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Colored floral organs influence pollinator behavior and pollen transfer in *Commelina communis* (Commelinaceae)[1]

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Visual floral guides such as colored anthers, lines, dots, and UV-absorption patterns on petals are commonly observed in insect-pollinated angiosperms. Floral guides that are known to enhance foraging efficiency of visitors on flowers thus promote return visits (foraging facilitation hypothesis, which predicts that visitors will discriminate against flowers with inferior floral guides). In this study, we experimentally examined the hypothesis that floral guides also prevent pollen-theft behavior by floral visitors (theft prevention hypothesis), which has rarely been tested. Nectarless flowers of *Commelina communis* have three types of brightly colored floral organs: large blue petals, rewarding yellow anthers, and nonrewarding yellow anthers. Colored floral organs were removed artificially from plants in two natural populations of *C. communis*. Removal of the nonrewarding yellow anthers diminished hoverings in front of flowers and tended to reduce the number of total floral visitor landings, supporting the foraging facilitation hypothesis. Additionally, removal of the rewarding yellow anther decreased the frequency of legitimate landings on flowers and the legitimate landing-to-total landing ratio, which is consistent with the theft prevention hypothesis. The nonrewarding anthers and the rewarding yellow anthers were shown to play an important role in increasing visitor landings and orienting floral visitors toward a landing point appropriate for pollination, respectively. We also showed that the absence of yellow anthers decreased both pollen dispatch from brown anthers and receipt by stigmas in *C. communis*. These findings support both the foraging facilitation hypothesis and the theft prevention hypothesis.

Key words: anther differentiation; *Commelina*; floral guide; pollen theft; zygomorphic flower.

Flowering plants are sessile; as a consequence, they require pollen vectors for sexual reproduction. The current diversity of floral characteristics such as shape, size, color, and scent is thought to have evolved under natural selection mediated by animal vectors of pollen. Flowers usually have complex floral attractants, such as olfactory and visual signaling cues, which orient flower-visiting animals toward floral rewards. Floral fragrance and showy petals attract visitors (potential pollinators) over long distances; subsequently, pollen odor and color, anthers, lines, dots, UV-absorption patterns, and petal micro-texture act as floral guides directing visitors to rewards (Sprengel, 1793; Kevan and Lane, 1985; Bergstrom et al., 1995; Lunau, 2000, 2001).

Visitors are believed to prefer flowers with floral guides that increase the efficiency of foraging and locating rewards. Flowers with superior floral guides are visited by pollinators more frequently and consequently set more seed (Waser and Price, 1983, 1985). Hereafter, we refer to this hypothesis as the "foraging facilitation hypothesis" of floral guide function; this hypothesis has been examined primarily using artificial flowers (Lunau et al., 1996; Kandori and Ohsaki, 1998; West and Laverty, 1998; Lunau, 2000; but see Waser and Price, 1983, 1985).

Animals often land on flowers and forage floral rewards without touching anthers or stigmas (Inouye, 1980; Irwin and Maloof, 2002). Although theft (robbing) behavior may increase foraging efficiency for the visitor, it often decreases plant fitness (Irwin and Brody, 1999, 2000; Maloof and Inouye, 2000). It is therefore reasonable to propose that plants would have a counterstrategy to avoid nectar or pollen theft by floral visitors. In addition to attracting pollinators, floral guides are believed to control pollinator landing behaviors, thus enhancing legitimate contact with mating-related anthers and stigmas during foraging. In this study, we tested the hypothesis that floral guides function to reduce theft behavior by controlling a visitor's landing position on a flower, hereafter referred to as the "theft prevention hypothesis." This hypothesis does not exclude the foraging facilitation hypothesis, but does lead to a different prediction. The foraging facilitation hypothesis predicts that floral guides increase the number of landings (visits) per flower, whereas the theft prevention hypothesis predicts that floral guides increase the ratio of legitimate landings (for pollination success) to total landings on a flower. This aspect of floral guide function has rarely been examined experimentally because most studies of floral guides have used artificial flowers with simple structures.

Bilaterally symmetrical (zygomorphic) flowers are thought to have evolved from the primitive, radially symmetrical (actinomorphic) form (Tucker, 1984) under selection favoring pollinator specificity (Neal et al., 1998). Zygomorphic flowers are suitable for testing the theft prevention hypothesis because their anthers and stigmas are often in specific positions (such as at the top or bottom of a flower, or inside guard petals, e.g., Orchidaceae, Fabaceae, and Scrophulariaceae), to control the visitor's landing site and movement on a flower (Ushimaru and Hyodo, 2005).
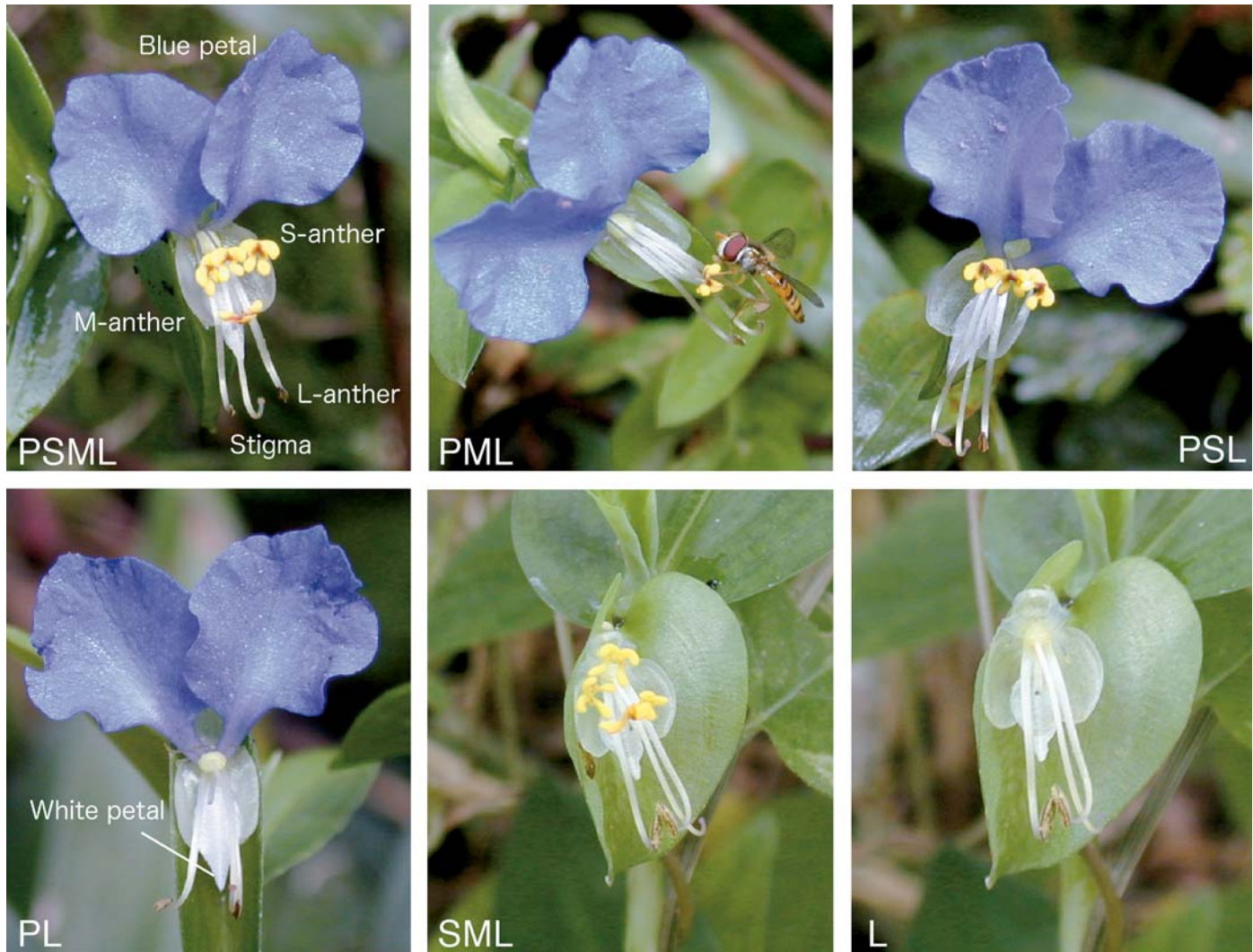
Fig. 1.   Artificially manipulated flowers exposed to natural pollinator visits (refer to the text for abbreviations). *Commelina communis* has several colored floral organs that differ in function: blue petals, three yellow S-anthers and one yellow M-anther. One petal is white and reduced in size.

Commelina communis L. (Commelinaceae) is an ideal test subject for the theft prevention and foraging facilitation hypotheses because its zygomorphic flowers have several monochromatic attraction-related organs, for example, blue petals and both rewarding and nonrewarding yellow anthers, which are thought to differ in function (Fig. 1; Vogel, 1978; Faden, 1992; Ushimaru et al., 2003b; Hrycan and Davis, 2005). Moreover, these organs are easily detached (Fig. 1). The flower of *C. communis* has three types of stamens: two long stamens, one medium length stamen, and three short stamens (staminoides) (Fig. 1; Ushimaru et al., 2003b). The anthers of the long stamens (L-anther) and medium length stamen (M-anther) produce fertile pollen; the anthers of the short stamens (S-anther) produce only a small amount of sterile pollen and function only in display (Morita and Nigorikawa, 1999; see also Hrycan and Davis, 2005). To the human eye, the M- and S-anthers are bright yellow and more conspicuous than the brown L-anthers (Faden, 1992). In *C. communis*, the filaments of long stamens typically become elongate and appear to function as a landing platform equivalent to the floral lower lips of the Orchidaceae (Figs. 1, 2). The two types of yellow anthers are considered to function as floral (pollen) guides to floral visitors in *Commelina* (Lunau, 2000; Hrycan and Davis, 2005). *Commelina communis* flowers do not produce nectar, and pollen is the only floral reward for floral visitors such as other Commelinaceae species (Faden, 1992). It has been noted that pollen from the L-anthers contributes to outcrossing, whereas pollen from the M-anthers functions mainly as a reward for insects visiting *Commelina* species (Vogel, 1978; Faden, 1992; Morita and Nigorikawa, 1999; Hrycan and Davis, 2005). Ushimaru et al. (2003a, b) suggested that the position of fertile stigmas may have evolved to maximize reception (from visiting insects) of pollen grains from L-anthers that are of the same length as the stigmas in perfect flowers.

Examining the role of S- and M-anthers and blue petals in controlling floral visitor behavior would be one step toward understanding the evolution of complex floral attraction, especially for widespread colored anthers in angiosperms. Here, to test the foraging facilitation hypothesis and the theft prevention hypothesis, we examined the effects of floral guides

Fig. 2.   Landing by a syrphid fly (*Episyrphus balteatus*) on a flower of *Commelina communis*. The fly is touching the long stamens and stigma with its abdomen and carrying pollen (arrow) while feeding on the pollen of a medium length stamen (legitimate landing).

on the visiting behaviors of insects by experimentally manipulating the morphology of *Commelina* flowers that were exposed to natural floral visitors. We divided visitor behaviors into three categories, i.e., approach, hovering, and landing (legitimate landings and others) to clarify the function of each floral guide in controlling each categorized behavior. The foraging facilitation hypothesis predicts that the removal of yellow anthers will decrease the total landing number and the landing-to-approach ratio, whereas the theft prevention hypothesis predicts that this removal will change the landing positions of visitors and increase pollen theft behaviors (decrease legitimate landings and the ratio of legitimate landings to total landings). In another field experiment, we tested whether the yellow S- and M-anthers removal decreases pollen transfer in *C. communis*. We discuss (in the context of our results) the functional differentiation found in three types of anthers in *C. communis*.

MATERIALS AND METHODS

Species and sites—*Commelina communis* is an annual, andromonoecious herb, that is natively distributed in temperate northeastern Asia, often growing around rice fields and along roadsides. One plant usually has many inflorescences, in which perfect and staminate flowers open at sunrise each day and remain open until noon. An inflorescence rarely has more than one open flower.

Commelina communis is self-compatible and can have delayed autogamy and bud pollination (Morita and Nigorikawa, 1999). The pistils and long stamens roll up when anthesis terminates, stimulating autonomous self-pollination through L-anther–stigma contact (Morita and Nigorikawa, 1999).

However, the high ratios of pollen per ovule (2100–2500) in perfect flowers, which are greater than those of facultatively xenogamous species (Cruden, 1977), and the existence of staminate flowers, both suggest that pollinator-mediated outcrossing is important for this species (Morita and Nigorikawa, 1999).

In 2002, we conducted field experiments in two populations: one in Yamanaka, Kyoto (35°02′ N, 135°49′ E), and the other in Kazagashira Park, Nagasaki (33°44′ N, 129°53′ E), Japan, to examine the effect of colored organs on the behavior of floral visitors. The population at Kazagashira Park was larger than that at Yamanaka.

Diverse insect groups (syrphid flies and social and solitary bees) have been reported to visit *C. communis* flowers (Tanaka, 1978; Ushimaru and Hyodo, 2005). This is consistent with a previous report on visitors to Commelinaceae species (Faden, 1992).

At Yamanaka, native syrphid flies (*Episyrphus balteatus* in most cases) were the exclusive visitors to *Commelina* flowers, whereas Japanese honey bees (*Apis cerana japonica*) and syrphid flies (*E. balteatus*) were observed visiting flowers at Kazagashira Park.

In 2004, another field experiment was conducted to examine the effect of yellow anthers on pollen transfer in Iwakura, Kyoto (35°05′ N, 135°47′ E). Most floral visitors were *E. balteatus*; visiting bumblebees (*Bombus diversus*) were rarely observed at this site.

Field experiment—Floral visitor behaviors—Six flower types were prepared artificially and exposed to natural insect visits (Fig. 1): PSML, intact flowers as controls; PML, flowers without S-anthers; PSL, flowers without the M-anther; PL, flowers with neither S- nor M-anthers; SML, flowers without large blue petals; and L, flowers without petals and S- and M-anthers. In these treatments, we did not remove the stamen filaments. In *C. communis*, one petal is reduced in size and inconspicuous, as in other zygomorphic Commelinaceae flowers (Fig. 1; Faden, 1992). We did not modify this small petal.

We used digital video cameras to record floral visitor behavior on these flowers. In each experimental trial, we arbitrarily chose a flower patch, and haphazardly selected and manipulated three to six flowers, including more than three of the manipulated flower categories (with at least one control in every session), for a single 15-min videorecording. Ushimaru et al. (2003b) found that the blue petals are larger in perfect flowers (11.8–13.9 mm mean length) than in staminate flowers (10.6–13.0 mm). Therefore, we used only perfect flowers for the trials to exclude sex differences in flower size. We did not account for the position of flowers within inflorescences. Video cameras were set up 50–100 cm away from the experimental flowers. For each trial, we used a new patch of flowers. We made all recordings (44 at Yamanaka from 9 to 19 September and 36 at Kazagashira Park from 12 to 18 September) during the morning (0700–1015 hours). No insect visits were observed during the videorecording in approximately 20% of the trials (25% for the Yamanaka population; 19.4% for the Kazagashira Park population).

Behavior categorization—We categorized floral visitor behavior into three groups: (1) Approaching: finding the flower and approaching from the front. (2) Hovering with regard to syrphid flies, hovering in front of the flower and repeatedly collecting pollen from the M- or L-anthers (flies may have been assessing the amount of pollen available). For honey bees, typical collecting behavior was not conspicuous, but they were observed hovering in front of the flowers. In most cases, the video image quality was not adequate to distinguish antennal contact with floral organs. (3) Landing, actually alighting on flowers.

We further divided landings into legitimate and other landing types (Fig. 2; Ushimaru and Hyodo, 2005). In a legitimate landing, insects clasped onto filaments of L- or M-anthers or onto small white petals and foraged or collected pollen grains from the M-anther, simultaneously touching L-anthers or stigmas or both with their abdomens (Figs. 2 and 3, left). In other landing types, syrphid flies clung to the L-anthers (L-anther landing) and directly ingested pollen (Fig. 3, middle), or landed on blue petals (petal landings) and foraged pollen while rarely touching mating-related organs (Fig. 3, right); honey bees collected pollen from L-anthers while touching stigmas only infrequently. L-anther landings were frequently observed and would be expected to affect the efficiency of pollen transfer.

For syrphid flies on PMSL, PML, PSL, and PL flowers, we used video films of landings to determine the anthers on which the insects fed. We did not investigate the amount of pollen that they consumed on the anthers.

Behavior analyses—We counted the number of behaviors seen in each recording session. If we observed a repetition of hoverings and landings by a

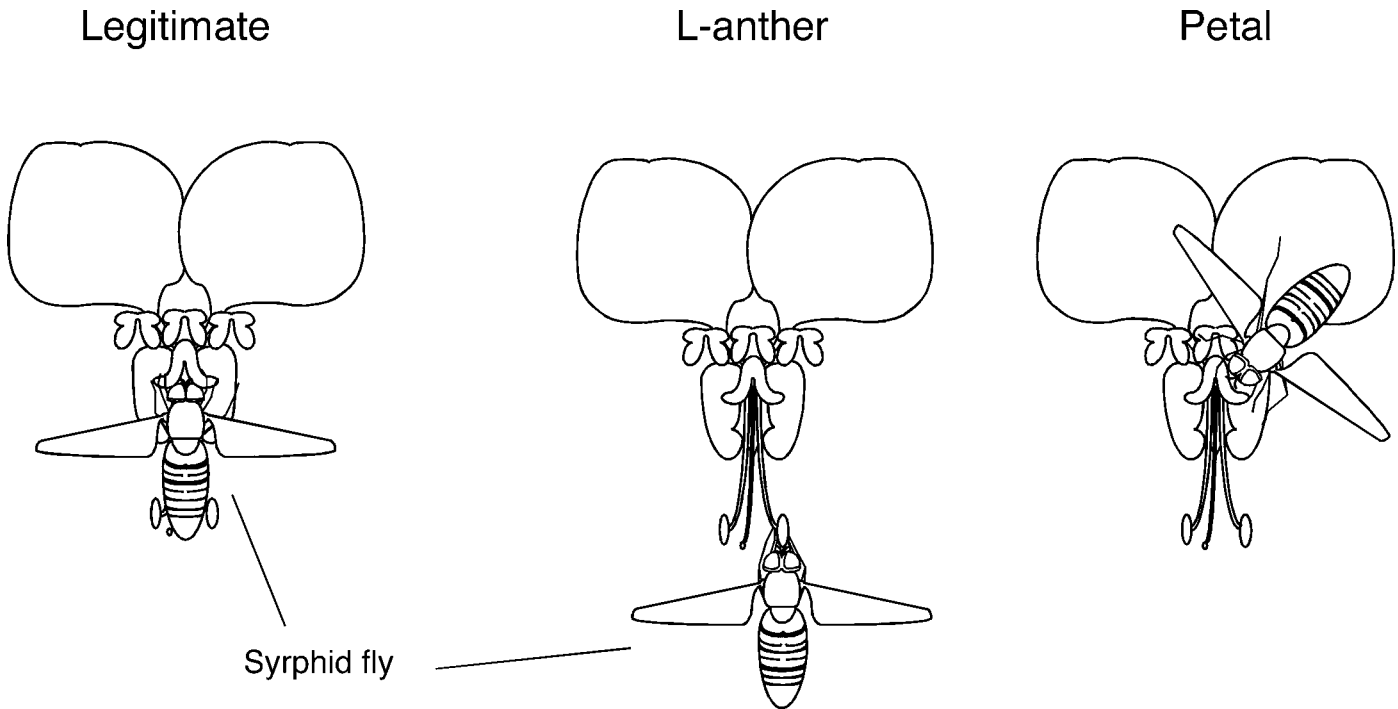# Legitimate   L-anther   Petal

Fig. 3.   Illustrations of legitimate, L-anther and petal landings of a fly on *Commelina communis*.

syrphid fly (i.e., approach, hovering, landing, hovering, landing) in a single visit, we designated it as one hovering and one landing. When both legitimate and other landings were observed in a single bout, we used the ratios of each to the total number of landings as the behavior numbers. For example, if one legitimate landing and one L-anther landing were seen, we designated the frequencies as 0.5 and 0.5, respectively. For the analyses, we used recordings containing at least a single approach of a syrphid fly or honey bee to an experimental flower, which thus provided 33 and 20 records for syrphid flies at Yamanaka and Kazagashira Park, respectively, and 18 records for honey bees at Kazagashira Park. At Kazagashira Park, both syrphid flies and honey bees visited experimental flowers in nine records, and 11 records for only syrphid flies and nine records for only honey bees (a total of 29 records were used in analyses). At each site, a single bumblebee (*B. diversus*) was observed visiting the flowers during the experiment, but these data were ignored in the analyses.

First, we examined the effects of petal, S-, and M-anther on the frequency of approach behaviors by syrphid flies and honey bees per flower per 15-min interval using generalized linear models (GLM) with Gaussian errors (identity link). Site (Yamanaka or Kazagashira Park) and flower morphology (presence or absence of petal, S-, and M-anther) were independent variables for syrphid flies. For honey bees, flower morphology (presence or absence of petals and S-, and M-anthers) was the single independent variable. In these models, we used data from all six types of experimental flowers.

We then examined two hypotheses relating to the function of floral guides, excluding data for SML and L flowers for comparison of hovering and landing behaviors (i.e., we used PSML, PML, PSL, and PL flower data for the test). To test the two hypotheses, we again used GLM with Gaussian errors (identity link) to examine the effects of S- and M-anthers (presence or absence) on the hovering, landing, legitimate landing, and L-anther landing behaviors of syrphid flies and honey bees at each site.

To determine the behaviors that were affected by a specific floral guide, we also compared the ratios of hoverings to approaches (total number of hoverings/total number of approaches), landings to hoverings (total number of landings/total number of hoverings), and legitimate landings to landings (total number of legitimate landings/total number of landings) between control flowers and flowers without S- or M-anthers or both for each insect at both sites. We also compared the ratios of landings to approaches between control flowers and anther-removed flowers. A chi-square test and Fisher's exact test were applied to these comparisons. Significance levels were adjusted with a sequential Bonferroni test (Rice, 1989). The foraging facilitation hypothesis predicts that

yellow anther removal will decrease the ratios of hoverings to approaches and landings to approaches, whereas the theft prevention hypothesis predicts that the ratios of legitimate landings to total landings should decrease with the removal of the yellow anthers.

Pollen transfer—We examined the effect of yellow anther removal on pollen transfer from dehiscent L-anthers to the stigmas by conducting a field experiment at Iwakura (35°05′ N, 135°47′ E) from 13 to 15 September 2004. We collected the stigmas and L-anthers of 10 newly opened flowers (nonvisited) at 0600 hours when no syrphid fly visits were observed. We also prepared 26 PSML and 14 PL flowers at 0600 hours and exposed them to natural insect visits until 1000 hours on the same day to examine the effect of illegitimate (mostly L-anther) landings on pollen transfer, which often occurred on flowers without both S- and M-anthers. The stigmas and L-anthers from these experimental flowers were then collected and stored separately in 0.1 mL of 70% ethanol. We counted the pollen on stigmas using a light microscope (×85). Because pollen grains detached easily from anthers in the solution, we estimated the number of pollen grains per anther by counting the pollen numbers under the microscope in three replicate 5.0-μL drops from a single preserved sample. *Commelina* has both large and small grains, but only the large grains were counted because the small grains are less abundant and generally not viable (Hrycan and Davis, 2005). We then compared the average number of pollen grains on the stigma and L-anthers among nonvisited, PSML, and PL flowers using one-way ANOVA and the Tukey–Kramer method.

RESULTS

Frequency of insect approaches—The GLM analyses indicated that the presence of petals had a significant effect on the frequencies of approach behaviors for syrphid flies and honey bees (Table 1). Flowers without petals received fewer approaches by both insect groups than did other flower types (Fig. 4). For syrphid flies, the number of floral approaches was lower at Kazagashira Park than that at Yamanaka (Fig. 4, Table 1). The removal of S- and M-anthers did not decrease the approach frequencies of either insect group.

TABLE 1. Results of generalized linear models (GLM) analyses for approach behavior of syrphid flies and honey bees to *Commelina communis*. ** $P < 0.01$, *** $P < 0.001$.

Insect Factor	Coefficient	SE	t	P
Syrphid fly				
Site	0.5340	0.2680	2.94	**
Petal	1.4839	0.1819	7.06	***
S-anther	0.3229	0.1917	1.68	0.094
M-anther	0.0530	0.2102	0.28	0.781
Intercept	−0.3764	0.2680	−1.40	0.162
Honey bee				
Petal	1.6108	0.3091	5.21	***
S-anther	0.2221	0.2699	0.81	0.418
M-anther	−0.0011	0.2731	−0.004	0.997
Intercept	−0.1051	0.3485	−0.30	0.764

Frequency of insect hovering behaviors—The S-anthers had significant effects on the hovering frequency by syrphid flies at Yamanaka (Table 2). The absence of S-anthers decreased the number of hoverings (Fig. 5). By contrast, S-anther removal did not have a significant effect on hovering behavior by syrphid flies and honey bees at Kazagashira Park (Table 2). Hovering behavior was not influenced by the presence of M-anthers (Table 2, Fig. 5).

Frequency of insect landings—The number of landings tended to be low for flowers lacking S- or M-anthers or both (Fig. 6). The GLM analyses revealed that S- and M-anthers had marginally significant effects on the number of landings for syrphid flies at Yamanaka and at Kazagashira Park, respectively (Table 2). The landing frequency of honey bees was not significantly affected by the absence of yellow anthers (Table 2).

Flowers missing M-anthers received significantly fewer legitimate landings by syrphid flies and honey bees than other experimental flower categories at both sites (Table 2, Fig. 7). At Yamanaka, S-anthers had a marginally significant effect on the number of legitimate landings for syrphd flies. The presence of S-anthers did not have a significant effect on legitimate landings for syrphid flies and honey bees at Kazagashira Park.

The absence of M-anthers significantly increased the number of L-anther landings by honey bees at Kazagashira Park (Fig. 8, Table 2). For syrphid flies, both S- and M-anthers had no significant effect on the number of L-anther landings (Table 2).

The four ratios—At Yamanaka, the hovering-to-approach ratio for syrphid flies was significantly lower for flowers without yellow anthers than for controls (Table 3). Thus, the removal of both S- and M-anthers decreased hoverings by syrphid flies. This ratio did not differ significantly between PSML and PML flowers and between PMSL and PSL flowers (Table 2). At Kazagashira Park, the hovering-to-approach ratio for syrphid flies was higher for PSML flowers than for PML, PSL, or PL flowers, although the differences were not statistically significant (Table 3). Honey bees tended to hover in front of most flowers that they approached, regardless of the presence of yellow anthers (Table 3).

At each site, approximately 60% of the PSML flowers whose pollen was collected during hovering, experienced
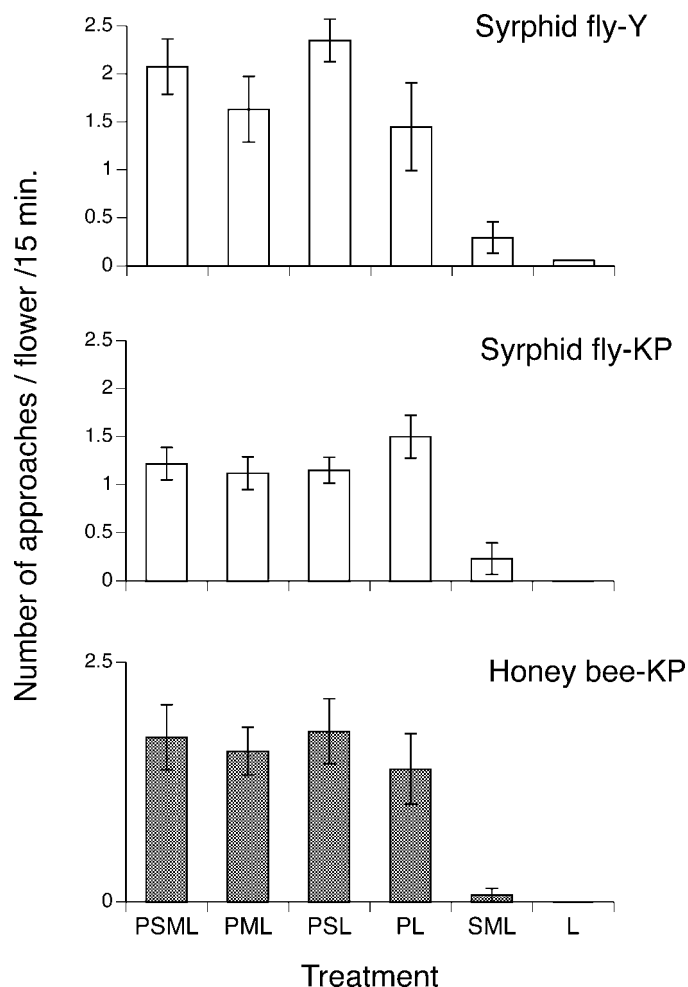

Fig. 4. The frequency of approaches to experimental flowers of *Commelina communis* by syrphid flies and honey bees. Y, Yamanaka; KP, Kazagashira Park. Refer to the text for the flower type abbreviations.

sequential landings by syrphid flies (Table 3). The landing-to-hovering ratio for syrphid flies did not differ between controls and PML flowers, between control and PSL flowers, nor between control and PL flowers (Table 3). At Kazagashira Park, this ratio for syrphid flies was 1.17 on PSL flowers because once one syrphid fly landed on a flower without hovering. For honey bees, this ratio was significantly lower for PL flowers than for PSML flowers ($\chi^2 = 5.67$, df = 1, $P = 0.017$), but the difference was only marginally significant after Bonferroni correction (Table 3).

The landing-to-approach ratio of syrphid flies was about 0.4 for control flowers, which was higher than for PL flowers but not significantly; the ratios for PML and PSL flowers fell between the values for PSML and PL flowers (Table 3). A similar trend was observed for honey bees, although the absolute values of the ratios were much higher than those for syrphid flies (Table 3). For honey bees, this ratio was significantly lower for PL flowers than for PSML flowers ($\chi^2 = 4.91$, df = 1, $P = 0.027$), but the difference was only marginally significant after Bonferroni correction (Table 3).

At Yamanaka, the legitimate landing-to-landing ratio for syrphid flies was significantly higher for PSML flowers than

TABLE 2. Results of GLM analyses on the number of hovering and landing behaviors of syrphid flies and honey bees at Yamanaka (Y) and Kazagashira Park (KP) to study the effects of removal of the yellow anthers of *Commelina communis*. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Behavior Insect site	Factor	Coefficient	SE	t	P
Hovering					
Syrphid fly Y	S-anther	0.8789	0.3244	2.71	**
	M-anther	−0.0717	0.3231	−0.22	0.82
	Intercept	0.8573	0.2982	2.86	**
Syrphid fly KP	S-anther	0.1249	0.1765	0.71	0.48
	M-anther	−0.1003	0.1752	−0.57	0.59
	Intercept	0.5032	0.1576	3.19	**
Honey bee KP	S-anther	0.4968	0.3463	1.43	0.16
	M-anther	−0.0909	0.3412	−0.27	0.79
	Intercept	0.6734	0.3128	2.15	*
Landing					
Syrphid fly Y	S-anther	0.4126	0.2342	1.76	0.08
	M-anther	−0.1392	0.2332	−0.60	0.55
	Intercept	0.5329	0.2152	2.48	*
Syrphid fly KP	S-anther	−0.0058	0.1402	−0.041	0.97
	M-anther	−0.2584	0.1392	−1.86	0.07
	Intercept	0.4283	0.1252	3.42	**
Honey bee KP	S-anther	0.2836	0.2793	1.02	0.31
	M-anther	−0.3521	0.2751	−1.28	0.21
	Intercept	1.0584	0.2522	4.20	***
Legitimate landing					
Syrphid fly Y	S-anther	0.2327	0.1213	1.917	0.06
	M-anther	−0.2717	0.1208	−2.25	*
	Intercept	0.2803	0.1115	2.51	*
Syrphid fly KP	S-anther	0.0952	0.093	1.03	0.31
	M-anther	−0.2301	0.092	−2.51	*
	Intercept	0.2327	0.083	2.82	**
Honey bee KP	S-anther	0.3091	0.2558	1.21	0.23
	M-anther	−0.6456	0.2520	−2.56	*
	Intercept	1.0146	0.2310	4.39	***
L-anther landing					
Syrphid fly Y	S-anther	0.1059	0.138	0.77	0.45
	M-anther	−0.1198	0.138	−0.87	0.39
	Intercept	0.2794	0.1270	2.20	*
Syrphid fly KP	S-anther	−0.1238	0.09	−1.38	0.17
	M-anther	−0.0038	0.089	−0.04	0.97
	Intercept	0.1837	0.08	2.29	*
Honey bee KP	S-anther	−0.0255	0.133	−0.19	0.85
	M-anther	0.2935	0.131	2.24	*
	Intercept	0.0439	0.1200	0.37	0.72

TABLE 3. The ratios of hovering to approach, landing to hovering, landing to approach, and legitimate landing to landing for floral visitors to four types of experimental flowers of *Commelina communis*. Refer to the text for the flower type abbreviations.

Site Insect	Flower type	Ratio h/ap	land/h	land/ap	l-land/land
Yamanaka					
Syrphid fly	PSML	0.79	0.58	0.46	0.54
	PML	0.65	0.50	0.32	0.45
	PSL	0.79	0.43	0.34	0.31
	PL	0.41***	0.67	0.28	0.00*
Kazagashira Park					
Syrphid fly	PSML	0.61	0.59	0.36	0.75
	PML	0.32	1.17	0.37	0.57
	PSL	0.35	0.38	0.13	<u>0.67</u>
	PL	0.38	0.33	0.13	<u>0.00</u>
Honey bee	PSML	0.97	0.77	0.75	0.96
	PML	0.86	0.84	0.73	1.00
	PSL	0.88	0.68	0.59	0.74*
	PL	1.00	0.44[a]	0.44[a]	0.38**

Note: ap = total no. approaches; h = total no. hoverings; land = total no. landings; l-land = total no. legitimate landings. Underlines mean the denominator is <5 and the value was not statistically compared with that of controls. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$

[a] $P < 0.10$ after Bonferroni correction.

Pollen transfer—We found a significant difference in the number of pollen grains on stigmas among nonvisited, PSML, and PL flowers (Table 4). One nonvisited flower had 23 pollen grains on its stigma, and the remaining flowers had 0–2 pollen grains per stigma, indicating that bud pollination rarely occurred in the Iwakura population. PSML flowers received significantly more pollen grains on the stigma than nonvisited flowers. The average pollen number on the stigma for PL flowers was approximately half that of PSML flowers and did not differ significantly from that of nonvisited flowers.

The number of remaining pollen grains on the L-anthers was highest in nonvisited flowers, lowest in PSML flowers, and intermediate in PL flowers (Table 4). A significant difference was observed in the remaining pollen number between nonvisited and PSML flowers.

DISCUSSION

Effects of yellow anthers on insect landing frequency and position—The foraging facilitation hypothesis predicts that floral visitors will discriminate against flowers with inferior floral guides and land less frequently on these flowers. Our results are partly consistent with these predictions. Syrphid flies and honey bees landed twice as many times on control flowers than on PL flowers, and the landing-to-approach ratio was higher for PSML flowers (controls) than PL flowers, although these were not significant trends. The GLM analyses revealed that the presence of either S- or M-anthers had a marginally significant effect on the landing frequency for syrphid flies. For honey bees, however, neither S- nor M-anthers significantly influenced the landing frequency.

The experimental results also support the theft prevention hypothesis, which predicts that the removal of floral guides will decrease the number of legitimate landings and the legitimate landing-to-total landing ratio. Legitimate landings by syrphid

for PL flowers; no significant differences in this ratio were observed between PSML and PML or between PSML and PSL flowers (Table 3). At Kazagashira Park, a similar tendency was observed. The behavior of the honey bees again was similar to that of the syrphid flies, and the differences were significant between PSML and PL flowers and between PSML and PSL flowers (Table 3).

Syrphid fly foraging on yellow anthers—We found that 57 and 83% of syrphid flies landing on control flowers foraged pollen from M-anthers (or sometimes S-anthers) at Yamanaka and Kazagashira Park, respectively. About 55% of the syrphid fly feeding on PML flowers at both sites was on M-anthers; about 73 and 67% of the syrphid fly feeding on PSL flowers at both sites was on S-anthers at Yamanaka and Kazagashira Park, respectively. At both sites, syrphid flies foraged pollen from L-anthers or sought fallen pollen on the surface of petals on PL flowers.

Syrphid fly-Y

Syrphid fly-KP

Honey bee-KP

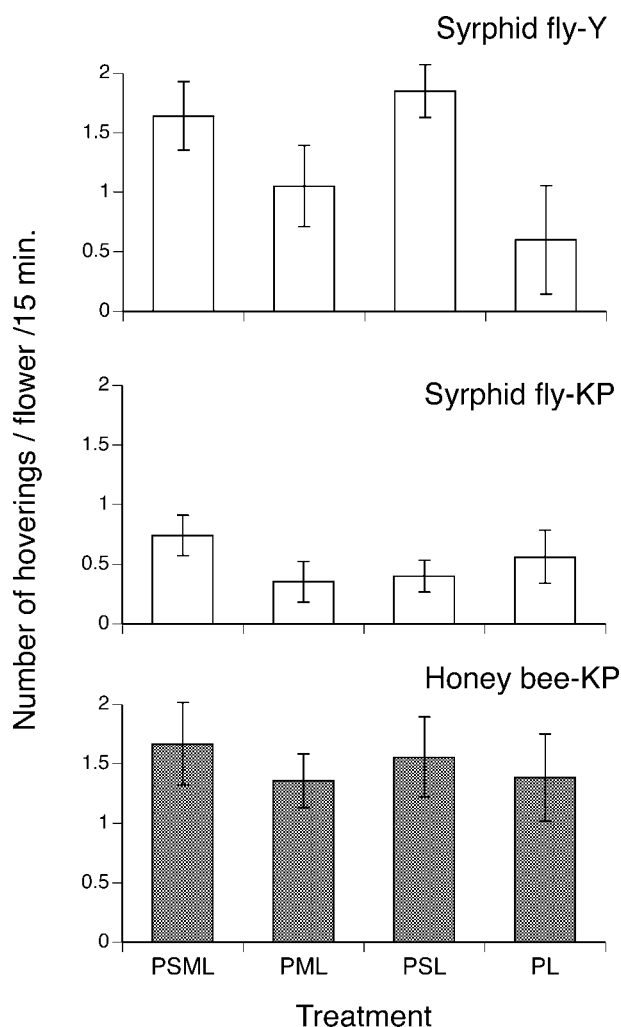Number of hoverings / flower /15 min.

Treatment

Fig. 5. The frequencies of hovering behaviors on experimental flowers by syrphid flies and honey bees. Y, Yamanaka; KP, Kazagashira Park. Refer to the text for the flower type abbreviations.

Syrphid fly-Y

Syrphid fly-KP

Honey bee-KP

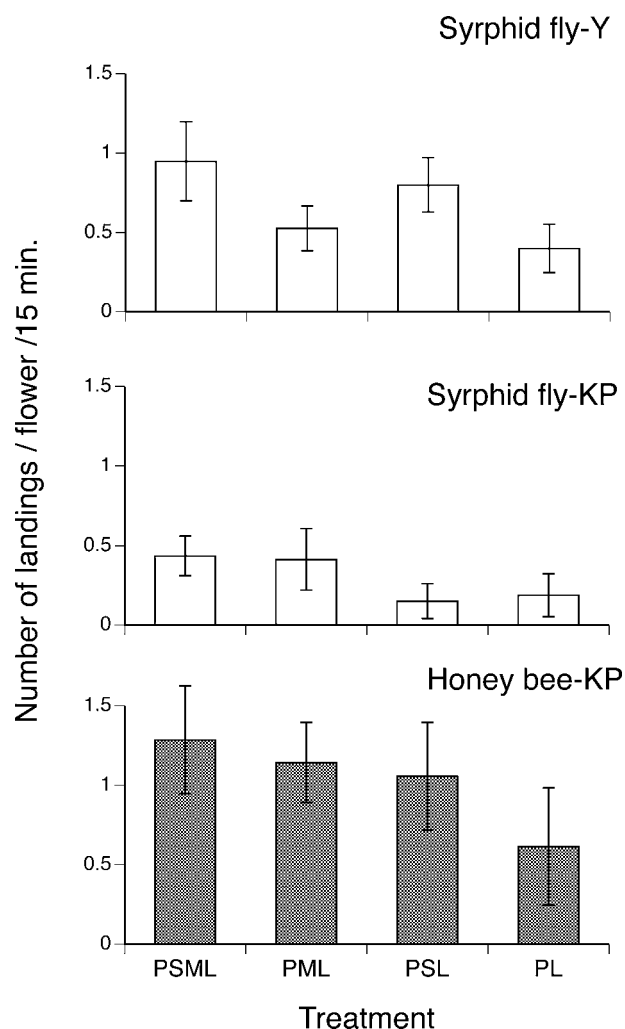Number of landings / flower /15 min.

Treatment

Fig. 6. The frequencies of landings on experimental flowers by syrphid flies and honey bees. Y, Yamanaka; KP, Kazagashira Park. Refer to the text for the flower type abbreviations.

flies and honey bees were significantly fewer on flowers without M-anthers than on other flower types. The legitimate landing-to-landing ratios for syrphid flies and honey bees tended to be lower for flowers without M-anthers. Thus, the rewarding M-anthers of *C. communis* played a vital role in orienting a floral visitor toward a legitimate position.

All landings of syrphid flies on PL flowers were, of course, nonlegitimate. Furthermore, even for PSML flowers, about 20–40% of the landings by syrphid flies were on L-anthers. Neither S- nor M-anthers had significant effects on the number of L-anther landings by syrphid flies, suggesting that the presence of S- and M-anthers did not decrease pollen-theft behaviors by syrphid flies. L-anther landings by honey bees significantly decreased with the presence of M-anthers, although honey bees sometimes collected pollen on L-anthers of PSML flowers at Kazagashira Park. Thus, the pollen of L-anthers was often collected by syrphid flies and honey bees for food. M-anthers produced fewer pollen grains on average than L- anthers; S-anthers produced no fertile pollen (Morita and Nigorikawa, 1999). Because pollen is a costly reward (Petanidou and Vokou, 1990), the pollen of some plants is less accessible as

food (Lunau, 2000). Although the presence of M-anthers increased legitimate landings that would promote outcross pollination, both S- and M-anthers, which provide low rewards, could not perfectly reduce predation of valuable pollen on L-anthers in this species. This finding is partly inconsistent with the statements of previous studies (Vogel, 1978; Faden, 1992; Morita and Nigorikawa, 1999; Hrycan and Davis, 2005).

Discrimination by insects against flowers without yellow anthers—As we reported in the previous section, flowers without S- or M-anthers or both tended to receive fewer insect landings. Although this pattern was found for both syrphid flies and honey bees, the prelanding reactions to experimental flowers differed between sites and between insect groups.

As expected under the foraging facilitation hypothesis, we frequently observed syrphid flies leaving the area in front of anther-removed flowers immediately after approaching at Yamanaka. The frequency of hoverings was significantly decreased in syrphid flies in the absence of S-anthers. Furthermore, the ratio of hovering-to-approach for syrphid flies was lower for flowers lacking S- or M-anthers or both than
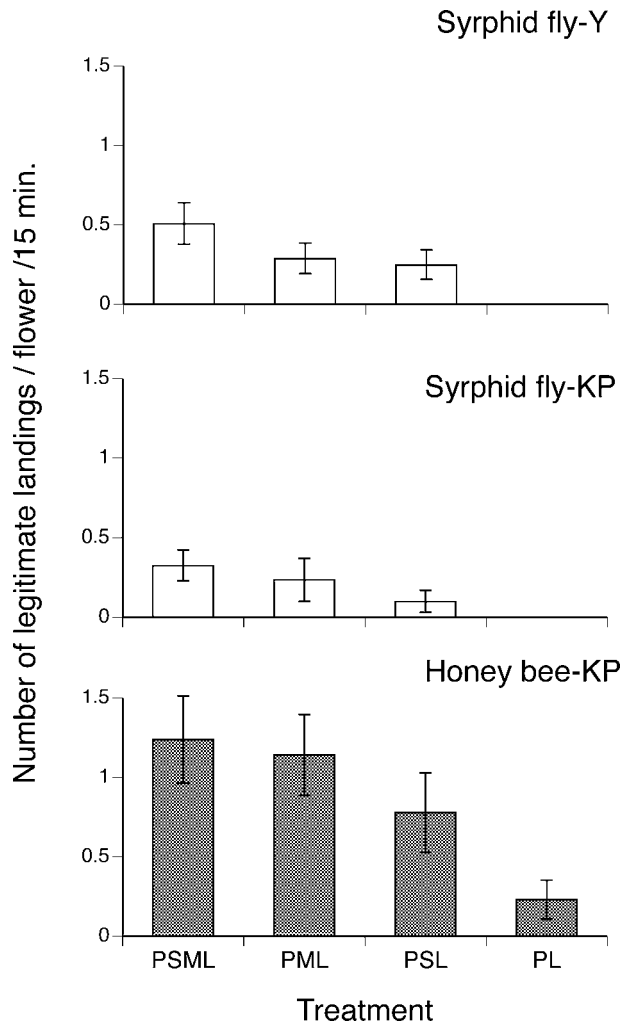
Fig. 7. The frequencies of legitimate landings on experimental flowers by syrphid flies and honey bees. Y, Yamanaka; KP, Kazagashira Park. Refer to the text for the flower type abbreviations.
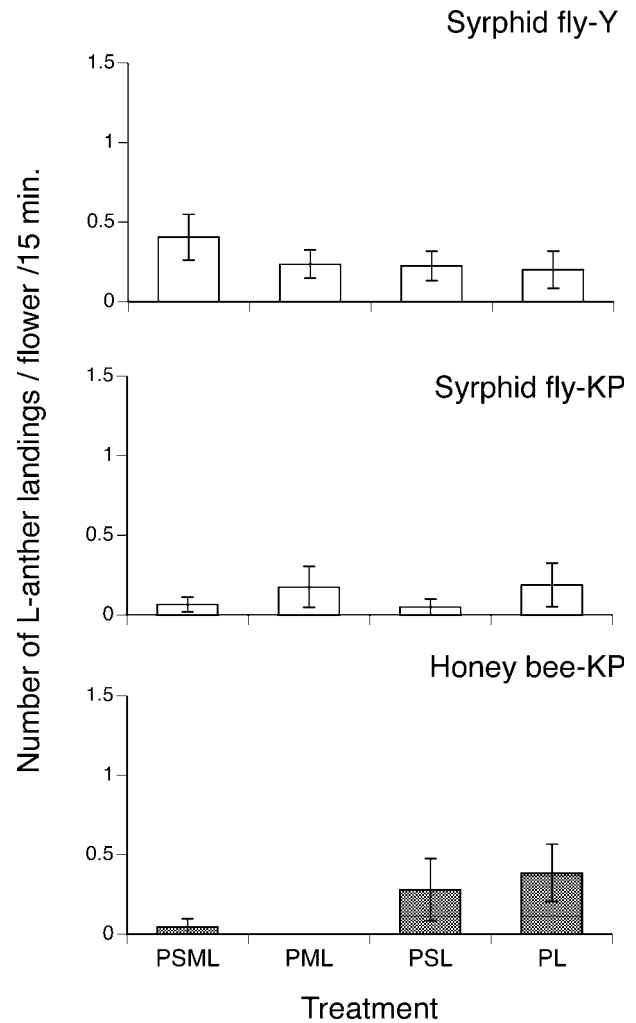

Fig. 8. The frequencies of L-anther landings on experimental flowers by syrphid flies and honey bees. Y, Yamanaka; KP, Kazagashira Park. Refer to the text for the flower type abbreviations.

for PSML flowers. Thus, yellow anthers, especially S-anthers, are signals that can trigger hovering for pollen assessment. UV-absorbing yellow pollen and anthers are prevalent in entomophilous flowers and induce specific responses by insect pollinators (Lunau, 2000). Syrphid flies (*Eristalis tenax*) innately extend their proboscises toward artificial UV-absorbing yellow spots (Lunau and Wacht, 1994; Lunau, 2000). In *C. communis*, as in the related *C. coelestis*, no anther types exhibit strong UV-reflection (Lunau, 2000; Ushimaru et al., unpublished data). Syrphid fly species visiting *C. communis* flowers might react to the UV-absorbing yellow color of S- and M-anthers. The nonrewarding S-anthers are more striking than the rewarding M-anthers and may strengthen this signaling function. Hovering by syrphid flies was also observed with PL flowers, meaning that the flies collected pollen on brown L-anthers. We also frequently observed syrphid flies collecting pollen on blue petals during hovering. This indicates that yellow and UV-absorbing color of the pollen grains is sensed by syrphid flies from a short distance.

At Kazagashira Park, the landing frequency of syrphid flies tended to decrease in the absence of M-anthers, and the

landing-to-hovering ratio was lower for flowers lacking M-anthers than for other experimental flower categories, suggesting that, after hoverings, syrphid flies at this site discriminated against flowers that had no rewarding pollen. This possibility will be examined in a field experiment in which the amount of pollen is regulated. Thus, we found an intersite difference in syrphid fly response to two types of yellow anthers.

Bees had antennal responses to both the pollen guides of real flowers and to the dummy anthers of artificial flowers (Lunau, 2000 and references therein). The quality of the video-recordings was too poor to examine antennal responses to experimental flowers by honey bees during hovering. Although not statistically significant, sequential landing was observed more frequently on PSML, PML, and PSL flowers than on PL flowers. The landing of related bumblebees is triggered by the scent of pollen, which is sensed using antennae (Lunau, 1992). The loss of S- and M-anthers might have made it more difficult for honey bees to perceive the pollen scent.

Blue petals as distant attractants—The artificial removal of the blue petals dramatically reduced approaches by syrphid

TABLE 4.   Mean number (±SD) of pollen grains on stigmas and L-anthers of different types of flowers of *Commelina communis*. The number of pollen grains was compared among three flower types using one-way ANOVA and the Tukey–Kramer method.

Flower type	No. pollen grains					
	Stigma	Min.–Max.	N	L-anther	Min.–Max.	N
Nonvisited	$2.9^A \pm 7.10$	0–23	10	$922.7^A \pm 150.7$	307–1880	10
PSML	$27.0^B \pm 24.6$	0–95	20	$292.0^B \pm 287.1$	0–873	20
PL	$14.9^{AB} \pm 16.5$	0–61	14	$596.2^{AB} \pm 491.2$	27–1880	14
F	5.33**			7.37**		

Note: Mean values with the same capital letter are not significantly different at $P = 0.05$. Refer to the text for the flower type abbreviations.

flies and honey bees. Waser and Price (1985) showed that bumblebees discriminated against albino flowers with an inferior nectar guide in *Delphinium* and visited common blue flowers more frequently. Syrphid flies and honey bees did not seem to discriminate among PSML flowers and PML, PSL, or PL flowers of *C. communis* during approach. This strongly suggests that the insects searched for flowers at a distance, using only the blue petals as a signal. A significant difference was observed between sites in the approach frequencies of syrphid flies. The occurrence of social bees such as honey bees affects the abundance of other floral visitors (Roubik, 1978, 1996; Kato et al., 1999). At Kazagashira Park, the presence of honey bees might reduce the visiting frequency of syrphid flies through resource competition. This idea should be investigated in a multipopulation comparison of pollinator compositions in *C. communis* (Kato et al., 1999).

Effects of yellow anthers on pollen transfer—In the Iwakura population, we found that control flowers had significantly more pollen grains on the stigma and fewer pollen grains remaining on the L-anthers than nonvisited flowers, whereas flowers without yellow anthers had intermediate values. This suggests that the absence of both S- and M-anthers impaired pollen dispatch and receipt, and hence, male and female fitness. Further research on the effect of yellow anthers relative to brown anthers on seed production and siring should be examined.

According to the results for PL flowers, L-anther landings contributed to pollen transfer. Considering the results of floral visitor response to experimental flowers, landing frequency seems to be responsible for the difference between PSML and PL flowers in pollen transfer frequency. However, it was difficult in this study to determine whether total landing frequency more strongly affected pollen dispatch and receipt than the ratio of legitimate landing. This uncertainty arises because we did not directly observe floral visitor behavior at Iwakura. In future research, both insect behaviors and pollen transfer in the same population should be examined.

The roles of floral guides in the process of pollinator orientation have been rarely investigated experimentally with real flowers (cf. Waser and Price, 1985). This may be because real flowers can have several functionally different guides within a single petal (Sprengel, 1793), making it difficult to experimentally remove a specific guide to examine its function. Based on our results, S-anthers would function to facilitate foraging efficiency, whereas M-anthers control the landing position of visitors. Thus, the two types of yellow anther may have different functions as floral guides. To test both the foraging facilitation hypothesis and the theft prevention hypothesis together, we need to examine additional species

of real flowers with floral guides that can be experimentally treated (removed or painted; e.g., Waser and Price, 1985).

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