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Dimorphic Stigmas, Pollen Accumulation, and Seed Production in the Gynodioecious Plant *Arenaria merckioides* var. *chokaiensis* (Caryophyllaceae)

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Abstract. In the gynodioecious plant *Arenaria merckioides* var. *chokaiensis*, stigma length is significantly longer in female flowers than in hermaphrodite flowers. To elucidate the functional significance of dimorphic stigmas we determined the number of pollen grains actually deposited on the stigmas and fruit and seed set percentages of hermaphrodite and female plants in a natural population. The number of pollen grains on the stigma was much less in female flowers than in hermaphrodite flowers, so the longer stigmas of the female plants are unlikely to increase the ability to trap pollen grains over the hermaphrodite plants in field. Fruit set percentage and average number of seeds per flower in the female plants were usually larger than those in the hermaphrodite plants. Lower seed set in the hermaphrodite plants is in contrast to the greater amount of pollen deposited on their stigmas. The reason may be due to selfing, because fruit and seed set percentages of the self-pollinated hermaphrodite flowers are usually less than in cross-pollinated flowers.

Key words: *Arenaria merckioides* var. *chokaiensis* - Caryophyllaceae - dimorphic stigmas - gynodioecy - protandry - self-compatibility

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Gynodioecy is characterized by the occurrence of the two sexually different plants within a single species: hermaphrodite and female plants, and has been reported in various species of the angiosperms (Darwin, 1877; Bawa and Beach, 1981; Richards, 1986; Kikuzawa, 1989; Delph, 1996). Although the female plants lack fertile pollen grains and depend on pollen vectors for pollination (Bawa. 1980; Bawa and Beach, 1981), their seed production is usually higher or to equal to seed production in hermaphrodite plants in natural populations, as recorded in various gynodioecious species (Richards, 1986; Sugawara, 1993; Sugawara *et al.*, 1994; Puterbaugh *et al.*, 1997). It has been argued that female plants can allocate more resources to seed production, since female plants, which have no fertile pollen grains and usually smaller flowers than hermaphrodite plants (Delph, 1996), are less costly (Bawa and Beach, 1981; Ashman, 1994). It is also stated that pollen availability limits seed production (Stanton, 1987), so female plants are presumed to have some mechanism for increasing pollen availability (Shykoff, 1992).

In a few gynodioecious species of Caryophyllaceae, it has been reported that

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stigmas in female plants are longer than those in hermaphrodite plants and thus may be superior in their ability to trap pollen grains (Dulberger and Horovitz, 1984). In *Silene acaulis*, however, no available evidence was observed to support that idea (Shykoff, 1992). By contrast, it was reported in *Monarda fistulosa* (Labiatae) that the longer stigmas increased pollen trapping ability (Cruden *et al.*, 1984).

Our previous study (Sugawara and Horii, 1995) showed that *Arenaria merckioides* var. *chokaiensis* (Caryophyllaceae) is gynodioecious, and that stigma length differs between hermaphrodite and female plants. In the present study, we aim to elucidate the functional significance of dimorphic stigmas. For

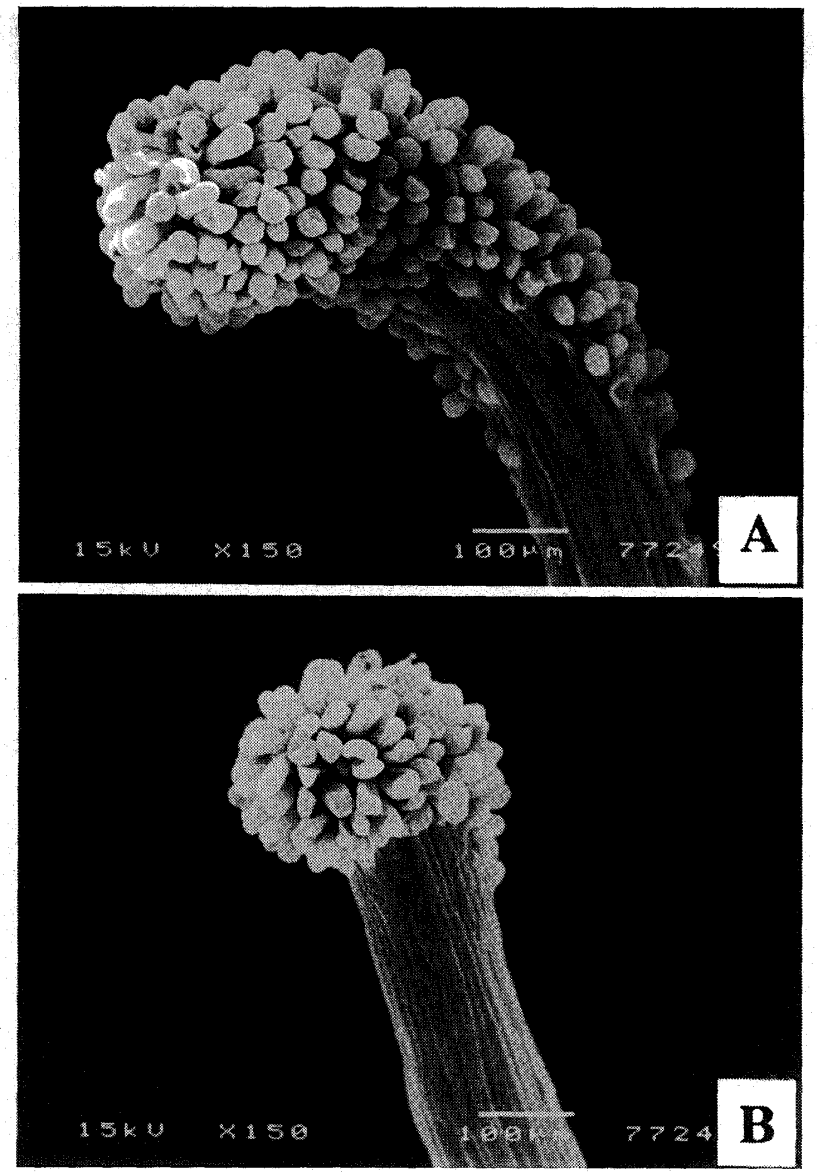

FIG. 1. Scanning electron micrographs showing stigmas of the female (A) and hermaphrodite (B) flowers in *Arenaria merckioides* var. *chokaiensis*.

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TABLE 1. Comparison of pistils between hermaphrodite (H) and female (F) plants in *Arenaria merckioides* var. *chokaiensis*

Sexual form	N	Style length Mean ± SD (mm)	Stigma length Mean ± SD (mm)
H	42	3.42 ± 0.52	0.66 ± 0.35
F	36	3.13 ± 0.43	1.09 ± 0.30

N: Number of flowers examined.

this question, the number of pollen grains actually deposited on the stigmas of female flowers was compared with the number deposited on hermaphrodite flowers. Subsequently, fruit and seed production in the two forms was examined in a natural population and the results were compared with the figures from the pollen deposition study. We also conducted a pollination experiment to confirm the probability of self-compatibility in the hermaphrodite plants.

Materials and Methods

Arenaria merckioides Maxim. var. *chokaiensis* (Yatabe) Okuyama is restricted to the alpine zone of Mt. Chokai in northern Honshu (Okuyama, 1966; Kitagawa, 1982). Field observations were made along the Yasu-shindo trail. The plants occur in large disjunct clumps and bear creeping rhizomes. Three study sites (each 3m x 5m quardrate) located at different altitudes (1750m, 1900m, 2000m) were delineated along the Yasu-shindo trail. The flowers open from July to August and set fruit in September. Hermaphrodite flowers are protandrous, and their styles and anthers are spatially separated at flowering (Sugawara and Horii, 1995).

To reconfirm the difference in pistil morphology between hermaphrodite and female plants, two flowers per plant (clump) were fixed with FAA, and their style and stigma lengths were measured at × 30 magnification. In many species of Caryophyllaceae the stigma is usually borne along the entire length of the style (Dulberger and Horovitz, 1984; Sugawara, 1993; Sugawara *et al.*, 1994). In the plants we examined the stigma is restricted to the distal adaxial part of the style (Fig. 1). The length of the stigma was measured by using a camera lucida on the adaxial side of the style that developed stigmatic papillae (Sugawara and Horii, 1995).

To count pollen grains on the stigmas, a few female flowers were randomly collected from the plants (clumps) at each site. All styles (usually three styles) within a flower were mounted on a glass slide, stained with cotton blue solution, and mounted with a coverglass. Subsequently, the coverglass was sealed with clear enamel nail polish. The number of pollen grains deposited on the stigmas was counted with the use of a microscope. To estimate fruit and seed set per plant (clump), 10 to 20 stems per plant were collected at random, and all flowers borne on those stems were examined.

To confirm the probability of self-compatibility and degree of seed production in the hermaphrodite plants, experimental pollinations were made

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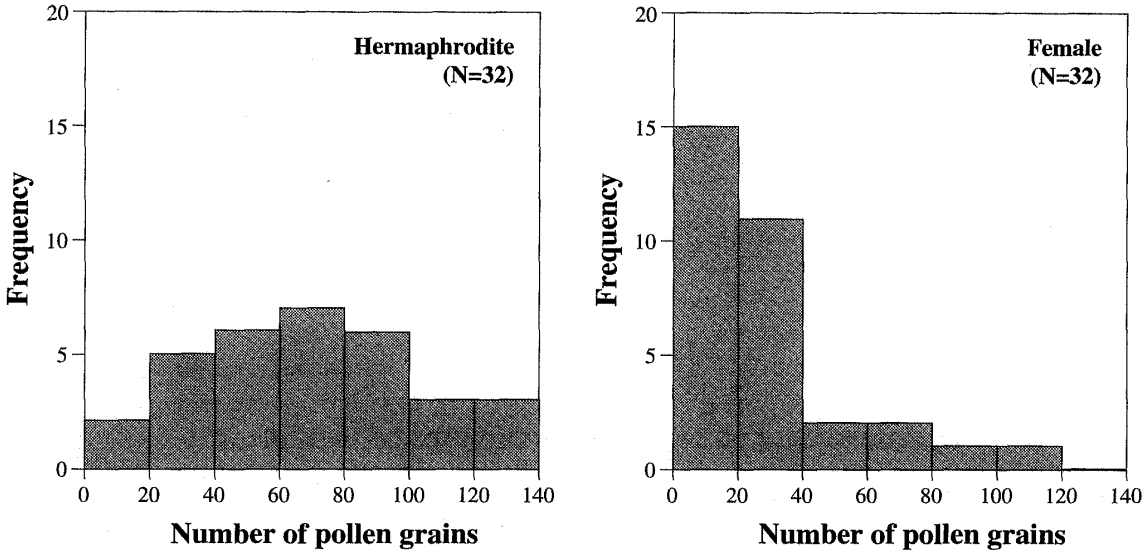

FIG. 2. Frequency distribution of number of pollen grains deposited on stigmas of hermaphrodite and female flowers. The data were totaled from the three sites.

using four individuals that were transplanted from the field into the experimental garden of Shinshu University, Matsumoto.

Results

Difference in style and stigma length between hermaphrodite and female flowers

The style length was shorter in the flowers of female plants than in flowers of hermaphrodite plants (*t*-test, $p<0.05$; Table 1). In contrast, the length of the stigma in female plants was significantly longer than in hermaphrodite plants (*t*-test, $p<0.001$; Table 1; see also Fig. 1), which is in agreement with a previous report (Sugawara and Horii, 1995). These results clearly show that dimorphism in stigma length is one of the characteristics of the gynodioecious plant *Arenaria merckioides* var. *chokaisensis*.

Comparison of pollen deposition on stigmas of hermaphrodite and female flowers

The number of pollen grains deposited on the stigmas of hermaphrodite and female flowers examined at the three study sites is summarized in Table 2. The frequency distribution of the number of pollen grains in the two sexual forms from the three study sites are shown in Fig. 2. The number of pollen grains deposited on the stigmas of female flowers was usually less than in hermaphrodite flowers. The histogram represents L-shaped distribution (Fig. 2, right). Although the sample sizes are insufficient, the hermaphrodite flowers tended to have more pollen grains on their stigmas than did the female flowers at all three sites (see Table 2).

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TABLE 2. Number of pollen grains deposited on the stigmas of hermaphrodite (H) and female (F) flowers at three sites of the Chokai population of *Arenaria merckioides* var. *chokaiensis*

Study sites (altitude)	Sexual form	No. of pollen grains		
		N	Mean	95 % CI
Site A (1750m) [1]	H	10	62.8	47.2 - 78.3
	F	10	18.4	5.5 - 31.3
Site B (1900m) [2]	H	11	74.5	52.7 - 96.2
	F	11	32.4	11.8 - 52.8
Site C (2000m) [3]	H	11	70.1	42.8 - 97.4
	F	11	25.6	5.5 - 45.7
Average in three sites[4]	H	32	69.3	57.7 - 80.9
	F	32	25.7	16.0 - 35.3

N: Number of flowers examined.
Mann-Whitney U-test, [1]U=5.0, P<0.001; [2]U=18.5, P<0.01; [3]U=19.5, P<0.01; [4]U=143.5, P<0.001.

Comparison of fruit and seed set percentages of hermaphrodite and female plants in the field

In 1996 we compared fruit and seed set percentages between hermaphrodite and female plants at three study sites at different altitudes (Table 3). The average ovule number per flower did not differ significantly between hermaphrodite and female plants at the three study sites (*t*-test, p=0.38), although it was varied between plants. In each of the three study sites, percentage of fruit set in the female plants was usually higher than in hermaphrodite plants. At site C the fruit set percentage of the female plants reached 100 %. Overall, the female plants tend to produce more fruits than hermaphrodite plants. The average number of seeds produced per flower in the female plants was usually higher than in the hermaphrodite plants.

Experimental pollination in hermaphrodite and female plants

Results of the experimental pollination in female and hermaphrodite plants are shown in Table 4. It is clear that the female plants exhibit no apomixis, since none of the bagged female flowers produced fruits and seeds. The hermaphrodite plants are self-compatible, because they produce fruits and seeds by hand self-pollination. In the hermaphrodite plants, 54 % of the hand self-pollinated and 87 % of the hand cross-pollinated flowers set fruits. A significant difference in the average number of seeds per flower was found between self- and cross-pollinated plants (Mann-Whitney U-test, p<0.001). However, there was no significant difference in the average number of seeds per fruit between the two different treatments (Mann-Whitney U-test, p=0.06).

Discussion

It was confirmed that the number of pollen grains actually deposited on the stigmas of female flowers is less than in hermaphrodite flowers in a natural

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TABLE 3. Fruit and seed set in hermaphrodite and female plants at three different sites on Mt. Chokai.

Study site (altitude)	Individual number	Sexual form	No. of flowers/ 10 stems	No. of ovules/flower (Mean ± SD)	Fruit set (%)	No. of seeds per flower (Mean ± SD)	No. of seeds per fruit (Mean ± SD)
Site A (alt. 1750m)							
	1	H	14	10.4 ± 2.1	76.4	2.4 ± 2.1	3.4 ± 1.5
	2	H	19	11.4 ± 1.3	31.0	1.0 ± 1.8	3.3 ± 1.6
	3	H	17	11.8 ± 0.9	48.1	1.5 ± 1.9	3.2 ± 1.3
	4	F	26	13.1 ± 2.5	77.9	3.8 ± 2.9	4.8 ± 2.3
Site B (alt. 1900m)							
	1	H	21	15.4 ± 2.0	52.4	1.2 ± 1.3	2.4 ± 0.7
	2	H	26	15.3 ± 3.2	69.2	1.7 ± 1.6	2.4 ± 1.3
	3	H	30	13.9 ± 2.4	61.3	1.7 ± 1.6	2.7 ± 1.1
	4	H	25	16.4 ± 2.3	70.4	2.4 ± 1.8	3.4 ± 1.0
	5	H	33	16.2 ± 2.7	75.8	2.9 ± 2.2	3.8 ± 1.6
	6	H	15	16.1 ± 2.7	50.0	1.2 ± 1.5	2.4 ± 1.1
	7	F	24	12.8 ± 2.4	100.0	3.6 ± 2.0	3.6 ± 2.0
Site C (alt. 2000m)							
	1	H	23	12.8 ± 1.9	62.2	2.2 ± 2.3	3.6 ± 1.9
	2	H	20	14.3 ± 2.7	67.9	3.2 ± 2.9	4.7 ± 2.2
	3	H	26	14.1 ± 2.4	62.3	2.5 ± 2.6	4.0 ± 2.1
	4	H	15	13.8 ± 2.4	85.7	3.5 ± 2.2	4.0 ± 1.9
	5	F	26	14.4 ± 2.9	80.8	5.4 ± 3.7	6.7 ± 2.9
	6	F	28	11.9 ± 2.5	84.3	3.3 ± 2.3	3.9 ± 2.0
	7	F	26	13.6 ± 2.4	83.3	4.8 ± 2.9	5.8 ± 2.0
Average in three sites		H (N=13)	21.8	13.9 ± 1.9	62.5	2.1 ± 0.8	3.3 ± 0.7
		F (N=5)	26.0	13.2 ± 0.9	85.3	4.2 ± 0.9	5.0 ± 1.3

TABLE 4. Hand pollination experiments in female and hermaprodite flowers of *Arenaria merckioides* var. *chokaiensis*

Treatment	No. of individuals examined	No. of flowers examined	No. of flowers fruiting	Fruit set (%)	Mean ovule number per flower	No. of seeds per flower Mean ± SD	No. of seeds per fruit Mean ± SD
<Female>							
Bagged	2	7	0	0	-	0	0
<Hermaphrodite>							
Bagged	3	18	0	0	13.7	0	0
Self-pollination	4	61	33	54.1	12.9	1.3 ± 1.6	2.3 ± 1.4
Cross-pollination	3	23	20	87.0	14.4	2.9 ± 2.3	3.4 ± 2.1

population. The longer stigmas of the female flowers are unlikely to increase the ability to trap pollen grains over the hermaphrodite flowers. The larger number of pollen grains on stigmas of the hermaphrodite flowers may reflect geitonogamous pollination, because many flowers open at one time on a single plant (clump) and the insect pollinators such as drone flies (mainly *Eristalomyia*, Syrphidae), hover flies (Syrphidae), musceid flies (Anthomyiidae) and blow flies (*Calliphora*, Calliphoridae) often move to neighbouring flowers of the same

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plant (Sugawara and Horii, 1995).

As revealed by experimental pollinations, the hermaphrodite plants are self-compatible. Hermaphrodite plants might be expected to produce more seeds than female plants because of selfing. The present data however indicate that the average number of seeds per hermaphrodite flower is far fewer than in the female plants. Why is the number of pollen grains deposited on the stigma not correlated with seed production in hermaphrodite plants ? It is possible that reduced seed production in hermaphrodite plants may be caused by selfing, because fruit and seed set percentages of the self-pollinated hermaphrodite flowers are usually less than in cross-pollinated flowers. More detailed investigations are necessary to understand the effect of selfing on seed production in hermaphrodite plants.

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摘　　要

菅原　敬[1]，堀井雄治郎[2]：雌性両全性異株植物チョウカイフスマ (ナデシコ科) における柱頭の二型性，花粉捕獲量，種子生産に関する研究

チョウカイフスマ (*Arenaria merckioides* var. *chokaiensis*) は秋田・山形県境に位置する鳥海山高山帯に固有なナデシコ科の多年草であるが，その性表現は雌個体と両性個体からなる雌性両全性異株 (gynodioecy) であることが知られている。この植物では性型の分化に伴い，柱頭の長さにも二型性の分化が生じ，雌花は両性花よりも著しく長い柱頭をつける。この二型性柱頭の機能的意味を探るため，鳥海山野外集団において実際の花粉付着量，果実や種子の生産量を調査した。また実験園へ移植した両性個体を用いて自家不和合性の程度を調べた。その結果，雌花は両性花よりも有意に長い柱頭をもつにもかかわらず，実際柱頭に付着する花粉粒は両性花において多いという傾向が見られた。野外では雌花の長い柱頭は花粉捕獲のうえで実際有利に働いているとはいえないようである。また，野外での結果率や結実率 (種子数／花) は両性個体より雌個体においてむしろ高いという傾向がみられた。この結果は花粉付着量と対応しないが，自家受粉による両性花の結実率が他家受粉による両性花の結実率よりも著しく低いという結果を考慮すると，野外における両性個体の結実率の低さには自殖による影響が現れている可能性がある。
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