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Authors: Dohzono, Ikumi, Takami, Yasuoki, and Suzuki, Kazuo

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## Is Bumblebee Foraging Efficiency Mediated by Morphological Correspondence to Flowers?

Ikumi Dohzono<sup>1,4</sup>, Yasuoki Takami<sup>2</sup> and Kazuo Suzuki<sup>3,5</sup>

<sup>1</sup>Makino Herbarium, Graduate School of Science, Tokyo Metropolitan University, 1-1 Minami-Osawa, Hachioji, Tokyo 192-0397, Japan. <sup>2</sup>Graduate School of Human Development and Environment, Kobe University, 3-11 Tsurukabuto, Nada, Kobe 657-8501, Japan. <sup>3</sup>Department of Mathematical and Natural Sciences (Life Science), Faculty of Integrated Arts and Sciences, University of Tokushima, 1-1 Minami-Josanjima-cho, Tokushima 770-8502, Japan. <sup>4</sup>Present address: Graduate School of Human Development and Environment, Kobe University, 3-11 Tsurukabuto, Nada, Kobe 657-8501, Japan. <sup>5</sup>Deceased in Sep. 2004. Corresponding author email: [ikumi.dohzono@gmail.com](mailto:ikumi.dohzono@gmail.com)

**Abstract:** Preference for certain types of flowers in bee species may be an adaptation for efficient foraging, and they often prefer flowers whose shape fits their mouthparts. However, it is unclear whether such flowers are truly beneficial for them. We address this issue by experimentally measuring foraging efficiency of bumblebees, the volume of sucrose solution consumed over handling time ( $\mu\text{L}/\text{second}$ ), using long-tongued *Bombus diversus* Smith and short-tongued *B. honshuensis* Tkalcu that visit *Clematis stans* Siebold et Zuccarini. The corolla tube length of *C. stans* decreases during a flowering period, and male flowers are longer than female flowers. Long- and short-tongued bumblebees frequently visited longer and shorter flowers, respectively. Based on these preferences, we hypothesized that bumblebee foraging efficiency is higher when visiting flowers that show a good morphological fit between the proboscis and the corolla tube. Foraging efficiency of bumblebees was estimated using flowers for which nectar quality and quantity were controlled, in an experimental enclosure. We show that 1) the foraging efficiency of *B. diversus* was enhanced when visiting younger, longer flowers, and that 2) the foraging efficiency of *B. honshuensis* was higher when visiting shorter female flowers. This suggests that morphological correspondence between insects and flowers is important for insect foraging efficiency. However, in contradiction to our prediction, 3) short-tongued bumblebees *B. honshuensis* sucked nectar more efficiently when visiting younger, longer flowers, and 4) there was no significant difference in the foraging efficiency of *B. diversus* between flower sexes. These results suggest that morphological fit between the proboscis and the corolla tube is not a sole determinant of foraging efficiency. Bumblebees may adjust their sucking behavior in response to available rewards, and competition over rewards between bumblebee species might change visitation patterns in the wild. Thus, the determinants of foraging efficiency and visitation frequency for bee pollinators may be more complex than previously thought.

**Keywords:** *Bombus diversus*, *Bombus honshuensis*, *Clematis stans*, flower morphological change, foraging efficiency, nectar removal rate

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## Introduction

Effective foraging is important, especially in social insects in which foragers need to maintain colonies that include many non-foragers such as larvae and nursing individuals.<sup>1</sup> Social insects have evolved a variety of foraging strategies, including information transfer systems based on chemical signals or dance languages.<sup>2</sup> Preference for certain types of flowers in bee species may be such an adaptation, which can contribute to the fitness of individuals or colonies by discriminating between the most profitable flowers and others.<sup>3</sup> Visitation patterns of bees to flowers, however, could be influenced by other ecological processes, such as competitive interaction with other species sharing common flower resources, suggesting that visitation patterns of bees may not simply reflect their own preferences.<sup>4,5</sup> Here we address this issue whether 'preferred' flowers are profitable for the foragers, by evaluating foraging efficiency of two bumblebee species that share flowers of a single plant species but differently 'prefer' its varied floral morphology.

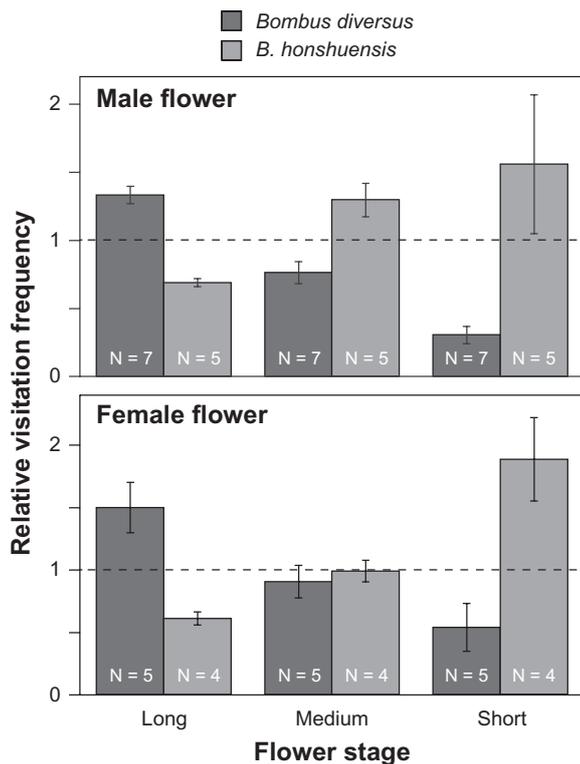
Many bee species are active foragers on floral resources (nectar and pollen) and therefore are the principal pollinators of many flowering plants. Such plant-pollinator relationships permit evolutionary modifications in insect foraging and plant reproductive tactics. Darwin hypothesized that nectar spurs and pollinator tongues (=proboscis) are engaged in a one-to-one coevolutionary race; the plants with the longest nectar spurs have a selective advantage because their reproductive organs optimally contact the pollinators, and thus they achieve the greatest reproduction, whereas pollinators with the longest tongues have a selective advantage because they obtain the largest food reward.<sup>6-10</sup> Pollination biologists have hypothesized that pollinator foraging efficiency depends on morphological correspondence between spur length and tongue length, by which the pollinator shows visitation preference for flowers with a good morphological fit.<sup>1,11-14</sup>

Bumblebee species (Hymenoptera, Apidae, genus *Bombus*) are often the principal pollinators of plant species with complex floral morphologies in temperate, arctic, and alpine zones in the Northern Hemisphere.<sup>1,15,16</sup> Bumblebees show differentiation in proboscis length,<sup>8,17</sup> whereby some bumblebee-specialized plants show conspicuous diversification in floral morphology and demonstrate morphological

correspondence between floral tube depth and the proboscis length of their particular bumblebee pollinator.<sup>17-20</sup> Thus, bumblebees and the flowers they visit provide opportunities for examining the effect of morphological correspondence between pollinators and flowers on pollinator foraging efficiency.

Graham and Jones<sup>4</sup> examined the relationship between visitation preference and foraging efficiency (nectar obtained per second spent in the flower) for two bumblebee species with different proboscis lengths. *Bombus appositus* Cresson, with a long proboscis, preferred a *Delphinium* sp. with a long spur, over an *Aconitum* sp. with a short spur, and foraged more efficiently from the former plant. In contrast, *B. flavifrons* Cresson, with a short proboscis, visited the latter flower species more frequently, but there was no significant difference in foraging efficiency between the two plant species. The authors concluded that *B. flavifrons* may be displaced from longer-spurred flowers by competition with *B. appositus*.

In the present study, we focus on a pair of species, *Bombus diversus* Smith and *B. honshuensis* Tkalcu, which have long and short proboscis lengths, respectively,<sup>17,19</sup> and which occur sympatrically on Honshu, the main island of Japan. Despite their differences in proboscis length, these two bumblebee species both visit the tubular flowers of *Clematis stans* Siebold et Zuccarini (Ranunculaceae). The floral morphology of this plant species changes temporally: the corolla tube length from the floral opening to the nectar repository decreases during the flowering period (3 to 4d). This length reduction results in flowers with various corolla tube lengths coexisting within a population and even within an inflorescence.<sup>19,21</sup> The pattern of visitation frequency to these flowers differs between the bumblebee species: long-tongued *B. diversus* frequently visits longer and younger flowers, whereas shorter-tongued *B. honshuensis* visits shorter and older flowers, irrespective of flower sex (Fig. 1).<sup>19</sup> Additionally, *C. stans* is sexually dimorphic in corolla tube length: male flowers are longer than female flowers, and the mean lengths of male and female flowers may correspond to the proboscis lengths of *B. diversus* and *B. honshuensis*, respectively.<sup>21</sup> Both bumblebee species prefer male flowers to female flowers because male flowers provide pollen resources in addition to nectar. Besides the effect of pollen resources on their



**Figure 1.** Relative visitation frequency of two bumblebee species to three flower stages (long, medium, short) of *Clematis stans* in the wild: *Bombus diversus* and *B. honshuensis* preferred longer and shorter flowers, respectively (modified from 19). Relative visitation frequency is a proportion of observed to expected number of visits at a certain type of flowers within a quadrat. Expected number of visits were derived from the number of each type of flowers in the quadrat under the assumption of no visitation preference (ie, random visit), which is scaled to unite and indicated by dashed lines. Weighted means (by total visits of the day)  $\pm$  1SE are shown. All the comparisons between flower stages are statistically significant (Kruskal-Wallis test,  $P < 0.05$ ) except the case that *B. diversus* visited female flowers ( $P = 0.063$ ).

visitation preferences, it is possible that the efficiency of nectar foraging *per se* depends on sexual difference in corolla tube length. Here we hypothesize that bumblebee foraging efficiency is higher when visiting flowers that show a good morphological fit between the proboscis and the corolla tube. Our a priori predictions are that 1) the foraging efficiency of *B. diversus* is higher in longer, younger flowers, whereas that of *B. honshuensis* is higher in shorter, older flowers, and that 2) the foraging efficiency of *B. diversus* is higher in longer, male flowers, whereas that of *B. honshuensis* is higher in shorter, female flowers. We tested these predictions by quantifying foraging efficiency using flowers with a controlled nectar quality and quantity in an experimental enclosure, and examined the effects of proboscis length and floral morphology (calyx tube length) on the foraging efficiency of the two bumblebee species.

## Material and Methods

### Bumblebees and flowers

Workers of *B. diversus* and *B. honshuensis* were collected from wild populations at Gotenba, in the foothills of Mt. Fuji, Shizuoka Prefecture (1300 m a.s.l.), and Kazuma, Nishitama-gun, Tokyo Prefecture (1000 m a.s.l.), respectively. These bumblebees were housed individually in polypropylene boxes (5 × 5 × 3 cm) for identification of individual bees. They were stored at 5–7 °C with moderate humidity maintained with damp cotton to suppress bee activity and avoid excess energy consumption. The experimental bees were fed a few drops of 20% sucrose water solution at 0900 and 2100 daily. These methods followed those of Matsumura and Washitani,<sup>22</sup> with a few modifications.

*Clematis stans* is a dioecious semi-arboreal plant, that is widely distributed throughout Honshu. The flowers are pendulous and apetalous. The four sepals are appressed to each other on the margins to form a calyx tube. Male flowers bloom for 3 days (occasionally 4) while female flowers bloom for 4 days (occasionally 5). During flowering, in both male and female flowers, the four sepals gradually curl up and become separated from each other, starting at the top, so that the tube becomes shortened and the styles or stamens become disclosed.<sup>19,21</sup> Inflorescences of *C. stans* were gathered from the wild population at Gotenba. These inflorescences were kept in a cool dark conditions.

### Foraging efficiency

To quantify the foraging efficiency of bumblebees in semi-natural conditions, we constructed an experimental enclosure using nylon mesh (1.8 × 1.8 × 2.0 m) on the campus of Tokyo Metropolitan University, Japan (140 m a.s.l.), wherein bumblebees were allowed to visit *C. stans* inflorescences. The inflorescences were cut at a slant and placed into floral form (OASIS, Smithers-Oasis Company, Cuyahoga Falls, USA) and supplied with water. The six inflorescences were arranged equidistantly in a row (20 cm apart). Male and female inflorescences were located at random, and included flowers with various lengths. Several flowers in each inflorescence were tagged arbitrarily (experimental flowers), removed the nectar using a capillary, and added 2  $\mu$ L of 20% sucrose solution (natural sugar concentration in *C. stans* is 10%–30%).<sup>21</sup> The calyx tube length



of these experimental flowers was measured using a digital caliper to the nearest 0.1 mm immediately after each experiment. At the start of each experiment, the lid of the bee box was opened in the enclosure and a bouquet of *C. stans* flowers (containing approximately 50 flowers) was served close to the bee to stimulate foraging. When the bee began to forage on the flowers, it was brought near other training flowers; the bee was then allowed to visit flowers freely. Twenty percent sucrose was added to some of the training flowers. Training was continued until bees voluntarily foraged among the flowers. Then, the bee was introduced to the experimental flowers using the bouquet. Subsequent visits to experimental flowers were recorded using a digital video camera (Panasonic NV-MX2000, Matsushita, Osaka, Japan), and the volume of sucrose solution remaining in experimental flowers at the end of the experiment was measured using a 2  $\mu$ L capillary (to the nearest 0.001  $\mu$ L). The bee was allowed to forage until it flew repeatedly to the walls of the enclosure. Then, the bee was caught and immediately transferred to the bee box and stored in a cooler with ice packs. Individual bees were used repeatedly during the experimental period, which included 18–20, 27, 28, and 30 August and 2, 3, and 6–9 September in 2001. Experiments were performed using seven *B. diversus* individuals and eight *B. honshuensis* individuals (Table 1). All of the individuals were used more than twice and visited more than three flowers per trip; a total of 306 visits were recorded (Table 1). There was no case of repeated visitation to one flower within a single foraging trip. The repeated use of individual bees is justified because it mimics their natural foraging behavior in the wild: individuals move from flower to flower in an inflorescence over the flowering period. The effect of repeated use was treated appropriately in statistical analyses (see below). After all of the experiments were completed, proboscis lengths (prementum and glossa) were measured to the nearest 0.1 mm using digital calipers.

Foraging efficiency was estimated as the ingestion rate, which was calculated as the volume of sucrose solution consumed over handling time ( $\mu$ L/second). Handling time was defined as the time spent on a flower, from the moment when the proboscis was extended for insertion into the corolla tube until the proboscis was pulled out of the corolla tube; handling time was

measured from video recordings (1/30 second unit). The proboscis of bumblebees consists of the prementum and glossa, and they show two types of sucking behavior: extension of both the prementum and the glossa or extension of the glossa alone.<sup>1,17,23</sup> Although it was often difficult to discriminate between the two types of sucking behavior on video recordings, we recorded these behaviors whenever possible.

## Statistical analyses

We examined the determinants of foraging efficiency using generalized linear mixed models by restricted maximum likelihood estimation (REML-GLMMs; Gaussian error). First, four GLMMs for foraging efficiency were constructed for each combination of bumblebee species and flower sex using the following independent variables: proboscis length, calyx tube length, interaction of proboscis length and calyx tube length (fixed effect), and bee identity (random term). The interaction term was included to examine the possible effect of correspondence between the proboscis and calyx tube lengths within each bumblebee species,<sup>24</sup> and the random term was to control for the repeated use of individual bumblebees. In addition, to examine the effect of variation in sucking behavior (extending the prementum or not), sucking behavior was added to the above four models as an additional independent variable; these models were constructed using cases in which sucking behavior was successfully recorded.

Second, two GLMMs for foraging efficiency were constructed for each bumblebee species by including flower sex, calyx tube length, their interaction term (fixed effect), and bee identity (random term) as independent variables, to examine differences in foraging efficiency between male and female flowers. The interaction term was included to detect possible differences in the effect of calyx tube length on foraging efficiency between flower sexes.

Finally, we examined whether foraging efficiency differs between bumblebee species by constructing GLMMs for each flower sex and including bee species, calyx tube length, and their interaction term as independent variables. The interaction term was included to detect possible differences in the effect of calyx tube length on foraging efficiency between bumblebee species. All measurements and foraging efficiency were  $\log_{10}$ -transformed before statistical analyses using JMP 6.0J.<sup>25</sup>

**Table 1.** Records on experimental bumblebee individuals.

Individual no.	Length of mouthparts (mm)			Locality	Number of flowers visited	
	Prementum	Glossa	Proboscis		Male flower	Female flower
<b><i>Bombus diversus</i></b>						
D1	3.7	7.5	11.2	Gotenba	21	0
D2	3.7	7.1	10.8	Gotenba	6	4
D3	3.9	9.0	12.9	Gotenba	5	0
D4	4.8	6.9	11.7	Kazuma	8	6
D5	4.5	7.3	11.8	Kazuma	14	31
D6	4.1	9.5	13.6	Kazuma	17	5
D7	4.2	5.5	9.7	Gotenba	14	36
<b><i>Bombus honshuensis</i></b>						
H1	3.1	4.5	7.6	Gotenba	7	15
H2	2.8	6.0	8.8	Gotenba	12	19
H3	3.1	4.4	7.5	Gotenba	3	0
H4	2.2	5.9	8.1	Kazuma	0	13
H5	3.8	5.0	8.8	Kazuma	7	5
H6	3.5	4.2	7.7	Kazuma	19	8
H7	3.1	6.0	9.1	Kazuma	12	5
H8	3.2	4.5	7.7	Kazuma	17	0

## Results

### Variation in proboscis length, calyx tube length, and sucking behavior

The *B. diversus* individuals used had longer proboscises than the *B. honshuensis* individuals [mean  $\pm$  1SD (mm), proboscis length: *B. diversus*,  $11.67 \pm 1.30$  (N = 7), *B. honshuensis*,  $8.16 \pm 0.64$  (N = 8), ANOVA for log-transformed scores,  $F_{1,13} = 21.2$ ,  $P = 0.0005$ ; glossa length: *B. diversus*,  $7.54 \pm 1.34$  (N = 7), *B. honshuensis*,  $5.06 \pm 0.78$  (N = 8),  $F_{1,13} = 52.0$ ,  $P < 0.0001$ , Fig. 2]. Calyx tube length was longer for male flowers visited by bumblebees than for female flowers [mean  $\pm$  1SD (mm), male:  $12.22 \pm 1.84$  (N = 165), female:  $8.70 \pm 1.26$  (N = 147), ANOVA for log-transformed scores,  $F_{1,310} = 384.6$ ,  $P < 0.0001$ , Fig. 2]. The mean calyx tube length of male flowers corresponded to the mean proboscis length of *B. diversus*, while the mean calyx tube length of female flowers corresponded to the mean *B. honshuensis* proboscis length (Fig. 2). These results indicate that our a priori assumptions in correspondence between the proboscis lengths and calyx tube lengths are met in the present study (see Introduction).

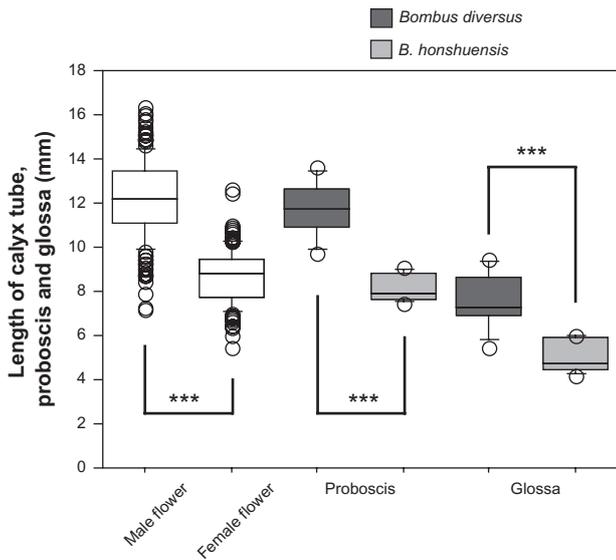
Variation in sucking behavior (extending the prementum or not) was recorded for 54 and 99 visits to male and female flowers, respectively. When visiting male flowers, individuals of both bumblebee species frequently extended their prementum when the

calyx tube length was longer than the bumblebee's proboscis length (Fig. 3A). Since the proboscis length of *B. honshuensis* was shorter than the calyx tube length of most male flowers (Fig. 2), they frequently pushed their heads into the calyx tube. When visiting female flowers, bumblebees seldom extended their prementum because the calyx tube was shorter than their proboscis (Fig. 3B).

### Foraging efficiency

When visiting male flowers, long-tongued *B. diversus* individuals sucked nectar significantly more efficiently when the calyx tube was longer as predicted, although there was no significant interaction between their proboscis length and the length of the calyx tube (Table 2). This was also true for visits by *B. diversus* individuals to female flowers. However, counter to our predictions, short-tongued *B. honshuensis* individuals sucked nectar significantly more efficiently from longer calyx tubes, and there was no significant interaction between proboscis length and calyx tube length (Table 2). No significant effect was detected for visits by *B. honshuensis* individuals to female flowers.

We found no significant effect of sucking behavior (extending the prementum or not) on foraging efficiency when *B. diversus* individuals visited male flowers or when *B. honshuensis* individuals visited either male or female flowers (GLMM for log-transformed



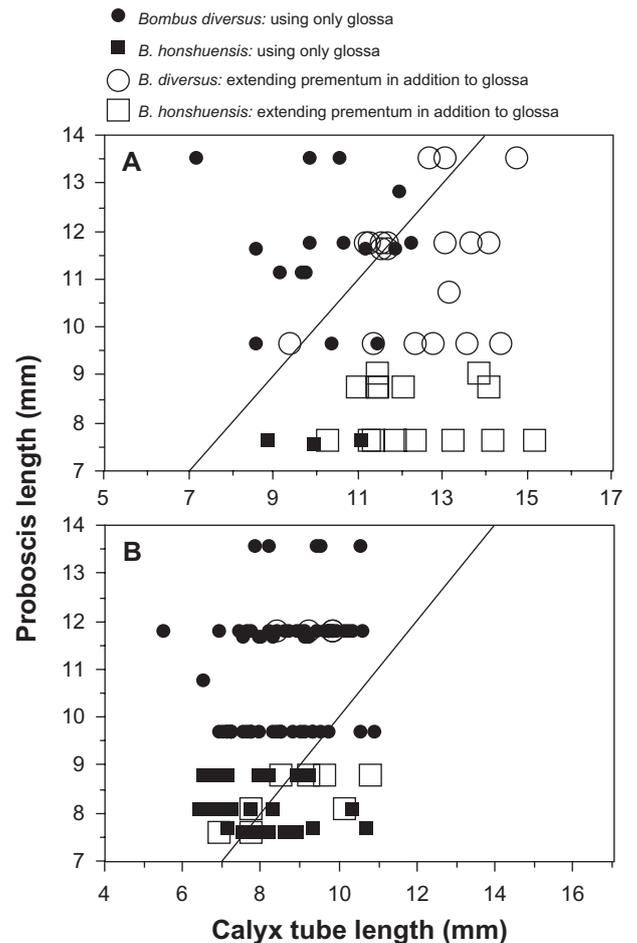
**Figure 2.** Variations in the calyx tube length of *Clematis stans* and in the proboscis and glossa lengths of the bumblebees. Boxes, lines in boxes, whiskers and circles show the quartiles, medians, 10% (90%) percentiles and outliers, respectively.

**Note:** \*\*\* $P < 0.001$ , see the text in detail.

scores, *B. diversus* to male flower:  $b = -0.08 \pm 0.05$  (SE),  $F_{1,31.4} = 2.51$ ,  $P = 0.12$ ; *B. honshuensis* to male flower:  $b = 0.00 \pm 0.12$ ,  $F_{1,10} = 0.00$ ,  $P = 0.99$ ; *B. honshuensis* to female flower:  $b = -0.03 \pm 0.08$ ,  $F_{1,31.5} = 0.13$ ,  $P = 0.72$ ). When *B. diversus* individuals visited female flowers, extension of the prementum tended to impede foraging efficiency ( $b = -0.15 \pm 0.07$ ,  $F_{1,61.1} = 3.85$ ,  $P = 0.054$ ). We did not include proboscis length and its interaction with calyx tube length in the GLMMs because the effects were not significant in the above analyses. This was also the case in the following analyses.

The foraging efficiency of *B. diversus* did not differ by flower sex; there was no significant interaction between calyx tube length and flower sex, and longer calyx tubes allowed higher foraging efficiency in both sexes (Table 3). By contrast, as predicted, the foraging efficiency of *B. honshuensis* was significantly higher with female flowers; there was no significant interaction between calyx tube length and flower sex, and longer calyx tubes allowed higher foraging efficiency in both sexes (Table 3).

With male flowers, the foraging efficiency of *B. diversus* was significantly higher than that of *B. honshuensis*, and the longer calyx tubes resulted in consistently higher foraging efficiencies in both species (Table 4). However, interspecific differences



**Figure 3.** Variation in the calyx tube length of flowers and the proboscis length of bumblebees, with respect to bumblebee's foraging behavior (extending prementum or not). **A)** male flowers; **B)** female flowers. Lines indicate that calyx tube length equals proboscis length (ie,  $y = x$ ), the right-under side of which means that the calyx tube is relatively longer than the proboscis, where bumblebees are likely to extend the prementum in addition to the glossa.

in foraging efficiency were not detected with female flowers (Table 4). Note that a large amount of non-explained variations is remained in our models as indicated by relatively low  $R^2$  values (0.11–0.28).

## Discussion

### Morphological correspondence and foraging efficiency

Morphology of flowers has been noticed as an important factor determining foraging efficiency of their pollinators. In tubular flowers, the longer calyx tubes may hinder the pollinators to access the nectar stored in the deeper site, or decrease their foraging efficiency, probably resulting in non-random visitation patterns that short-tongued pollinators avoid long-tubed flowers.<sup>1,8</sup> It has also been found

**Table 2.** Effects of calyx tube length and proboscis length on foraging efficiency of each bumblebee species visiting each flower sex. Results of generalized linear mixed model analyses for log-transformed scores are shown; non-integer degrees of freedom are due to REML method.

Independent variables	<i>b</i>	SE	df	<i>F</i>	<i>P</i>
<b><i>Bombus diversus</i></b>					
Male flower ( <i>N</i> = 85, Model <i>R</i> <sup>2</sup> = 0.18)					
Calyx tube length	1.003	0.436	1,76.4	5.29	<b>0.024</b>
Proboscis length	-0.611	1.241	1,3.9	0.24	0.65
Calyx tube length*proboscis length	-6.834	9.700	1,76.8	0.50	0.48
Female flower ( <i>N</i> = 82, Model <i>R</i> <sup>2</sup> = 0.11)					
Calyx tube length	1.266	0.564	1,76.6	5.03	<b>0.028</b>
Proboscis length	-0.793	1.335	1,2.3	0.35	0.61
Calyx tube length*proboscis length	5.861	12.499	1,77.3	0.22	0.64
<b><i>Bombus honshuensis</i></b>					
Male flower ( <i>N</i> = 77, Model <i>R</i> <sup>2</sup> = 0.28)					
Calyx tube length	1.368	0.545	1,71.0	2.51	<b>0.014</b>
Proboscis length	0.112	1.967	1,4.9	0.00	0.96
Calyx tube length*proboscis length	-19.006	17.700	1,71.6	1.15	0.29
Female flower ( <i>N</i> = 65, Model <i>R</i> <sup>2</sup> = 0.20)					
Calyx tube length	0.540	0.651	1,59.3	0.69	0.41
Proboscis length	-1.578	2.572	1,4.0	0.38	0.57
Calyx tube length*proboscis length	-7.557	23.271	1,58.4	0.11	0.75

that long-tongued pollinators tended to prefer long-tubed flowers, although they seemed to be able to suck the nectar from short-tubed flowers as well.<sup>4,8</sup> To address this, we hypothesized that the foraging efficiency of insect pollinators depends on their morphological correspondence to subject flowers. Our experimental quantification of bumblebee foraging efficiency showed mixed results. The following results supported our hypothesis: 1) the foraging efficiency of *Bombus diversus*, with a long proboscis, was enhanced when visiting younger, longer flowers, and 2) the foraging efficiency of *B. honshuensis*, with a short proboscis, was higher when visiting shorter, female flowers. However, contrary to our predictions,

3) short-tongued *B. honshuensis* individuals sucked the nectar more efficiently when visiting younger, longer flowers, and 4) there was no significant effect of flower sex on the foraging efficiency of *B. diversus*. These results suggest that bumblebees can not forage effectively in short flowers although they seem to be easier to handle, and correspondence between proboscis length and calyx tube length is not the sole determinant of foraging efficiency in bumblebees.

Other aspects of floral morphology may influence bumblebee foraging efficiency. Female *C. stans* flowers possesses a pistil that is relatively more slender than the bulk of stamens in male flowers, offering relatively wider spaces within the calyx tube and

**Table 3.** Effects of flower sex and calyx tube length on foraging efficiency of each bumblebee species. Results of generalized linear mixed model analyses for log-transformed scores are shown; non-integer degrees of freedom are due to REML method.

Independent variables	<i>b</i>	SE	df	<i>F</i>	<i>P</i>
<b><i>Bombus diversus</i> (<i>N</i> = 167, Model <i>R</i><sup>2</sup> = 0.13)</b>					
Calyx tube length	1.062	0.353	1,159.3	9.05	<b>0.0031</b>
Flower sex (female/male)	0.056	0.037	1,157.5	2.28	0.13
Calyx tube length*flower sex	0.074	0.350	1,158.2	0.04	0.83
<b><i>Bombus honshuensis</i> (<i>N</i> = 142, Model <i>R</i><sup>2</sup> = 0.22)</b>					
Calyx tube length	0.976	0.412	1,135.8	5.61	<b>0.019</b>
Flower sex (female/male)	0.175	0.042	1,137.4	16.96	<b>&lt;0.0001</b>
Calyx tube length*flower sex	-0.416	0.417	1,136.3	0.99	0.32



**Table 4.** Effects of bumblebee species and calyx tube length on the foraging efficiency on each flower sex. Results of generalized linear mixed model analyses for log-transformed scores are shown; non-integer degrees of freedom are due to REML method.

Independent variables	<i>b</i>	SE	df	<i>F</i>	<i>P</i>
<b>Male flower (N = 162, Model R<sup>2</sup> = 0.28)</b>					
Calyx tube length	1.215	0.351	1,154.3	11.98	<b>0.0007</b>
Bumblebee species ( <i>diversus/honshuensis</i> )	0.109	0.039	1,10.9	7.81	<b>0.018</b>
Bumblebee species*calyx tube length	-0.223	0.351	1,154.3	0.40	0.53
<b>Female flower (N = 147, Model R<sup>2</sup> = 0.16)</b>					
Calyx tube length	0.892	0.420	1,139.5	4.51	<b>0.036</b>
Bumblebee species ( <i>diversus/honshuensis</i> )	0.013	0.048	1,8.0	0.07	0.80
Bumblebee species*calyx tube length	0.311	0.420	1,139.5	0.55	0.46

possibly allowing bumblebees to forage more freely. If bumblebees can suck nectar more easily from female flowers, two of the results for *B. diversus*, 1) and 4) described above, are not contradictory because this effect would compensate for the possible reduction in *B. diversus* foraging efficiency with female flowers. This idea is also congruent with result 2): *B. honshuensis* foraged more efficiently on female flowers. During foraging, individuals of this short-tongued species often inserted their heads into calyx tubes, in addition to extending their proboscis, suggesting that the space in the floral tubes was important for this short-tongued forager. These arguments illuminate another aspect of morphological correspondence between foragers and flowers and its effect on foraging efficiency, although further studies are necessary to discriminate between the effects of calyx tube length and floral space.

One very unexpected result was that the short-tongued *B. honshuensis* individuals foraged more efficiently on longer male flowers [result 3) above]. Despite the fact that their proboscises were shorter than the calyx tubes of male flowers (Fig. 2), individuals frequently extended their prementum (Fig. 3) and inserted their head into the calyx tube, which may have allowed them to suck nectar. However, why longer flowers that appear to be more difficult for foraging by short-tongued bumblebees resulted in higher foraging efficiencies remains to be explained. One hypothesis is that bumblebees can adjust their sucking speed in response to flower age, reflecting resource availability. In *C. stans*, flowers with longer calyx tubes are newly opened and are more likely to contain nectar.<sup>21</sup> During an individual flowering period, calyx tube length decreases; nectar is continuously secreted

but is often consumed by other pollinators, suggesting that the volume of nectar in shorter, older flowers is more unpredictable. Although bumblebees require extra energy to extend their prementum in addition to the glossa when sucking from longer flowers,<sup>1</sup> newly opened flowers with much higher nectar content might offset such costs. It remains to be examined whether bumblebees change their foraging behavior, and if so, what cues (calyx tube length, odor, UV reflectance etc.) are used by them as an indicator of flower age and/or resources.

#### Foraging efficiency and visitation frequency

In the field, *B. diversus* and *B. honshuensis* individuals more frequently visited longer and shorter male *C. stans* flowers, respectively (Fig. 1).<sup>19,21</sup> This visitation ‘preference’ by *B. diversus* individuals was almost congruent to their foraging efficiency, as discussed above. However, the visitation pattern of *B. honshuensis* individuals in the wild largely contradicted their apparent foraging efficiency. The less efficient nectar foraging in short male flowers by *B. honshuensis* could be compensated by foraging pollen in some extent. Our analysis of interspecific differences in nectar-foraging efficiency showed that *B. diversus* can forage more efficiently from male flowers than can *B. honshuensis* (Table 4). This finding may explain the visitation pattern of *B. honshuensis*, which is suboptimal with respect to its foraging efficiency. These two bumblebee species may be potential competitors in the wild because they occur sympatrically and share flower resources. Since *B. honshuensis* exhibits lower foraging efficiency in at least male flowers, they may be displaced from longer flowers by competition with *B. diversus*. Although the mechanism involved in this possible competitive

exclusion is unclear, it is known that resource competition between bumblebee pollinators can influence visitation frequency and preference through signs that remain on previously visited flowers.<sup>5,26</sup>

Evidence for competitive exclusion between short- and long-tongued species was also reported by Graham and Jones,<sup>4</sup> who concluded that short-tongued *B. flavifrons* individuals can be displaced from longer-spurred plant species by competition with long-tongued *B. appositus* individuals. The long-tongued bumblebee visits long-spurred flowers more frequently and forages more efficiently therein, while the short-tongued species visits short-spurred flowers more frequently despite it can forage in both long- and short-spurred species with equal efficiency. The present study corroborates this hypothesis based on the result of an experiment that used a single plant species with varied floral morphology, which allowed us to exclude effects from unexamined differences between plant species. Thus, competition between pollinator species can be an important factor affecting visitation frequencies, in addition to foraging efficiency.

In conclusion, divergence in visitation ‘preference’ between pollinator species has been seen as a process of specialization in pollination systems and is thought to be mediated by morphological correspondence between flowers and pollinators, which enhances pollinator foraging efficiency as well as plant reproduction.<sup>1,4,13,27</sup> We performed experimental analyses of foraging efficiency using two bumblebee species and one plant species with varied floral morphology, and showed that morphological correspondence between insects and flowers can be an important factor affecting foraging efficiency. Additionally, our data also suggest that other factors, possibly behavioral adjustments and resource competition between insect pollinators, might also affect their foraging efficiency and visitation frequency. Thus, although bee pollinators often prefer the flowers that show a good morphological fit to their mouthparts, such a visitation pattern does not ensure that their foraging efficiency is maximal in those flowers.

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## Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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