sarasan, V. (2017). Collecting near mature and immature orchid seeds for ex situ conservation: 'in vitro collecting' as a case study. *Bot Stud* 58**,** 34.

Kui, L., Chen, H., Zhang, W., He, S., Xiong, Z., Zhang, Y., Yan, L., Zhong, C., He, F., Chen, J., Zeng, P., Zhang, G., Yang, S., Dong, Y., Wang, W., and Cai, J. (2017). Building a Genetic Manipulation Tool Box for Orchid Biology: Identification of Constitutive Promoters and Application of CRISPR/Cas9 in the Orchid, Dendrobium officinale. *Frontiers in Plant Science* 7.

Lau, S.E., Mazumdar, P., Hee, T.W., Song, A.L.A., Othman, R.Y., and Harikrishna, J.A. (2015a). Crude extracts of bacterially-expressed dsRNA protect orchid plants againstCymbidium mosaic virusduring transplantation fromin vitroculture. *The Journal of Horticultural Science and Biotechnology* 89**,** 569-576.

Lau, S.E., Schwarzacher, T., Othman, R.Y., and Harikrishna, J.A. (2015b). dsRNA silencing of an R2R3-MYB transcription factor affects flower cell shape in a Dendrobium hybrid. *BMC Plant Biol* 15**,** 194.

Lee, S.-H., Li, C.-W., Liau, C.-H., Chang, P.-Y., Liao, L.-J., Lin, C.-S., and Chan, M.-T. (2015). Establishment of an Agrobacterium-mediated genetic transformation procedure for the experimental model orchid Erycina pusilla. *Plant Cell Tiss Organ Cult (PCTOC)* 120**,** 211-220.

Lu, H.C., Hsieh, M.H., Chen, C.E., Chen, H.H., Wang, H.I., and Yeh, H.H. (2012). A high-throughput virus-induced gene-silencing vector for screening transcription factors in virus-induced plant defense response in orchid. *Mol Plant Microbe Interact* 25**,** 738-746.

Magrini, S., De Vitis, M., Torelli, D., Santi, L., and Zucconi, L. (2018). Seed banking of terrestrial orchids: evaluation of seed quality in Anacamptis following 4-year dry storage. *Plant Biol (Stuttg)* 0.

Mehrotra, S., and Goyal, V. (2014). Repetitive sequences in plant nuclear DNA: types, distribution, evolution and function. *Genomics Proteomics Bioinformatics* 12**,** 164-171.

Popova, E., Kim, H.H., Saxena, P.K., Engelmann, F., and Pritchard, H.W. (2016). Frozen beauty: The cryobiotechnology of orchid diversity. *Biotechnol Adv* 34**,** 380-403.

Schofield, E., Jones, E.P., and Sarasan, V. (2018). Cryopreservation without vitrification suitable for large scale cryopreservation of orchid seeds. *Bot Stud* 59**,** 13.

Su, C.L., Chao, Y.T., Yen, S.H., Chen, C.Y., Chen, W.C., Chang, Y.C., and Shih, M.C. (2013). Orchidstra: an integrated orchid functional genomics database. *Plant Cell Physiol* 54**,** e11.

Yan, L., Wang, X., Liu, H., Tian, Y., Lian, J., Yang, R., Hao, S., Wang, X., Yang, S., Li, Q., Qi, S., Kui, L., Okpekum, M., Ma, X., Zhang, J., Ding, Z., Zhang, G., Wang, W., Dong, Y., and Sheng, J. (2015). The Genome of Dendrobium officinale Illuminates the Biology of the Important Traditional Chinese Orchid Herb. *Mol Plant* 8**,** 922-934.

Yuan, Y., Jin, X., Liu, J., Zhao, X., Zhou, J., Wang, X., Wang, D., Lai, C., Xu, W., Huang, J., Zha, L., Liu, D., Ma, X., Wang, L., Zhou, M., Jiang, Z., Meng, H., Peng, H., Liang, Y., Li, R., Jiang, C., Zhao, Y.,

Nan, T., Jin, Y., Zhan, Z., Yang, J., Jiang, W., and Huang, L. (2018). The Gastrodia elata genome provides insights into plant adaptation to heterotrophy. *Nature communications* 9**,** 1615-1615.

Zhang, G.-Q., Xu, Q., Bian, C., Tsai, W.-C., Yeh, C.-M., Liu, K.-W., Yoshida, K., Zhang, L.-S., Chang, S.-B., Chen, F., Shi, Y., Su, Y.-Y., Zhang, Y.-Q., Chen, L.-J., Yin, Y., Lin, M., Huang, H., Deng, H., Wang, Z.-W., Zhu, S.-L., Zhao, X., Deng, C., Niu, S.-C., Huang, J., Wang, M., Liu, G.-H., Yang, H.-J., Xiao, X.-J., Hsiao, Y.-Y., Wu, W.-L., Chen, Y.-Y., Mitsuda, N., Ohme-Takagi, M., Luo, Y.-B., Van De Peer, Y., and Liu, Z.-J. (2016). The Dendrobium catenatum Lindl. genome sequence provides insights into polysaccharide synthase, floral development and adaptive evolution. *Scientific Reports* 6**,** 19029.

Zhang, G.Q., Liu, K.W., Li, Z., Lohaus, R., Hsiao, Y.Y., Niu, S.C., Wang, J.Y., Lin, Y.C., Xu, Q., Chen, L.J., Yoshida, K., Fujiwara, S., Wang, Z.W., Zhang, Y.Q., Mitsuda, N., Wang, M., Liu, G.H., Pecoraro, L., Huang, H.X., Xiao, X.J., Lin, M., Wu, X.Y., Wu, W.L., Chen, Y.Y., Chang, S.B., Sakamoto, S., Ohme-Takagi, M., Yagi, M., Zeng, S.J., Shen, C.Y., Yeh, C.M., Luo, Y.B., Tsai, W.C., Van De Peer, Y., and Liu, Z.J. (2017). The Apostasia genome and the evolution of orchids. *Nature* 549**,** 379-383.