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Pollination and Breeding system of *Ranunuclus japonicus* Thunb. in Japan

(Penyerbukan dan system reproduksi *Ranunuculus japonicus* Thunb. di Jepang)

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Abstrak

Penelitian tentang penyerbukan dan sistem reproduksi Ranunculus japonicus *telah dilakukan di kebun botani Universitas Osaka City, Katano, Osaka, Japan. Hasil penelitian menunjukkan bahwa putik* R. japonicus *lebih dahulu masak daripada benang sarinya* (protogyny). *Pada tumbuhan ini terjadi ketidakcocokan silang sendiri* (self-incompatible). *Tumbuhan ini tidak mengalami penyerbukan sendiri* (selfing) *dan juga tidak membentuk biji tanpa pembuahan (agamospermy), tetapi melakukan penyerbukan silang* (out-crossing) *dan membutuhkan perantara* (pollinator) *untuk pembentukan bijinya*.

Kata kunci: *out*-crossing, protogyny, *Ranunculus japonicus*, self-incompatible, *system reproduksi*

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Introduction

Ranunculus is the largest genus in the family *Ranunculaceae* with great diversities (Tamura, 1980; Kadota, 1990). Many of them are familiar spring wild flowers in temperate regions, with an estimated 600 species distributed in all continents (Tamura, 1967), of which about 200 species are distributed in the temperate and cold regions in the world wide, mainly in Asia and Europe, about 80 species in mainland of China, 11 species in Taiwan (Yang & Huang, 1996) and more than 26 species in Japan (Ohwi & Kitagawa, 1983).

R. japonicus is belonging to sect. *Acris* which distributed in wide-range in Japan (Hara & Kurosawa, 1956; Ohwi & Kitagawa, 1983). The basic chromosome number of *R. japonicus* is x = 7 (Hara & Kurosawa, 1956; Kurita, 1966; Goephert, 1974). Based on the karyological evidence, Hara and Kurosawa (1956) presumed that *R. japonicus* is the ancestor of some species of sect. *Acris* in Japan. If Hara and Kurosawa's (1956) presumption is true, *R. japonicus* played important role for evolutionary of *Ranunculus* in Japan.Analysis of breeding system is an important step for understanding the evolutionary process, genetic diversity of populations or species (Sutar *et al.*, 2000), and conservation process (Richard, 1996).

In general, outcrossing species usually contain high genetic diversity within population or species, and less genetic divergence among populations (Hamrick & Godt, 1990; Richter *et al.*, 1994), whereas selfing species consist of uniform individuals genetically and have substantial level of genetic divergence (Affre *et al.*, 1997; Maki *et al.*, 1999). Thus, to understand the genetic diversity of populations, how this species reproduct next generations should be investigated. For this reason, I carried out crossing test to clarify breeding system of *R. japonicus.*

Materials and Methods

Observations of floral behavior of *R. japonicus*

Observation of floral behavior were carried out at Botanical gardens Osaka City University using 150 flowers of 43 individuals collected from Mt. Ontake, Nagano Pref. and Mt. Ibuki, Shiga Pref., Japan. *R. japonicus* is a perennial herb, with bright yellow, radical shape flowers on a compound cymoses inflorescences. The flower consist 5 sepals, 5-7 petals with a nectary at the base, many stamens and many free carpels with one ovule.

The flowers were numbered and the condition of petals and stamens were observed daily at 7.00 a.m., 10.00 a.m., 12.00 a.m., and 3.00 p.m. Receptivity of stigmatic area was examined by following procedure. Flower buds were bagged. Flower or flower buds were fixed with FAA (formaldehyde : acetic acid : 50% ethanol = 5 : 5 : 90) after 4 hours from hand-pollinated. They were soak in 1N NaOH about 8 hours and stained 0.1 % anilin blue solution in 0.1 N K_2PO_4 and observed with the fluorescence microscope (Martin, 1958). If pollen tube grew, stigmas were judged as on the phase receptivity.

Breeding tests

To examine breeding system (whether *R. japonicus* is sexual reproduction or agamospermy ,producing seed without sex); self-compatible or self-incompatible; autogamy or xenogamy), crossing test were performed as follows: 1). Control, the flowers were left in natural condition; 2). Bagging, the flowers were bagged with a nylon mesh bag (50 mm diameter) before anthesis; 3). Emasculation, before anthesis all stamens were emasculated and bagged; 4). Self-pollination, stigmas were pollinated with the pollens from the same individual; 5). Cross-pollination, stigmas were pollinated wit the pollen from different individuals in the same population (Cross-pollination 1) and different population (Cross-pollination 2).

After three weeks from treatment fruit were harvested. Frequency of seed set was estimated as number of fertile achenes per total achenes in a head. Fertile Achenes are characterized by rather swollen and more bigger achenes and contain endesperm in the seed.

Results and Discussions

1. Results

R. japonicus started anthesis at 10.00 a.m.-12.00 a.m. and closed at about 3.00 p.m. until 5.00 p.m. Petals continued to open in the morning to midday and close in the evening for 3 to 4 days (Fig. 1. Sepals became recurved at noon of the first day and did not close again. At the third to fourth morning the petals reopened but did not close in the evening. Sepals dropped at fifth to sixth day and all petals dropped on the sixth to seven day. The stamen are arranged in five whorls. About one hour after petals opened on the first day of anthesis, the outer stamens began dehisced. The remaining stamens dehisced successively until the fourth to fifth day. In the fifth to seventh evening, all pollen grains within stamens appeared to have been dispersed. Stigmatic area has been receptive 12 hours before anthesis and continued for about the following fifth to seventh day judging from observations of pollen grain germination (Fig. 2). These observation indicated that *R. japonicus* is protogynous. Crossing test were carried out to examine self-incompatibility of *R. japonicus*. The fact that emasculation test resulted in 0 % seed set (Table 1) and indicated no possibility of agamospermy in *R. japonicus*. Seed set of cross pollination 1 and 2 were 75% and 91%, respectively (Table 1). The low seed set resulted from self pollination showed that *R. japonicus* was almost self-incompatible.

2. Discussions

Plant breeding system and mode of pollination have been identified as a major factor influencing genetic diversity and population structure of populations (Hamrick and Godt, 1990; Akimoto et. al. 1998). It is generally to say that out-crossing plant species has higher level of genetic variations than selfing species (Hamrick et. al. 1979; Hamrick and Godt, 1990). Hence, breeding system of the *R. japonicus* must be recognized before assessing its genetic variation.

The result of observations concerning floral behavior and flowering phenology of *R. japonicus* clearly showed that this species perform dichogamy and protogyny. Dichogamy, the phenomenon that timing of anther's dehiscence shifts from stigma's receptivity, is a important factor of breeding system which generally assumed to be an adaptation to prohibit self-pollination and promote cross-pollination (Proctor et. al., 1996). The Stigmas have already been receptable pollens at the flowers buds stage when anthers still do not dehisce (Fig.1). Protogyny is also found in *R. acris* (Wyatt, 1983).

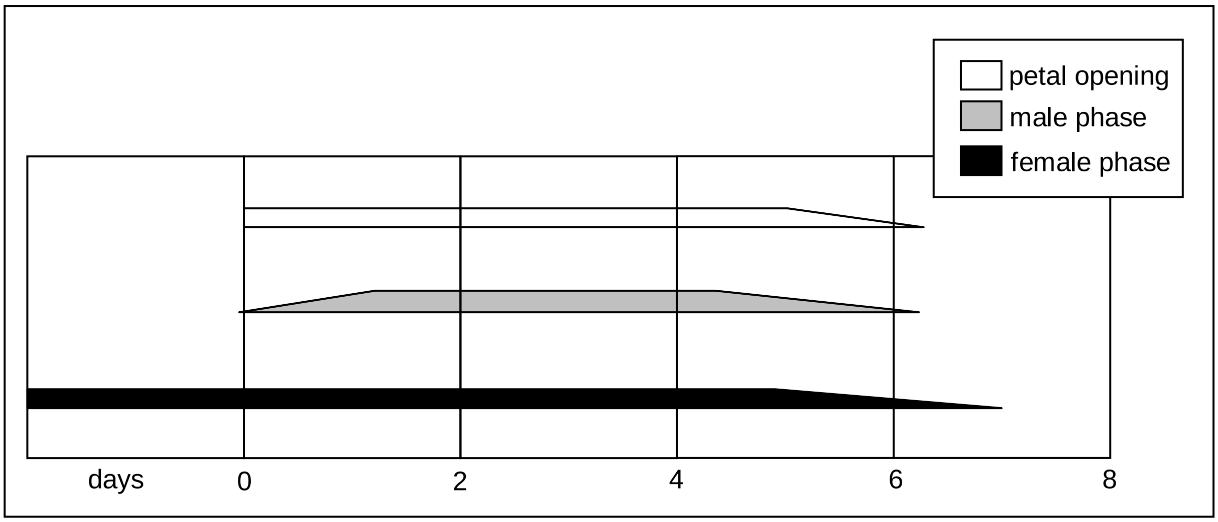

Figure. 1. Floral behavior of Ranunculus japonicus. *0 at x axis: 0 a.m. at the first day anthesis. Oblique line: the possibility of beginning or finishing time of petal movement, pollen exclusion and/or stigma receptivity*

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Table 1. Results of crossing test in R. japonicus

No.	Treatment	Number of sample	Seed set (%) Mean ± SD
1	Control	47	47.9 ± 28.5
2	Bagging	25	0 ± 0
3	Emasculation	20	0 ± 0
4	Self-pollination	16	7.1 ± 18.7
5	Cross-pollination 1	22	75.0 ± 24.0
6	Cross-pollination 2	20	90.9 ± 11.5

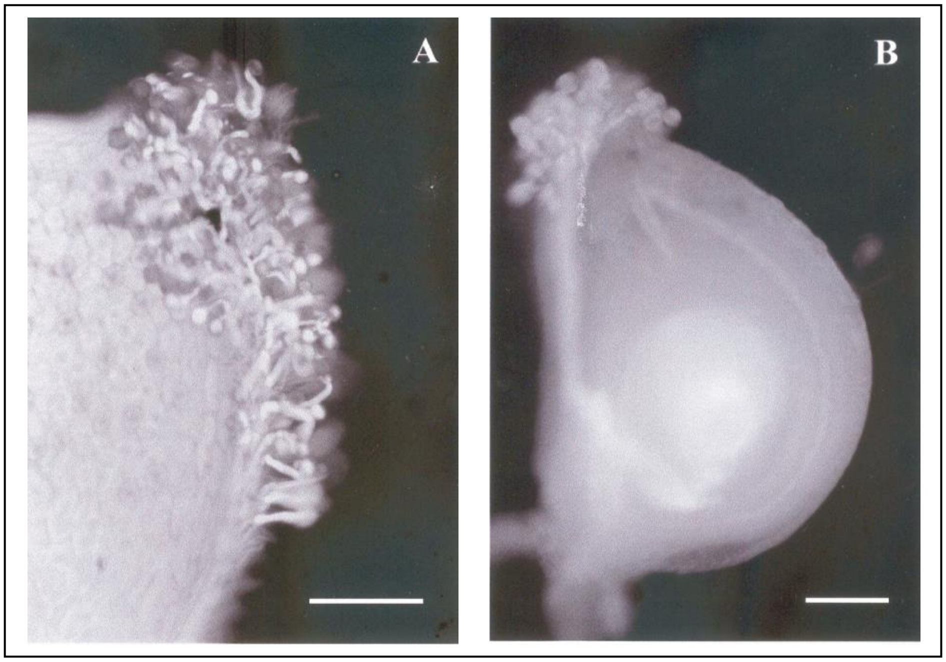

Fig. 2. Fluorescence microphotographs of stigmatic area of *Ranunculus japonicus*.
Bar: 200 μm. A: Numerous pollen tubes germinated on stigmatic area. B: Pollen tubes grew toward embryo.

Further, *R. japonicus* was self-incompatible and out-crossing, judging from the results of crossing test (Table 1) and field observation of flower visitors. Self-incompatibilty is also known from R. acris (Osterbye, 1977). Moreover, floral characters of *R. japonicus*, such as; many flowers, rotate corolla, numerous stamens, anthers do not dehisce in the bud, stigma receptivity and anthers dehiscence asynchronous, many ovules per flower, nectar present etc (Ornduff, 1969; Wyatt, 1983), and strong UV absorption of all part of flowers (Utech and Kawano, 1975) indicated that this species is complete out-crossing species and expected to maintain a higher genetic diversity (Hamrick and Godt, 1990). It is clarified that *R. japonicus* has a higher level of genetic diversity (Syamsuardi, 2002; Syamsuardi and Okada, 2002) than other plants with similar reproductive and phytogeographical traits (Hamrick and Godt, 1990).

Conclusions

Judging from the observation of floral behavior and results of breeding test, I concluded that : 1). *R. japonicus* exhibited *protogyny* and *self-incompatible*. 2). There is no possibility of spontaneous *autogamy* (*selfing*) in *R. japonicus*, and pollen vectors are required for seed set. 3). *R. japonicus* performs *out-crossing* for its seed production.

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