Heterostyly on Japanese Islands

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Abstract:

Heterostyly is a genetically controlled floral polymorphism that promote outbreeding. Although many studies on heterostyly have been done in Japan, there have been no comprehensive analysis nor review. Here I present the list of heterostyly in the native flora of Japan through reviewing references. By using this list, I tested the difference in occurrence of heterostylous species among subgroups of islands. Then I also tested the difference in growth forms and a dispersal mode between northern and southern part of Japanese islands. Finally, I briefly reviewed studies on heterostyly in Japan, special emphasis on maintenance and breakdown of heterostyly in the wild population.

After the examination of the literature and screening process, 37 heterostylous species were recognized in Japan (0.74％), which is slightly lower than the world estimate (1%, Renner 2014). There was no significant difference of occurrence among three geographical areas. More woody and zoochory (dispersal by animals) species in the southern islands compared to temperate islands. This is the first report of the species-based estimate of the proportion of heterostyly in Japan.

***Key words*: Japanese Islands, heterostyly, distyly, tristyly,**

I. Introduction

Flower and the plant breeding systems are very diversified, and they provide many interesting topics in the field of ecology and evolutionary biology. Heterostyly is one of the most complex breeding systems in the flowering plants. Heterostyly is a genetically controlled floral dimorphism (distyly), or trimorphism (tristyly) that promote outbreeding, known from 28 families (reviewed in Vuilleumier 1967; Ganders 1979b; Barrett 1992; Barrett and Shore 2008). Since Charles Darwin published the seminal paper (1862) and the book “The different flowers in the same species” (1877), heterostyly has attracted the attention of researchers in the field of plant ecology and evolutionary biology (Weller 2009; Barrett 2010). Heterostyly is one of the most complex breeding systems in the flowering plants, yet heterostyly evolved at least 23 times independently in the different lineages (Lloyd and Webb 1992, Barrett 2019).

Heterostyly includes distyly and tristyly, of which distyly is more common (Fig. 1). A distylous population is composed of two morphs; one is the short-styled morph (S-morph) with short styles and long anthers, and the other is the long-styled morph (L-morph) with long styles and short anthers. These two morphs usually have reciprocal positions in stigma and anther heights, and usually occur in equal numbers within a population. Distylous species usually possess an incompatibility system that prevents self-fertilization and intramorph-fertilization (heteromorphic incompatibility). This herkogamous floral dimorphism is generally thought to be controlled by “a heterostylous gene” (Barrett and Shore 2008).

It is generally thought that heterostyly is rare or absent on remote oceanic islands (Pailler *et al*. 1998b, Watanabe and Sugawara 2015), despite the fact that outcrossing is advantageous for avoiding inbreeding depression (Barrett 1996). Besides Renner (2014) estimated the heterostyly is 1% of the world flora without actual counting, there has been no appropriate estimate nor comparative study about the occurrence of heterostyly in the certain geographical area.

Japanese Islands is one of the most well surveyed area in the world in terms of flora and fauna. Japanese Islands are composed of ca. 1500 islands, stretching from subtropical to cool/temperate forest (Fig. 2). Over 6000 native flowering plant taxa have been described. In this paper, I divine Japanese Islands into three groups: Japanese archipelago, which includes four major islands (Honshu, Hokkaido, Kyushu and Shikoku) and the small islands nearby; the Ryukyu Islands; Ogasawara Islands.

Here I review the occurrence and nature of heterostyly in the native flora on Japanese Islands: Japanese archipelago and two southern islands (the continental Ryukyu and the oceanic Ogasawara Islands). Firstly, I list the species which are reported as heterostyly, and potentially heterostylous species. Secondary, I test if there is any geographical tendency of occurrence, growth form and taxonomic distribution among “Japanese archipelago”, “Ryukyu Islands”, and “Ogasawara Islands” using the list above. Thirdly, I briefly review the heterostylous species in Sothern islands in Japan. Finally, I suggest the future research possibly done using Japanese native plant species.

II. Heterostylous species in Japan

Methods of screening

To make the list of heterostylous species in Japan, I used the list of heterostylous genera in the world ("the world heterostyly list") based on Naiki (2012). Naiki (2012) listed 199 genera of 28 families which include at least one heterostylous species. Then, I listed all genera in the flora of Japan which appeared in "the world heterostyly list". Then I checked all species in the genera using the primary references ("Flora of Japan" and "World flowers of Japan"), and other references (secondary reference) to check if the species are heterostyly or not. Secondary references are obtained mainly from systematic literature review on heterostyly in Japan by Watanabe (2022), but also all other available sources were used. For the detail of the screening process, please refer to supplementary information and TableS1 and TableS2.

Taxonomic distributions

After the screening process above, 37 species of 16 genera in 7 faimiles are recognized as heterostylous (Table 1). Of them all, only two *Lythrum* species are tristylous, and other 35 species are distylous. Twelve species (in the genera *Damnacanthus, Guettarda, Leptodermis, Mitchella, Ophiorrhiza, Pseudopyxis, Psychotria*) are in Rubiaceae, and 11 species are Primulose (genus *Primula* in the Primulaceae). These two Families contain 23 species, which makes more than half of the whole list (62%).

Geographical distributions

Heterostylous species makes 0.74% of the Japanese flora (37 of 5016 flowering plant species), which is little less than Renner's (2014) estimated (1%). In total of 37 species in Japan: 29 species in the Japanese archipelago (0.61%), 13 species in the Ryukyu Islands (0.85%), and two species (0.52%) in the Ogasawara Islands (Table2). Although, rarity of the heterostyly in the oceanic islands has been reported, there is no significant difference in the frequency of heterostyly in these three island groups (Fisher's exact test followed by Bonferroni adjustment, $P > 0.3$).

Primulose are occurring only in the Japanese archipelago and consist 45% of the species, whereas half of the heterostylous species in the Ryukyu Islands and both two heterostylous species in the Bonin Islands are Rubiaceae (Fig. 3). Six species (*Persicaria chinensis, P. japonica, P. odorata* subsp*. consicua, Scoepfia jasminodora, Damnacnathus indicus, and Psychotria serpens*) are occurring both in the Japanese archipelago and the Ryukyu Islands.

Growth form and dispersal mode in three islands

Heterostylous species found in Japanese islands are mostly herbs, while mostly woody species in the Ryukyu Islands, and both two heterostylous species in the Bonin Islands are wood (One is woody liana (*Psychotria boninensis*) and the other is subcanopy tree (*Psychotria homalosperma*) (Fig. 4). Most heterostylous species in Japanese archipelago are not dispersal by animals, while mostly dispersal by birds in the Ryukyu islands and the Bonin Islands. This tendency is also recognized in world wide (Barrett and Richards 1990). These results may be due to the taxonomic differences; Primulose consists of herbs and mostly not dispersal by animals, whereas Rubiaceae mostly consists of woody species and dispersal by birds. But we can also reflect the tendency of the geographical distributions of heterostylous species in the world.

III. Recent progress in the studies of heterostyly in southern Japanese islands

Since the study of distyly in *Pseudopyxis* (Puff 1989), more than 80 research papers on heterostyly in native plants in Japan have been published through the year 2020 (Watanabe 2022). The number of publications on heterostyly has been increasing in recent years, from 2 in the decade of 1980 to 28 and 30 in the decade of 2000 and 2010, respectively (Watanabe 2022). In terms of content, in the early years, there were some basic studies on heterostylous plants in the Japanese archipelago, but later applied and conservation biological studies targeting some model plants such as *Primula*, *Nymphoides* increased. More recently, there has been an increase in the number of natural history studies on the discovery and ecology of heterostyly in the plants of the Ogasawara Islands and the Ryukyu Islands. Here, I review the recent remarkable studies heterostyly in the southern Japanese Islands, especially in terms of the maintenance and breakdown of heterostyly.

Breakdown of heterostyly accompanied with polyploidization

In studies of *Primula sieboldii*, distyly collapses into a monomorphy when there are few pollinators (Washitani et al. 1994, Washitani 1996). This is because autochthonous monomorphy has an advantage in reproduction in the absence of a pollinator.

On the other hand, some studies with Japanese heterostylous species showed that heterostyly breakdown via completely different pathway. In *Damnacanthus* (Rubiaceae), it has been shown that distyly is maintained in the diploid (2n=22), but becomes monomorphic in the tetraploid (2n=44, Naiki & Nagamasu 2004). Similarly, in *Ophiorrhiza japonica*, distyly is maintained in the diploid (2n=22), but becomes monomorphic in the tetraploid (2n=44, Nakamura et al. 2003, 2007).

Although, it is shown that polyploidization is not necessary caused breakdown of heterostyly from meta-analysis (Naiki 2012), link between polyploidization and breakdown of heterostyly provides a them for further exploration.

Evolution of dioecy from distyly

Besides many examples of evolutionary breakdown of heterostyly into monomorphy, there are about ten cases that are thought to have evolved into dioecy from the world at the time of 2015 (Watanabe & Sugawara 2015). Among them, *Mussaenda parviforia* (Naiki and Kato 1999) and *Psychotria asiatica* (synonym to *P. rubra*, Watanabe et al. 2014a) were studied in the Ryukyu Islands. Both species retain the complementary positioning of organs as in distyly, but S-styled plants lost female function and L-styled plants lost male function, and thus considered to be functionally dioecious. The evolutionary pathway and factors are still unclear. In *Pschotria* in the Hawaiian Islands, it is assumed that distyly evolved once to monomorphy and then again to dioecy (Watanabe 2021). It is possible that the same thing happened in *M. parviforia* and *P. asiatica. P. manillensis*, which is closely related to *P. asiatica*, is known to have evolved into Polygamomonoecy, which is unusual for a distyly derived species (Watanabe et al. 2021).

IV. Future Prospects

1. Hidden heterostyly and breakdown of heterostyly

The present list is only based on the literature data that exist to date, so it is possible that some heterostylous plants not in the list would be discovered. In fact, *Jasminum superfluum* was discovered as distyly during the present literature review (Watanabe 2022). The most likely candidates would be those plants in the same genus in the list (Table 1 and S2). They need to be examined in detail in the future. There may also be species that were once heterostyly but have now evolved into monomorphy or dioecy. Furthermore, some of the species and genera on this list may have different sexual expressions within a species in different locations. It would be worthwhile to expand the scope of the survey to the surrounding areas based on this list.

2. Maintenance of heterostyly and its fitness

As noted by Barrett (2019), there are still many unknowns as to how Heterostyly is maintained. It would be meaningful to study the maintenance and fitness of heterostyly using species in Japan. In particular, *P. homalosperma*, *Guettarda speciosa* and *Jasminum superfluum*, which are thought to be “moth pollination” (and have long flower tubes (>20mm)), have an inaccurate reciprocal position of the floral organs, and it would be important to investigate the adaptive significance of this misalignment. In Japan, where there are many islands, it would be advantageous to conduct comparative studies on how flowers are adapted to different pollinators in each island's fauna.

3. Comparative study with other area

This time, we succeeded in listing heterostyly plants in the Japanese flora, with a frequency of 0.7%, little less than previously thought for the world flora. On the other hand, we also compared the number of species in the oceanic Ogasawara Islands, and the continental Ryukyu Islands, however, it is undeniable that the number of samples was still small. The trend of heterotypic flowering columnar plants in the temperate and subtropical regions, depending on latitude, seemed to be in line with the global trend. However, it is expected that comparisons with other regions will confirm the generality of this point as well.

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Table 1. Heterostylous species on Japanese Islands

Family	Genus	N_sp[a]	Species	Japan	Ryukyu	Ogasawara	type	WFJ[c]
Lythraceae								
	Lythrum	2/2	*L. anceps*	Ind			tristlyly	y
			L. salicaria	Ind			tristlyly	y
	Pemphis	1/1	*P. acidula*		Ind		distyly	n
Menyanthaceae								
	Menyanthes	1/1	*M. trifoliata*	Ind			distyly	y
	Nephrophyllidi	1/1	*N. crista-galli* subsp. *japonicum*	Ind			distyly	y
	Nymphoides	2/3	*N. peltata*	Ind			distyly	n
			N. indica	Ind			distyly	y
Oleaceae								
	Forsythia	2/2	*F. japonica*	**End**			distyly	n
			F. togashii	**End**			distyly	n
	Jasminum	1/1	*J. superfluum*		**End**		distyly	n
Polygonaceae								
	Persicaria	3/30+	*P. chinensis*	Ind	Ind		distyly	y
			P. japonica	Ind	Ind		distyly	y
			P. odorata subsp. *consicua*	Ind	Ind		distyly	y
Primulaceae								
	Primula	11/14	*P. cuneifolia*	Ind			distyly	y
			P. nipponica	Ind			distyly	y
			P. modesta	Ind			distyly	y
			P. sorachiana	Ind			distyly	y
			P. seiboldii	Ind			distyly	y
			P. jesoana	Ind			distyly	y
			P. kisoana	Ind			distyly	y
			P. reinii	**End**			distyly	y
			P. tosaensis	**End**			distyly	y
			P. hidakaensis	**End**			distyly	y
			P. takedana	**End**			distyly	y
Rubiaceae								
	Damnacanthus	3/5	*D. biflorus*		**End**		distyly	y
			D. indicus	Ind	Ind		distyly	y
			D. okinawensis		**End**		distyly	y
	Guettarda	1/1	*G. speciosa*		Ind		distyly	n
	Leptodermis	1/1	*L. pulchella*	**End**			distyly	y
	Mitchella	1/1	*M. undulata*	Ind			distyly	y
	Ophiorrhiza	2/5	*O. japonica*		Ind		distyly	y
			O. kuroiwae		Ind		distyly	n
	Pseudopyxis	1/2	*P. depressa*	**End**			distyly	n
	Psychotria	3/5	*P. serpens*	Ind	Ind		distyly	y
			P. boninensis			**End**	distyly	y
			P. homalosperma			**End**	distyly	n
Schoepfiaceae								
	Schoepfia	1/1	*S. jasminodora*	Ind	Ind		distyly	n

a, number of (heterostylous species/all species in the genus) in Japan.
b, End, endmic to the area; Ind, Indigenous to the area.
c, presence of discription on heterostyly in Wild Flowers of Japan

Table 1 (continue). Heterostylous species on Japanese Islands

Growth form	Flower color	Flower tube[d] (mm)	Pollinator	Seed dispersal	Fruit type	Fruit color	Reference for studies in Japan
herb	pink			non zoochory	capsule		
herb	pink	6-8		non zoochory	capsule		
shrub	white	3	bees, butterfly	non zoochory	capsule		Sugawara et al. 2018
herb	white			non zoochory	capsule		
herb	white			non zoochory	capsule		
herb	yellow		butterfly, fly, wasp	non zoochory	capsule		Marui & Washitani 1993, Nishihiro et al. 2001, 2009
herb	white (yellow center)		butterfly, fly, bee	non zoochory	capsule		Shibayama & Kadono 2003a, b
tree	yellow	7*		non zoochory	capsule		
tree	yellow			non zoochory	capsule		Takashima & Suehiro 2006
woody liana	white	20-30*	moth, butterfly	bird	freshy fruit	black	Watanabe 2022
herb	white	2.5-4		bird	hy fruit (ach	black	
herb	white	2.5-3		non zoochory	achene		Hirtsuka & Nakao 1996; Nishihiro and Washitani 1998a, b; Ishikawa et al. 2010
herb	pink	2.5-4.7		non zoochory	achene		
herb	pink	7-8		non zoochory	capsule		
herb	white	4		non zoochory	capsule		
herb	pink	6		non zoochory	capsule		Shimono et al. 2007; Kameyama et al. 2015; Moriwaki et al. 2020
herb	pink	6-7		non zoochory	capsule		
herb	pink	10-13	bumble bee	non zoochory	capsule		
herb	pink	12-14		non zoochory	capsule		
herb	pink	10-12		non zoochory	capsule		Arima et al. 2019
herb	pink	8-10		non zoochory	capsule		Rechards 2003
herb	pink	12-15		non zoochory	capsule		Rechards 2003
herb	pink	6-7	hoverfly,	non zoochory	capsule		Rechards 2003; Yamamoto et al. 2018
herb	white	6-8	bee, hoverfly	non zoochory	capsule		Rechards 2003; Yamamoto et al. 2018
shrub	white	8-13		bird	freshy fruit	red	Naiki and Nagamasu 2003, 2004
shrub	white	10		bird	freshy fruit	red	Naiki and Nagamasu 2003, 2004
shrub	white	8-13		bird	freshy fruit	red	Naiki and Nagamasu 2003, 2004
tree	white	20-40*	moth	non zoochory	drupe		Hoshino et al. 2019
shrub	pink	15-22		non zoochory	capsule		Puff 1989b
herb	white	10		bird	freshy fruit	red	Kawano et al. 2005
herb	white	10-15		non zoochory	capsule		Nakamura et al. 2007; Nakagawa and Naiki 2014
herb	white	1.5-2.5		non zoochory	capsule		Flora of Taiwan
herb	white	2-3		non zoochory	capsule		Puff 1989a, Puff 1989b
woody liana	white	2.5-3.5	wasp, butterfly, bee	bird	freshy fruit	white	Sugawara et al. 2013, 2016
woody liana	white	4	(honeybee)	bird	freshy fruit	white	Kondo et al. 2007; Sugawara et al. 2014
tree	white	15-25*	moth, (honeybee)	bird	freshy fruit	black	Watanabe et al. 2014; Watanabe et al. 2018
shrub/tree	white (green at base)	5-8*	moth, bees	bird	freshy fruit	red	Ogiwara 2018

d, From description in "wild flowers of Japan", except for the ones with *

Table 2. Number of heterostylous species in each area.

	Number of flowering plant species	Number of heterostylous species	Occurrence	Area (km^2)
Japanese Arch.	4790	28	0.58%	376,000
Ryukyu Isl.	1532	13	0.85%	2,281
Ogasawara Isl.	382	2	0.52%	104
Total (Japan)	5016	37	0.74%	378,000

There is no significant difference in the occurrence of heterostyly in each area
(Fisher's exact test followed by bonfferoni adjustment).

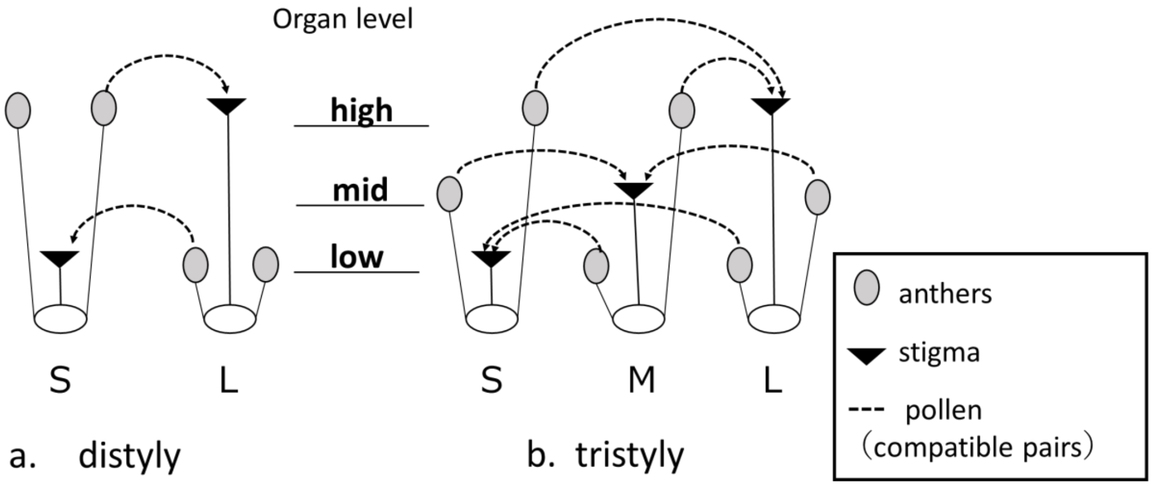

Fig. 1 Morphology of heterostylous flowers, distyly (a) and tristyly (b).
Compatible pairs are shown with dashed line.

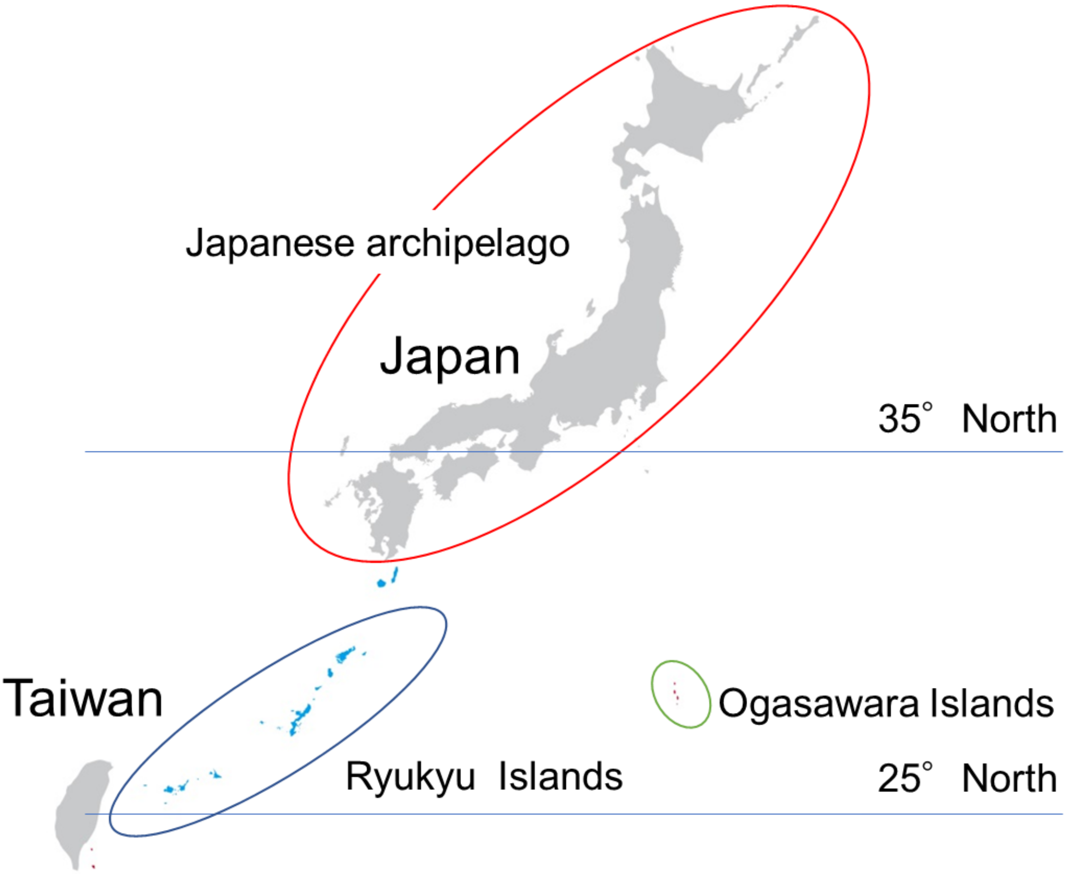

Fig. 2. Japanese Islands: Japanese archipelago, Ryukyu Islands, and Ogasawara Islands

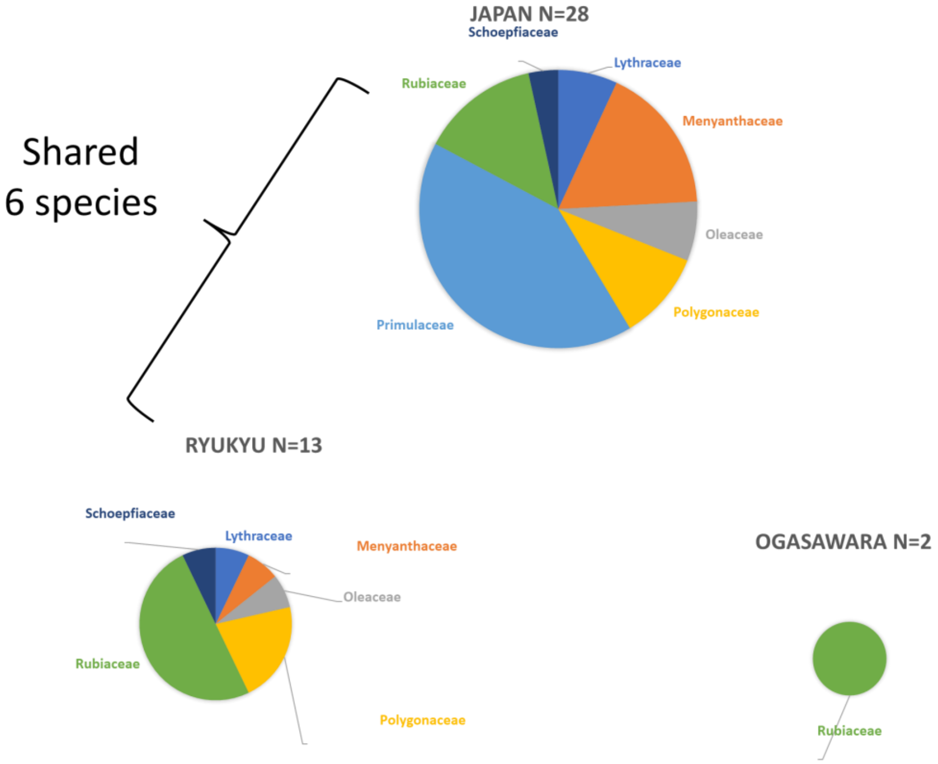

Fig. 3. Geographical and taxonomical distributions of heterostylous species in Japan

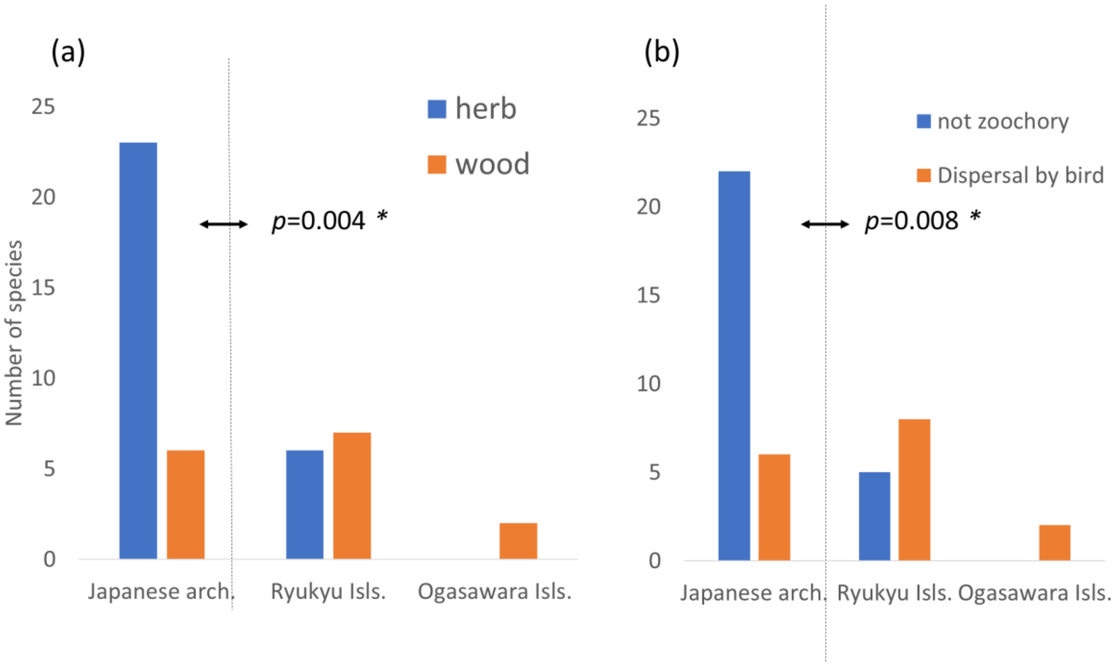

Fig. 4. Growth form (a) and dispersal mode (b) of heterostylous species on Japanese islands.
Significantly more herb species (p=0.004) and more species not zoochory (p=0.008) in Japanese archipelago after Fisher's exact test.