Oecologia (2008) 156:341–350
DOI 10.1007/s00442-008-0991-4

PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Combined effects of inflorescence architecture, display size, plant density and empty flowers on bumble bee behaviour: experimental study with artificial inflorescences

Hiroshi S. Ishii · Yuimi Hirabayashi · Gaku Kudo

Received: 27 July 2007 / Accepted: 28 January 2008 / Published online: 19 February 2008

Abstract Pollen dispersal by pollinators is governed by the extent to which diverse effects on pollinator behaviour act independently or augment or moderate each other. Using artificial inflorescences, we assessed the behavioural responses of bumble bees to inflorescence architecture (raceme, panicle, and umbel), inflorescence size (7 or 13 flowers), inter-inflorescence distance and the proportion of empty flowers per inflorescence. The advantage of large inflorescences in terms of attractiveness was larger for racemes and umbels than for panicles, whereas the effect of inter-inflorescence distance on the number of successive probes was smaller for racemes than for panicles and umbels. The number of flowers probed per visit increased almost proportionally with display size when fewer flowers were empty, whereas the number increased less when many flowers were empty. Our results suggest that display size and the spatial arrangement of flowers and nectar within inflorescences can contribute to efficient pollination by affecting pollinator behaviour interactively.

Keywords Artificial flower · *Bombus* · Nectar · Pollinator

Communicated by Jeff Corner.

H. S. Ishii (✉)
Department of Ecosystem Studies,
Institute of Agricultural and Life Sciences,
The University of Tokyo, Tokyo 113-8657, Japan
e-mail: aishii@mail.ecc.u-tokyo.ac.jp

Y. Hirabayashi
Obihiro Centennial Museum, Obihiro 080-0846, Japan

G. Kudo
Graduate School of Environmental Earth Science,
Hokkaido University, Sapporo 060-0810, Japan

Introduction

The mating outcomes of animal-pollinated plants depend strongly on pollinator behaviour within and between plants (Harder et al. 2004). In plants that display multiple flowers simultaneously, all open flowers can act together to attract pollinators, enhancing pollen export, import, and potential mate diversity (reviewed by Harder and Barrett 1995). Large floral displays also allow self-pollination among a flowers (geitonogamy: Harder and Barrett 1995), which reduces the amount of pollen that would otherwise be exported to other plants (pollen discounting: e.g. Harder and Barrett 1995) and, in self-compatible species, decreases both the production of outcrossed seeds (seed discounting: Lloyd 1992) and the average offspring performance due to inbreeding depression (Charlesworth and Charlesworth 1987). The consistency of the movement patterns of pollinators within inflorescences can create variation in the mating environment among flowers, which may select for gradients in sex allocation among flowers (Brunet and Charlesworth 1995; Kudo et al. 2001) or even segregation of the sex roles within inflorescences (Jordan and Harder 2006). Given these diverse influences of pollinator behaviour on plant mating, the individual and joint effects of plant traits on these behaviours should strongly affect reproductive performance in plant populations.

Of the plant traits identified as significant influences on pollinator behaviour, the number of flowers open at once (display size) has received the most attention (Harder et al. 2004). Plants that display many flowers typically attract more pollinators than those that have small displays, and pollinators tend to visit more flowers on large displays. This effect can be modified by flower density; Fishbein and Venable (1996) found that *Asclepias tuberosa* inflorescences that had intermediate flower densities attracted more pollinators than those that had the same number of flowers at higher or lower densities. Interestingly, the number of probes received by individual flowers often varies little with display size, because the proportion of open flowers probed by individual pollinators typically declines with increasing display size (reviewed by Ohashi and Yahara 2001). This equalisation of visitation to individual flowers is indicative of an ideal free distribution (Fretwell and Lucas 1970) in which consumers distribute themselves among resource patches such that all individuals realise an equal rate of resource intake (for examples involving pollinators see Dreisig 1995; Robertson and Macnair 1995; Ishii and Harder 2006).

The behaviour of pollinators while visiting inflorescences depends on both the three-dimensional arrangement of flowers (inflorescence architecture) and the distribution of nectar and/or pollen among flowers (Harder et al. 2004). Hainsworth et al. (1983) found that hummingbirds probed fewer flowers during visits to artificial hemispheric inflorescences than during visits to one- or two-dimensional inflorescences. In contrast, Jordan and Harder (2006) found that the number of flowers visited by bumble bees varied weakly among racemes, panicles, and umbels, although these different architectures affected the consistency of foraging paths within inflorescences.

Variation in nectar availability within inflorescences, including the occurrence of empty flowers, usually decreases the number of flowers probed by nectar-collecting pollinators (e.g. Biernaskie et al. 2002; Smithson and Gigord 2003). Hirabayashi et al. (2006) reported that this effect was common to all three types of artificial inflorescences, i.e. racemes, panicles, and umbels. In addition to the effects of intrinsic plant traits, particularly nectar secretion rates, the recent visitation history on each flower affects the nectar standing crop per flower. Maximum nectar accumulation typically occurs prior to pollinator activity (Cruden et al. 1983), after which nectar standing crops decrease rapidly (e.g. McDade and Weeks 2004). The effects of such temporal variation on pollinator service have received little attention.

Plant dispersion also affects the behaviour of pollinators. According to optimal diet theory, individual pollinators should probe a larger proportion of flowers with increasing inter-plant distances or decreasing inter-flower distances within plants (e.g. Pyke 1982); several studies have supported this prediction (Zimmerman 1981; Klinkhamer and de Jong 1990). Additionally, Ohashi and Yahara (1999) predicted that as the cost of inter-plant movement increases, the relative attractiveness of large displays should lessen and the number of successive flower probes should increase more rapidly with display size. Subsequent observations of bumble bee visitations to *Cirsium purpuratum* supported these predictions (Ohashi and Yahara 2002).

Despite the diversity of influences on pollinator behaviour, their effects have been examined largely in isolation, even though the extent to which these influences act independently or augment or moderate each other governs both pollinator foraging returns and pollen dispersal. Therefore, to evaluate the comprehensive effects of inflorescence characteristics on bumble bee behaviour, we conducted a factorial experiment with artificial inflorescences of different architectures, sizes, and spatial arrangements. In addition, to examine the effects of variable nectar states created by sequential bee visits, bees were allowed to repeatedly probe nonreplenishing flowers. We were primarily interested in the ability of inflorescence characteristics to improve the pollination success of animal-pollinated plants; thus, we did not consider additional effects of floral design such as the colour, shape, and size of individual flowers (see Ishii and Harder 2006). Our experiment specifically assessed the combined effects of inflorescence traits on (1) the relative attractiveness of large inflorescences, (2) the number of flower probes during an individual inflorescence visit, (3) the number of probes per flower, and (4) pollinator movement within inflorescences. Based on these results we discuss the pollination consequences of inflorescence characteristics for bee-pollinated plants.

Materials and methods

Experiment

We studied bee behaviour during August 2003 in a 4 × 5 × 2 m (width × depth × height) screened cage erected in an outdoor courtyard. The experiment involved workers from two captive colonies of *Bombus hypocrita* subsp. *sapporoensis* Cockerell, which were founded by over-wintered queens caught in May 2003 in a deciduous forest in Sapporo, Hokkaido, Japan.

We used two sizes of three commonly occurring inflorescence types (Fig. 1): vertical (raceme), conical (panicle) and horizontal circular (umbel) arrays. For each type, small inflorescences consisted of seven flowers, whereas large inflorescences had 13 flowers. Each artificial flower was constructed from 1 cm of the closed end of a 1.5-ml Eppendorf centrifuge tube painted yellow. Flowers were arrayed at 2-cm intervals on green inflorescence axes. The floor of the experimental cage was also green, so that the branches did not affect attraction.

During an experimental trial, we arranged small and large inflorescences of one inflorescence type in a 6 × 4 grid (Fig. 1). The inter-inflorescence distance (i.e. the distance between the centres of adjacent inflorescences; InfD in Fig. 1) was 8, 16, 24, or 32 cm during a trial; however, because of the flower arrangements within inflorescences,

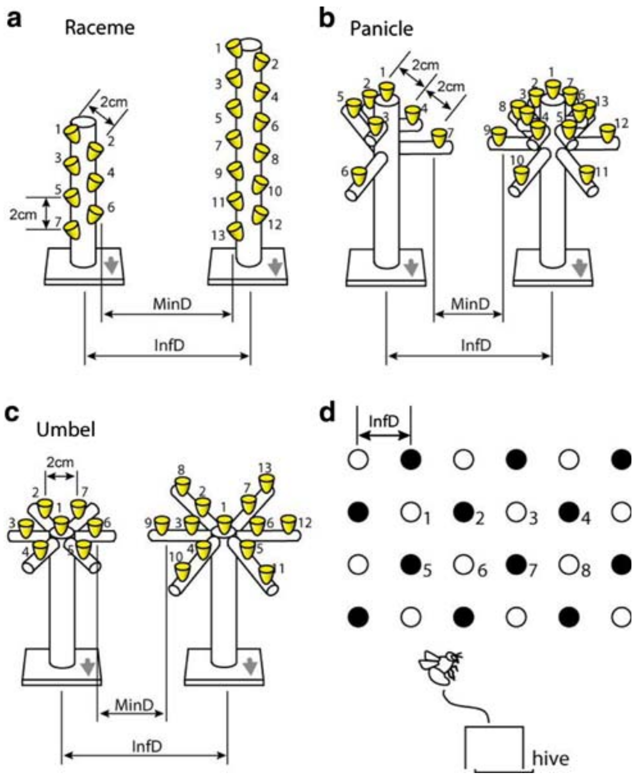

Fig. 1 Aspects of the study design, including the characteristics of inflorescences and foraging area. We constructed small and large inflorescences for each inflorescence type (**a** raceme, **b** panicle and **c** umbel); the three types were tested individually. *Filled* and *unfilled circles* in the foraging area represent small and large inflorescences, respectively. Only the eight inner inflorescences (**d**; inflorescences *1–8*) were considered in the analyses. *Arrows* at the bases of inflorescences indicate the direction of the beehive. We defined the inter-inflorescence distance (InfD) as the distance between the centres of adjacent inflorescences, and the minimum distance (MinD) as the minimum distance between flowers of adjacent inflorescences

the distances between distal flowers of adjacent inflorescences (MinD in Fig. 1) were closer than the InfD by 1.4 cm for racemes, 5.7 cm for panicles and 6.0 cm for umbels. We present results for the effect of inter-inflorescence distances (see below), which did not differ qualitatively from those for minimum distances.

All flowers were initially provided with 1 μl of 30% sucrose solution (nectar), but as each inflorescence received visits during the trial, its nectar status declined. This experiment mimicked situations in which plants receive visits rapidly in comparison to the nectar secretion rate, so that the maximum nectar accumulation occurs prior to pollinator activity (Cruden et al. 1983), but flowers are rapidly emptied once pollinators begin to forage (e.g. McDade and Weeks 2004). To consider the effect of nectar distribution within an inflorescence on bee behaviour, we classified each inflorescence according to its nectar status, assuming that a flower that had been probed at least once did not contain nectar (empty flower). Specifically, the nectar class of each inflorescence was defined as 5, 4, 3, 2 and 1 if the number of empty flowers was 0, 1–2, 3–4, 5–6 and 7, respectively, for small inflorescences, or 0, 1–4, 5–8, 9–12 and 13, respectively, for large inflorescences. Bees tended to visit the 16 outer inflorescences in the 6 × 4 grid more often than the eight inner inflorescences, so we only considered behaviour during visits to the inner inflorescences to exclude the edge effect. Each trial continued until the eight inner inflorescences had received a total of 30 visits.

We randomly selected two or three of approximately 20 foraging bees for each trial. Other bees were caged during measurements, so bees rarely simultaneously visited an inner inflorescence. Each day, approximately 3–5 bees died and were replaced; thus, >50 individuals were used during the ten-day experimental period. Prior to the experiment, each bee was trained repeatedly on a mixed array of all types of artificial inflorescences, of which inter-inflorescence distances were 16 or 24 cm, to preclude the effect of learning for any particular inflorescence type. Each test bee was pre-trained with other forager bees, so that they experienced the accidental bias of nectar volume due to other bees, as expected in nature.

The experiment lasted ten days, and we tested one set of the 12 types of array (three inflorescence types × four distances) each day. For five sets of trials, we increased the inter-inflorescence distance between trials (8 to 16 to 24 to 32 cm), whereas we used the opposite order for the remaining five trials (32 to 24 to 16 to 8 cm). The test order of the three inflorescence types was assigned randomly. All flowers were exchanged before each new trial to exclude the effects of scent marks (Goulson et al. 1998).

During a trial the behaviour of bees was recorded from various directions using four video cameras to avoid blind spots. Based on these recordings, we counted the number of visits to each inflorescence and number of flower probes during each visit, measured the duration of each inflorescence visit, and recorded the order of each visit out of the 30 inflorescence visits in a trial. The order of a visit represents the nectar status of the inflorescence patch, because the mean nectar standing crop per inflorescence is expected to decrease as the order of the visit increases. In total, we observed 3,600 visits (10 sets × four distances × three inflorescence types × 30 visits) to 960 inflorescences (10 sets × four distances × three inflorescence types × eight inflorescences) and 16,061 probes to 9,600 flowers.

Bees sometimes returned to flowers that they had probed during the same inflorescence visit (re-inspecting) and either probed (re-probing) or, most often, simply looked inside (scanning) the flower. Although scanning does not directly affect pollination, this behaviour, as well as re-probing, can affect the bee's instantaneous rate of energy gain on an inflorescence because the bee requires additional flights between flowers. To assess such effects on other behavioural responses by bees, we recorded the number of re-inspections (re-probing + scanning) on previously visited inflorescences. We also recorded the landing and leaving positions for visits on large inflorescences to characterise the movement patterns of bees on each inflorescence type. Because bees usually started probing the bottom flowers on racemes and the outer flowers on panicles and umbels, we defined the six bottommost flowers in racemes and the six outermost flowers in panicles and umbels of large inflorescences as "start positions".

Data analyses

All statistical analyses involved repeated-measures, generalised linear models (McCullagh and Nelder 1989: Genmod procedure of SAS ver. 9.1, SAS Institute Inc., Cary, NC, USA, 2004), which used generalised estimating equations with an exchangeable variance–covariance matrix (compound symmetry) to account for correlated responses within days or within inflorescences (Liang and Zeger 1986). Tests of the effects involved the generalised score statistic, *T*, which follows the χ^2 distribution (Rotnitzky and Jewell 1990). These analyses considered error distributions that reflected the characteristics of the observations, as well as appropriate transformations (link functions) of the means of the dependent variables to linearise their relationships to the independent variables, all of which are identified in the relevant table captions. Most analyses included inflorescence type and display size as categorical independent variables and inter-inflorescence distance (ln transformed) as a continuous covariate. We did not include inflorescence type in the analysis for the number of probes received by individual flowers because we analysed responses for each inflorescence type separately to incorporate the effect of flower position, as defined in Table 1. Display size was not an independent variable in the analysis for the probability of visits to large inflorescences because it was included as a dependent variable. The analysis of the probability of landing and leaving from start positions was performed only for large inflorescences. Some analyses also included the order of individual visits (1–30), nectar class, and number of probes to nectar flowers as continuous covariates. Bees never re-inspected flowers until they probed more than three flowers, so only inflorescence visits with more than three probes were used to analyse the number of re-inspections per visit. In the analysis of the duration of inflorescence visits, the number of probes to nectar flowers was ln-transformed before analysis. Given the ln transformation of the covariate in the model with a log-link function, a partial regression coefficient of $b > 1$ indicated an accelerating, increasing relationship of visit duration to the number of probes to nectar flowers, whereas $0 < b < 1$ indicated a decelerating relationship. We tested this expectation by determining whether the 95% confidence interval for the partial regression coefficient included 1.

We initially considered all possible interactions among independent variables in every analysis, but the models were then simplified by backward elimination of nonsignificant interactions ($\alpha = 0.05$). Back-transformation of results from these analyses resulted in asymmetrical standard errors (Figs. 2, 3, 4).

Table 1 Categories of flower position assigned to each flower within an inflorescence

Inflorescence type	Flower position		Flower position category
	Small inflorescence	Large inflorescence	
Raceme	1, 2	1, 2, 3, 4	Upper
	3, 4, 5	5, 6, 7, 8, 9	Middle
	6, 7	10, 11, 12, 13	Lower
Panicle	1	1	Upper
	2, 3, 4	2, 3, 4, 5, 6, 7	Middle
	5, 6, 7	8, 9, 10, 11, 12, 13	Lower
Umbel	1	1, 2, 3, 4, 5, 6, 7	Inner
	2, 3, 4, 5, 6, 7	8, 9, 10, 11, 12, 13	Outer

The numbers indicating different flower positions are illustrated in Fig. 1

Results

Bees consistently preferred large inflorescences to small ones, but this preference differed among inflorescence types (Table 2). The probability of visits to large inflorescences was significantly greater for racemes [0.57, 0.55–0.58 (least square mean, 95% confidence interval of the mean)] and umbels (0.59, 0.57–0.60) than for panicles (0.53, 0.52–0.54; based on Dunn-Šidák multiple comparisons, $\alpha = 0.05$). In contrast, we found no significant effects of either inter-inflorescence distance or order of inflorescence visits. The consistent difference in the attractiveness of large inflorescences during experimental trials indicated that relative attractiveness did not depend on the nectar status of an inflorescence patch.

Bees generally probed fewer flowers per inflorescence visit on small, closely spaced inflorescences with a large proportion of empty flowers than on large widely spaced inflorescences with a small proportion of empty flowers (Table 2; Fig. 2a,b). However, the effect of distance on the number of successive probes differed among inflorescence types, with a smaller effect on racemes than for panicles and umbels (based on Dunn–Šidák multiple comparisons, $\alpha = 0.05$; Fig. 2a). Display size and nectar class also had interacting effects on the number of probes per visit. The number of probes increased almost proportionally with

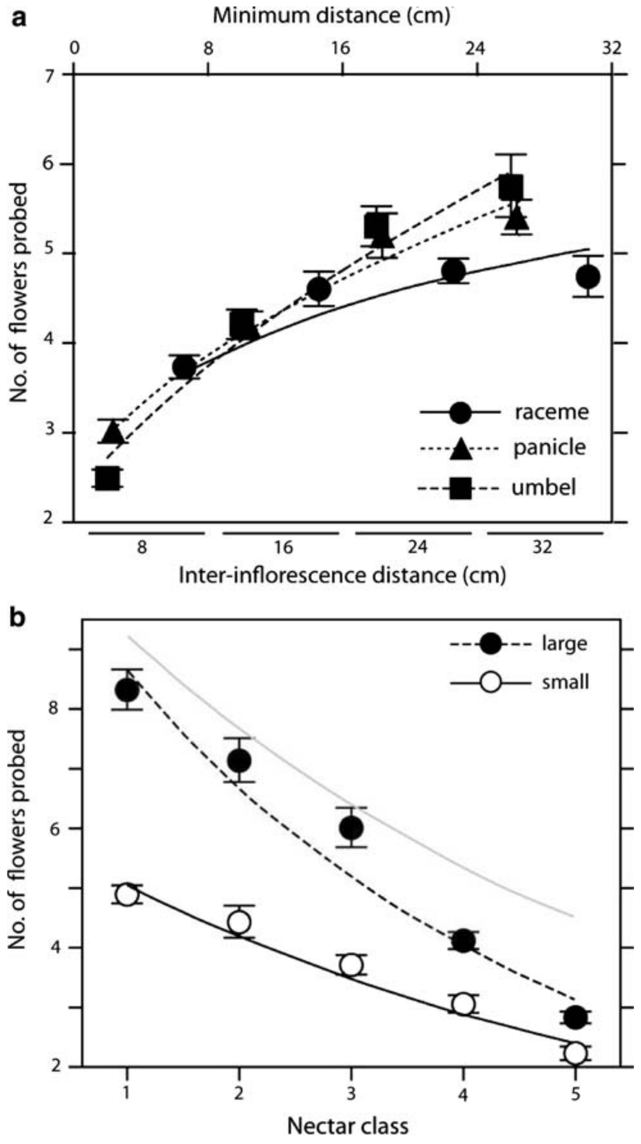

Fig. 2 Interacting effects of **a** inter-inflorescence distance and inflorescence type and **b** nectar class and display size on the mean (±SE) number of flowers probed per inflorescence visit (see Table 2 for statistical details). *Lines* indicate the partial regression relationships based on analyses of ln-transformed data. In **b**, the *grey line* is elevated above the regression for small displays by the ratio of flower numbers for large and small inflorescences (13/7). Observations for large displays below this line indicate that the number of probes increased less than proportionately with flower number

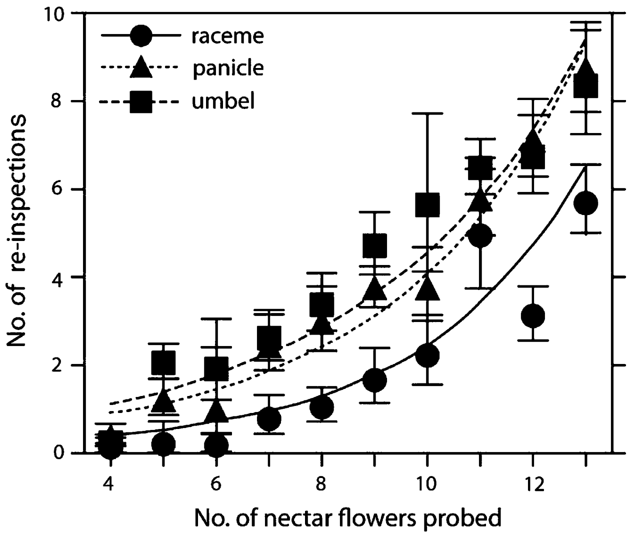

Fig. 3 Relationships of the mean (±SE) number of re-inspections per inflorescence visit to the number of rewarding flowers (i.e. nectar flowers) previously probed for each inflorescence type. *Lines* indicate the partial regression relationships based on the ln-transformation of the dependent variable

display size when inflorescences contained few empty flowers, whereas it increased less than proportionally with display size when most flowers were empty (Fig. 2b).

Despite the difference in the relative attractiveness of large inflorescences among inflorescence types, the number of probes received by individual flowers did not differ significantly between display sizes (Table 3). We observed similar results for all distances (no display size × distance interaction) for all inflorescence types. The number of probes per flower increased with inter-inflorescence distance. On racemes and panicles, the effect of flower position was significant because lower and upper flowers received more probes than did middle flowers, although the differences involved <10% additional probes. On umbels, the number of probes per flower did not differ between inner and outer flowers.

Bees re-inspected 37.1% ($N = 6{,}051$ flowers) of probed flowers at least once during inflorescence visits, although only 5.7% involved re-probing. The number of re-inspections increased as bees probed more flowers per inflorescence (Table 4; Fig. 3). This accelerating relationship indicated that the probability of re-inspection increased with the number of nectar flowers probed. Bees re-inspected more flowers on umbels and panicles than on racemes, although the partial regression coefficient for the number of nectar flowers probed was slightly smaller for umbels ($b \pm \mathrm{SE} = 0.432 \pm 0.016$) than for other inflorescence types (panicles: 0.502 ± 0.020; racemes: 0.496 ± 0.019; Dunn–Šidák multiple comparisons with $\alpha = 0.05$). In contrast, the partial regression coefficient for small inflorescences ($b \pm \mathrm{SE} = 0.679 \pm 0.045$) greatly exceeded that for large inflorescences (0.275 ± 0.016), and bees re-inspected more flowers on small inflorescences after probing a given number of nectar flowers.

Not surprisingly, bees spent longer visiting inflorescences on which they probed many nectar flowers (Table 4; Fig. 4a). The partial regression coefficient of ln (nectar flowers) (1.14, 95% confidence interval = 1.05–1.23) significantly exceeded 1, indicating an accelerating relationship of total visit duration to the number of nectar flowers

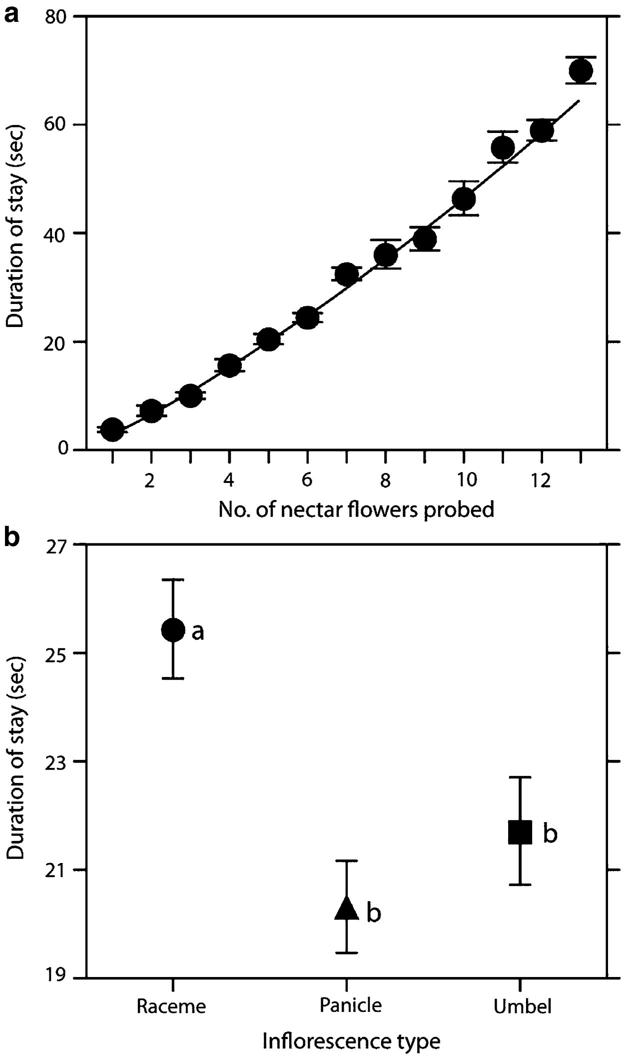

Fig. 4 Effects of **a** the number of rewarding flowers probed and **b** inflorescence type on mean (±SE) duration of stay per inflorescence visit. The *solid curve* in **a** depicts the partial regression relationship based on ln-transformation of the dependent and independent variables. In **b**, *letters* indicate the outcomes of of Dunn–Šidák multiple comparisons; means that share the same letter do not differ significantly ($\alpha = 0.05$)

probed. Consequently, the rate of nectar ingestion by a bee (i.e. the amount of nectar ingestion per unit time) decreased as the bee probed more nectar flowers. After accounting for the effect of the number of nectar flowers probed, we found that bees spent longer visiting racemes than panicles and umbels (Fig. 4b) and spent more time on small inflorescences than on large ones.

Although the probability of a bee beginning its visit to a flower at a start position differed significantly among inflorescence types (Table 5), significantly more than one-half of bees initially visited start positions in all inflorescence types [raceme: 78.3%, 74.7–81.5% (least square mean, 95% confidence interval of the mean), panicle: 86.9%, 84.0–89.5%; umbel: 84.4%, 81.7–86.7%]. This trend was more obvious for larger inter-inflorescence distances and when inflorescences contained relatively few empty flowers. In contrast, the probability of leaving from a start position greatly differed among inflorescence types. Less than one-half of bees left from start positions on racemes (25.1%, 21.6–29.1%), whereas more than one-half of bees left from start positions on panicles (60.1%, 55.9–64.2%) and umbels (66.0%, 62.0–69.8%). Although we used only large inflorescences in this analysis, bees behaved similarly on small inflorescences.

Discussion

We addressed the behavioural responses of bees to both previously studied inflorescence characteristics and to the novel aspect of the three-dimensional arrangement of flowers. In general, our results are consistent with previous studies: large displays attracted more bees than small displays (e.g. Ohashi and Yahara 2001); bees probed more flowers on large inflorescences than on small ones, but the proportion of flowers that they probed either did not differ or decreased with display size (Fig. 2b, Ohashi and Yahara 2001); the number of probes received per flowers varied little with display size (Table 3, e.g. Ishii and Harder 2006); bees tended to probe more flowers per inflorescence with increasing inter-inflorescence distance (Fig. 2a, e.g. Klinkhamer and de Jong 1990); encounters with empty flowers decreased the number of flowers probed per visit (Fig. 2b, e.g. Pyke 1982); and inflorescence architectures affected the consistency of foraging paths within inflorescences (Jordan and Harder 2006). Beyond the context of these known responses, we now discuss the new perspectives revealed by our experiments, and consider their implications for plant evolution.

Relative attractiveness of large displays

Bees exhibited a stronger preference for large inflorescences, especially when visiting racemes and umbels, regardless of the nectar status of the inflorescence patch or inter-inflorescence distance. According to optimal diet theory, pollinators prefer larger displays because of the decreased cost of inter-inflorescence flight to extract a given amount of nectar (e.g. Robertson and Macnair 1995; Ohashi and Yahara 1999). However, this explanation cannot apply to the observed effects of inflorescence architecture on bees' relative preference for large displays, because the density of flowers increased with flower number for panicles, but was constant for racemes and umbels (Fig. 1). Thus, the flight-cost reduction of large inflorescences provided by panicles should have exceeded that provided by racemes and umbels, but bees responded more strongly to

Table 2 Effects of inflorescence characteristics and inter-inflorescence distance on the relative attractiveness of large displays and the number of flowers probed per inflorescence visit

Dependent variable	Independent variable	*df*	*T*
Probability of visits to large inflorescences (relative attractiveness of large inflorescences)	Inflorescence type	2	8.25*
	ln (distance)	1	2.31
	Order of visits (1–30)	1	<0.01
Number of flowers probed per inflorescence visit	Inflorescence type	2	8.81*
	Inflorescence size	1	9.62**
	Nectar class	1	9.40**
	ln (distance)	1	9.75**
	Inflorescence type × ln(distance)	2	8.66*
	Inflorescence size × nectar class	1	7.82**

In each inflorescence visit, a bee chose either large or small inflorescence (dichotomous data) and then probed one to several flowers in succession within an inflorescence (count data). Thus, the analysis of the probability of visits to large inflorescences assumed binomial errors and a logit-link function, and the analysis of the number of flowers probed per inflorescence visit (aggregately distributed) used a negative-binomial response distribution and a log-link function

$^*P < 0.05$; $^{**}P < 0.01$

Table 3 Effects of inflorescence size, flower position and inter-inflorescence distance on the number of probes per individual flower for each inflorescence type

Inflorescence type	Independent variable	*df*	*T*
Raceme	Inflorescence size	1	0.08
	Flower position	2	9.09*
	ln (distance)	1	12.62***
Panicle	Inflorescence size	1	0.43
	Flower position	2	21.56***
	ln (distance)	1	69.18***
Umbel	Inflorescence size	1	2.51
	Flower position	1	1.31
	ln (distance)	1	89.24***

The analysis used a Poisson error distribution and a log link function

$^*P < 0.05$; $^{***}P < 0.001$

Table 4 Effects of inflorescence characteristics and inter-inflorescence distance on the number of re-inspected flowers and duration of inflorescence visits

Dependent variable	Independent variable	*df*	*T*
Number of re-inspections	Inflorescence type	2	8.22*
	Inflorescence size	1	7.80**
	Number of probes to nectar flower	1	9.13**
	ln (distance)	1	1.16
	Inflorescence type × nectar flower	2	7.25*
	Inflorescence size × nectar flower	1	8.17**
Duration of stay per visit	Inflorescence type	2	8.77*
	Inflorescence size	1	6.59*
	ln (number of probes to nectar flower)	1	9.71**
	ln (distance)	1	3.82†

The number of re-inspections was aggregately distributed and was analysed using negative-binomial error and a log-link function. The duration of inflorescence visits was analysed assuming a gamma error distribution and a log-link function

† $P < 0.1$; $^*P < 0.05$; $^{**}P < 0.01$

display size for the latter two architectures. Alternatively, our large racemes and umbels might have been more attractive than small inflorescences, simply because they presented proportionately larger display areas, whereas the display area appeared nearly constant for panicles across inflorescence size. Fishbein and Venable (1996) demonstrated that the attractiveness of inflorescences increased with display area when flower density was high, even if flower number was constant. Thus, for a given flower density, an increase in total display area may improve the attractiveness, probably because the maximum distance from which an object can be easily detected varies positively with its area (Giurfa et al. 1996).

We found no effect of inflorescence spacing on the relative attractiveness of large inflorescences, whereas Ohashi and Yahara (2002) reported that bumble bees exhibited a weak preference for large displays of *Cirsium purpuratum* when they were widely spaced. This discrepancy likely reflects the large difference in plant density between the two studies. Ohashi and Yahara (2002) studied plants that were separated by 1.3–10.2 m, whereas ours were separated by 0.08–0.32 m. As the distance between plants increases, inter-plant flights more strongly affect the energy budget of a bee relative to intra-plant flights (Ohashi and Yahara 1999). Moreover, when the inter-plant distance is large, the limited resolution of the eyesight of bees (Giurfa et al. 1996) may not allow them to detect more distant plants that

Table 5 Effects of inflorescence characteristics and inter-inflorescence distance on the probability that a bee began or ended inflorescence visits on flowers in start positions

Dependent variable	Independent variable	*df*	*T*
Landed on start positions	Inflorescence type	2	6.12*
	Nectar class	1	7.33**
	ln (distance)	1	6.07*
Left from start positions	Inflorescence type	2	9.66**
	Nectar class	1	1.89
	ln (distance)	1	2.97†

The analysis incorporated binomial error and a logit-link function

† $P < 0.1$; *$P < 0.05$; **$P < 0.01$

have larger displays. The range of inter-plant distance in our experiment was likely too short to detect such effects.

Successive probes within inflorescences

Bees probed fewer flowers on racemes than on panicles and umbels, particularly when the inflorescences were more separated (Fig. 2a). Using similar artificial inflorescences, Hirabayashi et al. (2006) did not detect significant effects of inflorescence architecture on the number of successive probes within inflorescences, which were arranged at 20-cm intervals. However, our experiment included longer inter-inflorescence distances and thus detected an interactive effect of inter-inflorescence distance and inflorescence architecture on successive probes. Energy-based hypotheses (e.g. Pyke 1982) propose that bees leave inflorescences when their instantaneous rates of energy gain decrease below the average rate of energy gain for the habitat. The instantaneous rate of energy gain usually decreases as bees probe more flowers on an inflorescence and the chance of re-probing (or re-inspecting) increases (Fig. 3, Ohashi and Yahara 2002). However, re-inspection occurred less often on racemes than on panicles and umbels because directional movement by bees on racemes allowed them to avoid re-inspection. For this reason, a bee's instantaneous rate of energy gain on racemes would be constant while moving upward and would then suddenly decline once reaching the top flowers. This sharp transition may explain why the number of successive visits on racemes was less sensitive to the inter-inflorescence distance. However, even though bees re-inspected fewer flowers on racemes, they took longer to probe the same number of nectar flowers on this inflorescence type than on panicles and umbels (Fig. 4b), probably because the upward movement against gravity required additional time. Consequently, bees likely realised a lower average rate of energy gain per flower on racemes than on panicles and umbels, which may be why bees generally probed fewer flowers on the former than on the latter inflorescence types.

The number of flowers probed per visit increased almost proportionally with display size when fewer flowers were empty, whereas it increased less strongly when many flowers were empty (Fig. 2b). Ohashi and Yahara (1999) proposed that the decline in the proportion of flowers probed with increasing display size results from an increased chance of re-probing (or re-inspecting) with the duration of pollinator visits and bees' limited memory for spatial information on previously probed flowers. However, this hypothesis cannot explain the effect of nectar class on the proportion of flowers visited. Instead, this response suggests that bees leave an inflorescence after they have encountered a few empty flowers (Dreisig 1989; Kadmon and Shmida 1992; Smithson and Gigord 2003). In natural habitats, recently visited individual plants tend to have a higher proportion of empty flowers (at least for a while), which may allow bees to determine whether a particular inflorescence is currently rewarding based on their experience in probing only a few flowers. We propose that the tendency of bees to probe proportionally fewer flowers on larger displays in natural conditions largely reflects responses to the existence of empty flowers.

When bees re-inspected flowers, they usually scanned, but did not re-probe. Thus, bees appeared to discriminate between nectar and empty flowers before re-probing, possibly by the scent of nectar (Dornhaus and Chittka 1999) or scent marks that bumble bees leave on visited flowers (Goulson et al. 1998).

Movement within inflorescences

On racemes, bees usually started from lower flowers and left from upper flowers, whereas on panicles and umbels, they usually started and left from outer flowers. Thus, movements from outer to inner flowers and from inner to outer flowers occurred frequently on panicles and umbels. Although bees sometimes began inflorescence visits on flowers in non-start positions, especially when the inter-inflorescence distance was short or most flowers were empty, the arrival and departure positions were relatively consistent across experimental treatments. Jordan and Harder (2006) reported similar results for racemes, umbels, and panicles with somewhat different arrangements of flowers, indicating that the contrasting movement patterns are specific to each inflorescence type. We also observed that bees re-inspected flowers more often on panicles and umbels than on racemes (Fig. 3), also indicating less constant movement on panicles and umbels. Upper and lower flowers of racemes and panicles received slightly more probes than middle flowers (Table 3) because bees often skipped middle flowers when they encountered empty flowers at the start positions.

Consequences of bee behaviour for plant evolution

Our experiment revealed that the spatial arrangement of flowers, display size, and the proportion of empty flowers acted in concert to affect various aspects of pollinator responses. Such interactive effects likely influence the evolution of inflorescence characteristics through effects on plant mating. For example, the effect of display size on relative attractiveness varied with inflorescence architecture, and effect on the number of flower probes per inflorescence visit varied with the proportion of empty flowers. Although the distribution of empty flowers reflected the visitation history by bees, such a behavioural response to empty flowers may affect the selection of plant strategies. Thus, the nature of selection to enhance the benefits of large displays for pollinator attraction while limiting the mating costs of within-inflorescence self-pollination will depend on the inflorescence architecture and the proportion of empty flowers. This expectation is consistent with previous observations that among plant species, the percentage of flowers that do not secrete nectar is positively correlated with the number of open flowers per inflorescence (Thakar et al. 2003; Tindall 2006). Similarly, inflorescence type and inter-inflorescence distance had interacting effects on the number of flowers probed; thus plant density could affect natural selection on inflorescence architecture. Although developmental and/or genetic constraints may restrict the evolutionary modification of fundamental inflorescence structure (Prusinkiewicz et al. 2007), selection can probably modify the three-dimensional arrangement of flowers through simple changes in the relative lengths of inflorescence branches and/or flower pedicels (Jordan and Harder 2006). Furthermore, typical patterns of pollinator movements on a specific inflorescence type likely create an evolutionary association between inflorescence type and position-dependent sex allocation or sexual segregation within inflorescences (Harder et al. 2004; Jordan and Harder 2006).

Additional research is necessary to assess the generality of our results. For example, all flowers in our study initially contained the same nectar volume, although several studies have observed a gradient of nectar distribution within vertical inflorescences (e.g. Best and Bierzychudek 1982). Several studies have demonstrated the limited effects of nectar gradients on the tendency for upward movement by bees (Waddington and Heinrich 1979) and on the number of successive probes per visit (Hirabayashi et al. 2006); whereas others have reported that these gradients affect the location at which bees begin foraging (Best and Bierzychudek 1982). In addition, unlike the consistent upward movement by bees on vertical inflorescences (e.g. Waddington and Heinrich 1979), hummingbirds move equally up and down (Harder et al. 2004). Thus, pollinator responses to inflorescence characteristics may differ among plant–pollinator combinations. Furthermore, our experiment eliminated the effects of floral design on bee responses, whereas traits of individual flowers such as colour, shape, and size also modify the performance of the entire floral display. Ishii and Harder (2006) demonstrated that reductions in individual flower size decreased the attractiveness of *Delphinium* inflorescences, but increased the number of probes per visit. Kudo and Harder (2005) also suggested evolutionary associations between flower and inflorescence characteristics. The assessment of such interactive effects among floral and inflorescence traits would also help to elucidate the function and evolution of inflorescence diversity.

Acknowledgments We thank L.D. Harder for his critical comments on the manuscript and assistance with statistical analysis. In addition, two anonymous reviewers provided valuable comments on the manuscript. We also thank the member of Regional Ecosystems in Hokkaido University for building the flight cage, and A.M. Ishii for collecting data. This research was funded by a fellowship of the Japan Society for the promotion of Science to H.S.I. (no. 0124) and a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology, Japan to G.K. (no. 15370006). The experiment complies with the laws of Japan, the country in which it was performed.

References

Best LS, Bierzychudek P (1982) Pollinator foraging on foxgloven (*Digitalis purpurea*): a test of a new model. Evolution 36:70–79

Biernaskie JM, Cartar RV, Hurly TA (2002) Risk-averse inflorescence departure in hummingbirds and bumble bees: could plants benefit from variable nectar volumes? Oikos 98:98–104

Brunet J, Charlesworth D (1995) Floral sex allocation in sequentially blooming plants. Evolution 49:70–79

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst 18:237–268

Cruden RW, Hermann SM, Peterson S (1983) Pattern of nectar production and plant-pollinator coevolution. In: Bentley B, Elias TS (eds) The biology of nectarie. Columbia University Press, New York, pp 80–125

Dornhaus A, Chittka L (1999) Evolutionary origins of bee dances. Nature 401:38

Dreisig H (1989) Nectar distribution assessment by bumblebees foraging at vertical inflorescences. Oikos 55:239–249

Dreisig H (1995) Ideal free distributions of nectar foraging bumblebees. Oikos 72:161–172

Fishbein M, Venable DL (1996) Evolution of inflorescence design: theory and data. Evolution 50:2165–2177

Fretwell SD, Lucas HL (1970) On territorial behavior and other factors influencing habitat distribution in birds. Acta Biotheor 19:16–36

Giurfa M, Vorobyev M, Kevan P, Menzel R (1996) Detection of colored stimuli by honeybees: minimum visual angles and receptor specific contrast. J Comp Physiol A 178:699–709

Goulson D, Hawson SA, Stout JC (1998) Foraging bumble bees avoid flowers already visited by conspecifics or by other bumblebees species. Anim Behav 55:199–206

Hainsworth FR, Mercier T, Wolf LL (1983) Floral arrangements and hummingbird feeding. Oecologia 58:225–229

Harder LD, Barrett SCH (1995) Mating cost of large floral displays in hermaphrodite plants. Nature 373:512–515

Harder LD, Jordan CY, Gross WE, Routley MB (2004). Beyond floricentrism: the pollination function of inflorescences. Plant Spec Biol 19:137–148

Hirabayashi Y, Ishii HS, Kudo G (2006) Significance of nectar distribution for bumblebee behavior within inflorescences, with reference to inflorescence architecture and display size. Ecoscience 13:351–359

Ishii HS, Harder LD (2006) The size of individual *Delphinium* flowers and the opportunity for geitonogamous pollination. Funct Ecol 20:1115–1123

Jordan CY, Harder LD (2006) Manipulation of bee behavior by inflorescence architecture and its consequence for plant mating. Am Nat 167:496–509

Kadmon R, Shmida A (1992) Departure rules used by bees foraging for nectar: a field test. Evol Ecol 6:142–151

Klinkhamer PG, de Jong TJ (1990) Effects of plant size, plant density, and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). Oikos 57:399–405

Kudo G, Harder LD (2005) Floral and inflorescence effects on variation in pollen removal and seed production among six legume species. Funct Ecol 19:245–254

Kudo G, Maeda T, Narita K (2001) Variation in floral sex allocation and reproductive success within inflorescences of *Corydalis ambigua* (Fumariaceae): pollination efficiency or resource limitation? J Ecol 89:48–56

Liang KY, Zeger SL (1986) Longitudinal data analysis using generalized linear models. Biometrika 73:13–22

Lloyd DG (1992) Self- and cross-fertilization in plants. II. The selection of self-fertilization. Int J Plant Sci 153:370–380

McCullagh P, Nelder JA (1989) Generalized linear models. 2nd edn. Chapman and Hall, London

McDade LA, Weeks JA (2004) Nectar in hummingbird-pollinated neotropical plant II: interactions with flower visitors. Biotropica 36:216–230

Ohashi K, Yahara T (1999) How long to stay on, and how often to visit a flowering plant? A model for foraging strategy when floral displays vary in size. Oikos 86:386–392

Ohashi K, Yahara T (2001) Behavioral responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. In: Chittka L, Thomson JD (eds) Cognitive ecology of pollination. Cambridge University Press, Cambridge, pp 274–296

Ohashi K, Yahara T (2002) Visit larger displays but probe proportionally fewer flowers: counterintuitive behaviour of nectar-collecting bumble bees achieves an ideal free distribution. Funct Ecol 16:492–503

Pyke GH (1982) Foraging in bumblebees: rule of departure from an inflorescence. Can J Zool 60:417–428

Prusinkiewicz P, Erasmus Y, Lane B, Harder LD, Coen E (2007) Evolution and development of inflorescence architecture. Science 8:1452–1456

Robertson AW, Macnair MR (1995) The effects of floral display size on pollinator service to individual flowers of *Mysotis* and *Mimulus*. Oikos 72:106–114

Rotnitzky A, Jewell NP (1990) Hypothesis testing of regression parameters in semiparametric generalized linear models for cluster correlated data. Biometrica 77:485–497

Smithson A, Gigord LDB (2003) The evolution of empty flowers revisited. Am Nat 161:537–552

Thakar JD, Knute K, Chauhan AK, Watve AV, Watve MG (2003) Nectarless flower: ecological correlates and evolutionary stability. Oecologia 136:565–570

Tindall JR (2006) The incidence and functional significance of nectarless flowers. M.Sc. thesis, University of Calgary, Calgary

Waddington KD, Heinrich B (1979) The foraging movements of bumblebees on vertical inflorescences: an experimental analysis. J Comp Physiol 134:113–117

Zimmerman M (1981) Optimal foraging, plant density and the marginal value theorem. Oecologia 49:148–153