Temporal variation in floral display size and individual floral sex allocation in racemes of Narthecium asiaticum (Liliaceae)

Temporal variation in floral display size and individual floral sex allocation in racemes of *Narthecium asiaticum* (Liliaceae)[1]

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We analyzed the effects of temporal variation in floral display size (number of flowers open at one time on a plant) on the rate of pollen removed and receipt of individual flowers for the sequential blooming plant *Narthecium asiaticum* (Liliaceae). Because of the acropetal blooming of this species, the display sizes when upper flowers opened was much greater than the display sizes when lower flowers opened. Our experiments revealed that large displays lead to a high rate of pollen removal from individual flowers, though they do not lead to a high rate of ovule fertilization. Consequently, the rate of pollen grains removed per flower by pollinators was greater in upper flowers than in lower flowers. The pattern of sex allocation in individual flowers within a raceme was consistent with such variation. Namely, both maleness [stamen mass/(stamen mass + pistil mass)] and pollen : ovule ratio were larger in upper flowers. We suggest that the temporal variation in display size, in addition to such factors as dichogamy and pollinator directionality, also produces variation in the probability of successful pollen transfer from individual flowers that may cause the variation in the sex allocation of individual flowers.

Key words: display size; individual flowers; Liliaceae; mating environment; *Narthecium asiaticum*; sex allocation; temporal variation.

Recently, some researchers have noticed the variation in sex allocation among flowers within individual plants of hermaphrodites (Brunet, 1996; Nishikawa, 1998; Kudo and Molau, 1999; Ashman and Hitchens, 2000). To understand the evolution of plant reproductive strategy, it is important to investigate such variation in sex allocation among individual flowers because the male and female contributions to the fitness are the sum of the male and female contributions by individual flowers (Stanton and Galloway, 1990; Brunet and Charlesworth, 1995). Brunet and Charlesworth (1995) proposed that such variation in sex allocation might be selected if probability of successful pollen donation and receipt of individual flowers vary among individual flowers. For example, if pollen grains from differently positioned flowers have different opportunities for siring offspring, the fitness contribution through male functions can vary among flowers and some flowers may evolve to specialize as males. Such variations, which we term variations in the mating environment of flowers, following Brunet and Charlesworth (1995), are produced by dichogamy and pollinator directionality (movement of pollinators along inflorescence) (Darwin, 1877; Pellmyr, 1987; Brunet and Charlesworth, 1995; Brunet, 1996). In protandrous plants, for example, flowers that open early may experience a mating environment in which the available ovule : pollen ratio is low compared to that of later flowers. In this case, early flowers are predicted to be less male-biased than later flowers. Brunet (1996) reported that this prediction is consistent with the empirical observation for the protandrous plant *Aquilegia caerulea*.

However, the effects of display size (number of flowers open at one time on a plant) on the variation of the mating environment of individual flowers have not yet been considered, though the display size of a plant may vary temporally, namely, in sequentially blooming plants, the display size for flowers that open early may be smaller than for those that open late. We propose that the temporal variation in display size also causes a difference in mating environments among flowers because the display size has important effects on pollinator behavior and, consequently, on plant fitness as follows. First, a large display size often causes successive probes by a pollinator within a plant (Geber, 1985; Klinkhamer, de Jong, and de Bruyn, 1989; Robertson, 1992; Harder and Barrett, 1995), which results in an increase in geitonogamous pollination (Richads, 1986; de Jong, Waser, and Klinkhamer, 1993; Snow et al., 1996; Ishii and Sakai, 2001b). Second, larger displays often attract more pollinators (Thomson, 1988; Klinkhamer, de Jong, and de Bruyn, 1989; Kevan et al., 1990; Klingkhamer and de Jong, 1990; Ohara and Higashi, 1994; Dreisig, 1995; Robertson and Macnair, 1995; Ohashi and Yahara, 1998; Ishii and Sakai, 2001a). This promotes cross-pollination due to increased pollen receipt, pollen removal, and/or potential mate diversity (Harder and Barrett, 1996). Thus, if the display size when individual flowers open differs between flowers, the amount of geitonogamous pollination of individual flowers and/or the numbers of pollen grains dispersed from and deposited on individual flowers may vary among flowers.

Furthermore, it is likely that the shape of fitness curves as a function of floral display size differs between male and female functions, i.e., larger flowers or inflorescences disperse more pollen grains per flower, although they do not set more seeds or fruits per flower (Bell, 1985; Cruzan, Neal, and Willson, 1988; Mitchell, 1993; Vaughton and Ramsey, 1998; but see Johonson, Delph, and Elderkin, 1995; Morgan and Schoen, 1997). This may be because the female function (fertilization

of ovule) is almost completely satisfied by a single pollinator visit, or a very few visits, while successful male function (dispersal of pollen) requires repeated visits. Thus, if the display size of a plant varies temporally, the male success might vary among flowers, whereas most flowers achieve sufficient female success irrespective of their display size. This situation may also select for the difference in sex allocation among flowers.

In this paper, we examine the variation of mating environment and sex allocation of individual flowers with respect to temporal variation in display size of racemes for the sequential blooming plant *Narthecium asiaticum* (Liliaceae). In this species, because pollen grains are removed and stigmas are pollinated immediately after the anthesis before the drastic temporal change in display size (Ishii and Sakai, 2001a), we could easily examine the effect of display size on the rate of pollen removal and ovule fertilization when each flower opened. In addition, dichogamy and pollinator directionality were not found in this species (Ishii and Sakai, 2001a). Thus, *N. asiaticum* is a good subject for investigating the effect of display size on the mating environments of individual flowers. We address the following questions: (1) Does the temporal variation in floral display size produce a variation in the mating environments among flowers? (2) Does the sex allocation of individual flowers depend on the mating environments among flowers? Based on these data, we discuss the strategy involved in the sex allocation of individual flowers in a raceme in relation to the display size of the raceme.

MATERIALS AND METHODS

Narthecium asiaticum is an herbaceous clonal perennial that grows on mountainous marshlands of northern Japan. During July and August, a sexual flowering plant produces several racemes. Each raceme bears 21.9 ± 3.5 yellow flowers (mean ± SD, N = 325 in 1998 observation) that open sequentially from bottom to top (acropetal-blooming). Each flower is almost homogamous, as most of the pollen grains are removed and stigmas are pollinated a few days after anthesis (Ishii and Sakai, 2001a). Though this plant species is self-compatible, they seldom fertilize autonomically, as shown by the fact that seed : ovule ratios of bagged flowers were extremely small (Ishii and Sakai, 2001a). After the flowering period, tepals discolor to gray, gradually change to green, and remain on plants until fruits mature in August or September. Since discolored flowers do not contribute to the attractiveness of racemes (Ishii and Sakai, 2001a), we defined the display size of a raceme as the number of yellow flowers in the raceme on the day when the focal flower opened. Flowers do not secrete nectar, and their reward for pollinators is pollen. Their main pollinators are syrphid flies (*Eristalis* sp. and *Metasyrphus* sp.) and most of them visit only one flower per visit to a raceme (Ishii and Sakai, 2001a). However, the number of pollinator visits per flower is great when the display size of a raceme is large because large display size attracts more pollinators.

The experiments were conducted in Tashiro Marsh (40°42′ N, 140°35′ E, 570 m above sea level) located on Mt. Hakkoda, Towada-Hachimantai National Park, northern Honshu, Japan, in 1998 and 1999. The study sites we chose in this marshland were dominated by *N. asiaticum*.

Phenology of flowers—To examine the phenology of flowers in 1998 we randomly selected 96 racemes and recorded the date of the anthesis of the flower in each of three positions: the third flower from the bottom (lo flowers hereafter), the middle flower (mi flowers), and the third flower from the top (up flowers). In this and the following experiments, to distinguish the subject flowers within a raceme from others, we marked lo, mi, and up flowers by tying a thread around the peduncle of each flower used in the experiments.

Rate of pollen removal—To examine the effects of flower position and display size on the rate of pollen removal, we conducted the following treat-
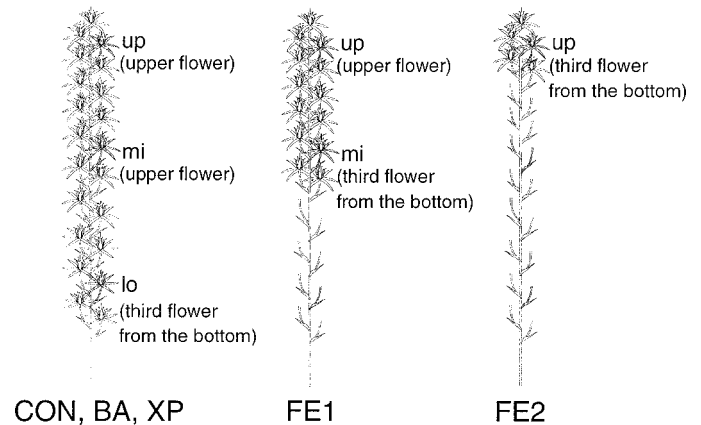

Fig. 1. Diagrams of experiment groups (see MATERIALS AND METHODS: *Rate of pollen removal*). In our experiments, we examined the third flower from the bottom (lo), the middle flower (mi), and the third flower from the top (up) of each sample raceme. CON, BA, XP experiments were conducted for racemes in which no flowers were removed. Flowers of FE1 and FE2 racemes were removed so that mi and up flowers became the third flowers from the bottom, respectively. Adapted from Ishii and Sakai (2001a).

ments for subsamples of marked racemes (Fig. 1). The first treatment was to expose racemes to natural pollination (CON); the second was to bag racemes to prevent visits by pollinators (BA); the third was to expose racemes to natural pollination and to remove about half of the flowers of each raceme just before flowering so that the mi flowers became the third flower from the bottom (FE1); and the fourth was to expose racemes to natural pollination and remove most flowers of each raceme just before flowering so that the up flowers became the third flower from the bottom (FE2).

In 1998, we conducted CON and BA treatments, and in 1999, we conducted all treatments (>20 samples were used for each treatment). The rate of pollen removal of the flowers in each of the three positions was determined by collecting all stamens from a sample of flowers at different stages of development between full buds and 7-d-old flowers in 1998 and full buds and 3-d-old flowers in 1999. Each sample was diluted with 10 mL of 0.1% saline and the number of pollen grains in four 0.25-mL subsamples was counted with stereoscope microscope (Model DAL, Kenis, Japan). The effect of manipulation on the number of remaining pollen grains within flowers of the same age was analyzed using Mann-Whitney U tests for each comparison pair. Their levels of significance were adjusted by the sequential Bonferroni test (Rice, 1989). Data from CON and BA and the data from the 1-d-old flowers of FE1 and FE2 treatments in this experiment were also used in a previous study (Ishii and Sakai, 2001a).

Rate of ovule fertilization—To examine the effects of flower position and display size on the time course of pollen deposition leading to fertilization (rate of ovule fertilization), we covered the pistil of sample flowers of lo, mi, and up positions at different stages that had been exposed to natural pollination. These experiments were conducted on 1-d-old to 7-d-old flowers of CON treatment in 1998 and 1-d-old to 3-d-old flowers of CON, FE1, and FE2 treatments in 1999 not used in the above experiment. In these experiments, we covered the pistils with tubes made of surgical tape. We closed the ends of the tubes and were careful not to touch their stigmas. To examine the degree of pollinator limitation in flowers in the three positions, we also conducted cross-pollination to all flowers of a raceme immediately after anthesis (XP).

We harvested all capsules when they matured and calculated seed : ovule ratio for each sample (>22 samples were used for each treatment). The differences in seed : ovule ratio between capsules of these treatments and those of cross-pollinated flowers (XP) were examined using Mann-Whitney U tests and Kruskal-Wallis tests. Data of CON and XP treatments in this experiment were also used in our previous study (Ishii and Sakai, 2001a).
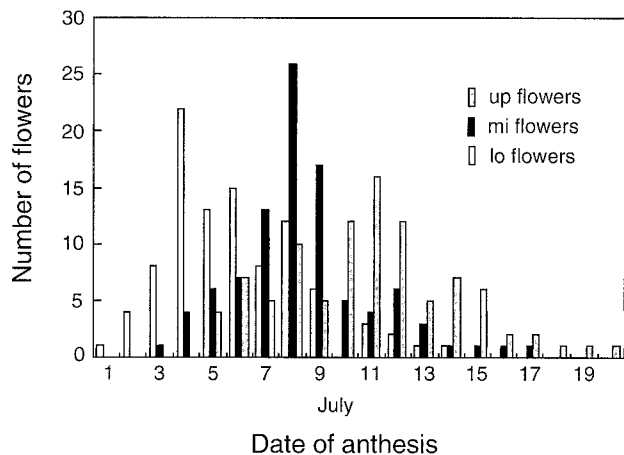
Fig. 2. Phenology of flowers in each of the three positions.

Temporal variation in floral display size—In 1999, to quantify the display size of a raceme when a flower in each of three positions was open, we recorded the overall raceme size (number of whole flowers in a raceme) and the number of yellow flowers on the day when each of the three positions opened for 24 randomly selected racemes of CON treatment. We first analyzed the correlation between the overall raceme size and the display sizes when lo, mi, and up flowers opened by using the Spearman's rank correlation tests and then compared the display size when a flower in each of three positions opened by using the Freedman tests.

To confirm the effects of flower removal treatment on the display size, we also recorded the number of yellow flowers on the day when mi and up flowers opened for FE1 (mi and up flowers) and FE2 (up flowers only) treatment, respectively. We used a one-way ANOVA to compare the display sizes of racemes when mi or up flowers opened among treatments.

Variation in floral sex allocation within racemes—In 1999, to examine the variation of floral sex allocation within racemes, we collected flowers of lo, mi, and up positions on the date of their anthesis. We recorded the dry mass of the stamens, pistils, and corollas of flowers from each position after they had been dried in an oven for >3 d. We then calculated the ratio of stamen mass/(stamen mass + pistil mass) for each flower to determine the maleness of the flower. We also collected full buds of lo, mi, and up positions and counted the numbers of pollen grains and ovules for each bud. We then calculated the pollen : ovule ratios (P : O) of flowers from each position. These data and seed set data of CON were analyzed with a Freedman test.

RESULTS

Phenology of the flowers—Though the flowering peak of mi and up flowers was 4 d and 7 d later than that of lo flowers, respectively, the date of the anthesis of these flowers overlapped each other: lo, mi, and up flowers opened during 1–14, 3–17, and 5–20 July, respectively (Fig. 2). This overlap was because individual racemes, in which most of flowers open within 1 wk, grew in succession during the first half of July.

Temporal variation in floral display size—We found no significant correlation between the overall raceme size and the display sizes when lo, mi, and up flowers opened with Spearman's rank correlation tests ($r_s = -0.135$, $P = 0.516$, $N = 24$; $r_s = 0.215$, $P = 0.303$, $N = 24$; $r_s = 0.315$, $P = 0.131$, $N = 24$, respectively). On the other hand, the display sizes of a raceme when lo flowers opened was much smaller than the display sizes when mi and up flowers opened (Table 1), because of the acropetal blooming of this species.

Display size of CON and FE1 racemes when mi flowers opened were 10.12 ± 0.39 and 4.24 ± 0.24 yellow flowers in the raceme, respectively (mean $\pm$ 1 SE; $F_{1,48} = 223.28$, $P < 0.0001$), and display size of CON, FE1, and FE2 when up flowers opened were 11.67 ± 0.90, 9.21 ± 0.53, and 4.20 ± 0.12 yellow flowers in the raceme, respectively ($F_{2,70} = 41.04$, $P < 0.0001$). These results indicate that display size of a raceme when mi and up flowers opened became small if their positions were manipulated so that they became the third flower from the bottom because of the acropetal blooming of this species.

Rate of pollen removal and ovule fertilization—In up and mi flowers, the numbers of pollen grains remaining in 1-d-old (in both years) and 3-d-old (in 1999) flowers were significantly fewer in the CON treatment than in the BA treatment (Fig. 3). Thus, the rates of pollen removal from up and mi flowers were higher if flowers were exposed to natural pollination (CON treatment) than if pollinator visits were prevented (BA treatment). Because the loss of pollen grains in the BA treatment was due to wind and gravity, the difference between the number of remaining pollen grains in the uncovered flowers and those in the covered flowers can be attributed to pollen removal by pollinators. When the positions of these flowers were

TABLE 1. Various characteristics of each positioned flower. Data are presented as means ± 1 SE. Freedman tests were applied to each row.

| Character | Flower position | | | N | χ^2 | P |
	lo	mi	up			
Display size of raceme[a]	3.80 ± 0.18	10.12 ± 0.39	11.67 ± 0.90	24	33.25	<0.0001
Flower mass (0.1 mg)	30.28 ± 0.88	26.76 ± 1.08	22.40 ± 5.24	25	36.14	<0.0001
Stamen mass (0.1 mg)	5.92 ± 0.25	5.40 ± 0.30	4.96 ± 0.26	25	8.88	0.0118
Pistil mass (0.1 mg)	6.36 ± 0.21	5.04 ± 0.30	3.88 ± 0.28	25	30.66	<0.0001
Corolla mass (0.1 mg)	18.00 ± 0.60	16.32 ± 0.62	13.56 ± 0.69	25	29.66	<0.0001
Maleness[b]	0.48 ± 0.01	0.52 ± 0.01	0.57 ± 0.02	25	17.24	0.0002
Number of pollen grains	61 027.2 ± 274.6	58 356.4 ± 289.3	55 550.0 ± 251.8	25	3.44	0.1791
Number of ovules	30.68 ± 0.79	28.08 ± 0.90	21.64 ± 0.81	25	43.38	<0.0001
Pollen : ovule ratio	2001.4 ± 88.0	2094.8 ± 88.9	2657.5 ± 168.4	25	16.64	0.0002
Number of seeds	18.50 ± 2.47	15.79 ± 2.64	6.71 ± 1.39	24	11.81	0.0027
Seed : ovule ratio	0.598 ± 0.073	0.516 ± 0.074	0.285 ± 0.057	24	13.4	0.0012
Fruit mass (0.1 mg)	42.46 ± 2.80	39.38 ± 2.86	25.92 ± 1.95	24	9.52	0.0073

[a] Display size of raceme (CON) when a flower in each of the three positions is open.
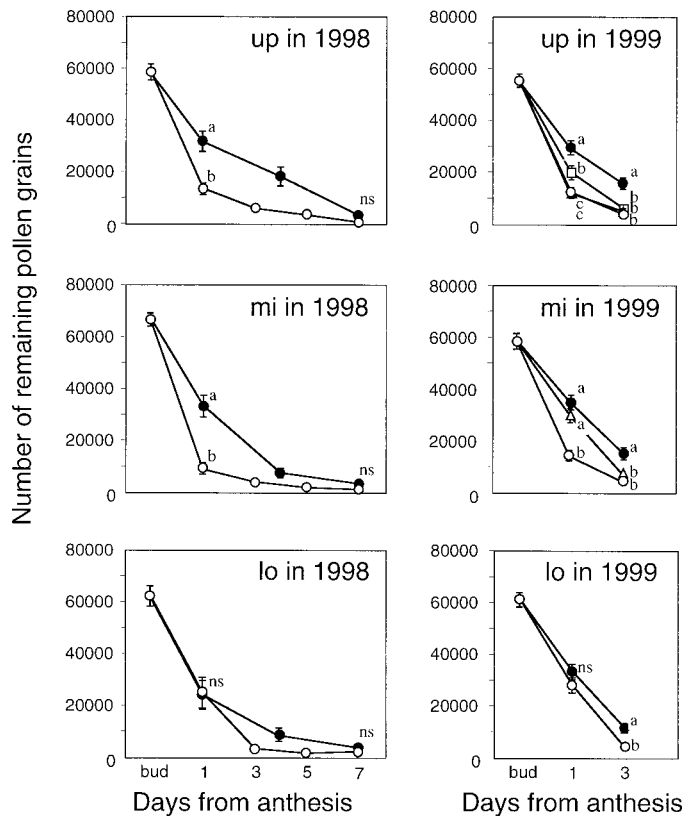[b] Maleness = stamen mass/(stamen mass + pistil mass).

Fig. 3. The mean number of pollen grains remaining per flower in full buds to 7-d-old flowers in 1998 and full buds to 3-d-old flowers in 1999: ○ = CON; ● = BA; △ = FE1; □ = FE2. Error bars represent the standard errors and each data point contains >20 samples. For each sample date of each figure, means with different letters differ significantly at $P = 0.05$ with Mann-Whitney U test. The levels of significance were adjusted by the sequential Bonferroni test.
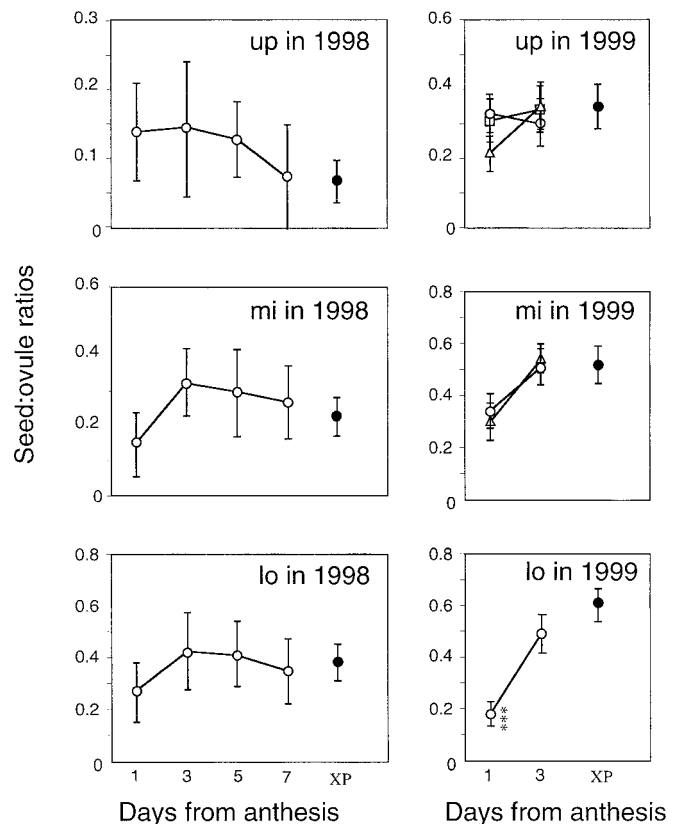
Fig. 4. The mean seed : ovule ratio of flowers bagged on different days from anthesis, including 1-d-old to 7-d-old flowers in 1998 and 1-d-old to 3-d-old flowers in 1999: ○ = CON; ● = XP; △ = FE1; □ = FE2. Error bars represent the standard errors and each data point contains >22 samples. Seed : ovule ratios between the treatments and flowers in XP did not differ significantly ($P > 0.05$) except for lo flowers at day 1 in 1999, which were significantly less (***$P < 0.001$; Mann-Whitney U tests). Seed : ovule ratios between these treatments also did not differ significantly ($P > 0.05$) except for lo flowers in 1999 ($P < 0.001$) with Kruskal-Wallis test.

manipulated as the third flower from the bottom (up and mi flowers in FE2 and FE1 treatments, respectively), the number of pollen grains in 1-d-old (in both years) flowers become significantly greater than that for the CON treatment (Fig. 3). This implies that the proportion of pollen grains removed by pollinators from up and mi flowers decreased if their positions were manipulated so that they became the third flowers from the bottom. In lo flowers (the third flower from the bottom), the number of pollen grains remaining in 1-d-old flowers (in both years) was not significantly different between the CON and the BA treatments, though the number of pollen grains remaining in 3-d-old flowers (in 1999) was significantly less in the CON treatment than in the BA treatment. This implies that the proportion of pollen grains removed by pollinators from lo flowers in the CON raceme was small. These results imply that the proportion of pollen grains removed by pollinators was small in the third flower from the bottom irrespective of their original positions, i.e., the proportion of pollen grains removed by pollinators was small when display size of a raceme was small.

On the other hand, for flowers in all positions (up, mi, and lo flowers), the seed : ovule ratio was not significantly different ($P > 0.05$) between flowers whose pistils were covered on different days from anthesis and flowers artificially cross-pollinated immediately after the anthesis (XP treatment), except for lo flowers, whose pistils were covered on the first day

from anthesis in the 1999 experiment (Fig. 4). These results imply that these flowers acquire enough pollen grains to set as many seeds as cross-pollinated flowers (XP treatment) within a few days of the anthesis, irrespective of their original and manipulated positions. This means that the ovule fertilization rate is less affected by display size than the pollen removal rate.

Variation in floral sex allocation within racemes—The dry mass of all parts of flowers (stamen, pistil, and corolla) decreased significantly in the order of lo, mi, and up flowers (Table 1). The sex allocation ratio was also different among flowers, i.e., maleness [stamen mass/(stamen mass + pistil mass)] significantly increased in the order of lo, mi, and up flowers. The number of pollen grains per flower was not significantly different among flower positions, whereas the number of ovules per flower significantly decreased in the order of lo, mi, and up flowers. Consequently, the P : O ratio increased in this order of flowers. The number of seeds per flower, seed : ovule ratio, and dry fruit mass also significantly decreased in this order.

DISCUSSION

In sequential blooming plants, the display size of a plant varies temporally, i.e., the display size when each flower opens varies among the flowers within the same plant. Such variation may produce differences in probability of successful pollen donation and receipt of individual flowers and select for variation in sex allocation among individual flowers. In *N. asiaticum*, flowers open acropetally and the display size is larger when upper flowers open than when lower flowers open (Table 1). The frequency of pollinator visits per flower is greater when the display size is large (Ishii and Sakai, 2001a). Consequently, the amount of pollen grains removed per flower by pollinators was greater in upper and middle flowers than in lower flowers (Fig. 3). The rate of pollen removal depended on the manipulated flower positions, which in turn depended on the floral display size, implying that the high rates of pollen removal in upper flowers are associated with the large display size when these flowers open. In this plant, because the date of the anthesis of the flowers in different positions overlap each other (Fig. 2), pollen from flowers at different positions should compete for the same ovules. Accordingly, it is likely that a greater proportion of pollen grains removed from the upper and the middle flowers leads to a greater proportion of successful pollen of those flowers. On the other hand, seed : ovule ratios did not greatly differ among treatments (Fig. 4), implying that the effect of display size on the fertilization rate of the ovule is small. This may be because the female function (fertilization of ovule) is almost completely satisfied by a single pollinator visit, or a very few visits, as flowers exposed to pollination for only 1 d set as many seeds as did the artificially pollinated flowers (Fig. 4). These results imply that male and female gain curves as a function of display size differs, i.e., the male success increases as the display size becomes large, whereas the female success does not depend on the display size. In such a situation, it is advantageous that flowers that open when the display size is large are more male-biased than flowers that open when the display size is small. This prediction is almost consistent with the pattern of sex allocation that we found in individual flowers, i.e., both maleness [stamen mass/(stamen mass + pistil mass)] and P : O ratio were larger in upper flowers than in lower flowers (Table 1). We suggest that the temporal variation in display size produces variation in the probability of successful pollen transfer from individual flowers and may cause (part of) the variation in the sex allocation of individual flowers.

A number of studies have reported that display size also affects the amount of geitonogamous pollination and, in turn, the degree of pollen discounting and self-pollination (reviewed in de Jong, Waser, and Klinkhamer, 1993). The cost of pollen discounting and self-pollination depends on various factors such as pollen carry-over (Robertson, 1992; Johnson and Nilsson, 1999), level of self-incompatibility (Galen and Gregory, 1989), and inbreeding depression (reviewed in Barrett and Harder, 1996). Hence, the differences in the frequency of geitonogamous pollination among individual flowers, which is mediated through the temporal variation in display size, may also affect the shape of male and female gain curves as a function of display size. In *N. asiaticum*, however, the effects of display size on geitonogamous pollination would be small because most pollinators leave a raceme after probing only one flower, irrespective of the display size of the raceme (Ishii and Sakai, 2001a). On the other hand, as mentioned in the introduction, neither dichogamy nor pollinator directionality, which

produce variation in the mating environment of flowers, were found in this species (Ishii and Sakai, 2001a). Thus, these factors are also not responsible for the variation in the mating environment of flowers in this species.

Seed : ovule ratio and the dry mass of reproductive structures such as flowers and fruits decreased acropetally (Table 1). Neither an increase in the frequency of geitonogamous self-pollination (Harder and Barrett, 1995) nor a decrease in the number of the potential mates in the population (Berry and Calvo, 1991; Goldingay and Whelan, 1993; Brunet and Charlesworth, 1995) are responsible for this pattern in seed : ovule ratios because the seed : ovule ratios of upper flowers still remained low when they were cross-pollinated (Fig. 4). This pattern in seed : ovule ratios may exist because the upper flowers are specialized as males (Brunet and Charlesworth, 1995) as shown by the fact that the number of pollen grains removed by pollinators was greater in upper flowers, and both maleness [stamen mass/(stamen mass + pistil mass)] and P : O ratio increased acropetally. Another hypothesis to explain these patterns in the reproductive structures is that flowers that develop earlier or closer to the source organs (e.g., leaves) are able to appropriate more reproductive resources through sink–source relationships than flowers that develop later or distant flowers (Lee, 1988; Wolfe, 1992; Diggle, 1995, 1997; Sakai and Harada, 2001) or that later flowers will have less time in which to obtain additional resources to support flowers and fruit development (Lee, 1988). However, resource competition and the effect of shortage in time for seed development alone can hardly explain the patterns of resource allocation in *N. asiaticum* because (1) the seed : ovule ratio remained low even when lower flowers were removed (Fig. 4), similar to the findings reported by Berry and Calvo (1991) (note that flowers were not pollen limited though they opened when display size was small) and (2) the desolate season for leaves (October–November) is much later than the fruit season (August) in *N. asiaticum*. These patterns might be attributable to the intrinsic (architectural) limitations (Diggle, 1995 and references therein). For example, if later flowers contain less vascular tissue and if the quantity of vascular tissue influences flower and fruit development, the mass of such reproductive structures should decreased acropetally. Ashman and Hitchens (2000) suggested that these variations in resource availability among individual flowers might also cause the selection for variation in sex allocation: flowers with reduced resources available become relatively more male. Thus, acropetal increase in maleness and P : O ratio in this species might be selected by additional effects of temporal variations in the mating environment and in the resource availability among individual flowers.

In this study, we revealed that the temporal variation in display size causes the difference in mating environments among flowers. The rate of pollen removal was higher in the upper flowers associated with larger display size, though the rate of ovule fertilization was not. In *N. asiaticum*, the pattern of sex allocation within a raceme was consistent with such variation in the mating environment of individual flowers, i.e., upper flowers were more male biased than lower flowers. Such functionalization of individual flowers may lead to the evolution of female sterility in upper flowers or male sterility in lower flowers (Brunet and Charlesworth, 1995). This is consistent with several studies on andromonoecism in which male flowers were found typically open after hermaphroditic flowers (Primack and Lloyd, 1980; Thomson and Barrett, 1981; Baertin, 1982; Solomon, 1985; Diggle, 1991; Emms, 1996). Thus,

temporal variation in display size may also play a role in the evolution of breeding systems of plants through the temporal variation in the mating environment.

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