Postprint of a Study on Floral Characteristics and Breeding System of the Androdioecious Chionanthus retusus

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Abstract

Androdioecy is a rare breeding system in nature. The pollination biology characteristics and breeding system of the androdioecious plant Chionanthus retusus were investigated through field observations and artificial pollination experiments. The results showed that the stamen development process in male flowers and hermaphroditic flowers of C. retusus was essentially consistent, with both producing functional pollen grains. In hermaphroditic flowers, the two carpel primordia fused and differentiated to form a pistil, whereas in male flowers, the two carpel primordia fused to form an empty chamber that ceased development until complete degeneration. The pistil was protogynous, with a long stigma receptive period; pollen remained viable after anther dehiscence, maintaining viability above 10% for approximately 2 weeks at room temperature. Chionanthus retusus is pollinated by both wind and insects (primarily thrips and hoverflies). After 30 days of controlled pollination, the natural control fruit set rate was 34.36%; hermaphroditic flowers exhibited no apomixis, were self-compatible, but the autonomous selfing fruit set rate was only 10.70%; under artificial pollination, the outcrossing fruit set rate was significantly higher than that of selfing (geitonogamy); sexual reproduction was limited by pollinators; and it represents a mixed mating system. This study confirms that C. retusus is another functional androdioecious species in the Oleaceae family, which relies on male plants to increase the quantity and quality of outcross pollen to avoid inbreeding depression, while the self-compatibility of hermaphroditic flowers ensures reproductive success. The pistil degeneration in male flowers of C. retusus further demonstrates from another perspective that androdioecy in Oleaceae represents a transitional state from hermaphroditism to dioecy.

Full Text

Preamble

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Floral Syndrome and Reproductive Strategy of an Androdioecious Species, *Chionanthus retusus* (Oleaceae)

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Abstract

Androdioecy is a rare reproductive strategy in plants. We investigated the pollination biology and breeding system of the androdioecious species *Chionanthus retusus* through field observations and artificial pollination experiments. The stamen development process was similar in male and hermaphroditic flowers, both producing functional 2-cell pollen grains. During early pistil development, two carpel primordia appeared inside the stamens of both flower types. In hermaphroditic flowers, these two carpels fused and differentiated into a functional ovary and stigma, whereas in male flowers, the fused carpels formed a cavity that ceased development and eventually degenerated. The species is protogynous, with stigmas remaining receptive throughout anthesis. Pollen viability was maintained after anther dehiscence. *C. retusus* is pollinated by both wind and insects (primarily Thripidae and Syrphidae), but the spontaneous selfing fruit set rate was only 10.70%. Under artificial pollination, cross-pollination fruit set was significantly higher than self-pollination ($P < 0.05$). No apomixis was observed. These results confirm that *C. retusus* is a functionally androdioecious species in the Oleaceae family, employing a mixed mating system. The species relies on male individuals to increase the quantity and quality of outcross pollen to avoid inbreeding depression, while the self-compatibility of hermaphroditic flowers ensures reproductive success. The pistil degeneration in male flowers provides further evidence that androdioecy in Oleaceae represents a transitional state from hermaphroditism to dioecy.

Keywords: *Chionanthus retusus*; androdioecy; flowering dynamics; pollination mechanisms

1. Study Materials and Locations

We selected four *C. retusus* populations for this study: Jigong Mountain Nature Reserve, Tongbai Huaiyuan Scenic Area, Zhengzhou Green Expo Park, and Henan University campus. The first two are wild populations, while the latter two are cultivated introductions. Geographic locations and climatic characteristics of each population are detailed in Table 1.

1.1 Flowering Dynamics and Floral Morphological Structure Observations

We tracked flowering dynamics of *C. retusus* on Henan University campus, randomly selecting 10 male and 10 hermaphroditic individuals. From each plant, we measured dimensions of floral parts (calyx, corolla tube, petals, stamens, and pistils) from 30 fully opened flowers using vernier calipers. The flowering process was divided into five stages: (1) bud stage, (2) pre-flowering stage (inflorescence expanding with leaves until petals begin to unfold), (3) full bloom (petals uncoiling from spiral folds, nearly all flowers fully open), (4) late flowering stage (some petals beginning to wilt, yellow, or abscise), and (5) post-flowering stage. Starting from bud initiation, we collected flower buds from both male and hermaphroditic plants daily. Conventional paraffin sectioning was employed for anatomical observations, with stereomicroscope or scanning electron microscopy imaging when necessary, using hematoxylin staining.

2. Breeding System Experiments

2.1 Stigma Receptivity, Pollen-Ovule Ratio, and Outcrossing Index (OCI)

Stigma receptivity was tested daily at noon using the hydrogen peroxide reaction method (OCI = 4:11:22) [**?**]. Pollen viability at different stages was assessed through in vitro culture. We selected 30 unopened flowers (with undehisced anthers) from each population for both flower types. Ovule numbers were counted under a dissecting microscope from 30 hermaphroditic flowers per population to calculate the pollen-ovule (P/O) ratio.

2.2 Pollinator Observations

We selected three plants with abundant flowers from each population to observe and photograph visiting insects, recording visitation times and behaviors. Insects were captured, preserved as specimens, and identified by experts. Wind-mediated pollen dispersal was detected using the gravity slide method: five relatively isolated trees at Zhengzhou Green Expo Park were selected, with petroleum jelly-coated slides placed at 1, 2, 4, 8, and 16 m from the trees in four cardinal directions. Slides were collected after 24 hours and *C. retusus* pollen grains were counted.

2.3 Controlled Pollination Experiments

We selected vigorous hermaphroditic plants from each population for the following treatments: (1) Open pollination (control); (2) Bagged without pollination (to test for apomixis); (3) Bagged with net covering (to test wind pollination efficiency while excluding insects); (4) Spontaneous self-pollination (bagged, unmanipulated); (5) Artificial geitonogamy (pollen from same plant, different flower); (6) Artificial xenogamy using hermaphroditic pollen; (7) Artificial xenogamy using male pollen. Each treatment included 30 replicates per population, with only 10 flowers retained per inflorescence. Pollen tube growth was observed by fixing pistils at 20 min, 40 min, 1 h, 2 h, 4 h, 8 h, 16 h, and 32 h after pollination, staining with decolorized aniline blue solution, and examining under fluorescence microscopy. Fruit set was recorded one month after pollination, calculated as: fruit set rate (%) = (number of fruits / number of treated flowers) × 100%.

3. Data Analysis

Floral morphological data were analyzed using two-way ANOVA in SPSS 19.0, with Duncan’ s method for multiple comparisons. Fruit set data were arcsine-transformed before analysis. Significance was set at $P < 0.05$. Data were processed using Excel.

1. Flowering Dynamics and Floral Characteristics of *C. retusus*

C. retusus has a calyx length of 2.35$±0.35*mmandcorollatubelengthof*2.63±$0.39 mm. On Henan University campus, flower bud differentiation occurred in the previous year’ s June-July, with flowers expanding in late March. Flowering time varied annually due to weather conditions. In 2014, buds emerged on March 20-23; in 2015, they emerged on March 12-14, followed by continuous cloudy and rainy weather; in 2016, they emerged on March 20-23.

Comparative analysis of 11 floral traits between male and hermaphroditic flowers across the four populations revealed significant inter-population differences in anther length and width. The two wild populations (Jigong Mountain and Tongbai) showed significantly lower values for multiple floral traits. Within populations, significant sexual dimorphism was observed only in corolla tube length and pollen number per flower, with male flowers producing significantly more pollen than hermaphroditic flowers. Other traits showed no significant differences between flower types.

[Figure 1: see original paper]

2. Floral Anatomical Observations

Scanning electron microscopy and paraffin sections revealed that pollen grains from both flower types were elliptical with reticulate surface ornamentation. The developmental process from bud initiation to stamen differentiation was essentially identical in both male and hermaphroditic flowers, producing normal 2-cell pollen grains after anther dehiscence.

During pistil differentiation, two carpel primordia emerged at the base inside the stamens of hermaphroditic flowers, subsequently fusing and differentiating into a functional pistil with ovary, style, and stigma. Male flowers also initiated carpel primordia, but after fusion they formed a central cavity that arrested development and eventually degenerated.

[Figure 2: see original paper]

3. Breeding System

3.1 Pollen Viability, Stigma Receptivity, P/O Ratio, and OCI

Pollen from both male and hermaphroditic flowers showed high germination rates (>80%) within two days of anther dehiscence when cultured in vitro, gradually declining thereafter. After 10-14 days at room temperature (20-25°C), germination rates dropped to 34.36%. ANOVA revealed no significant difference in pollen germination rates between genders ($P = 0.277$). Both aniline blue staining and SEM observations confirmed pollen tube growth on stigmas, with tubes penetrating papillar cell gaps and reaching ovules after 16 hours, demonstrating that pollen from both flower types is functionally male.

The benzidine-peroxide test showed that hermaphroditic flower stigmas were receptive during the pre-flowering stage when pollen was still inactive, confirming protogyny. The overlapping period of male and female gamete viability within the same flower was 3-5 days. The average P/O ratio for hermaphroditic flowers was 40,000-60,000 pollen grains per flower with a fixed ovule number of 2, yielding a P/O ratio of 10,000-15,000. According to Cruden' s [?] criteria, this indicates a facultative outcrossing breeding system.

3.2 Pollination Vectors

Gravity slide detection confirmed airborne pollen dispersal, with wind carrying substantial amounts of *C. retusus* pollen beyond 16 m. Insect visitors included Thripidae and Syrphidae. Thrips remained inside corolla tubes for extended periods, with their bodies completely covered in *C. retusus* pollen under stereomicroscope observation. Syrphid flies visited only briefly in sunny weather. The significant differences in fruit set between net-covered, control, and spontaneous selfing treatments demonstrated that both wind and insects play important roles in pollination.

3.3 Controlled Pollination

In controlled pollination experiments, emasculated and bagged hermaphroditic flowers failed to set fruit, confirming the absence of apomixis. ANOVA of fruit set rates across treatments showed significant differences among treatments ($F = 374.808$, $P < 0.01$). Spontaneous selfing and geitonogamy yielded fruit set rates of 10.70% and 24.67%, respectively, significantly lower than the control (34.36%), indicating self-compatibility but pollinator limitation. Cross-pollination treatments (xenogamy with pollen from males or hermaphrodites) achieved fruit set rates of 36.94% and 41.11%, respectively, significantly higher than selfing treatments and the control, demonstrating outcrossing advantage.

[Figure 5: see original paper]

Population-level analysis revealed that Tongbai had significantly lower fruit set in open pollination, net-covered, and emasculated treatments compared to other populations, suggesting compromised pollination vectors at this site. The Tongbai study site was located in a mountain valley under a cliff, while Jigong Mountain was situated on a secondary peak near the main summit—essentially a wind gap. The two cultivated sites were in plain areas. Despite these microenvironmental differences, artificial pollination fruit set showed no significant inter-population variation, indicating that Tongbai's reduced natural fruit set resulted from pollination limitation rather than genetic factors.

1. Sexual System and Pollination Mechanism of *C. retusus*

Our survey of multiple *C. retusus* populations identified only male and hermaphroditic individuals, with no true female flowers or monoecious plants, consistent with reports by Soejima et al. [**?**] and Ma et al. [**?**]. Combined with our controlled pollination results, we confirm that *C. retusus* is functionally androdioecious.

Floral traits are influenced by both genetic control and environmental conditions [**?**]. While all populations contained both sexual morphs with stable sex expression across years, significant inter-population variation in sepal, petal, and anther dimensions indicated strong environmental effects, consistent with Song et al.' s [**?**] observations across Korean populations. However, we found minimal sexual dimorphism in floral traits, differing from previous reports—a discrepancy likely attributable to differences in plant age, habitat conditions, or sample sizes.

Despite large floral displays and high pollen production in both morphs, *C. retusus* is self-compatible, contrasting with the typical association between large floral displays and obligate outcrossing [**?**]. The high P/O ratio may compensate for pollen loss during collection by inefficient pollinators [**?**]. The absence of nectar glands, consistent with Song et al. [**?**], resulted in limited pollinator attraction. However, artificial pollination achieved uniform fruit set across populations, while Tongbai showed significantly lower natural pollination success, indicating pollinator limitation at this site.

Wind plays a crucial role in pollination, as evidenced by airborne pollen detection and significantly higher fruit set in net-covered treatments compared to bagged treatments (which excluded both wind and insects). The combination of wind and insect pollination, coupled with protogyny and self-compatibility, characterizes *C. retusus* as having a mixed mating system that ensures reproductive assurance when pollinators are scarce [?].

Both male and hermaphroditic pollen showed identical morphology and in vitro germination rates, and both could fertilize ovules in controlled crosses, confirming functional androdioecy. While both selfing and outcrossing were compatible, outcrossing produced significantly higher fruit set, generating genetic diversity and avoiding inbreeding depression. The enclosed position of reproductive organs within the corolla tube makes autonomous selfing unlikely, but self-compatibility provides reproductive insurance during periods of pollinator scarcity or rainy weather. Ongoing research is investigating whether hybridization or selfing predominates in natural populations.

2. Maintenance and Evolution of Androdioecy in *C. retusus*

Androdioecy is a rare breeding system requiring specific conditions for evolutionary maintenance. Theoretical models predict that male individuals must have at least twice the pollination advantage of hermaphrodites to compensate for their inability to set seed, assuming strong inbreeding depression [?]. In our study populations, male proportions varied widely (39.5%-57.6%), often falling below the theoretical threshold, a pattern also observed in other androdioecious Oleaceae species [?]. However, our data may not reflect natural conditions due to severe wild resource destruction and human disturbance, particularly given the extremely high market value of seeds.

The evolutionary origin of androdioecy remains debated. Traditional hypotheses propose derivation from dioecy through females gaining male function [?], while alternative models suggest evolution from hermaphroditic ancestors via female-sterile mutants [?]. Molecular phylogenetic studies of ash trees (*Fraxinus*) support the latter, indicating androdioecy as a transitional state from hermaphroditism to dioecy, with concurrent shifts from insect to wind pollination [?].

Our comparative developmental analysis of male and hermaphroditic flower buds in *C. retusus* reveals that both morphs initiate carpel primordia, but male flowers abort this development, forming a degenerated cavity. This pistillode presence in male flowers, combined with wind-insect mixed pollination, supports Wallander’ s [?] conclusion that androdioecy in Oleaceae represents an evolutionary transition from hermaphroditism to dioecy.

References

[1] Dellaporta SL, Calderon-Urrea A. Sex determination in flowering plants. Plant Cell, 1993, 5(10): 1241-1251. [2] Anderson GJ, Symon DE. Functional dioecy and andromonoecy in solanum. Evolution, 1989, 43(1): 204-219. [3] Schlessman MA, Lowry PP, Lloyd DG. Functional dioecism in the New Caledonian endemic *Polysacias pancheri* (Araliaceae). Biotropica, 1990, 22(2): 133-139. [4]

Cane JH. Reproductive role of sterile pollen in *Saurauia* (Actinidiaceae), a cryptically dioecious Neotropical tree. Biotropica, 1993, 25(4): 493-495. [5] Ishida K, Hiura T. Pollen fertility and flowering phenology in an androdioecious tree, *Fraxinus lanuginosa* (Oleaceae), in Hokkaido, Japan. International Journal of Plant Sciences, 1998, 159(6): 941-947. [6] Liston A, Rieseberg LH, Elias TS. Functional androdioecy in the flowering plant *Datisca glomerata*. Nature, 1990, 343(6259): 641-642. [7] Akimoto J, Fukuhara T, Kikuzawa K. Sex ratios and genetic variation in a functionally androdioecious species, *Schizopepon bryoniaefolius* (Cucurbitaceae). American Journal of Botany, 1999, 86(6): 880-886. [8] Vassiliadis C, Saumitou-Laprade P, Lepart J, Viard F. High male reproductive success of hermaphrodites in the androdioecious *Phillyrea angustifolia*. Evolution, 2002, 56(7): 1362-1373. [9] Nishide M, Saito K, Kato H, Sugawara T. Functional androdioecy in *Morinda umbellata* subsp. *boninensis* (Rubiaceae), endemic to the Bonin (Ogasawara) Islands. APG Acta Phytotaxonomica Et Geobotanica, 2009, 60(2): 61-70. [10] Choudhury BI, Khan ML, Dayanandan S. Functional androdioecy in critically endangered *Gymnocladus assamicus* (Leguminosae) in the Eastern Himalayan Region of Northeast India. PLoS One, 2014, 9(2): e87287. [11] Zhou XJ, Ma L, Liu WZ. Functional androdioecy in the rare endemic tree *Tapiscia sinensis*. Botanical Journal of the Linnean Society, 2016, 180(4): 504-514. [12] Flora of China. Science Press, 1992. [13] Saeki I. Application of aerial survey for detecting a rare maple species and endangered wetland ecosystems. Forest Ecology and Management, 2005, 216(1/3): 283-294. [14] *Chionanthus retusus* faces survival crisis. 2013-04-03. [15] Seedling cultivation techniques of *Chionanthus retusus*. Modern Agricultural Science and Technology, 2012, (13): 181-181, 183-183. [16] Isolation and identification of flavonoid chemical components from *Chionanthus retusus* flowers, 2014, 35(1): 74-78. [17] Kwak JH, Kang MW, Roh JH, Choi SU, Zee OP. Cytotoxic phenolic compounds from *Chionanthus retusus*. Archives of Pharmacal Research, 2009, 32(12): 1681-1687. [18] Choi KS, Kim YH, Kim SO, Shin KO, Chung KH. Effect of intake of sponge gourd (*Luffa cylindrica*) seed oil and Yukdomok *Chionanthus retusa* L. seed oil on lipid levels of blood and organs of mice. Food Science and Biotechnology, 2013, 22(3): 757-763. [19] Arias RS, Techen N, Rinehart TA, Olsen RT, Kirkbride JH, Scheffler BE. Development of Simple Sequence Repeat Markers for *Chionanthus retusus* (Oleaceae) and effective discrimination of closely related taxa. Hortscience, 2011, 46(1): 23-29. [20] Chien CT, Kuo-Huang LL, Shen YC, Zhang RC, Chen SY, Yang JC, Pharis RP. Storage behavior of *Chionanthus retusus* seed and asynchronous development of the radicle and shoot apex during germination in relation to germination inhibitors, including abscisic acid and four phenolic glucosides. Plant and Cell Physiology, 2004, 45(9): 1158-1167. [21] Soejima A, Maki M, Ueda K. Genetic variation in relic and isolated populations of *Chionanthus retusus* (Oleaceae) of Tsushima Island and the Tôno region, Japan. Genes & Genetic Systems, 1998, 73(1): 29-37. [22] Song JH, Oak MK, Hong SP. Morphological traits in an androdioecious species, *Chionanthus retusus* (Oleaceae). Flora-Morphology, Distribution, Functional Ecology of Plants, 2016, 223: 129-137. [23] Dafni A. Pollination Ecology: A Practical Approach. Oxford: Oxford University Press, 1993. [24] Studies on flowering and seedling characteristics of *Chionanthus retusus* [D]. Shandong Agricultural University, 2007. [25] Barrett SCH, Case AL, Peters GB. Gender modification and resource allocation in subdioecious *Wurmbea dioica* (Colchicaceae). Journal of Ecology, 1998, 87(1): 123-137. [26] Lloyd DG, Webb CJ. Secondary sex characters in plants. The Botanical Review, 1977, 43(2): 177-216. [27] Shea MM, Dixon PM, Sharitz RR. Size differences, sex ratio, and spatial distribution of male and female water tupelo, *Nyssa aquatica* (Nyssaceae). American Journal of Botany, 1993, 80(1): 26-30. [28] El-Keblawy A, Freeman DC. Spatial segregation by gender of the subdioecious shrub *Thymelaea hirsuta* in the Egyptian Desert. International Journal of Plant Sciences, 1999, 160(2): 341-350. [29] Delph LF, Wolf DE. Evolutionary consequences of gender plasticity in genetically dimorphic breeding systems. New Phytologist, 2005, 166(1): 119-128. [30] Pannell J. Mixed genetic and environmental sex determination in an androdioecious population of *Mercurialis annua.* Heredity, 1997, 78(1): 50-56. [31] Walsh D. Sex lability discovered in *Atriplex vesicaria* Hew. ex Benth. (Chenopodiaceae). Journal of Arid Environments, 2005, 60(2): 201-210. [32] Willson MF. Sexual selection in plants. The American Naturalist, 1979, 113(6): 777-790. [33] Wyatt R. Pollinator-plant interactions and the evolution of breeding systems. In: Read L, ed. Pollination Biology. Orlando: Academic Press. 1983. [34] Cruden RW. Pollen-Ovule Ratios: A conservative indicator of breeding systems in flowering plants. Evolution, 1977, 31(1): 32-46. [35] Preston RE. Pollen-ovule ratios in the Cruciferae. American Journal of Botany, 1986, 73(12): 1732-1740. [36] Pellmyr O. Pollination ecology of *Cimicifuga arizonica* (Ranunculaceae). Botanical Gazette, 1985, 146(5): 404-412. [37] Fausto JAJr, Eckhart VM, Geber MA. Reproductive assurance and the evolutionary ecology of self-pollination in *Clarkia xantiana* (Onagraceae). American Journal of Botany, 2001, 88(10): 1794-1800. [38] Charlesworth D. Androdioecy and the evolution of dioecy. Biological Journal of the Linnean Society, 1984, 22(4): 333-348. [39] Bawa KS, Beach JH. Evolution of sexual systems in flowering plants. Annals of the Missouri Botanical Garden, 1981, 68(2): 254-274. [40] Fritsch P, Rieseberg LH. High outcrossing rates maintain male and hermaphrodite individuals in populations of the flowering plant *Datisca glomerata.* Nature, 1992, 359(6396): 633-636. [41] Pannell JR. The evolution and maintenance of androdioecy. Annual Review of Ecology and Systematics, 2002, 33(1): 397-425. [42] Lepart J, Dommée B. Is *Phillyrea angustifolia* L. (Oleaceae) an androdioecious species? Botanical Journal of the Linnean Society, 1992, 108(4): 375-387. [43] Dommée B, Geslot A, Thompson JD, Reille M, Denelle N. Androdioecy in the entomophilous tree *Fraxinus ornus* (Oleaceae). New Phytologist, 1999, 143(2): 419-426. [44] Ross MD. Five evolutionary pathways to subdioecy. The American Naturalist, 1982, 119(3): 297-318. [45] Wallander E, Albert VA. Phylogeny and classification of Oleaceae based on rps16 and trnL-F sequence data. American Journal of Botany, 2000, 87(12): 1827-1841. [46] Xu YC, Zhou LH, Hu SQ, Hao RM, Huang CJ, Zhao HB. The differentiation and development of pistils of hermaphrodites and pistillodes of males in androdioecious *Osmanthus fragrans* L. and implications for the evolution to androdioecy. Plant Systematics and Evolution, 2014, 300(5): 843-849. [47] Wallander E. Systematics of *Fraxi-*

nus (Oleaceae) and evolution of dioecy. Plant Systematics and Evolution, 2008, 273(1/2): 25-49.

Note: Figure translations are in progress. See original paper for figures.