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Flower orientation enhances pollen transfer in bilaterally symmetrical flowers

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Abstract

Zygomorphic flowers are usually more complex than actinomorphic flowers and are more likely to be visited by specialized pollinators. Complex zygomorphic flowers tend to be oriented horizontally. It is hypothesized that a horizontal flower orientation ensures effective pollen transfer by facilitating pollinator recognition (the recognition-facilitation hypothesis) and/or pollinator landing (the landing-control hypothesis). To examine these two hypotheses, we altered the angle of *Commelina communis* flowers and examined the efficiency of pollen transfer, as well as the behavior of their visitors. We exposed unmanipulated (horizontal-), upward-, and downward-facing flowers to syrphid flies (mostly *Episyrphus balteatus*), which are natural visitors to *C. communis*. The frequency of pollinator approaches and landings, as well as the amount of pollen deposited by *E. balteatus* decreased for the downward-facing flowers, supporting both hypotheses. The upward-facing flowers received the same numbers of approaches and landings as the unmanipulated flowers, but experienced more illegitimate landings. In addition, the visitors failed to touch the stigmas or anthers on the upward-facing flowers, leading to reduced pollen export and receipt and supporting the landing-control hypothesis.

Collectively, our data suggested that the horizontal orientation of zygomorphic flowers enhances pollen transfer by both facilitating pollinator recognition and controlling pollinator landing position. These findings suggest that zygomorphic flowers which deviate from a horizontal orientation may have lower fitness because of decreased pollen transfer.

Keywords *Commelina communis*, pollen transfer, pollinator specialization, horizontal flower orientation, zygomorphic flower

Introduction

Pollinator specialization is presumed to be an essential selective force for floral diversity in angiosperms (Darwin 1862; Grant and Grant 1965; Stebbins 1970; Fenster et al. 2004; Kay et al. 2006). Many reproductive characteristics, such as floral colors and scents, long corolla tubes, nectar spurs, and flowering phenology, are adaptations to specific pollinator groups (Nilsson 1998; Johnson and Steiner 2000). In particular, bilaterally symmetrical (zygomorphic) flowers have been repeatedly derived from radially symmetrical (actinomorphic) flowers (Neal et al. 1998 and references therein; Wolfe and Krstolic 1999; Sargent 2004). Fenster et al. (2004) reanalyzed Robertson’s (1928) data and found that, compared to actinomorphic flowers, zygomorphic flowers were more frequently visited by only one functional group of pollinators, suggesting that a specialized pollination system is prevalent in zygomorphic flowers.

Neal et al. (1998) reported that the *en face* surface of zygomorphic flowers is usually vertical or oblique (i.e., the orientation of the main floral axis is nearly horizontal, Fig. 1) and two hypotheses have been proposed to explain this horizontal flower orientation (Neal et al. 1998; Ushimaru and Hyodo 2005). First, zygomorphic flowers are usually more complex than actinomorphic flowers. Because of this morphological complexity, zygomorphic flowers should facilitate recognition and memorization of complex floral patterns by specialized pollinators (Neal et al. 1998; West and Laverty 1998). Facilitating recognition, in turn, allows the plant to receive repeated pollinator visits. Neal et al. (1998) argued that the horizontal orientation of the floral axis in zygomorphic flowers could restrict the approach course of visiting insects to facilitate their recognition of complex floral patterns (the recognition-facilitation hypothesis;

originally introduced as the unequal image projection hypothesis in Neal et al. 1998). Second, the morphological complexity of zygomorphic flowers should restrict pollinators to landing sites where they contact the anthers and stigmas correctly, ensuring pollen transfer (Ushimaru et al. 2007). The horizontal flower orientation can control access to landing sites on zygomorphic flowers (the landing-control hypothesis; Ushimaru and Hyodo 2005; see also the natural position hypothesis and the pollen position hypothesis in Neal et al. 1998). Under both of these hypotheses, zygomorphic flowers facilitate their own pollination by orientating their main axis horizontally.

Despite the hypothesized importance of a horizontal orientation for pollen transfer in zygomorphic flowers, its significance for successful pollen export and receipt has not been tested in the field. The effects of flower angle on pollinator behaviors, pollen export and receipt, and fruit set have been examined mainly in upright-oriented or pendant actinomorphic flowers (Fulton and Hodges 1999; Tadey and Aizen 2001). Fulton and Hodges (1999) showed that artificial changes in floral angle diminished pollinator visitation in *Aquilegia pubescens*, but they did not examine pollen transfer. Tadey and Aizen (2001) revealed that changes in flower (inflorescence) angle reduced pollen receipt on the stigmas in *Tristerix corymosus*, but the number of pollinator visits and pollen export were unaffected. In zygomorphic flowers, our previous study revealed a significant effect of flower angle on pollinator landings in *Commelina communis*, supporting the landing-control hypothesis (Ushimaru and Hyodo 2005). However, we did not examine pollen transfer. Thus, our understanding of the adaptive significance of flower orientation, especially in terms of pollen transfer in zygomorphic flowers, is still incomplete; further examination is needed to elucidate how the artificial manipulation of flower orientation alters pollinator behaviors (approach and landing frequency and landing site) and how pollen transfer is affected by these behavioral changes.

Here, we report the results of a field experiment in which we examined two major hypotheses concerning the function of horizontal orientation on pollen transfer in zygomorphic flowers: the recognition-falicitation hypothesis and the landing-control hypothesis. Note that these two hypotheses are not mutually exclusive but do lead to different predictions for several aspects of the pollination process, which allows us to determine the relative importance of the two hypothesized processes in the pollination success of zygomorphic flowers. The recognition-facilitation hypothesis predicts that changes in the floral angle upward or downward should reduce the number of approaches by visitors. In comparison, the landing-control hypothesis predicts that changes in the floral angle will impair the function of landing-specialized organs, which may increase visitor landings on the floral organs unsuitable for pollination (i.e., illegitimate landings) (Ushimaru and Hyodo 2005). The recognition-facilitation hypothesis and the landing-control hypothesis both predict that pollen transfer decreases in experimental flowers through decreased visitation frequency and through decreased frequency of legitimate landings, respectively. We experimentally altered the orientation of *C. communis* flowers to examine how the floral angle affects the approach and landing behavior of, and pollen transfer by, insect pollinators. Based on our results, we discuss the validity of the recognition-facilitation and landing-control hypotheses in horizontally oriented zygomorphic flowers.

Materials and methods

Study species

Commelina communis L. (Commelinaceae) is an annual, andromonoecious herb found throughout temperate northeast Asia, often growing around or near rice fields and roads. A single plant usually has several inflorescences in which perfect flowers bloom before staminate flowers. Each inflorescence contains three or four flower buds inside the bract. In most cases, one flower per inflorescence opens each day at sunrise and lasts until noon of the same day.

The flowers of *C. communis* have three types of stamens: two long brown stamens, one medium-length yellow stamen, and three short yellow stamens. Anthers of the long (L-anther) and medium-length (M-anther) stamens produce fertile pollen, whereas anthers of the short stamens (S-anther) produce only a small amount of sterile pollen (Morita and Nigorikawa 1999). Pollen production on the M- and L-anthers does not differ between perfect and staminate flowers and among different-positioned flowers (Morita and Nigorikawa 1999). The flowers of *C. communis* do not bear nectar; thus, pollen is the only floral reward for their pollinators. Pollen from the L-anthers is believed to contribute to outcrossing, whereas pollen from the M-anthers functions mainly as a reward for pollinators (Vogel 1978; Faden 1992). In *C. communis*, filaments of the long stamens are typically elongate, and they seem to function as a landing platform (Fig. 1; Ushimaru and Hyodo 2005; Ushimaru et al. 2007). Ushimaru et al. (2003a, b) suggested that the position of the stigmas evolved to maximize the receipt of pollen grains from the L-anthers, which are situated at the same height in perfect flowers (Fig. 1).

Commelina communis is self-compatible and exhibits both delayed autogamy and infrequent bud pollination within a single flower (Morita and Nigorikawa 1999; Ushimaru et al. 2007). The pistils and long stamens roll up at the end of anthesis, facilitating contact between the L-anther and stigma and subsequent autonomous self-pollination (Morita and Nigorikawa 1999). However, the fact that the pollen:ovule ratio in the perfect flowers is within the range of those in other facultatively xenogamous species (Cruden 1977; Morita and Nigorikawa 1999) and staminate flowers are present in addition to perfect flowers suggests that pollinator-mediated outcrossing occurs in *C. communis*. Syrphid flies (*Episyrphus balteatus*) are the exclusive visitors to the flowers of *C. communis* in many fields, although bee species, such as bumblebees, honeybees, and small solitary bees, sometimes visit as well (Ushimaru and Hyodo 2005; Ushimaru et al. 2007; Uchihashi H and Ushimaru A unpublished data).

Field experiment

We conducted a field experiment using a population of *C. communis* in Iwakura, Kyoto, Japan (35^{o}05'N, 135^{o}47'E), in September 2004. Native syrphid flies (*E. balteatus*) were frequent visitors to the flowers of *C. communis* plants, and some smaller syrphid species visited the flowers infrequently. We experimentally prepared three types of flowers that differed in terms of their floral angle (Fig. 1):

(1) ‘Unmanipulated’: intact flowers with angles of 0 to 20°;
(2) ‘Up’: flowers whose faces were turned upward; and
(3) ‘Down’: flowers whose faces were turned downward.

We used only perfect flowers in our experiment to avoid the effect of sexual differences in petal size on pollinator behavior (Ushimaru and Hyodo 2005). For each trial, we arbitrarily chose three neighboring perfect flowers from a single flower patch, which consisted of one to three individuals. We then manipulated the orientation of the flowers (Unmanipulated, Up, or Down) before pollinators had access to them and observed the approach and landing behavior of the pollinators for 15 min. It was technically difficult to change the stalk angle of an individual flower inside a bract; therefore, we leaned the plant stems and fixed them to create Up flowers, and we bent the inflorescence stalks with staples to create Down flowers (Fig. 1); the angles of these flower types were approximately 90° and –90°, respectively. We often removed a single leaf to maintain a space in front of Down flowers. In our previous study, the leaf removal treatment did not induce discrimination among experimental flowers by syrphid flies (Ushimaru & Hyodo 2005) and seemed not to affect their landing behavior. All trials were conducted in the morning (06:00–09:30 h), and a new flower patch was used for each trial. In total, 57 trials were conducted during the experimental period, which allowed us to examine 57 Unmanipulated, 57 Up, and 57 Down flowers.

Pollinator behavior

We first analyzed two types of pollinator behavior: approaching and landing. Approaching occurred when pollinators found flowers and approached them from the front. Landing was defined as a pollinator landing on any part of a flower after approaching it. We then split pollinator landing events into the following three types (Ushimaru and Hyodo 2005):

(1) Legitimate landing: the pollinator held on to the filaments of the L- and M-anthers or a small white petal beside the anthers and foraged or collected pollen grains from the M-anther, while simultaneously touching the L-anthers or stigma with the ventral surface of its abdomen (Fig. 1);

(2) L-anther landing: a syrphid fly hung onto the L-anthers and ingested pollen from them directly (Fig. 1); and

(3) Petal and side landing: the pollinator foraged for pollen from the M- or S-anthers after landing on the blue petals or on the bracts or sepals at the side of the flower. Because the L-anthers and sitgma are at the bottom of a flower, syrphid flies on the blue petals and bracts rarely touch these organs in Up flowers (Fig. 1, Ushimaru et al. 2007). For

Down flowers, they mostly land on the back side of the blue petals and never touch the L-anthers and the stigma.

In our analyses, we considered data from 57 observations. We counted the number of approaches and landings by pollinators on each flower type during each observation period. During the experiment, we recorded 254, 34, 4, 2, and 2 approaches by *E. balteatus*, smaller syrphid flies, *Bombus diversus*, a small-sized bee, and a hawkmoth, respectively; however, we analyzed only the data for *E. balteatus* and other small syrphid flies. Most *E. balteatus* individuals consistently visited *Commelina* flowers and infrequently visited flowers of other species during the morning at the study site (Ushimaru A., personal observation).

Episyrphus balteatus individuals were sometimes observed landing on a flower repeatedly (e.g., petal landing -> legitimate landing -> L-anther landing) during a single visitation, but we counted this as one landing. When both legitimate and other landings were observed in a single bout, we used the first landing for analyses. For example, when a syrphid fly was observed to land on the legitimate position and then on the L-anther in a single bout, we defined the landing type as legitimate landing. The landing repetitions were seen 15 times out of a total of 124 landings of *E. balteatus* and only once out of 23 landings of the smaller syrphid flies.

To test the recognition-facilitation and landing-control hypotheses, we analyzed how changes in flower orientation changed the behavior of visitors. We first compared the number of approaches and the total number of landings between Unmanipulated and manipulated (Up or Down) flowers using generalized linear models (GLMs) with Poisson error (logarithmic link). For these analyses, the number of behaviors (approaches and landings) were treated as the response variable and the treatment type as a fixed effect. We analyzed the behavior of *E. balteatus* and the smaller syrphid fly species separately. A significant decrease in the numbers of approaches and landings in the Up and Down flowers relative to the Unmanipulated flowers would support the recognition-facilitation hypothesis.

To test the effect of flower orientation on whether landing occurs after approach or not, a GLMM analysis with binomial errors and logit-link function was applied. The model has two explanatory variables, treatment (Unmanipulated, Up, and Down) as a fixed effect, and flower identity as a random term, because flower identity was the source of replication. In the analyses, we only used data from flowers that received one or more approaches. The behavior of *E. balteatus* and the smaller syrphid fly species were separately analyzed. The recognition-facilitation hypothesis predicts no difference in the occurence of landing after approach among experimental flowers, whereas the landing-control hypothesis predicts significant decreases in the occurence of landing after approach in the Up and Down flowers relative to the Unmanipulated flowers (Ushimaru and Hyodo 2005).

We then used GLMMs with binomial errors and the logit-link function to examine the effect of treatment (Up or Down) on legitimate landing. In the models, the presence of legitimate landing (presence, 1; non-legitimate landing, 0) was considered a response variable. The explanatory variables were treatment (fixed effect) and flower identity (random term). We only used data of flowers that received one or more landings in the analyses. We also compared the ratios of three landing types (legitimate, L-anther, and petal and side landings) to the total observed landings between Unmanipulated and treated (Up or Down) flowers using chi-squared and Fisher's exact tests. We did not apply these analyses to data from the smaller syrphids because of the small sample size. Significant decreases in the number of legitimate landings both in the Up and Down flowers would provide support for the landing-control hypothesis.

Pollen transfer

We examined the effect of flower orientation on pollen removal and deposition. We collected the M- and L-anthers and stigmas from the Unmanipulated, Up, and Down flowers (n = 26 for each), which were exposed to pollinators from bud break until 10:00 h. The sampled flowers were randomly selected from the flowers for which pollinator behaviors were observed. Additionally, we collected the M- and L-anthers and stigmas from ten non-visited flowers (i.e., newly opened flowers) at 06:00 h in order to compare pollen transfer with the Unmanipulated flowers. We stored each sample separately in 0.1 mL 70% ethanol. Because the pollen grains detach easily from the anthers in solution, we estimated the number of pollen grains per anther by counting the number of grains in three 5.0-μL aliquots per sample under a light microscope (x85). We directly counted the number of pollen grains on the stigmas in each sample using the microscope. *Commelina* species have both large and small pollen grains, but because the small grains are less abundant and generally not viable (Hrycan and Davis 2005; Ushimaru et al. 2007), we counted only the number of large grains. Male reproductive success has often been estimated by counting the number of pollen grains remaining on the anthers (e.g. Ishii and Sakai 2002; Ishii 2004).

We then compared the number of pollen grains on the M- and L-anthers and stigmas of the Non-visited, Up, or Down flowers to those of the Unmanipulated flowers using generalized linear models (GLMs) with Poisson error (logarithmic link). Statistical analyses were done using the statistical software package R (R Development Core Team 2005).

Results

Pollinator behavior

Compared to the Unmanipulated flowers, approaches by *E. balteatus* to the experimental flowers decreased only in the Down treatment (GLM, Up: $b = -0.253 \pm 0.149$, $z = -1.70$, $P = 0.089$; Down: $b = -0.372 \pm 0.154$, $z = -2.41$, $P = 0.016$; a negative value for b implies that the treatment has a negative effect on the number of behaviors; Fig. 2). A significant decrease in the frequency of landings and in the occurence of landing after approach was also found only in the Down flowers (landing: GLM, Up: $b = -0.097 \pm 0.197$, $z = -0.49$, $P = 0.62$; Down: $b = -0.944 \pm 0.257$, $z = -3.67$, $P < 0.001$; landing after approach: GLMM, Up: $b = 0.313 \pm 0.361$, $z = 0.87$, $P = 0.39$; Down: $b = -1.134 \pm 0.415$, $z = -2.74$, $P = 0.006$).

Legitimate landings decreased significantly in the Up and Down flowers relative to the Unmanipulated flowers (GLMM, Up: $b = -3.077 \pm 0.857$, $z = -3.59$, $P < 0.001$; Down: $b = -1.746 \pm 0.778$, $z = -2.24$, $P = 0.025$). For the Up flowers, L-anther landings, and petal and side landings were observed less and more frequently than for the Unmanipulated flowers, respectively ($\chi^2 = 70.78$, $df = 2$, $P < 0.001$; Fig 3). The ratio of L-anther landings to total landings was unchanged but that of petal and side landings increased in the Down flowers (Fisher's exact test, $P < 0.001$; Fig. 3).

We found no significant difference in the number of approaches and landings, and the proportion of approaching to landing by smaller syrphid flies between experimetal flowers (GLMM, $P > 0.05$ for all fixed effects). We observed only 23 landings by smaller syrphid flies (five, six, and 12 landings on the Unmanipulated, Up, and Down flowers, respectively), and most of them were petal and side landings.

Pollen transfer

Seventy-four percent of pollen grains were removed from the M-anthers of Unmanipulated flowers during the experimental period (Unmanipulated vs. Non-visited, GLM, $b = 1.362 \pm 0.029$, $z = 46.23$, $P < 0.001$; Fig. 4A). Significantly fewer pollen grains were removed from the M-anthers of Up and Down flowers, relative to Unmanipulated flowers (Up: $b = 0.838 \pm 0.027$, $z = 30.66$, $P < 0.001$; Down: $b = 1.015 \pm 0.027$, $z = 38.103$, $P < 0.001$). This indicates that manipulation of floral orientation decreased pollen removal from the M-anther (41% and 29% removed from the Up and Down flowers, respectively; Fig. 4A).

Seventy-one percent of pollen grains were removed from the L-anthers in the Unmanipulated flowers during the experimental period (Unmanipulated vs. Non-visited, GLM, $b = 1.221 \pm 0.016$, $z = 77.28$, $P < 0.001$; Fig. 4B). Significantly fewer pollen grains were removed from the L-anthers of Up flowers, compared to Unmanipulated flowers (30% removed; $b = 0.858 \pm 0.014$, $z = 60.49$, $P < 0.001$), whereas there was no difference in the amount of pollen removed from the L-anthers between the Down and Unmanipulated flowers (70% removed in the Down flowers: $b = 0.029 \pm 0.017$, $z = 1.71$, $P = 0.087$; Fig. 4B).

The stigmas of the Unmanipulated flowers received about 20 pollen grains during the experimental period (Fig. 4C), and this increase from the Non-visited flowers was significant (GLM, $b = -2.030 \pm 0.190$, $z = -10.67$, $P < 0.001$). The stigmas of the Up and Down flowers received significantly fewer pollen grains than those of the Unmanipulated flowers (Up: $b = -0.420 \pm 0.066$, $z = -6.34$, $P < 0.001$; Down: $b = -0.402 \pm$

0.066, $z = -6.10$, $P < 0.001$).

Discussion

We examined two hypotheses regarding the adaptive significance of the horizontal orientation in zygomorphic flowers: the recognition-facilitation hypothesis and the landing-control hypothesis. These hypotheses predict different patterns of pollen transfer success: the former predicts that pollen transfer will be correlated with total visitation frequency, whereas the latter predicts association with the frequency of legitimate landings. The results of our experiments using zygomorphic *C. communis* flowers that were mainly visited by *E. balteatus* support both hypotheses, although there was more support for the landing-control hypothesis.

We found support for the recognition-facilitation hypothesis, but only from the comparison between horizontally oriented and downward-facing flowers. The total frequencies of pollinator approach and landing, and the proportion of approaches to landings decreased only in the Down flowers relative to the Unmanipulated flowers (Fig. 2), while the recognition-facilitation hypothesis predicted a decrease in both the Up and Down treatments. Moreover, the recognition-facilitation hypothesis predicts that the number of pollinator visits should be reflected in pollen transfer success: i.e., pollen export and receipt would decrease only in the Down flowers. However, our results showed that pollen removal from the M- and L-anthers and pollen deposition on the stigma consistently decreased in the Up flowers (Fig. 4). Although the Down flowers also experienced decreased pollen removal from the M-anther and pollen deposition on the stigma, there was no difference in pollen removal from the L-anther between the Down and Unmanipulated flowers (Fig. 4b). Thus, the recognition-facilitation hypothesis is only weakly supported for the stages of pollen export and receipt.

Results of the landing type analyses strongly supported the landing-control hypothesis. The manipulation of flower angle significantly changed the composition of landing types and decreased the frequency of legitimate landings in the Up and Down flowers (Fig. 3). The landing-control hypothesis predicts that the frequency of legitimate landings should be reflected in the pollen transfer success, that is, pollen export and receipt should also decrease in the Up and Down flowers. As expected, pollen removal from the M- and L-anthers and pollen deposition on the stigma decreased in both the Up and Down flowers compared to the Unmanipulated flowers. Pollen removal from the L-anthers in the Down flowers, however, showed no difference to the Unmanipulated flowers (Fig. 4). No difference in pollen removal from the L-anthers between the Down and Unmanipulated flowers can be explained by our finding that the Down flowers experienced relatively frequent L-anther landings (Fig. 3) and pollen predation from the L-anthers. As a result, the amount of pollen removal was almost equal to the Unmanipulated flowers. Since our experiments cannot discriminate between pollen removal for pollen predation and for plant reproduction, our data on pollen removal may include these two effects. This suggests that the amount of pollen effectively transferred for plant reproduction could be overestimated, especially in the Down flowers. Meanwhile, the stigmas of the Up and Down flowers received significantly fewer pollen grains than the Unmanipulated flowers. This result indicates that illegitimate (L-anther, petal, and side) landings contribute less to pollen deposition. Collectively, our results suggest that manipulation of flower orientation results in a decrease in legitimate landings relative to illegitimate landings, and, consequently, reduced pollen export and receipt, providing more support for the landing-control hypothesis at the stage of pollen transfer.

Traits that increase pollen export and receipt are generally favored by natural selection (Harder and Wilson 1994; 1998). Pollen transfer efficiency depends on the physical fit between pollinators and the mating-related floral organs (i.e., anthers and stigmas), which are usually in specific positions in zygomorphic flowers (e.g., at the top or bottom of the flower or inside the guard petals or corolla tubes; Nilsson 1988; Harder and Barrett 1993; Johnson and Steiner 1995; Wilson 1995; Ushimaru and Nakata 2001). Our results demonstrate that landing on a legitimate position is important for successful pollination in *C. communis*, presenting the evidence for the functional importance of the structure and orientation of zygomorphic flowers in terms of controlling the landing sites of visitors on the flower.

The adaptive significance of flower orientation has been investigated in terms of pollinator attraction (Kevan 1975; Kudo 1995; Huang et al. 2002; Patino et al. 2002; Ushimaru et al. 2006), but few studies have focused on the efficiency of pollen transfer (e.g., Tadey and Aizen 2001; Ushimaru et al. 2006). We found that flower angle strongly influenced pollinator behaviors and consequent pollen transfer in a zygomorphic species, suggesting the importance of flower orientation in specialized pollination systems. In plants with actinomorphic flowers, flower angle affects pollinator behavior and/or pollination efficiency in a specialized (hawkmoth and humming bird) pollination system (Fulton and Hodges 1999; Tadey and Aizen 2001), but not in a generalized pollination system (Huang et al. 2002). Thus, further research is warranted to test the generality of the landing-control hypothesis, which should focus on the function of flower orientation in controlling pollinator landing behavior and pollen transfer efficiency, with respect to both floral symmetry (zygomorphic or actinomorphic) and pollination systems (specialized or generalized). We predict that in zygomorphic plants with a generalized pollination system, flower orientation would less strictly regulate pollinator landing and pollen transfer because of the diverse reponses of different pollinators (which may differ in body size and foraging behavior) to the flower orientation.

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Figure legends

Fig. 1

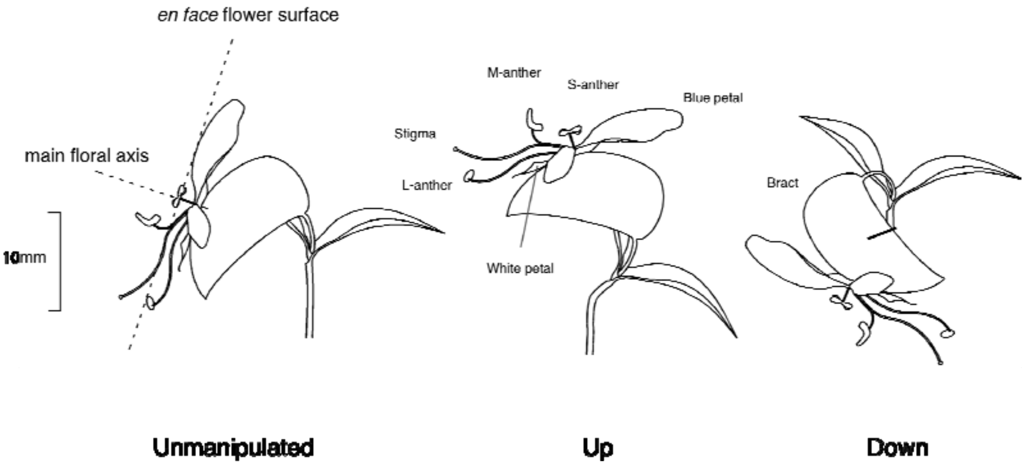
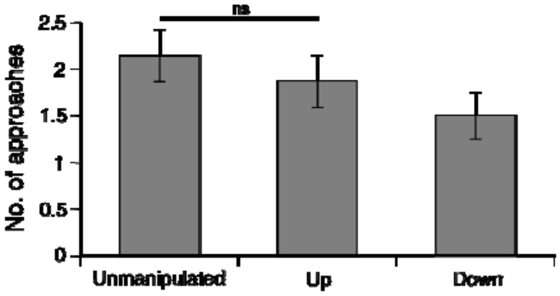


Fig. 1. Side views of experimental flowers of *Commelina communis*: intact (Unmanipulated), upward-facing (Up), and downward-facing (Down) flowers. Main floral axis of Unmanipulated and Manipulated (Up and Down) flowers is nearly horizontal and vertical, respectively. Positions of the stigma, S-, M- and L-anthers, and blue and white petals within a flower are illustrated. The plant stem was bent and fixed to prepare Up flowers. Bracts of Down flowers were fixed with staples.

Fig. 2

(a) Approach



(b) Landing

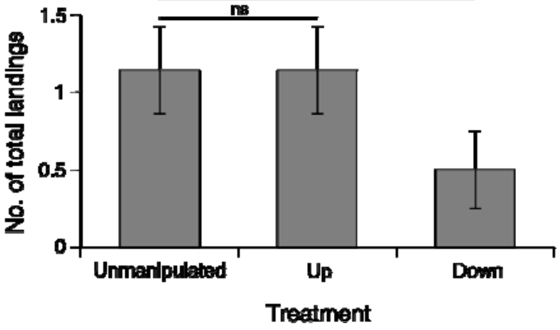


Fig. 2. Mean number of a) approaches to and b) total landings per flower (per 15 min, n = 57) by *Episyrphus balteatus* on the experimental (Unmanipulated, Up, and Down) flowers of *Commelina communis*. Bars show standard errors. * $P < 0.05$, *** $P < 0.001$, ns P > 0.05 by GLM or GLMM (see text).

Fig. 3

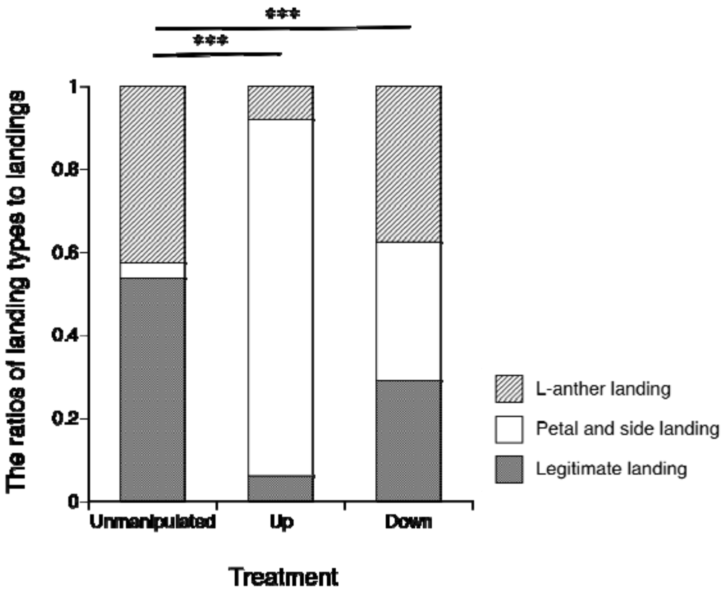


Fig. 3. The ratio of legitimate, L-anther, and petal and side landings to total landings by *Episyrphus balteatus* for the experimental (Unmanipulated, Up, and Down) flowers. The number of total landings for the Unmanipulated, Up, and Down flowers were 54, 49, and 21, respectively. *** $P < 0.001$ by chi-squared test (Unmanipulated vs. Up) and Fisher's exact test (Unmanipulated vs. Down).

Fig. 4

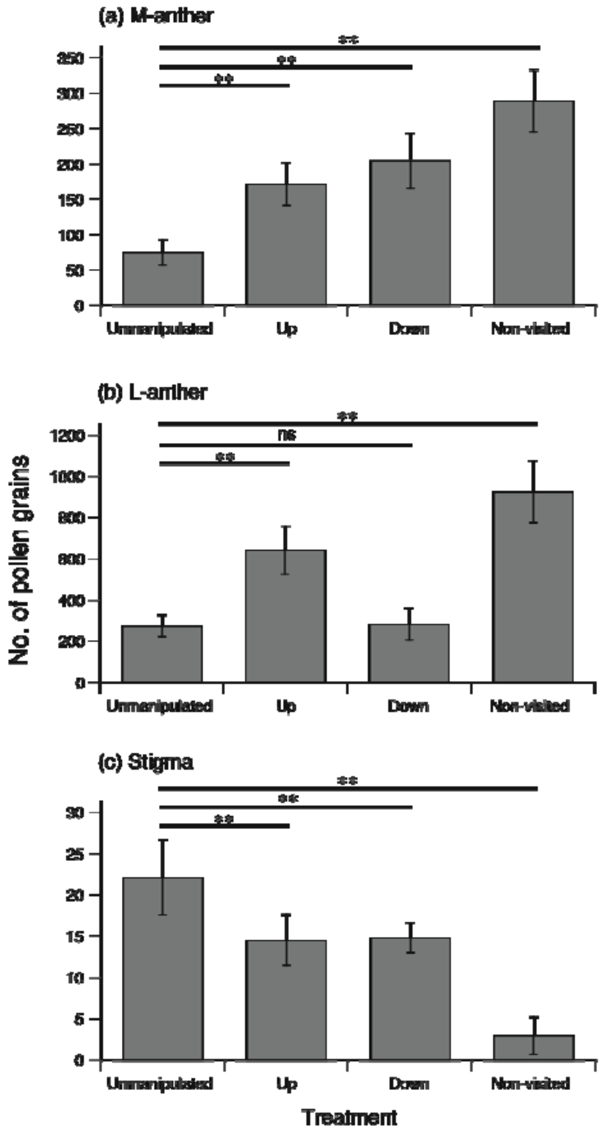


Fig. 4. Mean number of remaining pollen grains on the a) M-anther and b) L-anther, and pollen deposited on the c) stigma for the experimental (Unmanipulated, Up, and Down) and non-visited flowers. Bars show standard errors. ** *P* < 0.01, ns *P* > 0.05 by GLMs (see text).