Floral and Reproductive Biology of Gynodioecious
Dianthus superbus L. var. superbus (Caryophyllaceae)

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Abstract. Two populations of Dianthus superbus L. var. superbus were investigated in Aomori Prefecture to determine the extent of differences between hermaphrodite and female plants in floral morphology, reproductive ability of the flowers, and fruit and seed set in the field. The hermaphrodite flowers were protandrous, but partially self-compatible in pollination experiments, and morphologically characterized by larger petals and a longer calyx tube. The female flowers had smaller petals, shorter filaments, and abortive anthers. No significant difference was found between the genders in ovule number per flower, fruit set percentage, and in the kinds of insects visiting the flowers. However, a notable difference in flowering phenology was found between the two different types of flowers; the stigmas of the female flowers mature earlier than stigmas in hermaphrodite flowers, and the flowering period of female flowers is obviously longer than for hermaphrodite flowers. Earlier maturation of the stigma and longer female flowering may enhance the chance of pollination in female flowers. However, it was revealed from the field observations that seed set percentages in hermaphrodite flowers were significantly higher than in female flowers.

Key words: Caryophyllaceae, Dianthus superbus, gynodioecy, floral biology

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Dianthus L. (Caryophyllaceae) is a large genus, comprising more than 300 species, and is widely distributed in temperate regions of the northern hemisphere (Kitagawa, 1982). In many species of the genus, plants with female and hermaphrodite flowers are known (Tutin, 1964). Dianthus superbus L., a perennial herbaceous species, is gynodioecious (Yahara, 1988). Female flowers are usually smaller and do not produce pollen, and they may represent an adaptative form that compensates for their unisexual nature (Richards, 1986). However, no studies have addressed the exact nature of hermaphrodite and female plants in this species, and no information is available for the species on pollination and breeding systems. In this paper we document the floral characteristics of Dianthus superbus var. superbus and discuss the reproductive ability and pollination systems of hermaphrodite and female plants.

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Materials and Methods

*Dianthus superbus* is widely distributed throughout Japan from Kyushu to Hokkaido, and commonly occurs in lowlands, in mountain grasslands and in higher, rocky mountains. The species varies in floral morphology with particular respect to degree of petal serration, calyx-tube length, and number of bracteoles, and it is often subdivided into three varieties: var. *longicalycinus* (Maxim.) Williams, var. *speciosus* Reichb., and var. *superbus* (see Kitagawa, 1982). Despite frequent difficulties in distinguishing those infraspecific taxa, we assigned plants of the two *Dianthus* populations examined to var. *superbus* on the basis of the calyx-tube length (see Imai, 1981; Kitagawa, 1982).

Field observations of the plants were made at two different localities in Aomori Prefecture, northernmost Honshu: (1) Zubonmori (40° 43’N, 140° 0’E; alt. 10 m) near the sea, and (2) Takanagane (40° 40’N, 140° 24’E, alt. 120 m), the eastern foot of Mt. Iwaki (Fig. 1). The areas of the study plots were ca. 10 × 20 m at Zubonmori, and ca. 20 × 20 m at Takanagane. In the Zubonmori population, the plants grow on the lower slope of a sandy hill with *Rosa rugosa* Thunb., *Seseli libanotis* Koch var. *japonica* H. Boiss., *Galium verum* Linn. var. *asiaticum* Nakai, and *Adenophora triphylla* (Thunb. ex Murray) A. DC. var. *japonica* (Regel) Hara. In the Takanagane population they occur in a grassland with perennial herbs such as...
as Miscanthus sinensis Anderss., Artemisia japonica Thunb., Picris hieracioides Linn. var. glabrescens (Regel) Ohwi, and Sanguisorba tenuifolia Fisch.

Stems of the plants are often branched at the base. For examination of floral morphology and reproductive ability, erect stems with open flowers were randomly collected from different clones of two populations. The flowers were preserved in FAA (formalin-acetic acid-alcohol). One flower per stem was examined to determine petal length, petal width, and calyx-tube length. In addition, the number of ovules per ovary was counted. To check fertility of pollen grains, two anthers from each flower were crushed on a glass slide, stained with cotton blue solution, kept overnight, and examined the next day.

Several plants from different clones were transplanted into the experimental garden of Hirosaki University, Hirosaki, and were examined the following year to determine the sexual form of the flowers. Furthermore, the pollination experiments were conducted on those flowers.

To estimate the fruit- and seed-set in natural populations, several stems per clone were marked with color tape and collected at random, and all flowers borne on those stems were examined.

Insects visiting the flowers of these plants were observed for three days (6 hours in total, in late July in 1991 and 1992) in each population. Insects visiting the flowers were caught for identification.

Voucher specimens of the plants and flower visitors from the two populations were deposited in the Herbarium of Shinshu University, Matsumoto.

Results and Discussion

Sex expression in natural populations

Dianthus superbus var. superbus usually bears several flowers per stem in a cymose inflorescence; each flower has a long calyx-tube, five purplish petals, ten stamens with long filaments, and a compound ovary with two free and elongated styles.

In the two populations examined, two different sexual forms of flower, i.e., hermaphrodite and female flowers, were commonly found (Fig. 2). Sexual forms of the flowers in the same inflorescence and on the same erect stem were mostly consistent, although inflorescences rarely possess both hermaphrodite and female flowers. Plants transplanted from the field into the experimental garden mostly had one of the two different flower types for two years (1991 and 1992). Therefore, the species is certainly regarded as gynodioecious, as mentioned earlier by Yahara (1988).

If one clone is considered to be one individual plant, the percentage of female plants was 14 % (5 / 35) at Takanagane, and 19 % (8 / 43) at Zubonmori in 1992.

Floral characteristics and floral biology of hermaphrodite and female plants

In Table 1, petal length, petal width, calyx-tube length, and pollen fertility in the hermaphrodite and female flowers is summarized. Although
flower size is somewhat different between the two populations (flowers from Zubonmori are slightly smaller than those from Takanagane), the female flowers are significantly smaller in petal length, petal width and calyx-tube length than the hermaphrodite flowers in both populations, as in other gynodioecious species such as *Iris douglasiana* (Uno, 1982) and *Moehringia lateriflora* (Sugawara, 1993). Hermaphrodite flowers produced a large number of pollen grains. The mean number of pollen grains per anther was 3311 (n = 10, s.d. = 631), and almost all grains (more than 92%) were fertile in the two populations. The female flowers also produce many pollen grains (i.e., 2954 [n = 5, s.d. 563] in the mean number of pollen grains), but they are mostly sterile (see Table 1).

The hermaphrodite flowers are protandrous. As they open, the stamens elongate and reach the entrance of the perianth-tube. The anthers gradually dehisce longitudinally. At this stage, however, the styles still remain within the perianth-tube and are positioned below the anthers, and their stigmatic papillae are not developed (Figs. 2A, 3A). Two to three days later, the styles gradually elongate, and their distal parts begin to coil with the stigmatic papillae being differentiated and receptive to pollen
TABLE 1. Petal length and width, calyx-tube length, and pollen fertility of hermaphrodite and female flowers in two populations of Dianthus superbus var. superbus

<table>
<thead>
<tr>
<th>Character</th>
<th>Hermaphrodite Mean ± S.D.</th>
<th>Female Mean ± S.D.</th>
<th>H/F</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petal length (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takanagane</td>
<td>52.1 ± 3.9 (N = 19)*</td>
<td>42.7 ± 3.3 (N = 19)</td>
<td>1.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Zubonmori</td>
<td>47.4 ± 3.7 (N = 45)</td>
<td>37.9 ± 1.8 (N = 14)</td>
<td>1.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Petal width (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takanagane</td>
<td>20.8 ± 3.1 (N = 19)</td>
<td>16.9 ± 3.2 (N = 19)</td>
<td>1.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Zubonmori</td>
<td>16.9 ± 2.3 (N = 45)</td>
<td>14.3 ± 1.6 (N = 14)</td>
<td>1.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Calyx-tube length (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takanagane</td>
<td>31.8 ± 1.8 (N = 19)</td>
<td>29.7 ± 1.2 (N = 19)</td>
<td>1.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Zubonmori</td>
<td>30.5 ± 2.6 (N = 45)</td>
<td>27.8 ± 1.1 (N = 14)</td>
<td>1.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Pollen fertility (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Takanagane</td>
<td>94.6 ± 5.9 (N = 23)</td>
<td>0.1 ± 0.2 (N = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zubonmori</td>
<td>92.2 ± 9.9 (N = 30)</td>
<td>0.2 ± 0.6 (N = 7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*: Number of flowers examined

FIG. 3. Buds of hermaphrodite and female flowers of Dianthus superbus var. superbus. A: Hermaphrodite flower; styles within perianth tube undeveloped. B: Female flower, styles already elongated. gl, gland; p, petal; s, stamen; st, style. Scale bar represents 5 mm.
grains. The stigmatic papillae extend to the base of the style. As the flowers (petals) wilt, the stigmas also begin to droop. In the experimental garden, the flowering period of the hermaphrodite flowers was 7.3 days (n = 12, s.d. = 1.3, range 6–9 days), which included 4.2 days for the female stage (n = 12, s.d. = 1.2, range 3–6 days).

In the female flowers, on the other hand, the stamens abort early in flower development; the filaments fail to elongate and the anthers develop poorly (Fig. 2B). The styles elongate while the flowers are still closed. The stigmas appear to mature much earlier than in hermaphrodite flowers (Fig. 3). The stigmas of the female flowers do not wither during the flowering period, and the stigmatic papillae appear to be active. The flowering period of the female flowers was 7.1 days (n = 12, s.d. = 1.0, range 5–8 days) in the experimental garden.

It is noteworthy that the stigmas of the female flowers mature earlier than those of the hermaphrodite flowers, and the duration of their female phase is obviously longer than those of hermaphrodite’s. These flowering habits may enhance the chance of pollination in the female flowers.

**Pollination experiments and observation on flower visitors in the field**

Results of the pollination experiments in hermaphrodite and female flowers are presented in Table 2. No apomixis occurred in the female flowers, because none of the bagged female flowers produced fruit and seeds. Fruit set percentages of bagged hermaphrodite flowers and of the artificially self-pollinated hermaphrodite flowers whose stigmas had not elongated beyond the perianth tube were 42.9 % and 31.6 %, respectively. In artificially self-pollinated hermaphrodite flowers whose stigmas had elongated beyond the perianth tube, there was 100 % fruit set. Fruit set percentage in cross-pollinated hermaphrodite flowers was 68.2 %, obviously higher than in both the control (43.9 %) and in the bagged hermaphrodite flowers (42.9 %). The seed set percentages in self and cross pollination experiments are significantly higher than in the controls (Mann-Whitney U-test, p < 0.01). These pollination experiments indicate that the her-

<table>
<thead>
<tr>
<th>Flower form</th>
<th>Control</th>
<th>Bagged</th>
<th>Self-pollination</th>
<th>Cross-pollination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F.S.</td>
<td>S.S.</td>
<td>F.S.</td>
<td>S.S.</td>
</tr>
<tr>
<td>Herma-</td>
<td>43.9</td>
<td>15.8 ± 3.2</td>
<td>42.9</td>
<td>6.5 ± 7.0</td>
</tr>
<tr>
<td>phrodite</td>
<td>(N = 389)</td>
<td>(N = 178)</td>
<td>(N = 21)</td>
<td>(N = 9)</td>
</tr>
<tr>
<td>Female</td>
<td>42.8</td>
<td>15.3 ± 4.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(N = 365)</td>
<td>(N = 144)</td>
<td>(N = 19)</td>
<td>(N = 19)</td>
</tr>
</tbody>
</table>

F.S.: Fruit set percentage  #: Stigma extruded from a perianth-tube
S.S.: Seed set percentage  #: Stigma within a perianth-tube
N: Number of flowers examined
maphrodite flowers are partially self-compatible, despite their protandrous nature, and are partly dependent on pollinators, as in the female flowers.

Flowers of both sexual forms are fragrant, and produce nectar from yellowish nectaries situated at the base of the filaments (see Figs. 2, 3). We saw no major differences in insect visitors between the two types of flowers. Hover flies (Syrphidae), small bees (Halictus tsingtouensis and Lasioglossum affine), and butterflies (mainly Ochlodes venatus venatus and Papilio machaon hippocrates) were often found to visit those flowers. Rarely, bumblebees (Bombus diversus diversus) and hawkmoths (Macroglossum stellatarum) also visited the flowers.

Among those insects, the small bees often landed on the purplish petals of the open flowers (Fig. 4A), and entered the perianth-tube to suck nectar. They touched the anthers and / or stigmas, and actually loaded pollen grains on their bodies. In a few instances, the small bees visited half-open flowers and forcibly entered the flowers. The butterflies also landed on the upper surface of the petals and sucked nectar from the base of the perianth-tube. Both the small bees and butterflies probably play a major part in pollination in this species. The hover flies also visited the flowers and often fed on pollen grains. They appeared to be less important as pollinators.

Comparisons of flower numbers, ovule numbers, and fruit and seed set percentages between two different sexual forms under natural conditions

No significant differences were found in the numbers of flowers per erect stem and ovule number per flower between the hermaphrodite and female flowers examined in the two populations (t-test, p > 0.05; see Table 3). No major differences were found in fruit set percentages between the two types of flowers. The hermaphrodite flowers, however, show a significantly higher percentage of seed set than the female flowers in the two populations (t-test, p < 0.001).

In several gynodioecious species examined so far, seed set percentages in female flowers are usually higher, or nearly equal to those in the her-
TABLE 3. Comparison of flower number per stem, ovule number per flower, fruit and seed set percentages between hermaphrodite and female flowers in two populations of *Dianthus superbus* var. *superbus* examined in 1992

<table>
<thead>
<tr>
<th>&lt;Population&gt;</th>
<th>Flower form</th>
<th>Number of stems examined</th>
<th>Number of flowers</th>
<th>Mean number of flowers/stem (S.D.)</th>
<th>Mean number of ovules/flower (S.D.)</th>
<th>Fruit set %</th>
<th>Seed set % (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;Takanagane&gt;</td>
<td>Hermaphrodite</td>
<td>37</td>
<td>144</td>
<td>3.9 (2.3)</td>
<td>128.9 (26.4)</td>
<td>68.8</td>
<td>38.1 (23.2)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>17</td>
<td>75</td>
<td>4.9 (4.7)</td>
<td>132.8 (20.0)</td>
<td>70.1</td>
<td>18.5 (9.9)</td>
</tr>
<tr>
<td>&lt;Zubonmori&gt;</td>
<td>Hermaphrodite</td>
<td>207</td>
<td>773</td>
<td>3.7 (2.7)</td>
<td>119.3 (22.2)</td>
<td>59.2</td>
<td>41.5 (22.1)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>50</td>
<td>180</td>
<td>3.6 (2.6)</td>
<td>120.4 (21.1)</td>
<td>55.6</td>
<td>30.3 (22.1)</td>
</tr>
</tbody>
</table>

Hermaphrodite flowers in natural populations (Uno, 1982; Richards, 1984; Sugawara, 1993). However, the results obtained in this study from the two populations is in disagreement with those in other plants; seed set percentages in hermaphrodite flowers are significantly higher than in female flowers. This phenomenon may be due to higher self-pollination among the hermaphrodite flowers on the same plant, since each plant usually possesses several open flowers on the same stem and their flowers are self-compatible. More intensive investigations including on estimation of the selfing rate in hermaphrodite flowers and the availability of pollinators within a population are needed to clarify the above question.

Gynodioecy has generally been thought to represent a preceding stage in the evolution toward dioecy (see Richards, 1986), and indeed some species appear to represent such a condition (Tsukui and Sugawara, 1992). It is very important, however, to clarify whether the inheritance of male sterility is under cytoplasmic control or not; if male sterility is due to cytoplasmic inheritance, then gynodioecy would not evolve toward dioecy (Ross, 1978; Bawa, 1980). Investigation on the genetic system of male sterility in *Dianthus superbus* var. *superbus* is now in progress.

This study was largely undertaken in Hirosaki University. We are grateful to Prof. S. Sato of Hirosaki University and Dr. M. Maki of the University of Tokyo for advice during the course of this study. Our thanks are also due to Prof. H. Tobe of Kyoto University for critically reading the manuscript, to Dr. M. Yamada of the Aomori Apple Experimental Station for identification of the small bees, and to Mr. K. Suzuki of Tohoku Women's College for identification of the butterflies.

References


SUGAWARA et al.: Reproductive biology of Dianthus


摘 要
菅原 純1・中村真子2・神林真理2・星 秀章2・三上美代子2: 雌性両全性異株エゾカワラナデシコの花生態及び繁殖に関する研究

エゾカワラナデシコ (ナデシ科) には両性花をつける株に混じって雌花のみをつける株が見られることが知られている。しかし、このような雌雄性の分化 (雌性両全性異株性) くなった場合、この植物の両性花と雌花との間で花の形態や開花習性、授粉や交配にかかわる特性にどのような差異が生じているのか、また野外での種子や果実の形成、花粉媒介者はどのようなものか、などについてはほとんど知られていない。そこで、性型の異なる二つの花の基本的特性を明らかにした。野外での授粉や繁殖の様子を観察することを目的に、青森県内の2つの集団を用いて調査を進めてきた。両性花と雌花との間には、花の付属器官 (花弁やがくなど) における大きさの違いが認められるが、開花習性の上でもいくつかの興味深い違いが認められた。その一つは、花柱発達時期 (雌性期) のずれである。雌花では、開花時にすでに花柱を高く伸ばして柱頭組織を発達させ、授粉可能な状態にあるが、両性花では雌性期が開花から2、3日後であった。もう一つは、開花期間における雌性期の長さで、雌花では両性花よりもかなり長い雌性期をもっていることが明らかになった。これらは、雌花の受粉の機会を高めているように思われる。しかし、野外での果実あたりの種子の生産数は必ずしも両性花よりも高くなく、同様な性型を示す他の植物とはやや異なる状況であった。

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