

Multiple *Plantago* Species (Plantaginaceae) Modify Floral Reflectance and Color in Response to Thermal Change

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Abstract:

- *Premise of the study:* Understanding how plant reproduction responds to temperature has become increasingly important because of global climate change. Temperature-sensitive plasticity in floral reflectance is likely involved in some of these responses. Such plasticity, which underlies thermoregulatory ability, affects reproductive success in *Plantago lanceolata*. To see whether other *Plantago* species also show thermal plasticity in reflectance, we measured plasticity in *P. lagopus*, *P. coronopus*, *P. major*, *P. subulata*, *P. albicans*, *P. tomentosa*, *P. maritima*, and *P. weldenii*.
- *Methods:* We induced plants to flower at two temperatures in growth chambers and recorded floral reflectance (362–800 nm).
- *Key results:* All species were thermally plastic in visible and near-IR regions. Species and populations differed in response. Some showed greater variation in reflectance at warm temperature, while the reverse was true for others. Plasticity was greatest in the *P. lanceolata* clade. Cosmopolitan species were not more plastic than were geographically restricted species.
- *Conclusions:* The data suggest that (1) thermal plasticity is an ancestral trait for *Plantago*, (2) plasticities in visible and near-IR regions have evolved along different pathways within the genus, and (3) phylogenetic history partially explains this evolutionary divergence. Our data combined with those of previous studies suggest that global climate change will modify floral reflectance and color in many plant species. These modifications are likely to affect plant reproductive success.

Keywords: flower color | global warming | phenotypic plasticity | Plantaginaceae | *Plantago* | reflectance | temperature

Article:

Understanding how temperature affects the reproductive success of plant populations has become increasingly important in the face of global climate change. For this reason, many researchers have examined the associations between reproductive phenologies of plant populations and their local seasonal temperature patterns (e.g., Badeck et al., 2004; Sherry et al., 2007; Bertin, 2008; Körner and Basler, 2010). In contrast, much less is known about the thermal responses of other reproductive traits. Some researchers have shown that the thermal environment influences flower and fruit anthocyanin content in a variety of angiosperm taxa, including some crop species. Typically, warmer temperatures reduce anthocyanin content in reproductive tissue (e.g., Rabino and Mancinelli, 1986; Dela et al., 2003; Stiles et al., 2007). Consequently, when flowers and fruits develop at warmer temperatures, the color is less intense. Warmer temperatures can lower the expression of some anthocyanin biosynthetic pathway genes (e.g., Shvarts et al., 1997; Dick et al., 2011; Lai et al., 2011; Lin-Wang et al., 2011; Azuma et al., 2012).

In *P. lanceolata*, thermally sensitive anthocyanin production explains the phenotypic plasticity in floral reflectance in the visible (VIS) region (Stiles et al., 2007). Warmer temperatures reduce anthocyanin production in flowers, which increases reflectance in the VIS region, i.e., results in more lightly colored flowers. Moreover, warming also induces greater floral reflectance in the near-IR (NIR) region in *P. lanceolata*. The importance of these thermal responses is that floral reflectance influences the absorption of incoming solar radiation during the day. Reduced reflectance increases absorption and, consequently, helps to warm reproductive tissues during cool portions of the reproductive season (Lacey and Herr, 2005). Conversely, increased reflectance helps to cool tissues during warm periods. The temperature of reproductive tissues can affect reproduction generally (e.g., Hedhly et al., 2009), and temperature-sensitive plasticity, i.e., thermal plasticity, in reflectance affects reproductive success in *P. lanceolata* (Lacey et al., 2012).

The links between temperature and anthocyanin content, anthocyanin content and floral reflectance, temperature and floral reflectance, and floral reflectance and reproductive success suggest that the thermal environment could influence reproductive success via its effects on floral reflectance in many plant species. Currently, however, the connection between temperature and floral reflectance has been reported only for *P. lanceolata*. Therefore, to test whether floral reflectance is temperature-sensitive in other *Plantago* species, we measured the thermal sensitivity of reflectance in eight additional species of *Plantago*: *P. lagopus*, *P. coronopus*, *P. major*, *P. subulata*, *P. albicans*, *P. tomentosa*, *P. maritima*, and *P. waldenii* (Fig. 1; modified from Rønsted et al., 2002). For simplicity, we will refer to these species by their specific epithet. The study addressed several questions: (1) Does temperature affect floral reflectance in these species? (2) Do species differ in their thermal response? (3) Do thermal responses show evidence of evolutionary divergence within the genus? (4) Do phylogenetic history and geographic distribution help explain the divergence? Relevant to this last question, we tested two hypotheses: (1) Species closely related to *lanceolata* exhibit greater plasticity than do more distantly species; *lanceolata* is already known to be highly plastic. (2) Cosmopolitan species exhibit greater plasticity than do more geographically restricted species.

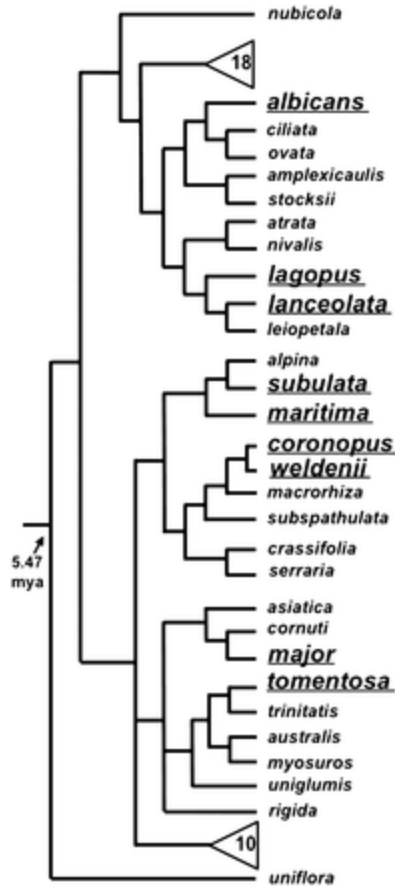


Fig. 1. Phylogenetic tree modified from Rønsted et al. (2002) to show the evolutionary relationships among the *Plantago* species used in this study. The larger font highlights the species used in this experiment. Numbers in the triangles represent the number of species examined by Rønsted et al. (2002) but not identified in this figure.

MATERIALS AND METHODS

Study species and seed sources

All species examined grow vegetatively as herbaceous rosettes, though they differ in leaf shape and size. All produce small flowers, tightly packed together in spikes, which are borne at the ends of stalks arising from leaf axils (Rahn, 1996). Previous studies have shown that in *lanceolata*, reflectance of a spike and its constituent flowers is fixed by ambient temperature at the time of spike development. However, reflectance is reversible at the individual plant level through the production of new flowers/spikes, which are formed at different temperatures throughout the reproductive season. Most *lanceolata* plants partially thermoregulate reproduction by producing poorly reflective (and darker) spikes/flowers in the spring and fall, when it is cool, and highly reflective (and lighter) spikes/flowers in the summer, when it is warm. (Lacey and Herr, 2005). Genetic variation in plasticity is explained by variation in ability to reduce reflectance in both the VIS and NIR regions at cool temperatures. Reduced reflectance in the VIS region darkens flowers.

The study reported here sampled three clades in the genus *Plantago* (Plantaginaceae), as described by Rønsted et al. (2002). *Plantago albicans* L., *P. lagopus* L., and *P. lanceolata* L. reside in what we will call the *lanceolata* clade (Fig. 1). *Plantago lagopus* is more closely related phylogenetically to *lanceolata*. *Plantago coronopus* L., *P. weldenii* Rchb. [synonym = *coronopus* subsp. *weldenii* (Rchb.) Arcang.], *P. subulata* L., and *P. maritima* L. belong to a second clade, which we will call the *coronopus* clade. *Plantago major* L. and *P. tomentosa* Lam. are found in a third clade, the *major* clade, which is more closely related to the *coronopus* clade than to the *lanceolata* clade.

All species are native to Europe except *tomentosa* (Rahn, 1996). *Plantago lagopus*, *subulata*, *albicans*, and *weldenii* are geographically restricted to the Mediterranean region. *Plantago coronopus* is native to Europe, western Asia, and North Africa, but is now established in North America and Australia. *Plantago major* and *lanceolata* are native to Eurasia, but are now naturalized throughout the temperate regions of the world. Thus, *coronopus*, *major*, and *lanceolata* have effectively cosmopolitan distributions in temperate regions. *Plantago maritima*, a highly salt-tolerant species, is also widely distributed in temperate regions. *Plantago tomentosa* is found in South America (Rahn, 1996).

Seeds were kindly sent to us by Dr. Dirk Albach and were collected from plants growing in the Mainz and Berlin Botanical Gardens, Germany and from natural populations encountered during European travels (Table 1). The original source for some species from the Mainz Botanical Garden is unknown. In these cases, we treated seeds as having come from a single population. Samples of *coronopus*, *major*, and *lagopus* came from 3, 2, and 2 populations, respectively. *Plantago coronopus* and *major* populations were combined for statistical analyses and also analyzed separately. The four *lagopus* plants were analyzed as belonging to one population because of the small sample size. Sample sizes (assumed different genotypes) per population = 2–15 plants (Table 1). Sample sizes were determined by the number of seeds that germinated per species.

Experimental design

Seeds were sown onto standard potting soil in small pots in a growth chamber set at 20°C, 12-h day/15°C, 12-h night. When seedlings were large enough, they were transplanted to 4-inch pots, and pots were randomly placed in the growth chamber. Light levels within the chamber averaged 330 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Plants were watered and fertilized 3×/wk. After several months, we changed the photoperiod to 15°C, 16-h day/10°C, 8-h night to induce flowering at a cool temperature. As plants began flowering, we collected one spike per plant and scanned it twice using a Shimadzu (Kyoto, Japan) spectrophotometer with an integrating sphere (see Methods in Lacey and Herr, 2005). Percentage reflectance of flower buds on a spike was recorded from 362 to 800 nm. At that stage, stigmas, flower petals, and anthers have not yet emerged from the sepals and subtending bracts.

Table 1. Sources of *Plantago* species and populations used for study shown by number of maternal families sampled for study, number of genotypes per family, and species/population symbols used in text and subsequent tables.

Symbol	Species	No. of maternal families	No. of genotypes	Seed Source
PLG	<i>P. lagopus</i>	2	4	Mainz Botanical Garden (original source of one genotype = Mallorca; others unknown)
PC1	<i>P. coronopus</i>	unk	6	Mainz Botanical Garden (original source unknown)
PC4	<i>P. coronopus</i>	1	13	Mallorca, Spain
PC11	<i>P. coronopus</i>	3	15	Bulgaria
PM1	<i>P. major</i>	unk	9	Mainz Botanical Garden (original source unknown)
PM10	<i>P. major</i>	unk	3	Bulgaria
PS	<i>P. subulata</i>	1	5	Mainz Botanical Garden (original source = Mont Rose, Marseilles, France)
PAB	<i>P. albicans</i>	2	6	Mallorca, Spain
PT	<i>P. tomentosa</i>	unk	7	Berlin Botanical Garden (original source = Sierra del Volcán, Balcarce Partido, Buenos Aires, Argentina)
PMR	<i>P. maritima</i>	1	2	Mainz Botanical Garden (original source = South Africa)
PW	<i>P. weldenii</i>	2	7	Berlin Botanical Garden (original source = Evia Island, Greece)
PL(IA)	<i>P. lanceolata</i>	10	10	Aprilia, Italy
PL(GJ)	<i>P. lanceolata</i>	10	10	Jena, Germany

- *Notes:* For seed source: unk = unknown. Maternal family = no. of maternal parent plants from which we germinated seeds. Genotype number = no. of experimental plants used per maternal parent. Samples for *P. coronopus* and *P. major* came from 3 and 2 sources, respectively. Data were analyzed for each *P. coronopus* and *P. major* population individually and for *P. coronopus* and *P. major* populations combined by species (PC and PM, respectively). *Plantago lanceolata* genotypes are random samples of genotypes used by Lacey et al., 2010.

After data had been collected for a plant in the cool-temperature treatment, we clipped all existing spikes, placed the plant in a warm-temperature chamber at approximately the same light level (27°C, 16-h day/20°C, 8-h night). Spikes were removed from the plant again after 5 d, and

then we allowed plants to produce new spikes, one of which was scanned. The fertilizer schedule remained the same, but plants in the warm chamber were watered more often to prevent desiccation. Previous experiments with these chambers indicate that any uncontrolled chamber effects on floral reflectance in *P. lanceolata* are negligible relative to thermal effects (E. P. Lacey, unpublished data). Geographic distributions of our experimental species and previous data for *lanceolata* suggest that the range of temperatures used in this experiment are typical of those experienced by all species during their natural reproductive seasons (cf. Lacey et al., 2010).

Data analysis

Statistical analyses were performed using SAS version 9.2 (PROC MIXED) to measure the effects of species and population nested within species on warm-temperature and cool-temperature reflectances at 550 nm and at 800 nm (VIS and NIR regions, respectively), and on plasticity at these wavelengths. Floral reflectance plasticity for each replicate plant was calculated as the absolute difference (magnitude of change) in percentage reflectance between the two temperature treatments. Thus, the individual plant was the experimental unit. Dependent variables were analyzed separately. We used the Tukey-Kramer procedure for pairwise means comparisons, which adjusts for multiple comparisons.

To explore whether intraspecific variation in reflectance was greater at warm vs. cool temperature, we calculated the coefficients of variation (CV) for warm- and cool-temperature reflectances at 550 and 800 nm for each species and population. A permutation test was used to assess statistical evidence that CV_{cool} is different from CV_{warm} . Under the null hypothesis that the distributions at warm and cool temperatures are identical, observations obtained within a unit are equally likely to be associated with warm or cool temperature. Thus, under the null hypothesis, the observed test statistic is assumed to be one of 2^n equally likely possible outcomes. A p -value for the observed test statistic can be calculated as the percentage of the 2^n possible outcomes at least as extreme as the observed value. The test statistic employed was $\max(CV_{cool}, CV_{warm})/\min(CV_{cool}, CV_{warm})$, which creates a two-sided p -value for testing for evidence that CV_{cool} is different from CV_{warm} . The program PopTools version 3.2 (Hood, 2011) was used to estimate the p -values, using 10000 randomly selected outcomes.

For our analyses, we included floral/spike reflectance data (same temperature treatments) previously collected from two European populations of *lanceolata* (Lacey et al., 2010). Because floral reflectance plasticity in this species declines with decreasing latitude, we chose a Mediterranean population and a northern European population, the latter located at approximately the same latitude as Mainz, where some of the experimental seeds of non-*lanceolata* species had been produced (Table 1). So that sample sizes of the *lanceolata* populations would resemble sample sizes for the other species, we randomly chose 10 genotypes per population to include in the analyses reported here.

To test whether phylogenetic history influenced reflectance plasticity, we contrasted *albicans* and *lagopus*, in the *lanceolata* clade, with species in the other clades (see Fig. 1). For the geographically “restricted” vs. “cosmopolitan” species comparison, we contrasted *albicans*, *lagopus*, *subulata*, and *weldenii* with *coronopus*, *major*, and *maritima*. *Plantago tomentosa* was not included in this latter contrast because it is not native

to Europe, as are the others, and because we could not find information about its geographical distribution in South America.

RESULTS

Spectrophotometric measurements showed that temperature affected floral reflectance in all species except *tomentosa* (Fig. 2). Species resembled each other in that the strongest thermal effects were in the visible (VIS) and near-IR (NIR) regions. However, the responses to thermal change in these regions differed across species and populations.

In the VIS region, warm-temperature reflectances were similar in value for all species, except *tomentosa*, whose reflectance was significantly lower (*tomentosa* mean $550_{\text{warm}} = 23.9\%$, means for other species = 30–39%; for all pairwise comparisons with *tomentosa*, $p < 0.0025$; Appendix S1, see Supplemental Data with the online version of this article). Reducing temperature strongly reduced the percentage reflectance in *albicans*, *subulata*, *major*, *lagopus*, and the *P. coronopus* population 1 (PC1). Reflectance plasticity in these species (= the difference between warm and cool percentage reflectance values) was statistically significant, i.e., the 95% CI did not include zero (Table 2). Thermal plasticity was highest in the PC1 population (Fig. 2: thick solid line = $PC1_{\text{cool}}$) and in *P. albicans*. The percentage reduction in reflectance from warm- to cool-temperature was -35% and -30% , respectively, which exceeded the reduction in both *lanceolata* populations. Thermal plasticity in *lagopus* approached that of the northern *lanceolata* population and surpassed that of the southern population. Other plastic species, including the two *maritima* plants, resembled the southern *lanceolata* population. Plasticity was visually apparent in *lagopus* and *albicans*, due to dark pigment deposition in subtending bracts at cool temperature (Fig. 3: plates 1–3). Also, floral bracts occasionally reddened at cool temperature on some plants in population PC1.

The NIR region showed greater variation than did the VIS region with respect to warm-temperature reflectance. Flowers in the *coronopus* PC1 and *weldenii* populations were the most reflective (mean $800_{\text{warm}} = 91.7\%$ and 90.9% , respectively), and *albicans* and *tomentosa* flowers were the least reflective (mean $800_{\text{warm}} = 77.1\%$ and 77.8% , respectively; Fig. 2). Other species and populations were intermediate in value. Floral reflectance in *weldenii* was significantly greater than in *albicans*, *tomentosa*, *coronopus*, and *major* (Appendix S1: adjusted pairwise comparisons: $P < 0.04$). The floral reflectance of *albicans* was significantly lower than that of all other species (adjusted pairwise comparisons: $P < 0.035$) except *tomentosa* and *lagopus* ($p > 0.16$). Among *coronopus* populations, PC1 flowers were significantly more reflective than were PC4 and PC11 flowers (mean reflectance at 800_{warm} for PC1, PC4, and PC11 = 91.1% , 82.9% , and 84.1% , respectively; Fig. 2: thin solid line = $PC1_{\text{warm}}$; pairwise comparisons in Appendix S1).

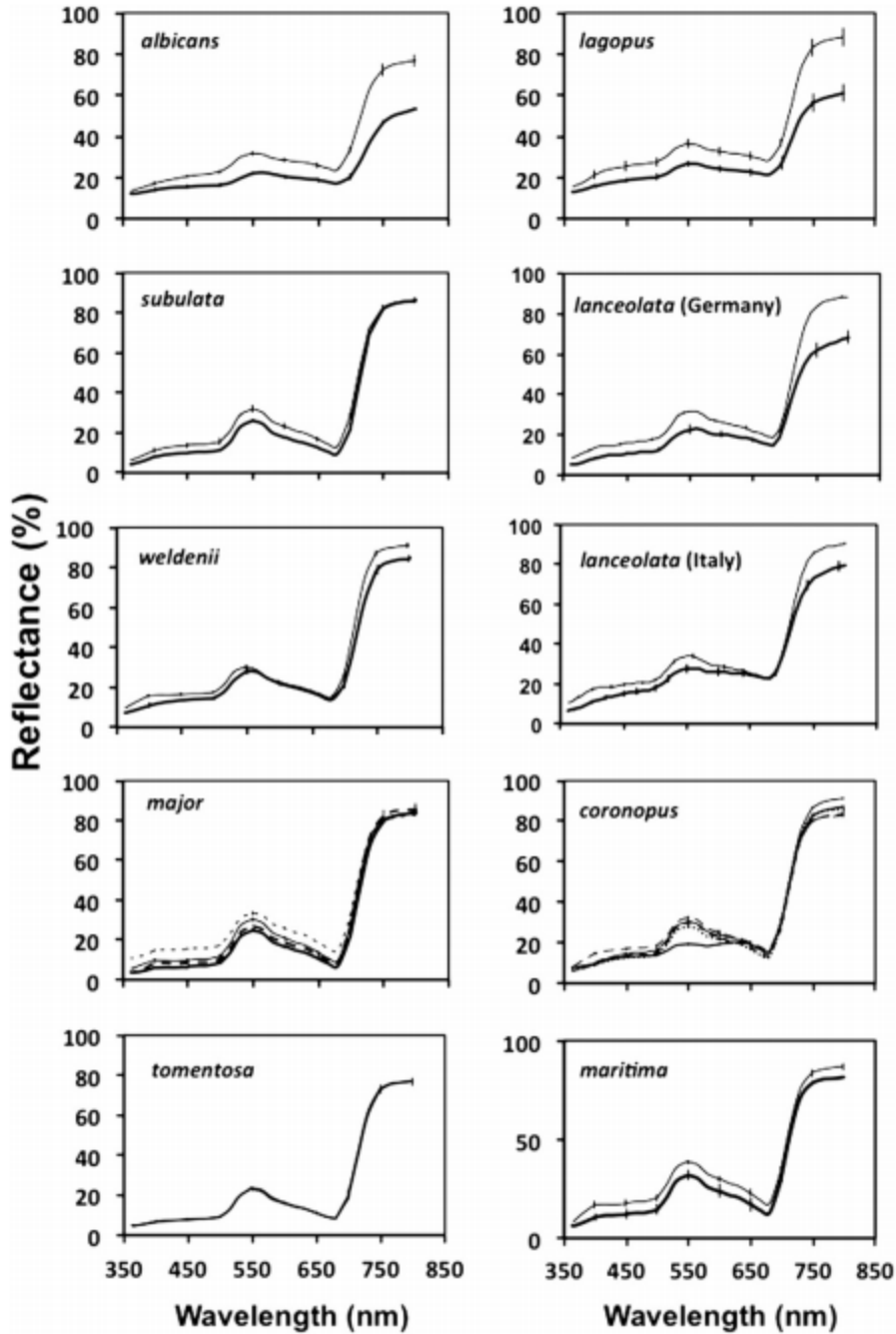


Fig. 2. Mean percentage reflectance of flower buds on spikes produced at warm and cool temperature shown by species and population within species. Temperature: cool = thick line, warm = thin line; Populations: PC1 = solid, PC4 = dashed, PC11 = dotted, PM1 = solid line, PM10 = dashed. Population symbols identified in Table 1. Vertical bars show ± 1 SE.

Floral reflectance in the NIR region was much lower at cool temperature than at warm temperature in *albicans*, *lagopus*, and *weldenii*, and reflectance plasticity was statistically significant in these species (Fig. 2, Table 2). The responses to thermal change in *albicans* and *lagopus* were significantly greater than in all other species, with the percent

decline in mean reflectance from warm to cool temperature being –31% in both *albicans* and *lagopus*. Although the plasticity in *weldenii* was statistically significant, it did not differ statistically from the plasticity in less plastic species (Appendix S1: for all adjusted pairwise comparisons: $P > 0.28$). No *coronopus* or *major* population showed evidence of plasticity in this region.

Table 2. Mean percentage reflectance at cool temperature, mean thermal plasticity, and mixed-model analysis t value for plasticity shown for each *Plantago* species and population at 550 nm and 800 nm.

Species	N	Wavelength 550 nm			Wavelength 800 nm		
		Percentage reflectance	Plasticity	t value	Percentage reflectance	Plasticity	t value
PL(GJ)	10	22.71	9.26***	5.50	68.45	20.27***	10.33
PL(IA)	9	27.68	6.37***	3.59	79.86	10.58***	5.11
PAB	6	22.14	9.62***	4.42	53.39	23.8***	9.39
PC [†]	34	27.3	3.29***	4.75	85.26	–0.37	0.40
PLG	4	26.72	9.66**	3.05	61.17	27.33***	7.38
PM ^{††}	12	25.21	5.88***	3.46	84.32	–0.72	–0.65
PMR	2	31.76	6.83 [†]	1.81	81.3	5.4	1.23
PS	5	25.99	5.88*	2.47	86.13	0.34	0.12
PT	10	24.1	–0.13	–0.08	77.89	–0.01	–0.00
PW	7	28.12	2.18	1.08	84.43	6.47**	2.76
PC1	6	19.34	10.55***	4.85	87.26	3.8	1.50
PC4	13	30.14	2.39	1.26	83.46	–0.53	–0.31
PC11	15	28.02	1.17	0.85	86.02	–1.91	–1.19
PM1	9	24.69	5.6**	3.15	83.87	–0.08	–0.04
PM10	3	26.77	6.71*	2.18	85.7	–2.61	–0.73

Notes: Species and population abbreviations identified in Table 1. [†]PC populations (1 + 4 + 11) combined; ^{††}PM populations (1 + 10) combined; Adjusted P values: * <0.05, ** <0.01, *** <0.001, [†]0.073 (marginally significant).

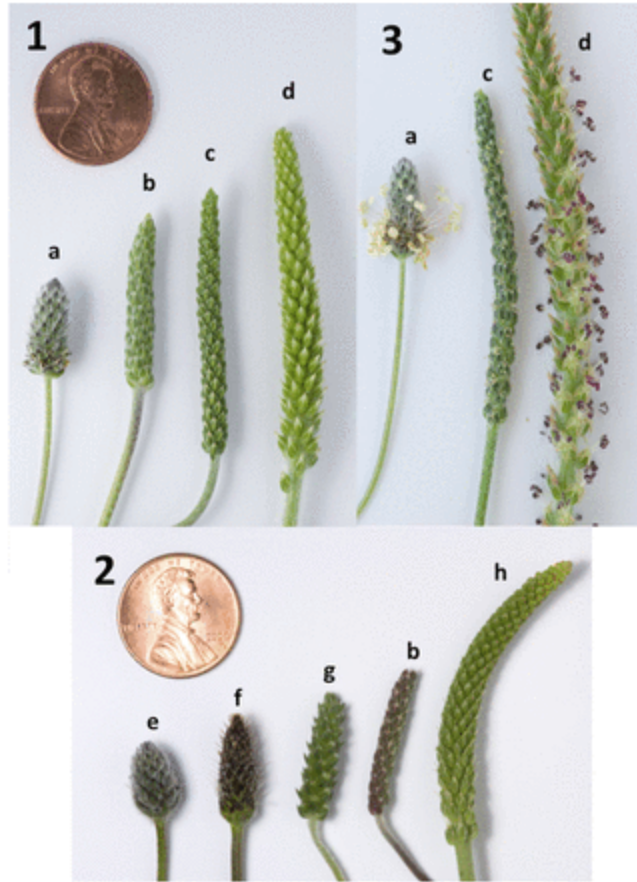


Fig. 3. Flowers on spikes of *Plantago* species produced in the cool temperature treatment, shown in color. Plates 1, 2. Spikes with flower buds. Plate 3. Spikes with some flowers in the male or female phases of anthesis. (a) *P. albicans*, (b) *P. coronopus*, (c) *P. weldenii*, (d) *P. tomentosa*, (e) *P. lagopus*, (f) *P. lanceolata*, (g) *P. subulata*, (h) *P. major*. U.S. penny diameter = 19 mm.

Intraspecific variation in reflectance at both 550 and 800nm was much greater at cool temperature than at warm temperature in *lanceolata*. (In this study, German population at 550 nm: $CV_{warm} = 8.58$, $CV_{cool} = 35.19$, $CV_{cool}/CV_{warm} = 4.10$, $P = 0.002$; at 800 nm: $CV_{warm} = 2.00$, $CV_{cool} = 15.56$, $CV_{cool}/CV_{warm} = 7.79$, $P = 0.002$; Italian population at 550 nm: $CV_{warm} = 12.31$, $CV_{cool} = 27.48$, $CV_{cool}/CV_{warm} = 2.23$, $P = 0.199$; at 800 nm: $CV_{warm} = 1.88$, $CV_{cool} = 9.59$, $CV_{cool}/CV_{warm} = 5.10$, $P = 0.013$.) Other species did not follow this pattern (Fig. 4). In the NIR region for *albicans*, variation was significantly greater at warm temperature (at 550 nm: $CV_{warm} = 8.12$, $CV_{cool} = 12.03$; at 800 nm: $CV_{warm} = 10.11$, $CV_{cool} = 3.67$, $CV_{warm}/CV_{cool} = 2.75$, $P = 0.029$). In the VIS region for *subulata* and the *coronopus* population PC1, there was greater intraspecific variation at warm temperature than at cool temperature, but the differences were not statistically significant (*subulata* at 550 nm: $CV_{warm} = 12.84$, $CV_{cool} = 8.57$, $CV_{warm}/CV_{cool} = 1.498$, $P = 0.69$; PC1 at 550 nm: $CV_{warm} = 22.39$, $CV_{cool} = 11.76$, $CV_{warm}/CV_{cool} = 1.904$, $P = 0.38$).

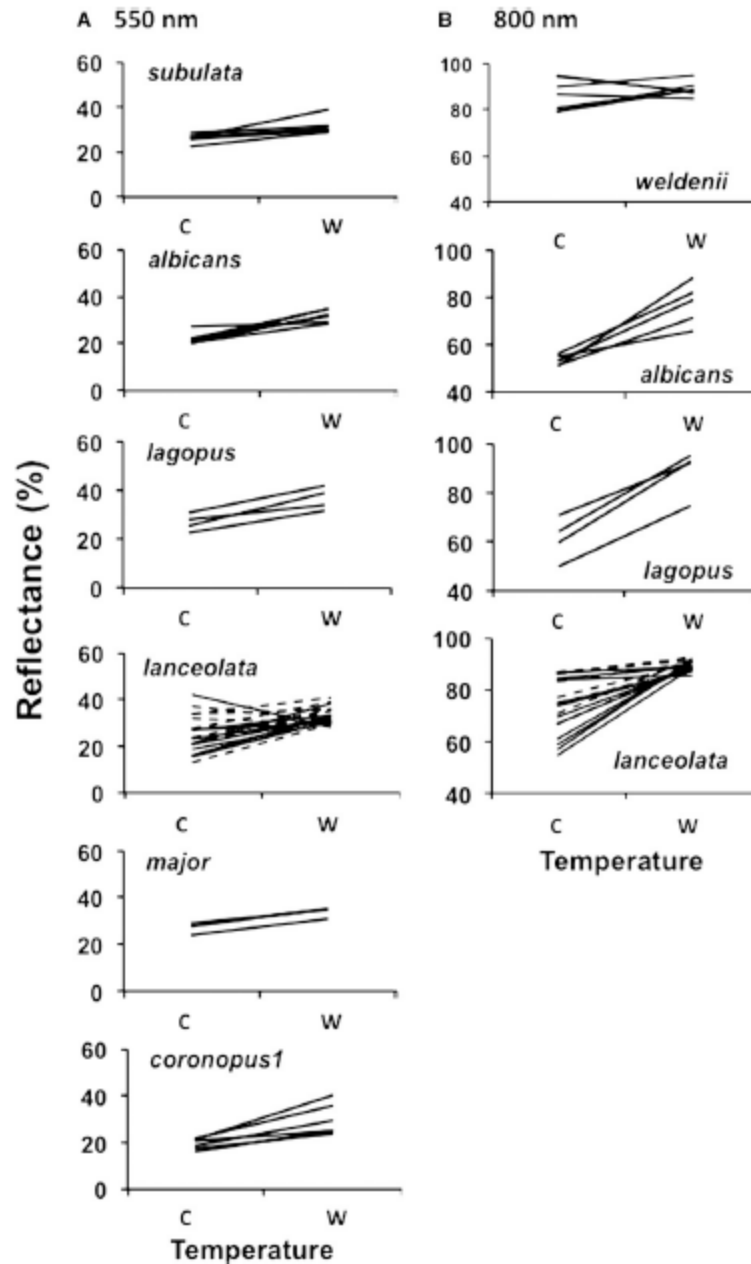


Fig. 4. Thermal reaction norms for species and populations exhibiting statistically significant plasticity in floral reflectance. Each line represents a single experimental plant. (A) 550 nm wavelength, (B) 800 nm wavelength. C = cool, W = warm. For *lanceolata* populations: PL(IA) = dashed lines, PL(GJ) = solid lines. Population symbols identified in Table 1.

The closest relatives of *lanceolata*, that is, *albicans* and *lagopus*, showed significantly greater plasticity than did more distantly related species in both the VIS and NIR regions (Figs. 2, 4; group means for plasticity₅₅₀ ± 1 SE: closer species = 9.64 ± 1.18, farther species = 3.42 ± 0.58; estimated difference = 5.66, $t = 3.39$, $P < 0.01$; group mean for plasticity₈₀₀ ± 1 SE: closer = 25.21 ± 2.28, farther = 0.52 ± 0.58; estimated difference = 23.71, $t = 13.21$, $P < 0.0001$). Cosmopolitan species did not differ significantly from geographically restricted species with

respect to plasticity in the VIS region (group mean plasticity₅₅₀ ± 1 SE: cosmopolitan = 4.09 ± 0.74, restricted = 6.41 ± 1.05; estimated difference = 1.49, $t = 0.95$, $P = 0.34$). However, they were less plastic than were restricted species in the NIR region (mean plasticity₈₀₀ ± 1 SE: cosmopolitan = -0.22 ± 0.62, restricted = 13.60 ± 2.65; estimated difference = 13.01, $t = 7.72$, $P < 0.0001$).

Also, when beginning the experiment, we had not considered the possibility that plants might alter anther color in response to temperature change. Therefore, we were surprised to see temperature-induced changes in anther color in three species. For all *tomentosa* plants, all newly emerging anthers were purple at cool temperature (Fig. 3, plate 3), but yellow at warm temperature. Most anthers were purple on some *major* plants at cool temperature, but yellow at warm temperature, and some *coronopus* plants produced some red anthers at cool temperature, but always produced yellow anthers at warm temperature. All other species produced only yellow anthers (e.g., Fig. 3, plate 3).

DISCUSSION

Our data show that temperature-induced modifications of floral reflectance are not unique to *lanceolata*. All examined species, except *tomentosa*, modified their floral spectra, i.e., reflectance and/or color, in response to thermal change. Flowers produced at warm temperature were more reflective than were flowers produced at cool temperature. Species showed the strongest thermal responses in the VIS or NIR region, or in both. These similarities suggest that thermal sensitivity in reflectance is an ancestral trait for the genus *Plantago*.

Nineteenth-century European naturalists observed that floral color in many herbaceous angiosperms darkens or intensifies with increasing latitude and altitude (Bonnier and Flahault 1878a, b; Flahault, 1878; Pellat, 1878; von Marilaun et al., 1894), and *lanceolata* shows this same pattern (Lacey et al., 2010). Such clinal variation could be explained either by genetically fixed population differences in floral color (i.e., reflectance in the VIS spectral region) or by population differences in level of thermal plasticity in the VIS, or both. Thus far, data support the latter explanation. In *lanceolata*, clinal variation in floral reflectance plasticity explains the clinal variation in mean floral color/reflectance per population (Lacey et al., 2010), and the *Plantago* species examined here also show thermal sensitivity in color. Additionally, temperature influences anthocyanin production (i.e., color) in other angiosperm taxa (e.g., Rabino and Mancinelli, 1986; Shvarts et al., 1997; Dela et al., 2003; Dick et al., 2011; Lai et al., 2011; Lin-Wang et al., 2011; Azuma et al., 2012). Together these studies support the hypothesis that thermal plasticity explains much of the latitudinal and altitudinal variation in floral color and reflectance in angiosperms. Additional studies will be needed to determine the relative contributions of plasticity. Such studies would help shed light not only on the effects of past climate but also on possible effects of current climate change.

Within the genus *Plantago*, species showed a variety of thermal responses. Although we cannot exclude parental environmental effects as a possible explanation for this variation, this explanation seems unlikely. Many seeds for the study were collected from plants of different species that had been grown in the same garden for at least one generation (Table 1), and this would have reduced parental effects arising from the original source locations for the seeds. For

similar reasons, parental effects do not likely explain the thermal responses in *lanceolata* (Lacey et al., 2010).

Two observations indicate that thermal responses have diverged among clades (Fig. 1). First, members of the *lanceolata* clade (*albicans*, *lagopus*, and *lanceolata*) responded most strongly to temperature change; they showed the greatest thermal plasticity. Second, this was the only clade to show significant thermal responses in both VIS and NIR regions. The responses of *albicans* and *lagopus* were more similar to *lanceolata*'s than were responses of other species. Consistent with these observations, we saw no evidence that the *lanceolata* clade altered anther color. In contrast, we observed that all *tomentosa* plants modified anther color. *Plantago major* and *coronopus* modified anther color, at least partially, as well as reflectance of flower buds.

There is also evidence of species' divergences within clades. Floral reflectance of European and U. S. genotypes of *lanceolata* is highly variable at cool but not warm temperature in the VIS and NIR regions (Lacey et al., 2010, 2012). This pattern is predicted if the intensity of selection acting on reflectance is weaker and/or more variable, either spatially or temporally, at cool temperatures than at warm temperatures. For this reason, we asked whether other *Plantago* species also show greater variability at cool temperature. They did not. Most strikingly, *albicans*, also in the *lanceolata* clade, showed statistically greater variability at warm temperature in the NIR region. *Plantago subulata* and the *coronopus* population PC1 showed a tendency for greater variability at warm temperature in the VIS region. If we had larger sample sizes for these populations, we might have detected statistically significant differences. Greater variation at warm temperature is predicted if the intensity of selection acting on reflectance is weaker and/or more variable at warm temperatures. A third pattern, a nonsignificant difference in variation between temperatures was observed in the other species and populations. We suspect that species-specific differences in the intensity of local selection pressures at warm and at cool temperatures explain these within-clade differences. Additional studies of these species would help to test this hypothesis.

Additional evidence for divergence at the species level comes from the observation that within the *coronopus* clade, species differed in the spectral region that responded to temperature change. Whereas *subulata* and *coronopus* PC1 exhibited thermal plasticity only in the VIS region, *weldenii*, formerly considered a subspecies of *coronopus*, exhibited plasticity only in the NIR region. This difference indicates that thermal plasticity in these two regions can evolve separately. What might favor the evolution of plasticity in one region over another is currently unclear. *Plantago* species are primarily wind-pollinated or selfing. Therefore, pollinators are not likely to be selective agents. Light quality and intensity might be selective factors, however. Reflectance influences the internal temperature of reproductive tissues when there is incoming solar radiation. On a sunny day, most incoming solar radiation hitting the Earth's surface lies in the NIR and IR regions (Gates, 1980). However, on cloudy days clouds capture so much NIR and IR solar radiation that the proportional representation of VIS radiation hitting the Earth's surface is greatly increased. Thus, cloudy regions may more strongly select for plasticity in the VIS region.

Because phenotypic plasticity in reflectance declines with decreasing latitude and altitude of European populations of *lanceolata* (Lacey et al., 2010) and because plasticity has been shown to be more adaptive in cool and short rather than warm and long reproductive seasons (Lacey et al., 2012), we had hypothesized that other species restricted to the warm Mediterranean region would show less reflectance plasticity than would species whose geographic ranges were broader and extended farther north. This hypothesis was not supported, in large part because two Mediterranean species in the *lanceolata* clade, *albicans* and *lagopus*, are highly plastic. At this point, phylogenetic history explains more of the observed diversity in thermal response than does a species' geographic distribution. We realize, however, that the number of species we examined represents a small proportion of species in the genus. Perhaps more importantly, for most species we sampled only one population. For the two species for which we had more than one sample population, *P. coronopus* populations did differ in thermal response. Therefore, had we been able to sample more populations of the other species, especially from different latitudes and altitudes of the cosmopolitan species, we might have found stronger support for the “geographic distribution” hypothesis. Expanding the study to include more species and more populations per species would allow one to test this hypothesis more rigorously.

Current global thermal trends are likely to alter the reproductive success in crop and noncrop plant species because temperature can influence the thermal microenvironment of flowers and developing fruits. The thermal microenvironment can affect gamete and offspring development, which can ultimately affect seed number, quality, and germination, and offspring fitness, independent of pollination mechanism (e.g., Akpan and Bean, 1977; Elgersma et al., 1989; Lacey and Herr, 2000, 2005; Hedhly et al., 2009; Whitney et al., 2011). Additionally, the thermal microenvironment can affect pollinator attraction in insect-pollinated species, which can sometimes affect reproductive success (e.g., Dyer et al., 2006; Faegri and van der Pijl, 1979; Fenster et al., 2004; Herrera, 1995; Kevan, 1975; Norgate et al., 2010; Rands and Whitney, 2008; Sapir, 2006; Seymour and Matthews, 2006; Whitney et al., 2008). Consequently, we need to understand, much better than we do currently, the mechanistic pathways by which temperature influences the thermal microenvironment and the subsequent effects on plant reproduction.

Additionally, we need to better understand temperature-sensitive plasticity in traits that can affect the thermal microenvironment: the extent of plasticity among seed plants, the fitness effects, and the mechanistic pathways underlying plasticity. On the basis of previous observations and the data presented here, we hypothesize that at least one of these plastic pathways involves anthocyanin biosynthesis and floral reflectance. Floral reflectance in UV and VIS regions influences pollinator attraction. Reflectance in multiple regions, including the NIR, influence internal flower and fruit temperatures. These combined effects are likely to influence reproductive fitness. Thus, clarifying the links between temperature, anthocyanin biosynthesis, floral reflectance, and reproductive success should help us better understand how plant species in natural and agricultural settings respond to seasonal variation in temperature during reproductive seasons and to global climate change in the longer term.

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