

Large and abundant flowers increase indirect costs of corollas: a study of coflowering sympatric Mediterranean species of contrasting flower size

Alberto L. Teixido & Fernando Valladares

Oecologia

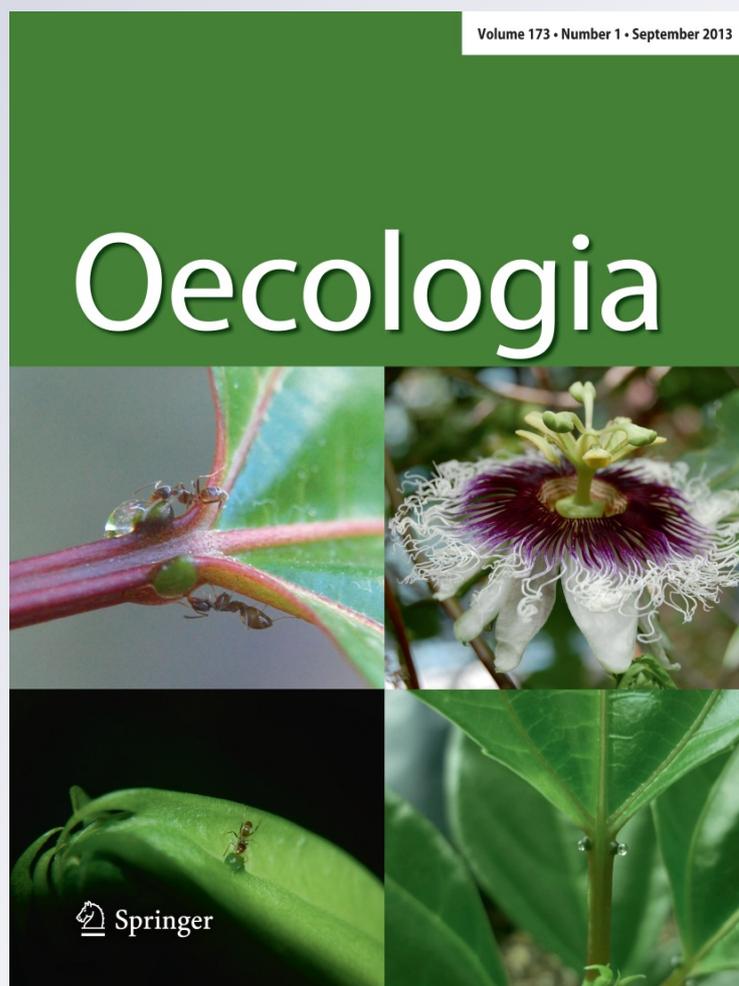
ISSN 0029-8549

Volume 173

Number 1

Oecologia (2013) 173:73-81

DOI 10.1007/s00442-013-2609-8



Your article is protected by copyright and all rights are held exclusively by Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your article, please use the accepted manuscript version for posting on your own website. You may further deposit the accepted manuscript version in any repository, provided it is only made publicly available 12 months after official publication or later and provided acknowledgement is given to the original source of publication and a link is inserted to the published article on Springer's website. The link must be accompanied by the following text: "The final publication is available at link.springer.com".

Large and abundant flowers increase indirect costs of corollas: a study of coflowering sympatric Mediterranean species of contrasting flower size

Alberto L. Teixido · Fernando Valladares

Received: 15 May 2012 / Accepted: 29 January 2013 / Published online: 13 February 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract Large floral displays receive more pollinator visits but involve higher production and maintenance costs. This can result in indirect costs which may negatively affect functions like reproductive output. In this study, we explored the relationship between floral display and indirect costs in two pairs of coflowering sympatric Mediterranean *Cistus* of contrasting flower size. We hypothesized that: (1) corolla production entails direct costs in dry mass, N and P, (2) corollas entail significant indirect costs in terms of fruit set and seed production, (3) indirect costs increase with floral display, (4) indirect costs are greater in larger-flowered sympatric species, and (5) local climatic conditions influence indirect costs. We compared fruit set and seed production of petal-removed flowers and unmanipulated control flowers and evaluated the influence of mean flower number and mean flower size on relative fruit and seed gain of petal-removed and control flowers. Fruit set and seed production were significantly higher in petal-removed flowers in all the studied species. A positive relationship was found between relative fruit gain and mean individual flower size within species. In one pair of species, fruit gain was higher in the large-flowered species, as was the correlation between fruit gain and mean number

of open flowers. In the other pair, the correlation between fruit gain and mean flower size was also higher in the large-flowered species. These results reveal that Mediterranean environments impose significant constraints on floral display, counteracting advantages of large flowers from the pollination point of view with increased indirect costs of such flowers.

Keywords *Cistus* · Floral display · Fruit production · Petal removal · Relative gain of fruits

Introduction

Pollinator attractiveness plays a decisive role in the reproductive ecology of entomophilous plants. Large floral display (i.e., number and size of open flowers) has been broadly associated with greater pollinator attraction and, thus, an increase in both male and female fitness (Stanton et al. 1986; Young and Stanton 1990; Kudoh and Wigham 1998; Thompson 2001; Harder and Johnson 2005; Arista and Ortiz 2007). Many studies have found pollinator-mediated phenotypic selection towards large floral displays (Galen 1989; Herrera 1993; Conner and Rush 1997; Hodgins and Barrett 2008; Nattero et al. 2010). However, the production of larger displays requires greater investment in biomass and water (Galen 1999; Halpern et al. 2010), and maintaining them is more costly due to high respiration and transpiration rates (Vemmos and Goldwin 1994; Galen et al. 1999; Galen 2000). In some cases, the size and number of flowers may be limited by abiotic factors such as water stress (Galen 1999; Caruso 2006).

From a resource economy perspective, the resources invested in floral structures, such as carbon, nutrients and water, can be considered direct costs (Chapin 1989;

Communicated by Christina Caruso.

A. L. Teixido (✉) · F. Valladares
Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, Tulipán s/n, Móstoles, 28933 Madrid, Spain
e-mail: alberto.teixido@urjc.es

F. Valladares
Museo Nacional de Ciencias Naturales, Consejo Superior de Investigaciones Científicas, MNCN-CSIC, Serrano 115 dpdo, 28006 Madrid, Spain

Ashman and Schoen 1997). Since these resources occur in limited amounts in natural populations and constrain plant development, survival and reproduction, differential resource allocation is often found among plant functions or structures (Chapin 1989). Therefore, a fundamental tenet of plant resource economy is that direct costs translate into indirect costs (Chapin 1989), i.e., negative effects on floral functions other than pollinator attractiveness, such as reproductive output. Indirect costs have been shown for nectar production (Pyke 1991) and floral longevity (Ashman and Schoen 1997; Castro et al. 2008). Andersson (1999, 2000, 2001, 2005), who was a pioneer in the assessment of the indirect costs of floral attractiveness, reported that fruit and seed production increased significantly in perianth/ray removal treatments compared to non-manipulated control plants under controlled garden conditions. However, whether these indirect costs are dependent on floral display remains to be explored. If indirect costs were found to increase with floral display, dry mass and water investment-based arguments to explain the potential advantages of smaller displays under stressful conditions would be supported (Galen 1999; Caruso 2006).

The objective of this study is to evaluate the indirect costs of floral display in two pairs of coflowering sympatric species of *Cistus* differing in flower size and to explore the influence of climatic conditions on these costs. *Cistus* is a large-flowered genus widely distributed in Mediterranean ecosystems (Arrington and Kubitzki 2003). We chose this study system due to the interspecific differences in fitness and floral physiological costs that these coflowering sympatric species of contrasting flower size may present. Differences in flower size in sympatric species contribute to differential pollinator visitation rates and pollen transfer (Aigner 2005; Vespineri and Pacini 2010; see also Bosch 1992 for *Cistus*) and may decrease the reproductive success of smaller-flowered species (reviewed by Jones 2001). However, floral costs may be lower in smaller-flowered species, allowing them to allocate greater resources to reproductive output. In Mediterranean ecosystems, high temperatures and water shortages constrain plant reproduction (Larcher 2000; Thompson 2005; Aragón et al. 2008). Under such stressful conditions, large flowers are expected to have higher maintenance costs (Galen 2000, 2005; Elle and Hare 2002). We specifically evaluated the direct costs of corolla production and indirect costs of corollas at three different levels: at the individual level (between flowers whose petals had been removed and non-manipulated control flowers), within populations (between plants differing in floral display), and between populations (between coflowering sympatric species of contrasting flower size).

Specifically, we hypothesized that: (1) a large amount of resources in terms of dry mass, N and P is directly

allocated to corollas relative to the other floral structures; (2) corollas entail sizeable indirect costs in terms of fruit set and seed production; (3) indirect costs increase with flower size; (4) indirect costs increase with the number of open flowers; (5) indirect costs differ between each of the coflowering sympatric pairs of species of contrasting flower size; and (6) local climatic conditions influence indirect costs.

Materials and methods

Species and study area

Cistus (Cistaceae) is one of the most characteristic genera of the Mediterranean flora as part of the shrub layer of sclerophyllous forests. Flowers, which have five pink or white petals of 2–12 cm diameter, are hermaphrodite, self-incompatible and last for 1 day (Bosch 1992; Herrera 1992; Muñoz-Garmendía and Navarro 1993). Flowers open synchronously within populations each day. Fruits are globular, woody 4- to 15-mm capsules containing numerous seeds (approx. range: 100–1,000) which are 1–2 mm long (Muñoz-Garmendía and Navarro 1993). We chose two pairs of coflowering sympatric *Cistus* species differing in flower size in two different ecological ranges in central Spain. The first pair of species was composed of *C. albidus* and *C. ladanifer* (hereafter, *C. albidus*–*C. ladanifer*; mean flower size \pm SD: 5.30 ± 0.49 vs. 9.19 ± 0.77 cm, respectively, $n = 250$ flowers per species). This pair of species was monitored in San Agustín de Guadalix (hereafter, lower site: 740 m; $40^{\circ}41'10''N$, $3^{\circ}36'00''W$) in April 2011. This area has limestone and gypsum soil and is covered by open scrubland vegetation on a south-facing slope. The climate is dry, with an annual mean precipitation and temperature of 567 mm and $13^{\circ}C$, respectively (Ninyerola et al. 2005; $n = 20$ years). The second pair of species was composed of *C. laurifolius* and *C. ladanifer* (hereafter, *C. laurifolius*–*C. ladanifer*; mean flower size \pm SD: 5.95 ± 0.46 vs. 9.15 ± 0.71 cm, respectively, $n = 250$ flowers per species). This pair of species was monitored in Puerto de Canencia (hereafter, higher site: 1,300 m; $40^{\circ}49'20''N$, $3^{\circ}45'50''W$) in June 2011. This area has granite soil and is covered by a sparse wood on a south-facing slope. The climate is subhumid with an annual mean precipitation and temperature of 867 mm and $9^{\circ}C$, respectively (Ninyerola et al. 2005; $n = 20$ years).

Resource allocation to corollas

During the flowering peak of each species, 15 similar-sized plants were haphazardly selected at each site. *C. ladanifer* was only monitored at the lower site. Two flower buds

were randomly harvested from each plant and placed in 70 % ethanol. In the laboratory, each flower was divided into pedicel, sepals, petals, carpels and stamens. All portions were oven-dried for 2 days at 60 °C and weighed to the nearest 0.1 mg with a microbalance (MX5; Mettler-Toledo International, Greifensee, Switzerland). To determine N and P content, 15 corollas and calyces were randomly selected per species. When necessary, the pedicels and sexual structures were pooled to obtain a weight ≥ 2.5 mg. They were then digested with sulphuric acid and analysed for total N and P content using a SKALAR San++ Analyzer (Skalar, Breda, The Netherlands).

Experimental design

During the coflowering peak, another set of ten similar-sized plants of each species was randomly selected and tagged at each site. Every day, we selected ten flowers on each of the previously selected plants, removed the petals on five flowers (petal-removed flowers) and left the other five flowers unmanipulated as controls (control flowers). A total of 25 petal-removed and 25 control flowers were randomly selected and tagged on each plant (i.e., 50 flowers per plant). Petal removal was carried out at pre-dawn using a pair of tweezers on flowers whose petals were fully developed. All petal-removed and control flowers were hand-pollinated to avoid differences in pollen receipt between treatments. Hand-pollination was carried out with a fresh pollen mixture collected from five different individuals within a 5-m radius of the recipient flower. Out-cross pollen was deposited on the stigma with a paintbrush 3 h after anthesis to ensure stigmatic receptivity (Herrera 1992). Flower diameter of control flowers was recorded (to the nearest centimeter) as well as the total number of open flowers on each plant each day. In early July, all ripe fruits were picked before seed dispersal, and fruit set (%) was calculated for petal-removed and control flowers and per plant as the number of fruits set over the total number of tagged flowers. Ten fruits of both petal-removed and control flowers were randomly selected per plant to record the mean number of seeds per fruit.

Statistical analysis

To determine the significance of indirect costs of corollas in terms of fruit set, we fitted Generalized Linear Mixed Models (GLMMs) considering species (fixed factor), plant (random factor nested within species), and petal removal (fixed factor) as independent variables, and the interaction species \times petal removal for each pair of species. Significant interaction indicates a different effect of petal removal on fruit set between coflowering species. We assumed a binomial error distribution with a logit link function and

analysed the model using restricted maximum likelihood (REML) estimation. In GLMMs, the response variable, which is a (link) function from the original data, is a linear combination of fixed and random factors (Littell et al. 1996). Since our data set had a nested structure and our experimental units (flowers) were not fully independent, we considered variation within plants by including plant within species as a random factor in our model. In this way, we included a statistical control of this source of variation. Since our model is a mixed one, by including the term plant nested in species we are partialling out this effect and then we can focus on the variation of interest: species variation, petal removal variation and the interaction between these two fixed predictors. Additionally, since the assignment of the number of degrees of freedom of residuals is critical when one faces a complex experimental design we followed Satterthwaite's method to determine the number of degrees of freedom of residuals (Littell et al. 1996; Quinn and Keough 2002).

To determine the significance of indirect costs of corollas in terms of mean seed number per fruit, we fitted General-linear model ANOVAs, considering species (fixed factor), plant (random factor nested within species), and petal removal (fixed factor) as independent variables, and the interaction species \times petal removal for each pair of species. Significant interaction indicates a different effect of petal removal on seed number between coflowering species.

We calculated the relative gain (RG_i) of fruits and seeds for petal-removed and control flowers for each plant of each species using the formula:

$$RG_i = (\text{petal-removed}_i - \text{control}_i) / \text{control}_i$$

where petal-removed_i and control_i are fruit set and mean seed number per fruit of plant i in petal-removed and control flowers, respectively. To determine whether indirect costs increase with floral display and whether these costs differ significantly between sympatric species of contrasting flower size, we assessed the effect of species and floral display on the relative gain of fruits and seeds by conducting two ANCOVAs for each pair of species, where the dependent variables were relative gain of fruits and relative gain of seeds, respectively. We included species (fixed factor), flower size and number of open flowers (covariates), and the interactions species \times flower size and species \times number of open flowers. Significant interactions indicate differences in regression slopes between relative gain of fruits and/or seeds with mean individual flower size and/or number of open flowers between coflowering species.

To determine the effect of local climatic conditions on the indirect costs of corolla production in the two *C. ladanifer* populations, we tested the effects of population (fixed factor), plant (random factor nested within populations), petal removal (fixed factor) and the interaction

population × petal removal on fruit set and mean seed number. We then assessed the effect of population and floral display on the relative gain of fruits and seeds by conducting two additional ANCOVAs, where the dependent variables were relative gain of fruits and relative gain of seeds, respectively. We included population (fixed factor), flower size and number of open flowers (covariates), and the interactions population × flower size and population × number of open flowers.

GLMMs were performed using the GLIMMIX Macro of SAS (SAS Statistical Package 1990; SAS Institute, Cary, NC, USA). ANOVAs and ANCOVAs were performed with R (R Development Core Team 2010).

Results

Resource allocation to corollas

Resource allocation to corollas differed among the studied species (Table 1). In *C. ladanifer*, the production of large showy corollas required a high resource investment, which was up to five times greater than that found in the other *Cistus* species. Thus, floral attractiveness is more costly in terms of C, N and P in this species. However, *C. ladanifer* corollas had lower N and P concentrations than the other two *Cistus* species. Overall, the studied species invested approximately 27 % of their floral biomass, 23 % of N and 24 % of P in corolla production.

Effect of petal removal on fruit set and seed production

Fruit set differed significantly between petal-removed and control flowers and between plants in both pairs of species (Table 2). In all cases, fruit set was significantly higher in petal-removed flowers (Fig. 1a, b). Seed production was significantly greater in the large-flowered species and differed between plants within species (Fig. 1c, d; Table 3). Mean seed number was also greater for petal-removed flowers in all species. Differences in mean seed number between petal-removed and control flowers were only significant in *C. laurifolius*–*C. ladanifer* (Table 3), but the

increase in seed production was similar for petal-removed flowers in both species (Species × Petal removal was non-significant, Table 3). Regarding the relative gain index (RG_i), all species showed a mean gain in fruit set and seed number for petal-removed relative to control flowers (range: 0.11 ± 0.07–0.33 ± 0.20 for fruit set and 0.10 ± 0.07–0.16 ± 0.12 for seed number). In the specific analysis of *C. ladanifer*, fruit set was significantly different between petal-removed and control flowers and between plants with differences increasing at the higher site (mean 80.8 vs. 91.6 %, lower vs higher site, respectively). However, differences between control and petal-removed flowers were similar between populations (Table 4). Seed production was significantly different among plants with differences decreasing at the higher site (mean 978.4 vs. 655.8, lower vs. higher site, respectively). Mean seed number was not significantly affected by petal removal (Table 4).

Effects of floral display on fruit set and seed production

Large-flowered species tended to have greater fruit set in petal-removed compared to control flowers (15 vs. 22 % for *C. albidus*–*C. ladanifer* and 9 vs. 12 % for *C. laurifolius*–*C. ladanifer*), but these differences were only significant in *C. albidus*–*C. ladanifer* (Species × petal removal marginally significant, Table 2). The relative gain index (RG_i) of fruits had a significant positive relationship with mean flower size (Table 5). In *C. albidus*–*C. ladanifer*, species had a significant effect on fruit set, with a relative gain of fruits of up to 66 % in the larger-flowered species. In *C. laurifolius*–*C. ladanifer*, the larger-flowered species had a higher slope in the relationship between relative gain of fruits and flower size (Species × flower size significant, Table 5; Fig. 2a). Number of flowers did not significantly affect relative gain of fruits in any case. However, in *C. albidus*–*C. ladanifer*, the species with a greater number of open flowers had a higher slope in the relationship between relative gain of fruits and flower number (Species × no. of flowers significant, Table 5; Fig. 2b). With regard to seed production, the relative gain index was similar in all the studied species. In the analysis of *C. ladanifer*, flower size was similar between populations

Table 1 Species differences in diameter $\bar{x} \pm SD$ ($n = 250$ flowers per species and site) and resource allocation to corollas (% of the floral biomass) in terms of dry mass, nitrogen and phosphorous ($n = 15$ flowers) in the three studied *Cistus* species

Species	Site	Diameter (cm)	Dry mass (mg)	Nitrogen (μg)	Phosphorous (μg)	[Nitrogen]	[Phosphorous]
<i>C. albidus</i>	–	5.30 ± 0.49	14.9 ± 2.8 (20.9)	242.7 ± 4.6 (18.4)	14.5 ± 1.0 (19.7)	116.3 ± 2.2	3.1 ± 0.2
<i>C. ladanifer</i>	Lower	9.19 ± 0.77	65.1 ± 8.0 (35.3)	896.0 ± 391.3 (27.8)	57.3 ± 35.2 (28.6)	98.3 ± 42.9	2.8 ± 1.7
<i>C. ladanifer</i>	High	9.15 ± 0.71	–	–	–	–	–
<i>C. laurifolius</i>	–	5.95 ± 0.46	13.2 ± 2.3 (27.0)	213.1 ± 50.2 (22.8)	11.9 ± 3.4 (25.2)	115.3 ± 27.2	2.9 ± 0.8

Percentage (%) of allocated resources is in parentheses. Concentrations are given in mmol/g dry mass. Allocation of resources to corollas in *C. ladanifer* was only quantified in one population

Table 2 Generalized linear mixed model for fruit set

Effect	<i>C. albidus</i> – <i>C. ladanifer</i>				<i>C. laurifolius</i> – <i>C. ladanifer</i>			
	<i>df</i>	Estimate ± SD	Test value	<i>P</i>	<i>df</i>	Estimate ± SD	Test value	<i>P</i>
Random plant (species)	19, 978	0.589 ± 0.234	2.33	0.010	19, 978	0.502 ± 0.240	2.09	0.018
Fixed species	1, 18	0.136 ± 0.412	0.14	0.731	1, 18	0.056 ± 0.404	0.12	0.756
Petal removal	1, 978	1.248 ± 0.231	33.82	<0.001	1, 978	1.425 ± 0.350	27.79	<0.001
Species × petal removal	1, 978	−0.738 ± 0.265	3.48	0.051	1, 978	−0.408 ± 0.463	0.78	0.379

Plant (species), as random factor, was tested with Wald *z* test, and fixed factors with Type III *f* tests

Fig. 1 Comparison of **a, b** fruit set and **c, d** mean seed number of control and petal-removed flowers and coflowering species. Values are mean ± SE (*n* = 250 for fruit set; *n* = 100 for mean seed number)

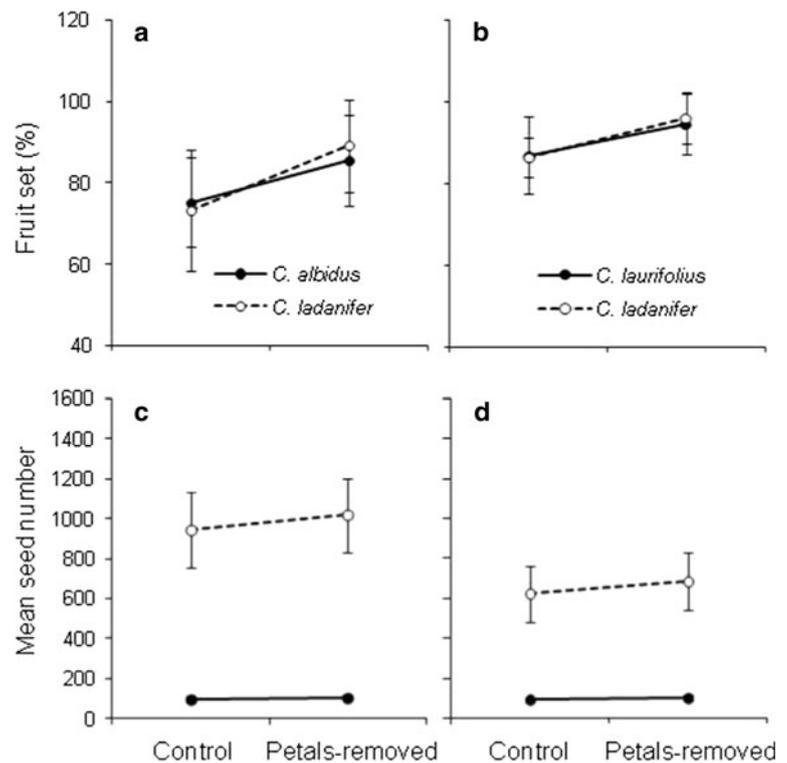


Table 3 Results of GLM for effect of species, plant and petal removal on mean seed number in each pair of sympatric species

Effect	<i>C. albidus</i> – <i>C. ladanifer</i>				<i>C. laurifolius</i> – <i>C. ladanifer</i>			
	<i>df</i>	MS	<i>F</i>	<i>P</i>	<i>df</i>	MS	<i>F</i>	<i>P</i>
Species	1, 18	4.42 × 10 ⁷	296.05	<0.001	1, 18	1.95 × 10 ⁷	222.36	<0.001
Plant (species)	19, 349	1.73 × 10 ⁵	17.94	<0.001	19, 349	9.38 × 10 ⁴	12.80	<0.001
Petal removal	1, 349	9.31 × 10 ²	0.10	0.755	1, 349	4.71 × 10 ⁴	6.42	0.012
Species × petal removal	1, 349	5.23 × 10 ³	0.55	0.461	9, 349	2.16 × 10 ⁴	1.95	0.107

Species and petal removal are fixed factors and plant is a random factor. *n* = 200 (100 control flowers and 100 petal-removed flowers)

(*F*_{1,19} = 0.01, *P* = 0.980). The relative gain of fruits was significantly higher at the lower site, which has the most stressful climatic conditions (mean 0.33 vs. 0.12, lower vs. higher site, respectively; Table 6). Relative gain of fruits also increased with flower size, but was similar between populations. Relative gain of seeds was not significantly affected by population, flower size or flower number (Table 6).

Discussion

This study found differences in direct costs of corollas among the three *Cistus* species of contrasting flower size. The largest-flowered species, *C. ladanifer*, allocated the greatest amount of resources to corollas in terms of dry mass, N and P. We also found sizeable indirect costs of

Table 4 Results for effect of population, plant within populations, petal removal and the interaction between population and petal removal on fruit set and mean seed number in *C. ladanifer*

Effect	Fruit set ^a			Mean seed number ^b		
	df	Test value	P	df	F	P
Population	1, 18	12.64	<0.001	1, 18	19.14	<0.001
Plant (population)	19, 978	53.24	<0.001	19, 349	11.87	<0.001
Petal removal	1, 978	33.11	<0.001	1, 349	1.42	0.235
Population × petal removal	1, 978	0.14	0.712	1, 349	6.13	0.348

^a Generalized linear mixed model: the effects of plant (random factor) were tested with Wald z tests, whereas the effects of population, petal removal (fixed factor) and the interaction between population and petal removal were tested with Type III f tests

^b GLM

Table 5 Results of GLM for effects of species, flower size, number of flowers and species × flower size and species × number of flowers interactions on relative gain of fruits and seeds in each pair of sympatric species using ANCOVAs

Effect	RG _i fruits								RG _i seeds							
	<i>C. albidus</i> – <i>C. ladanifer</i>				<i>C. laurifolius</i> – <i>C. ladanifer</i>				<i>C. albidus</i> – <i>C. ladanifer</i>				<i>C. laurifolius</i> – <i>C. ladanifer</i>			
	df	MS	F	P	df	MS	F	P	df	MS	F	P	df	MS	F	P
Species	1, 19	0.06	4.68	0.044	1, 19	0.00	0.39	0.602	1, 19	0.01	1.32	0.301	1, 19	0.02	1.78	0.219
Flower size	1, 19	0.07	7.32	0.012	1, 19	0.03	6.35	0.020	1, 19	0.05	3.26	0.080	1, 19	0.03	3.45	0.079
No. of flowers	1, 19	0.02	1.40	0.162	1, 19	0.00	0.04	0.862	1, 19	0.00	0.01	0.899	1, 19	0.03	3.46	0.079
Species × flower size	1, 19	0.02	1.31	0.215	1, 19	0.01	4.90	0.045	1, 19	0.01	2.45	0.102	1, 19	0.01	1.99	0.206
Species × no. of flowers	1, 19	0.02	5.08	0.042	1, 19	0.00	0.02	0.876	1, 19	0.00	0.04	0.846	1, 19	0.03	3.27	0.086

Species is a fixed factor and flower size and number of flowers are covariates

floral display in terms of fruit production at three different levels. First, we recorded indirect costs of corollas in the three species. We also verified that indirect costs increased with flower size within populations. Third, and most interestingly, we confirmed that indirect costs differed between pairs of species of contrasting flower size. In *C. albidus*–*C. ladanifer*, the indirect costs of fruit production increased in the large-flowered species. The relationship between relative gain of fruits and number of flowers was greater in the species displaying more flowers. In *C. laurifolius*–*C. ladanifer*, small increases in flower size entailed greater increases in relative gain of fruits in the large-flowered species.

The studied species invested smaller amounts of resources to corollas compared to other studies, although the data obtained for large-flowered *C. ladanifer* were similar to those of other large-flowered Mediterranean species. Cruden and Lyon (1985) found that the dry mass of corollas of several xenogamous species was higher than that of autogamous species (ranging from 0.31 to 17.91 mg), and this represented approximately 41 % of all floral biomass, thus being the heaviest structure. Likewise, corollas represented 61 % of floral biomass in the large-flowered self-incompatible tropical tree *Ipomoea wolcottiana* (Parra-Tabla and Bullock 2000). In a study on the large-flowered Mediterranean herbaceous perennial

Paeonia cambessedesii, Méndez and Traveset (2003) found that corollas represented an average of 153 mg of dry mass (31 % of floral biomass), about 2,000 µg of N (19 % of floral biomass) and about 317 µg of P (21 % of floral biomass).

The relevance of indirect costs was dependent on the magnitude of the relative gain of fruits and seeds of petal-removed compared to control flowers. Previous studies have obtained contrasting results. In a ray removal experiment in *Achillea ptarmica*, Andersson (1999) reported a high significant relative gain of fruit set (38 %). However, other studies have obtained non-significant results (Andersson 2000, 2001). The significant relative gain of fruits (11–33 %) and seeds (10–16 %) obtained in our study falls into the lower range recorded in Andersson's studies, while the results obtained for *C. ladanifer* (the largest-flowered species) at the lower site fall into the higher range. Several mechanisms related to resource availability and allocation might be involved in determining the indirect costs of floral display. For example, when the perianth was experimentally removed from *Nigella sativa* plants at the bud stage, a high proportion of biomass and nutrients was reallocated to seed production (Andersson 2005). The corollas of the species used in this study showed remarkable differences in direct costs in terms of floral dry mass (range: 21–35 %; Table 1). Furthermore,

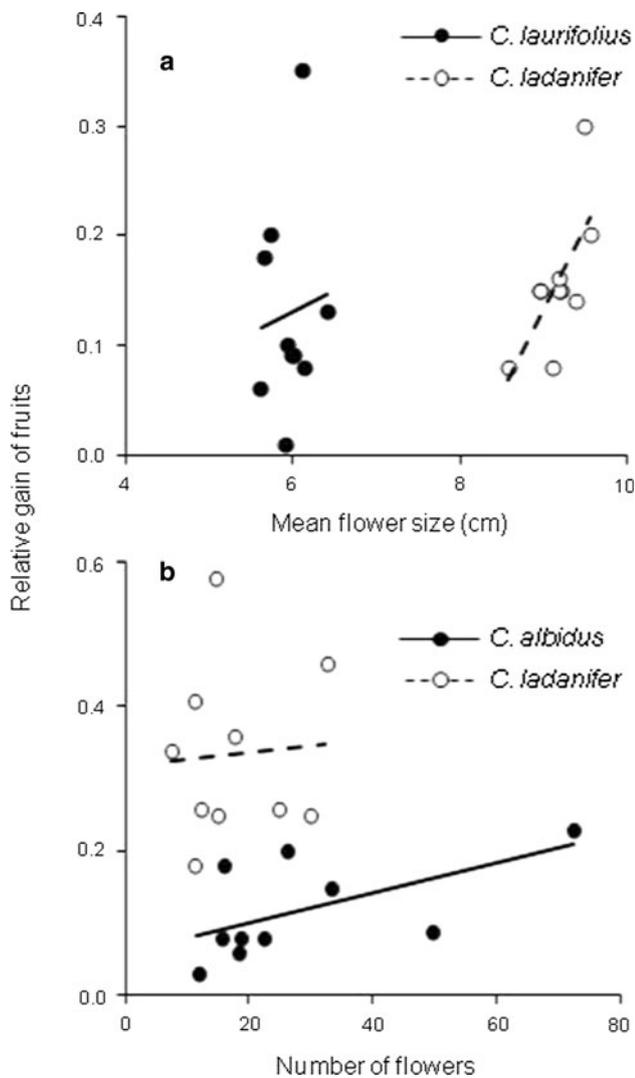


Fig. 2 Relationship between relative gain of fruits and **a** individual mean flower size and **b** individual mean number of open flowers for petal-removed and control flowers

petals differed in their content of floral N (18–28 %) and floral P (20–29 %). However, as we removed fully-developed petals, the reallocation of biomass and nutrients was equally prevented in all species.

Indirect costs of corollas can also be derived from the need for maintenance from resources such as water. Andersson (2000, 2001) found that the water regime did not significantly affect fruit set or seed viability under greenhouse conditions. However, this could be far more relevant in large-flowered plants under hot and dry Mediterranean conditions, which strongly limit reproduction (Thompson 2005; Aragón et al. 2008), as well as fruit and seed production (Konsens et al. 1991; Galen 2000; Fang et al. 2010). Although we do not have information for all the studied species, we know that high temperatures and large flower size increase water costs by transpiration in corollas

Table 6 Results of GLM for effects of population, flower size, number of flowers and population × flower size and population × number of flowers interactions on relative gain of fruits and seeds in *C. ladanifer*

Effect	RG _i fruits			RG _i seeds		
	df	F	P	df	F	P
Population	1, 19	8.59	0.010	1, 19	8.75	0.367
Flower size	1, 19	9.49	0.007	1, 19	3.32	0.087
No. of flowers	1, 19	0.24	0.634	1, 19	0.03	0.857
Population × flower size	1, 19	0.09	0.774	1, 19	0.58	0.464
Population × no. of flowers	1, 19	0.32	0.579	1, 19	0.00	0.992

Population is a fixed factor and flower size and number of flowers are covariates

in *C. albidus* and *C. ladanifer* in our study area. The larger corollas of *C. ladanifer* had significantly higher transpiration rates than those of *C. albidus* (mean ± SD: 64.1 ± 45.6 and 17.6 ± 6.4 g H₂O m⁻² h⁻¹, respectively). Transpiration rates increased significantly at higher temperatures, especially in *C. ladanifer*. Overall, daily flowering involved 7.6 ± 2.4 g H₂O plant⁻¹ day⁻¹ in *C. albidus* versus 56.7 ± 41.7 g H₂O plant⁻¹ day⁻¹ in *C. ladanifer*, indicating that corolla transpiration can be up to 50 % that of leaf transpiration on an organ surface area basis (A.L. Teixido and F. Valladares, unpublished data). This could be the main mechanism marking the differences in indirect costs between these two species. Likewise, although the *C. ladanifer* populations did not differ in flower size, differences in maintenance costs of corollas due to higher water use in the drier and hotter conditions at the lower site may explain the differences in fruit production between populations.

Indirect costs for fruit set increased with flower size, but did not increase for number of seeds in all three studied *Cistus* species. Most interestingly, indirect costs in terms of fruit production were greater in the larger-flowered species in the pair *C. albidus*–*C. ladanifer*. Moreover, the relationship between relative gain of fruits and flower size was also greater in the larger-flowered species in the other co-flowering pair. Contrary to our expectations, number of flowers did not have a significant effect on indirect costs. This is surprising, because number of flowers has been previously associated with a decrease in fruit production due to resource depletion (Montalvo and Ackerman 1987). However, in one pair of species, the relationship between relative gain of fruits and number of open flowers was greater in the species displaying more flowers. Although a correlation between indirect costs and floral display can be expected if resource allocation or transpiration underlie indirect costs of corollas, to our knowledge these

experimental results are novel. In the coflowering pair *C. albidus*–*C. ladanifer*, transpiration rates increased with flower size and were up to three times greater in larger-flowered *C. ladanifer* (A.L. Teixido and F. Valladares, unpublished data). Thus, increases in indirect costs in larger displays and their underlying mechanisms deserve further study.

The pattern of resource investment between attractiveness and offspring could be an important selective factor on plant reproductive function (Ashman and Schoen 1996; Galen 1999, 2005). In particular, the magnitude of fruit and seed gain in petal-removed flowers compared to the investment in attractiveness in control flowers could determine selective pressures on floral display. In the studied *Cistus* species, a higher relative gain of fruits in petal-removed compared to control flowers in larger-flowered individuals suggests relevant selective pressures towards smaller flowers in our study populations. Likewise, a greater relationship between relative gain of fruits and floral display between coflowering *Cistus* species of contrasting flower size could counteract the positive effect of greater pollinator attractiveness in the larger-flowered species. Climate conditions can also play a role in creating differences in flower size by limiting resource allocation between corollas and fruit and seed production. Our analysis of *C. ladanifer* found both lower fruit set and higher relative gain of fruits at the lower site, indicating that drier and hotter conditions in this population may impose constraints on flower size through female fitness. However, we did not detect a significant relative gain in number of seeds with increasing floral display. This may be due to the positive relationship between flower size and ovule number in Cistaceae (Herrera 1992). Another important aspect in assessing fitness effects of indirect costs is compensatory resource reallocation to the remaining seeds. As Andersson (2005) found indirect costs of floral attraction structures in terms of mean seed mass and germination rates, it would be useful to include estimates of seed mass and viability in future studies.

In conclusion, our results support the notion that corollas in *Cistus* imply relevant indirect costs in terms of fruit and seed production. Furthermore, indirect costs increased with mean flower size among individuals within populations and in larger-flowered individuals of coflowering sympatric congeneric species differing in flower size. Hence, since the petals were removed once fully expanded and they did not use resources that would otherwise have been invested in corolla production to set fruits and seeds, we propose that petal-removed flowers increased their reproductive output by saving water and other resources that would have been used for the functional and physiological maintenance of corollas. Minimizing floral water loss by reducing corolla size should be potentially advantageous for plants

living in resource-limited environments (Galen 2000, 2005). The short floral longevity of the three studied species, which would also mitigate maintenance costs, must be relevant in the pattern of resource investment between attractiveness and fitness under stressful conditions (Ashman and Schoen 1996; Galen 2005). In this context, we suggest that a short floral longevity is key to balance pollinator attraction with flowering costs and that indirect costs limit pollinator-mediated phenotypic selection on corolla size through fruit and seed production in large-flowered species inhabiting Mediterranean environments, which may explain the rarity of large flowers in these hot and dry environments. However, more research is needed to quantify the effects of floral display, longevity and maintenance costs on the magnitude of indirect costs and the balance between pollinator attractiveness and indirect costs in coflowering sympatric species of contrasting flower size.

Acknowledgments A. Traveset and J. M. Iriondo provided constructive comments during earlier versions of the manuscript. We also thank to R. Aceves, E. Barahona, F. Castellanos, C. de Castro, J. Iglesias and H. Lantarón for fieldwork support. We are also grateful to Y. Valiñani and E. Galisteo for lab assistance and Lori J. De Hond for linguistic assistance. This study was supported by CONSOLIDER MONTES project (CSD2008-00040) of the Spanish Ministerio de Ciencia e Innovación. A.L.T. held a PDI fellowship at Rey Juan Carlos University, Spain. The experiments comply with the current laws of Spain.

References

- Aigner PA (2005) Variation in pollination performance gradients in a *Dudleya* species complex: can generalization promote floral divergence? *Funct Ecol* 19:681–689
- Andersson S (1999) The cost of floral attractants in *Achillea ptarmica* (Asteraceae): evidence from a ray removal experiment. *Plant Biol* 1:569–572
- Andersson S (2000) The costs of flowers of *Nigella degenii* inferred flower and perianth removal experiments. *Int J Plant Sci* 16:903–908
- Andersson S (2001) Fitness consequences of floral variation in *Senecio jacobaea* (Asteraceae): evidence from a segregating hybrid populations and a resource manipulation experiment. *Biol J Linn Soc* 74:17–24
- Andersson S (2005) Floral costs in *Nigella sativa* (Ranunculaceae): compensatory responses to perianth removal. *Am J Bot* 92:279–283
- Aragón CF, Escudero A, Valladares F (2008) Stress-induced dynamic adjustments of reproduction differentially affect fitness components of a semi-arid plant. *J Ecol* 96:222–229
- Arista M, Ortiz PL (2007) Differential gender selection on floral size: an experimental approach using *Cistus salviifolius*. *J Ecol* 95:973–982
- Arrington JM, Kubitzki K (2003) Cistaceae. In: Kubitzki K, Bayer C, Stevens PF (eds) The families and genera of vascular plants, vol V. Springer, Berlin, pp 62–70
- Ashman TL, Schoen DJ (1996) Floral longevity: fitness consequences and resource costs. In: Lloyd DG, Barrett SCH (eds) Floral

- biology: studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York, pp 112–139
- Ashman TL, Schoen DJ (1997) The cost of floral longevity in *Clarkia tembloriensis*. *Evol Ecol* 11:289–300
- Bosch J (1992) Floral biology and pollinators of three co-occurring *Cistus* species (Cistaceae). *Bot J Linn Soc* 109:39–55
- Caruso CM (2006) Plasticity of inflorescence traits in *Lobellia siphilitica* (Lobeliaceae) in response to soil water availability. *Am J Bot* 93:531–538
- Castro S, Silveira P, Navarro L (2008) Effects on pollination on floral longevity and costs of delaying fertilization in the out-crossing *Polygala vayredae* Costa (Polygalaceae). *Ann Bot* 102:1043–1048
- Chapin FS III (1989) The cost of tundra plant structures: evaluation of concepts and currencies. *Am Nat* 133:1–19
- Conner JK, Rush S (1997) Measurements of selection on floral traits in black mustard, *Brassica nigra*. *J Evol Biol* 10:327–335
- Cruden RW, Lyon DL (1985) Patterns of biomass allocation to male and female functions in plants with different mating system. *Oecologia* 66:299–306
- Elle E, Hare JD (2002) Environmentally induced variation in floral traits affects the mating system in *Datura wrightii*. *Funct Ecol* 16:79–88
- Fang X, Turner NC, Yan G et al (2010) Flower numbers, pod production, pollen viability, and pistil function are reduced and flower and pod abortion increased in chickpea (*Cicer arietinum*L) under terminal drought. *J Exp Bot* 61:335–345
- 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 (1999) Why do flowers vary? The functional ecology of variation in flower size and form within natural plant populations. *Bioscience* 49:631–640
- 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
- Galen C (2005) It never rains but then it pours: the diverse effects of water on flower integrity and function. In: Reekie E, Bazzaz FA (eds) Reproductive allocation in plants. Elsevier, San Diego, pp 77–95
- Galen C, Sherry RA, Carroll AB (1999) Are flowers physiological sinks or faucets? Costs and correlates of water use by flowers of *Polemonium viscosum*. *Oecologia* 118:461–470
- Halpern SL, Adler LS, Wink M (2010) Leaf herbivory and drought stress affect floral attractive and defensive traits in *Nicotiana quadrivalvis*. *Oecologia* 163:961–971
- Harder LD, Johnson SD (2005) Adaptive plasticity of floral display size in animal-pollinated plants. *Proc R Soc Lond B* 272:2651–2657
- Herrera J (1992) Flower variation and breeding systems in the Cistaceae. *Plant Syst Evol* 179:245–255
- Herrera C (1993) Selection on floral morphology and environmental determinants of fecundity in a hawk moth-pollinated violet. *Ecol Monogr* 63:251–275
- Hodgins KA, Barrett SCH (2008) Natural selection on floral traits through male and female function in wild populations of the heterostylous daffodil *Narcissus triandrus*. *Evolution* 62:1751–1763
- Jones KN (2001) Pollinator-mediated assortative mating: causes and consequences. In: Chittka L, Thompson JD (eds) Cognitive ecology of pollination: animal behaviour and floral evolution. Cambridge University Press, Cambridge, pp 259–273
- Konsens I, Ofir M, Kigel J (1991) The effect of temperature on the production and abscission of flowers and pods in snap bean (*Phaseolus vulgaris* L.). *Ann Bot* 67:391–399
- Kudoh H, Wigham DF (1998) The effect of petal size manipulation on pollinator/seed-predator mediated female reproductive success of *Hibiscus moscheutos*. *Oecologia* 117:70–79
- Larcher W (2000) Temperature stress and survival ability of Mediterranean sclerophyllous plants. *Plant Bios* 134:279–295
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) SAS system for mixed models. SAS Institute, New York
- Méndez M, Traveset A (2003) Sexual allocation in single-flowered hermaphroditic individuals in relation to plant and flower size. *Oecologia* 137:69–75
- Montalvo AM, Ackerman JD (1987) Limitations to fruit production in *Ionopsis utricularioides* (Orchidaceae). *Biotropica* 19:24–31
- Muñoz-Garmendía F, Navarro C (1993) Cistaceae. In: Castroviejo S, Aedo C, Gómez-Campo M et al (eds) Flora Iberica. CSIC, Madrid, pp 318–436
- 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
- Ninyerola M, Pons X, Roure JM (2005) Atlas climático digital de la Península Ibérica. Metodología y aplicaciones en bioclimatología y geobotánica. Universidad Autónoma de Barcelona, Barcelona, ISBN 932860-8-7. <http://opengis.uab.es/wms/iberia/>. Accessed 15 Sept 2011
- Parra-Tabla V, Bullock SH (2000) Phenotypic natural selection on flower biomass allocation in the tropical tree *Ipomoea wolcottiana* Rose (Convolvulaceae). *Plant Syst Evol* 221:167–177
- Pyke GH (1991) What does it cost a plant to produce floral nectar? *Nature* 350:58–59
- Quinn G, Keough M (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge
- R Development Core Team 2010 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0. <http://www.R-project.org>
- Stanton ML, Snow AA, Handel SN (1986) Floral evolution: attractiveness to pollinators increases male fitness. *Science* 232:1625–1627
- Thompson JD (2001) How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? *Oecologia* 126:386–394
- Thompson JD (2005) Plant evolution in the Mediterranean. Oxford University Press, New York
- Vemmos SN, Goldwin GK (1994) The photosynthetic activity of Cox's orange pippin apple flowers in relation to fruit setting. *Ann Bot* 73:385–391
- Vespirini JL, Pacini E (2010) Pollination ecology in sympatric winter flowering *Helleborus* (Ranunculaceae). *Flora* 205:627–632
- Young HJ, Stanton ML (1990) Influences of floral variation on pollen removal and seed production in wild radish. *Ecology* 71:536–554