

Elevational Floral Size Variation in *Prunella vulgaris*

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Abstract

Floral size is potentially influenced by local pollinators' body size. As pollinator fauna and size often change with elevation, correlation between elevational variation of pollinator size and floral size is expected. We investigated the variation of floral size in *Prunella vulgaris* L. (Lamiaceae) and the variation of their pollinator fauna along an elevational gradient. We measured the floral size of four traits: corolla length (CL), corolla tube length (CTL), corolla width (CW), and calyx length (CAL), in 23 populations, and found that CL and CTL were negatively correlated with elevation, and CW and CAL were not. Six bumblebee species visited the flower, and the visiting bee fauna differed among populations; the smallest and the largest bumblebee species visited the high elevational range (above 1800 m a. s. l.) populations, and the largest and the second largest bumblebee species visited the middle elevational range (1400 - 1800 m a. s. l.). Although abiotic factors can potentially affect floral size, the fact that we do not find an elevational decrease in CW and CAL suggests that the elevational change in *P. vulgaris*'s CL and CTL reflects the local pollinator size.

Keywords

Bumblebee, Flower Size, Geographic Trait Variation, Pollinator Fauna

1. Introduction

Geographical changes in floral size have been reported by many studies [1]-[3]. As the match between floral size and pollinator size is important for plants to achieve reproductive success [4] [5], selection by differently-sized pollinators potentially causes variation in floral size [6] [7]. Thus, geographical changes in floral size are expected to be mediated by geographical changes in pollinator fauna (and the resultant changes in pollinator size)

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[8]-[10]. As changes in pollinator fauna along an altitudinal gradient can be potential selective pressures on floral size, not a few studies have investigated altitudinal variation of floral size in relation to pollinator size (e.g. [11]) although the number of sampling sites and their altitudinal range are often limited.

In this study, we investigated the variation of floral size in *Prunella vulgaris* in 23 populations and the variation of its pollinator fauna along an elevational gradient. In the study area, six bumblebee species were observed to visit *P. vulgaris* flowers, from the largest to the smallest, *Bombus consobrinus* (Bc), *B. diversus* (Bd), *B. ussurensis* (Bu), *B. honshuensis* (Bh), *B. beaticola* (Bb), and *B. hypocrita* (Bhy). As these species have different body sizes and different elevational distributions, they are expected to exert selective pressure on *P. vulgaris* floral size in this system.

2. Materials and Methods

2.1. *Prunella vulgaris* and Study Sites

Prunella vulgaris L. (Lamiaceae) subsp. *asiatica* (Nakai) H. Harais is found throughout the temperate northern hemisphere, including Japan. In the study area, Nagano, Japan, *P. vulgaris* is found over a wide range of elevation (1300 - 2200 m a. s. l.). *Prunella vulgaris* is a perennial herb with protandrous purple flowers on inflorescences blooming in June to August (Figure 1). It grows in open habitat and is mainly pollinated by bumblebees. This study was carried out in wild populations of *P. vulgaris* on Mt. Norikura and in Utsukushigahara highland, Kaidakougen highland, and Kashima in Nagano, central Japan (Table 1, Figure 2). We grouped the study sites

Table 1. Study sites of *Prunella vulgaris*.

Site	Elevation (m)	Latitude (°N)	Longitude (°E)	Observed bumblebee species*	Date of census
N1	1360	36.152	137.628	—	Jul.14
N2	1440	36.122	137.630	Bd (3.04), Bu (0.15), Bh (1.52), Bhy (0.02)	Jul.14
N3	1450	36.113	137.630	Bc (0.07), Bd (0.04), Bh (0.51)	Jul.16
N4	1460	36.108	137.626	Bd (0.52)	Jul.16
N5	1734	36.111	137.606	None	Aug.5
N6	1800	36.113	137.598	Bc (3.75), Bb (0.42)	Aug.8
N7	1975	36.118	137.591	Bc (5.09), Bb (0.93)	Aug.2
N8	1995	36.121	137.588	Bc (5.49), Bb (0.09)	Aug.2
N9	2011	36.122	137.587	Bc (11.9), Bb (0.65), Bhy (0.02)	Aug.2
N10	2037	36.122	137.587	Bc (31.72), Bb (2.19)	Jul.16
N11	2050	36.123	137.585	Bc (2.38), Bb (2.06), Bhy (0.42)	Aug.8
N12	2137	36.122	137.580	Bc (0.77), Bd (2.04), Bb (0.26)	Aug.26
U1	1040	36.260	138.011	—	Jul.2
U2	1652	36.247	138.055	—	Jul.12
U3	1665	36.246	138.056	Bc (9.04), Bh (0.1)	Jul.26
U4	1705	36.249	138.065	Bc (0.66), Bd (0.2)	Jul.12
U5	1906	36.253	138.080	—	Jul.26
U6	1935	36.231	138.105	—	Jul.26
U7	2014	36.227	139.455	Bc (6.2), Bh (0.07), Bb (0.42)	Jul.26
KS1	1028	36.606	137.777	—	Jul.11
KS2	1300	36.597	137.804	—	Jul.11
K1	1210	35.906	137.563	—	Jul.14
K2	1340	35.971	137.544	—	Jul.14

*Observations of bumblebee species (visiting frequency per flower per 10 h) were made between 09:00 and 14:00 on sunny days during the peak flowering season within an observation quadrat (1 m × 2 m) in each population for at least 1 h. Bc: *Bombus consobrinus*; Bd: *B. diversus*; Bu: *B. ussurensis*; Bh: *B. honshuensis*; Bb: *B. beaticola*; Bhy: *B. hypocrita*; —: not censused; None, no bees were observed. According to Nagano *et al.* (2014), the pollinators' mean mouthpart lengths (distance from the base of the foreleg to the tip of the galea) in the Mt. Norikura area are: Bc, 20.3 mm; Bd, 16.3 mm; and Bb, 10.9 mm.

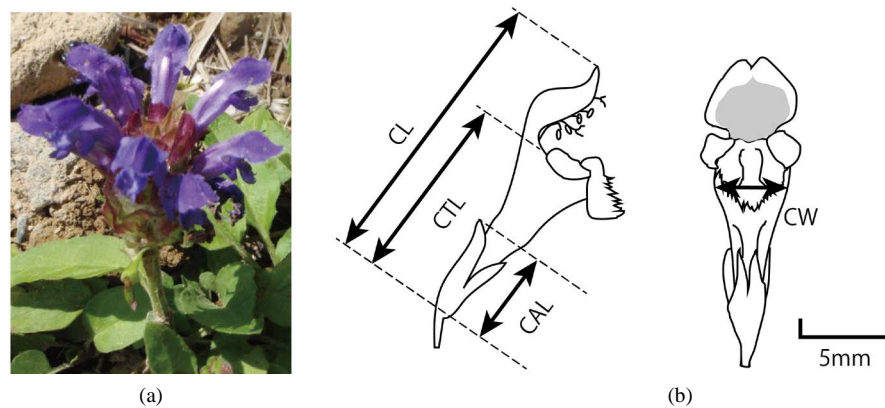


Figure 1. (a) An inflorescence of *Prunella vulgaris*. (b) The floral traits measured. CL, CTL, CW, and CAL indicate “corolla length”, “corolla tube length”, “corolla width” and “calyx length”, respectively.

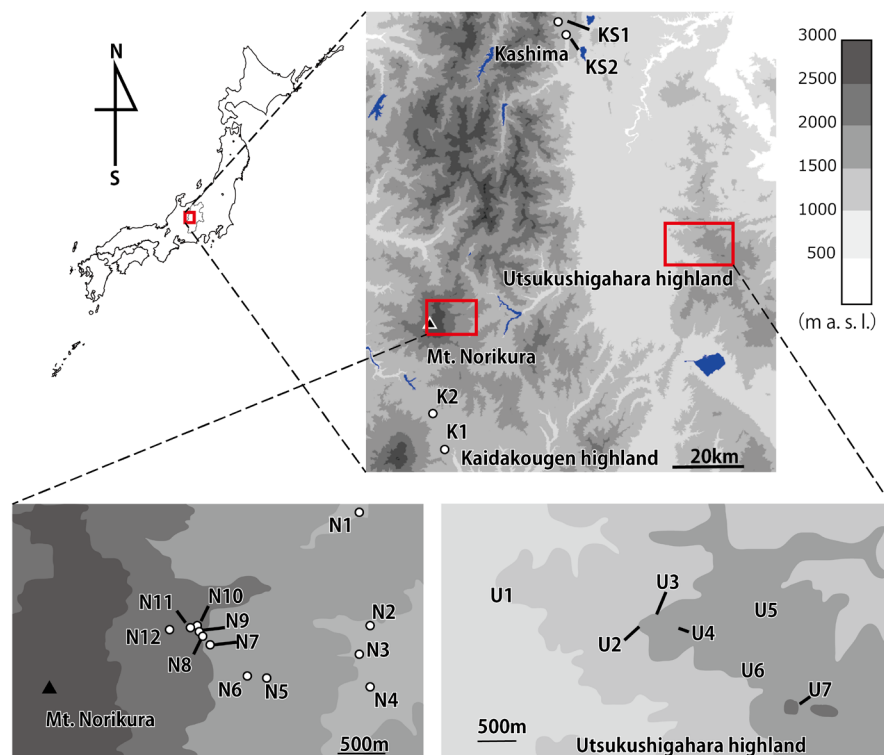


Figure 2. Locations of the study sites.

into three elevational ranges (<1400 m a. s. l., 1400 - 1800 m a. s. l., and >1800 m a. s. l.). In the study area, tree vegetation zone in 800 - 1400 m a. s. l. include montane deciduous broad-leaved forests whereas the upper elevational ranges include subalpine coniferous forests, and the smallest pollinator bumblebee species occur mostly in >1800 m a. s. l. (**Table 1**).

2.2. Measurement of Floral Size

During summer 2013, we measured the floral size of *P. vulgaris* in 23 populations (**Table 1**, **Figure 2**). In the peak flowering season of each population, 7 to 30 flowers were picked from each population (the lowest flower was picked from each randomly selected inflorescence) and stored in 70% ethanol. After one month preservation in 70% EtOH, we measured their corolla length (CL), corolla tube length (CTL), corolla width (CW), and

calyx length (CAL) by digital caliper (0.01 mm precision) (Figure 1).

CW can potentially inhibit the bumblebees from entering the flower. CL and CTL may influence pollinator efficiency, because the match, or the lack of one, between flower and bee may affect pollen attachment to the bee's body. CAL may not influence pollinator efficiency but can potentially inhibit nectar robbing from the base of the corolla.

We compared the four floral traits among the three elevational ranges (<1400 m a. s. l., 1400 - 1800 m a. s. l., and >1800 m a. s. l.), and among populations within each of the three elevational ranges by using one-way ANOVA and Tukey's HSD post hoc test. These analyses were performed with JMP ver. 9.0.2 software (SAS Institute, Japan). We tested the correlation of the floral sizes against elevation by using Pearson's correlation test.

2.3. Visiting Bumblebees

We observed visitors to *P. vulgaris* flowers in 14 populations (Table 1) in 2013. In each *P. vulgaris* population, we set an observation quadrat (1 m × 2 m), counted open flowers, and conducted observations for 1 to 2 h. The observations were made between 09:00 and 14:00 on fine days during the peak flowering season.

3. Results

3.1. Variation of *P. vulgaris* Floral Size along Elevational Gradient

Floral sizes differed among populations (Figure 3). Corolla length and corolla tube length significantly correlated with elevation (CL, $r = -0.349$, $P < 0.0001$; CTL, $r = -0.226$, $P < 0.0001$), but corolla width and calyx length did not correlate with elevation (CW, $r = -0.030$, $P = 0.545$; CAL, $r = -0.092$, $P = 0.067$).

Each floral dimension varied among the three elevational ranges (<1400 m a. s. l., 1400 - 1800 m, and >1800 m) (Figure 4; CL: $F = 34.76$, $P < 0.001$; CTL: $F = 15.55$, $P < 0.001$; CW: $F = 3.95$, $P = 0.0199$; CAL: $F = 7.61$,

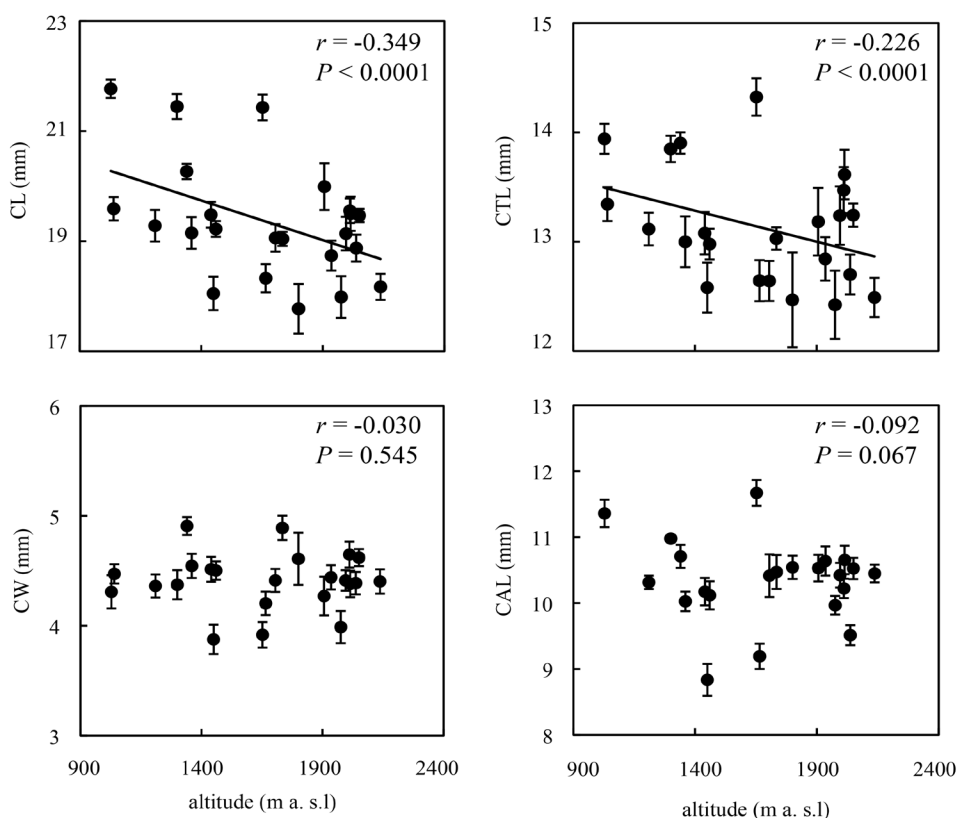


Figure 3. Elevational variation (mean \pm SE) of floral sizes (CL, CTL, CW and CAL, see Figure 1). Each circle represents a population.

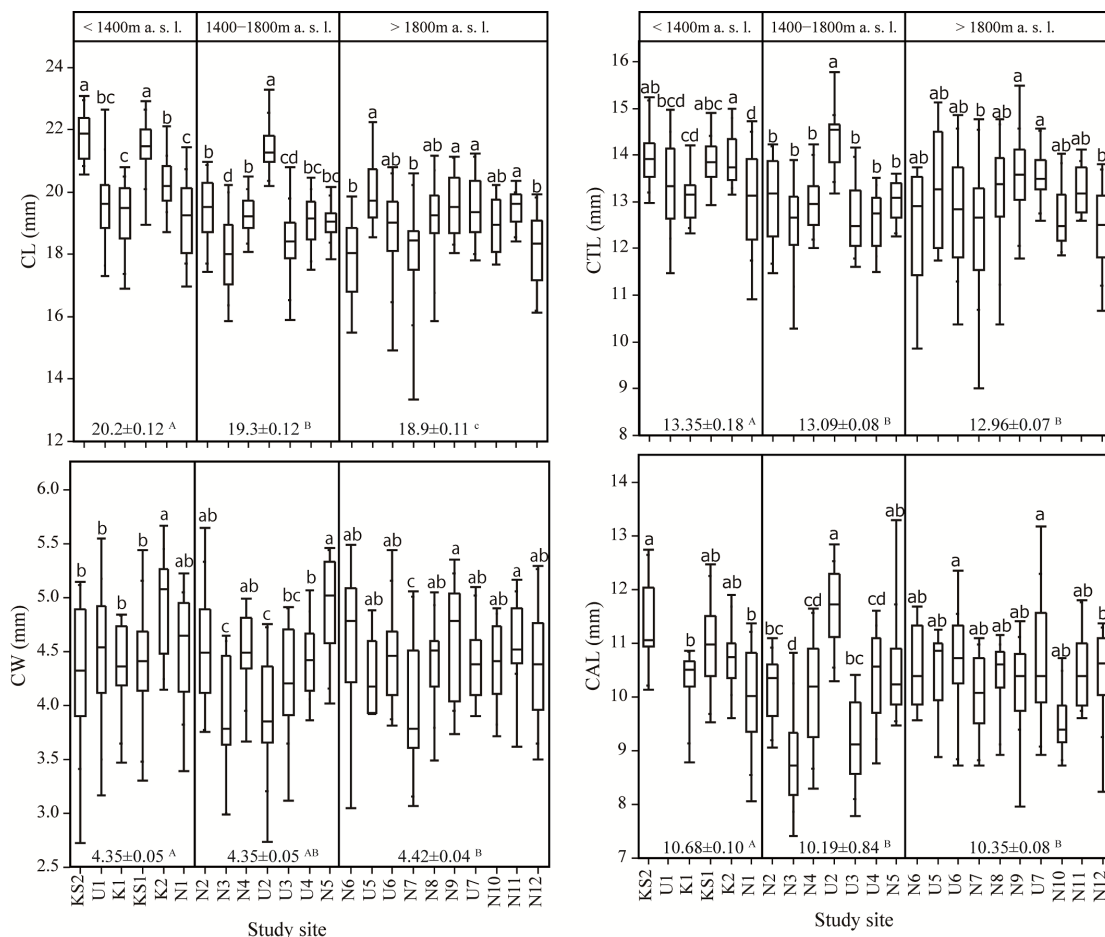


Figure 4. Box and whisker plots of *Prunella vulgaris* floral traits: CL, CTL, CW, and CAL indicate “corolla length”, “corolla tube length”, “corolla width” and “calyx length”, respectively. The bottom line in each box plot is the 25th percentile, the midline is the median, and the top is the 75th percentile. The whiskers extend to the lowest datum and the highest datum. Each floral dimension varied among the three elevational ranges (mean \pm SD indicated at the bottom, $P < 0.005$; one-way ANOVA, see text for results). The different uppercase letters indicate significant differences between elevational ranges ($P < 0.05$; Tukey’s HSD post hoc pairwise comparisons). Each floral dimension also varied among populations within each of the three elevational ranges ($P < 0.005$; one-way ANOVA, see text for results). Different lowercase letters indicate significant differences between the values within each of the three elevational ranges ($P < 0.05$; Tukey’s HSD post hoc pairwise comparisons).

$P < 0.001$), and among populations within each of the three elevational ranges (Figure 4; CL: $F = 21.94$, $P < 0.001$ at < 1400 m; $F = 30.37$, $P < 0.001$ at $1400 - 1800$ m; $F = 5.47$, $P < 0.001$ at > 1800 m; CTL: $F = 7.01$, $P < 0.001$ at < 1400 m; $F = 16.75$, $P < 0.001$ at $1400 - 1800$ m; $F = 3.17$, $P = 0.0016$ at > 1800 m; CW: $F = 4.33$, $P = 0.0012$ at < 1400 m; $F = 10.70$, $P < 0.001$ at $1400 - 1800$ m; $F = 2.82$, $P = 0.0042$ at > 1800 m; CAL: $F = 9.43$, $P < 0.001$ at < 1400 m; $F = 25.00$, $P < 0.001$ at $1400 - 1800$ m; $F = 2.93$, $P = 0.0031$ at > 1800 m).

3.2. Visiting Bumblebees

Bombus consobrinus (the largest bumblebee species in Japan) and *B. diversus* (the second largest species in Japan) were found at middle elevations ($1400 - 1800$ m a. s. l.; N2, N3, N4, U3, and U4; Table 1). *Bombus beaticola* (the smallest species in Japan) and *B. consobrinus* were found at over 1800 m a. s. l. (N6, N7, N8, N9, N10, N11, N12, and U7; Table 1).

4. Discussion

The results showed that *P. vulgaris* displays elevational/geographic variation in floral size. The pattern of varia-

tion was different among the four floral traits: corolla length and corolla tube length were both negatively correlated with elevation, but corolla width and calyx length did not correlate with elevation (**Figure 3**).

Corolla length (CL) and corolla tube length (CTL) may be under a selection regime correlated with elevation. Abiotic factors such as temperature, soil moisture, and insolation can potentially affect floral size [12]. However, we did not find any elevational decrease or increase in corolla width (CW) and calyx length (CAL); this fact suggests that clinal changes in abiotic factors may not be responsible for the floral variation, so other factors may be involved. When a bumblebee is collecting floral nectar of *P. vulgaris*, pollen grains attach to the dorsal side of its thorax or head. Therefore, if the bee proboscis is too long or too short relative to the corolla length or the corolla tube length, the pollination efficiency may decrease. Thus, CL and CTL are expected to be affected by the local bee proboscis length. CL and CTL were smaller in the high elevation range (>1800 m a. s. l.), where the smallest bumblebee species *B. beaticola* with short proboscis and the largest bumblebee species *B. consobrinus* with long proboscis visited *P. vulgaris* flowers (**Table 1**). On the other hand, CL and CTL were larger in middle elevation range, where the smallest species, *B. beaticola*, was absent and the two large *Bombus* species (*B. consobrinus* and *B. diversus*) frequently visited *P. vulgaris* flowers (**Table 1**). These results suggest that the elevational change in *P. vulgaris* corolla length and corolla tube length are affected by the local pollinator size, although the relative importance of the largest and the smallest bumblebee species as *P. vulgaris* pollinators in the high elevation range is unknown and should be explored in future studies. Further, the among-population variations in floral sizes within the same elevational range (**Figure 4**) suggest that a fine-scale difference in pollinator fauna may influence local floral size.

5. Conclusion

Prunella vulgaris displays elevational/geographical variation in floral size. Corolla length (CL) and corolla tube length (CTL) were negatively correlated with elevation, while corolla width (CW) and calyx length (CAL) were not. Six bumblebee species visited the flower, and the visiting bee fauna differed among populations. The smallest and the largest bumblebee species visited the high elevational range (above 1800 m a. s. l.) populations and the largest and the second largest bumblebee species visited the middle elevational range (1400 - 1800 m a. s. l.). These results suggest that local pollinator size rather than elevational abiotic change affects corolla length of *Prunella vulgaris*.

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