Pollination ecology of the red *Anemone coronaria* (Ranunculaceae): honeybees may select for early flowering

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ABSTRACT

Large red bowl-shaped flowers characterize the Mediterranean “poppy guild” plants, and were suggested to reflect convergence for beetle pollination. However, the earliest-blooming species in this guild, *Anemone coronaria* (L.), starts flowering about a month before beetle emergence. Early flowering can be adaptive if the plant receives sufficient pollination by other means during this period. We investigated *A. coronaria*’s pollination prospects throughout its flowering season by monitoring its flowering phenology, the composition of the surrounding insect community, and insect visitors. Clear protogyny precluded self pollination, and anthesis occurred gradually over several days. Released pollen was quickly collected by insects, suggesting no major role for wind pollination. Beetles, flies and bees were trapped at the study site throughout the flowering period. Honeybees were the main anemone visitors during the first seven weeks of flowering, and were joined by Glaphyrid beetles in the remaining three weeks. Early- and late-blooming flowers had similar female reproductive success. We propose that effective pollination by honeybees may allow anemones to bloom in early spring and thereby reduce competition for pollinators with later-blooming species. Our results support previous evidence for pollination of red flowers by bees, and for the importance of generalization in pollination interactions in heterogeneous environments.

Keywords: beetle pollination; generalization; insect community; phenology; poppy guild
INTRODUCTION

The eastern Mediterranean species of anemone, poppy, ranunculus and tulip bloom in succession between January and May. They have red, large bowl-shaped flowers with a black center, radial symmetry, weak scent and filamentous stamens. These traits have been suggested to reflect convergent evolution for pollination by beetles. Glaphyrid beetles of several species visit the “red poppy guild” flowers frequently, and are active during most of the flowering period of the red bowl-shaped flowers (Dafni et al., 1990, Bernhardt, 2000).

Availability of suitable pollinators is considered an important evolutionary force that shapes floral phenologies (Brody, 1997). From this point of view, the flowering schedules of “red poppy” flowers are expected to synchronize with the beetles’ activity season. However, this is not the case for the common anemone, the earliest-blooming species within the “red poppy guild”. Anemones start flowering in January, under cool and rainy weather conditions, several weeks before the emergence of the first Glaphyrids. Wind pollination and pollination by solitary bees were observed in previous studies (Dafni et al., 1990, Horovitz, 1991). The time course for these pollination vectors was not described in relation to the anemones’ flowering phenology, though. The discrepancy between the phenology of anemones and that of their beetle pollinators raises questions regarding the costs and benefits of such early blooming. The reproductive success of plants that flower early in the season is often limited by pollen availability, due to insufficient pollinator service (Baker, Barrett & Thompson, 2000). This cost may be balanced by reduced seed herbivory of early-flowering plants (Brody, 1997). In the early-flowering Narcissus longispathus, flowers maintain a warm microclimate and remain open for many days. This enables them obtain adequate pollination (Herrera, 1995). In Daphne laureola, a long blooming season may compensate for pollinator limitation associated with early flowering
(Alonso, 2004). Early flowering may also reduce competition for pollinators with other species within the guild, thus serving as a mechanism of niche separation (Ashton, Givnish & Appanah, 1988, Stone, Willmer & Rowe, 1998).

In the present study, we describe flowering phenology, sex ratio and seed set of an Israeli population of *A. coronaria* in relation to the composition of the local insect community and of insects observed on the flowers. We addressed the following questions:

1. What are the anemone’s main pollen vectors during its flowering season?
2. What are the rewards offered to insect pollinators?
3. Is pollination efficiency reduced during the first weeks of flowering, when beetles are absent?

**METHODS**

Observations were carried out in several anemone populations in central Israel during January – March of 2008. Anemone densities and sex ratios, and insect assemblage composition were monitored in one population (Haruvit) weekly throughout the flowering season, totaling 10 days of observations. Sampling in additional populations served to establish the plants’ flowering phenology, pollen release schedule and the frequency of insects sheltering overnight within flowers.

**Flowering phenology and population sex ratios**

We counted the number of open flowers within two 10×10 m permanent plots at the Haruvit study site once a week. Anthers of female-phase flowers are positioned parallel to the plane of the petals, while the anthers of male-phase flowers face upward, that is their position is
perpendicular to the petals. We used anther position as a field marker for floral sex-phase, after confirming that the change in anther orientation is synchronous with the beginning of anthesis. We measured the diameters of the red corolla, the white ring at its base, and the black center containing the reproductive organs (the torus), in a sample of 103 flowers. We monitored the population sex ratio by recording the sex phase (male, female or transition, i.e. anthers partially erect) of 100 randomly chosen flowers at weekly intervals. We noted the sex-phase of 60 marked buds at daily intervals at the beginning of the flowering season to establish the duration of the female- and male-phases per individual. We also estimated the proportion of dehisced anthers per flower at daily intervals. We counted the number of flowers per plant in a separate sample of 41 plants.

**Monitoring of the insect assemblage at the study site**

We set up colored water traps to monitor the composition of the insect assemblage at our main study site. Traps consisted of 20 red, 20 white and 20 yellow bowls filled with water and a few drops of colorless detergent. They were placed in alteration, in two groups of 30 traps, along a dirt road at the study site. The distance between neighboring traps was 1 m. Peak reflectance wavelengths were 640, 560 and 420 nm for the red, white and yellow traps, respectively. We placed the traps on the same days and hours as for anemone observations, i.e. once a week during the morning hours. We emptied the traps once an hour. Trapped arthropods were recorded according to the following functional groups: Glaphyrid male and female beetles, non-Glaphyrid beetles, large flies (body length > 6 mm), small flies, wasps, bees, butterflies, ants, mosquitoes and unidentified small (<2 mm) arthropods.
**Determination of pollination vectors**

We counted insect visits to male-phase and female-phase flowers on 10 observation days at weekly intervals. We made between 3 and 13 10-minute counts every day, resulting in 28 counts of male-phase flowers, and 23 counts of female-phase flowers. We scanned a small (1-15 individuals) group of flowers on each count, and noted the number and type of insect visitors to the flowers. We divided the number of visits by the number of observed flowers to obtain per-flower visit rates.

Glaphyrid beetles often remained on a single flower for several minutes. We sampled their behavior by scanning 533 flowers that contained beetles on 5 sampling days. We recorded the following data: the sex-phase of the flowers, the number and sex of the beetles, and their activity (resting, feeding, mating or in transition to another flower). Beetles were identified to species by Dr. Guido Sabatinelli, Dr. Vladimir Chikatnov and Oz Rittner.

Flowers closed for the night in the early afternoon. We noted the numbers and functional group of sheltering insects in 1100 closed flowers at the Haruvit site on 6 days.

**Determination of seed mass**

We marked 25 female-phase flowers once a week. Three weeks later, we harvested the plants that meanwhile set fruit. The dry mass of 15 "fruits" (including all the seeds and their ripe receptacle) was highly correlated with their seed number (Pearson’s correlation coefficient = 0.82). We therefore determined the dry weight of the collected fruit, and treated it as a substitute for seed number, an estimate of female fitness.
Data analysis

We used replicated G-tests for goodness of fit to determine whether the frequencies of beetles on male- vs. female-phase flowers conformed to the flowers' sex ratio. Floral sex ratios varied over the season, hence the expected proportion of beetles on male-phase and female-phase flowers varied as well. We used the population sex ratio on each sampling day to calculate the expected numbers of beetles on the two sex phases.

RESULTS

Flowering phenology and population sex ratios

Plants in the study population produced an average (±SD) of 2.83±1.11 protogynous flowers (n=41). The female phase was much shorter in duration than the male phase. A short transition phase, marked by partly erect anthers just before anthesis, occurred in a few of the flowers (Fig. 1). Anthesis progressed gradually, over most of the male-phase duration (Fig. 2). Male-phase flowers had significantly larger corollas than female-phase flowers (n=66 male-phase flower, 37 female-phase flowers, t_{101}=8.04, P<0.001). A white ring at the base of the corolla was conspicuous in male-phase flowers and nearly absent from female-phase flowers, thus the difference in ring diameter was highly significant as well (t_{101}=5.21, P<0.001). The size of the torus did not differ significantly between male-phase and female-phase flowers (t_{101}=0.33, P=0.37, Fig. 3). The population sex-ratio was female-biased in the first week of blooming, but male-biased during most of the blooming season (Fig. 4).
Insects in traps

The number of individuals caught per hour fluctuated over the study period, and was generally higher in yellow and white traps than in the red ones (Fig. 5). The main identified functional groups caught in the traps were beetles, small flies, large flies and bees. Insects smaller than 2 mm were not identified, but comprised a large proportion of the trapped individuals. The frequencies of these functional groups in the weekly catches of each trap type are depicted in Fig. 6. Glaphyrid beetles, mainly of the species *Pygopleurus israelitus* were trapped starting on the fifth week of the study, almost exclusively in the red traps. Glaphyrids of two additional species, *Eulasia genei* and *Pygopleurus orientalis*, were trapped in very low numbers. Male Glaphyrids were trapped more frequently than females throughout the study. The frequencies of other beetles, flies and bees showed no clear temporal trend. Non-glaphyrid beetles were collected from white, yellow and red traps, while flies and bees were mainly caught in white and yellow traps. Trapped bees mainly belonged to small solitary species. Very few honeybees were caught in white and yellow traps, and none in red traps. No individuals of *Anthophora spp.*, the local dominant genus of solitary large bees, were trapped.

Vectors for pollination

Anthers dehisced gradually during the morning hours (9 am-noon). When the weather allowed insect activity, the pollen was promptly collected by foragers. On cold and rainy days, the pollen remained on the anthers. Freshly released pollen grains of picked flowers remained in sticky clumps on the anthers and were not dispersed by wind within a 90-minute observation session.
Honeybees and Glaphyrid beetles were the main foragers on anemones. Honeybees were dominant in the first seven weeks of flowering: Out of 64 10-minute pollinator counts carried out during this period, honeybees were the only visitors in 59 cases, while beetles were observed in only 3 cases. On weeks 8-9 of blooming, eighteen additional 10-minute counts were conducted. We recorded honeybees as the only visitors in 5 counts, beetles in 6 counts, and both types of visitors in 5 counts. In the remaining two counts, the visitors were flies. On week 10, only three flowers were still in bloom in both plots combined, and no insect visitors were observed on them. Thus, the visit rates of honeybees to anemones decreased towards the end of the season, while the beetles’ visit rates increased (Fig. 7). This increase is consistent with the data obtained from the traps.

Honeybees collected pollen from the flowers. They visited male-phase flowers at a mean (±SD) rate of 0.27±0.06 visits/flower/minute, while female-phase flowers were visited at a rate of 0.11±0.02 visits/flower/minute. This difference was statistically significant (t_{44}=3.03, P=0.002). The bees also made significantly longer visits to male-phase flowers than to female-phase flowers (mean±SD visit durations: 8.40±40.25 s in male-phase flowers, 2.05±1.10 s in female-phase flowers; t_{20}=4.42, P<0.001).

In the survey of beetle behavior we recorded 536 male and 182 female beetles on 533 anemones (491 flowers were in male phase and 32 were in female phase). Beetles rested, fed (n=234) and mated (n=103) on the anemone flowers. The frequency of beetles on male-phase flowers was significantly higher than the proportion of male flowers in the field (replicated G-test for goodness of fit, G_p=87.64, df=1, P<0.001). Beetle frequency on male-phase flowers in relation to floral sex ratio varied significantly among the five days of sampling (G_H=50.15, df=4, P<0.001).
134 beetles, 9 flies, 8 large bees and two small bees sheltered overnight in 125 out of the 1150 surveyed flowers.

**Seed mass**

Mean seed mass per flower was $0.14\pm0.02$ g / flower for flowers that bloomed during weeks 1-7 (n=55) and were mainly visited by honeybees. Mean seed mass for flowers that bloomed in weeks 8-9 (n=73), and which were also visited by beetles, was $0.15\pm0.02$ g / flower. The two means did not differ significantly ($t_{111}=0.47$, $P=0.64$).

**DISCUSSION**

Our observations allowed us to address our three study questions. First, we observed that much of *A. coronaria*’s flowering in our study population occurred before the emergence of GLaphyrid beetles, in agreement with earlier studies (Dafni et al, 1990). During this period, as well as during the beetles’ activity period, honeybees extensively visited anemones. Second, bees and beetles obtained pollen as food reward from the flowers. Beetles also mated on the flowers, and we found strong evidence for the use of flowers as overnight shelters by potential pollinators as described for other species (Sapir, Shmida & Ne’eman, 2006). Thus, anemone flowers combine food, mating sites and night shelters as their rewards to pollinators. Finally, plants that bloomed early in the season had similar seed sets as late-season bloomers, suggesting that honeybee pollination did not reduce their female fitness as compared with pollination by beetles. The pollination services of honeybees may thus be sufficient to maintain early-flowering phenotypes in the population.
The observations on the phenology of individual plants confirm that the male- and female phases of *A. coronaria* are clearly separated in time, ruling out self-fertilization. This is consistent with previous phenological studies (Horovitz, 1991) and with the high genetic variability recorded within wild anemone populations (Yonash et al., 2004). In spite of the strict protogyny, 30% of the flowers were in their male phase already in the first week of flowering, probably due to the short duration of the female phase. The much longer duration of the male phase also accounts for the male-biased sex ratio found during most of the blooming period (Fig. 3). Also in concurrence with previous work (Horovitz, 1991), we found gradual release of pollen during the flowers’ male-phase. Pollen dosing has been proposed to favor plant reproduction when pollinator visit rates are high, pollen removal by the pollinators is rapid and pollen deposition on recipient stigmas is low (Thomson et al., 2000). These conditions may apply for honeybees foraging on anemones in early spring, and may be less applicable for the mess-and-soil foraging of beetles when pollinators are abundant. Thus, gradual pollen presentation by anemones may enhance their reproductive success at the beginning of the flowering season when wild pollinators are scarce.

Our phenological data differs from previous observations in two important points. First, we observed anther dehiscence and pollen release during morning hours, while Horovitz (1991) reports that it occurs during the night. This discrepancy may result from observations on field populations (in the present study) vs. on picked flowers of domesticated varieties (by Horovitz). Second, anthesis started immediately after the female phase in most flowers monitored in the present study. In agreement with this observation, we found low proportions of transition-phase flowers in the weekly surveys of sex ratio. Horovitz (1991), on the other hand, reports a 10-day transition period between the female and the male sex-phases. This may correspond to the period
of incomplete anthesis that we observed (Fig. 2). The male phase was of much longer duration than the female phase (Fig. 1). Male-phase flowers offered pollen as food rewards to pollinators, whereas female-phase flowers provided no food reward. These asymmetries seem to indicate higher resource allocation to pollinator attraction in the male phase of *A. coronaria* than in the female phase, as predicted by sexual selection theory (Bell, 1985, Carlson, 2007). Both honeybees and beetles indeed visited male-phase flowers more than expected according to their abundance. Honeybees also made longer visits to male-phase flowers than to female-phase flowers.

Additional selective forces that favor early flowering in anemone are a matter of speculation. One possible advantage of early flowering is reduced competition for pollinators with other plant species that bloom later in spring. In particular, early flowering may enable temporal niche partitioning between anemones and poppies, both red bowl-shaped flowers. The similarities in visual displays and pollinators between the two species may lead to inter-specific pollen transfer and reduced fertility if they flower synchronously (Stone et al., 1998). Other possible advantages to early flowering include reduced competition for sunlight (Ne’eman, pers. comm.) and/or for nutrients.

Another interesting point concerns the morphology and visual display of anemone flowers, which do not seem to be adapted to pollination by honeybees. In particular, bee-pollinated flowers often possess bilateral symmetry, provide nectar and pollen rewards, and possess colors that reflect light in the wavelength range of 300-600 nm. Anemone flowers strongly deviate from this syndrome, being radially symmetric, nectarless and red in color (peak reflectance at >700 nm). A possible explanation is that the shift to early flowering and honeybee pollination in anemones is evolutionarily recent (perhaps in response to modern high-density
beekeeping), and that flower morphology and display have not yet coevolved in response. The observation that honeybees nevertheless effectively pollinate such flowers is interesting from two points of view. First, it contributes to an increasing list of bee-pollinated red flowers, contradicting the view that such flowers are invisible to bees. Honeybees may react to non-red visual cues in anemones, such as the black spot or the white ring at the center of the flower, or they may perceive parts of the red spectral reflectance (Chittka et al., 1997, Rodríguez-Gironés & Santamariá, 2004). More generally, honeybee foraging on anemones exemplifies the limitations in the concept of pollination syndromes for predicting the pollinator range of plant species (Waser et al., 1996). Bernhardt (2000) lists additional examples of plants that possess adaptations for beetle pollination, but have other pollinators as well. Moreover, specialized “beetle flowers” actually consist of four overlapping patterns of floral presentation. It is therefore not surprising that some of these characters allow pollination by other insects as well (Bernhardt, 2000). We suggest that being pollinated by honeybees, in addition to beetles, may provide a selective advantage to the common anemone, by relaxing potential constraints on its flowering phenology.

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REFERENCES


FIGURE LEGENDS

Fig. 1: Mean durations of male, transition and female flower sex phases.

Fig 2: Mean (SD) duration of flowering with 10, 30, 50, 70, 90 and 100% dehisced anthers in 60 male-phase *A. coronaria*.

Fig. 3: Parameters of the visual display components in male- and female-phase flowers, recorded on the third week of flowering. The white ring surrounds the black center containing the reproductive organs (torus). Its diameter was defined as twice the width of the white band that extends beyond the torus.

Fig. 4: Anemone population density and sex ratio at the Haruvit study site during the flowering season. Flowers that were in transition between female and male-phase were added to the male-phase category.

Fig. 5: Mean per/trap hourly catches in the traps over the study period. Error bars are 1 SD.

Fig. 6: Proportions of the main insect functional groups that were caught in the red (top), yellow (middle) and white (bottom) traps over the study period. The total weekly catch also included small numbers of wasps, ants, mosquitoes and butterflies. These are not plotted for graphical clarity.

Fig. 7: Mean visit rates (number of visits / flower/ 10 minutes) for honeybees and Glaphyrids during the flowering season.
Fig. 1

Duration (d)

Flower sex phase

- Female
- Transition
- Male
Fig. 2
Fig. 3
Fig. 4

![Graph showing Proportion male-phase flowers and No. flowers over Week of flowering for Sex ratio, No. flowers plot 1, and No. flowers plot 2.]
Fig. 6

![Bar chart showing the proportion of trapped individuals over 10 weeks for different categories: Bees, Small flies, Large flies, Non-galphryid beetles, Galphryid females, Galphryid males, and those less than 2 mm.](image-url)
Fig. 7

![Bar chart showing the number of visits per flower over 10 minutes for Honeybees and Gaphyrids over different weeks of flowering.]