

Romina Malerba · Julieta Nattero

Pollinator response to flower color polymorphism and floral display in a plant with a single-locus floral color polymorphism: consequences for plant reproduction

Received: 16 February 2011 / Accepted: 28 November 2011
© The Ecological Society of Japan 2011

Abstract Variation in flower color, particularly polymorphism, in which two or more different flower color phenotypes occur in the same population or species, may be affected or maintained by mechanisms that depend on pollinators. Furthermore, variation in floral display may affect pollinator response and plant reproductive success through changes in pollinator visitation and availability of compatible pollen. To assess if flower color polymorphism and floral display influences pollinator preferences and movements within and among plants and fitness-related variables we used the self-incompatible species *Cosmos bipinnatus* Cav. (Asteraceae), a model system with single-locus flower color polymorphism that comprises three morphs: white (recessive homozygous), pink (heterozygous co-dominant), and purple (dominant homozygous) flowers. We measured the preferences of pollinators for each morph and constancy index for each pollinator species, pollination visitation rate, floral traits, and female fitness measures. Flower color morphs differed in floral trait measures and seed production. Pollinators foraged nonrandomly with respect to flower color. The most frequent morph, the pink morph, was the most visited and pollinators exhibited the highest constancy for this morph. Moreover, this morph exhibited the highest female fitness. Pollinators responded strongly to floral display size, while probed more capitulum from plants with large total display

sizes, they left a great proportion of them unvisited. Furthermore, total pollinator visitation showed a positive relation with female fitness. Results suggest that although pollinators preferred the heterozygous morph, they alternate indiscriminately among morphs making this polymorphism stable.

Keywords Attraction traits · *Cosmos bipinnatus* · Female fitness · Pollinator visitation rate · Pollinator preference

Introduction

There is a wealth of information about how floral traits influence components of a plant's reproductive success and mating pattern. Traits such as floral display size, corolla size, flower color, and signal reward influence the first step of the pollination process (pollinator attraction) (e.g., Hannan 1981; Hodges 1985; Campbell 1989; Galen 1989; Real and Rathcke 1991; Mitchell 1993; Gigord et al. 2001; Medel et al. 2003; Mitchell et al. 2004; Nattero et al. 2010), are expected to evolve in situations of floral variants (e.g., Galen 1989; Johnston 1991; Medel et al. 2003; Nattero et al. 2010), and induce alterations in pollinator behavior that may influence components of plant reproductive success and mate diversity (Harder and Barrett 1996).

Variation in flower color, particularly polymorphism, in which two or more different flower color phenotypes occur in the same population or species, may be affected or maintained by mechanisms that depend on pollinators. Pollinator behavior can induce changes in plant fertility, outcrossing rates, and pollen flow between morphs, which would result in variation in reproductive success and morphological evolution of populations (e.g., Proctor and Yeo 1973; Kay 1978; Mogford 1978; Waser and Price 1981; Brown and Clegg 1984; Stanton et al. 1989; Jones and Reithel 2001). In populations polymorphic for flower color, pollinator behavior directly affects mating pattern

R. Malerba · J. Nattero
Instituto Multidisciplinario de Biología Vegetal, CONICET,
Universidad Nacional de Córdoba, Casilla 495,
X5000JJC Córdoba, Argentina

Present address:

R. Malerba (✉) · J. Nattero
Cátedra de Introducción a la Biología, Facultad de Ciencias
Exactas, Físicas y Naturales, Universidad Nacional de
Córdoba, Avda. Vélez Sarsfield 299, piso 5, X5000JJC
Córdoba, Argentina
E-mail: rominamalerba@gmail.com
Tel.: + 54-351-4332100
Fax: + 54-351-4332100

among floral morphs through the influence of pollinators on the quantity and quality of pollen export and receipt. Polymorphism can be maintained by selection favoring the heterozygote (heterozygosity advantage), in which the heterozygote genotype has a higher relative fitness than homozygotes. There are many putative examples of genes where heterozygote advantage acts to maintain genetic variation in natural populations (e.g., Kalmus 1945; Gemmill and Slate 2006 and literature cited therein). Kay (1978) proposed different hypotheses to explain how flower color polymorphism can be maintained in a population by selective action of pollinators, but did not consider the effect of other interactions on the selection of morphs. Briefly, Kay (1978) postulated the following hypotheses: (1) pollinators alternate indiscriminately among morphs and there is an excess of heterozygotes in the population, (2) at least one pollinator prefers one of the morphs: in this case polymorphism would be stable only if the heterozygous morphs were preferred over the homozygous ones, (3) two or more species of pollinators differ in their preferences for the morphs: in this case, a fluctuating polymorphism could be maintained if the relative number of pollinators with different preferences changed from 1 year to another.

Faced with variation in flower color within a plant population, pollinators may ignore the variation, treating plants randomly with respect to flower color, or they may adjust their behavior as follows: one of the morphs may receive more pollinator visits, or more flower visits per plant visit (bout length) than the other morphs (Waser 1986). If pollinators truly ignore the variation, i.e., the morphs are visited in proportion to their representation in the population and the sequences of plants visited are random with respect to the polymorphism, then the mating pattern among morphs will be random in the population.

A pollinator visiting a plant of one morph is more likely to move to another of the same morph than would be expected based on morph frequencies in the population (Waser 1986). The tendency of most pollinators to restrict their visits to a subset of the available flowers thereby encompassing both assortative transitions among plants and nonrandom preferences is described as floral constancy (Waser 1986). Individuals over-visiting one morph are likely to pick up mostly that morph's pollen and carry it to stigmas primarily of the same morph, and other individuals are likely to do the same for the other morph(s). Selecting a single morph type (constancy) minimizes the cost of alternating between flower types. This view of flower constancy involves a trade-off between the costs of alternating between flower types and the costs of specializing in a single flower type (Gegear and Thomson 2004).

Floral display size, measured as total display size or as floral display area, is important in attracting pollinators and can be expressed as the number and size of pollination units. Variation in floral display may affect pollinator response and plant reproductive success through changes in pollinator visitation and availability

of compatible pollen (Holland et al. 2002; Ghazoul and Shaanker 2004; Maron and Crone 2006). Several studies have reported a positive relationship between floral display size and frequency of pollinator visits (e.g., Klinkhamer and de Jong 1990; 15 out of 17 studies reviewed by Ohashi and Yahara (1999, 2002). Once at a plant, pollinators are likely to probe several flowers in sequence (Klinkhamer et al. 1989; Mitchell et al. 2004) but may probe a decreasing proportion of the flowers in the display (Mitchell et al. 2004; Grindeland et al. 2005). Several studies reported that pollinators prefer to visit large flowers over small ones (e.g., Galen 1989; Campbell 1991; Vaughton and Ramsey 1998) probably because flower size is often associated with nectar and pollen reward (e.g., Galen 2000; Fenster et al. 2006). Large floral display size promotes increased pollen receipt or removal and is likely to enhance potential mate diversity (Wilson and Thomson 1991; Harder and Barrett 1996; Jones and Reithel 2001; Engel and Irwin 2003).

One way to understand how flower color polymorphism influences pollinator preferences and plant reproduction is to estimate pollinator movements within and among plants at each flower color morph (visits observed, constancy index) and evaluate its influence on female fitness. Moreover, we were interested in understanding how total display size and floral area, affect pollinator movements within and among plants (pollinator visitation rate, bout length, proportion of visited flowers) and female fitness. For this, we choose *Cosmos bipinnatus* Cav. (Asteraceae), a plant with a simplified model system with a single-locus floral color polymorphism. In this species, flower color polymorphism is reflected in white, pink, and purple ligulate flower phenotypes. This character is determined by a locus with multiple alleles (Crowe 1954; Howlett et al. 1975). *C. bipinnatus* has been previously described as strictly self-incompatible and depending exclusively on pollinator activity for seed formation (Crowe 1954). Studies addressing the influence of pollinator preferences on the maintenance of flower color polymorphism have received little attention. Moreover, the effects of floral display size on plant fitness are still scarce and very little is known about how these variables affect visitation rate.

Specifically, we aimed at evaluating (1) whether flower traits vary among flower color morphs, (2) whether pollinators prefer any of the flower color morphs, and (3) whether attraction traits, such as total display size and floral area, affect pollinator movements within and among plants and female fitness.

Materials and methods

Plant natural history and study site

Cosmos bipinnatus Cav. (Tribu Heliantheae, Family Asteraceae) is an erect annual herb that grows up to 2 m

tall, and is native to Central America but naturalized in Europe, Bolivia, Peru, and Argentina. The plants are highly self-incompatible (Crowe 1954) and fruit formation depends exclusively on pollinator activity. Capitulum are lateral or terminal, heterogamous, with eight ligulate flowers in the periphery and there are about 60 tubular flowers in the center (Fig. 1a). Tubular flowers are perfect and offer nectar as a reward, whereas ligulate flowers are sterile and only fulfill an attraction function. The flowers last about 3 days, have diurnal anthesis, and are scentless. *Cosmos bipinnatus* is characterized by a series of flower color polymorphisms of the ligulate flowers that include white, pink, and purple phenotypes (Fig. 1a). This character is determined by a locus with multiple alleles (Crowe 1954; Howlett et al. 1975). This locus is co-dominant, purple flowers being the dominant homozygous state, pink flowers the heterozygous co-dominant state, and white flowers the recessive homozygous state.

This study was conducted from March to May, 2007, in a population located in Villa Giardino, (Córdoba province, Argentina, 31°02'58.83"S; 64°29'24.66"W). In Córdoba province, this species grows at up to 1,000 m a.s.l. and is typically found in the surroundings of the mountain regions. In this area, the flowering season extends from the end of February up to May, is the same for all the morphs and across the whole of the population. The population studied has at least 20 years of establishment (Nattero J, pers. obs.). In this population, plants grew naturally in plots (small piece of ground ranging from 20 × 20 m up to 100 × 100 m) distant for

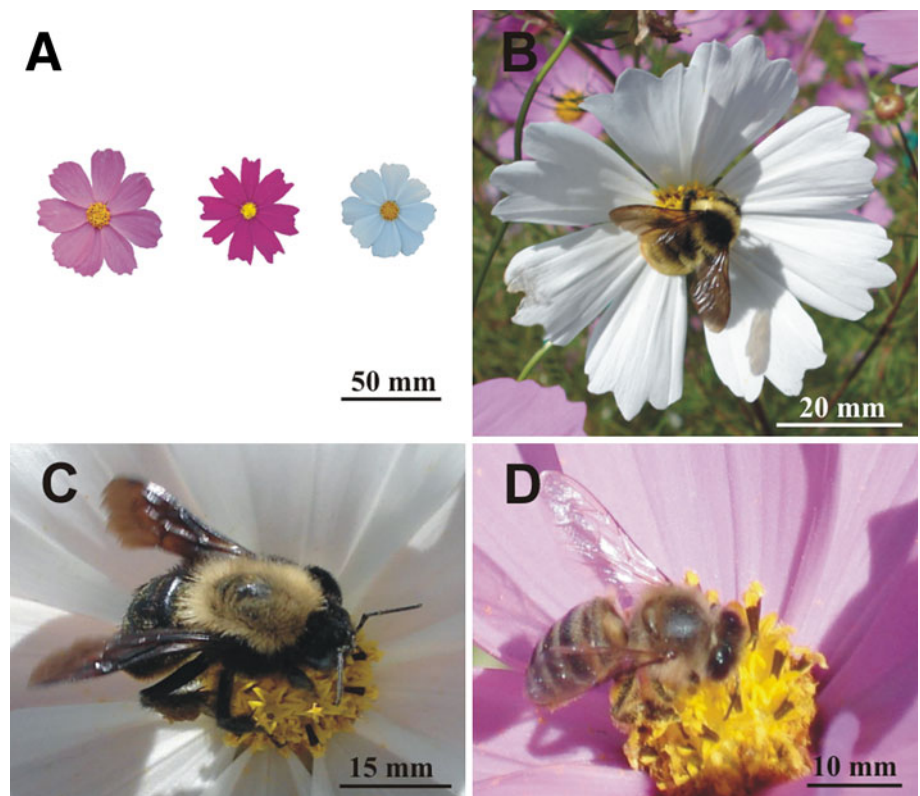
more than 50 m from each other. The whole population is constituted with about eight plots. Plots were distributed in wasteland between dwellings, ranging from about 100 to thousands of plants at each plot. In general, the pink morph is dominant related to its spatial frequency, with a frequency at the population level of about 60%. Morph frequency does not vary spatially at the population level, we only detected a few plots (about three) with dominance of purple morph and none of the plot exhibit dominance of white morph.

We estimated pollinator preferences in two natural plots that differed in the frequency of flower color morphs related to its spatial frequency. In plot A, the pink morph was dominant (0.60:0.25:0.15 pink, purple, and white, respectively). In plot B, the purple morph was dominant (0.30:0.60:0.10 pink, purple, and white, respectively). Both plots were located at the center of the populations, with a size of approximately 20 × 20 m, each containing about 100 plants. At each plot, morphs were haphazardly distributed and no color morphs were clustered.

Pollinators

We estimated pollinator preferences from partial preferences of individuals for each color morph in the two natural plots (A and B) described above. We did not make experimental plots because the goal of this study was to determine whether natural variation in frequency of flower color morphs influences pollination and

Fig. 1 Visual description of the study system. **a** Capitulum of the three flower color morphs. **b** The bumblebee pollinator *Bombus opifex* visiting a *Cosmos bipinnatus* capitulum. **c** The bumblebee *Bombus bellicosus* visiting *Cosmos bipinnatus* capitulum. **d** The honeybee *Apis mellifera* visiting a *Cosmos bipinnatus* capitulum



reproduction. Pollinator observations were performed during 9 days distributed throughout flowering period (about 3 months). In addition, we studied pollinator behavior for the different flower color morphs in several plots of the population. For each observation day, we recorded the identity of the visiting pollinator, the number of open capitulums per plant, and the number of times each plant was visited. We observed all study plants in the plot simultaneously for pollinator visits, totaling 560 observation minutes. From these data, we calculated: average of capitulum display size per plant (total display size), total number of visited capitulums per plant, and pollinator visitation rate (number of visits/number of open capitulum per plant), bout length (number of capitulums probed during a single plant visit), and the proportion of flowers visited (bout length divided by floral display size).

Trait measures

We sampled plants from each color morph at random to measure discal and radial capitulum areas. In 100 plants per morph, capitulum characters were measured in two capitulums per plant using digital photographs.

We estimated two measures of female fitness for each individual plant: fruit set and seed number per capitulum. Fruit set, measured as the proportion of fruiting and nonfruiting flowers, assess pollination intensity as pollinated flowers per capitulum, whereas seed number, measured as the number of seeds per capitulum, assesses mating quality. In two capitulums per plant and for the 100 plants per morph, we counted the number of fruiting and non-fruiting tubular flowers to estimate the proportion of fruit set and the number of seeds per capitulum.

Data analysis

We performed one-way ANOVA to determine possible variations in fitness measures or capitulum traits among flower color morphs and to check for differences in bout length among flower morphs. We tested the

assumptions of normality for all traits using the Shapiro–Wilk test.

We conducted a Chi-square goodness-of-fit test to determine possible preferences of pollinators for one of the flower morphs at the population level. We also used this test to evaluate preferences of different pollinators for flower color morphs in the natural plots (A and B) and preferences of pollinators for one of the flower morphs regardless of the pollinator identity.

To calculate a constancy index, we used the plot A described above and followed the path of pollinator bees. When we found a bee visiting the plot, we traced the bee and recorded the sequence of individual plants and the number of flowers per plant the bee visited until it left the plot. Flower constancy is defined as the tendency to visit one flower type sequentially while bypassing other equally or more rewarding flower types (Waser 1986). In one of the study plots, we used the measure of constancy proposed by Geager and Thompson (2004): constancy index, $CI = (c - e) / (c + e - 2ce)$, where c is the proportion of moves between the same color, and e is the expected proportion of moves between the same color based on the overall frequency of each color selected in a given plot. Possible values range from -1 (complete inconstancy) to 0 (random foraging) to $+1$ (complete constancy).

We used linear regression to test the prediction that the pollinator visits observed, visitation rate, and bout length were associated linearly with total display size. We also used linear regressions to analyze the relationship between seed per capitulum and visits observed and visitation rate. A multiple regression was used to evaluate the influence of radial and/or discal capitulum area on visitation rate of pollinators.

Results

Differences in floral display traits and fitness measures among the three flower color morphs

Capitulum traits involved in attraction (discal and radial area) and seeds per capitulum showed a wide variation among flower color morphs (Table 1). Fruit set did not

Table 1 Mean \pm SD for three capitulum traits and two fitness measures in a flower color polymorphic population of *Cosmos bipinnatus*

Flower and fitness traits	Flower color morphs			ANOVA Among flower color morphs
	Pink $x \pm SD$ ($n = 100$)	Purple $x \pm SD$ ($n = 100$)	White $x \pm SD$ ($n = 100$)	
Number of opened capitulums	5.70 \pm 0.12 ^b	4.01 \pm 0.19 ^a	6.98 \pm 0.35 ^c	$F_{(2,1022)} = 42.93; p < 0.0001$
Disc area (mm ²)	99.33 \pm 26.84 ^c	88.75 \pm 22.12 ^b	71.29 \pm 17.39 ^a	$F_{(2,298)} = 40.26; p < 0.0001$
Radial area (mm ²)	2,705.50 \pm 833.71 ^c	2,259.51 \pm 701.20 ^b	2,010.26 \pm 626.33 ^a	$F_{(2,298)} = 24.44; p < 0.0001$
Seeds per capitulum	38.32 \pm 0.24 ^c	36.98 \pm 0.23 ^b	33.22 \pm 0.30 ^a	$F_{(2,298)} = 9.21; p < 0.0001$
Fruit set	0.62 \pm 0.14	0.63 \pm 0.15	0.62 \pm 0.19	$F_{(2,298)} = 0.28; p = 0.7545$

The ANOVA column shows results of comparisons between morphs. Different letters indicate significant differences between populations in a Tukey a posteriori test at the $p < 0.05$ level
 n number of plants

show differences among morphs (Table 1). The white morph exhibited the smallest values of discal and radial areas and seed number, and the highest values of total display size (Table 1). The highest values of discal and radial area corresponded to the pink morph (Table 1).

Pollinators' behavior and preferences for flower color morphs

Bombus bellicosus was by far the most frequent visitor in the three morphs (Fig. 2). The Chi-square goodness of fit test showed that the pink morph was the most preferred one (Table 2). *B. bellicosus* was the most frequent visitor in the plot dominated by the pink morph (Table 3). In this plot, the three pollinators preferred one of the flower morphs: *A. mellifera* and *B. opifex* preferred the pink floral morph, whereas *B. bellicosus* preferred the white floral morph; however, none of the pollinators preferred the purple morph (Table 3). In the plot dominated by the purple morph, *B. bellicosus* was the most frequent visitor in the three morphs (Table 3). In this plot, this bee was the only one that showed preference for one of the morphs, selecting the pink morph over the other morphs (Table 3). In the evaluation of visits to each morph regardless of pollinator identity, the Chi-square test

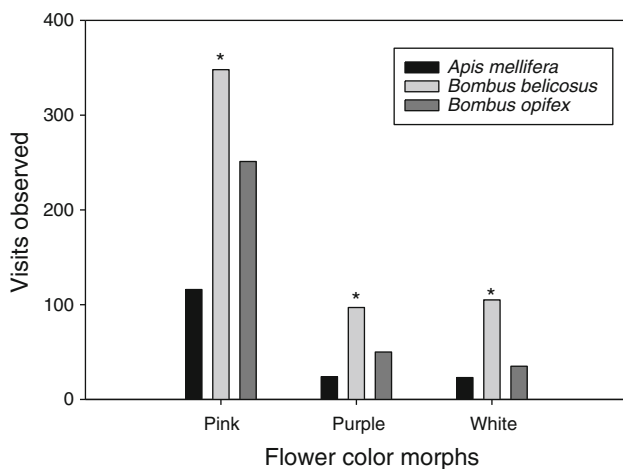


Fig. 2 Total pollinator visitation observed for each pollinator on each flower color morph in a polymorphic population of *Cosmos bipinnatus*. Asterisks indicate rejection of a homogeneity G ($p < 0.0001$) in the visit number of the three pollinators within each color morph

showed, in both plots, preference for the dominated flower morph, among one of the others two (Table 3).

There was an overall bias in the number of pollinator visits to purple, pink, and white morphs. The most frequently visited morph is the one that presented the highest constancy index for the three bee pollinators, indicating that bees foraged mostly on the pink morph (Table 4). *B. bellicosus* presented the highest constancy for the pink morph, whereas *B. opifex* exhibited the lowest one (Table 4). All pollinator species showed a high inconstancy for the white morph (Table 4).

Pollinator movements and its relation to total display size, floral area, and fitness measures

Three bee species belonging to two genera accounted for the total of visits observed in the three morphs: two native bumblebees, *Bombus opifex* and *Bombus bellicosus* and the honeybee *Apis mellifera* (Fig. 1b–d).

We recorded a total of 1,352 capitulums visited during the 560 observation minutes. The visits observed varied greatly among morphs (Table 2), the pink morph being the one that received the highest number of visits (68%). The purple and white morphs had 16% of the total visits each.

Bees responded strongly to total display size; the total number of visits observed on plants increased with high capitulum number, and this relation was significant for the pink and purple morphs (Fig. 3). Flower visitation rate decreased whereas total display size increased, considering both the entire population ($\beta = -0.25 \pm 0.06$; $p < 0.0001$) and the three morphs separately (Fig. 4); this relation was significant in all cases except for the white morph separately.

Seeds per capitulum increased linearly and significantly with observed visits per capitulum ($\beta = 0.37 \pm 0.18$; $p < 0.05$), whereas visitation rate did not show a significant association with seeds per capitulum ($\beta = 2.33 \pm 1.04$; $p < 0.05$).

Bout length (number of capitulums probed during a single plant visit) varied significantly among color morphs ($F_{(1020,2)} = 4.067$; $p = 0.0165$), the white morph being the one that had the longest bout length (1.44, 1.34, and 1.24 for white, pink and purple, respectively). In the three morphs, bout length showed a positive and significant relation with floral display size (Table 5), whereas the proportion of flowers visited

Table 2 Total pollinator visitation observed and expected on each flower color morph in a polymorphic population of *Cosmos bipinnatus*

Flower color morphs						Chi-square value
Pink		Purple		White		
Observed visits	Expected visits	Observed visits	Expected visits	Observed visits	Expected visits	
715	539	171	309	163	201	68.35**

Significant Chi-square value indicated differences in pollinator visits among flower color morphs

** $p < 0.001$

Table 3 Pollinator visits observed and expected in two natural plots with different flower color arrangement in a *Cosmos bipinnatus* population

Pollinator	Flower color morphs						Chi-square values
	Pink		Purple		White		
	O	E	O	E	O	E	
Total visits observed/morph (A)	443	335	70	141	122	81	44.32***
<i>Apis mellifera</i>	116	112.72	24	24.94	23	30.78	6.08*
<i>Bombus bellicosus</i>	167	181.18	22	40.09	73	49.48	33.48***
<i>Bombus opifex</i>	160	145.22	24	32.13	26	39.66	15.35**
Total visits observed/morph (B)	134	28	169	48	4	4	10.23**
<i>Apis mellifera</i>	44	29.75	39	51	2	4.25	5.16
<i>Bombus bellicosus</i>	73	57.75	90	99	2	8.25	6.02*
<i>Bombus opifex</i>	17	19.95	40	34.2	0	2.85	3.54

(A) Plot dominated by pink flower morph. (B) Plot dominated by the purple flower morph. Significant Chi-square values indicated differences in total visits observed per morphs and per pollinator per morph

O observed visits, E expected visits

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 4 Average constancy index \pm SD of each pollinator bee species to each flower color morph in a natural plot of *Cosmos bipinnatus*

Pollinators	Average constancy index		
	Flower color morphs		
	Pink	Purple	White
<i>Apis mellifera</i>	0.507 \pm 0.566	-0.374 \pm 0.73	-0.979 \pm 0.06
<i>Bombus bellicosus</i>	0.510 \pm 0.630	-0.510 \pm 0.63	-0.975 \pm 0.06
<i>Bombus opifex</i>	0.396 \pm 0.629	-0.409 \pm 0.71	-0.952 \pm 0.109

Frequency of morphs in the plot studied was 0.81:0.16:0.03 for pink, purple, and white morphs, respectively. Possible values range from -1 (complete inconstancy) to 0 (random foraging) to +1 (complete constancy)

showed a negative and significant relation with floral display size (Table 5). This means that compared to small floral displays, in large floral displays, pollinators visit more flowers in sequence but probe a decreasing proportion of the flowers in the display.

The multiple regression between visitation rate and radial and discal capitulum area showed that only the area of tubular flowers (radial area) was positively and significantly associated ($\beta = 0.03 \pm 0.02$; $p < 0.05$), indicating that plants with big radial area, where reward is located, showed a high number of pollinator visitations.

Discussion

The results of the present study indicate that flower color morphs of *C. bipinnatus* present ample and significant variations in flower traits. Regarding seed production, the pink morph was the one that produced the highest number of seeds but the white morph was the

one that produced the highest number of capitulum per plant, which would be compensating for the high seed production of the pink morph.

In the present work, pollinators did not ignore the variation in flower color polymorphism and foraged nonrandomly with respect to flower color, since they preferred the pink morph. A nonrandom pattern of pollinator visitation in polymorphic populations has been previously reported (e.g., Levin 1969, 1972; Kay 1978; Mogford 1978; Brown and Clegg 1984; Jones and Reithel 2001). In this study, *Bombus bellicosus* was the most frequent pollinator and mostly preferred the pink morph. This flower color polymorphism could be maintained by selection favoring the heterozygote (heterozygosis advance), since this morph exhibited the highest female fitness of the three morphs. Although pollinators visited all flower morphs, the most frequent morph, the pink morph, was the most visited one, and pollinators exhibited the highest constancy for this morph. Levin (1972) suggested that pollinators choose disproportionately the most common floral morph, even if nectar rewards are the same. So, pollinator behavior would lead to common-morph advantage among floral morph, i.e., a positive frequency dependent selection (e.g., Mogford 1978; Eppersson and Clegg 1987). Smithson (2001) predicted a positive correlation between morph frequency and relative fitness of plant population that exhibited flower color polymorphism either in corolla color or in the presence of corolla spot. Considering Kay (1978) hypotheses, our results seem to respond to the first one, i.e., pollinators alternate indiscriminately among morphs and there is an excess of heterozygotes in the population. Through their behavior, pollinators can directly affect the mating pattern among flower morphs by inducing changes in fertility, outcrossing rates, and pollen flow between morphs; such changes would result in variation in reproductive success of the different morphs. The

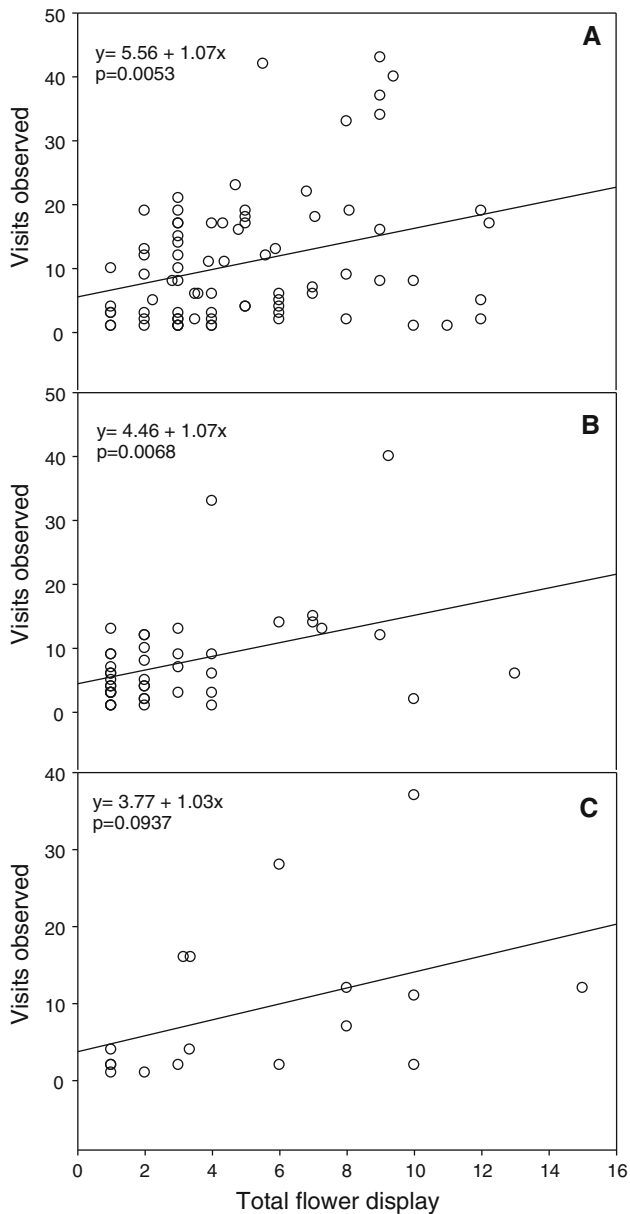


Fig. 3 Relationship between total display size and the pollinator visits observed for the three flower color morphs of a *Cosmos bipinnatus* population. **a** Pink morph. **b** Purple morph. **c** White morph

nonrandom pollinator visitation in flower colors may affect pollinator visitation rates and, in turn, plant reproductive success.

Bees visiting *C. bipinnatus* showed a high foraging constancy for the pink morph and a high inconstancy for the white morph. Previous studies found that bumblebees tend to be constant for a flower morph when inter-flower distances or flight times are short (Gegear and Thomson 2004) and because the pink morph is the most frequent, flight distance between pink capitulum would be shorter than between any capitulum of the other two morphs. Preliminary results obtained in this population indicate that there is a threshold beyond which pollinators change floral morph on their way,

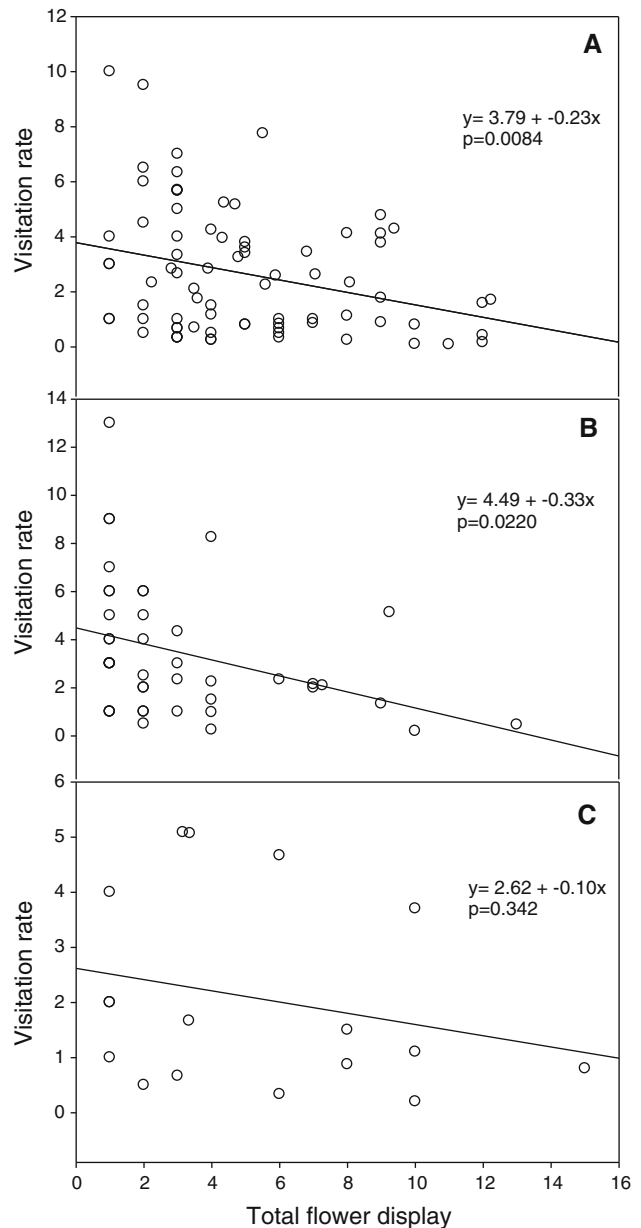


Fig. 4 Relationship between total display size and pollinator visitation rate for the three flower color morphs of a *Cosmos bipinnatus* population. **a** Pink morph. **b** Purple morph. **c** White morph

probably because the flowers of a certain morph become non-rewarding. This was already observed in other species in which pollinators learn to avoid a flower morph when it is not rewarding any more (Smithson and Macnair 1997).

In this *C. bipinnatus* population, of the three morphs, the pink morph is the one that receives the highest number of visits and the white morph has the longest bout length. These results showed consequences in female fitness, which were reflected in each morph: the pink morph presented the highest seed number and the white morph the lowest one. This means that a high bout length does not translate into a high seed number per

Table 5 Results of the linear regression ($\beta \pm \text{SE}$) between floral display size and bout length (number of capitulum probed during a single plant visit) and the proportion of flowers visited (bout length divided by floral display size)

Flower and fitness traits	Flower color morphs		
	Pink $\beta \pm \text{SE}$	Purple $\beta \pm \text{SE}$	White $\beta \pm \text{SE}$
Bout length	0.54 \pm 0.18**	0.96 \pm 0.31***	0.45 \pm 0.18*
Proportion of flowers visited	-7.49 \pm 0.36***	-6.19 \pm 0.34***	-9.36 \pm 0.09***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

capitulum. Pollinators visiting *C. bipinnatus* probed more capitulum from plants with large total display sizes but left a great proportion of them unvisited. Empirical evidences support that bout lengths usually increase with plant size, but this is often accompanied by a decrease in the proportion of flowers visited (references in Mitchell et al. 2004). This tendency has been repeatedly observed in other plant-pollinator systems (e.g., Ohashi and Yahara 1998, 2001, 2002; Goulson 2000; Mitchell et al. 2004; Grindeland et al. 2005). Large displays can maximize the benefits of their attractiveness (maximal pollen export) when visitation sequences are short, whereas small displays maximize pollen export (and partly compensate for their inevitable disadvantage) when a larger fraction of available flowers are visited (Klinkhamer et al. 1994). The optimal fraction of flowers visited per approach therefore decreases with the total number of open flowers available (Iwasa et al. 1995). In most studies, these factors have resulted in a constant relationship between display size and flower visitation rate (e.g., Robertson and Macnair 1995; Ohashi and Yahara 2002; Mitchell et al. 2004), whereas in *C. bipinnatus*, flower visitation rate decreased with increasing total display size. This tendency has also been observed in other plant-pollinator systems (e.g., Ohashi and Yahara 2001; Grindeland et al. 2005). One possible explanation for the lower number of flowers being probed by bees in plants with large floral display than in plants with small floral displays could be that the variation in per flower or per capitulum reward level increases with total display size (Biernaskie and Cartar 2004).

The total pollinator visitation observed showed a positive and significant relation with seeds per capitulum, indicating that the consecutive and non-consecutive visits to the same plant result in high seed number per capitulum; also, because mean bout length in this system is relatively low (1.5 capitulum per plant), in a high number of the visits observed bees may carry foreign pollen, which may increase females fitness (outcross pollen deposition).

The present results show that the area of tubular flowers was positively associated with visitation rate, whereas the area of ligulate flowers did not show a significant association with this variable. Pollinators prefer visiting larger flowers to small ones (e.g., Galen 1989; Campbell 1991; Vaughton and Ramsey 1998) probably because flower size is often associated with reward (e.g.,

Galen 2000; Fenster et al. 2006). In heterogamous capitulum of Asteraceae, reward is located mostly in the radial area, In *C. bipinnatus*, the number of ligulate flowers and therefore the limb area of the tubular flowers vary among plants (Malerba R, 2007, unpublished data), indicating that the area of ligulate flowers is highly variable among plants and pollinators may use this area as a cue for flower reward.

In this study, we examined whether flower color polymorphism influences pollinator preferences and movements within and among plants and fitness-related variables in a plant with a simplified model system with a single-locus floral color polymorphism. In general, we found that pollinators did not ignore variation in flower color polymorphism and foraged nonrandomly with respect to flower color. The most frequent morph, the pink morph, was the most visited and pollinators exhibited the highest constancy for this morph. Moreover, this morph exhibited the highest female fitness suggesting that this polymorphism could be maintained by selection favoring the most common floral morph. Our results stress that bees responded strongly to floral display size, while pollinators probed more capitulum from plants with large total display sizes, they left a great proportion of them unvisited. Furthermore, total pollinator visitation showed a positive relation with female fitness, indicating that the consecutive and non-consecutive visits to the same plant result in high seed number per capitulum.

Acknowledgments We are grateful to M. Calumi who assisted us in fieldwork. We would also thank two anonymous reviewers for their useful comments. JN is fellow researcher from CONICET.

References

- Biernaskie JM, Cartar RV (2004) Variation in rate of nectar production depends on floral display size: a pollinator manipulation hypothesis. *Funct Ecol* 18:125–129
- Brown BA, Clegg MT (1984) Influence of flower color polymorphism on genetic transmission in a natural population of the common morning glory, *Ipomoea purpurea*. *Evolution* 38:796–803
- Campbell DR (1989) Measurements of selection in a hermaphroditic plant: variation in male and female pollination success. *Evolution* 43:318–334
- Campbell DR (1991) Effects of floral traits on sequential components of fitness in *Ipomopsis aggregata*. *Am Nat* 137:713–737
- Crowe LK (1954) Incompatibility in *Cosmos bipinnatus*. *Heredity* 8:1–11

- Engel EC, Irwin RE (2003) Linking pollinator visitation rate and pollen receipt. *Am J Bot* 90:1612–1618
- Fenster CB, Cheely G, Dudash MR, Reynolds RJ (2006) Nectar reward and advertisement in hummingbird-pollinated *Silene virginica* (Caryophyllaceae). *Am J Bot* 93:1800–1807
- Galen C (1989) Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. *Evolution* 43:882–890
- Galen C (2000) High and dry: drought stress, sex-allocation trade-offs, and selection on flower size in the alpine wildflower *Polemonium viscosum* (Polemoniaceae). *Am Nat* 156:72–83
- Gegeer RJ, Thomson JD (2004) Does the flower constancy of bees reflect foraging economics? *Ethology* 110:793–805
- Gemmell NJ, Slate J (2006) Heterozygote advantage for fecundity. *PLoS ONE* 1:e125
- Ghazoul J, Shaanker RU (2004) Sex in space: pollination among spatially isolated plants. *Biotropica* 36:128–130
- Gigord LB, Macnair MR, Smithson A (2001) Negative frequency-dependent selection maintains a dramatic flower color polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soò. *Proc Natl Acad Sci USA* 98:6253–6255
- Goulson D (2000) Why do pollinators visit proportionally fewer flowers in large patches? *Oikos* 91:485–491
- Grindeland JM, Sletvold N, Ims RA (2005) Effects of floral display size and plant density on pollinator visitation rate in a natural population of *Digitalis purpurea*. *Funct Ecol* 19:383–390
- Hannan GL (1981) Flower color polymorphism and pollination biology of *Platystemon californicus* Benth (Papaveraceae). *Am J Bot* 68:233–243
- Harder LD, Barrett SCH (1996) Pollen dispersal and mating patterns in animal-pollinated plants. In: Lloyd DG, Barrett SCH (eds) *Floral biology: studies on floral evolution in animal-pollinated plants*. Chapman and Hall, New York, pp 140–190
- Hodges CM (1985) Bumblebee foraging: the threshold departure rule. *Ecology* 66:179–187
- Holland JN, DeAngelis DL, Bronstein JL (2002) Population dynamics and mutualism: functional responses of benefits and costs. *Am Nat* 159:231–244
- Howlett BJ, Knox RB, Paxton JD, Heslop-Harrison J (1975) Pollen-wall proteins: physicochemical characterization and role in self-incompatibility in *Cosmos bipinnatus*. *Proc R Soc Lond B* 188:167–182
- Iwasa Y, de Jong TJ, Klinkhamer PGL (1995) Why pollinators visit only a fraction of the open flowers on a plant: the plant's point of view. *J Evol Biol* 8:439–453
- Johnston MO (1991) Natural selection on floral traits in two species of *Lobelia* with different pollinators. *Evolution* 45:1468–1479
- Jones KN, Reithel JS (2001) Pollinator-mediated selection on a flower color polymorphism in experimental populations of *Antirrhinum* (Scrophulariaceae). *Am J Bot* 88:447–454
- Kalmus H (1945) Adaptive and selective responses of a population of *Drosophila melanogaster* containing e and e+ to differences in temperature, humidity, and to selection for development speed. *J Genet* 47:58–63
- Kay QO (1978) The role of preferential and assortative pollination in the maintenance of flower colour polymorphisms. In: Richards AJ (ed) *The pollination of flowers by insects*. Academic Press, London, pp 175–190
- Klinkhamer PGL, de Jong TJ (1990) Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Boraginaceae). *Oikos* 57:399–405
- Klinkhamer PGL, de Jong TJ, de Bruyn GJ (1989) Plant size and pollinator visitation in *Cynoglossum officinale*. *Oikos* 54:201–204
- Klinkhamer PGL, de Jong TJ, Metz JAJ (1994) Why plants can be too attractive: a discussion of measures to estimate male fitness. *J Ecol* 82:191–194
- Levin DA (1969) The effect of corolla color and outline on inter-specific pollen flow in *Phlox*. *Evolution* 23:444–445
- Levin DA (1972) Low frequency disadvantage in the exploitation of pollinators by corolla variants in *Phlox*. *Am Nat* 106:453–460
- Maron JL, Crone E (2006) Herbivory: effects on plant abundance, distribution and population growth. *Proc R Soc Lond B* 273:2575–2584
- Medel R, Botto-Mahan C, Kalin-Arroyo M (2003) Pollinator-mediated selection on the nectar guide phenotype in the Andean monkey flower, *Mimulus luteus*. *Ecology* 84:1721–1732
- Mitchell RJ (1993) Adaptive significance of *Ipomopsis aggregata* nectar production: observation and experiment in the field. *Evolution* 47:25–35
- Mitchell RJ, Karron JD, Holmquist KG, Bell JM (2004) The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. *Funct Ecol* 18:116–124
- Mogford DJ (1978) Pollination and flower color polymorphism, with special reference to *Cirsium palustre*. In: Richards AJ (ed) *The pollination of flowers by insects*. Academic Press, London, pp 191–199
- Nattero J, Cocucci A, Medel R (2010) Pollinator-mediated selection in a specialized pollination system: matches and mismatches across populations. *J Evol Biol* 23:1957–1968
- Ohashi K, Yahara T (1998) Effects of variation in flower number on pollinator visits in *Cirsium purpuratum* (Asteraceae). *Am J Bot* 5:219–224
- Ohashi K, Yahara T (1999) How long to stay on, and how often to visit a flowering plant? A model for foraging strategy when floral displays vary in size. *Oikos* 86:386–392
- Ohashi K, Yahara T (2001) Behavioral responses of pollinators to variation in floral display size and their influences on the evolution of floral traits. In: Chittka L, Thomson JD (eds) *Cognitive ecology of pollinators*. Cambridge University Press, New York, pp 274–296
- Ohashi K, Yahara T (2002) Visit larger displays but probe proportionally fewer flowers: counterintuitive behaviour of nectar-collecting bumble bees achieves an ideal free distribution. *Funct Ecol* 16:492–503
- Proctor M, Yeo P (1973) *The pollination of flowers*. Ed Collins, London
- Real LA, Rathcke BJ (1991) Individual variation in nectar production and its effect on fitness in *Kalmia latifolia*. *Ecology* 72:149–155
- Robertson AW, Macnair MR (1995) The effects of floral display size on pollinator service to individual flowers of *Myosotis* and *Mimulus*. *Oikos* 72:106–114
- Smithson A (2001) Pollinator preference, frequency dependence, and floral evolution. In: Chittka L, Thomson JD (eds) *Cognitive ecology of pollination*. Cambridge University Press, New York, pp 237–258
- Smithson A, Macnair MR (1997) Frequency-dependent selection by pollinators: mechanisms and consequences with regard to behaviour of bumblebees *Bombus terrestris* (L.) (Hymenoptera, Apidae). *J Evol Biol* 9:571–588
- Stanton ML, Snow AA, Handel SN, Bereczky J (1989) The impact of flower-color polymorphism on matting patterns in experimental population of wild radish (*Raphanus ristrum* L.). *Evolution* 43:335–346
- Vaughton G, Ramsey M (1998) Floral display, pollinator visitation and reproductive success in the dioecious perennial herb *Wurmbea dioica* (Liliaceae). *Oecologia* 115:93–101
- Waser NM (1986) Flower constancy: definition, cause and measurement. *Am Nat* 127:593–603
- Waser NM, Price MV (1981) Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. *Evolution* 35:376–390
- Wilson P, Thomson JD (1991) Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology* 72:1503–1507