Supporting Information

Article title: Flower orientation influences floral temperature, pollinator visits, and plant fitness
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Fig. S1 Comparison of average temperatures and solar radiation in Davis, CA and Charlottesville, VA. (a) Average monthly temperatures. (b) Average hourly temperatures in the month of July at the two sites. For (a) and (b), inner shaded region shows 95% confidence interval while outer shaded regions depict mean maximum and minimum temperatures. (c) Average daily solar irradiance (global horizontal irradiance) at the two sites from May through October; shaded regions indicate the mean +/- the standard error of the mean. For (b) and (c), time 0 = midnight. Data are from the National Solar Radiation Database field stations nearest the two field sites, 2014 – 2016.

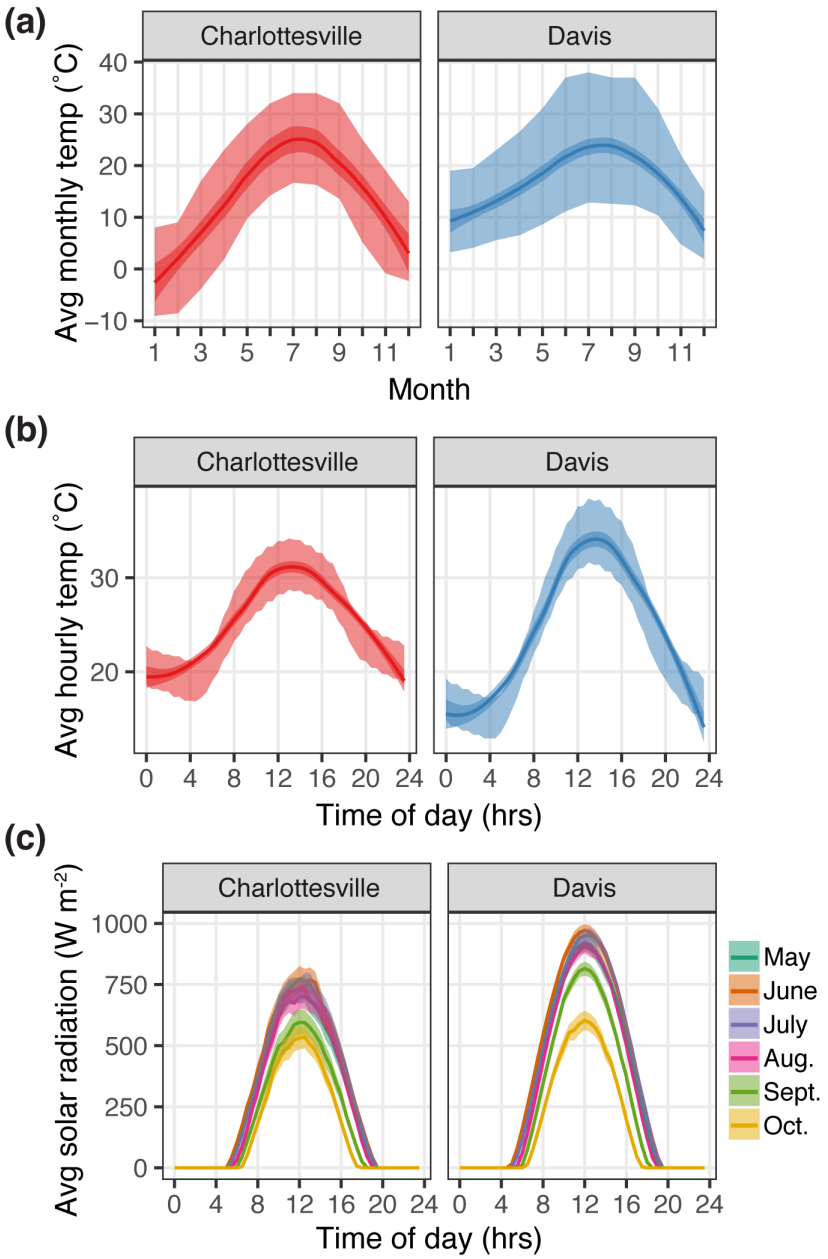


Fig. S2 No difference in the average seed number between east- and west-facing capitula in Davis, CA (a) and Charlottesville, VA (b). Means +/- the standard error of the mean are shown. The difference in the number of seeds between early- planted (May - July) and late-planted (July - September) sunflowers in Davis may be due to different GHI (global horizontal irradiance), with the late-planted set having similar numbers of seed to those planted in Charlottesville (c).

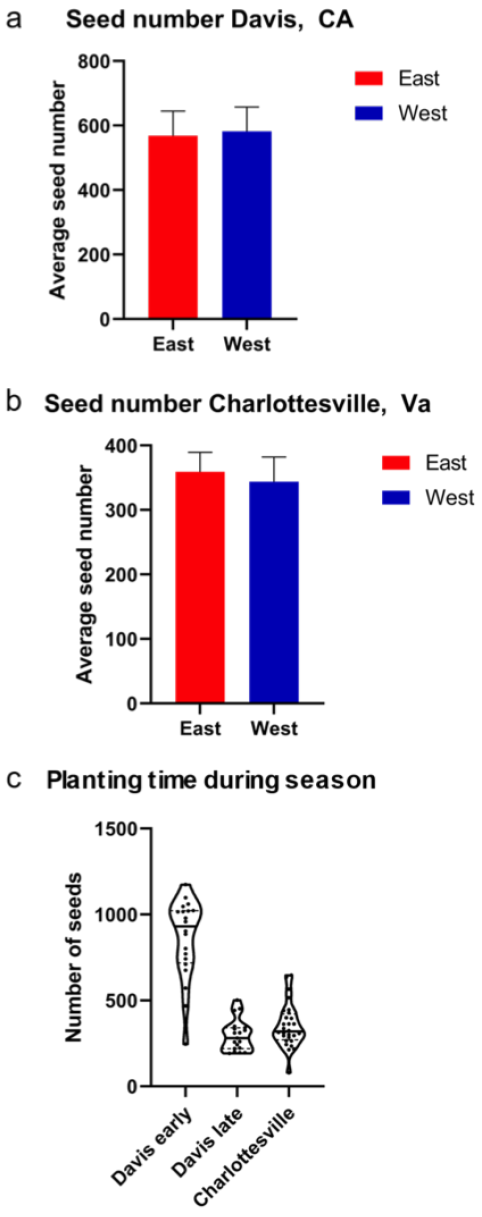


Fig. S3 No difference in seed weight (a) or width (b) between east- and west-facing capitula in Charlottesville, VA. No significant difference observed with a linear model with trial and year as random effects and direction as a fixed effect. Box edges represent the 75th and 25th percentile with the mid-line in the box represents the median and the whiskers represent the largest or smallest value within 1.5 times the interquartile range.

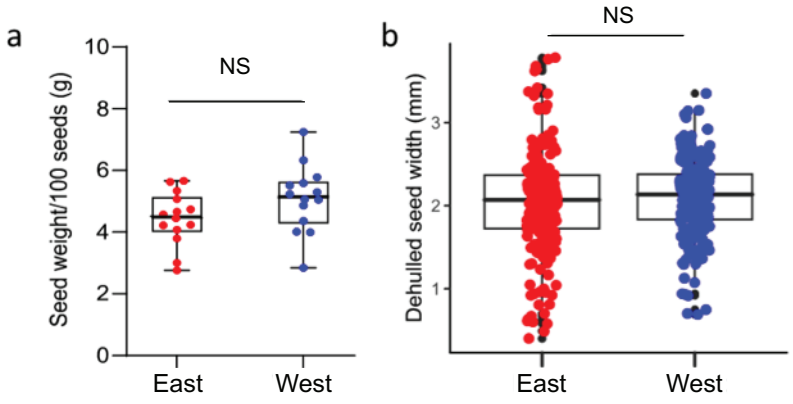


Fig. S4 Capitulum orientation affects the timing of insect visitation and pollen presentation by wild sunflower plants. (a) For two accessions derived from wild *H. annuus* populations, east-facing capitula (red) attract more insects during the hourlong interval from ZT1 to ZT2 than west-facing capitula (blue). In addition, for both the Oklahoma (b) and Texas (c) accessions, the daily timing of pollen release by florets in the pseudo-whorl entering staminate phase is advanced for east-facing capitula compared to west-facing capitula.

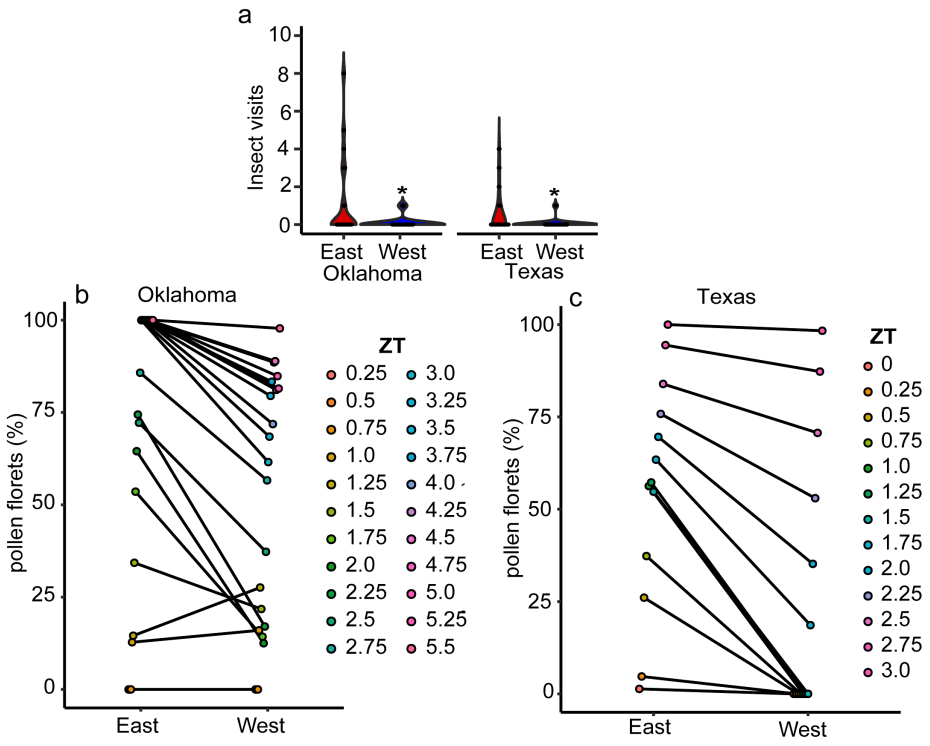


Fig. S5 Average temperature changes on the east- and west-facing sunflower capitula over a 24 hr period in Davis, CA (a and b) and Charlottesville, VA (c and d). Lines represent averages of temperatures measured over several days for multiple plants in each location with error bars representing SE.

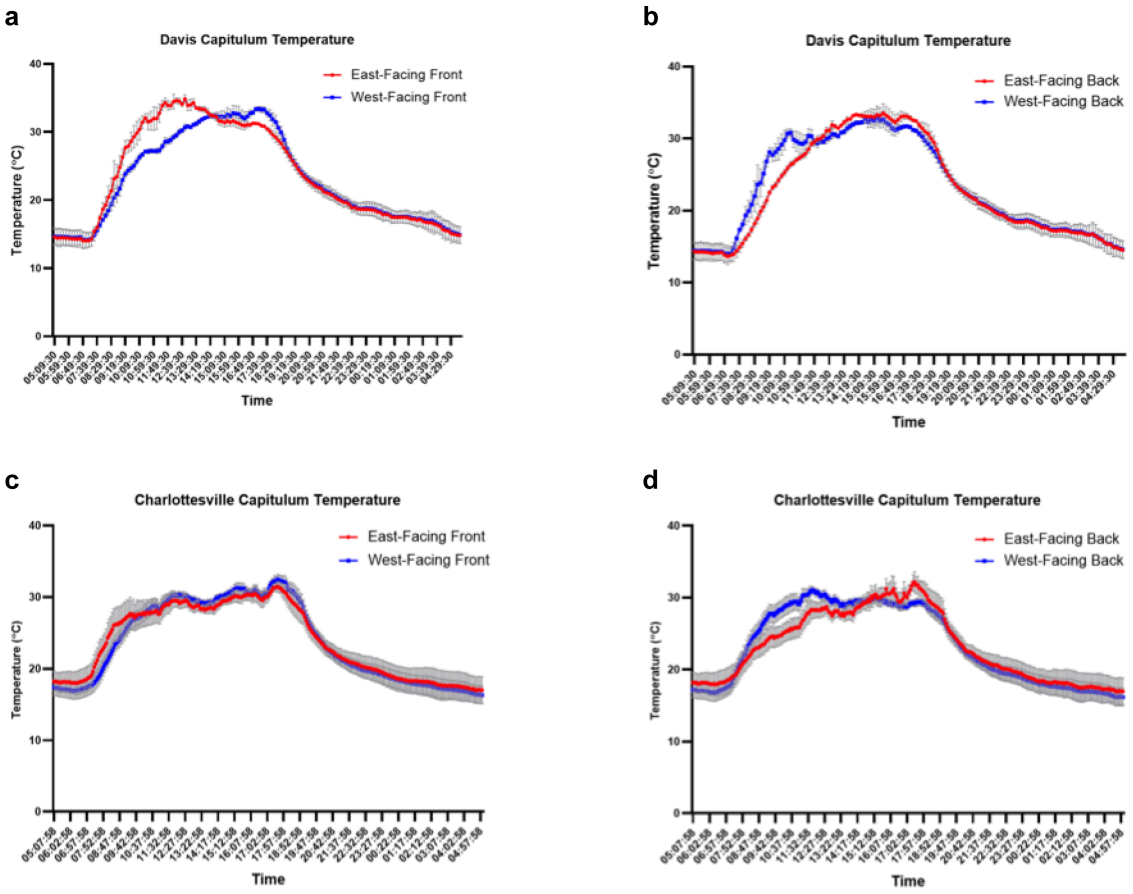


Fig. S6 Bayesian modeling of style growth. (A) Plots of actual and predicted growth. Each subplot is a different experimental day. Points represent averaged observations across the florets observed for a given treatment. Dashed lines represent predicted growth from the Bayesian growth model. Time 0 corresponds to ZT = -1. (B) and (C) parameter estimates for growth rate (k) and inflection point are plotted, +/- the 95% confidence intervals.

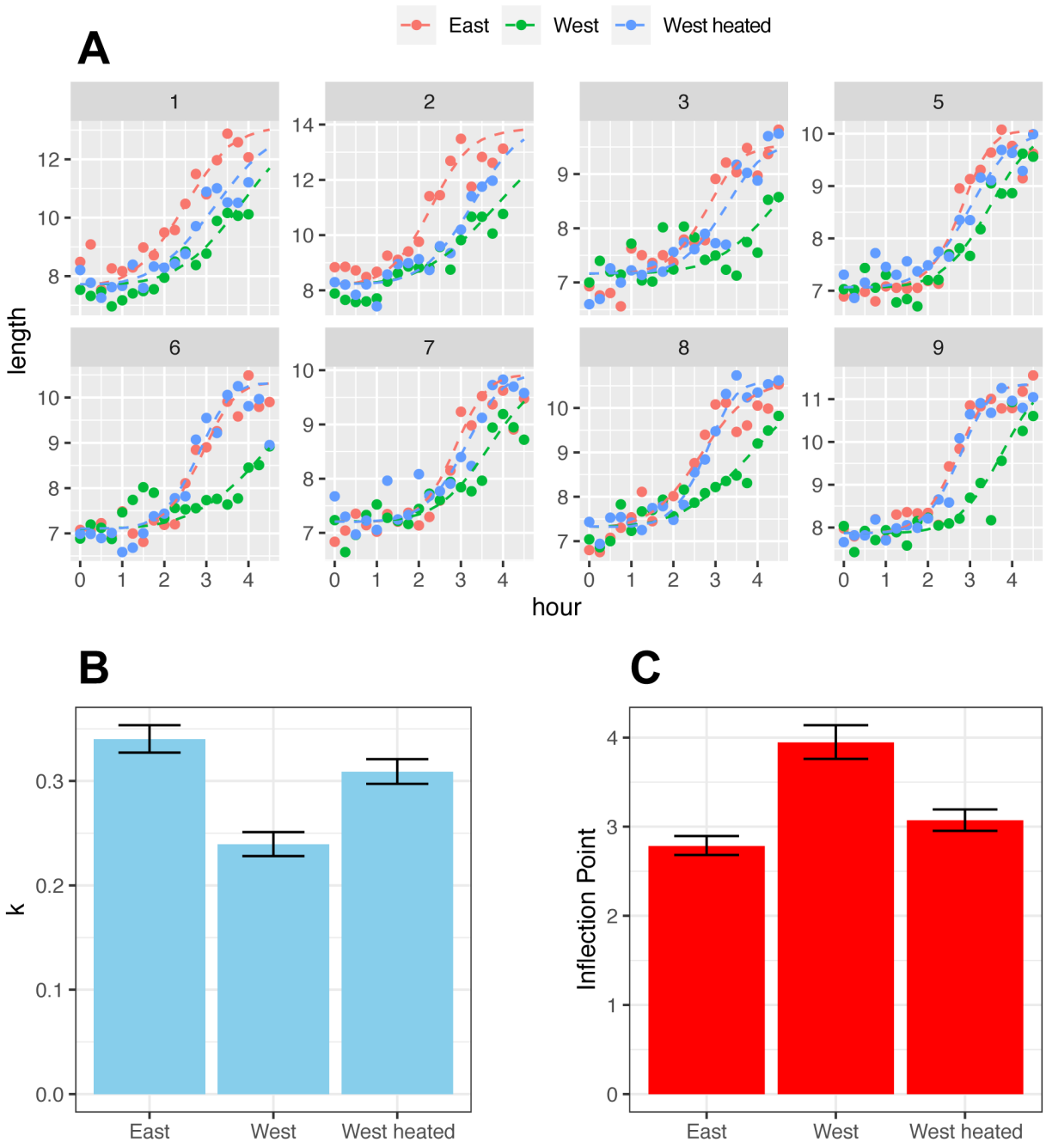


Fig. S7 Bayesian modeling of anther growth. (A) Plots of actual and predicted growth. Each subplot is a different experimental day. Points represent averaged observations across the florets observed for a given treatment. Dashed lines represent predicted growth from the Bayesian growth model. Time 0 corresponds to ZT = -1. (B) and (C) parameter estimates for growth rate (k) and inflection point are plotted, +/- the 95% confidence intervals.

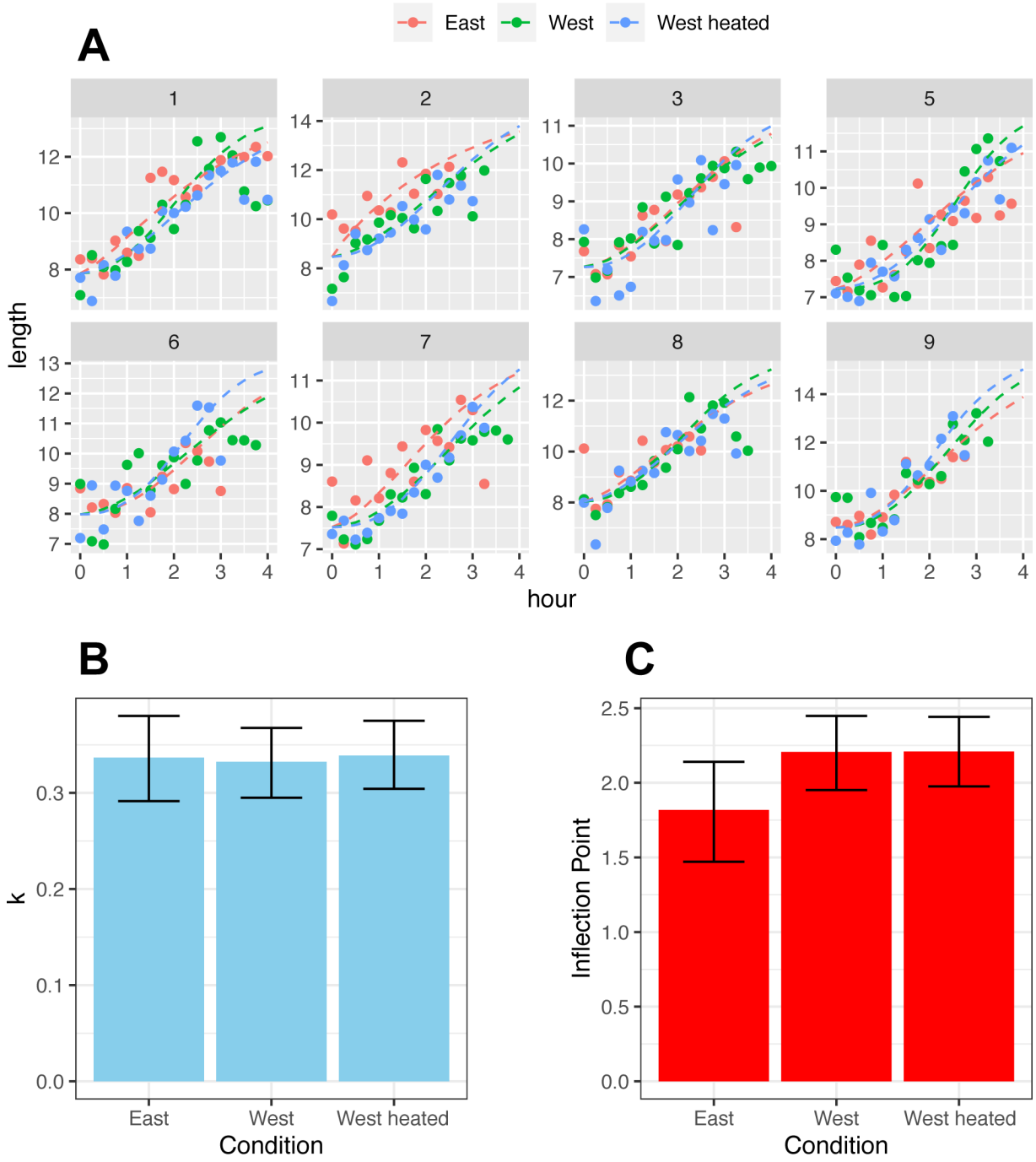


Fig. S8 Capitulum orientation has larger effects on floral temperature in Davis, CA than in Charlottesville, VA. Cumulative temperature of the heads over the morning from 8:49 – 11:49 (a, c) or afternoon from 15:39 – 18:39 (b, d) period on the fronts of east- and west- facing capitula (EF, WF) or the backs of east- and west-facing capitula (EB, WB) in Davis, CA (a and b) and Charlottesville, VA (c and d). Box edges represent the minimum and maximum values and the box mid-line represents the mean.

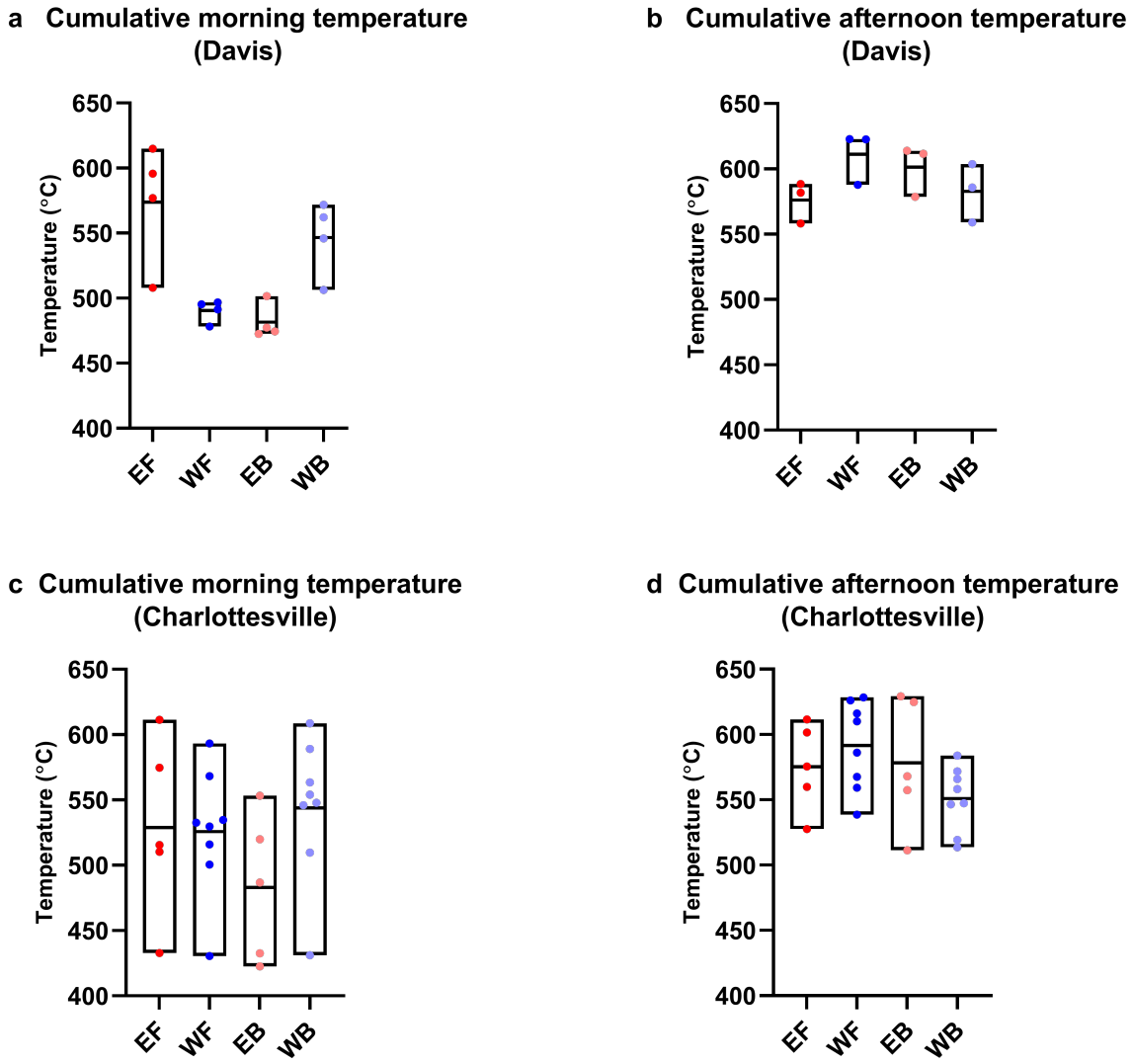
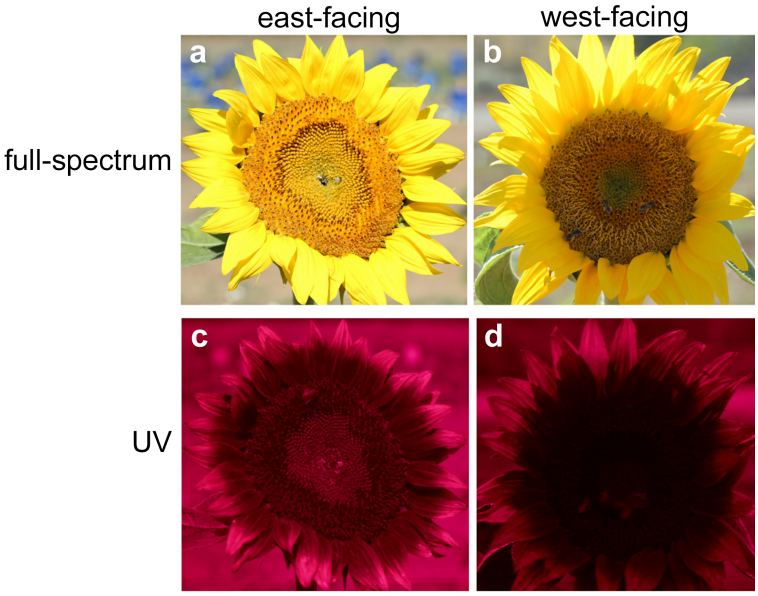


Fig. S9 Capitulum orientation in the morning changes sunflower visual aspects in both the visible and UV ranges of the spectrum. Photographs of east-facing (a, c) and west-facing (b, d) capitula were taken at ZT3 using a full-spectrum (400 – 700 nm) digital camera (a, b) or one modified to only detect light in the UV-A portion of the spectrum (350 – 400 nm).



Methods S1 Detailed description of plant growth conditions in field and in controlled environment chambers.

Plants were grown at the University of California, Davis Agronomy fields for the natural environment, pollinator, and environmental manipulation experiments. Seeds were germinated in growth chambers (Conviron, Winnipeg, Manitoba (MB), Canada) with 16 h days, 8 h nights at constant 25°C, and seedlings with 2-4 true leaves were moved to the glasshouse and transplanted into 19-liter pots with standard potting mix and one tablespoon of Osmocote Smart-Release Plant Food for Flowers and Vegetables (Scotts, Marysville, OH, USA). Seedlings were left for 2-3 d in a semi-covered shed for hardening and then relocated to the field site. At the field site, pots were half buried in the ground to maintain moisture and temperature during growth. Field-grown plants were drip irrigated every second or third day and were supplemented with MiracleGro (Scotts Miracle-Grow Company, Marysville, OH, USA) as required. Field trials were conducted in the summer months from May – October in 2015-2017. In controlled environment experiments, plants remained in plant growth chambers (Conviron, Winnipeg, MB, Canada) from germination to flowering. Chamber plants were watered every second or third day with a standard nutrient water mix. Upon flowering plants were moved to a second chamber for analysis under the indicated temperature regimes. In Charlottesville, Virginia (VA), seeds were first scarified and germinated in mid-May to early June in 2014 on moist filter paper in dishes before sowing in cell packs in the University of Virginia greenhouse for two to three weeks. Seedlings were then transferred to 19-liter paint buckets filled with topsoil mixed with 10% compost. *H. annuus* accessions from Oklahoma (ANN1272; USDA-GRIN ID: PI 468486) and Texas (ANN1811; USDA-GRIN ID: 659440) seeds were germinated on filter paper for one week and then transplanted into the soilless potting mix for seedling establishment. After three weeks, seedlings were planted into 19-liter buckets filled with a blend of sandy loam, organic compost, and mulch at the field site. Each bucket received 7.6 liters of water by drip irrigation each morning. Individual placement was randomized within a larger grid of domesticated sunflowers; plants were spaced 0.9 meters apart within rows and 1.5 meters between rows. For the experiments conducted under controlled conditions, plants were maintained in a large walk-in growth chamber (long-day conditions, fluence rate at head

between 180 and 280 µmol m^{-2} s^{-1} depending on plant height; light was provided by metal-halide and incandescent bulbs, 25 °C). A single capitulum was heated as described for field experiments and imaged alongside a non-heated capitulum to measure pollen extrusion, anther elongation and style elongation. Temperatures of individual capitula were monitored using thermocouple sensors as described above

Methods S2 Experimental methods for siring experiment.

Seeds from cytoplasmic male sterile line CMS HA89 (USDA Germplasm Resources Information Network ID: PI 650572) and fertile cultivars RHA 279 (PI 599763), R-188 (PI 607921), and RHA 397 (PI 597374) were germinated in several rounds from mid-May to mid-June in 2014 to ensure sufficient plants of multiple genotypes were co-flowering with CMS HA89 to allow for multiple siring trials. The three fertile genotypes were chosen from a larger set of 288 accessions based on their similarity in days to flowering and disk diameter in previous association mapping field trials of in Iowa, Georgia, and British Columbia (Mandel *et al.*, 2013). All heads were covered with bags constructed from Delnet 336 micron mesh (Delstar Technologies, Inc., Carol Stream, IL; PQ218) prior to anthesis to ensure that plants were pollinated only during a siring trial.

We performed seven experimental trials from July 25th to August 6th in 2014. Each trial involved four CMS HA89 plants, which were grouped with one facing each cardinal direction (Fig. 3a). This group was encircled at a distance of 3 m in each cardinal direction by pairs of bagged fertile plants from two different genotypes, one whose disks were oriented East in all pairs and another whose heads were oriented West (Fig. 3a and 3b). During each trial, the four CMS plants and the eight fertile plants were unbagged from 8am to noon on the day of the trial. In the three instances where it was possible based on co-flowering with enough additional CMS plants, we did a second trial with new CMS plants and the same sets of fertile genotype parent plants switched to face the opposite direction. All three pairwise comparisons of the fertile genotypes were tested in at least one trial, and reciprocal tests with the orientations of the sire genotypes switched were performed with the same sires for two of the three genotype

combinations. Both reciprocal tests were not completed for one genotype combination because we lacked enough remaining plants of each genotype that overlapped in flowering period to do so, despite our efforts to select lines with similar flowering times and multiple staggered germination rounds. The heads of all other flowering plants at the field site were bagged during the trials to prevent pollination of the CMS plants by plants not involved in the experiment.

The heads of CMS plants were harvested at maturity and dried in an oven at 40ºC after harvest. A subsample of seeds from all four CMS plants were germinated and grown in the greenhouse as above for several weeks for paternity analysis (n = 18 – 77 seeds per trial, mean = 52). We extracted DNA from leaf tissue of these seedlings and from seedlings of the CMS and three fertile parent genotypes using a modified CTAB protocol, and we screened six M13-tagged microsatellite markers (Tang *et al.*, 2002; Heesacker *et al.*, 2008) for parent diagnostic length polymorphisms by capillary sequencing on an ABI3130 instrument (Applied Biosystems). We then assessed the paternity of the CMS progeny with the two most diagnostic markers (primers and sequences indicated below). To test whether the ratio of progeny sired on CMS plants by east- and west-facing fertile parents differed from the expected 1:1 ratio, we performed chi-squared tests for each trial. We also tested the goodness-of-fit across all seven trials using a pooled G-test. Sequences of the primers used for genotyping are:

HT668:
Forward primer – CACGACGTTGTAAAACGACAGAACAACAAAGCAGCACCAAT
Reverse primer – CACCACCATAATCCTCATTTCC

ORS887:
Forward primer – CACGACGTTGTAAAACGACTCGAAAACGACTAATCCAACTTTC, Reverse primer – GAGCATGAACAAGAATTGACACA

Methods S3 Bayesian modeling of anther and style growth.

To determine if there were differences in the kinetics of style or anther growth in plants east-facing, west-facing, or artificially heated west-facing capitula, we fit a Weibull growth model (Weibull, 1951; Yang *et al.*, 1978) using the BRMS (Bürkner, 2017, 2018) package in R (R Core Team, 2021) as an interface to STAN (Stan Development Team, 2021a, 2021b). The model was parameterized as:

$$\mathrm{L_t} = L_{max} - (L_{max} - L_{min})^{e^{(-k \cdot t)^{\delta}}}$$

where L_t is the length at time t, L_{max} and L_{min} are the upper and lower asymptotes, k is the growth rate, and δ is related to the inflection point of the growth curve. In this parameterization the inflection point, I on the x-axis can be calculated as:

$$\mathrm{I} = \frac{1}{\mathrm{k}} \cdot \left(\frac{\delta - 1}{\delta}\right)^{\frac{1}{\delta}}$$

Observed style lengths shrank after the maximum length was reached, but this shrinkage cannot be fit with the Weibull model. Therefore, any datapoints occurring after the maximum length was obtained were set to equal the maximum length.

Leave one out information criteria was used to compare models. In the best-fit model for style growth, L_{max} and L_{min} were modelled with growth trial (i.e. day) as a random effect predictor, whereas k and δ were modeled with treatment as a fixed effect predictor and floret as a random effect predictor. To enable comparisons between anthers and styles, the same model was used for modeling anther growth.

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Table S1. Summary table of experiments and measurements.

Experiments	Location	Measurements	Field Season	Trial	Figure representing results
Seed traits (East vs West)	Davis, CA	Seed traits: • Capitulum diameter • Seed width • Seed weight • Seed number	2014, 2015, 2016	Trial 1 – mid-May germination Trial 2 – early June germination	Figure 2 and Figure S2
Seed traits (East vs West)	Charlottesville, VA	Seed traits: • Seed width • Seed weight • Seed number	2014	Trial 1 – early June planting Trial 2 – late June planting	Figure S2 and S3
Siring success	Charlottesville, VA	Offspring genotype: • Genotypes at two microsatellite markers	2014	Siring Trials 1 to 7	Figure 3a and Figure 3b
Pollinator visits (East, West and West Heated)	Davis, CA	Insect count time series: • Number of insects counted over 20 min at each time point • Temperature of plants	2016	Trial 1 and 2	Figure 3c and 3d
Pollen emergence (East, West and West Heated)	Davis, CA	Imaged flowers over time series: • counted florets with pollen	2017	Trial 1 and 2	Figure 4a and Figure 4b
Style and anther elongation (East, West and West Heated)	Davis, CA	Imaged florets and styles over time series • Measured lengths in images • Temperature of plants	2017	Trial 1 and 2	Figure 5a, Figure 5b, Figure 5c, Figure S6 and Figure S7
Style elongation and pollen emergence (Heated and Unheated)	Davis, CA	Imaged flowers over time series: • counted florets with pollen Imaged florets and styles over time series • Measured lengths in images • Temperature of plants	NA (Chamber experiment)	NA (Chamber experiment)	Figure 5d, Figure 5e and Figure 5f
Capitulum orientation wild *H. annuus* (East vs West)	Davis, CA	Imaged capitula every 5 minutes • Counted pollinator visits in all frames from ZT1 to ZT2	2019	Trial 1 – August germination	Figure S4a, Figure S4b and Figure S4c
East vs West	Davis, CA	Temperature logged every 10	2014	Trial 2	Figure S5a, Figure S5b,

temperatures (Front and Back)		minutes			Figure S8a and Figure S8b
East vs West temperatures (Front and Back)	Charlottesville, VA	Temperature logged every 5 minutes	2014	Trial 1 and 2	Figure S5c, Figure S5d, Figure S8c and Figure S8d
UV morning imaging (East vs West)	Davis, CA	Full spectrum images UV only images	2016	Trial 1	Figure S9

Table S2. Detailed statistical analysis of siring success of differentially oriented *Helianthus annuus* cultivars.

Trial	Parent Line		*y-axis indicator	Sire Genotype		E/W Ratio	Seedling Count	# Heads	d.f.	X^2	**X^2 *P-value*	G-statistic	**G-stat *P - value*
	East	**West**		**East**	**West**								
Siring 1	RHA 279	R-188	[b]S1 S2	27	8	3.38	35	3	1	10.31	**0.001**	4.730	**0.030**
Siring 2	R-188	RHA 279	[b]S2 S1	35	23	1.52	58	3	1	2.48	0.115	1.086	0.297
Siring 3	RHA 397	RHA 279	S3 S1	36	41	0.88	77	4	1	0.32	0.569	0.141	0.707
Siring 4	RHA 279	RHA 397	S1 S3	59	12	4.92	71	4	1	31.11	**0.000**	14.728	**<0.001**
Siring 5	R-188	RHA 397	S2 S3	43	24	1.79	67	4	1	5.39	**0.020**	2.372	0.123
Siring 6	R-188	RHA 279	[a]S2 S1	27	14	1.93	41	3	1	4.12	**0.042**	1.821	0.177
Siring 7	RHA 279	R-188	[a]S1 S2	15	3	5.00	18	2	1	8.00	**0.005**	3.793	*0.051*
Total G				242	125	3.38	367	23	7			28.6726298	**<0.001**
Pooled G									1			16.485266	**<0.001**
Heterogeneity G									6			12.1873638	0.058

* Indicator represented in y-axis of bar graph in Figure 3

***P*-values lower the 0.05 indicated by bold lettering in the X^2 P-value and G-stat *P*-value columns