J. Plant Res. **106**: 213-217, 1993

Pollination Biology of the Saprophytic Species *Petrosavia sakuraii* (Makino) van Steenis in Central Japan

Hiroshi Takahashi[1], Eiji Nishio and Hirotaka Hayashi

Department of Biology, Faculty of Education, Gifu University, Gifu, 501-11 Japan

Pollination biology of the saprophytic species *Petrosavia sakuraii* was investigated in Central Japan. The flower opens at any time of the day, and the anthers of outer stamens dehisce after about one day and those of the inner stamens after two days. The stigmata on the semicarpous (nearly apocarpous) pistil, which are already receptive when the flower opens, are situated in the center of the flower and move gradually outwards to touch the anthers of outer stamens about five days after anthesis. The breeding experiments show that the stigmata are receptive at least for five days, and many seeds are produced through autonomous self-pollination. That means the nearly apocarpous pistil, which is in a primitive condition, is adapted to the autonomous self-pollination. The selfing rate including insect-mediated self-pollination is very high, and this sexual-reproductive system seems favorable to the saprophytic plant which is probably severely limited in its resources. However, cross-pollination also may be performed by the small bees of Lasioglossum and some other insects, even though they do not so frequently visit the flowers and the out-crossing rate is low.

Key words: *Petrosavia sakuraii* — Pollination — Protogyny — Saprophyte — Self-pollination

Petrosavia is a saprophytic genus, which has been considered a rather primitive taxon in monocotyledons, because the pistil composed of three carpels is semicarpous (nearly apocarpous) though the outside of the ovary is in the perigynous condition in *P. sakuraii*. Nevertheless, a general consensus on its taxonomic position has not been obtained, though it is included often in Liliaceae (Krause 1930; Melchior 1964; Hutchinson 1973; Cronquist 1981; Dahlgren *et al*. 1985). The floral and pollination biologies will provide valuable information about its floral morphology and other areas. *Petrosavia sakuraii* (Makino) J.J. Smith ex van Steenis [syn. *Protolirion sakuraii* (Makino) Makino; cf. Ohba (1984)] is a rare plant occuring in Japan, Taiwan and south China (Chen 1980). We investigated the pollination biology of this species in central Japan. Obligatorily heterotrophic plants like saprophyte or some parasites are commonly minor understories and often grow on dark forest floors where other understories are very few. They usually do not develop into large plants probably because of disuse of photosynthetic organs and/or resource limitations. These growing habitats and habits might cause some distinct pollination syndromes in them. However, only a few investigations on pollination in these plants have been reported. *Rafflesia* species, which are parasitic, produce large and showy flowers disproportionate to the vegetative organs. The stenchy flowers of *R. pricei* attract carrion flies using both optical and olfactory cues. The flies are tricked into pollination without any rewards (Beaman *et al*. 1988).

Petrosavia sakuraii grows on the floors of mixed forests of deciduous and evergreen broad-leaved trees (Watanabe 1944; Mizuno *et al*. 1974) or coniferous forests (Akazawa and Tamura 1924; Ohba 1984) in central Japan. The plants are very small, mostly 10-20 cm tall, and the flowers are 2-3 mm in diameter. The plant is entirely whitish light-brown.

Materials and Methods

All investigations were made at Kukuri, Kani City, Gifu Prefecture in Japan, in 1984, 1986, 1987 and 1991. Phenological observations were performed in 1984 and 1986. The blooming sequence was observed in mid- and late July of both years. About 110 hours including night time were spent collecting foraging insects.

Breeding experiments were performed as follows: in 1984 artificial cross-pollination in first day flowers, bagging the emasculated flowers (apomixis test), bagging the intact flowers (selfing test), open pollination in emasculated flowers and open pollination in intact flowers; in 1986 artificial cross-pollination in second and third day flowers and artificial self-pollination in second and third day flowers as well as the same experiments as in 1984. The whole plant with flowers for artificial pollination, apomixis test or selfing test was bagged with a thin cloth before the first flower opened. The anthers in the flowers for artifi-

---

[1] Authot for correspondence and reprints.

cial cross-pollination and the other emasculated flowers were cut off after the flowers opened and before the anthers dehisced, because forced emasculation before the flowers opened would fatally damage them. In the experiment of open pollination in emasculated flowers, all flowers on a plant were used for the same experiment to test the degree of cross-pollination. Capsules were collected when they began to open, and seeds in them were counted.

The pollen/ovule ratio was calculated from 18 flowers. To estimate the total number of pollen grains in a flower, all grains in the anthers of one outer and one inner stamen were counted, and the value was multiplied by three. The ovules were counted under direct observation.

UV absorption in the overground part of the plant including the flowers was investigated with the same methods use by Takahashi (1984, 1987).

The voucher specimens of plants and of Hymenoptera are preserved in the Herbarium of Gifu University and those of Diptera are in the National Institute of Agricultural Sciences.

Results

Phenology

The aerial stems in most plants began to appear during late May and early June in both 1984 and 1986. The first flowers opened on the 10th and the 12th of July and the last ones on the 7th and the 11th of August in 1984 and 1986, respectively. We could not detect any kinds of scent from the flowers. The fruits matured and opened from late September to late October in both 1984 and 1986.

Blooming sequence

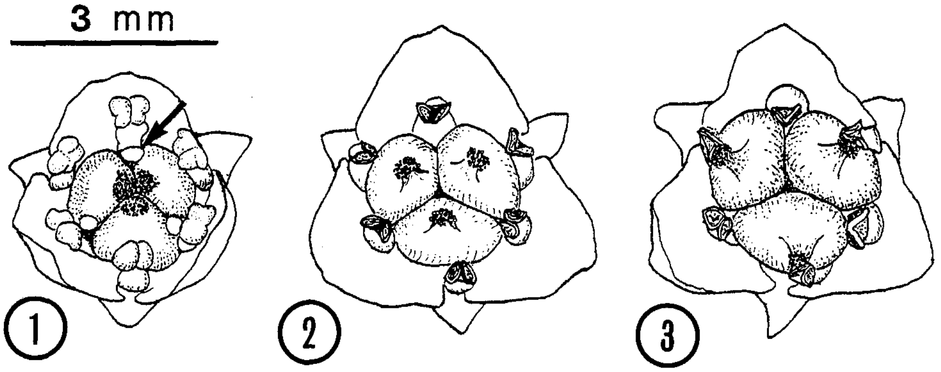
The flowers of *Petrosavia sakuraii* do not appear to open at a definite time of day; some flowers begin to open in the daytime and the others at night. The anthers of three outer stamens dehisce about 24 hours after the flowers open, and those of the three inner ones usually begin to dehisce further about 24 hours later. The stigmata already appear receptive when the flowers open. The three styles, which are the upper parts of the nearly free carpels, extend upwardly, and their stigmata are close to one another during the first and second days (Fig. 1). The ovaries grow obviously in the inner sides after the second day. The three styles consequently gradually separate from one another (Fig. 2), and finally the stigmata on them come in contact with the anthers of outer stamens on the fifth day of anthesis (Fig. 3). Many pollen grains remain in most anthers of the flowers of five days or more. The perianth and the filaments remain permanently.

Insect visitors and their behavior

Insects foraging on the flowers of *Petrosavia sakuraii* are shown in Table 1. Ants of *Paratrechina flavipes* were the most abundant on the flowers. They climb the stem to the flowers and put their head into the flower to suck nectar secreted between the carpels and the inner filaments. They lift their head and move on the flower to forage for nectar on the next nectary. Ants frequently

Table 1. Insects foraging on the flowers of *Petrosavia sakuraii*.

- Hymenoptera
  - Braconidae
    - 1 species
  - Formicidae
    - *Paratrechina flavipes*
    - *Camponotus obscuripes*
    - *C. tokioensis*
  - Halictidae
    - *Lasioglossum*
      - 3 species
  - Ichneumoidae
    - 2 species
- Diptera
  - Sarcophagidae
    - *Parasarcophaga albiceps* (Meigen) ?
  - Syrphidae
    - *Baccha maculata* Walker
    - *Eumerus iaponicus* Matsumura



Figs. 1-3. Top views of the first day (Fig. 1), the third day (Fig. 2) and the fifth day (Fig. 3) flowers of *Petrosavia sakuraii*. The arrow indicates a drop of nectar.

touch the anthers and stigmata during this behavior. *Paratrechina flavipes* has body hairs, and some pollen grains were observed on them under binocular. The ants do not visit flowers of 5 days or more in which nectar apparently is not secreted. They move from flower to flower on a plant, but it is uncertain whether they often visit another plant immediately after visiting one plant. *P. flavipes* foraged on the flowers also at night. A few ants of *Camponotus obscuripes* and *C. tokioensis* sucked nectar in the flowers in the manner similar to that of *Paratrechina flavipes.*

A few small bees of *Lasioglossum* (Halictidae), Braconidae and Ichneumoidae foraged for nectar on the flowers ; they touched the anthers and the filaments, and could be pollinators of *Petrosavia sakuraii*. They visited some other plants after foraging on a plant. A few kinds of Diptera also suck nectar and touch the anthers and the stigmata.

Breeding experiments

Results of the experiments in 1984 and 1986 are shown in Tables 2 and 3. The carpels of *Petrosavia sakuraii* usually remain on the plants even though seeds are not produced in them probably because of non-pollination. Therefore, we did not regard the ovary containing no seeds as a fruit.

Although almost all flowers under study produced fruits except for the bagged flowers without stamens, the seed number in the fruits varied. The average seed numbers in open-pollinated flowers with stamens were about 98 and 139 in 1984 and 1986, respectively, which were much higher than in any other experiments. Seeds produced in the flowers emasculated and open-pollinated were rather few averaging (about 13 and 28).

Pollen/ovule ratio

The mean number of pollen grains was 7844 (SD=776) and that of the ovules was 147 (SD=28). The mean pollen/ovule ratio was 54.6 (SD=7.4).

UV absorption by plant

The overground parts of the plant including the flowers absorb much UV light throughout (Figs. 4 and 5).

Discussion

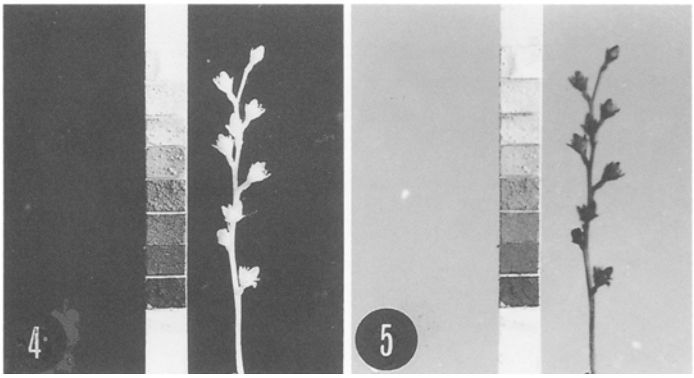
Petrosavia sakuraii bears fruits containing many seeds in almost all the flowers. The self-compatible plants seem to produce many seeds through autonomous self-pollination in spite of dichogamy. It is remarkable that the semicarpous (nearly apocarpous) pistil, which is usually considered to be in primitive condition, is adaptive to this pollination system. Some pollinators, however, are needed for more seed production. The open-pollinated flowers produced the most seeds in the experiments. Nevertheless, the pollinators do not seem to very effectively cross-pollinate them. The emasculated and open-pollinated flowers, whose plants had no flowers with

Table 2. Results of pollination experiments in *Petrosavia sakuraii* in 1984.

	Number of samples	Number of flowers fruited	%	Number of seeds in a fruit (mean±SD)
Cross-pollination in first day flower	38	36	94.7	20.5±16.3
Bagging emasculated flower	36	0	0	—
Bagging untreated flower	50	50	100	59.5±32.7
Open pollination in emasculated flower	28	28	100	13.0± 6.5
Open pollination in untreated flower	50	49	98	98.1±31.7

Table 3. Results of breeding experiments in *Petrosavia sakuraii* in 1986.

	Number of samples	Number of flowers fruited	%	Number of seeds in a fruit (mean±SD)
Cross-pollination in first day flower	34	33	97.1	61.6±28.8
Cross-pollination in second day flower	30	30	100	52.1±34.5
Cross-pollination in third day flower	29	29	100	42.1±33.9
Self-pollination in second day flower	27	27	100	54.7±32.2
Self-pollination in third day flower	23	23	100	45.6±22.5
Bagging emasculated flower	34	0	0	—
Bagging untreated flower	40	40	100	75.8±26.3
Open pollination in emasculated flower	34	34	100	28.0±11.5
Open pollination in untreated flower	40	40	100	138.5±41.6



Figs. 4-5. Photographs of *Petrosavia sakuraii* under visible light (Fig. 4) and UV light (Fig. 5).

anthers, produced only a small number of seeds. This suggests that the self-pollination is mediated also by the pollinators. The P/O of *P. sakuraii* (54.6) also suggests that the seed production depends mostly on self-pollination; it is considerably lower than the mean value (168.5) in the facultatively autogamous species examined by Cruden (1977), and is near their minimum.

The pollination syndrome of saprophytic plants should be affected by resource limitations. Obligate autogamy must be the most economical sexual reproduction except cleistogamy since it will not require much resource allocation to pollen and attractants such as perianth and nectar. *Petrosavia sakuraii* seems to have a reasonable reproductive system under severely limited resources. The relatively large pistil containing many ovules would suggest that the plant has a greater relative investment in the female structure such as is observed shown in some plant species with high selfing rates (Schoen 1982; Lovett Doust and Cavers 1982; Cruden and Lyon 1985; Charnov 1987; Inoue 1990). Nonetheless, the mature seeds are quite small (Makino 1903; Watanabe 1944), and probably germinate with the help of fungi, in terms of the limited resources. *P. sakuraii*, however, has not forsaken efforts at cross-pollinating, though the outcrossing rate is small. The flower is protogynous even if it is incomplete. It secretes nectar for pollinators, and the apparently small amount of nectar seems enough for the small pollinators. The small bees of *Lasioglossum* visit the flowers for nectar (and pollen) and their long distant flights in which they transport much pollen on their body should be significant for their pollen dispersal, though their visitation frequency is low. The quite small, plain and odorless flowers of *Petrosavia sakuraii* almost completely absorbing UV light may optically attract insects with a rather great effect due to the sharp contrast of their whitish color with the dark or brown background of the forest floor.

The bees of Braconidae and Ichneumonidae and some kinds of flies also can be pollinators of *Petrosavia sakuraii*. The ants of *Paratrechina flavipes*, which are the most abundant visitors to the flowers, as well as to *Camponotus obscuripes* and *C. tokioensis*, apparently contribute to their self-pollination. However, Beattie *et al.* (1984, 1985) and Hull and Beattie (1988) showed that the surface secretions of some ants seriously reduced pollen viability in some plant species, and ant pollination may not necessarily result in seed production. The careful investigations indicated by Peakall *et al.* (1991) are required to ascertain whether the ants are efficient pollinators or not.

Protogyny in *Petrosavia sakuraii* is noteworthy also in a phylogenetic context. Willemstein (1987) insisted that protogyny was found mainly in canthalophilous plants as well as anemophilous plants in which protogyny was more common (see also Lloyd and Webb 1986). Canthalophily is common in primitive angiosperms (Faegri and van der Pijl 1971; Bernhardt and Thien 1987), although Thien (1974) and Willemstein (1987) considered that the *Magnolia* was specialized for beetle pollination. Bernhardt and Thien (1987) found that the protogynous condition was dominant in relict angiosperms regardless of their primary pollinators. It is unclear whether the monocotyledonous *Petrosavia sakuraii*, which is not pollinated by beetles, derives its protogyny from the early angiosperms, but it is remarkable that such plant which appears to have been little specialized in the floral morphology even under saprophytic condition and which has a nearly apocarpous pistil whose flower is generally regarded as a primitive one is protogynous.

We thank Dr. N. Fukuhara of the National Institute of Agricultural Science for the identification of Diptera, Professor K. Yamauchi of Gifu University for the identification of Hymenoptera, and Professor S. Kawano of Kyoto University for his helpful suggestions.

References

Akazawa, T. and Tamura, M. 1924. *Protolirion sakuraii* occurring in Kyoto. Acta Phytotax. Geobot. **15**: 138 (in Japanese).

Beaman, R.S., Dicker, P.J. and Beaman, J.H. 1988. Pollination of *Rafflesia* (Rafflesiaceae). Amer. J. Bot. **75**: 1148-1162.

Beattie, A.J., Turnbull, C., Knox, R.B. and Williams, E.G. 1984. Ant inhibition of pollen function: a possible reason why ant pollination is rare. Amer. J. Bot. **71**: 421-426.

Beattie, A., Turnbull, C., Hough, T., Jobson, S. and Knox, R.B. 1985. The vulnerability of pollen and fungal spores to ant secretions: evidence and some evolutionary implications. Amer. J. Bot. **72**: 606-614.

Bernhardt, P. and Thien, L.B. 1987. Self-isolation and insect pollination in the primitive angiosperms: new evaluations of older hypotheses. Pl. Syst. Evol. **156**: 159-176.

Charnov, E.L. 1987. On sex allocation and selfing in higher plants. Evol. Ecol. **1**: 30-36.

Chen, S. 1980. *Petrosavia. In* Flora Reipublicae Popularis Sinicae, vol. 14, pp. 12-13. Science Press, Peking (in Chinese).

Cronquist, A. 1981. An Integrated System of Classification of Flowering Plants. Columbia University Press, New York.

Cruden, R.W. 1977. Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants. Evolution **31**: 32-46.

Cruden, R.W. and Lyon, D.L. 1985. Patterns of biomass allocation to male and female functions in plants with different mating systems. Oecologia **66**: 299-306.

Dahlgren, R.M.T., Clifford, H.T. and Yeo, P.F. 1985. The Families of the Monocotyledons. Springer-Verlag, Berlin.

Faegri, K. and van der Pijl, L. 1971. The principles of Pollination Ecology. 2ed. Pergamon Press, Oxford.

Hull. D.A. and Beattie, A.J. 1988. Adverse effects on pollen exposed to *Atta texana* and other North American ants: implications for ant pollination. Oecologia **75**: 153-155.

Hutchinson, J. 1973. The Families of Flowering Plants, 3 ed. Oxford University Press, London.

Inoue, K. 1990. Dichogamy, sex allocation, and mating system of *Campanula microdonta* and *C. punctata*. Plant Species Biol. **5**: 197-203.

Krause, K. 1930. Liliaceae. In A. Engler and K. Plantle, ed., Die Natürlichen Pflanzenfamilien, 2ed. vol. 15a, pp. 227-386, Engelmann, Leipzig.

Lovett Doust J. and Cavers, P.B. 1982. Biomass allocation in hermaphroditic flowers. Can. J. Bot. **60**: 2530-2534.

Lloyd, D.G. and Webb, C.J. 1986. The avoidance of interference between the presentation of pollen and stigmas in angiosperms I. Dichogamy. New Zeal. J. Bot. **24**: 135-162.

Makino, T. 1903. Observations on the flora of Japan. Bot. Mag. Tokyo **17**: 144-152.

Melchior, H. 1964. Liliiflorae, *In* H. Melchior, ed., A. Engler's Syllabus der Pflanzenfamilien vol. 2., pp. 513-543. Gebrüder Borntraeger, Berlin.

Mizuno, M., Tanaka, T., Hukuhara, H. and Suzuki, T. 1974. On the habitat of *Protolirion sakuraii* Dandy. J. Geobot. **21**: 70-80 (in Japanease with English summary).

Ohba, H. 1984. A review of *Petrosavia* (Liliaceae), with special reference to the floral features. Journ. Jap. Bot. **59**: 106-110 (in Japanese with English summary).

Peakall, R., Handel, S.N. and Beattie, A.J. 1991. The evidence for, and importance of, ant pollination. *In* C.R. Huxley and D.F. Cutler, ed., Ant-Plant Interactions, pp. 421-429. Oxford University Press, Oxford.

Schoen, D. 1982. Male reproductive effort and breeding system in an hermaphrodite plant. Oecologia **53**: 255-257.

Takahashi, H. 1984. The floral biology of *Tricyrtis latifolia* Maxim. (Liliaceae). Bot. Mag. Tokyo **97**: 207-217.

Takahashi, H. 1987. A comparative floral and pollination biology of *Tricyrtis flava* Maxim., *T. nana* Yatabe and *T. ohsumiensis* Masamune (Liliaceae). Bot. Mag. Tokyo **100**: 185-203.

Thien, L.B. 1974. Floral biology of *Magnolia*. Amer. J. Bot. **61**: 1037-1045.

Watanabe, K. 1944. Morphologisch-biologische Studien über *Miyoshia Sakuraii* Makino. Journ. Jap. Bot. **20**: 85-93 (In Japanese with Germany summary).

Willemstein, S.C. 1987. An Evolutionary Basis for Pollination Ecology. Leiden University Press, Leiden.

(*Received October 7, 1992: Accepted April 8, 1993*)