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ORIGINAL ARTICLE

Large flowers tend to be short-lived in Mediterranean ecosystems: Insights from three *Cistus* species

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Abstract

Larger and longer lived flowers receive more pollinators, but may also involve increased water maintenance costs under hot, dry environments. Hence, smaller and/or short-lived flowers may buffer such costs. We surveyed floral longevity in three large-flowered Mediterranean *Cistus* species. We hypothesize that: (1) in *Cistus*, floral longevity decreases with increasing air temperature and flower size; (2) in *C. ladanifer*, flower size and longevity increase along an altitudinal gradient; (3) floral longevity is differentially affected by temperature rather than flower size along the gradient; (4) under similar temperature, floral longevity decreases with flower size. For each species, we evaluated the effects of flower size and air temperature on floral longevity. Specifically, floral longevity was surveyed along an altitudinal gradient in the largest flowered species *Cistus ladanifer*. Floral longevity in *Cistus* species lasted < 1 d and was affected by air temperature, independently of flower size. In *C. ladanifer*, flower size increased along the gradient but floral longevity decreased. Still, floral longevity decreased with increasing air temperature and, to a lesser extent, with flower size. Together, our findings show a triangular relationship among air temperature, flower size and floral longevity with margins for plasticity to accommodate pollinator attraction with the costs of large-flowered Mediterranean plants.

Keywords: Air temperature, altitudinal gradient, *Cistus ladanifer*, floral maintenance costs, floral longevity, flower size

Flower size is closely related to pollinator attraction and, consequently, to reproductive success of plants (Stanton et al. 1986; Kudoh & Wigham 1998; Arista & Ortiz 2007). In agreement with this, a growing body of studies has documented pollinator-mediated phenotypic selection towards larger flowers (e.g. Galen 1989; Conner & Rush 1997; Medel et al. 2003; Hodgins & Barrett 2008). However, large flowers are associated with greater requirements of biomass and water for production of floral structures (Galen 1999; Elle & Hare 2002; Teixido & Valladares 2013), as well as with larger maintenance costs due to high respiration and transpiration rates (Vemmos & Goldwin 1994; Galen et al. 1999; Teixido & Valladares 2014). In this context, Galen (1999) pointed out that a unilateral view of the evolution of flower size from a pollinator perspective was probably over-simplistic and smaller flowered individuals can persist in natural plant populations.

Floral longevity, i.e. the length of time that flowers remain open and functional, favours the

processes of pollen removal and deposition on the flower, so longer lived flowers increase the opportunity for reproductive success (Primack 1985; Ashman & Schoen 1994, 1996). Nevertheless, floral longevity also entails costs (Ashman & Schoen 1997; Castro et al. 2008). From an evolutionary perspective, floral longevity must balance the rates of pollen deposition and removal against floral maintenance costs, so that an optimal floral longevity will tend to minimize costs and to maximize the fitness rates (Ashman & Schoen 1994). Many biotic and abiotic factors have been suggested to affect floral longevity (reviewed by Primack 1985; Ashman & Schoen 1994; Sargent & Roitberg 2000). For example, floral senescence is induced by pollen deposition on stigma (Proctor & Harder 1995; Sargent & Roitberg 2000; Giblin 2005). Likewise, environmental conditions such as altitude and air temperature significantly influence floral longevity, with higher altitudes and lower temperatures increasing flower lifespan (Yasaka et al. 1998; Blionis et al. 2001; Giblin

2005). Floral longevity may also be determined by flower size, with larger flowers lasting shorter periods (van Doorn & Schröder 1995).

However, a major question that remains open is to disentangle the triple association between climate, flower size and longevity. In a classical review, Primack (1985) reported that global ecosystems differ markedly in floral longevity. Shortest lived flowers predominate in both dry and humid tropical forests, whereas longest lifespans correspond to cold and alpine habitats. Relative to flower size, global humidity gradients are the factors controlling this trait, with larger flowers generally occurring in world's wet forests (Endress 1994; Thompson 2005). High temperatures of dry and hot environments cause a significant increase of floral maintenance costs to keep a physiologically optimal temperature, and also, water shortage can prevent this homeostasis by means of an inefficient transpirational cooling (Galen 2005). Acting together, heat and drought can disrupt the normal performance of flowers, affecting fruit and seed production (Konsens et al. 1991; Galen 2000; Aragón et al. 2008; Fang et al. 2010). Given that flower size and longevity also increase the rates of floral costs, the stressful conditions of hot and dry ecosystems may make small, short-lived flowers, potentially advantageous for plants living in these environments (Galen 2000, 2005; Elle & Hare 2002; Teixido & Valladares 2013).

In addition, because flowers also commonly show smaller sizes under hotter and drier sites by local adaptation within species (Barrett et al. 2004; Herrera 2005; Thompson 2005) and floral longevity increases under lower temperatures among populations (Blionis et al. 2001; Giblin 2005), it would be interesting to disentangle whether flower size and air temperature differentially affect floral longevity and what would be the net outcome in a population with the highest temperatures and the smallest flowers. Air temperature and flower size can influence both pollinator availability and floral production and maintenance costs, with important potential effects on floral longevity (Ashman & Schoen 1994, 1996). For example, air temperature and flower size increase pollinator activity (Obeso 1992; Blionis and Vokou 2001; Nattero et al. 2010). Then, as pointed out above, less pollination probability selects for larger flowers to better attract pollinators when there is pollinator limitation and also for longer floral life as probability of fertilization decreases. Following this assumption, longevity of flowers increases with increasing size, where investment in producing flowers is high and pollination probability is low, thus preventing waste of resources invested in floral production (Primack 1985; Ashman & Schoen 1994). In this regard, a negative correlation between both floral traits would simply break down. More-

over, an alternative hypothesis exists about the relationship between flower size and longevity. Thus, larger flowers would tend to last less time open based on the arguments to explain the balance of costs/fitness rates, since flower size increases pollen transfer and floral maintenance costs (Ashman & Schoen 1994, 1996). If floral longevity were found to decrease in larger flowered plants under the same temperature conditions, flower size-based assumptions would be supported.

Mediterranean climates involve high temperatures and water shortages, constraining plant reproduction by speeding up development, shortening flowering duration (Larcher 2000; Thompson 2005; Aragón et al. 2008) and, occasionally, delaying the initiation of flowering until the rainy season to maximize water use efficiency (Blionis et al. 2001; Verdú et al. 2002). Even though a reduced corolla size minimizes water loss under hot and dry conditions (Galen 2000, 2005; Teixido & Valladares 2014), several common Mediterranean species do exhibit large flowers. Most of these species show adaptations to prevent overheating and excessive water loss, such as nocturnal flowering and pollination (Fleming et al. 2001; Rhizopoulou et al. 2006), but little is known about the role of floral longevity.

In this paper, we evaluated the effects of flower size and air temperature on floral longevity in three *Cistus* species (Cistaceae), a common, large-flowered, pollinator generalist and self-incompatible Mediterranean genus (Bosch 1992; Talavera et al. 1993, 2001; Guzmán et al. in press). First, we conducted a study of floral longevity in the three species to assess interspecific variability in this trait. Second, for *Cistus ladanifer* (L.), the largest flowered species (Herrera 1992; Talavera et al. 1993; Guzmán et al. 2011), we carried out an observational survey of floral longevity along an altitudinal gradient. The altitudinal study was intended to reflect a gradient in flower size and air temperature. This gradient may also involve differences in pollinator environment affecting the floral traits of interest. However, we did not record the effect of pollination on floral longevity, pollinator visit rates among individuals or pollinator-mediated phenotypic selection on flower size among populations. Although the effects of the pollination system are, therefore, not included in our hypotheses, the importance of these effects will be addressed in the discussion. Focused on the potential relationship between flower size, floral longevity and temperature, we specifically hypothesize that (1) in the three species, floral longevity decreases with increasing daily mean air temperature and flower size within each population, tending to be shorter in hotter days and in larger flowered individuals; (2) in *C. ladanifer*, flower size and longevity increase along an altitudinal gradient due to decreasing mean air

temperature during the flowering period; (3) although flower size may reduce floral longevity, flower lifespan is differentially affected by mean air temperature during the flowering period, so floral longevity decreases at lower altitude wherein flowers are smaller but climatic conditions are hotter and drier; and (4) under the same daily mean air temperature conditions, floral longevity decreases with flower size.

Materials and methods

Species and study area

Cistus (Cistaceae) is a characteristic genus of shrubs from the Mediterranean area, being typical elements of sclerophyllous forests (Muñoz-Garmendía & Navarro 1993). Flowers have five pink or white petals and range 2–12 cm in diameter (Herrera 1992). Flowers open synchronously within populations each day. Flowering phenology ranges from late March to late June. All species have homogamous and polliniferous flowers with outcrossing breeding systems dependent on pollinators, mainly bees, beetles and flies (Bosch 1992; Herrera 1992; Talavera et al. 1993). Fruits are globular woody 4–15 mm capsules containing numerous seeds (approximate range: 100–1000) which are 1–2 mm long (Muñoz-Garmendía & Navarro 1993). We chose three species of *Cistus* species differing in flower size in different ecological ranges (Table I). Flowers of *Cistus ladanifer* are the largest in the family (Herrera 1992; Talavera et al. 1993; Guzmán et al. 2011).

The study was conducted from March to June of 2009 in a south-north altitudinal gradient from 725 to 1380 m a.s.l. in the Madrid province, central Spain (39°53'–41°09'N, 3°03'–4°34'W). A total of six different sites were used to study floral longevity of the three *Cistus* species (Table I). Madrid province is characterized by a Mediterranean climate with a SE-NW humidity gradient due to altitude. Study sites included dry and subhumid climate with annual average rainfalls ranging approximately 600–

900 mm and mean annual temperatures ranging 9–14°C (Ninyerola et al. 2005, $N = 20$ years).

Floral longevity in Cistus

For the study of floral longevity in the three *Cistus* species, one population per species was selected (Table I). Populations were similar in orientation (south), slope (0–10°) and tree canopy cover (0–10%). Sunny and southern slopes were chosen to remove as much as possible differences in environmental conditions. Ten similar plants were randomly selected and tagged per species. In each plant, 5 flowers per day, adding up to 25 flowers per plant, were randomly selected and tagged during the flowering peak. Every day, we monitored flowers once between 14:00 and 15:00 h and once again between 19:00 and 20:00 h. Pollination is only diurnal, and those flowers that remain open between 19:00 and 20:00 h start closing and reopen at dawn the next morning. Thus, floral longevity was recorded as fraction of days following the next criteria: 0.5 d for petals falling off before 14:00 or 15:00 h of the first day; 1 d when falling off after 15:00 h but before 19:00 or 20:00 h of the first day; 1.5 d for petals falling off before 14:00 or 15:00 of the second day, and so on. It is worth noting that this protocol readily allowed differentiating longevities depending on the fraction of the day. As a general rule, under warm and sunny days suitable for pollinator activity, flowers lasted until mid-day and, only under cloudy, cold and/or rainy days, flowers lengthened their lifespan during the afternoon and/or following days.

We also estimated flower size as diameter of the corolla (to the nearest mm). When a flower remained open several days, the average diameter was used. Air temperature (°C) in each population was also recorded every 15 min during 24 h/day along the study period with data loggers (Pro H8032, Onset Hobo, MA, USA) located 1 m above the ground and in the sun during the daytime. Then, we recorded mean air temperature during the flowering period (as

Table I. Location, ecological data (altitude is given in m a.s.l.), mean flower size (cm \pm SD), mean floral longevity (d \pm SD) and mean air temperature (°C \pm SD) during the flowering period (overall average) of the three *Cistus* species of used in the study.

Species	Location	Altitude	Climate	Flower size/floral longevity	Air temperature
<i>Cistus albidus</i> L.	San Agustín de Guadalix (40°41'N, 3°36'W)	740	Dry 567 mm, 13°C	4.77 \pm 0.52/0.86 \pm 0.31	14.51 \pm 5.98
<i>Cistus ladanifer</i> L.	Monte Valdelatas (40°32'N, 3°41'W)	725	Dry 520 mm, 14°C	7.26 \pm 0.74/0.89 \pm 0.33	14.33 \pm 5.87
<i>Cistus laurifolius</i> L.	Puerto de Canencia (40°49'N, 3°45'W)	1380	Subhumid 867 mm, 9°C	5.89 \pm 0.40/0.60 \pm 0.16	18.21 \pm 4.65

Note: Column of climate also shows the annual mean rainfall (mm) and the annual mean temperature (°C) (Ninyerola et al. 2005, $N = 20$ years). Nomenclature follows Muñoz-Garmendía and Navarro (1993). $N = 100$ flowers for all species.

overall average) and related floral longevity to daily mean air temperature at each population and species. Because temperature is associated with altitude and is not an independent factor in this study, it may be confounded with site and/or species. We are also aware that each species was represented by one site only. Therefore, temperature cannot be partitioned from the effect of species or site, and we did not make any interpretation on temperature as a factor affecting floral longevity among species.

Floral longevity of Cistus ladanifer along an altitudinal gradient

For *C. ladanifer*, four populations covering its altitudinal range in central Spain were chosen (Table II). This range was suitable to reproduce a gradient of temperature and also a gradient in flower size. Populations were selected following the same criteria as for the other *Cistus* regarding orientation, slope and tree canopy cover. At each population, 10 similar plants > 1 m high and with more than 75 flower buds were randomly selected and tagged before the beginning of the flowering in a plot of 20 m × 20 m. For each plant, total size – estimated as the volume of an ellipsoid ($4/3 \pi abc$) – onset and duration of flowering were recorded. Plant size averaged $1.03 \pm 0.37 \text{ m}^3$ and was similar among populations (Kruskal–Wallis test, $\chi^2_{3,9} = 1.432$, $p = 0.698$). Flowering duration was the number of days the plant remained in bloom. We divided the flowering duration of each plant into three periods: (1) early, from the flowering beginning until the time at which the plant opened more than 10 flowers per day; (2) peak, estimated as the span at which the plant opened more than 10 flowers per day; and (3) late, from the time at which the plant opened less than 10 flowers per day until the last open flower. At each period, between 8 and 22, 36 and 48, and 9 and 14 flowers, respectively, were randomly selected

and tagged per plant, for a sample of between 63 and 74 flowers per plant and a total sample size of 645 flowers at 725 m, 701 at 940 m, 706 at 1120 m, and 671 at 1300 m (total $N = 2723$ flowers). Flowers were monitored twice daily as pointed out above and floral longevity (fraction of days) and flower size (diameter – to the nearest mm) recorded. Air temperature (°C) in each population and its relationship with flower size and longevity were also recorded as described above.

Statistical analysis

To determine the effect of flower size and air temperature on floral longevity in the three *Cistus* species, we fitted generalized linear mixed models (GLMMs), one for each species, considering plant (random factor), flower size and daily mean air temperature (fixed factors). For all models, we assumed a Poisson error distribution with a logit link function for floral longevity and used the restricted maximum likelihood (REML) estimation.

In the specific study of *C. ladanifer*, we determined whether flower size and floral longevity vary among populations and flowering phenology. We also determined whether floral longevity varies with air temperature and/or flower size along an altitudinal gradient. We tested the effects of population (random factor), plant within population (random factor), flowering beginning, duration and period (fixed factors) on flower size and floral longevity by fitting GLMMs. In the case of floral longevity, flower size and air temperature were included as covariates. We assumed a normal error distribution with an identity link function for flower size and a Poisson error distribution with log canonical link function for floral longevity. For all models, we used the REML, and because our data were unbalanced, we used Satterthwaite's method to determine the approximate denominator degrees of freedom of residuals (Littell

Table II. Location, ecological data (altitude is given in m a.s.l.), mean flower size (cm ± SD), mean floral longevity (d ± SD) and mean air temperature (°C ± SD) during the flowering period (overall average) of study sites of *Cistus ladanifer*.

Study sites	Altitude	Climate	Substrate	Vegetation cover	Flower size/floral longevity/air temperature
Monte Valdelatas (40°32'N, 3°41'W)	725	Dry 520 mm, 14°C	Clay and sand	<i>Quercus ilex</i> and <i>Pinus pinea</i> interspersed in a shrub matrix	7.03 ± 1.34/0.98 ± 0.45/14.23 ± 6.21
La Pedriza (40°44'N, 3°52'W)	940	Subhumid 771 mm, 12°C	Clay and sand	<i>Q. ilex</i> among boulders and rocks	8.40 ± 0.79/1.92 ± 0.86/12.35 ± 6.89
Vista Real (40°44'N, 3°57'W)	1120	Subhumid 820 mm, 11°C	Granite	<i>Juniperus oxycedrus</i> among boulders and rocks	8.12 ± 0.76/0.87 ± 0.21/17.28 ± 6.53
Puerto Canencia (40°50'N, 3°46'W)	1300	Subhumid 865 mm, 9°C	Granite	Sparsely wood with <i>P. sylvestris</i> and <i>Q. pyrenaica</i>	8.56 ± 0.69/0.89 ± 0.23/16.38 ± 6.71

Note: Column of climate also shows the annual mean rainfall (mm) and the annual mean temperature (°C) (Ninyerola et al. 2005, $N = 20$ years).

et al. 1999; Quinn & Keough 2002). Due to the complex structure of the models, the relatively large number of potential explanatory variables and the use of population as a random factor, we did not consider any interaction between fixed factors and population. All GLMMs were performed using the GLIMMIX Macro of SAS (1990).

We subsequently examined whether flower size itself affects floral longevity independently of air temperature. Thus, we tested whether floral longevity decreases in larger flowered plants under the same daily mean air temperature conditions. We analysed differences in floral longevity between the populations of *C. ladanifer* with the most contrasted differences in flower size (i.e. 725 and 1300 m; Table II). Then we chose sunny days with no precipitation with the most frequent similar daily mean air temperature conditions and between both populations during the flowering peak, which included 6 d at 725 m and 5 d at 1300 m. Daily mean air temperature (\pm SD) for these 11 d varied $<1^\circ\text{C}$, ranging between 13.49 ± 7.63 and $14.63 \pm 7.93^\circ\text{C}$, where the total daily mean air temperature was $14.04 \pm 0.40^\circ\text{C}$ (14.08 ± 0.40 and $13.99 \pm 0.43^\circ\text{C}$ at 725 and 1300 m, respectively; non-significant differences in daily mean air temperature between populations; Student's t -test_{1,9} = -0.22, $p = 0.621$). Then, to determine whether floral longevity decreases with flower size, we assessed the influence of daily mean individual flower size on the daily mean individual floral longevity by means of Spearman's rank correlations.

To determine differences in flower size and its percentage of variation in *C. ladanifer* along an altitudinal gradient, we fitted a general linear model ANOVA with populations and plants within populations as random factors. Because flowering period significantly influenced flower size (see Table V), leading to a high variation in flower size within plants (48% of the variance in flower size), we subsequently tested differences in flower size at early, peak and late periods, respectively, by fitting three general linear model ANOVAs with populations and plants within populations as random factors. ANOVAs were performed with R (R Development Core Team 2010).

Results

Floral longevity in three Cistus species

Although floral longevity in the three *Cistus* species differing in flower size ranged 0.5–3 d, mean floral longevity was <1 d for all the species (Table I). We did not detect any significant effect of intraspecific variation in flower size in our survey of floral longevity in any of the three *Cistus* species (Table III).

Table III. GLMM for floral longevity of each species.

Effect	<i>C. albidus</i>			<i>C. ladanifer</i>			<i>C. laurifolius</i>					
	df	Estimate \pm SD	Test value	p	df	Estimate \pm SD	Test value	p	df	Estimate \pm SD	Test value	p
Plant	1, 9	0.06 \pm 0.03	1.66	0.145	1, 9	0.06 \pm 0.02	2.79	0.131	1, 9	0.05 \pm 0.02	1.72	0.138
Flower size	1, 249	-0.01 \pm 0.02	0.98	0.671	1, 249	-0.02 \pm 0.02	1.14	0.520	1, 249	-0.01 \pm 0.02	0.81	0.705
Air temperature	1, 249	-0.24 \pm 0.10	5.41	0.026	1, 249	-0.31 \pm 0.09	6.56	0.021	1, 249	-0.16 \pm 0.07	2.23	0.121

Note: Analysis was based on a GLMM with Poisson errors with log link for floral longevity using REML estimation. Random effect (plant) was tested with Wald Z -tests and fixed effects (flower size and air temperature) with Type III F -tests. Significant p -values are marked in bold. $N = 250$ flowers per species.

We only detected a significant negative effect of daily mean air temperature on floral longevity in *C. albidus* and *C. ladanifer*, but we did not detect any effect in *C. laurifolius*, which flowered under the highest and the more homogeneous temperatures (Tables III; see also Table S1).

Floral longevity of Cistus ladanifer in an altitudinal gradient

Flower size ranged between 4.2 and 10.8 cm and significantly differed among populations, among plants within populations and among flowering periods (Table IV). Mean flower size (\pm SD) increased about 23% with altitude (7.0 \pm 1.1 cm at 725 m vs. 8.6 \pm 0.8 cm at 1300 m; Table II). Most variation in flower size occurred within plants (Table V), but this was caused by a general decrease in flower size from the beginning to the end of the flowering period. Within each flowering period (early, peak or late), most variation in flower size occurred between populations (peak and late) or between plants within population (early) (Table V).

Longevity of individual flowers in *C. ladanifer* ranged from 0.5 to 5 d, though longevities between 4 and 5 d only occurred at 940 m. Differences in floral longevity were significant among populations, among plants within-populations and among flowering periods (Table IV). Floral longevity was not influenced by flower size, but it was influenced by mean air temperature along the altitudinal gradient, significantly decreasing with increasing mean air temperature during the flowering period (Table IV). However, floral longevity did significantly decrease in larger flowered individuals under similar daily mean air temperature ($r_s = 0.63$, $p < 0.001$, $N = 110$; Figure 1).

Discussion

The three *Cistus* species investigated showed a uniformly short (<1 d) floral longevity, especially under the most stressful conditions (i.e. higher temperatures). However, floral longevity showed certain plasticity in response to air temperature. A negative relationship between flower size and

Table IV. GLMMs for flower size and floral longevity (2706 flowers from 40 plants in 4 populations).

Effect	Flower size				Floral longevity			
	df	Estimate \pm SD	Test value	<i>p</i>	df	Estimate \pm SD	Test value	<i>p</i>
Random								
Population	–	0.24 \pm 0.10	2.25	0.012	–	–0.09 \pm 0.02	2.01	0.022
Plant (population)	–	0.18 \pm 0.05	3.87	<0.001	–	0.01 \pm 0.00	2.82	0.002
Fixed								
Flowering beginning	1, 9.9	0.01 \pm 0.02	0.17	0.690	1, 18.4	0.01 \pm 0.00	4.04	0.059
Flowering duration	1, 33.7	–0.06 \pm 0.04	2.59	0.117	1, 35	0.01 \pm 0.00	0.21	0.649
Flowering period	1, 2661	–0.75 \pm 0.04	1344.39	<0.001	1, 2567	–0.07 \pm 0.03	5.60	<0.001
Flower size	–	–	–	–	1, 1474	0.00 \pm 0.01	0.05	0.818
Air temperature	–	–	–	–	1, 151	–0.16 \pm 0.05	6.60	<0.001

Note: Analyses were based on two GLMMs with normal errors with identity link for flower size and with Poisson errors with log link for floral longevity using REML estimation. Random effects were tested with Wald *Z*-tests and fixed effects with Type III *F*-tests. Significant *p*-values are marked in bold.

Table V. Results for all the ANOVAs testing for the effects among populations, within populations and flowering periods on flower size.

Variation measures	Factor	df	MS	<i>F</i>	<i>p</i>	% of variation
Among and within populations	Population	3	297.8	19.9	<0.001	34.68
	Plant (population)	35	14.9	25.5	<0.001	16.94
	Error (within plant)	2665	0.6			48.38
Early period	Population	3	48.7	10.3	<0.001	37.04
	Plant (population)	35	4.9	23.9	<0.001	38.27
	Error (within plant)	525	0.2			24.69
Peak period	Population	3	159.0	15.6	<0.001	38.55
	Plant (population)	35	10.2	32.9	<0.001	25.31
	Error (within plant)	1680	0.3			36.14
Late period	Population	3	96.9	43.9	<0.001	65.19
	Plant (population)	35	2.2	6.7	<0.001	12.59
	Error (within plant)	385	0.3			22.22

Note: Percentage of variation of each factor is also shown. Significant *p*-values are marked in bold.

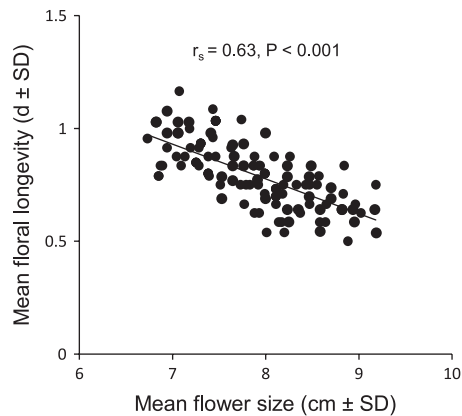


Figure 1. Spearman's rank correlation between mean flower size and mean floral longevity under similar daily mean air temperature between populations at 725 and 1300 m during the flowering peak.

longevity was also found in our study with *C. ladanifer*, confirming the expected effects of flower size and air temperature on floral longevity in our study system. We also found that flower size is smaller under the more xeric conditions between populations, but contrary to our expectations, floral longevity decreased with increasing altitude, i.e. at decreasing mean air temperature of the site during the flowering period. Later in the text, we discuss these findings and their implications for floral ecology and evolution.

Floral longevity of several days are not unusual in Mediterranean ecosystems (Petanidou et al. 1995; Blionis et al. 2001; Berjano et al. 2009). More than 60% of the species in a phryganean Mediterranean ecosystem had average floral longevity higher than 2 d (Petanidou et al. 1995). Nevertheless, floral longevity widely differed between families and Cistaceae in that study also showed floral longevity of approximately 1 d (Petanidou et al. 1995). Phylogenetic constraints could account for this pattern. Several families typically show 1-d flowers (e.g. Commelinaceae, Convolvulaceae, Pontederiaceae: Primack 1985; Acanthaceae: Endress 1994; Turneraceae: Arbo 2007). Alternatively, low floral longevity found for *Cistus* in our study could be due to climatic conditions, such as high temperature (our populations were also located at south-facing slopes). High temperatures could limit floral longevity by increasing the physiological costs of floral maintenance, especially in large flowers (Galen 2005). In addition, a high activity of pollinators (mediated or not by high temperature and/or large-sized flowers) could lead to a rapid and effective pollination (van Doorn 1997; Ishii & Sakai 2000). Which of these mechanisms is responsible for our results is difficult to establish from observational evidence alone. Nevertheless, our specific study on *C. ladanifer* provided evidence against strict phylo-

genetic constraint and supported a combined action of flower size and air temperature, which could lead to an efficient pollinator service at the expense of elevated floral maintenance costs.

We found variation in floral longevity in *C. ladanifer* along an altitudinal gradient. In general, floral longevity has been found to increase with increases in altitude (Blionis et al. 2001; Giblin 2005). This has been related to lower temperatures during the flowering period occurring at higher altitudes, since lower temperature has been significantly associated with low pollinator activity and longer lived flowers (Yasaka et al. 1998; Blionis & Vokou 2001; Blionis et al. 2001; Giblin 2005). In our study, the altitudinal gradient was intended to reflect a gradient in temperature and flower size; thus, higher altitude was expected to be related to lower temperature at flowering date and larger flowered individuals. Although we did find the expected pattern along the altitudinal gradient relative to flower size (i.e. larger flowers at higher altitude), we found the opposite pattern relative to floral longevity. However, the expected relationship between floral longevity with flower size and air temperature still held because local temperature at the time of flowering was higher at higher elevations.

We did not detect any effect of intraspecific variation in flower size on floral longevity along the altitudinal gradient. We surveyed floral longevity twice a day, but narrower variations in a few hours could be recorded. We only detected an effect of air temperature, with higher temperatures limiting floral longevity to about 0.5 d (see *C. laurifolius*, Table I). Experimental surveys with plants differing in flower size under controlled temperature conditions and/or lack of pollinator visits would be suitable to examine whether flower size also affects floral longevity (Ishii & Sakai 2000; Harder & Johnson 2005; Steinacher & Wagner 2010). Although we lack experimental data of this kind, our study system allowed us to confirm effects of flower size on floral longevity independently of air temperature. We are aware that there are differences in precipitation between considered populations (Table II). This sample heterogeneity could jeopardize the reliability of our analysis and subsequent results. However, we only chose sunny days and the hypothesized differences in the relationship floral longevity versus flower size still remain. Studies focused on the relationship between flower size and longevity are scant (but see Ashman & Schoen 1994; van Doorn & Schröder 1995), but the inverse relationship between temperature and floral longevity has been found in many field studies (Sargent & Roitberg 2000; Rathcke 2003; Giblin 2005) and also under controlled conditions avoiding the confounding effects of varying pollinator activity (Michaud 1990; Yasaka et al. 1998).

Further work is required to disentangle the proximate mechanisms of the patterns reported here, but flower size- and temperature-mediated increases in both pollinator availability and in transpiration rates are likely involved. The positive relationship between flower size or air temperature with pollinator visit rates has been broadly reported (e.g. Obeso 1992; Herrera 1995; Blionis & Vokou 2001; Nattero et al. 2010). As a consequence, a rapid and effective pollination of flowers decreases their longevity (Ishii & Sakai 2000; Arathi et al. 2002; Giblin 2005), whereas lack of pollinator visits lead to extended floral longevity (Ishii & Sakai 2000; Blionis & Vokou 2001; Steinacher & Wagner 2010). Although we lack data of pollinator visit rates, it can be assumed that pollinators differentially visit larger corollas in our study system, as described in *C. albidus* (Bosch 1992), *C. ladanifer* (Barrio & Teixido *in press*) and other generalist large-flowered *Cistus* (*C. libanotis*: Talavera et al. 2001).

Otherwise, floral physiological costs in terms of water could affect floral longevity in our study system (see Galen 2005). It is known that decreasing water availability reduces floral longevity in *Mimulus* (Arathi et al. 2002). In our study area, larger flowered individuals of *C. ladanifer* tended to spend more water per unit area ($R^2 = 0.28$; $p = 0.001$), whereas daily flowering under high temperatures involves 56.7 ± 41.7 g H₂O/plant/d, up to 50% that of leaf transpiration on an organ surface area basis (Teixido & Valladares 2014). These elevated water costs can also involve higher indirect costs in terms of fruit and seed production (i.e. opportunity costs, Gulmon & Mooney 1986; see also Chapin 1989). Under experimental conditions, we detected higher indirect costs in terms of fruit production in larger flowered individuals of *C. ladanifer* (Teixido & Valladares 2013). This means that direct resource allocation to larger corollas entails negative effects on floral functions other than pollinator attractiveness, such as reproductive output. In this context, short-lived flowers would support the arguments to explain the costs/fitness balance of large flowers under stressful conditions (Ashman & Schoen 1994; Galen 2005).

Other alternative plausible explanations may apply to floral longevity patterns found in our study. Overall, differences in biotic and abiotic factors among populations could also explain differences in floral longevity in *C. ladanifer* along an altitudinal gradient. For example, *C. ladanifer* and *C. laurifolius* co-flower in sympatry at high altitude. In this regard, vegetation cover and co-flowering species richness has been positively correlated to floral longevity in a community context in *Mimulus guttatus* (Arceo-Gómez & Ashman 2014). Because we have found the opposite pattern, we could rule

out the effects of vegetation cover on the basis of pollinator competition. Still, vegetation coverage may have other effects on floral longevity that we have not measured. Alternatively, abiotic factors such as substrate type and/or resource availability may likewise affect floral longevity. For instance, differences in the substrate together with different potentially vegetation cover lead to strong differences in water availability for plants. As pointed above, water shortage reduces floral longevity so, since floral longevity was shorter at high altitude, it is feasible to think that water availability decreases at this site. Beyond differences in substrate and vegetation coverage, this pattern is also consistent with differences in mean air temperature during the flowering period, since higher temperatures decrease water availability. Following these assumptions and together with the elevated floral water costs described above for our study system, we suggest that air temperature may be the most feasible pattern affecting floral longevity. Nevertheless, our design did not allow separating all these covarying factors, and interpretations should be made with caution. Further research based on experimental designs is largely needed to draw reliable conclusions.

Which are the evolutionary implications for flower size and longevity in *Cistus*? The negative phenotypic correlation found in our study suggests that large flowers would tend to be short-lived in Mediterranean environments. Nevertheless, we found plasticity in both floral traits, which decreased within-plant along the flowering season. This pattern may be explained by the sequential resource adjustment hypothesis (Lloyd 1980; Wolfe 1992). Otherwise, the variation observed in flower size along an altitudinal gradient could, in principle, result from adjustments to the actual thermal and pollination environments faced by the plants (Obeso 1992; Thompson 2005). However, it is not possible to establish whether this pattern results from simple scaling effects or it is due to other evolutionary drivers on flower size.

In conclusion, our survey of three *Cistus* species showed a uniformly short floral longevity. Nevertheless, our specific study on *C. ladanifer* suggests that floral longevity was not phylogenetically constrained but changed depending on air temperature and flower size. Together, these findings show a triangular relationship among air temperature, flower size and floral longevity with margins for plasticity for the two traits to accommodate pollinator attraction with the costs of large flowers for Mediterranean plants. Future works should focus on addressing cost- and pollinator-mediated phenotypic selection on flower size and longevity to confirm this hypothesis.

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