Doctoral Thesis (Abridged)

博士論文（要約）

Diversification of the sexual systems of *Psychotria* species on the islands of East Asia

（東アジアの島嶼におけるボチョウジ属植物の性表現の多様化）

The University of Tokyo

東京大学

Kenta Watanabe

渡邊　謙太

Summary

Chapter 1: General introduction

Oceanic islands, which have never been connected to any continental landmass, generally have flora/fauna and ecosystems different from the continental islands/fragments and continents. For plant breeding systems, many major oceanic islands have a high proportion of dioecism, although many researchers pointed that the self-compatible plants, rather than the self-incompatible ones, would be favored in their establishment after long-distance dispersal. Lately, researchers have noticed that this high proportion of dioecism on the oceanic islands is attributed to not only the colonization of dioecious ancestors, but also the autochthonous evolution of dioecism and adaptive radiation of the dioecious species, and this phenomenon is still regarded as one of the major issues in the evolutionary biology. Meanwhile, distyly is thought to be rare on the oceanic islands, although this breeding system is a highly efficient outbreeding system. However, only a handful of studies have investigated the occurrence and biology of distyly on the oceanic islands.

The genus *Psychotria* (Rubiaceae) is the third largest genus in flowering plants; it comprises more than 1800 species, including the largest number of distylous species, and occurs widely in the tropical and subtropical islands and continents of the world. However, almost no reproductive study has been performed on any island in the world.

The subtropical islands of Japan and Taiwan are the northern limit of the genus distribution in the western Pacific, and six *Psychotria* species occur in the oceanic Bonin (Ogasawara) Islands, Lanyu and Ludao Islands, and the continental Ryukyu Islands. Although the breeding systems of these species have never been documented, they seemed to be distyly or its derivative breeding systems from my preliminary observation. These species could offer an excellent opportunity to study the patterns of occurrence, maintenance, and breakdown of distylous breeding systems both on the oceanic and continental island groups. If we consider the fact that distyly is rare on the oceanic islands, the breeding systems of two species on the remote oceanic Bonin Islands could be changed from distylous to non-distylous, while three species on the continental islands maintain distylous breeding systems. Meanwhile, *P. cephalophora* on oceanic Lanyu island could maintain distyly because Lanyu island is geographically very close to Taiwan with rich biota.

The aims of the present study were to understand the reproductive nature of these *Psychotria* species on the oceanic and continental islands of Japan and Taiwan, and to review the occurrence, maintenance, and evolutionary modification of heterostyly on the oceanic islands based on the studies conducted worldwide. For this purpose, first, I investigated the breeding systems and pollination of four *Psychotria* tree species (*P. homalosperma*, *P. cephalophora*, *P. rubra*, and *P. manillensis*) in Japan and Taiwan (Chapter

2–6). Second, I presented an overview of the studies on heterostyly on oceanic islands, and discussed the occurrence and rarity of the heterostyly on islands. Finally, I discussed the maintenance and evolutionary modification of the distylous breeding system on islands (Chapter 7).

Chapter 2: Distyly and incompatibility of *Psychotria homalosperma*, an endemic plant in the oceanic Bonin (Ogasawara) Islands

Psychotria homalosperma in the Bonin Islands was found to be morphologically and functionally distylous with self- and intramorphic incompatibilities. This is the first report of a distylous species from the Bonin Islands together with the liana species *P. boninensis*.

Chapter 3: Recent pollinator shift promotes unidirectional pollen flow in distylous tree *Psychotria homalosperma* endemic to the oceanic Bonin Islands

I found that *P. homalosperma* was originally adapted to moth pollinators with floral traits, including the chemical composition of floral scent. However, the introduced honeybees visited flowers most frequently, whereas the moths visited only several times in more than 100 hours. This is probably because of the disruption of pollinator fauna caused by the anthropogenic impacts on the island. Honeybees could carry pollen grains unidirectionally

from the short- to the long-styled morph, resulting in higher fruit set in the long-styled morph. This result inferred that the maintenance of heterostyly on oceanic island is difficult partly because the insect fauna is vulnerable in such areas.

Chapter 4: Distyly and floral morphology of *Psychotria cephalophora* on the oceanic Lanyu (Orchid) Island

Psychotria cephalophora on Lanyu Island was found to be morphologically and functionally distylous, with self- and intramorphic incompatibilities. I could not perform pollinator observations for this species; many fruit and juveniles in the field indicated its successful reproduction.

Chapter 5: Dioecy derived from distyly in *Psychotria rubra* in the Ryukyu Islands

Psychotria rubra in the continental Ryukyu Islands was found to be functionally dioecious with male and female plants. Because the flowers of *P. rubra* still had distylous morphological traits, the dioecism of *P. rubra* was thought to have probably evolved from distyly. The evolution of dioecy from distyly has not been frequently reported, and plant-pollinator interactions (such as pollinator shift from long- to short proboscis or tongue) can be related to this evolution.

Chapter 6: Polygamous breeding system identified in *Psychotria manillensis* in the Ryukyu Islands

The breeding system of *Psychotria manillensis* in the continental Ryukyu Islands was found to be polygamous mainly with monoecious, female, and some male and hermaphroditic plants. To my knowledge, this is the first report of monoecism not only in the genus *Psychotria*, but also in all heterostylous species groups. It is very difficult to explain the evolutionary pathway from distyly to monoecy only by plant-pollinator interactions. *P. manillensis* is octoploid (2n=84). If we consider that its closely related ally, *P. rubra*, is tetraploid (2n=42), the evolution of monoecism in *P. manillensis* can be related to polyploidization.

Chapter 7: General discussion

Sexual systems and reproduction of Psychotria *in East Asia*

The results of the current field investigations were contradictory to my hypothesis: two species on the oceanic islands showed distyly, while the other two species on the continental Islands were dioecious and polygamous with monoecism. Together with our separate studies on the breeding systems of the two liana species (*P. boninensis* and *P. serpens* in the Bonin and the Ryukyu Islands, respectively), the breeding systems of all six *Psychotria* species in

Japan and Taiwan were revealed.

Psychotria homalosperma and *P. boninensis* are the first examples of distyly on the oceanic Bonin Islands. In *P. homalosperma*, the observed phenomena of pollen limitation and unidirectional pollen flow caused by the introduced honeybees might partly explain why this species is now facing extinction. *Psychotria rubra* is the second example of dioecy in the genus, following the Hawaiian species. Monoecism in *P. manillensis* is a unique example, because there has been no report of monoecism in the distylous species groups. These evolutionary modifications of the breeding systems are probably caused both by plant-pollinator interactions and genetic modifications such as polyploidization.

Occurrence of heterostyly on oceanic islands in the world

Only 11 examples of distyly on oceanic islands have been reported till date.However, the present study was an opposite case. However, if we pay more effort to examine the plant breeding systems on oceanic islands, we might be able to find more examples since three new examples were found in this study.

There are three possible difficulties for heterostylous species to colonize, evolve and maintain its sexual system on oceanic islands:1) plants with sexual dimorphism are generally difficult to colonize the remote oceanic islands, 2) autochthonous evolution of distyly on island is extremely difficult compared to the evolution of dioecism, and 3) changes of plant-pollinator

interactions can cause extinction or evolutionary modification of distyly to the other breeding systems on islands.

Unidirectional pollen flow by the introduced honeybees found in *P. homalosperma* (Chapter 3) suggested the possibility that the unstable pollinator fauna on the oceanic islands could cause the breakdown of heterostyly. Meanwhile, the oceanic islands with different areas, altitudes, and distances from the continent have different situations with the maintenance of distyly. For example, the oceanic Lanyu Island, where *P. cephalophora* grows, is close to Taiwan, and its ecosystem is more like that of continental islands (Chapter 4). Meanwhile, heterostyly could breakdown even on the continental islands, probably because of several reasons such as the pollinator shift, polyploidization, and small populations on the islands (Chapter 5 and 6). To understand the island-heterostyly relationship, we need to study many cases in different island environments and integrate them.

Future directions

To understand the general patterns of the maintenance and evolutionary modifications of heterostyly on islands, focusing on the particular species group with many species on different types of islands, is one of the most effective strategies. The genus *Psychotria* can probably be a good system for this purpose, when we combine its phylogeny, history with the ecosystems of the islands. We can also deepen our understanding of the diversification of

the breeding systems starting from distyly in the flowering plants using this system. Furthermore, this study can contribute to understand how this large genus diversified all over the world tropics.

Acknowledgements

I am very grateful to Dr. Motomi Ito of the General Systems Studies, Graduate School of Arts and Sciences, the University of Tokyo for advising this doctoral thesis and gave me this special opportunity to complete it.

I would like to express my deepest appliciation to Dr. Takashi Sugawara of Makino Herbarium, Tokyo Metropolitan University, for advising this doctoral works, and for his incredible kindness and warm encouragement on my study. This work would not be here without him. He taught me how to observe the nature and to write scientific papers very patiently. His passion and knowledge always inspired me; I was so lucky that I could explore the wonderful nature of the flowering plants with him for more than a decade.

I would like to thank my committee members, Dr. Nobumitsu Kawakubo of Graduate School of Applied Biological Sciences; Dr. Masakazu Shimada and Dr. Takehito Yoshida of the General Systems Studies, Graduate School of Arts and Sciences, the University of Tokyo for their truly important comments on my Doctoral thesis.

I would like to thank my mentors and collaborators, I couldn’t finish this work without them. Drs. Hidetoshi Kato, Michiyo Wakabayashi and Noriyuki Murakami supervised my works when I was at Makino Herbarium.Drs. Akira Shimizu, Koh Nakamura helped me in the field and gave me a lot of useful comments on my manuscripts, and their comments

improved them very much. Dr. Eiske Kuraya helped chemical analysis and let me continue my study in Okinawa College. In Taiwan, Drs. TY Aleck Yang, Ching I-Peng, Chien-Fan Chen, Tai-liang Huang, Wei-Yu Wang helped me in the field and had important discussion.

In the Bonin (Ogasawara) Islands, Drs. Yuka Kato (Chiba), Taku Fujita showed me the nature and the fields of Ogasawara Islands. Drs. Naoki Kachi, Kenji Hata, Katsuyouki Wada, Miho Wada, Hajime Suzuki, Naoko Suzuki, Kazuo Horikoshi, Makoto Inaba, Haruki Karube, Keiichi Matsumoto gave me incredible advices for the study. I had nice discussion with Drs. Norimasa Sugita, Shizuka Tsuneki, Kyoko Sugai, Kenichi Yokoi. Mrs. Keiko Saito (Miyashita), Kyohei Shoji, Akihiro Nojima and Hoho Yamamoto (Takayama) helped my field works.

In the Ryukyu Islands, Drs. Masatsugu Yokota, Tetsuo Denda, Tokushiro Takaso gave me important advices. Drs. Shunichi Matsumura and Akiyo Naiki taught me plant names and we had important discussion on the evolution and diversity of plants. My field work wouldn’t be successfully done without such knowledge. On Amami Island, Mrs. Nana Goto (Okuyama) and Mitsutake Tabata helped me in the field.

I would like to thank Drs. Satoshi Shinonaga, Nobuo Tsurusaki, Shuhei Niitsu, Utsugi Jinbo, Shuji Okajima for their identification of the flower visitors. I thank Ms. Miwa Kobayakawa for assistance with SEM observations, Drs. Hiroshi X. Chiura, Masako Hara,

Nobuhiro Ogawa, Keiichi Kogure and Yoshiro Watanabe for their help with using FE-SEM at Atmosphere and Ocean Research Institute of the University of Tokyo.

I thank Drs. Donald R. Drake and Akira Iguchi for giving me important comments on my manuscripts and improving English. I appreciate Drs. Emiko Oguri and Takaya Iwasaki giving me precious help in DNA analysis of Japanese *Psychotria*. I thank Drs. Jean-Yves Meyer, David Lorence, Charlotte M. Taylor, John R. Paul, SA. James, Barbara Kennedy for providing us valuable information and/or help for collecting literatures. I thank Drs. Ikumi Dozono, Saeko Nakamura, Yoshirou Tosaka, Sho Tomita (Hoshi), Yuki Sakurazawa, Mihoko Takahashi for their incredible advices in the lab at Tokyo Metropolitan University.

I thank people at my work place (Okinawa College, National Institute of Technology; Okinawa Kosen), I couldn't finish this work without their understanding and helps. Former president Dr. Shigeru Ito gave me a chance to challenge many new studies, current president Dr. Yasunori Ando let me continue this work. Dr. Tomoyasu Yara gave me a chance to continue the work and always encouraged me. I had good discussion with Drs. Hideyuki Yamshiro and Naoko Isomura, which inspired me a lot (although most of our discussion was about marine creatures).

Tokyo prefecture, Ministry of Environment and Forest, Okinawa prefecture, Nago and Naha cities and Nakijin son kindly provided permits for this study. This work was partly

supported by JSPS Kakenhi grant (22923008, 22570096, 23923008, 24923009, 26840130); the Environment Research and Technology Development Fund (4-1402) of the Ministry of the Environment, Japan; the Collaborative Research grant of Tropical Biosphere Research Center, University of the Ryukyu; the special study grant of Okinawa College, National Institute of Technology. Herbarium Pacificum of Bishop Museum (BISH) kindly provided images of the Pacific *Psychotria* specimens. Most of the speciesmens used in this study were deposited at Makino Herbarium (MAK).

I thank my friends of the diving team Snuck Snufkin for helping to keep my interests both in marine creatures and in flowering plants. I had fastidious discussion with my friends when I was in Tokyo Metropolitans University: Ryouichi Oomuta, Akiko Ono and many others. I thank Kenji Morii and Takanobu Okamoto for saving my old research materials from the messy warehouse.

I sincerely dedicate this thesis to Misato Ishikawa (Fujita) and Taku Fujita, who helped my work and always encouraged me. Sadly, Misato passed away in 2011, Taku in 2017. I won’t forget Misato’s words that she would help my field work in Hawaii someday.

I deeply appreciate Chihiro Nishihara (Watanabe) that she has been a good partner both in science and in private. She is a great naturalist as well as an artist; she always

inspires me and supports my works. She drew many beautiful flowers for me, and sometimes helped me making the figures. I thank Masayo Maki (Watanabe), she supported me all through my difficult days. Mahalo, Kaiju, our son, I hope you'll be a true naturalist. We know that you have a special sense of wonder.