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ORIGINAL ARTICLE

Pollination-associated shortening of the functional flower lifespan in an alpine species of *Alstroemeria* and the water content of flowers

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Abstract

Pollination-induced flower senescence is expected in species of dry habitats with large long-lived water-demanding flowers as means for reducing floral maintenance costs. We investigated this hypothesis in *Alstroemeria umbellata*, an alpine species of the semiarid central Chilean Andes. Pollinator-excluded flowers were submitted to hand cross-pollination and manual pollen removal and monitored twice daily to assess the time spans of four floral stages and two expressions of flower longevity. Wilting and floral stage duration responses in open-pollinated flowers were studied. Ramet-level floral and leaf water content were quantified. Pollen removal had no effect on any floral trait analyzed. Hand cross-pollination reduced the functional flower lifespan from 7.5 to 6.7 days and the female stage from 3.4 to 1.6 days, but did not have a clear effect on the total flower lifespan (9.3 days). Counterintuitively, the length of the dehydration stage increased following pollination. No effect of pollination was detected in naturally pollinated flowers. Inflorescences contained > 3.5 g of water, > 3 times more than the ramet leaves, with > 50% of floral water housed in the turgid tepals. Although inflorescences contain much more water than the leaves, based on the open-pollination results, the amount of tepal water saved through pollination-associated floral senescence under natural circumstances is likely to be far less than the ~ 11% predicted by the manipulative experiment. Knowledge of tepal and leaf transpiration rates and the water content of underground plant parts is desirable to arrive at a more precise assessment of the impact of pollination-associated floral senescence on the water balance in *A. umbellata*.

Keywords Flower longevity · Pollination · Floral water content · Floral maintenance costs · Central Chilean alpine · Andes

Introduction

Pollinator attraction depends on the maintenance of petal turgor at least in flowers where the petals have an attraction function. Maintenance of turgor in flowers comes at the expense of water drawn from the xylem or phloem of other plant organs and depends on a flower´s hydraulic properties, corolla cuticle properties, the pectin content of flowers and environmental conditions affecting transpiration (Galen et al. 1999; Chapotin et al. 2003; Feild et al. 2009; Lambrecht et al. 2011; Lambrecht 2013; Roddy and Dawson 2012; Teixido and Valladares 2014; Roddy et al. 2016; Zhang et al. 2017; Teixido et al. 2019). Flowers also entail carbon and respiratory costs (Ashman and Schoen 1997; Roddy et al. 2016; Dudley et al. 2018). In view of these various costs, it has been argued that the plastic cessation of the flower lifespan should be favored once a flower has completed its sexual functions, thereby lowering floral maintenance costs with respect to those incurred in unpollinated flowers that

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remain open for a longer period of time (Webb and Littleton 1987; Ashman and Schoen 1994; van Doorn 1997).

The plastic cessation of the flower lifespan once a flower has completed its sexual functions could be expected in above treeline habitats (alpine) characterized by semiarid and desert climates. First, water becomes limiting in midsummer and warm and dry conditions produce strongly desiccating conditions for flowers. Second, large long-lived flowers have been documented in both wet and semiarid alpine areas (Steinacher and Wagner 2010; Torres-Díaz et al. 2011; Pacheco et al. 2016; Arroyo et al. 2017). In relative terms, large long-lived flowers are expected to be more prone to floral water loss than small short-lived flowers (Galen et al. 1999; Teixido and Valladares 2014; Teixido et al. 2016), although, clearly, there could be trade-offs in terms of flower number. Third, resource allocation to flowers in relation to leaves increases with increasing elevation (Fabbro and Körner 2004; Körner 2021). Thus, the flowers of alpine species are likely to be more demanding on a plant´s water budget than their lowland counterparts. Such high floral water demands would further favor the plastic cessation of the flower lifespan in semiarid and arid alpine species as a means of reducing floral water maintenance costs.

In this study, we focus on *Alstroemeria umbellata*, an alpine species restricted to the semiarid Andes of central Chile. In general, species of *Alstroemeria* are characterized by large, long-lived, animal-pollinated flowers (Aizen and Basilio 1995, 1998; Buzato et al. 2000; González et al. 2015). *Alstroemeria umbellata* flowers in the middle of summer, fully 2–3 months after snowmelt, which is somewhat later than the average species found at the elevation where it grows (Arroyo et al. 1981). At this time of the season, soils in the alpine belt become very dry (Cavieres et al. 2006; Sierra-Almeida and Cavieres 2010; Sierra-Almeida et al. 2016). We submitted pollinator-excluded flowers of *A. umbellata* to experimental pollen saturation and complete pollen removal both separately and in combination. Concomitantly, we followed the responses of flowers exposed to open pollination. We quantified the water content of flowers, whole inflorescences and leaves at the individual ramet level. Based on the experimental and open-pollination results, we estimated the % of floral water loss that could be avoided through earlier floral senescence and related it to the overall water budget.

Materials and methods

Study species and site

Alstroemeria umbellata Meyen (Alstroemeriaceae) is a fleshy rhizomatous perennial herb found mostly between 2500 and 3000 m a.s.l. on scree slopes (Fig. 1a). It is characterized by attractive pink flowers (Fig. 1b). Individual plants form loose clumps of ramets. Each ramet supports a single terminal inflorescence (umbel) typically containing 4–6 tightly clustered flowers (Fig. 1c). The flowers are visited by the native bee, *Megachile semirufa* (Megachilidae) (Fig. 1c) and the high-elevation hummingbird, *Oreotrochilus leucopleurus*. *Alstroemeria umbellata* is self-compatible with some capacity for autonomous selfing (Supplementary Material 1, Table S1).

Flowers are characterized by sequential hermaphroditism whereby the male stage (Fig. 2a, b) precedes the female stage (Fig. 2c). The sexual stages are separated by a short neutral stage. Dehiscence of the 6 anthers is staggered over a number of days. During the male and neutral stages, the style elongates out of the corolla followed by separation of

Fig. 1 **a** *Alstroemeria umbellata* in its natural habitat showing ramets with their single inflorescences. **b** Cluster of inflorescences on a second plant. **c** *Megachile semirufa* (Megachilidae) visiting a male-stage inflorescence with six flowers. All images were taken on the study site, 2700 m a.s.l. in the central Chilean Andes, 33° S

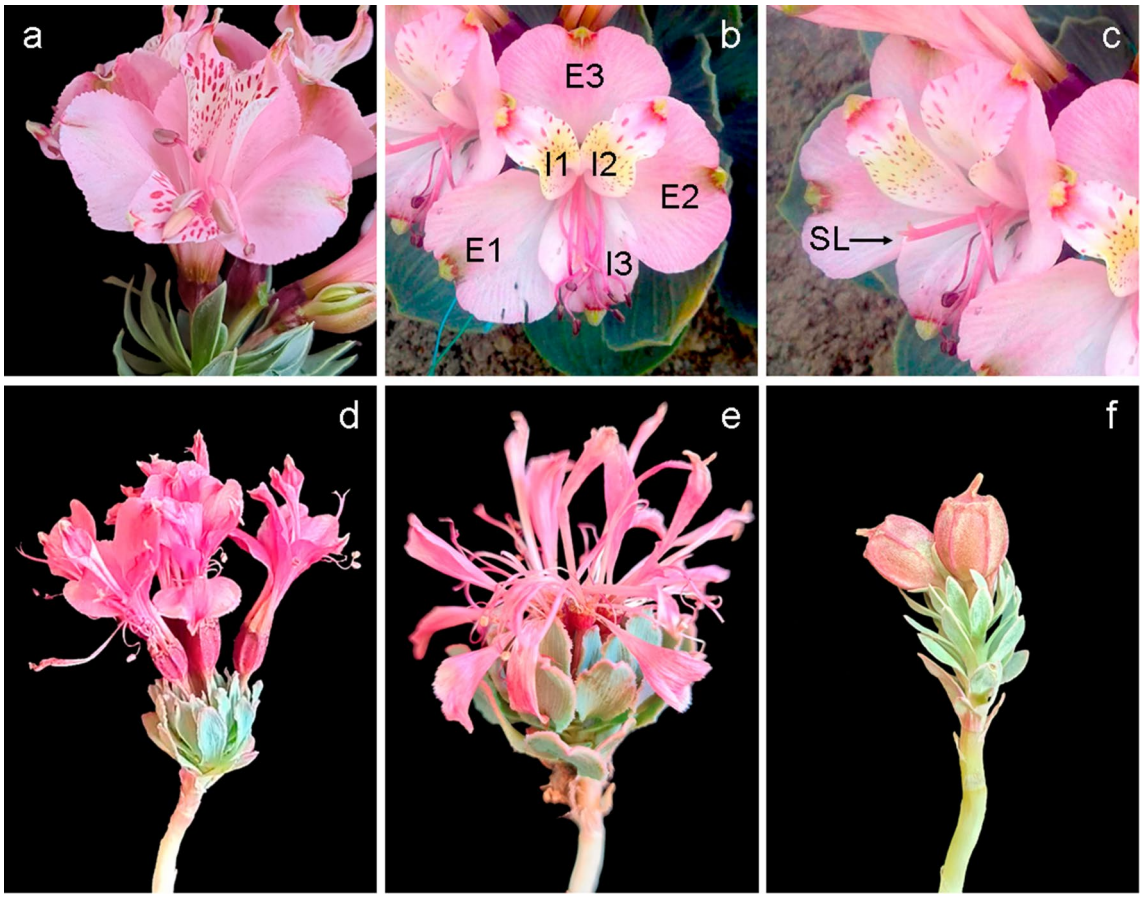

Fig. 2 Stages of floral development and fruits in *Alstroemeria umbellata*. **a** Early male-stage flower showing three closed and three dehisced anthers. **b** Late male-stage flower showing the elongating style and identification of the 6 individual tepals. E1, E2, E3, external tepal whorl; I1, I2, I3, internal tepal whorl. **c** Female-stage flower showing the three open receptive stigmatic lobes (SL). **d** Inflorescence with flowers in an advanced stage of dehydration. **e** Inflorescence with the flowers beginning to undergo tepal abscission. **f** Fruits in the early stage of development

the three stigmatic lobes. Prior observations showed abundant peroxidase activity on the inner surface of the open stigmatic lobes. During the dehydration stage, the tepals become erect and darker in color (Fig. 2d) to finally abscise (Fig. 2e). Flowers were not seen to be visited by pollinators at this stage. Therefore, in this paper we distinguish between the *functional flower lifespan* defined as when the tepals are turgid, and the *total flower lifespan* up until when the tepals abscise (Fig. 2e). Two- and 6-day-old pollinator-excluded flowers contained nectar volumes averaging 1.50 µl ($N=26$ flowers on 12 inflorescences) and 1.38 µl ($N=24$ flowers on 10 inflorescences), respectively. The fruit (Fig. 2f) is a trilocular capsule containing large globose seeds.

Work was conducted in the Andes east of Santiago in the Valle Nevado area (33°21′37.36″ S, 70°15′31.37″ W, 2700 m a.s.l.). Soil water potential measured at 2900 m a.s.l. decreases from −1.6 MPa in November to −4.5 MPa in March (Sierra-Almeida et al. 2009). Less than 5% of annual precipitation is received from January through March (Supplementary Material 1, Fig. S1). The plants studied occur on the upper reaches of a large steep scree slope (Fig. 1a). The manipulative experiment and work on the open-pollinated flowers took place in January 2020. Sampling for water content was undertaken in January 2022.

Pollen-import and pollen-export experiment

Well-developed flowers buds were selected on 59 inflorescences found on 41 clumps. The buds were randomly assigned to four treatments: (1) un-manipulated control (CON) ($N=71$); (2) hand cross-pollination (HCP) ($N=73$); (3) hand cross-pollination and manual pollen removal (PRP) ($N=69$); (4) manual pollen removal without hand cross-pollination (PRW) ($N=70$). Hand cross-pollination took place when the three stigmatic lobes first opened and were humid. The same pollen source collected from several plants growing at least five meters from the experimental plants each day was used for HCP and PRP. Stigmas were pollen-saturated

usually three times and minimally twice. Removal of pollen from the anthers was with a fine camel-hair paint brush. All inflorescences were pollinator-excluded with pollination bags from the bud stage to the end of the experiment. Plants exposed to passing animals were protected with large wire netting exclosures to prevent damage.

Floral observations and manipulations were undertaken twice daily during the following time periods: 9–14 h ("morning" block) and 14–19 h ("afternoon" block) (Standard Time) over 14 continuous days. During each time period, we recorded the sexual status of the flower and whether the flower was turgid or dehydrating and/or undergoing tepal abscission. Eleven days into the experiment, due to a logistic problem, some plants were observed only in the morning and others could not be observed that day. At this point, all of the experimental flowers had lost turgor. All flowers were examined for fruit formation. Well-developed and/or clearly aborted seeds, as seen under a binocular microscope, were considered as evidence of successful pollination.

Floral responses in flowers exposed to open pollination

We marked two well-developed buds per inflorescence ($N = 42$) on 21 additional plants. These inflorescences were neither bagged nor caged so as not to restrict visits by bees and hummingbirds. All flowers were observed as per in the manipulative experiment and assessed for fruit formation. Assessment of the amount of pollen removed by pollinators in these flowers was not undertaken.

Environmental variables

Hourly temperature (°C) and relative humidity (%) were recorded with a HOBO U23 Pro v2 battery-powered sensor (Onset Computer Corp., Cape Cod, MA, USA). The sensor was placed 15 cm above-ground level to represent the height of the inflorescences and protected from solar radiation with an inverted white cardboard cup.

Water content of flowers and leaves

Twenty-five ramets (1 per plant) containing male-stage and female-stage flowers (see Fig. 2 for stages), respectively, were excised around midday. The material was transported to a field lab over commercial ice packs. All leaves of each ramet were separated from the stem, discarding the latter. The total number of flowers per inflorescence was recorded. Following measurement of floral width between the tips of tepals E1 and E2 (Fig. 2b), two flowers per inflorescence ($N = 50$ per stage) were individually separated into the tepals and remaining floral parts. All material was immediately weighed on a field balance (Ohaus Scout STX223 model, 220×0.001 g). The material was reweighed on a precision balance (Denver Instrument Company) in g to four decimal places, following drying in a laboratory oven for 3 days at 70 °C. To estimate the amount of water lost during tepal dehydration, 47 dehydrating flowers derived from 25 additional inflorescences (1 per plant) were harvested and processed.

Data analysis

All statistical analyses and preparation of figures (excepting images) were carried out in R version 3.6.3 (R Core Team 2020).

Pollen-import and pollen-export experiment and open pollination

From the detailed field observations on the experimental flowers, we calculated the time spans for the following floral stages and flower longevity: (1) Tepals fully turgid (= functional flower lifespan); (2) Male stage; (3) Neutral stage; (4) Female stage; (5) Tepal dehydration. Total flower lifespan (6) was also calculated. To evaluate the effect of pollination in the open-pollination flowers, time spans were obtained for 1, 4, 5 and 6.

Prior to analysis, some of the experimental flowers were eliminated due to natural infestation by *Aphis alstroemeriae* (Aphididae), severe inflorescence drying, floral developmental problems and the loss of few flowers at the fruiting stage. Fruits formed in some CON and PRW flowers. Fruits failed to form in some HCP and PRP flowers where they was expected. These two groups of flowers were eliminated to prevent dilution of the potential effects of pollen-export and import. Pollination may be successful but still not lead to fruit and seed formation if fertilization is inhibited in some other way. Examination of pollen tube growth on stigmas and in styles, necessary to ascertain this, is not easily accomplished when flowers must be left intact for monitoring. By eliminating HCP and PRP flowers that failed fruit, we took a conservative approach. Some additional experimental and open-pollinated flowers affected by the observation gap on day 11 were also eliminated in the analyses of the later floral stages. In 2.1% of flowers, tepal abscission occurred very rapidly. In these, we assigned the dehydration stage 0 days. Final samples sizes are given in Tables 1 and 2.

The effect of treatment in the manipulative experiment was analyzed with One-Way ANOVA followed by pairwise post hoc comparisons. The morning and afternoon observation periods were considered as two half days expressed as fractions of a full day. In view of lack of normality and non-homogeneous variances, data were analyzed using the welch_anova_test and games_howell_test functions in the "rstatix" version 0.4.0 package (Kassambara 2020). To

Table 1 Time spans for two measures of flower longevity and four floral stages (in days) in *A. umbellata* and results of ANOVA to detect the effect of different treatments on these

Flower longevity/floral stage	Treatments								Num. df	Den. df	*F*	*p*
	CON		HCP		PRP		PRW					
	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*	Mean ± SD	*N*				
Functional fl. Lifespan	7.4 ± 1.2^{a}	37	6.8 ± 0.7^{b}	50	6.5 ± 0.8^{b}	53	7.6 ± 1.2^{a}	48	3	93.9	12.47	**0.000**
Dehydration stage	1.3 ± 0.8^{a}	22	1.9 ± 0.8^{b}	37	2.0 ± 0.8^{b}	34	1.4 ± 0.8^{ab}	33	3	62.7	5.33	**0.002**
Total flower lifespan	9.2 ± 1.0^{ab}	22	8.8 ± 0.6^{b}	37	8.7 ± 0.6^{b}	34	9.4 ± 0.8^{a}	33	3	59.6	7.46	**0.000**
Male stage	3.6 ± 0.7^{a}	37	3.6 ± 0.7^{a}	49	3.5 ± 0.7^{a}	53	3.6 ± 0.7^{a}	48	3	98.4	0.32	0.81
Neutral stage	0.8 ± 0.6^{a}	36	0.8 ± 0.7^{a}	49	0.7 ± 0.6^{a}	53	0.6 ± 0.7^{a}	48	3	98.3	0.67	0.58
Female stage	3.1 ± 1.3^{a}	33	1.6 ± 0.7^{b}	49	1.5 ± 0.7^{b}	52	3.6 ± 1.4^{a}	46	3	85.3	41.1	**0.000**

CON, control; HCP, hand cross-pollination; PRP, manual pollen removal combined with hand cross-pollination; PRW, manual pollen removal without hand cross-pollination. *N*, number of flowers. Functional fl. Lifespan, functional flower lifespan. Significant treatment effects ($p < 0.05$) are shown in bold. Different letters indicate significant differences between treatments according to the Games–Howell test. See Methods for definition of floral stages and Fig. 2 for stages

Table 2 Results of *t*-tests to detect differences in the time spans of different floral stages (in days) for flowers exposed to open pollination that formed (FRU) and did not form fruits (WFR)

Flower longevity/floral stage	FRU		WFR		Df	*t*	*p*
	Mean ± SD	*N*	Mean ± SD	*N*			
Functional flower lifespan	6.6 ± 0.9^{a}	19	6.4 ± 0.9^{a}	15	30.2	0.665	0.511
Dehydration stage	1.4 ± 0.6^{a}	17	1.6 ± 0.7^{a}	13	24.1	− 0.977	0.338
Total flower lifespan	8.1 ± 0.7^{a}	17	8.3 ± 0.9^{a}	13	22.8	− 0.520	0.608
Female stage	3.4 ± 1.1^{a}	20	2.7 ± 1.4^{a}	15	25.5	1.51	0.143

The same letters indicate non-significant differences for fruiting and non-fruiting flowers at $p < 0.05$ (Welch *t*-test)

test for the effect of pollination in the open-pollination plants, flowers were separated into fruiting versus non-fruiting flowers. Differences between the two categories were analyzed with the *t*-test for independent samples with Welch´s adjustment using the "rstatix" package, version 0.4.0 (Kassambara 2020).

Temperature can affect flower longevity (e.g., Arroyo et al. 2013; Teixido and Valladares 2015; Dudley et al. 2018). When pollen-import floral senescence is present, non-pollinated flowers will start dehydrating later than pollinated flowers. Thus, in a field experiment, pollinated and non-pollinated flowers could experience different temperature conditions. The same would be true for the female stage in a species with sequential hermaphroditism if pollen removal shortens the male stage. Such temporal differences could lead to spurious results if the effect of temperature is not taken care of. The functional flower lifespan and the total flower lifespan are measured as of the moment the flower buds open. Although there will always be some differences in the time flower buds open, even when all are marked on the same day as in the present case, in a well-randomized experiment, the small differences will be spread equally over all treatments such that spurious effects are unlikely.

To assess the effect of temperature and its interaction with treatment, the data were analyzed with ANCOVA. The initial models included treatment, temperature and treatment x temperature. Temperature was mean hourly temperature per half day. The first half day was defined as the duration of the "morning" observation block plus the preceding 6 h. The second half day included the "afternoon" observation block plus the following 6 h. Under these conventions, all 24 h were covered. The best-fit models were selected according to the Akaike Information Criterion (AIC), the amount of variance explained, and the variance inflation factor (VIF). Analyses were carried out using the stepAIC and vif functions in the "MASS" and "car" packages, respectively (Venables and Ripley 2002; Fox and Weisberg 2019). In general, the residuals of the fitted models, the pollination treatments and the covariate were not normal; therefore, the probability values were estimated using a permutation test with the aovp function of the "lmPerm" package (Wheeler and Torchiano 2016).

Water content of flowers and leaves

Absolute amount of water was calculated from the fresh and dry weights. Percent water content was obtained by dividing

the difference between fresh weight and dry weight by fresh weight. Prior to analysis, percentage values were arcsine transformed. Differences between male- and female-stage flowers were analyzed with *t* tests or the Mann–Whitney *U* test, depending on data normality.

Results

Pollen-import and -export floral responses in experimental flowers

The weather was mostly warm and dry (Fig. 3a) except for a light late afternoon shower toward the end of the experiment. At this stage the great majority of the tepals had fallen. Hourly relative humidity and temperature were significantly negatively correlated (Fig. 3b). However, as seen by the spread around the trend line in Fig. 3b, over short time scales, there were times when high temperatures were associated with low relative humidity and vice versa.

ANOVA showed that treatment had a highly significant effect on the time spans of the functional flower lifespan, dehydration stage, total flower lifespan and female stage but not on the duration of the male and the neutral stages (Fig. 4, Table 1). The two treatments involving pollination, independently of pollen removal, significantly shortened the functional flower lifespan (Fig. 4a). Overall, the tepals remained turgid for a mean 6.7 days in the two pollination treatments versus 7.5 days in those lacking pollination (Table 1). In parallel to these results, the stigma lobes remained open significantly less time in the pollinated flowers (Table 1, Fig. 4f). Contrary to expectation, the dehydration stage, which never exceeded a mean of two days (Table 1), was significantly longer in the pollinated flowers (Fig. 4b). For the total flower lifespan, in marked contrast to the functional flower lifespan, the results for pollination alone and in combination with pollen removal were not significantly different from the control, although PRP and PRW were different (Fig. 4c). Overall, the total flower lifespan lasted an average of 9.3 days in the two treatments where hand pollination had not been performed (Table 1). Compared to the control, pollen removal had no significant effects (Fig. 4a–f). Nor did it magnify the pollination effect when combined with the latter.

ANCOVA showed temperature had a significant effect on four of the six variables (Supplementary Material 1, Table S2). The best ANCOVA models for the functional flower lifespan, total flower lifespan, and female stage, failed to include the interaction term between temperature and treatment. Therefore, the conclusions drawn from the respective ANOVAs (Fig. 4, Table 1) can be confidently put down to an effect of pollination. In contrast, the best model for the dehydration stage included a significant temperature x treatment interaction. Therefore, in this case, we cannot be sure that the significant effect of pollination as per the ANOVA results is totally independent of temperature. The last result was not unexpected given that the non-pollinated and pollinated flowers would have experienced different temperature conditions.

The counterintuitive results for the dehydration stage raised the question as to whether other factors beyond temperature are involved. Figure 5 shows the relationship between length of the dehydration stage and when dehydration began in relation to the start of the experiment. Dehydration time became progressively shorter as the tepals began to dehydrate later in both the pollinated and unpollinated flowers. The slopes and intercepts of the regression lines for the two sets of flowers were not significantly different (Chow's test: $F_{2,12} = 3.132$, $p = 0.08$).

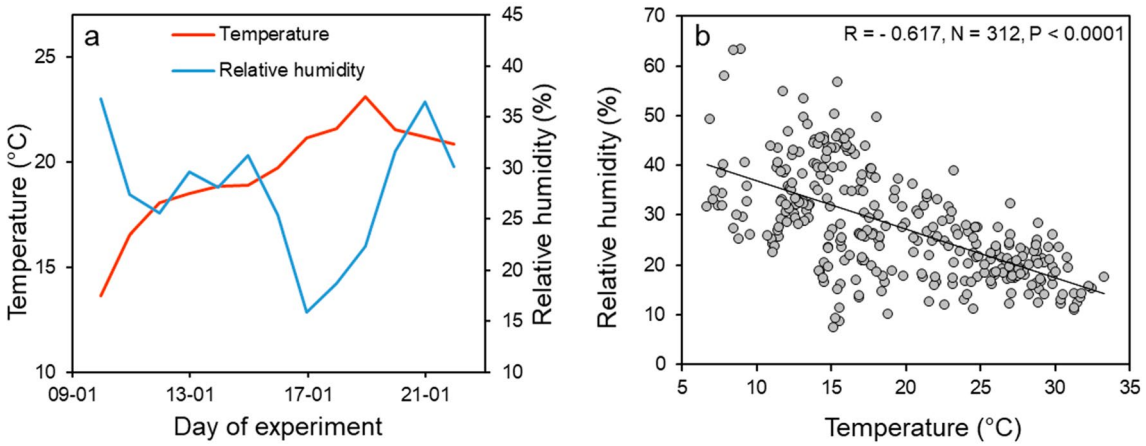

Fig. 3 **a** Mean daily temperature and relative humidity at 15 cm a.g.l recorded at 2700 m a.s.l. in January 2020. **b** Relationship between hourly temperature and relative humidity over the same period. Data are from the day the first flower opened to when the last flower lost its tepals

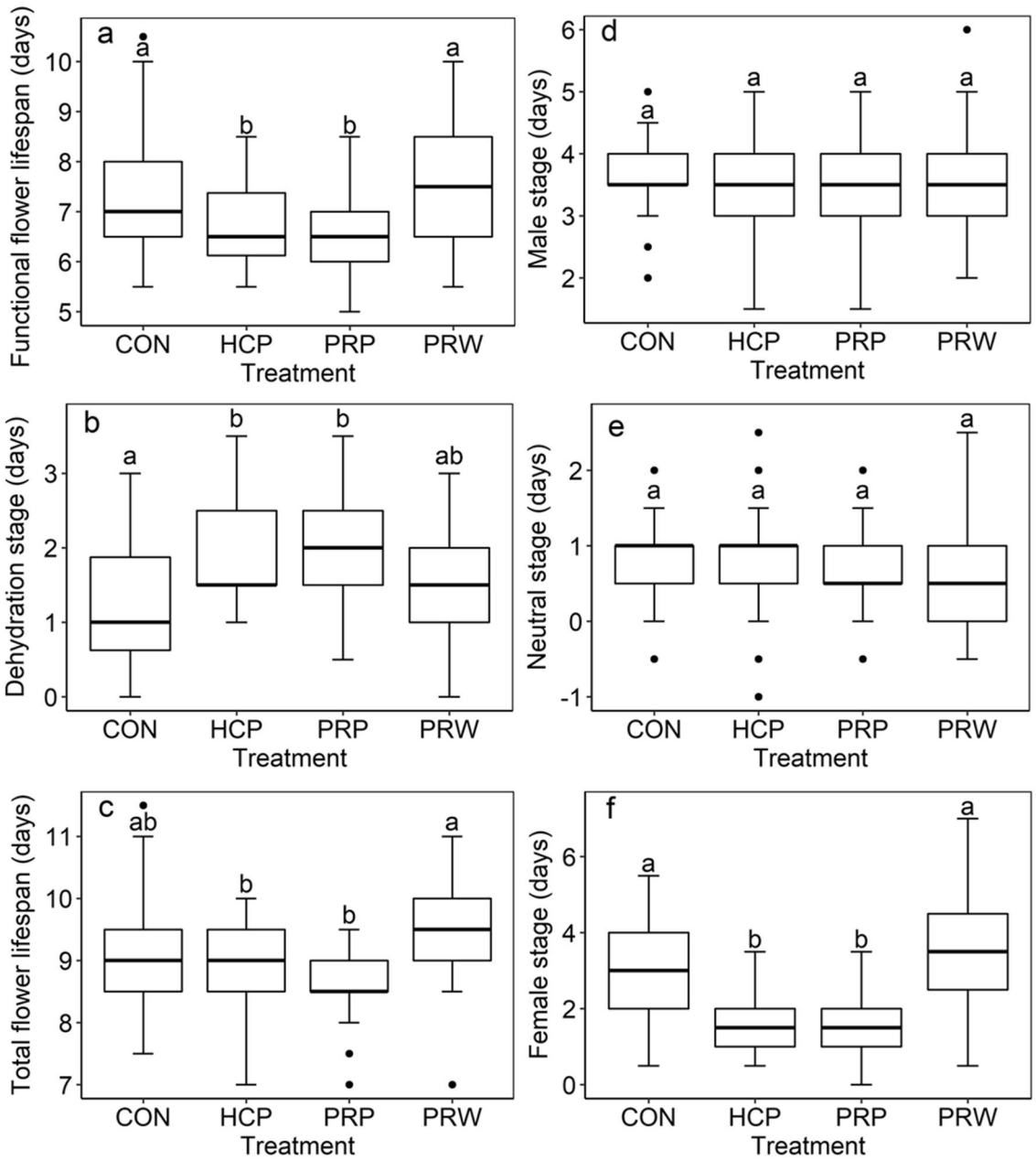

Fig. 4 Results of the ANOVA for the effect of different treatments on two measures of flower longevity and the time spans of four floral stages. CON, control; HCP, hand cross-pollination; PRP, manual pollen removal combined with hand cross-pollination; PRW, manual pollen removal without hand cross-pollination. See Table 1 for ANOVA statistics and sample sizes. Different letters indicate significant differences between treatments ($p < 0.05$) according to the Games–Howell test

Floral responses in flowers exposed to open pollination

Fruit set occurred in 52.6% of flowers exposed to open pollination. Open pollination, as indicated by fruit set, had no significant effects on any of the variables (Table 2). Temperature showed some effects (Supplementary Material 1, Table S3). However, a significant interaction between temperature × fruiting only appeared in the best model in the case of the female stage. The latter indicates fruiting and non-fruiting female stage flowers were affected differently by temperature (Table 2). This cautions against accepting the results of the *t*-test in Table 2 for this floral stage at face value.

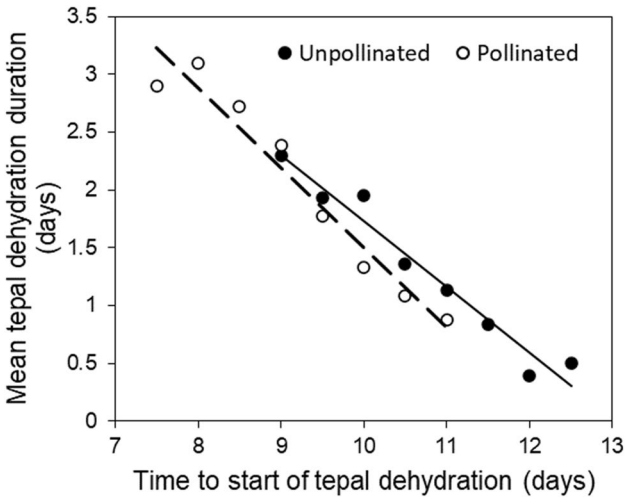

Fig. 5 Relationship between mean time span of tepal dehydration and days after the beginning of the experiment to when flowers began to dehydrate in hand-pollinated (HCP + PRP) and unpollinated flowers (CON + PRW). The beginning of the experiment was the day the first flowers became fully open. The slopes and intercepts of the two regression lines are not significantly different (see text for statistical details)

Water content of flowers and leaves

The single inflorescence borne on a ramet contained over three times as much water as the complete set of leaves on the ramet (Table 3). Over half of the water contained in a flower was found in the tepals. The percentage of water in whole flowers and the tepals was over 91% and higher than in leaves (84.8–87.6%) (Table 3). Percentage water was somewhat higher in male- compared to female-stage tepals and whole flowers. Also, frontal flower width was larger in female flowers. The tepals of a single flower at the advanced dehydration stage contained 0.19 ± 0.03 g (Mean ± SD) of water. This boils down to 0.14 g (42.4%) less water than contained in the tepals of the turgid female-stage flowers. The dehydration stages lasted around 1.5 days in the open-pollinated plants (Table 2). The amount of water lost per day in a dehydrating inflorescence thus is around 0.09 g multiplied by the mean number of flowers per inflorescences (5.6) which gives 0.50 g. Comparison with water contained in the leaves of a ramet (Table 3) shows the amount of water lost by the inflorescence in one day is very high.

Discussion

Pollen-export and -import induced floral senescence

Hand cross-pollination in *A. umbellata* produced a shortening of the functional flower lifespan and the female stage. The reduction in the functional flower lifespan is the equivalent of a 10.7% decrease over that in non-pollinated flowers. These findings contrast with the lack of a pollination effect on flower longevity in *Alstroemeria aurea* (Aizen and Basilio 1998), a species found in much wetter areas in the southern Andes. Results for *A. umbellata* and *A. aurea*, taken together, are consistent with the prediction that pressures to plastically reduce the flower lifespan after pollination will be greater in species of dry habitats than in species of wet habitats so as to economize on plant water. However, as will be shown below, the total amount of floral water saved under natural conditions of pollination in *A. umbellata* via this mechanism is expected to be far less than predicted by the manipulative experiment.

Manual pollen removal had no effect on flower longevity and thus provided no potential benefits in terms of economizing on floral water. Floral design constraints could be relevant in explaining the different responses of the female and male stages in *A. umbellata* flowers. The stigmatic lobes open and become receptive only after a long period of stylar elongation which occurs coeval with the male and neutral stages. Pollen-export reduction of the male stage, and, by default the functional flower lifespan, would lower the amount of time the stigma lobes remain open, thereby diminishing the adaptive value of the long-lived flowers—to buffer species against uncertain pollination. In contrast, a shortened functional flower lifespan following pollination would have no negative consequences for either female or male fitness. Male fitness depends on events that occur before stigma receptivity. On the other hand, once a stigma has been pollinated, there is little advantage in the flower remaining open in a species like *A. umbellata*. Male fitness is benefited when pollinator visits are spread out

Table 3 Water metrics for tepals, whole inflorescences and leaves of *A. umbellata*

Stage	FFW (cm)	Water content (g) (Mean ± SD)				Water (%) (Mean ± SD)		
		Tepals	Flowers	Inflorescence	Leaves	Tepals	Flowers	Leaves
M	3.32 ± 0.40^{a}	0.35 ± 0.06^{a}	0.64 ± 0.08^{a}	3.77 ± 1.54^{a}	1.19 ± 1.78^{a}	91.91 ± 0.96^{a}	92.70 ± 0.86^{a}	84.82 ± 14.37^{a}
F	3.51 ± 0.39^{b}	0.33 ± 0.06^{a}	0.62 ± 0.08^{a}	3.28 ± 1.04^{a}	0.87 ± 0.67^{a}	91.40 ± 0.71^{b}	92.13 ± 1.78^{b}	87.55 ± 1.19^{a}

M, male; F, female; FFW, frontal flower width. Data are for flowers with turgid tepals. Leaves refer to the complete set of leaves on the ramet that bore the single inflorescence. Different letters indicate significant differences according to the Mann–Whitney *U* test or *t*-test ($p < 0.05$)

(pollen-donation hypothesis) (Broyles and Wyatt 1990) as would be the case in *Alstroemeria* flowers with staggered anther dehiscence. This could place an additional constraint on the alteration of the male stage.

Studies on flower longevity usually consider the total flower lifespan. In terms of the benefits of pollen-export and -import induced floral senescence in relation to floral water maintenance costs, of interest here, in *A. umbellata* the functional flower lifespan, defined as when the corolla is turgid, is more relevant than the total flower lifespan, defined up to the point when the tepals finally abscise. By studying the two expressions of flower longevity, we found the tepals in pollinated and non-pollinated flowers abscised around the same time, independently of whether dehydration began earlier (as in the pollinated flowers) or later (as in non-pollinated flowers). This could reflect a fortuitous climatic event (like strong wind gusts) loosening the tepals in all flowers, causing those of the later dehydrating non-pollinated flowers to fall earlier than expected. However, it could also indicate that the earlier wilting tepals in pollinated flowers were forced to "wait longer" to undergo abscission until a preprogramed signal was issued to shut the flower down. This could imply different processes are involved in terminating the functional flower lifespan following pollination vs the total flower lifespan. Wilting (dehydration under our nomenclature) and tepal abscission in virgin flowers of *Alstroemeria* are ultimately a product of Programmed Cell Death (PCD) (Chanasut et al. 2003; Wagstaff et al. 2003, 2005; Breeze et al. 2004). Despite the huge amount of work on cultivated varieties of *Alstroemeria,* to our knowledge, PCD has not been investigated in pollinated flowers of *Alstroemeria*. This is critical point for understanding the responses of our species to pollination.

Role of pollination-associated floral senescence in lowering floral maintenance costs

To evaluate the floral maintenance hypothesis (Webb and Littleton 1987; Ashman and Schoen 1994), information is needed on: (1) how much water is at stake in flowers, and; (2) whether flowers under natural conditions of pollination close as fast as they do when submitted to experimental hand pollen cross-pollination on the first day the stigma becomes receptive. With respect to the first point, the single inflorescence per ramet in *A. umbellata* was shown to contain over three times as much water as contained in all leaves on the subtending ramet. Thus, flowers contain a significant proportion of above-ground plant water and a much larger volume of water that could be lost to transpiration than in the leaves. How much of the water is actually lost will depend on factors such as the presence of stomata on the tepals and whether they are functional, and cuticle properties. There are of course flowers that contain much more water than those of *A. umbellata*, as for example the giant flowers of *Magnolia grandiflora* (Feild et al. 2009) and large flowers of neotropical species of *Kielmeyera* (Teixido et al. 2019). However, it is unknown what proportion of the total water in the branches of these species is found in flowers versus leaves.

With respect to the second point, the 10.7% decrease in the potential duration of the turgid tepals following hand cross-pollination signifies that an equivalent of ~ 11% of the water transpired by the tepals of non-pollinated flowers would be saved through pollination-induced floral senescence. To arrive at the amount of water saved and its physiological relevance, data are required on leaf and tepal transpiration rates on ramets whose flowers have been pollinated and not pollinated. This was beyond the scope of the present study. The amount of water lost per day by an inflorescence in the advanced dehydrating stage was very high indicating fairly high floral transpiration rates under the prevailing climatic conditions. Nevertheless, under real-world natural pollination conditions, far less than ~ 11% of tepal water is likely to be saved through pollination-induced floral senescence in our species. This may be deduced from the results for the open-pollinated flowers where no significant effect of fruiting was found on the functional flower lifespan. These results tell us that no water would be saved at all through the floral senescence mechanism in some years or, perhaps, during part of the flowering season.

The contrasting projections arising from the manipulative experiment and the open-pollinated flowers beg an explanation. Fruit set in the open-pollination flowers (52.6%, $N = 38$) was significantly lower than in the total sample of experimental hand cross-pollinated flowers (85.3%, $N = 120$) (Test for Proportions, $p = 0.00017$), indicating the existence of pollen limitation. As a consequence, many of the flowers that were open-pollinated, unlike the experimental flowers, probably had to wait some time until they were adequately pollinated. Gradual pollen deposition has been documented in a number of outcrossing species with fairly long-lived flowers in alpine ecosystems (Wagner et al. 2016; Arroyo et al. 2017) and both the flower lifespan and stigma longevity have been shown to be sensitive to pollen dosage levels and pollinator abundance (Clark and Husband 2007; Castro et al. 2008; Spigler 2017; Trunschke and Stöcklin 2017). Such effects would tend to explain the non-significant difference in the duration of the functional flower lifespan in the flowers observed under open pollination that did and did not fruit. The take home message is that manipulative experiments conducted in species with long-lived flowers will tend to overestimate the true reduction in water and other maintenance costs attained through pollination-induced flower senescence under natural conditions of pollination.

Conclusions

Although *A. umbellata* flowers contain a large proportion of above-ground plant water and experimental pollination reduces the functional flower lifespan, considerable saving of floral water on account of the possession of pollination-associated floral senescence under natural circumstances is only expected in very exceptional pollination years. Effectively, our species is endowed with a mechanism for lowering floral water maintenance costs, but taking full advantage of it will not always be possible. That the functional and total flower lifespans of pollinated flowers were decoupled suggested different mechanisms might be involved in tepal abscission and the loss of turgor following pollination. Perhaps, the loss of turgor after pollination is due to the flow of water to the tepals being diverted to the ovaries and their developing zygotes. Detailed physiological work using stable isotope labeling to learn where incoming water from the xylem (or phloem) ends up after pollination is required to evaluate this hypothesis. It would also be useful to know how much water occurs in the underground rhizomes.

Finally, our work underscores the importance of incorporating environmental variables into analyses of flower longevity to avoid spurious conclusions. Monitoring flowers more frequently than once a day is advisable given that the effects of pollen import and export on floral senescence in many species are likely to be subtle. Taking the precaution of eliminating fruiting flowers in treatments where hand cross-pollination had not been performed and vice versa likewise increases the probability of detecting subtle effects by eliminating unwanted noise. Lastly, if we had not separated the functional and total flower lifespans, their tendency to be decoupled in pollinated flowers would not have been discovered. To advance in our understanding of the fascinating subject of floral maintenance costs, more work is needed in the domain of plant physiology. Alpine plants, in general, are a good place to start given their high resource allocation to flowers versus in stems and leaves.

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Author contributions MTKA and AS-A conceived the research. MC-D, VR and MTKA carried out the field experiments and observations. MTKA, PMV and PJ-A undertook the water sampling in flowers. VR and PJ-A sampled the nectar. The data were analyzed by MC-D, ÍT and MTKA. MTKA, MC-D, ÍT and PJ-A prepared the first version of the paper, which was revised and improved by the coauthors. All authors read and approved the final version of the paper.

Availability of data and materials All data generated or analyzed during this study are included in this published article [and its supplementary information files; see Supplementary Material 2 for original data for this study].

Declarations

Conflict of interest The authors declare that the corresponding author is part of the Editorial Board of Alpine Botany. There are no other conflicts of interest.

Ethical approval This study does not involve research on human participants or animals.

Consent to participate Not applicable.

Consent for publication Not applicable.

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