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**RED ANEMONE GUILD FLOWERS AS FOCAL  
PLACES FOR MATING AND FEEDING OF  
MEDITERRANEAN GLAPHYRID BEETLES**

by

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# **Red anemone guild flowers as focal places for mating and feeding of Mediterranean glaphyrid beetles**

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## SUMMARY

Several species of glaphyrid beetles forage and mate on Mediterranean red bowl-shaped flowers. In red anemones and poppies in Israel, female beetles occupy only a subset of the flowers, do not aggregate, and are hidden below the petals. This raises the question how males find their mates.

The possibility that males and females orient to similar plant-generated cues, thereby increasing their mate encounter prospects, was investigated. Beetle attraction to red models increased with display area in previous studies. Choice tests with flowers and with models indicate that both male and female beetles prefer large displays to smaller ones. In anemones, beetles rest, feed and mate mainly on male-phase flowers, which are larger than female-phase flowers. Poppies that contain beetles are larger than the population average. These findings support the hypothesis that males and females meet by orienting to large red displays. Corolla size correlates with pollen reward in both plant species, suggesting that visits to large flowers also yield foraging benefits. Male beetles often jump rapidly among adjacent flowers. In contrast to the preference for large flowers by stationary individuals, these jumps sequences are random with respect to flower sex-phase (in anemone) and size (in poppy). They may enable males to detect females at close range. We hypothesize that males employ a mixed mate-searching strategy, combining orientation to floral signals and to female-produced cues. The glaphyrids' preference for large flowers may have selected for extraordinarily large displays within the "red anemone" pollination guild of the Levant.

Key words: beetle pollination; co-evolution; display size; mate searching; red flowers.

## INTRODUCTION

Insects frequently court and mate at their feeding sites. In some species, males establish territories or aggregations around food resources, and wait for receptive foraging females to arrive (Thornhill & Alcock 1983). In other cases, males actively seek out mating partners on food sources (O'Toole & Raw 1991; Proctor, Yeo & Lack 1996). Individuals may find their mates by orienting to the females' preferred feeding habitats, or to signals produced directly by potential mating partners. Orientation to female foraging sites may involve similar food preferences by males and females. For example, both males and females of the Scarab beetle *Autoserica insanabilis* (Brenske, 1894) (sub *Maladera matrida*) are attracted to volatiles emitted by their food plant, where they form mating aggregations (Harari, Ben-Yakir & Rosen 1994). Orientation to female-produced cues often involves pheromone communication, visual or auditory signals (Bailey, 1991) or the combination of more than one sensory modality (e.g. Szentesi, Weber & Jermy 2002). In some insect species orientation to feeding sites and pheromonal communication may be used as alternative strategies: In burying beetles, for example, males either locate carcasses that serve as feeding and oviposition sites for females, or emit sex pheromones to attract mates (Eggert 1992). Furthermore, secondary metabolites from a food plant may be incorporated into sex pheromones, and chemicals from host plants often synergistically enhance the response of an insect to sex pheromones (Reddy & Guerrero 2004).

Flowers provide a food resource and a mating site for their insect pollinators (Thornhill & Alcock 1983; Faegri & van der Pijl 1979; Barth 1985). Receptive females on flowers can be regarded as a resource that is highly dispersed in space. Finding a female within a field of flowers may be difficult for males, especially if the density of females is low in comparison to the density of flowers. The prospects for finding a mate may improve if both males and females orient to a subset of the flowers, and visit them preferentially. This could be the case for some species of Glaphyridae beetles (Scarabaeoidea), whose adults feed on floral pollen and mate on flowers. Several species of these beetles, which strongly rely on visual cues to find flowers, are dominant pollinators of plants with red bowl-shaped flowers in the Eastern Mediterranean region.

The food plants of this beetle guild (henceforth named the Levant glaphyrids) include *Anemone coronaria*, *Ranunculus asiaticus*, *Tulipa agenensis* (= *T. systole* = *T. montana*), *Adonis* spp., *Glaucium* spp. and *Papaver* spp. (Shmida 1981; Dafni *et al.* 1990). Male and female beetles may enhance their chances of encountering potential mates by orienting to a restricted number of flower species, in response to specific floral cues. Moreover, the Levant glaphyrids have been shown to land on large red flower models more often than on smaller ones (Dafni 1997). This raises the possibility that both male and female beetles preferentially visit large flowers. Such joint preference may further increase the beetles' prospects for finding a mating partner. It may also be a profitable foraging strategy, if large flowers are more rewarding than small ones.

The family Glaphyridae includes 5 genera and about 190 species in the Palearctic region, and one genus with nine species in the Nearctic region. In the Levant the family is represented by 3 genera and about 45 species. Thus the Eastern Mediterranean is rich in glaphyrid species, and is a possible center of speciation for some of them.

Glaphyrid beetles are active diurnal fliers, and are often observed hovering near flowers or foliage, or flying over sandy areas. Adults of many species are brightly colored and hairy and often possess markings and coloration resembling bees and bumblebees. Glaphyridae generally feed on a large variety of flowers. The vast majority of *Pygopleurus* feed on red bowl-shaped flowers, while some species of the genus *Eulasia* also feed on Compositae (including Asteraceae). *Glaphyrus* species and *Eulasia* subgenus *Trichopleurus* Motschulsky 1860 species feed mainly on violet spiny flowers such as *Onopordum* and on yellow *Centaurea*. However, not all species feed on flowers, and the species of the genus *Anthypna* (not present in the Levant) are never found on flowers.

In the present study, the density and aggregation level of female beetles on flowers were characterized, as a measure for the probability of males to find mating partners by chance. Using this data, one can calculate whether the frequency of male-female couples in flowers is compatible with random choice of flowers by both sexes. Next, the hypothesis that mate-finding within a patch of red bowl-shaped flowers is based

on beetle attraction to large floral displays was addressed, by asking the following questions:

1. Do male and female beetles have a similar preference to large flowers, and large flower models, in choice tests?
2. Do beetles rest, feed and mate on large flowers more frequently than on small ones, and do flower-size choices depend on beetle sex?
3. Does the red display size correlate with the amount of food rewards in the flowers?

Similar landing, feeding and mating preferences in beetles of both sexes are expected if mate-finding is based on orientation to flower-produced cues by both males and females. If males use female-produced signals for mate-finding, on the other hand, then they are expected to orient to flowers that contain females, regardless of those flowers' displays. In addition, a positive correlation between the red display size and the flowers' pollen content would suggest that the beetles' orientation to large flowers also allows them to obtain higher food rewards.

## METHODS

### **Study sites and species**

Common anemones *Anemone coronaria* (Ranunculaceae) were observed at one study site in the Judean foothills of Israel in 2008 and 2009. Common poppies *Papaver rhoeas sensu lato* (Papaveraceae) were observed at a site in Israel's coastal plain in the same years. This group of poppy species includes *P. subpiriforme* = *P. umbonatum*, *P. polytrichum* and *P. carmeli* recorded from Israel. *P. humile* can also be regarded as a subunit of the *P. rhoeas* complex, but was treated here as independent taxonomic entity due to its distinct yellow sap. A choice experiment with real flowers (Experiment 1) was conducted at the University of Haifa – Oranim campus in northern Israel. A choice experiment with flower models (Experiment 2) was conducted at the anemone and poppy field sites. Anemones bloomed from mid-January to early March, and poppies bloomed throughout March. Glaphyridae activity started in mid-February. Generally, the phenology of emergence of adult beetles is synchronized with the flowering phenology of

beetle-pollinated flower species. Therefore, different flower species are often visited by different beetle species (Dafni *et al.* 1990). Accordingly, several glaphyrid species were encountered during the study. *Pygopleurus israelitus* (Muche, 1963) was the main beetle visitor on anemone, and *Eulasia japhoensis* (Petrovitz, 1972) was the main beetle visitor on poppies. In addition, *Eulasia genei* (Truqui, 1848) and *Pygopleurus orientalis* (Petrovitz, 1958) were occasionally observed on anemone. *Eulasia dilutipennis* (Reitter, 1890) infrequently visited poppies.

### **Observations of stationary beetles**

The densities of male and female beetles were determined in 600 anemones along arbitrary transects on five days of sampling between 0900-1400 h. 818 additional flowers that contained stationary beetles were scanned on eight observation days. The following data were recorded in the sample of anemones occupied by beetles: the sex-phase of the flowers, the number and sex of the beetles, and their activity (resting, feeding, or mating). The sex-phase of the flowers is indicative of their display size, since male-phase flowers have significantly larger corollas (perianth segments) than female-phase flowers in this protogynous species (Keasar *et al.*, unpublished). The proportion of male-phase flowers in the population was estimated by noting the sex-phase of 100 random individuals on each sampling day. These data were used to test whether the proportion of male-phase flowers occupied by beetles differs from their frequency in the population.

In the simultaneously hermaphrodite poppy flowers, beetle occupancy in 721 randomly selected flowers was recorded on three days of observation. As with the anemones, beetle activity was noted in 251 occupied flowers on three additional sampling days. Here the length of the largest petal in each flower, rather than floral sex-phase, was recorded. Petal lengths were noted in 150 additional randomly sampled poppies (50 per day). After confirming that flower sizes did not significantly differ among the samples (one-way ANOVA,  $F_{2,149}=0.102$ ,  $P=0.90$ ), the data from the three sampling dates were combined, and the mean flower size in the population was calculated. The mean sizes of flowers with mating beetles, with non-mating beetles, or with no beetles were compared.

### **Observations of jump sequences**

Individuals that made fast series of transitions among closely located flowers were observed. In anemones, 28 rapidly jumping beetles were followed until they settled on a flower or disappeared from view, on two observation days (6 and 22 sequences on days 1 and 2 of observation, respectively). The sex of the beetle and the sex-phase of the flowers visited within each sequence were noted. The floral sex-phase ratio (the proportion of male-phase flowers) was recorded in a random sample of 100 flowers on each observation day, to determine whether beetles visit one of the sex-phases preferentially during jump sequences. In two additional days of observation, the length of the largest petal was measured for 18 (day 1) and 21 (day 2) flowers visited during rapid jump sequences, each performed by a different individual. The mean size of visited flowers was compared with the average size of 100 randomly selected flowers on each observation day. For 12 additional beetles, the duration of stays on flowers, and transitions to the next flower were recorded during parts of jump sequences (from sighting until the end of the jump sequence, or until the beetle disappeared from sight). Beetle sex and flower sex-phase were recorded for these jump sequences as well. These observations yielded time records for 59 flower visits and 60 transitions.

In poppies, the sizes of 109 flowers visited during rapid jump sequences were recorded on three days of observation (n=8, 50 and 51 flowers on days 1, 2 and 3, respectively). The sizes of 50 randomly chosen flowers were recorded on each day as well. The size of flowers visited during sequences of jumps was compared to the flower size in the random population sample on each day. Additionally, 16 individuals were followed during parts of rapid jump sequences. Beetle sex, the duration of the observed sequence, and the number of flowers visited were recorded for each sequence. These observations were used to calculate the mean duration of a visit, together with the subsequent transition to the next flower, during jump sequences.

### **Experiment 1: Choice experiment with flowers**

The choices of male and female field-collected beetles that were presented with small and large poppies under controlled conditions were recorded, with and without



potential mates. In the first stage of the experiments, twenty male beetles were introduced into a 35×75×39 cm glass terrarium, and twenty females were introduced into another terrarium of the same size. Each terrarium contained 10 small (3.66±0.50 cm diameter, mean ±SD, n=20) and 10 large (6.58±0.48 cm diameter, n=20) cut poppy flowers. The flowers were placed in water-containing test-tubes in a checkerboard array of 12×28×9 cm. Stem lengths were adjusted so that all flowers were of equal height, since target height has been shown to affect the landing choices of Glaphyridae (Dafni & Potts 2004). The location of all individuals (on small flowers, large flowers or elsewhere in the terrarium) was recorded one hour later. In the second phase of the experiment, the males were transferred into the females' terrarium without interfering with the females' location. The location of all beetles was again recorded one hour later. These experiments were replicated seven times, on five observation days, during the beetles' peak activity hours (10 am - 2 pm). Beetles were housed at 4 C, and were not fed, between different days of the experiment. Preliminary experiments indicated high beetle survival and high remating frequencies under these conditions. Therefore, some individuals were used in more than one replicate.

### **Experiment 2: Choice experiments with models**

The landing frequencies of male and female beetles on red models of two sizes were recorded. Models were polymer discs glued onto transparent plastic plates of 17 cm diameter. The plates were filled with water and a small amount of colorless and odorless detergent, thus functioned as water traps. To study the effect of the red display size, red models of 7.2 cm diameter and 5.4 cm diameter were set up. These sizes correspond to the mean diameters of male-phase and female-phase anemones, respectively (Kearar, Shmida & Zylbertal submitted). 20 models of each display (40 models for each experiment) were placed in alteration at the study sites during observation hours. The sex and numbers of beetles trapped in the water were recorded once an hour and the traps were emptied. 12 replicates of the experiments were set up on four observation days, nine replicates at the anemone site and three at the poppy site.

### **Correlations between display size and food rewards**

*A. coronaria* and *P. humile* produce ample amounts of pollen as food reward for insect visitors. The correlation between petal length and dry anther mass (in anemone), or the dry mass of released pollen (in poppy) was determined. Anthers and pollen were dried at 70 C for 24 hours prior to weighing.

### **Data analysis**

The expected proportion of flowers harboring a male together with a female beetle, assuming random choice of flowers, was calculated as the product of the proportions of flowers that housed a single male and a single female. Direct estimation of these proportions from the samples of randomly selected flowers might be unreliable when the proportion of flowers occupied by beetles is low, as it is based on small samples. Therefore, the conditional probabilities, (single male| occupied flower) and (single female| occupied flower) were estimated from the sample of flowers occupied by beetles (n=818 for anemone, n=189 for poppy). The proportions of a single male on a flower, or a single female on a flower, were calculated from these probabilities using Bayes' theorem.

In the choice experiment with flowers (Experiment 1), the numbers of male beetles on large and small flowers in the first phase of each replicate were treated as paired observations. A one-way Wilcoxon matched-pairs signed rank test was used to test whether the difference between these observations was greater than 0. The females' choices, and the choices of each sex in the second phase, were tested in a similar manner. The Wilcoxon matched-pairs test was also used to determine whether males occupied female-containing flowers more than flowers without females in the second phase of the experiment. The preference of male and female beetles to large vs. small models was tested in the same way. The Bonferroni correction was applied to these tests, since they involve testing of two hypotheses using the same data set.

Replicated G-tests for goodness of fit were employed to determine whether the frequency of beetles on male- vs. female-phase anemone 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. The population sex-phase ratio on each sampling day was used to calculate the expected numbers of beetles on the two sex phases.

To test for sex-phase preferences during jump sequences in anemones, the frequency of male-phase flowers in the population on each observation day was sampled. This sex-phase ratio was treated as the success probability in a Bernoulli experiment. It was used to calculate the probability for obtaining the observed proportion of male-phase flowers within each sequence of visits under a random binomial choice model. t-tests were employed to compare the size of flowers visited during jump sequences with the population means in both anemones and poppies.

## RESULTS

### **Observations of stationary beetles**

#### The distribution of beetles among flowers

Under field conditions, beetles occupied 7.5% and 19.7% of the sampled anemone and poppy flowers, respectively. 98.0% of the occupied anemones and 87.3% of the occupied poppies contained one or two individuals. Male beetles were more abundant than females both in anemones and in poppies. The frequency of flowers that contained a male and a female beetle together was 18 times higher than expected by chance for anemone, and 5-fold higher than random expectation for poppy (Table 1).

Table 1: Parameters of beetle distribution among anemone and poppy flowers. A small proportion of the occupied flowers contained >2 beetles, or two beetles of the same sex. These are not listed here.

	<i>A. coronaria</i>	<i>P. humile</i>
Proportion of flowers occupied by beetles	0.075	0.197
No. individuals per occupied flowers	1.29±0.51	1.78±0.83
Proportion of flowers with one male beetle	0.033	0.049
Proportion of flowers with one female beetle	0.015	0.040
Proportion of flowers with a mating couple	0.018	0.104
Proportion of flowers with a non-mating couple	0.000	0.004
Expected proportion of flowers with couples, random choice	0.001	0.002

#### Foraging and mating in flowers

In anemones, beetle sex was independent of floral sex-phase ( $\chi^2=1.11$ ,  $df=1$ ,  $P=0.29$ ), that is male and female beetles did not differ in their degree of preference for male-phase flowers. However, the frequency of beetles on male-phase flowers was significantly higher than the proportion of male-phase flowers in the population (replicated G-test for goodness of fit,  $G_p=87.64$ ,  $df=1$ ,  $P<0.001$ ). This indicates that both male and female Levant glaphyrids preferentially occupied male-phase flowers, rather than flowers in the female phase. Couples of mating beetles were observed in 18.4% of the occupied flowers. 72.3% of the flowers with mating couples were in their male phase. 15% of the male beetles, and 40% of the female beetles observed in occupied flowers were in copulation. 40% of the males and 58% of the females were observed feeding on pollen while on the flowers. Female beetles fed while mating more than males. These frequencies of feeding-mating combinations were significantly influenced by beetle sex (Table 2 top, test for independence:  $\chi^2=90.42$ ,  $df=3$ ,  $p<0.001$ ).

Poppies that contained glaphyrids had significantly larger corollas (mean±SD 28.29±3.91 mm,  $n=237$ ) than the population mean (26.28±3.81 mm,  $n=150$ ,  $t_{385}=4.99$ ,

P<0.001). Flowers that contained mating couples, which comprised 43% of the occupied flowers, had significantly larger corollas than flowers that contained non-mating individuals ( $t_{97}=2.22$ ,  $P<0.028$ ). Accordingly, the frequency of mating on larger-than-average flowers was significantly higher than on flowers that were smaller than the population mean ( $\chi^2=27.54$ ,  $df=3$ ,  $P<0.001$ ).

51% of male beetles in occupied poppies, and 58% of the females, were in copulation. 1% of the males and 4% of the females fed on the anthers. The remaining individuals rested on the petals or on the flowers' reproductive organs. As in anemone, mating-feeding frequencies on the flowers significantly depended on beetle sex (Table 2 bottom, test for independence:  $\chi^2=15.85$ ,  $df=3$ ,  $p=0.001$ ).

Table 2: Frequencies of feeding on flowers by mating and non-mating beetles observed on anemones (top) and poppies (bottom).

	Male beetles		Female beetles	
	No.	Proportion	No.	Proportion
Mating & feeding	24	0.04	50	0.18
Mating & not feeding	59	0.11	62	0.22
Not mating & feeding	191	0.36	113	0.40
Not mating & not feeding	261	0.49	57	0.20
Total	535	1	282	1

	Male beetles		Female beetles	
	No.	Proportion	No.	Proportion
Mating & feeding	0	0	8	0.04
Mating & not feeding	108	0.51	100	0.54
Not mating & feeding	1	0.01	6	0.03
Not mating & not feeding	101	0.48	70	0.38
Total	210	1	184	1

### **Observations of jump sequences**

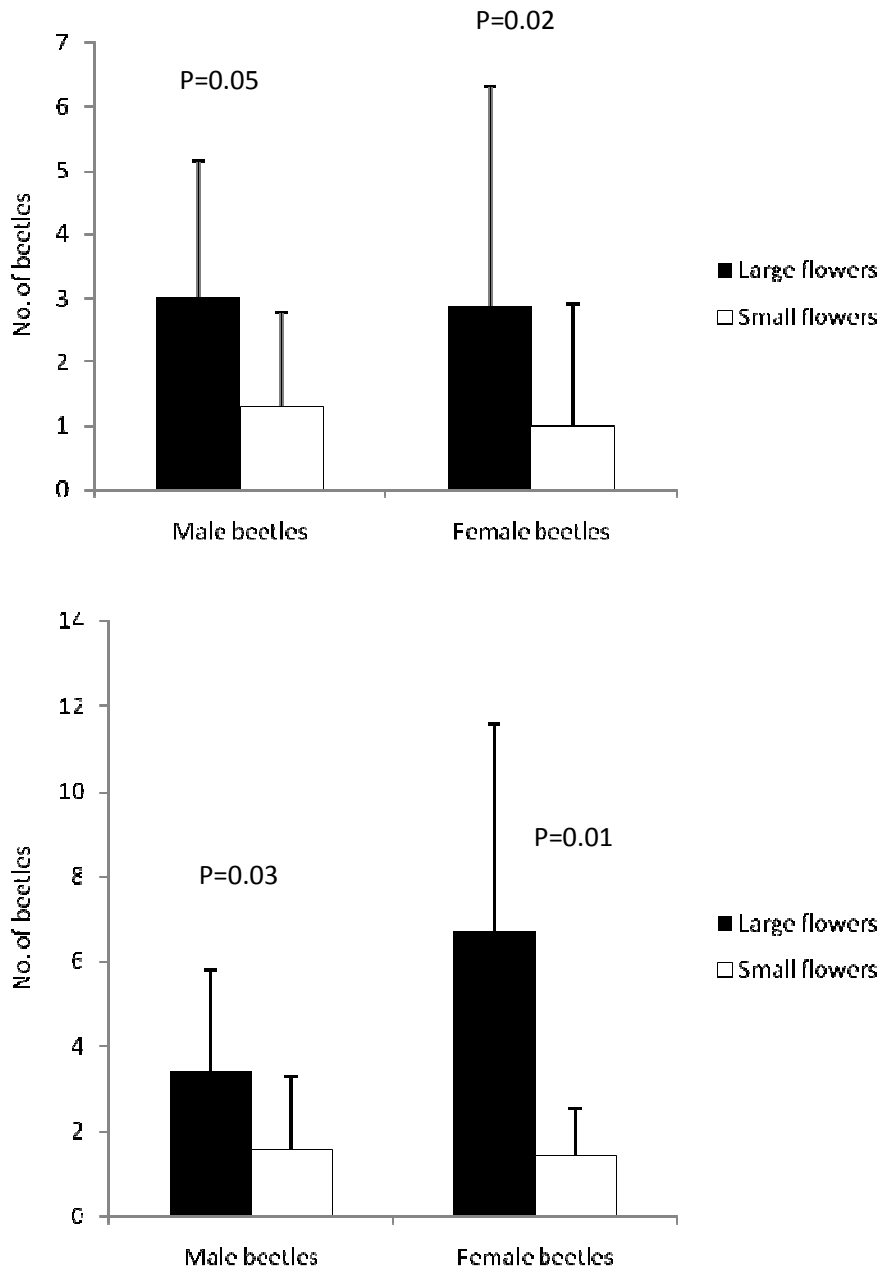
In anemones, 25 incomplete sequences of rapid transitions among flowers (“jumps”) performed by males, and three sequences of jumps conducted by females were recorded. The mean $\pm$ SD number of recorded visits per sequence was 7.11 $\pm$ 4.83, and the proportion of male-phase flowers visited per sequence was 0.95 $\pm$ 0.10. The probability that these proportions of male-phase flowers were visited as a result of random choices exceeded 0.05 in 23 out of 24 sequences, and averaged 0.64 $\pm$ 0.29. Thus the beetles’ choice of floral sex-phase did not deviate from random expectation. The mean petal size of flowers visited during jump sequences was not larger than the population mean (one-tailed t-tests;  $t_{116}=0.36$ ,  $p=0.35$  for day 1,  $t_{119}=1.17$ ,  $p=0.12$  for day 2). Jumping individuals spent 5.64 $\pm$ 15.01 s on a flower ( $n=59$ ), and 2.20 $\pm$ 6.48 s in transition between successive flowers ( $n=60$ ).

In poppies, all of the 16 recorded visit sequences were by male beetles. Flowers that were visited within sequences of rapid jumps did not significantly differ in size from the population mean ( $t_{257}=1.23$ ,  $P=0.22$ ). The number of recorded visits per sequence was 7.19 $\pm$ 4.51, and the mean combined time for a visit+transition to the next flower was 5.86 $\pm$ 2.42 s.

### **Experiment 1: Choice experiment with flowers**

The number of male and female beetles on large flowers was significantly higher than on small ones, when beetles of each sex were tested separately (Fig. 1, top; Wilcoxon one-tailed matched-pair signed rank tests,  $n=7$ : males -  $Z=1.63$ ,  $p=0.05$ , females -  $Z=2.06$ ,  $p=0.02$ ). After males were introduced into the females' terrarium, the same trend was observed (Fig. 1, bottom; males -  $Z=1.89$ ,  $p=0.03$ , females -  $Z=2.20$ ,  $p=0.01$ ). The males' preference for large flowers misses the statistical significance threshold of 0.025 after the Bonferroni correction in this case, while the females' preference remains statistically significant. After entering the females' terrarium, the mean ( $\pm$ SD) number of males found on flowers occupied by a female was 1.43 $\pm$ 1.27, while the mean number of males on flowers not occupied by females was 1.29 $\pm$ 1.38. These means did not differ significantly ( $Z=0.11$ ,  $p=0.45$ ).

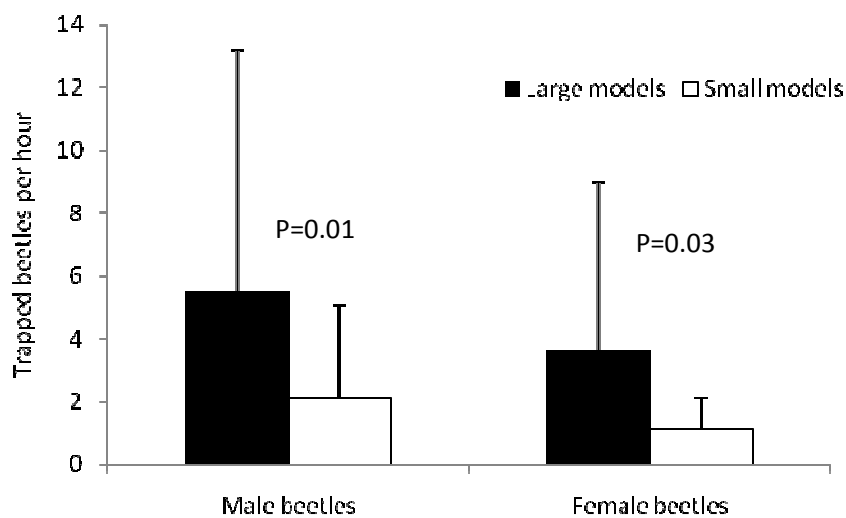
Fig. 1: Mean number of male and female glaphyrids on large and small poppies, 1 h after introduction into an experimental terrarium. Top: First phase of the experiment, each terrarium contained beetles of one sex only. Bottom: Second phase, males were introduced into the females' terrarium. Error bars are 1 SD.



## Experiment 2: Choice experiment with models

Both male and female beetles were significantly more abundant on large models than on small ones (Fig. 2, Wilcoxon matched-pairs signed rank tests,  $n=12$ ; males –  $Z=2.32$ ,  $p=0.01$ , females –  $Z=1.99$ ,  $p=0.03$ ).

Fig. 2: Mean (SD) number of male and female glaphyrids collected per hour from 20 large (black bars) and 20 small (white bars) water traps.  $n=12$  one-hour trapping sessions, conducted on four days.



## Correlations between display size and food rewards

Petal length and anther mass were highly correlated in anemone (Spearman's rank correlation,  $n=10$ ,  $r=0.74$ ,  $p=0.02$ ). Petal length and pollen mass showed a similar high correlation in poppy (Pearson's correlation,  $n=78$ ,  $r=0.81$ ,  $p<0.001$ ).

## DISCUSSION

Our survey of beetle occupancy in two species of red bowl-shaped flowers revealed that most of the flowers (>90% in anemone and >80% in poppy) did not contain any glaphyrids. Occupied flowers did not hold aggregations of beetles during the observation periods. This dispersed distribution probably reduces the beetles' chances of



randomly encountering their conspecifics on flowers. Nevertheless, a much higher proportion of the occupied flowers contained mating couples than expected by chance, suggesting that mate location involves non-random search. Our study aimed to investigate the hypothesis that mate-finding involves attraction of males and females to the same plant signals. In agreement with this hypothesis, both male and female beetles landed on large red displays in choice experiments with flowers (Experiment 1) and with models (Experiment 2). Moreover, males in the flower choice experiment selected flowers indiscriminately in respect to the presence of females. Glaphyrid-containing flowers were more likely to be male-phase (and thus with a larger red display) than expected by chance in anemone, and were larger than the population mean in poppy. This observation is also congruent with a preference of both beetle sexes for larger displays.

In addition to the above evidence for male and female orientation to visual plant signals, we also found some support for the hypothesis that males orient to cues directly emitted by their potential mates. Rapid jump sequences were mostly performed by male beetles, occurred in high-density patches that contained well-developed flowers, and were random in respect to flower size within patches. A possible interpretation of these observations is that jumping males move randomly and rapidly among flowers until they meet a female, or perceive a female-emitted cue (chemical, visual or other) at close range. This may cause them to remain longer on a flower occupied by a female. Jumping males were repeatedly observed landing on a flower, circling its reproductive organs, and stopping when they encountered another individual. They then inspected the stationary individual briefly, copulated with it if it was female and continued jumping if it was male. Thus the mating location in these cases was largely determined by the females' feeding / resting preferences. We therefore suggest that males of Levant glaphyrids employ a mixed mate-searching strategy, which combines waiting for females at large red floral display signals, and rapidly jumping in search of mates among flowers of various sizes.

The beetles fed and mated on the flowers. Interestingly, the proportions of females that mated and/or fed on flowers were higher than the proportions of males. The higher frequency of mating by females may be attributed to the male-biased sex ratio

observed on the flowers (Table 2) and on the models (Fig. 2). The higher proportion of feeding females, as compared to males, may reflect differences in nutritional requirements between the sexes. In addition, the mating position constrains the movement of males more than the movement of females, and may interfere with feeding by mating males. This may explain the lower frequency of combined mating and feeding observed in males as compared to females (Table 2).

The attraction of glaphyrid beetles to large red models in our experiment agrees with previous studies (Dafni *et al.* 1990; Dafni 1997). These studies also found increased attraction to models with a black spot at their center, another feature common to flowers of the red anemone guild. A preference for black spots against an orange background was also reported in some species of monkey beetles (Scarabaeidae: Melolonthinae: Hopliini) (Johnson & Midgely 1997; Klunen *et al.* 2007), but not in others (Johnson & Midgely, 2001). The **presence** of a black spot at the flower's center may help beetles discriminate between flowers of the red bowl-shaped guild (which all possess black spots) and species outside of this guild. The **size** of the black spot, on the other hand, varies very little among conspecific anemones (Keasar *et al.* unpublished). We therefore do not consider it a likely cue for guiding the beetles' landing choices between individuals of a single species within the red bowl-shaped guild. This prediction requires experimental testing.

The beetles' preferences for large red floral displays may directly affect their reproductive prospects. Orientation to large displays is also likely to provide foraging benefits, since petal size is strongly correlated with pollen mass in both study species. A dual role of flowers, as feeding and mating sites for beetles, has been suggested in previous studies (Gottsberger 1977; Barth 1985; Bernhardt 1996; Goldblatt, Bernhardt & Manning 1998). The mating and foraging benefits gained by the glaphyrids may, in turn, provide an indirect selective pressure for large displays in their food plants.

The red anemone guild comprises some of the species from six plant genera (*Anemone*, *Tulipa*, *Ranunculus*, *Adonis*, *Glacium* and *Papaver*) that belong to three phylogenetically distant families (Ranunculaceae, Liliaceae and Papaveraceae). Flowers of this guild are characterized by large, red bowl-shaped petals that surround a large black mass containing the anthers and the female reproductive organs. The flowers are

insect-pollinated, and offer only pollen as food reward to visitors. The uniqueness of the red anemone guild species is due to the rarity of large red floral displays among the congeners of the guild members, as well as in the Mediterranean flora (Shmida 1981; Dafni & Shmida 1989). Many species of *Anemone*, *Tulipa*, *Ranunculus*, *Adonis*, *Glacium* and *Papaver* distributed outside of the Near East have much smaller flowers that are usually not red. In addition, red flowers are extremely rare in the East Mediterranean flora: Only six species, in addition to members of the "red anemone guild", reflect in the red wavelength (Menzel & Shmida 1993). This pattern concurs with the absence of pollination by hummingbirds in the Near East (the pollination of the tropical tree parasite *Loranthus acaciae* by the sunbird *Nectarina osea* is a notable exception; Vaknin, Yom-Tov & Eisikowitch 1996).

These observations fuel the hypothesis that selection for the unique phenotype of the "red anemone guild" has involved co-evolution with glaphyrid pollinators in the Eastern Mediterranean. Two related genera in the family Glaphyridae, *Pygopleurus* and *Eulasia*, are important pollinators of red anemone guild flowers. The following indirect evidence hints that these genera have been selected for pollination of anemone-guild flowers in the Levant:

(a) Primitive glaphyrid genera (e.g. *Glaphyrus*) often feed on whole flowers. In contrast, the species of *Pygopleurus* and *Eulasia* that visit the "red anemone guild" flowers do not feed on ovaries and petals, i.e. they provide better pollination service, and less flower destruction, from the plants' point of view.

(b) Primitive glaphyrid genera, as well as other Mediterranean flower beetles (such as *Oxythyrea abigail* Reiche & Saulcy, 1856, and *Tropinota* spp.) often forage on species within the spiny Asteracea, and generally do not feed on red flowers. *Pygopleurus* and early-emerging species of *Eulasia*, on the other hand, specialize on red bowl-shaped flowers. Unlike other flower-feeding beetles, these species are also strongly attracted to red models (Dafni *et al.* 1991; Dafni 1997). In the only glaphyrid species that has been studied neurophysiologically, a red receptor is present in the visual system (Martinez *et al.*, submitted).

(c) The Near East and Asia Minor (Turkey) is the putative center of speciation of *Pygopleurus* and *Eulasia*, where they have diverged into more than 30 species.

Two additional observations provide circumstantial support for selection on floral traits for pollination by the Levant Glaphyridae in the "red anemone guild":

(d) Members of this guild offer pollen as the only food reward to pollinators (a common adaptation for beetle pollination), whereas other species of *Tulipa* and *Ranunculus* also possess nectaries (Arber 1936; Dahlgren 1992).

(e) Flowers of the "red anemone guild" close at night, while many of their congeners remain open (pers. observation). Closing of the petals at night protects flowers from cold and rain, but also provides an overnight shelter for potential pollinators. Indeed, Glaphyridae regularly shelter at night within the closed flowers of *Anemone* (Keasar *et al.* unpublished).

In conclusion, we propose that the Levant glaphyrids' preference for large red displays provides a selective pressure for the evolution of these unique displays in the flowers of the "red anemone guild", and possibly also for additional floral traits, such as a black central mass and copious pollen. Beetles that are attracted to large red floral displays enhance their mate-finding prospects since such displays, being extremely rare in the near East, form a well-defined and distinct niche. In addition, such flowers provide beetles with food and overnight shelters.

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