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Behavioural Thermoregulation of Flowers via Petal Movement

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ABSTRACT

Widely documented in animals, behavioural thermoregulation mitigates negative impacts of climate change. Plants experience especially strong thermal variability but evidence for plant behavioural thermoregulation is limited. Along a montane elevation gradient, *Argentina anserina* flowers warm more in alpine populations than at lower elevation. We linked floral temperature with phenotypes to identify warming mechanisms and documented petal movement and pollinator visitation using time-lapse cameras. High elevation flowers were more cupped, focused light deeper within flowers and were more responsive to air temperature than low; cupping when cold and flattening when warm. At high elevation, a 20° increase in petal angle resulted in a 0.46°C increase in warming. Warming increased pollinator visitation, especially under cooler high elevation temperatures. A plasticity study revealed constitutive elevational differences in petal cupping and stronger temperature-induced floral plasticity in high elevation populations. Thus, plant populations have evolved different behavioural responses to temperature driving differences in thermoregulatory capacity.

1 | Introduction

Behavioural thermoregulation—flexibility in response to the thermal environment which alters organismal temperature—reduces physiological stress caused by climate change (Beever et al. 2017; Kearney, Shine, and Porter 2009). While plants have the capacity to respond rapidly to environmental change (Silvertown and Gordon 1989) and thermoregulate (Mahan and Upchurch 1988; Michaletz et al. 2015), the study of behavioural thermoregulation has been almost entirely restricted to animals with the obvious capacity for movement and migration (Kearney, Shine, and Porter 2009). Plant behaviour, however, is common. Individual plants exhibit physical and physiological responses to external stimuli (Karban 2008; Silvertown and Gordon 1989) like physical touch (Braam 2005), mechanical injury (Armbruster and Muchhala 2020) and light (van Loon 2016). Because plants are largely sessile, with limited migratory capacity compared to many animals, they experience substantial thermal variability. Thus, behavioural thermoregulation may be crucial for the persistence of plants under altered climatic regimes and the increased frequency of extreme weather events.

In flowering plants, reproduction is particularly temperature sensitive. First, gametophytes (pollen and ovules) are temperature-sensitive with viability declining at low and high temperature extremes (Heiling and Koski 2024; Sherer, Heiling, and Koski 2024). Second, in animal-pollinated plants (~90% of angiosperms, Ollerton, Winfree, and Tarrant 2011), pollination is temperature dependent (Herrera 1995; Bishop and Armbruster 1999). For instance, most insect pollinators require

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a minimum threshold for activity, so they may favour warmer flowers under cool conditions (McCallum, McDougall, and Seymour 2013). However, pollination can decline at extremely high temperatures. In fact, some pollinators must take 'cooling flights' during foraging (Corbet and Huang 2016), so flowers that are too warm could experience poor pollination services. Thus, the effect of floral temperature on pollinator recruitment is likely dependent upon air temperature.

Barring plants with thermogenic flowers, most flowers must absorb or release thermal and solar radiation to warm or cool their floral environment, respectively (van der Kooi, Kevan, and Koski 2019). Solar tracking (heliotropism), a well-studied phenomenon whereby flowers orient to the sun, impacts floral temperature, insect visitation (Atamian et al. 2016) and seed production (Stanton and Galen 1989). Mechanisms of floral cooling, or those that limit the accumulation of solar heat, are less studied than warming but should be especially important for reproductive output under global change (Haverkamp et al. 2019; Creux et al. 2021). Evaporative cooling (Galen 2006; Patiño and Grace 2002) and shading of reproductive structures by petals (Karban, Rutkowski, and Murray 2023) may enhance reproduction under high heat.

The cupped, parabolic shape of many flowers focuses solar radiation reflected from petals onto thermally sensitive gametophytes (pollen and ovules) which should increase gametophyte viability and pollinator recruitment in cold environments (Kevan 1975). Following this logic, flowers with less cupped petals should reflect solar radiation outside of the floral microenvironment, reducing heat accumulation. Many angiosperms open and close petals repeatedly throughout the floral lifespan (van Doorn and Kamdee 2014; van Doorn and van Meeteren 2003), often in response to changes in temperature or light (Trivellini et al. 2016). Such responses to external environmental cues should alter the focus of solar radiation and modify floral temperature. Flower petals may therefore act as adjustable 'solar reflectors' (Kevan 1975), providing a mechanism of behavioural thermoregulation. A similar strategy is employed by butterflies whereby the angle at which wings are held during basking modifies the intensity of light focused onto the body (Barton, Porter, and Kearney 2014; Kevan and Shorthouse 1970; Kingsolver 1985). The role of petal angle in shaping floral temperatures is not well studied, but differences between populations in constitutive petal angle, or the capacity to adjust petal angle could contribute to differential floral thermoregulation.

The influence of petal angle on floral temperature could depend on several additional floral traits (van der Kooi, Kevan, and Koski 2019). For instance, flower colour influences the absorption of solar radiation, with lighter more reflective flowers having lower temperatures (Lacey et al. 2010). Long-wavelength reflection/absorption (NIR-IR) should be most important for organismal temperature because heat-carrying capacity increases with wavelength (Medina et al. 2018; Stuart-Fox, Newton, and Clusella-Trullas 2017). Size attributes, like petal surface area and mass, may also affect floral temperature—larger surfaces should capture more solar radiation, while higher mass may increase heat retention. While associations between floral traits and temperature have been surveyed for different species (Shrestha et al. 2018), to date, there have been no evaluations of whether floral trait variation among populations influences floral temperature. Population-level differences in temperature-induced plasticity for flowering time (Anderson et al. 2012; Ramirez-Parada et al. 2024), flower longevity (Arroyo et al. 2013) and size (Wiszniewski et al. 2022) are well documented, but we know little about population differentiation in temperature-induced plasticity in petal movement. Identifying population-level differences in floral traits that may impact floral temperature, and temperature-induced plasticity can provide insight into whether such differences are consistent with local adaptation.

Silverweed cinquefoil (*A. anserina*) is a temperate perennial herb widely distributed in the northern and southern hemispheres. Petals open and close diurnally and persist for 2 days. Petal movement is manifested through the alteration of petal angle and corresponding changes to flower width and depth (Figure 1A). More cupped petals (Figure 1B) should focus light inside the flower, whereas flatter petals (Figure 1C) should reflect light outside the flower (Kevan 1975). Previous work spanning a montane elevation gradient in southwestern Colorado demonstrated that floral interiors of this species warmed further above external flower-level air temperature during peak solar radiation in populations > 3000 m than those < 2600 m (Heiling and Koski 2024). Enhanced warming at high elevation brings the floral environment closer to the thermal optimum for pollen viability, while reducing heat accumulation at low elevation mitigates pollen overheating. Here, we address whether floral traits predicted to be associated with floral warming differed between low and high elevation populations in the field. We then associated phenotypic variation with the magnitude of warming and assessed the impact of warming on pollinator visitation. Finally, we tested whether there is a genetic contribution to elevational divergence in warming-associated floral traits and quantified temperature-induced floral plasticity. We address the following:

1. Do traits with the potential to influence floral temperature differ between low and high elevation populations?
2. Does air temperature affect the expression of potential thermoregulatory floral traits?
3. Which axes of floral trait variation are associated with warming?
4. Does floral warming impact pollinator visitation?
5. Are elevational differences in thermoregulatory traits genetic and differentially plastic?

2 | Materials and Methods

2.1 | System

Argentina anserina (= *Potentilla anserina*, Rosaceae) propagates vegetatively via stolons and sexually via flowers with gametophytic self-incompatibility (Cisternas-Fuentes et al. 2023). Its generalist bee and fly-pollinated (Koski and Ashman 2015) flowers are uniformly yellow in the visible spectrum but have UV-absorbing petal bases and UV-reflective petal tips (Koski and Ashman 2013). Petals begin to close in the afternoon on the first day of anthesis. They reopen on the second day until abscission. We focused on an elevation gradient in the San Juan

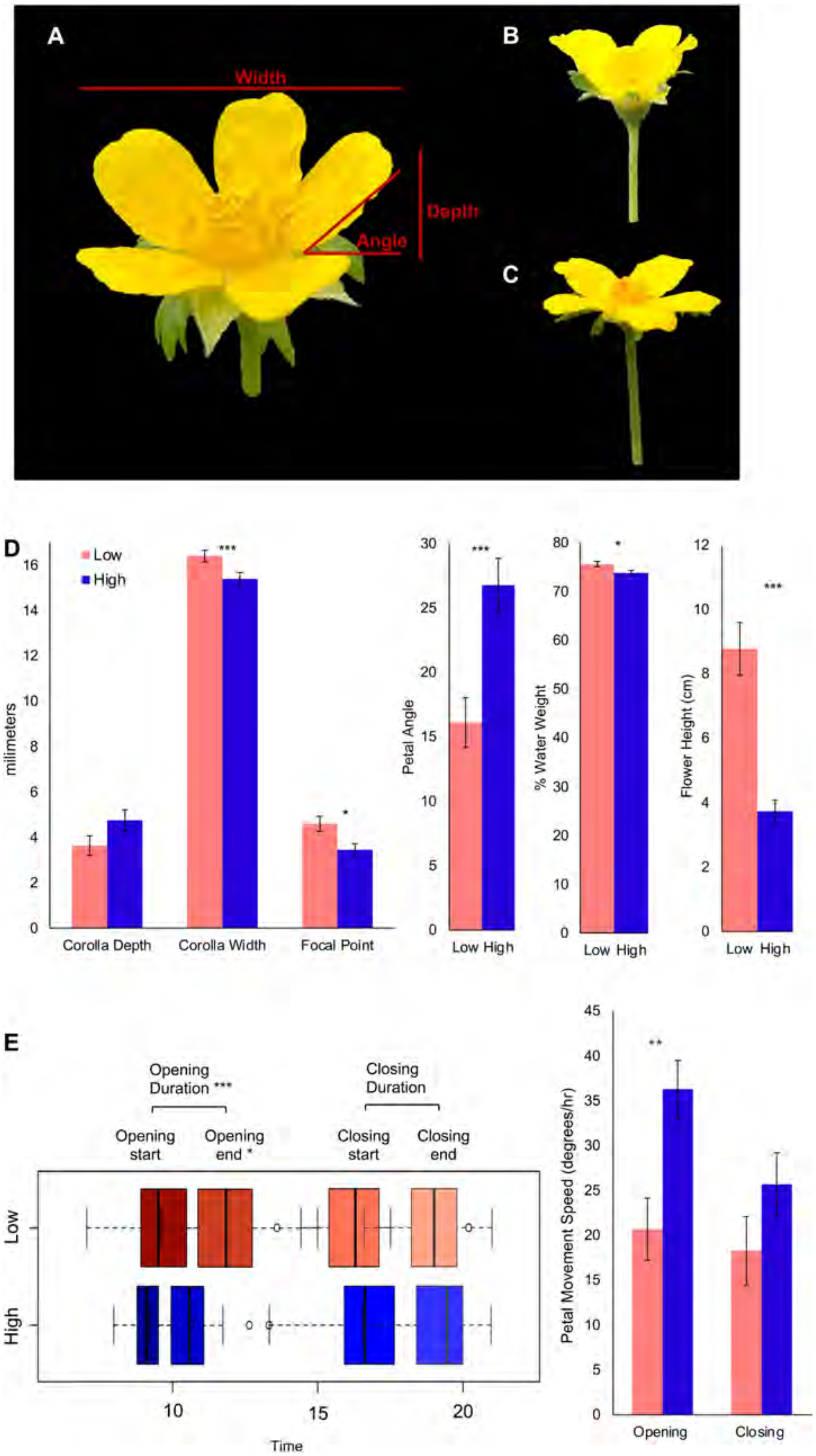

FIGURE 1 | (A) *Argentina anserina* flower showing floral dimensions measured in this study (petal angle, floral opening width and depth) which impact the focal point of light after it is reflected from petals. A flower with more cupped petals (B) focuses light deeper within the flower compared to a flower with flatter petals (C). Floral dimensions, the focal point of light, % water weight and flower height in low and high elevation populations measured in the field at mid-day (D). Floral opening and closing timing and speed metrics (E). Means and standard errors for panel (D) and movement speeds (E) are estimated marginal means from models accounting for population identity nested in elevation and petal size and day of year. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.0001$.

Mountains of Colorado over which populations span ~1000 m. The thermal optimum for pollen germination and tube growth in high elevation populations (> 3000 m) is ~4°C higher than lower elevation populations (< 2600 m) despite low elevations experiencing higher temperatures (Heiling and Koski 2024).

2.2 | Floral Traits and Temperature in the Field

We measured floral traits potentially associated with floral warming in four high (> 3000 m) and five low elevation populations (< 2600 m) during peak flowering in 2021 (Table S1). We measured traits on the first day of anthesis and floral temperature on 3–7 days per population. Sampling dates were similar between low (June 22–July 15) and high elevation (June 23–July 19). Between 11:00 and 14:00, we measured petal angle, length and width and corolla depth and diameter on an average of 62.4 flowers/population (range 38–95, $N = 562$). Petal angle was measured in the field with a protractor where 90° was an upright petal, 0° was a flat petal, and a negative angle was a reflexed petal. On an average of 29.4 flowers/population (range 8–45, $N = 265$), we affixed k-type thermocouple probes (Omega Engineering, Norwalk CT, USA) in flowers to record the temperature experienced by reproductive structures (hereafter, flower temperature). Probes were attached to pedicels with tape and bent into flowers between two petals so that petal movement was unobstructed (Heiling and Koski 2024). On flowers for which we measured temperature, we measured floral height with a ruler (cm). We attached a thermocouple probe to a florist stick ~2 cm from each flower at flower-height to measure flower-level air temperature (hereafter, air temperature). Probes were attached to 8-port Omega data loggers.

We logged temperature for ~24 h in 1 min intervals and binned flower and air temperature into 4 h blocks representative of diurnal changes in temperature and floral anthesis (06:01–10:00, 10:01–14:00, etc.; see Heiling and Koski 2024). We measured ΔT as flower temperature minus air temperature. Positive values indicate the floral microenvironment is warmer than the surrounding air, whereas negative values indicate it is cooler.

For an average of 33 flowers/population (range 22–50), we measured the water content of flowers destructively. Thus, temperature was not recorded. We collected flowers and weighed them to 0.001 g with portable scales (Gemini-20, American Weigh Scales, Cumming GA, USA). We dried flowers and weighed them to 0.001 g in the lab and calculated % water weight as (wet mass—dry mass/wet mass) × 100.

We modelled the focal point of light reflected within the corolla treating the bowl-shaped flower as a parabola (Kevan 1975) using

$$f = x^2 / 4a$$

where x is the corolla opening radius, and a is the corolla depth. Because depth must be ≥ 1 for focal point estimates, we bound depth < 1 to 1. We did not calculate the focal point of flowers with reflexed petals.

On an average of 19.4 flowers/population (range 13–23, $N = 175$), we destructively measured UV colour patterning and spectral reflectance. We removed three petals/flower, flattened them on tape and photographed them using a UV-sensitive digital camera. We used ImageJ to measure the following traits: petal area, UV-absorbing area, % UV absorbing area and the angle of the UV-absorbing area (see Koski et al. 2022). On one petal/flower, we measured spectral reflectance with a UV-NIR Ocean Optics spectrometre at the petal base and apex. We focused on NIR reflectance because NIR has a stronger capacity to impact floral temperature than UV/VIS. We used the *pavo* package in R (Maia et al. 2019) to measure peak % reflectance between 800 and 900 nm as an estimate of NIR reflectance.

2.3 | Petal Opening, Closing, Movement and Pollinator Visitation in the Field

We tracked the timing and speed of floral opening/closing using Brinno (Taipei City, Taiwan) time-lapse cameras (TLCs). In each population, two to three cameras captured petal movement on one flower or a patch of flowers. TLCs were focused on first-day flowers at mid-day and photographed flowers every 10 s for ~24 h. This captured floral closure on the first day of anthesis and opening on the second day. We recorded time for the following: closure onset, closure end, opening onset and opening end. We measured the petal angle at full openness and full closure. We measured opening and closing speed as the degrees moved per hour. We captured data from 64 high elevation and 31 low elevation flowers. Sample sizes for timing metrics varied due to flowers being obscured by vegetation or moisture. Because estimating opening/closing speeds (°/h) required all temporal and angle metrics on a given flower, speed metrics had the lowest sample size (high $N = 17$, low $N = 14$).

To assess whether individual flowers altered petal angle in concert with temperature, and how floral warming impacted pollinator visitation we used TLC data. This data set was finer-grained than the one linking mid-day petal angle with 4 h average mid-day air temperature, allowing us to track individual flower responses to temperature. A subset of flowers was monitored with both TLCs and thermocouples in 2021 ($N = 14$ low elevation, $N = 19$ high elevation) and 2022 ($N = 6$ low elevation, $N = 10$ high elevation). We paused videos every time a pollinator contacted the focal flower. At the onset of visitation, we measured petal angle, and timestamped flower and air temperature. Pollinator visitation occurred from 08:12 to 20:28 and 08:46 to 20:42 at low and high elevations, respectively. We recorded 152 visits at low and 190 at high elevation in 2021 and 240 visits at low and 90 visits at high in 2022. Angle was measurable from TLCs during the visit for only 167 instances (43%) at low elevation (range = 1–52, mean = 9.06/flower) and 150 instances (54%) at high elevation (range 1–21; mean = 4.84/flower). In most cases, visitor identity was unclear from the footage, so we did not consider taxonomic identity.

2.4 | Growth Chamber Study Design

We assessed whether elevational differences in floral traits in the field were maintained in a common environment and tested for temperature-induced phenotypic plasticity. We established two treatments in Percival growth chambers based on high and low elevation field temperatures. The warm treatment was set to 28/15°C day/night and the cool to 14/1°C day/night with 14 h daylengths. The day and night setpoints for the warm treatment were the average maximum and minimum June–July temperatures of low elevation sites, respectively (Heiling and Koski 2024). Daytime setpoint for the cool treatment was between the mean and maximum high elevation temperature, and nighttime was the long-term minimum. Relative humidity was 32/50% day/night in both treatments. Plants were collected from 4 low and 4 high elevation populations in 2019 and 2020 and maintained in the greenhouse. Twice per year, plants were subject to a 4°C vernalisation for ~1 month. After vernalisation, plants were moved to the greenhouse for ~4 months set to 18.3°C under which they grew above-ground tissue, flowered and senesced.

We chose 34 plants from low and 48 plants (hereafter, genotypes) from high elevation populations and cloned each at least twice from vegetative runners to reduce maternal environmental effects (Hill and Mackay 2004). After flower buds formed, we placed one clone per genotype into the warm treatment and the other into the cool treatment. On the first day of anthesis, we measured petal angle, corolla diameter, corolla depth, petal length and petal width on at least one flower per plant from 11:00 to 13:00. We measured all dimensions on the same flower the next day. We collected an additional day one flower and photographed three petals with a UV-sensitive camera to measure UV-absorbing area, UV proportion and the angle of the UV-absorbing area. On one petal per flower, we measured spectral reflectance. The experiment was conducted in Spring 2022 and 2023 to obtain floral dimensions, UV floral traits and reflectance properties from at least one flower per genotype × treatment. For the second experimental replicate, we switched temperature treatments and all associated plants between chambers to minimise chamber effects. For 15 genotypes (7 low, 8 high), only one clone flowered. In total, we collected data from 147 plants.

2.5 | Analyses

We conducted analyses in R v. 4.3.2. For all models, we treated elevation as a binary factor (low vs. high), and population identity was nested in elevation as a random effect.

Question 1: We modelled petal angle, floral diameter, floral depth, focal point of light, floral height, and % water weight as a function of elevation (low vs. high) with petal size (length × width) and day of year (DOY) as covariates. DOY accounts for changes in solar angle over the season and daily differences in wind and clouds which impact environmentally sensitive floral traits. Field data were collected after the summer solstice such that increasing DOY is associated with reduced solar declination (Cornwall et al. 2024).

UV patterning traits and petal NIR reflectance were modelled similarly but DOY was not included because each population was sampled only 1 day. Petal area (mm^2) was a covariate for UV absorbing angle and NIR reflectance. Petal movement and timing data were modelled using a similar model structure but without DOY because some populations were only sampled on 1 or 2 days, and without petal size as it was not measured. Focal point, flower height and UV absorbing area were modelled with a Gamma distribution and a log link due to skewed distributions (*glmer*), while all other traits were modelled with a Gaussian distribution (*lmer*).

Question 2: We modelled each trait as described above but included mean air temperature from 10:00 to 14:00, and its interaction with elevation. Because air temperature was not recorded for flowers that were destructively sampled (% water weight, UV patterning and reflectance), we did not test whether air temperature impacted these traits. For petal opening time and speed, we used the average flower-level external temperature from 10:00 to 14:00 within a population on the sampling day as temperature for all flowers, and for closing time and speed traits, we used temperature from 14:01 to 18:00.

To test whether individual flowers differed in responsiveness to air temperature, we modelled petal angles from TLC videos as a function of elevation, air temperature at the 1 min scale and their interaction. Year and flower identity nested in population were included as random terms. Flower identity was included because repeated measurements were made on most flowers.

Question 3: Petal angle was correlated with flower depth ($r = 0.74$, $p < 0.0001$), diameter ($r = -0.47$, $p < 0.0001$) and the focal point of light ($r = -0.59$, $p < 0.0001$). We chose petal angle as an indicator of petal cupping to determine if it impacted ΔT at mid-day (10:00–14:00). We modelled ΔT as a function of elevation, petal angle, air temperature and all interactions. Flower height, petal size and DOY were covariates.

Because we measured petal UV patterning and reflectance destructively, we could not relate air temperature with these traits. Therefore, we tested for correlations between population-level mean trait values and population-level average ΔT from 10:00 to 14:00.

Question 4: We modelled visits to each flower as a function of elevation, ΔT, and air temperature with all possible interactions using a binomial distribution. DOY and day of floral anthesis (first or second) were included as covariates, and flower identity nested in population were random effects. Day of anthesis was included because first-day flowers may have more floral resources than second-day flowers due to resource use by pollinators on the first day.

Question 5: We constructed a linear model for each floral dimension trait, UV patterning trait and petal NIR reflectance measured in the growth chambers. Each trait was modelled as a function of elevation, temperature treatment and their interaction, with petal size as a covariate. Genotype nested in population and experimental replicate (year) were included as random terms. For all traits, a Gaussian distribution was used (*lmer*) but a negative binomial fit better for focal point of light which was overdispersed (*glmer.nb*).

We calculated the change in petal angle, flower depth, flower width and focal point over the floral lifespan by subtracting the first-day trait value from the second day. This estimated how much floral cupping changed over the flower lifespan. We modelled phenotypic change scores as a function of elevation, treatment, the trait value on day one and all two-way interactions. The trait value on day one was included because the potential change for a given trait depends on the initial trait value. For example, petals held at 80° on day one have further to fall than petals that start at 20°. Petal size was included as a covariate and genotype nested in population, and experimental replicate were random terms.

3 | Results

3.1 | Putative Floral Thermoregulatory Traits Differ Between Low and High Elevation Populations

Petals at high elevation had a 66% higher angle during mid-day than low elevation, which was associated with a 6% narrower corolla opening, and a 25% lower focal point of light within flowers (Table S2; Figure 1D). Together, comparing corolla depth and focal point shows that light was focused inside the corolla at high elevation, but reflected outside of the corolla at low elevation (Figure 1D). Both percent water weight and flower height were higher in low elevation populations (Figure 1D).

The absolute and proportional area of petal UV absorption, and the angle of the UV-absorbing pigmented area were larger in high elevation populations (Table S2; Figure S1). NIR reflectance tended to be higher in high elevation populations but not significantly so (Table S2; Figure S1).

High and low elevation flowers initiated anthesis at the same time, but high elevation flowers finished opening earlier than low, resulting in a reduction in time to complete anthesis (Table S2; Figure 1E). The speed of opening (°/h) was 75% faster in high elevation populations (Table S2; Figure 1E). The onset and completion of floral closure, and floral closing speed were not significantly different between low and high elevations (Table S2; Figure 1E).

3.2 | Floral Morphology Responds More Strongly to Temperature at High Elevation Than Low

Higher mid-day air temperature was associated with petal flattening in high, but not low elevation populations, indicated by elevation-by-temperature effects on petal angle (Figure 2A; Table S3) and corolla diameter (Figure S2B; Table S3). Corolla depth was lower at higher temperatures in all populations (Figure S2A). Petal cupping decreased the focal point of light in high elevation populations (Figure 2B). Despite temperature effects on traits, overall elevational differences were maintained for petal angle, focal point of light and corolla diameter (Table S3).

Air temperature had little influence on the timing and speed of opening/closing (Table S4). Petal angle, however, was more responsive to temperature at high elevation than low (Figure S3; Table S5). High elevation flowers reduced petal angle by 10° for every 5°C increase in air temperature (Figure S3). Low elevation flowers only reduced petal angle by <4° for every 5°C increase in air temperature (Figure S3).

3.3 | Petal Cupping Warms Flowers at High Elevation but Not Low

Petal angle was positively associated with ΔT at high elevation but there was no relationship at low (Figure 2C; Table S6). For every 20° increase in petal angle, there was 0.462°C increase in ΔT at high elevation (Figure 2C). In the greenhouse, a thermal image shows that cupping increases temperature of the pollen and ovule environment within the flower (Figure 2D). Neither flower height nor petal size influenced ΔT (Table S6). There were no correlations between population-level average mid-day ΔT and UV patterning traits ($r = 0.29$–0.54, all $p > 0.12$), NIR reflectance ($r = 0.27$–0.32, all $p > 0.40$), or percent water weight ($r = -0.24$, $p = 0.54$) implicating petal angle as the primary thermoregulatory trait.

3.4 | Warming Enhances Pollinator Visitation, Especially Under Cold High Elevation Conditions

The likelihood of receiving a pollinator visit was higher for flowers warming further above air temperature (ΔT effect; $\chi^2 = 36.7$, $p < 0.0001$), but the benefit of warming for visitation was stronger at high elevation than low (Figure 3; Elevation × ΔT; $\chi^2 = 11.61$, $p < 0.001$). Finally, the interaction between air temperature and floral warming differed across elevation (Elevation × ΔT × Air Temp.; $\chi^2 = 13.77$, $p < 0.001$). At high elevation, floral warming had a strong positive effect on visitation during cool air temperatures but no effect during high air temperatures (Figure 3).

3.5 | High Elevation Populations Have Constitutively More Cupped Petals and Stronger Temperature-Induced Plasticity Than Low

Temperature effects on floral dimensions and the focal point of light were pervasive, with flowers flattening in the warm growth chamber treatment which increased the focal point of light (Figure S4; Table S7). On the first day of anthesis, high elevation flowers were more cupped than low in both treatments (Figure S4A), indicating a genetic basis for the elevational differences recorded in the field. On the second day of anthesis, high elevation populations had a steeper reaction norm for petal angle than low driving a modest elevation-by-treatment interaction (Figure S4B; Table S7). Additionally, low elevation flowers were wider than high, especially in the cold (Figure S4C). Most UV pigmentation and NIR reflectance traits were not impacted by elevation or temperature (Table S7). Elevational differences in plasticity were not detected for most traits measured on a single day (Table S7). However, differential plasticity was detected for the focal point of light on the second day (Figure S4D; Table S7). The focal point increased ~12-fold from low to high temperature for high elevation populations, but only ~6-fold for low. In the cold treatment, the focal point was significantly lower in high elevation flowers than low ($p = 0.032$, Figure S4D).

Petal mobility, measured as the phenotypic change from the first to second day, was impacted by temperature, elevation-of-origin, and their interaction (Table S8; Figure 4). High elevation flowers became more cupped over their lifespan in the cold treatment but flatter in the warm treatment (Figure 4A).

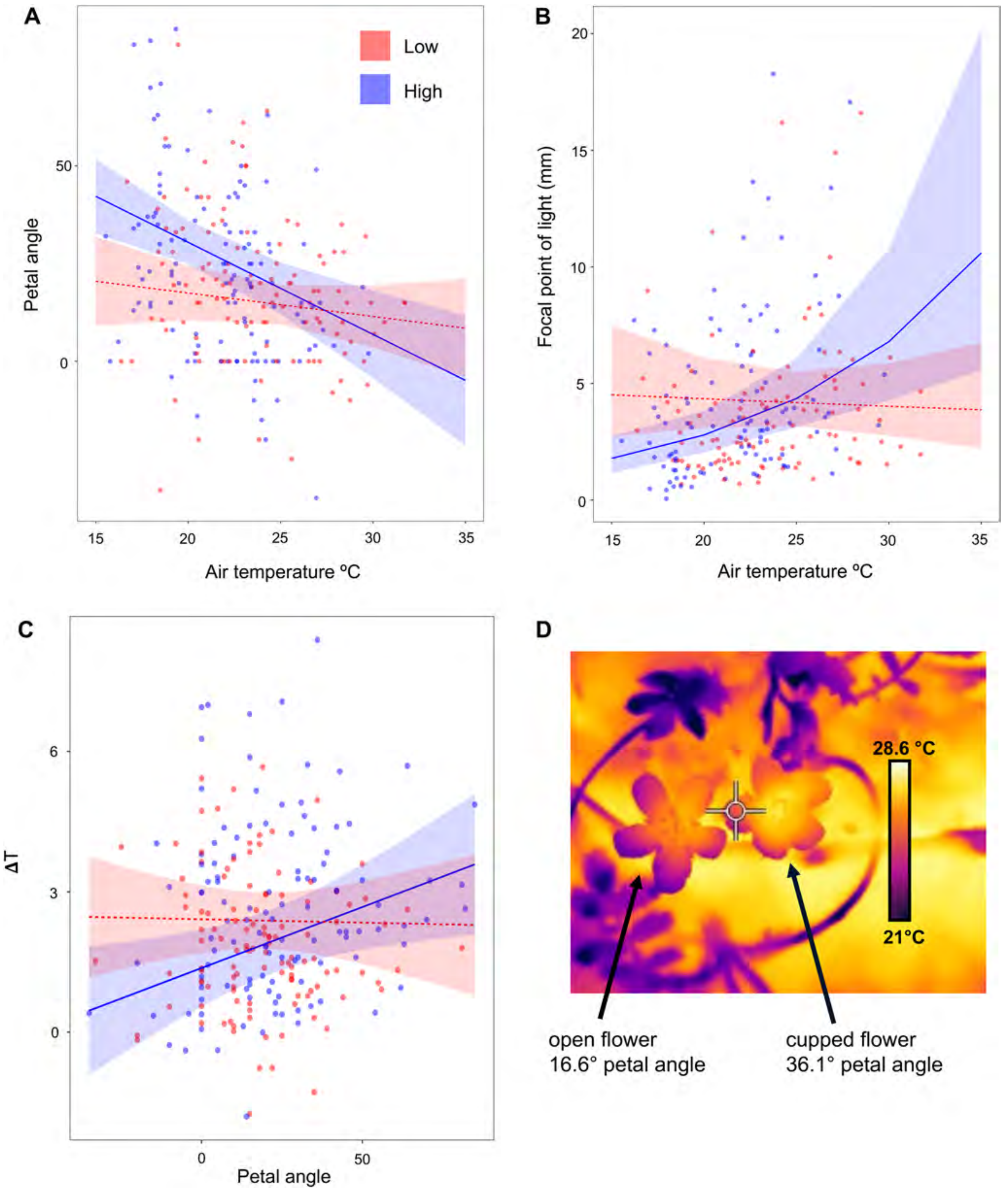

FIGURE 2 | The effect of 4 h average flower-level air temperature at mid-day on petal angle (A) and the focal point of light (B) for low and high elevation populations. Petal angle impacted floral warming (ΔT; flower temperature—air temperature) in high but not low elevation populations (C). Lines depict estimated marginal effects and standard errors from full models including petal size and day of year as covariates with population identity nested in elevation as a random term. Elevation × Temperature effects were significant for panels (A)–(C) (Tables S3 and S6). (D) Is a thermal image of a *Argentina anserina* flowers from separate plants taken in a greenhouse. The flower on the left has a lower angle ('open') and the flower on the right has a higher angle ('cupped').

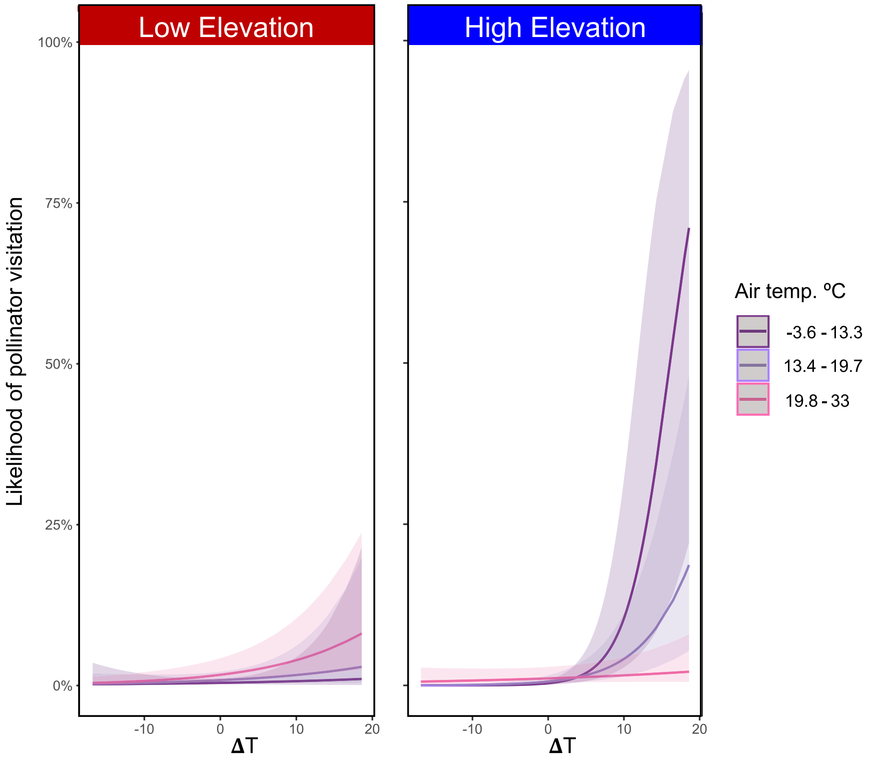

FIGURE 3 | The effect of floral warming (ΔT) and air temperature on the likelihood pollinator visitation at low and high elevations. Traces are predicted probabilities of a visit being recorded at the 1 min interval from a model of visitation as a binomial factor and population nested in elevation and flower identity nested in population as random terms. Elevation × ΔT × Air Temperature $\chi^2 = 13.88$, $p = 0.0002$.

There was a concordant increase in floral depth in the cold and a decrease in depth in the warm treatment for high elevation flowers, but this response was weaker for low elevation (Figure 4B). Low and high elevation populations did not alter the focal point of light over the floral lifespan in the cold. High elevation flowers increased focal point more than low in the warm treatment (Figure 4C). Thus, temperature-induced petal movement was stronger in high elevation populations than in low.

4 | Discussion

We demonstrate for the first time that populations have evolved differential temperature-induced petal mobility, which impacts floral temperature. Given the influence of temperature on gametophyte performance and pollinator visitation, regulating the floral microenvironment should be imperative for plant reproduction under warming and more extreme thermal conditions. Most mechanisms of plant thermoregulation are considered passive, whereby plant functional traits differ across temperature gradients in a manner that enhances performance under local conditions (Michaletz et al. 2015). However, our study offers an example of active behavioural thermoregulation in plants akin to classic examples in lepidopterans (Barton, Porter, and Kearney 2014; Kevan and Shorthouse 1970; Kingsolver 1985) where the alteration of solar reflectors—wings in the case of lepidoptera but petals in flowering plants—modifies the focus of solar radiation on thermally sensitive structures.

High elevation flowers of *A. anserina* were previously shown to warm more above air temperature than lower elevation populations in the San Juan Mountains of Southwest Colorado (Heiling and Koski 2024). Here, we dissected the mechanisms underlying differential warming. We found support for elevational differences in many traits that could impact floral temperature including floral dimensions, the focal point of light reflected from petals, petal pigmentation patterning, NIR reflectance and opening speed. However, petal dimensions dictated by petal angle had the clearest impact on floral warming at high elevation. More cupped flowers focused solar radiation deeper within flowers and increased mid-day floral warming. Surprisingly, petal cupping at low elevation was unassociated with floral warming. While petal angle was higher on average at high elevation, the range of angles was similar between low and high elevation populations. Thus, our failure to detect a positive association between petal angle and warming at low elevation was not due to limited phenotypic variation. Instead, environmental change with elevation may drive the differences in the petal angle-warming relationship. Total solar irradiance increases with elevation (Blumthaler, Ambach, and Ellinger 1997). Thus, reduced availability of solar heat energy at lower elevations could limit the ability of floral cupping to capture heat. Floral cupping increases floral warming in the high arctic (Kevan and Shorthouse 1970), but here we demonstrate population-level variation in the effect of floral cupping on floral warming.

Petals in high elevation populations also tracked air temperature—cupping when cooler and flattening when warmer. Low elevation petals however did not track temperature as closely. This result was supported by a stronger relationship between petal angle and flower-level temperature at mid-day, and stronger temperature tracking recorded by time-lapse cameras at high elevations. It was recapitulated in the growth chamber where high elevation flowers exhibited stronger plasticity for

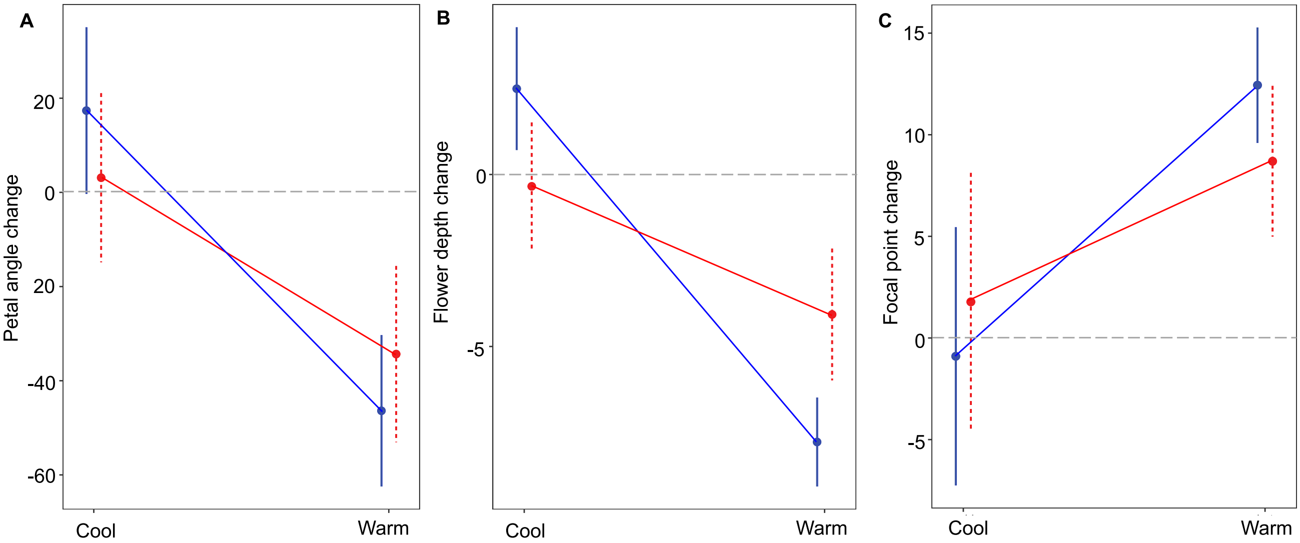

FIGURE 4 | Temperature-induced reaction norms of floral trait change across the floral lifespan in low (red) and high (blue) elevation populations. Changes in petal angle (A), floral depth (B) and focal point of light (C) exhibited differential plasticity between elevation clusters indicated by significant elevation × treatment effects (Table S8). Dashed horizontal lines indicate no change across the floral lifespan. Points and standard errors are estimated marginal means from mixed effects linear models.

petal movement than low elevation populations. To our knowledge, this is the first example of population-level divergence for a plant thermoregulatory mechanism that employs movement (e.g., petal movement, heliotropism). However, patterns of population differentiation for temperature-induced plasticity of petal reflectance that are consistent with adaptive thermoregulatory plasticity have been revealed in *Plantago* (Lacey et al. 2010; Lacey, Lovin, and Richter 2012). The more rapid and reversible movements of petal angle found in *A. anserina*, are likely to provide a more temporally fine-tuned mode of thermoregulation than the upregulation of floral pigments required for the alteration of petal reflectance.

Elevational differences in floral traits affecting temperature (petal angle, depth) and overall petal mobility were maintained in common growth conditions. This supports a genetic basis underlying elevational differences in floral thermoregulation. Two lines of evidence suggest that the evolution of stronger behavioural thermoregulation at high elevation is likely adaptive in both abiotic (thermal) and biotic (pollinator) alpine environments. First, the thermal optimum for pollen at high elevation far exceeds temperatures experienced by flowers but low elevation populations experience temperatures nearer their thermal optimum (Heiling and Koski 2024). Thus, selection favouring warming should be stronger at high elevation. Second, floral warming enhanced pollinator visitation at high elevation, especially when air conditions were cool. However, at low elevation, pollinator visitation simply increased under warmer conditions, and the impact of floral warming on visitation was independent of air temperature. Together, results suggest that selection should favour more fine-tuned floral thermoregulation at high elevation (i.e., selection on thermoregulation should be dependent on air temperature). In montane systems pollinators are often more limiting for sexual reproduction at higher elevations (Arroyo, Armesto, and Primack 1985) and pollinators preferred warmer flowers under cool conditions in our study. Thus, the selection of traits to attract pollinators (like temperature; Seymour, White, and Gibernau 2003) may be stronger in cooler environments. If pollination limits reproduction less at low elevations, or if pollinators are less reliant on basking in flowers for warmth (Sapir, Shmida, and Ne'eman 2006), then pollinator-mediated selection on floral temperature could simply be weaker. While visitation rates do not differ across the elevation gradient of *A. anserina*, fruit and seed set to decline with elevation (Cisternas-Fuentes and Koski 2024) supporting the idea that pollinator-mediated selection on female reproduction may be stronger at high elevation.

Petal movements are a widespread phenomenon in plants that have the potential to mitigate the negative impacts of thermal stress on pollen and ovules, and plant–pollinator mutualisms. Our work supports that petal cupping can finely tune the temperature of the floral microenvironment. Petal movements are likely to thermoregulate more rapidly than other mechanisms (e.g., altering pigment production), and in a less energetically costly manner than endothermic reactions. Thus, as populations expand into novel thermal ranges (Corlett and Westcott 2013) and experience novel temperatures in their contemporary range, petal movements may be crucial for maintaining reproductive output.

Author Contributions

M.H.K.: project design and management, funding acquisition, data collection, data analysis, manuscript writing. J.M.H.: project design and management, data collection, data curation, manuscript editing. J.S.A.: project design, data collection, data curation, manuscript editing.

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Data Availability Statement

All data and code are available upon Dryad doi: 10.5061/dryad.jdfn2z3jc.

Peer Review

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References

Anderson, J. T., D. W. Inouye, A. M. McKinney, R. I. Colautti, and T. Mitchell-Olds. 2012. "Phenotypic Plasticity and Adaptive Evolution Contribute to Advancing Flowering Phenology in Response to Climate Change." *Proceedings of the Royal Society B: Biological Sciences* 279: 3843–3852.

Armbruster, W. S., and N. Muchhala. 2020. "Floral Reorientation: The Restoration of Pollination Accuracy After Accidents." *New Phytologist* 227: 232–243.

Arroyo, M. T. K., J. J. Armesto, and R. B. Primack. 1985. "Community Studies in Pollination Ecology in the High Temperate Andes of Central Chile II. Effect of Temperature on Visitation Rates and Pollination Possibilities." *Plant Systematics and Evolution* 149: 187–203.

Arroyo, M. T. K., L. S. Dudley, G. Jespersen, D. A. Pacheco, and L. A. Cavieres. 2013. "Temperature-Driven Flower Longevity in a High-Alpine Species of *Oxalis* Influences Reproductive Assurance." *New Phytologist* 200: 1260–1268.

Atamian, H. S., N. M. Creux, E. A. Brown, A. G. Garner, B. K. Blackman, and S. L. Harmer. 2016. "Circadian Regulation of Sunflower Heliotropism, Floral Orientation, and Pollinator Visits." *Science* 353: 587–590.

Barton, M., W. Porter, and M. Kearney. 2014. "Behavioural Thermoregulation and the Relative Roles of Convection and Radiation in a Basking Butterfly." *Journal of Thermal Biology* 41: 65–71.

Beever, E. A., L. E. Hall, J. Varner, et al. 2017. "Behavioral Flexibility as a Mechanism for Coping With Climate Change." *Frontiers in Ecology and the Environment* 15: 299–308.

Bishop, J. A., and W. S. Armbruster. 1999. "Thermoregulatory Abilities of Alaskan Bees: Effects of Size, Phylogeny and Ecology." *Functional Ecology* 13: 711–724.

Blumthaler, M., W. Ambach, and R. Ellinger. 1997. "Increase in Solar UV Radiation With Altitude." *Journal of Photochemistry and Photobiology. B* 39: 130–134.

Braam, J. 2005. "In Touch: Plant Responses to Mechanical Stimuli." *New Phytologist* 165: 373–389.

Cisternas-Fuentes, A., and M. H. Koski. 2024. "Effective Population Size Mediates the Impact of Pollination Services on Pollen Limitation." *Proceedings of the Royal Society B: Biological Sciences* 291: 20231519.

Cisternas-Fuentes, A., R. Dwyer, N. Johnson, L. Finnell, J. Gilman, and M. H. Koski. 2023. "Disentangling the Components of Pollen Limitation in a Widespread Herb With Gametophytic Self-Incompatibility." *American Journal of Botany* 110: e16122.

Corbet, S. A., and S.-Q. Huang. 2016. "Small Bees Overheat in Sunlit Flowers: Do They Make Cooling Flights?" *Ecological Entomology* 41: 344–350.

Corlett, R. T., and D. A. Westcott. 2013. "Will Plant Movements Keep up With Climate Change?" *Trends in Ecology & Evolution* 28: 482–488.

Cornwall, C., A. Horiuchi, and C. Lehman. 2024. NOAA Solar Position Calculator. Accessed June 20, 2024. https://gml.noaa.gov/grad/solcalc/azel.html.

Creux, N. M., R. I. Brown, A. G. Garner, et al. 2021. "Flower Orientation Influences Floral Temperature, Pollinator Visits and Plant Fitness." *New Phytologist* 232: 868–879.

Galen, C. 2006. "Solar Furnaces or Swamp Coolers: Costs and Benefits of Water Use by Solar-Tracking Flowers of the Alpine Snow Buttercup, *Ranunculus adoneus*." *Oecologia* 148: 195–201.

Haverkamp, A., X. Li, B. S. Hansson, I. T. Baldwin, M. Knaden, and F. Yon. 2019. "Flower Movement Balances Pollinator Needs and Pollen Protection." *Ecology* 100: e02553.

Heiling, J. M., and M. H. Koski. 2024. "Divergent Gametic Thermal Performance and Floral Warming Across an Elevation Gradient." *Evolution* 78: 665–678.

Herrera, C. M. 1995. "Microclimate and Individual Variation in Pollinators: Flowering Plants Are More Than Their Flowers." *Ecology* 76: 1516–1524.

Hill, W., and T. Mackay. 2004. "D. S. Falconer and Introduction to Quantitative Genetics." *Genetics* 167: 1529–1536.

Karban, R. 2008. "Plant Behaviour and Communication." *Ecology Letters* 11: 727–739.

Karban, R., D. Rutkowski, and N. A. Murray. 2023. "Flowers That Self-Shade Reduce Heat Stress and Pollen Limitation." *American Journal of Botany* 110: e16109.

Kearney, M., R. Shine, and W. P. Porter. 2009. "The Potential for Behavioral Thermoregulation to Buffer "Cold-Blooded" Animals Against Climate Warming." *Proceedings of the National Academy of Sciences* 106: 3835–3840.

Kevan, P. G. 1975. "Sun-Tracking Solar Furnaces in High Arctic Flowers: Significance for Pollination and Insects." *Science* 189: 723–726.

Kevan, P. G., and J. D. Shorthouse. 1970. "Behavioural Thermoregulation by High Arctic Butterflies." *Arctic* 23: 268–279.

Kingsolver, J. G. 1985. "Thermal Ecology of *Pieris* Butterflies (Lepidoptera: Pieridae): A New Mechanism of Behavioral Thermoregulation." *Oecologia* 66: 540–545.

Koski, M. H., and T.-L. Ashman. 2013. "Quantitative Variation, Heritability, and Trait Correlations for Ultraviolet Floral Traits in *Argentina anserina* (Rosaceae): Implications for Floral Evolution." *International Journal of Plant Sciences* 174: 1109–1120.

Koski, M. H., and T.-L. Ashman. 2015. "An Altitudinal Cline in UV Floral Pattern Corresponds With a Behavioral Change of a Generalist Pollinator Assemblage." *Ecology* 96: 3343–3353.

Koski, M. H., L. M. Finnell, E. Leonard, and N. Tharayil. 2022. "Elevational Divergence in Pigmentation Plasticity Is Associated With Selection and Pigment Biochemistry." *Evolution* 76: 512–527.

Lacey, E. P., M. E. Lovin, and S. J. Richter. 2012. "Fitness Effects of Floral Plasticity and Thermoregulation in a Thermally Changing Environment." *American Naturalist* 180: 342–353.

Lacey, E. P., M. E. Lovin, S. J. Richter, and D. A. Herington. 2010. "Floral Reflectance, Color, and Thermoregulation: What Really Explains Geographic Variation in Thermal Acclimation Ability of Ectotherms?" *American Naturalist* 175: 335–349.

Mahan, J. R., and D. R. Upchurch. 1988. "Maintenance of Constant Leaf Temperature by Plants—I. Hypothesis-Limited Homeothermy." *Environmental and Experimental Botany* 28: 351–357.

Maia, R., H. Gruson, J. A. Endler, and T. E. White. 2019. "pavo 2: New Tools for the Spectral and Spatial Analysis of Colour in r." *Methods in Ecology and Evolution* 10: 1097–1107.

McCallum, K. P., F. O. McDougall, and R. S. Seymour. 2013. "A Review of the Energetics of Pollination Biology." *Journal of Comparative Physiology. B* 183: 867–876.

Medina, I., E. Newton, M. R. Kearney, R. A. Mulder, W. P. Porter, and D. Stuart-Fox. 2018. "Reflection of Near-Infrared Light Confers Thermal Protection in Birds." *Nature Communications* 9: 3610.

Michaletz, S. T., M. D. Weiser, J. Zhou, M. Kaspari, B. R. Helliker, and B. J. Enquist. 2015. “Plant Thermoregulation: Energetics, Trait–Environment Interactions, and Carbon Economics.” *Trends in Ecology & Evolution* 30: 714–724.

Ollerton, J., R. Winfree, and S. Tarrant. 2011. “How Many Flowering Plants Are Pollinated by Animals?” *Oikos* 120: 321–326.

Patiño, S., and J. Grace. 2002. “The Cooling of Convolvulaceous Flowers in a Tropical Environment.” *Plant, Cell & Environment* 25: 41–51.

Ramirez-Parada, T. H., I. W. Park, S. Record, C. C. Davis, A. M. Ellison, and S. J. Mazer. 2024. “Plasticity and Not Adaptation is the Primary Source of Temperature-Mediated Variation in Flowering Phenology in North America.” *Nature Ecology & Evolution* 8: 467–476.

Sapir, Y., A. Shmida, and G. Ne'eman. 2006. “Morning Floral Heat as a Reward to the Pollinators of the *Oncocyclus irises*.” *Oecologia* 147: 53–59.

Seymour, R. S., C. R. White, and M. Gibernau. 2003. “Heat Reward for Insect Pollinators.” *Nature* 426: 243–244.

Sherer, T. N., J. M. Heiling, and M. Koski. 2024. “Floral Thermal Biology in Relation to Pollen Thermal Performance in an Early Spring Flowering Plant.” *Plant Biology* 26: 811–820.

Shrestha, M., J. E. Garcia, Z. Bukovac, A. Dorin, and A. G. Dyer. 2018. “Pollination in a New Climate: Assessing the Potential Influence of Flower Temperature Variation on Insect Pollinator Behaviour.” *PLoS One* 13: e0200549.

Silvertown, J., and D. M. Gordon. 1989. “A Framework for Plant Behavior.” *Annual Review of Ecology and Systematics* 20: 349–366.

Stanton, M. L., and C. Galen. 1989. “Consequences of Flower Heliotropism for Reproduction in an Alpine Buttercup (*Ranunculus adoneus*).” *Oecologia* 78: 477–485.

Stuart-Fox, D., E. Newton, and S. Clusella-Trullas. 2017. “Thermal Consequences of Colour and Near-Infrared Reflectance.” *Philosophical Transactions of the Royal Society, B: Biological Sciences* 372: 20160345.

Trivellini, A., G. Cocetta, D. A. Hunter, P. Vernieri, and A. Ferrante. 2016. “Spatial and Temporal Transcriptome Changes Occurring During Flower Opening and Senescence of the Ephemeral Hibiscus Flower, *Hibiscus rosa-sinensis*.” *Journal of Experimental Botany* 67: 5919–5931.

van der Kooi, C. J., P. G. Kevan, and M. H. Koski. 2019. “The Thermal Ecology of Flowers.” *Annals of Botany* 124: 343–353.

van Doorn, W. G., and C. Kamdee. 2014. “Flower Opening and Closure: An Update.” *Journal of Experimental Botany* 65: 5749–5757.

van Doorn, W. G., and U. van Meeteren. 2003. “Flower Opening and Closure: A Review.” *Journal of Experimental Botany* 54: 1801–1812.

van Loon, L. C. 2016. “The Intelligent Behavior of Plants.” *Trends in Plant Science* 21: 286–294.

Wiszniewski, A., E. Uberegui, M. Messer, et al. 2022. “Temperature-Mediated Flower Size Plasticity in *Arabidopsis*.” *iScience* 25: 105411.

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Additional supporting information can be found online in the Supporting Information section.