Preliminary Study on Interspecific Hybridization Directionality in Rhododendron Postprint

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Abstract

Abstract: To investigate the directionality characteristics of interspecific hybridization in Rhododendron, a preliminary study on related directional issues was conducted based on 186 hybrid combinations with clear selfing characteristics, comprising 5 subgenera, 3 sections, 12 subsections, 32 species, and 33 reciprocal cross combinations within the genus. The results indicated that: (1) The ratio variation of interspecific hybridization in Rhododendron from bidirectional fertility → unidirectional sterility → bidirectional sterility aligns with the parental systematic positions from primitive → evolved and the genetic relationships between parents from close → distant. Interspecific hybridization within the primitive subgenus Hymenanthes is easier than within the evolved subgenus Rhododendron, and due to the close genetic relationship between these two subgenera, the fertility ratio of inter-subgeneric hybridization between them is also higher than that of other inter-subgeneric crosses; (2) On the evolutionary dimension, unidirectional sterility in interspecific hybridization of this genus generally lacks a fixed parental combination direction, but hybrid combinations within subgenus Hymenanthes (Intra-subgen. Hymenanthes), within subgenus Rhododendron (Intra-subgen. Rhododendron), and between subgenus Tsutsus × subgenus Pentanthera exhibit certain directional tendencies; (3) Interspecific hybrid fertility is correlated with parental combination patterns differing in selfing characteristics, with the general fertility tendency being SC×SC > SI×SC SC×SI > SI×SI. The reproductive evolutionary direction proceeds from self-compatibility to self-incompatibility, accompanied by strengthened outcrossing reproductive isolation; (4) Cytological sterility and polyploidy are two important causes of unidirectional sterility, and the involvement of SI parents substantially increases the sterility ratio in interspecific hybridization.

Keywords: hybridization directionality, reciprocal cross, compatibility, sterility, Rhododendron

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Full Text

A Preliminary Study on the Directionality of Interspecific Hybridization in *Rhododendron*

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Abstract

To investigate the directional characteristics of interspecific hybridization in *Rhododendron*, we conducted a preliminary study on hybrid directionality based on 33 reciprocal cross combinations and 186 hybrid combinations with clear selfing properties, involving 5 subgenera, 3 sections, 12 subsections, and 32 species. The results showed that: (1) The transition rate from bidirectional fertility to unidirectional sterility to bidirectional sterility in interspecific crosses of *Rhododendron* correlates positively with both the phylogenetic position of the parents (from primitive to advanced) and the genetic distance between them (from close to distant). Interspecific hybridization occurs more readily within the primitive evergreen subgenus *Hymenanthes* than within the more advanced subgenus *Rhododendron*. Because these two subgenera share a close phylogenetic relationship, the fertility rate of inter-subgeneric crosses between them is also higher than that of other inter-subgeneric combinations; (2) Along the evolutionary dimension, unidirectional sterility in interspecific hybrids generally shows no fixed parental combination pattern, though certain hybrid combinations within subgenera and between subgenus *Tsutsusi* and subgenus *Pentanthera* exhibit distinct tendencies; (3) Hybrid fertility correlates with the selfing characteristics of the parental combinations, with the general fertility trend being SC×SC > SI×SC SC×SI > SI×SI. The reproductive evolutionary direction proceeds from self-compatibility to self-incompatibility, accompanied by strengthened outcrossing reproductive isolation; (4) Cytoplasmic sterility and polyploidy are two important causes of unidirectional sterility, and the involvement of SI parents substantially increases the rate of hybrid sterility.

Keywords: hybrid directionality, reciprocal cross, compatibility, sterility, *Rhododendron*

Introduction

Directionality refers to the parental combination pattern in hybridization, specifically the response of compatibility or fertility to normal and reciprocal crosses (Meng, 1997). Previous studies have demonstrated three outcomes in interspecific hybridization within *Rhododendron*: bidirectional fertility, unidirectional sterility, and bidirectional sterility (Williams et al., 1990; Rouse et al., 1993). Tom et al. (2007) reported three patterns in reciprocal crosses between different subgenera: some showed bidirectional fertility, such as subgenus *Tsutsusi* × subgenus *Rhododendron*; some exhibited unidirectional sterility, such as subgenus *Tsutsusi* × section *Vireya* and subgenus *Tsutsusi* × subgenus *Hymenanthes*; and others displayed bidirectional sterility, such as subgenus *Pentanthera* × subgenus *Tsutsusi*, although seeds obtained with subgenus *Pentanthera* species as maternal parents could germinate, they failed to produce green seedlings. These inter-subgeneric crosses encounter both prezygotic and postzygotic barriers, yet viable hybrids or embryos suitable for rescue can still be obtained.

Ma et al. (2010) confirmed natural hybridization between *R. cyanocarpum* from subsection *Thomsonia* and *R. delavayi* from a different subsection within the same subgenus *Hymenanthes*, with asymmetric bidirectional mating possible. Kenji et al. (2000, 2006), Akihide et al. (2004), and Kaori et al. (2008) all noted unidirectional sterility in crosses between subgenus *Tsutsusi* (e.g., *R. eriocarpum*) and subgenus *Pentanthera* (e.g., *R. japonicum* f. *flavum*). Akihide et al. (2004) investigated crosses between 13 species of subgenus *Tsutsusi* and subgenus *Pentanthera*, finding that pre-fertilization incompatibility manifested as blocked pollen tube growth preventing entry into ovules, while post-fertilization sterility appeared as ovule non-development, seed non-germination, and seedling death. Among 22 incompatible combinations, 15 showed prezygotic incompatibility, 6 postzygotic incompatibility, and 1 developmental disorder, concluding that incompatibility variation exists between subgenera while compatibility differences exceed taxonomic boundaries. Liu et al. (2010) also found differences in fruit set rates between reciprocal crosses of *Rhododendron* cultivars. Zhuang (2018a, 2018d, 2018c) similarly observed varying degrees of unidirectional sterility in intra-subgenus *Hymenanthes*, intra-subgenus *Rhododendron*, and inter-subgeneric hybridizations.

Currently, research on hybrid directionality among subgenus *Hymenanthes*, subgenus *Rhododendron*, and subgenus *Azaleastrum* remains limited, particularly regarding the distribution characteristics of hybrid directionality and its relationship with taxonomic groups and parental selfing properties. This study aims to analyze the distribution characteristics of cross-compatibility among five subgenera including subgenus *Hymenanthes*.

Materials and Methods

1.1 Materials

Building upon previous studies on intra-subgenus *Hymenanthes* (Zhuang, 2018a), intra-subgenus *Rhododendron* (Zhuang, 2018b), inter-subgeneric hybridization of different *Rhododendron* subgenera (Zhuang, 2018c), and their compatibility (Zhuang, 2018d, 2018e), this study investigated the directionality of 33 complete reciprocal cross combinations and 186 hybrid combinations with clear selfing properties (Zhuang, 2017b), involving 32 *Rhododendron* species across 5 subgenera, 3 sections, and 12 subsections.

1.2 Methods

Based on comprehensive fertility assessments of relevant combinations (Zhuang, 2018a, 2018b, 2018c), and referencing the *Rhododendron* classification system (Chamberlain et al., 1996) along with studies on floristics and evolution (Min and Fang, 1990; Fang and Min, 1995) and cytogenetics and reproductive biology, we conducted three types of analyses.

1.2.1 Analysis of Taxonomic Group Combinations and Hybrid Directionality Distribution We categorized the fertility of 33 reciprocal cross combinations into three types—bidirectional fertility, unidirectional sterility, and bidirectional sterility—to analyze the directional distribution patterns within or between subgenera and subsections.

1.2.2 Analysis of Unidirectional Sterility Types Using knowledge from systematics and genetics, we classified and analyzed the unidirectional sterility observed in complete reciprocal crosses to explore distribution patterns and potential causes.

1.2.3 Analysis of Self-Compatibility Types and Hybrid Fertility Distribution Utilizing self-compatibility (SC or SI) data for 32 species (Zhuang, 2017b) and relevant theoretical frameworks, we examined fertility differences and variation patterns under four combination conditions: SC×SC, SC×SI, SI×SC, and SI×SI.

Results and Analysis

This study primarily focused on interspecific hybridization within subgenus *Hymenanthes*, within subgenus *Rhododendron*, and between these two subgenera, with limited investigation of directionality among these subgenera plus subgenus *Azaleastrum*, subgenus *Tsutsusi*, and subgenus *Pentanthera*.

2.1 Taxonomic Group Combinations and Hybrid Directionality Distribution

Data from 33 reciprocal cross combinations revealed distinct patterns in the directional distribution of hybridization within and between subgenera and subsections of *Rhododendron* (Table 1). The transition from bidirectional fertility → unidirectional sterility → bidirectional sterility generally corresponds to the sequence: intra-subgenus *Hymenanthes* → intra-subgenus *Rhododendron* → subgenus *Hymenanthes* × subgenus *Rhododendron* → other inter-subgeneric crosses. Notably, intra-subgenus *Hymenanthes* showed the highest proportion of bidirectional fertile combinations without any bidirectional sterile cases. Within this pattern, crosses within subsection *Fortunea* may have higher bidirectional mating capacity than those between subsection *Fortunea* and subsection *Argyrophylla*. The "other inter-subgeneric crosses" category contained no bidirectional fertile combinations, with one pair each showing bidirectional sterility between subgenus *Hymenanthes* and subgenus *Azaleastrum*, and between subgenus *Hymenanthes* and subgenus *Tsutsusi*. Hybridization patterns within subgenus *Rhododendron* and between subgenus *Hymenanthes* and subgenus *Rhododendron* fell between these extremes, exhibiting all directionality types.

2.2 Taxonomic Group Combinations and Unidirectional Sterility

Sporadic studies on unidirectional sterility in *Rhododendron* have suggested that crosses between more primitive taxa as maternal parents and more advanced taxa as paternal parents often show compatibility or fertility, while reciprocal crosses are incompatible or sterile (Akihide et al., 2006; Kenji et al., 2006; Nobuo et al., 2003; Rouse et al., 1993; Williams et al., 1990), though this remains inconclusive. Examining 14 unidirectional sterile hybrid combinations revealed three preliminary patterns:

2.2.1 Intra-subgeneric crosses between different taxonomic groups and species showed unidirectional compatibility when primitive species served as maternal parents. This pattern was observed in two combinations within subgenus *Hymenanthes* (*R. oreodoxa* × *R. hunnewellianum* and *R. irroratum* × *R. delavayi*) and two within subgenus *Rhododendron* (*R. polylepis* × *R. ambiguum* and *R. rigidum* × *R. ambiguum*). Notably, *R. ambiguum* in subgenus *Rhododendron* is a secondarily evolved polyploid species. Additionally, the combination *R. simsii* × *R. molle* between two subgenera displayed this pattern of normal cross fertility with reciprocal cross sterility.

2.2.2 Reciprocal crosses between subgenus *Hymenanthes* and subgenus *Rhododendron* both exhibited unidirectional sterility, thus not fully conforming to the pattern of unidirectional fertility with primitive taxa as maternal parents. Combinations fitting this pattern

included *R. argyrophyllum* subsp. *omeiense* × *R. augustinii*, *R. argyrophyllum* subsp. *omeiense* × *R. ambiguum*, and *R. floribundum* × *R. polylepis*. *R. hunnewellianum* × *R. moupinense* and *R. floribundum* × *R. liliiflorum* may also belong to this category. Combinations not fitting the pattern included *R. polylepis* × *R. davidi*, *R. liliiflorum* × *R. argyrophyllum* subsp. *omeiense*, and *R. augustinii* × *R. rex*, as well as *R. polylepis* × *R. rex*, *R. polylepis* × *R. glischrum*, and *R. rubiginosum* × *R. hunnewellianum*.

2.2.3 Some parental combinations in unidirectional sterility showed certain tendencies but require further verification. The cross *R. decorum* × *R. molle* (subgenus *Hymenanthes* × subgenus *Pentanthera*) fits the pattern of unidirectional compatibility with primitive species as maternal parents, though previous research indicates these subgenera are bidirectionally fertile (Tom et al., 2007). Additionally, our study lacks reciprocal cross data for the fertile normal cross of *R. decorum* × *R. molle*, which would also belong to this category. Conversely, *R. stamineum* × *R. davidi* and *R. simsii* × *R. liliiflorum* showed unidirectional fertility with advanced taxa as maternal parents, as might *R. simsii* × *R. augustinii*. However, further research on these taxonomic combinations is needed to draw definitive conclusions.

2.3 Relationship Between Selfing Characteristics and Hybrid Fertility

Previous studies have indicated a connection between parental self-compatibility and unidirectional incompatibility in hybrids (Meng et al., 1997). Among the five *Rhododendron* subgenera examined, *R. stamineum* (subgenus *Azaleastrum*), *R. simsii* (subgenus *Tsutsusi*), and *R. molle* (subgenus *Pentanthera*) are self-incompatible (SI) species, while most species in subgenera *Hymenanthes* and *Rhododendron* are self-compatible (SC).

Analysis of 186 hybrid combinations revealed an overall fertility trend of SC×SC > SI×SC   SC×SI > SI×SI (Table 2). However, intra-subgenus *Hymenanthes* crosses showed a pattern of SI×SC > SC×SC   SC×SI > SI×SI. Furthermore, under SC×SC combinations, intra-subgenus *Hymenanthes* crosses showed higher fertility than inter-subgeneric crosses between this subgenus and subgenus *Rhododendron*. In other *Rhododendron* crosses excluding intra-subgenus *Hymenanthes* combinations, fertility followed the trend SC×SI > SI×SC > SI×SI. These results demonstrate that crosses between SC parents generally outperform those involving SI parents, which in turn outperform SI×SI combinations. Notably, SI×SC combinations within subgenus *Hymenanthes* showed unexpectedly high fertility. Under SC×SC conditions, intra-subgenus *Hymenanthes* hybridization was superior to its inter-subgeneric hybridization with subgenus *Rhododendron*, while in other cases, maternal parents of the SC type showed higher fertility.

The 14 unidirectional sterile reciprocal combinations (Table 1) were distributed across all combination types: SC×SC (8 pairs), SI×SC (2 pairs), SC×SI (2 pairs), and SI×SI (2 pairs). Since all these combination types simultaneously showed varying degrees of fertility or sterility (Table 2), the fertility—particularly unidirectional fertility—among *Rhododendron* taxa appears more complex than anticipated.

Unidirectional incompatibility or sterility in *Rhododendron* does not fully conform to the rule that "ancient self-compatible species follow the SI×SC incompatibility rule" (Lewis & Crowe, 1958), particularly for intra-subgenus *Hymenanthes* hybridization. The hypothesis that a control gene *UI* determines SI×SC reciprocal cross patterns (Liu et al., 1997) offers limited explanatory power. Only *R. flavum* (SI type) may lack the *UI* gene, thus showing bidirectional compatibility with *R. polylepis* (SC type). *R. delavayi* may carry the *UI* gene, showing unidirectional compatibility as maternal parent in crosses with *R. irroratum*. However, *R. simsii* (SI type) shows compatibility with *R. liliiflorum* (SC type) but incompatibility with *R. decorum* (SC type) when serving as maternal parent, a pattern that cannot be explained by the *UI* gene hypothesis.

Conclusions and Discussion

Based on our investigation of hybrid directionality in *Rhododendron*, we propose the following preliminary conclusions:

3.1 The transition rate from bidirectional fertility to unidirectional sterility to bidirectional sterility in interspecific hybridization of *Rhododendron* correlates positively with the phylogenetic position of parents (from primitive to advanced) and their genetic distance (from close to distant)

Research demonstrates that the primitive evergreen subgenus *Hymenanthes* (Min and Fang, 1990; Fang and Min, 1995), particularly within subsection *Fortunea*, exhibits a higher proportion of bidirectional fertility than the more advanced subgenus *Rhododendron*, confirming that primitive subgenera hybridize more readily (Williams et al., 1990; Richard et al., 2010; Milne et al., 2003; Zhuang, 2018a). The higher proportions of bidirectional and unidirectional fertility in subgenus *Hymenanthes* × subgenus *Rhododendron* crosses compared to other inter-subgeneric combinations (Zhuang, 2018b, 2018c) further indicates that directional characteristics correlate with parental genetic relatedness.

3.2 Along the evolutionary dimension, unidirectional sterility in interspecific hybridization generally shows no fixed parental combination pattern, though exceptions exist within certain subgenera and between subgenera

Except for crosses within subsection *Fortunea* that showed no unidirectional sterility, all four combination types involving SC and SI parents in intra-subgeneric and inter-subgeneric crosses exhibited varying degrees of unidirectional sterility (Table 2). Thus, no fixed parental combination pattern exists overall. However, unidirectional compatibility with primitive species as maternal parents was evident in intra-subgenus *Hymenanthes*, intra-subgenus *Rhododendron*, and subgenus *Tsutsusi* × subgenus *Pentanthera* crosses. Simultaneously, some advanced subgenus *Rhododendron* species as maternal parents showed unidirectional fertility with certain primitive subgenus *Hymenanthes* taxa. Existing hypotheses on unidirectional sterility (Lewis & Crowe, 1958; Meng, 1997) cannot fully explain these phenomena in *Rhododendron*.

3.3 Interspecific hybrid fertility correlates with parental selfing characteristics, with reproductive evolution proceeding from self-compatibility to self-incompatibility accompanied by strengthened outcrossing reproductive isolation

The general fertility trend SC×SC > SI×SC SC×SI > SI×SI shows varying fluctuations in intra-subgeneric and inter-subgeneric crosses. Since primitive *Rhododendron* taxa are predominantly SC while advanced taxa are mainly SI, this trend reflects consistent evolutionary relationships between hybrid directionality and systematic evolution. Specifically, SI×SC reciprocal crosses show significantly reduced fertility compared to SC×SC crosses, while SI×SI combinations exhibit the lowest fertility. Combined with previous reports (Williams et al., 1990; Rouse et al., 1993; Ng & Corlett, 2000; Jose et al., 2002; Escaravage & Wagner, 2002; Nathannel et al., 2006; Zhang et al., 2007; Akira, 2010), we infer that *Rhododendron* reproductive evolution proceeds from self-compatibility to self-incompatibility (Zhuang, 2017b), accompanied by strengthened reproductive isolation among taxa and species (Zhuang, 2018d, 2018e).

3.4 Cytoplasmic sterility and polyploidy are two important causes of unidirectional sterility, and SI parent involvement substantially increases hybrid sterility rates

Numerous studies on subgenus *Tsutsusi* × subgenus *Pentanthera* crosses have demonstrated reciprocal cross cytoplasmic sterility (Kenji et al., 2000, 2006; Akihide et al., 2006; Nobuo et al., 2008). The reciprocal cross sterility observed in our *R. simsii* × *R. molle* combination likely shares this mechanism. Additionally, our study shows that the polyploid species *R. ambiguum* as maternal parent produced unidirectional sterility in crosses with *R. polylepis*, *R. rigidum*, and *R. argyrophyllum* subsp. *omeiense*, while serving as paternal parent yielded fertile crosses, indicating that polyploid maternal parents represent another important cause of unidirectional sterility (Zhuang, 2018b, 2018c). SI parent involvement drastically increases hybrid sterility rates, with the increase exceeding two-fold when both parents are SI (Table 2), though the underlying genetic mechanisms require further investigation.

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