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ORIGINAL ARTICLE

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Effects of number of flowers per raceme and number of racemes per plant on bumblebee visits and female reproductive success in *Salvia nipponica* (Labiatae)

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Abstract We studied the adaptive arrangement of raceme flowers in dense and sparse habitats of *Salvia nipponica*. We recorded bumblebee visits and collected mature seeds to estimate outcrossing rates from allozyme genotypes. To examine the resource investment in flowering stalks, we measured their length and dry mass. We found that a greater number of open flowers in a raceme enhanced bumblebee visits and successive probings in both density plots. However, greater height of a raceme did not enhance these in plots of either density. In the high-density plot, both a greater mean number of open flowers in a raceme and a greater number of flowering racemes on a plant enhanced bumblebee visits, successive probings, and also enhanced outcrossing rate in spite of an increase in successive probings. Although the number of flowers per raceme had a greater positive effect on seed-to-ovule ratios than did the number of racemes, the number of flowers per raceme may be constrained because a longer flowering stalk needs greater buckling strength. Hence, it may be advantageous for plants in high-density plots to increase both number of flowers per raceme and number of racemes. In the low-density plot, a greater number of flowering racemes on a plant enhanced pollinator visits, but a greater mean number of open flowers in a raceme did not. Hence, it may be advantageous for plants to increase the number of racemes while keeping the numbers of flowers per raceme small. Thus, the adaptive number of flowers per raceme and number of racemes per plant may differ between high- and low-density plots.

Keywords Bumblebee pollination · Floral display · Outcrossing rate · Plant density · Number of flowers per raceme and number of racemes

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Introduction

In animal-pollinated plants, the number of open flowers, the number of flowering racemes on a plant, and the height of racemes strongly affects attraction of pollinators and the pattern of their movements within a plant. Many studies have examined the effects of these factors on pollination, as explained below.

Many authors have reported that a greater number of open flowers in a raceme increases pollinator visits to the raceme (Ohara and Higashi 1994; Harder and Barrett 1995; Ishii and Sakai 2001; but see Robertson and Macnair 1995), which results in high seed-to-ovule ratios (Ohara and Higashi 1994) and high genotypic diversity in seed progenies (Palmer et al. 1988). However, geitonogamy occurs in racemes with a large number of open flowers because pollinators probe flowers successively within such racemes, which leads to self-fertilization and reduces outcrossing siring success due to pollen discounting (Harder and Barrett 1995; Harder et al. 2000). On the other hand, a greater number of flowering racemes in a plant has also been found to increase pollinator visits to the plant (Goulson et al. 1998; Williams et al. 2001).

Tall racemes may attract some pollinator species (Donnelly et al. 1998; but see Peakall and Handel 1993) owing to their visibility and accessibility. Local plant density is an important factor that affects pollination as well. Tall racemes are more advantageous in a dense habitat compared to in a sparse habitat (Ehrlén et al. 2002). However, it is costly to develop a tall raceme because, as compared with height, the diameter of a flowering stalk must increase more than proportionally.

What then is the optimal arrangement of raceme flowers? What is the optimal number of flowers per raceme, and number and height of racemes in a plant under limited availability of resources? Fishbein and Venable (1996) manipulated flowers in a raceme keeping the total number of simultaneously open flowers constant and demonstrated that plants that have an intermediate number of open flowers in a raceme and an intermediate number of flowering racemes were visited most frequently. However, they did not examine how the number of open flowers in a raceme and the number of flowering racemes on a plant affected the number of successive probes by pollinators and the outcrossing rate of the plant. Moreover, they did not reveal whether the attractiveness was derived from the number of open flowers in the raceme or the number of flowering racemes on the plant or both. In addition, although the height of a raceme often strongly correlates with the number of open flowers, few studies have distinguished the attractiveness of height from that of the number of open flowers (but see Ishii 2004).

Using a natural population of *Salvia nipponica*, we investigated how pollinator visitation responded to the number of open flowers in a raceme, the number of flowering racemes on a plant, and the height of racemes. We also studied the resulting seed production and outcrossing rate. We compared the foraging behavior of pollinators between dense and sparse plots. Based on these data, we examined the adaptive arrangement of raceme flowers in *S. nipponica*.

Materials and methods

Study species and site

Salvia nipponica Miq. is a self-compatible, perennial herb sage that commonly grows on the forest floor of the mountainous region of the temperate zone in Japan. In Miyagi Prefecture, including our study area, the flowering season extends for about 2 weeks into September, and pollination is almost exclusively performed by the bumblebee *Bombus diversus* Smith. They visit mainly for nectar, but also accumulate a small pollen load. Each plant has from one to about six flowering stalks, and each flowering stalk has one raceme. Each raceme produces up to ca. 40 yellow lip-shaped flowers. Flowers bloom sequentially, and each flower lasts 1 or 2 days, though a few flowers last as many as 4 days. The corolla of a flower drops off when its floral longevity is finished. The number of open flowers in a raceme tends to increase as the number of flowers in the raceme increases, and the number of flowering racemes on a plant (i.e., the number of racemes that have at least one open flower) tends to increase as the number of racemes on the plant increases. Autonomous selfing (autogamy) does not occur, and bumblebee visits are necessary for ovule fertilization (Y. C. Miyake, unpublished data). More detailed information of this species can be found in Ohashi (2002).

During 2001–2003, we studied a population on the forest floor of the cool temperate forest on Mt. Kagitori, Miyagi Prefecture, northern Honshu, Japan. This forest is dominated by coniferous *Pinus densiflora* with some deciduous trees such as *Quercus serrata* and *Acer sieboldianum*.

Bumblebee observation

To record the number of bumblebee visits to the study site, we set up two types of plots (1×2 m each): a high-density plot (during 2001–2003, containing 44–48 plants) and a low-density plot (in 2002 and 2003, containing 16 plants). The distances between the two plots were about 15 and 50 m in 2002 and 2003, respectively. We marked all racemes in the plots with plastic tags and counted the number of racemes on each plant and the number of flowers in each raceme. We recorded the number of open flowers in each raceme every day during the flowering season. We also measured the height of each raceme (i.e., distance from the ground to the top open flower) in the plots in 2003 (note that we regarded the height of a raceme as being the height of the top open flower since the height to the top open flower and that to the top flower, which may or may not be open, are strongly correlated).

Observation of bumblebee visits and their movements within a plant was carried out on sunny and relatively warm days in both the high-density plot (15–17 September 2001; 9, 12, 15, 17 and 18 September 2002; and 5, 7, 10, 12, 14 and 17 September 2003) and the low-density plot (15, 19, 20 and 22 September 2002, and 10–12 September 2003) for 80–120 min during 0900–1200 hours each day. For each bumblebee visiting the plots, we recorded which racemes were visited and how many probes were made on the same raceme. From this data, we calculated visitation rates (the number of visits to a raceme per hour and the number of visits to a plant per hour) and the number of successive probes to a plant (i.e., the mean number of probes per visit to a plant).

We also examined the differences in the distribution of the number of flowers in a raceme between the high- and low-density plots using a Kolmogorov-Smirnov two-sample test.

Effects of the number of open flowers and height of a raceme on bumblebee visits

Since the number of open flowers in a raceme correlates with its height, these two effects on bumblebee visits need to be evaluated separately. To examine the effects of the number of open flowers and height of a raceme on attraction to bumblebees, we divided the racemes in each plot into two classes according to the number of open flowers (one or two, and more than three) based on the median (two open flowers in a raceme). These two classes were each further divided into three height classes [short (1–15 cm), medium (16–25 cm), and tall (over 26 cm)]. The visitation rate (the number of visits per hour) to a raceme and the number of successive probes (per visit to a raceme) were each compared between the two classes of the number of open flowers and between the three height classes in each class of the number of open flowers using Mann-Whitney *U*-tests adjusted with sequential

Bonferroni tests at a significance level of $P = 0.05$. Racemes that were not visited during the observation were excluded from calculation of the number of successive probes.

Effects of the number of open flowers in a raceme and the number of flowering racemes on a plant on bumblebee visits

Factors that affect attraction to bumblebees were examined by a multiple regression analysis with visitation rate (the number of visits per hour) to a plant as the criterion variable and the mean number of open flowers in a raceme on a plant and the number of flowering racemes on a plant as the explanatory variables. Likewise, factors that affect the movement of bumblebees within a plant were examined by a multiple regression analysis with the number of successive probes (per visit to a plant) as the criterion variable and the mean number of open flowers in a raceme on a plant and the number of flowering racemes on a plant as the explanatory variables.

Seed production

In the high-density plot, seed number was measured ca. 2 weeks after flowering. Seeds were produced in the bottom of the hypanthium, which enabled us to count the number of swelling ovules even before full maturity. In Labiatae, each flower produces four ovules, and thus the number of ovules produced is easily estimated by counting the number of flowers produced. From those data, we calculated seed-to-ovule ratios (note that the seed number includes both fully matured seeds and seeds aborted during development). We collected seeds when they had fully matured, but some seeds had already dropped off of the hypanthiums because of their open structure. In the low-density plot, seeds suffered heavy predation, and we did not collect seed-production data for this plot.

The effect of bumblebee visits on seed production was examined by a multiple regression analysis with the seed-to-ovule ratio as the criterion variable and the mean number of flowers per raceme on a plant and the number of racemes on a plant as the explanatory variables.

Outcrossing rates

To estimate outcrossing rates from allozyme genotypes of seeds, we conducted starch gel electrophoresis on 354 progenies from 35 open-pollinated plants. Mature seeds were collected in mid-October 2003 from the high-density plot used for bumblebee observation (seeds were not collected from the low-density plot due to heavy predation), and seeds with full contents were air-dried and stored at 4°C for ca. 10 days before extraction and electrophoresis. Although 30 isozymes were initially screened, only three polymorphic loci showed clear staining and polymorphic patterns: diaphorase (DIA; EC 1.6.4.3), esterase (EST; EC 3.1.1), and phosphoglucose isomerase (PGI; EC 5.3.1.9).

To examine the effects of the number of open flowers in a raceme and the number of flowering racemes on a plant on outcrossing rates, the 35 sample plants were divided into the following classes: the mean number of open flowers on a plant was divided into five classes (1–2, –3, –4, –5, and over 5), the mean number of open flowers in a raceme was divided into six classes (1–1.25, –1.5, –2, –2.5, –3, and over 3), and the mean number of flowering racemes on a plant was divided into four classes (1, –1.5, –2, and over 2). We estimated the outcrossing rate for each class using Ritland's (2002) multilocus maximum likelihood program, MLTR (version 2.4). The standard errors of the multilocus outcrossing rate were calculated from the standard deviations of 1,000 bootstrap estimates, where the unit of resampling was the family. To detect if there were significant differences among classes, outcrossing rates were analyzed with *t*-tests adjusted with sequential Bonferroni tests at a significance level of $P = 0.05$.

Resource investment in flowering stalks

To examine the relationship between the resource investment in the flowering stalks of a plant and the flower number of the plant, we collected flowering stalks after seed maturation from the high-density plot in 2002 and 2003 ($n = 58$, and $n = 85$, respectively). A flowering stalk was clipped at the highest node with a pair of leaves beneath a raceme. After eliminating all hypanthiums, peduncles and folioles from a flowering stalk, we measured the length of the flowering stalks. Next, they were weighed after drying in an oven at 75°C for 1 week.

Results

Relationships between the number of flowers per raceme and the number of racemes on a plant

There was no significant relationship between the mean number of flowers per raceme on a plant and the number of racemes on a plant (linear regression: $n = 115$, $R^2 = 0.001$, $P = 0.71$ during 2001–2003). The mean number of flowering racemes on a plant (during its flowering period) increased with an increase in the number of racemes on a plant (linear regression: $Y = 0.554 + 0.423X$, $n = 127$, $R^2 = 0.769$, $P < 0.0001$ during 2001–2003). Likewise, the mean number of open flowers in a raceme (during its flowering span) increased with an increase in the number of flowers in the raceme (linear regression: $Y = 1.331 + 0.051X$, $n = 347$, $R^2 = 0.288$, $P < 0.0001$ during 2001–2003). In each analysis shown above, incomplete data sets (e.g., due to predation) were excluded.

Frequency distribution of the number of flowers in a raceme

Figure 1 shows the relative frequencies of the number of flowers in a raceme in each raceme-number class in the high- and low-density plots. In each plot, the majority of the flower numbers ranged from 1–15 (the data during the observed years are pooled). The distribution of the number of flowers in a raceme was significantly different between the high- and low-density plots [Kolmogorov-Smirnov two-sample test (without grouping by the number of racemes): $\chi^2=7.897$, $P=0.0386$]. In detail, for the class of the number of racemes = 1 or 2, the distribution ranged over a greater number of flowers in the high-density plot than in the low-density plot (Fig. 1; $\chi^2=10.62$, $P=0.0099$), while for the class of the number of racemes = 3 or 4, the distributions were similar for the two different densities (Fig. 1; $\chi^2=2.794$, $P=0.49$).

Effects of the number of open flowers and height of a raceme on bumblebee visits

In the high-density plot, racemes with a large number of open flowers (over 3) were visited more frequently and successively probed more than those with a small number of open flowers (1 or 2) (Fig. 2). On the other hand, the visitation rate to a raceme and the number of successive probes were not significantly different between the height classes within each class of the number of open flowers (Fig. 2). Thus, the number of open flowers, rather than height, affected bumblebee visits to racemes in the high-density plot. In contrast, in the low-density plot, neither height nor the number of open flowers affected the visitation rate (Fig. 2). However, racemes with a large number of open flowers (over 3) were successively probed more than those with a small number of open flowers (1 or 2).

Effects of the number of open flowers in a raceme and the number of flowering racemes on a plant on bumblebee visits

In the high-density plot, the visitation rate to a plant significantly increased with an increase in both the mean number of open flowers in a raceme and the number of flowering racemes on a plant in 2001 and 2003, though there was no significant relationship in 2002 (Table 1). Likewise, the number of successive probes significantly increased with an increase in both the mean number of open flowers in a raceme and the number of flowering racemes on a plant in 2001 and 2003, though there was no significant relationship in 2002 (Table 2).

In the low-density plot, the visitation rate to a plant significantly increased with an increase in the number of flowering racemes on a plant in 2002 and 2003 (Table 1), but was independent of the mean number of open flowers in a raceme. The number of successive probes significantly increased with an increase in both the mean number of open flowers in a raceme and the number of flowering racemes on a plant (Table 2).

Effects of the number of open flowers in a raceme and the number of flowering racemes on a plant on female reproductive success

Seed-to-ovule ratios increased significantly with an increase in both number of flowers per raceme and number of racemes per plant (Table 3). The standard regression coefficient of the number of flowers per raceme was about twice as large as that of the number of racemes, indicating that the effect of the number of flowers per raceme on seed-to-ovule ratios was large compared with that of the number of racemes on a plant.

Outcrossing rates increased significantly with an increase in the mean number of open flowers on a plant (Fig. 3). This indicates that seeds from a plant with a small number of open flowers are sired by both cross- and self-pollen, but that those from a plant with a large number of open flowers are almost all sired by cross-

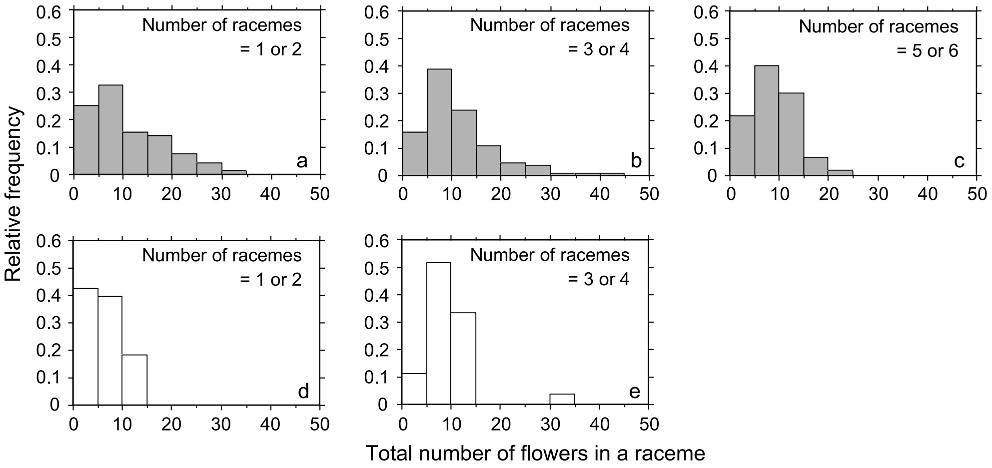

Fig. 1 Frequency distributions of the number of flowers in a raceme. In the high-density plot (**a**–**c**), the data during 2001–2003 are pooled [$n=63$, 40, and 12 plants for the number of racemes = 1 or 2 (**a**), 3 or 4 (**b**), and 5 or 6 (**c**), respectively]. In the low-density plot (**d** and **e**), the data in 2002 and 2003 are pooled [$n=21$ and 8 plants for the number of racemes = 1 or 2 (**d**), and 3 or 4 (**e**), respectively]

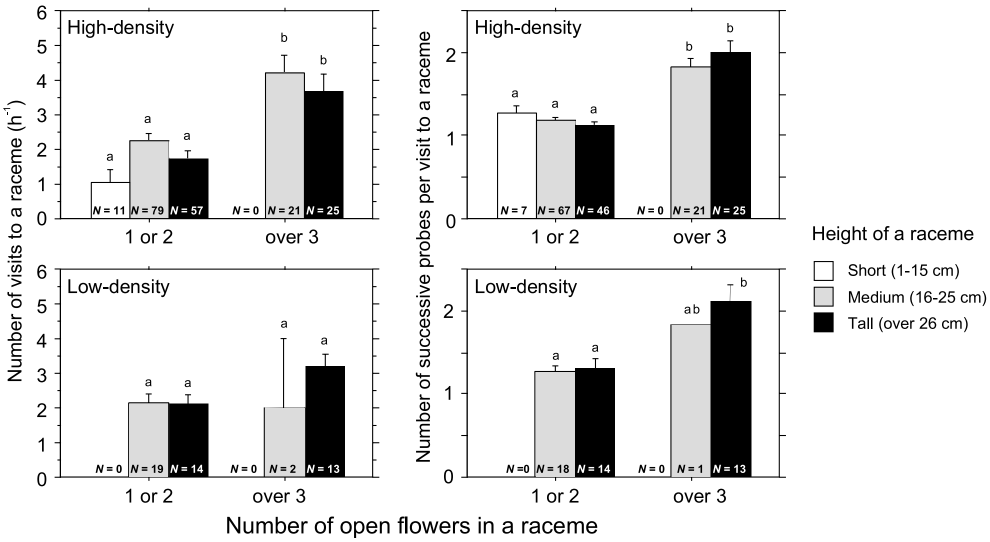

Fig. 2 The number of bumblebee visits to a raceme per hour and the number of successive probes per visit in the high- and low-density plots in 2003. Racemes were divided into two classes according to the number of open flowers in a raceme (1 or 2, and over 3) and three height classes [short (1–15 cm), medium (16–25 cm), and tall (over 26 cm)]. *Different letters* indicate a significant difference between the number of open flowers and height classes based on Mann-Whitney *U*-tests adjusted with sequential Bonferroni tests at a significance level of $P = 0.05$

pollen. When the number of open flowers on a plant was divided into the number of open flowers in a raceme and the number of flowering racemes on a plant, outcrossing rates were found to increase with an increase in both factors (Fig. 3).

Resource investment in flowering stalks

The total dry mass of flowering stalks of a plant increased with an increase in the number of flowers on a plant (linear regression: $n = 39$, $R^2 = 0.771$, $P < 0.0001$ in 2002; $n = 37$, $R^2 = 0.864$, $P < 0.0001$ in 2003), but was independent of the number of racemes on a plant (Table 4). Also, in each raceme, the dry mass of a flowering stalk increased linearly with an increase in the number of flowers (Fig. 4). However, the dry mass increased exponentially with an increase in length of a flowering stalk (Fig. 4). This indicates that a flowering stalk requires more than a proportional resource investment to increase its height.

Discussion

Effects of the height, the number of open flowers in a raceme and the number of flowering racemes in the high- and low-density plots on bumblebee visits

Bumblebees were attracted by the number of open flowers, rather than by the height, of a raceme in *S. nipponica* (Fig. 2). However, Ishii (2004) detected a

Table 1 Multiple regressions for the number of visits to a plant per hour as the criterion variable in the high- and low-density plots

Density	Year	Source	Regression coefficient	SE	Standard regression coefficient	*t*	*P*
High	2001[a]	Constant	0.253	0.753		0.336	0.7376
		Size	0.756	0.329	0.220	2.299	0.0241
		Number	0.974	0.206	0.452	4.720	< 0.0001
	2002 [b]	Constant	1.475	0.328		4.501	< 0.0001
		Size	0.078	0.134	0.046	0.579	0.5635
		Number	−0.003	0.095	−0.002	−0.027	0.9788
	2003 [c]	Constant	−0.086	0.404		−0.212	0.8326
		Size	0.750	0.144	0.376	5.222	< 0.0001
		Number	1.099	0.211	0.375	5.211	< 0.0001
Low	2002 [d]	Constant	0.098	0.785		0.125	0.9011
		Size	0.173	0.196	0.140	0.880	0.3858
		Number	0.899	0.308	0.465	2.922	0.0064
	2003 [e]	Constant	0.848	0.734		1.155	0.2583
		Size	0.289	0.257	0.193	1.126	0.2702
		Number	0.699	0.297	0.403	2.351	0.0263

Size is the mean number of open flowers in a raceme of a plant; *number* describes the number of flowering racemes on a plant

[a] $R^2 = 0.273$, $n = 83$, $P < 0.0001$

[b] $R^2 = 0.002$, $n = 171$, $P = 0.8399$

[c] $R^2 = 0.335$, $n = 136$, $P < 0.0001$

[d] $R^2 = 0.223$, $n = 34$, $P = 0.0201$

[e] $R^2 = 0.222$, $n = 30$, $P = 0.0336$

Table 2 Multiple regressions for the number of successive probes per visit to a plant as the criterion variable in the high- and low-density plots

Density	Year	Source	Regression coefficient	SE	Standard regression coefficient	t	P
High	2001[a]	Constant	−0.362	0.278		−1.301	0.1968
		Size	0.635	0.121	0.447	5.235	<0.0001
		Number	0.404	0.076	0.442	5.296	<0.0001
	2002 [b]	Constant	1.291	0.218		5.916	<0.0001
		Size	−0.102	0.089	−0.091	−1.142	0.2551
		Number	0.024	0.063	0.031	0.387	0.6993
	2003 [c]	Constant	−0.112	0.137		−0.823	0.4122
		Size	0.382	0.049	0.477	7.855	<0.0001
		Number	0.545	0.071	0.464	7.629	<0.0001
Low	2002 [d]	Constant	−0.182	0.480		−0.379	0.7074
		Size	0.244	0.120	0.299	2.032	0.0508
		Number	0.670	0.188	0.525	3.564	0.0012
	2003 [e]	Constant	−0.553	0.389		−1.419	0.1682
		Size	0.640	0.135	0.564	4.744	<0.0001
		Number	0.656	0.155	0.503	4.235	0.0003

Size is the mean number of open flowers in a raceme of a plant; *number* describes the number of flowering racemes of a plant

[a] $R^2 = 0.434$, $n = 83$, $P < 0.0001$

[b] $R^2 = 0.008$, $n = 171$, $P = 0.5189$

[c] $R^2 = 0.526$, $n = 136$, $P < 0.0001$

[d] $R^2 = 0.334$, $n = 34$, $P = 0.0018$

[e] $R^2 = 0.655$, $n = 30$, $P < 0.0001$

Table 3 Multiple regressions for seed-to-ovule ratio as the criterion variable in the high- and low-density plots

Source	Regression coefficient	SE	Standard regression coefficient	t	P
Constant	0.159	0.082		1.941	0.0594
Size	0.017	0.004	0.523	4.013	0.0003
Number	0.052	0.024	0.286	2.192	0.0342

Size is the mean number of open flowers in a raceme on a plant; *number* describes the number of flowering racemes on a plant

$R^2 = 0.327$, $n = 43$, $P = 0.0004$

preference of pollinators for higher-positioned flowers when the number of open flowers in a raceme was kept constant. This disagreement might be due to the difference in pollinator species; e.g., syrphid flies probed just one flower per visit to a raceme in Ishii (2004), while in our study, bumblebees probed open flowers successively. Bumblebees might prefer racemes with a large number of open flowers due to this behavior of successive probing. Separating the effect of the number of open flowers from that of raceme height would be important in each case since interaction between a plant species and the behavior of its pollinators is diverse. We concentrated on the number of open flowers, rather than the height, of a raceme since height had a smaller effect on pollinator visits in our study.

In the low-density plot, only an increase in the number of flowering racemes on a plant enhanced the visitation rate to a plant (Table 1), indicating that horizontal expansion by branching might be functional when the plant stands apart from its neighbors. This result may be related to the range of the number of flowers in a raceme: the number of flowers in a raceme was small (it ranged from 1–15) in the low-density plot (Fig. 1). In the high-density plot, however, increases in both the mean number of open flowers in a raceme and the number of flowering racemes on a plant enhanced attractiveness to bumblebees (except for 2002) (Table 1). Thus, some plants had racemes with many flowers without an increase in the number of flowering racemes, while others showed an increase in the number of racemes, although the number of flowers per raceme remained small (Fig. 1).

Effects of the number of open flowers in a raceme and the number of flowering racemes on a plant on female and male reproductive success

The effect of the number of flowers per raceme on the seed-to-ovule ratios was greater than that of the number of racemes (Table 3) though the seed-to-ovule ratio increased with an increase in both number of flowers per raceme and number of racemes in a plant. Thus, a raceme with many flowers is more advantageous than a raceme with few flowers for enhancing seed production.

Both the mean number of open flowers in a raceme and the mean number of flowering racemes contributed to the outcrossing rate of the plant. A plant with a small number of open flowers was visited less frequently (Table 1), and the number of successive probes was also smaller (Table 2), which resulted in the production of seeds sired by both cross- and self-pollen. On the other hand, a plant with a large number of open flowers was visited frequently (Table 1) and probed successively (Table 2). Successive probing of pollinators within a plant has been assumed to be costly, and the negative effects of a large number of open flowers have been discussed in previous studies (Klinkhamer and de Jong 1993; Harder and Barrett 1995; Robertson and Macnair 1995; Rademaker et al. 1997; Rademaker et al. 1999; Ohashi and Yahara 2002). However, our study showed that even though a plant was probed successively and received geitonogamous pollen, almost all seeds produced by the plant were sired by cross-pollen (Fig. 3). Thus, the negative effect of successive probing within a plant was far less than the positive effect of frequent visits to a plant, which should result in a great amount and diversity of pollen. *Salvia nipponica* may have a mate-selection mechanism against self-pollen so as to maximize the outcrossing rate, as shown by Carthew et al. (1996) for plants of the genus *Banksia*. Such selected high-quality pollen might also have enhanced seed-to-ovule ratios in our study. Further experiments, e.g., examining pollen competition by depositing cross- and self-pollen on a stigma, would be necessary to confirm this prediction.

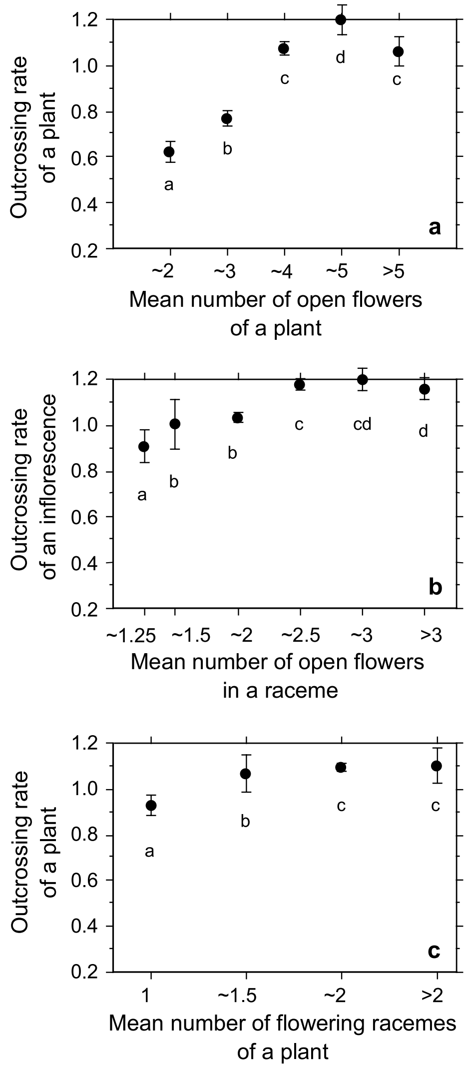

Fig. 3 Dependence of outcrossing rates (±1 SE) in the high-density plot in 2003 on the mean number of open flowers on a plant (**a**), the mean number of open flowers in a raceme (**b**), and the mean number of flowering racemes on a plant (**c**). *Different letters* indicate a significant difference between the classes based on *t*-tests adjusted with sequential Bonferroni tests at a significance level of $P = 0.05$

It is also important to consider the effect of the number of open flowers on the potential of outcrossing siring success because increased geitonogamy should result in reduced pollen export to other plants. For example, Harder et al. (2000) experimentally demonstrated a negative correlation between outcrossing siring success and the number of self-fertilized seeds per raceme, indicating the presence of pollen discounting. Although we have no data on outcrossing siring success in *S. nipponica*, the estimated proportion of pollen carry-over was 0.924 (Ohashi 2002), i.e., only 7.6% of pollen on the body of a bumblebee is deposited (or lost) during a single visit to a flower. This may suggest that the proportion of pollen exported to other plants is large and that pollen discounting is small even in a raceme with a large number of open flowers.

Costs of increasing the number of flowers per raceme

A flowering stalk required more than a proportional resource investment as it extended vertically (Fig. 4). This indicates that although a great number of flowers per raceme is advantageous to enhance seed production, vertical elongation of a flowering stalk is constrained in terms of resource investment. Givinsh (1982) showed in

Table 4 ANCOVA analysis of factors affecting the total dry mass of flowering stalks of a plant

Year	Source	df	SS	*F*	*P*
2002	Number of racemes	4	5,691.82	1.20	0.3318
	Number of flowers	1	25,907.73	21.85	< 0.0001
	Number of flowers × number of racemes	4	3,554.29	0.75	0.5664
	Error	29	34,380.12		
2003	Number of racemes	3	3,570.49	2.33	0.0947
	Number of flowers	1	29,661.43	58.15	< 0.0001
	Number of flowers × number of racemes	3	1,110.40	0.73	0.5450
	Error	29	14,793.71		

Covariate is the number of flowers on a plant and the main effect is the number of racemes on a plant
$R^2 = 0.327$, $n = 43$, $P = 0.0004$

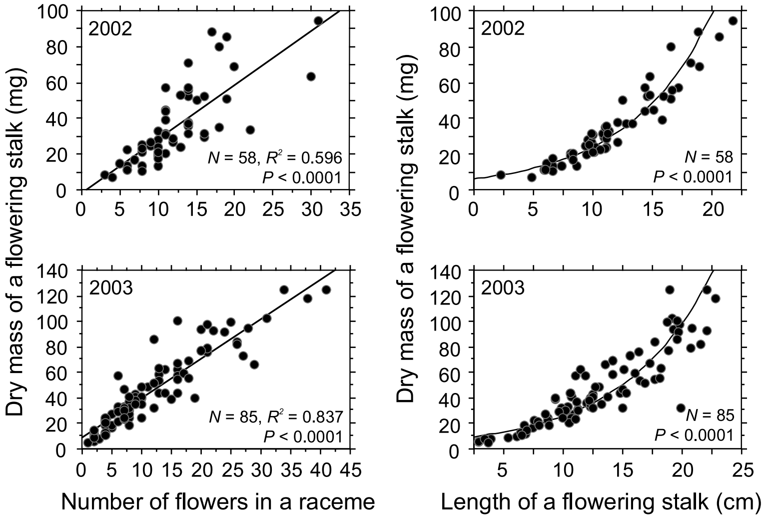

Fig. 4 Relationship between the number of flowers in a raceme and the dry mass of a flowering stalk and between the length of a flowering stalk and the dry mass of a flowering stalk in 2002 and 2003

his models that taller plants had to divert more resources into support tissues to increase buckling strength, so that allocation to other organs such as foliage should be reduced. In addition, several studies have suggested the risk of concentration of many flowers in a single large raceme. Dispersing flowers to more than one raceme might be important to distribute the risk of accidental injury of a flowering stalk (Wyatt 1982) and damage from herbivores (Brody and Mitchell 1997), as well as to moderate resource competition between flowers or fruits within the same raceme (Stebbins 1974).

Conclusion

A greater number of open flowers, rather than greater height, of a raceme can contribute to increasing seed-to-ovule ratios and the outcrossing rate through its attractiveness to bumblebees in a high-density plot. The only factor that constrains its vertical elongation to produce a raceme with many flowers is the cost of resource investment in the flowering stalk. In the high-density plot, it was advantageous for a plant to increase both number of flowers per raceme and number of racemes under the constraint of resource availability. In a low-density plot, increasing the number of racemes while keeping flower numbers small is advantageous since only an increase in the number of flowering racemes enhances bumblebee visits. Thus, the optimal number of flowers per raceme and number of racemes per plant may differ between high- and low-density plots. Further data on female reproductive success in low-density plots is needed.

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References

Brody AK, Mitchell RJ (1997) Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. Oecologia 110:86–93

Carthew SM, Whelan RJ, Ayre DJ (1996) Experimental confirmation of preferential outcrossing in *Banksia*. Int J Plant Sci 157:615–20

Donnelly SE, Lortie CJ, Aarssen LW (1998) Pollination in *Verbascum thapsus* (Scrophulariaceae): the advantage of being tall. Am J Bot 85:1618–25

Ehrlén J, Käck S, Ågren J (2002) Pollen limitation, seed production and scape length in *Primula farinosa*. Oikos 97:45–51

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

Givnish TJ (1982) On the adaptive significance of leaf height in forest herbs. Am Nat 120:353–381

Goulson D, Stout JC, Hawson SA, Allen JA (1998) Floral display size in comfrey, *Symphytum officinale* L. (Boraginaceae): relationships with visitation by three bumblebee species and subsequent seed set. Oecologia 113:502–508

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

Harder LD, Barrett SCH, Cole WW (2000) The mating consequences of sexual segregation within inflorescences of flowering plants. Proc R Soc Lond B Biol Sci 267:315–320

Ishii HS (2004) Increase of male reproductive components with size in an animal-pollinated hermaphrodite, *Narthecium asiaticum* (Liliaceae). Funct Ecol 18:130–137

Ishii HS, Sakai S (2001) Effects of display size and position on individual floral longevity in racemes of *Narthecium asiaticum* (Liliaceae). Funct Ecol 15:396–405

Klinkhamer PGL, De Jong TJ (1993) Attractiveness to pollinators: a plant's dilemma. Oikos 66:180–183

Ohara M, Higashi S (1994) Effects of inflorescence size on visits from pollinators and seed set of *Corydalis ambigua* (Papaveraceae). Oecologia 98:25–30

Ohashi K (2002) Consequences of floral complexity for bumblebee-mediated geitonogamous self-pollination in *Salvia nipponica* Miq. (Labiatae). Evolution 56:2414–2423

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

Palmer M, Travis J, Antonovics J (1988) Seasonal pollen flow and progeny diversity in *Amianthium muscaetoxicum*: ecological potential for multiple mating in a self-incompatible hermaphroditic perennial. Oecologia 77:19–24

Peakall R, Handel SN (1993) Pollinators discriminate among floral heights of a sexually deceptive orchid: implications for selection. Evolution 47:1681–1687

Rademaker MCJ, de Jong TJ, Klinkhamer PGL (1997) Pollen dynamics of bumble-bee visitation on *Echium vulgare*. Funct Ecol 11:554–563

Rademaker MCJ, de Jong TJ, van der Meijden E (1999) Selfing rates in natural populations of *Echium vulgare*: a combined empirical and model approach. Funct Ecol 13:828–837

Ritland K (2002) Extensions of models for the estimation of mating systems using *n* independent loci. Heredity 88:221–228

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

Stebbins GL (1974) Flowering plants: evolution above the species level. Belknap, Cambridge

Williams CF, Ruvinsky J, Scott PE, Hews DK (2001) Pollination, breeding system, and genetic structure in two sympatric *Delphinium* (Ranunculaceae) species. Am J Bot 88:1623–1633

Wyatt R (1982) Inflorescence architecture: how flower number, arrangement, and phenology affect pollination and fruit-set. Am J Bot 69:585–594