The signaling function of an extra-floral display: what selects for signal development?

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The vertical inflorescences of the Mediterranean annual *Salvia viridis* carry many small, colorful flowers, and are frequently terminated by a conspicuous tuft of colorful leaves ('flag') that attracts insect visitors. Insects may use the flags as indicators of food rewards in the inflorescences below, as long-distance cues for locating and choosing flowering patches, or both. Clipping of all flags from patches of inflorescences in the field reduced the number of arriving insects, but not the total number of inflorescences and flowers visited by them. The number of flowers visited per inflorescence increased with inflorescence size, and inflorescence and flower visits rates significantly increased with patch size. Six percent of the plants in the study population did not develop any flag during blooming, yet suffered no reduction in seed set as compared to flag-bearing neighboring individuals. Removal of flags from all inflorescences in a patch reduced seed set in comparison with untreated controls, while flag clipping from ten randomly selected inflorescences in a patch did not decrease seed production. These results suggest that flags signal long-distance information to potential pollinators (possibly indicating patch location or size), while flower-related cues may indicate inflorescence quality.

Plants that do not develop flags probably benefit from the flag signals displayed by their neighbors, without bearing the costs of signal production. Greenhouse-grown *S. viridis* plants allocated a low proportion of their biomass to flags. Plants grown under water stress did not reduce biomass allocation to flags as compared to irrigated controls. Water loss rates of picked flags were lower than those of picked leaves. These findings suggest that the expenses of flag production and maintenance are modest, reducing the selective advantage of individuals that do not carry flags. We discuss additional potential evolutionary mechanisms that may select for flag production.

The conspicuous visual displays of flowering plants attract pollinating insects and promote plant reproductive success (Faegri and van der Pijl 1979, Chittka and Thomson 2001, Schaefer et al. 2004). Most of these displays are located in the corolla, and surround the reproductive organs of a single flower. In some cases, however, prominent visual signals are located at a distance from the flowers (extra-floral displays), while floral corollas are rather small and unremarkable (Heywood 1978, Proctor et al. 1996, Borges et al. 2003). 'Flags', large tufts of colorful bracts at the top of upright inflorescences, provide a striking example of extra-floral displays.

Flags have long been claimed to attract insect visitors, but the empirical support for this claim is scant and equivocal (Herrera 1997, Higginson et al. 2006, 2007, Keasar et al. 2006). Evidence for the pollination role of flags has been found in the Mediterranean annual *Salvia viridis* (Lamiaceae). In this species, experimental clipping of flags from inflorescences in a field population reduced insect visitation (Keasar et al. 2006). This study also showed that flag size in greenhouse-grown plants correlates positively with the number of open flowers per inflorescence, and

with their nectar content. A possible interpretation of these findings is that flags function as signals of plant quality ('selective cues') to foraging insects (Cohen and Shmida 1993). Such signals may be very effective from the plant's point of view, as each flag advertises the quality of a whole inflorescence, rather than of a single flower (Faegri and van der Pijl 1979, Gottsberger and Hartmann 1988). We designate this possible selective advantage of flag displays as the 'quality signaling' hypothesis.

A second possibility as to the adaptive function of flags is that they increase the long-distance visibility of *S. viridis* plants to visitors (the 'detection' hypothesis) (Herrera 1997). That is, flags may function as 'detective cues', or as signals of patch size, rather than indicating the quality of individual plants. Under this interpretation, plant quality may be signaled by other cues, such as plant height, number of flowers, flower size or additional visual cues associated with the flowers. Such is the case for the fruit clusters of *Pistacia terebinthus*, where conspicuous, unripe fruits increase the long-distance detectability of the whole cluster to seed dispersers (Fuentes 1995). An additional example is the extra-floral bract display in *Mussaenda frondosa*,

which may make the low-density plants more conspicuous to butterfly visitors (Borges et al. 2003). The 'detection' hypothesis and the 'quality signaling' hypothesis are not mutually exclusive, and flags may in fact signal both patch location at long distances, and plant quality from short distances.

The two hypotheses diverge in the predicted direction of selection that acts on flag display. The 'quality signaling' hypothesis views flags as a secondary sexual trait. The theory of sexual selection is increasingly applied to the evolution of secondary displays in plants (Skogsmyr and Lankinen 2002, Delph and Ashman 2006). Sexual selection is predicted to favor exaggerated (super-stimulus) secondary displays, such as large flags, in all individuals, since each plant would receive more pollinator visits if it carried larger flags. However, in contrast with this prediction, flag size in *S. viridis* is variable and correlates positively with plant size, suggesting that the flag signal provides honest information on plant quality. Such honest communication can be maintained under sexual selection if the flag signals are costly to produce and/or maintain (Zahavi and Zahavi 1997). In particular, honest signaling can evolve if flag expression inflicts condition-dependent costs on the plant (David et al. 2000, Kotiaho 2002, Cotton et al. 2004), i.e. higher costs to poor-condition plants. Condition-dependent expression may enable good-condition plants to produce large flags, and benefit from increased attraction of potential pollinators. In poor-condition plants, the cost of flag production may limit flag size, leading to reduced visitor attraction. The 'detection' hypothesis, on the other hand, views the production of flags as a trait that is expressed by some of the plants, but benefits all the individuals in the patch through enhanced attraction of potential pollinators. Individual selection should therefore favor 'hitchhiker' individuals that do not carry flags, and grow in the proximity of flag-producing neighbors.

The two hypotheses thus differ in their predictions regarding the following points:

(a) The effects of flag clipping: the 'quality signaling' hypothesis predicts that flag clipping will affect within-patch foraging, i.e. that visitors will make fewer visits to clipped inflorescences after entering a patch. The 'detection' hypothesis predicts that insects will approach un-flagged patches less often than control patches. That is, that flag clipping would affect the movement of potential pollinators between patches. Effects on foraging between and within patches are expected if flags function both as quality signals and as detection cues.

(b) The fitness of un-flagged individuals: the 'quality signaling' hypothesis predicts lower fitness to flagless individuals than to flag-producing plants, because flagless individuals are predicted to receive fewer insect visits. The 'detection' hypothesis predicts no fitness advantage to flag-carrying plants over rare flagless individuals that are interspersed within the same patch.

(c) The costs of flags: the 'quality signaling' hypothesis predicts a high physiological cost to flag production/maintenance, and therefore condition-dependent expression of flags. Hence, individuals under stress

are expected to allocate fewer resources to flags than individuals in good condition. The 'detection' hypothesis predicts a group-level benefit to flag-producing patches, but no individual benefit to any plant within a patch from carrying a large flag. Thus, this hypothesis does not assume selection for excessively large flag signals, and does not require costly signaling to maintain their reliability. Allometric growth may account for the positive correlation between flag size and plant size, but this relationship is not predicted to be condition-dependent under the 'detection' hypothesis.

We evaluated these predictions using *Salvia viridis* as our experimental model. The test of the first prediction consisted of comparing insect visits to patches of *S. viridis* in a natural field population, before and after clipping of their flags. To test the second prediction, we compared female fitness (estimated by seed set) between flag-producing and non-producing plants from the same field population. We also assessed the effect of clipping flags of some inflorescences, and of all the inflorescences within a patch, on seed set. Tests of the third prediction focused on estimating the costs of the flag displays to *S. viridis* plants. To this end, we determined the biomass allocated to flags, flowers and foliage in greenhouse-grown plants at different stages of flowering and under two water regimes. Finally, we assessed the rate of mass loss in flags as an indication of water evaporation, a likely maintenance cost of display organs in arid environments (Galen et al. 1999).

Salvia viridis is a common spring-flowering species in Mediterranean and Irano-Turanian steppe grasslands. The protandrous flowers are arranged in whorls around each stem, and flowering progresses from the bottom of the inflorescences upwards. Inflorescence height is 19.40 ± 0.80 cm (mean $\pm$ SE, n $=40$). Inflorescences carry 3.92 ± 0.27 (SE) whorls on average (n $=26$ inflorescences from our field population). Each whorl can develop up to six flowers. 2.58 ± 0.05 flowers are open per inflorescence at peak bloom (n $=729$ inflorescences). The upper lip of the corolla is dark purple while the lower lip is light purple or white (Feinbrun-Dothan 1978). Flag-like bract clusters, composed of several colorful (purple, pink or white) leaves, develop at the top of inflorescences in some of the individuals. The proportion of flag-bearing individuals varies among populations (Keasar et al. 2006). The first flags usually develop on the main inflorescence. Secondary inflorescences that develop later sometimes carry flags as well. The plant grows in dense patches and is mainly pollinated by bees, though self fertilization is possible (unpubl.). Each flower produces up to four seeds. The closed, dry seed pods remain on the plant for 2–7 months before dispersal.

Methods

Field study

We conducted field observations in two large populations of *S. viridis* in central Israel in the spring of 2007 and 2008. We used plants that grew in dense patches for observations

and manipulations. Blooming patches were surrounded by young, non-blooming annuals of similar height, which formed a distinct border around the patches. The diameter of the observed patches was 30–35 cm, and the distances between them were at least 7 m. The number of inflorescences per patch ranged from 8 to 200. This variability allowed us to evaluate the effect of the number of inflorescences per patch on the behavior of visiting insects. Due to the high density of blooming plants per patch, we were not able to determine which inflorescence belonged to what plant, or whether a sample included plants with several inflorescences. We therefore used inflorescences as our observational units. We estimated the proportion of flag-bearing inflorescences in the population in a random sample of 372 inflorescences in full bloom from several patches. We recorded flag length and the number of flag leaves for an additional 285 inflorescences from several patches, as an estimate of the population distribution of flag size.

The effects of flag clipping

We observed insect activity on intact inflorescences, and following removal of their flags, during three days of observation. For each observation (n = 11) we chose a distinct patch of blooming *S. viridis*, and recorded the combined number of primary and secondary inflorescences. We observed each patch for ten minutes for insect visits. We counted the number of insect arrivals into the patch from surrounding areas. After the insect initiated foraging in the patch, we recorded the number of open flowers and visited flowers, flag length and the number of flag leaves for each inflorescence that received visits. We noted whether the visitor was a honeybee, a small solitary bee (smaller than a honeybee), a large solitary bee (larger than a honeybee), a fly, a lepidopteran or a beetle. We also recorded how many inflorescences each individual visited before leaving the patch. Return visits to a previously visited inflorescence were scored as an additional visited inflorescence. At the end of the ten-minute period, we clipped all flags from the tops of the inflorescences in the patch. We then observed the patch for insect visits for ten more min. We noted the number and type of visitors, the number of arrivals into the patch, the number of inflorescences and flowers per inflorescence visited by each potential pollinator in the clipped inflorescences. We treated the number of insect arrivals into the patches as a measure of between-patch behavior, and the number of inflorescences/flowers visited per 10-min period as measures of within-patch behavior. Flag-clipped inflorescences were not used in further observations. In a previous study, clipping and re-attaching of flags to inflorescences did not reduce insect visit rates, as compared to visit rates on intact inflorescences (Keasar et al. 2006). We therefore expected no effect of the clipping manipulation per se on the foraging rates of visitors.

The fitness of un-flagged individuals

We marked 60 flag-carrying plants, and 60 plants that did not carry flags when in full bloom, during the 2007 season. These plants grew in twenty patches that we did not

manipulate. The typical number of flagless plants per patch was 1–4. At the end of the blooming season, but before seed dispersal, we recovered the surviving plants. We counted the number of whorls, number of flowers per whorl, and number of seeds per flower for the two largest inflorescences of each plant. The patch identity of the recovered plants was not recorded. The mean number of seeds per flower was used as an estimate of female reproductive success of non-manipulated plants with and without flags in the field.

In 2008, we performed flag-clipping manipulations in six patches that were not used for behavioral observations. In three patches, flags were removed from ten inflorescences per patch, and these inflorescences were marked. In each of the three other patches, we clipped the flags from all the inflorescences, and marked ten inflorescences. As controls, we also marked ten inflorescences in each of three *S. viridis* patches that were not manipulated. At the end of the flowering season we collected the marked inflorescences, and determined the number of whorls, number of flowers per whorl, and number of seeds per flower in each of them.

The costs of flags

We characterized biomass allocation to flag production throughout blooming, and tested the effects of water stress on flag development, in greenhouse-grown plants. Potted *S. viridis* plants for both purposes were grown in a commercial nursery from seeds collected at the Hebrew University's botanic garden. Shortly before blooming, they were transferred into a 6×8 m experimental greenhouse. The plants were exposed to ambient light–dark and temperature conditions, and were watered 180 ml week^{-1} through drip irrigation between 5 March and 5 April 2007. Irrigation volume was gradually increased to 550 ml week^{-1} during April, as the weather became drier and hotter. The study was terminated at the end of April, when flowering ended.

For the determination of biomass allocation to flags, we harvested plants at the opening of their first flowers, and a week, two weeks and three weeks (peak blooming) later. We harvested 20 plants at each time point, and recorded the dry weight of above-ground vegetative parts (stems and leaves), reproductive organs (flowers and buds), and flags for each of them. We used an analytic scale, sensitive to 10^{-4} g, for biomass determination. This repeated harvesting allowed us to detect possible fluctuations in biomass allocation to flags with flowering phase.

To characterize flag development under water stress, we grew plants under a standard or a restricted irrigation regime (n = 40 for each treatment). Control plants received 100 ml water per irrigation, five times a week, while water-stressed plants received 70 ml, five times per week. In 2007 we started the restricted irrigation regime when the flags started developing, to reduce mortality of young plants (and hence unintended selection for drought-resistant pheno-types). Thus, the restricted irrigation treatment typically started a few days before opening of the first flowers (45-day old plants), and continued until the end of blooming (75-day old plants). At the end of flowering, we determined the number of flowers and flags per plant, and the dry mass of the flags, flowers and seeds, and above-ground vegetative

parts. Initial sample size was 40 for both treatments, but two control plants and one water-stress plant were lost to insect herbivory after the end of blooming.

In the 2008 season we repeated both irrigation treatments as above, but started the irrigation restriction two weeks before the onset of flag development (30-day old plants). This was done to test whether early water stress might affect flag development more strongly than stress induced at a later developmental stage. Initial sample size was 50 plants per treatment, but mortality of two plants from the control treatment reduced this sample to 48 at the end of the experiment.

To estimate whether maintenance of flags is associated with water-loss costs, we picked five flags and five green leaves from potted plants, and immediately sealed the injured tissue with hot wax. We determined the wet mass of the flags and leaves, and placed them in a drying oven at 27°C and 70% relative humidity (typical ambient conditions in spring in the Mediterranean) for six h. During drying, we repeatedly weighed the flags and leaves. We calculated the proportion of the original mass retained in the flags and leaves at each weighing.

Data analysis

The effects of flag clipping
In the clipping experiment, we treated observations of the same patch, before and after flag removal, as paired. Using one-tailed Wilcoxon matched-pairs signed-rank tests, we tested whether flag clipping reduced the number of insect arrivals into the patch, the number of visited inflorescences and the number of visited flowers per 10-min observation period. We employed linear regression to test for the effects of the total number of inflorescences per patch (patch size) on the number of visitors, visited inflorescences and visited flowers per observation period. We tested this effect separately for the patches prior to flag clipping, and after flag clipping. One outlying observation, contributed by a single honeybee that visited a manipulated patch, was excluded from the regression analysis. We used stepwise forward regression (inclusion criterion 0.05, exclusion criterion 0.1) to test the effects of flag length, number of leaves per flag, and the number of open flowers on the number of successive visits by each visitor on an inflorescence.

The fitness of un-flagged individuals
The number of seeds per flower in non-manipulated plants with and without flags was compared via a t-test. This analysis does not take into account possible effects of patch of origin on seed-set in flagged versus flagless plants. The

fact that both types of plants were marked in a large number of patches (n = 20) reduces this possible confound. In the flag-clipping manipulation, we used a linear mixed model to evaluate the effects of treatment (three levels) and patch (nine levels) on the mean number of seeds per flower. Patch was considered the subject variable, and was defined as a nested random factor within treatment in the analysis. Treatment was defined as the fixed factor.

The costs of flags
We employed t-tests to compare biomass allocation to flags, reproductive tissue and vegetative tissue in water-stress versus control plants. We compared the allocation of dry biomass to these functions, as well as the proportion allocated to each function out of the total biomass. In the drying experiment, we fit linear regressions to describe the relationship between drying time and biomass of flags and leaves. We tested whether leaves and flags desiccate at similar rates by comparing the regression slopes.

SPSS ver. 15.0 was used for all statistical analyses.

Results

The effects of flag clipping

Of the 372 inflorescences sampled at full bloom 93.8% carried a flag. Mean ± SD flag length was 12.4 ± 3.8 mm. The mean ± SD number of leaves per flag was 5.06 ± 1.47 (n = 285 inflorescences from the flag-clipping manipulation). Flag length and the number of flag leaves were significantly correlated (Pearson's correlation coefficient = 0.44, p < 0.001, n = 285 inflorescences). In accordance with previous observations (Keasar et al. 2006), the number of flag leaves was also significantly correlated with the number of open flowers in the inflorescence (correlation coefficient = 0.17, p < 0.001, n = 382).

Honeybees were the most frequent visitors (76.48% of all visits), followed by large solitary bees (17.79%), small bees (3.16%), flies (1.58%) moths (0.59%) and beetles (0.40%). The number of insects that visited *S. viridis* patches was significantly higher prior to flag clipping than after their removal. Inflorescence and flower visit rates were not significantly reduced after flag clipping (Table 1). Thus, insects may have performed longer foraging bouts in flag-clipped plants than in intact ones. Inflorescence and flower visit rates significantly increased with patch size (Fig. 1, top and middle). The number of visiting insects per observation, on the other hand, was not significantly affected by patch size (Fig. 1, bottom).

Table 1. Parameters of insect visits to 11 *S. viridis* patches before and after flag removal. The mean ± SE number of inflorescences per patch was 74.36 ± 16.57. n denotes the number of non-tied ranks in Wilcoxon matched-pairs signed-rank tests for the effects of flag clippings. p-values for these tests are reported.

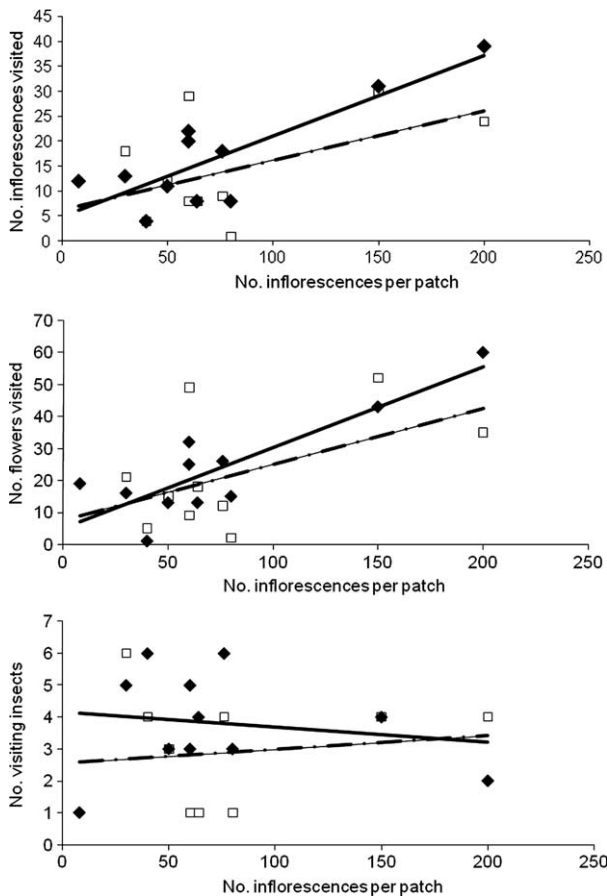|	No. of inflorescences visited	No. of flowers visited	No. of visitors
Before flag removal	16.91 ± 3.20	23.91 ± 4.92	3.82 ± 0.48
After flag removal	15.91 ± 3.37	26.36 ± 6.82	2.73 ± 0.54
n	9	11	8
p	0.37	0.45	0.04

Figure 1. The effect of patch size on the number of visited inflorescences (top), visited flowers (middle) and visiting insects (bottom). Full symbols denote intact patches, empty symbols denote patches after removal of all flags. Regression equations are: inflorescence visit rates: $y = 0.161x + 4.958$, $r^2 = 0.69$ (intact); $y = 0.099x + 6.274$, $r^2 = 0.26$ (flag removal); flower visit rates: $y = 0.253x + 5.095$, $r^2 = 0.73$ (intact); $y = 0.174x + 7.664$, $r^2 = 0.27$ (flag removal); number of visiting insects: $y = -0.005x + 4.171$, $r^2 = 0.03$ (intact); $y = 0.004x + 2.551$, $r^2 = 0.02$ (flag removal).

Flag-bearing inflorescences that were visited by insects had a mean length of 12.2 ± 2.4 mm and 4.9 ± 2.1 leaves ($n = 260$). We tested whether insects preferentially visited inflorescences with large flags by comparing these values with the data set of flag sizes, which was recorded without observations of insect visits. Flags of inflorescences that received insect visits did not significantly differ in size from the population means (two-tailed t-tests: $t_{543} = 0.40$, $p = 0.69$ for flag length; $t_{515} = 1.23$, $p = 0.22$ for number of leaves). The number of open flowers, but not flag length or flag leaf number, significantly affected the number of flowers visited per inflorescence (stepwise regression, $r^2 = 0.121$, $p < 0.001$).

The fitness of un-flaged individuals

We recovered 49 of the flag-carrying and 11 of the marked, non-manipulated plants that did not develop flags (the remaining plants were grazed by sheep). The two groups of plants did not significantly differ in the number

of seeds per flower at the end of the blooming season ($t_{58} = 0.01$, $p = 0.99$). Clipping of all flags in a patch resulted in a lower number of seeds per flower, compared to clipping of 10 plants per patch and to no flag clipping at all (mixed model: $F_{2,6.01} = 5.53$, $p = 0.043$ for the effect of treatment; Fig. 2).

The costs of flags

As flowering progressed in the greenhouse-grown plants, increasingly more biomass was allocated to the production of flowers and seeds, while the fraction of biomass allocation to leaves and stems decreased. Allocation to flags fluctuated, and accounted for 0.5% or less of the plants' total biomass during the first four weeks of blooming (Table 2). Water stress significantly reduced the biomass of vegetative and reproductive tissue, but not of flags (Table 3, top). Restricted irrigation that was applied at the onset of flag development (in 2007) did not reduce the proportions of biomass allocated into vegetative, reproductive and flag tissue. Water restriction that was applied two weeks before flag development (in 2008) significantly increased the proportion of biomass allocated into flag production, but did not affect the proportional allocation into vegetative and reproductive organs (Table 3, bottom).

In the drying experiment, both flags and leaves lost mass at a constant rate during six h after picking, probably through water evaporation (Fig. 3, regression equations: relative mass $= 1 - 0.00004 \times$ time, $r^2 = 0.95$ for flags, relative mass $= 1 - 0.00005 \times$ time, $r^2 = 0.92$ for leaves). The rate of mass loss was significantly higher for leaves
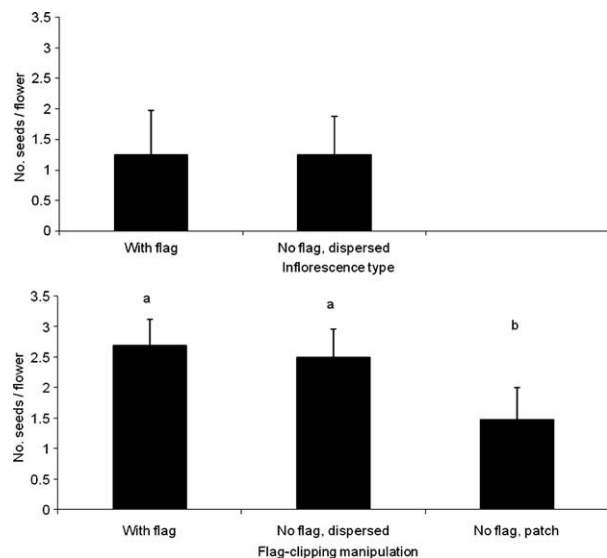

Figure 2. Mean (SD) number of seeds per flower in inflorescences with and without flags. Top: non-manipulated plants ($n = 49$ for flagged inflorescences, $n = 11$ for unflagged. Unflagged inflorescences were dispersed within several patches of flag-carrying plants. Bottom: Flag-removal manipulations. Flags were either removed from a whole patch ($n = 20$ marked inflorescences) or from 10 random individuals within a patch ($n = 26$). Flags were not manipulated in a group of control plants ($n = 28$). Different letters denote significant differences in Tukey's post-hoc test.

Table 2. Mean±SD dry biomass of flags, flowers and buds, and vegetative tissue in greenhouse-grown plants. Plant samples (n=20 per sample) were harvested at the beginning of blooming, and one, two and three weeks later. In the two first weeks, the mean mass of flags was lower than 0.001 g.

Week of blooming	Dry biomass (g) of					
	Vegetative tissue		Flowers and buds		Flags	
	gram	% of total	gram	% of total	gram	% of total
1	0.226±0.133	92.2	0.019±0.014	7.8	0.000±0.000	0.0
2	0.317±0.088	87.3	0.045±0.022	12.4	0.000±0.001	0.3
3	0.412±0.125	74.9	0.135±0.042	24.5	0.003±0.002	0.5
4	0.505±0.147	66.3	0.255±0.154	33.5	0.002±0.001	0.3

than for flags (t-test for comparison of regression slopes, $t_8 = 2.55$, $p = 0.014$).

Discussion

The results of the field manipulation are in line with the 'detection' hypothesis, which proposes that flags increase the visibility of *S. viridis* patches to insects from large distances, while flower-related cues are used for making foraging choices within patches (Lewis and Lipani 1990, Armbruster et al. 2005). This interpretation is supported by the following additional lines of evidence: (a) flag size in insect-visited inflorescences did not significantly differ from the population mean, suggesting no preference for inflorescences with large flags within a patch. (b) clipping of flags from all inflorescences in a patch reduced female fitness, while unflagged individuals surrounded by flag-carrying neighbors did not suffer from reduced seed sets. This implies that the selective benefit of flags is most pronounced at the patch level. Nevertheless, the number of open flowers correlated with the sizes of the flags in our field population. This suggests that flag size may also indicate the food potential of an inflorescence, as predicted by the 'quality signaling' hypothesis. Asynchronous development of bracts

and floral rewards can reduce the reliability of the signal for insects, though (Keasar et al. 2006).

Our findings resemble the results of flag-clipping manipulations in the perennial shrub *Lavandula stoechas* (Herrera 1997): both studies provide evidence for increased insect attraction to flags at a large distance, but not when they forage within a patch. Our present results also generally agree with a previous flag manipulation study with *S. viridis* (Keasar et al. 2006), which demonstrated a significant decrease in the number of visitors following flag clipping.

Experimental flag clipping in patches of *S. viridis* in the field reduced the rate of insect arrivals into the patches, but not the rate of visits to inflorescences or flowers within patches. This implies that fewer insect individuals visited patches after flag removal, but that each of them made a longer bout of visits before leaving the patch. The increased bout lengths may reflect the foragers' response to higher nectar and pollen yields in less-visited patches after flag clipping. Patch size correlated with per-patch visit rates, in agreement with previous reports (Goulson et al. 1998, Grindeland et al. 2005) (Fig. 1).

Our experiment did not control for possible location learning by the foraging bees. Foragers may have relied on their previous experience and spatial memory, rather on the flag displays, to locate the clipped patches of *S. viridis*. Spatial memory is well-developed in bees, and can guide

Table 3. Mean±SD dry biomass (top), and proportions of biomass allocation to flags, flowers and buds, and vegetative tissue (bottom) in plants grown without irrigation restriction and under water stress in the greenhouse. Irrigation restriction was initiated at the start of flag development in 2007, and two weeks prior to flag development in 2008. Plants were harvested at the end of flowering. Test statistics refer to comparisons between the two irrigation treatments.

| Irrigation schedule	Dry biomass (g) of		
	Vegetative tissue	Flowers and seeds	Flags
No restriction (2007 season)	1.26±0.52	1.41±0.53	0.02±0.02
Restricted (2007 season)	1.05±0.41	1.22±0.48	0.02±0.01
Test statistics (2007 season)	$t_{75}=1.92$, $p=0.03$	$t_{75}=1.68$, $p=0.049$	$t_{75}=0.52$, $p=0.60$
No restriction (2008 season)	1.90±1.58	2.48±1.19	0.16±0.14
Restricted (2008season)	1.32±0.48	1.25±0.52	0.14±0.12
Test statistics (2008 season)	$t_{96}=5.38$, $p<0.001$	$t_{96}=6.65$, $p<0.001$	$t_{96}=0.78$, $p=0.22$
Irrigation schedule	Proportion of dry biomass allocated to		
	Vegetative tissue	Flowers and seeds	Flags
No restriction (2007 season)	0.46±0.07	0.53±0.07	0.01±0.01
Restricted (2007 season)	0.46±0.05	0.53±0.05	0.01±0.01
Test statistics (2007 season)	$t_{75}=0.12$, $p=0.45$	$t_{75}=0.20$, $p=0.42$	$t_{75}=0.74$, $p=0.23$
No restriction (2008 season)	0.47±0.19	0.49±0.20	0.04±0.03
Restricted (2008 season)	0.49±0.12	0.46±0.14	0.05±0.05
Test statistics (2008 season)	$t_{96}=0.51$, $p=0.45$	$t_{96}=1.01$, $p=0.31$	$t_{96}=2.11$, $p=0.04$
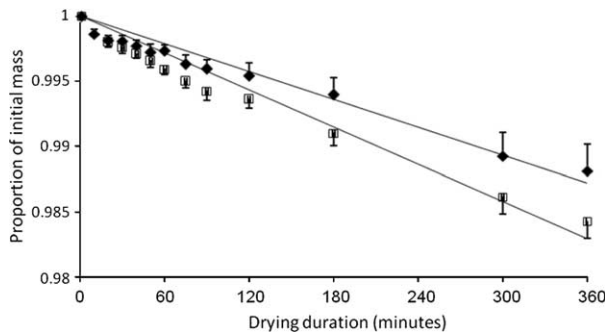
Figure 3. Proportional loss of mass of flags (full symbols) and leaves (empty symbols) during six h of drying at 27°C and 70% RH. Error bars indicate SEs.

foraging choices (Wehner and Menzel 1990), hence our observation procedure may have underestimated the effects of flag removal on the frequency of visitor arrivals. Flag removal nevertheless significantly reduced visitor arrivals, suggesting that the location effect was not dominant.

Our data also show that the costs of flags, at least in terms of biomass, are quite modest. Water limitation reduced overall plant size, indicating that the treatment plants were stressed. Nevertheless, the relative biomass allocation into flag expression was unaffected in the first study season, and even increased in the second. In addition, the rate of water loss from flags was lower than from leaves, suggesting a low cost to flag maintenance from a water balance perspective. These results, similarly to the results from the field manipulations, are compatible with the 'detection' hypothesis, and do not suggest that flags are condition-dependent costly handicap displays. It should be noted, however, that we did not measure other possible expenses of flag production (such as pigment production costs), or indices of male fitness.

Seed set in unflagged individuals that were interspersed in flag-carrying patches was similar to the seed set of inflorescences with flags. This finding may indicate that these flagless inflorescences received sufficient pollination, and did not suffer fitness costs. This begs the question how flag production is maintained in natural populations, if the benefits of this trait are shared by hitchhikers that do not produce flags. One possible mechanism is kin selection: if individuals in a patch are genetically related, then flag-producing individuals may gain indirect fitness from the increased reproductive success of their neighbors. Such indirect benefits may select for an altruistic trait (such as flag production), if the associated costs to altruists are low enough (de Jong and Klinkhamer 2005). Relatedness among individuals in a patch is expected in synaptospermic taxa such as *Salvia*, which disperse their seeds in synchrony to a short distance (Zohary 1950). Kin recognition, and traits that benefit genetic relatives, have been previously described in plants (Dudley and File 2007, Kobayashi and Yamamura 2007). The possibility of kin selection in our study system can be tested by assessing the relatedness of individuals within *S. viridis* patches, and by measuring the currently unknown heritability of the flag production trait.

Another possibility is that flag producers are not really altruists, because they increase their individual fitness through additional benefits, which are not related to

female reproductive success, and which may be frequency-dependent. Protection from radiation, herbivory and rain were suggested as possible survival advantages of extra-floral displays (Galen and Cuba 2001, Armbruster 2002, Sun et al. 2008). Our low recovery rate of marked, flagless plants at the end of the flowering season suggests that their survival may indeed be low as compared to flag producers. This possibility can be tested by a more rigorous and larger-scale comparison of the survival of plants with and without flags under field and laboratory conditions. These investigations are currently underway.

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