Gyn dioecy and floral dimorphism of *Glechoma longituba* (Nakai) Kuprian. (Lamiaceae) in Korea

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**ABSTRACT:** The present study was focused on the careful investigation of *Glechoma longituba* flowers collected from four populations in Korea to clarify their precise sexual systems. All floral characters of this taxon were examined by using a stereo microscopy. The nutlet size and production were compared between two distinct morphs (i.e., hermaphrodites and females), and pollen viability test of hermaphrodite and intermediate phenotype (i.e., individuals with a mixture of females and perfect flowers) was also observed by light microscopy (LM). All of the investigated floral characters of *G. longituba* are significantly different from one sexual morph to another. Hermaphrodite plants were larger than the female ones in corolla width and length, calyx length, filament length, anther length, and style length (all \( P < 0.0001 \), respectively). However, the nutlet width of the female plants was larger than those of the hermaphrodites \( (P = 0.013) \). Nutlet production of females was higher than hermaphrodites in Na-Ju population \( [H \text{ (Hermaphrodite): ca. 17.76\%}, \text{F (Female): ca. 37.45\%}] \), but in Mun-San population there was no significant difference between these sexual morphs \( (H: \text{ca. 57.52\%}, \text{F: ca. 53.16\%}) \). We have found significant differences between the fertile pollen grain of hermaphrodite and sterile pollen grain of intermediate phenotype based on pollen size and viability. The results of the present study suggest that compensation (flower size dimorphism, seed set, and pollen viability) is one of the main mechanisms in order to maintain female plants in gyn dioecious *G. longituba*.

**Keywords:** Floral dimorphism, *Glechoma longituba*, Gyn dioecy, Lamiaceae, Pollen viability, Reproductive compensation.

Gyn dioecy is an interesting breeding system, which includes both hermaphrodite and female plants within a population (Darwin, 1877). The presence of gyn dioecy species is relatively common in the Lamiaceae, e.g., ca. 57% of species in this family is gyn dioecious (Owens and Uber- Jiménez, 1992). Two taxa of the genus *Glechoma* [G *hederacea* L., *G longituba* (Nakai) Kuprian.] in the Lamiaceae have already reported to be gyn dioecious (Slade and Hutchings, 1989; Widén and Widén, 1999; Zhang et al., 2008a, b; Zhang et al., 2011; Bai et al., 2011). *G. longituba* is a clonal herbaceous perennial plant that grows in shady habitats including woodlands and grasslands and which is mainly distributed in China, Korea, and Russia. Flowers are zygomorphic and few flowers are second axillary whorls. Corolla has strongly divided two-lipped and it is longer than calyx (Harley et al., 2004; Jang, 2008).

Sexual morph of gyn dioecy was represented by floral dimorphism, particularly in flower size. In general, size of hermaphrodite plants is larger than female flowers (Baker, 1948; Lloyd and Webb, 1977; Eckhart, 1999). Since the female flowers produce no pollen grains at all, and their degenerated stamens were not only smaller but also sometimes occurred at lower positions inside of corolla than the normal stamens of hermaphrodite flowers (Kawakubo, 1994).

Gyn dioecy is sometimes more complicated according to the presence of flowers with different degrees of male sterility. For example, some of which come together with female flowers and hermaphrodite flowers within individual, i.e., intermediate phenotype. The presence of intermediate phenotype has been observed both in natural populations and in cultivation (Ågren and Wilson, 1991; Koelewijn and Van Damme, 1996; Lafuma and Maurice, 2006; Zhang et al., 2008b). This phenomenon was regarded as the gradual changes from unstable gyn dioecy to stable gyn dioecy (Ross, 1977; Charlesworth and Charlesworth, 1978). This situation is unlike what has been reported for most other gyn dioecious taxa, both females and hermaphrodites are more labile in their sex

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expression (Widen and Widen, 1999; Zhang et al., 2008a). It is, therefore, needed to examine the exact breeding system of *G. longituba* more carefully.

The aims of this paper were (1) to examine floral characters in detail by using a stereo-microscopy, (2) to compare nutlet production and size measurement between two different morphs, and (3) to observe pollen viability of intermediate phenotype in four populations of Korea. In addition, we asked what developmental processes account for differences in relative flower size and nutlet size between female and hermaphrodite. We also discussed the effect of the female advantage over hermaphrodites by comparing reproductive traits for the maintenance of gynodioecy in this species.

**Materials and Methods**

The present study is based on living materials, which were taken in Korea populations (Mt. Cheong-Gye: 37°24'50"N, 127°01'32"E, 163 m elevation, Hong-Neung: 37°35'29"N, 127°02'32"E, 141 m elevation, Mun-San: 37°57'47"N, 126°56'20"E, 68 m elevation, and Na-Ju: 35°02'35"N, 126°42'48"E, 72 m elevation). To compare the floral morphological differences between the two sexual types and among populations, we selected two natural populations (Mun-San and Na-Ju), which were located in different habitats, and two cultivated populations (Cheong-Gye and Hong-Neung) for comparing the natural populations. Population Mun-San was located on a sunward slope whereas Na-Ju was located on a shaded slope. Flowers and nutlets were collected and preserved in formalin-acetic-acid-alcohol (FAA), and examined with the stereo-microscope (Olympus SZ405).

The flower and nutlet size of all collected samples was measured by using digital calipers (Cd-15cp). Data for nutlet production were measured from ten individuals of each sex form in two studied populations (Mun-San and Na-Ju). To test the self-incompatibility, ten individuals from each of hermaphrodite and intermediate form from Na-Ju population were used for each self pollination treatment by hand. Flowers were bagged after treatment to prevent access to pollinations and predators. For pollen viability test, anthers were put in aniline blue dye solution. The states of stained pollen grains were photographed by LM. A MANOVA was used to compare floral character such as corolla, calyx, upper and lower lip, filament, anther, ovary, stigma, style, and nutlet size as well as between and among populations. The significance level was set at 0.05. All measurements were analyzed by MANOVA using the SPSS software package (Release 12.0.0, 2003, SPSS Institute, USA), and all prepared vouchers are deposited in KHUS (The Herbarium of Kyung Hee University, Seoul).

**Results**

Two distinct sex forms (hermaphrodite, Fig. 1A; female, Fig. 1C) were found in *Glechoma longituba* in Korea natural population. However, sometimes an intermediate phenotype (i.e., individuals with a mixture of females and hermaphrodite flowers) was also found in the investigated population (Fig. 1B). Unfortunately, we could not be able to check the frequency of sex morphs in studied populations with standard approach this time. Thus, future studies should perform the long time

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**Fig. 1.** Photographs of *G. longituba* (Nakai) Kuprian. A. Hermaphrodite flowers (Na-Ju population); B. Intermediate phenotype (Na-Ju population; black arrow indicates perfect flower within intermediate and white arrow indicates female flower within intermediate individual); C. Female flowers (Na-Ju population); D. Flower size dimorphism between hermaphrodite (Jang 041501, Na-Ju; KHUS) and female (Jang 041502, Na-Ju; KHUS); E. Fertile pollen grains of hermaphrodite; F. Sterile pollen grain of intermediate phenotype.
Table 1. Flower measurements of the females and hermaphrodite flowers of *Glechoma longituba*. Differences between means were analyzed by MANOVA (unit: all in mm, *P* < MANOVA for means; N, number of measured individual; N.S., non-significance; -, not investigated).

<table>
<thead>
<tr>
<th></th>
<th>Female (mean ± S.D.)</th>
<th>Hermaphrodite (mean ± S.D.)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mun-San</td>
</tr>
<tr>
<td>Corolla length</td>
<td>10</td>
<td>10.16 ± 0.37</td>
</tr>
<tr>
<td>Corolla width</td>
<td>10</td>
<td>9.32 ± 0.97</td>
</tr>
<tr>
<td>Calyx length</td>
<td>10</td>
<td>5.98 ± 0.68</td>
</tr>
<tr>
<td>Calyx width</td>
<td>10</td>
<td>5.29 ± 0.29</td>
</tr>
<tr>
<td>Upper lip width</td>
<td>10</td>
<td>3.23 ± 0.60</td>
</tr>
<tr>
<td>Lower lip width</td>
<td>10</td>
<td>4.19 ± 0.70</td>
</tr>
<tr>
<td>The longest filament</td>
<td>10</td>
<td>0.44 ± 0.41</td>
</tr>
<tr>
<td>The shortest filament</td>
<td>10</td>
<td>0.26 ± 0.14</td>
</tr>
<tr>
<td>The longest anther</td>
<td>10</td>
<td>0.34 ± 0.20</td>
</tr>
<tr>
<td>The shortest anther</td>
<td>10</td>
<td>0.29 ± 0.16</td>
</tr>
<tr>
<td>Ovary length</td>
<td>10</td>
<td>0.38 ± 0.04</td>
</tr>
<tr>
<td>Stigma length</td>
<td>10</td>
<td>0.88 ± 0.15</td>
</tr>
<tr>
<td>Style length</td>
<td>10</td>
<td>9.47 ± 0.92</td>
</tr>
<tr>
<td>Nutlet length</td>
<td>10</td>
<td>1.58 ± 0.08</td>
</tr>
<tr>
<td>Nutlet width</td>
<td>10</td>
<td>1.03 ± 0.05</td>
</tr>
</tbody>
</table>
monitoring of each sex forms in the expanded populations.

Floral organs of hermaphrodites in *G. longituba* were significantly larger than those of females (corolla length and width, upper and lower lip width, calyx length, filament length, anther length, style length, respectively; all $P < 0.0001$, except calyx width $P = 0.002$, ovary and stigma length $P = N.S.$, Fig. 1D, Table 1). The size of upper and lower lip in *G. longituba* was clearly differed from two sexual morphs. Width of the upper lip in hermaphrodites was ca. 1.43 times wider than that of females, and also the lower lip was ca. 1.49 times wider than that of female, respectively. Style length of hermaphrodites was longer than that of females (Fig. 1D, Table 1). Stamens of hermaphrodite flowers were clearly exerted from the corolla tube (Fig. 1A), whereas those of female flowers were not exerted (Fig. 1C). Width and length of nutlet in female flowers were longer than those of hermaphrodites (Table 1, Fig. 2).

Nutlet production ratio of females was higher than that of hermaphrodites in Na-Ju population (Fig. 2; H: Hermaphrodite: 0.37-45%, F: Female: 0.37-45%), but in Mun-San population was no significant difference between these sexual morphs (Fig. 2; H: ca. 0.52%, F: ca. 0.53%). The size of nutlet in female flowers was longer than those of hermaphrodites (Fig. 2). The hand-pollinated individuals in Na-Ju population did not produce nutlets at all, thus it seems to be self-incompatibility (Jang and Hong, pers. obs.).

Intermediate phenotype of *G. longituba* occurred in three populations (Mt. Cheong-Gye, Mun-San, and Na-Ju). During anther dehiscence, the fertile pollen grains were predominated in hermaphrodite (Fig. 1E), while in perfect flowers of the intermediate phenotype, pollen grains were smaller, with abnormal sterile pollen grains (Fig. 1F).

**Discussion**

Observations of *Glechoma longituba* in Korea revealed the presence of two distinct sexual morphs in which the population contains female and hermaphroditic individuals, i.e. gynodioecy.

Floral organs of *Glechoma longituba* were significantly larger in hermaphrodites than those of females in the populations studied (Table 1). In other gynodioecious taxa in angiosperms (ca. 131 species from 30 families), an overwhelming majority (98%) exhibits larger hermaphrodites than female flowers (Delph, 1996). According to Baker (1948), female flowers may be one-third to one-half size of flowers on hermaphrodite plants. Flowers of hermaphrodites in *G. hederacea* L. (the closely related species of *G. longituba*; cf. Jang, 2008; Jang and Hong, 2010) were ca. 1.45 times larger than those of female flowers (Delph, 1996). The ratios of flower size differences were similar with our results that corolla length of hermaphrodites was ca. 1.77 times larger than those of female flowers in four populations (cf. Table 1). Variation
in the pattern of flower size dimorphism was related to both the protective and attractive functions (Lloyd and Webb, 1977). Following an extensive survey of populations in China, Zhang et al. (2008b) and Bai et al. (2011) also reported that several reproductive characteristics (corolla length and mass, seed set and mass per each sex forms) were significantly different between hermaphrodites and female flowers. The hermaphroditic flower usually contains viable pollen and the flower of them must enclose both anthers and ovaries, whereas female do not produce pollen grains and only enclose ovaries. Hence corollas on hermaphroditic flowers may be larger than those on females (Delph, 1996 and references therein). This floral size dimorphism can result either as a reduction in female flower size that allows reallocation to greater fruit and seed production, or as an increase in hermaphroditic flower size resulting from the increased importance of pollinator attraction and pollen export for hermaphroditic flowers (Miller and Venable, 2003).

Upper and lower lip size difference in G. longinuba was clearly observed between two sexual morphs (P < 0.0001; cf. Fig. 1D; Table 1). Ten taxa of visiting insects as a pollinator (e.g., Amegilla zonata, Andrena spp., Anthophora plumipes, Apis cerana, Habropoda omeiensis, Liathrus spp., Megachile spp., Xylocopa sinensis; and two kinds of flies: Mesembrio flaviceps, Eristalinus tarsalis) to flowers of G. longinuba were reported in Chinese populations (Bai et al., 2011), hermaphroditic flower must increase surface area of lips, which may produce more nectar reward and more visual (Eckhart, 1999). Externally, stamens of hermaphrodite are clearly exerted from the corolla tube (Fig. 1A), whereas ease of female, stigma is exerted from the corolla tube (Fig. 1C). Exerted stigma of female may be making initial contact with flower visitor and previously deposited pollen grain. It is, therefore, promoted to outbreeding (Spira, 1980). Outcrossing advantage of females is a major role in the preservation of gynodioecious populations, but that females must also have other selective advantages such as reproductive compensation (Sun and Ganders, 1986).

On average, nutlet size of female was larger than those of hermaphrodite (Fig. 2). Female plants had a larger nutlet size, and produced more nutlet than hermaphrodite plants, as is often reported in several gynodioecious species (Delph and Lloyd, 1991; Hong and Moon, 2003; Shykoff et al., 2003; Zhang et al., 2008b). Female plants may have an increased female function compared to hermaphrodite because they do not allocate resources to male function such as pollen production (Hong and Moon, 2003; Lafuma and Maurice, 2006). Nutlet production of female was higher than that of hermaphrodite in Na-Ju, however in Mun-San was no clear difference between these sexual morphs (Fig. 2). The habitat of the Na-Ju was relatively wetter than Mun-San (Jang, per obs.). In Plantago lanceolata (Plantaginaceae), the relative seed fertility of hermaphrodites and females varies among natural populations (Van Damme and Van Delden, 1984) and female frequencies sometimes could also be correlated with ecological factors (e.g., conditions of habitat, cf. Krohme et al., 1980).

In the studied populations, pollen grains of hermaphrodites are well-stained and thus are fertile (Fig. 1E), while the intermediate individuals which produce sterile pollen grains (Fig. 1F). Ubers-Jiménez and Hidalgo-Fernández (1992) found three basic floral sex forms (i.e., hermaphrodite, female, intermediate flowers) in Rosmarinus officinalis L. (Lamiaceae), among them intermediate flower was produced collapsed pollen grains (i.e., sterile). As in other species of the gynodioecious plants (Koelwijn and Van Damme, 1996; Lafuma and Maurice, 2006; Rodríguez-Riallo and Dafni, 2007), G. longinuba reveal intermediate phenotype (Fig. 1B). On the other hand, Widén (1992) put this intermediate individual into the category of the hermaphrodite. Thompson et al. (2002) mentioned that intermediate individuals may influence the expression of sexual dimorphism, which is maintained at relatively high frequencies or almost absent in population. However this phenomenon still remains a puzzling question.

In conclusion, the results of the present study are indicated in context of the mechanisms maintaining gynodioecy in the studied populations. In this taxon, hermaphrodites produce larger flowers than females, on the other hand, nutlet size of hermaphrodite is smaller than female's one. Floral dimorphism, nutlet production, and pollen viability may allow compensation of female fitness.

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