Plant Species Biology (2000) 15, 281–288

REGULAR ARTICLE

Reproductive biology of a North American subalpine plant: *Corydalis caseana* A. Gray ssp. *brandegei* (S. Watson) G. B. Ownbey

JOAN E. MALOOF
Salisbury State University, Department of Biological Sciences, Salisbury, Maryland 21801, USA (Email: jemaloof@ssu.edu)

Abstract

***Corydalis caseana* ssp. *brandegei* (Fumariaceae) is a perennial plant that grows in moist, subalpine regions of south central Colorado, USA. Prior to this study, nothing was known of its reproductive biology. The most numerous visitors (59%), and the only known pollinators, were long-tongued bumblebees (*Bombus appositus*). Twenty-nine percent of visits were from short-tongued nectar-robbing bumblebees (*Bombus occidentalis*). Hummingbirds also visited the flowers but they did not pollinate them. *Corydalis caseana* flowers remained open and in good condition for approximately 4 days. During that time, in the absence of visitors, nectar containing 35% sugar accumulated at a rate of approximately 1 μL per day. *Corydalis caseana* has a mixed-mating system. It is self-fertile, but the self-fertilized flowers produce fewer seeds per fruit than the outcrossed flowers (a mean of 2.9 compared with a mean of 4.7). Results suggest a possibility of inbreeding depression.**

Keywords: Bombus, breeding system, *Corydalis*, nectar, pollination.

Received 27 November 1999; accepted 30 June 2000

Introduction

Corydalis caseana A. Gray (Fumariaceae) is an herbaceous, perennial plant that usually grows in or near a source of fresh water such as a small creek or snowmelt drainage. The mature plant ranges from less than 0.5 m to over 2 m tall, but it is most commonly approximately 1 m tall. It has glaucous, dissected, leaves and multiple inflorescences of pink to white flowers. There are five subspecies of *C. caseana* scattered across the mountainous areas of the western United States. All of the subspecies are geographically separated from each other, and differ morphologically in minor, but perceptible ways, such as branching of the inflorescence, height at maturity, typical flower color and the size and shape of the outer petals. These details are described by Stern (1998) and Ownbey (1947).

Prior to this study, nothing was known about either the breeding system or the pollinators of *Corydalis caseana* (A. Gray) ssp. *brandegei* (S. Watson). In this study I determined the phenology of the flowers, the breeding system, the identity of the major flower visitors, pollinators and nectar robbers, the rate and amount of nectar produced and the concentration of the nectar.

Materials and methods

The plant

The subspecies I studied, *C. caseana brandegei*, grows in central Colorado, USA, most commonly around altitudes of 3000 m. The plant is rate, but where it does occur, it is locally abundant, sometimes forming large, almost monospecific, patches containing thousands of plants. With the onset of winter, all above-ground parts die back. In spring, as the snow melts, new shoots emerge. Seedlings germinate in the spring and develop a taproot that grows larger every year. Each year a taller, single stem with more leaflets is produced until the plant is mature enough to begin flowering. Eventually the large taproot will give rise to more stems, and these too must mature before they begin flowering. A similar pattern has been described in a closely related species, *Corydalis aquae-gelidae* (Goldenberg 1992), which begins flowering about 7 years after germination (Goldenberg & Zobel 1997). An old plant may have up to 20 stems emerging in close proximity to each other. A typical mature stem will have a terminal racemose inflorescence that has up to 70 flowers and numerous secondary racemes that may have from 5 to 40 flowers each.

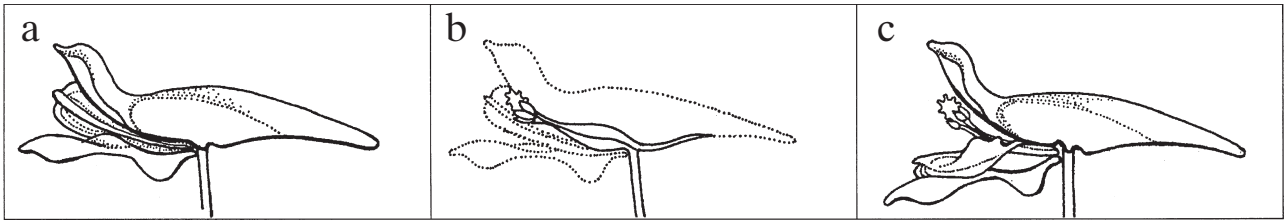

Fig. 1 Flower structure of Corydalis caseana. (a) Outer view of an open flower, (b) cut-away view showing location of the reproductive organs within the inner petals. The nectary is projecting to the right of the pedicel, inside the nectar spur. (c) View of flower from the outside showing the inner petals depressed and the reproductive organs exposed as they would be during a visit from a pollinator. Actual length of flower is 2 cm. (Drawn by Heather O'Connor.)

Figure 1 shows the structure of the flowers. The corolla has four petals. The inner petals are fused at the tip and they conceal the anthers and stigma. The anthers are appressed to the subplanate stigma. The outer petals are rotated 90° from the inner petals and form the lips. The upper petal extends posteriorly to form a nectar spur. When large visitors, such as bumblebees, collect nectar from the front of the flower, the fused inner petals are depressed and the reproductive organs are exposed through an open slit (Fig. 1). The inner petals have a hinge-like structure to facilitate this action. After the visitor leaves, the inner petals resume their original position.

Fruits dehisce explosively as soon as the seeds mature, approximately 20 days after pollination.

Study site

This research was conducted in the vicinity of the Rocky Mountain Biological Laboratory in Gothic, Colorado (38°50′N, 106°50′W). Most observations were carried out in Washington Gulch (2940 m, 38°56′N, 107°01′W) in a subalpine meadow approximately 2 km from the laboratory. Additional pollinator observations were conducted at Kebler Pass (38°50′N, 107°06′W), 16 km from the laboratory, in a series of eight meadows beginning at the top of the pass (altitude 3050 m) and continuing 880 m in a north-west direction to an altitude of 2975 m.

Determination of flower phenology

In 1996, a representative sample of eight inflorescences on eight different plants was chosen for this study. The plants were located within 10 m of each other at the Washington Gulch site. Each inflorescence had between 10 and 20 flower buds. Every bud or flower ($N = 101$) was observed on 25, 26, 27, 28, 29 and 30 June and on 1, 2, 3, 5, 7, 9, 11, 12, 15 and 17 July. The condition of each flower was described according to the following categories: (i) closed (bud stage); (ii) open, without holes from nectar robbers; (iii) open, with holes from nectar robbers; (iv) flower with brown spots or wilted; (v) flower fallen, no fruit forming; and (vi) fruit forming. All of the observed flowers were in bud stage when the study began, and all were either in fruit or had fallen from the plant when the study ended.

Stigma receptivity and pollen longevity were determined by bagging inflorescences still in bud from different plants (four for stigma receptivity and 16 for pollen longevity), and performing controlled pollinations when the inflorescences reached peak bloom. The stigmas of four flowers (on the same inflorescence) that had been open for only 1 day were treated with pollen from four flowers on another plant that had been open 1, 2, 3 and 4 days, respectively. Toothpicks were used to transfer the pollen. Stigmas from 2, 3 and 4 day-old flowers were treated in a similar manner. Treatments were replicated 4 times using a different male donor for each replicate. Two flowers on each inflorescence were left untreated as controls for detecting self-fertilization. Jeweler's tags were used to label the treatment regime of each flower. Inflorescences were rebagged immediately after treatment. Fruit set was used as an indicator of stigma receptivity and pollen longevity. Counts of fruit set per 4 replicates were organized in a 4×4 contingency table using age of stigma as columns and age of pollen as rows. Fisher's exact test was used to analyze the table (Ghent 1972).

Evaluation of the breeding system

Traditional breeding system studies usually involve emasculating the flowers (Schoen & Lloyd 1992), but in the case of *C. caseana* this is impractical. Because the anthers are appressed to the stigma, and the anthers dehisce prior to, or simultaneously with, the opening of the outer petals, anthers cannot be removed from an open flower without moving pollen onto the stigma. Removing the anthers from a bud would require cutting open both the outer and the inner petals; the validity of pollination treatments after such damage would be dubious. For that reason my outcrossing treatments did not include emasculation. It is possible, in fact likely, that some self-pollen would become attached to the stigma during outcrossing

treatments. Therefore I labeled this treatment outcross + self-pollinated.

Fourteen plants, each with a minimum of six flowering stems, were selected for analysis of the breeding system. On each plant four inflorescences were identified for experimental treatments; each inflorescence was then randomly assigned to the treatment of: (i) self-pollinated, (ii) outcross + self-pollinated, (iii) open-pollinated, and (iv) non-pollinated. All 4 treatments were performed on all 14 plants. Inflorescences ($N = 56$) were bagged with green nylon netting while the flowers were in bud stage to exclude pollinators. On the day of the treatment the bags were removed and the pedicels of four open flowers (standardized by flower age and position) on each experimental inflorescence were marked with black ink pens (Sanford, Sharpie; Bellwood, Illinois, USA). Pilot studies showed that marking a pedicel in this manner did not affect normal development. Each of the four flowers was treated in an identical manner.

For the self-pollination treatment the inner petals were depressed (as shown in Fig. 1), a toothpick was used to press the anthers against both sides of the stigma, and the netting was replaced. For the outcross + self-pollination treatment fresh pollen, collected from more than 10 plants located 100–200 m from the experimental plant, was mixed and applied to both sides of the stigma, again using a toothpick. The netting was replaced. For the open-pollination treatment the netting was simply removed, and for the non-pollinated treatment the netting was removed for approximately 30 s and then replaced.

After 16 days the fruits were collected and dissected to determine seed set. The seeds in each fruit were counted under a dissecting microscope, and transferred to a coin envelope. Seeds inside the envelope were oven dried at 40°C for 72 h and weighed using an electronic analytical balance (Denver Instrument Company, Arvada, Colorado, USA) to determine mean seed weight per fruit. The seed weight per fruit for each of the four treated flowers on an inflorescence was then pooled to determine the mean seed weight per inflorescence.

Results were analyzed by two-way (treatment and plant) ANOVA (Underwood 1997; p. 387). A separate analysis was carried out for each of the dependent variables: (i) percentage fruit set per inflorescence, (ii) mean number of seeds per fruit per inflorescence, and (iii) mean seed weight per inflorescence. Differences between treatment means for each variable were compared by the Tukey test (Zar 1984).

Pollinator observations

Pollinator species assemblages may vary due to time of day, time of season, or the location (e.g. Herrera 1988). In order to get a broad idea of the visitors to *C. caseana* flowers, I made observations throughout the flowering season, at different times of the day (between 09.00 and 16.00 hours), and in two different locations during 1996. Observations were made at Washington Gulch on 24, 25, 26 and 30 June ; 1, 2, 5, 7, 9, 10 and 12 July and 7 and 9 August. Observations were made at Kebler Pass on 11, 16, 19 and 23 July and 4 August. Observations were made by visually scanning each patch of plants in a study area. If a pollinator was noted it was identified, by close visual examination in the field, to species (and caste in the case of bumblebees) and mode of foraging behavior – legitimate, primary nectar robber or secondary nectar robber (*sensu* Inouye 1983).

Pollinator effectiveness

To determine if flower visitors were indeed pollinators, 50 inflorescences still in bud were covered with bags made from green mesh netting that excluded visitors. When the inflorescences were in bloom, the bags were removed and the pedicels of all open flowers on the inflorescence were marked (as in the breeding experiments). After a visitor foraged on an experimental inflorescence, the identity of the visitor and the number of flowers visited on the inflorescence was recorded on a jeweler's tag. This method was preferable to tagging the individual flowers visited because hummingbird visits were so swift that it was difficult to see exactly which flowers had been visited. After the 4 h observation period the inflorescences were rebagged to prevent subsequent visits. Unvisited inflorescences were considered to be controls and were also rebagged. Sixteen days later the inflorescences were collected and brought to the laboratory where fruit set and seed set were determined. Seed set data are for number of seeds per fruit formed.

Nectar concentration, volume, and accumulation

All nectar measurements were taken by inserting a 10 µL micropipette tube (Microcaps, Drummond Broomall, Pennsylvania, USA) into the spur of the flower. For ease of handling, flowers were first removed from the plant. Care was taken not to pierce the corolla or otherwise contaminate the nectar with cell sap. Nectar was drawn into the tube by capillary action. Volume was determined by measuring the length of the filled tube with a digital micrometer (Mitutoyo, Utsunomiya-shi, Japan) and converting the length measurement to microliters. To measure nectar concentration, the contents of the micropipette tube were emptied onto a portable Bellingham & Stanley refractometer (Tunbridge Wells, UK) modified to handle small volumes. Reported concentrations are from the 106 flowers sampled on 2 and 3 July 1996, during sunny weather, that had over 2 µL of nectar.

The standing crop of nectar was measured by running a 35 m transect parallel to the length of a *C. caseana* patch. At each 5 m interval, one flower was collected from each of eight different plants. Sampled flowers were all approximately 3 days old and located on a terminal inflorescence. To determine mean nectar accumulation per flower in the absence of visitors, I enclosed two patches of *C. caseana* plants in screen tents (3.9 m × 2.7 m × 2 m). In each patch 10 inflorescences were tagged. At 0 h (immediately after enclosure), 24 h, 48 h, 72 h and 120 h, four flowers from each inflorescence were removed and nectar volume was measured ($N = 80$). I determined nectar accumulation by averaging the volume in the four flowers sampled per inflorescence, and using those means for each of the 20 inflorescences to calculate the grand means and variance at each time period.

Results

Flower phenology

Flowers begin blooming on the terminal raceme first, from the bottom to the top (acropetally). On large, terminal racemes, the bottommost flowers may be producing fruits while the uppermost flowers are still in bud. In 1996–1998, in the locations of this study, flowering began between 14 and 20 June. When an individual flower opened it remained in good condition for approximately 4 days (3.6 ± 1.3 days, mean ± SD; $N = 101$). The flower then developed brown spots that grew larger each day; this phase lasted for another 4 days (3.8 ± 1.2) until the corolla completely wilted or fell from the inflorescence revealing a developing fruit or a vacant pedicel.

There is no apparent spatial or temporal separation of female and male function in *C. caseana*. In the stigma receptivity and pollen longevity experiment at least one flower (from the 4 replicates) produced fruit in each of the treatments (Table 1). The stigmas are receptive from the day the flower opens (day 1) at least until the flower begins to brown (day 4). Likewise, pollen age (between 1 and 4 days) did not affect fruit set, and pollen age was independent of stigma age in determining fruit set. The flowers used to measure pollen longevity were not emasculated; therefore self-pollen the same age as the stigma would have been available in each treatment in addition to the applied pollen. Despite this confounding factor, I have shown that 1-day-old pollen is viable on a 1-day-old stigma, and 4-day-old pollen is viable on a 4-day-old stigma, and this also applies to situations intermediate to these. Therefore, it seems likely that the pollen remains viable from the time of anthesis, at least until the flower begins to brown.

Table 1 Stigma receptivity and pollen longevity treatments

Pollen	Stigma First day	Second day	Third day	Fourth day
First day	4	3	2	3
Second day	3	2	1	2
Third day	1	1	2	1
Fourth day	1	1	4	2

Numbers represent the total number of fruits set from the four replicates of each treatment combination. The day the flower opened was considered the first day.

Evaluation of breeding system

The flowers that were bagged, but not hand-pollinated, did not set fruit. This treatment was removed from subsequent analyses. The remaining three treatments: self-pollinated, outcross + self-pollinated and open-pollinated, showed significant differences in fruit set (ANOVA, $F_{2,26} = 12.95$, $P < 0.005$). In the open-pollination treatment an average of 91% ($N = 14$) of the treated flowers in an inflorescence set fruit (Fig. 2). This was significantly different from the fruit set in the self-pollinated and outcross + self-pollinated treatments (Tukey test). In the flowers that were treated with self-pollen, 42% set fruit, compared to 46% in the flowers receiving outcross + self-pollen. These values were not significantly different from each other.

In addition to differences in the number of fruits produced per flower, there were also significant differ-

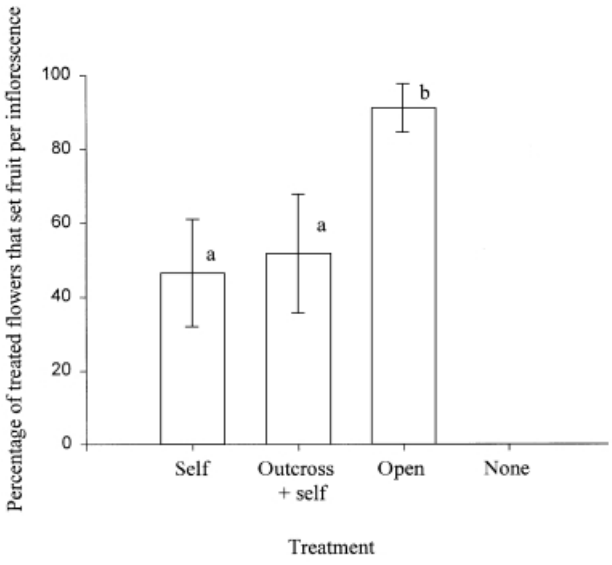

Fig. 2 Fruit set from various pollination treatments (bars are ±2 SE). Treatments with the same letter are not significantly different from each other.

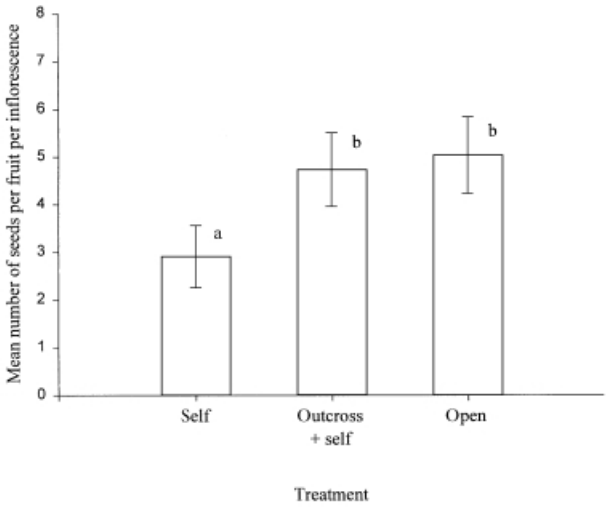

Fig. 3 Seed set from various pollination treatments (bars are ±2 SE). Treatments with the same letter are not significantly different from each other.

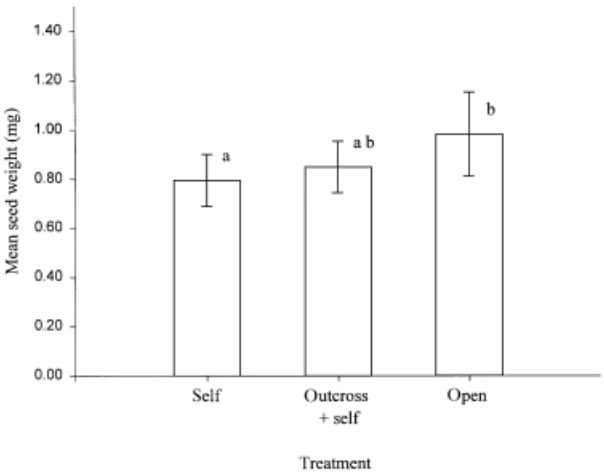

Fig. 4 Mean seed weight per inflorescence from various pollination treatments (bars are ±2 SE). Treatments with the same letter are not significantly different from each other.

ences in the number of seeds produced per fruit in the various treatments (ANOVA, $F_{2,26} = 10.89$, $P < 0.005$). Open-pollinated and outcross + self-pollinated flowers produced more seeds per fruit (5.0 ± 0.8 and 4.7 ± 0.8, mean ± 2 SE, respectively) than selfed flowers (2.9 ± 0.65; Fig. 3).

Treatment effects on mean seed weight were significant ($F_{2,26} = 3.75$, $P = 0.037$). Mean seed weight from the open pollinated inflorescences (0.985 ± 0.199 mg, $N = 14$) was greater than from either the self-pollinated (0.796 ± 0.106 mg) or the outcross + self-pollinated (0.850 ± 0.105 mg) inflorescences (Fig. 4). Although the difference between the self-pollinated and outcross + self-pollinated treatments was not significant, the difference between the self-pollinated and the open-pollinated flowers was (Tukey test).

Pollinator observations

Two hundred and fourteen visitors were observed on the *C. caseana* flowers, summarized in Table 2. The majority of the visitors (59%) were *Bombus appositus*, a long-tongued bumblebee. *Bombus appositus* usually entered the flowers through the front to collect nectar legitimately. In the process of nectar collection, the ventral surface of the bee came into contact with the anthers of the flower. Consequently, pollen would collect on the bee and the bee would occasionally land, or hover, and groom the pollen into her pollen baskets. Therefore, *B. appositus* foraging in this manner, collected nectar and pollen simultaneously. In many hundreds of observations from this and other studies on *C. caseana*, I have never seen *B. appositus* foraging solely for pollen. I captured four bees, removed their pollen loads, and released the bees. In each case more than 99% of the pollen they were carrying was from *C. caseana*. In 8% of the pollinator observations, however, smaller *B. appositus* individuals – presumably workers – collected nectar through existing holes in the nectar spur. Using a pre-made hole in such a manner is called secondary nectar robbing (Inouye 1983). Bees foraging in this way did not contact the anthers, therefore they did not collect pollen.

The second most common visitor (29%) was *Bombus occidentalis*, a short-tongued bumblebee. Most of these bees behaved as primary nectar robbers – biting a hole in

Table 2 Breakdown of pollinator visitors ($N = 214$) to *Corydalis caseana*, late June to early August 1996, Washington Gulch and Kebler Pass, Gunnison County, Colorado, USA

Pollinator visitors	Proportion of total (%)
Bombus appositus bumblebee	**59%**
Queens, visiting legitimately	50% (107)
Workers, secondary nectar robbing	8% (17)
Workers, visiting legitimately	1% (2)
Bombus occidentalis bumblebee	**29%**
Workers, primary nectar robbing	25% (54)
Workers, secondary nectar robbing	3% (7)
Queens, primary nectar robbing	1% (2)
Bombus flavifrons bumblebee	**8%**
Workers, secondary nectar robbing	7% (15)
Workers, visiting legitimately	1% (1)
Selasphorus platycercus and *Selasphorus rufus* Broad-tailed and Rufous Hummingbirds	**3%** (7)
Papilio zelicaon Gothic swallowtail butterfly	**1%** (2)

the back of the corolla and inserting their proboscis through the hole to collect nectar. Once holes were made by *B. occidentalis*, either *B. appositus* (a long-tongued bee), *B. occidentalis* (a short-tongued bee), or *B. flavifrons* (a medium-tongued bee), could use the holes to collect nectar. The effects of corolla perforation ('robbing') on forager behavior and seed production are covered in detail elsewhere (Maloof 2000).

Three percent of the visitors were hummingbirds (*Selaphorus rufus* and *Selaphorus platycerus*) and on two occasions the Gothic Swallowtail butterfly (*Papilio zelicaon*) was observed collecting nectar from *C. caseana*.

From 1996 to 1998 I also made casual observations of visitors to other *C. caseana* populations. At one population in Elkton, approximately 2 km north of the Washington Gulch study site, hummingbirds were more prevalent than they were in the regular study sites. At a population in Yule Basin (39.00°N, 107.06°W; 3346 m) *Bombus nevadensis* and *B. kirbyellus*, other long-tongued bumblebees, were observed foraging legitimately, and probably pollinating, alongside *B. appositus*. In the same area, but at a higher elevation (3474 m) a hawkmoth (*Hyles lineata*) was observed collecting nectar from *C. caseana*. Another subspecies, *Corydalis caseana* ssp. *cusikii*, was observed growing by Mores Creek Summit in the Boise National Forest, Idaho. Of the 15 visitors observed there on 24 June 1997, 10 were *B. appositus* (legitimate pollinators), four were *B. occidentalis* (nectar robbers), and one was a hummingbird. I found it interesting that this suite of visitors was similar to the suite observed in the study sites of *C. caseana* ssp. *brandegei*, approximately 1000 km away.

Pollinator effectiveness

Queens of *B. appositus* visited 5 of the 50 experimental inflorescences. Between 2 and 5 flowers were visited on each inflorescence (mean 3.6). None of the unvisited control flowers set fruit, but 100% of the visited flowers set fruit. Mean seed set was 3.9 seeds per fruit ($N = 16$, $R = 1$–7). Migratory Rufous Hummingbirds were also observed visiting five inflorescences. They visited between three and 15 flowers on each inflorescence (mean 9.6). None of the flowers visited by the hummingbirds set fruit.

Nectar volume, accumulation and concentration

Standing crop nectar volume, measured 9 July 1996, at 09.30 hours, ranged from 0.0 to 3.12 µL. Mean nectar volume for the standing crop was 0.60 ± 0.73 µL (mean ± SD; $N = 64$). Figure 5 shows the temporal change in mean nectar volume over a 120 h period.

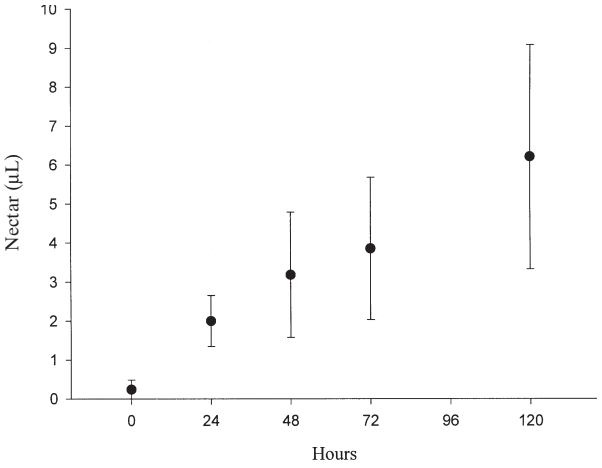

Fig. 5 Nectar accumulation in *Corydalis caseana* protected from visitors. Each observation is a mean of volumes from four flowers on the same inflorescence; each point (●) represents the grand mean of 20 observations (bars are ±1 SD).

Flowers had a mean sugar concentration of 35 ± 7.5% (mean ± SD; $N = 106$; $r = 19$–50%).

Discussion

This is the first study conducted of flower longevity in *C. caseana*. The flowers remain open, in good condition, for approximately 4 days; they then develop brown spots and drop, or wilt, after another 4 days. Similarly, *C. cava* flowers have a life-span of approximately 9 days (Olesen 1996). The flowers of *C. ambigua* last from 2 to 25 days depending, in part, on the air temperature and whether or not the flowers have been pollinated (Yasaka *et al.* 1998).

The results of this study indicate that the pollen is viable, and the stigmas are receptive, for at least the first 4 days that the flowers are open. There have been no other studies on pollen viability or stigma receptivity in *Corydalis*.

The flowers that were bagged, but not hand-pollinated, did not set fruit. This is consistent with the findings of Lloyd & Schoen (1992) that autonomous modes of self-pollination are rare in families with bilaterally symmetrical flowers such as the Fumariaceae (the family to which *C. caseana* belongs). Likewise, in the pollinator effectiveness observations, flowers that received no visits did not produce fruits or seeds.

The results of the breeding study indicate that *C. caseana* has a mixed breeding system; it is capable of pollinator mediated self-fertilization as well as outcrossing. In self-fertile species we often see spatial or temporal separation of male and female function, presumably as

a means to prevent autogamy (pollen transfer within a single flower) and promote outcrossing. But in the case of *C. caseana*, a self-fertile species, anthers dehisce pollen onto a receptive stigma. How can the evolution of this mating system be explained? It appears that *C. caseana* is selecting for outcrossing by requiring a visit from a pollinator before fertilization can occur. It is unclear exactly why a pollinator visit is required for fertilization—perhaps there is a stigmatic cuticle that must be ruptured, as in the case of *Medicago* spp. (Kreitner & Sorensen 1985), before the pollen tubes can enter the stigma. Whatever the exact mechanism is, it must promote outcrossing, for wherever there are pollinators there is likely to be at least some non-self pollen, and if this outcross pollen has some advantage in rate of germination or tube growth, then fertilization by outcross pollen could be expected to occur, even in the case of a stigma with abundant amounts of self-pollen (see Spira *et al*. 1992). Additional research is needed on these questions.

The populations studied during this research do not appear to be pollen limited. Fruit set in open pollination treatments was higher (91%) than fruit set in the hand-pollinated trials (46%), a typical measure of pollen limitation. Why did the open-pollinated (control) flowers produce more fruits than the selfed or outcrossed flowers pollinated by hand? Young & Young (1992) found similar results in 17% of the cases they studied. Among the possible causes listed in their study are: (i) at high densities (such as those created by hand-pollination) pollen grains or pollen tubes may interfere with each other, (ii) peak stigma receptivity may be missed by the experimenter, or (iii) the bagging process itself may reduce seed set. The open-pollinated flowers were left unbagged after treatment so perhaps multiple visits, by pollinators, over a longer time period were more effective at targeting peak stigma receptivity than this experimenter's one-time pollen application. I don't have any reason to believe that the bags interfere with fruit set, seed number, or seed weight, but it is possible. The self- and outcross-pollinated flowers were treated identically in every respect except for the pollen they received, so comparisons between those two treatments are uncomplicated by possible bagging effects.

Although the flowers are self-fertile the self-pollinated flowers exhibited the lowest values for every parameter measured (fruit set, seed number, seed weight), suggesting lower fitness due to inbreeding depression. However, of the three parameters measured, only the difference in seed number was statistically significant.

In 1996 *B. appositus* was the most numerous visitor and the only known pollinator in the study sites. *Bombus occidentalis*, the nectar robber, was the second most numerous visitor (Table 2). Drawing on what is known of other plant-pollinator relationships, I would expect the exact numbers and perhaps even the composition of the pollinator community to change through space and time (e.g. Heinrich 1976; Herrera 1988; Traveset *et al*. 1998). I have used 1996 as a 'snapshot' from which I have made the following assumptions: (i) long-tongued bumblebees are important pollinators of *C. caseana*, and (ii) *C. caseana* sometimes shows evidence of high rates of robbing. Additional experiments carried out at these study sites in 1997 and 1998 (Maloof 2000) lend support to these assumptions. In each of those years *B. appositus* was, again, the dominant visitor and at least 40% of the flowers were robbed.

Because none of the 48 flowers visited by Rufous Hummingbirds set fruit, I do not consider them to be pollinators of *C. caseana*. More extensive studies should be done, however, to be certain. It is possible that only a very small percentage of their visits are effective, and there were no effective visits in my sample. During the pollinator observation study resident Broad-tailed Hummingbirds were observed visiting the flowers. However, in the pollinator effectiveness study no Broad-tailed Hummingbirds visited the experimental inflorescences, consequently, I cannot say for certain that Broad-tailed hummingbirds are not pollinators. Broad-tailed Hummingbirds generally have beaks in the same size range as those of the Rufous Hummingbirds; the average beak length in the two species differs by only 1 mm (Calder & Calder 1992; Calder 1993) therefore I would expect similar results from both species.

The nectar produced by *C. caseana* rewards the pollinators that are essential for fertilization and subsequent seed set. This nectar contains a mean of 35% sugar, and in the absence of visitors it accumulates at a rate of approximately 1 µl per day. Flowers protected from visitors for 120 h had a mean nectar volume of 6.22 ± 2.88 µl (mean ± SD; $N = 80$). In comparison, flowers recently exposed to visitors contained a mean nectar volume of 0.23 ± 0.25 µl, Fig. 5). Zimmerman (1988) also measured the nectar standing crop in *C. caseana*; on 5 August 1985, at 04.00 hours, and it was 0.27 ± 0.45($N = 103$). The difference between nectar volumes in unvisited and visited flowers indicates that visitors are removing most of the nectar that the flowers produce.

The results of this study indicate that *C. caseana* is dependent upon long-tongued bumblebee pollinators for reproduction, and the pollinators are abundant and effective.

Acknowledgements

This study was made possible by financial support from Earthwatch and its research corps, a Sigma-Xi grant in

aid of research, and a Faculty Development grant from Salisbury State University.

The author thanks David Inouye's Rocky Mountain Wildflower Earthwatch teams of 1996, 1997 and 1998 for their assistance with collecting data in the field. Special thanks to Heather O'Connor for illustrating the flower.

References

Calder W. A. (1993) *Rufous Hummingbird*. *The Birds of America 53*. Academy of Natural Sciences, Philadelphia.

Calder W. A. & Calder L. L. (1992) *Broad-tailed Hummingbird*. *The Birds of America 16*. The Academy of Natural Sciences, Philadelphia.

Ghent A. (1972) A method for exact testing of 2x2, 2x3, 3x3 and other contingency tables, employing binomial coefficients. *The American Midland Naturalist* **88**: 15–27.

Goldenberg D. M. (1992) Ecology of *Corydalis aquae-gelidae*, a rare riparian plant. MSc Thesis. Oregon State University, Corvalis (Unpubl.).

Goldenberg D. M. & Zobel D. B. (1997) Allocation, growth and estimated population structure of *Corydalis aquae-gelidae*, a rare riparian plant. *Northwest Science* **71**: 196–204.

Heinrich B. (1976) Resource partitioning among some eusocial insects: Bumblebees. *Ecology* **57**: 874–889.

Herrera C. M. (1988) Variation in mutualisms: The spatio-temporal mosaic of a pollinator assemblage. *Biological Journal of the Linnean Society* **35**: 95–125.

Inouye D. W. (1983) The ecology of nectar robbing. In: *The Biology of Nectarines* (eds B. Bentley & Elias T.) pp. 153–173. Columbia University Press, New York.

Kreitner G. L. & Sorensen E. L. (1985) Stigma development and the stigmatic cuticle of *Medicago scutellata*. *Canadian Journal of Botany* **63**: 813–818.

Lanza J., Smith G. C., Sack S. & Cash A. (1995) Variation in nectar and composition of *Impatiens capensis* at the individual, plant, and population levels. *Oecologia* **102**: 113–119.

Lloyd D. G. & Schoen D. J. (1992) Self- and cross-fertilization in plants. II. The selection of self-fertilization. *International Journal of Plant Science* **153**: 370–380.

Maloof J. E. (2000) The ecological effects of nectar robbers, with an emphasis on the reproductive biology of *Corydalis caseana*. PhD Dissertation. University of Maryland, College Park Bell and Howell Information and Learning, Ann Arbor, Michigan, USA.

Olesen J. M. (1996) From naïveté to experience: Bumblebee queens (*Bombus terrestris*) foraging on *Corydalis cava* (Fumariaceae). *Journal of the Kansas Entomological Society* **69**(Suppl.): 274–286.

Ownbey G. B. (1947). Monograph of the North American species of *Corydalis*. *Annals of the Missouri Botanical Garden* **34**: 187–259.

Schoen D. J. & Lloyd D. G. (1992) Self- and cross-fertilization in plants. III. Methods for studying modes and functional aspects of self-fertilization. *International Journal of Plant Science* **153**: 381–393.

Spira T. P., Snow A. A., Whigham D. F. & Leak J. (1992) Flower visitation, pollen deposition, and pollen-tube competition in *Hibiscus moscheutos* (Malvaceae). *American Journal of Botany* **79**: 428–433.

Stern K. R. (1998) *Corydalis*. In: *Flora of North America North of Mexico*, *Vol. 3* (ed. Flora of North America Editorial Committee) pp. 348–351. Oxford University Press, New York.

Traveset A., Willson M. F. & Sabag C. (1998) Effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra Del Fuego: A disrupted mutualism. *Functional Ecology* **12**: 459–464.

Underwood A. J. (1997) *Experiments in Ecology*. Cambridge University Press, Cambridge, UK.

Yasaka M., Nishiwaki Y. & Konno Y. (1998) Plasticity of flower longevity in *Corydalis ambigua*. *Ecological Research* **13**: 211–216.

Young H. J. & Young T. P. (1992) Alternative outcomes of natural and experimental high pollen loads. *Ecology* **73**: 639–647.

Zar J. H. (1984) *Biostatistical Analysis*, *2nd Edn*. Prentice Hall, Englewood Cliffs.

Zimmerman M. (1988) Pollination biology of montane plants: Relationship between rate of nectar production and standing crop. *The American Midland Naturalist* **120**: 50–57.