Factors Influencing Cross Barriers in Interspecific Hybridizations of Water Lily

Chunqing Sun¹, Zhihu Ma¹, Zhenchao Zhang, Guosheng Sun, and Zhongliang Dai²
Department of Vegetables, Zhenjiang Agricultural Research Institute, Jurong 212400, P.R. China

ABSTRACT. In interspecific hybridizations of water lily (Nymphaea), the existence of cross barriers makes it difficult to obtain hybrids and seriously influences the utilization of admirable characters from tropical water lilies. To determine the causes, pollen viability, pistil receptivity, and embryo and endosperm development were investigated in three water lily crosses, including Nymphaea odorata ‘Peter Slocum’ × Nymphaea micrantha (PM), ‘Peter Slocum’ × Nymphaea gigantea (PH), and ‘Peter Slocum’ × Nymphaea colorata (PC). The results indicated that the viability of pollen grains was 17.3% for ‘Peter Slocum’, 19.3% for N. colorata, 10.3% for N. micrantha, and 17.6% for N. gigantea. In the self-pollinated ‘Peter Slocum’, the number of germinated pollen grains on stigmas peaked at 12 hours after pollination (HAP), indicating its good pollen germinability. However, only a few pollen grains germinating on the stigma between 2 and 24 HAP in the crosses of PM, PH, and PC. In addition, a high percentage (81.2%) of normal embryos developed to different stages within 20 d after pollination in the self-pollinated ‘Peter Slocum’. But only 3.5% and 3.7% of normal globular embryos were observed in the PC and PM combinations, respectively. Moreover, no normal embryos were observed in the PH cross. At the same time, no seeds were obtained in PM, PC, and PH crosses. The results suggest that preferfertilization barriers existed in the PH cross, whereas pre- and postfertilization barriers existed together in the PC and PM crosses. These may be the main causes resulting in the failure of interspecific hybridizations in water lily.

Water lilies are important flowers that are widely cultivated in gardens for both ornaments and water purification (Huang et al., 2009). The genus Nymphaea could be classified into six subgenera, i.e., Anecphya, Brachyceras, Confluentes, Lotos, Hydrocallis, and Nymphaea, with the former five belonging to tropical water lilies and the last one being hardy water lily. The subgenera Anecphya, Brachyceras, Nymphaea, and Confluentes bloom during daytime (0600 to 1400 HR), whereas Lotos and Hydrocallis bloom in nighttime (1900 to 1000 HR) (Péeter et al., 2011; Songpanich and Hongtrakul, 2010). In addition, hardy water lilies usually bloom for 3 d, which gives them higher ornamental value than those tropical water lilies.

To obtain desired traits of interest in cultivars, breeders have tried to make interspecific hybridizations. In the past, interspecific hybridizations in water lily have been performed for the hope of creating a blue hardy water lily hybrid, but most of them are failed to success (Songpanich and Hongtrakul, 2010). Over a period of more than 100 years of hybridization, the new-generation of hybridizers still endeavors to create a hardy blue-flowered water lily. In fact, we have also strived to create a hardy blue water lily hybrid through the infusion of germplasms from tropical water lilies with several colors, including blue, in subgenera Anecphya, Confluentes, and Brachyceras. In most interspecific crosses, however, the existence of reproductive barriers makes it difficult to obtain the hybrids and influences the utilization of excellent traits from tropical water lilies. Few studies have been taken to investigate the cross barriers in water lily; therefore, the factors affecting seed set in interspecific crosses are still unclear.

In general, the features of parental reproductive systems, a series of events in the process of pollination and fertilization, and embryogenesis are closely related to efficiency of interspecific hybridization. Consequently, the parental reproductive systems and the sexual reproduction process have been studied in many plants, such as Dendranthema grandiflorum, Phaseolus vulgaris, Nelumbo nucifera, Fragaria anansa, and Jasminum sambac (Deng et al., 2017; Marta et al., 2004; Ndoutoumou et al., 2007; Sun et al., 2010; Teng et al., 2012). These studies have successfully uncovered the factors that affect the seed set and efficiency of hybridization. Building on these previous studies, we set out to systematically study the reproductive processes in three interspecific crosses between a hardy water lily and three tropical water lily species, including pollen viability, pollen tube behavior on stigmas, and embryo development. The aim of this study was to identify the main reasons that may affect the breeding efficiency of water lilies, thus moving one step closer to overcome the reproductive barriers and create a hardy blue water lily hybrid.

Materials and Methods

EXPERIMENTAL MATERIALS. ‘Peter Slocum’, a hardy water lily in Nymphaea subgenus, has a high self-seeds rate; thus, 20 flowering individuals were used as female parents. Three species (five individuals each) from the blue-flowered water lily subgenera, Anecphya (N. gigantea) and Brachyceras (N. micrantha and N. colorata), were used as male parents. The plants were grown in ponds in the town of Xingxiang, Zhenjiang, Jiangsu Province, China (lat. 31°59’N, long. 119°17’E). ‘Peter Slocum’ has a chromosome number of 84 (2n = 6x). Nymphaea gigantea has a chromosome number
224 \( (2n = 16x) \), whereas \( N. \) colorata has a chromosome number of 28 \( (2n = 2x) \) and \( N. \) micrantha has a chromosome number of 56 \( (2n = 4x) \) (Pellicer et al., 2013). Three crosses were made among four taxa: PM, PH, and PC. In addition, to investigate the percentage of normal pistils just before pollination, the self-compatibility of ‘Peter Slocum’ was determined.

The taxa used in the present study were selected because of two main reasons. First, the ornamental qualities of ‘Peter Slocum’, \( N. \) micrantha, \( N. \) colorata, and \( N. \) gigantea are complementary. For example, ‘Peter Slocum’ flowers are pink and \( N. \) colorata flowers are blue. If the two taxa could cross successfully, it would be possible to obtain a hardy blue-flowered water lily hybrid. Second, preliminary studies have shown that no seeds obtained from the crosses; therefore, a detailed study on the factors that influenced the seed set of these crosses is valuable.

**Determination of pollen viability.** Pollen grains from newly split anthers were collected from \( 0900 \) to \( 1000 \) HR on sunny days. The samples were placed on a glass slide coated with culture medium including 100 g L\(^{-1}\) polyethylene glycol (PEG4000), 0.8 g L\(^{-1}\) CaCl\(_2\)\(\cdot\)2\(\mathrm{H}_2\mathrm{O}\), 10 mg L\(^{-1}\) \(\mathrm{H}_3\mathrm{BO}_3\), 0.9 g L\(^{-1}\) \(\mathrm{KNO}_3\), and 300 g L\(^{-1}\) sucrose and then incubated at 26 °C. After 6 h of culturing, pollen viability was determined from 10 optical fields (at least 50 pollen grains in each optical field) with a microscope (BX41; Olympus, Shanghai, China). The viable pollen grains were scored when the pollen tube length was longer than its radius (Sun et al., 2015). Each experiment was repeated three times and a Tukey’s test was performed to detect the significance of difference.

**Artificial pollination.** The pistil of water lily matures 1–2 d before stamen; thus, we only bagged the flowers of the female parent 2 d before their pistils matured (Huang et al., 2009). The best time for pollination ranges from \( 0800 \) to \( 1000 \) HR on sunny days (Huang et al., 2009). When the stigmas of female flowers were full of bright mucus, the stigmas were pollinated with fresh pollen grains from male parents. After pollination, the female flowers were bagged again. One hundred flowers per cross were pollinated artificially. One month after pollination, \( \approx 10 \) water lily seed pods were randomly chosen for seed collection. The crosses were performed from late September to early Oct. 2015, when the average temperature was \( \approx 27 \) °C with a range of 26 to 30 °C.

**Pollen tube behavior on stigmas after pollination.** Twenty pistils per cross were fixed in formalin-acetic acid-alcohol (FAA; 90 mL 70% ethanol : 5 mL formalin : 5 mL acetic acid) at 2, 6, 12, and 24 h after pollination and then stored at 4 °C until use. The pistils were softened overnight in 1 mol L\(^{-1}\) NaOH and then washed in distilled water three times. After that, the samples were stained with 0.01% aniline blue (18% glycerol + 0.1 mol L\(^{-1}\) \(\mathrm{K}_3\mathrm{PO}_4\)), squashed, and then observed under a fluorescence microscope (Axioplan 40; Carl Zeiss, Shanghai, China). Digital images were captured using a camera (Axiocam MRC 5; Carl Zeiss). In addition, 20 pistils per cross at the same time points were fixed in 2.5% glutaraldehyde (0.1 M phosphate buffer, pH = 7.2). The pistil samples were then dehydrated in an ethanol series (40%, 70%, 90%, and 100%, for 15 min in each solution), subjected to critical point drying, and coated with gold for scanning electron microscope (S-3000N; Hitachi, Beijing, China) analysis. Images were processed digitally. If pollen grains did not germinate on stigmas, they are much easily washed away during the processes of fixing in FAA, softening in NaOH and washing in distilled water. Therefore, pollen observed on stigmas can be judged as germinated ones with penetrating into the stigmatic tissues.

**Examination of embryo development.** We collected 210 ovaries from five plants (two flowers per plant) for each parental combination at 2, 5, 8, 12, 15, and 20 d after pollination. The samples were stored in FAA at 4 °C for later examination of embryo and endosperm development. These samples were dehydrated through a graded series of ethanol solutions (70%, 85%, 95%, and 100%, for 5 min in each solution), infiltrated with xylene, and embedded in paraffin wax. Sections were cut to a thickness of 8–10 μm using a microtome (RM2016; Leica, Shanghai, China), stained in Heidenhain’s hematoxylin, and were observed and photographed under a Zeiss Axioplan 40 microscope.

**Statistical analysis.** The data were subjected to a one-way analysis of variance using SPSS (version 16.0; IBM Corp., Armonk, NY). The means were compared using the Bonferroni \( t \) test with \( a = 0.05 \) (Type I error rate).

### Results

**Pollen viability.** Pollen grains began to germinate after 2 h of culture on the germination medium and the germination peak occurred at 6 h after culture. The viability of pollen grains was 17.3% for ‘Peter Slocum’, 19.3% for \( N. \) colorata, 10.3% for \( N. \) micrantha, and 17.6% for \( N. \) gigantea (Table 1).

**Pollen tube behavior on stigmas after pollination.** To the self-pollinated ‘Peter Slocum’, the number of germinated pollen grains on each stigma increased at 6 HAP; the highest number was attained at 12 HAP and it gradually decreased thereafter (Figs. 1A and 2A). For instance, the average number of germinated pollen grains on each stigma increased during 6 HAP in the ‘Peter Slocum’ self-cross, in which an average of 14.7 pollen grains per stigma germinated at 6 HAP. Several germinated pollen grains on each stigma (134) were observed at 12 HAP. Then, at 24 h, the number decreased to 42.3 (Table 2); however, the numbers of germinated pollen grains per stigma in the PM and PC crosses were drastically reduced compared with the self-pollinated ‘Peter Slocum’ (Table 2), and only a few pollen tubes penetrated the stigmatic tissue from 2 to 24 HAP. At 24 HAP, many pollen tubes grew abnormally (including splitting, convolution, coiling, and branching) on stigmas in the PM and PC crosses (Figs. 1B and C, 2B and C). In addition, no pollen tubes penetrated the stigmatic tissue between 2 and 24 HAP in the PH cross (Table 2). In PH, the accumulation of callose on the stigma surface hampered the germination of pollen grains, and abnormal growth of pollen tubes was usually observed (Figs. 1D and 2D).

**Embryo development, endosperm development, and seed set.** Zygote divided quickly and 80.9% of ovaries with normal globular embryos were observed 5 d after artificial pollination.

### Table 1. Pollen viability of four \( Nymphaea \) taxa determined with the method of germination in vitro.

| Taxon              | Pollen viability [mean ± SD (%)] |
|--------------------|----------------------------------|
| \( Nymphaea odorata \) ‘Peter Slocum’ | 17.3 ± 1.0 a                         |
| \( Nymphaea colorata \)                  | 19.3 ± 4.9 a                       |
| \( Nymphaea micrantha \)                 | 10.3 ± 4.0 a                       |
| \( Nymphaea gigantea \)                  | 17.6 ± 2.1 a                       |

*aValues followed by the same letter are not significantly different at \( a = 0.05 \) with the Bonferroni \( t \) test.*
in the self-pollinated ‘Peter Slocum’ (Fig. 3A; Table 3). At 15 d after artificial pollination, 79.1% of ovaries with normal ellipse-shaped embryos were observed (Fig. 3B; Table 3). Then, at 20 d after pollination, 83.7% of ovaries with normal diamond-shaped embryos were observed and endosperm degenerated (Fig. 3C; Table 3). In addition, only 3.5% and 3.7% of the ovaries with normal globular embryos were observed in the PC (Fig. 3D; Table 3) and PM crosses, respectively. It should be noted that in PH, no normal embryos were observed after pollination (Table 3).

No seeds were obtained in PM, PC, and PH crosses. But the self-seed rate was 68.7% ± 3.5% for N. colorata, 40.9% ± 5.1% for N. micrantha, and 74.3% ± 5.4% for N. gigantea. So it can be reasonably speculated that pollen tube behavior and embryo development after self-pollination of the three pollen parents were normal. In addition, the seed set rate of the self-pollinated ‘Peter Slocum’ was 76.4%, indicating that most pistils of the female parent were normal before pollination and pistil development did not significantly hamper seed set of the crosses.

**Discussion**

Successful sexual reproduction of interspecific hybridization depends on a series of events in the process of pollination and fertilization (Gao et al., 2015). From pollen tube growth to embryogenesis, any barriers in this process can result in the failure of hybridization. These barriers are divided into pre- and post-fertilization barriers, often described as stigma–pollen incompatibility and embryo abortion, respectively (Deng et al., 2010, 2017; Lee et al., 2008; Spielman and Scott, 2008). First, pollen grains with low viability pollinated a stigma may increase the risk of pollination failure and reduce seed set (Aleemullah et al., 2000; Deng et al., 2017; Huang et al., 2004; Wilcock and Neiland, 2002). For example, Koshy and Jee (2001) showed that low pollen viability is one of the main factors responsible for failure of seed set in Bambusa vulgaris. In the present study, the
The highest pollen viability of the four taxa was only 19.3% (Table 1). Our previous research indicated that poor pollen viability may have negative effects on seed set of the four water lily crosses (Sun et al., 2015); however, this was not indicated in this study. Specifically, the percentages of normal embryos at 5 d after artificial pollination were 80.9% and 3.5% for the 'Peter Slocum' self-pollination treatment and PC cross, respectively (Table 3). In addition, the corresponding highest pollen viabilities of male plants were only 17.3% ('Peter Slocum') and 19.3% (N. colorata) in the two aforementioned crosses (Table 1). In addition, the highest pollen viabilities of male plants were only 17.3% ('Peter Slocum') and 19.3% (N. colorata) in the two aforementioned crosses (Table 1). Furthermore, the fertilization rate of ovules has a close relationship with pollen viability, and is closely related to the absolute quantity of pollen grains germinating on stigmas (Jin et al., 2010; Sun et al., 2011). This information suggests that the large difference in percentages of normal embryos at 5 d after artificial pollination in the three crosses may be attributed to other factors, not to pollen viability. Therefore, we speculated that low pollen viability may not be the main factor influencing seed production of the three interspecific crosses in water lily. Second, pollen–pistil interaction is also a main factor influencing seed set of plant interspecific cross. Stigma–pollen incompatibility is often indicated by few pollen grains germination, extended and twisted pollen tubes, and deposited callose on the stigma surface (Sun et al., 2010, 2011). In the self-pollinated 'Peter Slocum', the number of germinated pollen grains on each stigma peaked at 12 HAP, but only a few pollen tubes penetrated the stigmatic tissue on each stigma between 2 and 24 HAP in the PM, PH, and PC crosses. The number of pollen germinated in the PM, PH, and PC crosses

Table 2. Pollen grains germinating on the stigma after pollination in four Nymphaea crosses.

| Time after pollination (h) | Self-pollinated Nymphaea odorata ‘Peter Slocum’ | ‘Peter Slocum’ × Nymphaea colorata | ‘Peter Slocum’ × Nymphaea micrantha | ‘Peter Slocum’ × Nymphaea gigantea |
|---------------------------|-----------------------------------------------|-----------------------------------|------------------------------------|-----------------------------------|
|                           | Pollen grains germinating [mean ± SD (no./stigma)] |                                   |                                    |                                   |
| 2                         | 3.0 ± 1.0 a                                   | 1.0 ± 1.0 b                       | 0.7 ± 1.2 b                       | 0 c                               |
| 6                         | 14.7 ± 4.2 a                                  | 2.7 ± 1.5 b                       | 3.0 ± 1.0 b                       | 0 c                               |
| 12                        | 134.0 ± 8.5 a                                 | 3.0 ± 1.0 b                       | 3.3 ± 1.5 b                       | 0 c                               |
| 24                        | 42.3 ± 11.7 a                                 | 1.3 ± 0.6 b                       | 1.7 ± 1.5 b                       | 0 c                               |

*Values followed by the same letter are not significantly different at a = 0.05 with the Bonferroni t test.

Fig. 3. Anatomical structure of the ovule of Nymphaea after pollination. (A) Globular embryo (Em), abundant perisperm, and endosperm (En) at 5 d after pollination in the self-pollinated Nymphaea odorata ‘Peter Slocum’. (B) Embryo at 15 d after pollination in the self-pollinated ‘Peter Slocum’. (C) Embryo, abundant perisperm, and little endosperm at 20 d after pollination in the self-pollinated ‘Peter Slocum’. (D) Globular embryo at 5 d after pollination in the ‘Peter Slocum’ × Nymphaea colorata cross. (E and F) At 15 d after pollination, nearly all the cells in the embryo and endosperm have degenerated in ‘Peter Slocum’ × Nymphaea micrantha and ‘Peter Slocum’ × N. colorata crosses (scale bar = 20 μm). De = degradative endosperm; Dm = degradative perisperm.
completely failed to meet the needs of fertilization because there are ≈200 ovules in a water lily inflorescence and increased the probability of fertilization failure. Moreover, in the PH cross, a large amount of callose was deposited on stigma, blocking the pollen grains from directly coming in contact with the stigma, which resulted in few pollen grains germinating on the stigma (Figs. 1D and 2D). Therefore, it is suggested that no seed set in the PC, PM, and PH crosses may be largely attributable to prefertilization barriers, i.e., the low numbers of germinated pollen grains and subsequent abnormal growth of their pollen tubes on the stigma. Some special pollination methods, such as gibberellic acid treatment, delayed pollination, and mentor pollen, may be used to overcome prefertilization barriers in water lily interspecific hybridizations, for these methods have been applied successfully to overcome prefertilization barriers in many other plants (Sun et al., 2011).

In addition to prefertilization barriers, successful interspecific hybridization is closely related to embryogenesis (Gao et al., 2017). Low seed set is often caused by postfertilization barriers, i.e., abortion or degeneration of several embryos during their development (Datson et al., 2006; Deng et al., 2012; Ndoutoumou et al., 2007; Yoon et al., 2006). In the present study, embryos aborted during their developmental processes in the PC and PM crosses; 3.5% and 3.7% of embryos were normal at 5 d after pollination in PC and PM crosses, respectively. However, at 15 d after pollination, no normal embryos were present. Such results clearly indicate that the postfertilization barrier (embryo abortion) was a main factor dramatically reducing seed set of both the PC and PM water lily crosses. The specific reasons for embryo abortion need to be further studied. According to previous research, we speculated that embryo abortion was caused by uncoordinated development of immature embryos and endosperm because the endosperm is nourishing during the development of the immature embryo (Hu, 2005). Embryo and ovary culture have been applied successfully to obtain hybrids in many interspecific hybridizations with postfertilization barriers (Deng et al., 2010; Tang et al., 2009). If embryo rescue are used in such interspecific hybridizations of water lily in the near future, it is very possible that more hybrids will be obtained.

Ploidy level and genetic relationship are both factors which lead to prefertilization and postfertilization barriers in plant interspecific cross. Clearly, the closer the parent’s relationship is, the higher of the success of the hybridization, and the more fertile hybrid will be. Thus, hybrids can be obtained where the parents have the same ploidy level or where one is tetraploid and the other hexaploid (Li et al., 2008); however, tetraploid × hexaploid or diploid × hexaploid crosses usually produce inviable progeny or are sterile (Li et al., 2008). Unsurprisingly, there are a small amount of embryos in PC and PM crosses in the present study, suggesting that it is distant from the male and female parent. Moreover, in the PH cross, no embryo has been obtained. We speculated that the main reason for the lack of embryo in PH cross may be large chromosomal differences, which leads to the unbalanced development of the embryo and the endosperm. Ploidy manipulation can provide a means to enable interspecific crosses where these are difficult to achieve because of differences in ploidy level between the prospective parents (Liu et al., 2011). Relevant examples of this have been provided in chrysanthemum [subtribe Chrysantheminae (Li et al., 2008)]. Thus, we will attempt to generate the bridging genotype required to make a successful cross between N. micrantha and ‘Peter Slocum’. For example, some N. micrantha (4x) will be doubled to 8x and then crossed with N. micrantha to create 6x plants, which would potentially be easier to cross with ‘Peter Slocum’.

In conclusion, prefertilization barriers existed in the PH cross, whereas pre- and postfertilization barriers existed together in the PC and PM crosses. Pollen viability of the male parent is not a major factor in the failure of hybridization. These findings will provide clues for overcoming prefertilization and postfertilization barriers and increasing seed set in the interspecific crosses in water lily; however, the causes of embryo abortion and stigma–pollen incompatibility remain unclear and require further investigation.

**Table 3. Percentage of normal embryo at three dates after pollination and seed set after artificial pollination in four Nymphaea crosses.**

| Cross                          | Time after pollination (d) | Proportion of ovaries with normal embryos [mean ± SD (%)] | Seed set after artificial pollination [mean ± SD (%)] |
|-------------------------------|---------------------------|----------------------------------------------------------|------------------------------------------------------|
| Self-pollinated *Nymphaea odorata* ‘Peter Slocum’ | 5                      | 80.9 ± 4.2 a'                                              | 76.4 ± 5.3 a                                         |
|                               | 15                      | 79.1 ± 2.6 a                                               |                                                      |
|                               | 20                      | 83.7 ± 4.5 a                                               |                                                      |
| ‘Peter Slocum’ × *Nymphaea colorata* | 5                      | 3.5 ± 0.7 b                                                | 0 b                                                  |
|                               | 15                      | 0 c                                                        |                                                      |
|                               | 20                      | 0 c                                                        |                                                      |
| ‘Peter Slocum’ × *Nymphaea micrantha* | 5                      | 3.7 ± 0.9 b                                                | 0 b                                                  |
|                               | 15                      | 0 c                                                        |                                                      |
|                               | 20                      | 0 c                                                        |                                                      |
| ‘Peter Slocum’ × *Nymphaea gigantea* | 5                      | 0 c                                                        | 0 b                                                  |
|                               | 15                      | 0 c                                                        |                                                      |
|                               | 20                      | 0 c                                                        |                                                      |

*Values followed by the same letter are not significantly different at α = 0.05 with the Bonferroni t test.

**Literature Cited**

Aleemullah, M., A.M. Haigh, and P. Holford. 2000. Anthesis, anther dehiscence, pistil receptivity and fruit development in the Longum group of *Capsicum annuum*. Austral. J. Expt. Agr. 40:755–762.

Datson, P.M., B.G. Murray, and K.R.W. Hammett. 2006. Pollination systems, hybridization barriers and meiotic chromosome behaviour in *Nemesia* hybrids. Euphytica 151:173–185.
Huang, Z.H., J.M. Zhu, X.J. Mu, and J.X. Lin. 2004. Pollen dispersion, Huang, G.Z., H.Q. Deng, Z. Li, and G. Li. 2009. Water lily. China Hu, S.Y. 2005. Reproductive biology of angiosperms. Higher Educa-
Gao, C., R. Yang, and D.Y. Yuan. 2017. Characteristics of developmental differences between fertile and aborted ovules in Camellia oleifera. J. Amer. Soc. Hort. Sci. 140:12–18.
Gao, C., D.Y. Yuan, Y. Yang, B.F. Wang, D.M. Liu, and F. Zou. 2015. Pollen tube growth and double fertilization in Camellia oleifera. J. Amer. Soc. Hort. Sci. 140:12–18.
Hu, S.Y. 2005. Reproductive biology of angiosperms. Higher Education Press, Beijing, China.
Huang, G.Z., H.Q. Deng, Z. Li, and G. Li. 2009. Water lily. China Forestry Press, Beijing, China.
Huang, Z.H., J.M. Zhu, X.J. Mu, and J.X. Lin. 2004. Pollen dispersion, pollen viability and pistil receptivity in Leymus chinensis. Ann. Bot. 93:295–301.
Jin, B., L. Wang, J. Wang, N.J. Teng, X.D. He, X.J. Mu, and Y.L. Wang. 2010. The structure and roles of sterile flowers in Viburnum macrocephalum f. keteleeri (Adoxaceae). Plant Biol. 12:853–862.
Koshy, K.C. and G. Jee. 2001. Studies on the absence of seed set in Bambusa vulgaris.Curr. Sci. 82:375–378.
Lee, C.B., L.E. Page, B.A. McClure, and T.P. Holtsford. 2008. Postpollination hybridization barriers in Nicotiana section Alatae. Plant Reprod. 21:183–195.
Li, J., S.M. Chen, F.D. Chen, and W.M. Fang. 2008. Karyotype and meiotic analyses of six species in the subtribe Chrysantheminae. Euphytica 164:292–301.
Liu, S.Y., S.M. Chen, Y. Chen, Z.Y. Guan, D.M. Yin, and F.D. Chen. 2011. In vitro induced tetraploid of Dendranthema nankingense (Nakai) Tzvel. shows an improved level of abiotic stress tolerance. Scientia Hort. 127:411–419.
Marta, A.E., E.L. Camadro, J.C. Díaz-Ricci, and A.P. Castagnaro. 2004. Breeding barriers between the cultivated strawberry, Fragaria ×ananassa, and related wild germplasm. Euphytica 136:139–150.
Ndoutoumou, P.N., A. Toussaint, and J.P. Baudoin. 2007. Embryo abortion and histological features in the interspecific cross between Phaseolus vulgaris L. and P. coccineus L. Plant Cell Tissue Organ Cult. 88:329–332.
Pellicer, J., L.J. Kelly, C. Magdalena, and I.J. Leitch. 2013. Insights into the dynamics of genome size and chromosome evolution in the early diverging angiosperm lineage Nymphaeales (water lilies). Genome 56:437–448.
Péter, P., K.M. Kinga, S. István, V. Ildikó, H. Jaakko, C. István, M.G. Ahmad, D. Kincsó, and T. János. 2011. Genetic variability of thermal Nymphaea (Nymphaeaceae) populations based on ISSR markers: Implications on relationships, hybridization, and conservation. Plant Mol. Biol. Rpt. 29:906–918.
Songpanich, P. and V. Hongtrakul. 2010. Intersubgeneric cross in Nymphaea spp. L. to develop a blue hardy waterlily. Scientia Hort. 124:475–481.
Spielman, M. and R.J. Scott. 2008. Polyspermy barriers in plants: From preventing to promoting fertilization. Plant Reprod. 21:53–65.
Sun, C.Q., F.D. Chen, N.J. Teng, Z.L. Liu, W.M. Fang, and X.L. Hou. 2010. Factors affecting seed set in the crosses between Dendrantha grandiflorum (Ramat.) Kitamura and its wild species. Euphytica 171:181–192.
Sun, C.Q., Z.Z. Huang, Y.L. Wang, F.D. Chen, N.J. Teng, W.M. Fang, and Z.L. Liu. 2011. Overcoming pre-fertilization barriers in the wide cross of chrysanthemum by using special pollination techniques. Euphytica 178:195–202.
Sun, C.Q., Z.H. Ma, G.S. Sun, Z.L. Dai, N.J. Teng, and Y.P. Pan. 2015. Cellular mechanisms of reproductive barriers in some crosses of water lily (Nymphaea spp.) cultivars. HortScience 50:30–35.
Tang, F.P., F.D. Chen, S.M. Chen, N.J. Teng, and W.M. Fang. 2009. Intergeneric hybridization and relationship of genera within the tribe Anthemideae Cass. (I. Dendranthema crassum (Kitam.) Kitam. × Crossostephium chinense (L.) Makino). Euphytica 169:133–140.
Teng, N.J., Y.L. Wang, C.Q. Sun, W.M. Fang, and F.D. Chen. 2012. Factors influencing fecundity in experimental crosses of water lily (Nelumbo nucifera Gaertn.) cultivars. BMC Plant Biol. 12:82.
Wilcock, C. and R. Neiland. 2002. Pollination failure in plants: Why it happens and when it matters. Trends Plant Sci. 7:270–277.
Yoon, J.B., D.C. Yang, J.W. Do, and H.G. Park. 2006. Overcoming pre-fertilization reproductive barriers and the underlying cytological mechanism in crosses among three petal-types of Jasminum sambac and their relevance to phylogenetic relationships. PLoS One 12:e0176026.