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TEMPERATURE-LIMITED FLORAL LONGEVITY IN THE LARGE-FLOWERED MEDITERRANEAN SHRUB *CISTUS LADANIFER* (CISTACEAE)

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Premise of research.   Larger and longer-lived flowers are more frequently pollinated, but they are associated with high maintenance costs due to greater water usage. The Mediterranean climate limits plant reproduction and maximizes resource use efficiency, so smaller and short-lived flowers are potentially advantageous under hot and dry conditions.

Methodology.   We experimentally evaluated the relationship between the floral longevity of *Cistus ladanifer*, a large-flowered Mediterranean shrub, and pollination, flower size, and temperature at two different altitudes with contrasting climatic conditions. We hypothesized that flower size and longevity would be reduced in the drier and hotter conditions of the lower-altitude site. In addition, we expected that floral longevity would decrease with experimental pollen deposition, flower size, and temperature. We pollinated and capped flowers to evaluate the variation in floral longevity by comparing actual and potential floral longevity at low and high altitudes.

Pivotal results.   In agreement with our hypothesis, flower size was observed to increase with altitude, but, contrary to expectations, floral longevity decreased at the high altitude since temperature increased during the flowering time. Floral longevity tended to be short but depended on pollination, such that potential floral longevity was greater than actual floral longevity. Capped flowers lasted longer (1.31 ± 0.65 d) than control (0.95 ± 0.39 d) or hand-pollinated (0.60 ± 0.08 d) flowers. At the high altitude, larger flowers and higher temperatures resulted in a reduction in both the observed and potential floral longevity, but temperature had a differential effect in limiting potential floral longevity.

Conclusions.   Our experimental survey reported short-lived flowers and limitations in floral longevity when flowers were larger and temperatures were higher. Overall, the results reveal an important effect of temperature on increasing floral maintenance costs, imposing selective pressures on flower size, and longevity in large-flowered Mediterranean plants.

Keywords: actual floral longevity, altitude, floral maintenance costs, flower size, pollen deposition, potential floral longevity.

Online enhancements: appendix tables.

Introduction

Floral longevity, the length of time that flowers remain open and functional, favors the processes of pollen removal and deposition on the flower (Primack 1985; Ashman and Schoen 1994). Although longer-lived flowers increase the opportunity for reproductive success, floral longevity also entails costs (Ashman and Schoen 1997; Castro et al. 2008). The evolution of floral longevity is thought to be shaped by natural selection to

maximize pollen transfer while minimizing floral maintenance costs (Ashman and Schoen 1994, 1996). For example, longer flower life spans have been reported to be associated with infrequent pollination, while a reduction in floral longevity is induced by pollination (van Doorn 1997; Sargent and Roitberg 2000; Giblin 2005; Weber and Goodwillie 2013). Likewise, floral longevity increases with higher altitude and greater water availability and/or under cooler temperatures (Yasaka et al. 1998; Arathi et al. 2002; Vesprini and Pacini 2005; Arroyo et al. 2013; Jorgensen and Arathi 2013).

The vast majority of studies examining variations in floral longevity have focused on alpine ecosystems in several different regions of the world, which tend to have a low frequency of pollinators under unsuitable weather conditions (e.g., Blionis

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and Vokou 2001; Giblin 2005; Steinacher and Wagner 2010; Arroyo et al. 2013). In these stochastic unpredictable environments, temperature-driven potential floral longevity can be prolonged to assure reproduction when pollinators are absent, which contrasts with the shorter actual floral longevity that has been measured in pollinated flowers (Steinacher and Wagner 2010; Arroyo et al. 2013). Other than such studies, many determinants of variation in floral longevity remain poorly understood, particularly those associated with hot and dry ecosystems. Under such conditions, transpirational cooling is critical for maintaining plant organs at an optimal physiological temperature (Erickson and Markhart 2002; Galen 2005). Thus, water shortage can prevent the normal development of flowers, affecting both fruit and seed production (Galen 2005; Fang et al. 2010). If temperature increases pollinator visitation, an early and efficient pollination would shorten actual floral longevity in self-incompatible species or those with low reproductive assurance, thus allowing a higher resource allocation to develop seeds. However, if late pollination occurs, a prolonged floral longevity could reduce such a resource allocation to develop seeds (Ashman and Schoen 1997; Ishii and Sakai 2000; Castro et al. 2008). In this context, an optimal regulation of floral longevity may buffer costs of delaying fertilization by means of a fast fitness accrual, a scenario in which short-lived flowers should predominate in these environments (Primack 1985; Ashman and Schoen 1994; Galen 2005).

In addition, two other aspects of ephemeral flower life span remain understudied. First, although flower size is known to influence floral longevity (Stratton 1989; van Doorn and Schröder 1995; Arroyo et al. 2013), much remains to be learned about the relationship between floral longevity and flower size variation in a given species. Floral longevity may increase with size among different populations where investment in producing flowers is high and pollination probability is low. However, a negative correlation would be expected within populations based on the arguments used to explain floral longevity as a resource allocation strategy, as larger flowers require more resources for maintenance and pollinator attraction (Ashman and Schoen 1994, 1996). Second, it is unclear whether flower size, temperature, and/or precipitation differentially affect floral longevity, even in absence of pollination, and what the net outcome would be in a population with the hottest and driest conditions and the smallest flowers.

A model scenario to examine those understudied determinants of variation in floral longevity and to evaluate whether fast fitness accrual rates entail short-lived flowers under hot and dry conditions is the Mediterranean region. High temperatures and water shortage over the summer generally characterize the climate in this region (Blondel and Aronson 1999; Quézel and Médail 2003; Thompson 2005). These harsh conditions strongly constrain plant survival and reproduction (Larcher 2000; Thompson 2005; Aragón et al. 2008). Phenologically and morphophysiologically conservative resource use strategies and water use efficiency appear to be adaptative and to play a key role in these environments (Joffre et al. 1999; Valladares et al. 2000, 2008; Thompson 2005). A fundamental question in Mediterranean ecology is how plants tolerate these stressful conditions and are able to flower and fruit. For example, a reduced flowering period, a delay in floral initiation until the rainy season, and a low investment in nectar reward

are common phenomena in many Mediterranean plants (e.g., Herrera 1985; Verdú et al. 2002; Aragón et al. 2008). In highly self-incompatible entomophilous plants, reproduction requires that pollinators are attracted by a flower, and so flower size is an important trait in their reproductive ecology. Larger flowers in the Mediterranean zone have higher pollinator visit rates and greater reproductive success (e.g., Herrera 1996; Thompson 2001; Arista and Ortiz 2007; Barrio and Teixido 2014). However, the benefits resulting from the attractiveness of larger flowers can be offset by greater resource costs associated with the increases in water use for floral development and maintenance (Galen et al. 1999; Elle and Hare 2002; Teixido and Valladares 2014). As a consequence, Mediterranean environments may constrain flower production, making small flowers potentially advantageous. As an alternative explanation to selective pressures, phenotypic plasticity in flower size may also result in reduced forms in hotter and drier sites (Barrett et al. 2004; Herrera 2005).

Despite such assumptions, several common Mediterranean species such as rockroses (*Cistus* spp.) exhibit large flowers. These diurnal-pollinated and spring-flowering shrubs experience elevated floral physiological costs in terms of water and carbon, especially larger-flowered species under high temperatures (Teixido and Valladares 2014). A direct investment of these resources in floral attractiveness is accompanied by indirect costs in terms of fruit and seed production (Teixido and Valladares 2013). In this regard, elevated costs of flower size may involve low margins of variation in floral longevity and counteract the benefits associated with pollen transfer. Therefore, potential floral longevity in the absence of pollination may also be similar to actual floral longevity. This idea would be congruent with a predominance of short-lived flowers under hot and dry environments, in agreement with a fast fitness accrual model.

In this study, we evaluated the combined effects of pollination, flower size, and temperature on floral longevity in *Cistus ladanifer*, a large-flowered, generalist, and self-incompatible Mediterranean shrub (Talavera et al. 1993). This species occurs along an altitudinal range with significant differences in flower size, ranging from 5 to 11 cm in diameter (Teixido et al. 2011). Experimentally, we assessed the longevity of pollination-excluded, unmanipulated, and hand-pollinated flowers from two different altitudes. The altitudinal study was intended to reflect differences in flower size and temperature, and higher altitude was expected to be associated with lower temperature and larger flowers. Altitude may also be related to differences in the pollination environment affecting floral longevity. Although we did not record pollinator visitation in this study, we observed that visit rates were constant and that differences in the composition of pollinators do not affect pollen limitation or female fitness between altitudes (Barrio and Teixido 2014). We hypothesized that (1) flower size and longevity are reduced under drier and hotter conditions (i.e., at the lower altitude); (2) floral longevity decreases with experimental pollen deposition and increases when pollination is prevented, such that actual floral longevity is shorter than potential floral longevity at both altitudes; (3) for all treatments and at each altitude, floral longevity decreases with flower size and temperature; and (4) temperature differentially affects floral longevity, such that higher temperatures may

limit this trait independent of pollination, thus matching potential with actual floral longevity.

Material and Methods

Species and Study Area

Cistus ladanifer (Cistaceae) is a shrub 100–250 cm in height that inhabits acid and dry soils in warm open areas of the western Mediterranean region. Flowering phenology spans from March to June, and each plant produces white flowers, often exhibiting dark-colored spots at their bases. The flowers of this species are the largest in the family and are homogamous, polliniferous, and self-incompatible; secrete some nectar (Herrera 1992; Talavera et al. 1993); and are mainly pollinated by bees, beetles, and flies (Talavera et al. 1993). Flower opening occurs synchronously at dawn each day. The study was conducted from April to June of 2009 at two different altitudes (high and low) in the Madrid province, central Spain (lat. 39°53′–41°09′N, long. 3°03′–4°34′W). These altitudes exhibited clear differences in air temperature and humidity. The experimental design to study flower size and longevity (table 1) involved three replicate populations at each of the two altitudes. All populations had a similar orientation (south), slope (0°–10°), and tree canopy cover (0%–10%). Sunny and southern slopes were chosen to remove environmental effects as much as possible. We note that the sympatric species *Cistus laurifolius*, which is found at the high-altitude site, does not coflower or overlap with the flowering of our study species.

Floral Longevity Response

A total of 10 similar plants >1 m high was randomly selected from each population and tagged during the flowering peak (approximately 2 wk) in a 20 × 20-m plot. All plants were at a similar phenological stage when sampled, and there was no overlap between altitudes at flowering time. Air temperature (°C), air relative humidity (%), soil moisture (%), and solar irradiance (μmol PAR m^{-2} s^{-1}) were recorded at the site of each population. Sensors were used for air temperature and relative humidity (Hobo H08-032-08 datalogger; Onset, Pocasset, MA) located 1 m above the ground. Readings from each sensor were recorded every 30 min over the period of study. Soil moisture was recorded at 3–5 points below the canopy in the understory of each plant, and sensors were calibrated based on integrated TDR technology (ECH$_2$O EC-20; Decagon Devices, Pullman, WA). Solar irradiance was recorded every 30 min with a quantum sensor (QSO-SUN; Apogee, Logan, UT). All microclimatic variables were significantly correlated with each other between the two altitudes (tables A1, A2, available online). Air temperature was positively correlated with solar irradiance and negatively correlated with air relative humidity and soil moisture. As a consequence, we used only air temperature as a predictor variable to explain variation in floral longevity. We then recorded mean air temperatures at each altitude during the respective sampling time and related floral longevity to daily mean air temperature.

We also evaluated the relationship between variation in floral longevity and pollination by conducting three different treatments, each using 12 randomly selected individual flowers per plant: hand-pollinated flowers with xenogamous pollen, pollination-excluded flowers by capping of stigmas (hereafter termed capped flowers), and unmanipulated naturally pollinated control flowers. Hand-pollinated and control flowers were used to assess actual floral longevity, whereas capped flowers were used to determine potential floral longevity. Hand pollination was carried out with a fresh pollen mixture collected from 10 different individuals within a 10-m radius of the recipient flower (Giblin 2005; Castro et al. 2008). Using a paintbrush, we then deposited outcross pollen on the stigma 2 h after anthesis to ensure stigmatic receptivity (Herrera 1992) until the stigmatic area was fully covered. Capping was carried out after flowers were totally open to avoid damage. We note that capping took place early in the morning and, subsequently, flowers did not receive any pollinator visits, so no pollen was deposited on stigma. The caps, which were made of straw and sellotape, were attached to

Table 1

Location and Ecological Data Related to the *Cistus ladanifer* Experimental Study Sites

Study sites	Altitude (m)	Climate	Substrate	Vegetation cover
Lower-altitude sites:				
Monte del Pardo (lat. 40°34′N, long. 3°42′W)	732	Dry 533 mm, 14°C	Clay and sand	Dehesa with *Quercus ilex* and *Pinus pinea* interspersed in a shrub matrix
Monte del Pardo (lat. 40°33′N, long. 3°42′W)	741	Dry 529 mm, 14°C	Clay and sand	Dehesa with *Q. ilex* and *P. pinea* interspersed in a shrub matrix
Monte Valdelatas (lat. 40°32′N, long. 3°41′W)	725	Dry 520 mm, 14°C	Clay and sand	Dehesa with *Q. ilex* and *P. pinea* interspersed in a shrub matrix
Higher-altitude sites:				
Becerril de la Sierra (lat. 40°43′N, long. 3°58′W)	1163	Subhumid 844 mm, 11°C	Granite	Patchy scrubland with *Cistus laurifolius* and *Juniperus oxycedrus*
Navacerrada (lat. 40°43′N, long. 4°00′W)	1176	Subhumid 878 mm, 11°C	Granite	Patchy scrubland with *C. laurifolius* and *J. oxycedrus*
Navacerrada (lat. 40°43′N, long. 4°00′W)	1199	Subhumid 878 mm, 11°C	Granite	Patchy scrubland with *C. laurifolius* and *J. oxycedrus*

Note.   Climate column shows the annual mean rainfall (mm) and the annual mean temperature (°C; Ninyerola et al. 2005; *n* = 20 yr).

cover the stigma. Flowers were not bagged to avoid elevated floral transpiration rates that might affect floral longevity. The presence of the caps did not alter visitation of the flowers by pollinators (A. L. Teixido, personal observation). Thus, flowers were visited by pollinators, and pollen removal might also affect the potential floral longevity (e.g., Ishii and Sakai 2000). We have taken this assumption into consideration in our discussion.

Treatments were conducted between April 20 and May 3 at the low-altitude site and between May 26 and June 8 at the high-altitude site. Capping was carried out between 0700 and 0900 hours, before pollinator activity and any pollen deposition on stigmas, and hand pollination was carried out between 0900 and 1100 hours. Overall, we selected and tagged 36 flowers per plant, 12 per treatment, giving a sample size of 1080 flowers per altitude (total $n = 2160$ flowers). We also measured the diameter of the corolla (centimeter to the nearest millimeter) using a ruler as a proxy of flower size to examine its relationship with floral longevity. Corolla diameter (cm) and flower area (cm²) are strongly correlated in the study species (Teixido and Valladares 2014). When a flower remained open for several days, the average diameter was used in the analysis.

To record floral longevity, we monitored flowers once between 1400 and 1500 hours and again between 1900 and 2000 hours each day. Pollination occurs diurnally, and those flowers that remain open between 1900 and 2000 hours start closing and reopen at dawn the next morning. Thus, floral longevity was recorded as a fraction of days according to the following criteria: 0.5 d for petals falling off before 1400 or 1500 hours of the first day, 1 d when falling off after 1500 hours but before 1900 or 2000 hours of the first day, 1.5 d for petals falling off before 1400 or 1500 hours of the second day, and so on. This protocol thus allowed a determination of the flower longevities based on the fraction of the day. As a general rule, during warm and sunny days that were suitable for pollinator activity, flowers lasted until midday, and only under cloudy, cold, and/or rainy days did flowers have an extended life span during the afternoon and/or following days.

Statistical Analysis

We used two different generalized linear mixed models (GLMMs) to model the variability of flower size as a function of altitude (fixed factor) and the variability of floral longevity as a function of altitude and treatment (fixed factors). In the first GLMM, modeling the variability of flower size, we included population within an altitude and plant within a population as random factors. In the second GLMM, modeling the variability of floral longevity, we included population within an altitude and plant within a population as random factors, flower size and temperature as covariates, and the interactions altitude × treatment, flower size × altitude, flower size × treatment, temperature × altitude, and temperature × treatment. A significant altitude × treatment outcome indicates a different effect of treatments on floral longevity between altitudes, whereas the other interactions indicate differences in regression slopes between floral longevity with flower size and/or temperature between altitudes and/or treatments. We assumed a normal error distribution with an identity link function for flower size and a Poisson error distribution with a logit link function for

floral longevity. The assumptions of normality and homogeneity of variance were tested using Shapiro-Wilk's and Levene's tests, respectively. For all models, we used the restricted maximum likelihood test, and to assess significant differences among treatments at each altitude for floral longevity, their means were compared using post hoc HSD (honest significant distance) tests. All the computations were performed using the GLIMMIX macro function of SAS (SAS statistical package; SAS Institute, Cary, NC).

For the 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. 1999). 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 population as a random factor in our model. In this way, we included a statistical control for this source of variation. Since our model is mixed, by including the term plant nested in population, we partition out this effect and can then focus on the variation of interest—variation in altitude, treatment, flower size, and temperature—and the interactions between altitude and treatment, altitude and flower size, altitude and temperature, treatment and flower size, and treatment and temperature. Additionally, since the assignment of the number of degrees of freedom of residuals is critical when using 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).

Given the reported importance of flower size and temperature, we specifically examined how these factors influenced floral longevity. For flower size, we correlated individual mean flower size with individual mean floral longevity (i.e., mean flower size and longevity per plant) for each treatment. We used a Spearman's rank correlation since our data for floral longevity did not satisfy the assumptions of normality and homogeneity of variance. For temperature, we related mean longevity of flowers measured every day with mean air temperature in the days those flowers remained open for each treatment. We used nonlinear regressions as the best-fitting analysis for our data. The sample size comprised 24 data points for all regressions since we conducted the experiment over a period of 24 d with 12 d/altitude.

Although we included the interactions between altitude and treatment with flower size and temperature in our full GLMM modeling of variability in floral longevity, there may have been confounding effects on evaluating the relationship between flower size and temperature and floral longevity since higher temperatures were present at the altitude containing larger flowers (see "Results"). To partition out these possible effects, we subsequently analyzed the specific effects of flower size and temperature for each treatment and altitude separately. Thus, we could address two questions: first, whether temperature (rather than flower size) differentially affected floral longevity, reducing the impact of this trait and matching actual with potential flower life span, and second, whether under similar temperature conditions occurring at each altitude having larger flowers significantly reduced both actual and potential floral longevity. At each altitude, we used Spearman's rank correlations for flower size and nonlinear regressions for temperature, as explained above. All these analyses were performed using STATISTICA v12.0 (Statsoft, Tulsa, OK).

Results

Temperature, Flower Size, and Longevity

Contrary to our expectations, all the measured microclimatic variables corresponded with hotter and drier conditions at the high altitude during the flowering time (table A1). Mean temperature ($\pm$ SD), recorded over 12 d at each altitude, averaged $14.1° \pm 2.8°C$ and $17.2° \pm 3.4°C$ at the low and high altitudes, respectively, and this difference was significant (fig. 1A). *Cistus ladanifer* flower size ranged from 5.9 to 10.8 cm and was significantly greater (by about 12%) at the higher altitude (mean $\pm$ SD: 7.3 ± 1.3 cm at the low altitude and 8.2 ± 1.1 cm at the high altitude; fig. 1B). Flower size also differed significantly between populations within each altitude and between plants within populations (table 2). Longevity of individual flowers ranged from 0.5 to

3 d, though longevities of 3 d occurred only in control and capped flowers. Mean actual floral longevity ($\pm$SD), as measured in control flowers, averaged 1.05 ± 0.48 and 0.86 ± 0.35 d at the low and high altitude, respectively (fig. 1C).

Effects of Altitude and Treatments on Floral Longevity

Overall, floral longevity differed significantly between altitudes and treatments, flower size, and temperature, as well as the interactions altitude $\times$ treatment and altitude $\times$ temperature, with marginal differences in altitude $\times$ flower size (table 2). For all treatments, flowers at the high altitude lasted for a shorter time than those at the low altitude. At both altitudes, actual floral longevity was shorter than potential floral longevity. Thus, hand-pollinated flowers were the shortest lived and capped flowers the longest lived, whereas control flowers showed intermediate longevities (fig. 2). However, between altitudes, the variation in floral longevity among treatments was significantly variable (altitude $\times$ treatment significant; table 2). For example, at the high altitude, potential floral longevity, as measured in capped flowers, was similar to actual floral longevity, as measured in control flowers at the low altitude (i.e., flowers that lasted approximately 1 d; fig. 2).

Additionally, the reduction in floral longevity at the high altitude was also apparent through a diminished response of floral longevity. In this regard, capped flowers lasted for 13% longer and hand-pollinated flowers a 14% shorter time than control flowers at the high altitude, whereas at the low altitude, capped flowers lasted 31.5% longer and hand-pollinated flowers a 24% shorter time than control flowers (fig. 2). Therefore, actual and potential floral longevity were more similar at the high than the low altitude, and potential floral longevity was prolonged under cooler conditions, which was also associated with smaller flowers. The reduction in the variation of floral longevity, as well as the difference between actual and potential floral longevity, was also noticeable across the distribution of the frequency of flower life spans per treatment (fig. 3). Approximately 87% of the hand-pollinated flowers at the high altitude lasted 0.5 d, while only 80% did so at the low altitude. For control flowers, 19% and 32% lasted >1 d at the high or low altitudes, respectively. Last, 69% of the high-altitude flowers showed a potential floral longevity of <1 d and only 14% >2 d, compared with 31% and 35%, respectively, of flowers at the low altitude.

Effects of Flower Size and Temperature on Floral Longevity

In accordance with the effect of altitude, analyses of the relationship between flower size or temperature and floral longevity revealed that floral longevity significantly decreased with flower size and temperature for all treatments (fig. 4). As reported by our full model, these relationships tended to be constant, independent of treatment (treatment $\times$ flower size and treatment $\times$ temperature were not significant; table 2). We determined that temperature and flower size had different effects on floral longevity. Daily mean temperature showed a nonlinear relationship with flower life span, skewed toward flowers lasting 0.5 d under high temperatures (approximately >18°C) independent of pollination, such that potential floral longevity, as measured in capped flowers, was limited and

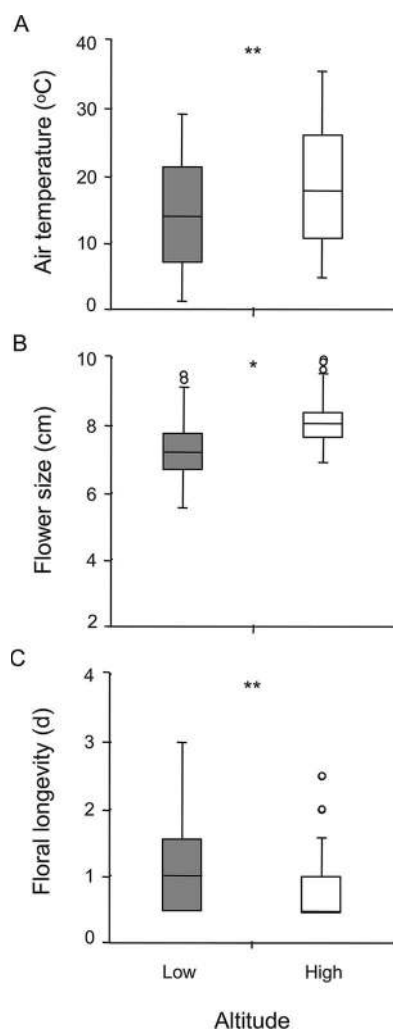**Fig. 1** Box plots of variation in temperature (°C), *Cistus ladanifer* flower size (cm), and floral longevity (d) at each altitude. Median values are indicated by a horizontal line. One asterisk, $P < 0.05$; two asterisks, $P < 0.01$.

Table 2

Generalized Linear Mixed Model (GLMM) of Flower Size and Floral Longevity (2160 Flowers from 60 Plants in 6 Populations at 2 Altitudes)

Effect	Flower size				Floral longevity			
	df	Estimate $\pm$ SD	Test value	P	df	Estimate $\pm$ SD	Test value	P
Random:								
Population (altitude)	5, 54	1.27 $\pm$ .24	5.21	.006	5, 54	.01 $\pm$ .00	.98	.163
Plant (population)	55, 2114	9.36 $\pm$ 1.17	2.31	<.001	55, 2094	.01 $\pm$ .00	1.01	.159
Fixed:								
Altitude	1, 4	1.54 $\pm$ .09	99.32	<.001	1, 4	−.28 $\pm$ .04	16.58	.015
Treatment	...	...	...	...	2, 660	−.27 $\pm$ .02	344.55	<.001
Altitude × treatment	...	...	...	...	2, 660	−.24 $\pm$ .03	34.96	<.001
Flower size	...	...	...	...	1, 2094	−.14 $\pm$ .01	96.32	<.001
Temperature	...	...	...	...	1, 2094	−.23 $\pm$ .04	209.71	<.001
Altitude × flower size	...	...	...	...	1, 2094	−.06 $\pm$ .03	3.57	.064
Altitude × temperature	...	...	...	...	1, 2094	−.09 $\pm$ .01	4.51	.021
Treatment × flower size	...	...	...	...	1, 2094	−.04 $\pm$ .03	2.89	.097
Treatment × temperature	...	...	...	...	1, 2094	−.05 $\pm$ .03	3.30	.081

Note.   Analyses were based on GLMM with normal errors and identity link using restricted maximum likelihood estimation. Random effects were tested with Wald Z-tests and the fixed effect with Type III F-tests. Significant P values are underlined.

strongly correlated with actual floral longevity (fig. 4A). Otherwise, flower size did not limit potential floral longevity, in contrast with the effects of temperature. Thus, larger flowers showed some variation in floral longevity, and even actual floral longevity, as measured in control flowers, did not correlate with the actual floral longevity of hand-pollinated flowers (fig. 4B).

Our analyses also partially distinguished the possible confounding effects of flower size and temperature due to the presence of both larger flowers and higher temperatures at the high altitude. Moreover, these analyses accounted for the interactions between altitude and flower size or temperature. At the low altitude and for the three treatments, flower size

Fig. 2   Differences in *Cistus ladanifer* floral longevity in the interaction altitude × treatment. Different letters indicate significant differences among treatments using an unequal HSD post hoc test ($P <$ 0.05). Columns = mean values; bars = SE.

and temperature did not have any effect on floral longevity (tables 3, 4). However, at the high altitude, larger flowers and higher temperatures resulted in shorter-lived flowers. Once again, temperature had a stronger effect than flower size on floral longevity, significantly reducing the duration of the flowers in all three treatments (table 3). In contrast, larger flowers reduced the potential floral longevity and actual floral longevity, as measured in control flowers, but did not affect hand-pollinated flowers (table 4).

Discussion

Our experimental survey of *Cistus ladanifer* populations represents an important contribution to understanding how abiotic factors coupled with pollen deposition and flower size can play a role in the amount of time flowers remain open, which has important consequences for plant reproduction. Our data show how altitude has unexpected effects when phenological differences in plants at higher elevations blooming later correspond with higher temperatures compared to lower elevations. We describe here the variation in floral longevity in relation to pollination, flower size, and temperature. Most interestingly, higher temperatures limited floral longevity to less than 1 d, independent of pollination. Overall, our results provide empirical evidence that large flowers tend to be short lived under hot and dry conditions.

However, an important caveat is that we lack data regarding the temporal variation in floral longevity and pollinator visits. Likewise, the correlation reported for the microclimatic variables measured might not be consistent in multiple years. Indeed, differences in the pollination environment may affect floral longevity (Ishii and Sakai 2000; Blionis and Vokou 2001; Arroyo et al. 2013). These differences may also show temporal variation in relation to changes in climatic conditions and ultimately influence floral longevity (Blair and Wolfe 2007). Although we did not record pollinator visitation or any temporal variation in this study, we still report the importance of pollination on variation in floral longevity. Additionally, our results support the assumption for fast fitness accrual and ephemeral
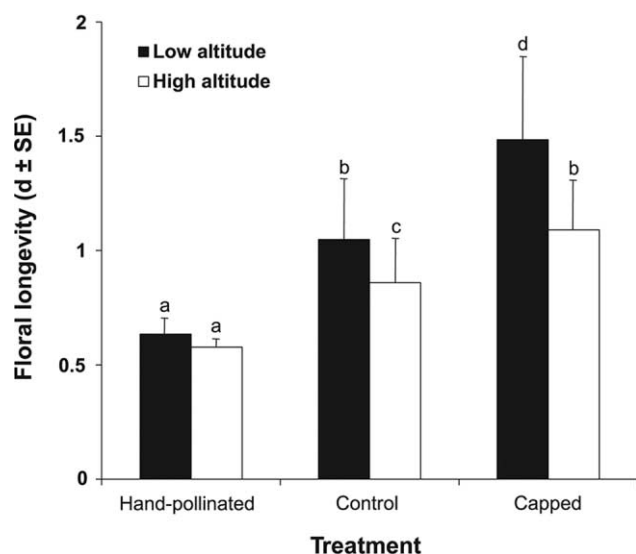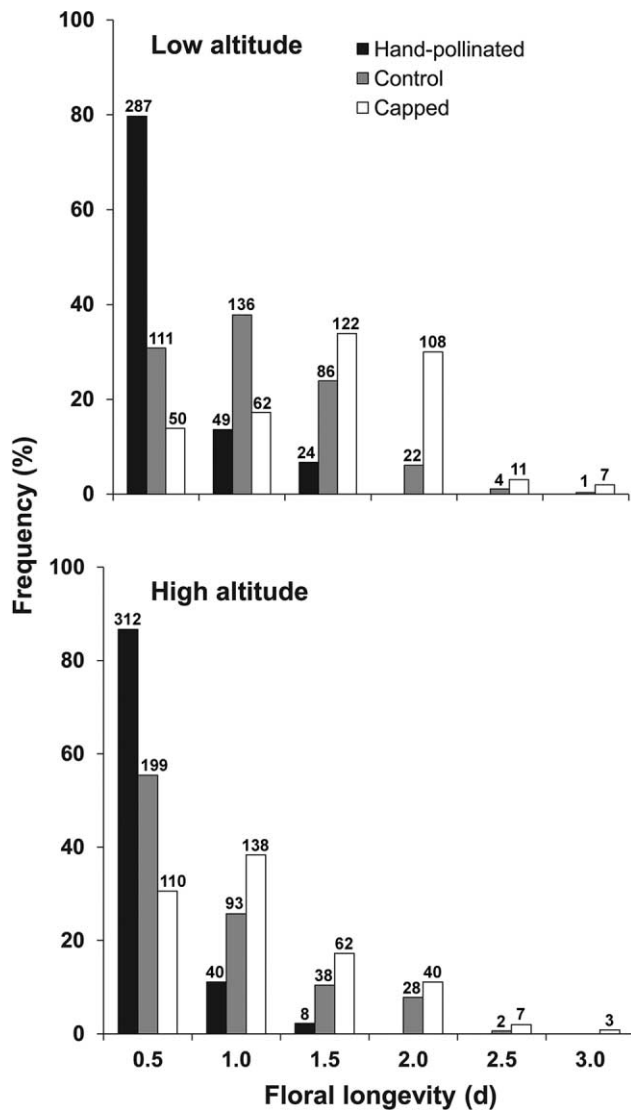
Fig. 3 Frequency (%) of different *Cistus ladanifer* floral lon-gevities (0.5–3.0 d) per treatment and altitude. Absolute numbers of flowers are shown above each bar.

A negative relationship between floral longevity and pollen deposition has been found in many other studies, where pollination of flowers decreased their longevity (Clayton and Aizen 1996; Ishii and Sakai 2000; Arathi et al. 2002; Giblin 2005; Arroyo et al. 2013), while lack of pollinator visits led to extended floral longevity (Ishii and Sakai 2000; Blionis and Vokou 2001; Steinacher and Wagner 2010; Arroyo et al. 2013). This pattern may be related to the importance of fe-male function in resource allocation and the costs of pro-longed floral longevity to fruit and seed production (Ashman and Schoen 1997; Castro et al. 2008). Therefore, the limita-tion of floral longevity to 0.5 d in pollinated *C. ladanifer* flowers is in accordance with the elevated floral costs of this
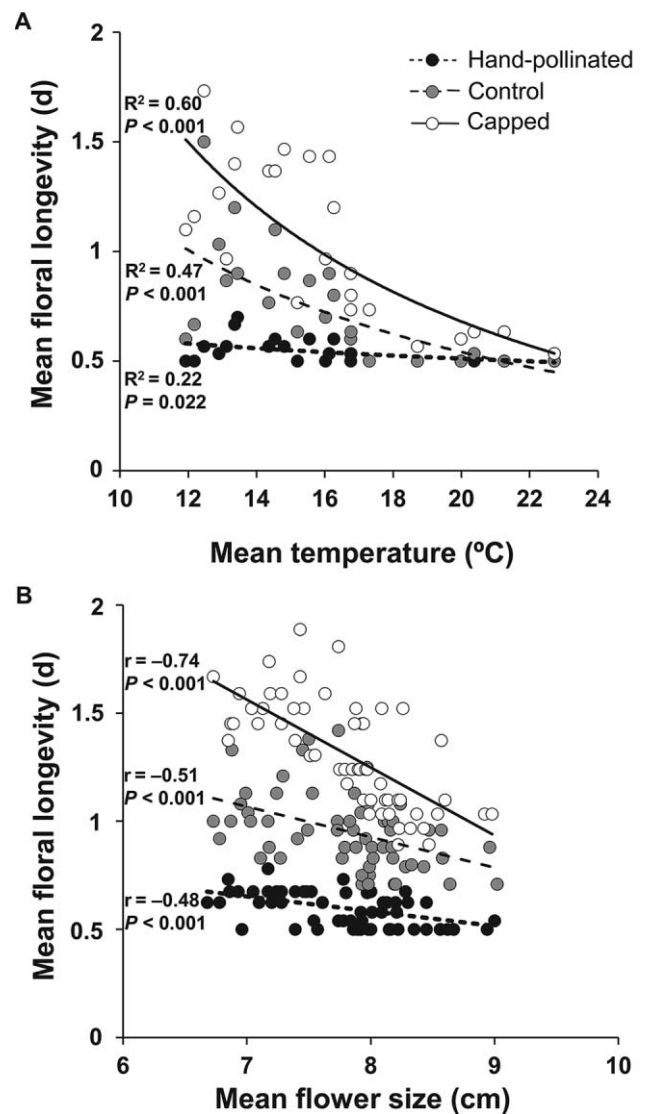

Fig. 4 Differences in *Cistus ladanifer* floral longevity among treatments at different temperatures (*A*) and flower sizes (*B*). Circles denote the mean floral longevity of flowers measured each day versus the daily mean temperature (*A*; $n = 24$ d/treatment) and of all in-dividuals sampled versus individual mean flower size (*B*; $n = 60$ in-dividuals).

floral longevity in large-flowered plants blooming under a hot and dry environment.

The short floral longevity observed in our study contrasts with floral longevities of several days in Mediterranean eco-systems (Petanidou et al. 1995; Blionis et al. 2001; Berjano et al. 2009; Marques and Draper 2012). However, Petanidou et al. (1995) reported that members of the Cistaceae also show floral longevities of 1 d. Phylogenetic constraints may account for this difference, as several families have flowers that typi-cally last 1 d (e.g., Commelinaceae, Convolvulaceae, Pontede-riaceae [Primack 1985]; Acanthaceae [Endress 1994]; Turner-aceae [Arbo 2007]). We propose, however, that there is an alternative plausible explanation to phylogeny in our experi-mental system: the combined effects of pollination, flower size, and environmental (temperature) influences.

Table 3

Linear Regressions of Each Treatment at Each Altitude for Mean Floral Longevity (d) against Mean Temperature (°C)

	Low altitude						High altitude					
Treatment	R^2	$\beta_0 \pm$ SD	$\beta_1 \pm$ SD	df	F	P	R^2	$\beta_0 \pm$ SD	$\beta_1 \pm$ SD	df	F	P
Hand-pollinated	.01	1.12 ± .21	−.00 ± .02	1, 11	.070	.797	.56	1.21 ± .05	−.01 ± .00	1, 11	12.885	.005
Control	.02	1.53 ± .47	−.01 ± .03	1, 11	.182	.679	.60	2.50 ± .35	−.08 ± .02	1, 11	14.697	.003
Capped	.06	2.20 ± .55	−.03 ± .04	1, 11	.581	.463	.67	2.93 ± .36	−.09 ± .02	1, 11	19.991	.001

Note. Significant P values are underlined. $n = 12$ data for all regressions.

species, which reduce the fruit and seed production (Teixido and Valladares 2013, 2014). Our data support the general trend of a pollination-mediated reduction in floral longevity and evolution of short-lived flowers by mean fast fitness accrual rates that may minimize floral maintenance costs.

The observed differences in flower life span among treatments and altitudes and their interaction represent an important data set in the context of understanding the ecology and evolution of floral longevity. Altitude may involve differences in the pollination environment that affect floral longevity. Although we did not record pollinator visitation, our pollination treatments were designed to accurately assess the potential and actual floral longevity at both sites. From an evolutionary perspective, variation between actual and potential floral longevity reveals the importance of this trait in the reduction of the balance between maintenance cost and fitness rates (Ashman and Schoen 1994, 1996). At the low altitude, prolonged potential floral longevity may ameliorate slow pollination at reduced maintenance cost rates under cooler temperatures. Likewise, increases in effectiveness and/ or visit rates of pollinators, together with increases in floral maintenance costs, may account for shorter actual floral longevity of open-pollinated control flowers at higher elevation. Consequently, the potential for fitness gain in a short time would exceed the costs associated with floral maintenance (i.e., fast fitness accrual). For example, if we assume identical costs at each altitude, fitness accrual is faster at the high altitude and actual floral longevity decreases since visitation would be higher and/or more effective. Similarly, assuming identical pollinator activity, increased costs at the high altitude would also involve faster fitness accrual as they may exceed potential fitness gain (Ashman and Schoen 1994, 1997). The effects of flower size and temperature (regardless of pollination) on floral longevity that are revealed in our study contribute to distinguishing between these models.

Table 4

Spearman's Rank Correlations of Each Treatment at Each Altitude for Mean Floral Longevity (d) against Mean Flower Size (cm)

Altitude	Hand-pollinated	Control	Capped
Low	−.002	−.020	−.044
High	−.179	−.368*	−.797**

Note. Significant P values are underlined. $n = 12$ data for all correlations.
* $P = 0.046$.
** $P < 0.001$.

Here we provide new insights into the underinvestigated relationship between flower size and longevity. Contrary to our expectations, floral longevity was shorter at the high altitude where flowers were larger. This suggests a negative effect of flower size on floral longevity at a species level, although the potential confounding effects of temperature are likely to be important. We did not detect the patterns of shorter floral longevity in sites with higher temperatures and smaller flowers. We hypothesized that flower longevity would be less at the low altitude despite flowers being smaller, as a consequence of the higher temperature. Given our results, it is difficult to distinguish the effects of flower size and temperature. Acting together, both factors may affect floral longevity and limit its response by reducing potential floral longevity at high altitudes. Alternatively, we expected that, within populations, flower size would reduce floral longevity independent of temperature. This was found to be the case only at the high-altitude site (i.e., under higher temperatures and for larger flowers). One explanation is that smaller-flowered individuals suffered higher pollen limitation levels at this site, thus reducing the amount of pollen deposition on stigmas and prolonging floral longevity. This would be in accordance with the results reported by Barrio and Teixido (2014), where at higher elevations, C. ladanifer flowers were reported to be not only larger but also more pollen limited than at lower sites. Likewise, low levels of pollen limitation at lower altitude may explain the apparent absence of any relationship between flower size and longevity.

Ultimately, our results based on temperature in the absence of pollinator data may explain why both actual floral longevity, as measured in control flowers, and the potential floral longevity of capped flowers were shorter at the high altitude. Interestingly, although inverse relationships between temperature and floral longevity have been reported (Yasaka et al. 1998; Sargent and Roitberg 2000; Giblin 2005; Arroyo et al. 2013), we detected a limited effect on potential floral longevity with higher temperature. Further work is required to elucidate the proximate mechanisms of the patterns reported here, but temperature-mediated increases in transpiration and respiration rates are likely involved (see Teixido and Valladares 2014). In such a scenario, the theoretical expectation of a balance between costs and fitness rates would not be realized. Thus, elevated floral physiological costs may increasingly exceed fitness gain and even disrupt flower function independent of pollination. These factors are important for understanding the ecology and evolution of floral longevity and, specifically, for explaining why large flowers tend to be short lived under hot and dry environments.

There may be other explanations for the floral longevity relationships found in our study. For example, a relationship between temperature and flower life span may be affected by other correlated factors (e.g., time-related changes in temperature and plant resource status conditioning floral longevity), and biotic factors acting at the individual and/or population level may explain differences in floral longevity. In this context, variation in this trait may be associated with male function, with longer-lived flowers favoring higher amounts of pollen removal (Clayton and Aizen 1996; Ishii and Sakai 2000; Weber and Goodwillie 2007, 2013). If pollinator visitation varied between the two altitudes, this may have influenced potential floral longevity at both sites, although we note that it has been previously reported that pollinator visit rates are similar between altitudes (Barrio and Teixido 2014). Indeed, floral longevity seems to be more related with pollen deposition than with pollen removal (Proctor and Harder 1995; Martini et al. 2003), and our data based on hand-pollinated flowers support this conclusion. Likewise, both the number of displayed flowers and flower position in the inflorescence have been reported as factors that affect floral longevity (Stpiczyńska 2003; Harder and Johnson 2005). It would be interesting to analyze the role of these traits in terms of pollinator service and floral maintenance costs.

In conclusion, our study of *C. ladanifer* populations provides new insights into the evolution of floral longevity and the combined effects of pollination, flower size, and temperature on this trait. The results showed an altitudinal variation in flower size and floral longevity that was short but not fixed. The main conclusions are that pollen deposition reduces floral longevity and that this response is strongly limited at sites where flowers are larger and temperatures during the flowering time are higher. Together, all these results suggest an important effect of temperature on increasing maintenance costs in large-flowered Mediterranean plants. We propose that further research on floral longevity should focus on sympatric coflowering species with contrasting flower size and patterns of temporal variation beyond patterns recorded among populations. Moreover, it would be interesting to analyze differences in pollinator environments as well as the effects of the floral longevity response on physiological maintenance costs of flowers and, ultimately, on female fitness accrual rates.

Acknowledgments

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