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## Effects of inflorescence size on visits from pollinators and seed set of *Corydalis ambigua* (Papaveraceae)

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**Abstract** Female reproductive success (seed set) of a spring ephemeral plant, *Corydalis ambigua* Cham. et Schlecht (Papaveraceae) was investigated in relation to inflorescence size and foraging behavior (frequency and duration of visitations) by pollinators (namely, overwintered queens of *Bombus hypocrita sapporensis*) by detailed daily observations of a natural population. Pollination experiments indicated that *C. ambigua* is self-incompatible and that seed set was significantly affected by the behavior of the pollinating queens. Plants with larger inflorescences were visited more often than those with fewer flowers. Fecundity also increased with increasing size of inflorescences. Visitation time (duration of foraging) rather than the frequency of visitations (number of visits) was critical for higher fecundity. Seed production was strongly enhanced by a few long visits (of more than 60 s), and seemed to be independent of large numbers of short visits (of less than 60 s). Hence, plants with larger inflorescences, which provide a conspicuous signal to pollinators and offer greater rewards in terms of nectar, received longer visits by *B. hypocrita sapporensis* queens and those plants exhibited higher fecundity.

**Key words** Bumblebee · *Corydalis ambigua*  
Female reproductive success · Inflorescence size  
Pollinator visitation

### Introduction

For any outcrossing entomophilous plant, transport of pollen by pollinators has important consequences for re-

productive success. Accordingly, the type, abundance and efficiency of pollinators have a significant influence on flower morphology (Grant and Grant 1965) and on the energetic cost to the plant, which are reflected in flower size, production of nectar and odor, and the pollen-ovule ratio (Heinrich 1975; Cruden 1977; Solbrig and Rollins 1977). Among floral characteristics, the inflorescence size (number of flowers per inflorescence) has been shown to be important for the attraction of pollinators (Willson and Rathcke 1974; Willson and Price 1977; Shaffer and Shaffer 1979; Thomson et al. 1982). It is generally accepted that pollinators preferentially visit large inflorescences with many flowers that provide a strong signal to attract pollinators, and offer greater rewards in terms of pollen and/or nectar. However, inflorescence size must represent a trade-off between the resources available for the production of the attractive organs and those available for the maturation of fruits and future reproduction (Willson et al. 1979; Schemske 1980; Wyatt 1982; Firmage and Cole 1988). In order to explain the evolution of inflorescence size, several hypotheses have been proposed, and inflorescences containing different numbers of flowers have been shown to be differentially successful as pollen recipients (female function) and/or pollen donors (male function) in many hermaphroditic plant species (Willson and Price 1977; Schemske 1980; Montalvo and Ackerman 1987; Sutherland 1987; Firmage and Cole 1988; Ackerman 1989; Campbell 1989a, b; Broyles and Wyatt 1990; Rodriguez-Robles et al. 1992).

*Corydalis ambigua* Chem. et Schlecht (Papaveraceae) is a perennial herb that grows predominantly in deciduous forests in the northern parts of Japan. The plant generally produces a single inflorescence per individual. The flowers are strongly zygomorphic, light blue with a deep blue palate, and arranged in racemose inflorescences of 1 to about 15 flowers (Ohwi 1975). Each flower has a spur in which nectar collects. The plants flower from the end of April to early May, before the expansion of the leaves of the canopy trees. Each flower remains in anthesis for 3–4 days. During its short flowering period, this plant is

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exclusively visited and pollinated by overwintered queens of the bumblebee, *Bombus hypocrita sapporensis* Cockerell (Sakagami 1951; Higashi et al. 1988). This specific plant-pollinator interaction during the short flowering period in early spring allows us to observe details of the behavior of the bumblebees, and to examine the precise effectiveness of visitations by the pollinator in terms of subsequent seed set of *C. ambigua*.

In this study, we examined the way in which inflorescence displays affect visitations by pollinators and subsequent seed set (female reproductive success), as well as visitation frequency and the duration of visitations that affect the seed set.

## Materials and methods

### Study site

The field study was conducted in Nopporo Forest Park, near Sapporo (43° 25' N, 143° 32' E), from the end of April (flowering) to the middle of June (fruiting) in 1986. The overstories of the study site were *Acer mono*, *Quercus mongolica* var. *grosseserrata*, *Ulmus davidiana* var. *japonica*, and the main associated herbaceous species were *Adonis ramosa*, *Trillium kamtschaticum*, *Symplocarpus reinfolium* and *Veratrum grandiflorum*.

### Breeding system

Pollination experiments were conducted in order to characterize the breeding system of *C. ambigua*. Since the stigmatic area is in contact with anthers with pollen grains, emasculation without damage to stigmata is technically difficult. Hand-pollination was carried out without emasculation. Whole inflorescences of 24 individuals, i.e. 212 flowers, were bagged with nylon bags prior to anthesis. A total of 148 flowers on 21 individuals were hand-pollinated using pollen from distant plants. In addition, 90 flowers on 16 individuals were geitonogamously pollinated, and 158 flowers on 30 individuals were chosen as controls. In mid-June, all individuals in the pollination experiments were collected and seed set was examined.

To determine the pollen/ovule ratio (P/O ratio: Cruden 1977), 20 flowers were collected from five individuals before dehiscence of anthers. The number of ovules per flower was counted under a dissecting microscope. Pollen grains were washed from anthers with 95% ethanol, mounted in glycerine jelly with gentian violet, and counted under a microscope. The P/O ratio was then calculated.

### Observation of pollinators

To investigate the visitation by pollinators and subsequent effects on seed set of the plants, a 5 × 5 m plot was established on the forest floor at a site where *C. ambigua* was dominant, and 224 individuals of *C. ambigua* within the plot were marked with sequentially numbered sticks before anthesis (29 April). After plants started to flower (3 May), all the visitations of queens of *B. hypocrita sapporensis* to each plant, and the duration of each visitation, were recorded by following the bumblebees within the plot from sunrise (about 0420 hours) to sunset (about 1840 hours). Since many bumblebees visited the plot simultaneously, visitation times were conveniently classified into two categories: namely, less than and more than 60 s. Observations were made daily for 7 days (but not on 7 May, when it rained) until all the flowers had withered and no further bumblebees visited the plot (10 May). At fruiting in mid-June, marked individuals within the plot were collected and their seed set was examined.

## Results

### Floral display and seed sets

Inflorescence size varied from 1 to 13 flowers per individual, and the mean inflorescence size was 4.8 flowers with a standard deviation of 2.4 ( $n=224$ , Fig. 1a). The seed-setting rate per individual [seed-ovule (S/O) ratio: ratio of total number of seeds produced per individual to total number of ovules per individual] varied from 0 to 86.1%, with an average of  $12.9 \pm 1.1\%$  ( $\pm$ SE). However, the most common inflorescence size did not show the greatest success in seed production. Seed-setting rates increased with increasing numbers of flowers per individual (Fig. 1b:  $y=1.20x+10.02$ ,  $P<0.025$ , ANOVA; Sokal and Rohlf 1981). Individuals with only one flower each did not produce any seeds at all.

### Breeding system

Each flower on inflorescences of *C. ambigua* had an average of  $9.7 \pm 0.3$  ovules and about 14,000 pollen grains per flower. The P/O ratio of about 1,400 is consistent

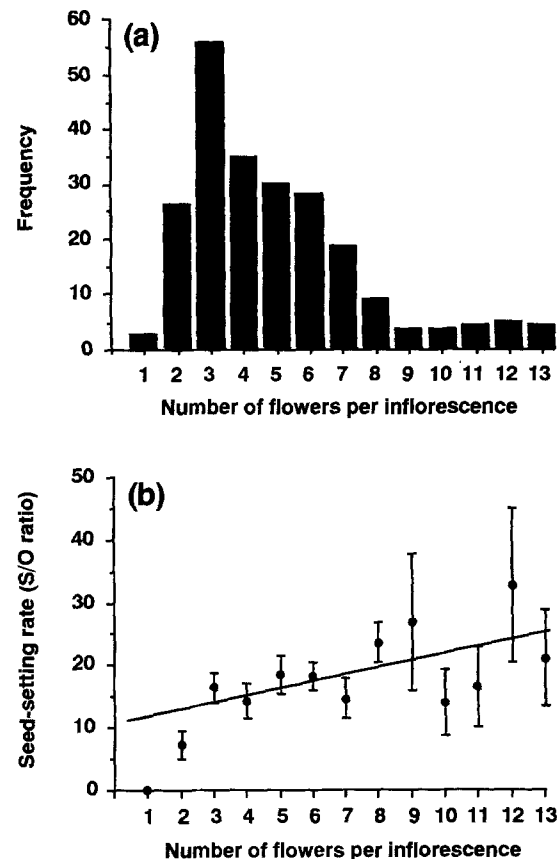
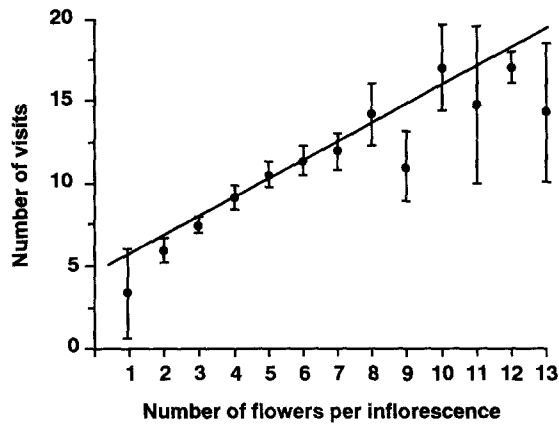


Fig. 1a, b Frequency histogram for inflorescence size (numbers of flowers per individual) within the study plot (a), and seed-setting rates (means  $\pm$  SE) for each inflorescence size (b). Seed-setting rates are expressed as arcsine-transformed values

**Table 1** Results of pollination experiments

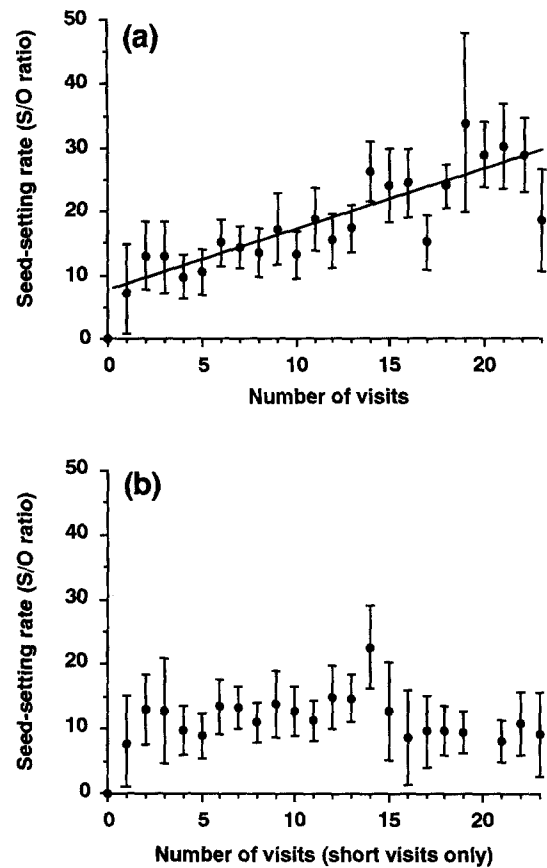
|                           | Number of plants (flowers) examined | Seed-setting rate (%) (mean±SE) |
|---------------------------|-------------------------------------|---------------------------------|
| Control                   | 30 (158)                            | 20.1±3.8                        |
| Bagged                    | 24 (212)                            | 0                               |
| Artificial pollination    |                                     |                                 |
| Cross pollination         | 21 (148)                            | 65.7±3.3                        |
| Geitonogamous pollination | 16 (90)                             | 1.6±1.1                         |

**Fig. 2** Relationship between the inflorescence size and the frequency of visitation by bumblebees (means±SE)

with a xenogamous breeding system (Cruden 1977). The pollination experiments also suggested that *C. ambigua* is self-incompatible and pollen limited, and that fecundity is strongly affected by the efficiency of visitation by *B. hypocrita sapporensis* queens (Table 1). Of marked individual plants left under open pollination conditions, 83.3% (25/30) set fruit; the mean number of seeds produced per plant was  $13.8 \pm 3.5$  and the average seed-setting rate was  $20.1 \pm 3.8\%$ . Individuals with bagged inflorescences produced no seeds. Very few seed set (7/90 flowers) were recognized after artificial geitonogamous pollination. By contrast, seed production was significantly enhanced by cross-pollination; all of the treated individuals (21/21) set fruit, and they showed significantly higher seed-setting rates ( $65.7 \pm 3.3\%$ ) than naturally pollinated plants ( $P < 0.001$ , *t*-test).

#### Visitation frequency

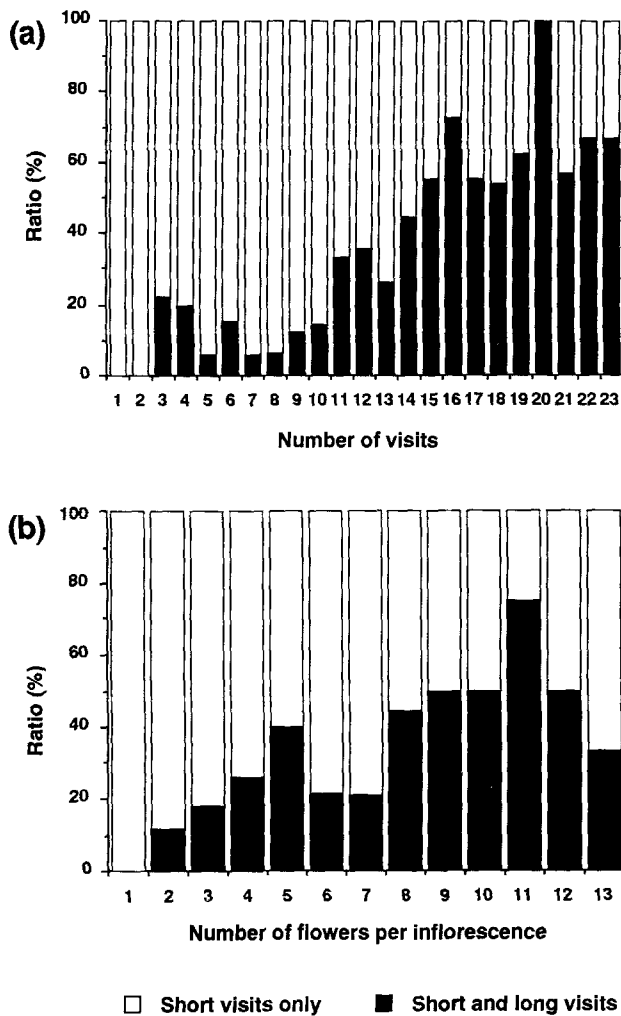
During the entire flowering season, there were 19 visits by honeybee (*Apis mellifera* L.) workers and 467 visits by bumblebee (*B. hypocrita sapporensis* Cockerell) queens to the  $5 \times 5$  m plot. Each plant was visited by bumblebees 0–23 times, with a mean of  $9.4 \pm 0.3$  times. Average numbers of visits by bumblebees varied with the number of flowers per plant, and the individuals with larger numbers of flowers were visited many times (Fig. 2:  $y = 1.07x + 4.47$ ,  $P < 0.01$ , ANOVA; Sokal and Rohlf

**Fig. 3a, b** Relationship between the frequency of visitation by bumblebees (a total visits; b short visits only) and the seed-setting rate (means±SE). Seed-setting rates are expressed as arcsine-transformed values

1981). Furthermore, the plants that received more visits from bumblebees had higher seed-setting rates (Fig. 3a). The results gave a significant linear regression equation in this case also ( $y = 0.81x + 7.98$ ,  $P < 0.01$ , ANOVA; Sokal and Rohlf 1981). Four plants (three plants with one flower and one plant with two flowers) that had received no visitations by bumblebees set no seeds, as we expected from our bagging experiment (cf. Table 1).

#### Duration of visitations

Of 224 plants, 162 individuals received short visits (of less than 60 s) only, and 58 individuals received not only short visits, but also long visits (of more than 60 s). As mentioned previously, four individuals received no visits from bumblebees. Numbers of short and long visits varied from 1 to 23 and from 1 to 4, respectively. It is of great interest that seed-setting rates of individuals that received a long visit at least once ( $25.29 \pm 2.83\%$ ,  $n = 58$ ) were significantly higher than those of individuals that received short visits only ( $8.85 \pm 0.86\%$ ,  $n = 162$ ;  $P < 0.001$ , *t*-test). Furthermore, among the plants that received short visits only, seed-setting rates were not significantly dif-



**Fig. 4a, b** Proportion of *Corydalis ambigua* individuals that received short visits (of less than 60 s) only and long visits (of more than 60 s) at least once, in relation to the total number of visits by bumblebees (a), and the inflorescence size (b)

ferent, regardless of the number of visits, indicating that even individuals that received many short visits did not have higher seed-setting rates than the ones that were visited on fewer occasions (Fig. 3b:  $F=0.51$ , ANOVA).

Although the individuals visited on  $\leq 2$  occasions were ones that had received only short visits, individuals with more than two visits included those that received long visits (Fig. 4a). More than 50% of individuals that received more than a total of 15 visits received long visits at least once, and all individuals visited on 20 occasions received long visits. It is very important to note here that the ratio of the individuals that received long visits increased with increases in the total number of visits ( $y=3.56x-7.15$ ,  $r=0.866$ ,  $P<0.001$ ). Furthermore, the relative number of long visits varied with the number of flowers per plant. The individuals with more flowers were visited many times (Fig. 2) and the proportion of individuals that received long visits also increased with increases in the number of flowers per plant ( $y=$

$4.05x+5.54$ ,  $r=0.779$ ,  $P<0.001$ ; Fig. 4b). Consequently, it appeared that individuals with more flowers tended to receive longer visits and showed higher fecundity (Fig. 1b).

## Discussion

It has commonly been observed that plants with larger inflorescences receive more visits from pollinators than plants with smaller ones (Willson and Price 1977; Schemske 1980; Udovic 1981; Thomson et al. 1982; Cole and Firmage 1984; Schmid-Hempel and Speiser 1988). It has also been demonstrated that increasing the size of the floral display leads to greater production of seeds, because many pollinators preferentially visit larger floral displays (Harper 1977; Stephenson 1979; Willson et al. 1979; Wyatt 1980; Shannon and Wyatt 1986). In the case of *C. ambigua*, plants with larger numbers of flowers also received more visits from *B. hypocrita sapporensis* queens and showed higher fecundity than plants with fewer flowers. However, the duration of visitation (foraging time) rather than the frequency of visitation (number of visits) was critical for higher fecundity. Higashi et al. (1988) reported that *C. ambigua* is exclusively pollinated by the robber-like activities of *B. hypocrita sapporensis* queens, which rarely collect nectar through the front of the flowers, but commonly do so by using perforations made in the spurs by themselves, or by predecessors. That is, while they are searching for flowers with nectar, perforating the spurs and absorbing the nectar, the bumblebees open the inner petals that enclose the anthers and stigma. Bumblebees that stay longer at flowers should deposit more pollen grains, as a result of the increased chance of a stigma touching a polleniferous area of the bumblebee's body. A positive relationship between nectar volume and handling time suggests that large nectar volumes have a very strong effect on the time spent at flowers of various species (Thomson and Plowright 1980; Hodges and Wolf 1981; Harder 1982, 1983; Thomson 1986). Accordingly, although visitation frequency is sometimes emphasized as a source of enhanced production of seeds, longer visits are very important in *C. ambigua*, and increase the chances that the pollinators will open more flowers and touch stigmata and anthers. Inflorescences with many flowers, which provide more nectar rewards, receive longer visits and more pollen grains, and are thus associated with higher fecundity.

Numbers of visits are generally accepted as contributing not only to opportunities for increased reception of pollen from different individuals, but also to chances for pollen export. As Willson and Price (1977) and Wolfe (1987) demonstrated in *Asclepias*, production of fruit and seed may not always increase with increasing inflorescence size and visitation frequency, whereas pollen removal is enhanced. Sutherland (1987) and Thomson (1988) also reported that pollinators preferentially visited at the male phase in *Yucca mekelveyana* and *Aralia*

*hispidata*, respectively, and that those flowers contributed as pollen donors. We did not measure male reproductive success in the present study. Plants with one flower, however, were never visited by bumblebees and never produced any seeds at all, an indication that these plants never acted as pollen donors (the male function) either. That is, even if flowers participate in the male function, a certain number of flowers for attracting pollinators is essential. Frequent visitations by pollinators must simultaneously promote removal of pollen from anthers, because stigmatic areas and pollen mature synchronously in *C. ambigua*. Accordingly, in the case of *C. ambigua*, plants with many flowers and subsequent larger seed sets (female success) also seem to contribute to greater export of pollen (male success). In fact, Schemske (1980) found that both pollen dispersal and production of fruit increased as a function of the number of flowers per raceme in the orchid *Brassavola nodosa*. Similarly, in other flowering plants, it has been demonstrated that the evolution of larger floral displays is the result of selection for increased overall reproductive success rather than male success only (Campbell 1989a, b; Broyles and Wyatt 1990). *C. ambigua* individuals with more flowers, which provide a larger signal for attraction of pollinators and offer greater nectar rewards, received many and longer visits by *B. hypocrita sapporensis* queens and, subsequently, seemed to exhibit higher reproductive success.

Although the pollinators tend to select inflorescences with larger displays, the predominant size of inflorescences was relatively small under the natural conditions: the mode and mean number of flowers per plant were 3 and 4.8, respectively (Fig. 1a). Willson et al. (1979), Schemske (1980), Udovic (1981) and Firmage and Cole (1988) discussed how inflorescence size is determined by a compromise between resource allocation and pollinator limitation, and between the present and future years' reproduction/survival for perennial plant species. Since each organism allocates limited amounts of available resources for growth, maintenance and reproduction (Cody 1966), a larger inflorescence entails a larger total cost to the plant. In perennial plants, high levels of fecundity, i.e. high energy consumption by fertilized ovules, in one year may deplete plant resources and lead to a deficiency in resources in subsequent years (Janzen et al. 1980). *C. ambigua*, a typical spring ephemeral plant, grows in deciduous forests using prevernal sunlight for photosynthesis during the short period before closure of the forest canopy (Kawano 1975). It requires a long period of time to reach sexual maturity with a gradual annual increment in individual size (e.g. leaf and bulb size) that is achieved by storing photosynthates in the bulb every year. Hence, although larger inflorescences would provide both increased male and female contributions, there must be a limit to the resources available for development of fruit, as well as conservation of resources for future years' reproduction. In this regard, it is important to note that the plants subjected to artificial outcrossing still had seed-setting rates of far below 100%, which may

suggest that limited resources are a strong selective factor. Although there are obvious selective advantages to larger inflorescences, limited availability of resources may reduce the advantages of large inflorescences. In order to clarify whether there is an energetic trade-off in *C. ambigua*, demographic information on individual plants, together with the monitoring of flower numbers and fecundity levels from year to year, must be obtained.

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