



Red anemone guild flowers as focal places for mating and feeding by Levant glaphyrid beetles

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Several species of glaphyrid (Scarabaeoidea: Glaphyridae) 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 of how males find their mates. In the present study, we investigated the hypothesis that males and females orient to similar plant-generated cues, thereby increasing their mate encounter prospects. Previous studies have demonstrated that beetle attraction to red models increases with display area. Choice tests with flowers and with models indicate that both male and female beetles prefer large displays. 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. By contrast to the preference for large flowers by stationary individuals, these jump 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. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, 99, 808–817.

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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

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females of the Scarab beetle *Maladera (Cephaloserica) insanabilis* (Brenske, 1894) (syn. *Maladera matrida* Argaman, 1986) 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 (Szentesi, Weber & Jermy, 2002). In some insect species, orientation to feeding sites and pheromonal communication may be used as alternative strategies. For example, in burying beetles, 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 may provide both 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 compared to the density of flowers. The prospects for finding a mate 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), in which adults feed on floral pollen and mate on flowers. Several species of these beetles, which rely strongly on visual cues to find flowers, are dominant pollinators of plants with red bowl-shaped flowers in the Eastern Mediterranean region (Shmida, 1981; Dafni *et al.*, 1990). The food plants of this beetle guild (hereafter named the Levant glaphyrids) include *Adonis* spp., *Anemone coronaria*, *Ranunculus asiaticus* (Ranunculaceae), *Tulipa agenensis* [= *T. Montana*, *T. systole* (Liliaceae)], *Glaucium* spp., and *Papaver* spp. (Papaveraceae) (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 a 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 five genera and approximately 190 species in the Palaearctic region,

and one genus with nine species in the Nearctic region (Löbl & Smetana, 2006). In the Levant, the family is represented by three genera and approximately 45 species. In the absence of phylogenetic studies for Glaphyridae, this large number of species supports the speculation that the Near East has been a centre of speciation for some genera.

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 coloured 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, whereas some species of the genus *Eulasia* also feed on Compositae (including Asteraceae). *Glaphyrus* species and *Eulasia* subgenus *Trichopleurus* species feed mainly on violet spiny flowerheads such as *Onopordum* and on yellow *Centaurea* (both Asteraceae). However, not all species feed on flowers, and the species of the genus *Ant-hypna* (not present in the Levant) are never found on flowers (G. Sabatinelli, unpubl. observ.).

In the present study, the density and aggregation level of female beetles on flowers were characterized as a measure of the probability of males finding mating partners by chance. These data are used to 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 investigating: (1) do male and female beetles have a similar preference for 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; and (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 containing females, independently 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.

MATERIAL AND METHODS

STUDY SITES AND SPECIES

Common anemones *A. coronaria* (Ranunculaceae) were observed at Haruvit forest in the Judaean foot-

hills of Israel (elevation 150 m) in 2008 and 2009. Common poppies *Papaver subpiriforme* (sect. *Rheadium*; Carolan, Hook & Cha, 2006) (Papaveraceae) were observed near Kibbutz Givat Brenner in Israel's coastal plain (elevation 60 m) in the same years. 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. The identification of Glaphyridae was based on Baraud (1989, 1990). Male beetles could be easily recognized in the field by the reddish colour of their last two sternites. Voucher specimens are housed at the Department of Zoology, Tel Aviv University.

OBSERVATIONS OF STATIONARY BEETLES

The densities of male and female beetles were determined in 600 anemones along arbitrary transects on 5 days of sampling between 09.00 h and 14.00 h. The beetles generally remained on the flowers during examination. This facilitated their counting, and reduced the risk that a disturbed beetle would fly off and be counted more than once along a transect. Eight hundred and eighteen additional flowers that contained stationary beetles were scanned on eight observation days to increase the sample size for beetle-containing flowers. Accordingly, all the beetle-containing flowers within two 10 × 10-m plots (originally set up to study the flowering phenology of *A. coronaria*) were recorded. The data taken for these anemones comprised: 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 (and quicker to record than display size) because male-phase flowers have significantly larger corollas (perianth segments) than female-phase flowers in this protogynous species (T. Keasar, A. Shmida & A. Zylbertal,

unpubl. data). The proportion of male-phase flowers in the population was estimated by noting the sex-phase of 100 individuals from the 10 × 10-m plots 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 flowers along arbitrary transects was recorded on 3 days of observation. As with the anemones, beetle activity was noted in 251 occupied flowers on three additional sampling days. The length of the largest petal in each flower was recorded. Petal lengths were noted in 150 additional arbitrarily selected poppies (50 per day). These flowers were sampled along transects as well. After confirming that flower sizes did not significantly differ among the samples (one-way analysis of variance: $F_{2,149} = 0.102$, $P = 0.90$), 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 nonmating beetles or with no beetles were compared.

OBSERVATIONS OF JUMP-FLIGHT SEQUENCES

Individuals that made fast series of transitions among closely located flowers were observed. In anemones, 28 beetles that made rapid jump-flights were followed until they settled on a flower or disappeared from view, on two observation days (six 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-flight 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-flight 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-flight sequences (from sighting until the end of the jump-flight sequence or until the beetle disappeared from sight). Beetle sex and flower sex-phase were recorded for these jump-flight 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-flight sequences were recorded on 3 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 jump-flights was compared with the flower size in the random population sample on each day. Additionally, 16 individuals were followed during parts of rapid jump-flight 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-flight sequences.

GUT CONTENTS OF MALE AND FEMALE BEETLES

The composition of pollen grains in the guts of 30 male and 73 female glaphyrids was determined as an additional indicator of their feeding choices. Beetles were collected from anemones, poppies, and ranunculus in four sites in northern Israel: Haifa University (six males and 11 females on ranunculus), Carmel Park (ten males and eight females on ranunculus), Aloney Abba (two males and 13 females on anemone) and Mt. Giloba (23 males and 41 females on poppy). The beetles were kept at -20°C until dissection, and then washed thrice in ethanol 70% to remove pollen grains from the cuticle. Their abdomens were removed, dissected into a drop of water, stained with methylene blue and examined under a light microscope. The dissected individuals were sexed by their internal genitalia. The species composition of pollen grains in the beetles' guts was determined according to pollen morphology by comparison with a reference collection. The number of grains per sample was scored as trace (< 50), low (51–500) or high (> 500).

CORRELATIONS BETWEEN DISPLAY SIZE AND FOOD REWARDS

Anemones and poppies produce ample amounts of pollen as food reward for insect visitors. The correlation between petal length and dry anther mass was determined. Anthers and pollen were taken from flowers shortly before they opened, and were dried at 70°C for 24 h prior to weighing.

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 $35 \times 75 \times 39$ -cm glass terrarium, and twenty females were introduced into another terrarium of the same size. Each terrarium contained ten small (diam-

eter 3.66 ± 0.50 cm, mean \pm SD, $N = 20$) and ten large (diameter 6.58 ± 0.48 cm, $N = 20$) cut poppy flowers. The flowers were placed in water-containing test tubes in a checkerboard array of $12 \times 28 \times 9$ cm. Stem lengths were adjusted so that all flowers were of equal height because 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 1 h 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 recorded again one hour later. These experiments were replicated seven times, on five observation days, during the beetles' peak activity hours (10.00 h to 14.00 h). 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 in diameter. The plates were filled with water and a small amount of colourless and odorless detergent, thus functioning as water traps. To study the effect of the red display size, red models (peak reflectance 640 nm) of 7.2 cm in diameter and 5.4 cm in diameter were set up. These sizes correspond to the mean diameters of male-phase and female-phase anemones, respectively (T. Keasar, A. Shmida & A. Zylbertal, unpubl. data). Twenty models of each display (40 models for each experiment) were placed in alteration at ground level along a dirt road at the study sites during observation hours. The distance between neighbouring traps was 1 m. The sex and numbers of beetles trapped in the water were recorded once every hour and the traps were emptied. Twelve replicates of the experiments were set up on four observation days: nine replicates at the anemone site and three at the poppy site.

STATISTICAL 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 because 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 (McCarthy, 2007).

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 versus small models was tested in the same way. The Bonferroni correction was applied to these tests because they involve the 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- versus 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-flight 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. This 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-flight 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, respectively, 7.5% (anemone) and 19.7% (poppy) of the sampled anemone and poppy flowers, respectively. Some 98.0% of the occupied anemones and 87.3% of the occupied poppies contained just one or two individuals. The maximum number of beetles per flower was four in anemone and seven in poppy. 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-fold higher than expected by chance for anemone and five-fold higher than random expectation for poppy (Table 1).

Foraging and mating in flowers

In anemones, beetle sex was independent of floral sex-phase ($\chi^2 = 1.11$, d.f. = 1, $P = 0.29$), i.e. 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, $G_p = 87.64$, d.f. = 1, $P < 0.001$). This indicates that both male and female Levant glaphyrids preferentially occupied male-phase flowers. Couples of mating beetles were observed in 18.4% of the occupied flowers. Some 72.3% of the flowers with mating couples were in their male phase. Fifteen percent of the male beetles and 40% of the female beetles observed in occupied flowers were in copulation. Forty percent of the males and 58% of the

Table 1. Parameters of beetle distribution among anemone and poppy flowers

	<i>Anemone coronaria</i>	<i>Papaver subpiriforme</i>
Proportion of flowers occupied by beetles	0.075	0.197
Number of individuals per occupied flowers (mean \pm SD)	1.29 \pm 0.51	1.78 \pm 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 nonmating couple	0.000	0.004
Expected proportion of flowers with couples, random choice	0.001	0.002

A small proportion of the occupied flowers contained > 2 beetles, or two beetles of the same sex. These are not listed here.

Table 2. Frequencies of feeding on flowers by mating and nonmating beetles observed on anemones and poppies

	Male beetles		Female beetles	
	<i>N</i>	Proportion	<i>N</i>	Proportion
<i>Anemone coronaria</i>				
Mating and feeding	24	0.04	50	0.18
Mating and not feeding	59	0.11	62	0.22
Not mating and feeding	191	0.36	113	0.40
Not mating and not feeding	261	0.49	57	0.20
Total	535	1	282	1
<i>Papaver subpiriforme</i>				
Mating and feeding	0	0	8	0.04
Mating and not feeding	108	0.51	100	0.54
Not mating and feeding	1	0.01	6	0.03
Not mating and not feeding	101	0.48	70	0.38
Total	210	1	184	1

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 (test for independence: $\chi^2 = 90.42$, d.f. = 3, $P < 0.001$; Table 2).

Poppies that contained glaphyrids had significantly larger corollas (mean \pm SD = 28.29 \pm 3.91 mm, $N = 237$) than the population mean (26.28 \pm 3.81 mm, $N = 150$, $t_{385} = 4.99$, $P < 0.001$). Flowers containing mating couples, which comprised 43% of the occupied flowers, had significantly larger corollas than flowers containing nonmating 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 ($\chi^2 = 27.54$, d.f. = 3, $P < 0.001$).

Fifty-one percent of male beetles in occupied poppies, and 58% of the females, were in copulation. One percent 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 depended significantly on beetle sex (test for independence: $\chi^2 = 15.85$, d.f. = 3, $P = 0.001$; Table 2).

OBSERVATIONS OF JUMP-FLIGHT SEQUENCES

In anemones, 25 incomplete sequences of rapid transitions among flowers ('jump-flights') performed by males, and three sequences of jump-flights 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 27 out of 28 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-flight 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 jump-flights 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.

GUT CONTENTS OF MALE AND FEMALE BEETLES

A high (> 500) number of pollen grains was found in the guts of most of the dissected beetles. More than 95% of all pollen grains examined originated from large red bowl-shaped flowers (anemone, ranunculus and poppy). In 40% of the individuals, a single type of pollen was detected, while the remaining samples contained pollen from two to three species of red flowers. We conclude that both male and female glaphyrids feed on pollen of red bowl-shaped flowers, and do not specialize on any single species within this guild.

CORRELATIONS BETWEEN DISPLAY SIZE AND FOOD REWARDS

Petal length and anther mass were positively correlated in anemone (Spearman's rank correlation,

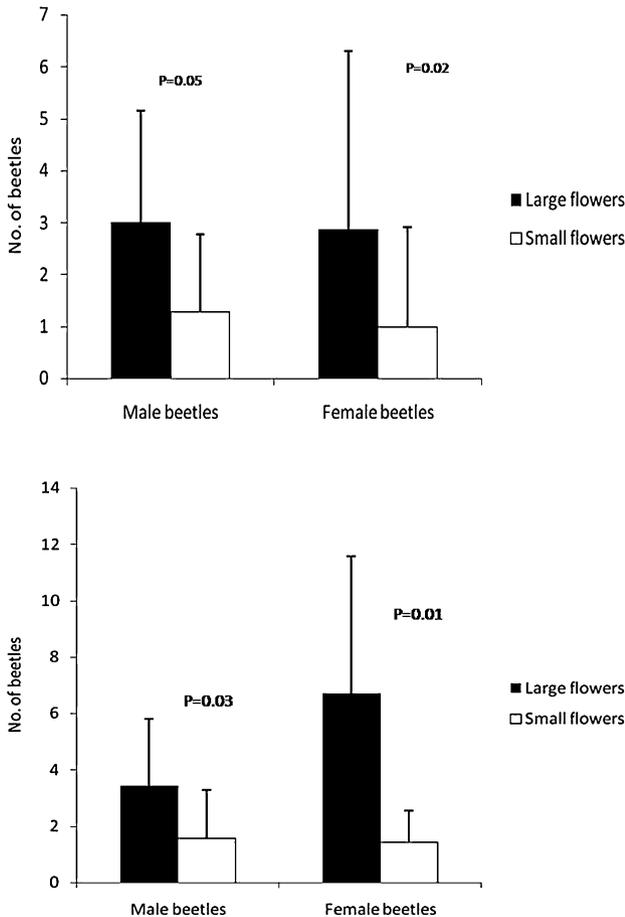


Figure 1. Mean number of male and female glaphyrids on large and small poppies, 1 h after introduction into an experimental terrarium. Top: first phase of the experiment, each terrarium contained beetles of one sex only. Bottom: second phase, males were introduced into the females' terrarium. Error bars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

$N = 10$, $r = 0.74$, $P = 0.02$) and in poppy (Pearson's correlation, $N = 50$, $r = 0.45$, $P < 0.001$).

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 (Wilcoxon one-tailed matched-pair signed rank tests, $N = 7$: males, $Z = 1.63$, $P = 0.05$, females, $Z = 2.06$, $P = 0.02$; Fig. 1). After males were introduced into the females' terrarium, the same trend was observed (males, $Z = 1.89$, $P = 0.03$, females, $Z = 2.20$, $P = 0.01$; Fig. 1). The male preference for large flowers misses the statistical significance threshold of 0.025 after the Bonferroni correction in this case, whereas female preference remains statistically significant. After entering the females' terrarium, the mean \pm SD

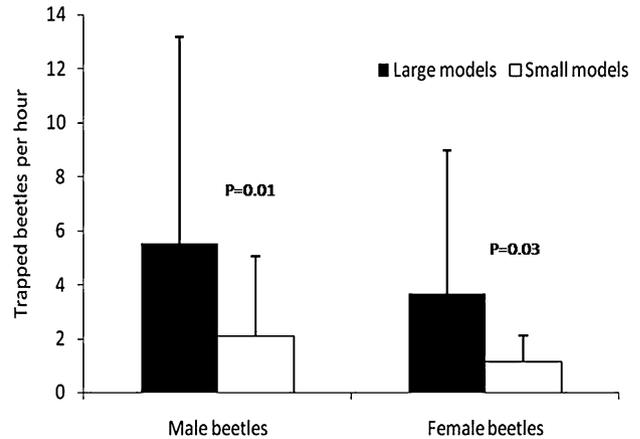


Figure 2. Mean \pm SD number of male and female glaphyrids collected every hour from 20 large (black bars) and 20 small (white bars) water traps ($N = 12$ 1-h trapping sessions, conducted on 4 days).

number of males found on flowers occupied by a female was 1.43 ± 1.27 , whereas the mean number of males on flowers not occupied by females was 1.29 ± 1.38 . These means did not differ significantly ($Z = 0.11$, $P = 0.45$).

EXPERIMENT 2: CHOICE EXPERIMENT WITH MODELS

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

DISCUSSION

The 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, with the great majority of occupied flowers containing just one or two beetles. This dispersed distribution probably reduces the beetles' chances of randomly encountering 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. The present study aimed to investigate the hypothesis that mate-finding involves attraction of males and females to the same plant signals. In support of this hypothesis, both male and female beetles preferentially selected 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 with respect to the presence of females.

Glaphyrid-containing flowers were more likely to be male-phase (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 but 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. Significantly, 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 females observed feeding compared to males may reflect differences in nutritional requirements between the sexes. In addition, the mating position constrains the movement of males more than it does 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 compared to females (Table 2). Nevertheless, the gut contents analyses revealed that male beetles feed on the pollen as well.

The attraction of glaphyrid beetles to large red models in our experiment is concordant with previous studies (Dafni *et al.*, 1990; Dafni, 1997). These studies also found increased attraction to models with a black spot at their centre, 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 (Scar-

baeidae: Melolonthinae: Hopliini) in South Africa (Johnson & Midgley, 1997; van Klunen *et al.*, 2007), but not in others (Johnson & Midgley, 2001). The presence of a black spot at the flower's centre 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 (T. Keasar, A. Shmida & A. Zylbertal, unpubl. data). 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 because petal size is positively correlated with pollen mass in both study species. A dual role of flowers as feeding and mating sites for beetles has been documented in previous studies (Gottsberger, 1977; Crowson, 1981; 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 species from six plant genera (*Anemone*, *Tulipa*, *Ranunculus*, *Adonis*, *Glaucium* and *Papaver*) from 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 a result of 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*, *Glaucium*, 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 near absence of red flowers pollinated by birds 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* (Baraud, 1990), are important pollinators of red anemone guild flowers. The indirect evidence outlined below hints that these genera have been selected for pollination of anemone-guild flowers in the Levant:

1. Other glaphyrid genera (e.g. *Glaphyrus*) often feed on whole flowers. By 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).
2. Other glaphyrid genera, as well as other Mediterranean flower beetles [such as *Oxythyrea abigail* Reiche & Saulcy, 1856, and *Tropinota* spp. (Scarabaeidae)] often forage on spiny species of Asteraceae, 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.*, 1990; Dafni, 1997). In the only glaphyrid species that has been studied neurophysiologically, a red receptor is present in the visual system (J. Martinez, M. Vorobyev, J. Schorn, A. Shmida & R. Menzel, unpubl. data).
3. The Near East and Asia Minor (Turkey) is the putative centre of speciation of *Pygopleurus* and *Eulasia*. This assumption is based on the high species richness of glaphyrids in the Near East, in the absence of a phylogeny for the Glaphyridae.

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

4. 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).
5. Flowers of the 'red anemone guild' close at night, whereas many of their congeners remain open (pers. observation). Closing of the petals at night not only 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* (T. Keasar, A. Shmida & A. Zylbertal, unpubl. data).

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 because such displays, being extremely rare in the near East, occupy a well-defined and distinct niche. In addition, such flowers provide beetles with food and overnight shelters.

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